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Degradation of the Herbicide Propanil in Distilled Water

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Propanil (3',4' - dichloropropionanilide) is classified as an acylanilide which belongs to the class of phenylamides. It is a selective contact herbicide which is used for post-emergence control of weeds on rice, potatoes and tomatoes (Hill and Wright 1978, Royal Soc. Chemistry 1983). In soils,microorganisms are responsible for the degradation of acylanilides to the corresponding aniline (Matsunaka 1971). The microorganisms were identified as Pseudomonas striata, Fusarium solani and species of Penicillium and Pulullaria (Bartha and Pramer 1970). Propanil biodegradation has been demonstrated in soils and it was shown that 3,4 - dichloroaniline (DCA) was the common intermediate metabolite following the microbiological degradation of propanil as well as several phenylamide herbicides (Bartha and Pramer 1967, Chisaka and Kearney 1970, Cripps and Roberts 1978, Lanzilotta and Pramer 1970a, 1970b). Other studies have been carried out on the metabolism of 3,4 - dichloroaniline by soil microorganisms (Bartha and Pramer1970, Kearney and Plimmer1972, You and Bartha 1982a, 1982b) and one of the major biodegradation products of DCA is 3,3',4,4' - tetrachloroazobenzene (TCAB). The bulk of DCA becomes covalently bound to humic materials in soils (Bartha 1971), and is thus slowly degraded (You and Bartha 1982a). However, it was shown that DCA mineralization was stimulated by the addition of an unchlorinated compound such as aniline (You and Bartha 1982b).

Few studies have been carried out on the persistence of propanil in aquatic environments. El Dib and Aly (1976) have reported that chemical hydrolysis of phenylamide herbicides was of minor significance with regard to the degradation of these herbicides. At 20°C and pHs commonly found in natural waters, the herbicides, including propanil, were stable for more than four months.During a study of chemical hydrolysis of propanil at alkaline pHs and high temperatures (Dahchour and Sabadie unpublished data) it was discovered that this herbicide was relatively rapidly degraded in non-sterile distilled water at neutral pH. A study was undertaken to isolate the microorganisms responsible for propanil biodegradation is distilled water.

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MATERIALS AND METHODS

The herbicide under study was 3',4'-dichloropropionanilide (Propanil). It was purchased from Rohm and Haas (Philadelphia, PA, USA).

Distilled water was obtained from the distillation apparatus and routinely stored in a polyethylene carboy. It was buffered to pH 7.0 with phosphate buffer ($Na_2HPO_4.12H_2O$ - KH_2PO_4). The final molarity of the buffer was 0.03 M.

Erlenmeyers containing 100 ml of buffered distilled water were amended with 50 ppm (w/v) of propanil and incubated at 28°C on a rotary shaker until all the propanil was degraded to 3'4' dichloroaniline. For experiments dealing with abiotic degradation of propanil, the buffered distilled water containing 50 ppm propanil was autoclaved for 20 min at 120°C (it was observed that only 3% of propanil was degraded following autoclaving). In some experiments, propanil biodegradation was carried out in a minimal mineral growth medium described by Pochon and Tardieux (1962). This enrichment medium was amended with 50 ppm of propanil as the sole source of carbon and energy.

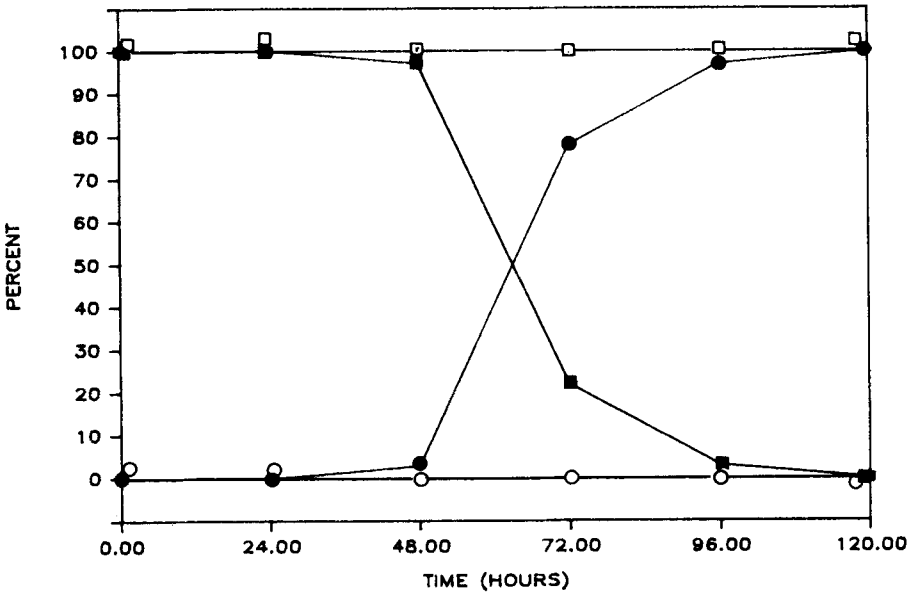
Two ml aliquots were sampled at given time periods and were extracted with 5 ml hexane solution. The mixtures were vortexed for approximately 1 min and then centrifuged. An aliquot of the upper hexane phase was sampled and analyzed via HPLC. The HPLC apparatus had a constametric I pump and a spectromonitor III detector with an NH_2 spherisorb column at 236 nm wavelength. The eluent mixture used was isooctane-ethanol (97:3 v/v).

RESULTS AND DISCUSSION

In the course of an investigation of the chemical degradation of propanil at high temperatures (Dahchour and Sabadie, unpublished data), it was discovered that this herbicide was biodegraded in distilled water buffered to pH 7 with 0.03 M phosphate buffer. Figure 1 shows the biodegradation of 50 ppm propanil in non-sterile, buffered distilled water at 28°C. The lag phase for the initiation of propanil degradation was approximately 48 hours and the herbicide could no longer be detected after 120 hrs. Figure 1 also shows the formation of 3,4 dichloroaniline during the time period under study. No degradation was, however, observed in an autoclaved solution of 50 ppm propanil in buffered distilled water for up to 10 days. It was thus suspected that biodegradation was implicated in propanil disappearance and subsequent formation of 3,4 dichloroaniline.

The effect of pH (pH 5 - pH 8) on propanil biodegradation was investigated and the results are displayed in Figure 2. Although we observed a slower propanil degradation at pH 5, the herbicide was totally degraded at all pH values following incubation for 120 hrs at 28°C.

Figure 1. Propanil degradation in distilled water.



■ Propanil remaining in distilled water.
□ Propanil remaining in autoclaved distilled water.
● DCA formed in distilled water.
○ DCA formed in autoclaved distilled water.

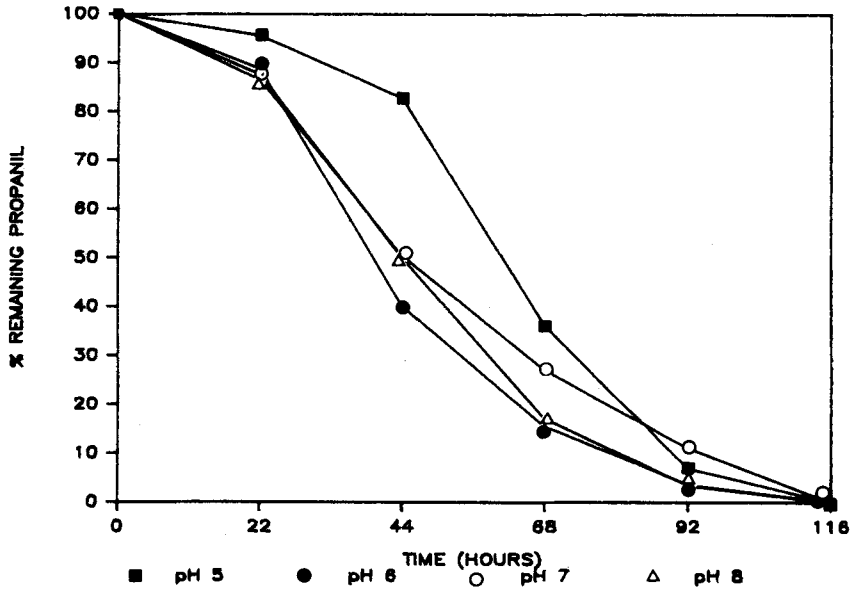


Figure 2. Effect of pH on propanil degradation in distilled water.

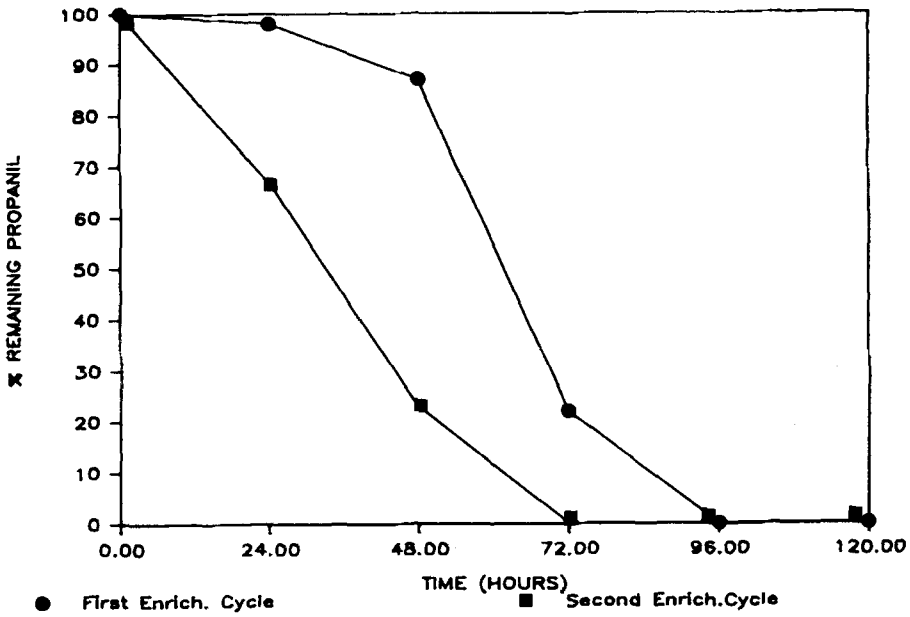


Figure 3. Enrichment for propanil-degrading microorganisms.

Enrichment for propanil-degrading microorganisms was undertaken by introducing 2 ml of inoculum from the previous experiment into 100 ml of minimal growth medium (Pochon and Tardieux 1962) supplemented with 50 ppm of propanil as a sole source of carbon and energy. The next enrichment cycle was undertaken when all the propanil has been degraded to 3,4 dichloroaniline. Figure 3 shows the extent of propanil degradation during two successive enrichment cycles. It was observed that the lag period for initiation of herbicide degradation was reduced from 48 h (see Figure 1) to less than 24 hrs. At the end of the second enrichment cycle, the remaining propanil was 2% after 72-hr incubation period as compared to 22% in the original inoculum (Fig. 1 and 3).

To isolate the microorganisms responsible for propanil degradation, aliquots (0.1 ml) of microbial suspensions from the previous experiment were spread on nutrient agar and incubated at 28°C for 48 hrs. Other aliquots were also spread on minimal growth medium containing 50 ppm propanil as the sole source of carbon and energy and which has been solidified with 2% agar. The plates were incubated at 28°C for up to 5 days. In both media, two types of bacterial colonies were observed: large colonies of 3 to 5 mm diameter and smaller colonies of approximately 1 mm diameter. The two colony types were separately reisolated on nutrient agar and sent to the Pasteur Institute (Lyon, France) for identification. The two bacteria responsible for propanil biodegradation in buffered distilled water

were Pseudomonas putida (large colonies) and Streptococcus avium (small colonies). It was observed, however, that propanil biodegradation occurred only in the presence of the mixture of both bacterial types. When incubated separately in the presence of the herbicide, neither Ps. putida nor S. avium was able to degrade propanil.

At the GERAP (University of Perpignan) the distilled water is routinely stored in a polyethylene carboy. It was of interest to investigate whether the contamination source was the carboy or the distillation apparatus. Water samples were then taken from the carboy and also directly from the distillation unit in sterile erlenmeyers.The water was buffered to pH 7 with phosphate buffer and spiked with 50 ppm of propanil. The biodegradation of the herbicide was then monitored using HPLC, for up to 96 hrs. It was observed that propanil degradation occurred also in the water sample taken from the distillation unit.

The relatively rapid disappearance of propanil in non-sterile laboratory-distilled water at pH 7 was of great interest since this herbicide is relatively stable at temperatures and pHs found in natural waters (El-Dib and Aly 1976). Our experiments were carried out in the dark since propanil may undergo photodecomposition as reported by Moilanen and Crosby (1972). Through enrichment experiments we have shown that biodegradation was responsible for the disappearance of propanil in approximately 120 hrs. The growth of propanil-degrading microorganisms in buffered distilled water was a proof that the herbicide was used as a source of C and N. Isolation of propanil-degrading microorganisms revealed two types of bacterial colonies (Communication from Pasteur Institute, Lyon, France): Type 1 colonies consisted of gram negative rods, oxidase +, catalase +, arginine dihydrolase + and with growth on glucose and lactate. They were identified as Pseudomonas putida. Type 2 colonies were smaller in size and consisted of gram + cocci, catalase-, ADH-, esculine +, VP+ and pyrrolidonylarylamidase +. Type 2 was identified as Streptococcus avium.

Ps. putida was isolated from soil and was able to grow on o-nitrophenol and m-nitrophenol as the sole source of C and N (Zeyer and Kearney 1984). It was also shown that this bacterial species slowly degraded 3,4-dichloroaniline, the intermediate product formed during propanil degradation. DCA failed to serve as the sole substrate but its degradation was stimulated in the presence of aniline (You and Bartha 1982a, 1982b). In our study, DCA was also not degraded under our experimental conditions and within the time period (up to 120 hrs) under study.
The unusual finding is the concurrent isolation of Streptococcus avium in the enrichment broth. This bacterium is a fecal species which occurs in chicken feces (Jones 1978). Even more surprising was the finding that propanil degradation occurred only in the presence of a mixture of both Ps. putida and S. avium. Lappin et al(1985) recently reported the biodegradation of the herbicide mecocrop by a microbial community from wheat rhizosphere. The community was composed of two Pseudomonas species,Alcaligenes, Flavobacterium and Acinetobacter

calcoaceticus. Other beneficial interactions were found to occur during the biodegradation of other halogenated compounds (Bordeleau and Bartha 1968, Senior et al 1976).

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