PESTICIDE POLLUTION IN SURFACE AND GROUND WATER OF AN AGRICULTURAL AREA, KUMLUCA, TURKEY

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ABSTRACT

PESTICIDE POLLUTION IN SURFACE AND GROUND WATER OF AN AGRICULTURAL AREA, KUMLUCA, TURKEY

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Concentrations of 17 organochlorine and 14 organophosphorus pesticides were measured in 27 ground and 11 surface water samples collected from a heavily agricultural area, Kumluca, in spring and fall seasons of 2005. The samples were preconcentrated by Solid Phase Extraction. GC-ECD and GC-NPD systems were used for quantitative determination of organochlorine and organophosphorus pesticides respectively.

The quality check/quality assurance tests were performed by the analysis of field and laboratory blanks, standard reference materials, spiked control and sample matrices, surrogate standards, sampling and analysis replicates. It is observed that, sample matrix lowers average percent recoveries from 89% to 76%.

The uncertainties of measurements were calculated to determine major factors affecting the analysis results. It was observed that uncertainty arising from extraction procedure was generally the highest.

The most commonly observed pesticide was endosulfan (70%) and chlorpyriphos (53%) for organophosphorus and organochlorine pesticides. The highest average concentration was observed for heptachlor (26 ng/L) and fenamiphos (184 ng/L).

Generally pesticides were detected more often in surface waters, where the concentrations were also higher. The concentrations of organophosphorus pesticides in spring, and organochlorine pesticides in fall season were higher.

The high occurrences and detection of degradation products of chlorinated pesticides clearly indicate their intense use before 1980s. It is shown that, in Kumluca environment, degradation of these pesticides mostly occurs in surface waters.

It is observed that agricultural activities affect water quality in the region. The total concentration limit (500ng/L) was exceeded for 27% of surface and 14% of ground water samples, at least once in both seasons. The legal limit for a single pesticide (100ng/L) was exceeded by 32 % of surface, 24 % of ground water samples.

Keywords: Pesticide, Ground Water Pollution, Surface Water Pollution, Solid Phase Extraction

ÖZ

KUMLUCA TARIM BÖLGESİNİN YERALTI VE YÜZEY SULARINDAKİ PESTİSİT KİRLİLİĞİ

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Tarım faaliyetlerinin yoğun olarak sürdürüldüğü Kumluca bölgesinden, 2005 yılı ilkbahar ve sonbahar dönemlerinde toplanan 27 yeraltı ve 11 yüzey suyu örneğinde 17 organoklorlu ve 14 organofosforlu pestisit derişimleri ölçülmüştür. Örnekler Katı Faz Ekstraksiyonu yöntemiyle önzenginleştirilmiş ve organoklorlu pestisitler için GC-ECD, organofosforlu pestisitler için GC-NPD sistemleri kullanılarak analitik tayinleri yapılmıştır.

Arazi ve laboratuar kör numuneleri, standart referans maddeleri, eklenmiş kontrol matriksi, eklenmiş örnek matriksi, vekil standartları, örnekleme ve analiz tekrarları kullanılarak kalite kontrol/kalite güvence testleri uygulanmıştır. Sonuçlar örnek matriksin ortalama yüzde geri kazanımını %89'dan %76'e düşürdüğünü göstermiştir.

Analiz sonuçlarını etkileyen ana etkenleri belirlemek için ölçümlerin belirsizlikleri hesaplanmıştır. Ekstraksiyon prosedürünün belirsizliğe etkisinin en yüksek olduğu belirlenmiştir.

Organoklorlu pestisitler arasında en çok gözlemlenen edosulfan (%70) olurken, organofosforlular arasında en çok gözlemlenen chlorpyriphos (%53) olmuştur. En yüksek ortalama derişimler heptachlor (26 ng/L) ve fenamiphos (184 ng/L) için belirlenmiştir.

Pestisitlerin yüzey sularında bulunma oranlarının ve derişimlerinin daha yüksek olduğu gözlemlenmiştir. Organofosforlu pestisitlerin derişimleri ilkbahar döneminde, organoklorluların ise sonbahar döneminde yüksektir.

Klorlu pesitisitlerin bozunma ürünlerinin yüksek miktarlarda bulunması 1980 öncesinde bu pestisitlerin yoğun olarak kullanıldığını göstermektedir. Kumluca çevresinde pestisitlerin bozunma oranının yüzey sularında yüksek olduğu tespit edilmiştir.

Tarımsal faaliyetlerin bölgedeki su kalitesini etkilediği gözlemlenmiştir. Yüzey suyu örneklerinin %27'sinde ve yeraltı suyu örneklerinin %14'ünde her iki mevsimde en az bir kere yasal toplam derişim limiti (500ng/L) aşılmıştır. Yüzey suyu örneklerinin %32'sinde ve yeraltı suyu örneklerinin %24'ünde tek bir pestisit için limit (100ng/L) aşılmıştır.

Anahtar Kelimeler: Pestisit, Yeraltı Suyu Kirliliği, Yüzey Suyu Kirliliği, Katı Faz Ekstraksiyonu

Anneme ve kızkardeşime…

To my mother and sister…

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LIST OF ABBREVATIONS

ASE	Accelerated Solvent Extraction
DDD	Dichloro diphenyl dichloroethane
DDE	Dichloro diphenyl dichloroethylene
DDT	Dichloro diphenyl trichloroethane
DDVP	Dichlorvos
DI	Deionized Water
ECD	Electron Capture Detector
EPA	(U.S.) Environmental Protection Agency
EU	European Union
FPD	Flame Photometric Detector
GC	Gas Chromatography
HCH	Hexachlorocyclohexane
HPLC	High Performance Liquid chromatography
LOD	Limit of Detection
LOQ	Limit of Quantification
MAE	Microwave Assisted Extraction
MS	Mass Spectrometry

NIST	National Institute of Standards and Technology
NPD	Nitrogen Phosphorus Detector
OCPs	Organochlorine Pesticides
OPPs	Organophosphorus Pesticides
PAHs	Polycyclic Aromatic Hydrocarbons
PCBs	Polychlorinated biphenyls
PFE	Pressurized Fluid Extraction
POPs	Persistent Organic Pollutants
QA	Quality Assurance
QC	Quality Control
SFE	Supercritical Fluid Extraction
SPE	Solid Phase Extraction
SPME	Solid Phase Micro-Extraction
SRM	Standard Reference Material
USGS	United States Geological Survey
WHO	World Health Organization
VOCs	Volatile Organic Compounds

CHAPTER 1

INTRODUCTION

Pollution means the introduction by man, directly or indirectly, of substances or energy into the environment resulting in deleterious effects of such a nature as to endanger human health, harm living resources and ecosystems, and impair or interfere with amenities and other legitimate uses of the environment. In all cases of pollution, there is a source of pollutants, the pollutants themselves, the transport medium and a target or receptor, which includes ecosystems, individual organisms and structure. Pollution can be classified in several ways according to source (e.g. agricultural pollution), the media effected (e.g. water pollution) or by the nature of the pollutant (e.g. pesticide pollution) (Alloway and Ayres, 1997).

In most cases, air pollution is the form of pollution which causes people the most concern. It is usually obvious by its effects on the eyes and nostrils and also causes obvious toxicity symptoms on vegetation. Water pollution is the second most noticeable type of pollution, especially when it affects drinking water supplies. In contrast, the soil pollution is often far less conspicuous but it is still very important. As a result of the adsorptive and buffering properties of the soil, some pollutants have long half-lives and so accumulate in the soil. As soil is difficult to remediate, the polluted soil may have effects lasting for centuries. The threats that environmental pollution poses to human health, food, environment and welfare leads the need for a greater understanding of environment and pollution (Hill, 2004).

1.1. Introduction to Water Systems

Water covers about 71 % of the global surface therefore the water bodies comprises a major part of the environment. Of this water mass, 97 % is in the oceans or seas, 2 % is in the form of ice and only almost 1 % exists mostly as groundwater, than in lakes and rivers. Only this small portion of whole water supply in the earth is available for human use. Worldwide, agriculture accounts most of (65 %) the water used, and agricultural demand is growing as population continues to increase. The industry or power generation has 25 %, and domestic use has 10 % share of the total water consumption by human. (ImpEE Project, 2006)

Water is constantly moving within and above the earth in a cycle called "the hydrological cycle" (Figure 1.1). There are six major components of this cycle: evapotranspiration, condensation, precipitation, infiltration, percolation and run-off. Evapotranspiration is the combined effect of evaporation from surface water and transpiration from the plants, producing the water vapor. Condensation is the formation of clouds from water vapor which leads to precipitation. The entry of the precipitated water into the soil is infiltration, by which constitutes the source of water to sustain the growth of vegetation and ground water supply to wells, springs and streams. Percolation is the downward movement of water through soil and rock. The terms infiltration and percolation are often used interchangeably. The rainwater that does not infiltrate into the soil directly reaches the surface water, by the run-off to rivers and lakes (Ground Water-Primer-Hydrological Cycle, n.d.).

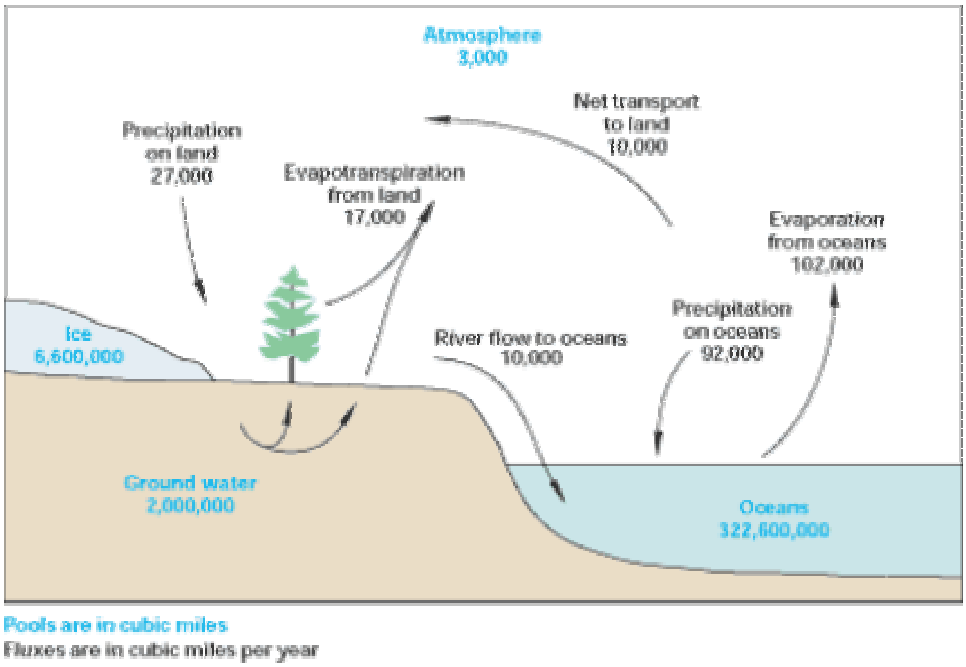


Figure 1.1. The Hydrological Cycle (From USGS Circular 1139, 1998)

Some of the runoff carried to the ocean directly in surface waters but much of the water falling on land percolates into permeable rock layers. As the water in form of precipitation seep into the ground, it first enters a zone where the voids contain both air and water, referred as “unsaturated zone” or “vodase zone”. The upper part of this zone supports plant growth and called as “root zone”. Although a considerable amount of water can be present in the unsaturated zone, this water can not be pumped by wells. The water content in this zone is held by surface adhesive forces and it rises above the water table by capillary action. Water moves from unsaturated zone into the “saturated zone”, where all available spaces are filled with water. It is within this saturated zone that the term “groundwater” is correctly applied. The upper surface of the saturated zone is referred to as “water table” (USGS Circular 1139, 1998).

Streams and other surface-water bodies may either gain water from ground water or lose (recharge) water to ground water. Streams commonly are a significant source of recharge to ground water downstream from mountain

fronts and steep hill slopes in arid and semiarid areas and in karst terrains, areas underlain by limestone and other soluble rocks (USGS, Circular 1186, 1999).

Groundwater is a widely distributed natural resource found beneath the earth's surface. Usable groundwater available to supply wells and springs comes from geologic formations called aquifers, which are underground layer of water bearing permeable rock or unconsolidated (loose) materials from which groundwater can be extracted using a water well. Aquifers are composed of various materials such as rock, sand, and gravel that reflect local geology. Some consist of unconsolidated deposits of sand, clay, silt, or gravel containing water in the voids between particles and rock fragments. Other aquifers occur as cracks in bedrock or consolidated (solid) materials such as igneous rock (granite, basalt), sedimentary rock (limestone, siltstone, sandstone), or metamorphic rock (slate) (Whitford et al., 2004).

1.2. Water Pollution

The earth's water supply remains constant but man is capable of altering the hydrological cycle. Population increases, rising living standards, industrial and economic growth have place greater demands on natural environment. Man-made activities can create an imbalance in the hydrologic cycle and can affect the quality and quantity of natural water resources available.

The water quality is of great concern not only for the health of aquatic ecosystems, but also for human health and welfare. The major man made water pollutants arise from industrial and mining activities which pollute the water by discharges of a variety of toxic materials and exposed soil. Discharge of sewage represents the major global source of pollution reduces the dissolved oxygen content, upsetting the biological balance of the water systems. Agriculture can foul surface and ground waters with excess nutrients and

poisonous chemicals. Petroleum spills kill or adversely affect aquatic organisms besides birds and mammals. Urban storm water runoff, which contains all the debris of a city, introduces some organic and inorganic chemicals into water bodies. Fallout from the atmosphere is another source of water pollution (Weiner, 2000; Spiro and Stigliani 2003). The sources of water pollutants are summarized in Table 1.1.

Table 1.1. Summary of Water Pollutants
(Compiled from Weiner (2000) and Spiro and Stigliani, (2003))

Source	Pollutants
Agriculture (Growing Crops)	Fertilizers (nutrients), pesticides, suspended soil
Agriculture (Animal Operations)	Animal wastes, nutrients (pathogens), suspended soil
Construction and Mining	Acids, heavy metals, oil/grease, debris, soil, SO_4^{2-}, CN^-
Sewage and Waste Water	Organic wastes, detergents, nutrients (pathogens), HPO_4^{2-}, NO_3^-, Cl^-, SO_4^{2-}
Industrial Effluents (Chemical, electrical, metallurgical, etc.)	Metals, acids, solvents (VOCs), PAHs, PCBs, organometals, detergents
Petroleum Discharge	Petroleum products, solvents
Urban Storm Runoff	Suspended soil, oil/grease, heavy metals, salts, PAHs, bacteria, animal wastes
Leachate from Landfills	Metals, acids, organic chemicals, microorganisms
Atmospheric Fallout	Heavy metals, NO_x, SO_4^{2-}, pesticides, PAHs
Radioactive Wastes	Radioactive substances like U and Th

In considering the effects on water quality, “point sources” and “non-point sources” of pollution should be distinguished. Point source is any single identifiable source from which pollutants are discharged, such as a pipe or a factory. The majority of pollution episodes though arise from point sources and this type of sources are easily identified and controlled. Non-point sources are harder to identify precisely and include agricultural or urban runoff and emissions from transport vehicles. The progress made in controlling point sources has drawn attention to non-point sources, which account for an increasing fraction of the total pollutant load (Spiro and Stigliani 2003).

Natural waterways normally contain micro-organisms, which enable them to undergo self-purification. Similarly, as the rainwater or streams percolates down and replenish groundwater, the soil absorbs and detoxifies many pollutants. As rivers are moving bodies of water, any pollution in these medium can be discharged into the sea (Wright, 2003; Hill 2004).

Surface pollutants, dissolved in water, percolate down through the soil. How much pollutant reaches groundwater depends on soil type, pollutant characteristics, and the distance to the water source. Contamination sources include many types of runoff, agricultural and urban, chemical spills, and landfill leachate-anything that may percolate through the soil into groundwater. Sewage from improperly installed or maintained septic systems and confined animal operations can contaminate groundwater. Petrochemical from leaking underground storage tanks can contaminate too. Groundwater often has detectable levels of pesticides. A detectable dose not necessarily indicates a problem, but does indicate a need for ongoing monitoring and efforts to prevent further pollution (Hill, 2004).

1.3. Organic Pollutants

Organic substances consist of a potentially large group of pollutants, particularly in urban environments. Even at low levels, some of these organic pollutants are toxic and can be hazardous to human health, particularly if the exposure is long term.

Considering their chemical and physical properties, they can be grouped into different classes, such as volatile organic compounds (VOCs), polycyclic aromatic hydrocarbons (PAHs), polychlorinatedbiphenyl (PCBs), polychlorinated dibenzodioxins (PCDD-dioxins), polychlorinated dibenzofurans (PCDF-furans) and pesticides.

Persistent organic pollutants (POPs) are a group of substances that are toxic, bioaccumulative, persist in the environment and can be transported to remote regions of the globe. POPs are environmentally stable, so they may be found in air, water, soil, sediments and biota of different regions of the globe where they have never been used. They are also fat soluble substances; therefore they can bioaccumulate through food chain, causing adverse effects to human health (UNEP, 1999).

Due to the global dimensions of the potential impacts POPs related problems can only be handled on the basis of international agreements. In December 2000, representatives of 122 countries finalized a treaty, the “Stokholm Convention” by the United Environmental Program (UNEP). Here, it has been defined a list of 12 high priority POPs, the so called “dirty dozen”. These include 8 chlorinated pesticides (aldrin, chlordane, DDT, dieldrin, endrin, heptachlor, mirex and toxaphene), 2 industrial chemicals (PCBs and hexachlorobenzene-HCH) and 2 unwanted byproducts of combustion and industrial processes (dioxins and furans). The convention includes instruments for the total elimination of these 12 POPs on a global scale.

1.4. Pesticides

A pesticide is any substance or mixture of substances that kill pest, or inhibits in some way its development. Under the UK Food and Environmental Protection Act of 1985, a pesticide is defined as “any substance, preparation or organism prepared or used, to protect plants or wood or other plant products from harmful organisms; to regulate the growth of plants; to give protection against harmful creatures; or to render such creatures harmless”. A pest is a living organism that is not required in some place because of its detrimental effects (Wright, 2003).

Extensive pesticide use throughout the world is needed to increase the agricultural productivity to meet the increasing demand for food production. Pesticides have widely been used also in public-health reasons to fight with disease carrying organisms, such as mosquitoes, flies and rats which lead to spread of malaria, yellow fever and plague (Hill, 2004)

Pesticides are classified according to their target organism. A pesticide that kills insects is an *insecticide*. The one killing unwanted plants is a *herbicide*. The other pesticide types and target organisms are given in Table 1.2. The pests attacking agricultural crops are insects, weeds, rodents, and disease causing organisms including fungi and bacteria.

Table 1.2. Common Pesticides and Target Organisms

Pesticide	Target
Acaricide	Mites and ticks
Biocide	Microorganisms (bacteria, viruses)
Fungicides	Fungi
Herbicide	Plants (weeds)
Insecticide	Insects and related animals
Nematicide	Nematodes (worms)
Rodenticide	Rodents (rats, mice)

The use of pesticides in agriculture and other areas is not a recent concern. For thousands of years, people looked for means to keep their crops from insects eating them, the weeds choking them, or the fungi making them inedible. Chemicals known to have pesticide activity such as lime, sulfur, nicotine and kerosene have extensively been used until 1800s. Later, inorganic chemicals containing arsenic, mercury, lead, cupper and even hydrogen cyanide came into use (Hill, 2004). However, attempts to combat insect pests were relatively ineffective until the development of modern chemical pesticides. Para, para-dichlorodiphenyltrichloroethane (DDT) was the first of these chemicals, patented as insecticide. During World War II, DDT was used to control typhus and malaria outbreaks, due to its relatively long persistence, cheap cost and effect on wide range of insects. Saving millions of additional lives through disease vector control, its discoverer was awarded the Nobel Prize in 1948 (Spiro and Stigliani 2003).

Use of DDT and related chlorohydrocarbon insecticides rapidly accelerated during 1940s and subsequent decades. Food production was increasing rapidly along with an exponential rise in the use of pesticides and fertilizers and little attention was given to the consequences of their accumulation in the environment, or of the toxicity of their degradation products. However, DDTs success came at a price; ecological implications of DDT and insecticide use have arisen. The book *Silent Spring* by Rachel Carson (1962) focused attention

on effects of pesticide pollution. The title dramatically refers to a scenario of a spring without birdsong because most birds had been killed by pesticides or their residues. This book had a major influence on policy makers and environmental chemists and it initiated a large research area through out the world (Connell, 2005). This has resulted in the use of environmentally acceptable but more expensive pesticides such as organophosphorus compounds, carbamates and pyrethroids.

1.5. Types of Pesticides

Pesticides are classified according to the type of pest they control. Another way for the classification is based on chemical structures, considering the functional groups in their molecular structures, such as organochlorine (OCPs) and organophosphorous (OPPs) pesticides. This classification also determines the methods of sample preparation and analysis. The main types of compounds used as pesticides are shown in Table 1.3. A detailed and well arranged list of pesticides with different chemical classes is available on internet (Pesticide Classification, n.d.).

In nature, the plants have some sort of self-protection against pests. For example, *pyrethrum* is a natural insecticide found in the flowers of certain plants. Today, these flowers contain up to 3% pyrethrins and used to fight against insecticides. The clarification of the structure of natural pyrethrins ha made possible the synthesis of related compounds, “synthetic pyrethhroids”, processing similar insectical activity but being more stable to moisture and light (Connell, 2005).

Table 1.3. The Main Types of Pesticides (Pesticide Classification, n.d.)

Pesticide	Chemical Class	Example
Insecticides	Organochlorines	DDT, Lindane, Aldrin
	Organophosphorus	Parathion, Malathion
	Carbamates	Carbaryl, Aldicarb
	Pyrethroids	Cypermethrin, Permethrin
Herbicides	Phenoxy Componds	Dichlorprop, MCPA
	Carbamates/Thiocarmamets	Chlorprocarb, Molinate
	Urea Compounds	Linuron, Azimsulfuron
	Amides/Anilines	Dimethenamid, Alachlor
Fungicides	Cu-Compounds	$Cu(OH)_2$, $CuSO_4$
	Dicarboximides	Captan, Folpet
	Dithiocarbamates	Mancozeb, Thiram
	Benzimidazoles	Benomyl, Carbendaizm

Pesticides constitute a wide range of research area. In the scope of this work, chlorinated and phosphorus pesticides, which are both insecticides will be discussed. Therefore, other types of pesticides will no longer be included in the text.

1.5.1. Organochlorine Pesticides (OCPs)

This group of substances is referred to as chlorinated hydrocarbons, chlorohydrocarbons of organochlorines. DDT is the most well known member of this group. Lindane, aldrin and heptachlor are other chlorinated pesticides once widely used. Most of them have been banned or restricted in developed countries due to their environmental persistence, damage to animal populations and ability to bioaccumulate in animal fat.

1.5.1.1. Chemical Structure of Organochlorine Pesticides

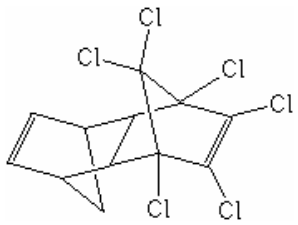
DDT is produced from the reaction of chloral with chlorobenzene in sulfuric acid. DDT is biodegraded to DDE under aerobic conditions and to DDD under anaerobic conditions (Zhou et al., 2006). It was soon discovered that other organochlorine molecules, quite different than DDT, were also insecticide

properties. Several of these were products of reaction between hexachlorocylopentadiene and an olefinic molecule through Diels-Alder condensation. Combination with bicyloheptadiene leads to aldrin, which on epoxidation leads to formation of dieldrin. A similar relationship found between heptachlor and its more active epoxide (Spiro and Stigliani 2003).

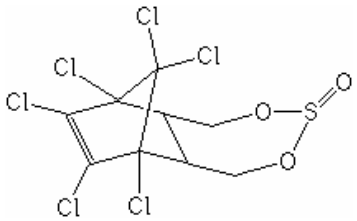
Hexachlorocyclohexane (HCH) is obtained by the addition of chlorine to benzene ring activated by UV radiation. In theory, there are eight isomers in which chlorine atoms occupy different positions about cyclohexane ring. The product, technical-grade HCH consist principally five isomers: α-HCH (60-70 %), β-HCH (5-12 %), γ-HCH (10-15 %), δ-HCH (6-10 %), ε-HCH (3-4 %). This mixture is marketed as an inexpensive insecticide, but since γ-HCH is the only isomer that exhibits strong insecticidal properties, it has been common to refine it from the technical HCH and market it under the name "lindane" (Willett et al., 1998). Chemical structures of some OCPs studied in this work and their chemical identities are given in Figure 1.2 and Table 1.4 respectively.

In this work, *p-p'*- isomers of DDT, DDD and DDE were studied. Therefore, throughout the text, DDT, DDD and DDE refers to *p-p'*- isomers of these compounds.

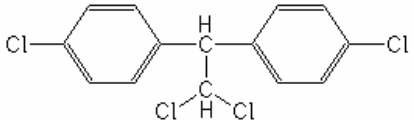
Aldrin



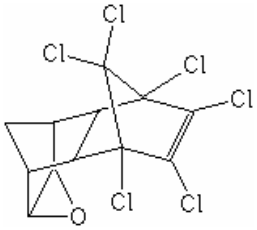
Endosulfan



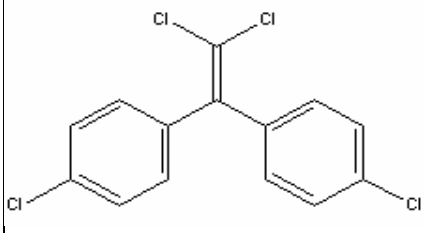
DDD



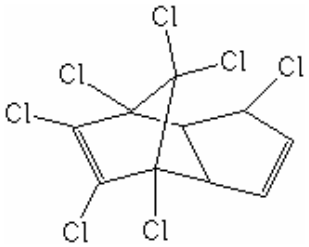
Endrin



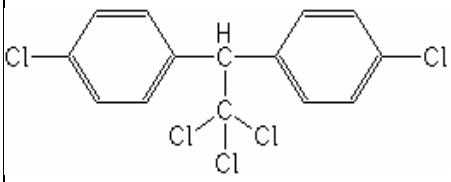
DDE



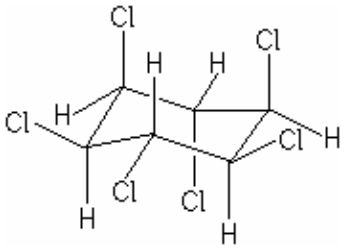
Heptachlor



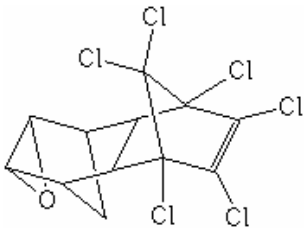
DDT



Lindane



Dieldrin



Methoxychlor

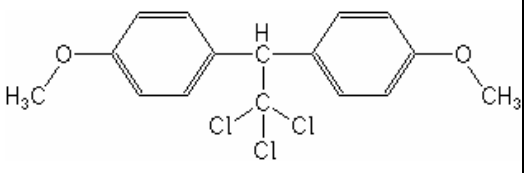


Figure 1.2. Molecular structures of some Organochlorine Pesticides

Table 1.4. Chemical Identities of Organochlorine Pesticides

Pesticide Name	CAS-Number	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
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
DDT	50-29-3	$C_{14}H_9Cl_5$	1,1,1-trichloro-2,2-bis(4-chlorophenyl)ethane
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-oktahydrogen-endo,exo-1,4:5,8-dimethanonaftalen
α-Endosulfan	959-98-8	$C_9H_6Cl_6O_3S$	1,4,5,6,7,7-hexachlor-,cyklický sulfit,endo-5-norbornen-2,3-dimethan
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-oktahydrogen-endo,endo-1,4:5,8-dimethanonaftalen
γ-HCH	58-89-9	$C_6H_6Cl_6$	1α,2α,3β,4α,5α,6β-hexachlorocyclohexane
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

1.5.1.2. Physical and Chemical Properties of Organochlorine Pesticides

There is limited range of bond types present in this group of pesticide formulations. These are 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 dipole moments which are relatively low. Considering the whole molecular structures, these make the compounds in this group tend to have low polarity, being fat soluble or lipophilic and having low solubility in water (Connel, 2005). In Table 1.5, some physical and chemical properties of this type of pesticides are given.

1.5.1.3.Interaction of Organochlorine Pesticides with Environment

Besides the selective toxicity to insects, the choice of DDT and other OCPs as was based on their chemical and physical properties: They are chemically stable and degrades only slowly under environmental conditions, so each application is effective for a long time. They have very low solubility in water (less than 1 mg/L for DDT at 20ºC), therefore they are not readily washed away. These characteristics make OCPs persistent (Wright, 2003). Most of the compounds in this group persist for long periods of time in soil, and exhibits long half lives (Table 1.5). All degradation pathways for the compounds involve hydrolysis and oxidation at various stages.

1.5.1.4. Mode of Action of Chlorinated Pesticides

Being hydrophobic and fat soluble, DDT readily penetrates the waxy outer coating of insects and once introduced into body, it quickly paralyzes the insect. DDT acts by binding to the nerve cells of insects in a way that it holds open the molecular channels that admit sodium ions, which in turn lead to uncontrolled firing of the nerves. DDT's toxicity to mammals and human is low, as animals absorb much less of the chemical in their tissues (Spiro and Stigliani, 2003).

Table 1.5. Physical and Chemical Properties of Organochlorine Pesticides (Compiled from PAN Pesticide Database, n.d.; ARS Pesticides Database, n.d.; EXTOXNET Pesticide Information Profiles, n.d.)

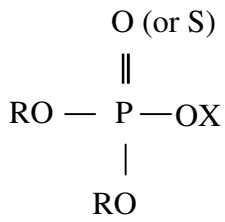
	Molecular Weight	Physical State	Water Solubility mg/L	Vapor Pressure (mPa)	log Kow	Log Koc	Soil Half Life (days)
Aldrin	365	S	0.03	3.10	7.4	4.7	365
DDD	320	S	0.05	0.14	6.2	5.4	5694
DDE	318	S	0.14	0.86	6.9	5.9	5694
DDT	354	S	0.04	0.02	1.0	5.6	5694
Dieldrin	381	S	0.25	0.05	6.2	4.1	1000
α-Endosulfan	407	S	0.32	0.83	3.1	4.1	43
End sulfate	423	S	0.22		3.7	4.1	
Endrin	381	S	0.23	0.02	5.3	4.0	4300
γ-HCH	291	S	7.30	5.61	3.8	3.1	423
Heptachlor	373	S	0.06	53.05	5.5	4.4	250
Methoxychlor	346	S	0.10	0.35	4.3	4.9	170

1.5.2. Organophosphorus Pesticides (OPPs)

This group of pesticides was intensely investigated during World War II, for use as military gases. These insecticides first developed as the nerve gas chemical-warfare agents. They were considered quite unsuitable for agricultural use due to their high mammalian toxicity. After the recognition of environmental problems that became apparent with OCPs, a great deal of attention has been focused on organophosphate group for development as commercial pesticides. In recent years, this group of pesticide is the most widely used one all around the world (Connell, 2005).

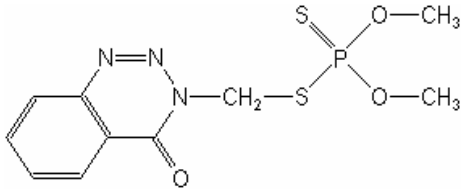
1.5.2.1.Chemical Structure of Organophosphorus Pesticides

The organophosphate pesticides (OPPs) have the following general formula:

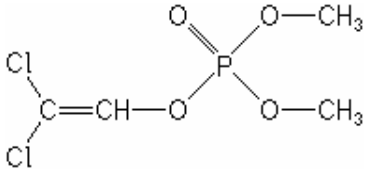


The two R groups are usually methyl or ethyl groups. The oxygen atoms can be replaced by sulfur atoms. Although the group has a common core structure, there is considerable diversity due to variations in the attached chemical groups. There are aliphatic, aromatic and heterocyclic derivatives of these compounds (Connell, 2005). The structures of OPPs studied in this work and chemical identities are given in Figure 1.3 and Table 1.6 respectively.

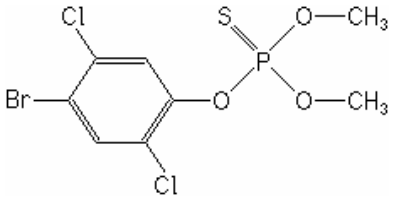
Azinphos-Methyl



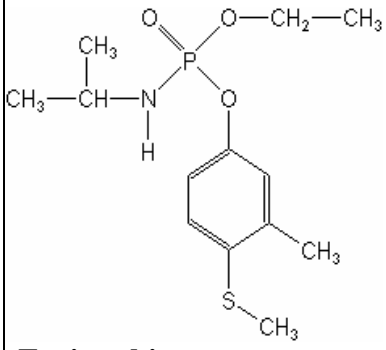
Dichlorvos



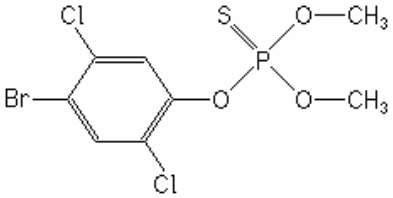
Bromophos-Methyl



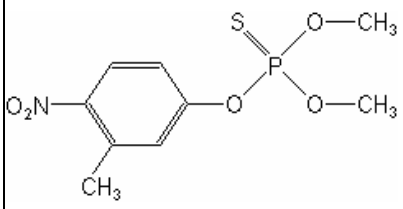
Fenamihos



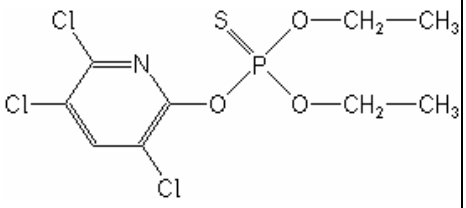
Bromophos-Ethyl



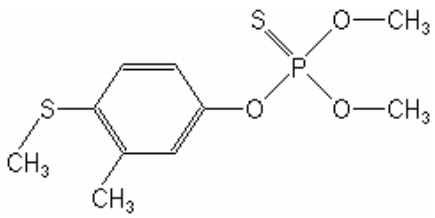
Fenitrothion



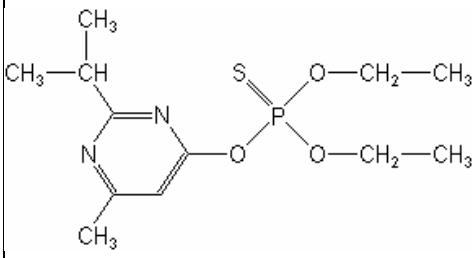
Chlorpyriphos



Fenthion



Diazinon



Malathion

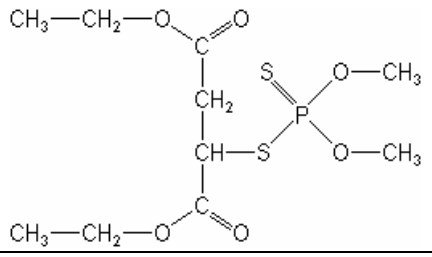


Figure 1.3. Chemical Structures of Organophosphorus Pesticides

Figure 1.3. Continued

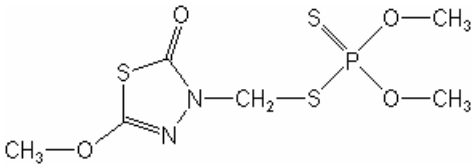
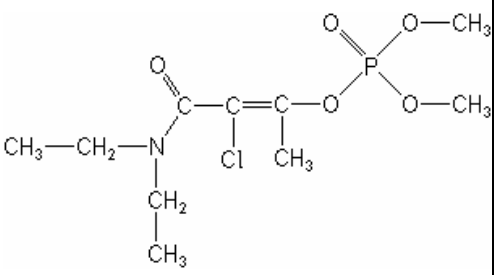
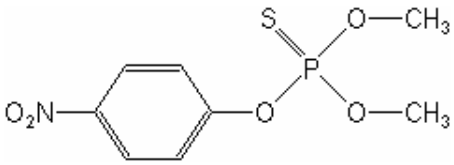
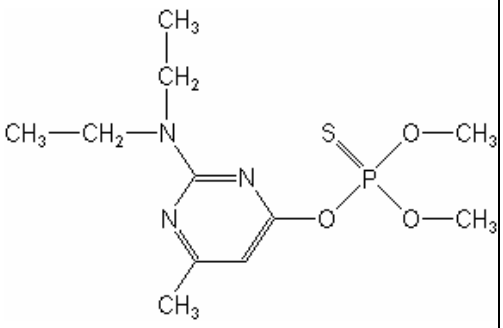
Methidathion	**Phosphamidon**
	
Parathion-Methyl	**Pirimiphos-Methyl**
	

Table 1.6. Chemical Identities of Organophosphorus Pesticides

Pesticide Name	CAS Number	Formula	IUPAC Nomenclature
Azinphos-methyl	86-50-0	$C_{10}H_{12}N_3O_3PS_2$	*S*-(3,4-dihydro-4-oxobenzo[*d*]-[1,2,3]-triazin-3-ylmethyl) *O,O*-dimethyl phosphorodithioate
bromophos-ethyl	4824-78-6	$C_{10}H_{12}BrCl_2O_3PS$	*O*-4-bromo-2,5-dichlorophenyl *O,O*-diethyl phosphorothioate
bromophos-methyl	2104-96-3	$C_8H_8BrCl_2O_3PS$	*O*-4-bromo-2,5-dichlorophenyl *O,O*-dimethyl phosphorothioate
Chlorpyrifos	2921-88-2	$C_9H_{11}Cl_3NO_3PS$	*O,O*-diethyl *O*-3,5,6-trichloro-2-pyridyl phosphorothioate
Diazinon	333-41-5	$C_{12}H_{21}N_2O_3PS$	*O,O*-diethyl *O*-2-isopropyl-6-methylpyrimidin-4-yl phosphorothioate
Dichlorvos	62-73-7	$C_4H_7Cl_2O_4P$	2,2-dichlorovinyl dimethyl phosphate
Fenamiphos	22224-92-6	$C_{13}H_{22}NO_3PS$	(*RS*)-ethyl 4-methylthio-*m*-tolyl isopropylphosphoramidate
Fenitrothion	122-14-5	$C_9H_{12}NO_5PS$	*O,O*-dimethyl *O*-4-nitro-*m*-tolyl phosphorothioate
Fenthion	55-38-9	$C_{10}H_{15}O_3PS_2$	*O,O*-dimethyl *O*-4-methylthio-*m*-tolyl phosphorothioate
Malathion	121-75-5	$C_{10}H_{19}O_6PS_2$	diethyl (dimethoxythiophosphorylthio)succinate
Methidathion	950-37-8	$C_6H_{11}N_2O_4PS_3$	*S*-2,3-dihydro-5-methoxy-2-oxo-1,3,4-thiadiazol-3-ylmethyl *O,O*-dimethyl phosphorodithioate
Parathion-methyl	298-00-0	$C_8H_{10}NO_5PS$	*O,O*-dimethyl *O*-4-nitrophenyl phosphorothioate
Phosphamidon	13171-21-6	$C_{10}H_{19}ClNO_5P$	(*EZ*)-2-chloro-2-diethylcarbamoyl-1-methylvinyl dimethyl phosphate
Pirimiphos-methyl	29232-93-7	$C_{11}H_{20}N_3O_3PS$	*O*-2-diethylamino-6-methylpyrimidin-4-yl *O,O*-dimethyl phosphorothioate

1.5.2.2. Physical and Chemical Properties of Organophosphorus Pesticides

The defining chemical structure of organophosphate pesticides contains one P=O and three P-O bonds. P-O bond has similar polarity to O-H bond, thus it is polar. At the same time, the molecule usually contains a range of other bond types, including O-alkyl, which is relatively of low polarity. These compounds generally have greater water solubility and lower lipophility than OCPs. However, depending on the R and X groups, the OPPs can have a wide range of physicochemical properties (Connell, 2005). The properties of OPPs studied in this work are given in Table 1.7.

Table 1.7. Physical and Chemical Properties of Organophosphorus Pesticides (Compiled from PAN Pesticide Database, n.d.; ARS Pesticides Database, n.d.; EXTOXNET Pesticide Information Profiles, n.d.)

	Molecular Weight	Physical State	Water Solubility (mg/L)	Vap Pres (mPa)	log Kow	Log Koc	Soil Half Life (days)	Water Half Life (days)
Azinphos-me	317	S	29	0.03	2.69	3.0	10	19
Bromohos- et	394	L	2	6.1	5.68	3.8	8	-
Bromohos- me	366	S	40	17	4.88	3.0	-	-
Chlorpyrifos	351	S	1.18	2.50	5.50	3.8	30	58
Diazinon	304	L	40	0.10	3.30	3.2	40	138
Dichlorvos	221	L	8000	2100	1.90	1.7	10	4
Fenamiphos	303	S	329	6.27	3.25	2.2	50	300
Fenitrothion	277	L	30	18.60	3.43	3.3	4	36
Fenthion	278	L	9.3	4	4.84	3.1	34	41
Malathion	330	L	130	5.3	2.70	3.2	30	6
Methidathion	302	S	240	186	4.72	2.6	7	26
Parathion-me	263	S	55	1.3	3.43	3.7	12	45
Phosphamdon	300	L	1.0×10^6	2.93	0.795	1.5	17	48

L: Liquid, S: Solid

1.5.2.3. Interaction of Organophosphorus Pesticides with Environment

This group of pesticides is chemically reactive. They are susceptible to hydrolysis. The half lives in soil are considerably less than OCPs. Their lack of persistence in soil indicates low persistence in biota, and together with their moderate water solubility and low lipophilicity, leads to a lack of bioaccumulation capacity (Connell, 2005).

1.5.2.4. Mode of Action of Organophosphorus Pesticides

The organophosphates work by inhibiting the enzyme called *acetycholinesterase*, which hydrolyzes the neurotransmitter *acetylcholine*. Neurotransmitters are molecules that are released by a nerve cell in order to fire an adjacent nerve cell by diffusing across the gap between the cells, called the *synapse*, and binding to receptors of the second cell. There are many kinds of neurotransmitter molecules, but the one responsible for firing motor nerve cells in higher life forms is acetylcholine. Once acetylcholine binds to its receptor, a motor nerve cell will continue to fire until the acetylcholine is broken down by acetylcholinesterase, which is present in the synapse. If the acetylcholinesterase is inhibited, then the nerve firing continues uncontrollably, leading to paralysis and death (Spiro and Stigliani 2003).

Toxicity of OPPs is much lower than the nerve gases but higher than OCPs. Some of the most widely used ones, like parathion and malathion are highly toxic and can cause death and injury to many agricultural workers. Thus, the environmental advantage of these nonpersistent agents is counterbalanced by their health impacts on agricultural workers. Due to these health concerns, the use of chlorpyriphos, previously most widely used household insecticide, has been banned and use of methyl-parathion has restricted in U.S. (Spiro and Stigliani 2003).

1.6. Pesticide Use in World Agriculture

Currently, world population is 6.4 billion and growing constantly with an annual rate of 1.2 %, mostly in low-income countries. By the year 2050, the world population is estimated to reach 9.1 billion. The 95 % of population increase will take place in developing countries, where daily average food consumption is already been limited, representing an increase in the number of undernourished for the near future. For the present time, to lessen poverty and undernourishment the production of more food and ensure food security regionally is the primary challenge for policy makers. For the long term, the challenge is to feed growing population. Therefore, future growth of the agriculture is essential at global level (UN World Water Development Report-2, 2006).

Besides other possible means, such as increasing agricultural land, improved soil and water management, development and use of genetically modified organisms (GMOs), the immediate response to the need for the increasing production of food is more intensive use of agrochemicals. These include chemical fertilizers and pesticides. Increased pesticide use has adverse affects on environment, food and human health. Although less harmful formulations are found and used as pesticides in developed countries in scope of "green chemicals", developing countries still use and invest on OCPs such as DDT and HCHs as they are cheap, easy to synthesize and even offered by developed countries (Carvalho, 2006).

In 1990s, the global pesticide consumption was about 2.5 million tons, accounting a market of US$ 25 billion per year. In the first half of 2000s, the annual global pesticide expenditure was about US$ 30 billion for about 3.0 millions of tons, of which 24% is consumed in the USA alone, 45% in Europe and 25% in the rest of the world. (Gupta, 2004; Mansour 2004). Herbicides accounted for the largest portion of total use (36 %), followed by insecticides

(25 %), fungicides (10 %) ant others (29 %). Globally, OPP use makes up 40 % of total insecticide use, followed by carbamates (20.4 %), pyrethroids (18.4) and OCPs (6.1 %). Globally, more than 60 % of pesticides are used for the production of vegetables and cereals. (Eddleston et al., 2002 ; Gupta, 2004; Mansour, 2004)

Only 25 % share of pesticide market belongs to developing countries, where 58 % of the world's agricultural land is located. Pesticide use in developing countries has an increasing trend. Besides the cumulative application amount, the quantity of pesticide applied per acre of land is also increasing. In addition, farmers use higher concentrations of pesticides, with increased frequency of application and they mix several pesticides to combat pesticide resistance by pests. While the majority of pesticides used in developed countries are herbicides, the bulk of pesticides used in developing countries are insecticides. Furthermore, the insecticides used in developing countries often consist of OCPs (Gupta, 2004, Mansour, 2004).

1.6.1. Pesticide Use in Turkey

Agriculture is an important economic sector in Turkey, although its share is decreasing over time. In 2003, agriculture contributed 11 % gross domestic product (GDP) and 10.7 % of total exports (OECD in Figures, 2005). More than 40% of the total population is engaged in agriculture, operating 4 million farm holdings and the arable and permanent croplands makes up 30 % of total land area of the country (Özkan et al., 2002).

In Turkey, throughout 20 years, although there were some deviations from the trend due to economical floatation and epidemiologic effects of insects and plant diseases, the pesticide use has increased from 37 ktons in 1985 to 44 ktons in 2005 (Data from Turkish Statistical Institute). However pesticide consumption in Turkey accounts for just 1 % of the world's total.

The detailed pesticide use in Turkey is given in Figure 1.4, where the percentage consumption of different groups of pesticides is given in comparison with global ratio. As seen, insecticides are the most widely used pesticide group as in the other developing countries. Organophosphorus pesticides represent 41 % of the insecticides in Turkey. Among the OPPs, mostly used ones are methamidophos, chlorpyriphos, parathion-methyl, dichlorvos and azinphos-methyl (Delen et al, 2005).

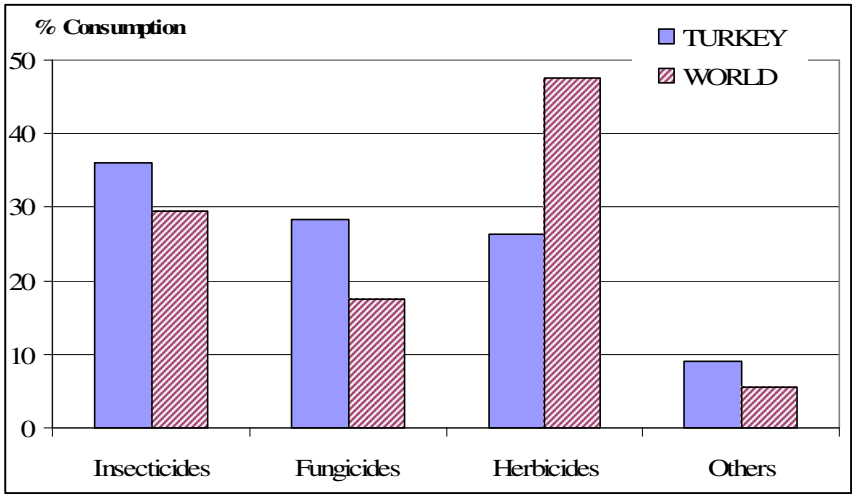


Figure 1.4 Pesticide Consumption for Different Groups
(World data from Gupta, (2004), Turkey data from Turkish Statistical Institute, for 2005)

1.7. Environmental Fate of Pesticides

The environment is not static and materials are constantly being transported between atmosphere, hydrosphere and lithosphere. At each stage of the transportation, the concentration of the substances will be altered by phase transfer, dilution, adsorption, transformation and degradation.

Contaminants in the environment are driven to change by physical forces, chemical changes and biological activity. Physical forces move the contaminants to new locations without significant change in their chemical properties. Chemical changes include the reactions of the contaminants within the medium or with other contaminants. Biological activity is the breakdown of the contaminant molecules via biological processes, which are a special kind of chemical change. There are three possible naturally occurring fates of pollutants in the environment (Weiner, 2000):

1. All or a portion of the pollutant might remain unchanged in their present location.

2. All or a portion of the pollutant might be carried elsewhere by transport processes.

a) Movement to other phases (air, water, soil) by volatilization, dissolution, adsorption.
b) Movement within the same phase under gravity, diffusion and advection.

3. All or a portion of the pollutant might be transformed into other chemical species by chemical reaction or biological processes.
a) *Weathering:* Pollutants undergo a series of environmental chemical changes by processes such as oxidation-reduction, hydration, hydrolysis, complexation, acid-base reactions, and photolysis reactions.
b) *Aerobic/Anaerobic Biodegradation:* Pollutants are altered structurally by biological processes.
c) *Bioaccumulation:* Pollutants accumulate in plant and animal tissues to higher concentrations.

Considering the pesticides, the fate processes can be beneficial; they can move a pesticide to the target area or destroy its potentially harmful affects. However, they can also be detrimental, leading to reduced control of a target pest, injury of non-target plants and animals, and environmental damage.

Once pesticide is introduced to the environment by application, accidental release or waste disposal, it becomes distributed among four major compartments of environment: air, water, soil and biota. The distribution is determined mainly by adsorption, transport and transformation/degradation processes. Apart from other transport processes, adsorption is of special importance as it influences how much of the pesticide is free to enter into other processes. Movements to other phases occur by runoff, leaching, volatilization and uptake by plants. Transformation includes chemical and photodegradation, biodegradation and bioaccumulation (Environmental Fate of Pesticides, n.d.). These processes are demonstrated in Figure 1.5.

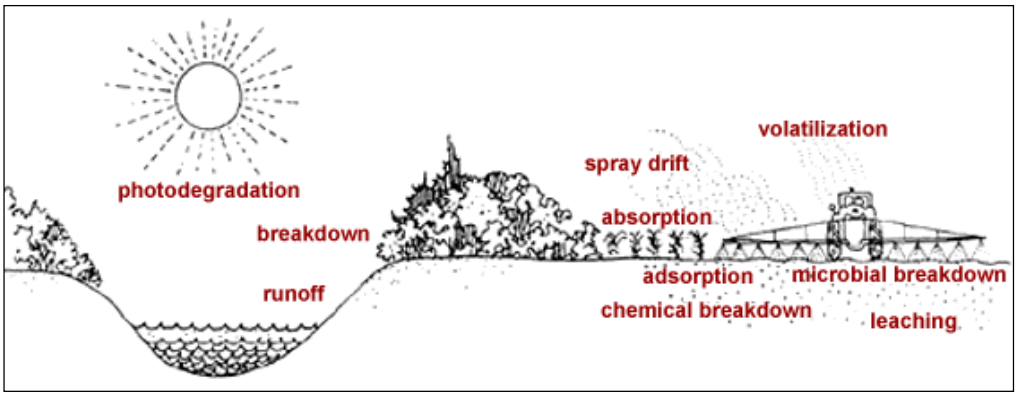


Figure 1.5. Fate Processes of Pesticides in Environment (Environmental Fate of Pesticides, n.d.)

1.7.1. Pesticide Transportation in Environment

Adsorption is a reversible process that binds pesticides to the surface of soil particles or sediments. Pesticides vary in their tendency to adsorb to soil particles. To measure the extent of adsorption, soil-water partition coefficient (K_D) is used, which is the ratio of the compound's concentration in soil (C_S) to its concentration in water (C_W):

$$K_D = C_S / C_W$$

Many soil properties influence pesticide adsorption, such as structure, texture, pH and moisture content (Connel, 2005).

Soil has different physical and chemical properties; therefore K_D values are extremely site specific. To overcome this variability, soil-water partition coefficients are generally expressed in terms of soil organic carbon (K_{OC}) rather than total soil mass. It is the normalization of the K_D to total organic carbon content.

$$K_{OC} = K_D / f_{OC}$$

Where, f_{OC} is the fraction of organic matter in soil. (Weiner, 2000) The K_{OC} values for the chlorinated and phosphorus pesticides studied have been given in Table 1.5 and Table 1.7 for respectively.

Besides the soil properties, the adsorption process also depends on the properties of the pesticides. The polarity of pesticide correlates with its solubility; the more polar the pesticide, the more soluble it is.

Another valuable environmental characteristic of the organic chemicals is the *octanol-water partition coefficient,* K_{OW}, which is the ratio of the concentration in n-octanol to that in water.

$$K_{OW} = C_O / C_W$$

The significance of octanol is that, it is a useful surrogate for the weakly polar organic matter present in soils and the lipid tissue of biota.

The water solubility of a pesticide and K_{OC} values are inversely related; pesticides that are typically not very water soluble has high K_{OC} values. Similar to K_{OC} values, compounds with low water solubility has high K_{OW} and tend to partition strongly into organic-rich environmental phases, leading sorption to soil or sediments and accumulation in biota, rather than being carried by runoff or leaching. (Weiner, 2000)

Pesticides can be moved by runoff when they are either has high water solubility. The amount of pesticides in runoff water depends on site related factors, climatic factors and pesticide interactions between soil and water. Pesticides carried by runoff can pollute drainage ditches, ponds, streams, rivers and lakes. Additionally, water bodies can be polluted by the improper washing away of spillages and leaks, and by the illegal dumping of pesticides. (Wright, 2003).

Leaching is the movement of pesticides through the soil via soil water. Pesticides with high water solubility and low adsorption coefficient tend to move with water in the soil if they are persistent to degradation during the movement. Soil factors affecting leaching are texture and organic matter content of the soil (Gevao and Jones, 2002). Similar to runoff, frequent and heavy rainfall leads to transport of pesticide pollutants through leaching.

Volatilization is another type of transport process. It is high when the pesticide has high vapor pressure and there is high temperature, low relative humidity and air movement. *Spray drift* is the airborne movement of spray droplets from the application site. This process depends on the spray droplet size, wind speed and application height (Gevao and Jones, 2002).

Absorption or uptake transfers the pesticides to plants, animals and microorganisms. Once absorbed by plants, pesticides may be broken down via biochemical processes or they may remain until tissue decay or harvest (Gevao and Jones, 2002).

1.7.2. Degradation of Pesticides

Pesticide degradation process can break down pesticide molecules into simpler, smaller and generally less toxic compounds. This could be rearrangement of the molecule into another form or it could be the addition or loss of functional groups by environmental processes. Various degradation processes can safely reduce pesticide concentrations after the target pests have been controlled, thus minimizing problems with persistence, accumulation and associated environmental effects. There are three main types of pesticide degradation: Photodegradation, chemical degradation and biotic degradation (Connel, 2005).

Photodegradation is the breakdown of pesticides due to exposure to radiation on the surface soil, on foliage and even in the air. This processes have been widely studied in the literature as it has been proved to be a promising method for the treatment of waste water contaminated with pesticides (Herrmann and Guillard, 2000; Burrows et al., 2002; Devipriya and Yesodharan, 2005).

Chemical degradation is the breakdown of pesticides by processes that do not involve living organisms. The reactions of pesticides in environment can be classified as neutral and electron transfer (redox) reactions. The neutral

reactions include the nucleophilic substitution (including hydrolysis), dehydrohalogenation, rearrangement and addition (including hydration). Some pesticides are susceptible to oxidation and reduction reactions which occur predominantly in aerobic and anaerobic conditions, respectively. Some OPPs may undergo rapid oxidation in aerobic soils and some OCPs and various pesticides with nitro-groups undergo anaerobic degradation (Gevao and Jones, 2002).

The microbially mediated breakdown of pesticides has been identified to be more important in degradation compared to chemical and physical means. The rate of degradation is a function of the pesticide properties (structure, solubility, concentration etc.) and the environmental conditions affecting the fate processes and microorganisms in the system (Gevao and Jones, 2002).

1.8. Pesticides in Water

Applications of pesticides to cropland can result in significant additions of contaminants to water resources. Some pesticides are only slightly soluble in water and may adsorb to soil particles instead of remaining in solution, such as OCPs. These compounds are less likely to cause contamination of ground water. Other pesticides, such as some OPPs having high water solubilities are detected in low, but significant, concentrations in both ground water and surface water (USGS circular 1139, 1998).

Solubility of the pesticide can not be enough to estimate the behavior of the pesticides in groundwater. Gustafson (1989) has developed an assessment method to rank pesticides for their potential to move toward groundwater on the basis of the adsorption coefficient (Koc) and the soil half-life (DT_{50}) of the compound. From this observation, a groundwater ubiquity score is derived: the GUS score.

GUS = log (DT_{50}) x [4 - log10 (Koc)].

The pesticide movement rating is derived from the GUS. Movement ratings range from extremely low to very high. Pesticides with a GUS less than 0.1 are considered to have an extremely low potential to move toward groundwater. Values of 1.0-2.0 are low, 2.0-3.0 are moderate, 3.0-4.0 are high, and values greater than 4.0 have a very high potential to move toward groundwater. The ratings for OCPs and OPPs studied are given in Table 1.8.

Table 1.8. Pesticide Properties Indicating Environmental and Health Effects (From Vague at al., 1994)

Common Name	Pesticide Movement Rating	WHO Classification
Aldrin	Very Low	O
Azinphos-methyl	Low	I B
Chlorpyrifos	Very Low	II
DDT	Extremely Low	II
Diazinon	Low	II
Dichlorvos	Extremely Low	I B
Dieldrin	Extremely Low	O
Endosulfan	Extremely Low	II
Endrin	Extremely Low	O
Fenamiphos	High	I B
Fenitrothion	Very Low	II
Fenthion	Low	II
Heptachlor	Extremely Low	O
Lindane	Moderate	II
Malathion	Extremely Low	III
Methamidophos	Moderate	I B
Methidathion	Low	I B
Methoxychlor	Extremely Low	U
Methyl parathion	Very Low	I A
Phosphamidon	High	I A
Pirimiphos-methyl	Low	III

Explanations for Classification: I A: Extremely Hazardous, I B: Highly Hazardous, II: Moderately Hazardous, III: Slightly Hazardous, U: Unlikely to Present Acute Hazard in Normal Use, O: Obsolete as Pesticide, not Classified

In addition to the non-point sources of water contamination by pesticides, point sources of contamination are common in agricultural areas where the farms are concentrated in small areas. These are due to spillage and washing water when equipment is cleaned on site; spillage whilst transferring pesticides from containers to applicators or whilst mixing; pesticide storage areas where the cleaning up of spillage is not correctly carried out; the improper washing out and disposal of contaminated containers; and the improper disposal of excess pesticides. Some point sources are controlled by discharge consents, e.g. from manufacturing companies (Wright, 2003).

Ground water contamination presents special concern, because even pesticides that are short lived in surface water may degrade very slowly in ground water and ground water is much more harder to clean up (Hill, 2004).

1.9. Effect of Pesticides

Of all the environmental contaminants, pesticides have properly been the most widely criticized due to their direct use in natural systems (Connel, 2005). The nature of pesticide usage often requires broad distribution over large areas of crops, finally affecting human and environment.

1.9.1. Effect on Environment

Concentrations of OCPs, such as DDT and HCH, have been declining over the past decade in environmental waters, as regulations to restrict their use have been put in place. Such compounds are the focus of major global studies (Ueno et al., 2003; Lia and Macdonald, 2005), because they are harmful to aquatic biota, persistent in ecosystems, and their derivatives can bio-accumulate in food chains, having potentially significant impacts on animals at the top of these chains. Studies undertaken in the northern rivers of Russia clearly show the degree of decline in both river water quality and Burbot fish (Lota lota)

(Zhulidov et al., 2002). Similarly, HCH concentrations in China have exhibited a significant decline over time. However, because of their persistence, the impacts of DDT and other OCPs continue to be seen for many years after their use has been discontinued (UN World Water Development Report-2, 2006).

Another interesting and unpredictable effect of uncontrolled pesticide use has been observed for DDT; the biochemical effect of this neurotoxic chemical. DDT is the prime substance that enters birds and is not a strong disrupter of breeding success. However, its metabolic product DDE is very powerful in this area. It has been shown that, DDE interference with the endocrine system (a complex array of glands and organs that control the hormones in the circulatory system) has disturbed the avian hormonal system of certain species of birds, such as the peregrine falcon, that controls calcium deposition during egg formation. As a result, birds having high levels of DDT lay eggs with shells that are too thin to endure until hatching (Spiro and Stigliani, 2003; Connel, 2005).

It should be noted that, the pesticide pollution can be more important than industrial pollution in certain parts of the world affecting soil, water and air. Therefore, the monitoring and control of pesticide use has gained a significant importance, especially in the last decades.

1.9.2. Effect on Human

By their nature, most pesticides create some risk of harm to humans, animals, or the environment because they are designed to kill or otherwise adversely affect living organisms. Pesticides can enter the human body orally, dermally or by inhalation.

The toxicity of a pesticide is its capacity or ability to cause injury or illness. The two types of toxicity are acute and chronic. Acute toxicity of a pesticide

refers to the chemical's ability to cause injury to a person or animal from a single exposure, generally of short duration. Acute toxicity is measured as the amount or concentration of a toxicant required to kill 50 % of the animals in a test population. This measure is usually expressed as LD_{50} (lethal dose 50) or LC_{50} (lethal concentration 50) (Pesticide Toxicity, n.d.).

The lower the LD_{50} or LC_{50} of a pesticide product, the greater its toxicity to humans and animals. Pesticides with a high LD_{50} are the least toxic to humans if used according to the directions on the product label.

World Health Organization (WHO) has classified the pesticides according to the LD_{50} values of pesticides for oral and dermal exposure, for the rats since these determinations are standard procedures in toxicology. The classification involves the grouping of pesticides into: Ia-"extremely hazardous", Ib-"highly hazardous", II-"moderately hazardous", III-"slightly hazardous" and "active ingredients unlikely to present acute hazard". (WHO, 2004). The WHO recommended classification of pesticides studied in this work is given in Table 1.8.

The chronic toxicity of a pesticide is determined by subjecting test animals to long-term exposure to the active ingredient. Any harmful effects that occur from small doses repeated over a period of time are termed chronic effects. Some of the suspected chronic effects from exposure to certain pesticides include birth defects, production of tumors, blood disorders, decrease in fertilization and neurotoxic effects (nerve disorders). The chronic toxicity of a pesticide is more difficult to determine through laboratory analysis than acute toxicity (Tielemans et al., 1999).

It should be noted that, pesticides are the most important method of self poisoning in many rural regions and associated with high rate of death. WHO estimates that, three million pesticide poisoning cases occur world wide every

year and over 500 000 people died from self-harm in Southeast Asia and the Western Pacific during 2000 alone (Eddleston et al., 2002).

1.10. Determination of Organic Pollutants

The environmental pollution, which is a result of human activities, has initiated the development of legislative measures. The assessment of the efficiency of environmental protection policies requires applicable and reliable data on the concentrations of pollutants in the environment. Micropollutants were the largest problems encountered as there are many different compounds each at very low concentration levels, in a wide range of complex matrices. The need for reliable data on occurrence of micropollutants in the environment was an important motivation for the development of modern analytical techniques and procedures. In this development processes, two major areas can be distinguished for trace organic analysis: First one is analytical separation and detection and the second is sample preparation. In the first field, remarkable processes have been achieved during several decades. However, the developments for the second field had to wait until highly sensitive analytical systems had become a common standard. It was realized that any mistake occurring in sampling and sample preparation steps can lead to substantial error in the final result regardless of the excellent performance of the state-of-the-art analytical technique used (Liska, 2000).

It is become possible to identify and determine a large variety of organic environmental pollutants (pesticides, PCBs, PAHs, VOCs, phenols, phthalate esters, benzidines, nitrosamines etc.) which exist at trace levels in the presence of thousands of other organic compounds. Advances both in techniques of separation (high-resolution gas and liquid chromatography), in methods of identification (computerized mass spectrometry and selective detectors) and the introduction of hyphenated techniques have been key factors in this achievement. The result has been a dramatic increase in the number researches

dealing with the identification and analysis of organic compounds in the environment in general.

Following the rapid development of analytical techniques, increasing demands are placed on sample quality, and thus the extraction as a sample preparation tool. Trends in analytical extraction have been moved toward less organic solvent consumption, faster extraction time, improved quantification (by means of higher recoveries, better reproducibility, lower method detection limits), easy to use systems and automation (Raynie, 2004).

1.10.1. Extraction Methods

The basic concept of a sample preparation method is to convert a real matrix into a sample in a format that is suitable for analysis. This can be achieved by employing a wide range of techniques. Extraction of organic pollutants from environmental matrices aims the followings (Smith, 2003):

- To convert the analyte into a suitable form for separation and detection.
- The removal of potential interferents for either separation or detection, from the bulk of the matrix, thereby increasing the selectivity of the method.
- To increase the concentration of the analyte and hence the sensitivity of the assay.
- To provide robust and reproducible method that is independent of variations in the same sample matrix type.

Although many traditional sample preparation methods are still in use the trends in recent years have been moved towards (Smith, 2003):

- The ability of smaller initial sample sizes even for trace analysis.
- Greater specificity or greater selectivity in extraction.

- Increased potential for automation or for on-line methods reducing manual operations.
- A more environmental friendly approach (green chemistry) with less waste and the use of small volumes or no organic solvents.

These goals are being achieved in a number of different ways and are still the subject of active research (Smith, 2003).

Over the past several decades, time consuming manual methods have been used for sample preparation. Most of the time, about 60 % of a typical chromatographic analysis is spent on sample preparation, requiring more time than collection, analysis and data evaluation. This step is also the major source for error in chromatographic analysis, which accounts 30 % of error generated (Settle, 1997).

Classical extraction procedures consume large amounts of solvents, thus themselves creating environmental and occupational hazards, and often provide very little selectivity. During the volume reduction step of most extraction procedures, the solvents are frequently disposed to atmosphere (Pawliszyn, 2003). For the determination of the organic pollutants in water samples, methylene chloride is removed as much as 5-10 million L per year by the US Superfund Contact Laboratory Program alone. Methylene chloride removes ozone from upper atmosphere and is suspected carcinogen. As a result of the Pollution Prevention Act of 1990, the US Environmental Protection Agency (EPA) has taken action to reduce the use of methylene chloride in their current analytical methods (Thurman and Snavely, 2000).

The analytical community responded to this challenge by increasing research on solid phase extraction (SPE) and supercritical fluid extraction (SFE) as less solvent consuming alternatives to liquid-liquid extraction and Soxhlet extraction, respectively. The development of new technologies, such as

pressurized fluid extraction (PFE), hot-solvent extraction (accelerated solvent extraction-ASE), microwave assisted extraction (MAE) and microextraction approaches such as solid phase microextraction (SPME) also reduced solvent use, time and labor consumption for extraction (Pawliszyn, 2003).

For the extraction of organic compounds from solid matrices, Soxhlet extraction has been used traditionally. In this method the solvent is continuously recycled through the sample in a closed system for some hours, leaching out analytes. However, the analyte must be stable in refluxing boiling solvent. Less efficient methods include shaking the sample manually or automatically in hot or cold solvents for prolonged periods. Sample is than filtered, decanted or centrifuged. This technique is often called as *shake/filter method*. The subsequent steps for both extractions involve the evaporation of the solvent and concentration of the sample. All these procedures are often time consuming and require the use of significant amounts of sample and large volumes of organic solvents (Settle, 1997).

The most recent methods involve the instrumental extraction techniques. These processes aim to reduce the amount of solvent and sample, to reduce the time required involve and to enhance selectivity. The extraction can be speeded up by heating or agitating the sample (PFE and MAE) or by using alternative solvent which has higher diffusion rate (SFE) (Smith, 2003).

Pressurized fluid extraction (PFE) is commercialized as accelerated solvent extraction (ASE). In this technique, organic solvents are used at elevated temperatures above their atmospheric boiling points by employing heat and increased pressure. The solvent remains as liquid but has enhanced solvation power and lower viscosity and hence higher diffusion rate. These changes increase the extraction rate and procedures, which have taken many hours by Soxhlet refluxing, can be carried out in minutes on a smaller sample. It requires

a smaller fraction of organic solvent and more concentrated. Moreover, ASE system is able to carry out multiple extractions at the same time (Smith, 2003).

Microwave assisted extraction (MAE) is similar to PFE, and involves liquid solvents heated. In the case of MAE, the heating is due to irradiation with microwave energy, which results in more rapid heating. The sample and solvent are subject to irradiation in either a sealed vessel (pressurized MAE) or an open vessel (atmospheric MAE). The solvent or the sample must possess a dipole to absorb microwave energy. The development of this system followed the development of microwave digestion for inorganic analytes (Raynie, 2004). MAE also allows multiple extractions.

SFE involves carbon dioxide as primary extracting solvent. The carbon dioxide is pressurized above 75 atm, where the gas is used as a supercritical fluid. Through alteration of the applied temperature-pressure combination, some alteration of the solvent properties is achieved (low viscosity, high diffusion rate). Initial limitations of the technique centered on its inability to extract polar molecules from matrix. Solvent polarity is modified through addition of organic co-solvents such as methanol. This technique is an environmentally friendly as it uses carbon dioxide as solvent (Dean and Xiong, 2000).

Extractions from solids may also be accelerated through application of ultrasonic energy through water. It is based on the enhancement of mass exchange in pores of the solid particles when exposed to ultrasound. This technique can be applied to a wide range of samples, resulting in high extraction efficiencies in relatively shorter time with high reproducibility (Banjoo and Nelson, 2005; Babic et al, 1998).

Common and well established methods for the determination of semi volatile organic compounds are based on the use of either liquid-liquid extraction (LLE) or solid phase extraction (SPE). In the LLE, typically a volume of 500-

1000 mL sample is extracted with a non-polar organic solvent (100-250 mL) and the extraction has to be repeated 2-3 times to achieve a high recovery. (EPA Method 3510C). There are disadvantages of LLE: The resulting sample usually includes matrix interferences, therefore further clean-up stage is required. It is laborious, time-consuming, subject to problems arising from the formation of emulsions and requires use of large volumes of organic solvents which are evaporated during the process and pose a risk for both laboratory workers and the environment. In addition, recoveries of many polar analytes are low due to their relatively high solubility in water. This technique is also difficult to automate. Alternatively, solid phase trapping methods such as SPE and SPME has been developed. (Sabik et al., 2000, van der Hoff and Zoonen, 1999, Pichon, 2000, Poole, 2003).

SPE is a widely used sample preparation technique for the isolation and preconcentration of selected analytes from a liquid phase. In SPE, typically an aqueous sample is passed through a small tube filled with porous solid particles such as poly(styrene-divinylbenzene) or silica. Alternatively, a membrane disk containing sorbent particles may be used. The organic analytes are transferred to solid phase where they are retained during the extraction process. The sample is than isolated from the solid phase and the analytes eluted by a small volume of an organic solvent, after a brief wash. Then the elute can be analyzed by gas or liquid chromatography. It is now the most common extraction technique in many areas of chemistry including environmental, pharmaceutical, clinical, food and industrial chemistry. A high level of automation is also possible in the applications of this technique (Poole, 2003, Fritz and Macka, 2000).

More recently, SPME was developed for the need of fast, solvent-free, simple and easy to automate extraction techniques. It has been evaluated for the extraction of a wide variety of pesticides, PAHs, PCBs and other solutes from water samples. SPME is based on the sorption (partitioning) of the analytes

present in the water sample onto a small layer of stationary phase coated onto a syringe-like device. The main advantage of this method is its simplicity, besides the SPME device, only standard GC instrumentation is required. The main disadvantage is that, since this method is based on partitioning equilibrium, extraction is in some cases incomplete which render quantitation difficult. Each analyte should be individually calibrated and the extraction yield should be determined for each analyte. SPME is especially suited as a rapid screening method (Baltussen et al., 1998).

1.10.2. Solid Phase Extraction

Solid phase extraction has several important advantages as an extraction technique for the analysis of organic contaminants in the environment:

1. SPE is faster and requires less manipulation. A sample can be quickly passed through a SPE cartridge or disk with a gentle suction and extracted substances can be easily washed from the solid phase by a small volume of organic solvent. By contrast, simple solvent extraction requires a considerable amount of manipulation in adding extractive liquid, shaking and separation of the phases. During the extraction, emulsion formation is possible and it should be broken to complete the extraction. These processes are time consuming and the manipulations clearly decrease the reproducibility of the technique. The environmental studies require the analysis of large number of samples, in a reproducible way. Considering these necessities, the SPE technique seems to be much appropriate for environmental analysis. Moreover, SPE steps can be automated readily.

2. Besides the medium exchange of the analytes from sample matrix into a solvent suitable for instrumental analysis, the SPE technique has also assist the matrix simplification. By the choice of a proper SPE sorbent

material, the selective extraction of target compounds from bulk of the matrix can be possible. It is clear that, the more selective the SPE step is, the more sensitivity obtained. Hence, the new types of sorbents such as selective immunosorbents or molecular imprinted polymers have been introduced in that way (Pichon, 2000). In fact, SPE technique is commonly used as a clean-up step after the extraction of solid samples such as sediments, soils and foods employing hand packed, normal phase materials such as silica or Fluorisil. In these applications, the principal role of SPE was the retention of unwanted components from the sample such as polar non volatile compounds.

3. SPE requires much less amount of organic solvent. Disk extraction has been reported to use 90 % less solvent than LLE and up to 20 % less solvent than cartridge (Sabik et al., 2000). In an environmental pollution research, use of SPE provides an environmentally friendly way of extraction.

4. SPE provides higher concentration factors. In environmental samples, the organic contaminants usually found in very trace amounts. The transfer of an analyte from 1 L of water sample into 1 mL of solvent leads to a concentration factor of 1000, which makes the determination of the analytes more reliable. This property makes SPE more advantageous over SPME, in which such a concentration can not be obtained.

5. Environmental researchers have studied the potential use of SPE disks for temporary pesticide storage, field extraction of pesticides and shipping pesticides from one location to another. Temporary storage on C_{18} worked well and enhanced the stability of most compounds compared with storage in water at 4 °C (Senseman et al., 2003). Mattice et al. (2002) tested a field extraction manifold using C_{18} disks. They

have found comparable recoveries from field extractions compared with sample collection followed by laboratory extraction. By the use of SPE as a storage and transport media, many problems associated with delivery of water samples, such as storage stability, bottle breakage, and high shipping charges can be eliminated.

1.10.2.1. Reversed Phase Solid Phase Extraction

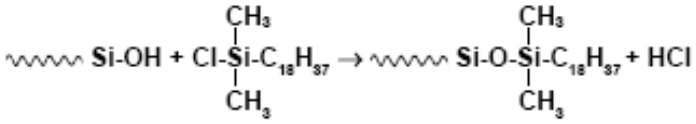
Developing a SPE method requires understanding of the interactions between the analytes and the sorbents. Sorbent-analyte interactions basically fall into three categories: ion exchange, normal phase and reversed phase. Besides the general classification of SPE types, there are also compound specific sorbents such as immunosorbents or molecular imprinted polymers (Fritz, 1999).

In ion exchange mode, solid particles contain cation- or anion-exchange groups that retain ionic analytes or ionic products of analytes converted through a change in pH. The retained analytes are desorbed by an acidic or basic eluent. The normal phase SPE is used to isolate polar compounds from a non-polar sample which were eluted by a polar solvent such as water or alcohol. Alumina and various types of silica gels are often used in normal phase SPE. Reversed phase separations involve a polar or moderately polar sample matrix and a non-polar stationary phase. The analyte of interest is typically mid- to nonpolar (Fritz, 1999).

The sorbents used in SPE include graphitized carbon black (GCB), reversed-phase (RP) materials (modified silica gels) and polymeric materials (Weigel et al., 2001).

Several SPE materials, such as the alkyl- or aryl-bonded silicas are in the reversed phase category. Here, the hydrophilic silanol groups at the surface of the raw silica packing (typically 60Å pore size, 40µm particle size) have been

chemically modified with hydrophobic alkyl or aryl functional groups by reaction with the corresponding silanes.

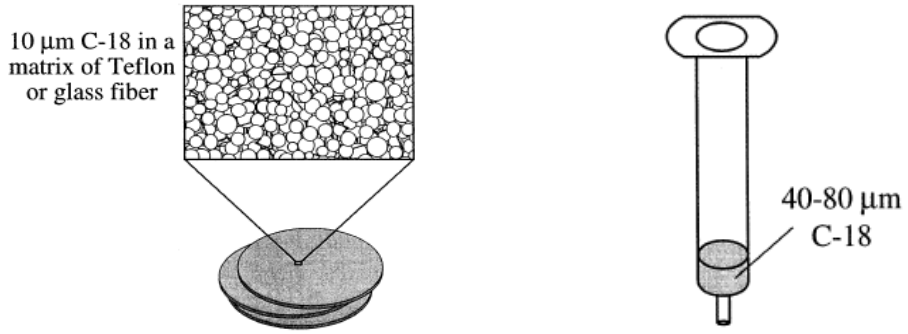


Retention of organic analytes from polar solutions (e.g. water) onto these SPE materials is due primarily to the attractive forces between the carbon-hydrogen bonds in the analyte and the functional groups on the silica surface. These nonpolar-nonpolar attractive forces are van der Waals forces, or dispersion forces. To elute an adsorbed compound from a reversed phase SPE tube or disk, a non-polar solvent is used (SUPELCO Technical Resources, 1998).

Since the retention mechanism is primarily controlled by hydrophobic interactions between analyte and the carbonaceous moieties of the sorbent, a relation can be established between the retention factor of the analyte and its K_{OW} value. It has been shown that the use of C_{18} silica is well appropriate for the trace enrichment of compounds with log K_{OW} values higher than 2 and application of C_{18} silica to the multiresidue extraction of moderately polar and non-polar analytes has been described for monitoring purposes (Pichon, 2000).

1.10.2.2. Solid Phase Extraction Apparatus

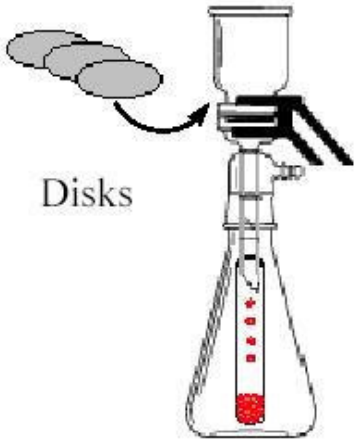
There are two main formats of SPE material in the market: Cartridge and disk format. These devices use the same sorbent technology. The forms of SPE are presented in Figure 1.6, together with the apparatus for extractions via disks.



Disk Type

Cartridge Type in Syringe Barrel Format

a) Forms of SPE



b) SPE Apparatus for Disk Extraction

Figure 1.6. The Forms of SPE Apparatus
a)Forms of SPE (From Thurman and Snavely, 2000),
b) SPE Apparatus for Disk Extractions (Lab_Environ_Tech, n.d.)

SPE cartridges are available in packed-tube form with a wide range of sizes (1-60 mL) and sorbent types. The particle size of the packing material varies but typically averages 40-50 μm in diameter. The selection of optimum cartridge size depends on the concentration of the analyte in the sample, sample volume, final volume after elution. In general, the mass of the retained compounds should be less than 5 % of the mass of the sorbent and the elution volume should be 2-5 times the bed volume of the cartridge (Sabik et al., 2000). SPE cartridges are popular, easy to use and work well for many purposes (Fritz, 1999).

Resin loaded disks are produced by the embedment of the sorbent such as C_{18} into a web of polytetrafluoroethylene (PTFE) or glass fiber matrix. Glass fiber disks are thicker and more rigid with faster flow rates. The sizes of the sorbent particles impregnated (8 μm in diameter) in the disks are smaller than those found in cartridges. The short sample path and small particle promote partitioning and allow efficient trapping of analytes with a relatively high flow rate through the sorbent. Figure 1.7 compares the particle sizes and flow path for disk and cartridge forms. For the same bed height for a disk and a cartridge, the disk has many more particles and much more tortuous path of flow, which means there is considerably more surface area available and the kinetics of sorption is much quicker. Hence, a smaller mass of sorbent is required to process a similar volume of sample, permitting the use of smaller volumes of elution solvent (Sabik et al., 2000, Thurman and Snavely, 2000). Moreover, use of SPE disks also gives lower interference levels when compared to conventional SPE cartridges with polyethylene frits (Tolosa et al., 1996).

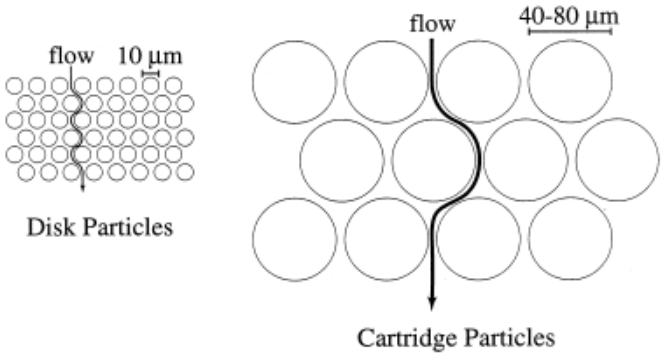


Figure 1.7. Comparison of Particle Sizes for Disk and Cartridge Formats (From Thurman and Snavely, 2000)

The disks are available in different sizes (25, 47 and 90 mm diameter). The most frequently used size is 47 mm, suitable for 0.5-1.0 L of water sample volumes and can be used with flow rates up to 200 mL/min (Fritz, 1999).

The main difficulties encountered with any kind of bonded silica phase are caused by the presence of suspended particles in the sample. The particles of alkyl bonded silica act as a mechanical filter retaining particles of suspended sediment giving a loss in flow rate. This is very inconvenient when large volumes of sample are processed. To solve this problem, acidification to pH 2 can be applied to solubilize insoluble salts of aluminum, magnesium and calcium salts. However, such extreme pH conditions are not recommended as they may alter the chemical stability of target compounds and the performance of disks. Therefore, filtering the water samples containing particulate matter is performed before the extraction (Viana et al., 1996).

1.10.2.3. Solid Phase Extraction Procedure

A typical SPE procedure consists of four main steps: (1) Conditioning, (2) Adsorption, (3) Washing, (4) Elution. In Figure 1.8, these steps are shown for cartridges to visualize the processes occurring, which are same for SPE disks (Fritz, 1999, SUPELCO Technical Resources, 1998).

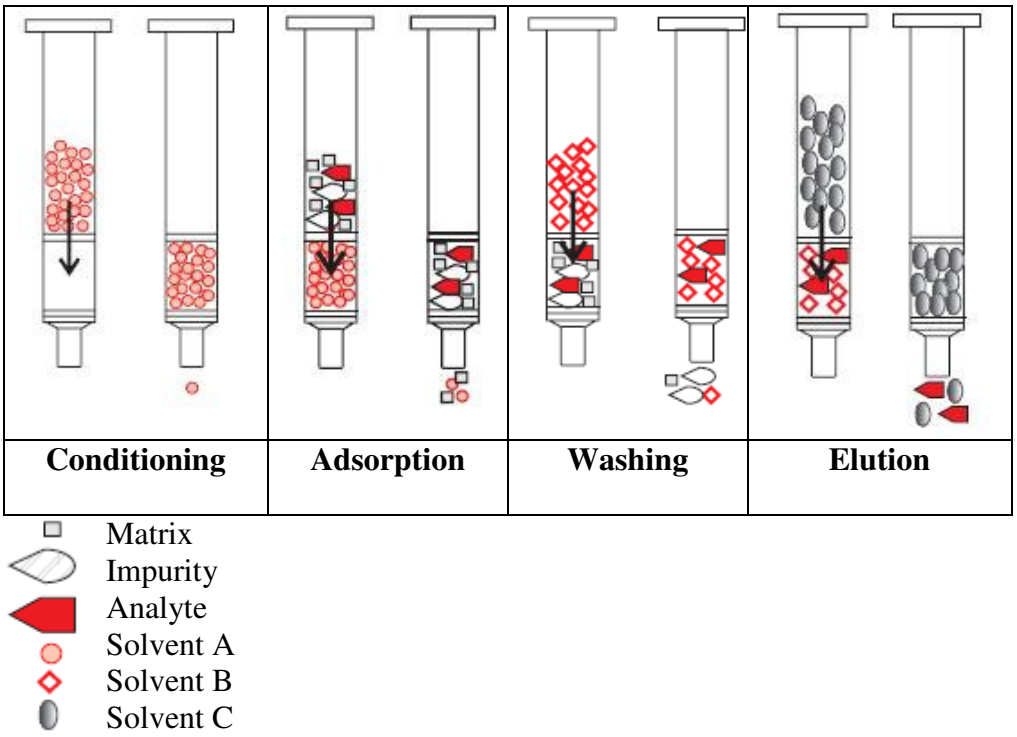


Figure 1.8. Steps for SPE Procedure (from Supelco Technical Resources, 1998)

Before extraction of the analytes, the sorbent bed must be prepared so that it will make intimate and effective surface contact with the sample. Wetting the sorbent by passing a small volume of organic solvent allows the bonded alkyl chains, which are twisted and collapsed on the surface of the silica, to be solvated so that they spread open to form a bristle. This ensures good contact between the analyte and the sorbent in the adsorption of the analyte step. It is also important that the sorbent remains wet in the following steps, oyherwise poor recoveries can result (Dean, 1998).The presence of air prevents efficient interfacial contact between the liquid and solid phases.

The aqueous sample to be extracted is passed through the column or disk under gentle vacuum. After the adsorption of the analytes on the sorbent, it is rinsed with a suitable solvent to remove unwanted extraneous material. The liquid used for rinsing should not elute the analytes (Dean, 1998).

In the elution step, the adsorbed analytes are removed from the solid sorbent and are returned to a liquid phase that is suitable for analytical measurements. The selection of the elution solvent is important, as it should elute the analytes of interest completely using as small volume as possible (Fritz, 1999). Strong and weak elution solvents for adsorbed compounds in SPE are described in Table 1.9.

Table 1.9 Elution Strengths of Solvents (from SUPELCO Technical Resources, 1998)

Polarity			**Solvent**	**Miscible in Water?**
Nonpolar	Strong Reversed Phase	Weak Normal Phase	Hexane	No
			Isooctane	No
			Carbon tetrachloride	No
			Chloroform	No
			Methylenechloride	No
			Tetrahydrofuran	Yes
			Diethyl ether	No
			Ethyl acetate	Poorly
			Acetone	Yes
			Acetonitrile	Yes
			Isopropanol	Yes
	Weak Reversed Phase	Strong Normal Phase	Methanol	Yes
			Water	Yes
Polar			Acetic acid	Yes

Two small aliquots of eluting solvent generally recover the compounds of interest more efficiently than one larger aliquot. Best recovery of analytes can be obtained when each aliquot remains in contact with the tube packing or disk for 20 seconds to 1 minute. Slow or dropwise flow rates in this step are beneficial (SUPELCO Technical Resources, 1998).

1.10.2.4. Applications of Solid Phase Extraction for Pesticide Analysis

SPE is a widely used technique for the extraction of different classes of pesticides from drinking water (Quayle et al., 1997; Ballesteros and Parrado, 2004; Rodrigues et al., 2007), ground water (Vassilakis et al., 1998; Hernandez et al., 2001; Marin et al., 2006), surface water (Wolska et al., 1999; Bagheri et al., 2000; Zhou et al., 2006) and rain water (Coupe et al., 2000; Nyangababo et al., 2005).

Pesticides are routinely determined in vegetables, fruits and food products. SPE is used also for the extraction of different classes of pesticides from liquid foods, such as fruit juices (Khrolenko et al., 2002), wine (Jimenez et al., 2001; Miliadis et al., 1999), and oil (Barrek et al., 2003; Sanchez et al., 2006). Before SPE can be applied to a solid matrix such as fruits and vegetables, a separate homogenization step and often, filtration, sonication, centrifugation are required (Pico et al., 2007). However, SPE can still find application for the pesticide extraction from fruits and vegetables (Stajnbaher and Zupancic-Kralj, 2003; Juan-García et al., 2005).

In the analysis of pesticides, SPE is used not only for extraction but also used for clean up after the extraction of fruits and vegetables (Schenck et al., 2002; Sharif et al, 2006), meat and fatty matrices (Juhler, 1997; Kuivinen and Bengtsson, 2002), soil and sediment samples (Bester and Huhnerfuss, 1997; Dabrowska et al., 2003).

The extraction of pesticides from biological fluids can also be successfully performed by SPE such as urine, serum and blood samples (Lacassie et al., 2001; Pitarch et al., 2001).

1.10.3. Analysis Methods

The use of pesticides leads to adverse effects on both human and environment. Legislations were set in USA, European Union and other countries to regulate pesticide residues in food and food products, drinking water and environment. The legislations and the risks to human health has brought the requirement of the detection of pesticide compounds in a variety of matrices; food, drinking water, ground and surface water, soil, human serum, urine, tissues etc. In addition, the actual state and the transformation products of the pesticides in these matrices should be extensively monitored. It should be noted that, the analytes (pesticides) are often not expected to be detected in the samples and the regulations set very low limit concentrations. Therefore scientists are forced to develop simple, fast, selective, sensitive and reliable sample preparation and analysis systems for the determination of variety of pesticides in numerous types of matrices.

For the analysis of pesticides, multiresidue methods (MRMs), which are capable of simultaneously determining more than one residue in a simple analysis, have been developed. Multi-class MRMs involve residues of various classes of pesticides and selective MRMs concern multiple residues of chemically related pesticides (Ahmed, 2001).

The most common instrumental methods for trace analysis of pesticides involve Gas Chromatography (GC) with specific detectors such as Electron Capture Detector (ECD), Nitrogen Phosphorus Detector (NPD), Flame Photometric Detector (FPD); High Performance Liquid Chromatography with UV and fluorescence detectors; and Mass Spectrometry for both separation techniques. For the analysis of pesticides in different chemical classes, different analytical techniques are widely used and these are summarized in Table 1.10.

Table 1.10. Methods of Analysis for Different Classes of Pesticides

Type of Pesticides	Method of Analysis
Chlorinated Pesticides	GC-ECD, MS
Phosphorus Pesticides	GC-NPD, MS
Nitrogen Containing Pesticides	GC-NPD, MS
Herbicides	HPLC-UV, MS
Carbamates	HPLC-UV, MS

GC is preferably used for the analysis of volatile and thermally stable pesticides, including OCP and OPPs. HPLC is used for the analysis of thermally labile and polar compounds which are not suitable for GC analysis. These include the carbamates, urea- and phenoxyacid herbicides, benzimidazoles, etc (Balinova, 1996). The limitations of HPLC include expensive instrumentation and operation, and the lack of a sensitive universal detector (Ahmed, 2001).

1.10.3.1 Gas Chromatography in the Analysis of Pesticides

Gas Chromatography (GC) is a widely used technique in environmental analysis, due to its high resolution power and selectivity, good accuracy and precision, wide dynamic concentration range and high sensitivity (Santos and Galceran, 2002). The analysis of pesticides residues by GC is well documented, and have been developed for 500-600 different compounds used world wide (Fifield and Haines, 2000). Most standard multiresidue methods are based on GC for determination of pesticide residues (AOAC Int. Official Method 990.06, 2000; AOAC Int. Official Method 985.22, 2000; EPA Method 8081 B,1998; EPA Method 8141 B, 1998).

Compounds which are gaseous or having low boiling points (up to 300ºC) may be separated and determined by GC. The liquid samples to be analyzed are usually prepared in a readily volatile solvent and introduced to GC system with

a syringe into injection port or inlet, heated usually at least 50 ºC above the column temperature. For packed columns, the inlet is a relatively simple device; samples are generally injected directly into a short portion of tubing at the column head, which does not contain any stationary phase. For capillary columns, samples are injected into a tube (glass sleeve or liner) separate from the column in a heated block with an inert gas stream. There, the liquid sample is vaporized instantly (flash vaporization) without decomposition, mixed homogenously with the carrier gas and finally transferred to the column in the vapor phase (Fifield and Haines, 2000).

For a capillary column, required injection volumes are on the order of 0.10 nL to prevent overloading of the column. This is achieved by splitting the carrier-gas stream into two highly unequal parts with the split valve; a small portion is swept to the column and the remainder is vented out. Splitless injection is widely used for trace analysis for which maximum sensitivity is desirable, where the sample is vaporized and slowly transferred onto the column when split valve is closed. For the analysis of pesticides, splitless injection is generally preferred (Santos and Galceran, 2002; Ahmed, 2001).

Split/splitless inlets are available for capillary GC and a schematic diagram is given in Figure 1.12. The GC system used in this study for the analysis of OCP and OPP compounds has this type of inlet.

The selection of the GC stationary phase depends on the nature of the analytes. For the analysis of OCPs, non-polar stationary phases such as DB-1 and DB-5 are widely used. For the analysis of OPPs, the column selection should be carefully done considering the polarity of the analytes. Besides DB-1 and DB-5, DB-170, DB-1701 and other chemically bonded phases available in the market can be used (Ahmed, 2001, van der Hoff and van Zoonen, 1999).

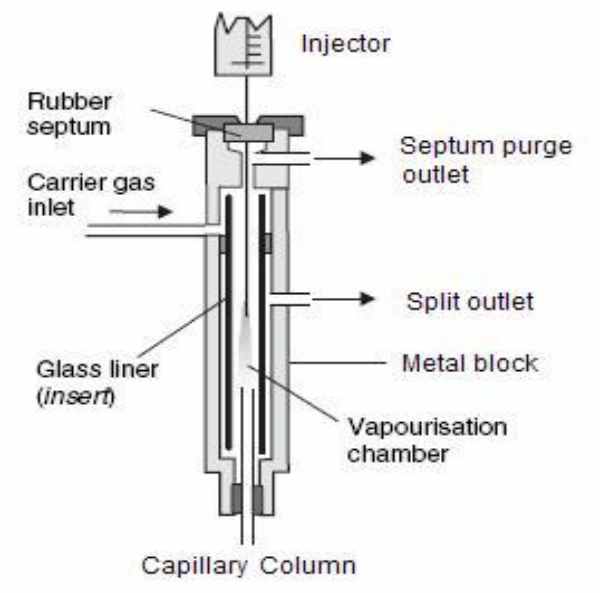


Figure 1.9. Split/Splitless Injector (from Rouessac and Rouessac, 2007)

After the separation of the analytes by GC system, the final determination can be achieved by a series of selective detectors, such as FPD, ECD or NPD. FPD is a highly selective detector for sulfur and phosphorus compounds; it is based on element specific luminescence produced when sulfur or phosphorus compounds are burnt in a hydrogen rich flame. These emission bands for S_2 for sulfur and HPO for phosphorus species are detected at 394 and 526 nm, respectively (van der Hoff and van Zoonen, 1999). The detailed information for ECD and NPD detectors are given in the subsequent sections.

Modern analytical systems use GC with MS detection. GC-MS is the most powerful technique available for the analysis of trace organic materials, with its ability to detect great number of compounds with high selectivity, good sensitivity and its versatility. It combines a high performance separation method with a high performance measuring technique. However, in pesticide analysis, the use of GC-MS is restricted to a confirmation technique as a consequence of its higher detection limits achieved in general with quadrupole analyzers operating in full scan mode (Fernandez-Alba et al., 1998).

1.10.3.2 Electron Capture Detector

In the 1960s, a real advance in pesticide analysis by GC was introduced by the invention of ECD. It is the first selective detector with extremely high sensitivity for halogenated compounds, enabling simultaneous analysis of various chlorinated pesticides, at detection limits hundred times lower than available flame detectors (van der Hoff and van Zoonen, 1999).

This is the most widely used of several detectors which employ a β-ray ionizing source. A typical ECD diagram is shown in Figure 1.10. As the nitrogen gas, as carrier or make-up, flows through the detector a tritium or ^{63}Ni source ionizes the gas forming "slow" electrons which migrate towards the anode wire under an applied potential difference of 20–50 V. The flow of "slow" electrons constitutes a steady current while only carrier gas is present. If a solute with a high electron affinity is eluted from the column, some of the electrons are "captured", reducing the current in proportion to its concentration. The detector is very sensitive to compounds containing halogens and sulphur, anhydrides, peroxides, conjugated carbonyls, nitrites, nitrates and organometallics, but is virtually insensitive to hydrocarbons, alcohols, ketones and amines. The electron capture detector is particularly useful in the analysis of halogen-containing pesticides which can be detected in the sub-picogram range. Although it is the most sensitive available, its linear range is restricted to only 10^2 or 10^3 and it is sensitive to temperature changes. The carrier gas must be exceptionally pure as the presence of oxygen, air and water affect the detector performance, besides causing column bleed. Halogenated solvents should be avoided in sample preparation (Fifield, and Haines, 2000).

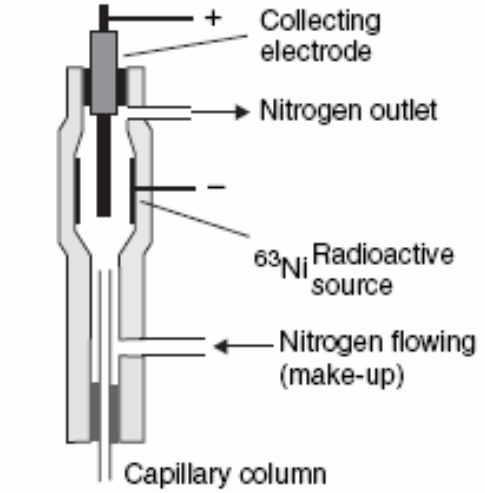


Figure 1.10 Design of Electron Capture Detector (from Rouessac and Rouessac, 2007)

1.10.3.3 Nitrogen Phosphorus Detector

Since its introduction in 1964 the nitrogen phosphorus detector (NPD) (also called as thermionic detector) has been successfully used for the detection of phosphorus compounds, particularly pesticides, and has been developed for the analysis of nitrogen containing compounds such as drugs. The NPD is another sensitive, but, in this case, a specific detector. It is a modified Flame Ionization Detector (FID) with an alkali metal bead (rubidium or cesium silicate) inside a heater coil placed between the flame tip and a collector electrode (Scott, 1996). The diagram of a NPD is presented in Figure 1.11.

A sufficient flame temperature vaporizes the alkali metal salt and generates a stable population of alkali metal ions necessary for the therimonic process. The NPDs use an electrically heated temperature controlled glass bead which contains the alkali as a rubidium silicate which is thermally stable. The combustion products of nitrogen and phosphorus compounds interact with the alkali metal ions by a complex series of reactions, which produce thermionic electrons. These are collected and give rise to the increase in current (Braitwaite and Smith, 1999).

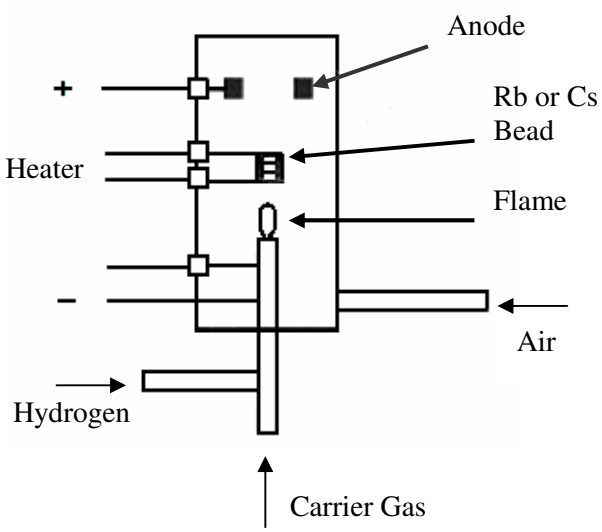


Figure 1.11. Diagram of Nitrogen Phosphorus Detector (from Scott, 1996)

When compared with a standard flame ionization detector, NP detector is approximately 50 times more sensitive to N-containing compounds and 500 times more sensitive to P-containing compounds. The NPD has a linear range of 10^5 (Braitwaite and Smith, 1999).

The main disadvantage of this detector is that its performance deteriorates with time. The alkali used as the bead is usually a silicate and it is demonstrated that the loss in response was due to water vapor from the burning of hydrogen. This converts the alkali silicate to the hydroxide and free silica. Unfortunately, at the normal operating temperature of the bead, the alkali hydroxide has a significant vapor pressure and consequently, the rubidium or cesium iscontinually lost during the operation of the detector. Finally all the alkali is evaporated, leaving a bead of inactive silica. This is an inherent problem with all NP detectors and as a result the bead needs to be replaced regularly if the detector is in continuous use (Scott, 1996).

1.10.4. Quality Control

The Quality Control and Quality Assurance practices in chemical analysis are getting increasingly important for policy makers and the customers who require reliable data from an analysis in decision making such as to take management actions or to have investments.

Obtaining reliable data is extremely crucial for environmental analysis where a set of environmental samples are collected for a specified point at a certain time and the chemical analysis of contaminants is carried out using various techniques in the laboratory. The sampling and analysis are conducted to meet certain objectives that have been formulated to address specific questions and problems. As a result of the analysis, management actions are taken, which include reduction of discharge of contaminants, restrictions to use agricultural chemicals and change in chemical manufacturing methods, etc. These actions need to be based on accurate information; otherwise significant economic costs and damage to environment can occur. To be sure the chemical analysis information is as correct as possible; a system of quality assurance/quality control is needed (Cornell, 2005).

Quality assurance (QA) includes the activities by which it is shown that the analysis meets satisfactory standards by comparison with standards, setting acceptable variability and detection limits, and that appropriate control procedures in place. Quality control (QC) is a management system of laboratory procedures and day-to-day activities to control and assess the results obtained.

Typical QA/QC procedures are as follows (Connell, 2005; EPA 8000C):

1. *Blanks (Field and Laboratory):* These are the samples that are collected or prepared in such a way to represent the levels of contaminants in reagents used in the laboratory, contamination on equipment used for sampling, and so on. The concentration of the analyte in the blank should not be higher than the method detection limit or 5 % of the measured concentration.

2. *Calibration of Equipment and Analytical Procedure:* Sampling or analytical equipment should be calibrated before use and the precision and detection limit should be known. Recalibration must take place when the performance changes o the point that accepted performance criteria can not be achieved and after significant maintenance activities.

3. *Laboratory Control Samples:* Laboratory control sample(s) should be analyzed with each batch of samples processed to verify that the precision and bias of the analytical process are within control limits. The results of the laboratory control sample(s) are compared to control limits established for both precision and bias. These are the samples held by the laboratory of known composition, often from previous analyses.

4. *Standard Reference Materials:* Standard reference materials (SRM) are used as laboratory control samples and are obtained from an external authoritative source that certifies the composition of the material. They should be analyzed as the laboratory control samples.

5. *Replicates:* The duplicate (two) or replicate (more than two) preparation or analysis of the same sample provides the precision associated with these laboratory procedures. To determine the precision

of sample collection procedures, duplicate or replicate samples should be collected during the sampling.

6. *Matrix Spikes:* The chemicals of interest can also be added to in known concentrations to a matrix of the environmental sample, known as matrix spike. The matrix spike sample is prepared and analyzed as the samples and its purpose is to determine the bias resulted from the sample matrix.

7. *Surrogate Recovery:* It is the substance similar to the target analyte(s) in chemical properties and behavior in the analytical process, but which is not normally found in the environmental samples. This substance is added to the sample aliquot in known amount(s) before sample preparation and is measured as analyte. The purpose of a surrogate is to monitor sample preparation performance with each sample. For example, for the extraction of organic compounds the percent recoveries of surrogate compounds are calculated for each sample and ongoing recovery of extraction procedure is monitored. The recovery of the surrogate must lie within 99 or 95 % confidence interval around the mean surrogate recovery.

8. *Consistency of Analysis:* The results from analysis of a known reference material can provide a useful control on the analysis results. The results of the analyses on a regular basis over time can be plotted as a chart. When a significant number of analyses have been carried out, the charts can indicate the deviations from control limits.

Laboratories can contact other laboratories conducting the same analyses and organize an interlaboratory calibration program. Laboratories can be registered or accredited by appropriate organizations through on-site evaluation by independent assessors (Connell, 2005).

1.11. Literature Review

In the literature, the studies about the subject of "pesticides" are well documented and widely presented as these useful man made chemicals have adverse effects on human health and environment. The materials cover books, data bases, analytical methods, and journal articles. The books and data bases involve the general information about the pesticide properties, environmental fate, toxicity and their use statistics. The analysis of the pesticides have been performed not only in research laboratories, but also performed routinely in governmental agencies for many decades, due to legislative actions. Therefore, there are well established analytical methods for pesticide residue analysis in different matrices. It should be noted that, some of these sources can be accessed easily through the internet, including general information, pesticide properties data bases, regulations, test methods, fact sheets for pesticide use, safety and etc.

The subjects of the publications can be divided into four groups; analytical chemistry studies for food, environmental and biological samples, environmental studies (determination of pesticides as pollutants, monitoring, modeling, fate and removal processes), health effects (including epidemiology and toxicity studies) and pesticide use policies (including the statistics, legislative actions and regulations, discussions and comparisons of policies). In the scope of this work, among the wide range of literature resources, the journal articles will be summarized only of which are about the analytical chemistry studies on the determination of pesticides in water samples and environmental measurements in aqueous matrices.

The studies about pesticides performed in Turkey will be summarized separately in another sub-section. Only the studies related with the pesticide determinations in environmental samples and analytical chemistry will be presented.

1.11.1 Analytical Chemistry Studies

Analytical chemistry studies involve the method development / validation for extraction and determination of pesticides. SPE is the most widely used technique for the extraction of pesticides from water samples in the literature. Although in most of the studies cartridges were used (Aguilar et al., 1997; Wolska et al., 1999; Vidal et al., 2000; Hernandez et al., 2001; Sosa et al., 2003; Ballesteros and. Parrado, 2004; Rubio et al, 2007), the application of the disks were also documented (Albanis et al., 1998; Golfinopoulos et al., 2003; Leandro et al., 2006).

Aguilar et al. (1997) has determined 17 pesticides in different chemical groups, containing OCPs (including α, γ and δ HCH, heptachlor and heptachlor-endo epoxide, aldrin, dieldrin, α and β endosulfan) and OPPs (including malathion) via SPE-GC-ECD and SPE-GC-MS systems. For SPE, cartridges with ethyl-vinylbenzene-divinybenzene copolymer were used. The parameters affecting SPE process were optimized; elution solvent (first hexane, then ethyl acetate), pH of the medium (~6), addition of NaCl (15 g/L) and sample volume (500 mL). The percent recoveries were ranging between 40-106 %. It was shown that, GC-ECD system has better limit of detection (LOD) values (0.2-1.0 ng/L) than GC-MS system with SIM mode (20-100 ng/L).

Pocurull et al. (1998) has determined almost the same 17 pesticides in water by on-line coupling of SPE to GC-MS through an on-column interface. A precolumn packed with polystyrene-divinylbenzene copolymer was selected for the SPE process. The parameters affecting the transfer of the analytes from the precolumn to the GC system (flow-rate, temperature and solvent vapor exit time) were optimized. The use of the MS detector under SIM acquisition enabled the analytes to be quantified at sub µg/L levels with only 10 mL of sample, and the LODs were between 2 and 20 ng/L. The linearity of the

response was obtained in the range of 0.01-10 µg/L. The reproducibility of the measurements was lower than 21 % for SIM mode. The method was applied to the determination of the pesticides in tap and river water samples.

SPE-GC-ECD, SPE-GC-MS (in SIM mode) and GC-MS-MS systems were employed for the identification of 12 pesticides, covering OCPs (including dieldrin) and OPPs (including parathion-methyl, fenitrothion and malathion) in water samples by Vidal et al. (2000). C_{18} cartridges were used and the SPE procedure was optimized in terms of the breakthrough volume and the saturation concentration. Different volumes (100-600 mL) of the water samples were spiked with pesticides standards to determine breakthrough volume and 500 mL was chosen as the optimum volume of the sample to use. The saturation concentration was not reached to the tested highest standard (1600 ng/L) for most of the pesticides, except dieldrin (200 ng/L) and buprofezin (400 ng/L). The LOD values provided by three analysis systems were comparable being in the range of ng/L and GC-ECD system were generally lower and GC-MS system has mostly higher LODs than other systems. To study the extraction efficiency of the analytes, three 500 mL aliquots of Milli-Q water was spiked to contain100 ng/L of each target pesticide. Good recoveries (76-122 %) were obtained for all pesticides, except captan (142 %) by using GC-ECD system.

An automatic method for the determination of 13 OCPs in water samples was developed by Colume et al. (2001). The analytes were preconcentrated onto a C_{18} column and subsequently eluted with ethyl acetate. GC-ECD was used for separation and selective detection. The LODs of the analysis was ranging from 0.01 to 0.1 ng/mL, and the RSD values for the measurements were between 4 and 6 %. The average recovery at a fortification level of 2 ng/mL was 92%. The method was used to screen OCPs in natural waters collected near agricultural areas and also to tap waters.

Another recent publication is about the use of SPE in the analysis of 8 OPPs (including diazinon, parathion-methyl, malathion, fenthion and methidathion) including in natural and drinking waters (Ballesteros and. Parrado, 2004). In this study, the pesticides were extracted through a continuous system consists of two injection valves, a pump and an adsorbent column where the pesticides were preconcentrated and subsequently eluted with ethyl acetate. Various sorbent materials were assayed and C_{18} was found to provide the best results, with percent recoveries ranging from 96.8 % to 99.5 %. The whole extract was collected in a glass vial and introduced to GC-NPD system for the analysis. Here, the authors claim that, this system can easily be coupled to GC with the introduction of an injection valve, becoming an on-line system.

SPE-GC-MS system was used for the determination of 96 pesticides, with OCP (including aldrin, HCH isomers, DDE, DDT, dieldrin, endosulfan, β-endosulfan and endosulfan sulfate, heptachlor, its endo epoxide and methoxychlor), OPPs (including azinphos-methyl, chlorpyriphos, fenitrothion, malathion, pirimiphos-methyl), and their transformation products in drinking water (Leandro et al., 2006). The SPE medium was C_{18} disks and MS was operated in SIM mode. The percent recoveries were in the range of 60-116 % and the RSD values were lower than 20 %. These results demonstrate that, SPE can be successfully applied for the simultaneous analysis of various classes of pesticides.

SPME is the most frequently reported technique in recent years due to its advantages over SPE and LLE as being a solvent free, fast, simple, robust and easily to automate technique. Dugay et al. (1998), Valor et al. (2001), Gonçalvez and Alpendurada (2002a), and Perez-Trujillo et al. (2002) has studied the effects of different SPME coatings on the extraction efficiencies of different classes of pesticides, including OCPs, OPPs, triazines and pyrethroids in water samples. These studies have shown that, fibers containing divinylbenzene (DVB) provides higher extraction efficiencies. Gonçalvez and

Alpendurada (2002b) have later detailed their work on fibers and by comparing three different DVB containing coatings, they have found the more suitable fiber for each class of pesticides. Afterwards, the same authors have developed an analytical procedure for multipesticide residue analysis in water samples using SPME-GC-MS-MS technique which has quantitative and qualitative capabilities (Gonçalvez and Alpendurada, 2004).

Dong et al.(2005) has used headspace (HS) SPME for the determination of 11 OCPs by GC-ECD whereas Sakamoto and Tsutsumi (2004) has demonstrated that HS-SPME coupled to GC-MS system can be applied for the analysis of multi-class pesticides (174 pesticides) in aqueous samples.

Lambropoulou et al. (2000) has compared the two methods for the analysis of 10 OPPs (including parathion-methyl, fenitrothion, malathion, fenthion, bromophos-methyl, bromophos-ethyl and fenamiphos) in natural waters using SPE, with C18 disks (requiring 1000 mL of sample) and SPME with polyacrylate (PA) fiber (requiring 2.5 mL of sample). The analysis system was GC-FPD. The LODs obtained were similar for both methods; 0.01-0.07 μg/L for SPE and 0.01-0.05 μg/L for SPME. The recoveries of SPME (86.2-119.7 %) were slightly higher than the recoveries provided by SPE (60.7-104.1 %). The authors have proposed the use of SPME technique as an alternative technique to SPE, especially when the sample volume is limited. In the same work, the analysis system, GC-FPD has compared with GC-MS in SIM mode and it was found that GC-FPD has slightly lower LODs than GC-MS system. The proposed methods were applied to the trace level screening determination of insecticides in river water samples originating from different Greek regions.

The SPE can also be applied in LC based procedures. A very rapid, multi-residual, sensitive and specific procedure for determining 35 pesticides in environmental ground and surface water in was proposed by Hernandez et al. (2001). The method was based on the use of SPE combined on-line (LC)

electrospray (ESI) tandem mass spectrometry (MS–MS). Simultaneous target analysis of 29 pesticides (1 fungicide, 16 insecticides, 10 herbicides and 2 acaricides) and 6 metabolites with positive or negative ionization was performed by the direct injection of only 1.3 mL of filtered water sample, with a total analysis time of 18 min. A C_{18} cartridge was used for the extractions and the SPE–LC–MS–MS method was validated. The percent recoveries were in the range of 65-116 % for ground and 50-115 % for surface water samples at 100 ng/L fortification level. The LOD values were between 0.5-60.1 ng/L, and the method was stated to be precise with RSD values lower than 15 %.

1.11.2 Environmental Measurements in Aqueous Samples

Senseman et al. has studied the pesticide pollution of groundwater (1997a) and surface water (1997b) in Arkansas, USA. The groundwater study involves the 2-year monitoring of selected sampling sites, where pesticides were mixed, loaded or rinsed. The authors aimed to assess the temporal groundwater quality, regarding pesticide contamination at these point sources. At the beginning of the study, the information about the pesticide use has been obtained from volunteer farmers by questionnaires. The 16 sampling sites located in 11 countries in Arkansas were representing varying agricultural situations, applications and management schemes. 80 samples were collected 5 times in 1990-1991 period. Samples were extracted with SPE disks and analyzed by GC-ECD, HPLC-UV for 17 pesticides (including azinphos-methyl and parathion-methyl) commonly used. The percent recoveries were ranging from 82 % to 98 %, with RSD values below 7 %. The limit of quantification (LOQ) values were between 0.1-1.0 µg/L. Only 14 samples were detected to contain 8 different pesticides, single or multiple and only three detections were above advisory levels. The pesticide's proximity to the wells during mixing, rinsing or loading was considered to be a greater influence on temporary contamination than the chemical or site specific characteristics.

In the surface water study, the water quality of selected lakes, rivers and streams of Arkansas was measured with respect to pesticides. The study was based on monitoring of 59-62 sampling sites for 3 year period (1989-1991). Totally, 485 samples were collected in 8 sampling time. The same analytical methods were used for the detection of the same 17 pesticides, with the same LOQ values in the previous study. The percent recoveries were ranging from 72-98 %, with RSD values below 6 %. 256 samples were detected to contain 14 different pesticides, alone or mixed and a total of 5 % of detections were above the health advisories. Spring and summer samples provided 73 % of the detections and rivers/streams were responsible for 62 % of the detections.

The studies performed to evaluate pesticide exposure both in Portuguese surface and ground water, from 1983 to 1999, showed that some of the monitored pesticides were present at different concentration levels (Cerejeira et al., 2003). During the study period, different extraction and analysis methods have been used; liquid-liquid extraction, SPE, SPME, GC-ECD, GC-NPD, GC-MS for both analysis and confirmation. Lindane, α-BHC, β-BHC, δ-BHC, hexachlorobenzene, heptachlor, heptachlor-epoxide, aldrine, DDE, DDD, endrine, dieldrine, α- and β-endosulfan, dimethoate, diazinon, atrazine, simazine, molinate, chlorfenvinphos, propanil and its metabolite 3,4-dichloraniline (DCA), ethyl-parathion, alachlor, metolachlor, MCPA, bentazone and 2,4-D were monitored in surface water. All of the mentioned pesticides were detected except the HCH isomers, cyclodiens, DDT and derivatives, probably due to their agricultural interdiction. In some of the samples, the concentrations of pesticides were higher than the maximum admissible concentration (0.1 μg/L); 32 μg/L (for chlorfenvinphos izomers), 48 μg/L (for molinate). Residues of each pesticide showed a seasonal variation of concentration with the highest levels registered in spring, after pesticide treatments. The monitored herbicides (alachlor, atrazine, metolachlor, metribuzine and simazine) in ground water were all detected at different exposure levels in several agricultural areas. The herbicides more frequently

detected were atrazine (64%), simazine (45%) and alachlor (25%). As in the surface waters, there were some concentration extremes for ground water samples. For example, alachlor, atrazine, metolachlor, metribuzine and simazine have reached the maximum values of 13, 30, 56, 1.4 and 0.4 μg/L, respectively.

Golfinopoulos et al. (2003) has applied SPE with C_{18} disks for the determination of 20 OCPs (including all of the OCPs studied in this work) in the surface waters of Northern Greece. After the extraction, the final determinations were performed by a GC-ECD system. The percent recoveries, for 0.4 μg/L fortification level, were ranging from 50 % (for aldrin) to 145 % (for endosulfan sulfate). The RSD values were lower than 14 %, and the highest LODs was obtained was 0.020 μg/L for β-HCH. The SPE-GC-ECD system was used for the seasonal monitoring of OCPs in four river and five lake samples for a period of two years, 1996-1998. The most commonly detected OCPs were isomers of HCH, aldrin, dieldrin and endosulfan sulfate. In some cases the concentrations were higher than the limit value of 100 ng/L set by European Union (EU Council Directive 98/83/EC, 1998), especially for HCH and aldrin. The occurrences of these compounds in Greek surface waters were attributed to intense agricultural activity and transboundary pollution. This study clearly shows the effectiveness of SPE technique for the routine determination of OCPs in environmental water samples.

A survey undertaken in Kanpur, northern India, has shown the presence of high concentrations of both organochlorine and organophosphorous pesticides in the surface and ground water samples (Sankararamakrishnan et al., 2005). Liquid–liquid extraction of followed by GC-ECD was used for the determination of these compounds. Percent recoveries were ranging between 58.5 % (parathion-methyl) and 110.8 % (γ-HCH) and the maximum LOD was 0.04 μg/L for malathion. Among the various pesticides analyzed, high concentrations of γ-HCH (0.26 μg/L) and malathion (2.61 μg/L) were detected in the surface water

samples collected. In the ground water samples collected from the various hand pumps located in agricultural and industrial areas, apart from γ-HCH and malathion, dieldrin was also detected. The maximum concentration values of γ-HCH, malathion and dieldrin were 0.90, 29.84 and 16.23 μg/L, respectively. Pesticides like DDE, DDT, aldrin, ethion, parathion-methyl and endosulfan were not detected in both the surface and ground water samples. However, Shukla et al. (2006) has found the concentrations of DDT, lindane, α- and β-endosulfan in ground waters of Hyderabad city of India higher than the qualitative target set by European countries. This was explained by the possible transfer of OCPs from agricultural and health protection activities carried out and in near Hyderabad. These results presented were alarming for the health of the human beings in the region.

The levels of 13 OCPs in surface water and sediments from Quintang River in East China were investigated by Zhou (2006) to evaluate their potential pollution and risk. In 2005, a total of 180 surface water samples from 45 points and 48 sediment samples from 19 points were collected along the river in four seasons. For the extraction of water samples, SPE cartridges and for the sediment samples, ultrasonic extractions were used. The analyses were performed by GC-ECD system. The percent recoveries for water samples were between 76-87 %, RSD values were below 6 % and LODs were lower than 0.15 ng/L. The concentrations of DDT isomers were much lower than HCH isomers and other OCPs. Although they have never been used in large amounts the region, aldrin, dieldrin, endrin, heptachlor and heptachlor epoxide has detected in most of the water samples. This has been explained by the atmospheric transport from developing countries around the tropical belt. The concentrations of OCPs were ranging from 7.64 to 269 ng/L. The maximum concentrations were observed for the sampling points around the center of the river, which were subjected to farmland runoff along the riverside. The seasonal variations indicate higher concentrations in July and October, which was explained by wet deposition and the transport through soil eroding to

waters with much rainfall in these seasons. In contrast, the concentrations of OCPs in sediments collected in spring were higher than summer and autumn samples. The dominance of γ-HCH in the most sediment samples reflected the recent use of lindane.

1.11.3. Pesticide Studies in Turkey

Barlas (1999) has determined the OCP residues in water, sediment and fish (adipose tissue) samples in upper Sakarya River basin. The samples were collected once in four months between 1995 and 1996. A GC-ECD system was used for the analysis. It was observed that the sampling points subject to discharge of agricultural wastes were more polluted than others. The degradation products were observed in higher concentrations than parent compounds, in all types of samples. A seasonal trend was also observed for the different types of samples, with higher concentrations in fall and summer months. This variation was related to the application and transport processes. The OCP levels in sediment samples were higher than water quality criteria. The DDT metabolites were dominant in fish tissue, indicating OCP pollution in the food chain.

Turgut (2003) was determined the residues of OCPs and heavy metals in surface water of Küçük Menderes River, Turkey. The samples were collected between 2000 and 2002 from selected three sampling points. After liquid-liquid extraction, OCPs were determined by means of GC-ECD system. The results have shown that Küçük Menderes River was still polluted with OCPs despite the bans on their use over a long time. The concentrations of OCPs have shown a seasonal trend, but the tendency was not same for all pesticides. DDD was observed to be the dominant among DDT compounds. The observed concentrations were mostly lower than the data in literature.

The OCP concentrations in surface sediments, sea and river water were studied in mid-Black Sea region of Turkey by Bakan and Arıman (2004). The sea sediment and water samples were collected from 6 points in December 1998, and river samples were collected from 8 points in April 1999-2000. The sampling points were located along the coast of Samsun city, stated to be hotspots of pollution. Soxhlet and liquid-liquid extractions, followed by a clean-up step, were used to recover the pesticides from sediment and water samples, respectively. The quantification of the analytes was achieved via a GC-ECD system. Among 15 target analytes, only aldrin, δ-HCH and heptachlor epoxide were determined in water samples. The residues of DDT, DDD, DDE, aldrin, lindane, dieldrin, heptachlor epoxide, α- and δ-HCH were detected in sediment samples. The high frequency of observation of aldrin in both types of samples has shown the widespread contamination among Turkish Black Sea coast. The concentrations of OCPs in sediments were compared with literature data and the concentrations of total HCH and DDT isomers were reported to be higher than the values recorded for different parts of the world.

Erkmen and Kolankaya (2006) have determined the OCP residues in surface water, sediment and fish samples in Meriç Delta to assess the extent of contamination and evaluate the toxicological significance of the residues. The samples collected from eight points from May 2002 to August 2003 were extracted with liquid-liquid and soxhlet extraction methods, and the concentrations of 20 OCPs were determined by a GC-ECD system. The most commonly observed OCPs in the samples were α-HCH, β-HCH, DDE, DDT, β-endosulfan, heptachlor endo epoxide and endrin ketone. In general, the OCP concentrations in fish samples were generally higher than the values for water and sediments. The predominance of α-HCH and β-HCH in all types of samples were attributed to the use of HCH in the region. The high concentrations of DDE in sediment samples were related to historical use of DDT. It was concluded that, Meriç Delta is contaminated with low levels of OCPs.

An analytical method for the simultaneous determination of four fungicides (folpet, chlorothalonil, quinomethionat, tetradifon) and one herbicide (trifluralin) in fruit juices has been developed by Topuz et al. (2005). C_{18} solid-phase extraction cartridges were used for the preconcentration of target pesticides form 25 g apple, cherry and peach juice samples. The pesticides were separated and quantified by HPLC-DAD system. The LOD values were between 0.5–1 μg/kg. Recoveries from spiked samples were ranging from 93.8% to 99.5% and % RSD values were less than 3.4% in the concentration range of 1–16 μg/kg. The developed method has been tested on canned pure apple, cherry juices and peach nectar manufactured in Turkey. The pesticide residues in these samples were below the limits of detection.

Yenisoy-Karakaş (2006) has developed rapid extraction methods for the determination of 16 OCPs in fresh vegetables by applying ultrasonic extraction with dichloromethane and ethyl acetate, followed by florisil clean-up. It was observed that the extraction efficiencies were better with ethyl acetate, being 78-107% for cucumber samples. The procedures were validated with the parameters of accuracy, precision, recovery, detection limits and selectivity. The result has shown that the methodologies developed can be an alternative for laboratories where new extraction techniques, such as SPE, SFE and SPME, are unavailable. The author has further calculated the uncertainties of the measurements. The major uncertainty sources for two methods were decided as standard preparation and repeatability, final volume of the extract, sample weight and recovery. The expanded uncertainties (with a coverage factor of 2) were ranging between 5.2 and 16% without including the recovery correction. When this factor was included, the expanded uncertainties were ranging between 6.4 and 21%. It was recommended to use recovery data of different types of samples separately to correct the results.

1.12. Objectives of the Study

The aim of this thesis can be summarized as follows:

- Setting up a quality control/quality assurance program for pesticide analysis in water samples.
- Optimization of SPE technique for pesticide extraction from ground and surface water samples.
- Optimization of GC-NPD and GC-ECD systems for the analysis of organophosphorous and organochlorine pesticides, respectively.
- Analysis of the pesticides in ground and surface water samples of Kumluca region with the desired quality control practices.
- Determination of the major components of uncertainties in the measurements.
- Study of the general pattern of total, organophosphorous and organochlorine pesticide concentrations, together with their occurrences in Kumluca surface and ground water samples.
- Determination of the extent of pesticide pollution in the region.
- Study of seasonal trends in pesticide pollution.
- Study of the spatial distribution of pesticide pollution in the region.

CHAPTER 2

EXPERIMENTAL

In this chapter the study area, the field works, instrumentation, analysis methods and extraction procedure will be explained.

2.1. Study Area

The study area covers a part of Kumluca-Finike plain, in Antalya city. Antalya has a population of almost 1.7 millions. Agriculture and tourism with small to medium scale industry are the major means of subsidence in the region. Antalya is the leading region for tourist attraction in the country. On the average, 6 million tourists visit Antalya city and surroundings every year. The 20 % of the city area is used for agriculture (Antalya Governorship, n.d.). Agricultural activities are based on fresh vegetables and orchards. Large areas to the west of the city are orchards and vegetable fields. Mostly tomatoes, green pepper and eggplant are cultivated in the greenhouses. These agricultural areas with greenhouses constitute 33 % of the greenhouses in Turkey (Antalya Agricultural Master Plan, 2002).

The Kumluca-Finike plain is in between 36˚00'-37˚00' latitude and 30˚00'-31˚00' longitudes. The total area of the plain is 102 km^2, of which 56 km^2 belongs to Finike and 46 km^2 belongs to Kumluca districts. The study was mostly concentrated in Kumluca where there are intensive greenhouse activities. Kumluca, lies at the Mediterranean cost 90 km west of Antalya city.

The district is surrounded by the south edges of West Taurus mountain chain and Mediterranean Sea. In Kumluca, there is no industrial activity but two settlement centers exists; namely Kumluca and Finike. The economy is based on agriculture with mostly greenhouses and than the citrus gardens being the main investments. The agricultural production in the region is so important that it makes 1/3 of total country agricultural production (Antalya Agricultural Master Plan, 2002).

There were approximately 300 dug wells in the plain, in 1978 according to General Directorate of State Hydrologic Works. However, currently the number is about 3000 according to the regional governmental authorities (personal communication). The well depths change between 10-15 m and the groundwater depths are between 24-180 m (Günay, 2003). According to General Directorate of State Hydrologic Works (1978) in Kumluca, the precipitation events change the groundwater depths by 4-5 m in coastal parts, where the change is 1-2 m for the inner parts of the plain. The flow of groundwater is generally in the direction of north to south.

In Kumluca, the total amount of water withdrawn from the groundwater sources for drinking and potable water is 3.34×10^6 m^3/year (Data from Turkish Statistical Institute, 2004). In Finike, the groundwater recharge and discharge rates are 56×10^6 m^3/year, whereas in Kumluca, the rates are 8×10^6 m^3/year. In the plain, the streams, Göksu (Karasu) and Alakır have discharge rates of 4.5 and 2.3 m^3/s, respectively (Günay, 2003).

In Kumluca, 17 000 ha area is used for agriculture. Table 2.1 summarizes the land use and agricultural production in the region. Although most of the agricultural area is used for cereal production (31 %) for the cultivation of mainly wheat, barley and corn, the fruits and vegetables are main products. The

vegetables grown are tomato, pepper and eggplant in descending order, and they are mostly cultivated in greenhouses (98.8 %) rather than open fields. The citrus fruits make up 94 % of the total fruit production. The other agricultural products are industrial plants, indoor plants, feed and oil-grains (Data from Turkish Statistical Institute for 2000).

Table 2.1. Land Use and Agricultural Production in Kumluca (Data from Turkish Statistical Institute for 2000)

	Land Use (ha)	**Production (tons)**
Cereals	5 250	11 620
Fruits	3 933	129 600
Vegetables	3 744	476 000
Others (with fallowing)	4 073	2 610

For many years, fertilizers and different classes of pesticides, including chlorinated and phosphorous pesticides, have been applied in the region. These chemicals contaminated the air, soil and ground water for many years. This is the first study for the determination of pesticide pollution in the region.

2.2. Sampling Strategy

In environmental studies, the sampling site selection is the most important step, in order to achieve the goals of the study. The samples should represent the entire study region and the data obtained should be informative and reliable enough for further evaluations and decisions.

To investigate the study region, in July 2004, first field trip to Kumluca has been arranged and the sampling strategy was developed. Accordingly, the study region was determined covering 40 × 36 km area and it was divided into grids and in total, 40-50 sampling points were defined. As the north of the

region is mountainous, most of the sampling points were selected in south part, where the agricultural activities are mostly concentrated.

In this field trip, it was recognized that, not only the ground waters but also the surface waters should be studied to evaluate the water pollution in terms of the pesticides. The surface waters were also subject to pollution and their discharge to sea could pose a risk to the Mediterranean Sea.

A photographical image (from Google Maps) of the region is given in Figure 2.1. In this figure, each sampling point can be seen one by one. Moreover, in the figure, the intense greenhouse constructions can be seen near the coast, spreading through the north.

Two sampling periods were decided for pesticide analysis, spring and fall period of the year. In spring period, the pesticides are applied heavily because of increased production at this season, so this period represents the polluted period of the year. In fall season, the pesticides are not applied heavily and for most of the greenhouses the crops are removed to left the soil rest. Therefore the samples would represent the background levels of the pesticides.

The identities of OCPs to be quantified were decided from literature, and most common 17 OCPs were selected. For OPPs, the crops cultivated in greenhouses in Kumluca and the pesticides used for that crops were investigated. Information from Protection and Control General Headquarters and from City Agriculture Headquarter of Antalya was obtained for the most common OPPs in the region and 14 of those were decided as analytes.

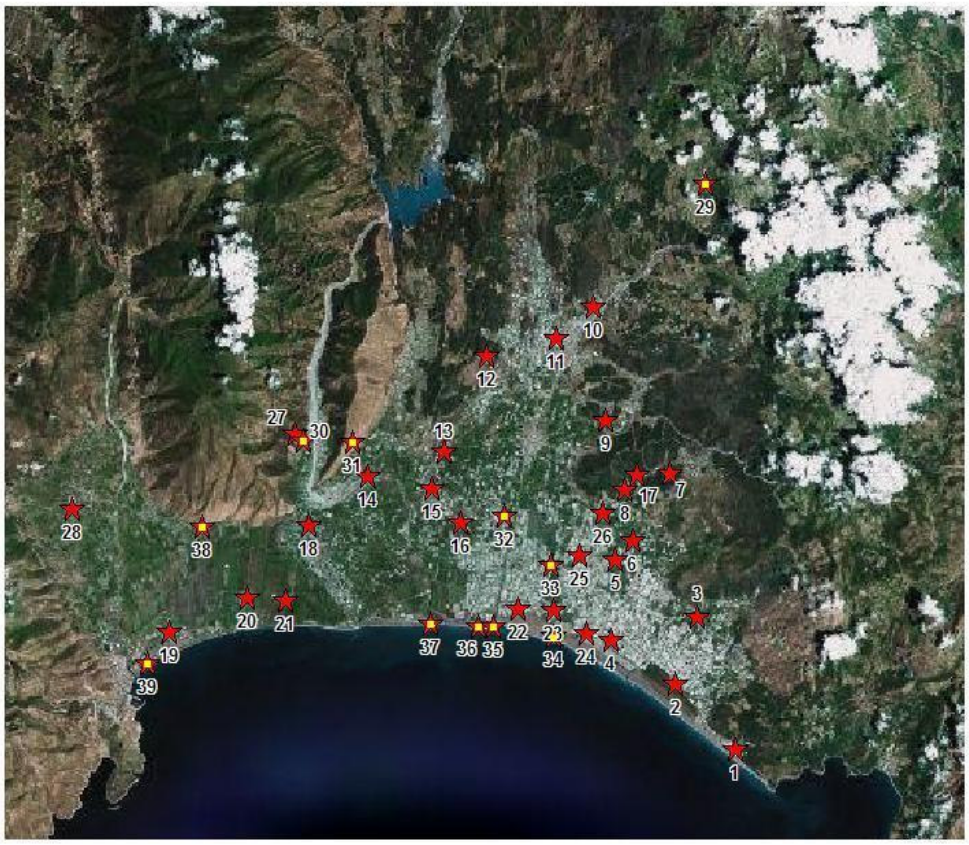


Figure 2.1. Sampling Points

2.3. Sample Collection

Sampling points were located using the geographical positioning system (GPS). At the groundwater sampling sites, the wells were flushed for 3 min before sample collection. Water samples were collected into 1 L amber glass bottle, which were previously cleaned as explained in Section 2.4.2. The pH, salinity and conductivity of the water samples were measured at site and the samples were kept at 4°C until the analysis. The pH values of the samples were not altered as the addition of acids or bases may affect the target analytes. It should be noted that the pH of the samples were in the extraction pH range (0-10) of the disks, as stated by the manufacturer.

During each sampling program, field blank samples were prepared by deionized water at 5 sampling points.

The ground waters were sampled from the wells of the greenhouses. The well waters which were sampled are being used for irrigation purposes only and not for human consumption or drinking. The surface waters were collected from the surface of rivers in the region, from the source, from mid-point and where they reach to sea, aiming to follow the discharge pattern of the pesticides to the sea.

The first sampling program was performed for spring season samples, between May 4-6, 2005 at 39 points. Ground water samples were collected from 28 wells. In total, 49 bottles of groundwater samples were collected, with two replicates for 21 points. The surface water samples were collected from 11 sampling points with two replicates except for one sampling point.

The fall season samples were collected between October 10-11, 2005 from 38 sampling points, 11 of which were surface waters. Almost all samples were collected as two replicates except for 6 wells.

The sampling stations, together with the information about the points are given in Table 2.2 and Table 2.3 for ground and surface waters respectively.

Table. 2.2. Sampling Stations for Ground Water Samples

S. Pt	Site	Agr. Structure	Well Depth (m)	Coordinates	
				N	E
1	Aktaş	GH	10	36º 16.943'	30º 21.297'
2	Pamukalanı	GH	7	36º 18.082'	30º 20.181'
3	Ilıca	GH	28	36º 19.233'	30º 20.787'
4	İncekum	GH	6	36º 18.765'	30º 18.890'
5	Çörüş	GH	8	36º 20.194'	30º 19.161'
6	B.Orta M.	GH + O	10	36º 20.542'	30º 19.561'
7	Beşikçi Uç	GH	13	36º 21.727'	30º 20.514'
8	Beşikçi	GH	22	36º 21.405'	30º 19.519'
9	Sarıkavak	GH	19	36º 22.621'	30º 19.263'
10	Toptaş	GH	15	36º 24.650'	30º 19.115'
11	Kanlıkavak	GH + O	24	36º 24.038'	30º18.350'
12	Sarıcasu	GH	10	36º 23.697'	30º 15.864'
13	Hacıevler	O	9	36º 21.972'	30º 15.742'
14	Salur	O	10	36º 21.495'	30º 14.071'
15	Hızırkahya	GH	15	36º 21.327'	30º 15.401'
16	Çaydağıldığı	GH	20	36º 20.750'	30º 15.938'
17	Şirlengiç	GH	9	36º 21.688'	30º 17.800'
18	F.Yarbaşı	GH	9	36º 20.615'	30º 12.704'
19	F. Kum Mah.	GH	10	36º 18.686'	30º 09.484'
20	Meysan	-	6	36º 19.318'	30º 11.238'
21	F. Orta Mah	O	12	36º 19.286'	30º 12.048'
22	Karşıyaka M.	GH	55	36º 19.273'	30º16.968'
23	Bağlık	GH	100	36º 19.287'	30º17.722'
24	Resiller M.	GH	90	36º 18.886'	30º18.375'
25	Çörüş	O	75	36º 20.248'	30º18.414'
26	Şirlengiç	GH	60	36º 20.993'	30º18.990'
27	F. Hasköy	GH	100	36º 22.202'	30º12.606'
28	F. Turunçova	GH	100	36º 20.764'	30º07.679'

S. Pt: Sampling point, GH: Greenhouse, O: Orchard

Table 2.3. Sampling points for Surface Water Samples

S. Pt.	Site	Area Description	Coordinates	
			N	E
29	İncircik	Spring	36º 26.788'	30º21.910'
30	Alakır Stream	Mid. Point	36º 22.093'	30º 12.771'
31	Göksu Stream	Spring	36º 22.104'	30º13.805'
32	Gavur Stream	Mid. Point	36º 20.877'	30º16.902'
33	Akmaz	Mid. point	36º 20.066'	30º17.768'
34	Akmaz Deresi	Discharge point to sea	36º 18.813'	30º 17.682'
35	Gavur Çayı	Discharge point to sea	36º 18.960'	30º 16.422'
36	Göksu Çayı	Discharge point to sea	36º 18.961'	30º16.110'
37	Alakır Çayı	Discharge point to sea	36º 18.978'	30º15.078'
38	F. Zengeder	Spring	36º 20.523'	30º10.429'
39	F. Tatlısu	Discharge point to sea	36º 18.117'	30º08.958'

2.4. Reagents and Materials

C18 Solid Phase Extraction disks (ENVI discs) were purchases from Supelco. A Millipore filtration apparatus was used with a vacuum pump. All the solvents used were chromatographic grade and purchased from Merck Company.

The organophosphorus pesticide standards were purchased from Dr. Ehrenstorfer (Ausburg, Germany) as certified neat standards. The stock solutions were prepared as 1000 µg/mL in acetone. Working standard solutions were prepared by combining them and diluting with acetone. For chlorinated pesticides, certified standard solution 1000 µg/mL (in toluene/hexane) of 17 target pesticide was also purchased from Dr. Ehrenstorfer (Ausburg, Germany). The working standard solutions were prepared in acetone with appropriate dilutions. Hamilton gas-tight glass syringes (500, 100 and 10 µL) were used for the preparation of the standard solutions in 2 mL amber vials. All the stock, intermediate and working standard solutions were stored in refrigerator.

Pentachloronitrobenzene (ChemService) and the mixture of 2,4,5,6-tetrachloro-m-xylene and decachlorobiphenyl (ChemService) were used as internal and surrogate standards, respectively, for chlorinated pesticides. For organophosphorus pesticides, triphenyl phosphate (ChemService) and tributyl phosphate (ChemService) were used as internal and surrogate standards, respectively.

For chlorinated pesticides, the Standard Reference Materials (SRMs) were purchased from National Institute of Standards and Technology (NIST); NIST SRM 2261 (Chlorinated Pesticides in Hexane), NIST SRM 2273 (Chlorinated Pesticides (DDTs) and Metabolites in Isooctane) and NIST SRM 2275 (Chlorinated Pesticide Solution-II in isooctane). However, some of the target analytes were not present in each of these pesticide mixtures. Therefore, to check the accuracy of the analysis continuously, another standard solution from AccuStandards, EPA Method 508/608 Pesticide Standard Solution was used, as it contains all the analytes, except methoxychlor.

For organophosphorous pesticides, SRMs from NIST were not available in the market. Therefore, the calibration curves were verified by using 3 different pesticide mixture standard solutions (Mix Standart Solution-167, Mix Standart Solution-154, Mix Standart Solution-64) obtained form Dr. Ehrenstorfer. These solutions contain all the phosphorus pesticides, except fenamiphos.

The SRM solutions were also diluted with acetone before use, in order to bring the analyte concentrations into working range.

2.4.1. Preparation of Na_2SO_4 and Glass Wool

Anhydrous sodium sulfate (Na_2SO_4) was purchased from J.T. Baker Company and used to dry the extracts. Although it is purchased in extra-pure grade, it was cleaned before use. For that purpose, Na_2SO_4 was put in a glass column and sequentially washed twice with hexane and dichloromethane. The volume of the solvent used for each washing was twice 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 225ºC overnight. Dry Na_2SO_4 was then transferred to an amber glass bottle with Teflon lined cap and stored in a desiccator.

Glass wool used was also cleaned before use. A quantity of a glass wool was compressed into a glass column and washed sequentially with hexane and dichloromethane and treated as Na_2SO_4.

2.4.2. Cleaning of Glassware

Since the amount of analytes was very low in the samples, extreme precautions were taken to eliminate the contamination. All the glassware used were rinsed with acetone and hexane, washed with detergent and hot water, following several rinses with tap water and deionized water successively and dried.

2.5. Instrument and Apparatus

A HP (Hewlett Packard) 6890 series gas chromatograph, coupled with two split/splitless injectors was used for the chromatographic separation of the analytes. The GC system was equipped with micro-cell Electron Capture Detector (μ-ECD) in which ^{63}Ni source was used to produce thermal electrons.

The instrument was also coupled with a Nitrogen Phosphorus Detector with cesium silicate bead. The ECD and NPD detectors were used for the detection of organochlorine and organophosphorus pesticides respectively.

For the separation of chlorinated pesticides, a fused silica HP-5 capillary column (Agilent Tech.) coated with cross-linked (5%-phenyl)-methylpolysiloxane with a length of 30 m × 0.32 mm id and a film thickness of 0.25 μm was used. A non polar fused silica capillary column, 30 m × 0.25 mm id and a film thickness of 0.25 μm coated with cross-linked dimethylpolysiloxane, HP-1 MS (Agilent Tech.), was used for the separation of organophosphorus pesticides. For both inlets, a 4 mm id., deactivated glass liner (Agilent Tech.) packed with glass wool was used to prevent contamination of the analytical column from sample particulates and pieces of septum. The configuration of the GC system is given in Figure 2.2.

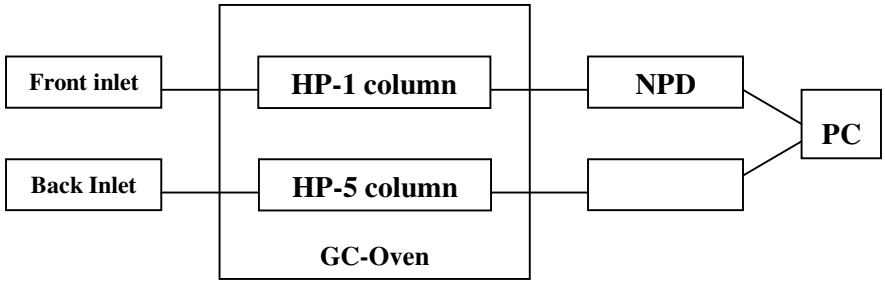


Figure 2.2. Configuration of the Analysis System

2.6. Optimization of Analysis Systems

The instrumental parameters were adjusted to give the highest signal for the analytes with minimum time for running of the analysis and between the runs.

2.6.1. Optimization of GC-ECD System

For the optimization of GC-ECD system, the parameters studied were the detector temperature, inlet temperature and the flow of make-up gas. During the optimization studies, 100 ng/mL standard containing all the analytes, surrogates and internal standard was injected twice.

Inlet and detector temperatures were decided according to results given in Figure 2.3 and Figure 2.4 respectively. Make-up flow was a little bit difficult to adjust, because, although the signal intensities were increased with higher N_2 flow rates, the background was also increased. The optimum flow was chosen as 30 mL/min.

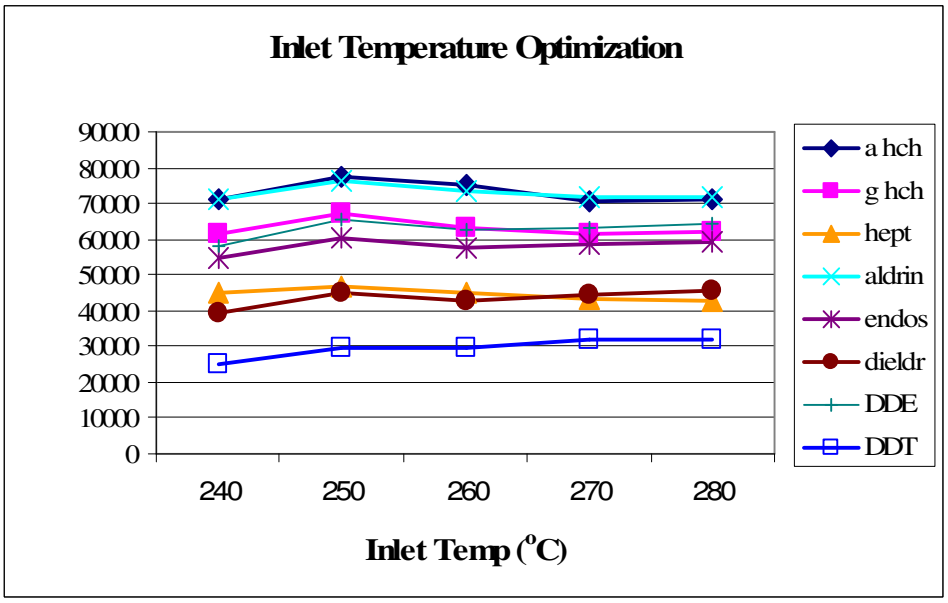


Figure 2.3. Inlet Temperature Optimization for GC-ECD

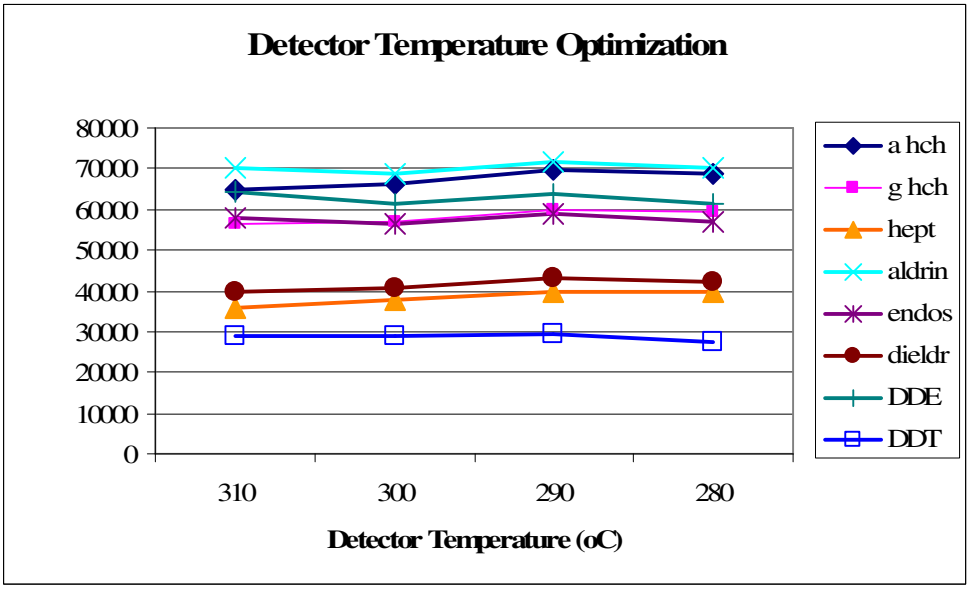


Figure 2.4. Detector Temperature Optimization for GC-ECD

The temperature program has also been developed to achieve the separation of all the analytes, as shown in Figure 2.5 for 100 ng/mL OC mixture. The program has a short run time, and has a high initial temperature which minimizes the cool-down time between runs.

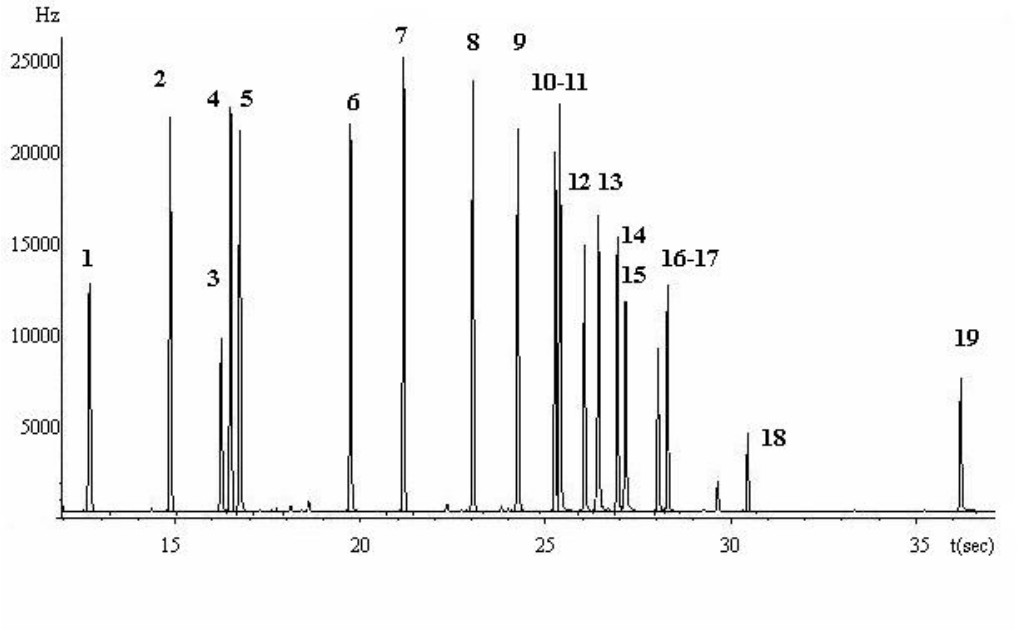


Figure 2.5. Chromatogram of 100 ng/mL Organochlorine Pesticide Mixture; **1.** 2,4,5,6-tetrachloro-m-xylene (Surrogate Standard 1), **2.** a-hch, **3.** b-hch, **4.** g-hch, **5.** Pentachloronitrobenzene (Internal Standard), **6.** Heptachlor, **7.** Aldrin, **8.** Heptachlor-endo Epoxide, **9.** Endosulfan, **10.** Dieldrin, **11.** DDE, **12.** Endrin, **13.** b-Endosulfan, **14.** DDD, **15.** Endrin Aldehyde, **16.** Endosulfan Sulfate, **17.** DDT, **18.** Methoxychlor, **19.** Decachlorobiphenyl (Surrogate Standard 2)

The optimized operating parameters for GC-ECD system is given in Table 2.4, including the oven temperature program.

Table 2.4. Operating Parameters for GC-ECD system

Column	HP-5 (30 m × 0.32 mm id, 0.25 μm)
Oven Temperature	80ºC -150ºC at 10ºC/min, wait for 5 mins, 150-275ºC at 5ºC/min, wait for 3 min (total run time = 40 minutes)
Carrier Gas	Ultra pure He at 35 cm/sec, in constant flow mode
Inlet	Splitless, 250ºC
Injection	Manual, 1.0 μL
Detector	290 ºC, Constant column and make-up (N_2) flow 30 ml/min

2.6.2. Optimization of GC-NPD System

For NPD Detector, the most critical instrumental parameter is the "Adjust Offset" value which is the baseline signal produced by the voltage applied to the bead. Default value for adjust offset is 50 pA, suggested operating range is 30 to 60 pA, and allowable range is 10 to 99 pA. Use of 50 pA increases sensitivity but reduces bead life. Lower settings reduce sensitivity and increase bead life. As higher adjust values decreases the lifetime of the bead, a value of 45 pA was chosen to work with the NPD.

The detector temperature should be greater than the highest oven ramp temperature. With higher detector temperatures, less bead heating voltage is required for higher analyte signals. However, again, the bead life decreases with increasing detector temperatures, therefore, a value of 330°C was chosen.

Inlet temperature was optimized with 1.0 µg/mL standard solution containing all the analytes, surrogate and internal standards. The standard solution was injected twice and the results are given in Figure 2.6. The optimum value was decided as 250ºC.

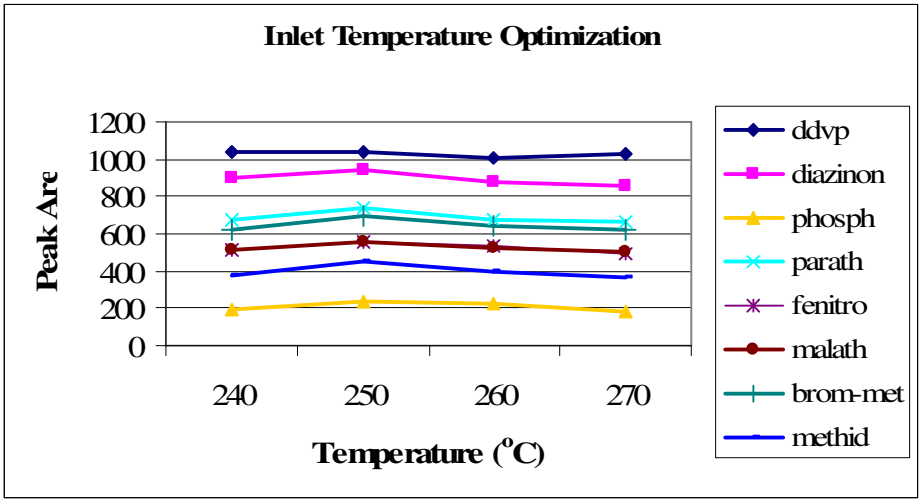


Figure 2.6. Inlet Temperature Optimization for GC-NPD

The temperature program was optimized considering high resolution of the analyte peaks and minimum run time. The Figure 2.7 shows the chromatogram of 1.0 µg/mL standard solution, which was obtained using the optimized temperature program.

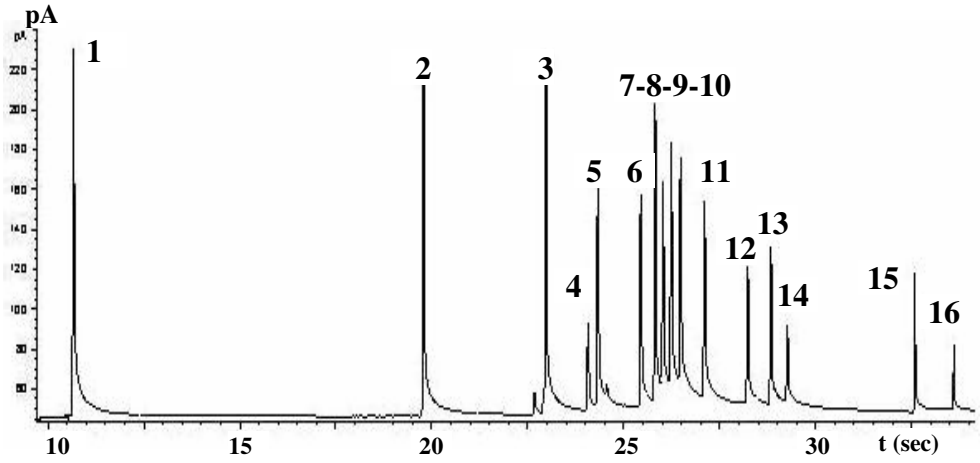


Figure 2.7. Chromatogram of 1.0 µg/mL Organophosphorus Pesticide Mixture **1.** Dichlorvos (DDVP), **2.** Tributyl Phosphate (Surrogate Standard) **3.** Diazinon, **4.** Phosphamidon, **5.** Parathion-methyl, **6.** Fenitrothion **7.** Pirimihos, **8.** Malathion, **9.** Fenthion, **10.** Chlorpyriphos, **11.** Bromohos-methyl **12.** Methidathion, **13.** Bromophos-ethly **14.** Fenamiphos, **15.** Triphenyl Phosphate (Internal Standard) **16.** Azinphos-methyl.

The optimized operating parameters for GC-NPD system is summarized in Table 2.5, including the oven temperature program.

Table 2.5. The Optimized Method for GC-NPD System

Column	HP-1 MS (30 m × 0.25 mm id, 0.25 μm)
Column Temperature	50ºC -100ºC at 10ºC/min, 100-220ºC at 5ºC/min, wait for 1 min, 220-280ºC at 30ºC/min, wait for 4 min (total run time = 36 minutes)
Carrier Gas	Ultra Pure He at 25 cm/sec, constant flow
Inlet	Splitless, 250ºC
Injection	Manual, 1.0 μL
Detector	330 ºC, Constant column and make-up (N_2) flow 3.0 ml/min

2.7. Application of Solid Phase Extraction

The SPE procedure used in this work was based on EPA METHOD 525.2 (1995). However, some modifications were done in this procedure. Different conditioning solvents were tested to obtain higher recoveries of pesticides from water samples. The procedures tested are given in Table 2.6. For that purpose, 1.00 L deionized water sample was spiked with chlorinated pesticides together with surrogates at a concentration of 100 ng/L and extracted accordingly. Duplicate extractions and blank samples were performed for each procedure.

Table 2.6. Extraction Procedures Used for SPE Optimization

No	Extraction Procedure
1	**Conditioning** 10.0 mL hexane:acetone + 10.0 mL MeOH + 10.0 mL DI **Elution** 2 × 10.0 mL hexane:acetone
2	**Conditioning** 10.0 mL ethylacetate + 10.0 mL MeOH + 10.0 mL DI **Elution** 2 × 10.0 mL ethylacetate
3	**Conditioning** 10.0 mL DCM + 10.0 mL MeOH + 10.0 mL DI **Elution** 2 × 10.0 mL DCM
4	**Conditioning** 10.0 mL DCM:ethylacetate (1:1)+ 10.0 mL MeOH + 10.0 mL DI **Elution** 2 × 10.0 mL DCM:ethylacetate (1:1)
5	**Conditioning** 10.0 mL acetone + 10.0 mL MeOH + 10.0 mL DI **Elution** 2 × 10.0 mL DCM:ethylacetate (1:1)

The percent recoveries of the chlorinated pesticides are presented in Figure 2.8. From the results, it seems that the recoveries are close to each other. The accepted criteria for the demonstration of the capability of sample preparation methods are 70-130 % for percent recoveries (EPA Method 8000C). However, the same study could not be applied for organophosphorous pesticides due to time limitation for the sampling. Considering these limits, Procedure-3 was selected for further studies including the phosphorous pesticides.

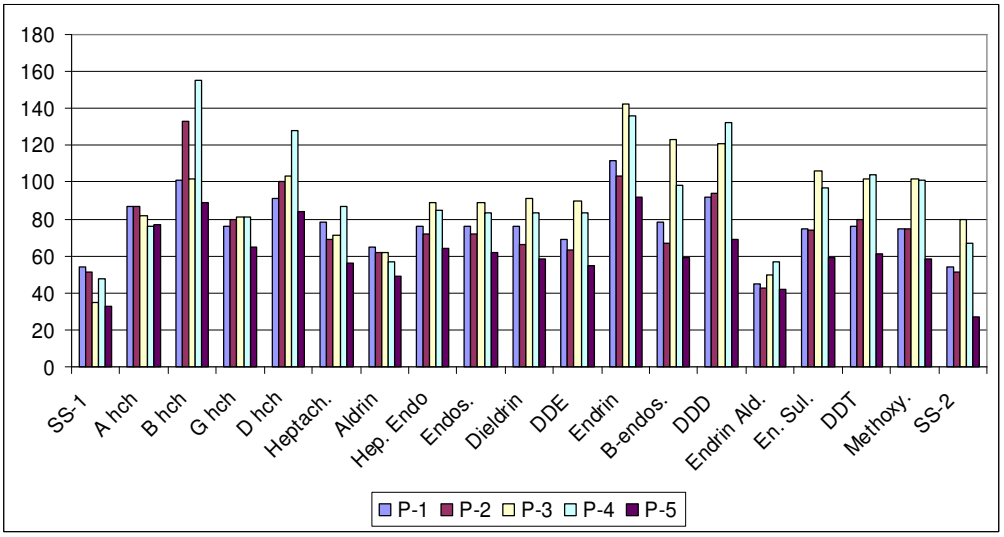


Figure 2.8. Comparison of Different SPE Procedures

The procedure used for SPE optimization and sample extractions was summarized in Figure 2.9. For the extraction, 1.00 L of water sample was used. After addition of surrogates and methanol, the sample was passed through a disk with chemically bonded C_{18} organic phase, which was conditioned before with organic solvents. The analytes and surrogates were trapped on the disk. These organic compounds were eluted from the disk with small quantities of dichloromethane (DCM). The extract was than passed through anhydrous sodium sulfate column to remove any water residues left. The solvent was evaporated by gentle stream of nitrogen near to dryness and the volume was completed to 1.0 mL with acetone after the addition of internal standards. The details of the steps were given in the following paragraphs:

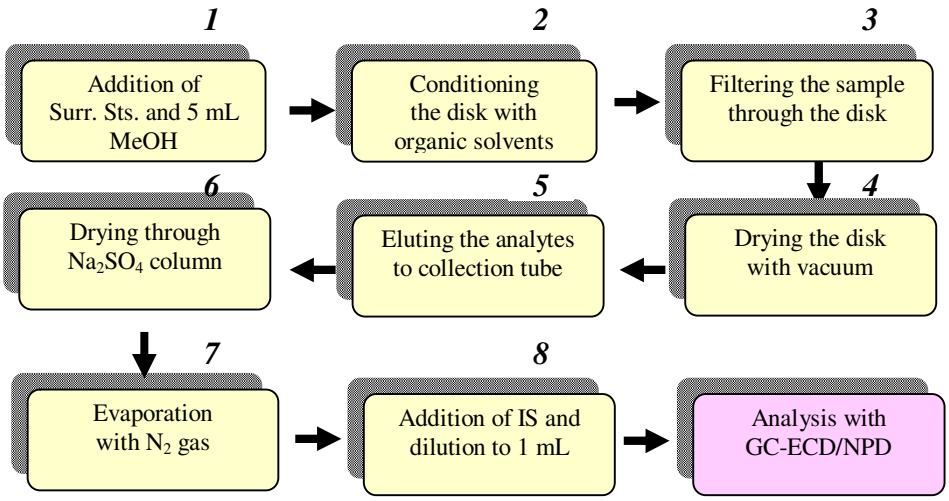


Figure 2.9. Flow Diagram of the Extraction Procedure

Step 1: Surrogate standard is an organic compound which is similar to the target analyte(s) in chemical composition and behavior in the analytical process. It is extremely unlikely to be found in any sample and it is added to the sample aliquot in known amounts before extraction or other processing, and is measured with the same procedures used to measure other sample components. The purpose of using the surrogate is to monitor the experimental performance with *each* sample (EPA Method 525.2). The recovery of the surrogate standard indicates unusual matrix effects, gross sample processing errors, etc. Surrogate recovery is evaluated for acceptance by determining whether the measured concentration falls within the acceptance limits (70-130 % according to EPA Method 8000C). The percent recovery of a surrogate is calculated as follows:

Recovery (%) = Concentration Found / Concentration Added × 100

In this study, tributyl phosphate was used as the surrogate for organophosphorus pesticides. This surrogate was spiked as being 0.50 µg/L in a 1.00 L sample. For chlorinated pesticides, the surrogates were 2,4,5,6-tetrachloro-m-xylene and decachlorobiphenyl, which were spiked to 1.00 L sample solution with a final concentration of 0.10 µg/L.

The addition of methanol to the sample before the extraction is required to allow a better extraction (Albanis et al., 1998; Lambropoulou et al., 2000; Golfinopoulos et al., 2003). The methanol modifier maintains the conditioning of the surface of the disk through the extraction. As suggested by the manufacturer, methanol was added to the sample to a final concentration of 0.50 %.

Step 2: The surface of the disk should be conditioned before sample extraction by organic solvents. This wetting step also provides the cleaning of the extraction medium. In this study, 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. The solvent was then drawn by vacuum. Beginning with the conditioning step, it is critical that the disk not go dry until extraction is completed. Therefore, the surface of the disks was not allowed to contact with air and a thin layer of the solvent or water was left before the following steps. Methanol and water was also kept in contact for 90 seconds and drawn by vacuum sequentially.

Step 3: The samples were then loaded on the disk and passed through with application of vacuum at a rate of 50-250 mL/min depending on the particulate matter of the water samples. Sample particulates may clog the solid phase

media and result in extremely slow sample extractions. Use of an appropriate filter aid will result in shorter extractions. However, it is also recommended to include any particulates in the original sample aliquot that is extracted, as some of the analytes may be associated with particulate matter in the sample (EPA Method 3535A). Therefore, in this study most of the samples were not filtered. However, for a few fall season samples, the filtration was required as the samples were with high particulate matter content. These samples were filtered through glass fiber filters (Cole Palmer, 90 mm, 2.7 µm pore size) after the addition of surrogate compounds. The filters were previously cleaned by washing with hexane and DCM and dried at 225ºC overnight.

Step 4: Following the sample extraction, the disc was dried under vacuum for about 10 minutes.

Step 5: The analytes trapped on the disk were eluted by 20 mL DCM into a collection tube placed inside the Erlenmeyer flask with vacuum. The solvent was added by 10+5+5 mL portions with a total contact time of 5 minutes.

Step 6: The extract in the collection tube was removed and dried by passing through a drying column of Na_2SO_4. The drying column was 1 cm diameter glass tube containing 5-7 g of pre-cleaned anhydrous Na_2SO_4. The column bed was wetted by 6.0 mL Ethyl Acetate, DCM (1:1) mixture before use. After passing the extract, the drying column was rinsed with 5.0 mL of the same mixture of the solvents and this portion was collected with sample extract.

Step 7: The collected extract was then placed in fume hood and dried under gentle stream of nitrogen. The extract was not let to dry completely.

Step 8: The internal standards were added and the final volume was completed to 1.0 mL with acetone.

2.8. Calibration of Analysis Systems

Quantitative analysis demands that an analytical measurement can be accurately and reliably related to the composition of the sample. This relationship can be established by means of calibration procedures. For a typical simple calibration, a range of standards is prepared containing varying amounts of the analyte. These are then analyzed by the analysis method and a calibration curve of signal versus amount of analyte is plotted. Results for unknowns are then interpolated from this graph (Fifield and Haines, 2000). In chromatographic analysis, the peak heights or peak areas of analytes from the chromatogram of the standards were used to plot the calibration curve.

The most commonly employed calibration procedures involve the use of external standards containing known concentrations of analytes. However, in chromatographic analyses, uncertainty associated with injection of a reproducible volume of a very small amount of sample (generally ~1.0 μL) with a microsyringe may be an important source of error. In addition to this, in gas-chromatography, the sample is introduced to a heated sample port, where evaporation from the needle tip may lead to large variations in injection volume. The highest precision for quantitative chromatography is obtained by the use of internal standards. In this procedure, a carefully measured and equal amount of an internal standard (a standard whose identity is different from the analytes and its signal is well separated from target analytes) is introduced to each standard, as well as samples. The ratio of analyte to internal standard peak areas (or heights) serves as the analytical parameter (Skoog et al., 1996).

The response factor is calculated as follows:

$RF = (A_S \times C_{IS}) / (A_{IS} \times C_S)$

where A_S and A_{IS} are the area (or height) for the analyte and internal standard, respectively and C_S and C_{IS} are their concentrations. To calculate the RF for the analytes, a standard solution is used and than the unknown concentration is calculated as follows (Braitwaite and Smith, 1999):

Unknown Concentration $(C_S) = (A_S \times C_{IS}) / (A_{IS} \times RF)$

However, this is a single-point internal standardization. To construct an internal standard calibration curve, it is necessary to prepare several standards containing different concentrations of analyte. A calibration curve is then plotted with amount ratio versus response ratio (Harvey, 2000).

In this study, internal standard calibration was used for quantification of both organochlorine and organophosphorus pesticides. Calibration parameters are given in Table 2.7. The standards were injected three times by 1.0 µL with 10 µL glass syringe. The average values of these replicates were used in calculations. Linear calibration curves with linear regression coefficients greater than 0.99 were obtained for all the analytes and surrogates. Calibration curves for both types of pesticides were given in Figures 2.10 and 2.11.

Table 2.7. Calibration Parameters for the Analysis

	Internal Standard (Concentration)	**Standard Concentrations**
OCPs	Pentachloronitrobenzene (100.0 ng/mL)	5.0-10.0-20.0-50.0-100.0-200.0 ng/mL
OPPs	Triphenyl phosphate (1.00 µg/mL)	0.05-0.10-0.20-0.50-1.00-2.00 µg/mL

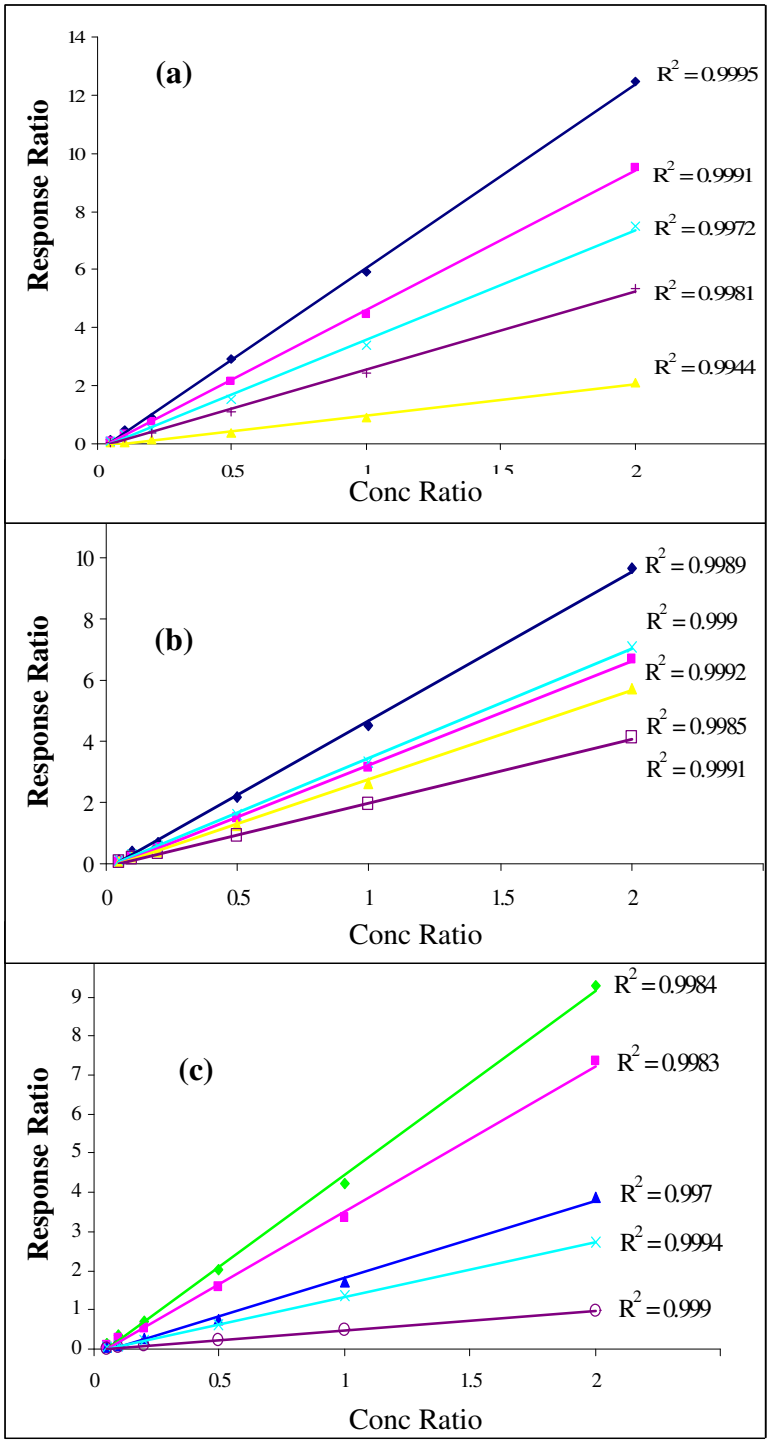


Figure 2.10. Calibration Curves for Organophosphorus Pesticides:

a) ◆ DDVP, ■ Surr Std, × Parathion, + Fenitrothion ▲Phosoamidon,
b)◆Diazinon, × Bromophos-Me, ■ Pirimiphos, ▲ Malathion, □ Bromophos-Et,
c)◆ Chlorpyriphos, ■ Fenthion,▲ Methidathion,×Fenamiphos, ○ Azinphos-Me

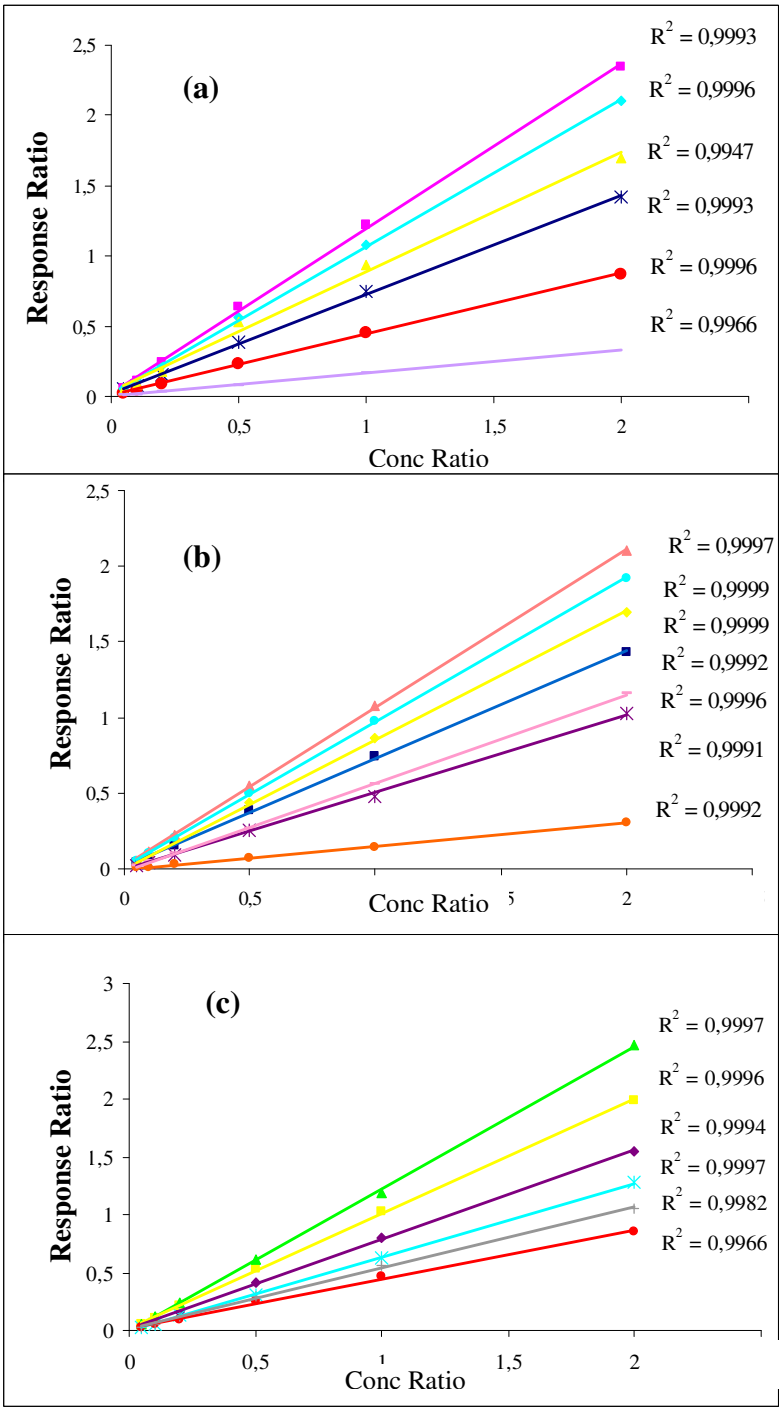


Figure 2.11. Calibration Curves for Organochlorine Pesticides:
a) ■α-hch, ◆ γ-hch, ▲δ-hch, *Tetrachloro-m-xylene(SS), ● β-hch, — Decachlorobiphenyl (SS), **b)** ▲heptachlor-endo, ● endosulfan, ◆ heptachlor, ■ beta endosulfan, — ddt, * endosulfan sulfate, ● methoxychlor, c) ▲ aldrin, ■ dde, ◆ dieldrin, * endrin, + ddd, ● endrin aldehyde

2.9. Analysis of the Samples

The 1.0 µL aliquot of the each sample was injected twice. The results were calculated from the average values of these replicates. Almost for each 10 samples, i.e. for each 20 injections, SRM and blank samples were analyzed. Final concentrations of the samples were obtained after the correction with percent recoveries obtained for each analyte.

The sample chromatograms obtained for GC-ECD and GC-NPD systems are given in Figure 2.12 and 2.13, respectively. As seen, some chromatograms have signals which were not qualified. These peaks may be due to the presence of other substances in the samples, containing chlorine or phosphorus. It should be noted that none of these peaks affected the quantification of the target analytes. The peak tailing observed for NPD detector is expected for phosphorus compounds (Agilent 6890, Gas Chromatography, Service Manual).

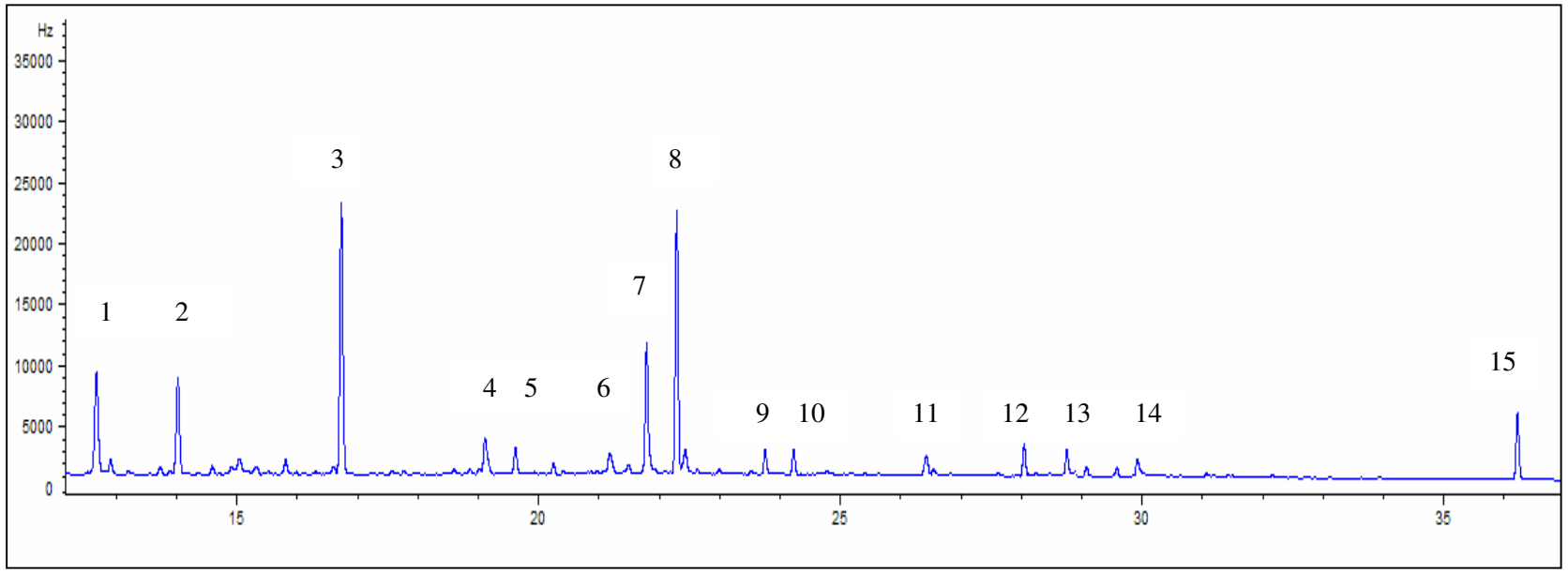


Figure 2.12. Sample Chromatogram for GC-ECD System.
1. SS(1), **2.** α-HCH, **3.** IS, **4.** δ-HCH, **5.** Heptachlor, **6.** Aldrin, **7.** Unknown, **8.** Heptachlor endoepxide, **9.** Unknown, **10.** Endosulfan, **11**. β-endosulfan, **12.** Endosulfan Sulfate, **13.** DDT, **14.** Methoxychlor, **15.** SS(2). Retention Times are Given in Table 2.8

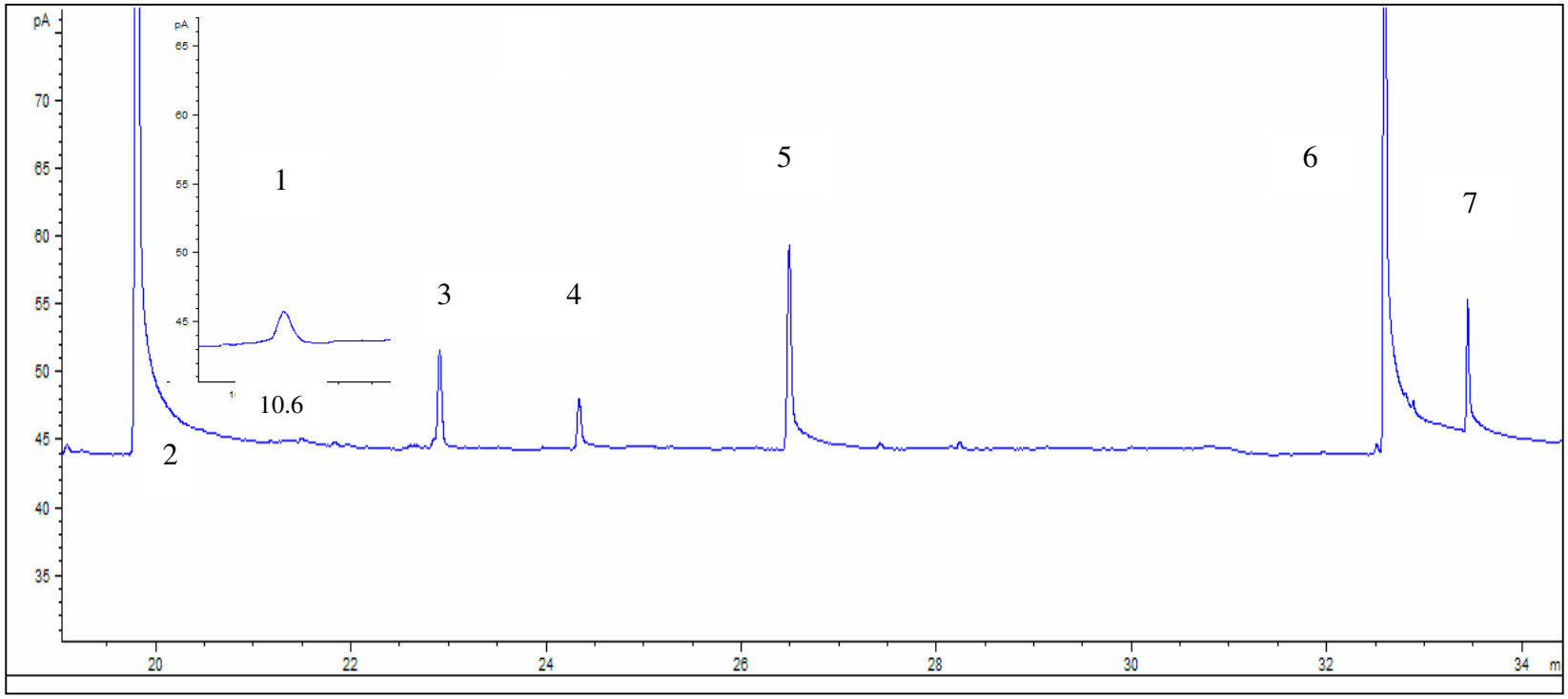


Figure .13. Sample Chromatogram for GC-NPD System.
1. Dichlorvos, **2.** SS, **3.** Diazinon, **4.** Parathion-methyl, **5.** Chlorpyriphos, **6.** IS, **7.** Azinphos-Methyl.
Retention Times are Given in Table 2.8

In environmental analysis, generally the samples contain the analytes in very low concentrations. Therefore, the analysis systems should be capable of measuring such low concentrations. In this study, pesticides were analyzed and they were expected to be “not present” in the samples. Moreover, the accepted health limits of these pollutants are as low as 0.1 µg/L. Therefore, the quantification was performed with specific detectors. To examine the capability of the systems for low concentrations, the detection limits were calculated for GC-ECD and GC-NPD systems. The limits of detection (LOD) were calculated experimentally and they were the concentrations of the analytes at which signal-to-noise ratio (S/N) is equal to 3. The calculation of S/N was performed by the instrument software. The LODs are presented in Table 2.8., together with the retention times of the analytes. It should be noted that, these values are instrumental detection limits, and the concentrations in real samples were measured after 1000 fold concentration of the analytes from 1.00 L to 1.0 mL.

Table 2.8. Limits of Detection and Retention Times of the Analytes

	RT (min)	**LOD (ng/mL)**
Organophosphorous Pesticides		
Dichlorvos	10.64	0.59
Tributylphophate (SS)	19.77	0.62
Diazinon	22.97	0.51
Phosphamidon	24.07	2.56
Parathion-methyl	24.32	0.82
Fenitrothion	25.44	1.08
Pirimiphos-methyl	25.82	0.95
Malathion	26.02	2.01
Fenthion	26.24	1.57
Chlorpyriphos	26.47	1.73
Bromophos-methyl	27.10	1.08
Methidathion	28.23	1.40
Bromophos-ethyl	28.83	1.32
Fenamiphos	29.26	2.60
Triphenylphosphate (IS)	32.58	1.22
Azinphos-methyl	33.48	2.34
Organochlorine Pesticides		
Tetrachloro-m-xylene (SS1)	12.67	0.03
A-HCH	14.05	0.01
B-HCH	16.23	0.03
G-HCH	16.48	0.02
PCNB (IS)	16.73	0.01
D-HCH	19.07	0.01
Heptachlor	19.72	0.02
Aldrin	21.15	0.01
Hep. Endo Epoxide	22.28	0.01
Endosulfan	24.24	0.02
Dieldrin	25.25	0.04
DDE	25.38	0.02
Endrin	26.04	0.03
B-endosulfan	26.42	0.02
DDD	26.93	0.06
Endrin Aldehyde	27.15	0.03
Endosulfan sulfade	28.03	0.04
DDT	28.82	0.03
Methoxychlor	29.95	0.12
Decachlorobiphenyl (SS2)	36.20	0.12

CHAPTER 3

RESULTS AND DISCUSSION

This chapter includes the results of quality check (QC) and quality assurance (QA) tests for the field and experimental work, calculation of uncertainties for the measurements, statistical evaluation of data set obtained, comparison of the results with the literature, evaluation of the seasonal variations of pesticide concentrations and spatial distribution of the pollution in Kumluca region.

3.1. Evaluation of QC/QA Tests

As in the other environmental studies, the field and experimental part of this study consists of three main steps; sampling, sample preparation and analysis. These processes may bring some errors, which may result from the sampling procedures, extraction apparatus, analysis systems or by personal factors, affecting the reliability of the data. To minimize or at least to determine the effect of these factors, a quality assurance program was followed during whole study from sampling to calculation of concentrations to obtain data which is scientifically valid, reliable and of known precision and accuracy. The quality assurance program includes the procedures and controls at each stage; sampling replicates, validation of extraction procedure, optimization and calibration of the analytical systems and monitoring of the stability of these systems.

To achieve the quality assurance program, operational day to day activities, quality control practices were performed, including blank analysis, calculation of recoveries for different matrix types, use of surrogates, demonstration of accuracy and precision of the measurements.

The quality assurance and quality control tests performed in this thesis during sampling, sample preparation and analysis steps are summarized in Table 3.1, together with the explanation of their use.

Table 3.1. Summary of Quality Check and Quality Assurance Tests

STEP	QC/QA TESTS	AIM
1 Sampling	Field Blanks	Check for Contamination During Sampling and Storage
	Sampling Replicates	Check for Reproducibility of Sampling
2 Sample Preparation	Laboratory Blanks	Check for Contamination During Sample Preparation
	Replicate Samples	Check for Precision of Extraction Procedure for Real Samples
	Spiked Control Matrix (Spiked DI)	Check for Extraction Efficiency and Precision of Extraction Procedure
	Spiked Sample Matrix	Check for Effect of Sample Matrix to Extraction Efficiency
	Use of Surrogate Standards	Monitoring Extraction Performance For Each Sample
3 Analysis	Use of Standard Reference Materials	Check for Accuracy and Precision of the Analysis Methods
	Replicate Analysis	Check for Precision of Each Measurement

As the extraction procedure and the optimization and detection limits of analysis systems have been presented in Chapter II, they will not be further discussed in the subsequent paragraphs.

3.1.1. QC/QA Tests during Sampling

3.1.1.1. Field Blanks

The field blanks were prepared with reagent water (DI) placed in sample containers in five sampling points, during each field work. The field blanks were treated as a sample in all respects, including exposure to sampling equipment, site conditions, storage and all analytical procedures. The purpose of it is to check the contamination of method analytes or other interferences in the field environment, equipment used, sampling containers and to check contamination during storage.

The concentrations of both Organochlorine Pesticides (OCPs) and Organophosphorus Pesticides (OPPs) were below the Limit of Detection of the analysis systems. Typical field blank chromatograms are shown in Figure 3.1. For ECD system, after the solvent peak, two major peaks, with retention times of 9.7 and 11.8 min. These peaks were observed for blanks and for all samples subjected to extraction procedure. These peaks were identified by GC-MS system, as being long chain hydrocarbons, which were eluted from SPE material. As the retention times of these peaks were far from than that of analyte peaks, and as they were not affecting the quantitative determination of pesticides, no action was taken to eliminate this carryover from extraction. The other three peaks were for two surrogates and the internal standard. A similar peak coming from the SPE material was also observed for NPD system. This is appearing as a small peak at retention time of 16.7 min, which is again far from the any analyte peaks. The later two peaks are for surrogate and internal standards, respectively.

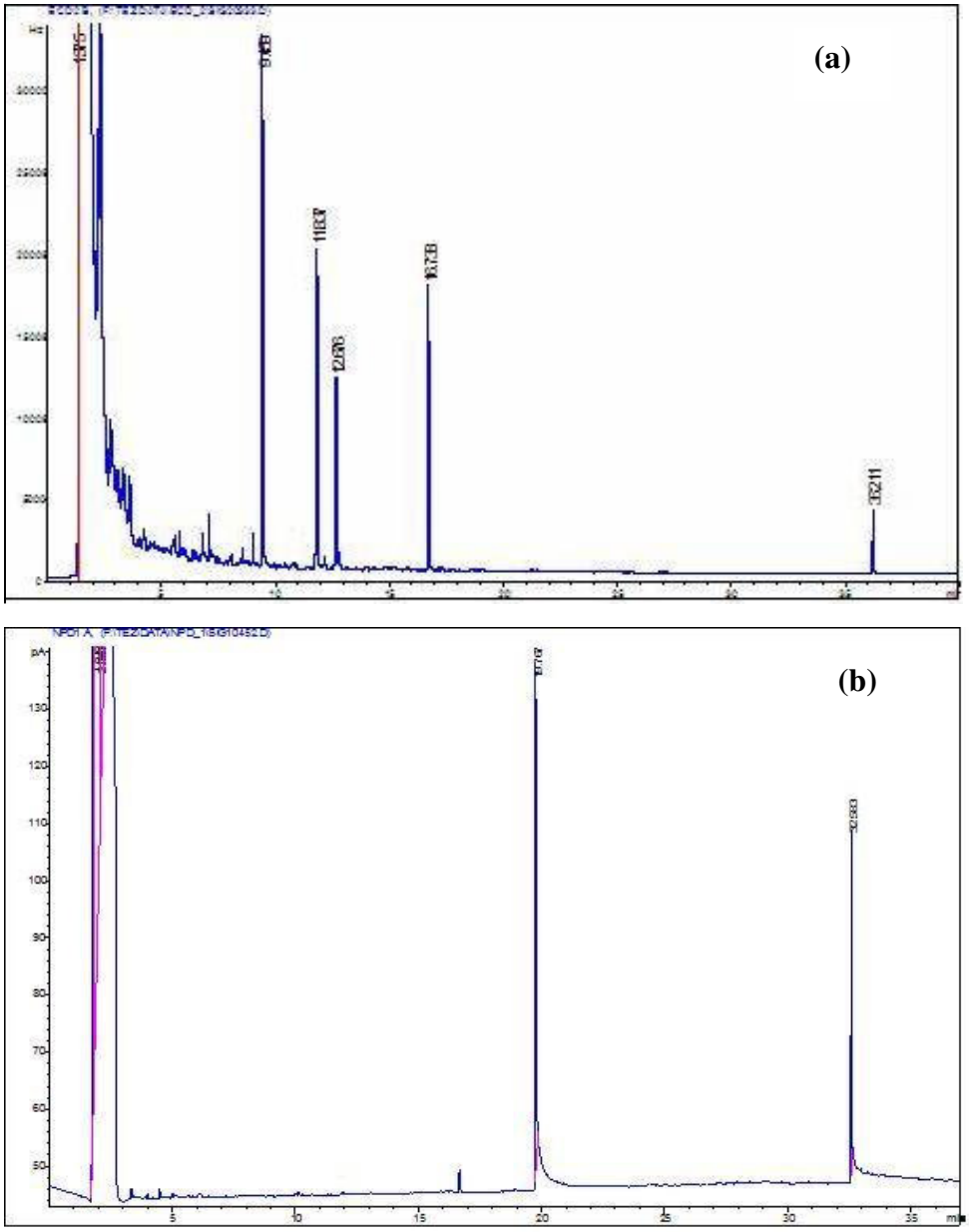


Figure 3.1. Typical Blank Chromatograms for Analysis Systems
(a) For GC-ECD, (b) For GC-NPD
(x-axis: Retention Time, y-axis; Instrumental Signal)

3.1.1.2. Sampling Replicates

To check the reproducibility of the sampling, i.e., whether the two replicates in the sample sampling point give the same results, replicate sampling from some of the sampling points was performed. From 77 of the samples in whole study, 63 of them were collected in two replicates. Among these, 15 set of replicate samples were used to calculate the sampling reproducibility. These replicates were treated as different samples during sample preparation and analysis steps. The duplicate sampling may seem providing insufficient degrees of freedom, but the methods commonly applied use one "duplicate" sample for each *set of samples*, to check the variance in the sampling and analysis techniques (EPA, SW-846, 1996; USGS TWRI Book 9, 1999). Moreover, in the literature, there is insufficient number of publications considering replicate sampling for pesticide analysis in environmental water samples.

The analysis results were expected to be similar in an acceptable sampling. Unfortunately, not all of the pesticides were observed in the replicate samples. The results are given in Table 3.2 showing the percent relative standard deviations (RSD) of the detected pesticide concentrations among the samples collected from the same sampling point. In the table, the number of data points evaluated and the ranges were also given. As seen, the averages of percent RSD values were almost below 10 %. This demonstrates a good agreement between the replicate sample collections.

Table 3.2 Evaluation of Sampling Replicates

	N	Av of RSD	Min	Max
ENDOSULFAN	9	11.7	0.1	22.9
DIELDRIN	5	10.9	0.5	21.6
B ENDOS.	1	7.1	7.1	7.1
DDD	3	9.2	0.2	26.8
EN. SULFATE	6	10.4	0.1	27.9
DDT	2	2.9	0.8	5.0
METHOXY.	2	9.3	0.2	18.4
DIAZINON	3	8.7	0.8	23.0
PHOSPHAM.	1	1.1	1.1	1.1
FENITROTHION	2	7.8	0.7	15.0
MALATHION	2	3.8	3.1	4.4
CHLOPYR.	4	0.8	0.3	2.0
FENAMIPHOS	2	9.1	4.9	13.2
AZINPHOS-ME	2	5.1	1.7	8.6

3.1.2. QC/QA Tests during Sample Preparation

3.1.2.1. Laboratory Blanks

The laboratory blank samples were prepared by an aliquot of reagent water (DI) that was treated exactly as a sample including exposure to all glassware, equipment, solvents, reagents, internal standards, and surrogates that were used with other samples. They were used to determine if method analytes or other interferences are present in the laboratory environment, reagents, or apparatus. For each set of 15 samples, one laboratory blank has been prepared together with the samples, making up a total of 10 for whole sample preparation period.

As in the field blanks, the laboratory blank samples had the analyte concentrations below the detection limits of the analysis systems for both chlorinated and phosphorus pesticides. This shows that the laboratory environment, the chemicals and the glassware used did not contribute to the observed pesticide concentrations in the samples.

3.1.2.2. Replicate Samples

The samples which were collected in two replicates and used for the evaluation of sampling efficiency also served for the estimation of reproducibility of the extraction procedures on real matrices. As it was explained in Section 3.1.1.2, the RSD values for the calculated concentrations were almost better than 10 %. Besides indicating good sampling reproducibility, these results also point out high precision for the extraction procedure, when applied for real matrices.

3.1.2.3. Spiked Control Matrix

The spiked control matrices were used to monitor the ongoing extraction efficiency and precision of the extraction procedures. The spiked control matrix was prepared by spiking 1 L of DI with the surrogates and the target chlorinated and phosphorus pesticides to make 0.1 and 0.5 μg/L in concentration, respectively. This solution was then treated as sample and extracted with the same procedure. For each set of 15 samples, one spiked control matrix has been prepared, making up 10 spiked control samples for both fall and spring sample's extractions.

The percent recoveries of the analytes were calculated using the formula given below:

$$Recovery\ (\%) = (C_s - C_u) / C_{Certified} \times 100$$

where; C_s = Measured concentration of spiked sample, C_u = Measured concentration of unspiked sample (original concentration of the analyte in the sample), $C_{Certified}$ = Nominal (theoretical) concentration increase that results from spiking of the sample with the standards.

As stated in Chapter I, Literature Review section, Solid Phase Extraction (SPE) is a commonly used technique in pesticide analysis. Different types of extraction medium, such as cartridges or disks, were used for the sample preparation of different classes of pesticides from aqueous samples. The extraction efficiencies vary according to the sorbent material-pesticide interaction, the sample matrix and the experimental procedure.

The percent recoveries obtained in this study for spiked control matrix were compared with similar studies and presented in Table 3.3 in which extraction of pesticides from water matrices was performed with SPE technique.

Golfinopoulos et al. (2003) and Zhou et al. (2006) have used C_{18} containing cartridges for the extraction of OCPs in surface waters. The analyses were performed by using a GC-ECD system. Liquid chromatography/atmospheric pressure chemical ionization mass spectrometry (LC-APCI-MS) was used for the trace determination of several OPPs in groundwater samples (Lacorte and Barcelo; 1996). This study involves online liquid-solid extraction step using C_{18} cartridges.

C_{18} disks were used by Tolosa et al. (1996) for the extraction of 11 organochlorine and 24 organophosphorus compounds. GC-ECD and GC-FPD systems were used for quantification.

Patsias and Papadopoulou-Mourkidou (1996) has developed a multiresidue method for the trace analysis of 96 pesticides from different classes in surface and ground waters. The pesticides were extracted by C_{18} containing cartridges and determined by Gas Chromatography-Ion Trap Mass Spectrometry (GC-IT-MS) system. A method based on semi-automated SPE using C_{18} bonded silica disks and GC-MS was developed and used in the detection and quantification of approximately 100 pesticides and transformation products in drinking water by Leandro et al. (2006).

Table 3.3. Comparison of Percent Recoveries with Literature
(Average recoveries are given with standard deviations in the parenthesis)

	A	**B**	**C**	**D**	**E**	**F**	**G**
OPPs	***N=3***	***N=5***	***N=9***	***N=3***	***N=3***	***N=5***	***N=10***
Azinphos-m	-	-	114 (22)	84 (13)	94 (2)	82 (7)	78 (13)
Bromophos-e	-	-	-	-	-	-	98 (5)
Bromophos-m	-	-	-	-	-	-	96 (7)
Chlorpyriphos	-	-	-	91 (12)	79 (4)	77 (9)	92 (6)
Diazinon	-	-	98 (23)	92 (12)	86 (3)	-	97 (6)
Dichlorvos	-	-	120 (31)	48 (19)	-	-	94 (8)
Fenamiphos	-	-	-	-	-	-	70 (15)
Fenitrothion	-	-	114 (22)	96 (16)	88 (5)	100 (3)	92 (8)
Fenthion	-	-	94 (30)	-	-	99(4)	61 (8)
Malathion	-	-	94 (18)	92 (12)	99 (2)	-	86 (9)
Methidathion	-	-	-	91 (13)	85 (3)	-	64 (10)
Parathion-m	-	-	104 (22)	82 (12)	97 (3)	-	93 (7)
Phosphamdon	-	-	-	-	-	-	100 (4)
Pirimiphos-m	-	-	-	-	78 (5)	92 (5)	91 (9)
OCPs							
a hch	100 (8)	76 (6)	-	-	67 (8)	76 (14)	86 (5)
b hch	122 (7)	87 (3)	-	-	86 (3)	79 (11)	89 (7)
g hch	100 (8)	83 (5)	-	110 (3)	83 (7)	94 (3)	90 (4)
d hch	104 (6)	85 (4)	-	-	89 (2)	76 (13)	95 (14)
Aldrin	50 (7)	78 (7)	-	-	59 (5)	67 (10)	73 (12)
b-endosulfan	95 (8)	-	-	105 (11)	92 (3)	100 (5)	95 (11)
Dieldrin	96 (7)	78 (4)	-	-	89 (1)	84 (8)	99 (7)
Endosulfan	100 (7)	-	-	88 (6)	99 (2)	82 (8)	89 (9)
Endosulfan sul.	145 (9)	-	-	115 (5)	89 (5)	84 (7)	90 (17)
Endrin	104 (7)	87 (6)	-	-	94 (1)	-	99 (11)
Endrin aldeh	101(10)	-	-	-	-	-	88 (13)
Hep. Endo Ep.	68 (7)	76 (3)	-	-	85 (2)	80 (12)	91 (8)
Heptachlor	75 (9)	75 (8)	-	-	66 (3)	70 (13)	87 (10)
Methoxychlor	95 (14)	-	-	-	82 (7)	91 (10)	96 (14)
p-p' DDD	80 (8)	79 (5)	-	108 (5)	72 (1)	-	97 (11)
p-p' DDE	58 (6)	80 (7)	-	67 (12)	57 (8)	92 (4)	81 (6)
p-p' DDT	64 (10)	87 (5)	-	102 (9)	72 (5)	82 (9)	97 (12)

A: Golfinopoulos et al. (2003),
B : Zhou et al. (2006),
C : Lacorte and Barcelo (1996),
D: Tolosa et al. (1996),
E: Patsias and Papadopoulou-Mourkidou (1996),
F: Leandro et al. (2006),
G : This Work

The percent recoveries obtained in this work is in good agreement with previous studies for both OPP and OCPs. The findings justify the common use of SPE technique for pesticide analysis in aqueous samples.

The percent recoveries of this study are between 61-100 %. They are generally in the limit of acceptance (70-130%) according to EPA, except for two OPPs, fenthion and methidathion which have 61 and 64 % recoveries, respectively. The recoveries were ranging between 61-100 % for OPPs, being highest for phosphamidon, and between 73-99 % for OCPs, being highest for dieldrin and endrin. Except aldrin, with 73% recovery, the OCPs have recoveries higher than 80%, whereas the values are more variable for OPPs. This is due to the higher variation in the physical and chemical properties of OPPs than that of OCPs, affecting the behavior of the analytes during extraction.

For spiked control matrix, the percent RSD values were lower than 10 %, except azinphos-methyl and fenamiphos for OPPs and lower than 15 % for OCPs, except for endosulfan sulfate. The percent RSD values for OCPs may seem high but in the analysis of organic pollutants, these values are common, such as the ones obtained by Lacorte and Barcelo (1996) and Tolosa et al. (1996). Considering that the extractions were performed with a high number of replicates in a long period of time for sample preparation, we can state that the precision of the extraction procedure was good and the extractions were performed with acceptable reproducibility.

The spiked control matrix study shows that, the SPE is a suitable technique for the extraction of OCPs and OPPs from aqueous samples and the procedure applied in this work was successfully validated.

3.1.2.4. Spiked Sample Matrix

To determine the effect of matrix on extraction performance, one replicate of collected 47 replicate samples were spiked with the surrogates and the target chlorinated and phosphorus pesticides to make 0.1 and 0.5 μg/L in concentration, respectively. The other replicate of these samples were spiked with only surrogates and left unspiked with respect to target analytes.

The percent recoveries of spiked sample matrices were presented in Table 3.4. The particulate content of the samples may affect the percent recoveries of SPE procedure. In Kumluca samples, the groundwater samples collected from wells and the surface water samples were different in their particulate matter content, representing two different water types. Therefore, in the table the percent recoveries were presented individually for spiked control matrix, ground and surface water samples.

Table 3.4. Percent Recoveries of Pesticides for Different Matrices

	Spiked Control Matrix N=10		Ground Water Samples N=31		Surface Water Samples N=17	
	Recovery %	RSD %	Recovery %	RSD %	Recovery %	RSD %
Organophosphorus Pesticides						
Azinphos-methyl	77.5	13.4	73.6	15.7	72.2	18.8
Bromophos-ethyl	98.1	5.3	89.7	10.8	82.6	21.7
Bromophos-methyl	96.4	6.8	92.2	8.7	86.4	19.3
Chlorpyrifos	92.2	5.9	78.4	6.6	71.7	12.4
Diazinon	97.2	6.1	90.3	7.8	79.0	12.8
Dichlorvos	94.2	8.0	91.3	9.2	83.6	13.0
Fenamiphos	70.0	14.5	64.7	17.4	63.9	13.9
Fenitrothion	92.2	8.2	85.8	8.7	81.7	14.5
Fenthion	61.4	8.1	58.7	15.4	53.9	11.7
Malathion	86.2	8.8	81.6	9.9	78.8	12.0
Methidathion	63.5	10.1	60.2	16.5	62.4	13.8
Parathion-methyl	92.5	7.4	89.3	10.3	83.5	15.7
Phosphamidon	100.4	4.3	97.1	12.4	96.2	16.9
Pirimiphos-methyl	90.5	8.6	84.2	9.6	78.2	13.0
Tributhylphosphate(SS)	100.5	2.9	97.6	6.9	95.3	7.7
Organochlorine Pesticides						
a hch	85.5	5.1	81.9	8.2	81.6	14.7
b hch	89.4	7.2	97.1	6.5	96.0	10.8
g hch	90.9	3.9	84.0	7.9	82.8	13.0
d hch	94.5	14.3	86.6	12.0	80.3	21.4
Aldrin	73.1	11.9	71.0	15.1	70.1	20.0
b-endosulfan	95.0	10.6	81.0	14.8	78.4	16.1
Dieldrin	98.7	7.3	85.6	13.3	81.7	17.6
Endosulfan	89.0	8.7	80.1	10.7	78.1	14.4
Endosulfan sulfade	89.7	16.8	83.3	12.9	78.0	18.8
Endrin	99.3	11.1	85.0	10.9	77.7	15.1
Endrin Aldehyde	88.1	12.7	71.9	12.1	70.3	16.3
Hep. Endo Epoxide	90.8	7.5	93.1	8.4	92.1	13.1
Heptachlor	87.4	10.3	71.2	13.3	64.3	22.5
Methoxychlor	96.0	13.7	71.4	16.4	66.9	24.3
p-p' DDD	97.2	10.7	81.6	11.0	80.1	15.6
p-p' DDE	80.5	6.3	84.9	10.6	75.9	17.9
p-p' DDT	97.4	12.1	77.6	18.6	73.6	23.4
Decachlorobiphenyl(SS)	76.1	8.7	75.8	8.2	70.6	9.3
Tetrachloro-m-xylene (SS)	90.2	9.8	83.3	7.8	82.1	8.2

SS: Surrogate Standard

As seen in the Table 3.4, the percent recoveries are decreasing with increase in the particulate matter content of the sample, which is highest in surface water samples and lowest in spiked control matrix (DI). For extractions from DI, the average percent recoveries for all pesticides is 88.9 %, with a RSD value of 9.2% , whereas for ground water samples, the average of recoveries is 81.4% with 11.7% RSD. The average of recoveries is further decreased to 77.5 %, with 16.3% RSD for surface water samples. In the case of heptachlor, for example, the recovery is decreased by 16 % for ground water than that of DI. Moreover, the recovery is further lowered by 7% when the matrix was surface water. As stated, the reason for the differences in recoveries is the different amount of particulate matter content of these three types of water samples.

It is known that dissolved organic carbon may bind the analytes carrying them through the SPE material, thereby not allowing them to attach the stationary phase. In addition, dissolved organic carbon may saturate the active sites of the sorbent, as the particles of the bonded silica phase act as a mechanical filter retaining particles of suspended sediment. This will also affect relative recoveries. Large humic molecules may hinder the penetration of the elution solvent to the sorbent bound analytes. These factors usually result in decrease in the recoveries (Ridal et al., 1997; Lyytikainen et al., 2003).

To resolve this problem, acidification to pH 2 is widely applied in order to solubilize the small particles of insoluble salts of Mg, Al and Ca (Viana et al., 1996). However, such extreme pH values are not recommended as the chemical degradation of the analytes is fastened.

In literature, similar results were obtained for the effect of sample matrix on extraction efficiencies; showing the lowered recoveries for real samples than that of pure water without any particulate matter content. Lambropoulou et al. (2000) has used C_{18} bonded silica disks for the extraction of OPPs from different types of water samples. The common target analytes in this work

were parathion methyl, fenitrothion, malathion, fenthion, bromophos methyl, bromophos ethyl and fenamiphos. The authors have reported recoveries between 93 and 101 % for distilled water, 70-95 % underground, 74-87 % for river, 65-77 % for lake water samples. The values were all higher in distilled water compared to underground and surface waters.

Another study represents the results of interlaboratory comparison tests for SPE disk extraction and GC based detections (Senseman et al., 2003). In this study, 6 out of 7 laboratories have reported lower extraction efficiencies for pesticides in surface waters than in deionized water.

In Table 3.4., it is seen that the percent RSD values are the highest for surface water samples, ranging from 10.8 to 24.3 %, when compared with the ground water samples (with percent RSD values between 6.5-18.6%) and spiked control matrix (with percent RSD values between 3.9-16.8 %). The variations are due to the differences in particulate matter content among the samples collected, which are significantly changing for surface waters.

To evaluate the effect of matrix type on percent recoveries of chlorinated and phosphorus pesticides in water samples, a detailed discussion is necessary for obtained data set for Kumluca samples.

In this study, we wanted to confirm statistically the differences in between the recoveries obtained from different water samples. Therefore, the means of the percent recoveries were compared with one-way ANOVA test, using SPSS 13.0 software. It was observed that within 95.0% confidence interval, most of the pesticides have significantly different percent recoveries in different types of water samples, except for phosphamidon, malathion, methidathion, fenamiphos, azinphos-methyl, α-HCH, β-HCH, aldrin, heptachlor-endo, DDE and endosulfan sulfate. Further, Post-Hoc multiple comparison was used to test the difference between each pair of means. These comparisons were performed

for the average recoveries between the pairs of spiked control sample and well water samples; the spiked control sample and surface water samples; and surface and well water samples within a confidence level of 95 %.

The results of the comparisons for organophosphorus pesticides were presented in Table 3.5, for the pairs significant difference was observed. It should be reminded that the recoveries were decreasing in the order for DI (N=10), well water samples (N=31), and surface water samples (N=17) for all specified pesticides.

Table 3.5. Organophosphorus Pesticides with Significantly Higher Percent Recoveries for Different Types of Water Samples

Pairs Compared	Well Water	Surface Water
Spiked Control Matrix	Bromophos Ethly Chlorpyriphos Diazinon Fenitrothion Pirimiphos	Bromophos Ethly Chlorpyriphos Diazinon Fenitrothion Pirimiphos Bromophos Methly Dichlorvos Fenthion Parathion
Well Water	-	Bromophos Ethly Chlorpyriphos Diazinon Fenitrothion Pirimiphos Bromophos Methly Dichlorvos Fenthion Parathion

From the table one can easily observe that, among all the OPPs, bromophos-ethly, chlorpyriphos, diazinon, fenitrothion and pirimiphos have mostly been affected from sample matrix. The reason lies behind the solubilities of these pesticides; their solubilities (all below 70 mg/L) are lower than the other OPPs. As stated in Chapter I, the water solubility of a pesticide and K_{OC} values are inversely related. Pesticides with low water solubility have high K_{OC} values and tend to partition strongly into organic-rich environmental phases, leading sorption to particulate phase (Weiner, 2000). The log K_{OC} values of these pesticides were between 3.0-3.8, being the highest among all OPPs studied. As stated in previous paragraphs, the reason for the low recoveries of these pesticides in real environmental water samples is the particulate content in these samples, leading to the pesticide partitioning on the particulate phase rather than being trapped and eluted from extraction media.

In addition to these five pesticides, the recoveries of bromophos-methyl, dichlorvos, fenthion and parathion also differ between spiked control sample and surface water samples. Except dichlorvos, which has a solubility of 8000 mg/L, these pesticides also have solubilities lower than 100 mg/L and log K_{OC} values about 3.0.

The results of the statistical comparison tests for chlorinated pesticides are given in Table 3.6, where again significant differences between pairs were shown as in Table 3.5. The percent recoveries were decreasing in the order for DI, well water samples, and surface water samples for all the specified pesticides.

Table 3.6. Organochlorine Pesticides with Significantly Higher Percent Recoveries for Different Types of Water Samples

Pairs Compared	Well Water	Surface Water
Spiked Control Matrix	DDD DDT Dieldrin Endosulfan β-endosulfan Endrin Endrin Aldehyde γ-hch δ-hch Heptachlor Methoxychlor	DDD DDT Dieldrin Endosulfan β-endosulfan Endrin Endrin Aldehyde γ-hch δ-hch Heptachlor Methoxychlor
Well Water	-	Endrin

The OCPs; DDD, DDT, dieldrin, endosulfan, β-endosulfan, endrin, endrin aldehyde, γ-hch, δ-hch, heptachlor and methoxychlor have been affected from sample matrix indicated by the significant difference between recoveries of DI and real environmental aqueous samples. This can again be explained by the lower solubilities and higher log K_{OC} values of these pesticides. Slightly soluble analytes have been mostly associated with particulate matter and lead to the observed differences. In fact, all the 17 OCPs studied, have solubilities smaller than 1 mg/L and log K_{OC} values higher than 4.0 except γ-hch with a solubility of 7 mg/L and log K_{OC} value of 3.1. The 11 OCPs acting in identical manner in extraction can represent the behavior of OCPs.

Only endrin has different recoveries between ground and surface waters. It is clear that, whether the sample matrix contains low or high particulate matter content, OCPs have shown similar behavior, leading to a lowering in recoveries during SPE process.

3.1.2.5. Surrogate Standards

The surrogate standards were used to monitor the performance of sample preparation procedures for each sample. The application of surrogates in this work and calculation of their percent recoveries has been explained in Section 2.7. The surrogates were spiked to all samples, blanks, spiked control matrix and spiked sample matrix before the extraction, so as to give a final concentration of 0.5 µg/L for organophosphorus, 0.1 µg/L in chlorinated pesticides.

The percent recoveries of the surrogate standards are given in Table 3.4 together with the target analytes. The percent recoveries for surrogates used for chlorinated pesticides, decachlorobiphenyl and tetrachloro-m-xylene, were 74.2 and 85.2 % respectively. Tributyl phosphate, which was used as surrogate for phosphorus pesticides, has a percent recovery of 97.8 %. The percent recoveries of the surrogates were in the accepted limit, which is 70-130 % according to EPA (EPA Method 8000C). Moreover, the RSD values for all of the surrogate recoveries were lower than 10 %. This shows that, during the sample preparation steps the procedure was performed successfully.

3.1.3. QC/QA Tests during Analysis

3.1.3.1. Use of Standard Reference Materials

To check the accuracy of the measurements of GC-ECD system, which was used to determine the chlorinated pesticides, different SRMs (Standard Reference Material) from NIST were available in the market. It should be mentioned that, these SRMs does not related with real samples, but they have certain concentrations of some chlorinated pesticides in a suitable solvent. The analysis of these SRMs also used to determine the retention times of the analytes precisely. The studied Nist SRMs and the results are presented in

Table 3.7, shows the initial demonstration of the accuracy of the measurements, which compares the found concentrations with certified values. However, to check the ongoing accuracy, another standard solution from AccuStandards, EPA Method 508/608 Pesticide Standard Solution was used, as it contains almost all the analytes. The results of the analysis of this solution are presented in Table 3.8. The percent errors for 11 of the target chlorinated compounds were lower than 10 %, and all of them were below the 20 %. In the organic analysis, these results can be considered good and can be accepted as high accuracy.

Table 3.7. Initial Demonstration of the Accuracy of the Measurements with the Analysis of NIST SRMs (N=5).
Values in µg/mL.

	NIST-2261			**NIST-2273**			**NIST-2275**		
	Av.	**S.D.**	**Cert.**	**Av.**	**S.D**	**Cert.**	**Av.**	**S.D.**	**Cert.**
a hch							1.99	0.11	2.07
b hch							2.15	0.03	2.05
g hch	1.96	0.07	1.97						
d hch									
heptach	2.16	0.03	1.98						
aldrin									
hep-end	1.92	0.09	1.98						
endos							2.11	0.04	1.99
dieldrin	2.16	0.06	1.97						
dde	2.03	0.12	1.98	2.03	0.04	1.97			
endrin							2.19	0.11	2.01
b endos							2.09	0.08	2.03
ddd	1.78	0.22	1.99	1.79	0.05	2.01			
end ald									
end sulf							1.63	0.08	2.02
ddt	2.13	0.21	1.97	2.10	0.06	2.00			

Table 3.8. Demonstration of Ongoing Accuracy of the Measurements with the Analysis of EPA Method 508/608 Pesticide Standard Solution (N=31).

	Found (µg/mL)	**Certified (µg/mL)**	**St. Dev**	**% error**
a hch	95.6	100	4.7	-4.4
b hch	103.0	100	4.2	3.0
g hch	94.9	100	4.7	-5.1
d hch	80.4	100	5.4	-19.6
heptachlor	110.9	100	4.2	10.9
aldrin	91.7	100	5.4	-8.3
hep endo	92.7	100	5.8	-7.3
endos	168.5	200	6.8	-15.8
dieldr	226.1	200	20.0	13.1
dde	204.0	200	15.4	2.0
endrin	215.6	200	22.6	7.8
b endos	180.6	200	20.7	-9.7
ddd	664.1	600	60.6	10.7
en aldh	616.8	600	64.8	2.8
en sulfate	552.7	600	44.0	-7.9
ddt	536.4	600	55.8	-10.6

To check the accuracy of the measurements of GC-NPD system, SRMs (Standard Reference Material) from NIST were not available in the market. Therefore, the calibration curves, which were constructed by standard solutions prepared from pure solid or liquid standards, were verified by using 3 different pesticide mixture standard solutions (Mix Standart Solution-167, Mix Standart Solution-154, Mix Standart Solution-64) obtained form Dr. Ehrenstorfer. These solutions contain all the phosphorus pesticides, except fenamiphos. The concentrations of all analytes were 10 µg/mL in all standard solutions. The results of the analysis of these solutions are presented in Table 3.9. It can be concluded that the measurements were highly accurate for all of the analytes.

Table 3.9. Results of Standard Solutions for Accuracy Check

	MIX STD-167		MIX STD-154		MIX STD -64	
	N=18 (µg/mL)		N=14 (µg/mL)		N=13 (µg/mL)	
	Av	SD	Av	SD	Av	SD
DDVP	10,05	0,51			10,53	0,33
DIAZINON	10,62	0,49			10,01	0,51
PHOSPHAM					9,50	0,78
PARATHION			9,78	0,40		
FENITRO					9,90	0,40
PIRIM	10,23	0,56			10,42	0,38
MALATH	10,12	0,44	8,97	0,11	9,79	0,33
FENTHION			11,14	0,18		
CHLOPY	10,05	0,33			10,06	0,62
BROM-ME	9,66	0,34				
METHID	9,37	0,59				
BROM-ET	9,99	0,90				
AZIN-ME	9,61	1,05				

The EPA Method 508/608 Pesticide Standard Solution of OCPs and Mixed Pesticide Standard Solutions of OCPs were also used to monitor the stability of the GC-ECD and GC-NPD systems, respectively. For each 20 injections, EPA Pesticide Solution and at least one of the Mixed OCP Standard Solutions were analyzed. The results are given in Figure 3.2 and Figure 3.2 for OCPs and OPPs, respectively.

As seen from the figures, the readings of the solutions were constant throughout all the analysis period, in an acceptable degree of variation, within $\pm 2\sigma$ range. It can be concluded that the analysis systems were stable until the end of the analysis. Moreover, the calibration curves were still valid and were giving highly accurate results.

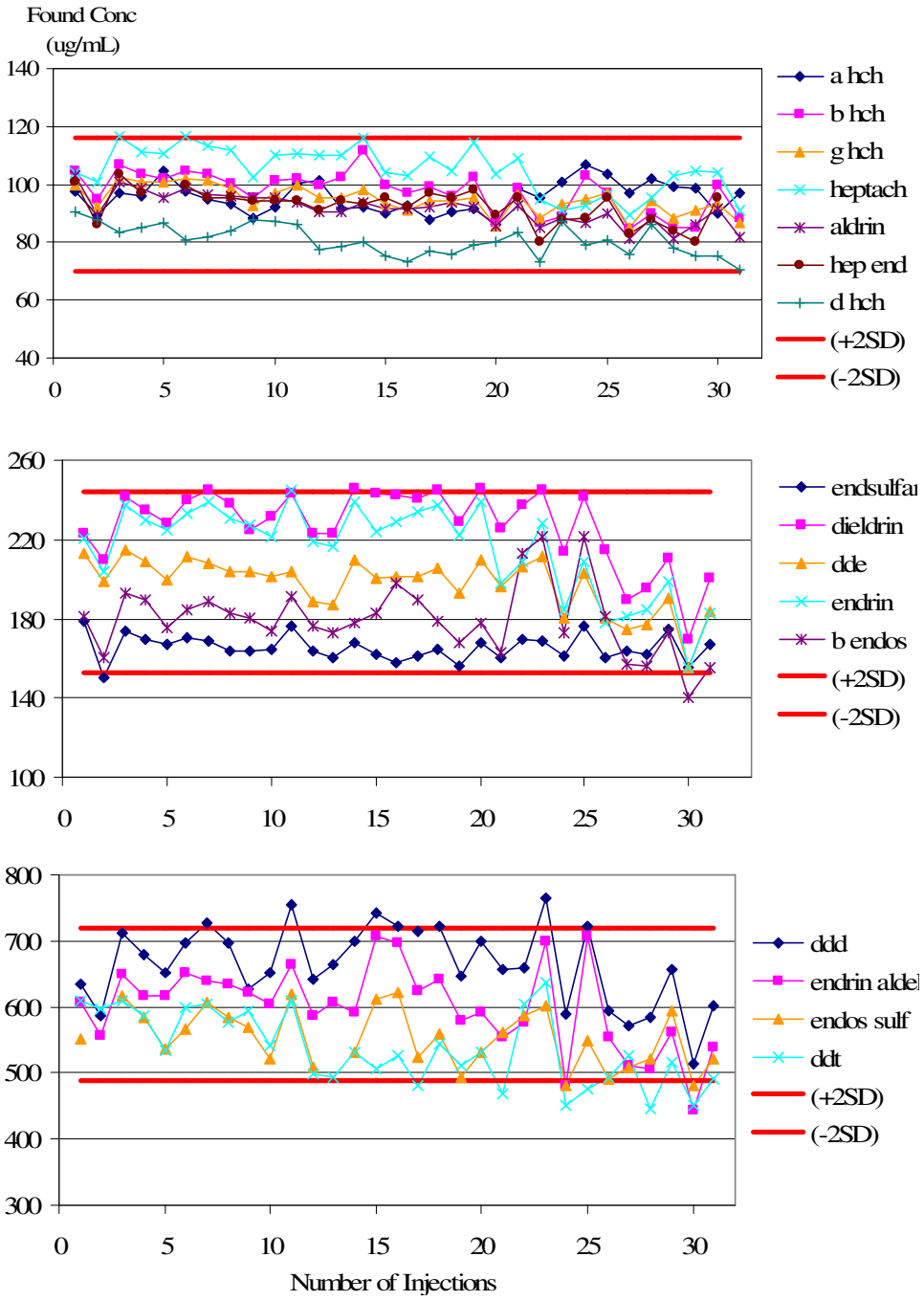


Figure 3.2. Monitoring the stability of GC-ECD System

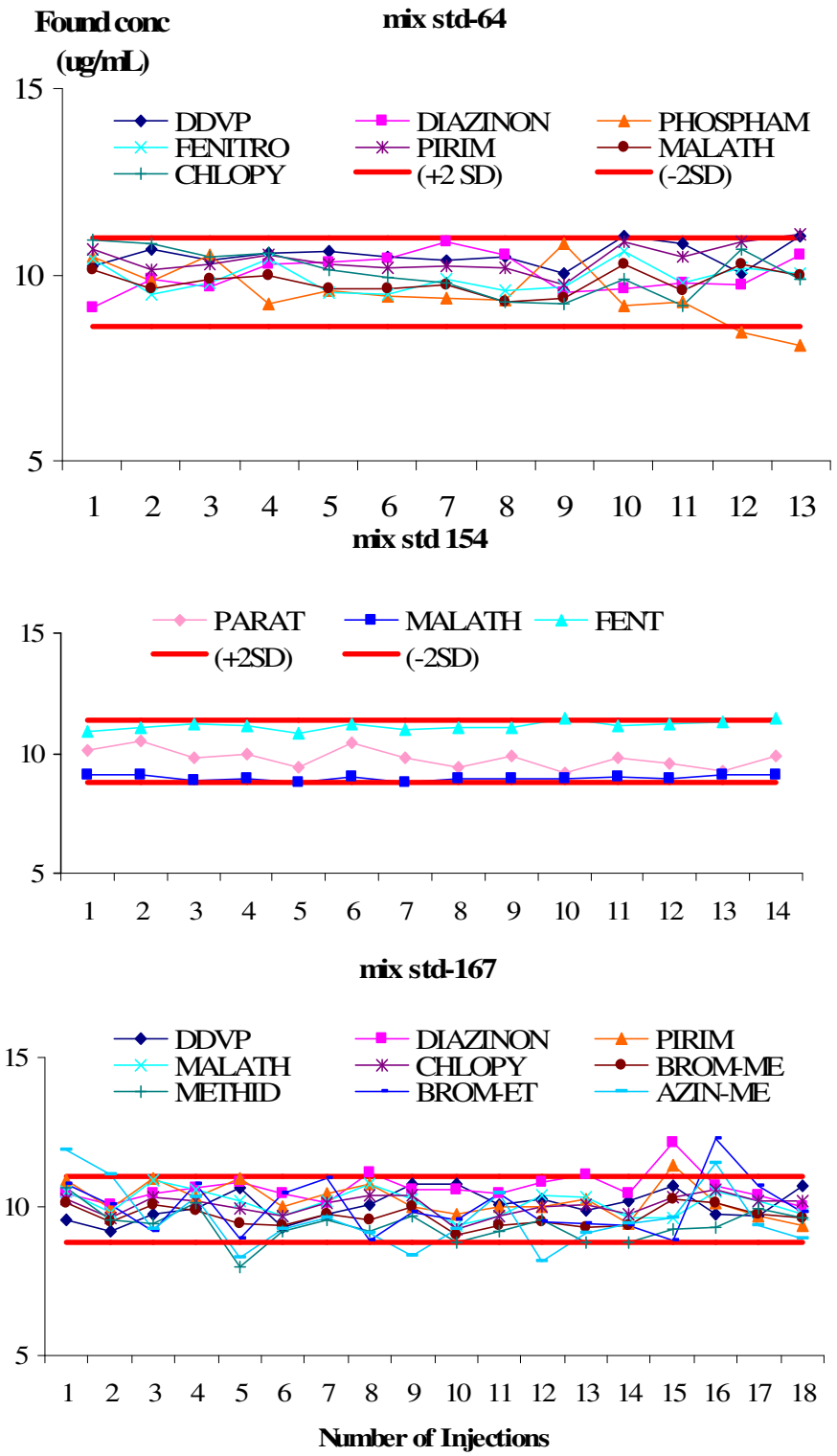


Figure 3.3 Monitoring the Stability of the GC-NPD System

3.1.3.2. Replicate Analysis

To check the repeatability of each measurement, the standards, SRMs, samples, spiked control matrices, spiked sample matrices and blanks were injected twice. All of the results presented in this work are the average results of these two replicates. The calibration curves were constructed by triple injection of the calibration standards. The RSD values for the replicate analysis for the samples are presented in Table 3.10. As seen, the repeatability of measurements is better than 10% for all of the analytes and samples and better than 5% for most of them. The use of internal standards may be the reason for this good precision in chromatographic analysis.

Table 3.10. Repeatability of Duplicate Measurements (N=40)

Pesticide	**Av. of % RSD**	**Pesticide**	**Av. of % RSD**
a hch	3.0	Azinphos-methyl	6.2
b hch	6.0	Bromophos-et	3.4
g hch	8.2	Bromophos-me	0.6
d hch	6.7	Chlorpyrifos	1.2
Aldrin	4.1	Diazinon	2.0
b-endosulfan	5.6	Dichlorvos	1.8
Dieldrin	5.4	Fenamiphos	5.2
Endosulfan	4.2	Fenitrothion	2.4
End. sulfade	5.6	Fenthion	-
Endrin	-	Malathion	2.2
Endrin Aldehyde	0.4	Methidathion	1.5
Hep.End Epox.	4.4	Parathion-methyl	0.9
Heptachlor	4.4	Phosphamidon	3.3
Methoxychlor	7.3	Pirimiphos-me	-
p-p' DDD	4.5	OPP Surr. Std.	2.3
p-p' DDE	5.9		
p-p' DDT	5.9		
OCP Surr. Std.	1.5		

3.2. Estimating the Uncertainty of the Measurements

Measurement uncertainty is a statistical parameter which describes the possible fluctuations of the result of a measurement (Meyer, 2007). In most cases, the *uncertainty* relates to the general concept of *doubt*. However, uncertainty of measurement does not imply doubt about the validity of a measurement; on the contrary, knowledge of the uncertainty implies increased confidence in the validity of a measurement (EURACHEM/CITAC Guide, 2000). A measurement result is complete only when accompanied by a quantitative statement of its uncertainty. The uncertainty is required in order to decide if the result is reliable for its intended purpose, to be able to compare different measurements, to establish traceability and to improve the analysis method.

The uncertainty of the measurement may arise from many possible sources such as uncertainties of masses and volumetric equipment, matrix effects and interferences, environmental conditions, approximations and assumptions, and random variations (EURACHEM/CITAC Guide, 2000).

The approach used for the estimation of uncertainty of measurements for the determination of pesticides in Kumluca environmental water samples was adapted from the guidelines of EURACHEM, which is a network of organizations in Europe with 32 member countries including Turkey, having the objective of establishing a system for the international traceability of chemical measurements and the promotion of good quality practices. Basic definitions and the approach used in the calculations are given in Appendix A.

3.2.1. Estimation of Uncertainty for Pesticide Analysis in Kumluca Environmental Water Samples

As stated, the EURACHEM guidelines were followed in the estimation of uncertainties in this study. The procedure explained in Appendix A will be followed and presented stepwise in the following sections.

3.2.2.1. Specification of the Measurand

The analytical procedure used is summarized by a flow diagram and presented in Figure 3.4. The measurand is defined as the pesticide concentration in the ground and surface waters of Kumluca.

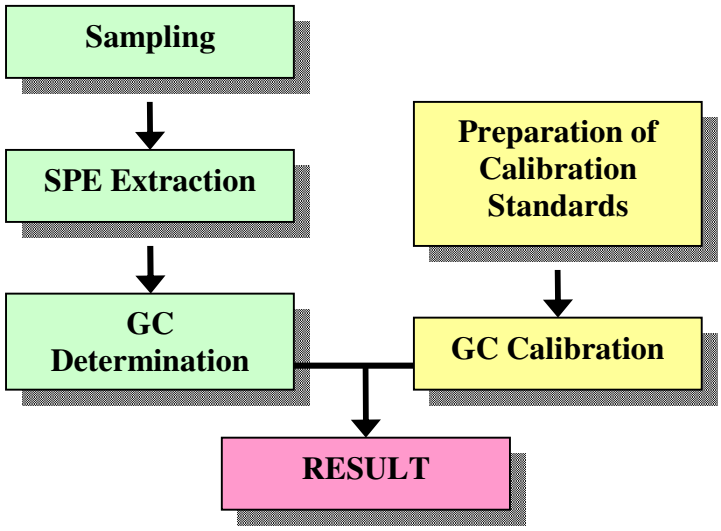


Figure 3.4. Flow Diagram of the Analytical Procedures

In Figure 3.5, the fishbone diagram is presented showing the factors affecting the calculation of analyte concentrations, which are the sources of measurement uncertainty.

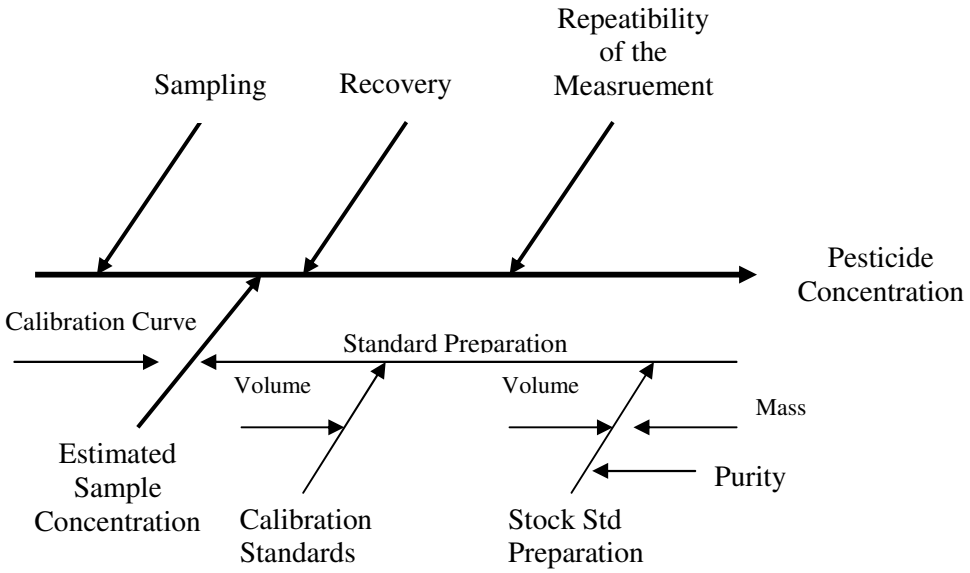


Figure 3.5. Fishbone Diagram for the Determination of Pesticides in Kumluca Environmental Water Samples

3.2.2.2. Identification of the Uncertainty Sources

The analyte concentrations in the samples were calculated with the formula given below:

$$C = SC \times F_{dil} \times \frac{1}{R} \qquad \text{(Eq.1)}$$

where,

C: final concentration of the analyte in the samples (ng/L)

SC: is the estimated analyte concentration obtained from the calibration curve in µg/mL for OPPs, ng/mL for OCPs

F_{dil}: Dilution factor arising from the 1 L sample to 1 mL after extraction procedure

R: Recovery of the analytes after extraction procedure

The uncertainty sources for the determination of pesticide concentrations are identified as, analyte concentration estimation, variation of the recovery of extractions, repeatability of the measurements. The effect of dilution factor operation on uncertainty calculations will be discussed in analyte concentration estimation factor.

The repeatability of the measurements should be included, which reflects the day-to-day variations of the analytical systems.

The sampling may bring some variation for the analysis results of field samples. To evaluate the effect of this variation, the uncertainty of the sampling is finally included in the uncertainty calculations. The combined uncertainty should include these sources;

$$u_{rel}(COM) = \sqrt{u_{rel}^2(smpl) + u_{rel}^2(SC) + u_{rel}^2(rep) + u_{rel}^2(R)} \qquad \text{(Eq.2)}$$

3.2.2.3. Quantification of Uncertainty Components

3.2.2.3.1. Uncertainty for Sampling, *u(smpl)*:

As stated in Appendix A.2.1., to calculate the uncertainty coming from sampling, the average of coefficient of variation values presented in Table 3.2 were used. The uncertainty arising from sampling replicates were calculated as being 0.021 for all pesticides.

3.2.2.3.2. Estimated Analyte Concentration in the Sample *u(SC):*

The main factors affecting the estimation of sample concentrations are the uncertainty arising from the preparation of the standards and the linear calibration curves.

$$u(SC) = \sqrt{u^2(stds) + u^2(cal)} \qquad \text{(Eq.3)}$$

The calculation of uncertainty components for the estimation of pesticide concentration is presented in the Appendix, A.2.2. Table 3.11 gives the calculated uncertainty components used and combined uncertainties for *u(SC)* for all pesticides.

Table 3.11. The Uncertainty Components and Uncertainty Values for the Estimation of the Pesticides Concentrations

	u(stds)	***u(cal)***	***u(SC)***
a hch	0.009	0.013	0.016
b hch	0.009	0.010	0.014
g hch	0.009	0.011	0.015
d hch	0.009	0.041	0.042
Aldrin	0.009	0.008	0.012
b-endosulfan	0.009	0.014	0.017
Dieldrin	0.009	0.012	0.015
Endosulfan	0.009	0.006	0.011
Endos. Sulf.	0.009	0.013	0.016
Endrin Ald.	0.009	0.031	0.032
Hep. Endo Ep.	0.009	0.008	0.012
Heptachlor	0.009	0.006	0.011
Methoxychlor	0.009	0.014	0.017
p-p' DDD	0.009	0.022	0.024
p-p' DDE	0.009	0.011	0.014
p-p' DDT	0,009	0.009	0.013
Azinphos-me	0.009	0.015	0.018
Bromophos-et	0.009	0.016	0.018
Chlorpyrifos	0.009	0.019	0.021
Diazinon	0.009	0.011	0.015
Dichlorvos	0.010	0.016	0.018
Fenamiphos	0.010	0.012	0.016
Fenitrothion	0.010	0.023	0.025
Malathion	0.009	0.021	0.023
Methidathion	0.009	0.035	0.037
Parathion-me	0.009	0.027	0.028
Phosphamidon	0.010	0.038	0.039

3.2.2.3.3. Repeatability of the Measurements, *u(rep):*

As stated in Appendix A.2.3, the uncertainty arising from the repeatability of the measurements are calculated using the CV (coefficient of variation) values for SRM readings, as these are the samples analyzed for the whole analysis period. The data set used in the calculation of *u(rep)* for all the pesticides is given in Table 3.12.

Table 3.12 Uncertainties for the Repeatability of Measurements

	CV	***N***	***u(rep)***
a hch	0.055	31	0.010
b hch	0.074	31	0.013
g hch	0.050	31	0.009
d hch	0.069	31	0.012
Aldrin	0.059	31	0.011
b-endosulfan	0.101	31	0.018
Dieldrin	0.084	31	0.015
Endosulfan	0.040	31	0.007
Endos. Sulf.	0.080	31	0.014
Endrin Ald.	0.108	31	0.019
Hep. Endo Ep.	0.063	31	0.011
Heptachlor	0.076	31	0.014
Methoxychlor	0.077	31	0.014
p-p' DDD	0.091	31	0.016
p-p' DDE	0.069	31	0.012
p-p' DDT	0.105	31	0.019
Azinphos-me	0.109	18	0.026
Bromophos-et	0.090	18	0.021
Chlorpyrifos	0.033	18	0.008
Diazinon	0.046	18	0.011
Dichlorvos	0.050	18	0.012
Fenamiphos	0.054	18	0.013
Fenitrothion	0.040	13	0.011
Malathion	0.043	18	0.010
Methidathion	0.016	14	0.004
Parathion-me	0.040	14	0.011
Phosphamidon	0.082	13	0.023

3.2.2.3.4. Recovery, $u(R_{av})$

The estimation of uncertainties for recovery component was given in Appendix A.2.4. The calculated uncertainty components and uncertainty values for recoveries for each pesticide are presented in Table 3.13

Table 3.13 Uncertainty Components and Uncertainty Values for the Extraction Recoveries of Pesticides

	R_{av}	$\frac{u(C_{Obs})}{C_{Obs}}$	$\frac{u(C_{Cert})}{C_{Cert}}$	$u(R_{av})$
a hch	0.810	0.022	0.014	0.021
b hch	0.938	0.012	0.014	0.017
g hch	0.830	0.018	0.014	0.019
d hch	0.824	0.027	0.014	0.025
Aldrin	0.707	0.028	0.014	0.022
b-endosulfan	0.758	0.035	0.014	0.028
Dieldrin	0.842	0.028	0.014	0.026
Endosulfan	0.717	0.027	0.014	0.022
Endos. Sulf.	0.818	0.022	0.014	0.021
Endrin Ald.	0.717	0.027	0.014	0.022
Hep. Endo Ep.	0.887	0.026	0.014	0.026
Heptachlor	0.723	0.022	0.014	0.019
Methoxychlor	0.732	0.025	0.014	0.021
p-p' DDD	0.800	0.027	0.014	0.024
p-p' DDE	0.817	0.024	0.014	0.023
p-p' DDT	0.768	0.026	0.014	0.022
Azinphos-me	0.720	0.033	0.014	0.026
Bromophos-et	0.873	0.029	0.014	0.028
Chlorpyrifos	0.765	0.019	0.014	0.018
Diazinon	0.868	0.024	0.014	0.024
Dichlorvos	0.892	0.022	0.015	0.024
Fenamiphos	0.657	0.030	0.015	0.022
Fenitrothion	0.841	0.019	0.015	0.020
Malathion	0.802	0.020	0.014	0.020
Methidathion	0.623	0.026	0.014	0.018
Parathion-me	0.878	0.025	0.014	0.025
Phosphamidon	0.968	0.028	0.015	0.030

The t-test is applied for the recoveries to see the deviation from unity, as explained in Appendix 2.4, When $t_{exp} > t_{crit}$, the recovery correction is included in the calculation of combined uncertainty (EURACHEM/CITAC Guide, 2000). The t_{crit} value for degrees of freedom of 47 at 95% confidence is 2.01. Except phosphamidon, it is observed that all the recoveries were significantly different than unity; therefore, recovery factor was included in the calculations. As significance has been detected, the observed concentration of the pesticide obtained was corrected with recovery. Therefore, uncertainty brought by this operation was included in the calculation of combined uncertainties.

3.2.2.4. Calculation of Combined Uncertainty

The combined uncertainty for the measurements is calculated by the Equation 2. Later, the corrected combined standard uncertainty is calculated as explained in Appendix 2.4. The comparison of the uncertainty contributions and the discussion about the estimation of uncertainty for the analysis of pesticides in Kumluca water samples will be presented in the following sections.

3.2.2.5. Calculation of Expended Uncertainty

After the recovery correction operation, the expended uncertainty *U(C)* is obtained by multiplying the corrected combined uncertainty ($u(COM)_{corr}$) with a coverage factor of 2, giving

$$U(C_{av}) = 2 \times u(COM)_{corr} \qquad \text{(Eq.4)}$$

3.2.2. An Example for the Estimation of Uncertainty: Estimation of Uncertainty for Dichlorvos

The two steps in the estimation of uncertainty are presented in Sections 3.2.2.1, (specification of the measurand) and 3.2.2.2 (identification of the uncertainty sources) are valid for dichlorvos (ddvp). Therefore, the calculations will be shown starting from third step; quantification of uncertainty components.

3.2.2.1. Quantification of Uncertainty Components for Dichlorvos

3.2.2.1.1. Uncertainty for Sampling, *u(smpl)* for Dichlorvos

As stated in Section 3.2.2.3.1,the *u(smpl)* is 0.021 for all pesticides.

3.2.2.1.2. Estimated Sample Concentration *u(SC)* for Dichlorvos

The *u(SC)* value given in Table 3.11 for ddvp is calculated using Equation 3., where the unceartainty for *u(stds)* are calculated as follows:

$$\frac{u(stds)}{C_{stds}} = \sqrt{\left(\frac{u(m)}{m}\right)^2 + \left(\frac{u(V_{100mL})}{V_{100mL}}\right)^2 + \left(\frac{u(P)}{P}\right)^2 + \left(\frac{u(V_{100\mu L})}{V_{100\mu L}}\right)_1^2 + \left(\frac{u(V_{500\mu L})}{V_{500\mu L}}\right)_1^2 + \left(\frac{u(V_{100\mu L})}{V_{100\mu L}}\right)_2^2 + \left(\frac{u(V_{500\mu L})}{V_{500\mu L}}\right)_2^2}$$

(Eq. 5)

The concentration of the standard solution was assumed to be the average of all calibration standard concentrations, which is 0.642 µg/mL.

Purity of ddvp is 98.5±1.0 %, leading to, $u(P) = 0.01/\sqrt{3} = 0.006$

The values and uncertainties for estimated sample concentration for ddvp are calculated as explained in Appendix A and summarized in Table 3.14.

Table 3.14. Values and Uncertainties for the Components of Estimated Sample Concentration Factor for Dichlorvos

Description	**Value *(x)***	**Standard uncertainty *u(x)***	**Relative Std. Uncertainty *u(x)/x***
mass	100 mg	0.173 mg	0.002
V_{100mL}	100 mL	0.499 mL	0.005
Purity	0.985	0.006	0.006
$(V_{100\mu L})_1$	20 µL	0.245 µL	0.012
$(V_{500\mu L})_1$	500 µL	1.162 µL	0.002
$(V_{100\mu L})_2$	64 µL	0.245 µL	0.004
$(V_{500\mu L})_2$	500 µL	1.162 µL	0.002

The values in the table are used for the calculation of *u(stds)* with Equation 5;

$$\frac{u(stds)}{0.642} = \sqrt{(0.002)^2 + (0.005)^2 + (0.006)^2 + (0.012)^2 + (0.002)^2 + (0.004)^2 + (0.002)^2}$$

u(stds)=0.010 µg/mL

For the calibration curve of ddvp, where n=6,

B_1= 6.313

B_0= -0.251

S= 0.122

S_{xx}= 2.832

The average concentration of ddvp calculated from calibration curve for sample extracts (p=13) is 0.097 µg/mL

$$u(cal) = \frac{S}{B_1}\sqrt{\frac{1}{p} + \frac{1}{n} + \frac{(c_0 - c_{av})^2}{S_{xx}}} \qquad \text{(Eq. 6)}$$

$$u(cal) = \frac{0.122}{6.313}\sqrt{\frac{1}{13} + \frac{1}{6} + \frac{(0.097 - 0.642)^2}{2.832}} = 0.011 \text{ µg/mL}$$

Combining *u(stds)* and *u(cal)* to give *u(SC)* (Eq. 3)*;*

$$u(SC) = \sqrt{0.010^2 + 0.011^2} = 0.015 \text{ µg/mL}$$

3.2.2.1.3. Repeatability of the Measurements, *u(rep)* for Dichlorvos

The mix-std 167 was used to calculate the repeatability of ddvp concentration measurements. As given in Table 3.12, the percent RSD obtained for this pesticide is 5.03%, for 18 measurements. Therefore,

$$u(rep) = 0.050 / \sqrt{18} = 0.012 \text{ µg/mL}$$

3.2.2.1.4. Recovery, *u(R)* for Dichlorvos

Combining the standard deviations for spiked and unspiked sample concentrations for ddvp, $u(C_{Obs})/C_{Obs}$ is obtained as 0.022. The $u(C_{Obs})/C_{Obs}$ contribution is calculated similar to the calculation of the *u(stds)*, using the formula given for OPPs in Appendix 2.2.1, and found to be 0.015. The percent recovery of ddvp was 89.2%. Therefore the $u(R_{av})$ is calculated as follows;

$$u_{rel}(R_{av}) = \frac{u(R_{av})}{R_{av}} = \sqrt{\left(\frac{u(C_{Obs})}{C_{Obs}}\right)^2 + \left(\frac{u(C_{Cert})}{C_{Cert}}\right)^2} \qquad \text{(Eq. 7)}$$

$\frac{u(R_{av})}{0.892} = \sqrt{(0.022)^2 + (0.015)^2}$, leading $u(R_{av})$=0.024, as given in Table 3.13.

The t-test is applied to decide weather the recovery factor should be included or not in combined uncertainty.

$t_{\exp} = \frac{|1 - 0.892|}{0.024}$ = 4.58. This value is higher than the critical value of 2.01; therefore the recovery factor will be included in further calculations.

3.2.2.2. Combined Uncertainty for Dichlorvos

First, the combined uncertainty is calculated with the recovery component, as given in Equation 2.

The components are summarized in Table 3.15., with average dichlorvos concentration of 0.097 µg/mL among all data set, without recovery correction.

Table 3.15. Uncertainty Components for Combined Uncertainty of ddvp

Description	**Value *(x)***	**Standard uncertainty *u(x)***	**Relative Std. Uncertainty *u(x)/x***
Sampling Rep.	1	0.021	0.021
SC	0.097µg/mL	0.015	0.155
Repeatability	1	0.012	0.012
Recovery	0.892	0.024	0.027

Using Equation 2, the combined uncertainty is calculated as follows;

$$u_{rel}(COM) = \sqrt{(0.021)^2 + (0.155)^2 + (0.012)^2 + (0.027)^2} = 0.159$$

Than the recovery correction operation is performed on combined uncertainty; as the final concentration (C_{corr}) is obtained after the recovery correction;

$$C_{corr} = C/R_{av} \qquad \text{(Eq.8)}$$

Using Equation 8, $C_{corr} = 0.098/0.892 = 0.110$ µg/L

The uncertainty for the operation in Equation 8 is calculated as follows;

$$\frac{u(COM)_{Corr}}{C_{Corr}} = \sqrt{\left(\frac{u(COM)}{C_{av}}\right)^2 + \left(\frac{u(R_{av})}{R_{av}}\right)^2} \qquad \text{(Eq. 9)}$$

$$u(COM)_{Corr} = 0.110 \times \sqrt{(0.159)^2 + (0.027)^2} = 0.018 \text{ µg/mL.}$$

3.2.2.3. Expanded Uncertainty for Dichlorvos

The expanded uncertainty (Eq. 4) can now easily be calculated, using a *k* value of 2, for 95 % confidence interval.

$U(C)=2\times u(COM)_{corr}= 2 \times 0.018 = 0.036$ µg/L

Therefore, the average concentration of dichlorvos in the samples should be reported as follows;

*Concentration of Dichlorvos: (0.110 ± 0.036) µg/mL**
** The reported uncertainty is an expanded uncertainty calculated for 95% confidence level*

3.2.3. Evaluation of Uncertainties of Pesticide Measurements in Kumluca Environmental Water Samples

The uncertainty components and calculated combined uncertainties for all pesticides analyzed in Kumluca environmental water samples are presented in Table 3.16. It should be emphasized here that, the uncertainties obtained for OCPs are for concentrations in the range of ng/L, whereas the ones for OPPs are in the µg/L levels. The higher combined uncertainty values for OCPs should be considered accordingly.

Table 3.16. Uncertainty Components Used for the Calculation of Combined Uncertainties

	u(smpl)	$\frac{u(SC)}{C_{Av}}$	***u(rep)***	$\frac{u(R_{av})}{R_{av}}$	***u(COM)$_{corr}$***
Organochlorine Pesticides					
a hch	0.021	0.003	0.010	0.026	0.294
b hch	0.021	0.004	0.013	0.018	0.121
g hch	0.021	0.015	0.009	0.023	0.052
d hch	0.021	0.039	0.012	0.031	0.083
Aldrin	0.021	0.001	0.011	0.031	0.617
b-endosulfan	0.021	0.005	0.018	0.037	0.278
Dieldrin	0.021	0.006	0.015	0.031	0.149
Endosulfan	0.021	0.010	0.007	0.030	0.235
Endos. Sulf.	0.021	0.001	0.014	0.026	0.716
Endrin Ald.	0.021	0.010	0.019	0.030	0.235
Hep. Endo Ep.	0.021	0.008	0.011	0.029	0.088
Heptachlor	0.021	0.001	0.014	0.026	1.177
Methoxychlor	0.021	0.010	0.014	0.029	0.120
p-p' DDD	0.021	0.014	0.016	0.030	0.109
p-p' DDE	0.021	0.009	0.012	0.028	0.094
p-p' DDT	0.021	0.003	0.019	0.029	0.283
Organophosphorous Pesticides					
Azinphos-me	0.021	0.193	0.026	0.036	0.026
Bromophos-et	0.021	0.352	0.021	0.032	0.021
Chlorpyrifos	0.021	0.367	0.008	0.023	0.028
Diazinon	0.021	0.273	0.011	0.028	0.021
Dichlorvos	0.021	0.153	0.012	0.026	0.017
Fenamiphos	0.021	0.130	0.013	0.034	0.026
Fenitrothion	0.021	0.466	0.011	0.024	0.030
Malathion	0.021	0.686	0.010	0.025	0.028
Methidathion	0.021	0.456	0.004	0.030	0.059
Parathion-me	0.021	0.417	0.011	0.028	0.032
Phosphamidon	0.021	0.558	0.023	0.031	0.040

(u(rep): Uncertainty for repeatability of the measurements; u(smpl): Uncertainty for sampling; u(SC): Uncertainty for estimated analyte concentration; u(R): Uncertainty for recovery, u(COM): Combined Uncertainty)

The data set in the table can be visualized in Figure 3.6 for OPPs.

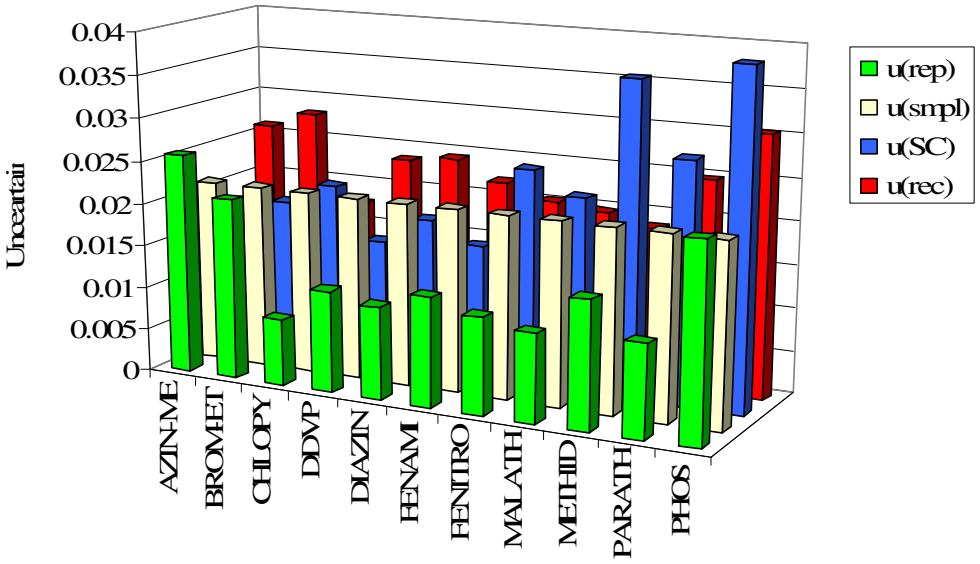


Figure 3.6. Uncertainty Components of OPPs
(u(rep): Uncertainty for repeatability of the measurements; u(smpl): Uncertainty for sampling; u(SC): Uncertainty for estimated analyte concentration; u(rec): Uncertainty for recovery)

From Figure 3.6., it is seen that the uncertainty arising from the repeatability of the measurements has generally the lowest contribution to total uncertainty. This is due to stability of GC-NPD system through all analysis period.

The uncertainty arising from the extractions, as indicated by recovery component is generally highest for OPPs. As seen in Table 3.4., the RSD values for recoveries are higher, especially in surface water samples. Therefore, the uncertainties of recovery component for some OPPs are high, such as azinphos-methyl, bromophos-ethyl and phospamidon.

Sampling replicates seem to have considerable contribution on total uncertainty. In Table 3.2, it is shown that the replicate samples have small

variation. As the number of data points (N=15) in the calculation of the uncertainty of this factor was low, its contribution became somewhat higher.

The high contribution of uncertainty arising from estimation of analyte concentration in total uncertainty of phosphamidon and methidathion arises from the calibration uncertainties. The calibration curves of these pesticides have linear regression coefficients of 0.9985 and 0.997, respectively (Figure 2.11). With these correlation factors, it is clear that the calibration curves obtained for these pesticides have poor fit on linearity among all OPPs, increasing the uncertainty for calibration.

The uncertainty components of OCPs are presented in Figure 3.7.

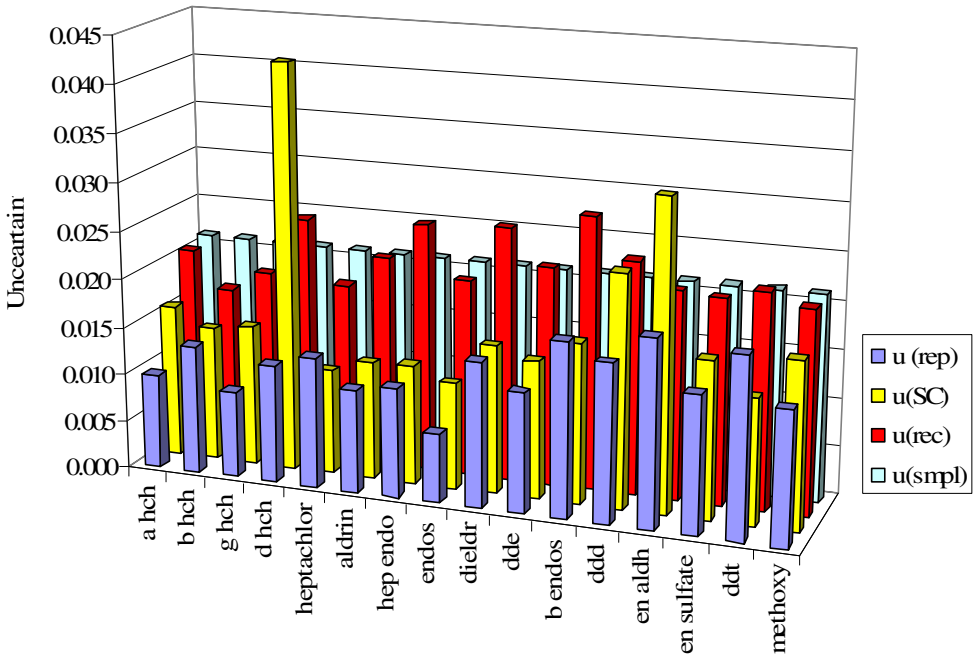


Figure 3.7. Uncertainty Components of OCPs
(u(rep): Uncertainty for repeatability of the measurements; u(smpl): Uncertainty for sampling; u(SC): Uncertainty for estimated analyte concentration; u(rec): Uncertainty for recovery)

For OCPs, the uncertainty from repeatability of the measurements has generally the lowest contribution to total uncertainty, similar to OPPs. The high uncertainties for estimation of sample concentrations for δ-HCH, endrin aldehyde and DDD are again due to calibration curves with poor fit on linearity. Sampling uncertainty has higher contribution to combined uncertainty than OPPs, as other factors have low contributions. The uncaertainty arising from extractions (*u(rec)*) is highest for β-endosulfan, heptachlor endo and dieldrin.

3.3. Evaluation of Data Set

In this section, produced data will be evaluated using different statistical treatments. In this way we will try to find the answers to the concentrations, occurrences, sources and effects of pesticides observed in surface and underground waters of Kumluca region.

3.3.1. Concentrations of Chlorinated Pesticides in Kumluca Environmental Water Samples

The summary of the data set obtained in whole study period for Organochlorine Pesticides (OCPs) in ground water (55 samples in total) and surface water (22 samples in total) samples are presented in Table 3.17. The values presented are for the concentrations above the Limit of Quantification, which is determined as the concentration at S/N value of 10.

Table 3.17. Summary Statistics for Organocholorine Pesticides (values in ng/L)

Pesticide	GW Av (% RSD) min-max N	SW Av (% RSD) min-max N	Pesticide	GW Av (% RSD) min-max N	SW Av (% RSD) min-max N
α-hch	5.7 (60.6) 0.2-10.1 11	8.2 (129.9) 0.4-32.6 8	Endrin	BLOQ	BLOQ
β- hch	3.6 (88.1) 0.6-10.8 15	2.7 (128.7) 0.2-8.6 5	Endrin Aldehyde	4.7 (191.2) 0.6-30.0 10	BLOQ
γ- hch	1.2 (82.0) 0.7-3.3 6	BLOQ	Hep. Endo Epoxide	1.0 (55.7) 0.4-2.2 12	3.4 (107.1) 0.3-8.3 6
δ- hch	1.9 (160.2) 0.3-6.5 4	0.5 (67.5) 0.2-0.9 3	Heptachlor	26.5 (150.5) 2.4-72.4 3	BLOQ
Aldrin	11.5 (166.1) 0.3-76.4 27	14.4 (198.7) 0.6-98.4 11	Methoxychlor	2.1 (40.2) 1.1-3.6 6	2.7 (95.2) 1.0-9.6 10
β-endosulfan	2.2 (176.7) 0.4-10.9 7	6.4 (146.2) 0.8-28.1 10	p-p' DDD	2.2 (74.5) 0.5-5.0 7	1.9 (69.2) 0.6-3.5 4
Dieldrin	1.9 (74.3) 0.5-3.9 14	5.3 (218.4) 0.5-37.9 10	p-p' DDE	1.5 (85.5) 0.4-3.2 4	2.5 (33.4) 1.8-3.6 4
Endosulfan	2.9 (125.8) 0.2-13.9 35	3.9 (125.3) 0.3-17.9 19	p-p' DDT	3.8 (107.9) 0.7-16.1 13	7.9 (213.4) 0.4-57.9 11
Endosulfan sulfate	4.8 (209.3) 0.5-52.5 28	31.7 (164.5) 0.6-188.1 20			

GW: Ground Water, SW: Surface Water, N: Number of Observations,
BLOQ: Below Limit of Quantification

The concentration levels (ng/L, ppt) presented here seems to be very low for any organic pollutant analysis with these analytical systems. Therefore, before discussing the actual levels of these pollutants, calculations of the concentrations will be explained.

The calibration curves were prepared with ng/mL (ppb) level concentrations. The samples were collected as 1 L and at the end of the extraction procedure the final volume was 1 mL. Therefore, the original samples have been concentrated 1000 fold, and the concentrations of the analytes were increased to the limits of quantification of the analysis systems.

The observed low concentration levels of OCPs in environmental water samples are very common in literature and also reported for different regions of the world (Iwata et al, 1995; Tanabe at al., 2001; Golfinopoulus et al, 2003; Risatto et al., 2006; Zhou et al., 2006).

The high percent RSD values indicate high variations from one sampling point to another on the observed concentrations. This is an expected trend in environmental studies, as the concentrations of the pollutants may vary widely from one sample to other, depending on the factors affecting the sampling region. As a matter of fact the reason why this study is undertaken is to understand these external factors. Considering the high variations and mostly the low concentrations, it can be stated that in Kumluca environmental water samples, the chlorinated pesticides were in very low concentrations, except a few extremes for certain sampling points.

In general, the concentrations of OCPs were lower than the maximum allowable limit for a single pesticide by EU directive (EU Council Directive 98/83/EC, 1998) which was 100 ng/L. The highest concentration was 188.1 ng/L and observed for endosulfan sulfate in a surface water sample in fall

season. The next pesticide with the high concentration was aldrin (98.4 ng/L) observed for a similar sample. This was also close to the limit value. However, these concentration extremes were not common and they should be handled carefully during the evaluation of the results.

Heptachlor seems to have the highest average concentration (26.5 ng/L) in ground water well samples. However, this value was due to a single sample with a high concentration (72.4ng/L) and the evaluation of this result will be given in the following paragraphs. Excluding this case, aldrin has the highest average concentration (11.5 ng/L) and the highest observed maximum concentration (76.4 ng/L) among all the other pesticides in the well water samples.

In surface water samples, besides the maximum concentration observed, endosulfan sulfate has also the highest average concentration (31.6 ng/L). The average concentration of this analyte in well waters is only 4.8 ng/L. This trend can be further generalized to all data set; the surface waters are more polluted than ground water. Only DDD, β and δ HCH has higher average concentrations in ground waters than surface waters.

Figure 3.8 shows the distribution of average OCP concentrations among surface and ground water samples for both sampling campaigns, which are given in Table 3.17.

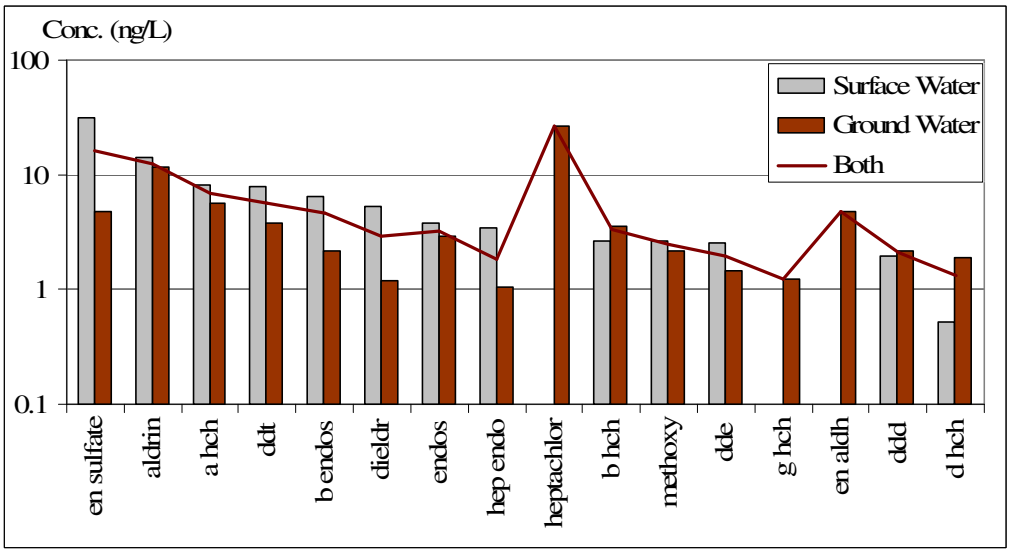


Figure 3.8. Average Concentrations of Organochlorine Pesticides in Different Types of Water Samples

The concentration of aldrin in surface waters is also high (14.4 ng/L). This pesticide has the highest concentration for ground water samples (11.5 ng/L) followed by α-HCH (Hexachlorocyclohexane) (5.7 ng/L). The well waters have also endosulfan sulfate and endrin aldehyde in relatively high concentrations.

The percentage of number of samples with a certain pesticide gives a general idea about the pollution and helps to evaluate the observed concentrations. The percent occurrences, i.e. the frequency of detection, of OCPs for all study period were calculated and given in Figure 3.9, where the values are presented for each type of water samples.

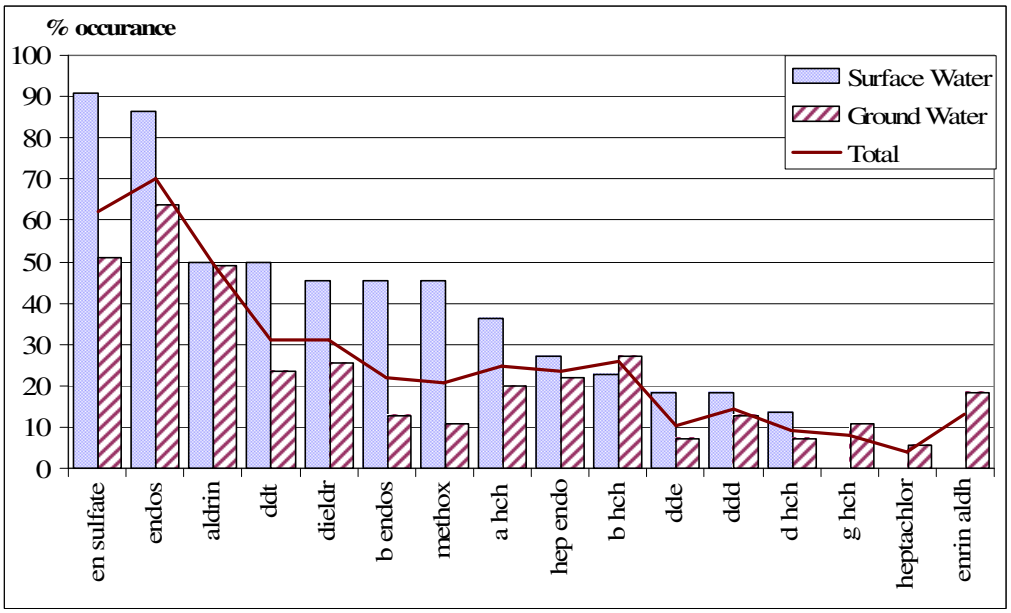


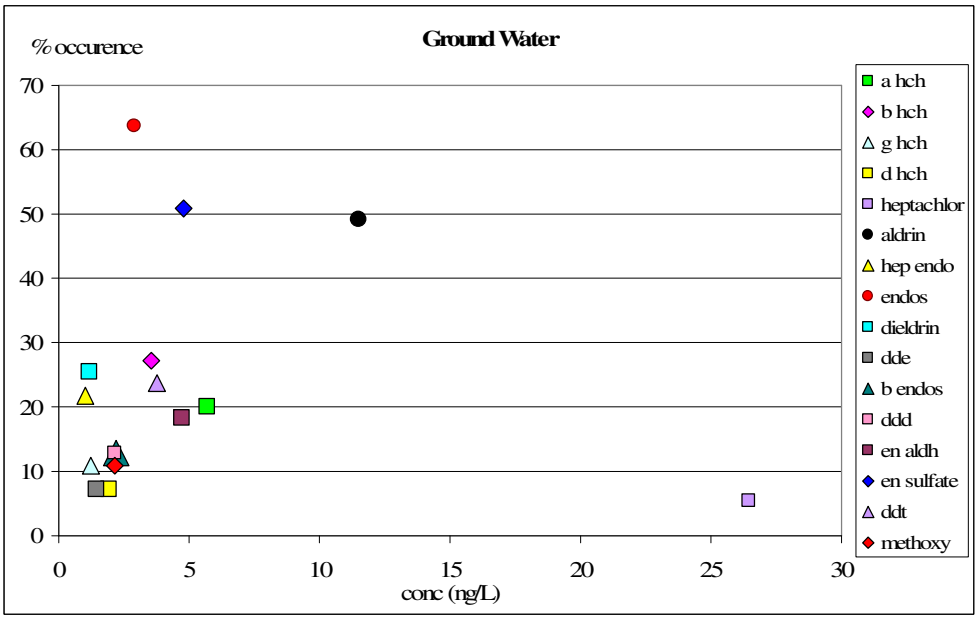
Figure 3.9 Percent Occurrences of Organochlorine Pesticides

From Figure 3.9, it can be seen that heptachlor has the lowest percent occurrence (5 %) for well samples. This value means that this pesticide was observed only in 3 samples. However, among all the chlorinated pesticides, it has the highest average for well samples (26.5 ng/L). This is due to the high concentration (72.4 ng/L) observed for a single sample in fall season and it shows a clear indication that this pesticide has applied around the well, maybe for domestic purposes as there is strict regulations for the application of the most of the chlorinated pesticides in agricultural products. Without this extreme, the average value would be 3.5 ng/L.

Pesticides were detected more frequently in surface water samples than in ground water samples. In Kumluca surface water samples, endosulfan sulfate and endosulfan were the most commonly observed pesticides with percent occurrences of 91 % and 86 %, respectively. These values were lowered to 51% for endosulfan sulfate and 64% for endosulfan in ground water samples, which are still the highest among other OCPs. Aldrin has been detected in similar frequencies for both surface (50 %) and well (49 %) samples. As it is related with endosulfan, the occurrences of β-endosulfan should also be noted; this pesticide was detected in 45 % of surface, and only 13 % in ground water samples.

The high occurrence of endosulfan sulfate in surface waters indicates that, the observed high concentrations are not extreme cases and the average concentrations were common levels of this analyte.

Figure 3.10 combines the average concentrations and percent occurrences. In ground water samples, the extreme case for heptachlor has already been described. Aldrin has a similar trend with endosulfan and endosulfan sulfate with high occurrences and moderate concentrations. Dieldrin, heptachlor endo epoxide, α-HCH, β-HCH, DDT and endrin aldehyde has relatively high percent occurrences but low concentrations. β-Endosulfan, DDD, δ-HCH, γ-HCH, methoxychlor and DDE have both low detection of frequencies and concentrations.



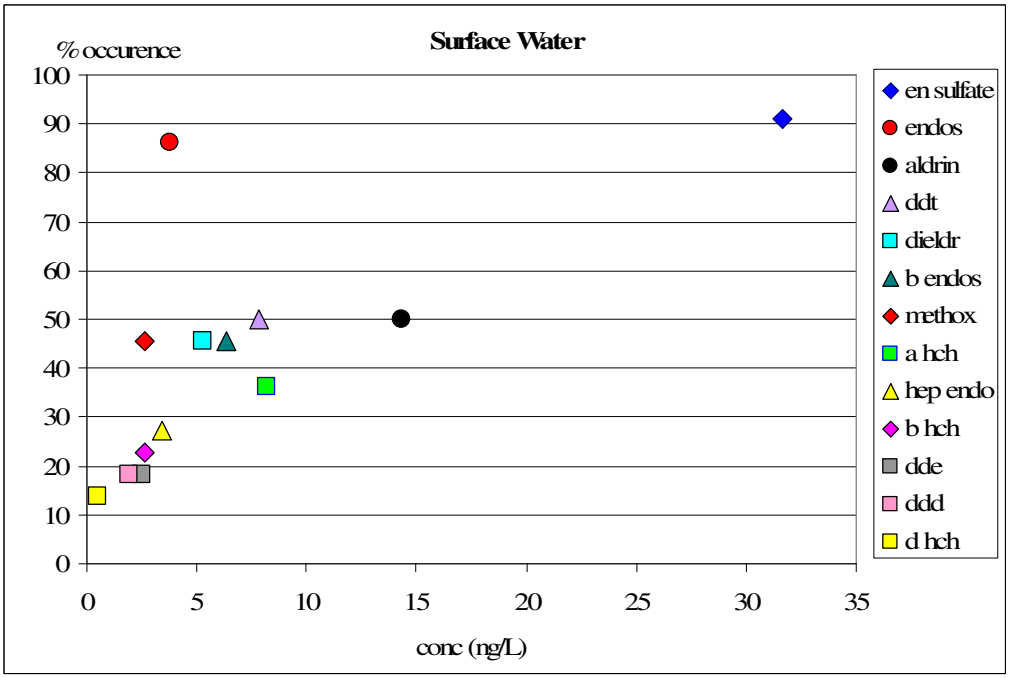


Figure 3.10. Average Concentrations and Percent Occurrences of OCPs

In surface waters, endosulfan sulfate displays an extraordinary case with high percent occurrence and concentrations. Endosulfan also has high occurrence but with low concentrations. Aldrin with slightly high concentration has moderate percent occurrence. Methoxychlor, dieldrin, β-endosulfan, DDT and α-HCH have moderate occurrence and low concentrations. The other pesticides have both low percent detections and concentrations.

Endosulfan and its Derivatives

Endosulfan is used in the cultivation of vegetables, fruits, paddy, cotton, tea, coffee, tobacco and timber crops. Worldwide use of endosulfan increased with the bans or restrictions in use of the more persistent organochlorine pesticides like DDT and endrin. It should be noted that, although the use of most of the chlorinated pesticides were banned in our country since 1980s, the use of endosulfan is still legitimate. In Kumluca, it is still commonly used as insecticide for tomato production in both greenhouses and orchards.

Technically endosulfan is a mixture of two isomers, alpha (α)-endosulfan and beta (β)-endosulfan in the ratio 8:2. Endosulfan can be broken down by photolysis, hydrolysis and bio degradation to endosulfan sulphate. This is the main degradation product of both isomers, which is equally toxic and itself is more persistent in the environment (Usha and Harikrishnan, 2005). In water endosulfan has a half life of 35 to 150 days (Romeo and Quijano, 2000), whereas the endosulfan sulfate with a half life higher than 200 days (Guerin, 2001).

In Figure 3.10, it is seen that α-endosulfan and endosulfan sulfate have high occurrences in both types of water samples (86 and 91 % for surface, 64 and 51 % for ground water samples, respectively). This is due to wide application of endosulfan in Kumluca. However, they have moderate concentration levels (below 5 ng/L) in ground water samples. Although it is detected in 45% surface

water samples, and 13 % of well samples the concentrations of β-endosulfan in both types of water samples were also moderate (lower than 6.5 ng/L). This is due to low solubility and high Koc values of these substances. Once released, they tend to accumulate onto soil instead of being dissolved. However, endosulfan sulfate has higher concentration levels in surface water samples. This indicates the degradation of endosulfan via photolysis or microorganisms in surface waters and surface run off from fields after degradation, as it has longer half life than its parent compound.

The relative concentration of the degradation product endosulfan to total endosulfan compounds (α- and β- isomers and endosulfan sulfate) can be used to assess the degradation or how recent the endosulfan contamination occurred. The concentration ratio of (endosulfan sulfate)/(total endosulfan compunds) lower than 0.5 indicates the recent use of endosulfan, at least as far as its half life (150 days).

In 24 of all ground water samples, endosulfan sulfate and at least one of the endosulfan isomer were detected. In surface waters, the number of samples in which the parent compounds and endosulfan detected together was 20. These data points are used to calculate the ratio between the concentrations of endosulfan sulfate to total endosulfan in order to assess the recent or past use of endosulfan in Kumluca. The results are given in Figure 3.11 where the concentration ratio was plotted against number of samples.

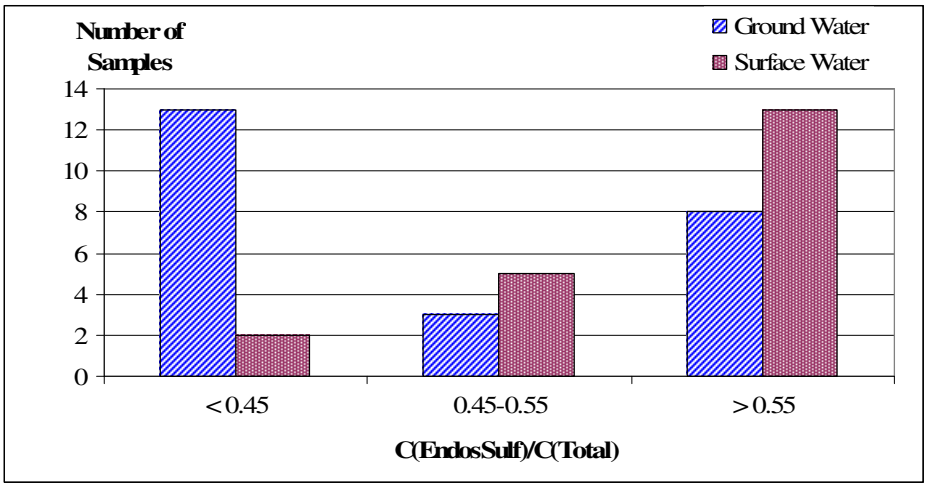


Figure 3.11. Ratio of Total Endosulfan to Endosulfan Sulfate

In 14 of the ground water samples the ratio was equal or lower than 0.5. This indicates definite use of endosulfan around these wells at least as far as its half life. As this pesticide has been used commonly more than a decade in the region, slightly higher ratios than 0.5 does not mean that endosulfan was not applied; instead they may reflect the cumulative effect of pollution, with higher endosulfan sulfate concentrations. In 8 of the samples, the dominant endosulfan sulfate concentrations indicate the past (longer than 150 days) use of endosulfan.

In most of the surface water samples, the ratio is higher than 0.5. This is due to the conversion of endosulfan to endosulfan sulfate by photo- and bio-degradation (Awasthi et al., 2003) in Kumluca surface waters.

Aldrin and Dieldrin

Aldrin and dieldrin are insecticides with similar chemical structures. They are both included in the high priority list of POPs. From the 1950s until 1970, aldrin and dieldrin were widely used pesticides for crops like corn and cotton. Because of concerns about damage to the environment and potentially to human health, their use has been banned since 1980s in our country. As a

result, the primary source of aldrin and dieldrin to the environment should be the past agricultural use (U.S. Department of Health and Human Services, 2002a).

Aldrin was classified as moderately persistent meaning its half-life in soil ranged from 20-100 days. Sunlight and bacteria change aldrin to dieldrin. They bind tightly to soil and slowly evaporate to the air. Dieldrin in soil and water breaks down very slowly (U.S. Department of Health and Human Services, 2002a).

As seen in Figure 3.10, similar to endosulfan sulfate, aldrin also has high occurrence in surface water (50 %) and ground water (49 %) samples and relatively high average concentrations for surface water (14.4 ng/L) and ground water (11.5 ng/L). However, dieldrin has moderate detection frequencies (45 % for surface, 25 % for ground water) and slightly lower concentrations (5.3 g/L for surface, 1.2 g/L for ground water). This shows the slow degradation of aldrin to dieldrin in the region.

According to local authorities, aldrin has been widely used in the region for cotton production before 1990s. At almost all points where data is available (8 surface, 8 ground water data points) concentration ratio of aldrin to dieldrin is higher than unity. The local authorities strictly claim that aldrin has not been used since 1990s in the region. Therefore, the observed presence of aldrin can be explained by its wide use in the past and slow degradation to dieldrin in Kumluca environment.

In literature, the higher concentrations of aldrin than dieldrin are commonly reported in environmental water samples for Turkey and different parts of the world, where use of aldrin has been banned in for decades (Ayas et al., 1997; Espigares et al., 1997; Hung and Thiemman, 2002; Golfinopoulos et al., 2003; Bakan and Arıman, 2004; Erkmen and Kolankaya, 2006).

Hexachlorocyclohexane (HCH) Isomers

HCHs are one of the most widely used and most readily detected organochlorine pesticides in environmental samples. The relatively high volatility of HCH has led to global transport, even into formerly clean locations such as the Arctic (Walker et al.1999). When compared with other OCPs, lindane has highest solubility and lowest Koc values, indicating its higher potential for the contamination of water sources.

Compositions of the isomers are considered as a useful tool for understanding the formulation types, origin, transport pathways, etc (Iwata et al., 1995). This tool is widely used in the pollution studies in sediments and soils, by evaluating the distribution of the isomers in samples (Doong et al., 2002; Zhang et al., 2004; Zhou et al., 2006). However, in this study, the data set produced is not available to derive such conclusions sample by sample, as there are missing points. These isomers were not detected together in most of the samples. Therefore, at least, to characterize the general situation in Kumluca, the following conclusions will be based on the average concentrations of the pollutants among the samples.

The distribution of HCH isomers in Kumluca samples are presented in Figure 3.12. as seen, δ-isomer has similar contribution for both types of water samples. For ground water samples, the major isomer is β-HCH (53 %). The α-isomer makes up 38 % of this type of samples. It is seen that γ-isomer and some portion of β -isomer is converted to α-isomer in surface water samples.

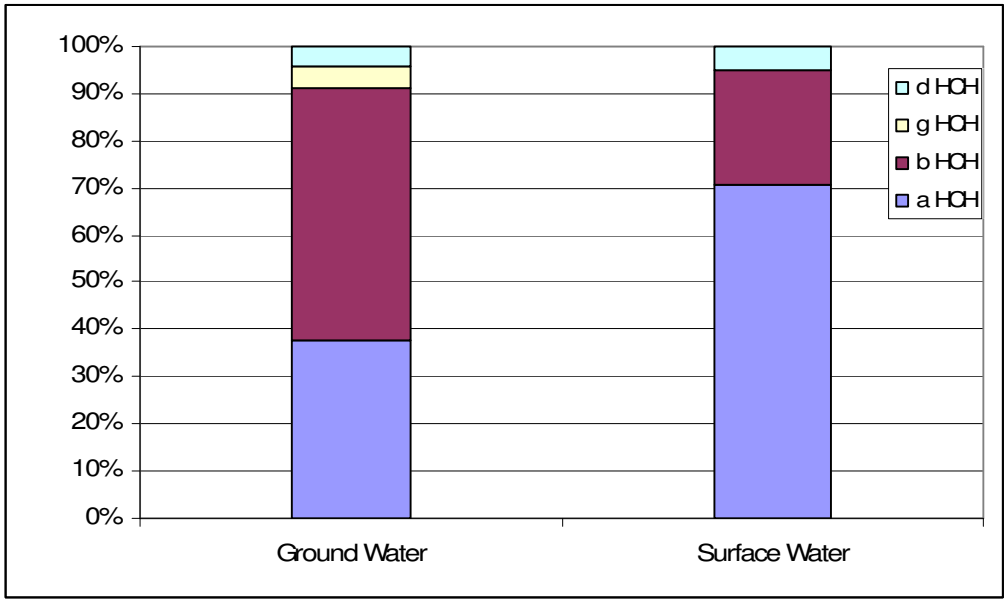


Figure 3.12. Distribution of HCH Isomers in Kumluca Water Samples

The presence of β-HCH in ground water samples indicates biological degradation of lindane use in the past and its leaching through soil into ground waters (Willett et al., 1998; Doong et al, 2002). The α- isomer is the second mostly observed product of lindane in ground water samples. γ -HCH is not observed in surface waters, but the major HCH constituent is α-HCH. The dominant presence of α-HCH has observed in surface waters indicates photo degradation of lindane (Willett et al., 1998; Rissato et al., 2006). Moreover, the δ-HCH has equal contribution for both types of water samples.

The obtained results generally show that, the HCH pollution in the region is due to the past use of this group of OCPs, as the major contribution is from degradation products of γ-HCH, namely the α-, β- and δ- isomers.

Dichloro Diphenyl Trichloroethane (DDT) and Its Derivatives (DDD and DDE)

DDT was the first synthetic pesticide of the modern age. Although it had found wide applications, it has later created widespread concern as an environmental hazard and toxicant. It is highly persistent in the environment, with a reported half life of between 2-15 years and it is immobile in most soils. Routes of loss and degradation include runoff, volatilization, photolysis and biodegradation (aerobic and anaerobic), which generally occur very slowly. Breakdown products (also called as metabolites) in the environment are DDE (dichloro diphenyl dichloroethylene) and DDD (dichloro diphenyl dichloroethane), which are also highly persistent and have similar chemical and physical properties (U.S. Department of Health and Human Services, 2002b).

The relative concentration of the metabolites can be used to assess the possible degradation mechanism and how recent the DDT contamination occurred. Since DDT can be biodegraded under aerobic conditions to DDE and under anaerobic conditions to DDD, and the ratio of (DDD+DDE) / total DDTs > 0.5 can be thought to be subjected to a long term weathering showing that the degraded derivatives (p,p-DDD and p,p-DDE) formes a significant proportion of total DDTs (Hong et al, 1999; Doong et al, 2002; Zhou et al., 2006).

Similar to HCHs, the concentration of DDT and its degradation products in Kumluca water samples are evaluated by using the average concentrations, as a general interpretation.

From Table 3.17 it is observed that, in the surface waters the average concentration of DDE (2.5 ng/L) is higher than DDD concentration (1.9 ng/L), indicating aerobic degradation is dominant. In ground waters, due to anaerobic degradation, the average concentration of DDD is higher (2.2 ng/L) than that of DDE (1.5 ng/L).

In Kumluca samples, the (DDD+DDE) / total DDTs ratio is 0.4 and 0.5 for surface and ground waters, respectively. If the two extreme concnentrations of DDT (16.1 ng/L for ground and 57.9 ng/L for surface waters) is excluded the values are increased to 0.6. This approach clearly demonstrates the DDT residues were due to the past use of DDT in the region, before 1980s.

Heptachlor and Heptachlor Epoxide

Similar to other OCPs, heptachlor is also has low water solubility and high affinity to partition in soil. It is readily converted to its degradation product, heptachlor epoxide in soil, plants and animals. Both of these substances are resistant to degradation in the environment and can be transported (U.S. Department of Health and Human Services, 1993).

In Kumluca ground waters, heptachlor epoxide occurred more frequently than its parent compound. This can be attributed to degradation of heptachlor. Moreover, there was no detection of heptachlor in surface waters. Without the extreme case explained before, heptachlor and its epoxide have very low concentrations.

Endrin and Endrin Aldehyde

In the samples analyzed, the concentration of endrin was below the limit of quantification, which is 0.1 ng/L for real samples. Its degradation product, endrin aldehyde was observed in ground waters with relatively low concentrations, with an average of 4.7 ng/L. This indicates the past use of endrin, which is no longer a pollutant in Kumluca region. The reason of endrin aldehyde detection only in well samples points out slow leaching from deep soil layers, which are protected from surface run-off.

The high occurrences of OCPs and detection of degradation products in Kumluca environmental water samples clearly indicate their intense use before 1980s. As the OCPs are persistent in the environment, their residues can still be found in soil, water and sediments. Other than this study, chlorinated pesticides are still being detected in aquatic environments in different regions of our country (Barlas 2002; Turgut, 2003; Bakan and Arıman, 2004).

Except few extreme cases, they have low concentrations, and therefore they do not pose a risk on water pollution. However, these types of pesticides have tendency to attach soil particles and accumulate. Therefore further studies required to determine the OCPs in soils and sediments to evaluate the OCP pollution in the region.

3.3.2. Concentrations of Organophosphorus Pesticides in Kumluca Water Samples

The summary of the data set obtained for organophosphorus pesticides for both ground water (N=55) and surface water (N=22) samples are presented in Table 3.18.

Table 3.18 Summary Statistics for Organophosphorus Pesticides (values in ng/L)

Pesticide	**GW** **Av (% RSD)** **Range** **N**	**SW** **Av (% RSD)** **Range** **N**
Azinphos-methyl	100.0 (32.0) 70.3 -178.8 12	184.1 (101.8) 85.1-564.9 6
Bromophos-ethyl	71.6 (42.5) 46.9-124.5 6	42.1 (65.3) 13.2-60.5 4
Bromophos-methyl	BLOQ	BLOQ
Chlorpyrifos	68.8 (22.9) 56.7-119.0 27	85.8 (50.8) 51.0-193.4 14
Diazinon	68.6 (76.8) 16.3-234.9 13	85.0 (58.6) 15.7-176.8 12
Dichlorvos	103.5 (35.6) 73.5-174.5 7	116.3 (91.2) 67.6-322.2 6
Fenamiphos	191.2 (45.6) 111.4-394.8 8	155.2 (16.6) 140.5-168.8 2
Fenitrothion	60.5 (39.3) 35.7-89.8 4	68.4 (56.0) 35.2-123.7 4
Fenthion	BLOQ	BLOQ
Malathion	38.8 (8.6) 35.6-44.6 7	49.6 (18.1) 43.3-56.0 2
Methidathion	BLOQ	128.8 (25.2) 109.7-166.2 3
Parathion-methyl	73.4 (34.6) 56.8-124.2 6	79.4 (38.9) 52.2-123.7 8
Phosphamidon	64.7 (27.2) 33.8-79.0 9	88.7 (31.5) 55.8-123.5 4
Pirimiphos-methyl	BLOQ	BLOQ

BLOQ: Below Limit of Quantification,
GW: Ground Water, SW: Surface Water

The values presented are for the concentrations above the Limit of Quantification. As seen, the concentrations were almost ten times higher when compared to chlorinated pesticides. Bromophos methyl, fenthion and pirimiphos methyl have not been detected in any of the samples.

At the first glance, the most striking feature of the table is; some of the OPPs in Kumluca water samples are higher than maximum allowable concentration of a single pesticide (100 ng/L). The acute effects of phosphorus pesticides are more severe than other types of pesticides. Therefore, their presence in high concentrations poses a risk for human health. It is clear that, in Kumluca region, the sampled wells and surface waters are polluted with these pesticides, and they should not be consumed for domestic purposes.

The highest observed concentration (565 ng/L) was for azinphos-methyl in a surface water sample in fall season. This value is as high as 2 SD (standard deviation) than the mean value for surface waters. Azinphos-methyl is commonly used in the region for the control of insecticides in greenhouses and orchards. The high concentration is due to surface run off to the rivers after the extensive use and spray drift during the application of pesticide around the sampling point. However, it should be emphasized that in the region, the disposal of empty containers of pesticides or other agricultural chemicals onto surface waters is a common practice among farmers. Moreover, they have been easily recognized during the field trips. This pollution may also lead to the observed extreme concentration.

The second highest concentration (395 ng/L) was observed for fenamiphos in a well in spring season. This value is also higher than the mean value by 2 SD. Fenamiphos is used for the control of tomato root nemotodes, and others for tomatoes, pepper and cucumber, which are currently cultivated in the region. This pesticide is one of the most commonly used OPPs from September to May and it has relatively high solubility (330 mg/L). The observed concentration of

this insecticide reflects the pollution of the well due to the leaching into ground water.

To figure out the values given in Table 3.18, average concentrations of OPPs for different types of water samples are given in Figure 3.13.

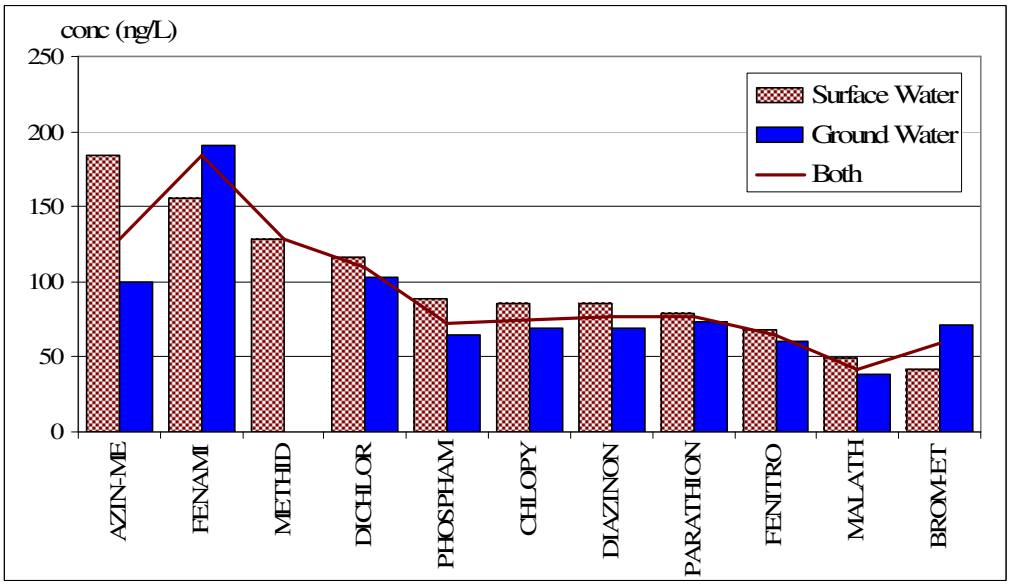


Figure 3.13. Average Concentrations of Organophosphorus Pesticides in Different Types of Water Samples

Similar to OCPs, the surface water concentrations were generally higher than ground water samples. The maximum average concentration was for azinphos methyl (184.1 ng/L). Fenamiphos, methidathion and dichlorvos also have high average values. Methidathion and dichlorvos are well known and commonly used pesticides in the region as stated by local authorities.

The highest average concentrations (191.2 ng/L) for ground water samples were observed for fenamiphos. Moreover, all the observed concentrations for this pesticide in ground water samples were higher than the limit value. The average concentrations for dichlorvos (103.5 ng/L), and azinphos-methyl (100.0 ng/L) were also high when compared to the rest of OPPs. However, in

order to evaluate this high average values, the percent occurrences should be considered.

The percent occurrences of OPPs are given in Figure 3.14. Similar to OCPs, generally, the OPPs are also detected more frequently in surface water samples. The mostly observed pesticide was chlorpyriphos with a percent occurrence of 64 and 49 % for surface and well water samples respectively. Chlorpyriphos is another mostly used pesticide in the region for both greenhouses and orchards. This is followed by diazinon with percent occurrences of 55 % and 24 % for surface and ground waters, respectively. Diazinon is also commonly used, especially in spring season, for vegetables in greenhouses. Azinphos methyl has also considerably high frequency of detection in ground water samples (22%).

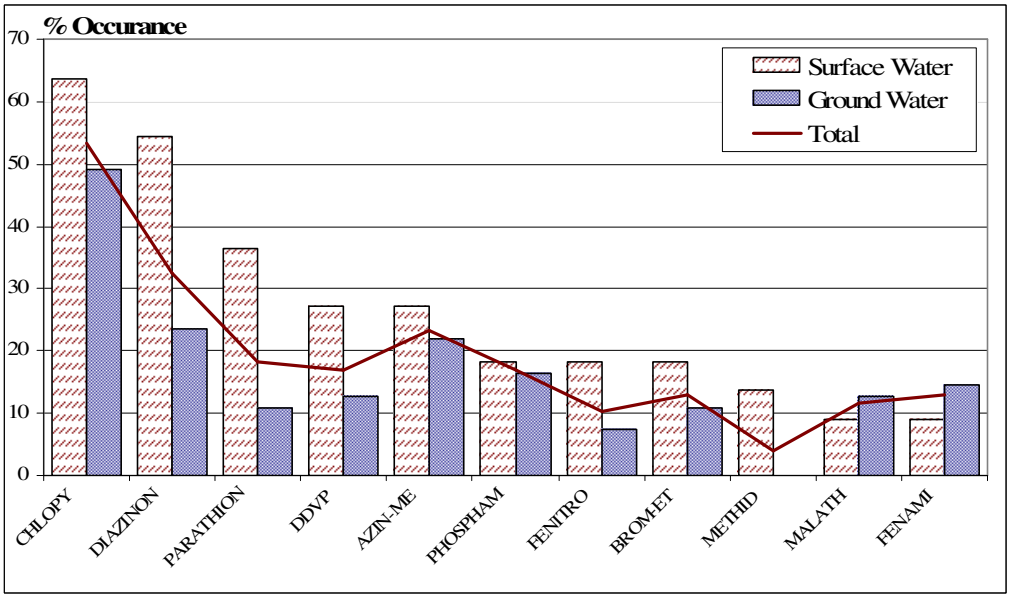


Figure 3. 14. Percent Occurrences of OPPs

In Figure 3.15, the average concentrations of OPPs were compared with percent occurrences. In ground waters, fenamiphos, dichlorvos and azinphos-methyl have low percent occurrences (lower than 40 %) and high average concentrations (higher than 100 ng/L). In contrast, chlorpyriphos has high

occurrence in low concentrations. As stated, all these four pesticides are commonly used in the region, together with methidathion and diazinon according to local authorities.

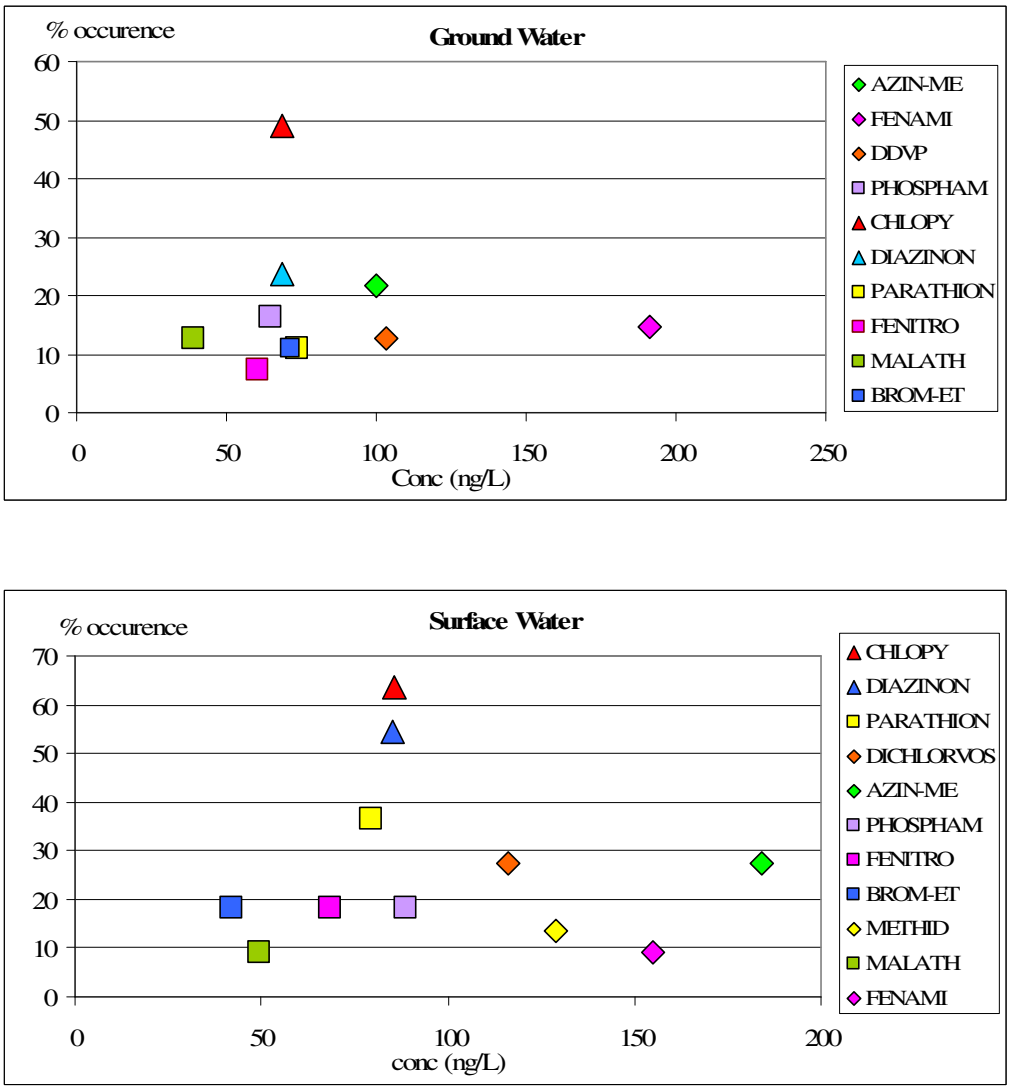


Figure 3. 15. Average Concentrations and percent Occurrences of OPPs

Before discussing the observed concentrations and percent occurrences, it is necessary to mention the pesticide use trends in the region. Table 3.19 gives the application field and extend of use of pesticides, together with the pesticide

properties given in Table 1.7. It should be noted that, there is no record of pesticide use in the region and the amounts (high/low) are given by the District Agriculture Directorate by personal communication. It is also stated by the local authorities that, the pesticides are mostly applied in spring season.

Table 3.19. Pesticide Properties and Use Trends in Kumluca (from Plant Protection Products, 2002 and information from District Agriculture Directorate)

	Water Solubility (mg/L)	**Water Half Life (days)**	**Field Applied**	**Extend of Use**
Azinphos-me	29	19	GH+O	High
Bromohos- et	2	-	GH+O	Low
Chlorpyrifos	1.18	58	GH+O	High
Diazinon	40	138	GH	High
Dichlorvos	8000	4	GH	High
Fenamiphos	329	300	GH	High
Fenitrothion	30	36	O	Low
Malathion	130	6	GH+O	Low
Methidathion	240	26	O	High
Parathion-me	55	45	GH+O	Low
Phosphamdon	1.0×10^6	48	O	Low

High water solubility (330 mg/L) and long half life in water (300 days) of fenamiphos explain the observed high concentrations. The longer half life of fenamiphos may also lead to accumulation in waters, increasing its concentrations. However, when compared with other pesticides, the use of fenamiphos is limited, while the other pesticides are used in both greenhouses and orchards than fenamiphos, which is used only in greenhouses. This leads lower occurrence of this pesticide in wells among others.

Dichlorvos has short half life in water, (4 days) and high solubility (8000 mg/L). The high solubility of this substance explains the high concentrations, and low occurrences are due to degradation of these pesticides, although it is

widely used. Once applied, dichlorvos readily enters to surface waters, however as it is not stable, the detected concentrations reveals the recent use. It should be stated that in the region, the pesticide applications are started by March and the sampling was performed in May.

The case for azinphos methyl is somewhat different. This pesticide has low water solubility (30 mg/L) and short half life (19 days). Knowing the wide use in the region, the high concentrations and low occurrences may be attributed to recent and intensive use of this pesticide, around the sampled wells.

Chlorpyriphos is widely applied in greenhouses and orchards leading the higher occurrences due to surface run off and spray drift from fields to rivers through channels. However it has low solubility (1 mg/L). This explains the high occurrence and low concentrations of this pesticide.

Although diazinon is also a well known and preferred insecticide used mostly in greenhouses, it has low occurrence and low concentration in ground waters. The seasonal comparisons will be given in the following sections, but it is notable that, the concentrations of diazinon in fall season samples (with an average value of 56 ng/L) are lower than the spring season average (89 ng/L), lowering the average values obtained for both seasons. Diazinon is the single OPP that has high difference in concentrations for both seasons.

The use of parathion-methyl, bromophos-ethyl, phosphamidon, malathion and fenitrothion in the region is limited, and these pesticides have low concentrations and low occurrences in both surface and ground water samples.

Similar conclusions for surface waters can be drawn for fenamiphos, azinphos methyl and dichlorvos which have high concentrations (> 100 ng/L) with low percent occurrences (< 40%). In surface waters, methidathion, which is absent in the wells is also observed. This pesticide similar properties (high solubility,

240mg/L) and short half life (26 days), and have similar behavior in surface waters with dichlorvos. Chlorpyriphos still has high occurrence and low concentrations in ground water samples. However, the frequency of diazinon has increased from 24 % in well waters to 55 % in surface waters. As stated, the surface waters are subject to pollution more than ground waters due to surface run off and spray drift from application fields, and disposal of empty containers.

3.3.3. Total Pesticide Concentrations

In order to evaluate the pesticide pollution in Kumluca samples, the concentrations of all OCPs and all OPPs detected in the samples are summed up to obtain the total pesticide concentrations. The average value of the total concentrations observed in both sample types in whole study period is 221.1 ng/L. The average of total concentrations is 171.1 ng/L in ground, and 339.8 ng/L in surface waters.

Although the difference between the total concentrations of pesticides in different types of water samples was obvious, we wanted to show it statistically by using t-test. The t-test results show that, these ground and surface water concentrations are significantly different than each other in 95% confidence. This difference is expected, as the surface waters are subject to pollution more than ground waters.

Figure 3.16 presents the distribution of total pesticide concentrations among the samples.

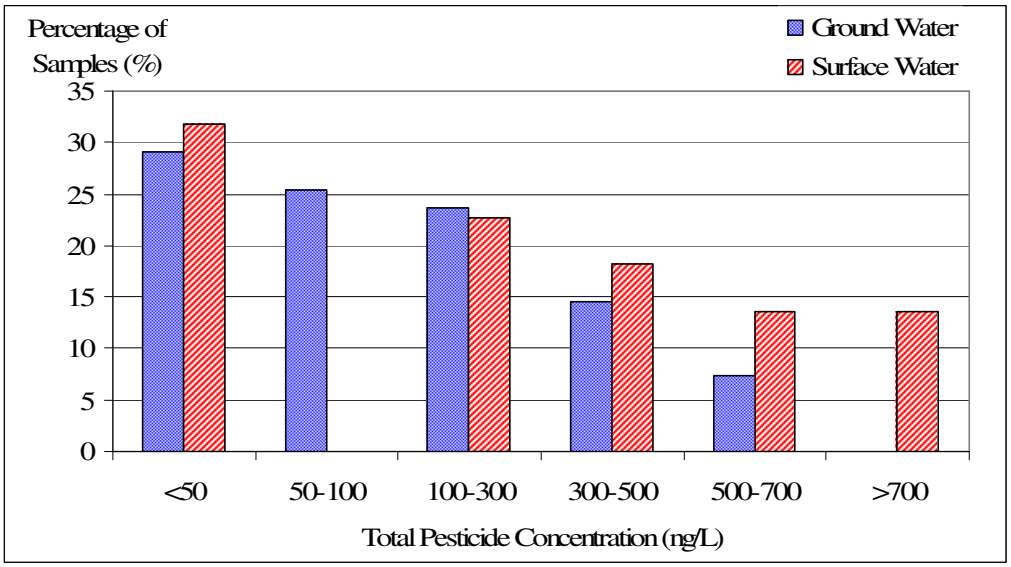


Figure 3.16. The Frequency of Samples as a Function of Total Pesticide Concentrations.

The total concentration of detected pesticides in water intended for human consumption should be maximum 500 ng/L, according to EU regulations (EU Council Directive 98/83/EC, 1998). As clearly seen in the figure, there are well and surface water samples exceeding this limit. Among wells, 4 samples, among surface waters, 6 samples have high concentrations, making up the 13 % of the total samples.

Similar to single pesticide concentrations, the total concentrations are higher in surface waters, as 27 % of surface water samples exceed the limit value. As explained before, the reason for surface water pollution is the run-off from the fields after application and the disposal of empty containers into streams. The percentage of well water samples with total concentrations higher than 500 ng/L is only 7% considering both seasons. However, this represents 14 % of the sampling points, where the total concentration limit is exceeded at least once.

The figure shows that, although some samples exceed the limit value, most of the samples are below that. In fact, 71 % of all samples have total concentrations below 300 ng/L; 78 % of ground and 54 % of surface water samples have pesticide concentrations below 200 ng/L. The 48% of all the samples have total concentrations even lower than 100 ng/L.

There are limited numbers of studies about agricultural pollution in Kumluca, concerned about the effect of fertilizer use (Muhammetoğlu and Yardımcı, 2006; Kaplan et al., 1999), and vulnerability of soil to pesticides (Uslu, 2007). Unfortunately, there is "*no*" record of pesticide use among the farms in Kumluca. The findings of this study can only expose the fact that the surface and ground waters are being affected by the uncontrolled pesticide applications.

3.4. Comparison with Literature Data

To assess the pollution level, it is necessary to compare the obtained data with similar studies performed at different regions. The pesticide concentrations in ground and surface waters of Kumluca will separately be compared with literature data.

As stated in Chapter I, the use of chlorinated pesticides are banned in most of the countries. Being in the POP group, they have still environmental concern all around the world. However, in developing countries, such as India, they are widely used as insecticides both in agriculture and in the control of malaria. Therefore, especially in the far East region, the OCP pollution in the environment has been widely studied, especially in China (Hong et al., 1999; Zhang et al., 1999; Wang et al., 2003; Xue et al., 2005; Zhou et al., 2006; Xu et al., 2007), in India (Singh and Gupta, 2002; Sarkar et al., 2003; Singh et al., 2005; Sankararamakrishnan et al., 2005), in Taiwan (Doong et al, 2002; Hung

et al., 2007), in Philippines (Bouman et al., 2002), and in Vietnam (Nhan et al., 1999; Hung and Thiemann, 2002). The data for India, China, Japan and Philippines are included with this respect.

However, for the OPPs studied in this work, the research is highly scarce. Again as stated in Chapter I, the trends in pesticide use in developing countries are different than that of developed countries, the herbicides are more commonly used than insecticides in countries such as USA, UK, Germany and France. Being in the Mediterranean region and similar climate zones and cultivations, there are few studies available concerning about similar OPPs in Greece, Spain, Egypt and even Portugal, with Turkey.

The OCP and OPP levels in ground water samples in Kumluca was compared with similar data sets obtained for different parts of the world and presented in Figure 3.17.

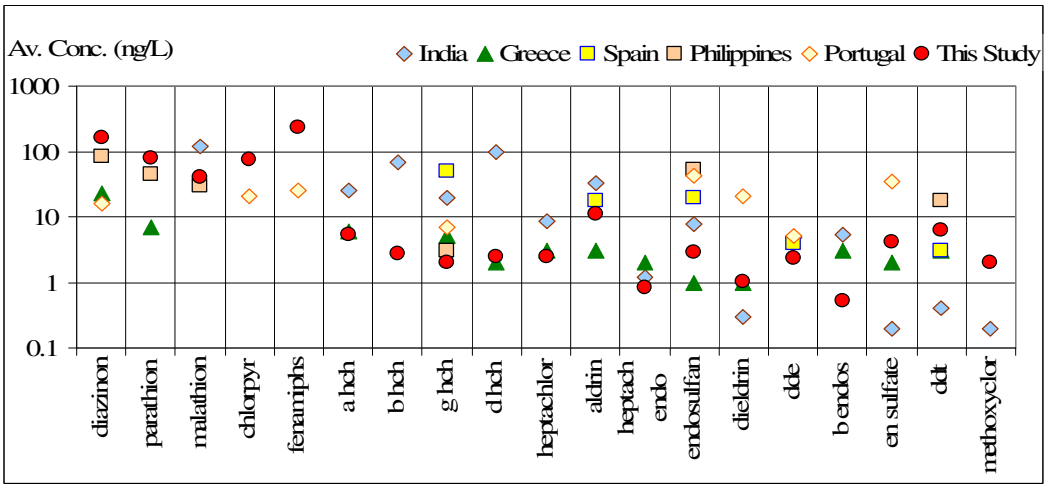


Figure 3.17. Comparison of Ground Water Pesticide Concentrations with Literature

Although there are numerous studies about pesticide pollution, few of them are concentrated on the ground water pollution. The studies presented in Figure 3.17 were selected as common pesticides are studied in ground water samples with this study.

The Indian OCP data was for ground water samples in Unnao district (Singh et al., 2005). Total 96 samples were collected in October-November of 2003 and after liquid-liquid extraction, the determinations were performed by GC-ECD system. Sankararamakrishnan et al. (2005) has studied OCP and OPP pollution in both surface and ground water of Kanpur region, where OCP concentrations were much lower than Unnao district. As the number of OCPs analyzed was lower, only the OPP data (available just for malathion) of this study was used in Figure 3.17.

Bouman et al. (2002) has studied nitrate and pesticide contamination in 54 wells from 1989 to 2000, under the rice based production systems in Luzon, Philipines. The common pesticides studied were DDT, diazinon, endosulfan, endrin, lindane, malathion and parathion. The data used in Figure 3.17 was for 1989-1990 period.

A pesticide monitoring program of was conducted in Portugal, with a network comprised 23 sampling points sampled every 3 months during a 2-year period (Azevedo et al., 2000). 42 pesticides of different chemical classes, including lindane, diazinon, chlorpyriphos, endosulfan, endosulfan sulfate, fenamiphos and DDE, were analyzed by solid-phase microextraction (SPME) and gas chromatography with electron-capture detection-thermoionic specific detection (GC-ECD-TSD) or mass spectrometry (GC-MS).

SPME-GC-ECD was used for the determination of OCPs in ground waters of Spain (Perez-Trujillo et al., 2002). After the optimization, the experimental procedure was applied to polluted ground water samples. Among the 12 analytes studied, the pesticides, with detectable concentrations were presented in Figure 3.17.

Vassilakis et al. (1998) has evaluated the use of C-18 bonded porous silica for the extraction of 32 pesticides in different chemical classes, including OCPs, from surface and ground waters. GC-ECD was used for the analysis of OCPs. The authors have applied the developed analytical methodology to real samples; 30 ground water samples collected monthly from Lassithi Plateau, Crete Island, Greece, where there is unsustainable agricultural activities.

The concentration levels of OCPs in Kumluca water samples are generally agree with literature values. The OPPs concentrations may seem higher; however it should be noted that the number of publications about this type of pesticides is limited, and further comparisons are required.

The comparison of pesticide concentrations in surface waters of Kumluca with similar studies at different parts of the world and Turkey is presented in Figure 3.18.

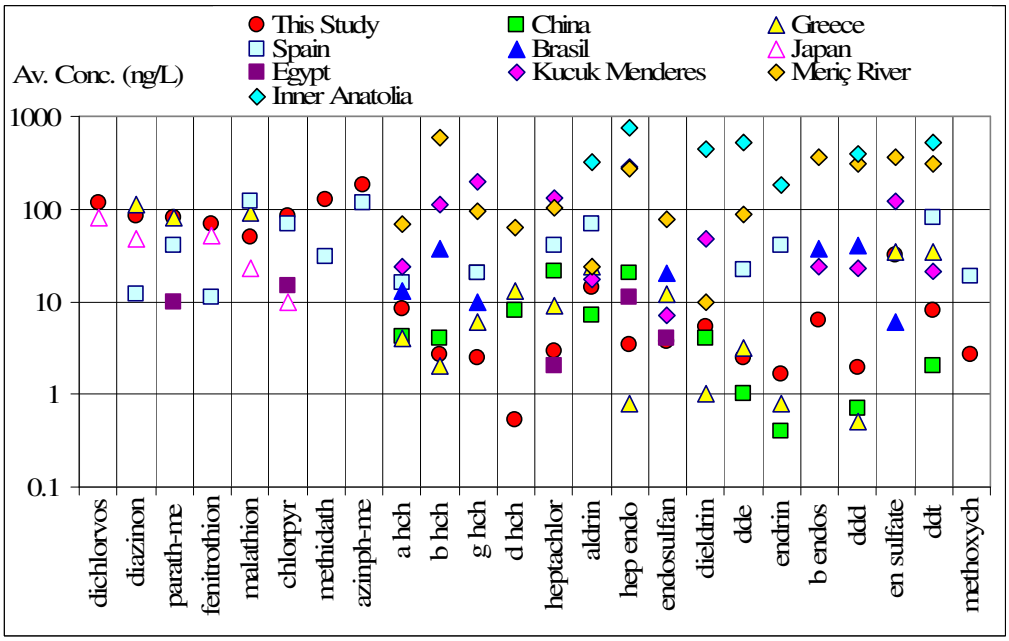


Figure 3.18 Comparison of Surface Water Pesticide Concentrations with Literature

Zhou et al. (2006) has studied the distribution of 13 OCPs in surface water and sediment of Qiantang River, in East China to evaluate their potential pollution and risks. From 45 sampling points, 180 surface water samples were collected regularly during 2005. The data used in Figure 3.18 is for October samples. SPE-GC-ECD system was used for the extraction and analysis of the samples.

Greece data presented in Figure 3.18 was compiled from different studies. The concentrations of diazinon and parathion-methyl were reported for Kalamas River, a river in Western Greece in the vicinity of agricultural areas, in January and December of 2000 (Lambropoulou et al 2002). After application of SPME, GC-FTD and GC-MS systems were used for the analysis.

The OCPs concentrations for Greece surface waters were for Loudias (Axios and Evros Rivers, all in Northern Greece where there were intense agricultural activities. Loudias samples were collected May 2006 to April 2007 period

(Albanis et al., 1998; Konstantinou et al, 2006); and Axios and Evros River samples were collected in June 1996-June 1998 period (Golfinopoulos et al., 2003). The SPE-GC-ECD system was used for the extraction and analysis in these studies.

The Spanish surface water data was obtained from different sources for different pesticides. Espigares et al. (1997) has studied both OCP and OPP pollution in Guadalquivir River and streams reaching to this river. From 22 sampling points, samples were collected from May 1989 to March 1990. SPE-GC-ECD system was used for the extraction and analysis of 8 OCPs and 6 OPPs. Claver et al. (2006) has studied the pesticide pollution in Ebro River, around agricultural areas. Analyses were performed by SPE-GC-MS system. Among 44 substances found in Ebro River samples, 6 of them (parathion-methyl, γ- and δ-HCH, heptachlor, DDE and methoxychlor was presented in Figure 3.18. Planas et al. (2006) was also used SPE-GC-MS system for the analysis of 32 pesticides in 93 Spanish surface water samples. The results for 5 OPPs common with our work were used in the comparisons.

The data obtained for Brazil was for samples collected around agricultural fields and forests in 2005 (Rissato et al., 2006). After liquid liquid extraction, 18 OCPs and 7 PCBs were analyzed by GC-MS system. The Shinano River, the largest river in Japan, was sampled between April and August of 2006 (Tanabe et al., 2001). The concentrations of 48 pesticides with different chemical classes and 6 metabolites were determined by SPE-GC-MS system. The Egypt data in figure 3.18 was obtained for an agricultural land, El-Haram, Giza (El-Kabbany et al., 2000), with samples collected from surface waters in April-May 1996. Again, SPE-GC-MS system was used for the analysis of 11 pesticides including two common OPP and three OCPs with the current study.

Turgut (2003) was determined the residues of OCPs in surface water of Küçük Menderes River, Turkey. From selected three sampling points, surface waters were collected between 2000 and 2002. After liquid-liquid extraction, OCPs were determined by means of GC-ECD system.

The concentrations of OCPs in Meriç River were determined by Erkmen and Kolankaya (2006). The results presented are the average values of 8 sampling points, which were sampled between May 2002 and August 2003. Inner Anatolia data is the average concentrations of OCPs in 5 lakes, sampled between April 1998 and October 1999 (Barlas, 2002). In both of these studies, the OCPs were quantified by GC-ECD after liquid-liquid extraction.

The OCP concentrations observed in inner Anatolian lakes, Küçük Menderes and Meriç Rivers are mostly very high when compared to Kumluca data. It can be stated that, the Kumluca seems unpolluted with respect to OCPs, among other regions of Turkey. However, the OCP concentrations in Kumluca surface water samples are generally in good agreement with the observed concentrations in different parts of the world.

The concentrations of diazinon, parathion methyl and malathion are also comparable with literature findings. Kumluca surface waters contain slightly higher concentrations of dichlorvos, fenitrothion and chlorpyriphos.

From Figure 3.18, it can easily be observed that, the concentrations of azinphos-methyl and methidathion are extremely higher. As stated in Section 3.3.2, the percent occurrence of methidathion was only 14 % in surface waters, meaning detection only in three sampling points. These detections were for spring season. Therefore, it would not be logical to extrapolate these results to all data set and state the methidathion pollution in Kumluca samples. In contrast, azinphos-methyl was observed in both seasons with total percent

occurrence of 27%. Knowing the high application rates in the region, it is clear that azinphos-methyl is a potential pollutant in Kumluca waters.

3.5 Seasonal Variations of Pesticide concentrations

The critical factors for the time interval between the application of pesticides and their occurrences in surface and ground waters include the physical and chemical properties of pesticides besides the soil type, application amount, size of the water table, and meteorological conditions (Capel et al., 2001; Konstantinou et al., 2006).

This study undertakes sampling and analysis of Kumluca environmental water samples in spring and fall seasons of 2005. Up to now, the discussions were based on all data set covering the both seasons. In this section, the results will be presented in more detail, considering the seasonal behaviors of total pesticide concentrations and individual pesticides.

3.5.1. Seasonal Variations of Total Pesticide Concentrations

Figure 3.19 compares all data set with respect to both season and water type. It should be first noted that, all spring season samples have an average total concentration of 237.1 ng/L, and it is 203.4 ng/L for the fall season samples. Although spring season samples seems to have higher average total pesticide concentrations, in 95 % confidence level, t-test reveals no difference between these two means. In spring season, the averages of total concentrations in well and surface waters are 191.1 and 354.3 ng/L and in fall season, 150.1 and 325.4 ng/L, respectively. Again, there is no difference observed at 95 % confidence between the means of total pesticide concentrations in same sample types among the two seasons.

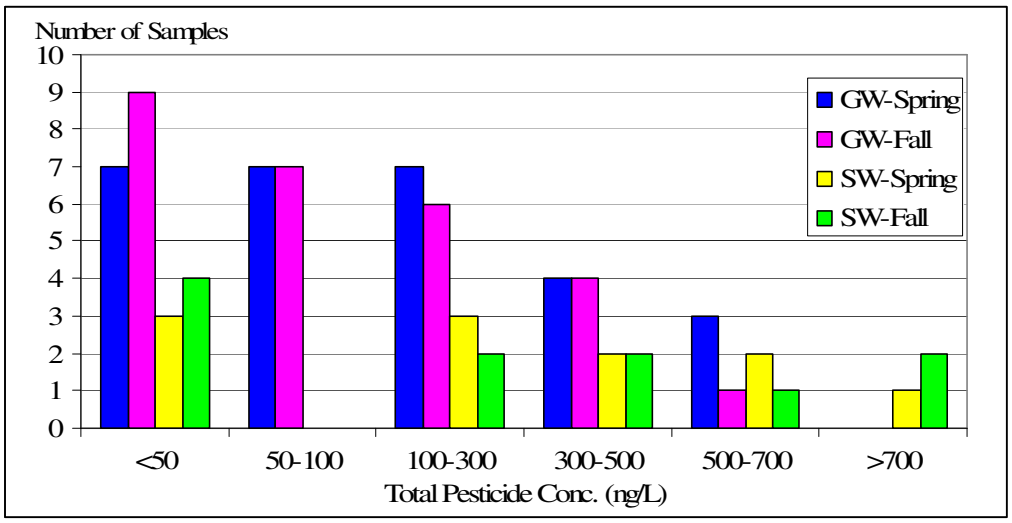


Figure 3.19. Number of Samples with Varying Total Concentrations
GW: Ground Water, SW: Surface water

In Section 3.3.3, the general distribution of total pesticide concentrations in two different types of water samples were discussed, for whole study period. As stated in that section, there are samples with total concentrations higher than allowable maximum value of 500 ng/L, according to EU regulations (EU Council Directive 98/83/EC, 1998). Considering this limit and the pattern in Figure 3.19., it can be stated that, slightly higher number of samples are polluted in spring season, for ground waters. Surface waters have similar pollution levels in both seasons.

It may be surprising to see these lower concentrations of fall season samples. They were expected to be higher, as the water levels in the reservoirs are lower in this season. However, the percentage of samples with concentration levels below 300 ng/L is similar for both seasons, around 70 %. The reason is the higher application rates of pesticides in Kumluca in spring season.

The 6 of surface water concentrations, lower than 50 ng/L are observed for water springs for both seasons, with total pesticide concentrations even lower than 10 ng/L.

In Figure 3.20, Box-Whisker plots of total OCP and OPP concentrations in different types of water for both seasons are given. As seen, the total OPP concentrations are higher in spring season. The reason is the higher application rate of OPPs in spring season in Kumluca, as stated before. In contrast, the OCP concentrations are higher in fall season. As most of the OCPs are not currently used, they are not introduced in water systems anymore. The residue concentrations are lowered in spring season as the amount of water in the wells and surface waters are high, diluting the pollutants.

Similar to total pesticide concentrations, t-test was applied in 95% confidence to compare the total OPP concentrations in two seasons. It is seen that, although spring samples seem to have higher total OPP concentrations, the difference is not significant in ground and surface waters.

The total OCP concentrations seem to be high in fall season for both well and surface waters. However, t-test in 95% confidence shows that, the difference is not significant among seasons, as it is the case for OPPs.

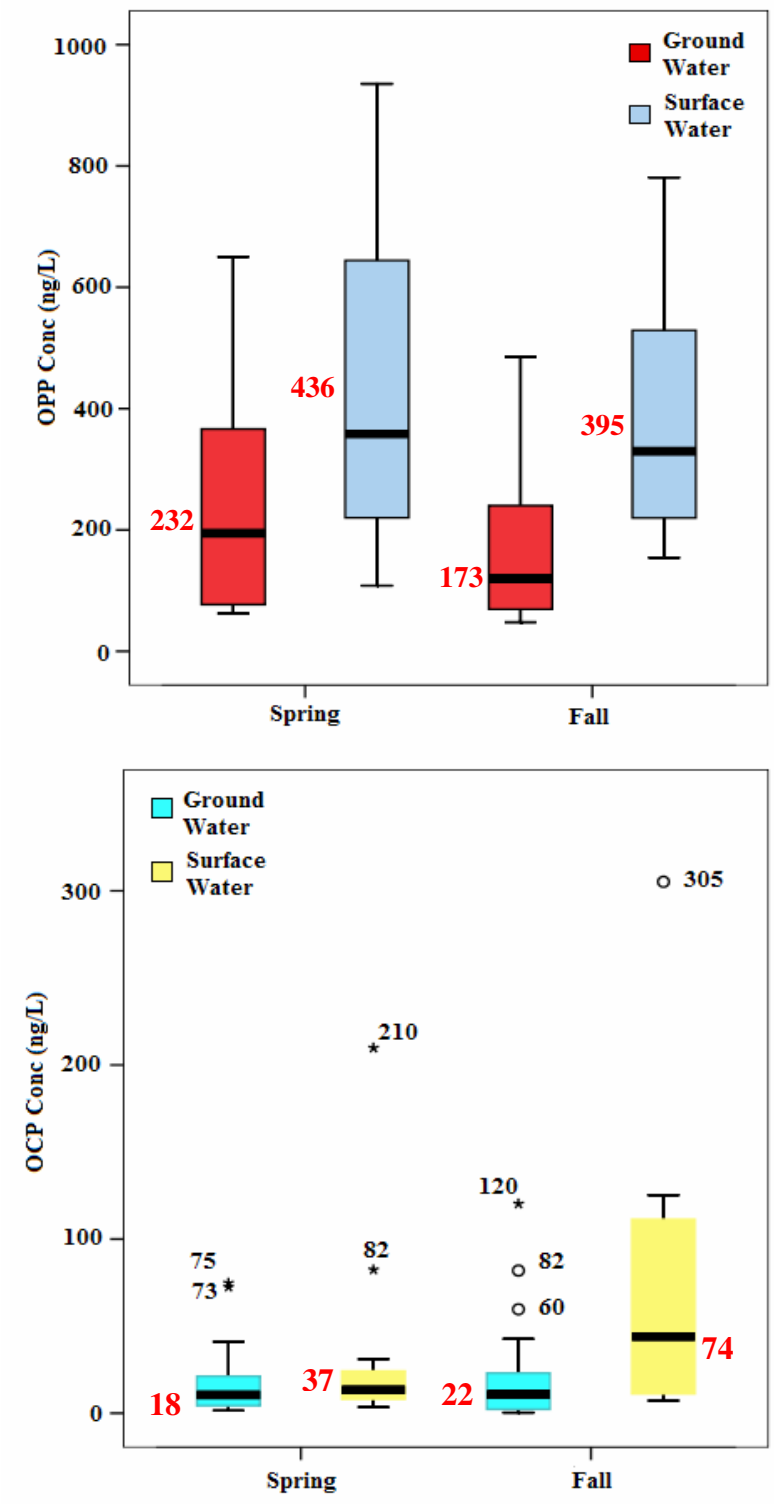


Figure 3.20. Box-Whisker Plots of Range of Total OPP and OCP Concentrations for Both Types of Waters in Different Seasons
(* ; extremes, ○; autliers. Mean values are given in red)

The amount of rain after the application of pesticides also effects their concentrations in surface and well waters (Castilho et al., 2000; Lambropoulouet al., 2002; Konstantinou et al., 2006), increasing the surface run-off and leaching. In Kumluca, the daily average rain amount between January and May 2005 is 32.6 mm/day, between June and October 2005 is 7.2 mm/day. As seen, before the first sampling campaign, the probability of pesticides to reach ground water sources and surface waters is higher. This may result in the increased concentrations of OPPs, which have higher mobility in soil, in spring season for both types of waters.

3.5.2. Seasonal Variations of Individual Pesticides in Ground Waters

The seasonal differences of total pesticide, total OPP and total OCP concentrations were discussed in previous section. Although the total concentrations of OPPs seem to be higher in spring season and of OCPs in fall season, this general conclusion may be incomplete without the information about every single pesticide.

To itemize the findings, the seasonal variations for individual pesticide concentrations are presented in Figure 3.21 for ground waters, together with the percent occurrences. For the construction of the figure, only the pesticides observed in both seasons were considered. The excluded cases are as follows: Parathion-methyl was observed only in spring season in 14 samples, 8 of which were surface waters. All three detections of malathion were again among fall season surface water samples. The δ-HCH was measured in 7 spring samples, three of which are surface waters. The DDD was only observed in 7 wells and 3 surface water samples.

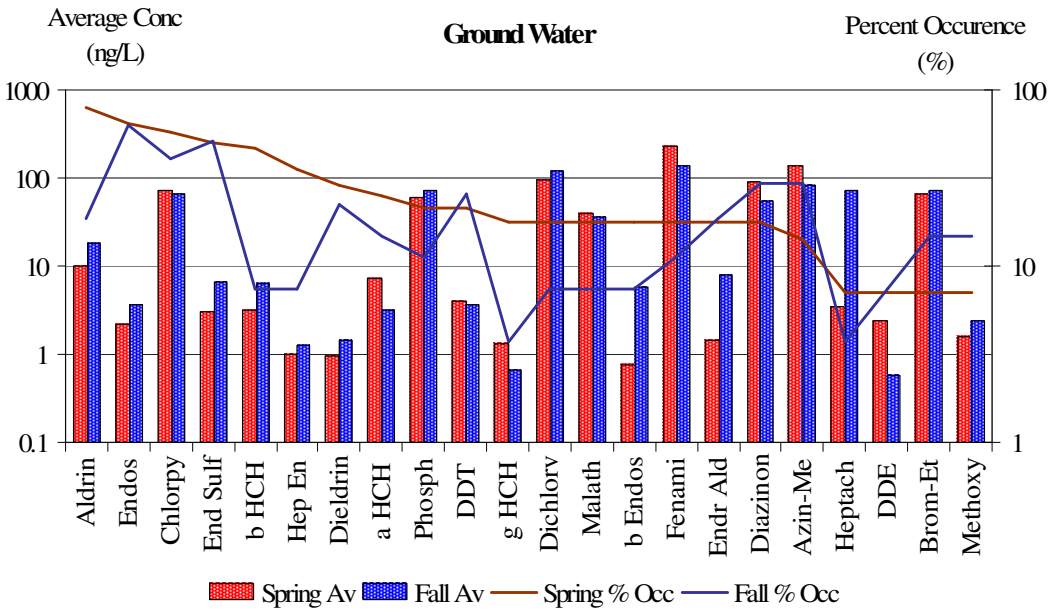


Figure 3.21. Seasonal Variations of Individual Pesticides in Ground Water Samples

The concentrations and percent occurrences of OPPs can be explained by their use in Kumluca, as their life times are short. The observed high concentrations and percent occurrences are due to high application rates, as explained in Section 3.3.2. As mentioned in Section 3.3.1, the detections and concentrations of OCPs are due the use of these pesticides before 1980s, in the region.

In ground waters, the OPPs have generally low concentrations in fall season, except dichlorvos, phosphamidon and bromophos ethyl. For OCPs, only α-HCH, γ-HCH and DDE have lower concentrations in fall season; others are higher as mentioned in the previous section.

In spring season the pesticides with the highest concentrations are fenamiphos and azinphos methyl, being 223.9 and 135.4 ng/L respectively. These values are higher than the limit value of 100 ng/L set by EU directives (EU Council

Directive 98/83/EC, 1998), for a single pesticide. It should be noted that, dichlorvos, with a concentration of 97.6 ng/L is also indicating pollution in spring season. Among OCPs, the highest concentrations are observed for aldrin (9.9 ng/L) and α-HCH (7.2 ng/L).

In fall season, the highest concentrations were observed for fenamiphos and dichlorvos, with 136.8 and 118.3 ng/L, respectively. The concentration of azinphos-methyl was lowered to 82.4 ng/L. Heptachlor seems to have an average concentration of 72.4 ng/L. However, that is a single observation of this pesticide in fall season. Excluding this case, in fall season the highest OCP concentration is obtained for aldrin and endrin aldehyde, with 18.4 and 8.0 ng/L, respectively.

Especially in ground waters, it is observed that the percent occurrences in fall season are generally lower for both OCP and OPPs (Figure 3.21). As OPPs are applied less frequently in fall season, this trend is expected for these types of pesticides. However, it is surprising to observe the lower number of detections for OCPs in fall season, which have generally higher concentrations in this season.

The lower percent occurrences for fall season may be due to the insufficient mixing of the underground water tables. The high amount of water in the water reservoirs in spring season may lead to mixing of the aquifers, homogenizing the pollution, giving high percent occurrences. However with the low levels of water, the aquifers may be present as separated with less amount of mixing, keeping the pollutant non-dispersed.

In well waters, the most commonly observed pesticides in spring season are chlorpyriphos (57 %) and phosphamidon (21 %) among OPPs; aldrin (79 %) and endosulfan (64 %) among OCPs.

Among OPPs, chlorpyriphos is still the most frequently detected pesticide in fall season, with 41 %, followed by, diazinon and azinphos methyl with 30 % occurrence. In fall season, endosulfan and its degradation product, endosulfan sulfate has highest percent occurrences, above 50 %.

3.5.3. Seasonal Variations of Individual Pesticides in Surface Waters

Figure 3.22 shows the seasonal variations of individual pesticides in surface waters, together with the percent occurrences.

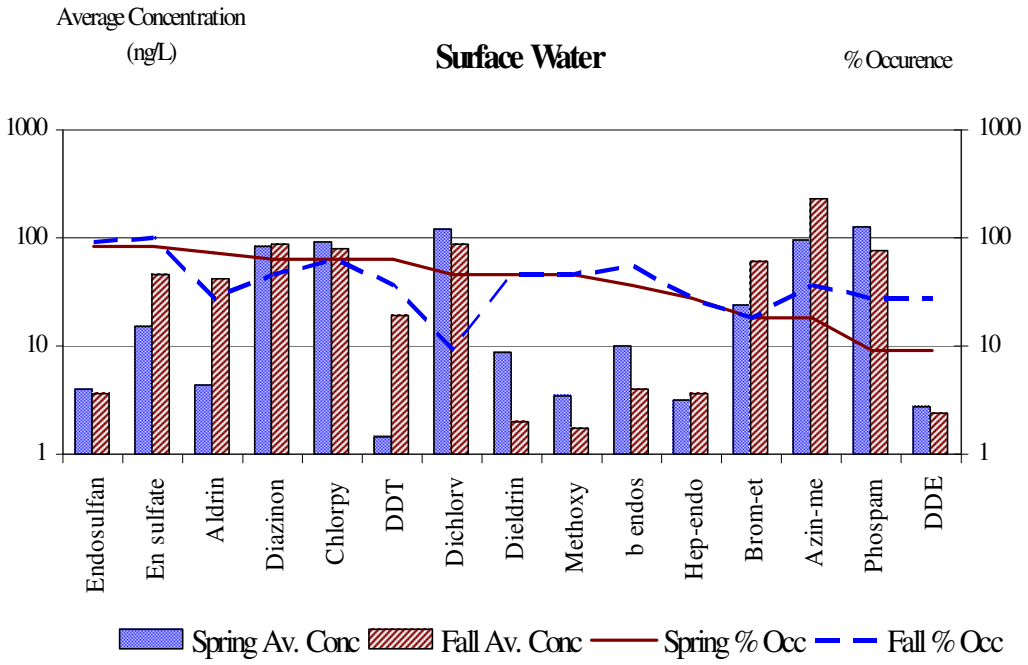


Figure 3.22 Seasonal Variations of Individual Pesticides in Surface Water Samples

As stated before, the surface waters are more polluted than ground waters in Kumluca. This can easily be recognized from the Figure 3.22, with high number of pesticides having concentrations above or close to limit value of 100 ng/L.

In spring season, the highest concentrations are observed for phosphamidon (123.5 ng/L) and dichlorvos (122.4 ng/L). These two pesticides have highest solubilities among other OPPs; the solubility of phosphamidon is 1,0 X 10^6 mg/L and it is 8000 ng/L for dichlorvos. This trend supports the suggested contribution of rain events on the observed concentrations of soluble pesticides, increasing their surface run-off into the rivers.

In spring season, the concentrations of azinphos methyl (93.9 ng/L) and chlorpyriphos (91.3 ng/L) are also close to the limit value. The concentration of endosulfan sulfate (15.1 ng/L) is the highest among OCPs, in spring season surface samples. One of its parent compound, β-endosulfan has a concentration of 9.8 ng/L.

In fall season, azinphos-methyl has an average concentration of 229.3 ng/L, followed by diazinon with 87.6 ng/L. Phosphamidon, dichlorvos and chlorpyriphos also have concentrations above 70 ng/L. Similar to ground waters, the concentration of endosulfan sulfate in surface water samples are higher (45.2 ng/L) in fall season.

As seen from Figure 3.22, in almost all surface water samples, endosulfan and its degradation product, endosulfan sulfate is observed for both seasons. As stated before, this is due to wide application of endosulfan in the region. Chlorpyriphos is detected in 64 % of surface waters in both spring and fall season. Similarly, diazinon has high detection frequencies; 64 % in spring, 46 % in fall seasons.

The extend of pesticide pollution in Kumluca well waters are summarized in Table 3.20, considering the limits set by EU directives (EU Council Directive 98/83/EC, 1998), for a single pesticide (100 ng/L).

Table 3.20. Number of Sampling Points with Concentration Values Higher than the Limit for Individual Pesticides

Season	**Spring**		**Fall**	
Sample Type	**GW**	**SW**	**GW**	**SW**
Azinphos-methyl	4	1	-	3
Bromophos-ethyl	-	-	1	-
Chlorpyrifos	2	3	-	1
Diazinon	1	2	-	1
Dichlorvos	1	1	2	-
Fenamiphos	5	-	3	2
Fenitrothion	-	1	-	-
Fenthion	-	-	-	-
Malathion	-	-	-	-
Methidathion	-	3	-	-
Parathion-methyl	1	3	-	-
Phosphamidon	-	1	-	-
Endosulfan Sulf	-	-	-	3

In ground water samples, the limit value is exceeded 20 times for the analyzed pesticides. Although it is not reflected in the table, in spring season, 9; in fall season 4 ground water samples contain at least one pesticide higher than this limit. We can state that, 24% of the ground water samples the concentrations for single pesticides reflects certain pollution. In surface waters, the limit value exceed 25 times., for 4 sampling points in spring and 3 sampling points in fall seasons. These make up 32 % of the surface water samples.

3.5.4. Environmental Behavior of Organochlorine Pesticides

The seasonal differences among parent compound and degradation products may give information about the behavior of OCPs in Kumluca environment. The OPPs can not be taken into consideration as their lifetimes are short to show a seasonal change.

Kumluca shows typical Mediterranean climate characteristics, with hot, dry summers and cool wet winters. As stated, the sampling campaigns were performed at the end of spring and fall seasons. Therefore, the spring samples represent the pesticide behavior after a wet winter and fall samples represent their behavior after a dry and hot summer season.

The most commonly observed OCP in the region is endosulfan. It is observed in 70% of all samples in both seasons. Its degradation product, endosulfan sulfate has a percent occurrence of 62%. The β-isomer of endosulfan is observed only 22 % of all samples.

Similar to discussions in Section 3.3.1, the concentration ratio of total α- and β-endosulfan to endosulfan sulfate may give information about the degradation pathway of this pesticide in Kumluca environment. Now, the ratios will be compared between two season samples. When we look at the ground waters, the ratio is 1.0 and 1.4 for spring and fall seasons. Whereas, it is 0.9 and 0.2 in spring and fall seasons for surface waters. As seen, in spring season, endosulfan and its derivatives have similar concentration patterns in surface and ground waters, as this pesticide is currently applied in most of the farms. However, the lowest ratio in fall season clearly demonstrates its degradation to endosulfan sulfate in surface waters, which are open to atmosphere and subject to sunlight.

Similarly, the concentration ratios of aldrin to dieldrin for ground waters are 10 and 13; for surface waters, 0.5 and 20 in spring and fall, respectively. As stated in Section 3.3.1, aldrin has slow degradation in Kumluca. However, it is seen that the degradation product, dieldrin is dominant in surface waters in spring. This is due to the almost ten times higher solubility of dieldrin than aldrin. In spring, as the pollutants are subject to run-off from soil due to rain events, the dieldrin is dissolved more and polluted the surface waters.

The concentration ratio of DDT and its derivatives in ground waters are 1.7 and 1.3, in surface waters, 0.5 and 1.4 for spring and surface waters, respectively. Here, the extreme concentration (57.9 ng/L) observed for DDT in one of fall season surface water samples was excluded. Similar to dieldrin, DDE has higher water solubility than its parent compound DDT, therefore, it is dominant in surface waters in spring.

These findings show that, in Kumluca environment, the degradation of OCPs mostly occurs in the surface waters. Here, it should be noted that, the data set obtained for HCH isomers is not suitable to drive such conclusions, as they are not observed together for *both* seasons in water samples.

3.6 Spatial Distribution of Pesticides

In order to evaluate the pesticide pollution in Kumluca region, finally, the spatial variation of the target analytes will be presented. For this purpose, the contour maps for the distribution of total pesticide concentrations will be demonstrated, and the relationship between the observed concentrations and the characteristics of the sampling points will be discussed in this section. In these discussions, the seasonal trends will also be considered for a complete evaluation of the data set obtained.

The general pattern and seasonal variations of the total pesticide concentrations have been shown in previous sections. To visualize their spatial distribution, the maps were drawn by using MapInfo Professional 7.5 SPC program, with Vertical Mapper VM 1.51 utilities.

3.6.1. General Distribution of Total Pesticide Concentrations

Figure 3.23 shows the distribution of the total OPP concentrations among all the samples in spring and fall seasons. It should be noted that, the contribution of OCPs on total concentrations are almost negligible when compared to OPP concentrations, as shown in Section 3.5.1.

From the figure the higher OPP pollution in the spring season is clearly seen. As stated before, the reason is the higher application rate of these pesticides in this season. As the half lives of the pesticides are mostly shorter than the period between two sampling campaigns, the fall season concentrations are lowered.

However, the pollution near the coast is also obvious for both seasons. This is due to the streams, discharging to see among these polluted points.: Akmaz (34), Gavur (35), Göksu (36) and Alakır (37).

The spatial variations of total OCP concentrations are shown in Figure 3.24. As seen, in contrast to OPPs, OCP concentrations are higher in fall season. Moreover, the OCPs are more homogenously detected in Kumluca region. The coastal site is more polluted also with OCPs, when compared to inner parts. In fact, the green houses are concentrated at the regions shown to be polluted in Figure 3.24 (Karşıyaka, Resiller, Beykonak and Mavikent).

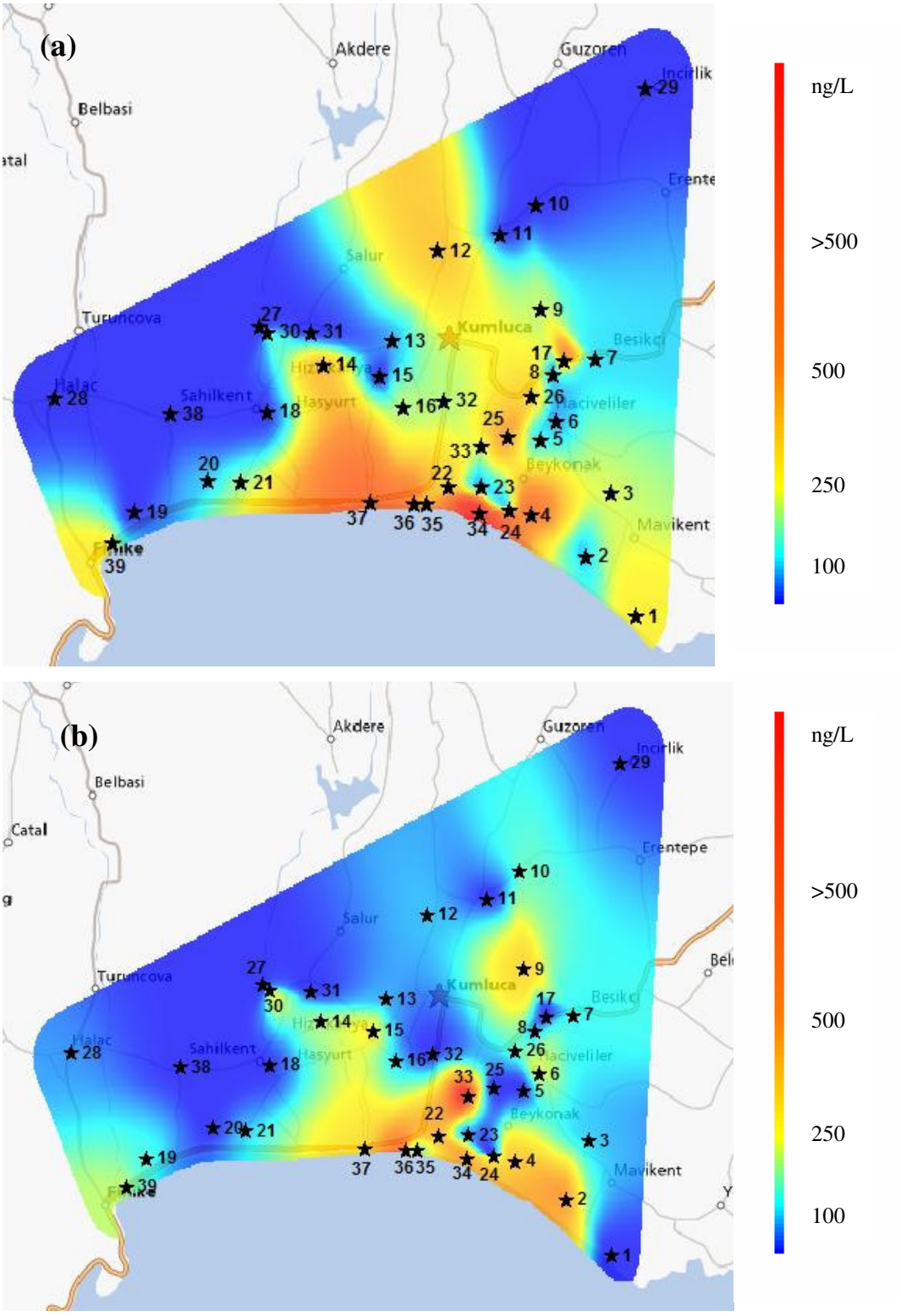


Figure 3.23. Counter Maps for OPP pollution in both Season; (a) Spring, (b) Fall Seasons

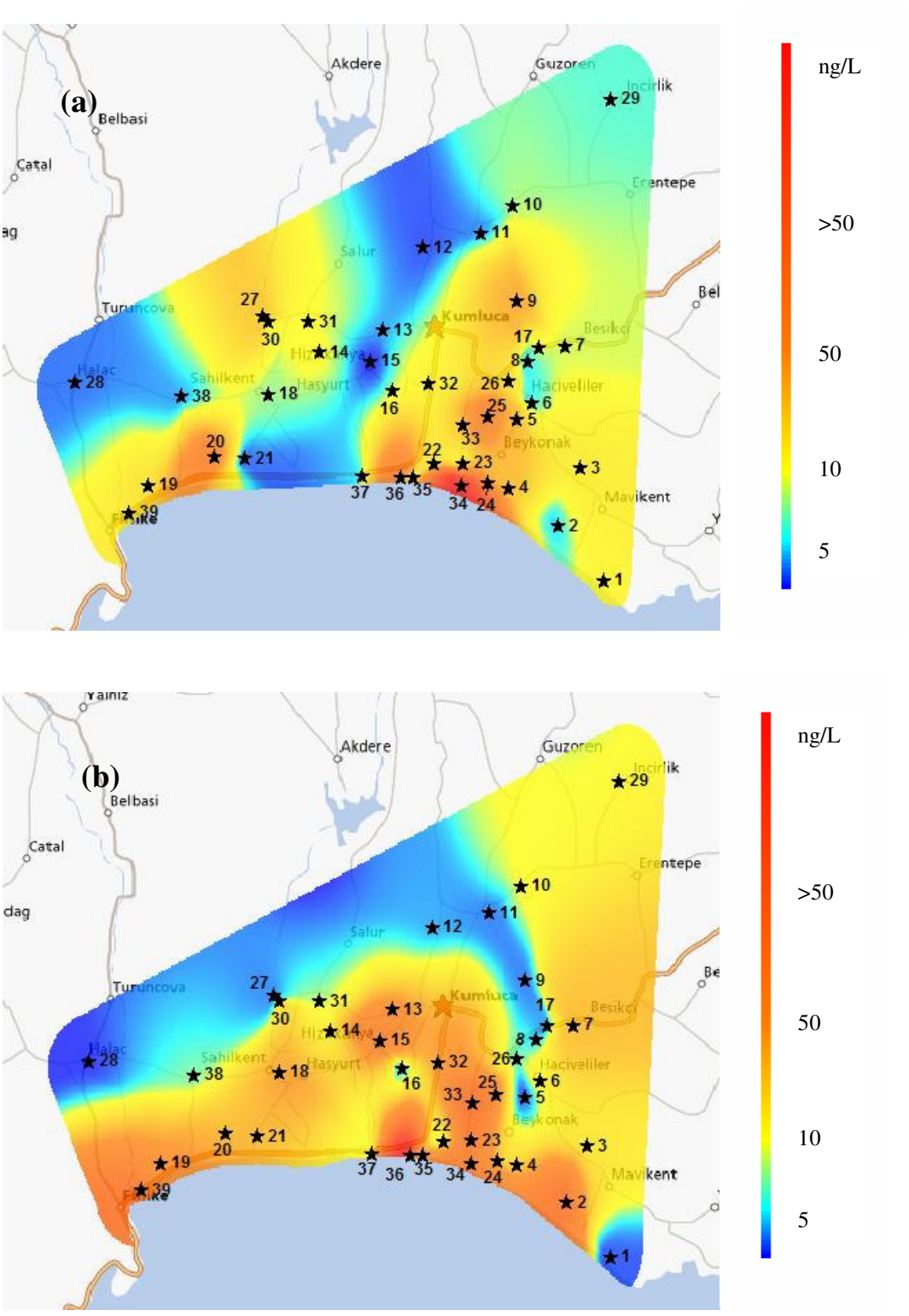
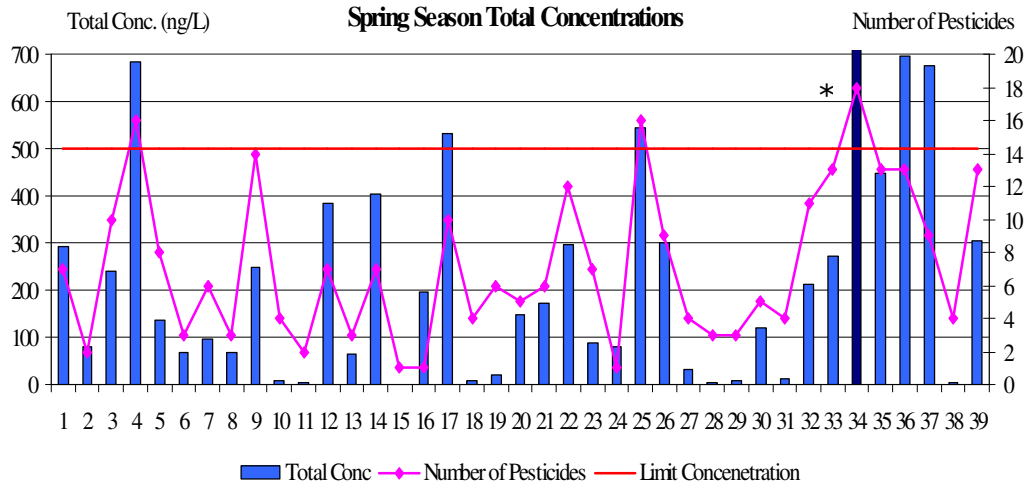


Figure 3.24. Counter Maps for OCP pollution in both Season; (a) Spring, (b) Fall Seasons

Figure 3.25 shows the variation of total pesticide concentrations in every sampling point in spring and fall seasons. In this figure, the number of pesticides detected in every point is stated to give general information about the pollution. Moreover, the limit value (500 ng/L) set by EU directives (EU Council Directive 98/83/EC, 1998), is also shown. It should be noted that, the contribution of OCPs on total concentrations are almost negligible when compared to OPP concentrations, as shown in Section 3.5.1.

The information about the sampling points has been given in Table 2.2 and Table 2.3 for ground and surface waters, respectively. It should be reminded that, the sampling point numbers which are higher than 28 represent the surface waters.

From the figure, the higher pollution levels in surface waters can easily been observed for both seasons. Sampling points 29, 31 and 38 are water springs of streams in the region and the concentrations of the pesticides are very low compared to other sampling points, in both seasons.



* Sampling Point 34 is out of range with a total concentration of 1145 ng/L

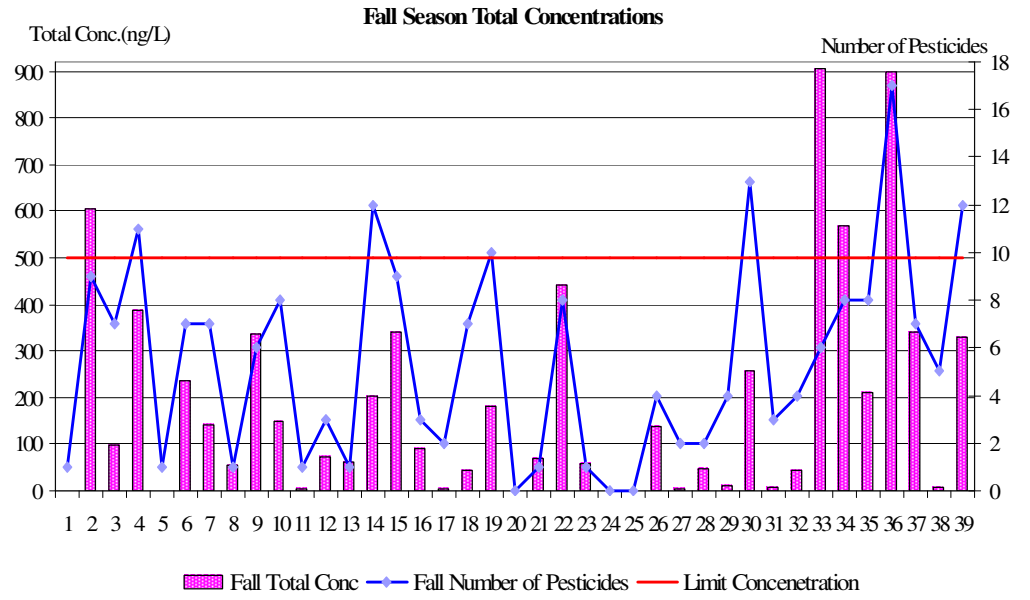


Figure 3.25. Spatial Variation of Total Pesticide Concentrations among Sampling Points

3.6.2. Pesticide Pollution in the Wells

There are some wells exceeding the limit for total pesticide concentration (500 ng/L) set by the EU directives (EU Council Directive 98/83/EC, 1998), as seen in Figure 3.25. For spring season, these are sampling points 4, 17 and 25, for fall season it is only 2.

The sampling point 4 belongs to a well in a greenhouse where paper was cultivated in sampling season. In spring, the total number of pesticides detected at this point is 16, 7 of which were phosphamidon, malathion, chlorpyriphos, bromophos ethyl, fenamiphos and azinphos methyl among OPPs. It should be noted that, this point also exceeded the concentration limit of a single pesticide (100 ng/L) for fenamiphos and azinphos methyl. During the sampling, it was recorded that, the pesticide applications have been performed just one week before sampling. The results obtained clearly indicate the effect of this application in the well.

In the fall season, in addition to diazinon; phosphamidon, chlorpyriphos and bromophos ethyl were also detected. In fall season, the concentration of bromohos ethyl was higher than 100 ng/L and the concentration of chlorpyriphos was close to this limit with a value of 93 ng/L.

In both seasons, the OCPs, α- and γ-HCH, heptachlor endo-epoxide, dieldrin, endosulfan and endosulfan sulfate were detected, with generally higher concentrations in fall season. These findings clearly show the pollution due to agricultural activities for this well.

The sampling point 17 also belongs to the well of a greenhouse, in which tomato was cultivated. Similar to previous case, this well also exceeds the limit of 100 ng/L for fenamiphos and azinphos methyl. Besides these, at this sampling point, 8 more pesticides were detected, 4 of which are OPPs. In fall season, only two OCPs were detected, aldrin and endosulfan, which were also observed in spring season.

The sampling point 25 was a well in an orchard. In spring season, 5 OPPs and 11 OCPs were detected in this well. Among OPPs, the concentrations of parathion, chlorpyriphos and azinphos methyl was higher than the limit value and the concentration of diazinon (94 ng/L) should also be emphasized. However, in fall season, none of the pesticides were detected.

In sampling point 2, which belongs to a green house where paper was cultivated, the concentrations of dichlorvos and fenamiphos were higher than, and that of azinphos methyl (96 ng/L) was very close to the limit value in fall season. Among OCPs, endrin aldehyde, heptachlor, endosulfan and its degradation product, endosulfan sulfate were detected. In spring season, only dichlorvos and endrin aldehyde were observed. The detection of endosulfan (13 ng/L) and its sulfate derivative (5 ng/L) indicates the application of this pesticide between two sampling campaigns.

In fact, this findings correlate well with the pesticide use trends in the region. In the region, the use of pesticides is not controlled. The farmers are not aware of their needs and they have a tendency to apply different formulations at the same time, as indicated by the higher number of OPPs observed in the same wells for the same season.

Figure 3.26 shows the concentrations of mostly observed OPPs (chlorpyriphos, azinphos methyl and diazinon) in the ground water samples for both seasons.

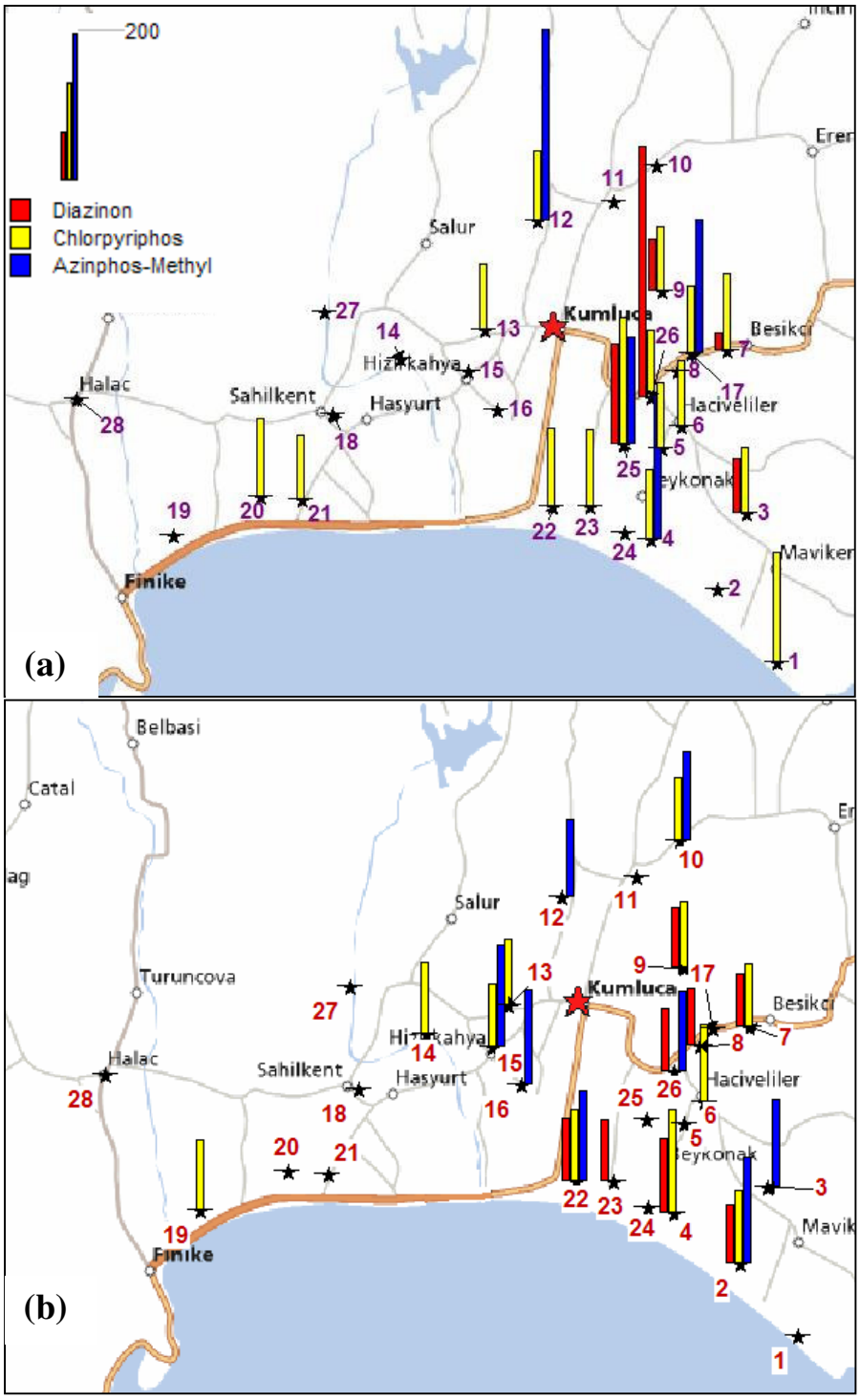


Figure 3.26. Distribution of Selected OPPs among Wells for (a) Spring and (b) Fall Seasons

As seen, the Hacıevler, Beykonak and Mavikent regions of Kumluca, where the green houses are concentrated, is more polluted than other sites.

The higher concentrations of these OPPs in spring season can clearly be observed in the figure. From the figure it is also recognized that the OPPs detected at high concentrations in spring was disappeared in fall season, such as chlorpyriphos at sampling points 1, 20 and 21; azinphos methyl at 4 and 25; diazinon at 3,4 and 25. This is due to short half life of these pesticides, after their application, they have high concentrations in the wells. However until the second sampling campaign, they have decomposed and have not been observed in fall season.

Another important conclusion picked up from data set is about the degradation pattern of fenamiphos. This pesticide was detected in 5 wells (sampling points 4, 12, 16, 22) in spring season, and only in a single well (sampling point 22) in fall season. All these detections were higher than the limit value (100 ng/L) for a single pesticide.

The hydrolysis half life of fenamiphos is stated as 300 days (PAN Pesticide Database, n.d.), leading the formation of fenampiphos sulfoxide as the main degradation product (Patterson et al., 2000; Megharaj et al., 2003). However, Lacorte et al. (1995) has reported a half life of 1.8 days in filtered estuarine water. Rate of degradation for fenamiphos vary significantly and show dependency on individual site conditions (Patterson et al., 2002). The time period between two sampling campaigns was 165 days. Roughly, if we assume complete degradation after 4 half-life period, the half life of fenamiphos would be lower than 41 days.

The findings of this work show that, to evaluate the pesticide pollution in the region, a monitoring program is necessary. This program should be conducted more frequently, in shorter time intervals and should include the analysis of soil and sediments. Besides these, the pesticide application records should also be obtained.

The distribution of most frequently observed OCPs in both seasons are presented in Figure 3.27. For fall season, the concentrations are generally higher. Similar to OPPs, in the spring season, more OCPs were detected in the wells. As explained before, the reason may be the mixing of water tables at this season.

Some OCPs detected in spring season seems to be disappeared in fall season for some sampling points. For example aldrin was detected in sampling points 11, 19, 20, 27 and 28, where in fall season, this pesticide was not detected. Knowing that, aldrin is a persistant pesticides when compared to OPPs, this finding may be surprising. However, as stated, the fate of a pecticide in environment depends on physical and chemical properties of the pesticide (the solubility of pesticides, their Koc values) the environmental conditions (soil properties, water flow, and temperature). In spring season, the precipitation and irrigation may desorp the pollutants which have bonded to soil more than in fall season, in addition to leading the mixing of water tables.

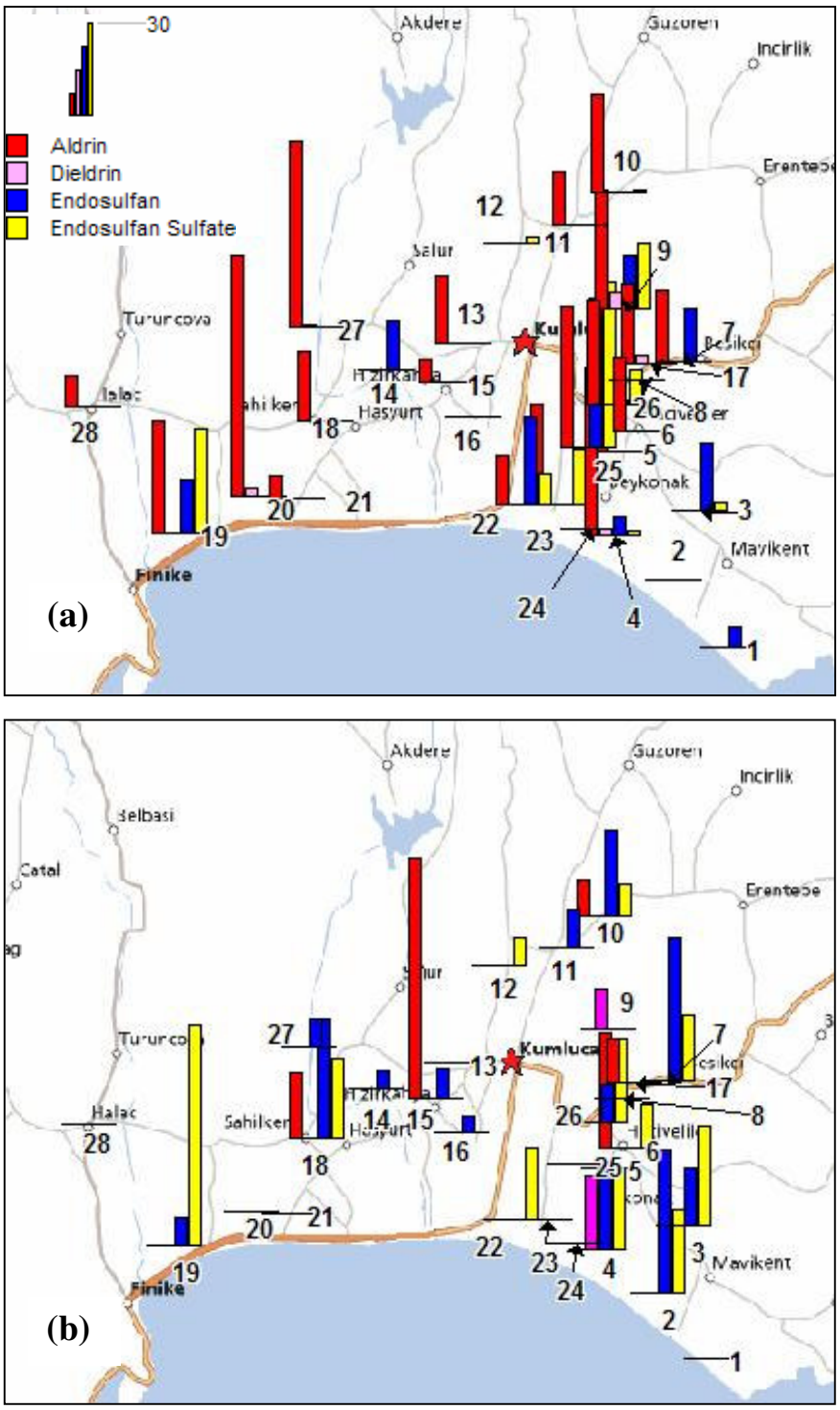


Figure 3.27. Distribution of Selected OCPs among Wells for (a) Spring and (b) Fall Seasons

From the data set obtained, it is seen that in some wells, similar OCPs were detected. For example, in sampling points 1 and 3, the pesticides observed were similar; α-HCH, β-HCH, heptachlor endo, endosulfan and endosulfan sulfate were detected in both wells. The concentration ratio of β-HCH/α-HCH is 0.13 and 0.19 for sampling points 1 and 3 respectively. Considering their closeness to each other, we can suggest a connection of ground water tables between these points.

Similarly, for both sampling points 4 and 9, α-HCH, γ-HCH, aldrin, heptachlor endo, dieldrin, DDT, endosulfan and endosulfan sulfate were detected. The concentration ratio of γ-HCH/α-HCH is 0.12 and 0.09 for sampling points 4 and 9, respectively.

Sampling points 22 and 25 have also α-HCH, γ-HCH, δ-HCH, aldrin, heptachlor endo, DDT, endosulfan and endosulfan sulfate. However, for a certain statement, these findings should be supported with the measurement of other parameters, such as the ions.

The aim of this work was just to detect the pesticide pollution in Kumluca surface and ground waters. Therefore, the findings may be used as supplementary data for the studies about behavior of these pollutants in water systems and for the studies about hydro-geological characteristics of the aquifers in the region.

The comparison of the total concentrations among wells with different depths gives information about the pesticide behavior. The pesticides may reach to shallow aquifers more easily than deeper wells, where the pesticides may absorb to soil before they reach to water tables. The depths of the wells for each sampling point are given in Table 2.2. Figure 3.28 shows the variation of total pesticide concentrations with well depths. The sampling points from 22 to 28 have depths higher than 50 m.

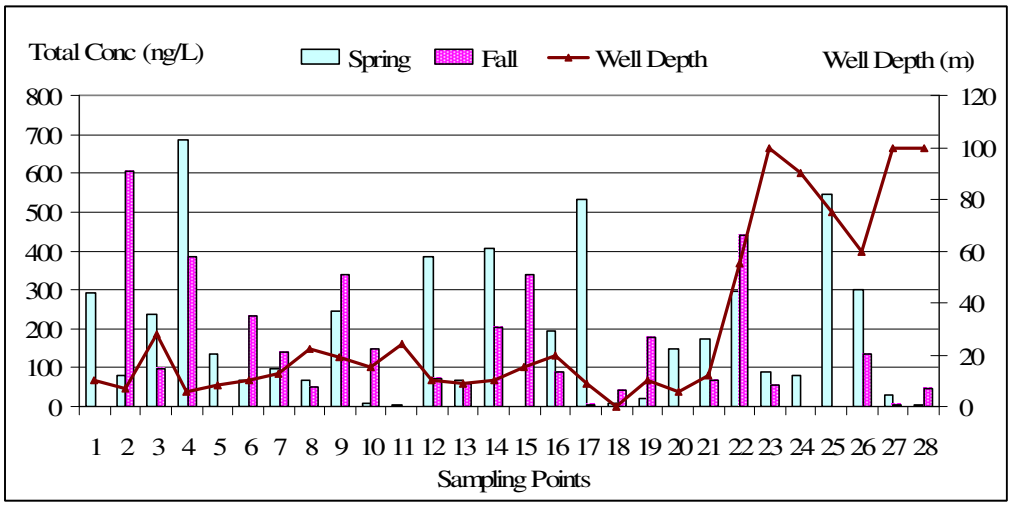


Figure 3.28. Well Depth and Total Pesticide Concentrations

As seen, the total concentrations of pesticides in shallow wells are generally higher, as expected. When the well depth is higher than 80 m, the total pesticide concentrations are significantly lowered. However, for sampling point 22 (Karşıyaka), with a depth of 55 m, the total pesticide concentrations for both seasons are considerably high. The observed high total concentration in spring season is due to the cumulative effect of 12 different pesticides with mostly lower than limit concentrations, except fenamiphos (204 ng/L). For fall season, the number of detected pesticides is 8 for this point, with again low concentrations except dichlorvos (115 ng/L) and fenamiohos (111 ng/L). This is due to high application rate of various pesticides around this sampling point.

In sampling point 25, the well depth is 75 m and the total pesticide concentration is only high in spring season, due to 16 different pesticides, mostly with low concentrations, similar to sampling point 22.

In sampling point 26, where the well depth is 60 m and 9 pesticides were detected. However, only diazinon with a concentration of 235 ng/L is dominating over the others, increasing the total concentration up to 302 ng/L. However, for fall season, the total pesticide concentration is lowered to 137 ng/L with diazinon concentration of 57 ng/L.

3.6.3. Pesticide Pollution in the Surface Waters

The samples from surface waters of Kumluca were collected from three springs (sampling points 29, 31 and 38), mid-points (30, 32, 33), and their discharge points to the sea (34, 35, 36, 37, 39). The Incircik spring (sampling point 29) flows through Gavur stream (32 and 35). Goksu spring (31) feeds also Gavur stream and through irrigation channels, it reaches to Akmaz stream (33 and 34). The water from an irrigation dam, in northern part of Kumluca, reaches to sea through Alakır stream (30 and 37). Besides these, in Finike, there is one stream sampled, named as Tatlısu (39), with its spring in Zengeder (38).

As stated in previous sections, the percent occurrences and concentrations are higher for surface waters than ground waters. From Figure 3.25, it is seen that for some sampling points (32, 34, 35 and 37), the spring season total concentrations are higher than fall season. These are streams flowing through dense green house regions and subject to pesticide more in spring season.

The distribution of total pesticide concentration through the streams is given in Figure 3.29.

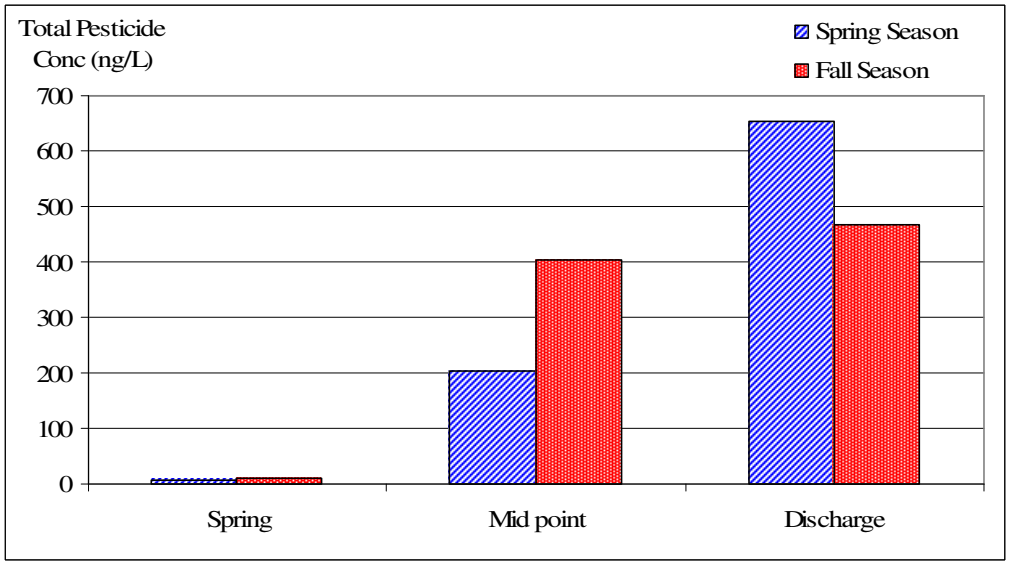


Figure 3.29. Total Pesticide Concentrations through Streams

From the figure, it is seen that the pesticides used in the fields are carried to the sea through these streams, with increasing total concentrations. The main mechanism for this carryover is surface run off and spray-drift. The high application rates and observed precipitation events in spring season are increasing the pollution in surface waters, when compared to fall season.

The spatial distribution of total concentrations for OPPs and OCPs in surface waters are shown in Figure 3.30.

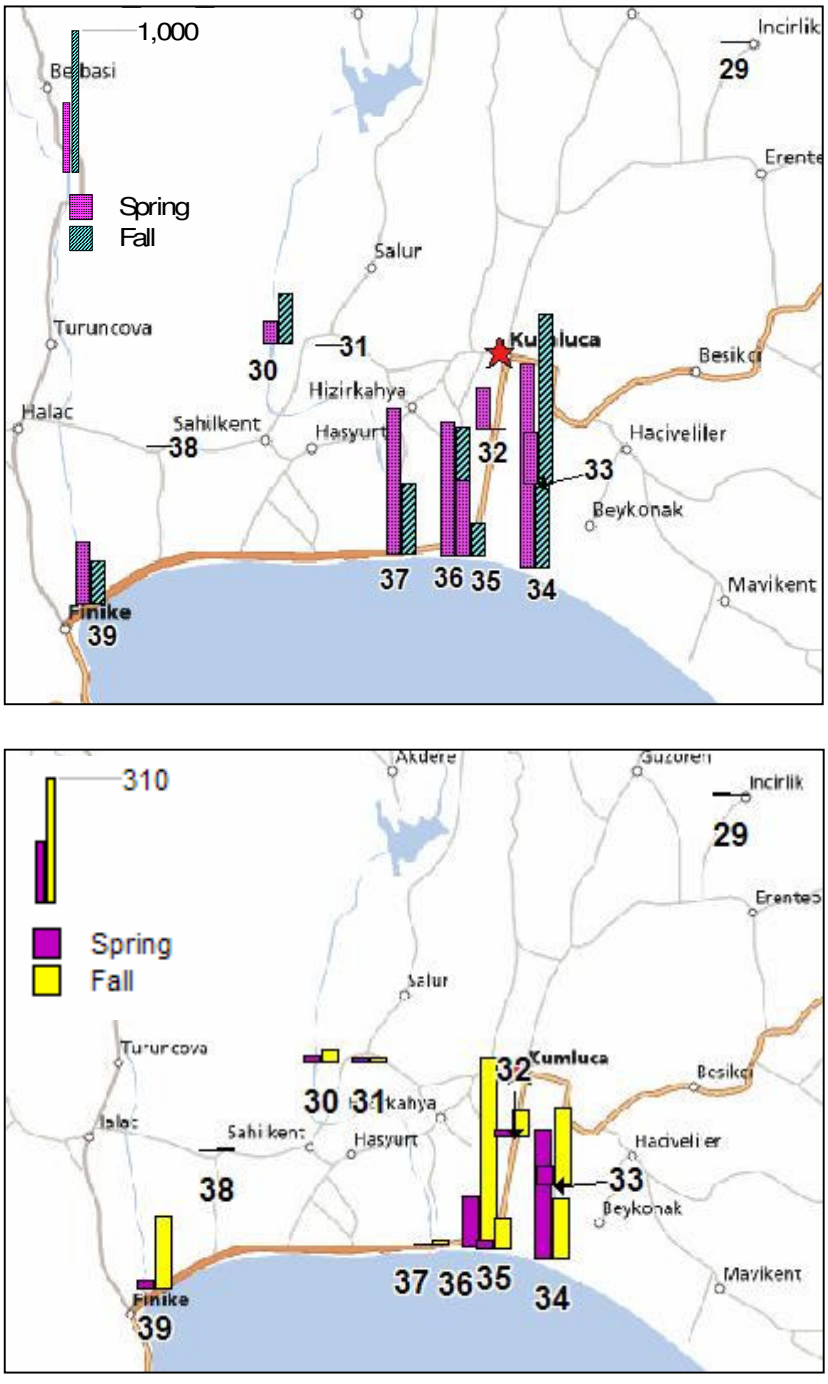


Figure 3.30. Spatial Distribution of Total Concentrations of OPP (a) and OCP (b) Pollution in Surface Waters

The Akmaz stream (33) flows through a dense green house region, where the OPP concentrations were highest among wells (Figure 3.26). The observed high concentrations in this stream are due to the application of OPPs around this stream. As seen the discharge point (34) is the most pollutant one, indicating a high discharge rate to sea from this point. It should be noted that, in the region, there are numerous irrigation channels and cracks flowing among the farms to the streams. They all have contribution to the pollution here besides the sampling point 33.

Similarly, the OPPs were carried through Alakir (30) and Gavur (32) streams to sea at the points 37 and 35, respectively. This is indicated by increasing total OPP concentrations from middle parts to discharge points.

From the figure, it is also seen that, the OCP concentrations are also increasing in the streams through their way to the sea.

CHAPTER 4

CONCLUSIONS

This study covers analysis of pesticides in environmental waters of an important agricultural area of our country, Kumluca for the first time. Moreover, among similar studies conducted in Turkey, this study brings a new perspective in the analysis of high number of pesticides with detailed analytical chemistry approaches.

Surface and well water samples in Kumluca, a distinct of Antalya in Turkish Mediterranean coast, were analyzed for 17 organochlorine (OCP) and 14 organophosphorus pesticides (OPP). The water samples from domestic wells and surface waters were collected in spring and fall seasons of 2005. In total, 140 samples were collected from 39 points, 11 of which were surface waters. The pesticides in water samples were extracted with Solid Phase Extraction (SPE) technique by means of extraction disks. The analysis were performed by GC-ECD and GC-NPD systems for chlorinated and phosphorus pesticides, respectively. The SPE procedure and the analysis systems were optimized before their use for real samples.

The quality check (QC) and quality assurance (QA) practices were applied during sampling, sample preparation and analysis steps. The results of QC/QA tests reveal that, the laboratory and field environment did not contribute to observed pesticide concentrations. Analyses of the sampling replicates have demonstrated a good agreement between the replicate sample collections.

The sample extractions were performed mostly with acceptable recoveries for spiked control matrix which were ranging in between 61-100 %. They were generally in the limit of acceptance (70-130%) according to EPA, except for two OPPs, fenthion and methidathion which have 61 and 64 % recoveries, respectively. Moreover, high reproducibility of the SPE procedure applied was shown by RSD values better than 15 % for spiked control matrix. The surrogate recoveries were in between 70-130 % showed that each extraction was performed successfully. These results indicate that the SPE technique was appropriate for the extraction of chlorinated and phosphorus pesticides from aqueous matrices.

The SPE procedure was applied to spiked sample matrix, in order to determine the effect of matrix on extraction performance. The percent recoveries are decreasing with increasing amount of particulate matter content in the samples. The lowest recoveries were obtained for surface water samples, with an average of 77.5 %. For ground water samples, the average of recoveries is 81.4%, and highest were obtained for spiked control matrix with 88.9 %. These differences were statistically proven. The particulate matter content was also affecting the reproducibility of the samples, the percent RSD values are the highest for surface water samples (16%), when compared with the ground water samples (12%) and spiked control matrix (9%).

The standard reference materials (SRM) were continuously analyzed to check both the accuracy of the measurements and the stability of the analysis systems. The relative percent errors for the SRMs were almost below 15 %, indicating high accuracy for almost all of the target pesticides. Moreover, it is shown that the analysis systems were stable during the analysis period, as the results of the SRM analysis were in the limit of $\pm 2\sigma$ (standard deviation) from the averages.

The uncertainties of the measurements were calculated with an approach based on EURACHEM/CITAC guidelines. The main uncertainty components were decided as sampling, recovery, estimated analyte concentration in the sample and repeatability of the measurements. It was shown that, for both OCP and OPPs, the uncertainty arising from the reproducibility of the measurements were lowest almost for all pesticides, whereas the uncertainty arising from recoveries were generally the highest. It was observed that, to reduce the uncertainty arising from sampling replicates, the number of these samples should be higher.

In Kumluca water samples, among two types of pesticides, the OCPs were generally observed more frequently observed. In both sample types and in both seasons the most frequently detected pesticides, were as follows; among OCPs, endosulfan (70%), endosulfan sulfate (62%) and aldrin (49%); among OPPs, chlorpyriphos (53%), diazinon (33) and azinphos methyl (23%). The percent occurrences of most of the pesticides were higher in surface waters. In spring season, the frequencies of detection were generally higher for all pesticides, in surface and ground waters. The OPPs are more frequently applied in spring, and the rain events can carry the pesticides to ground waters by leaching and to streams by surface run-off. For OCPs, the reason of high detection was due to sufficient mixing of water sources in spring season.

The concentrations of OCPs were almost 10 times lower than OPPs, as they are not currently used in the region and as their solubilities are much lower than OPPs. The highest concentration among OCPs was 188 ng/L and observed for endosulfan sulfate in a surface water sample in fall season. In the wells, aldrin has the highest concentration (76 ng/L). The highest average concentrations were also observed for these pesticides; in surface waters for endosulfan (32 ng/L) and in well water for aldrin (12 ng/L).

Although they have low concentrations, the high occurrences of OCPs and detection of degradation products in Kumluca environmental water samples clearly indicate their intense use before 1980s, except endosulfan which is still used.

Some of the OPPs in Kumluca water samples have concentrations higher than maximum allowable concentration of a single pesticide (100 ng/L). The acute effects of phosphorus pesticides are more severe than other types of pesticides. Therefore, their presence in high concentrations poses a risk for human health. The highest OPP concentration was 565 ng/L for azinphos-methyl in a surface water sample in fall season. This pesticide has also the highest average concentration for surface waters (184 ng/L). In the well waters, fenamiphos has the highest observed (395 ng/L) and average (191 ng/L) concentration.

In general, the pesticide concentrations were higher in surface water samples. The surface waters are subject to pollution more than ground waters due to surface run off and spray drift from application fields. The disposal of empty containers to open streams was another pollution source for surface waters in Kumluca region.

The analysis results of Kumluca water samples reveal that, agricultural activities affect the water quality in the region. The total concentration limit of 500 ng/L, set by the EU regulations, was exceeded for 27% of surface and 14% of ground water samples, at least once in both seasons. The limit for a single pesticide (100 ng/L) was exceeded by 32 % of surface, 24 % of ground water samples.

The total OPP concentrations for both types of water samples were higher in spring; the total OCP concentrations were higher in fall season. However, these differences were shown to be insignificant.

These findings show that, in Kumluca environment, the degradation of OCPs mostly occurs in the surface waters. Moreover, the half life of fenamiphos was suggested to be lower than 41 days in Kumluca environment, although it is stated to be 300 days.

The pesticide pollution was higher in Beykonak and Mavikent regions of Kumluca, where the green houses were concentrated. It was shown that the pesticide pollution in the rivers (Akmaz, Alakır, Göksu and Gavur) were increasing through the sea. This is due to the carryover of the pollutants via surface run off and spray-drift from application areas through irrigation channels and cracks flowing among the farms to the streams.

CHAPTER 5

RECOMMENDATIONS FOR FUTURE STUDIES

In this study, it was observed that the environmental waters in Kumluca were polluted by pesticides due to intense agricultural activities. Kumluca is a very important agricultural area for our country. The results obtained in this thesis are valuable for the sustainability of the agriculture in the region. However, there are some further studies required to see the complete picture of the pesticide pollution in Kumluca.

The first and the most important data set required are the pesticide use trends in the region. The presented pollution patterns should be correlated with defined pesticide application records answering the questions; which pesticides are used, when, how much and how.

It is clear that, although the work in this study provides the first, basic and informative data on pesticide pollution in Kumluca waters, the further studies should be conducted using these findings within a monitoring program. This program should include sampling for a longer time with shorter intervals, to evaluate the pesticide pollution.

Besides these, to evaluate the environmental behavior of the pesticides, the monitoring program should include the determination of pesticides in soil, sediment and vegetable samples. The determination of metals and organometallic compounds may also help to assess the environmental pollution in the region for a sustainable agricultural planning. The data in this work further may be used for risk assessment calculations.

The findings in this thesis work may be used for, or supported, by the studies about hydro-geological characteristics of the aquifers in the region; the groundwater table depths, hydrological properties, flow patterns and depth of sampled wells.

Another data required to complete the pesticide pollution assessment in environmental waters of Kumluca is the flow regime of surface waters. Further studies should be performed considering the pattern of surface water flow, with seasonal variations.

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APPENDIX A

ESTIMATION OF UNCEARTAINTY IN MEASUREMENTS

A.1. Basic Definitions and Background Information

The International Organization for Standardization (ISO) has developed a detailed guide for the calculation of uncertainties. The document "Guide to the Expression of Uncertainty in Measurement", the so-called "GUM" was published in 1993 (corrected and reprinted in 1995) with a number of detailed examples. It defines the term "measurement uncertainty" as follows: "Parameter, associated with the result of a measurement that characterizes the dispersion of the values that could reasonably be attributed to the measurand". The parameter may be "standard deviation" or the width of a confidence interval.

The main approaches to calculate the uncertainty are "bottom-up" and "top-down" methods. The former considers each individual step for measurement process, and combines them to give a final uncertainty. This approach was proposed by ISO and it is the favorite overwhelmingly, being central to many associations such as EURACHEM, American Association for Laboratory Accreditation (A2AL) (Vanatta and Coleman, 2007). On the other hand, the

"top-down" approach uses validation data and proficiency testing results to estimate uncertainty in measurements. A disadvantage of this method is that no information is available about the variation of uncertainty and no corrective actions can be performed to improve the analytical methods used (Quintana et al., 2001). The approach used in this work for the estimation of uncertainties in the measurements was "bottom-up" approach, based on the guidelines of EURACHEM. The guidelines presented in "EURACHEM/CITAC Guide, Quantifying Uncertainty in Analytical Measurement", which was based on ISO Guide, was adapted for the estimation of uncertainty of measurements for the determination of pesticides in Kumluca environmental water samples.

In bottom-up approach, the main steps in the estimation of uncertainty are;

1. Specification of the measurand
2. Identification of uncertainty sources
3. Quantification of uncertainty components
4. Calculation of combined uncertainty
5. Calculation of expanded uncertainty

1. Specification of the Measurand: The measurand should be well defined and relationship between the input quantities and the measurand should be stated. A flow diagram of the procedures followed will be helpful showing the steps. An Ishikawa diagram or cause-effect diagram (also termed as fishbone diagram) is also a useful tool to identify the influence parameters (Meyer, 2007). By drawing such a structure, one can identify, sort and discuss these parameters. A fishbone diagram for this study will be presented in the subsequent sections.

2. Identification of Uncertainty Sources: In the estimation of overall uncertainty, each source should be specified carefully and treated separately. The each separate contributor is called "uncertainty component". Uncertainty component is known as "standard uncertainty" ($u(x)$) when it is expressed by

standard deviation. For the measurement uncertainty of *y*, the total uncertainty is called as "combined standard uncertainty" and donated by $u_c(y)$. It is an estimated standard deviation equal to the positive square root of the sum of variances for all uncertainty components (EURACHEM/CITAC Guide, 2000).

3. Quantification of uncertainty components: In the case of the uncertainty component evaluated experimentally, the standard deviation (*s*) is directly used in combination. These type of contributors are called Type-A, and $u(x) = s$.

The other components, which can also be characterized by standard deviations, are evaluated from assumed probability distributions are called Type-B. The evaluations of distributions are based on experience, general knowledge of the behavior and property of relevant materials or instruments, previous measurements, manufacturer's specifications, calibration data etc.

Rectangular, triangular and normal distributions are mostly used. "Rectangular" or "uniform" distribution is used to model cases where the probability of obtaining any value between two limits is equal to the probability of obtaining any other value. The uncertainty is obtained by the formula, $u(x) = \frac{a}{\sqrt{3}}$, where, ± a is the containment limits. It may be the case that there is a tendency for the values of the uncertainty contributor to be near the center of the distribution. An estimate should be made as "triangular distribution", for which the uncertainty can be calculated by $u(x) = \frac{a}{\sqrt{6}}$.

When an estimate is made from repeated observations of a randomly varying process, the "normal distribution" is assumed and the uncertainty is given directly in the form of standard deviation, s, or coefficient of variation, CV $u(x)=s$ (Adams, 2002).

EURACHEM offers to use $u(x)=s/\sqrt{n}$, where n is the number of measurements, for the uncertainties of run to run variations when an analytical procedure is applied for "long term" preferably with high number of replicates, such as in in-house validation studies. By this way, the variations among these replicates are corrected for the uncertainty of the single values.

4. Calculation of Combined Uncertainty: Following the estimation of individual components of uncertainty, the next stage is the calculation of combined uncertainty. In general, the mathematical model will be a function of several input quantities showing how the measurement result is obtained from the input quantities or components. If the input quantities are designated as x_1, x_2 ,…,x_n , then the functional relationship between the measurement result y and the input quantities x can be written as

$$y = f(x_1, x_2, \ldots, x_i) \qquad \text{(Eq.A.1)}$$

This function is to be understood in the broadest possible context as including every possible source of variation in the measurement result (Adams, 2002). The uncertainty of the measurement result y (u_y) arises from the uncertainties of the input estimates x_i ($u(x_i)$) in the equation above. Once all of the values of the uncertainty contributors have been estimated and reduced to one standard deviation, the square root of the sum of the squares of the uncertainty estimates give the combined standard uncertainty;

$$u_c(y) = \sqrt{u(x_1)^2 + u(x_2)^2 + \ldots + u(x_i)^2} \qquad \text{(Eq.A.2)}$$

The combination of input parameters requires a detailed quantitative model of the experimental procedure. When it is possible to establish a mathematical model between the parameters, the law of propagation of uncertainties is used for the calculation of resultant uncertainty:

For models involving only the sum or difference of the quantities, such as *y=(p+q+…),* the combined uncertainty is calculated by the equation above. For the models involving only a product or quotient, such as *y=p/(q×r×..),* the combined uncertainty is given by,

$$u_c(y) = y\sqrt{\left(\frac{u(p)}{p}\right)^2 + \left(\frac{u(q)}{q}\right)^2 + \ldots} \qquad \text{(Eq.A.3)}$$

For the models involving both sum or difference and multiplications or divisions, the mathematical model is broken down to expressions which consists only the operations explained above. The partial uncertainties are combined according to the rules explained (EURACHEM/CITAC Guide, 2000).

5. *Calculation of Expanded Uncertainty:* The final stage is to multiply the combined standard uncertainty by the chosen coverage factor *(k)* in order to obtain expanded uncertainty. The expanded uncertainty is required to provide an interval which may be expected to cover a large fraction of distribution of values attributed to the measurand. In choosing a value for *k*, the level of confidence required the knowledge of the distribution of measurements and the knowledge of the number of measurements should be considered. For most purposes, it is recommended that the *k* is set to 2 for 95% confidence interval with relatively high degrees of freedom (higher than six) (EURACHEM/CITAC Guide, 2000).

A.2. Calculation of Uncertainty Components for Pesticide Analysis in Kumluca Water Samples

The combined uncertainty for the measurements is modeled by the following formula, considering the experimental steps used for the determination of pesticide concentrations in Kumluca water samples.

$$u_{rel}(COM) = \sqrt{u_{rel}^2(smpl) + u_{rel}^2(SC) + u_{rel}^2(rep) + u_{rel}^2(R)} \qquad \text{(Eq.A.4)}$$

The calculation of uncertainties for each component is given in the following sections.

A.2.1. Uncertainty for Sampling, *u(smpl)*:

As stated in Section 3.1.1.2, sampling replicates were used to check variability in sampling. To calculate the uncertainty coming from sampling, the average of percent RSD or CV (coefficient of variation) values presented in Table 3.2 are used. The uncertainty arising from sampling replicates were calculated with the formula,

$$u(smpl) = CV_{av} / \sqrt{N} \qquad \text{(Eq.A.5)}$$

where N is equal to 15.

The percent CV values are considered instead of standard deviations to normalize the variation for different sample concentrations (EURACHEM/CITAC Guide, 2000). Moreover, average of the coefficient of variations (CV_{av}=0.83) is used, as there are pesticides for which the data is not available and for some pesticides, the values are calculated for single sampling points, which brings insufficient degrees of freedom. The CV_{av} is not directly used, but divided by a factor of √N, to correct the long term deviation on the

uncertainty of single values. Therefore, for all the pesticides, u(sampl) is calculated using Equation A.5 as follows,

$$u(smpl) = 0.083 / \sqrt{15} = 0.021$$

A.2.2. Uncertainty for Estimated Sample Concentration, *u(SC)*

The uncertainties arising from preparation of standards and from the linear calibration curves affects the estimation of analyte concentrations in the samples. Therefore, a mathematical model can be drawn as follows;

$$u(SC) = \sqrt{u^2(stds) + u^2(cal)} \qquad \text{(Eq.A.6)}$$

The obtained concentrations from calibration curve (C) were multiplied by dilution factor (DF) to calculate the concentration in 1.0 L sample.

$$C \times DF = C_{corr} \qquad \text{(Eq.A.7)}$$

This operation would be reflected in uncertainty calculations as follows;

$$u(SC)_{corr,rel} = \sqrt{\left(\frac{u(SC)}{C}\right)^2 + \left(\frac{u(DF)}{DF}\right)^2} \qquad \text{(Eq.A.8)}$$

Here the dilution factor is not conversion of analyte concentration in exactly 1.0 mL extract to the concentration in exactly 1.0 L sample. It covers the conversion of the concentration of the analyte detected in approximately 1.0 mL extract, obtained from approximately 1.0 L sample, to a concentration for exactly 1 L. As the internal standard calibration was used, there would be no uncertainty arising from the volume of 1.0 mL of extract. However, as the samples were collected with bottles and their volumes are measured in the

laboratory by using 1000 mL graduated cylinders, an uncertainty from this step may be expected.

The number of measurements for the volume measurements with 1000 mL graduated cylinder were higher than 100. Therefore the CV values may be used for the estimation of uncertainty arising from dilution factor, *u(DF)*. The CV value for the volume measurement of 100 samples was 0.006. For the calculation of *u(DF)*, this value should be divided by $\sqrt{100}$, giving *u(DF)*=0.0006. Moreover, the actual DF values are very close to 1 L. Therefore, the contribution of this conversion operation of (*u(DF)/DF)* on $u(SC)_{corr}$ calculation is negligible, leading, $u(SC)=u(SC)_{corr}$.

A.2.2.1. Uncertainty Arising From Standard Preparation; *u(stds)*

The operations in standard preparation are the stock standard preparation and further dilutions of these to working standards (calibration standards).

The stock standard is the 10 µg/mL mixture standard for 14 OPPs, prepared from 1000 µg/mL single standard of each OPP. The 1000 µg/mL single standard solutions of OPPs were prepared from neat standards, by weighing 0.1 g of neat pesticide and diluting them to 100 mL in volumetric flasks with acetone. The preparation of stock standard solutions can be expressed in the formula;

$$C_{stock} = \frac{m}{V_{100mL}} \times purity \times \frac{(V_{100\mu L})_1}{(V_{500\mu L})_1} \qquad \text{(Eq.A.9)}$$

where,

C_{stock}: The concentration of OPPs in standard mixture solution (10 µg/mL)

m: mass of neat standard

V_{100mL}: Final volume for single standard (100 mL)

$V_{100\mu L}$: The volume taken from single stock to prepare 10 µg/mL standard mixture solution (20 µL) with 100 µL injector

$V_{500\mu L}$: Final volume of standard mixture solution (2 mL), obtained with 500µL injector

Purity: The certified purity of neat standards by the supplier (%)

The calibration standards were prepared by further dilution of 10 µg/mL stock solution with 100 and 500 µL injectors.

$$Cstds = Cstock \times \frac{(V_{100\mu L})_2}{(V_{500\mu L})_2} \qquad \text{(Eq.A.10)}$$

The C_{stds} is the concentration of calibration standards, changing for each calibration level. The deviations for each calibration standards will be reflected in calibration uncertainty, therefore average concentrations of the calibration standards (64 ng/mL for OCPs, 0.64 µg/mL for OPPs) are used in this operation.

Combining Equation A.9 and Equation A.10, the overall uncertainty for the standard preparation will be;

$$\frac{u(stds)}{C_{stds}} = \sqrt{\left(\frac{u(m)}{m}\right)^2 + \left(\frac{u(V_{100mL})}{V_{100mL}}\right)^2 + \left(\frac{u(P)}{P}\right)^2 + \left(\frac{u(V_{100\mu L})}{V_{100\mu L}}\right)_1^2 + \left(\frac{u(V_{500\mu L})}{V_{500\mu L}}\right)_1^2 + \left(\frac{u(V_{100\mu L})}{V_{100\mu L}}\right)_2^2 + \left(\frac{u(V_{500\mu L})}{V_{500\mu L}}\right)_2^2}$$

(Eq.A.11)

For the preparation of OCP standards, 1000 µg/mL mixture solution was used. Successive dilutions were performed to obtain 100 µg/mL (dilution 1), 1 µg/mL (dilution 2) and calibration (dilution 3) standard solutions, using the 100 and 500 µL injectors. Therefore, the combined uncertainty for the OCP standards will be,

$$\frac{u(stds)}{C_{stds}} = \sqrt{\left(\frac{u(P)}{P}\right)^2 + \left(\frac{u(V_{100\mu L})}{V_{100\mu L}}\right)_1^2 + \left(\frac{u(V_{500\mu L})}{V_{500\mu L}}\right)_1^2 + \left(\frac{u(V_{100\mu L})}{V_{100\mu L}}\right)_2^2 + \left(\frac{u(V_{500\mu L})}{V_{500\mu L}}\right)_2^2 + \left(\frac{u(V_{100\mu L})}{V_{100\mu L}}\right)_3^2 + \left(\frac{u(V_{500\mu L})}{V_{500\mu L}}\right)_3^2}$$

(Eq. A.12)

a) Mass, u(m):

The weighing procedure is a weight by difference of the tare and gross weight. Each of them is subject to run to run variability and the uncertainty of the calibration of the balance. The calibration itself has two uncertainty sources, the sensitivity and the linearity. As the weighing is done on the same scale over a small range of weight, the sensitivity contribution is neglected. The balance linearity contribution has to be counted twice, one for tare and one for gross weight as each one is an independent observation and the linearity effects are not correlated (EURACHEM/CITAC Guide, 2000).

$$u(m) = \sqrt{2u^2(bal - cal) + u^2(bal - rep)} \qquad \text{(Eq.A.13)}$$

where

u(bal-cal): uncertainty component for balance calibration

u(bal-rep): uncertainty component for repeatability of weight measurements

The manufacturer certificate quotes 0.2 mg for the linearity and 0.1 mg for the repeatability. Assuming rectangular distribution for these contributors and using Equation A.13,

$$u(m) = \sqrt{2 \times \left(\frac{0.2}{\sqrt{3}}\right)^2 + \left(\frac{0.1}{\sqrt{3}}\right)^2} = 0.173 \text{ mg}$$

b) Uncertainty for Volumetric Flasks, $u(V_{100mL})$:

The volume of the solution contained in the volumetric flask is subject to three major sources of uncertainty:

- The uncertainty in the certified internal volume of the flask; $u(V_{100mL}\text{-}cal)$
- Variation in filling the flask; repeatability; $u(V_{100mL}\text{-}rep)$
- The flask and solution temperatures differing from the temperature at which the volume of the flask was calibrated; $u(V_{100mL}\text{-}temp)$

$$u(V_{100mL}) = \sqrt{u^2(V_{100mL} - cal) + u^2(V_{100mL} - rep) + u^2(V_{100mL} - temp)} \quad \text{(Eq.A.14)}$$

- Calibration:

The manufacturer quotes a volume of the flask of 100 mL±0.1 mL measured at a temperature of 20°C. The value given without distribution information, so an assumption is necessary. Here, the standard uncertainty is calculated assuming a triangular distribution (EURACHEM/CITAC Guide, 2000);

$$u(V_{100mL}\text{-}cal) = \frac{0.10mL}{\sqrt{6}} = 0.041 \text{ mL}$$

- Repeatibility:

The uncertainty due to variations in filling is estimated by filling and weighting the 100 mL flask for 10 times. This experiment has yielded a standard deviation (s) of 0.44 mL. This value is directly used as standard uncertainty.

$u(V_{100mL}\text{-}rep)=s=$ 0.440 mL

- Temperature:

The manufacturer cites that the flask has been calibrated at a temperature of 20°C. The uncertainty from the temperature effect can be calculated from the estimate of the temperature range and coefficient of the volume expansion. A variation of ±4°C from calibration temperature is assumed in this work. The volume expansion of the solvent (acetone) used for the dilutions is considerably larger than that of the flask, therefore only the former needs to be considered. The coefficient of volume expansion for organic solvents are almost 10^{-3}°C^{-1} around 20°C (Meyer, 2007).

The volume variation due to temperature becomes,

Volume Variation (T) = $\pm (100\times 4.00 \times 10^{-3})= \pm$ 0.400 mL (Eq.A.15)

The standard uncertainty is calculated using the assumption of rectangular distribution for the temperature variation (EURACHEM/CITAC Guide, 2000),

$$u(V_{100mL}\text{-}temp)= \frac{0.400mL}{\sqrt{3}}=0.231 \text{ mL}$$

The three contributions are combined to give the standard uncertainty of the 100 mL volumetric flask with the Equation A.17;

$$u(V_{100mL}) = \sqrt{0.041^2 + 0.440^2 + 0.231^2} = 0.499 \text{ mL}$$

c) Purity, u(P):

The purities of OPPs are quoted in the supplier's certificate as % values together with their tolerance values. These values are included in the uncertainty calculations with an assumption of rectangular distribution. To obtain the standard uncertainty *u(P)*, the purity values has to be divided by √3.

d) Uncertainty for Volume Taken with 100 µL injector ; u($V_{100\mu L}$):

The single standards were combined to give a final standard solution of mixture of all OPPs. To prepare this solution, 20 µL of the single standards were taken with 100 µL injector and diluted to 2 mL with acetone using 500 µL injector. Further, this mixture standard was used to prepare calibration standards by using these injectors.

The uncertainty of the volume taken is arising from the uncertainty of 100 µL injector used. Similar to the volumetric flasks, the uncertainty of this operation depends on the factors of calibration, repeatability and temperature variation and calculated as in Equation A.17.

- Calibration:

The manufacturer quotes an accuracy of ±1% of volume for the injectors. Assuming a triangular distribution,

$$u(V_{100\mu L}\text{-}cal) = \frac{0.01\mu L}{\sqrt{6}} = 0.004\ \mu L$$

- Repeatability:

The uncertainty due to variations in use of this injector is estimated by weighting the dispensed water for 10 times. This experiment has yielded a standard deviation of 0.083 µL. This value is directly used as standard uncertainty.

$u(V_{100\mu L}\text{-}rep)=s=0.083$ µL

- Temperature:

The volume variation due to temperature is calculated similar to flask calculations, and the standard uncertainty is calculated using the assumption of rectangular distribution for the temperature variation; giving $u(V_{100\mu L}\text{-}temp)$ is 0.231 µL

The three contributions are combined to give the standard uncertainty of the 100 µL injector similar to Eq.A.14;

$$u(V_{100\mu L}) = \sqrt{0.004^2 + 0.083^2 + 0.231^2} = 0.245 \text{ µL}$$

e) Uncertainty for Final Dilution with 500 µL injector; $u(V_{500\mu L})$:

After combining the single standards for OPPs, 500 µL injector was used to dilute the constituents to the final volume. The uncertainty for 500 µL injector is calculated similar to 100 µL injector, giving $u(V_{500\mu L})=1.162$ µL.

A.2.2.2. Uncertainty Arising From Linear Calibration Curve; *u(cal)*

The concentrations of pesticides were calculated using six-point internal standard calibration curves obtained for GC-ECD and GC-NPD systems. Each calibration standard was injected three times and the linear least squares fitting procedure was performed. The calibration curves have the general formula:

$$A=B_1 \times C + B_0 \qquad \text{(Eq.A.16)}$$

Where,

A: Peak area, C: Concentration, B_1: Slope, B_0: Intercept

According to EURACHEM, the uncertainty sources arising from the estimation of concentration of analytes are;

- Random variations in measurement of A, affecting both calibration standards and analyte concentrations.
- Random effects resulting in errors in the assigned reference values of calibration standard concentrations.
- Constant unknown offsets for the values of standard concentrations and corresponding peak areas, such as serial dilution of calibration standards.
- Deviation from linearity.

EURACHEM suggests calculation of uncertainty associated with linear square fitting procedure (*u(cal)*) to estimate the analyte concentration (c_0) as follows;

$$u(cal) = \frac{S}{B_1}\sqrt{\frac{1}{p}+\frac{1}{n}+\frac{(c_0 - c_{av})^2}{S_{xx}}} \qquad \text{(Eq.A.17)}$$

where, S: Residual Standard Deviation $S = \sqrt{\frac{\sum_{j=1}^{n}\left[A_j - \left(B_0 + B_1 \times c_j\right)\right]^2}{n-2}}$

p: Number of measurements to determine c_0

n: Number of measurements for the calibration

S_{xx}: $S_{xx} = \sum_{j=1}^{n}\left(c_j - c_{av}\right)^2$

j: index for the number of measurements to obtain calibration curve

A.2.3. Estimation of Uncertainty for Repeatability of the Measurements; *u(rep):*

The % RSD (or CV, coefficient of variation) values for SRM readings are used as these are the samples analyzed for the whole analysis period (N=30 for OCPs, N=18 for OPP mix-std 167, N=13 for OPP mix-std 64, N=14 for OPP mix-std 154) to monitor the stability of analysis systems. The uncertainty arising from this component is calculated as follows,

$u(rep) = CV / \sqrt{N}$, where N is the number of analysis replicates of SRMs. CV is used instead of standard deviations to normalize the deviation of different concentrations of SRMs and the samples (EURACHEM/CITAC Guide, 2000). For the pesticides, methoxychlor and fenamiphos, the SRM results were not available, therefore the average of CV values of the results were used for the calculations.

A.2.4. Estimation of Uncertainty for Recovery, $u(R_{av})$

The recoveries of the analytes were calculated using the formula given in Section 3.1.2.3:

$$Recovery = (C_s - C_u) / C_{Certified} \quad \text{(Eq.A.18)}$$

Where, $(C_s - C_u)$ can be defined as observed concentration; $C_{observed}$.

The uncertainties of the average recoveries are calculated by using the law of propagation as follows;

$$\frac{u(R_{av})}{R_{av}} = \sqrt{\left(\frac{u(C_{Obs})}{C_{Obs}}\right)^2 + \left(\frac{u(C_{Cert})}{C_{Cert}}\right)^2} \quad \text{(Eq.A.19)}$$

where R_{av} is the average recovery and $u(R_{av})$ is the uncertainty of this parameter. As the sample matrix affects the recovery of extractions, the average recoveries (R_{av}) from ground water (for which N=31) and surface water (N=17) are used.

The $u(C_{Obs})$ can simply be calculated using the formula,

$$u(C_{Obs}) = \sqrt{u^2(Cs) + u^2(Cu)} \quad \text{(Eq.A.20)}$$

where $u(Cs)$ and $u(Cu)$ are the uncertainties for the spiked and unspiked sample concentrations, respectively. They are calculated as;

$$u(Cs \text{ or } Cu) = s / \sqrt{N}\ , \quad \text{(Eq.A.21)}$$

where *s* is the standard deviation of the concentrations and *N* is the number of samples used for the calculation.

As the same standard solution was used to spike the samples and to prepare calibration standards, as explained in A.2.2.1, the $u(C_{Cert})$ for OPPs is calculated using Equation A.11 and for OCPs using Equation A.12.

The t-test is applied for the recoveries to see the deviation from unity. When $t_{exp} > t_{crit}$, the recovery correction is included in the calculation of combined uncertainty (EURACHEM/CITAC Guide, 2000).

$$t_{\exp} = \frac{|1 - R_{av}|}{u(R_{av})} \qquad \text{(Eq.A.22)}$$

The t_{crit} value for degrees of freedom of 47 at 95% confidence is 2.01. In case of the detection of significance, the concentration of the pesticide obtained should be corrected with recovery. This brings another operation for uncertainty calculations:

$$C_{corr} = C_{av} / R_{av} \qquad \text{(Eq.A.23)}$$

where C_{av} is the average concentration obtained for all data set.

The contribution of this operation to combined uncertainty is;

$$u_{rel}(COM)_{Corr} = \sqrt{u_{rel}^2(COM) + u_{rel}^2(R_{av})} \qquad \text{(Eq.A.24)}$$

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PUBLICATIONS

1. Güllü, H. G., Ölmez, İ., Öztaş, N.B., Tuncel, G., 2003. Doğu Akdeniz'deki Atmosferik Eser Element Konsantrasyonları: Zamana Bağlı Değişimleri Etkileyen Faktörler, Çevre Bilim ve Teknoloji, 1 (3), 41-49.

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ATTENDED MEETINGS

1. Öztaş N.B., Tuncel S.G., "Bebek Mamasında Arsenik ve Selenyumun Hidrür Jeneratörlü ICP-AES ile Tayini", XII. Ulusal Kimya Kongresi, Edirne, Türkiye, 7-11 Eylül, 1998.

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3. Öztaş N.B., Tuncel S.G., "Atmosferik Partiküllerdeki Eser Elementlerin Sudaki Çözünürlüğü", XIV. Ulusal Kimya Kongresi, Diyarbakır, Türkiye, 10-15 Eylül 2000.

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10. Öztaş-Emek, N.B., Tuncel, S.G., "Sampling and Analysis Methodologies for Pesticides in Groundwater Samples", 1st International Conference on Air Pollution and Combustion, Ankara, Turkey, June 22-25, 2005.

11. Öztaş-Emek, N.B., Tuncel, S.G., "Pesticide Pollution in surface and Ground Water of an Agricultural Area, Kumluca, Turkey", 16th Regional Conference on Clean Air and Environment in Asian pacific Area, Tokyo, Japan, August 2-4, 2005.

12. Tuncel, S.G., Öztaş-Emek, N.B., Erduran, M.S., "Air and Groundwater Pollution in Turkish Mediterranean Coast", Workshop on Agricultural Air Quality: State of the Science, Washington DC, USA, June 5-8, 2006.

13. Öztaş, N.B., Tuncel, S.G., "Application of Quality Assurance and Quality Control Practices for the Analysis of Pesticides in Environmental Water Samples", Colloquium Spectroscopicum Internationale XXXV, Xiamen, China, September 23-27, 2007.

CONFERENCE ORGANIZATION

Member of Organizing Committee, 1st International Conference on Air Pollution and Combustion, Ankara, Turkey, June 22-25, 2005.

FIELD OF INTEREST

Analytical Chemistry, Environmental Chemistry, Atmospheric Pollution, Water Pollution, Archaeometry.