EVALUATION OF PERSISTENT ORGANIC POLLUTANTS (POPs) IN BALIKESIR DAM LAKE SEDIMENTS

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

BY

PINAR GÖKMEN

IN PARTIAL FULFILLMENT OF THE REQUIREMENTS
FOR
THE DEGREE OF MASTER OF SCIENCE
IN
CHEMISTRY

JANUARY 2011

Approval of the thesis:

EVALUATION OF PERSISTENT ORGANIC POLLUTANTS POPS IN BALIKESİR DAM LAKE SEDIMENTS

submitted by **PINAR GÖKMEN** in partial fulfillment of the requirements for the degree of **Master of Science in Chemistry Department. Middle East Technical University** by.

Prof. Dr. Canan ÖZGEN
Dean. Graduate School of **Natural and Applied Sciences**
Prof. Dr. İlker ÖZKAN
Head of Department, **Chemistry**
Prof. Dr. Semra TUNCEL
Supervisor **Chemistry Dept. METU**

Examining Comitee Members:
Prof. Dr. Semra TUNCEL
Chemistry Dept., METU
Prof. Dr. Osman Yavuz ATAMAN
Chemistry Dept., METU
Prof. Dr. Ali GÖKMEN
Chemistry Dept., METU
Prof. Dr. Gülen GÜLLÜ
Environmental Engineering Dept. Hacettepe University
Assist. Prof. Dr. Eftade E. GAGA
Environmental Engineering Dept. Anadolu University

Date: 07.01.2011

.

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

Name, Lastname : Pınar Gökmen

Signature :

ABSTRACT

EVALUATION OF PERSISTENT ORGANIC POLLUTANTS (POPs) IN BALIKESİR DAM LAKE SEDIMENTS

Gökmen, Pınar
M. Sc., Department of Chemistry
Supervisor: Prof. Dr. Semra Tuncel
January 2011, 98 pages

In this study, the Persistent Organic Pollutants (POPs) specifically; 17 Organochlorine Pesticides (OCPs) and 19 Polycyclic Aromatic Hydrocarbons (PAHs) were evaluated in the sediment samples of Balıkesir (İkizcetepeler) Dam Lake. Sixteen sampling points were chosen for determination of concentrations of OCPs and PAHs After ultrasonic bath extraction of the sediment samples GC-MS was used as analytical tool. Extraction efficiencies changes from 63.8 to 87.4% depending on the type of the POPs. Average OCP concentration was found in the range of 3.33-379 µg/kg a, average PAH concentration was found in the range of 3.28-32.9 µg/kg. Pollution maps regarding OCP and PAH distributions were drawn and the correlation between these two pollutant types was investigated. The quality control (QC) and quality assurance tests were applied by the analysis of standard reference materials (SRMs), surrogate standards and analysis replicates.

Keywords: Persistent Organic Pollutants(POPs), Organochlorine Pesticides (OCPs), Polycylic Aromatic Hydrocarbons (PAHs), Gas Chromatography-Mass Spectrometry (GC-MS)

ÖZ

BALIKESİR İKİZCETEPELER BARAJ GÖLÜ SEDİMANLARINDA KALICI ORGANİK KİRLETİCİLERİN DEĞERLENDİRİLMESİ

Gökmen, Pınar
Yüksek Lisans, Kimya Bölümü
Tez Yöneticisi: Prof. Dr. Semra Tuncel
Ocak 2011, 98 sayfa

Bu çalışmada Balıkesir İkizcetepeler Baraj Gölü sediman örneklerinde kalıcı organik kirleticilerin özellikle 17 organoklorlu pestisitin ve 19 çok halkalı aromatik hidrokarbonun değerlendirilmesi yapılmıştır. Organoklorlu pestisitlerin ve çok halkalı aromatik hidrokarbonların konsantrasonlarının tayini için 16 örnekleme noktası seçilmiştir. Sediman örneklerinin ultrasonik banyo özütlemesinden sonra, Gaz Kromatografisi-Kütle Spektrometresi analitik cihaz olarak kullanılmıştır. Ekstraksiyon verimleri kalıcı organik kirleticinin türüne göre %63.8 ile %87.4 arasında değişmektedir. Ortalama organoklorlu pestisit konsantrasyonu 3.33-379 µg/kg aralığında, ortalama çok halkalı aromatik hidrokarbon konsantrasyonu da 3.28-32.9 µg/kg aralığında bulunmuştur. Organoklorlu pestisit ve çok halkalı aromatik bileşiklere ait kirlilik haritaları çizilmiş ve bu iki kirletici çeşidi arasındaki korelasyon araştırılmıştır. Kalite kontrol ve kalite güvence testleri standart referans maddeleri, vekil standartları ve analiz tekrarlarıyla uygulanmıştır.

Anahtar Kelimeler: Kalıcı organik kirleticiler, Organoklorlu pestisitler, Çok halkalı aromatik hidrokarbonlar, Gaz kromatografisi- Kütle spektrometresi

To my family

ACKNOWLEDGEMENTS

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

I would like to thank to Tansel TOPAL and Nur Banu ÖZTAŞ for their endless support and help during this study.

I would like to thank to my dear lab mate Hazal AYDİŞ for her endless support and other labmates Yunus ERDAL, Emin TAYLAN for their help during this challenging period.

I would like to thank to TÜBİTAK for funding this study.

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

TABLE OF CONTENTS

ABSTRACT .... **iv**

ÖZ .... **v**

ACKNOWLEDGEMENTS .... **vii**

LIST OF TABLES .... **xiii**

LIST OF ABBREVIATIONS .... **xv**

CHAPTERS **1**

1.INTRODUCTION **1**

1.1 Purpose of the study 1

1.2 Importance of Sediments as Environmental Matrices and Persistent Organic Pollutants as Contaminants 2

1.3 Pesticides 3

1.3.1 Organochlorine Pesticides 3

1.3.1.1 Properties of Organochlorine Pesticides, Their Transport and Interaction with Environment 4

1.3.2 Pesticide Use in Turkey 7

1.3.3 Effect of Pesticides 9

1.4 Polycyclic Aromatic Hydrocarbons as Pollutants 10

1.4.1 Chemical and Physical Properties of Polycyclic Aromatic Hydrocarbons 11

1.4.2 The Effects of Polycyclic Aromatic Hydrocarbons on Health 13

1.4.3 Sources of Polycyclic Aromatic Hydrocarbons 14

1.4.4 Polycyclic Aromatic Hydrocarbons in Sediment 15

1.5 Analytical Methodologies for the Determination of OCPs and PAHs in Sediment 16

1.5.1 Extraction Methodologies 16

1.5.1.1 Ultrasonic Bath Extraction 16
1.5.2 Analyses Techniques 17
1.5.2.1 Gas Chromatography 17
1.5.2.2 Mass Spectrometry 17
1.6 Literature Search 19
2.EXPERIMENTAL 24
2.1 Study Area 24
2.2 Sampling Strategy 25
2.3 Sample Collection 26
2.4 Reagents and Materials 28
2.5 Preparation of Na_2SO_4, Florisil and Glass Wool 29
2.6 Cleaning of Glassware 30
2.7 Instrument and Apparatus 30
2.8 Optimization of GC-MS system 30
2.9 Calibration of the Analysis Systems 35
2.10 Application of Ultrasonic Bath Extraction 39
2.10.1 Ultrasonic Bath Extraction for Organochlorine Pesticides 39
2.10.2 Ultrasonic Bath Extraction for Polycyclic Aromatic Hydrocarbons 41
2.11 Analysis of the Samples 41
3.RESULTS AND DISCUSSION 45
3.1 Method Validation 45
3.1.1 Quality Assurance (QA) and Quality Control (QC) Tests During Extraction of the Samples 45
3.1.2 Quality Assurance (QA) and Quality Control (QC) Tests During The Analyses 47

3.2 Comparison of Performance Characteristics of the Method with the Literature for Organochlorine Pesticides 58
3.3 Evaluation of the Data Set 63
3.3.1 Concentrations of Organochlorine Pesticides and Polycyclic Aromatic Hydrocarbons in İkizcetepeler Dam Lake sediment samples 63
3.3.2 Pollution Distributions 72
4.CONCLUSION 82
REFERENCES 84
APPENDIX A 91

LIST OF FIGURES

FIGURES

Figure 1.1 Chemical structures of some OCPs 5

Figure 1.2 Chemical structures of 19 PAH compounds... 12

Figure 1.3 Scheme of a gas chromatograph 17

Figure 1.4 Scheme of a mass spectrometer 18

Figure 2.1 Sampling points of İkizcetepeler Dam Lake...26

Figure 2.2 The schematic representation of a van Veen grab sampler taking a sample 27

Figure 2.3 GC-MS Chromatogram (SIM Mode) of Standard Containing 1 μg/L OCPs and internal standard 33

Figure 2.4 GC-MS Chromatogram (SIM Mode) of Standard Containing 1 mg/L PAH and Surrogates 34

Figure 2.5 Calibration curves of pesticide ions... 37

Figure 2.6 Calibration curves for PAHs.. 38

Figure 2.7 Calibration curves for PAHs (continued) 39

Figure 2.8 The sample chromatogram showing OCPs obtained from GC-MS system for the sediment from sampling point 4 43

Figure 2.9 The GC-MS chromatogram (SIM Mode) of Ultrasonic Bath Extracted İkizcetepeler Dam Lake Sediment 44

Figure 3.1 Demonstration of the accuracies for OCPs in SRM 2261, SRM 2273, SRM 2275...50

Figure 3.2 The ongoing accuracy of SRM 2261, SRM 2273 and SRM 2275 during the sample injection periods for organochlorine pesticides 51

Figure 3.3 Demonstration of Repeatability of the Measurements with the Analysis of EPA Method 508-Chlorinated Pesticide Mix 1- Std. Solution, 1.00 mg/L (N=3)..... 55

Figure 3.4 Average Concentrations of Organochlorine Pesticides in İkizcetepeler Dam Lake Sediments 65

Figure 3.5 Average Concentrations of Polycyclic Aromatic Hydrocarbons in İkizcetepeler Dam Lake Sediments 71
Figure 3.6 Distribution of total OCPs in the sampling region 73
Figure 3.7 Distribution of selected OCPs in the sampling region 75
Figure 3.8 Distribution of total PAHs in the sampling region 78
Figure 3.9 Distribution of selected PAHs in the sampling region 80
Figure A.1 The distribution of Hexachlorocyclohexane (HCH) isomers in İkizcetepeler dam lake sediment samples 91
Figure A.2 The distribution of Hexachlorocyclohexane (HCH) isomers in İkizcetepeler dam lake sediment samples (continued) 92
Figure A.3 The distribution of cyclodienes in İkizcetepeler dam lake sediment samples 93
Figure A.4 The distribution of cyclodienes in İkizcetepeler dam lake sediment samples (continued) 94
Figure A.5 The distribution of cyclodienes in İkizcetepeler dam lake sediment samples (continued) 95
Figure A.6 The distribution of Endosulfan and its derivatives in İkizcetepeler dam lake sediment samples 95
Figure A.7 The distribution of Endosulfan and its derivatives in İkizcetepeler dam lake sediment samples (continued) 96
Figure A.8 The distribution of Dichloro Diphenyl Trichloroethane (DDT) and Its Derivatives (DDD, DDE and Methoxychlor) in İkizcetepeler dam lake sediment samples 97
Figure A.9 The distribution of Dichloro Diphenyl Trichloroethane (DDT) and Its Derivatives (DDD, DDE and Methoxychlor) in İkizcetepeler dam lake sediment samples (continued) 98

LIST OF TABLES

TABLES

Table 1.1 Persistence of Various Chlorohydrocarbon Pesticides 6

Table 1.2 Pesticide consumption in Turkey between 1979 and 2002 (in tons) 7

Table 1.3 European countries' pesticide consumption according to the averages per hectare between the years 1993-1995 8

Table1.4 Toxicity of Various Chlorohydrocarbon Pesticides................................... 10

Table 2.1 The coordinates of the sampling points..27

Table 2.2 Operating parameters of GC-MS system for OCP determination 31

Table 2.3 Operating Parameters of GC-MS system for PAH determination............. 31

Table 2.4 Adjustment of SIM parameters for OCP determination 32

Table 2.5 Adjustment of SIM parameters for PAH determination 32

Table 2.6 Calibration parameters for the determination of OCPs and PAHs 36

Table 2.7 Retention times and ions of the analytes.. 42

Table 3.1 Surrogate recoveries of the standards used for ultrasonic bath extractions47

Table 3.2 Measurements with the analysis of NIST SRMs 2261, 2273, 2275 (N=3) 49

Table 3.3 Method performance results for Chlorinated Pesticide Mix 1 53

Table 3.6 Limit of Detection (LOD) and Limit of Quantification (LOQ) values of OCPs for GC-MS, Values in (µg/kg)... 57

Table 3.7 Limit of Detection (LOD) and Limit of Quantification (LOQ) values of PAHs for GC-MS, Values in (µg/kg) 57

Table 3.8 Some information about the methods used for comparison....................... 59

Table 3.9 Comparison of percent recovery values of this study with five different studies.. 60

Table 3.10 Comparison of limit of detection (LOD) values of this study with four different studies 61

Table 3.11 Comparison of percent relative standard deviation values of this study with three different studies 62

Table 3.12 Summary Statistics for Organocholorine Pesticides 64

Table 3.13 Distribution of the organochlorine pesticide pollution of the sampling points according to the subgroups 66
Table 3.14 The information about the studies about organochlorine pesticides which are performed in Turkey 67
Table 3.15 The comparison of concentration levels of organochlorine pesticides found in the studies performed in Turkey 68
Table 3.16 Concentrations of PAHs in sediment samples of six sampling points..... 70

LIST OF ABBREVIATIONS

ACE: Accelerated Solvent Extraction
ANOVA: Analysis of Variance
BLOQ: Below Limit of Quantification
DDT: Dichlorodiphenyltrichloroethane
DDE: Dichlorodiphenylethane
DDD: Dichlorodiphenyldichloroethane
ECD: Electron Capture Detector
GC: Gas Chromatography
GPS: Global Positioning System
HCH: Hexachlorocyclohexane
HSSPME: Head Space Solid Phase Micro Extraction
K_{ow}: Octanol-water Coefficient
LD_{50}: Lethal Dose 50%
LC_{50}: Inhalation Toxicity
LOD: Limit of Detection
LOQ: Limit of Quantification
MS: Mass Spectrometry
NIST: National Institute of Standards and Technology
OCPs: Organochlorine Pesticides
PAHs: Polycylic Aromatic Hydrocarbons
PCBs: Polychlorinated Biphenyls
POPs: Persistent Organic Polutants
QA: Quality Assurance
QC: Quality Control
SIM: Selected Ion Monitoring
S/N: Signal to Noise Ratio
SRM: Standard Reference Material
TOC: Total Organic Carbon

UBE: Ultrasonic Bath Extraction

USEPA: United States Environmental Protection Agency

WHO: World Health Organization

% RSD: Percent Relative Standard Deviation

CHAPTER 1

INTRODUCTION

1.1 Purpose of the study

Our group has an extensive experience in POPs analyses, extraction, and their evaluation as pollutants and their sources. Obviously, method (extraction and analyses) performance varies depending on the matrices even if everything else is the same.

In above studies different extraction methodologies are applied to aerosol, water and sediment samples. Nevertheless, in none of them pesticides were determined in sediment matrix. Therefore, above studies were used as guidance in this study for the following purposes:

- Application of ultrasonic bath extraction method for the determination of OCPs and PAHs in the sediment samples of İkizcetepeler Dam Lake
- Analysis of the sediment samples in terms of OCP and PAH concentrations using GC-MS system
- Validation of used extraction and analyses methods
- Investigation of the extent of pollution due to OCPs and PAHs by drawing pollution maps
- Investigating the analogy between the distribution of these two persistent organic pollutants and their chemical structure and possible sources

1.2 Importance of Sediments as Environmental Matrices and Persistent Organic Pollutants as Contaminants

Sediment is moved from land into the lakes, rivers or streams by natural events and it is an organic solid matter. Surface sediments are nutrition source for biological life, a transporting agent for pollutants and main source for settling organic and inorganic matter. The composition of the sediments serves as an important criterion for the identification of long-term water quality. The pollution of sediments has natural and anthropogenic components. The anthropogenically introduced components by far exceed the natural component in heavily polluted sediments and constitute a hazard to the marine ecosystem because of their bioavailability and this damage is cumulative. When plant and fish deaths occur, the water way cannot break down wastes and materials that are washed into it in natural ways. These materials begin to accumulate and form another sink of pollutants, chemicals such as some pesticides, phosphorus, ammonium, metals, polycyclic aromatic hydrocarbons are transported with sediment in an adsorbed state, and chemical sediment forms as minerals settle from water that contain lots of dissolved particles (Algan A.O. 1999).

The chemicals are resistant to photochemical, biological and chemical degradation for long periods in the environment are called persistent organic pollutants (POPs) (Vagi, et al. 2007).

Persistent organic pollutants include organochlorine pesticides (OCPs), polycyclic aromatic hydrocarbons (PAHs) and polychlorinated biphenyls (PCBs). These chemicals are originated from anthropogenic processes and various routes lead these compounds to be introduced into the environment. Despite the fact that the registered usage of POPs has been withdrawn for many years by numerous countries, they persist at considerable levels in different environmental compartment worldwide. Persistent organic pollutants are considered hazardous to the environment, due to their toxic, mutagenic, and carcinogenic characteristics. Since POPs are adsorbed on the surface of the particles related to the organic content of the solid phase matrix and can be accumulated to the sediments, the concentration of POPs in aquatic environments, regarding the contaminant loading, are considered to be informative on the anthropogenic (of human origin) impact on the environment (Doong R. 2008).

1.3 Pesticides

Agrochemicals designed to eliminate the attack of various pests on agricultural crops are called as pesticides. A pest is usually defined as any living organism interfering with the human activity in a negative way. Specifically, the growth of agricultural crops is inhibited by the major pests, which are insects, fungi, and weeds.

Dichlorodiphenyltrichloroethane (DDT), a pesticide, is fatal for insects and this property was first discovered by Muller, and this discovery is considered as the beginning of the modern era of synthetic pesticides. Although the thousands of molecules with insecticidal activity have been synthesized after this discovery, a few of them have found commercial success (Kaushik P. 2007). Pesticides are grouped in two classes as organochlorine pesticides and organophophorus pesticides. Because of their persistence in the environment, their usage was limited by laws in developed countries. However, developing countries still use pesticides, especially organochlorine pesticides since they are cheap and easy to synthesize.

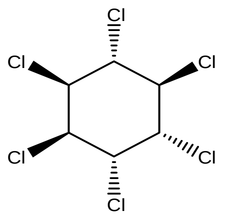
1.3.1 Organochlorine Pesticides

This cluster of pesticides is referred as the chlorinated hydrocarbons, the chlorohydrocarbon or the organochlorines. Dichlorodiphenyltrichloroethane (DDT) is the most known member of the chlorinated pesticides. Its history and use have had an important effect on the use of pesticides. Its use slowly expanded and reached to various parts of agriculture (Connell 2005).

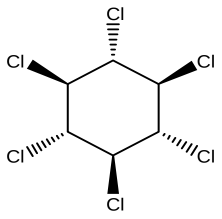
Due to their lipophilic nature, hydrophobicity, and low chemical and biological degradation rates, organochlorine pesticides (OCPs) have led to their accumulation in the biological tissues and subsequent magnification of concentrations in the organisms due to the progression up the food chain (Hernandez R.A. 2004).

1.3.1.1 Properties of Organochlorine Pesticides, Their Transport and Interaction with Environment

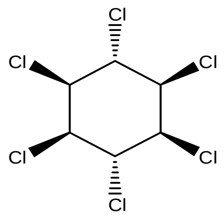
The bond types present in the organochlorine pesticides is limited. These are C---C (aromatic), C=C, C-H, and C-Cl, with lesser numbers of C-C. The symmetrical bonds, C-C (aromatic), C-C, and C=C, have dipole moments close to zero. C-H and C-Cl have dipole moments 0.4 and 0.5 Debyes respectively, though these are relatively low. This indicates low polarity of the compounds in this group due to their inclination to have low dipole moments. These properties cause the compounds in this group be fat soluble or lipophilic and therefore have a low solubility in water. The lipophilicity of these compounds is shown by the octanol-water coefficient (K_{ow}). This value lies between 470 and 2.300.000 (Connell 2005). The chemical structures of some OCPs are shown in Figure 1.1.



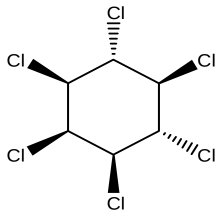
Alpha-HCH



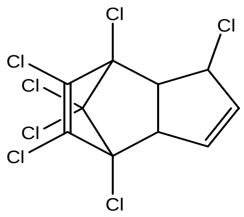
Beta-HCH



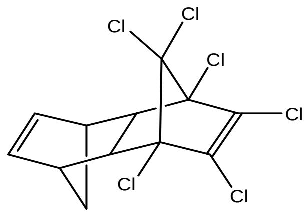
Gamma-HCH



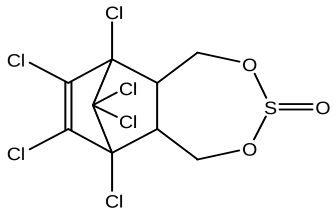
Delta-HCH



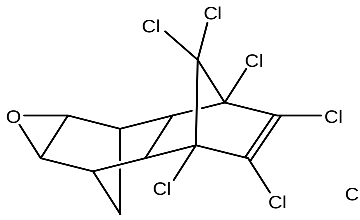
Heptachlor



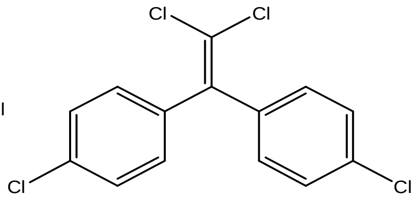
Aldrin



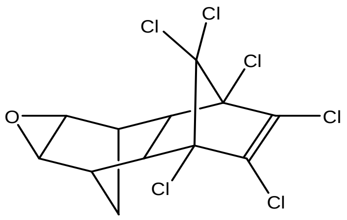
Endosulfan



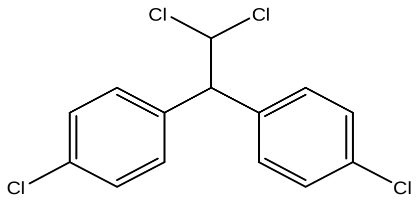
Dieldrin



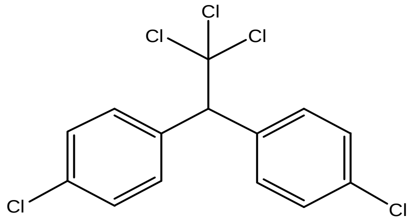
p,p'-DDE



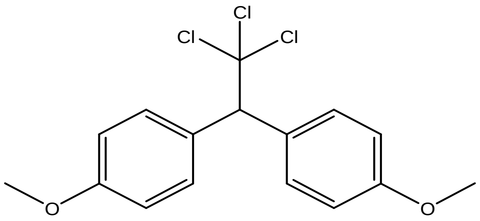
Endrin



p,p'-DDD



p,p'-DDT



Methoxychlor

Figure 1.1 Chemical structures of some OCPs

The chlorohydrocarbon pesticides are relatively resistant to the attacks of abiotic or biotic agents in the environment because of the limited range of bond types present. Therefore, environmental degradation proceeds at a relatively slow rate. Most compounds in this group persist for long periods in soil. The half-lives approach to many years as illustrated in Table 1.1.

Table 1.1 Persistence of Various Chlorohydrocarbon Pesticides

Compound	Half-life Range (soil, years)
p,p'-DDT	2.0-15.6
Dieldrin	0.5-3.0
Lindane	0.04-0.7
Aldrin	0.06-1.6

(Connell 2005)

As seen in the Table 1.1, the half-lives can range from 0.06 to 15.6 years owing to the various conditions in soil such as soil moisture and temperature (Connell 2005).

Pesticides, a group of persistent organic pollutants (POPs), are observed in air, water, sediments, and biota in many areas throughout the world in trace concentrations. The capacity for long-range movement across continents and oceans is the particular feature of POPs that is of interest. Such that, the pesticides bioaccumulate to enormous concentrations, by evaporation into the atmosphere and dissolution in ocean water, and reveal the adverse effects to the environment previously noted. The POPs must be persistent since the transport process takes a reasonable period to realize. They must be capable of being vaporized especially at a slow rate and must have a Henry's law constant between 10^{-5} and 10^{-2} atoms m^3 mol^{-1}, with a vapor pressure greater than 1000 Pa. Furthermore, they must be bioaccumulative with log K_{ow} values between 2 and 5.5 (Connell 2005).

1.3.2 Pesticide Use in Turkey

Pesticide production and use have increased in the last 40 years throughout the world, the global pesticide market was valued as 30 billion $ in 1996. In the last ten years, the rate of pesticide sales has slowed down in highly developed and industrialized countries. however, it still grows very quickly in developing countries which are becoming more dependent on pesticide**.** Since Turkey has the largest agricultural land among the European countries, it is considered as an agricultural country (Yazgan M. 2003).

The pesticide consumption in Turkey has increased by 45.29% in 2002 with respect to 1979. Despite this increase, the average consumption value is low relative to the developed countries. The consumption in the western and southern coastal regions of Turkey is higher than the average amount, since these are the sites that intensive agriculture is applied (Delen Nafiz 2005).

The number of registered pesticides used is 2000, and the number of active ingredients available is around 300, according to the officially stated data by Ministry of Agriculture and Rural Affairs on pesticides by year 2000 (Yazgan M. 2003). The Table 1.2 shows the pesticide consumption between 1979 and 2002 in Turkey.

Table 1.2 Pesticide consumption in Turkey between 1979 and 2002 (in tons)

Pesticide	**1979**	**1987**	**1994**	**1996**	**2002**
Insecticides	2287.658	3303.446	2064.991	3027.380	2250.898
Acaricides	203.107	240.360	192.279	223.857	296.809
Fumigants	315.665	322.227	530.738	1076.661	1559.489
Fungicides	1537.315	2611.960	2201.406	2951.191	1964.292
Herbicides	2451.977	3495.044	3902.588	3643.971	3697.397
Total	8395.848	12112.267	10871.792	13797.488	12198.917

(Yazgan M. 2003)

As seen from the Table 1.2, as well as some fluctuations in 22 years time there is an increase in the total consumption of pesticides in Turkey. Despite the stable consumption rate of pesticides in recent years, it had a high growth rate, 3.4% in 1983-1993 and 18 % in 1993-1995 annually. The consumption of pesticides decrease from 12146 tons to 11516 tons in a period between 1983-1995. In other words, the consumption of pesticides in Turkey decreased by 5 % in the 12 years period. If we make a comparison between Turkey's and European countries' pesticide consumption, European countries' pesticide consumption according to the averages per hectare between the years 1993-1995 is given in Table 1.3.

Table 1.3 European countries' pesticide consumption according to the averages per hectare between the years 1993-1995

Country	**Pesticide Consumption(kg/ha)**
Germany	2.6
Austria	4.0
Belgium	1.2
Denmark	1.7
Finland	1.2
France	5.6
Holland	13.8
England	6.4
Ireland	8.0
Spain	2.3
Sweden	4.4
Italy	9.3
Portugal	6.0
Greece	13.5

(Yazgan M. 2003)

As can be seen from this table, the consumption is the least in Belgium and Finland and the most in Holland and Greece as interpreted, Turkeys' consumption lies between 400 and 700 g/ha annually, however. These values indicate that pesticide consumption is lesser in Turkey than that of in Europe. However, it should be noted that, this consumption in Turkey is heterogenic in such a manner that Aegean and Mediterranean Provinces have more than 1/3 of the total consumption whereas Eastern and Southeastern Provinces have a portion of only 10% (Yazgan M. 2003).

Turkey, with 125 countries, signed The Stockholm Convention on Persistent Organic Polluters on 22 and 23 May 2001. By this convention, withdrawal and reduction of the releases of twelve POPs including some pesticides such as DDT, aldrin, endrin, dieldrin, heptachlor, hexachlorobenzene, chlordane, mirex and toxaphene. By 1970s, Turkey started to ban the use of pesticides in the group of POPs. In addition to this, their production, import, export, and use are regulated by law. In 1971, dieldrin was banned and in 1979 aldrin, heptachlor, endrin were banned (Dağlı 2007).

1.3.3 Effect of Pesticides

The mode of action of this group is still under investigation. They are active on nerve system, produce tremors pursued by the loss of movement, convulsions, and death.

Lethal Dose 50% $(LD)_{50}$:The LD value is the statistics of the estimation of a pesticide that would kill 50% of the test animals (usually rats, mice, rabbits) in a determined time interval (24 hours to seven days). Since pesticides can enter the body by three different routes (oral, dermal, respiratory) of exposure, the lethal dose or concentration for each route must be measured. Pesticides with low LD values (0-10) are extremely toxic. Smaller the LD_{50} value, more toxic the pesticide. Only a very small amount of these pesticides are sufficient to be harmful to the living organisms. Inhalation Toxicity $LC_{50}$is the pesticide concentration in the air which will kill 50% of the test animals by breathing in period of time and it is expressed in parts per million(ppm)(mg/m^3) . LD_{50} and LC_{50} are used to acquire information about acute toxicity. Chronic toxicity should also be taken into account since acute toxicity cannot be used to get complete information (Connell 2005). Toxicity of some organochlorine pesticides in accordance with these toxicity values are given in Table 1.4.

Table1.4 Toxicity of Various Chlorohydrocarbon Pesticides

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

(Connell 2005)

1.4 Polycyclic Aromatic Hydrocarbons as Pollutants

Another important class of POPs in the environment is Polycyclic Aromatic Hydrocarbons (PAHs). Polycyclic aromatic hydrocarbons, which are organic compounds, contain two or more fused aromatic rings of hydrogen atoms. The combustion of fossil fuels causes the formation of these pollutants and they are always found as a mixture of individual compounds. They are not soluble in water at all. Most of the PAHs, which have low vapor pressure in the air, are absorbed on particles. Polycyclic aromatic hydrocarbons may undergo photodecomposition under exposure to ultraviolet light from solar radiation, when they are dissolved in water or adsorbed on particulate matter.

Polycyclic aromatic hydrocarbons can react with pollutants in the atmosphere in this order: ozone,nitrogen oxides,sulfur dioxide, yielding diones, nitro- and dinitro-PAHs, and sulfonic acids. Some microorganisms in the soil may also degenerate PAHs. Polycyclic aromatic hydrocarbons are biodegradable under aerobic conditions and not easily decomposed to hydrolysis (Kielhorn J. 1998).

1.4.1 Chemical and Physical Properties of Polycyclic Aromatic Hydrocarbons

The most common features for this class are; having high melting and boiling points, low vapor pressures, and low water solubilities.

Polycyclic Aromatic Hydrocarbons in Soil and Sediment

There are PAHs almost in all types of the soils. Polycyclic Aromatic Hydrocarbons are firmly tied to the soil and sediment particles and they are nearly static. Polycyclic Aromatic Hydrocarbons in soils are believed to be an outcome of a local or long-range air transport and sequential deposition. In urban soils and adjoining near shore sediment, PAH levels are the highest because there are many antropogenic activities in urban sites. Public wastewater disposals, exhaust gases of the automobiles, irrigation with coke oven effluent, leachate from coal storage sites and use of contaminated soil compost and fertilizers are more obvious reasons of PAHs in soils. As soils cannot be separated from high ways, they are naturally prone to be contaminated by vehicle exhaust and wearing of tires and asphalt,
Lastly, high PAH levels are expected to be found in the soils around landfill sites and industrial sites, like wood-preserving, coking, and former gas manufacturing plants. Because PAHs are attached to soil particles and do not easily fade, PAHs in surface water sediments can be found a lot and they are certainly major causes of contamination in surface waters (Kielhorn J. 1998). The chemical structures of 16 PAH compounds are shown in Figure 1.2.

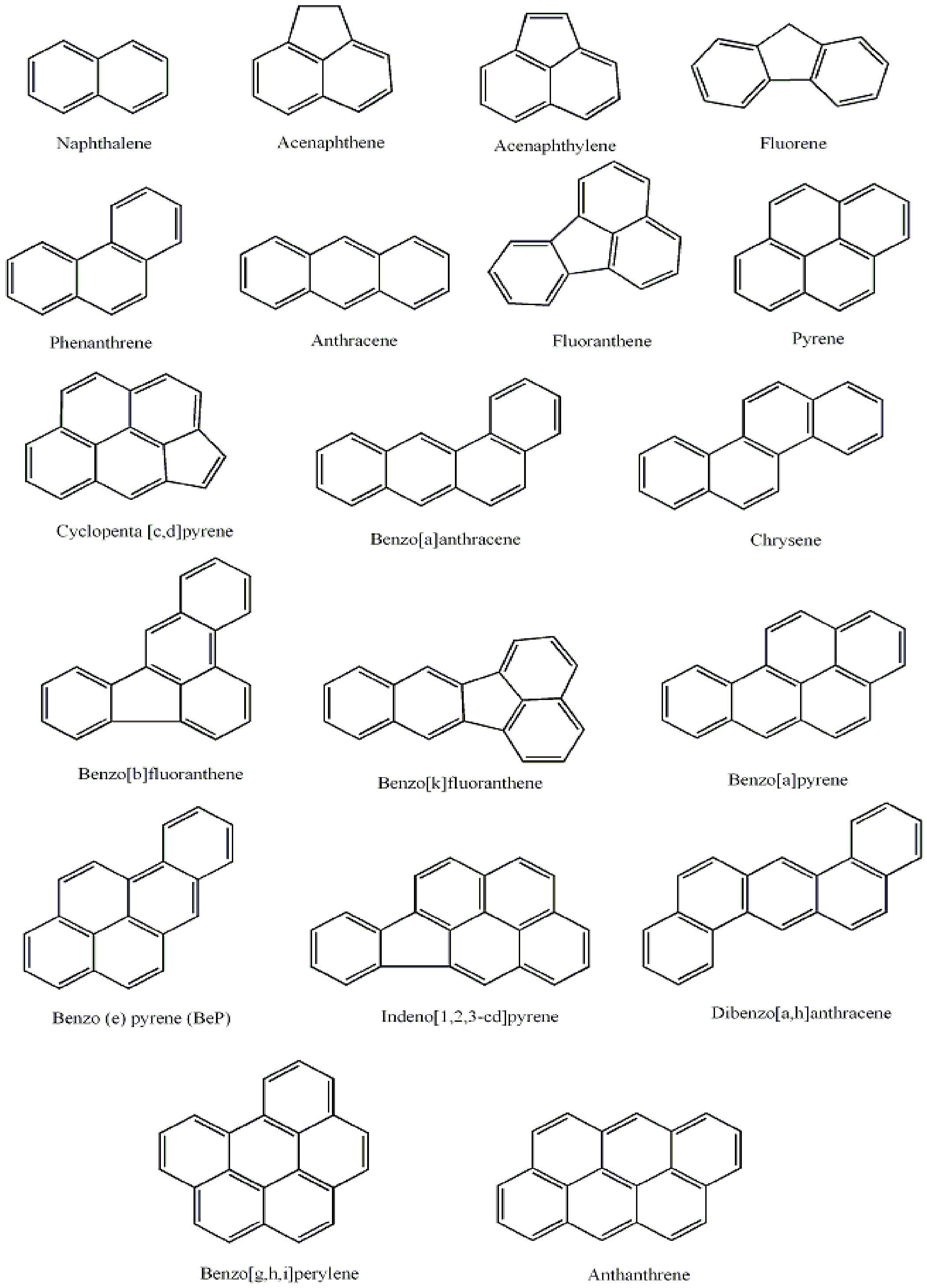


Figure 1.2 Chemical structures of 19 PAH compounds

1.4.2 The Effects of Polycyclic Aromatic Hydrocarbons on Health

The forms of releasing of PAHs into the air water and soil are burning, industrial processes and use of some household products. They cannot be faded in the environment in the short term. The type and extent of exposure and the concentration of PAHs are effective on the health impacts of PAHs

The chronic or long-term health effects of PAHs

The results of chronic and long-term exposure to PAHs on health are cataracts, kidney and liver damages, and jaundices. Redness and skin inflammation may be the result of repeated exposure. If inhaled or ingested, a lot of red blood cells can breakdown. Laboratory studies show that when animals exposed to different levels of some PAHs for long periods, stomach cancer from ingestion of PAHs in food , lung cancer from inhalation and skin cancer from touching. It is indicated from the long-term studies that when workers are exposed to mixtures of PAHs and other workplace chemicals, they are have a tendency to have lung, skin, bladder and gastrointestinal cancers.

At the same time, asthma like symptoms, lung function abnormalities and weakened immune function are identified in these studies. Even though, it should not be forgotten that there are other reasons of having cancer, so PAHs are not the only sources.

Any other health effects of PAHs

Again in laboratory studies it is seen that, difficulty in reproducing occured as the mother mice ingested a specific PAH known as benzo(a)pyrene at high levels during its pregnancy. In addition, the offspring showed the effects of this exposure. These effects are birth defects and lower body weight. In humans whether these effects may be seen is not known (Department of Health, 2009).

1.4.3 Sources of Polycyclic Aromatic Hydrocarbons

Polycyclic aromatic hydrocarbons can be formed in various processes:

Combustion: Pyrogenic PAH are mainly non-alkylated compounds, often dominated by 4–6 rings PAH, e.g. pyrene and benzofluoranthenes. The source of these compounds may be both natural (e.g. forest fires, volcano eruptions, etc.) and anthropogenic (wood ovens, industry, etc.). In the marine environment, pyrogenic PAH may originate from emissions from shipping, flaring at offshore oil installations or long-range transport from land areas.

Oil formation: Petrogenic PAH vary strongly in their composition, each type of oil having its specific profile; high amounts of lighter PAH with a high degree of alkylation are typical, e.g.alkylphenanthrenes. In the marine environment, the sources of these compounds may be both natural, such as oil seeps from oil reservoirs or hydrocarbon source rocks, and anthropogenic, due to oil spills.

Biological processes: Biogenic PAH formed in recent biological processes such as microbial activity in plant detritus, e.g. perylene, are not as strongly varied in chemical structure as petrogenic PAH due to restricted pathways of biosynthetic reactions. These compounds of natural origin have been observed to dominate PAH composition in sediments in some places, e.g. Northern Barents Sea, or Dvina Bay of the White Sea (Boitsov S. 2009).

By pyrolytic processes, particularly the incomplete combustion of organic materials and natural gas during industrial and other human activities, vehicle traffic and cooking cause the formation of PAHs, a lot of heterocyclic aromatic compounds (e.g. carbazole and cridine), and nitro-PAHs (WHO Regional Office for Europe 2000).
Polycyclic aromatic hydrocarbons are produced by natural and anthropogenic processes. Natural sources of petrogenic PAHs arise from oil seepages and erosion of

petroliferous rocks since natural sources of PAHs from combustion or pyrolysis (i.e. pyrogenic sources) include PAHs from incomplete (i.e. insufficient oxygen availability) combustion of wood and biomass via forest and grass fires. One of the most widespread categories of pyrogenic PAH inputs relates to the high temperature combustion of motor (automobile) and power plant (coals and petroleum) fuels. The combustion processes introduce large quantities of PAHs globally but more concentrated amounts especially in urban areas. Burning of wood is one of the prominent sources of atmospheric pyrogenic PAHs. Other crucial sources in indoor air include environmental tobacco smoke, gas cooking and heating appliances. Although mobile sources (vehicular exhaust) are often the most frequent atmospheric sources, in urban or suburban areas stationary sources are the reasons for approximately 80% of total PAH emissions annually (World Health Organization 1998).

1.4.4 Polycyclic Aromatic Hydrocarbons in Sediment

Polycyclic aromatic hydrocarbons in the marine environment are represented by a complex mixture of compounds consisting of 3 or more fused aromatic rings and may include alkyl chains; similar type of compounds including only 2 aromatic rings (e.g. naphthalene), unsaturated rings (e.g. acenaphthene) and heteroatoms such as sulphur (e.g. dibenzothiophene) are often also considered as PAH.

Simoneltite, a substituted PAH compound, is also found abundantly where organic conifer residues exist. These specific, singular PAHs are found in coastal sediments around the world.

Polycyclic aromatic hydrocarbons are lipophilic and can be taken up by biota. They can be toxic to marine organisms, including acute toxicity and carcinogenicity, particularly so for some PAH of higher molecular weight (Boitsov S. 2009).

1.5 Analytical Methodologies for the Determination of OCPs and PAHs in Sediment

1.5.1 Extraction Methodologies

For the extraction of organic pollutants ultrasonic bath extraction, Soxhlet extraction, solid phase micro extraction, accelerated solvent extraction, supercritical fluid extraction are the most popular extraction techniques.

1.5.1.1 Ultrasonic Bath Extraction

Ultrasonic bath extraction method is used for separating nonvolatile and semivolatile organic matters from soils, sludges and sediments by providing effective contact of the sample matrix with the extraction solvent.

It is crucial to remove moisture from the sample to obtain high extraction efficiency for ultrasonic bath extraction, as in other extractions with organic solvents. So as to dry the solid particles and allow them to contact the solvent it is mandatory to mix the sample with Na_2SO_4. The sample flasks are set into a rack in the ultrasonic bath after the samples are suspended in the extraction solvent; waves formed owing to the sonication disrupt the sample particles and agitate the solution. This process takes merely a few minutes but successive extraction may be rarely needed to achieve high extraction efficiencies. The sample is centrifuged or filtered by vacuum for separation from the extract. Then, the extract is concentrated and cleaned up for the analysis (United States Environmental Protection Agency 1996).

1.5.2 Analyses Techniques

1.5.2.1 Gas Chromatography

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

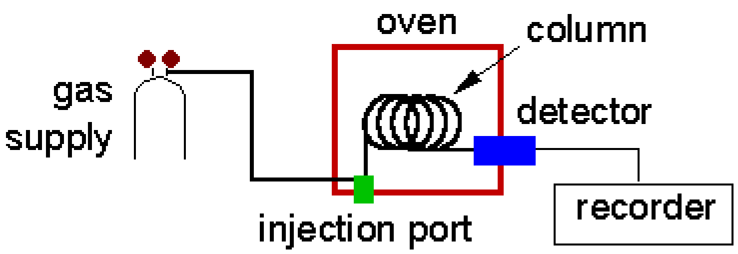


Figure 1.3 Scheme of a gas chromatograph
(Tissue 1996)

1.5.2.2 Mass Spectrometry

The major information used to identify the compound when gas chromatography used is the retention time. However, when two or more compounds have very close retention times identification becomes very difficult. If this is the case, mass spectrometer is used to acquire the structural information regarding the compound.

A molecule is ionized and separated into its fragments in a mass spectrometer, by determination of each fragment ion molecular masses occurs. The pattern displayed by the mass spectrometer is considered as the fingerprint of the molecule since a unique set of fragments compose each molecule (Kebbekus M. 1998). All sort of mass spectrometers are operated by producing and sorting ions with respect to their mass and charge. The space through which these ions flow must be isolated from other gases; so, a vacuum system is crucial. An ion source converting the molecules of the sample into ions which contain fragments of the original molecule must also be provided. In the analyzer, the ions are sorted by their mass and charge and sent to the detector (Kebbekus M. 1998). A mass spectrometer system is shown in Figure 1.4.

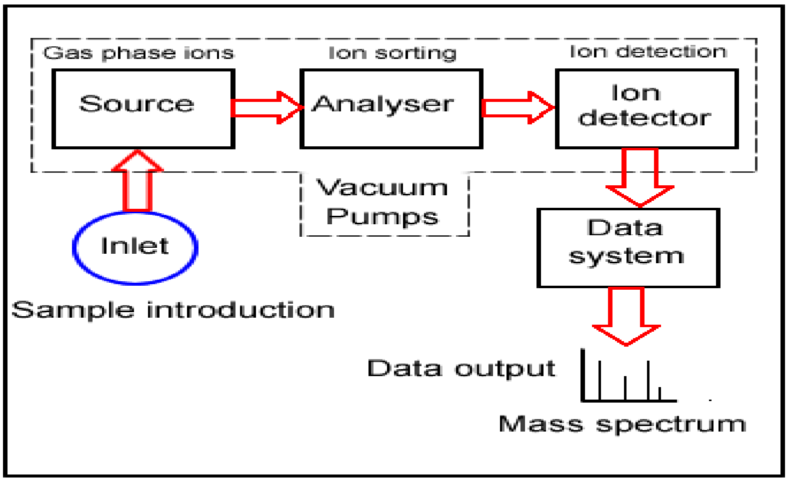


Figure 1.4 Scheme of a mass spectrometer
(University of Hull Web Page 2009)

1.6 Literature Search

It was stated in the study of Kaushik (2007) that the structure, the polarity, the solubility of the pesticides and the nature of the substituents to the parent molecule and the relative positions of them in space is effective on the toxicity of the pesticide. Lethal Dose 50%, $(LD)_{50}$, value was utilized to determine the relationship between toxicity and structure of the pesticide (Kaushik P. 2007).

Doong et. al. collected 19 sediment samples from five sampling stations along Wu-Shi river. Extractions of the samples were done by Soxhlet apparatus and the extracts were cleaned up by Florisil SPE cartridge. Gas chromatography with ECD detector and PTE-5 fused capillary column was used for determination of the concentration of pesticides in the sediment samples. After total organic carbon (TOC) corrections, the OCP concentrations are found in the range of 1.73-71.9 µg/g- OC. The analytical methods used in this study, proved that they were adequate to determine OCP residues in sediments since the recoveries of OCPs in the sediments were in the range of 70–124% (Doong, et al. 2002).

Optimization studies for a rapid method for the analysis of 17 organochlorine pesticides, including HCHs, aldrin, heptachlor epoxide, endosulfan I, dieldrin, endrin, endosulfan II, DDT derivatives, endrin aldehyde, endosulfan sulfate, methoxychlor and endrin ketone, were performed by ultrasonic solvent extraction of sediment samples by Vagi et. al. (2007). The analytical measurements were done by GC-ECD system with a fused-silica capillary column (30 m*0.53 mm id) coated with 0.50 µm chemically bonded HP-608 phase. Among the recovery studies performed at different fortification levels, the highest recovery values results from sediment samples spiked with concentrations of 50 ng g^1 and when they were extracted two times by ultrasonic bath with 5 mL of dichloromethane for 20 minutes (Vagi, et al. 2007).

Soxhlet extraction was used to examine the OCP contamination in 55 surface sediment samples from Bohai Sea by (Hu, et al. 2009). Concentrations of total dichlorodiphenyltrichloroethanes (DDTs) and hexachlorocyclohexanes (HCHs) in Bohai ranged from 0.24 to 5.67 ng g^{-1} and 0.16 to 3.17 ng $g–^{1}$, respectively. The compounds were identified with respect to retention times by Agilent 5975 GC-MSD system and HP-5890 Series G GC-ECD system with HP-5 capillary column determined OCPs. It was stated in the paper that; because of different physical and chemical properties of HCHs and DDTs and amounts of their production and usage in the past, the distribution pattern of was different from each other.

The purpose of the study carried out by Sapozhnikova et. al. was to determine 6 organophosphorus pesticides, 12 chlorinated pesticides, and 55 polychlorinated biphenyl (PCB) congeners in sediments and fish tissues in the Salton Sea and evaluate the relative ecological risk of these compounds. After ultrasonic extraction with hexane and clean up procedure from EPA method 3550B, HP-6890 gas chromatograph, equipped with an electron capture detector (ECD) and a DB-5MS fused silica capillary column (60 m· 0.32 mm· 0.25 lm) and a confirmation column from the quantitation column (DB-35) was utilized to confirm chemical identity. The levels of some of the chemicals, some of them are lindane, dieldrin, dichlorodiphenylethane (DDE) and total PCB concentrations, were found to be higher than the threshold limits (Sapozhnikova, Bawardi and Schlenk 2004) .

Turgut (2002) conducted a research investigating the pollution levels of OCPs of the water samples from Küçük Menderes River in Turkey. HP-5890 gas chromatograph with ECD detector is the system used to detect OCPs and AAS was used to detect metal pollution. In spite of the fact that, OCPs were banned over a long time ago. this river was still polluted with these pesticides. The highest concentration belonged to heptachlor epoxide with 281 ng/L. Nevertheless, the residues were lower than most polluted water from other sources in the world. The residues for the studied surface water metals were low except for Ni, Cu, and Zn which are varied from not detected to 0.258 mg/L (Turgut 2003).

Page et. al.(2006) conducted a research in 2003 in order to identify the distribution and concentrations of PAHs in sediments in Prince William Sound (PWS), Alaska where the Exxon Valdez oil spill occurred in 1989. From nine sites 181 samples were taken for investigation of total PAH concentration by immunochemical analysis. Results show that the concentration of total PAH was in a range between 20 ppb and 1320 ppm. There existed also PAHs with 2-3 ring indicating petroleum and 4–6 ring indicating combustion products that are not related to the 1989 Exxon Valdez oil spill are the contaminants (Page, et al. 2006).

A research done by Hinga (2003) after the North Cape No .2 fuel oil spill showed that, degradation of low molecular weight PAHs correlated with the total organic carbon (TOC) in the sediment. The results of the samples taken in 2.5-5 months intervals that low molecular weight PAHs remained where TOC levels are low (Hinga 2003).

Yim et. al. (2007) collected 117 sediment samples through Korea coast in order to identify the concentration and distribution of PAHs. The range of PAH concentrations are 8.80–18 500 ng/g. The pollution levels due to PAHs are high in industrialized and urbanized regions. Statistical measurements displays that major sources of PAHs were pyrogenic petrogenic, PAH contamination was determined as high in Youngil Bay, and a specific management was recommended. HP 5890 GC with HP 5972 MSD equipped with DB-5MS capillary column with dimensions of 30 m × 0.25 mm internal diameter was used for analytical measurements (Yim, Hong and Shim 2007).

The study was conducted by Qiao et. al. (2006) in 2003 by collecting 25 surface sediment samples from Meiliang Bay, Taihu Lake, China. GC-MS was used to determine the concentrations of some PAHs which are USEPA priority pollutants. Total concentrations were ranged from 1207 to 4754 ng/g dry weight; the ones with the highest concentration were from northern site and the southern part has PAHs with lower concentrations (Qiao, et al. 2006). Moreover, found PAH levels were higher than river sediments in China and lower than samples from urban areas and harbors. PAHs are mostly from the high-temperature pyrolytic process according to the observed molecular indices; on the other hand, in harbors, the reason of contamination is petrogenic process. The levels of PAHs in Meiliang Bay, except some northern sites, should not exert adverse biological effects according to the sediment quality guideline (SQGs) of the United States but, the PAH levels at northern sites 21 and 22 exceeded the health limits and could cause acute illnesses (Qiao, et al. 2006).

Klanova et. al. (2008) conducted a research with 25 samples for POPs namely; OCP, PAH and PCB contamination in soil and sediments of James Ross Island, Antarctica. Buchi System B-811 automatic extractor was used for the extraction of soil, sediment and polyurethane foam filters from passive air samplers. Analyses of the samples were performed by GC-ECD (HP 5890) and GC-MS (HP 6890-HP 5975) for PCBs, OCPs and 16 US EPA PAHs. Polychlorinated biphenyl concentrations were in range between 0.51 and 1.82 ng g^{-1} in soils, 0.14 and 0.76 ng g^{-1} in sediment samples; OCP concentrations were in range between 1.00 and 5.02 ng g^{-1} in soils, 1.33 and 1.91 ng g^{-1} in sediment samples; PAH concentrations were in range between 34.9 and 171 ng g^{-1} , and in sediment samples 1.4 and 205 ng g^{-1}. Occurrence of less chlorinated PCBs, low mass PAHs and more volatile chemicals showed that the most probable contamination source is the atmospheric transport from Africa, South America, and Australias' inhabited areas (Klánová, et al. 2008).

Allan L. (2007), presented a data set on the occurrence of POPs in different environmental matrices of Singapore such as seawater, sediments, biota and mangrove habitats. Data included the concentrations of polycyclic aromatic hydrocarbons (PAHs), organochlorine compounds (OCPs), polychlorinated biphenyls (PCBs) and polybrominated diphenyl ethers (PBDEs) in 22 samples from both the northeastern and southwestern regions of Singapore's coastal environment The total PAH concentration measured between 12.6 mg g^{-1} and 93.85 mg g^{-1}.

Concentrations of total PCB concentrations were in the range of 1.4 ng g^{-1} and 329.6 ng g^{-1}, the concentration range of total HCHs (sum of a-, b-, g- and d-HCH) were 3.3 ng g^{-1} and 46.2 ng g^{-1} and total DDT concentrations ranged from 2.2 ng g^{-1} to 11.9 ng g^{-1} in Singapore's coastal sediments. These results displays that the contamination level in the sediments of Singapore's marine environment is categorized as moderate if compared with that of other locations in Asia (Allan 2007).

Zhang et. al. (2009) overviewed a research in the samples of sediments and mollusks of the Bohai Sea including six types of POPs which are PAHs, DDTs, HCHs, PCBs and PCDD/Fs. The recent usage of DDTs, HCHs and PCBs were investigated by the compositions of the sediments. Some POPs such as DDTs and HCHs are still in use or remain in the environment of the undeveloped countries since the production was banned very late. The researches indicated that industrialized countries stopped the use of the pesticides in last twenty years for agricultural purposes and in the disease control like malaria, typhus, and cholera unlike undeveloped countries (Zhang, Song and Yuan 2009).

CHAPTER 2

EXPERIMENTAL

2.1 Study Area

The study area is a dam lake called İkizcetepeler in Balıkesir city/Turkey. İkizcetepeler Dam Lake was completed on Kille Çayı in between 1986-1991, for irrigation, drinking water, and flood prevention purposes. It is a lake with a surface area of 9.6 km^2 and located at 52.0 m above the river and it is the drinking water and irrigation water source of the area. İkizcetepeler Reservoir, is only 25 km far away from the city therefore, it is under the influence of the urban polluted air. In addition to the dense traffic on Balıkesir-İzmir-Bursa highway which is passing over the lake, there exists the organized industrial zone of the city that is constructed between the city center and dam lake. Because of the above surroundings, the dam lake is a good receptor for pollution and worthed to study. Figure 2.1 shows the sampling region in Balıkesir.

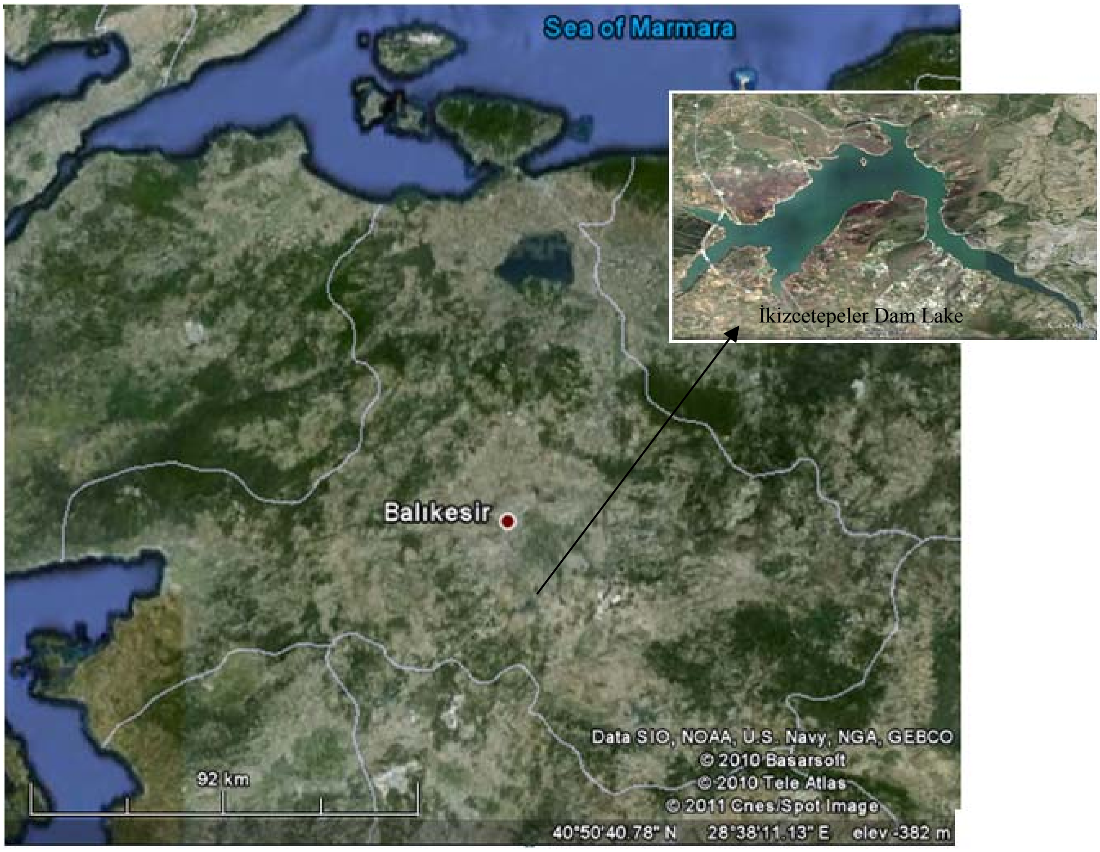


Figure 2.1 Sampling region

2.2 Sampling Strategy

Sampling is one of the fundamental steps of environmental analyses as the samples should represent the pollution of the entire region. Several different sampling strategies could be used depending on the purpose of the research and topography of the region. In this research Grid Method is used. In this approach, the study area is divided into equal squares and the samples are taken from the centers of each square. The reservoir was divided into grids of 0.123 km^2 and total 44 sampling points were determined. Sixteen sampling points for OCPs and for PAHs were chosen for the determination of the OCP and PAH pollution distribution. Figure 2.1 shows the sampling points for OCPs and PAHs.

Figure 2.2 Sampling points of İkizcetepeler Dam Lake

2.3 Sample Collection

The coordinates of the 16 sampling points were determined by using Global Positioning System (GPS). The samples were collected from the lake in 15-16 September, 2009 by using van Veen Grab sampler. The schematic representation of a van Veen grab taking a sample is shown in Figure 2.2.

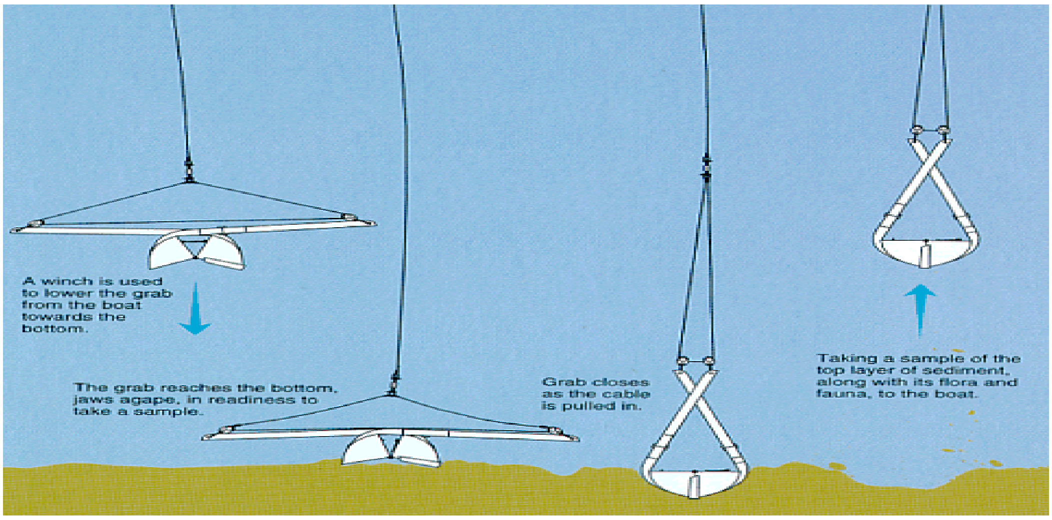


Figure 2.3 The schematic representation of a van Veen grab sampler taking a sample (Geo Seabed Instruments 2006)

The coordinates of the sampling points are shown in Table 2.1. The weight of the samples which were taken from 44 sampling points ranged between 200 grams and 500 grams and all of them were wrapped with Aluminum foil and transferred to nylon bags.

Table 2.1 The coordinates of the sampling points

Sample	**Coordinates**	**Sample point**	**Coordinates**
1	N 39 28 57.5	9	N 39 28 27.7
	E 27 56 20.9		E 27 56 14.7
2	N 39 28 40.4	10	N 39 28 04.1
	E 27 56 37.0		E 27 55 43.1
3	N 39 28 55.3	11	N 39 27 13.0
	E 27 56 33.3		E 27 55 11.8
4	N 39 28 32.2	12	N 39 27 48.3
	E 27 56 58.8		E 27 54 59.6
5	N 39 28 11.6	13	N 39 27 33.5
	E 27 57 02.6		E 27 54 24.2
6	N 39 27 57.7	14	N 39 28 07.8
	E 27 57 14.7		E 27 54 23.4
7	N 39 27 22.5	15	N 39 28 03.7
	E 27 57 52.5		E 27 55 06.5
8	N 39 27 08.4	16	N 39 28 38.4
	E 27 58 19.7		E 27 55 40.5

2.4 Reagents and Materials

The organochlorine pesticide standards (EPA Method 508-Chlorinated Pesticide Mix 1, 1000µg/ml), internal standards (Accustandard, Pentachloronitrobenzene, 1.0 mg/ml) and surrogate standards (2,4,5,6-Tetrachloro-m-xylene, 10 ng/µl and Decachlorobiphenyl, 0.5 mg/ml) were purchased from Dr. Ehrenstorfer(Ausburg, Germany). The intermediate standard solutions were prepared from the stock solutions with appropriate dilutions with hexane.

PAH standard solutions (PAH-Mix 68, 100.00 mg/L) and deutorated surrogate standards (Internal Standards Mix 25, 500.00 mg/L) were also purchased from Dr. Ehrenstorfer(Ausburg, Germany). The intermediate standards solutions were prepared from the stock solutions with appropriate dilutions with dichloromethane. Standard reference materials (SRM 2261, SRM 2275, and SRM 1597) were purchased from National Institute of Standards (NIST).

All the solvents used for OCP and PAH determination were chromatographic grade and purchased from Merck Company (Germany).

All the stock, intermediate and standard solutions were stored in refrigerator. Hamilton gas tight glass syringes (500, 100, 10 ml) were used for the preparation of the standards into 2 ml amber vials(Supelco). Ultrasonic extractions were performed by Branson ultrasonic bath, Heidolph rotary evaporator (Laborota 4000) was used to evaporate the solvents of both standards and samples. A Supelco minivap evaporator was used to reduce the volumes of extracts. The extracted samples were transferred to 2 ml amber glass vials for further reduction of the volume.

2.5 Preparation of Na_2SO_4, Florisil and Glass Wool

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

Florisil was used for clean-up after the extraction. It is a polar mixture of Magnesium Oxide-Silicon Dioxide (Magnesium Silicate) in 15:85 ratio. It is used to separate the polar compounds from the sample. Although Florisil was purchased in extra pure grade from J.T. Baker Company, it was cleaned and then conditioned just like as Na_2SO_4. For conditioning a glass column was filled with Florisil and acetone-hexane (1:3) mixture was eluted through the column. A beaker was used to transfer the washed Florisil and it was covered with solvent rinsed aluminum foil and conditioned at 225 °C overnight. Dry Florisil was transferred to an amber glass bottle with a Teflon lined cap and stored in desiccator until the extractions.

Glass wool was used to fill the tip of the column and purchased from Supelco. It was also cleaned before use. A quantity of a glass wool was compressed into a large glass column and washed sequentially hexane and dichloromethane and treated like Na_2SO_4 and storred in a desiccator.

2.6 Cleaning of Glassware

The samples contain the analytes in very small amounts therefore; to eliminate the contamination extreme precautions were taken. All the glassware used was washed with detergent and hot water first. Then, several rinses with tap water and deionized water are followed. Dicloromethane, hexane, and acetone are used for rinsing at last. All of the glassware was oven dried.

2.7 Instrument and Apparatus

An HP (Hewlett Packard) 6890 series gas chromatograph coupled with HP 5973 mass spectrometer was used for the analysis. Instrument is also equipped with Flame Ionization and Nitrogen Phosphorus detectors. A 30m, 0.32 mm id., 0.25mm film thickness, crosslinked 5% Phenyl methyl siloxane, HP 5MS, capillary column (Agilent Tech.) was used for the separation of PAHs and OCPs throughout the study.

2.8 Optimization of GC-MS system

The parameters of GC-MS for PAH and OCP determination were formerly optimized by Tansel Topal and Nur Banu Öztaş, respectively. Table 2.2 and Table 2.3 show the operating parameters of GC-MS used for detecting PAH and OCP concentrations in the sediment samples respectively. GC-MS chromatograms of OCP and PAH standards are given in Figure 2.3 and Figure 2.4 respectively.

Table 2.2 Operating parameters of GC-MS system for OCP determination

Injector:	Splitless
Inlet temperature:	250 °C
Column:	HP-5 MS (5 % Phenyl Methyl Siloxane 30.0m*250mm*0.32 mm)
Oven temperature:	80° C-150 ° C at 10 ° C/min. wait for 5 mins. 150 ° C-275 ° C at 5 ° C/min. wait for 3 mins.
MS source temperature:	290 °C
MS quadrupole temperature:	150 °C
Injection volume:	1 µl
Carrier gas:	Ultra purified Helium, 99.999%. 1ml/min

Table 2.3 Operating Parameters of GC-MS system for PAH determination

Injector:	Splitless
Inlet temperature:	280 °C
Column:	HP-5 MS (5 % Phenyl Methyl Siloxane. 30.0m*250mm*0.32 mm)
Oven temperature:	50° C at 4 min. 10 ° C/min 300 °C at 10 min
MS source temperature:	230 °C
MS quadrupole temperature:	150 °C
Injection volume:	2 µl
Carrier gas:	Ultra purified Helium, 99.999%, 1ml/min

(Topal 2011), (Öztaş 2008)

Gas chromatography equipped with a mass spectrometer allows chemists to detect very small quantities of the contaminants in the environmental matrices, but Selected Ion Monitoring (SIM) mode improves the sensitivity of the measurements by limiting the mass of the ions detected to one or more specific fragment ions of known mass. Therefore, selectivity is increased in the SIM mode whereas in scan mode there are many noises originating from the ions that are not in concern. The SIM windows for OCP and PAH determination is given in Table 2.4 and Table 2.5 and sample chromatograms for the standards used for OCP and PAH determination are given in Figure 2.3 and Figure 2.4, respectively.

Table 2.4 Adjustment of SIM parameters for OCP determination

Windows	**Time Period (min)**	**Ions Monitored**
1	3-15	63,82,126,127,128,129, 151,152,153,154,172, 206,208,209,244,330
2	15-30	82,166,172,178, 202,228,244,252,330
3	30-45	82,172,244,252, 276,278,330

Table 2.5 Adjustment of SIM parameters for PAH determination

Windows	**Time Period (min)**	**Ions Monitored**
1	3-21	91,100,109,111,136,181,183, 207,209,219,237,244,249 263,265,272,273,274,295
2	21-26.40	67,79,81,195,207,235,237,239, 241,246,248,250,261,263,265,277 316,318,345,347,351,353,355
3	26.40-40	165,169,199,212,227,229,235,237, 250,272,274,387,426,497,499

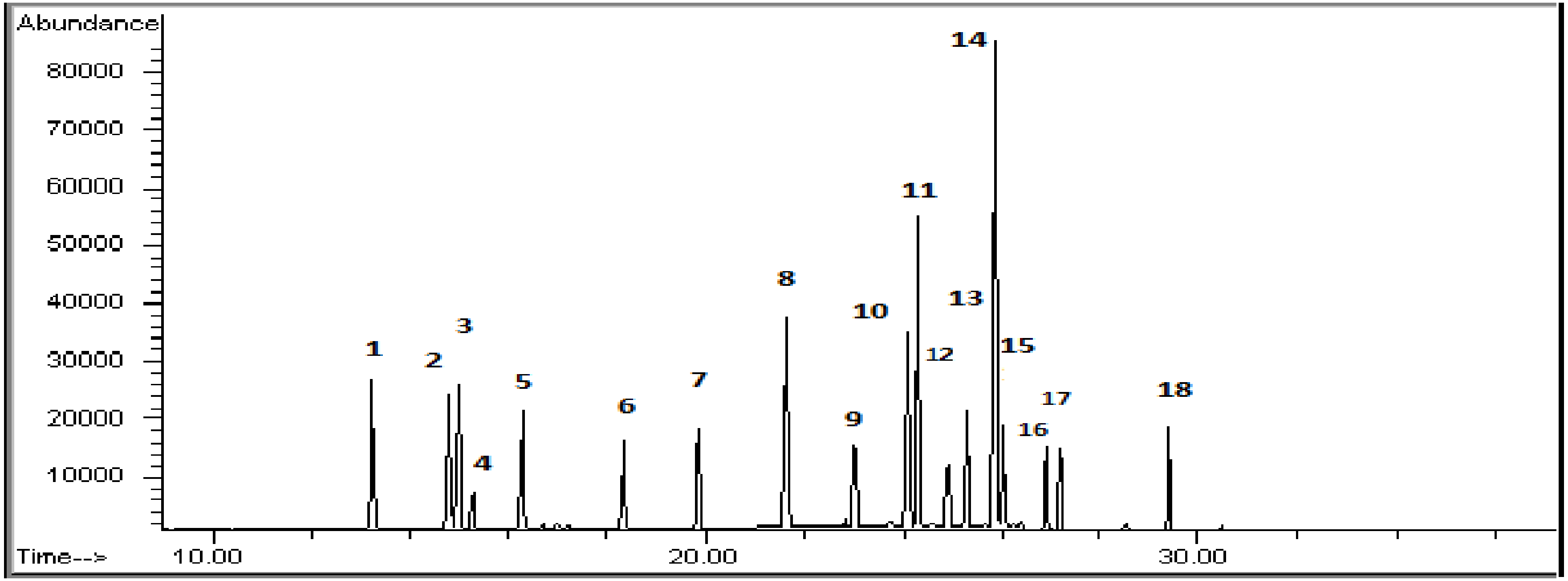


Figure 2.4 GC-MS Chromatogram (SIM Mode) of Standard Containing 1 µg/L OCPs and internal standard

1. Alpha-HCH **2.** beta-HCH **3.** gamma-HCH **4.** Pentachloronitrobenzene(IS) **5.** delta-HCH **6.** Heptachlor **7.** Aldrin **8.** Heptachlorepoxide **9.** Endosulfan **10.** Dieldrin **11.** 4,4’-DDE **12.** Endrin **13.** Endosulfan II **14.** 4.4’-DDE **15.** Endrin Aldehyde **16.** Endosulfan sulfate **17.** 4.4’-DDT **18.** Methoxychlor

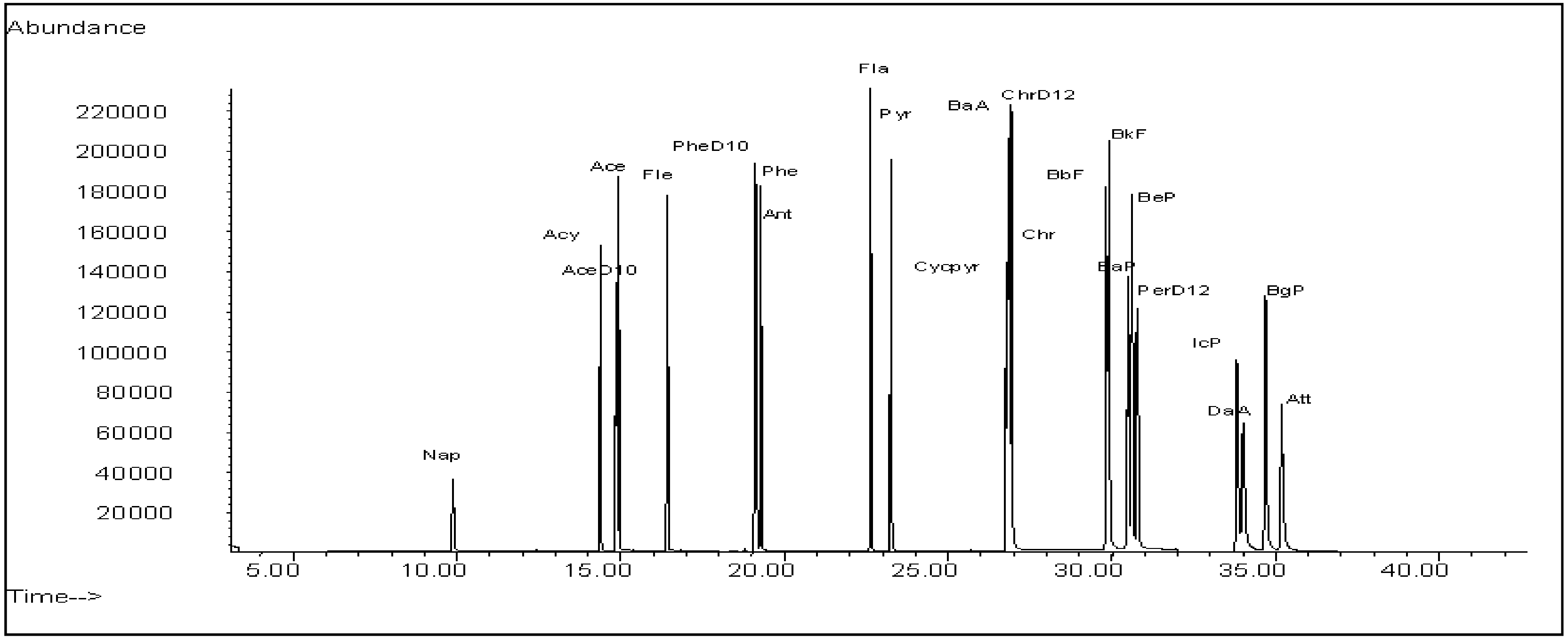


Figure 2.5 GC-MS Chromatogram (SIM Mode) of Standard Containing 1 mg/L PAH and Surrogates

2.9 Calibration of the Analysis Systems

Quality control studies for analytical measurements are performed in terms of accuracy and precision.

Before determination of the unknown concentration of the analytes a calibration curve is prepared external standards with known concentrations of the analytes are mostly used for calibration of the analysis systems, but sample injection gives some uncertainty to the measurements since it is performed with a small volume of sample (generally 1.0 or 2.0 μL). Use of internal standards can serve a solution for this problem. Internal standard addition involves the mixing of the internal standard with the samples and calibration standards. The ratio of the areas of the analyte peaks of the sample to that of internal standard is used as the analytical parameter.

In this study, internal standard calibration method was used for quantification of organochlorine pesticides. Table 2.6 shows the calibration parameters for the determination of OCPs and PAHs.

Table 2.6 Calibration parameters for the determination of OCPs and PAHs

	Internal Standard (Concentration, µg/ mL)	**Surrogate Standard (Concentration, µg/ mL)**	**Standard Concentrations, µg/ mL**
OCPs	Pentachloronitrobenzene(PCNB) (0.50)	SS1:2,4,5,6-Tetrachloro-m-xylene SS2:Decachlorobiphenyl (0.10, 0.20, 0.25, 0.50, 1.0)	0.10, 0.20, 0.25, 0.50, 1.0
PAHs	-	Acenaphtene D10, Phenanthrene D10, Chrysene D12, Perylene D12	0.1, 0.25, 0.50, 0.75, 1.00, 2.50, 5.00

Internal standard addition method was applied during the determination of OCPs in sixteen sediment samples. Calibration curves for OCPs and PAHs are shown in Figure 2.5 and Figure 2.6, respectively.

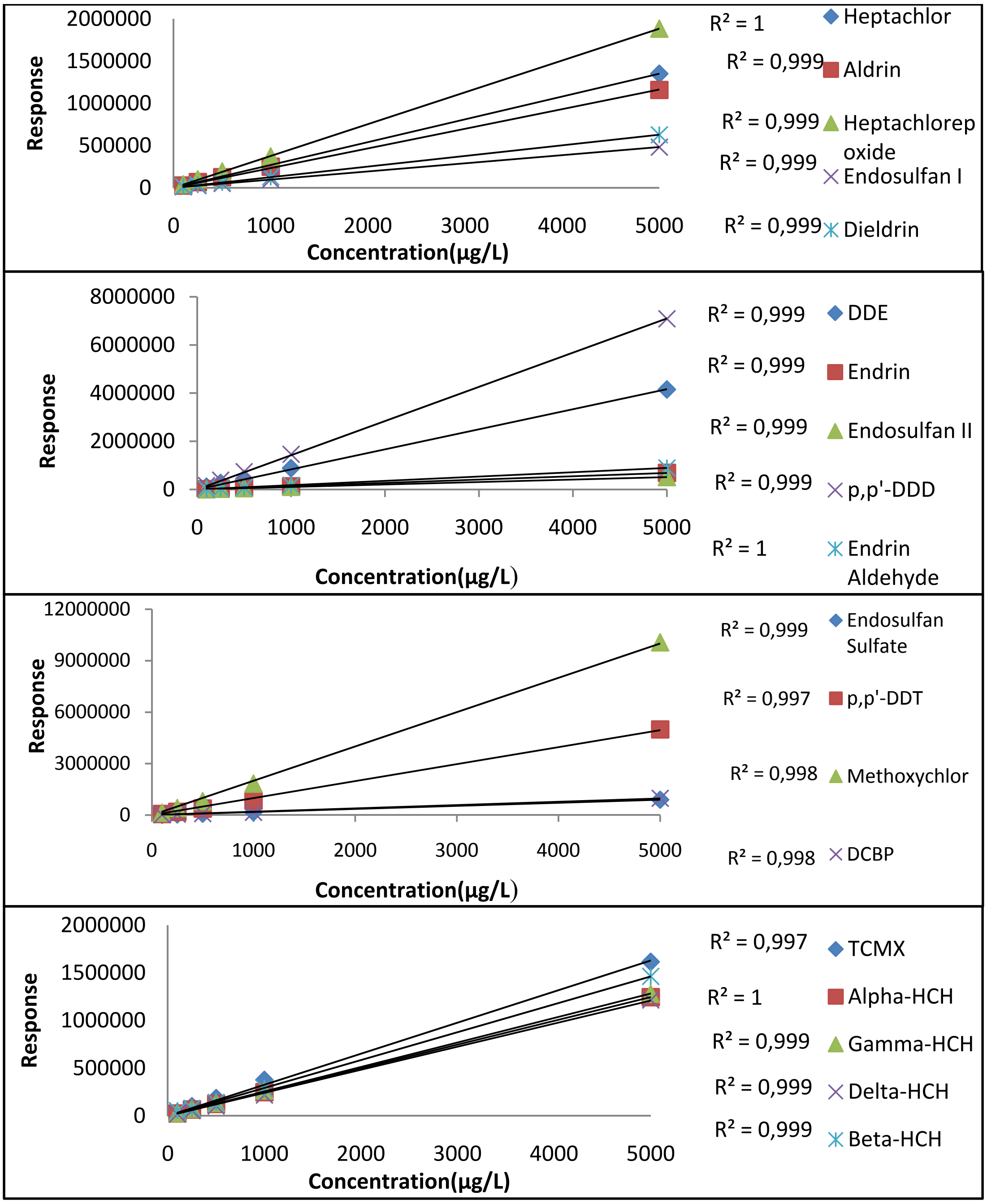


Figure 2.6 Calibration curves of pesticide ions

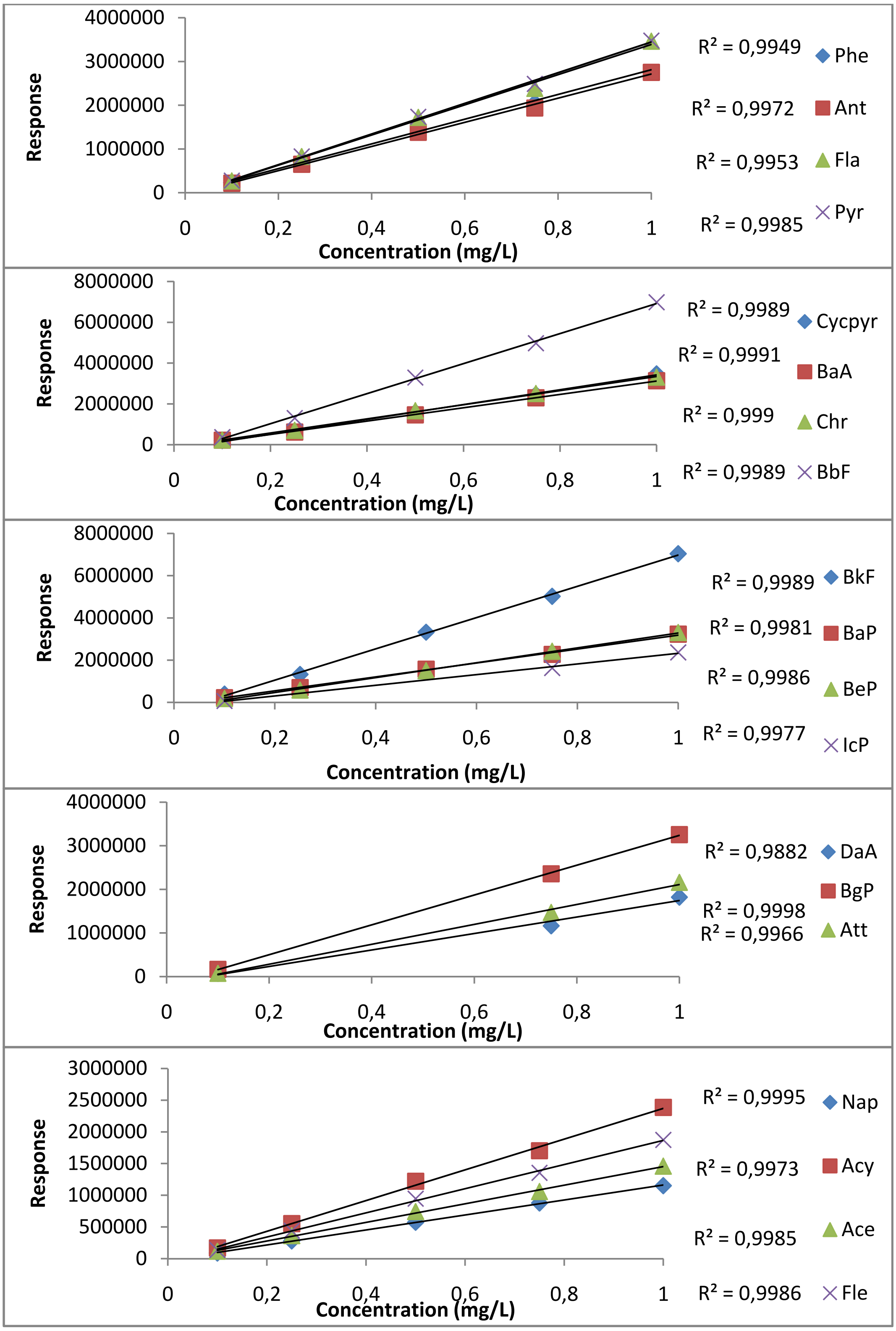


Figure 2.7 Calibration curves for PAHs

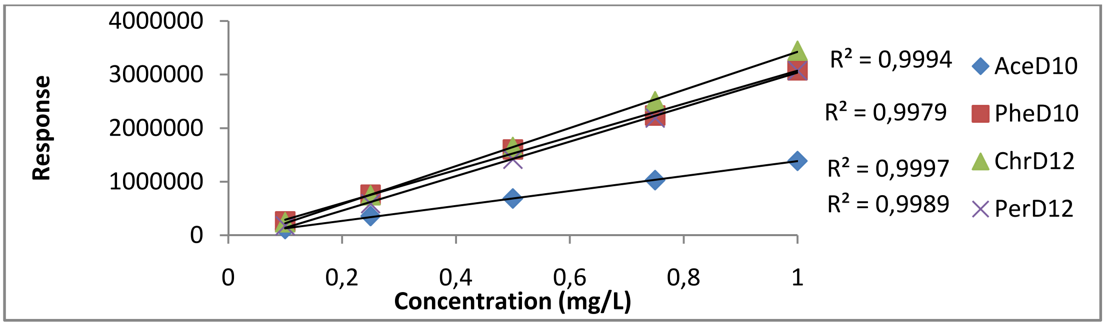


Figure 2.8 Calibration curves for PAHs (continued)

As seen from the Figure 2.5 and Figure 2.6 coefficient of determination, R^2, values obtained were greater than 0.988 for PAHs and 0.99 for OCPs.

2.10 Application of Ultrasonic Bath Extraction

2.10.1 Ultrasonic Bath Extraction for Organochlorine Pesticides

The moisture contents of the sediment samples were determined since the samples were in a form similar to mud. For this purpose, 9 grams of the sediment from the different parts of the sample were weighed. This portion of the sample was dried in an oven overnight at 150 ^{0}C. Moisture content of the wet sediment samples was calculated according to the formula given below;

$$\textit{Water content (\%)} = \frac{M_{cws} - M_{cs}}{M_{cws} - M} \times 100$$

Where;

$\mathbf{M_{cws}}$ = mass of container and wet sample

$\mathbf{M_{cs}}$ = mass of container and dry sample

$\mathbf{M_{c}}$ = mass of container

It was found that the average water content of the sediment samples is 56%. A classification method based on the water content describes the sediment as dry if the moisture content is lower than 20 weight%, wet if the moisture content is between 20 and 30 weight%, very wet if the moisture content is between 30 and 40 weight%, and saturated if the moisture content is more than 40 weight% (Adam, et al. 2006)). According to this classification the sediment samples obtained from the İkizcetepeler Dam Lake are saturated type since water content is higher than 40 % . The corrections were done after the analyses of the samples considering this classification.

Two grams of sediment samples were weighted in an amber glass bottle with Teflon cap and 100 µL of surrogate standards 2,4,5,6 Tetrachloro-m-xylene and Decachlorobiphenyl with a concentration of 1.0 µg/L were added. Sixty milliliters of hexane : acetone mixture(3:1) was added to the bottle and closed for ultrasonic bath extraction for two hours. The extracts were eluted through Na_2SO_4 column. prepared as mentioned previously, to eliminate the water and then cleaned up with Florisil column, preparation was similar with Na_2SO_4 column. to remove the polar compounds that interfere with the analytes. The mixture was evaporated with rotary evaporator and 100 µL internal standard (Pentachloronitrobenzene) having a concentration of 0.5 µg/L was added. Afterwards, the final solution was preconcentrated to 1 mL with a minivap evaporator under nitrogen gas and taken into a 2 ml amber glass vial and kept in refrigerator at 4 ^{0}C before the analysis with GC-MS (Manirakiza, et al. 2001).

2.10.2 Ultrasonic Bath Extraction for Polycyclic Aromatic Hydrocarbons

Nine grams of the wet sediment was weighed to an extraction vessel (50 mL beaker) After spiking 1ml of surrogate including 5 ppm of Ace D10, Phe D10, Chr D12 and Per D12 in dichloromethane, 10 ml of DCM and 10 ml of acetone was added. The sample was extracted by sonication for 15 minutes then the extract was taken.

After getting all of the extract, a sodium sulfate column was prepared by plugging it with glass wool. The column was 20 cm in length and washed with DCM and acetone two times before the elution started. The total extract was taken with Pasteur pipette and eluted form the Na_2SO_4 column in order to get rid of the water residue and also the impurities.

After eluted, the extract was dried with rotary evaporator and the volume was reduced to 1 ml under nitrogen gas. The final solution was taken into a 2 ml amber glass vial and kept in refrigerator at 4 ^{0}C before the analysis with GC-MS (Topal 2011).

2.11 Analysis of the Samples

The 2.0 μL aliquot of the sample is taken with the Agilent gas tight glass syringes and injected three times to the injection port of GC-MS system. The average values of these three replicates are used as the results. For all sixteen sediment samples used for OCP determination and six samples used for PAH determination, blank and SRM samples were analyzed. After the correction with the percent recoveries obtained for each analyte, the final concentrations were calculated. Table 2.7 shows the retention times and ions used to determine the analytes for OCP and PAH determination.

The sample chromatograms obtained from GC-MS systems for OCPs and PAHs are given in Figure 2.7 and Figure 2.8, respectively.

Table 2.7 Retention times and ions of the analytes

	RetentionTime(min)	Target and confirmation Ions
Organochlorine Pesticides		
2,4,5,6 Tetrachloro-m-xylene (SS1)	11.016	207,209,244,136
alpha-HCH	13.228	181,183,219,109
beta-HCH	14.737	181,183,219,109
gamma-HCH	14.954	181,183,219,111
Pentachloronitrobenzene (IS)	15.228	237,265,249,295
delta-HCH	16.245	181,183,219,109
Heptachlor	18.314	272,100,273,274
Aldrin	19.783	263,265,66,91
Heptachlorepoxide	21.549	353,355,351,81
Endosulfan	22.973	237,195,241,207
Dieldrin	24.007	263,265,261,79
p.p’-DDE	24.201	246,318,248,316
Endrin	24.813	263,265,261,81
Endosulfan II	25.207	195,237,241,207
p.p’-DDD	25.790	235,237,165,199
Endrin aldehyde	25.962	345,347,250,67
Endosulfan sulfate	26.865	272,387,274,229
p.p’-DDT	27.156	235,237,165,199
Methoxychlor	29.397	227,229,212,169
Decachlorobiphenyl (SS2)	35.186	497,499,426,427
Polycyclic Aromatic Hydrocarbons		
Naphthalene	9.917	128, 127, 129
Acenaphtylene	14.415	152, 151, 153
Acenaphtene D10 (SS1)	14.878	162,164,160,163
Acenaphtene	14.970	153, 154, 152
Fluorene	16.444	166,165,163
Phenanthrene D10 (SS2)	19.113	188,189,184,187
Phenanthrene	19.182	178,176,179
Anthracene	19.308	178,176,179
Fluoranthene	22.663	202,200,203
Pyrene	23.263	202,200,203
Cyclopenta (c,d)pyrene	26.795	226,224,227
Benzo(a)anthracene	26.858	228,226,229
Chrysene D12 (SS3)	26.892	240,236,241,239
Chrysene	26.966	228,226,229
Benzo(b)fluoranthene	29.841	252,250,253,251
Benzo (k) fluoranthene	29.910	252,250,253,126
Benzo (a) pyrene	30.504	252,250,253
Benzo (e) pyrene	30.624	252,253,250
Perylene D12 (SS4)	30.767	264,260,265,263
Indeno(l,2,3-c,d) pyrene	33.911	276,277,277,274,138
Dibenz (a,h) anthracene	34.082	278,276,277,139
Benzo (g,h,i) perylene	34.797	276,277,274,138
Anthanthrene	35.214	276,277,274,275

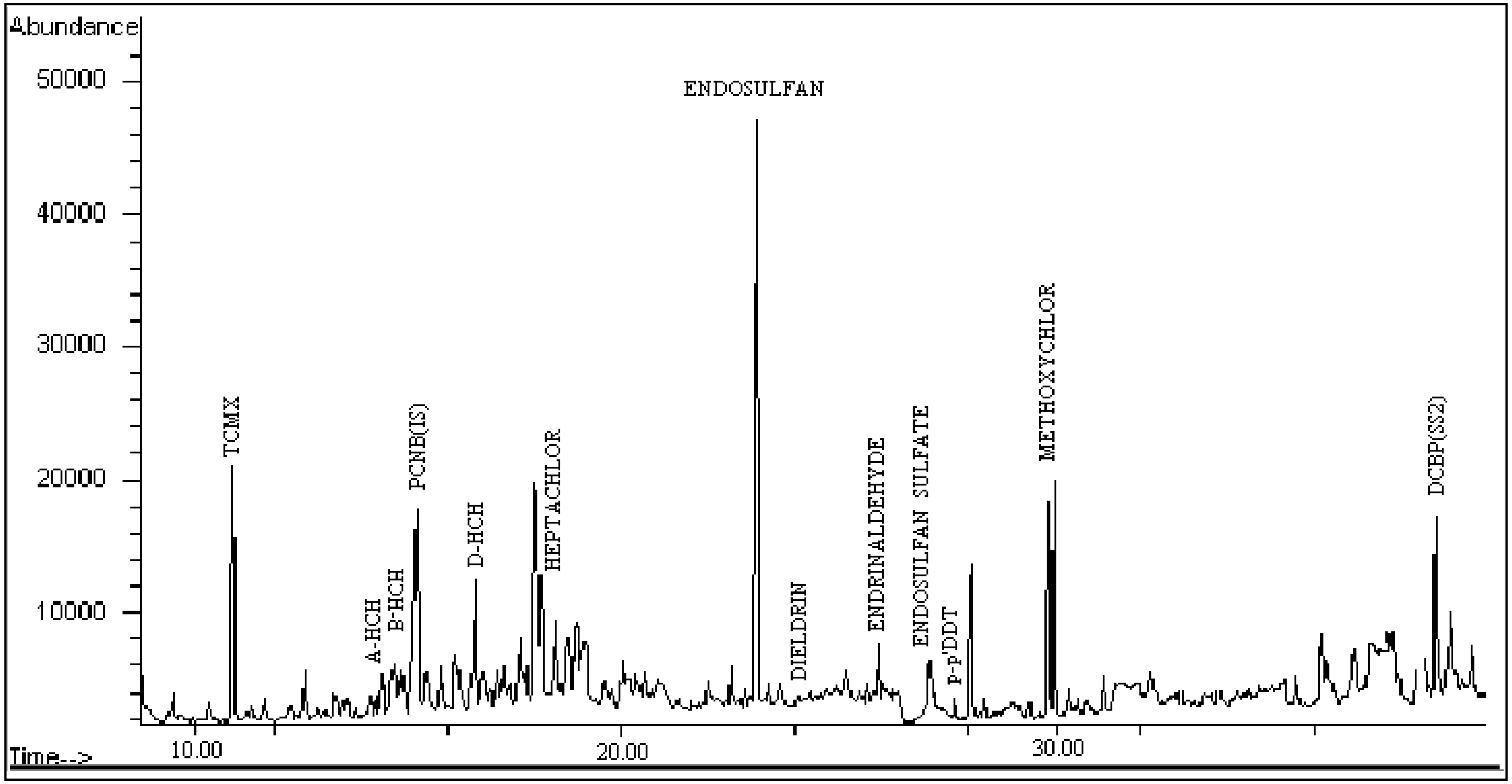


Figure 2.9 The sample chromatogram showing OCPs obtained from GC-MS system for the sediment from sampling point 4

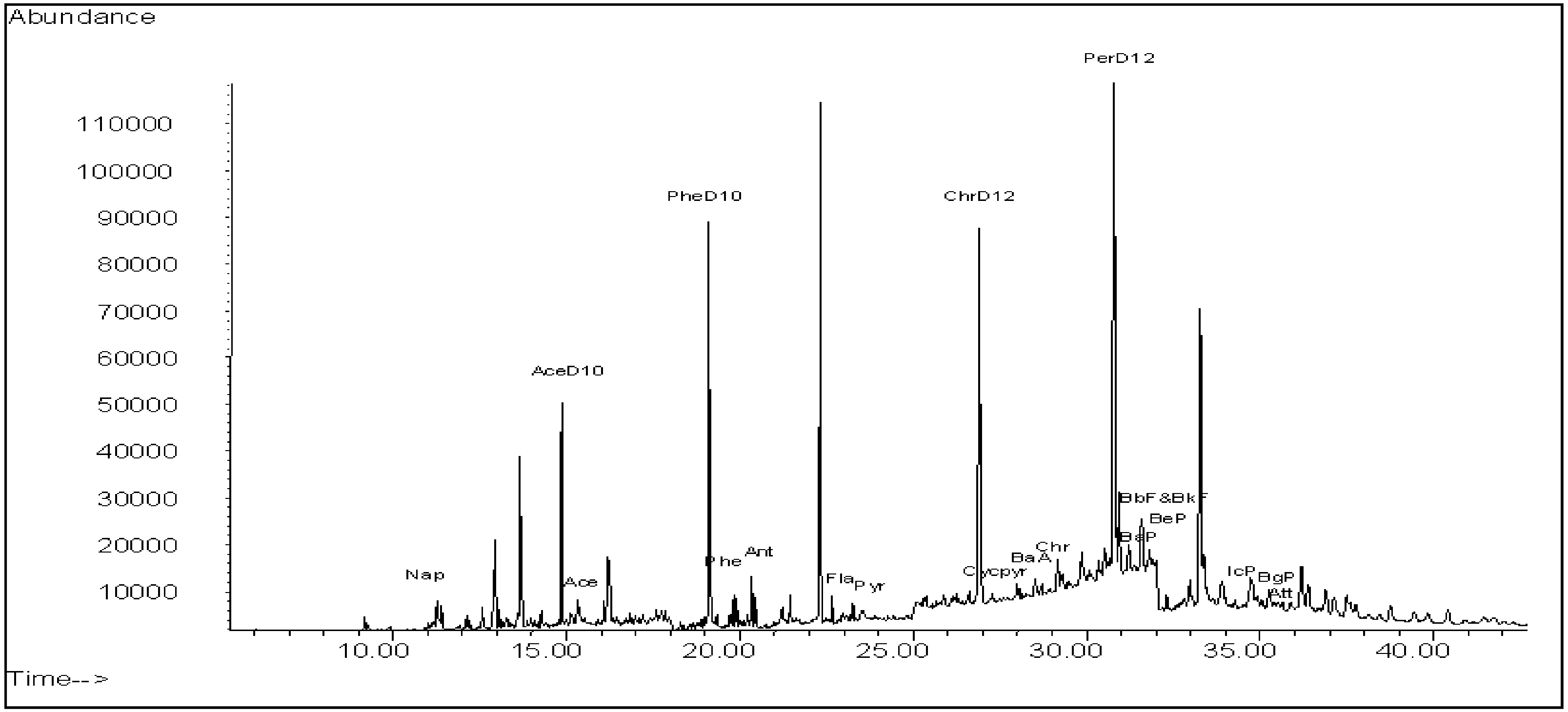


Figure 2.10 The GC-MS chromatogram (SIM Mode) of Ultrasonic Bath Extracted İkizcetepeler Dam Lake Sediment

CHAPTER 3

RESULTS AND DISCUSSION

3.1 Method Validation

In this chapter the Quality Assurance (QA) and Quality Control (QC) tests during extraction process, during the analyses and evaluation of the data set and data interpretation of Organochlorine Pesticides (OCPs) and Polycyclic Aromatic Hydrocarbons (PAHs) are discussed.

3.1.1 Quality Assurance (QA) and Quality Control (QC) Tests During Extraction of the Samples

Before the discussion on the produced data set, it is essential to investigate efficiency of extraction procedure, as the concentrations are very low in environmental samples. Possible contaminations from the laboratory conditions and the equipments used during the experimental procedure cause deviations from the real concentrations of the analytes. Addition of standards with known amounts before the extraction procedure is the common technique to determine the efficiency of the method. As it was mentioned before, extraction methodologies are extensively studied in our group for both pesticides (Öztaş 2008) and PAHs (Gaga 2004), (Topal 2011). Therefore, the extraction methods, which were recommended by previous studies of our group for the sediment matrices, were used in this study. It is called as surrogate standard addition method. Surrogates are organic compounds that are not naturally found in environmental samples but have similar chemical composition and behavior in the analytical method. Surrogate standard addition method was used for extraction of sediments for both OCP and PAH determinations. The surrogate standards namely are Tetrachloro-m-xylene and Decachlorobiphenyl for OCPs; Acenaphthene D10, Chrysene D12, Perylene D12, Phenanthrene D10 for PAHs. Table 3.1 shows the percent recoveries obtained for these surrogate standards used before the ultrasonic bath extractions. Percent recoveries were calculated as:

$$\% \text{ Recovery } = {(Cs - Cu)}/_{Cn} \times 100$$

where C_s: measured concentration of the spiked sample aliquot

C_u: measured concentration of the unspiked sample aliquot

C_n: nominal (theoretical) concentration increase that results from spiking the sample, or the nominal concentration of the spiked aliquot (United States Environmental Protection Agency 2003)

Percent surrogate recoveries are acceptable when they are in 70-130 % range according to United States Environmental Protection Agency (USEPA) standards (United States Environmental Protection Agency 2003). As it is illustrated in the Table 3.1, the recoveries obtained in this study are in the acceptable range. The concentrations of the analytes in the sediment samples were computed according to the corrections done in terms of recovery of the surrogates.

The stability of the instrument should also be checked because of the fact that some errors may occur during the analysis. Internal standard addition method was used in case there were errors originating from the instrument and standard reference materials (SRMs) were used to determine the accuracy of the system. Results of the accuracy checks with SRMs will be discussed in Section 3.2.

Table 3.1 Surrogate recoveries of the standards used for ultrasonic bath extractions

Surrogate	Used for	% Recovery
2,4,5,6 Tetrachloro-m-xylene **Decachlorobiphenyl**	OCP	70.3±18.2
Acenaphthene D10	PAH	67.2±9.41
Phenanthrene D10		63.8±8.42
Chrysene D12		87.4±28.8
Perylene D12		77.8±21.7

3.1.2 Quality Assurance (QA) and Quality Control (QC) Tests During The Analyses

As mentioned above, internal standard addition method using SRMs in replicate injections are used for quality control checks during the analyses.

An internal standard is spiked in the sample vial at a known and fixed concentration to every single sample extract with the purpose of evaluating the variations of the analysis system. For determination of OCPs, Pentachloronitrobenzene was used as the internal standard with a fixed concentration of 500 µg/L. This concentration of Pentachloronitrobenzene was also spiked to the calibration standards.

Standard reference materials (SRMs) are used to check the accuracy of the measurements in analytical studies. Since there is no standard reference material including all of the analyzed OCPs of concern, three different SRM standards were separately used namely SRM 2261 (Chlorinated Pesticides in Hexane), SRM 2273(Chlorinated Pesticides (DDTs) and Metabolites in Isooctane), SRM 2275(Chlorinated Pesticide Solution-II in Isooctane), and their errors in percent are shown in Table 3.2. All of the SRM checks were done by three replicate injections with certain amount of internal standard (500 µg/L) standard deviations between these three replicate injections are also shown in Table 3.2. The last column in Table 3.2 shows the ratio of certified concentrations to found concentrations. Ideally, this ratio should be one but in our case for some parameters, we observed greater than one and for the others less than one. The same results are also explained in terms of percent errors. The percent errors for six 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.

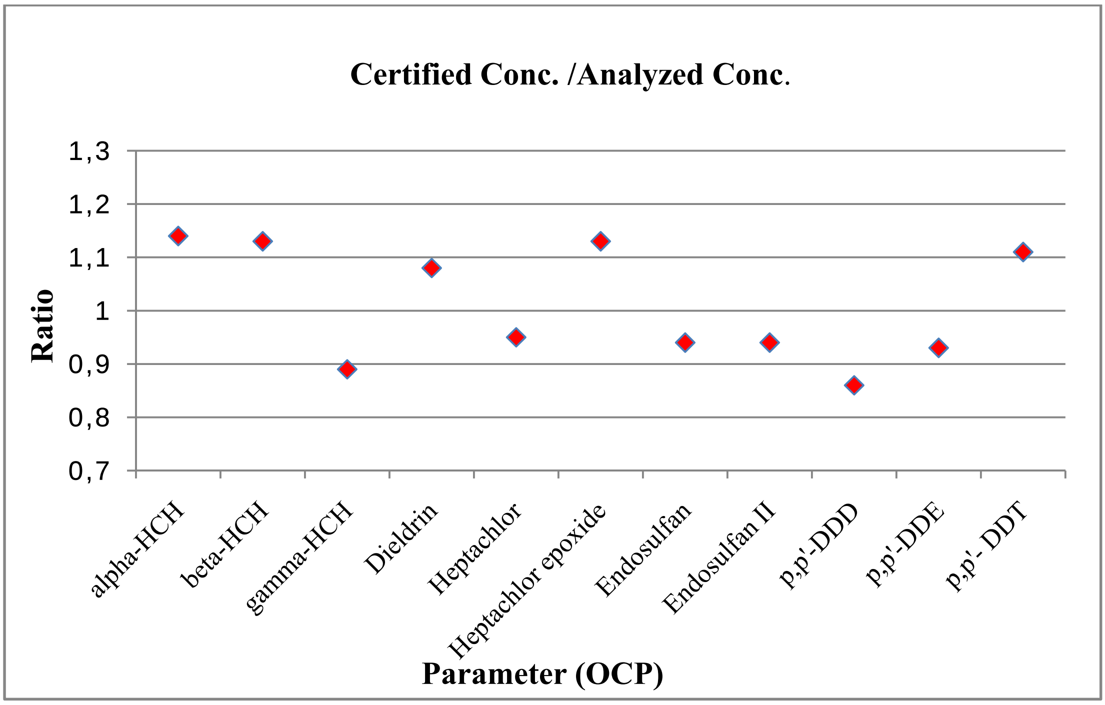
Table 3.2 Measurements with the analysis of NIST SRMs 2261, 2273, 2275 (N=3)

Conc. (mg/L) Parameter	**NIST SRM 2261**				**NIST SRM 2273**				**NIST SRM 2275**				
	Av. (mg/L)	**S.D. (mg/L)**	**Cert. (mg/L)**	**%Err**	**Av. (mg/L)**	**S.D (mg/L)**	**Cert (mg/L)**	**%Err.**	**Av. (mg/L)**	**S.D (mg/L)**	**Cert (mg/L)**	**%Err.**	**Ratio(Certified Conc.**
alpha-HCH									1.81	0.02(3)	2.07	-12.6	1.14
beta-HCH									1.82	0.07(3)	2.05	-11.3	1.13
gamma-HCH	2.21(3)	0.22(3)	1.97	12.1									0.89
Dieldrin	1.83(3)	0.06(3)	1.97	-6.99									1.08
Heptachlor	2.09(3)	0.17(3)	1.98	5.72									0.95
Heptachlor epoxide	1.75(3)	0.09(3)	1.98	-11.7									1.13
Endosulfan									2.12	0.14(3)	1.99	6.50	0.94
Endosulfan II									2.15	0.09(3)	2.03	5.90	0.94
p,p'-DDD					2.39(3)	0.09(3)	2.06	-17.5					0.86
p,p'-DDE					2.16(3)	0.08(3)	2.01	7.38					0.93
p,p'- DDT					1.78(3)	0.13(3)	1.97	-9.44					1.11

* The numbers in the parentheses are the number of injections

Best accuracies were obtained for Heptachlor, Endosulfan and Endosulfan II, next to these parameters was Dieldrin. The poorest accuracy was obtained for alpha-HCH and p,p'-DDD. The same data are shown as line graph in Figure 3.1.

Figure 3.1 Demonstration of the accuracies for OCPs in SRM 2261, SRM 2273, SRM 2275



The ongoing accuracy during the sample injection periods are shown in Figure 3.2 for organochlorine pesticides in order to observe the fluctuation, if exists, in the accuracy. Figure 3.2 shows some of the results of the SRM values obtained during eight analyses days.

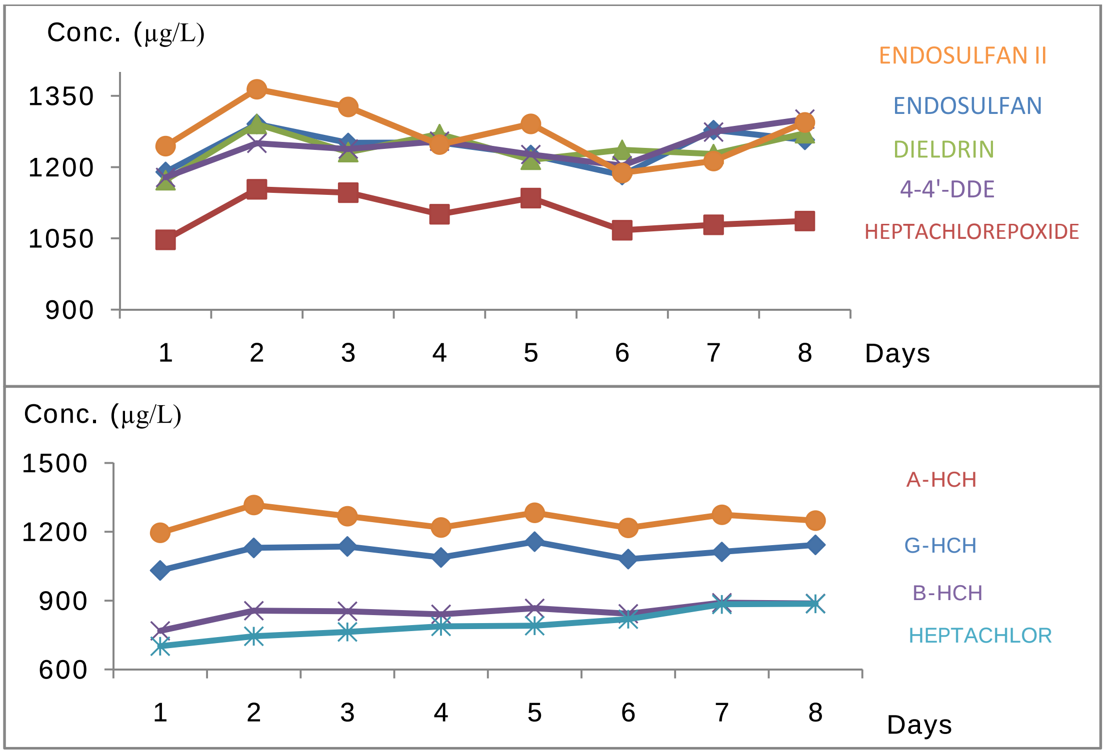


Figure 3.2 The ongoing accuracy of SRM 2261, SRM 2273 and SRM 2275 during the sample injection periods for organochlorine pesticides

As seen from the Figure 3.2, the readings of the solutions were almost constant throughout all the analysis period, in an acceptable degree of variation. It can be concluded that the analysis systems were stable until the end of the analysis. As seen from the figures, the readings of the solutions were constant throughout all the analysis period, in an acceptable degree of variation, within ± 2σ range. Moreover, the calibration curves were still valid and were giving highly accurate results during the analyses period.

An organochlorine pesticide mixture standard (EPA Method 508-Chlorinated Pesticide Mix 1-Standard Solution) containing all of the analytes and internal standard was injected as 23 replicates for seven days. Table 3.3 shows the average found concentration of the analytes in this pesticide standard mixture, percent relative bias and also the percent relative standard deviation between these injection replicates.

Percent recovery values are higher than 70 % except p,p'-DDT and methoxychlor with 41.2 % and 37.5 % recovery. The reason for this difference may be because of the solvent exchange of the mixture before the analysis with the instrument. For endrin and gamma-HCH, the percent recoveries are the highest.

The percent relative standard deviations (% RSD) values are generally smaller than 10% if we do not consider DDT, DDE and methoxychlor. 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 Zhou et. al. (2006) and Hung et al. (2006). Environmental conditions, the concentrations of compounds, and experimental operation of individuals are the main factors influencing the % RSD values (Wang, et al. 2007).

The comparisons of the method performance values obtained in this study are present in section 3.2. In spite of the long period of time for sample preparation with a high number of replicates since there are sixteen sediment samples and ultrasonic bath extraction were applied for two hours for each sample, it can be stated that the precision of the analysis technique is good and has acceptable reproducibility.

Table 3.3 Method performance results for Chlorinated Pesticide Mix 1

Compound	**Certified Conc. (mg/L)**	**Found Conc. (mg/L)**	**Bias (%)**	**RSD (%)**	**% Recovery**
Alpha-HCH	1.00	1.17	16.6	5.22	117
Beta-HCH	1.00	0.850	-14.9	5.82	85.1
Delta-HCH	1.00	0.900	-9.82	7.22	90.2
Gamma-HCH	1.00	1.11	11.0	4.93	111
Heptachlor	1.00	0.790	-20.3	7.46	79.7
Aldrin	1.00	0.130	25.3	5.54	125
Dieldrin	1.00	0.120	23.9	5.78	124
Endrin	1.00	0.790	-20.1	5.16	79.9
Endrin Aldehyde	1.00	1.03	3.90	5.14	104
Heptachlor	1.00	1.10	10.2	4.84	110
Endosulfan	1.00	1.24	24.1	6.26	124
Endosulfan II	1.00	1.27	27.1	5.47	127
Endosulfan	1.00	0.710	-29.4	10.2	70.6
p.p'-DDD	1.00	1.24	24.3	6.88	124
p.p'-DDE	1.00	1.24	24.1	11.9	124
p.p'-DDT	1.00	0.410	-58.8	40.6	41.2
Methoxychlor	1.00	0.370	-62.5	32.1	37.5

Replicate measurements were also applied to check the repeatability of each measurement. There could be fluctuations in the measurements due to the reasons like the high temperature of the injection port evaporating the sample in the needle of the injector and the error associated with the injection of a small portion of the sample (2µL). As mentioned above, an organochlorine pesticide mixture standard (EPA Method 508-Chlorinated Pesticide Mix 1- Standard Solution) containing 1.00 µg/L of all of the analytes and internal standard was injected as 23 replicates for seven days (three injections for 6 days and five injections for the first day) to monitor the variations.

The standard deviations (in µg/L) of for each of the analyte during seven days are expressed as bar graphs in Figure 3.3. According to this figure, standard deviation of each analyte alters from day to day. Considering that all of the injections were done by the same operator applying the same conditions, with the same instrument, the standard deviations could be the reason of a systematic error caused by the instrument. If the first group of pesticides in Figure 3.3 are considered, standard deviations are below 0.10 µg/L for all analytes. In the first group, the values reach their maxima in 6th day except Heptachlor epoxide having its maximum at day 2. Endosulfan II has the highest standard deviation with 0.093 µg/L in this group, which is at day 6. The best repeatibilities were obtained for alpha-HCH in 2nd (0.005 µg/L) and 3rd day (0.005 µg/L), Heptachlor epoxide in 5th day (0.008 µg/L). In the second group of pesticides, Endosulfan has the highest standard deviation during the analyses of 7th day and 6th day follows it with the same value as Dieldrin. For the second group presented in Figure 3.3 injections of 4th and 6th days yielded high fluctuations in the concentrations. The poorest repeatability was obtained for Endosulfan in this group, and this value is the maximum value observed for the whole injection period. In the third group presented in Figure 3.2, best repeatabilities were obtained during the injections performed at day 2. The lowest value belongs to beta-HCH with 0.007 µg/L in 3rd day, 0.008 µg/L 2nd day and p, p'- DDD follows it with 0.008 µg/L standard deviation in 2nd day. The poorest repeatabilities obtained for the third group were for both p, p'- DDD and gamma-HCH with 0.086 µg/L standard deviation.

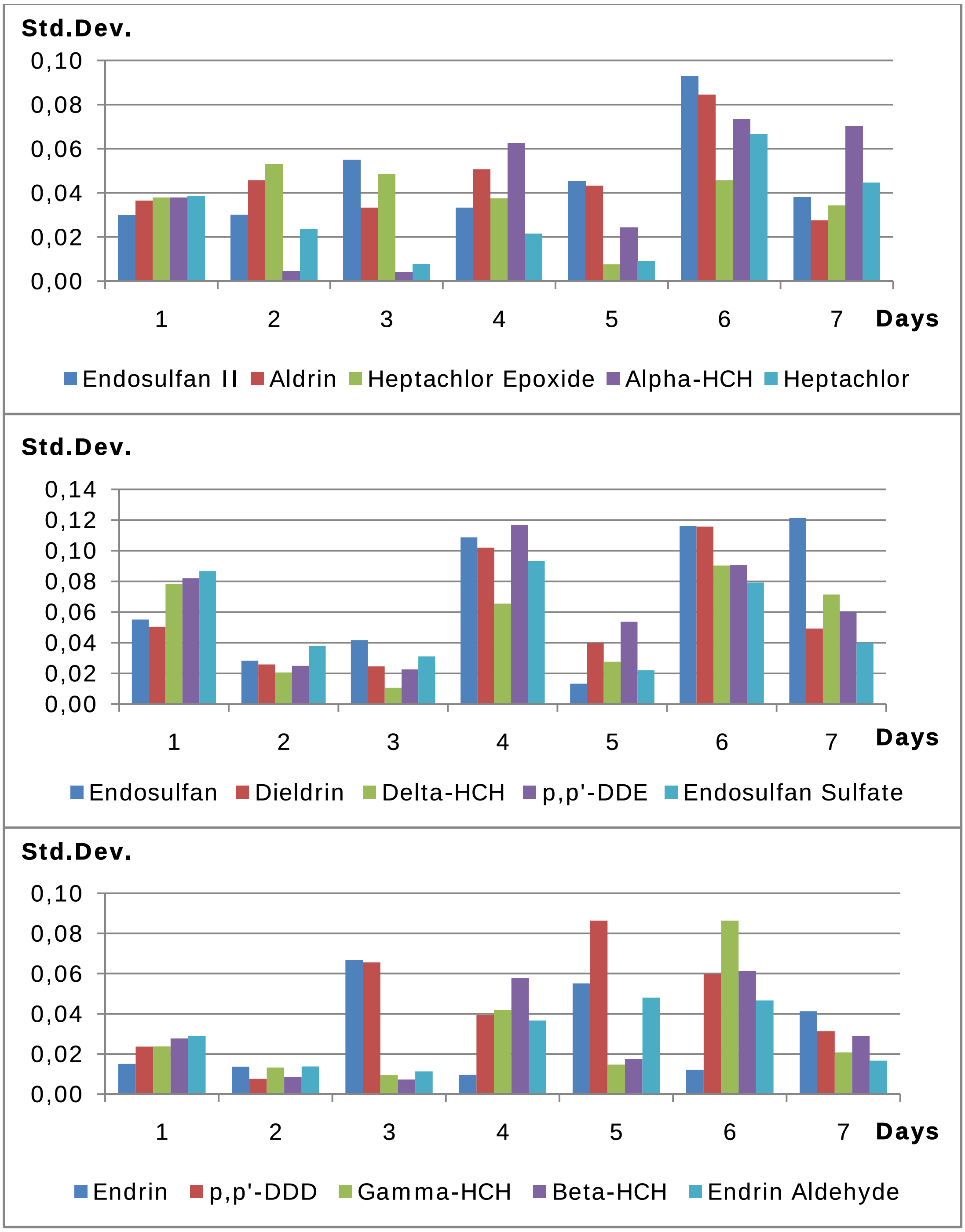


Figure 3.3 Demonstration of Repeatability of the Measurements with the Analysis of EPA Method 508-Chlorinated Pesticide Mix 1- Std. Solution, 1.00 mg/L (N=3)

In environmental analyses, the analysis system should be capable of detecting very low concentrations of the concerned analytes, as the pollutants if present are generally in very low concentrations. In order to check the capability of the analysis systems, limit of detection (LOD) and limit of quantification (LOQ) values were calculated with the instrument software. For an analytical procedure, the smallest quantity that can be detected with an acceptable certainty is called limit of detection. When we used the LOD values, it resulted in high errors in the measurements. Since the LOD value was not considered as acceptable for quantitative analysis, the LOQ value was used to obtain statistical separation of the blank measurement and true analyte signal distributions. Thus, the limit of quantification of a given procedure refers to the smallest concentration, which can be analyzed quantitatively with reasonable reliability (Mocak, et al. 1997).

The values shown in Table 3.6 and Table 3.7 are the LOD and LOQ values and calculated as the concentrations of the analytes at which the signal to noise (S/N) ratio is equal to 3 and 10, respectively. The lowest LOD value is for p,p'-DDD with 0.033µg/kg. Therefore, the GC-MS instrument used for this study can detect p, p'-DDD at lower concentrations than the other organochlorine pesticides with 0.033 µg/kg LOD value. The highest LOD value is for Endosulfan Sulfate with 0.463 µg/kg. In other words, detection of endosulfan sulfate cannot be performed below the concentration 0.463 µg/kg which is the LOD value of this target analyte. The LOQ value is the highest for Endosulfan Sulfate and lowest for p, p'-DDD. The found concentrations of Heptachlor epoxide are below limit of quantification value, 0.643 µg/kg, for all of the sediment samples. For the polycyclic aromatic hydrocarbons, pyrene has the lowest detection limit with 0.0120 µg/kg and Dibenz (a.h) anthracene has the highest detection limit with 0.390 µg/kg. The comparison of these LOD values of this study with the other studies in the literature is present in section 3.6.

Table 3.4 Limit of Detection (LOD) and Limit of Quantification (LOQ) values of OCPs for GC-MS, Values in (µg/kg)

OCPs	LOD	LOQ	OCPs	LOD	LOQ
Alpha-HCH	0.220	0.733	**Endrin**	0.221	0.736
Gamma-HCH	0.392	1.31	**Endosulfan II**	0.409	1.36
Delta-HCH	0.204	0.681	**p,p’-DDD**	0.0330	0.110
Beta-HCH	0.280	0.933	**Endrin Aldehyde**	0.275	0.916
Heptachlor	0.160	0.532	**Endosulfan Sulfate**	0.463	1.54
Aldrin	0.256	0.852	**p,p’-DDT**	0.325	1.084
Heptachlor Epoxide	0.193	0.643	**Methoxychlor**	0.147	0.490
Endosulfan I	0.284	0.947	**Decachlorobiphenyl**	0.192	0.639
Dieldrin	0.424	1.41	**2,4,5,6Tetrachloro-m-xylene**	0.426	1.421
p,p’-DDE	0.065	0.218			

Table 3.5 Limit of Detection (LOD) and Limit of Quantification (LOQ) values of PAHs for GC-MS, Values in (µg/kg)

PAHs	LOD	LOQ	PAHs	LOD	LOQ
Naphthalene	0.098	0.327	**Chrysene**	0.215	0.717
Acenaphtylene	0.056	0.187	**Benzo(b)fluoranthene**	0.124	0.413
Acenaphtene	0.025	0.083	**Benzo (k) fluoranthene**	0.072	0.240
Fluorene	0.037	0.123	**Benzo (a) pyrene**	0.074	0.247
Phenanthrene	0.039	0.130	**Benzo (e) pyrene**	0.07	0.233
Anthracene	0.025	0.083	**Indeno(l.2.3-c.d) pyrene**	0.193	0.643
Fluoranthene	0.024	0.080	**Dibenz (a.h) anthracene**	0.39	1.300
Pyrene	0.012	0.040	**Benzo (g.h.i) perylene**	0.164	0.547
Cyclopenta(c.d)pyrene	0.112	0.373	**Anthanthrene**	0.238	0.793
Benzo(a)anthracene	0.049	0.163			

3.2 Comparison of Performance Characteristics of the Method with the Literature for Organochlorine Pesticides

It is essential to compare the obtained data with related studies present at different sampling regions in order to evaluate the level of the pollution in concern. The values compared were obtained by injecting EPA Method 508-Chlorinated Pesticide Mix 1 for 23 injections in 7 days.

The use of chlorinated pesticides is banned in most of the countries as mentioned in Chapter I, but they are still being used in agriculture in developing countries. The studies related to the OCP pollution in the environment have been continued especially in the far East region specifically in China (Xue, Zhang ve Xu 2006); (Zhou, et al. 2006), (Carvalho, et al. 2008) Tan et. al., 2009, (Hu, et al. 2009)), in Taiwan (Doong R. 2008); (Hung, et al. 2007)) and in Korea (Lee, Tanabe ve Koh 2001), (Hong, et al. 2003), (Kim, et al. 2008)). The data for China, Taiwan and Portugese are used for comparison with ours in terms of percent recoveries, limit of detections and percent relative standard deviations. Moreover, information about the extraction and analysis methods of compared data are also provided. The studies used for comparison are expressed as Method 1 (Zhou, et al. 2006), Method 2 (Doong R. 2008), Method 3 (Doong, et al. 2002), Method 4 (Hung, et al. 2007), Method 5 (Carvalho, et al. 2008). Table 3.8 shows the analytical methodologies used for these methods.

Table 3.6 Some information about the methods used for comparison

Method	Extraction Method	Analysis Method	Author	Year
Method 1	Ultrasonic bath extraction	GC-ECD	Zhou et. al.	2006
Method 2	Soxhlet extraction	GC-ECD	Doong et. al.	2008
Method 3	Soxhlet extraction	GC-ECD	Doong et. al.	2002
Method 4	Accelarated Solvent Extraction	GC-ECD	Hung et. al.	2006
Method 5	Head Space Solid Phase Micro Extraction	GC-MS	Carvalho et. al.	2008

Percent recovery values of this study and four different studies are shown in Table 3.7. As can be seen from the table, the values are variable from one target analyte to another. The recoveries for this study and Methods 1, 2, 3, 4 are generally close to each other and recoveries for our research exist in the values between the other five methods. For Method 2, since Soxhlet extraction and GC-ECD was used during the study the recovery values are relatively high compared to the other methods. In terms of the extraction procedure used for the sediment samples, the most similar study with our research is Method 1. However, since the analysis was performed by GC-ECD instrument the recovery values are a bit higher than that of ours. For Method 3, the extraction technique is solid phase extraction and the analysis technique is performed by GC-ECD. If we compare the ranges of the percent recovery values of Method 3 (70-116) and our research (73-129) by excluding the data of p,p’-DDT and methoxychlor, they are very similar to each other. It can be concluded that, ECD detector is more sensitive than MS detector by interpreting these values. Accelerated

Solvent Extraction (ACE) and GC-ECD were the techniques used for Method 4 and recovery values are very close to the recoveries of Method 3.

Table 3.7 Comparison of percent recovery values of this study with five different studies

Percent Recovery Values					
Organochlorine Pesticides	**Method 1**	**Method 2**	**Method 3**	**Method 4**	**This study**
alpha-HCH	90	99	85	88	83
beta-HCH	88	106	104	111	115
gamma-HCH	85	99	8 3	90	89
delta-HCH	83	90	93	88	109
Heptachlor	90	101	85	110	75
Aldrin	88	92	116	88	120
Heptachlor epoxide	89	95	89	97	90
Endosulfan	-	98	92	-	76
Dieldrin	92	99	98	91	76
p,p'-DDE	106	86	85	87	129
Endrin	90	82	70	92	120
Endosulfan II	-	91	96	93	73
p,p'-DDD	90	109	103	105	76
Endosulfan sulfate	-	92	-	-	76
p,p’-DDT	85	120	96	87	159
Methoxychlor	-	120	124	-	163

Method 1: Zhou et. al., 2006, Method 2: Doong et. al., 2008, Method 3: Doong et. al., 2002, Method 4: Hung. et al., 2006, Method 5: Carvalho et. al., 2008.

It is known in literature that GC-MS is the most powerful analysis technique for organic pollutants, however, detection limits of the ECD detector is known to be lower than that of MS detector in organochlorine pesticide analyses therefore ECD is more sensitive in pesticide analyses. On the other hand, the selectivity of GC-MS is generally better than GC-ECD system for these pollutants. Detection limits of this study and four different studies are shown in Table 3.8. Limit of detection values of this study are generally better than the limits of Method 1, which uses the same extraction and analysis technique as ours. Method 4 has the lowest detection limits with ACE and GC-ECD. Method 5 has also very low detection limits because of HSSPME and GC-MS.

Table 3.8 Comparison of limit of detection (LOD) values of this study with four different studies

Values in μg/kg

LOD					
Organochlorine Pesticides	**Method 1**	**Method 3**	**Method 4**	**Method 5**	**This study**
alpha-HCH	0.10	0.12	0.12	0.04	0.22
beta-HCH	0.40	0.35	0.04	-	0.93
gamma-HCH	0.18	0.22	0.09	0.11	0.39
delta-HCH	0.24	0.09	0.04	-	0.20
Heptachlor	0.30	0.15	0.03	0.11	0.16
Aldrin	0.36	0.05	0.02	0.05	0.26
Heptachlor epoxide	0.20	0.15	0.02	0.01	0.19
Endosulfan	-	0.08	-	-	0.28
Dieldrin	0.15	0.12	0.02	0.04	0.42
p.p'-DDE	0.12	0.12	0.02	0.005	0.07
Endrin	0.16	0.22	0.04	0.04	0.22
Endosulfan II	-	0.16	0.04	-	0.41
p.p'-DDD	0.16	0.18	0.03	0.04	0.03
Endosulfan sulfate	-	-	-	-	0.46
p.p'-DDT	0.46	0.18	0.02	0.04	0.33
Methoxychlor	-	0.30	-	-	0.15

Method 1: Zhou et. al., 2006, Method 2: Doong et. al., 2008, Method 3: Doong et. al., 2002, Method 4: Hung. et al., 2006, Method 5: Carvalho et. al., 2008.

The relative standard deviation (RSD) is an indication of precision and is calculated to compare the uncertainty between different measurements. Percent relative standard deviations of this study and three different studies are shown in Table 3.9. The percent RSD data of this study are generally close to the other studies except data for p.p'-DDT, and methoxychlor. Method 4, which is performed by ACE and GC-ECD, has the lowest %RSD values for almost all analytes. If we consider the data of p.p'-DDT, and methoxychlor as outliers, the precision of our GC-MS instrument is relatively better than Method 5 which includes GC-MS system.

Table 3.9 Comparison of percent relative standard deviation values of this study with three different studies

RSD (%)				
Organochlorine Pesticides	**Method 1**	**Method 4**	**Method 5**	**This study**
alpha-HCH	7	1.5	9	5.2
beta-HCH	5	5.4	-	5.8
gamma-HCH	8	2.7	7	4.9
delta-HCH	6	1.1	-	7.2
Heptachlor	4	20.0	17	7.5
Aldrin	9	3.7	9	5.5
Heptachlor	10	1.2	12	4.8
Endosulfan	-	-	-	6.3
Dieldrin	7	2.7	14	5.8
p.p'-DDE	8	3.4	8	11.9
Endrin	9	1.3	16	5.2
Endosulfan II	-	1.2	-	5.5
p.p'-DDD	6	11.6	17	6.9
Endosulfan	-	-	-	10.2
p.p'-DDT	11	11.6	14	40.6
Methoxychlor	-	-	-	32.1

Method 1: Zhou et. al., 2006, Method 2: Doong et. al., 2008, Method 3: Doong et. al., 2002, Method 4: Hung. et al., 2006, Method 5: Carvalho et. al., 2008.

3.3 Evaluation of the Data Set

In this part of the discussion, the produced data for the organochlorine pesticides (OCPs) and polycyclic aromatic hydrocarbons (PAHs) in sediment samples obtained from Balıkesir-İkizcetepeler Dam Lake will be presented and discussed in terms of environmental impact using statistical calculations.

3.3.1 Concentrations of Organochlorine Pesticides and Polycyclic Aromatic Hydrocarbons in İkizcetepeler Dam Lake sediment samples

The data set obtained in whole study period for Organochlorine Pesticides (OCPs) in 16 sediment samples are displayed in Table 3.10. The values presented are for the concentrations above the Limit of Quantification, which is determined as the concentration at S/N value of 10. There are sixteen samples concerning both OCP and PAH contamination. As mentioned before in the experimental section; during the extraction period, 100 micro liters of each two surrogate standards (1000ppb) were added to the sample and 100 µL (500 ppb) of internal standard was added before the preconcentration of the extract to 1.0 mL. Injection of each sixteen sample solution in determination of OCPs was performed as three replicates in order to evaluate the precision of the data set.

Table 3.10 Summary Statistics for Organocholorine Pesticides

Values in µg/kg

Pesticide	Average %RSD min-max	Pesticide	Average %RSD min-max
alpha-HCH	3.62 117 0.680-11.4	Heptachlor epoxide	BLOQ
beta-HCH	379 108 9.42-1630	Endosulfan	16.3 177 1.22-91.5
gamma-HCH	45.2 142 1.82-158	Endosulfan II	25.8 58.8 11.1-50.8
delta-HCH	122 112 12.3-158	Endosulfan Sulfate	227 47.5 74.6-422
Aldrin	90.8 127 0.710-283	p.p'-DDD	3.33 102 0.070-9.18
Dieldrin	87.2 172 0.330-509	p.p'-DDE	7.14 133 0.110-22.8
Endrin	84.6 150 1.00-306	p.p'- DDT	91.3 115 20.0-346
Endrin Aldehyde	68.8 88.7 29.6-157	Methoxychlor	12.4 185 0.160-78.2
Heptachlor	66.6 252 0.300-606		

BLOQ:Below the Limit of Quantification

It can be interpreted from Table 3.10 that beta-HCH has the greatest average concentration in sediment samples, Endosulfan Sulfate follows it, and heptachlor epoxide concentration was observed as below limit of quantification. Percent relative standard deviations, which show fluctuation from one sampling point to another, are quite high as expected as the sampling points are affected differently from contributing sources. Sampling points 13 and 6 have OCP concentration of 2388.6 µg/kg and 236.8 µg/kg, respectively. If we look at the average concentration of each measured pesticide, beta-HCH and Endosulfan Sulfate show highest concentration. These data are shown as bar graph in Figure 3.4.

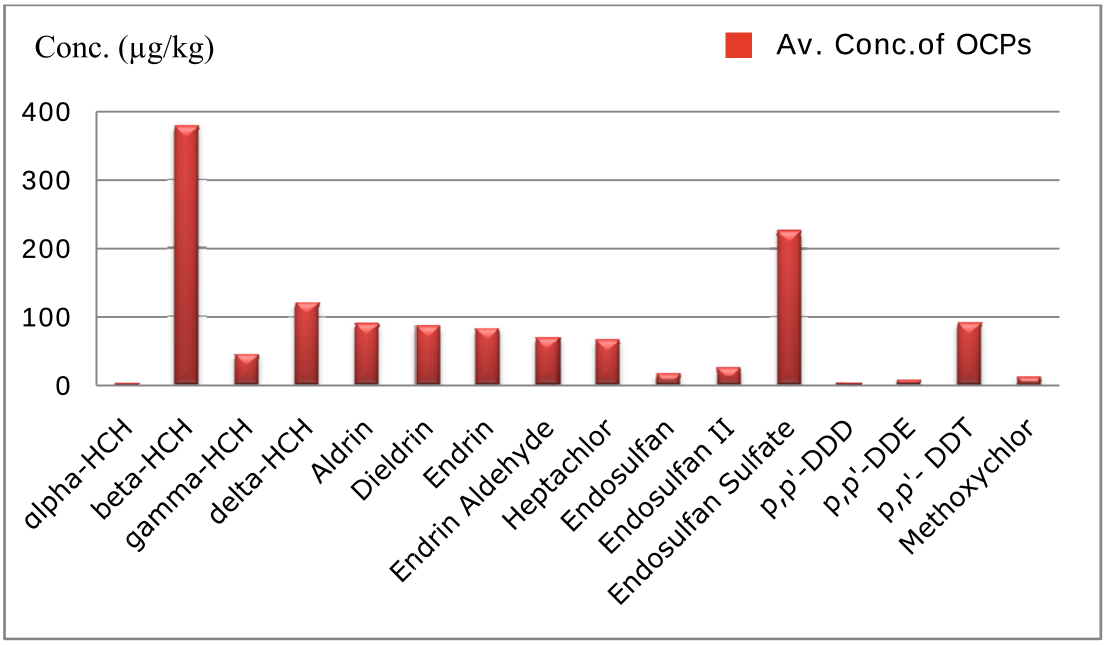


Figure 3.4 Average Concentrations of Organochlorine Pesticides in İkizcetepeler Dam Lake Sediments

When we group the pollutants like in Table 3.11, it can be seen that Cyclodienes subgroup (Aldrin, Dieldrin, Endrin, Endrin Aldehyde, Heptachlor) are considered, they have almost the same average concentration. Endosulfan Sulfate has the highest average concentration among the Endosulfans subgroup (Endosulfan, Endosulfan II, Endosulfan Sulfate). Lastly, p,p'-DDT is the prominent OCP in DDTs subgroup (p,p'-DDD, p,p'-DDE, p,p'-DDT, Methoxychlor).

Table 3.11 Distribution of the organochlorine pesticide pollution of the sampling points according to the subgroups
Concentrations in µg/kg

OCP classes	**OCPs in the groups**	**Average Conc±stdev**
HCHs	alpha-HCH, beta-HCH, delta-HCH, gamma-HCH	476± 490
Cyclodienes	Heptachlor, Heptachlor epoxide, Aldrin, Dieldrin,Endrin, Endrin Aldehyde	248± 360
Endosulfans	Endosulfan I, Endosulfan II, Endosulfan sulfate	230±135
DDTs	p,p'-DDD, p,p'-DDE, p,p'-DDT, Methoxychlor	105±116

If the organochlorine pesticides in concern are grouped according to their pesticide classes, then the results could be interpreted straightforwardly. Table 3.11 was prepared in order to enable the interpretation of the concentrations for OCPs according to their subgroups. If the average concentrations of the OCPs in these subgroups are taken into account, then it can be observed that the dam lake is polluted with HCHs mostly. DDT and its derivatives were banned earlier, and this impact is seen on the average total concentration of OCPs in DDTs subgroup as 105 µg/kg.

It is displayed in Table 3.11 that the deviations of the concentrations between the samples obtained from different sampling points are high. The percent relative standard deviation (%RSD) is generally high in environmental studies since the pollution level may not be the same in different locations due to different sources effective near the environment of the study area.

The concentrations found in this study are compared with two different studies, which are performed in Turkey. Table 3.12 shows the information about these studies.

Table 3.12 The information about the studies about organochlorine pesticides which are performed in Turkey

Study	**Extraction Method**	**Analysis Method**	**Sampling Region**	**Author**	**Year**
Study 1	Soxhlet Extraction	GC-ECD	Black Sea	(Bakan and Ariman 2004)	2004
Study 2	Soxhlet Extraction	GC-ECD	Meriç Delta	(Erkmen and Kolankaya 2006)	2006
Study 3	Soxhlet Extraction	GC-ECD	Lake Uluabat	(Barlas, Çok and Akbulut 2006) .	2008

The concentration levels obtained by these three studies are designated in µg/kg in Table 3.13. This table shows that the pollution levels in Black Sea and Meriç Delta are generally lower than Uluabat Lake and İkizcetepeler Dam Lake. It is seen in Table 3.13 that study 1 has generally lower analyte concentrations in the sediment samples from Black Sea than this study except for alpha-HCH, Heptachlor epoxide and p.p'-DDD. Study 2, which was about the sediment samples of Meriç Delta, includes the lowest concentrations for all analytes, however , Heptachlor epoxide was below limit of quantification in our study. Study 3 has the closest values to our study in OCP pollution. Except for beta-HCH and Aldrin the results of the study of Uluabat Lake sediment have higher OCP concentrations. In Uluabat Lake Heptachlor epoxide was found as 697.53 µg/kg whereas in İkizcetepeler Dam Lake the concentration of this analyte was below limit of quantification.

Table 3.13 The comparison of concentration levels of organochlorine pesticides found in the studies performed in Turkey

Organochlorine Pesticide	**Study 1**	**Study 2**	**Study 3**	**This Study**
alpha-HCH	5	0.46	466.2	3.62
beta-HCH	-	0.85	309.7	379
gamma-HCH	18.5	0.32	127.5	45.2
delta-HCH	13.83	0.91	-	122
Heptachlor	-	0.34	-	66.6
Heptachlor epoxide	12	0.65	697.53	nd
Aldrin	61	0.69	73.87	90.8
Dieldrin	21.86	0.23	-	87.2
Endrin	-	0.34	199.65	84.6
Endrin Aldehyde	-	nd	-	68.8
Endosulfan	-	0.59	nd	16.3
Endosulfan II	-	0.80	57.88	25.8
Endosulfan Sulfate	-	0.38	-	227
p.p'-DDD	47.5	0.88	-	3.33
p.p'-DDE	7	0.72	448.87	7.14
p.p'- DDT	24.5	0.70	331.27	91.3
Methoxychlor	-	nd	-	12.4

The second pollutant in concern is distributed among the sixteen sampling points as shown in Table 3.14. Sixteen sediment samples taken from these sixteen sampling points were extracted after the addition of 1.0 mL of 1.0 µg/L of surrogate mix, as explained in experimental section. The results were obtained after injection of the 2 µL of the aliquot of the sample extract. Injection of each sixteen sample solution in determination of PAHs was performed as three replicates in order to evaluate the precision of the data set. The values shown in Table 3.14 are for the PAH concentrations which are higher than the Limit of Quantification (LOQ) and LOQ value is determined as the concentration at S/N values of 10. The analytes are sorted according to their number of rings in the chemical structure. According to the Table 3.14 the highest average concentration belongs to the Benzo(e)pyrene pollution (23.5 μg/kg) and also it has the highest observed maximum concentration (122.6 μg/kg). Dibenzo(ah)anthracene has the smallest average concentration (3.28 μg/kg) and Dibenzo(ah)anthracene, Benzo(k)fluoranthene, Benzo(e)pyrene have the smallest observed minimum concentration (2.92 μg/kg). As in the case of OCP distribution in the lake, the PAH distribution leads to high %RSDs. The reason for this irregular distribution is that the sampling points near the highway have higher traffic-caused PAH contamination. In the table, the concentration of Acenaphtylene is not displayed since it is below the limit of quantification.

The sum of concentrations of the PAHs with more than three rings is greater than the sum of 2-3 ring PAH in all sampling points.

Table 3.14 Concentrations of PAHs in sediment samples of six sampling points
Concentrations in µg/kg

PAHs	**# of Rings**	**Average** **%RSD** **Min-max**	**PAHs**	**#of Rings**	**Average** **%RSD** **Min-max**
Naphthalene	2	20.2 61.1 6.09-42.6	**Cyclopenta(cd)pyrene**	5	4.21 54.1 2.93-11.7
Acenaphtylene	3	BLOQ	**Benzo(b)fluoranthene**	5	9.67 28.6 5.84-14.6
Acenaphtene	3	4.18 87.8 6.09-12.2	**Benzo(k)fluoranthene**	5	9.12 34.8 2.92-14.6
Fluorene	3	4.95 102.7 6.09-12.2	**Benzo(a)pyrene**	5	6.39 33.33 5.84-11.7
Phenanthrene	3	18.2 71.3 8.29-49.7	**Benzo(e)pyrene**	5	23.5 133.6 2.92-122.6
Anthracene	3	17.4 73.6 8.29-49.7	**Dibenzo(ah)anthracene**	5	3.28 63.9 2.92-5.84
Fluoranthene	4	10.8 27.5 5.86-17.6	**Indeno(1.2.3-cd)pyrene**	6	16.1 60.8 8.76-20.43
Pyrene	4	7.88 42.4 2.93-14.6	**Benzo(ghi)perylene**	6	19.2 40.1 8.76-38.0
Benzo(a)anthracene	4	5.31 54.1 2.93-11.7	**Anthanthrene**	6	20.8 71.9 5.84-58.4
Chrysene	4	10.1 31.8 5.86-14.7			

BLOQ:Below Limit of Quantification

Figure 3.5 shows the average concentrations of PAHs in the sediments of sampling region.

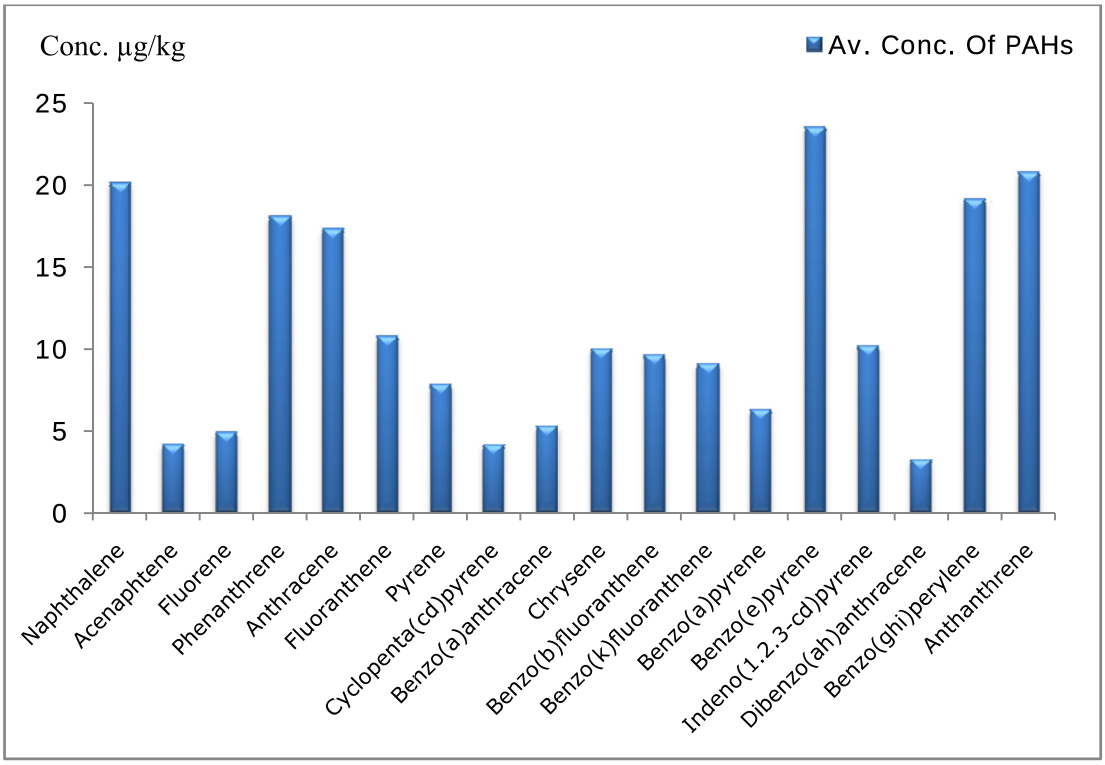


Figure 3.5 Average Concentrations of Polycyclic Aromatic Hydrocarbons in İkizcetepeler Dam Lake Sediments

Benzo(e)pyrene has the highest concentration in the sediment samples and Anthanthrene follows it. Dibenzo(ah)anthracene has the least concentration in the sediment samples.

3.3.2 Pollution Distributions

In order to evaluate the pesticide pollution in İkizcetepeler region, the variation of the target analytes will be presented. For this purpose, the pollution 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. The pollution maps were also drawn by using MapInfo Professional 7.5 SPC program, with Vertical Mapper VM 1.51 utilities.

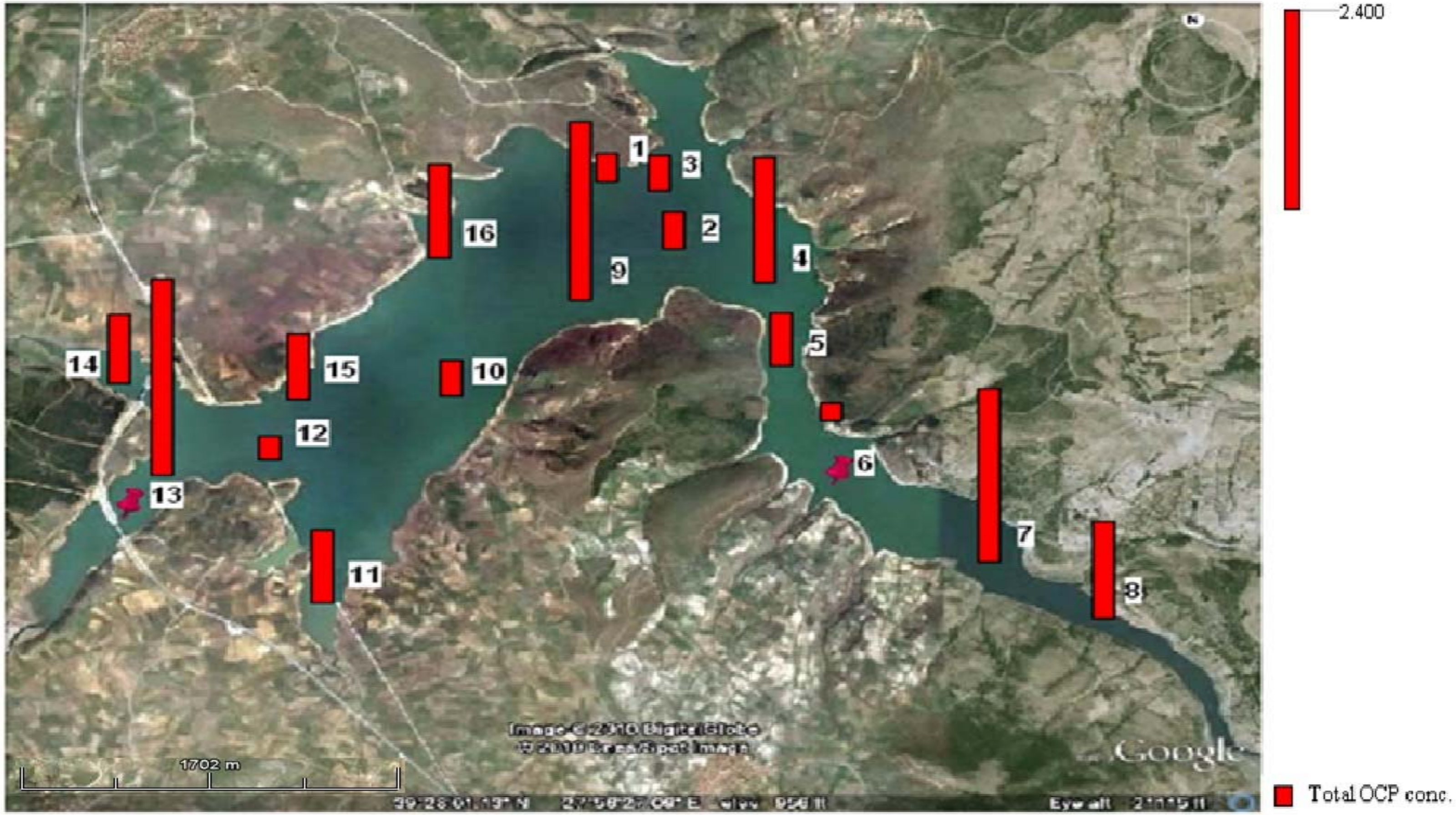


Figure 3.6 Distribution of total OCPs in the sampling region

Figure 3.6 shows the distribution of total OCP concentration for each of the sixteen sampling points. According to this figure, sampling point 13 has the highest total concentration and the dominant pollutant beta-HCH has the greatest share with concentration 1630 μg/kg. Dieldrin is the prominent pollutant in sampling point 9, which is the second most polluted point in the lake.

Dieldrin belongs to the cyclodienes subgroup and has a hexachlorocyclopentadiene group, which is the primary chemical moiety that shows toxicidal activities (Kaushik P. 2007). Sampling point 7 follows 9 with beta-HCH as the dominant pesticide. In environmental samples Hexachlorocyclohexanes are one of the most widely used and detected organochlorine pesticides. In this group, gamma-HCH is the most toxic isomer to insects, which is 500–1000 times active compared to the δ-isomer, whereas the β isomer is non-toxic (Kaushik P. 2007).

In this study, heptachlor epoxide concentration was found as below limit of quantification (BLOQ) therefore a pollution map regarding this persistent organic pollutant was not drawn.

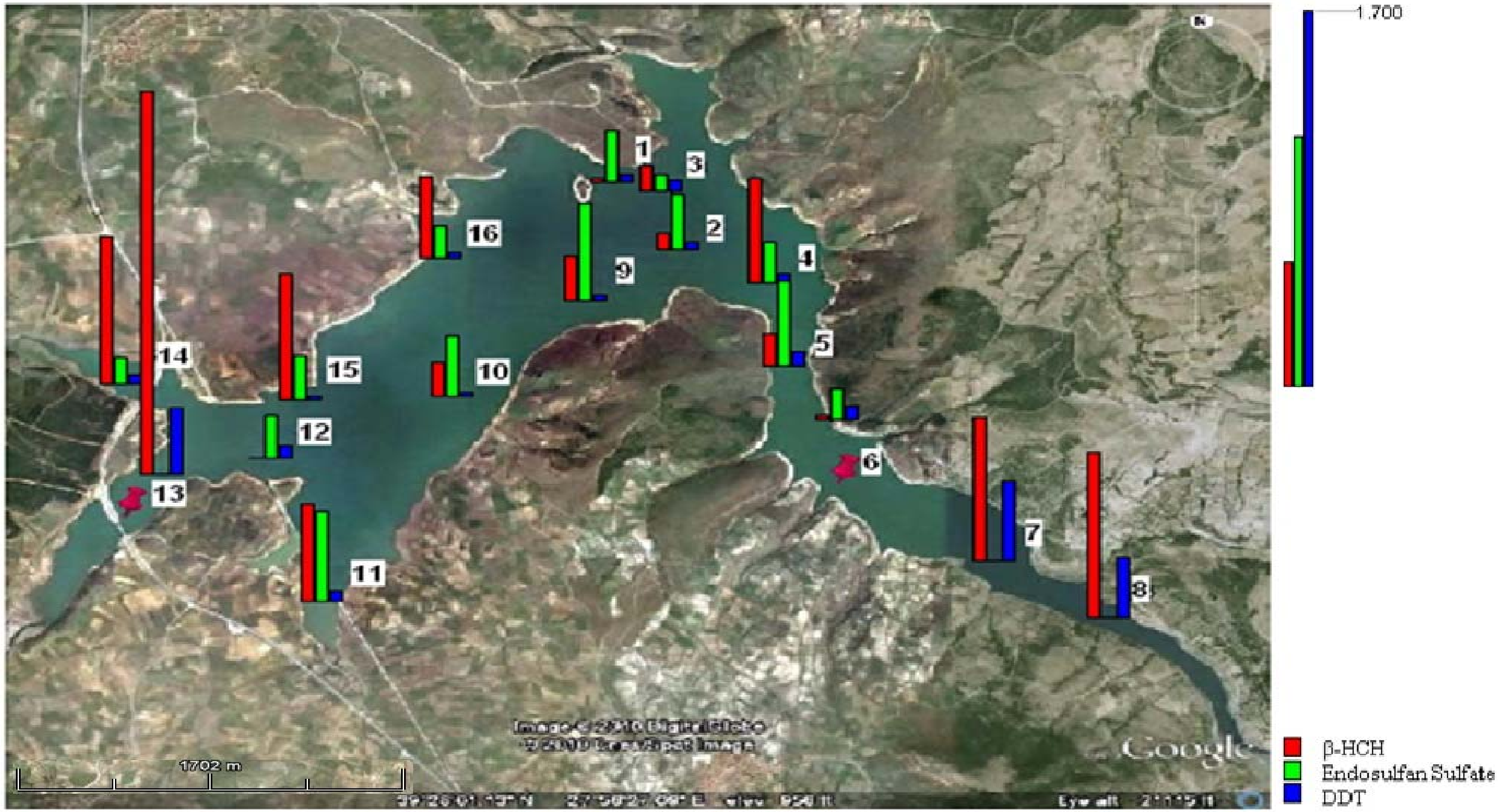


Figure 3.7 Distribution of selected OCPs in the sampling region

Figure 3.7 shows the distribution of three selected pesticides (β-hexachlorocyclohexane, Endosulfan Sulfate and p,p'- Dichloro Diphenyl Trichloroethane) having the highest concentration in the sediments. According to this figure, beta-HCH and p, p' DDT exist in all of the samples.

Beta-Hexachlorocyclohexane pollution reaches to maximum at 13th sampling point. In this subgroup, gamma-HCH is the most toxic isomer to insects, which is 500–1000 times active compared to the δ-isomer, whereas the β isomer is non-toxic. The exact configuration and the stereochemistry exhibited by γ isomer fit perfectly at the target site of the pores of lipoprotein structure of nerve of insect. Other HCH isomers do not show toxicidal properties against pests because they do not have such configuration as gamma-HCH has (Kaushik P. 2007).

Sampling point 7 has the highest average concentration of p,p' DDT and 8th sampling point, which is next to 7th sampling point, has the second highest average concentration of DDT. The oral Lethal Dose, 50% (LD_{50}) of Dichloro Diphenyl Trichloroethane (DDT) to rats is 300 mg/kg (Gaines, 1969). The other DDT isomers have higher LD_{50} values than DDT isomer indicating DDT is the most toxic in its subgroup and was found in all of the sixteen sediment samples. The most toxic one of all DDT has the highest average concentration. The reasons for this are the half-life of DDT is 2-15 years, the most widely used pesticide in Turkey, and it degrades to its degradation products such as DDD and DDE. DDD and DDE show very similar distributions around the sampling area. Under aerobic conditions DDE, under anaerobic conditions DDD is the degradation product (Doong, et al. 2002); (Zhou, et al. 2006). Since the average concentration of DDE is higher than DDD concentration, it can be concluded that the degradation occurred aerobically mostly.

Endosulfan Sulfate exists mostly in the sampling points that are at the south side of the lake on the other hand west sides have no pollution due to this pesticide. Endosulfan sulfate has an oral LD_{50} value of 18 mg/kg in rats which is the smallest value in Endosulfans subgroup. Since as the LD_{50} value decreases toxicity increases, endosulfan sulfate is the most toxic in this subgroup. The dominant pollutant in endosulfans subgroup is endosulfan sulfate in addition it distributed all around the sampling region.

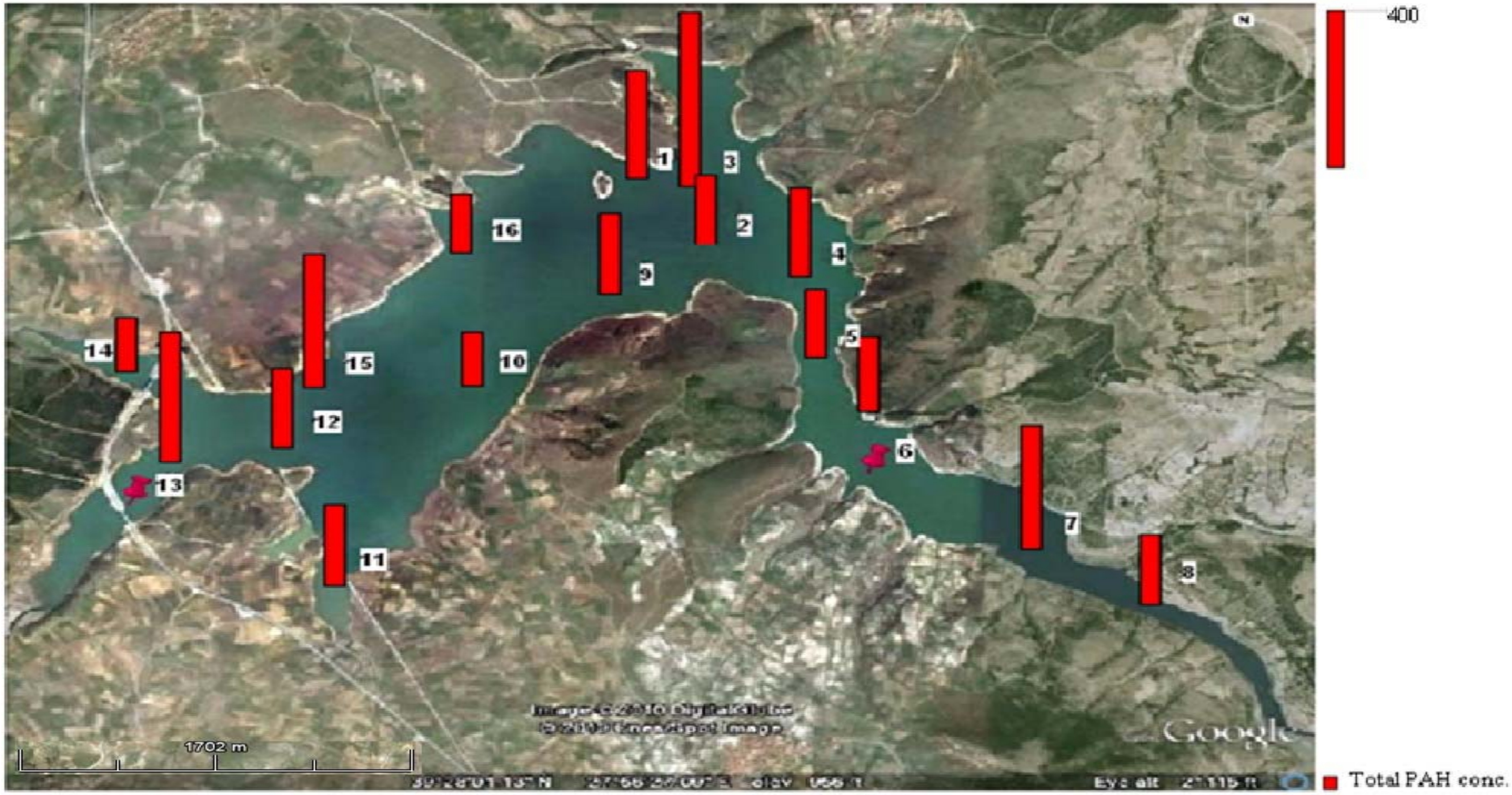


Figure 3.8 Distribution of total PAHs in the sampling region

In Figure 3.8, distributions of total concentrations of PAHs in the sixteen sediment samples are shown. Sampling point 3 has the highest PAH concentration with 391.4 μg/kg. In this sampling point Phenanthrene and Anthracene are the dominant PAHs with concentration 49.7 μg/kg. These PAHs are 3 rings PAHs, abundant in petrogenic sources mainly in petrol spills. Sampling points 7, 13, 15 have the second mostly polluted sediments with very close concentrations. Generally, higher molecular weight PAHs were concentrated around the highway region indicating the source for this pollution is traffic.

If Figure 3.6 and Figure 3.8 are compared, it will be observed that sampling points 4, 7, 8, 11, 13, 14 have very similar distributions for both OCP and PAH compounds. However, in the north of the lake the average concentrations are very different for Figure 3.6 and Figure 3.8.

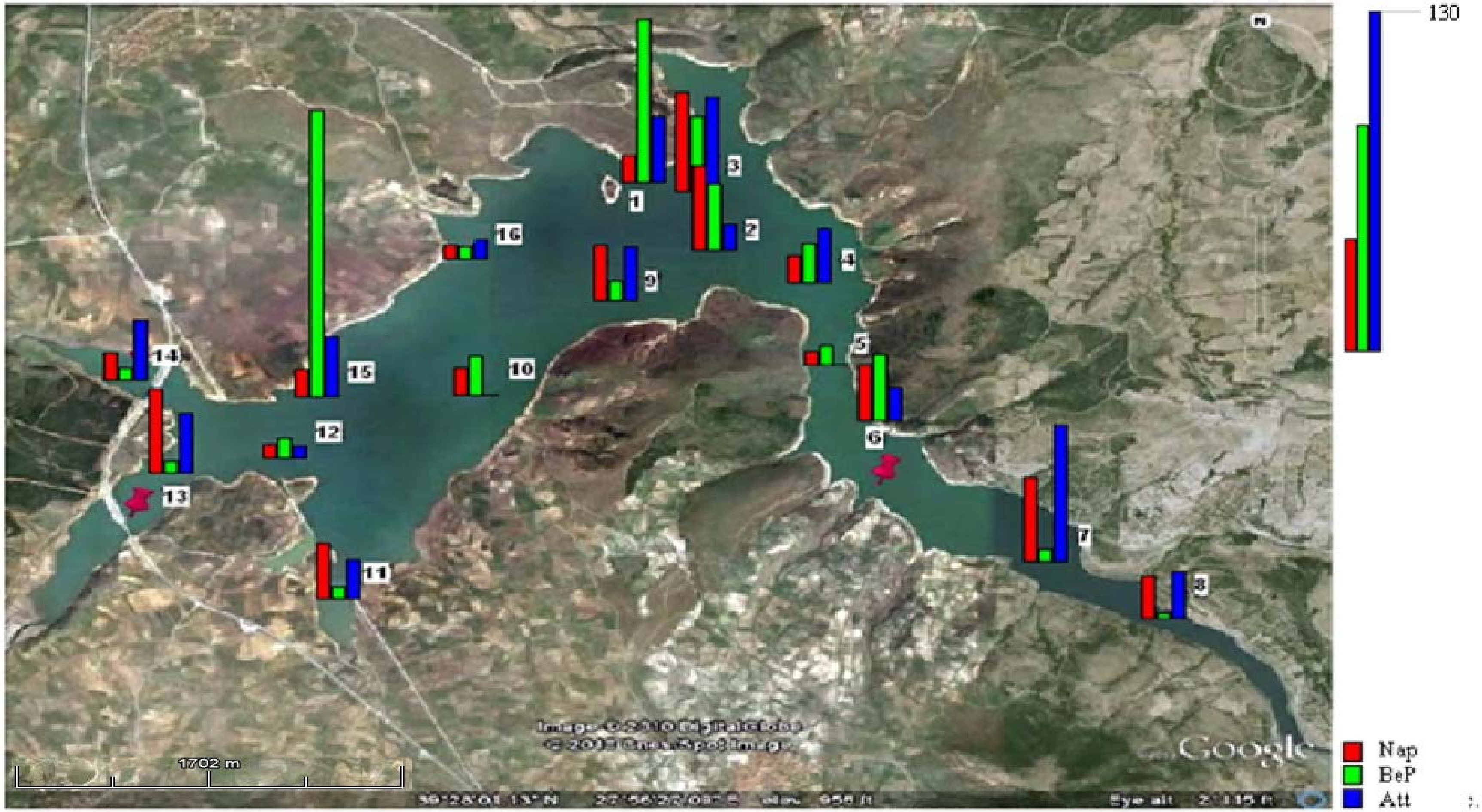


Figure 3.9 Distribution of selected PAHs in the sampling region

Figure 3.9 shows the distribution of three selected PAHs having the highest concentrations in sampling region. As seen in this figure, Benzo(e)pyrene (BeP) exists in sixteen samples and also having a maximum value in sampling point 15. Naphthalene (Nap) is found in all of the samples and highest at point 3.

Benzo(e)pyrene (BeP) is a 3-ring PAH and fossil fuel, petroleum or coal are the sources of this petrogenic PAH. BeP distributed mostly in the north side of the lake. This may be the result of a residential area constructed at this side.

Anthanthrene is a 6-ring, pyrogenic PAH resulting from forest and grass fires and fossil fuel combustion. Sampling points near the highway have higher Anthanthrene pollution proving that highway is a source for this kind of pollution.

The pollution maps of the sampling region regarding the individual pesticide are given in the Appendix part.

CHAPTER 4

CONCLUSION

This study is about the method validation (extraction and analyses) and environmental impact evaluation of pesticides and PAHs in sediment samples of a dam lake in Balıkesir, called İkizcetepeler, for the first time. Sediment samples in Balıkesir Dam Lake, used for irrigation, drinking water, and flood prevention purposes, were analyzed for 17 organochlorine pesticides (OCP) and 19 polycyclic aromatic hydrocarbons (PAH). The sediment samples were collected from the lake in 15-16 September, 2009 by using van Veen Grab sampler. The pesticides in sediment samples were extracted with Ultrasonic Bath Extraction (UBE) technique. The analyses were performed by GC-MS systems for chlorinated pesticides and polycyclic aromatic hydrocarbons. The quality control (QC) and quality assurance (QA) tests were applied during sample preparation and analysis. The laboratory and field environment did not contribute to observed pesticide concentrations according to the results of QC/QA tests.

The surrogate recoveries were in between 70-130 % showed that each extraction was performed successfully for OCPs. These results indicate that the UBE technique was appropriate for the extraction of chlorinated pesticides in sediment samples.
The accuracy of the measurements and the stability of the analysis systems were checked with the standard reference materials (SRMs). The percent errors for the SRMs were below 17.5 %, indicating high accuracy for almost all of the target analytes. Furthermore, since the results of the SRM analysis were in the limit of $+ 2\sigma$ (standard deviation) from the averages, it is shown that the analysis systems were accepted as stable during the analysis period.

The LOD and LOQ values were calculated as the concentrations of the analytes at which the signal to noise (S/N) ratio is equal to 3 and 10, respectively. The lowest LOD value in pesticides is for p,p'-DDD with 0.033µg/kg. The highest LOD value is for Endosulfan Sulfate with 0.463 µg/kg. The LOQ value is the highest for Endosulfan Sulfate and lowest for p, p'-DDD. The found concentrations of Heptachlor epoxide are below limit of quantification value, 0.643 µg/kg, for all of the sediment samples. For the polycyclic aromatic hydrocarbons, pyrene has the lowest detection limit with 0.0120 µg/kg and Dibenz (a.h) anthracene (DaA) has the highest detection limit with 0.390 µg/kg.

The highest average concentration among OCPs was 379 µg/kg and observed for beta-HCH in sediment samples. The highest average concentrations were also observed for these pesticides; for endosulfan (227 µg/kg) and for gamma-HCH (122 µg/kg). The pollution of organochlorine pesticides in the lake was studied by grouping these chemicals in terms of their chemical structures. The HCH subgroup was found to be the dominant one whereas DDT subgroup showed the lowest average concentrations. Although, use of gamma-HCH and DDT had been banned since 1985 in Turkey (Güler, 2010), this study indicates their presence in a drinking water source.

With respect to PAH concentration Benzo(e)pyrene had the highest concentration (23.5 µg/kg) which is high for drinking water source. We attribute the above observation to the influence of the heavy traffic on Balıkesir-İzmir-Bursa highway which passes nearby to dam lake.

The pollution maps were drawn and used to identify the correlation between OCP and PAH pollution in the sediment samples. According to the results, it will be observed that sampling points 4, 7, 8, 11, 13, 14 have very similar distributions for both OCP and PAH pollution. However, in the north side of the lake the average concentrations are very different for two persistent organic pollutants.

REFERENCES

Adam, Stefanie, Ilse Vitse, Chris Johannsen, ve Jaak Monbaliu. «Sediment Type Unsupervised Classification of the Molenplaat, Westerschelde Estuary, the Netherlands.» *EARSeL eProceedings*, 2006: 146-160.

Algan A.O., Çağatay M.N., Sarıkaya H.Z., Balkıs N., Sarı E. «Pollution Monitoring Using Marine Sediments: A Case Study on the Istanbul Metropolitan Area.» *Tr. J. of Engineering and Environment Science*, 1999: 39-48.

Allan, Legge H. «Persistent Organic Pollutants in Singapore's Marine Environment.» *Developments in Environmental Sciences(Persistent Organic Pollutants in Asia: Sources, Distributions, Transport and Fate)* içinde, yazan Jeffrey Philip Obbard, Oliver Wurl Wurl ve Stéphane Bayen, 657-720. Amsterdam: Elsevier, 2007.

Bakan, Gülfem, ve Sema Ariman. «Persistent Organochlorine Residues in Sediments Along the Coast of Mid-Black Sea Region of Turkey.» *Marine Pollution Bulletin* , 2004: 1031-1039.

Barlas, Nurhayat, İsmet Çok, ve Nuray Akbulut. «The Contamination Levels of Organochlorine Pesticides in Water and Sediment Samples in Uluabat Lake, Turkey.» *Environmental Monitoring and Assessment*, 2006: 383-391.

Boitsov S., Jensen H.K.B. Klungsoyr J. «Natural Background and Anthropogenic Inputs of Polycyclic Aromatic Hydrocarbons (PAH) in Sediments of South-Western Barents Sea.» *Marine Environmental Research*, 2009: 236-245.

Carvalho, Pedro N., Pedro Nuno R. Rodrigues, Filipe Alves, Rafael Evangelista, Maria Clara P. Basto, ve M. Teresa S.D. Vasconcelos. «An Expeditious Method for the Determination of Organochlorine Pesticides Residues in Estuarine Sediments Using Microwave Assisted Pre-extraction and Automated Head Space Solid-Phase Microextraction Coupled to Gas Chromatography–Mass Spectrometry.» *Talanta* , 2008: 1124–1129.

Connell, D. W. *Basic Concepts of Environmental Chemistry.* New York: Lewis Publishers, 2005.

Dağlı, S. «Stocholm Convention and Current Situation on POPs in Turkey.» *The Fate of Persistent Organic Pollutants (Pops) in the Environment.* İstanbul: Springer, 2007. 21.

Delen Nafiz, Durmuşoğlu E., Güncan A., Güngör N., Turgut C., Burçak A. «Türkiye'de Pestisit Kullanımı, Kalıntı ve Organizmalarda Duyarlılık Azalışı Sorunları.» *Türkiye Ziraat Mühendisliği 6. Teknik Kongre.* Ankara: Ziraat Mühendisleri Odası, 2005. 1-21.

Department of Health,. *Polycyclic Aromatic Hydrocarbons (PAHs): Health effects.* Public Health Fact Sheet, Adelaide: Department of Health, Government of South Australia, 2009.

Doong R., Lee S., Lee C., Sun Y., Wu S. «Characterization and Composition of Heavy Metals and Persistent Organic Pollutants in Water and Estuarine Sediments from Gao-ping River,Taiwan.» *Marine Pollution Bulletin*, 2008: 846-857.

Doong, R, Lee S, Sun Y, ve Wu S. «Composition and Distribution of Organochlorine Pesticide Residues in Surface Sediments from the Wu-Shi River Estuary, Taiwan.» *Marine Pollution Bullettin*, 2002: 246-253.

Erkmen, Belda, ve Dürdane Kolankaya. «Determination of Organochlorine Pesticide Residues in Water, Sediment, and Fish Samples from the Meriç Delta, Turkey.» *Intern. J. Environ. Anal. Chem.*, 2006: 161-169.

Gaga, Eftade O. «Investigation of Polycyclic Aromatic Hydrocarbon (PAH) Deposition in Ankara.» Ph.D.Thesis, Ankara, 2004.

Geo Seabed Instruments. 2006. http://www.geosi.no/no/equipment/grabb.htm (accessed 1 2, 2011).

Hernandez R.A., Tovilla H.C. Malo E.A., Bello M R. «Water Quality and Presence of Pesticides in a Tropical Coastal Wetland in Southern Mexico.» *Marine Pollution Bulletin*, 2004: 1130-1141.

Hinga, K. R. «Degradation rates of low molecular weight PAH correlate with sediment TOC in marine subtidal sediments.» *Marine Pollution Bulletin*, 2003: 466-474.

Hong, Sang Hee, Un Hyuk Yim, Won Joon Shim, Jae Ryoung Oh, ve In Sook Lee. «Horizontal and Vertical Distribution of PCBs and Chlorinated Pesticides in Sediments from Masan Bay, Korea.» *Marine Pollution Bulletin*, 2003: 244-253.

Hu, Limin, Gan Zhang, Binhui Zheng, Yanwen Qin, Tian Lin, ve Zhigang Guo. «Occurrence and Distribution of Organochlorine Pesticides (OCPs) in Surface Sediments of the Bohai Sea, China.» *Chemosphere*, 2009: 663-672.

Hung, Chin-Chang, et al. «Relationships Between Pesticides and Organic Carbon Fractions in Sediments of the Danshui River Estuary and Adjacent Coastal Areas of Taiwan.» *Environmental Pollution*, 2007: 546-554.

Kaushik P., Kaushik G. «An Assessment of Structure and Toxicity Correlation in Organochlorine Pesticides.» *Journal of Hazardous Materials*, 2007: 102-111.
Kebbekus M., Mitra S. *Environmental Chemical Analysis* . Newark: CRC Press, 1998.

Kielhorn J., Boehncke A. «Polynuclear Aromatic Hydrocarbons in Drinking Water.» *WHO Guidelines for Drinking-water Quality*, 1998: 1-27.

Kim, Min-Sun, Tae Woo Kang, Heesoo Pyo, Junheon Yoon, Kyunghee Choi, ve Jongki Hong. «Determination of Organochlorine Pesticides in Sediment Using Graphitized Carbon Black Solid Phase Extraction and Gas Chromatography/Mass Spectrometry.» *Journal of Chromatograhy A*, 2008: 25-33.

Klánová, J., N. Matykiewiczová, Z. Mácka, P. Prosek, K. Láska, ve P. Klán. «Persistent Organic Pollutants in Soils and Sediments from James Ross Island, Antarctica.» *Environmental Pollution*, 2008: 416-423.

Lee, K. T., S. Tanabe, ve C. H. Koh. «Distribution of Organochlorine Pesticides in Sediments from Kyeonggi Bay and Nearby Areas, Korea.» *Environmental Pollution*, 2001: 207-213.

Manirakiza, P., A. Covaci, S. Andries, ve P. Schepens. «Automated Soxhlet Extraction and Single Step Clean-Up for the Determination of Organochlorine Pesticides in Soil by GC-MS or GC-ECD.» *Environmental Analytical Chem*, 2001: 25-39.

Mocak, J., A. Bond, M. S. Mitchelland, ve G. Scollary. «A statistical overview of standard (IUPAC and ACS) and new procedures for determining the limits of detection and quantification: Application to voltammetric and stripping techniques .» *Pure & Appl. Chern*, 1997: 297-328.

Öztaş, Nur Banu. «Pesticide Pollution in Surface and Ground Water of an Agricultural Area, Kumluca, Turkey.» Ph.D. Thesis, Ankara, 2008.

Page, David S., John S. Brown, Paul D. Boehm, Edward A. Bence, ve Jerry M. Neff. «A Hierarchical Approach Measures the Aerial Extent and Concentration Levels of PAH Contaminated Shoreline Sediments at Historic Industrial Sites in Prince William Sound, Alaska.» *Marine Pollution Bulletin*, 2006: 367-379.

Pietersen, J., ve G. Daminaov. *Guide To Practical Statistics.* Pretoria: HSRC Press, 1998.

Qiao, Min, Chunxia Wang, Shengbiao Huang, Donghong Wang, ve Zijian Wang. «Composition, Sources, and Potential Toxicological Significance of PAHs in the Surface Sediments of the Meiliang Bay, Taihu Lake, China.» *Environment International*, 2006: 28-33.

Sapozhnikova, Y., O. Bawardi, ve D. Schlenk. «Pesticides and PCBs in Sediments and Fish from the Salton Sea, California, USA.» *Chemosphere*, 2004: 797-809.

Tissue, Brian M. *Science Hypermedia Home Page.* June 13, 1996. http://elchem.kaist.ac.kr/vt/chem-ed/sep/gc/gc.htm (accessed 1 19, 2011).

Topal, Tansel. «Investigation of Extraction Methodologies for Quantitative Determination of Polycyclic Aromatic Hydrocarbons in Sediments.» Ph.D. , Ankara, 2011.

Turgut, C. «The Contamination with Organochlorine Pesticides and Heavy Metals in Surface Water in Küçük Menderes River in Turkey.» *Environment International*, 2003: 29-32.

United States Environmental Protection Agency. *Determinative Chromatographic Separations (Method 8000C).* Method, Washington: USEPA Publications, 2003.

United States Environmental Protection Agency. *Ultrasonic Bath Extraction (Method 3550B).* Washington, D.C.: USEPA Publications, 1996.

University of Hull Web Page. 2 11, 2009. http://www.hull.ac.uk/chemistry/masspec3/principlesofms.html (accessed 6 25, 2010).

Vagi, M. C., Petsas A.S., Kostopouloua M.N., Karamanoli M.K., ve T. D. Lekkas. «Determination of Organochlorine Pesticides in Marine Sediments Samples Using Ultrasonic Solvent Extraction Followed by GC/ECD.» *Desalination*, 2007: 146-156.

Wang, W., B. Meng, X. Lu, Y. Liu, ve S. Tao. «Extraction of Polycyclic Aromatic Hydrocarbons and Organochlorine Pesticides from Soils: A Comparison Between Soxhlet Extraction, Microwave-assisted Extraction and Accelerated Solvent Extraction Techniques.» *Analytica Chimica Acta*, 2007: 211-222.

WHO Regional Office for Europe. *Air Quality Guidelines for Europe.* Copenhagen: WHO Regional Publications, 2000.

World Health Organization. *Polynuclear Aromatic Hydrocarbons in Drinking Water.* Geneva: Office of Publications, WHO, 1998.

Xue, Nandong, Daren Zhang, ve Xiaobai Xu. «Organochlorinated pesticide multiresidues in surface sediments from Beijing Guanting reservoir.» *Water Research*, 2006: 183-194.

Yazgan M., Tanık A. «Obsolete Pesticides in Turkey.» 2003.

Yim, U. H., S. H. Hong, ve W. J. Shim. «Distribution and Characteristics of PAHs in Sediments from the Marine Environment of Korea.» *Chemosphere*, 2007: 85-92.

Zhang, Peng, Jinming Song, ve Huamao Yuan. «Persistent Organic Pollutant Residues in the Sediments and Mollusks from the Bohai Sea Coastal Areas, North China: An Overview.» *Environment International*, 2009: 632-646.

Zhou, Rongbing, Lizhong Zhu, Kun Yang, ve Chen Yuyun. «Distribution of organochlorine pesticides in surface water and sediments from Qiantang River, East China.» *Journal of Hazardous Materials*, 2006: 68-75.

APPENDIX A

A.1. Pollution Maps Regarding Organochlorine Pesticides in İkicetepeler Dam Lake

Hexachlorocyclohexane (HCH) Isomers

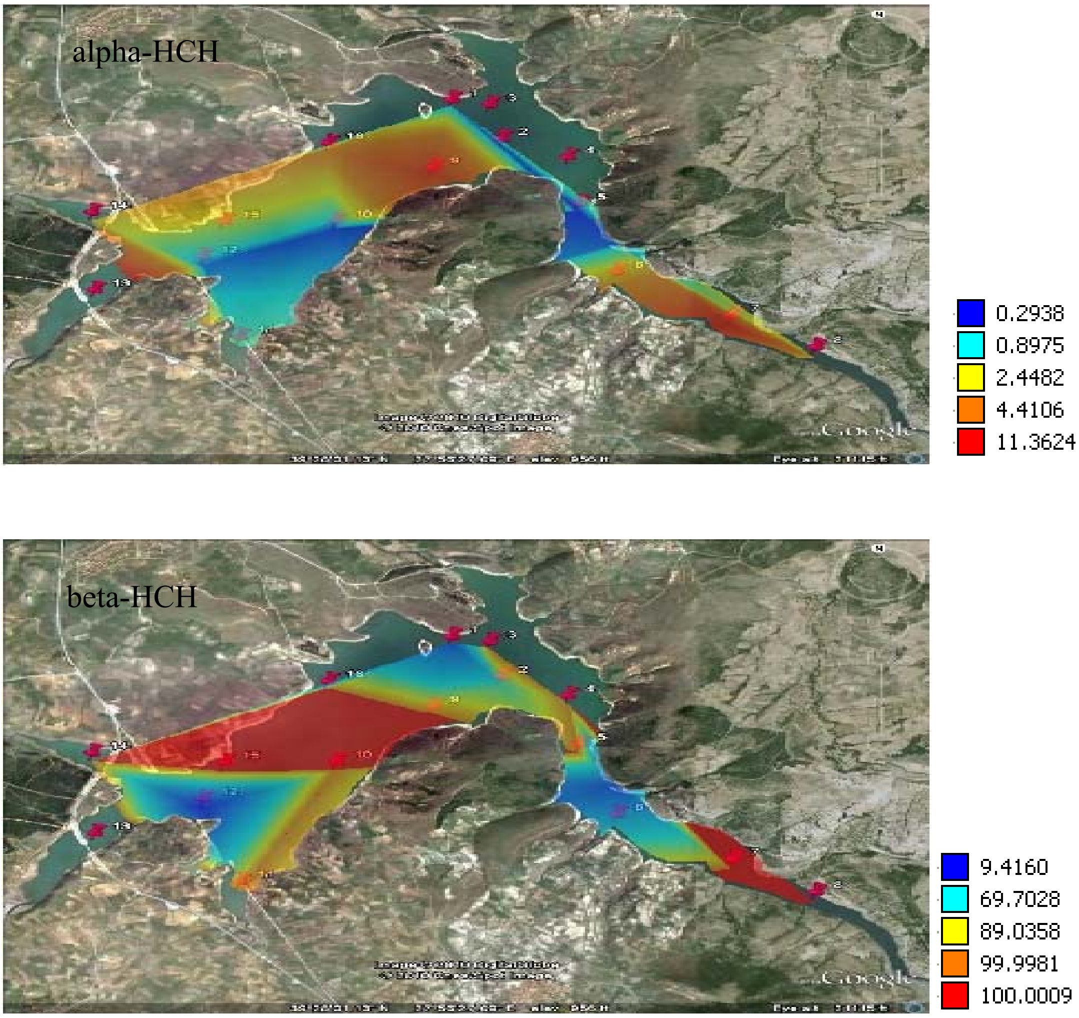


Figure A.1 The distribution of Hexachlorocyclohexane (HCH) isomers in İkizcetepeler dam lake sediment samples

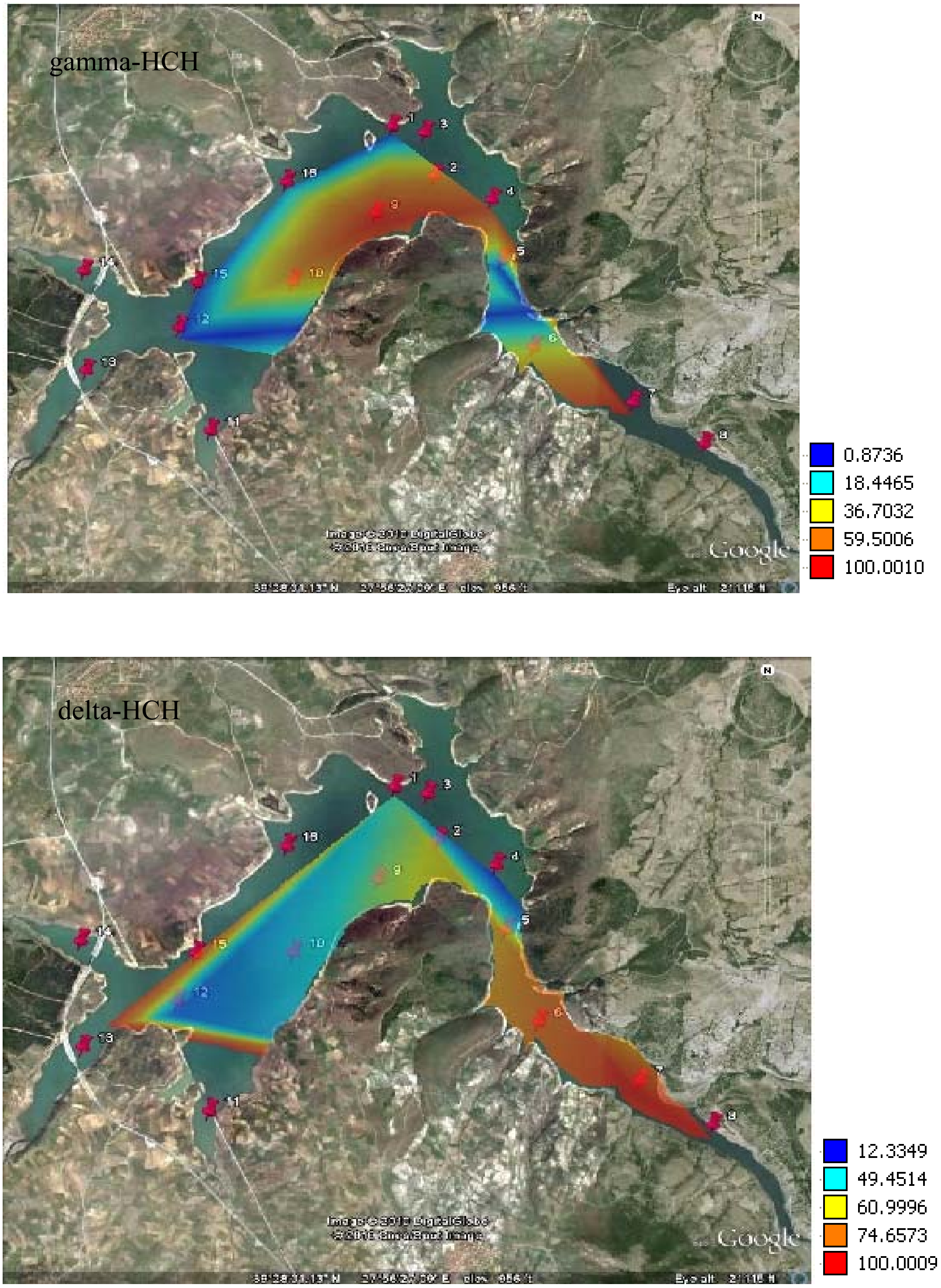


Figure A.2 The distribution of Hexachlorocyclohexane (HCH) isomers in İkizcetepeler dam lake sediment samples (continued)

Cyclodienes

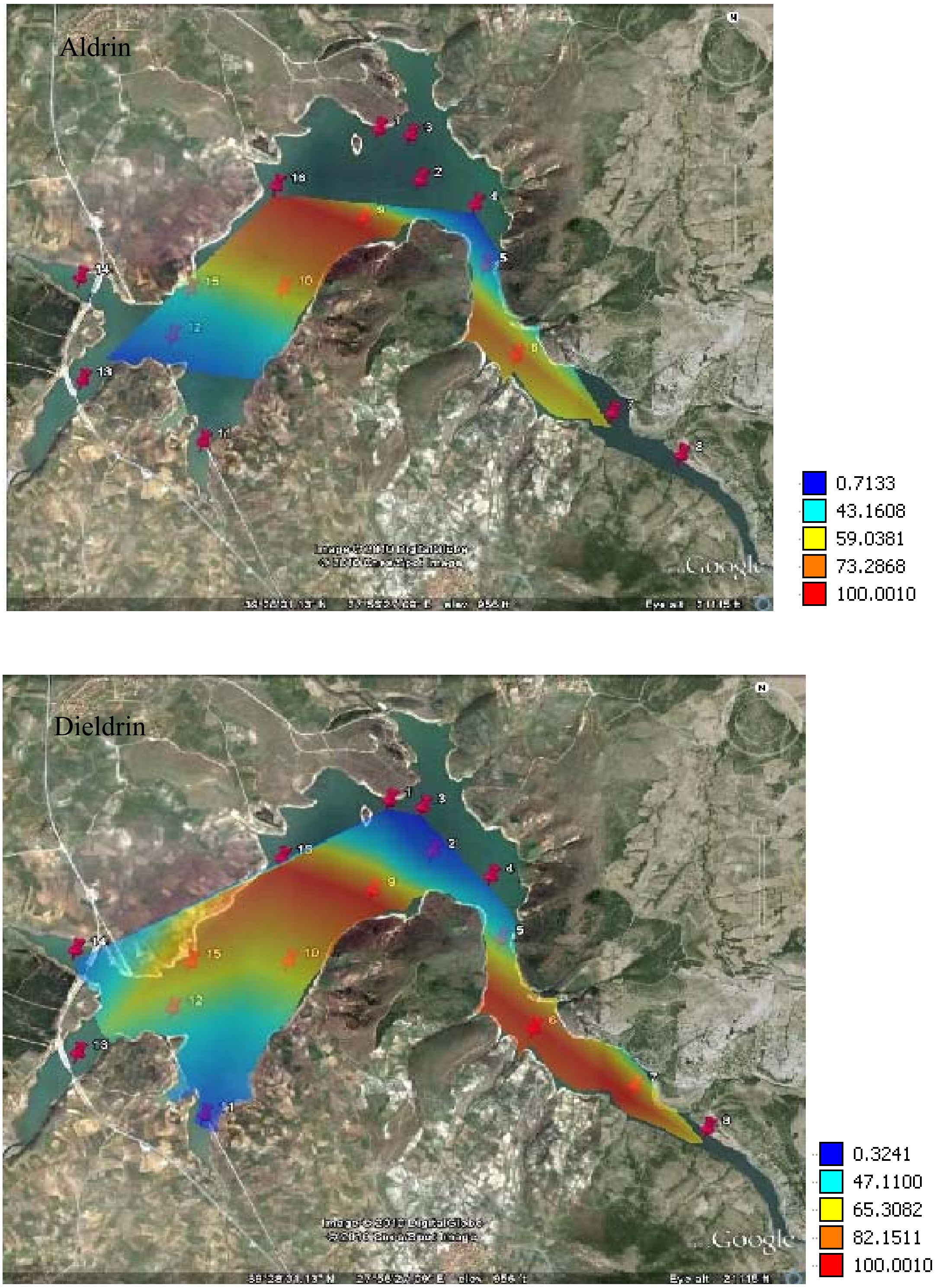


Figure A.3 The distribution of cyclodienes in İkizcetepeler dam lake sediment samples

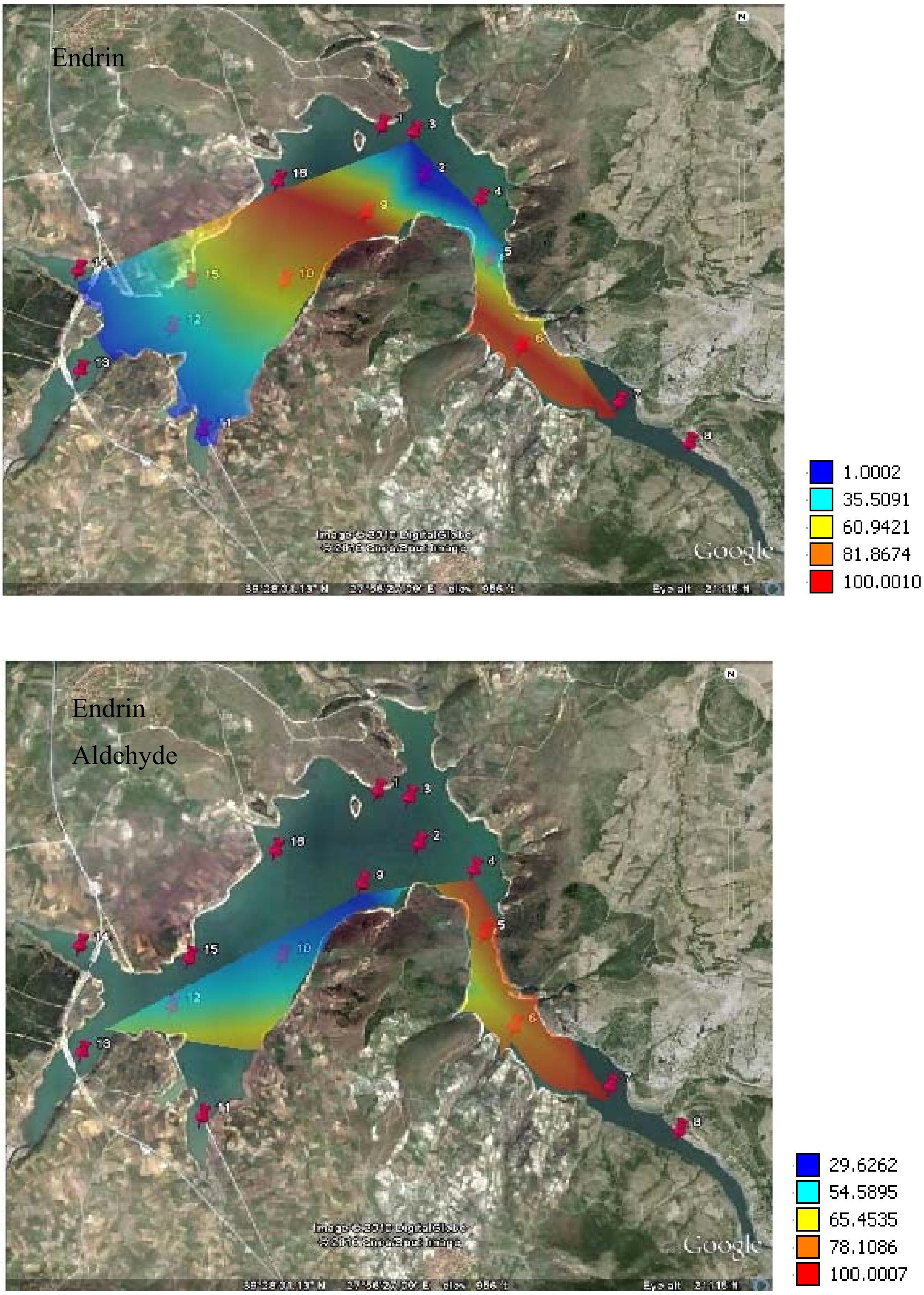


Figure A.4 The distribution of cyclodienes in İkizcetepeler dam lake sediment samples (continued)

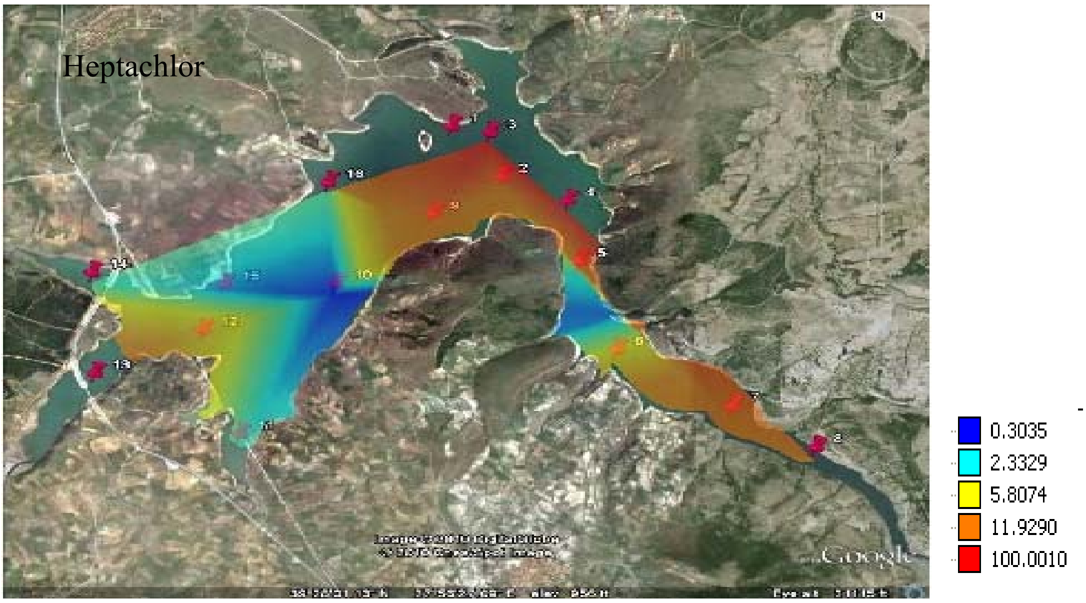


Figure A.5 The distribution of cyclodienes in İkizcetepeler dam lake sediment samples (continued)

Endosulfan and Its Derivatives

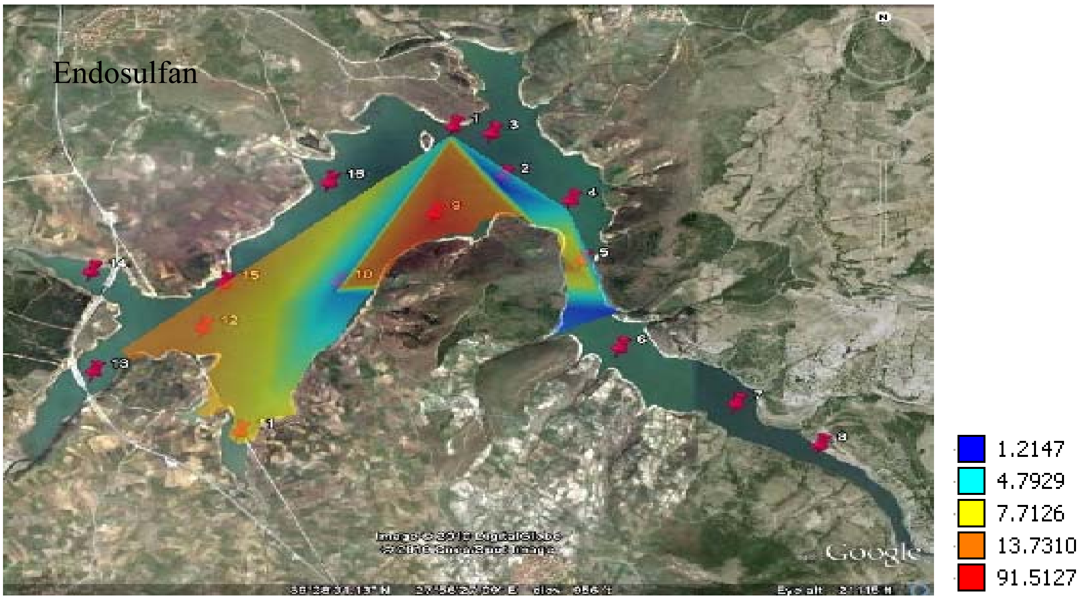


Figure A.6 The distribution of Endosulfan and its derivatives in İkizcetepeler dam lake sediment samples

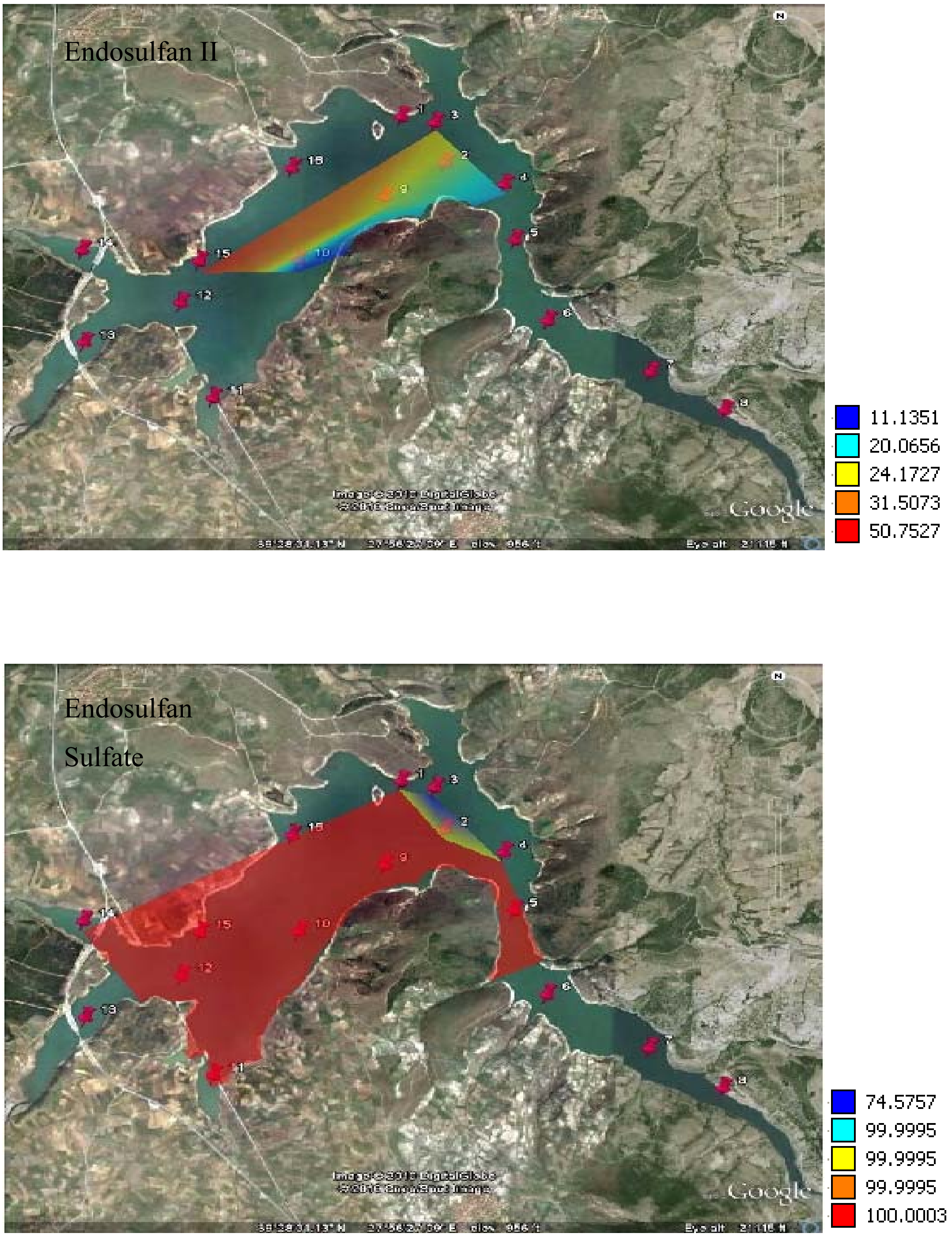


Figure A.7 The distribution of Endosulfan and its derivatives in İkizcetepeler dam lake sediment samples (continued)

Dichloro Diphenyl Trichloroethane (DDT) and Its Derivatives (p,p'-DDD, p,p'-DDE and Methoxychlor)

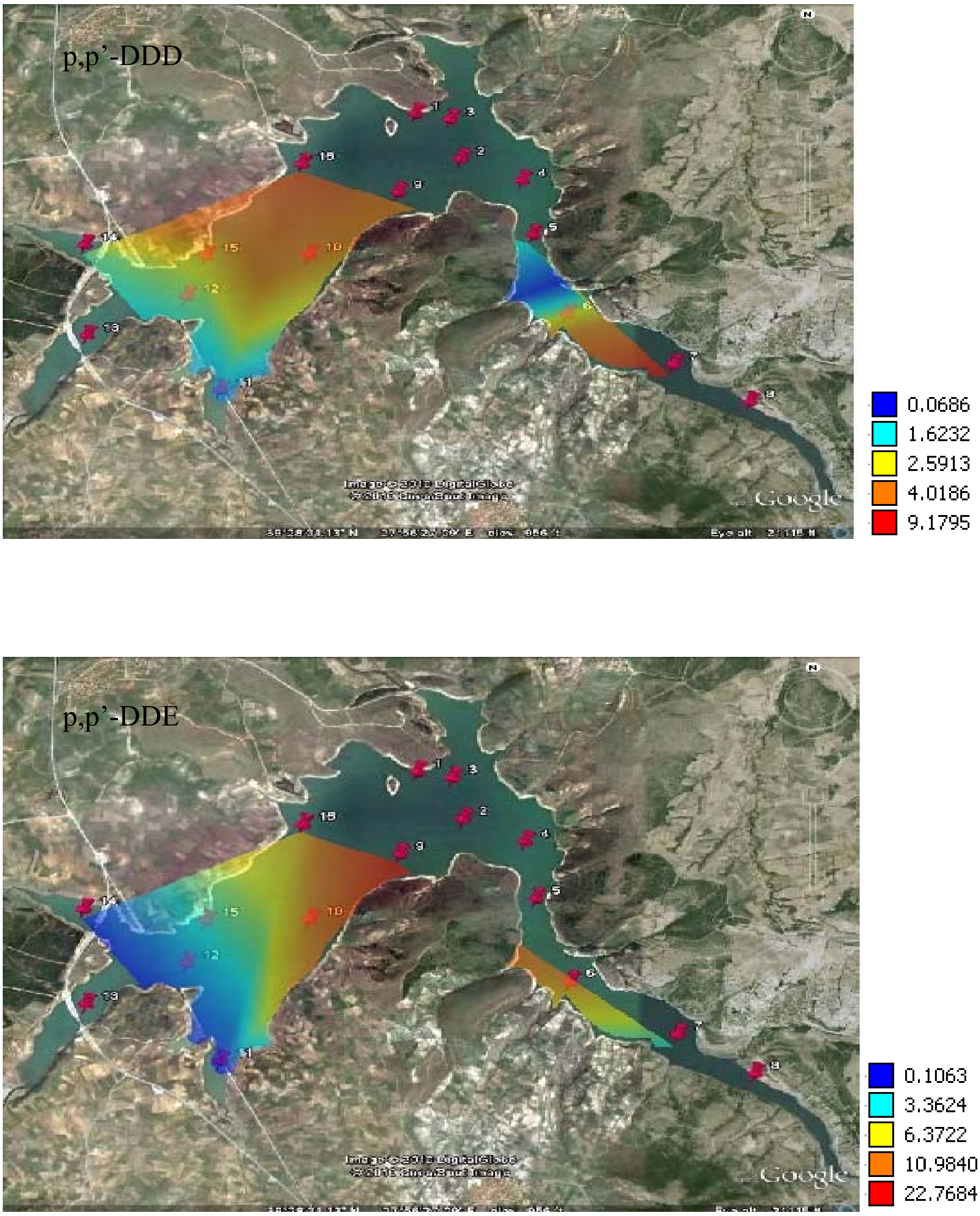


Figure A.8 The distribution of Dichloro Diphenyl Trichloroethane (DDT) and Its Derivatives (DDD, DDE and Methoxychlor) in İkizcetepeler dam lake sediment samples

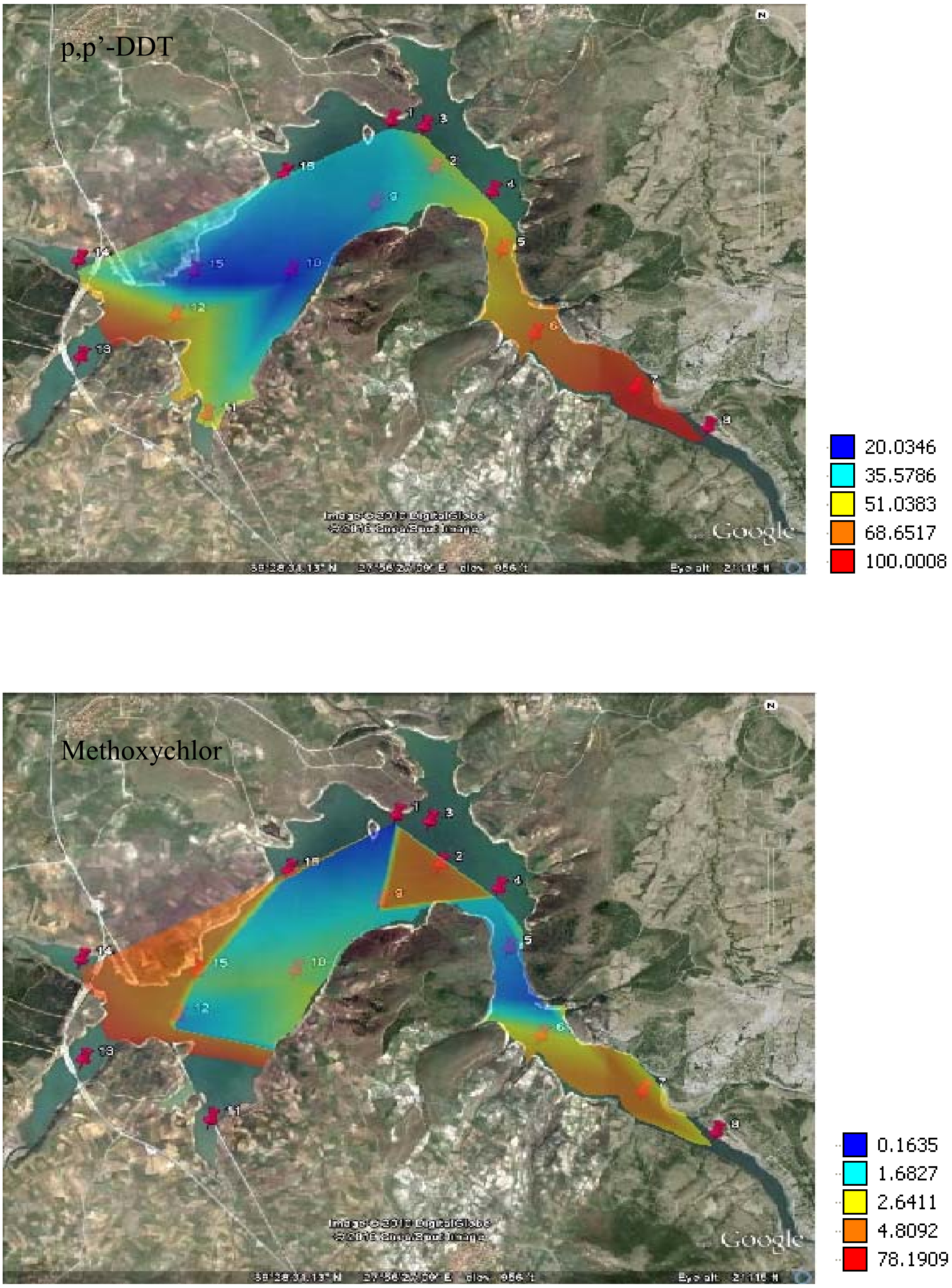


Figure A.9 The distribution of Dichloro Diphenyl Trichloroethane (DDT) and Its Derivatives (DDD, DDE and Methoxychlor) in İkizcetepeler dam lake sediment samples (continued)