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Solid-Phase Treatment of a Pentachlorophenol-Contaminated Soil Using Lignin-Degrading Fungi

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The abilities of three lignin-degrading fungi, *Phanerochaete chrysosporium, Phanerochaete sordida,* and *Trametes hirsuta,* to deplete pentachlorophenol (PCP) from soil contaminated with PCP and creosote were evaluated. A total of seven fungal and three control treatments were examined for their effect on the soil PCP concentration over eight weeks in two complementary field treatability studies at the Brookhaven Wood Preserving Facility in Brookhaven, MS. The fungi were applied to the soil as pure or mixed cultures at several different inoculum loading levels (inoculum:soil, w/w, dry). Inoculation of soil that contained 672 μg g^{-1} PCP and 4017 μg g^{-1} total measured polynuclear aromatic components of creosote with *P. sordida* at 10% resulted in the greatest decrease in PCP concentration (89%). PCP decreases by *P. chrysosporium* (67%–72%) or *T. hirsuta* (55%) at the same inoculum loading level were less extensive. The results of this study demonstrate that with further development, bioaugmentation using lignin-degrading fungi has the potential to be a viable treatment option for the remediation of PCP-contaminated soils.

Introduction

The ability of lignin-degrading fungi to transform and in many cases completely mineralize a wide variety of hazardous organic compounds has generated interest in using these organisms in the treatment of hazardous materials. We have focused on the development of a soil-remediation technology that is based on the pollutant-degrading abilities of these fungi. Results of previous work have demonstrated that inoculation of soils contaminated with the wood preservative pentachlorophenol (PCP) with selected fungi causes a significant decrease of the chemical in a variety of soils under both laboratory (1, 2) and field conditions (3). Field application of the fungal technology for soil remediation has also been demonstrated for the removal of polyaromatic hydrocarbons (PAH) from soil at the site of a former gas works installation (4). Significant removal, up to 80% of the total, of 16 priority pollutant PAHs was reported. Additional demonstrations of the efficacy of the fungal treatment in different soil types and on a variety of contaminants are necessary for the further development of this technology.

The objective of the present work was to evaluate the ability of several lignin-degrading fungi to deplete PCP and PAH Components of creosote in soil from a waste sludge pile that contained K001 sludge. This material is described as bottom sediment, sludge from the treatment of wastewaters from wood preserving processes that use creosote or PCP (5). The study was conducted at the Brookhaven Wood Preserving Facility, originally the Escambia Treating Co., that operated a pole treatment facility from 1946 to 1986. The facility is located on a 27.5-ha tract just north of Brookhaven, MS. In January 1986, the facility was sold to the employee-owned firm of Brookhaven Wood Preserving Inc. Escambia Treating Co. retained ownership of 6 ha that included a hazardous waste management unit (HMWU) that accumulated K001 sludge and a solid waste management unit.

The HWMU is an unlined impoundment constructed of a 4–7-ft earthen dike. The impoundment was used for evaporation of process wastewater from 1972 to 1984. The HWMU covers about three acres and contains a waste sludge pile at the southwestern corner. Escambia Treating Co. also formerly utilized two surface impoundments, a condenser pond and a sludge waste pit, which were located near the wood treating process area. Soil and sludge from these impoundments were excavated to a depth of 5 m, and the material was placed in the southwest corner of the HMWU. This excavated material constitutes the bulk of the waste sludge pile. Here we report the results of the effects of several fungal and control treatments on PCP concentrations in soil excavated from the waste sludge pile.

Experimental Section

Soil Characterization and Preparation. For determination of PCP concentrations in the waste sludge pile, soil samples were taken to a depth of 30 cm and were analyzed in duplicate for PCP using the protocol outlined in the sample analysis section. A composite sample consisting of soil from each of the sample locations was also collected and analyzed by the USDA Forest Service Soil and Plant Analysis Lab, Berea, KY, for soil chemical characteristics.

For the study, soil was excavated from the upper 40 cm of the section of the waste sludge pile from which samples were initially taken. The excavated soil was sieved through a mechanical sieve (Read Screen All RD408) to exclude materials >1.9 cm in diameter.

Fungi and Inocula. The fungi evaluated in this study were Phanerochaete chrysosporium Burds. (BKM-F-1767), Phanerochaete sordida (Karst.) Erikss. and Ryv., and Trametes hirsuta (Wulf.: Fr.) Pila't. Fungal strains used in this study were obtained from the culture collection of the Center for Forest Mycology Research, Forest Products Laboratory (FPL), Madison, WI.

Fungal inocula were produced by the L. F. Lambert Spawn Co., Inc., Coatesville, PA, using pure culture slants of each fungus provided by FPL. The inocula consisted of pure cultures of each fungus grown on a sterile nutrient-fortified grain-sawdust mixture (proprietary formulation: further referred to as "standard substrate"). The inocula, packaged in autoclavable bags that contained a microporous filter to allow adequate air exchange, were

Table I. Composition of Treatments in Completely Random Design (CRD) and Balanced Incomplete Block Design (BIB) Studies

		loading level (%)[a]	
CRD treatments	inoculum	inoculum	wood chips
1	*P. chrysosporium*	5	2.5
2	*P. chrysosporium*	10	2.5
3	*P. sordida*	10	2.5
4	*P. chrysosporium* and *T. hirsuta*	5 5	2.5
5	standard substrate	10	2.5
6	no amendement		
BIB treatments			
7	*P. chrysosporium*	10	2.5
8	*P. chrysosporium*	13	2.5
9	*P. chrysosporium*	10 (day 0) 3 (day 14)	2.5
10	*T. hirsuta*	10	2.5
11	chips only		2.5

[a] Loading levels are defined as dry weight of inoculum, standard substrate, or wood chips to dry weight of soil on a percentage basis.

delivered to the study area in a refrigerated truck. Each bag contained approximately 3 kg of inoculum. Three-kilogram bags of sterile standard substrate without fungus were also provided for use in a control treatment.

Chemicals. Pentachlorophenol (purity, 98%); $NA_2S_2O_6$, technical grade; and NA_2SO_4, anhydrous (purity, 99%) were obtained from Aldrich Chemical Co., Milwaukee, WI. Acetone and hexane were Burdick and Jackson brand high-purity solvents obtained from Baxter Healthcare Corp., McGraw Park, IL. Pentachloroanisole (PCA) was prepared by the reaction of PCP with diazomethane in ether.

Plot Construction and Preparation. An 18 m × 24 m area with an approximate 3% slope along the short side was constructed outside the HWMU, using a sandy clay material. Three 3-m wide drainage swales with 10% slopes from outer edge to nadir were cut into the incline. Eleven 3 m × 3 m plot borders with 0.70-m-high vertical side walls were constructed of no. 14 galvanized sheet metal. Five of the plots were subdivided into four 1.5 m × 1.5 m squares using 0.40-m-high sheet metal walls. The upper edges of these interior walls were flush with the upper edges of the exterior walls. The plot borders were placed over the drainage swales and sunken into the sandy clay material so that the tops of the side walls were level. Each plot was lined with 4-mil polyethylene sheeting and drained using a 3-m-long piece of 3.8-cm perforated PVC pipe that was placed on the liner in the middle of the drainage swale and connected to a drainage hose through a hole in the front wall of the plot. The drainage pipe was completely covered with gravel which was further covered with sand to form a level surface. This left approximately 45 cm to the top of the plot border. Each plot was then filled to a depth of 25 cm with sieved soil from the sludge pile. There was approximately 2.2 t (dry weight) of soil in each plot.

Experimental Design. The study was designed as two complementary experiments in which a total of seven fungal and three control treatments were evaluated for their effect, over 2 months, on the PCP concentration in the soil. In the first experiment, six treatments were evaluated in a completely random design (CRD) (Table I). Each of these treatments were randomly assigned and applied to the soil in one 3 m 3 m plot (Figure 1). During treatment application, the soil in each of the CRD plots

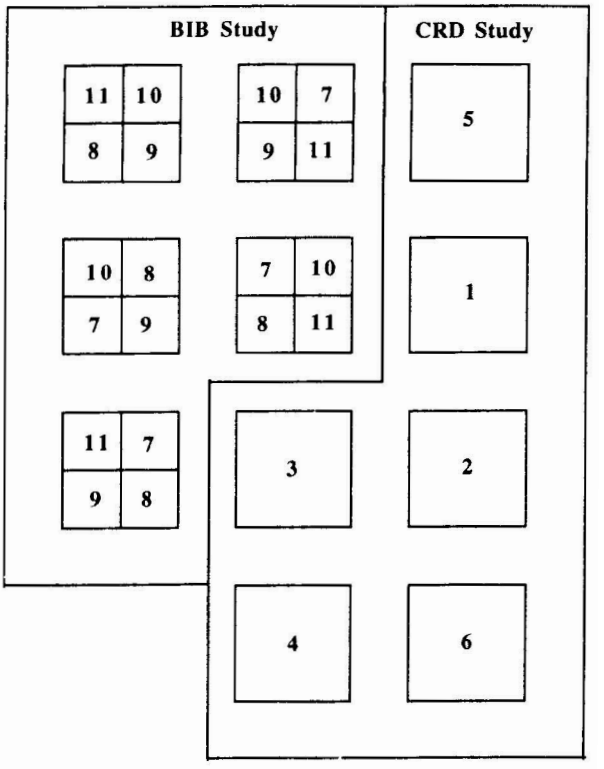

Figure 1. Arrangement of plots in the completely random design study and the balanced incomplete block design study. Numbers within plots refer to the treatment as assigned in Table I.

was physically divided into four 1.5 m × 1.5 m sections. These sections were then treated as replications during maintenance and sampling.

In the second experiment, five treatments were evaluated in a balanced incomplete block design (BIB). For this experiment each of the five 3 m × 3 m plots, hereafter referred to as blocks, were divided into four 1.5 × 1.5 m squares (Figure 1). Each treatment was randomly assigned to one square in each of four out of the five blocks. Thus, each treatment was replicated four times.

Treatment Application. On the day prior to application of fungal inocula and standard substrate, sterile aspen wood chips were tilled into the soil to a depth of 20 cm in all plots at a rate of 2.5% (w/w, dry weight basis), except soil in the nonamended treatment plot. Three days before application, the chips were sterilized by fumigating with methyl bromide. One day after chip application, the standard substrate and the three fungal inocula were tilled into the soil in assigned plots to a depth of 20 cm. All tilling was accomplished with a 9 HP rototiller. The rototiller was washed thoroughly between tillage of the different fungal and control treatments to prevent contamination. The integrity of the CRD sections was maintained over the duration of the study by tilling each one separately. The water content of the soil in each plot was adjusted to 20% immediately after treatment application.

Sampling. Soil samples were taken with a soil core sampler to a depth of 20 cm. The soil from each core was homogenized, and a subsample was taken to fill a 40-mL amber-glass sample container. Residual soil and chips were returned to the square or section from which they were taken. Soil and chip samples were stored at −20°C. Soil samples were taken 1 day after the soil was loaded into the plots (sample time 1); 1 day after chip application

(sample time 2); and 1, 7, 14, 28, and 56 days after application of fungal and control treatments (samples times 3–7). For the CRD study, five random soil samples were taken from each whole plot for samples 1 and 2. In addition, one pooled chip sample per plot, with the exception of the nonamended treatment plot, was obtained by randomly selecting chips from each of the five soil samples taken during sample 2. Three random soil samples were taken from each section for samples 3–7. For the BIB study, three random soil samples were taken from each square at each sample time. Beginning with sample 2 for the BIB study and sample 3 for the CRD study, one pooled chip sample per square or section, respectively, was obtained by randomly selecting chips from each of the three soil samples. The soil in each plot was rototilled to a depth of 20 cm immediately before sampling.

Plot Maintenance. Soil water content was determined gravimetrically daily and maintained, when necessary, at a minimum of 20% by the application of tap water. Soil and air temperatures were monitored daily at 8:00 a.m., 12:00 p.m., and 4:00 p.m. The soil in each plot was mixed once a week, using a combination of tilling and manual shoveling, to provide aeration.

Sample Analysis. Two 5-g subsamples per each soil sample and two 1-g subsamples per chip sample were analyzed. Chips were ground in a commercial coffee grinder prior to extraction and subsampling. Soil or chip samples to be analyzed were placed in 25 × 150 mm culture tubes with Teflon-lined screw caps. Approximately 100 mg of $Na_2S_2O_6$ was added to each tube. Soil and chip samples were then extracted for 1 h on a rotating tumbler shaker with two 20-mL volumes of a mixture of *n*-hexane–acetone (1:1) acidified to pH 2 with concentrated H_2SO_4. The extracts were pooled in a clean 18 × 150 mm culture tube, dried by passing them through a column of Na_2SO_4, and collected in a second culture tube. The Na_2SO_4 was prepared by muffling for 4 h at 400 °C. All glassware were muffled for 1 h at 450 °C prior to use. Culture tubes containing the extracts were placed in a Turbovap LV evaporator (Zymark Corp., Hopkinton, MA) held at 35 °C and the extracts evaporated to approximately 5 mL under nitrogen. The 5 mL, with hexane rinse, was then filtered through a Milex-SR 0.5-μm filter unit (Millipore, Bedford, MA) into a 10-mL volumetric flask. The extract volume was adjusted to slightly less than 10 μL with hexane. The extract was then filtered again through a Milex-SR 0.5-μm filter unit into a second 10-mL volumetric flask and brought to volume adjusted with hexane. Extracts were stored at −20 °C under nitrogen in amber vials fitted with Teflon-lined screw caps. Recoveries of PCP, based on recovery of [^{14}C]PCP spiked into soil samples prior to extraction averaged 80%.

Extracts were analyzed by gas chromatography for PCP and pentachloroanisole (PCA). Pentachlorophenol was analyzed as the trimethylsilyl derivative and quantitated using authentic derivatized standards. The derivatizing reagent was Sylon BTZ (Supelco Inc., Bellefonte, PA). Pentachloroanisole was quantified nonderivatized with authentic standards. Analyses of extracts were performed on Hewlett Packard Model 5890A or 5890II gas chromatographs equipped with ^{63}Ni electron capture detectors, Model 7673A autosamplers, and split–splitless capillary column injection ports. Operating temperatures for both instruments were injector 220 °C and detector 300 °C; carrier gas, He; and makeup gas, N_2. The columns were

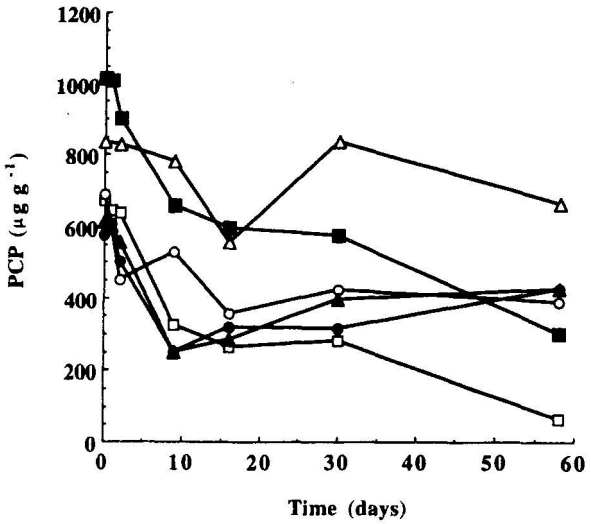

Figure 2. Concentration of FCP (μg g^{-1}) over time in soil receiving CRD treatments. Symbols represent soil inoculated with *P. chrysosporium* 5% (●), *P. chrysosporium* 10% (■), *P. sordida* 10% (□). *P. chrysosporium* 5%, and *T. hirsuta* 5% (▲) or soil receiving standard substrate 10% (O) or no amendments (Δ).

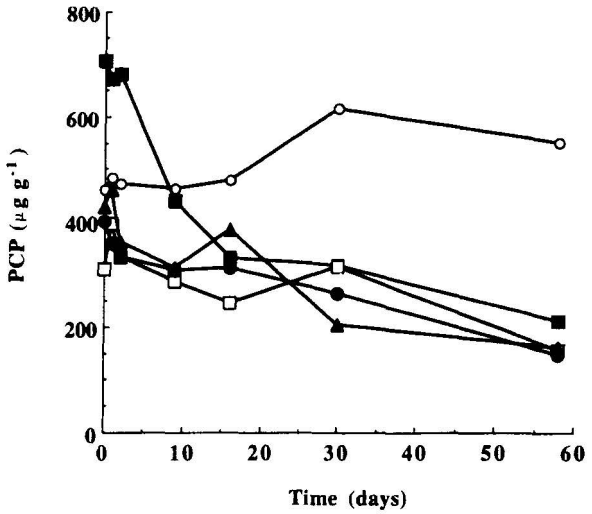

Figure 3. Concentration of FCP (μg g^{-1}) over time in soil receiving BIB treatments. Symbols represent soil inoculated with *P. chrysosporium* 10% (■), *P. chrysosporium* 13% (□), *P. chrysosporium* 10% day 0 followed by 3% day 14 (▲), *T. hirsuta* 10% (●), or soil amended with chips only (O).

30 m × 0.321 mm DB-5 fused-silica capillary columns, film thickness 0.25 μm (J & W Scientific, Folsom, CA). Injections were splitless with split off for 1 min. The temperature program was initial temperature 60 °C; hold for 1 min; ramp A, 10 °C min^{-1} for 9 min (60 °C to 150 °C); ramp B, 2 °C min^{-1} for 20 min (150 °C to 190 °C); and hold at 190 °C for 5 min.

Statistical Analysis. Initial PCP concentrations varied greatly among treated soils (see Figures 2 and 3). Therefore, the percentage of PCP remaining in the soil after 56 days was used as the dependent measure of interest to evaluate treatment effects on the PCP concentration. The concentration of PCP at sample 3 was used as the initial level because it was the first sample collected after treatment application. For the CRD study, the mean percentage PCP was obtained for each section by averaging the six values (two subsamples per three samples for each section) from each section to adjust for the non-normal distribution of PCP concentration. The statistical equality

Table II. Effect of CRD and BIB Treatments on Percentage of PCP Remaining in Soil after 56 Days[a]

treatment	initial PCP concn (μg g^{-1})	PCP remaining (%)[b]
Completely Randomized Design		
P. chrysosporium (5%)	576	85c
P. chrysosporium (10%)	1016	33ab
P. sordida (10%)	672	11a
P. chrysosporium (5%) and *T. hirsuta* (5%)	615	77bc
standard substrate (10%)	687	86c
no amendments	736	85c
Balanced Incomplete Block Design		
P. chrysosporium (10%)	705	28a
P. chrysosporium (13%)	311	48a
P. chrysosporium (10%, day 0) (3%, day 14)	428	45a
T. hirsuta (10%)	399	45a
chips only (2.5%)	458	114b

[a] If the ANOVA showed a significant difference among treatment means, Tukey's multiple comparison test was used to determine treatment differences. [b] Treatment means within experimental design followed by the same letter are not significantly different (a = 0.05).

of CRD treatments was tested using an analysis of variance (ANOVA) (a = 0.05) on the section means. Similarly for the BIB study, the mean percentage PCP was obtained for each square by averaging the six values (two subsamples per three samples for each section) from each square to decrease non-normality and the possible influence of outliers, and the average was adjusted for blocks. This adjustment was necessary because each treatment occurred in only four out of the five blocks *(6).* An ANOVA (a = 0.05) was performed to test for equality of mean response of each BIB treatment. If by ANOVA either CRD or BIB treatment means were shown to be significantly different, a Tukey multiple comparison test (a = 0.05) was performed to determine which treatments were different from the others.

Results

Chemical Characteristics in Sludge Pile Soil. Soil from the waste sludge pile was a clay with the following chemical characteristics: pH = 3.8; CEC 8.87 mequiv 100 g^{-1}; base saturation 54.8% ; 0.04% total nitrogen, and 2.17% total carbon. The concentration of PCP in the samples taken to a depth of 30 cm, before soil was excavated from the waste sludge pile averaged 133 μg g^{-1} and ranged from 15 to 342 μg g^{-1}.

Soil Pentachlorophenol Concentration. The initial PCP concentration in soil after treatment application (sample time 3) in the CRD plots averaged 717 μg g^{-1} and ranged from 576 μg g^{-1} in soil inoculated with *P. chrysosporium* at 5% to 1017 μg g^{-1} in soil inoculated with the same fungus at 10% (Figure 2). After 56 days, only 11% (89% loss) of the PCP remained in soils inoculated with *P. sordida* (Table 11). This loss was significantly greater than in soils receiving other fungal or control treatments, except for soils inoculated with *P. chrysosporium* at 10% (Table II). Inoculation of the soil with *P. chrysosporium* at a rate of 10% resulted in a greater PCP decrease than in soils inoculated with the same fungus at a rate of 5% alone or in combination with *T. hirsuta.* This difference

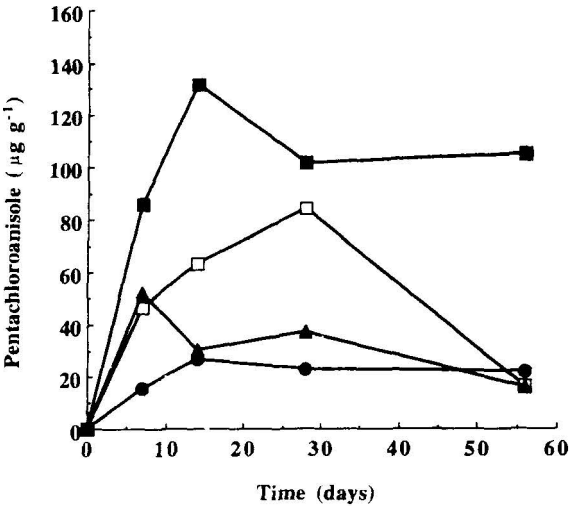

Figure 4. Concentration of PCA μg g^{-1}) in soil in the CRD study. Symbols represent soil inoculated with *P. chrysosporium* 5% (●), *P. chrysosporium* 10% (■), *P. sordida* 10% (□), *P. chrysosporium* 5%, and *T. hirsuta* 5% (▲).

was not significant, however, due to the extreme variability of the data. Decreases in the PCP concentration over the 8-week period in soils receiving either of the control treatments were slight. Thus, the PCP decreases observed in the fungal inoculated soils can be attributed, in large part, to the activity of the fungi.

Initial PCP concentrations (sample time 3) in soil in BIB plots averaged 436 μg g^{-1} and ranged from 333 μg g^{-1} in soils inoculated with *P. chrysosporium* at 13% to 681 μg g^{-1} in soils inoculated with *P. chrysosporium* at 10% (Figure 3). In soils inoculated with *P. chrysosporium* at 10% there was a steady decline in the PCP concentration resulting in a 72% decrease (28% remaining) after 56 days (Table II). This percentage decrease was comparable to the 68% decrease observed for the same treatment in the CRD experiment. The percentage of PCP remaining in soils receiving the other fungal treatments (treatments 8–10), after 56 days, was greater than that in soils inoculated with *P. chrysosporium* at a rate of 10% (Table II). However, differences in percentage PCP remaining among fungal treatments were not statistically different. No decrease in PCP concentration was observed in soils amended with chips only. After 56 days, the percentage PCP remaining in fungal inoculated soils was statistically less than that in the soil amended with chips only.

Soil Pentachloroanisole Concentration. Pentachloroanisole was not detected in initial soil samples (sample times 1 and 2) or at any time in samples taken from soils receiving control treatments. Pentachloroanisole was observed to accumulate in all fungal treated soils. Accumulation of PCA was coincident with PCP depletion (Figure 4). In both studies, the greatest amount of PCA accumulated in soils inoculated with *P. chrysosporium* at 10% (Figure 4, data from BIB study not shown). Accumulation of PCA was least in soils inoculated with *T. hirsuta* at 10% (data not shown) or *P. chrysosporium* at 5% (Figure 4). After peak accumulations, there were decreases in soil PCA concentrations in all fungal inoculated plots except those treated with P. chrysosporium at 5% or *T. hirsuta* at 10%, where PCA accumulations were negligible. The greatest PCA decrease (81%), after an initial accumulation, was observed in soil inoculated with *P. sordida* where the PCA concentration decreased

from 84 to 16 μg g^{-1} between day 28 and day 56 (Figure 4). Conversion to and accumulation of PCA only accounted for approximately 3% of the overall 89% PCP decrease in soils inoculated with this fungus. In contrast, 10% in the CRD study and 15% in the BIB study of the PCP decrease in soils inoculated with *P. chrysosporium* at 10% was due to transformation to and accumulation of PCA.

Chip Pentachlorophenol Concentration. Aspen chips were incorporated as part of the treatments because of their capacity to adsorb PCP (3) and as an additional substrate for the fungi. Although PCP was adsorbed rapidly by the chips after they were incorporated into the soil, the amount adsorbed did not cause a significant change in the soil PCP concentration. After 24 h, PCP concentrations averaged 50 and 84 μg g^{-1} in chips from CRD and BIB soils, respectively. After 56 days, the average PCP concentration in chips from CRD soils was 73 μg g^{-1} except in chips from the soils inoculated with *P. chrysosporium* at 5% (165 μg g^{-1}) and soils amended with the standard substrate (126 μg g^{-1}). The PCP concentration in chips from BIB soils, after 56 days, averaged 55 μg g^{-1} except in chips from soils receiving the chips-only treatment. The PCP concentration of these chips was 335 μg g^{-1}. Regardless of treatment, the total amount of PCP adsorbed by the chips after 56 days accounted for less than 1% of the total PCP present in the soil. Thus, loss of PCP due to adsorption to chips did not add significantly to the overall decreases.

Soil Temperature. In general, soil temperatures were greater in soils receiving the fungal treatments or the standard substrate than in the nonamended soil or soils receiving the chips-only treatment. During the first 41 days of the study, soil temperatures recorded at noon, fluctuated around an average of 22 °C after which there was a precipitous drop to an average of 8 °C for the last 15 days of the study.

Discussion

The results of this field study confirm those of an earlier field demonstration *(3)* of the ability of the lignin-degrading fungi *P. sordida* and *P. chrysosporium* to cause significant decreases of soil PCP concentrations in solid-phase treatment. In the present study, inoculation of soil that was contaminated by 672 μg g^{-1} PCP and creosote (total of 15 measured PAHs ca. 4017 μg g^{-1}) *(6),* with *P. sordida* resulted in an 89% decrease in the PCP concentration after8 weeks. Significant removalof PCP by lignin-degrading fungi, under field conditions, has now been demonstrated in a strongly acidic (pH 3.8) clay soil in the present study and in a strongly alkaline (pH 9.6) sandy gravel soil *(3).* These results suggest that utilizing lignin-degrading fungi for remediation of PCP-contaminated soil is a robust treatment technology which, after further development, may be applicable for remediation over a broad range of soil physical and chemical conditions.

In the present study, the residual PCP concentration in soil inoculated with *P. chrysosporium* was 64 μg g^{-1}. In a previous study, the average residual soil PCP concentration was 32 μg g^{-1} *(3).* Although remedial goals are site specific, these residual concentrations are greater than the suggested risked-based concentrations for PCP of 24 μg g^{-1} for commercial/industrial soil and 14 μg g^{-1} for residential soil *(8).* The extent of PCP removal was probably somewhat limited by the extremely low soil temperatures encountered during the present and previous field *(3)* investigations. Low soil temperature would restrict fungal growth and activity and, therefore, the length of the treatment period. However, a fraction of the residual PCP may have simply been unavailable to the fungi. In addition to providing environmental conditions that optimize fungal growth and activity, the development of techniques for enhancing the availability of contaminants to fungal degradative processes is necessary to facilitate more extensive contaminant removal.

Of the three tested fungi, *P. sordida* was superior in its ability to deplete PCP in soil from the waste sludge pile. The results obtained for decreases of PCP by *P. sordida* are consistent with the results of a previous study in which the inoculation of soil contaminated with 250–400 μg g^{-1} PCP with either *P. chrysosporium* or *P. sordida* resulted in an overall decrease of 88–91% of PCP in 6.5 weeks *(3).* In the present study, P. chrysosporium caused only 67–72% depletion of PCP. The difference in percentage PCP decreases caused by the two fungi in the present study was not caused by differences in initial concentrations because they were similar for soil in the BIB study inoculated with *P. chrysosporium* 10% (705 μg g^{-1}) and soil in the CRD study inoculated with *P. sordida* 10% (673 μg g^{-1}). Rather, the difference in the extent to which the fungi caused decreases in PCP concentrations may have been due to the ability of *P. sordida* to tolerate lower soil temperatures than *P. chrysosporium.* This is supported by *(i)* the substantially lower temperature optimum for growth of *P. sordida* (30 °C) *(2)* compared to *P. chrysosporium* (40 °C) *(9)* and *(ii)* the greater PCP decrease (44% decrease) in soil inoculated with *P. sordida* at 10% compared to that in soil inoculated with *P. chrysosporium* at 10% (29% decrease in the CRD treatment) during the last four weeks of the study when soil temperatures varied between 20 and 5 °C. In addition to contaminant-degrading ability, the ability to tolerate fluctuations in temperature and other environmental variables are important criteria for selecting fungi for use in the field.

Some of the decrease in PCP concentrations in fungal inoculated soils was caused by methylation, producing PCA. This would be an undesirable transformation if the PCA was not also degraded. However, after an initial accumulation phase, the concentration of PCA was decreased, greatly in soils inoculated with *P. sordida* and moderately in soil treatments containing *P. chrysosporium.* Methylation of PCP and other chlorinated phenols has been observed previously in liquid cultures of *P. chrysosporium (10, 11)* and in laboratory and field soils *(2, 3)* inoculated with *P. chrysosporium* or P. sordida. In liquid cultures of P. chrysosporium, approximately 16 % of PCP was converted to PCA, the only chlorinated transformation product found, but most of the PCP was degraded to nonchlorinated products *(11).* The rate of disappearance of PCA from these cultures was slow relative to the rapid rate of PCA removal observed in cultures that contained only the chloroanisole as substrate *(11).* Therefore, in both soil and liquid fungal cultures, methylation appears to compete with other PCP transformation reactions (e.g., oxidation) and slows the rate of PCP degradation. In laboratory soil cultures inoculated with *P. chrysosporium,* the amount of soil-bound versus an organic extractable PCP-transformation product, later identified as PCA *(2),* was greatly influenced by soil type *(1).* If the conditions

that favor methylation can be identified, it may be possible to enhance the efficiency of PCP degradation by manipulating soil conditions to limit methylation and favor oxidative PCP degradation.

Stimulation of indigenous contaminant-degrading microbes through nutrient amendments has generally been a more popular approach to soil remediation than bioaugmentation–the addition of previously-cultured contaminant-degrading microbes to contaminated soil. The use of bioaugmentation has been proposed for soils not supporting indigenous populations of contaminant-degrading microbes or for soils where environmental parameters cannot be manipulated to stimulate growth of these organisms. These situations are not uncommon, and examples of soils not supporting populations of indigenous PCP-degrading microbes have been reported *(12,23)*. The lack of a significant decrease in the PCP concentration in control soils in the present study suggests that Brookhaven and other similar sites may be good candidates for remediation using fungal bioaugmentation.

The results of this study and previous field *(3)* and laboratory investigations *(1, 2)* demonstrate that bioaugmentation using lignin-degrading fungi has the potential to be a viable treatment option for remediation of PCP-contaminated soils. Commercial application of this technology will require further developments in inoculum formulation, in production and application, and in enhancing the efficiency of contaminant removal.

Acknowledgments

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