

Detection of Endosulfan Residues in the Soil of Jordan Valley

By

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This thesis was successfully defended on 07/07/2004 and approved by

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DEDICATION

To my father, my mother, my brothers, and my sister, for
their encouragement with love and respect.

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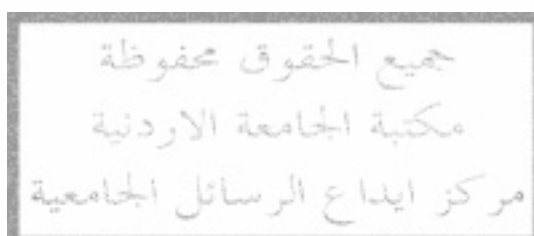
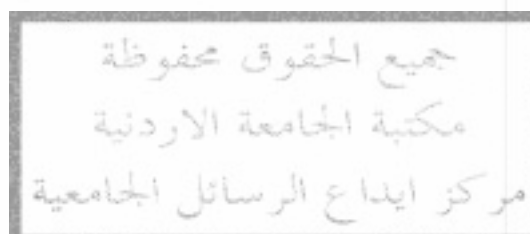


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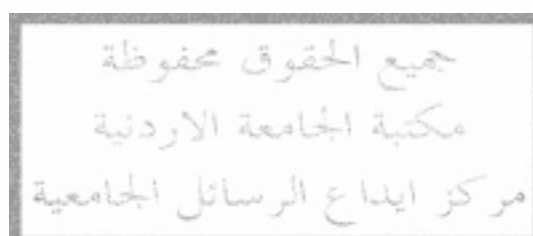
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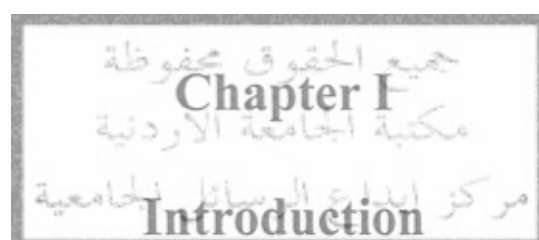
Abbreviations

GC/MS	Gas chromatography/mass spectrometry
ppb	Part per billion
ppm	Part per million
LD₅₀	Lethal Dose which kills 50% of treated organisms
LC₅₀	Lethal Concentration which kills 50% of treated organisms
α	Alpha
β	Beta
μ	Micron (e.g. μm = micrometer)
EPA	Environmental Protection Agency
CAS	Chemical Abstracts Services
EC	Electrical Conductivity
SG	Specific Gravity

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Detection of Endosulfan Residues in the Soil of Jordan Valley**By****Nader Mahmoud Eisa Sadeq****Supervisors****Dr.Yacoub Batta and Dr.Nidal Zatar*****ABSTRACT***

One hundred sixty soil samples were collected from ten separate locations in the Jordan Valley extending from Pardala (north) to Jericho city (south). The residues of the most widely used insecticide (endosulfan) in the collected soil samples at different depths (from 0 to 30 cm and 30 to 60 cm) were detected using gas chromatography/mass spectrometry. The endosulfan residues in the collected samples has been determined and compared . On the other hand, the endosulfan residues in the soil at the two depth levels has been determined in order to obtain a correlation between the pesticide residues and soil depth. Results have indicated that mean values of the pesticide residues (α and β -isomers) in the locations of northern part of Jordan Valley were higher than that in the southern part. For the same location, residues in the soil at 0 to 30 cm soil depth were always higher than that at 30 to 60 cm depth, significant differences in this respect were obtained. Comparison of the levels of total pesticide residues determined in the soil of Jordan Valley during this study with those conducted on the same pesticide in other countries demonstrated the presence of higher pesticide residue levels under our conditions, therefore the dose and frequency of pesticide application should be reduced.



1.1 Background

Jordan valley is considered the most important agricultural area in Palestine where the most important crops are grown, many agricultural insect pests are attacking these crops, so many farmers use pesticides in large quantities to control these insect pests.

Biodegradation of pesticides used for control insect pests may occur in the environment where these pesticides are applied. Different routes of pesticide metabolism may happen according to the type of pesticide and the prevailing environmental conditions. Some pesticides such as Lindane[®], Thionex[®], D.D.T. and many other organochlorine insecticides may remain undegraded in the soil for 7 years (Anonymous, 1991).

The long persistent pesticides may be transported from one place to another by wind and rain. Although they are not easy to be dissolved in water, some root crops such as carrot could absorb pesticides from soil and store them in the cortex or in other parts of fleshy roots (Anonymous, 1991).

Beside its ability to accumulate in the vegetative tissues, the intensive use of pesticides may cause the appearance of resistant strains of insects that don't be affected by pesticide treatment.

Some pesticides specially organochlorine insecticides cause environmental pollution when they are leached by rain to the ground water and pollute it, also these pesticides affect the environment because they contain recycled chemical compounds that increase chloride and nitrate concentrations in the environment.

Nowadays, pesticides are major sources of pollution to the soil and ground water because just about 0.05 % of them reaches the pests while the rest remains in the soil causing pollution to the environment (Anonymous, 1991).

1.2 Pesticides

In general, there are more than 865 active ingredients registered as pesticides in the world. They are formulated into thousands of pesticide products and are available in the market. Of these about 350 pesticides are used in food protection (Wasserman et al., 1982).

Pesticides may pose risk to human health and the environment if improperly handled. This risk could come from many of sources such as, direct exposure through improper use (U.S. EPA, 1986), by entering the body through indirect exposure through residual pesticides in food or inhalation and skin by contact pesticide for skin (U.S. EPA, 1995).

1.2.1 Chemical classification of pesticides

Pesticides include chemical and non-chemical types such as natural products (e.g. avermectin or vertamic[®]), cyclic or aromatic compounds (e.g. azocyclotin or peropal[®]), pyrothroid (e.g. cypermethrin or cymbush[®]), thiadiazin compounds (e.g. buprofezin or applaud[®]), metal organic compounds (e.g. aluminum phosethyle or aliette[®]), neretoxin derivatives (e.g. thiocyclam hydrogen oxalate or evisect[®]), diary tetrazine (e.g. clofentezine or apollo[®]), organic fungicide (e.g. metalaxyl or ridomil[®]), organic phosphate (e.g. malation or malathion[®]), and organichlorine compounds (e.g. endosulfan or thionex[®]) (Williams et al., 1988).

1.2.2 Hazard identification of pesticides

The first step in the risk assessment process is to identify the potential health effect that may occur from different types of pesticide exposure (Jorgensen et al 1991). Generally, for human health risk assessments, many toxicity studies are conducted by pesticide companies in dependent laboratories and evaluated for acceptability by EPA conditions (U.S. EPA 1990). EPA evaluates pesticides for wide range of adverse effects , from eye and skin irritation to cancer and birth defect , in laboratory animals. EPA may also consult the public literature or the sources of supporting information on any aspect of the chemical (Smith et al., 1991).

1.3 Pesticide used in the study (Endosulfan)

Looking at its chemical structure, endosulfan is an organochlorine insecticide that characterized by its strong hydrogen bond between molecules in sulfur group and its structure from benzene ring is characterized to be very stable (non degraded). This structure increases its persistence in the environment.

Endosulfan (benzadioxathiepin oxide) is a chlorinated hydrocarbon. Technical grade endosulfan is a mixture of two different isomers of the same chemical (α and β). These isomers are present in a mixture with a ratio of 70 % to 30 %, respectively (kimber et al., 1995).

Endosulfan sulfate is a reaction product found in technical endosulfan. It is also found in the environment due to photolysis and in organisms as a result of oxidation by biotransformation.

Endosulfan is available as an emulsifiable concentrate (9-34%), water dispersible powder or wettable powder (1-50 %), dust, granules, and as ultra-low-volume formulation (kimber et al., 1995). The wettable powder is frequently packaged in water soluble bags. It was registered under one of the following trade names: Thiodan[®], Phaser[®], Thionex[®], Fan[®], Cekulfan[®], Endocel[®], Endohan[®], Endosol[®], Endostar[®], Cyclodan[®], Thiomul[®], Derisulfan[®], Malix[®] and Insectophene[®] (Smith et al., 1991).

Endosulfan is characterized by a high level of persistence in the environment (Garabrant et al., 1984), and its ability to bioaccumulation in animal tissues, it is insoluble in water and usually highly soluble in fat. Endosulfan is a non-systematic insecticide, it has also an acaricidal effect by contact and stomach poison action. It is used to control aphids, thrips, beetles, foliar feeding larvae, mites, borers, cutworms, bollworms, bugs, whitefly and leafhoppers on citrus, deciduous small fruits, coffee, tea, fiber crops, ornamentals, tobacco and vegetables. It can also be used as a wood preservative and for control of tsetse fly (Carey and Douglas, 1971).

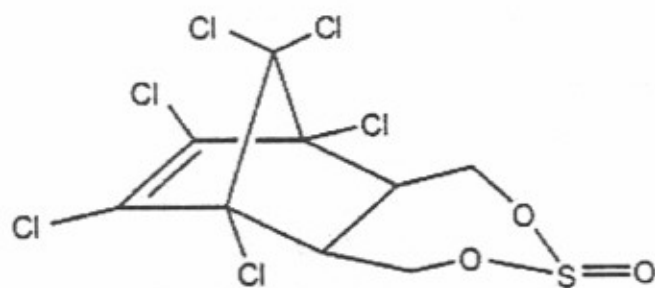
1.3.1 Chemical identification of endosulfan (Wasserman, et al,1982).

Chemical name: Endosulfan

Chemical formula: $C_9H_6Cl_6O_3S$.

CAS registry: 115-29-7

Chemical structure :



endosulfan
(115-29-7)
(stereochemistry unspecified)

6,7,8,9,10,10-hexachloro-1,5,5a,6,9,9a-hexahydro-6,9-methano-2,4,3-benzo(e)-dioxathiepin-3- oxide. Endosulfan technical; 5-norbomene-2,3-dimethanol-1,4,5,6,7,7-hexachlorocyclic sulfite.

1.3.1.1 Chemical identification of α -endosulfan

Chemical name: α -Endosulfan

Synonyms: Endosulfan I , Endosulfan A

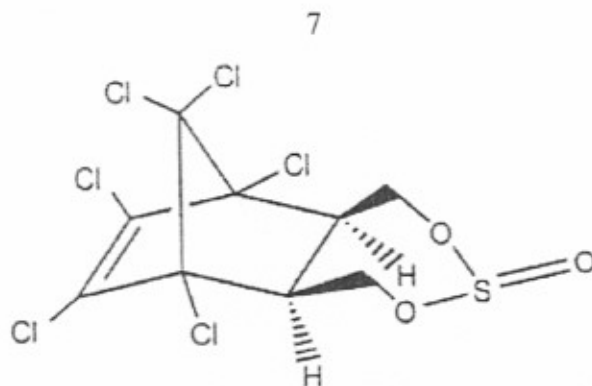
6,9-methano-2,4,3-dioxathiepin, 6,7,8,9,10,10-hexachloro-1,5,5a,6,9,9a-hexahydro-3- oxide (3 α , 5a β , 6 α , 9a α , 9 β) .

Registered trade name α -Benzoepin, α -Thiodan, Thionex[®].

Chemical formula: C₉H₆Cl₆O₃S .

CAS registry: 959-98-8

Chemical structure:



alpha-endosulfan

(959-98-8)

1.3.1.2 Chemical Identification of β -Endosulfan

Chemical name: β -Endosulfan

Synonyms: Endosulfan II, Endosulfan B

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6,7,9,10,10-hexachloro-1,5,5a,6,9,9a-
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hexahydro,6,9-methano-2,4,3- Benzodioxathiepin

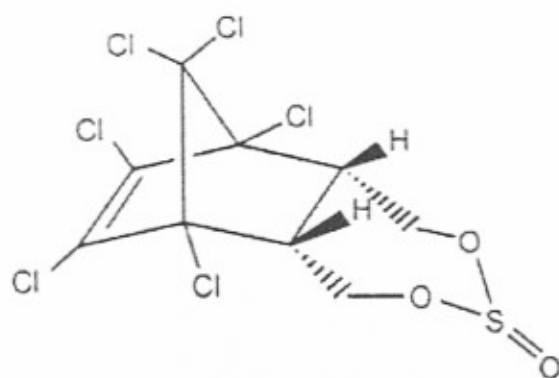
- 3 -oxide(3 α ,5 α ,6 β ,9 β ,9 α) .

Registered trade name: α -Benzoepin , α -Thiodan , Thionex[®] .

Chemical formula: C₉H₆Cl₆O₃S .

CAS registry : 33213-65-9

Chemical structure:



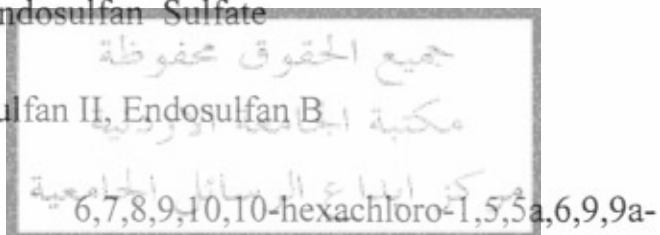
beta-endosulfan

(33213-65-9)

1.3.1.3 Chemical identification of endosulfan sulfate

Chemical name: Endosulfan Sulfate

Synonyms: Endosulfan II, Endosulfan B



hexahydro,6,9-methano-2,4,3- Benzodioxathiepin

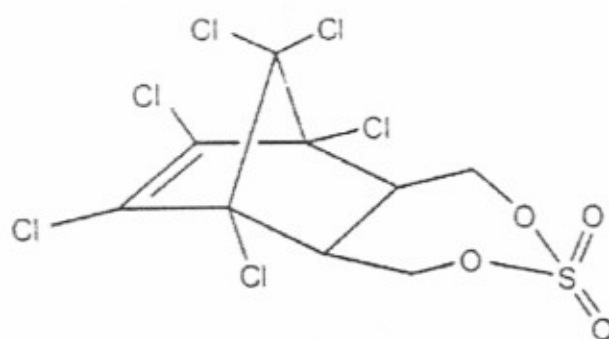
-3,3-dioxide .

Registered trade name: NO Data

Chemical formula: $C_9H_6Cl_6O_4S$.

CAS registry: 1031-07-8

Chemical structure



endosulfan sulfate

(1031-07-8)

1.4 Evaluation of overall environmental effects of endosulfan

Degradation of endosulfan in the soil and water may occur by photolysis, and by other chemical reactions. Biotransformation is governed by a wide range of climatic factors and the type of microorganisms present (Smith et al., 1991). Endosulfan is hazardous in acute overexposure for some aquatic species, especially fish. There has been large-scale field experience with endosulfan without any long-term adverse effects on the environment (Goebel et al., 1982).

Careful application to avoid overexposure of non-target organisms does not eliminate mortalities in local fish populations when endosulfan is applied to wet land areas at recommended rates. Because there is little or no biomagnifications, endosulfan when applied at recommended rates, is not hazardous to terrestrial animals (Buhler, 1989).

α -isomer dissipated more rapidly in the soil than the β -isomer. β -isomer is more strongly adsorbed on soil than α -isomer. The results of field studies confirmed that α -isomer has a shorter half-life time (60 days) than β -isomer (900 days) (Carey and Douglas, 1971).

Endosulfan sulfate (the major degradation product in the soil) accumulates at a rate comparable to the rate of loss of α and β -isomers of endosulfan. Endosulfan sulfate tends to be more stable than either of the two isomers, but none of the three compounds is subjected to leaching in soil because they are not soluble in water (Carey and Douglas, 1971).

The degradation of endosulfan, which was substantially reduced when the compound was incorporated into soil, halts during winter time. Increase the temperature up to 50 °C during summer time affect an increase in rate of degradation of endosulfan. Soil samples under variety of conditions also affect endosulfan degradation and demonstrates that the percentage of endosulfan diol is increased in the flooded soil samples and that a lower percentage of the sulfate is observed (Carey and Douglas, 1971).

Both α and β -endosulfan are fairly resistant to Photodegradation, but the two dominant by-products, endosulfan sulfate and endosulfan diol, are susceptible to photolysis. Technical endosulfan is sensitive to moisture, acids, alkaline condition and will undergo slow hydrolysis producing sulfur dioxide (SO₂) and endosulfan alcohol via the intermediate endosulfan sulfate (Goebel et al., 1982).

In the soil and on plant surfaces, endosulfan sulfate is the primary degradation product of endosulfan , with lesser amounts of endosulfan diol and endosulfan lactone being produced. Although sunlight may be involved in the initiation of sulfate production, it is felt that thermolysis was the principle formation mechanism. In aquatic environments (water and sediment), endosulfan diol was present together with smaller amounts of the sulfate and other compounds (Smith et al., 1991).

As a result of the non solubility of endosulfan in water it has the affinity for lipids that present in most related organochlorine compounds (Hudson et al.,1984). Consequently, biomagnification and accumulation of endosulfan in food chains. The typical response for most organisms exposed to endosulfan at below lethal levels, is to accumulate the compound up to a toxic level , but clear the residues fairly rapidly once the source of contamination is removed. The higher the exposure level, the longer it takes to reach a plateau or a toxic level and the higher the plateau is, this response was demonstrated in mussels, fish and algae (Pasivirta, 1991).

Endosulfan sulfate in generally is the only by-product detected in tissue of animals exposed to endosulfan. In cattle, the concentration factors were small (0.5 in milk, 0.05 in muscle tissue, and 0.15 in fat), and residues cleared quite rapidly when endosulfan was removed from the diet. Other diet studies have produced similar results in sheep and dogs. Up to our knowledge, no reports of endosulfan residues in human adipose tissue or breast milk are available (U.S. EPA, 1986).

In plants sprayed with endosulfan, initial residues on fruits and vegetables can vary from about 1 to 100 mg/kg; after 1 week, residues generally decrease to 20% or less of the initial amount (kimber et al., 1995).

1.4.1 Breakdown in soil

Endosulfan is moderately persistent in soil. The two isomers have different degradation times in soil. The half-life for α -endosulfan is 35 days, compared to 150 days for β -endosulfan under neutral conditions

(pH=7). The two isomers persist for longer time under more acidic conditions. Half-life in sandy loam soil is reported to be between 60 and 800 days(U.S. EPA, 1995).

The degradation rate depends on the pH of the soil, for instance the rate of degradation in alkaline condtions is higher that in acidic conditions (Carey and Douglas, 1971).

Jayakumar (2000)has analyzed three samples of soil for the presence of endosulfan using gas chromatographic methods of analysis. The obtained results showed the amounts of α and β -endosulfan residues were 3 mg/kg and 34.86 mg/kg in the first sample, respectively. The second sample showed an amounts of 1.53 mg/kg and 2.64 mg/kg, respectively while the third sample showed an amounts of 1.91 mg / kg and 19.79 mg / kg, respectively. The obtained results indicated that the persistence of β -endosulfan in the soil is more than α -endosulfan (Carey and Douglas, 1971).

Addition of endosulfan into the soil appears to reduce the rate of degradation of other organochlorine pesticides already present in the soil, either because endosulfan reduces the populations of microorganisms, or because of reduction of the activity of microorganisms responsible for degradation of the other organochlorines (U.S. EPA, 1995).

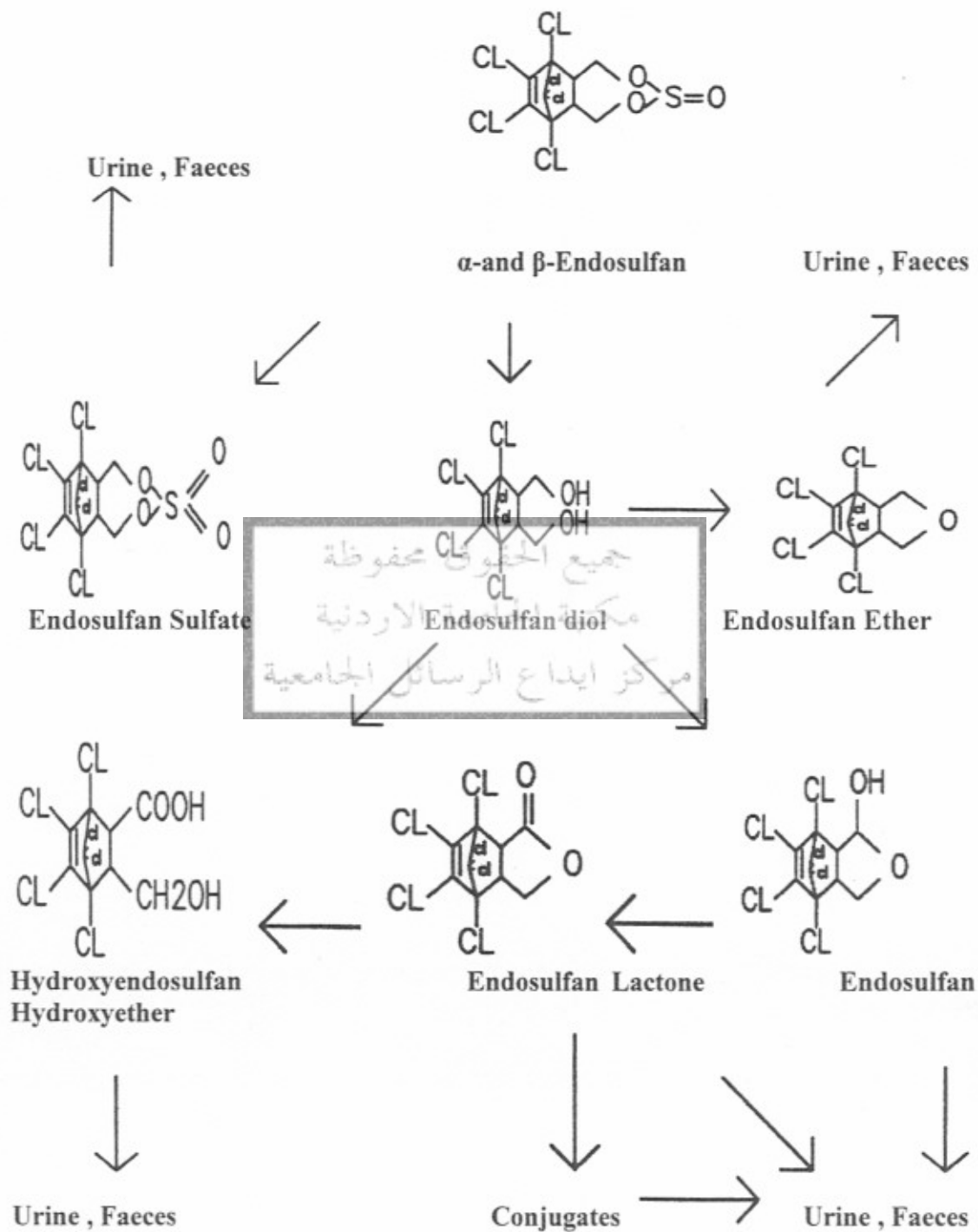
Endosulfan was found to be broken down in the soil by fungi and bacteria . It does not easily dissolve in water. It has a moderate capacity to adhere or adsorb to soil particles. It is not likely to be very mobile or to pose a threat to groundwater (Carey and Douglas, 1971).

Early work by Bayer company, indicated that α -endosulfan dissipated more rapidly in the soil than β -endosulfan, the results of field studies confirmed that α -isomer has shorter half-life (60 days) than β -isomer of endosulfan (900 days) .

1.4.2 Biotic degradation and bioaccumulation

Two biotransformation pathways have been described for endosulfan degradation in mammals. The first pathways involves hydrolysis and oxidation to various sulfur free metabolites, namely endosulfan diol, endosulfan ether, hydroxy –endosulfan ether, and endosulfan lactone which is also converted to endosulfan hydroxylactone in insects only, and polar conjugates (Wauchope et al., 1992). The second pathway involves the formation of various sulfur-containing metabolites namely endosulfan sulfate, endosulfan sulfuric acid ester and endosulfan bicarbonic acid . The proposed metabolic pathway is described in figure (1).

Figure1. Proposed metabolic pathway for endosulfan (Carey & Douglas, 1971)



1.4.3 Breakdown of endosulfan in vegetation

Endosulfan is often applied to crops using sprayers. It is usually breaking down within a few weeks. The degradation product (endosulfan sulfate) has been observed in several field studies involving plants. The sulfate is more persistent than the parent compounds (α and β -endosulfan) (Carey and Douglas, 1971).

On plant surfaces endosulfan sulfate is the primary degradation product of endosulfan with lesser amounts of endosulfan diol and endosulfan lactone being produced. Although sunlight and temperature may play a role in the reaction perhaps in starting the process by the initiation of sulfate production, on most fruit and vegetables, 50 % of the parent residue is lost within three to seven days. Endosulfan and its degraded products have been detected in vegetables (0.0005 – 0.013 ppm), initial residues on fruits and vegetables can vary from about 1 to 100 mg/kg. After one week, residues generally decrease to 20 % or less of the initial amount (kimber et al., 1995).

1.4.4 Breakdown in water

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Endosulfan contamination does not appear to be widespread in the aquatic environments but has been found in agricultural run-off and rivers in the industrial areas where it is manufactured or formulated. The estimated half-life of both isomers of endosulfan range from 4 to 15 days in river water (Williams et al., 1988).

In normal water (pH = 7), with normal oxygen saturation, the half-life was profoundly affected by pH and oxygen content. A drop in either of these two parameters inhibited endosulfan degradation under anaerobic

conditions. At (pH 7), the half-life decreases to approximately 5 weeks while at pH 5.5, the half-life was nearly 5 months . Studies on water samples taken from lakes and rivers showed that approximately 15 % of the samples contained endosulfan at levels ranging from 0.005 to 0.06 $\mu\text{g/L}$ (U.S. EPA, 1994).

In recent work in western Canada, endosulfan was found at a level of 0.011 $\mu\text{g/L}$ in one out of 1400 surface water samples, indicating that the water contamination by this insecticide is very limited (National Research Council of Canada, 1975).

1.5 Toxicological effects

Endosulfan is highly toxic to mammals, since the oral LD_{50} to rats is 40 – 50 mg/kg . The dermal LD_{50} range from 130 to 181 mg/kg . α -endosulfan has oral LD_{50} to rats 76 mg/kg, but the oral LD_{50} of β -endosulfan is 240 mg/kg. The inhalation LC_{50} is 350 mg/m^3 for male rats and 80 mg/m^3 for female rats when exposed for 4 hours (U.S.EPA,1994)

Endosulfan is highly toxic to aquatic fauna , both vertebrate (fish , amphibians), and invertebrate (molluscs , insects , gastropods) are susceptible with LC_{50} in the level of 1 ppb for many species (Jorgensen et al., 1991).

1.6 Geographical and climatological characteristics of soil in J.V.

According to the data obtained from Palestinian Ministry of Agriculture (2002), the soil in southern part of Jordan Valley is an organic soil and in the northern part of Jordan Valley is clay soil, The average temperature during summer is 31.52°C, while during winter it is 18.67 °C.

The average annual rainfall in northern part of J.V is 253 mm, while in southern part of J.V is 153 mm and the average annual relative humidity during the summer is 45%. The electrical conductivity of soil in northern part of Jordan Valley is 8 millmose/cm, while the electrical conductivity of soil in southern part of Jordan Valley is more than 10 millmose/cm. The specific gravity of soil in northern part of Jordan Valley at 17.5 °C is 2.7, while in southern Jordan Valley at 21.1°C is 2. The details of geographical and climatological characteristics of soil in J.V are shown in figure (3) and Table (1).

Figure2. Annual rainfall in Jordan Valley in the years from to 2002 (Palestinian Ministry of agriculture, 2002).

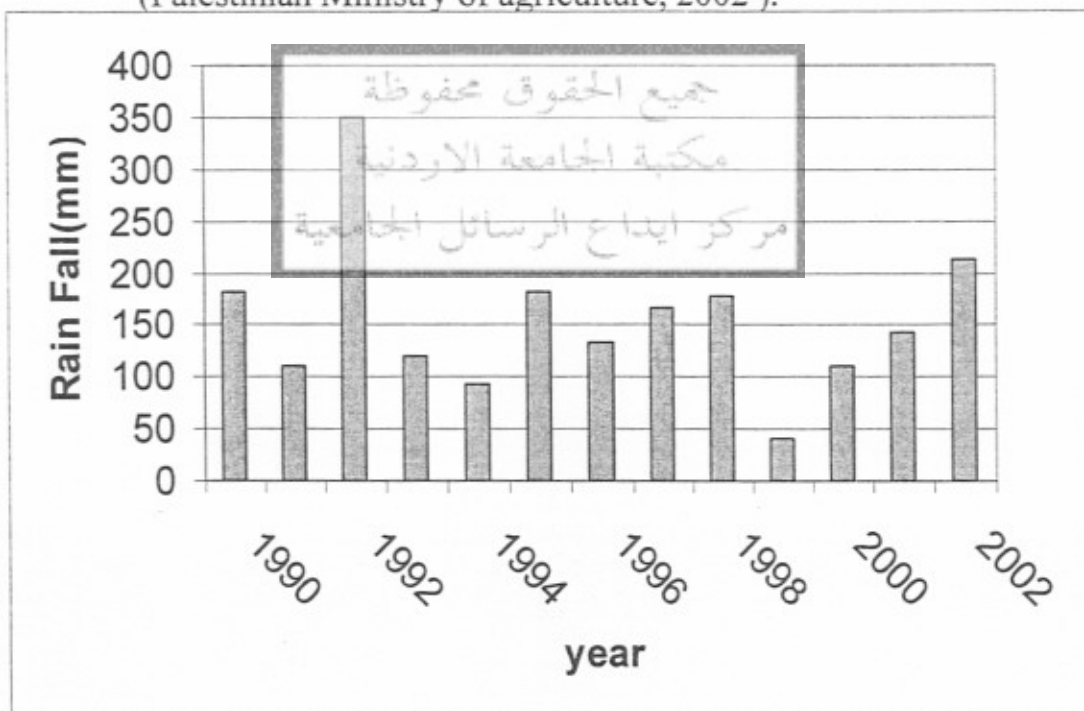


Table1. Geographical and climatological characteristics of Jordan Valley
(Palestinian Ministry of agriculture, 2002)

Location	Soil type	Average annual rainfall (mm)	Average annual temperature (°C)	Soil characteristics		
				EC	SG	pH
BARDALA	Clay	350	23	8	2.7	7.2
EIN AL BETA	Clay	350	23	8	2	7.8
MARJ NAJA	Organic	260	31.52	>10	2	7.8
AZ ZUBEIDAT	Organic	260	31.52	>10	2	7.8
MARJ AL GHAZAL	Organic	260	31.52	>10	2	7.8
AI JIFTLIK	Organic	150	31.52	>10	2	7.8
FASAYIL	Organic	150	31.52	>10	2	7.8
AL AUJA	Organic	150	31.52	>10	2	7.8
AN NUWEIMA	Organic	150	31.52	>10	2	7.8
JERICHO CITY	Organic	150	31.52	>10	2	7.8

1.7 Objective of the study

Due to the intensive use of the pesticide (endosulfan) especially in Jordan Valley to control many insect pests mentioned above this study aimed at determining the residual quantities of endosulfan in the soil important of this agricultural area.

Chapter II
جميع الحقوق محفوظة
مكتبة الجامعة الأردنية
Materials and Methods
مركز أبحاث الرسائل الجامعية

2.1 Equipment used in the study

2.1.1 Field equipment

- 1- Auger used for collection of the soil samples from different locations.
- 2- Polyethylene bags .
- 3- Sieve (U.S standard , 2 mm stainless steel)

2.1.2 Laboratory equipment

Soxhlet extraction apparatus , complete with 125-ml round bottom flask, siphon capacity 100 mL (33×80 mm thimble), and regulated heating mantle.

2.1.3 Gas chromatography/mass spectrometer (GC/MS)

The GC/MS system used during the present work consists of GC-17A gas chromatograph (shimadzu), QP 5000 mass spectrometer, AOC-17 autosampler, and data handling system. Capillary column: 30 m × 0.25 mm, 0.25 µm film DB-SMS (J&W Scientific).

Operating conditions : injection at 250°C, helium carrier gas at 1 ml/min at 25°C, split less injection. Temperature program : 100 °C, ramp at 5 °C/min to 320 °C then hold 10 min. at 320 °C. Endosulfan standard solutions and soil samples were analyzed by GC/MS in the selected ion monitoring mode.

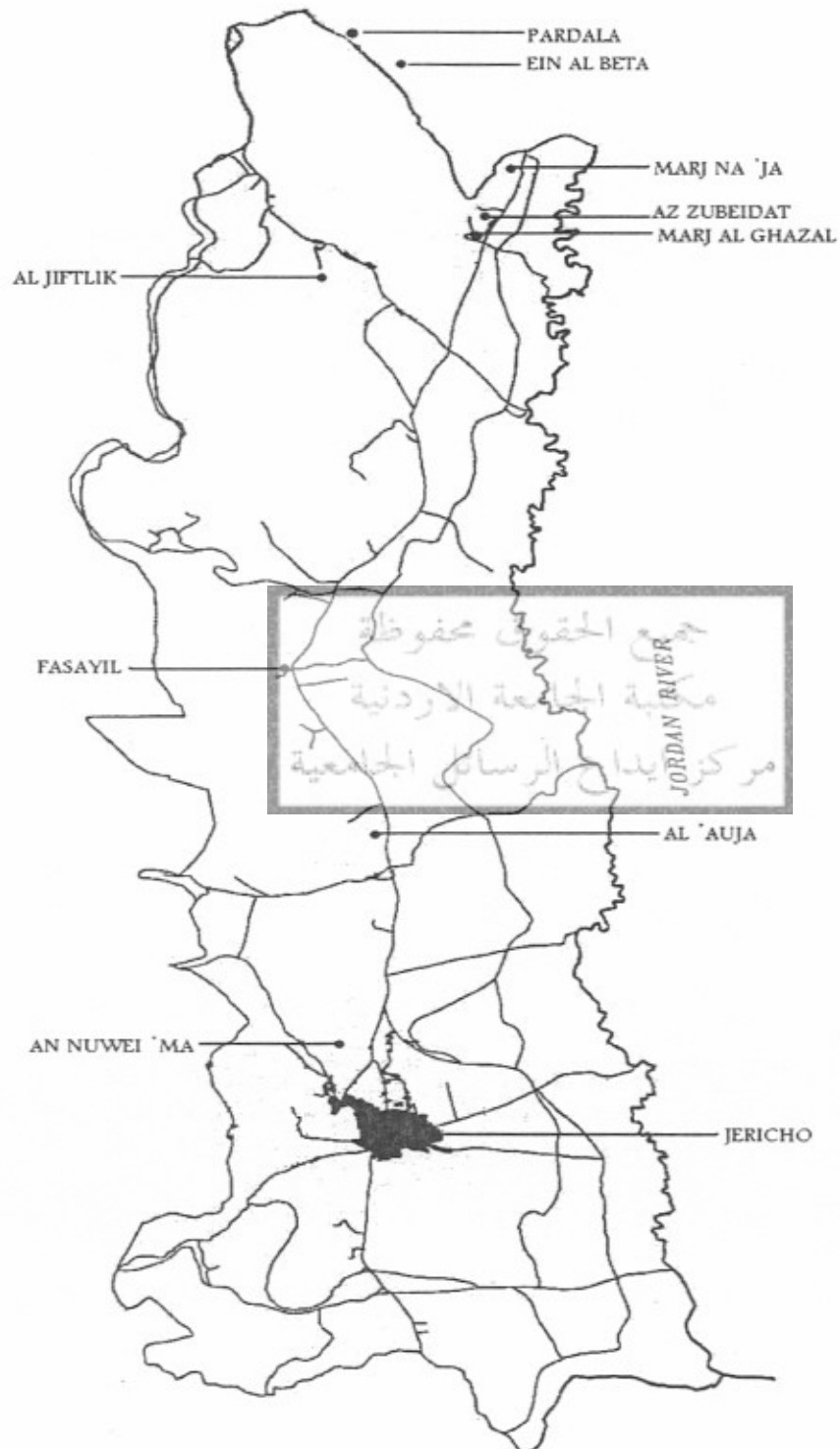
2.2 Soil samples used in the analysis

2.2.1 Source and type of soil samples

Soil samples were collected from ten locations in the Jordan Valley. These locations extend from northern part to southern part of the Jordan Valley, they include Ein-Albeda, Bardala, Marj-Naja, Marj-Al Ghazal, Az-Zubadat in northern part of Jordan Valley, and Al-Jiftlik, Fasayil, Al-Auja, An-nwema and Jericho city in southern part of Jordan Valley as shown in figure (2). The geographical area in which the locations are present is bordered by Nablus in the north, dead sea in the south, Jordan river in the east and Ramalla in the west.

The locations from which the soil samples were collected are situated in agricultural areas (cultivated with different types of crops). The farmers in this agricultural area usually apply large quantities of endosulfan (Thionex®) for control of many insect pests attacking cultivated crops. The formulation of pesticide used is the emulsifiable concentrate (EC).

Figure3. Locations in the Jordan Valley from which the soil samples were collected.



2.2.2. Collection of soil samples

Two types of soil samples were collected for analysis depending on the depth level of the soil. The first type of soil samples was collected at a depth level 0 to 30 cm , while the second types were collected at a depth level 30 to 60 cm. Eight samples of each type were collected from each location, they were mixed after collecting then grinded and sieved.

About 1 kg of sieved soil sample was kept in a polyethylene bag and then stored in the refrigerator at 2-5 °C till analysis for endosulfan residues is performed . The number of locations that were used in the present study were ten locations. The number of samples collected from each location were sixteen samples and the representative samples from each locations were two.

2.2.3 Preparation of soil samples for GC/MS analysis

Two grams of each soil sample were weighed out accurately, then placed on a filter paper, and then inserted into the extraction thimble after folding. Thimble was placed in soxhlet, supporting with spiral. a 100 mL of (1:1 acetone-hexane) mixture were transferred into the round-bottom flask, few anti bumping chips were added to the flask.

The sample was refluxed for five hours. The heat was adjusted so that extractor siphons approximately thirty time per hour. The flask was removed, and the solvent was evaporated on steam path. The residue then was dissolved in 2 ml dichloromethane and transferred into gas chromatography column for analysis.

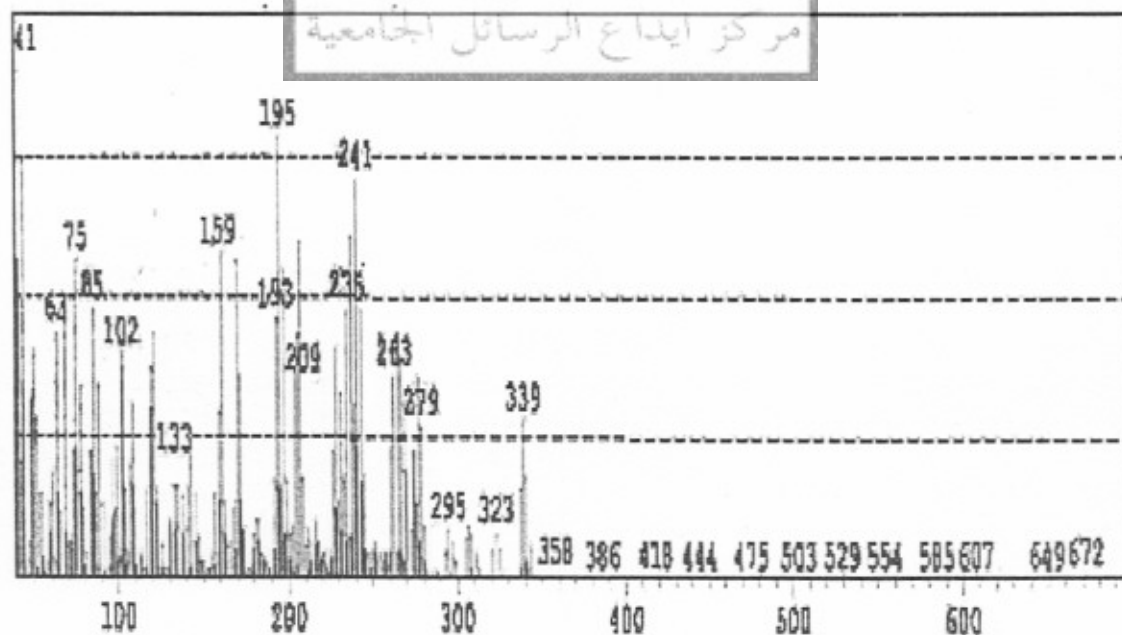
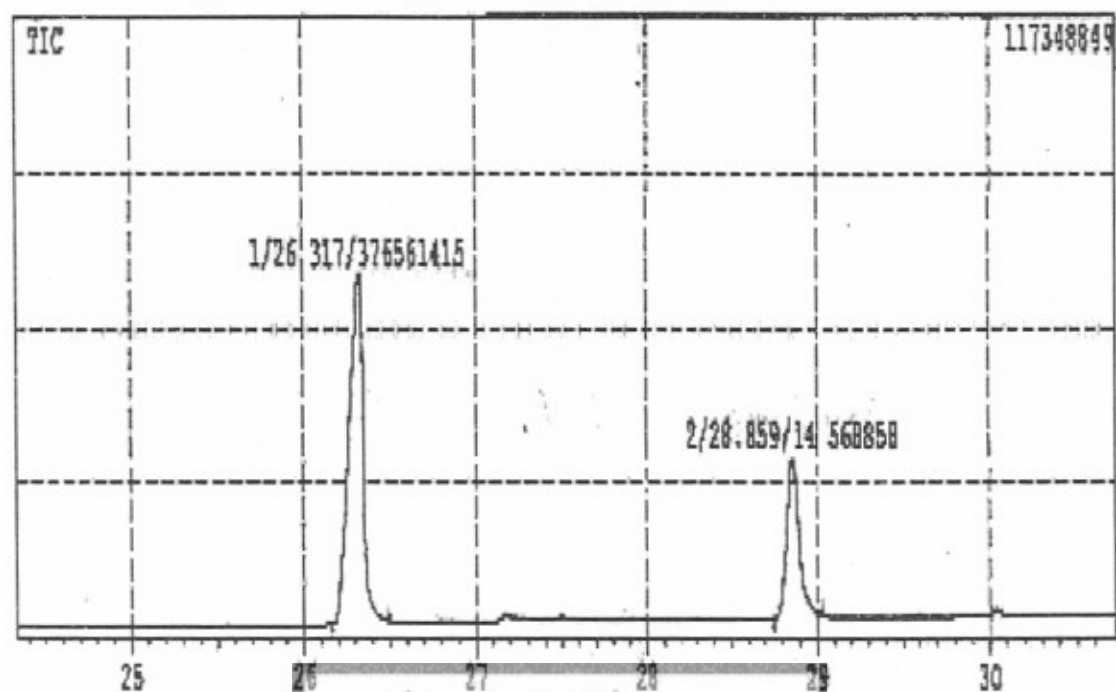
2.3 Preparation of endosulfan standard solution

1000 ppm standard solution of endosulfan was prepared by diluting 28.57 mL of endosulfan (original concentration = 35g/L) to one liter with hexane. Then 100 ppm solution was prepared by diluting 10 mL of endosulfan standard solution to 100 ml with hexane. The percentage of endosulfan concentration in the standard solution was 70% for α -endosulfan and 30 % for β -endosulfan.

2.4 Retention time of α and β -endosulfan

Two mL of 100 ppm standard solution of endosulfan containing α and β isomers were injected into the GC/MS column and then the retention time was monitored in the chromatogram. The results obtained are presented in figure (4). It can be seen that the retention time of α -endosulfan is 26.3 min., while that of β -endosulfan it is 28.8 min.. Figure (5) showed typical results for GC/MS in the selected ion monitoring mode for the soil sample contaminated with α and β -endosulfan

Figure4. GC/MS chromatogram of 100 ppm standard endosulfan solution analyzed according to the recommend procedure



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The readings monitored from gas chromatography/mass spectrometer after injection were used in calculation of α - and β -endosulfan residues in the soil samples collected from different locations.

The α and β -endosulfan in the soil samples were calculated using the following equation :

α -endosulfan residue (ppm) =

$$\frac{St_{\alpha} \times A_{\alpha}(\text{sample}) \times \text{Volume of extract (mL)}}{A_{\alpha}(\text{standard}) \times \text{Mass of soil sample (g)}}$$

B-endosulfan residue (ppm) =

$$\frac{St_{\beta} \times A_{\beta}(\text{sample}) \times \text{Volume of extract (mL)}}{A_{\beta}(\text{standard}) \times \text{Mass of soil sample (g)}}$$

Where :

St_{α} : Concentration of α -endosulfan in the standard solution (ppm)

St_{β} : Concentration of β -endosulfan in the standard solution (ppm)

$A_{\alpha}(\text{sample})$: Area under the peak of α -endosulfan in the soil sample extract.

$A_{\alpha}(\text{standard})$: Area under the peak of α -endosulfan standard solution.

$A_{\beta}(\text{sample})$: Area under the peak of β -endosulfan in the soil sample extract.

$A_{\beta}(\text{standard})$: Area under the peak of β -endosulfan standard solution.

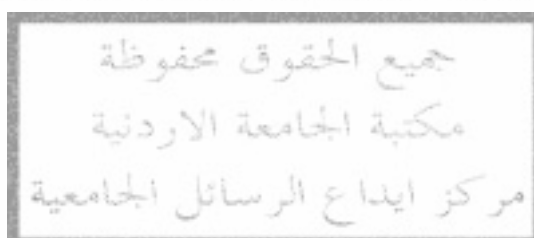
For example, for the residues of α -endosulfan of in Az-zubeidat location equal to:

$$\alpha\text{-endosulfan residue (ppm)} = \frac{.7 \times 7410 \times 2}{383 \times 2.267} = 11.943 \text{ ppm}$$

Residues of β -endosulfan at depth level 0-30 cm using similar steps as indicated above and considering the ratio of β -endosulfan in active ingredient 30 % was calculated in the same way as α - endosulfan.

2.6 Statistical analysis

Statistical analysis was performed using statistical package for social sciences (SPSS). The T-test was used to test the significance of each of the factors that was associated with the residues of endosulfan to find if there is a correlation between residue of endosulfan & depth level. All significance tests used were one-way and were considered statistically significant if the observed significance level (p value) was < 0.05 .



Chapter III

Results

جميع الحقوق محفوظة
مكتبة الجامعة الاردنية
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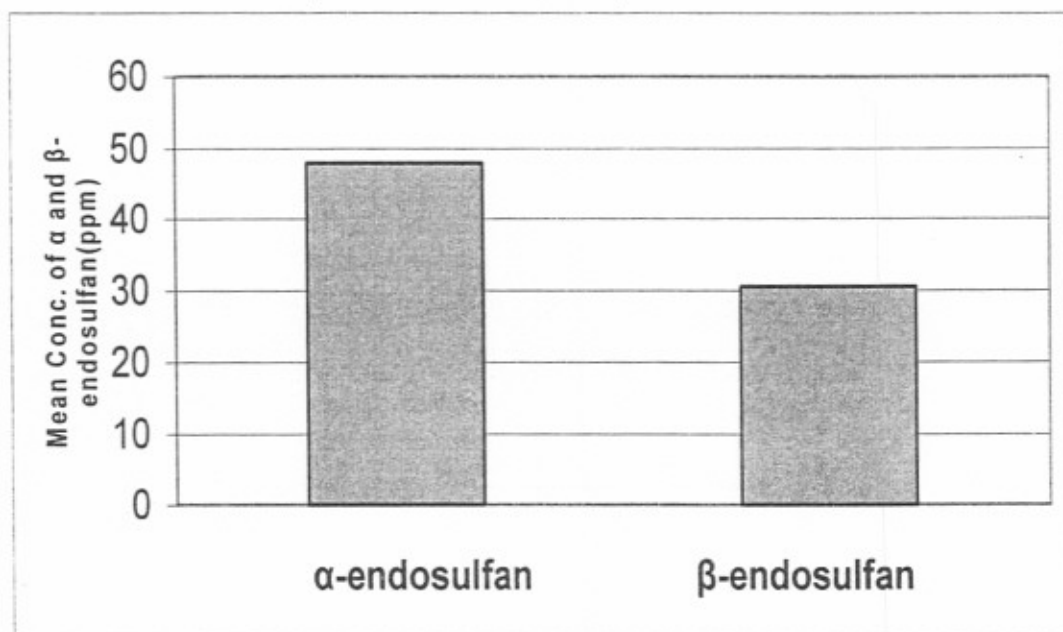
3.1 Residues of α and β -endosulfan in Jordan Valley soil at 0-30 cm deep

The obtained results are presented in Table (2) and figure (6) . Results indicate that α and β -endosulfan were detected in the soil samples of seven locations out of the ten locations subjected to our study. On the other hand, only α -endosulfan was detected in the soil of two locations while no endosulfan residues was detected in soil of Bardala.

Table2. Residues of α and β -endosulfan in the Jordan Valley soil at 0-30 cm and 30-6- cm deep for all locations

LOCATION	Concentration of endosulfan (ppm)			
	Depth level			
	0-30 cm	0-30 cm	30-60 cm	30-60 cm
	α - endo.	β - endo.	α - endo.	β - endo.
BARDALA	0	0	0	0
EIN ALBEDA	1.737	0	3.974	2.747
MARJ NAJA	120.134	50.829	63.769	35.852
AZ ZUBADAT	11.575	7.497	0	0
MARJ AL GHAZAL	6.838	10.738	6.855	0
AL JIFTLIK	7.554	0	5.598	0
FASAYIL	71.837	36.327	0	0
AL AUJA	185.378	85.456	16.924	6.710
AN NUWEIMA	51.071	40.082	0	0
JERICO CITY	23.528	20.817	15.995	7.385
TOTAL RESIDUES OF ENDOSULFAN	479.652	251.746	113.115	52.694

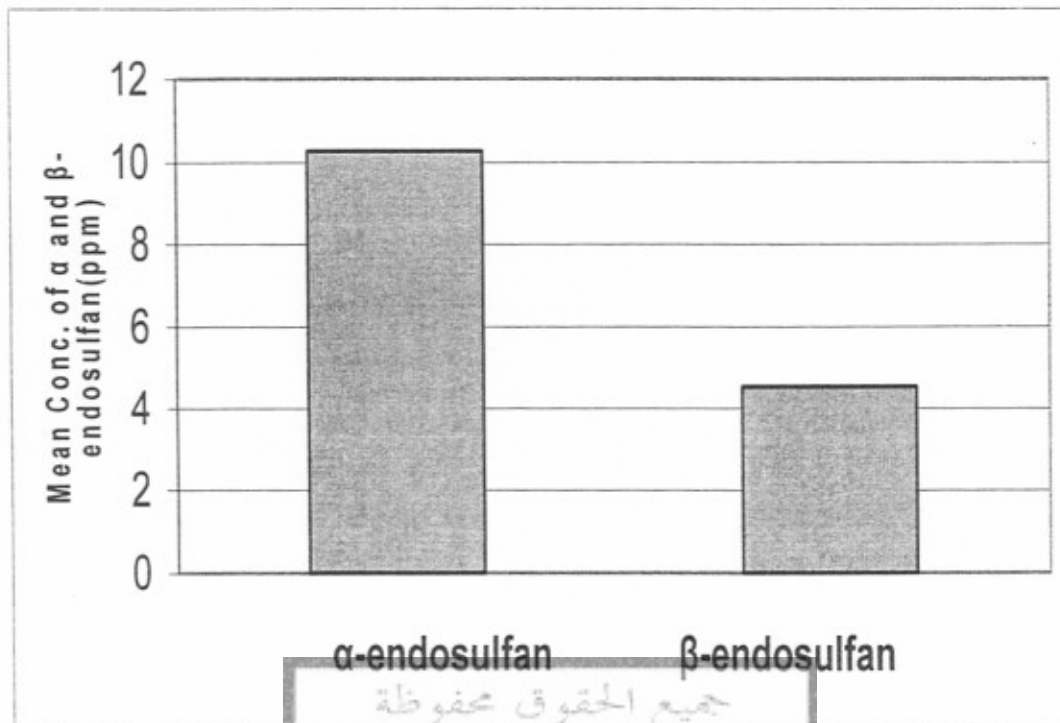
Figure6. Mean values of residues of α and β -endosulfan in all location of Jordan Valley soil at 0-30 cm deep



3.2 Residues of α and β -endosulfan in Jordan Valley soil at 30-60 cm deep.

The collected samples from the ten locations at a depth level 30-60 cm were subjected to the soxhlet extraction and GC/MS analysis for quantitative determination of α and β -endosulfan. The obtained results are presented in Table (2) and figure (7). It can be seen from the results that both α and β -endosulfan were detected in the soil samples of four locations out of the ten locations subjected for study. On the other hand, α -endosulfan was detected in the soil of two locations, while neither α nor β -endosulfan were detected in the soil samples of four locations out of the ten locations subjected for our study as shown in Table 2.

Figure7. Mean values of residues of α and β -endosulfan in Jordan Valley soil at 30-60 cm deep

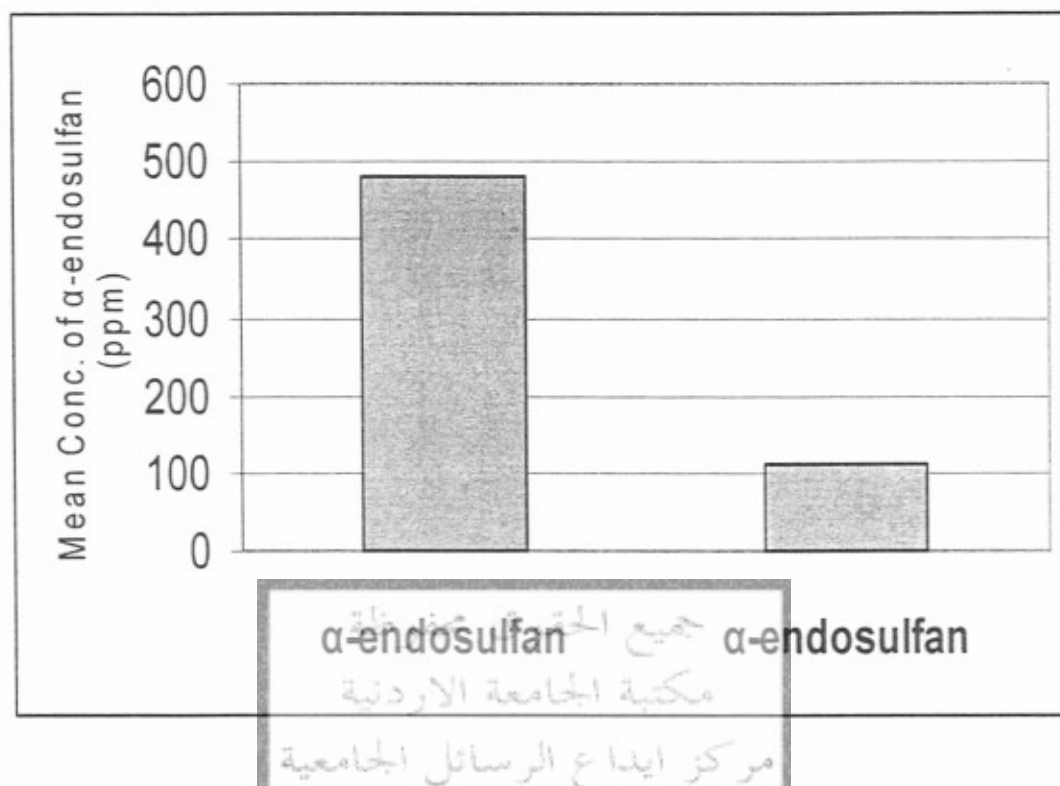


3.3 Comparison of the concentrations of α -endosulfan in soil samples collected at 0-30 cm and 30-60 cm deep .

The α -endosulfan residue in the soil samples collected from the ten locations at a depth level of 0-30 cm were compared with the residue in the soil samples collected at a depth level of 30-60 cm . The results are presented in Table (2) and figure (8). It is evident that the concentration of α -endosulfan at a depth level of 0-30 cm is generally higher than that at 30-60 cm with the exception of samples collected from Marj Al-Ghazal and Ein Al Bada. In the samples of soil of three locations α -endosulfan was detected at 0-30 cm depth level while no detection was found at 30-60 cm.

Finally α -endosulfan has not been detected in the soil samples of Bardala at the two depth levels.

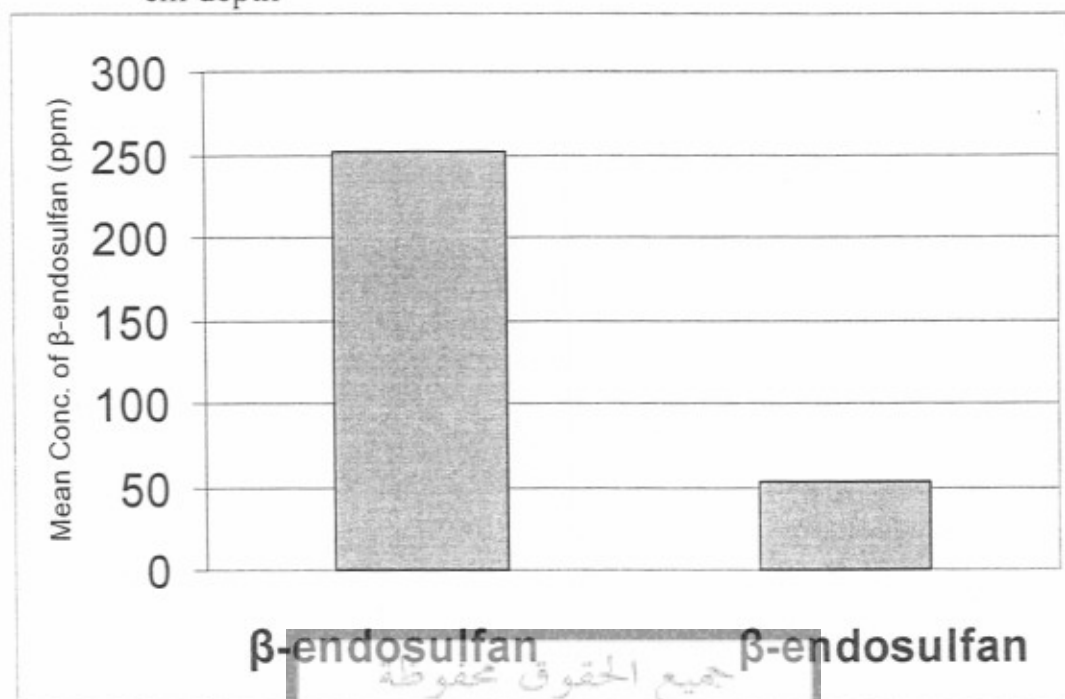
Figure8. Comparison of the mean values of concentrations of α -endosulfan in soil samples collected from all locations at 0-30 cm and 30-60 cm deep



3.4 Comparison of the concentrations of β -endosulfan in soil samples collected at 0-30 cm and 30-60 cm depth

The β -endosulfan residue in the soil samples collected from the ten locations at depth level of 0-30 cm were compared with those obtained for samples collected at a depth level of 30-60 cm. The obtained results are presented in Table (2) and figures (9). Results show higher concentration of β -endosulfan at a depth level of 0-30 cm in the soil samples of seven locations. In the soil of Ein Al-Beta, β -endosulfan has not been detected at 0-30 cm depth level, but it was detected (concentration of 2.747 ppm) at a depth level of 30-60 cm. The soil of Bardala and Al-Jiftilk location were found to be free from any contamination with β -endosulfan.

Figure9. Comparison of mean values of concentrations of β -endosulfan in soil samples collected from all locations at 0-30 cm and 30- 60 cm depth



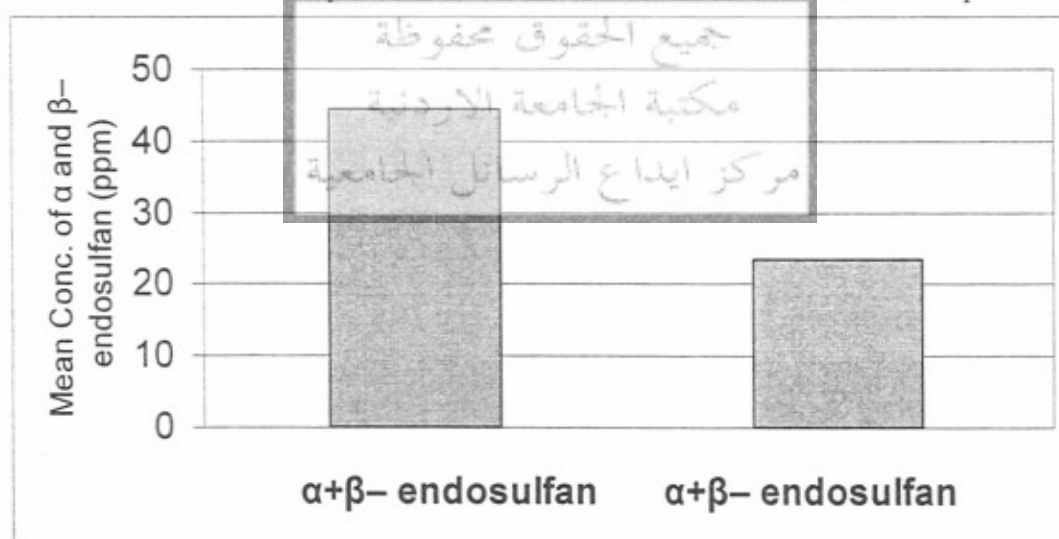
3.5 Comparison of the total concentrations of α and β - endosulfan in soil samples collected at 0-30 cm and 30-60 cm deep.

The total residue of α and β -isomers in the soil samples collected from the ten locations in Jordan Valley at 0-30 cm and 30-60 cm deep was determined using the recommend GC/MS method of analysis. The obtained results are presented in Table (6) and figure (10). Total α and β - endosulfan concentration at a depth level of 0-30 cm is generally much higher than that at 30-60 cm depth level, except the soil sample of Bardala which was found to be free from any contamination with endosulfan at the two depth levels. α and β -endosulfan was detected in the soil of three locations (Az-Zubidat, Fasayil and An Nuweima) at depth level of 0-30 cm while no detection was observed at a depth level of 30-60 cm of the same locations.

Table3. Comparison of the concentrations of α and β -endosulfan in soil samples collected at 0-30 cm and 30-60 cm deep

Location	Concentration of the total α and β -endosulfan (ppm)	
	Depth level	
	0-30 cm	30-60 cm
BARDALA	0	0
EIN AL BETA	1.737	6.721
MARJ NAJA	170.962	99.621
AZ ZUBEIDAT	19.072	0
MARJ AL GHAZAL	17.576	6.855
AI JIFTLIK	7.554	5.598
FASAYIL	108.164	0
AL AUJA	270.834	23.634
AN NUWEIMA	91.153	0
JERICO CITY	44.345	23.380
AVERAGE	73.1397	16.5809

Figure10. Comparison of the concentrations of α and β -endosulfan isomers in soil samples collected at 0-30 cm and 30-60 cm deep



3.6 Comparison of the total concentrations of α and β -endosulfan isomers in soil samples collected from locations of northern and southern parts of Jordan Valley

Comparison between α and β - endosulfan isomers or total endosulfan residues in the locations of north Jordan Valley with those of south Jordan Valley are presented in Table (4) and figures (11 and 12). The results show that the residues of total endosulfan isomers at depth level of

0-30 cm were higher in the locations of south J.V than in the locations of north J.V.(average concentration of α and β -endosulfan isomers per location = 104.1 ppm in south J.V and 41.87 ppm in north J.V). On the other hand, the total endosulfan residues at depth level of 30-60 cm were found to be higher in two locations of south Jordan Valley (Al-Jiftlik and Jericho City), while the residues were higher in two locations of north Jordan Valley (Ein Al-Beda and Marj Naja).The residues of total endosulfan have not been detected at 30-60 cm in two locations (Bardala and Az Zubidat)from north J.V and two locations (Fasyil and An Nuweima) from south J.V as shown in Table (7). The results show that the residues of total endosulfan residues isomers at depth level of 30-60 cm were higher in the locations of north J.V than in the locations of south J.V. (average concentration of α and β -endosulfan isomers per location = 22.639 ppm in north J.V and 10.522 ppm in south J.V).

Table4. Comparison of the concentrations of total endosulfan in soil samples collected from all locations of northern and southern part of Jordan Valley

Northern part of J.V			Southern part of J.V		
Depth level (Total endosulfan in ppm)			Depth level (Total endosulfan in ppm)		
0-30 cm	30-60 cm		0-30 cm	30-60 cm	
Locations	Total Endo. (ppm)	Total Endo. (ppm)	Locations	Total Endo. (ppm)	Total Endo. (ppm)
BARDALA	0	0	AL JIFTLIK	7.554	5.598
EIN AL BETA	1.737	6.721	FASAYIL	108.164	0
MARJ NAJA	170.962	99.621	ALAUJA	270.834	23.634
AZ- ZUBEIDAT	19.072	0	AN NUWEI MA	91.153	0
MARJ AL GHAZAL	17.576	6.855	JERICHO CITY	44.345	23.380
Average	41.869	22.639	Average	104.41	10.522

Figure11. Comparison of mean value of concentrations of total endosulfan in soil samples collected from all locations of northern and southern parts of Jordan Valley from 0-30 cm deep

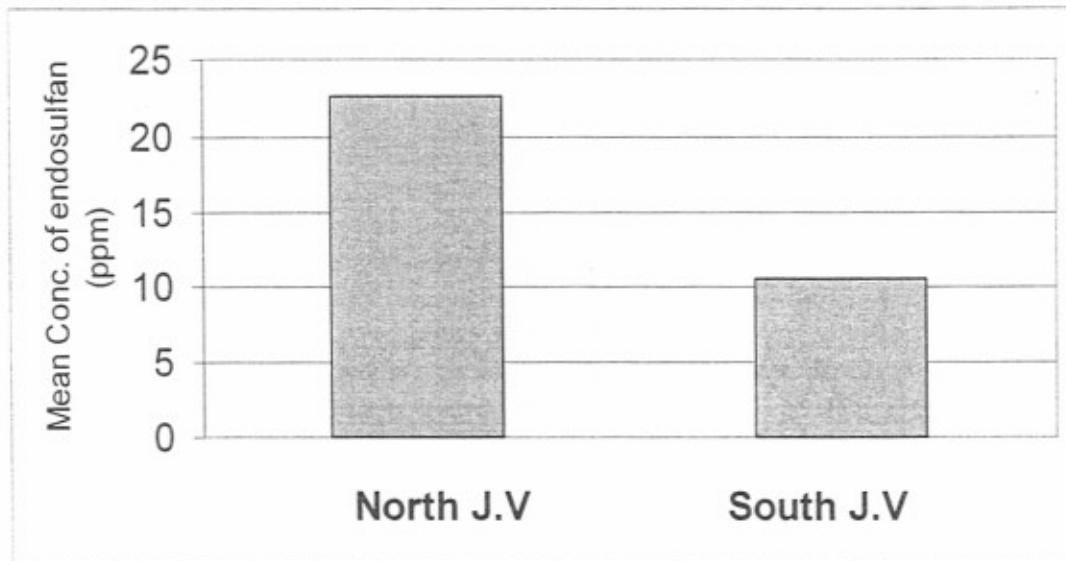
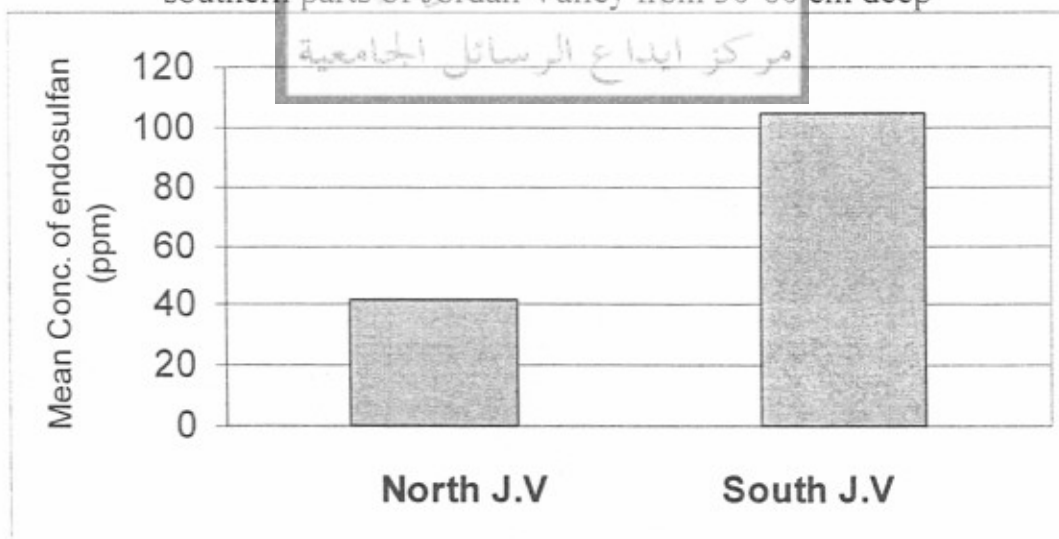


Figure12. Comparison of mean value of concentrations of total endosulfan in soil samples collected from all locations of northern and southern parts of Jordan Valley from 30-60 cm deep



Chapter IV
جميع الحقوق محفوظة
مكتبة الجامعة
Discussion
مركز ايداع الرسائل الجامعية

It is well-known that Jordan Valley is one of the most fertile and cultivated areas in Palestine. Many types of fruits and vegetables are usually cultivated in this area such as cucumbers, tomatoes, squashes eggplants, and peppers, etc.. Excessive amount of pesticides are being used in order to protect the crops from insect pest attack. Due to its organochlorine-origin, endosulfan is banded or restricted in many countries of the world but it is still largely used in Palestine. Results obtained and presented in Table (2) show the presence of high level of endosulfan residues (α and β -isomers) in the soil of many locations in Jordan Valley. This high residue level is due to the ability of the two isomers for persistence and bioaccumulation. The highest concentration of α -endosulfan at depth level of 0-30 cm was detected in Al-Auja due to many cultivated crops per year and the extensive use of pesticides especially endosulfan to control insect pests that are attacking many crops cultivated in this area, while Bardala soil was found to be free from α and β -endosulfan. This can be probably attributed to the soil type (clay soil), average rainfall, moisture and biodegradation in the soil.

Comparison between the residue levels of α and β -isomers at a depth level of 0-30 cm shows that the concentrations of α -endosulfan in the soil of most locations was higher than β -endosulfan in the same locations. This phenomenon could be attributed to the difference in degradation time of the two isomers in the soil, the composition ratio of α and β -isomers in the prepared endosulfan (70% and 30% , respectively) (Goebel, et al., 1982).

The half-life of α -endosulfan was reported to be 35 days, compared to 150 days for β -endosulfan at soil (pH = 7) (Carey & Douglas, 1971).

According to the half-life, the concentration of β -endosulfan in the soil should be higher than that of α -endosulfan, but the higher ratio of α -endosulfan in the applied formulation (70%) was the real reason behind the higher concentration of α -endosulfan in the soil at a depth level of 0-30 cm.

Comparison between the residue levels of α and β -endosulfan in the soil at depth level of 30-60 cm (Table 3) showed higher concentration of α -endosulfan. These results could be attributed to the compared pesticide. In six locations out of ten, the results revealed that there was no detection of β -endosulfan in the soil.

Comparison of α and β -endosulfan between the depth levels 0-30 cm and 30-60 cm shows higher residue levels of both isomers at depth level 0-30 cm than at 30-60 cm (Table 2). These results are in agreement with the results reported by Cary (1971) who have indicated that endosulfan is not easily dissolving in water and it has a moderate capacity to be adsorbed to soil particles. They concluded that endosulfan is not likely to be very mobile in the soil.

It could be concluded that the degree of soil contamination with α and β -endosulfan is affected by many factors such as the ratio of α and β -endosulfan in the applied formulation, depth level of soil, temperature, humidity, half-life of α and β -isomers, type of the soil, amount of applied formulations and the intervals between the application rates of the pesticides. It is almost impossible to obtain a correlation between all of the above mentioned factors and the degree of contamination of the soil in the studied locations.

Comparison between concentrations of α and β -isomers or total endosulfan in the ten locations studied in our research shows that the concentrations of total endosulfan in the locations of South Jordan Valley are higher than that in locations of North Jordan Valley at a depth level of 0-30 cm (Table 4, figure 11 & 12). These results can be attributed to the type of soil in the locations which influence the degree of adsorption of endosulfan by the soil particles; the soil type in North Jordan Valley (Pardala) is clay but in the other locations it is organic.

On the other hand, temperature and soil moisture were found to have an affect on the rate of degradation of the endosulfan molecules. The average temperature in the Jordan Valley during summer is 31.52°C. This temperature is not enough for degradation of endosulfan which requires higher temperature to degraded.

In conclusion, the overall results obtained in this study on the mean residue level per location of α and β endosulfan or total endosulfan in the soil of Jordan Valley (73.273 ppm at soil depth of 0-30 cm) was higher than that in the other soils reported by Jayakumar (2000) (34.89 ppm) . This indicates the presence of large quantities of the pesticide residues in the soil of Jordan Valley, therefore efforts should be made to decrease the frequency and quantity of pesticide application under our conditions.

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Appendix

جميع الحقوق محفوظة
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مركز ايداع الرسائل الجامعية

Table B1. T-test between depth level 0-30 cm to 30-60 cm

Area	Paired Differences					T-calculated	d.f	T-table	Sig. (2-tailed)
	Mean	Std. Deviation	Std. Error Mean	95% Confidence Interval of the Difference					
				Lower	Upper				
Depth (0-30cm)-(30-60cm)	56.558	78.2898	24.7574	.5537	112.56	2.285	9	1.697	0.048

T-calculated greater than T-table there is a difference toward residue from two depth levels, so the depth level affects the residues.

Table B2. Effect of Place on residue of endosulfan (0-30cm), F-Test when $\alpha = 5\%$

Source of variation	DF	SS	MS	F- value	P- value
Due to places	9	35896	3988	4.90	0.010
Due to Errors	10	8134	813		
Total	19	44031			

Table B3. Effect of Place on residue of endosulfan (30-60cm), F-Test when $\alpha = 5\%$.

Source of variation	DF	SS	MS	F-value	P-value
Due to places	9	4201.9	466.9	9.00	0.001
Due to Errors	10	518.8	51.9		
Total	19	4720.7			

جميع الحقوق محفوظة
مكتبة الجامعة الاردنية
مركز ايداع الرسائل الجامعية

Table B4. F-test to know the difference between northern part and southern part of J.V (depth level 0-30cm)

No.	Place	Mean	
1	BARDALA	0.00	*
2	EIN AL BETA	0.87	*
3	MARJ NAJA	85.48	*
4	AZ- ZUBEIDAT	9.54	*
5	MARJ AL GHAZAL	8.79	*
6	AI JIFTLIK	3.78	*
7	FASAYIL	54.00	*
8	ALAUJA	135.42	*
9	AN NUWEI MA	45.58	*
10	JERICO CITY	22.17	* -----+-----+-----+----- 0 70 140 Pooled StDev = 28.52

* = there is the difference to the greater mean

Table B5. F-test to know the difference between northern part and southern part of J.V (depth level 30-60cm)

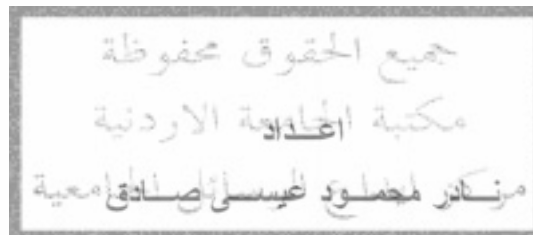
No .	Place	Mean	
1	BARDALA	0.00	*
2	EIN AL BETA	3.361	*
3	MARJ NAJA	49.810	
4	AZ- ZUBEIDAT	0.000	*
5	MARJ AL GHAZAL	3.427	*
6	AI JIFTLIK	2.799	*
7	FASAYIL	0.00	*
8	ALAUJA	11.817	*
9	AN NUWEI MA	0.00	*
10	JERICO CITY	11.690	*

---+---+---+---+
 0 20 40 60
 Pooled StDev = 7.203

* = there is the difference to the greater mean

جامعة النجاح الوطنية
كلية الدراسات العليا

قياس كمية المتبقيات من مبيد اندوسلفان في تربة غور الأردن



اشراف
د. يعقوب بطه و د. نضال زعتر

قدمت هذه الأطروحة استكمالاً لمتطلبات درجة الماجستير في العلوم البيئية بكلية الدراسات
العليا في جامعة النجاح الوطنية في نابلس، فلسطين.

2004

قياس كمية المتبقيات من مبيد اندوسلفان في تربة غور الاردن

اعداد

نادر محمود عيسى صادق

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الملخص

تم في هذا البحث جمع 160 عينة تربة من عشرة مواقع منفصلة في غور الأردن امتدت من بردلا شمالاً الى مدينة أريحا جنوباً. تم قياس كمية المتبقيات للمبيد الأكثر استعمالاً لمكافحة الحشرات وهو اندوسلفان (تيونكس) في عينات التربة التي جمعت من المناطق الزراعية المعاملة بالمبيد على عمق صفر إلى 30 سم و من عمق 30 إلى 60 سم، وتم استعمال طريقة التحليل بواسطة جهاز التحليل الكروماتوغرافي (GC/MS) لقياس كمية المتبقيات. أظهرت النتائج التي تم الحصول عليها أن معدل كميات المتبقيات لهذا المبيد كانت أعلى في مواقع المنطقة الشمالية لغور الأردن منه في مواقع المنطقة الجنوبية. أما بالنسبة للمواقع نفسها فقد كانت كمية المتبقيات أعلى عند العمق صفر إلى 30 سم منه على العمق 30 إلى 60 سم. وقد تبين أن هناك فروقات معنوية بين كمية المتبقيات وعمق التربة. كما تبين ان متبقيات المبيد في جنوب غور الأردن أعلى من شماله. ونتيجة لهذا البحث نوصي بتقليل استخدامه.