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Bioavailability of Bentazon Residues in a German and Korean Agricultural Soil

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독일과 한국토양중에서 Bentazon 잔류물의 생물에 의한 이용

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Abstract

Maize plants, grown on a German soil and a Korean soil which had been treated with benzene-ring-labelled ^{14}C-Bentazon (5.02mg/kg) immediately before planting (T-0), took up 36.0~42.8% of the radioactivity present during a 21 day growing period. Plants grown on the same soils (4.79~4.84mg/kg) which had been treated with Bentazon and pre-incubeted for 105 days absorbed 8.2~14.2% (T-1) of the radioactivity. Plants grown in soils (5.56~7.95mg/kg) treated with Bentazon which had been incubated for 105 days and then exhaustively extracted with distilled water and/or 0.01 M $CaCl_2$ to produce non-extractable residues (T-2) took up 1.8~2.3% of the radioactivity. The distribution of the absorbed radioactivity ranged from 2.7 to 9.7% in shoots and from 90.3 to 97.3% in roots. Extraction of maize roots revealed that 39.1~51.3% of the radioactivity was bound in T-0 and 55.7~63.1% was bound in T-1. This suggests that polar metabolites and parent Bentazon might be present as conjugates.

Introduction

In recent years, quite a few workers have paid much attention to the plant uptake of soil-bound residues of pesticides. Fuhremann and Lichtenstein[1] used methyl-[^{14}C] parathion bound residues to study uptake by oat plants. The bioavailability of bound dinitroaniline herbicides to soybean plants was investigated by Helling and Krivonak[2]. Führ and Mittelstaedt[3] reported on the availability of ^{14}C-methabenzthiazuron bound residues to maize plants. Oat plants were used to evaluate the bioavailability of bound ^{14}C-prometryn residues in sol by Khan[4].

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Roberts and Standen[5] reported the uptake of the radioactivity by wheat grown in soil containing bound residues of ^{14}C-cypermethrin. More recently, the biovailability of bound ^{14}C-simazine residues was investigated by Kloskowski and Führ[6] again using maize plants. In the present investigation, the bioavailability of bound Bentazon residues to maize plants was studied. Very little has been reported concerning the bioavailability of bound residues of this herbicide. With a view to comparing differences in Bentazon absorption by maize plants, from different soil treatment protocols resulting in differing Bentazon availabilities, three soil treatments were adopted. These included fresh ^{14}C-Bentazon applied immediately before transplanting, aged ^{14}C-Bentazon which had been incubated in soil, and non-extractable ^{14}C-Bentazon residues which had been formed by pre-incubation in soil followed by an exhaustive aqueous extraction.

Materials and Methods

Chemicals

Uniformly benzene-ring-labelled ^{14}C-Bentazon (specific activity: 10.81mCi/mMol) and cold Bentazon (>99.5% purity) were provided by the BASF Corporation, Limburgerhof, West Germany. The purity was confirmed by thin-layer chromatography followed by autoradiography prior to use.

Soils used

The physico-chemical characteristics of the German parabraun and Korean soil are as follows: sand, 7.0 and 64.4%; silt, 85.3 and 31.2%; clay, 7.7 and 4.4%; textural class, sandy loam both; pH (KCl), 7.1 and 5.9; carbon, 1.10 and 4.11%; total nitrogen, 0.05 and 0.06%; maximum water-holding capacity, 44 and 46g/100g soil, respectively.

Treatments of ^{14}C-Bentazon in soil

For each soil, three treatments of ^{14}C-Bentazon were undertaken (Table 1). Treatment 0 (T-0) constitutes the soils which were treated with ^{14}C-Bentazon immediately before planting maize plants. Treatment 1 (T-1) includes the soils which had been treated with ^{14}C-Bentazon and incubated for 105 days. After incubation, the soils were air-dried before transplanting the maize plants. Treatmeat 2 (T-2) comprises the soils which were treated with ^{14}C-Bentazon, incubated for the same period of time, and then exhaustively extracted with distilled water and/or 0.01 M $CaCl_2$ solution. After extraction, the soils were also air-dried for the subsequent plant experiment. The total amounts of Bentazon applied were calculated on the basis of the degradation rate during the aerobic incubation, phytotoxicity to maize plant, and extractability in the solvent to be used for extraction. The estimation was based on a preliminary degradation experiment in accordance with BBA-regulations[7].

According to the preliminary experiment, the initial radioactivities and active ingredient concentrations for the T-1 treatment of the Parabraun and Korean soil for incubation were 25 μCi (5.51mg/kg) and 25.13μCi (5.51mg/kg), respectively, for 5kg of soil on a dry weight basis.

For the T-2 treatment of the parabraun and Korean soil, the initial radioactivities were 100μCi (25.05 mg/kg) and 100.5μCi (25.05mg/kg) for 5kg of soil on a dry weight basis, respectively. The soils were aerobically incubated in desiccators, being flushed

Table 1. Three treatments with different backgrounds of ^{14}C-Bentazon residues used in the growing of maize plant (1.5kg soil/pot)

Treatment	Description	Parabraun soil		Korean soil	
		mg/kg	μCi/pot	mg/kg	μCi/pot
T-0	Bentazon added freshly	5.02	5.7	5.02	4.5
T-1	Bentazon aged by pre-incubation for 105 days	4.84	6.7	4.79	6.5
T-2	Bentazon aged as in T-1 but extracted exhaustively with water	5.56	6.8	7.95	9.6

with CO_2-free air. Throughout the incubation, the moisture contents were maintained at 50% of the maximum water-holding capacity of each soil.. Incubation was at 23±1°C for 105 days. The $^{14}CO_2$ evolved during incubation was absorbed in 1N NaOH and measured at weekly intervals.

Desorption experiment

10g of the air-dried soils of each treatment were extracted with 50ml of distilled water for 16.5 hrs on a shaker. The soil suspension was then centrifuged at 18,000g for 20min. and the supernatant was collected. The same procedure was repeated 5 times for complete extraction. The radioactivity of the combined supernatant and that of the residual soil were measured.

Extraction of the roots of maize plant

1g of the freeze-dried root harvested from T-0 and T-1 of each soil was extracted consecutively with two 50-ml aliquots of MeOH in a sonicator. The extract was separated from root tissues by centrifugation and the radioactivity was measured. The residual root was air-dried and the radioactivity measured by combustion followed by liquid scintillation counting.

Results and Discussion

Uptake of ^{14}C-Bentazon by maize plant

Table 2 shows the weights of maize plants at harvest after 21 days of growth. Plants grown in the T-2 Korean soil (7.95mg/kg), which had a relatively higher concentration of Bentazon than all other treatments (Table 1), did not exhibit any symptoms of phytotoxicity during the growing period. The ranges of fresh weights of shoots and roots for all plants were 73.7~136.3g and 19.3~39.5g, respectively. The total uptake of ^{14}C-Bentazon by maize plants from T-0, T-1, and T-2 treatments in parabraun soil was 36.0, 14.2, and 2.3% of the radioactivities in the soil at the beginning of the plant experiment, respectively (Table 3). In the case of Korean soil, the total uptake was 42.8, 8.2, and 1.8%, respectively (Table 3). The big differences in the uptake of ^{14}C-Bentazon between the the treatments resulted from the fact that Bentazon in all three treatments had different origins of soil residues, even though the initial concentrations were similar. Table 4 presents more detailed results of the plant experiment. Expressing these percentage figures as uptake ratios, they are 16 : 6 : 1 and 24 : 5 : 1 for T-0, T-1, and T-2 in parabraun and

Table 2. Growth state of maize plant at harvest after 21 days of growing in soil containing freshly applied and aged Bentazon residues

Soil	Tseatment	Fresh weight(g/4 plants)*** Shoot	Root
Parabraun	Extracted control*	96.0	19.3
	Un-extracted control**	133.0	34.2
	T-0	119.0	30.2
	T-1	136.3	34.7
	T-2	131.0	32.4
Korean	Extracted control*	94.4	26.2
	Un-extracted control**	92.3	29.8
	T-0	73.7	38.0
	T-1	98.1	39.5
	T-2	88.5	33.7

* Extracted with distilled water and/or 0.01 M $CaCl_2$ solution (Control for T-2)
** Control for T-0 and T-1
*** Average values of 2 or 3 replicates, depending on treatments

Table 3. Uptake of ^{14}C-activity and its distribution between shoots and roots of maize plants after growing for 21 days in soils containing fresh (T-0), aged (T-1), and bound (T-2) ^{14}C-Bentazon residues. ^{14}C-activity in soil on 0 day=100%

Soil	Treatment	^{14}C-activity in plants(%)	Distribution(%)* Shoot	Root
Parabraun	T-0	36.0	5.9	94.1
	T-1	14.2	5.7	94.3
	T-2	2.3	9.6	90.4
Korean	T-0	42.8	2.7	97.3
	T-1	8.2	9.3	90.7
	T-2	1.8	5.0	95.0

* Radioactivity in the plants=100%

Table 4. Results of the plant experiment for the bioavailability of ^{14}C-Bentazon residues in soil. ^{14}C-activity before planting=100%

Soil	Treatment	Mineralization to $^{14}CO_2$(%)	Residue in soil (%)	Maize uptake(%)		Recovery (%)
				Shoot	Root	
Parabraun	T-0	0.93	51.95	2.13	33.89	88.90
	T-1	1.70	80.33	0.81	13.40	96.24
	T-2	1.93	95.54	0.22	2.06	99.75
Korean	T-0	0.62	55.56	1.16	41.66	99.00
	T-1	1.24	87.71	0.77	7.47	97.19
	T-2	0.41	97.53	0.09	1.70	99.73

All values are the averages of three replicates.

Table 5. Uptake of ^{14}C-Bentazon by maize plant for 21 days, calculated on the basis of the ^{14}C-Bentazon concentrations applied initially to the soil. The concentrations of T′-1 and T′-2 are those before the pre-incubation was started. as distinguished from T-1 and T-2.

Soil	Treatment	Initial conc. (mg/kg)	Total plant uptake (%)	Uptake ratio
Parabraun	T-0	5.02	36.02	72.0
	T′-1*	5.51	12.47	24.9
	T′-2**	25.05	0.50	1
Korean	T-0	5.02	42.82	75.1
	T′-1*	5.51	7.15	12.5
	T′-2**	25.05	0.57	1

* includes 105 days' aerobic pre-incubation.
** 105 days' aerobic pre-incubation followed by an exhaustive extraction with water.

Table 6. Behavior of ^{14}C-Bentazon throughout the pre-incubation and plant growing. Applied ^{14}C-activity=100% Average data of 3 replicates

Soil	Treatment	Pre-incubation (105 days), ^{14}C lost prior to plant growing by		Maize plant growing (21 days), ^{14}C found as			
		$^{14}CO_2$(%)	Extraction(%)	$^{14}CO_2$(%)	Plant uptake(%)	Residue in soil (%)	Recovery (%)
Parabraun	T-0	—	—	0.93	36.02	49.70	86.65
	T′-1*	8.98	—	1.49	12.47	70.49	93.43
	T′-2**	3.44	74.37	0.43	0.50	21.20	99.94
Korean	T-0	—	—	0.62	42.82	55.55	98.99
	T′-1*	8.79	—	1.07	7.15	76.15	93.16
	T′-2**	3.18	65.08	0.13	0.57	32.07	101.03

* Followed by 105 days' aerobic pre-incubation.
** Followed by 105 days' aerobic pre-incubation plus an exhaustive aqueous extraction.

Korean soil, respectively. These ratios become even greater, if the plant uptake of ^{14}C-activity is calculated on the basis of the ^{14}C-Bentazon applied initially to the soil of the treatments T-1 and T-2 before the pre-incubation experiment was started (Table 5). That is, as seen in this table, the ratios of 72 :

25 : 1 and 75 : 13 : 1 resulted in the parabraun and Korean soil, respectively. Table 6 shows this result more explicitly, in connection with the data of the pre-incubation in the previous paper.

Kloskowski and Führ[6] reported that the maize plant grown on parabraun soil containing freshly added ^{14}C-simazine, ^{14}C-simazine aged for 108 days, and bound ^{14}C-simazine residues took up 20, 10, 0.08% of the applied radioactivity, respectively. Formerly, Führ and Mittelstaedt[3] reported that maize plants grown for 29 days in extracted soil treated with methabenzthiazuron absorbed 2.4% of the bound ^{14}C in soil. It was reported that the shoot contained 70% and the root 30% of the total plant radioactivity. Khan[4] observed that the oat plants took up 0.5% of the previously soil-bound ^{14}C, when grown in a mixture of a soil containing ^{14}C-prometryn bound residues with silica sand. The shoots and roots also contained 70% and 30%, respectively.

Conversely, ^{14}C-dinitroaniline-bound residues were distributed between roots and greens as 76% and 24%, respectively[2]. In addition, Worobey[11] and Still[12] also observed that TCAB (3, 3′, 4, 4′-tetrachloroazobenzene) was distributed much more in the roots than in the shoots of soybean plants. The detailed results on the uptake of ^{14}C-Bentazon by maize plant from soil (Table 4) disclosed that ^{14}C-Bentazon absorbed by roots was translocated into the shoots only to a limited extent. As can be seen in Table 3, the distribution of ^{14}C-Bentazon between shoots and roots was in the range of 2.7∼9.6% and 90.4∼97.3%, respectively. Mine et al.,[15] in the paper on the mechanism of Bentazon selectivity, reported that rice and *Cyperus serotinus* plants took up ^{14}C-Bentazon from 3 ppm aqueous solution and readily translocated it to stems, sheaths, and leaves. Rice plants were shown to have an especially high concentration of ^{14}C-activity in the sem. Our results and those cited above suggest that the translocation of ^{14}C-Bentazon can differ from plant to plant, and the absorption and translocation can be different depending on the method of application. The maize plant is known to have the smallest uptake of ^{14}C-Bentazon among the plants tested[15].

Mineralization of ^{14}C-Bentazon to $^{14}CO_2$ during maize growing

During the 21 day plant growth period, the mineralization of the benzene ring carbons of Bentazon in T-1 and T-2 of parabraun soil amounted to 1.7 and 1.9% of the radioactivity present in the soil at the beginning of this plant experiment, respectively. These were higher by a factor of 2 than that observed in T-0 where Bentazon had been freshly added to the soil (Fig. 1). If the results were to be expressed as percentage of ^{14}C-applied originally to the soils of T-1 or T-2 before the pre-incubation was started, the mineralization of aged Bentazon residues would be much highers as compared with T-0 containing the freshly added ^{14}C-Bentazon (Table 6). This result can be explained by the presumption that the soil microorganisms responsible for the degradation of the chemical had already adapted to the environment and Bentazon during the 105 days of pre-incubation, as was also observed by Kloskowski and Führ[8] in a similar experiment with ^{14}C-simazine. Especially, in the case of T-2, most of Bentazon (74.37%) was removed by the extraction to prepare a soil containing bound residue (Table 6). Therefore, the activity of microorganisms was not retarded by the high concentration of Bentazon. The high percentage of silt (85.3%) and clay (7.7%) in the parabraun soil could rule out the possibility of washing-out of the microorganisms during extraction. Meanwhile, in the case of Korean soil, the amount of $^{14}CO_2$ evolved in T-1 was by factors of 2 and 3 greater than those observed in T-0 and T-2, respectively. These results are different from those of parabraun soil. The smallest amount of $^{14}CO_2$ evolved in T-2 is believed to derive from the fact that the Korean soil has a higher percentage of sand (64.4%) than the parabraun soil (7.0%). Even if the Korean soil has a higher amount of carbon (4.11%) than parabraun soil (1.10%), which implies higher microbial activity, most of the microorganisms may have been washed out during the extraction with water, to the degree that the microbial activity was far less than that in T-0 where they just started to adapt to the environment. If this assumption is accurate, then it is not surprising that the greatest $^{14}CO_2$ evolution

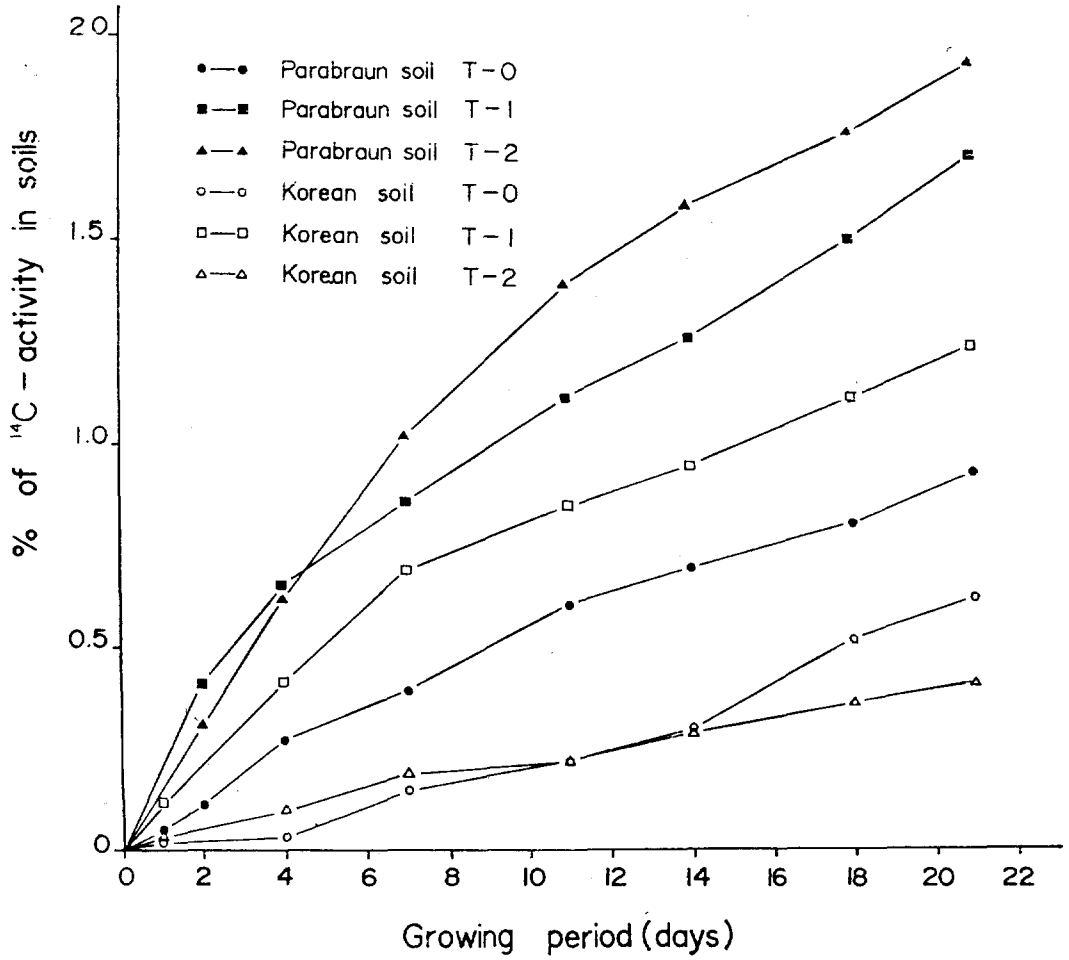

Fig. 1. Evolution of $^{14}CO_2$ during the growing period of maize plants. Radioactivity in soils at the start of the plant test=100

occurred in T-1 where the microorganisms had adapted to the environment during the pre-incubation period.

Desorption experiment with water

Table 7 shows the differences in the water-extractable ^{14}C of the three soil treatments after growing maize plants for 21 days. In T-0 where ^{14}C-Bentazon had been freshly added, the water-extractable ^{14}C was greatly reduced in both soils to 19.6~28.7% from almost 100% of the radioactivity prior to planting. However, considering the amounts of 36~43% taken up by maize plant, the water-extractable ^{14}C should be much greater, since the plant could absorb only the water-extractable or waterdesorbable ^{14}C. In T-1, the water-extractable ^{14}C was also decreased, but not so much as in T-0. The decrease of T-2 was far less than that of T-0 or T-1. Meanwhile, the distribution of the water-extractable ^{14}C between aqueous and ethyl acetate phase is noteworthy. In table 7, it is obvious that the radioactivity in the aqueous phase increases as a result of time plus the presence of maize plants. The increase in aqueous soluble material means that polar degradation products were formed during these periods.

Change in the non-extractable ^{14}C residues

The non-extractable ^{14}C residues formed in each treatment of the two soils prior to the plant expe-

Table 7. Comparison of water-extractable ^{14}C-from each treatment of the two soils between before and after maize planting.

^{14}C-activity before planting=100%

Soil	Treatment	Planting*	Maize plant uptake(%)	Water-extractable (%)	Distribution of ^{14}C after partitioning (%)	
					Aqueous phase	Ethyl acetate
Parabraun	T-0	B	—	100.07	1.13	98.94
		A	36.02	19.61	3.58	16.03
	T-1	B	—	41.71	6.46	35.25
		A	14.21	15.12	5.73	9.39
	T-2	B	—	16.77	0.72	16.05
		A	2.28	11.65	4.38	7.27
Korean	T-0	B	—	104.15	1.79	102.36
		A	42.82	28.69	5.69	23.00
	T-1	B	—	29.29	4.45	24.84
		A	8.24	17.94	4.89	13.05
	T-2	B	—	17.71	1.53	16.18
		A	1.79	16.38	3.67	12.71

* B : Before planting
A : After planting

Table 8. Change in the non-extractable ^{14}C applied as ^{14}C-Bentazon and formed in each treatment of the two soils after 21 days of maize plant growth.

^{14}C-activity before planting=100%

Soil	Treatment	Planting*	Non-extractable bound residue(%)	Fulvic acid (%)	Humic acid (%)	Humin (%)
Parabraun	T-0	B	2.38	1.09	0.66	0.92
		A	29.95	9.77	5.60	14.39
	T-1	B	46.55	14.20	10.17	22.18
		A	56.09	17.70	12.70	28.55
	T-2	B	81.81	23.71	17.85	38.46
		A	78.24	21.90	16.37	35.65
Korean	T-0	B	3.33	2.30	0.15	1.28
		A	23.27	10.14	5.55	10.90
	T-1	B	56.69	24.42	10.95	21.32
		A	62.27	22.54	13.93	25.48
	T-2	B	83.31	33.01	21.06	36.95
		A	73.76	26.43	18.23	29.10

* B : Before planting
A : After planting

riment increased during the growth of maize plants for 21 days (Table 8). In T-0 in both soils, the non-extractable ^{14}C increased by 19.9~27.6% during this period, whereas the increase was 9.5~11.9% in T-1. In contrast, in T-2 in both soils the non-extractable ^{14}C decreased by 3.6~9.6% from the level before planting.

This result indicates that the non-extractable ^{14}C applied as ^{14}C-Bentazon can be partially released from soil binding sites during the growing of plants. Apparently even after 105 days of incubation the fixation process of Bentazon in soil is still going on. The roots of the plant could take up part of the released ^{14}C-activity as evidenced in Table 7. The ^{14}C-activities distributed in fulvic acid, humic acid, and humin showed a similar tendency.

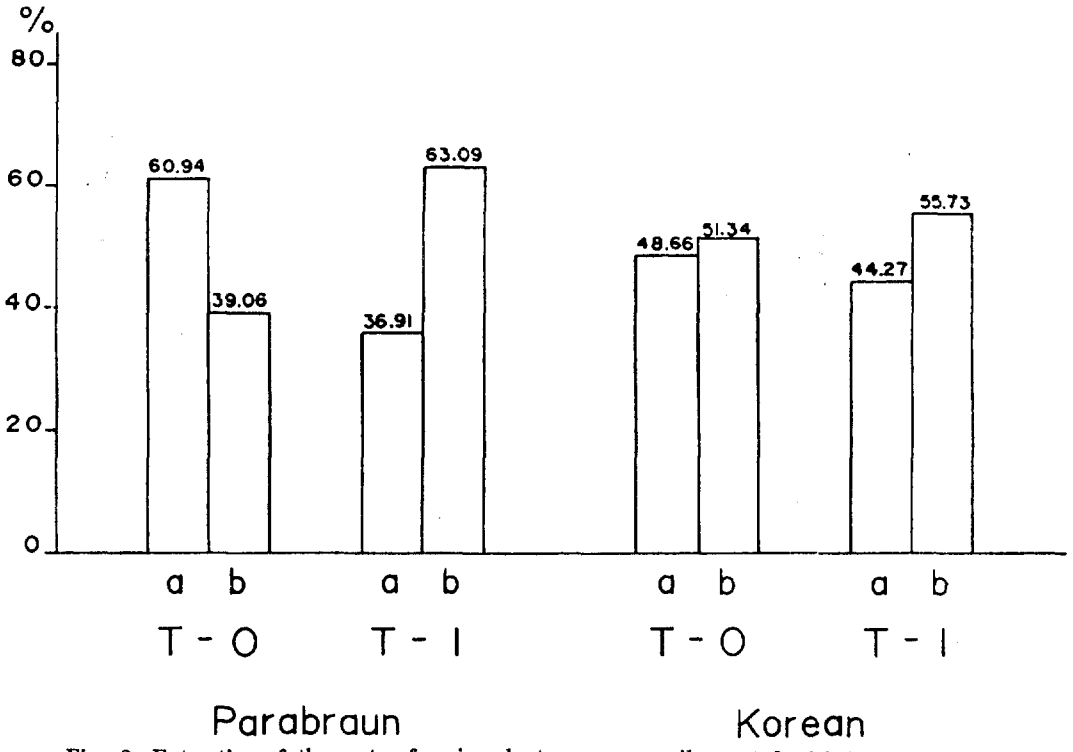

Fig. 2. Extraction of the roots of maize plant grown on soils treated with ^{14}C-Bentazon
Root : 1g
Solvent : MeOH (100ml×2)
a : Extracted (%)
b : Root-remaining(%)

Extraction of the roots of maize plants

Fig. 2 shows the results of the extraction of maize plant roots. The uptake of ^{14}C-radioactivity by maize plants in T-2 was so small that the sample from T-2 was not considered. In T-1. less radioactivity was extracted with MeOH than in T-0 for both soils. This result may be due, in T-1 soil, to the formation of polar products such as 6-hydroxy-Bentazon which had been incubated previously for 105 days. The polar products absorbed directly from soil or formed in plant could be further metabolized and/or incorporated into higher molecular weight constituents such as lignin, cellulose, starch, and protein[13]. The fact that much of the radioactivity remained in the roots after extraction also indicates the formation of some conjugates. The metabolites present in the methanol extracts of the roots were not identified owing to the difficulties encountered in separating these very polar substances. Previously, Otto and Drescher[14] also reported water-soluble metabolites of ^{14}C-Bentazon in foliar and root-treated soybeans after 50 and 28 days, respectively. According to Mine et al.[15], the major metabolite in rice treated with ^{14}C-Bentazon was 6-(3-isopropyl-2, 1, 3-benzothiadiazin-4-one-2, 2-dioxide)-O-β-glucopyranoside. Again, Otto et al.[13] showed that the methanol extract of rice plants treated with ^{14}C-Bentazon contained polar conjugates of 6-hydroxy Bentazon.

要 約

Benzene 環이 균일하게 標識된 ^{14}C-Bentazon(5.02 mg/kg)을 재배직전에 처리한 독일 및 한국의 토양(T-0)에서 21일간 생육한 옥수수는 처리된 방사능의 36.0~42.8%를 흡수하였다. 반면 ^{14}C-Bentazon으로 처리하고 105일 동안 전배양(pre-incubation)한 토양(4.79~4.84mg/kg) (T-1)에서는 8.2~14.2%를 흡수하였으며 추출이 불가능한 잔류물을 만들기 위하여 105일 동안 전배양한후 증류수와 0.01 M $CaCl_2$ 수용액으로 방사능을 완전히 추출한 토양(5.56~7.95 mg/kg) (T-2)에서는 1.8~2.3%를 흡수하였다. 흡수된

방사능의 지상부와 뿌리간의 분포는 각각 2.7~9.7% 와 90.3~97.3%이었다. 옥수수 뿌리를 유기용매로 추출한 결과 T-0 에서는 39.1~51.3%, T-1 에서는 55.7~63.1%가 뿌리에 강력하게 흡착 또는 결합되어 있음을 알 수 있고 이것은 Bentazon 과 그의 극성대사물질들이 접합체의 형태로 뿌리에 존재함을 암시해 준다.

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