

Altered Propanil Biodegradation in Temporarily Air-Dried Soil

Temporary air drying of soil severely reduced its capacity to form 3,3',4,4'-tetrachloroazobenzene from 3,4-dichloroaniline. The cleavage of the parent herbicide 3',4'-dichloropropionanilide (propanil)

was less affected. These findings provide a new interpretation for a recent report that questioned the role of 3,4-dichloroaniline as the prime precursor of 3,3',4,4'-tetrachloroazobenzene residues.

Formation of 3,3',4,4'-tetrachloroazobenzene (TCAB) residue was reported from several 3,4-dichloroaniline (DCA) based herbicides in the course of their biodegradation in soil (Bartha and Pramer, 1967, 1969; Bartha, 1968). Free DCA was the first detectable cleavage product, and it was proposed that DCA combined with its peroxidase-generated labile oxidation product to form the azo residue (Bordeleau and Bartha, 1970). Belasco and Pease (1969), however, reported that while 3',4'-dichloropropionanilide (propanil) gave rise to substantial amounts of TCAB, DCA formed only very small quantities. On this basis, they concluded that DCA is probably not the prime precursor of TCAB. As the cited part of this report was in direct conflict with data obtained in our laboratory, a reason was sought for this discrepancy. The experiments in question were conducted in almost identical manners, but while our laboratory used freshly collected moist soil samples, Belasco and Pease employed one with a lower moisture content and an unspecified storage history. Therefore, the possible effect of soil moisture content on propanil and DCA transformations was investigated.

EXPERIMENTAL

Propanil and DCA were purified and applied to soil as described earlier (Bartha, 1968). TCAB was synthesized according to Corbett and Holt (1963). Nixon sandy loam (pH 5.5) was collected in the month of August to a 15 cm depth from a silage corn field of the Agricultural Experiment Station, New Brunswick, N.J. The collected soil was immediately passed through a sieve with 2.8 mm diameter openings. One portion of the sieved soil was enclosed in a polyethylene bag to prevent loss of moisture; the other portion was spread out on a tray and allowed to air dry. Both samples were stored at 27° C for 3 days. Moisture content of each soil was calculated from weight loss at 100° C in 24 hr. All soil weights are given on this oven-dry basis. Fifty gram aliquots of moist and air-dry soil were treated each with 25 mg of propanil or 25 mg of DCA (500 ppm). The

treated samples were moistened to 60% of their water holding capacity, were placed in polyethylene film-covered 600 ml beakers, and were incubated with daily aeration for 1 week at 27° C. Extraction and analysis for propanil, DCA, and TCAB residues by quantitative gas chromatography were performed as reported earlier (Bartha, 1968). Cell-free peroxidase activity of moist-stored and air-dry soil samples were assayed according to Bartha and Bordeleau (1969). The molarity of the phosphate buffer used in this assay was increased from 0.05 to 0.20. Separate experiments established the latter buffer concentration as optimal for elution of peroxidases from Nixon sandy loam.

RESULTS AND DISCUSSION

The soil sample, when collected, contained 184 mg of water per g of fresh soil (28% of the water holding capacity). Air-drying in the laboratory reduced the moisture content to 19 mg per g (3% of the water holding capacity). In the temporarily air-dried soil, propanil degradation was only mildly affected (Table I). In 1 week, 85% of the added propanil was degraded, compared to 92% in moist-stored soil. However, TCAB formation was 92% inhibited by temporary

Table I. Residues after 7 Days in Moist-Stored and in Temporarily Air-Dried Nixon Sandy Loam, Treated each with 25 mg of Propanil or 25 mg of DCA

Soil	Propanil (mg)	DCA (mg)	TCAB (mg)
Moist-stored	1.7	2.7	2.7
Air-dry	4.1	5.2	0.1
Moist-stored		3.7	12.5
Air-dry		15.0	1.3

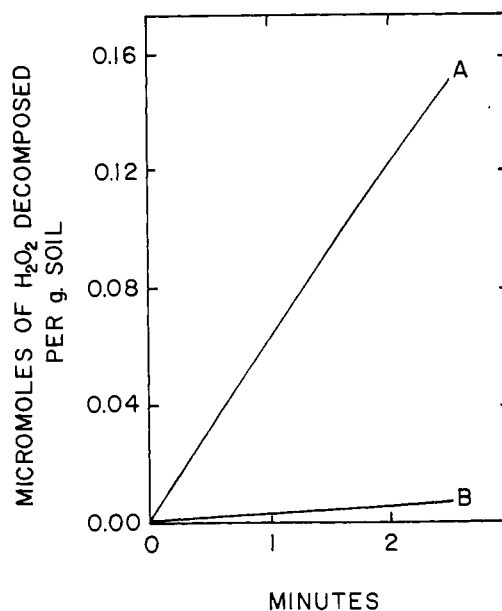


Figure 1. Effect of air drying on soil peroxidase activity. Millipore-filtered extracts of moist-stored (A) and air-dried (B) Nixon sandy loam were spectrophotometrically assayed for peroxidase activity at 460 nm wavelength and 25° C. Reaction mixture: 2.7 ml soil extract in 0.2 M (pH 6.0) phosphate buffer, 0.3 ml 0.06% H₂O₂ in the same buffer, and 0.05 ml 5% 3,3'-dimethoxybenzidine in methanol. Light path 1 cm

air-drying, causing doubled DCA accumulation. Similarly, TCAB formation was 90% inhibited and DCA-persistence was substantially increased by temporary air-drying in the DCA-treated soil samples. TCAB yields were consistently higher from DCA than from propanil.

As peroxidases were implicated in biochemical aniline transformations in soil (Bartha *et al.*, 1968), cell-free peroxidase activities of moist-stored and air-dry soil were compared (Figure 1). Activity in the latter was 96% lower.

Propanil, though subject to rapid biodegradation, is chemically stable and has low volatility (Herbicide Handbook, 1967). In contrast, DCA is subject to chemical oxidation, reacts with the soil organic matter (Linke and Bartha, 1970), and has high volatility. By neglecting to use fresh soil, Belasco and Pease (1969) created conditions under which DCA-losses by nonbiochemical mechanisms predominated. In consequence, TCAB yields from DCA were extremely low, leading the authors to the conclusion that DCA was not a direct precursor of TCAB. It seems reasonable to assume that in the time required for the release of DCA from the chemically stable propanil, a partial recovery of the original peroxidase activity occurred and allowed substantial amounts of TCAB to be formed.

Beyond the attempt to resolve a specific controversy, this report stresses the need to conduct biodegradation studies in biochemically intact soil samples.

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