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Degradation of Endosulfan in Different Soils by Indigenous and Adapted Microorganisms

A. Özyer, N. G. Turan, Y. Ardalı

***Abstract*—**The environmental fate of organic contaminants in soils is influenced significantly by the pH, texture of soil, water content and also presence of organic matter. In this study, biodegradation of endosulfan isomers was studied in two different soils (Soil A and Soil B) that have contrasting properties in terms of their texture, pH, organic content, etc. Two *Nocardia* sp., which were isolated from soil, were used for degradation of endosulfan. Soils were contaminated with commercial endosulfan. Six sets were maintained from two different soils, contaminated with different endosulfan concentrations for degradation experiments. Inoculated and uninoculated mineral media with *Nocardia* isolates were added to the soils and mixed. Soils were incubated at a certain temperature (30 °C) during ten weeks. Residue endosulfan and its metabolites' concentrations were determined weekly during the incubation period. The changes of the soil microorganisms were investigated weekly.

***Keywords*—**Endosulfan, biodegradation, *Nocardia* sp., soil, organochlorine pesticide.

I. Introduction

THE organochlorine pesticides are regarded as pollutants throughout the world. The indiscriminate use of these pesticides in agriculture, forestry and public health has left considerable residues and toxic metabolites in the environment [1]. Chlorinated organic pesticides are one of the major groups of chemicals responsible for environmental contamination. Many chlorinated pesticides are highly toxic to both human health and the environment [17]. Using organochlorine pesticides except endosulfan and endosulfan parathion-methyl were forbidden in Turkey but the effects of these pollutants are continuous.

Endosulfan is a chlorinated cyclodiene insecticide currently used throughout the world for the control of numerous insects in a wide variety of food and nonfood crops [17]. Endosulfan comprise two parent isomers, the α-, and β-endosulfan or endosulfan I and II, respectively. Because of such abundant usage and the potential for environmental transport, endosulfan contamination is found throughout the environment. Endosulfan isomers have a wide distribution in the environment and they have been detected in soils and sediments at considerable distances from the point of their original application [1], [14], [17].

Although the metabolites of endosulfan; endosulfan sulfate, endosulfan diol, endosulfan ether, endosulfan hydroxy ether and endosulfan lactone have been shown to occur; only the endosulfan sulfate metabolite is significant as a residue (Fig. 1).

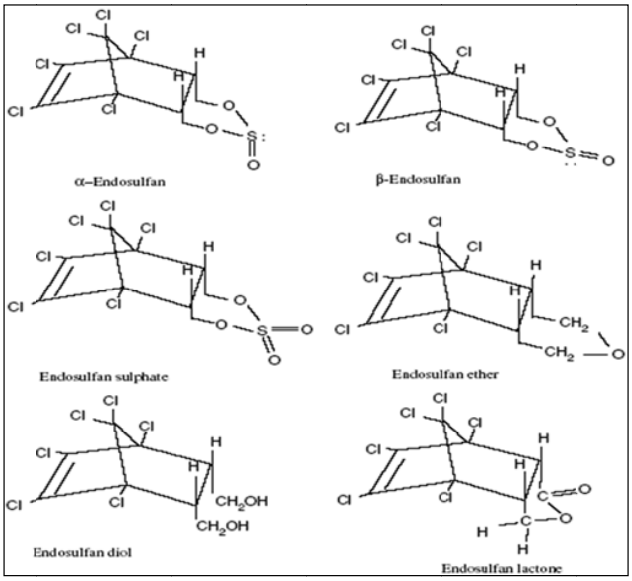

Fig. 1 Structures of endosulfan isomers and their metabolites produced during microbial degradation

Microbial degradation of endosulfan may play an important role in detoxifying the endosulfan-contaminated sites in the environment. Many bacteria and fungi such as *Corynebacterium* sp., *Nocardia* sp., *Mycobacterium* sp., *Pseudomonas fluorescens, Penicilium* sp., *Aspergillus* sp., *Phanerochaete chrysosporium* have been reported to be endosulfan degraders [2]-[4], [6], [7], [9]. Recent reports indicated that microbial conversion of endosulfan to endosulfan diol by hydrolytic pathway is a detoxification process whereas endosulfan sulfate was found to be a terminal degradation product [5], [11]. For the determination of the metabolic pathways and endosulfan degradation capacities of *Nocardia* sp., soil isolates were taken from the family of *Leguminaceace (Acasia dealbata* L.*)* and was from Ataş oil Refinery soil, Mersin.

Ayşe Özyer is with the Republic of Turkey Ministry of Environment and Urbanization, Ankara, Turkey (e-mail: arslana@omu.edu.tr).

Gamze Turan and N. Yüksel Ardalı are with the Ondokuz Mayıs University, Engineering Faculty, Environmental Engineering Department, Kurupelit, 55200 Samsun, Turkey (e-mail: gturan@omu.edu.tr, yuksel.ardali@omu.edu.tr).

II. Procedure for Analysis

A. Chemicals

The organochlorine pesticide endosulfan and its metabolites were purchased from Dr. Erhenstorfer GmbH. The pesticides and the metabolites studied include α-endosulfan, β-endosulfan, endosulfan sulfate, endosulfan diol, and endosulfan ether and endosulfan lactone. Commercial endosulfan was purchased from a local market. All other reagents used were of the highest analytical grade and were employed without further purification.

B. Soil Properties and Soil Studied

Soil A which contains clay 29%, silt 31%, sand 40%, organic matter 2.45% at pH 6.76 was taken from Kurupelit Campus of Ondokuz Mayis University and Soil B which contains clay 34%, silt 19%, sand 46%, organic matter 1.53% at pH 7.85 was taken from Atakum Region Collected soils were air dried at room temperature and sieved through a 2 mm screen. Solutions for batch tests with concentrations ranging between 1.25-5.00 gL^{-1} were prepared with commercial endosulfan (Korsulfan, 35%WP). 60 ml test solutions were added to 150 g of each soil and mixed thoroughly. After air drying for 24-48 h, the soils were pulverized and used for degradation studies.

C. Growing Bacteria

In this study, each of *Nocardia* isolates was grown separately in nutrient broth medium at 30 °C for a week. The cultures were transferred in proportion 10% to a mineral medium prepared as (KH_2PO_4, 2.3 g; Na_2HPO_4, 2.7 g; NH_4Cl, 1.0 g; $MgSO_4 \times 7H_2O$, 0.5 g; $NaHCO_3$, 0.4 g; $CaCl_2 \times 2H_2O$, 0.01 g; Fe(NH_4) citrate, 0.05 g; dissolved in 980 ml of distilled water and the medium pH was adjusted to 6.8).

D. Biodegradation of Endosulfan in Soil

Degradation of endosulfan was performed in a set of 500 ml Erlenmeyer flasks containing 150 g soil spiked with technical grade endosulfan dissolved in water. The specified concentrations of endosulfan were added to the set of all soil. The study was performed with the different endosulfan concentrations. As a result, 2.50 g/L, 2.50 g/L, 5.00 g/L, 5.00 g/L, 2.50 g/L and 5.00 g/L endosulfan solutions were added to the soils as namely 2A, 5A,7B, 10B, 3A and 8B, respectively. Inoculated and uninoculated mineral mediums were mixed with soil and incubated at 30°C. To set 2A and 7B inoculated 45 ml mineral medium containing *Nocardia isolate* EL025, and to set 5A and 10B inoculated 45 ml mineral medium containing *Nocardia isolate* EL045 were added and mixed. In soil 2A and 7B indigenous soil microorganisms and *Nocardia isolate* EL025 and in soil 5A and 10B indigenous soil microorganisms and *Nocardia isolate* EL025 degradation capacity of endosulfan were investigated. To investigate only the indigenous soil microorganisms' degradation capacity of endosulfan, 45 ml uninoculated mineral medium were added to the soils 3A and 8B.

E. Extraction and Analysis of Soil Applied Endosulfan

Soil samples were taken weekly for two months. Soil samples were prepared for sox let extraction by using USEPA Method 3500 and USEPA Method 3540. Each soil sample (5 g) was spiked with internal standards: 2, 4, 5, 6-Tetrachloro-m- xylene and decachlorobiphenyl. These standards were used to quantify the overall recovery of the procedures. Soil samples were sox let extracted for 16-24 hours with 200 ml hexane- acetone (1:1, v/v). The extracts were combined and concentrated to about 10 ml using a rotary evaporator and were evaporated to 1-2 mL in a water bath. Florisil clean-up (USEPA Method 3620) was employed to sample extracts. The cleanup and fraction were performed by passing the extract through a Florisil (20 g) column which had been activated. From this column three fractions were collected and all the fractions were concentrated about 1 ml. After concentration, endosulfan and its metabolites in the extract were determined by a GC/ECD system (Fisions HRGC Mega Series II).

F. Analytical Techniques

The instrument for gas chromatography was a Fisions HRGC Mega Series II with equipped with electron capture detector (ECD) and a DB-5 capillary column. The oven temperature was programmed for an initial temperature of 180 C (hold 2 min) and raised to 250 C (hold 2 min) at 10 min^{-1} and then finally raised to 270 C (hold 2 min) at 20 min^{-1}. The temperature of injector port and the detector block was maintained at 250 C and 300 C, respectively. Under these analytical conditions, the retention times for α-endosulfan, β-endosulfan, endosulfan sulfate, endosulfan ether, and endosulfan diol and endosulfan lactone were 10.85, 12.22, 13.27, 7.80, 8.35 and 9.81 min, respectively (Fig. 2).

III. Results and Discussion

Soil moisture content and soil temperature are the two most influential factors on the degradation rates and patterns of both α- and β-endosulfan isomers, as well as on the formations and dissipation of their primary degradation product endosulfan sulfate [12], [14], [15]. Our experiments were performed at optimum soil moisture content (70% for soil A, 55% for soil B) and optimum soil temperature (30 ˚C). The degradation of endosulfan in the soil was determined by monitoring its metabolites. A sequential identification of the primary and secondary metabolic products enabled us to elucidate the metabolic pathways utilized for the degradation of this pesticide. These results suggest that *Nocardia* isolates process two major enzymatic activities oxidation and hydrolysis that participate in the metabolism of this compound. Microbial degradation of both α-endosulfan and β-endosulfan were observed for ten weeks. Substantial (nearly 50%) degradation of the α-endosulfan was seen in two weeks for all soil samples except uninoculated soil 3A and almost in all of the soil samples 80% of endosulfan was the degraded by the tenth week.

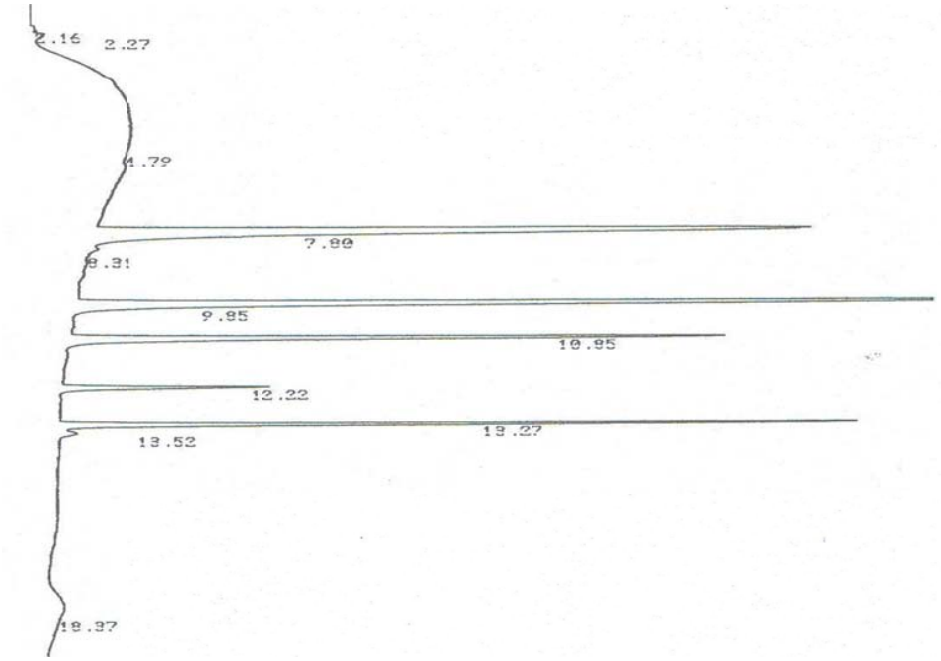

Fig. 2 Standard chromatogram of endosulfan and its degradation products

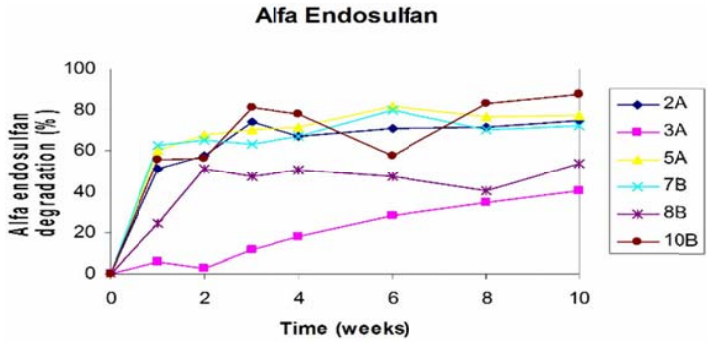

Fig. 3 α-Endosulfan degradation in all experiments during 10 weeks

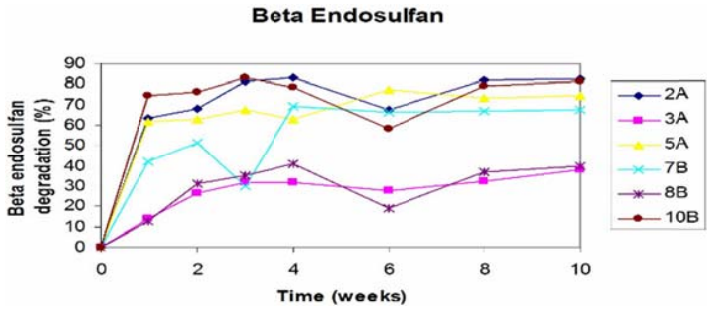

Fig. 4 β-Endosulfan degradation in all experiments during 10 weeks

The degradation of β-endosulfan is very fast in first 2 weeks for all soil samples like α-endosulfan and average half-life of β-endosulfan around 7 days. In soil 8B, nearly 30% of β-endosulfan degradation was observed. This is indication of absence of *Nocardia isolates* in the soil retard degradation of endosulfan. As shown in Figs. 3 and 4, the indigenous microorganisms did not play an important role in endosulfan degradation.

The microbial metabolism of endosulfan results in formation of various intermediate products before its complete degradation. The data obtained from gas chromatographic analyses were given in Tables I, II, and III for soil 2A, 7B, and 10B, respectively.

TABLE I
RESIDUAL α- ENDOSULFAN AND ITS METABOLITES IN SOIL 2A DURING TEN WEEKS OF INCUBATION (2,5 GL^{-1} ENDOSULFAN, *NOCARDIA EL025*)

Time (week)	α-Endosulfan (mg/kg)	β-Endosulfan (mg/kg)	Endosulfan sulfate (mg/kg)	Endosulfa ndiol (mg/kg)	Endosulfan lacton (mg/kg)	Endosulfan ether (mg/kg)
0	84,442	357,073	2,268	-	-	-
1	41,255	130,196	2,390	0,653	-	-
2	35,950	113,714	0,8003	0,872	1,195	0,811
3	21,734	66,591	3,268	5,128	2,024	1,248
4	27,542	60,010	1,254	18,754	7,703	1,340
6	24.595	115,593	11,863	50,622	7,811	1,013
8	24,017	64,243	12,702	276,221	6,465	1,238
10	21,082	63,045	14,775	383,934	8,306	1,732

TABLE II
RESIDUAL α-ENDOSULFAN AND ITS METABOLITES IN SOIL 7B DURING TEN WEEKS OF INCUBATION (5,00 GL^{-1} ENDOSULFAN, *NOCARDIA EL025*)

Time (week)	α-Endosulfan (mg/kg)	β-Endosulfan (mg/kg)	Endosulfan sulfate (mg/kg)	Endosulfan diol (mg/kg)	Endosulfan lactone (mg/kg)	Endosulfan ether (mg/kg)
0	163,736	326,838	-	-	-	-
1	61,199	189,523	0,517	1,118	2,191	1,277
2	55,921	157,999	9,713	32,985	3,084	1,912
3	59,563	228,500	17,976	87,098	6,381	2,243
4	53,805	99,854	4,487	129,546	1,962	5,468
6	33,035	110,42	16,907	179,756	7,784	6,127
8	48,091	107,309	16,651	190,510	7,125	9,239
10	45,257	105,415	17,002	186,321	10,163	10,105

TABLE III
RESIDUAL α- ENDOSULFAN AND ITS METABOLITES IN SOIL 10B DURING TEN WEEKS OF INCUBATION (5,00 GL^{-1} ENDOSULFAN, *NOCARDIA EL045*)

Time (week)	α-Endosulfan (mg/kg)	β-Endosulfan (mg/kg)	Endosulfan sulfate (mg/kg)	Endosulfan diol (mg/kg)	Endosulfan lactone (mg/kg)	Endosulfan ether (mg/kg)
0	183,712	559,310	0,137	-	-	-
1	81,689	142,446	0,304	35,627	2,477	1,328
2	79,873	134,329	1,045	67,014	1,856	1,421
3	34,272	95,347	2,088	100,038	0,616	1,404
4	39,469	118,989	3,318	134,230	2,671	3,366
6	78,459	233,511	5,860	179,094	4,106	5,087
8	30,563	116,871	6,105	287,653	5,980	10,763
10	22,242	105,154	6,663	301,269	8,453	18,765

The degradation of α-endosulfan and β-endosulfan was found as 75,034% and 82,34%; 77,78% and 74,3%; 72,36% and 67,75%; 87,89% and 81,19% for 2A, 5A, 7B and 10B soils, respectively. During the degradation, the formation of endosulfan sulfate, endosulfan diol, endosulfan lactone and endosulfan ether was 97, 94%, 88, 18%, 70, 7% and 92, 9% for soil 10B, respectively. In contrast, in the same type soil 8B the formation of endosulfan sulfate and endosulfan lactone was less than that of inoculated. In the present study, uninoculated controls retained 54% of α-endosulfan and 40% β-endosulfan in soil 8B after 10 weeks, indicating that little chemical degradation or volatilization of endosulfan had occurred.

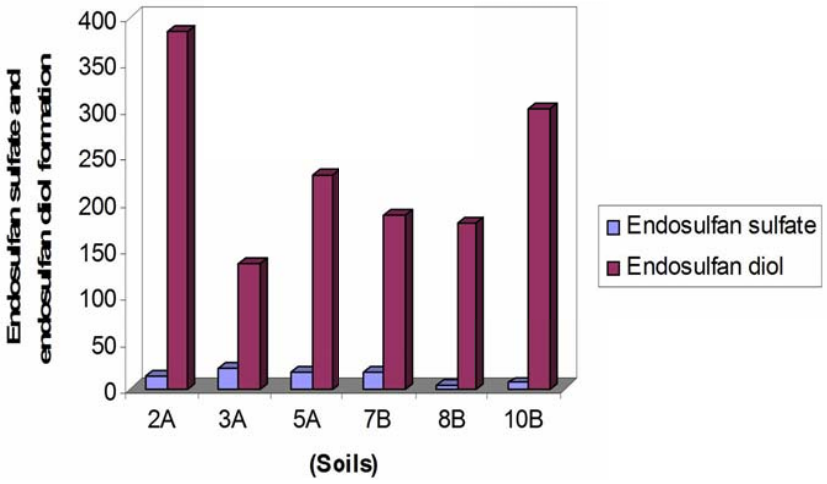

Fig. 5 Production of endosulfan metabolites endosulfan diol, endosulfan sulfate after ten weeks

As shown in Fig. 5, *Nocardia* isolates degrade α- and β-endosulfan to the non-toxic metabolite endosulfan diol. Endosulfan diol was the major degradation product in all soil experiments. Accumulation of endosulfan sulfates a metabolite known to be formed primarily by biological activity. In comparison to previous reports on the microbial metabolism of endosulfan, our results indicate that *Nocardia* isolates are unique in its capabilities to both oxidize and directly hydrolyze the parent form of this pesticide. While several bacteria and fungi have been shown to metabolize endosulfan, it has been determined most produce endosulfan sulfate as the major metabolic product and thus favor on oxidative rather than hydrolytic mechanism for metabolism of this pesticide. Soil bacteria are capable of metabolizing endosulfan and produce predominantly endosulfan diol, favoring hydrolysis [8], [10], [13], [16].

IV. CONCLUSION

The degradation of endosulfan was faster in soil A than in soil B, probably because of their different properties. During

the degradation, the formation of endosulfan sulfate was much higher than that of the formation of endosulfan diol. Under inoculated conditions, the degradation of endosulfan isomers was increased and this could be due to high bioavability of endosulfan. The results showed that *Nocardia* spp. can be effectively used for the treatment of endosulfan contaminated water and soil and they have valuable applications for endosulfan degradation in polluted sites.

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