Relation between the Degradation of DDT and the Iron Redox System in Soils

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Four soils varying in organic matter and free iron contents were treated with DDT at 200 ppm, amended with the enzyme urease, and water-logged at 35°C from 3 to 28 days. The rates of degradation of DDT were related to the rates of formation of ferrous iron in the amended soils. A relation was also obtained between the redox potential and the DDT degradation in the soils. With an *in vitro* iron redox system, 20% of the original DDT was converted to DDD after 7 days. It was concluded that DDT underwent an irreversible redox type of reaction. A mechanism is proposed for the degradation of DDT whereby electrons furnished by the reduced organic substrate are transferred to the DDT molecules *via* the Fe^{2+} ions thus initiating a free radical reaction in the absence of oxygen.

Microbial decomposition of DDT under anaerobic conditions has been demonstrated as the major pathway of degradation of DDT in soils (Guenzi and Beard, 1968; Plimmer *et al.*, 1968). Very little attention has been directed toward the influence of various chemical changes that occur in reduced soils on the degradation of DDT.

When soils are flooded or water-logged in the presence of easily decomposable organic matter, the reduction of ferric to ferrous iron has been shown to be one of the most significant chemical changes (Starkley and Wight, 1945; Jeffrey, 1961). Similarly the reduction of DDT, specifically the conversion of DDT to DDD, was found to be accelerated under the same conditions (Ko and Lockwood, 1968).

The objective of the present study was to define the relationship between the degradation of DDT and the formation of ferrous iron on amended and unamended soils to increase our understanding of the mechanism of the degradation of DDT under anaerobic conditions. The iron system was selected for this study because it has been postulated that the iron (III–II) couple is the dominant redox system in reduced soils (Takai and Kamura, 1966; Ponnamperuma *et al.*, 1967).

Determinations of ferrous iron and redox potentials were utilized to compare the state of reduction in the soils with the rate of DDT degradation. Also, the degradation of DDT was studied with an *in vitro* iron redox system under N_2.

MATERIALS AND METHODS

Soil Treatment. The experiment was conducted in the laboratory on four agricultural soils collected from different regions of the United States. The soil series and pertinent soil data are shown in Table I.

One-hundred-and-fifty grams of each soil based on the oven-dry weight was passed through a 20-mm sieve and collected on a sheet of Teflon wrap. Quantities of DDT (30 mg) (Montrose Chemical Corp., 99.9% purity) were dissolved by 15 ml of acetone in 25-ml volumetric flasks. The DDT solutions were made to volume with distilled water and transferred to the soils with a 9-in. Pasteur pipette (Fisher Co.). The wet soils, containing 200 ppm of DDT, were mixed thoroughly with a spatula and the solvents were allowed to evaporate for 1 hr. Afterwards, the soils were weighed again and transferred to 2-qt Mason jars, where they were mixed for 2 hr. The spiked soils were then passed through a riffle (Fisher Co.) and divided into 10- to 12-g subsamples, in 4-oz serum bottles.

High-purity urease (Sigma Co.) recrystallized from jack bean meal was added to the soils at 3.0% of the oven-dry weight. Urease solutions were made in distilled water and added in 20- to 25-ml aliquots sufficient to submerge the soils.

The four urease-amended water-logged soils in the serum bottles were capped with rubber stoppers, placed in a constant temperature chamber, and incubated at 35°C from 3 to 28 days. Unamended water-logged soils were incubated simultaneously as controls.

DDT Analysis. The DDT and metabolites were extracted from the soils by adding 100 ml of hexane:isopropyl alcohol (1:1) to the serum bottles and shaking the soil suspensions for 1 hr on a mechanical shaker. After the liquid and solid phases had separated, the extracts were decanted into 250-ml volumetric flasks. A second extraction was necessary for quantitative recovery. After the extracts were combined and made up to volume in the 250-ml volumetric flasks, 5-ml aliquots were transferred to 100-ml volumetric flasks and diluted with hexane. Assays of residues of DDT and DDD were conducted on a Micro-Tek Model MT 2000 gas chromatograph equipped with ^{63}Ni electron capture detector. An Infotronic Digital Integrator Model CRS-100 was used for peak area quantitation. The operating temperatures of various gc components were as follows: inlet, 235°C; oven, 200°C; detector, 285°C. The chromatographic column, a 180 cm × 3 mm (i.d.) glass U-tube, was packed with an equal weight mixture of 10% DC-200 and 15% QF-1 on 100–120 mesh Gas Chrom Q. The carrier gas was argon:methane (95%:5%), which had a flow rate of 120 ml per min.

Redox Potential Measurements. The redox potential measurements were made on urease-amended and unamended soils, which were water-logged in 2-oz glass jars with plastic screw caps. Two black-coated platinum microelectrodes, a glass microelectrode, and a 1 *N* KCl calomel reference microelectrode were used for these measurements. The microelectrodes were fitted into 12.5-mm holes drilled into a plastic cap of one of the glass jars. Silicone bouncing putty (General Electric Co.) was spread around the microelectrodes to exclude air from the system. All measurements were conducted with this single unit of microelectrodes, which were successively changed from sample to sample in a plexiglas glove box under N_2. The microelectrodes were rinsed, dried, and exposed to the air before each measurement, as recommended by Bohn (1968). Measurements of the redox potentials with the two platinum microelectrodes were very consistent and reproducible from soil to soil. All redox potential

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Table I. Description of the Four Soils Used

Soil series	Location	pH	Organic matter, %	Free iron, %	Mechanical analysis		
					Sand, %	Silt, %	Clay, %
Drummer	Illinois	6.2	4.64	0.65	11.7	54.0	34.3
Houston	Texas	7.5	2.68	0.41	9.1	37.5	52.6
Muskingum	Ohio	5.0	1.72	1.28	43.8	40.6	15.6
Cecil	S. Carolina	5.4	0.1	1.02	48.5	17.1	13.8

Table II. Percent DDT and DDD Recovery from Urease-Amended and Unamended Water-Logged Soils at 35 °C

Time (days)	Drummer		Houston		Muskingum		Cecil	
	% DDT	% DDD	% DDT	% DDD	% DDT	% DDD	% DDT	% DDD
Urease-amended soils[a]								
3	88	8	88	19	85	13	87	10
7	64	27	71	28	87	21	86	19
14	26	43	55	39	65	33	72	23
21	12	61	39	49	35	58	72	32
28	6	61	23	59	28	52	54	32
Controls-unamended soils[a]								
3	90	4	96	3	92	3	102	3
7	84	4	78	3	88	3	87	3
14	74	10	88	5	95	11	92	3
21	65	21	92	12	87	26	92	7
28	53	29	76	15	70	29	88	11

[a] Soils contained initially 200 ppm of DDT.

measurements were conducted on a research model Beckman pH meter at 25°C.

Ferrous Iron. Quantitative determinations of Fe^{2+} ions brought into solution during the incubation period were made on the urease-amended and unamended water-logged soils. The method followed in this analysis was essentially the same as described by Black (1965). The Fe^{2+} ions were extracted by adding 500 ml of 1 *N* NH_4OAc to the water-logged soils and shaking for 15 min. The soil mixtures were centrifuged for 5 min at approximately 1800 rpm. The liquid fraction of the sample was assayed for soluble Fe^{2+} ions by the *O*-phenanthroline method. The colorimetric analysis was performed on a Bausch & Lomb spectrophotometer at 510 mμ. A minimum of two determinations was made for this experiment.

in vitro **Iron System.** Twenty-five milliliters of a 0.4 *M* solution of $FeSO_4$, which was allowed to stand for 15 min after preparation, was added to 10 g of autoclaved quartz sand that contained 200 ppm of DDT in serum bottles. With the addition of 10 ml of 0.1 *N* NaOH, a dark green precipitate, $Fe_3(OH)_8$, ferrosoferric hydroxide, was formed as described by Arden (1950). In this system the $Fe_3(OH)_8$–Fe^{2+} couple existed and the oxygen was displaced by nitrogen in order to maintain the reduced forms of iron. Untreated sand samples, submerged with 10 ml of H_2O, were used as controls. Duplicate iron-containing samples and controls were incubated at two different temperatures, 35 °C and 55 °C, for 7 days.

RESULTS AND DISCUSSION

Rate of Degradation. The percent recovery of DDT and DDD (Table II) shows that the rate of DDT degradation was faster where urease, an easily oxidizable organic substrate, was present. Guenzi and Beard (1968) first reported similar observation in soils with alfalfa amendments. The most extensive degradation of DDT in the present study occurred on the amended Drummer soil, where 61% of the original DDT was converted to DDD and 6% remained as DDT after 28 days. The 61% recovery as DDD on this soil was comparable to the 65% recovery as DDD from the alfalfa-amended water-logged soils reported by Ko and Lockwood (1968). DDT degradation was least on the Cecil soil, where only 32% of the original DDT was converted to DDD and 54% remained as DDT after 28 days. The different rates of DDT degradation obtained with these four soils despite equivalent urease amendments indicate the influence of some effect in the soil other than microbial activity.

Considerable DDT degradation also occurred in the unamended water-logged soils (Table II). Again, DDT degradation was highest in the Drummer soil, where the recovery was 53% as DDT and 29% as DDD. It contrast, recovery in the Cecil soil was 88% as DDT and 11% as DDD. The fact that, of the four soils used, the organic matter content was highest for Drummer and lowest for Cecil soil (Table I) indicates that the degradation of DDT was related to the organic matter content. Therefore, it was concluded that the organic matter was producing a physicochemical effect in the soil that increased the degradation of DDT.

Redox Potential and Iron System. In searching for the specific physicochemical effects that organic matter caused in water-logged soils, it was observed that the redox potentials were lower in the amended soils than in the unamended soils (Figure 1). The redox potentials were near −250 mV in the amended soils and near −20 mV for all the unamended soils except Drummer, which produced a moderately low potential of −90 mV after 28 days. It was concluded from these results that a relation existed between the low redox potentials and the rapid degradation of DDT. The rate of degradation of DDT

Table III. Formation of Soluble Ferrous Iron (ppm) in Urease-Amended and Unamended Water-Logged Soils at 35°C

Time, days	Drummer[a]		Houston[a]		Muskingum[a]		Cecil[a]	
	Amended	Unamended	Amended	Unamended	Amended	Unamended	Amended	Unamended
3	375	63	96	7	656	29	228	6
7	794	82	181	6	702	274	392	24
14	762	87	305	25	893	105	425	23
21	672	277	375	5	895	361	459	11
28	463	321	439	3	415	229	322	21

[a] Means of two determinations.

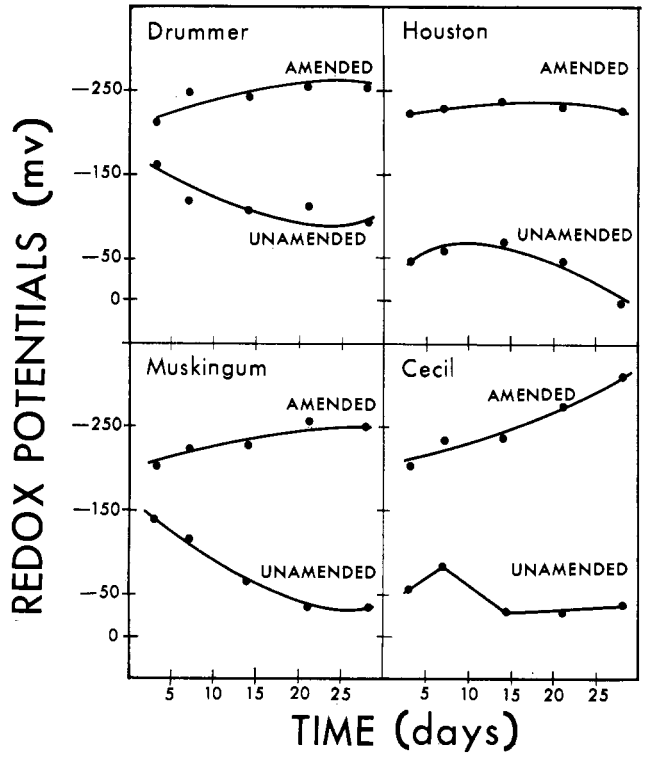

Figure 1. Changes in redox potentials of water-logged soils

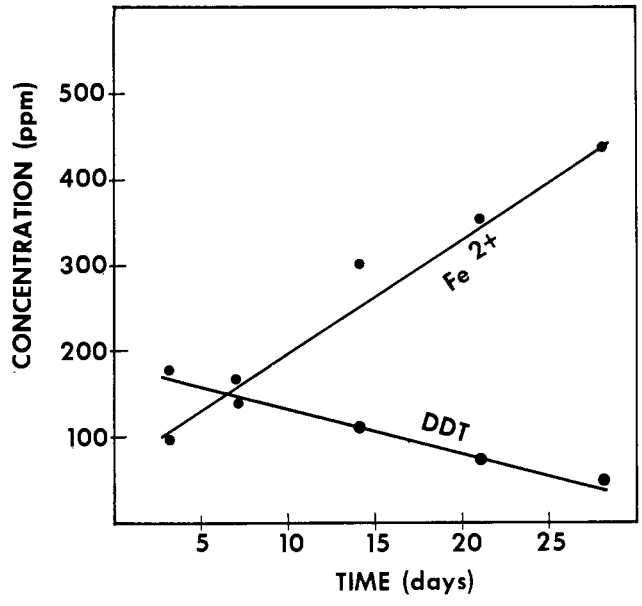

Figure 2. Relations between DDT and Fe^{2+} concentrations in amended Houston soil as a function of time

appeared to be dependent upon the state of reduction or the reducing intensities in the soil. No evidence of this relation has been published in the literature to the author's knowledge.

Many investigators have used redox potential measurements to study the state of reduction in water-logged soils (Aomine, 1962; Ponnamperuma, 1965) and some evidence indicates that the oxidation–reduction mechanisms include chemical as well as biological processes. In a submerged soil containing easily oxidizable organic matter, aerobic microorganisms utilize most of the available oxygen within a few hours to produce anaerobic conditions. Under this reduced environment, facultative and obligate anaerobes further oxidize the organic matter. Inorganic soil components, such as nitrates and sulfates, which become acceptors of terminal electrons in facultative respiration, are reduced. The reduction of ferric iron in water-logged soils has been attributed to microbial activity, but Takai and Kamura (1966) have reported evidence of the chemical reduction of iron with reduced organic products that resulted from the anaerobic decomposition of the organic amendment. Other workers in basic organic chemistry research have demonstrated the reduction of ferric iron to ferrous with various types of sugars (Orr and Williams, 1951). In the mechanisms, it was suggested that sugar and ferric iron formed a complex that resulted in the formation of sugar free radicals, which subsequently reduced the remaining ferric iron. The formation of the reduced iron seems to be one of the most significant influences on the state of reduction in the water-logged soils.

The concentrations of ferrous iron formed on the amended and unamended soils as a function of time are shown in Table III. On the four soils, there was a marked difference in the accumulation of ferrous iron on the amended as compared to the unamended soils after 28 days. The Drummer had the highest accumulation of ferrous iron and the Cecil had the least on the amended soils at the end of the incubation period. The decline in recovery at 28 days in amended Drummer and amended and unamended Muskingum might be attributed to precipitation of Fe^{2+} as iron sulfide. Sulfides are the products of sulfate reduction in water-logged soils. In plotting the changes in concentrations of Fe^{2+} ions and DDT on the amended Houston soil as a function of time, linear relationships were obtained as shown in Figure 2. Plotted data from the other three soils gave nonlinear results; however, the rates of the reaction for Fe^{2+} and DDT on the four amended soils appear to be between zero-order and first-order kinetics.

The results obtained from the *in vitro* iron system (Table IV) conclusively demonstrate that the degradation of DDT occurs in the presence of an iron redox system. The $Fe_3(OH)_8$–Fe^{2+}

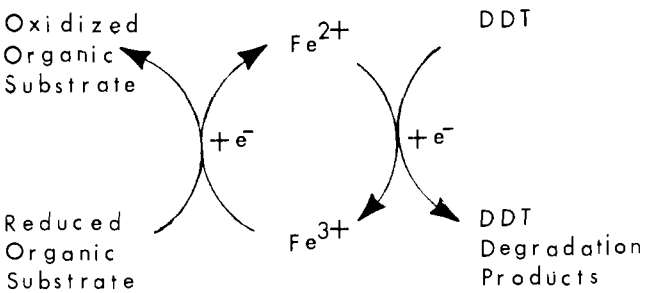

Figure 3. Proposed mechanism for the degradation of DDT in water-logged soils

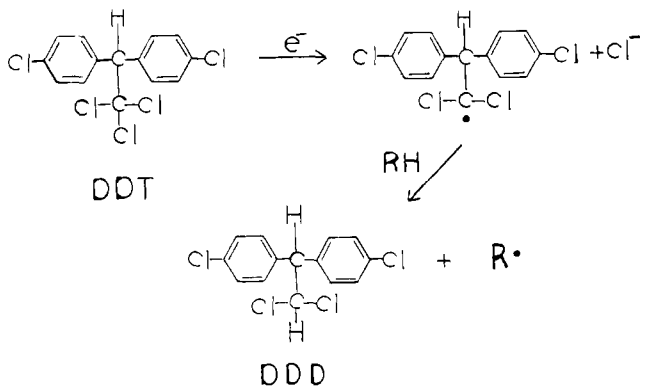

Figure 4. Proposed free radical mechanism for the formation of DDD

couple is one iron redox system that Ponnamperuma (1965) has predicted to be present in reduced soils from thermodynamic calculations. In the iron system, the percent of DDT converted to DDD was approximately equal at 35°C and 55°C; however, a significant difference in the DDT recovery was obtained at the two temperatures. No explanation was found for this observation. The total recovery for the controls at 35°C and 55°C ranged between 94% and 85%, which were acceptable for the relatively high incubation temperatures.

Mechanism of Degradation. The conclusion drawn from the empirical evidence obtained in this investigation is that DDT undergoes an irreversible redox type of reaction under anaerobic conditions. The proposed mechanism for the degradation of DDT is shown in Figure 3. The Fe^{3+} ion gains an electron from the metabolized organic substrate and becomes a Fe^{2+} ion, an electron donor. The electron is transferred then to the DDT molecule, which exhibits strong electron affinity because of the chlorine atoms. When a molecule such as DDT captures a free electron, it dissociates into a chloride ion and a free radical. This has been found to occur in electron capture detectors (Lovelock, 1962; Gregory, 1968) and in reactions with near thermal electrons (Durbin *et al.*, 1970). The general reaction for this dissociation process is given by

$$R - Cl + e^- \longrightarrow R\cdot + Cl^-$$

Consequently, a free radical mechanism is proposed for the formation of DDD (Figure 4). The first reaction of the proposed mechanism is the dissociation of the DDT molecule into a chloride ion and the 1,1-dichloro-2,2-bis(*p*-chlorophenyl) ethane radical, which was proposed by Miller and Narang (1970) in a photo-induced electron transfer mechanism with DDT. In the next reaction, the free radical abstracts a proton from the donor RH, which could be ethanol, acetaldehyde, or any of the proton donors produced in the anaerobic decomposition of added organic matter (Burge, 1971; Takai and Kamura, 1966). The abstraction of a proton from the water molecule also appears likely in this reaction mechanism.

Table IV. Degradation of DDT (200 ppm) at Two Temperatures with an *in vitro* Iron Redox System

Treatment[a]	Temperature	Percent recovery DDT	DDD	Total
$Fe_3(OH)_8$–Fe^{2+}	35°C	56	19.5	76
Control	35°C	92	2.1	94
$Fe_3(OH)_8$–Fe^{2+}	55°C	38	20	58
Control	55°C	83	1.5	85

[a] 7 days of incubation.

CONCLUSION

The evidence obtained in this study demonstrates that the iron redox system in reduced soils is capable of degrading DDT. Other redox systems in reduced soils are expected to perform the same function with the mechanism postulated in this work, because the transfer of electrons to DDT is the essential process for degradation. This chemical model shows one reason why DDT does not degrade under aerobic conditions: no free electrons are available for transfer.

No practical chemical process can be predicted at this stage that will speed up the degradation of DDT in aerobic soils, but it is speculated that high-voltage electron dischargers may be effective.

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