

Original Articles

Effects of Herbicide Butachlor on Nitrogen Transformation of Fertilizers and Soil Microbes in Water-logged Soil*

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Butachlor [2-chloro-2',6'-diethyl-N-(butoxymethyl)acetanilide] was applied at the recommended rate, and at ten and fifty times the recommended rate to samples of silty clay loam and then compared with untreated samples incubated at 15°C or 30°C under water-logged conditions. The effects of the herbicide on ammonification and nitrification were studied by adding 100 ppm $\text{NH}_2\text{-N}$ as $\text{CO}(\text{NH}_2)_2$ or $\text{NH}_4\text{-N}$ as $(\text{NH}_4)_2\text{SO}_4$ respectively, at pH 6.8 by the addition of CaCO_3 to the soil. Butachlor at all doses inhibited the rate of ammonification and stimulated the rate of nitrification slightly at 30°C when pH was adjusted from the original 4.9 to 6.8. At ten and fifty times the recommended rate, butachlor delayed the maximum rate of ammonification of $\text{CO}(\text{NH}_2)_2$, and at the fifty fold dose inhibited the nitrification of $(\text{NH}_4)_2\text{SO}_4$ at 30°C when pH was not adjusted. The effects of butachlor on ammonification and nitrification were not significant compared to those of the control at 15°C. One of the major metabolites of butachlor by soil microbes, 2-chloro-2',6'-diethylacetanilide, delayed the rate of utilization of $\text{NH}_4\text{-N}$, and inhibited NO_2^- oxidation at 1,000 ppm concentration. Butachlor mixed with diphenyl ether type herbicides, nitrofen (2,4-dichlorophenyl 4'-nitrophenyl ether), chloronitrofen (2,4,6-trichlorophenyl 4'-nitrophenyl ether and chlomethoxyphenyl ether) at either normal or ten fold the recommended rate, did not significantly affect the rate of ammonification and nitrification. Cation exchange capacity of the soil was not affected by the application of butachlor after incubation for 7 weeks. Butachlor applied at the normal rate stimulated soil respiration in the initial stage, but at ten and fifty times the recommended rate, inhibitory phenomena were observed. The inhibitory effects seemed to be transitory and disappeared rapidly. Application of butachlor at all rates increased the population of fungi, actinomycetes and bacteria compared to those of the control after incubation for one week. The higher doses of butachlor caused the population of soil microbes to be greater than those of the control up to 4 weeks after incubation.

INTRODUCTION

Butachlor [2-chloro-2',6'-diethyl-N-(butoxymethyl)acetanilide, Machete®] is a pre-emergence herbicide which has been officially recommended in Taiwan since 1971 for the control of weeds of transplanted rice in

paddy fields. This herbicide has also been recommended for the control of weeds of direct seeded rice in wet fields and spinach fields since 1973, and in seed beds of rice since 1978. At the present time, it is still one of the most important and popular herbicides used in rice fields in this country. Butachlor has also been extensively used in rice fields in Japan, South Korea, India and the Philippines. Several diphenyl ether type herbicides such as nitrofen (2,4-dichlorophenyl 4'-nitrophenyl

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ether, NIP), chloronitrofen (2,4,6-trichlorophenyl 4'-nitrophenyl ether, CNP), chlomethoxynil (2,4-dichlorophenyl 4'-nitro-3'-methoxyphenyl ether, X-52®), and oxyfluorfen (2-chloro-4-trifluoromethylphenyl 4'-nitro-3'-ethoxyphenyl ether, Goal®) have been mixed with butachlor and these mixtures have also been officially recommended for the control of weeds of paddy fields in Taiwan since 1976.

In order to study the behavior and fate of this herbicide after its application in paddy rice fields and to further seek a safe and more correct use, a series of experiments has been done on its photodecomposition as thin film on glass,¹⁾ degradation by soil microbes,²⁾ and degradation and dissipation in paddy fields,³⁾ both under controlled laboratory conditions and in a field situation. Because butachlor is mainly used in paddy fields, the effects of this herbicide on nitrogen transformation of fertilizers (namely on urea and ammonium sulfate) and on soil microbes, including ammonification, nitrification, combined effects with three diphenyl ether type herbicides, nitrofen, chloronitrofen and chlomethoxynil, soil respiration, cation exchange capacity of the soil and microbial population in water-logged soil were studied in this investigation.

In spite of a large number of papers which have been published on herbicidal effects on non-target soil microorganisms,⁴⁾ little information has been published on the relationship between butachlor and fertilizers. Only Kim⁵⁾ has reported on the influence of this herbicide on the mineralization of nitrogen fertilizers in upland soil at $20 \pm 1^\circ\text{C}$. He found that butachlor did not affect the hydrolysis of urea to ammonia or the rate of nitrification at the recommended rate, but at ten and fifty times the recommended rate, depressed urea hydrolysis resulted in reduction of ammonia and inhibited the oxidation of nitrite; these depressive effects, however, were found to be transitory.

MATERIALS AND METHODS

1. Materials

Butachlor of 99.2% purity was obtained from Monsanto Co., St. Louis, Mo., U.S.A. Nitrofen, chloronitrofen and chlomethoxynil were extracted with acetone from commercial

granular products and purified by recrystallization from dilute alcohol. 2-Chloro-2',6'-diethylacetanilide was synthesized as described in the previous paper.¹⁾ The silty clay loam with clay 17%, silt 68%, sand 15% and organic matter 1.7%, and pH 4.9 (H₂O) was collected from the Experimental Farm of National Taiwan University, Taipei, Taiwan.

2. Methods

Butachlor at the normal rate, namely, the recommended rate of 6 ppm equivalent to 1.5 kg ai/ha, and at ten and at fifty times the recommended rate was dissolved in acetone and mixed with 12 g of a fresh soil sample in 250 ml flask. The soil pH (H₂O) was adjusted to 6.8 by the addition of calcium carbonate. After evaporating the solvent, urea or ammonium sulfate was added at the rate of 100 ppm NH₂-N or NH₄-N. Ten milliliters of distilled water was added to the flask to make a flooded condition. It was then covered with a paraffin film to retain the moisture and incubated at 15°C or 30°C. Control experiments without the herbicide were carried out simultaneously with and without urea or ammonium sulfate. After the indicated periods, the soil muds were shaken with 90 ml of 2 M KCl for one hr on a rotary shaker and were allowed to stand overnight. NH₄-N was determined by distilling an aliquot of the extract added with magnesium oxide into 2% boric acid using a semimicro Kjeldahl apparatus. The distillate was titrated with standardized 0.0158 N-H₂SO₄ solution according to the method of Bremner and Keeney,⁶⁾ NO₂-N was determined by the 1-naphthylamine and sulfanilic acid method.⁷⁾ A second distillation with Devarda's alloy powder for the aliquot used in the NH₄-N determination gave the value of NO₂-N plus NO₃-N. The value for NO₃-N was obtained by subtracting NO₂-N from the above value.⁶⁾ Experiments were carried out in a similar manner with: 2-chloro-2',6'-diethylacetanilide at 10, 100 and 1,000 ppm; the combined effects with three diphenyl ether type herbicides at the recommended rates, namely, butachlor 0.9 kg ai/ha plus nitrofen 0.9 kg ai/ha equivalent to 3.6 plus 3.6 ppm, butachlor 0.9 kg ai/ha plus chloronitrofen 1.5 kg ai/ha equivalent to 3.6

plus 6 ppm, butachlor 0.75 kg ai/ha plus chlo-methoxynil 1.05 kg ai/ha equivalent to 3 plus 4.2 ppm; and ten fold the recommended rates. To estimate the effects of butachlor on soil respiration, the herbicide was added at the described levels to 100 g of fresh soil with 20 ml of 2% glucose solution and incubated at 30°C. The evolved CO₂ was absorbed by barium hydroxide and was titrated with oxalic acid. Effects of butachlor on cation exchange capacity were determined by the ammonium saturation and acidified sodium chloride leaching method.⁸⁾ Effects on change of soil microbial population were carried out by a serial dilution plate method with the selective media for fungi,⁹⁾ actinomycetes,¹⁰⁾ and bacteria.¹¹⁾

RESULTS AND DISCUSSION

1. Effects on Ammonification

In all treatments, butachlor inhibited the rate of ammonification of urea slightly during the initial two days in water-logged soil at 30°C when soil pH (H₂O) was adjusted from the original 4.9 to an optimum 6.8 by adding calcium carbonate. When butachlor was applied at ten and fifty times the recom-

mended rate, the maximum rate of ammonification was delayed if pH was not adjusted. The rates of ammonification slowed down at the lower temperature, but pH had no significant effect (Table 1). It was reported that few herbicides have an inhibitory effect on ammonifying microorganisms, but three major groups of herbicides, the phenoxy-acids, triazines and urea-type compounds, are mainly stimulative in their effects on these organisms.⁴⁾

2. Effects on Nitrification

Some stimulative effects were observed on the nitrification of ammonium sulfate after incubating for two weeks at 30°C with pH 6.8. NO₂⁻ was detected after one week but had disappeared after two weeks in all treatments. Because nitrifying bacteria are not best suited to an acidic soil at pH 4.9, the rates of nitrification were slower than those of pH 6.8 at 30°C. No significant effect on nitrification was demonstrated at normal and ten fold the recommended rate, but at fifty fold, an obvious inhibitory effect was observed after 6 weeks. The rates of nitrification were much slower

Table 1 Effects of butachlor on ammonification of 100 ppm NH₂-N applied with urea in water-logged soil.

Butachlor added	Temperature (°C)	pH	NH ₄ -N formed after incubation (ppm)*				
			1	2	Days 4	7	14
Control (No N added)	30	6.8	1.1	8.9	8.9	11.1	12.2
	30	4.9	0	0	0	0	1.1
	15	6.8	0	5.5	3.3	1.1	3.3
	15	4.9	5.5	0	0	0	0
Control (NH ₂ -N added)	30	6.8	86.0	101.8	73.0	27.1	16.6
	30	4.9	83.0	78.5	79.6	58.6	16.6
	15	6.8	40.9	56.4	76.3	94.0	96.2
	15	4.9	52.0	71.9	81.8	94.0	87.4
Normal (6 ppm)	30	6.8	85.4	86.3	77.4	34.3	14.4
	30	4.9	96.2	89.6	76.3	47.6	18.8
	15	6.8	29.9	58.6	74.1	91.8	94.0
	15	4.9	47.6	71.9	100.7	98.4	85.2
10 Fold (60 ppm)	30	6.8	91.2	90.7	78.5	27.7	15.5
	30	4.9	71.9	85.2	89.6	79.7	78.5
	15	6.8	40.9	60.8	80.7	98.4	87.4
	15	4.9	47.6	67.4	98.4	98.4	100.7
50 Fold (300 ppm)	30	6.8	86.3	86.3	76.3	19.4	6.6
	30	4.9	74.1	83.0	85.2	87.4	47.5
	15	6.8	40.9	63.0	78.5	96.2	96.2
	15	4.9	47.6	69.7	94.0	85.2	91.8

* Mean of duplicate.

Table 2 Effects of butachlor on nitrification of 100 ppm $\text{NH}_4\text{-N}$ applied with ammonium sulfate in water-logged soil.

Butachlor added	Temperature (°C)	pH	$\text{NO}_3\text{-N}$ and $\text{NO}_2\text{-N}^*$ formed after incubation (ppm)**						
			1	2	4	Days 7	14	28	42
Control (No N added)	30	6.8	4.4	2.2	8.9	23.2	16.6	37.6	67.5
	30	4.9	8.9	6.6	11.1	18.8	17.7	28.8	44.2
	15	6.8	5.5	5.5	7.7	10.0	10.0	10.0	8.9
	15	4.9	3.2	8.9	11.1	6.6	0	0	0
Control ($\text{NH}_4\text{-N}$ added)	30	6.8	7.5	8.3	28.4	69.8 (1.04)	87.5	116.1	126.1
	30	4.9	9.9	10.0	25.4	23.2	69.7	90.7	98.4
	15	6.8	2.2	18.8	20.0	21.1	21.1	23.3	27.6
	15	4.9	2.2	16.6	15.5	16.6	17.7	11.1	10.0
Normal (6 ppm)	30	6.8	8.3	16.6	18.9	72.5 (1.09)	111.7	104.0	131.6
	30	4.9	6.6	14.4	18.8	28.8	47.5	60.8	111.7
	15	6.8	6.5	15.5	19.9	16.0	10.0	12.1	14.4
	15	4.9	6.6	21.0	20.0	21.0	15.5	16.6	19.9
10 Fold (60 ppm)	30	6.8	9.4	14.4	20.2	88.4 (1.18)	96.3	117.2	125.0
	30	4.9	6.6	11.1	12.2	16.6	27.7	27.7	91.8
	15	6.8	4.4	12.2	18.8	25.4	17.8	15.5	12.2
	15	4.9	4.4	15.5	10.0	11.1	17.7	14.4	12.2
50 Fold (300 ppm)	30	6.8	2.5	11.1	19.9	74.1 (1.12)	99.6	109.5	85.4
	30	4.9	5.5	12.2	12.2	23.2	24.3	23.0	12.2
	15	6.8	5.4	12.2	15.5	18.8	13.3	17.7	16.6
	15	4.9	3.2	18.8	10.0	12.2	15.5	17.7	13.3

* $\text{NO}_2\text{-N}$ was not detected in any treatments except in the case of 30°C at pH 6.8, expressed in parenthesis.

** Mean of duplicate.

and pH had no significant effect at the lower temperature (Table 2).

Tena *et al.*¹²⁾ also demonstrated that an analogous herbicide of butachlor, alachlor [2-chloro-2',6'-diethyl-N-(methoxymethyl)-acetanilide, Lasso®] showed a stimulatory action on nitrification at 50 and 100 ppm, but inhibitory effects were observed with higher concentrations at 500 and 1,000 ppm. In considering the overall effects on nitrification, most of the herbicides had a negligible effect at normal field rates and were only effective against nitrification when the soil pH was below 7.0. Oxidation of NO_2^- to NO_3^- appeared to be more sensitive than oxidation of NH_4^+ to NO_2^- .⁴⁾

3. Combined Effects on Ammonification and Nitrification with Diphenyl Ether Type Herbicides

Three diphenyl ether type herbicides, nitrofen, chloronitrofen and chlomethoxynil, were

mixed with butachlor separately and the combined effects on ammonification and nitrification were investigated. The effects of these combined herbicides were not found to be significant at normal and ten fold the recommended rates compared to those of the control. 2-Chloro-2',6'-diethylacetanilide at 1,000 ppm concentration inhibited the NO_2^- oxidation. About 4 ppm of NO_2^- still remained in the soil after incubating for 7 weeks. The rates of ammonification and nitrification were obviously affected by 2-chloro-2',6'-diethylacetanilide at 1,000 ppm (Tables 3 and 4). Undoubtedly, this is an extremely high concentration; it could not happen under actual field conditions.

4. Effects on Soil Respiration

Butachlor stimulated soil respiration at the normal rate in the initial stage for about one week, but at ten and fifty times the recommended rate, the inhibitory effects were observed only

Table 3 Effects of butachlor mixed with diphenyl ether type herbicides and 2-chloro-2',6'-diethylacetanilide on ammonification of 100 ppm $\text{NH}_4\text{-N}$ applied with urea in water-logged soil under 30°C at pH 6.8.

Herbicide added	$\text{NH}_4\text{-N}$ formed after incubation (ppm)*				
	1	2	Days 5	7	14
Control (No N added)	0	0	0	0	7.7
Control ($\text{NH}_2\text{-N}$ added)	78.5	86.3	58.6	24.3	3.3
Butachlor-Nitrofen (Normal) (3.6+3.6 ppm)	74.1	90.1	49.8	19.9	1.1
Butachlor-Nitrofen (10 Fold) (36+36 ppm)	80.7	84.1	62.5	40.9	0
Butachlor-Chloronitrofen (Normal) (3.6+6 ppm)	76.3	85.6	49.8	0.5	0
Butachlor-Chloronitrofen (10 Fold) (36+60 ppm)	80.7	86.3	53.1	8.9	0
Butachlor-Chlomethoxynil (Normal) (3+4.2 ppm)	80.7	87.9	59.7	1.1	2.2
Butachlor-Chlomethoxynil (10 Fold) (30+42 ppm)	83.0	88.5	43.7	10.0	0
2-C** (10 ppm)	83.0	82.4	63.0	16.6	3.3
2-C (100 ppm)	81.8	85.2	67.5	27.7	2.2
2-C (1,000 ppm)	81.8	86.8	81.8	78.5	52.0

* Mean of duplicate, ** 2-C: 2-Chloro-2',6'-diethylacetanilide.

Table 4 Effects of butachlor mixed with diphenyl ether type herbicides and 2-chloro-2',6'-diethylacetanilide on nitrification of 100 ppm $\text{NH}_4\text{-N}$ applied with ammonium sulfate in water-logged soil under 30°C at pH 6.8.

Herbicide added	$\text{NO}_3\text{-N}$ and $\text{NO}_2\text{-N}$ formed after incubation (ppm)*							
	Days							
	7		14		28		49	
	NO_3^-	NO_2^-	NO_3^-	NO_2^-	NO_3^-	NO_2^-	NO_3^-	NO_2^-
Control (No N added)	13.3	0	21.0	0	53.0	0	53.1	0
Control ($\text{NH}_4\text{-N}$ added)	74.6	1.73	108.4	0	104.9	0	139.4	0
Butachlor-Nitrofen (Normal) (3.6+3.6 ppm)	83.6	1.59	108.4	0	112.8	0	134.9	0
Butachlor-Nitrofen (10 Fold) (36+36 ppm)	72.5	1.74	100.6	0	110.6	0	132.7	0
Butachlor-Chloronitrofen (Normal) (3.6+6 ppm)	80.2	1.59	101.8	0	112.8	0	140.4	0
Butachlor-Chloronitrofen (10 Fold) (36+60 ppm)	78.2	1.47	95.7	0	109.5	0	146.0	0
Butachlor-Chlomethoxynil (Normal) (3+4.2 ppm)	79.1	1.60	108.4	0	109.5	0	140.4	0
Butachlor-Chlomethoxynil (10 Fold) (30+42 ppm)	82.6	1.49	106.2	0	123.9	0	136.0	0
2-C** (10 ppm)	75.7	1.80	102.9	0	118.3	0	147.1	0
2-C (100 ppm)	80.4	1.47	97.3	0	99.5	0	146.0	0
2-C (1,000 ppm)	74.5	1.00	103.1	1.42	98.2	5.70	121.9	4.20

* Mean of duplicate, ** 2-C: 2-Chloro-2',6'-diethylacetanilide.

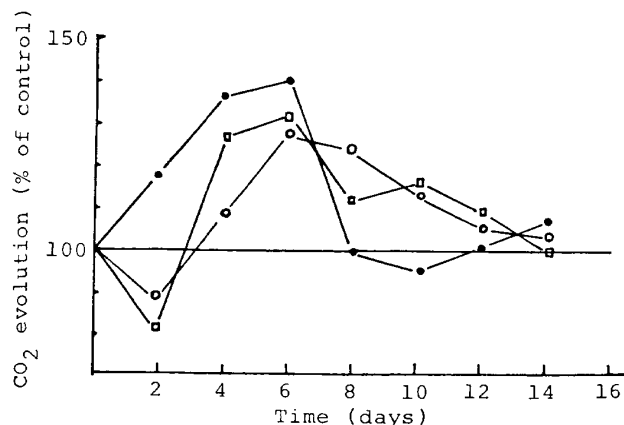


Fig. 1 Effect of butachlor on CO_2 output in water-logged soil amended with glucose at 30°C .

●: normal rate (6 ppm), □: 10 fold, ○: 50 fold.

in the initial three days. These inhibitory effects seemed to be transient and disappeared quickly (Fig. 1). Since the microbial biomass is a major contributor to soil respiration, it is pertinent to obtain some idea of the likely rate of degradation of the chemical beforehand. This may contribute significantly to CO_2 -evolution (or O_2 -uptake), especially where high rates of the compound are applied to the soil. In general, most herbicides have a slightly inhibitory effect on respiratory activity in soil.⁴⁾

5. Effects on Soil Cation Exchange Capacity

Cation exchange capacity was almost unaf-

Table 5 Effects of butachlor on cation exchange capacity of soil under water-logged conditions at 30°C .

Butachlor added	Cation exchange capacity (meq/100 g soil)*
Control	6.18
Normal (6 ppm)	6.68
10 Fold (60 ppm)	6.72
50 Fold (300 ppm)	6.42

* Mean of duplicate.

ected by application of the different amounts of butachlor to the soil at 30°C under water-logged conditions after incubation for 7 weeks (Table 5). Since the cation exchange capacity is an index of soil fertility, it seemed that application of butachlor would not affect the fertility-holding ability of the soil.

6. Effects on Soil Microbial Population

The population of fungi, actinomycetes and bacteria was increased by the addition of butachlor to the soil after incubation for one week. It seemed that the higher doses of butachlor caused the population of soil microbes to remain greater than that of the control up to 4 weeks after incubation (Table 6). As reported in the previous paper,²⁾ the half life of butachlor in viable soil was about 11 days, and butachlor seemed to be utilized as an energy source by soil microbes; the

Table 6 Effects of butachlor on microbial population in water-logged soil at 27°C .

Butachlor added		Microbes in one gram soil after incubation*				
		1	7	Days 14	21	28
Control	Fungi ($\times 10^4$)	25.2	22.8	50.8	38.4	29.6
	Actinomycetes ($\times 10^6$)	18.9	16.3	19.3	38.4	25.2
	Bacteria ($\times 10^6$)	38.4	25.6	24.4	32.0	34.0
Normal (6 ppm)	Fungi ($\times 10^4$)	32.8	23.2	93.2	21.0	22.4
	Actinomycetes ($\times 10^6$)	17.8	15.8	55.6	38.8	27.0
	Bacteria ($\times 10^6$)	46.4	81.6	76.0	28.4	44.0
10 Fold (60 ppm)	Fungi ($\times 10^4$)	28.0	27.6	64.4	49.6	41.6
	Actinomycetes ($\times 10^6$)	16.0	18.6	80.8	30.4	103.5
	Bacteria ($\times 10^6$)	39.2	95.6	106.8	33.6	81.2
50 Fold (300 ppm)	Fungi ($\times 10^4$)	22.8	30.0	60.8	49.2	36.4
	Actinomycetes ($\times 10^6$)	14.1	24.7	45.4	46.8	46.2
	Bacteria ($\times 10^6$)	39.2	190.0	159.2	110.0	98.5

* Mean of colonies of 5 plates.

results of this experiment well agreed with the tendency of microbial population change. In general, few herbicides have any great or prolonged adverse effect on the total microbial population of soils. The usual pattern is a decrease in total numbers in the initial stage, followed by a return to normal or even an increase in numbers. Such effect may be due to a disruption of the rhizosphere and non-rhizosphere microbes imposed by the killing of the vegetative cover followed by utilization of the dead plant material, which allows for increased populations.⁴⁾

This investigation indicated that butachlor used at the recommended rate did not change the soil properties significantly. These observations also demonstrated that butachlor may not cause a serious problem of environmental pollution.

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REFERENCES

- 1) Y. L. Chen & C. C. Chen: *J. Pesticide Sci.* **3**, 143 (1978)
- 2) Y. L. Chen & T. C. Wu: *J. Pesticide Sci.* **3**, 411 (1978)
- 3) Y. L. Chen & J. S. Chen: *J. Pesticide Sci.* **4**, 431 (1979)
- 4) I. R. Hill & S. J. L. Wright: "Pesticide Microbiology," Academic Press, London, p. 321, 1978
- 5) M. K. Kim: *Hanguk Sikmul Poho Hakhoe Chi* **16**, 149 (1977) (in Korean); *Chem. Abstr.* **88**, 11628 (1978)
- 6) J. M. Bremner & D. R. Keeney: *Soil Sci. Soc. Am. Proc.* **30**, 577 (1966)
- 7) J. R. Norris & D. W. Ribbons: "Methods in Microbiology," Vol. 3B, Academic Press, London, p. 31, 1972
- 8) C. A. Black, D. D. Evans, L. E. Ensiminger, J. L. White & F. E. Clark: "Methods of Soil

Analysis," Part 2, American Society of Agronomy and American Society for Testing and Materials, Madison, p. 894, 1965

- 9) L. F. Johnson: *Phytopathology* **47**, 630 (1957)
- 10) J. D. Farley: *Phytopathology* **57**, 809 (1967)
- 11) E. Kuster & S. T. Williams: *Nature* **202**, 928 (1964)
- 12) A. M. Tena, L. R. Garrido & P. J. Soriano: *An. Edafol. Agrobiol.* **35**, 1115 (1976) (in Spanish); *Chem. Abstr.* **87**, 917 (1977)

要 約

湛水土壤中におけるチッ素の形態変化および土壌微生物におよぼす除草剤ブタクロールの影響

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除草剤ブタクロールを一般使用量, 10 倍量および 50 倍量で湛水土壤中に施用し, 尿素および硫酸施用時のチッ素の形態変化におよぼす影響を対照区と比較した結果, pH 6.8, 30°C ではブタクロールはいずれの濃度においても尿素のアンモニア化成と硫酸の硝酸化成をわずかに促進することが認められたが, pH 4.9 の場合には 10 倍量および 50 倍量においては尿素のアンモニア化成を遅延させ, 50 倍量では硫酸の硝酸化成を阻害することがわかった. 15°C においては対照区とほとんど差異はなかった. ブタクロールの土壌微生物による主要代謝物の一つである 2-chloro-2',6'-diethylacetanilide は 1,000 ppm の濃度においてはアンモニア態チッ素の利用を遅延させ, 亜硝酸態チッ素の酸化を阻害した. ブタクロールと三種類のジフェニルエーテル系除草剤 NIP(nitrofen), CNP(chloronitrofen), およびクロメトキシニル(X-52)とのそれぞれの混合剤では一般使用量および 10 倍量ではアンモニア化成および硝酸化成に著しい影響を与えない. 土壌の塩基置換容量はブタクロールを使用後 7 週間後においても大した変化はなく, 一般使用量では初期に土壌の呼吸の促進を認めたが 10 倍量および 50 倍量では一時的阻害現象が認められた. ブタクロールの添加はいずれの濃度においても 1 週間後には対照区よりも多い土壌微生物の存在が認められ, 4 週間後には高濃度においてより高い数値を示した.