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In Situ Depletion of Pentachlorophenol from Contaminated Soil by *Phanerochaete* spp.

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The ability of two white rot fungi to deplete pentachlorophenol (PCP) from soil, which was contaminated with a commercial wood preservative, was examined in a field study. Inoculation of soil containing 250 to 400 μg of PCP g^{-1} with either *Phanerochaete chrysosporium* or *P. sordida* resulted in an overall decrease of 88 to 91% of PCP in the soil in 6.5 weeks. This decrease was achieved under suboptimal temperatures for the growth and activity of these fungi, and without the addition of inorganic nutrients. Since the soil had a very low organic matter content, peat was included as a source of organic carbon for fungal growth and activity. A small percentage (8 to 13%) of the decrease in the amount of PCP was a result of fungal methylation to pentachloroanisole. Gas chromatographic analysis of sample extracts did not reveal the presence of extractable transformation products other than pentachloroanisole. Thus, when losses of PCP via mineralization and volatilization were negligible, as they were in laboratory-scale studies (R. T. Lamar, J. A. Glaser, and T. K. Kirk, Soil Biol. Biochem. 22:433–440, 1990), most of the PCP was converted to nonextractable soil-bound products. The nature, stability, and toxicity of soil-bound transformation products, under a variety of conditions, must be elucidated before use of these fungi in soil remediation efforts can be considered a viable treatment method.

Recently we reported that a white rot basidiomycete, *Phanerochaete chrysosporium* Burds., is capable of depleting pentachlorophenol (PCP) from three soils in the laboratory (19). Depletion was found to result mainly from transformation of PCP to nonvolatile products. The nature of these products—whether they were soil bound or extractable—was greatly influenced by soil type. Results of other laboratory investigations have demonstrated that 2,4,5-trichlorophenoxyacetic acid (25) and fluorene (13) are also depleted from soil by *P. chrysosporium*. Our objective is to determine the feasibility of using white rot fungi to remediate field soils that are contaminated with hazardous organic compounds. Towards this end, we have now completed a field study to determine whether the PCP-depleting activity of *P. chrysosporium* and another white rot fungus with PCP-degrading ability, *Phanerochaete sordida* (Karst.) Erikss. & Ryv. (R. T. Lamar, M. J. Larsen, and T. K. Kirk, submitted for publication), would also be expressed under field conditions. This paper summarizes that study.

The study was conducted in Oshkosh, Wis., at the site of a former "tank farm" where aboveground storage tanks were in service from approximately 1972 until 1984. The tank farm area measured ca. 14 by 30 m and was surrounded by a concrete berm that was 30.5 cm high and 30.5 cm thick (Fig. 1). The tanks were situated on a ca. 30-cm-thick gravel bed that overlaid a very strongly alkaline (pH 9.6) gravelly sand. Three 56,700-liter vertical steel tanks were on the west end of the storage area and were used to store a commercial wood preservative product. This product contained 84% mineral spirits, 0.8% to 1% paraffin wax, 10% alkyd varnish, and 5% technical-grade PCP (4.3% PCP) as the active ingredient. All tanks were removed from the site by 1984.

A preliminary screening of soil samples taken from the tank farm area for PCP and mineral spirits contamination was performed by Ayres Associates, Madison, Wis. Soil samples were collected to a depth of 30 cm below the soil surface. Sample locations were chosen on the basis of the reported pattern of material storage at the site. All samples suspected to be contaminated with wood preservative—those taken from the centers of the former 56,700-liter tank locations and from outside the north and west sides of the berm—were found to contain mineral spirits in concentrations ranging from 860 to 6,730 μg g^{-1} and PCP in concentrations ranging from 73 to 227 μg g^{-1}. The area within the berm occupied by the tanks, where the wood preservative was stored, was designated the study area. This area ran the width of the berm and 6.4 m down the length of the berm (Fig. 1).

The primary objective of this study was to determine, under field conditions, the ability of two white rot basidiomycetes to deplete PCP from the upper 30 cm of the contaminated soil. Pentachloroanisole (PCA) was also quantified since both *P. chrysosporium* and *P. sordida* are known to methylate PCP to PCA (Lamar et al., submitted). In this paper, we report on the effects of inoculating the contaminated soil with either of the two fungi on the concentration of PCP and PCA and on survival of the fungi during a 6.5-week period.

MATERIALS AND METHODS

Determination of PCP distribution. The PCP distribution was determined by a systematic sampling of the soil in the study area (Fig. 2). Soil samples were taken with a soil core sampler (7.6 by 15 cm) to a depth 15 to 30 cm below the soil surface. For sample locations 1 to 20, cores were collected to a depth of 30 cm. For sample locations 21 to 50, cores were collected to a depth of 15 cm. Soil cores were divided into 7.5-cm-long segments, and the soil from each segment was mixed thoroughly before being stored in 60-ml amber glass jars (I-CHEM Research, Hayward, Calif.). Samples were stored at −20°C. Analytical procedures are described below.

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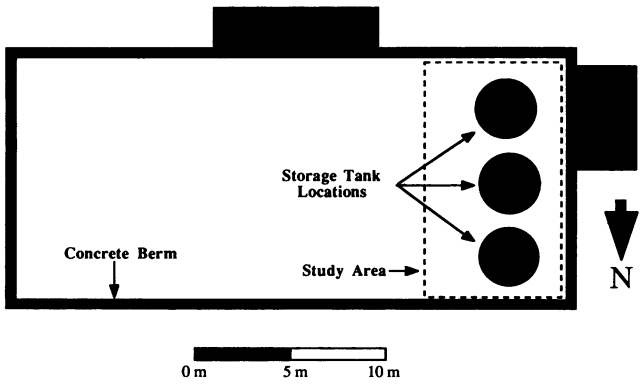

FIG. 1. Outline of tank farm area showing locations of study area and storage tanks reported to store commercial wood preservative that contained PCP as the active ingredient.

Fungi. *P. sordida* (Karst.) Erikss. & Ryv. and *P. chrysosporium* (BKM F-1767, ATCC 24725) were obtained from the Center for Forest Mycology Research, Forest Products Laboratory, Madison, Wis. *P. chrysosporium* and *P. sordida* were grown on 2% malt agar slants at 39 and 30°C, respectively, for 1 week and then stored at 4°C. Conidia from these slants were used to inoculate 2% malt agar inoculum plates. Inoculum plates were held at 39°C for *P. chrysosporium* and 30°C for *P. sordida* for 1 week before use.

Chemicals. Pentachlorophenol (purity, >99%); 2,6-dibromophenol (purity, 98%); 2,4,6-tribromophenol (purity, 99%); $Na_2S_2O_4$, technical grade (purity, ~85%); and Na_2SO_4, anhydrous (purity, 99%), were obtained from Aldrich Chemical Co., Milwaukee, Wis. Acetone and hexane were B & J brand high-purity solvents obtained from Baxter Healthcare Corporation, McGaw Park, Ill. Pentachloroanisole was prepared by reaction of PCP with diazomethane in ether. Silane-treated glass wool was obtained from Supelco, Inc., Bellefonte, Pa.

Inoculum preparation. Inoculum consisted of aspen (*Populus tremuloides* Michx.) chips (ca. 1.5 by 0.5 by 0.25 cm) that were thoroughly grown through with *P. chrysosporium* or *P. sordida*. Inoculum was prepared by sterilizing chips by autoclaving them at 121°C for 1 h in autoclavable bags (VWR Scientific, Philadelphia, Pa.). The moisture content of the chips was then adjusted to 60% by the addition of sterile, distilled water, and the chips were inoculated by exposing them to pieces of malt agar from inoculum plates. The bags were sealed with clamps fitted around black rubber stoppers. The stoppers were fitted with inlet-outlet ports that were protected from contamination by sterile, glass wool traps. The inoculated chips were kept at 39°C for *P. chrysosporium* and 30°C for *P. sordida* for 6 weeks prior to use. The atmosphere of each bag was evacuated with air for 15 min twice daily.

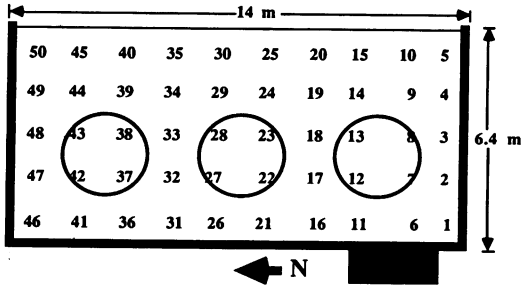

FIG. 2. Outline of study area indicating locations from which soil samples were taken to determine initial distribution of PCP in upper 30 cm of soil.

TABLE 1. Treatment composition

Treatment	Component[a]			
	Inocula		Sterile chips	Organic matter
	P. chrysosporium	*P. sordida*		
Fungal treatments				
A1	+	−	−	+
A2	−	+	−	+
Controls				
B	−	−	+	+
C	−	−	+	−
D	−	−	−	+
E	−	−	−	−

[a] +, Included in treatment; −, not included in treatment. All treatments included rototilling.

Soil preparation. Soil samples were submitted to the U.S. Department of Agriculture Forest Service's Plant, Soil and Water Laboratory for determination of chemical characteristics. Prior to soil preparation, the layer of gravel covering the contaminated soil in the study area was piled on an adjacent area. The gravel pile was covered with a polyethylene tarpaulin to prevent further contamination of underlying soil from gravel leachate. Results from the initial sample revealed that the PCP concentration in the soil varied greatly among sample locations and with soil depth. Also, areas that contained high concentrations of PCP also contained high concentrations of mineral spirits. Preliminary experiments demonstrated that the mineral spirits completely prevented the growth of *P. chrysosporium* (unpublished results). Therefore, the study area was rototilled and the soil was physically mixed to a depth of 30 cm to equalize the concentration of PCP in the soil and to facilitate volatilization of the mineral spirits to a concentration that would allow fungal growth.

After the soil was thoroughly mixed, nine plot borders were established in a three-by-three configuration at the southern end of the study area. Plot borders were constructed of galvanized steel and measured 1 m by 1 m by 35 cm. Plot borders were worked into the soil surface and then filled with soil from outside the plot borders to a depth of approximately 25 cm. There was approximately 370 kg (dry weight) of soil per plot.

The soil within each plot was then sterilized by fumigating with BROM-O-GAS (methyl bromide, 98%, chloropicrin, 2%; Great Lakes Chemical Corp., West Lafayette, Ind.). One canister of BROM-O-GAS was loaded onto a trigger mechanism, which was placed in a hole excavated in the center of each plot. Plots were covered with polyethylene tarpaulins, and the sides of each cover were sealed by placing soil around the edges. The BROM-O-GAS was released by pushing the canisters onto the trigger mechanism. The plots were left covered for 2 days, after which time the covers were removed.

Experimental design. Six types of treatment plots were established (Table 1). The study was set up as a completely randomized design, with treatments randomly allocated to experimental plots. Because of the limited test plots available, only the two fungal treatments, A1 and A2, were replicated. Inocula (i.e., chips containing either *P. chryso-*

sporium or *P. sordida*) and sterile chips were both applied at a rate of 3.35% (based on dry weight of soil). Preliminary experiments revealed that the fungi would not grow in the soil without the addition of some source of organic matter. In separate laboratory studies, several organic materials were added to soil samples from the field test site and from which the mineral spirits had been volatilized, to evaluate their potential to stimulate fungal growth. Growth of *P. chrysosporium* was greatest when peat moss (Premier Brands, Inc., Stamford, Conn.) was used. This peat was therefore included in the field study. The peat had a pH value of ca. 4.0 and a cation exchange capacity of 120 meq 100 g^{-1}, and contained 38% C, 0.4% N, 20 μg of P_2O_5 g^{-1}, 100 μg of K_2O g^{-1}, 1,000 μg of Ca g^{-1}, and 500 μg of Mg g^{-1}. Peat was applied at a rate of 1.93% (dry weight basis). Treatments were applied by rototilling the inoculum, sterile chips, and peat into the upper 30 cm of soil. After treatment application, the soil-water potential in each plot was adjusted to ca. −0.05 MPa with tap water and the plots were protected with polyethylene covers to prevent excessive moisture loss. The covers were kept in place except when soil samples were taken and when the soil-water potential was adjusted.

Plot maintenance and sampling. Tensiometers (Irrometer Co., Riverside, Calif.) were installed in several plots throughout the study area, and soil-water potential was monitored daily and maintained at approximately −0.05 MPa. Soil temperature in one plot, at a depth of 15 cm, was monitored continuously with a PTC recording thermometer (Pacific Transducer Corporation, Los Angeles, Calif.) for the first 4 weeks of the study. Soil temperature data are reported for each 12-h period beginning with noon the day the study was installed. Data for daily noon air temperatures were obtained from the Oshkosh Air Traffic Control Tower, Whitman Field, Oshkosh, Wis.

The day the treatments were installed was designated day zero. Soil sampling for PCP and PCA analysis was performed before (day −4, or 4 days before treatment application) and after (day −2) fumigation and 1, 8, 15, 22, 29, and 46 days after treatment application. Fumigation had no effect on the concentrations of PCP and PCA in the soil. A separate experiment showed that PCP is not methylated by methyl bromide under application conditions. Therefore, only data from day −4 are reported. Three soil samples were taken per plot for the first three sample times. Five soil samples per plot were taken at all other sample times. Before sampling, the soil in each plot was mixed thoroughly. Then, 60-ml amber glass jars with Teflon closures (I-CHEM Research) were filled with soil taken from random locations throughout the plot. Samples were stored at −20°C.

Analytical procedures. For analysis of soil samples for PCP and PCA, two 5-g subsamples from each soil core were placed in culture tubes (25 by 150 mm) with Teflon-lined screw caps. An additional sample was taken to determine moisture content gravimetrically. Culture tubes were prepared before extraction by muffling for at least 30 min at 450°C. Approximately 50 to 100 g of sodium dithionite was added to each tube prior to extraction. 2,4-Dibromophenol, at 100 ppm, was added as a surrogate to the initial soil samples used to determine the distribution of PCP in the study area. Recoveries of 2,4-dibromophenol averaged 94%. Soil samples were extracted twice for 1 h on a rotating tumbler-shaker with 20 ml of a mixture of *n*-hexane–acetone (1:1) acidified to pH <2 with concentrated H_2SO_4. The combined extracts were then dried by passing them through a column of anhydrous Na_2SO_4. Drying columns (18 by 240 mm) were muffled at 450°C for 30 min, and Na_2SO_4 was muffled at 400°C for 4 h prior to use. Drying columns were prepared by placing a plug of silane-treated glass wool (Supelco) in the bottom of the drying tube and then adding 10 to 15 cm of anhydrous Na_2SO_4. The columns were washed with three 5-ml volumes of hexane before the sample was loaded onto the column. Each sample was loaded onto the column, and the extracts were collected in clean culture tubes that were prepared as described above. After elution of the sample, the column was rinsed with one 5-ml volume of hexane to ensure collection of the entire sample. Finally, the combined extracts for each sample were heated to ca. 60°C and evaporated to <5 ml under a gentle stream of nitrogen. The final volume of each extract was then adjusted to 10 ml with hexane in a volumetric flask.

Extracts were analyzed by gas chromatography-electron capture detection. 2,4,6-Tribromophenol was used as an internal standard for quantification of PCP. PCA was quantified by external standard. Gas chromatographic analyses of extracts were performed on a Hewlett-Packard model 5890 gas chromatograph equipped with a ^{63}Ni electron capture detector, a model 7673A autosampler, a model 3396A reporting integrator, and a split-splitless capillary column injection port. Operating temperatures were 220°C (injector) and 300°C (detector); the carrier gas was He, and the make-up gas was N_2. The column was a DB-5 fused silica capillary column (30 m by 0.321 mm; film thickness, 0.25 μm; J & W Scientific, Folsom, Calif.). The temperature program was as follows: initial, 60°C; hold for 1 min; split off for 0.5 min; ramp A, 10°C min^{-1} for 9 min (60 to 150°C); ramp B, 2°C min^{-1} for 20 min (150 to 190°C); hold at 190°C for 5 min, split off for 1 min.

Determination of fungal survival. To determine the survival of *P. chrysosporium* and *P. sordida* over the course of the investigation, chips from soil samples from each sample period beginning with day 1 and collected from plots receiving treatments A1, A2, B, and C were examined for the presence of *P. chrysosporium* or *P. sordida*. Chips from plots receiving treatments B and C (controls) were checked to determine whether these plots had become contaminated with either of the fungi. The presence of *P. chrysosporium* was determined by aseptically placing chips on plates containing a medium developed for the isolation of this fungus from soil (11) and then incubating at 39°C. Presence of *P. sordida* was checked by aseptically placing chips on plates containing peptone-glucose nutrient agar and incubating at 30°C. After 3 days of incubation, each plate was checked for the presence of the fungi.

Statistical analysis. We assumed that the variability of the replicated treatments was the same as that of the nonreplicated treatments, making the within-treatment variability the appropriate error term to test for differences in treatments. The measurement at day 1 was considered the base level since it was the first reading after treatment application. Different initial levels of PCP were found in the different plots. Therefore, for PCP analysis, percent reduction of PCP from the day 1 reading was used as the dependent measure of interest. For analysis of PCA, percent conversion of PCP to PCA was used as the dependent measure of interest. The amount of PCP found in the soil at the day 1 reading was also used as the basis for determining percent conversion of PCP to PCA. We were interested in comparing treatments after each sample period for both PCP and PCA. Thus, an analysis of variance (ANOVA) was performed separately for each time period after day 1 to test for equality of mean response of each treatment. If by ANOVA, treatment means were shown to be significantly different, a Tukey multiple com-

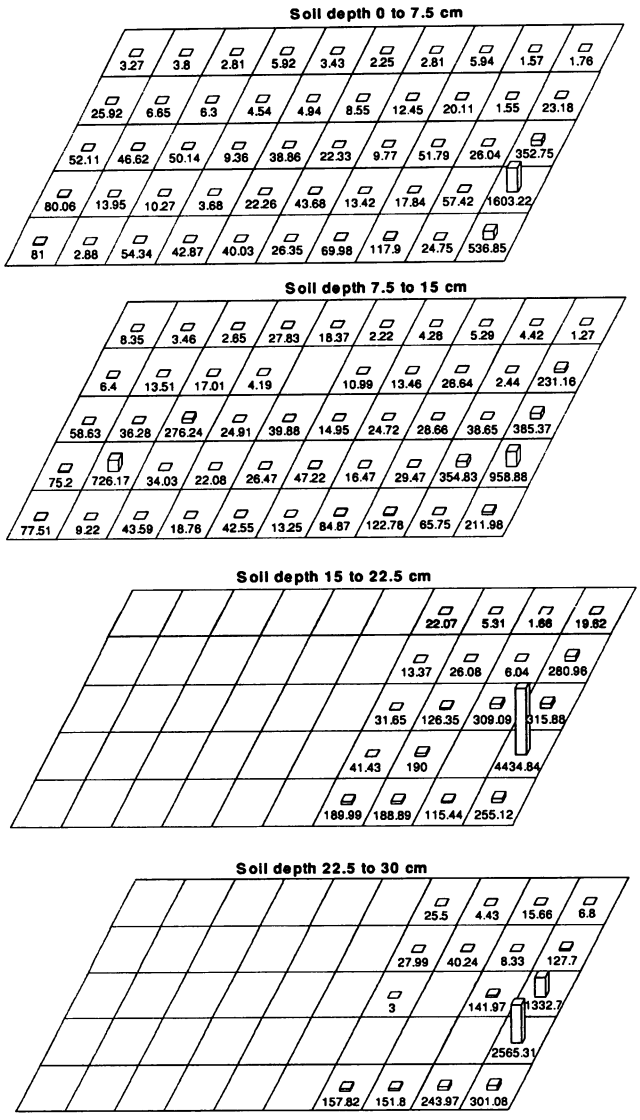

FIG. 3. Concentration of PCP (in micrograms gram^{-1}) in the soil.

parison test was performed to determine which treatments were different from others.

RESULTS

Initial distribution of PCP. PCP was found at every sample location and every depth at concentrations ranging from 1 to 4,435 μg g^{-1} (Fig. 3). Concentration of PCP in the soil tended to be greater at sample locations in the areas that were beneath the former storage tank locations. However, the highest concentrations of PCP were found at the southwest corner of the study area, particularly at sample location 2, where the PCP concentration ranged from 959 to 4,435 μg g^{-1}. The PCP concentration was generally highest at the west end of the study area and decreased towards the east. Also, PCP concentration tended to increase with depth to 23 cm then to decrease slightly at 23 to 30 cm.

Soil chemical characteristics. The soil was very strongly alkaline and calcarious and contained adequate amounts of inorganic nutrients (with the exception of nitrogen and potassium) to support a satisfactory level of microbial activity. Analysis of the soil after fumigation showed that it had a pH value of 9.6, 0.14% S, 8.95% C, 17.22 meq of cation exchange capacity 100 g^{-1} (extract was 1 N NH_4Cl), and 381.0 eq of $CaCO_3 \cdot H_2O$ extraction revealed the following (in micrograms gram^{-1}): Cl, 113.0; NO_2, 0.20; NO_3, 0.30; SO_4, 238.0; and NH_4, 0.13. HCl extraction (0.1 M) revealed the following (in milliequivalents 100 g^{-1}): Ca, 59.88; Mg, 32.96; K, 0.14; and Na, 0.20. The following were also present in micrograms gram^{-1}): B, 8.26; Si, 129.49; Zn, 5.03; P, 63.35; Fe, 83.51; Cu, 1.19; Mn, 49.45; Al, 21.83; Ni, 3.21; Ti, 0.24; Cr, 0.67; and Pb, 24.55. The soil was almost totally devoid of organic matter. Most carbon was in the form of calcium and magnesium carbonates and was thus unavailable as an energy source for fungal growth and activity. The addition of the peat moss to the soil increased the level of available carbon, potassium, and, to a lesser extent, nitrogen.

Effect of inoculation on concentration of PCP and PCA. Inoculation with either *P. chrysosporium* or *P. sordida* or application of chips resulted in a rapid ca. 35% decrease in the average amount of PCP in the soil (Fig. 4). This decrease was probably due to adsorption of PCP by the chips. Adsorption of PCP by bark chips has been reported previously (3). No similar decrease was observed in soil from plots receiving chips plus peat, peat only, or no treatment. The lack of a large decrease in the amount of PCP in soil treated with chips plus peat may have been due to an inhibition of chip adsorption of PCP by the peat.

After treatment application, there was a dramatic further decrease in the amount of PCP in soil that was inoculated with either *P. chrysosporium* or *P. sordida*, but not in soil receiving control treatments (Fig. 4). The greatest decrease in soil receiving the fungal treatments occurred between days 1 and 22 and resulted in average decreases of ca. 51 and 77% of the PCP in soil inoculated with *P. chrysosporium* and *P. sordida*, respectively (Table 2). Depletion of PCP occurred at a much lower rate during the last 3.5 weeks of the study (Fig. 4a). After 46 days, the amount of PCP in soil inoculated with *P. chrysosporium* had decreased by ca. 86% and that in soil inoculated with *P. sordida* by ca. 82% (Table 2). There was never a significant difference in the percentage of decrease of PCP caused by inoculation with *P. chrysosporium* compared to that caused by inoculation with *P. sordida*, which indicates that under the conditions encountered during the study, the two fungi had similar abilities to deplete PCP in the soil. The percentage of decrease of PCP in soil receiving the fungal treatments was greater than that in control treatments beginning on day 15 (Table 2). This difference became statistically significant at day 29 and indicates that the dramatic decrease of PCP in soil inoculated with *P. chrysosporium* or *P. sordida* was due to activity of the fungi.

Some PCA was in the soil at the beginning of the study (Table 3). PCA increased between days 1 and 22 in soil that received the fungal treatments (Fig. 5). A similar increase was not observed in soil receiving any of the control treatments, indicating that the increase of PCA was due to the activity of *P. chrysosporium* or *P. sordida*. Only a small percentage of the total decrease of PCP in the soil receiving the fungal treatments could be attributed to methylation to PCA. In soil inoculated with *P. chrysosporium*, the amount of PCA rapidly increased during the first 15 days after inoculation, which resulted in an average 13% conversion of PCP to PCA (Table 3). After day 15, the amount of PCA in the soil inoculated with *P. chrysosporium* did not change

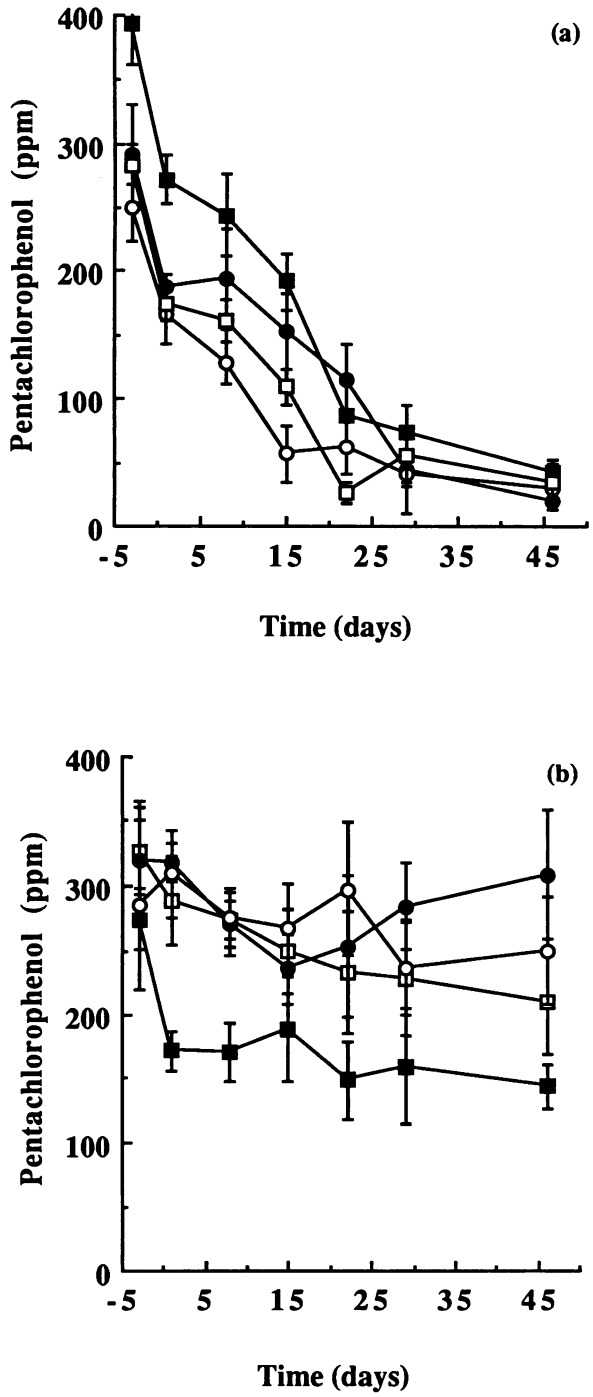

FIG. 4. Concentration of PCP (in micrograms gram^{-1}) in soil inoculated with *P. chrysosporium* (●○) or *P. sordida* (■□) (panel a) or soil receiving chips (■), chips and peat (□), peat (●), or no treatment (○) (panel b). Fungal treatments consisted of wood chips that were thoroughly grown through with either *P. chrysosporium* or *P. sordida* and had an application of peat. Chips and peat were added to the soil at rates of 3.35 and 1.93% (wt/wt of soil, dry weight), respectively.

significantly. In soil inoculated with *P. sordida*, the increase in the amount of PCA was less rapid and resulted in an average of only ca. 8% conversion of PCP to PCA by day 22.

The percentage of transformation of PCP to PCA by *P. chrysosporium* was significantly greater than that by *P. sordida* beginning on day 15 and continued to the end of the study (Table 3). This difference was due to the greater amount of PCA produced by *P. chrysosporium* in the plot that had the lowest initial concentration of PCP. In this plot, the final concentration of PCA in the soil was ca. 32 μg g^{-1} compared to an average of ca. 18 μg of PCA g^{-1} in the soil in the other plot inoculated with *P. chrysosporium* and in the two plots inoculated with *P. sordida* (Fig. 5).

TABLE 2. Results of ANOVA for decrease in PCP in PCP-contaminated soil[a]

Treatment	PCP decrease (%) at various days after treatment[b]				
	8	15	22	29	46
A1	9.06a	33.27a	76.46a	70.45a	82.25a
A2	9.71a	42.15a	50.55a	75.89a	85.75a
B	4.86a	13.67a	19.38a	20.85b	27.46b
C	0.50a	−10.00a	13.21a	7.09b	16.13b
D	15.30a	26.13a	20.63a	10.73b	3.02b
E	10.87a	13.80a	3.76a	23.82b	19.12b
P[c]	0.5849	0.5146	0.3829	0.0133	0.0116

[a] If the ANOVA showed a significant difference among treatment means, Tukey's ω multiple comparison test was used to determine treatment differences.

[b] Treatment means within a column followed by the same letter are not significantly different at α = 0.05. Refer to Table 1 for a description of treatments.

[c] *P* equals the probability of a larger *F* value for difference among treatment means.

Survival of fungi. Survival of *P. chrysosporium* and *P. sordida* in the soil in which they were inoculated and fungal contamination of soil that was treated with chips only or a combination of chips and peat was assessed by determining whether the fungi could be reisolated from chip samples. Samples were collected with the soil samples for PCP analysis at each sample time. *P. chrysosporium* was isolated from chip samples collected on days 1, 8, 15, and 46 from soil inoculated with the fungus but not from those chips collected on days 22 and 29 from the same soil. *P. sordida* was isolated from chip samples collected at all sample times from soil in which it was inoculated. Neither of the fungi was isolated from chips collected from plots receiving the chips only or chips plus peat treatments at any sample time. Also, neither fungus was isolated from chips collected from plots into which the other fungus had been inoculated. Thus, there was

TABLE 3. Results of ANOVA for percentage of PCP converted to PCA[a]

Treatment	% of PCP converted to PCA at various days after treatment[b]					
	1	8	15	22	29	46
A1	1.30a	5.19a	13.10a	13.43a	12.99a	14.06a
A2	0.82a	3.66ab	6.61b	8.32b	9.43b	9.07b
B	0.79a	0.69c	1.37c	1.16c	1.09c	0.69c
C	1.30a	1.24bc	2.25c	1.60c	1.41c	1.48c
D	0.51a	0.47c	0.85c	2.14c	0.59c	0.55c
E	0.62a	0.55c	0.94c	1.88c	0.78c	0.68c

[a] If the ANOVA showed a significant difference among treatment means, Tukey's ω multiple comparison test was used to determine treatment differences.

[b] Values given are (micrograms of PCA gram^{-1} on day *x*/micrograms of PCP gram^{-1} on day 1) × 100. Treatment means within a column followed by the same letter are not significantly different at α = 0.05. Refer to Table 1 for a description of treatments.

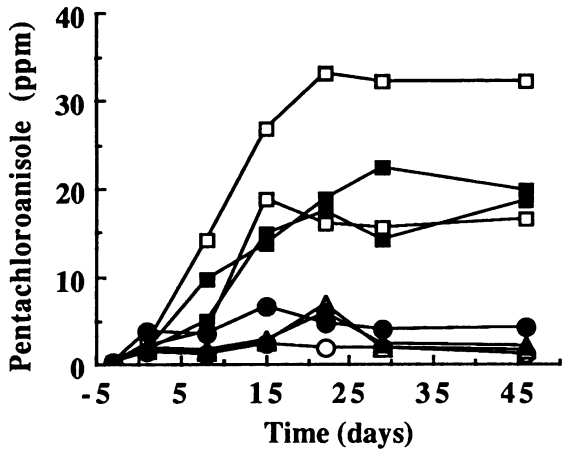

FIG. 5. Concentration of PCA (in micrograms gram^{-1}) in soil inoculated with *P. chrysosporium* (■) or *P. sordida* (□) or soil receiving chips (○), chips and peat (●), peat (△), or no treatment (▲). Both replicates of fungal treatments are shown.

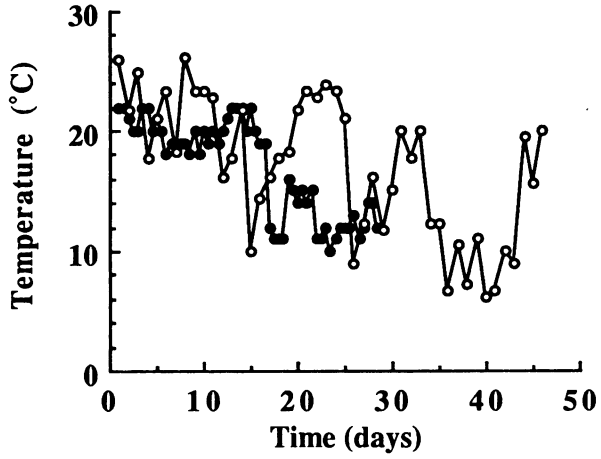

FIG. 6. Soil temperature at a depth of 30 cm, recorded at midnight and noon (●), and air temperature recorded at noon (○).

no cross-contamination by either fungus of fungal treatment plots or contamination of control plots receiving treatments that included chips.

During the course of the study, mycelial masses resembling those of *P. chrysosporium* and *P. sordida* were visually observed in the soil in plots receiving the fungal treatments. Most of the mycelia were associated with chips that were located next to the sides of the plot borders. A similar phenomenon was observed when *P. chrysosporium* was grown in soil in petri plates in which most of the visible growth occurred at the edges of the plates (20). This phenomenon might indicate a growth response to greater oxygen content around the edges of both the plot borders and the petri plates.

The optimum temperatures for growth of *P. chrysosporium* and *P. sordida* are 40°C (6) and 28°C (Lamar et al., submitted), respectively. Soil temperatures taken at a depth of 15 cm indicated that during the study, temperatures were well below the optimum temperature for growth of either fungus (Fig. 6). Throughout the first 4 weeks of the study, soil temperature ranged from 22 to 10°C. During the first 2 weeks, the average soil temperature was ca. 20°C. During the second 2 weeks the average soil temperature decreased to ca. 14°C as a result of a large drop in air temperature during day 14 of the study (Fig. 6). Air temperatures recorded at noon each day were quite variable but were also usually well below the optimum for growth of the fungi.

DISCUSSION

In this paper, we report a successful field-scale investigation that demonstrates the depletion of PCP in contaminated soil by using the degradative abilities of white rot fungi. Inoculation of a soil that had been sterilized by fumigating with methyl bromide and that contained 250 to 400 μg of PCP g^{-1} with either *P. chrysosporium* or *P. sordida* resulted in an overall decrease of 88 to 91% of PCP in the soil in 6.5 weeks. This decrease was achieved under suboptimal temperatures for the growth and activity of these fungi and without the addition of inorganic nutrients other than those contained in the peat. The rates of PCP depletion obtained in this investigation compare favorably with rates reported for other field-scale studies in which the microbial removal of chlorinated phenols (CPs) from soil was investigated. Approximately 80% of the CPs were removed in 4 months in a CP-contaminated soil (200 to 300 μg of CPs g^{-1}) that was composted with ash and softwood bark, fertilized with inorganic nutrients, and inoculated with a mixed culture of chlorophenol-degrading bacteria (28). The amount of PCP in a soil composted with manure and containing 70 to 80 μg of PCP g^{-1} was decreased by 95% over a 4-month period, apparently by indigenous PCP-degrading microbes (18).

Temperature and availability of nutrients and oxygen have all been shown to exert profound influences on the rate of microbial PCP degradation (4, 7, 27). During the course of our field study, the soil, except for the soil surface, was not aerated. Also, soil and air temperatures were suboptimal for fungal growth. The estimated half-life of PCP in the field soil inoculated with *P. chrysosporium* or *P. sordida* averaged 21 days. In a laboratory study, the estimated half-lives of PCP in sterile soils that initially contained 50 μg of PCP g^{-1} and to which *P. chrysosporium* was introduced ranged from ca. 0.5 to 4 days depending on soil type (19). That study was conducted in aerated soil microcosms under optimal temperature and moisture conditions for growth of *P. chrysosporium*. Comparison of the laboratory and field studies suggests that rates of depletion in field soils could be greatly enhanced by regulating environmental conditions to favor optimal fungal growth and activity.

Bark chips were found to be essential for the degradation of PCP by a mixed bacterial culture at concentrations higher than 2.7 μg g^{-1} (3). The bark chips adsorbed PCP reversibly, thus detoxifying the medium and allowing PCP degradation by the bacteria to proceed at concentrations beyond 54 μg g^{-1}. The rate of PCP mineralization in soil by *Flavobacterium* sp. was found to vary inversely with PCP concentration (7). As the initial concentration of PCP was increased from 10 to 100 μg g^{-1}, the rate of mineralization was decreased. At 500 μg of PCP g^{-1}, mineralization by *Flavobacterium* sp. was completely inhibited. Thus, the initial concentration of PCP in contaminated media has a significant influence on the ability of microorganisms to metabolize the compound. On the first day after treatment application, we observed a large decrease in the PCP concentration in the soil due to adsorption of the compound by chips. Therefore, the concentration of PCP to which the fungi were exposed in the soil was decreased and might have allowed more rapid PCP depletion

to proceed at higher concentrations than would have occurred if chips were not included in the treatments.

In field-scale investigations, it is difficult to determine the fate of a xenobiotic and what proportion of a xenobiotic is allocated to various fates by degradative processes. However, results from our laboratory studies on the fate of PCP in soils inoculated with *P. chrysosporium* or *P. sordida* have demonstrated that *P. chrysosporium* depleted PCP in three soils primarily by converting it to nonvolatile products (19). In that study, less than 4% of the PCP was lost via mineralization and volatilization. Soil type greatly influenced the nature of the nonvolatile products—that is, whether they were soil bound or extractable. In a later study, PCA was identified as the major extractable transformation product in a PCP-contaminated soil inoculated with either *P. chrysosporium* or *P. sordida* (Lamar et al., submitted).

In the field soil, a small percentage of the decrease in the amount of PCP was a result of fungal methylation of PCP to PCA. Both bacteria (1, 2, 4, 7, 14, 21, 22, 26, 28) and fungi (8, 9, 12) methylate CP compounds to the corresponding methylated derivatives. Studies in which the relative toxicities of PCP and PCA to selected microbes have been evaluated suggest that microbial methylation may function as a detoxification mechanism in procaryotes. For example, several strains of *Rhodococcus* sp. were sensitive to PCP but not to the same concentration of PCA (15). Pentachloroanisole was less toxic than PCP to 17 bacterial strains with the exception of *Streptomyces* spp. (24), to 16 strains of blue stain and wood-rotting fungi (23), and to *Trichoderma* spp., *Penicillium* spp., and *Cephaloascus* spp. (8). Methylation of CP compounds increases their lipophilicity and thus their tendency to bioaccumulate (22, 28). Thus, for eucaryotes, anisoles may be more toxic than phenols (28). However, we found that only ca. 9 to 14% of the PCP was converted to PCA. Thus, methylation was not the major route of PCP depletion in the contaminated soil. Also, a laboratory study showed that PCA is gradually degraded by the fungus (Lamar et al., submitted).

Gas chromatographic analysis of sample extracts did not reveal the presence of extractable transformation products other than PCA. Thus, when losses of PCP via mineralization and volatilization were negligible, as they were in the laboratory-scale studies, most of the PCP was converted to nonextractable soil-bound products. The nature of these products is not known. However, like naturally occurring phenolic compounds that arise from microbial synthesis or degradation of plant phenolic polymers, phenolic or other aromatic intermediates formed during degradation of xenobiotics in soil probably become incorporated into soil humus through enzymatic oxidative coupling or polymerization reactions (5). Evidence for enzymatic coupling reactions was obtained by Bollag and Liu (5). They reported that chlorophenol-syringic acid hybrid polymers were produced when *Rhizoctonia praticola* laccase, a phenol-oxidizing enzyme, was exposed to syringic acid, a humus constituent, and CP. Similarly, oxidation of PCP in the field soil by ligninolytic enzymes of *P. chrysosporium* or *P. sordida* might have resulted in polymerization reactions, perhaps by quinonoid intermediates (17), resulting in irreversible binding to organic matter.

The stability of xenobiotic-humic acid hybrid polymers under natural conditions is not known. However, work with artificially produced humic acid-xenobiotic hybrid polymers suggests that xenobiotics bound to humic materials through enzymatic polymerization reactions are relatively stable. Microbial release of catechol, a natural humus constituent, and PCP and other CP from synthetic humic acid polymers was limited, and most released products were mineralized abiotically or by microbes (10). In the same study, most of the PCP (78.9%) remained bound in the precipitated polymer. Chlorinated phenols and several other xenobiotics bound in humic acid-xenobiotic hybrid polymers were mineralized in liquid cultures of *P. chrysosporium* at about the same rate as the carbon from the humic compound (16). Experimental evidence indicates, therefore, that release of CP from humic acid-CP hybrid polymers is slow and unlikely to adversely affect the environment.

The results of this field investigation indicate that the use of white rot fungi to deplete PCP in contaminated soils has potential. However, before use of these fungi in soil remediation efforts can be considered a viable alternative, the nature, stability, and toxicity of the soil-bound transformation products, under a variety of conditions, must be elucidated.

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