INVESTIGATION OF EXTRACTION METHODOLOGIES FOR QUANTITATIVE DETERMINATION OF POLYCYCLIC AROMATIC HYDROCARBONS IN SEDIMENTS

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INVESTIGATION OF EXTRACTION METHODOLOGIES FOR QUANTITATIVE DETERMINATION OF POLYCYCLIC AROMATIC HYDROCARBONS IN SEDIMENTS

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ABSTRACT

INVESTIGATION OF EXTRACTION METHODOLOGIES FOR QUANTITATIVE DETERMINATION OF POLYCYCLIC AROMATIC HYDROCARBONS IN SEDIMENTS

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The extraction procedures for the determination of polycyclic aromatic hydrocarbons (PAH) concentrations in sediment samples had been developed by using GC-FID and GC-MS. The optimized methods were soxhlet extraction, ultrasonic bath extraction and solid phase micro extraction (SPME). In order to search out the main factors affecting extraction efficiencies of the methods, factorial design was used. The best extraction method was chosen and optimum values for main factors were selected for the development of the extraction method for PAH determination in sediment samples. The accuracy of the method was verified by analyzing NIST SRM 1597 (complex mixture of polycyclic aromatic hydrocarbons from coal tar). The selectivitiy and sensitivity obtained were quite adequate for the determination of PAHs in sediment sample. The best extraction and analysis methods were then applied to determine 16 PAHs in sea sediments from Ölüdeniz Lagoon, Muğla, Turkey and 19 PAHs in İkizcetepeler Dam Lake, Balıkesir, Turkey sediments to illustrate the capability of the selected extraction and analysis method to detect PAHs and to determine the status of the contamination.

Keywords: Polycyclic Aromatic Hydrocarbons, Sediment, Chemometrics, Gas Chromatography Flame Ionization Detector (GC-FID).

ÖZ

SEDİMANLARDAKİ POLİSİKLİK AROMATİK HİDROKARBONLARIN TAYİNİ İÇİN ÖZÜTLEME TEKNİKLERİNİN ARAŞTIRILMASI

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Sediman örneklerinde bulunan polisiklik hidro karbonların gaz komatografisi alevli iyonlaştırma dedektörü ve kütle dedektörü kullanılarak tayin edilmesi için özütleme metotları geliştirilmiştir. Optimize edilen metotlar sokslet, ultrasonik banyo ve katı faz mikro özütlemedir. Özütleme verimini etkileyen ana faktörleri araştırmak için faktöriyel dizayn kullanılmıştır. En iyi özütleme metodu bulunmuş ve ana faktörler için optimum değerler seçilerek, sediman örneklerindeki polisiklik hidro karbonların tayini için özütleme metodu geliştirilmiştir. Metodun doğruluğu NIST SRM 1597'nin (kömür katranındaki polisiklik hidrokarbonların komplex karışımı) analizi ile saptanmıştır. Metodun seçiciliği ve hassasiyeti, sedimanda bulunan polisiklik hidrokarbonların tayini için uygun olarak bulunmuştur. En iyi özütleme ve analiz metodu, seçilen özütleme ve analiz metodunun polisiklik hidrokarbonları tayin etmedeki kabiliyetini göstermek ve kirletici durumu tayin etmek için Muğla, Ölüdeniz'den toplanan deniz sedimanlarındaki 16 polisiklik hidrokarbonun ve Balıkesir, İkizcetepeler Barajından toplanan sedimanlardaki 19 polisiklik hidrokarbonun tayin edilmesinde kullanılmıştır.

Anahtar Kelimeler: Polisiklik Aromatik Hidrokarbonlar, Sediman, Kemometrik, Gas Kromatografisi Alevli İyonlaştırma Dedektörü.

To My Family

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LIST OF ABBREVIATIONS

BBD	Box–Behnken Designs
CCD	Central Composite Designs
DI	Deionized Water
DM	Doehlert Matrix
EPA	Environmental protection Agency
FID	Flame Ionization Detector
GC	Gas Chromatography
HPLC	High Performance Liquid Chromatograph
LOD	Limit of Detection
LOQ	Limit of Quantitation
MS	Mass Spectrometry
nd	No Date
NIST	National Institute of Standarts and Technology
PAH	Polycyclic Aromatic Hydrocarbons
QA	Quality Assurance
QC	Quality Control
SPME	Solid Phase Micro Extraction
SRM	Standart Reference Material
USEPA	United States Environmental Protection Agency
TLC	Thin Layer Chromatography
SSJ/LIF	Supersonic Jet / Laser Induced Fluorescence
GC/FT-IR	Gas Chromatography/Fourier Transfrom Infra Red Spectrometry

CHAPTER 1

INTRODUCTION

1.1.What is Sediment ?

Sediment is mineral or organic solid matter which is deposited by washing or blowing from land into the lakes, rivers or marine environment. It can be a dangerous pollutant since contaminants including heavy metals and toxic chemicals can be transported with it. Fine sediment can blanket the bottoms of lakes and rivers. This is dangerous for aquatic life such as fish eggs, insects, fish gills and therefore for ecosystem since food chain is disrupted. Suspended sediment in water can also be detrimental before it settles down. It makes water turbid and high turbidity makes water aesthetically unpleasant. Suspended sediment also creates thermal pollution by darkening water. This lead to water absorbs more solar radiation and therefore water temperature raises to the higher points which is a dangerous situation for aquatic life. In addition, sediment prevents light from reaching aquatic plants, therefore plant growth is hindered. The oxygen level drops down and fish may die. This type of damage has further results. When plants and fish died, wastes and materials that are naturally carried can not be broken down and they accumulate by forming another source of pollution.

There are five main sediment sources which include:

1. Terrigenous sediment(carried from land)
2. Biogenic sediment(comes from plants and animals)
3. Hydrogenous sediment (formed on sea floor by precipitation by chemical or biochemical reactions in water)

4. Volcanogenic sediment (come from volcanic eruption particles)
5. Cosmogenous sediment (comes from outer space particles)

It is difficult to decide that sediment pollution is good or bad since there are several sediment quality criteria (Pinet, 2000).

1.1.1. Sediment Pollution

The agriculture has an important effect on water quality, but anthropogenic erosion and sedimentation are more important issues than agriculture. Sediment pollution is investigated under two headings:

1. Physical Pollution: Top soil loss by erosion which causes turbidity and ecological problems.

2. Chemical Pollution: Some chemicals like phosphorus, chlorinated pesticides and most metals are adsorbed on silt and clay part of sediment and are carried into the aquatic system.

The main sources of sediment pollution are rainfall, soil permeability, infiltration, surface runoff, interflow, groundwater and snowmelt. (http://www.fao.org/docrep/w2598e/w2598e05.htm).

There are alot of inorganic and organic pollutants exist in sediment. Among these organic pollutants, the most important one is polycyclic aromatic hydrocarbons (PAHs) and it was explained below.

1.2. Polycyclic Aromatic Hydrocarbons (PAHs)

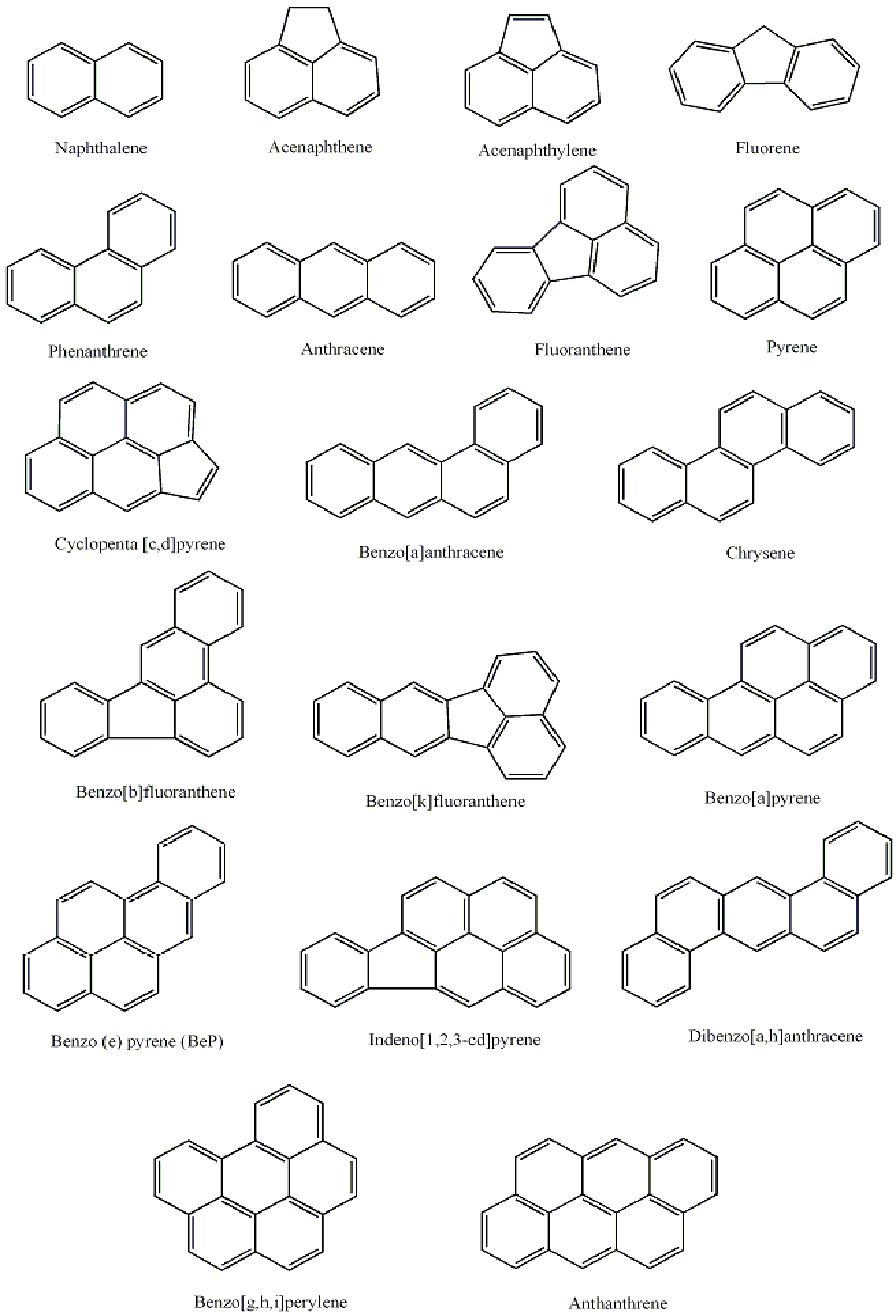
PAHs are chemicals which are produced by the incomplete burning of coal, oil, gas, wood and other organic substances. Their numbers are more than 100 and they generally occur as complex mixtures. Most PAHs occur naturally, and they are manufactured as individual compounds for research purposes. They are generally colorless or yellow-green and they may have pleasant odor. The small number of PAHs are used in medicines, dyes, plastics, pesticides and asphalt.

They may also exist in crude oil, coal, coal tar and creosote. They are found in the air, water, and soil in environment. Figure 1.1 shows the general structures of some important PAHs including 16 EPA PAHs.

These 16 EPA PAHs are; Naphthalene (Nap), Acenaphtylene (Acy), Acenaphtene (Ace), Fluorene (Fle), Phenanthrene (Phe), Anthracene (Ant), Fluoranthene (Fla), Pyrene (Pyr), Benzo*(a)*anthracene (BaA), Chrysene (Chr), Benzo*(b)*fluoranthene (BbF), Benzo*(k)*fluoranthene (BkF), Benzo*(a)*pyrene (BaP), Indeno*(1,2,3–cd)*pyrene (IcP), Dibenzo*(a,h)*anthracene (DaA), Benzo*(g,h,i)*perylene (BgP).

These 16 PAHs were chosen as EPA pollutant since;

1) They are mostly known
2) They are more harmful
3) They mostly exist in environment



Naphthalene
Acenaphthene
Acenaphthylene
Fluorene
Phenanthrene
Anthracene
Fluoranthene
Pyrene
Cyclopenta [c,d]pyrene
Benzo[a]anthracene
Chrysene
Benzo[b]fluoranthene
Benzo[k]fluoranthene
Benzo[a]pyrene
Benzo (e) pyrene (BeP)
Indeno[1,2,3-cd]pyrene
Dibenzo[a,h]anthracene
Benzo[g,h,i]perylene
Anthanthrene

Figure 1.1. The General Structures of 19 PAHs

1.2.1. PAHs in Environment

PAHs enter the environment in variety of ways. For example, they enter to air from volcanoes, forest fires, residential wood burning, exhaust of automobiles and trucks. They also enter to surface water from discharges of industrial plants and waste water treatment plants. In addition, they enter to soils at hazardous waste sites from storage containers if there is any escape. The movement of PAHs in the environment depends on mainly two properties; (1) how easily they dissolve in water (2) how easily they evaporate into the air. The PAHs, generally do not easily dissolve in water, and in air; they exist as vapors or they stuck to the surfaces of small solid particles by which way they can travel long distances. They return to earth from air by rain or particle settling. Some PAHs escape from surface waters into the atmosphere by evaporation, but most of them stick to solid particles and settle down to the bottoms of rivers, lakes and seas as sediment. The PAHs most likely stick to solid particles in soil and some PAHs goes from surface soils to air by evaporation. Some PAHs in soils also diffuse into the underground water. The PAH concentration in living organisms such as plants and animals on land or in water can be much higher than the PAH concentration in soil or water. Breakdown of PAHs in air , to longer-lasting products occurs by reacting with sunlight and some chemicals. This process takes days to weeks. Breakdown in soil and water also occurs by the actions of microorganisms and takes weeks to months.

1.2.2. Effects of PAHs

It may be exposed to PAHs at home, outside, or at workplace. However, it is exposed to a mixture of PAHs, instead of an individual one.

In the environment, such as in air, it is most likely to be exposed to vapors or dust particles including PAHs. The main sources of PAHs in environment are; cigarette smoke, exhausts, asphalts, coal, residential wood and coal burning, agricultural burning, hazardous wastes and municipal and industrial waste incineration.

Background PAH levels in air are 0.02–1.2 ng/m^3 and 0.15–19.3 ng/m^3 for rural sites and urban sites respectively. In soil where coal, wood, gasoline, or other products were burned, it is possible to be exposed to PAHs. The other PAH sources in soil are hazardous waste sites, gas factory sites and sites where wood-preserving facilities are performed. PAHs can also be found in drinking water, for example, background levels of PAHs in some drinking waters in United States are 4-24 ng/L.

1.2.3. Healths and PAHs

PAHs may enter the body through lungs from air by cigarette, wood, coal and industrial smoke. Hazardous waste sites are also another source. However, the process of absorption of PAHs by lungs is still unknown. In addition to inhaling by air, PAHs can be absorbed by drinking or swallowing. While they are swallowed absorption process is generally slow. PAHs may enter the body also by skin contact with e.g. soil, crankcase oil and other products containing high level of PAHs. Absorption rate of PAHs by eating, drinking and skin contact depends on the presence of other compounds existing in environment. PAHs can enter tissues containing fat and mostly stored in kidneys, liver and fat.While, small fraction is deposited in spleen, adrenal glands, and ovaries. PAHs stored in tissues are changed into less or more harmful substances. Studies showed that PAHs no longer stay in the body and disposed in a few days together with the fece or urine.

Studies with animals also showed that fighting with disease after short or long term exposure, PAHs may have some harmful effects on skin, body fluids, and the body's system. These effects were not explained for people. EPA explained that BaA, BaP, BbF, BkF, Chr, DaA, and IcP are probable human carcinogens and Acy, Ant, BgP, Fla, Fle, Phe, and Pyr are not human carcinogen. Ace is not classified as carcinogen by EPA.

Once enter the body, PAHs are transformed into chemicals that have ability to attach substances in the body. These substances are then measured in the body tissues and blood. Urine is also another form of body fluid where PAHs or their metabolites can be measured. (http://www.atsdr.cdc.gov/ToxProfiles/tp69.pdf).

1.2.4. Extraction Methods for PAHs in Sediments

The PAHs in sediments can be extracted with solvent extraction techniques including soxhlet and ultrasonic bath extractions. In addition, a novel technique; solid phase extraction (SPME) was improved to reduce solvent amount. In this study soxhlet, ultrasonic bath and SPME (with two modes, direct SPME and headspace SPME) were used. All these works are discussed below.

1.2.4.1. Soxhlet Extraction

Soxhlet extraction is an old fashioned method and used for comparisons with new extraction methods. It mainly consists of four parts; a reservoir part for solvents, body part for placing an extraction thimble, heating source and water cooled reflux condenser. Organic solvents are used for removal of organic compounds from solid samples. An operation of soxhlet extraction is simple; the same amount of solid sample and anhydrous sodium sulfate are placed in a cellulose extraction thimble which is placed in an extraction body (above solvent flask) and washed with organic solvents in bottom flask, several times. This extraction body is fitted to a reflux condenser through which condensed solvent falls into the cellulose thimble. As solvent level reaches the top of the body part, it syphons over into the solvent flask and it is said that one cycle is completed.

1.2.4.2. Ultrasonic Extraction

Ultrasonic bath extraction (sonication) is mainly based on using sound waves for agitation of sample in organic solvents. By this way, organic analytes are separated from solid matrices and taken into an organic extraction solvents. It is important to cover all of samples in glass container with the proper organic solvents to make an extraction efficient. The sonication time is generally between 3-15 minutes. After an extraction finished, extraction solvent in the glass container is taken either directly or after filtration /centrifugation. Then fresh solvent is added and this process is repeated three times (total extraction time is in the range of 9-45).

Finally all three extracts are combined to obtain final solution. Anhydrous sodium sulphate is either combined with the solid samples in same amount or drying column is prepared and final solution is filtered throuh this column (Dean, 2003).

1.2.4.3. SPME

The SPME is an extraction method in which analytes are adsorbed on the surface of coated silica fiber and then desorbed into a proper instrument (e.g. GC-MS, HPLC, LC-MS) for separation, identification and quantification. Since in natural waters and waste waters organic analyte concentrations are very small, preconcentration step is necessary. Nowadays, SPME is also used as a preconcentration method.

Although variety of stationary phases exist, the most important three phases for PAH analysis were given in Table 1.1.

Table 1.1. SPME Fiber Coatings

7 µm polydimethylsiloxane (bonded)
100 µm polydimethylsiloxane (non-bonded)
85 µm polyacrylate (partially cross-linked)

(Pawliszyn, 1997)

There are two main types of SPME; (1) Direct SPME, (2) Headspace (HS) SPME as explained below;

Direct SPME: In this mode of extraction, the coated fiber is directly immersed in a sample and analytes are transferred from sample to extracting fiber. When equilibrium is reached between the sample matrix and coated fiber, extraction is said to be completed.

Headspace SPME: In this extraction mode, an aqueous or solid sample is placed in a closed container having a headspace (empty volume), and coated fiber is kept above the sample (in headspace part of the container), without touching to the sample matrix. Analytes are firstly transferred from solid or liquid sample matrix to the headspace (gas phase) and then from headspace to the fiber coatings until equilibrium is reached.

The selection of fiber type, generally depends on the chemical properties of the analytes. The adsorption principle is simply; 'similar attracts similar' (Pawliszyn, 1997).

These three extraction methods which were explained above, were optimized by using chemometrics.

1.3. What is Optimization and Chemometrics ?

Optimization is performed to improve the performance of a system to get the maximum benefit. This term is mostly used in analytical chemistry and it is applied to the experimental procedure to obtain the best efficiency.

In analytical chemistry, when old fashioned technique is used, optimization is performed by changing one variable (affecting experimental response) and keeping rest of them constant. However this old technique has some disadvantages; for example, interactive effects (interaction of variables with each other) are not included. In addition, the number of experiments required are very large in this technique, and more reagents, time and expenses are required. All the mentioned problems are solved by using multivariate statistical techniques (Bezerra et al., 2008). In these statistitical techniques two variables exist; (1) response and (2) factor. When this technique is used; minimum and maximum values of factors are selected and their effects on experimental response is investigated. To investigate the importance of the factors full factorial and fractional factorial designs are used. More complex designs including Doehlert matrix (DM), Central Composite designs (CCD) or Box–Behnken designs (BBD) are used to determine optimum experimental conditions (Tarley et al., 2009).

In this study 2-level 3-factor (2^3) full factorial CCD design including eight experiments was used and it was explained below (Brereton, 2003).

1.4. Chemometrics Used for Optimization of the Extraction Methods

Every extraction method in analytical chemistry, need to be optimized before used. In this study, for the optimization of extraction methods, chemometrics (factorial design) was used. By using 2^3 factorial design (screening design), three factors and their effects on response (extraction efficiency) were investigated. Analysis results were given as pareto chart and response surface charts.

Pareto charts searchs which factor has significant importance on experimental response. Importance of factors are shown with the bars on pareto charts and as length of this bar increases importance of the factor also increases. A vertical line on chart represents the 0.005 critical value for student's t test. If any bar exceeds this line, it means that, it is significantly important at 95 % confidence level according to the student's t test. (http://www.statgraphics.com/How%20To%20Perform%20an%20Optimization%20Experiment.pdf).

In addition X axis represents standardized regression coefficient (regression coefficient divided by standart error), Y axis represents regression coefficient for linear terms (A, B, C) and product terms (AB, AC, BC).

Response Surface Methodology (RSM) is a polynomial equation fitted to a experimental data and response surface charts explain the meaning of them. By using response surface charts, the levels of the factors at the maxima (at which response is the highest) can be found. And these levels of the factors are said to be optimum conditions.

In this study first factorial design was used and equation obtained was the linear regression equation which is shown as Y=a + bX + e. In this equation;

Y: product (extraction efficiency in this study)
X: factor level
a: intercept
b: slope
e: error

(http://www.stats.gla.ac.uk/steps/glossary/paired_data.html#regeqn)

The experiments for the extraction of samples were carried on by creating screening design (2^3) with the help of chemometric program (statistical software; statgraphics plus). There were mainly three factors at two level (min-max) as can be seen from Table.

Factorial design was involving total of 8 runs. The values -1 and +1 values represents the factor levels which were low and high values of the factors respectively. Using factorial design results which were found in terms of surrogate recoveries (or peak area) pareto charts and estimated response surface charts were drawn. They were used in order to decide the signifance of the main effects. The factorial design for the extractions was shown in Table 1.2.

Table 1.2. Screening Design (2^3) for the Extractions

Run	Factor A	Factor B	Factor C
1	-1	-1	1
2	1	-1	1
3	-1	-1	-1
4	1	-1	-1
5	1	1	-1
6	-1	1	-1
7	1	1	1
8	-1	1	1

(Brereton, 2003)

1.5. Analytical Methods for Determining PAHs in Sediment Samples

It is difficult to separate PAHs from sediment matrix because of the complexity of the sample matrix. Sometimes, even clean-up step may not be enough and besides analytes, large number of other compunds were also observed.

A simple PAH analysis in sediment sdample includes three basic steps; (1) extraction with a suitable method (removing PAHs from sample matrix-sediment), (2) clean-up (removing impurities from extract), (3) identification and quantification of the analytes-PAHs. Among the used sediment extraction methods, the most important three are; soxhlet, ultrasonic and SPME. The mostly used extracting solvents are; dichloromethane, hexane and acetone, benzene, cyclohexane, chloroform. For clean-up procedures; silica, alumina, fluorisil and sephadex are the most common examples. And the instrumental methods generally used are; as can be seen from the Table 1.3, Thin Layer Chromatography (TLC), GC/MS, High Performence Liquid Chromatography/Diode Array Detector/Mass Spectrometer (HPLC/DAD/MS), Supersonic Jet / Laser Induced Fluorescence (SSJ/LIF), Gas Chromatography /Flame Ionization Detector (GC/FID) and Gas Chromatography/ Fourier Transfrom Infra Red Spectrometry (GC/FT-IR) (http://www.atsdr.cdc.gov/ToxProfiles/tp69.pdf).

Table 1.3. Analysis Methods for Determining PAHs in Sediment Samples

Extraction method	Analytical Method	Detection Limit	Percent Recovery
Exraction with DCM, clean-up with alumina column	TLC,GC-MS	$2x10^{-4}$- $27x10^{-4}$ mg/kg	86-89
Freeze drying, sieving and homogenization, extraction with DCM, clean-up with silica gel followed by sephadex column	HPLC/DAD/MS	$1x10^{-12}$ mg/kg range	No data
Extraction of dry sample with DCM, injection into super critical fluid extracting system	GC-MS	$28x10^{-4}$- $73x10^{-4}$ mg/kg	91-97
Extraction of dried sample with benzene; clean-up with silica gel and alumina column	GC-FID, GC-MS	$14x10^{-6}$- $93x10^{-6}$ mg/kg	76-110
Direct sampling on sediment in sample insert of SSJ/LIF	SSJ/LIF	1.8 mg/kg BaP, 0.4 mg/kg Pyr	No data
Extraction by sonication, clean-upwith mini silica column	Spectrofluorometry	$8x10^{-9}$- $45x10^{-7}$ mg/L	80-95
Freeze drying, extraction with chloroform-MeOH (2:1),concentration on crude extract, clean-up by TLC followed by HPLC	GC-FID	$12x10^{-5}$-$46x10^{-5}$ mg/kg	51-100
Extraction in organic solvent	GC/FT-IR	$1x10^{-5}$-$6x10^{-5}$ mg/kg	No data
Extraction of dried sample with DCM, clean-up with activated copper column followed by sephadex	GC-FID, GC-MS	$3x10^{-5}$-$9x10^{-5}$ mg/kg	99-113

(http://www.atsdr.cdc.gov/ToxProfiles/tp69.pdf)

In this study ultrasonic bath was used for extraction (organic solvents were DCM, acetone and hexane), GC-MS/GC-FID were used as analytical methods and they were explained below in detail.

1.6. Gas Chromatography

Since 1940's gas chromatography (GC) has been widely used in analytical and environmental studies. The GC is preferred since it is sensitive, fast and has a lot of stationary phases. Among disadvantages, the most important one is that; sample introduced should be both thermally stable and volatile since the sample is introduced into GC in gas phase. Applications areas are petrochemistry, environment and pharmaceuticals etc.

It has several components such as injector, column and detector. The mobile phase is gas and generally called as carrier gas. The flow of carrier gas affects the retention times of the analytes and is kept constant during the whole analysis.

Analysis starts with the injection of liquid or gaseous sample. It is vaporized in the injector and swept with the carrier gas into the column whose length changes between 1-100 m. The column contains stationary phase and it is placed in oven whose temperature is controlled with oven temperature program. After passing through the column, it reaches to the detector where identification and quantification is done. Detectors are generally chosen depending on the analyte and mass selective detector (MSD) or flame ionization detector (FID) for PAH analysis. They were explained below.

1.6.1. Flame Ionization Detector (FID)

This detector is very common for determination of organic analytes and is destructive. Flame ionization detector has several components such as stainless steel or alumina body fitted to a flame ignition coil, collector electrode and polarized jet which is insulated electrically from the body (Figure 1.2). When gas exits from the column end (with the organic compounds swept), it passes from the small burner where it is combined with the hydrogen (fuel gas) and air (oxidant) and combusted. Combustion of organic compounds in flame creates charged particles which creates small current btw two electrodes (the burner and the collector electrode). Collector electrode collects the generated ionization current and electrometer converts this to the signal by amplifying it.

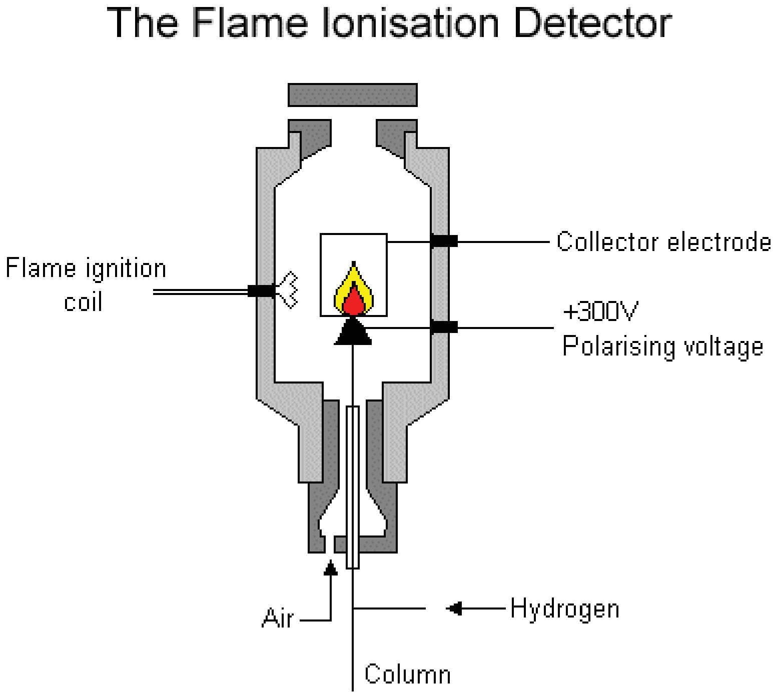


Figure 1.2. The FID
(Kitson et al., 1998)

1.6.2. Mass Selective Detector (MSD)

When an analyte exit GC column;

1) It is bombarded with electrons in ion source and produce ion fragments,
2) These fragments passes through lenses and accelerated into quadrupole by applying RF frequency and DC voltage to both ends of the quadrupole,
3) Fragments are separated according to the mass to charge (m/z) ratios and only specific masses reaches to the electron multiplier and mass detector,
4) MS gives a mass spectrum based on the abundances of the ionized fragments

This spectrum can be either ‘full scan or sim (selected ion monitoring)’ mode. In GC-MS full scan mode; all of the compunds in sample matrix are observed in spectrum. This mode is used when unknown compunds exist in sample and GC-MS confirmation is required. In GC-MS sim mode; only specific analytes (which are looked for in a sample) are observed with higher sensitivity (sensitivity in GC-MS sim mode is greater 10-100 times than that of GC-MS full scan). Generally 2-4 ions are observed for each compound and the observed ratios are specific to each analyte. This makes identification of the compounds easier. In order to increase sensitivity mass scan rate and dwell time (analysis time to look at each mass) should be adjusted. Sensitivity in sim mode is higher since unwanted ions are eliminated therefore matrix interference is overcomed. This also increases selectivity (http://www.caslab.com/News/gcms-full-scan-vs-cgms-sim.html).

1.7. Polycyclic Aromatic Hydrocarbon Sources

There are hundred of PAH present in nature. They are produced either naturally or anthropogenically. There are mainly four types of PAHs; diagenic, petrogenic, pyrogenic and biogenic.

1. Diagenic PAHs: These PAHs are formed at low temperature (< 70 ºC) in a relatively rapid process (days to years). Organic matters (after deposited in sediments) are transformed (by biomolecules) into PAHs. Examples of diagenic PAHs are; perylene, retene, phe and chr.
2. Petrogenic PAHs: These PAHs are produced in a slow manner in a long-term process. At moderate temperature (100-300 ºC) fossil fuel, petroleum or coal is formed. Petrogenic PAHs are rich in 2-3 ringed PAHs.
3. Pyrogenic PAHs: These PAHs are produced rapidly at high temperatures (> 500 ºC). Incomplete (when there is a small amount of oxygen) combustion of organic biomass produces pyrogenic PAHs. The examples of incomplete combustion are; forest and grass fires which are natural sources, and fossil fuel combustion which are anthropogenic source. Pyrogenic PAHs are highly enriched with 4,5 and 6 ringed PAHs.
4. Biogenic PAHs: These PAHs are produced by biosynthesis of plants and animals. As a result biosynthesis either individual PAHs or simple mixtures are formed. Retene, C_4-Phe isomer and simoneltite (Sim) are the examples to the biogenic PAHs.

Among the mentioned PAHs above, some of them are coming from natural sources and some of them are coming from anthropogenic sources. For example, diagenesis and biosynthesis PAHs come from natural sources. However pyrogenic and petrogenic PAHs have both natural and anthropogenic sources. For example, natural sources of petrogenic sources are; oil seepages and erosion of petroliferous shales. The anthropogenic source for these PAHs is; accidential petroleum spillages. The natural sources of pyrogenic PAHs are; forest and grass fires. The anthropogenic sources for pyrogenic PAHs are high temperature combustion of motor, shipping and power-plants fuels.

Combustion processes are the main sources of PAHs in urban regions. Other source of pyrogenic PAHs in atmosphere are residential wood burning (largest contribution) and at home; tobacco smoke, unwented radiants, space heaters, gas cooking and heating systems.

Although 80 % of total PAH emissions are coming from stationary sources, in urban and suburban areas, mobile sources (vehicle exhaust) are the major sources. Other pyrogenic sources are; high temperature processing of fossil fuel, coal (coal tar, creosote), emission from aluminum smelters (using Horizonta Stud Soderbeg process).

What determines whether PAHs are petrogenic or pyrogenic or whether sources are natural or anthropogenic, is the formation temperature. Since degree of alkylation is inversely proportional to the formation temperature. For petrogenic PAHs, main feature is that alkylated PAHs > parent PAHs. However the major property for pyrogenic PAHs is that parent PAHs >> alkylated PAHs.

Source Type Differentiation (Petrogenic vs. Pyrogenic)

The PAH compounds are classified as pyrogenic, petrogenic or mixed according to the Fossil Fuel Pollution Index. Pyrogenic source samples are generally rich in non-alkylated PAH rather than alkylated PAHs as in petrogenic source materials. Although pyrogenic sources generally include 4-6 rings PAHs, petrogenic sources include 2-3 ring PAHs. Some of the PAHs like Phe have both pyrogenic source and petrogenic source, therefore it is said to be originated from mixed source. In order to determine whether the source is petrogenic or pyrogenic, ratios of the analytes were used for example, high molecular weight PAHs (4-5 rings) versus low molecular weight PAHs (2-3 rings) (HMWPAH/LMWPAH) ratio is used for the source identification (Boehm P.D., 2006).

1.8. Methods Used for the Source Determination of PAHs in Sediments

For the source determination of PAHs in sediment samples mainly diagnostic ratios, factor analysis and hierarchical cluster analysis methods were applied. They were explained below.

1.8.1. Diagnostic Ratios of PAHs

Diagnostic ratio is useful tool for the source identification of PAHs in sediment samples. Table 1.4 shows the range of diagnostic ratios for PAH sources (pyrolytic and petrogenic origins of PAHs). Characteristic values of selected molecular ratios are given in Table. The concentration ratio of low molecular weight PAHs to high molecular weight PAHs (LPAHs/HPAHs) is a ratio of sum of Nap, Ace, Acy, Fle, Phen and Ant concentrations against sum of Fle, Pyr, BaA, Chr, BbF, BkF, BaP, IcP, DbA and BgP concentrations.

Table 1.4. The Range of Diagnostic Ratios for PAH Sources

	LPAHs /HPAHs	**Fla /(Fla+Pyr)**	**Ant /(Phe+Ant)**
Pyrolytic origin	<1	>0.4	>0.1
Petrogenic origin	>1	<0.4	<0.1

(Liu et al., 2009)

Pyrogenic sources, such as combustion-derived particles present in urban atmospheric dust, were poor in low molecular weight 2-3 rings PAHs (Low Molecular Weight PAHs: LPAHs) and enriched in high molecular weight 4-6 rings PAHs (High Molecular Weight PAHs: HPAHs), which led to LPAHs/HPAHs < 1. Petrogenic sources, such as fuel oil or light refined petroleum products, were dominated by LPAHs and had LPAHs/HPAHs > 1. Some researchers thought that those PAHs in sediments with the ratio of anthracene to anthracene plus phananthrene; $Ant/(Ant + Phe) < 0.1$ were mainly from petroleum contamination (petrogenic source), while those with $Ant/(Ant + Phe) > 0.1$ were mainly from combustion sources (pyrogenic source).

Some researchers believed that PAHs in sediments with the ratio of fluoranthene to fluoranthene plus pyrene; $Fla/(Fla + Pyr) < 0.4$ means petroleum contamination, while $Fla/(Fla + Pyr) > 0.5$ means PAHs are mainly from combustion of grass, wood and coal and $0.4 < Fla/(Fla + Pyr) < 0.5$ from combustion of petroleum (Liu et al., 2008).

1.8.2. Factor Analysis (FA)

Factor Analysis (FA) is a collection of methods which are used for examining how underlying constructs affects the responses. There are mainly two classes for FA; exploratory FA and conformatory FA. In this study exploratory FA (EFA) was applied. The EFA is used to determine the pattern of the constructs affecting responses.

The "Common Factor Model" as illustrated in Figure 1.3, is a basis for FA. According to this model each response (Measure 1-5) is partially affected both by underlying common factors (Factor 1-2) and unique factors (E 1-5). In addition the power of the relationships between factors and measures differs.

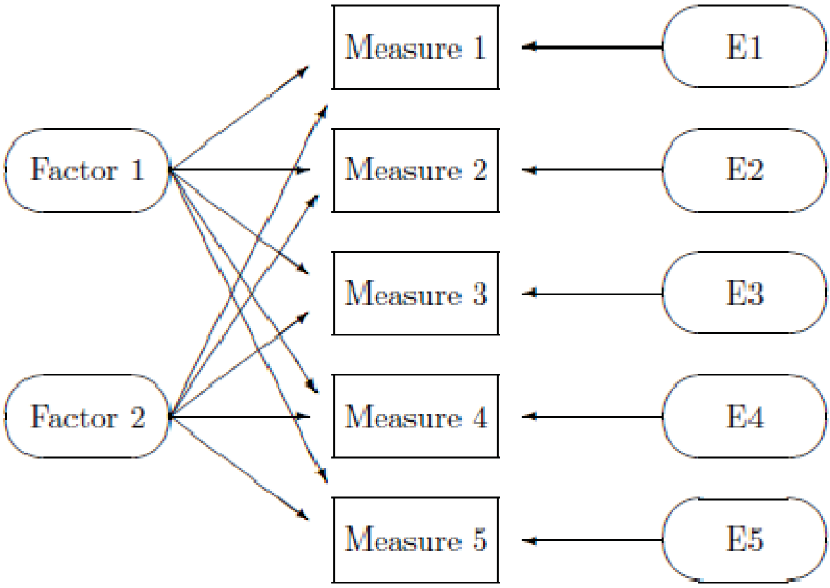


Figure 1.3. Common Factor Model

The FA is applied by searching the correlations between the measures. For example, although high correlation between measures (positive or negative) indicates that measures are effected by same factors, measures that are relatively uncorrelated are affected by different factors.

The Goals of EFA

1. To decide the number of common factors
2. To find out the power of the relationship between the factors and measures
3. To recognize the nature of the constructs underlying responses
4. To find which set of items are in question
5. To show the dimensionality of measurement scale
6. To decide which properties are the most important when items are classified
7. To create factor scores that represents values of constructs

How EFA is Performed?

The EFA is performed in seven steps;

1. Collecting measurements.
2. Getting correlation matrix.
3. Selecting factors. If there are k measures, there can be maximum k factors. There are different methods for deciding factor numbers. For example according to "Kaiser Criterion", factor number is equal to the number of eigenvalues of the correlation matrix which are greater than1. According to the "Scree Test", eigenvalues are plotted in descending order and the number of factors are decided according to the number of eigenvalues greater than 1.
4. Extracting initial set of factors. This step is achieved by computer. There are a lot of methods of extraction which are maximum likelihood, principal component and principal axis extraction. Among them mostly maximum likelihood is preferred.
5. Rotating factors to a final solution. There are a lot of ways of defining factors. Interpretations of some definitions are easier. For example by rotating factors, factor solution equal to one is obtained in initial extraction and this is the simplest interpretation. There are a lot of ways for rotation but in theory all of them try to make factors highly responsive to a small set of items. There are two types of rotations; orthogonal rotation and oblique rotation. Varimax is the best among orthogonal rotations and there are three common oblique rotations; Direct Quartimin, Promax and Harris-Kaise Orthoblique.
6. Interpretation of factor scores. The measures will be linearly related with the factors. The power of this relationship between measures and factors is explained with the factor loadings which is the result of rotation. Interpretation of factor loadings can be done by using standardized regression coefficient. Factor is defined by considering the possible theoretical constructs that is responsible for the pattern of positive and negative loadings. To make interpretation easier, all the loadings are multiplied with -1 and by this way scale of the factor is reversed and it turns from unfriendliness to friendliness.

7. Constructing factor scores for further analysis. When extra analysis are needed by using factors as variables, factor scores should be constructed. Factor score is a linear combination of the measures and is weighted by the factor loadings. Factors are idealized by giving positive 1 to strongly positive loadings, negative 1 to strongly negative loadings and zero to intermediate loadings. The factor scores like the other varibles, can be used in analysis and they will be collinear with the measures. (DeCoster, 1998).

In this study, while performing FA (EFA) Kaiser Criterion and Varimax Rotation were used.

1.8.3. Hierarchical Cluster Analysis (HCA)

The HCA is a method of determining underlying object structures by agglomerative methods (associates) or divisive methods (dissociates). It is finished when all of the objects were processed.

The agglomerative method starts with each object at different cluster and then combine the clusters. At the final, all the clusters are reduced and all objects are collected under one cluster. However the divisive method starts with one cluster having all of the objects, and then creates smaller clusters until having separate clusters for each object. When defining HCA method two options exist; type of similarity between objects and linkage technique. Firstly by constructing similarity matrix, value of the similarity between objects is determined. There are many options for this but the most common ones are; "Euclidian Distance" and "Correlation Coefficient". Next objects are grouped or ungrouped. For this reason agglomerative method is used and objects are connected to each other forming groups. The first connection is done between the most similar objects. After first group is created, similarity between new group and the rest of the objects is defined. For this reason, number of techniques are used. Among them the most commons are; complete linkage (furthest neighbor), single linkage (nearest neighbor), average-linkage (between groups and within groups), centroid method and Ward's linkage.

In this study, the samples were hierarchically clustered using weighted average linkage between the groups and Pearson correlation for the cluster intervals. The Pearson's correlation is applied to determine a correlation between at least two objects. Pearson value of 0 means no correlation and 1 means perfect correlation. For the high correlations, value of 0.80 is used (http://academic.uofs.edu/department/psych/methods/cannon99/level2a.html).
The average group linkage is one of the linkage method and it is used for calculating distance between the average values of the two clusters (http://www.statistics.com/resources/glossary/a/avggrplnkg.php).

After determining similarity measure and linkage method, the agglomeration of objects follows the order of larger similarity. By hierarchical clustering, structure called as dendogram is obtained, and in dendogram each linkage in cluster is shown by a connection line. As a result of different similarity measure and linkage techniques, different dendograms are obtained. But it is good to use different methods and compare the results. Each method has its own advantages and disadvantages (Almeida et al., 2007).

1.9. Literature Review

PAHs are anthropogenic (formed in a result of human activities) organic pollutants and they exist commonly in the environment. They are priority of concern since they are carcinogen and very common in the environment. They enter the environment from different ways such as; oil spills, industrial discharges, municipal and urban runoff and atmospheric precipitation. In marine environment, PAHs are partition into sediment since they are hydrophobic. Therefore while determining PAH concentration in marine or other aquatic environments sediment pollution need to be investigated (Xu et al., 2007).

There are a lot of sediment pollution study and they were mentioned in literature review part. This part was studied under two headings; analytical methods and marine & lake studies.

1.9.1. Analytical Methods

An extraction procedure including fast automated extraction clean-up procedure was investigated for the analysis of organic pollutants such as polychlorinated dibenzo-p-dioxins (PCDDs), polychlorinated dibenzofurans (PCDFs) and coplanar polychlorinated biphenyls (cPCBs) in biological fluids. Soxhlet extraction and solid phase extraction were compared with this new method. By using certified reference values, repeatibility, reproducibility, accuracy and recovery values were also compared. Total analysis time and cost were lowered by using simultaneous sample preparation units. Sample amount was large compared with the other methods. This new method is suitable for the areas where fast sample preparation is needed e.g. emergency foodstuff analysis and epidemiological studies (Focant and Pauw, 2002).

As an example to coupled online studies, supercritical fluid chromatography clean-up system was coupled to GC-MS by using cryo-trap cell. This system was used for the analysis of organic pollutants in environmental samples. High performance liquid chromatography (HPLC) packed column with 100 mg Florisil was used for clean-up system. As a result of this coupling, high boiling compounds effect on chromatographic efficiency was reduced. Recovery values for pollutants were higher than 75 % and RSD was 15 % (Fuoco et al.,1999).

To investigate the effect of sorbent modification on sorptive behaviour of PAHs by synthetic chemical thermal weathering, this study was conducted. Clean soil samples which were sandy-clay-loam, extracted with soxhlet and sorptive behaviour of PAHs were investigated by considering quantity/quality changes in soil organic matter (SOM) and clay minerals. Differences in sorption capacity was found to be related with beginning PAH concentrations. When PAH concentration was above critical limit, qualitative modifications in sorbents facilitated an opposite trend. The factors leading different PAH sorptive phenomena were; sorbent swelling, removal of competing compounds, changes in surface characteristics by Soxhlet and increased concentration gradient effects (Hwang and Cutright, 2004).

A new technique, fluidized-bed extraction for the analysis of PAHs, was developed and compared with the classical technique, soxhlet extraction. This new technique was optimized by using surface response design. During the optimization, parameters such as extraction cycles and holding time were considered. By using surface response design, effects of these parameters on extraction performance were investigated (Gfrerer et al.,2002).

Analysis of 16 PAHs in sediment porewater samples from Mersey Estuary, UK, was achieved by using SPME coupled with GC-MS method. For the SPME analysis, first analytes were sorbed on a fiber and then desorbed from fiber to the GC inlet. The SPME efficiency was found to be mostly depend on factors such as exposure time and use of agitation (King et al., 2004).

The volatile and semivolatile pollutants in soil samples were analyzed by using HSSPME. The soil samples were placed in a solvent and SPME fiber was kept above the samples, in headspace. After analytes are adsorbed on the fiber, they were desorbed from the fiber to the GC inlet. The factors, influencing the distribution of PAHs in different SPME phases such as soil-solvent-headspace-fiber coating, were searched. For the analysis of volatile organic compounds (VOCs), addition of water, headspace volume and addition of salt were found as important factors. Effect of adsorption temperature was also investigated and HSSPME was performed at both 95 ºC and 20 ºC. The result of the comparison showed that, there was no big difference between them (Lompart et al., 1999).

A new method for the GC-MS analysis of PAHs in marine sediments, was developed. The combination of microwave-assisted extraction with micellar medium and SPME was used during the extraction of marine sediment samples. Among the SPME fibers, it was found that polyacrylate (PA) fiber had the highest PAH recovery value. By using this new method equilibrium times of SPME were lowered and higher reproducibilities were obtained in micellar medium. The limits of detection (LOD) were found in the range of 0.28-7.66 µg/L. After method was developed it was applied to the analysis of PAHs in certified reference material, SRM 1941a. The PAH recoveries were found in the range of 58.6-111 (Pino et al., 2003).

A new SPME method, for the GC-MS analysis of PAHs in environmental solid samples such as seaweed, humic substances and soil, was developed. This method has advantages like having short extraction times and high sensitivities. For the SPME analysis internal calibration was used and total analyte concentration was based on partitioning behaviour of already existing and spiked pollutants. The limits of detection were in the range of 0.001-0.1 mg/kg on a dry weight basis (Cam et al., 2004).

There are variety of methods for the extraction and analysis of PAHs and other organic pollutants in different samples. Among the extraction methods, soxhlet and ultrasonic extractions were used for the extraction of solid samples and liquid-liquid extraction was used for the extraction of aqueous samples. However most of these methods require large amount of organic solvents and involve several steps which may lead to loss of analytes. They are also time consuming. The relatively new method SPME, is more advantageous than the other methods for the analysis of PAHs. Experiments were carried to find out the optimum operation conditions of SPME. Investigated parameters were; temperature, equilibration time, salinity and compound concentration. After the optimization of the method, it will be apply to the analysis of PAHs in sediment samples (King et al., 2003).

A manual SPME method was used for the analysis of 24 organic compounds in waste water of chemical plant in northern Germany. Method precision, linearities of the calibration curves and limits of detection were found. Among the SPME parameters, methanol content, pH, salt amount, compound concentration were investigated. Internal standards were also investigated among the several organic compounds. Developed automated SPME-GC system will be used at on site at industrial wastewater purification plant (Grote et al., 1999).

The several methods were compared and extraction efficiencies were determined in less or heavily contaminated soil and sediment samples. The methods compared were; the German VDLUFA method, shaking method (using ISO method -A), soxhlet method (using ISO method-B) and ultrasonic method. Recoveries of 16 PAHs both in low or high-PAH contaminated soils were found. In addition, extraction efficiencies were determined in three sediment and five soil samples. For the less polluted samples no difference was found between the methods. However for the heavily contaminated samples, extraction efficiency was found as; VDLUFA method > ISO-A>ultrasonic method>ISO-B. Effect of soil mositure on extraction efficiency was also investigated and it was found that it depends on used solvent and PAH content of samples. For the ultrasonic extractions of moist samples, dichloromethane/acetone (5:1) solvent pair was recommended (Song et al., 2002).

1.9.2. Marine & Lake Sediment Studies

There are a lot of studies related with PAH analysis in sediment samples. Some of these studies were mentioned below.

Distribution and sources of PAHs (22) were determined by using GC-MS in selected ion monitoring mode. Sediment samples were collected from Stagnone coastal lagoon at Marsala in Italy. The PAH concentrations were found in the range of 72-18381 µg/kg. The source of the PAHs is mainly pyrogenic. Since sediment samples contain trace amount of perylene, biogenic source was the another source. To differentiate anthropogenic and biogenic sources, cluster analysis was performed (Culotta et al., 2006).

GC-MS analysis of 14 PAHs was performed in bottom sea sediments from Gemlik Bay, Marmara Sea, Turkey. It was established that PAH content is free from organic carbon content but slightly increase with silt/clay ratio. Therefore PAH concentrations were determined by direct input instead of sediment type. The highly polluted areas were found to be affected by tourism, discharges from river, surface run-off, domestic discharges, industrial discharges, contaminants from ships. In order to differentiate PAH sources different ratios such as; Phe/Ant, Fla/Pyr, BaA/Chr, LPAHs/HPAHs were used. As a result of using these ratios, it was established that along the highly populated eastern and southern coasts pyrogenic source is the main source (Unlu and Alpar, 2006).

The PAH analysis was done in 13 sediment samples collected from South-Western Barents Sea, Norway. The PAH concentration was found as generally lowand this is the sign of low entrance of petroleum hydrocarbons to this region. The most common source in the area was petrogenic. However in fjord regions, pyrogenic source was dominant especially upper sediment layers. This was the sign of human contribution which was low. At open sea, especially in deeper sediments, petrogenic PAH was dominant. In order to determine oil-related hydrocarbons caused by the natural leakages, normalisation to total organic carbon (TOC) was done (Boitsov et al., 2009).

The PAH analysis was performed in water, mussel and sediment samples, by the regional monitoring program for water quality in San Francisco estuary since 1993. During the time period of 1993-2001, few significant fluctuation was found in PAH concentrations determined water, sediment and muscle samples. Water PAH concentration did not show any difference in wet and dry season. Total maximum PAH concentration was 10,700 kg/yr. The PAH pathways and their contributions were; storm water runoff (≈51 %) > tributary inflow (≈28%) > wastewater treatment plant (≈10 %) > atmospheric deposition (≈8 %) > dredged material disposal (≈2%). NOAA suggested that in order to protect estuarine fish, PAH level in sediment shouldn't be greater than 1 mg/kg. However in 11 stations among 26, this threshold was exceeded by more than 50 %. According to the modeling the most common loss pathway of PAH was the degradation of sediments (Oros et al., 2007).

Sediment trap study was also performed and for this reason, from two different depths (250 and 2850 m) of the open Mediterranean Sea, the sinking particles were collected for one year and analyzed for PAHs. Total PAH concentrations at 250 m and 2850 m were 593 ± 284 ng/g and 551 ± 198 ng/g respectively. They were very close to each other. Total PAH flux at 250 m and 2850 m were 73 ± 58 ng $m^{-2}d^{-1}$ and 53 ± 39 ng m^{-2} d^{-1} respectively. Total PAH fluxes were also very similar. There were some factors affecting PAH flux which were downport transport processes and sinking particles nature. Temporal analysis of PAHs also revealed that during winter pyrolytic PAH increases (Bouloubassi et al., 2006).

Since PAHs are carcinogen and exist commonly in environment, their distributions and human health risks were under investigation. Therefore a lot of studies were conducted related with the levels and toxicity of PAHs in last years. According to this study, higher molecular weights were related with particles and it is possible to remove them by dry deposition. On the other hand, lower molecular weight compounds mostly exist in gas phase and therefore they are transformed and removed by photochemical degradation (Nikolaou et al., 2009).

The sediments collected from Gaoping estuary were analyzed for PAHs and according to diagnostic ratios, petrogenic source was found as the main source. However, pyrogenic source was the main source for sediments collected from Kaohsiung coast. According to the principal component analysis automobile emissions and coal burning were the dominant sources in Kaohsiung coast. Diagenetic PAH contribution was also detected with the high diagnostic ratios of Perylene/penta aromatic PAH isomers. According to diagnostic ratios it was found that some ratios were beter than the others. For example, it was found that for determining petrogenic PAHs, Phe/Ant ratio was more useful than the MethylPhe/Phe. In addition for determination of pyrogenic source, rather than BaP/BeP and BbF/BkF, BaA/Chr and IcP/BgP were preferred (Jiang et al., 2009).

An analytical procedure for determination of PCBs, coplanar PCBs and PAHs in the same biota and sediment samples, was developed. This method includes several steps such as; solvent extraction, purification, separation by alumina and silica column. By using this method even trace amounts (as low as 1 pg/g for coplanar PCBs) were determined with a good precision (20 %) (Madoulet et al., 2000).

In 1995 and 1998, total hydrocarbons and PAH concentrations were determined in sediments collected from Venice Canal, Italy. It was found that distribution of PAHs were similar and did not show any variation with the location of the samples and years. Main source in the area was detected as atmospheric deposition of petrochemical combustion products when PAH alkyl homolog distributions were investigated. However in some sampling points, low concentration of two-ring naphtalene homologous series revealed that the other source was low fresh oil input (Wetzel and Vleet, 2003).

The water samples were collected from the lower Mississipi River in April and Nowember 1999, and from the Gulf of Mexico in April 1999. PAH concentration in lower Mississipi River showed that PAH particle-water distribution was mainly depend on seasonal discharge. PAH concentrations in river samples collected in Nowember, showed that main source was combustion-driven processes which was understood by black carbon abundance. In order to constrain PAH sources into the gulf, three PAHs (Ant, BaA and BgP) were isolated in the river and gulf and were fit into a mass balance model (Mitra and Bianchi, 2003).

The PAH concentrations were determined in 57 trout in 7 mountain lakes in Europe and one remote lake in Greenland. The general PAH distribution was found as very similar; Phe had the highest concentration and Fla & Pyr had also very high concentrations (Vives et al., 2004).

Distant from point sources in Eastern Mediterranean Sea, marine sediment (coastal), air and seawater samples were collected at 6 sampling points. The PAH, black carbon (BC) and organic carbon (OC) analyses were performed in collected samples. The BC concentration was in the range of 0.3-5.6 mg g^{-1} and OC concentration was in the range of 2.9-21.4 mg g^{-1} on a dry weight basis (Tsapakisa et al., 2003).

US Steel Corp. Plant contaminated the Black River, OH with PAHs coming from coke ovens. When this plant was closed in 1983, by dredging of sediments in

1989 and 1990, PAH concentrations were lowered. This decrease in PAH concentration was determined and by using chemical mass balance modeling PAH sources were identified (Gu et al., 2003).

The PAH analysis by GC-MS were performed in collected 33 sediment samples from Hsinta Harbour and neighboring coastal areas. Total parental and alkylated PAH concentrations (30 samples) were in the range of 98.1-3382 ng/g on a dry weight basis. The main source was found as petrogenic caused by disposal of motor oil and it was identified by diagnostic ratios of MethylPhe/Phe > 2 and low Phe/Ant at inner harbour stations. Rather than MethylPhe/Phe ratio, 4,6-dimethyldibenzothiophene/3,6-dimethylPhe (4,6-C2D/3,6-C2P) ratio was found as more helpful in source identification. Sediments were divided into three groups by cluster analysis; off-shore group, near-shore group and inner harbour group. While characterizing sediments, three diagnostic ratios were very useful; 4,6-C2D/3,6-C2P (indicates petrogenic source), BaA/Chr (indicates pyrogenic source) and Per/PPAH (indicates biogenic source). Enrichment of pyrogenic and petrogenic PAHs showed that sediments were mixed with biogenic (natural) PAHs (Fang et al., 2003).

The sources and concentration of 16 PAHs in sediment cores collected from Ya-Er Lake, Hubei, China, were determined. The analysis method used includes the steps; ultrasonic extraction, gel-permeation clean-up and analysis by HPLC with fluorescence detector. The total PAH concentration was determined as 68-2242 mg/kg in sediment samples (Chen et al., 1997).

1.10. Objective of the Study

The aim of this study was to develop optimized extraction methods for the analysis of PAHs in sediment samples by using GC-FID & GC-MS. The used extraction methods were SPME, soxhlet and ultrasonic bath. Sediment samples were collected since sediment acts as a contaminant trap and when extracted with the most proper method it is so proper to monitor level of PAH contamination.

The sediment samples used for the optimization of the extraction methods were collected from Ölüdeniz Lagoon. The best extraction method was used for the analysis of the sediment samples collected from Ölüdeniz lagoon and Balıkesir, İkizcetepeler dam lake. The PAH concentration level in collected sediment samples were investigated. And these concentrations were used as a database to determine the level of pollution in this area.

CHAPTER 2

EXPERIMENTAL

2.1. Materials and Method

As mentioned in the introduction part (Table 1.3) several extraction methods exist for the PAH analysis in sediment samples and analyses can be achieved by variety of the instruments. In this study three extraction methods; soxhlet, ultrasonic bath and solid phase micro extraction were investigated. And the analyses of PAHs was done with GC-FID and/or GC-MS.

2.1.1. Reagents

All chemicals used during the study were of analytical reagent grade. Organic solvents; dichloromethane (DCM), acetone and hexane were bought from Merck. Three PAH standard solutions were used: Restek 16 PAH standards (ISO 9001 registered in DCM, 2000 ug/mL), EPA 610 Polynuclear Aromatic Hydrocarbons Mix (in methanol:DCM having a range of 10-1000 ppm, Supelco). Standards (except SPME) for the calibration curve were prepared diluting them with DCM (Merck, HPLC-grade). Standard reference materials (SRM) were bought from National Institute of Standards and Technology and they were; SRM 1941b (marine sediment), 1597a (complex mixture of PAHs from coal tar prepared in DCM). The GC-FID surrogate solution which includes three surrogates Nitrobenzene D5, p-Terphenyl D14, 2-Fluorobiphenyl (1000 mg/L, in DCM) was bought from Dr. Ehrenstorfer. The GC-MS surrogate solution mix which includes Acenaphtene-D10 (Ace-D10), Phenanthrene-D10 (Phe-D10), Chrysene-D12 (Chr-D12) and Perylene-

D12 (Per-D12) (1000 mg/L, in Acetone) was bought also from Dr. Ehrenstorfer. All the gases; Nitrogen, Helium, Hydrogen and dry air, were the high purity (99.999 %). The sodium sulphate (Na_2SO_4, Merck, Darmstad, Germany) was used for the removal of the extracted samples and it was also analytical reagent grade. High purity glasswool was used during the extraction of sediment samples. Cellulose extraction thimbles (Schleicher & Schuell Microscience 603, 33 x 80 mm, ref. no. 10350240) were used for soxhlet apparatus. Alconox detergent powder (Supelco Cat. No. 1104) was used for the cleaning of all glassware used in laboratory.

2.1.2. Instruments and Apparatus

The PAH analysis was performed with Agilent Hewlett Packard (HP) 6890 GC system equipped with a flame ionization detector. The system has also 5973 mass selective detector, μ-electron capture detector (μ-ECD), nitrogen-phosphorus detector (NPD) and TD-4 short path thermal desorption unit. The column: HP-5, 5% Phenyl Methyl Siloxane, 30 m x 0.25 μm film thickness x 0.32 mm internal diameter were used for the analysis of polycyclic aromatic hydrocarbons. An inlet liner (5062-3587) 78.5 mm x 6.5 mm OD tapered (wool packed) splitless Agilent/HP model was installed to GC. Agilent helium purifier (RMSH-2) was also used for the purification of the all gases. Supelco (23168) thermogreen LB-2 (11 mm pre-drilled septa) was used as a septa during the SPME injections. SGE microsyringe for GC were used for the preparation of standards and sample introduction.

Heidolph rotary evaporator (Laborota 4000 efficient) was used for reducing the organic solvent volumes to the desired amounts. In addition mini-vap evaporator with 6 ports (Catalog No: 22970, Cat. No: 22971) was used during evaporation of standards. Bransonic ultrasonic cleaner (Model B-2200 E4, 205 W, 220V) with a power supply 50-60 Hz and working frequency 47 Hz and a heater (velp scientifica ,V ~ ± 4) for soxhlet extraction system was also used. Stuart scientific oven 252 D and sensitive analytical balance (Mettler AJ100) were used during the sample preparation. Deionized water (ultrafiltered type 1 water) was supplied by Barnstead nanopure ultrapure water system. Nalgene bottles for solvent wash (Cat. No. 2421-0500) was used as solvent container in laboratory.

The SPME apparatus (fiber assembly Catalog No:57330-U bought from Supelco Bellefonte, PA), SPME inlet guide (Catalog No: 57356-U, Supelco, Bellefonte, PA) and three tips including fibers with different pore size (7-100 μm PDMS and 85 μm PA) were bought from Supelco.

Preparation of Reagents and Apparatus

Sodium sulphate (anhydrous extra pure, Merck) and glass-wool were pre-cleaned by transferring them to a large glass column (1 L capacity or larger) and washing sequentially with hexane/DCM (for Ölüdeniz samples) or acetone/DCM (for İkizcetepeler samples), according to the solvents of the ultrasonic extraction, before use. Sodium sulphate was activated in an oven at 400°C for 4 h. Glass-wool was conditioned overnight at 225°C in an oven. Cellulose extraction thimbles were cleaned with proper solvents (used in soxhlet) prior to the use with soxhlet extraction system.

All glasswares were cleaned with detergent in hotwater and rinsed with DI . After rinsing with DCM/acetone, they were dried in an oven overnight. They were cleaned before and after used, during the whole analysis.

2.2. Optimization of Instrumental Parameters

Before starting analysis with GC-MS and GC-FID, the optimum parameters were determined and these parameters were used during the whole analysis. These parameters include gas chromatography parameters and detector parameters. For example for the column selection two different capillary columns; HP-5 MS, 5% Phenyl Methyl Siloxane, 30 m x 0.25 μm film thickness x 0.25 mm internal diameter and HP-5, 5% Phenyl Methyl Siloxane, 30 m x 0.25 μm film thickness x 0.32 mm internal diameter were tried for the analysis of PAHs, and HP-5 column was preferred since the column having 0.25 mm internal diameter, did not allow higher carrier gas (He) flow rates (bigger than 1 mL/min). On the other hand higher flow rate was necessary for obtaining better response (higher peak area). Therefore, during the optimization studies HP 5 - 30m × 0.32 mm × 0.32 μm column was used both for GC-MS and GC-FID. Both of the instruments were used in the splitless mode since when the sample contains very small amounts of analyte e.g. an analyte mass close to the detection limit of the method, splitless injection is used. If a split injection is used then there won't be enough analyte injected on-column to detect therefore for trace analysis, therefore split injection was not preferred.

The optimization was done using one-variable-at-a-time technique. In this technique while one parameter is changed, others are kept constant. The surrogate and PAH standards were used during the optimization of instrumental parameters and peak areas were used for the comparison of different parameters.

2.2.1. Optimization of the GC-FID Parameters

Before starting routine analysis with GC-FID, some important instrumental parameters were optimised. Among these parameters; GC parameters were injection volume, inlet temperature, carrier gas (He) flow rates and oven temperature program parameters such as initial oven temperature, ramp rate and initial time. The detector parameters were make-up gas flow rate and flame ionisation detector (FID) temperature. Studies related with these optimizations were explained below.

Optimization of GC-Parameters

Injection volumes between 0.5-2 μL were tried in order to select best value. According to the Figure 2.1, it can be seen that 2 μL was the best volume for injection.

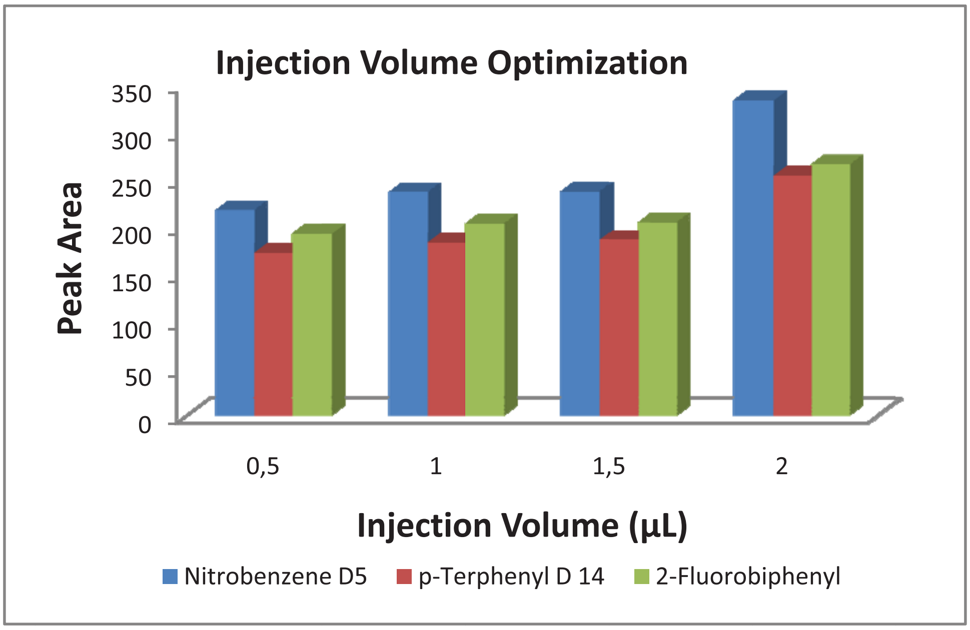


Figure 2.1. Injection Volume Optimization for GC-FID

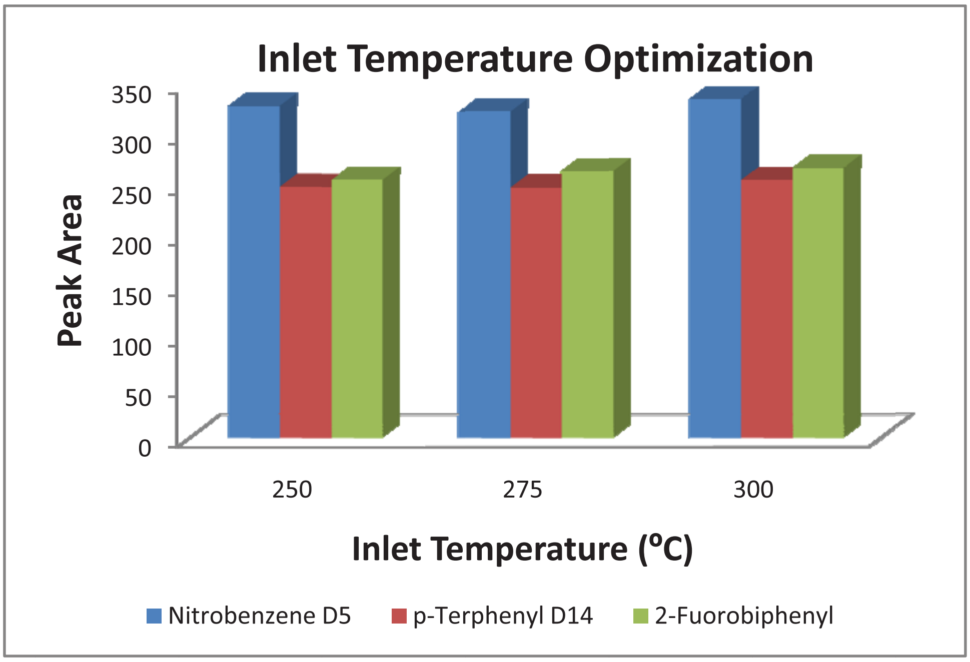


Figure 2.2. Inlet Temperature Optimization for GC-FID

Inlet temperature was also optimized by changing inlet temperature between 250-300 °C and keeping the rest of the parameters constant (Figure 2.2). For the three temperatures efficiency was the same, therefore middle value, 280 °C was chosen as an optimum value.

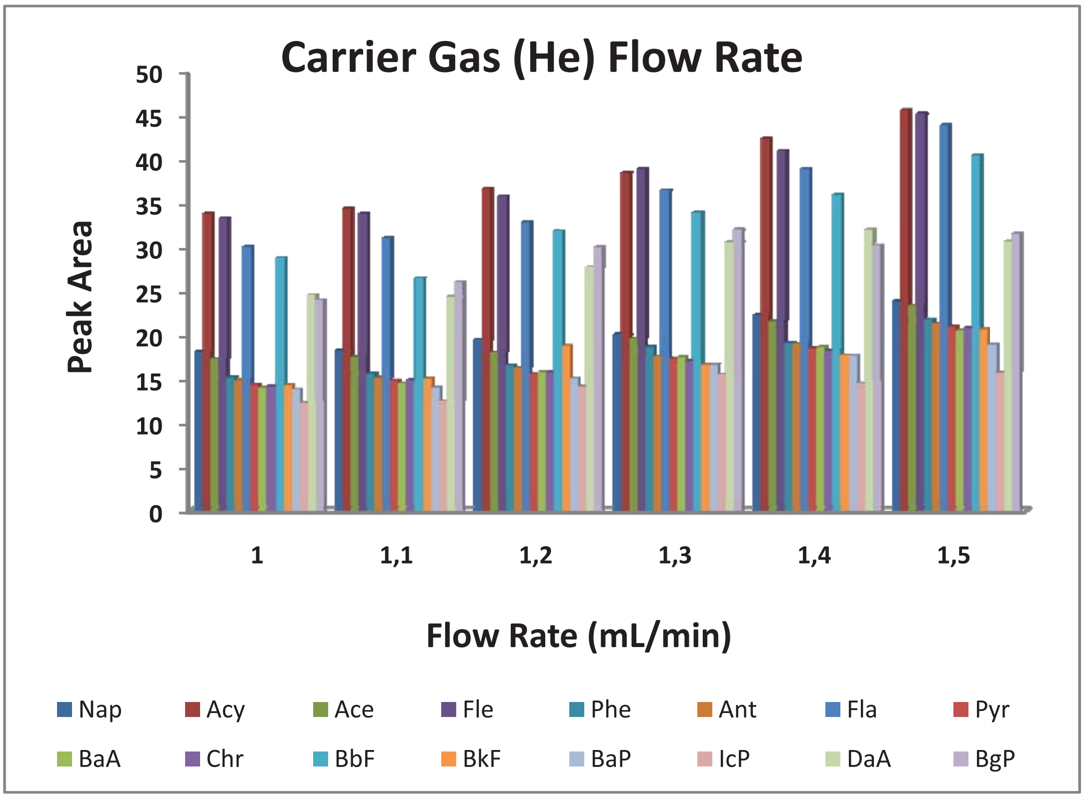


Figure 2.3. Optimization of the Carrier Gas (He) Flow Rate for GC-FID

Carrier gas (He) flow rates were changed between 1-1.5 mL/min and responses were observed (Figure 2.3). It was discovered that resolution was the same in this range. Therefore, a flow rate giving the highest peak area (1.5 ml/min) was chosen as the optimum value. With this flow rate, total analysis time was 40.75 min.

Temperature program for GC oven was also optimised for obtaining the best resolution and efficiency during the quantization step. Special attention was given to the initial oven temperature, oven ramp rate and and initial time.

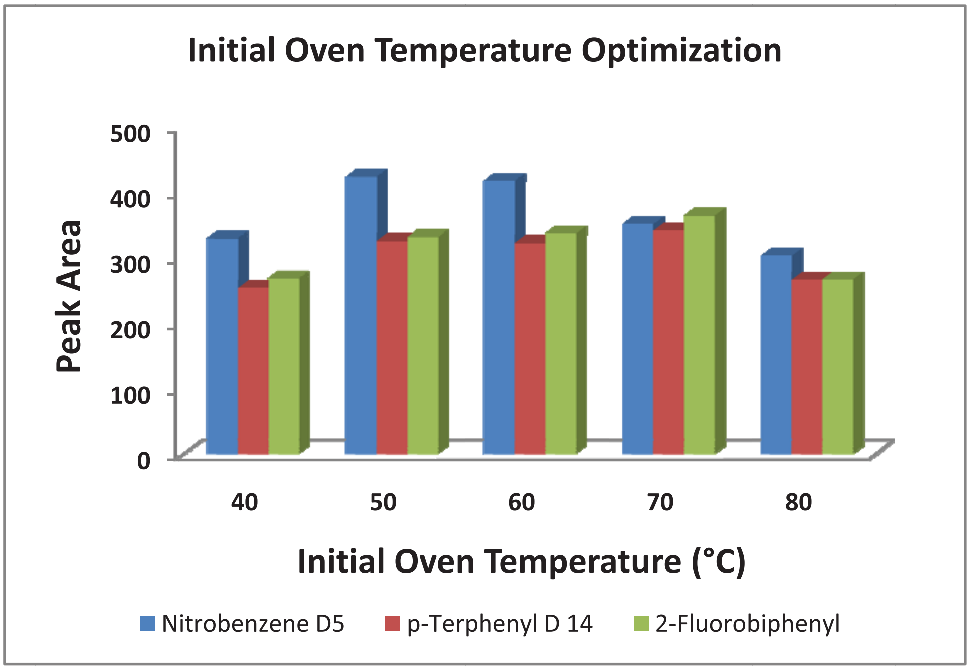


Figure 2.4. Optimization of Initial Oven Temperature for GC-FID

As can be seen from Figure 2.4, different initial oven temperatures between 40-80 °C were tried. Since inital oven temperature shouldn’t be below boiling point of solvent (DCM) temperatures started from 40 °C. At 60 °C and above, a split in Nap peak was observed, therefore temperature points above 50 °C was not proper. Best efficiency was obtained for all surrogate compounds when 50 °C was chosen as an initial temperature.

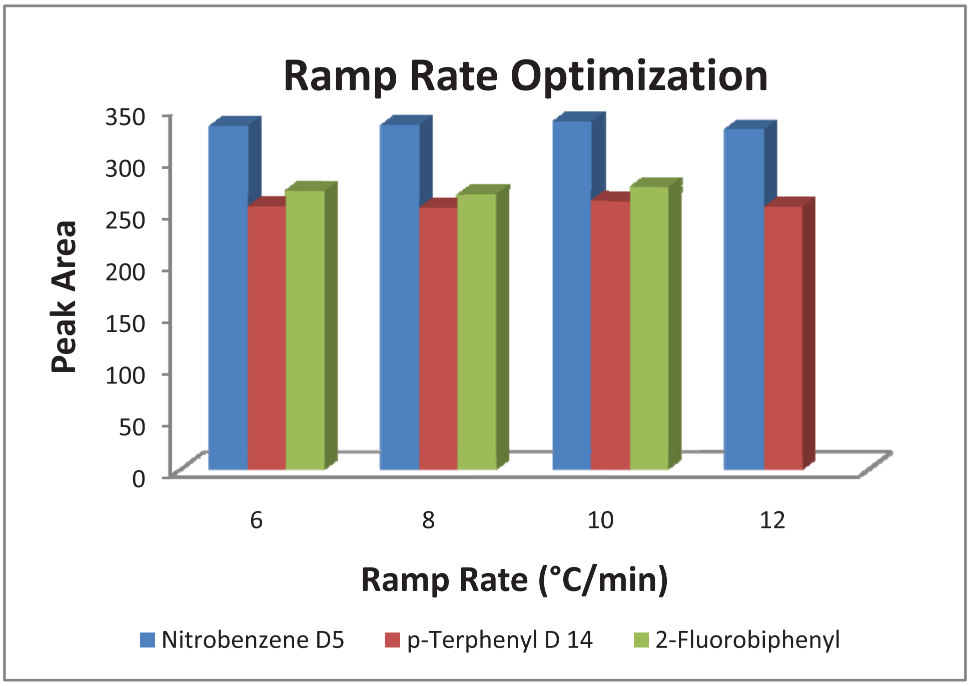


Figure 2.5. Optimization of the Oven Ramp Rate for GC-FID

Usually higher ramp rate is necessary for increasing the peak areas but there is a limitation that ramp rate shouldn't be too steep. If it is too steep, this may lead to an increase in the background level and small peaks are lost. Variety of the ramp rates between 6-12 °C /min were tried and it was seen that there was no big difference between them. Therefore 8 °C/min was chosen as a ramp rate since at this ramp rate, 40 min of analysis time (neither short nor long) and a good resolution was obtained (Figure 2.5).

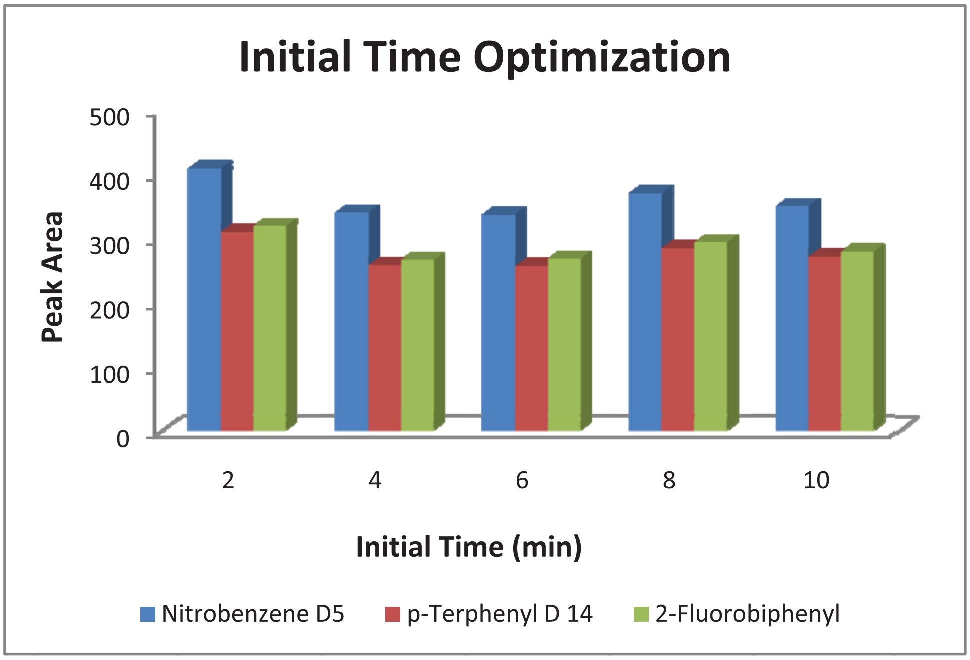


Figure 2.6. Optimization of the Initial Time (min) in Oven Temperature Program

Initial time in GC oven temperature program was also optimized (Figure 2.6) and it was found that after 2 minutes peak areas are slightly decreasing and staying constant until 10 minutes.Therefore initial time of 2 min was found as the best value.

According to the optimization results; the optimised final GC oven temperature program started at 50°C, held for 2 min, and increased to 280 °C at a rate of 8 °C min^{-1}. The final temperature was held for 12 min at 280°C.

Optimization of Detector (FID) Parameters

Among the FID parameters, Hydrogen (H_2, fuel gas) flow rate and detector temperature were investigated. The air flow rate (400 mL/min) and make-up gas (He) flow rate (20 mL/min) were taken from the literature.

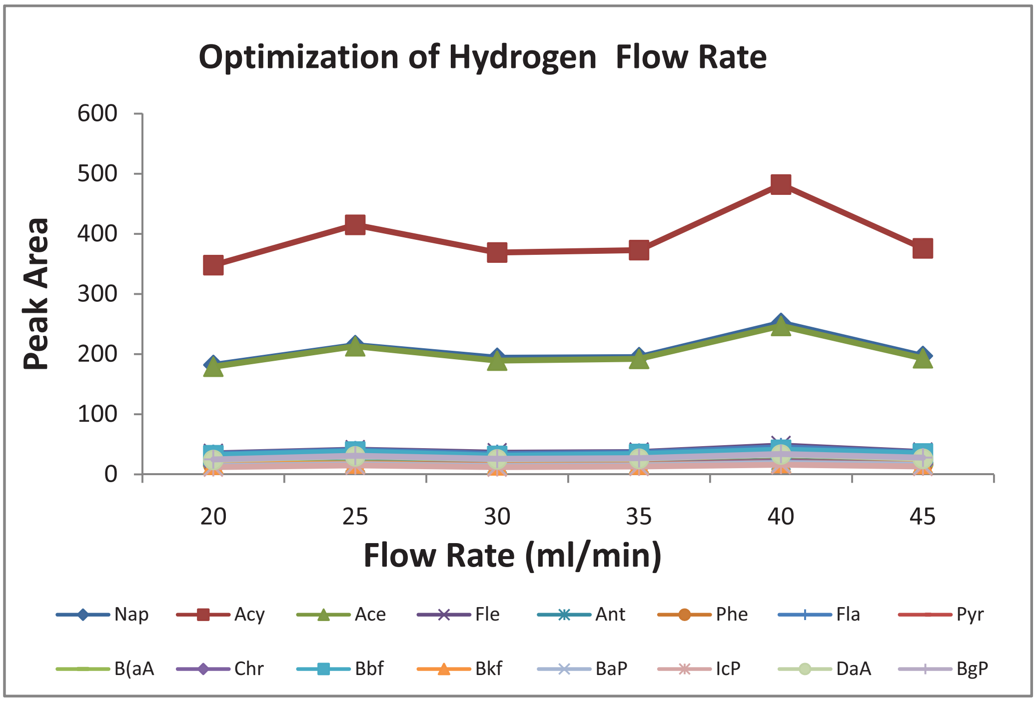


Figure 2.7. Optimization of the Hydrogen Flow Rate Optimization

Hydrogen flow rate was changed between 20-45 ml/min and 40 mL/min was chosen as the optimum value according to the observed PAH peak areas. When this value was chosen, both low background and the higher ionization efficiency were observed (Figure 2.7).

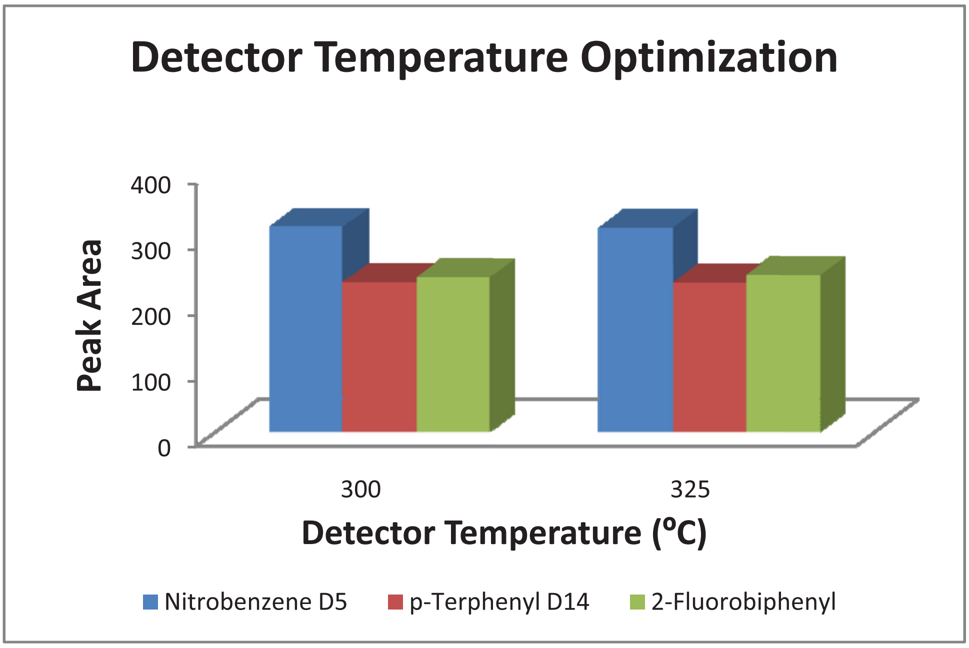


Figure 2.8. Optimization of Detector Temperature for GC-FID

For FID detector temperature optimisation two different detector temperatures (300 °C and 325 °C) were experimented (Figure 2.8). As can be seen from the Figure, there is no difference on the performance of the detector in between 300 °C and 325 °C. Since the detector temperature should be at least 20 °C higher than the last oven temperature, the points below 300 °C were not selected and also the temperatures higher than the 325 °C were not necessary, as the points above 325°C do not lead to bigger differences in the peak areas. Therefore, 325 °C was chosen as the optimum detector temperature.

GC-FID Stability

Stability of the instrument within a day was investigated by making successive two injections within a day (Figure 2.9). It was found that during the same day repeatability is very high. Therefore if standards and samples were injected in the same day, there is no need to use internal standard and external calibration curve was preferred.

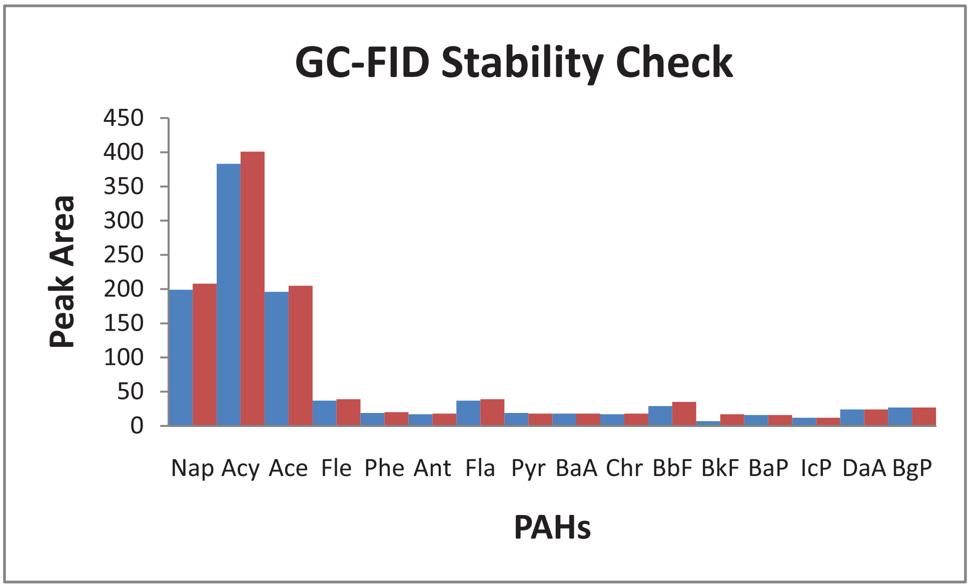


Figure 2.9. GC-FID Stability Check Within A Day

Table 2.1. GC-FID Conditions for Determination and Quantification of PAHs

Agilent 6890 GC with FID	
Column type	HP-5 5% Phenyl Methyl Siloxane
Length	30 m
Internal diameter (ID)	0.32 mm
Film thickness	0.25 µm
Injection	Splitless, 2 µl
Injector temp.	280 °C
Carrier gas	Helium
He flow	1.5 ml min^{-1}
He Flow (make-up gas)	20 ml/min
H_2 flow	40 ml min^{-1}
Air	400 ml min^{-1}
Linear velocity	27 cm s^{-1}
Make up gas	Hydrogen
FID Temperature	325°C

Optimized GC conditions for determination and quantification of PAHs was given in Table 2.1.

2.2.2. Optimization of GC-MS Parameters

For GC-MS, the optimized parameters for GC-FID were used. In order to see any difference caused by the detector, injection volume and He flow rate optimizations were repeated and it was discovered that GC parameters optimized for FID detector are also valid for MS detector. The other mass parameters such as MS source temperature and MS quadrupole temperature values were taken from the instrument guide book. The stability of the GC-MS was checked using surrogate solutions.

Optimization of GC Parameters

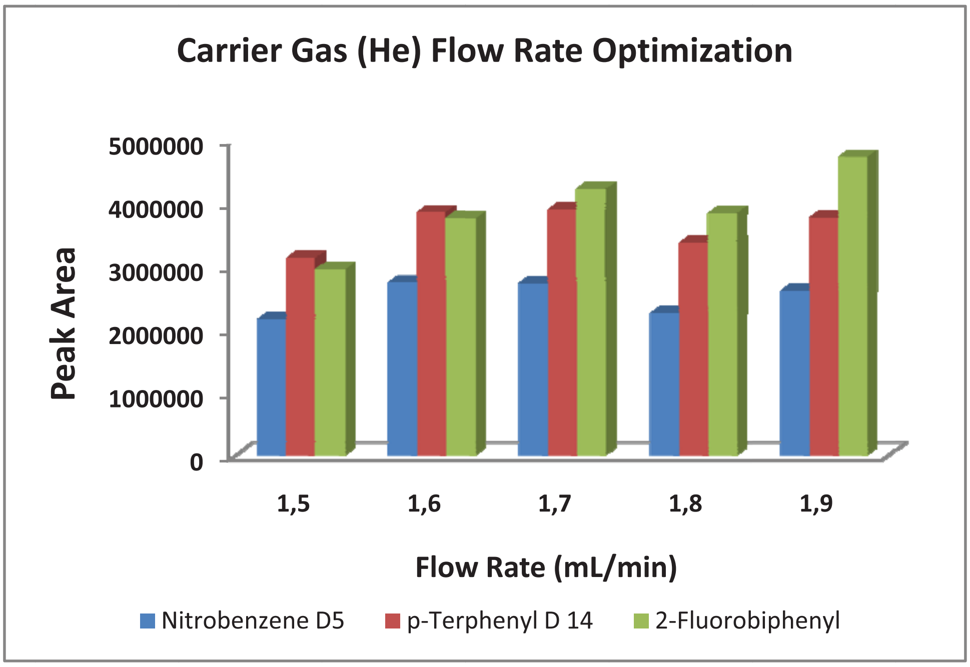


Figure 2.10. He Flow Rate (mL/min) Optimization for GC-MS

Carrier gas (He) flow rate was changed between 1.5-1.9 ml/min. It was observed that as He flow rate increases peak areas (response of the instrument) also increase (Figure 2.10). On the other hand resolution becomes worse. Since at low flow rates, better resolution is obtained, 1.5 mL/min was chosen as a He flow rate.

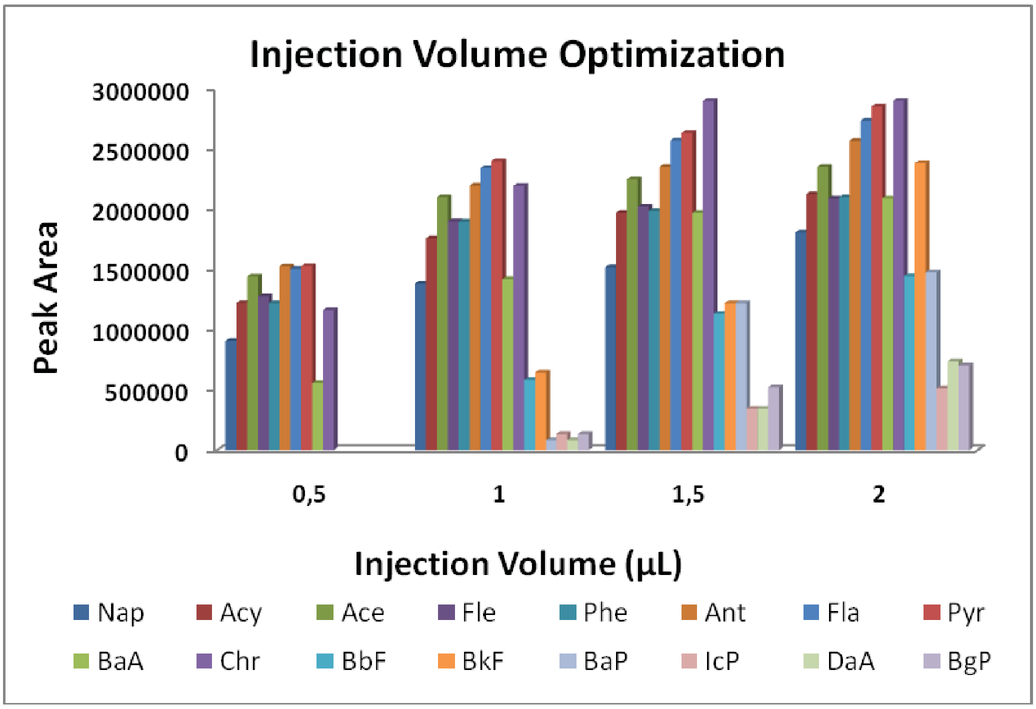


Figure 2.11. Injection Volume Optimization for GC-MS

The PAH standard was used for the optimization of injection volumes (µL). Injection volume was changed between 0.5-2 µL and it was observed that peak areas increase with increasing injection volume (Figure 2.11). Therefore 2 µL was chosen as an optimum injection volume.

GC-MS Stability Test

In order to decide to the type of calibration curve (internal or external) stability of GC-MS was checked by using 1 mg/L surrogate mix. When the instrument is highly stable (repeatibility is high) external curve is used for calculations. However when the stability is bad internal calibration curve is used using internal standards. As can be seen from the Figure 2.12, surrogate averages within the given time interval are; 1.09 ± 0.15 mg/L for Ace D10, 1.14 ± 0.19 mg/L for Phe D10, 1.05 ± 0.24 mg/L for Chr D12 and 1.32 ± 0.32 mg/L for Per D12 that is; instrument is highly stable (no big fluctuations were observed in the surrogate concentrations, low standard deviations) and there is no need to use internal standard for the given time interval (40 days; total analysis time). Therefore external calibration curves were used for the quantification of PAHs with GC-MS.

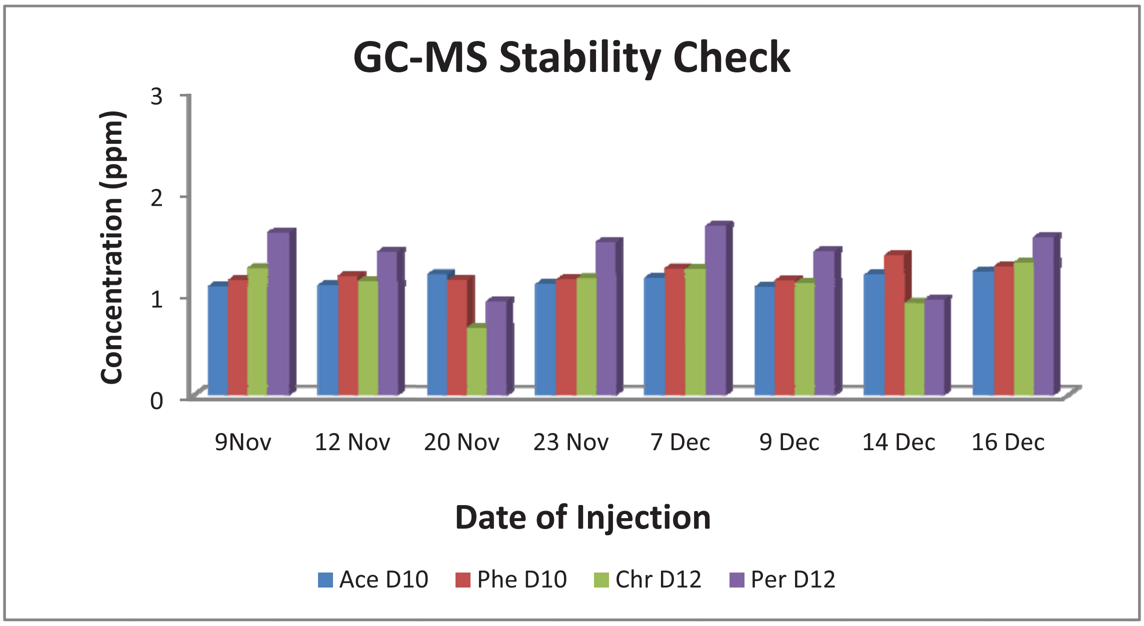


Figure 2.12. GC-MS Stability Check Between Days

Optimized GC parameters were given in Table 2.2.

Table 2.2. GC-MS Conditions for Determination and Quantification of PAHs

Agilent 6890 GC with 5973 MSD	
Injector:	Splitless
Inlet temperature:	280 °C
Column:	HP-5 (5 % Phenyl Methyl Siloxane, 30.0m*250mm*0,32 mm)
Oven temperature:	50° C at 2 min, 8 °C/min 280 °C at 12 min
MS source temperature:	230 °C
MS quadrupole temperature:	150 °C
Injection volume:	2 µl
He flow rate:	1.5 mL/min

Table 2.3. Target Ions and Confirmation Ions for PAHs and Surrogates with GC-MS

No	**Component**	**Target Ion**	**Confirmation Ions**
1	Nap	128	127,129
2	Acy	152	151,153
3	**Ace D10***	162	164,160,163
4	Ace	153	154,152
5	Fle	166	165,163
6	**Phe D10***	188	189,184,187
7	Phe	178	176,179
8	Ant	178	176,179
9	Fla	202	200,203
10	Pyr	202	200,203
11	Cycpyr	226	224,227
12	BaA	228	226,229
13*	**Chr D12***	240	236,241,239
14	Chr	228	226,229
15	BbF	252	250,253,251
16	BkF	252	250,253,126
17	BaP	252	250,253
18	BeP	252	253,250
19	**Per D12***	264	260,265,263
20	IcP	276	277,274,138
21	DaA	278	276,277,139
22	BgP	276	277,274,138
23	Att	276	277,274,275

***Surrogate Standard**

Table 2.3 lists the abbreviations, target ions and confirmation ions for all PAHs and surrogates determined by using HP 6890 GC coupled with 5973 series mass selective detector.

2.3. Sampling Areas and Sample Collection

Sediment samples were collected from two areas; Ölüdeniz Lagoon in Muğla and İkizcetepeler Dam Lake in Balıkesir.

2.3.1. Ölüdeniz Lagoon

Ölüdeniz Lagoon was the first sampling area and it is in Fethiye, Muğla, Turkey (Figures 2.13). It is one of the most important tourist attractions due to its beautiful beach and spectacular setting. The width and length of the lagoon is ~ 650 and 1300 m, respectively and the water circulation is provided by a narrow (~60 m) strait (maximum depth is 7 m). Despite protection efforts, the number of hotels and guesthouses has increased in recent years around this charming lagoon. Consequently, the region is exposed to increased domestic pollution. In addition, many tourist or sportive boats visiting the lagoon may be another source for organic or oil related pollution. Due to the low water circulation and accumulation of sediment coming with the seasonal streams, Oludeniz Lagoon is also under the risk of transforming into land (Tuncel et al., 2007).

Figure 2.13. Sampling Points Inside and Outside the Ölüdeniz Lagoon

The sediment samples (N=68) were collected in 20-22 March, 2003 from Ölüdeniz Lagoon which is located on the intersection of coastal lines of Mediterranean Sea and Aegean Sea in Turkey (Figure 2.13). Although 68 sediment samples were collected, for the PAH analysis totally 5 points were chosen from the lagoon and the positions of these points were given in Table 2.4. Among the analyzed sediment samples, three of them (1,3 and 4) were collected from the inside and two of them (2 and 5) were collected from outside of the Lagoon. The rest were used for the preparation of the homogenized sample which was necessary for the optimization of extraction methodology.

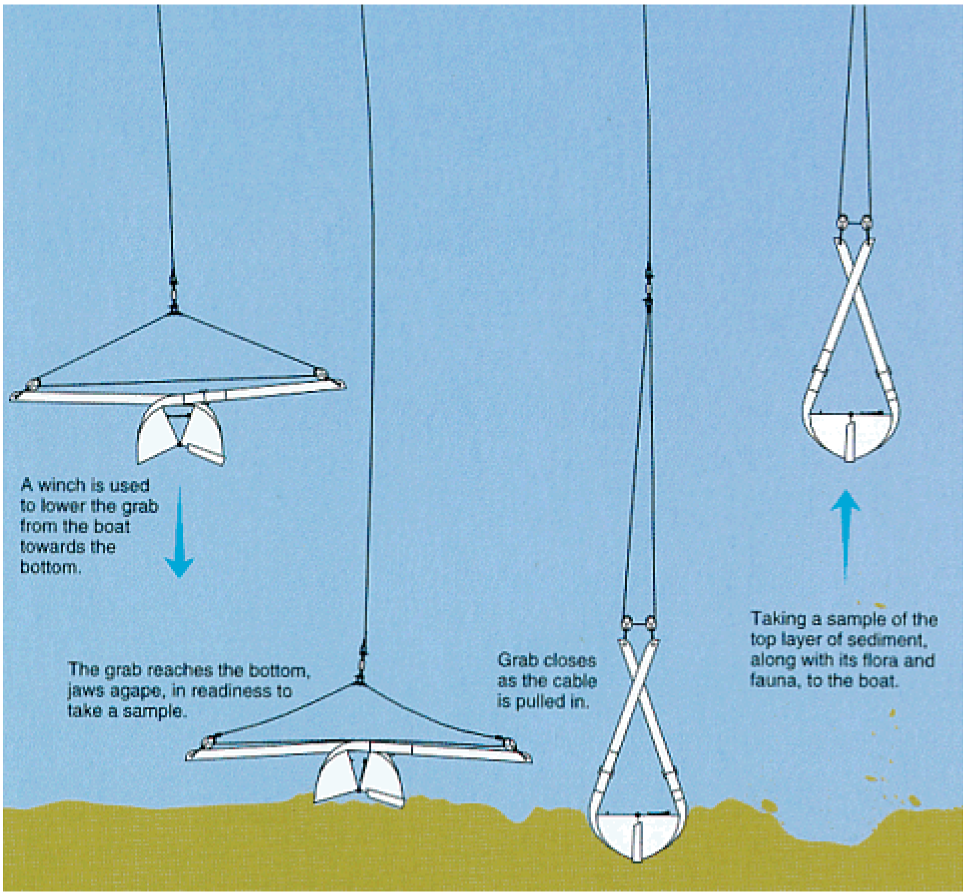
The geographical positions of the analyzed sediment samples were given in Table 2.4.

Table 2.4. Geographical Positions of the Points for Ölüdeniz Lagoon

Sample No	Coordinates
S1	N 36 33 248 E 029 06 755
S2	N 36 32 758 E 029 06 620
S3	N 36 32 286 E 029 06 574
S4	N 36 33 161 E 029 06 418
S5	N 36 32 710 E 029 06 609

Sediment samples inside the lagoon were collected by using Van Veen grab sampler (Fig. 14) but the outside of the lagoon (where the use of Van Veen grab sampler is difficult) scuba diver was employed. The Van Veen grab (Figure 2.14) is a light weight sediment sampler designed to take large samples in soft bottoms. Its long lever arms and the sharp cutting edges on the bottom of the scoops, enable it to cut deeply into the softer bottoms. The weighted jaws, chain suspension, and doors and screens allow flow-through during lowering to the bottom and assure vertical descent where strong underwater currents exist. The relatively large surface area and the strong closing mechanism allow the jaws to excavate relatively undisturbed sediments. When the powering cable is slowly made taut, the chains attached at the top of the release exert great tension on the long arms extending beyond the jaws, causing them to lift, dip deeper into the sediment, and trap material as they tightly close. When the grab settles on the bottom, the flaps fall back and cover the screens completely, preventing any loss of sediment during retrieval (http://www.rickly.com/as/bottomgrab.htm).

The collection of the surface sediment samples with Van Veen grab sampler was explained on the Figure 2.14. And according to the explanations there are 4 steps for the surface sediment collection: First; grab is lowered from the boat to the bottom, second; it reaches to bottom, third; grab closes as the cable pulled in, last; a sample of the top layer of sediment along with its flora and fauna is taken to the boat.



http://www.aims.gov.au/pages/reflib/bigbank/images/vanveen-480.gif.

Figure 2.14. Van Veen Grab Sampler

The top 0-1 cm surface layer was collected and collected sediment samples (100-250 g) were wrapped with DCM washed aluminum foils. Then they were placed in locked HDPE nylon bags and stored in a refrigerator at -20 °C. Empty aluminum foils and bottles, that were treated as samples, were used as field blanks.

2.3.2. İkizcetepeler Dam Lake

İkizcetepeler Dam Lake is situated 20 km south of Balıkesir province. It was constructed between the years 1986-1992 across Kille stream and is fed by Akçaköy, Taşköy and Kozludere creeks. The lake is used to irrigate Pamukçu and Aslıhantepecik plains and is also used for drinking water (Alper et al., 2007). The surface of the reservoir of İkizcetepeler is 9.6 km^2, the overall volume totals 164,56 hm^3. The minimum code (water level above sea level) is 49.75 m and the maximum code is 175 m. According to the measurements taken in April 2000, the maximum depth was 47.0 m. The lake's largest water supply stream is Kille stream, which is connected to the other streams, namely, the Akcaköy, the Tasköy and the Kozludere (Torcu-Koç et al., 2008). The İkizcetepeler dam lake has been spreaded over a broad land including an old willage (Selimiye), agricultural fields and the old Balıkesir-İzmir highway. Presentdays, it is carried out agricultural applications around it and the new Balıkesir-İzmir highway passes over it. Drinking water for Balıkesir City has been supplied from Ikizcetepeler Dam Lake since 2003 (Şahin İ. Et al., 2004).

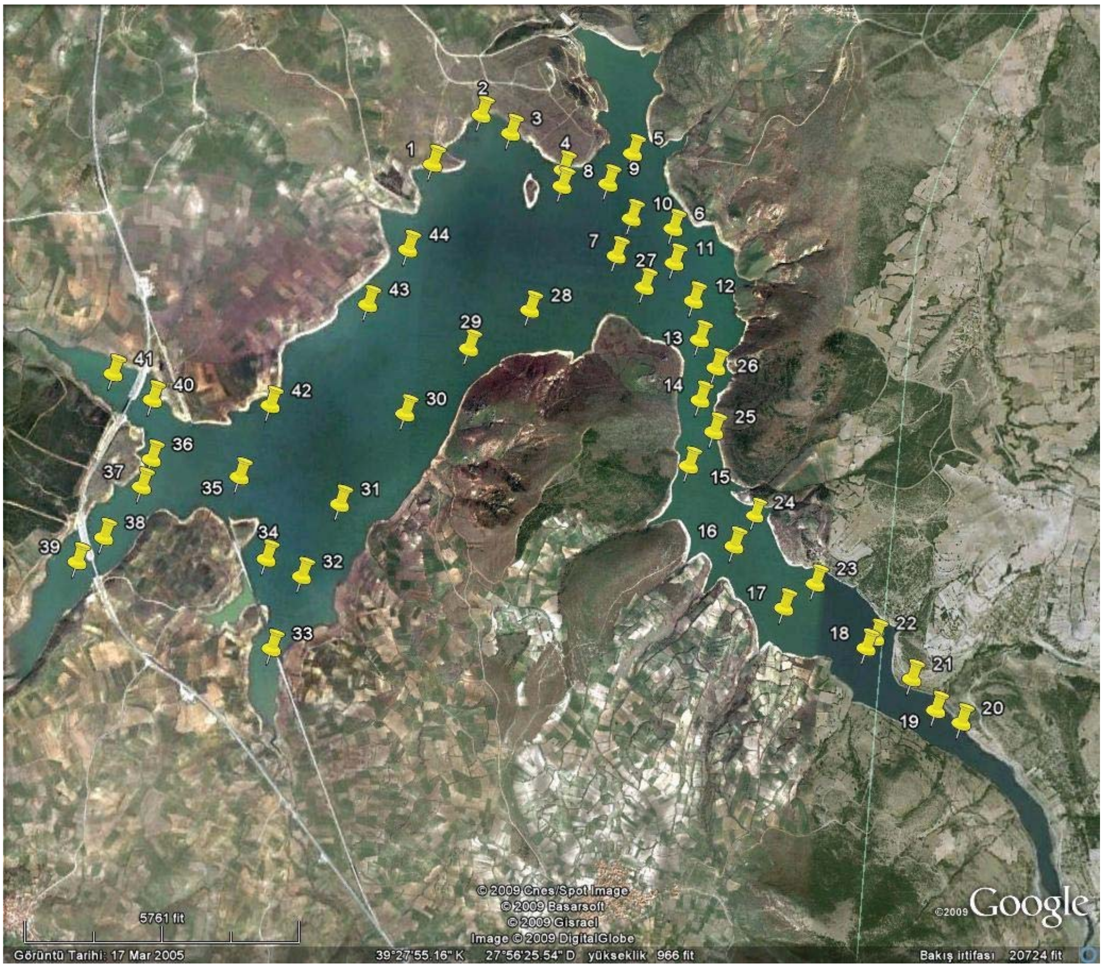


Figure 2.15. Sampling Points Inside the İkizcetepeler Dam Lake

Figure 2.15 shows the 44 sampling points inside the İkizcetepeler Dam Lake.

The sediment samples (N=44) were collected from İkizcetepeler Dam Lake, Balıkesir, in 15-16 September, 2009. The geographical positions of the analyzed sediment samples were given in Table 2.5.

Table 2.5. Geographical Positions of the Points for İkizcetepeler Dam Lake

Sample No	Coordinates	Sample No	Coordinates
S1	N 39 28 56.5 E 27 55 45.5	**S23**	N 39 27 35.1 E 27 57 37.5
S2	N 39 29 07.6 E 27 55 57.6	**S24**	N 39 27 48.7 E 27 57 19.8
S3	N 39 29 04.3 E 27 56 05.9	**S25**	N 39 28 05.2 E 27 57 06.9
S4	N 39 28 57.5 E 27 56 20.9	**S26**	N 39 28 18.6 E 27 57 06.2
S5	N 39 29 02.0 E 27 56 39.3	**S27**	N 39 28 34.3 E 27 56 45.2
S6	N 39 28 47.0 E 27.56 51.8	**S28**	N 39 28 27.7 E 27 56 14.7
S7	N 39 28 40.4 E 27 50 37.0	**S29**	N 39 28 18.7 E 27 55 59.0
S8	N 39 28 54.1 E 27 56 20.9	**S30**	N 39 28 04.1 E 27 55 43.1
S9	N 39 28 55.3 E 27 56 33.3	**S31**	N 39 27 44.2 E 27 56 27.3
S10	N 39 28 48.4 E 27 56 40.3	**S32**	N 39 27 28.5 E 27 55 18.5
S11	N 39 28 39.8 E 27 56 40.3	**S33**	N 39 27 13.0 E 27 55 11.8
S12	N 39 28 32.2 E 27 56 58.8	**S34**	N 39 27 31.6 E 27 55 08.6
S13	N 39 28 24.2 E 27 57 01.8	**S35**	N 39 27 48.3 E 27 54 59.6
S14	N 39 28 11.6 E 27 57 02.6	**S36**	N 39 27 50.6 E 27 54 35.5
S15	N 39 27 57.7 E 27 57 00.8	**S37**	N 39 27 33.5 E 27 54 33.5
S16	N 29 27 57.7 E 27 57 14.7	**S38**	N 39 27 33.5 E 27 54 24.2
S17	N 39 27 29.6 E 27 57 29.4	**S39**	N 39 27 28.0 E 27 54 17.5
S18	N 39 27 22.5 E 27 57 52.5	**S40**	N 39 28 03.0 E 27 54 34.6
S19	N 39 27 10.6 E 27 58 12.6	**S41**	N 39 28 07.8 E 27 54 23.4
S20	N 39 27 08.4 E 27 58 19.7	**S42**	N 39 28 03.7 E 27 55 06.5
S21	N 39 27 16.8 E 27 58 05.4	**S43**	N 39 28 26.3 E 27 55 30.8
S22	N 39 27 24.7 E 27 57 37.5	**S44**	N 39 28 38.4 E 27 55 40.5

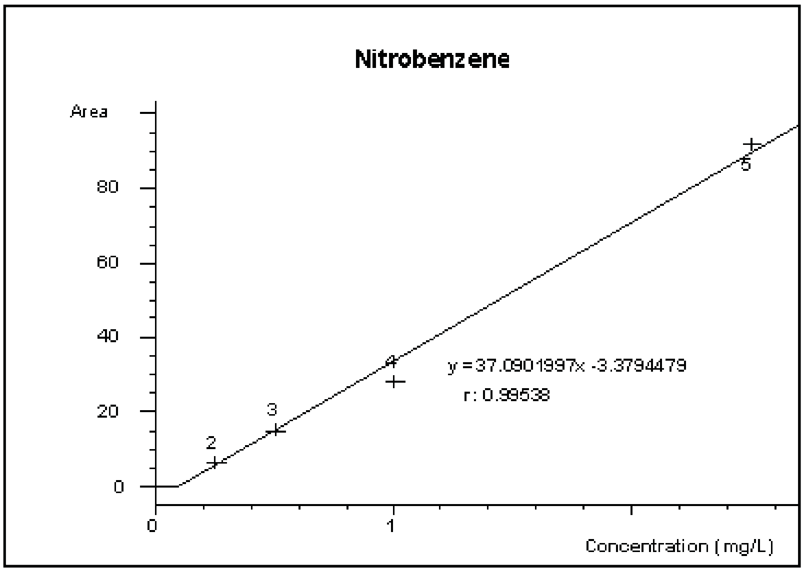
2.4. Validation of Analyses Methodologies

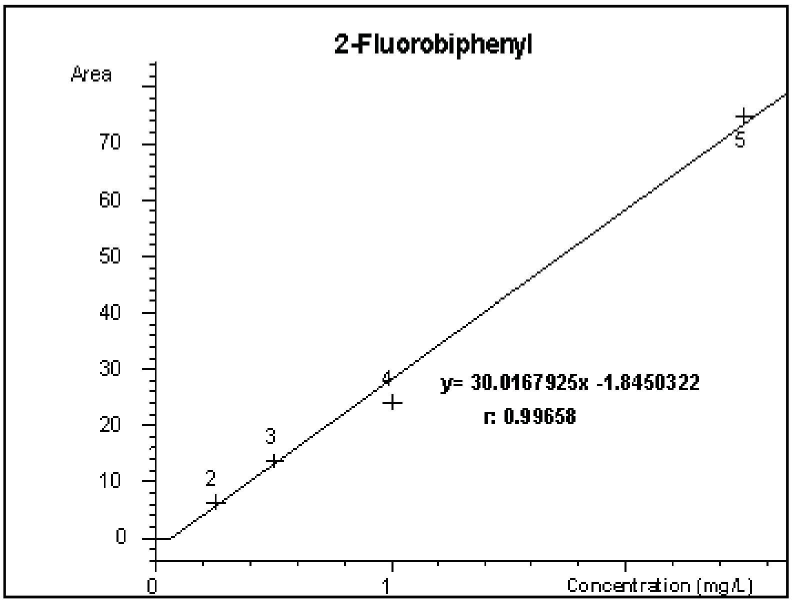
Both the features of GC-MS and GC-FID were given below since both of them were used during the analyses.

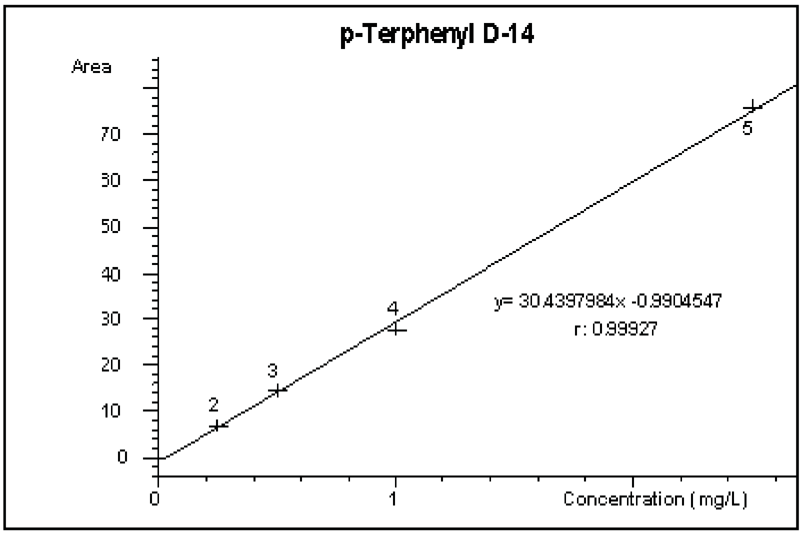
2.4.1. GC-FID Analysis

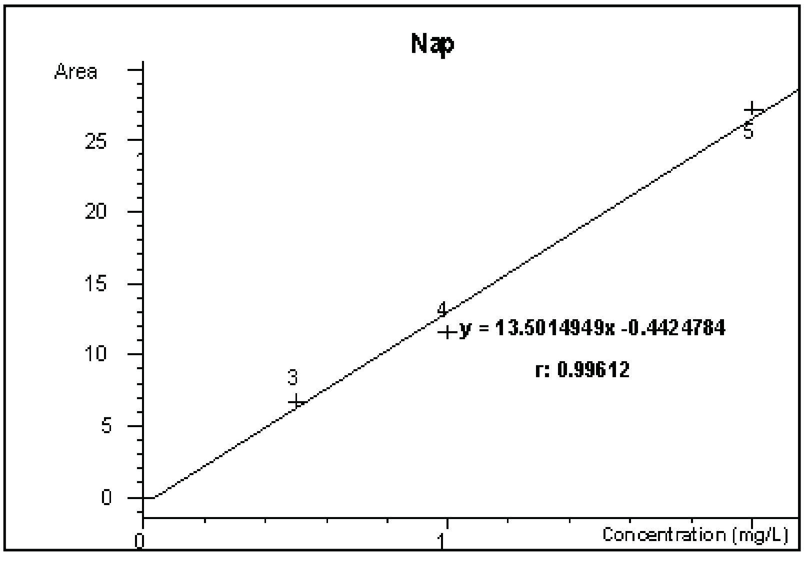
Calibration Curves for GC-FID Analysis

The calibration curves (Figure 2.16) were prepared for the analysis of Ölüdeniz sediment samples for the 16 PAHs and three surrogates.




2-Fluorobiphenyl
Area
70
60
50
40
30
20
10
0
0
1
Concentration (mg/L)
2
3
4
5
y= 30.0167925x -1.8450322
r: 0.99658


p-Terphenyl D-14
Area
70
60
50
40
30
20
10
0
0
1
Concentration (mg/L)
2
3
4
5
y= 30.4397984x -0.9904547
r: 0.99927


Nap
Area
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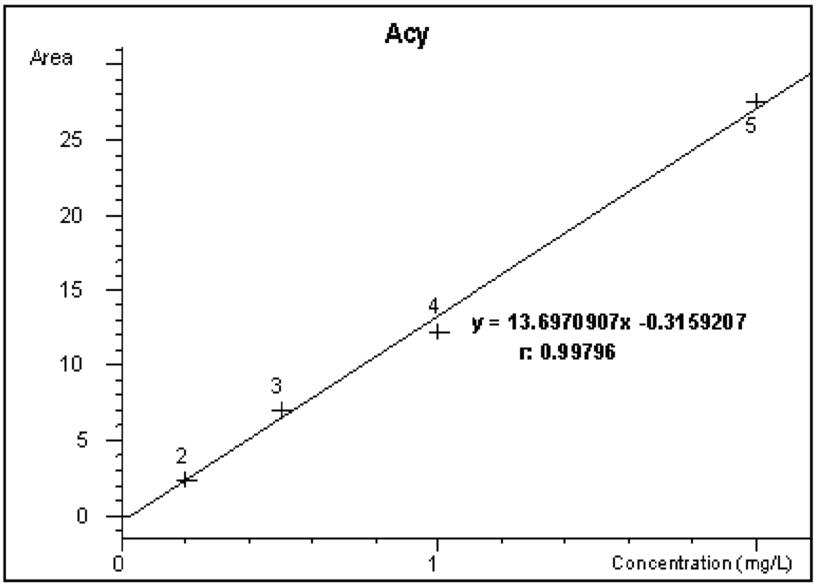
20
15
10
5
0
0
1
Concentration (mg/L)
3
4
5
y = 13.5014949x -0.4424784
r: 0.99612


Acy
Area
25
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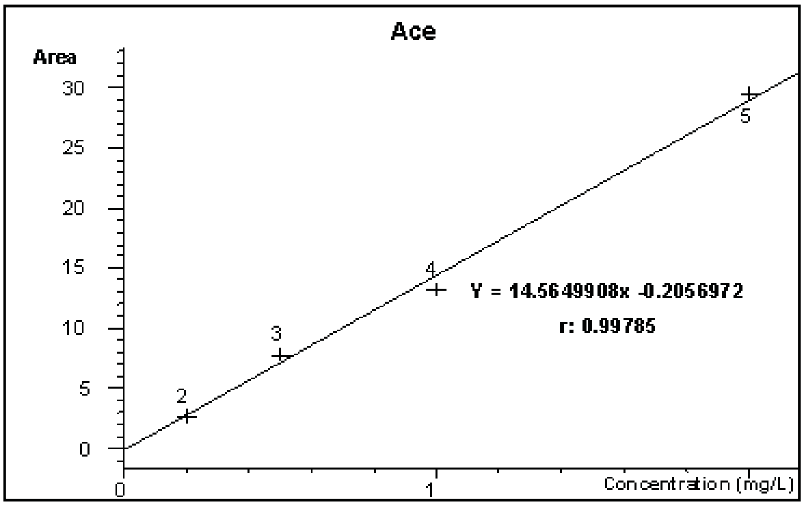
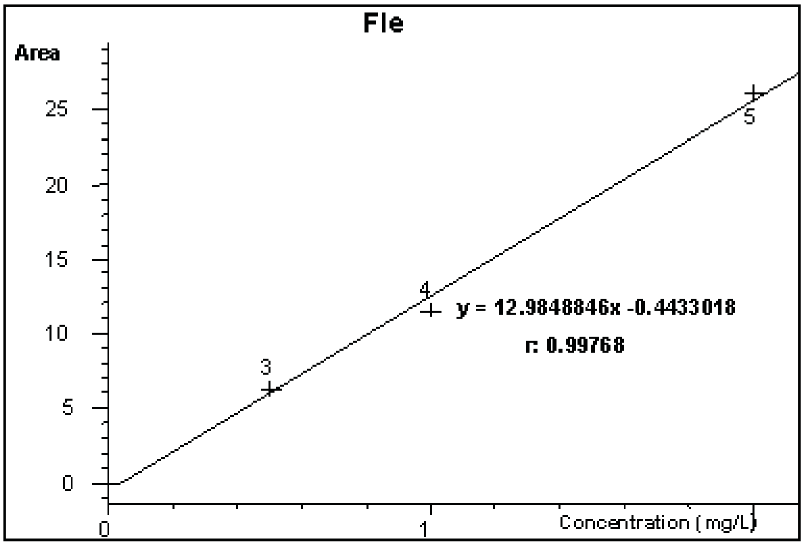
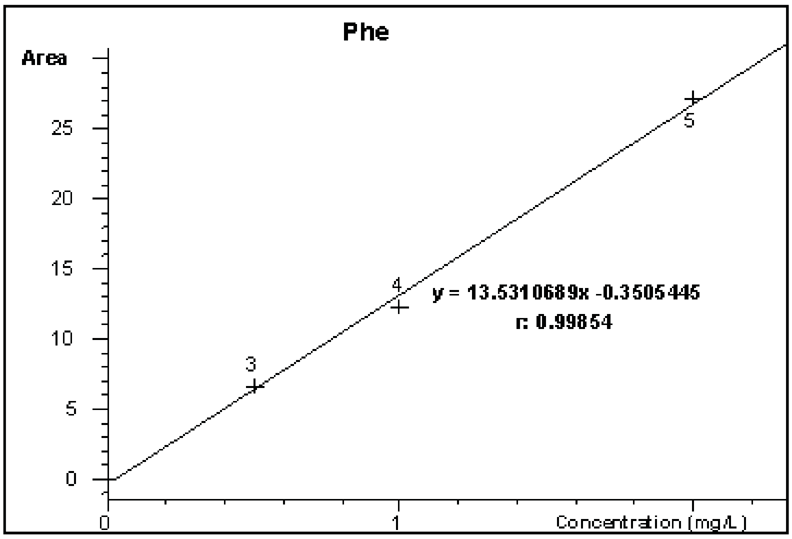
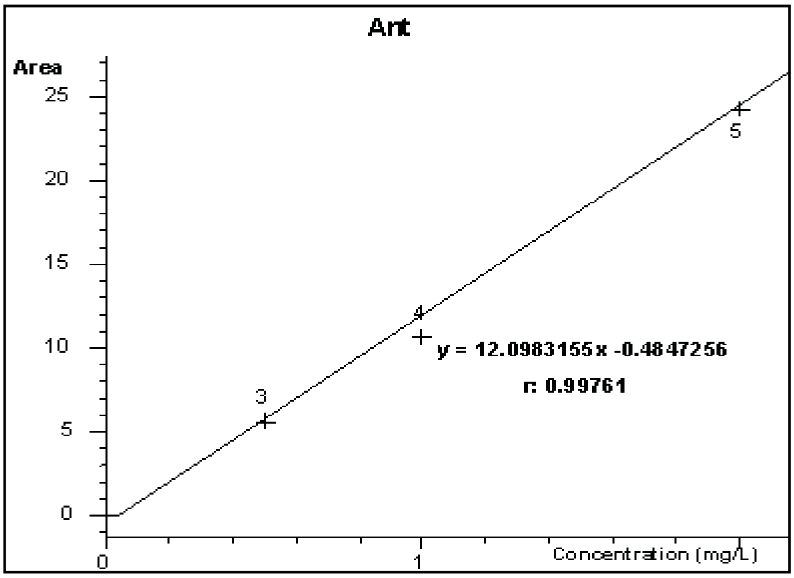
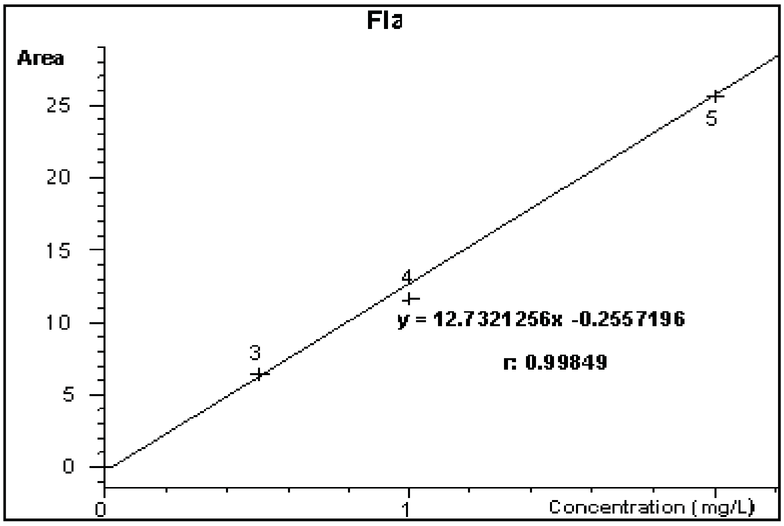
5
0
0
1
Concentration (mg/L)
2
3
4
5
y = 13.6970907x -0.3159207
r: 0.99796

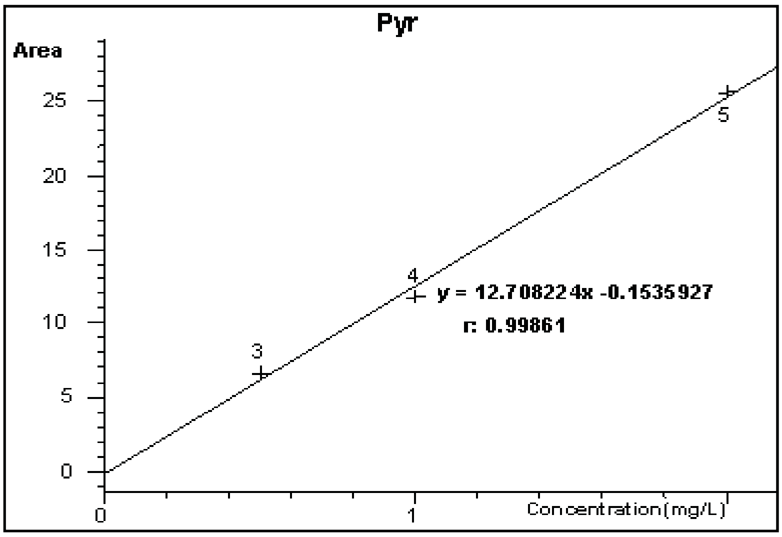

Ace
Area
30
25
20
15
10
5
0
0
1
Concentration (mg/L)
2
3
4
5
Y = 14.5649908x -0.2056972
r: 0.99785

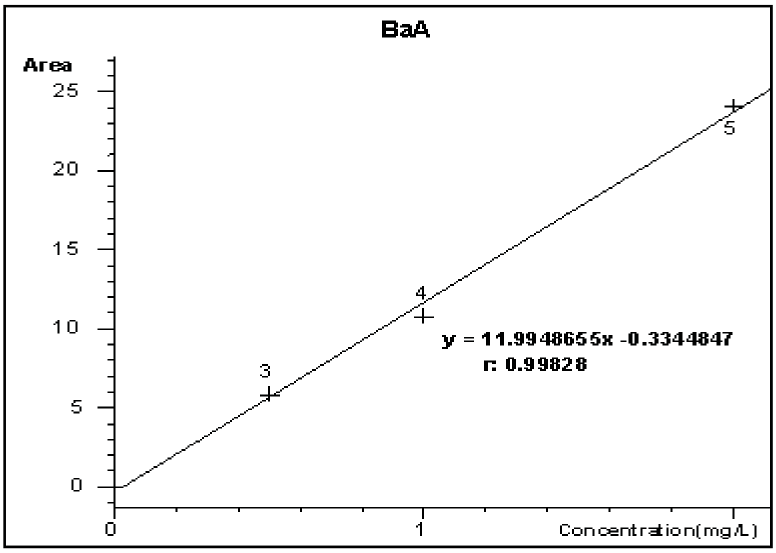

Fle
Area
25
20
15
10
5
0
0
1
Concentration (mg/L)
3
4
5
y = 12.9848846x -0.4433018
r: 0.99768

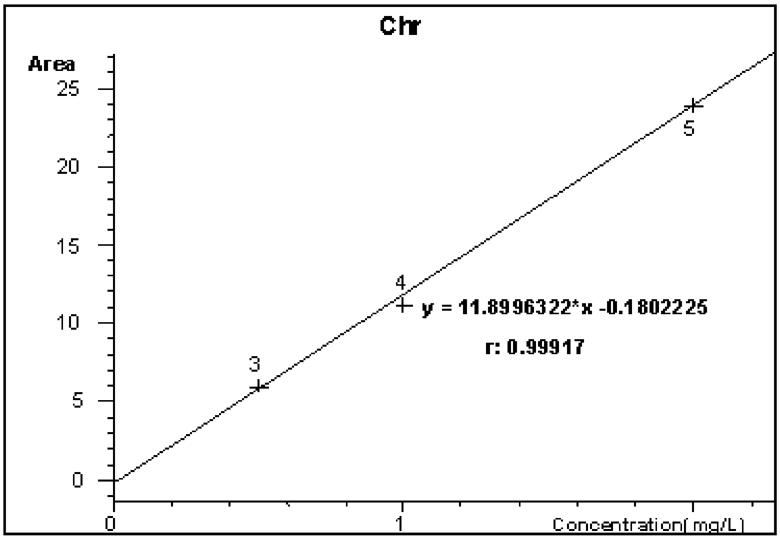

Phe
Area
25
20
15
10
5
0
0
1
Concentration (mg/L)
3
4
5
y = 13.5310689x -0.3505445
r: 0.99854

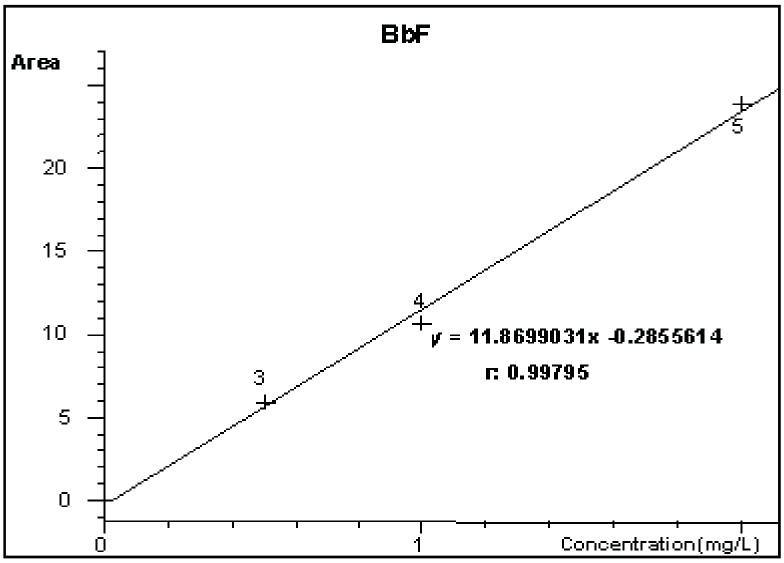

Ant
Area
25
20
15
10
5
0
0
1
Concentration (mg/L)
3
4
5
y = 12.0983155x -0.4847256
r: 0.99761

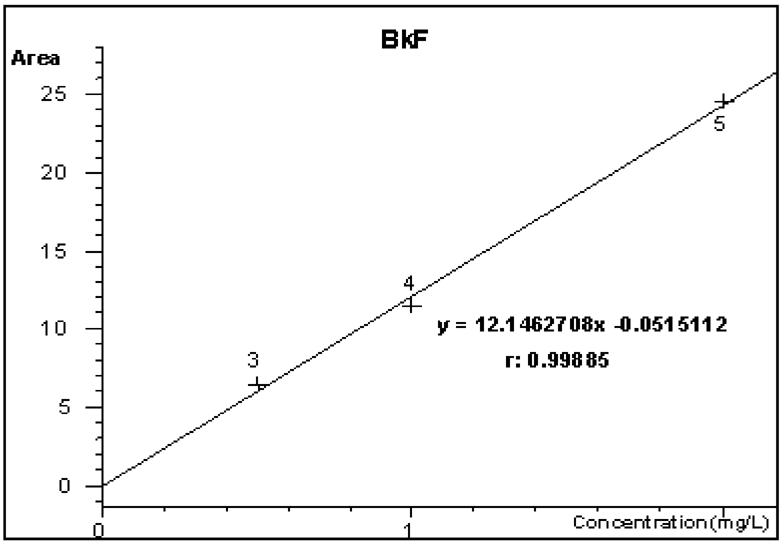

Fla
Area
25
20
15
10
5
0
0
1
Concentration (mg/L)
3
4
5
y = 12.7321256x -0.2557196
r: 0.99849

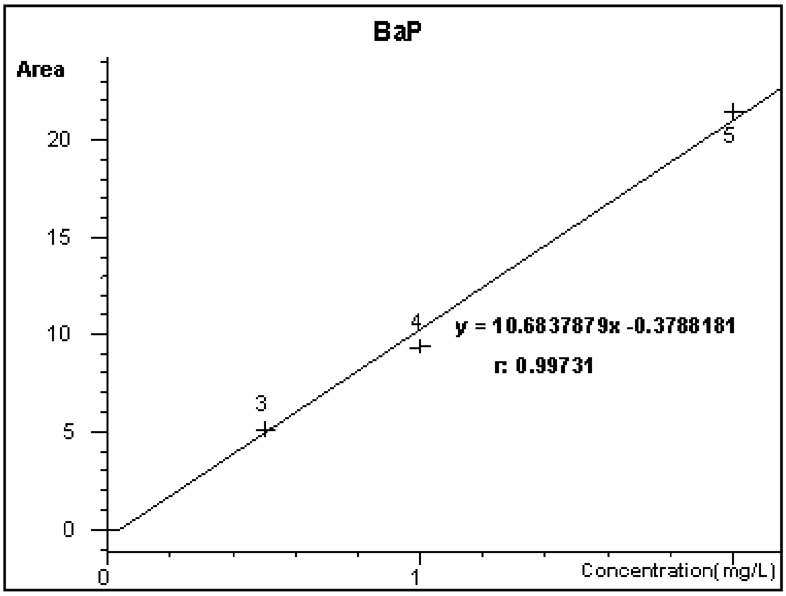

Pyr
Area
25
20
15
10
5
0
0
1
Concentration(mg/L)
3
4
5
y = 12.708224x -0.1535927
r: 0.99861


BaA
Area
25
20
15
10
5
0
0
1
Concentration(mg/L)
3
4
5
y = 11.9948655x -0.3344847
r: 0.99828


Chr
Area
25
20
15
10
5
0
0
1
Concentration(mg/L)
3
4
5
y = 11.8996322*x -0.1802225
r: 0.99917


BbF
Area
20
15
10
5
0
0
1
Concentration(mg/L)
3
4
5
y = 11.8699031x -0.2855614
r: 0.99795


BkF
Area
25
20
15
10
5
0
0
1
Concentration(mg/L)
3
4
5
y = 12.1462708x -0.0515112
r: 0.99885


BaP
Area
20
15
10
5
0
0
1
Concentration(mg/L)
3
4
5
y = 10.6837879x -0.3788181
r: 0.99731

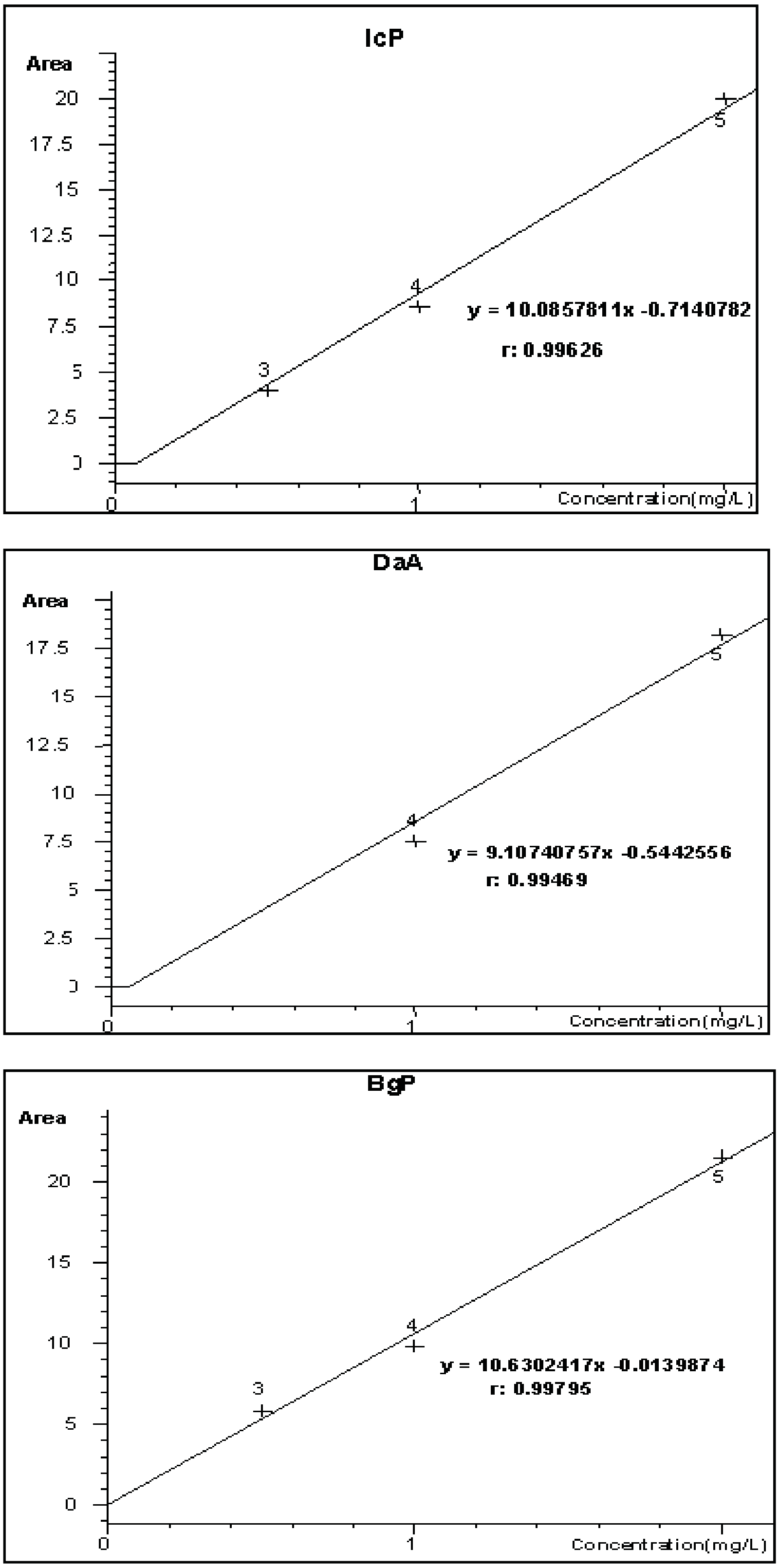


Figure 2.16. PAH (n=16) Calibration Curves for GC-FID

The retention times and LODs of the each target analytes for GC-FID were determined and given in Table 2.6. By GC-FID, each target compound was identified by the retention time of the analyte and the width of the retention time window was 0.01 min to identify the target compounds. The peak areas were integrated by chemstation and manually adjusted if necessary.

The PAH calibration curves were obtained with the standards having a range of 0.05-2 mg/L. The response curves for 16 PAHs were linear with correlation coefficients around 0.99. The concentration range of the surrogate standards was 0.1-2.5 mg/L. The limits of detection ranged from $6.0\ x10^{-3}$-$28\ x10^{-3}$ mg/L as can be seen from the Table 2.6. To identify the minimum detection amount of the analytes, the standard having the lowest concentration was injected. The detection limits were determined based on the concentration (or amount) of an analyte which gave a signal three times the background noise. The GC-MS have lower detection limits than GC-FID. Therefore, GC-MS was used when the analyte concentrations below the detection limits of GC-FID.

Table 2.6. Retention Time and LOD of PAH for GC-FID

No	**Name**	**Retention Time (min)**	**LOD (mg/L)**
1	Nap	11.63	$11.00\ x10^{-3}$
2	Acy	16.03	$7.000\ x10^{-3}$
3	Ace	16.55	$16.00\ x10^{-3}$
4	Fle	17.99	$9.000\ x10^{-3}$
5	Phe	20.67	$14.00\ x10^{-3}$
6	Ant	20.80	$10.00\ x10^{-3}$
7	Fla	24.09	$6.000\ x10^{-3}$
8	Pyr	24.69	$13.00\ x10^{-3}$
9	BaA	28.19	$14.00\ x10^{-3}$
10	Chr	28.29	$14.00\ x10^{-3}$
11	BbF	31.13	$9.000\ x10^{-3}$
12	BkF	31.20	$16.00\ x10^{-3}$
13	BaP	32.01	$19.00\ x10^{-3}$
14	IcP	36.09	$28.00\ x10^{-3}$
15	DaA	36.31	$14.00\ x10^{-3}$
16	BgP	37.23	$14.00\ x10^{-3}$

LOD:Limit of Detection

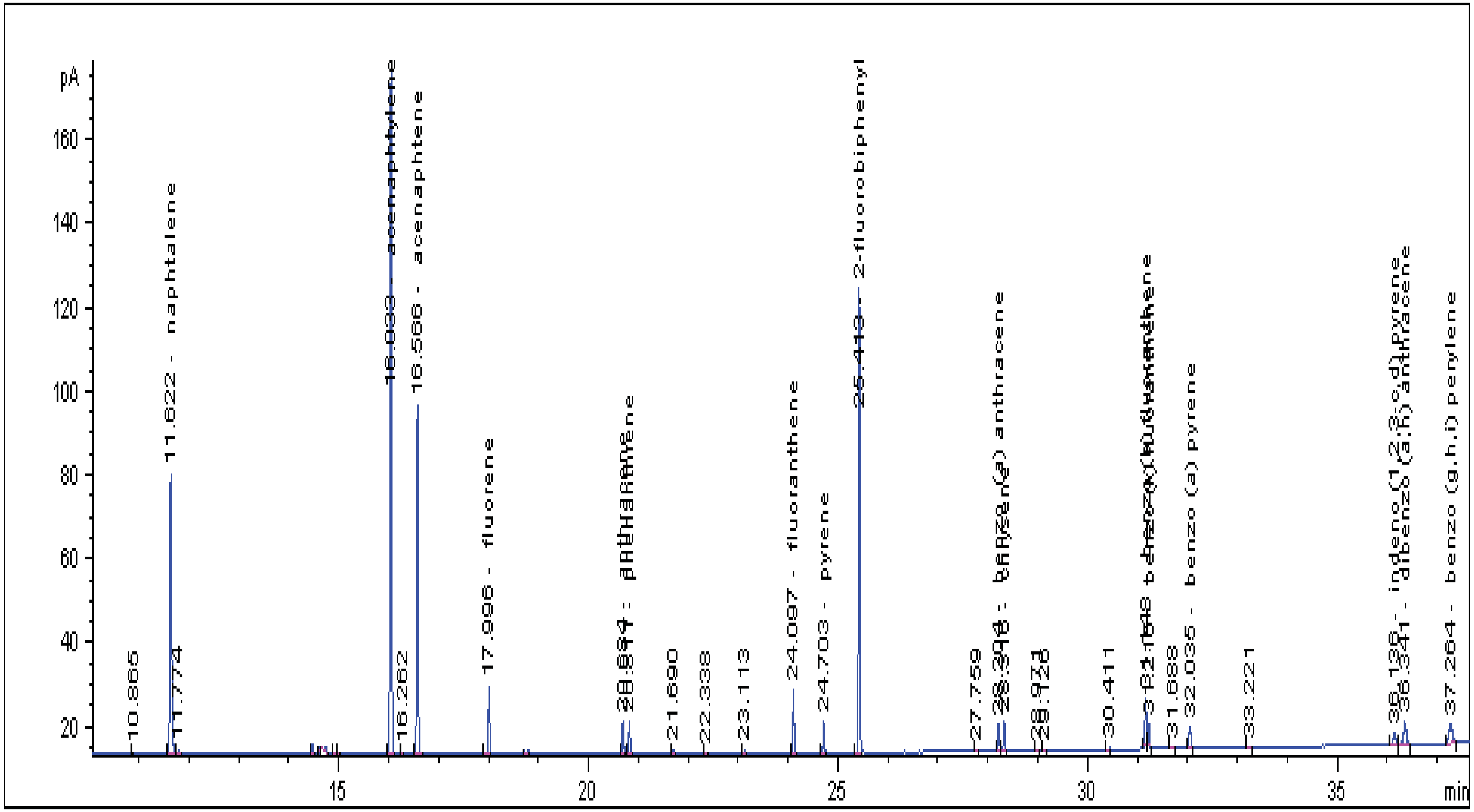


Figure 2.17. GC-FID Chromatogram of Standard Containing 1-10 mg/L PAH and 6.5 mg/L Surrogate (2-Fluorobiphenyl)

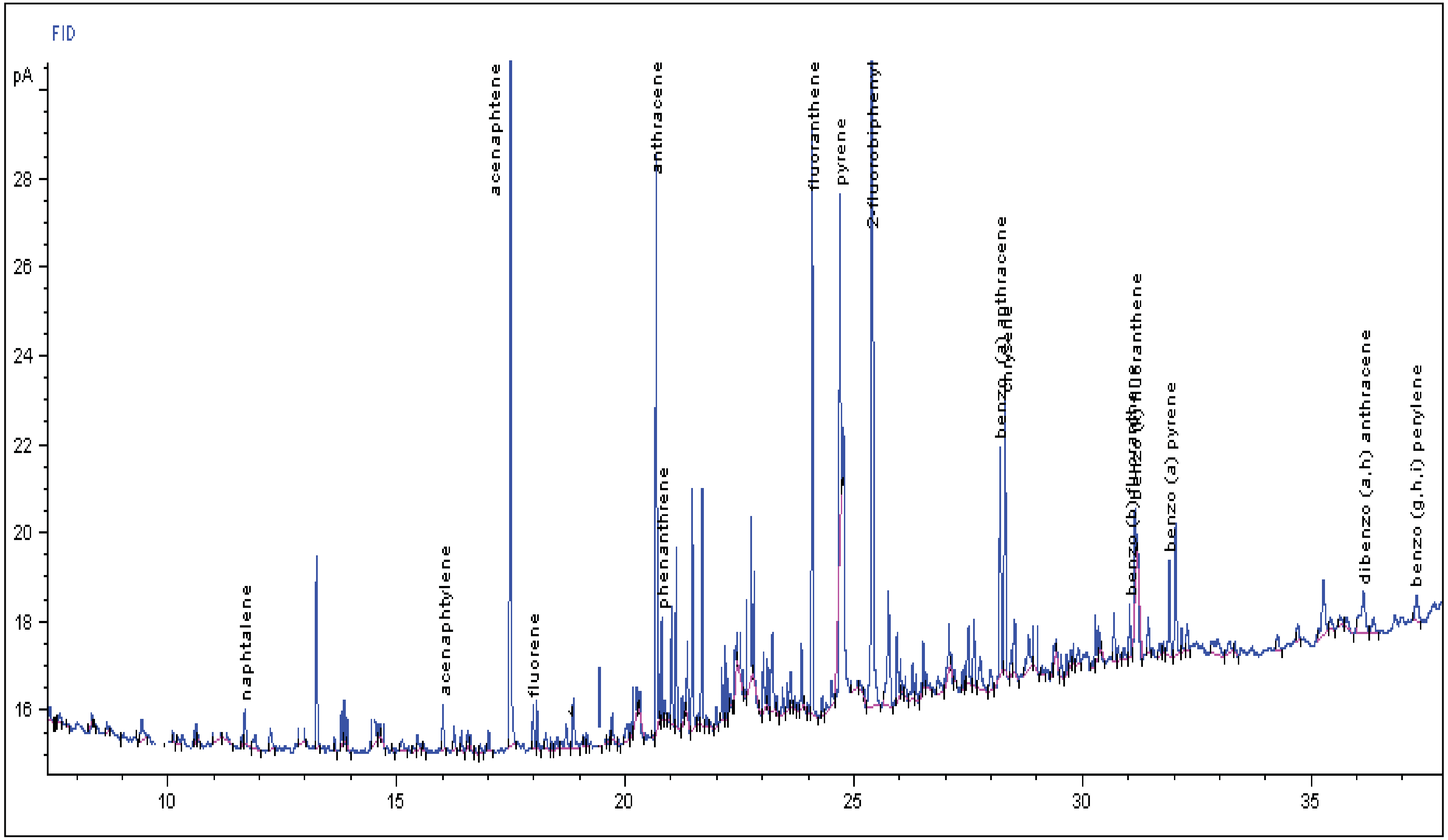


Figure 2.18. GC-FID Chromatogram of Surrogate Spiked (6.5 mg/L 2-Fluorobiphenyl) Ultrasonic Bath Extracted Ölüdeniz Sediment Sample

Sample chromatogram for both standart and the sample were given in Figure 2.17 and 2.18 respectively. The standart (Figure 2.17) was containing 1-10 mg/L PAH and 6.5 mg/L surrogate (2-Fluorobiphenyl). The sediment sample (Figure 2.18) was from Ölüdeniz Lagoon and extracted with ultrasonic bath method after surrogate adition (6.5 mg/L 2-Fluorobiphenyl). As can be seen from the figures sample chromatogram was very clean and no clean-up step was necessary after ultrasonic bath extraction. In addition no baseline increment was observed in the sample chromatogram like standart chromatogram. Therefore it could be said that no matrix effect was observed.

2.4.2. GC-MS Analysis

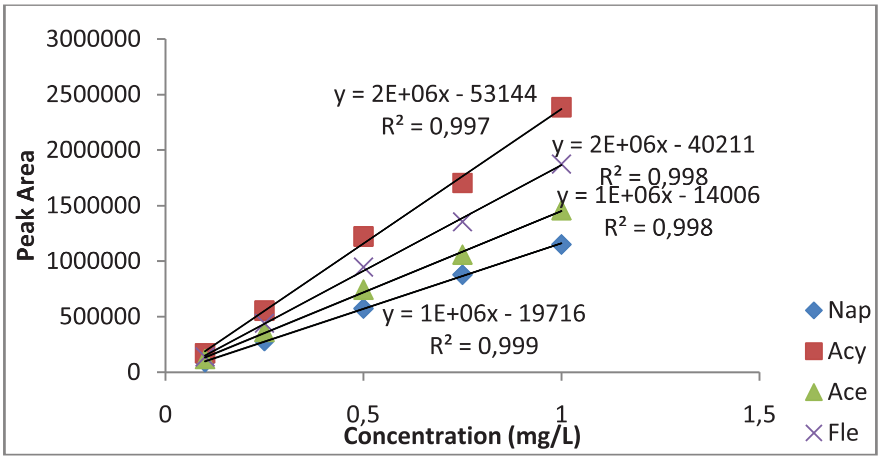
Gas chromatography mass spectrometer was used in selected ion monitoring (explain) (SIM) mode and five SIM windows were created by using the retention time, target and confirmation ions of the PAHs. Table 2.7 shows GC-MS SIM parameters for the PAHs. GC-MS parameters are the same in each window.

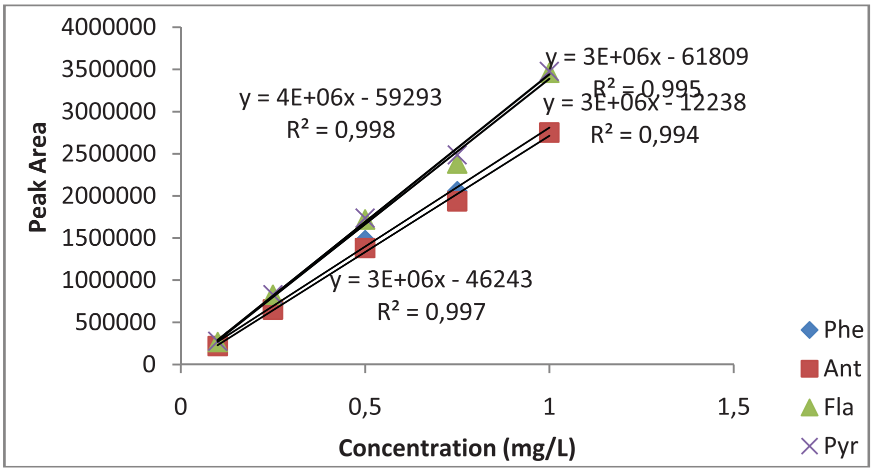
Table 2.7. GC-MS SIM Parameters for PAHs

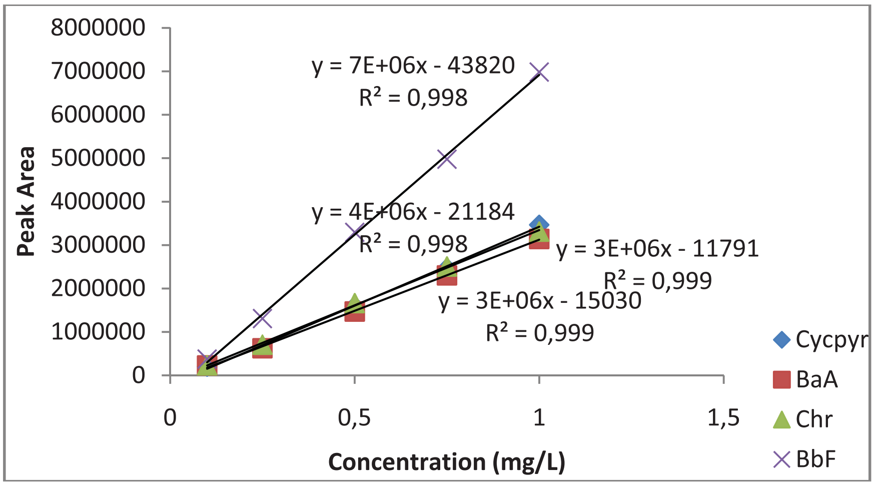
Window	Time	Ions
1	6-18	127,128,129,151,152,153,154,158,160,162,163,164,165,166
2	18-25	160,176,178,179,184,188,189,200,202,203
3	25-32	120,126,224,226,227,228,229,236,240,241,250,252,253,260,261,264,265
4	32-42	128,139,274,275,276,277,278

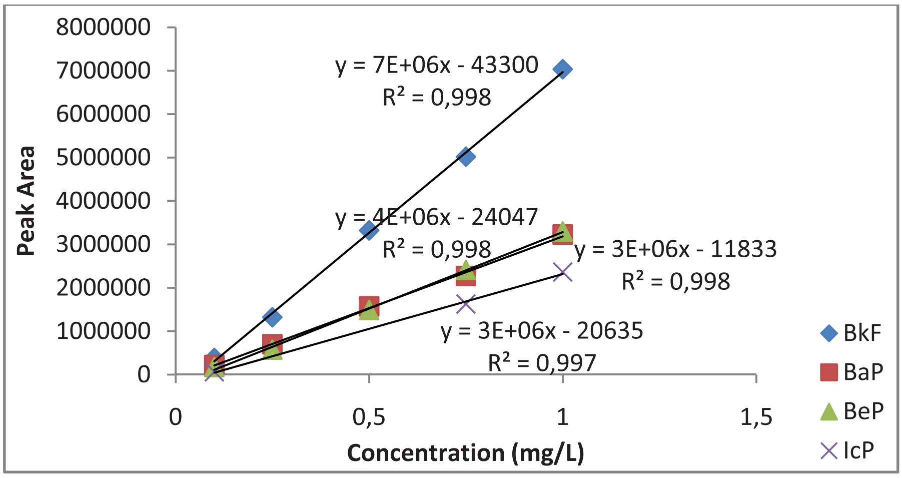
Calibration Curves for GC-MS Analysis

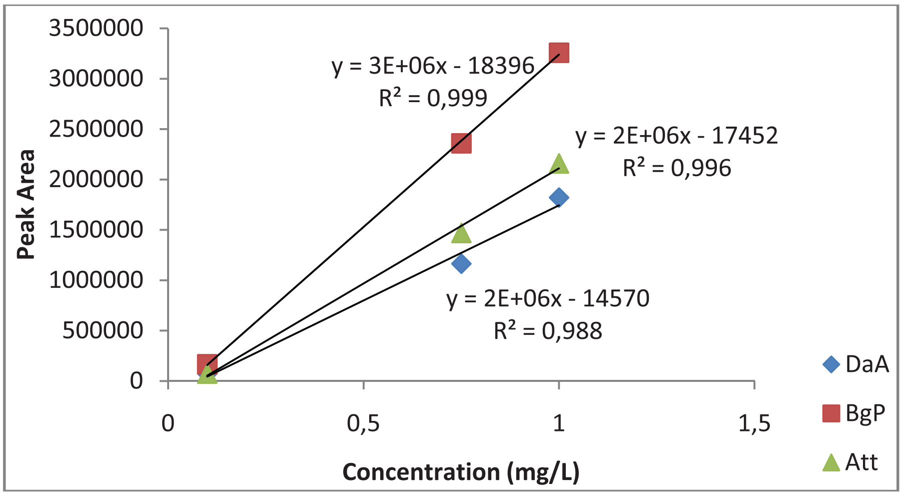
The calibration curves prepared for the analysis of 19 PAHs in sediment samples were given in Figure 2.19.


3000000
2500000
2000000
1500000
1000000
500000
0
Peak Area
y = 2E+06x - 53144
R² = 0,997
y = 2E+06x - 40211
R² = 0,998
y = 1E+06x - 14006
R² = 0,998
y = 1E+06x - 19716
R² = 0,999
0
0,5
1
1,5
Concentration (mg/L)
◆ Nap
■ Acy
▲ Ace
× Fle


4000000
3500000
3000000
2500000
2000000
1500000
1000000
500000
0
Peak Area
y = 4E+06x - 59293
R² = 0,998
y = 3E+06x - 61809
R² = 0,995
y = 3E+06x - 12238
R² = 0,994
y = 3E+06x - 46243
R² = 0,997
0
0,5
1
1,5
Concentration (mg/L)
◆ Phe
■ Ant
▲ Fla
× Pyr


8000000
7000000
6000000
5000000
4000000
3000000
2000000
1000000
0
Peak Area
y = 7E+06x - 43820
R² = 0,998
y = 4E+06x - 21184
R² = 0,998
y = 3E+06x - 11791
R² = 0,999
y = 3E+06x - 15030
R² = 0,999
0
0,5
1
1,5
Concentration (mg/L)
◆ Cycpyr
■ BaA
▲ Chr
× BbF





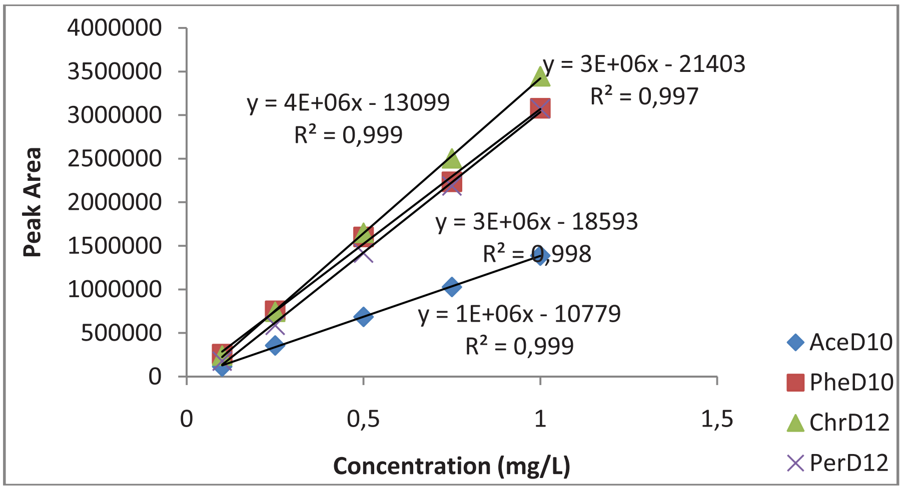


Figure 2.19. PAH (n=19) Calibration Curves for GC-MS

For the GC-MS analysis of sediment samples; the standard calibration curves (Figure 2.19) were prepared by 0.1-1 mg/L PAH standards. Table 2.8 shows retention times and limit of detections (LODs) of PAH for GC-MS. The range of r values lies between 0.99 and 1. They showed good linearity. Detection limits are varied between $12x10^{-6}$-$390x10^{-6}$ mg/L. Detection limits of the each target analytes for GC-MS were determined after qualitative analysis of samples and standards. The peak areas were integrated by chemstation and manually adjusted if necessary. To identify the minimum detection amount of the analytes, the standard having the lowest concentration or the least polluted sample was injected. The limit of detection was found by considering the concentration (or amount) of an analyte (each PAHs) which gave a signal three times the background noise.

Table 2.8. Retention time and LOD of PAHs and Surrogates for GC-MS

No	Name	Retention Time (min)	LOD (mg/L)
1	Nap	9.900	$98.00x10^{-6}$
2	Acy	14.39	$56.00x10^{-6}$
3	Ace D10	14.85	$56.00x10^{-6}$
4	Ace	14.94	$25.00 x10^{-6}$
5	Fle	16.43	$37.00x10^{-6}$
6	Phe D10	19.09	$80.00 x10^{-6}$
7	Phe	19.15	$39.00 x10^{-6}$
8	Ant	19.28	$25.00 x10^{-6}$
9	Fla	22.64	$24.00 x10^{-6}$
10	Pyr	23.25	$12.00 x10^{-6}$
11	Cycpyr	26.78	$112.0 x10^{-6}$
12	BaA	26.85	$49.00 x10^{-6}$
13	Chr D12	26.89	$70.00 x10^{-6}$
14	Chr	26.95	$215.0 x10^{-6}$
15	BbF	29.82	$124.0 x10^{-6}$
16	BkF	29.89	$72.00 x10^{-6}$
17	BaP	30.47	$74.00 x10^{-6}$
18	BeP	30.61	$70.00 x10^{-6}$
19	Per D12	30.78	$81.00 x10^{-6}$
20	IcP	33.84	$193.0 x10^{-6}$
21	DaA	34.01	$390.0 x10^{-6}$
22	BgP	34.70	$164.0 x10^{-6}$
23	Att	35.19	$238.0 x10^{-6}$

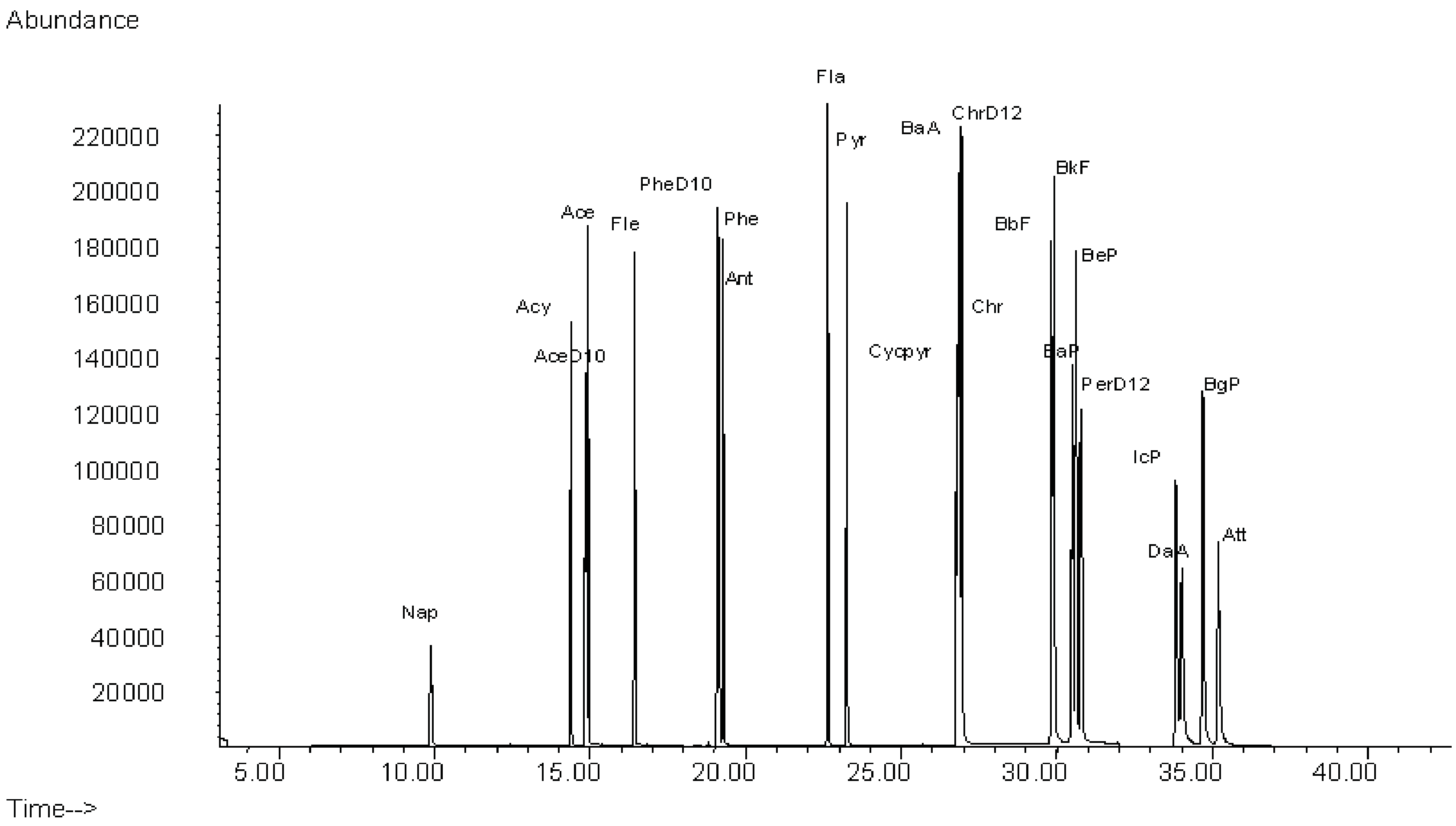


Figure 2.20. The GC-MS Chromatogram (SIM Mode) of Standart Containing 1 mg/L PAH and Surrogates

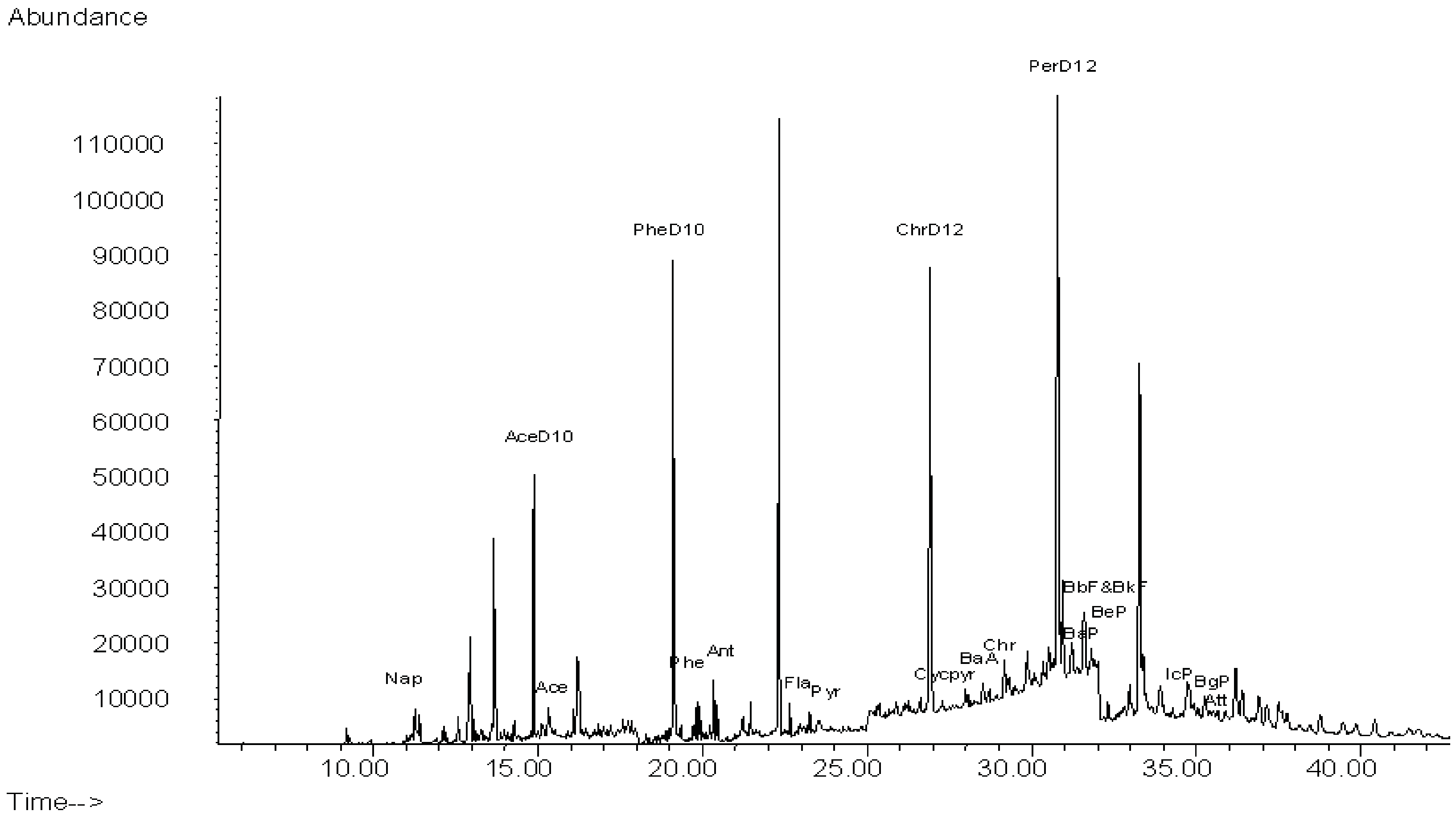


Figure 2.21. The GC-MS chromatogram (SIM Mode) of Ultrasonic Bath Extracted İkizcetepeler Sediment Sample

The GC-MS chromatograms both for standart and sample were given in Figure 2.20 and 2.21 respectively. The sediment sample was from İkizcetepeler Dam Lake, Balıkesir and extracted with optimized ultrasonic bath extraction method after addition of 1 mg/L surrogate mix (Ace D10, Phe D10, Chr D12, Per D12). The standart was containing 1 mg/L PAH and 1 mg/L surrogate mix.

2.5. Quality Assurance and Quality Control

The method used in this study was verified using NIST SRM 1597a (complex mixture of PAHs from coal tar prepared in DCM) as a reference both for GC-MS and GC-FID. Since two different SRM 1597a were bought in different years, PAH compositions are different in Table 2.9 and 2.10.

Table 2.9. Certified Concentrations (mg/L) and % Recoveries of PAHs in SRM 1597a (complex mixture of PAHs from coal tar prepared in DCM) for GC-MS

SRM 1597a	**Certified Conc. (mg/L)**	**Found Conc. (mg/L)(n=4)**	**% Error**	**% Recovery**
Nap	896 ± 87.0	479 ± 10.7	-46.6	53.4
Acy	229 ± 6.00	184 ± 6.85	-19.5	80.5
Ace	6.63 ± 0.230	7.90 ± 0.258	19.2	119
Fle	126 ± 3.00	104 ± 5.65	-17.8	82.2
Phe	395 ± 6.00	325 ± 7.54	-17.8	82.2
Ant	93.0 ± 2.60	76.9 ± 2.88	-17.4	82.6
Fla	284 ± 6.00	257 ± 10.1	-9.67	90.3
Pyr	209 ± 6.00	197 ± 3.16	-5.74	94.3
BaA	85.3 ± 2.00	105 ± 41.6	23.5	124
Chr	57.6 ± 4.60	76.5 ± 31.3	32.7	133
BbF	57.5 ± 3.80	54.8 ± 3.35	-4.70	95.3
BbK	35.8 ± 0.400	53.6 ± 2.86	49.7	150
BeP	43.8 ± 0.900	33.1 ± 1.75	-24.5	75.5
BaP	81.3 ± 1.20	43.2 ± 2.86	-46.9	53.1
BgP	43.9 ± 0.500	33.5 ± 3.24	-23.7	76.3
IcP	48.3 ± 0.700	41.8 ± 5.69	-13.5	86.5
DaA	6.03 ± 0.350	5.80 ± 0.909	-3.81	96.2

Table 2.9 shows the certified concentrations and % recoveries of PAHs in SRM 1597a (mg l^{-1}) for GC-MS. As can be seen % recoveries are lying between 53,4 and 150 and % errors are in the range of 3.81- 49.7.

The concentrations and recoveries of PAHs for GC-FID were shown in Table 2.10. It can be seen that recoveries of individual PAH ranged from 80.1 % to 125 % and % errors are in the range of 1.40 – 25.0.

Table 2.10. Certified Concentrations (mg/L) and % Recoveries of PAHs in SRM 1597a (complex mixture of PAHs from coal tar prepared in DCM) for GC-FID

SRM 1597a	**Certified Conc. (mg/L)**	**Found Conc. (mg/L)(n=3)**	**% Error**	**% Recovery**
Nap	1000 ± 50.0	1017 ± 60.0	1.70	102
Phe	400 ± 4.00	500 ± 76.0	25.0	125
Fla	278 ± 4.00	284 ± 218	2.10	102
Pyr	204 ± 3.00	230 ± 8.40	13.0	113
BaA	85.3 ± 3.40	89.4 ± 8.50	4.80	105
Chr	62.0 ± 1.00	74.3 ± 34.0	20.0	120
BeP	82.9 ± 5.30	81.7 ± 49.0	-1.40	98.6
IcP	52.1 ± 4.00	56.4 ± 7.80	8.20	108
BgP	46.5 ± 6.70	37.2 ± 5.50	-20.0	80.1

Table 2.11. Comparison of Accuracy Values of GC-FID and GC-MS by SRM 1597a

SRM 1597a	**GC-FID % Error**	**GC-MS % Error**	**Which detector is more suitable?**
Nap	**1.70**	-46.6	FID
Phe	25.0	**-17.8**	MS
Fla	**2.10**	-9.67	FID
Pyr	13.0	**-5.74**	MS
BaA	**4.80**	23.5	FID
Chr	**20.0**	32.7	FID
BaP	**-1.40**	-46.9	FID
IcP	**8.20**	-13.5	FID
BgP	**-20.0**	-23.7	FID

Accuracy values of GC-FID and GC-MS were compared in Table 2.11. The columns 2 and 3 shows % errors of GC-FID and GC-MS, respectively in measuring SRM 1597a PAH concentrations. According to the last column it is suitable to analyze Phe and Pyr with GC-MS and Nap, Fla, BaA, Chr, BaP, IcP and BgP with the GC-FID.

CHAPTER 3

RESULTS AND DISCUSSION

3.1. Application of Chemometrics

A chemometric approach was used for the optimization of the three selected sediment extraction methods; soxhlet, ultrasonic bath and SPME. The 2^3 factorial design (including 8 runs) was used for the optimization of each extraction methods.

3.1.1. Factorial Design for Extraction Methods

Factor levels for soxhlet, ultrasonic bath, direct SPME and HS-SPME extractions were given in Tables 3.1, 3.2, 3.3, 3.4 respectively. There were three factors; A, B, C and two levels; low (-1, minimum) and high (+1,maximum) in the 2^3 factorial design.

Table 3.1. Factor Levels in the Screening Design (2^3) for Soxhlet

Factor	Key	Low	High
Time (h)	A	6	24
Solvent volume (ml)	B	200	500
Amount of sediment (g)	C	10	50

As can be seen from the Table 3.1 for soxhlet extraction, the most important three factors were considered; extraction time, volume of the solvent used and the amount of the extracted sediment. They were investigated at two levels; low and high. Low and high values were determined by considering EPA method 3540 C and they were given in the Table 3.1 clearly. Temperature was kept constant (temperature should be higher than the solvents boiling points) during the experiments and adjusted to extract the sample for 6-24 hours at 4-6 cycles/hour.

Table 3.2 . Factor Levels in the Screening Design (2^3) for Ultrasonic Bath Extraction

Factor	**Key**	**Low**	**High**
Time (min)	A	15	45
Solvent volume (ml)	B	20	50
Amount of sediment (g)	C	1	10

Table 3.2 shows the factors and levels for the ultrasonic bath extraction. The factors were time, solvent volume and the amount of the sediment as considered also in soxhlet extraction. Extractions were made in the room temperature. Low and high values were based on the information given in EPA Method (EPA SW-846-3550C).

Table 3.3. Factor Levels in the Screening Design (2^3) for the Direct SPME

Factor	**Key**	**Low**	**High**
Adsorption Temperature (ºC)	A	20	80
Inlet Temperature (ºC)	B	220	280
Desorption Time (min)	C	5	20

At fixed adsorption time: 60 min

Table 3.3 shows the important three factors for SPME and their low and high values. The factors were adsorption temperature, inlet temperature and desorption time. Adsorption time was tried to be kept at maximum; 60 min during the optimization. The SPME manual and the information about the fiber properties were considered for the determination of factor levels. For example proper desorption inlet temperature can not be too low and too high. In addition since an equilibrium was reached after a while and values (concentrations, peak areas etc.) stay constant, longer desorption and adsorption times were unnecessary. After an adsorption step the SPME syringe was immediately transferred to the inlet for desorption and the moisture in the needle was neglected.

Table 3.4. Factor Levels in the Screening Design (2^3) for the HSSPME

Factor	Key	Low	High
Adsorption Temperature (ºC)	A	20	80
Inlet Temperature (ºC)	B	220	280
Adsorption time (min)	C	30	60

At fixed desorption time: 20 min.

Table 3.4 also shows the factors; adsorption temperature, inlet temperature, adsorption time and their levels for HSSPME. Instead of adsorption time, in case of SPME optimization, optimized value of desorption time; 20 min. was used and kept constant during the optimization of HSSPME. While choosing low and high levels for each factor, literature studies were considered.

After factorial design was created for the extraction methods, firstly homogenized sediment sample (mixture of the 68 Ölüdeniz sediments) was prepared. Secondly, water content of the sediment samples were determined by using homogenized sediment samples in order to give concentrations of PAHs in sediments on a dry weight basis. Thirdly, most suitable extraction solvent was selected. And finally, optimization experiments for the extraction methods were completed and results were evaluated by using chemometric approach. Details of these steps were given below.

Homogenized Sample Preparation

Before starting to the optimization of the each extraction methods homogenized sediment sample was prepared from the Ölüdeniz sediment samples. For the homogenization of the sample each sample was opened and 100 grams were taken from each 68 Ölüdeniz sediment samples and put in a bottle having a capacity of 5.0 L and then they were mixed. The prepared sample was kept in refrigerator at +4 ºC. The samples were mixed with sodium sulphate prior to extraction with soxhlet or ultrasonic bath methods.

Water Content Determination

A small portion of wet homogenized sediment sample (5-10 g) was taken in a container and weighed with container (M_{cws}). It was dried in an oven overnight and weighed with container after drying (M_{cs}). The container (where we put the sediment sample) was also weighed (M_c). The average moisture content was calculated for the 10 samples, with the formula given below.

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

Where;

M_{cws} = mass of container and wet sample

M_{cs} = mass of container and dry sample

M_c = mass of container

In numerator water amount is found by subtracting M_{cs} from $M_{cws.}$ In denominator amount of wet sediment is determined by subtracting M_c from M_{cws}. Finally by taking the ratio of them and multiplying with 100, % water content is determined. By the subtraction of average moisture content from the wet sediment samples, dry weights of the sediment samples were calculated.

Solvent Selection for Ultrasonic Bath and Soxhlet Extraction Methods

The solvent types were chosen by considering some factors such as suitability to analytes, solvents of the standard, toxicity and cost. Therefore three solvents were investigated; dichloromethane (DCM, solvent of the standards), acetone (Ac, least toxic solvent) and hexane (Hex, suitable to analytes). The most proper (which extracts better than the others) solvent was selected amongst: hexane/acetone (Hex/Ac), dichloromethane/acetone (DCM/Ac) and dichloromethane (DCM). Solvents were selected under the EPA guidance (EPA 3540C and 3550B). Exchange solvent was chosen as DCM which is compatible with the solvent of the calibration standards. The comparison was done according to the % surrogate recovery. As a surrogate, the mixture including 1. Nitrobenzene D5, 2. p-Terphenyl D14, 3. 2-Fluorobiphenyl, was used. The short explanation of this part was given below.

The most traditional soxhlet extraction method was used for the solvent selection. The factors which were solvent volume, sediment amount and the time, were used at their maximum values; 250 ml, 80 g and 24 h respectively . The number of replicate was three. The surrogate mix (1 ml of 10 mg/L) was added to the sediment sample prior to extraction in order to decide which solvents recovery is the highest after the extraction with soxhlet. Final extract obtained was reduced to 1 ml and the solvent was DCM. According to average surrogate recovery values: DCM/Ac was chosen as the best solvent pair for soxhlet extraction and it was also used in ultrasonic bath extractions (Figure 3.1, Table 3.5).

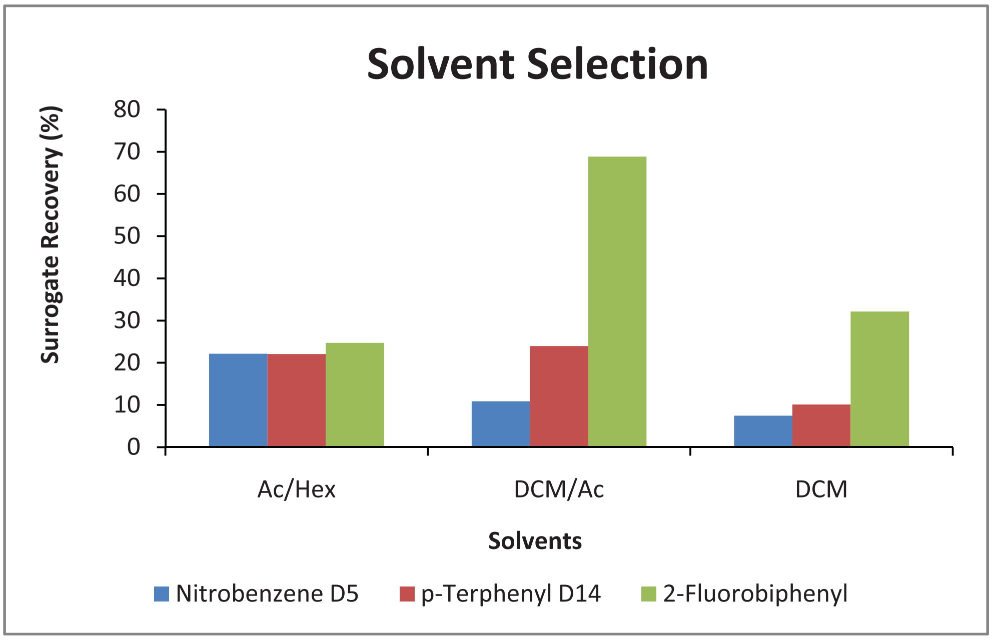


Figure 3.1. Selection of the Most Proper Solvent For the Soxhlet Extraction

As can be seen from the Figure 3.1 and Table 3.5, the best surrogate recovery (35 %) was obtained with DCM/Ac. Therefore the best solvent pair was DCM/Ac. Except Nitrobenzene D5, other two surrogates, p-Terphenyl D14 and 2-Fluorobiphenyl gave the best result with DCM/Ac. In addition to recovery values, DCM/Ac was also compatible to solvent of the standards which was DCM. Moreover, DCM/Ac was less toxic than DCM since it was diluted (1:1) with the less toxic Ac.

Table 3.5. Percent Surrogate Recoveries of Selected Solvents in Soxhlet Extraction

	Nitrobenzene D5	**p-Terphenyl D14**	**2-Fluorobiphenyl**	**Average % Recovery**
Acetone/Hexane (Ac/He)	22	22	25	23
Dichloromethane/ Acetone (DCM/Ac)	11	24	69	35
Dichloromethane (DCM)	7	10	32	16

Since 2-Fluorobiphenyl seems more suitable surrogate than the others this surrogate was added the Ölüdeniz sediment samples prior to the ultrasonic bath and soxhlet extractions. Soxhlet extraction procedure for solvent selection was shown in Figure 3.2.

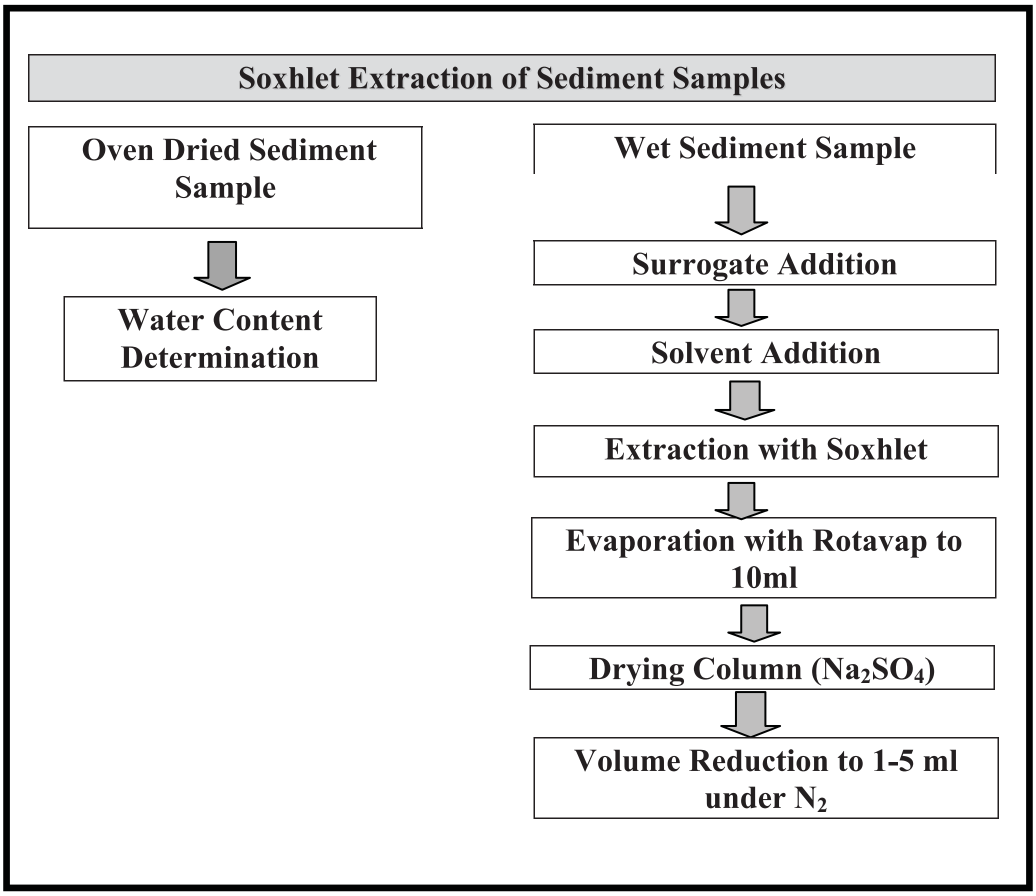


Figure 3.2. Soxhlet Extraction Procedure for Solvent Selection

3.2. Results of Chemometrics Application

All the optimizations were done by using GC-FID. The results were given below.

3.2.1. Solid Phase Micro Extraction (SPME)

The SPME fiber in conjuction with GC was used in water and sediment analysis. The influence of various parameters on PAH extraction efficiency by SPME was throughly studied. Extraction efficiencies of the three SPME fibers; PDMS (7,100) and 85 µm PA, were compared for the analysis of 16 EPA priority PAHs. The PA provided highest extraction efficiency both in direct SPME application and the HS analysis. The fibers were conditioned in the hot injector port of the GC according to the instructions provided by the manufacturer (0.5 h at 250 ºC for the 100 µm PDMS fiber, 1 h at 300 ºC for the 7 µm PDMS fiber and 2 h at 300 ºC the PA fiber) in order to reduce background, make cleaning and perform a blank analysis (http://www.labplus.co.kr/tech/upload/SPME%20guide.pdf)

The kinetics of the extraction process determines the speed of extractions. Since the polimeric structures of the each fiber was different from another (Table 1.1), they have different kinetics, therefore different conditioning times.

Before starting optimization of the SPME and HSSPME, the most suitable fiber was selected among PDMS (7,100) and 85 µm PA. After selecting the best fiber, factorial design experiments for SPME and HSSPME were completed. Details of these studies were given below.

Fiber Selection for SPME and HSSPME Analysis

The most proper fiber was selected for both SPME and HSSPME. For the SPME; acetone was used as a solvent (by considering the polarity of the fibers). Since the solvent that is proper for three of the fiber does not exist, the 7 µm PDMS was elected during the fiber selection part of the SPME analyses. For the HSSPME, SRM 1941b (Marine Sediment) was used as a soil matrix sample (by considering the effect of fiber size on extractions) and three of the fibers were compared. Three replicates measurement was done for each fiber.

The GC-FID conditions was the same with the Table 2.1 in experimental part except that the inlet temperature. Other experimental parameters were taken from the literature. Inlet temperature of 220 °C was used since it was advised in the SPME manual. Same liner was used for both direct SPME and HSSPME.

Fiber Selection for Direct SPME

The SPME procedure consisted of the immersion of the fibers for a certain time in amber vials capped with PTFE coated septa. After that, the fiber was subjected to desorption in the GC injector. Possible carryover was removed by keeping the fiber in the injector for an additional time with the injector in splitless mode. Reinserting the SPME fiber after the run did not show obvious carryover. Morever, blanks were run periodically during the analysis to confirm the absence of contaminants. Response areas of PAHs and surrogates were used for the comparison.

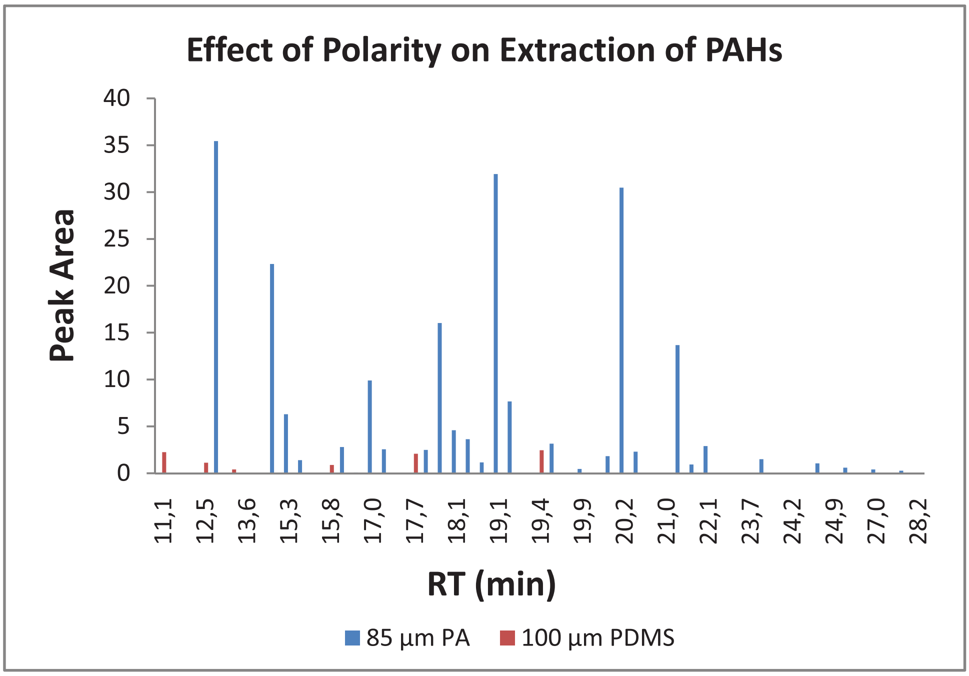


Figure 3.3. Fiber Selection for Direct SPME

The solutions (nine replicates each have the same concentrations of PAHs and surrogates) were prepared in Ac. The concentrations were 0.5 mg/L for surrogate and 0.5 mg/L for PAHs. The best fiber was selected according to the extraction efficiency. The analyses with the Ac solutions, were done by directly immersing fiber into a solution with an adsorption depth of 3 cm and desorption depth of 2.5 cm at room temperature; 25 °C. The adsorption time was 45 minutes and desorption time was 10 minutes.. The results were given in Figure 3.3. Peak areas were used to draw Figure 3.3 and the best fiber was selected according to the highest peak area.

According to the peak aras given in the Figure 3.3, 85 µm PA had better efficiency than 100 µm PDMS. Since Ac was not proper solvent for 7 µm PDMS, it was not used. The 100 µm PDMS gave poorer adsorption profile, therefore the polarity of this fiber was less proper to adsorb PAHs from Ac samples.

Fiber Selection for HSSPME

In order to select best fiber for the extraction of PAHs from solid matrices a different set of experiment was performed. Amount of 0.05 gram of SRM 1941b were introduced into a 4 ml PTFE amber vials and then 0.2 mg/kg PAH and 0.4 mg/kg surrogate were added. The vials were placed in water bath at 80 ºC for 45 min before the analysis. A desorption time of 10 min at 220 ºC was used for quantitative desorption of all analytes studied and no carry over effect was observed. The PAH and surrogate peak areas were compared and the results were shown in Figure 3.4.

According to the peak areas given in the Figure 3.4, 85 µm PA had the best efficiency. The order of the fibers according to the higher peak area, therefore better adsorption capacity, was given like; 85 µm PA > 100 µm PDMS > 7 µm PDMS. The 7 µm PDMS gave the poorest adsorption profile, therefore the polarity of this fiber was not so proper to adsorb PAHs from SRM 1941b samples. Although having the same polarity, 100 µm PDMS was relatively better than 7 µm PDMS since its capability of collecting analytes (PAHs) was better than the capability of 7 µm PDMS.

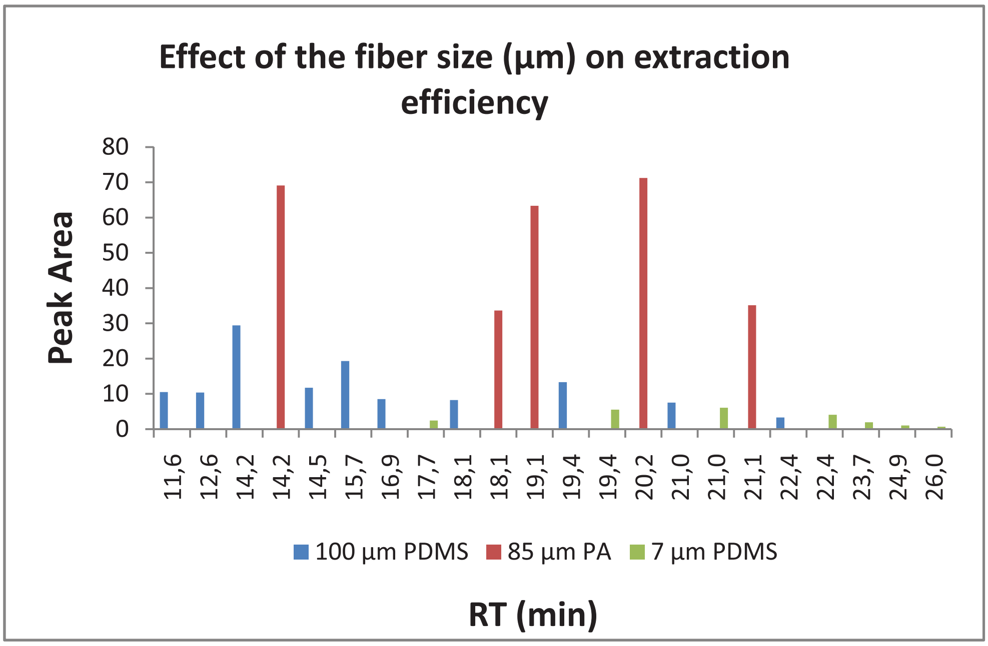


Figure 3.4. Fiber Selection for HSSPME

During the HSSPME analysis the sampling depths for both adsorption and immersion were 2.5 cm for all fibers. The water bath (100 ml beaker was used for the homogenized heating of the sample) was used to keep temperature constant (80 °C) during the adsorption of the PAHs and surrogates onto the fiber. The adsorption time and the desorption time were also the same with direct SPME, 45 minutes and 10 minutes respectively.

By using the HSSPME datas, detection limits for three fibers were determined separately. Table 3.6 shows the detection limits found for three fibers. As can be seen from the Table, in general LOD of 85 µm PA < 100 µm PDMS < 7 µm PDMS. Bold numbers in Table 3.6 shows the lowest detection limits of the analyst.

Table 3.6. Detection Limits (mg/kg) of GC-FID for Three Fibers (7 µm and 100 µm PDMS, 85 µm PA) (n=4)

PAHs	100 µm PDMS LOD (mg/kg)	7 µm PDMS LOD (mg/kg)	85 µm PA LOD (mg/kg)
Nap	209 x10^{-3}	216 x10^{-3}	**102x10^{-3}**
Fle	**197 x10^{-3}**	305 x10^{-3}	611 x10^{-3}
Phe	273 x10^{-3}	503 x10^{-3}	**6.30 x10^{-3}**
Ant	231 x10^{-3}	694 x10^{-3}	**16.9 x10^{-3}**
Fla	64.5 x10^{-3}	75.8 x10^{-3}	**2.90 x10^{-3}**
Pyr	227 x10^{-3}	124 x10^{-3}	**61.9 x10^{-3}**
BaA	231 x10^{-3}	294 x10^{-3}	**24.9 x10^{-3}**
Chr	237 x10^{-3}	336 x10^{-3}	**24.6 x10^{-3}**
BbF	180 x10^{-3}	332 x10^{-3}	**22.2 x10^{-3}**
BkF	485 x10^{-3}	701 x10^{-3}	**21.0 x10^{-3}**
BaP	121 x10^{-3}	651 x10^{-3}	**21.7 x10^{-3}**
BgP	639 x10^{-3}	1066 x10^{-3}	**22.0 x10^{-3}**
IcP	257 x10^{-3}	1071 x10^{-3}	**14.3 x10^{-3}**
DaA	243 x10^{-3}	1212 x10^{-3}	**26.4 x10^{-3}**

*Bold numbers are the lowest detection limits for the PAHs

Table.3.7. Detection Limits of the GC-MS and GC-FID Determined by 85 µm PA Fiber Extracted From SRM 1941b (marine sediment)

PAHs	85 µm PA with GC-FID LOD (mg/kg)	85 µm PA with GC-MS LOD (mg/kg)
Nap	102 x10^{-3}	**6.20 x10^{-3}**
Fle	611 x10^{-3}	**0.860 x10^{-3}**
Phe	6.30 x10^{-3}	**4.90 x10^{-3}**
Ant	16.9 x10^{-3}	**1.50 x10^{-3}**
Fla	**2.90 x10^{-3}**	6.50 x10^{-3}
Pyr	61.9 x10^{-3}	**5.00 x10^{-3}**
BaA	24.9 x10^{-3}	**2.70 x10^{-3}**
Chr	24.6 x10^{-3}	**3.27 x10^{-3}**
BbF	22.2 x10^{-3}	**5.71 x10^{-3}**
BkF	21.0 x10^{-3}	**2.70 x10^{-3}**
BaP	21.7 x10^{-3}	**4.00 x10^{-3}**
BgP	22.0 x10^{-3}	**2.40 x10^{-3}**
IcP	14.3 x10^{-3}	**4.00 x10^{-3}**
DaA	26.4 x10^{-3}	**0.383 x10^{-3}**

*Bold numbers are the lowest detection limits for the PAHs

For the best fiber, 85µm PA, beside GC-FID detection limits, GC-MS detection limits were also determined and the results were given in Table 3.7. For the analysis with GC-MS, optimized conditions in Table 2.2 was used. According to the results except Fla, PA fiber gave lower detection limits with GC-MS especially with Fle and DaA. Since in general GC-MS has lower detection limits and identification of the analytes is quite easy with GC-MS, the rest of the study including factorial analysis with SPME and HSSPME, was conducted with GC-MS.

After selection of the best fibers for seawater and sediment samples, 2^3 screening analyses which contain 8 runs for sediment samples and 8 runs for water samples, were designed. This time more representative samples were prepared in the same 4 ml vials; 8 water (prepared from deionized water) solutions containing 1 mg/L PAH and 2 mg/L surrogate and 8 sediment samples spiked with 0.5 mg/kg PAH and 1 mg/kg surrogate. These analyses (SPME and HSSPME) were done only with using the best fiber; 85 µm PA fiber, for each sample (Water-Ac and sediment).

3.2.1.1. Direct SPME

Since the screening analysis include 8 runs, the same number of samples were prepared from the deionized water and the design was shown in Table 3.8. Average peak areas found for surrogates were also given in Table 3.8.

Samples were prepared with adding 200 µl of 10 mg/L stock surrogate solution (prepared in Ac) and 200 µl of 5 mg/L PAH stock solution (prepared in Ac) to 4 ml vials and diluting to 1 ml with deionized water by adding a 600 µl with a pipette (0.5-5 ml, brand). The solutions were % 40 acetone (v/v) and they were containing 1 mg/L (1 µg) PAH and 2 mg/L (2 µg) surrogate.

Table 3.8 shows the factorial analysis for SPME of water samples. The average surrogate peak areas were used to draw pareto and response surface chart.

Table 3.8. Factorial Design for SPME Analysis of Water Samples

SPME	**Factor A** **$T_{adsorption}$ (°C)**	**Factor B** **T_{inlet} (°C)**	**Factor C** **$t_{desorption}$ (min)**	**Average Surrogate Peak Area**
1	**Room T**	**220**	**20.0**	**690**
2	80.0	220	20.0	365
3	Room T	220	5.00	406
4	80.0	220	5.00	412
5	80.0	280	5.00	251
6	Room T	280	5.00	410
7	80.0	280	20.0	465
8	Room T	280	20.0	551

*Bold line is the optimum values of the factors.

The results of the analysis shown in Table 3.8 produces the main effect and two factor interactions Pareto charts (P=95 %) shown in Figure 3.5 for three selected factors that affects the extraction efficiency. Pareto chart also show the minimum t-value (at the 95 % confidence interval) 12.8 as a vertical black line and a parameter having a higher value than ± t was assumed as significant.

According to the chart all the factors and interactions were below the line at minimum t-value, therefore there was no significantly important factor affecting analysis performance and they are significantly different from zero at 95 % confidence level. However the most important factor was adsorption time which was slightly more important than the adsorption temperature. The inlet temperature had the least importance. The order of the importance of the factors can be given as; adsorption time > adsorption temperature > interaction of adsorption temperature and adsorption time > inlet temperature >interaction of inlet temperature and adsorption time > interaction of adsorption temperature and inlet temperature. The negative or positive effects of the factors on the surrogate recovery was explained with the formula given below.

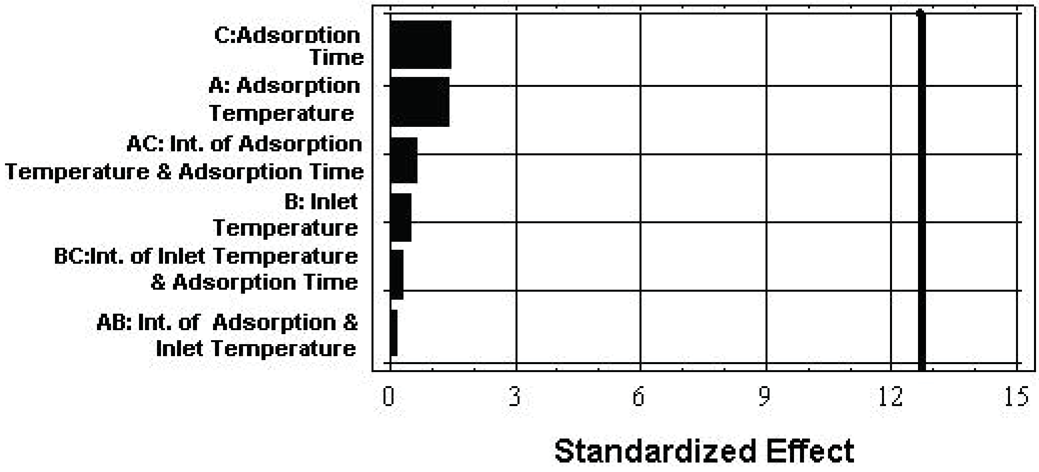


Figure 3.5. Standardized Pareto Chart for Direct SPME Water Analysis

Surface response analysis for SPME analyses was given in Figure 3.

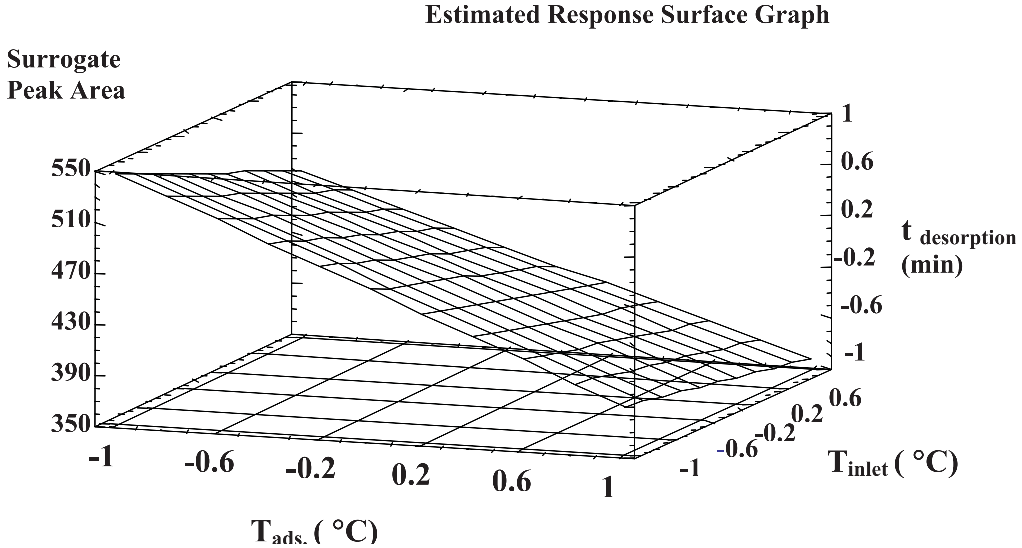


Figure 3.6. Response Surface for Direct-SPME

As can be seen from the estimated response surface graph maximum surrogate peak area (average of the peak areas of 3 surrogates; Nitrobenzene D5, p-Terphenyl D14, 2-Fluorobiphenyl) was observed when factor A; $T_{adsorption}$ (°C) and B; T_{inlet} (°C) were at their lowest levels (-1); room temperature and 220 respectively and Factor C; $t_{desorption}$ (min) was in its maximum level (+1); 20 (1st run). The experimental value at best conditions was 690 (shown as bold in Table 3.8, first row). The lowest peak area was found when factor A and factor B were (+1) and factor C was (-1) (5th run).

As can be seen from direct-SPME model given below, Factor A and B were affecting extraction efficiency negatively. On the other hand factor C had positive effect on the extraction efficiency. According to the model (like in pareto chart); Factor C > Factor A > Factor B in terms of contribution to the extraction efficiency. Effects of the interactions were also observed clearly from the model. Interaction of A and B was the least important.

Extraction Efficiency= 443.75 - 70.5 x Factor A - 24.5 x Factor B + 74.0 x Factor C + 9.25 x Factor A x Factor B - 32.25 x Factor A x Factor C +14.75 x Factor B x FactorC.

By using the equation above, the surrogate recovery (average of 1st,Nitrobenzene D5 and 2nd, p-Terphenyl D14 and 3rd, 2-Fluorobiphenyl) which was obtained when optimized values of the factors (A= -1, B= -1 and C= +1) were used, was found as 639.5 in terms of peak area. Estimated values from the model are consistent with those obtained experimentally (690). This area was necessary for the calculation of the percent surrogate recovery of HSSPME.

3.2.1.2. Headspace SPME

A portion of the previously prepared homogenized sediment sample was dried in room temperature for dryness for overnight. And then it was grinded to remove any stone present. Nearly 2 g was weighed and placed in 4 ml vials. They were spiked with a 200 µl solution containing 10 mg/L surrogate and 5 mg/L PAH in acetone. Final spiked sediments were containing 0.5 mg/kg (1 µg) PAH and 1 mg/kg (2 µg) surrogate.

Table 3.9 **.** Factorial Design for HSSPME Analysis of Sediment Samples

HSSPME	**Factor A** $T_{adsorption}$ **(°C)**	**Factor B** T_{inlet} **(°C)**	**Factor C** $t_{adsorption}$ **(h)**	**Average Surrogate Peak Area**
1	Room T	220	1.00	11.0
2	80.0	220	1.00	287
3	Room T	220	0.500	27.0
4	80.0	220	0.500	303
5	80.0	280	0.500	389
6	Room T	280	0.500	223
7	**80.0**	**280**	**1.00**	**549**
8	Room T	280	1.00	274

*Bold line is the optimum values of the factors.

Analyses were performed with sediment samples (2g) in 4 ml amber vials as shown. Factorial design for HSSPME was shown in Table 3.9. Average surrogate peak areas (average of the two surrogates which were Nitrobenzene D5, p-Terphenyl D14) were found at the end of each run and they were given in the Table 3.9. These values were used for drawing pareto and surface response charts by using SPSS software.

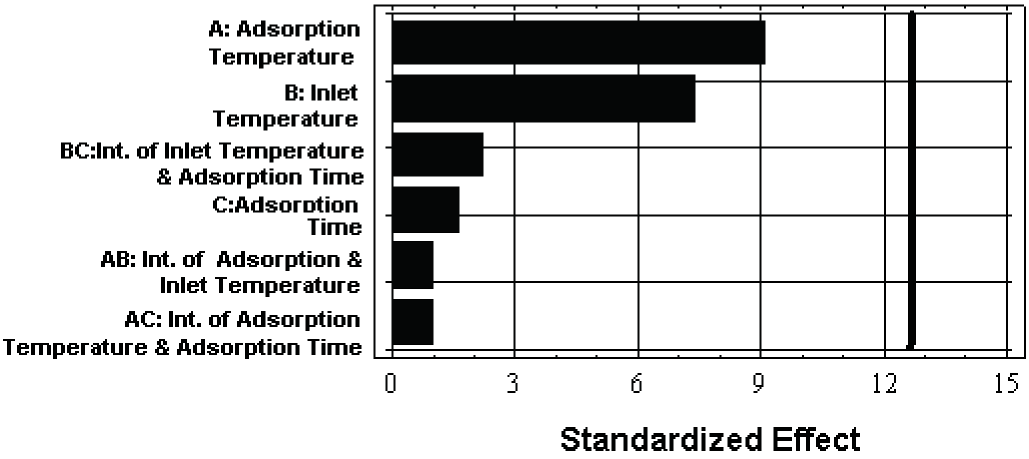


Figure 3.7. Standardized Pareto Chart for Sediment HSSPME Analysis

Pareto chart for the HSSPME analysis of sediment samples, was also shown in Figure 3.7. As can be seen from the Figure 3.7, no factor was significantly important (all are below the minimum t value at 12,8) and they are significantly different from zero at 95 % confidence level. However Factor A (Adsorption temperature) was relatively more important than the others. The order of the importance of the factors were; adsorption temperature > GC inlet temperature > adsorption time. The interaction of factors were also shown in the chart. For example the interaction of inlet temperature & adsorption time was more important (nearly twice as much as important) than the interaction of the adsorption temperature & inlet temperature and the interaction of the adsorption temperature & adsorption time. In addition interaction of the adsorption temperature & inlet temperature and the interaction of the adsorption temperature & adsorption time had nearly the same importance. Response surface graph for the HSSPME analyses was given in Figure 3.8.

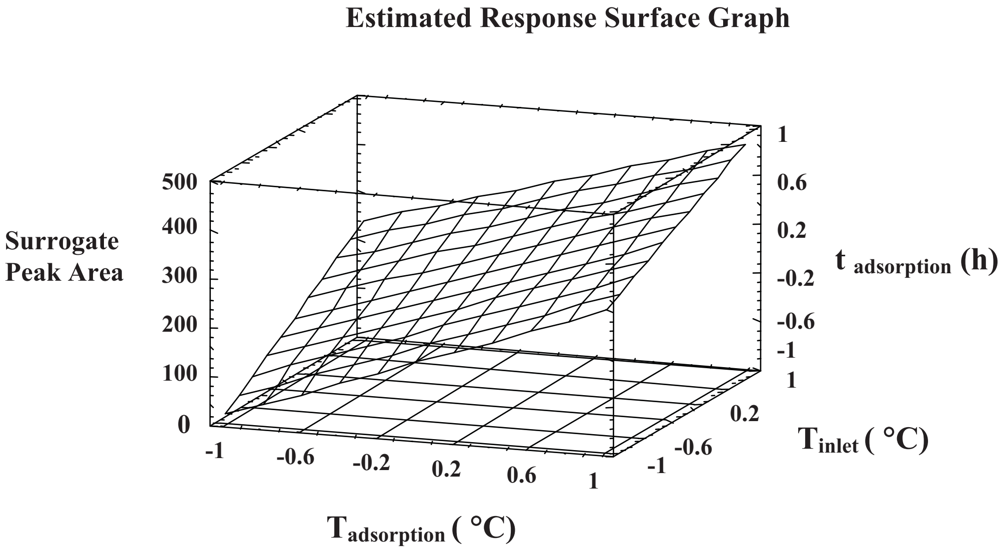


Figure 3.8. Response Surface for HSSPME Sediment Analysis

As can be seen from the estimated response surface graph maximum surrogate peak area (average of the peak areas of 2 surrogates; Nitrobenzene D5, 2-Fluorobiphenyl) was observed when Factor A; $T_{adsorption}$ (°C), B; T_{inlet} (°C) and C; $t_{adsorption}$(h) were at their maximum values (+1) which were 80, 280 and 1 respectively (7th run). The experimental peak area at this best condition is 549. The lowest recovery value was observed when all factors were -1; that is when they were at their minimum values; 3rd run as given in Table 3.9.

As can be seen from the created HSSPME model given below, factor A, B and C have positive contribution on the extraction efficiency. Among the factors, the greatest contribution was belong to $T_{adsorption}$ and smallest contribution was from $t_{adsorption}$. Effect of the interactions was also seen from the model. For example interaction of factor A and B negatively contributed to the extraction efficiency however interaction of factor A and C positively contributed and the amount of their contributions are nearly the same. According to this model, all the factors should be in high level.

Surrogate Peak Area = 257.875 + 124.125 x Factor A + 100.875 x Factor B + 22.375 x Factor C - 13.875 x Factor A x Factor B + 13.625 x Factor A x Factor C.

Two µg surrogate was used for both HSSPME and Direct-SPME. In order to calculate % surrogate recovery of HSSPME, optimum peak area (optimum peak area determined from HSSPME), was divided into the 639.5 (optimum peak area determined from direct-SPME) and multiplied with 100 as is given in the formula below.

% Surrogate Recovery = Found Optimum Peak Area of Added Surrogate (HSSPME) *100/ Found Optimum Peak Area of Added Surrogate (Direct-SPME)

The optimum HSSPME surrogate peak area (average of 1st,Nitrobenzene D5 and 2nd, p-Terphenyl D14) was obtained when optimized values of the factors (A= +1, B= +1 and C= +1) were used in surrogate peak area formula and it was found as 505. The percent surrogate recovery, 79 % was found using above equation (by dividing optimum peak area obtained by HSSPME;505 into optimum peak area determined from direct-SPME; 639,5 and multiplying with 100). The estimated theoretical value at optimum conditions; 505 is consistent with experimental value; 549 (7th run in Table 3.9).

3.2.2. Soxhlet Extraction

The experimental design parameters and their elemental responses were shown on Table 3.10. According to the previous results, DCM/Ac was used as a solvent. In order to obtain the best sensitivity for all the PAHs studied, recoveries of the 3rd surrogates (2-Fluorobiphenyl, 10 mg/L) were used.

Table 3.10. Factorial Design for Soxhlet Analysis of Sediment Samples

Run No	**Time (h) Factor A**	**Solvent (ml) Factor B**	**Sediment (g) Factor C**	**Surrogate Recovery (%)**
1	6.00	500	10.0	9.68
2	6.00	200	10.0	10.9
3	6.00	500	40.0	17.3
4	24.0	200	40.0	42.7
5	24.0	200	10.0	6.45
6	24.0	500	10.0	7.66
7	6.00	200	40.0	11.3
8	**24.0**	**500**	**40.0**	**61.6**

*Bold line is the optimum values of the factors.

First, as a result of the each run, concentrations of the surrogates (which was added as 10 mg/L before the extractions) were found. Then, in order to calculate % recovery, each concentration (found as a result of each run), was divided into the 10 and multiplied with 100 as is given below.

% Recovery = Found Concentration of Surrogate *100/ Added Concentration of Surrogate (10 mg/L)

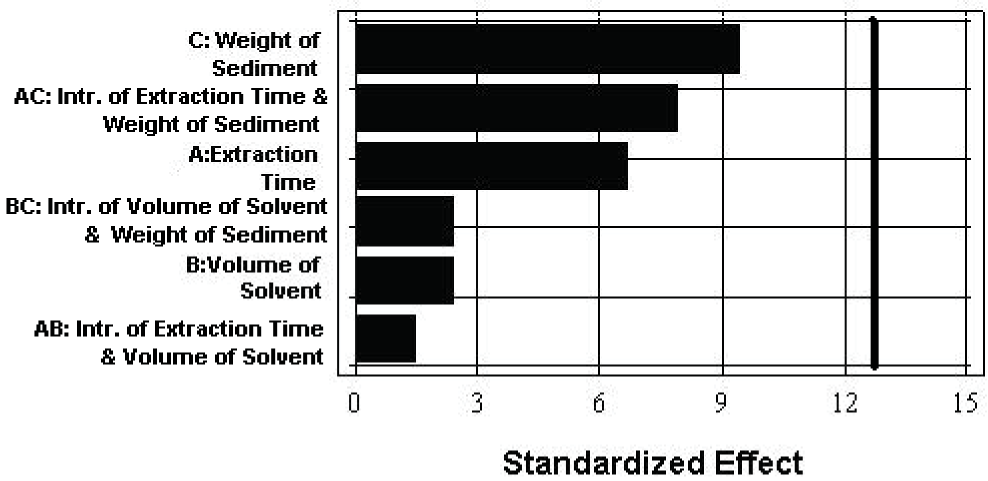


Figure 3.9. Standardized Pareto Chart for Soxhlet Analysis

According to the standardized pareto chart (Figure 3.9), sediment amount was the most important than the others. The least important factor was the solvent amount. The importance of the factors and their interactions were; Sediment amount > Interaction of extraction time and sediment amount > Extraction time > Interaction of solvent volume and sediment amount >Solvent volume > Interaction of extraction time and solvent volume. No one of the factors or interactions was significantly important and they are significantly different from zero at 95 % confidence level.

Response surface analysis results were shown in Figure 3.10. For the surrogate recovery calculations, surrogate 3 (2-Fluorobiphenyl) was used. The maximum surrogate recovery was observed when factor A; Time (min), B; Solvent (ml) and C; Sediment (g) were at their maximum level (+1) which were 24, 500, 40 respectively (8th run). The surrogate recovery at best conditions was 61.6 %. The minimum surrogate recovery was observed when all factors were at their low level (-1) (2nd run).

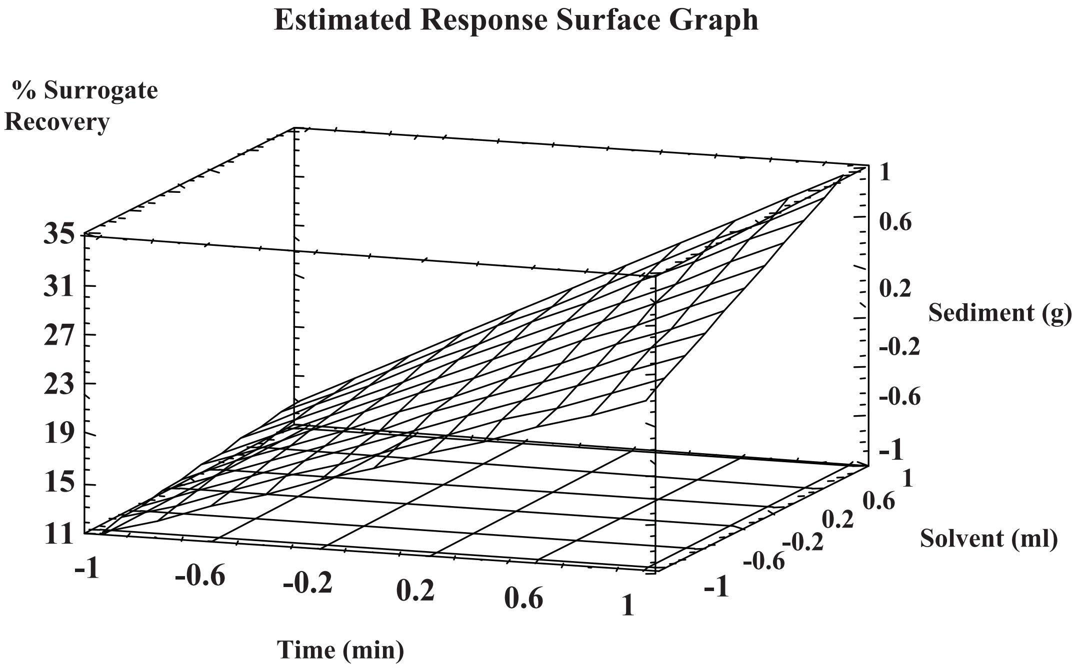


Figure 3.10. Response Surface for Soxhlet Extraction

Soxhlet extraction model was given below. According to the model all the factors and the interactions contributed to surrogate recovery positively and the importance of the factor contributions were Factor C > Factor A > Factor B. Interaction of the A and B was the least important.

Extraction Efficiency = 20.955 + 8.655 x Factor A + 3.1125 x Factor B + 12.285 x Factor C + 1.9025 x Factor A x Factor B + 10.27 x Factor A x Factor C + 3.1125 x Factor B x Factor C.

The surrogate recovery (3rd, 2-Fluorobiphenyl) was obtained as 60 %, when optimized values of the factors (A= +1, B= +1 and C= +1) were used by using the equation above. This estimated value (60) is consistent with the experimental value 61.6 % (8th row in Table 3.10).

3.2.3. Ultrasonic Bath Extraction

According to previous solvent selection analyses results, DCM/Ac was used as a solvent for ultrasonic bath extractions and the same 2^3 factorial design with soxhlet extraction (with values adapted to ultrasonic bath) was applied to experiments (Table 3.11).

Surrogate efficiencies (3rd surrogate; 2-Fluorobiphenyl) were determined as in the Soxhlet extraction, and used for drawing pareto chart. The results of surrogate recoveries were given in Table 3.11.

Table 3.11. Factorial Design for Ultrasonic Bath Analysis of Sediment Samples

Run No	Factor A Time (min)	Factor B Solvent (ml)	Factor C Sediment (g)	% Surrogate Recovery
1	45.0	50.0	1.00	101
2	15.0	50.0	1.00	48.8
3	45.0	20.0	10.0	78.2
4	45.0	50.0	10.0	31.5
5	15.0	50.0	10.0	87.1
6	45.0	20.0	1.00	69.8
7	15.0	20.0	1.00	96.4
8	**15.0**	**20.0**	**10.0**	**62.5**

*Bold line is the optimum values of the factors.

According to pareto analysis in Figure 3.11 sediment and time relationship is more important and extraction time had the less importance among the other factors. There was no significantly important factor or interaction and they are significantly different from zero at 95 % confidence level. The order of the importance of the factors was; Interaction of time and sediment > Sediment amount > Solvent amount > Extraction time > Interaction of time and solvent > Interaction of solvent and sediment.

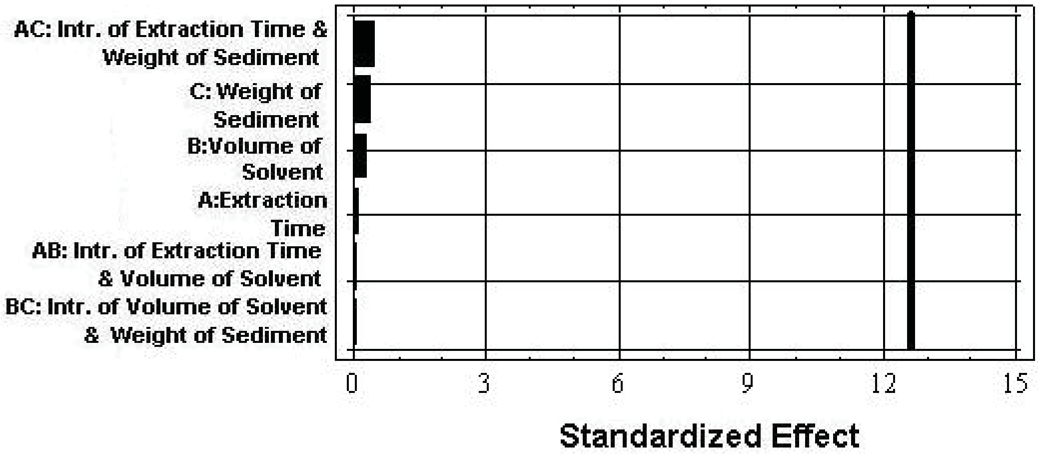


Figure 3.11. Standardized Pareto Chart for Ultrasonic Bath Analysis

Estimated response surface analysis was shown in Figure 3.12. For the surrogate recovery; surrogate 3 (2-Fluorobiphenyl) was used. The maximum was observed when factor A: time (min) and B: solvent (ml) were at their low level (-1) and factor C: sediment amount (g) was at its maximum level (+1): which were 15, 20 and 10 respectively (8th run). The surrogate recovery was 62.5 % at these best conditions. The minimum was observed when factor A and B were at their maximum values (+1) and factor C was in its minimum value (-1) (1st run).

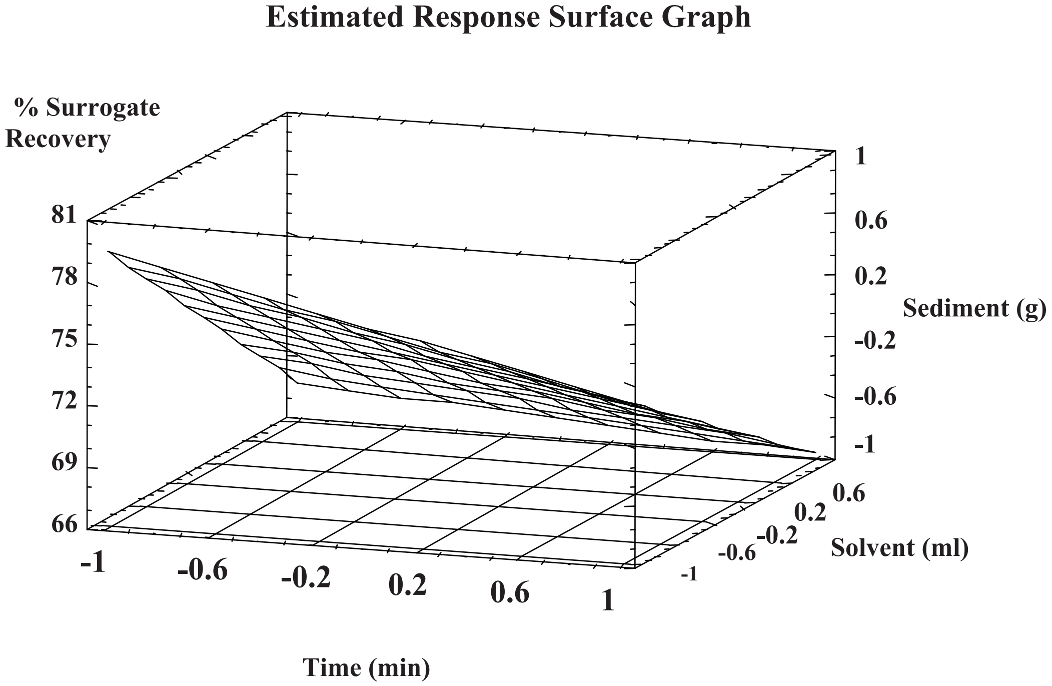


Figure 3.12. Response Surface for Ultrasonic Bath

The model for ultrasonic bath extraction was given below. As can be understood from ultrasonic bath extraction model, all factors contributed negatively and the contribution of the factors were Factor C > Factor B > Factor A. Interaction of A and C was the most important one amongst all.

Extraction Efficiency = 71.9375 - 1.7625 x Factor A - 4.7875 x Factor B - 7.1125 x Factor C + 0.9625 x Factor A x Factor B - 8.2125 x Factor A x Factor C -0.7375 x Factor B x Factor C.

By using the equation above, the surrogate recovery (3rd, 2-Fluorobiphenyl) which will be obtained when optimized values of the factors (A = -1, B = -1 and C = +1) were used, was found as 81 %. The estimated value (81 %) is slightly different from the experimental value 62.5 %.

3.3. Comparison of the Extraction Techniques

Three extraction techniques (ultrasonic bath, soxhlet and HSSPME) for the PAH analysis in sediment samples were optimized and recoveries for the best optimum values of the factors were calculated using the model created from estimated response surface charts. According to the results percent recoveries of the optimized extraction methods were; 81 for ultrasonic bath, 79 for HSSPME and 60 for soxhlet.

According to this result, in terms of extraction efficiency, ultrasonic bath was offered for the extraction of sediment samples for the analysis of the PAHs but HSSPME may also be used since it had nearly the same recovery with ultrasonic bath extraction. In addition it has low solvent consumption and it is less time consuming compared to ultrasonic bath extraction. Moreover, HSSPME did not require any extra cleaning or drying step. Soxhlet had the smallest surrogate recovery however it may also be used with the less polluted samples which require higher amount of sediment samples.

3.4. Application of the Method to Real Samples

The best extraction method (ultrasonic bath) was used for the extraction of sediment samples collected from Ölüdeniz Lagoon and İkizcetepeler Dam Lake. Details were given below.

3.4.1. Ölüdeniz Samples

Since analysis of the Ölüdeniz sediment samples were done before the optimization of the extraction methods, ultrasonic bath method based on EPA 3550 B was used for the extraction of 5 sediment samples. Solvents were also chosen according to the EPA 3550 B method and Hex - DCM (1:1) pair was used as a solvent.

The extraction efficiency was calculated by using surrogate spike containing 2-Fluorobiphenyl in DCM and it was found as 91 % (very good). The procedure for the extraction of sediment was summarized below.

The water content of the wet Ölüdeniz samples (which were sandy in appearance) was determined as in 'water content' determination part of this Chapter. The average moisture content of the samples was ranged between 3-4 % (n=10) Since water content determines the sediment type it could be be said that, Ölüdeniz sediments were dry type sediment since water content is less then 20 % (Adam S. et al., 2006).

Wet sediment sample was used for the ultrasonic extraction (when wet sediment is dried, volatile and semivolatile organic compounds evaporate). Five gram (air dried) sediment was weighted into an extraction vessel. After spiking with surrogate standard, 1ml of 6.5 mg/L 2-Fluorobiphenyl (in DCM), the Ölüdeniz sediment sample was extracted in the 50 ml beaker for about 10 minutes with an 10 ml of DCM/Hex mixture (1:1, v/v). The same procedure was repeated with a mixture of Hex (5 mL) and DCM (5 mL) twice more for a total volume of 30 ml.

After getting the extract it was eluted from sodium sulphate column. Sodium sulphate column was 20 cm in length, plugged with glass wool and pre-washed twice with Hex and DCM.

After dried, extracts were preconcentrated under the stream of Nitrogen (N_2) and volume was reduced to 1 ml. The final solution was kept in a 2 ml amber glass vials tapped with a teflon-lined cap and preserved in a refrigerator at 4 °C until the analysis with GC-FID. The quantification was made against an external standard analytical curve, constructed with solutions prepared from the Supelco PAH standard in DCM: Methanol (1:1).

In order to make a precision comparison between GC-MS and GC-FID, three selected sediments among five extracted sediments, were analyzed with both GC-FID and GC-MS at the same time, with similar conditions and the results were explained below.

3.4.1.1. Comparison of GC-MS and GC-FID Methods

In order to make a precision comparison between GC-MS and GC-FID, three selected sediments among five extracted sediments, were analyzed with both GC-FID and GC-MS at the same time, with the same column and instrumental conditions; including He flow rate, inlet temperature, injection volume, liner and temperature program. By considering accuracy and precision values of the methods (GC-MS and GC-FID), the best detector (MS or FID) was chosen. The comparison of mean concentrations of PAHs detected with both GC-FID and GC-MS was presented in Figure 3.13. According to the Figure, except Ant, BaA, BbF and BkF; GC-FID measurements were higher. However Nap and Acy were observed only in GC-MS measurements.

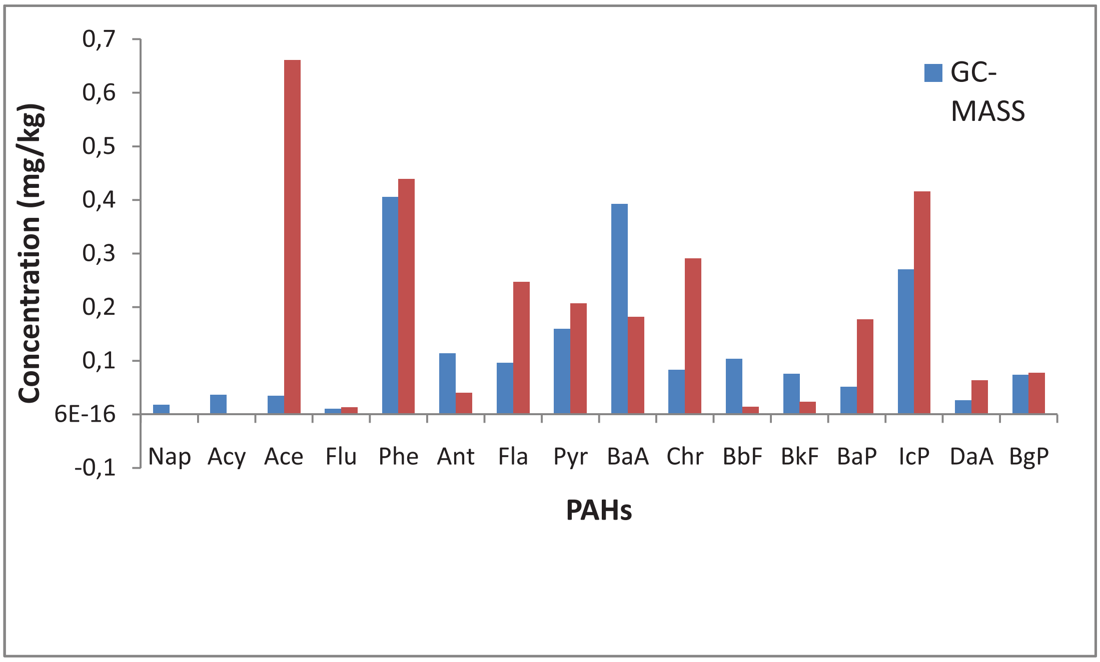


Figure 3.13. Comparison of Mean Concentrations of Selected Sediment Samples (n=3) Analyzed both GC-FID and GC-MS at the Same Time with the Same Conditions

Table 3.12 shows the comparison of mean concentrations and precisions of 3 sediment samples analyzed both GC-MS and GC-FID.

Table 3.12. Comparison of the Precisions of GC-MS and GC-FID (n=3)

PAHs	**GC-MS Mean Conc. (mg/kg)**	**GC-MS Precision Std dev.**	**GC-FID Mean Conc. (mg/kg)**	**GC-FID Precision Std dev.**
Nap	0.0177	0.00410	ND	ND
Acy	0.0370	0.0124	ND	ND
Ace	0.0347	**0.00790**	0.662	0.480
Fle	0.0101	**0.00440**	0.0132	0.00860
Phe	0.406	0.517	0.439	**0.380**
Ant	0.114	0.0730	0.0405	**0.0583**
Fla	0.0960	**0.141**	0.248	0.338
Pyr	0.160	**0.223**	0.207	0.249
BaA	0.393	0.602	0.182	**0.232**
Chr	0.0830	**0.115**	0.291	0.250
BbF	0.104	0.131	0.0144	**0.0147**
BkF	0.0759	0.0898	0.0231	**0.0239**
BaP	0.0516	0.0679	0.178	ND
IcP	0.271	0.341	0.416	ND
DaA	0.0268	**0.0242**	0.0634	0.0559
BgP	0.0740	0.0744	0.0776	**0.0655**

ND: Not Detected

*Bold numbers show that better precision values.

According to the Table 3.12 both methods were good in terms of precision (GC-MS is better for Ace, Fle, Fla, Pyr, Chr, DaA and GC-FID is better for Phe, Ant, BaA, BbF, BkF, BgP). However, quality assurance studies in Chapter 2, showed that accuracy values for GC-FID were better than GC-MS (Table 2.10 and 2.9 respectively). Therefore GC-FID analyses results were used for the Ölüdeniz sediment samples.

3.4.2. İkizcetepeler Samples

İkizcetepeler sediment samples were analyzed after optimization of the extraction methods were completed. According to the extraction efficiencies ultrasonic bath extraction was found as the most suitable method for the analysis of sediment sample. Therefore it was used for the extraction of 44 sediment samples collected from İkizcetepeler dam lake. During solvent selection DCM/Ac pair gave the best result therefore it was used as a solvent pair. Other extraction parameters were assigned by considering surface response area chart prepared for ultrasonic bath extraction.

As can be seen from Table 3.13; for the GC-MS analysis of İkizcetepeler sediment samples, Ace-D10 and Phe-D10 were used for the recovery calculations of 3 rings PAHs, Chr-D12 for 4 rings PAHs and Per-D12 for the 5 and 6 rings PAHs. Average percent surrogate recoveries (n=26) in İkizcetepeler sediment samples were also given in Table. Recovery of surrogates for PAH analysis in İkizcetepeler samples by GC-MS were given in Table 3.13; 41 % for Ace D10, 60 % for Phe D10, 85 % for Chr D12 and 86 % for Per D12. The average % recovery of the four surrogates were 68 % with real samples and 81 % in theory (62.5 % at best experimental conditions of ultrasonic bath extraction; Table 3.11). Therefore it could be said that ultrasonic bath model (calculating % recoveries of PAHs) is working with 84 % efficiency.

Table 3.13. Surrogates (with % Recovery) for the Analysis of PAHs in Sediment Samples with GC-MS and Their Representative PAHs

Surrogates (% Recovery)	**Representative PAHs**
Ace D10 (% 41)	**3 rings PAHs** Nap, Acy, Ace, Fle
Phe D10 (%60)	**3 rings PAHs** Ant, Phe
Chr D12 (% 85)	**4 rings PAHs** Fla, Pyr, Cycpyr, BaA, Chr
Per D12 (% 86)	**5-6 rings PAHs** BbF, BkF, BaP, BeP, IcP, DaA, BgP, Att

The procedure for the extraction of sediment was summarized below.

For determination of water content; firstly two homogenized part of the İkizcetepeler sediment sample (which were mud, clay type in appearance) were taken and weighed nearly around 9 gr. First part was dried in an oven overnight for moisture content determination. Moisture content was calculated with the formula given previously. Moisture content of the wet samples were ranged from 56 % (which shows sediments were saturated type since water content is higher then 40 %) (Adam S. et al., 2006).

For the ultrasonic bath extraction, nine gram wet sediment was weighted into an extraction vessel (50 ml beaker). After spiking with surrogate standard (1ml surrogate mix; Ace D10, Phe D10, Chr D12 and Per D12 in DCM), the sample was extracted in extraction vessel for about 15 minutes with 20 ml DCM-Ac mixture (1:1).

For drying step, the sodium sulphate column that was used also for Ölüdeniz sediments, was used after washing twice with Ac and DCM (1:1). After dried, extracts were preconcentrated under the stream of Ni and volume was reduced to 1 ml and the final solution was kept in a 2 ml amber glass vials tapped with a teflon-lined cap and preserved in a refrigerator at 4 °C until the analysis with GC-MS. The quantification was made against an external standard analytical curve, constructed with solutions prepared from the PAH-Mix 68 in Cyclohexane (100 mg/L, Dr. Ehrenstorfer). The calibration curves were given in Figure 2.19.

3.5. Polycyclic Aromatic Hydrocarbons (PAHs) Observed in Ölüdeniz Sediment Samples

Prior to the each extraction known amount of surrogate standard (6.5 mg/L of 2-Fluorobiphenyl) was added to the each sample and according to the average surrogate recovery value, PAH concentrations in Ölüdeniz sediment samples were corrected. The PAHs in Ölüdeniz sediment samples were analyzed by using GC-FID (with the previously optimized conditions, Table 2.1) and corrected average PAH concentrations of five sediment samples and their standard deviation values (Stdev) were given in Table 3.14. The average total PAH (∑PAH) concentration in Ölüdeniz sediment samples was found as 1.85 ± 1.39 mg/kg (n=5).

The most of the individual PAH concentrations, in this study were above LOD with the exception of Nap and Acy. They were not detected in any sediment samples. For the Nap and Acy, GC-MS (has lower detection limits) was used.

Table 3.14. The Corrected Average Concentration (mg/kg) and Stdev. of PAHs in Ölüdeniz Sediments

PAHs	**Average PAH Conc. ± Stdev (mg/kg)**
Nap	ND
Acy	ND
Ace	0.620 ± 0.346
Fle	0.0120 ± 0.0080
Phe	0.278 ± 0.347
Ant	0.0360 ± 0.0480
Fla	0.299 ± 0.213
Pyr	0.191 ± 0.178
BaA	0.123 ± 0.193
Chr	0.515 ± 0.493
BbF	0.0140 ± 0.0100
BkF	0.0250 ± 0.0220
BaP	0.121 ± 0.080
IcP	0.270 ± 0.207
DaA	0.0630 ± 0.056
BgP	0.0780 ± 0.065
∑PAH (n=5)	1.85 ± 1.39

ND: Not Detected

The same data was also given as bar graph in Figure 3.14. Ace, Chr, Fla, Phe and IcP have the highest concentrations among the 16 PAHs analyzed. It can be said that, sediments were contaminated with the PAHs in moderate level.

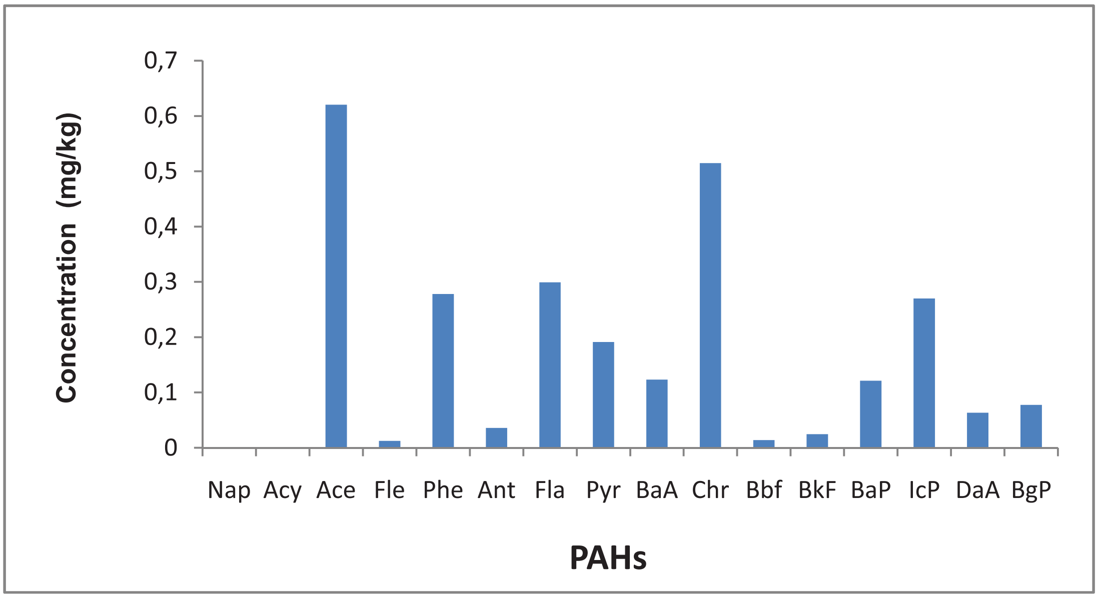


Figure 3.14. Average Concentration (mg/kg) of PAHs in Ölüdeniz Sediment Samples (n=5)

Total PAH concentrations in five Ölüdeniz sediment samples were calculated separately and were given in Figure 3.15. The average total PAH (∑PAH**)** concentration in Ölüdeniz sediment samples was found as 1.85 ± 1.39 mg/kg (n=5) as given in Table. Total PAH concentrations in samples 2 and 4 are above the average value (showed as a line at x axis, 1.85) and the rest are below the average value (Figure 3.15).

Sample 4 had the highest concentration which was close to the mouth of the Lagoon. Samples; 1 and 3 were collected inside lagoon and exhibited similar concentrations. They were collected near to the hotel around the lagoon. Therefore hotel can not be the source of contamination in the lagoon. However motor boats which were close to mouth of the lagoon could be the source as can be understood from the sample 4 concentration. The samples; 2 and 5 were collected from the points close to each other (they were collected just from outside of the Lagoon).

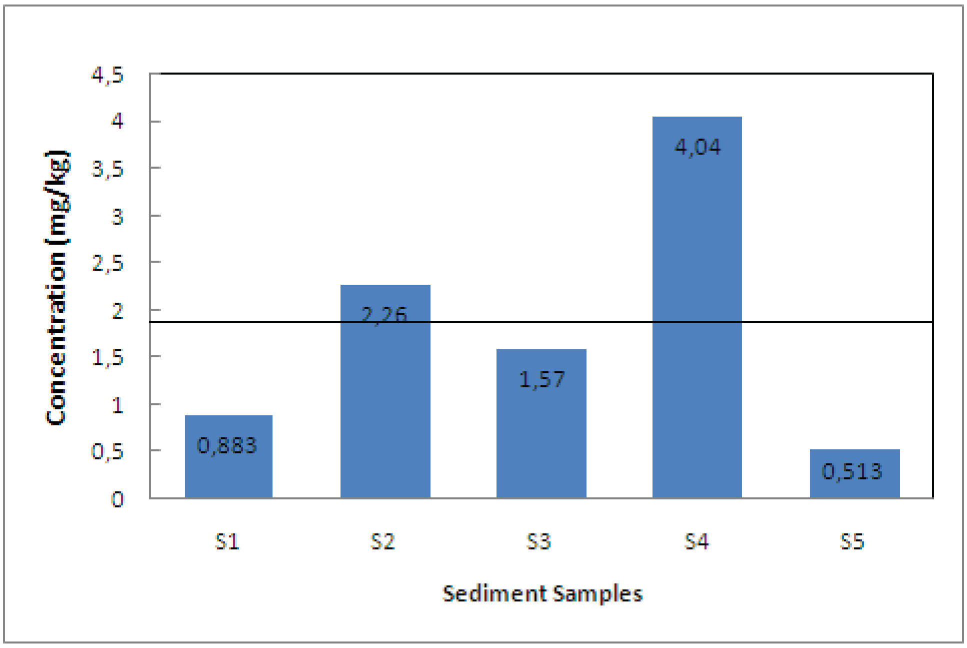


Figure 3.15. ∑PAH Concentrations (mg/kg) in Ölüdeniz Sediment Samples (n=5)

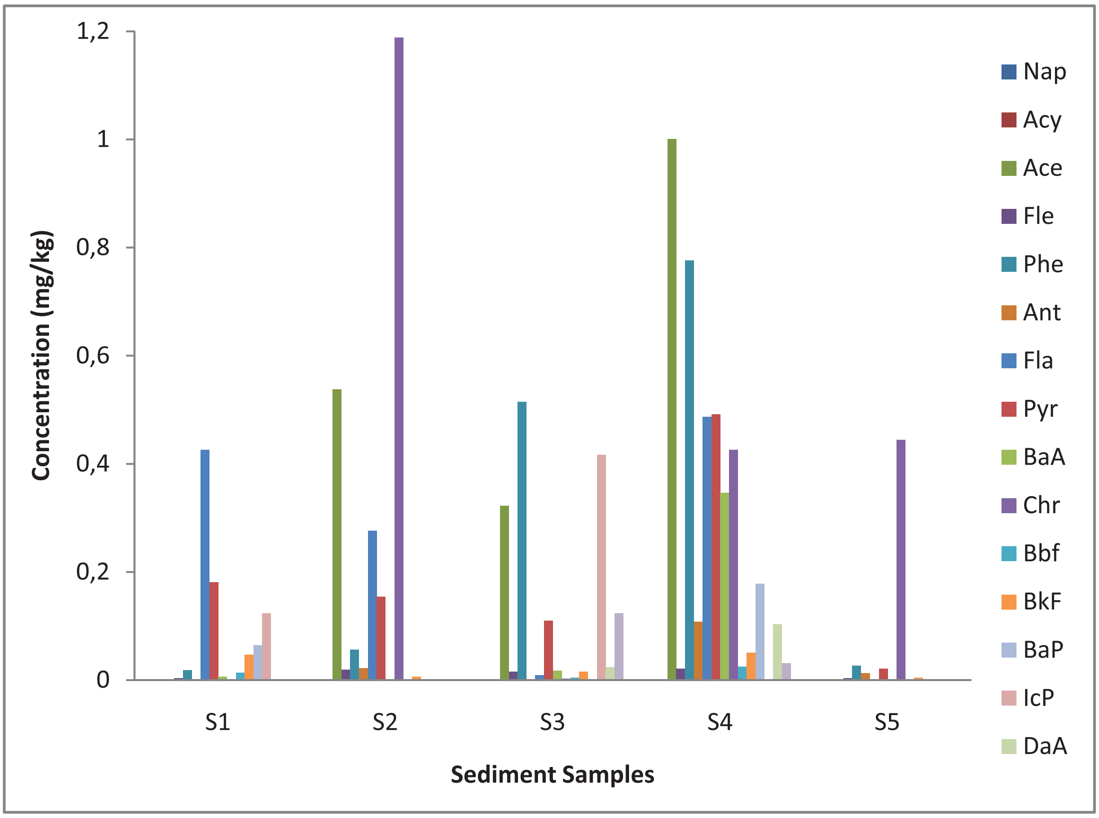


Figure 3.16. Individual PAH Concentrations (mg/kg) in Ölüdeniz Sediment Samples (n=5)

Individual PAH concentrations were drawn (Figure 3.16) and it was found that; in all samples Ace and Phe had the highest concentration and Ant, BbF had the lowest concentration. This result could be an indication of common source. And this source may be the motor boats.

3.6. Polycyclic Aromatic Hydrocarbons (PAHs) Observed in İkizcetepeler Sediment Samples

Prior to the each extraction known amount of surrogate standard (1 mg/L of surrogate mix; Ace D10, Phe D10, Chr D12, Per D12) was added to the İkizcetepeler sediment samples (n=26) and according to the average surrogate recovery 19 PAH concentrations in İkizcetepeler sediment samples were corrected.

The PAHs in İkizcetepeler sediment samples were analyzed by using GC-MS (with the previously optimized conditions, Table 2.2) and corrected average PAH concentrations of 44 sediment samples and their standard deviation values (Stdev) were given in Table 3.15. The average total PAH (∑PAH) concentration in İkizcetepeler Dam Lake sediment samples was found as 0.19 ± 0.07 mg/kg (n=5).

As can be seen from both Table 3.15 and Figure 3.17, the highest concentrations were observed for Nap and BeP and it was 0.020 mg/kg. Cycypyr has the lowest concentration which was 0.004 mg/kg. General concentration profile was as following; Nap=BeP > Phe=Ant > BgP=Att > IcP > Fla > Chr=BbF > BkF=BaP=Fle=Pyr > Ace > Acy > DaA=BaA > Cycpyr.

Table 3.15. The Corrected Average Concentration (mg/kg) and Stdev of PAHs in İkizcetepeler Dam Lake Sediments

PAHs	Average PAH Conc. ± Stdev (mg/kg)
Nap	0.020 ± 0.011
Acy	0.0060 ± 0.0010
Ace	0.0070 ± 0.0020
Fle	0.0080 ± 0.0030
Phe	0.018 ± 0.011
Ant	0.018 ± 0.011
Fla	0.010 ± 0.003
Pyr	0.0080 ± 0.0030
Cycpyr	0.0040 ± 0.0020
BaA	0.0050 ± 0.0020
Chr	0.0090 ± 0.0040
BbF	0.0090 ± 0.0030
BkF	0.0080 ± 0.0030
BaP	0.0080 ± 0.0030
BeP	0.020 ± 0.023
IcP	0.012 ± 0.005
DaA	0.0050 ± 0.0050
BgP	0.017 ± 0.007
Att	0.017 ± 0.012
∑PAH	**0.19 ± 0.07**

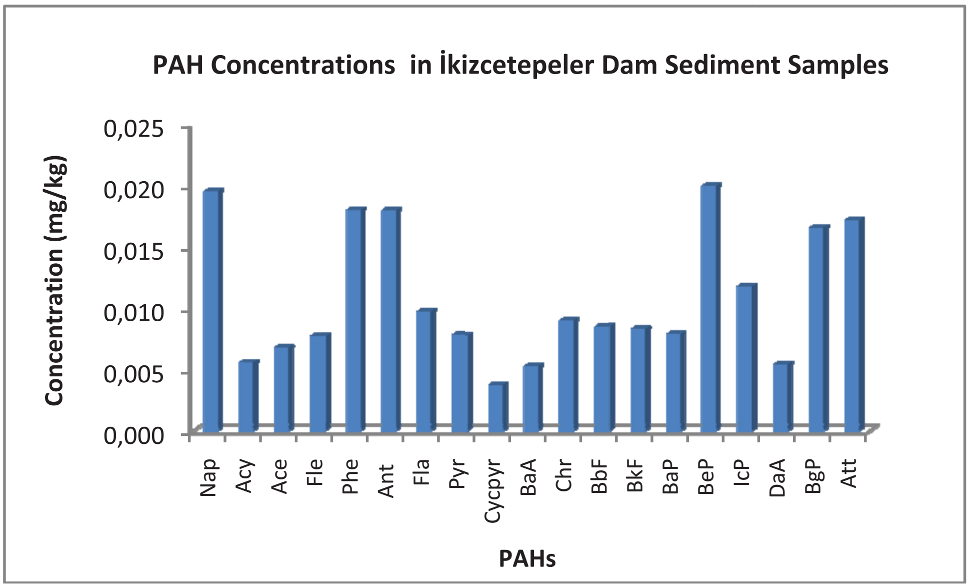
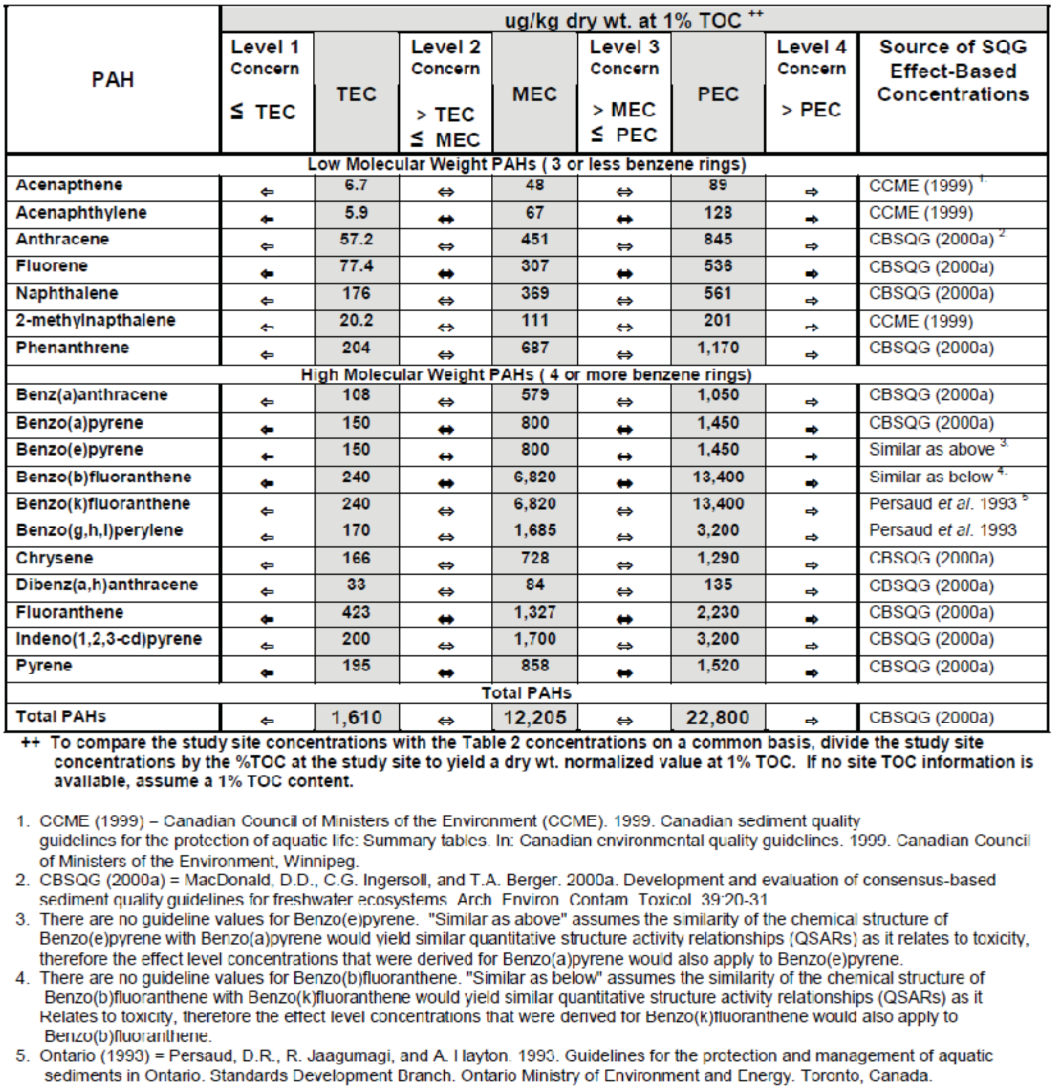


Figure 3.17. PAH Concentrations (mg/kg) in İkizcetepeler Dam Sediment Samples

3.7. Sediment Quality Determinations for Ölüdeniz and İkizcetepeler Sediments

In order to determine the quality of the sediments collected from Ölüdeniz and İkizcetepeler, Table 3.16 explaining the recommended sediment quality values for 18 PAHs was used. There are four levels for the PAH pollution on Table 3.16. The individual and total PAH concentrations were considered in Table in order to decide the level of pollution in sediment samples.

Table 3.16. Recommended Sediment Quality Guideline Values for PAHs and Associated Levels of Concern to be Used in Doing Assessments of Sediment Quality



PAH	ug/kg dry wt. at 1% TOC ++							
	Level 1 Concern ≤ TEC	TEC	Level 2 Concern > TEC ≤ MEC	MEC	Level 3 Concern > MEC ≤ PEC	PEC	Level 4 Concern > PEC	Source of SQG Effect-Based Concentrations
Low Molecular Weight PAHs (3 or less benzene rings)								
Acenapthene	⇦	6.7	⇔	48	⇔	89	⇨	CCME (1999) [1]
Acenaphthylene	←	5.9	↔	67	↔	128	→	CCME (1999)
Anthracene	⇦	57.2	⇔	451	⇔	845	⇨	CBSQG (2000a) [2]
Fluorene	←	77.4	↔	307	↔	536	→	CBSQG (2000a)
Naphthalene	⇦	176	⇔	369	⇔	561	⇨	CBSQG (2000a)
2-methylnapthalene	⇦	20.2	⇔	111	⇔	201	⇨	CCME (1999)
Phenanthrene	⇦	204	⇔	687	⇔	1,170	⇨	CBSQG (2000a)
High Molecular Weight PAHs (4 or more benzene rings)								
Benz(a)anthracene	⇦	108	⇔	579	⇔	1,050	⇨	CBSQG (2000a)
Benzo(a)pyrene	←	150	↔	800	↔	1,450	→	CBSQG (2000a)
Benzo(e)pyrene	←	150	↔	800	↔	1,450	→	Similar as above [3]
Benzo(b)fluoranthene	←	240	↔	6,820	↔	13,400	→	Similar as below [4]
Benzo(k)fluoranthene	⇦	240	⇔	6,820	⇔	13,400	⇨	Persaud *et al.* 1993 [5]
Benzo(g,h,I)perylene	⇦	170	⇔	1,685	⇔	3,200	⇨	Persaud *et al.* 1993
Chrysene	⇦	166	⇔	728	⇔	1,290	⇨	CBSQG (2000a)
Dibenz(a,h)anthracene	⇦	33	⇔	84	⇔	135	⇨	CBSQG (2000a)
Fluoranthene	←	423	↔	1,327	↔	2,230	→	CBSQG (2000a)
Indeno(1,2,3-cd)pyrene	⇦	200	⇔	1,700	⇔	3,200	⇨	CBSQG (2000a)
Pyrene	←	195	↔	858	↔	1,520	→	CBSQG (2000a)
Total PAHs								
Total PAHs	⇦	1,610	⇔	12,205	⇔	22,800	⇨	CBSQG (2000a)

++ To compare the study site concentrations with the Table 2 concentrations on a common basis, divide the study site concentrations by the %TOC at the study site to yield a dry wt. normalized value at 1% TOC. If no site TOC information is available, assume a 1% TOC content.

1. CCME (1999) – Canadian Council of Ministers of the Environment (CCME). 1999. Canadian sediment quality guidelines for the protection of aquatic life: Summary tables. In: Canadian environmental quality guidelines. 1999. Canadian Council of Ministers of the Environment, Winnipeg.
2. CBSQG (2000a) = MacDonald, D.D., C.G. Ingersoll, and T.A. Berger. 2000a. Development and evaluation of consensus-based sediment quality guidelines for freshwater ecosystems. Arch. Environ. Contam. Toxicol. 39:20-31
3. There are no guideline values for Benzo(e)pyrene. "Similar as above" assumes the similarity of the chemical structure of Benzo(e)pyrene with Benzo(a)pyrene would yield similar quantitative structure activity relationships (QSARs) as it relates to toxicity, therefore the effect level concentrations that were derived for Benzo(a)pyrene would also apply to Benzo(e)pyrene.
4. There are no guideline values for Benzo(b)fluoranthene. "Similar as below" assumes the similarity of the chemical structure of Benzo(b)fluoranthene with Benzo(k)fluoranthene would yield similar quantitative structure activity relationships (QSARs) as it Relates to toxicity, therefore the effect level concentrations that were derived for Benzo(k)fluoranthene would also apply to Benzo(b)fluoranthene.
5. Ontario (1993) = Persaud, D.R., R. Jaagumagi, and A. Hayton. 1993. Guidelines for the protection and management of aquatic sediments in Ontario. Standards Development Branch. Ontario Ministry of Environment and Energy. Toronto, Canada.

(http://dnr.wi.gov/org/aw/rr/technical/cbsqg-interim-final.pdf)

According to the consensus-based sediment quality guidelines (CBSQGs) three classes (explaining toxicity level) exist; TEC, MEC and PEC. The TEC (threshold effect concentration); lower concentration at which toxicity to benthic-dwelling organisms are predicted to be unlikely. The PEC (probable effect concentration); upper concentration at which toxicity to benthic-dwelling organisms are predicted to be probable. The MEC (midpoint effect concentration); is a concentration midway between the TEC and PEC concentrations (TEC + PEC / 2 = MEC). There are four levels which are; Level1 (≤ TEC), Level 2 (TEC < Level 2 ≤ MEC) , Level 3 (MEC< Level 3 ≤ PEC) and Level 4 (> PEC).

To compare the study site concentrations with the Table concentrations on a common basis, study site concentrations should be divided by the study site % total organic carbon (TOC) concentrations to yield a dry wt. normalized value. When no site TOC information is available % TOC is assumed as 1. In this study no site TOC information was available therefore study site PAH concentrations were divided by 1 % TOC.

All PAH concentrations in Ölüdeniz sediment samples are in Level1, except Ace (Level 2) and Acy (Level 2).

3.8. Comparison of PAH Concentrations in Ölüdeniz Lagoon and İkizcetepeler Dam Lake

In order to observe the difference between Ölüdeniz Lagoon and İkizcetepeler Dam, average PAH concentrations were given in Table 3.17. The comparison was made by taking the ratio (**$C_{Ölüdeniz}$ / $C_{İkizcetepeler}$**) of the concentrations for each analyte.

Table 3.17. PAH Concentrations (mg/kg) and Concentration Ratios of Ölüdeniz Lagoon to İkizcetepeler Dam Lake

PAHs	Ölüdeniz Lagoon Conc. (mg/kg)	İkizcetepeler Dam Conc. (mg/kg)	$C_{Ölüdeniz}$ / $C_{İkizcetepeler}$
Ace	062 ± 0.35	0.0070 ± 0.0020	91
Fle	0012 ± 0.008	0.0080 ± 0.0030	2
Phe	0.28 ± 0.35	0.018 ± 0.011	15
Ant	0.036 ± 0.048	0.018 ± 0.011	2
Fla	0.30 ± 0.21	0.010 ± 0.003	31
Pyr	0.19 ± 0.18	0.0080 ± 0.0030	24
BaA	0.12 ± 0.19	0.0050 ± 0.0020	23
Chr	0.52 ± 0.49	0.0090 ± 0.0040	57
Bbf	0.014 ± 0.010	0.0090 ± 0.0030	2
BkF	0.025 ± 0.022	0.0080 ± 0.0030	3
BaP	0.12 ± 0.08	0.0080 ± 0.0030	15
IcP	0.27 ± 0.21	0.012 ± 0.005	23
DaA	0.063 ± 0.056	0.0050 ± 0.0050	12
BgP	0.078 ± 0.065	0.017 ± 0.007	5

By using these ratios another Figure was prepared in order to see the difference in concentrations. As can be seen from Figure 3.18, nearly all ratios are much higher than 1. Especially Ace and Chr concentrations of Ölüdeniz lagoon are much higher than those of İkizcetepeler Dam. However concentrations of Fle, Ant, BbF, BkF and BgP are closer to each other.

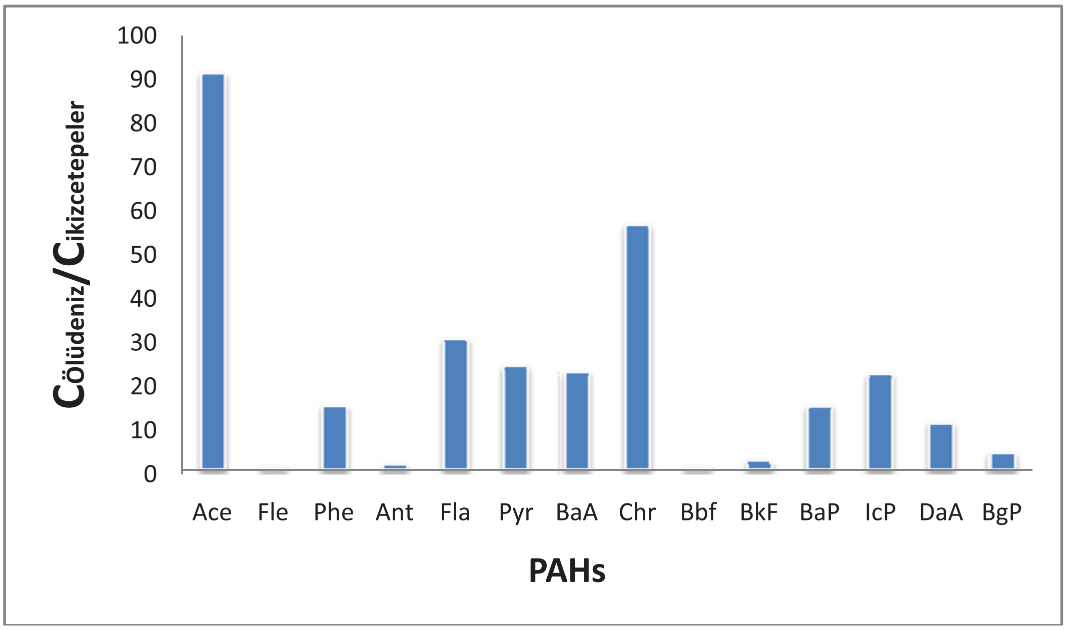


Figure 3.18. Concentration Ratios of Ölüdeniz and İkizcetepeler Sediments

Total PAH concentrations in 44 İkizcetepeler sediment samples were calculated separately and were given in Figure 3.19. The average total PAH (∑PAH**)** concentration in Ölüdeniz sediment samples was found as 0.19 ± 0.07 mg/kg (n=44) as given in Table. Total PAH concentrations in samples; 1, 2, 4, 6, 9, 12, 13, 15, 18, 23, 24, 37, 38, 39, 40, 42 are above the average value (showed as a line at x axis, 0.19) and the rest are equal to or below the average value (Figure 3.19).

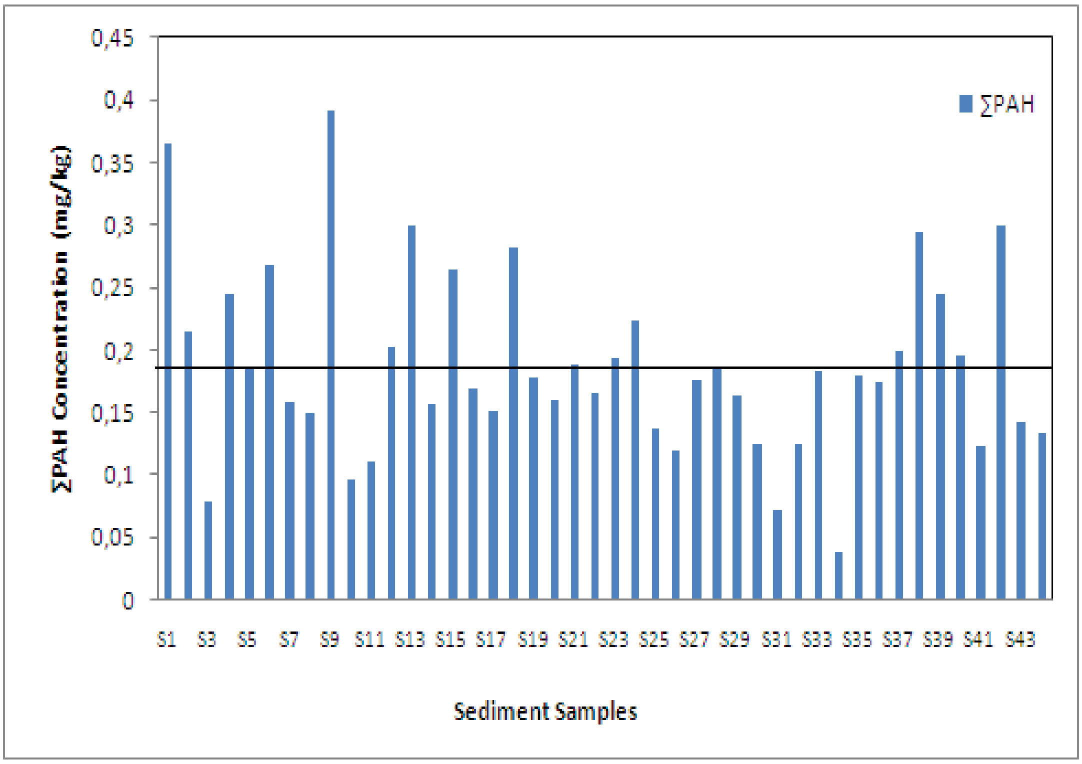


Figure 3.19. ∑PAH Concentrations in İkizcetepeler Sediment Samples (n=44)

Another comparison was made between the average concentrations of Lagoon and Dam Lake in Figure 3.20. This Figure was necessary to observe any existing similarity or difference in the fluctuation of PAH concentrations in sediment samples. Any similarity in fluctuations refers to having same kind of PAH source and any difference refers to they may differ in PAH source. The Figure 3.20 showed that Fle, Phe, Pyr, BaA, Chr, BbF, BkF, BaP, IcP and DaA are showing the same variation therefore this analytes may have the same sources in both areas.

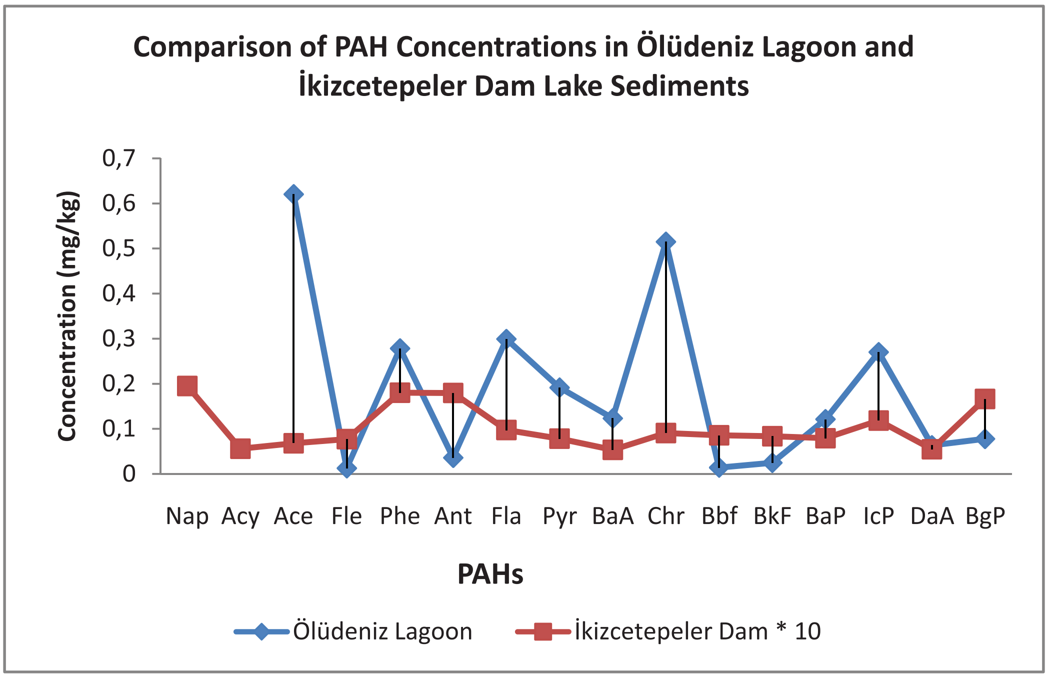


Figure 3.20. Comparison of PAH Concentrations in Ölüdeniz Lagoon and İkizcetepeler Dam

3.9. Determination of PAH Sources for the İkizcetepeler Lagoon

3.9.1. Source Estimates from Cluster Analysis

Cluster analysis was performed in order to classify İkizcetepeler sediment samples. The result of cluster analysis was shown in the hierarchical dendogram in Figure 3.21.

```
      H I E R A R C H I C A L   C L U S T E R   A N A L Y S I S

 Dendrogram using Average Linkage (Between Groups)

                      Rescaled Distance Cluster Combine

   C A S E    0         5        10        15        20        25
  Label  Num  +---------+---------+---------+---------+---------+

          40   -+-+
          42   -+ |
          29   ---+-+
           4   ---+ +-----------+
          26   ---+ |           |
           2   -+---+           +-----+
          43   -+               |     |
           8   -------+---+     |     +-+
          39   -------+   +-----+     | |
           3   -----------+           | |
          30   -----------------------+ +-------------------+
           5   -+-+                     |                   |
          11   -+ +-----------+         |                   |
           7   ---+           +---------+                   | 2
          16   -------+-------+                             +---+
          17   -------+                                     |   |
          31   -----------------+-----------------------+   |   |
          34   -----------------+                       |   |   |
          18   -+---------+                             |   |   |
          41   -+         +-------+                     +---+   |
          28   -----------+       +---------+           |       |
          27   -------------------+         |           |       |
          13   -----------+---------+       +-----------+       |
          15   -----------+         |       |                   |
          22   ---+---+             +-------+                   |
          23   ---+   +---------+   |                           |
          32   -------+         |   |                           |
          33   -------------+-+ +---+                           |
          37   -------------+ | |                               |
           6   ---+-+         | |                               |
          20   ---+ +---+     +-+                               |
          21   -----+   |     |                                 |
          19   ---+-+   +---+ |                                 |
          38   ---+ |   |   | |                                 |
           9   -----+-+ |   +-+                                 |
          24   -----+ +-+   |                                   |
           1   -------+     |                                   |
          12   -------------+                                   |
          35   -------+---------+                               |
          44   -------+         +-------+                       |
          14   -----------------+       +---+                   |
          10   -------------------------+   +-------+         1 |
          36   -----------------------------+       +-----------+
          25   -------------------------------------+
```

Figure 3.21. Hierarchical Cluster Analysis for İkizcetepeler Sediment Samples

According to hierarchical cluster analysis sediment samples were divided into two cluster. The first cluster includes the samples; 35, 44, 14, 10, 36, 25. The samples in this cluster are lower ∑PAH value than the average ∑PAH (0.19 mg/kg). The samples in this cluster are also poor in low molecular weight PAH and rich in 5-6 rings PAH, therefore they may be mainly traffic sourced (pyrogenic source).

The second cluster includes the samples; 40, 42, 29, 4, 26, 2, 43, 8, 39, 3, 30, 5, 2, 11, 7, 16, 17, 31, 34, 18, 41, 28, 27, 13, 15, 22, 23, 32, 33, 37, 6, 20, 21, 19, 38, 9, 24, 1, 12. The samples in second cluster are equal or higher than the average ∑PAH (0.19 mg/kg) value, in addition they are rich in both 3-4 and 5-6 rings PAHs which may indicate that both traffic and coal combustion (pyrogenic source) may be the common source of PAH pollution. Some samples; 38, 37, 28, 24, 23, 21, 18, 16, 15, 13, 19, 5, 2 have high Nap content therefore they may be petrogenic sourced samples.

3.9.2. Diagnostic Ratios of PAHs

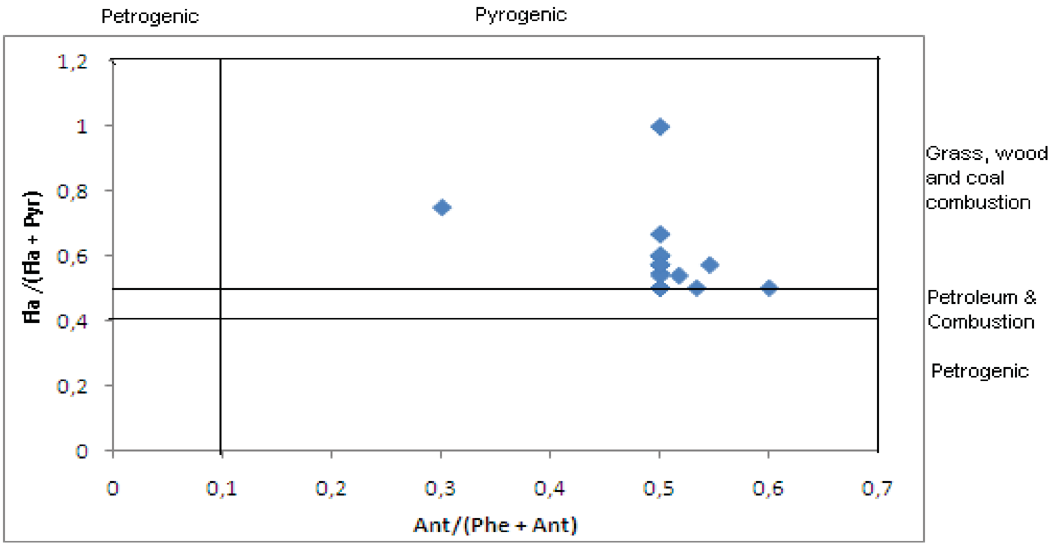


Figure 3.22. PAH Cross Plots for the Ratios of Ant/(Ant + Phe) vs. Fla/ (Fla + Pyr)

According to the Figure 3.22, PAHs in sediments with the ratio of Ant / (Ant + Phe) > 0.1 were typical of combustion sources (pyrogenic source). In addition, PAHs in sediments with the ratio of Fla/(Fla + Pyr) > 0.5 indicates PAHs are mainly from combustion of grass, wood and coal and 0.4 < Fla/(Fla + Pyr) < 0.5 from combustion of petroleum.

With the data at hand, it can be concluded that pyrogenic origins (mostly grass, wood and coal combustion + slightly petroleum combustion) were the dominant sources in İkizcetepeler Dam.

While diagnostic ratios of PAHs is widely applied to identify sources in various environments, their use is limited due to a lack of reliability. More sophisticated statistical approaches are needed. Factor analysis can provide information on source contributions and performed to identify and apportion PAH sources in air, soil and sediment.

3.9.3. Factor Analysis (FA) for İkizcetepeler Samples

The purpose of FA is to represent the total variability of the original PAH data with a minimum number of factors. By critically evaulating the factor loadings, as estimate of the chemical source responsible for each factor can be made (Liu et al., 2008). The rotated factors of 18 normalized PAHs (by assuming % TOC content in İkizcetepeler sediment is 1) from the İkizcetepeler sediments were presented in Table 3.18.

The communalities found are close to 1 as can be seen from the Table. Table 3.18 also shows the number of factors having eigen value greater than 1, their % variance and cumulative % values. There are 5 factors whose eigen values are greater than 1. The rest of the factors have eigen values less than 1. According to Table 3.18 the five factors account for 76 % of the variability in the data. Factor 1 explains 35 % of the total variance, is dominated by Fla, Pyr, Chr, BbF, BkF, BaP, IcP (HPAHs; 4-6 rings). Factor 2 contributing 17 % of total variance, and it is highly weighted by

Nap, Phe and Ant (LPAHs; 2-3 ring). Factor 3 explains 8.6 % of total variance and it is dominated by Ace and BaA. Factor 4 explains 7.9 % of total variance and Factor 5 explains 7.2 % of total variance and dominated by Cycpyr.

Table 3.18. Rotated Component Matrix of 18 PAHs from the İkizcetepeler Sediment

Factor Analysis Results							
No	**PAH**	**1**	**2**	**3**	**4**	**5**	**Communalities**
1	Nap	0.12	**0.73**	0.050	-0.14	0.16	0.59
2	Ace	0.06	0.25	**0.76**	0.19	0.070	0.68
3	Fle	0.24	0.080	0.27	0.45	-0.45	0.54
4	Phe	0.32	**0.85**	0.19	0.030	-0.040	0.86
5	Ant	0.33	**0.85**	0.16	0.040	-0.16	0.88
6	Fla	**0.83**	0.33	0.21	0.020	0.020	0.84
7	Pyr	**0.83**	0.19	0.020	-0.06	-0.30	0.82
8	Cycpyr	0.16	0.09	0.010	0.040	**0.86**	0.77
9	BaA	0.23	0.030	**0.71**	-0.13	-0.14	0.59
10	Chr	**0.83**	0.18	0.14	0.16	0.06	0.78
11	BbF	**0.93**	0.22	0.030	0.14	0.10	0.94
12	BkF	**0.88**	0.23	0.080	0.06	0.16	0.86
13	BaP	**0.83**	0.080	0.32	-0.15	0.040	0.82
14	BeP	0.040	-0.30	0.37	0.44	0.31	0.51
15	IcP	**0.88**	0.16	0.070	-0.11	0.010	0.82
16	DaA	0.50	0.17	0.060	-0.63	0.13	0.70
17	BgP	0.63	0.53	-0.15	0.33	0.090	0.82
18	Att	0.34	0.53	-0.060	0.61	0.13	0.79
Estimated Source		Pyrogenic	Petrogenic	Unknown 1	Unknown 2	Unknown 3	
Variance (%)		35	17	8.6	7.9	7.2	
Total Variance		35	52	61	69	76	
Rotation Method: Varimax with Kaiser Normalization **Bold Loadings > 0.70**							

Factor 1, corresponding to the first group, represent a pyrogenic source; Factor 2, corresponding to the second group, represents petrogenic source PAHs. Factor 3,4 and 5 corresponding 3rd,4th and 5th group respectively, represent an unknown source.

The results indicate that pyrogenic (35 %) and petrogenic (17 %) sources are the important sources of PAHs, but can not differentiate the two subsets of pyrogenic sources even if the number of factors is five. Since pyrogenic sources are the main PAH source in İkizcetepeler sediments, the unknown sources can be ignored. Therefore Ace, Cycpyr and BaA were removed from the data matrix and FA was performed again in order to further investigate the pyrogenic sources of PAHs.

Table 3.19. Rotated Component Matrix of 15 PAHs from the İkizcetepeler Sediment

		Factor	**Analysis**	**Results**	
No	**PAH**	**1**	**2**	**3**	**Communalities**
1	Nap	0.132	**0.700**	-0.183	0.542
2	Fle	0.183	0.169	0.505	0.317
3	Phe	0.309	**0.860**	-0.012	0.835
4	Ant	0.311	**0.863**	-0.007	0.841
5	Fla	**0.829**	0.374	0.107	0.838
6	Pyr	**0.800**	0.246	0.031	0.702
7	Chr	**0.798**	0.256	0.262	0.771
8	BbF	**0.883**	0.292	0.231	0.919
9	BkF	**0.855**	0.289	0.157	0.839
10	BaP	**0.882**	0.097	-0.015	0.787
11	BeP	0.061	-0.254	0.571	0.394
12	IcP	**0.887**	0.192	-0.010	0.824
13	DaA	0.602	0.105	-0.565	0.692
14	BgP	0.522	0.615	0.282	0.729
15	Att	0.206	0.637	0.530	0.729
Estimated Source		Pyrogenic	Petrogenic	Unknown 1	
Variance (%)		39.8	22.3	9.69	
Total Variance		39.8	62.0	71.7	
Rotation Method: Varimax with Kaiser Normalization **Bold Loadings > 0.70**					

The rotated factors of the 15 PAHs without Ace, Cycpyr and BaA were shown in Table 3.19. There were three factors accounting for 71.7 of the variability in the data. The results are very similar to the previous FA analysis results (Table 3.18). Among three factors; Factor 1 is responsible for 39.8 of the total variance and heavily weighted in Fla, Pyr, Chr, BbF, BkF, BaP, IcP (HPAHs; 4-6 rings) as in the case of previous FA. Factor 2 contributing 22.3 % of total variance, and it is highly weighted by Nap, Phe and Ant (LPAHs; 2-3 rings) like in previous FA. Factor 3 explains 9.69 of total variance.

Pyrogenic sources. such as the combustion-derived particles present in urban atmospheric dust, were depleted in low molecular weight 2-3 rings PAHs (LPAHs) and enriched in high molecular weight 4-6 rings PAHs (HPAHs). Petrogenic sources, such as fuel oil or light refined petroleum products, were dominated by LPAH (Liu et al.. 2008).

Therefore, Factor 1, corresponding to the first group, represent a pyrogenic source (coal combustion;4 ring PAH and traffic related pollution;5-6 rings PAHs); Factor 2, corresponding to the second group, represents petrogenic source PAHs. Factor 3, corresponding 3rd group respectively, represent an unknown source.

In brief, coal combustion, traffic related pollution and petroleum spillage are the major sources in İkizcetepeler Dam Lake.

3.10. Pollution Maps

Geographical information systems (GIS) software. Mapinfo 7.5 was used for the construction of pollution maps of PAHs in Ölüdeniz Lagoon and İkizcetepeler Dam Lake. Distribution maps of PAHs were drawn using “triangulation with smothing” interpolation approach. According to this approach a triangular mesh using each data point for the triangle vertices is created. Cell values are then calculated based on the three data points that make up the triangle in which the cell falls. Therefore unknown values are predicted with the help of known values at neighbouring points.

3.10.1. Ölüdeniz Sediment Samples

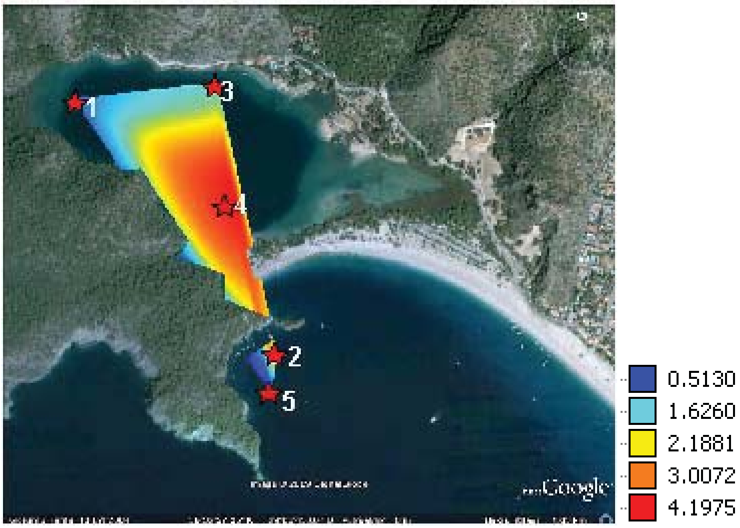
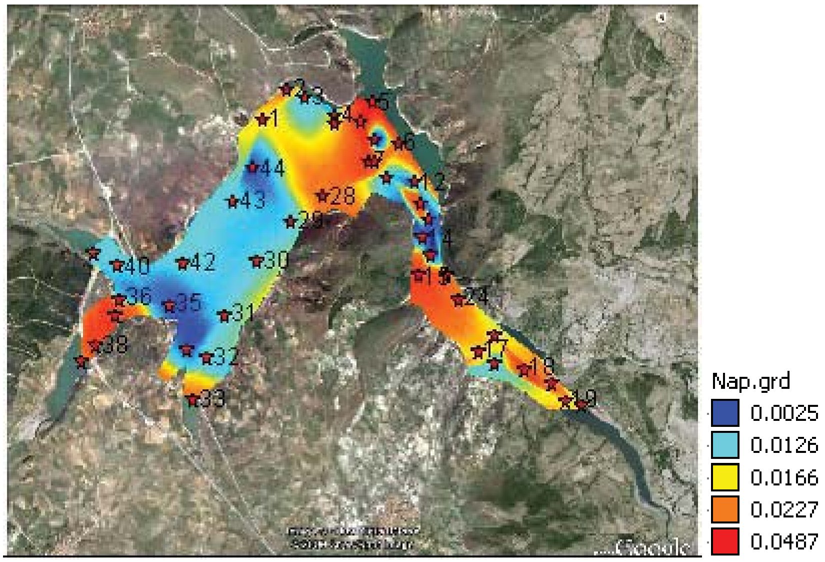
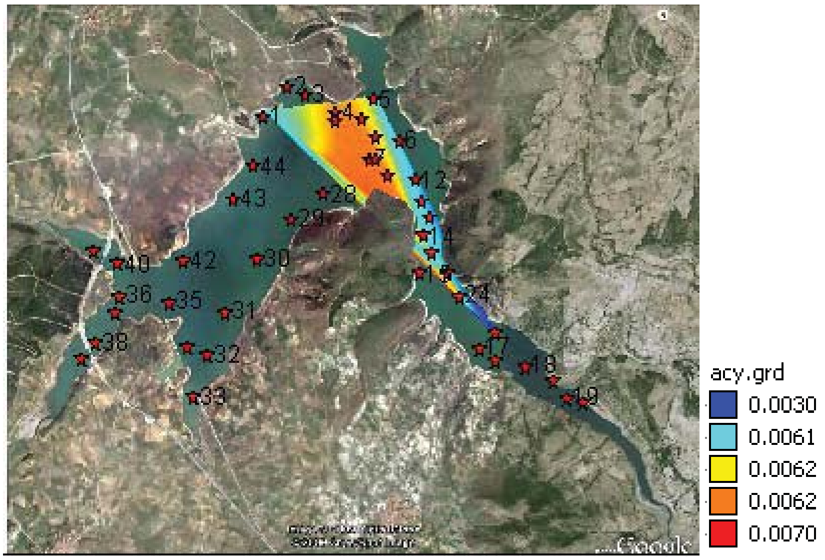


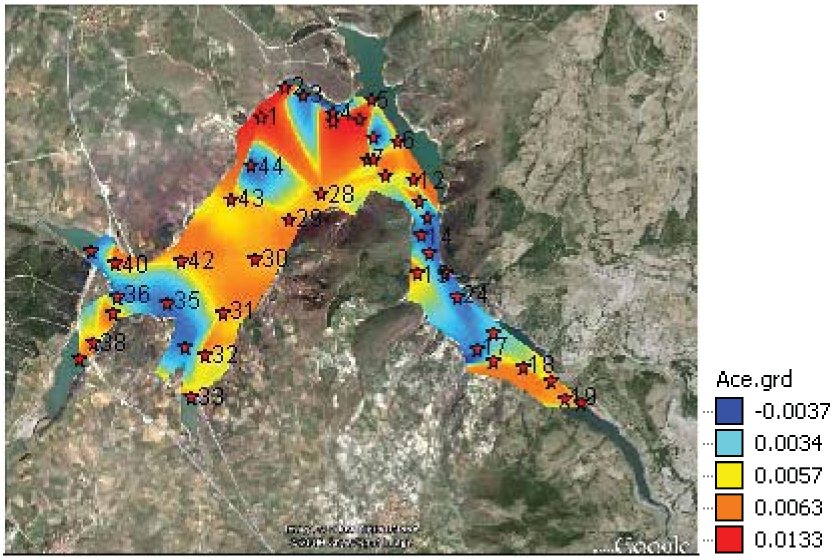
Figure 3.23. Pollution Maps of Sum PAH (n=16) Concentrations (mg/kg) in Ölüdeniz Lagoon

From the Figure 3.23, it can be understood that, PAHs are heavily concentrated in the middle of the lagoon rather than the coastal lines.

3.10.2. İkizcetepeler Sediment Samples



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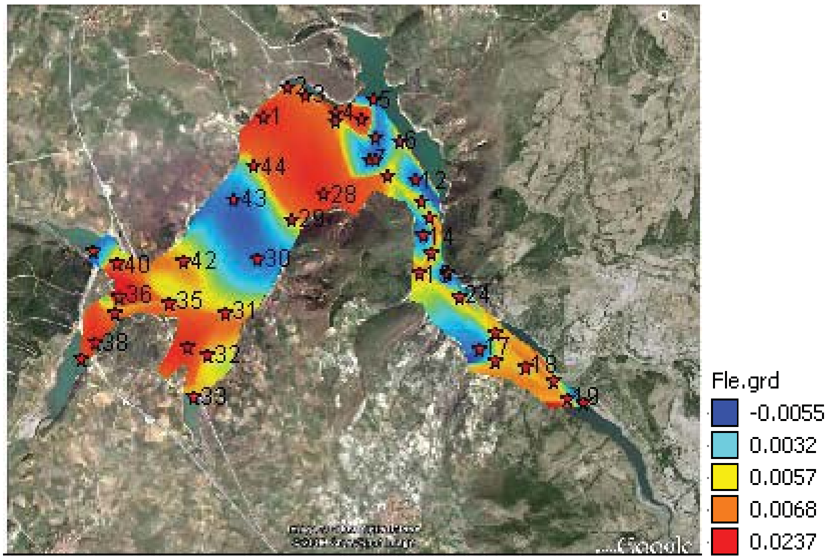
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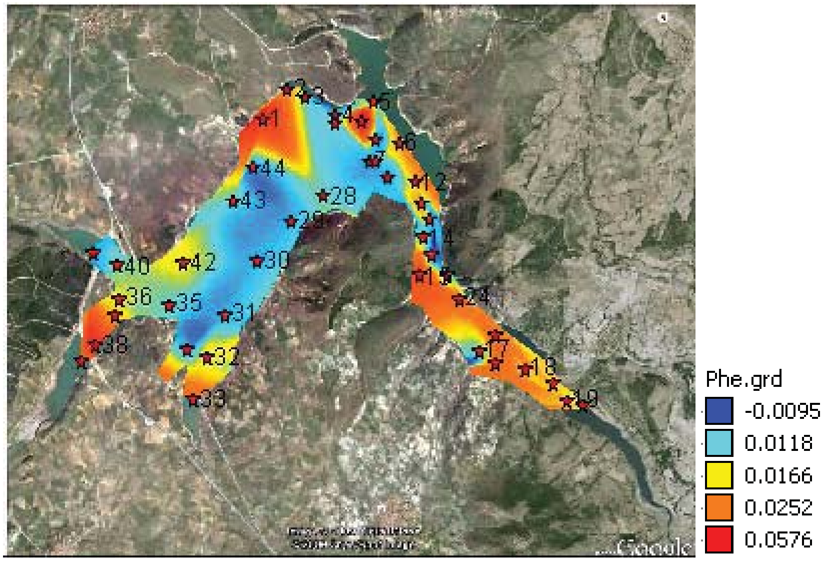
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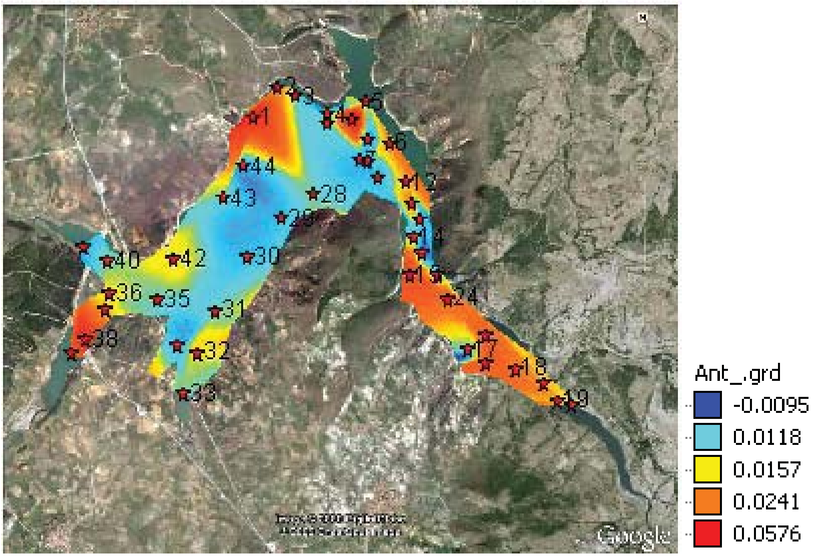
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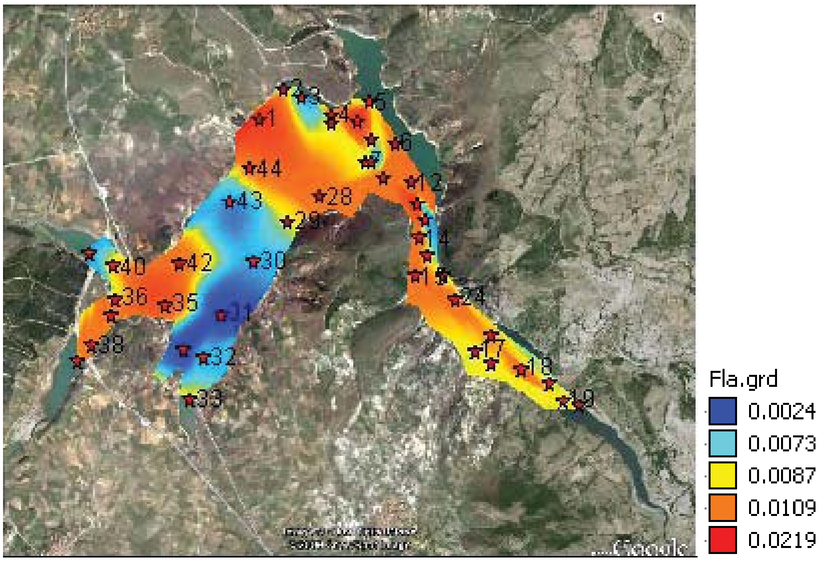
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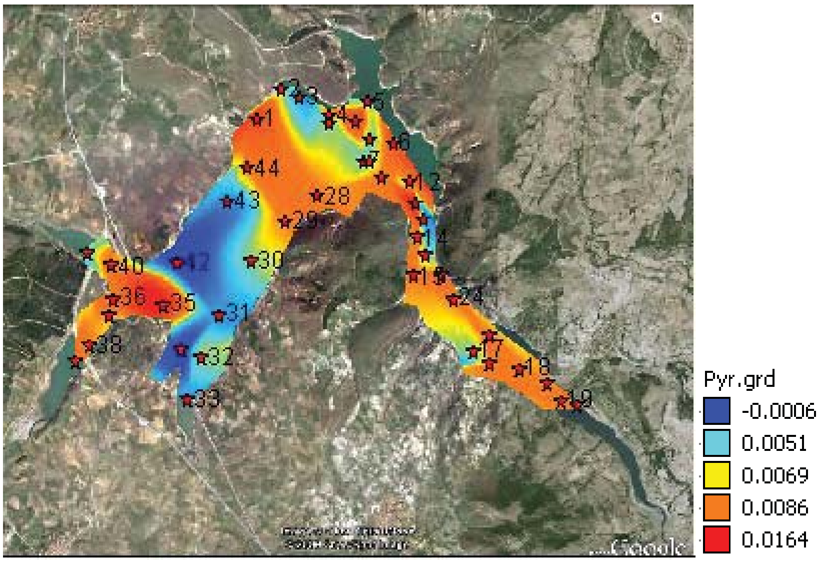
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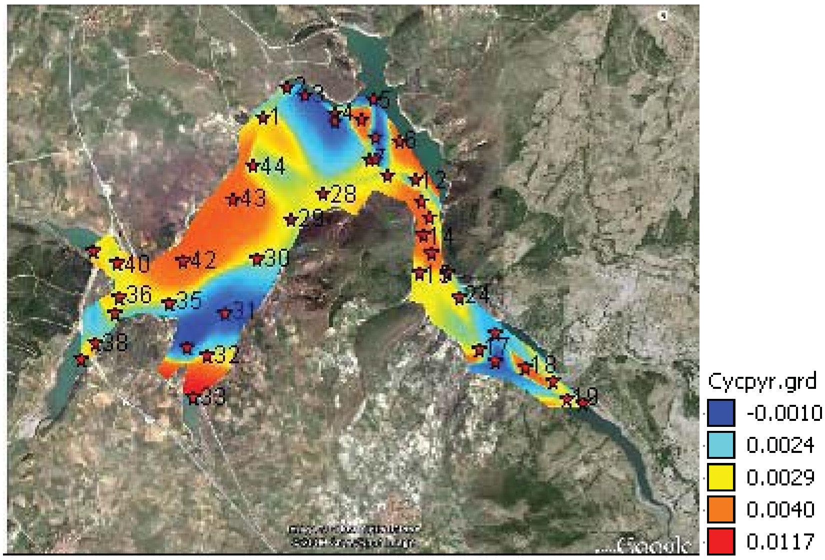
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Cycpyr.grd
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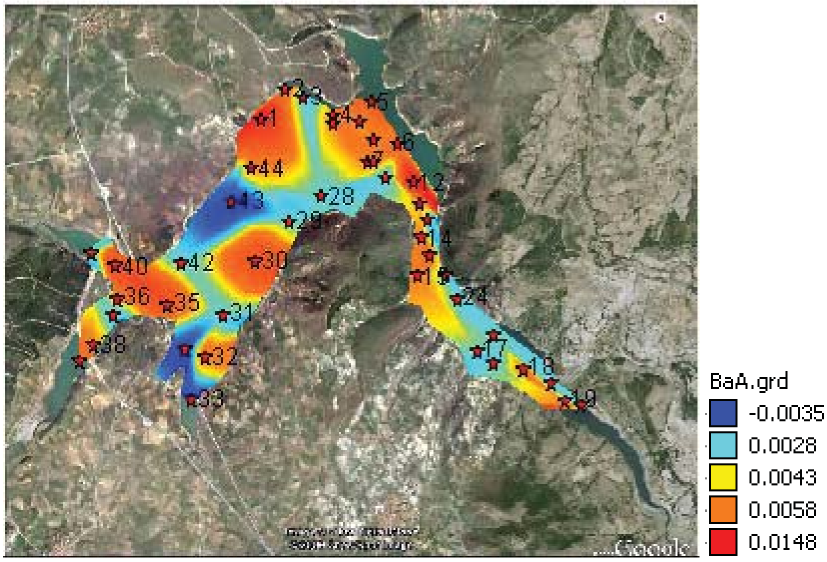
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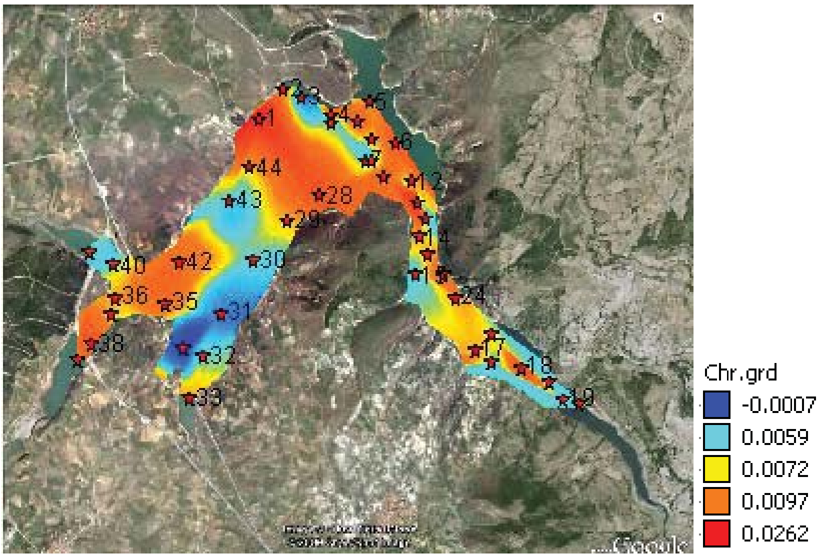
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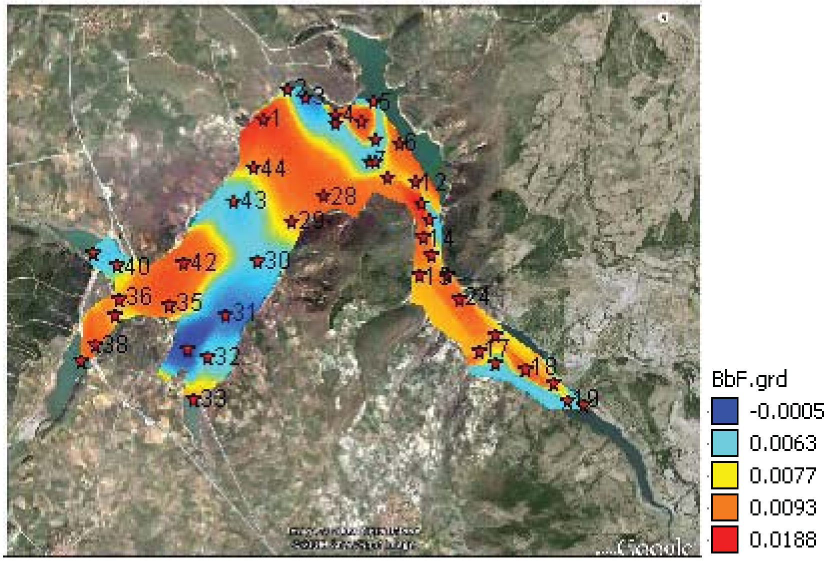
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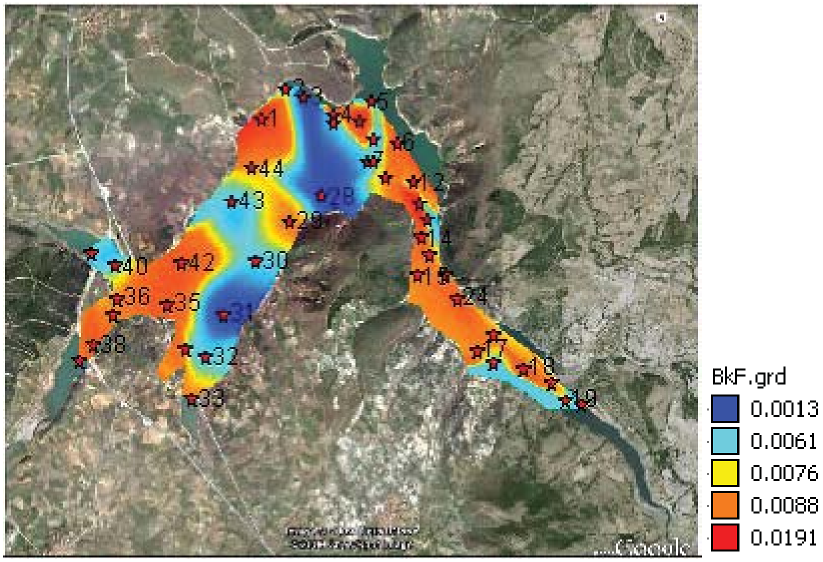
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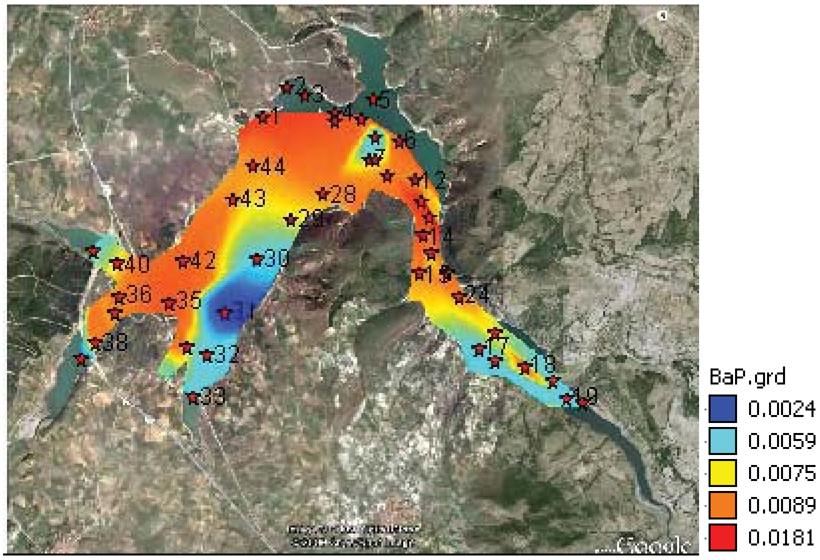
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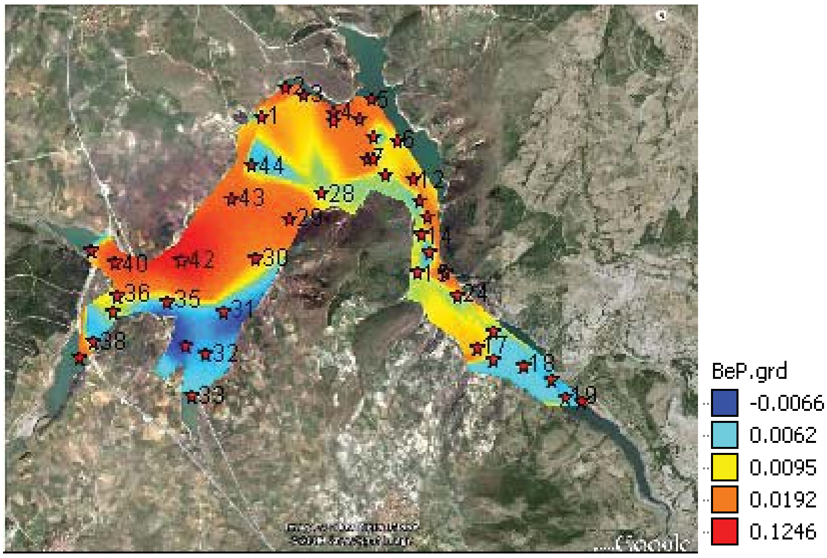
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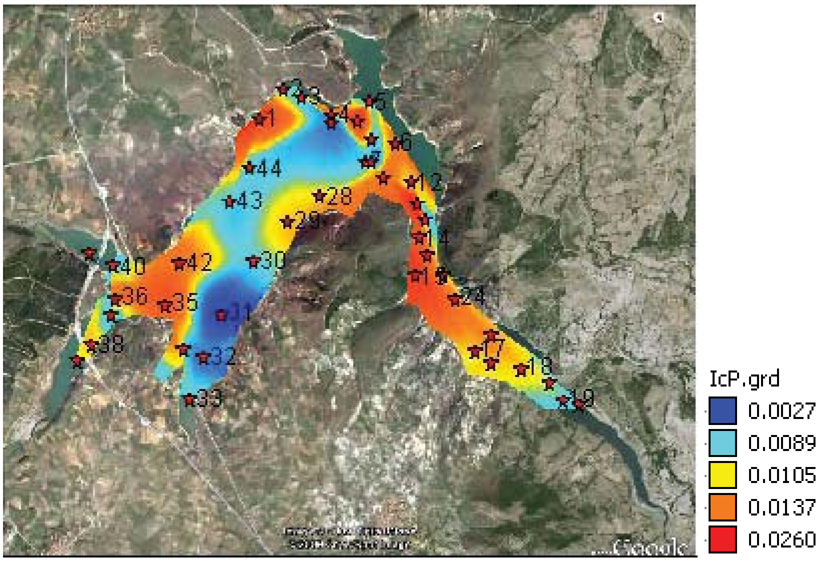
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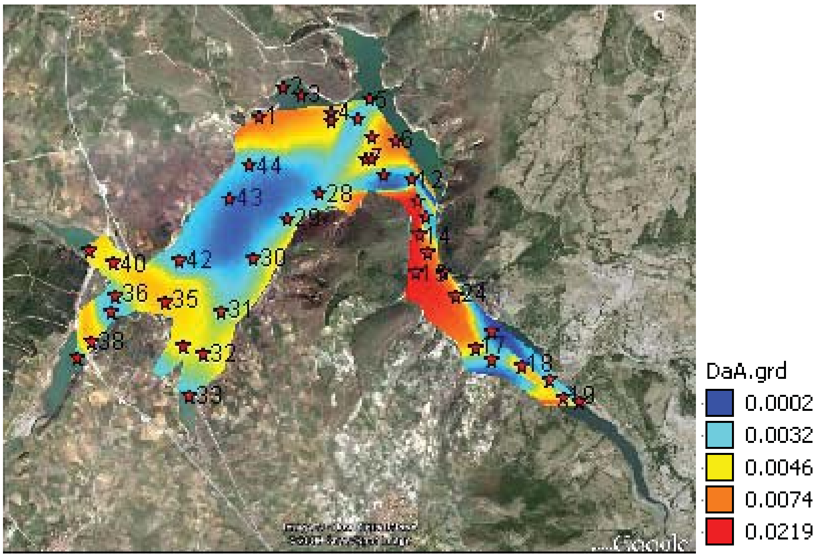
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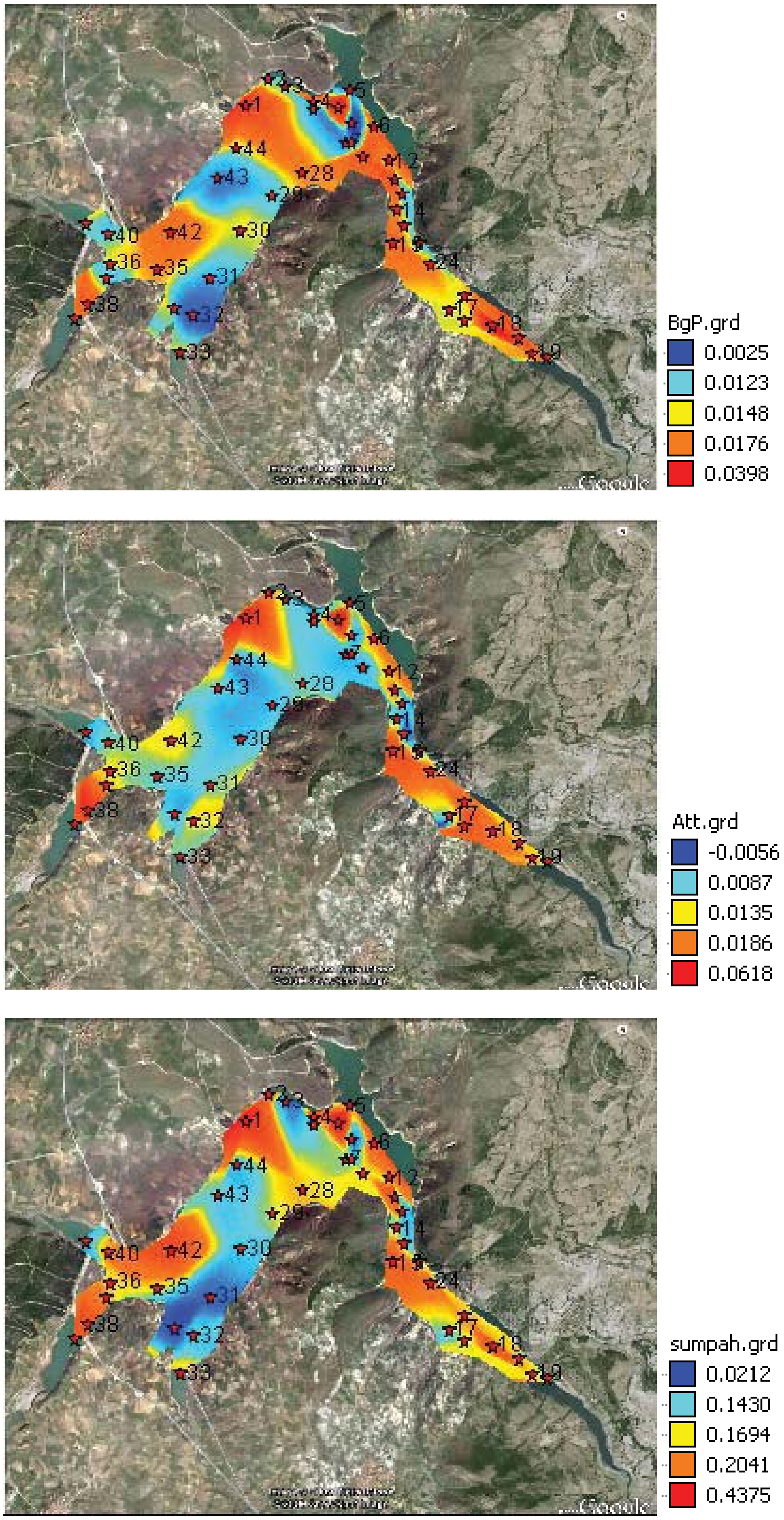

Figure 3.24. Pollution Maps of PAHs (n=19) Concentrations (mg/L) in İkizcetepeler Dam Lake

The PAH concentrations were given as mg/kg in all maps. If it is looked at maps (Figure 3.24) separately, it can be seen that Ant and Phe (3 rings PAHs, abundant in petrogenic sources mainly in petrol spills), which are LPAHs, have completely the same PAH distributions and accumulated on the west end (near to the highway) and east side of the dam. The BbF, BkF, Chr (4-5 rings) which are HPAHs, (have pyrogenic sources; the combustion of coal, wood, vehicle fuel and waste tire) have similar PAH distributions and their concentrations are higher in east. Although most PAHs were highly concentrated on east BeP, BaP and Cycpyr are highly concentrated in west and as is known these PAHs are pyrogenic and sourced from traffic. The Acy (3-rings PAH, LPAH. petrogenic source) has the least observed PAH and accumulated in north and east of the dam lake like Nap.

In general, for all PAHs, lower concentrations were observed in the west of the dam lake far from the highway passing over the dam lake and in northeast side of the dam. Moderate-high concentrations were observed near to the highway in the west of the dam, in the east and northwest of the dam. Although highway is passing from the west of the lagoon, overall concentrations in east are higher from the west except the region around the highway. Generally higher molecular weight PAHs were concentrated around the highway region which indicates that PAHs in this region may be sourced from the traffic.

CHAPTER 4

CONCLUSIONS

In this study, PAH compounds in Ölüdeniz and İkizcetepeler sediment samples were determined with high recoveries by using HP 6890 series GC coupled to a flame ionization detector and mass detector respectively.

Three extraction methods (ultrasonic bath, soxhlet, SPME) for the sediment samples were optimized by using chemometric approach. By using pareto charts (SPSS) importance of the factors affecting extraction efficiency were investigated. No one of the factors was found as statistically significant by using t-test. By using surface response graphs best optimum values were chosen for each extraction methods and methods were compared according to surrogate recovery results.

The optimization studies of extraction methods showed that ultrasonic bath extraction was the best in terms of surrogate recovery (81 %) but HSSPME extraction (79 % recovery) was also applicable to extraction of PAHs from sea sediments and it was a good alternative to the ultrasonic bath for the moderately or less polluted samples. Soxhlet had the poorest extraction efficiency (60 % recovery). Therefore ultrasonic bath extraction method with optimized parameters was used for the analysis of Ölüdeniz and İkizcetepeler sediment samples. In addition, optimized soxhlet extraction system might also be used for the analysis of sediment samples for the less polluted samples.

Concentrations of 16 PAHs in sediments were generally moderate in Ölüdeniz sediment samples. Ace and Chr were dominant ones among the sixteen

PAHs analysed and their concentrations were 0.620 and 0.515 mg/kg relatively. Total sum was 1.854 ± 1.393 mg/kg for 14 PAHs in five sediment samples. The Nap and Ace in Ölüdeniz sediment samples could not be detected with GC-FID because the concentrations of these PAHs were below the detection limit of GC-FID. Since GC-MS had lower detection limits it is a good alternative for the analysis of Nap and Ace in less polluted sediment samples.

The concentrations of 19 PAHs in İkizcetepeler Dam sediment samples were quite low. The highest concentrations were observed for Nap and Bep and they were 0,020 mg/kg. The Cycypyr had the lowest concentration which was 0.004 mg/kg. The sum PAH concentrations was 0.187± 0.074 mg/kg.

When the sum PAH concentrations of Ölüdeniz Lagoon and İkizcetepeler Dam was compared it was seen that concentrations in İkizcetepeler are much lower than Ölüdeniz. For most of the PAHs the fluctuations in the concentrations are the same, that is Ölüdeniz and İkizcetepeler may have the same kind of PAH source. Since they are far from each other and no distinguishable source is situated around them, atmosphere may be the only common source for both of them.

According to first FA analysis of İkizcetepeler samples, the five factors account for 76 % of the variability in the data. Factor 1 (pyrogenic; coal combustion + traffic) explains 35 % of the total variance, Factor 2 (petrogenic) contributing 16.93 % of total variance and Factor 3 (unknown 1) explains 8.6 % of total variance, Factor 4 (unknown 2) explains 7.9 % of total variance and Factor 5 (unknown 3) explains 7.2 % of total variance.

In order to further investigate the pyrogenic sources of PAHs, second FA was performed by removing Ace, Cycpyr and BaA from the data matrix. According to second FA results; there were 3 factors account for 71.7 % of the variability in the data. Factor 1 (explains 39.8 % of the total variance), represents a pyrogenic source (coal combustion; 4 rings PAH and traffic related pollution; 5-6 rings PAHs), Factor

2 (explains 22.3 of the total variance) represents petrogenic source PAHs and Factor 3 (explains 9.69 of the total variance), represents an unknown source.

To sum up the FA and diagnostic analyses results indicated that pyrogenic (the most common) and petrogenic sources are the important sources of PAHs in İkizcetepeler dam lake.

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1. Tuncel S.G., Tuğrul S., Topal T. (2007) A Case Study on Trace Metals in Surface Sediments and Dissolved İnorganic Nutrients in Surface Water of Ölüdeniz Lagoon-Mediterranean, Turkey. **Water Research, 41**, 365-372.

ATTENDED MEETINGS

1. Participation to Chemistry 98 Congress, Poster; "Topal, T., Tuncel S., "Major Elements in Human Milk", Kimya 98, Edirne, Turkey, September, 1998.

2. Participation to Science Education Congress," Oral Presentation; Topal T., Geban Ö.,Ertepınar, H., "Analogies in Acid-Base Chemistry", KTÜ, Trabzon, Turkey, October, 1998.

3. Participation to Chemistry 99 Congress; Samsun, Turkey, September,1999.

4. Participation to "Colloquium Spectroscopicum Internationale XXXI", METU, Ankara, Turkey, September, 1999.

5.Participation to " Third Mediterranean Basin Analytical Chemistry Congress", Poster; Topal T.,Tuncel S., " Water Quality in Ölüdeniz Region; Chemical and Ecological Parameters" Antalya , Turkey, June, 2000.

6.Participation to Kimya 2000 Kongresi, Oral Presentation, Diyarbakır, Turkey, September, 2000.

7.Participation to Colloquium Spectroscopicum Internationale XXXII", Heavy Metals in Surface Sediments of Ölüdeniz Lagoon of theTurkish Aegean Coast, South Africa, July, 2001.

8.Best Poster award in Colloquium Spectroscopicum Internationale XXXII, South Africa, July,2001.

9.Participation to EUROTRAC TOR-2 Workshop program, Ankara, Turkey, September, 2001.

10.Participation to Second International Symposium on Air Quality Management, Istanbul, Turkey, September, 2001.

11.Participation to 3rd Aegean Analytical Chemistry Days, September, 2002, Greece, Poster presentation.

12.Participation to Eurotrac-2 Symposium, Germany, March 2002, Poster presentation, “Study of Trace and Heavy Metals in Rural and Urban Aerosol of Uludağ and Bursa (Turkey)”.

13.Participation to international congress held in Antalya by MESAEP organizing comitte, a poster presentation, Antalya, Turkey, 2004.

14.Participation to the workshop: ‘’POPs’’, Berchtesgaden (Germany), organized by the MESAEP,2004.

FIELD OF INTEREST

Analytical Chemistry, Environmental Chemistry, Water Pollution, Marine Chemistry, Atmospheric Pollution.