DOKUZ EYLÜL UNIVERSITY
GRADUATE SCHOOL OF NATURAL AND APPLIED SCIENCES

ASSESSMENT OF ALIPHATIC AND POLYCYCLIC AROMATIC HYDROCARBON LEVELS IN SELECTED FISH SPECIES FROM İZMİR BAY

by
Enis DARILMAZ

October, 2010
İZMİR

ASSESSMENT OF ALIPHATIC AND POLYCYCLIC AROMATIC HYDROCARBON LEVELS IN SELECTED FISH SPECIES FROM İZMİR BAY

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We have read the thesis entitled **“ASSESSMENT OF ALIPHATIC AND POLYCYCLIC AROMATIC HYDROCARBON LEVELS IN SELECTED FISH SPECIES FROM İZMİR BAY”** completed by **ENİS DARILMAZ** under supervision of **PROF. DR. FİLİZ KÜÇÜKSEZGİN** and we certify that in our opinion it is fully adequate, in scope and in quality, as a thesis for the degree of Doctor of Philosophy.

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CONTENTS

Page

ASSESSMENT OF ALIPHATIC AND POLYCYCLIC AROMATIC HYDROCARBON LEVELS IN SELECTED FISH SPECIES FROM İZMİR BAY

ABSTRACT

In this thesis, polycyclic aromatic hydrocarbons (PAHs) and aliphatic hydrocarbons were analyzed in red mullet (*Mullus barbatus*) and in annular sea bream (*Diplodus annularis*) from İzmir Bay (Eastern Aegean). This is the first time that a complete set of such data has been collected and evaluated. These species were selected because of they are common, widely distributed and eaten. They were sampled by trawling and analyzed seasonally in 2000-2001 and in 2004-2005 at five locations. Naphthalene was found to be the dominant PAH. Red mullet showed higher concentrations of PAHs and aliphatics than annular sea bream. PAH levels in red mullet ranged from 202 to 556 $ngg^{-1}dw$ and in annular sea bream from 78.7 to 415; aliphatics levels ranged from 834 to 2420 and from 436 to 1724 $ngg^{-1}dw$, respectively. The molecular ratios, used to identify sources of PAHs and aliphatics in fish of the İzmir Bay, showed pyrolitic inputs for PAHs, but biogenic and anthropogenic inputs for aliphatics. Carcinogenic Benzo(a)Pyrene was detected in most fish samples at levels between 22.2 and 64.1 $ngg^{-1}dw$. Statistical analysis showed no correlation of PAHs and aliphatics regarding extractable organic matter (HEOM). No significant temporal, inter-species, differences for total aliphatics ($p<0.05$) was shown by the ANOVA test; a significant, temporal, PAHs difference was detected for red mullet. The average İzmir Bay PAH contamination level was within the “moderate” category. Results indicate the need for systematic monitoring of the study area, in order to provide accurate assessment and risk management for human health.

Keywords: Polycyclic aromatic hydrocarbons, aliphatic hydrocarbons, red mullet, annular sea bream, molecular ratios, İzmir Bay (Eastern Aegean)

İZMİR KÖRFEZİ'NDEN SEÇİLEN BALIK TÜRLERİNDE ALİFATİK VE POLİSİKLİK AROMATİK HİDROKARBON SEVİYELERİNİN DEĞERLENDİRİLMESİ

ÖZ

Bu tezde İzmir Körfezi'nden trolle yakalanmış olan barbun (*Mullus barbatus*) ve isparoz (*Diplodus annularis*) balıklarında ilk defa detaylı olarak polisiklik aromatik hidrokarbonlar (PAHs) ve alifatik hidrokarbonlar analiz edilmiştir. Körfezde sıklıkla bulunması ve tüketilmesi nedeniyle bu türler seçilmiştir. Örnekler trolle körfezdeki beş bölgeden 2000-2001 ve 2004-2005 yıllarında mevsimsel olarak toplanmış ve analiz edilmiştir. Naftalen polisiklik aromatic hidrokarbonlar içinde en baskın bileşiktir. Barbundaki PAH ve alifatik hidrokarbon konsantrasyonları isparozdan daha yüksek bulunmuştur. PAH konsantrasyonları barbun ve isparoz balıklarında sırasıyla 202-556 ile 78,7-415 ng/g, alifatik hidrokarbonlar ise 834-2420 ile 436-1724 ng/g kuru ağırlık aralığında ölçülmüştür. İzmir Körfezi'ndeki balıklarda ölçülmüş olan PAHs ve alifatik hidrokarbonların kaynaklarını belirlemek amacıyla moleküler oranlar kullanılmış olup PAHs'ın pirolitik, alifatiklerin ise biyojenik ve antropojenik kaynaklı olduğu bulunmuştur. Balıkların çoğunluğunda kanserojenik özelliği olan Benzo(a)Piren bileşiği 22,2 ile 64,1 ng/g kuru ağırlık aralığında ölçülmüştür. Yapılan istatiksel analiz sonucunda ekstrakte edilebilen organic madde ile PAHs ve alifatik hidrokarbonların arasında bir ilişki bulunmamıştır. Barbun ve isparozda zamana bağlı olarak alifatik hidrokarbon konsantrasyonlarında anlamlı bir değişim ($p<0.05$) bulunmaz iken barbunda PAH konsantrasyonlarında zamana bağlı anlamlı bir değişim ANOVA testi ile saptanmıştır. İzmir Körfezi'ndeki PAH kirlenmesi orta seviyededir. Sonuçlar insan sağlığı için doğru bir yaklaşım ve risk değerlendirmesi açısından sistematik olarak izlenmenin gerekliliğini işaret etmektedir.

Anahtar sözcükler: Polisiklik aromatik hidrokarbonlar, alifatik hidrokarbonlar, barbun, isparoz, moleküler oranlar, İzmir Körfezi (Ege Denizi)

CHAPTER ONE
INTRODUCTION

In environment, the persistent organic pollutants (POPs) are photochemical, biological and chemical degradation resistant at different degrees; they bioaccumulate through the food chain causing human health and environmental damages. The United Nations Environment Programme (UNEP) choose 12 POPs, all chlorine-containing organic compounds, as priority pollutants due to their impact on the human health and environment. Polychlorinated biphenyls, organochlorine insecticides and polycyclic aromatic hydrocarbons were also included in the list (Euro Chlor, 1998–1999). They are characterized by low water solubility and high lipid solubility that increase with increased chlorine atoms. These characteristics and their persistence lead to bioaccumulation in fatty tissues (Perugini et al., 2004).

Polynuclear aromatic hydrocarbons (PAHs) are wellknown environmental pollutants at low concentrations and are included in the European Union and US Environmental Protection Agency (EPA) priority pollutant list due to their mutagenic and carcinogenic properties (Nieva-Cano et al., 2001). They are generated by incomplete combustion of organic materials arising in part from natural combustion such as forest fires and volcanic eruptions (Grova et al., 2002). Anthropogenic sources such as industrial production, transportation and waste incineration generate significant levels of PAHs (Baek et al., 1991; Lorber et al., 1994; Yang et al., 1998). Petroleum production, import and export of petroleum products also contribute a lot to the extent of PAH contamination especially in the marine samples (Baek et al., 1991; Lorber et al., 1994; Nwachukvu, 2000; Nwachukvu et al., 2001). Several PAHs are known to be potential human carcinogens; these include benz[a]anthracene, chrysene, benzo[b]flouranthene, benzo[k]flouranthene, benzo[a]pyrene and benzo[ghi]perylene (Anyakora et al., 2005; IARC, 1983). Major routes of exposure to PAHs are from inhaled air and especially by the ingestion of contaminated food. PAHs in the atmosphere enter into the aquatic environment being readily taken up and bioaccumulated by aquatic organisms. Because of hydrophobicity of PAHs, marine sediments concentrate the pollutants, especially in coastal waters where fish and molluscs are particularly prone to exposure and accumulation (Pena et al., 2006).

İzmir Bay is one of the great natural bay areas of the Mediterranean and compares well with similar coastal areas in the world. It consists of three parts: the Inner, the Middle and the Outer bays with the total surface area of over 500 square kilometers and a water capacity of 11.5 billion cubic meters. It has a total length of 64 kilometers and opens in the Aegean Sea.

Trace metal levels have been investigated extensively in marine organisms in İzmir Bay (Kucuksezgin & Balci, 1994; Kucuksezgin et al., 2002; 2006; Parlak & Demirkurt, 1990). Petroleum hydrocarbons have been studied in sediments from İzmir Bay (Darilmaz & Kucuksezgin, 2007). A few data are available on POPs concentrations in marine organisms (Uluocak & Egemen, 2005). The aim of this study was to evaluate the level, distribution and trend of aliphatic and polycyclic aromatic hydrocarbons in two marine fish species, red mullet (*Mullus barbatus*) and annular sea bream (*Diplodus annularis*) in different sites at İzmir Bay. As the species selected are regularly used in the Turkish diet, it will be important to know the pollutants data in edible fish with the aim of protecting consumers.

CHAPTER TWO
HYDROCARBONS

In early days of chemistry science, chemical compounds were divided into two groups: inorganic and organic, depending on their original source. Inorganic compounds were obtained from minerals, while organic compounds were obtained from living organisms and contained carbon. However, now organic compounds can be produced in the laboratory. Those organic compounds that contain only elements of carbon (C) and hydrogen (H) are called hydrocarbons, and they form the largest group of organic compounds. There might be as many as several thousand different hydrocarbon compounds in petroleum reservoir fluids. Hydrocarbon compounds have a general closed formula of CxHy, where x and y are integer numbers. Generally, hydrocarbons are divided into four groups: (1) paraffins, (2) olefins, (3) naphthenes, and (4) aromatics. Paraffins, olefins, and naphthenes are sometime called aliphatic versus aromatic compounds. The International Union of Pure and Applied Chemistry (IUPAC) is a nongovernment organization that provides standard names, nomenclature, and symbols for different chemical compounds that are widely used (IUPAC, 2003).

Paraffins are also called alkanes and have the general formula of C_nH_{2n+2}, where n is the number of carbon atoms. Under standard conditions of 20°C and 1 atm, the first four members of the alkane series (methane, ethane, propane, and butane) are in gaseous form, while from C_5H_{l2} (pentane) to n-hexadecane ($C_{16}H_{36}$) they are liquids, and from n-heptadecane ($C_{17}H_{38}$) the compounds exist as waxlike solids at this standard temperature and pressure.

Olefins are another series of noncyclic hydrocarbons but they are unsaturated and have at least one double bond between carbon-carbon atoms. Compounds with one double bond are called monoolefins or alkenes, such as ethene (also named ethylene: $CH_2{=}CH_2$) and propene or propylene ($CH_2{=}CH{-}CH_3$) (Jahn et al., 1998).

Naphthenes or cycloalkanes are ring or cyclic saturated hydrocarbons with the general formula of C_nH_{2n}. Cyclopentane (C_5H_{10}), cyclohexane (C_6H_{12}), and their derivatives such as n-alkylcyclopentanes are normally found in crude oils.

Aromatics are an important series of hydrocarbons found in almost every petroleum mixture from any part of the world. Aromatics are cyclic but unsaturated hydrocarbons that begin with benzene molecule (C_6H_6) and contain carbon-carbon double bonds. The name aromatic refers to the fact that such hydrocarbons commonly have fragrant odors. (Riazi, 2005).

2.1 Petroleum Hydrocarbons

Of contaminants that can enter a watershed from human activity, organic pollutants such as polycyclic aromatic hydrocarbons (PAHs), polychlorinated biphenyls (PCBs), and organochlorine pesticides (OCPs) are among the most toxic to aquatic life and the most persistent. Released into the environment through either combustion or weathering of oil products, some PAHs are known carcinogens and mutagens (Barron et al., 2004). Polycyclic aromatics, because they are larger and heavier than monocyclic aromatics, are released from oil films and droplets at progressively slower rates with increasing molecular weight and can persist in sediments and tissues for several years (Short et al., 2004).

2.1.1 Chemical and Physical Properties of Petroleum Hydrocarbons

Hydrocarbons are compounds composed of carbon and hydrogen alone. They may be classified two main groups:

1. Aromatic hydrocarbons, which contain ring systems with delocalised electrons, e.g. benzene.
2. Non-aromatic hydrocarbons, which do not contain such a ring system. Included here are alkanes, which are fully saturated hydrocarbons, alkenes, which contain one or more double bonds, and alkynes, which contain one or more triple bonds (Walker, 2001).

Aromatic compounds contain one or more aromatic rings, which have a stable alternate double-bond configuration. Single-ring aromatics in crude oil include benzene, toluene, ethylbenzene, and xylenes. Naphthalene, anthracene, pyrene, and coronene have two, three, four, and seven rings, respectively, and belong to the polynuclear aromatic group of compounds (PAH) some of which are carcinogenic. In addition to pure hydrocarbons, some organic compounds in crude oil contain small amounts of oxygen, nitrogen, and sulphur (Wright, 2001). The nomenclature of PAH compounds has been ambiguous in the past due to different peripheral numbering systems. The currently accepted nomenclature is shown in Figure 2.1 (Eisler, 2000).

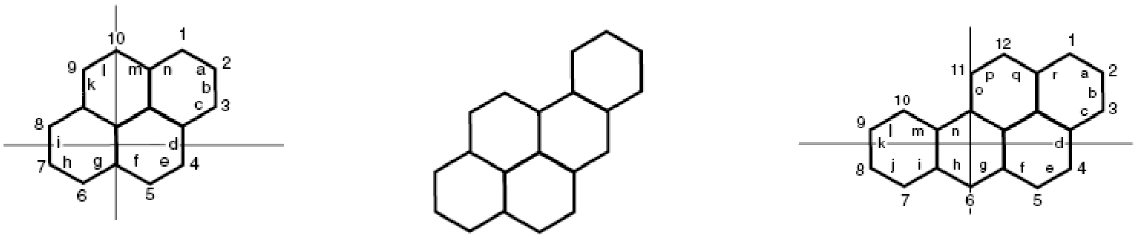

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Figure 2.1 Nomenclatures of PAHs (modified from Grimmer 1983; Lee & Grant 1981). The PAH formula is oriented so that the greatest number of rings are in a horizontal row and a maximum number of rings are above and to the right of the horizontal row. The first carbon atom that belongs to the uppermost ring and is not engaged in ring fusion with another ring is given the number C-1; numbering continues in a clockwise direction, omitting those carbon atoms that do not carry a hydrogen atom. The bond between C-1 and C-2 is designated as side “a”; other peripheral sides continue in clockwise direction in alphabetical order. Examples are: **(1)** pyrene (correctly oriented, numbered, and lettered), **(2)** benzo[*a*]pyrene (not oriented correctly), and **(3)** benzo[*a*]pyrene (correctly oriented, numbered, and lettered)

Physical and chemical characteristics of PAHs generally vary with molecular weight. With increasing molecular weight, aqueous solubility decreases, and melting point, boiling point, and the log K_{ow} (octanol/water partition coefficient) increase (Table 2.1), suggesting increased solubility in fats, a decrease in resistance to oxidation and reduction, and a decrease in vapor pressure. Accordingly, PAHs of different molecular weight vary substantially in their behavior and distribution in the environment and in their biological effects (Eisler, 2000).

The compounds range from naphthalene ($C_{10}H_8$, two rings) to coronene ($C_{24}H_{12}$, seven rings). Common PAH compounds include six two-ring compounds (biphenyl, naphthalene, 1-methylnaphthalene, 2,6-dimethylnaphthalene, and acenaphthene); three-ring compounds (flourene, pyrene, and benz[a]anthracene); four-ring compounds (fluoranthene, pyrene, and benz[a]anthracene); and five-ring compounds (chrysene, benzo[a] pyrene, benzo[e]pyrene, perylene and dibenzo[a,h]anthracene).

Table 2.1 Some Physical and Chemical Properties of Selected PAHs

Compound	Number of Rings	Molecular Weight	Melting Point (ºC)	Solubility in Water (mg/l)	Log K_{ow}
Nap	2	128	80	30.0	3.37
Ant	3	178	216	0.07	4.45
BaAe	4	228	158	0.014	5.61
BaP	5	252	179	0.0038	6.04
BPer	6	276	222	0.00026	7.23

The low molecular weight PAH (LMWPAH) compounds, containing two or three rings, are acutely toxic but noncarcinogenic to a broad spectrum of marine organisms. The high molecular-weight PAH (HMWPAH) compounds, containing four, five, and six rings, are less toxic but have greater carcinogenic potential. Hence, the LMWPAH compounds are sometimes classified separately from the HMWPAH varieties. Examples of LMWPAH compounds that tend to be toxic are anthracene, fluorene, naphthalene, and phenanthrene. HMWPAH compounds that are carcinogenic include benzo(a)pyrene, benzo(c)phenanthrene, dibenzo(a,i)pyrene, and 3-methylcholanthrene (Kennish, 1997).

2.1.2 Sources of Petroleum Hydrocarbons

The principal routes of entry of PAHs into estuarine and marine environments include atmospheric deposition, discharges of domestic and industrial wastes, runoff from land, and spillage and seepage of fossil fuels. Endogenous sources of PAHs (i.e., biosynthesis) in these environments may be significant only in anoxic sediments, and reliable estimates of the global input of PAHs by this pathway generally are lacking. Atmospheric deposition is a major route of PAH entry into marine waters, as is the discharge of domestic and industrial wastes. Nearly all PAHs in atmospheric fallout are associated with airborne particulate matter and aerosols. Rain, dry fallout, and vapour phase deposition represent the principal atmospheric processes responsible for the flux of PAHs on the world's oceans. Air masses passing over highly industrialized regions often accumulate high PAH loads (20 to 30 ng/m^3). Rates of chemical degradation and photo oxidation, together with particulate settling rates, largely control the residence time of PAHs in the atmosphere (Kennish, 1997).

Table 2.2 Major Sources of PAHs in Atmospheric and Aquatic Environments (Modified from Edwards, 1983; Lo & Sandi, 1978; Neff, 1979; and Sims & Overcash, 1983

Ecosystem and Sources	Annual Input (metric tons)
ATMOSPHERE	
Total PAHs	
Forest and prairie fires	19,513
Agricultural burning	13,009
Refuse burning	4769
Enclosed incineration	3902
Heating and power	2168
Benzo[a]pyrene	
Heating and power	
Worldwide	2604
U.S. only	475
Industrial processes (coke production)	
Worldwide	1045
U.S. only	198
Refuse and open burning	
Worldwide	1350
U.S. only	588
Motor vehicles	
Worldwide	45
U.S. only	22
AQUATIC ENVIRONMENTS	
Total PAHs	
Petroleum spillage	170,000
Atmospheric deposition	50,000
Wastewaters	4400
Surface land runoff	2940
Biosynthesis	2700
Total benzo[a]pyrene	700

About 43,000 metric tons of PAHs are discharged into the atmosphere each year, and another 230,000 tons enter aquatic environments (Table 2.2). Anthropogenic activities associated with significant production of PAHs include: coke production in the iron and steel industry; catalytic cracking in the petroleum industry; the manufacture of carbon black, coal tar pitch, and asphalt; heating and power generation; controlled refuse incineration; open burning; and emissions from internal combustion engines used in transportation. Thus, the formation of PAHs in the environment is due to an endogenous synthesis by microorganisms, algae, and macrophytes that provide natural background, and to a second process that is connected to human-controlled, high-temperature (>700°C) pyrolysis of organic materials, to open burning, and to natural volcanic activities.

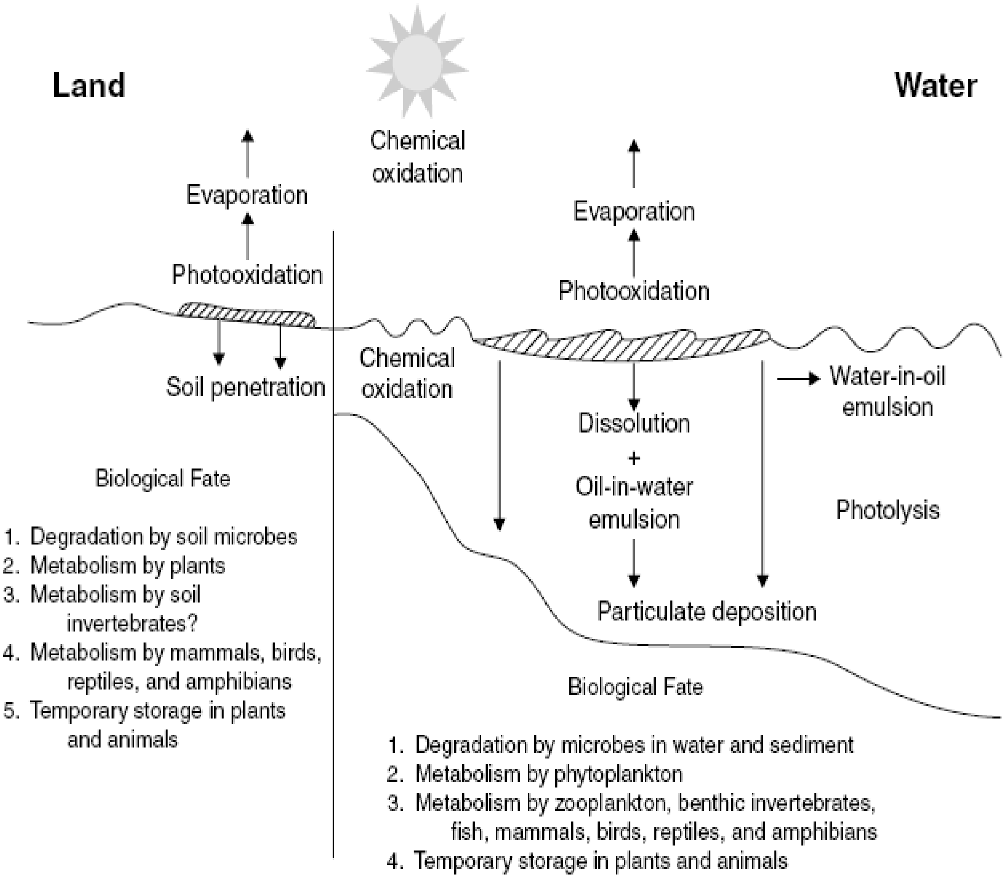


Figure 2.2 Chemical and biological fate of petroleum and PAHs in water and on land (Albers, 2003)

The discovery in fossil fuels of complex mixtures of PAHs spanning a wide range of molecular weights has led to the conclusion that, given sufficient time (i.e., millions of years), pyrolysis of organic materials at temperatures as low as 100 to 150°C can also lead to production of PAHs (Neff, 1985). The majority of PAHs entering aquatic environments remains close to sites of deposition, suggesting that lakes, rivers, estuaries, and coastal marine environments near centers of human populations are the primary repositories of aquatic PAHs (Neff, 1979). Crude and refined oil products begin to change composition on exposure to air, water, or sunlight (Atlas & Bartha, 1973) (Figure 2.2).

Table 2.3. Suggested PAHs sources based on the ratios of some PAH compounds

Ratio Source	LMW/HMW	Phe/Ant	Flt/Pyr	Flt/(Flt+Pyr)	BbF/BaP	BkF/BaP
Pyrolytic	<1	<10	>1	>0.5	<0.5	<0.5
Petrogenic	>1	>15	<1	<0.5	>0.5	>0.5
References	Tam et al. (2001)	Baumard et al. (1998a,b)	Baumard et al. (1998a,b)	Budzinski et al. (1997) and Gogou et al. (1998)	El Deeb et al. (2007)	El Deeb et al. (2007)

The fingerprints of PAHs from pyrolytic or petrogenic origin may be used to differentiate these two origins by using molecular indices based on ratios of selected PAH concentrations. The interest of these indices relies on the fact that during low temperature processes such as those occurring during catagenesis of organic matter leading to the formation of the petroleum, the PAH distribution is governed by thermodynamic properties. On the contrary, for high temperature processes, such as pyrolysis of organic matter, their distribution is rather governed by kinetic characteristics. Hence, PAH distribution and more precisely isomeric compound or alkylated PAH relative concentrations are temperature dependent (Alberty & Reif, 1988) and depend on the processes that generated these compounds. Some characteristic values of these indices are given in Table 2.3. These indices allowed the scientists to determine with accuracy the origin of PAHs from various environments (Budzinski et al., 1997; Gogou et al., 1998, Baumard et al., 1998a,b; Tam et al., 2001; El Deeb et al., 2007).

2.1.3 Effects to Fish

Adult and juvenile fish, larvae, and eggs are exposed to petroleum through contact with whole oil, dissolved hydrocarbons, particles of oil dispersed in the water column, or ingestion of contaminated food and water (Bowman & Langton, 1978).

Death of fish in natural habitat usually requires a heavy exposure to petroleum. Consequently, it is unlikely that large numbers of adult fish inhabiting large bodies of water would be killed by the toxic effects of petroleum. Fish kills usually are caused by large amounts of oil moving rapidly into shallow waters (Hampson & Sanders, 1969; Teal & Howarth, 1984). However, fresh and weathered crude oils and refined products vary considerably in their composition and toxicity, and the sensitivity of fish to petroleum differs among species. Petroleum concentrations (total petroleum hydrocarbons) in water of less than 0.5 ppm during long-term exposure (Woodward et al., 1983) or higher concentrations (several to more than 100 ppm) in moderateor short-term exposures can be lethal (Anderson et al., 1987; Barnett & Toews, 1978; Hedtke & Puglisi, 1982; Little et al., 2000). Sublethal effects begin at concentrations of less than 0.5 ppm and include changes in heart and respiratory rates, gill structural damage, enlarged liver, reduced growth, fin erosion, corticosteriod stress response, immunosuppression, impaired reproduction, increased external and decreased internal parasite burdens, behavioral responses, and a variety of biochemical, blood, and cellular changes (Carls, et al., 1998; Gregg, et al., 1997; Khan, 1999; Kuehn, et al., 1995; Moles & Norcross, 1998; Thomas & Budiantara, 1995; Willette, 1996).

In short-term exposure trials (24 to 96 h) on selected species of fish, individual PAH compounds had LC_{50} values in water ranging from 1.3 to 3400 ppb. The primary target organ for toxic action is the liver. Sublethal effects on eggs, larvae, juveniles, and adult fish are generally similar to those previously described for exposure to fresh or weathered petroleum and separate aromatic fractions but with greater emphasis on neoplasm induction and DNA alteration (Albers, 2003).

Aquatic organisms that metabolize PAHs to little or no extent, such as algae, molluscs and the more primitive invertebrates (protozoans, porifers and cnidaria) accumulate high concentrations of PAHs, as would be expected from their log K_{ow} values, whereas organisms that metabolize PAHs to a great extent, such as fish and higher invertebrates, accumulate little or no PAHs (WHO, 2003). Biomagnification (the increase in concentration of a substance in animals in successive trophic levels of food chains) of PAHs has not been observed in aquatic systems and would not be expected to occur, because most organisms have a high biotransformation potential for PAHs. Organisms at higher trophic levels in food chains show the highest potential for biotransformation (WHO, 1998).

CHAPTER THREE
CHARACTERISTICS OF İZMİR BAY

3.1 Study Area

The bay of İzmir and the surrounding coastal area has been under intensive examination for some time and for various purposes. Early planning for the Metropolitan area was developed at the beginning of the 1970’s and this originated a number of assessments including technical and financial evaluations for the construction of environmental infrastructures. Sufficient understanding has also been developed on the nature, quality and relationships between the waters of the bay and land activities. At the same time significant advances were made worldwide in the economic assessment of the benefits, often expressed as avoided damage, of environmental protection measures.

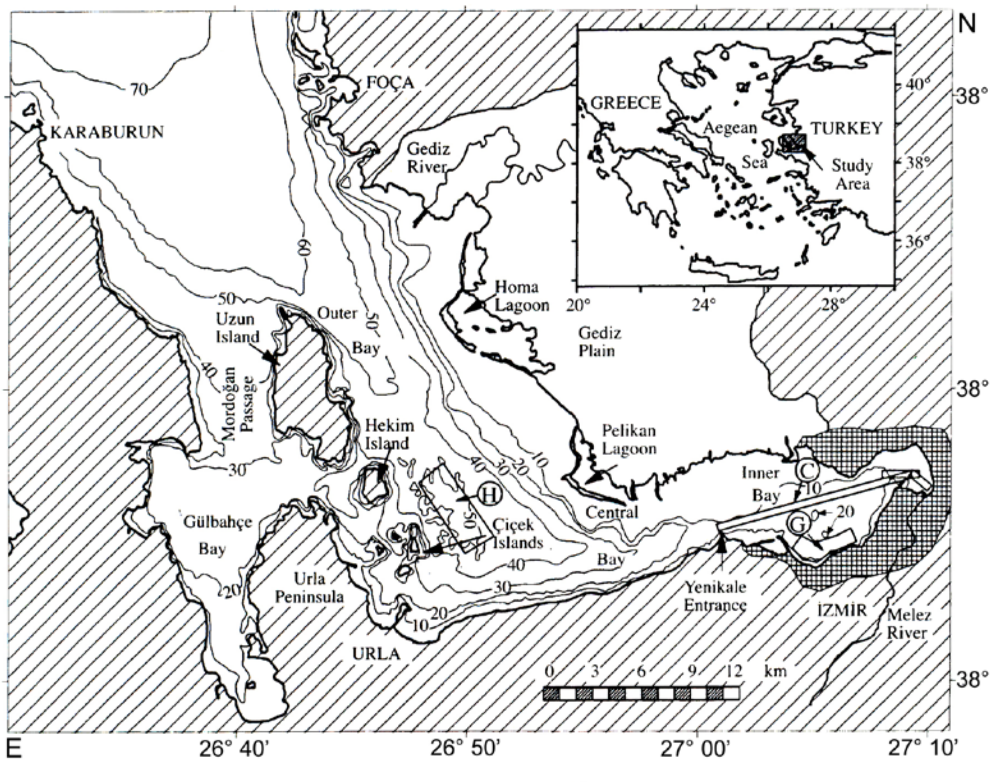


Figure 3.1 Location map of the study area (showing present-day bathymetry). C-Entry Canal; G-Göztepe Bay dump site; H-Hekim Island dump site (from Aksu et al., 1998). Isobaths are in meters.

The city of İzmir is built around the perimeter of the inner part of the bay. The shallow sill depth at the Yenikale entrance and the shallow water depths of the Inner Bay prevent large container ships to enter the İzmir Harbor. Between 1930 and 1976 the innermost harbor region was dredged and ~2.8 x 10^6 m^3 of excavated sediments were dumped on the sea-bed at Göztepe Bay dump site (Figure 3.1) Subsequent excavations between 1976 and 1990 produced ~9.0 x 10^6 m^3 of sediments, which were also dumped on the sea-bed at the Hekim Island dump site.

During the last *ca.* 30 years a large number of studies have been carried out on the physical, chemical and biological oceanography of İzmir Bay, but no published data are available on petroleum hydrocarbon concentrations in marine biota from the İzmir Bay. Aksu et al. (1998) have investigated congeners of PAH in surface sediments from the inner part of the Bay. This is the first time that the complete set of such data has been collected and evaluated in fish.

Table 3.1 Pathways of pollutants in İzmir Bay (from UNEP, 1993a)

Pollution arising from domestic and industrial wastes	50 %
Pollution due to flood water	15 %
Pollution due to transport of chemicals used in agriculture by surface and drainage waters	10 %
Pollution transported by rivers and streams	10 %
Pollution due to erosion	8 %
Pollution caused by ship traffic and bay activities	4 %
Others	3 %

İzmir Bay (western Turkey) is one of the great natural bays of the Mediterranean. The Bay divided into three zones: Inner Bay, Middle Bay, and Outer Bay according to the topographical, hydrological and ecological features of İzmir Bay. The main urban conurbation around the bay is the İzmir Metropolitan Municipality, covering 88000 hectares and population of close to 3 million inhabitants. İzmir is an important industrial, commercial and cultural city center. Industrial activities cover a large range of industries including food processing, tanneries, paint, chemicals, textile and petroleum refining. Various pollution sources and their contribution to the observed pollution levels are given in Table 3.1. As shown in table, the main sources of pollution in the bay are domestic and industrial effluents, which account for 50% of the observed organic pollution.

The bay has a total surface area of over 500 km^2, water capacity of 11.5 billion m^3, a total length of 64 km and opens in the Aegean Sea. A 13 m deep sill, the Yenikale Strait, separates the Middle Bay from the Inner Bay. The Gediz River, which flows to the Outer Bay, is the biggest river in the İzmir Bay. The Inner Bay is heavily polluted by nutrients and organic material (UNEP, 1993a).

The Inner Bay extends from the head of the Bay to the Yenikale lighthouse. The water volume of the Inner Bay is $6x10^8$ m^3, average water depth is about 7 m (depth changes between 0 m and 20 m). The Inner Bay is heavily polluted by organic material and nutrients, however metal concentrations were not high enough to indicate heavy metal pollution. The main source of pollution is streams that flow to the bay and organic materials were measured in the Inner Bay coastal stations that are located in the area of discharge points. The north part of the Inner Bay had been filled by alluvion of Gediz River just released in the west of Karsıyaka until the second half of nineteen century. Due to that, the İzmir Harbour had encountered a shoaling problem and the entrance of Gediz River had been moved toward the Outer Bay. The depth increase steeply from the inshore to the offshore that the ships must follow a shallow channel with a depth of 10 m between the old Gediz Delta and Narlıdere (UNEP, 1993a).

The Middle Bay extends from Yenikale lighthouse to the Kokola point. The water volume of the Middle Bay $9x10^8$ m^3, average water depth is about 16 m. Surface area of the Middle Bay is relatively 57 km^2. The Middle Bay is a passage zone with pollutant concentrations intermediate between Outer and Inner Bays, which are a clear indication of spreading pollution in the bay. Because of the shallow nature of the Inner and Middle Bays, water exchange and self-purification capacities are very limited (UNEP, 1993a). The Outer Bay is extends from Kokola point to the mouth of the bay. The water volume of the Outer Bay 1x10^{10} m^3, average water depth is about 49 m. Surface area of the Outer Bay is relatively 417 km^2. Pollution in the Outer Bay is not significant; this part of the bay is relatively clean according to the most of the pollution indicators show that (UNEP, 1993a).

Gediz River is the second biggest river in the Aegean Region and main fresh water source of the İzmir Bay. It flows to the Outer Bay. Gediz River area is approximately 18000 km^2 and annual average flow is estimated to be 2.33x10^9 m^3 (UNEP, 1993a).

3.2 Geological Characteristics of İzmir Bay

The İzmir Bay is shallow marine basin influenced by freshwater inflow, bottom sediment resuspension and increasing pollution. The surficial sediments can be subdivided into seven zones on the basis of grain-size composition. The western part of the Outer Bay consists of silty and muddy sand, whilst eastern part of the Outer Bay covered silt mud fractions. The most of the Middle Bay is covered by sandy silt. In between Middle and Inner Bays sea bottom floored by silt sized sediments (Duman et al., 1999).

Two large barrier islands encircling the Homa and Pelikan lagoons and several smaller sand bars are also composed of medium-sorted sands, and are formed through winnowing and reworking of progradational channel mouth deposits by waves and long-shore currents following delta abandonment. Silty sands and sandy-silts also occur along the nearshore regions of western Outer Bay between the northern tip of Uzun Island and west of the Çiçek Island. Clayey-silts are essentially restricted to the southern edges of the Middle Bay. Several small ephemeral rivers discharge relatively minor quantities of sediments along this shoreline, particularly during the rainy winter and spring months. Fine-grained sediments occur predominantly within the deeper water segment of Outer Bay as well as throughout Inner Bay.

CHAPTER FOUR
MATERIAL AND METHODS

4.1 Sampling and Biological Characteristics of Fish Species

Sampling took place seasonally in 2000-2001 and 2004-2005 periods, at five locations shown in Figure 4.1. *Mullus barbatus* and *Diplodus annularis* were collected by trawling from İzmir Bay (Foca, Gediz, Uzunada, Gulbahce, Guzelbahce).

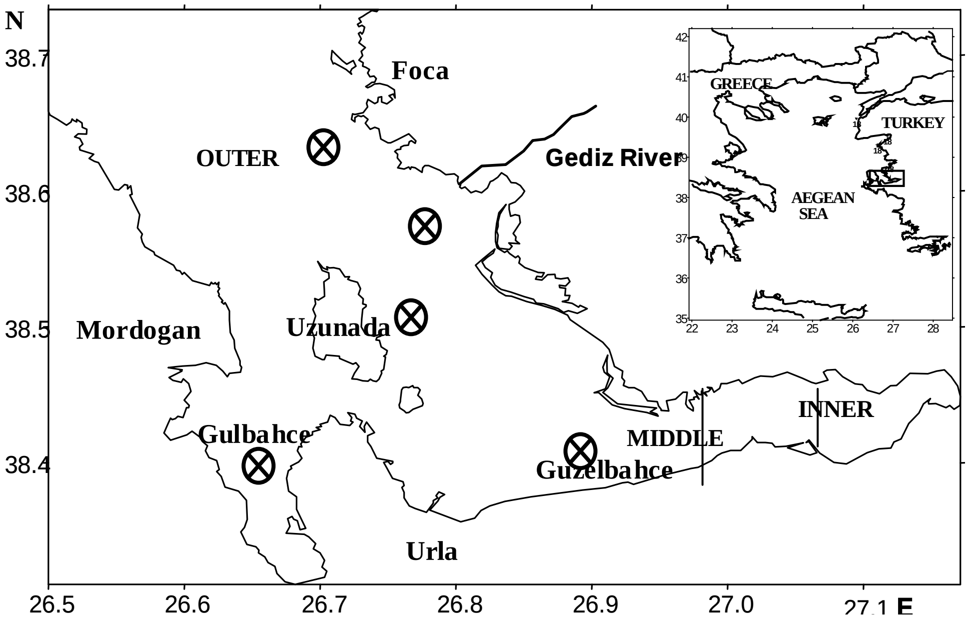


Figure 4.1 Sampling areas in the İzmir Bay (⊗)

The two species are characteristic of İzmir Bay and occur along the coast with abundance. Muscle tissue of the samples was removed in the field using surgical sheets, wrapped in aluminum foil and preserved at -20°C until analysis. The samples were analyzed for aliphatic hydrocarbons and polycyclic aromatic hydrocarbons. The numbers of sampled and main biometric parameters are represented in Table 4.1.

Table 4.1 Main biometric parameters of fish species during sampling periods

Sampling	***Sampling***	***Fork Length (mm)***		***Body Weight (g)***	
Periods	***Number***	***Mean±S.D.***	***Range***	***Mean±S.D.***	***Range***
Mullus barbatus					
November 2000	46	152±4.29	111-202	64.5±5.60	22-140
January 2001	37	149±3.68	124-198	58.9±4.38	32-126
April 2001	11	162±4.19	145-184	73.5±5.18	56-102
August 2001	13	163±3.85	145-184	75.1±4.59	58-102
November 2004	51	143±3.56	86-177	59.7±3.75	8-118
February 2005	112	130±2.39	92-195	39.1±2.47	12-120
April 2005	31	123±3.89	97-165	32.1±4.06	12--84
August 2005	42	150±1.04	140-169	57.5±1.51	42-82
September 2005	32	151±4.27	121-185	57.5±5.25	11-100
Diplodus annularis					
November 2000	33	123±0.80	116-138	43.9±1.12	36-64
January 2001	36	125±2.12	105-150	47.7±2.65	26-88
April 2001	7	146±1.21	141-150	69.4±2.95	60-84
August 2001	18	134±2.92	121-150	56.4±3.31	40-83
November 2004	15	113±0.58	110-116	30.0±0.78	26-36
February 2005	56	126±2.16	110-168	42.1±2.56	24-104
April 2005	20	119±1.23	110-129	35.5±0.99	28-42
August 2005	31	111±1.08	100-122	28.7±0.93	20-38
September 2005	26	132±1.06	120-140	48.2±1.44	36-60

***Diplodus annularis* (Linnaeus, 1758)** (Fishbase.se, 2010)

Environment: Benthopelagic; brackish; marine; depth range 0 - 90 m.

Distribution and Biology: Eastern Atlantic: Madeira and Canary islands. Also found along the coast of Portugal northward to the Bay of Biscay, Mediterranean, Black Sea and Sea of Azov. Inhabit chiefly Zostera seagrass beds but also found on Posidonia beds and sandy bottoms, rarely on rocky bottoms. Carnivorous, feed on worms, crustaceans, mollusks, echinoderms and hydrozoans. The sexes are separated, although these fish are potential hermaphrodites; certain individuals are protandric.

***Mullus barbatus* (Linnaeus, 1758)** (Fishbase.org, 2010)

Environment: Demersal; marine; depth range 10 - 328 m, usually 100 - 300 m.

Distribution and Biology: Eastern Atlantic: British Isles (occasionally Scandinavia) to Dakar, Senegal, Canary Islands, Mediterranean and Black Sea. Also known from the Azores. Found on gravel, sand and mud bottoms of the continental shelf. Depth range from 10-300 m and up to depth of 328 in the eastern Ionian Sea. Feeds on small benthic crustaceans, worms and mollusks. Marketed mainly fresh.

4.2 Analytical Procedures

Reagents and chemicals were of analytical or chromatographic grade. n-hexane, dichloromethane, methanol were purchased from Merck. The target aliphatic and PAH compounds investigated in this study included for aliphatics: n-Decane (n-C10), n-Dodecane (n-C12), n-Tetradecane (n-C14), n-Hexadecane (n-C16), n-Heptadecane (n-C17), Pristane (Pri), octadecene-1 (C18-1), n-octadecane (n-C18), Phytane (Phy), n-Eicosane, (n-C20), n-Heneicosane (n-C21), n-Docosane (n-C22), n-Tetracosane (n-C24), n-Hexacosane (n-C26), n-Octacosane (n-C28), n-Triacontane (n-C30), n-Dotriacontane (n-C32), n-Tetratriacontane (n-C34) and for PAHs: naphthalene (Nap), 1-methyl naphthalene (1-m-Nap), 1-ethyl naphthalene (1-e-Nap), acenaphthylene (Acy), acenaphthene (Ace), fluorene (Flu), phenanthrene (Phe), 2-methyl phenanthrene (2-m-Phe), 1-methyl phenanthrene (1-m-Phe), 3,6-dimethyl phenanthrene (3,6-dm-Phe), anthracene (Ant), fluoranthene (Flt), pyrene (Pyr), 1-methyl pyrene (1-m-Pyr), benzo[a]anthracene (BaA), chrysene (Chr), benzo[b]fluoranthene (BbF), benzo[k]fluoranthene (BkF), benzo[e]pyrene (BeP), benzo[a]pyrene (BaP), Perylene (Per), indeno[1,2,3-c,d]pyrene (IP), dibenzo[a,h]anthracene (dBA) and benzo[g,h,i]perylene (BPer). Working standards were prepared by dilution with hexane. Quantitative determinations were performed by means of five internal standards: n-C19 d40, n-C32 d66, Hexamethylbenzene, Cadalene, Naphthalene-d8.

4.2.1 Aliphatic and Polycyclic Aromatic Hydrocarbons Analysis

Prior to extraction, about 2.5-3g of freeze-dried fish samples placed into the microwave extraction tubes and internal, non-naturally occurring aliphatic and PAH standards (n-C19 d40, n-C32 d66, Hexamethylbenzene, Cadalene: 1,6-dimethyl-4-(1-methylethyl) naphthalene and Naphthalene-d8) are added for the quantitative calculations. Extraction is realised with 30 ml methanol within the following cycle:

- Power of the microwaves: 1600 watts
- Temperature increase to 115 °C in 10 minutes.
- Extraction maintained at 115 °C for 20 minutes
- Cooling to ambient temperature within one hour.

All samples are extracted in a microwave extraction system for 30 minutes, using 30 ml methanol according to (UNEP, 1993b). After the extraction is completed, 5 ml of 2 M KOH are added to the flask and the extraction is continued for saponify the lipids.

- Power of the microwaves: 1600 watts
- Temperature increase to 90 °C in 5 minutes.
- Extraction maintained at 90 °C for 10 minutes
- Cooling to ambient temperature within one hour.

The content of the extraction flask is transferred into a separatory funnel with 30 ml of water (distilled and extracted with hexane) and extracted with 90 ml of hexane and re-extracted again twice with 50 ml of hexane. Then all hexane extracts are combined, filtered through glass wool and dried with anhydrous sodium sulfate.Then the extract is concentrated to 15 ml in rotary evaporator. The evaporation is achieved to 1 ml under a gentle stream of nitrogen. Extracts are then separated into two fractions, using a column chromatography on 5 g of deactivated silica (bottom), 10 g of deactivated alumina and 1 g of anhydrous Na_2SO_4 (top). The alumina and silica are precleaned by soxhlet extraction, first for 8 hours with methanol and then for 8 hours with hexane and then dried. Before use, they are activated at 200 ºC for 4 hours and partially deactivated with 5% water. The sample is added on top of the column. A first fraction is eluted with 20 ml hexane. The second saturated fraction is eluted with 30 ml solution of hexane:dichloromethane (90:10). All groups are collected in Kuderna-Danish, and first concentrated to 15 ml using Kuderna-Danish heater then using a gentle stream of nitrogen gas to 1 ml. The first group has saturated aliphatic hydrocarbons, the second group has unsaturated and aromatic hydrocarbons. Detection limits of aliphatic and polycyclic aromatic hydrocarbons are shown in Table 4.2.

Table 4.2 Detection limits of aliphatic and polycyclic aromatic hydrocarbons (ng/g)

Detection Limits			
n-C10	7.23	**Nap**	4.76
n-C12	6.48	**1-m-Nap**	4.89
n-C14	7.29	**1-e-Nap**	3.97
n-C16	6.21	**Ace**	6.11
n-C17	7.38	**Acy**	4.15
Pri	6.45	**Flu**	5.36
C18-1	7.08	**Phe**	5.88
n-C18	7.27	**Ant**	5.72
Phy	8.98	**2-m-Phe**	9.47
n-C20	5.96	**1-m-Phe**	9.16
n-C21	2.01	**3,6 dm-Phe**	9.71
n-C22	6.09	**Flt**	6.79
n-C24	6.19	**Pyr**	6.99
n-C26	6.10	**1-m-Pyr**	9.19
Squ	9.10	**Chr**	8.97
n-C28	6.24	**Per**	9.79
n-C30	6.26	**BaA**	6.29
n-C32	6.95	**BbF**	6.68
n-C34	7.89	**BkF**	6.96
		BeP	6.32
		BaP	8.39
		IP	5.91
		dBA	7.96
		BPer	5.26

Chromatograms of aliphatic and aromatic hydrocarbons for selected sampling points and standards are given in Figure 4.2, 4.3, 4.4, 4.5 4.6, 4.7, 4.8 and 4.9.

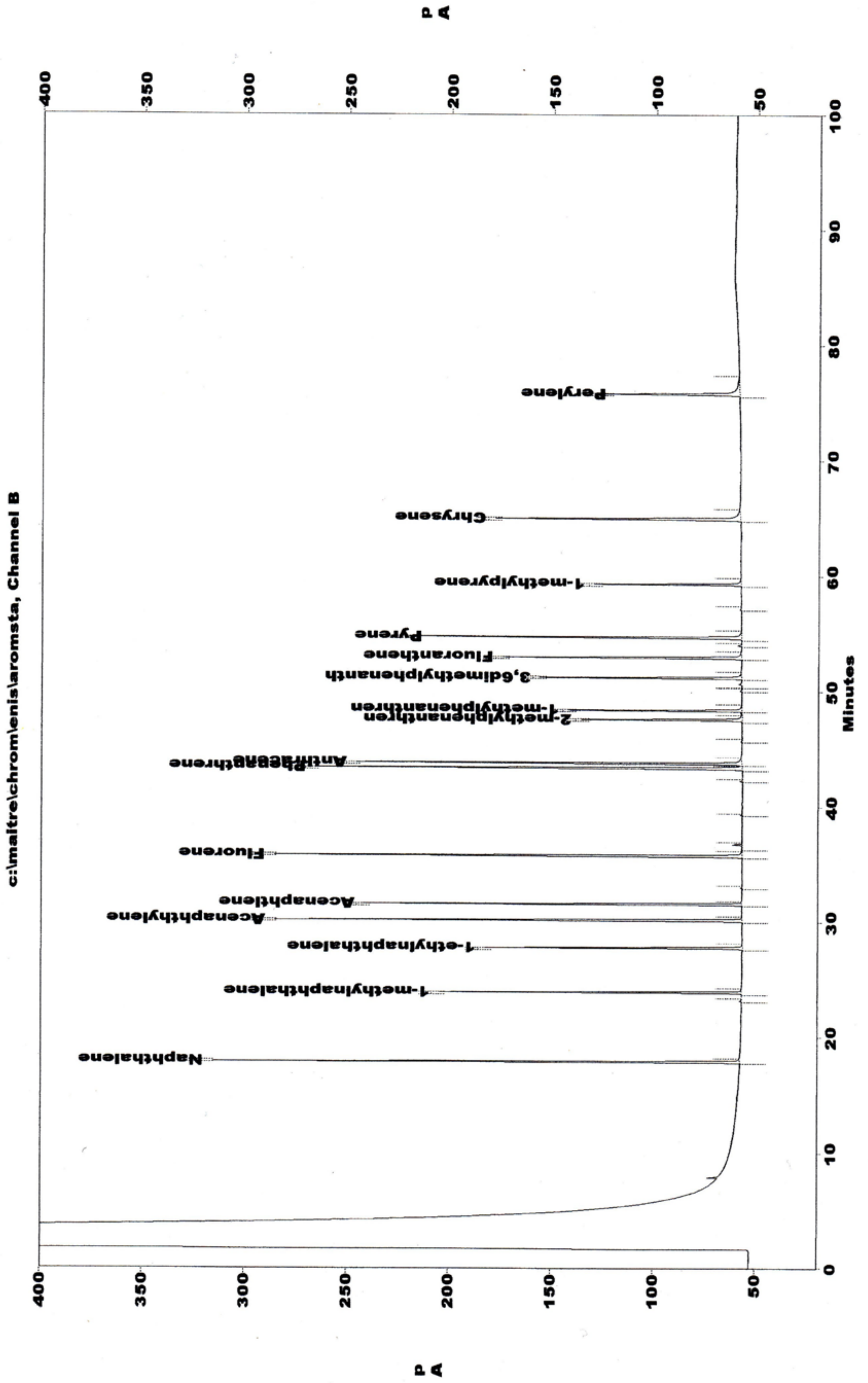


Figure 4.2 Chromatogram for standard polycyclic aromatic hydrocarbons

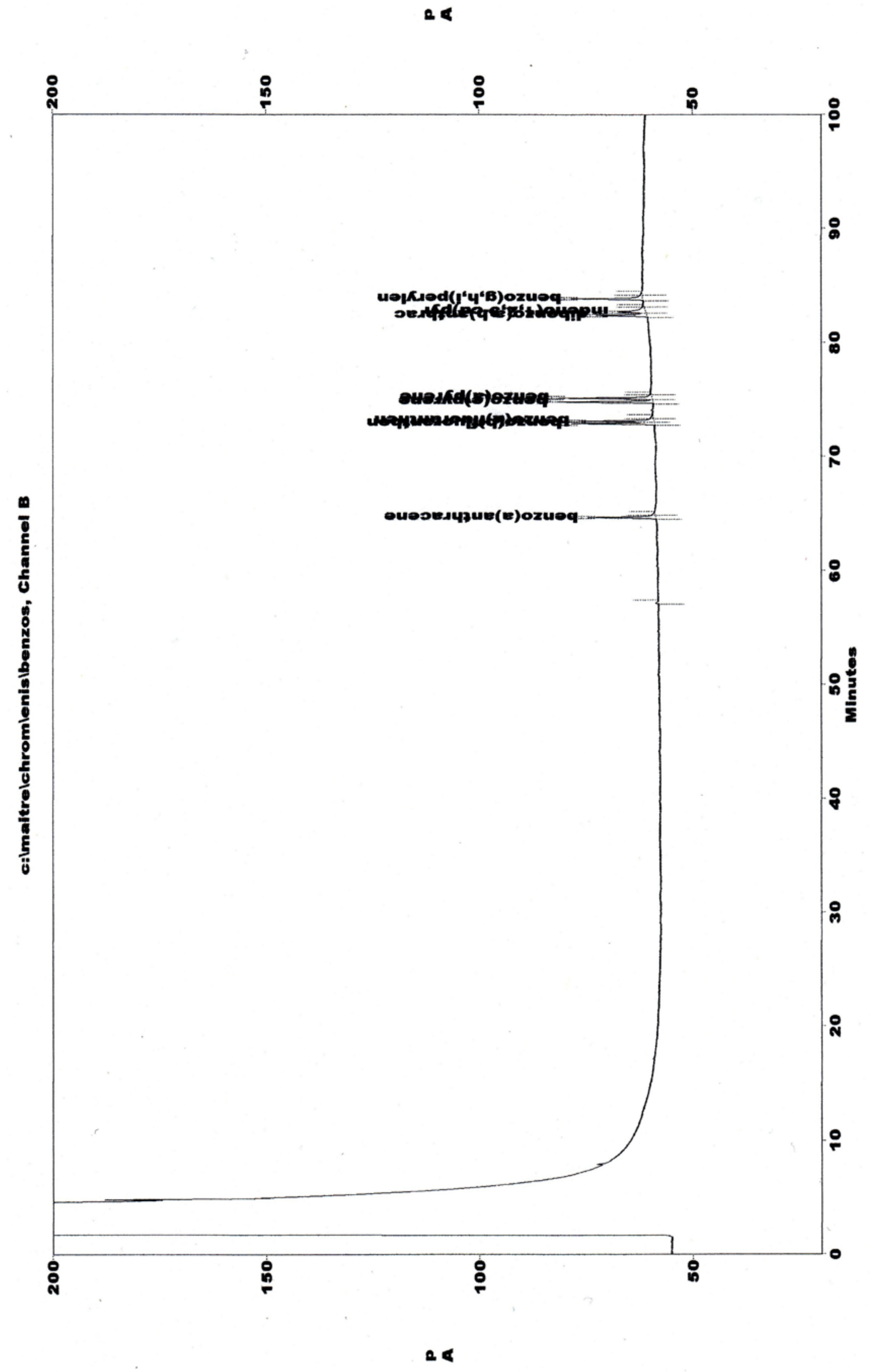


Figure 4.3 Chromatogram for standard benzo compounds

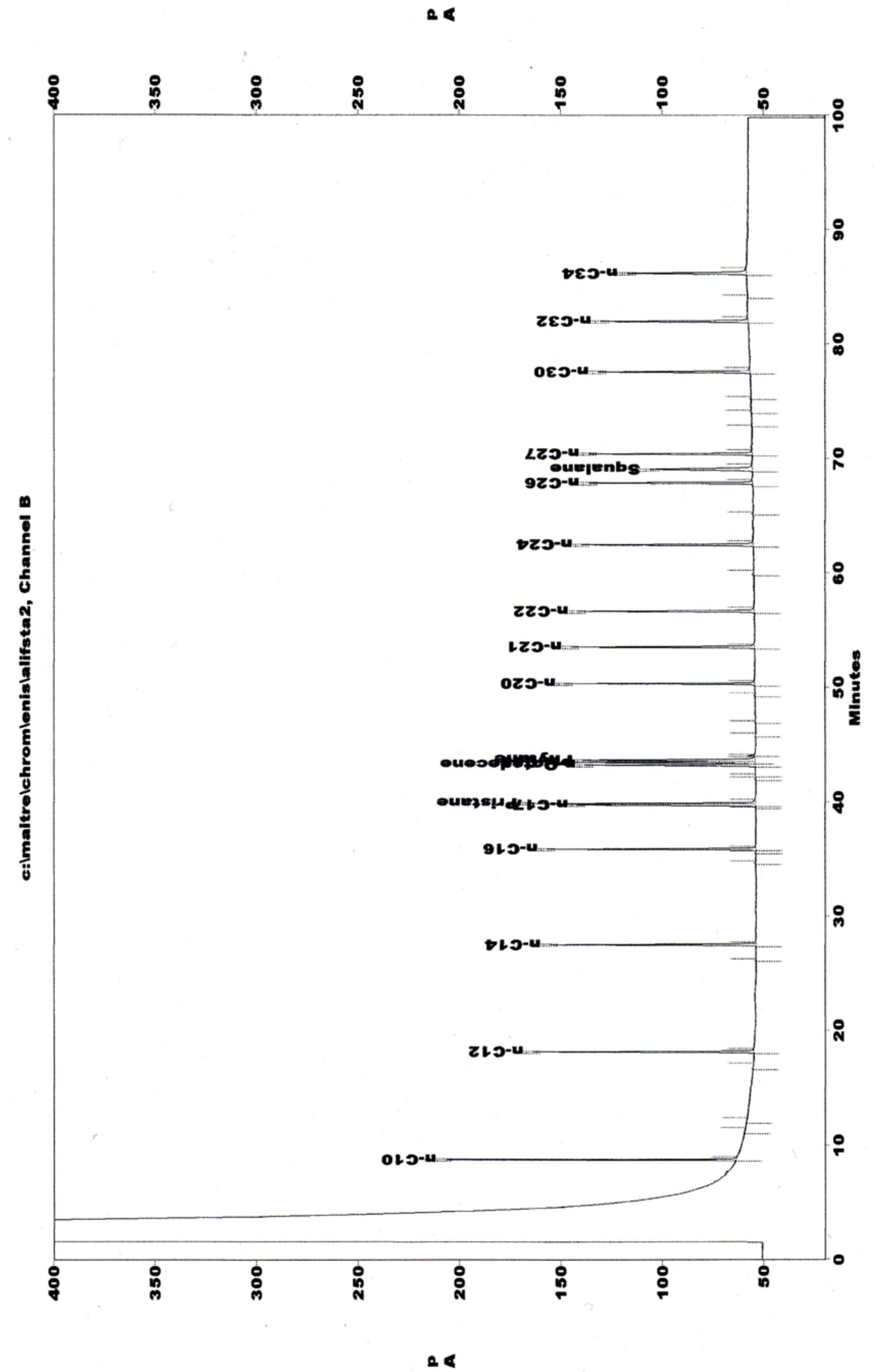


Figure 4.4 Chromatogram for standard aliphatic hydrocarbons

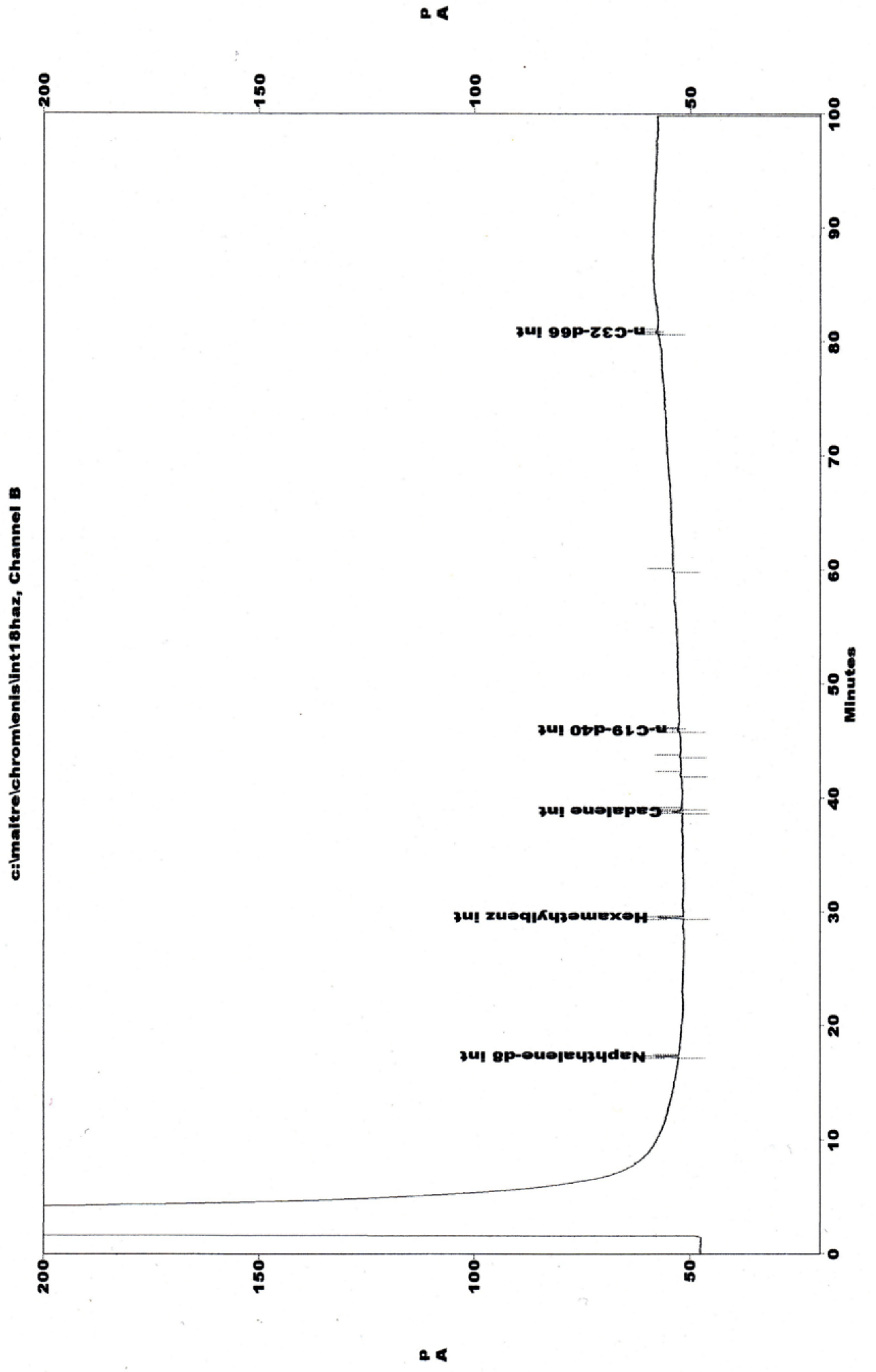


Figure 4.5 Chromatogram for internal satandards

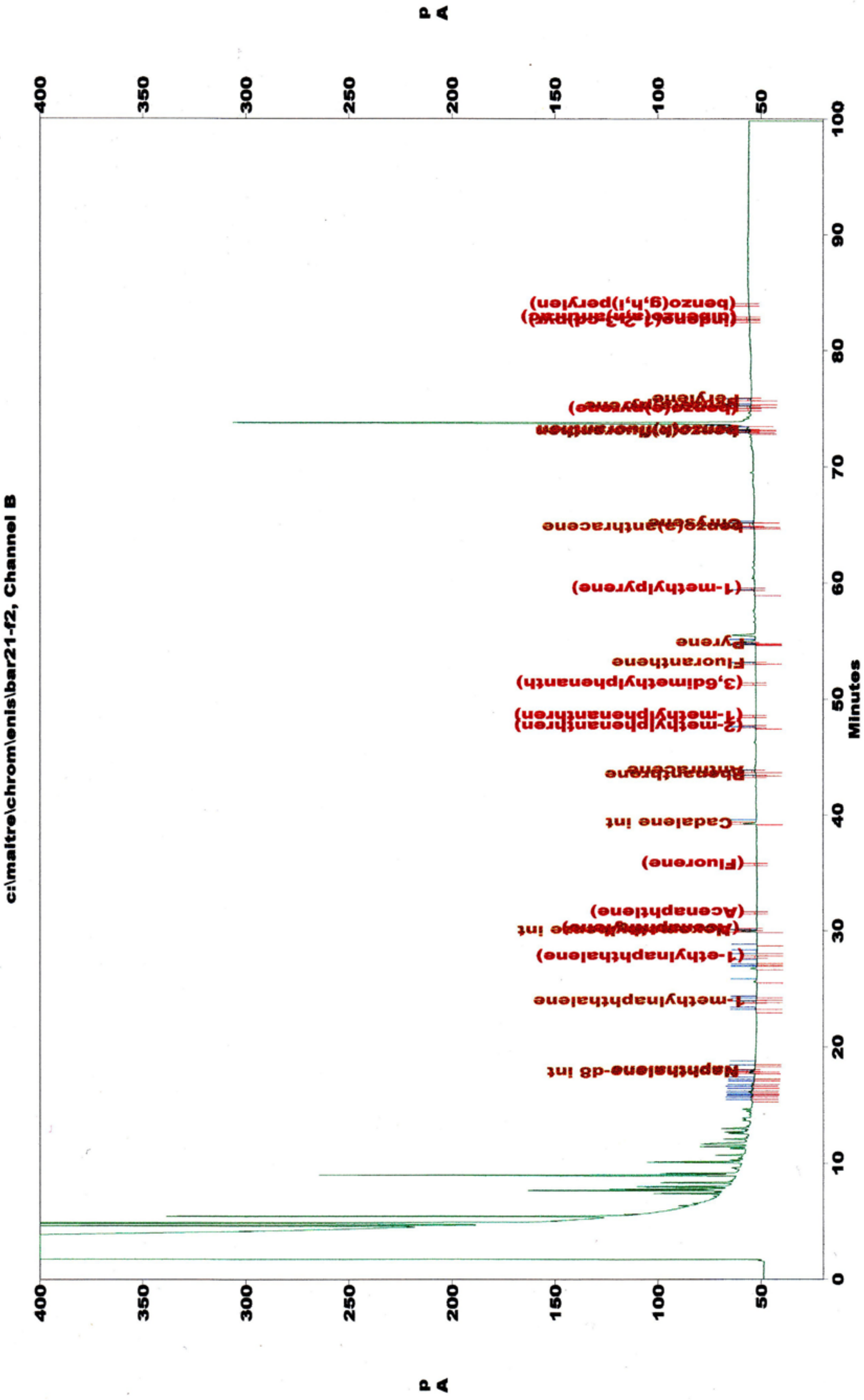


Figure 4.6 Chromatogram of PAHs for *Mullus barbatus*

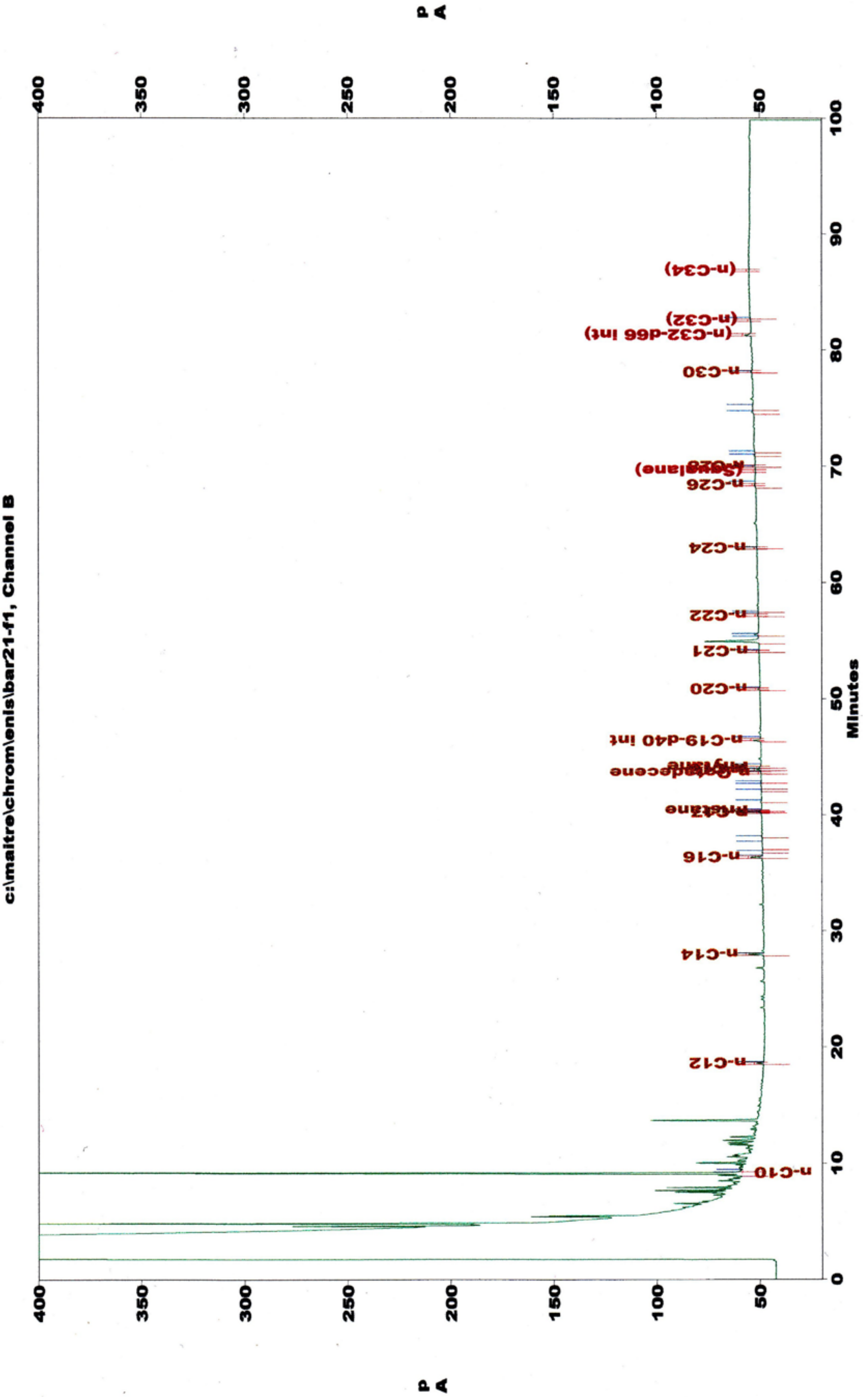


Figure 4.7 Chromatogram of aliphatic hydrocarbons for *Mullus barbatus*

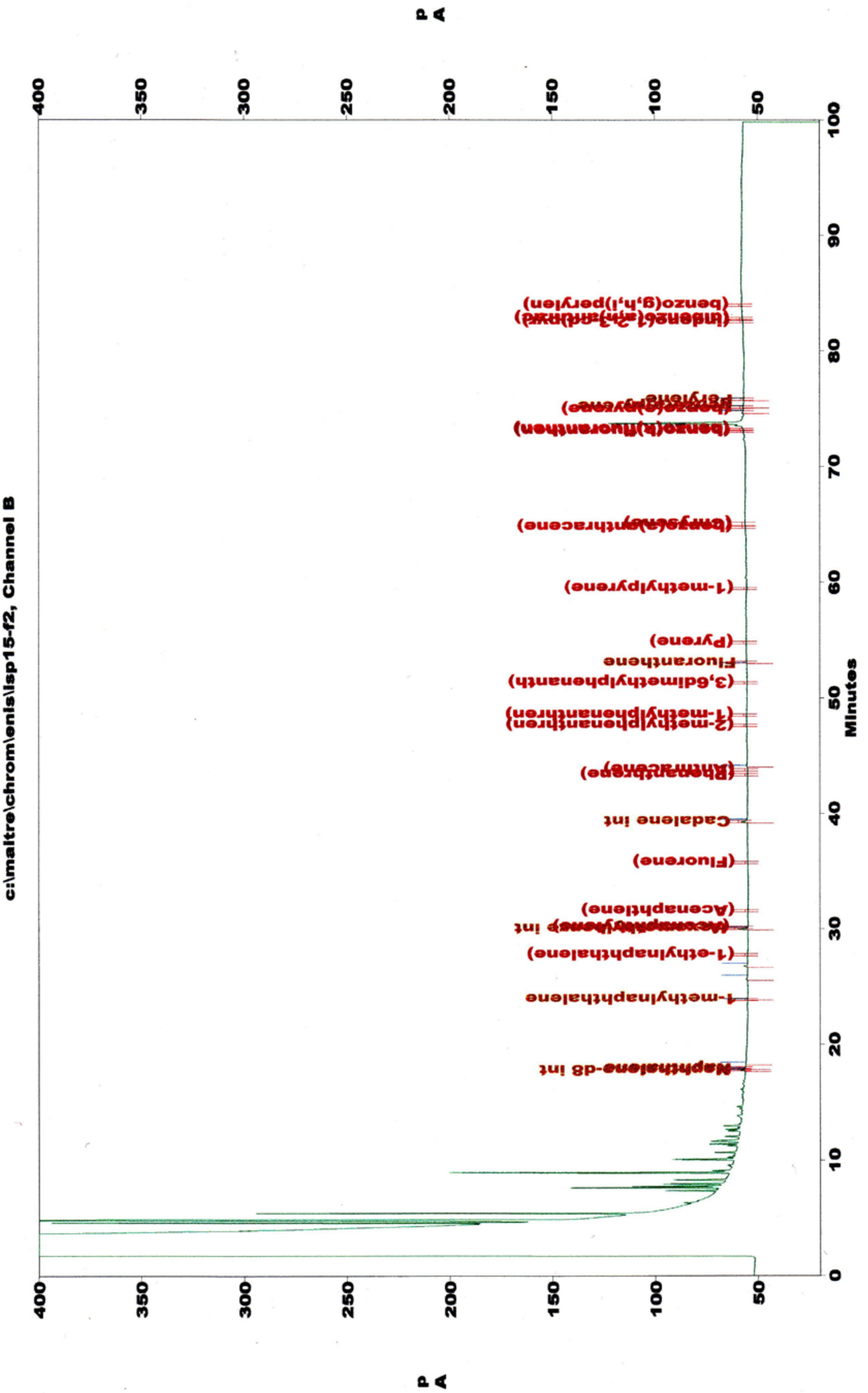


Figure 4.8 Chromatogram of PAHs for *Diplodus annularis*

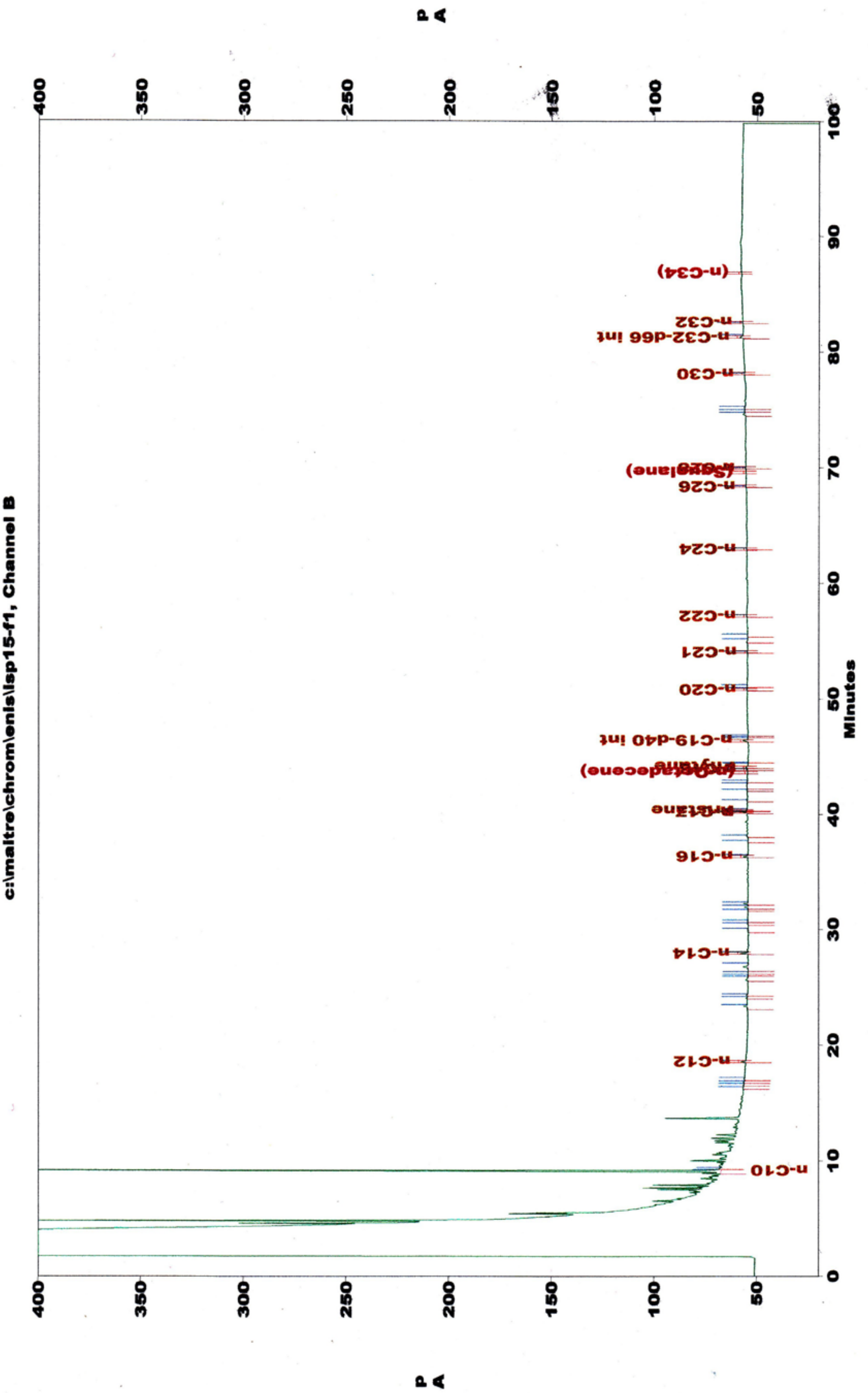


Figure 4.9 Chromatogram of aliphatic hydrocarbons for *Diplodus annularis*

4.2.2 Gas Chromotography Conditions for Petroleum Hydrocarbons

DB-5MS Column 30m x 0.25mm, film thickness 0.25 µm

Carrier gas: Nitrogen (99.99 % pure), flow rate: 1.6 ml/min

Make-up gas flow rate: 20 ml/min

FID Detector temperature: 300°C

Injector temperature: 270°C

Temperature program: Initial temperature 60°C, increases 3°C/min and after reaching to 300°C, analysis continues isothermally for 15 min.

4.3 Quality Assurance

For every set of 10 samples, a procedural blank and spike sample consisting of all reagents is run to check for interference and cross contamination. The aliphatic hydrocarbons and PAHs recoveries are determined relative to the ratio of direct injection of extract and the working standards prepared in hexane. Also, the quality of the analytical data is assured using the reference materials of IAEA-435 tuna fish sample (from the International Laboratory of Marine Radioactivity, IAEA, Monaco) for petroleum hydrocarbons. The recoveries for fish fell within a fairly narrow range, for internal standards between 67.0-87.1%.

4.4 Statistical Analyses

Statistical analyses will perform using STATISTICA for Windows, Release 6.0, and Copyright StatSoft, Inc. 1995. Pearson’s Product-Moment Correlation test will use to check for significant relationships aliphatic, polycyclic aromatic hydrocarbons and extractable organic matter in muscle. In all case, the level of significant was set at $p< 0.05$. One-way analysis of variance (ANOVA) will utilise to investigate effect of sampling periods on variations in aliphatic and polycyclic aromatic hydrocarbons concentrations in fishes from İzmir Bay.

Hierarchical cluster analysis (HCA) which identifies homogenous groups of samples will engage according to the complete linkage between groups of stations in fish samples using PRIMER statistical package. Prior to analysis the raw data were transformed using the log(x+1) transformation.

Multivariate statistical analyses have been largely used as a tool to assess environmental data. In this study, principal component analysis (PCA) was applied to the fish samples. Standardizing data is reasonable when the variables are in different units or the variance of the different columns of raw data is substantial. The data were standardized to eliminate scaling before PCA processing.

CHAPTER FIVE
RESULTS and DISCUSSION

5.1 Aliphatic Hydrocarbons

The aliphatic hydrocarbons concentrations are reported in Table 5.1 and Table 5.2 for *Mullus barbatus* and *Diplodus annularis* during 2000, 2001, 2004 and 2005. The total mean concentrations (Table 5.1 and Table 5.2) ranged from 0.83 to 2.42 µg g^{-1} and 0.44 to 1.72 µg g^{-1} in *Mullus barbatus* and *Diplodus annularis*, respectively. The concentrations of total aliphatics were generally higher than 1 µg g^{-1} dry wt in İzmir Bay. Higher and lower concentrations occurred at sites Gediz and Foca for red mullet, at sites Foca and Gediz for annular sea bream. The recorded concentrations in red mullet were higher than annular sea bream (Figure 5.1).

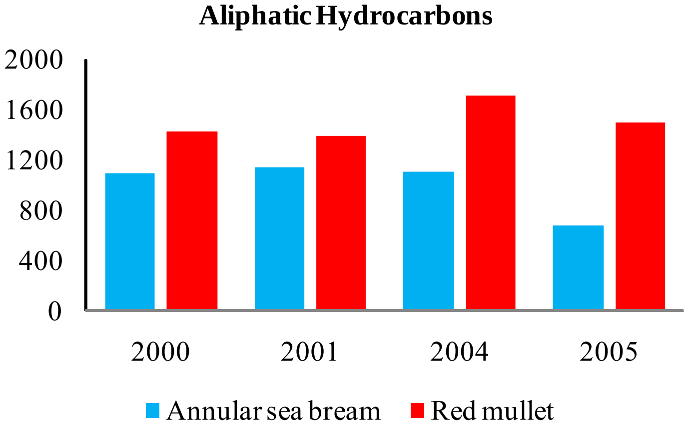


Figure 5.1 Annual aliphatic hydrocarbons concentrations (ng/g dw) in fish species

The ranges, mean ±standard error of individual compounds were reported for red mullet and annular sea bream in Table 5.3 and Table 5.4. Higher concentrations occurred for n-C10, n-C14, n-C16, n-C17, n-C18, n-20 and n-C21 at Gediz; n-C12, Pri, n-C26 and n-C28 at Foca; n-C22, n-C24, Squ, n-C30, n-C32 and n-C34 at Guzelbahce; C18-1 and Phy at Gulbahce in red mullet. Lower concentrations were observed for n-C10 in Guzelbahce; n-C12, n-C17, Pri and Phy in Foca; n-C14, n-C16, n-C18-1, n-C18, n-C21, n-C22 and n-C24 in Uzunada; n-C20 in Gediz. High concentrations are probably related to fisheries and anthropogenic activities from İzmir Bay.

Table 5.1 Concentrations of Aliphatics and Aromatic Hydrocarbons (ng g^{-1} dw), extractable organic matter (mg g^{-1}) and selected molecular ratios in *Mullus barbatus*

	Compounds	Gulbahce				Uzunada			Foca			Guzelbahce		Gediz	
		2000	2001	2004	2005	2000	2001	2005	2000	2001	2004	2004	2005	2004	2005
Aliphatics	nC17	74.0	61.0	168	103	51.0	56.0	98.7	49.6	61.7	46.0	121	165	190	62.0
	Pri	138	152	171	185	52.5	83.1	112	212	156	47.4	128	170	203	64.5
	nC18	88.3	83.0	92.3	48.0	25.2	43.8	63.8	73.3	28.0	37.1	73.0	34.0	97.3	56.2
	Phy	96.3	94.8	114	153	47.6	51.9	72.5	127	120	45.9	76.2	65.0	102	61.3
	Pri/Phy	1.88	1.49	1.44	1.41	1.10	1.60	1.71	1.66	1.29	1.03	1.68	2.62	2.00	1.06
	nC17/Pri	0.61	0.60	0.98	0.73	0.97	0.67	0.87	0.24	0.45	0.97	0.95	0.97	0.94	0.94
	nC18/Phy	0.88	0.87	0.79	0.60	0.53	0.84	0.92	0.58	0.23	0.81	0.96	0.52	0.96	0.91
	Σ(C10-C34)	1387	1425	1371	1167	711	1149	1320	1409	877	720	1394	826	2107	1669
	Σ Aliphatics	1666	1731	1889	1563	841	1293	1525	1793	1169	834	1710	1075	2420	1843
Aromatics	Nap	304	282	245	197	239	323	218	321	288	109	205	350	266	234
	1-m-Nap	15.3	18.5	21.1	14.2	16.4	22.3	10.9	13.5	16.8	8.52	27.9	31.7	21.3	17.4
	1 e-Nap	8.89	nd	13.8	2.06	nd	nd	4.64	16.4	9.62	nd	nd	nd	nd	nd
	Phe	18.0	18.6	nd	4.59	nd	15.0	14.7	11.3	nd	nd	nd	nd	8.06	nd
	Ant	43.0	50.9	nd	nd	nd	78.8	nd	30.6	nd	nd	nd	nd	43.4	nd
	2-m-Phe	14.0	nd	nd	6.16	nd	nd	nd	7.72	nd	nd	nd	nd	10.3	nd
	1-m-Phe	7.30	nd	nd	nd	nd	nd	nd	21.1	nd	nd	18.0	nd	nd	nd
	Flt	4.34	5.73	nd	nd	nd	11.7	2.36	8.07	nd	nd	nd	nd	9.42	23.6
	Pyr	3.79	4.15	nd	nd	nd	10.5	1.84	4.10	nd	nd	nd	nd	7.80	13.5
	BbF	3.86	nd	nd	nd	nd	7.78	1.97	3.47	nd	nd	7.44	nd	nd	nd
	BkF	nd	nd	nd	nd	nd	7.83	1.62	4.60	nd	nd	8.69	nd	nd	nd
	BeP	3.29	4.12	nd	6.97	12.4	nd	6.58	5.04	nd	36.9	nd	nd	7.30	nd
	BaP	42.5	54.4	39.4	44.1	59.0	56.0	45.4	57.4	64.1	47.9	45.7	42.2	40.4	nd
	Per	21.6	21.6	7.76	6.12	32.0	23.6	31.1	20.9	54.3	nd	nd	24.5	25.1	20.6
	Σ PAHs	489	460	327	281	359	556	339	525	433	202	313	448	439	309
	LMW/HMW	5.68	4.30	5.89	4.08	2.47	3.74	2.89	4.07	2.66	1.39	4.05	5.72	3.88	5.82
	BeP/BaP	0.08	0.08	-	0.16	0.21	-	0.14	0.09	-	0.77	-	-	0.18	-
	Phe/Ant	0.42	0.37	-	-	-	0.19	-	0.37	-	-	-	-	0.19	-
	Flt/Pyr	1.14	1.38	-	-	-	1.11	1.28	1.97	-	-	-	-	1.21	1.76
	Flt/(Flt+Pyr)	0.53	0.58	-	-	-	0.53	0.56	0.66	-	-	-	-	0.55	0.64
	HEOM	97.1	132	118	53.6	52.0	167	94.8	174	149	95.0	114	56.0	103	45.2

Table 5.2 Concentrations of Aliphatics and Aromatic Hydrocarbons (ng g^{-1} dw), extractable organic matter (mg g^{-1}) and selected molecular ratios in *Diplodus annularis*

		Gulbahce			Uzunada			Foca		Guzelbahce	Gediz	
		2000	2001	2005	2000	2001	2005	2000	2001	2005	2004	2005
Aliphatics	**Aliphatics**											
	nC17	65.8	44.3	54.7	62.1	39.5	51.5	51.5	56.7	78.0	60.3	38.8
	Pri	73.3	54.0	57.9	64.2	47.7	55.7	55.1	65.4	80.9	64.7	41.7
	nC18	55.4	18.3	43.3	38.8	31.7	40.0	50.7	44.1	50.6	57.2	29.8
	Phy	71.4	41.2	53.9	43.3	34.3	50.3	52.3	55.4	60.4	61.6	32.4
	Pri/Phy	1.03	1.31	1.07	1.48	1.39	1.11	1.05	1.18	1.34	1.05	1.29
	nC17/Pri	0.90	0.82	0.95	0.97	0.83	0.92	0.93	0.87	0.96	0.93	0.93
	nC18/Phy	0.78	0.44	0.80	0.90	0.92	0.80	0.97	0.79	0.84	0.93	0.92
	Σ(C10-C34)	941	1097	368	1078	441	950	884	1562	587	953	362
	Σ Aliphatics	1085	1196	489	1204	540	1059	1006	1724	753	1112	436
Aromatics	**Aromatics**											
	Nap	288	120	208	254	269	160	nd	243	127	172	18.1
	1-m-Nap	18.7	3.96	8.12	8.89	26.6	14.3	nd	17.5	9.37	17.5	2.45
	1 e-Nap	nd	nd	nd	nd	20.5	12.4	nd	10.5	nd	nd	4.71
	Phe	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd
	Ant	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd
	2-m-Phe	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd
	1-m-Phe	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd
	Flt	12.6	4.76	9.08	nd	nd	nd	17.8	22.0	nd	nd	10.7
	Pyr	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd
	BbF	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd
	BkF	nd	nd	14.0	nd	nd	nd	15.3	6.15	nd	nd	nd
	BeP	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd
	BaP	58.0	43.1	50.5	45.4	38.0	40.7	25.0	22.2	35.8	42.2	34.8
	Per	38.0	27.8	41.0	44.0	23.6	8.39	31.0	35.3	13.1	24.1	8.00
	Σ PAHs	415	200	331	352	377	236	89.1	357	185	256	78.7
	LMW/HMW	2.82	1.25	1.91	2.94	5.13	4.40	nd	3.17	4.45	2.86	0.48
	Flt/Pyr	3.61	1.36	2.59	-	-	-	5.08	6.30	-	-	3.06
	HEOM	74.0	51.2	26.8	42.0	60.0	32.4	31.0	60.1	32.3	76.0	13.7

Table 5.3 The ranges and mean±SE of individual aliphatic and aromatic compounds for *Mullus barbatus*

Aliphatics	Range	Mean±SE	Aromatics	Range	Mean±SE
n-C10	20.0-605	260±45.4	**Nap**	109-350	256±16.8
n-C12	14.7-190	120±13.4	**1-m-Nap**	8.52-31.7	18.3±1.68
n-C14	101-458	224±24.7	**1-e-Nap**	nd-16.4	3.96±1.55
n-C16	23.0-272	124±17.9	**Ace**	nd	
n-C17	46.0-190	93.4±13.2	**Acy**	nd	
Pri	47.4-212	134±14.6	**Flu**	nd	
C18-1	8.89-188	31.8±12.6	**Phe**	nd-18.6	6.44±2.02
n-C18	25.2-97.3	60.2±6.62	**Ant**	nd-78.8	17.6±7.08
Phy	45.9-153	87.7±8.80	**2-m-Phe**	nd-14.0	2.72±1.27
n-C20	21.7-133	56.9±8.30	**1-m-Phe**	nd-21.1	3.32±1.92
n-C21	17.8-134	54.2±8.84	**3,6-dm-Phe**	nd	
n-C22	13.7-55.8	31.5±3.48	**Flt**	nd-23.6	4.66±1.82
n-C24	37.1-211	89.7±11.3	**Pyr**	nd-13.5	3.26±1.18
n-C26	0-92.4	32.9±6.29	**1-m-Pyr**	nd	
Squ	0-91.7	19.7±6.54	**BaA**	nd	
n-C28	0-230	61.1±15.1	**Chr**	nd	
n-C30	0-80.7	27.3±5.27	**BbF**	nd-7.78	1.75±0.76
n-C32	0-76.4	9.00±6.27	**BkF**	nd-8.69	1.62±0.82
n-C34	0-77.9	9.18±6.40	**BeP**	nd-36.9	5.90±2.60
			BaP	nd-64.1	45.6±4.07
			Per	nd-54.3	20.6±3.81
			IP	nd	
			dBA	nd	
			Bper	nd	

In annular sea bream, maximum concentrations were found for n-C10, n-C16, C18-1, n-C21, n-C22, n-C26 and n-C28 at Foca; n-C14 at Uzunada; n-C17 and Pri at Guzelbahce; n-C12, n-C18, n-C20, n-C24, Squ, n-C30 and n-C32 at Gediz; Phy at Gulbahce.

Table 5.4 The ranges and mean±SE of individual aliphatic and aromatic compounds for *Diplodus annularis*

Aliphatics	Range	Mean±SE	Aromatics	Range	Mean±SE
n-C10	nd-262	260±78.3	**Nap**	nd-288	169±29.2
n-C12	nd-338	85.8±27.3	**1-m-Nap**	nd-26.6	11.6±2.44
n-C14	55.8-224	132±14.4	**1-e-Nap**	nd-20.5	4.38±2.13
n-C16	8.15-100	49.6±8.26	**Ace**	nd	
n-C17	38.8-78.0	54.8±3.53	**Acy**	nd	
Pri	41.7-80.9	60.0±3.38	**Flu**	nd	
C18-1	nd-29.7	7.14±2.97	**Phe**	nd	
n-C18	18.3-57.2	41.8±3.56	**Ant**	nd	
Phy	32.4-71.4	50.6±3.60	**2-m-Phe**	nd	
n-C20	18.3-52.7	31.3±3.12	**1-m-Phe**	nd	
n-C21	18.4-55.4	33.0±3.64	**3,6-dm-Phe**	nd	
n-C22	nd-64.3	18.5±5.17	**Flt**	nd-22.0	7.00±2.42
n-C24	13.3-104	56.3±9.88	**Pyr**	nd	
n-C26	nd-75.0	23.6±7.30	**1-m-Pyr**	nd	
Squ	nd-33.0	7.93±3.66	**BaA**	nd	
n-C28	nd-126	15.7±11.4	**Chr**	nd	
n-C30	nd-54.6	21.3±5.54	**BbF**	nd	
n-C32	nd-58.8	14.1±5.75	**BkF**	nd-15.3	3.23±1.80
n-C34	nd		**BeP**	nd	
			BaP	22.2-58.0	39.6±3.12
			Per	8.00-44.0	26.7±3.83
			IP	nd	
			dBA	nd	
			Bper	nd	

Generally the recorded individual aliphatic hydrocarbons concentrations were higher for red mullet than the recorded for annular sea bream. The values of n-C17 and n-C18 ranged between 46-190 and 25-97 ng.g^{-1} for red mullet; 39-78 and 18-57 ng.g^{-1} for annular sea bream in the study area, respectively. High concentrations for n-C17 at Gediz site in 2004 and at Guzelbahce site in 2005 for red mullet and annular sea bream, respectively; for n-C18 at Gediz site in 2004 for both species.

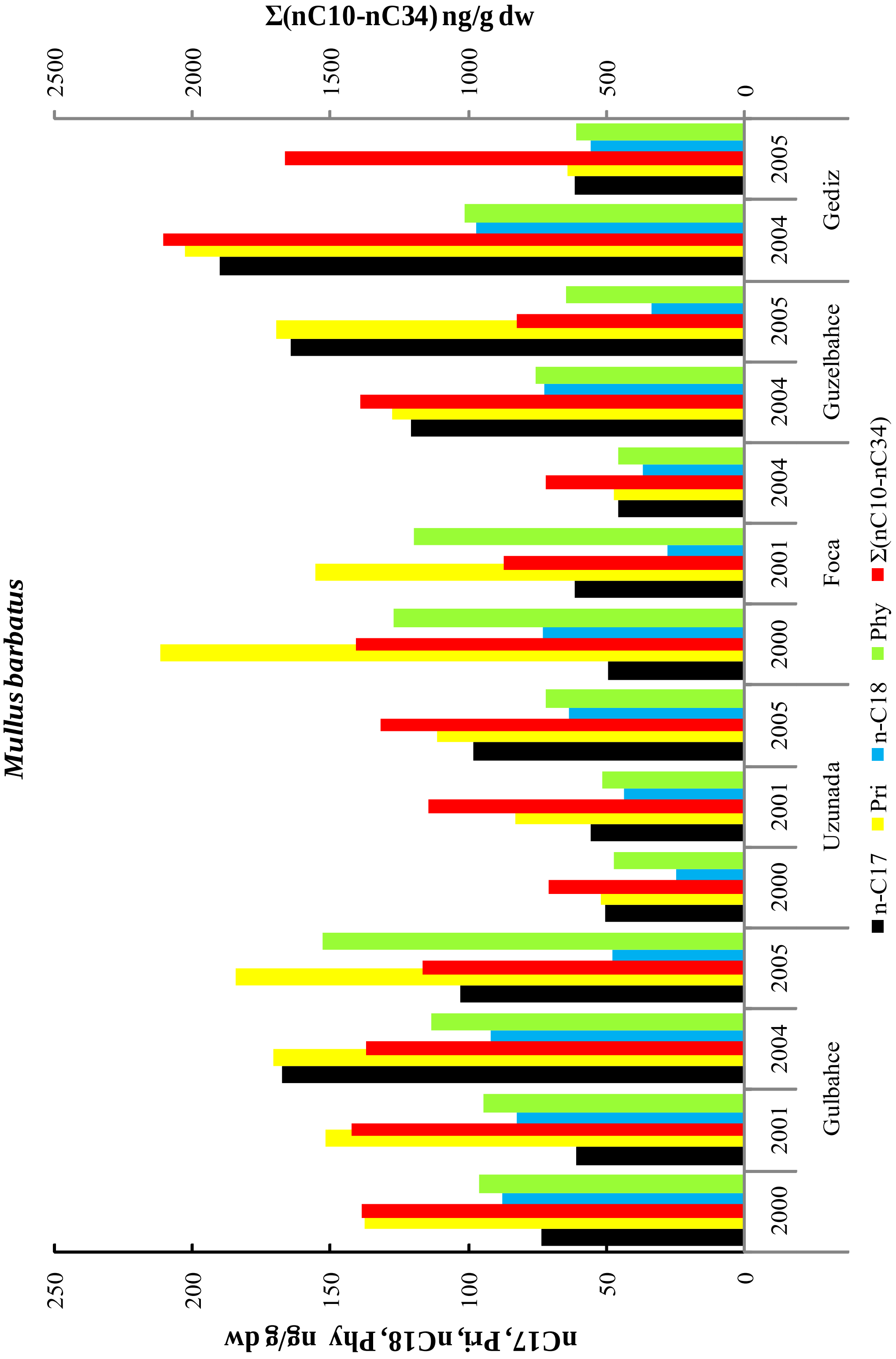


Figure 5.2 Selected aliphatic hydrocarbon concentrations for *Mullus barbatus* from İzmir Bay

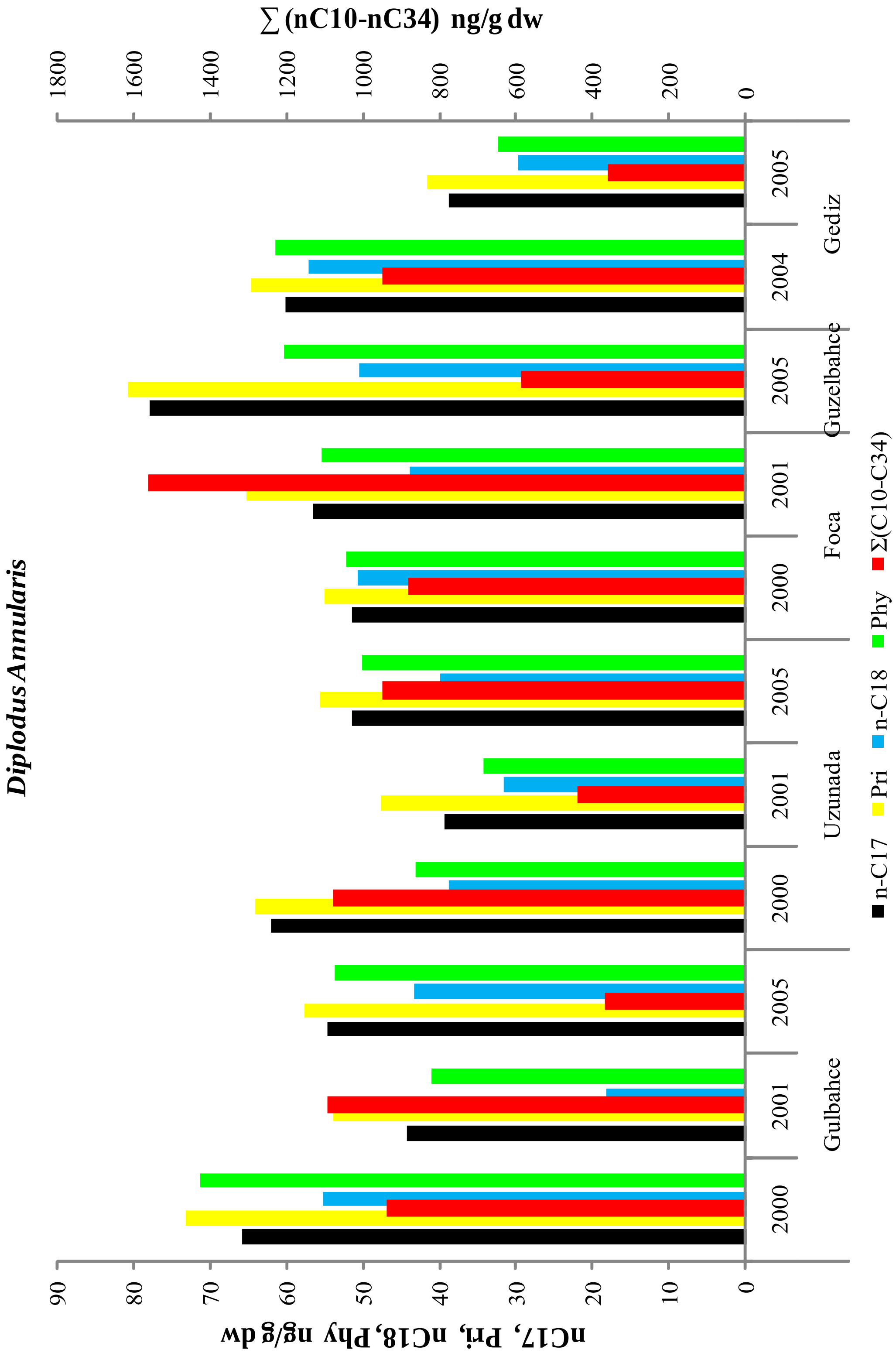


Figure 5.3 Selected aliphatic hydrocarbon concentrations for Diplodus annularis from İzmir Bay

Some selected aliphatic hydrocarbon (n-C17, Pri, Phy, n-C18, ΣC10-C34) concentrations during sampling periods for red mullet and annular seabream were given Figure 5.2 and 5.3.

The values of Pri and Phy ranged between 47-212 and 46-153 $ng.g^{-1}$ for red mullet; 42-81 and 32-71 $ng.g^{-1}$ for annular sea bream in the study area, respectively. High concentrations for Pri at Foca site in 2000 and at Guzelbahce site in 2005 for red mullet and annular sea bream; for Phy at Gulbahce site in 2005 and at Gulbahce site in 2000 for both species, respectively.

In general, pristane has a biochemical origin while phytane comes from geochemical processes. Pristane (C19) and phytane (C20) are often considered as good indicators of petroleum contamination. The ratio of pristane to phytane varies between oils reflecting the depositional environment of the original source. As a rule, a high ratio of pristane to phytane or the predominance of a single isoprenoid (such as pristane) indicates a biogenic source (UNEP/IOC/IAEA, 1992).

For both fish species Pri/Phy ratios were >1 indicating the biogenic source. The ratio of Pri/Phy range from 1.03 to 2.62 and 1.03 to 1.48 for red mullet and annular sea bream, respectively. The highest values were found in Gulbahce region during 2004 for red mullet and in Uzunada region during 2000 for annular sea bream.

The origin of these two isoprenoid hydrocarbons, C17 and C18 are plankton (Lacaze, 1980), algae (Blumer et al., 1971) and bacteria (Albaiges et al, 1984). The ratio of nC17/pristane and nC18/phytane are often used as qualitative measures of biodegradation (Ezra et al., 2000), because pristane and phytane are relatively persistent biomarkers. As the fuel contaminant is biodegraded, bacteria preferentially consume the nC17 and nC18 compounds, resulting in a relative enrichment of pristane and phytane in the residue. The relative nC18/Phy and nC17/Pri ratios at all stations ranged from 0.23-0.96 and 0.24-0.98 for red mullet, 0.44-0.97 and 0.82-0.97 for annular sea bream, respectively.

Table 5.5 Occurrence of PAHs and Aliphatics in fish from different worldwide field collection (ng g^{-1})

Species	**Location**	**Total PAHs**	**Total Aliphatics**		**Reference**
Epinephelus coioides	Gulf and the Gulf of Oman	11.8-115	120-1500	dw	Tolosa et al., 2005
Solea solea	Egyptian Mediterranean Sea	1768	319	ww	El Deeb et al, 2007
Donax truaulus	Egyptian Mediterranean Sea	1137	22	ww	El Deeb et al, 2007
Peneaus japonicas	Egyptian Mediterranean Sea	923	307	ww	El Deeb et al, 2007
Diplodus vulgaris	Egyptian Mediterranean Sea	1509	326	ww	El Deeb et al, 2007
Mullus barbatus	Aegean Sea	30-460	-	ww	Kucuksezgin et al., 1999
Anguilla anguilla)	France	314-1984	-	dw	Buet et al., 2006
Mullus barbatus	Spain	3.1	-	ww	Llobet et al., 2006
Mullus barbatus	Adriatic-Italy	2.28-6.66	-	ww	Corsi et al., 2002
Mullus barbatus	Catalan Mediterranean coast	50-190	-	ww	Porte & Albaiges, 1993
Mullus surmuletus	Catalan Mediterranean coast	40-100	-	ww	Porte & Albaiges, 1993
*Thunnus thynn*us	Catalan Mediterranean coast	40-150	-	ww	Porte & Albaiges, 1993
Mullus barbatus	Saros Bay, NE Aegean	121	-	dw	IMST-165, 2009
Merliccius merliccius	Saros Bay, NE Aegean	97	-	dw	IMST-165, 2009
Merlangius merlangus	Saros Bay, NE Aegean	104	-	dw	IMST-165, 2009
Mullus barbatus	Adriatic Sea, Italy	16.5	-	ww	Perugini et al., 2007
Scomber scombrus	Adriatic Sea, Italy	63.3	-	ww	Perugini et al., 2007
Merluccius merluccius	Adriatic Sea, Italy	44.1	-	ww	Perugini et al., 2007
Oreochromis mossambicus	Hong Kong, China	15.1 to 92.5	-	ww	Kong et al., 2005
Mullus barbatus	Adriatic Sea	78.77-124.28	-	ww	Torre et al., 2010
Mullus barbatus	İzmir Bay, Aegean Sea	202-556	834-2420	dw	*This study*
Diplodus annularis	İzmir Bay, Aegean Sea	78.7-415	436-1724	dw	*This study*

Σ(C10-C34) concentrations range between 711-2107 for red mullet, 362-1562 ng g^{-1}dw for annular sea bream. The highest values of Σ(C10-C34) were observed in Gediz and Foca for red mullet and annular sea bream, while the lowest values were found in Uzunada and Gediz for red mullet and annular sea bream, respectively. The ratios of Σ(C10-C34)/TOTAL ALIP range between 0.72-0.91 for red mullet and 0.71-0.91 for annular sea bream (Table 5.1 and Table 5.2).

Aliphatics and PAHs concentrations in marine biota around the world are shown in Table 5.5. Aliphatic hydrocarbon concentrations in fish from İzmir Bay were lower than those in North Western Mediterranean coast (Villeneuve et al., 1999), Cartagana Bay (Parga-Lozano et al., 2002), Gulf of Naples (Amodio-Cocchieri & Cirillo, 2003), Gulf of Oman (Tolosa et al., 2005), and higher than those in Egyptian Mediterranean Sea (El Deeb et al., 2007). Total hexane extractable organic matter (HEOM) ranged from 45 to 174 (mg g^{-1}) in red mullet, from 14 to 76 (mg g^{-1}) in annular sea bream from İzmir Bay (Table 5.1 and Table 5.2).

5.2 Polycyclic Aromatic Hydrocarbons

The PAHs concentrations in samples collected in different periods are reported in Table 5.1 and Table 5.2. The total mean concentrations ranged from 202 to 556 ng g^{-1} dw and 78.7 to 415 ng g^{-1} dw in *Mullus barbatus* and *Diplodus annularis*, respectively. The concentrations of total aromatics were generally lower than 1 µg g^{-1} dry wt in İzmir Bay. Higher and lower concentrations occurred at sites Uzunada and Foca for red mullet, at sites Gulbahce and Gediz for annular sea bream. The recorded concentrations in red mullet were higher than annular sea bream during sampling periods (Figure 5.4).

In red mullet, the specific PAH Ace, Acy, Flu, 3,6-dm-Phe, 1-m-Pyr, BaA, Chr, IP, dBA and BPer were under the detection limits. Individual PAH compounds ranged between for Nap 109-350; 1-m-Nap 9-32; 1-e-Nap nd-16; Phe nd-19; Ant nd-79; 2-m-Phe nd-14; 1-m-Phe nd-21; Flt nd-24; Pyr nd-14; BbF nd-8; BkF nd-9; BeP nd-37; BaP nd-64 and Per nd-54 ng g^{-1}dw. Higher concentrations occurred for Nap, 1-m-Nap and BkF at Guzelbahce; 1-e-Nap, 1-m-Phe, BeP, BaP and Per at Foca; Phe and 2-m-Phe at Gulbahce; Ant and BbF at Uzunada; Flt and Pyr at Gediz.

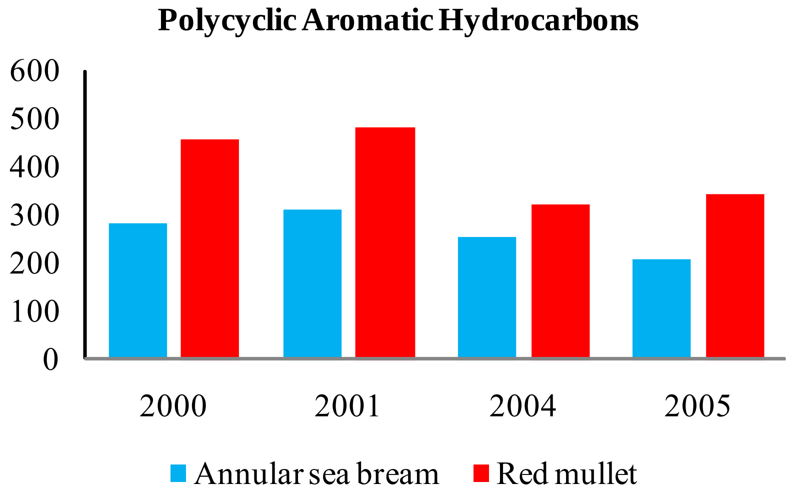


Figure 5.4 Annual Polycyclic Aromatic Hydrocarbons concentrations (ng/g dw) in fish species

In annular sea bream, the specific PAH Ace, Acy, Flu, Phe, Ant, 2-m-Phe, 1-m-Phe, 3,6-dm-Phe, Pyr, 1-m-Pyr, BaA, Chr, BbF, BeP, IP, dBA and BPer were not detected. Individual PAH compounds ranged between for Nap nd-288; 1-m-Nap nd-27; 1-e-Nap nd-21; Flt nd-22; BkF nd-15; BaP 22-58 and Per 8-44. Higher concentrations occurred for Nap and BaP at Gulbahce; 1-m-Nap, 1-e-Nap and Per at Uzunada; Flt and BkF at Foca.

Some selected aromatic hydrocarbons (1-m-Nap, BeP, BaP, Per, Nap) concentrations during sampling periods for red mullet and annular seabream were given Figure 5.5 and 5.6.

Naphthalene is the dominant PAH for both fish species. According to Thomann & Komlos (1999), among PAHs with Log K_{ow} less than 5, no metabolism was documented for naphthalene, phenanthrene, and anthracene in fish. Therefore, direct exchange with the water through the gills served as higher levels of naphthalene in red mullet and annular sea bream.

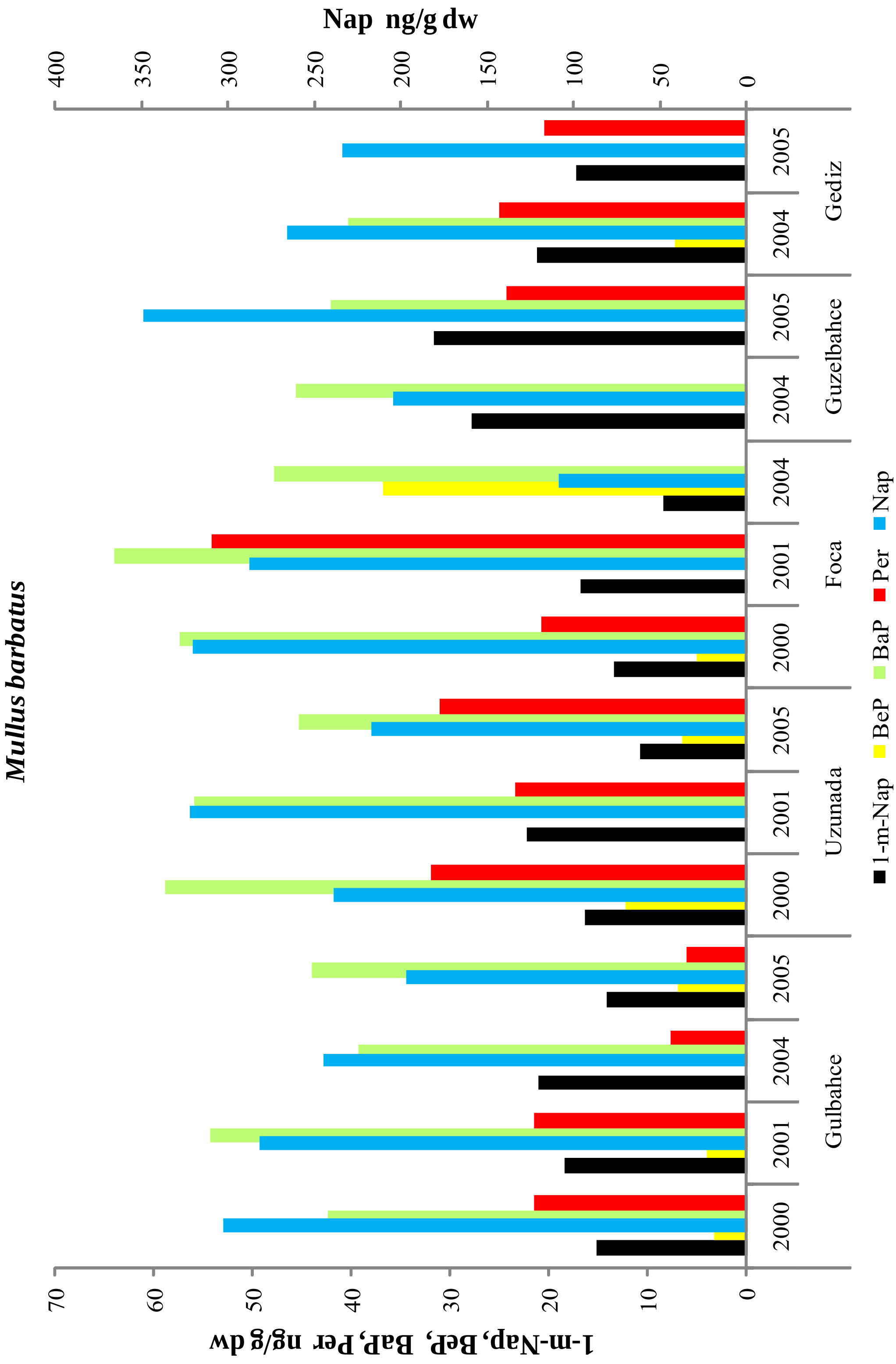


Figure 5.5 Selected aromatic hydrocarbon concentrations for red mullet from İzmir Bay

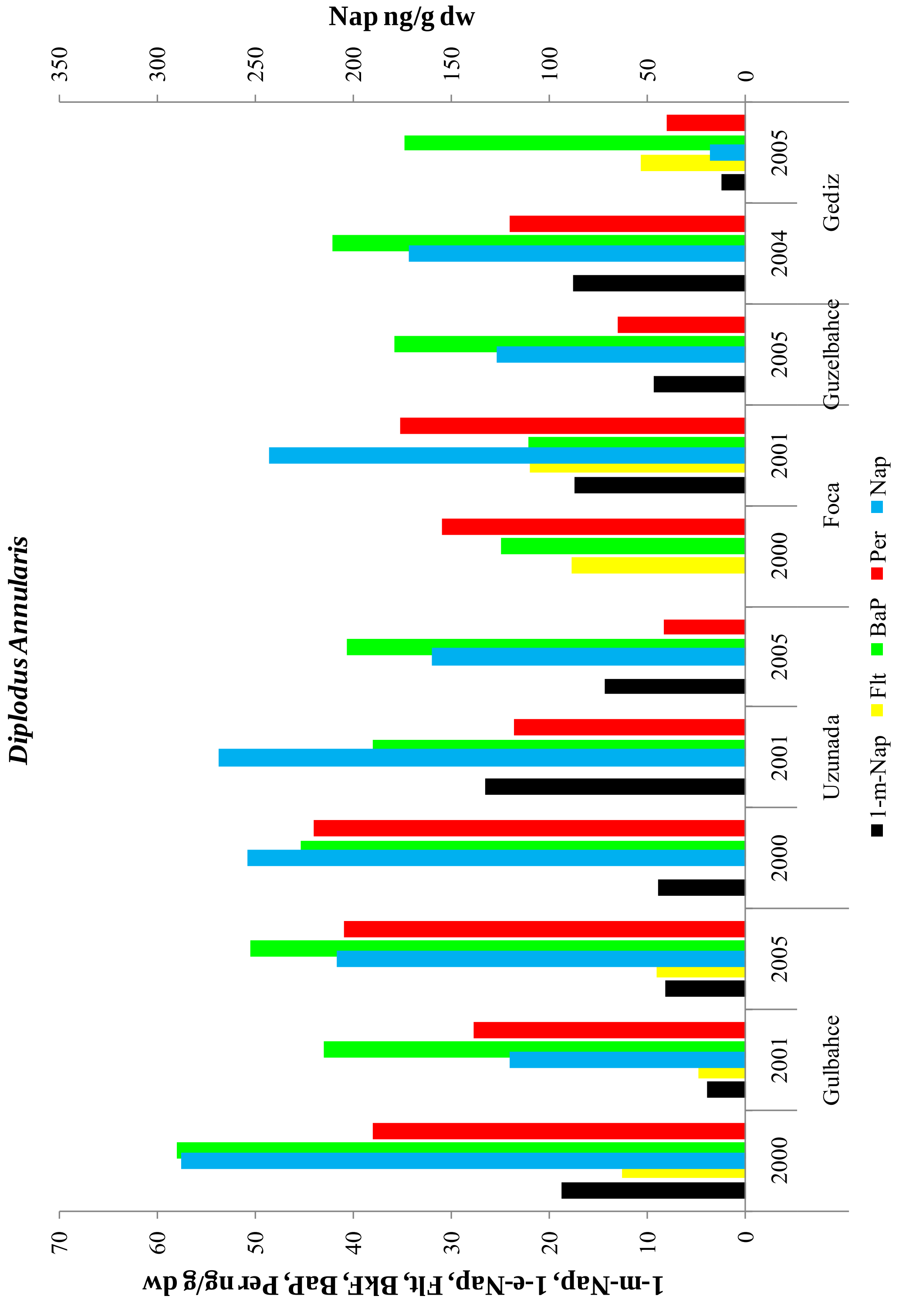


Figure 5.6 Selected aromatic hydrocarbon concentrations for annular seabream From İzmir Bay

Composition pattern of PAHs in red mullet were dominated by 2 and 5 ring PAHs along the contamination gradient. The percentage ratio of 2-ring (Nap, 1-m-Nap and 1-e-Nap), 5-ring (BbF, BkF, BeP, BaP and Per), 3-ring (Phe, Ant, 2-m-Phe and 1-m-Phe), 4-ring (Flt and Pyr) to total PAHs were 71.4%, 20.2%, 6.4%, 1.9%, respectively in the bay. 2 and 5 ring PAHs were accounting for about 91.6% of ∑PAH, while 3 and 4 ring PAHs were less abundant (8.4%).

Composition pattern of PAHs in annular sea bream were dominated by 2 and 5 ring PAHs along the contamination gradient. The percentage ratio of 2-ring (Nap, 1-m-Nap and 1-e-Nap), 5-ring (BkF, BaP and Per), 4-ring (Flt) to total PAHs were 63.1%, 32.5%, 4.4%, respectively in the bay. 2 and 5 ring PAHs were accounting for about 95.60% of ∑PAH, while 4 ring PAHs were less abundant (4.4%).

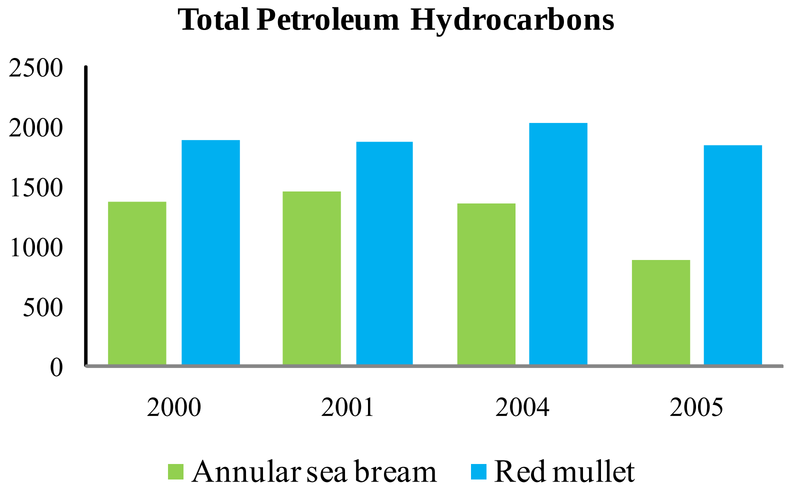


Figure 5.7 Annual Total Petroleum Hydrocarbons concentrations (ng/g dw) in fish species

Mean concentrations of total hydrocarbon for İzmir Bay are given in Figure 5.7. In terms of total hydrocarbons, concentrations range from 1036 to 2860 and 514 to 2081 ng g^{-1} dry wt for red mullet and annular sea bream, respectively. Higher concentrations were found for red mullet than annular sea bream. The highest total hydrocarbon levels were found at Gediz 2004 for red mullet and at Foca 2001 for annular sea bream.

The molecular ratios were calculated to precise the pyrolytic or petrogenic processes generating PAH (Baumard et al., 1999; Maldonado et al., 1999; Tolosa et al., 2004). PAH molecular indices have been widely used to identify potential PAH sources in marine environment samples; these are largely based on the differences in thermodynamic stability observed in compounds (Readman et al., 1987). Two different categories have been defined: Phe/Ant > 10 for the petrogenic sources and Phe/Ant < 10 for the dominance of pyrolytic sources (Budzinski et al., 1997). In general, a ratio of Phe/Ant of <10, Flt/Pyr of >1 and Flt/(Flt+Pyr)>0.5 suggests that PAH contamination arises from pyrolytic origins (Table 2.3); all samples were characterized as Phe/Ant < 10 and Flt/Pyr > 1 in red mullet. Equivalent values for the (Phe/Ant), (Flt/Pyr) and Flt/(Flt + Pyr) are presented in Table 5.1. In annular sea bream, when values for Pyr and BbF were not detected, a value of half the detection limit was used for ratios calculation. The higher Flt/Pyr ratios from İzmir Bay indicate that PAH pollution was contributed by pyrolitic input (Table 5.2). This is likely due to the shipping activities, fisheries and residential heating by the coal. El Deeb et al. (2007) reported a method using the ratios BbF/BaP and BkF/BaP (pyrolitic<0.5; petrogenic>0.5) to indicate the sources of PAHs. All the BbF/BaP and BkF/BaP ratios lower than 0.5 suggesting pyrolitic origin of PAHs in both species.

The choice of ratio is based on the fact that petrogenic contamination distribution is characterized by the predominance of low molecular weight PAHs, while high molecular weight PAHs dominate in pyrolytic contamination distribution (Soclo et al., 2000). The abundance ratio of two and three ring hydrocarbons to four to six ring hydrocarbons (LMW/HMW) can be used to distinguish petrogenic and pyrolytic sources (Tam et al., 2001). The ratios of LMW/HMW were lower than 1, suggesting significant PAH inputs from pyrolytic sources (Table 2.3). On the contrary higher (>1) LMW/HMW ratios in recorded samples from İzmir Bay indicate that the major PAH pollution was contributed by petrogenic in red mullet and annular sea bream except for Gediz in 2005, this is likely due to the higher Nap concentrations (because of the low K_{ow} value and the more water soluble compound compared to the other PAHs).

PAHs concentrations in fish species around the world are shown in Table 5.5. Polycyclic aromatic hydrocarbon concentrations in fish from İzmir Bay were lower than those in Abu Qir Bay (El Deeb et al., 2007), Bahia Blanca Estuary (Arias et al., 2009) and higher than those in Adriatic Sea (Perugini et al., 2007), Gulf of Oman (Tolosa et al., 2005), Catalonia (Llobet et al., 2006), Mai Po Marshes Nature Reserve (Liang et al., 2007), Pearl River delta (Kong et al., 2005).

For PAHs concentrations if we used classification approach generated by Baumard et al. (1998a) [low (0-100 ng/g dw), moderate (>100-1000 ng/g dw), high (>1000-5000 ng/g dw) and very high (>5000 ng/g dw)] our results showed that the average PAHs contamination levels was within the “moderate” category in the study area.

5.3 Statistical Analysis

Total hexane extractable organic matter (HEOM) ranged from 45 to 174 (mg g^{-1}) in red mullet, from 14 to 76 (mg g^{-1}) in annular sea bream from İzmir Bay (Table 5.1 and 5.2). Total aliphatics and total aromatic hydrocarbon concentrations in fish showed no relation to HEOM.

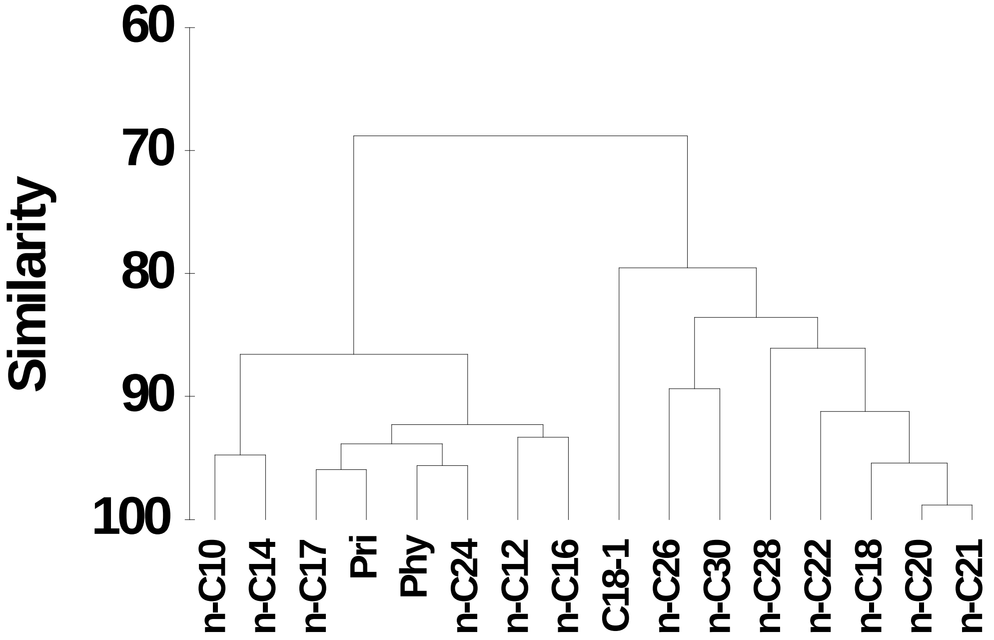
The ANOVA test showed no significant temporal differences for total aliphatics ($p<0.05$) in *Mullus barbatus* ($p=0.7923$, $F=0.3461$, df=3) and *Diplodus annularis* ($p=0.1715$, $F=1.9279$, df=3). The ANOVA test showed the significant temporal differences for total PAHs in *Mullus barbatus* ($p=0.029$, $F=3.6313$, df=3), while no significant differences were detected in *Diplodus annularis* ($p=0.6051$, $F=0.6343$, df=3).

Cluster analysis was performed to identify the complete linkage groups of individual aliphatics in *Mullus barbatus* and *Diplodus annularis*. The data set normalized using log (x+1) transformation. Results are shown in the hierarchical dendogram for İzmir Bay in Figure 5.8, which distinguished the aliphatics in to two major groups in *Mullus barbatus*. The first group generally contained high molecular weight; the second included lower molecular weight of aliphatics. Three large clusters of aliphatics with sub-groups were identified in *Diplodus annularis*. The first group contains maximum concentrations of nC10, the second group included nC12, nC14, nC16, nC17, Pri, nC18, Phy, nC20, nC21, nC24; and the third indicates other aliphatics in *Diplodus annularis* (Figure 5.9).

Cluster analysis was performed to identify the homogeneous groups of individual PAHs occurring in *Mullus barbatus*. Results are shown in the hierarchical dendogram in Figure 5.10, which distinguished the 14 individual PAHs into two major groups. The first major group included 1-m-Phe, BbF and BkF, which are mainly high molecular weight PAHs with 3-5 rings. The second major group included two subgroups. The first subgroup contained Nap, 1-m-Nap, Per and BaP, two of them including 5 rings. BaP is usually detected in pyrogenic sources, e.g., combustion of coal, wood, vehicle fuel and waste tire (Arias et al., 2009), and although it cannot be explained why Nap was classified in this group. The second subgroup included PAHs (Flt, Pyr, Phe, Ant, 2-m-Phe and BeP) with 3-5 rings except 1-e-Nap.

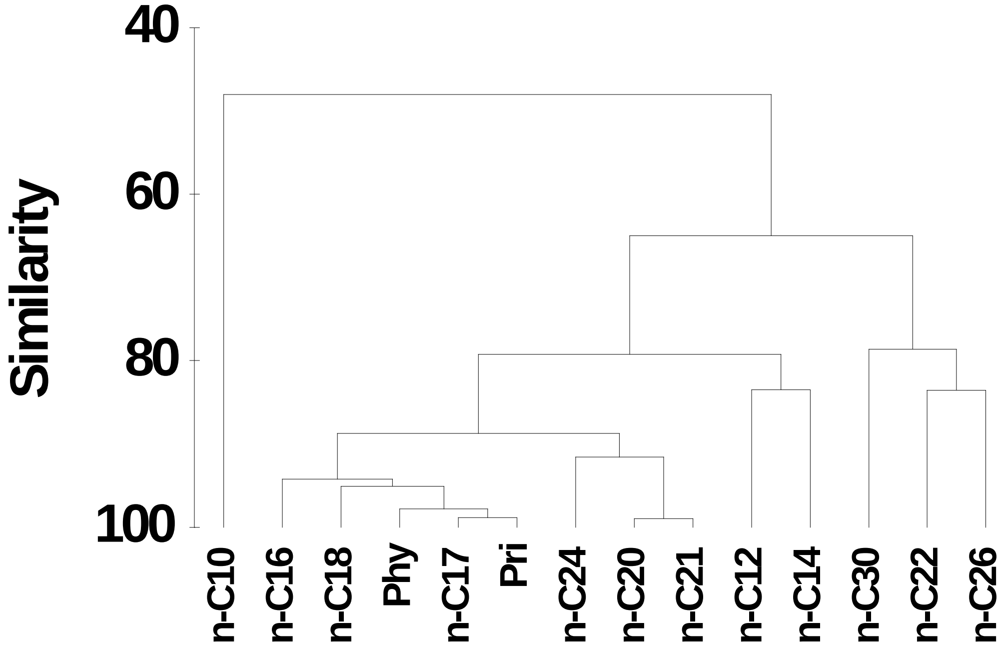
Two clusters with sub-groups could be distinguished, coinciding with the previously defined classes in *Diplodus annularis*. The first group (G1) represents Flt and BkF; the second (G2) contains Nap, 1-e-Nap, 1-m-Nap, BaP and Per indicate 2 and 5 rings (Figure 5.11).

In this study, principal component analysis (PCA) was applied to the fish samples. The first step in the multivariate statistical analysis was application of PCA with the aim to group the individual aliphatic hydrocarbons. The PCA results were generally similar to that of the cluster analysis in the previous paragraph. The PCA calculation for aliphatic compounds resulted in three principal components describing almost 90.2 % of the total variability. Principal components described giving account for 65.6 %, 15.3 % and 9.3% respectively of the total variance in *Mullus barbatus*. nC18, nC18-1, Phy, nC20, nC21, nC22, nC26, nC28 and nC30 in the positive part of PC1 were grouped together. nC12, nC14, nC16, nC17, Pri and nC24 are well differentiated by the higher scores on the second component in the negative part. PC3 represented nC10 in the negative part (Figure 5.12).



G1 G2

Figure 5.8 Hierarchical dendogram for aliphatic hydrocarbons in *Mullus barbatus* from İzmir Bay



G1 G2 G3

Figure 5.9 Hierarchical dendogram for aliphatic hydrocarbons in *Diplodus annularis* from İzmir Bay

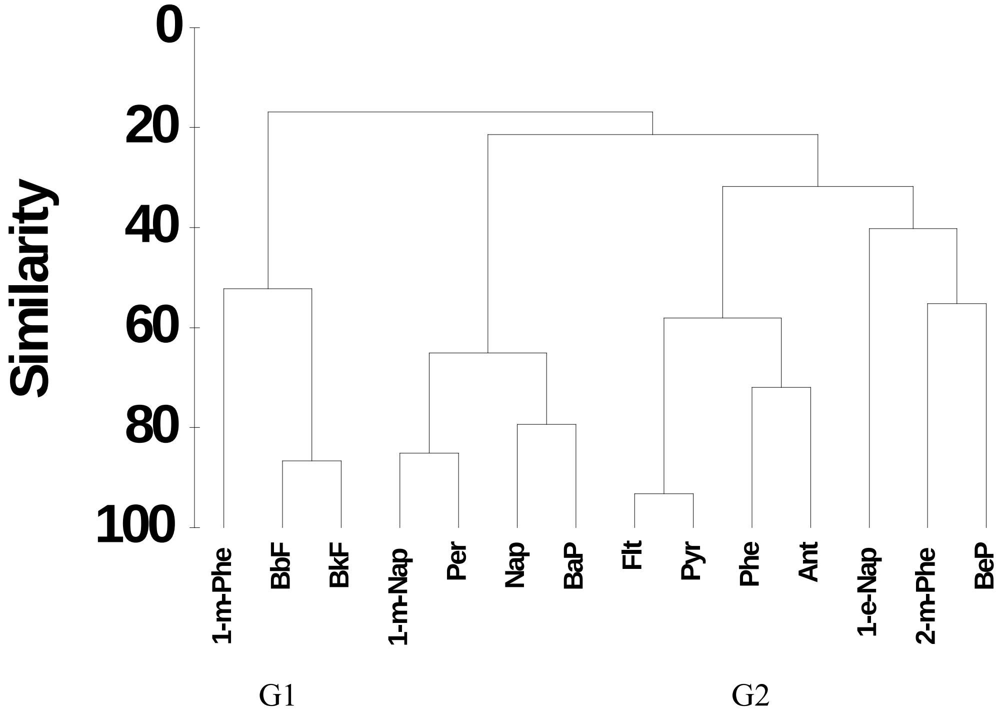


Figure 5.10 Hierarchical dendogram for PAHs in *Mullus barbatus* from İzmir Bay

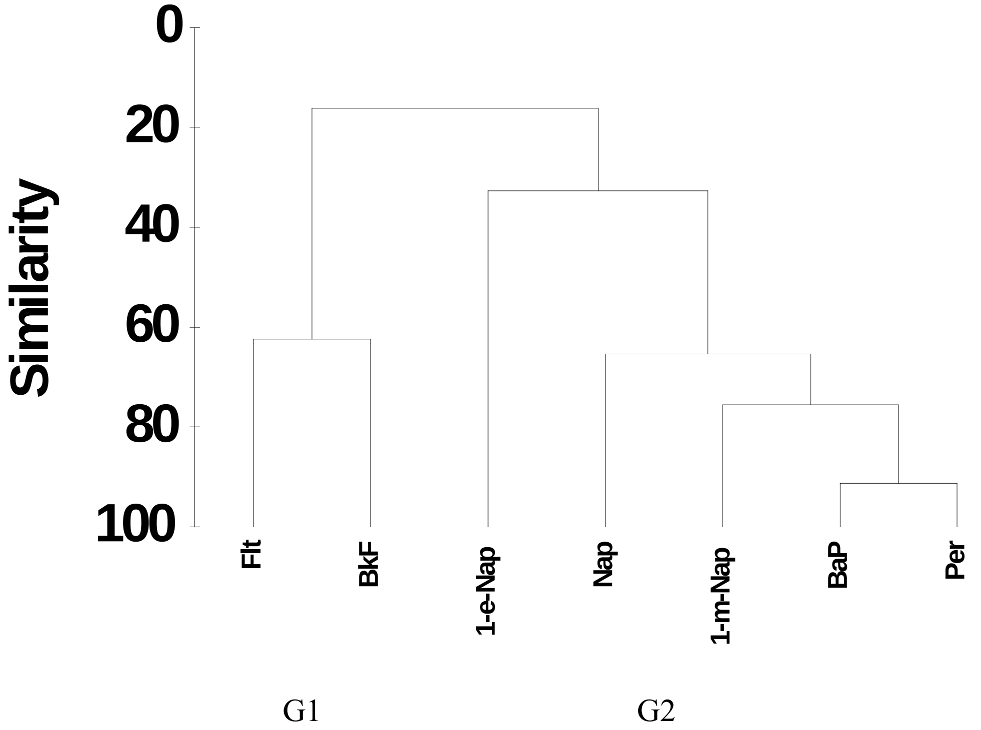


Figure 5.11 Hierarchical dendogram for PAHs in *Diplodus annularis* from İzmir Bay

The first three principal components explained 92.4 % of the total variability in *Diplodus annularis* (Figure 5.13). Those three principal components were highly weighted due to the contributions of aliphatic hydrocarbons. After auto scaling, two significant components were identified giving account for 55.4 % and 26.6 % of the total variance, respectively. The third component takes into account only 10.5 % of the total variance. The horizontal axis PC1 correlated with nC12, nC16, nC18, nC20, nC22, nC26, nC30 in the positive part. The vertical axis PC2 represented nC14, nC17, Pri, Phy and nC24 in the positive part. PC3 represented nC10 in the positive part.

In *Mullus barbatus*, the first three principal components explained 99.3 % of the total variance with the values for PC1, PC2 and PC3 being 97.2%, 1.5 % and 0.6 %, respectively. The first component was mainly positively related to 1-m-Nap, Ant, 2-m-Phe, 1-m-Phe, Flt, Pyr, BbF, BkF, Bep, Phe and Per, whereas the second component was positively related to BaP. PC3 represented Nap in the positive part (Figure 5.14). Consequently PC1 was defined as a general traffic emission/fossil fuel combustion component. Flt and Pyr are typical markers for pyrolysis or in complete combustion. On the other hand, focusing at fuel combustion emission profile predominantly contains Phe, Flt and Pyr (Wang et al., 2009). 2-m-Nap, Phe and Ant belong to the LMW PAHs with 2-3 rings or alkyl-substituted PAHs, which are abundant in petrogenic sources mainly caused by petroleum spills (Arias et al., 2009).

The score plot of first two components allows for the characterization of the aromatic compounds according to the first and second component. In *Diplodus annularis*, three principal components explained 99.8 % of the total variance with the values for PC1, PC2 and PC3 of 83.3 %, 12.6 % and 3.9 %, respectively. The scatter plot of the component scores on PC1/PC2 is depicted in Fig. 5.15, which clearly shows that component 1 was represented by compounds 1-m-Nap, 1-e-Nap, BkF, Flt and Per in the positive part. BkF is a component of fossil fuels and a portion of BkF is associated with its combustion (Kavouras et al., 2001). PC2 represented Nap in the positive part.

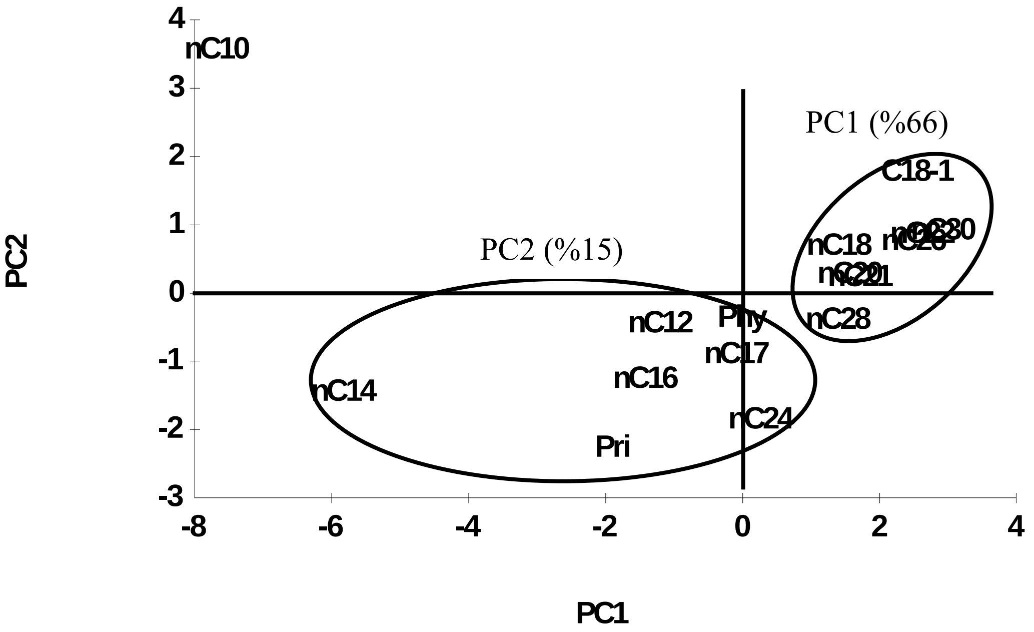


Figure 5.12 Principal component analysis loading plots for *Mullus barbatus* collected in the different sites of İzmir Bay for aliphatics

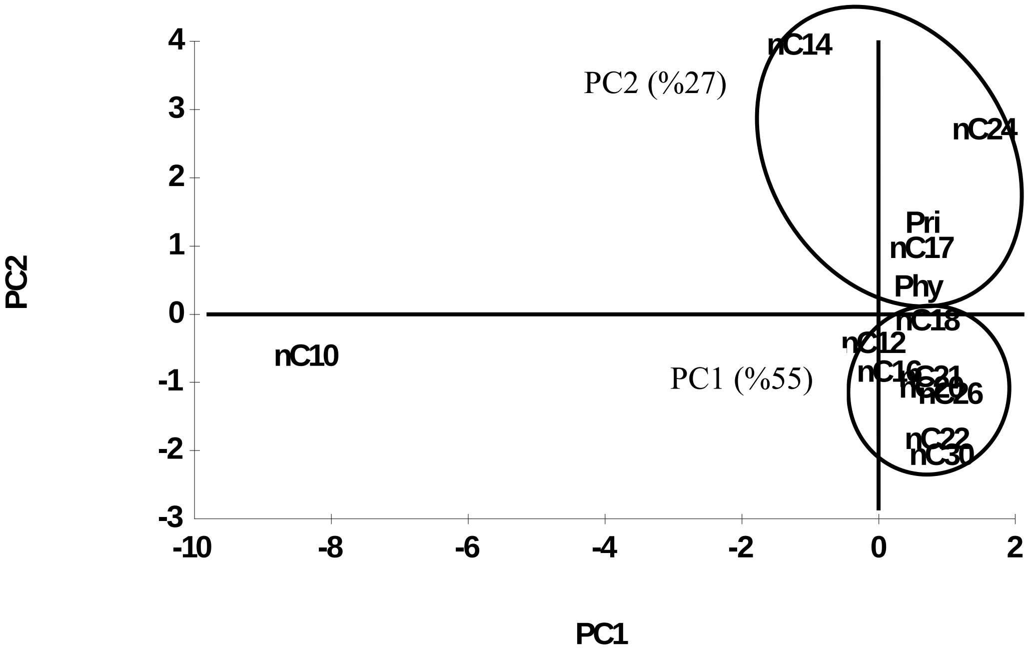


Figure 5.13 Principal component analysis loading plots for *Diplodus annularis* collected in the different sites of İzmir Bay for aliphatics

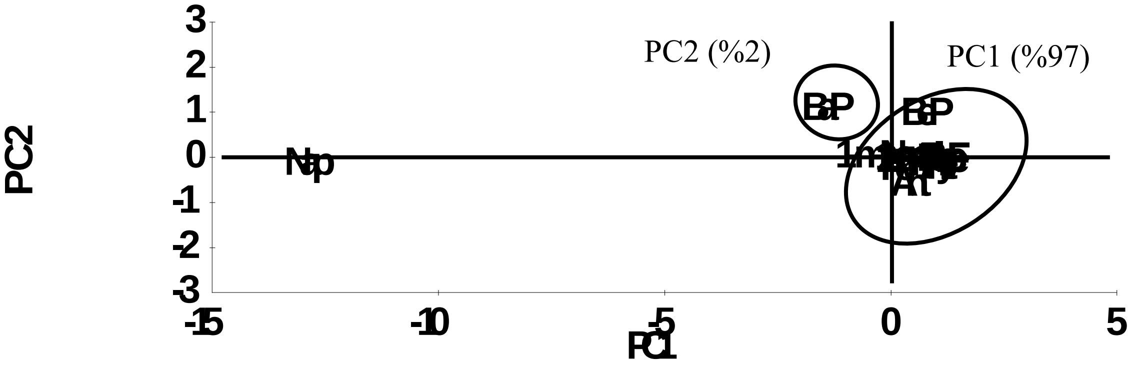


Figure 5.14 Principal component analysis loading plots for *Mullus barbatus* collected in the different sites of İzmir Bay for PAHs

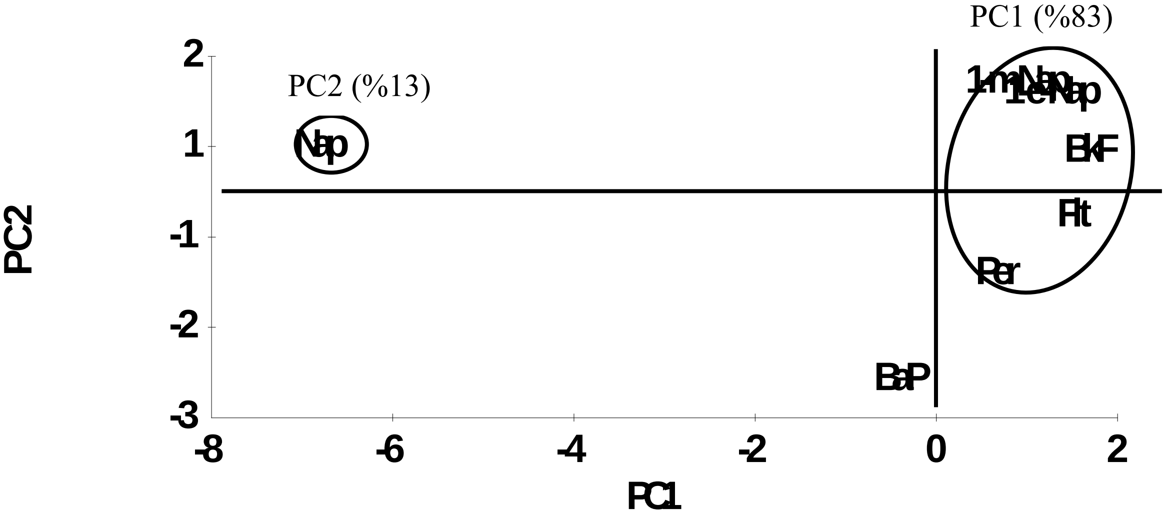


Figure 5.15 Principal component analysis loading plots for *Diplodus annularis* collected in the different sites of İzmir Bay for PAHs

Nap exhibit high pollution extent and was clearly separated from the other compounds. BaP is well differentiated by the higher scores on the third component in the positive part. BaP is associated with traffic emission/fosil fuel combustion component. In general the heavier PAHs are the result of combustion/pyrolitic processes and are absent in crude oil or refined products (Wang et al., 1999).

Studies from the 1960s onward began to report PAHs pollutes sites and since then, PAHs concentrations that may cause adverse effects in marine organisms are incipiently reported around the world. Since February 2005 the European Regulation 208/2005/EC fixed at 5 ng g^{-1} wet weight the maximum admissible concentrations for BaP in crustaceans and cephalopods, at 2 and 10 ng g^{-1} wwt (wet weight) in fish muscle and in shellfish, respectively (Commission Regulation, 2005). The highest values were observed in Gulbahce for annular sea bream and Foca for red mullet. In present study average value of dry weight to wet weight about 0.20 because of that all samples analyzed showed a value of BaP for red mullet and annular sea bream from İzmir Bay being well below the values established by the law.

CHAPTER SIX
CONCLUSIONS

The present work represents the first detailed study of the distribution and origin of petroleum hydrocarbons in fish from the İzmir Bay, an area economically important for the western part of Turkey due to maritime, industrial and tourism activities. This investigation showed petroleum hydrocarbons in the bay consist in a mixture of compounds from natural and anthropogenic sources.

In this study, principal component analysis (PCA) was applied to the fish samples. Both HCA and PCA could differentiate two and three groups with subgroups of aliphatics and aromatics for two species. The ANOVA test showed no significant temporal and spatial differences for total aliphatics and PAHs in *Diplodus annularis,* while significant temporal differences were observed for PAHs in *Mullus barbatus*.

Naphthalene is the dominant PAHs for both fish species. In the present study, among PAHs with low Log K_{ow}, no metabolism was documented for naphthalene in fish. Therefore, direct exchange with the water through the gills served as higher levels of naphthalene in two species.

Considering concentration ratios between the different PAHs, it was possible to indicate pyrolysis processes as the most probable source of contamination. The ratios of pristane and phytane were higher than 1 in two species, indicating a high contribution from biogenic hydrocarbons.

Levels of total PAHs in fish at various sampling locations of the İzmir Bay were found moderate concentrations, registering a wide spectrum of molecular weights. The levels of BaP all the fish samples were below the guideline of 10 ng g^{-1} ww reported by European Commission (Commission Regulation, 2005).

The levels of petroleum hydrocarbons in two species (red mullet and annular sea bream) were comparable to the concentrations observed in relatively unpolluted areas elsewhere in the world. Regular monitoring of the area and more specific analyses are recommended in İzmir Bay. Further studies should be necessary to improve the state of knowledge on the relative potency of individual PAHs and estimate their carcinogenic toxicity.

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