BIOSECURITY OF AGRICULTURAL PRODUCT THROUGH SPECIATION OF PESTICIDES: AYAŞ TOMATO

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JANUARY 2014

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A THESIS SUBMITTED TO
THE GRADUATE SCHOOL OF NATURAL AND APPLIED SCIENCES
OF
MIDDLE EAST TECHNICAL UNIVERSITY

BY

K M SIFATULLAH

IN PARTIAL FULFILLMENT OF THE REQUIREMENTS
FOR
THE DEGREE OF MASTER OF SCIENCE
IN
EARTH SYSTEM SCIENCE

JANUARY 2014

Approval of the thesis:

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I hereby declare that all information in this document has been obtained and presented in accordance with academic rules and ethical conduct. I also declare that, as required by these rules and conduct. I have fully cited and referenced all materials and results that are not original to this work.

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ABSTRACT

BIOSECURITY OF AGRICULTURAL PRODUCT THROUGH SPECIATION OF PESTICIDES: AYAŞ TOMATO

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January 2014, 93 pages

Speciation of chlorinated pesticides in soil-water-tomato plant is researched in connection with food security. For this purpose 16 soil, 16 tomato and 4 irrigation water samples were collected from selected two fields in Ayaş, Ankara, Turkey and analyzed with GC-MS system. Soil and tomato samples were extracted by using ultrasonic bath. On the other hand, water samples were extracted by using solid phase extraction. The extraction recoveries were determined as %64.88, %42.03, and %53.36 for tomato, water, and soil respectively. The concentration ranges of pesticides in soil, water and tomato samples are 3.799-219.1µg/kg, 0.1877-8.005µg/L and 8.302-23.03 µg/kg respectively. According to quality control/ quality assurance (QC/QA) studies, % error values are changing between % 5.90-55.6 and calibration curves has good linearity (R^2= 0.99). LOD values are 0.370-25.7 µg/kg for soil 0.680-23.7 µg/kg for water and 0.840-94.6 µg/kg for tomato. The order of pesticide contamination is like soil>water>tomato except Endrin aldehyde. For the comparison of the average pesticide concentrations with literature data, ANOVA analysis was utilized and according to results, Turkey is in middle of the countries in pollution level. According to MRL values, water used for irrigation is slightly contaminated with chlorinated pesticides. The investigation of pesticide transition from soil to water reveals that the highest % transfer ratio for pp-DDT (%69) and lowest ratio is for Endrine aldehyde (%37). For soil to tomato transfer the values are

% 42 (alpha-HCH) and %38(Endrine aldehyde). It was observed that from red and green tomato samples, the longer the tomato stayed in field (red tomato) the more pesticides are seen on tomato. The found concentrations will be a database for chlorinated pesticide pollution level in Ayaş-Turkey.

Keywords: Organochlorine Pesticides (OCPs), Tomato, Soil, Water, Gas Chromatography-Mass Spectroscopy (GC-MS).

ÖZ

TARIM ÜRÜNLERİNİN İÇERDİĞİ TARIM İLAÇLARI TÜRLERİ BAKIMINDAN BİYOGÜVENLİĞİ: AYAŞ DOMATESİ

Sifatullah, KM
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Ortak Tez Yöneticisi. Prof. Dr. Gürdal Tuncel
Ocak 2014, 93 sayfa

Klorlu pestisitlerin toprak, su ve domateste ayrı ayrı miktarları araştırılmış ve gıda güvenliğiyle ilişkilendirilmiştir. Bu amaçla 16 toprak, 16 domates ve 4 adet tarla su örneği Ayaş, Ankara, Türkiye mevkiindeki 2 adet tarladan toplanmış ve GC-MS ile analiz edilmiştir. Su örnekleri katı faz tekniğiyle özütlenmiştir. Toprak ve domates örnekleri ise ultrasonik banyo tekniğiyle özütlenmiştir. Özütleme verimi toprak, su ve domates örnekleri için sırasıyla %53.36, %42.03 ve %64.88 tür. Toprak, su ve domates örnekleri için konsantratsyon aralığı sırasıyla 3.799-219.1 µg/kg, 0.1877-8.005 µg/L and 8.302-23.03 tür. Kalite kontrol / kalite güvence araştırması sonuçlarına göre, hata sonuçları % 5.90-55.6 arasında değişmektedir, and kalibrasyon eğrilerinin linearitesi 0.99 civarındadır. Tayin sınırı değerleri su için 0.680-23.7 µg/L, domates için 0.840-94.6 µg/kg ve toprak için 0.370-25.7 µg/kg dır. Endrin aldehit hariç diğer klorlu pestisit kirliliği bakımından sıralama toprak>su>domates şeklindedir. Bulunan ortalama pestisit konsantrasyonlarını diğer ülkelerle karşılaştırmak amacıyla Anova analizi yapıldı ve sonuçlara göre Türkiye'nin sıralamada ortalarda yer aldığı belirlenmiştir. Maksimum artık seviyesi karşılaştırmalarına göre sulama suları bir miktar kirlenmiştir. Topraktan suya pestisit transferinin incelendiği araştırmaya göre suya en yüksek oranda pp-DDT(%69) and düşük oranda ise endrin aldehit (%37) geçmektedir. Bu oranlar topraktan domatese olan geçişler için maksimum % 42(alfa-HCH) ve minimum %38(Endrin aldehit) şeklindedir. Domates tarlada ne kadar uzun kalırsa o kadar çok pestisite rastlandığı gözlenmiştir kırmızı dometes uzun sure tarlada kalimş. Bulunan konsantrasyonlar Türkiye -Ayaş bölğenin pestisit kirliliği seviyesi açısından bir veribankası niteliği taşımaktadır.

Anahtar Kelimeler: Organokolorlu pestisit, Domates, Toprak, Su, Gaz komotografisi-kütle spektroskofisi.

To my family

ACKNOWLEDGEMENTS

I would like to express my appreciation to my supervisor Prof. Dr. Semra TUNCEL and Prof. Dr. Gürdal TUNCEL for their support and guidance throughout this study.

I would like to thank to Dr. Tansel TOPAL and Pınar GÖKMEN for their endless support and help during this study.

I would like to thank to my dear lab mates Hazal AYDİŞ and Damla ŞENLİK for her support and help during this challenging period.

I would like to thank all Bangladeshi students at METU and Turkey for their moral support.

Lastly, my deepest gratitude goes to my dear mother and my family who always supported me.

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LIST OF ABBREVIATIONS

ACE: Accelerated Solvent Extraction
BLOQ: Below Limit of Quantification
DDT: Dichlorodiphenyltrichloroethane
DDE: Dichlorodiphenylethane
DDD: Dichlorodiphenyldichloroethane
DOC: Dissolve Organic Carbon
EAF: The European Federation of Agricultural workers
EC: The European Commission
ECD: Electron Capture Detector
EFSA: The European Food safety Authority
EPPO: The European and Mediterranean Plant Protection Organization
EU: European Union
FAO: Food and Agricultural Organization
GC: Gas Chromatography
GPS: Global Positioning System
HCH: Hexachlorocyclohexane
HSSPME: Head Space Solid Phase Micro Extraction
IPM: Integrated Pest Management
IUPAC: International Union of Pure and Applied Chemistry
K_{ow}: Octanol-water Coefficient
LD_{50}: Lethal Dose 50%
LC_{50}: Inhalation Toxicity
EC_{50}: half maximal effective concentration
LOD: Limit of Detection
LOQ: Limit of Quantification
MRL: Maximum Residue Level
MS: Mass Spectrometry
OCPs: Organochlorine Pesticides

POPs: Persistent Organic Pollutants
QA: Quality Assurance
QC: Quality Control
SIM: Selected Ion Monitoring
S/N: Signal to Noise Ratio
SPE: Solid Phase Extraction
SPM: Suspended Particulate Matter
SRM: Standard Reference Material
TUIK: Turkiye Istatistik Kurumu
TURKSTAT: Turkish statistics institutes
UBE: Ultrasonic Bath Extraction
UNCTAD: United Nations Conference on Trade and Development
USA: The United States of America
USEPA: United States Environmental Protection Agency
WHO: World Health Organization
% RSD: Percent Relative Standard Deviation

CHAPTER 1

INTRODUCTION

1.1. Introduction

Food safety is gaining importance as a global issue and the farmers are worried to provide quality and cheap food for consumers. Focus on the food safety is not only concerns of the rich countries or poor countries it is a concerned for every citizens. There should be a plan for food and water safety for the whole World population. Governments in many countries have established new institutions, standards, and methods for regulating food safety and have increased investments in hazard control. Food safety is gaining full attention due to several worldwide trends affecting food systems and scarcity of quality food. The urbanization in developing countries, migration of people, live animals, and food products changes the food production patterns. Illness due to contaminated food was the most widespread health problem in the modern World, and an important cause of reduced economic productivity stated by Food and Agriculture Organization of the United Nations (FAO) and the World Health Organization (WHO) in 1983. The FAO/WHO-sponsored International Conference on Nutrition recognized that hundreds of millions of people suffer from communicable diseases caused by contaminated food and drinking water in 1992, (Karim, 1997). The U.N. Conference on Environment and Development in 1992 recognized that food was a major vehicle for the communication of environmental contaminants both chemical and biological to human. In the conference they urged the World and governments to take measures to prevent or minimize these food safety threats. The World Health assembly, the top governing body of WHO adopted a common resolution recognizing food safety as an essential public health functions in 2000. Food should not be considered only an agricultural or trade commodity, but also a public health issue. Along the entire food chain from farm to table food safety must keep in mind. The government, industry, and consumers must share equal responsibilities for food safety issue. The over use of synthetic chemicals to control pests and diseases has become widespread in the 20th century especially after the Second World War. An increase in food production has been seen with the increase of World population and demand for food. However, the quality of food over this time has been decreased and farmers aim to get more and more yield. Synthetic pesticides reached in the market to fulfill the needs for farmer for maximum yield of crops. Agrochemicals used to eliminate the attack of various pests on agricultural crops are called as pesticides. In agriculture pest is defined as any living organism interfering with the agricultural activity in a negative way. The major pests hamper the growth of agricultural crops, are insects, fungi, and weeds. Pesticides are grouped in many classes. The organochlorine pesticides is chlorine contained pesticides. They persistence in the environment, and laws are implemented to limit their usage. However, developing countries still use pesticides, especially organochlorine pesticides since they are cheap and easy to access. Organochlorine pesticides (OCPs) are constant contaminants in the environment. OCPs are known as hydrophobic compounds and have low aqueous solubility, which causes their adsorption or sorption to dissolve organic carbon (DOC) and suspended particulate matter (SPM) on the surfaces.

1.1.1. Importance and types of Pesticides

Pesticide is substance or mixture of substances used for preventing, destroying or controlling pest, including human or animal disease. Plants or animals causing harm during the agricultural production, processing, storage, transport or marketing of food therefore, pesticides are used widely. Pesticides are also used as a plant growth regulator, defoliant, desiccant or agent for thinning fruit or preventing the premature fall of fruit. They are applied to crops either before or after harvest to protect the product from deterioration during storage and transport (FAO, 2005). Pesticides became very important for the agricultural yield. Farmer takes risk in order to grow more food and high quality food to serve the people demands in the market and aims to earn more. Nowadays, the overuse of pesticides becomes a big concerned for the consumers. After the Second World War excess pesticides was used for agriculture in addition to cure human and animal diseases. Among them most important ones are herbicides, insecticides and fungicides.

1.1.2 Herbicides

Herbicides used in early stage of growing season for weed control. They may also be used at the end of the growing season before planting a new crop. Herbicides can be used to target to a particular type of plant control. Nowadays, some crops are seen which are genetically engineered to be resistant to the effects of herbicides. Modern synthetic herbicides have low mammalian toxicity, because they are designed to mainly affect specific metabolic pathways within plants. The important class of herbicides chemicals is the triazines, the ureas, and the sulfonylureas use in plant protection.

1.1.3. Insecticides

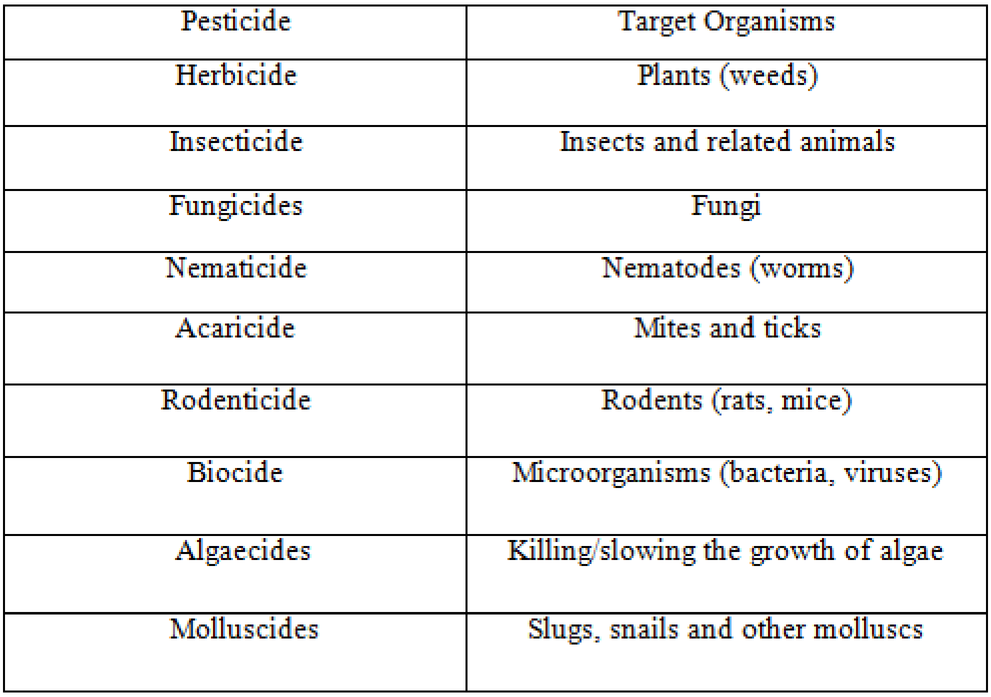
Insecticides are another important chemical class commonly referred to as organochlorines, organophosphorus compounds, carbamates, pyrethroids, insect growth regulators, and the nicotinyl chloronicotinyl compounds. A common term used for the persistent organochlorine pesticides is organochlorines. It includes the cyclodienes, DDT and DDT derivatives compounds, lindane and the hexachlorocyclohexanes, and toxaphene and they were commonly used synthetic insecticides. After World War II the excess use of chemicals were generally long-acting. Because of the high fat solubility and chemical stability in environment and in agricultural products, bioaccumulation can take place over time.

The volatility of pesticides changes region to region and with the temperature. They can also travel long distances. These organochlorine pesticides have been banned for agricultural use in Turkey, EU and USA because of the concerns about environmental persistence, bioaccumulation, and trans-boundary movement.

1.1.4. Fungicides

Fungicides are another important group of pesticides next to herbicides and insecticides. The chemical classes of fungicides are the azoles compounds. However, for herbicides and insecticides, there is a different range of chemical types. Fungicides are two types protectant and systemic. Fungicides protect the plant or fruit against infection at the site of application and do not penetrate the tissue. Systemic fungicides penetrate the plant and prevent disease from developing on parts of the plant. They often control disease by eradication of the fungus. The different pesticides types and target organisms are shown in the Table 1.1.

Table :1.1. Pesticides and Target Organisms



Pesticide	Target Organisms
Herbicide	Plants (weeds)
Insecticide	Insects and related animals
Fungicides	Fungi
Nematicide	Nematodes (worms)
Acaricide	Mites and ticks
Rodenticide	Rodents (rats, mice)
Biocide	Microorganisms (bacteria, viruses)
Algaecides	Killing/slowing the growth of algae
Molluscides	Slugs, snails and other molluscs

1.2. Organochlorine Pesticides (OCPs)

Organochlorine (OCPs) pesticides is one of the important type of pesticides. The long life duration in the environment is due to the properties of pesticides with lipophilic nature, hydrophobicity, and low chemical and biological degradation. Organochlorine pesticides (OCPs) can also be accumulating in the biological tissues and which can increase the pesticides concentrations in food chain. Organochlorine pesticides are called with three names as chlorinated hydrocarbons, the chlorohydrocarbon, and organochlorine. Dichlorodiphenyltrichloroethane (DDT) is the most well known member of the chlorinated pesticides. DDT is biodegraded to DDE under aerobic conditions and to DDD under anaerobic conditions (Zhou, 2006). In the history the use of DDT has an important place. During the Second World War the excess use was observed and the book titled “Silent Spring” by Rachel Carson (RFF) drew the attention of the effects of pesticides on birds. During Second World War pesticides were used to cure diseases then slowly became important for agriculture (Connell 2005).

When the stucture of pesticide is considered the types of bonds that can be seen are as follows C:C (aromatic), C=C, C-H, C-Cl and lesser number of C-C. Among them only C-H and C-Cl bonds have low dipole moments. This makes the compounds in this group to have low polarity, being fat soluble or lipophilic and having low solubility in water. Tables 1.2 and 1.3 shows the scientific IUPAC names and chemical physical properties of pestisides.Physical states of almost all pestisides are solid and low solibulity in water. (Connel, 2005).

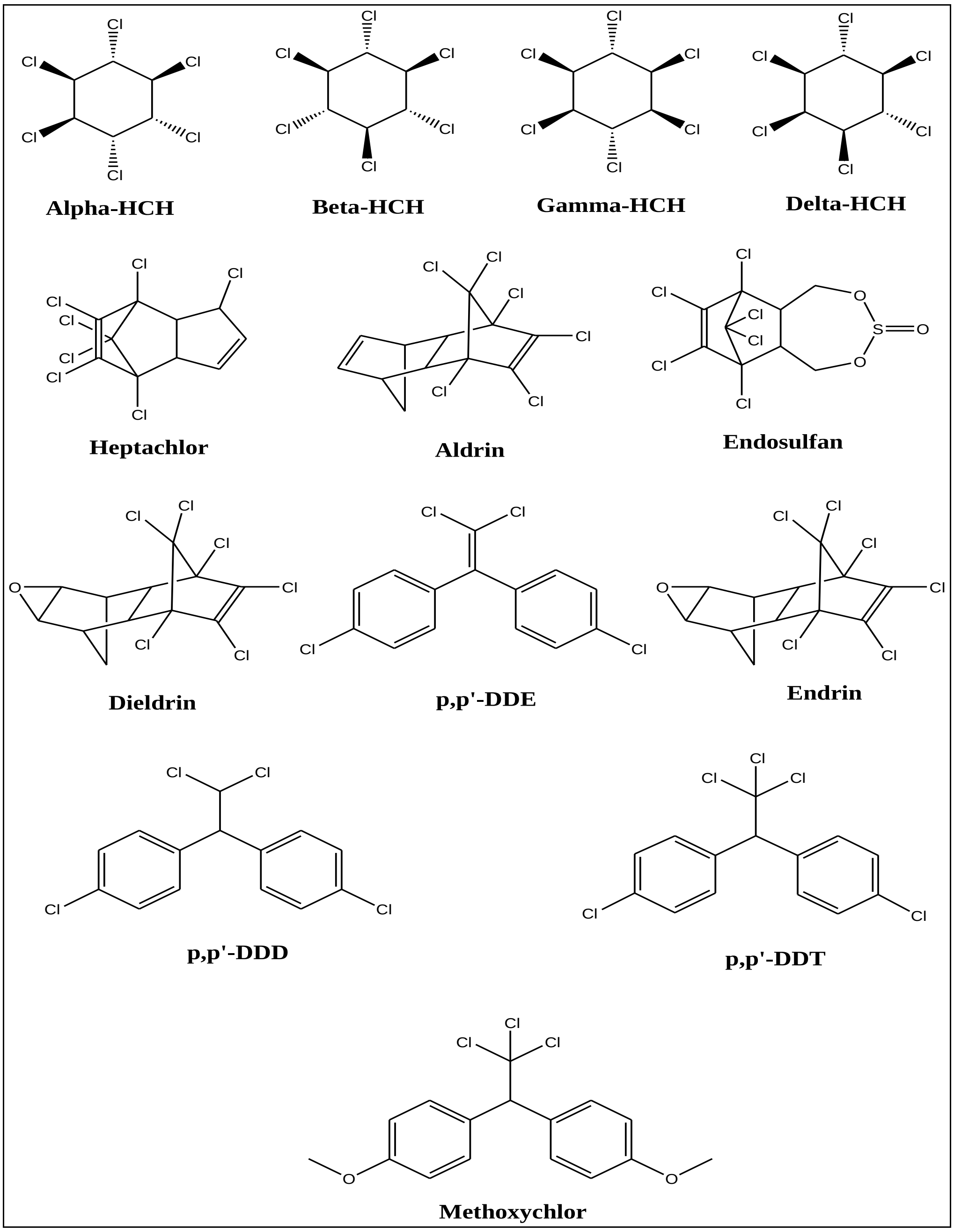


Figure :1.1 Chemical Structure of Organochlorine Pesticides

Table :1.2. Chemical Identities of Organochlorine Pesticides

Pesticide Name	**CASNumber**	**Formula**	**IUPAC Nomenclature**
Aldrin	309-00-2	$C_{12}H_8Cl_6$	1,2,3,4,10,10- hexachloro-1,4,4a,5,8,8a-hexahydro-1,4:5,8-Dimethanonaphthalene
DDT	50-29-3	$C_{14}H_9Cl_5$	1,1,1-trichloro-2,2-bis(4-chlorophenyl)ethane
DDD	72-54-8	$C_{14}H_{10}Cl_4$	1,1-dichloro-2,2-bis(4-chlorophenyl)ethane
DDE	72-55-9	$C_{14}H_8Cl_5$	1-chloro-4-[2,2-dichloro-1-(4-chlorophenyl)ethenyl]benzene
Dieldrin	60-57-1	$C_{12}H_8Cl_6O$	1,2,3,4,10,10-hexachlor-6,7-epoxy-1,4,4a,5,6,7,8a-oktahydrogenendo, exo-1,4:5,8-dimethanonaftalen
Endosulfan	959-98-8	$C_9H_6Cl_6O_3S$	1,4,5,6,7,7-hexachlor-,cyklický sulfit,endo-5-norbornen-2,3-dimethan
HCH	58-89-9	$C_6H_6Cl_6$	1a,2a,3b,4a5a,6b-hexachlorocyclohexane
Endrin	72-20-8	$C_{11}H_8Cl_6O$	1,2,3,4,10,10-hexachlor-6,7-epoxy-1,4,4a,5,6,7,8,8a-oktahydrogenendo, endo-1,4:5,8-dimethanonaftalen
Heptachlor	76-44-8	$C_{10}H_5Cl_7$	1,4,5,6,7,8,8-heptachloro-3a,4,7,7a-tetrahydro-4,7-methanoindene
Methoxychlor	72-43-5	$C_{16}H_{15}Cl_3O_2$	1,1,1-trichloro-2,2-bis(4-methoxyphenyl)ethane

1.2.1. Physical and Chemical Properties of Organochlorine Pesticides

Table :1.3. Physical and Chemical Properties of Organochlorine Pesticides

Pesticide	Molecular Weight(g/mol)	Physical State	Water Solubility mg/L	VaporPressure (mPa)	log Kow	Log Koc	Half Life (days)
Aldrin	365	S	0.03	3.10	7.40	4.70	365
DDT	354	S	0.04	0.02	1.00	5.60	5694
DDD	320	S	0.05	0.14	6.20	5.40	5694
DDE	318	S	0.14	0.86	6.90	5.90	5694
Dieldrin	381	S	0.25	0.05	6.20	4.10	1000
Endosulfan	407	S	0.32	0.83	3.10	4.10	43
Endo- sulfate	423	S	0.22		3.70	4.10	
Endrin	381	S	0.23	0.02	5.30	4.00	4300
g-HCH	291	S	7.30	5.61	6.80	3.10	423
Heptachlor	373	S	0.06	53.05	5.50	4.40	250
Methoxychlor	346	S	0.10	0.35	4.30	4.90	170

1.3. Effect of Pesticides to the Environment and Human Health

The evaporation of pesticides into the atmosphere and dissolution in water, and expose the adverse effects to the environment. Pesticides as Persistent organic pollutants (POPs) are observed in air, water, soil, plant and biota in many environmental areas in trace amount. The declining of bird populations by Rachel Carson in her classic book “Silent Spring” and this topic was addressed in the very first paper in Environmental Pollution in 1970 (Prest et al., 1970). DDE a metabolic breakdown product of DDT, can affect eggshell thickness birds of prey ([Ratcliffe, 1967], [Ratcliffe, 1970] and [Pearce et al. 1979]). The reproductive system of animal can be affected, most of the pesticides are suspected to be carcinogens.

In time interval of 24 hours to seven days period Lethal Dose 50% $(LD)_{50}$ level is used as indicator for health risk. The statistics estimation of a pesticide, that would kill 50% of the test animals is $(LD)_{50}$ value usually rats, mice, rabbits are used to test. In three ways pesticides can enter the body by oral, dermal, respiratory. The concentration for each route must be measured. The LD values (0-10) are extremely toxic. Smaller the LD_{50} value, which indicates more toxicity of the pesticide. Only small amount of these pesticides are enough to be harmful to the living organisms.

The pesticide concentration in the air which will kill 50% of the test animals by breathing in a certain period of time and it is expressed in parts per million (ppm) (mg/m^3) is called Inhalation Toxicity LC_{50} level. LD_{50} and LC_{50} are used to get information about acute toxicity. Chronic toxicity should also be taken into care since acute toxicity does not provide complete information. EC_{50} represents the concentration of a compound where 50% of its maximal effect is observed. The EC_{50} represents the concentration of a compound where 50% of the population exhibits a response, after specified exposure duration (Connell 2005). Toxicity of some organochlorine pesticides in accordance with these toxicity values are given in Table1. 4.

Table: 1.4. Toxicity levels of Chlorohydrocarbon Pesticides.

Compound	**LD_{50}(mg/kg body rats)**	**LC_{50}(estuarine fish µg/l;96 h)**	**EC_{50}(Daphnia, µg/l;48 h)**
p,p'-DDT	115	0.4-89	0.36
Dieldrin	50	0.9-34	250
Lindane	125	9-66	460
Aldrin	50	5-100	28

(Connell 2005)

Understanding the environment includes knowledge of surroundings, water, air, soil, and their interrelationship and the relationships between them and any living organisms (FAO, 2005). Natural environment is not interfered by human and not made, changed by any interference. Nonnatural environment is one changed, modified, disturbed, or created by our human activities (Johnson ,1997). Organochlorine Pesticides are chemically stable substances and degrade slowly under natural environmental conditions. Pesticides are effective in the environment for a long time. The misuse of pesticides, or overuse, can contaminate environment. Especially water, air and soil, may be affected seriously which may cause decline on plants and wildlife, and a loss of biodiversity.

The exposure of pesticides is considered to be harmful for human health and the exposure levels of pesticides must be controlled in order to be safe. The exposure can be direct or indirect. The industrial workers, operators in particular farmers who use them may expose directly. The indirect exposure can be observed by consumers, residents and bystanders, where pesticides activity is going on such as agriculture, landscaping, and on sports grounds, and for public building maintenance, road and railway side weed control, lawn care, and etc.

A survey on the effects of pesticides on workers and operators include acute headaches, vomiting, stomach-aches, and diarrhea was observed by The European Federation of Agricultural Workers' Unions (EAF). Chronic health impairment such as cancer, birth defects, reproductive problems, and sensitisation can be the cause of low constant exposure for long-term. Generally due to a lack of awareness, pesticides exposure may exceed safe levels. The educational background Knowledge on pesticides may help to get rides of the pesticide risk.

1.3.1. The health risks from long-term exposure to pesticides and their residues

Daily exposure and societal concerns about the possible dangers posed by pesticides or agrochemicals can help people from serious health problem. Pesticides are unique and use widely to kill or control microorganisms and pest mostly in agricultural purpose. Agricultural or veterinary chemicals introduced for sale and use, regulatory agencies are required to assess, among a range of other investigations usually conducted by sponsor companies. The studies conducted for pesticides are detailed and extensive toxicological testing, Toxicological investigations on organisms and animals in the environment, field practice to determine likely dermal and inhalation exposure of agricultural workers, bystander exposure, residues in crops, in order to estimate the possible dietary exposure of the general population to the chemical.

1.4. Use of pesticides in Turkey

Agriculture play an important role in the economy sector for all nations. In 2010, it represented 10.1% of GDP and 24.7% of employment (TURKSTAT) in Turkey. Turkey is a major World producer and exporter of agricultural products such as fruit and vegetables. Social and economic development of rural areas is a major challenge for coming years. In Turkey more than 40% of the total population is engaged in agriculture, working more than 4 million farm holdings.The arable and permanent croplands makes up 30 % of total land area of the country (Özkan, 2002). The total pesticide use in Turkey 1 % of the world's total amount. The consumption vary region to region it was seen that the western and southern coastal regions of Turkey use higher than the average amount. These areas are used for intensive agriculture (Nafiz 2005). According to the officially stated data by Ministry of Agriculture and Rural Affairs on pesticides by year 2000 there are 2000 registered pesticides used with 300 active ingredients (Yazgan, 2003). Table 1.5 shows the pesticides consumption from 1997-2012.

Table: 1.5. The pesticides consumption between 1997 and 2012 in Turkey.

Year	Pesticides consumption (tones)	Pesticides used (g/ha)
1997	13083.00	703.00
2000	12458.00	683.00
2004	13146.00	726.00
2006	18258.00	1047.00
2007	18944.00	1118.00
2008	20032.00	1209.00
2009	15412.00	950.00
2010	20121.00	1234.00
2011	26770.00	1703.00
2012	25534.00	1308.00

1.5. Rules and Regulation of pesticides residues with food safety

Chemical substances used in agricultural production to control pest and improve the yield of agricultural products. However, many chemicals stay as contaminant in agricultural products. Pesticides residues are among the most important toxic substance in food products that raised the concern of consumers. Pesticides are used against creatures that are believed to be harmful for the agriculture. Control and monitoring activities need to be conducted annually at regularly schedule times when the food safety issue is concerned. In other words, There should be a plan for the chemical materials will be examine for food safety and how many different products will be checked for pesticides residue in a specific zone (Şik, 2013).

The European Commission took an important step to ensure food safety in the European Union. The new rules were set on Maximum Residue Levels (MRLs) for pesticides in agricultural products. Regulation (EC) No 396/2005 is the result of a considerable joint effort by the Commission, The European Food Safety Authority (EFSA) and the Member States. Androulla Vassiliou said pesticide residues in food should be as low as possible and have no harmful effect for consumers. Regulation (EC) No 1107/2009 to regulate plant protection products in Europe and Directive 2009/128/EC to regulate the sustainable use of pesticides in Europe besides Statistics Regulation 1185/2009/EC concerning statistics on pesticide. Machinery Directive amendment 2009/127/EC was set to certify new equipment for pesticides spray. European Pesticide Legislation and Guideline work includes: Setting maximum residue levels (MRLs), the European and Mediterranean Plant Protection Organisation (EPPO) for Classification and labelling of plant protection products.

A maximum residue level (MRL) is the highest possible level of a pesticide residue that is legally tolerated in food and feed. The EU Regulation covers approximately 1100 pesticides currently or formerly used in agriculture in or outside the EU and 315 agricultural products are listed for MRLs. These MRLs also apply to processed products, adjusted to take account of dilution or concentration during processing. EFSA is responsible for the safety assessment, based on the properties of the pesticide, the maximum levels expected on food. The Commission is responsible to carry out inspections in the Member States to assess and audit their control activities.

1.6. Food safety and pesticide use in Turkey

The Ministry of Food, Agriculture and Animal Husbandry is responsible for all controlling, monitoring and auditing duties in relation to agricultural and food products. Pesticides residues in food are tested in the Provincial Food Control Laboratories and Food Control Branch Directorates functioning under this ministry. There are 41 active Food Control Laboratories conducting pesticides residue analysis in Turkey. A fine is imposed, if pesticide residue limits exceed by law is found during analysis conducted in these labs. Before tackling pesticides residues problem, there should not be doubt in the amount of pesticides used and current legislation.

When comparison is made with other countries Turkey is using around 700 grams of pesticides per hectare (Tiryaki, 2010). Netherlands, which has the highest pesticide use in Europe, uses nearly 13 kilograms and Finland; use the lowest pesticide with 1.2 kilograms per hectare. From this perspective, the amount of pesticides used in Turkey seems quite low. However, this is not the case. The pesticide usage varies from region to region. For example, pesticide usage in Antalya, where fresh vegetable-fruit production is widespread, is twice the amount of the Netherlands, with approximately 26 kilograms per hectare (Anonymous, 2011).

According to data collected in 2011, 44.7 million tons of fresh fruits and vegetables were produced in Turkey and 7.2 per cent was exported (Anonymous, 2012). 93 percent of products produced are consumed internal and pesticides residue control over them is inadequate.

Toxicological tests essentially to determine the specific toxic chemical levels in a food product, in order to understand the harmful effect for human health. Therefore, they should try to find out the MRL value of a toxic chemical in food products can be. In recent years, studies conducted suggest that the small amounts of residues of pesticides are harmful to health even though they are below MRL values (Mckinlay, 2008), (Mnif, 2011). There are many of varieties of pesticides used in agriculture and it is quite possible that multiple pesticide residues can be observed in food products.

1.7. Extraction Techniques

Extraction techniques play a important role in analytical chemistry. The development of instrumental analysis techniques has grown interest with the development of new technologies, such as the transistor and microprocessor control. The importance of extraction technology has been recognized for its role in the generation of quality analytical information in past 15 years.

1.7.1. Extraction Methodologies

The extraction of organochlorine pesticides in tomato, soil and waters samples with these extractions methodologies of Ultrasonic Bath extraction (UBE), solid phase micro extraction, Soxhlet extraction, accelerated solvent extraction, supercritical fluid extraction and liquid- liquid extraction are possible. The most popular extraction techniques used in Tomato and soil is Ultrasonic Bath Extraction (UBE) and for water Solid Phase Extraction (SPE) techniques are used.

1.7.1.1. Ultrasonic Bath Extraction

The liquid (solvent) compresses and stretches, the cavitation bubbles are formed and it can behave in two different ways. One way is forming stable cavitation bubbles with fairly low ultrasonic intensities (1–3 W/ cm^2). This is oscillating about some equilibrium size for many acoustic cycles. In the second way is transient cavitation bubbles formation by using sound intensities in excess up to 10 W/ cm^2. Transient bubbles expand through a few acoustic cycles to a radius of at least twice their initial size, before collapsing violently on compression. The main source of the chemical and mechanical effects of ultrasonic energy is transient bubble collapsing. Each time collapsing of a bubble can be considered as a microreactor in which temperatures can reach up to several thousands degrees and pressures can reach higher than one thousand atmospheres instantly.

Nonvolatile and semi volatile organic matters can be separated from soils, tomatoes samples by using Ultrasonic bath extraction method. Effective contact of the sample matrix with the extraction solvent is required during the extraction process. It is very important to remove moisture contamination from sample matrix in order to obtain high extraction efficiency for ultrasonic bath extraction. The anhydrous Na_2SO_4 is used to dry the samples during ultrasonic bath extraction and to remove the moisture. This process may take few minutes. The sample is then centrifuged or filtered by vacuum to get the extract and residue. Finally, the extract is concentrated using rotary evaporator and cleaned up for the analysis (United States Environmental Protection Agency 1996).

1.7.1.2. Solid phase extraction (SPE)

The mechanism(s) of SPE is to understand the interaction between the sorbent and analyte of interest. The knowledge of the hydrophobic, polar and ionogenic properties of both the solute and the sorbent will help to chose proper SPE disk/syringe barrels-cartridge for the extraction. The common mechanisms in SPE are based on van der Waals forces (“non-polar interactions”), hydrogen bonding, dipole-dipole forces (“polar interactions”) and cation-anion interactions (“ionic interactions”). Solid phase extraction is performed using either silica-based or organic resin-based sorbents, with suitable physical characteristics and chemical properties of the matrixes.

Solid phase extraction (SPE) is a useful technique for water samples preparation. The problems associated with liquid-liquid extraction can be prevented with SPE. The incomplete phase separations, less quantitative recoveries, use of expensive, breakable glassware, and disposal of large quantities of organic solvents can be prevented by using SPE. SPE is used most often to prepare liquid samples and extract semi volatile or nonvolatile analytes. SPE products are excellent for sample extraction, concentration, and cleanup. They are available in a wide variety of chemistries, adsorbents, and sizes. Selecting the most suitable SPE products can make easy for the extraction of sample. Figure 1.2 shows a schematic diagram of SPE.

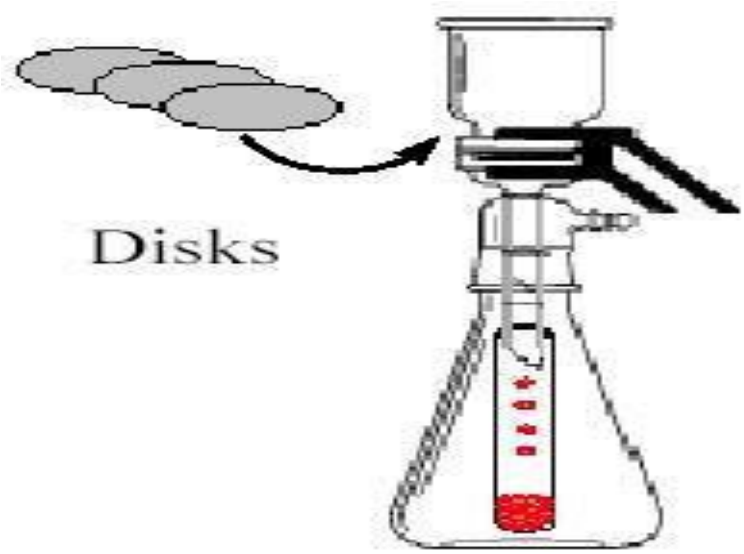


Figure: 1.2. Apparatus of Solid Phase Extraction

1.7.2. Analyses Techniques

1.7.2.1. Gas Chromatography

Gas chromatography is used for wide range of compounds; because of its sensitively and selectively. Helium, hydrogen or nitrogen gases are often used as the eluent in gas chromatography. Gas chromatography is combined with different detectors for different purpose. The sample is separated in terms of volatility differences of the components of the sample and also the differences between their interactions with the stationary phase. Either liquids or solids are used as stationary phases contained in the column with an internal diameter of 100 µm to 4 mm. A detector, an injection system, a temperature controlled column are the three essential elements of the chromatographic system (Kebbekus M. 1998). Figure: 1.3.Shows a Typical Gas Chromatography.

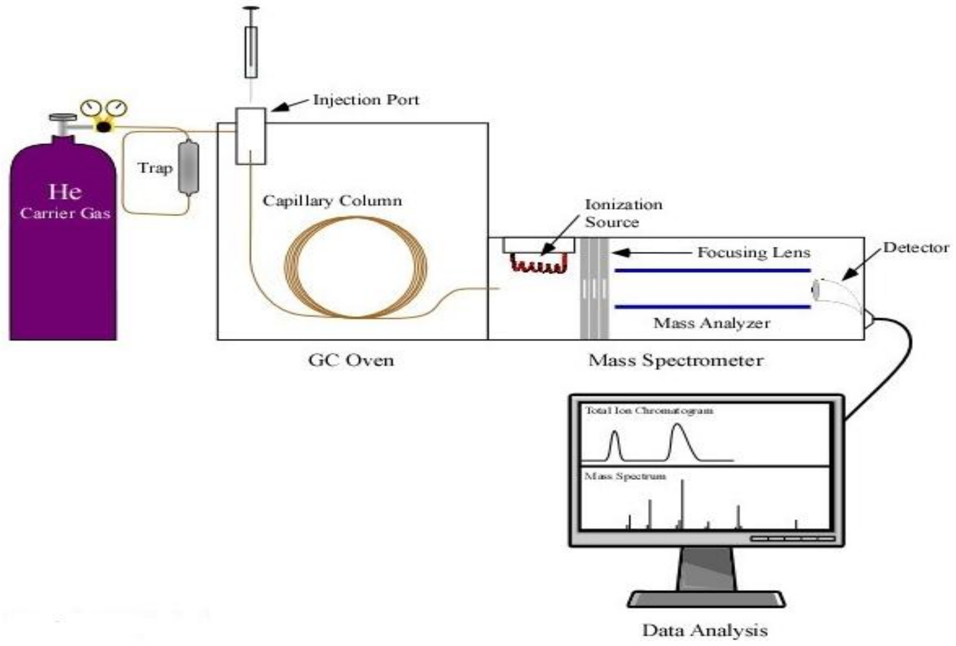


Figure: 1.3. Gas Chromatography (HP-6890)

1.7.2.2. Mass Spectrometry

The retention time play an important role to identify the compound. However, when two or more compounds have very close retention times identification becomes very difficult. If this is the case, mass spectrometer is used to acquire the structural information regarding the compound. The information provide by mass spectroscopy is by ionizing molecule and separated into different fragments in a mass spectrometer, by determination of each fragment ion molecular masses occur. The pattern displayed by the mass spectrometer is considered as the fingerprint of the molecule. The unique set of fragments composes for each molecule help to identify the proper molecule (Kebbekus M. 1998). All sort of mass spectrometers are operated by producing and sorting ions with respect to their mass and charge ratio. The space through which these ions flow must be isolated from other gases; so, a vacuum system is necessary. An ion source for converting the molecules of the sample into ions and an analyzer to sorted their ions by mass and charge ratio and sent to the detector is needed (Kebbekus M. 1998). A mass spectrometer system is shown in Figure

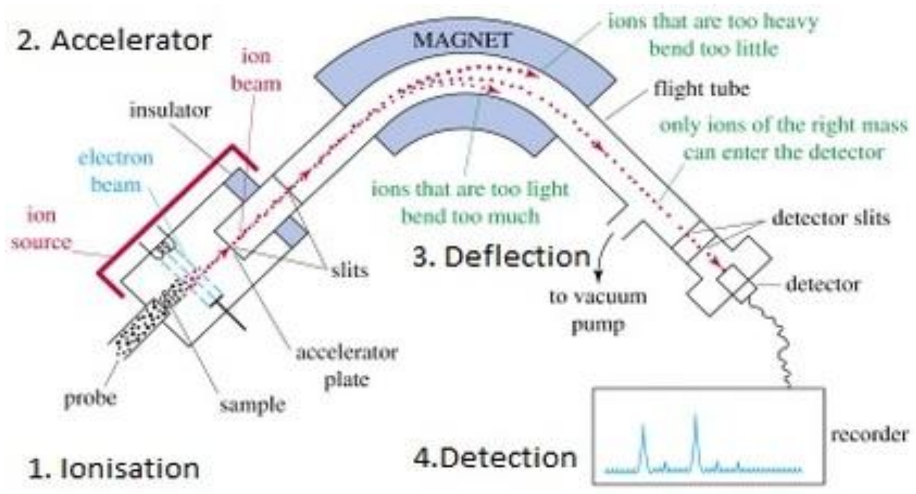


Figure: 1.4. Mass Spectrometer (HP-5973)

1.8. Aim of the study

The aim of this study is about the validation of method in the aspect of extraction and analyses and environmental impact evaluation of pesticides in soil, water and tomato samples. The aim was also to determine the biosecurity of agricultural product in Ayaş region by speciation of chlorine pesticides from water-soil-tomato. Chlorine pesticides speciation was determined by using GC-MS. Produced data were used as a database for pesticides pollution in Ayaş region, Turkey. In this way, the extent of bio safety of the agricultural product in the Ayaş region of Ankara was determined. Due to pesticides contamination in agricultural product and rejection of agricultural products from different countries Turkey’s economic loss was compared based on the pesticides results found in the study. The level of pesticides residue in agricultural product in Ayaş-turkey was compared with different countries around the world.

1.9. Literature Review

Doong.et.al (2001) determined organochlorine pesticides (OCPs) and their metabolites in sandy soil samples by using 100-mm polydimethylsiloxane (PDMS) and 65-mm PDMS–divinylbenzene for good extraction efficiency. The authors optimized the developed procedures involving “fiber selection, temperature effect, absorption time, soil matrix and the addition of solvents” of different polarity. He used Soxlet extaction for soil and HS-SPME procedure was applied to the analysis of OCPs in certified reference material (CRM) of soil. He compared with Soxhlet extraction procedure with HS-SPME.

Ozcan. et. al (2009) developed an ultrasonic extraction procedure for the determination of different organochlorine pesticides (OCPs) in soil and analysed by gas chromatography (GC-ECD). The procedure used for extraction of soil samples as was 5-min sonication for an acetone–petroleum ether (1/1, v/v) solvent. The authors compared the performance of the procedure with traditional shaking flask and Soxhlet extraction procedure and ultrasonic extraction procedure was better for extraction. The proposed procedure requires small volumes of solvent and sample.

Zhang. et. al (2005) determined prohibit organochlorine pesticides(DDT and HCH concentrations) in Taihu Lake region with 30 soil samples in China. The study conducted to see the distribution and contamination levels of OCPs within the study area. The authors plotted distribution graph for HCH and DDT patterns as well as the degradation ratios between the parent substances and their isomers.

Yawar. et. al. (2011) determined pesticides residues in locally produced vegetables. 200 samples of eight different vegetables were analyzed by gas chromatography coupled with mass selective detector (GC–MSD). The authors used the results to have information on the current pesticide contamination level in commonly used vegetables in Pakistan locally produced vegetables.

Goncalves. et. al.(2004). Developed a suitable methodology for pesticide residue analysis in soil samples based on ultrasonic extraction (USE) and gas chromatography–mass spectrometry (GC–MS). From an intensive horticulture area in North of Portugal soil samples were collceted and this methodology was applied in monitoring soil samples.

Turgut.et.al (2003) determined the OCPs and investigating the pollution levels of OCPs in the water samples in a river in Turkey by using HP-5890 gas chromatograph with ECD detector. OCPs were banned over a long time ago in turkey. However, the presence of the pesticides is seen in surface water samples.

Yazgan.et.al. (2005) based on the Toxicity Human health Persistency (THP) Hazard Rating System, and a consumption factor authors calculated the environmental risk of pesticides. The authors method were based on the basic properties of pesticides rather than on the processes that occur both on land and in water are applied to soil samples. This method may be used as a practical quantitative tool to generate significant findings to aid the selection of the most environmentally friendly substitute pesticides against a certain pest.

Gun.et.al.(2009) a study was done by Gun to infrom farmers on various issues related to pest applications in vegetable cultivation in greenhouses. Though, Farmers were aware of the excessive and inappropriate use of pesticides. They also knew that it can damage crops and might be harmful to human health. However, their knowlege about the relationship between the environment and agricultural pesticide use was not clear.

Gambacorta.et.al.(2004) a study conducted for the use of pesticides on Tomato plants when fruits were about to ripeness(e.g. Funcicides). The authors extracted pesticide residues and analysised by gas and liquid chromatography. The findings of pesticides indicated that there was a need for careful control of the spraying doses of this fungicide, in particular on varieties of fresh tomato.

Wu.et.al.(2013) A study was conducted for organochlorine pesticide (OCP) species to identify their possible sources, and estimate health risk of drinking the shallow ground water in China. From the study carcinogenic risk values for pesticides in the shallow groundwater in majority area were found to be posing a serious cancer risk to the consumers.

Zhang.et.al.(2013) The reuse of wastewater for agricultural irrigation becomes a well practice in china due to shortage of water resources and sustainable preservation of croplands and pasture land. However, many contaminants have been also introduced into the soil groundwater systems such as persistent organochlorine pesticides (OCPs). OCPs analysis in groundwater showed that the major influence factors on the distribution of OCPs in groundwater systems attribute to the flow field of groundwater and to the current pesticide use for crops.

Öztas.et.al(2008) studied organochlorine and organophosphorus pesticides in ground and surface water samples collected from intense agricultural area, Kumluca,Antalya for spring and fall seasons of 2005. She used Solid Phase Extraction method to extract the samples and GC-ECD and GC-NPD systems to quantitatively determine of organochlorine and organophosphorus pesticides.

Gokmen.et.al. (2011) studied Organochlorine Pesticides (OCPs) and Polycyclic Aromatic Hydrocarbons (PAHs) in the sediment samples of Balıkesir (İkizcetepeler) Dam Lake. She used an ultrasonic bath extraction method to extract OCPs and PAHs in sediment samples and GC-MS was used for the analysis.

CHAPTER 2

EXPERIMENTAL

2.1. Study Area

The study area is Ayaş region in Ankara city/Turkey. Ayaş is a town located in the central Anatolia region of Turkey district of Ayaş is 58 km from Ankara. Which is very rich for historical monuments. In 2012 census, population of Ayaş is 13087 of which 9,749 live in the urban center of Ayaş and 3,338 lives in rural area. The district covers an area of 1,112 km^2 (429 sq mi). The average elevation of Ayaş is 910 m (2,986 ft). The district is known for its tasty tomatoes, mulberry trees, and its healing mineral water spas, both for drinking and bathing. An annual mulberry festival take place in the town every year. Besides the taste of tomato, among the furits and vegetables tomato exports to other countries is highest for Turkey. They gain a huge amount of foriegn currency by exporting tomato to Russia and Europe. The quality is know by the consumers for it different taste.However. when it comes for importing to foriegn country, the safety and quality of product play an importantant role. The study will give us a general view for Ayaş tomato for the quality and safety purpose.

Figure: 2.1. Sampling region Ayaş

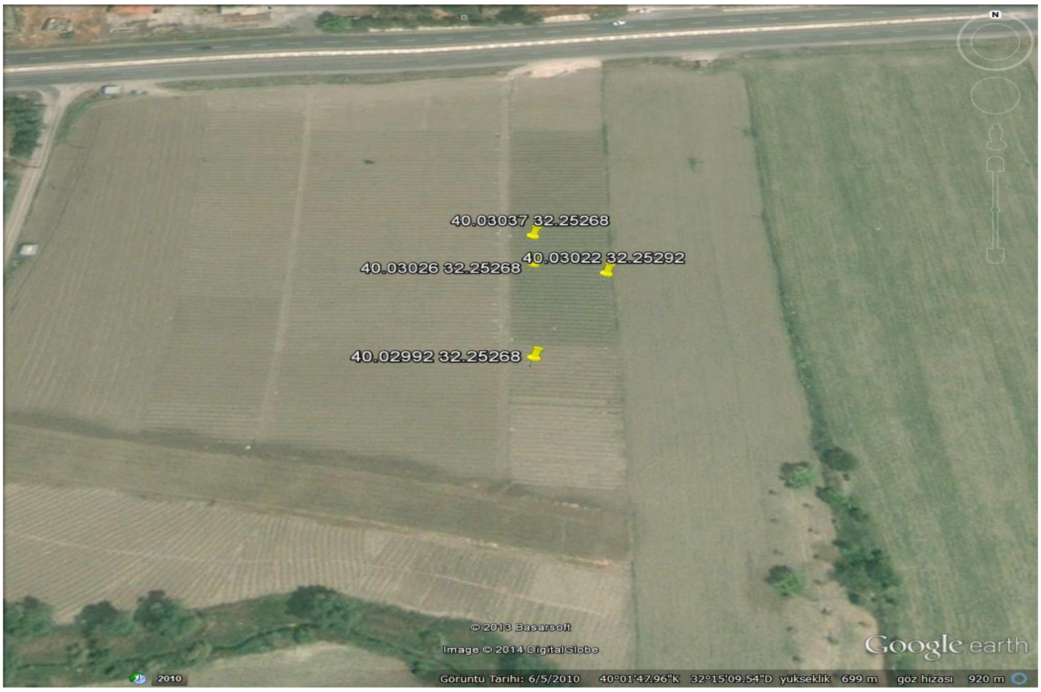


Figure: 2.2. Sampling points for Red Tomatoes

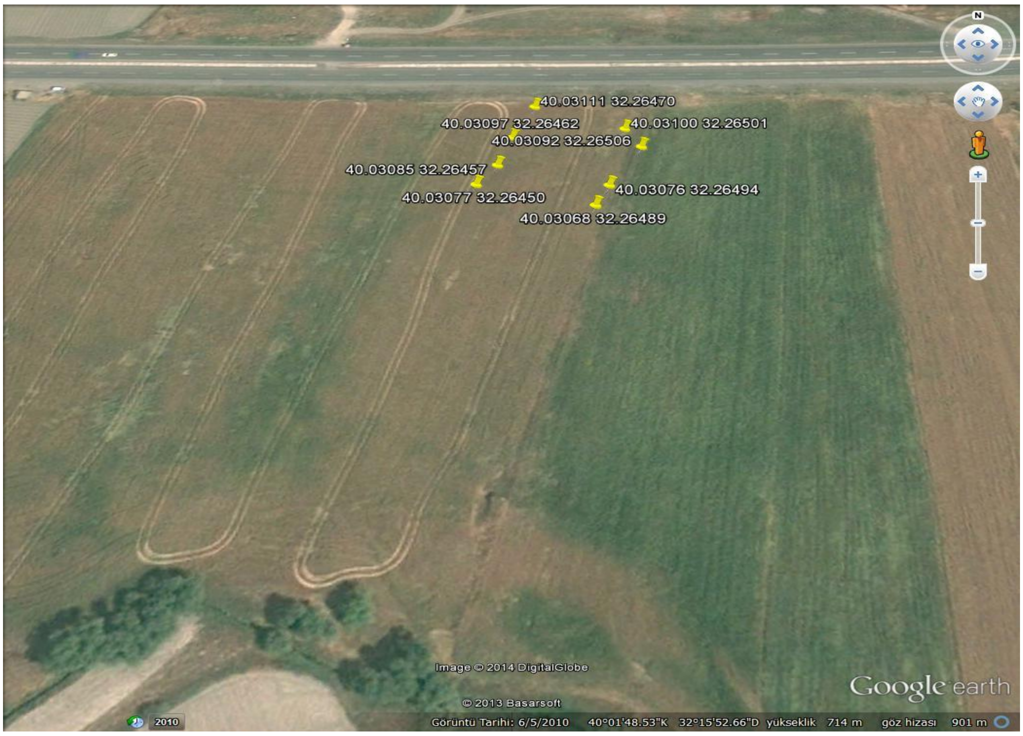


Figure: 2.3. Sampling points for Green Tomatoes

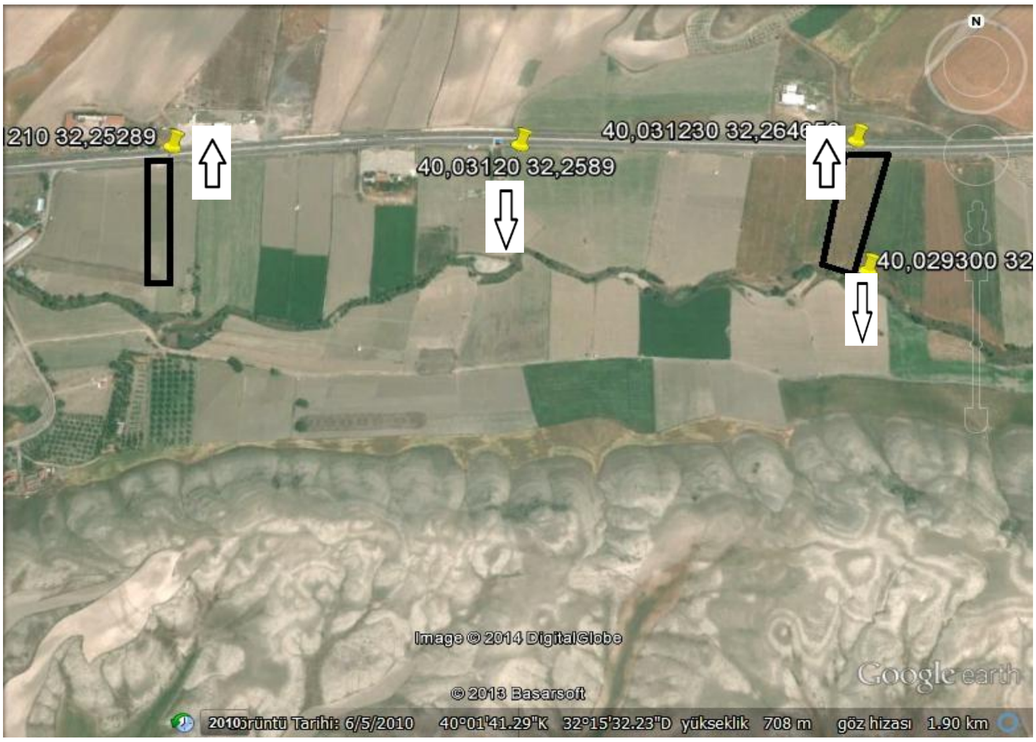


Figure: 2.4. Sampling points for Water

2.2 Sampling Strategy

Sampling is one of the important steps of environmental analyses as the samples should represent the pollution of the entire region. Several different sampling strategies could be used depending on the purpose of the research and topography of the region. In this study Grid Method is used. In this approach, the study area is chosen with 2 fields one with $500m^2$ and another $1000m^2$. Samples were collected by dividing into equal squares and the samples are taken from the centers of each square. 16 sampling points were determined for tomatoes and soil samples and four sampling pints for irrigated water samples.

2.3 Sample Collection and coordinates

The coordinates of the 16 sampling points were determined by using Global Positioning System (GPS). The transparent poly bags are used for collecting soil and tomato samples and polyethylene bottles are used to collect water samples. The samples were collected from Ayaş in 24 April, 2013. The coordinates of the sampling points are shown in Tables 2.1, 2.2, and 2.3 and Google earth map images figure 2.1shows the two fields, figure 2.2 shows the red tomato sampling points, figure 2.3 shows green tomato sampling points and figure 2.4 shows the sampling coordinates of irrigated water with downward arrows shows the entrance of the irrigated water and upward arrows show exit of water.

Table: 2.1 sampling points for Tomato and Soil. Field 1 (Red Tomatoes)

Point	**N**	**E**
1	40.03023	32.25295
2	40.03037	32.25268
3	40.03022	32.25292
4	40.03026	32.25268
5	40.03010	32.25296
6	40.03010	32.25265
7	40.02991	32.25298
8	40.02992	32.25268

Field 1 sampling coordinates for tomato and soil samples

Table: 2.2. Sampling points for Tomato and Soil. Field 2 (Green Tomatoes)

Point	**N**	**E**
1	40.03111	32.26470
2	40.03128	32.26501
3	40.03097	32.26462
4	40.03092	32.26506
5	40.03085	32.26457
6	40.03076	32.26494
7	40.03077	32.26450
8	40.03068	32.26489

Field 2 sampling coordinates for tomato and soil samples

The sampling of irrigation water from ground is given in the table 2.3. The entry and exit points are written for the sample points. Irrigation water was pumped out from the ground and at the point of pump is written as entry sampling point. The fields are watered by drop-drop system (small holes pipe line) and at the end of the pipe line the water coming out of pipe as mention as Exit sampling point. The depth of the water reservoir was not known and fields were 1.5 km away each other. The flow direction was from entrance to exit.

Table: 2.3. Sampling points for irrigation water from ground.

Point	**N**	**E**
1(Entry)	40.03120	32.25890
2 (Exit)	40.031210	32.25289
3 (Entry)	40.029300	32.264620
4 (Exit)	40.031230	32.264650

2.4 Reagents and Materials

The organochlorine pesticide standards (EPA Method 508-Chlorinated Pesticide Mix 1, 1000ng/µl), internal standards (Pentachloronitrobenzene, 1.0 mg/ml) and surrogate standards (2,4,5,6-Tetrachloro-m-xylene, 200ng/ml and Decachlorobiphenyl, 200ng/ml) were purchased from Dr. Ehrenstorfer (Ausburg, Germany). The intermediate standard solutions were prepared from the stock solutions with appropriate dilutions with Acetone, Hexane and cyclohexane.

All the stock, intermediate and standard solutions were stored in refrigerator. Hamilton gas tight glass syringes (500, 100, 50 and 10 µl) was used for the preparation of the standards into 1.5 ml amber vials (Supelco). Ultrasonic extractions were performed by Branson ultrasonic bath, Rotary evaporator (Laborota 4000) was used to evaporate the solvents of both standards and samples. The extracted samples were transferred to 1.5 ml amber glass vials for further reduction of the volume.

2.4.1 Preparation of Na_2SO_4 and Glass Wool

Na_2SO_4 was purchased in extra pure grade from the company J.T. Baker Company. Sodium sulfate (Na_2SO_4) was used for column packing to dry the extracts. Na_2SO_4 was put in a glass column and washed two times with acetone and two times with dichloromethane. The volume of solvent used for each washing is two times of the estimated volume of the Na_2SO_4 in the column. Washed Na_2SO_4 was transferred to a large beaker, covered loosely with solvent rinsed aluminum foil and conditioned at 225 °C overnight. Glass wool was used to fill the tip of the column and purchased from Supelco. A quantity of a glass wool was compressed into a large glass column and washed sequentially hexane and dichloromethane and treated like Na_2SO_4 and stored in a desiccators.

2.4.2 Cleaning of Glassware

Extreme precautions were taken to eliminate the contamination. All the glassware used was washed with Alconox and hot water first. Then, several rinses with tap water and deionized water are followed. Dichloromethane, Hexane, and Acetone are used for rinsing at last. All of the glassware was oven dried at 100 degree Celsius.

2.5 Instrument and Apparatus

An HP (Hewlett Packard) 6890 series gas chromatograph coupled with HP 5973 mass spectrometer was used for the analysis. A 30m, 0.25 mm id., 0.25mm film thickness, crosslinked 5% Phenyl methyl siloxane, HP 5MS, capillary column (Agilent Tech.) was used for the separation of OCPs throughout the study.

2.6 Analysis with GC-MS system

Gas chromatography equipped with a mass spectrometer allows chemists to detect very small quantities of the contaminants in the environmental matrices, but Selected Ion Monitoring (SIM) mode improves the sensitivity of the measurements by limiting the mass of the ions detected to one or more specific fragment ions of known mass. Therefore, selectivity is increased in the SIM mode whereas in scan mode there are many noises originating from the ions that are not in concern. The parameters of GC-MS for OCP determination were formerly optimized by Pinar Gökmen and Nur Banu Öztaş, respectively. Table show the operating parameters of GC-MS used for detecting OCP concentrations in the sediment samples respectively. GC-MS SIM windows parameter of OCP standards are given in tables 2.4, 2.5 and 2.6 and chromatograms Figures are given in figure 2.5,2.6,2.7,2.8,2.9and 2.10 respectively .

Table: 2.4. SIM parameters for OCP determination Tomato

Windows	**Time Period (min)**	**Ions Monitored**
1	3-15	109, 111, 136, 181, 183, 207, 209, 219, 235, 237, 244, 249, 265, 295
2	15-30	66, 79, 81, 100, 165, 195, 199, 227, 229, 235, 237, 241, 246 , 248, 261, 263, 265, 272, 273, 274, 316, 318, 330, 345, 347, 351, 353, 387
3	30-40	426, 427, 497, 499

Table: 2.5. SIM parameters for OCP determination soil

Windows	**Time Period (min)**	**Ions Monitored**
1	3-21	74, 91, 100, 109, 111, 136, 181, 183, 207, 209, 219, 235, 237, 244, 249, 263, 265, 274, 273, 274, 295
2	21-28	67, 74, 79, 81, 165, 199, 207, 229, 235, 237, 239, 241, 246, 248, 250, 261, 263, 265, 272, 274, 277, 316, 318, 345, 351, 353, 355, 387
3	28-40	169, 212, 227, 229, 426, 427, 497, 499

Table: 2.6. SIM parameters for OCP determination water

Windows	**Time Period (min)**	**Ions Monitored**
1	3-18	91, 100, 109, 111, 136, 181, 183, 207, 209, 219, 237, 244, 249, 263, 265, 272, 273, 274, 295
2	18-28	67, 79, 81, 195, 207, 235, 237, 239, 241, 246, 248, 250, 261, 263, 265 ,277, 316, 318, 345, 347, 351, 353, 355
3	28-40	165, 169, 199, 212, 227, 229, 235, 237, 250, 272, 274, 387, 426, 497, 499

2.7 GC- MS Chromatograms for Standard and samples

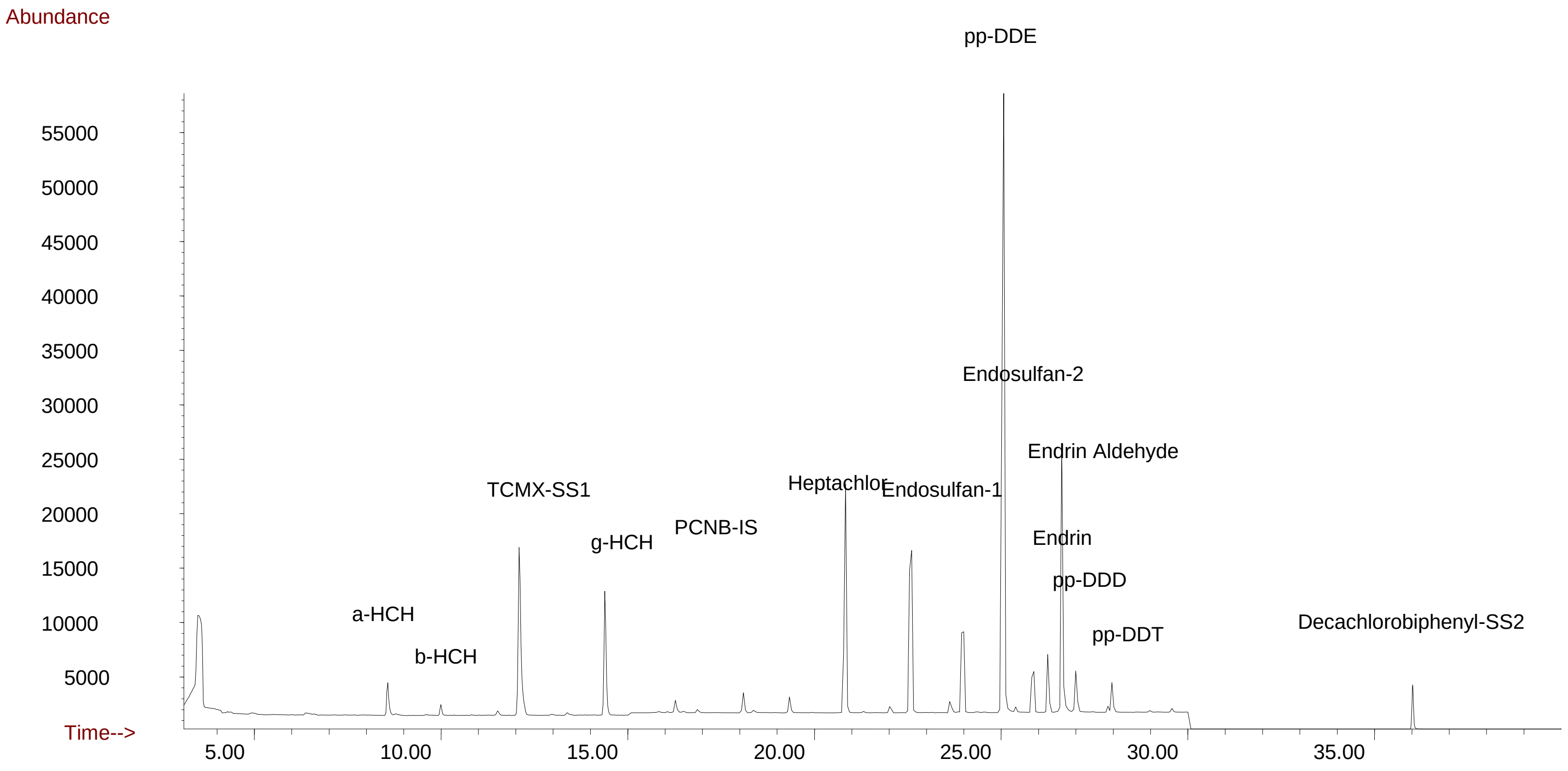


Figure: 2.5. Chromatogram of 5.00ppm Standard for Tomato

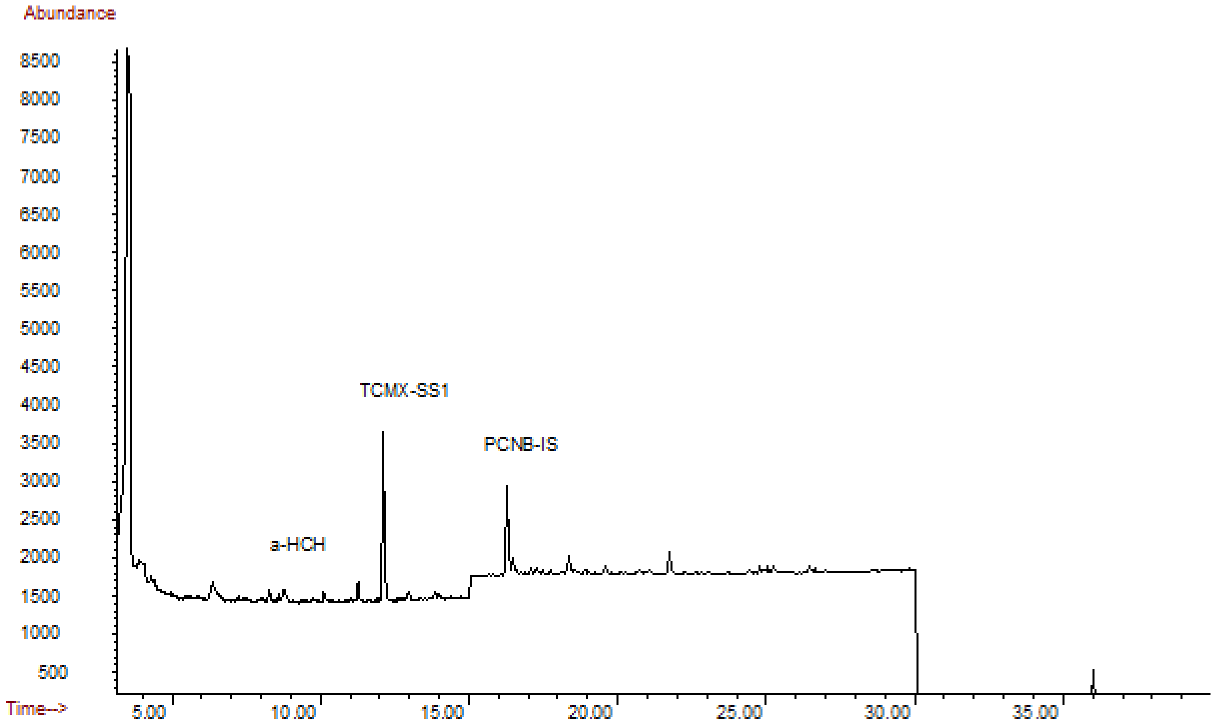


Figure: 2.6. Chromatogram of tomato sample 1

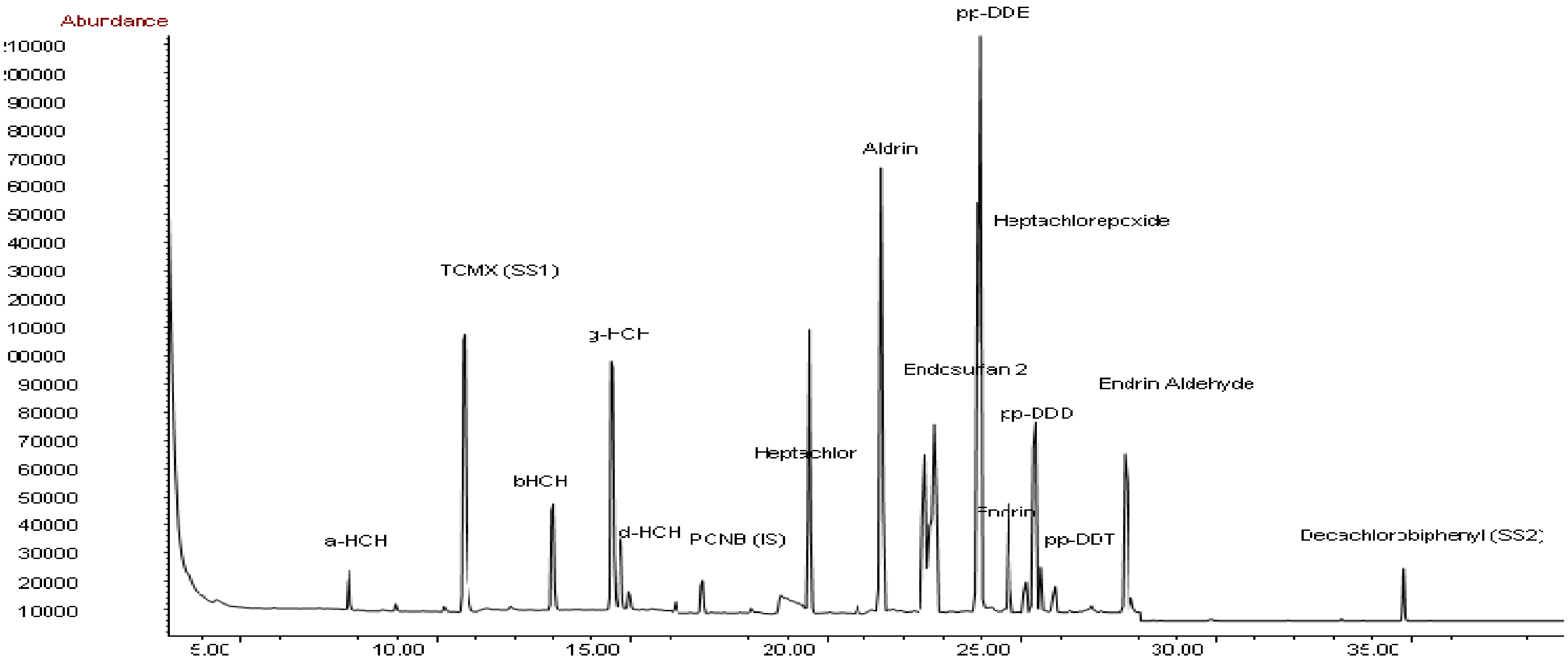


Figure: 2.7. Chromatogram of 5.00ppm Standard for Soil

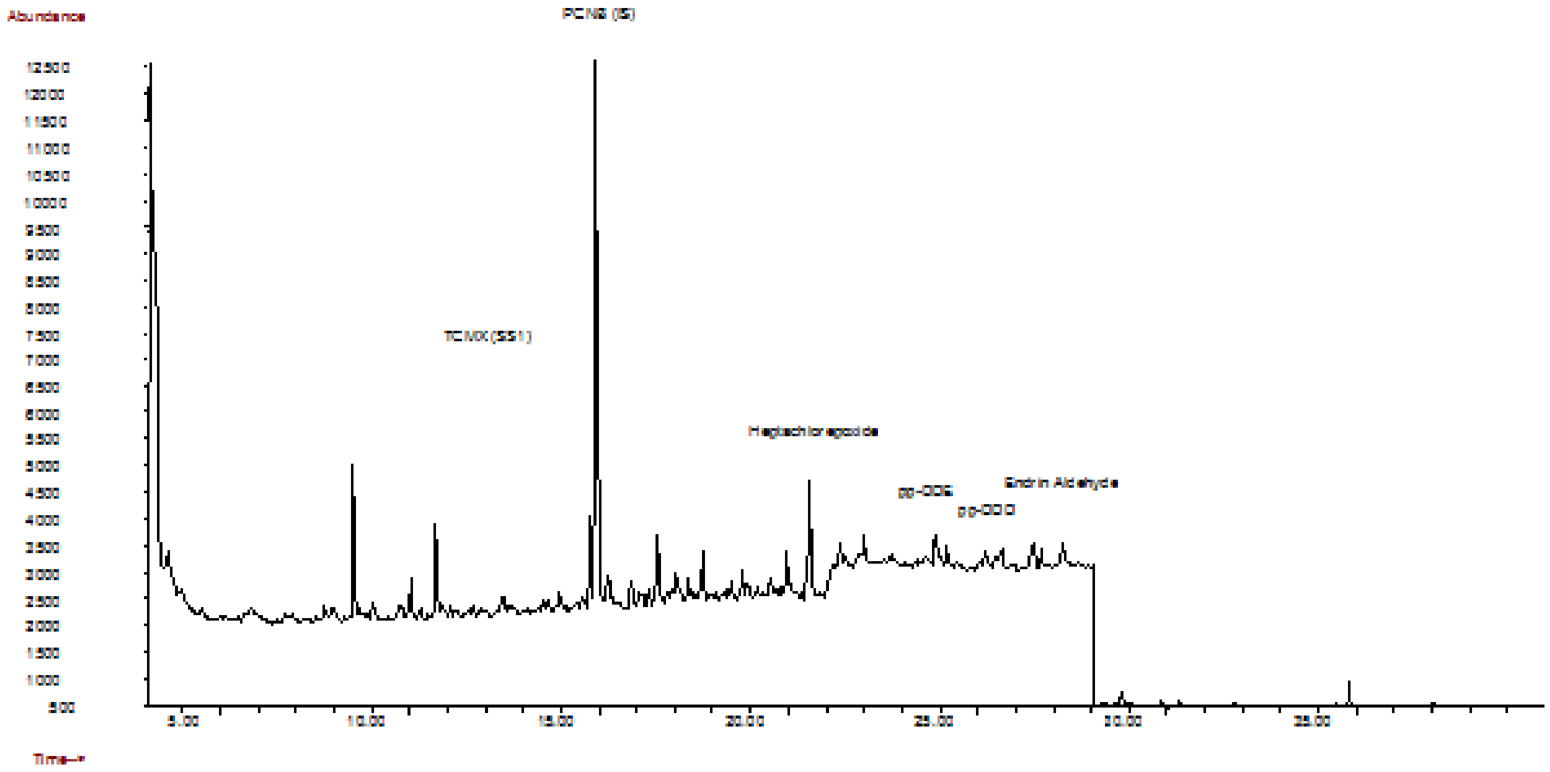


Figure: 2.8. Chromatogram of Soil sample 2

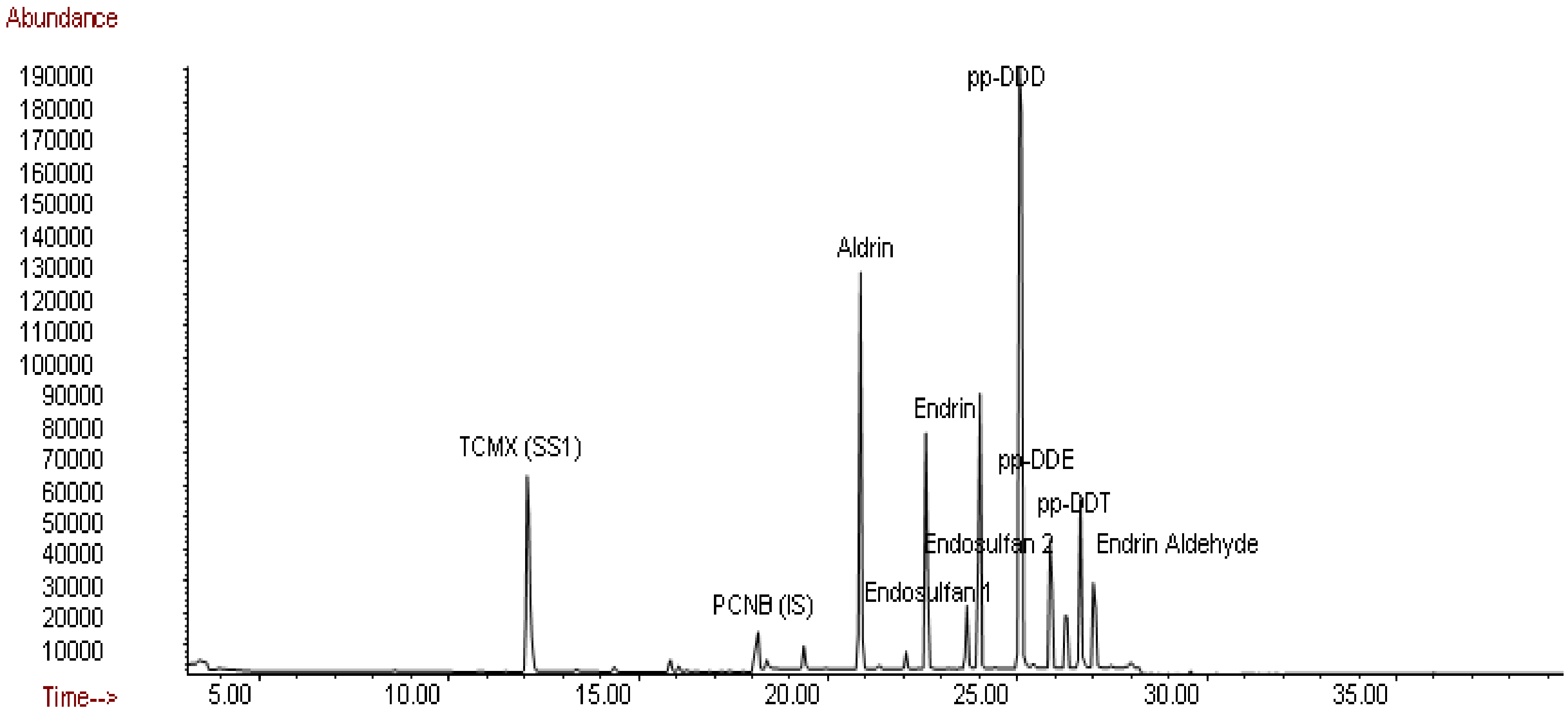


Figure: 2.9. Chromatogram of 5.00ppm Standard for Water

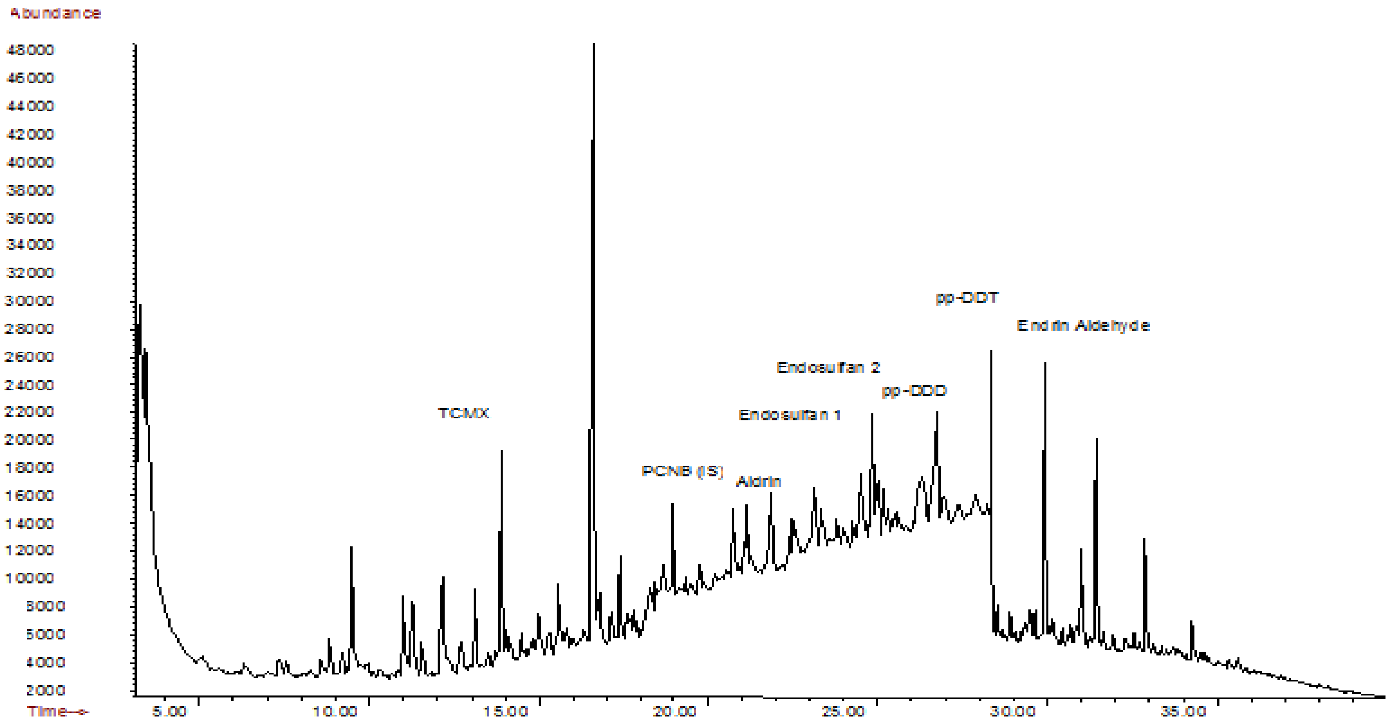
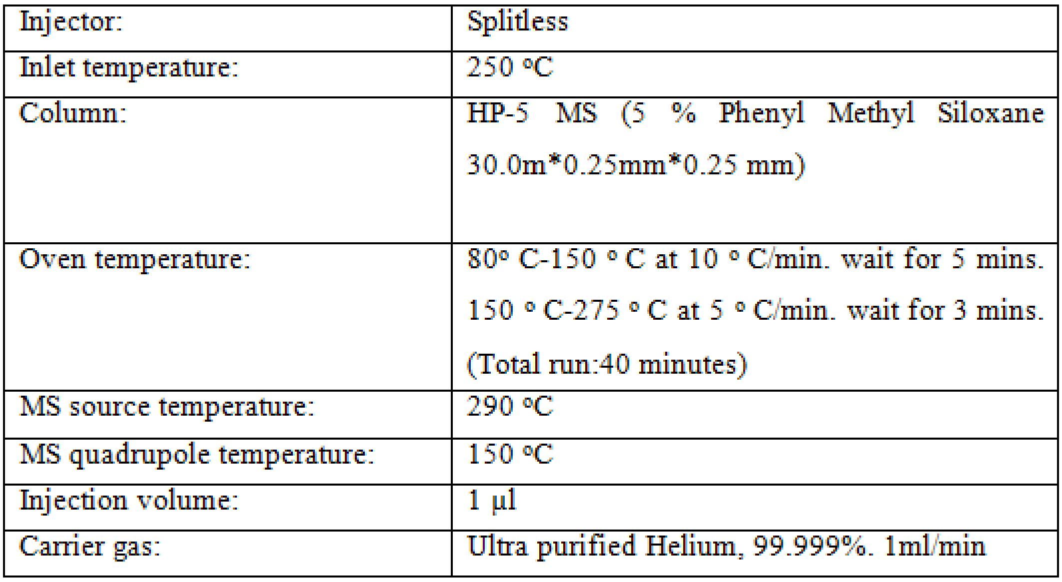


Figure: 2.10. Chromatogram for water sample

The operating parameters are given in the table 2.7. The optimization was done by Nur Banu Öztaş and Pınar Gökmen for determination of OCPs in water and Sediments samples.

Table: 2.7. Operating parameters of GC-MS system for OCP determination



Injector:	Splitless
Inlet temperature:	250 °C
Column:	HP-5 MS (5 % Phenyl Methyl Siloxane 30.0m*0.25mm*0.25 mm)
Oven temperature:	80° C-150 ° C at 10 ° C/min. wait for 5 mins. 150 ° C-275 ° C at 5 ° C/min. wait for 3 mins. (Total run:40 minutes)
MS source temperature:	290 °C
MS quadrupole temperature:	150 °C
Injection volume:	1 µl
Carrier gas:	Ultra purified Helium, 99.999%. 1ml/min

(Gökmen 2011), (Öztaş 2008)

The identification of OCPs was done with retention time, target and conformation ions. They are given in the tables 2.8, 2.9 and 2.10 for tomato, soil and water samples.

Table: 2.8. Retention times and ions of the analytes (Tomato)

Name of Pesticide	**Target and Confirmation Ions**	**Ret. Time (min)**
alpha-HCH	181,183,207,111	8,576
beta-HCH	181,183,207,111	9,998
2,4,5,6 Tetrachloro-m-xylene (SS1)	207,209,244,136	12,093
gama-HCH	181,183,207,111	14,389
Pentachloronitrobenzene (IS)	237,265,263,235	16,276
Heptachlor	272,100,273,274	19,333
Aldrin	263,266,261,66	20,834
Endosulfan I	81,237,235,263	22,55
Heptachlorepoxide	353,351,263,318	22,604
Endosulfan II	195,241,237,209	23,944
p.p’-DDE	318,316,246,248	25,07
Endrin	263,265,261,345	25,875
p.p’-DDD	195,237,241,235	26,25
Endrin aldehyde	345,248,347,246	27,001
p.p’-DDT	235,237,165,248	27,966
Decachlorobiphenyl (SS2)	426,499,497,427	36,024

The retention time in minutes and the target and confirmation ions are given in the table above for tomato analysis.

Table: 2.9. Retention times and ions of the analytes (Soil)

Name of Pesticide	**Target and Confirmation Ions**	**Ret. Time (min)**
alpha-HCH	181,183,219,109	8,956
2,4,5,6 Tetrachloro-m-xylene (SS1)	207,209,244,136	10,681
beta-HCH	181,183,219,109	12,942
gamma-HCH	181,183,219,111	14,475
delta-HCH	181,183,219,109	14,705
Pentachloronitrobenzene (IS)	237,265,249,295	14,935
Heptachlor	272,100,273,274	18,078
Aldrin	263,265,66,91	19,573
Heptachlorepoxide	353,355,351,81	21,386
Endosulfan II	237,195,241,207	22,764
p.p’-DDE	246,318,248,316	23,913
Endrin	263,265,261,81	24,66
p.p’-DDD	235,237,165,199	25,522
Endrin aldehyde	345,347,250,67	25,809
p.p’-DDT	235,237,165,199	26,843
Methoxychlor	227,229,212,169	29,109
Decachlorobiphenyl (SS2)	497,499,426,427	34,825

The retention time in minutes and the target and confirmation ions are given in the table above for soil analysis.

Table: 2.10 Retention times and ions of the analytes (Water)

Name of Pesticide	Target and Confirmation Ions	Ret. Time (min)
2,4,5,6 Tetrachloro-m-xylene (SS1)	209,244,208,172	12,107
Pentachloronitrobenzene (IS)	265,263,267,237	18,387
Aldrin	263,265,267,79	20,857
Endosulfan I	195,263,265,237	22,064
Endosulfan II	237,217,235,183	22,581
p.p'-DDE	176,248,246,250	23,672
Endrin	241,277,265,263	24,017
p.p'-DDD	318,246,248,281	25,108
p.p'-DDT	235,237,165,199	26,659
Endrin aldehyde	67,209,185,250	27,004

The retention time in minutes and the target and confirmation ions are given in the table above for water analysis.

2.8. Calibration of the Analysis Systems

In this study, internal standard calibration method was used for quantification of organochlorine pesticides. Table 2.11 shows the calibration parameters for the determination of OCPs. The figures 2.11, 2.12 and 2.13 show the calibration curves and linearity values for Pesticides.

Table: 2.11. Calibration parameters for the determination of OCPs

Concentration, (µg/ mL)	**Internal Standard Pentachloronitro-benzene (PCNB)**	**Surrogate Standard** **SS1:2,4,5,6-Tetrachloro-m-xylene** **SS2: Decachlorobiphenyl**	**Standard Concentation (µg/ mL)**
0.25	1.00	0.25	0.25
0.50	1.00	0.50	0.50
1.00	1.00	1.00	1.00
2.50	1.00	2.50	2.50
5.00	1.00	5.00	5.00

Internal standard addition method was applied during the determination of OCPs in sixteen tomatoes, soils and four water samples.

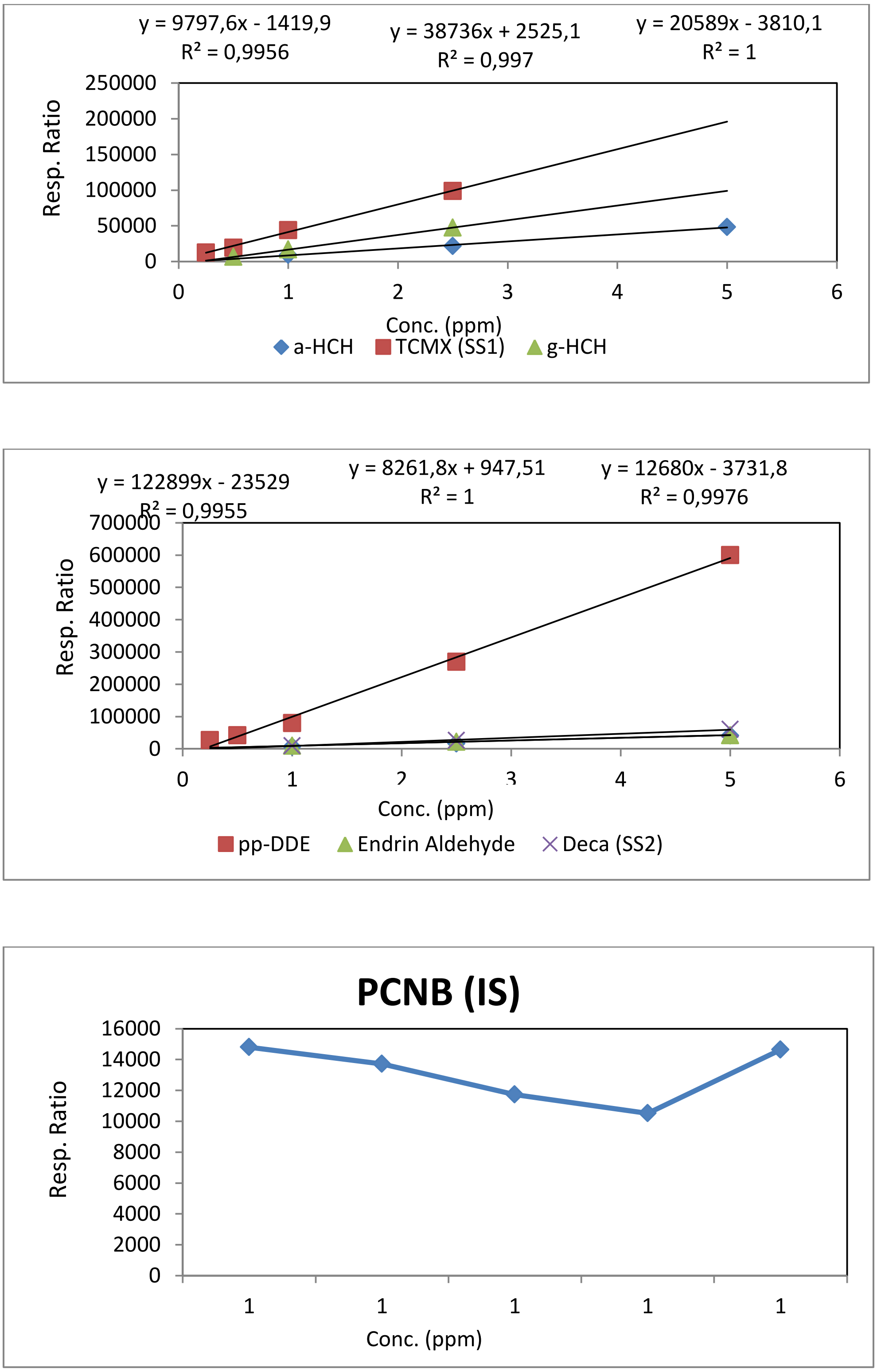


Figure: 2.11.Calibration curves for OCPs in Tomato

The linearity's are changing between 0.97-1.00

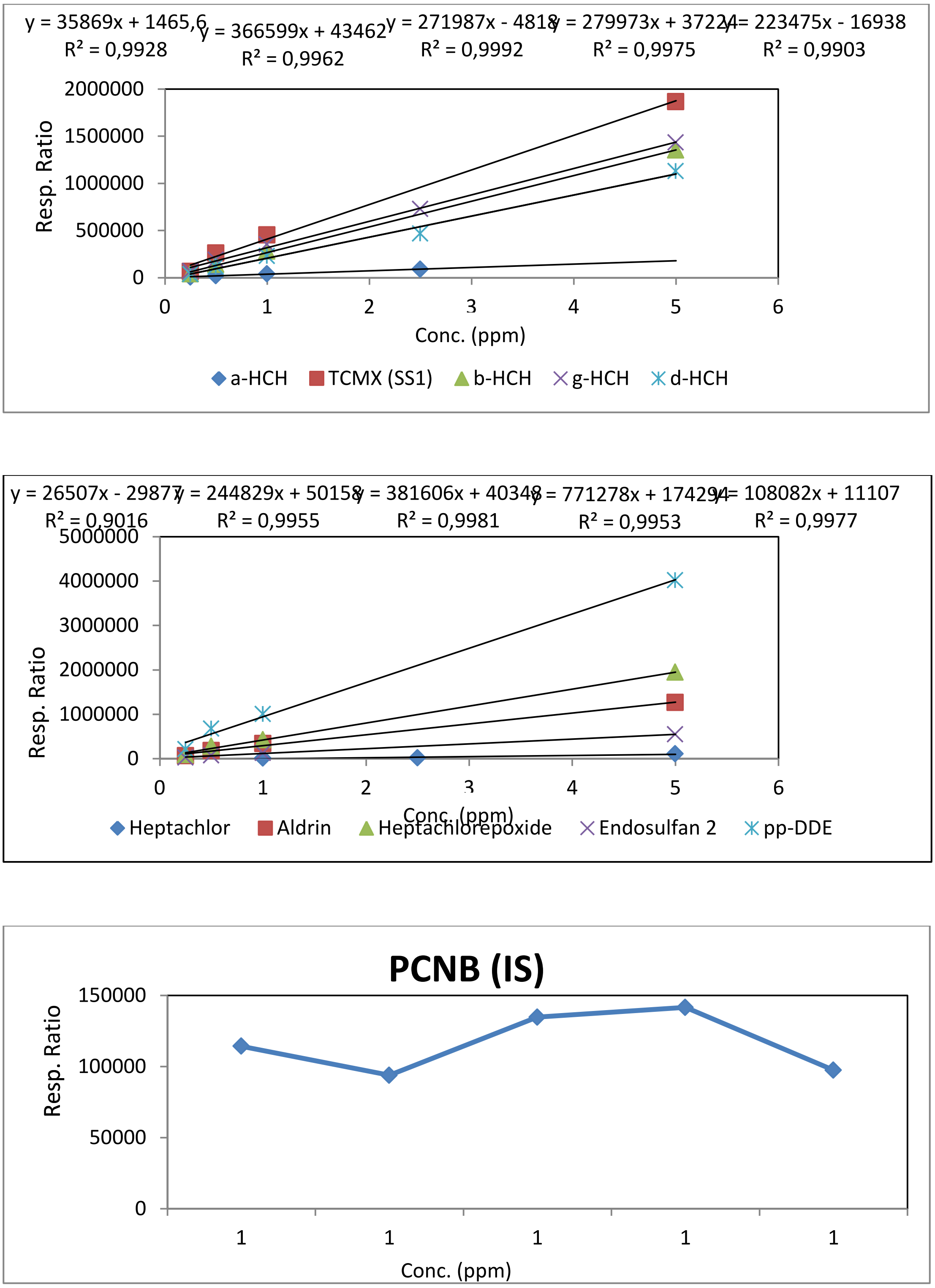


Figure: 2. 12. Calibration curves for OCPs in soil

The linearity’s are changing around 0.99.

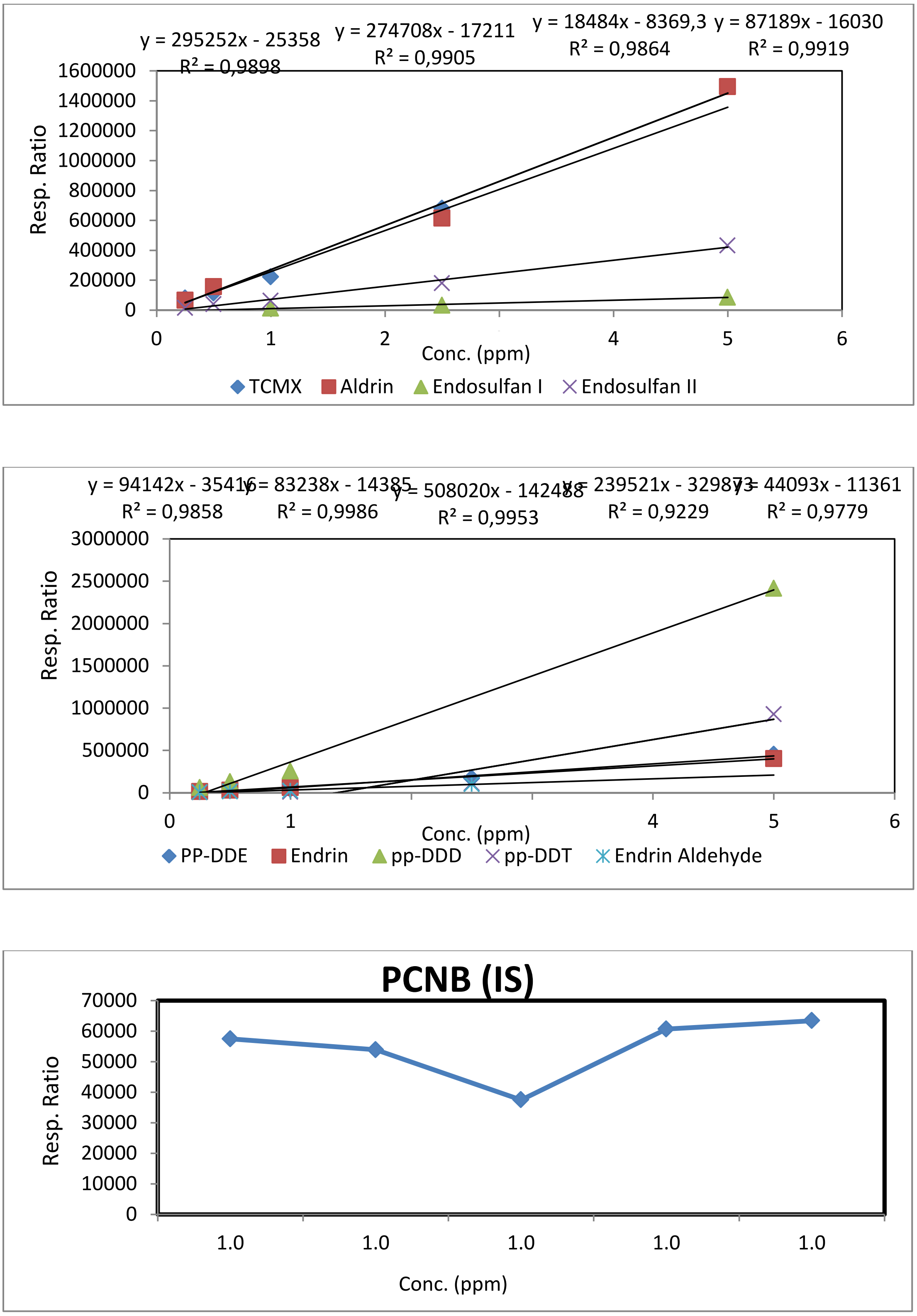


Figure: 2.13. Calibration curves for OCPs in Water

The linearity’s are changing between 0.92-0.99.

2.9 Extraction of samples

Tomato (Ultrasonic Bath Extraction for Organochlorine Pesticide)

Tomato extraction was performed according to the reported method (Arrebola ,2003) A tomato was blend and an aliquot of 15g of blend tomato paste sample were weighed and mixed with 30 ml of dichloromethane and addition of 1.0 mL of 1.0 µg/mL surrogate standards (2,4,5,6 Tetrachloro-m-xylene and Decachlorobiphenyl) mixed for 2–3min with a glass budged. After mixing, 30g anhydrous sodium sulfate were added and allowed to rest for 2 min in an Ultrasonic bath at 40^{o} C, after the sonication filtered above solution through a Buchner funnel and then again filtered above filtrate through filter paper with anhydrous sodium sulfate. Internal standard 1.0mL (Pentachloronitrobenzene) was added then final filtrate was evaporated to dryness in a rotary evaporator, and dried residue was again dissolved with 10ml of cyclohexane. By this way final extract was subjected to GC–MS for analysis.

Soil (Ultrasonic Bath Extraction for Organochlorine Pesticide)

Soil samples are crushed in a mortar and two grams of soil samples were weighted in an amber glass bottle with Teflon cap and 1.0 mL of 1.0 µg/mL surrogate standards (2,4,5,6 Tetrachloro-m-xylene and Decachlorobiphenyl) were added. Then sixty milliliters of hexane: acetone mixture (3:1) was added to the bottle and closed for ultrasonic bath extraction for two hours at room temperature. The extracts were firstly eluted through Na_2SO_4 column to eliminate the water. The extract was evaporated with rotary evaporator and 1.0mL internal standard (Pentachloronitrobenzene) having a concentration of 1.0 µg/ml was added. Afterwards, the final solution was pre concentrated to 1 mL with evaporating under pure nitrogen gas and taken into a 1.5 ml amber glass vial and kept in refrigerator at 4 ^{0}C before the analysis with GC-MS (Manirakiza, et al. 2001).

Water (Solid phase Extraction)

Water samples are filtered with filter paper. 200 ml water was taken to a beaker and the surrogates standards were 2,4,5,6- tetrachloro-m-xylene and decachlorobiphenyl, added to 200ml sample solution with a final concentration of 1ppm of 1.0 ml.
Then the solid phase extraction disk surface is conditioned in order to extract organic solvent. The conditioning was performed by sequential addition of 10.0 mL DCM, 10.0 mL methanol and 10.0 mL deionized water. After DCM, addition of methanol and water helps to exchange the medium to match the sample matrix.

After the addition of DCM, the solvent was retained on the disk for 90 seconds to allow the interaction with disk surface. Methanol and water was also kept in contact for 90 seconds and drawn by vacuum sequentially.

The samples were then loaded on the disk and passed through with application of vacuum pump. In this study all of the samples were filtered.

The analytes trapped on the disk were eluted by 20 mL DCM inside the Erlenmeyer flask with vacuum. The solvent was added by 10+5+5 mL portions with a total contact time of 5 minutes. The extract was removed and dried by passing through a drying column of Na_2SO_4. The column bed was wetted by 6.0 mL DCM mixture before use. After passing the extract, the drying column was rinsed with 5.0 mL of the same solvents and this portion was collected with sample extract. The collected extract was then dried in rotary evaporator and then dried in fume hood under gentle stream of nitrogen.

The internal standards were added and the final volume was completed to 1.0 mL with acetone.

For quality control studies, a QA/QC procedure including Standard reference materials (SRMs), Surrogate standards and internal standards materials are used.

Three SRM standards were used namely SRM 2261 (Chlorinated Pesticides in Hexane), SRM 2273(Chlorinated Pesticides (DDTs) and Metabolites in Isooctane), SRM 2275(Chlorinated Pesticide Solution-II in Isooctane).

CHAPTER 3

RESULTS AND DISCUSSION

3.1. **Validation of Method**

The Quality Assurance (QA) and Quality Control (QC) tests during extraction process, during the analyses and evaluation of the data set and data interpretation of Organochlorine Pesticides (OCPs) were discussed.

3.1.1. **Quality Control and Quality Assurance Analysis**

The stability of the instrument was checked because of the fact that some errors may occur during the analysis. Internal standard addition method was used in case there were errors originating from the instrument and standard reference materials (SRMs) were used to determine the accuracy of the system. The accuracy of the system was given in table 3.1.

Table: 3.1.Percent errors and standard deviations of SRMs for OCPs

Organochlorine Pesticide	**USED SRM**	**Certified Values(µg/L)**	**Found Conc. ±Stdev (µg/L)**	**% Error**
AlphaHexachlorocyclohexane (A-HCH)	SRM2275	2070±100.00	1809.33±16.0300	-12.5900
GammaHexachlorocyclohexane (G-HCH)	SRM 2261	1972±10.00	2210.35±222.690	12.0900
BetaHexachlorocyclohexane (B-HCH)	SRM2275	2054±83.00	1821.44±74.2200	-11.3200
HEPTACHLOR	SRM 2261	1977±15.00	2090.05±167.300	5.72000
HEPTACHLOREPOXIDE	SRM 2261	1977±17.00	1746.29±92.7000	-11.6700
ENDOSULFAN	SRM2275	1987±46.00	2116.21±135.850	6.50000
DIELDRIN	SRM 2261	1972±13.00	1834.22±63.6200	-6.99000
Dichlorodiphenyldichloroethylene (p, p’-DDE)	SRM 2273	2009±54.00	2157.36±7.90000	7.38000
ENDRIN	SRM2275	2006±68.00	1363.39±31.1500	-32.0300
ENDOSULFAN II	SRM2275	2031±48.00	2150.89±9.23000	5.90000
Dichlorodiphenyldichloroethane (p, p’-DDD)	SRM 2273	2006±52.00	2399.18±91.1500	-17.4700
ENDOSULFAN SULFATE	SRM2275	2019±60.00	897.347±58.2900	-55.5500
Dichlorodiphenyltrichloroethane (p, p’-DDT)	SRM 2261	1967±12.00	1781.29±127.340	-9.44000

According to table % error values are changing from 5.90000-55.6000.

3.1.2. Limit of Detection (LOD) and Limit of Quantification (LOQ)

The LOD and LOQ for the pesticides were calculated for each program. 5 ppm standard was used for the calculations. LOD was calculated by determining the concentration where S/N ratio is 3 using GC-MS software. LOQ was calculated by multiplying LOD with three. Slight difference in LOD and LOQ values are caused by the different GC-MS programs and exchange solvents. According to LOQ values in tables some pesticides concentrations are below LOQ and neglected. LOD values are as 0.680-23.7 µg/kg for water, 0.840-94.6µg/kg for tomato, 0.370-25.7 µg/kg for soil. LOQ values are the three times of these values. Values under LOQ were identified as BLOOQ

The lowest order of detection and lowest order of quantization for soil is given in the table 3.2.

Table: 3.2 Limit of Detection (LOD) and Limit of Quantification (LOQ) values organochlorine Pesticides (OCPs) for Soil Samples

Soil		
Name of Pesticide	**LOD (ppb)**	**LOQ (ppb)**
alpha-HCH	25.7	76.9
2,4,5,6 Tetrachloro-m-xylene (SS1)	4.80	14.4
beta-HCH	2.06	6.17
gamma-HCH	0.330	1.00
delta-HCH	1.75	5.25
Pentachloronitrobenzene (IS)	25.2	75.5
Heptachlor	4.70	14.1
Aldrin	1.89	5.68
Heptachlorepoxide	0.500	1.51
Endosulfan II	5.65	16.9
p.p’-DDE	3.35	10.1
Endrin	0.370	1.11
p.p’-DDD	8.78	26.4
Endrin aldehyde	8.39	25.2
p.p’-DDT	0.800	2.39
Methoxychlor	4.51	13.5
Decachlorobiphenyl (SS2)	0.710	2.13

The lowest order of detection and lowest order of quantization for water is given in the table 3.3.

Table: 3.3. Limit of Detection (LOD) and Limit of Quantification (LOQ) values organochlorine Pesticides (OCPs) for Water Samples

Water		
Name of Pesticide	**LOD (ppb)**	**LOQ (ppb)**
2,4,5,6 Tetrachloro-m-xylene (SS1)	3.84	11.5
Pentachloronitrobenzene (IS)	16.6	49.8
Aldrin	1.91	5.72
Endosulfan I	16.2	48.7
Endosulfan II	2.76	8.28
p.p’-DDE	4.39	13.2
Endrin	4.55	13.6
p.p’-DDD	0.680	2.04
p.p’-DDT	23.7	71.1
Endrin aldehyde	5.18	15.6

The lowest order of detection and lowest order of quantization for Tomato is given in the table 3.4.

Table: 3.4. Limit of Detection (LOD) and Limit of Quantification (LOQ) values organochlorine Pesticides (OCPs) for Tomato Samples

Tomato		
Name of Pesticide	**LOD (ppb)**	**LOQ (ppb)**
alpha-HCH	10.20	30.45
beta-HCH	43.10	129.0
2,4,5,6 Tetrachloro-m-xylene (SS1)	18.70	56.00
gamma-HCH	10.70	32.20
Pentachloronitrobenzene (IS)	94.60	284.0
Heptachlor	26.30	78.90
Endosulfan I	0.8400	2.530
Endosulfan II	7.290	21.90
p.p’-DDE	7.390	22.20
Endrin	8.710	26.10
p.p’-DDD	13.10	39.30
Endrin aldehyde	7.930	23.80
p.p’-DDT	13.30	39.80
Decachlorobiphenyl (SS2)	4.850	14.60

3.1.3. Extraction Recoveries

Percent recoveries were calculated as:

$$\% \text{ Recovery} = \frac{Cs}{Cn} \times 100$$

Where C_s: measured concentration of the spiked sample aliquot

C_n: nominal (theoretical) concentration of the spiked aliquot (United States Environmental Protection Agency 2003)

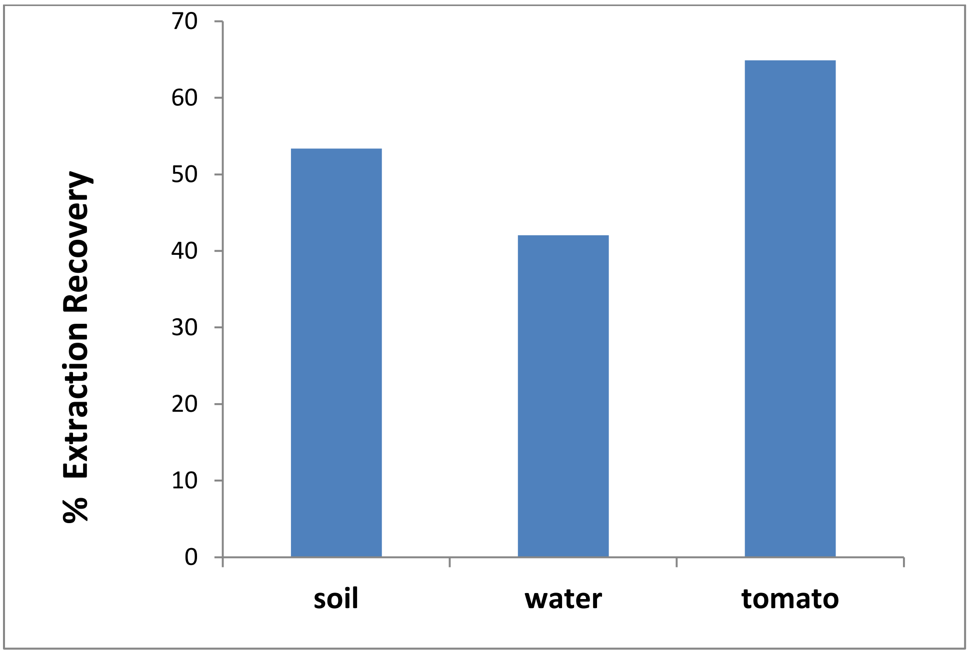


Figure: 3.1. The Comparison of Extraction Recoveries of Tomato, Water and Soil Samples

Since extraction methods for soil and tomato was the same (53.36 and 64.88 respectively), their extraction recovery values are similar. Small difference is caused by different exchange solvents and matrix. Extraction recovery for water (42.03) is little less than soils a tomato.

3.2. Evaluation of Data Set

3.2.1. Soil Analysis

16 soil samples were collected from 16 sampling points. Each soil sample was extracted twice and analyzed separately. Their averages were used for drawing figure. There were 2 sampling field. Sampling points 1-8 were in the Field 1. The rest 9-16 points were in the Field 2. According to figure 3.2 second field is more polluted. Pollution level is high in soil sampling points especially 5-8 in field 1 and similarly the pollution level is high in sampling points 15 and 16 in Field 2. However in Field 2 all sampling points are polluted with higher concentrations. The sum concentrations of pesticides are shown in figure 3.2 names of individual pesticides are given in figure 3.3.

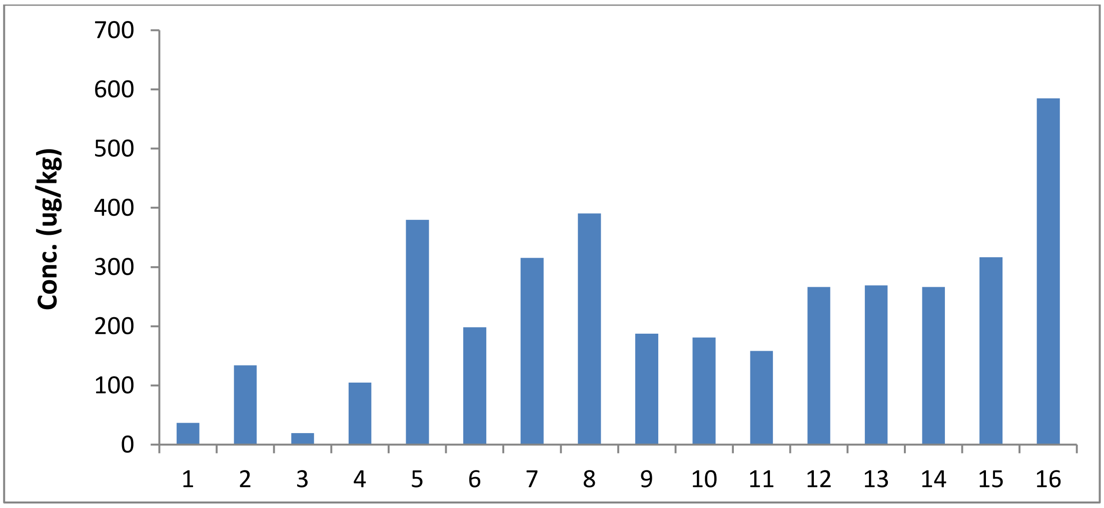


Figure: 3.2. Sum of Pesticide Concentrations in Soil Samples

Figure3.3 shows individual pesticide concentrations in each sampling point. Gamma-HCH, pp-DDT and alpha- HCH has the highest concentration among pesticides in soil. In field 1 the fluctuation between the pesticides concentrations are less compared to filed 2. There was no background soil sample analysis because the possibility of finding pure pesticides free soil is difficult. Background soil samples away from sampling points can give different soil characteristics.

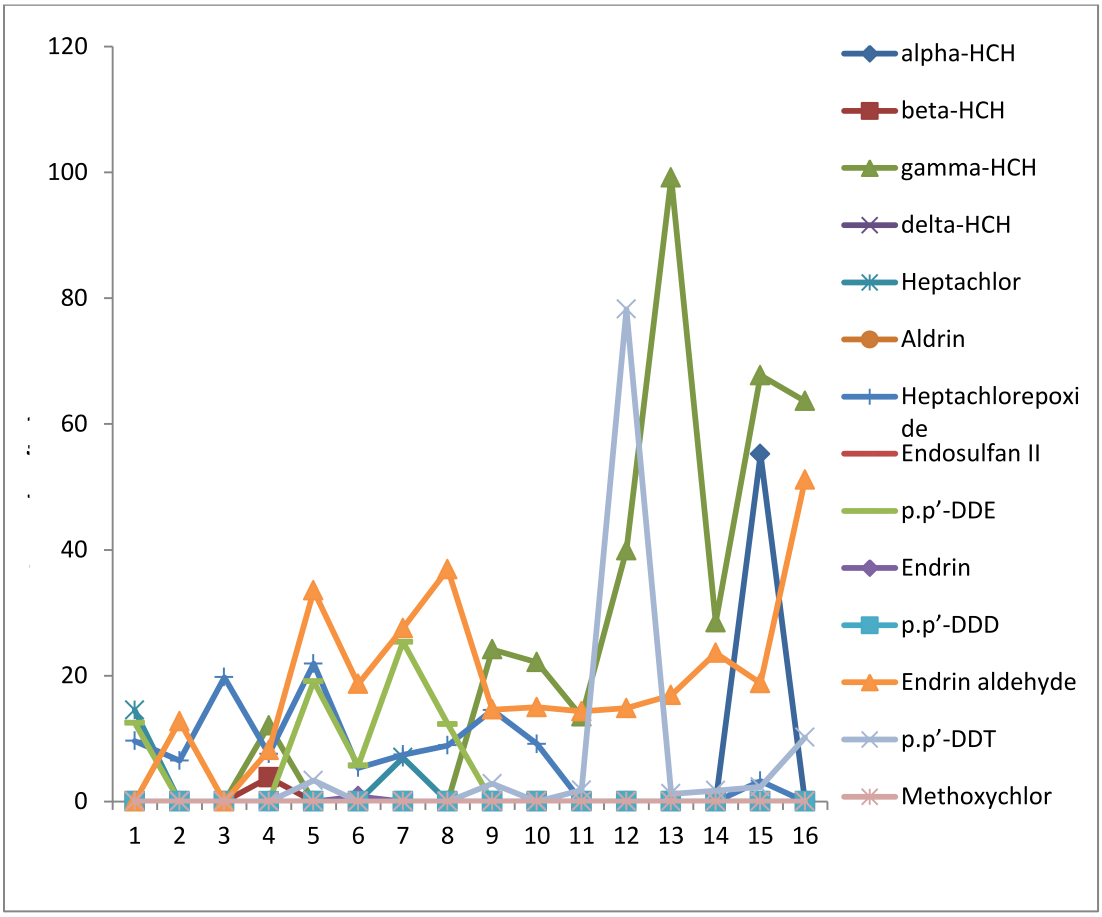


Figure: 3.3. Pesticide Concentrations in Soil Samples

3.2.2. Water Analysis

Two different fields were chosen for sample collection and 2 different pumps were used to pump out water for irrigation. For each field two water samples were collected at the origin of the pump (as entry) and the end of pipeline (as exit) of water samples. Samples are given number as 1 and 2 for field 1. Number 1 is used as entry water sample and 2 as exit water sample for filed 1. Number 3 as entry water sample and number 4 as exit water sample for field 2 were written. In addition 2 blank samples (deionized water) were analyzed and their averages were taken which is sample number 5. According to results no pesticide was found in entrance water 1. In blank very small amount (pp'-DDD) was observed which may be a contamination. The sampling points pesticides are shown in figure 3.4 and individual pesticides are shown in figure 3.5.

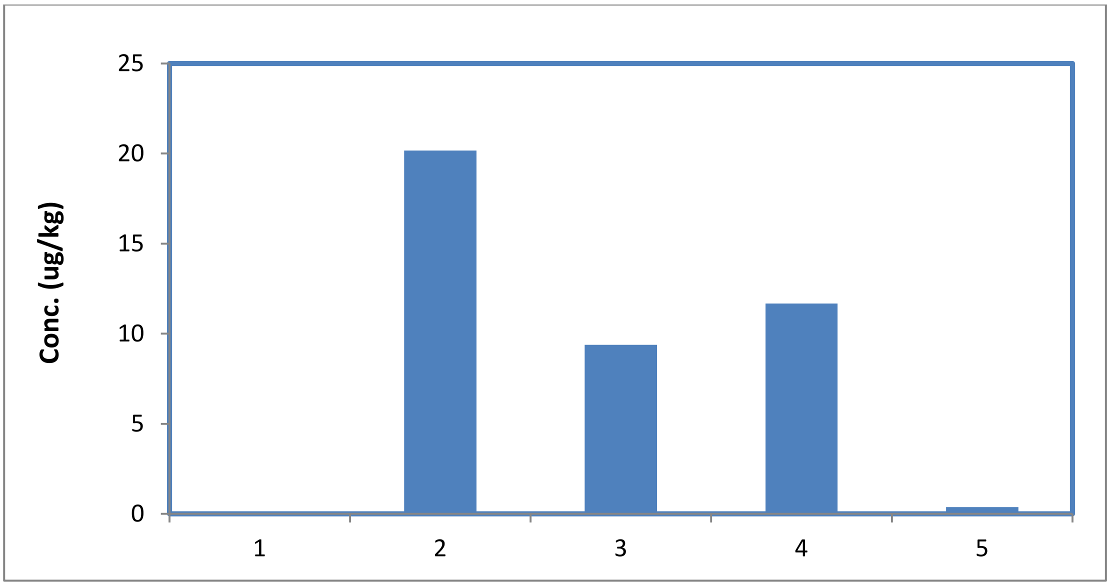


Figure: 3.4. Sum of Pesticide Concentration in Water Samples

According to Figure: 3.5 it shows that Endrine Aldehyde and Endosulfan I have the highest amount among the pesticides in all water samples. The fluctuations of the rest of pesticides are similar. The fluctuations of Endosulfan I and Endosulfan II are quiet same.

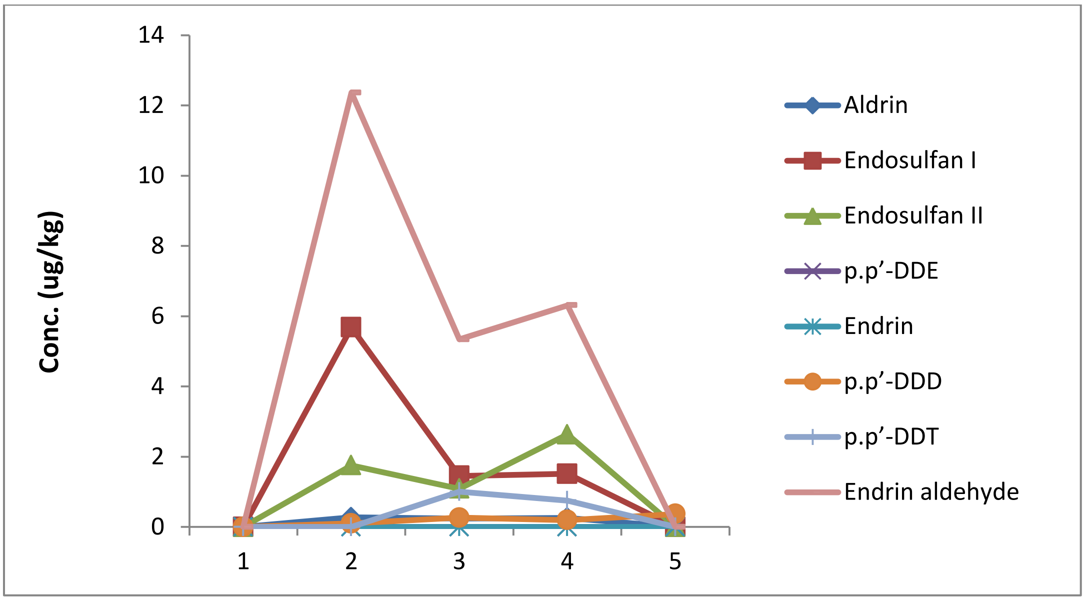


Figure: 3.5. Pesticide Concentrations in water Samples

3.2.3. Tomato Analysis

16 tomato samples were collected from Field 1 (1-8) and Field 2 (9-16). Each sample was extracted twice. In Field 1, there are some pesticides in tomato samples. These tomato samples were grown and red in color. However, no pesticide was observed in green (Ungrown) tomato samples collected from Field 2. The lowest concentrations was seen in sample points 5,7,8 and highest concentration was seen in sample point 3 in field 1. The concentrations of the rest sampling point in field 1 are almost similar. The sum concentrations of pesticides are shown in figure 3.6 names of individual pesticides are given in figure 3.7.

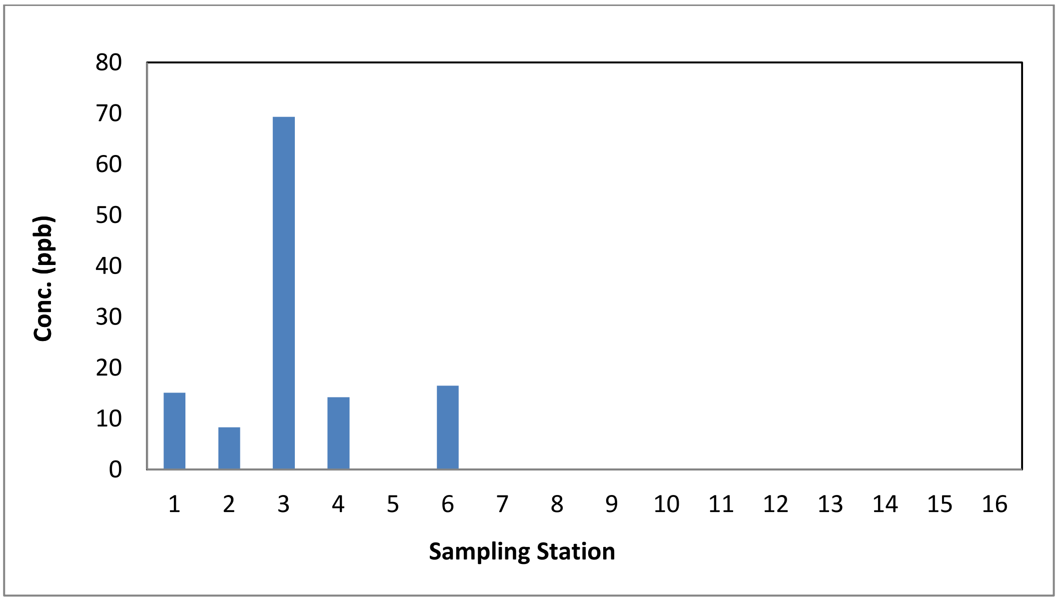


Figure: 3.6. Sum of Pesticide Concentrations in Tomato Samples

As can be seen from the Figure 3.7 only alpha-HCH and Endrine Aldehyde were observed in tomato samples collected from Field 1. Alpha-HCH was observed in 4 sampling points and only in second sampling point Endrin aldehyde was seen.

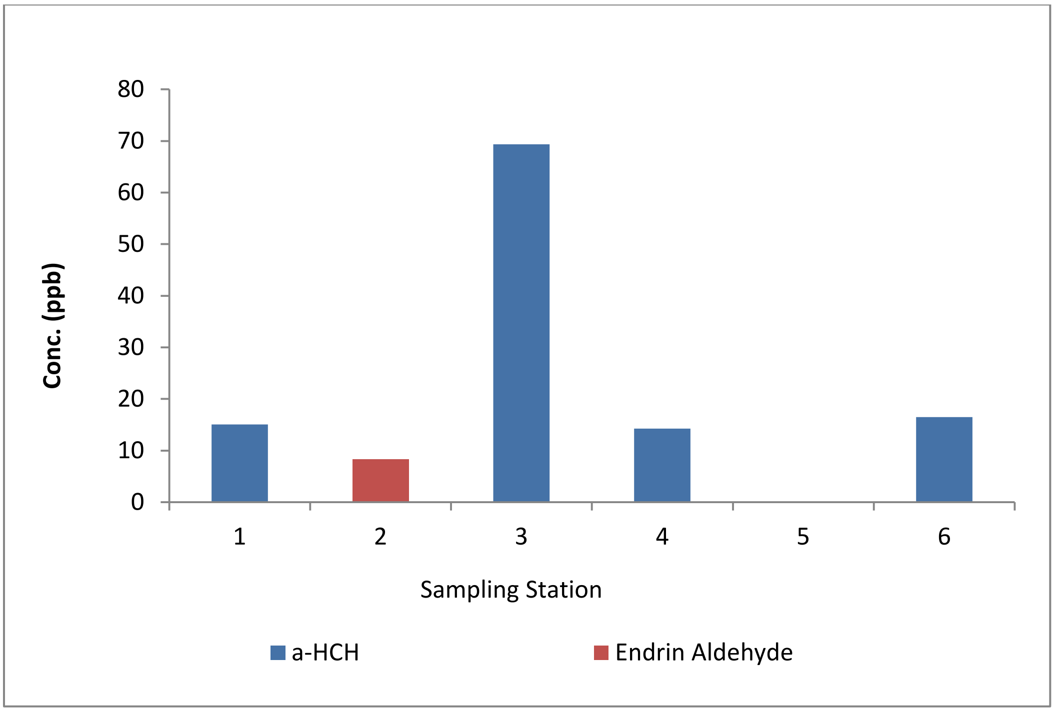
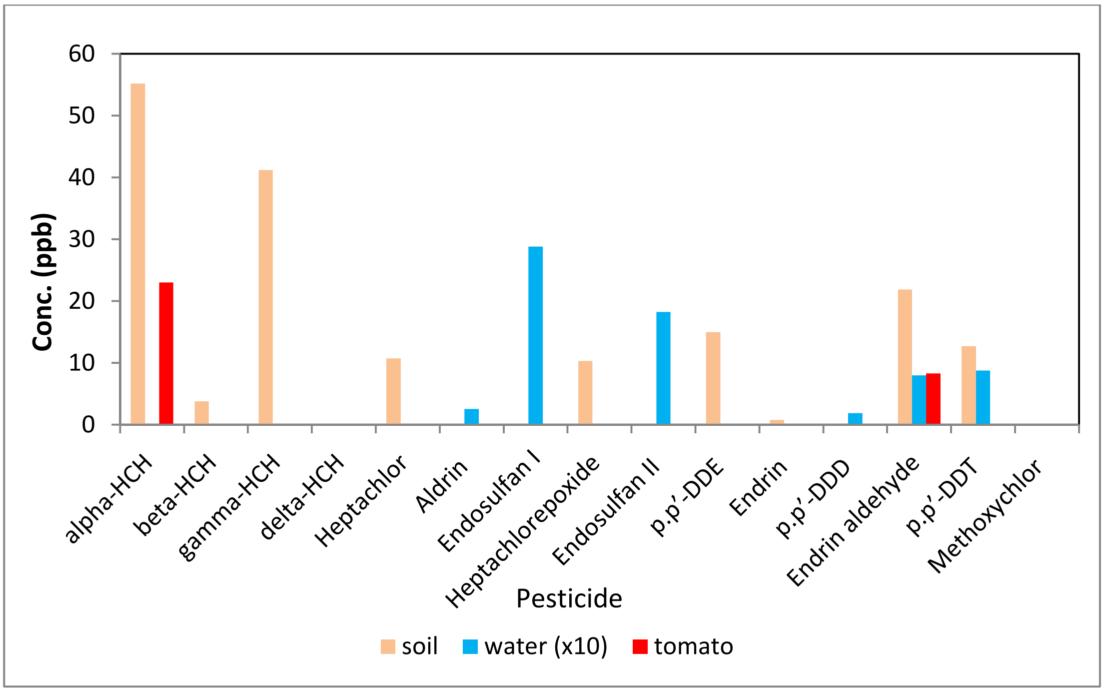


Figure: 3.7. Pesticide Concentrations in Tomato Samples

3.3.1. Comparison of Soil, Water and Tomato Pesticide Concentration

As can be seen from the figure 3.8 in exit water samples Endosulfan I, Endosulfan II, Endrin Aldehyde, pp-DDT and pp-DDD are seen. Secondly, in soil samples alpha-HCH and gamma-HCH is seen with highest concentration. In tomato only alpha HCH and Endrin Aldehyde were observed. And the level of Endrin Aldehyde in tomato and water samples is observed at the same amount. Since pesticides concentrations in waters are low (except Endrin Aldehyde) they were multiplied with 10. Endrin aldehyde concentration is high in soil so it is divided by 10.



Endrin aldehyde was divided by 1/10 for soil and except endrin aldehyde all water values were multiplied by 10.

Figure: 3.8. Comparison of Pesticide Concentrations in Soil, Water and Tomato Samples

Table 3.5 shows individual pesticides concentrations with their standard deviations in soil water and tomato samples Pesticide concentration range in soil was found as 3.799-219.1 µg/kg, and pesticide concentration range in water was found as 0.1877-8.005 µg/L, for tomato range is 8.302-23.03 µg/kg The cells with BLOQ some pesticides are seen however due to small amount they could not be calculated. According to the Table 3.5 order of pesticide contamination were soil>water>tomato except Endrin aldehyde. For Endrin aldehyde order changed as soil, tomato and water from highest to lowest concentration.

Table: 3.5. Average pesticide concentrations (ppb) in soil, water and tomato samples

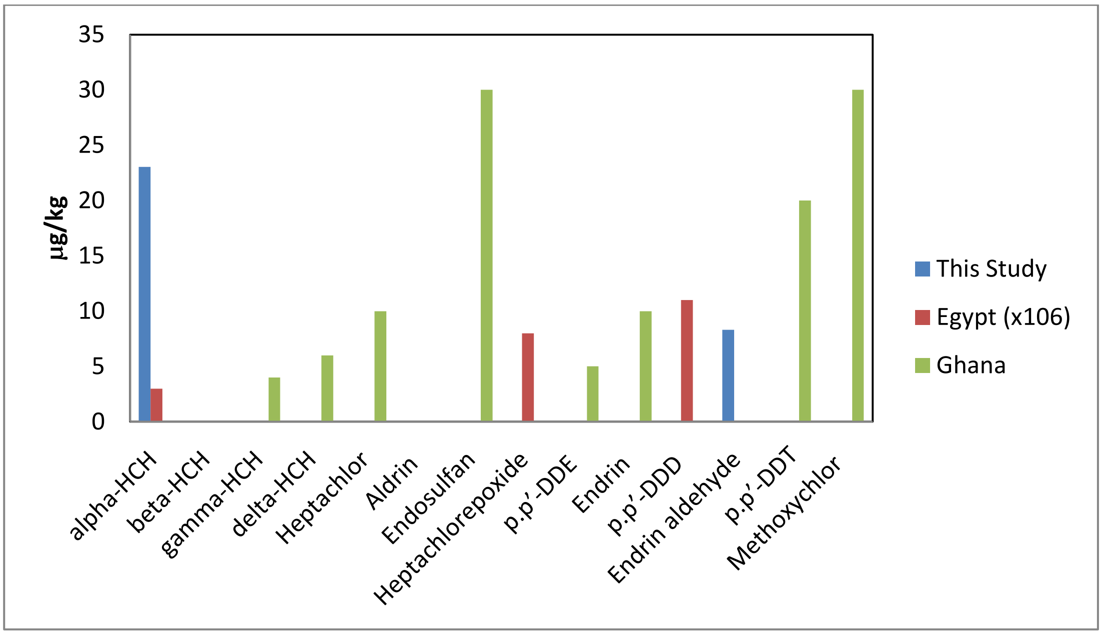
	Soil (µg/kg)	**Water (µg/L)**	**Tomato (µg/kg)**
alpha-HCH	55.19	BLOQ	23.03±26.73
beta-HCH	3,799	BLOQ	BLOQ
gamma-HCH	41.19 ± 29.59	BLOQ	BLOQ
delta-HCH	BLOQ	BLOQ	BLOQ
Heptachlor	10.73 ± 5.314	BLOQ	BLOQ
Aldrin	BLOQ	0.2539±0.01816	BLOQ
Endosulfan I	BLOQ	2.882 ± 2.424	BLOQ
Heptachlorepoxide	10.35 ± 5.915	BLOQ	BLOQ
Endosulfan II	BLOQ	1.826 ± 0.7769	BLOQ
p.p'-DDE	14.99 ± 7.494	BLOQ	BLOQ
Endrin	0.8152	BLOQ	BLOQ
p.p'-DDD	BLOQ	0.1877±0.08120	BLOQ
Endrin aldehyde	219.1 ± 116.9	8.005 ± 3.808	8.302
p.p'-DDT	12.70 ± 26.64	0.8792±0.1786	BLOQ
Methoxychlor	BLOQ	BLOQ	BLOQ

3.4.1. Comparison of Fields

In field 1, pesticide was observed in tomato samples very rarely. However no pesticide was observed in field 2. In field 2 soil samples were more polluted however tomato and water is less polluted. Therefore transition from soil to water and tomato is less in Field 2 since tomatoes are not grown like in Field 1.The longer the tomato stayed in field the more pesticides are seen. The fields were 1.5 km away each other and the pesticides sprayed twice before harvesting.

3.5.1. Literature Comparison

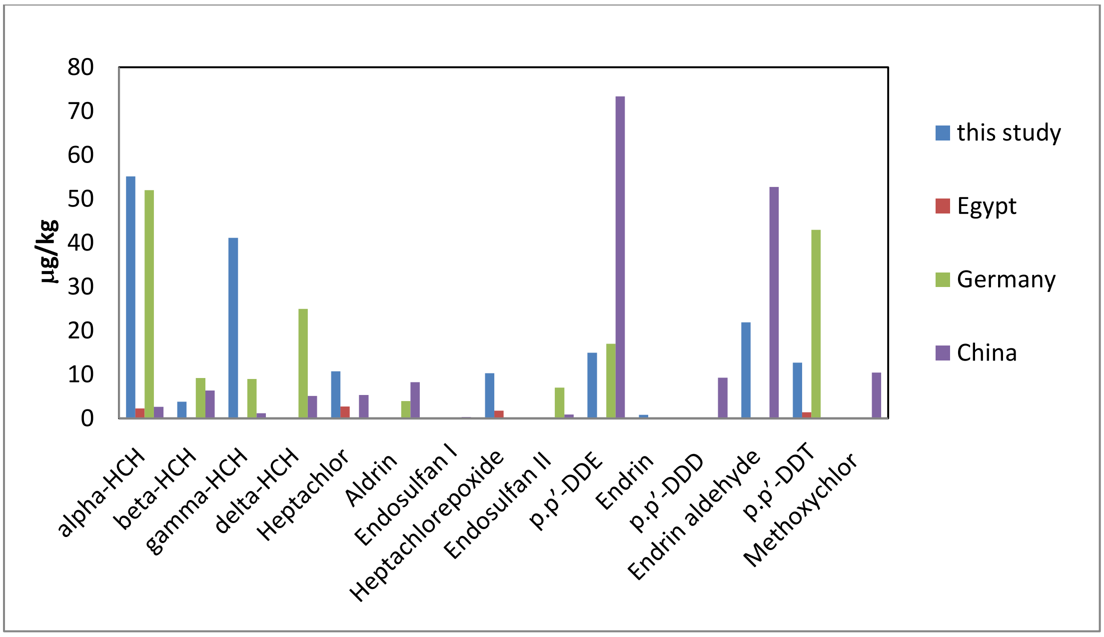
In Ghana gamma-HCH, delta-HCH and PP-DDE concentrations divided by 10. These pesticides have the highest concentrations. In Turkey also alpha-HCH has the highest concentration. In turkey Endrine aldehyde was observed but other countries observed very less. As can be seen from the figure 3.9 Ghana has the highest and Egypt was the lowest concentration and Egypt concentrations were multiplied with 10^6 .



Egypt:Abou-Arab, 1999, Ghana: Bempah et al., 2011

Figure: 3.9. The pesticides concentration in tomato from different countries.

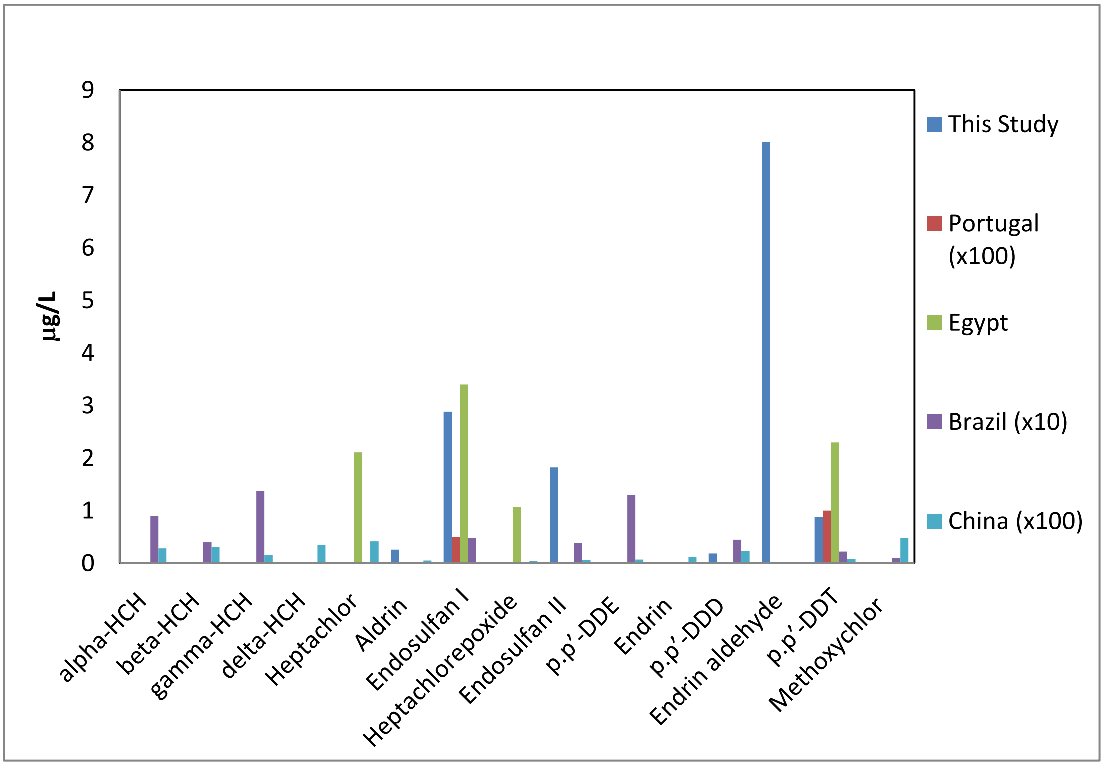
It can be seen from the figure 3.10 Turkey is in between the countries in terms of soil pollution with pesticides. Egypt has the lowest concentration in their soil. In China, soil is mostly polluted. Germany is also moderately polluted.



Egypt Ahmad et al., 1998, Germany: Kiersch et al., 2010, China: Zhou et al., 2013.

Figure: 3.10. The pesticides concentration in soil from different countries.

As can be seen from the figure 3.11 there is a slight difference between the countries. Turkey is mostly polluted with Endrin Aldehyde. Pollution level in Egypt is closer to Turkey. Portugal and China is the least polluted countries. Brazil is moderately polluted. In Egypt heptachloroepoxide concentration has the highest value and the concentration is divided by 10. In turkey Endrine aldehyde has the highest concentration among other countries. In Egypt Heptachlor, Endosulfan I, Heptachloroepoxide and pp-DDT were observed in highest amount. The concentration of pesticides in China, Brazil and Portugal are relatively small. Brazil concentrations were multiplied with 10, Portugal and China concentrations were multiplied with 100.



Portugal:Pinto et al., 2010, Egypt: El-Kabbany et al., 2000, China: Zhang et al., 2013, Brazil: Rissato et al., 2006

Figure: 3.11. The pesticides concentration in water from different countries.

3.6.1. ANOVAs Analysis

ANOVAs test was applied to understand whether there is a significant difference between the means of countries. For the comparison a common pesticide analytes (heptachloroepoxide) was chosen for soil samples is chosen. With the help of statgraphics program ANOVAs test is performed. According to ANOVAs test results it was found that F-Ratio is 6.96 and P-value is 0.0128. Since the P-value of the F-test is less than 0.05, there is a statistically significant difference between the means of the 4 variables at the 95.0% confidence level.

For ANOVAs analysis of water sample pp-DDT was chosen as a common analyte. The ANOVAs results show that the F-ratio is 4.21 and p-value is 0.072. Since the P-value of the F-test is greater than or equal to 0.05, there is not a statistically significant difference between the means of the 3 variables at the 95.0% confidence level.

For ANOVAs analysis of tomato sample alpha-HCH was chosen as a common analyte. The ANOVAs results show that the F-ratio is 35.96 and p-value is 0.0005. Since the P-value of the F-test is less than 0.05, there is a statistically significant difference between the means of the 3 variables at the 95.0% confidence level (Shegunova.2006).

3.7.1. Maximum Residue Level (MRL) values comparison with European Union (EU)

Maximum residue level (MRL) is the maximum amount of residue legally permitted on food. They act as an indicator of the correct use of pesticides, and ensure compliance with legal requirements for low residues on unprocessed food. MRLs are trading standards used to ensure that imported and exported food test whether food is safe to eat. In this study I found pesticides concentrations as 23.03 µg/kg for alpha HCH and 8.302 µg/kg for Endrine aldehyde. Table 3.6 shows The EU MRL values.

Table: 3.6. Maximun Residue Level (MRL) values for Tomato

	Tomato (µg/kg)	**MRL (EU)***
alpha-HCH	23.03±26.73	
beta-HCH	BLOQ	
gamma-HCH	BLOQ	0.00001
delta-HCH	BLOQ	
Heptachlor	BLOQ	0.00001
Aldrin	BLOQ	0.00001
Endosulfan	BLOQ	0.00005**
Heptachlorepoxide	BLOQ	
Endrin	BLOQ	0.00001
Endrinaldehyde	8.302	
DDT	BLOQ	0.00005***
Methoxychlor	BLOQ	0.00001

* EU pesticides database

**Sum of endosulfanI,II andEndosulfansulfate

***Sum of pp-DDE,pp-DDDandpp-DDT,op-DDT

From the table 3.6 MRL value for individual pesticide is 0.00001 µg/kg for tomato. However, there is exception for Endosulfan and DDT. For these pesticides the MRL values are 0.00005 they are Sum values of their derivatives. For example Endosulfan is sum of Endosulfan I, Endosulfan II and Endosulfan sulfate. DDT is the sum of pp-DDT, op-DDT, PP-DDD and pp-DDE.

From table 3.7 it can be seen that instead of using individual MRL values for pesticides in water. It was given only the total pesticides MRL value which is 0.5 for EU. The total concentration of pesticides in our samples is calculated as 14.04 and this value is much higher than maximum residue limit mention in the table.

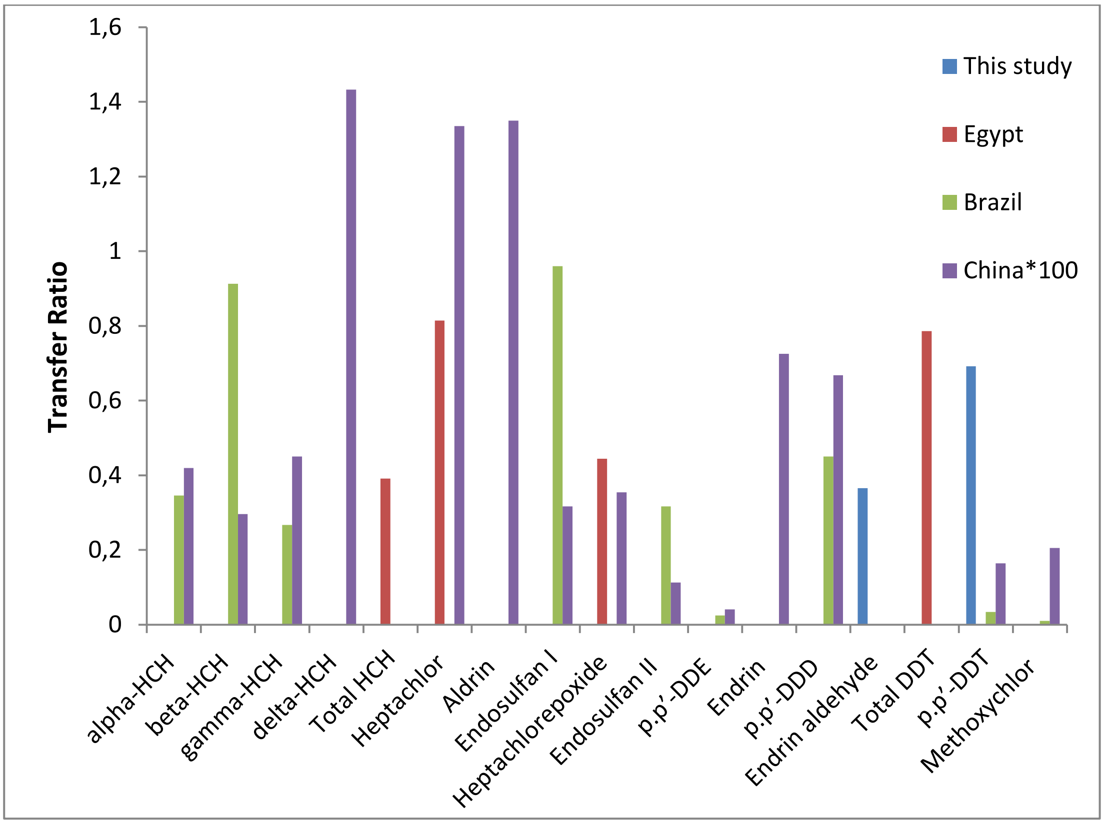
Table: 3.7. Maximum Residue Level (MRL) values for Water

	Water (µg/L)	**MRL (µg/L)***
alpha-HCH	BLOQ	
beta-HCH	BLOQ	
gamma-HCH	BLOQ	
delta-HCH	BLOQ	
Heptachlor	BLOQ	
Aldrin	0.2539±0.01816	
Endosulfan I	2.882 ± 2.424	
Heptachlorepoxide	BLOQ	
Endosulfan II	1.826 ± 0.7769	
p.p'-DDE	BLOQ	
Endrin	BLOQ	
p.p'-DDD	0.1877±0.08120	
Endrin aldehyde	8.005 ± 3.808	
p.p'-DDT	0.8792±0.1786	
Methoxychlor	BLOQ	
Total Pesticide	14.04	0.5

*http://www.europarl.europa.eu/sides/getAllAnswers.do?reference=E-2013-008794&language=EN

3.8.1. Soil to Water transition

As can be seen from the Figure 3.12 only Endrin Aldehyde and pp-DDT were observed in both soil and water samples and soil/water transfer is the highest (% 69) in DDT and the lowest (% 37) in Endrin Aldehyde. In China water is least contaminated from soil however more variety of pesticide transfer were observed. Brazil and Egypt has similar transfer ratios.



Egypt:Kiersch et al. 2010, Brazil: Rissato et al. 2006, China: Zhang et al. 2013

Figure: 3.12.Soil to Water transition

3.8.2. Soil to Tomato transition

Since only two pesticides were observed tomato, only their ratios were found. According to Figure 3.13 soil/tomato transfer is the highest for a-HCH (% 42), and the lowest for Endrin Aldehyde (% 38). For Endrin Aldehyde the ratio is similar to soil/water transfer was observed.

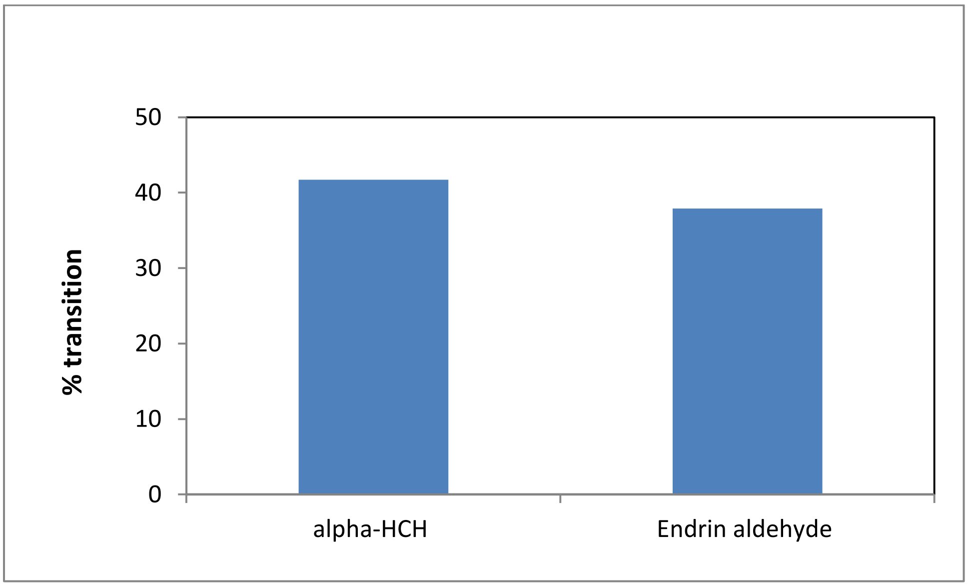


Figure: 3.13. Percent transition from soil to tomato

3.9.1. Health risk estimation

Health guidelines: (a) hypothetical body weight of 10 kg for children and 70 kg for adults; and (b) maximum absorption rate of 100% and bioavailability rate of 100%. Food consumption rate for fruits in Turkey was assumed as 0.250 kg/person/day. For each type of exposure, the estimated lifetime exposure dose (mg/kg/day) was obtained by multiplying the residual pesticide concentration (mg/kg) in the food of interest times the food consumption rate (kg/day), and dividing the product by the body weight (kg). The hazard indices for adults and children were calculated as ratios between estimated pesticide exposure doses, and the reference doses which are considered to be safe levels of exposure over the life time. Risk estimations were calculated as based on US Environmental Protection Agency's method. The health risk for tomato consumption was compared for Endrin Aldehyde and it was seen in the table 3.8 that for Children it is harmful but for adult it does not affect so much.

Table: 3.8. Health Risk for Tomato Consumption

Pesticides	***Refereence dose(mg/kg/day)**	**Estimated dose(mg/kg/day)**	**Hazard index**	**Health risk**
g-HCH	0.0030			
d-HCH	0.0030			
Heptachlor	0.0001			
Heptachloroepoxide	0.0001			
Endrin Aldehyde	0.0002	0.2965(adult) 2.07×10^{-4} (children)	0.1483 1.037	No Yes
pp-DDT	0.0200			

*Bempah.et.al(2011)

We have analysis soil, water and tomato for pesticides residues and we have gathered some information on effect of pesticides on environmental and human health. Now let's see how they affect the economy of a nation as well as farmers income. From table 3.9 it can be seen that there is an increase in tomato production around the World. If we consider 2000 there is an increase of %39 in tomato production until 2009.

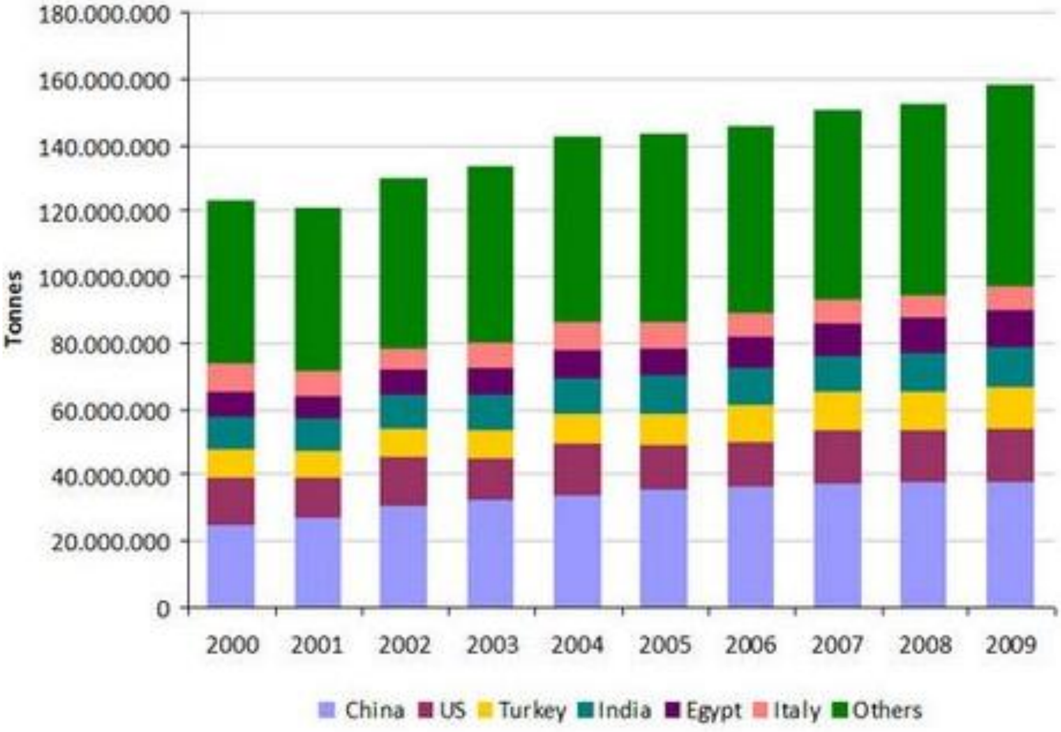
3.10.1 Tomato Production in the World

Table: 3.9. Annual Worldwide Production of Tomatoes from 2000-2009.

Year ending	**Tomato Production (tons)**
31-12-2000	110,017,091
31-12-2001	107,955,919
31-12-2002	116,265,102
31-12-2003	119,097,733
31-12-2004	127,644,308
31-12-2005	127,929,037
31-12-2006	130,226,252
31-12-2007	137,291,870
31-12-2008	142,153,859
31-12-2009	152,956,115

Source: UNCTAD

According to the figure 3.14 China has the highest production trends the others countries follows a similar trends with china. The US, Turkey, India, Egypt and Italy have an increasing trend in production of tomatoes. In addition their production in all years is similar.



Source: UNCTAD

Figure: 3.14. World production of tomato: 2000-2009

From the table 3.10 it can be said that the highest production of Tomato is in China and the position of Turkey is 4th in the world after USA and India.

Table: 3.10. Top 10 Countries producing tomato.

Rank	Country/Region	Tomato production (tonnes)
1	China	33,911,702
2	United States	13,718,171
3	India	10,965,355
4	Turkey	10,313,000
5	Egypt	9,204,097
6	Italy	5,976,912
7	Iran	4,826,396
8	Spain	3,922,500
9	Brazil	3,867,655
10	Mexico	2,936,773

Source: FAOSTAT , 2010.

The significant trading partners for the major exporters are shown in Figure: 3.15. Mexico, Morocco and Turkey with their trading partners; USA, Canada, El Salvador, Spain, France, Russia, Romania and Bulgaria; the most valuable relationship is between Mexico and the US. France is Morocco while Russia obtains most of its imports from Turkey.

Figure: 3.15. Mexico. Morocco and Turkey Trading partner map

The large amount of exported tomato from Mexico is to USA and Morocco to France and Turkey to Russia. The highest export tomato earning is 1563944005.00 dollar from Mexico to USA. Turkey has 248007706.00 dollars trade amount.

Table: 3.11. Mexico. Morocco and Turkey Trade volume

Map legend		
Country	**Trading partner/trade value($)**	
Mexico	USA	1,563,944,005,
	Canada	24,443,080
	El Salvador	6,854,805
Morocco	France	498,917,034
	Russia	20,772,120
Turkey	Russia	248,007,706
	Bulgaria	65,087,479
	Romania	42,468,289

3.10.2 Tomato trades in the world and economic earnings for the top 10 countries

The trade values from tomato Turkey is in 3rd after Mexico and Morocco. The highest mean price per kg is in Italy with $2.23/kg and Turkey with $ 0.83/kg. The lowest mean price per kg is in Jordan $ 0.63 can be seen from table 3.12.

Table: 3.12. Top ten exporters of tomatoes in 2010

Country	**Trade value ($)**	**Trade quantity (kg)**	**Mean price per kg**
Mexico	1,595,315,056	1,509,615,649	$1.06
Morocco	571,284,039	784,964,560	$0.73
Turkey	476,873,744	574,278,907	$0.83
USA	373,626,415	224,278,636	$1.67
Canada	356,415,730	166,869,630	$2.14
France	355,117,720	189,462,000	$1.87
Italy	287,182,488	128,797,318	$2.23
Belgium	281,623,333	191,100,924	$1.47
Jordan	232,376,618	371,257,022	$0.63
Israel	73,635,000	66,567,807	$1.11

Source: UNCTAD

The highest importer of tomato and highest spending on tomato is in USA. The highest mean price per kg is paid by Sweden with $2.02 per kilogram and Russia Federation imports the cheapest tomato with $1.10 per kilogram.

From the above table 3.12 and table 3.13 it is seen that USA, France, Canada and Italy is in Top 10 Export and import Countries. We can see also from the table that they import with cheap price and export with high prices.

Table: 3.13. Top ten importers of tomatoes in 2010

Country	**Trade value ($)**	**Trade quantity (kg)**	**Mean price per kg**
USA	1,879,534,489	1,532,491,924	$1.23
Germany	1,334,184,919	681,215,500	$1.96
Russian Federation	773,582,210	699,282,212	$1.11
United Kingdom	670,071,371	384,601,843	$1.74
France	608,674,710	497,387,900	$1.22
Canada	302,014,382	193,586,938	$1.56
Sweden	173,124,806	85,683,000	$2.02
Belgium	145,690,695	77,338,097	$1.88
Italy	132,437,305	97,270,928	$1.36
Czech Republic	132,224,370	91,419,161	$1.45

Source: UNCTAD

3.11.1. Tomato production in Turkey

Table 3.14 represents the top 10 cities in Turkey production of tomatoes and first 5 cities are focusing on fresh tomato and the next 5 cities are focusing on processed tomato. The range of production of fresh tomato is; the highest is; %99.9 in Antalya and Mersin and lowest value is %17 in Balikesir. The range of production of processed tomato is ;the highest is %83 in Balikesir and the lowest is %0.1 in Antalya and Mersin. Although the production is less than Antalya and Mersin the fresh tomato production is the highest in Mugla (%100). The highest production of process tomato is produced in Bursa.

Table: 3.14. Top 10 cities producing Fresh and Processed Tomato in Turkey

No	**Provinces**	**Production in tons**	**Fresh tomato production %**	**processed tomato %**
1	Antalya	1666826	99.9	0.1
2	Mersin	771223	99.9	0.1
3	Mugla	451922	100	0
4	Tokat	525676	91.9	8.1
5	Canakkale	482100	53.8	46.2
6	Konya	241655	47	53
7	Bursa	907024	22	78
8	Manisa	694281	19	81
9	Balikesir	399994	17	83
10	Izmir	734670	29	71

Source: TUIK , 2001

3.12.1 The rejection of tomato commodities from different countries due to the pesticides residue.

Table: 3.15. RASFF data for Rejection of tomatoes from different countries

No	Year	Product category	Product	Pesticide catagory	Origin	Destination	Action taken
1	12.04.2012	Fruits and vegetables	Green pickled tomatoes	Pesticide Residue	Turkey	Greece	Destruction
2	08.03.2012	,,	Tomatoes	,,	Turkey	Denmark	Official detention
3	29.02.2012	,,	Chilled Tomatoes	,,	Turkey	Romania	Placed under customs seals
4	27.02.2012	,,	,,	,,	Turkey	Romania	Official detention
5	20.01.2012	,,	Tomatoes	,,	Turkey and Tunisia	Slovenia	Official detention
6	19.12.2011	,,	Chilled tomatoes	,,	Turkey	Austria	Informing recipients
7	20.05.2011	,,	Tomatoes	,,	,,	Romania	Informing authorities
8	11.05.2011	,,	,,	,,	,,	----	,,,
9	06.05.2011	,,	,,	,,	,,	----	Obsolete
10	07.02.2011	,,	Chilled tomatoes	,,	,,	-------	Obsolete

Source: Republic of Turkey Ministry of Food, Agriculture and Livestock

Table: 3.15. RASFF data for Rejection of tomatoes from different countries (continued)

No	Year	Product category	Product	Pesticide catagory	Origin	Destination	Action taken
11	28.01.2011	,,	Tomatoes	,,	Turkey	-------	Destruction
12	16.03.2010	,,	,,	,,	,,	Hungary	Withdraw from the market
13	23.02.2010	,,	Fresh Tomatoes	,,	Turkey-Germany(Via)	Austria	Destruction
14	06.06.2007	,,	,,	,,	Turkey	Austria , Germany	Obsolete

Source: Republic of Turkey Ministry of Food, Agriculture and Livestock

Turkey is among the top 10 producer of the fresh tomato and also earns a lot of money by exporting to other countries. From the above from table 3.15 it can be seen that some of Turkish origin produced is returned back to turkey from these countries according to the date given. The countries are Greece, Denmark, Romania, Slovenia, Austria, Hungary and Germany. The tomatoes were distracted or withdraw from market, official detention and also informed the recipients about the pesticides residue presence. The amount and the economic loss is not mention about the return of tomatoes from other countries. The pesticides residues contaminated products are destructed, official detained, withdrawn from market, placed under customs seals, informed the authority and inform the recipients some are obsoletes.

CHAPTER 4

CONCLUSION

4.1. Conclusion

The17 chlorinated pesticides were determined in soil, water and tomato samples collected from 2 different fields in Ayaş, Ankara. Fields were close to each other and totally 8 samples were collected from each field. They were all analyzed by using GC-MS. SRM 2275 and 2261 were used for accuracy check. According to accuracy studies, %error values are changing between % 5.90-55.6. Calibration curves has also good linearity (r= 0.99). LOD values are 0.680-23.7 µg/L for water, 0.840-94.6µg/kg for tomato, 0.370-25.7 µg/kg for soil. LOQ values are the three times of these values. Values under LOQ were identified as BLOOQ.

Water samples were extracted by using solid phase extraction. Tomato and soil samples were extracted by using ultrasonic bath. Each sample was extracted twice and their averages were taken. The extraction recoveries were %64.88, %42.03 and %53.36 for tomato, water, and soil respectively. The extraction methods used for tomato and soil samples were similar except exchange solvent. For tomato, cyclohexane was used, for soil samples hexane was used. Acetone was used for water samples.

Totally 16 soil sample were analyzed. The range of pesticides in soil samples is 3.799-219.1 µg/kg. According to analysis results, field 2 is more polluted. Gamma-HCH, pp-DDT and alpha- HCH has the highest concentration among pesticides is in soil.

Two water samples collected from each field (total=4). The range of pesticides in water samples is 0.1877-8.005µg/L. In both fields entrance waters are (1 and 3) less polluted. Endrine Aldehyde and Endosulfan I have the highest amount among the pesticides in all water samples.

Sixteen tomato samples were analyzed. The range of pesticides in tomato samples is 8.302-23.03 µg/kg. In Field 1, there are some pesticides in tomato samples. These tomato samples were grown and red in color. However no pesticide was observed in green (ungrown) tomato samples collected from Field 2. Only Alpha-HCH and Endrine Aldehyde were observed in tomato samples collected from Field 1.

When we investigate pesticide concentration in all kind of samples we found that, the order of pesticide contamination is like soil>water>tomato except Endrine aldehyde, for soil, tomato and water from highest to lowest concentration. When we made literature comparison, it was found that Turkey is in middle of the countries (ANOVA test result). According to MRL values, water is contaminated. Transition from soil to water and soil to tomato were also investigated. In Turkey the highest% transfer pp DDT (%69) and lowest ratio is for Endrin aldehyde (%37) while transferring from soil to water. Since only alpha-HCH and Endrine aldehyde are found in tomato samples, only two of them were investigated and their transitions were found as alpha-HCH (% 42) and Endrin aldehyde (%38) from soil to tomato respectively.

If we compare 2 fields, in field 1, pesticide was observed in tomato samples very rarely. However no pesticide was observed in field 2. Infield 2 soil samples were more polluted however tomato and water is less polluted. Therefore transition from soil to water and tomato is less in Field 2 since tomatoes are not grown like in Field 1.The longer the tomato stayed in field the more pesticides are seen.

There is an increase demand for fresh vegetables and it was seen that tomato production around the World is increasing. Turkey has also increased its trend in tomato production. The position of Turkey is 4th in the world after China, USA and India. The large amount of exported of tomato from Turkey to Russia with 248007706.00 dollars trade amount. Turkey is in 3rd after Mexico and Morocco exporting huge amount of tomato. The production of fresh tomato is especially the highest in Antalya and Mersin (%99.9). Therefore, it could be said that tomato production is very important for Turkey. For that reason among other vegetables, especially tomato was taken into consideration in this study. However, there are some problems pops up during the exportation, such as the rejection of tomato commodities from different countries due to the pesticides residue. This is the common problem in world actually. For that reason some precautions should be taken. For example, farmers should be informed about the over use of pesticides. In addition more efficient policies about pesticide use should be developed. Although chlorinated pesticides are banned in Turkey and around the world they can be still observed in environment. This persistence can be explained by long life-time of these species which are so dangerous.

Everyone must be careful and the analysis of the pesticides should be made with a special attention in the field before plantation and after harvesting. For that purpose, private and state owned accredited residue laboratories should be established in order to carry out regular pesticide residue analysis for agricultural products. And the cities, where tomato production is high and exportation is common, (e.g. Antalya, Mersin) should be preferred firstly to establish laboratories. However, besides fresh tomato we must not forget that there is a large market for process tomato too.

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