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Degradation of the Herbicide, TOK (2, 4-dichloro-4′-nitro diphenyl ether) in Soil

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除草劑 TOK의 土壤中 分解

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要 約

物理化學的 특성을 달리하는 청주토와 충주토에 除草劑 TOK를 一定 농도로 처리하여 담수상태로 一定期間 배양한 후 生成된 分解産物과 分解에 관여한 토양미생물에 관하여 硏究를 行한바 다음과 같은 結果를 얻었다.

1. TOK를 500ppm으로 청주토와 충주토에 처리하고 2, 4, 6個月間 30°C에서 배양시 主된 分解産物로 4-Chloro-4′-amino diphenyl ether, 2, 4-dichloro - 4′amino diphenyl ether(amino-TOK), N-[4′-(4-chlorophenoxy)] phenyl acetamide, 및 N-[4′-(4-Chlorophenoxy)] phenyl formamide의 順으로 生成되었다.
2. 上記 토양중에 상기와 같은 농도로 TOK를 처리하면 nitro基의 amino基로의 환원, 탈염소화, acetylation 및 formylation이 主로 일어나고 ether 結合의 붕괴는 일어나지 않았다.
3. pH가 보다 높고 토성이 Clay loam이고 C.E.C가 높은 충주토에서 TOK의分解는 더 용이 하게 일어났다.
4. 上記와 같은 변화가 생기면 TOK의 毒性은 현저히 감소되어 환경의 오염원으로서의 기능은 약화된다.
5. TOK를 처리한 토양으로부터 12균주의 細菌을 分離하였다.
6. 분리한 토양미생물의 순수배양액중에서 TOK를 배양시킨 결과 청주토에서 분리한 T-1-1균주는 거의 分解力이 없고 T-2-3 균주가 가장分解力이 우수한 것으로 보인다.
7. 분리한 균주에 의한 TOK의 分解는 主로 nitro 基의 amino基로의 환원이었다.
8. 충주토의 citrate buffer 추출액은 청주토의 citrate buffer 추출액보다 TOK를 amino-TOK으로 환원시키는 능력이 컸다.

Introduction

TOK(2, 4-dichloro-4′-nitrodiphenyl ether) is one of the most important herbicides being used in flooded rice fields in Korea. Nevertheless, the environmental fate of the diphenyl ethers including TOK has received relatively little attention. In general, substituted diphenyl ether compounds have excellent herbicidal activity and have been reported to function essentially as inhibitors of chloroplast noncyclic electron transport. The main site of action appears to be associated with light reaction II and the oxygen evolution pathway.[11]

Gutenmann et al[5] reported that 2, 4-dichloro-4′-amino-diphenyl ether(amino-TOK) was rapidly detected in rumen fluid as a metab-

olite of TOK in dairy cows. Crosby et al.[2] demonstrated that TOK was quickly photolyzed in water to 2,4-dichlorophenol and *p*-nitrophenol, and the nitro group also underwent both reduction(formation of amino×××TOK) and nucleophilic displacement by hydroxideions to give the corresponding phenol and nitrite ion.

Ichihashi[8] et al. compared the residual effects in soil of MO-338(2,4,6-trichloro-4′-nitrodiphenyl ether), TOK, and X-52 (2,4-dichloro-3′-methoxy-4′-nitro-diphenyl ether) by bioassay with barnyard grass and other weeds. They found that in enriched soil solution, the reduced amino derivatives formed from all these diphenyl ethers.

MO-338, TOK, X-52, and MO-500(2,4-dichloro-6-fluorophenyl-4-nitrophenyl ether) were compared by Kuwatsuka[9] as to their degradation in soils under flooded and upland conditions.

The fate of TOK in the foliage of some weeds was investigated by Hawton et al.[6] with the aid of C^{14}-TOK.

In the present investigation, as part of an effort to understand and elucidate what noxious materials can be formed in our environment from the applied chemicals, TOK was applied at a rather higher concentration of 500ppm to two Korean rice paddy soils with different physico-chemical properties and incubated for a certain period of time under flooded conditions for its metabolic products. The identities of the metabolites extracted from the soils were elucidated by means of TLC, GLC, and MS.

Materials and Methods

Sample Soils

The physico-chemical properties of Chong Ju and Chung Ju soils which were used are shown in Table 1.

Table 1. Physico-chemical properties of sample soils

Characteristics / Soils	Textural class	pH		Organic matter (%)	Available P_2O_5 (ppm)	Exchangeable (me/100g)				C.E.C. (me/100g)	Total nitrogen (%)
		H_2O (1 : 1)	1N-KCl (1 : 1)			K^+	Na^+	Ca^{++}	Mg^{++}		
Chong Ju	SiL	4.83	3.82	2.45	35	0.08	0.08	2.33	1.23	7.7	0.12
Chung Ju	CL	6.43	6.04	2.9	44.0	0.05	0.09	10.5	1.3	14.14	0.14

Purification of TOK[10]

TOK was supplied by the Rohm and Haas Chemical Company, as a technical grade and was purified by dissolving in ethanol and filtering by suction, the residue being discarded. The filtrate was evaporated to dryness on a rotary evaporator and the residue was dissolved in acetone and recrystallized from n-hexane. The compound was dissolved again in a mixture of benzene-chloroform(1 : 1, v/v) and column chromatographed using the benzene-chloroform as eluting solvent(Silica gel 0.2~0.5mm, 30~70mesh ASTM for column chromatography, Merck, Germany). With this column most of the colored material was removed. The eluates were collected,evaporated on a rotary evaporator and passed again through aluminum oxide column (Activity II-III, according to Brockmann, M. Woelm Eschwege, Germany). The eluates were evaporated on a rotary evaporator, dissolved in acetone, recrystallized from n-hexane, and washed with dilute hydrochloric acid and distilled water, and finally with n-hexane. More than 99% purity was obtained. The purity was confirmed by TLC,, GLC, and MS.

Preparation of amino-TOK[10]

Four milligrams of TOK were transferred

to a 50-m*l* round-bottomed flask, and 10m*l* of 95% ethanol, 10m*l* of distilled water, 2m*l* of 5N-hydrochloric acid and, 0.2g of zinc dust were added An air condenser was attached and the mixture boiled for 5 min. After cooling, the contents were transferred to a separatory funnel, made basic, and extracted with benzene. The benzene extract was evaporated to dryness in a rotary evaporator to give the amino-TOK crystals. The purity was confirmed by TLC, GLC, and MS.

Incubation of the soils treated with TOK

One hundred grams of wet sample soils and 50m*l* of distilled water were mixed up in 500 -m*l* Erlenmeyer flasks,respectively, to provide flooded conditions. Each 50 mg of TOK dissolved in a small amount of acetone was added to the soils to give 500 ppm concentrations. The mixtures were held at 30°C in an incubator for 2, 4, and 6 months, respectively. The control soils without TOK were held in like manner.

Extraction of soils treated with TOK

Incubated soil mixtures were extracted with five 60m*l* portions of methanol and four 60 m*l* portions of acetone continuously for complete recovery by agitating on a shaker for one hr each and centrifuging at 14,000g followed by filtration.

Thin-layer chromatography

TLC was performed with 5196494X, Art. 5554, DC-Alufolien, Kieselgel 60 F_{254} (25 Folien 20×20 cm), Schichtdicke 0.2mm, E. Merck, Darmstadt. A mixture of xylene-chloroform-acetone(40 : 25 : 35, v/v) was used as the developing solvent. The chromatograms were visualized under a UV lamp.

Gas-liquid chromatography

The analyses were performed with a Finnigan 9500 Gas Chromatograph equipped with a flame ionization detector. The column was a pyrex glass of 6mm(OD)×6ft packed with 5% SE-30 on Chromosorb W-HP. Operating parameters were as follows: helium carrier flow, 30 m*l*/min; oxygen, 400 m*l*/min; hydrogen, 40 m*l*/min; injector temp, 250°C; detector temp, 250°C; column temp was programmed from 90°C to 250°C at a rate of 6°C /min. The chart speed was 0.5 inch/min. The area under each chromatogram peak was measured with a planimeter. For GLC-MS, all analyses were made by temperature-programming from 100°C to 260°C at a rate of 10°C/ min. The other parameters were the same as above.

Mass spectrometry

Mass spectra were obtained by a combination of GLC-MS using a Finnigan 3200 Gas Chromatograph-Mass Spectrometer.The electron ionizing potential was 70 eV. A Finnigan MS Data System 6000 was used for the recordings of the mass spectra.

Isolation of microorganisms from TOK-treated soils

Microorganisms were isolated from Chong Ju and Chung Ju soils treated with 500 ppm of TOK, held in an incubator for two and four months, respectively, and extracted beforehand with organic solvents for collecting degradation products. The temporary designations are shown in Table 2.

Table 2. The designations of the isolates from the TOK-treated soils held for different incubation periods

Origin(soil)	Incubation period(month)	Designation
Chong Ju soil	2	T-1-1 T-1-2 T-1-3
	4	T-1-4 T-1-5 T-1-6
Chung Ju soil	2	T-2-1 T-2-2 T-2-3
	4	T-2-4 T-2-5 T-2-6

Since the isolates were selected and designated according to the morphological shapes of their colonies, some of them might be duplicated. The identification of the isolates was not intended in the present investigation.

Degradation of TOK in pure culture of

the isolates

Each 150 ml of the medium that has the composition shown in Table 3. put in a 500 ml incubation flask and inoculated with the isolates,was preincubated at 30°C for four days, respectively. Three milligrams of TOK dissolved in a small volume of acetone were added to each medium to provide a concentration of 20 ppm and incubated on a shaker for another 17 days. The incubated mixtures were extracted with two 60 ml portions of chloroform in a separatory funnel to recover the residual TOK and its metabolites. The chloroform ext racts were dried over anhydrous sodium sulfate and concentrated on a flash evaporator for TLC and GLC analyses.

Table 3. Composition of the medium used for the incubation of the isolates

Nutrient broth	10 g
Peptone	10 g
Sodium chloride	5 g
Distilled water	1,000 m*l*
pH	7.2

TOK-degradability of soil extracts

Preparation of citrate buffer: Buffer solutions of pH 4.83 and 6.43 were prepared with 0.1 M sodium citrate and 0.1 N hydrochloric acid. Chong Ju soil was extracted with the buffer of pH 4.83 and Chung Ju soil with pH 6.43.

Incubation of soils: One hundred grams of Chong Ju soil and 100 ml of the citrate buffer with pH 4.83 and 100 g of Chung Ju soil and 100 ml of the citrate buffer with pH 6.43 were incubated in duplicate at 30°C for 11 days. After incubation, the mixtures were centrifuged and the supernatants were collected through filtration as soil extracts.

Incubation of TOK with soil extracts: To 25 ml of each soil extract, 1 mg of TOK dissolved in a small amount of acetone was added to give a concentration of 40ppm. It was then incubated at 30°C for 21 days.

Extraction of degradation products: The incubation mixtures were extracted with two 50 ml portions of redistilled chloroform and the extracts were concentrated on a flash evaporator for TLC and GLC analysis.

Results and Discussion

Degradation of TOK in soils

The organic solvent extracts of Chong Ju and Chung Ju soils treated with TOK were subjected to TLC, GLC and MS. On TLC, the Rf values of TOK and amino-TOK were 0.96 and 0.70, respectively.The Rf values of the other metabolites could not be compared, because the authentic compounds were not available. The gas-liquid chromatograms of the solvent extracts of Chong Ju and Chung Ju soils treated with TOK and incubated for six months are shown in Fig. 1 and 2, respectively. On GLC, the retetnion times of the metabolites identified are shown in Table 4.

The identification of the metabolites of TOK was mostly done by means of GLC-MS. Since the gas-liquid chromatograms of the samples showed almost the same metabolites, the solvent extract of Chung Ju soil treated with TOK and incubated for two months

Table 4. The metabolites obtained from the soils treated with TOK

Metabolite	Retention time(min)
4-chloro-4′-amino diphenyl ether	17.6
2,4-dichloro-4′-amino diphenyl ether(amino-TOK)	20.1
2,4-dichloro-4′-nitrodiphenyl ether(TOK)	21.4
N-[4′-(4-chlorophenoxy)] phenyl formamide	23.6
N-[4′-(4-chlorophenoxy) phenyl acetamide	24

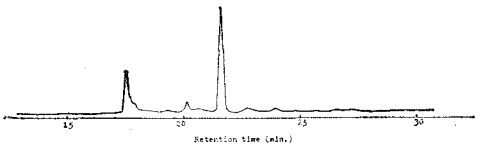

Fig. 1. Gas-liquid chromatogram of the solvent extract of Chong Ju soil treated with TOK and incubated for six months

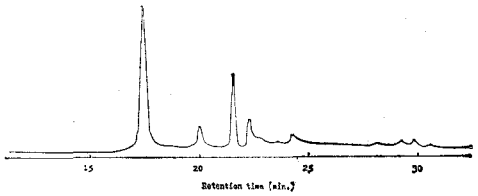

Fig. 2. Gas-liquid chromatogram of the solvent extract of Chung Ju soil treated with TOK and incubated for six months.

was subjected to GLC-MS. Fig.3 shows the gas-liquid chromatogram for MS, indicating the relative amounts of the metabolites and the peak numbers obtained with the MS data system.

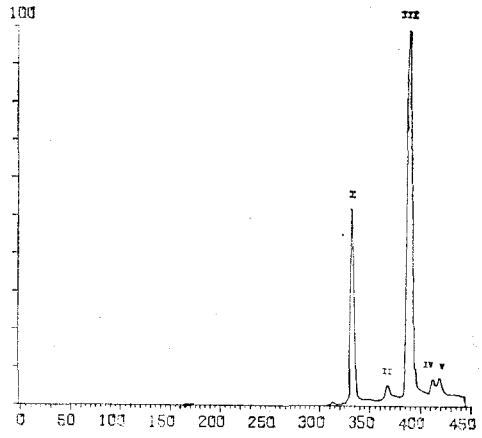

Fig. 3. Gas-liquid chromatogram for the mass spectra of the metabolities from Chung Ju soil treated with TOK and incubated for two months

The mass spectrum of each peak were obtained. The mass spectrum of metabolite I is shown in Fig. 4. As seen in Fig. 4, the molecular ion at m/e 219 and the M+2 peak showing the isotopic abundance suggest the chemical structure, 4-chloro-4'-amino diphenyl ether. The small peak at m/e 245 is thought

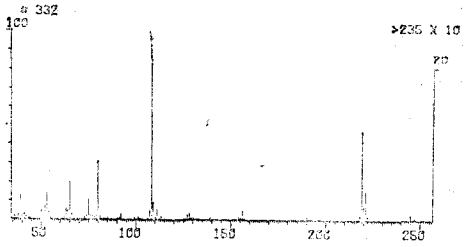

Fig. 4. Mass spectrum of metabolite I, 4-chloro-4'amino diphenyl ether

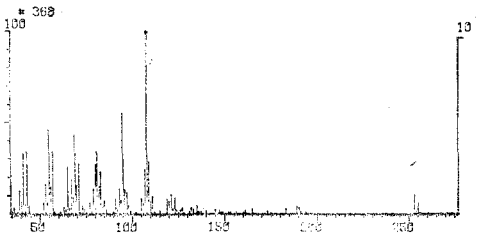

Fig. 5. Mass spectrum of metabolite II, amino-TOK(2,4-dichloro-4'-amino diphenyl ether)

to be an impurity. Fig. 5 shows the mass spectrum of metabolite II.

In Fig. 5, the molecular ion at m/e 253 and the istopic abundance ratio of the M+2 and M+4 peaks indicate the structure, 2,4-dichloro-4'-amino diphenyl ether(amino-TOK) possessing two chlorine atoms. Fig. 6 shows the mass spectrum of TOK which remains unchanged. The molecular ion appears at m/e 283.

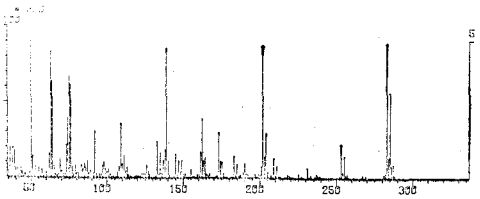

Fig. 6. Mass spectrum of metabolite III , the residual TOK

Fig. 7 is the mass spectrum of metabolite IV. In this spectrum, the molecular ion appears at m/e 247. The M+2 peak shows the presence of one chlorine atom. The suggested structure is N-[4'-(4-chloro-phenoxy)]phenyl formamide. Fig. 8 shows the mass spectrum of metabolite V. As seen in this spectrum,

the molecular ion at m/e 261 and the isotopic abundance at the M+2 peak suggest the structure, N-[4′-(4-chloro-phenoxy)] phenyl acetamide.

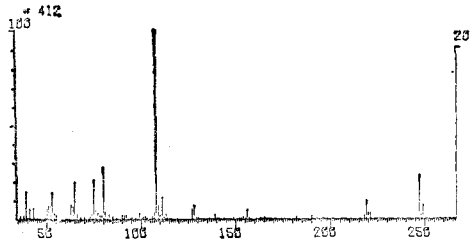

Fig. 7. Mass spectrum of metabolite IV, N-[4′-(4-chloro-phenoxy)] phenyl formamide

Fig.8. Mass spectrum of metabolite V, N-[4′-(4-chloro-phenoxy)] phenyl acetamide

The degradation of TOK in different soils as a function of the incubation periods is shown in Table 5.

Table 5. Comparison of the metabolites of TOK formed in soil with the residual TOK as an index as a function of the incubation periods, by means of GLC

Soil	Incubation period(month)	Metabolite*					
		I	II	III	III′	IV	V
Chong Ju soil	2	3.35	2.52	100			trace
	4	28.37	4.96	100			trace
	6	50.17	8.77	100			trace
Chung Ju soil	2	42.91	2.05	100		2.37	3.03
	4	62.96	23.93	100	4.62	trace	3.20
	6	287.72	28.94	100	32.89		13.15

* I : 4-Chloro-4′-amino diphenyl ether
II : 2,4-Dichloro-4′-amino diphenyl ether(amino-TOK)
III : 2,4-Dichloro-4′-nitro diphenyl ether(the residual TOK)
III′ : Unknown
IV : N-[4′-(4-chloro-phenoxy)] phenyl formamide
V : N-[4′-(4-chloro-phenoxy)] phenyl acetamide

As seen in Table 5, the degradation of TOK turns out to depend on the physico-chemical properties of the soils onto which the herbicide was applied. In Chong Ju soil, the major metabolites were 4-chloro-4′-amino diphenyl ether and 2,4-dichloro-4′-amino diphenyl ether(amino-TOK). Only trace amounts of N-[4′-(4-chloro-phenoxy)] phenyl acetamide were recognized. In other words, in Chong Ju soil, the replacement of one chlorine by hydrogen and the reduction of nitro group to amino group were the main features.

Meanwhile, in Chung Ju soil, the metabolites identified include 4-chloro-4′-amino diphenyl ether, amino-TOK, N-[4′-(4-chloro-phenoxy)] phenol formamide, N-[4′-(4-chloro-phenoxy)] phenyl acetamide, and some other unknowns, in addition to the residual TOK. The main features are also the replacement of one chlorine by hydrogen and the reduction of nitro group to amino group.

The degradation of TOK increases with incubation periods. Compared with the degradation in Chong Ju soil, TOK is more readily decomposed in Chung Ju soil as seen in Table 5.

In Table 1, the big differences between the two soils are that the pH's of Chong Ju and Chung Ju soils are 4.83 and 6.43, that the textural class silty loam and clay loam, that

the contents of Ca^{++} 2.33 and 10.5, and that the C.E.C. 7.7 and 14.14, respectively. Therefore, it is likely that the different rate of degradation of TOK in the two soils will be due to the different physico-chemical characteristics of the soils which, as a result, will affect the activity of the microorganisms involved in the degradation.

According to Kuwatsuka[9], the substituted dipenyl ethers, MO-338, TOK, X-52, and MO-500 produced their amino compounds only in flooded soils,the degradation was much slower under upland conditions than under flooded ones, and no amino derivatives were detected in soils under upland conditions. It was also shown that under flooded conditions, TOK and MO-500 were degraded much faster than MO-338 and X-52, and amino derivatives were the major metabolites in the earlier stages of incubation, whereas later, amino compounds were also metabolized to their compounds.

Based on our results, at a relatively higher concentration of 500ppm, TOK undergoes the reduction of nitro group to amino group followed by the replacement of one chlorine by hydrogen to give 4-chloro-4′-aminodiphenyl ether as the major metabolite in both soils.

In the light of the fact that the amounts of amino-TOK is less than those of metabolite I, 4-chloro-4′-amino diphenyl ether in all cases, it is quite evident that the ring chlorines will be replaced by hydrogen with time. The detection of metabolites IV and V indicates the formylation and acetylation of TOK in soils following the reduction of nitro group to amino group and the replacement of one chlorine by hydrogen.

In the metabolic studies of C^{14}-TOK in some weeds,Hawton et al.[6] concluded that at least two of the metabolites were lipid-TOK conjugates or TOK polymers and others may be formed by cleavage of TOK at the ether linkage. Similar results were obtained from another diphenyl ether, Fluorodifen(p-nitrophenyl-α,α,α-trifluoro-2-nitro-p-tolyl ether). Rogers[13] reported that soybean rapidly degraded Fluorodifen. That is, metabolism was indicated to involve limited reduction of the nitro substituents and rapid cleavage of the other linkage to form the corresponding phenolic derivatives.

Eastin[3] also suggested the reduction of nitro group to amino group and the cleavage of the ether linkage in Fluorodifen in susceptible cucumber seedlings.

In addition, Frear et al.[4] have shown that the diphenyl ether herbicide, Fluorodifen is readily metabolized in peas at the diphenyl ether linkage.

Bound residues of Nitrofen in cereal grains and in rice and wheat straw were also reported.[14,7] Gutenmann et al.[5] showed the amino-TOK as a TOK metabolite in rumen fluid of dairy cows. TOK can be also metabolized in vivo to the acetamide, 2,4-dichloro-1-(4-acetamidophenoxy) benzene.[1]

The principal metabolite of diphenyl ether fed to rabbits was 4-hydroxydiphenyl ether which was excreted mainly as a glucuronide. The derivatives 4-methoxy- and 4,4-dimethoxy diphenyl ether were converted to the corresponding hydroxy derivatives by the rabbit.[15]

In the photodecomposition of TOK, Nakagawa et al.[12] showed that TOK undergoes rapid cleavage of the ether linkage to form 2,4-dichlorophenol and p-nitrophenol. As other prominent products, they identified hydroquinone, 4-nitrocatechol, amino-TOK, 4,4′-bis(2,4-dichloro-phenoxy) azobenzene, and an artifact N-[4-(2′,4′-dichloro-phenoxy)] phenyl formamide. Other pathways involved the replacement of the ring chlorines by hydroxyl or hydrogen, ring hydroxylation, denitration by hydroxide prior to cleavage of the ether linkage, and possible intramolecular rearrangement of TOK to a biphenyl. Similar photolysis products of TOK were reported by Lee[10] at nearly the same time.

As reviewed above, even if the substituted diphenyl ether compounds could be cleaved at

the ether linkage in vivo or photochemically, in our experimental conditions in soil where somewhat higher concentrations of TOK were used, no noticeable metabolites resulting from the ether linkage cleavage were recognized. In the photodecomposition of TOK in aqueous suspensions under sunlight.Nakagawa et al.[12] reported the formation of N-[4-(2′,4′,-dichl-oro-phenoxy)] phenyl formamide as an artifact resulting from the methanol used.

However, in our investigation, acetylation and formylation during the degradation of TOK occurred in soils where no methanol was involved.

According to Yih et al.[16], in preemergence treatment, TOK is applied onto the surface of the soil. Incorporation of this compound into the soil drastically reduces its effectiveness as a herbicide.

Therefore, it is believed that the reduction of nitro group to amino group, dechlorination, formylation, and acetylation will bring about a decrease in the toxicity and effectiveness of TOK as a possible environmental contaminant.

Table 6. Comparison of the residual TOK and the metabolite, amino-TOK in pure culture of microorganisms isolated from the two soils

Origin (soil)	Designation of the isolates	Residual TOK(%)	Amino-TOK,metabolite(%)
Chong Ju soil	T-1-1	100	trace
	T-1-2	97.8	2.2
	T-1-3	98.92	1.08
	T-1-4	80	20
	T-1-5	41	59
	T-1-6	82.7	17.3
Chung Ju soil	T-2-1	97	3
	T-2-2	31.8	68.2
	T-2-3	0	100
	T-2-4	97.6	2.4
	T-2-5	30.4	69.6
	T-2-6	92	8

Degradation of TOK in pure culture of the microorganisms isolated

The GLC, results of the chloroform extracts of the incubation mixtures are presented in Table 6.

Since no metabolites other than amino-TOK were formed from TOK, the percentage was calculated from the combined total of TOK and amino-TOK. In the case of the isolate T-1-1, almost all TOK remained unchanged, whereas in the isolate T-2-3, amino-TOK was formed as much as 100%, no residual TOK remaining. These results indicate that the basic diphenyl ether moiety is thought to be quite stable to the microbial degradation. Even if the identities of the isolates are not disclosed at this time, they are thought to be all bacteria.

TOK-degradability of the soil extracts

Chong Ju and Chung Ju soils were extracted with the citrate buffers of pH 4.83 and 6.43, respectively, which are identical with those of the soils in use. TOK was added to the two buffers of pH 4·83 and 6.43 as controls and two soil extracts of the same pH, separately, to provide a conentraticon of 40 ppm and incubated. After 21 days of incubation at 30°C, the mixtures were extracted with chloroform and the concentrated extracts were subjected to GLC analyses.

Based on the GLC results, in the Chong Ju soil extract, the original TOK remained almost intact, whereas in the Chung Ju soil extract, 3.6% of amino-TOK was formed by the reduction of the nitro group on TOK. Still, 96.4% of the original TOK remained unchanged.

Even if it is not clear whether the transformation of nitro group to amino group is chemical or biological, it is of interest to note that Chung Ju soil with higher contents of organic matter, Ca, and C.E.C. besides higher pH than in Chong Ju soils, is more capable of degrading TOK than Chong Ju soil.

Abstract

TOK(2, 4-Dichloro-4′-nitrodiphenyl ether) was applied to two Korean soils possessing different physico-chemical properties at a cer tain concentration and incubated foı a certain time under flooded conditions. The metabolites and the soil microorganisms involved in the degradation of TOK are studied.

Chong Ju and Chung Ju soils treated with TOK at a concentration of 500 ppm and incubated for two, four, and six months at 30°C yielded 4-chloro-4′-amino diphenyl ether, 2,4-dichloro-4′-amino diphenyl ether(amino-TOK), N-[4′-(4-chloro-phenoxy)] phenyl acetamide, and N-[4′-(4-chloro-phenoxy)] phenyl formamide as the major metabolites. TOK underwent the reduction of nitrogroup to amino group, dechlorination, acetylation, and formylation. No cleavage at the ether linkage was recognized. TOK was more readily degraded in Chung Ju soil which is characterized by the higher pH (pH 6.43), clay loam in textural class, and the higher cation exchange capacity.

The toxicity of TOK as a possible environmental contaminant is expected to be considerably reduced as a result of the above degradation

Twelve strains of soil bacteria were isolated from the TOK-treated Chong Ju and Chung Ju soils.

As a result of the incubation of TOK in pure cultures of the isolates, T-1-1 strain isolated from Chong Ju soil had almost no degradability, whereas T-2-3 strain turned out to be the most potent.

The degradation of TOK by the isolates constituted mostly the reduction of the nitro group to amino group.

The citrate buffer extract of Chung Ju soil reduced TOK more readily to amino-TOK than that of Chong Ju soil.

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