Microbial Degradation of Paraquat Sorbed to Plant Residues

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It was demonstrated that paraquat (1,1′-dimethyl-4,4′-bipyridynium dichloride) sorbed to plant residues was degraded by natural microbial populations associated with plants and/or soil under laboratory conditions. The microorganisms associated with plants showed a higher degradation rate than those from soil. The degradation rate was higher in rice straw (C/N ratio = 53) than in dropwort (C/N = 20) and Chinese milk vetch (C/N = 17). Urea, ammonium chloride, and sodium nitrate suppressed the paraquat degradation rate by microorganisms associated with rice straw, suggesting a relationship to nitrogen metabolism. The degradation rate was much higher under aerobic conditions than under anaerobic conditions. When rice straw containing sorbed paraquat was placed on the surface of soil or mixed with soil, the herbicide was degraded under the former condition but not under the latter condition. Monopyridone (1′,2′-dihydro-1,1′-dimethyl-2′-oxo-4,4′-bipyridynium ion) and $^{14}CO_2$ were detected as metabolites in rice straw spiked with [*methyl*-$^{14}C_2$]-paraquat.

Keywords: *Microbial degradation; herbicide paraquat; plant residues; C/N ratio; plant-associated microorganisms; soil microorganisms; soil environment; aerobic conditions*

INTRODUCTION

The herbicide paraquat (1,1′-dimethyl-4,4′-bipyridynium dichloride), the active ingredient of Gramoxone, is a widely used nonselective herbicide. Paraquat is a divalent cation which is adsorbed very strongly to soil particles with negative charge, especially to clay minerals. Khan (1973) summarized that the strong adsorption is primarily due to the charge-transfer complex between paraquat and clays. Weber et al. (1965) concluded that the adsorption of paraquat to montmorillonite is primarily by the Coulombic force of ion exchange supplemented by van der Waals forces, the cation being held in the clay lattice with the plane of the ring parallel to the silicate sheets. The herbicide is quickly inactivated once it reaches the soil (Knight and Tomlinson, 1967). The adsorption of paraquat by clay colloids renders the herbicide unavailable for microbial degradative attack. Paraquat also seems to be stable to chemical degradation in soil (Hance, 1967); thus, it has been considered that paraquat remains unaltered in soil almost indefinitely.

To the contrary, Hance et al. (1980) and Constenla et al. (1990) reported that paraquat did not accumulate as much as expected in spite of repeated applications. Kanazawa (1990) reported that paraquat concentration in soil decreased after application was stopped. These results suggested that paraquat could be degraded by microorganisms. Although the possibility of microbial degradation of paraquat in soil was anticipated by some researchers (Burns and Audus, 1970; Fryer et al., 1975), no conclusive evidence could be found until now. Thus, it is of interest to know where paraquat is being decomposed microbiologically in the soil environment.

The main objective of this study was to identify a possible site of microbial degradation of paraquat in the soil environment. We describe here that the plant residues which sorb paraquat are possible sites for paraquat degradation in the soil environment.

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Table 1. Carbon and Nitrogen Contents of Plant Samples

sample	% C	% N	C/N ratio
rice straw	41.9	0.8	52
dropwort	37.4	1.9	20
Chinese milk vetch	43.4	2.5	17

MATERIALS AND METHODS

Chemicals. [*methyl*-$^{14}C_2$]Paraquat with a specific radioactivity of 6.2 mCi/mmol was used. Paraquat dichloride aqueous solution (51.5%) was donated by Zeneca Agrochemicals. Monopyridone (1′,2′-dihydro-1,1′-dimethyl-2′-oxo-4,4′-bipyridynium ion) was obtained from Prof. Kuwatsuka, Nagoya University. The stock solution of paraquat was stored in a freezer at −20 °C and diluted with appropriate solvents for use.

Plant Residues. Rice straw (*Oryza sativa* L. cv. Aoinokaze), dropwort (*Oenanthe javanica* DC), and Chinese milk vetch (*Astragalus sinicus* L.) were used as test plants for their considerable difference in carbon and nitrogen content. The rice straw was kept under air-dry conditions (septic) for 9 months after harvesting. The other two plant samples were collected from an experimental farm of Nagoya University in the spring, air-dried, chopped to less than 1 cm lengths, and then stored under septic conditions at room temperature until use (for 2 months).

Soil. Ibaraki paraquat-treated and -nontreated plot soils (Anthraquic Dystrandepts) were collected from the plow layer of a paddy field at the Research Institute of the Japan Association for Advancement of Phyto-regulators in Ibaraki Prefecture, Japan. The paraquat-treated plot had been treated with paraquat dichloride (960 g of active ingredient (ai)/ha, 4 L/ha as Gramoxone) once a year for 13 years. The paraquat-treated plot soil contained 13.0−14.9 mg/kg of soil of paraquat (oven-dry basis) and was used as the inoculum source of soil microorganisms. The nontreated plot soil had not been treated with any herbicides. This soil was used for the plant residue−soil system degradation experiment. The nontreated plot soil was screened through a 2-mm sieve and stored at 4 °C until use. The soil contained 25.9% clay, 28.2% silt, 29.8% fine sand, and 4.8% coarse sand. The cation-exchange capacity was 27.2

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cmol/kg of soil (dry weight basis). The carbon content was 5.9%, the nitrogen content 0.5%, the pH 6.1 (1:2.5 soil/water), and the maximum water holding capacity 105%.

Degradation Experiment Using the Plant Residue System. The microbial degradation of paraquat sorbed to plant residues was examined using three types of plant residues: rice straw, dropwort, and Chinese milk vetch. The chopped plant residue, 1 g, was placed in a 100-mL volume beaker and spiked with 154.5 μg of paraquat dichloride in 3 mL of sterile aqueous solution. Four conditions were devised to estimate the effects of microorganisms associated with plants and/or soil: (A) autoclaved plant residue as control, (B) autoclaved plant residue inoculated with a soil suspension to examine the capacity of soil microorganisms degrading paraquat, (C) intact plant residue to examine the capacity of microorganisms inhabiting the plant residues to degrade paraquat, (D) intact plant residue inoculated with a soil suspension to estimate the effects of combinations of microorganisms with two origins on paraquat degradation. In conditions A and B, plant residues containing paraquat were autoclaved at 1.2 kg/cm^2 for 15 min. Autoclaving had no effect on the recovery of herbicide. One milliliter of a suspension of treated plot soil with sterile water (soil/water = 1:100 w/v) was added to conditions B and D as inoculum after big soil particles were removed by precipitation for 30 min. All samples were incubated in the dark at 30−32 °C for designated periods and then were subjected to the analysis of remaining paraquat. The moisture of the sample was maintained by adding sterile water on the basis of weight loss.

Effects of other nitrogen sources on the paraquat degradation were examined using rice straw. One of the nitrogenous compounds, urea, ammonium chloride, or sodium nitrate (20 mg of N), was added to 1 g of rice straw as aqueous solution. This N addition lowered the C/N ratio of 20.

Anaerobic conditions were also used to examine the degrading capacity of anaerobic microorganisms. Containers, 100-mL volume Erlenmeyer flasks, were sealed with a double rubber cap, and the inside air was replaced with nitrogen. Then an oxygen absorbent, 5 mL of 10% pyrogallol in 2.5 N sodium hydroxide solution, was added to a small test tube in the container. The incubation was carried out as described above.

Degradation Experiment in the Plant Residue−Soil System. To examine the microbial degradation of paraquat sorbed to plant residues in the soil environments, 1 g of chopped rice straw containing sorbed paraquat was placed on the surface or mixed with 25 g of the nontreated plot soil and incubated as described above. The rice straw samples were prepared by mixing with paraquat solution (rice straw/solution = 1:3 w/v). The soil samples were preincubated for 10 days with 60% of maximum water holding capacity prior to addition of rice straw. The autoclaved plant residue−soil systems were prepared to assess the nonbiological degradation.

Determination of Paraquat. The basic method of analysis is that described by Calderbank and Yuen (1965) for extraction and cleanup and by Paschal et al. (1979) for determination of paraquat, with some modifications. Plant samples were refluxed with 18 N sulfuric acid and neutralized to a pH between 6.5 and 7.0 with 10 N sodium hydroxide. The neutral solution was passed through 10 mL of Dowex 50W-X8 (100−200 mesh) cation-exchange resin. The paraquat adsorbed to the resin was eluted with 150 mL of a mixed solvent, saturated ammonium chloride and acetonitrile (85 + 15 v/v), at a flow rate of 5 mL/min. Paraquat was determined by a high-performance liquid chromatograph with a UV detector (254 nm) and a μBondapak C_{18} reversed-phase column (4.0 × 25 mm). The mobile phase consisted of 10 mM 1-octanesulfonic acid sodium salt and 100 mM diethylamine in 20% acetonitrile−water; the pH was adjusted to 2.2 with phosphoric acid. In the case of the plant residue−soil mixture, samples were centrifuged for 15 min at 8000*g* after neutralization, and the supernatant was subjected to the cleanup using the cation-exchange column.

The recoveries of paraquat from plant residues were 95.5 ± 1.5% 3 h after paraquat spiking at application rates ranging from 51.5 to 515.0 μg/g of plant residues. There was no difference in recovery among the plant residues. The recovery from soil was 76.7 ± 2.7% 3 days after paraquat spiking at application rates ranging from 6.18 to 61.8 μg/g of soil. Recoveries from soil samples were low compared to those from plant samples but constant; thus, the paraquat amounts measured were not corrected for apparent method losses.

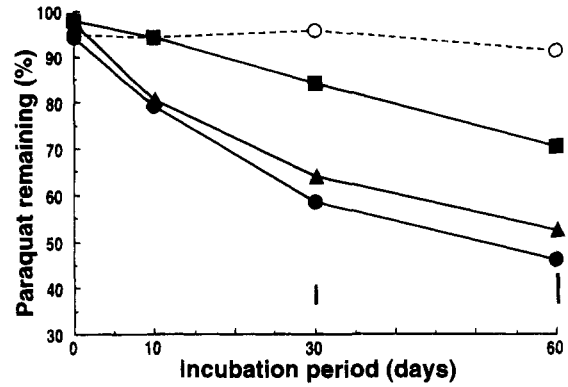

Figure 1. Degradation of paraquat sorbed to rice straw: (○) sterilized; (●) intact; (▲) intact with soil suspension; (■) sterilized with soil suspension. Vertical bars denote the least significant difference ($P < 0.05$).

Analysis of variance (F test) was performed on paraquat remaining (percent), and a least significant difference (lsd) was determined if the preliminary F test was significant.

Degradation Products Experiment. A 1-g sample of chopped rice straw was placed in a deep-bottom Petri dish and then spiked with 154.5 μg of [*methyl*-$^{14}C_2$]paraquat dichloride (6.2 mCi/mmol, equivalent to condition C). After the Petri dish was sealed by a plastic tape, the incubation was carried out as described above. The experimental method for determination of $^{14}CO_2$ evolved was adopted from the paper by Katayama et al. (1992) with some modification. The $^{14}CO_2$ evolved was trapped in a small vessel containing 5 mL of 5 N sodium hydroxide placed in the deep-bottom Petri dish. The sodium hydroxide solution was changed twice a week, and the radioactivity was measured by an Aloka 5100 liquid scintillation counter. To assess the nonbiological degradation, the autoclaved rice straw residue served as control. After incubation, the rice straw residue was ground using mortar and pestle and extracted with 30 mL of 1 N hydrochloric acid and methanol (1 + 1 v/v). The extract was filtered and the pH adjusted to 9.0 with 0.1 N sodium hydroxide. The alkaline solution was passed through a Sep-Pak C_{18} preactivated cartridge (Tsunoda, 1983). The radioactivity adsorbed to the cartridge was eluted with 2 mL of 0.1 N hydrochloric acid and 2 mL of H_2O, at a flow rate of 0.8 mL/min. Two eluates were combined and concentrated and then dissolved with methanol. Radioactivity was analyzed using a two-dimensional TLC system. A small aliquot of the methanol solution was spotted on a cellulose plate (Merck cellulose F, precoated, 0.1-mm thickness) with reference compounds by cochromatography and developed in a mobile phase of 4:1:2 1-butanol−glacial acetic acid−H_2O (first development) and 1:1:2:1 benzene−1-pentanol−methanol−0.01 N hydrochloric acid (second development) by a two-dimensional developing system. After developing, plates were then scanned with a BAS 2000 Radioanalytic Imaging System (Fujix), and R_f values of radiolabeled compounds were determined. The result showed 100.2% recovery.

RESULTS

Microbial Degradation of Paraquat Sorbed to Plant Residues. Figures 1−3 show the dissipation of paraquat sorbed to plant residues during incubation. Dissipation was observed under nonsterile conditions (B−D) but not under sterile conditions (A as control) in the three varieties of plant residues tested. However, the dissipation rates were different. The results suggested that the dissipation was due to the biological action.

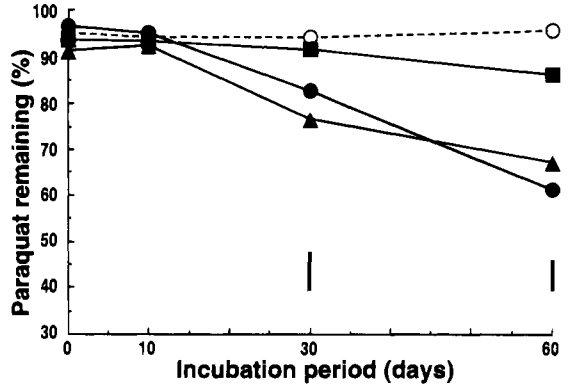

Figure 2. Degradation of paraquat sorbed to Chinese milk vetch. Symbols are the same as in Figure 1.

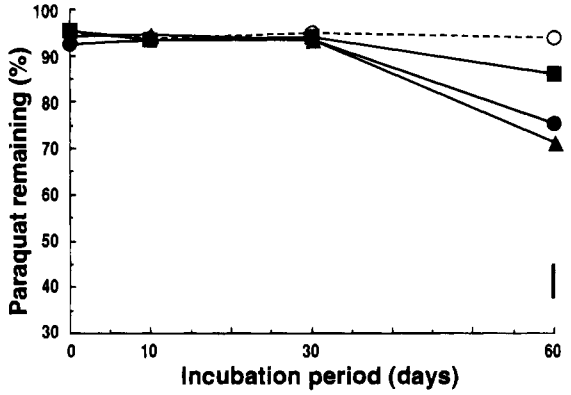

Figure 3. Degradation of paraquat sorbed to dropwort. Symbols are the same as in Figure 1.

Table 2. Changes of pH in Rice Straw Residue during Incubation

plant sample	condition[a]	incubation period 0 days	30 days	60 days
rice straw	A	5.5[b]	5.5	5.0
	B	6.3	7.7	8.1
	C	6.2	7.9	8.3
	D	5.5	8.2	9.0

[a] See Materials and Methods. [b] Rice straw–water (1:5 w/v).

The adsorption of paraquat to soil particles was tested under a condition comparable to conditions B and D. One milliliter of a soil suspension or sterile water (as control) was added, respectively, to 3 mL of paraquat solution containing 154.5 μg of paraquat dichloride. Both samples were preserved at 4 °C for 3 days. The HPLC results showed that only 2.1% of paraquat dichloride was adsorbed to the soil particles. This result suggested that the adsorption by soil particles added to conditions B and D had negligible effect on paraquat dissipation.

An inoculation of mixed microbial population from rice straw as an aqueous suspension to the autoclaved rice straw resulted in paraquat dissipation to the same extent as in the case of intact rice straw under condition C (data not shown).

Table 2 shows the increase of pH in rice straw residue from 5 to 9 under the nonsterile conditions during 60 days of incubation. No chemical degradation under the high pH conditions was observed at pH 9 of 0.1 M sodium borate under sterile condition, showing high chemical stability. In this experiment the amount of paraquat remaining was 89% at pH 9.0, compared to 92.1% remaining under neutral conditions. The stability of paraquat under weak alkaline conditions was also reported by Funderburk and Lawrence (1963).

Table 3. Remaining Percentage of Paraquat Sorbed to Rice Straw Supplemented with Nitrogenous Compounds after 30 Days of Incubation

nitrogenous compound added	paraquat remaining (%) condition A[a]	condition B[a]	condition C[a]	condition D[a]
none (control)	95.7	84.1	58.5	64.0
urea	89.6	83.5	79.7*[b]	78.0*
ammonium chloride	93.2	87.3	90.9*	86.8*
sodium nitrate	95.5	67.3*	74.8*	69.0
lsd ($P < 0.05$)		8.1	10.4	7.2

[a] See Materials and Methods. [b] The asterisk, *, denotes significant difference at 5% probability.

Degradation Products. In the experiment using [*methyl*-$^{14}C_2$]paraquat sorbed to rice straw, 0.2% of $^{14}CO_2$ was evolved after 10 days of incubation under the nonsterile condition but there was no evolution of $^{14}CO_2$ under condition A, confirming the microbial degradation of paraquat. On the two-dimensional TLC of the extract from rice straw incubated under nonsterile condition, only one spot was other than parent compound appeared to be radioactive. This metabolite migrated in the same manner as monopyridone (1′,2′-dihydro-1,1′-dimethyl-2′-oxo-4,4′-bipyridynium ion), which is the oxidized metabolite of paraquat. The metabolite was also fluorescent, which is a specific property of monopyridone. In this experiment, the distribution of radioactivity after 10 days of incubation was as follows: 57.7% paraquat, 4.3% monopyridone, 0.2% $^{14}CO_2$, and 38% unextractable radioactivity.

It is evident from these results that paraquat sorbed to plant residues was degraded by microbial actions during incubation.

Plant-Associated and Soil Microorganisms. Higher degradation rates of paraquat were observed under conditions C and D, where plant-associated microorganisms are present, than under condition B, where soil microorganisms were inoculated. The microorganisms associated with plant residues had a higher capability in paraquat degradation than soil microorganisms in the plant residues under the present experimental conditions. However, the degradation capacity would vary depending on conditions such as the size of the microbial biomass inoculated and the incubation temperature.

The combination of plant-associated and soil microorganisms neither enhanced nor suppressed the degradation rate of paraquat.

Different Degradation Rates among Plant Residues. Under all nonsterile conditions, the paraquat degradation rate was higher in rice straw than in the other two plant residues, in which lag phases were observed for the dissipation. The carbon to nitrogen ratio of rice straw was much higher than that of the other two plants (Table 1). It was suggested that the nitrogen content was closely related to paraquat degradation. This was also supported by the results described below.

Effects of Nitrogenous Compounds. The effects of nitrogenous compounds on the degradation of paraquat sorbed to rice straw are shown in Table 3. The degradation rates of paraquat by plant-associated microorganisms were suppressed by adding urea, ammonium chloride, or sodium nitrate. These findings indicated that paraquat degradation in rice straw residue by plant-associated microorganisms was sup-

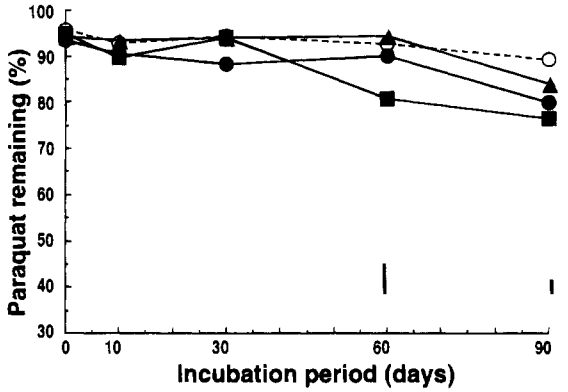

Figure 4. Anaerobic degradation of paraquat sorbed to rice straw. Symbols are the same as in Figure 1.

pressed by adding other nitrogenous compounds. The high carbon to nitrogen ratio was preferable for the degradation of paraquat on the plant residues. This explains the difference in the degradation rate among plant residues with different C/N ratios. The rice straw residues had the highest C/N ratio in the plant residues tested. The degradation rates of paraquat by soil microorganisms were not suppressed in the case of sodium nitrate, which was promoted for unknown reasons.

Anaerobic Degradation. Figure 4 shows the dissipation of paraquat sorbed to rice straw under anaerobic conditions. Significant dissipation of paraquat was observed under conditions B and C but not under condition D. The dissipation rates were far less than that under the aerobic conditions. In the case of condition C, the low dissipation rate is natural since aerobes are the dominant microorganisms on phylloplanes of fresh plants and on plant residues stored under air-dry conditions. In the case of condition B, it is suggested that the paraquat-degrading microorganisms are mainly aerobes in this soil.

Plant Residue–Soil System. Figure 5 shows that paraquat was significantly dissipated when rice straw sorbing paraquat was placed on the surface of soil but not when it was thoroughly mixed with soil. It is considered that the difference in dissipation rates between the two conditions was caused by the difference in the bioavailability of paraquat. It has been reported that paraquat adsorbed to the organic components in soil transfers to much stronger adsorption site on the clay minerals (Burns and Audus, 1970). Thorough mixing of rice straw with soil would promote paraquat translocation from rice straw to soil.

DISCUSSION

This study demonstrated that paraquat sorbed to plant residues was degraded by the natural microbial populations associated with three plant residues and a soil. The dissipation of paraquat was only observed in the nonsterile conditions. The effects of adsorption of paraquat to soil particles and of weak alkaline condition on the paraquat dissipation were negligible. The use of [*methyl*-$^{14}C_2$]paraquat showed the production of metabolites, $^{14}CO_2$ and monopyridone, in rice straw only under the nonsterile conditions.

It is generally considered that paraquat is not degraded by higher plants as shown in maize (Slade, 1966), alligatorweed and beans (Funderburk and Lawrence, 1964), and paraquat-resistant hairy fleabane (Norman et al., 1993). Smith et al. (1976) expected

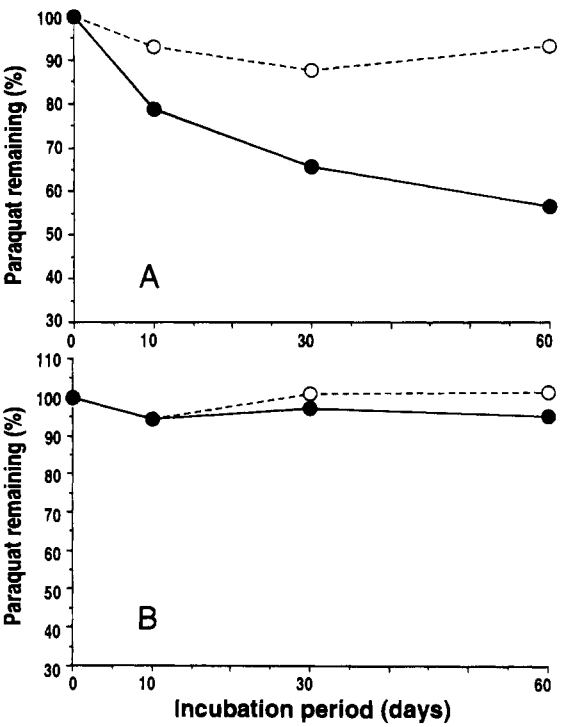

Figure 5. Degradation of paraquat sorbed to rice straw in the soil environment: (A) rice straw was placed on the soil surface; (B) rice straw was thoroughly mixed with soil; (○) sterilized; (●) nonsterilized.

paraquat degradation during the saprophytic colonization of plant material. However, no investigator has shown conclusive evidence for paraquat degradation.

Even when the plant residue (rice straw) was placed on the soil surface, paraquat dissipation was still observed. It is considered that this experimental condition is similar to the practical case whereby weeds are killed by application of paraquat and left on soils. It is also suggested that when the paraquat adsorption process to soil is slowed due to reasons such as the large bulk of plant residues, the dissipation of paraquat may occur even in the plant residue incorporated into soil. Thus, this study demonstrated for the first time that the plant residues in the soil environment are a possible site for the microbial degradation of paraquat and, at the same time, that degradation is a possible reason why paraquat did not accumulate as much as expected in spite of repeated application (Hance et al., 1980). However, Kanazawa (1990) reported a decrease in paraquat concentration in the field after stopping the application. This result suggested that the paraquat adsorbed to soil can be degraded. Our study demonstrated the microbial degradation of paraquat before adsorption to soil. In our experiment, however, paraquat sorbed to rice straw was not degraded when the rice straw was mixed with soil. So, the degradation of paraquat did not occur after adsorption to soil. The presence of other sites was suggested in the soil environment for paraquat degradation by microorganisms after adsorption.

The produced amount of $^{14}CO_2$ detected using [*methyl*-$^{14}C_2$]paraquat was rather small compared to the dissipation amount in the experiment using nonradioactive paraquat. The production of oxidized metabolite, monopyridone, suggested the accumulation of oxidized metabolites in plant residues, resulting in little production of $^{14}CO_2$. Imai and Kuwatsuka (1989) also detected monopyridone as a metabolite by fungal degraders and

the same disagreement between the dissipation amount and the produced amount of $^{14}CO_2$. It is known that the oxidized aromatic compounds tend to be polymerized abiotically (Wang et al., 1986). Further experiments will be required to gain an understanding of the disagreement.

The paraquat-degrading microorganisms in this study are considered in relation to saprophytes. Paraquat-degrading microorganisms were present in the three different plant residues as well as in soil with a history of paraquat application, suggesting a wide distribution of paraquat-degrading microorganisms. The degradation rate was faster in rice straw, which had a higher carbon to nitrogen ratio. This suggests a relationship between paraquat degradation and the nitrogen metabolism of responsible microorganisms. Some saprophytes, including a yeast *Lipomyces* sp., were reported as being capable of *in vitro* degradation of paraquat as nitrogen source (Baldwin et al., 1966; Tu and Bollen, 1968). The microflora colonizing plant residues may also change due to the carbon to nitrogen ratio or the kinds of plant materials.

The effects of sodium nitrate on paraquat degradation were different between conditions B (soil microorganisms) and C (plant-associated microorganisms) in Table 3, suggesting the presence of two different types of paraquat-degrading microorganisms. In the natural environment, both plant-associated and soil microorganisms are present in the plant residues left on the soil. In this study, the degradation rate was neither enhanced nor suppressed under the conditions of presence of both microorganisms. It can be proposed that both microorganisms work for paraquat degradation in the soil environment.

ACKNOWLEDGMENT

We thank Zeneca Agrochemicals for their offer of paraquat dichloride and the soil samples of long-term experimental plots and Dr. K. Nakamura, Saitama Agricultural Experimental Station, for his suggestions on the analytical method.

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Received for review August 4, 1994. Revised manuscript received March 13, 1995. Accepted March 16, 1995.[⊗]

JF940442S

[⊗] Abstract published in *Advance ACS Abstracts,* April 15, 1995.