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Environmental Contamination and Toxicology

Effects of Soil Moisture and Aeration on the Biodegradation of Pentachlorophenol Contaminated Soil

M. N. Miller,[1] G. W. Stratton,[2] G. Murray[3]

[1] Nova Scotia Department of Agriculture and Fisheries, 20 Tower Road, Truro, NS, Canada B2N 5E3
[2] Department of Environmental Sciences, Nova Scotia Agricultural College, Post Office Box 550, Truro, NS, Canada B2N 5E3
[3] Department of Environmental Sciences, Nova Scotia Agricultural College, Post Office Box 550, Truro, NS, Canada B2N 5E3

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Pentachlorophenol (PCP) has been extensively used throughout the world as a pesticide and general biocide in agriculture and industry (Megharaj 1998). Pentachlorophenol is toxic to humans, depending on concentration and route of exposure, and is listed as a priority pollutant by the U.S. Environmental Protection Agency and the European Community (Wild et al. 1993). The highest reported usage of PCP is for the treatment of utility poles in the wood preservation industry (McAllister et al. 1996). PCP is present as a contaminant in selected soils at concentrations as high as several thousand ppm (Valo et al. 1985). These levels are generally associated with sawmills and wood preservation sites. Removing PCP from contaminated soil reduces the risk of pollution, therefore, it is important to find effective methods of removal. Bioremediation is a rapidly developing technology with the potential to provide an efficient and economic means of removing organic pollutants from contaminated soils. One type of bioremediation technology is soil biopile treatment. A soil biopile is a controlled and engineered biological process in which contaminants are biodegraded by microorganisms under aerobic conditions (von Fahnestock et al. 1998). Biopiles are normally constructed on an impermeable base to reduce the potential migration of leachate to the environment. A piping network installed above the base is connected to a blower that facilitates aeration of the pile and another piping network is installed near the top of the pile to deliver moisture or nutrients, as required. A leachate collection system is also present.

The challenge in many biopile situations is to ensure that the microbes present are metabollically active and able to degrade the contaminants. Adequate moisture levels are required for microorganisms to maintain cell structure, transport nutrients, and carry out metabolic processes (von Fahnestock et al. 1998). Most microbial metabolism takes place intracellularly and microbes are limited to utilizing materials that can be transported across their cell membranes (von Fahnestock et al. 1998). A biopile must also be adequately aerated in order to enhance degradation of contaminants by the soil microorganisms. Aerobic microbes require oxygen as the terminal electron acceptor in aerobic respiration (Alexander 1977). Since soil aeration is highly dependent on soil moisture, it is important to study how these factors simultaneously influence degradation processes in a soil biopile. The objective of this study was to determine the effects that supplemental aeration and moisture have on the biodegradation of PCP within a soil biopile that was slated to

Correspondence to: G. W. Stratton

be constructed at a contaminated wood preservation site.

MATERIALS AND METHODS

A PCP contaminated sandy loam soil was collected from a wood preservation plant located in Central Canada at which a bioremediation soil biopile was slated to be constructed. The site has been in operation as a wood preservative facility since 1979 and the major soil contaminant present is PCP. The soil had a pH of 7.1, an organic matter content of 2.5%, a bulk density of 1.24 g cm^{-3}, a moisture content of 11.4%, and a 100% field capacity (FC) of 0.25 mL g^{-1}. The PCP concentration of the test soil was 1365 ± 214.55 μg g^{-1}. The experimental design was a two factor factorial with supplemental aeration and moisture as factors. Five liter pails were used to house 4 kg of contaminated soil with each treatment combination replicated three times. The supplemental aeration factor consisted of three levels, which were no supplemental aeration (control), 150 cm^3 min^{-1} of supplemental aeration pumped though for 1 h day^{-1} and 150 cm^3 min^{-1} of supplemental aeration pumped through for 2 h day^{-1}. The air was supplied by air pumps via 3 mm flexible plastic tubing and was controlled by electronic timers. The tubing was situated in a circular pattern in the middle of the 5 L pail with each end of the piping exiting at opposite sides of the pail. One end of the piping served as the inlet source while the other served as the exhaust. Small 0.5 mm diameter holes were placed in the plastic tubing to allow air to be released into the soil. Air flow was monitored with a Humonics Veri-Flow 500 Electronic Flowmeter on a daily basis. The moisture factor also consisted of three levels, which were 40, 60 and 80% of the soil's FC. Plastic tubing for moisture addition was also placed in a circular pattern on the top of the soil surface to simulate an irrigation unit. Small 2 mm diameter holes were placed in the 7 mm tubing in order to allow water to be added, as needed. The moisture was monitored daily with a Hydrosense™ soil reflectometry unit. In order to simulate a biopile, a plastic liner was placed on top of the soil and moisture irrigation unit to ensure that each treatment was self contained. There were only two sampling periods, time zero (before the liner was sealed) and after a 24 wk incubation. This was to more accurately mimic a biopile, where the soil is left undisturbed after the pile is constructed.

In order to analyze the soil for PCP,10.0 g oven dry weight equivalent of fresh soil samples were air dried, sieved (< 2mm), mixed with 10.0 g of anhydrous Na_2SO_4, and subjected to Soxhlet extraction using 300 mL 1:1 HPLC grade acetone: hexane (acidified with sulfuric acid, pH< 2) for 24 h. The Soxhlet extract was dried down using rotary evaporation and, after an initial dry down, the extraction solvents were exchanged for acetonitrile acidified with 1% acetic acid. Each sample was then dried down twice more using two 25 mL solvent transfers of acetonitrile acidified with 1% acetic acid. The dried residue was then reconstituted to a final volume of 10.0 mL using acetonitrile acidified with 1% acetic acid. All solvents used were HPLC-grade (Caledon Laboratories LTD., Georgetown, Ontario). Each sample was filtered using a 13 mm 0.45 μm Millex® syringe filter and analyzed by reverse-phase High Performance Liquid Chromatography with UV detection. Gradient elution was performed using acetonitrile and water both acidified with 1% acetic acid at a flow

Table 1. High pressure liquid chromatography gradient method.

Time of solvent	acidified H_2O in the	acidified acetonitrile in the	Gradient
initial	65	35	isocratic
4	65	35	isocratic
12	20	80	linear
15.5	20	80	isocratic
16.5	0	100	linear
17.5	0	100	isocratic
21.5	65	35	linear
28	65	35	isocratic

rate of 1 mL min^{-1} (Table 1). The retention time for PCP was 13.0 min. The instrumentation used included a Waters Model 484 UV detector at 280 nm, Model 600 Automated Gradient Controller and Model 600 multisolvent delivery system, a Hewlett Packard Model 3396A Integrator, and a Phenomenex EnviroSep-PP column kept at a constant temperature of 45°C. The detection limit for PCP was 0.3 μg mL^{-1}.

Chloride was extracted from soil samples equivalent to 10 g oven dry weight with 100 mL of glass distilled water on a multi wrist-action shaker for 1 h. The extracts were centrifuged for 45 min at 1300 x g. The level of Cl^{-1} was measured with an Orion Model 94-17B ion selective electrode, Model 90-02 reference electrode and Model 401 specific ion meter. The detection limit for Cl^{-1} was 2 mg L^{-1}.

Microbial enumerations were conducted on the indigenous soil microorganisms. Bacteria, actinomycetes, pseudomonads and fungi were enumerated according to standard methods using a soil dilution technique (Atlas 1982). Tryptic Soy Agar was the medium used for bacteria, Pseudomond Isolation Agar for pseudomonads, Chitin Agar for actinomycetes, and Rose Bengal Agar for fungi. Microbial enumerations were conducted at the beginning and end of the 24 wk incubation period.

In order to reduce the effects due to variation in the time zero analysis of PCP, the data for each sampling time were converted to a percentage of the time zero PCP for each level. Therefore, all time zero values were reported as 100%. Percent values less than 100 represent a decrease in PCP. All data were statistically analysed based on the two factor factorial design. After the initial assumptions of constant variance, normality, and independence were tested, the data for PCP concentration, Cl^{-1} ion concentration and microbial enumerations were analysed by Proc GLM using the SAS statistics software. The data for the Cl^{-1} ion concentration and the microbial enumerations were transformed using $\log_{10}$. The interaction of the factors moisture and aeration showed no significance difference so a Least Significant Difference (LSD) Test was conducted to determine where among the main effects treatments significant differences could be found ($\alpha = 0.05$) and, in turn, determine the appropriate factor level.

RESULTS AND DISCUSSION

Time zero (T_0) analysis for the microbial enumeration data was based on six randomly chosen soil sub-samples from the composite contaminated soil used in the experimental design. Neither pseudomonad nor actinomycete populations were detected after 24 wk in the three moisture level treatments for the control (no supplemental aeration). Statistical analysis using a two factor factorial revealed that the moisture factor levels had no significant effect on any microbial populations when comparing the T_0 cfu g^{-1} soil to T_F (at the end of 24 wk). However, the supplemental aeration factor was found to have a statistically significant effect on the fungi ($p = 0.0309$) and actinomycete ($p = 0.0390$) populations (data not shown).

When comparing actinomycete populations at T_0 versus T_F there was a statistically significant decrease observed for both levels of supplemental aeration and the control. For fungi, the treatments which received 150 cm^3 min^{-1} of supplemental aeration for 1 h d^{-1} elicited a significantly higher population. The bacterial ($p= 0.848$) and pseudomonad ($p= 0.0525$) levels were statistically the same at T_0 and T_F. Statistical analysis using a two factor factorial was also performed on the microbial enumeration data in order to compare T_F enumerations for each of the four microbial populations. The moisture factor had a significant effect ($p = 0.037$) only on bacterial populations. The bacteria numbers were significantly higher in treatments maintained at 60% FC (Fig. 1). This result is not surprising, since the optimal moisture content for aerobic bacterial activity in soil is approximately 50 to 75% of the soil's water holding capacity (WHC) (Alexander 1977). The interaction of supplemental aeration* moisture also revealed significant differences ($p = 0.0499$). The control treatment maintained at a FC of 80% was significantly lower in fungal populations than were all of the other treatments, which were statistically the same. This may have occurred because the population may not have been able to tolerate a high water content with no additional sources of oxygen.

Overall, most of the microbial populations experienced little or no change in number. The metabolites of PCP could be more toxic than PCP itself, therefore, as PCP is degraded the metabolites produced could have caused a reduction in microbial levels (Schonborn and Dumpert 1990). Even though there was little change in the microbial populations this does not mean that PCP could not be degraded by the surviving populations. The indigenous microbes must have been somewhat tolerant to PCP since the initial concentrations were relatively high. Briglia et al. (1990) found that certain microbes are more tolerant to PCP than others. They found that over an incubation time of 200 d the *Rhodococcus chlorophenolic PCP-1* cells used fluctuated a little but stayed fairly consistent in number. On the other hand, the PCP degrading activity of the inoculated *Flavobacterium* cells declined within 60 d in natural soil under the same conditions.

The concentration of PCP in the contaminated soil was analysed on two sampling dates during the 24 wk incubation period. In the experimental design, it was found that the moisture treatment factor had no significant effect on the concentration of

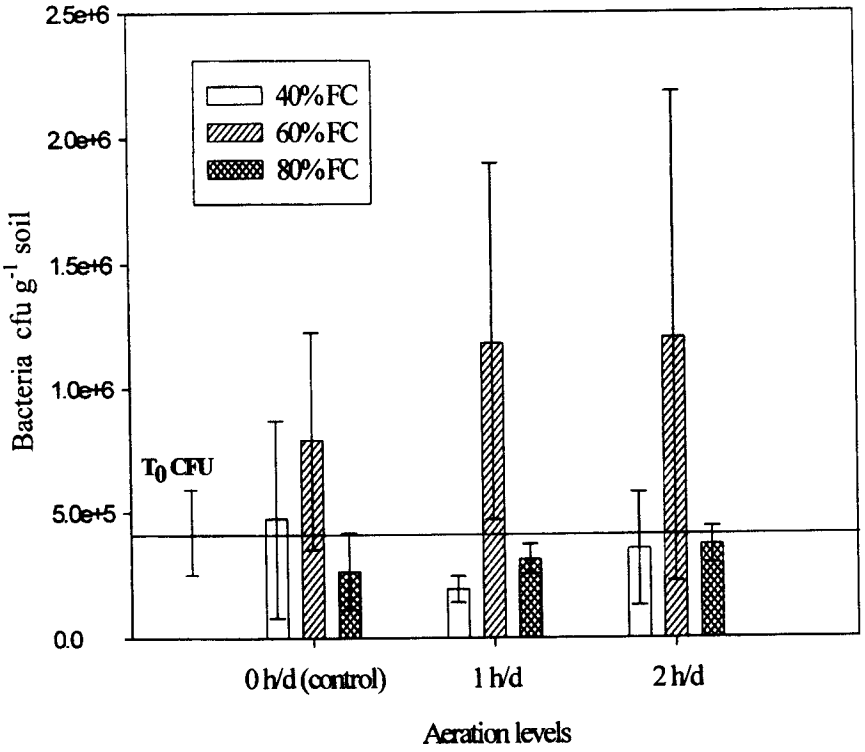

Figure 1. Effect of treatments on the number of bacteria (cfu g^{-1} soil) at each aeration and moisture level after the 24 wk incubation. Bars are standard errors. The horizontal line is the T_0 population level.

PCP in the soil over the 24 wk incubation. This may be due to the high variability in PCP levels within the contaminated soil samples. As noted in Fig. 2 the aeration level of 2 h d^{-1} exhibited a greater decrease in PCP concentration at an 60% FC, compared to 40 and 80% FC treatments. This response however, was not statistically significant across all treatment combinations. In general, biodegradation of contaminants is optimal at a soil moisture content between 30 and 80% of the soil's WHC (Dibble and Bartha 1979). Therefore, the results obtained were expected, since the treatments were designed to simulate the remediation process in a soil biopile. Studies have indicated that PCP can be degraded by soil microbes over a wide range of moisture contents. In one study, Crawford and Mohn (1985) found that with a *Flavobacterium* sp. in a sandy soil, a 15 to 20% soil water content was most effective at eliciting the degradation of PCP, although after 10 days at 50% water content mineralization activity increased and equaled that of drier soils. Steinle et al. (2000) reported that soil moisture had no statistically significant effect on the biodegradation of 2,6-dichlorophenol with the soil's WHC being either 30 or 90%. Briglia et al. (1992) found that there was no obvious relationship between the rate of mineralization of PCP and moisture content, although it was noted that no activity was observed in water logged soils.

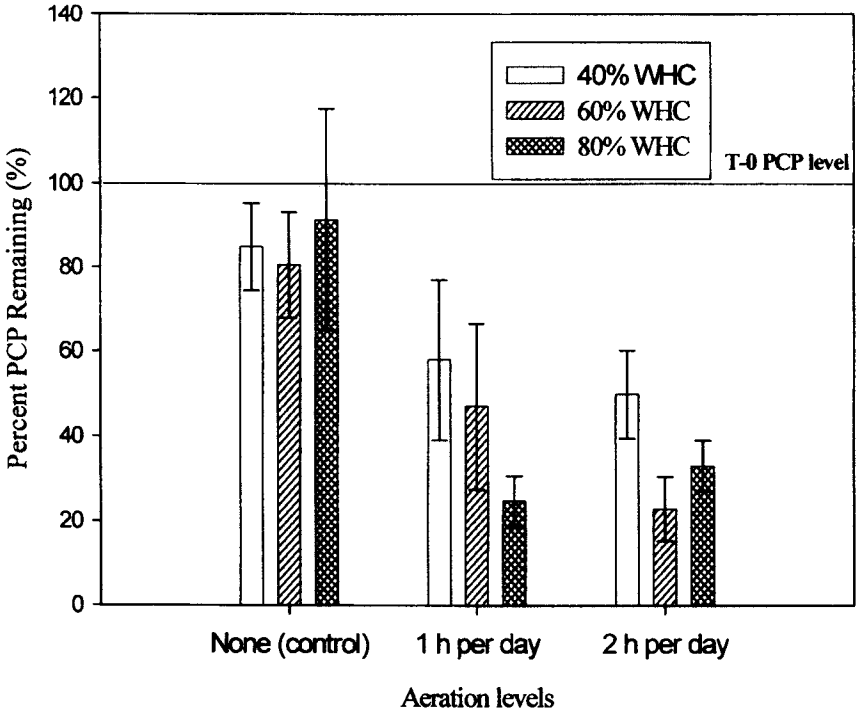

Figure 2. Percentage of PCP remaining in the soil for each aeration and moisture level after the 24 wk incubation. Bars are standard errors. The horizontal line is the T_0 PCP level (100%).

In contrast to soil moisture, supplemental aeration ($p = 0.0491$) had a significant effect on the PCP concentration in the present study (Fig. 2). When comparing T_0 PCP levels to T_F for the treatment with no supplemental aeration (control) there was an average 14% reduction in PCP. However, when comparing the T_0 PCP level to T_F with treatments receiving 150 cm^3 min^{-1} of supplemental aeration for 1 h d^{-1}, the PCP concentration was reduced by 57%, while with 150 cm^3 min^{-1} of supplemental aeration for 2 h d^{-1} the concentration of PCP was decreased by approximately 65% (Fig. 2). In order to get a statistically significant decrease of PCP, a minimum of 150 cm^3 min^{-1} of supplemental aeration for 2 h d^{-1} would be required. Reports have also indicated that biodegradation of PCP is more rapid under aerobic conditions (McAllister et al. 1996). Seech et al. (1991) found that the influence of soil solution oxygen level on PCP biodegradation appears to be organism dependent. In the remediation of PCP contaminated soil with *Flavobacterium sp.*, adequate oxygen availability and the availability of pathways for oxygen diffusion into the soil were found to be important factors.

Chloride ion release was not significantly different among the varying water contents. However, the 40% FC treatments appeared to release less Cl^- on average than the 60 and 80% FC treatments, although the trend was not statistically significant across all

treatment combinations (data not shown). In comparison, the least amount of PCP was degraded in the 40% FC treatments (Fig. 2) therefore, the Cl^- ion concentration would be expected to be lower in this treatment. The levels of supplemental aeration also had no statistically significant effect on the release of Cl^- ions in the soil, even though the data indicate that as the level of supplemental aeration increased the amount of Cl^- in the soil also increased. This corresponds with the data collected for the analysis of PCP concentrations. The amount of Cl^- ions released increased as the aeration level increased, while the PCP concentration decreased. This trend confirms that PCP was degraded, thereby releasing Cl^-.

In the present study bacteria populations were found to be higher in treatments maintained at a 60% FC therefore, the moisture content should be maintained at approximately 50 to 75% of the soil's WHC, since this is optimal for aerobic bacteria activity. Results from this study found, that in order to achieve a statistically significant decrease in the concentration of PCP, a minimum of 150 cm^3 min^{-1} of supplemental aeration for 2 h d^{-1} should be used. These factors should be continually monitored throughout the remediation process to ensure that the indigenous microorganisms can survive and degrade the target compound. There were no additional nutrients added to the contaminated soil in the present study, but more stimulation of the microbial populations and increased degradation of PCP may have occurred if the indigenous microbes were exposed to other nutrient sources at the beginning of the experiment. Numerous studies have shown the benefits of increased microbial degradation of PCP when supplemental nutrient sources are used (Briglia et al. 1990; Yu and Ward 1994). Soil biopiles are site specific and it is necessary to conduct preliminary studies on optimizing the conditions for microbial degradation to occur before a larger pilot scale remediation strategy is implemented. This research can be used as a basis for determining the types of preliminary studies that can be conducted.

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