**DOKUZ EYLÜL UNIVERSITY**
**GRADUATE SCHOOL OF NATURAL AND APPLIED SCIENCES**

# LEVELS OF ORGANOCHLORINE COMPOUNDS IN SEDIMENT AND FISH FROM EASTERN AEGEAN COAST AND USE OF POLYMERIC SORBENT FOR THE EXTRACTION OF DDT

**by**
**Shareef K. I. MUZYED**

**September, 2016**
**İZMİR**

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**by**
**Shareef K. I. MUZYED**

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**Ph.D. THESIS EXAMINATION RESULT FORM**

We have read the thesis entitled **"LEVELS OF ORGANOCHLORINE COMPOUNDS IN SEDIMENT AND FISH FROM EASTERN AEGEAN COAST AND USE OF POLYMERIC SORBENT FOR THE EXTRACTION OF DDT"** completed by **SHAREEF K.I. MUZYED** under supervision of **PROF. DR. FİLİZ KÜÇÜKSEZGİN and PROF. DR. NALAN TÜZMEN** and we certify that in our opinion it is fully adequate, in scope and in quality, as a thesis for the degree of Doctor of Philosophy.

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# LEVELS OF ORGANOCHLORINE COMPOUNDS IN SEDIMENT AND FISH FROM EASTERN AEGEAN COAST AND USE OF POLYMERIC SORBENT FOR THE EXTRACTION OF DDT

## ABSTRACT

Organochlorines (OCs) were determined in sediment and fish samples from two regions (İzmir and Çandarlı Bays) in eastern Aegean coast during 2014. The total organochlorine pesticides (OCPs) in sediments were in the range of 1.5-30 ng per g dry wt. The results generally indicated that the cyclodienes were the predominant OCPs in sediments. Aroclor1254 and 1260 were present in noticeably higher levels than OCPs at all sampling sites with average values of 45 and 59 ng per g dry wt, respectively. The highest levels of Aroclors and OCPs were found in samples collected from Nemrut site which can be attributed to industrial activities. According to sediment quality guidelines (SQGs), the concentrations of DDTs were higher than the values that may cause adverse biological risk in Nemrut site. Aroclor 1254 levels in sediment sample exceeded the threshold effect level (TEL), showing that adverse biological effect is expected occasionally at Nemrut site whereas they were below the probable effect level (PEL) at all stations. OCPs were detected in all fish samples (*Mullus barbatus)* collected from the sampling sites. The maximum values of total OCPs was found in fish collected from Gülbahçe site. The highest concentration of Aroclors was found in samples from Aliağa. According to related indices, results indicate no recent influxes of DDT in the sampling areas. The estimated daily intake of DDTs was below the acceptable daily intake (ADI) level recommended by FAO-WHO. Since removal of pesticides from environmental medium is very important, poly(HEMA-MAPA) nanospheres were used as adsorbent for DDT from standard solutions. Adsorption conditions including contact time, initial DDT concentration, temperature, amount of polymer and ionic strength were also optimized. It was concluded that poly(HEMA-MAPA) nanospheres can be effectively used as DDT adsorbent. For future studies, it seems possible for poly(HEMA-MAPA) nanospheres to be recomonded for DDT's removal from environmental matrices.

**Keywords:** Organochlorinated pesticides, polychlorinated biphenyls, sediment, fish, Eastern Aegean, nanostructures, adsorption, hydrophobic interaction chromatography

# DOĞU EGE KIYILARINDA BALIK VE SEDİMENTTEKİ ORGANOKLORLU BİLEŞİKLERİN SEVİYELERİ VE DDT'NİN EKSTRAKSİYONU İÇİN POLİMERİK SORBENT KULLANIMI

## ÖZ

Doğu Ege kıyılarında 2014 yılında iki bölgeden (İzmir ve Çandarlı Körfezleri) alınan sediment ve balık örneklerinde organoklorlu bileşikler tayin edilmiştir. Sedimentteki toplam organoklorlu pestisitler (OCP) gramda 1,5-30 ng kuru ağırlık arasındadır. Sonuçlar, siklodienlerin genellikle baskın OCP olduğunu göstermiştir. Aroklor1254 ve 1260 bütün örnekleme noktalarında OCP'den önemli ölçüde yüksek olup ortalama değerler sırasıyla gramda 45 ve 59 ng'dır. En yüksek Aroklor ve OCP seviyeleri yoğun endüstriyel aktivitelerden dolayı Nemrut bölgesinden toplanan örneklerde bulunmuştur. Sediment kalite rehberlerine göre, Nemrut bölgesinde DDT seviyeleri ters biyolojik risk oluşturan değerlerden yüksektir. Sediment örneklerinde Aroklor 1254 seviyeleri bütün noktalarda muhtemel etki değeri (PEL) altında bulunurken Nemrut bölgesinde eşik etki seviyesini (TEL) aşmaktadır. Bu da Nemrut bölgesinde ters biyolojik etkiyi göstermektedir. Toplanan bütün balık örneklerinde (*Mullus barbatus)* OCP saptanmıştır. Maksimum toplam OCP değerleri Gülbahçe bölgesinden toplanan örneklerde bulunmuştur. En yüksek Aroklor değeri Aliağa'da ölçülmüştür. İlgili indekslere göre, sonuçlar örnekleme alanlarında yeni DDT girdisinin olmadığını göstermektedir. DDT'lerin tahmini günlük alım değerleri (FAO-WHO)'nun tavsiye ettiği günlük kabul edilebilir seviyenin (ADI) altındadır. Pestisitlerin çevresel ortamdan uzaklaştırılmaları çok önemli olduğundan poly(HEMA-MAPA) nanokureleri DDT adsorbenti olarak kullanılmıştır. Temas zamanı, başlangıç DDT konsantrasyonu, sıcaklık, polimer miktarı ve iyonik siddeti içeren adsorpsiyon koşulları optimize edilmiştir. Sonuç olarak, poly(HEMA-MAPA) nanokureleri DDT adsorpsiyonunda etkin olarak kullanılabilir. Elde edilen sonuclar ışığında, gelecek çalışmalarda, çevresel matrislerden DDT'nin uzaklaştırılması için poly(HEMA-MAPA) nanokurelerinin kullanımı önerilir.

**Anahtar kelimeler:** Organoklorlu pestisitler, poliklorlu bifeniller, sediment, balık, Doğu Ege kıyıları, nanostructures, adsorpsiyon, hydrofobik etkili kromotografi

**CONTENTS**

**Page**

THESIS EXAMINATION RESULT FORM ..... ii
ACKNOWLEDGEMENTS ..... iii
ABSTRACT ..... v
ÖZ ..... vi
LIST OF FIGURES ..... x
LIST OF TABLES ..... xi

**CHAPTER ONE – INTRODUCTION ..... 1**

**CHAPTER TWO – PESTICIDES AND HYDROPHOBIC INTERACTION CHROMATOGRAPHY ..... 5**

2.1 Pesticides ..... 5
2.1.1 Organochlorinated Pesticides ..... 6
2.1.2 Physicochemical Properties and Environmental Partitioning for POPs ..... 7
2.1.2.1 Vapour Pressure ($P_{VP}$) ..... 11
2.1.2.2 Solubility and Hydrophopocity ..... 11
2.1.2.3 Octanol/Water Partition Coefficient-Kow (Log Kow) ..... 12
2.1.2.4 Bioavailability ..... 12
2.1.2.5 Soil Adsorption Coefficient $K_{OC}$/Kd ..... 13
2.1.2.6 Henry`s Law Constant-H` ..... 13
2.1.2.7 Persistence ..... 14
2.1.3 The Current Status of OCPs ..... 14
2.1.4 Toxicity of OCPs ..... 17
2.1.5 DDT and its Derivatives ..... 20
2.1.6 Input of Contaminants ..... 22
2.1.7 Movement of Pesticides ..... 23

2.1.7.1 Transformation ..... 23
2.1.7.2 Retention ..... 24
2.1.7.3 Uptake ..... 25
2.1.7.4 Transport ..... 25
2.1.8 Review of Literature ..... 26
2.2 Hydrophobic Interaction Chromatography (HIC) ..... 30
2.2.1 Definition and Principle of (HIC) ..... 30
2.2.2 The Effect of pH on HIC ..... 33
2.2.3 The Effect of Temperature on HIC ..... 33
2.2.4 The Effect of Salt on HIC ..... 34
2.2.5 Review of Literature ..... 35

**CHAPTER THREE – MATERIAL AND METHODS ..... 38**

3.1 Study Area ..... 38
3.2 Sample Collection ..... 39
3.3 Experiments ..... 40
3.3.1 Extraction of OCPs from Sediment Samples ..... 40
3.3.1.1 Cleaning of Reagents ..... 40
3.3.1.2 Extraction of Samples ..... 40
3.3.1.3 Concentration of the Extract ..... 40
3.3.1.4 Removal of Sulfur and Sulfur Compounds Using Copper ..... 41
3.3.1.5 Fractionation ..... 41
3.3.2 Extraction of OCPs from Fish ..... 42
3.3.2.1 Extraction of Freeze-dried Samples ..... 42
3.3.2.2 Concentration of the Extract ..... 43
3.3.2.3 Removal of Lipids by Concentrated Sulphuric Acid ..... 43
3.3.2.4 Fractionation ..... 43
3.3.3 Gas Chromotography Conditions for Halogenated Hydrocarbons ..... 43
3.3.4 Quality Assurance for OCPs ..... 44
3.3.5 Extraction of DDT Using Poly [HEMA-MAPA] Nanospheres ..... 44

3.3.5.1 Materials Used for the Extraction of DDT Using Poly[HEMA-MAPA] Nanospheres ..... 44
3.3.5.2 Adsorption Studies of p, p' DDT Standards ..... 46
3.4 Statistical Analysis ..... 47

**CHAPTER FOUR – RESULTS AND DISCUSSION ..... 48**

4.1 Organochlorine Pesticides in the Sediments from the Aegean Coast ..... 48
4.1.1 Organochlorine Levels in Sediment ..... 48
4.1.2 DDTs Composition and Source Identification in Sediments ..... 52
4.1.3 Potential Biological Effects of OCPs in Sediments ..... 53
4.1.4 Ecotoxicological Concerns for PCBs ..... 56
4.2 Organochlorines in Fish Samples from the Izmir and Çandarlı Bays ..... 58
4.2.1 Contamination Levels of Organochlorines in Fish ..... 58
4.2.2 Characteristics of OCP Contamination in Fish Samples ..... 60
4.2.3 Health Risks Assesment ..... 61
4.3 Statistical Analysis ..... 64
4.4 Use of Poly (HEMA-MAPA) Nanospheres as Adsorbent for DDT Standards ..
..... 67
4.4.1 Optimization of HPLC Method for DDT ..... 67
4.4.2 Optimization of DDT Adsorption ..... 69
4.4.2.1 Effect of Contact Time ..... 69
4.4.2.2 Effect of Polymer Amount ..... 69
4.4.2.3 Effect of Initial DDT Concentration ..... 70
4.4.2.4 Effect of Temperature ..... 71
4.4.2.5 Effect of Ionic Strength ..... 71

**CHAPTER FIVE – CONCLUSION ..... 73**

**REFERENCES ..... 76**

## LIST OF FIGURES

**Page**

Figure 2.1 Schematic representation of the formation of hydrophobic bond between two isolated side chains (leucine, alanine). The bond is formed through an approach of the two side chains until they contact, with a reduction of the number of nearest water neighbors. The extent of contact shown here is less than the maximum obtainable for the two side chains ..... 31
Figure 2.2 Illustration of the Hofmeister series (Porath, 1987) ..... 34
Figure 3.1 Biota (●) and sediment (▲) sampling sites in Izmir and Çandarlı Bays.. 39
Figure 3.2 The hypothetic structure of poly (HEMA–MAPA) nanospheres ..... 46
Figure 4.1 Concentrations of DDTs and PCBs in the sediments from the sampling sites. ERL and ERM values ..... 55
Figure 4.2 Factor analysis loading plots for sediment samples collected in the different sampling stations from the study area ..... 65
Figure 4.3 Factor analysis loading plots for fish samples collected in sampling sites of the Izmir and Candarli bays for organochlorine compounds ..... 66
Figure 4.4 The calibration curve of DDT standards at wavelength with 238 nm..... 68
Figure 4.5 Effect of contact time on the DDT adsorption (C: 0.5 mg/l, T: 25°C and polymer amount: 400µl) ..... 68
Figure 4.6 Effect of polymer amount (C: 0.5mg/L, T:25 °C, t: 60 min) ..... 69
Figure 4.7 Effect of initial DDT concentration effect (T: 25°C, 60 min and 400µl polymer) ..... 70
Figure 4.8 Effect of temperature (C: 0.5 mg/l, t: 60min and polymer amount 400µl)71
Figure 4.9 Effect of ionic strength (C: 0.5mg/l, T:25 °C, t: 60 min and polymer amount 400µl) ..... 72

## LIST OF TABLES

**Page**

Table 2.1 Classes of organic pesticides ..... 6
Table 2.2 Structures of some organochlorine pesticides of major environmental and toxicological concern..... 8
Table 2.3 Some OCPs last known uses..... 17
Table 2.4 Health hazards and exposure limits of common organochlorine Insecticides ..... 19
Table 2.5 Frequency of occurrence of pesticide residues found in total diet study foods by FDA in 2003 ..... 21
Table 2.6 Main sources of chlorinated pesticides to the various environmental vectors and compartments ..... 23
Table 4.1 Total concentration of DDT, cyclodienes, OCPs, Aroclors, EOM (extractable organic matter) and the selected ratios of chlorinated pesticides in sediments from Izmir and Çandarlı Bays ..... 48
Table 4.2 The concentrations of organochlorine compounds (ng $g^{-1}$ dry weight) in sediment samples from Izmir and Çandarlı Bays..... 51
Table 4.3 Assessments of potential biological risks of selected OCPs in the surface sediments of Izmir and Candarli Bays using two sediment quality guidelines (SQG) ..... 54
Table 4.4 The concentrations of organochlorine compounds (ng $g^{-1}$ dry weight) in fish samples from Izmir and Çandarlı Bays ..... 57
Table 4.5 Total concentration of DDTs and the ratios of DDT concentrations in biota samples collected from the sampling sites ..... 58
Table 4.6 Estimated daily intakes of PCBs and OCs in the fish samples by human (average body wt 70 kg) in İzmir and Çandarlı Bays (Eastern Aegean) and Acceptable Daily Intake (ADI) by FAO/WHO (1996b) (ng/kg body weight/day) ..... 61
Table 4.7 Product-Moment Pearson's correlation coefficients for organochlorines (ng $g^{-1}$ dry weight) and EOM (mg $g^{-1}$) ($p<0.05$) in sediment samples ..... 62

Table 4.8 Product-Moment Pearson's correlation coefficients for organochlorines (ng $g^{-1}$ dry weight) and lipid (%) ($p<0.05$) in fish samples ..... 63
Table 4.9 Variamax normalized factor loadings for sediment..... 64
Table 4.10 Variamax normalized factor loadings for fish ..... 67

# CHAPTER ONE
# INTRODUCTION

All over the world more than 6 billion people need food and water resources with severe consequences on the planet environmental conditions. Nowadays, the quality and safety of food attract great attention of the authorities and consumers. Meat and seafood are significant components of the human food. Meat is a good source for proteins, essential amino acids, elements and vitamins (Gold, 2004). However, the healthy image of meat falls into disrepute because of the contents of saturated fat, cholesterol, and the presence of chemical contaminants like, pesticides, polychlorinated biphenyls (PCBs), polycyclic aromatic hydrocarbons (PAHs) and dioxins (Higgs, 2000). On the other hand, seafood is regarded as safe for human consumption, because it is well known that they are able to prevent coronary heart diseases, diabetes, hypertension and cancer. Seafood contains low cholesterol levels, high quality proteins with all essential amino acids, polyunsaturated n-3 fatty acids, liposoluble vitamins and essential elements like calcium, selenium and iodine (Chen, Zhang, & Shrestha, 2006; Simopolpoulos, 1997). Therewithal, several seafood items are also systematically affected by the accumulation of chemical and microbiological contaminants, which can be extremely dangerous for human health (Marques et al., 2011).

In recent years, the contamination of the marine environment by chemical contaminants such as pesticides has risen due to the global increase of both, population and industrial development (Arellano et al., 1999). It is well known that pesticides are used to prevent and control harmful organisms being an acute tool to agricultural problems. Pesticides have also different usage areas such as wood preservation, disinfection or household uses (Pinto, Sontag, Bernardino, & Noronha, 2010). In spite of the several advantages, extensive usage of pesticides in agriculture leads to accumulation of pesticide residues in soil, water, and food therefore this unconscious usage generates a serious risk to human health and the environment worldwide (Bektasoglu, Ozkutuk, Ersoz, & Say, 2014).

Among pesticides, DDT, an important organochlorine pesticide, has been extensively used for the control of soil-dwelling insects and against diseases such as malaria and typhus all over the world. Although it has been prohibited in most countries due to their high toxic effects and persistence in the environment, recent reports showed that DDT and its main metabolites were important persistent organic compounds (POPs). POPs is the common name of a group of pollutants that are semivolatile, bioaccumulatative, persistent and toxic (Vallack et al., 1998; Jones & de Voogt, 1999). DDT and its main metabolites are suspected to be carcinogens and mutagens. It has been suggested that DDT can cause cancer and human beings may be badly affected through the food chain (Zhou, Wu, & Xie, 2013).The lipophilic nature, hydrophobicity and low chemical and biological degradation rates of organochlorine pesticides have led to their accumulation in biological tissues and subsequent magnification of concentrations in organisms progressing up the food chain (Swackhamer & Hites, 1988; Vassilopoulou & Georgakopoulous-Gregoriades, 1993). On the other hand, sedimentary hydrocarbons have received special attention because these compounds are readily adsorbed onto particulate matter, and bottom sediments ultimately act as a reservoir of hydrophobic contaminants (Volkman, Uoldsworth, Neill, & Bavor, 1992). From this point of view, it is of great importance to develop simple, rapid and sensitive analytical techniques for the detection of residual concentrations of POPs in the aquatic environments including the analysis of the water column, organisms and sediment.

As a part of the Eastern Mediterranean basin, the Aegean Sea is exposed to contamination from both natural and anthropogenic sources. There are a number of rivers along the eastern and western coast of the Aegean Sea through which a large amount of contaminants is being transported into the marine ecosystem thereby causing a great concern for marine pollution. The present environmental problems are due to untreated sewage discharge by coastal settlements, unmanaged shipping activity, river run-off (Gediz is the biggest river along the eastern Aegean), and dumping of toxic and industrial wastes from the western part of Turkey.

Several methods such as gas chromatography with electron capture detection (GC-ECD) (Hussen, Westbom, Megersa, Mathiasson, & Bjoklund, 2007; Surma-Zadora and Grochowalski, 2008), gas chromatography tandem mass spectrometry (GC-MS/MS) (Pinto et al., 2010) and gas chromatography/high performance liquid chromatography (GC/HPLC)-mass spectrometry (MS) (Valsamaki, Boti, Sakkas, & Albanis, 2006; Baugros, Giroud, Dessalces, Loustalot, & Oliv´e, 2008) have been used for the determination of DDT and its metabolites in environmental and food samples. However, DDT and its metabolites as all other organic contaminants are often present in marine environment in trace levels and also the sample matrices are very complicated. So, a preconcentration of the compounds present mostly at trace levels prior to analysis is absolutely necessary (Zhou et al., 2013; Verma & Murray, 2012).

The organochlorines present in the final extract need to be individually separated and detected by selective separation techniques in order to be correctly identified and quantified. As adsorption is one of the most effective and simplest approaches to removing toxic and recalcitrant pollutants from aqueous systems and activated carbon is one of the most widely used adsorbents for this purpose (Pan, Zhang, Lv, Zhang, & Zheng, 2009). In recent years, non-ionic polymer resins have been attracting much attention as an alternative to activated carbon for the removal of organic pollutants (Gusler, Browne, & Cohen, 1993).

Major objectives of the present study are;

- to investigate the occurrence and distribution patterns of organochlorine pesticides (OCPs) and polychlorinated biphenels (PCBs) in sediment and fish samples from Izmir and Çandarlı Bays in the Eastern Aegean coast, which gave the information about status of contamination,
- to assess the potential sources and using history of DDTs contaminants, if any, in sediment samples collected from Izmir and Candarli Bays using related indices,
- to provide a better understanding of recent distribution, possible sources as well as potential biological risk of DDTs, PCBs (Aroclors) in the study areas,

- to compare the measured contaminants' levels with those of other corresponding areas in the world,
- to estimate the extent of contamination of sediment and fish samples from the study areas, so this will be a useful reference for the development of any future studies,
- to test the adsorption ability of poly[hydroxyethyl methacrylate-methacryloylamido phenylalanine] (poly[HEMA-MAPA]) nanospheres toward organochlorine compounds (DDT was used as model) depending on their hydrophobic interaction properties,
- to optimize some factors affecting the adsorption efficiency including polymer amount, contact time, initial concentration of DDT, temperature, and ionic strength.

# CHAPTER TWO
# PESTICIDES AND HYDROPHOBIC INTERACTION CHROMATOGRAPHY

## 2.1 Pesticides

Therewithal the term 'pesticides' has been defined as any substance or mixture of substances, of chemical or biological origin, used by human society to mitigate or repel pests such as bacteria, nematodes, insects, mites, mollusks, birds, rodents, and other organisms that affect food production or human health. They usually act by disrupting some component of the pest's life processes to kill or inactivate it. Pesticides can be organic (some synthesized and some extracted from plants such as nicotine) or inorganic chemicals (e.g synthesized from inorganic salts of arsenic compounds) (Castilho, Fenzl, Guillen, & Nascimento, 2000). Pesticide compounds are characterized by their toxicity, volatility, as well as by their capacity to interfere with cell biochemistry when accumulated in organic tissues chemically. Pesticides consist of different elements or atoms bonded together, so that the smallest unit of any pesticide is a molecule. The elements that are often used in pesticide construction are;

- Carbon (C)
- Hydrogen (H)
- Nitrogen (N)
- Chlorine (Cl)
- Sulfur (S)

In addition, there are metallic and semi metallic elements such as iron, copper, zinc, mercury, arsenic and others that may include in some pesticides. Essentially all of the pesticides are organic compounds that contain carbon in their molecules. A very few contain no carbon and are termed inorganic compounds (Ware, 1986). Organic pesticides are generally classified by use, i.e, insecticide, nematocide, miticide, rodenticide, herbicide, fungicide, etc. A more appropriate classification

would be by chemical species. Classification of organic pesticides appears in Table 2.1 (Ciaccio, 1972).

### *2.1.1 Organochlorinated Pesticides*

Generally, the organochlorine pesticides(OCPs) are hydrophobic substances, with low water solubility, frequently at the μg or ng per liter level. Most of the OC pesticides have octanol water partition coefficients (Kow) with log Kow comprised between 3.5 and 6 and, thus, are very soluble in lipids. As a consequence, these pesticides are highly concentrated by living organisms and it's levels can biomagnify along the food chain (Noble, 1993). Also OCPs are known for their environmental persistence and global concerns. Residues of OCPs continue to detect in many areas (Doong, Sun, Liao, Peng, & Wu, 2002).

Table 2.1 Classes of organic pesticides

| |
|---|
| **A. Chlorinated hydrocarbons** |
| 1. Class I: oxygenated compounds (Dieldrin, methoxychlor, endrin) |
| 2. Class II: benzenoid, nonoxygenated compounds (BHC, DDD (TDE), DDT, perthane) |
| 3. Class III: nonoxygenated, nonbenzenoid compounds (Aldrin, chlordan, heptachlor, strobane, toxaphene) |
| **B. Organophosphorus compounds** |
| 1. Aliphatic derivatives (Demeton, dimethoate, ethion, malathion, phosdrin, phorate) |
| 2. Aromatic derivatives (Trithion, diazinon, EPN, fenthion, parathion, runnel) |
| **C. Herbicides, fungicides, nematocides etc**. |
| 1. Phenoxyalkyl acids (2,4-D; 2,4,5-T, 2(2,4,5-TP) |
| 2. Substituted ureas (Fenuran, manuran, diuron) |
| 3. Substituted carbamates (IPC, CIPC, EPTC) |
| 4. Symmetrical triazines (Simazine, atrazine) |
| 5. Substituted phenols (PCP, DNBP, DNC) |

OCPs are of the most important persistent organic pollutants (POPs) and have been of great concern around the world owing to their chronic toxicity, persistence

and bioaccumulation (Willett, Ulrich, & Hites, 1998). POPs can be broadly classified into two groups: polycyclic aromatic hydrocarbons and halogenated hydrocarbons. These compounds pose great threats to ecosystems and human health. Although the application of these chemicals has been banned or restricted in many countries especially the developed ones, some developing countries are still using these compounds because of their low cost and versatility in industry, agriculture and public health (Tanabe, Iwata, & Tatsukawa, 1994; Sarkar, Nagarajan, Chaphadkar, Pal, & Singbal, 1997).

OCPs may differ widely in their chemical structures and, therefore, in their physical and toxicological properties. Some of the most well-known pesticides of this class, many of which listed as United States Environmental Protection Agency (EPA) priority environmental pollutants, may be structurally classified into the following five types (Patnaik, 2007):

1. Hexachlorooctahydronaphthalene type (e.g., Aldrin, Dieldrin, Endrin, ketone, and Endrin aldehyde)
2. Chlorinated camphene type (e.g., Heptachlor, Chlordane, Heptachlor epoxide and Toxaphene)
3. Chlorinated diphenylethane type (e.g.,DDT, DDD, Methoxychlor and Perthane)
4. Hexachlorocyclohexane type (e.g., Lindane and it's α, β, and γ-isomers)
5. Chlorinated pentacyclodecane type (e.g., Mirex & Kepone). Table 2.2 shows the chemical structures of common organochlorine pesticides (Mrema, Colosio, & Rubino, 2014)

### *2.1.2 Physicochemical Properties and Environmental Partitioning for POPs*

The physical properties of greatest importance are water solubility, vapour pressure, octanol water partition coefficient ($K_{OW}$), Henry's law constant (H), and the organic carbon water partition coefficient ($K_{OC}$). Persistence in the environment is the other important property of a substance since transport can extend the range of exposure to persistent substances far beyond the immediate area of use and/or release.

Table 2.2 Structures of some organochlorine pesticides of major environmental and toxicological concern

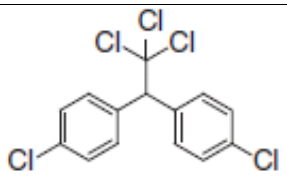
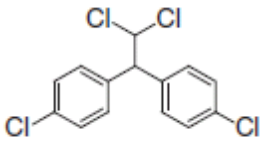
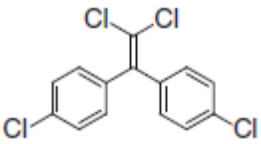
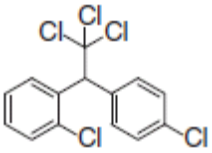
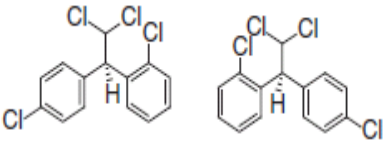
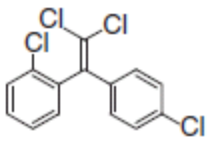
| Description of substance | Chemical structure |
|---|---|
| ***1. Dichlorodiphenyl-ethanes*** | |
| *p,p'* - DDT<br>IUPAC: 1,1,1-trichloro-2,2-bis(p-chlorophenyl)ethane<br>CAS No.: 50-29-3<br>Molecular formula: C14H9Cl5; Formula weight: 354.49 |  |
| *p,p'*-DDD<br>IUPAC: 1,1-dichloro-2, 2-bis (4-chlorophenyl)ethane<br>CAS. No.: 72-54-8<br>Molecular formula: C14H10Cl4; Formula weight: 318.03 |  |
| *p,p'*-DDE<br>IUPAC: (1,1-dichloro-2, 2bis (4-chlorophenyl) ethylene)<br>CAS No.: 72-55-9<br>Molecular formula: C14H8Cl4; Formula weight: 320.05 |  |
| *o,p'*-DDT<br>IUPAC: 1,1,1-trichloro-2-(o-chlorophenyl)-2-(p chlorophenyl)-ethane<br>CAS No.: 789-02-6<br>Molecular formula: C14H9Cl5; Formula weight: 354.49 |  |
| *o,p'*-DDD (Mitotane)<br>IUPAC: 1,1-dichloro-2-(o-chlorophenyl)-2-(p chlorophenyl)ethane<br>CAS No.: 53-19-0<br>Molecular formula: C14H10Cl4; Formula weight: 320.05 |  |
| *o, p'*-DDE<br>IUPAC: 1,1-dichloro-2-(o-chlorophenyl)-2-(p-chlorophenyl)ethylene<br>CAS No.: 3424-82-6<br>Molecular formula: C14H8Cl4; Formula weight: 318.03 |  |

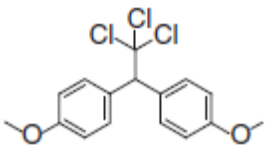
Table 2.2 Structures of some organochlorine pesticides of major environmental and toxicological concern (cont.)

Methoxychlor

IUPAC: 1,1,1-Trichloro-2,2-bis(4-methoxyphenyl)ethane

CAS No.: 72-43-5

Molecular formula: C16H15Cl3O2; Formula weight: 345.65

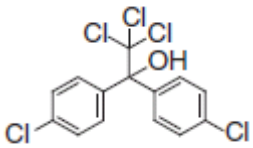


Dicofol (Kelthane)

IUPAC: 2,2,2-trichloro-1,1-bis(4-chlorophenyl)ethanol

CAS No.: 115-32-2

Molecular formula: C14H9Cl5O; Formula weight: 370.49



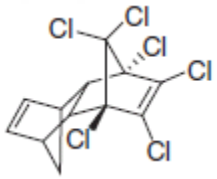
***2. Cyclodienes***

Aldrin

IUPAC: 1,2,3,4,10,10-Hexachloro-1,4,4a,5,8,8a-hexahydro-1,4:5,8-dimethanonaphthalene

CAS No.:309-00-02

Formula: C12H8Cl6; Formula weight: 364.92

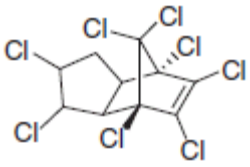


Chlordane

IUPAC: 1,2,4,5,6,7,8,8-octachloro-2,3,31,4,7,71-hexahydro-4,7-methano-1H-indene Cl

CAS No.: 12789-03-6

Molecular formula: C10H6Cl8; Formula weight: 409.78

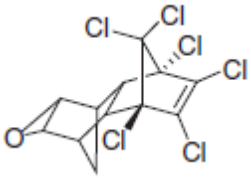


Dieldrin

IUPAC: 3,4,5,6,9,9-Hexachloro-11,2,21,3,6,61,7,71-octahydro-2,7:3,6-dimetanonapth[2,3-b]oxirene

CAS No.: 60-57-1

Molecular formula: C12H8Cl6O; Formula weight: 380.91



Endrin

IUPAC: 3,4,5,6,9,9,-Hexachloro-1a,2,2a,3,6,6a,7,7a-octahydro-2,7:3,6-dimethanonaphth[2,3-b]oxirene

CAS No.: 72-20-8

Molecular formula: C12H8Cl6O; Formula weight: 380.92

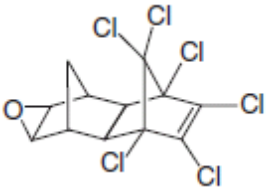


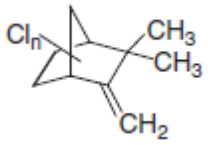
Table 2.2 Structures of some organochlorine pesticides of major environmental and toxicological concern (cont.)

Toxaphene

IUPAC: Toxaphene

CAS No.: 8001-35-2

Molecular formula: C10H10Cl8; Formula weight: 413.82

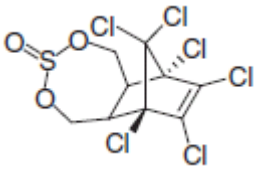



Endosulfan

IUPAC: 6,7,8,9,10,10-hexachloro-1,5,5a,6,9,9a-hexahydro-6,9-methano-2,4,3-benzodioxathiepine-3-oxide

CAS No.: 115-29-7

Molecular formula: C9H6Cl6O3S; Formula weight: 406.95

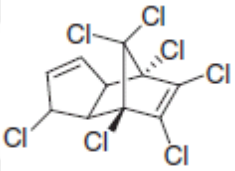



Heptachlor

IUPAC: 1,4,5,6,7,8,8-Heptachloro-3a,4,7,7a-tetrahydro-4,7-methanol-1H-indene

CAS No.: 76-44-8

Molecular formula: C10H5Cl7; Formula weight: 373.32



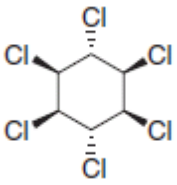

***3. Hexachlorocyclohexane isomers***

Lindane (g-HCH also known as g-BCH)

IUPAC: (1r,2R,3S,4r,5R,6S)-1,2,3,4,5,6 -hexachlorocyclohexane

CAS No.: 58-89-9

Molecular formula: C6H6Cl6; Formula weight: 290.83



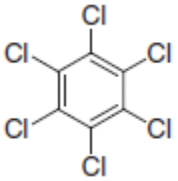

***4. Hexachlorobenzene***

Hexachlorobenzene

IUPAC: Hexachlorobenzene

CAS No.: 118-74-1

Molecular formula: C6Cl6; Formula weight: 284.78




***5. Others***

Mirex

IUPAC: 1,1a,2,2,3,3a,4,5,5a,5b,6-dodecachloroacta-hydro-1,3,4-metheno-1H-cyclobuta[cd]pentalene

CAS No.: 2385-85-5

Molecular formula: C10Cl12; Formula weight: 545.5

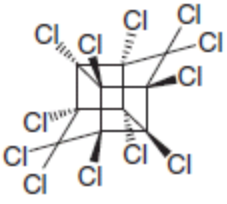
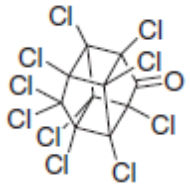


Table 2.2 Structures of some organochlorine pesticides of major environmental and toxicological concern (cont.)

| | |
|---|---|
| Chlordecone (Kepone)<br>IUPAC: 1,1a,3,3a,4,5,5,5a,5b,6-Decachlorooctahydro-2H-1,3,4-(methanetriyl)cyclobuta[cd]pentalen-2-one<br>CAS No.: 143-50-0<br>Molecular formula: C10Cl10O; Formula weight: 490.64 |  |

*2.1.2.1 Vapour Pressure ($P_{VP}$)*

The vapour pressure of a substance is the measure of how easy it can volatilise and turn into vapour (gas state). Pesticides with high vapour pressure need to be handled in such away so that the vapours do not escape into the atmosphere. A pesticide with low vapour pressure does not move into air, so there is a potential to accumulate in water if it is water soluble. If it is not water soluble, the pesticide may accumulate in soil or biota. Semi-volatility is one of the important characteristic of persistent organic pollutants. This property confers a degree of mobility through the atmosphere that is sufficient to allow relatively great amounts to enter the atmosphere and be transported over long distances. This moderate volatility does not result in the substance remaining permanently in the atmosphere where it would present little direct risk to humans and organisms in the environment. Thereby, these substances may volatilize from hot regions but will condense and tend to remain in colder regions.

*2.1.2.2 Solubility and Hydrophopocity*

Solubility is a measure of how easily cans a given material dissolve in a given solvent. Measurements of solubility are influenced by temperature, polarity and pH of the substance, hydrogen bonding and molecular size. Pesticides have a low solubility in water and therefore tend to adsorb on to particles, soil or living organisms. They tend to end up in the lipids of living organisms making them difficult to excrete and causing them to bioconcentrate. They also biomagnify which cause the top predators of the food chain to contain high levels of these compounds.

*2.1.2.3 Octanol/Water Partition Coefficient-Kow (Log Kow)*

Partition coefficient is a measured ratio (at equilibrium) of the dissolved mass of the substance between equal layers of *n*-octanol and water.

$$K_{ow} = \frac{\text{concentration in n-octanol phase}}{\text{concentration in water phase}} \quad (2.1)$$

$K_{ow}$ is considered to be a good indicator of bioaccumulation of pesticides in organisms and food chains. Pesticides with a positive correlation to Log $K_{ow}$ are more likely to have bioaccumulation effects to organisms and food chains. The parameter is also a good indicator of systemic mode of action of a pesticide. Pesticides with low $K_{ow}$ values (generally$\leq$2) indicate the likely systemic translocation of such pesticides or their metabolites in the plants transvascular system. Kow values are generally influenced by the polarity of the pesticide and the general physical factors. Polar pesticides tend to be more soluble in water and hence low values of $K_{ow}$. The $K_{ow}$ partition coefficient has been extensively used as an estimate of the bioconcentration factor (BCF). According to (Paasivirta, 1991) the BCF can be calculated by the use of: BCF=0.048 * $K_{ow}$

In general, $K_{ow}$ will increase when the following physical properties increase; molecular surface area, molar volume, molecular weight, and density (Mallhot & Peters, 1988).

*2.1.2.4 Bioavailability*

The distribution of contaminants in the aquatic environment depends on their characteristics, i.e. water solubility, molecular size and persistence. Also, it depends on the characteristics of the interactions between the organism and phases into which the contaminant is distributed. Thereby the bioavailability of the contaminant will be determined.

*2.1.2.5 Soil Adsorption Coefficient $K_{OC}/K_d$*

Adsorption of pesticides on soils and sediments is a main factor that determines processes. Most pesticides are non polar and hydrophobic meaning that they are not very soluble in water. The non polar pesticides tend to be pushed out of water onto soils and sediments which contain non polar organic matter. $K_d$ is called the sorption coefficient and it measures the amount of pesticides adsorbed onto soil per amount of water without considering the organic matter content of the soil. The values for $K_d$ varies greately because the organic matter content of the soil is not considered in the equation. The preffered parameter to determine the soil`s ability to adsorb pestcides is $K_{OC}$ since it considers the organic matter content of the soil. $K_{OC}$ is the ratio (at equilibrium) of the mass of a substance, adsorbed on to a unit mass of soil, relative to the mass of the substance remaining in water solution. $K_{OC}$ is also a unitless parameter and its value is dependent on the organic matter content of the soil, polarity of the chemical as well as soil pH.

$$K_d = \frac{\text{Concernration of chermical in soil}}{\text{concentration of chemical in water}} \tag{2.2}$$

$$K_{oc} = \frac{Kd * 100}{\%\ \text{organic carbon}} \tag{2.3}$$

Higher $K_{OC}$ values correlate to less mobile organic matters while lower $K_{OC}$ values correlate to more mobile organic chemicals.

*2.1.2.6 Henry`s Law Constant-H`*

Henry`s Law Constant (HLC) is a measure of the concentration of a chemical in air over its concentration in water. It expresses the tendency of a material to volatilise from aqueous solution to air. Chemicals with a high HLC tend to volatilize from water and be distributed in the atmosphere. Meanwhile, a chemical with a low HLC will tend to accumulate in water and soil, rather than volatilize. It is sometimes measured, but more usually calculated as following:

$$H` = \frac{16.04*P*M}{T*S} \quad (2.4)$$

where P = Vapour pressure,

M = Molecular mass

T = Temperature

S = Solubility

*2.1.2.7 Persistence*

Organochlorines are known as persistent compounds in the physical environment and in organisms. The persistence of the compound in nature is determined from its halflife that might range from months to years, decades or even centuries. Thus, persistence is controlled by several physical properties such as temperature, pH, moisture and light. Pesticides with long halflives are considered to have high contamination potential due to their life duration.

### *2.1.3 The Current Status of OCPs*

The first organochlorine synthesized was DDT. Although it was first synthesized by Zeidler in 1874, it was not produced or used for many years. Mueller rediscovered DDT in 1939 and won the Nobel Prize for his efforts in 1948. The first major uses for DDT were vector control of typhus and malaria and control of lice and other pests during World War II. During the late 1940s, other organochlorine compounds were synthesized and came into general use, thus introducing the synthetic insecticide era (Dikshith & Diwan, 2003). From the 1940s through the 1960s, organochlorine insecticides were used widely to control insect pests in both agricultural and domestic settings and for vector control of some diseases such as malaria, typhus, that affecting human health.

The organochlorine pesticides have similar uses and histories. The insecticide DDT was banned in the United States of America (USA) and other industrialized countries in the early 1970s. It remains in restricted use for malaria control in developing countries (Buser & Muller, 1995). Technical HCH is another heavily used and ubiquitous pesticide introduced in World War II, and consists of three major isomers: α-HCH, the most abundant (60–70%) and the only chiral isomer β-HCH (5–12%) and the most bioaccumulative isomer; γ-HCH or lindane (10–12%), the active insecticidal isomer; and small amounts of other isomers.

The technical product of HCH was banned in the US and Canada during the 1970s, in Europe in the 1970s and 1980s, in China in 1983, and in the former Soviet Union in 1990. However, it continued to be used past 1990 in India and some African and South American countries (Li, 1999). Toxaphene, a heavily used pesticide banned in the 1980s, is an extremely complex mixture of mostly chlorinated bornane compounds. Technical chlordane, a complex mixture of more than 140 components (Dearth & Hites, 1991), was widely used as an agricultural insecticide and as a termiticide from the 1950s to the 1980s, at which time active use was either phased out or severely restricted in most countries.

In Turkey, OCPs have been used since 1945, large quantities of these chemicals were used during 1960s and 1970s (Cok et al., 1997) but the use of OCPs, including DDT, aldrin, endrin, dieldrin, heptachlor, HCH, chlordane, lindane and toxophane for agricultural purposes has been severely restricted since 1983. In spite of this restriction, high residue concentrations for OCPs still exist in many areas in Turkey (Erkmen, Yerli, Erkakan, & Kolankaya, 2013).

Pesticide usage in Turkey have been controlled by Ministry of Agriculture and Rural Affairs (MARA). Registration Committee of MARA register agricultural pesticides after studying the physical and chemical properties, residues, biological activity, toxicological and ecotoxicological properties with internationally approved analysis methods and techniques. MARA also monitors registered pesticides from their production or import to their consumption. Pesticide retailers are constantly

controlled by the Provincial and County Directorates of MARA responsible from market control. MARA has also carried out some monitoring studies on pesticides mainly on food samples affected by pesticides. A total of 1231 commercial pesticides had been registered in 1995, the picture changed in 2004 as 485 active ingredient and 3,006 commercial pesticides were registered in Turkey. However, due to various reasons only 252 active ingredients containing registered pesticides are commercially available.

Records from Undersecretariat of Foreign Trade and Undersecretariat of Customs show the fact that POPs substances have not been imported or exported after they had been banned. Monitoring of chlorine containing pesticides has being performed on agricultural products, soil, and streams. No illegal use of such substances has been detected. POPs pesticides ban was started in Turkey by 1970s, and their use, production, import, and export are prohibited by law. Dieldrin was banned in 1971, aldrin, chlordane, heptachlor, endrin were banned in 1979 and toxaphene was banned in 1989. Moreover, registrations of plant protection products containing these active ingredients were canceled. Mirex and its products have never been registered in Turkey (Gonul, 2011).

There is no record of production of POPs pesticides in Turkey since their ban. After registration, plant protection chemicals were being prepared by using imported active ingredients. Production of those active substances in Turkey has never been an issue. As required by the Stockholm Convention, necessary arrangements are initiated for frequent inspection by MARA.

The last known uses for some of the OCPs are summarised in Table 2.3 (Mörner, 1996). Data on the use of certain pesticides are difficult to obtain and may be unreliable. The table provides some insight for what purposes the POPs pesticides have been or are being used. In high-income countries, production and use of the pesticides on the initially agreed list of POPs has, for all practical purposes, already ended except for some products for termite control. Their use in low-income countries has been reduced, often because of growing trade ban on agricultural

produce containing pesticide residues. However, DDT and possibly a few other POP pesticides are still used in a number of countries. A significant portion of this use is that of chlordane and heptachlor for termite controland of DDT for the control of malaria vectors.

Table 2.3 Some OCPs last known uses

| POP pesticide | Last known uses |
|---|---|
| Aldrin | Against termites and other soil pests, termites attacking building materials, in grain storage, and for vector control |
| Toxaphene | Control of insect pests in cotton and other crops |
| Chlordane | Against termites and other soil pests, termites attacking building materials |
| DDT | Control of medical and veterinary vectors, such as malaria-transmitting mosquitoes, plague-transmitting fleas and trypanosomiasis-transmitting tsetse flies |
| Dieldrin | Control of locusts, termites, human disease vectors |
| Endrin | Formerly used against insects and rodents. No current or recent uses are known |
| Heptachlor | Against termites and other soil pests, termites attacking building materials |
| HCB | Formerly used for seed treatment against fungal diseases, as well as for industrial purposes. No current or recent agricultural uses are known. |
| Mirex | Against leaf-cutting ants, termites in buildings and outdoors, and also as a fire retardant and for other industrial purposes |

### *2.1.4 Toxicity of OCPs*

It is well known that, the widespread use of organochlorine compounds have caused serious problems to man due to their bioaccumulation in several organs. The majority of these compounds are toxic in high levels and few are carcinogenic in small animal tests (Fytianos, Vasilikiotis, Weil, Kavlengis, & Laskaridis, 1985).

The most common effects of the wide spread of organochlorine compounds in the environment are birth defects, neurological effects, behavioral effects, reproductive effects and cancer (Windham, 2002).

Among 24 chemicals targeted by the Stockholm Convention, listed in the annexes of the convention, there are 15 OCPs: aldrin, endrin, dieldrin, chlordane,

chlordecone, DDTs, heptachlor, mirex, toxaphene, endosulfan and isomers, hexachlorobenzene (HCB), alpha-hexachlorocyclohexane (α-HCH), beta-hexachlorocyclohexane (β-HCH), lindane and pentachlorobenzene.

OCPs have broad spectrum insecticidal activity and primarily act as excitatory neurotoxins, by altering the movement of ions across the nerve cell membranes, thus changing theability of the nerve to fire. For example, DDT act through neurotoxicity by causing repetitive firing of nerve cells and makes them unable to fire in response to a signal. Lindane kills insects by stimulating the central nervous system (CNS) causing trembling, hyperexcitation, loss of coordination, paralysis and eventually death. Cyclodienes act by blocking the chloride channel of gamma aminobutyric acid (GABA) receptors and inhibiting GABA-ergic neurotransmission. Dicofol inhibits electron transport during energy-producing mitochondrial electron transport and oxidative phosphorylation. By interrupting this process the insects fail to store energy for the later use. Pentachlorophenol acts mainly through an inhibition of phosphorilative oxidation, with a consequent block in energy storage and the fungicide HCB acts through an inhibition of porphyrins metabolism (Mrema et al., 2014).

The diphenyl aliphatic organochlorines, such as DDT, affect the peripheral nerves and brain by slowing sodium ($Na^+$) influx and inhibiting potassium ($K^+$) outflow. This results in excess intracellular $K^+$ in the neuron, which partially depolarizes the cell.

Organochlorine insecticides can be absorbed orally and topically, with absorption being rapid due to the lipid solubility of these compounds and persist in the environment. Organochlorine insecticides are not highly volatile, so inhalation is not a normal route of exposure (Jaeger, Conolly, and Murphy, 1973). As a result bioaccumulation occurs in the food chain from the environment to animals and humans. With the ability of these compounds to persist in the environment and bioaccumulate, the organochlorine insecticides will continue to be an important toxicant for many years (Custer et al., 2005).

Table 2.4 Health hazards and exposure limits of common organochlorine insecticides

| **Organochlorine insecticides** | **Target organs**[a] | **OSHA-PEL**[b] **(mg m$^{-3}$)** | **ACGIH-TLV**[c] **(mg m$^{-3}$)** | **TLV-basis** |
|---|---|---|---|---|
| Aldrin | Central nervous system, liver, kidneys, skin | 0.25 (skin) | 0.05 | Central nervous system impairment; liver and kidney damage |
| Chlordane | Central nervous system, eyes, lungs, liver, kidneys | 0.5 (skin) | 0.5 | Liver damage; (skin)[e] |
| DDT | Eyes, skin, central nervous system, kidneys, liver, peripheral nervous system | 1 (skin) | 1 | Liver damage |
| Dieldrin | Central nervous system, liver, kidneys, skin | 0.25 (skin) | 0.25 | Liver damage; central nervous system convulsion; (skin) |
| Endrin | Central nervous system, liver | 0.1 (skin) | 0.1 | Liver damage; central nervous system impairment; headache; (skin) |
| Heptachlor | Central nervous system, liver | 0.5 (skin) | 0.05 | Liver damage; (skin) |
| Lindane | Eyes, skin, respiratory system, central nervous system, blood, liver, kidneys | 0.5 (skin) | 0.5 | Liver damage; central nervous system impairment; (skin) |
| Toxaphene | Central nervous system; skin | 0.5 (skin) | –[d] | - |

[a]National Institute for Occupational Safety and Health (NIOSH), NIOSH Pocket Guide to Chemical Hazards.

[b]OSHA-PEL: Occupational Safety and Health Administration – Permissible Exposure Limit based on 8-h time-weighted average.

[c]ACGIH-TLV: American Conference of Governmental Industrial Hygienists – Threshold Limit Value based on 8-h time-weighted average.

[d]Not available.

[e]The designation 'skin' refers to the potential significant contribution to the overall exposure by the cutaneous route, including mucous membranes and the eyes.

The acutely toxic dose varies considerably with the specific OC. In general the OCs are less acutely toxic than organophosphates or carbamates used for similar purposes. For example, the most potent OCs have a mammalian acute median lethal dose (LD50) of approximately 50 mg/kg body weight, 10-fold to 100-fold larger than the LD50 of the carbamate, aldicarb. Within the class, the cyclodienes (e.g., heptachlor, chlordane, and dieldrin) are generally most toxic, followed by the aryl hydrocarbons (e.g., lindane and methoxychlor), and finally by the diphenyl aliphatics (DDT, etc.). Because of their lipophilicity, both oral and dermal exposures may result in notable systemic effects. Their low volatility usually precludes significant respiratory exposure unless aerosolized (Raisbeck, 2006).

The acute health effects from the exposure of organochlorine insecticides include irritation of the nose, throat, and skin, causing burning, stinging, and itching as well as rashes and blisters (Table 2.4). Nausea, vomiting, dizziness, and diarrhea also have been observed (Peterson, Lewne, & Walum, 1981).

### *2.1.5 DDT and its Derivatives*

DDT (1,1,1-trichloro-2,2-bis(*p*-chlorophenyl)ethane) is a pesticide that was once widely used to control insects on agricultural crops and insects that carry diseases like malaria and typhus, but now it is only used in a few countries to control malaria. Technical-grade DDT is a mixture of three forms, *p,p'*-DDT (85%), *o,p*'-DDT (15%), and *o,o*'-DDT (trace amounts). The chemical formulas, structures, and identification numbers for *p,p'*-DDT, *p,p'*-DDE, *p,p'*-DDD, *o,p'*-DDT, *o,p'*-DDE, and *o,p'*-DDD are listed in Table 2.2. The latter five compounds are either impurities or metabolites of technical DDT.

All of these are white, crystalline, tasteless, and almost odorless solids. Technical-grade DDT may also contain DDE (1,1-dichloro-2,2-bis(*p*-chlorophenyl)ethylene) and DDD (1,1-dichloro-2,2-bis(*p*-chlorophenyl) ethane) as contaminants. DDD was also used to kill pests, but to a far lesser extent than DDT. One form of DDD (*o,p'*-

DDD) has been used medically to treat cancer of the adrenal gland. Both DDD and DDE are breakdown products of DDT.

DDT does not occur naturally in the environment. After 1972, the use of DDT was no longer permitted in the USA except in cases of a public health emergency. However, it is still used for controlling malaria in some areas of the world. The use of DDD to kill pests has also been banned in the United States.

When we refer to DDT, we are generally referring to *p,p'*-DDT, which was produced and used for its insecticidal properties. Although, technical grade DDT, the grade that was generally used as an insecticide, was composed of up to fourteen chemical compounds, of which only 65–80% was the active ingredient, *p,p'*-DDT. The other components included 15–21% of the nearly inactive *o,p'*-DDT, up to 4% of *p,p'*-DDD, and up to 1.5% of 1-(*p*-chlorophenyl)-2,2,2-trichloroethanol (Metcalf, 1995).

Table 2.5 Frequency of occurrence of pesticide residues found in total diet study foods by FDA in 2003[1]

| Pesticide | Total No. of Findings | Occurrence (%) | Range (ppm) |
|---|---|---|---|
| DDT | 123 | 12 | 0.0001–0.171 |
| Malathion | 71 | 7 | 0.0006–0.121 |
| Endosulfan | 70 | 7 | 0.0001–0.116 |
| Dieldrin | 64 | 6 | 0.0001–0.141 |
| Chlorpyrifos | 50 | 5 | 0.0002–0.110 |
| Carbaryl | 20 | 2 | 0.0003–0.190 |
| Methamidophos | 19 | 2 | 0.0002–0.123 |
| Diazinon | 19 | 2 | 0.0002–0.043 |
| Indane | 16 | 2 | 0.0001–0.007 |

[1]Based on four market baskets analyzed consisting of 1039 total items; only those found in more than 2% of the samples are shown.

However of its banded in the United States since 1972, DDT stills one of the best-known synthetic pesticides. DDT is a very non polar molecule so it has high lipid solubility. Since DDT is also extremely stable, it accumulates in animal tissues and

in the food chain. As illustrated in Table 2.5, DDT is still one of the most abundant pesticides found in total diet study foods by FDA in 2003 (Shibamoto & Bjeldanes, 2009).

### *2.1.6 Input of Contaminants*

Generally, there are three main routes; direct, riverine and atmospheric inputs by which contaminants (such as heavy metals, organic compounds and radioactivity) inter to the marine environment. The relative importance of individual input routes differs between and within regions, and also for individual contaminants. For large parts of the maritime area, especially the oceanic parts, the atmospheric transport source is prevailing.

Water is one of the primary pathways by which pesticides are transported from their application areas to other parts of the environment. Direct input of contaminants arises as a consequence of municipal and industrial outfalls in coastal waters and from offshore activities and dumping. The major impacts of direct inputs are likely to occur in coastal waters and especially in semi-enclosed areas and/or where water exchanges are low.

Riverine input is made up of the run-off from land and discharges into the rivers and their tributaries. Estimates of the flux of substances from riverine inputs are heavily dependent on river flow. Estuarine processes can also significantly modify the level of inputs to the marine environment. This makes it difficult to interpret trends and to make regional comparisons.

Atmospheric input is the dominant source to the marine environment for several substances including mercury, lead, POPs and some nitrogen compounds. Quantification of atmospheric input especially for POPs, is affected by large uncertainties. Some substances have a relatively short residence time in the atmosphere and are consequently deposited close to their sources, while others

(mercury, POPs) can be transported on a global scale through the atmosphere. Main sources of chlorinated pesticides are given in Table 2.6 (Gonul, 2011).

Table 2.6 Main sources of chlorinated pesticides to the various environmental vectors and compartments

| | Air sources | Water sources | Soil sources | Sea |
|---|---|---|---|---|
| Chlorinated Pesticides | -Agricultural usage<br>-Spraying/Land application<br>-Production | -Agricultural usage<br>-Run-0ff<br>-Production | -Stockpiles<br>-Production waste<br>-Misuse | -Agricultural run-off<br>-Major rivers and drains<br>-Atmospheric Deposition |

### *2.1.7 Movement of Pesticides*

The five processes about the pathways for (of) pesticide movement in the environment are;

- Transformation/Degradation
- Retention or Sorption
- Transport
- Uptake
- Volatilization

#### *2.1.7.1 Transformation*

One of the main concerns when dealing with pesticides is the transformation process. When pesticides enter the soil and groundwater they can be degraded, transformed, or stored by micro-organisms, plants, and animals. According to Shukla, "the term transformation includes all the changes in the chemical structure or composition of the pesticide" (Somasundaram & Coats, 1991).

There are three main transformation processes:

- Microbial processes
- Chemical processes
- Photochemical processes

When pesticides are absorbed into the soil they affect the metabolic processes in soil micro organisms. Microbes are a major controlling factor in the subsurface movement of pesticides because they break down the pesticide into $CO_2$ and $H_2O$ molecules. The five basic processes involved in the microbial transformation of pesticides are: biodegradation, polymerization or conjugation, cometabolism, accumulation, and secondary effects of microbial activity.

Major factors influencing microbial population are:

- Temperature
- Organic matter content
- Cation exchange capacity
- pH
- Aeration
- Soil moisture status

It is very difficult to distinguish between chemical transformations and microbial transformations.

Chemical transformation of pesticides takes place as soon as they enter the water. There are several physical and chemical properties that affect chemical reactions in water. These include pH, buffering, temperature, general acid and base catalysis, metal ions, dissolved organics and suspended solid, and the redox state of the water column. The type of soil to which the pesticide is applied also affects the chemical transformation. Redox and hydrolysis reactions in sediments are generally considered to be the most important modes of transformations. Photochemical transformation requires sunlight so it is considered as an important degradation pathway for many pesticides, especially those that are surface applied.

*2.1.7.2 Retention*

Retention, commonly known as adsorption is the soil's ability to retain or hold the pesticide on its surface. Several soil propereties affect the adsorption rate of a

pesticide as pH, moisture content, clay content, oxide content, cation exchange capacity, specific surface area, and organic matter content.

Retention affects the characteristics of the pesticides for several reasons. First, adsorption provides the pesticide with a place that it can collect and force chemical transformations. These chemical changes can create very complex problems that may make remediation very difficult. Second, adsorption can also affect the pesticide's ability to be transported. Simply, the more pesticide adsorped by the soil, the less that will be transported (Schreiber, Smith, & Cooper, 1996).

*2.1.7.3 Uptake*

Plant uptake is the process whereby pesticides are transported into and within the plant's structure. This process can be separated into two notable methodic pathways, sorption by the roots of the plant, and adsorption with subsequent movement to the plant's surface structure. The most important factor governing sorption and movement within the plant is the solubility of the pesticide in water. The content of the surrounding soil is also important to the plant uptake. For non-polar pesticides the volume of organic matter is particularly important. Other factors such as pH and clay and microbial activity are more important as the polarity of the pesticide increases. The accumulation of pesticide through plant uptake can have severe consequences through the food chain if the pesticide is translocated into the section of the plant that will be subsequently harvested.

*2.1.7.4 Transport*

A pesticide can be transported by different ways. The most common are 'mass flow' and 'diffusion'. Mass flow is when a pesticide is transported by a flow of water in which the pesticide has been retained and can transport the pesticide molecules very quickly over large distances. Mass flow in a soil is a function of the rate of water movement and the soil's characteristics. Diffusion occurs in a much more random manner than mass flow. It occurs when a pesticide travels from an area of

high concentration to an area of low concentration by way of random molecular movement. Due to its randomness, diffusion is a much slower process than mass flow. It is dependent upon the soil's characteristics and the molecular structure of the pesticide (Camp & Sadler, 1997).

### *2.1.8 Review of Literature*

Related works in the region concern in OCs in sediment, biota and water due to their toxicity and accumulation in biota and sediment, determination the levels of OCs in sediment and fish species have received considerable attention in different countries in the region and around the world. Such interest aimed at ensuring the safety of the food supply and minimizing the potential hazard effect on human health. Some of the important documented contributions relevant to the present study are as follows:

Kucuksezgin, Pazi, Gonul, & Duman (2016) determined the organochlorine (OCs) concentrations in sediments samples collected from different regions of northern coast of Cyprus. The results appeared that DDE was the most abundant compound among all OCs contaminants. The sediment quality guidelines (SQG) implied that, Aroclor1254 exceeded the TEL values, showing that adverse biological effects are expected occasionally at nearly half of the sampling points. However, DDTs were dominant and more ecotoxicological concern in the northern Cyprus. In addition, authors in their abstract mentioned to the probability of DDTs to impose ecologically hazardous impacts in the sedimentary environment at the study areas.

In another work, Kucuksezgin & Gonul (2012) investigated the levels of organochlorine pesticides and polychlorinated biphenyls in sediment from the Eastern Aegean coast. The results indicated that DDTs were the predominant contaminant in sediments. DDE was the most often found OCP at all stations. They attributed the sediment contamination by DDTs to the aged and weathered agricultural soils and to the transportation of these contaminants by surface run off from the rivers. Moreover, they pointed to the noticeably higher concentrations of

OCPs and PCBs at Izmir Inner Bay than the other sites. Also, according to established sediment quality guidelines, they concluded that the risk of adverse biological effects from the levels of PCBs at Candarli and Izmir Inner Bay sites should be significant.

Pazi, Kucuksezgin, & Gonul (2011) investigated the distribution and sources of organochlorinated contaminants in sediments from Izmir Bay (Eastern Aegean Sea). It was found that the sites of high concentrations of organochlorine compounds were associated with dense population, such as the harbour and the Gediz River estuary. ΣDDT concentrations and high DDT/(DDE + DDD)ratios in the Gediz River estuary indicated DDT usage. Also, it was concluded that the risk of adverse biological effects from such levels of OCPs and PCBs at most of the study sites was insignificant. But the higher concentrations in Inner bay and in the Gediz River estuary could cause biological damage.

Ayas, Barlas, & Kolankaya (1997) studied organochlorine pesticide residues in various environments and organisms in Göksu Delta (Turkey). Researcher confirmed that OC pesticide residues were accumulated more in adipose tissues than in liver of fish.

In a study performed by Pazi, Kucuksezgin, & Gonul (2012) to assess the occurrence and distribution of organochlorine residues in surface sediments of the Candarli Gulf (Eastern Aegean) results showed that 39% of the sites originated from recent DDT inputs. On the other hand they showed that, according to ERL/ERM guidelines, DDT and PCBs posed ecological risk to the bottom-dwelling consumers.

The contamination levels of organochlorine pesticides in water and sediment samples in Uluabat Lake (Turkey) were studied by Barlas, Cok, & Akbulut (2006). Results demonstrated that Uluabat Lake was still contaminated by organochlorine pesticides and their residues despite the existence of bans over a long time. Also it was shown that considerable amounts of organochlorine pesticides (HCB, p.p'-DDT, p.p'- DDE, α-, β- and γ -BHC, Aldrin, Heptachlorepoxide, Endrin, Endosulfan I and

II) were detected in water and sediment samples during one-year study in this lake and residue analyses showed that higher organochlorine pesticides and their residues were generally more accumulated in the sediment samples.

Eighteen fish species were examined by Kalyoncu, Agca, & Aktumsek (2009) to determine the levels some organochlorine pesticide residues in commercial fish species in Konya market, (Turkey). Results appeared that the 14 different organochlorine pesticides were detected in all fish species, except in trout, horse mackerel and bonito. Also, DDT and its metabolites and HCH were the predominant contaminants in fish muscles. The researchers concluded that the consumption of these fishes had no significant health risks for consumers.

Stefanelli et al. (2004) studied the organochlorine compounds in tissues of swordfish (Xiphias gladius) from Mediterranean Sea and Azores islands. Results showed that among organochlorine pesticides DDE, DDT and DDD (TDE) predominated with an overall range of 2.37–4734.56 ng/g ww. In particular p,p'-DDE had concentrations appearing up to 3900 ng/g, with the highest values found in fatty tissues, such as blubber. Stefanelli et al. (2004) concluded that organochlorine contaminant concentrations in tissues of swordfish from Mediterranean Sea decrease in the order blubber > liver > gonad > muscle. The factors affecting the level of contamination in tissues from marine biota are related to the fat content and the metabolic characteristics of the tissues.

Gomez-Gutierrez, Jover, Bodineau, Albaiges, & Bayona (2006) estimated the organic contaminant loads into the Western Mediterranean Sea. Results indicated that the levels of OCPs in particulate and dissolved phases. Furthermore, based on the contaminant concentrations and on hydrological data, contaminant discharges into the sea were estimated amounting in total to 167 and 1258 kg $year^{-1}$ of organochlorine compounds and polar pesticides, respectively. It was observed that PCBs, DDTs and HCB inputs were basically influenced by flood periods due to an increase in suspended particulate matter associated to runoff and sediment

resuspension. Also, it was concluded that DDT still predominates over its degradation products, indicating recent inputs of this banned product.

Bastürk, Dogan, Salihoglu, & Balkas (1980) determined levels of DDE, DDT and PCBs in four different commercial bony fishes as well as in shrimps, limpets and sediments obtained from the eastern Mediterranean coast of Turkey. Results showed that the PCB levels in living organisms and sediments were very low, and in most cases below the detection limits. However, the DDE and DDT values were relatively high compared to PCBs and there was a linear correlation between the organochlorine residue concentrations and the extractable organic material of the analysed samples.

Erkmen & Kolankaya (2006) determined organochlorine pesticide residues in water, sediment, and fish samples from the Meric Delta (Turkey). Results indicated that the concentration of organochlorine pesticides in fish samples were higher than in water and sediment samples. Researchers attributed this to the low water solubility of the organochlorine compounds, it is expected that any organochlorine pesticides present in the study area, will be preferably adsorbed to sediment or bioaccumulated in fish.The concentration levels of p,p'-DDT in all analysed fish samples were consistently higher than its metabolite p,p'-DDE, indicating a recent use of this organochlorine pesticide in the area.

Turgut (2003) performed a study to detect the contamination with organochlorine pesticides and heavy metals in surface water in Kucuk Menderes River in Turkey. The results showed that Kucuk Menderes River was still polluted with organochlorine pesticide despite the existence of bans over a long time. The concentrations of pesticides depended on the sampling season and the tendency was not the same for all detected pesticides. The DDTs (DDT, DDD, DDE) were detected in most water samples.

Fillmann et al. (2002) assessed the extent of contamination of the Black Sea by organochlorine compounds. In that study the DDE/DDT ratio was low indicating fresh inputs and hence current usage of DDT within the Black Sea region.

## 2.2 Hydrophobic Interaction Chromatography (HIC)

### *2.2.1 Definition and Principle of (HIC)*

Hydrophobic interaction chromatography (HIC) separates molecules based on their hydrophobicity. HIC is a useful separation technique for purifying proteins while maintaining biological activity due to the use of conditions and matrices that operate under less denaturing conditions. HIC has been used in biomolecules separation and purification for a long time. Nowadays, it has become a powerful method at the laboratory scale and in industry. HIC was first used in 1949 by Shepard and Tiseliusin who described the method as "salting-out" (Cronin, 2006). The term "hydrophobic interaction chromatography" was first used by Hjerten (1973). When salt was used to enhance hydrophobic adsorption, Porath proposed alternative name and described it as "salt-promoted adsorption chromatography" (Queiroz, Tomaz, & Cabral, 2001).

Hydrophobicity can be defined as the repulsion between a nonpolar compound and a polar environment as water (Fig. 2.1) (Nemethy & Scheraga, 1962) . When a non-polar compound is inserted into water, a decrease in entropy ($\Delta S<0$) can be observed this can be attributed to the overall increase in the degree of order of water molecules surrounding the hydrophobic group. Since the enthalpy change ($\Delta H$) is small compared with the $T\Delta S$ value, according to Equation 1, there is a positive change in free energy ($\Delta G>0$).

$$\Delta G=\Delta H-T\Delta S \qquad (2.5)$$

Therefore, because it is thermodynamically unfavorable, the process of dissolving of a non-polar compound in polar solvent such as water does not occur

spontaneously. On the other hand, if two or more non-polar molecules are inserted into polar medium like water these molecules will spontaneously associate together due to their hydrophobic interactions. An increase in entropy ($\Delta S>0$) is observed, resulting from a displacement of the ordered water molecules around the non-associated hydrophobic groups to the more unstructured bulk water (Uçar, 2012; Queiroz et al., 2001).

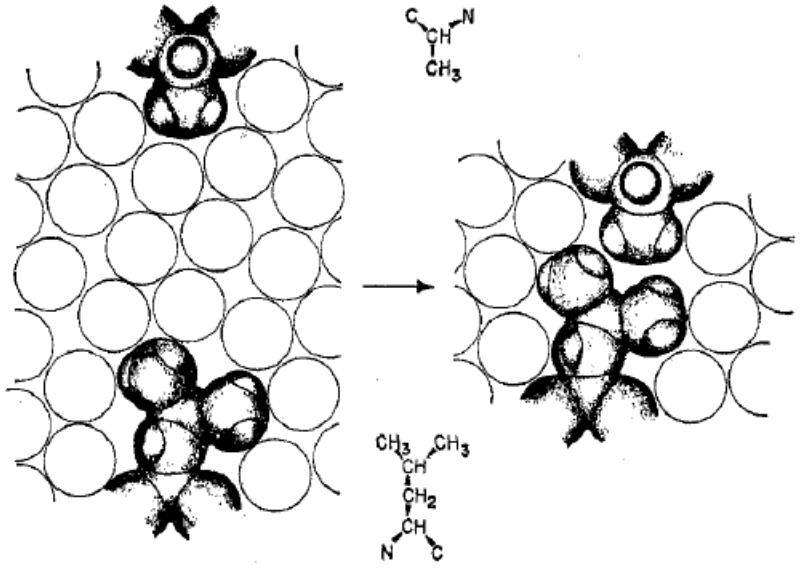


Figure 2.1 Schematic representation of the formation of hydrophobic bond between two isolated side chains (leucine and alanine). The bond is formed through an approach of the two side chains until they contact, with a reduction of the number of nearest water neighbors. The extent of contact shown here is less than the maximum obtainable for the two side chains (Nemethy and Scheraga, 1962)

This implies a negative change in free energy ($\Delta G<0$) and a thermodynamically favorable process, which means that the interaction between two or more hydrophobic molecules in aqueous solutions proceeds spontaneously and is mainly determined by the change in entropy (Lewin, 1974; Jennissen, 1976; Ochoa, 1978; Tanford, 1980; Shansky, Wu, Figueiroa, & Karger, 1990).

To obtain success in a chromatographic process for a specific application, two major elements have to be considered a fluid mobile phase and a stationary phase. The various kinds of stationary phases could differ on the ligand chain length, the type of ligand, ligand density and on the type of matrix or support. The most widely used ligands for HIC are linear chain alkanes with or without a terminal amino

group. Phenyl (and other aromatic groups) is also used as a ligand with good results due to mixed hydrophobic and aromatic (π- π) interactions. At a constant degree of substitution on the matrix the n-alkane ligands constitute a homologous series in a hydrophobicity scale (Tanford, 1972):

methyl < ethyl < propyl < butyl < pentyl < hexyl < heptyl < octyl

Ligands with linear chain alkanes (as butyl, octyl) and some aromatic groups (such as phenyl) are the most widely used ligands. The increase in the chain length of an alkyl ligand increases the strength of hydrophobic interaction between the non-polar and the resin; meanwhile, the specificity of the resin towards the adsorbed protein is changed (Er-el, Zaidenzaig, & Shaltiel, 1972). However, resolution decreases when chain length is higher (Queiroz et al., 2001). Due to the higher probability of forming multipoint attachment, the increase in the substitution degree of the resin leads to an increase in the binding capacity of the stationary phase; but it can be hard to elute the bound protein without denaturation (Hjerten, Rosengren, & Pahlman, 1974). Using the same type of ligand and the same type of base support, the selectivity of an HIC resin can be manipulated by changing the ligand density (Lin, Chen, Ruaan, & Huang, 2000).

The density of the ligands at all HIC gels has to be optimized, and not always the highest amount of immobilized ligand results in the best chromatographic performance. A very high density of ligands, even weak hydrophobic ones, will reduce the recovery and the resolution. Therefore, the immobilization technique for the production of HIC gels must be kept highly reproducible. The steric accessibility of the ligand itself is also directly related to a suitable capacity of an HIC gel since the ligand itself must interact in a selective mode with the compound to be isolated. Thus, spacers or linear polymer chains (tentacles) were introduced to enable an appropriate spacing of functional groups. In graft polymerizations which are a powerful modification methods, a flexible polymer chain can be covalently attached to chromatographic supports and thus provide favourable binding properties. In

contrast to spacers, such flexible polymer chains also result in minimized non-specific interactions with proteins.

The non-polar compound retention in HIC depends not only on the stationary phase but also on characteristics of the mobile phase, such as the type and concentration of the salt, pH, temperature and additives (Queiroz et al., 2001).

### *2.2.2 The Effect of pH on HIC*

Low pH strengthens the hydrophobic interaction and increase the retention of biomolecules. On the other hand, high pH weakens the hydrophobic interaction between biomolecules and the hydrophobic ligands, due to a change in the proteins charge and hydrophobicity (Hjerten 1973; Hjerten et al., 1974). Hydrophobic interactions are maximized when the electrostatic repulsion between the proteins and biomolecules and ligand reaches a minimum (Lienqueo et al., 2007). The control of pH value is one of the dominant factors in determining the retention of biomolecules in the column.

### *2.2.3 The Effect of Temperature on HIC*

Increasing the temperature enhances hydrophobic bonding while lowering the temperature reduces the hydrophobic interaction (Hjerten, 1973; Hjertén et al., 1974). When temperature is raised, the stability of nanoparticles is increased by van der waals and hydrogen bond interaction and thus enhance the hydrophobic interaction (Ochoa, 1978). As it was explained before, in the process of HIC entropy is always positive and free energy is negative during this spontaneous process. Thus, the higher the temperature, the larger the free energy and the longer the retention time in the column (Queiroz et al., 2001). In other words, the HIC is an entropy-driven process, as mentioned before, where the Gibbs free energy is giving according to the Eq. (1). Since $\Delta H$ may be a negative or small positive value, $\Delta G$ is controlled by a positive entropy change and thus increases with temperature. Moreover, by

increasing the temperature of nanoparticles their Brownian motion would be increased and thus enhance their hydrophobic interaction.

### *2.2.4 The Effect of Salt on HIC*

The desired precipitation and separation of proteins can be increased with the follow of Hofmeister series (Porath, 1987). The ion which is more close to the left-hand side of the series (such as $PO4^{-3}$, $NH4^{+}$ etc) can promote hydrophobic interactions and increase surface tension which increases the retention time and protein separation (Fig.2.2). Nevertheless, as ions move to the right-hand side of the series (such as $SCN^{-}$, $Ba^{+2}$ etc), the strength of hydrophobic interaction is decreased (Xia, Nagrath, Garde, & Cramer, 2004). In like manner, the retention of nanoparticles can follow the Hofmeister series which was used in protein separation.

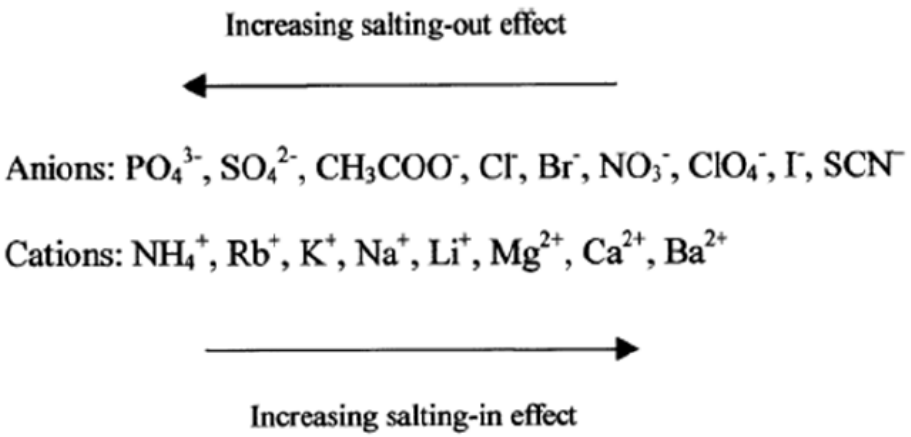



Figure 2.2 Illustration of the Hofmeister series (Porath, 1987)

The salts at the beginning of the series are called antichaotropic ions as they promote hydrophobic interactions and protein precipitation (salting-out effect). They are considered to be water structuring, whereas salts at the end of the series are called chaotropic ions as they randomize the structure of the liquid water and thus tend to decrease the strength of hydrophobic interactions and protein presipitation (salting-in) (Porath, 1987).

The concentration of salt used during HIC strongly influences not only the strength of hydrophobic interaction between the ligand and the biomolecules but also the selectivity of proteins adsorbed (Oscarsson & Karsnas, 1998). A moderately high salt concentration can increase ionic strength and surface tension, promoting the interaction between the biomolecules and the ligand attached onto the medium. The increase in retention time improves protein separation (Queiroz et al., 2001). For nanoparticles, the consideration for salt concentration is also important. Critical concentration for nanoparticles to form agglomeration is around 100 mM (Jiang, Oberdörster, & Biswas, 2009).

### *2.2.5 Review of Literature*

Environment pollution by pesticides during routine agricultural practices is a common and growing problem in major agricultural areas of the world .Several techniques for the removal of pesticides are known such as; various oxidation processes, membrane filtration, and adsorption onto different media are applied. Related to present study, some of researches utilized adsorbents for the extraction of pesticides from environmental matrices are given as following:

Jing, Yang, & Zongshan (2014) performed a study to remove organochlorine pesticides from aqueous systems by magnetic nanospheres coated with polystyrene ($Fe_3O_4$ @PS). The adsorption results showed that pesticides could be effectively adsorbed and the adsorption equilibrium time was less than 20 mins. The effect of salinity and humic acid was also studied and the results illustrated that they could be neglected under optimized conditions. The sorbent showed a good performance with more than 93.3% pesticides removal in treating actual water.

Uçar (2012) used a synthesized nanostructure (HEMA-MAT) polymer as adsorbent for deltamethrin pesticide from water samples. Maximum adsorption capacity was found to be 315.6 mg/g and the adsorbed amounts per unitmass of nanostructures reached to a plateau value at about 800 mg/l. It was observed that

deltamethrin in real water samples such as river and ground waters were determined and removed efficiently using these nanostructures.

Shahin (2004) carried out a research to determine some chlorinated pesticides levels in Jordanian ground and surface waters using a plainpolystyrene-divinylbenzeneresin as solid phase extraction medium. The results obtained from examining the effect of matrix on the extraction efficiency of pesticides indicated an increase in the concentration of the spiked pesticides in the field samples compared to MilliQwater, most probably due to matrix effects, i.e. presence of other organic compounds inthe water samples that modified the surface onto which the pesticides were adsorbed during the chemical analysis.

Kyriakopoulos, Doulia, & Anagnostopoulos (2005) used two porous polymeric adsorbents for the adsorption of some herbicides alachlor, amitrole, trifluralin and prometryn. The first adsorbent resins was the highly hydrophobic Amberlite XAD-4 (polystyrenedivinylbenzene copolymer) while the second was the functionalized more hydrophilic XAD-7(nonionic aliphatic acrylic polymer). Results approved that XAD-4 resin is generally more effective for hydrophobic herbicides, such as alachlor, prometryn and trifluralin, while XAD-7 resin is preferable for more hydrophilic molecules (such as amitrole molecule). The effect of low pH was positive in herbicides adsorption on both resins revealing the development of H-bonding and polar interactions at the herbicide–resin interface. Finally, it was concluded that the abnormal adsorption behaviour of alachlor and amitrole with temperature should be due to their high solubility in water.

Tepus, Simonic, & Petrinic (2009) compared between the nitrate and pesticide removal from ground water using novel adsorption resins (DowexOptipore L493 and Lewatit VP OC 1064 MD PH), and reverse osmosis (RO) and nanofiltration (NF) membranes. According to the results, the first of the mentioned adsorption resins (DowexOptipore L493) was successfully used for pesticides' removal among the tested adsorption media, whereas the removal of nitrates was unsuccessful. The initial concentration being about 0.2 µg/l for each measured pesticide.

Baruch-Teblum, Mastai, & Landfester (2010) performed a research aimed to design an efficient technology for water purification from organic contaminants by a new class of polymeric nanospheres based on cyclodextrins as building blocks. A series of cross-linked cyclodextrin polymeric nanospheres of different sizes were synthesized by a unique method, miniemulsion polymerization. These cyclodextrin nanospheres exhibit a high ability to absorb aromatic organic molecules such as toluene and phenol. Results showed that sorption experiments in solutions with high concentrations of corresponding organic molecules show a high adsorption capacity.

Youssef, Malhat, & Abd El-Hakim (2013) prepared and utilized a polysterene Nanocomposites Based on $TiO_2$ nanowires to remove organochlorine pesticide (OCPs) residues from contaminated water. Results showed that the pristine $TiO_2$ nanowires has a poor affinity for removing OCPs residues (from 8.82 to 70%), whereas polystyrene/TiO2-NWs nanocomposites are powerful material for removing OCPs residues (61.76 to 98.64%) because of the changing of $TiO_2$ nanowires surfaces from completely hydrophilic to hydrophobic structure as proven by contact angle results. These nanocomposites materials can be used for packaging application and the mainly application of these materials in a water filter, which may be a promising and economically feasible technology for wastewater purification.

# CHAPTER THREE
# MATERIAL AND METHODS

## 3.1 Study Area

Fish and sediment samples in recent study were collected from two regions in western Turkey, the first is Izmir Bay and the second is Çandarlı Bay (Fig. 3.1). Izmir Bay is one of the great natural bays of the Mediterranean. The Izmir Metropolitan Municipality has responsibility for an area covering 88,000 ha with a population of close to three million inhabitants. Izmir is an important industrial and commercial centre and cultural focal point. Pollution in the Outer Bay is not significant according to the most pollution indicators. The Gediz River, which flows into the Outer Bay, is the second biggest river along the Eastern Aegean coast and densely populated and includes extensive agricultural lands and numerous industrial areas. The Inner Bay is heavily polluted by nutrients and organic material (UNEP, 1993). The main sources of pollution are domestic and industrial effluents (Kucuksezgin, 2011).

Çandarlı Bay (38.67º -38.95ºN, 26.83º–27.08ºE) is a semi-enclosed bay in the eastern Aegean. Great industry settlements located in the coastal area of Çandarlı, have been discharging their solid and liquid wastes into Bakırçay or Çandarlı Bay after limited treatment. The intensive maritime traffic and untreated domestic discharges from 200,000 inhabitants around the bay area are the other factors that influence sea contamination (Pazi et al., 2012). Aliaga town located in the southern part of Çandarlı Bay, has been subjected to extensive industrial developments and is a ship dismantling area. There are iron and steel factories, coal storage yards, fuel storage yards, fertilizer factories, natural gas power plant, electrical substations, small industrial areas, and other medium and small establishments in the region (Pazi et al., 2012; Sponza & Karaoglu, 2002).The Nemrut Bay, the neighboring marine environment of Aliağa town, is a subsystem within the Çandarlı Bay ecosystem and connects to the northern Aegean Sea. The Nemrut Bay is considered with

anthropogenic pollution and sediments from the Nemrut Bay is highly contaminated by Hg, Pb, As and Zn (Esen, Kucuksezgin, & Uluturhan, 2010).

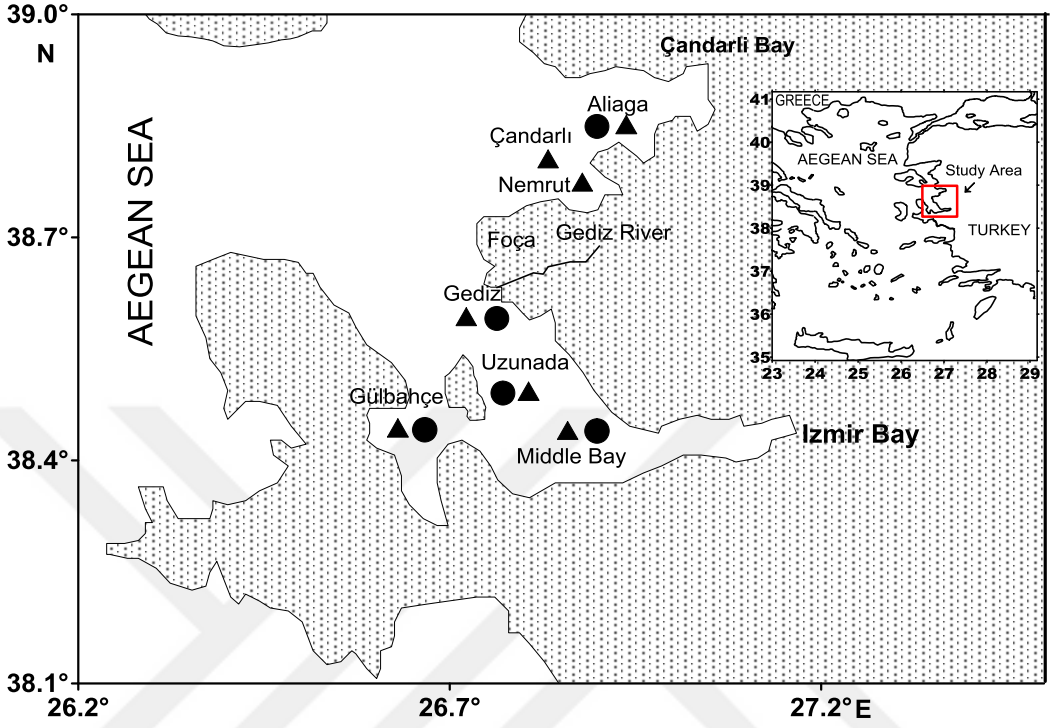



Figure 3.1 Biota (●) and sediment (▲) sampling sites in Izmir and Çandarlı Bays

### 3.2 Sample Collection

*Mullus barbatus* species were collected by trawling from five sites in İzmir (Gediz, Uzunada, Gulbahce, Middle Bay) and Çandarlı Bays during cruises of *R.V.* K. Piri Reis in 2014. Immediately, muscle tissues were dissected in the field and preserved at -20º until transferring to labs. Muscle tissues were freeze-dried using Labconco system and homogenized, then kept until chemical analysis.

Surface sediment samples were collected from the seven sampling sites (Gediz, Uzunada, Gülbahçe, Middle Bay, Çandarlı, Aliağa, Nemrut) using a box corer sampler, wrapped in aluminum foil and stored at -20ºC for subsequent analysis in order to avoid degradation in 2014. The samples were freeze-dried, hand-sieved

through 250 μm for grain size correction and homogenized, then kept until chemical analysis.

## 3.3 Experiments

### *3.3.1 Extraction of OCPs from Sediment Samples*

The methods used for the analysis of OCs in sediment are: "Manuals and Guidelines No 27. IOC, 1993", "Reference Method No 57, UNEP/IOC/IAEA/FAO: Contaminant monitoring programs using marine organisms: Quality assurance and good laboratory practice. (UNEP, 1990)".

#### *3.3.1.1 Cleaning of Reagents*

To avoid contamination, powdered or crystalline reagents such as anhydrous sulfate ($Na_2SO_4$), potassium hydroxide (KOH), glass wool were cleaned before use. They were extracted with mixture of hexane and dichloromethane (1:1) for 8 hours using soxhlet apparatus.

#### *3.3.1.2 Extraction of Samples*

Extraction of sediment samples (10 g) were extracted in microwave extraction system (1200W, temperature increases to 115ºC in 10 minutes and hold 20 minutes in this temperature) by adding 40 ml of a mixture of hexane/dichloromethane (1:1) and internal standards (PCB29, PCB198, Endosulfan Id4, ε-HCH; as recovery surrogates) to each sediment sample.

#### *3.3.1.3 Concentration of the Extract*

After extraction, the extracts were concentrated on a rotary evaporator to about 15 ml. The temperature of the water bath did not exceed 30°C. The extract was collected

in the graduated tube and adjusted to 6 ml by evaporating excess solvent under a gentle stream of clean dry nitrogen.

*3.3.1.4 Removal of Sulfur and Sulfur Compounds Using Copper*

40 g of copper powder were transferred in an erlenmeyer flask. Enough amount of concentrated HCl were added to cover the copper powder, the mixture was agitated, then sonicated for 10 minutes, after that it was agitated and put again in ultrasonic bath and sonicate for 10 min. The used HCl was thrown; some fresh HCl was added, transferred in ultrasonic bath and sonicated for 20 min. That procedure was repeated four times in total. The mixture was then washed with distilled water; agitated, discarded, water was added again, transferred in ultrasonic bath and sonicated for 15 min. The used water was discarded; the procedure was repeated up to pH neutral. The mixture was then washed with with acetone, agitated, transferred in ultrasonic bath and sonicated for 15 min. The procedure was repeated four times in total. Then the same procedure was used with hexane as a solvent. The active copper was kept in hexane to avoid Cu to be in contact with air (It was used immediately). Three to four Pasteur pipettes were transferred per sample in the flasks containing the hexane extracts. The copper was left to react with sulfur in samples all night. The presence of sulfur compounds in the sample will be detected by the tarnishing of the copper powder.

*3.3.1.5 Fractionation*

Before using, florisil was refluxed with mixture of hexane and dichloromethane (1:1) for 8 hours and it was then dried in a drying oven at 120ºC for four hours. Florisil was activated at 130ºC for 8 hours. A florisil column was used to separate extracted of each sample for three fractions.

The sulphur-free extracts were cleaned up and fractioned, using 17 g of florosil (bottom), 1 g of anhydrous sodium sulphate (top) in a column with 70 ml hexane (first fraction), 45 ml hexane-dichlorometane mixture (second fraction) and 70 ml

dichlorometane (third fraction). The first fraction contains the PCBs, pp' and op DDE and some other pesticides such as HCB, aldrin, heptachlor. The second fraction contains the DDTs, DDDs, most of the toxaphene and some pestisides as HCH isomers and chlordane components. The third fraction contains mainly dieldrin, endrin and endosulfan components. The eluates were collected and concentrated to 1 ml using nitrogen stream and injected to GC-MS. Same procedure was applied for both blanks and all samples. The detection limits for organochlorinated pesticides were 0.10-0.57 $pgg^{-1}$.

### *3.3.2 Extraction of OCPs from Fish*

*Mullus barbatus* fish species were collected, by trawling in April 2014 from five sites: four locations in İzmir Bay (Gediz, Uzunada, Gülbahçe, Middle Bay) and one in Çandarlı Bay. Immediately, muscle tissues were dissected in the field and preserved at -20º until transferring to our labs. Muscle tissues were freeze-dried using Labconco system and homogenized by blender until chemical analysis.

The methods used for the analysis of OCPs in fish are: "Reference Method No: 12 Rev.2, UNEP/FAO/ IAEA/IOC: Sampling of selected marine organisms and sample preparation for the analysis of chlorinated hydrocarbons. (UNEP, 1991)", "Reference Method No 6, UNEP/FAO/IOC/IAEA: Guidelines for monitoring chemical contaminants in the sea using marine organisms. (UNEP, 1993)", "Reference Method No 57, UNEP/IOC/IAEA/FAO: Contaminant monitoring programs using marine organisms: Quality assurance and good laboratory practice. (UNEP, 1990)".

#### *3.3.2.1 Extraction of Freeze-dried Samples*

5gr of dried biota sample were used for OCPs analysis. The sample was extracted with a microwave digestion system (1200W, temperature increases to 115ºC in 10 minutes and hold 20 minutes in this temperature) using mixture of 30 ml hexane/acetone (90:10). For recovery of the method, internal standarts (PCB29, PCB198, Endosulfan Id4, ε-HCH) were added to each sample.

*3.3.2.2 Concentration of the Extract*

After the extraction was completed, the extract was concentrated to about 10 ml with a rotary evaporator. The temperature of the water bath was not exceeding 30ºC. Then the extract was dried with sodium sulfate and concentrated with nitrogen down to 1 ml.

*3.3.2.3 Removal of Lipids by Concentrated Sulphuric Acid*

Whenever lipids present in significant amount, these compounds can interfere with gas-chromatographic separation. An appropriate volume of extract (100 µl) was taken to determine EOM (extractable organic matter) gravimetrically. To remove the lipid content of the extract which was more than 100-150 mg, concentrated sulfuric acid (about 5 ml) was used.

*3.3.2.4 Fractionation*

The samples were then transferred into florisil column which was precleaned with methanol and hexane and dried. Before fractionation, florisil was activated at 130ºC for 8 hours and deactivated with 0.5 % water. A florisil column separated the extractionof each sample for three fractions. The first fraction contains the PCBs, pp' and op DDEand some other pesticides such as HCB, aldrin, heptachlore. The second fraction contains the DDTs, DDDs, most of the toxaphene and some pestisides as HCH isomers and chlordane components. The third fraction contains mainly dieldrin, endrin and endosulfan components. A blank sample is prepared to determine any contamination during analyses and the same procedures are applied to the blank sample. The detection limits for organochlorinated pesticides were 0.10-0.57 $pgg^{-1}$.

***3.3.3 Gas Chromotography Conditions for Halogenated Hydrocarbons***

DB-5MS column 30m x 0.25mm, film thickness 0.25 µm
Carrier gas: Helium (99.99 % pure)

Inlet pressure: 13.728 kPa

Purge flow: 30 ml/min

Inlet temperature: 300ºC

Detector temperature: 250°C

Oven temperature program: Initially 70 °C for 2 min, then increased to 150 °C with a velocity of 25 °C/ min, increased to a temperature of 200 °C with a velocity of 3 °C/ min and up to 280 °C with a velocity of 8 °C/min held for 10 min.

MS Quad: 150ºC

MS Source: 230ºC

### *3.3.4 Quality Assurance for OCPs*

For every set of 10 samples, a procedural blank and spike sample consisting of all reagents is run to check for interference and cross contamination. The OCP recoveries are determined relative to the ratio of direct injection of extract and the working standards prepared in hexane. The recoveries fell with in a fairly narrow range, for internal standards between 70.0 and 96.1 %.

The final results are cited on a dry weight basis. Also, the quality of the analytical data is assured using the reference materials of IAEA-417 the sediment sample and IAEA-451 the fish sample (from the International Laboratory of Marine Radioactivity, IAEA, Monaco). The results for reference materials were given in Table 4.1 and 4.2. The whole methodology was verified on these reference materials, obtaining results in good agreement with the certified values.

### *3.3.5 Extraction of DDT Using Poly [HEMA-MAPA] Nanospheres*

#### *3.3.5.1 Materials Used for the Extraction of DDT Using Poly[HEMA-MAPA] Nanospheres*

*p,p'* DDT standard was supplied Sigma (St. Louis, USA). 2-Hydroxyethyl methacrylate (HEMA) were obtained from Sigma (St. Louis, USA) and distilled

under reduced pressure in the presence of hydroquinone inhibitor, and stored at 4 °C until use. Methanol used in HPLC analysis was HPLC grade and supplied from Merck A.G. (Darmstadt, Germany). All other chemicals were of the highest purity commercially available and were used without further purification. Ultra-pure water filtered by Millipore S.A.S 67120 whose quality management system is approved by the accredited registering body to the ISO 9001. Before use the laboratory glassware was rinsed with deionized water and dried in a dust free environment. Poly [HEMA-MAPA] nanospheres used in this study were supplied from the laboratory of Dr. Nalan Tuzmen and synthesized in the another study of her group (Kalburcu, Ozturk, Tuzmen, Akgol, & Denizli, 2014). Briefly, poly [HEMA-MAPA] nanospheres were synthesized as the following procedure.

Methacryloylamido phenylalanine (MAPA) was prepared as outlined in literature (Türkmen, Öztürk, Akgöl, Elkak, & Denizli, 2008; Oncel, Uzun, Garipcan, & Denizli, 2005; Say, Emir, Garipcan, Patir, & Denizli, 2003). L-Phenylalanine (5.0 g) and $NaNO_2$ (0.2 g) were dissolved in 30 ml of $K_2CO_3$ aqueous solution (5%, w/v). This solution was cooled to 0 °C. Methacryloyl chloride (4.0 ml) was slowly poured into this solution under nitrogen gas then this solution was magnetically stirred at room temperature for 2 h. At the end of this chemical reaction period, the pH of the solution was adjusted to 7.0 and subsequently the solution was extracted with ethyl acetate. The aqueous phase was evaporated using rotary evaporator. The residue (MAPA) was crystallized from ether and cyclohexane.

Poly(HEMA–MAPA) nanospheres were produced by surfactant free emulsion polymerization as outlined in (Kalburcu et al., 2014; Türkmen et al., 2008; Oncel et al., 2005; Say et al., 2003). The preparation method was as follows: the stabilizer, poly (vinyl alcohol) (PVAL) (0.5 g), was dissolved in 50 ml deionized water in the polymerization reactor to prepare the continuous phase. Then, the comonomer mixture [2-Hydroxyethyl methacrylate (HEMA) (0.6 ml) and methacryloylamido-phenylalanine (MAPA) (0.3 ml)] was added to this dispersion, which was mixed in an ultrasonic bath for about 30 minutes. Before polymerization, initiator [potassium peroxydisulfate (KPS) (0.44 ml)] was added to the solution and nitrogen gas was

blown through the medium for about 5 min in order to remove dissolved oxygen. Polymerization was carried out in a constant temperature (70°C) with shaking under nitrogen medium for 24 hours. After the polymerization, the polymeric nanospheres were cleaned by washing with ethanol and water several times to remove the unreacted monomers. For this purpose, the nanospheres were precipitated at the rate of 20,000 g for 1 hour in a centrifuge (Zentrifugen, Universal 32 R, Germany), the collected precipitate was resuspended in ethanol and water several times. After that poly(HEMA–MAPA) nanospheres were further washed with deionized water and used for the extraction of DDT via adsorption. Fig. 3.2 shows the hypothetic structure of poly(HEMA–MAPA) nanospheres. Characterization data of poly (HEMA-MAPA) nanospheres was given in the study of Kalburcu et al. (2014).

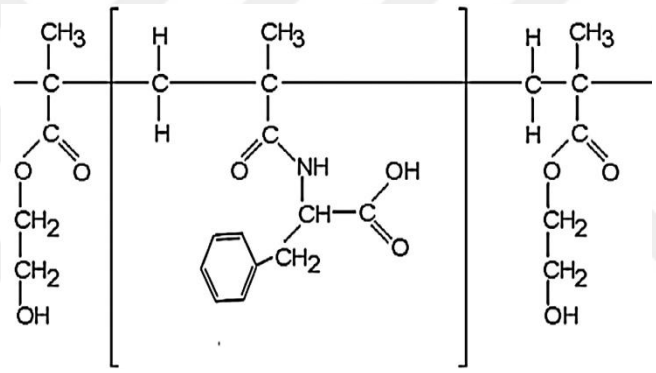


Figure 3.2 The hypothetic structure of poly(HEMA–MAPA) nanospheres (Kalburcu et al., 2014; Oncel et al., 2005; Say et al., 2003)

*3.3.5.2 Adsorption Studies of p, p' DDT Standards*

*p,p'* DDT standards adsorption studies were performed in a batch system with continuous stirring of the adsorption medium. Some variables such as amount of polymer, contact time between the poly[HEMA-MAPA] nanospheres and the DDT standard, initial DDT concentration, temperature and ionic strength were studied to optimize adsorption conditions. The amount of polymer was adjusted by using different amounts included 0, 50, 100, 200,400 and 500 µl. To determine the effect of contact time on adsorption, experiments were carried out at different times

including 0, 15, 30, 45, 60 and 90 min. Initial DDT concentration was changed between 0.1-500 mg/l. To determine the effects of temperature, temperature values were changed between 15$^{o}$C and 45$^{o}$C. NaCl concentrations were changed between 0, 0.005, 0.05 and 1M to study the effect of ionic strength. Determination of DDT was performed by dionex ultimate 3000 UHPLC (Thermo Scientific, United States of America) with Diode Array Detector and 3µm X 150mm Hypersil ODS C18 Columns (Thermo). Many trials were perfurmed in order to optimize the best conditions for DDT detection and quinitification and the one giving the best chromatography performance in terms of peak shape and retention time was selected. The mobile phase was detected as a mixture of double deionized water (A) and methanol (B). The conditions were as follow: A 10%, B 90% during the analyses. Other chromatographic conditions were as follows: flow rate, 0.8 ml/min; injection volume was 20 µl; pressure at isocratic system, 96 bar in 30$^{\circ}$ C and in wavelength of 238nm. The retention time of the DDT standard was 2.21 min at flowrate of 0.8 ml/min. Run times per sample were 3.6 min.

### 3.4 Statistical Analysis

The statistical analysis was done using the STATISTICA v.8.0 (STATSOFT) software package, and statistical significance was defined at the $p<0.05$ level. Product-Moment and Partial Correlations were calculated to verify existing relationships. Factor analysis, a multivariate analytical tool, was used to investigate the correlation of the determined parameters and facilitate the evaluation of pollution effects on the study area. This program based on Principal Component Analysis (PCA) as an extraction method and correlation matrix has been determined and Varimax Normalization rotation was performed.

# CHAPTER FOUR
# RESULTS AND DISCUSSION

## 4.1 Organochlorine Pesticides in the Sediments from the Aegean Coast

### *4.1.1 Organochlorine Levels in Sediment*

Sediment samples were collected from selected sampling points in İzmir and Çandarlı Bays. Samples were freeze dried and extracted using solvents. Details were given in "Material and Methods" section. The concentrations of organochlorine compounds in sediment samples from Izmir and Çandarlı Bays were given in Table 4.1 and Table 4.2.

Compounds identified included hexachlorocyclohexane (γ HCH), cyclodienes (aldrin, dieldrin, endrin, heptachlor), HCB, DDTs (dichlorodiphenyltrichloroethane) and its degradates (*p,p'*-DDE, *p,p'*-DDD, *p,p'*-DDT). The levels of organochlorines were generally low for all compounds by global standards (Fowler, 1990), including the relatively higher values for organochlorines found areas from the middle part of Izmir and Candarli Bays. The concentrations of ΣOCPs were in the range of 1.48-29.6 ng $g^{-1}$ with an average value of 6.91 ng $g^{-1}$ dry weight (dry wt) (Table 4.1).

Table 4.1 Total concentration of DDT, cyclodienes, OCPs, Aroclors, EOM (extractable organic matter) and the selected ratios of chlorinated pesticides in sediments from Izmir and Çandarlı Bays

| Compounds/Locations | Gediz | Uzunada | Gülbahçe | Middle Bay | Aliağa | Nemrut | Çandarlı |
|---|---|---|---|---|---|---|---|
| ΣOCPs (ng $g^{-1}$) | 1.48 | 3.62 | 1.71 | 1.88 | 6.52 | 29.6 | 3.51 |
| ΣDDTs (ng $g^{-1}$) | 0.29 | 0.88 | 0.16 | 0.46 | 0.96 | 19.2 | 0.58 |
| ΣCyclodienes (ng $g^{-1}$) | 1.16 | 2.69 | 1.51 | 1.24 | 4.96 | 8.93 | 2.71 |
| ΣAroclors[1] (ng $g^{-1}$) | 8.57 | 11.9 | 14.7 | 12.4 | 97.1 | 562 | 16.7 |
| EOM (mg $g^{-1}$) | 0.59 | 1.81 | 0.71 | 2.42 | 1.80 | 0.84 | 1.78 |
| DDD/DDE | 0.27 | 0.31 | 0.17 | 0.29 | 0.45 | 0.196 | 1.89 |
| (DDE+DDD)/ΣDDT | 0.96 | 0.97 | 0.90 | 0.96 | 0.98 | 0.97 | 0.95 |
| DDT/DDE | 0.050 | 0.045 | 0.13 | 0.059 | 0.032 | 0.033 | 0.15 |
| %DDT/ΣDDTs | 3.78 | 3.30 | 9.68 | 4.35 | 2.19 | 2.66 | 4.84 |

[1]ΣAroclors: Aroclor1254+1260

The results indicated that the cyclodienes were the predominant OCP contaminant in sediments of the Izmir and Çandarlı Bays. All of DDTs, HCB, HCH, cyclodienes and Aroclors were found in all sediment samples from both Izmir and Çandarlı Bays. Aroclors were the predominant compounds between all the contaminants from all sampling sites with average concentrations of 44.5 and 58.9 ng $g^{-1}$ (dry wt) for Aroclor1254 and Aroclor1260, respectively.

The OCPs concentrations ranged between 0.12-15.6 for *p,p'*-DDE, 0.07-5.92 for Dieldrin, 0.02-3.05 for *p,p'*-DDD, 0.24-1.08 for Endrin, 0.22-0.89 for Heptachlor, 0.014-1.37 for HCB, 0.017-1.07 for Aldrin, 0.011-0.51 for *p,p'*-DDT and 0.01-0.17 ng $g^{-1}$ (dry wt) for Lindane. The range of extractable organic matter were 0.59-2.42 mg $g^{-1}$ and the highest EOM was observed in the Middle Bay.

The mean concentrations were 2.54 for *p,p'*-DDE, 1.88 for Dieldrin, 0.58 for *p,p'*-DDD, 0.62 for Endrin, 0.55 for Heptachlor, 0.32 for HCB, 0.26 for Aldrin, 0.091 *p,p'*-DDT and 0.063 ng $g^{-1}$ (dry wt) for Lindane.

Concentrations of the total DDT (the sum of 3 isomers of DDT) ranged from 0.16 to 19.2 ng $g^{-1}$ (dry wt) with a mean value of 3.21 ng $g^{-1}$ dry wt. Highest ΣDDTs levels occurred at Nemrut Bay with 19.16 ng $g^{-1}$, followed by Aliağa with 0.96 ng $g^{-1}$ (dry wt), Uzunada and Çandarlı with 0.88 and 0.58 ng $g^{-1}$, respectively. Lower concentrations were observed at Middle Bay, Gülbahçe and Gediz regions (Table 4.1).

The concentrations of ΣCyclodienes (including heptachlor, aldrin, dieldrin, endrin) in the sediments from 7 sampling sites ranged between 1.16-8.93 ng $g^{-1}$ (dry wt) (Table 4.1). The concentration of heptachlor in sediment samples ranged from 0.22 Aliağa site to 0.89 ng $g^{-1}$ at site Gülbahçe. Maximum dieldrin level was found at Nemrut site (5.92 ng $g^{-1}$) while the minimum level was found at site Middle Bay with value of 0.07 ng $g^{-1}$ (Table 4.2). The mean concentration level of dieldrin found in the sediment samples from the seven sites in this study was 1.88 ng $g^{-1}$ (dry wt). Endrin was observed at all sampling locations and the cocentrations ranged from

0.24 to 1.08 ng $g^{-1}$ (dry wt). The maximum concentration of Endrin was found at Nemrut region while the minimum concentration was found at site Uzunada (Table 4.2). The mean concentration of Endrin in sediment samples in this study was 0.62 ng $g^{-1}$ (dry wt). The concentration of Lindane in sediment samples ranged from 0.01 at site Middle Bay to 0.17 ng $g^{-1}$ at Nemrut. The mean concentration of Lindane in sediment samples in this study was 0.063 ng $g^{-1}$ (dry wt).

The highest levels of HCB were measured at Nemrut site with value of 1.37 ng $g^{-1}$ while the lowest concentrations were in Gediz, Uzunada and Gülbahçe sites with values of 0.014, 0.019 and 0.017 ng $g^{-1}$, respectively. In this study the concentrations of HCB from Izmir and Çandarlı Bays were lower than those of Romania Coastline, Black Sea (5.3-23 ng $g^{-1}$) (Fillmann et al., 2002), Gulf of Lions, Northwest Mediterranean (0.2-1.5 ng $g^{-1}$), Ebro Po delta, Northwest Mediterranean (0.1-18.8 ng $g^{-1}$), Rhone prodelta, Mediterranean Sea (11-39 ng $g^{-1}$), Offshore Barcelona, Mediterranean Sea (0.4-2.9 ng $g^{-1}$) (Tolosa, Bayona, & Albaige´s, 1995), but they were slightly higher than those of Bahrain (0.005-0.027 ng $g^{-1}$), Oman (0.0006-0.010 ng $g^{-1}$), Qatar (0.0010-0.0054 ng $g^{-1}$), UAE (0.0009-0.0073 ng $g^{-1}$) (De Mora, Fowler, Tolosa, Villeneuve, & Cattini, 2005).

Tolosa et al. (2004); Camusso, Golossi, & Vignati (2002); Fillmann et al. (2002); Picer (2000); Panagiotopoulou, Tsipi, Hiskia, & Scoullos (1996); Tolosa et al. (1995); Albanis, Danis, & Hela (1995) reported a similar concentration levels of DDT (as ng $g^{-1}$ dry wt) in samples collected from Gulf of Elefsis, Greece (2.64-15); Mediterranean (3.5-12); the Northwest Russian Federation coast, Rhone Delta, France (5-15); Black Sea (3.3-12); Adriatic Sea (0.2-35); Caspian Sea, Azerbaijan (0.56-13); Northern Estuaries, Greece (0.3-60); Po River, Italy (3.9-19).

Aroclor 1254 and 1260 were present in noticeably higher concentrations than OCPs at both of Çandarlı and Izmir Bays. Aroclors are used as industrial chemicals and their probable source is industrial waste. Aroclor 1254 concentration in the sediments ranged from 3.35 at site Gediz to 215 ng $g^{-1}$ (dry wt) at Nemrut site.

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Table 4.2 The concentrations of organochlorine compounds (ng $g^{-1}$ dry weight) in sediment samples from Izmir and Çandarlı Bays

| Station | HCB | Lindane | Heptachlor | Aldrin | Dieldrin | Endrin | *p,p'*DDD | *p,p'*DDE | *p,p'*DDT | Aroclor 1254 | Aroclor 1260 |
|---|---|---|---|---|---|---|---|---|---|---|---|
| **Sediment** | | | | | | | | | | | |
| Gediz | 0.014 | 0.02 | 0.40 | 0.017 | 0.19 | 0.55 | 0.06 | 0.22 | 0.011 | 3.35 | 5.22 |
| Uzunada | 0.019 | 0.03 | 0.47 | 0.063 | 1.92 | 0.24 | 0.20 | 0.65 | 0.029 | 9.86 | 2.06 |
| Gülbahçe | 0.017 | 0.03 | 0.89 | 0.038 | 0.21 | 0.37 | 0.02 | 0.12 | 0.015 | 5.76 | 8.92 |
| Middle Bay | 0.17 | 0.01 | 0.55 | 0.019 | 0.07 | 0.60 | 0.10 | 0.34 | 0.020 | 10.38 | 2.04 |
| Aliağa | 0.45 | 0.15 | 0.22 | 0.062 | 4.07 | 0.61 | 0.29 | 0.65 | 0.021 | 56.9 | 40.2 |
| Nemrut | 1.37 | 0.17 | 0.86 | 1.07 | 5.92 | 1.08 | 3.05 | 15.6 | 0.51 | 215 | 347 |
| Çandarlı | 0.19 | 0.03 | 0.46 | 0.56 | 0.81 | 0.88 | 0.36 | 0.19 | 0.028 | 10.0 | 6.71 |
| **Mean** | 0.32 | 0.06 | 0.55 | 0.26 | 1.88 | 0.62 | 0.58 | 2.54 | 0.09 | 44.46 | 58.88 |
| **Reference Material** | | | | | | | | | | | |
| **Observed value** | | | | | | | | | | | |
| IAEA-417/1 | 0.98 | 0.55 | 2.01 | 1.81 | 1.92 | 5.73 | 20.0 | 13.0 | 16.7 | 514 | 207 |
| IAEA-417/2 | 0.87 | 0.46 | 2.00 | 1.79 | 2.04 | 5.90 | 20.4 | 12.9 | 16.4 | 405 | 226 |
| IAEA-417/3 | 0.95 | 0.48 | 2.10 | 1.80 | 2.14 | 5.81 | 20.6 | 13.1 | 17.3 | 446 | 218 |
| **Mean± Std.dev**. | 0.93±0.06 | 0.50±0.05 | 2.04±0.06 | 1.8±0.01 | 2.03±0.11 | 5.81±0.09 | 20.3±0.31 | 13±0.10 | 16.8±0.46 | 455±55 | 217±9.5 |
| **Certified value** | 0.9-1.5 | 0.39-0.69 | 1.1-2.9 | 1-2.2 | 1.2-3.4 | 4.3-9.9 | 18.1-23.9 | 12.1-15.9 | 15.8-22.2 | 310-520 | 110-230 |

The mean concentration of Aroclor 1254 for samples collected from the seven locations was 44.5 ng $g^{-1}$ (dry wt). The concentration of Aroclor 1260 in the sediments ranged from 2.04 at site Middle Bay to 347 ng $g^{-1}$ (dry wt) at Nemrut site. The mean concentration of Aroclor 1260 for samples collected from the seven locations was 58.9 ng $g^{-1}$ (dry wt).

It is concluded that, the highest concentrations of ΣAroclors and ΣOCPs were found in samples collected from Nemrut site due to industrial activities

### *4.1.2 DDTs Composition and Source Identification in Sediments*

The relative concentration of the DDT compound and its biological metabolites, DDD and DDE, can be used as indicative indices for assessing the possible contamination sources. Since DDT can be biodegraded under aerobic condition to DDE and under anaerobic condition to DDD, ratio of (DDE+DDD)/ΣDDT> 0.5 can be thought to be subjected to a long-term weathering (Hites & Day, 1992; Hong, Chen, Xu, Wang, & Zhang, 1999; Zhang, Min, Mai, Sheng Gy, & Wang, 1999).

Table 4.1 illustrates the relationship between (DDE+DDD)/ΣDDT and DDD/DDE in the surface sediments collected from Izmir and Çandarlı Bays. Ratios of (DDE+DDD)/ΣDDT were in the range of 0.90 -0.98 with entire values being >0.5. This infers that the degraded metabolites formed a significant proportion of total DDT compounds. Also, whole values of DDD/DDE ratio in Izmir Bay ranged between 0.17-0.31 and were lower than unity, indicating that the sediments was dominated by p,p'-DDE, the product of aerobic degradation of *p,p'*-DDT. This result showed that the sediments collected from Izmir and Çandarlı Bays were mainly under oxic conditions.

Also, the results obtained from the indices clearly indicate that DDTs in the sediments from this bay may be derived from the aged and weathered agricultural soils and transported by surface run-off from the rivers. Oxic conditions were particularly confirmed by the higher dissolved oxygen concentrations in the inner

part of Izmir Bay. It was due to the fact that in early 2000, a wastewater treatment plant (WTP) began operation to treat domestic and industrial wastes in the Izmir Metropolitan Municipality (Kucuksezgin, 2011).

Results in Table 4.1, shows the relative percentages of p,p'-DDT and it was found that p,p'-DDT levels didn't even exceed 9.68 % of the ΣDDTs at all stations. Given the relatively slow degradation rate of DDT in the environment (Woodwell, Craig, & Horton, 1971), such findings indicate early DDT influxes and, by implication, non-existing DDT use throughout the region. In addition, the ratio of p,p'-DDT/p,p'-DDE provides a useful index to assess whether the DDTs at a given site is fresh or aged, a value <0.33 generally indicative of an aged input (Stranberg et al., 1998). As shown in Table 4.1, aged mixture of DDT was evident through the entire of the Izmir and Çandarlı Bays. Aged DDT was observed in all sampling stations in the western part of the Turkish coastal zone. In summary, DDT contamination of both regions is not highlighted as a contemporary and ubiquitous problem.

### *4.1.3 Potential Biological Effects of OCPs in Sediments*

As shown in Table 4.3, two widely used sediment quality guidelines, i.e. the effects range-low value (ERL) and effects range-median value (ERM) guidelines (Long, MacDonald, Smith, & Calder, 1995; Long, Field, & MacDonald, 1998), as well as the probable effects level (PEL) guidelines and the threshold effects level (TEL) (CCME, 2002) were applied to evaluate the possible ecotoxicological risks of OCPs in the study areas.

Concentration levels of *pp'*-DDE and *pp'*-DDD were higher than ERL values at one site (Nemrut Region). Meanwhile, these compounds also exceeded the TEL value at the same site, but were significantly lower than the PEL and ERM values. The level of heptachlor was over the ERL value at 3 sites located in Nemrut, Gülbahçe and Middle Bay, whereas they were below the ERM, TEL and PEL values (Fig. 4.1).

Table 4.3 Assessments of potential biological risks of selected OCPs in the surface sediments of Izmir and Çandarlı Bays using two sediment quality guidelines (SQG)

| Chemical | Range ($ngg^{-1}$) | ER-L[1] | Above ER-L (%)[5] | ER-M[2] | Above ER-M (%)[e] | TEL[3] | Above TEL(%)[e] | PEL[4] | Above PEL (%)[5] |
|---|---|---|---|---|---|---|---|---|---|
| *p,p'*-DDT | 0.011- 0.51 | 1.0 | 0 | 7 | 0 | 1.19 | 0 | 4.77 | 0 |
| *p,p'*-DDE | 0.12- 15.6 | 2.2 | 14 | 27 | 0 | 2.07 | 14 | 374 | 0 |
| *p,p'*-DDD | 0.02- 3.05 | 2.0 | 14 | 20 | 0 | 1.22 | 14 | 7.81 | 0 |
| DDTs | 0.16-19.2 | 1.58 | 14 | 46.1 | 0 | 3.89 | 14 | 51.7 | 0 |
| Dieldrin | 0.07- 5.92 | NA | - | NA | - | 0.71 | 57 | 4.3 | 14 |
| γ-HCH | 0.01- 0.17 | NA | - | NA | - | 0.32 | 0 | 0.99 | 0 |
| Heptachlor | 0.22- 0.89 | 0.5 | 43 | 6 | 0 | 2.26 | 0 | 4.79 | 0 |
| PCBs[6] | 8.57-562 | 22.7 | 29 | 180 | 14 | 21.5 | 29 | 189 | 14 |
| Aroclor 1254 | 3.35-215 | NA | - | NA | - | 63.3 | 14 | 709.0 | 0 |

[1] Effect range-low value,[2] Effect range-median value,[3] Threshold effect level,[4] Probable effect level,[5] Percentage of samples above the corresponding levels,
[6] Sum of Aroclor1254 and Aroclor1260. NA: Not available

This suggested that the concentration levels of *p,p'*-DDE, *p,p'*-DDD and heptachlor at most sites other than Nemrut, Gülbahçe, Middle Bay sites were lower than the values that may cause adverse biological risk. For DDTs, although it was not above the ERM and PEL values, 14% of total sites in the study area (Nemrut site) were higher than ERL and TEL values (Table 4.3), suggesting that the exposure of DDTs may cause ecological risk on the neighboring benthic organisms.

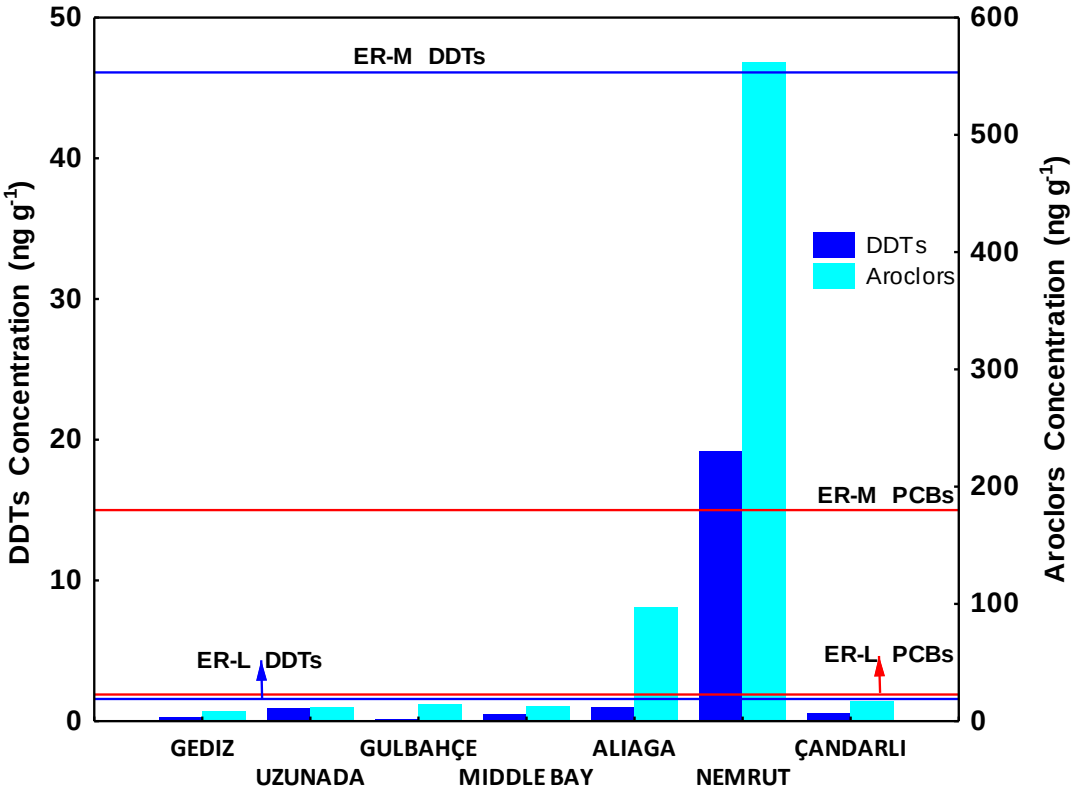



Figure 4.1 Concentrations of DDTs and Aroclors in the sediments from the sampling sites. ERL and ERM values (Long et al., 1995)

Therefore, DDTs could be the one-main species of OCPs with more ecotoxicological concern in Nemrut Region, while other OCPs in surface sediments would be less possible to cause adverse biological effect. Concentration levels of Dieldrin were above the TEL values in 4 sites (Aliağa, Nemrut, Çandarlı and Uzunada). However, the Dieldrin levels didn't exceed the PEL except in one site (Nemrut site) of these four sites.

### *4.1.4 Ecotoxicological Concerns for PCBs*

Sediment-bound PCBs can generally affect benthic organisms. To evaluate the ecotoxicological aspect of sediment contamination, some published sediment quality guidelines were applied in this study. Although the guidelines are limited in some cases, they provide useful indicators of the effects of PCB contamination in the absence of environmental assessment criteria for PCBs in Turkey.

The effect range low value (ERL, 22.7 ng $g^{-1}$ dw) suggests that PCBs can exert toxic biological effects on aquatic organisms, while the effect range median value (ERM, 180 ng $g^{-1}$ dw) indicates the high possibility of PCBs posing detrimental biological effects on aquatic organisms (Long et al., 1995). Based on Canadian quality guidelines for PCBs in marine sediment, PCB concentrations at the probable effect level (PEL, 189 ng $g^{-1}$ dw) frequently cause adverse effects on aquatic biota, whereas at concentrations corresponding to the threshold effect level (TEL, 21.5 ng $g^{-1}$ dw), the effects are negligible (CCME, 1999). PCBs were measured at all sites in this study. The total PCB concentrations of the samples collected from sampling sites exceeded the ERL values at Aliağa and Nemrut regions but these levels were above the ERM and PEL values in Nemrut site (Fig. 4.1).

According to Canadian quality guidelines for PCBs in sediment, Aroclor 1254 concentrations at the PEL (709 ng $g^{-1}$ dry wt) frequently cause adverse effects on aquatic biota, whereas at concentrations corresponding to the TEL (63.3 ng $g^{-1}$ dry wt), the effects are negligible (CCME, 1999). In this study, concentrations of Aroclor 1254 were higher than the TEL values in 14 % of sampling stations, whereas they were below the PEL value at all of the stations (Table 4.3). The Aroclor 1254 levels in sediment sample exceeded the TEL value, showing that adverse biological effect is expected occasionally at station Nemrut. However, Aroclor 1254 levels were below the TEL values at remaining stations demonstrated that adverse biological effects on benthic organisms are expected rarely Table 4.3).

Table 4.4 The concentrations of organochlorine compounds (ng $g^{-1}$ dry weight) in fish samples from Izmir and Çandarlı Bays

| Station | HCB | Lindane | Heptachlor | Aldrin | Dieldrin | Endrin | *pp'*DDD | *pp'*DDE | *pp'*DDT | Aroclor 1254 | Aroclor 1260 |
|---|---|---|---|---|---|---|---|---|---|---|---|
| **Fish (*M. barbatus*)** | | | | | | | | | | | |
| Gediz | 0.41 | 0.62 | 0.33 | 3.25 | 1.45 | 8.40 | 0.66 | 0.82 | 0.45 | 21.2 | 14.5 |
| Uzunada | 0.53 | 1.16 | 0.44 | 9.05 | 3.15 | 7.21 | 1.65 | 3.66 | 0.74 | 37.9 | 29.1 |
| Gülbahçe | 0.47 | 0.54 | 0.76 | 8.56 | 3.97 | 7.41 | 0.87 | 4.84 | 2.03 | 32.8 | 16.5 |
| Middle Bay | 0.12 | 0.17 | 0.13 | 0.55 | 0.09 | 3.07 | 0.16 | 0.79 | 0.23 | 15.9 | 19.2 |
| Aliağa | 0.31 | 0.57 | 0.08 | 1.03 | 0.59 | 3.26 | 0.31 | 0.37 | 0.25 | 44.2 | 95.4 |
| **Reference Material** | | | | | | | | | | | |
| **Observed value** | | | | | | | | | | | |
| IAEA-451/1 | 0.40 | 0.54 | 2.07 | 0.79 | 1.75 | 4.68 | 1.03 | 1.81 | 1.28 | 29.4 | 54.1 |
| IAEA-451/2 | 0.36 | 0.60 | 2.18 | 0.79 | 1.89 | 4.58 | 0.97 | 1.75 | 1.30 | 31.2 | 49.7 |
| IAEA-451/3 | 0.38 | 0.57 | 2.24 | 0.88 | 1.84 | 4.64 | 1.02 | 1.76 | 1.39 | 30.6 | 51.6 |
| Mean± Std.dev. | 0.38±0.02 | 0.57±0.03 | 2.16±0.09 | 0.82±0.05 | 1.83±0.07 | 4.63±0.05 | 1.01±0.03 | 1.77±0.03 | 1.32±0.06 | 30.4±0.92 | 51.8±2.21 |
| **Certified value** | 0.39 | 0.56 | 2.07 | 0.87 | 1.88 | 4.60 | 0.99 | 1.73 | 1.34 | 34.3 | 53.2 |

## 4.2 Organochlorines in Fish Samples from the Izmir and Çandarlı Bays

### *4.2.1 Contamination Levels of Organochlorines in Fish*

Table 4.4 illustrates the concentrations of OCs (ng $g^{-1}$ dw) in fish samples collected from five sites in Izmir and Çandarlı Bays. The nine OCPs (*p,p'*-DDE, *p,p'*-DDD, *p,p'*-DDT, lindane, HCB, dieldrin, endrin, heptachlor and aldrin) together with PCBs: such as commercial mixtures, Aroclor 1254 and 1260 were analyzed in *M. barbatus* samples.

The nine types of OCPs were detected in all fish samples collected from the five sampling sites in this study (Gediz, Uzunada, Gülbahçe, Middle Bay and Aliağa). The mean concentration of ΣOCPs was 17.1 ng $g^{-1}$ dry wt. The maximum concentration of ΣOCPs (29.45 ng $g^{-1}$) was found in fish samples collected from Gülbahçe site. The minimum concentration of ΣOCPs (5.31 ng $g^{-1}$) was found in samples collected from Middle Bay of Izmir.

Table 4.5 Total concentrations of DDTs and the ratios of DDT concentrations in biota samples collected from the sampling sites

| Compounds/ Locations | Foca-Gediz | Uzunada | Gülbahçe | Middle Bay | Aliağa |
|---|---|---|---|---|---|
| ΣDDTs (ng $g^{-1}$) | 1.93 | 6.05 | 7.74 | 1.18 | 0.93 |
| DDD/DDE | 0.81 | 0.45 | 0.18 | 0.20 | 0.84 |
| (DDE+DDD)/ΣDDT | 0.77 | 0.88 | 0.74 | 0.81 | 0.73 |
| DDT/DDE | 0.55 | 0.20 | 0.42 | 0.29 | 0.68 |
| DDT/ΣDDTs % | 23.3 | 12.2 | 26.2 | 19.5 | 26.9 |
| DDE/ΣDDTs % | 42.5 | 60.5 | 62.5 | 66.9 | 39.8 |
| DDD/ΣDDTs % | 34.2 | 27.3 | 11.2 | 13.6 | 33.3 |

The concentrations of *p,p'*-DDE were in the range of 0.37 at Aliağa site to 4.84 ng $g^{-1}$ dry wt at Gülbahçe site. The concentrations of *p,p'*-DDD were detected in the range of 0.16 in Middle Bay to 1.65 ngg$^{-1}$ dry wt at UzunadaThe concentration of

*p,p'*-DDT was in the range of 0.23 at samples from Middle Bay to 2.03 ng $g^{-1}$ dry wt at Gülbahçe site.

Concentrations of the total DDT (the sum of 3 isomers of DDT) ranged from 0.93 to 7.74 ng $g^{-1}$ dry wt with a mean value of 3.57 ng $g^{-1}$ dry wt (Fig. 4.5). Highest ΣDDTs levels occurred at Gülbahçe region with 7.74 ng $g^{-1}$ dry wt, followed by Uzunada region with 6.05 ng $g^{-1}$ dry wt, Gediz region and Middle Bay with 1.93 and 1.18 ng $g^{-1}$ dry wt, respectively. The lowest concentration was observed at Aliağa region with value of 0.93 ng $g^{-1}$ dry wt (Table 4.5).

The highest levels of HCB were measured at Uzunada with value of 0.53 ng $g^{-1}$ while the lowest concentration was found at Middle Bay with value of 0.12 ng $g^{-1}$, respectively. The average value of HCB in the five sampling sites in this study was 0.37 ng $g^{-1}$ dry wt.

The concentrations of Σcyclodienes (including heptachlor, aldrin, dieldrin, endrin) in the fish samples from five sampling sites ranged between 3.84–20.7 ng $g^{-1}$ dry wt (Table 4.4). The average value of cyclodienes in the five sampling sites in this study was 12.6 ng $g^{-1}$ dry wt. The concentration of heptachlor in fish samples ranged from 0.08 at Aliağa site to 0.76 ng $g^{-1}$ dry wt at Gülbahçe site. The mean value of heptachlor in fish samples from the five sampling sites according to this study was 0.35 ng $g^{-1}$ dry wt. Maximum dieldrin level was found at Gülbahçe site (3.97 ng $g^{-1}$) while the minimum level was detected at Middle Bay with value of 0.09 ng $g^{-1}$ dry wt. The mean concentration level of dieldrin found in the fish samples from the five sites in this study was 1.85 ng $g^{-1}$ dry wt. Endrin as well as other cyclodienes was observed in fish samples from all sampling locations, it's level ranged from 3.07 to 8.40 ng $g^{-1}$ dry wt. The maximum concentration of Endrin was found at Gediz region (due to agricultural activities), while the minimum concentration was found at the Middle Bay (Table 4.4). The average concentration of Endrin in fish samples in this study was 5.87 ng $g^{-1}$ dry wt. Maximum Aldrin level was found at Uzunada (9.05 ng $g^{-1}$) while the minimum level was found at Middle Bay with value of 0.55 ng $g^{-1}$ dry wt. The average concentration level of Aldrin found in the fish samples from the five

sites in this study was 4.49 ng $g^{-1}$ dry wt. The concentration of Lindan in fish samples ranged from 0.17 at Middle Bay to 1.16 ng $g^{-1}$ dry wt at Uzunada site. The average concentration of Lindane in fish samples in this study was 0.61 ng $g^{-1}$ dry wt (Table 4.4).

Aroclor 1254 concentration in this study ranged from 15.9 at Middle Bay to 44.2 ng $g^{-1}$dry wt at Aliağa site. The average level of Aroclor 1254 was 30.4 ng $g^{-1}$ dry wt Aroclor 1260 concentration ranged between 14.5 at Gediz and 95.4 ng $g^{-1}$ dry wt at Aliağa. The mean concentration of Aroclor 1260 was 34.9 ng $g^{-1}$dry wt. The highest concentration of ΣPCBs (140 ng $g^{-1}$) was found in samples from Aliağa, while the lowest concentration of ΣPCBs (35.1 ng $g^{-1}$) was found in fish samples collected from the Middle Bay (Table 4.4).

### *4.2.2 Characteristics of OCP Contamination in Fish Samples*

Table 4.5 presented the ratios of (DDE+DDD)/ΣDDT and DDD/DDE in fish samples collected from the selected sites in this study. The relative percentages of *p,p'*-DDT, *p,p'*-DDE and *p,p'*-DDD, highlights that *p,p'*-DDT does not exceed 27% of the ΣDDTs in samples. Considering the relatively slow degradation rate of DDT in the environment (Woodwell et al., 1971), the results indicate no recent identified influxes of DDT in the sampling area. Generally, the ratio of (*p,p'*-DDE+*p,p'*-DDD)/ ΣDDTs>0.5 indicates long-term weathering (Schmitt, Zajicek, & Peterman, 1990).

The (*p,p'*-DDE+ *p,p'*-DDD)/ΣDDTs ratios in this study was found to be >0.5 at the all samples collected from the five sites. The high (*p,p'*-DDE+*p,p'*-DDD)/ΣDDTs ratios point to no current DDT usage in these sites. Generally, this result indicates that DDTs input at the sampling sites was historical and significant degradation has occurred (Table 4.5). The DDD/DDE ratio could be an indicator of the aerobic/anaerobic conditions in the environment (Liu et al., 2008). Higher ratios of *p,p'*-DDE to *p,p'*-DDD in the samples indicated that DDT undergo aerobic transforming organisms (Zhou et al., 2008).

### *4.2.3 Health Risks Assesment*

A health risk assessment is a health questionnaire, used to provide individuals with an evaluation of their health risks and quality of life (Baker, Dejoy, & Wilson, 2007). According to this study, health risk assesment was analyzed about PCBs and OCPs due to consumed fish. Annual per capita fish consumption is 7.081 kg in Turkey (TÜİK, 2013). Comparing the annual average fish consumption per idividual in Turkey to the world consumption average its clearly appeared the very low consumption value in Turkey. The annual average fish consumption in the world is about 20.7 kg, meanwhile, this rate increases in the European Union countries to reach 25 kg (World Review of Fisheries and Aquaculture Part 1, 2012). Fish consumption per person is 0.89 kg monthly in West Mediterranean Region in Turkey.

Table 4.6 Estimated daily intakes of PCBs and OCs in the fish samples by human (average body wt 70 kg) in İzmir and Çandarlı Bays (Eastern Aegean) and Acceptable Daily Intake (ADI) by FAO/WHO (1996b) (ng/kg body weight/day)

| | Gediz | Uzunada | Gülbahçe | Middle Bay | Aliağa | ADI |
|---|---|---|---|---|---|---|
| ∑DDTs | 0.53 | 1.66 | 2.12 | 0.32 | 0.25 | 20000 |
| ∑PCBs[1] | 9.8 | 18.4 | 13.5 | 9.62 | 38.2 | - |
| ∑OCs | 14.3 | 26.0 | 21.6 | 11.1 | 40.1 | - |

[1]∑PCBs: ∑Aroclor1254+Aroclor1260

The estimated daily intake (EDI) of OCs and PCBs in fish samples in the study areas was given in Table 4.6. As shown in Table 4.6, the EDI of DDTs by the people were below the acceptable daily intake (ADI) recommended by the Food and Agriculture Organization of the United Nations/World Health Organization (FAO/WHO, 1996b) suggesting this intake would not pose a health risk in Izmir and Çandarlı Bays today.

Table 4.7 Product-Moment Pearson's correlation coefficients for organochlorines (ng $g^{-1}$ dry wt) and EOM (mg $g^{-1}$) ($p<0.05$) in sediment samples

| | HCB | γHCH | Hep | Ald | Dield | End | DDD | DDE | DDT | Aro 1254 | Aro 1260 | EOM |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| HCB | 1.00 | 0.86 | 0.36 | 0.86 | 0.89 | 0.79 | 0.96 | 0.95 | 0.95 | 0.99 | 0.97 | -0.20 |
| γHCH | 0.86 | 1.00 | 0.054 | 0.59 | 0.96 | 0.56 | 0.74 | 0.72 | 0.71 | 0.85 | 0.77 | -0.17 |
| Hep | 0.36 | 0.054 | 1.00 | 0.45 | 0.092 | 0.19 | 0.50 | 0.54 | 0.55 | 0.42 | 0.51 | -0.47 |
| Ald | 0.86 | 0.59 | 0.45 | 1.00 | 0.67 | 0.87 | 0.91 | 0.87 | 0.89 | 0.84 | 0.87 | -0.23 |
| Dield | 0.89 | 0.96 | 0.092 | 0.67 | 1.00 | 0.54 | 0.82 | 0.80 | 0.79 | 0.89 | 0.83 | -0.11 |
| End | 0.79 | 0.56 | 0.19 | 0.87 | 0.54 | 1.00 | 0.75 | 0.70 | 0.71 | 0.72 | 0.72 | -0.09 |
| DDD | 0.96 | 0.74 | 0.50 | 0.91 | 0.82 | 0.75 | 1.00 | 0.99 | 0.996 | 0.98 | 0.99 | -0.31 |
| DDE | 0.95 | 0.72 | 0.54 | 0.87 | 0.80 | 0.70 | 0.99 | 1.00 | 0.90 | 0.98 | 0.996 | -0.35 |
| DDT | 0.95 | 0.71 | 0.55 | 0.89 | 0.79 | 0.71 | 0.996 | 0.99 | 1.00 | 0.97 | 0.99 | -0.34 |
| Aro 1254 | 0.99 | 0.85 | 0.42 | 0.84 | 0.89 | 0.72 | 0.98 | 0.98 | 0.97 | 1.00 | 0.99 | -0.28 |
| Aro 1260 | 0.97 | 0.77 | 0.51 | 0.87 | 0.83 | 0.72 | 0.99 | 0.99 | 0.99 | 0.99 | 1.00 | -0.36 |
| EOM | -0.20 | -0.17 | -0.47 | -0.23 | -0.11 | -0.09 | -0.31 | -0.34 | -0.34 | -0.28 | -0.36 | 1.00 |

Table 4.8 Product-Moment Pearson's correlation coefficients for organochlorines (ng $g^{-1}$ dry wt) and lipid (%) ($p<0.05$) in fish samples

| | HCB | γHCH | Hep | Ald | Dield | End | DDD | DDE | DDT | Aro 1254 | Aro 1260 | Lipid |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| HCB | 1.00 | 0.85 | 0.72 | 0.87 | 0.86 | 0.82 | 0.87 | 0.70 | 0.57 | 0.51 | -0.15 | -0.15 |
| γHCH | 0.85 | 1.00 | 0.33 | 0.71 | 0.59 | 0.56 | 0.92 | 0.45 | 0.14 | 0.58 | 0.05 | -0.47 |
| Hep | 0.72 | 0.33 | 1.00 | 0.88 | 0.95 | 0.74 | 0.59 | 0.93 | 0.95 | 0.10 | -0.55 | 0.12 |
| Ald | 0.87 | 0.71 | 0.88 | 1.00 | 0.97 | 0.72 | 0.90 | 0.94 | 0.76 | 0.34 | -0.40 | -0.17 |
| Dield | 0.86 | 0.59 | 0.95 | 0.97 | 1.00 | 0.73 | 0.78 | 0.96 | 0.88 | 0.34 | -0.38 | 0.02 |
| End | 0.82 | 0.56 | 0.74 | 0.72 | 0.73 | 1.00 | 0.67 | 0.56 | 0.53 | -0.04 | -0.59 | -0.32 |
| DDD | 0.87 | 0.92 | 0.59 | 0.90 | 0.78 | 0.67 | 1.00 | 0.72 | 0.40 | 0.38 | -0.29 | -0.50 |
| DDE | 0.70 | 0.45 | 0.93 | 0.94 | 0.96 | 0.56 | 0.72 | 1.00 | 0.90 | 0.27 | -0.42 | 0.05 |
| DDT | 0.57 | 0.14 | 0.95 | 0.76 | 0.88 | 0.53 | 0.40 | 0.90 | 1.00 | 0.18 | -0.38 | 0.40 |
| Aro 1254 | 0.51 | 0.58 | 0.10 | 0.34 | 0.34 | -0.04 | 0.38 | 0.27 | 0.18 | 1.00 | 0.73 | 0.35 |
| Aro 1260 | -0.15 | 0.05 | -0.55 | -0.40 | -0.38 | -0.59 | -0.29 | -0.42 | -0.38 | 0.73 | 1.00 | 0.47 |
| Lipid | -0.15 | -0.47 | 0.12 | 0.17 | 0.02 | -0.32 | -0.50 | 0.05 | 0.40 | 0.35 | 0.47 | 1.00 |

### 4.3 Statistical Analysis

Matrices of Pearson's correlation coefficients were calculated for pairs of organochlorine compounds in sediment and fish (Table 4.7 and Table 4.8). Where as non-significant negative correlations were found between organochlorine levels and EOM in sediments, significant high correlations were observed between HCB and Aroclors and all organochlorine pesticides ($p<0.05$) except heptachlor (Table 4.7).

Positively high correlations were found in fish between HCB and lindane, heptachlor, aldrin, dieldrin, endrin, DDD, DDE; lindane and aldrin, DDD; heptachlor and aldrin, dieldrin, endrin, DDE, DDT; aldrin and all of OCPs; dieldrin and endrin, DDD, DDE, DDT; DDD and DDE; DDE and DDT; Aroclor 1254 and Aroclor 1260 for $p<0.05$ (Table 4.8). Negative low correlations were calculated were detected between lipid content and HCB, lindane, aldrin, endrin, DDD while lipid content and DDT, Aroclors were positively correlated. There is no significant correlations between aroclors and OCPs.

Table 4.9 Variamax normalized factor loadings for sediment

| Variable | Factor 1 | Factor 2 | Factor 3 |
|---|---|---|---|
| HCB | 0.688 | 0.131 | *0.707* |
| Lindane | *0.930*[1] | 0.009 | 0.297 |
| Heptachlor | -0.157 | *0.756* | 0.501 |
| Aldrin | 0.357 | 0.155 | *0.874* |
| Dieldrin | *0.910* | 0.006 | 0.381 |
| Endrin | 0.304 | -0.105 | *0.839* |
| DDD | 0.573 | 0.285 | *0.761* |
| DDE | 0.568 | 0.348 | *0.729* |
| DDT | 0.545 | 0.347 | *0.749* |
| Aroclor 1254 | *0.705* | 0.239 | 0.662 |
| Aroclor 1260 | 0.619 | 0.333 | *0.705* |
| EOM | -0.192 | *-0.885* | 0.051 |

[1] Italicized red numbers indicates significant loadings on the factors

In this study, Factor Analysis was performed to analyze the data. A factor matrix was obtained after Varimax rotation. The first three principal components explained 93.60 % of the total variability in the sediment samples. The factor loadings for sediments were given in Table 4.9.

The PCA crossplots were shown in Figure 4.2 for sediment from the sampling stations. The score plot of first three components allows for the characterization of organochlorines and EOM.

The first factor explains up to 75 % of the initial information and is mostly defined by the lindane, dieldrin, Aroclor 1254. The second factor (about 12 % of the initial variance) is mainly defined by heptachlor and EOM (two variables which clearly distinguishe from other variables, as can be seen in Fig. 4.2. The second factor correlated with heptachlor in the positive part and EOM in the negative part. The third factor is directly related to HCB, aldrin, endrin, DDD, DDE, DDT and Aroclor 1260.

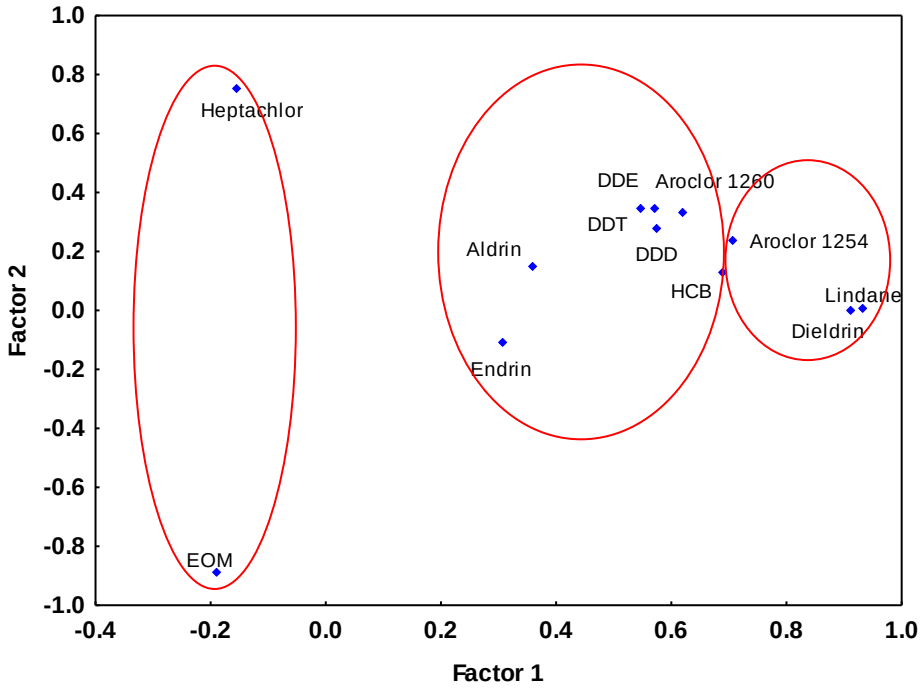



Figure 4.2 Factor analysis loading plots for sediment samples collected in the different sampling stations from the study area

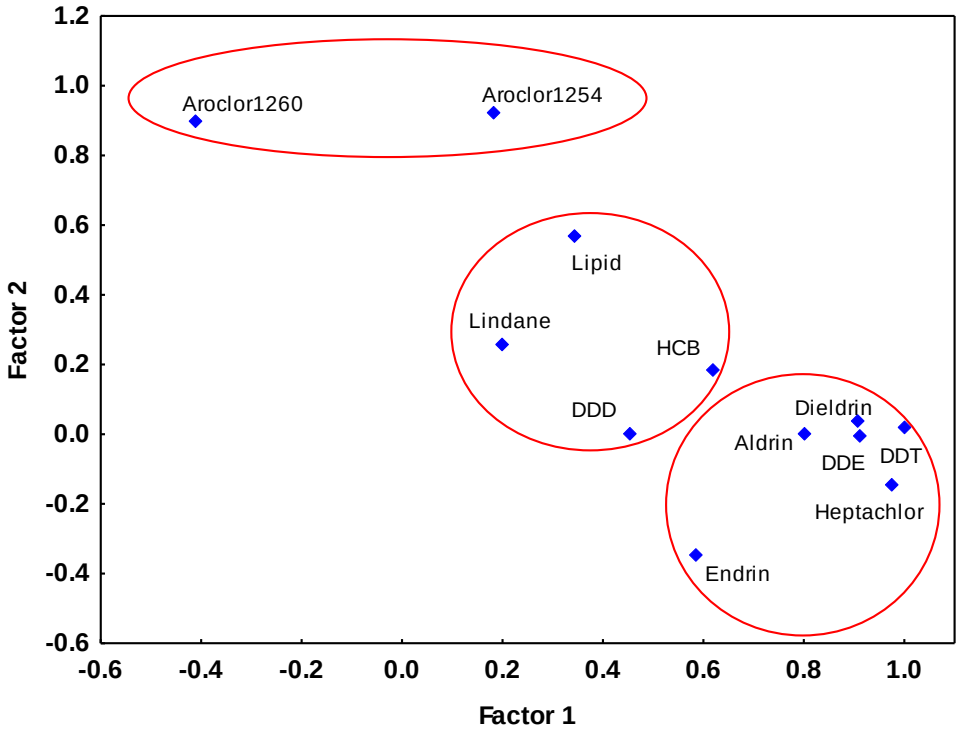



Figure 4.3 Factor analysis loading plots for fish samples collected in sampling sites of the Izmir and Candarli bays for organochlorine compounds

The first three principal components explained 96.10 % of the total variability in the fish samples. These three principal components were highly weighted due to the contributions of organochlorines and lipid content. The PCA crossplots were given in Figure 4.3 for fish samples from İzmir and Çandarlı Bays. The horizontal axis Factor 1 correlated with heptachlor, aldrin, dieldrin, endrin, *p,p'*-DDE, *p,p'*-DDT in the positive part and Aroclor 1254 and Aroclor 1260 were connected with the vertical axis Factor 2.

As it was shown in Table 4.10, HCB, lindane and *p,p'*-DDD were correlated and gave positive loadings while lipid showed negative loading on Factor 3. These results suggested that in fish samples; (1) heptachlor, aldrin, dieldrin, endrin DDE, DDT were correlted, (2) high correlations were observed between Aroclor 1254 and Aroclor 1260, (3) HCB, lindane and DDD were negatively correlated with lipid content.

Table 4.10 Variamax normalized factor loadings for fish

| Variable | Factor 1 | Factor 2 | Factor 3 |
|---|---|---|---|
| HCB | 0.618 | 0.189 | *0.717* |
| Lindane | 0.198 | 0.260 | *0.945* |
| Heptachlor | *0.971*[1] | -0.144 | 0.186 |
| Aldrin | *0.799* | 0.002 | 0.581 |
| Dieldrin | *0.905* | 0.043 | 0.422 |
| Endrin | 0.582 | -0.343 | 0.561 |
| *pp*DDD | 0.452 | 0.006 | *0.877* |
| *pp*DDE | *0.912* | -0.002 | 0.292 |
| *pp*DDT | *0.997* | 0.023 | -0.065 |
| Aroclor 1254 | 0.182 | *0.928* | 0.325 |
| Aroclor 1260 | -0.412 | *0.903* | -0.110 |
| Lipid | 0.343 | 0.574 | *-0.724* |

[1] Italicized red numbers indicates significant loadings on the factors

## 4.4 Use of Poly (HEMA-MAPA) Nanospheres as Adsorbent for DDT Standards

### *4.4.1 Optimization of HPLC Method for DDT*

The sensitivity of the method for DDT was determined by construction of a calibration curve in the low concentration region (10–1000 µg/l). The limit of detection was calculated as three times the ratio between the standard division (SD) and the slope of the low concentration curve (LOD = 3.SD /slope) and the limit of quantification as ten times the same ratio (LOQ = 10.SD /slope) (Trajkovska & Petrovska-Jovanovie, 2002). Under these chromatographic conditions DDT shows the best sensitivity. LOD and LOQ were calculated as 1.07 µg/l and 3.56 µg/l, respectively.

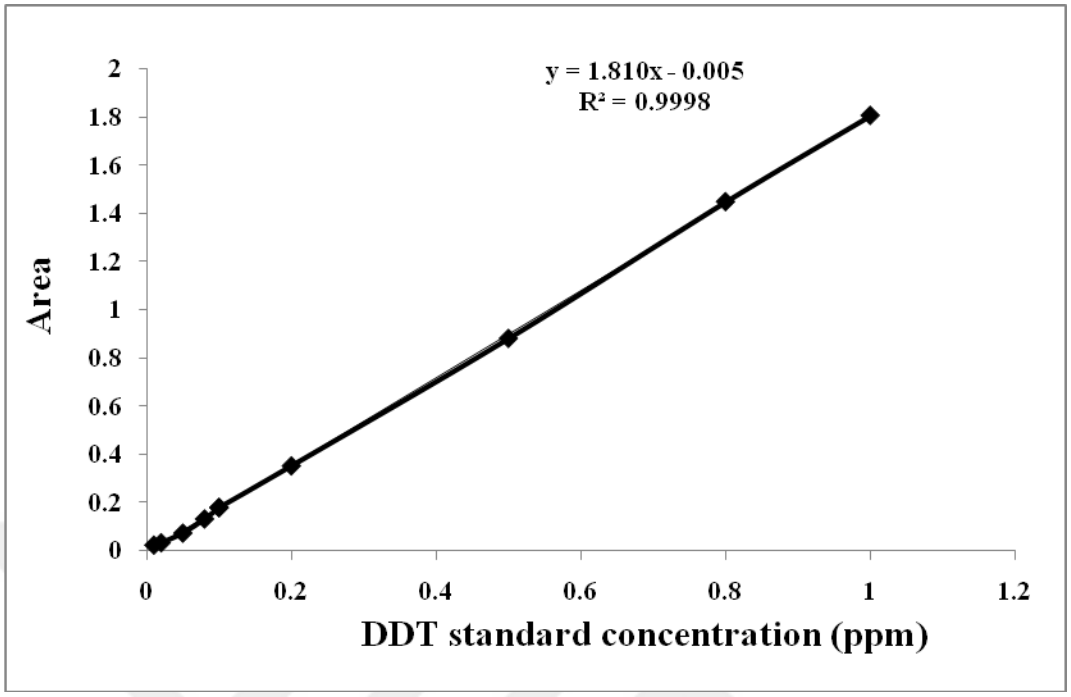



Figure 4.4 The calibration curve of DDT standards at wavelength with 238 nm

Figure 4.4 shows the calibration curve for DDT standards with range of 10 – 1000 µg/l at wave length of and 238nm. The coefficient of determination ($R^2$) for the calibration curve was 0.9998.

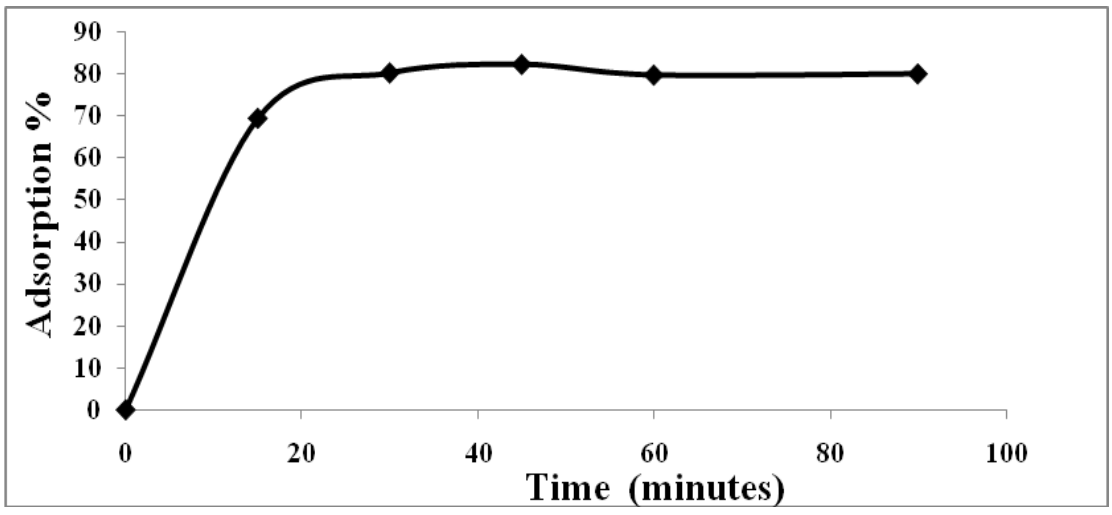



Figure 4.5 Effect of contact time on the DDT adsorption (C:0.5 mg/l, T:25°C and polymer amount: 400 µl)

### *4.4.2 Optimization of DDT Adsorption*

#### *4.4.2.1 Effect of Contact Time*

To determine the effect of contact time on DDT adsorption onto the poly (HEMA-MAPA) nanospheres, adsorption studies were performed from 0 to 90 min. As shown in Figure 4.5, DDT adsorption increased with time and reached a plateau of saturation at 30 min.

#### *4.4.2.2 Effect of Polymer Amount*

The concentration of poly (HEMA-MAPA) nanospheres in the adsorption medium was varied between 0µl to 500 µl. The results of the adsorption studies for poly (HEMA-MAPA) nanospheres are presented in Figure 4.6. We observed that the amount of adsorbed DDT increased with the increasing amount of polymer. Maximum adsorption capacity of the prepared nanostructures was found to be 82% and adsorbed amounts per volume unit of poly (HEMA-MAT) nanospheres reached a plateau at about 400 µl.

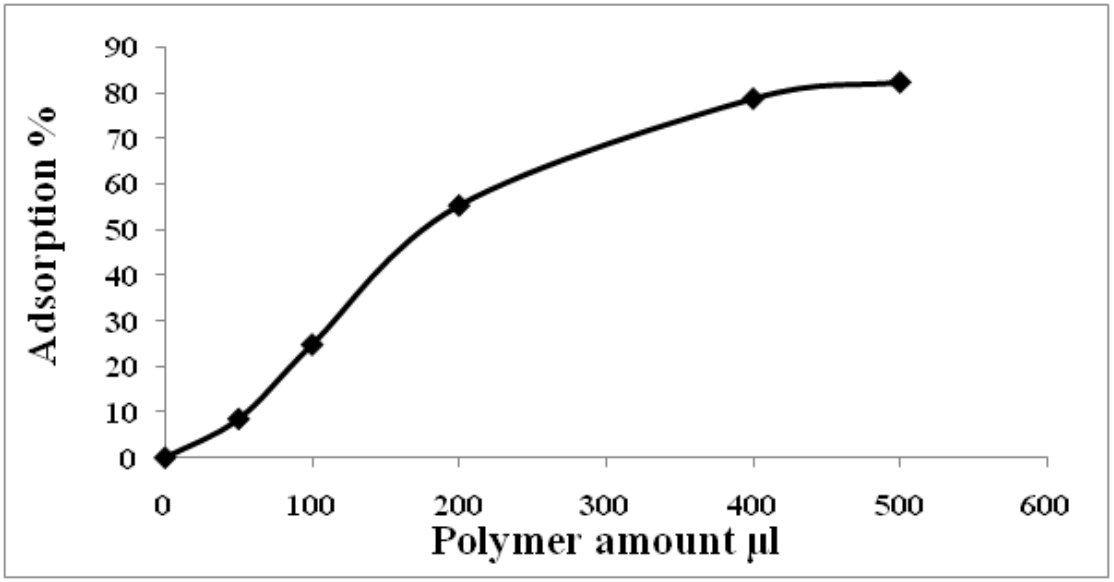



Figure 4.6 Effect of polymer amount (C: 0.5 mg/l, T:25 °C, t: 60 min)

*4.4.2.3 Effect of Initial DDT Concentration*

The concentration of DDT in the adsorption medium was varied between 0.1 and 500 mg/l. The results of the adsorption studies for poly (HEMA-MAPA) nanospheres are presented in Figure 4.7. We observed that the amount of adsorbed DDT increased with the initial DDT concentration.

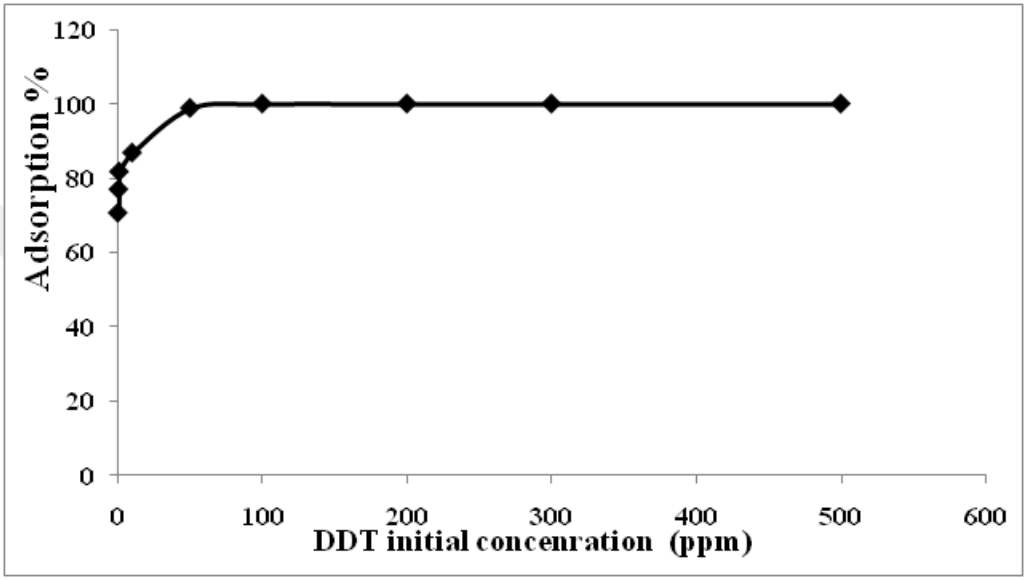



Figure 4.7 Effect of initial DDT concentration effect (T: 25°C, 60 min and 400 µl polymer)

Maximum adsorption effeciency of the prepared nanospheres was found to be 99% and adsorbed amounts per unit µl of poly (HEMA-MAPA) nanospheres reached a plateau at about 100 mg/l. These results agreed with some experiments in which cyclodextrin nanospheres were used for water purification from organic pollutants. By results it was concluded that sorption experiments in solutions with high concentrations of corresponding organic molecules show a high adsorption capacity until reaching the saturating state (Baruch-Teblum et al., 2010). Also Shahin (2004) attributed the increase of the adsorbent effeciency for the extraction of chlorinated pesticides from water field samples comparing to MilliQ water to the presence of organic compounds in the water samples that modified the surface onto which the pesticides were adsorbed during the chemical analysis.

*4.4.2.4 Effect of Temperature*

The adsorption studies were performed over a range of temperatures from 15 to 45°C. As seen in Figure 4.8, the adsorption capacity of the poly (HEMA-MAPA) nanospheres was found to have low affect with increasing the temperature between 15-35 °C when the temperature was rised to 45 °C the adsorped amount of DDT on poly (HEMA-MAPA) nanospheres increased about 10% than the amount that adsorped at 15 °C. This result is agreed with what we mentioned before in chapter 2. Briefly, as HIC is an entropy-driven and as it is well known, if temperature is increased entropy will be increased also and as aresult the HIC will increase (Queiroz et al., 2001). Moreover, according to equation (4.1) the capacity factor k' in HIC increases with increasing temperature (El Rassi, 1996):

$$\ln k' = \ln\varphi - \Delta G/RT \qquad (4.1)$$

where R is the gas constant, φ is the phase ratio and T is the absolute temperature.

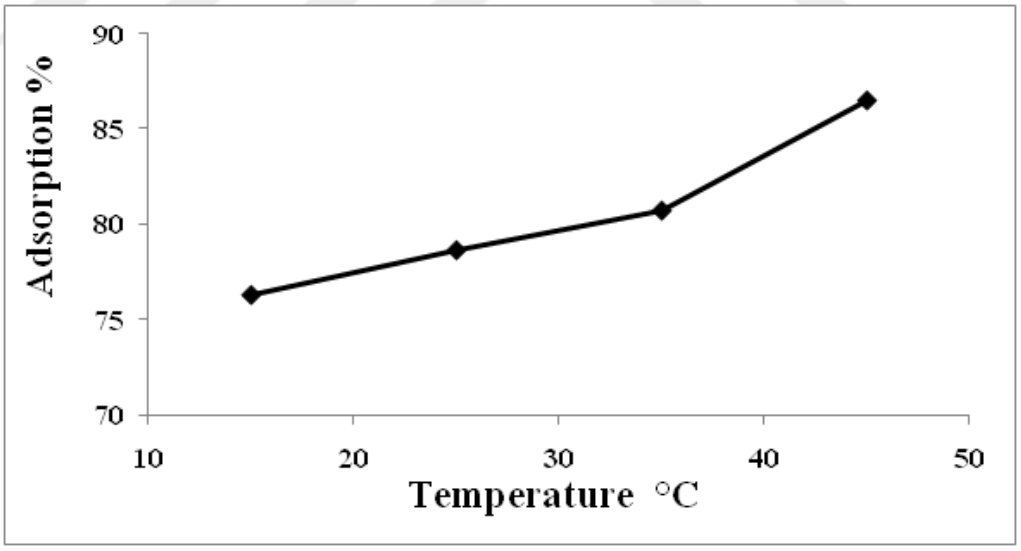



Figure 4.8 Effect of temperature (C: 0.5 mg/l, t: 60 min and polymer amount 400µl)

*4.4.2.5 Effect of Ionic Strength*

To determine the effect of ionic strength on DDT adsorption, adsorption studies were carried out in the range of 0.001- 0.1 M NaCl. As shown in Figure 4.9, the

adsorption capacity of poly (HEMA-MAPA) nanospheres was not significantly changed in the concentration range of 0.00 - 0.1M NaCl.

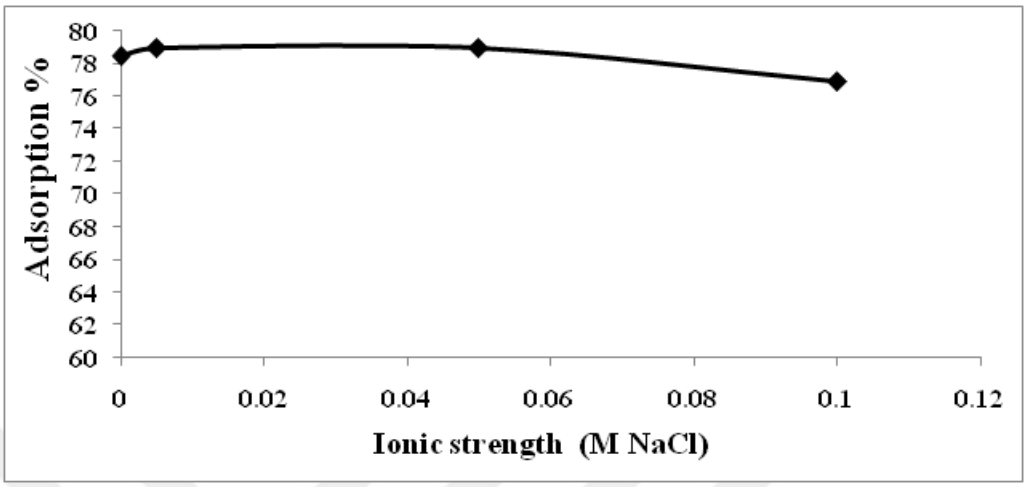



Figure 4.9 Effect of ionic strength (C: 0.5 mg/l, T:25 °C, t: 60 min, polymer amount 400 µl)

This can be attributed to the high binding capacity (high hydrophbocity) and selectivity of the Phenylalanine group in poly [HEMA-MAPA] nanospheres which may need no promoter like a salt to enhance the connection ability between it and the phenyl groups of the non-polar DDT compound, as that the feature structure of the adsorbent is a very important factor in determining the adsorption ability of any material. When Jing, Yang, & Zongshan (2014) used magnetic nanospheres coated with polystyrene (Fe3O4 @PS) to remove OCPs from aqueous systems by adsorption, the effect of salinity and humic acid on the adsorption efffeciency was also studied and the results illustrated that their effect could be neglected under optimized conditions.

# CHAPTER FIVE

# CONCLUSION

The present work investigated the levels of organochlorine pesticides (OCPs) and polychlorinated biphenels (PCBs) in sediment and fish samples from Izmir and Çandarlı Bays along the Eastern Aegean coast, an areas economically important for the western part of Turkey due to maritime, industrial and tourism activities.

The OCPs identified in this study included γ HCH, cyclodienes, HCB, DDTs in sediment. In addition to OCPs levels of PCBs including Aroclor1254 and Aroclor 1260 were measured in the sampling sites. Due to it's using as industrial chemicals Aroclors were the predominant compounds between all the contaminants from all sampling sites with average concentrations of 44.5 and 58.9 ng $g^{-1}$ dry wt for Aroclor 1254 and Aroclor 1260, respectively. Their probable source in marine sediment is industrial wastes. Cyclodienes were the predominant OCP contaminant in sediments of the Izmir and Çandarlı Bays. The levels of organochlorines in sediments were generally low for all compounds by global standards. The concentrations of ΣOCPs were in the range of 1.48-29.6 ng $g^{-1}$ dry wt with an average value of 6.91 ng $g^{-1}$ dry wt. The total DDT concentrations ranged from 0.16 to 19.2 ng $g^{-1}$ (dry wt) with a mean value of 3.21 ng $g^{-1}$ (dry wt) and the highest ΣDDTs levels occurred at Nemrut Bay with 19.16 ng $g^{-1}$. The highest concentrations of both of ΣPCBs and ΣOCPs found in sediment samples collected from Nemrut site of Çandarlı Bay can be attributed to industrial activities in that region.

Ratios of (DDE+DDD)/ΣDDT ratios ranged from 0.90 to 0.98 with entire values being >0.5. This result indicates that the degraded metabolites formed a significant proportion of total DDT compounds. Moreover, the relative percentages of *p,p'*-DDT didn't exceed 9.68 % of the ΣDDTs at all stations. Given the relatively slow degradation rate of DDT in the environment, such results indicate early DDT influxes and non-existing DDT use throughout the sampling sites. The presence of DDTs in the sediments from those bays seems to be derived from the aged and weathered agricultural soils and transported by surface run-off from the rivers.

According to sediment quality guidelines, the levels of *pp'*-DDE and *pp'*-DDD were higher than ERL values at Nemrut Region. Meanwhile, these compounds also exceeded the TEL value at the same site, but were significantly lower than the PEL and ERM values. DDTs could be the one-main species of OCPs with more ecotoxicological concern in Numrut Region, while other OCPs in surface sediments would be less possible to cause adverse biological effect.

The PCBs levels (Aroclors) in sediment were also compared to international quality guidelines in order to assess the propable ecotoxicological effect of these compounds. The total PCB concentrations of the samples collected from sampling sites exceeded the ERL value at Aliağa and Nemrut regions which suggests that PCBs can exert toxic biological effects on aquatic organisms. On the other hand these levels exceeded the ERM, PEL values in Nemrut site. Based on Canadian quality guidelines, Aroclor 1254 concentrations of the samples collected from sampling sites exceeded the TEL value at Nemrut region which implies that adverse biological effects is expected occasionally at this region.

As in sediment samples, the OCs were detected in all fish samples collected from the Gediz, Uzunada, Gülbahçe, Middle Bay and Aliağa sampling sites in this study. The maximum concentration of ΣOCPs was found in fish samples collected from Gülbahçe site. ∑Cyclodiens were the predominant contaminants in all fish samples, representing 53-89% to total OCPs. In all samples *p,p'*-DDE was the predominant DDT congener. The highest concentration of ΣPCBs was found in samples from Aliağa, while the lowest concentration of ΣPCBs was measured fish samples collected from the Middle Bay. The estimated daily intake for DDTs via the studied fish species by Eastern Aegean coast habitants was lower than the ADI recommended by FAO/WHO, proposing consumption of marine fish in Izmir and Çandarlı Bays might not pose a significant health risk to local residents in the coastal areas of the western Turkey.

In this study, hydrophobic nanospheres containing phenylalanine group prepared by the polymerization of HEMA and MAPA were used for the extraction of DDT via adsorption.

According to our results the poly[HEMA and MAPA] nanosphere has high capacity for the adsorption of DDT from it's standard solution via hydrophic interaction phenomenon due to the large surface area of these nanospheres. Rising the temperature of the medium to about 45 °C the adsorped amount of DDT on poly(HEMA-MAPA) nanospheres increased about 10% comparing to the amount that adsorbed at 15 °C. This result is attributed to the nature of HIC which is an entropy-driven phenomenon and as it is well known, If temperature is known an increase of entropy will be observed if the temperature increases. On the other hand, adsorption capacity of poly (HEMA-MAPA) nanospheres toward DDT standard was not significantly changed by changing the concentration of NaCl salt between 0.00 and 0.1M.

By increasing the initial concentration of DDT in the adsorption medium an increasing of of poly(HEMA-MAPA) nanospheres adsorption capacity was observed. Solutions with high concentrations of corresponding organic molecules show a high adsorption capacity until reaching the saturating state. On the other hand, the adsorption was relatively fast, and the time required to reach equilibrium conditions was about 30 min. As a result, the low cost, less labor intensiveness, high separation efficiency of poly(HEMA-MAPA) nanospheres make them most promising synthetic materials for the removal of DDT.

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