Surveying for Suspected DDT Contamination of Agricultural Soils in North Cyprus

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ABSTRACT

DDT is a very potent and cheap pesticide which was once widely used indoors and outdoors for pest control and in agriculture to increase crops. It was used throughout the world until evidence began to accumulate during the 1960s and 1970s about its toxic effects, its spread through the food chain, as well as its persistence in the environment. Its use was banned almost worldwide, starting with USA in 1972. Although its sale and use still remain prohibited, Government agencies can use DDT in times of outbreaks or epidemics. The commercial DDT is a mixture of 4,4'-DDT and its various isomers and degradation products, namely 2,4'-DDT and smaller amounts of 4,4-DDD, 2,4-DDD, 4,4-DDE and another isomers. The generic name however is simply DDT. Although banned in North Cyprus since the early 1980s, occasionally reports appear in the media about DDT tainted produce or contaminated agricultural fields. Most recently, in 2012, reports appeared about DDT contaminated potatoes destined for export. It was claimed that although prevented from export, the potatoes were quietly marketed locally. Also the identity of land on which these potatoes were grown was never made public by the authorities. In this work we aimed to determine whether DDT contamination can be detected and measured accurately and precisely in agricultural soils in North Cyprus. Soil samples from five potato fields were taken. At the time of sampling the fields had not been planted yet, so only soil samples were taken. These samples were analysed for DDT residues by HPLC with DAD-UV absorbance measurement. In all the samples measurable amounts of the degradation product 4,4'DDE were determined.

Keywords: DDT, Soils, Malaria Control, Residues, Degradation, Environmental Contamination, HPLC

ÖZ

DDT çok etkili ve ucuz bir pestisit olarak haşerelere karşı açık ve kapalı mekânlarda ve ürün artırmak için tarımsal alanlarda geçmişte yaygın bir şekilde kullanılmıştı. Zararlı etkileri, gıda zinciri yoluyla canlılarda yayılması ve doğada dayanıklılığı hakkında 1960/70'li yıllarda bulgular ortaya çıkmaya başlayana kadar tüm dünyada kullanılmaktaydı. Kullanımı 1972de Amerika'daki yasak ile başlayarak dünyanı birçok ülkesinde yasaklandı. Kullanım ve satışı hala yasak olmasına rağmen, acil durumlarda Hükümet kuruluşları DDT kullanabilirler. Ticari DDT'nin ana maddesi 4,4'-DDT olmakla birlikte çeşitli izomerler ve bozunma ürünleri de içerir. Bunlar 2,4'-DDT, 4,4-DDD, 2,4-DDD, 4,4-DDE ve benzeri maddelerdir. Ancak tümü DDT olarak anılır. Kuzey Kıbrıs'ta 1980'li yılların başından bu yana yasak olmasına rağmen, basında DDT kalıntısı içeren tarım ürünleri veya tarım toprakları hakkında zaman zaman haberler çıkmaktadır. Örneğin, 2012 yılında, ihracat edilecek olan bazı patateslerde sınırların üstünde DDT tespit edildiği ve ihracatının durdurulduğu; ancak bu patateslerin imha edilmeyip ülke içinde tüketildiği haberi yayınlandı. Ayrıca bu patateslerin yetiştirildiği tarlanın nerde olduğu açıklanmadı. Bu çalışmada, Kuzey Kıbrıs tarım arazilerinde DDT kalıntılarının tespit ve ölçümünün doğru ve hassas bir şekilde yapılıp yapılamayacağını anlamak hedeflenmiştir. Bu hedef doğrultusunda beş patates yetiştirilen tarladan toprak örnekleri alınmıştır. Örnekleme tarihinde tarlalarda ekili ürün olmadığından sadece toprak örnekleri alınmıştır. Bu örneklerdeki DDT kalıntıları, HPLC cihazı ve DAD-UV emilme ölçümü yöntemiyle analiz edilmiştir. Bütün örneklerde bozunma ürünü 4,4 'DDE ölçülebilir miktarda tespit edilmiştir.

Anahtar: DDT, Topraklar, Sıtma Kontrolü, kalıntı, Degradasyon/bozunma, Çevre kirlenmesi, HPLC

DEDICATION

To ...

My Dear father r.i.p. Jala Sadiq; he was a significant driving force in the continuation of my education and in my life, who offered me love, support and encouragement throughout the years.

My Dear mother, for her supporting and her magical love.

My respectable brothers and my sisters; your support always keeps me persistent

Best Regards

Hawar Hawezy

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p, p'- DDT: 1,1,1-trichloro-2,2-bis- (p-chlorophenyl) ethane

HPLC: High Performance Liquid Chromatography

UV: Ultraviolet light

WHO: World Health Organization

U.S.EPA: United States Environmental Protection Agency

p, p'- DDD: 1,1- Dichloro- 2,2- bis-(p-chlorophenyl) ethane

p, p'- DDE: 1,1-dichloro-2,2-bis (p-chlorophenyl) ethylene

o, p'-DDT: 1,1,1-trichloro-2-(o-chlorophenyl)-2-(p-chlorophenyl) ethane

o, o'-DDT: 1,1,1-trichloro-2,2-bis-(o-chlorophenyl) ethane

m, p'-DDT: 1,1,1-trichloro-2-(m-chlorophenyl)-2-(p-chlorophenyl) ethane

POP: Persistent organic pollutant

µm: Micrometres

PTFE: Polytetrafluoroethylene

rpm: Revolutions per minute

ppm: parts per million

µL: Microliter

mAU: milli absorbance units

R_t: Retention time

SD: Standard Deviation

RSD: Relative Standard Deviation

R^2: Correlation coefficient

ppb: parts per billion

Chapter 1

INTRODUCTION

The purpose of the present work was to survey some of the agricultural soils in the Beyarmudu region in North Cyprus for suspected contamination by the infamous pesticide 1,1,1- trichloro- 2,2- bis- (p- chlorophenyl) ethane (otherwise known as 4,4'-DDT) and its main isomer 2,4-DDT as well as the main degradation products of 4,4'-DDE and 4,4'-DDD using High Performance Liquid Chromatography, HPLC, with UV-Visible detection. The structure of 4,4'-DDT is shown in Figure 1

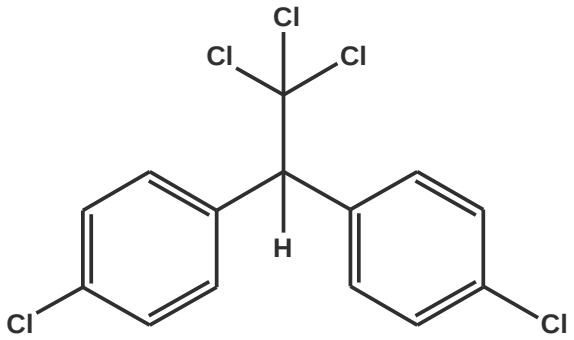


Figure 1: Structure of 4,4'-DDT; main component of DDT!

Two important factors have been the main motivation behind this work. The first is the widespread use and abuse of pesticides in agriculture in North Cyprus which frequently finds its way into the headlines of local newspapers, with news of exported agricultural produce being returned or never leaving the port because of too high pesticide content [1]. The second is the high incidence of cancer cases which

was reported by the ministry of health of TRNC as being somewhere between 170 to 200 per 100,000 among the local population compared to European Union's 278 and U.S.A's 322. Although the TRNC numbers are lower than Europe and USA, it must be remembered that the TRNC is a non-industrial country supposedly free of the most of the industrial pollution in the developed countries [2, 3].

Although use of DDT was banned in North Cyprus together with the rest of the world in 1988, there are occasional claims (in newspapers) of produce, notably potatoes, being tainted with unacceptable levels of DDT. Claims are also made that the problem is greater than that admitted officially [4].

Consequently, in the present work we sought to answer the following questions;

- Can we detect/identify and measure DDT constituents (4,4'-DDT, 2,4'-DDT, 4,4'-DDD and 4,4'-DDE) in our labs by HPLC with UV-Visible detection?
- How accurate and precise are the retention time, R_t, and peak area/height information for identifying components and determining them quantitatively? And what range of concentrations can we measure with this technique?
- Is the solvent system and the extraction regime used suitable to extract and determine quantitatively DDT components in the soils studied?
- How much DDT residue is present in the five soil samples?
- And finally, is there sufficient evidence to start a country wide survey for pesticide residues in the agricultural soils to determine the severity of the problem?

1.1 DDT

DDT was first made in 1874 by an Austrian chemist named Othmar Zeidler [5]. Later, in 1939, the Swiss chemist Paul Hermann Muller discovered that DDT possessed powerful insecticidal properties [6]. It quickly began to be used widely as a cheap and effective insecticide in agriculture and for improving human health by aiding the control of human diseases transmitted by insects (including controlling of malaria), until evidence of its toxicity to organisms was discovered in early [7]. Since than its use has been banned in many countries [8]. Only exception is in cases of emergencies when government authorities need to control outbreaks of certain diseases/ epidemics or particular insect infestation and some agricultural uses [6].
Its widespread and indiscriminate use in the past has led to many agricultural soils to have become contaminated with DDT. Although DDT decomposes slowly in soils, with reported half- life between 22 days to 30 years, there are reports that it still persists in some places [5].

Until now, many studies have shown that organochlorine pesticides are the most toxic compounds synthesized so far, with serious adverse effects to all living things and systems even at very low levels [9].

When people hear the word "pesticides", they consider it as a negative word. And many times, they'll think of DDT first. DDT has become the most notorious pesticide in the world because even though it is toxic and harmful, we still need and use it in malaria vector control [10, 11]. DDT is categorized as a persistent organic pollutant, POP. The toxicity of DDT is high and its degradability is low [12]. It was not until 1939 that its insecticidal properties were discovered by Muller. Because of this work

he won the Noble prize in Physiology or Medicine in 1948 even though its synthesis had been reported by Zeidler in 1874. In 1942 DDT was introduced commercially by Geigy and in 1943 it was being produced on an industrial scale [13]. As a result of its efficient and effective killing power against insects it was called the “atomic bomb” of pesticides [10].

DDT is the cheapest insecticide and the one which has the longest effectiveness after application against malaria vectors (6–12 months depending on dosage and substrate). Other insecticides have shorter effective lifetimes (pyrethroids: 4–6 months; organophosphates and carbamates: 2–6 months) [14].

DDT is currently manufactured in three countries; India, China and the Democratic People’s Republic of Korea [15].

1.2 Use of DDT

DDT had been used widely throughout the world [16]. Initially it was used for public health by the Allied army during World War II. It was mainly used to control the vector insects responsible for malaria, typhus, bubonic plague, sleeping sickness, and yellow fever, as well killing off body lice. In addition to its public health uses, growers used DDT on a variety of food crops, through massive aerial spraying programs in the United States and worldwide as shown in Figure 2 [17, 18]. When DDT was used in agriculture, huge increases in production were achieved. During 1972 to 1979, DDT was used to combat the pea leaf weevil and the Douglas-fir tussock moth; rabid bats; and plague-carrying fleas. Previously, we have used it to control mice, rats, bats and termites [10, 19, 20]. Public and household use also

brought significant improvements in human health and life quality by reducing housefly and cockroach numbers [7, 18].

DDT has three separate mechanisms in the case of malaria control: repellency, irritancy, and toxicity, which together are successful at halting the spread of the disease. Repellency is the most important mechanism, and along with DDT's long residual time, it makes DDT superior to other insecticides. In spite of its repellency qualities, it has been forgotten by the community of malaria-fighting [19].

Figure 2: Plane Spraying Alfalfa Fields in Imperial Valley with DDT. Picture by Loomis Dean

1.3 Current Usage Status of DDT

The use of DDT was banned in most countries during the 1970's and the ban continues to this day. However its use to control malaria was excluded from this ban [21, 22]. The Stockholm Convention on Persistent Organic Pollutants (POPs) prohibited the use of 12 industrial organic chemicals including DDT in 2004 [23].

The reason why DDT use is still permitted especially against malaria is because it is cheap and very active and long lasting. When DDT use was banned there were serious outbreaks of malaria, which were only brought under control after the re-use of DDT! So even though DDT is hazardous and can have a serious impact on wildlife, its use is accepted for malaria control. In 2006, the World Health Organization (WHO) gave its support for countries struggling with malaria for indoor use as well [21, 22, 24]. DDT is still used in the tropical regions of many countries such as Brazil, Colombia, Ecuador, Peru and Venezuela, India, China, South America, Africa, and Malaysia to control mosquito vectors of malaria [10, 13].

Although DDT has been banned in many countries for many years now, residues continue to be detected in soils, water, sediments and aquatic biota. It is known that DDT can be taken up by plant roots and transferred to different parts of its body such as leaves, fruit/ seeds and branches. In this way DDT can get in to the biosphere and move through the food chain [24, 25].

1.4 Physical and Chemical properties

DDT is a colorless, tasteless and almost scentless crystalline solid. It is very hydrophobic and highly lipophilic. It is essentially non polar, in other words, there are not positive and negative portions on DDT. Consequently it is very soluble in most organic solvents such as cyclohexanone, benzene, chloroform, petroleum solvents, ethanol but almost insoluble in water [5, 10, 26]. Hence it tends to accumulate in the fatty layers or tissues of organisms and it doesn't break down easily in the environment [18].

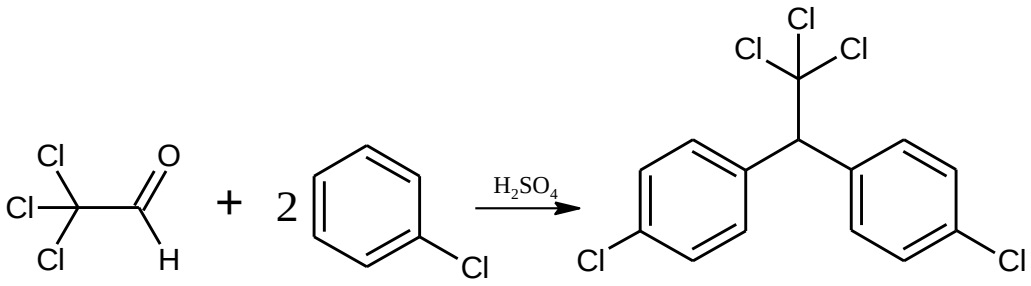
It can be broken down by microorganisms or by radiation from the sun. Also it may enter the air by evaporating from soil [27].

Table 1: Chemical Properties & Physical Properties of DDT

1,1,1-trichloro-2,2-bis-(p-chlorophenyl) ethane	
Molecular formula	$C_{14}H_9Cl_5$
Bioaccumulation Potential	Strong
Concentration in Gas Phase	1.9×10^{-6} mg/L
Solubility in Water	Very Low (0.001-0.04 mg/L)
Potential for Entry into Fresh water	Strong
Aquatic Toxicity	High
Aquatic Persistence	Prolonged
Density	0.99 g/cm³
Melting point	108.5 °C
Boiling point	260 °C (decomposes)
Formula Weight	322.25g/mole

1.5 Synthesis of DDT

DDT does not occur naturally but it is manufactured by the reaction of chloral (CCI_3CHO) with chlorobenzene (C_6H_5CI) in the presence of sulfuric acid, which acts as a catalyst [5]. The reaction is shown in Scheme 1



Scheme 1: Preparation of 1,1,1-trichloro-2,2-bis-(p-clorophenyl)ethane

1.6 Toxicity of DDT

DDT like the other pesticides plays an important role in the fight against many terrible diseases [28]. It affects humans through soil, water, air and food by different routes of exposure such as ingestion, inhalation or absorption from skin [29]. Application to food crops has a negative effect on human health. Its harmful effects are: acute neurologic toxicity, chronic neurodevelopment impairment and possibly dysfunction of the immune system [30]. It is also believed to cause liver and pancreatic cancer, leukemia, lymphoma and testicular cancers. Studies indicate that exposure to DDT before puberty causes breast cancer in adulthood. Thus, it is likely to affect reproductive health, such as birth outcomes. Exposure to DDT in the womb may affect neurodevelopment, leading to behavioral problem disorders during childhood and retarded psychomotor development. It may also affect thyroid hormone levels. Through breastfeeding, infants experience high DDT exposure. Because of the effect of DDT on early stages of the neural and physical development, Pre- and postnatal exposures are especially critical [23, 31]. Furthermore, it has adverse effects on the normal state of the endocrine system of humans and wildlife [11]. Other effects include chronic kidney diseases and sterility among males and females [29].

Research shows that children living in highly DDT-exposed areas have higher levels of serum DDT compared to those in less exposed areas, which correlates well with the DDT levels in soil and dust in their home environments [32].

Ingestion of DDT in humans will cause nausea, dizziness, confusion, and headaches. So, there are concerns that small amount of DDT found in soils can be transferred to

crops and may then be ingested by humans. High DDT concentrations tend to be lethal to adult animals by affecting their central nervous system [13].

The concentration of DDT is high in human milk especially. It tends to stay in our bodies because milk production depends strongly on the use of stored body fat [10]. Although no syndrome related to chronic DDT exposure is recognized in humans, evidence indicates that DDT may cause aplastic anemia and thrombocytopenia [18]. The World Health Organisation (WHO) has reported that almost three million pesticide poisonings occur every year, and causes 220,000 deaths worldwide [28].

1.7 Environmental Impact of DDT

Pesticides which remain unchanged after use/application to control insects can spread in the environment (soil, surface and underground waters) as well as enter the foods that we consume [33]. For example, DDT sprayed on crops and on forests for the control of insects, large quantities of DDT find their way into soil, air and water. The DDT in the soil and water can and have entered the food chain. DDT in waters have made their way in to birds via insects and fish. Also, DDT can become airborne from contaminated waters and soils and enter the atmosphere. In this way it is transported and distributed over large areas. The air DDT eventually will be deposited on to soils or water bodies. This cycle can be repeated several times. DDT sticks strongly to soil, and in general it remains in the outer layers of soil particle [34].

The length of time DDT remains in soil depends on many factors including temperature, type of soil, humidity and the presence of microorganisms. For example DDT degrades quickly in the humid tropics where the chemicals evaporate quicker

and where microorganisms bio-degrade them much faster. DDT also disappears faster when the soil is flooded with water or is wet/damp for prolonged periods [34].

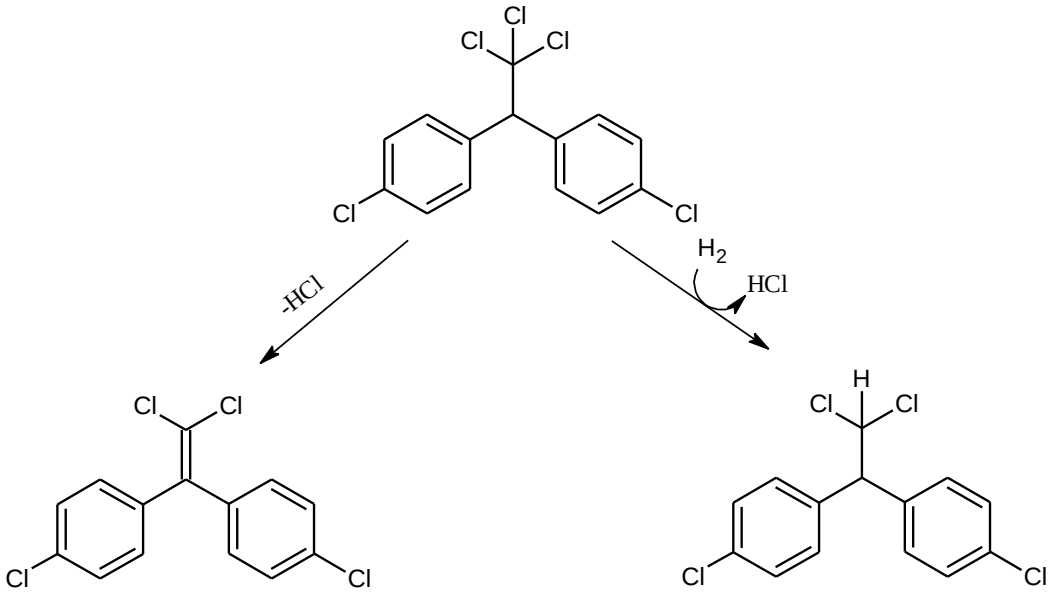
There is no consensus on the half-life of DDT in the various spheres of the environment. In some articles it is claimed to be approximately 2-15 years in the soil [17]. Yet in others DDT half-life in the soil is said to vary between 22 days to 30 years. Its half-life in aquatic environments is claimed by some to be about 150 years while the U.S. EPA (1989) report declares that DDT half-life in lake water is 56 days and in river waters it is 28 days [5,13,17]. In humans DDT is claimed to be very resistant to metabolism and elimination, and has an estimated half-life of 6 to 10 years [5]. Its half-life in the atmosphere is approximately 1.5-3 days [34].

1.8 Degradation of DDT

The degradation of DDT is very slow in soils with a DT_{50} of 3800 days [35] and therefore it may be necessary to actively clean-up DDT in contaminated soils using suitable techniques. Possible techniques or methods to reduce DDT concentrations is soils based on the observations that certain natural processes cause a decline in DDT concentrations in soil. These processes include:

a- Natural attenuation process based on physical and chemical processes such as photolysis, hydrolysis and volatilization.

b- Enhanced biodegradation through aerobic and anaerobic biodegradation [36]. The products of DDT transformation are DDE and DDD. The transformation of DDT into DDD under anaerobic condition occurs through reductive dechlorination and under aerobic condition occurs through elimination of HCI to form DDE [5] as shown in Scheme 2.



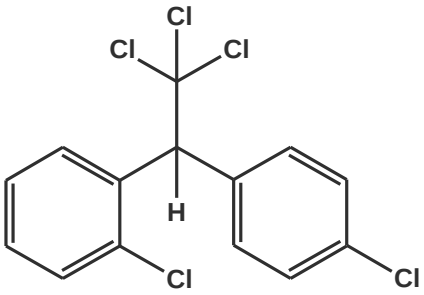
Scheme 2: Degradation of DDT to form DDD (right) and DDE (left)

DDT in acidic environment degrades very slowly, with a half-life of 12 years. Hydrolysis of DDT to DDE is base catalyzed with a half-life 81 days [37]. Ingested DDT is metabolized in humans to DDD and DDE slowly [18]. DDD enters the environment as a collapse product of DDT; DDE also enters the environment as contaminant or collapse product of DDT [34].

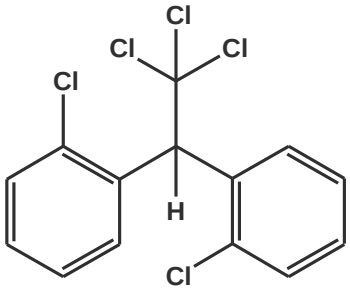
1.9 Commercial grade DDT

In general when we refer to DDT, we are referring to the specific isomer p, p'-DDT which is most prevalent in the environment, it accounted for approximately 85% of the total amount of DDT, DDE, or DDD found. In addition, Commercial grade DDT which is basically technical grade DDT, is composed of up to 14 chemical compounds, of which only 65-80% is the active p, p'- DDT. The other components in this grade include 15-21% of the almost inactive o, p'- DDT, up to 4% of p, p'- DDD, and up to 1.5% traces of o, o'-DDT and bis (p-chlorophenyl) sulfone. Up to 1% m, p'-DDT may be present in some technical DDT. Structures of these components are given in Figure 3 [34, 38].

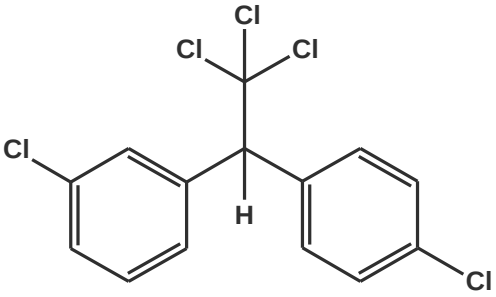
o, p'-DDT



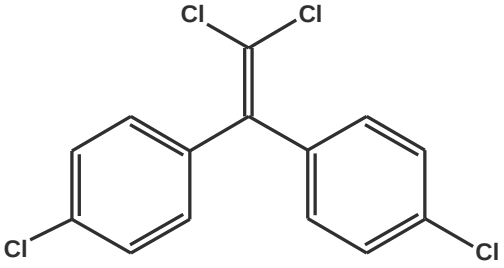
o, o'-DDT



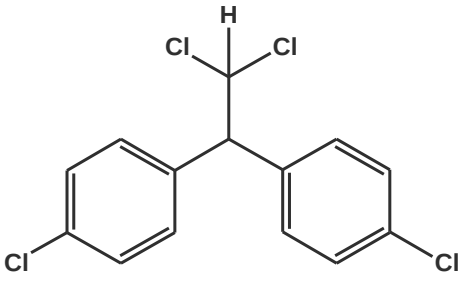
m, p'-DDT



DDE



p, p'-DDD



bis(p-chlorophenyl) sulfone

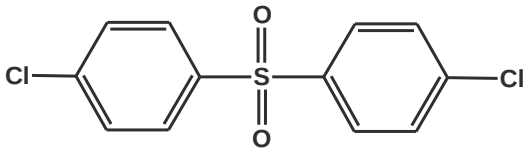


Figure 3: Chemical structures of the main components in commercial DDT

Chapter 2

EXPERIMENTAL

The details of the materials and instruments used; preparation of the soil samples for extraction (drying, grinding, sieving, and sub sampling); the extraction of the DDT from the soil samples with different solvents; and the analysis of the extracts by HPLC are presented in this chapter.

2.1 Materials

2.1.1 Chemicals and Reagents

Soil samples were stored in Kraft sample bags.

The solvents used for the soil extraction step − n-Hexane, methyl isobutyl ketone, acetone and methanol − were all Analytical Reagent (AR) grade purchased from Merck -Germany.

All glassware (borosilicate) was washed thoroughly with detergent, rinsed with tap water, and then rinsed with distilled water and finally air dried.

Solvent extracts of the soils were filtered through a Whatman No 41 ashless filter paper.

The acetonitrile used as one of the mobile phase solvents was HPLC grade LiChrosolv® Ph Eur, purchased from Merck-Millipore.

The water used as the second mobile phase solvent was ultrapure water obtained from an in-house reverse osmosis water purification system.

Methanol used to re-dissolve the extracts was HPLC grade LiChrosolv® Ph Eur purchased from Merck-Millipore.

The soil extracts, after being taken up into methanol, were stored in stoppered polyethylene sample tubes and stored in the freezer. Before HPLC analysis each extract was filtered through a 0.2 µm PTFE membrane filter (Whatman™ 50 mm In-Line Filters, PTFE, 0.2 µm) and then placed directly into the glass HPLC vials for injection into the HPLC.

The certified reference DDT standards used in this study were kindly provided by the TRNC State Laboratories in Nicosia. The original standards were obtained from Absolute Standards, Inc. The standards supplied to us were 10 ppm solution in HPLC grade methanol for 2,4-DDT; 4,4-DDT; 4,4-DDD; and 4,4-DDE. These standards were in stoppered glass sample bottles and were stored in the freezer at (-18C^{o}) when not in use. The part number and the lot number of each of the certified reference standards are given in Table 2.

Table 2: Part and lot numbers of the 1000 ppm Certified Reference Materials obtained from Absolute Standards, Inc. (www.absolutestandards.com).

Compound	**Part Number**	**Lot Number**
4,4'-DDD	70099	052010
4,4'-DDE	70100	101311
4,4'-DDT	70101	112911
2,4'-DDT	70102	081711

2.2 Instruments

For the extraction step of the soils with solvent, a flat-bed EYELA Multi- Shaker MMS 4010 mechanical shaker was used at a speed of 200 rpm.

The solvents from the extracts after filtration were removed under vacuum on a rotary evaporator with a water-bath set at a temperature of 30°C.

All the samples were analyzed with an Agilent Technologies 1260 Infinity series High Resolution Liquid chromatography, HPLC, system equipped with a reversed phase silica 15 cm capillary column (C18). The optimized operating parameters for the HPLC are given in Table 3.

Table 3: Optimized operating parameters for HPLC

Column Temperature	40°C	Kept constant
Mobile phase composition	80% Acetonitrile 20% UP Water	Isocratic elution
Flow rate	1.500 mL / minute	
Injection volume	50 µL	Needle wash with methanol in between sample injections
Detector wavelength	210 and 220 nm	

2.3 Methods

2.3.1 Study area and Sampling

The region of Beyarmudu was selected because it is a well-known region for potato growing. Soil samples were collected on 19 March 2014 from five different fields and labelled as A, B, C, D and E. All but one of the fields is regularly used for potato growing. Satellite image of the sampling locations as well as the coordinates were recorded via GPS with Google Maps®. The coordinates are given in Table 4. From each location approximately 3 kg of surface soil (topsoil – to 20 cm) was collected. All soils were dry and free from vegetation and stones.

Table 4: Sampling coordinates and field description

Sample label	Longitude	Latitude	Vegetation at time of sampling
A	35.060	33.739	Approximately 15 year old olive orchard
B	35.054	33.744	Planted with wheat
C	35.049	33.727	Recently ploughed, no vegetation.
D	35.043	33.716	Recently ploughed, no vegetation
E	35.077	33.721	Planted with potatoes

All the samples collected were placed in Kraft sample bags and sealed to avoid contamination, and were brought to the laboratory immediately.

2.3.2 Clean-up

Upon returning to the laboratory, the samples were removed from their bags and placed in clean trays and all non-soil materials such as stones, twigs vegetation and roots removed. The soil in the trays were covered with clean paper and air-dried at room temperature for two days. The air dried soils were then crushed and ground (gently) in a mortar with a pestle, and sieved with a Tyler 10 mesh sieve (diameter =1.651 mm) to remove large particles. The sieved soils were stored in clean labelled Kraft sample bags at room temperature until further use.

2.3.3 Solvent Extraction and Preparation for HPLC Analysis of the Soil Samples

DDT residues were extracted from the soils by solvent extraction at room temperature according to the following procedure.

1- For each soil, 50 gm of the air-dried and sieved soil was sub-sampled. The sub-samples were placed in to 250 mL conical flasks together with 50 mL of the extracting solvent. Three different solvents were tried. Solvents tried for the extraction were 1 to 1 acetone-Hexane mixture; methyl isobutyl ketone (MIBK); and methanol. Details of soil-solvent systems are given in Table 5.

2- The bottles were closed and shaken on the mechanical shaker (EYELA Multi-Shaker MMS 4010) for 10 hours at 180 rpm.

3- The extracting solvents were filtered through a Whatman number 41 filter paper and the supernatant collected. The extracted soil residues were returned to their conical flasks after filtration and 30 mL of fresh extracting solvent were added to the flasks.

5- The flasks were returned to the shaker and were extracted for a further one hour. After allowing the soil to settle for 10 minutes, the mixtures were filtered again; the soil on the filter paper washed with a few ml of fresh solvent and all the filtrates together with the initial extract were combined in a 250 mL round bottom flask. The round bottom flasks containing the filtrates were dried under vacuum on a rotary-evaporator at 30°C.

6- Once all the solvent was removed, the extracts in the round bottom flasks were then taken up with 6.00 mL of HPLC grade methanol and transferred to stoppered plastic sample tubes. These solutions were stored in the freezer at -18°C until analysis by HPLC.

7- Immediately before analysis, 1.5 to 2 mL of each of the soil extracts were filtered through a 0.20 µm PRFE membrane filters directly in to HPLC vials. The vials were then placed on the sample turret of the HPLC machine for analysis. When unused, all vials were stored in the freezer.

The extracts obtained from different soils with the various solvents used are listed in Table 5.

.

Table 5: Details of the solvent systems used for the soil extractions

Sample Label	Soil Sample	Extracting solvent	Comments
A1AcHx	A	1:1 Acetone- Hexane	Duplicated
A2AcHx	A	1:1 Acetone- Hexane	Replicate of A1AcHx
A1MIBK	A	Methyl isobutyl ketone	Duplicated
A2MIBK	A	Methyl isobutyl ketone	Replicate of A1MIBK
A1MeOH	A	Methanol	
B1MeOH	B	Methanol	
C1MeOH	C	Methanol	
D1MeOH	D	Methanol	
E1MeOH	E	Methanol	In triplicate
E2MeOH	E	Methanol	Replicate of E1MeOH
E3MeOH	E	Methanol	Replicate of E1MeOH

2.4 Analysis of the Extracts for DDT Residue by HPLC

The operating parameters for the HPLC instrument were optimized after some trial runs to those specified in Table 3. Then, with these settings, chromatograms of the standards and the samples were obtained. The diode array detector, DAD, was able to obtain a UV spectrum of the significant peaks as they eluted from the column. The HPLC gave information about the retention time (R_t) of each component; the shape of the peak as it eluted from the column; the peak height, peak area and peak base width; and also provided the UV spectrum of the component. These data enable, with some confidence, the identification and quantification of the unknown components by comparison of their R_t, peak area or height and peak UV spectrum with those for the standards.

2.4.1 Repeatability of Chromatograms

The repeatability of the chromatograms was assessed by measuring the same DDT standard a number of times. The chromatograms for the replicates were used to calculate the standard deviation and the relative standard deviation in the R_t of known DDT standard.

2.4.2 Reproducibility of Chromatograms

Reproducibility of the chromatograms was assessed by measuring the same standard on different days using the same operating parameters. Significant changes in R_t for a particular component meant that reproducibility was not very good, such that standards needed to be run alongside the unknowns.

2.4.3 Quantification from Peak area and Peak height

The 10 ppm DDT standards were measured separately and also as a combined 2.5 ppm solution made by mixing 500 µL of each standard in a vial. The peak height and/or peak areas of each component were then correlated with their concentration.

2.4.4 Identification and Quantification of DDT Residues in the Soil Extracts.

Chromatograms for each of the soil extracts were obtained together with chromatograms for the 10 and 2.5 ppm DDT standards. The retention time, Rt, peak area/height and UV spectrum were used to identify which DDT components –if any- were present in the extract and how much.

Chapter 3

RESULTS AND DISCUSSION

3.1 Chromatographic Data and the UV Spectrum of DDT Standards

The results obtained have been organized so as to provide answers to the original set of questions we posed in the beginning, namely;

- Can we detect/identify and measure DDT constituents (4,4'-DDT, 2,4'-DDT, 4,4'-DDD and 4,4'-DDE) in our labs by HPLC with UV-Visible detection?
- How accurate and precise are the retention time, R_t, and peak area/height information for identifying components and determining them quantitatively? And what range of concentrations can we measure with this technique?
- Is the solvent system and the extraction regime used suitable to extract and determine quantitatively DDT components in the soils studied?
- How much DDT residue is present in the five soil samples?
- Is there sufficient evidence to start a country wide survey for pesticide residues in the agricultural soils to determine the severity of the problem?

3.1.1 Retention Times and Peak Area/Height of DDT Standards

To answer the question "Can we detect/identify and measure DDT constituents (4,4'-DDT, 2,4'-DDT, 4,4'-DDD and 4,4'-DDE) in our labs by HPLC with UV-Visible detection?", we repeatedly analyzed four standard DDT samples at two different concentrations. The details of the replicated chromatograms of the DDT standards that were obtained on two different dates are:

1. Five replicate chromatograms of each of the 10 ppm 4,4-DDD, 4,4-DDT and 4,4-DDE standards, obtained on the same day (30 June 2014).
2. Triplicate chromatograms of the mixed DDT standards solution with 2.5 ppm of each also obtained on the same day (30 June 2014).
3. Triplicate chromatograms of the mixed DDT standards solution with 2.5 ppm of each obtained on another day (22 June 2014).

For each set, the mean, the standard deviation and the % relative standard deviation of the retention time (R_t) in minutes; the peak area and peak height in mAU for each component are given in Tables 6, 7 and 8.

The data presented here is for the concentrations of 10 ppm and 2.5 ppm. The blank (methanol) was taken as the zero ppm and gave zero peak area or height at the DDT retention times.

Table 6: The mean, standard deviation and relative standard deviation of Rt, peak area and peak height for the individual 10 ppm standards. N = 5. Analysis date is 30 June 2014

		R_t /minute	**Peak area**	**Peak height mAU**
4,4-DDD	**Mean**	3.094	1290	114.7
	SD	0.002	2.4	0.4
	RSD %	0.063	0.19	0.34
4,4-DDT	**Mean**	4.909	1226	96.6
	SD	0.005	5.0	0.3
	RSD %	0.098	0.41	0.36
4,4-DDE	**Mean**	6.139	1520	113.2
	SD	0.006	5.9	0.4
	RSD %	0.09	0.39	0.40

NOTE: The standard 2,4-DDT was not included in this run because it is not a main component of active DDT, it is present at a much lower concentration in commercial DDT than 4,4-DDT.

Table 7: The mean, standard deviation and relative standard deviation of R_t, peak area and peak height for each component in the 2.5 ppm standards mixture. N = 3. Analysis date is 30 June 2014

		R_t /minute	Peak area	Peak height mAU
4,4'-DDD	**Mean**	3.093	330	29.4
	SD	0.004	4.6	0.1
	RSD %	0.11	1.4	0.34
4,4'-DDT	**Mean**	4.916	311	25.1
	SD	0.008	7.6	0.4
	RSD %	0.16	2.5	1.5
2,4'-DDT	**Mean**	5.688	347	26.8
	SD	0.006	23.5	0.9
	RSD %	0.10	6.8	3.4
4,4'-DDE	**Mean**	6.135	378	28.9
	SD	0.008	19.1	0.6
	RSD %	0.12	5.1	2.1

Table 8: The mean, standard deviation and relative standard deviation of Rt, peak area and peak height for each component in the 2.5 ppm standards mixture. N = 3. Analysis date is 22 June 2014

		R_t /minute	Peak area	Peak height mAU
4,4'-DDD	**Mean**	3.064	334	29.3
	SD	0.021	3.6	0.1
	RSD %	0.69	1.1	0.4
4,4'-DDT	**Mean**	4.863	306	24.1
	SD	0.034	1.8	0.1
	RSD %	0.70	0.6	0.6
2,4'-DDT	**Mean**	5.628	329	25.2
	SD	0.037	4.2	0.1
	RSD %	0.66	1.3	0.5
4,4'-DDE	**Mean**	6.069	379	28.8
	SD	0.040	5.2	0.1
	RSD %	0.66	1.4	0.4

One of the actual chromatograms for the mixed 2.5 ppm standards is shown in Figure 4, to illustrate the shapes of the peaks as well as the quality of the chromatograms.

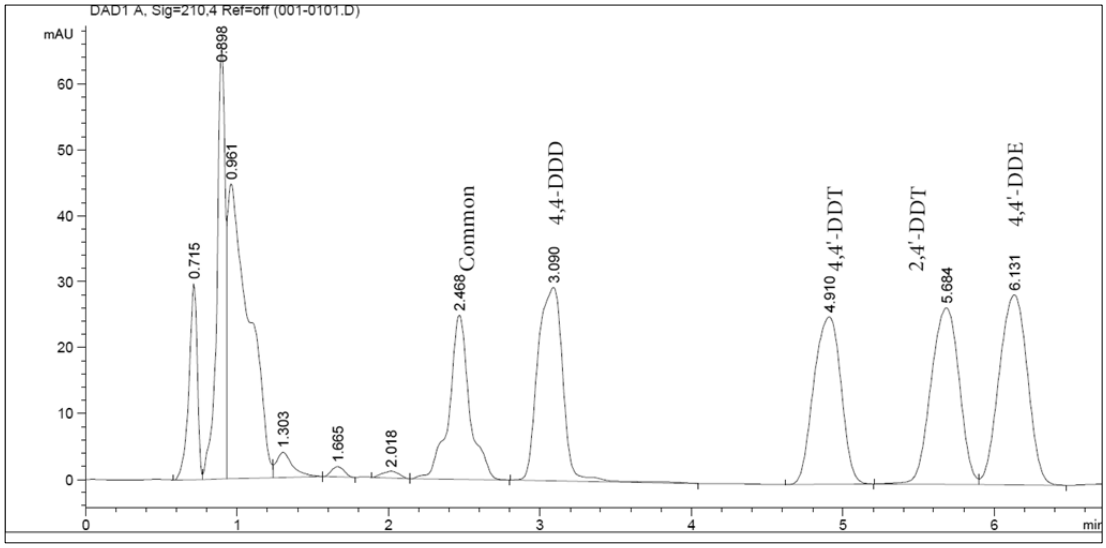


Figure 4: Chromatogram of the mixed DDT standards, 2.5 ppm of each component.

There was an unidentified peak with a retention time of around 2.5 minutes, present in all the chromatograms of the standards – whether individual or mixed. Visually the shape looks as if it is two superimposed peaks; one broad and the other narrow. The UV spectrum of this common peak is given in Figure 5. We were unable to identify this component ourselves and we could not find any reference to it in the literature. We may guess that it is an internal standard intentionally placed there or that it is one of the starting materials that has not been removed.

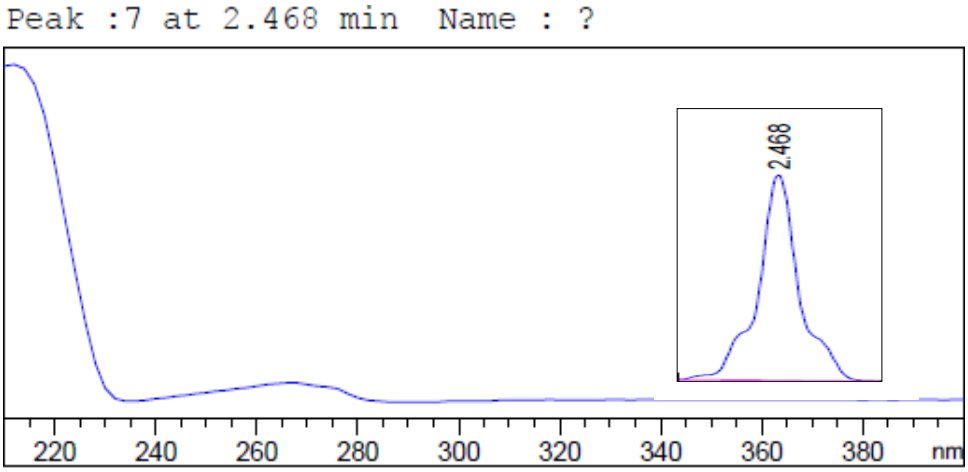
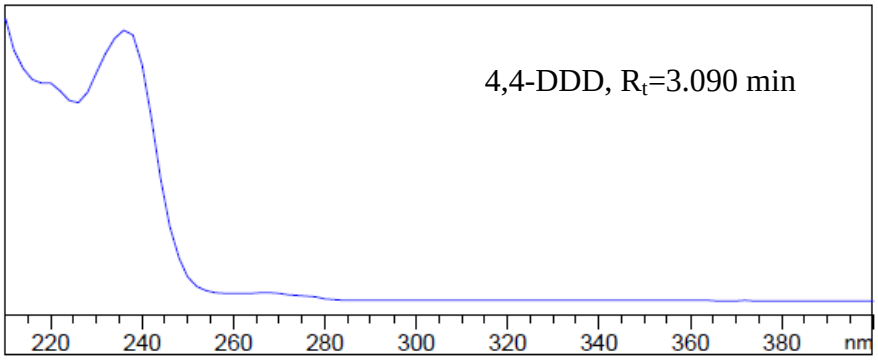
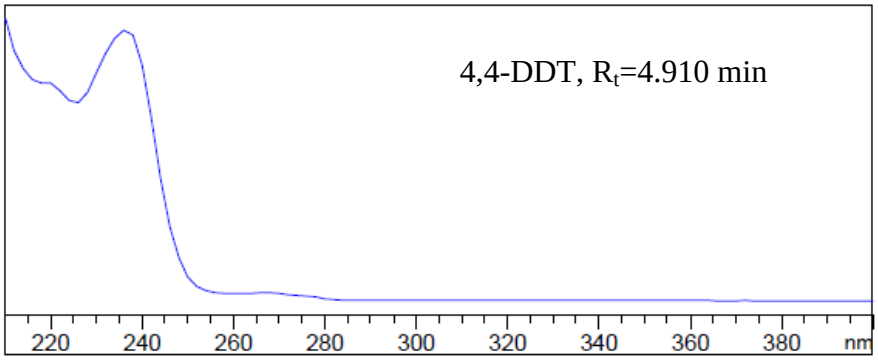


Figure 5: The UV spectrum of the unidentified common peak found in all DDT Standard Solutions. A cutout of the peak from the actual chromatogram is placed on the right. Compare it with the peak labelled "common" in Figure 4.

3.1.2 UV Spectrums of the DDT Standards

The UV spectrums of the four DDT standards obtained by the HPLC's DAD detector as each peak eluted, are given in Figure 6.





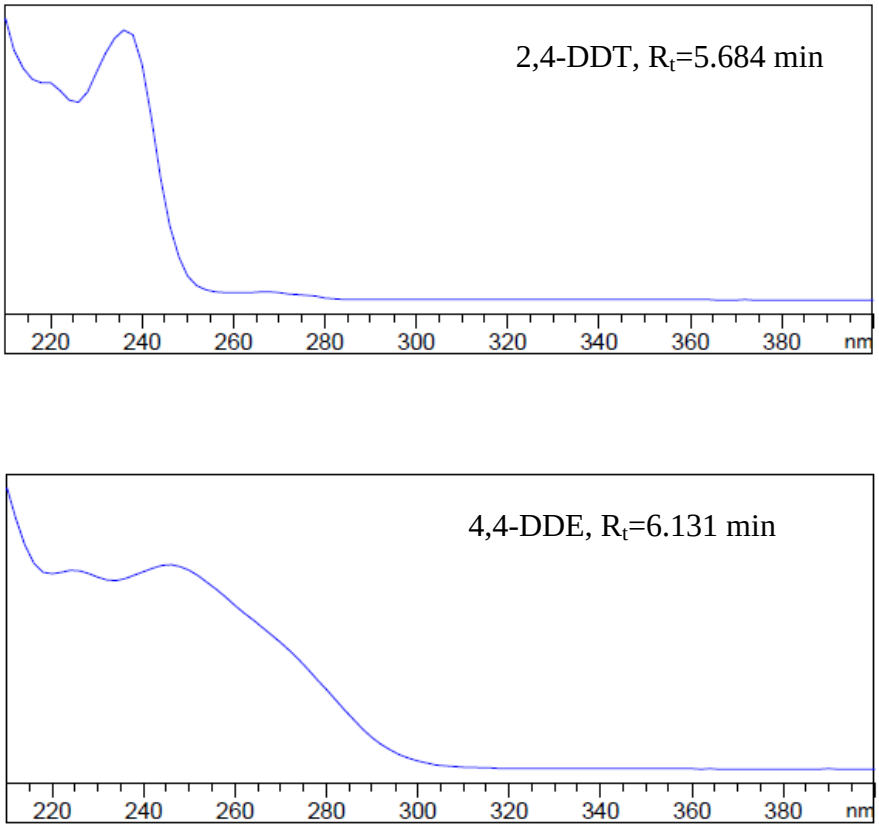


Figure 6: UV Spectrum of the four DDT standards obtained from the combined 2.5 ppm standard solution chromatogram.

It is interesting to mention here that with the exception of 4,4-DDT, we were unable to find any UV-Visible spectrums of the other components of DDT in the printed literature or on the web! Only one documented 4,4-DDT UV spectrum we found is produced below in Figure 7.

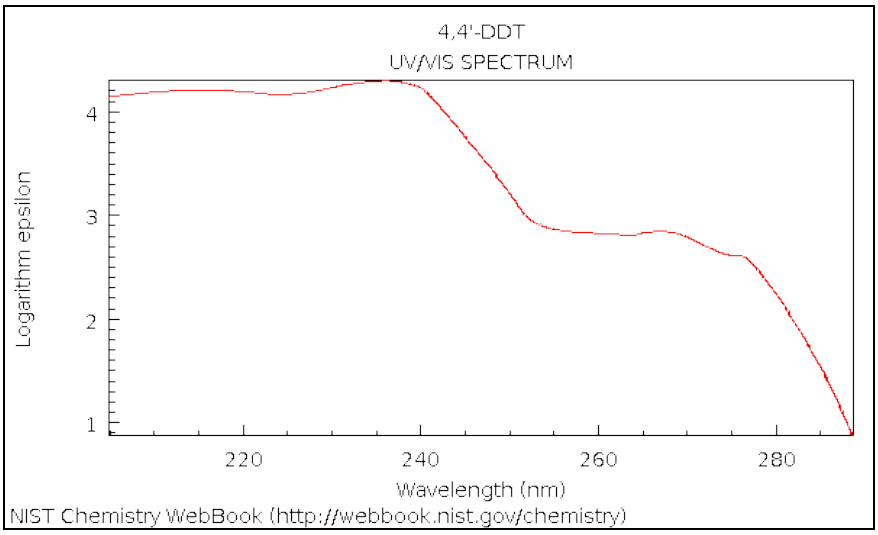


Figure 7: Published UV Spectrum of 4,4-DDT.

The absorbance spectrums of each of the DDT standards are all similar to each other, all absorbing strongly especially below 280 nm. In some reported HPLC methods for DDT determination, the suggested 254 nm detection wavelength appears to be very inappropriate as the absorptivity at this wavelength is not as great as that at lower wavelengths [39]. It was because of the spectrums that we decided to make measurements at 210 nm.

3.1.3 Repeatability and Reproducibility of Chromatograms

The data in the Tables 6, 7 and 8 above show that the retention times, peak areas and peak heights of the DDT components are very repeatable, when repeated on the same day. Also, very reproducible, when analysed on a different day. The differences in retention times and peak area/height are very small, all within 1% of each other. For example we have re-tabulated below the data for 4,4-DDE just to illustrate the point.

		R_t /minute	Peak area	Peak height /mAU
10 ppm 4,4-DDE 30 June 2014	**Mean (N=5)**	6.139	1520	113.2
	SD	0.006	5.9	0.4
	RSD %	0.09	0.39	0.40
2.5 ppm 4,4-DDE 30 June 2014	**Mean (N=3)**	6.135	378	28.9
	SD	0.008	19.1	0.6
	RSD %	0.12	5.1	2.1
2.5 ppm 4,4-DDE 22 June 2014	**Mean (N=3)**	6.069	379	28.8
	SD	0.040	5.2	0.1
	RSD %	0.66	1.4	0.4

The retention time for 4,4-DDE changes from 6.069 minutes to 6.131 or to 6.139 minutes. The biggest difference between these retention times is about 1%.

Similarly for peak areas we get 378 and 379 for 2.5 ppm and 1520 for 10 ppm. The peak areas are almost in the same ratio of 1 to 4 as their nominal concentration. In other words the peak area for 2.5 ppm standard is one quarter of the peak area for the 10 ppm solution. The same ratio is observed with peak heights, which are 28.8 and 28.9 mAU for 2.5 ppm and 113.2 mAU for 10 ppm. Again these values are in the ratio of 1 to 4.

What this data shows us is that individual DDT components can be identified by their retention time (in combination UV spectrum) with the HPLC and that peak area/height measured at a suitable wavelength is fairly proportional to its concentration. Although the peak areas or heights for the different components are similar, they are sufficiently different from each other to require separate calibration curve for each component obtained from its own standard. In other words, we should not use data for one component to calculate the concentration of another component.

Why this is so may be due to differences in the absorptivity of each DDT component or errors in the nominal concentration of the DDT standards.

3.1.4 Detection Limit

Since the largest standard deviation in peak area or peak height is around 5 %, we can assume a reasonable detection limit that corresponds to this percentage. That means that we should be able to detect and quantify with confidence when the peak area is greater than about 10 or the peak height is greater than about 1.0 mAU. In fact the HPLC system itself reports signals greater than 10 for area and 1.0 mAU for peak height as positive detection and not just noise.

3.1.5 Calibration Curves for the DDT Standards

Since the concentration and peak area/height data shows good repeatability and reproducibility, and also an almost linear relationship between them, the following calibration curves for each of the DDT standards were plotted.

Figures 8, 9, 10 and 11 are the calibration curves for 4,4 DDD, 2,4 DDT, 4,4 DDT and 4,4 DDE. The plots were made on Excel using three data points; 0, 2.5 and 10 ppm concentration on the horizontal axis and the corresponding absorbance as peak area measured at 210 nm on the vertical axis. Least squares line (through zero) along with its correlation coefficient, R^2, are also displayed on the plot.

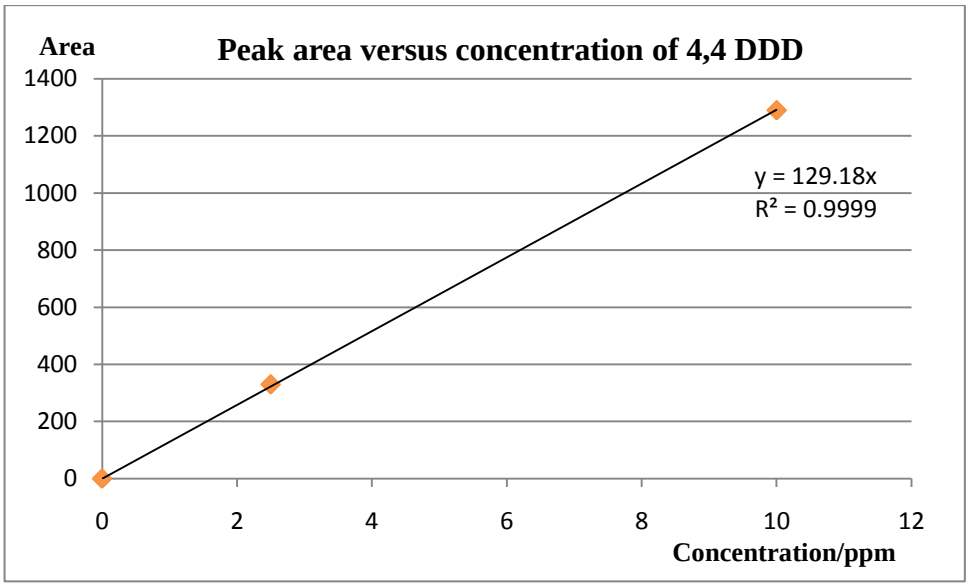


Figure 8: Calibration Curve for 4,4-DDD Standard

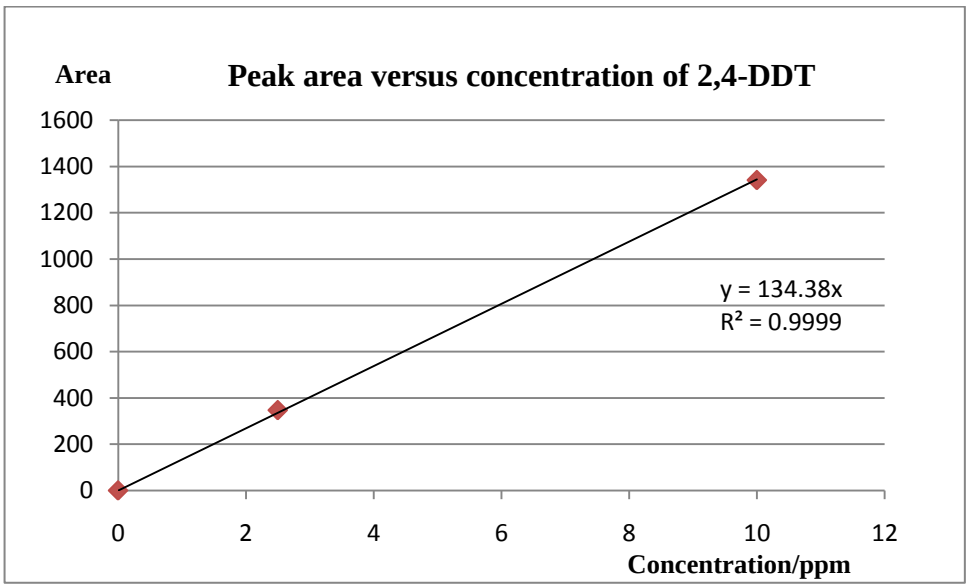


Figure 9: Calibration Curve for 2,4-DDT Standard

Note: Here, the 10 ppm for 2,4 DDT had done in different day.

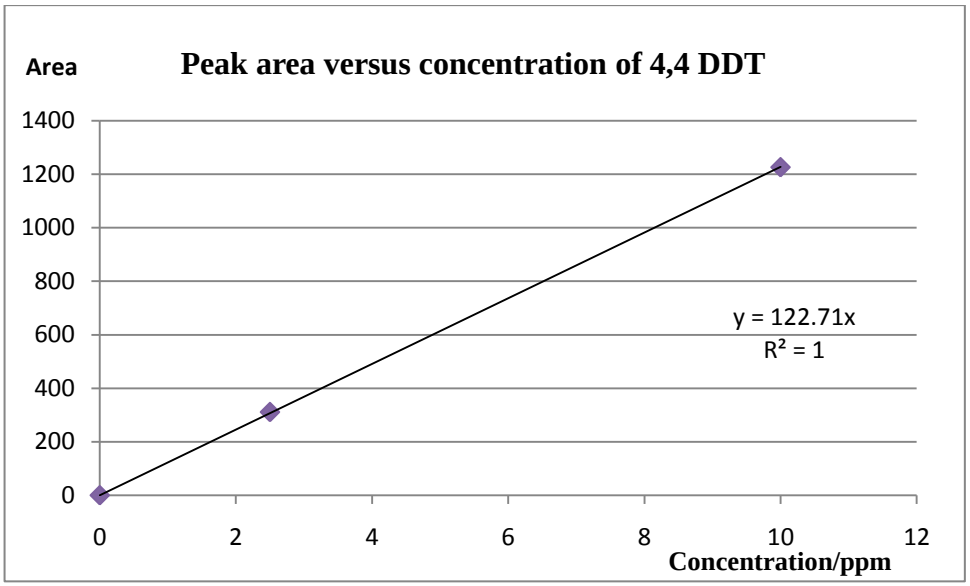


Figure 10: Calibration Curve for 4,4 DDT Standard

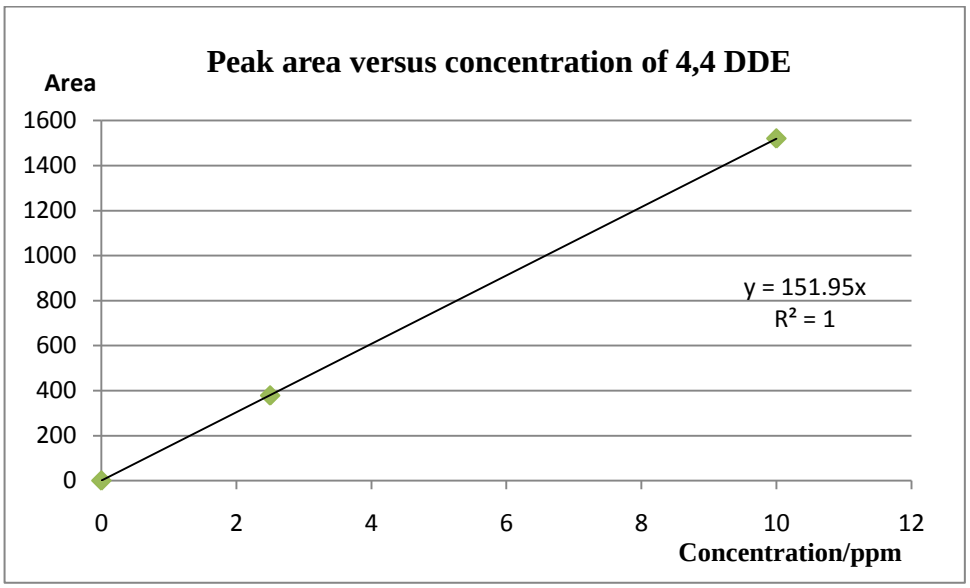


Figure 11: Calibration Curve for 4,4-DDE Standard

The fit of the least squares line for each plot is almost perfect with an R^2 value of 1.00 in all cases. These plots were subsequently used to determine the concentration of the residual DDT in the various soil extracts.

3.2 The Soil Extracts

First, the solvent system for soil extraction was decided upon. Then, using the solvent chosen, the soils were extracted and analyzed. One of the soils (E) was extracted in triplicate to assess the reproducibility of the sampling and extraction steps.

3.2.1 Solvent System for the Extractions

The air-dried and sieved soils were initially extracted with three different solvent systems. These solvents were:

- One to one mixture of acetone and n-hexane
- Methyl isobutyl ketone
- Methanol

To save time and avoid solvent wastage, not all the soil samples were tried. Soil sample A was extracted with each of the above solvents. Extractions were carried out in duplicate for AcHx and MIBK, but one time for MeOH and same procedure was followed for each. The only DDT component measurable was the 4,4-DDE component. The results are tabulated in Table 9.

Table 9: Extracting efficiency of different solvent systems.

Sample	**Rt**	**Peak area**	**Concentration /ppm**
4,4-DDE standard	6.003	381	2.5
A1AcHx	5.945	64	0.42
A2AcHx	5.960	83	0.54
A1MIBK	5.947	58	0.38
A2MIBK	6.002	88	0.58
A1MeOH	5.947	299	1.96

Based on these results it is clear that methanol was the most efficient extraction system for the soil. Methanol extracted 3 to 5 times more DDE than the other solvents. So the remaining extractions were all carried out with methanol.

3.2.2 The Methanol Soil Extracts

The soil samples were extracted with methanol (50 + 30 mL), filtered through a Whatman No 41 hardened ashless filter paper, the soil washed with portions of fresh methanol, and the entire methanol collected was evaporated in a round-bottom flask under vacuum on a rotary-evaporator at a temperature of 30°C. Once dried the residue in the flask was re-dissolved with 6.00 mL of HPLC grade methanol and transferred in to a stoppered sample bottle. When not used the extract solutions were kept in the freezer. Before analysis approximately one to two mL of each extract was filtered directly into the HPLC vials through a 0.20 µm Teflon membrane filter cartridge to get rid of any colloidal particles. The vials containing the extracts were then placed on the HPLC turret and analysed by automatic injection.

It was found that although a number of peaks were present in each chromatogram, the only DDT residue that could be positively identified by its retention time of 6.0 minutes and its characteristic UV spectrum was 4,4-DDE. To illustrate this point, the UV spectrum of 4,4-DDE standard and the UV spectrum of the peak at 6.072 minutes for sample E1 are given in Figure 12 by way of an example.

The remaining peaks in the soil extracts had retention times that were very different from the other three DDT standards, namely 44-DDD, 2,4-DDT and 4,4-DDT. Without further analysis it is not possible to identify any of the other peaks present in

the soil extracts. We can only suspect that they are residues of legal pesticides in current use.

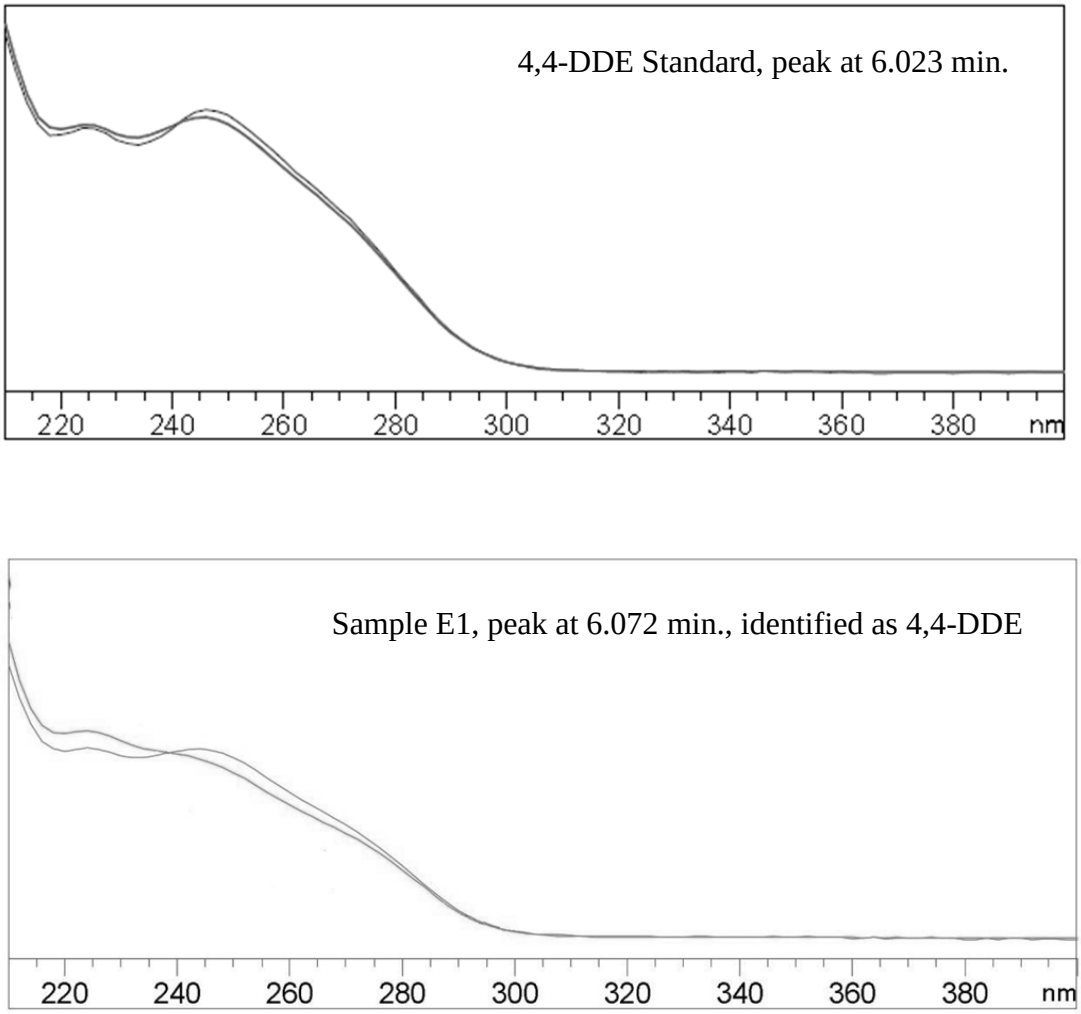


Figure 12: The UV Spectrums of 4,4- DDE standard (top); peak at 6.072 min of sample E_1 (bottom)

Table 10: The retention time, peak are, extract and calculated soil concentration of 4,4-DDE for each soil sample

Soil sample	R_t min	Peak Area	Component identified as 4,4-DDE	
			Extract Conc ppm	Soil Conc (calc.) ppm
A1MeOH	5.947	299	1.97	0.236
B1MeOH	6.015	184	1.21	0.145
C1MeOH	5.985	210	1.38	0.166
D1MeOH	6.117	536	3.53	0.424
E1MeOH	6.072	396	2.61	0.313
E2MeOH	6.091	345	2.27	0.272
E3MeOH	6.066	380	2.50	0.300

Note: E samples triplicate extraction and analysis

As for the 4,4-DDE results of the soils we sampled, there are no surprises since we know from the literature that commercial DDT under aerobic conditions degrades to 44-DDE rather than 4,4-DDD. So, since the field conditions where we sampled are clearly aerobic, any remnants of DDT would have degraded to 4,4-DDE.

We did not detect any 2,4 or 4,4-DDT indicating that at least in the areas where we have taken the samples DDT has not been in recent use. However, the presence of 4,4-DDE confirms that DDE was once used extensively in these fields.

The triplicate extraction of soil sample E as E1, E2 and E3 gave results that are quite close to each other. This indicates that the sub-sampling of the ground-sieved soil and its extraction steps are fairly consistent and reproducible. For heterogeneous materials such as soils it is not uncommon to have variations of more than 50 % between results. In this case the values for extracted DDE (2.27 ppm, 2.50 ppm and

2.61 ppm) were around 15 %. Therefore we have no reason to doubt the reliability of our method or measurements [40].

Finally, recent values of Serious Risk Concentration of DDT in soil according to RIVM report 711701 023 are given as 1 mg of 4,4-DDT/kg dry soil and 1.3 ppm of 4,4-DDE/kg dry soil. This means that the measured levels of DDE (and hence total DDT) in the Beyarmudu region appears to be reasonably low and acceptable, at least by today's standards.

3.3 Chromatographic Analysis of Samples

The actual HPLC chromatograms of the soil extracted samples with methanol are given in Figures 13 to 19.

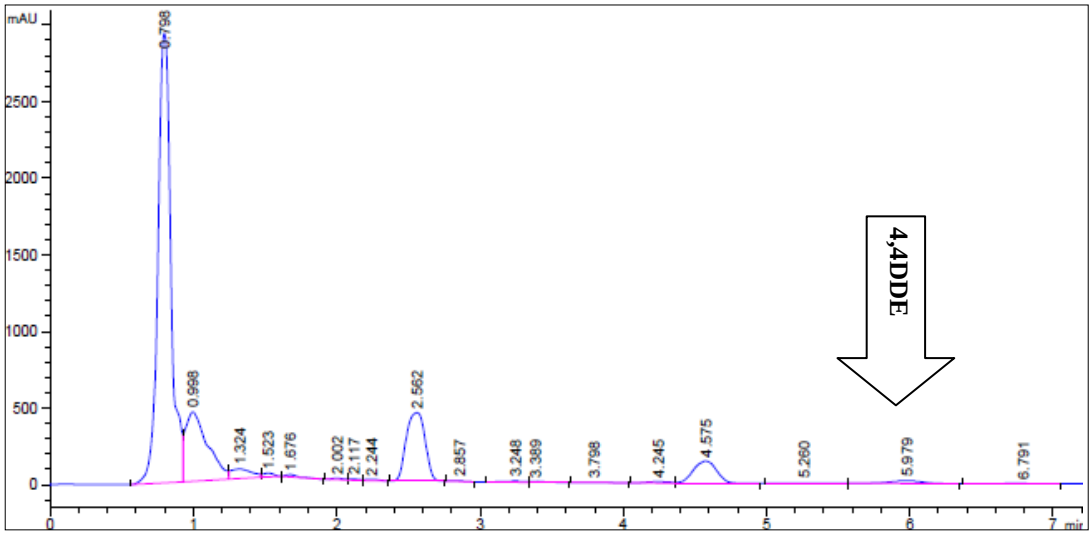


Figure 13: Chromatogram of Sample A

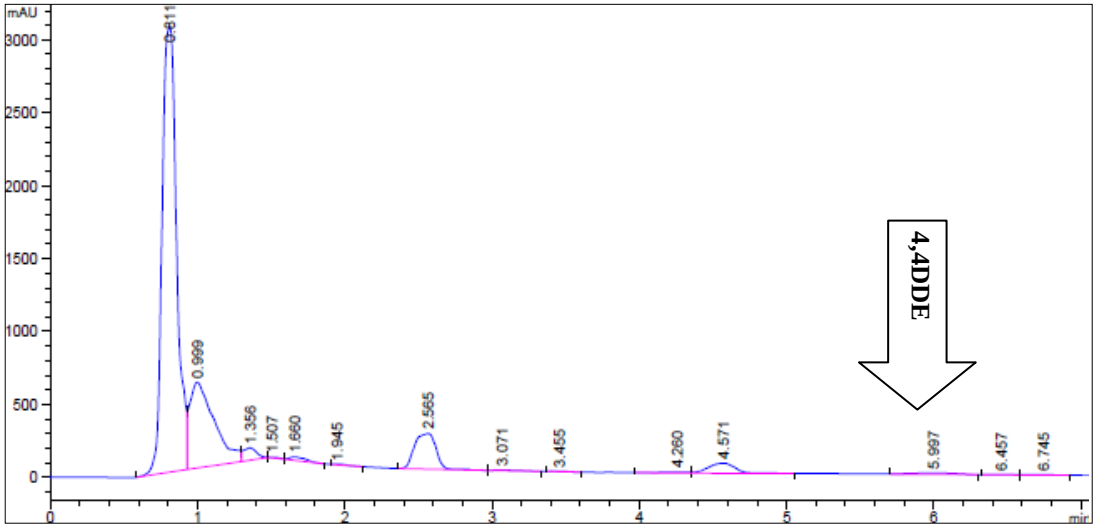


Figure 14: Chromatogram of Sample B

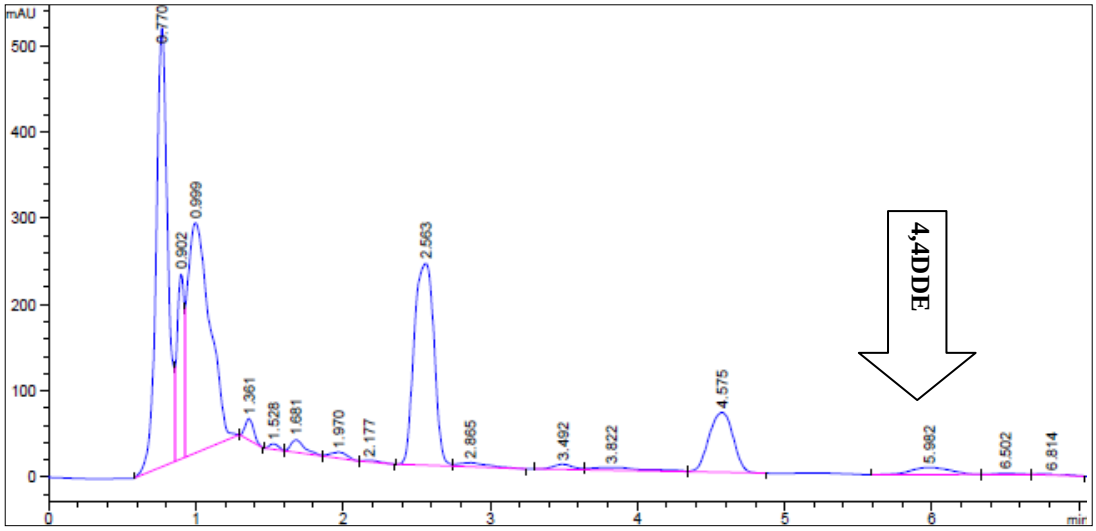


Figure 15: Chromatogram of Sample C

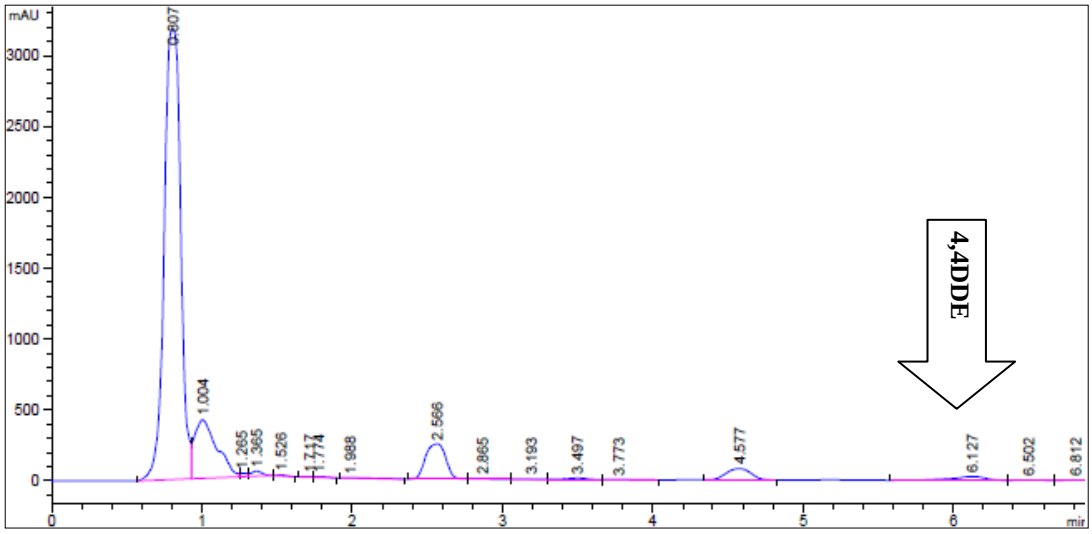


Figure 16: Chromatogram of Sample D

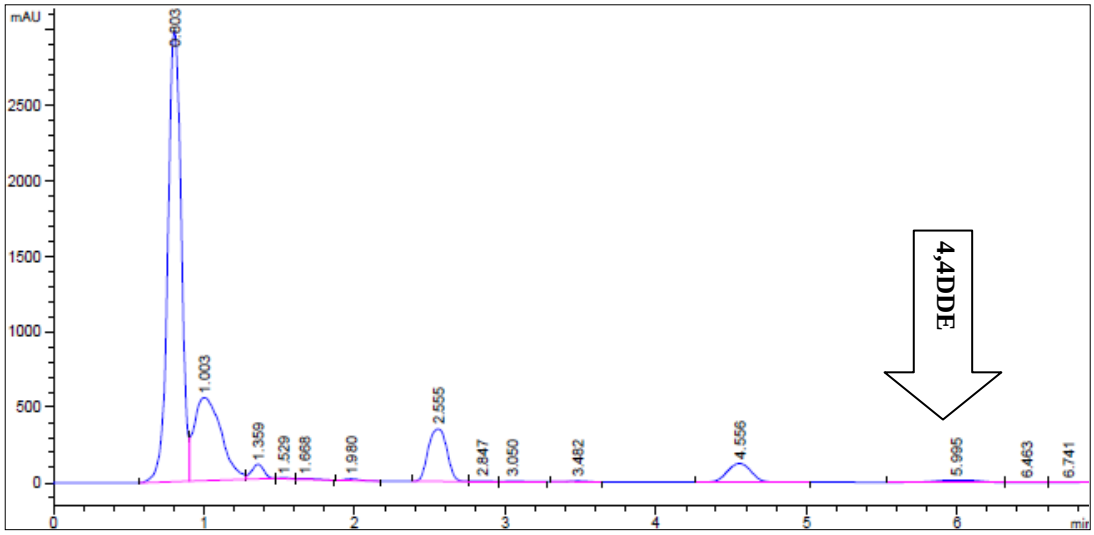


Figure 17: Chromatogram of Sample E_1

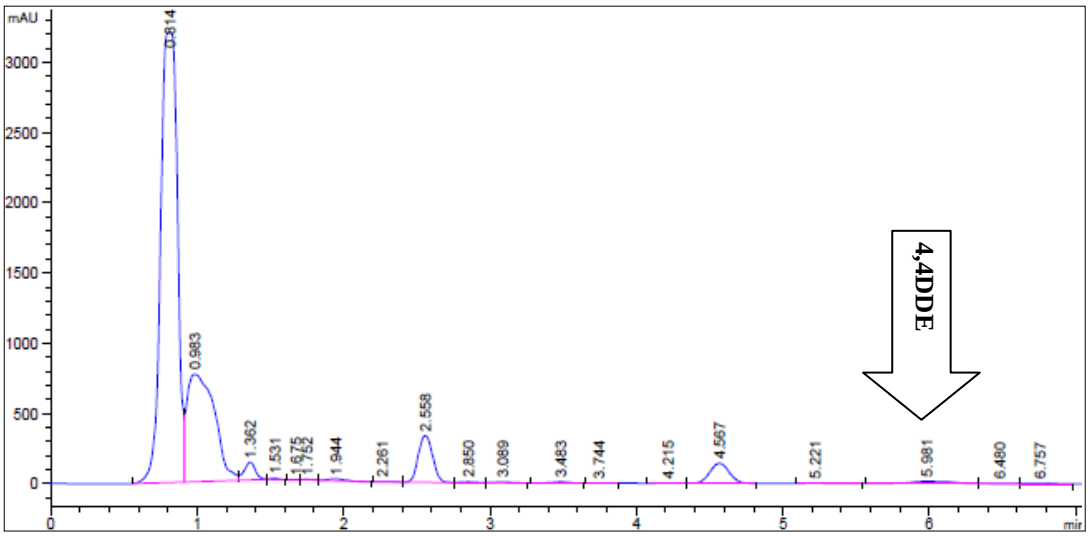


Figure 18: Chromatogram of Sample E_2

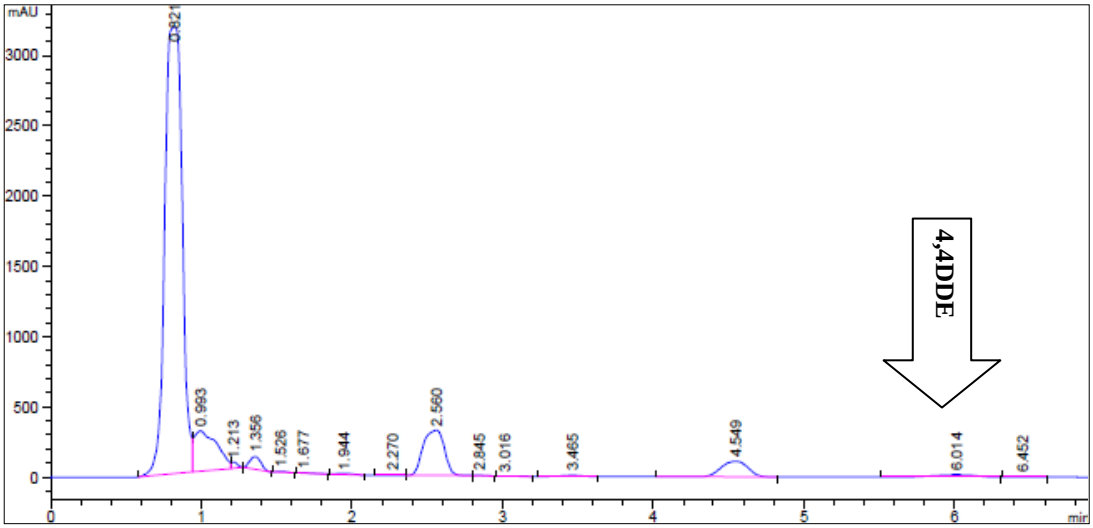


Figure 19: Chromatogram of Sample E_3

Chapter 4

CONCLUSION

In this work we tried to conduct a brief survey for DDT contamination of agricultural soils in Beyarmudu region of north Cyprus. The region is well known for high quality potato growing but is also suspected that the producers use excessive amounts of pesticides. As a result, our aim was to answer the thesis questions posed in the introduction. Based on our results we can conclude the following:

1. *Can we detect/identify and measure DDT constituents (4,4'-DDT, 2,4'-DDT, 4,4'-DDD and 4,4'-DDE) in our labs by HPLC with UV-Visible detection?*
2. *How accurate and precise are the retention time, R_t, and peak area/height information for identifying components and determining them quantitatively? And what range of concentrations can we measure with this technique?*

We have shown that we can indeed detect, conclusively identify and measure DDT constituents quantitatively. Our data for the 2.5 and 10 ppm standards gave very consistent and reproducible peaks with well-defined retention times and peak area or height, which correlates linearly with concentration. Additionally the UV spectrum provided by the diode array detector helps to confirm identity of the components in a given peak. Our results show that we can confidently measure concentrations of about 0.1 ppm DDT component in methanol solution. In this present work, because we used 50 g of dry soil, this corresponds to an actual soil concentration of $\frac{0.1\ ppm \times 6\ mL}{50\ g} = 0.012$ mg DDT/kg soil or 12 ppb of DDT in the soil. This level can be

reduced even further either by using a greater amount of soil for extraction or reducing the volume of solvent used to re-dissolve the filtered and evaporated soil extract. Also accuracy can be improved by doing standard addition to the samples to be analysed.

3. *Is the solvent system and the extraction regime used suitable to extract and determine quantitatively DDT components in the soils studied?*

The solvent system found to be the most efficient extracting agent amongst the three solvents tried was methanol. It extracted the greatest amount for each peak in the chromatogram. Methanol extracted 3 to 5 times more 4,4-DDE than the other solvents.

The consistency and the high degree of agreement between replicate extraction results also indicate that the extraction regime works well.

4. *How much DDT residue is present in the five soil samples? and*
5. *Is there sufficient evidence to start a country wide survey for pesticide residues in the agricultural soils to determine the severity of the problem?*

Using the current method, we were able to detect and measure only 4,4-DDE in the soils we sampled. The other components were either not present or below our detection limits. The concentration of 4,4-DDE in the soils sampled vary from 0.145 ppm for sample B to a maximum of 0.424 ppm for sample D. These concentrations are not higher than those prescribed by some European agencies. So based on these results and on the fact that no DDT could be detected; we can safely assume that use or application of DDT - at least in the areas sampled – has stopped and the residual 4,4-DDE that we measured is simply the degradation product of 4,4-DDT.

This however does not preclude the need for a broader more detailed survey of not just DDT but of the many other pesticides used in the country.

The methanol extracts of the soils we sampled show peaks with significantly large absorbance values. These peaks may correspond to other pesticides that are currently and legally being used. To identify and measure them will require standards of the currently legal and used pesticides to be obtained. With known standards, and face to face interviews with the producers, it should be possible to map the level and nature of pesticide “pollution” in north Cyprus.

REFERENCES

[1] Gunduzler, I. (2012). (Did we eat the poisoned potatoes) Zehirli patatesleri yedik mi?. Retrieved from http://haberkibris.com/bizi-kim-zehirliyor-2012-05-25.html. 25/05/2012

[2] Aydeniz, O. (2011). (Pesticides that are threatening our health) Sağlığımızı tehdit eden zehirler. Retrieved from http://www.kibrispostasi.com/index.php/cat/1/col/117/art/13865/PageName/KIBRIS_POSTASI. 23 Aralık 2011

[3] Yenidüzen. (2014). (Ministry of Health releases cancer incidence rates) Sağlık Bakanlığı kanser oranlarını açıkladı. Retrieved from http://www.yeniduzen.com/Haberler/haberler/saglik-bakanligi-kanser-oranlarini-acikladi/36761. 02 Nisan 2014

[4] Yenidüzen. (2012). (Was DDT imported) DDT İthal mı edildi?. Retrieved from http://haberkibris.com/ddt-ithal-mi-edildi-2012-05-26.html. 26/05/2012

[5] WIKIPEDIA, DDT. Retrieved from http://en.wikipedia.org/wiki/DDT.

[6] Carson, R. (2007). DDT. Retrieved from http://www.pbs.org/moyers/journal/09212007/profile2.html. September 21, 2007

[7] Chikuni, O., Nhachi, C. F. B., Polder, A., Bergan, S., Nafstud, I., & Skaare, J. U. (2002). Effects of DDT on paracetamol half- life in highly exposed mothers in Zimbabwe. *Toxicology Letters, 134,* 147-153.

[8] Feng, L., Fu, C., Yuan, D., & Miao, W. (2014). A P450 gene associated with robust resistance to DDT in ciliated protozoan, Tetrahymena thermophila by efficient degradation. *Aquatic Toxicology,* 149, 126-132.

[9] Veljanoska-Sarafiloska, E. M., Jordanoski, M., & Stafilov, T. (2013). Presence of DDT metabolites in water, sediment and fish muscle tissue from Lake Prespa, Republic of Macedonia. *Journal of Environmental Science and Health, Part B: Pesticides, Food Contaminants, and Agricultural Wastes, 48, 548-558.*

[10] CRUISING CHEMISTRY, Pesticides. Retrieved from http://people.chem.duke.edu/~jds/cruise_chem/.

[11] Younas, A., Hilber, I., Rehman, S., Khwaja, M., & Bucheil, T. D. (2013). Former DDT Factory in Pakistan revisited for remediation: severe DDT concentrations in soils and plants from within the area. *Environ Sci Pollut Res***,** *20,* 1966-1976.

[12] Xin, J., Liu, X., Liu, W., Jiang, L., Wang, J., & Niu, J. (2011). Production and use of DDT containing antifouling paint resulted in high DDTs residue in three paint factory sites and two shipyard sites, China. *Chemosphere, 84,* 342-347.

[13] Rios, L. E. (2010). Removal of DDT from Soil using Combinations of Surfactants. *University of Waterloo. Applied Science in Chemical Engineering*, 1-90.

[14] WHO (2006) Indoor residual spraying: *use of indoor residual spraying for scaling up global malaria control and elimination.* Global Malaria Programme. WHO/HTM/MAL/2006.1112. World Health Organization.

[15] Van den Berg, H. (2008). Global status of DDT and its alternatives for use in vector control to prevent disease. *Stockholm Convention on Persistent Organic Pollutants*, 1-31.

[16] Liu, C., Li, S., Gao, R., Dang, J., Wang, W., & Zhang, Q. (2014). Mechanism and kinetic properties of NO_3- initiated atmospheric degradation of DDT. *Journal of Environmental Sciences, 26*, 601-607.

[17] DDT. Technical in nature for individuals with a scientific background or familiarity with the regulation of pesticides; Oregon State University and the U. S. Environmental Protection Agency, *National Pesticide Information Center*, 1999, 1-6.

[18] Bioassays of DDT, TDE, and p,p'-DDE for possible carcinogenicity. *National Cancer Institute Carcinogenesis Technical Report Series, 1978, 131*, 1-117.

[19] Bate, R. (2007). The Rise, Fall, Rise, and Imminent Fall of DDT. *American Enterprise Institute for Public Policy Reasearch*, 14, 1-9.

[20] Gebremichael, S., Birhanu, T., & Tessema, D. A. (2013). Analysis of organochlorine pesticide residues in human and cow's milk in the towns of Asendabo, Serbo and Jimma in South-Western Ethiopia, *Chemosphere,* 90, 1652-1657.

[21] Muñoz-Arnanz, J., & Jiménez, B. (2011). New DDT inputs after 30 years of prohibition in Spain. A case study in agricultural soils from south- western Spain. *Environmental Pollution, 159,* 3640-3646.

[22] U.S Environmental Protection Agency, DDT- A Brief History and Status. Retrieved from http://www.epa.gov/pesticides/factsheets/chemicals/ddt-brief-history-status.htm.

[23] Channa, K., Röllin, H. B., Nøst, T. H., Odland, J. Ø., & Sandanger, T. M. (2012). Prenatal exposure to DDT in malaria endemic region following indoor residual spraying and in non- malaria coastal regions of South Africa. *Science of the Total Environment, 429,* 183-190.

[24] Humphries, M. S. (2013). DDT residue contamination in sediments from Lake Sibaya in northern KwaZulu- Natal, South Africa: Implications for conservation in a World Heritage Site. *Chemosphere, 93,* 1494-1499.

[25] Bashour, I. I., Dagher, S. M., Chammas, G. I., Lteif, A. E., & Kawar, N. S. (2004). DDT Residues in Lebanese Soils. *Journal of Environmental Science and Health, Part B: Pesticides, Food Contaminants, and Agricultural Wastes, 39*(2), 273-283.

[26] World Health Organization (WHO). (1989). DDT and its derivatives-environmental aspects, Environmental Health Criteria 83. World Health Organization, Geneva, Switzerland. Retrieved from http://www.inchem.org/documents/ehc/ehc/ehc83.htm.

[27] Eco-USA, DDT. Retrieved from www.eco-usa.net/toxics/chemicals/ddt.shtml.

[28] Wang, S., Wang, Z., Zhang, Y., Wang, J., & Guo, R. (2013). Pesticide residues in market foods in Shaanxi Province of China in 2010. *Food Chemistry, 138*, 2016-2025.

[29] Sinha, S. N., Bhatnagar, V. K., Doctor, P., Toteja, G. S., Agnihotri, N. P., & Kalra, R. L. (2011). A novel method for pesticide analysis in refined sugar samples using a gas chromatography-mass spectrometer (GC-MS/MS) and simple solvent extraction method. *Food Chemistry, 126*, 379-386.

[30] Domotorova, M., & Matisova, E. (2008). Fast gas chromatography for pesticide residues analysis. *Journal of Chromatography A, 1207*, 1-16.

[31] Gyalpo, T., Fritsche, L., Bouwman, H., Bornman, R., Scheringer, M., & Hungerbühler, K. (2012). Estimation of human body concentrations of DDT from indoor residual spraying for malaria control. *Environmental Pollution*, 169, 235-241.

[32] Van Dyk, J. C., Bouwman, H., Barnhoorn, I. E. J., & Bornman, M. S. (2010). DDT contamination from indoor residual spraying for malaria control. *Science of the Total Environment, 408*, 2745-2752.

[33] Camino-Sa´nchez, F. J., Zafra-Go´mez, A., Ruiz-Garcı´a, J., Bermu´dez-Peinado, R., Ballesteros, O., Navalon, A., & Vı´lchez, J. L. (2011). UNE-EN ISO/IEC 17025:2005 accredited method for the determination of 121 pesticide residues in fruits and vegetables by gas chromatography-tandem mass spectrometry. *Journal of Food Composition and Analysis, 24*, 427-440.

[34] ATSDR. Toxicological profile for DDT, DDE, and DDD. U.S. Department of Health and Human Services, Public Health Service. Agency for Toxic Substances and Disease Registry: Atlanta, (2002).

[35] Bashour, I. I., Dagher, S. M., Chammas, G. I., & Kawar, N. S. (2003). Comparison of Gas Chromatography and Immunoassay Methods for Analysis of Total DDT in Calcareous Soils. *Journal of Environmental Science and Health, Part B: Pesticides, Food Contaminants, and Agricultural Wastes, 38*(2), 111-119.

[36] Singh, S. P., Bose, P., Guha, S., Gurjar, S. K., & Bhalekar, S. (2013). Impact of addition of amendments on the degradation of DDT and its residues partitioned on soil. *Chemosphere, 92*, 811-820.

[37] PubChem Compound, DDT. Retrieved from http://pubchem.ncbi.nlm.nih.gov/summary/summary.cgi?cid=3036#x351.

[38] Fourth Annual Report on Carcinogens, DDT (Dichlorodiphenyltrichloroethane; 1,1,1-Trichloro-2,2-bis(*p*-chlorophenyl)ethane). Retrieved from http://dwb4.unl.edu/Chem/CHEM869E/CHEM869ELinks/ntp-server.niehs.nih.gov/htdocs/8_RoC/RAC/DDT.html. CAS No. 50-29-3

[39] GBC Application Note, Number A1 01-0331-00. "Determination of DDT by C18 RP-HPLC." Accessed July 2014 http://www.gbcscientific.com/appnotes/HPLC_app_note_001.pdf.

[40] Lijzen, J. P. A; Baars, A. J; Otte, P. F; Rikken, M. G. J; Swartjes, F. A; Verbruggen, E. M. J; & van Wezel, A. P. (2001). Technical evaluation of the Intervention Values for Soil/sediment and Groundwater. *RIVM research for man and environment*, 1-147. Accessed July 2014: http://rivm.openrepository.com/rivm/bitstream/10029/9660/1/711701023.pdf.

APPENDIX

Appendix A: Important equations and calculations used in this study.

In this study the standard deviations in Tables 6, 7 and 8 were calculated from the following equation:

$$s = \sqrt{\frac{\sum(\chi - \bar{x})^2}{N-1}} \quad \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots (1)$$

Where, s is the standard deviation.

x is the each value in the sample.

$\bar{x}$ *is the mean of the values.*

N *is the number of values.*

Percent Relative standard deviation, % RSD was calculated by the following equation:

$$\%\text{RSD} = \frac{S}{\bar{x}} \times 100\ \% \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots (2)$$

Where, s is the standard deviation.

$\bar{x}$ *is the mean of the values.*

In Table 10 the "DDE soil concentration" values were calculated from the extract concentrations that had been determined by HPLC. Since the 6 mL of the extract had been obtained from 50 grams of soil, the following equation was use to convert extract concentration in to:

$$C = \frac{C\ extract\ \times V}{m} \quad \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots (3)$$

Where, C is the soil concentration of DDE (or DDT) in the sample taken.

$c_{extract}$ *is the solution concentration of the extract that we found from the calibration curve.*

v is the volume of the methanol extract, in this instance 6 mL for all the extracts.

And m is the mass of soil that had been extracted, which was 50 grams in this study.

For example, the soil concentration for soil sample A, whose $c_{extract} = 1.97 ppm$

$$C = \frac{C\ extract\ \times V}{m} = \frac{1.97\ Mg/ml \times 6\ ml}{50\ g} = 0.236\ \mu g/g\ (or\ ppm)$$