Hydrolysis of 2,6-Dichlorobenzonitrile in Soils

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Adsorption of 2,6-dichlorobenzonitrile by samples of 34 New York surface- and subsurface-soils was highly correlated with their organic matter contents. Decomposition of 2,6-dichlorobenzonitrile in closed systems containing both nonsterile and azide-treated soils was slow. In contrast, the unhindered nitrile, 2,6-dichlorophenylacetonitrile was degraded rapidly in nonsterile soils but not in azide-treated soils. 2,6-Dichlorobenzonitrile was probably degraded by nonbiological hydrolysis and its rate of hydrolysis was inversely related to its partition coefficient, K. Adsorption by organic matter apparently protected the nitrile from hydrolysis. The product of chemical hydrolysis was shown by its infrared and mass spectra to be 2,6-dichlorobenzamide.

The herbicides 2,6-dichlorobenzonitrile (dichlobenil) and the related 2,6-dichlorothiobenzamide (chlorthiamid) are relatively new. The thiobenzamide is converted to the nitrile in soil (Beynon et al., 1966). Beynon et al. indicated that these compounds disappeared from soils fairly rapidly with an average half-life of about four weeks and with a range of 1 to 12 weeks depending on both the kind of soil and weather conditions. The concentration of the nitrile in treated ponds initially fell quickly but residues were still detectable after several months (Van Valin, 1966). In these investigations 2,6-dichlorobenzoic acid was expected as a metabolite, but it could not be detected.

Hindered nitriles are not hydrolyzed by the nitrilase enzyme system, 2-chlorobenzonitrile being unaffected by an enzyme preparation which converted unhindered nitriles to their corresponding acids without liberating the intermediate amide. Slow chemical hydrolysis of hindered nitriles *via* the amide was thought to occur in biological systems (Mahadevan and Thimann, 1964).

It seemed probable that 2,6-dichlorobenzonitrile would be hydrolyzed to 2,6-dichlorobenzamide in soil systems. This work was done to study the degradation of 2,6-dichlorobenzonitrile in soil, to investigate its adsorption by soil, and to determine whether or not adsorption affected the rate of hydrolysis.

MATERIALS AND METHODS

Reagents. Solvents used in the analytical determinations were distilled before use. 2,6-Dichlorobenzamide was prepared from 2,6-dichlorobenzonitrile by refluxing in acetic acid, concentrated sulfuric acid, and water (1:1:1) for 6 hours.

Chromatography. 2,6-Dichlorobenzonitrile and 2,6-dichlorophenylacetonitrile were determined using a Varian Aerograph, Model 200 gas chromatograph equipped with electron-capture detector and a $1/s$-inch O.D. $\times$ 8-foot stainless steel column packed with 10% DC 200 on Gas-Chrom Q (80 to 100 mesh). Temperatures used were: 200, 220, 200° C. for column, injector, and detector, respectively. The carrier gas was nitrogen; its flow rate was 20 ml. per minute.

Degradation Experiments. One milliliter of a 50 p.p.m. (w./v.) nitrile solution in 30/60 petroleum ether was added to 10 grams of soil in a 2-ounce jar. The soil was mixed several times while the solvent evaporated. The soils were then wetted to field capacity and caps put on the jars using an aluminum foil liner to prevent loss of nitrile. At the end of the incubation period, 10 grams of anhydrous sodium sulfate was added, mixed with the soil, and left to stand for 2 hours. The soil was then shaken with 25 ml. of ethyl acetate for 2 hours. The filtered extract was diluted with hexane and analyzed by gas chromatography. Recovery of the nitriles averaged over 90% by this procedure. Identical experiments were run on samples treated with 200 mg. of sodium azide per kilogram of soil.

Adsorption Experiments. Ten milliliters of a 20 p.p.m. (w./v.) 2,6-dichlorobenzonitrile solution in 1% ethanol was shaken with 1 gram of air-dry soil in a glass-stoppered flask for 2 hours. The soil was removed by centrifugation. Three milliliters of the supernatant was removed, sodium bicarbonate (0.2 gram) added to this solution, and the herbicide extracted with 3 ml. of 1-butanol. Concentration of the herbicide in the butanol phase was determined by measurement of absorption at 298 nm. against a blank treatment using a Beckman Model DB spectrophotometer.

Analytical data quoted in this study were from a collection of the major soils of New York (Cline, 1957).

RESULTS AND DISCUSSION

Adsorption of 2,6-Dichlorobenzonitrile by Soils. The properties of the surface- and subsurface-soils and the results of the adsorption experiments are given in Table I. K, the partition coefficient, was significantly correlated with organic matter content ($r = 0.93$). The relation between K and organic matter is described by the following equation:

$$K = 0.67 + 0.66(\text{OM})$$

Q the sorption per unit mass of organic matter (Furmidge and Osgerby, 1967) was very similar for all the surface soils. There was variation in Q for the lower horizons with a general tendency for Q to decrease and then increase moving down the profile.

Clay content appeared to have no influence on adsorption, the results for the Vergennes and Covington soils showing this clearly.

Persistence of 2,6-Dichlorobenzonitrile in Soils. The amounts of this herbicide found in samples of several surface soil (Ap) horizons after storage for two periods of time and with or without azide treatment are given in Table II. These data show that losses from all samples occurred slowly. Rates of loss from both nonsterile and azide-treated soils were very similar indicating a nonbiological process. Half-life was estimated from a plot of log concentration against time and was at least five months in these closed systems.

Table I. Adsorption of 2,6-Dichlorobenzonitrile by Soils

Soil Series	Horizon	Depth, Inches	pH	Clay, %	OM, %	K (µg./G./µg./Ml.)
Croghan loamy fine sand	Ap	0–7	5.4	3.0	4.6	3.7
	B_{21}	7–14	4.4	0.3	3.0	1.6
	B_{2h}	7–13	4.2	0.9	6.5	2.2
	B_{22ir}	13–25	4.6	0.6	0.5	0.4
	B_{23}	26–36	4.8	0.3	0.2	0.3
	B'_{2g}	67–72	4.6	4.0	0.2	0.6
Amenia silt loam	Ap	0–8	6.2	13.3	5.8	5.0
	B_{22}	14–22	6.4	14.1	1.2	1.2
	B_{23}	22–28	7.1	9.6	0.5	1.2
	C	28–34	7.4	9.7	0.2	0.8
Camroden silt loam	Ap	0–7	4.6	22.4	8.2	6.6
	A'_2	13–15	4.6	7.7	2.0	1.1
	B'_{2x}	15–27	5.6	9.5	0.4	1.9
Vergennes silt loam	Ap	0–7	4.6	33.6	4.9	4.5
	A_2	7–11	4.6	41.2	1.3	1.3
	B_{21t}	11–20	4.9	53.2	0.6	0.6
	B_{22t}	20–27	5.9	50+	0.4	1.3
Covington silty clay	Ap	0–8	5.7	36.1	9.2	6.7
	B_{21g}	8–12	6.5	34.3	1.0	1.3
	B_{22g}	12–19	6.7	57.2	0.7	1.3
Empeyville stony loam	Ap	0–6	4.9	9.0	9.6	7.8
	B_2	6–10	4.5	7.0	7.5	4.5
	A'_{21}	10–15	4.5	4.0	1.8	1.7
	A'_{22}	15–20	4.6	2.9	0.3	0.9
Vergennes clay	Ap	0–5	4.8	59.2	4.4	4.1
Troy gravelly silt loam	Ap	0–6	3.8	19.6	4.2	3.9
Lackawanna stony loam	Ap	0–5	4.6	...	9.6	6.0
Colonie loamy fine sand	Ap	0–11	5.0	3.4	1.6	1.9
Williamson silt loam	Ap	0–12	3.9	8.7	4.4	4.3
Granby fine sandy loam	Ap	...	6.8	6.5	5.2	5.1
Sodus gravelly loam	Ap	...	4.8	9.4	5.3	5.3
Lima gravelly silt	Ap	...	6.3	17.4	4.5	4.2
Howard gravelly loam	Ap	...	4.6	...	5.3	4.4
Mardin silt loam	Ap	...	5.9	...	5.0	4.5

Table II. Amounts of 2,6-Dichlorobenzonitrile and 2,6-Dichlorophenylacetonitrile in Surface Soils Treated with 5 µg of Herbicide per Gram of Soil

Soil	Time, Weeks	2,6-Dichlorobenzonitrile, µg. per Gram of Soil		2,6-Dichlorophenylacetonitrile, µg. per Gram of Soil	
		Nonsterile	Azide-Treated	Nonsterile	Azide-Treated
Croghan	1	...	...	3.8	4.5
	2	...	...	2.4	4.6
	5	...	...	1.3	4.4
	13	3.5	3.4	...	...
	26	1.9	2.0	...	...
Amenia	1	...	...	2.5	4.4
	2	...	...	1.7	5.2
	5	...	...	0.3	4.3
	13	3.7	3.5	...	...
	26	2.2	...	...	...
Camroden	13	4.1	4.0	...	...
	26	2.9	3.1	...	...
Vergennes	1	...	...	1.4	4.2
	2	...	...	0.4	4.9
	5	...	...	0.0	...
	13	3.2	3.4	...	...
	26	...	1.9	...	...
Covington	1	...	...	1.7	4.8
	2	...	...	0.4	4.9
	5	...	...	0.2	4.0
	13	3.7	4.0	...	...
	26	2.9	2.9	...	...
Empeyville	13	4.3	4.3	...	...
	26	3.1	3.3	...	...
Colonie	13	3.0	3.5	...	...

Table III. Amounts of 2,6-Dichlorobenzonitrile after 15 Weeks in Horizons of a Vergennes Silt Loam Soil Treated with 5 μg. of Herbicide per Gram of Soil

Horizon	2,6-Dichloro-benzonitrile μg. Herbicide per Gram Soil	K, $(\mu g/G)/$ $(\mu g./Ml.)$	OM, %
Ap	4.2	4.5	4.9
A$_2$	3.3	1.3	1.3
B$_{21t}$	2.6	0.6	0.6
B$_{22t}$	3.5	1.3	0.4

The conditions do not represent conditions in the field where vapor losses are thought to be significant (Furmidge and Osgerby, 1967) but the rate of loss may be a more realistic estimate of the rate of loss from the environment.

The results in Table II indicate that increasing adsorption of the herbicide lowers the rate of disappearance, the concentration of herbicide after six months being related directly to K. Data on the persistence of the herbicide in samples of different horizons of the same soil are shown in Table III. K rather than organic matter content was the factor which governed the loss of herbicide. This indicates that adsorption of the herbicide protects it from degradation.

Analyses of soil extracts by both thin-layer and gas chromatography indicated that the amide rather than the benzoic acid derivative accumulated as the herbicide disappeared. The presence of the amide was confirmed as follows. A large sample (800 grams) of Colonie soil was treated with 50 mg. of herbicide, wetted, and incubated for 5 weeks. The soil was then extracted with 80% ethanol which was evaporated. The residue obtained was extracted with hot benzene. The benzene was evaporated and the residue from it was extracted with 30% sulfuric acid. After neutralization and filtration, the aqueous solution was extracted with ether. This procedure effectively eliminated most of the natural materials which had been extracted from the soil, and the infrared spectrum of the residue, obtained by evaporation of the ether extract, was identical to that of synthesized 2,6-dichlorobenzamide. The mass spectrum of the product showed a parent ion molecular weight of 189 (the calculated value for $C_7H_5Cl_2NO$) and showed two chlorine atoms in the molecule. There were fragments with m/e values of 173 (loss of NH_2) and 145 (loss of $CONH_2$).

A sample of 2,6-dichlorobenzonitrile taken through the same acid treatment, neutralization, and ether extraction showed no evidence of hydrolysis.

Persistence of 2,6-Dichlorophenylacetonitrile in Soils. The amounts of this compound found in several surface (Ap) horizons during incubation are shown in Table II. The azide treatment prevented the rapid degradation which occurred in soils that were not treated with this inhibitor of biological activity. A comparison of these results with those already presented for 2,6-dichlorobenzonitrile showed that the unhindered nitrile disappeared 23 times faster than did the hindered nitrile. The rapid disappearance of the unhindered nitrile was probably caused by micro-organisms, whereas the slow disappearance of the hindered nitrile was primarily by nonbiological hydrolysis.

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