Microbial Decomposition of Diquat Adsorbed on Montmorillonite and Kaolinite Clays

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Nutrient solutions treated with tagged diquat [6,7-dihydrodipyrido(1,2-*a*:2′,1′-*c*)-pyrazidiinium dibromide] and soil microorganisms readily released $^{14}CO_2$. Sterile controls and standard solutions showed no nonbiological decomposition of diquat. Additions of montmorillonite clay in an amount calculated to adsorb one half of the ^{14}C-diquat reduced $^{14}CO_2$ evolution to approximately one half. When enough montmorillonite clay was added to adsorb all of the diquat, no $^{14}CO_2$ was detected. Additions of kaolinite clay to the nutrient solutions had no significant effect on the total diquat decomposed in these systems.

Knowledge concerning the microbial decomposition of pesticides which are applied to soils is of great importance. It is desirable that these compounds be decomposed by some agent, biological or nonbiological, and not accumulate and cause problems in the future. Several requirements must be fulfilled if a compound is to be decomposed by soil microbes (Alexander, 1965). Briefly, to be decomposed a pesticide must be metabolizable and available. The present study is most strongly concerned with availability. Several workers have shown that the dipyridylium herbicides can be microbiologically degraded (Baldwin *et al.*, 1966; Funderburk and Bozarth, 1967; Tu, 1966). These compounds ionize completely in aqueous solutions to yield organic cations which react very strongly with soil particulates, especially the clay minerals. The dipyridylium compounds, and all organic cationic pesticides, are readily inactivated once they reach the soil. However, inactivation and decomposition are not synonymous, and it is important to know if the compounds are being decomposed in the soil.

Considerable research has been performed on the interaction of pure organic compounds with clay minerals, especially montmorillonite, because of its expanding lattice structure and high cation exchange capacity. The adsorption of organic molecules by montmorillonite clay involves the entry of the organic molecules between the silicate sheets of clay, causing an expansion of the crystalline lattice structure. Observations have shown the presence of mono-, di-, and trimolecular layers of organic molecules in the expanded crystal lattice (MacEwan, 1948). Other investigators (Gieseking, 1939; Hendricks, 1941) showed that large aromatic organic compounds are adsorbed in the interlayer spaces of montmorillonite in various positions, and they are held by several types of adsorption forces. Several workers (Bower, 1949; Ensminger and Gieseking, 1942; Estermann *et al.*, 1959; Goring and Bartholomew, 1949; Pinck and Allison, 1951) showed that the adsorption of organic compounds by clay minerals influences their availability to soil microbes. The adsorption of organic compounds by clay minerals is dependent upon the chemical properties of the compounds and the types of clay minerals involved (Pinck *et al.*, 1961a, 1961b). The dipyridylium herbicides are strongly adsorbed in the interlayer spaces of montmorillonite clay and are not readily extractable using 1*M* salt solutions (Weber *et al.*, 1965). The adsorption of the dipyridylium herbicides by several clay minerals reduces their availability to plants (Coats *et al.*, 1966; Weber and Scott, 1966) and their photochemical decomposition by ultraviolet light (Funderburk *et al.*, 1966).

The present experiments were performed to determine the effects of two clay minerals on the microbial mineralization of ^{14}C-tagged diquat [6,7-dihydrodipyrido(1,2-*a*:2′,1′-*c*)-pyrazidiinium dibromide].

PROCEDURE

To determine whether soil microorganisms are active in decomposing diquat, 10-ml. aliquots of filtrate from a mixture of 10 grams of a Norfolk sandy loam soil and 250 ml. of distilled water were added to 500-ml. Erlenmeyer flasks containing 100 ml. of nutrient solution (0.25 gram of polypeptone and 0.15 gram of beef extract) and varying levels of ring-labeled ^{14}C-diquat. The polypeptone consisted of a mixture of casein (Trypticase) and animal tissue (Thiotone) peptones and was obtained from the Baltimore Biological Laboratory. The flasks were incubated at 25° C. Air was slowly bubbled through the nutrient solutions and then passed through a $CaCl_2$ dryer and finally into vials containing 10 ml. of a CO_2-trapping solution of 0.01*M* hyamine [*p*-(diisobutylcresoxyethoxyethyl)-dimethylbenzylammonium chloride] in methanol. The scintillators PPO (2,5-diphenyloxazole) and POPOP [2,2-*p*-phenylenebis(5-phenyloxazole)] in toluene were added to the vials containing the hyamine-carbonate-methanol mixture, and the vials were then counted in a Tri-Carb scintillation spectrometer for 10 minutes at 0° C. The $^{14}CO_2$ was collected for 8 hours each day; the counts were then multiplied by three to obtain c.p.m. per day. $^{14}CO_2$ was collected over a 24-hour period for random samples, and the counts obtained were in agreement with the standard 8-hour $^{14}CO_2$ collections. Rates of diquat employed were 10, 1, and 0.1 p.p.m. At the end of 10 days, the cumulative release of $^{14}CO_2$ for the high rate of diquat amounted to 6100 c.p.m., and the authors concluded that diquat was being decomposed. Experiments were then established to determine the effects of the clay minerals on the diquat mineralization. Flasks with actively growing microorganisms were used for

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inoculation in the experiments involving the clay minerals.

Previous studies (Weber *et al.*, 1965) showed that diquat was strongly adsorbed on the clay minerals montmorillonite and kaolinite to approximately their cation exchange capacity (C.E.C.). To determine the effects of microbial decomposition of diquat adsorbed on these two clay minerals, experiment 1 was established. Ten milliliters of deionized water containing 10 μc. (3.7 μmoles) of ^{14}C-diquat were added to varying amounts of each of the clay minerals and shaken for 1 hour. The samples were then centrifuged, and the amount of diquat present in the supernatant was determined spectrophotometrically employing a recording spectrophotometer equipped with a deuterium lamp. An analytical wavelength of 307 mμ was used. The samples were then resuspended and added to the 500-ml. flasks containing similar nutrient solutions as employed above. Microbes were introduced by adding 2 ml. of solution from flasks with actively growing microbes from the preliminary experiment discussed above. Sterile (autoclaved) controls containing 10 μc. of ^{14}C-diquat were included to detect possible nonbiological mineralization of the compound. Spectrophotometric analysis of autoclaved diquat solutions showed that the sterilization process had no effect on the herbicide. Samples were also included for background purposes. Duplicate, and in some cases triplicate, treatments were employed. Standard solutions of diquat which were analyzed weekly were included to determine if diquat was photochemically decomposed under these conditions. $^{14}CO_2$ was collected in the manner described above. The amounts of each clay mineral employed were based on their cation exchange capacities as determined by the ammonium acetate method of Jackson (1958) using Na as the saturating cation and NH_4 as the replacing cation. Two levels of each clay mineral were employed. The low level was calculated to adsorb one half of the diquat from solution and to reduce the free diquat available to the microorganisms by one half. The high rates of each clay mineral were calculated to adsorb all of the diquat from solution and make it necessary for the microbes to decompose the herbicide on the clay surfaces. The following calculations show how the amounts of each clay mineral employed were determined: For montmorillonite clay with a C.E.C. of 0.847 meq. per gram:

0.847 meq. per gram ÷ 2 (divalent cations) = 0.423 mmole per gram

3.7 μmoles ^{14}C-diquat ÷ 423 μmoles per gram = 8.7×10^{-3} gram

The high rate of montmorillonite employed for experiment 1 was 9.0 mg. For kaolinite clay with a C.E.C. of 0.051 meq. per gram:

0.051 meq. per gram ÷ 2 (divalent cations) = 0.025 mmole per gram

3.7 μmoles ^{14}C-diquat ÷ 25 μmoles per gram = 0.148 gram

The high rate of kaolinite employed for experiment 1 was 160 mg.

Experiment 2 was performed in a manner similar to experiment 1, except that the amounts of ^{14}C-diquat were doubled, and the amounts of each clay mineral were slightly more than doubled. Microbial inoculation was made by adding 2-ml. aliquots from solutions with actively growing microorganisms from experiment 1.

Experiment 3 was similar to experiment 2 except that the amounts of ^{14}C-diquat and clay were those employed in experiment 1. Inoculation was made employing solutions with actively growing microorganisms from experiment 2. At the end of experiment 3, the solutions were centrifuged, and isotopic determinations were made on the ^{14}C remaining in the supernatant and in the clay pellets. The clay pellets were suspended in a thixotropic gel, and the ^{14}C in the two fractions was determined using a naphthalene-dioxane mixture and employing PPO and POPOP as the scintillators according to the method of Bray (1960).

^{14}C-diquat was extracted from the clay pellets by use of solutions of $10^{-4}M$ paraquat (1,1′-dimethyl-4,4′-bipyridinium dichloride) (Weber *et al.*, 1968).

RESULTS AND DISCUSSION

The sterile control [10 μc. (3.7 μmoles) of ^{14}C-diquat] of experiment 1 yielded counts of 186 c.p.m. per 8-hour $^{14}CO_2$ collection, and the background treatments (nutrient solution only) gave counts of 100 c.p.m. for the same period. Both treatments gave similar results for all experiments. Chemical determinations of standard diquat solutions for the same period showed no loss of diquat resulting from nonbiological decomposition. All of the treatments in Table I are corrected according to the sterile control for the respective experiment. The acidity of the nutrient solutions ranged from pH 7 to 8.5.

Mineralization of diquat, as measured by $^{14}CO_2$ evolution, occurred in all treatments in experiment 1 (Table I). Additions of montmorillonite clay to the nutrient solutions effectively reduced $^{14}CO_2$ evolution as it was calculated to do. The 9-mg. rate of montmorillonite decreased $^{14}CO_2$ to a very low rate, but not to zero. Chemical determinations showed that 9 mg. of montmorillonite was not enough to adsorb all of the diquat in the solutions in experiment 1, and explain why the small amount of diquat mineralization resulted from this treatment. Results in experiments 2 and 3 show that when enough montmorillonite clay was added to adsorb all of the diquat from solution, $^{14}CO_2$ evolution did not occur and mineralization of diquat was inhibited. Additions of kaolinite clay to the nutrient solutions appeared to stimulate $^{14}CO_2$ evolution initially in experiment 1, but by the end of the 6-week period $^{14}CO_2$ evolution was similar for the no-clay and kaolinite clay treatments.

Microbial decomposition of diquat on the no-clay treatment of experiment 2 was approximately twice as high as for experiment 1. This resulted from the higher rate of diquat employed (7.4 *vs.* 3.7 μmoles for experiments 2 and 1, respectively). Additions of 10 mg. of montmorillonite to the flasks resulted in $^{14}CO_2$ evolution of one half to one third that of the no-clay treatment. The high rate of montmorillonite, which adsorbed all of the diquat from solutions, completely inhibited $^{14}CO_2$ evolution. Mineralization of diquat was very similar

Table I. Cumulative Release of $^{14}CO_2$ Resulting from Application of Tagged Diquat to Nutrient Solutions Containing Montmorillonite (M) and Kaolinite (K) Clays

Diquat Added, μmoles	Clay Added, Mg.	Radioactivity in Evolved $^{14}CO_2$, C.P.M.[a] 1 week	2 weeks	4 weeks	6 weeks	8 weeks
			Experiment 1			
3.7	0	1,450	15,800	89,700	178,000	
3.7	4 M	1,660	28,000	81,100	115,000	
3.7	9 M	90	1,510	4,020	5,310	
3.7	60 K	13,100	69,600	183,000	262,000	
3.7	160 K	12,300	72,900	139,000	180,000	
			Experiment 2			
7.4	0	2,090	8,710	191,000	424,000	
7.4	10 M	658	8,760	87,600	134,000	
7.4	20 M	0	0	0	0	
7.4	160 K	1,880	58,200	380,000	589,000	
7.4	340 K	775	65,000	426,000	628,000	
			Experiment 3			
3.7	0	37,000	80,800	159,000	217,000	254,000
3.7	5 M	16,300	27,300	44,300	60,200	72,800
3.7	10 M	0	0	0	0	0
3.7	80 K	38,800	58,700	106,000	150,000	187,000
3.7	170 K	24,400	46,000	94,500	152,000	204,000

[a] Corrected for $^{14}CO_2$ derived from sterile control (<200 c.p.m.).

on both kaolinite clay treatments and was from 50 to 100% higher than the no-clay treatment for experiment 2.

Experiment 3 yielded results very similar to experiments 1 and 2 (Table I). Additions of montmorillonite clay inhibited diquat mineralization by soil microorganisms. $^{14}CO_2$ evolution on the kaolinite clay treatments was similar to the no-clay treatment and indicated that diquat adsorbed on this clay mineral was completely available for microbial decomposition.

At the conclusion of experiment 3, isotopic ^{14}C determinations were made on the supernatant from centrifuged samples of the nutrient solutions. No diquat was present in the solutions containing the high level of montmorillonite clay, confirming that no free diquat was present in the solutions for the microbes to decompose (Table II). ^{14}C in the solutions containing the low rate of montmorillonite was approximately one half of that initially added, and confirms the calculations that 5 mg. of montmorillonite adsorbed one half of the diquat added and left one half free in solution to be decomposed. Isotopic determinations of ^{14}C-diquat in the clay pellets showed that virtually all (97%) of the diquat adsorbed by the high rate of montmorillonite was still present on the clay particles. Adsorption-desorption studies (Weber *et al.*, 1968) showed that diquat adsorbed on montmorillonite clay could not be displaced by $1M$ $BaCl_2$ solutions, but could be displaced by $10^{-4}M$ paraquat solutions. Diquat thus displaced with paraquat from the clay pellets in experiment 3 (Table II) yielded ultraviolet spectra identical with the pure compound in the range from 290 to 360 mμ. The inhibition of diquat mineralization from adsorption by montmorillonite clay is analogous to results obtained when cucumber plants were employed to determine whether paraquat adsorbed on the same clay mineral was available to plant roots (Weber and Scott, 1966).

Table II. Tagged Diquat Remaining in Nutrient Solutions and in Montmorillonite (M) and Kaolinite (K) Clay Pellets at Termination of Experiment 3

Diquat Added, μc.	Clay Added, Mg.	C^{14}-Diquat Present,[a] μc. In solution	In pellet[b]
10	0	8.4	0.4
10	5 M	5.1	4.5
10	10 M	0	9.7
10	80 K	8.1	1.0
10	170 K	5.4	3.6

[a] Corrected for background.
[b] Pellet contained microbial cells and clay.

Adsorption of ^{14}C-diquat by the kaolinite clay did not reduce the microbial decomposition of the herbicide in this system (Table I). In experiments 1 and 2, diquat mineralization in the solutions containing kaolinite clay was considerably higher in the first 3 weeks than in the solutions containing no clay, but not in experiment 3. Chemical determinations showed that the nutrient solutions displaced some of the diquat from the kaolinite clay particles, so it is not possible to distinguish whether the microbes decomposed only free diquat in solution or diquat adsorbed on the kaolinite clay surfaces. An analogous situation resulted in studies where paraquat was adsorbed on kaolinite clay and was found to be readily available to cucumber plants (Weber and Scott, 1966). One half of the added ^{14}C-diquat was present in the solutions where 170 mg. of kaolinite clay was calculated to adsorb all of the added diquat (Table II). Adsorption-desorption studies (Weber *et al.*, 1968) showed that deionized water did not displace diquat from kaolinite, but that $1M$ $BaCl_2$ solutions displaced 80 to 85% of the adsorbed herbicide.

These experiments indicate that diquat free in solution

was microbiologically decomposed, but diquat adsorbed in the crystalline lattice of montmorillonite clay particles was not available for mineralization. Only small amounts of the dipyridylium herbicides are applied to agricultural soils, so this phenomenon is at present only academic, but because the use of cationic pesticides is increasing, we need to be aware of this problem. To demonstrate this effect conclusively, it will be necessary to employ diquat adsorbed on montmorillonite clay particles in the soil system.

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