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Degradation of Dentachlorophenol in Simulated Lentic Environment

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Pentachlorophenol (PCP) and its salt, sodium pentachlorophenate, are widely used as biocides (BEVENUE and BECKMAN 1967). PCP is most commonly used as a fungicide-bactericide in the treatment of wood products (ARSENAULT 1967). The amount of PCP manufactured in the U.S. in 1976 exceeded 2 million metric tons (U.S. INTERNATIONAL TRADE COMMISSION 1976) and the widespread use of the chemical as well as its extreme toxicity to a number of different aquatic organisms (LC50's as low as 0.1 mg/ℓ for some species of fish) make it potentially dangerous environmental contaminant (GOODNIGHT 1942, CRANDALL and GOODNIGHT 1962, ANDERSON and WEBER 1975, CARDWELL et al. 1976).

PCP and some of its degradation products bioconcentrate in fish (GLICKMAN et al. 1977, PRUITT et al. 1977), and are among the phenolic compounds known to taint fish flesh even when present at levels far below those normally producing toxic effects (BOETIUS 1954, SHUMWAY and CHADWICK 1971). Because even low levels of PCP and some of its degradation products may have detrimental effects, it is important to estimate the persistence of PCP in different aquatic environments, factors affecting degradation, and the relative magnitude and formation of certain degradation products.

Previous studies have considered the effect of only single factors influencing the degradation of PCP, such as light (KUWAHARA et al. 1966, 1969), fungi (CSERJESI and JOHNSON 1972), anaerobic soil bacteria (KAUFMAN 1978), or aerobic microbial communities (REINER et al. 1978, WONG and CROSBY 1978). However, because different biological and chemical environments exist in natural aquatic ecosystems it is important to determine and compare differences in the degradation of PCP in the presence of multiple differences in commonly found aquatic habitats.

Tetrachlorophenol (TCP) and pentachloroanisole (PCA) were commonly formed in laboratory experiments and were major degradation products in the one environmental study that has been done (PIERCE et al. 1977). We followed the degradation of PCP, formation of TCP and PCA, and the distribution of metabolites into various components within each of four aquaria. It was our specific objective to determine what differences occurred in aquaria simulating four lentic environments: (1) the oxygen rich euphotic epilimnion, (2) oxygen rich euphotic littoral zone, (3) low oxygen, dark hypolimnion, and (4) benthic hydrosoil-hypolimnion.

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METHODS AND MATERIALS

Experimental design and sampling methods

The experimental design consisted of two 50-ℓ aquaria (1 and 2) that were illuminated by two 40-w fluorescent lights set 30 cm above the aquaria, and two 50-ℓ aquaria (3 and 4) that were completely covered with black plastic to exclude all light. Homogenized autoclaved hydrosoil from an uncontaminated pond was placed in two of the aquaria (2 and 4), and all aquaria were filled with pond water. Each aquarium was covered with a sheet of glass and the dark aquaria were sealed with a silicone rubber sealant. After 45 days the aquaria were treated with PCP as its sodium salt to produce a concentration of 2 mg/ℓ. Each aquarium was also innoculated with 5 μCi of uniformly ^{14}C ring-labeled PCP as its sodium salt (specific activity 11.67 mCi/mM). Measurements of pH (Beckman model 1009 pH meter) and dissolved oxygen concentration (DO) (YSI model 54 oxygen meter) were taken at 4-6 day intervals throughout the study. Water samples for PCP analysis were taken on days 1, 4, 10, 14, 21, 28, 35, 42, 68, 73, 106, 124, and 131 after treatment.

The study was terminated after 131 days. Samples of the hydrosoil, filamentous algae, biogenic sediment, and floating flocculent material in each aquaria were frozen. The water was siphoned from the aquaria into brown glass bottles and stored in a refrigerator at 4°C. We swabbed 800 cm^2 of the cover and sides of each aquarium with glass wool and methanol to remove any biogenic material or PCP adhered to the glass.

Reagents and apparatus

All organic solvents were glass distilled from Burdick and Jackson, Muskegon, Michigan; pentafluorobenzylbromide (PFBB), 18-crown-6-ether catalyst, diazomethane (Diazald^R), and the chlorophenols were obtained from Aldrich Chemicals, Inc., Milwaukee, Wisconsin. Radiolabeled pentachlorophenol (^{14}C-uniformly ring labeled) was obtained from Pathfinders Laboratories, Inc., St. Louis, Missouri. Potassium carbonate (K_2CO_3), potassium hydroxide (KOH), sodium hydroxide (NaOH), sodium sulfate (Na_2SO_4), and hydrochloric acid (HCl) were obtained from Mallinckrodt, Inc., St. Louis, Missouri. Pentachloroanisole was prepared by reacting PCP with diazomethane (HOWARD and UIP 1971). Silica gel was EM-60 (230-400 mesh) from E. Merck, Darmstadt, Germany. Scintillation fluid contained Beckman fluoralloy dry mix, dissolved in toluene; Triton X-100^R was used as a water solubilizer.

We analyzed the samples with a Tracor MT-220 gas chromatograph (GC) equipped with a linearized-electron capture detector and a 2-mm i.d. x 4-m glass GC column packed with 1.3% purified Apiezon-L on 80-100 mesh Chromsorb W HP (w.w) (JENSON and SUNDSTROM 1974). Argon/methane (95/5 v/v), delivered at 300 cc/min, was used for both the carrier and makeup gas. After sample injection the column temperature was held at 180°C for 2 min and then temperature programmed to 195°C at 3° C/min. Hydrosoil and biogenic materials were oxidized in a Biological Material Oxidizer, (R.J. Harvey Instrument Corp.) and ^{14}C collected in a CO_2 trap. A Beckman model 200-L liquid scintillation counter was

used for radiometric analyses.

Gas chromatographic analysis

A 100 mℓ water sample from each aquarium was treated with 10 mℓ of 1.0 N KOH in a 250 mℓ separatory funnel and was extracted with two 40 mℓ portions of dichloromethane. The extracts were combined, and dried with anhydrous Na_2SO_4, 3 mℓ of nonane was added, and the dichloromethane was removed by rotary evaporation. The residue was quantitatively transferred to a screw top test tube and diluted to 5 mℓ with hexane. This fraction contained the pentachloroanisole which was analyzed by GC as described. The previously extracted aqueous portion was acidified with 10 mℓ of 2.0 N HCl and subsequently extracted with two 40 mℓ portions of dichloromethane. The organic extracts were combined, dried, and reduced in volume to 0.5 mℓ by rotary evaporation. The residue was quantitatively transferred to a screw top test tube with 3 mℓ of acetone and the acetone was evaporated to approximately 1 mℓ with a stream of dry nitrogen. A solution containing 30 µℓ of 18-crown-6-ether (100 mg/mℓ in acetone, DAVIS 1977), 10 mg K_2CO_3, and 1 µℓ of PFBB reagent was added to the residue. The test tube was sealed with a teflon lined cap and maintained at 58°C for 30 min. The residue was cooled, 5 mℓ hexane was added and the solvent was evaporated with a stream of dry nitrogen to 0.5 mℓ. The residue was quantitatively applied to a silica gel column (0.5 cm i.d. by 11 cm) containing 1.4 g silica gel. The column was eluted with 6 mℓ of hexane which was discarded. PFB ethers were next eluted with 8 mℓ of 15% benzene in hexane (v/v), and analyzed by GC as described. Chemical analyses were rejected if recoveries were beyond the tolerance limits presented in Table 1. Hydrosoil samples were freeze dried and analyzed using a method described by RENBURG (1974).

Radiometric analysis

A 500-mℓ water sample was taken 131 days after pentachlorophenol addition, placed in a separatory funnel, adjusted to pH 12.0 with 1.0 N NaOH, and extracted with three 50-mℓ aliquots of isooctane. The isooctane fraction was then evaporated to 10-mℓ on a rotary evaporator, and 1.0 mℓ of the concentrate was analyzed by liquid scintillation for neutral compounds, such as pentachloroanisole. A 1.0-mℓ subsample of the previously extracted aqueous sample was also analyzed by liquid scintillation for polar degradation products.

The filamentous algae, biogenic sediment, and flocculent material were weighed, freeze dried, and reweighed to obtain a wet to dry conversion factor. The dried samples were then oxidized and the collected $^{14}CO_2$ was analyzed. We determined the form of the ^{14}C CO_2 activity in the hydrosoil in two different ways: (1) A subsample of hydrosoil from each aquarium was acidified with HCl, freeze dried, oxidized and the trapped ^{14}C CO_2 was counted. The acid conditions should have caused any carbonate forms of ^{14}C to be released from the sample; (2) A subsample of hydrosoil from each aquarium was extracted with three 50 mℓ portions of hexane, freeze dried, oxidized and the trapped ^{14}C CO_2 counted. If any organically extractable forms of ^{14}C were

present in the hydrosoil they should have been extracted by the hexane. The remaining ^{14}C was considered bound on clay particles or incorporated into the microbial community.

TABLE 1

Percent recovery of pentachlorophenol and four potential degradation products

Compound and concentration (µg/ℓ)	$\bar{x}$ (%)	SD	N	Tolerance limit*
Pentachlorophenol				
(0.01 µg/g)	82	13	6	±33
(0.1 µg/g)	87	7	4	±22
(1.0 µg/g)	87	5	6	±13
Pentachloroanisole				
(0.01 µg/g)	70	6	6	±15
(0.1 µg/g)	73	9	6	±23
(1.0 µg/g)	83	3	4	±10
2,3,4,5-Tetrachlorophenol				
(0.01 µg/g)	90	13	6	±33
(0.1 µg/g)	98	6	3	±26
(1.0 µg/g)	91	10	6	±26
2,3,4,6-Tetrachlorophenol				
(0.01 µg/g)	68	13	6	±33
(0.1 µg/g)	87	14	4	±45
(1.0 µg/g)	87	5	6	±13
2,3,5,6-Tetrachlorophenol				
(0.01 µg/g)	68	13	6	±36
(0.1 µg/g)	87	9	6	±23
(1.0 µg/g)	87	3	5	±10

(*) = $\bar{x} \pm$ S.D. (t_n-1) tolerance limit. Beyond this range analyses were rejected.

RESULTS AND DISCUSSION

The disappearance of PCP is potentially mediated by several environmental factors. Since different biological and chemical environments exist in lentic ecosystems, it is important to determine what factors are responsible for PCP degradation in different ecological zones. We consider the effects of DO, light, pH, and the presence of hydrosoil on PCP persistence. The DO in the aquaria kept in the dark (3 and 4) ranged from 0.0 to 1.0 mg/ℓ. In the aquaria subject to fluorescent lighting (1 and 2), the DO dropped to 50% saturation for three days following PCP addition; thereafter, the DO remained at the saturation level. The pH of the water in the dark aquaria was between 3.0 and 5.5 and was most frequently in the 4.9-5.1 range. In the lighted aquaria the pH ranged from 7.5 to 8.5 indicating that the PCP was in the phenate form. Hydrosoil pH measurements were between 6.0 and 7.5 in aquarium 2 and between 4.0 and 5.0 in aquarium 4.

PCP disappeared from the water in the aquaria at differential rates (Fig. 1). After 131 days, the concentration of PCP in the water was highest in aquarium 3. The analysis of PCP in the

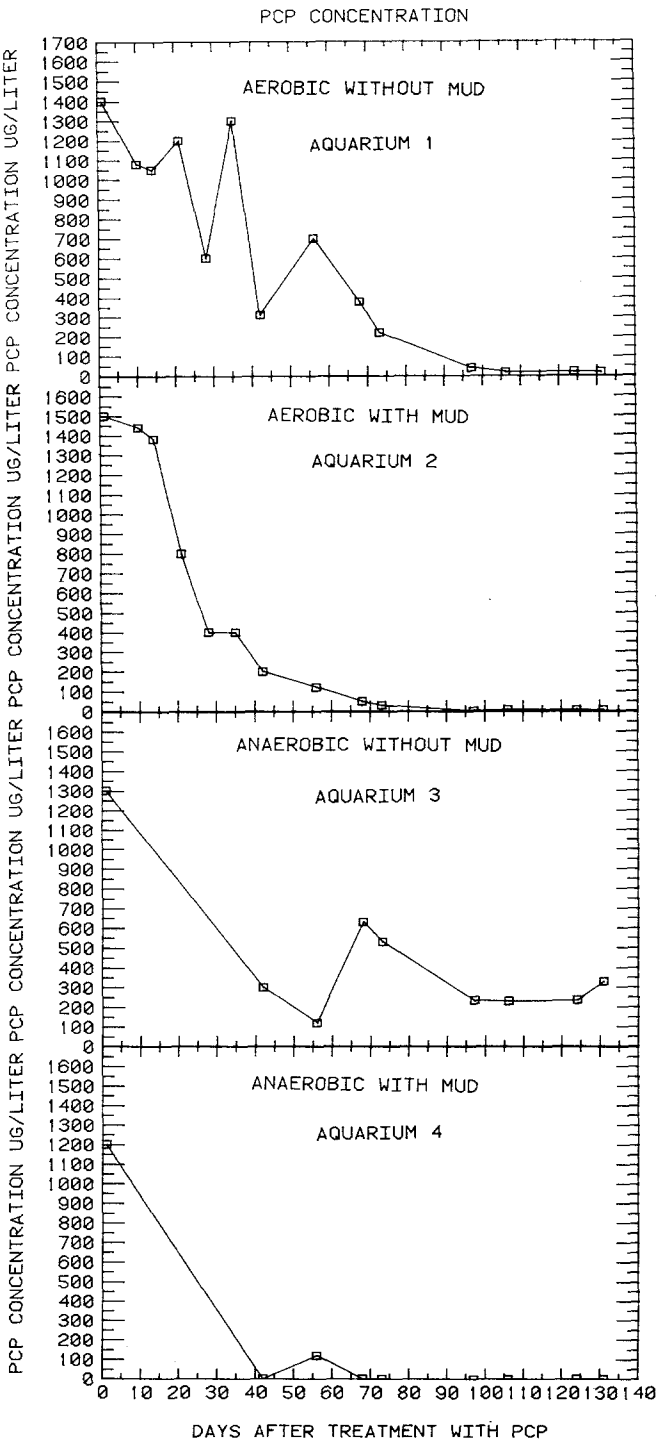

Figure 1. The disappearance of PCP in the water of four aquaria.

aquaria without mud (1 and 3) showed a fluctuating pattern between days 21 and 68. When the PCP recoveries from analysis of aquarium samples were compared to the tolerance limits in Table 1, it was apparent that the magnitude of these oscillations was greater than measurement error. Possibly, these fluctuations were due to the uptake and release of PCP by changing microbial populations. Similar fluctuations of PCP concentration in the water was not evident in aquaria with hydrosoil (2 and 4).

The half-life of the PCP concentration in water, calculated from an exponential decay function using a least squares fit, also shows that PCP was more persistent in aquarium 3 (Table 2).

The only degradation product found in the water consisted of trace amounts of PCA (2 µg/ℓ in aquaria 1 and 2). This product has been shown to be formed by fungal microorganisms in previous laboratory studies (CSERJESI and JOHNSON 1972). PCA has also been found in a contaminated lake (PIERCE et al. 1977) where PCA formation may be mediated by microbial communities inhabiting the euphotic epilimnion. No TPC's were detected in the water of any aquarium, indicating that TPC's either were not formed or were very short lived intermediates. At the end of the experiment, hydrosoil in aquaria 2 and 4 contained one TPC isomer (2,3,5,6-tetrachlorophenol) and trace amounts of PCP.

The ^{14}C activity of PCP and unknown degradation products were lowest in aquarium 1 (Table 3).

TABLE 2

Amount of PCP remaining at the end of the experiment in each of the four aquaria (100 mg PCP originally present) and half-life in the water

Aquarium No.	Water (mg PCP)	Half life-days	Hydrosoil (mg PCP)	Hydrosoil (mg TCP)	Total (mg PCP)
1*	0.95	18.6			0.95
2	0.21	13.9	0.03	0.04	0.24
3*	16	79.8			16
4	.005	12.8	0.04	0.04	.045

*These aquaria did not have hydrosoil

The percent ^{14}C remaining after 131 days was similar in aquaria 2, 3, and 4; however, the distribution of ^{14}C activity among components within the aquaria varied (Table 3). Significant proportions of ^{14}C activity were found in the hydrosoil of aquaria 2 and 4. The hydrosoil of aquarium 2 contained more than three times the ^{14}C activity of the hydrosoil of aquarium 4. These results indicate that there were substantial amounts of unidentified PCP degradation products in the hydrosoil of aquaria 2 and 4 which did not appear in the residue analysis (Table 2). These unidentified degradation products may be formed in the hydrosoil or in the overlying water for ^{14}C carbonates was negative. Only a small fraction (0.4% and 0.6%) ^{14}C activity was recovered from the hydrosoil by organic extraction indicating that over 99% was either bound to clay particles or incorporated into the microbial community. Algae, floating flocculent material, and biogenic sediment also had significant levels of ^{14}C activity (Table 3).

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TABLE 3

Percent of original ^{14}C activity recovered, and its distribution among the components in the aquaria

Component	Aquarium Number			
	1	2	3	4
Water				
Extract 1*	0	0	0	0
Extract 2*	24	23	58	38
Hydrosoil	NF	34	NF	10
Filamentous algae	12	NF	NF	NF
Flocculent material	NF	NF	0.2	13
Biogenic sediment	2.0	NF	1.0	NF
Aquarium sides	0.3	0.7	3.0	0
Aquarium cover	0	0	0	0
Total Activity recovered	38.3	57.7	62.2	61.0

NF - component not found
* See methods

Several conclusions can be drawn from this study. The persistence of PCP was associated with three environmental variables: (1) the absence of light and hydrosoil in aquarium 3; (2) pH near or below the pKa of 4.8 (ROBINSON and BATES 1966) for PCP; and (3) low oxygen concentration. This pattern of persistence may indicate the phenolic form of PCP is more persistent than the phenate salt. Pentachloroanisole was found only in aquarium 1 and 2, suggesting that light or an aerobic microbial community is responsible for formation of this degradation product. A comparison of the residual ^{14}C activity with the PCP and TCP remaining in the aquaria at the end of the experiments suggests that the predominant degradation products were not those we considered. Since light, high pH and DO were associated with the most rapid and complete breakdown, PCP may degrade to chlorinated benzoquiones and maleic acid (REINER et al. 1977, WONG and CROSBY 1977).

In simulated natural aquatic situations, several different degradation pathways of PCP take place. The conversion of PCP to TCP does not appear to be a major pathway in water but may be more important in hydrosoil. Our experimental results indicate that PCP may be most persistent in the deoxygenated hypolimnion water of lakes.

References to trade names do not imply Government endorsement of commercial products.

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