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Persistence of Pentachlorophenol in a Wastewater-Estuarine Aquaculture System

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This project was undertaken to examine the accumulation and elimination of pentachlorophenol (PCP) in an estuarine aquaculture system receiving sewage effluent as a nutrient source for raising shrimp.

Approximately 80 million pounds of PCP and its salts are used each year as biocides: primarily as wood preservatives with lesser applications as insecticides, fungicides, herbicides, algacides, disinfectants and in anti-fouling paint (Bevenue and Beckman, 1967; Cirelli, 1978; Wegman and Van den Broek, 1983). Widespread distribution of PCP in aquatic environments has been well documented, including surface waters and sediments (Pierce, 1978; Wegman and Hofstee, 1979; Pierce et al., 1980; Van Gelder, 1982; Wegman and Van den Broek, 1983), in rainwater and drinking water (Bevenue, 1972; Abrams et al., 1975), in wastewaters (Buhler et al., 1973; Chau and Coburn, 1974), and in aquatic organisms (Zitko et al., 1974; Kobayashi et al., 1976; Pruitt et al., 1977: Lu et al., 1978; Pierce and Victor, 1978; Van Gelder, 1982).

Various studies of PCP and its degradation products in aquatic systems have shown that PCP is rapidly accumulated in organisms and in sediment from the water column (Courtney and Denton, 1976; Pierce, 1978; Boyle et al., 1980; Pierce et al., 1980; Wegman and Van den Broek, 1983). Accumulation and depuration studies of PCP in aquatic organisms has shown that when contaminated organisms were placed in a clean environment, they generally exhibited the ability to cleanse themselves of most of the PCP within a few days (Pruitt et al., 1977; Borthwick and Schimmel, 1978; Schimmel et al., 1978; Hauch et al., 1980; Van Gelder, 1982). In contrast, PCP associated with sediment and detritus in freshwater systems was found to persist for months (Pierce, 1978; Pierce et al., 1980; Boyle et al., 1980). Lu et al. (1978) presented a classification scheme predicting enhanced solubility of PCP in estuarine water (pH 8). This enhanced solubility should lead to rapid

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reduction in PCP concentration by dilution and photochemical degradation (NRCC, 1982), thus precluding accumulation in sediment and aquatic organisms.

As part of a larger project investigating a shrimp aquaculture-wastewater system (Landau, 1983), this study provided the opportunity to examine the fate of PCP during chronic contamination of a simulated estuarine environment. The hypothesis tested was that PCP entering the estuarine water (pH 8) would be solubilized as the phenolate anion, reducing the potential for accumulation in sediment and shrimp feeding at the sediment surface.

MATERIALS AND METHODS

Three PVC-lined ponds, 10 m x 10 m x 0.5 m, received 12,500 L/day (turnover rate of once in four days) of water from the Indian River estuary in east central Florida. Post-larval shrimp, Penaeus vannamei Boon (19-23 mg) were stocked in each of the three ponds at a density of $24/m^2$. When the shrimp reached approximately 0.6 gm, about one month after stocking, those in the control pond (pond 1) began to receive prepared feed while those in the test ponds (ponds 2 and 3) began to receive a mixture of seawater and sewage effluent (9:1). The prepared feed was a pellet, Ralston Purina Experimental Marine Ration LF (25% protein, 5% crude fat, 5% crude fibre); the feeding schedule is discussed elsewhere (Landau, 1983). Secondary treated sewage effluent was pumped from the percolation pond of the activated sludge plant at the Harbor Branch Institute, Ft. Pierce, Florida. PCP was added manually to the test ponds by broadcasting 500 mg of Na-PCP salt in acetone initially, and 250 mg on alternate days thereafter to provide a theoretical concentration of 10 ug/l, averaged over the four-day turnover time (Seidler, 1983). The overflow from the test ponds was routed to a holding pond, which flowed into a swamp after removal of any residual PCP by charcoal filtration (Fig. 1) while pond 1 was discharged directly into the swamp. Pond 2 was used to study the decontamination of the system after termination of sewage and PCP; it received the treated sewage and PCP treatment for 89 days, followed by a 71-day decontamination, during which no more effluent or PCP were added but the prepared feed was fed to the shrimp. Pond 3 received PCP dosing continuously throughout the 160-day investigation period.

Surface water and surface sediment samples were collectd in glass containers from each of the ponds at least once every two weeks beginning June 25, 1982. Each water and sediment sample was a composite from a minimum of two locations in the pond. Sediment samples were always collected after water samples to avoid contamination of the water by suspended material. Shrimp were collected in baited fish traps during July, August and September. After collection, all materials were put on ice and transported for about one hour to the laboratory where they were kept refrigerated until extraction, which was performed no later than seven days after collection. Shrimp were dissected and divided into edible (ED) and nonedible (NED) tissue fractions.

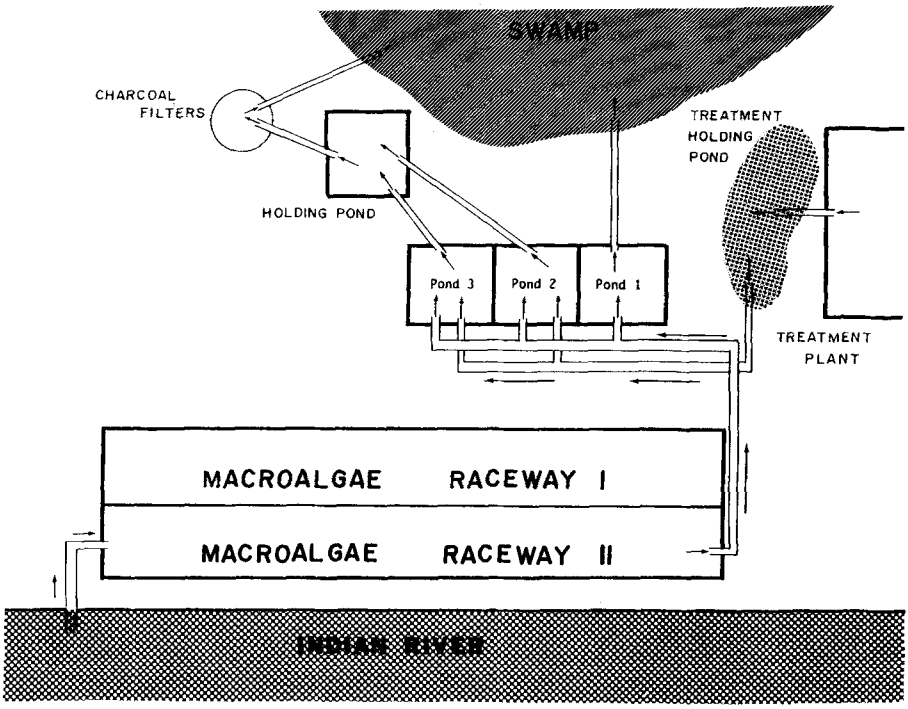

Figure 1. The wastewater-estuarine aquaculture facility.

The ED fraction consisted of the edible tail meat and the NED fraction contained the rest of the organism (i.e., exoskeleton, viscera, head).

Water samples were extracted following the procedure of Wegman and Hofstee (1979). One L sample was sonicated, placed in a separatory funnel and acidified to pH 2 with HCl. The acidified samples were then extracted twice with hexane and the combined extracts were transferred to screw-capped culture tubes and frozen. The organic phase was then decanted from the frozen aqueous layer and successively washed three times with 0.1 m Na_2CO_3 to recover PCP. The PCP washings were then combined and the hexane solution was discarded. Derivatization of the free phenols to the methyl ether was accomplished by adding redistilled acetic anhydride and hexane and thoroughly mixing at room temperature for five minutes. The hexane fraction was recovered and retained for analysis.

Sediments were air-dried at 30-35°C, reduced to powder in a stainless steel Waring blender and stored in the dark in tightly capped glass vials until extraction. The extraction method was adapted from Wegman and Van den Broek (1983). The powdered sediment was placed in a capped glass culture tube and extracted for 20 minutes in toluene by shaking. The mixture was then centrifuged and the toluene was decanted into a separatory funnel. The sediment was then reextracted two more times and the combined organic extracts

were washed three times with 0.1 M Na_2CO_3 which was then treated in the same manner as the water sample.

Freeze-dried shrimp samples were extracted in a soxhlet apparatus with hexane for six hours, washed twice with water, followed by two washings with 0.1 N NaOH. The two basic washings were combined and acidified to pH 2 with HCl and the PCP recovered by extraction with hexane. The PCP-containing hexane solution was then concentrated to ca. 1 ml on a rotary evaporation apparatus in preparation for gas chromatographic analysis.

Analyses of the sediment and water samples were performed with a Varian model 2440 gas chromatograph (GC) (Varian Associates, Sunnyvale, CA) with an electron capture detector. The 2 mm x 1 m glass column was packed with 3% SP-2100 on 80/100 mesh Suplecoport. The carrier gas was N_2. The identity of the PCP peaks was confirmed using a 1% SP-1240-DA packed column (Supelco, Inc., Belefonte, PA).

Analysis of the shrimp tissue was performed using a Varian model 6000 GC coupled to a VISTA-401 data system. Columns were glass capillary (SE-30, 0.25 mm x 30 m) and the carrier gas was N; an electron capture detector was used. Samples were introduced by direct splitless injection mode. The identity of PCP was confirmed by mass spectroscopy on select samples.

Since a normal distribution cannot be assumed for time-series data, nonparametic tests were employed to detect significant differences in data groups (Mann-Whitney U test) and significant positive correlations between data sets within a pond (Spearman rank correlation test).

RESULTS AND DISCUSSION

The control pond was used to establish background concentrations of PCP in water, sediment and shrimp (Fig. 2). Water from the test ponds (ponds 2 and 3, receiving PCP contamination) were found to contain PCP in concentrations significantly higher than background during the first 22 days of exposure (Fig. 2a). The concentration then diminished to background levels in water from both ponds through day 89, even though PCP was continuously added. After termination of PCP addition to pond 2 at day 89, the water PCP concentration remained at background levels. Water in pond 3, continuing to receive PCP contamination, exhibited an increase in concentration through day 125 and then diminished to background by the end of the experiment at day 160.

Sediment PCP concentrations persisted at elevated levels for a longer period of time than in water (Fig. 2b). PCP levels significantly higher than background were observed in sediment from both ponds through day 64. This was followed by a brief period of near-background levels around day 76 with the concentration increasing again through day 89. After termination of PCP addition

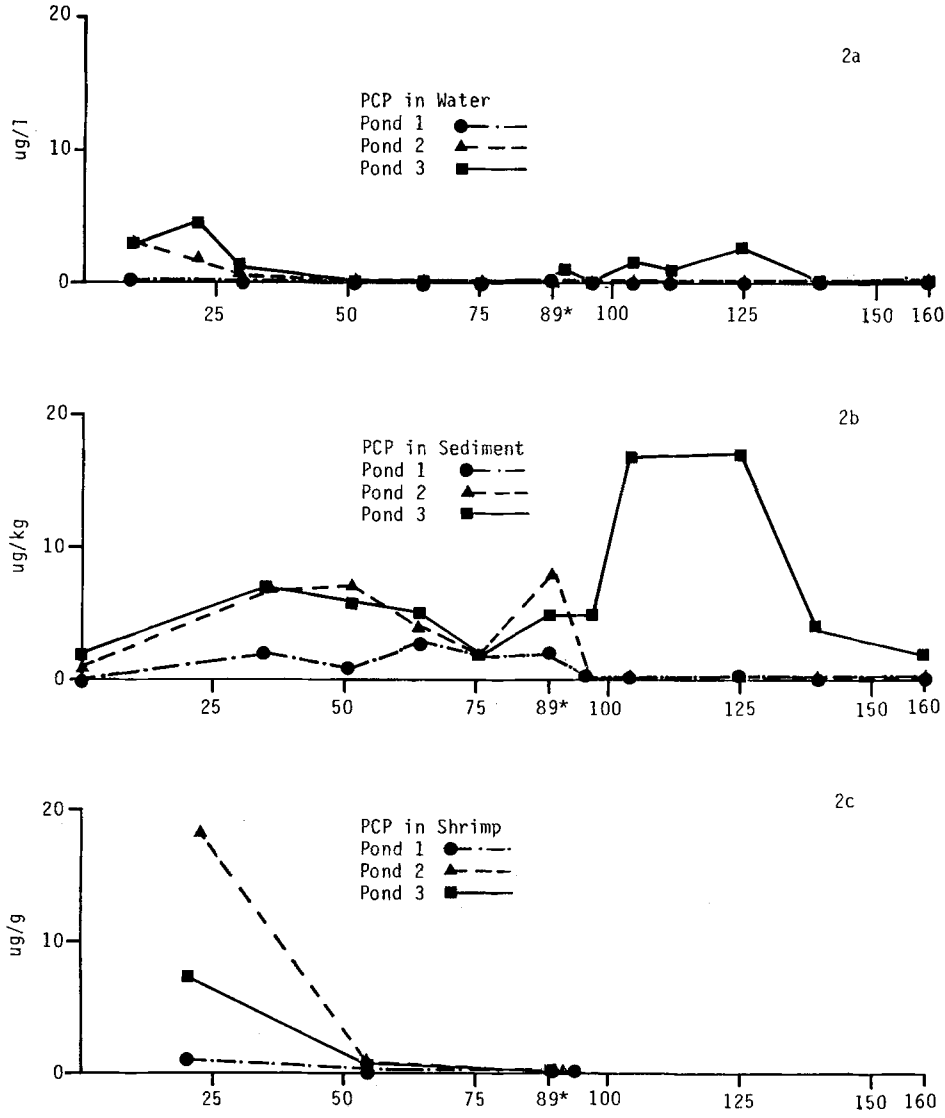

Figure 2. PCP concentrations in water, sediment and shrimp in Pond 1 (control), Pond 2 (89 day PCP exposure) and Pond 3 (160 day PCP exposure). *Termination of PCP to Pond 2 after 89 days.

to pond 2, the sediment PCP levels diminished to background within one week while sediment in pond 3 continued to exhibit high concentrations. As with water samples, sediment PCP diminished to background levels at the end of the study (day 160).

The PCP content of shrimp was determined through day 92 (Fig. 2c). Due to a similarity in PCP concentration trends in both ED and NED tissue, the data were averaged to give whole body shrimp PCP content. Although these analyses were not carried throughout the entire 160-day exposure study, they do show that shrimp contained significantly higher than background PCP levels at day 20. By day 55, however, levels had dropped to background and remained there through day 92.

Considering the many pathways for PCP loss from pond water, the observed concentrations of 3 to 5 ug/l in ponds 2 and 3 showed excellent agreement with the theoretical amount added (10 ug/l) during the first 22 days of the study. In addition to loss from the flow-through system, numerous physical, chemical and biological pathways such as sorption, photolysis, volatilization, microbial degradation, and bioaccumultion can account for PCP reduction.

At the pH of these ponds (8.0), 99.9 percent of added PCP theoretically would be dissolved as the phenolate form (Lu et al., 1978), implicating photolysis as the most significant degradation process. A PCP photolysis rate constant of $17.18\ cm\ d^{-1}$ has been reported for highly polluted water (pH 7.0) in Canada (NRCC 1982). Given that the effect of latitude is unimportant in the photolysis rate compared to season, pH, and light attenuation (NRCC 1982), the estimated half-life for dissolved PCP would be about two days for the 0.5 m-deep ponds. This is the same time required for 50 percent of the added PCP to be flushed from the ponds before another application was made. Thus, the declining aqueous concentration could be caused by photolysis and hydraulic turnover.

High concentrations of PCP found in surface sediment (concentration factor of 54 compared to PCP in water), however, indicate that incorporation into sediment was important even at pH 8. These findings indicate that although PCP solubility may be enhanced in estuarine water, accumulation in sediment did occur. Possible mechanisms for accumulation in sediment include adsorption to detritus settling to the bottom, assimilation into benthic algae and ingestion by zooplankton with subsequent deposition in fecal pellets. The rapid loss of PCP from sediment in pond 2 after termination of exposure would indicate a biological elimination process such as microbial degradation or active elimination from benthic algae rather than physical desorption from sediment particles. At this point, the solubility of PCP at pH 8 would enhance removal from the pond by dilution and photolysis as suggested by Lu et al. (1978) and the NRCC (1983).

The initially high PCP concentrations found in shrimp at day 20, reducing to and remaining at background from day 155 through 92, correlate with the PCP levels found in water but not in surface sediment, suggested that shrimp did not accumulate PCP through the benthic food chain.

The results of this study indicate that chronic influx of PCP to an estuarine environment at the 10 ug/l level lead to elevated values in water, sediment and shrimp. Shrimp did not reflect PCP concentrations in sediment, but more closely followed that found in the water column. Once the source of PCP contamination was removed however, no persistence was observed in water or sediment, indicating no long-term effects to the pond ecosystem.

Additional studies considering PCP degradation products and more intensive monitoring of these compounds in water, sediment and organisms are required to establish relative importance of various mechanisms responsible for PCP elimination from estuarine systems.

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