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Biodegradation and Photolysis of Pentachlorophenol in Artificial Freshwater Streams

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The biodegradation, photolysis, and adsorption of pentachlorophenol (PCP) in outdoor, aquatic environments were examined with man-made channels built by the U.S. Environmental Protection Agency at a field station on the Mississippi River near Monticello, Minn. Four channels were used, each channel being approximately 520 m long and receiving river water that flowed through the channels for about 10 h before reentering the river. The channels were dosed continuously during the summer of 1982 with various concentrations of PCP (approximately 0, 48, 144, and 432 μg/liter). We monitored the biotic and abiotic degradation of PCP in these channels for approximately 16 weeks. Photolysis of PCP was rapid at the water surface, but greatly attenuated with depth. Depending on sunlight conditions, photolysis accounted for a 5 to 28% decline in initial PCP concentration. Adsorption of PCP by sediment and uptake by biota accounted for <15% and probably <5% in unacclimated water. Microbial degradation of PCP became significant about 3 weeks after the initiation of dosing and eventually became the primary mechanism of PCP removal, accounting for a 26 to 46% (dose-dependent) decline in initial PCP. Most of the PCP-mineralizing microorganisms that developed in the channels were either attached to surfaces (e.g., rocks and macrophytes) or associated with surface sediments. Total bacterial numbers (direct microscopic counts) in the various channels were not affected significantly by PCP concentrations of micrograms per liter. Numerous strains of bacteria able to grow at the expense of PCP were isolated from the adapted channels. The experiments reported here will help predict the responses of flowing aquatic ecosystems to contamination by biocides such as pentachlorophenol.

Pentachlorophenol (PCP) is a general biocide. It first was introduced into the United States during the 1930s, and originally was employed as a wood preservative. It still is used for that purpose, but its uses have expanded to include applications as a fungicide, bactericide, herbicide, molluscicide, algicide, and insecticide. PCP is used by both agricultural and industrial groups, and it is available to the general public. Worldwide production of PCP is about 5×10^7 kg per year, of which about 2.3×10^6 kg is produced in the United States (7). About 80% of U.S. production is used for commercial wood treatment. PCP also may arise from environmental biotransformations of other commercial chemicals such as hexachlorobenzene and lindane, but the relative contribution of these sources to the overall biospheric burden of PCP is unknown.

PCP has been found in the atmosphere, even in pristine areas (~0.25 ng/m^3 in an uninhabited mountainous area) (7). Air concentrations can be as high as 160 μg/m^3 in rooms containing PCP-treated wood or paint (7). PCP also contaminates rain and snow (2). Streams commonly contain PCP concentrations of micrograms per liter (4), and ground water or drinking water contamination by PCP is fairly common in the United States (4). For example, in Minnesota there are at least seven cases of ground water contamination by PCP (Minnesota Pollution Control Agency, personal communication).

As Liu et al. (12) point out, very little information is available regarding PCP biodegradation in aquatic environments. Most of the studies that are available involve laboratory models of aquatic systems (3, 9, 12). Pierce et al. (15) studied the fate of PCP accidentally spilled into a freshwater stream-lake system. These authors primarily were interested in determining PCP persistence and the formation of PCP transformation products in the contaminated lake sedi-

ments, not in the response of the microbial flora to the sudden presence of PCP. A few authors have reported that natural freshwater microfloras may adapt to the presence of PCP and become effective at PCP mineralization (19, 20); however, no studies have been carried out under the highly controlled conditions of the study reported here.

As part of a larger study addressing the field applicability of water quality criteria (S. F. Hedtke and J. W. Arthur, submitted for publication), we have examined the biotic and abiotic degradation of PCP in an outdoor aquatic system. Since it is not practical to examine these processes in a natural water body, we have employed a number of man-made channels built by the U.S. Environmental Protection Agency at a field station near Monticello, Minn. Our studies show that if PCP is introduced into a flowing aquatic system, degradation of the pollutant occurs by both abiotic (primarily photolysis) and biotic (microbial degradation) processes. The biotic process requires a moderately long adaptive response by the aquatic microflora, but eventually becomes the predominant mechanism of PCP removal from the system.

MATERIALS AND METHODS

Facility. The Monticello Ecological Research Station, a field station of the U.S. Environmental Protection Agency Environmental Research Laboratory at Duluth, Minn., is located on 13.4 hectares of land leased from the Northern States Power Co. adjacent to the nuclear electric generating plant and the Mississippi river at Monticello, Minn. Geographically, this site is positioned about 70 km (ca. 45 miles) northwest of Minneapolis, Minn. A continuous flow of river water is provided by a pumping station adjacent to the power plant intake. The water is conveyed to the outdoor channels.

Outdoor experimental channels. The station has eight outdoor experimental channels each 520 m long with 0.14 hectares of water surface area. Each channel contains nine 30.5-m mud-bottomed pools (consisting of fine sandy loam to coarse sand) and alternating with eight 30.5-m gravel riffles. The diameter of the riffle rock is generally between 2 and 5 cm. An aerial view and a schematic representation of the channels were given by Nordlie and Arthur (14) and Arthur et al. (1), respectively. For additional physical descriptions of the channels the reader is referred to measurements reported by Stefan et al. (H. Stefan, J. Gulliver, M. Hahn, and A. Y. Fu, University of Minnesota project report no. 193, 1980; U.S. EPA Project no. EPA-600/3-81-008).

Four channels (numbers 5 to 8) were employed for the PCP study. River water was supplied to each channel at 0.8 m^3 (ca. 200 gallons) per min. The first channel pool served as an area for incoming river water sediment to settle. Actual study reaches in the channels started below the first pool and extended downstream to the upper end of pool 4. The riffles were 1.2 to 1.3 m wide and 10 to 16 cm deep. The pools measured 3.4 to 3.5 m wide and about 0.58 m deep. The plant and animal communities and water chemistries of the four channels were similar; thus comparisons between channels are valid.

Chemical toxicant. The PCP used for the outdoor study was supplied from Dow Chemical Co., Midland, Mich. as EC-7 (lot 12109-M). According to Dow Chemical, the EC-7 shipment had the following chemical characteristics: PCP, 93.7%; 2,3,4,6-tetrachlorophenol, 4.9%; 2,3,4,5-tetrachlorophenol, 0.9%; hexachlorodibenzo-*p*-dioxin, 2 ppm; octachlorodibenzo-*p*-dioxin, 40 ppm; hexachlorobenzene, 270 ppm. This formulation shipped from Dow Chemical Co. arrived as solid chunks. The solid was passed through a grinder and 10 mesh sieve for reduction into a powdered state.

Channel dosing. PCP actually metered into the channels was in the form of sodium pentachlorophenate. Conversion of PCP to the sodium salt was accomplished by adding and then blending 250 ml of sodium hydroxide (50% solution) with 879 g of EC-7. The sodium pentachlorophenate solution was conveyed from a storage tank to the channels by using controlled volume Milton Roy (mRoy) diaphragm pumps. Continuous, metered volumes of the toxicant were added to three channels; individual pumps were deployed for each channel. Pumps were equipped with back-pressure valves to stabilize pumping rate and prevent back syphoning. The rate of toxicant additions were controlled manually to achieve desired channel concentrations. Nominal sodium pentachlorophenate concentrations in channels 5, 6, and 8 were 144, 48, and 432 μg per liter, respectively. The high-dose channel was lethal to many aquatic organisms, including two species of fish (Hedtke and Arthur, submitted). Channel 7 served as the control (undosed) channel. The rate of additions of the toxicant solution to channels 5, 6, and 8 ranged from 67 to 70, 23 to 30, and 205 to 209 ml/min, respectively. PCP toxicant addition rates were measured with a stop watch and volumetric cylinder. Measurement accuracy was within ±1 ml. Typical toxicant flow rates into these respective channels were 69 ± 1, 26 ± 1, and 207 ± 1 ml/min. Channel dosing began on 3 June 1982 and was terminated on 26 August 1982 (channels 6 and 8) or 17 September 1982 (channel 5). Effluents from the channels were released to the river under permission of the Minnesota Pollution Control Agency.

Sampling for microbiological analysis. Samples were collected from four sources—channel water, sediments, macrophyte surfaces, and rock surfaces—approximately every 2 weeks between May and October 1982. Each of the channels was sampled at 12 locations on 10 occasions, of which 6 occasions were during the PCP dosing period. Water samples (4 liters) were taken in acid-washed, Nalgene bottles. Subsamples of 50 ml were taken from these bottles. Sediments were sampled by using a simple coring device (plastic cylinder, 3.5 cm in diameter). Only the upper 3 cm of sediment was collected routinely; additional sediment depths (3 to 5, 5 to 7, 7 to 10, and 10 to 15 cm) were sampled less often for chemical analysis of PCP concentration. Surfaces of aquatic plants were sampled by collecting approximately equal amounts (50 to 75 g) of two predominant species (*Potamogeton crispus* and *Elodea canadensis*). Attached silt and weakly attached bacteria were washed from the surfaces of a measured

weight (e.g., 25 g) of fresh material by using 250 ml of sterilized water from the control channel. For "rock" surface studies we used unglazed 6.25-cm^2 ceramic tiles (Stylon Co., Plymouth, Minn.), which were placed in the channel riffles in mid-May. After the tiles had become colonized, the attached microflora was scraped from the tiles with a sterile scalpel blade into 62.5 ml of control-channel water that had been autoclaved and filter sterilized.

Bacterial counts. Total bacterial counts were performed by using acridine orange staining and epifluorescence microscopy as summarized by Hobbie et al. (8). An additional procedure was required for acridine orange direct counts of the sediments to eliminate background staining of detritus (13). Samples were diluted to give stained cell counts in the range of 20 to 60 cells per microscope field. A Zeiss fluorescence microscope was used at ×1,000 magnification and with all light source conditions as specified by Hobbie et al. (8). At least 20 fields were counted for every sample.

Microorganisms able to mineralize PCP were enumerated using the radioisotopic most-probable-number procedure of Lehmicke et al. (11). Water from the undosed channel was used as the growth medium for most-probable-number replicates. The water was adjusted to pH 7.4 to 7.6 and sterilized after adding 32 to 50 μg of [*U*-^{14}C]PCP (18 mCi/mmol; radiochemical purity, ≥98%) obtained from California Bionuclear Corp. (Sun Valley, Calif.). Each replicate contained at least 4,800 dpm of [^{14}C]PCP. The growth medium (1 ml per replicate) was distributed into 4-ml, 50- by 13-mm biovials (Vanguard International, Inc., Neptune, N.J.) that were capped (after inoculation with 0.1 ml of natural sample) with gas-permeable caps (Fisher Scientific Co., Springfield, N.J.). These were placed into 20-ml plastic scintillation vials (Research Products International Corp., Mt. Prospect, Ill.) containing 1 ml of 1 N NaOH for trapping evolved $^{14}CO_2$. Sterile dilution water was used to determine background radiation or any [^{14}C]PCP that volatilized (or both). Incubation was in the dark at 20 to 24°C for 6 weeks. At this time, the biovials were removed and discarded; 4 ml of distilled water was added to each scintillation vial, followed by the addition of 10 ml of Aquasol-2 scintillation cocktail (New England Nuclear Corp., Boston, Mass.) to convert the contents to the gel phase for counting. After subtracting background counts, replicates evolving at least 3% of the total radioactivity were scored as positive. Most probable numbers were calculated from a table for 10-fold, five-replicate dilutions by the method of Rodina (17) and 95% confidence limits were determined by the method of Cochran (5).

PCP mineralization rates. Samples of sediments (top 3 cm of cores) were collected from dosed and undosed channels. Multiple cores were taken at each sampling site and homogenized to insure a representative sediment sample. Three grams of wet sediment was suspended in 30 ml of filter-sterilized control-channel water, and the slurry was placed into a sterile 125-ml serum bottle. A subsample of this slurry was analyzed by a gas chromatographic procedure to determine PCP concentration. Control replicates from the undosed channel site had PCP added to equal the concentration found in the dosed-channel samples. [*U*-^{14}C]PCP (approximately 400,000 dpm and 100 μg/liter) was then added to each replicate, giving a total PCP concentration of 125 μg per liter. Serum bottles were capped with 20-mm Teflon-silicone Tuf-bond discs (Pierce Chemical Co., Rockford, Il), Teflon side down, and sealed with 20-mm aluminum seals (Pierce) to make an airtight fit. Incubation was in the dark at 25°C with 100 rpm shaking to maintain aerobic conditions. Flushing of evolved $^{14}CO_2$ was performed daily to ensure ample aeration. Compressed air was used to displace the $^{14}CO_2$ into an ethanolamine-supplemented scintillation counting fluid for counting as described previously (6).

TABLE 1. Rate data for PCP photolysis as a function of depth in channels[a]

Water depth (cm)	3-h avg (10:00 a.m. to 1:00 p.m.)		24-h avg	
	k_{obs} (h^{-1})[b]	$t_{1/2}$ (h)[c]	k_{obs} (h^{-1})[b]	$t_{1/2}$ (h)[c]
0.5	0.996	0.70	0.229	3.02
3.8	0.286	2.40	0.165	4.20
7.2	0.186	3.73	0.0411	16.9
10.5			0.0252	27.5
13.8	0.072	9.63	0.0161	43.1
20.5		18.3		216
30.0		228		1,681
40.0		1,469		14,794
50.0		9,471		129,090

[a] Data are from 23 and 24 August 1982 under the following conditions: sky, mostly sunny; root-mean-square of solar radiation intensity, 1,217 for the 3-h period and 194 microeinsteins per m^2 per s for the 24-h period; initial PCP concentration, 128 μg/liter.

[b] Corrected for absorption of light by glass container. Uncertainty in k_{obs}, ±10%.

[c] Rates at depths below 13.8 cm were too slow to obtain accurate k_{obs} and $t_{1/2}$. Values of $t_{1/2}$ for depths below 13.8 cm were calculated via equation 1 by obtaining the empirical values of kI_0 and η from plots of $\ln k_{obs}$ versus z for depths ≤13.8 cm ($r^2 = 0.938$, $n = 4$ for 3-h average; $r^2 = 0.953$, $n = 5$ for 24-h average).

PCP analyses. PCP was extracted from water samples by acidifying to pH <2 with H_2SO_4 and shaking with hexane. Extraction from sediments was carried out by stirring 1 to 3 g of wet sediment in 25 ml of acetone acidified with H_2SO_4 for 2 h, followed by partitioning the mixture between equal volumes of 0.1 N HCl–0.1 N KCl and hexane. The hexane layer was then subjected to the ion-exchange cleanup procedure of Renberg (16). The moisture content of the sediment was determined with a parallel sample by heating at 105°C overnight. Spike recovery with [^{14}C]PCP was 98 to 99% from the channel water and 87 to 94% from the sediments. Full details of these procedures will be published elsewhere. The PCP in the hexane extracts was derivatized with diazoethane in hexane and injected into a gas chromatograph (Hewlett-Packard; 5840) equipped with a 6-ft (ca. 1.83-m) by 2-mm glass column (1.95% OV-210 and 1.5% OV-17 on Chrom W-HP, 100-200 mesh) and ^{63}Ni electron capture detector. Column conditions were as follows: 160°C for 7 min; bakeout at 250°C for 5 min; retention time of PCP, ca. 5.4 min. PCP concentrations were determined by comparison with standards. The percent standard deviation was 4 to 7% ($P < 0.05$) for water samples. The

mean percent standard deviation was 12% with a range of 4 to 26% for sediment samples.

Photolysis kinetics. The photolysis kinetics determinations were carried out in 20-ml borosilicate glass scintillation vials with aluminum foil-lined caps fastened to a Plexiglas rack. The rack was then placed in the channel pool in an unshaded spot in such a way that the vials did not shade each other. The depth of each vial was taken to be the depth of its center of gravity. A sample of 5 ml was withdrawn at 1, 3, and 24 h from each vial. During the sampling time the vials were protected from sunlight. Rate constants were corrected for apparent absorption of light by the glass by comparing rates (k) of samples in the vials with those in open shallow bowls; the k_{open}/k_{glass} ratio was 1.14.

The extinction coefficient of the channel water was determined by measuring the absorbance at 320 nm versus that of distilled water in 1-cm cells with a Bausch & Lomb Spectronic 710 spectrophotometer. Solar radiation was measured hourly with a radiometer (Lambda Instrument Co.; model LI-1905; active region, 400 to 700 nm) interfaced with a data logger. To calculate the relative solar radiation integral for a particular period of time, a computer-generated plot of intensity versus time was made on standard paper with standard axis dimensions, and the weight of the paper underneath the curve was measured to the nearest milligram.

PCP volatilization. Air-borne PCP was measured by pumping air at 228 ml/min from a 70-mm-diameter funnel placed ~3 cm from the water surface through all-glass tubing to two flasks (protected from light) in series equipped with dispersion tubes immersed in 0.1 N NaOH solution. The total pumped volume was 588 liters. At termination, the contents were acidified and extracted into hexane.

Biodegradation kinetics. Determinations of bacterial degradation kinetics in the channel water were carried out by monitoring the PCP concentration of 100-ml samples of channel water shaken at 100 rpm in the dark at 25 to 26°C in cotton-plugged 125-ml serum bottles. The controls consisted of channel water filtered through 0.2-μm Gelman Metricel filters. All samples were handled aseptically.

Chloride assay. Free chloride ion concentrations at ionic strengths less than 10^{-2} M were determined with an Orion model 94-17 chloride electrode, following instructions provided by Orion Research Inc. Free chloride ion concentrations measured ranged from 10^{-5} to 10^{-2} M. An Orion model 701 digital pH/mv meter was used with the Orion electrodes (model 90-02 double-junction reference electrode). The electrodes were calibrated daily against standard solutions of chloride. The electrode was checked hourly during an experiment; if necessary, a new calibration curve was constructed. All samples and standards were measured at ambient temperature with minimal magnetic stirring. Chloride determinations were used during studies of PCP mineralization by pure bacterial cultures.

RESULTS

Channels fed by Mississippi river water were dosed continuously during the summer of 1982 with PCP. One channel was undosed and served as a control. PCP was removed from the dosed channels by a combination of adsorption, volatilization, photolysis, and microbial degradation. Photolysis was rapid at the water surface, but greatly attenuated with depth (Table 1). Adsorption, sedimentation, and volatilization of PCP were not significant mechanisms of PCP removal from unacclimated (before week 3 of the study) channel water. Microbial degradation of PCP in the channels became significant about 3 weeks after the start of dosing. This was indicated by the following: (i) a sharp decline in the concentration of PCP down the length of all dosed channels between weeks 3 and 5; (ii) rapid microbial transformation of PCP in samples taken from dosed channels (but not the control channel) after about week 4 of the study (Fig. 1 and 2); (iii) the emergence in the dosed channels of bacterial degraders capable of mineralizing [^{14}C]PCP and $^{14}CO_2$ concomitant with the decrease in PCP concentration within the dosed channels (Fig. 2 and 3); and (iv) a large decline in sediment PCP concentrations between weeks 3 and 5 (Fig. 3A). Most-probable-number counts indicated that most of the PCP-mineralizing microorganisms that developed in the channels

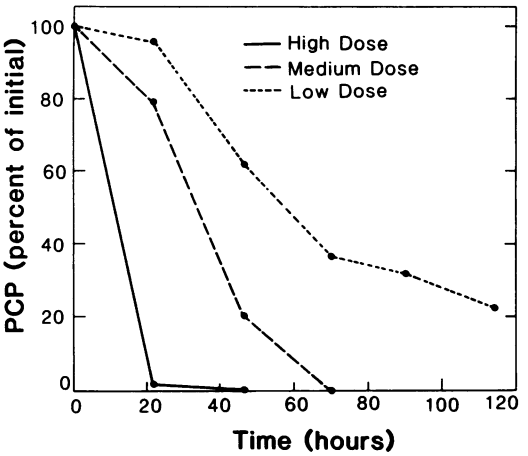

FIG. 1. Microbiological removal of PCP from PCP-acclimated channel waters. Samples described here were taken on day 46 of dosing at 137 m from the PCP injection point. Biodegradation kinetics were determined by gas-chromatographic analyses as described in the text. The results are averages of duplicates. Ambient initial PCP concentrations were used (i.e., 199, 76.0, and 32.6 μg/liter for the high-, medium-, and low-dose channels, respectively). Filter-sterilized controls did not degrade PCP. Similar experiments with PCP-spiked water samples from the control channel or from the dosed channels above the PCP injection point did not show PCP degradation within 2 weeks. The apparent dose response (increased degradation rates at higher PCP concentrations) shown was not consistently observed during repetitions of these experiments.

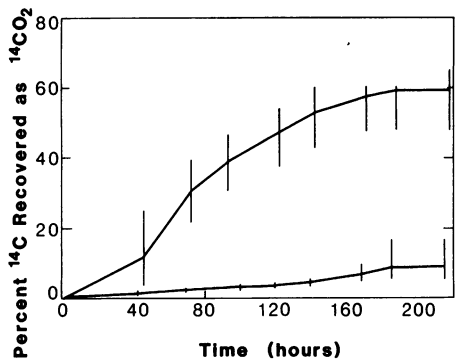

FIG. 2. Mineralization by PCP-acclimated channel sediments. Mineralization studies were performed as described in the text. Samples were taken from a dosed channel that had been exposed to 144 μg of PCP per liter for 99 days; control samples were not previously exposed to PCP. The lower line illustrates the lack of $^{14}CO_2$ evolution from control sediments. Bars show the range between replicate samples (three replicates per point). Initial concentrations of PCP were ca. 125 μg/liter.

were either attached to surfaces (rocks, macrophytes, and detritus particles), or were associated with surface sediments (Table 2). Our determinations of PCP losses from the channels after week 3 of the study indicated that photolysis caused 5 to 28% decline (Table 3 and below) in initial PCP concentrations down the channels, depending on sunlight conditions. Microbial degradation accounted for a 26 to 46% decline (Table 3). After the 3-week acclimation period, adsorption of PCP to surfaces and sediments presumably became more important as a mechanism of PCP transport to the microflora. We could not estimate accurately the quantitative importance of this unidirectional transport of PCP. It is reasonable to assume, however, that microbial removal of surface-adsorbed PCP (even if only small amounts are involved) would promote migration of PCP to surfaces by mass action.

The rate law for photolysis of PCP in the channel water is given by the following equation:

$$-\ln[PCP]/[PCP]_0 = k_{obs}t$$
$$k_{obs} = kI_0(e^{-\eta z}) \quad (1)$$

where I_0 is the solar radiation intensity at photochemically active wave lengths (near 320 nm for PCP) incident on the water surface, k is a constant of proportionality which includes the quantum yield of the reaction, η is the extinction coefficient of the channel water (which is the sum of η for dissolved and particulate absorbers), and z is the depth (21).

Rate constants (k_{obs}) and half-lives as a function of depth were measured on a mostly sunny day in August (Table 1); photolysis was greatly attenuated with depth. This attenuation is due to the extinction of light by suspended particles and dissolved materials in the channel water (21), since water does not appreciably absorb near the λ_{max} of PCP (320 nm). The measured extinction coefficient of the channel water at 320

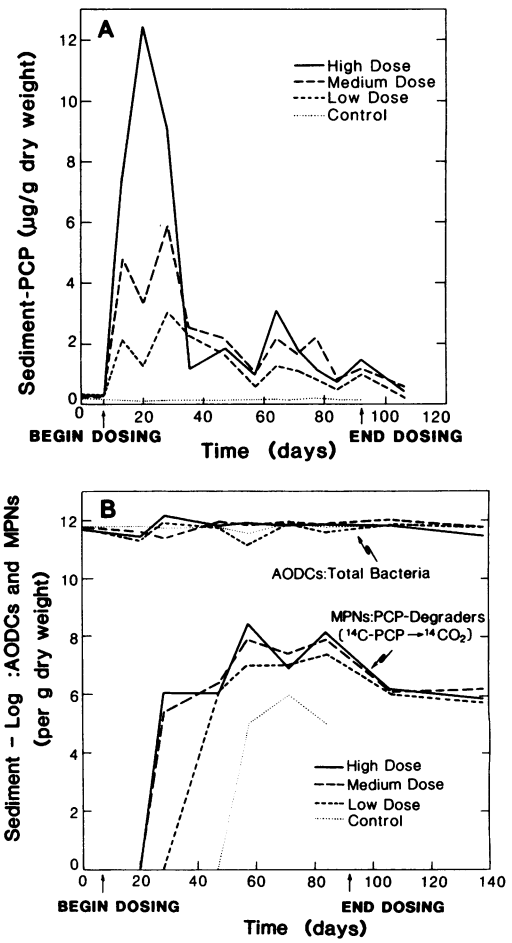

FIG. 3. Concomitant development of PCP-degrading microorganisms and PCP disappearance in PCP-dosed channel sediments. (A) Sediment PCP concentrations. (B) Sediment bacterial populations. PCP degraders appeared in the control channel after about 45 days, perhaps as a result of cross-contamination from other channels as a result of multidisciplinary sampling activities. It also is possible that these microorganisms may represent a natural input from the river (note the low background level of PCP in the control stream sediments). All samples were collected at points 115 m from the PCP injection point. For typical statistical variations in most probable numbers (MPN) and acridine orange direct counts (AODC), see Table 2.

TABLE 2. Microbial numbers in various locations of the high-dose (432 μg/liter) PCP channel (concentration range, 204 to 480 μg/liter)

Location	No. of PCP degraders[a]	95% confidence limits[b] (lower, upper)	Total no. of bacteria[c]	SD
Water column	6.5×10^0/ml	2.0×10^0, 2.1×10^1	9.3×10^6/ml	$\pm 1.4 \times 10^6$
Tile surfaces	2.5×10^3/cm^2	7.6×10^2, 8.3×10^3	1.4×10^8/cm^2	$\pm 1.7 \times 10^7$
Macrophyte surfaces	3.4×10^5/g[d]	1.0×10^5, 1.1×10^6	4.8×10^9/g[d]	$\pm 6.2 \times 10^8$
Sediments (0 to 3 cm in depth)	8.2×10^7/g[d]	2.5×10^7, 2.7×10^8	5.3×10^{11}/g[d]	$\pm 5.9 \times 10^{10}$

[a] Determined by the ^{14}C most-probable-number procedure (see the text). The numbers shown are averages of all sampling points for 22 July 1982.
[b] Determined by the method of Cochran (5).
[c] Determined by epifluorescence microscopy (see the text). The numbers are averages of all sampling points for 22 July 1982.
[d] Microbes per gram (dry weight) of tissue or sediment.

nm ranged from 9.5 to 19.0 m^{-1} over the summer.

The loss of PCP from the system can occur by export (volatilization), adsorption, chemical reaction (i.e., photolysis), and biodegradation. Volatilization loss in the form of vapor and aerosol was found to be ≤0.006% of the load. Loss due to adsorption to sediments and accumulation or transformation by plants and animals (hereafter referred to as "adsorption") was estimated to be ≤15% in the medium-dose channel. This was calculated from the 6:00 a.m. downstream gradient as a 3-day average in the early phase of the study (8 to 10 June, days 5 through 7), before bacterial degradation became significant. The residency time in the channels, determined with fluorescent dye, was approximately 10 h; this means that by 6:00 a.m. the photolysis-induced gradient from the previous day had been flushed out. More detailed studies presently in progress indicate that adsorptive losses of PCP may in fact be <5% (unpublished data). It is possible that adsorption losses declined during the summer as "saturation" of the sediments, etc., progressed; however, bacterial degradation of adsorbed PCP after channel acclimation might also promote transport of PCP to surfaces by a mass-action mechanism ($PCP_{water} \rightarrow PCP_{surfaces} \rightarrow CO_2$).

The 24-h average photolysis contribution was estimated to average 19% during days 5 through 7 (Table 3) by subtracting the adsorptive losses from the 24-h average total decline in the medium-dose channel. The relative contribution should be independent of initial concentration (equation 1). The value of 19%, the mean relative solar radiation integral (see above), and the channel water extinction coefficient for days 5 through 7 were used to calculate an empirical rate constant k for a 24-h period via equation 1. The extent of photolysis for other days, relative to days 5 through 7, was determined from the relative solar radiation integral and the channel water extinction coefficient η for the day, by the use of equation 1 and the empirically derived 24-h rate constant. These are listed in Table 3 for representative days. The solar radiation integral for the photoactive region (i.e., ~320 nm) was approximated by the area under the curve of radiation intensity (microeinsteins per square

TABLE 3. Photolytic and bacterial transformation losses in the channels on selected dates

Day of study	Relative solar radiation integral[a]	Sky condition[b]	% PCP decline from initial, 24-h avg[c]				
			Photolytic (all channels)		Bacterial, in channel:		
			Found	Calculated	High dose	Medium dose	Low dose
5	685	Sun	Mean 19		~0	~0	~0
6	259	Cl					
7	751	Sun					
24	789	Sun		28			
37	131	Cl		5			
53	276	Cl		7			
55	643	Sun		24	44	36	26
69	655	Sun		24	46	38	29
81	406	Pt Cl		16			

[a] See the text for a definition of relative solar radiation integral.
[b] Sun, Sunny; Cl, cloudy; Pt Cl, partly cloudy.
[c] See the text for methods of calculation.

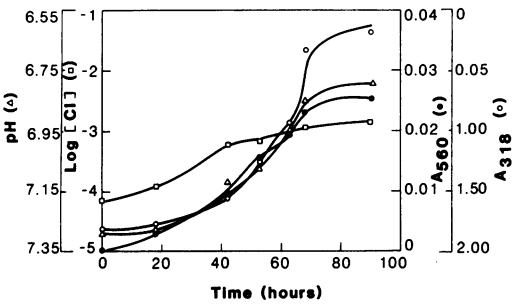

FIG. 4. Growth of a pure bacterial culture on PCP as a sole source of carbon and energy. Symbols: △, pH; □, log chloride ion concentration (molar); ●, absorbance at 560 nm (culture turbidity); ○, absorbance at 318 nm (PCP concentration). The bacterium was grown at 30°C in a defined mineral-salts medium (18, 19) containing PCP (100 mg/liter) as the only source of carbon and energy. PCP removal was complete in about 90 h, with all of the organic chlorine being released as chloride ions.

meter per second which was measured from 400 to 700 nm) versus time. This is likely to be valid to a first approximation, especially for clear days (10). For cloudy days we obtained rate constants obtained in vitro that were 32 to 35% smaller than predicted on this basis; this had been taken into account in the calculated values for cloudy days given in Table 3.

The contribution to PCP loss from bacterial transformation was estimated from diel PCP measurements in all three channels on two occasions, after bacterial degradation was well underway. The values listed in Table 3 were obtained by subtracting the calculated photolysis contribution for the day and the adsorption loss from the total 24-h average decline. The values obtained are somewhat lower than those calculated from the 6:00 a.m. (i.e., photolysis-absent) PCP measurements: 57 to 62 (high-dose channel), 49 to 50 (medium-dose channel), and 41 to 44% removal (low-dose channel). This could be due to an overestimation of adsorption or photolysis contributions (or both) or to diel fluctuations in the bacterial transformation rate due to swings in pH or other water chemistry. Channel monitoring over the summer indicated relatively stable PCP gradients after week 4 of the study, which suggests that the bacterial contributions calculated for these two occasions are applicable for much, if not all, of the period after week 4.

Confirmation of the importance of bacteria to PCP removal was obtained by our isolation from dosed channels of numerous pure cultures of bacteria that are able to use PCP as a sole source of carbon and energy for growth. One such organism (Fig. 4) grew on PCP at a concentration of 100 mg/liter, releasing all of the organically bound chlorine as chloride ions. With regard to total bacterial numbers in the channels, we observed no significant effect of PCP on total bacterial counts irrespective of channel treatment (*t*-test, 95% level). Direct epifluorescence counts of bacteria showed relatively constant levels of bacteria within the channels throughout the summer, as follows: water, 2.7×10^6 to 3.4×10^7 bacteria per ml; tile (rock) surfaces, 6.0×10^7 to 2.4×10^8 bacteria per cm^2; plant surfaces, 9.8×10^8 to 7.8×10^9 bacteria per g (dry weight); and sediments, 1.3×10^{11} to 1.5×10^{12} bacteria per g (dry weight).

After the dosed channels had become acclimated to PCP and PCP concentrations within the channels had decreased (by as much as 80%), the high-dose stream was no longer lethal to fish species that were quickly killed at the outset of dosing (Hedtke and West, personal communication). These data will be published separately.

DISCUSSION

The most important conclusions of our in vitro and in vivo studies are that in a continuously PCP-contaminated stream ecosystem, (i) photolysis of PCP in the near surface waters initially is the primary mechanism of PCP removal, (ii) after a period of weeks the aquatic microflora becomes adapted to PCP mineralization and supplants photolysis as the major PCP removal process, (iii) "attached" microorganisms are primarily responsible for PCP biodegradation, and (iv) total bacterial numbers are not significantly affected by PCP concentrations of micrograms per liter.

It is clear from these studies that when PCP enters most previously uncontaminated streams at levels of ≥400 ppb, fish kills probably will result. If contamination is long term at these levels, lethality to higher organisms will continue for a period of weeks. Eventually the microbial population in such a stream will become capable of PCP mineralization and decrease the PCP concentrations to levels that are not overtly toxic to higher animals like fish (although long-term effects might still be observed if PCP levels are not decreased sufficiently). However, it is important to note that our experiments were run during May to October. In the winter in northern climates stream temperatures are much lower, and microbial adaptive responses and biodegradation rates are much slower. Thus one might not expect to observe as rapid or complete a detoxification of PCP during winter months.

It also is apparent from our experiments that photolysis of PCP will contribute to an increasingly smaller extent to PCP removal as the average depth of a stream or river increases, due to the extinction of light. On the other hand,

biodegradation rates in the same stream may be expected to be similar to those in our channels, providing the surfaces available for microbial attachment (i.e., rocks, macrophytes, and surface sediments) remain in the same proportion to the water volume.

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