BIOREMEDIATION OF POLYCHLORINATED BIPHENYL AND PETROLEUM CONTAMINATED SOIL

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ABSTRACT: A six month study evaluated the effects of amendments to soil contaminated with polychlorinated biphenyls (PCBs) and petroleum hydrocarbons. The objective was to determine which amendments could enhance biodegradation of Aroclors 1242, 1248, and 1260 and reduce the concentration of total petroleum hydrocarbons (TPH). The amendments added were chicken manure, chicken manure plus 1,000 ppm biphenyl, kenaf, and kenaf plus white rot fungus (*Phanerochaete chrysosporium).* All treatments and control were replicated three times. Contaminated soil and amendments were place in 1.5 L amber dishes and kept in an environmental chamber. Moisture was adjusted every two days. Samples were collected at 45 day intervals for biological and chemical analysis. PCB concentrations for Aroclors 1242 and 1248 were significantly reduced in all treatments and the control by day 180. Only the kenaf plus white rot fungus treatment showed a significant decrease in Aroclor 1260 concentration from days 0 to 90, but increased by day 180. All treatments showed a significant reduction of TPH by the end of the study.

INTRODUCTION

Polychlorinated biphenyls (PCBs) are a widespread environmental pollutant. PCBs were produced as complex mixtures by the Monsanto Corporation from 1930 to 1977 (Hutzinger, 1974, Tryphonas, 1995) for a variety of industrial uses, including dielectric fluids in capacitors and transformers. Careless disposal practices and accidental spills have contaminated the soil, groundwater, lakes , and rivers in many areas of the world. PCBs may contain 209 possible congeners and are chemically and physically stable and resistant to degradation in the natural environment. The lipophilic properties of PCBs make them slightly soluble in water and readily soluble in oils which causes bioaccumulation in the fatty tissues of fish, birds, animals, and humans. Humans can be exposed through the ingestion of food or drinking water that has been contaminated by PCBs (Erickson, 1986).

Bioremediation of PCB contaminated soil is a difficult task due to the structure and level of chlorination and occurs primarily by co-metabolic mean (Raloff, 1987, Abramowicz, 1995, Fernandez et al., 1999, Smith et al., 1999). Only a select few fungi have been used to aerobically reduce the toxicity and quantity of the PCB or chlorinated phenyls (Bonaventura and Johnson, 1997, Bampus et al., 1987). PCB degrading microorganisms breakdown the less chlorinated congeners at a faster rate than the highly chlorinated congeners (Bokvajova and Burkhard, 1994). Degradation rates also increased when biphenyl was added to the contaminated soil. Biphenyl, which is unchlorinated PCB, can be an analogue enhancement to degrade PCBs because the

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microorganisms that degrade the biphenyl also cometabolize the more recalcitrant PCBs.

Degradation of Aroclors 1242, 1254, and 1260 was evaluated with varying nutrient levels and the addition of white rot fungus (*Phanerochaete chrysosporium*) (Yadav et al., 1995). Results showed that Aroclor 1242 degraded 56 to 60% regardless of media and nutrient contents. The data further showed that *Phanerochaete chrysosporium* was capable of transforming a variety of congeners with varying degrees and positions of chlorine substitutions. The congeners with *ortho*, *meta*, and/or *para* substitutions were degraded. *Phanerochaete chrysosporium* also showed substantial degradation of Aroclor 1260. This study concluded that the number and position of the chlorines, as well as the length of incubation, determined the amount of degradation.

The objective of this study was to determine the efficacy of white rot fungus (*Phanerochaete chrysosporium*), biphenyl, kenaf, and chicken manure for bioremediation of PCB and total petroleum hydrocarbon (TPH) contaminated soil.

MATERIALS AND METHODS

Soil contaminated with polychlorinated biphenyls and petroleum hydrocarbons was collected from a site in south Mississippi. The soil was spread on plastic and air dried under a hood for 24 hours. A 3.35 mm mesh screen separated the larger clods and trash. One thousand g of contaminated soil were combined with 1,500 g of clean soil and 500 g of sterile sand to improve soil porosity and reduce the contaminants concentration. The three soils were thoroughly hand mixed then put in a 3 L jar and placed on a rolling mixer for two hours. Two hundred g of the mixed soil were placed in 1.5 L amber glass dishes. The study consisted of five treatments that were replicated three times. The treatments were: 1.Control: 200 g of mixed soil/dish, 2.Chicken manure: 200 g of mixed soil amended with 5% (10 g) chicken manure (dry-weight basis)/dish, 3.Chicken manure + biphenyl: 200 g of mixed soil amended with 5% (10 g) chicken manure (dry-weight basis) and, 1000 ppm biphenyl/dish, 4. Kenaf: 200 g of mixed soil amended with 3% (6 g) kenaf/dish, 5. Kenaf + white rot fungus: 200 g of mixed soil amended with 3% (6 g) kenaf and white rot fungus (*Phanaerochaete chrysosporium*)/dish.

Fresh chicken manure was collected from a poultry farm and was spread on plastic to air dry under a hood for 24 hours. Moisture of the soil was adjusted to 70% of water holding capacity with deionized water. After drying the manure was placed in a grinder and ground into small fines. The core of kenaf was also dried and ground into small fines. White rot fungus was *Phanaerochaete chrysosporium* (PC, ATCC 24725), purchased from the American Type Culture Collection (Rockville, MD) and grown in potato dextrose broth. The white rot fungus was mixed with deionized water then ground in a blender for approximately 10 seconds. Thirty three mL of the solution were added to its treatment at day zero and every 30 days until day 150. After day 45 sampling, biphenyl was dissolved in acetone and added to the soil at a concentration of 1,000 mg/kg every 45 days until day 135. Acetone was used because it evaporates quickly. After moisture and amendments were added, the weights of the dishes were recorded. Moisture and aeration was provided every two days with deionized water and hand mixing. Each dish was placed in a Hotpack environmental chamber with 43.5 % relative humidity and at 28 C. A 35 g soil sample was taken at 45 day intervals for 180 days. EPA Method 3540 was used for soil extraction and the extracted samples were analyzed for PCBs by EPA method 8080 (U.S. EPA, 1986). Total petroleum hydrocarbon (TPH) concentrations were measured by modified Standard Method 5520-F (Clesceri et al., 1989). Complete randomized design with three replications for each treatment was used for this experiment. Mean comparisons were made using a least significant difference at the p = 0.05 probability level by the Statistical Analysis System (SAS) program (SAS Institute, Cary, N.C.).

RESULTS AND DISCUSSION

All treatments showed a significant decrease in concentration of Aroclors 1242 and 1248 (Figure 1). The chicken manure amended treatments showed faster degradation in the first 45 days compared to the other treatments. Overall, the chicken manure treatment showed the most degradation at day 180, but was not significantly different from the other treatments. This suggests, with proper moisture and mixing the concentration of Aroclors 1242 and 1248 will decrease, but the addition of nutrients will enhance degradation rates. This is in agreement with the findings of Bokvajova and Burkhard (1994).

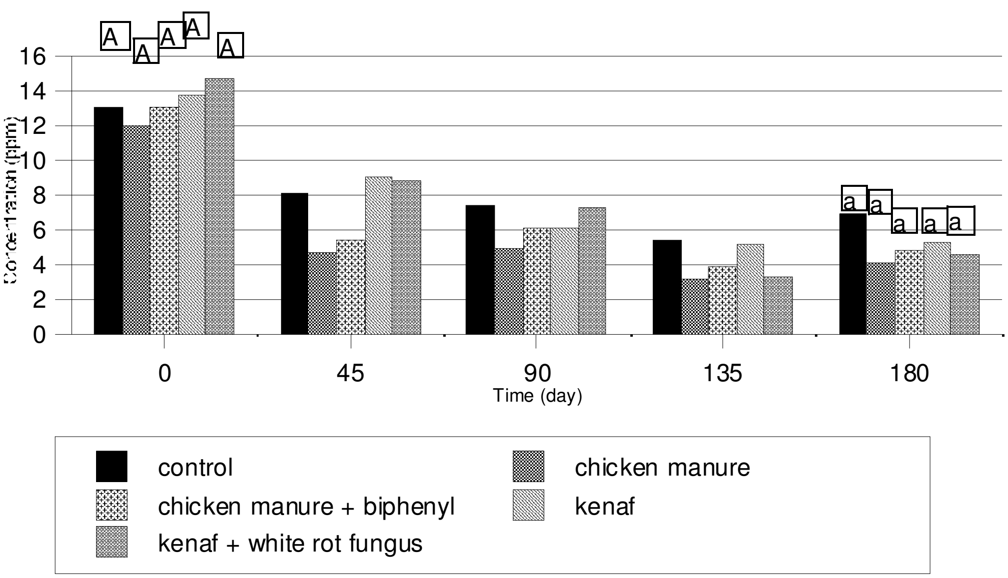

Figure 1. Degradation of Aroclors 1242 and 1248 in PCB contaminated soil. Means with the same letter indicate no significant difference at the P=0.05 probability level. Means with lower case letters indicate a significant difference between day 0 and 180.

Adding biphenyl or white rot fungus to enhance degradation does not seem to enhance degradation of the lower chlorinated PCBs. However, for the highly chlorinated Aroclor 1260, the kenaf plus white rot fungus treatments showed a significant decrease in concentration at day 90, but increased for days 135 and 180 (Figure 2). This may be attributed to procedure or heterogenicity of the soil

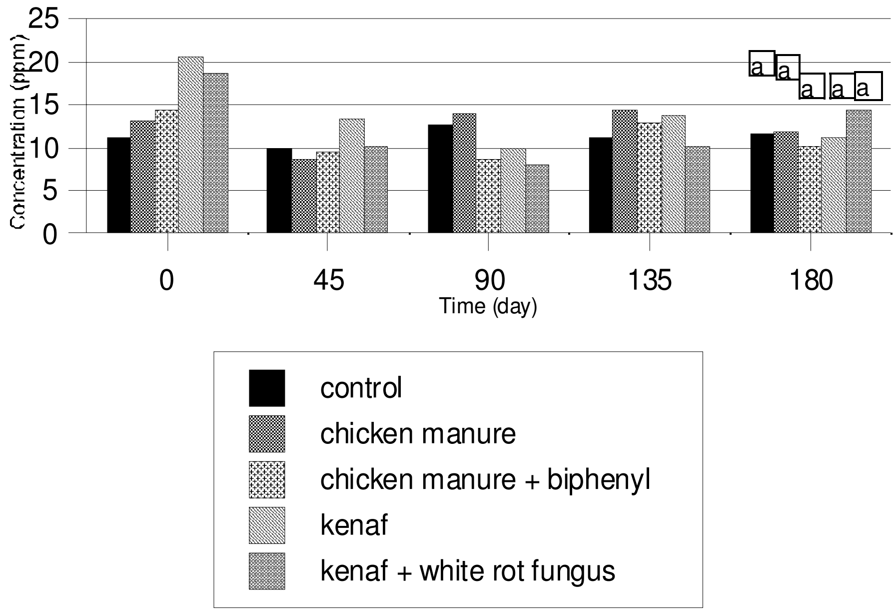

Figure 2. Degradation of Aroclor 1260 in PCB contaminated soil. Means with the same letter indicate no significant difference at the P=0.05 probability level.

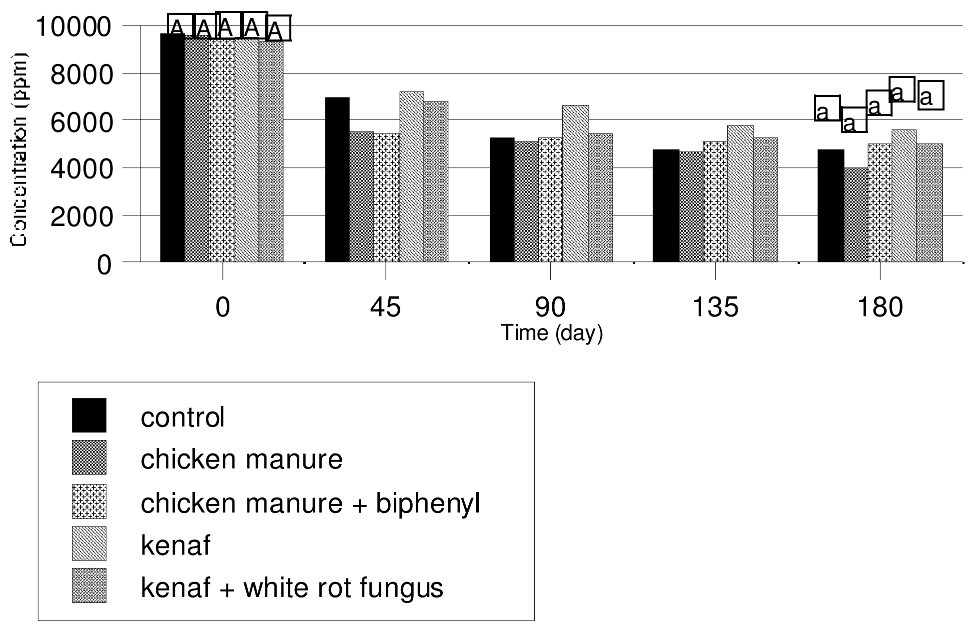

Figure 3. Concentration of total petroleum hydrocarbons from PCB contaminated soil. Means with the same letter indicate no significant difference at the P=0.05 probability level. Means with lower case letters indicate a significant difference between days 0 and 180.

since the increase was not significant. By day 180 there was no significant difference between any of the treatments. The chicken manure plus biphenyl, kenaf, and kenaf plus white rot fungus treatments showed lower concentration levels when compared to day 0, but were not significantly different.

Total Petroleum Hydrocarbons. All treatments showed a significant reduction of total petroleum hydrocarbon concentration by day 180 (Figure 3). The chicken manure treatment showed the lowest concentration, but was not significantly different than the other treatments. The results indicate that with sufficient moisture and aeration, the indigenous microorganisms are capable of degrading the petroleum hydrocarbons in PCB contaminated soil.

CONCLUSIONS

Adding chicken manure to PCB contaminated soil enhanced the degradation rate of Aroclors 1242 and 1248. Although the final concentration of all treatments was not significantly different, the chicken manure amended soil exhibited a lower concentration throughout the study. The results suggest that if the soil is provided adequate moisture and mixing, the concentration of lower chlorinated PCBs and total petroleum hydrocarbons (TPH) can be readily decreased. The contaminated soil in this study apparently contained a good population of indigenous microorganisms capable of degrading Aroclors 1242 and 1248 and TPH, but that might not always be true for all field studies. The addition of chicken manure as a nitrogen source may be necessary to increase microorganism populations at a contaminated site. Although the microbial count results on selective media is not presented here due to page limitations. The addition of chicken manure increased bacterial and fungal population.

Results for Aroclor 1260 showed no significant decrease from day 0 to day 180 for any of the treatments. The kenaf + white rot fungus treatment, however showed a significant decrease in concentration from day 0 to day 90, but concentration levels increased by day 180. No conclusive statement, therefore could be made based on these results.

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REFERENCES

Abramowicz, D.A. 1995. "Aerobic and anaerobic PCB biodegradation in the environment." *Environmental Health Perspectives*. 103(5) : 97-99.

Bampus, J.A., and S.D. Aust. 1987. “Bioremediation of DDT (1,1,1, - trichloro - 2,2 - bis (4 - chlorophenyls ethane) by the white rot fungus P. chrysosporium.” Appl. Environ. Microbiol. 53:2001-2008

Bokvajova, A. and J. Burkhard. 1994. “Screening and separation of microorganisms degrading PCBs.” *Environmental Health Perspectives*. 102(6-7):552-554.

Bonaventura, C. and F.M. Johnson. 1997. “Healthy environments for healthy people: bioremediation today and tomorrow.” *Environmental Health Perspectives.* 105:5-20.

Erickson, M.D. 1986. *Analytical Chemistry of PCBs*. p.1-53. Butterworth Publishers. Stoneham, Massachusetts.

Fernandez-Sanchez, J.M., R.A. Garciela, and V.R. Rodriguez. 1999. “Polychlorinated biphenyls transformation by bioaugmentation in solid structure.” In: *Bioremediation of Nitroaromatic and Haloaromatic compounds*. Alleman and Leeson (eds.) Battelle Press. Columbus, Ohia. pp. 174-178.

Hutzinger, O. 1974. *The Chemistry of PCBs*. p. 269. CRC Press. Cleveland, Ohio.

Raloff, J. 1987. “Detoxifying PCBs: everything from microbes to vitamin C is being considered in new approaches to degrade PCBs.” *Science News.* 132:154-155.

Smith, J.R., J.V. Fleckenstein, M. Mitraka., and U. Ghosh. 1999. “Long-term passive PCB/PAH bioremediation following active land treatment.” In: *Bioremediation of Nitroaromatic and Haloaromatic compounds.* Alleman and Leeson (eds.) Battelle Press. Columbus, Ohio. pp. 89-99

Tryphonas, H. 1995. “Immunotoxicity of PCBs (Aroclors) in relation to Great Lakes.” *Environmental Health Perspectives*. 103(9):35-46.

U.S. Environmental Protection Agency. 1986. Test methods for evaluating solid waste. SW-846. 3rd edition.

Yadav, J.S., J.F. Quensen III, J.M. Tiedje, and C.A. Reddy. 1995. “Degradation of polychlorinated biphenyl mixtures (Aroclors 1242, 1254, and 260) by the white rot fungus *Phanerochaete chrysosporium* as evidenced by congener-specific analysis.” Appl. Environ. Microbiol. 61(7):2560-2565.