

PESTICIDE RESIDUES IN GROUND WATER OF  
NORTHERN CYPRUS

A THESIS SUBMITTED TO  
THE GRADUATE SCHOOL OF APPLIED SCIENCES  
OF  
NEAR EAST UNIVERSITY

By  
BESTE OYMEN

IN PARTIAL FULFILLMENT OF THE REQUIREMENTS FOR  
THE DEGREE OF MASTER OF SCIENCE  
IN  
FOOD ENGINEERING

NICOSIA, 2014

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## ABSTRACT

A survey undertaken in Northern Cyprus has shown that pesticide residues are present in groundwaters. Liquid – liquid extraction followed by liquid chromatography with mass spectrometry was developed to monitor pesticides in groundwaters. Seventy compounds, including herbicides, fungicides, insecticides and some acaricides were surveyed to evaluate the quality of groundwaters.

Analysis which will be used for water samples are artichokes, parsley, peppers, eggplant, zucchini, lettuce, tomatoes, cucumbers, potatoes, leeks, lemon, watermelon, arugula, green beans, chard, peas, melon, spinach, okra, molehiya, gumbo, apricots, 40 different grapes of vineyards and citrus fruit grown in the area in the depth ranging from 10.5 to 105 meters, were collected from wells.

Twelve compounds included in this study (five insecticides (buprofezin, chlorpyrifos, diazinon, methidathion, imidacloprid), six fungicides (cyprodinil, difenoconazole, imazalil, iprodione, penconazole, propiconazole) and one herbicide (linuron)) were detected in one or more of the samples.

The insecticide chlorpyrifos and methidathion were compounds most frequently detected in water samples. Substances found in the highest concentration are methidathion, chlorpyrifos, difenoconazole and imazalil. Beside these substances, other detected pesticides are buprofezin, diazinon, penconazole, propiconazole, iprodione, imidacloprid and linuron.

The results reveal the presence of pesticides in some investigated samples. These results obtained in the European Union according to the limits for drinking water has been found to be below the limit.

**Keywords:** Pesticides, Groundwater, Liquid Chromatography, Mass Spectrometry, North Cyprus

## ÖZET

Kuzey Kıbrıs'ta gerçekleştirilen bu araştırma yeraltı sularında pestisit kalıntılarının mevcut olduğunu göstermiştir. Sıvı - kütle ekstrasyonu ile sıvı kromatografi ve ardından kütle spektrometresi yeraltı sularında pestisit kalıntılarını izlemek için geliştirilmiştir. Herbisitler, mantar, böcek ve bazı akarisitler de dahil olmak üzere yetmiş bileşik, yeraltı sularının kalitesini değerlendirmek için incelenmiştir.

Analiz için kullanılacak su örnekleri enginar, maydanoz, biber, patlıcan, kabak marul, domates, salatalık, patates, pırasa, limon, karpuz, roka, taze fasulye, pazı, böğrölce, kavun, ıspanak, bamya, molehiya, bamya, kayısı, üzüm bağları ve narenciye yetiştirilen 40 ayrı bölgede bulunan ve derinlikleri 10,5 ile 105 metre arasında değişen kuyulardan toplanmıştır.

Bu çalışmanın içerisinde yer alan oniki bileşik (beş insektisit (buprofezin, chlorpirifos, diazinon, metidation, imidacloprid), altı fungusit (cyprodinil, difenoconazole, imazalil, iprodione, penconazole, propiconazole) ve bir herbisit (linuron)) bir ya da daha fazla numunede tespit edildi.

Insektisit chlorpirifos ve methidathion su örneklerinde en sık tespit edilen bileşiklerdir. En yüksek konsantrasyonda rastlanan maddeler ise methidathion, chlorpyrifos, difenoconazole ve imazalil'dir. Bu maddelerin dışında buprofezin, diazinon, penconazole, propiconazole, iprodione, imidacloprid ve linuron tespit edilen diğer pestisitlerdir.

Sonuçlar, incelenen örneklerde bazı pestisitlerin varlığını ortaya koymaktadır. Elde edilen bu sonuçlar Avrupa Birliğinde içme suları için belirtilen sınır değerlerine göre limit altında olduğu tespit edilmiştir.

**Anahtar Kelimeler:** Pestisitler, Yeraltı Suyu, Kütle Spektrometresi, Sıvı Kromatografisi, Kuzey Kıbrıs

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## LIST OF ABBREVIATIONS

**DDT:** Dikloro difenil trikloroetan

**UK:** United Kingdom

**EU:** European Union

**USA:** United States of America

**EPA:** The United States Environmental Agency

**R<sub>1</sub>, R<sub>2</sub>:** Alkyl, alkoxy

**X:** Acid Radical

**O:** Oxygen

**S:** Sulphur

**P:** Phosphorus

**α-HCH:** Alpha Hexachlorocyclohexane

**γ-HCH:** Gamma Hexachlorocyclohexane

**pH:** Power of Hydrogen

**NO<sub>2</sub>:** Nitrogen Dioxide

**NH<sub>2</sub>:** Amine

**OH:** Hydroxide

**DDE:** Dichlorodipenyldichloroethylene

**H<sub>2</sub>O:** Water

**kg:** Kilogram

**ha:** Hectare

**mg:** Milligram

**L:** Liter

**mL:** Milliliter

**µg:** Microgram

**°C:** Centigrade heat unit

**mM:** Millimolar

**HPLC:** High Pressure Liquid Chromatography

**LC:** Liquid Chromatography

**MS:** Mass Spectrometry

**ESI:** Electro Spray Ionisation

**min:** Minute

**mm:** Millimetre

**µl:** Microliter

**sec:** Second

**DP:** Declustering Potential

**EP:** Entrance Potential

**CE:** Collision Energy

**CXP:** Cell Exit Potential

**Q1:** Precursor Ion

**Q3:** Product Ion

**Q2:** Collision Cell

**MRM:** Multiple Reaction Monitoring

**msn:** Milisecond

**cps:** Cycling per Second

**DA:** Dalton

**m:** Metre

**m/z:** Mass /Charge number of ions

**Ar:** Argon

**N<sub>2</sub>:** Nitrogen

**km:** Kilometer

**km<sup>2</sup>:** Square Kilometre

**TRNC:** Turkish Republic of Northern Cyprus

**m<sup>3</sup>:** Cubic meter

**CH<sub>2</sub>Cl<sub>2</sub>:** Dichloromethane

**S:** Sample

**ppm:** Parts per Million

**ppb:** Parts per Billion

**R<sup>2</sup>:** Regression

**LOD:** Limit of Detection

**ng:** Nanogram



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 material and results that are not original to this work.

Name, Last Name :

Signature :

Date:

## **CHAPTER 1**

### **INTRODUCTION**

One of the most important problems experienced across the globe, environmental pollution, due to industrialization and climate changes and to the reduction of farmland and the decrease in productivity gains compared with the increasing population, shows us that nutrition needs are not fulfilled. Existing agricultural fields are evaluated in the best possible way to find a method to increase the productivity. Chemical control method is one of the most preferred methods for this. Today if production is done without the use of pesticides 65% of the products quantity will be lost (Öztürk, 1997).

The use of chemical pesticides, ensures high quality and quantity of products, offers easy application, gives the result in a short time, can be applied to large areas and is relatively low cost. In addition to these advantages, however, the negative effects of pesticide residues in the environment should not be unnoticed. Unconscious use of pesticide can ruin the natural balance, can cause soil water and air pollution, leave residue in foods and create resistance of pests (Özkan et al., 2002).

When pesticides remain active in the environment for long along time, bioaccumulation trends and their impact on non-target species pose a great danger for ecosystem health. For this reason, pesticides require monitoring and surveillance in foods and environment for health protection, environmental assessment and pollution. The diagnosis, identification and concentration measurement of pollutants are not only important in pollution content and their effects, but are also important for understanding new pollution control precaution activities (Akbal and Onar, 2000).

When literature research was done they found out that no pesticide screening had been done in the groundwater of North Cyprus. Pesticides which will be screened in this study are the ones according to the statistics of the state laboratory in 2012-2013, the most extensive use of pesticide in North Cyprus. Artichokes, vegetables, potatoes, vineyards and citrus grown in 40 different regions are used in the analysis of groundwater.



**Table 1:** Pesticides which are highly concentrated in fresh fruit and vegetables in the Turkish Republic of Northern Cyprus (TRNC Governmental Chemistry Laboratory, 2012-2013)

Area	Product	Pesticide above limit
Mağusa	Parsley	Chlorpyrifos
Mağusa	Celery	Propargite
Yedidalga	Lemon	İmazalil
Gaziveren	Celery	Chlorpyrifos
Haspolat	Pepper	Acetamiprid
Tatlısu	Bell pepper	Etoxazole
Şehitler	Artichoke	Pyrimethanil
Alaniçi	Artichoke	Folpet
Tepebaşı	Chard	Chlorothanil
İskele	Artichoke	Folpet
Karşıyaka	Cos	Chlorpyrifos
Taşpınar	Strawberry	İmazalil
Çatakköy	Parsley	Chlorpyrifos
Tepebaşı	Tomato	Chlorothanil
Yıldırım	Parsley	Chlorpyrifos
Koruçam	Parsley	Malathion
Yuvacık	Artichoke	Iprodione
Alsancak	Kapia Biber	Acetamiprid
Güzelyurt	Jaffa	Methidation
Aydınköy	Mandarin	Methidation
Gaziveren	Mandarin	Methidation

The reason of this study is to evaluate the possible groundwater contaminations in Northern Cyprus.

The study shows in agricultural field, statistically the most widely used 72 pesticides in North Cyprus.

### **1.1. Definition and History of Pesticides**

Pesticide is the common name of chemicals that is used to reduce the devastating effects such as insects, rodents, weed, fungi like living forms, in human and animal body, on plants or living things around them, food resource production, damages and reduces during storage and consumption (Maister, 1999).

Pesticides, besides agricultural activities, can be used in different areas such as human health threats like malaria, significant health problems created by mosquitoes, household pest control, forestry, landscaping, fumigation protectionism, industrial pest control, construction, aquatic organisms control, food preservation and community hygiene. All mentioned in this wide scope and pesticides used for many years, today are spread into the environment and is one of the most dangerous pollutants (Güler, 1997).

The first synthesized organichlorine structured insecticide is the DDT (Dikloro Difenil Trikloroetan). Synthesized for the first time in 1847 by Othmar Zeid, its biological activity was shown by Paul Muller in 1936. It was first used against illnesses spread mechanically and biologically in the Second World War. After the use of synthetic and pesticide use of DDT the production and use of the synthetic pesticides has accelerated (Dağlıoğlu, 2004).

The first concerns of the environmental risks related to the use of pesticides came with the discovery of synthetic pesticides. For example in 1946, Cottam and Higgins studied the direct and indirect effects of the DDT in fish, birds and wildlife. However, the book published in 1962 “Silent Spring” written by an American writer Rachel Carson, aroused a great public interest about the risks posed by the use of pesticides in the environment. In her book Carson gathered attention for the first time in all its dimensions on how the unlimited use of pesticides, specially DDT, dieldrin and aldrin organochlorine pesticides had negative effects on fish and birds. Development of resistance to DDT, negative effects on non-target species such as accumulation in fatty tissues of living subjects were discussed.

As a result of research, due to physicochemical properties, permanent featured organochlorine pesticides led to numerous bird and fish deaths, and reached more

concentrated to the end of the food chain which is mankind. In later years, some countries brought restrictions; in some countries it is strictly prohibited. The use of DDT in the United States was banned in 1971, in the UK between the years of 1974 to 1984 went to voluntary abandonment, today is completely prohibited (Erdem, 2010).

Nowadays, especially in developed countries there is more conscious and controlled use of pesticides. To achieve this, the EU countries and the United States made many laws and as the official organizations, civil society organizations have a say in this direction too. In modern pesticide application a principle has been adopted that says it can be used in a level of not harming the environment and only when it is necessary. As a result of this, including the USA, in developed countries “low risk” or “environmentally friendly” pesticides have been preferable. For example, the United States Environmental Protection Agency (EPA), began to facilitate the registration of these pesticides and their use, and began to promote them (Türel and Tarakçı, 2009).

## **1.2. Classification of Pesticides**

Pesticides can be classified as follows according to various criteria (Erdem, 2010)

1. According to biological targets
2. According to the composition's active group substance
3. According to the Biological period

### **1.2.1. Pesticides to the Biological Target**

1. Kills insects (Insecticides)
2. Kills mushrooms (Fungicides)
3. Kills bacteria (Bactericidal)
4. Kills spiders and mites (Acaricides)
5. Kills weed (Herbicides)
6. Kills nematodes (Nematicides)

7. Kills rodents (Rodenticides)
8. Kills snails (Molluscicides)
9. Kills seaweeds (Algicides)

### **1.2.2. Pesticides According to the Active Substance Group**

1. Inorganic Pesticides
  - a) Arsenic pesticides
  - b) Mercury pesticides
  - c) Fluoride pesticides
  - d) Copper pesticides
  - e) Elemental sulfur
2. Synthetic organic pesticides
  - a) Organochlorine
  - b) Organophosphates
  - c) Organosulfur
  - d) Carbamates
3. Natural organic pesticides
  - a) Rotenone
  - b) Pyrethrum
  - c) Nicotine
  - d) Allethrin

### **1.2.3. Pesticides According to Their Biological Period**

1. Larvicides (kills larvae)
2. Ovicides (kills the eggs)
3. Adultisit (kills adult insects)
4. Ovalarvisit (kills eggs and larvae)

Herbicides in agrochemicals are on the top of the list with the proportion of 47 %. It is followed by a 29 % of insecticides and 19 % of fungicides. Herbicides and insecticides compromise a greater part of 70 % of its usage (Dağ et al., 2000).

### **1.3. The Mechanism Against the Formation of Resistance Against Pesticides**

Agricultural pests; climate change, disease, environmental factors such as competition with other species generation, while in the struggle to ensure the continuation of pesticides has been another factor compelling them. Powerful individuals, populations, constantly changing to adapt to genetically to these conditions have developed resistance (Cygler et al., 1993).

The development and formation of resistance is a selective event. By extracting the non resistant pesticides in a population of individuals, resistant individuals pass their genes to the next generation and by doing this the percentage of the resistance problem is increased in the population day by day. The frequent use of the pesticide in the same population makes the resistance faster (Kaygısız, 2003).

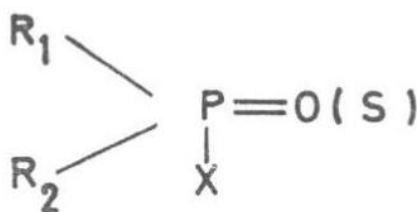
Every year the increase of the resistance to pesticides leads to the use of chemicals and creates problems like the extinction of not only pest species but also of the useful pests (Çakır and Yamanel, 2005).

The most important situation that creates problems to the resistance of the agricultural pests against pesticides, is that during the use for resistance of the pesticides, the insects that feed the same pesticide don't show the same resistance and this may give the pest a superiority and therefore their number starts to increase. Such situations can trigger the degradation of the ecological balance. To reduce the development of resistance, the usage of different drugs in the same area or mixing different pesticides, just make pests effective in their reproductive stages (Erdem, 2010).

#### 1.4. Organic Phosphorus Pesticides

Organophosphates, phosphorus-containing acids, ester thiolester or anhydride derivatives are used in agriculture, homes, gardens and veterinary pesticide. Pesticides with organophosphates were initially synthesized in the 1800s and in the 1930s its cholinergic effects were defined and its insecticidal properties were discovered. After a short time they found out that it can be used as warfare agent. After the Second World War pesticides have begun a large scale production (Demirdöğen, 2010).

Biologically active organic phosphorus compounds of the general structures are shown in the following scheme:



**Figure 1.4:** The organic phosphorus compounds of the general chemical structure  
(Vural and Güley, 1978)

Here  $R_1$  and  $R_2$  are alkyl, alkoxy, alkyl amino; X can be an acid radical (fluoride, nitrite, phenol, enol). Oxygen or sulphur is attached directly to phosphorus. In biological activity, the fifth link must be strong acid radical (Vural and Güley, 1978).

Despite being highly toxic, organophosphates are not usually environmentally persistent; sunlight, air and when in contact with soil they are broken down as hydrolysis. Thanks to these features, organophosphates are started to be used as an alternative to persistent organochlorines such as DDT, aldrin and dieldrin. The popularity of organophosphate pesticides increased when organochlorine pesticides were banned in the 1970s. Although organophosphates break faster than organochlorines, their acute toxicities are higher (Costa, 2006).

### 1.4.1. Mechanisms of Action

The mechanism of action of organophosphates is based on the suppression of acetylcholinesterase enzyme. Acetylcholine which is a neurotransmitter, acetylcholinesterase which breaks into choline and acetic acid, in the central and peripheral nervous system they are located in neuromuscular junction and in erythrocytes (Demirdöğen, 2010).

The organophosphate, inactivate the acetylcholinesterase enzyme by phosphorylating the hydroxyl group of serine amino acid in the active area. When the acetylcholinesterase enzyme is suppressed it starts to accumulate acetylcholine in the nervous system and as a result, muscarinic and nicotinic receptors are over stimulated. This case is called 'cholinergic syndrome'. Increase in the amount of acetylcholine in cholinergic nerve junctions, leads to smooth muscle contraction, and would lead to the secretion of glands. At junctions of skeletal muscle it can lead to an excessive amount of acetylcholine but can also paralyze the cell. The high amount of acetylcholine in the central nervous system, leads to sensory and behavioral disturbances, incoordination, the suppression of motor function and leads to respiratory insufficiency. Respiratory disorders associated with increased lung secretions are the most common cause of death seen in organophosphate intoxication (Demirdöğen, 2010).

Organophosphorus compounds which can be absorbed by inhalation and gastrointestinal tracks, can also be absorbed significantly through the skin. Organophosphorus insecticides, at the same time, by increasing the formation of free radicals it can reduce the antioxidants. Especially in individuals with low levels of cholinesterase, there can be increase in lipid peroxidation and decrease in total antioxidant capacity, degradation in erythrocyte membrane structure and there can be an increase in oxidative stress due to acetylcholinesterase inhibition (Yalvaç et al., 2004).

In the structure of many types of organophosphate pesticides there is a sulfur atom which is double bonded to phosphorus. To become toxic, they have to be transformed with metabolic activation into oxo, so in their structures, P=S group must be converted to P=O group. Because only the ones that are structured by P = O group with the organophosphate compounds may suppress the acetylcholinesterase. As a result of a biotransformation called

“Oxidative desulfuration” the P450 enzymes catalyze the microsomal cytochrome in the liver and the organophosphate becomes toxic (Demirdöğen, 2010).

## **1.5. Organic Chlorine Pesticides**

### **1.5.1. Structure and Effects**

The first synthetic organic insecticides which were used in agricultural war, are organochlorine pesticides which are the ones combined by carbon and chlorine (Tuncer, 2000).

Organochlorine insecticides can remain intact for a long time, can be soluble in lipid, their biotransformation and biological degradation is very slow, and for this reason various organisms are subjected to biomagnifications and this brings negative effects and shows us that through food chain, this has reached the mankind (Vural, 2005).

Organochlorine pesticides such as aldrin, dieldrin, endosulph and isodrine can remain stable in water even after many years (Golfinoupoulus et al., 2003).

Organochlorine insecticides are prepared by the chlorination of acyclic varied hydrocarbon. Due to its oil dissolution properties, its neural toxicities are high. Organochlorine insecticides are insoluble in water, but soluble in organic solvents, mineral, in plant and animal fats. These features make the organochloride insecticide remain in the environment for a long time and can cause accumulation in other tissues and in human and animal fats. Especially the accumulation in the milk of pets, makes it important in terms of human health. Today, used from this group, the most important insecticide is endosulfan (Dökmeci, 1994).



## 1.6 Transport of Pesticides

Pesticides first spread in the atmosphere from smoke machines or from compressed boxes by spraying into the air. The movement of pesticides in the atmosphere, are effected by factors such as the size of particles, the dispersed volume, air flow velocity and air temperature. Pesticides, binding with dust particles in the air, can go kilometers away and by combining with other chemicals in the air they can create secondary pollutants (Güler and Çobanoğlu, 1997).

The insecticides which mix with the atmosphere in particles or vapour, are cleaned by rain and therefore transported in stream, creek and lakes and then deposited in soil. Also by being attached to gas and other particles in the atmosphere it accumulates in soil and as a result it gets transported to surface and ground waters (Tunçbilek, 1998).

Pesticides applied directly to soil surface or plants, vaporization, runoff, soil penetration and adsorption like factors play a role. Evaporation is on the surface of soil, water and plants and the most important factor in the evaporation of pesticides is its vapor pressure. Furthermore, high temperature, low relative humidity and air movement are environmental factors that accelerate the evaporation. The evaporation of the pesticides is less likely to happen in strongly absorbed pesticides by soil particles. For this reason physical and chemical structure of the soil and the type of formulation of the pesticide are other factors affecting evaporation (Aksoy and Demirci, 2000).

In the transport of pesticides in surface, runoff, slope of the land and structure, soil moisture, and rainfall factors such as erosion status is effective. Pesticides, usually after medication, are transported more after heavy and continuous raining. Movement of pesticides into the soil from the soil surface is referred to as infiltration into the soil. This result of this leakage can bring pesticides to groundwater. Penetration into the ground depends on the physical and chemical properties of the pesticide. These properties can be listed as the rate of pesticides absorbed by soil particles, permeability of water through soil, the persistence and

duration of the pesticide, application dose, method and duration, soil tillage methods that alter the soil structure and rain or water on the area after it is sprayed (Aksoy and Demirci, 2000).

The adsorption of the organic soil and inorganic material of chemicals depends on their absorbed and absorbing properties. Pesticides, in their adsorption by soil system are indirectly effected by the physico-chemical structure of the pesticide, soil reaction, cation structures on the colloid change surface, soil water content and with the direct effect of heat, the soil's physical properties as substrate and outdoor climate conditions (Erdem, 2010).

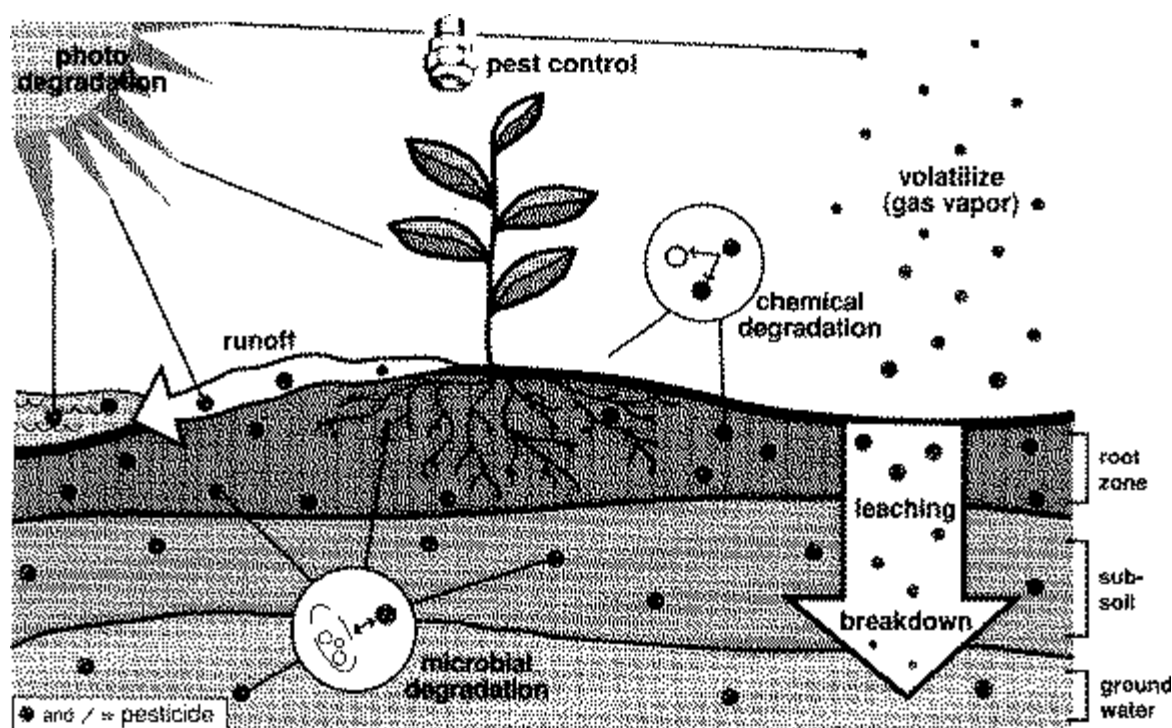
The absorption of the pesticides in target or non target organisms depend on; the active substance of the pesticide, formulation type, physical and chemical properties, dosage rate, rate of its fragmentation in nature, biochemical and physiological structure of target and non target organisms, environmental conditions and on physical and chemical structures of soil (Aksoy, 2000).

When insecticide is accumulated on soil surface, it evaporates and mixes again in the atmosphere. By doing this it moves through the soil surface until it is completely disintegrated (Tutkun, 1999).

Until the 1960s it was thought that pollution which was caused by pesticides were a local problem and it was believed that there was a very small shipment of insecticides which remained for a long time. When DDT and other organochlorine compounds were found in the Arctic and Antarctic fish and mammalian body this opinion has changed. They found out that insecticides moved with heavy wind and rain to places where it was not even sprayed. Nowadays, the atmosphere plays a big role in the transportation of the insecticides, their transportation to far away areas where it is not sprayed and their accumulation in those areas. Most of the oranochlorine compounds found ahead of the list of insecticides found in the atmosphere are DDT,  $\alpha$ -HCH,  $\gamma$ -HCH (lindane), heptachlor and dieldrin (Tunçbilek, 1998).

The undergo of accumulation and magnification and their accumulation in biotic and abiotic environment, form an important environmental problem in pesticides which enter the

food chain. The most important problem associated with the use of pesticides, is when, where, how it interacts and the difficulties in finding with certainty its harm for humans and nature when it is processed for a long time. Therefore within legal frames of nature, they imposed stricken limitations in the usage of pesticides which are time consuming and difficult to destruct in the nature (Klinhard, 1995).



**Figure1.6:** Pesticides fate processes (Wardworth, 1914)

The amount of pesticides used in pesticide application process, timing and method of application is very important in the formation of pesticide pollution problems. If pesticides are used more and more, there is a possibility of contamination of ground water. Rainfall and irrigation and groundwater flows resulting from this, plays an important role in the movement of pesticides (Close, 1993).

Pesticides can enter water bodies with spread in soil. This could be from direct flows from the soil surface or from houses, plants and agricultural regions. Some pesticides could seep into the ground water by water flow, by injecting it into the soil and by the washing from

rain and snow. Therefore the pesticides should always be used under control and water bodies should be done regularly. Before the control of pesticides and algae, surface water bodies and lakes should be carefully evaluated. If this evaluation is not done, pesticides can bring loss instead of benefits. It shouldn't be forgotten that too much fertilizer for agricultural purposes used in homes, through their rainwater gutters and underground water sources, might be a very easy reach. The watering of pesticides during field work and done next to wells which water is taken from it with containers, makes the surrounding area of the well a pesticide concentrated area. Washing the containers contaminated with pesticides by using the well water, may increase the situation (Güler and Çobanoğlu, 1997).

Groundwater and surface water contamination by pesticides is creating serious problems. In such cases, the unconscious use of pesticides that cause pollution must be controlled. The presence of contaminated groundwater also causes contamination and also will cause degradation (Kırımhan, 1997).

## **1.7. Decomposition of Pesticide Mechanism**

The events that cause decomposition of pesticides in soil systems are sequenced as photochemical decompositions, chemical decompositions and biological decompositions. The defined three separate decompositions can take place separate from each other or as a combination of three mechanisms.

### **1.7.1. Photochemical Decomposition**

Although the photochemical degradation of pesticides are mostly seen in air and water, the most important one is the one which happens in soil. Light energy is absorbed strongly by the soil and finally a photochemical decomposition takes place near the surface of the soil. This phenomenon is especially observed in dry soil and in sunlight exposure in a thin layer (Connell and Miller, 1984).

Because this type of decomposition is limited completely by the soil surface and not incorporated into soil after the capillary rise of water from the soil surface is likely to remain under the influence of compounds ( Haktanır and Arcak, 1998).

### **1.7.2. Chemical Decomposition**

Chemical reactions that create this by the soil components are classified as catalyzed and non catalyzed reactions. The first group entered the chemical degradation reactions are hydrolysis, oxidation, isomerization, ionization and consists of salt formation. Catalyzing events that are formed by soil, especially if soil is acid characterized, clay fraction plays an important role (Haktanır and Arcak, 1998).

Decomposition reactions catalyzed by compounds of land usually near the surface of clay minerals are associated with an increase in hydrogen ion concentration Furthermore soil components like iron oxides and amorphous alumina also catalyze the decomposition. On the other hand, organic substances in the environment may delay or stop the chemical decomposition. The exact mechanism of the catalyst effect of the soil has not been clarified. Catalytic effects depend largely on the nature of the pesticide. In the decomposition of a pesticide compound a soil factor that does a catalytic effect, can cause a retarding effect on another pesticide decomposition (Haktanir and Arcak, 1998).

### **1.7.3. Biological Decomposition**

This type of decomposition is controlled by microorganisms. This event is affected by factors like the soil temperature affecting the normal biologic effects in the soil, moisture content, presence of organic material and pH. Some polar groups which contain pesticide molecules form an impact point for the microorganisms. They are- OH-, -COO-, -NH<sub>2</sub> and – NO<sub>2</sub> groups. One of the intensely used and investigated chlorinated hydrocarbons like DDT, in the 1980s are highly resistant to soil decomposition. During the microbiological decomposition of DDT, DDE which is resistant in ambient conditions is formed ( Haktanır and Arcak, 1998).

### 1.8. Pesticide Effects on Soil, Water and Animals

Pesticides can be applied directly on soil or it reaches the soil in indirect ways like through air sprayed ones which are washed by rain and then reach the soil. By leaves falling in autumn and plant residues, many pesticides fall on soil (Pimentel, 1986).

As is well known, by soil microorganisms break down plant and animal residues, including particularly nitrogen, phosphorus, sulfur like nutrition elements, and creating CO<sub>2</sub> and H<sub>2</sub>O maintain balance. However, by fixing the free nitrogen in the atmosphere, it provides nitrogen to soil and plants. The soil microflora creates effective operations on soil but the effect of the insecticide prevents them from fulfilling their beneficial actions (Erdem, 2010).

By putting insecticides in agricultural areas, DDT and other like pesticides accumulate on soil. 15-20 kg/ha of DDT has been identified in the soil of normally medicated farmlands. Efficient, treated soils contain much more live elements so the accumulation of poisons in the soil is extremely dangerous. One kg of rich farm soil contain one trillion bacteria, fungi 200 million, 25 million algae, 15 million protozoa and includes many more living things. This is vital for soil organisms. These organisms provide the soil cycle, and activities. The accumulated effects of the poison to these organisms are not fully understood. However, an investigation in the US showed that some of the organochlorine hydrocarbons prevent the nitrification of the soil. In the soil which has been sprayed with toxaphene 10 years ago, termite do no longer live (Tuncer, 2000).

As well as insect pests, pesticides have a negative impact also on non-target organisms. But these effects may vary. In studies on the subject we have seen that the beneficial insects which we accept as predators and parasites are more affected from insecticides. Beneficial insects are killed directly and also the pests and their nutrients are effected indirectly because they are also killed. Many pesticides directly toxic to beneficial insects, is higher than that of the host (Tutkun, 1999).

The main factors affecting the persistence of pesticides in the soil; soil texture, soil temperature and humidity, soil organic matter, cation change capacity, soil pH, the pesticide's

vanishing, and adsorption capacity, receiving and washing of the pesticide with plant roots and soil microbial activity (Erdem, 2010).

Pesticides which from soil are carried to water in many ways have bad effects in various living things. For example in fish, it can cause the decrease of resistance to some illnesses, reproducing disorder and eating disorders. Some pesticides the slow development of fish larvae, cause damage to gills and the liver. Some pesticides also affect enzyme activity in fish. At the beginning fish showed resistance to the drugs, then they started to accumulate greater amounts of residues and the people and animals who eat these fish also get these residues and accumulations of the residues in their body increases (Zeren, 1978).

Some incorrect medicine application and unconsciously used pesticides in the pest control, causes a significant reduction especially in birds that feed from seeds, insectivorous and pray birds. The bird species most affected by pesticide residues are passerines. These are followed by fish characterized birds. In the same way, despite having a strong structure, eagles are one of the most affected bird species by pesticides (Erdem 2010).

Careless behavior of humans and animal behavior sometimes results with the poisoning of farm animals. Cattle, dogs, cats and horses are the most intoxicated animal (Sunding and Zivin, 2000).

Also decreased production yield and not benefiting essentially from the feed, results in situations like not gaining sufficient weight. Abortion and fertility disorders have been found to decrease in reproductive capacity (Erdem, 2010).

## **1.9. The Effects of Pesticides on Humans**

Pesticides have many negative effects on humans. The effects of pesticides can be different on every person. This is because there are many factors that determine the dose of exposure. Age, gender, race, socioeconomic status, diet, health status, length of exposure and

form, pesticide concentration, has a significant change on the influence and on people under the influence of pesticides (Güler and Çobanoğlu, 1997).

### **1.9.1. Acute Effects of Pesticides on People**

Acute effects of pesticides depending on irritation, dermatitis, systemic absorption can bring up to death. Respiratory and cardiovascular disease, are more susceptible to pesticides influences. Asthma or severe allergies are those with higher response level (Güler and Çobanoğlu, 1997).

In many countries in the world, pesticide poisoning can be of natural disaster. For example, in India in 1984 in the accident that took place at the Union Carbide pesticide factory, at least 200 000 people were poisoned and 2500 of them died. After 1970, in Asia with the so called “green revolution” where insecticides used as a result of an innovation made in the growing of rice, the death rate of men increased in 27 % (Ware, 1991).

### **1.9.2. Chronic Effects of Pesticides on People**

Chronic effects of pesticides on humans can be listed as cancer, birth defects, neurological effects, increase in epilepsy and parkinson, hypertension, reduced fertility and infertility (Tuncer and Ecevit, 1991).

Studies made on occupational and environmental influences of pesticides on people, show us that pesticides increase in the risk of cancer. Particularly non-Hodgkin lymphoma, leukemia, liver cancer, testicular cancer, brain cancer, lung cancer was found to be a significant increase in the risk. The groups seen with these disease are; agricultural workers, pest control operators and pesticide manufacturing workers. In New Zealand and Sweden tenon hodgkin lymphoma, in Australia, Finland and New Zealand, multiple myeloma, in England, Waller and Sweden testicular cancer, in Sweden liver cancer, in Italy brain cancer, in West Germany lung cancer growth was observed (Moses, 1989).



% 3-7 of infant morbidity and mortality in the United States are among birth defects. Discomfort in the arms and legs in the children of agricultural workers was found to be high (Moses, 1998).

### **1.10. Pesticide Use in the World**

In the world especially in the developed countries, in all countries until the beginning of the 1980s agricultural production, to increase the yield per unit area and in this way reducing the cost of production, principal agricultural policy has been the target. However, intensive use of pesticides on human health, natural resources, and direct and indirect negative effects, starting from the 1980s from developed countries, has emerged as the most important development and environment problem (Tanrıvermiş, 2000).

In the world in recent years due to the risks caused by pesticides, especially in developed countries has began to be used more deliberate and controlled. To ensure this, for example, in the countries of the European Union they issued many laws in the United States, as official organizations, also civil society organizations have a voice in this direction (Erdem , 2010).

Now in the developed countries, pesticides, in terms of environmental and health risks are seriously being evaluated. Therefore, they are in the direction that while using pesticides in a conscious and controlled way, on the other hand they want to limit the risk of the use of pesticides or to completely stop them (Delen, 2005).

In the drinking water of the European Union, for each concentration of organochlorine pesticide 0,1 ml/L, for total pesticide concentration 0,5 mg/L and for a maximum of aldin, dieldrin and for heptachlorine 0,3 mg/L limit values were determined. For surface water, 0,1 mg/L limit value was determined.

### **1.11. Literature Information**

Domagalski and Dubrovsky in 1992, examined pesticide residues in the ground waters of California, and found atrazine, bromacil, 2,4-DP, diazinon, dibromochloropropane, 1,2-dibromoeth, dicamba, 1,2-dichloropropane, diuron, prometone, prometryn, propazine and simazine.

When Lari et al. (2014), made a comparison on pesticides residues in water and agricultural fields, they came up with the results that ground water, compared to surface water is more contaminated with pesticides, and that groundwater was found to be contaminated by the least of isomer in HCL, endosulph, diclorovos and chlopyrifos.

Albanis et al. (1998), as a result of the work done in pesticide residues and metabolites found in the ground waters and surface waters in Imathia, they found; atrazine in ground waters, DEA, carbofuran, simazine, diazinon, parathionethyl and parathionmethyl.

Sankararamakrishnan et al. (2004), examined organochlorine and organophosphorus pesticides in ground waters and surface waters, and as a result to this study, in the ground waters they detected HCH (and its isomers), dieldrin and malathion.

Carejeira et al. (2003), studies in the ground waters of Portugal and they detected alachlor, atrazine, metachlor, metribuzine and simazine.



## CHAPTER 2

### MATERIALS AND METHODS

#### 2.1. Chemicals

Standards of pesticides with purity of minimum 98 % were purchased from Dr. Ehrenstorfer (Germany), Absolute Standards (USA) and they all had certificates.

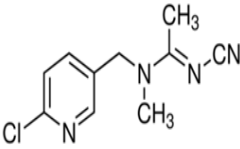
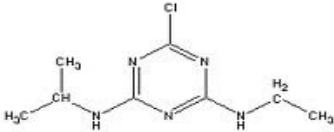
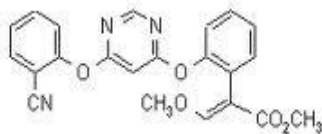
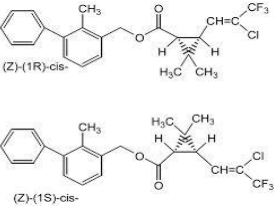
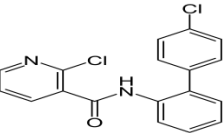
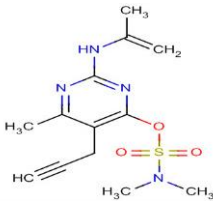
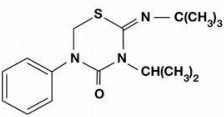
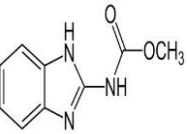
The compounds studied, belonging to several chemical classes, are listed in Table 2.1 including their use, water solubility and chemical structure.

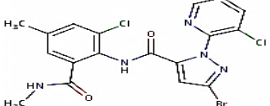
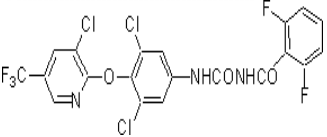
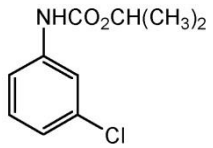
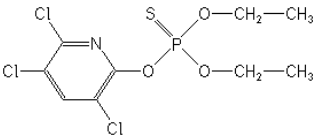
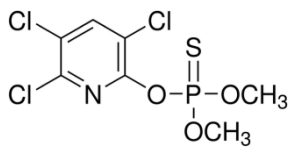
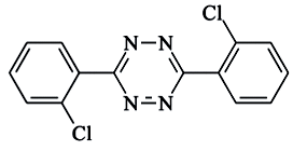
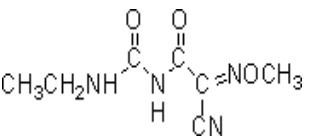
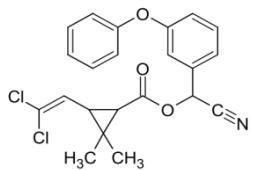
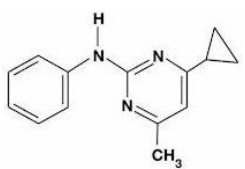
Stock standard solutions ( $1000 \mu\text{g mL}^{-1}$ ) for each of the analtes were first prepared by dissolving standards of pesticides in acetonitrile and stored in the dark at  $-18^\circ\text{C}$ . An intermediate standard solution ( $10 \mu\text{g mL}^{-1}$ ) was prepared by appropriate dilution of stock solutions in acetonitrile and this mixture was used as spiking solution for the aqueous calibration standards.

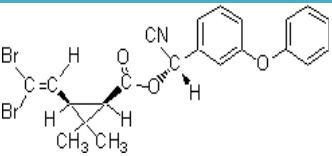
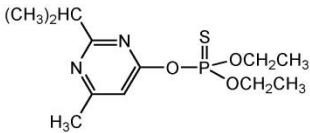
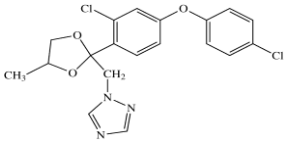
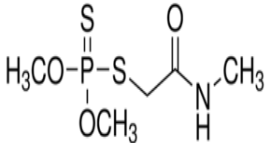
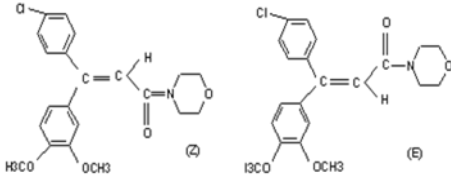
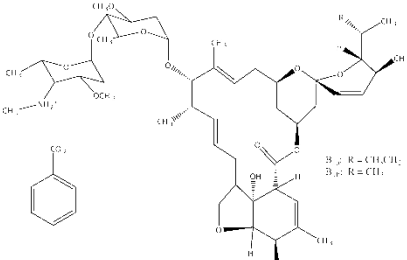

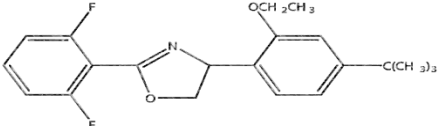
5mM ammonium format in water and 5 mM ammonium format in methanol were used as mobile phases. Ammonium format was from Fluka with 99 % purity.

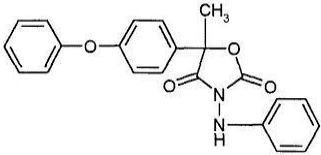
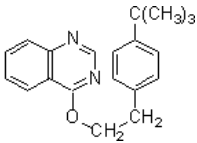
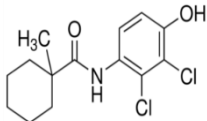
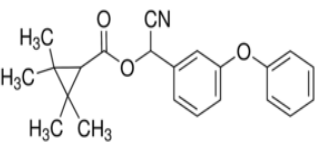
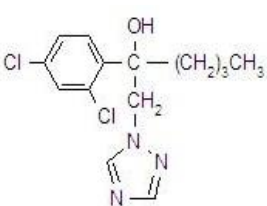
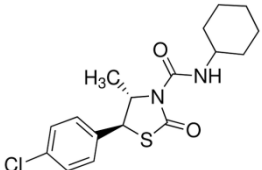
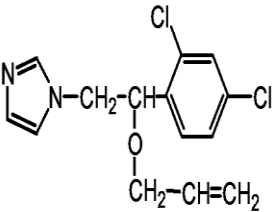
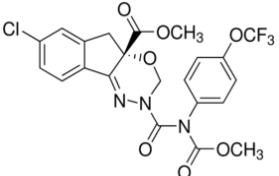
The organic solvents acetonitrile, methanol and dichloromethane were of HPLC grade and supplied by Merck (Germany). Ultra-pure quality water was obtained from a Milli-Q water purification system (Millipore, Milford, MA, USA).

**Table 2.1:** Pesticide group and physicochemical properties of pesticides selected for the study

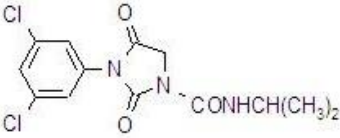
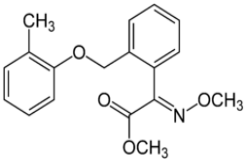
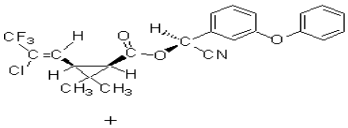
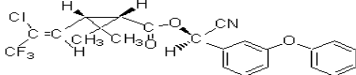
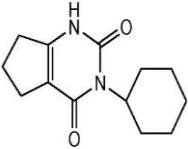
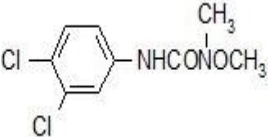
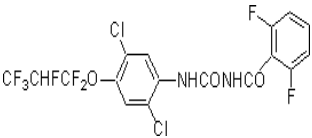
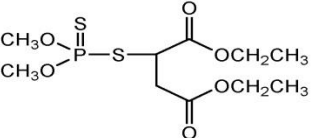
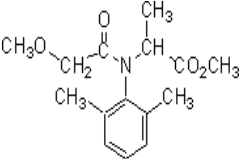
Pesticide group	Compound	Structure	Water solubility (mg/L)
Insecticide	Acetamiprid		4250 (25 °C)
Herbicide	Atrazine		33(22°C)
Fungicide	Azoxystrobin		6.70(20°C)
Insecticide, acaricide	Bifenthrin		<0,001 (20°C)
Fungicide	Boscalid		4.6(20°C)
Fungicide	Bupirimate		13.06 (20°C)
Insecticide,acaricide	Buprofezin		0.387 (20°C)
Fungicide	Carbendazim		8(24°C)

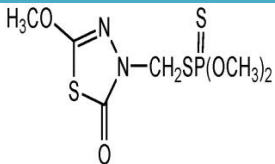
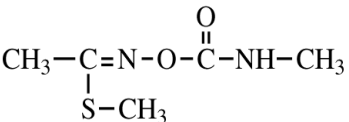
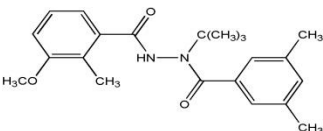
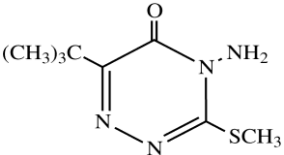
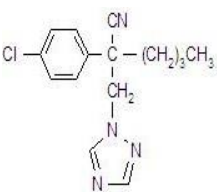
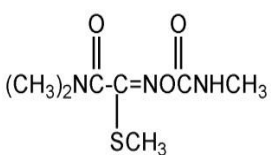
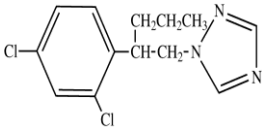
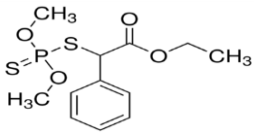
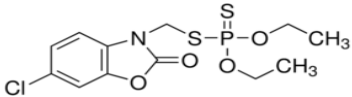
<b>Insecticide</b>	<b>Chlorantraniliprole</b>		0.9-1 (20°C)
<b>Insecticide</b>	<b>Chlorfluazuron</b>		0.012(20°C)
<b>Herbicide (plant grow regulator)</b>	<b>Chlorpropham</b>		89(25°C)
<b>Insecticide</b>	<b>Chlorpyrifos</b>		1.4 (25°C)
<b>Insecticide ,acaricide</b>	<b>Chlorpyrifos Methyl</b>		2.6 (20°C)
<b>Acaricide</b>	<b>Clofentezine</b>		0.0025 (22°C)
<b>Fungicide</b>	<b>Cymoxanil</b>		890(20°C)
<b>Insecticide</b>	<b>Cypermethrin</b>		0.004(20°C)
<b>Fungicide</b>	<b>Cyprodinil</b>		13(25°C)

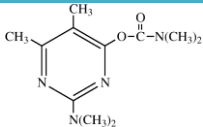
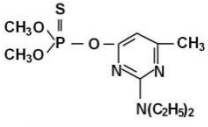
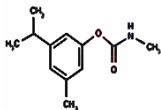
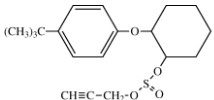
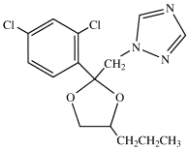
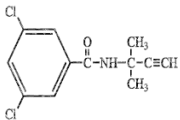
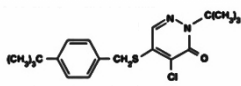
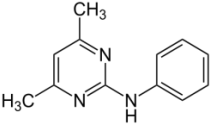
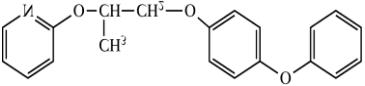
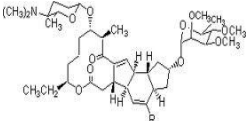
Insecticide	Deltamethrin		<0.0002 (25°C)
Insecticide,acaricide	Diazinon		60(20°C)
Fungicide	Difenoconazole		15(25°C)
Insecticide,acaricide	Dimethoate		39800(25°C)
Fungicide	Dimethomorph		49.2 (20°C)
Insecticide	Emamectin Benzoate		24 (25°C)
Fungicide	Epoxiconazole		6.63 (20°C)
Acaricide	Etoxyzazole		0.0754(20°C)

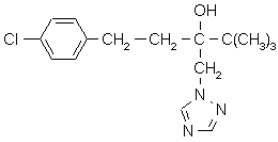
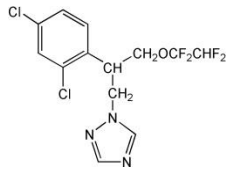
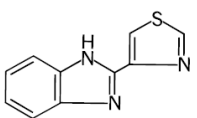
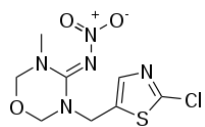
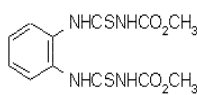
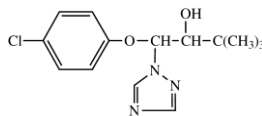
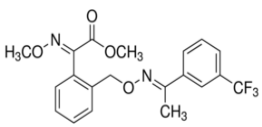
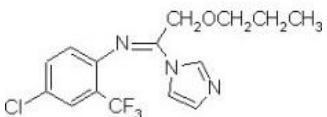
Fungicide	Famoxadone		0.052(20°C)
Acaricide	Fenazaquin		0.102 (20°C)
Fungicide	Fenhexamid		20(20°C)
Acaricide ,insecticide	Fenpropathrin		0.0141(25°C)
Fungicide	Hexaconazole		17(20°C)
Acaricide	Hexythiazox		0.41(20°C)
Fungicide	Imazalil		224(20°C)
Insecticide	Indoxacarb		0.20(25°C)



Fungicide,Nematicide	Iprodione		13(20°C)
Fungicide	Kresoxim-methyl		2(20°C)
Insecticide	Lambda – cyhalothrin	<p>(S) (Z)-(1R)-cis-</p>  <p>+</p>  <p>(R) (Z)-(1S)-cis-</p>	0.005(20°C)
Herbicide	Lenacil		3(20°C)
Herbicide	Linuron		63.8(20°C)
Insecticide ,acaricide	Lufenuron		0.048(25°C)
Insecticide ,acaricide	Malathion		145(25°C)
Fungicide	Metalaxy		8400 (22°C)

Insecticide ,acaricide	Methidation		200(25°C)
Insecticide ,acaricide	Methomyl		57900 (25°C)
Insecticide	Methoxyfenozide		3,3 (20°C)
Herbicide	Metribuzin		1050(20°C)
Fungicide	Myclobutanil		132 (20°C))
Insecticide, acaricide,nematicide	Oxamyl		280000(25°C)
Fungicide	Penconazole		73(25°C)
Insecticide, acaricide	Phenthoate		10(25°C)
Insecticide,acaricide	Phosalone		1.4 (20°C))

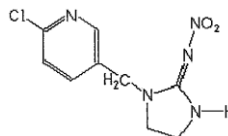
Insecticide	Pirimicarb		3100(20°C)
Insecticide,acaricide	Pirimiphos-methyl		10(20°C)
Insecticide	Promecarb		91(20°C)
Acaricide	Propargite		0.215(25°C)
Fungicide	Propiconazole		100(20°C)
Herbicide	Propyzamide		15(25°C)
Insecticide ,acaricide	Pyridaben		0.012(24°C)
Fungicide	Pirimethanil		121(25 °C)
Insecticide	Pyriproxyfen		0.367 (25 °C)
Insecticide	Spinosad		235(20°C)

Fungicide	Tebuconazole		36(20°C)
Fungicide	Tetraconazole		183.8(20°C)
Fungicide	Thiabendazole		30(20°C)
Insecticide	Thiometoxan		4100(25 °C)
Fungicide,wound protectant	Thiophanate – methyl		18.5(20°C)
Fungicide	Triadimenol		Isomer A: 56 Isomer B: 27 (20°C)
Fungicide	Trifloxystrobin		0.610 (25 °C)
Fungicide	Triflumizole		10.2 (20°C)

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**Insecticide**

**Imidacloprid**



61(20°C)

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## 2.2. Instruments

In a study conducted rotary evaporate vacuum (Buchi, Switzerland), separating funnel, measure, 250 mL volume balloon, general laboratory instruments and equipment, and LC-MS / MS instrument was used.

Liquid chromatography with MS or tandem MS (MS/MS) detection provides an improved sensitivity and selectivity for the analysis of pesticides which are to be scanned in this study.

Liquid chromatography with mass spectrometric detection (LC-MS) was carried out using a Shimadzu (Japanese) system equipped with a model AB-MSD Sciex multi solvent delivery and system coupled with a 4000Q Trap triple quadrupole mass spectrometer detector with an ESI interface and Analyst software as the data acquisition and processing system.



**Figure 2.2:** Liquid chromatography with mass spectrometer

### 2.3. Chromatographic Conditions

Shimadzu-ABS Sciex LC-MS/MS System parameters were as follows:

Flow: 0, 5 ml/min

Column: Synergi 4 $\mu$  Fusion –RP 80A (50 $\times$ 2.00 mm)

Run Time: 8 minutes

Mobile Phase: A: 5Mm Ammonium format in water

B: 5Mm Ammonium format in methanole

Shimadzu LC System Equilibration Time: 1.00 minutes

Shimadzu LC System Injection Volume: 20.00  $\mu$ l

**Table2.3:** LC- MS/MS Time Programming

Time	Module	Events	Parameter
5.00	Pumps	Pump B Conc.	95
6.00	Pumps	Pump B Conc.	95
6.50	Pumps	Pump B Conc.	5
8.00	Pumps	Pump B Conc.	5
8.01	System controller		Stop

**Pumps:**

Pump A Model: LC-20ADXR

Pump B Model: LC-20ADXR

Pumping Mode: Binary Flow

Total Flow: 0.5000 ml/min

Pump C Concentration: 5.0 %

B Curve: 0

Pressure Range (Pump A/B): 0-300 Bars

**Auto Sampler:**

Model: SIL-20A

Rinsing volume: 200µl

Needle stroke: 52mm

Rinsing speed: 35µl/sec

Sampling speed: 15.0 µl/sec

Purge time: 25.0 min

Rinse dip time: 0sec

Rinse mode: Before and after aspiration

Control vial needle stroke: 52 mm

**System Controller:**

Model: CEM-20A Lite

Power: on

Event 1: off

Event 2: off

**2.4. Mass Spectrometry Conditions**

The MS–MS parameters (declustering potential (DP), entrance potential (EP), Q1 mass, Q3 mass, collision energy (CE) and cell exit potential (CXP)) were optimized by infusing standards of each individual compound at 0,5 µg/mL of individual standard solution in full scan mode directly into the MS. Optimization was automatically done by the MS instrument. Values are reported in Table 2.4.

The mass spectrometry method properties were as follows:

**Period 1:**

Scans in period: 1580

Relative Start Time: 0.00msn

Experiments in Period: 1

Period 1 experiment 1:

Scan type: MRM

Scheduled MRM: yes

Polarity: Positive

Scan mode: N/A

Ion source: Turbo Spray

MRM detection window: 60 sec

Target scan time: 1.0000 sec

Resolution Q1: unit

Resolution Q3: unit



Intensity Thrs: 0.00 cps

Settling time: 700.0000 msn

MR pause: 5.0000msn

MCA: no

Step size: 0.00 DA

**Table 2.4:** Analytical conditions of the studied pesticides

<b>Pesticide</b>	<b>RT</b>	<b>Q1 Mass</b>	<b>Q3Mass</b>	<b>DP(V)</b>	<b>EP (V)</b>	<b>CE (V)</b>	<b>CXP(V)</b>
Acetamiprid1	3,3	223,063	126	66	10	31	8
Acetamiprid 2	3,3	223,063	99	66	10	55	6
Atrazine 1	4,6	216,2	174	66	10	25	12
Atrazine 2	4,6	216,2	104	66	10	41	18
Azoxystrobin 1	4,8	404,136	344,1	66	10	35	22
Azoxystrobin 2	4,8	104,136	372	66	10	21	10
Bifenthrin 1	6,2	440,071	181	41	10	19	14
Bifenthrin 2	6,2	440,071	166,1	41	10	59	14
Boscalid 1	5	342,992	307,1	86	10	29	8
Boscalid 2	5	342,992	139,8	86	10	31	10
Bupimate 1	5,3	317,184	166	86	10	35	14
Bupimate 2	5,3	317,184	108	86	10	37	8
Buprofezin 1	5,8	306,195	201	51	10	19	12
Buprofezin 2	5,8	306,195	115,9	51	10	23	8
Carbendazim 1	3,6	192,051	160	61	10	29	12
Carbendazim 2	3,6	192,051	132	61	10	45	10
Chlorantraniliprole 1	4,8	483,961	453	56	10	21	14
Chlorantraniliprole 2	4,8	482	284	74	10	57	4
Chlorfluazuron 1	6	539,9	383	91	10	47	10
Chlorfluazuron 2	6	539,9	158	106	10	47	10
Chlorpropham 1	5	231,007	172	36	10	17	14
Chlorpropham 2	5	231,007	154	36	10	31	12

<b>Pesticide</b>	<b>RT</b>	<b>Q1 Mass</b>	<b>Q3Mass</b>	<b>DP(V)</b>	<b>EP (V)</b>	<b>CE (V)</b>	<b>CXP(V)</b>
Chlorpyrifos 1	5,8	350	197,9	61	10	27	16
Chlorpyrifos 2	5,8	350	96,9	61	10	49	6
ChlorpyrifosMethyl 1	5,5	321,942	125	76	10	29	8
ChlorpyrifosMethyl 2	5,5	321,942	289,8	76	10	23	20
Clofentezine 1	5,5	303,082	138,1	71	10	21	10
Clofentezine 2	5,5	303,082	102,2	71	10	61	6
Cymoxanil 1	3,5	199,063	127,9	56	10	13	10
Cymoxanil 2	3,5	199,063	110,9	56	10	25	8
Cypermethrin 1	6	433	190,9	36	10	27	8
Cypermethrin 2	6	433	127	36	10	43	14
Cyprodinil 1	5,4	226,056	93,1	81	10	49	6
Cyprodinil 2	5,4	226,056	77	81	10	65	4
Deltamethrin 1	6	522,938	281	51	10	23	8
Deltamethrin 2	6	522,938	181	51	10	51	14
Diazinon 1	5,5	305,137	169	71	10	31	14
Diazinon 2	5,5	305,137	97	71	10	47	6
Difenoconazole 1	5,5	406	251	96	10	37	14
Difenoconazole 2	5,5	406	337	96	10	37	14
Dimethoate 1	3,3	230,067	198,9	56	10	15	16
Dimethoate 2	3,3	230,067	125	56	10	29	8
Dimethomorph 1	5	388,145	301,1	46	10	31	8
Dimethomorph 2	5	388,145	165,1	46	10	43	14
EnamectineBenzoate 1	5,8	886,538	158	111	10	49	12
EnamectineBenzoate 2	5,8	886,538	82,2	111	10	123	4
Epoxiconazole 1	5,3	330,058	101,1	71	10	69	8
Epoxiconazole 2	5,3	330,058	120,9	71	10	31	10
Etoxazole 1	6	360,063	141	66	10	41	12
Etoxazole 2	6	360,063	113,1	66	10	83	8
Famoxadone 1	5,4	392,123	331,1	51	10	13	16
Famoxadone 2	5,4	392,123	237,9	51	10	25	18
Fenazaquin 1	6,1	307,143	161	66	10	25	10

<b>Pesticide</b>	<b>RT</b>	<b>Q1 Mass</b>	<b>Q3Mass</b>	<b>DP(V)</b>	<b>EP (V)</b>	<b>CE (V)</b>	<b>CXP(V)</b>
Fenazaquin 2	6,1	307,143	147,1	66	10	29	12
Fenhexamid 1	5,1	302,108	97,2	81	10	35	6
Fenhexamid 2	5,1	302,108	55,3	81	10	69	8
Fenpropathrin 1	5,9	350,203	125,1	76	10	19	10
Fenpropathrin 2	5,9	350,203	97	76	10	45	6
Hexaconazole 1	5,3	313,989	70	61	10	45	4
Hexaconazole 2	5,3	313,989	159	61	10	41	12
Hexythiazox 1	5,8	353,1	228	66	10	23	16
Hexythiazox 2	5,8	353,1	168	66	10	37	12
Imazalil 1	5,4	297,077	158,9	76	10	31	12
Imazalil 2	5,4	297,077	200,8	76	10	27	16
Indoxacarb 1	5,6	528,142	203,1	86	10	57	14
Indoxacarb 2	5,6	528,142	56,1	86	10	57	4
Iprodione 1	5,2	330,1	244,9	61	10	21	14
Iprodione 2	5,2	332,1	246,9	61	10	21	14
KresoximMethyl 1	5,3	314,192	206	66	10	11	18
KresoximMethyl 2	5,3	314,192	116	66	10	19	8
Lamda Cyhalothrin 1	6	467,136	225	61	10	23	18
Lamda Cyhalothrin 2	6	467,136	141	61	10	59	8
Lenacil 1	4,6	235,3	153,2	41	10	23	4
Lenacil 1	4,6	235,3	136,2	41	10	43	4
Linuron 1	4,8	248,996	159,9	76	10	29	10
Linuron 2	4,8	248,996	181,9	76	10	25	16
Lufenuron 1	5,9	511,014	158	96	10	31	12
Lufenuron 2	5,9	511,014	141	96	10	67	10
Malathion 1	5,1	331,071	285	71	10	11	8
Malathion 2	5,1	331,071	127	71	10	19	10
Metalaxyl 1	4,6	280,183	220,1	46	10	19	16
Metalaxyl 2	4,6	280,183	160,1	46	10	33	14
Methidation 1	4,7	303,012	145,1	61	10	13	10
Methidation 2	4,7	303,012	85,2	61	10	31	14

<b>Pesticide</b>	<b>RT</b>	<b>Q1 Mass</b>	<b>Q3Mass</b>	<b>DP(V)</b>	<b>EP (V)</b>	<b>CE (V)</b>	<b>CXP(V)</b>
Methomyl 1	2,6	163,096	88	51	10	13	6
Methomyl 2	2,6	163,096	106	51	10	15	8
Metoxyfenozide 1	5,1	369,19	148,9	46	10	25	12
Metoxyfenozide 2	5,1	369,19	313,2	46	10	13	18
Metribuzin 1	4,1	215,034	187	66	10	27	16
Metribuzin 2	4,1	215,034	84	66	10	31	6
Myclobutanil 1	5	289,141	70,1	56	10	39	4
Myclobutanil 2	5	289,141	124,9	56	10	49	8
Omethoate 1	2	213,96	125	51	10	31	10
Omethoate 2	2	213,96	154,9	51	10	23	12
Oxamyl 1	2,4	237,15	72,1	31	10	31	4
Oxamyl 2	2,4	237,15	90,1	31	10	13	6
Penconazole 1	5,3	284,177	158,9	71	10	37	12
Penconazole 2	5,3	284,177	70,2	71	10	35	4
Phenthoate 1	5,4	321,04	79,1	66	10	61	12
Phenthoate 2	5,4	321,04	163,1	71	10	23	12
Phosalone 1	5,5	367,988	181,9	76	10	23	14
Phosalone 2	5,5	367,988	111	76	10	59	8
Pirimicarb 1	4,5	238,704	72,1	56	10	33	4
Pirimicarb 2	4,5	238,704	182,1	56	10	23	14
Pirimiphosmethyl 1	5,5	306,129	164,1	81	10	31	14
Pirimiphosmethyl 2	5,5	306,129	108	81	10	43	6
Promecarb 1	5	208,07	108,9	61	10	23	8
Promecarb 2	5	208,07	151,1	61	10	13	12
PropamocarbHcl 1	2	189,069	101,9	56	10	25	8
PropamocarbHcl 2	2	189,069	144	56	10	19	10
Propargite 1	5,9	368,169	231,2	51	10	15	14
Propargite 2	5,9	368,169	175,1	51	10	23	14
Propiconazole 1	5,4	342,06	159	86	10	43	12
Propiconazole 2	5,4	342,06	69,1	86	10	35	4
Propyzamide 1	5,1	256,352	173	91	10	35	14

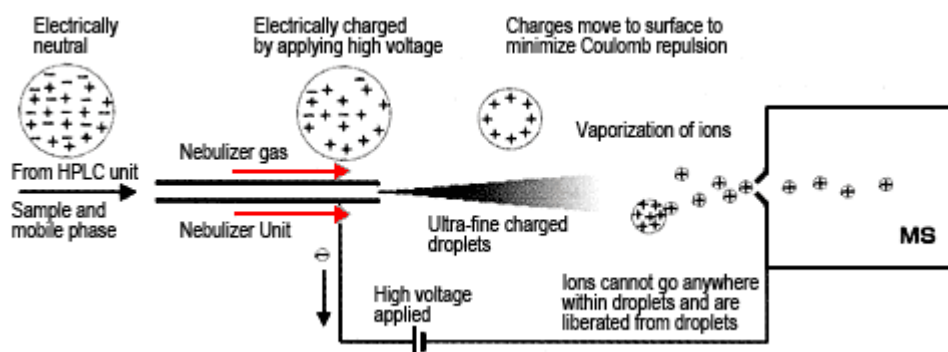
<b>Pesticide</b>	<b>RT</b>	<b>Q1 Mass</b>	<b>Q3Mass</b>	<b>DP(V)</b>	<b>EP (V)</b>	<b>CE (V)</b>	<b>CXP(V)</b>
Propyzamide 2	5,1	256,352	189,9	91	10	21	12
Pymethrozine 1	2,8	218	105	46	10	53	6
Pymethrozine 2	2,8	218	79	51	10	53	6
Pyridaben 1	6	365,126	309,1	56	10	19	8
Pyridaben 2	6	365,126	147,1	56	10	37	10
Pyrimethanil 1	4,9	200,18	82,1	76	10	37	6
Pyrimethanil 2	4,9	200,18	107	76	10	35	8
Pyriproxyfen 1	5,8	322,139	96	56	10	23	6
Pyriproxyfen 2	5,8	322,139	184,9	56	10	33	14
Spinosad 1	5,9	732,427	142	101	10	41	10
Spinosad 2	5,9	732,427	98	101	10	95	6
Tebuconazole 1	5,3	308,205	70,1	81	10	47	4
Tebuconazole 2	5,3	308,205	125	81	10	53	10
Tetraconazole 1	5,2	371,978	159	81	10	45	12
Tetraconazole 2	5,2	371,978	70	81	10	49	4
Thiabendazole 1	3,9	202,046	174,9	66	10	37	10
Thiabendazole 2	3,9	202,046	131	66	10	47	10
Thiomethoxan 1	2,6	292,015	181	66	10	33	10
Thiomethoxan 2	2,6	292,015	211	66	10	19	14
Thiophanatemethyl 1	4,1	342,906	151,1	61	10	27	12
Thiophanatemethyl 2	4,1	342,906	93	61	10	69	6
Triadimenol 1	5	296,127	70	51	10	31	4
Triadimenol 2	5	296,127	227,1	51	10	13	20
Trifloxystrobin 1	5,6	409,059	186	51	10	23	16
Trifloxystrobin 2	5,6	409,059	205,9	51	10	21	16
Triflumizole 1	5,6	346,066	277,9	46	10	15	16
Triflumizole 2	5,6	346,066	73,1	46	10	25	4
Imidacloprid 1	3,1	256,147	209,1	61	10	21	16
Imidacloprid 2	3,1	256,147	175,1	61	10	29	14

## 2.5. Shimadzu AB-MDS Sciex LC-MS/MS Working Principle

Liquid chromatography-mass spectrometry (LC-MS) is now a routine technique with the development of electro spray ionisation (ESI) providing a simple and robust interface. Coupling of MS to chromatographic techniques has always been desirable due to the sensitive and highly specific nature of MS compared to other chromatographic detectors. It can be applied to a wide range of biological molecules and the use of tandem MS and stable isotope internal standards allow shightly sensitive and accurate assays to be developed although some method optimization is required to minimize ion suppression effects. Fast scanning speeds allow a high degree of multiplexing and many compounds can be measured in a single analytical run (Pitt, 2009).

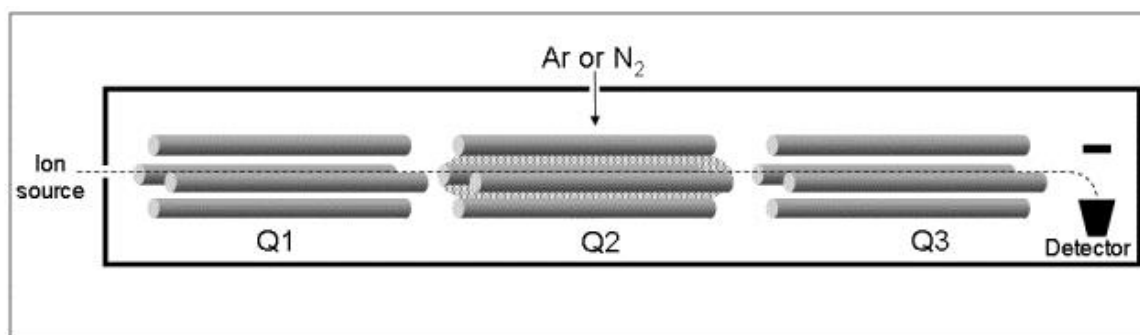
Mass spectrometers operate by converting the analyte molecules to a charged (ionised) state, with subsequent analysis of the ions and any fragmentations that are produced during the ionization process, on the basis of their mass to charge ratio ( $m/z$ ). Several different technologies are available for both ionization and ion analysis, resulting in many different types of mass spectrometers with different combinations of these two processes (Pitt, 2009).

Elektrospray Ionization Source, works well with moderately polar molecules and is thus well suited to the analysis of many metabolites, xenobiotics and peptides. Liquid samples are pumped through a metal capillary maintained at 3 to 5 kV and nebulized at the tip of the capillary to form a fine spray of charged droplets. The capillary is usually orthogonalto, or off-axis from, the entrance to the mass spectrometer in order to minimize contamination. The droplets are rapidly evaporated by the application of heat and dry nitrogen, and the residual electrical charge on the droplets is transferred to the analytes. The ionized analytes are then transferred into the high vacuum of the mass spectrometer via a series of small apertures and focusing voltages. The ion source and subsequent ion optics can be operated to detect positive or negative ions, and switching between these two modes within an analytical run can be performed (Pitt, 2009).



**Figure 2.5:** Evaporation of ions in electrospray ionization (Pitt, 2009)

The quadrupole analyzer consists of a set of four parallel metal rods (Figure 2.5). A combination of constant and varying (radio frequency) voltages allows the transmission of a narrow band of  $m/z$  values along the axis of the rods. By varying the voltages with time it is possible to scan across a range of  $m/z$  values, resulting in a mass spectrum. Most quadrupole analysers operate at  $<4000$   $m/z$  and scan speeds up to 1000  $m/z$  per second are common. They usually operate at unit mass resolution meaning that the mass accuracy is seldom better than 0.1  $m/z$  (Pitt, 2009).



**Figure 2.6:** A triple quadrupole mass spectrometer (Pitt, 2009)

Q1 and Q3 act as mass filters and can be independently fixed, scanned or stepped. Q2 is a collision cell that contains a low pressure inert gas (Pitt, 2009).

## 2.6. Sample Collection

Water samples are filled in amber glass bottles of 2 L they are washed with sample water three times and are filled till the top leaving it with no bubbles. Until the water is brought to the laboratory, samples are taken there in cooler containers at +4 ° C and they have been moved and analyzed immediately. Until their extraction, water samples are kept in the dark in the refrigerator at 4 ° C temperature.

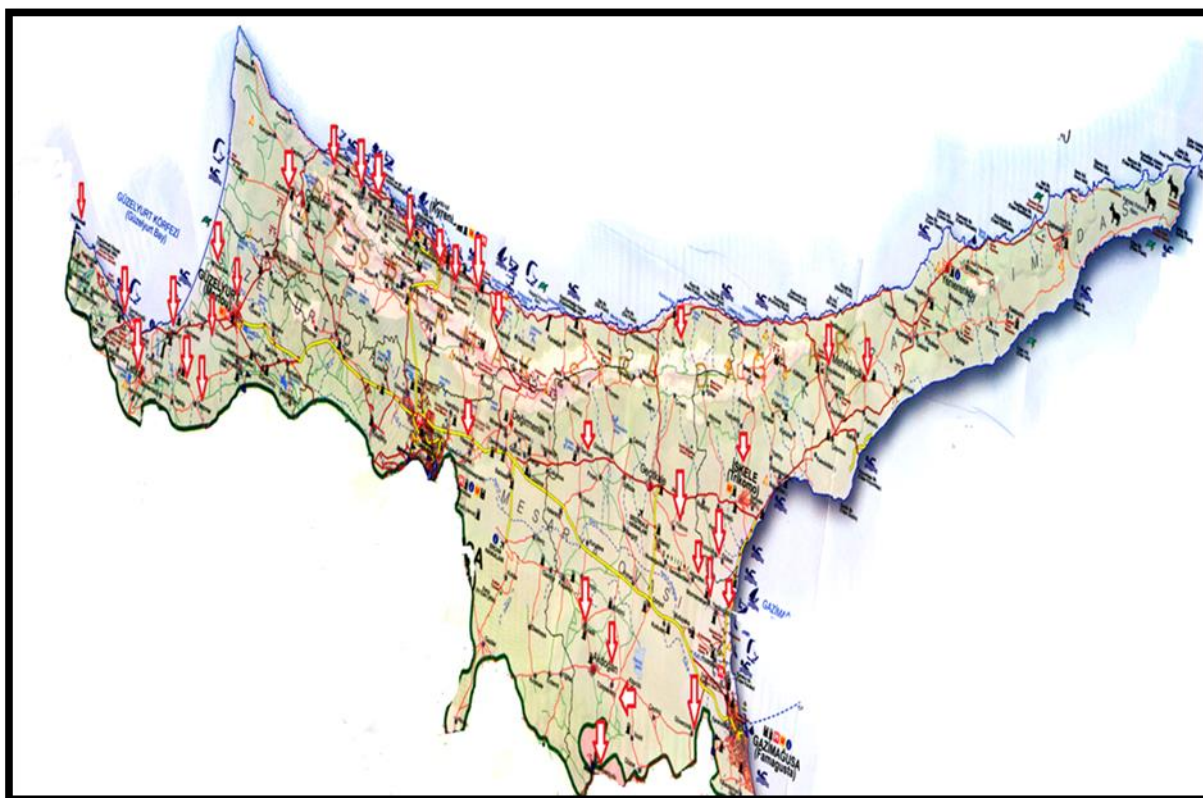
Water samples which will be used for the analysis on artichokes, parsley, peppers, eggplant, zucchini, lettuce, tomatoes, cucumbers, potatoes, leeks, lemon, watermelon, arugula, green beans, chard, peas, melon, spinach, okra, molehiya, gumbo, apricots, grapes vineyards and citrus fruit grown in 40 different regions, was collected from the depths of wells ranging from 10.5 to 105 meters ( Table 2.6 ).

**Table 2.6:** Characteristic of sampling point

<b>Sample No</b>	<b>Name of Territory</b>	<b>Well Depth(m)</b>	<b>Products growing in the agricultural field</b>
S1	Mormenekşe	81	Artichokes
S4	Yeniboğaziçi	10,5	Artichokes
S5	Serdarlı	25,5	Vegetables
S6	Mehmetçik	13,5	Vegetables
S7	Beyarmudu	24	Vegetables
S9	Büyükkonuk	24	Vegetables
S10	İskele	36	Artichokes
S11	Mehmetçik	42	Grape vines
S14	Pile	24	Vegetables
S15	Doğancı	15	Vegetables
S16	Lefke	15	Vegetables
S19	Güvercinlik	30	Vegetables
S21	Karşıyaka	12	Vegetables
S22	Ozanköy	10,5	Vegetables



<b>Sample No</b>	<b>Name of Territory</b>	<b>Well Depth(m)</b>	<b>Products growing in the agricultural field</b>
S23	Doğanköy	11,1	Vegetables
S24	Akdoğan	51	Vegetables
S25	Edremit	30	Vegetables
S26	Vadili	10,5	Vegetables
S27	Güzelyurt	105	Oranges
S28	Haspolat	15	Vegetables
S29	Türkmenköy	45	Vegetables
S30	Tatlısu	22,5	Vegetables
S32	Tatlısu	28,5	Vegetables
S31	Kuzucuk	24	Vegetables
S33	Lapta	24	Vegetables
S34	Atlılar	24	Vegetables
S36	Arapköy	30	Vegetables
S37	Çatalköy	25,5	Vegetables
S41	Yeşilyurt	31,8	Vegetables
S42	Tepebaşı	40	Vegetables
S43	Alsancak	24	Vegetables
S44	Yayla	24	Citrus fruit
S46	Yeşilırmak	10,5	Strawberry
S47	Taşpınar	50,4	Vegetables
S48	Ötüken	12	Vegetables
S51	Doğancı	60	Vegetables
S53	Güneşköy	11,4	Vegetables
S54	Yedidalga	39	Vegetables
S58	Yıldırım	24	Vegetables
S59	İskele	24	Artichokes



**Figure 2.7:** Sampling Points on Northern Cyprus Map

## **2.7. Description of Studied Area**

### **2.7.1. Geographical Location**

Cyprus, the Mediterranean's third largest island after Sicily and Sardinia is located in the North Eastern Mediterranean region. In its North 60 km away from Turkey, in its West 100 km away from Syria, 150 km away from Lebanon, in its South 330 km away from Egypt and respectively 370 km and 500 km away from the Mediterranean Islands of Crete and Rhodes. It is 227 km long in East-West direction, 97 km wide in North-South direction and its surface area is 9,125 km<sup>2</sup>. Beşparmak is in the North of the Island and Trodos which is the main water source of the area is in the South (Moments and Sites Cyprus, 1996).

### **2.7.2. Climate**

According to macro-climatic classification of Northern Cyprus it takes place in the so called 'semi-arid' climate zone. At the same time because it is on a Mediterranean island summer is hot and dry; little rainy and warm in winter. In Northern Cyprus the average annual air temperature is 19.0 ° C (The Weather Bureau Office of TRNC, 2014).

### **2.7.3. Economic Potential**

The main sectors that rely on the economy are mainly agriculture, tourism and education. In addition to these small businesses contribute to the economy.

#### **Agriculture**

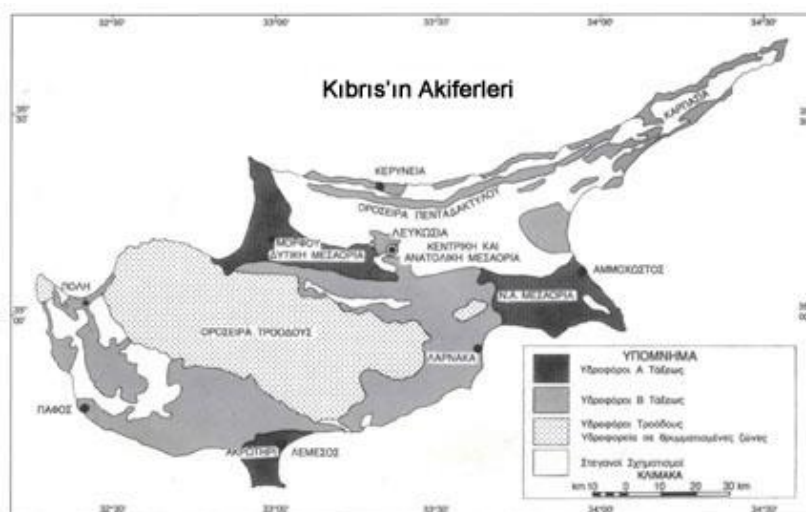
The island has a suitable climate for agriculture. The agricultural sector is the main production sector of TRNC. 41 % of annual exports of agricultural products show the potential and importance of agriculture in the country. In TRNC, there are 187,168 hectares of farmland in a total area of 329,89 hectars (Gültekin, 2006).

Citrus, potatoes, olives, carob, tobacco, almonds, sultanas and wine grapes, tropical fruits, such as bananas, kiwi and avocado, as are grown. In summer, in the plain areas, the temperature can rise above 40 °C. Streams that can be used for watering is not available in

agricultural areas. The water needed for the watering of agricultural areas, is provided from groundwater. In the plains of Mesarya, where annual rainfall is lower groundwater are not fed enough. So we are not rich in groundwater. Flat areas which are needed for agriculture, have most of the space in the country. At the beginning of these flat plains comes Mesarya and Güzelyurt. Material that comes eroded from Kyrenia- Karpaz and Trodos mountains, provide alluvial soil in the plain. Dry farming is done in these fertile plains. In Mesarya, where there are drought conditions, dry farming is done and in Güzelyurt which is rich of water resources they do irrigated farming (Mor and Çitci, 2006).

#### **2.7.4. Water Resources**

Turkish Republic of Northern Cyprus is one of the countries suffering from water shortages. Cyprus has a semi-arid climate and can not take regular rainfall. Water resources are mainly based on rainfall. The main source of water for are groundwater resources. Annual available water potential in the TRNC is 97 million / year. Of this amount, 80 million m<sup>3</sup> form underground water and springs, 17 million m<sup>3</sup> form surface water resources (Gültekin, 2006). A certain number of beds in Cyprus in groundwater (aquifers) are available, today, the needs of island residents of domestic use and irrigation water requirements are largely met from groundwater resources. The formation of this aquifer, the Kretase (90 years ago) while coming to the present days, the island's geological processes in this geological evolution, is based on geological factors. In Cyprus aquifers spread in the lateral direction and thickness according to their first-class and second class is divided into sub-sections, including (Figure 2.8). First-class aquifers are rather thick and give a widespread distribution in the lateral direction and need continuity (Cyprus geological heritage educational tool, 2004).



**Figure 2.8:** Aquifers of cyprus (underground water beds)

The second class aquifers thickness is consisting of highly variable permeable layer and emissions are limited in lateral direction (Cyprus geological heritage educational tool, 2004).

## 2.8. Sample Preparations

1 liter water sample is put into a separatory funnel. 60 ml of dichloromethane ( $\text{CH}_2\text{Cl}_2$ ) was added on it and after shaking vigorously for 3 minutes is waited for the phase separation. The lower phase (dichloromethane) is collected into 250 ml of volumetric flask. 60 ml of dichloromethane is added to the remaining water left in the separating funnel and after shaken well, the phase above is filtered with the same volumetric flask. Then, in the remaining water in a separating funnel 60 ml of is added then shaken thoroughly and waited for phase separation. The lower phase (final phase) filtered into the same volumetric flask. The solution collected in the volumetric flask is put to evaporator flask under vacuum at  $45^\circ\text{C}$  and number of revolutions per minute is to be 180 and evaporated to dryness. After the complete evaporation of the solution 2 ml of acetonitrile is added. Then filtered through filters of 0.45 mm are transferred to vials and injected to LC.



**Figure 2.7:** Sampling Points on Northern Cyprus Map