DOKUZ EYLÜL UNIVERSITY
GRADUATE SCHOOL OF NATURAL AND APPLIED SCIENCES

DISTRIBUTIONS OF PCB CONGENERS AND RISK ASSESSMENT IN BIOTA FROM İZMİR BAY, EASTERN AEGEAN

by
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December, 2018
İZMİR

DISTRIBUTIONS OF PCB CONGENERS AND RISK ASSESSMENT IN BIOTA FROM İZMİR BAY, EASTERN AEGEAN

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We have read the thesis entitled **"DISTRIBUTIONS OF PCB CONGENERS AND RISK ASSESSMENT IN BIOTA FROM İZMİR BAY, EASTERN AEGEAN"** completed by **ONUR YURDAKUL** 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 Master of Science.

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ABSTRACT

Fish are one of the biota species often used as an environmental bioindicator to monitor a level of pollution in the marine environment. The levels of PCBs in two fish species from the İzmir Bay was investigated. A total of 624 individuals of different fish species were sampled by trawling in İzmir Bay (Foca-Gediz, Uzunada, Gulbahce) in 2010,2011,2012,2013. The concentrations of PCBs in all samples ranged between bdl-34.3 nanogram in gram (dry weight) from all sites during sampling periods. The highest levels of PCBs were found in Gülbahçe for *D. annularis* and *M. barbatus* 2010 and 2011, respectively. PCB153 was the most frequently compound in both fish species during sampling periods.

PCA analysis was performed to evaluate the correlation of the determined variables in fish species. According to PCA analysis, the PCBs are not correlated with fish length. According to one-way ANOVA test, significant temporal variations were generally observed for PCBs, while non significant spatial variations were found among sampling sites. 6 PCB congeners were found as 0.59-6.64 nanogram in gram (wet weight, ww) and compared with iPCB6 limits of 75 nanogram in gram (ww) set by the Environmental Food Safety Authority. Accordingly, fish samples analysed did not exceed the safe limits of iPCB6 set by EU. The Estimated Daily Intake of 6 PCB congeners by the people were below the tolerable daily intake (TDI, 10 nanogram in kilogram bw/d) recommended by WHO (2003) suggesting this intake would not pose adverse effect to inhabitants of İzmir.

Keywords: PCB congeners, fish, *Mullus barbatus*, *Diplodus annularis*, health risk, İzmir Bay (Eastern Aegean)

DOĞU EGE, İZMİR KÖRFEZİ'NDE BİYOTADA PCB TÜRLERİNİN DAĞILIMI VE RİSK DEĞERLENDİRMESİ

ÖZ

Balık, deniz ortamında kirlilik seviyesinin izlenmesinde çevresel biyogösterge olarak sıkça kullanılan bir canlıdır. Bu çalışmada, İzmir Körfezi'nde iki balık türünde PCB seviyeleri araştırılmıştır. İzmir Körfezi'nde 2010-2013 yılları arasında yapılan seferlerinde üç bölgeden (Foça-Gediz, Uzunada, Gülbahçe) trol ile toplam 624 adet *Mullus barbatus, Diplodus annularis* örnekleri toplanmıştır. Bütün örnekleme dönemleri boyunca tüm bölgelerde PCB konsantrasyonları bdl ve 34,3 1 gramda nanogram (kuru ağırlık) arasında değişmektedir. En yüksek PCB konsantrasyonu Gülbahçe'de 2010 yılında *D. annularis* ve 2011 yılında *M. barbatus'*ta bulunmuştur. Örnekleme sırasında iki balık türünde de en sık rastlanılan bileşik PCB153'tür.

Balık türlerinde tayin edilen değişkenleri değerlendirmek için PCA analizi yapılmıştır. PCA analizine göre, PCB seviyeleri ile balık boyu arasında ilişki bulunmamıştır. One-way ANOVA testine göre örnekleme bölgeleri arasında anlamlı değişkenlik bulunmazken, genellikle zamana bağlı değişim anlamlıdır. Bu çalışmada, 6 PCB türü seviyesi 1 gramda 0,59-6,64 nanogram (yaş ağırlık) olarak bulunmuş olup Çevresel Gıda Güvenlik Otoritesinin (EFSA) kural olarak koyduğu 1 gramda 75 nanogram limit değeri ile karşılaştırılmıştır. Buna göre, analiz edilen balık örnekleri 6 PCB türü için EU'nun koyduğu güvenlik limitini aşmamıştır. Toplum tarafından 6 PCB türünün tahmin edilen günlük alım miktarı (EDI) WHO (2003)'ün tavsiye ettiği tolere edilebilir günlük alım miktarının (TDI, 1 kilogramda 10 nanogram bw/d) altında olup bu da İzmir'de yaşayanlar için olumsuz etkinin olmayacağını göstermektedir.

Anahtar Kelimeler: PCB türleri, balık, *Mullus barbatus, Diplodus annularis,* sağlık riski, İzmir Körfezi (Doğu Ege)

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CHAPTER ONE
INTRODUCTION

Polychlorinated biphenyls (PCBs) are aromatic, synthetic chemicals which do not occur naturally in the environment (World Health Organization (WHO), 2000). They consist of 209 synthetic halogenated aromatic hydrocarbons, are used extensively in the electricity generating industry as insulating or cooling agents in transformers and capacitors (Eisler, 2000). The biphenyl structure has two linked benzene rings in which some or all of the hydrogen atoms have been substituted by chlorine atoms. (WHO, 2000).

The synthesis of PCBs was first described in 1881 (Schmidt, & Schultz, 1881). Their wide industrial application, as commercial mixtures of individual congeners in products under various trade names (Aroclor, Clophen, Phenoclor, etc.), started from 1930. PCB congeners are highly lipophilic and their lipophilicity increases with increasing degree of chlorination. However, they have very low water solubilities. Congeners with a lower degree of chlorination are more volatile than those with a higher degree PCB congeners are colorless and often crystalline. Commercial PCB mixtures are clear to light yellow oils or resins and they do not crystallize, even at low temperatures (Eisler, 2000; WHO, 2000).

Due to the human activities and the chemical characteristics of PCBs are now distributed worldwide, and measurable concentrations occur in marine organisms from different regions around the world. They have low electrical conductivity, high thermal conductivity and high resistance to thermal degradation and fire resistant because of their high flash points. On the basis of these properties, PCB congeners have been used as dielectric isolators in electrical equipment (Eisler, 2000; WHO, 2000).

Although the use of PCBs were banned or, at least, intensely reduced in the last two decades, it is still frequently observed in environmental samples due to their highly persistence and accumulation within food chains. The presence of PCBs in the marine environment and related hazards were probably first identified in biologi-

cal samples in 1966 by Jensen. (Anonymous, 1966; Jensen, 1972). The distribution of PCBs has been extensively examined both experimentally and theoretically (Eisler, 2000; WHO, 2000).

The lipophylic contaminants like PCBs are very persistent, widely observed in the environment and show potential for bioaccumulation risk. Human exposure is possible by several routes: inhalation of air, dermal adsorption and food consumption. Dietary intake is the major route of uptake, contributing more than 90% of the daily exposure to lipophylic compounds (EPA, 1999; Schlummer et al., 1998, Bayarri et al., 2001).

Fish are one of the animal species often used as an environmental bioindicator to monitor a level of pollution in the marine environment. The bioaccumulation of PCBs in two most edible fish species from the İzmir Bay was monitored. The fish species, red mullet (*Mullus barbatus*), annular seabream (*Diplodus annularis*) were selected for their characteristic habitats and feeding behaviours to investigate their potential as bioindicators in study area. Since the species chosen are regularly consumed in the Turkish diet, it will be important to know the pollutants data in edible fish with the purpose of protecting the health of consumers.

İzmir Bay, which is one of the great bay of the Mediterranean compares well with similar coastal areas in the world. İzmir is an important industrial, commercial and cultural focal point. Industrial activities cover a large range of industries including food processing, paint, tanneries, textile, chemicals and petroleum refining. The Gediz River, which flows to the northern part of the bay, is the second biggest river in the western part of Turkey.

Several investigations have been achieved on the levels of nutrients and metals in the İzmir Bay (Parlak and Demirkurt, 1990; Kucuksezgin et al., 2001; Kucuksezgin et al., 2002; Kucuksezgin et al., 2006; Kucuksezgin et al., 2011a, Kucuksezgin et al., 2011b), however there is no published data on PCB congeners in fish from İzmir

Bay. Furthermore, this is a first comprehensive study to report on the concentrations of PCBs in biota samples.

The aim of this study is;

- to comparatively investigate the spatio-temporal distribution of PCB congeners in both fish species, red mullet (*Mullus barbatus*) and annular sea bream (*Diplodus annularis*) collected during the four year period from 2010 to 2013.
- to determine the interspecies difference among fish based on PCB levels,
- to estimate associated health risk for people consuming fish from İzmir Bay,
- to estimate the dietary exposure to ∑6 PCBs (non-dioxin like) in fish in order to assess the risk for human in İzmir.
- to compare the measured PCB congeners with those of other corresponding areas in the world,
- to assess the extent of contamination of fish and will be useful reference for the development of any future studies,

CHAPTER TWO
POLYCHLORINATED BIPHENYLS

2.1 POPs Characteristics and National/International Legislations

2.1.1 POPs Characteristics

The POPs are man made organic chemical substances which are consciously or unconsciously produced. Polychlorinated biphenyls (PCBs), pesticides and unintended by-products can be produced as industrial processes. They are persistent in the environment for long periods, and lead long term global pollution. POPs will always be detected in environmental samples. These pollutants cause several diseases but it is most diffucult to understand that ilness are directly traceble to exposure to a specific POPs. The fact that POPs rarely occur as a single compound.

POPs accumulate in fat-rich tissues due to their lipophilic charecteristics and POP levels bioconcantrete/biomagnify in food chain. Highest concentrations of POPs are determined in marine mammals therefore their immune system are affected and this immune disfunction increases mortality (WHO, 2008).

2.1.2 EU legislation

Countries of the world has called for actions to reduce and stop production, use and releases of POPs. Finally, two international legally obligating decisions have been taken:

- "The Protocol to the regional UNECE Convention on Long-Range Transboundary Air Pollution (CLRTAP) on POPs, opened for signatures in June 1998 and entered into force on 23 October 2003"
- "The global Stockholm Convention on POPs, opened for signatures in May 2001 and entered into force on 17 May 2004"

These desicions establish strict international systems for initial lists of POPs (16 in the UNECE Protocol and 12 in the Stockholm Convention). These decisions also include criterias for adding new chemicals into these lists. After these decisions some control measures were disclaimed:

- Restriction of POPs production and use,
- Export and import of the POPs were restricted,
- Safely handling of stockpiles (Stockholm Convention),
- Safely eliminating of wastes containing POPs,
- Reduction of emissions of unintentionally produced POPs such as furans and dioxins,
- New POPs noted to the Stockholm Convention in May 2009 and to the POP Protocol in December 2009

The EU has signed decions on POPs, with all 15 Member States. The EU approved the Protocol on 30 April 2004 and the Stockholm Convention on 16 November 2004 (http://ec.europa.eu/environment/archives/pops/index_en.htm).

Table 2.1 12 chemicals in Stockholm Convention (Harrad, 2010)

Currently listed	**Under consideration**
Aldrin	Chlordecone[a]
Chlordane	Endosulfan
DDT	Hexabromobiphenyl[a]
Dieldrin	Hexabromocyclododecane
Endrin	α-Hexachlorocyclohexane
Heptachlor	β-Hexachlorocyclohexane
Hexachlorobenzene	γ-Hexachlorocyclohexane (Lindane)[a]
Mirex	Octabromodiphenyl ether
Polychlorinated biphenyls	Pentabromodiphenyl ether[a]
Polychlorinated dibenzo-p-dioxins	Pentachlorobenzene
Polychlorinated dibenzofurans	Perfluorooctane sulfonate[a]
Toxaphene	Short chain chlorinated paraffins

[a]These chemicals were already recommended by the POPs Review Committee (POPRC) for listing under the Convention

The aim of the Convention is 'to protect human health and the environment from persistent organic pollutants'. And it is accepted that POPs are dangerous both for human and environment. Moreover, POPs transport by air, water, and migratory species, and are precipated far from their source, and they accumulate in environment. In 2008, there are 12 chemicals are listed under the Convention (Table 2.1).

2.1.3 POPs in Turkey and Legislation

Turkey signed and approved the Stockholm Convention (SC) in 2001 and 2009 respectively. The first National Implementation Planning (NIP), prepared with Global Environment Facility (GEF) assistance was developed by the Ministry of Environment and Forestry in the period 2007-2010 for 12 POPs, and officially forwarded to the SC's Secretariat on April 5, 2011. Then, Turkey has prepared draft of updated NIP with United Nations Industrial Development Organization (UNIDO) to give the status of POPs management and declare the new added POPs that came into force in 2010 (UNIDO, 2016). The updated NIP has been submitted to the Convention Secretariat in 2015. Turkey didn't declare acceptable purposes under the provisions of the Stockholm Convention (United Nations Development Programme, UNDP, 2015).

2.2 General Characteristics of PCBs

Polychlorinated biphenyls (PCBs), a group of 209 synthetic organochlorinated hydrocarbons, are used widely in the electricity producing industry as insulating agents or cooling systems in transformers, capacitors, surface coatings, inks and adhesive substances. Due to anthropogenic activities and the chemical properties of the production outcomes. PCBs are now distributed globally and PCB concentrations can be measured in biota. PCBs cause various effects such as death, failure of immunological factors during pregnancy, birth defects, liver diseases, wasting syndrome and body or brain tumors, PCBs bioaccumulate and biomagnify in the food web.

Polychlorinated biphenyls (PCBs) are a member of halogenated aromatic hydrocarbons (HAHs) and contain 209 isomers and congeners (PCB 1 to PCB 209) with different numbers and places of chlorine atoms substituted on the biphenyls. 1-10

chlorine atoms, which are substituted in biphenyls though constitute 10 different PCB homologues, specifically called mono-CBs, di-CBs, tri-CBs, tetra-CBs, penta-CBs, hexa-CBs, hepta-CBs, octa-CBs, nona-CBs and deca-CB. PCBs were commercially manufactured as complex mixtures since 1929 in throughout the world using different trade names such as Aroclor, Clophen, Phenoclor and it had been used widely as dielectric fluids in transformers, capacitor oils, hydraulic and heat exchange fluids, and lubricating and cutting oils (Frame et al., 1996).

All uses and producing of PCBs has been prohibited by legislation of the United States since 1979; then the concentrations PCB residues decreased in biota. But the current environmental load of PCBs in water, sediments, waste disposal areas and other PCB containers. More than 374 million kg PCB loads caused potential hazard for the human health associating with natural sources (Eisler, 1986; Hansen, 1987; Parkinson & Safe, 1987; Safe, 1984; Huckins et al., 1988; Tanabe, 1988; Skaare et al. 1991; Eisler & Belisle, 1996; Niimi, 1996).

2.3 Chemical and Physical Properties of PCBs

Polychlorinated biphenyls (PCBs) are aromatic, synthetic organic contaminants which do not observe naturally in the environment. They are made up of the two benzene rings linked by a single carbon-carbon bond with one to all ten of the hydrogen atoms have been replaced with chlorine atoms (WHO, 2000; Eisler, 2000). PCBs have been produced commercially since 1929. Polychlorinated biphenyls are highly lipophilic group of global pollutants and include 209 congeners (Figure 2.1). These congeners have extensively different toxicity and other biological effects in the environment (Kannan et al., 1989).

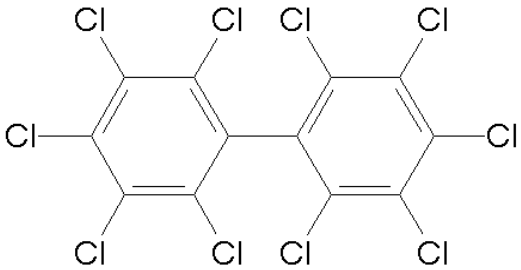


Figure 2.1 Configuration formula of PCB

PCBs are commercially produced by chlorination of a biphenyl with anhydrous chlorine using iron filings or ferric chloride as the catalyst. The purified product is a complex mixture of chlorobiphenyls including 18 to 79% chlorine. Ten possible degrees of chlorination of the biphenyl group contain 10 PCB congeners: mono-, di-, tri-, tetra-, penta-, hexa-, hepta-, octa-, nona-, and decachlorobiphenyl. Congener groups have different positional isomers. For instance, the tetrachlorobiphenyl and pentachlorobiphenyl congener groups include 30 and 46 probable isomers, respectively. All of the 209 probable isomers are not likely to be formed during the producing process. The most common PCB isomers have generally either an equal number of chlorine atoms on both rings, or a difference of one chlorine atom between rings. Chlorine substitution is preferred at the ortho and para positions; nevertheless, commercial PCBs are complex mixtures of isomers and congeners with no apparent positional choice for halogen substitution (Eisler, 2000)

The chemical formula of PCBs is $C_{12}H_{10-n}Cl_n$, where n ranges from 1 to 10. 209 congeners are theoretically possible, but only about 130 of congeners have been identified in commercial products. Ballschmiter and Zell suggested a numbering system for the PCB congeners which has been accepted by the International Union of Pure and Applied Chemists (IUPAC) (Ballschmiter & Zell, 1980). All PCB congeners are lipophilic and their lipophilicity increases with increasing chlorination degree. Though, they have very low water solubility. Congeners, which have a lower degree of chlorination are more volatile than those with a higher degree of chlorination. Pure definite PCB congeners have colourless and often crystalline structure.

Commercial PCB mixtures are clear to light yellow oils or resins and they do not crystallize, even at low temperatures. Due to high ignition point (170–380 °C), PCBs are fire resistant in practice. PCBs generate vapours which are heavier than air, but are not explosive. They have low electrical conductivity, high thermal conductivity and PCBs show high heat resistance. According to these properties PCBs are mainly used as dielectric isolators in electrical equipment.

Similar to other organochlorine compounds, most of the congeners are persistent and accumulate in the food chain. The distribution of PCBs was widely investigated in several countries of the world. The studies showed that air transportation is the main source of PCBs throughout the world. The high volatility of PCBs from landfills into the atmosphere and resistance to degradation at low incinerating temperatures, make atmospheric transportation the primary way globally.

PCBs are very persistent in the air, soil and water, especially those lacking adjacent unsubstituted positions on the biphenyl rings (e.g., 2,4,5-, 2,3,5- or 2,3,6-substituted on both rings). The half-lives of PCBs change between three weeks and two years in air according to the degree of chlorination. Photodegradation seemed to be important loss mechanism in air. Soils and sediments act as storage for PCBs because PCBs strongly attached to them. Estimated half-lives of PCBs are more than six years in soils and sediments and they have high resistance to degradation (UNEP/GEF, 2003). Because of their lipophilic property, PCBs accumulate in fatty tissues (Log Kow of tri- to hepta-CBs ranges from 5.6 to 7). The biological half-life of PCB 153 were more than ten and 12.4 years in eels and human body, respectively (Brown, 1994).

2.4 Sources and Routes to the Sea

2.4.1 Sources

There are a lot of potential PCB sources in addition to more commonly known sources such as capacitor oils and electrical transformer and fluorescent light bal-

lasts. PCBs containing products are used by people for years due to their durability. Therefore, materials including PCBs will continuously input to the wastes and use in the recycling operations. These materials are summarized in Table 2.2 (EIP Associates, 1997; EPA, 2002).

Table 2.2 The sources of PCBs in waste matters and recycling operations

Material or operation	Comments
Scrap metal recycling	Transformer shell salvaging; heat transfer and hydraulic equipment; and fluff (shredder waste from cars and appliances including upholstery, padding and insulation). Also present in non-ferrous metal salvaging as parts from PCB containing electrical equipment, and oil & grease insulated electrical cable.
Auto salvage yards, auto crushing	Hydraulic fluid, brake fluid, recycled oil, capacitors, and oil-filled electrical equipment such as some ignition coils.
Repair activities.	Shipyards (electrical equipment, hydraulic oil, paint, etc.), locomotive repair, heavy equipment repair facilities, auto repair, repair of manufacturing equipment, etc
Used oil	May be present in used oil from various sources including auto salvage yards, automotive and heavy equipment repair shops, hydraulic equipment repair, industrial machinery repair, etc. Because some PCBs have been mixed with used oil, some recycled oils currently in circulation may contain PCBs at concentrations generally <50 ppm. PCBs may also be present where used oil has been used for dust suppression/road oiling, weed control, energy recovery.
Recycled paper	Paper may contain PCBs where carbonless copy paper has been used in recycling. However, PCB concentrations have decreased over time as the volume of unrecycled carbonless copy paper is reduced. Recycled paper containing PCBs has historically been used for food packaging. PCB concentrations in food packaging are restricted to 10 ppm unless an impermeable barrier is present between the packaging and food product.
Effluent	PCBs may be in wastewaters from manufacturing facilities and equipment such as chemical and pesticide facilities, pulp and paper mills, cooling waters from vacuum pumps and electric power generation facilities where leaks have occurred, and condensate from vacuum pumps and natural gas pipelines. Significant cleanup activities have been performed at natural gas pipeline compressor stations from discharges of condensate to ground and storm drainage systems.
Asphalt roofing materials, tar paper, and roofing felt	Anticipated at generally very low concentrations where used oil containing PCBs has been used in asphalt mix.

Table 2.2 continues

Building demolition	Electrical equipment, joint caulking, oil & grease insulated cable, surface coatings as flame retardant and waterproofing.
Dredge spoils	From areas where contaminated sediments are present.
Landfills	Landfills Municipal and industrial solid waste; virtually all potential sources could be
Wastewater treatment plant sludge	Derived from atmospheric deposition and stormwater, water supply systems, leaks and spills, leaching from coatings and plastics containing PCBs, PCBs in food and human waste.

Oregon Department of Environmental Quality (DEQ) (2003)

PCBs do not easily degrade in the environment. They persist for long periods in between air, water and soil. PCBs can be transported long distances and have been observed in seawater and snow in regions far from where PCBs were found in the environment. In general, the low molecular weight PCBs can be carried easier than the higher form of PCBs. PCBs can accumulate in the several parts of plants and food crops. They are also taken up into the bodies of biota such as fish and mussels in the marine environment. People consume these fish and other organism as food and they ingest fish in which PCBs have bioaccumulated (EPA, 2017)

2.4.2 Routes

Due to their resistantance to degradation, highly chlorinated PCBs are persistant in the environment for years. However, the concentration of PCBs in the environment have decreased past two decades. Food (such as eggs, and animal fats—and some fish and wildlife) is the main source of exposure to PCBs for the general population. (ATSDR, 2000; Fisher 1999; Gunderson and Gunderson 1988; Hopf et al., 2009; ATSDR, 2014).

In aquatic environments, due to their high lipophilicity, PCBs are preferentially adsorbed to sediments. Sediment adsorption prevents the contamination of drinking-water supplies, and the partitioning of PCBs to sediments causes to become concent-

rated in benthic organisms. Bottom-feeder fish ingest and accumulate PCBs from sediment. These compounds resist to biodegradation and they become more concentrated through the food web from the bottom-feeding organisms. Consequently, PCB levels in aquatic organisms can be detected as much as one million times higher than the levels in the aquatic environment (ATSDR, 2000).

People living and working near PCB disposal facilities, or hazardous waste sites may receive higher PCB exposures than the general population. These exposures may be through ingestion, inhalation, or skin contact (ATSDR, 2000).

PCBs exposure mainly through the inhalation and dermal routes. Commercial PCB mixtures change from colorless to dark brown oils, and from viscous liquids to sticky resinous semisolids. Even if PCBs evaporate slowly at room temperature, a small rise in temperature increases the volatility of PCBs. Because of their highly lipophilic characteristic, PCBs also can be absorbed through the skin after contacting with contaminated equipment, water, or soil.

Infants with human milk as the dominant food source will consequently have a much higher PCB intake than adults. Monitoring programmes have showed a declining trend in PCB concentrations in breast milk during the last two decades. Additionally people who consume big amounts of contaminated food and those who work with PCBs or contaminated materials, people living or working in PCB-contaminated buildings may also be subject to high exposures. However, at present, elevated blood levels have not been detected in humans living in such contaminated buildings (Benthe et al., 1992).

2.5 Uses of PCBs

Manufacturing, processing, distribution and usage of PCBs were regulated in the framework of EPA actions. The Toxic Substances Control Act (TSCA) banned PCBs in 1979. Transformers and hydraulic fluids were realized as high-risk PCB sources

and were purposed for phasing-out. Certain present usages of PCBs are certified under 40 CFR Part 761 and are given in Table 2.3 (EPA, 2002).

PCBs, are intentionally manufactured chemicals, that were produced for decades before the prohibit in buying and selling in market and usage was adopted in 1985 due to their toxicity and bio-accumulative effects. These products are very persistent and can bioaccumulate in fatty tissue of biota, in soils, sediments and in the whole aquatic environment.

People have used in two different applications and these are given below (Official Journal of the European Communities, Community strategy for dioxins, furans and polychlorinated biphenyls/ C 322/02, 2001):

- Closed uses: dielectric fluids in electrical equipment such as transformers, capacitors, heat transfer and hydraulic systems.
- Open uses: as pesticide extenders, sealant, copy paper, industrial oils, paints, adhesives, plastics, flame retardants and to control dust on roads (EC, 2016).

The uses of PCBs are given in Table 2.3

Table 2.3 Uses of PCBs

Use	Comments
Transformers	Authorized use at any concentration though restrictions and regulatory requirements increase with higher PCB concentration thresholds.
Railroad transformers	Transformers used in locomotives and self-propelled railcars. Authorized use at < 1,000 ppm; < 50 ppm if transformer coil is removed at any time.
Heat transfer systems, hydraulic systems, mining equipment	Authorized use at < 50 ppm
Natural gas pipelines	Authorized at < 50 ppm, or at > 50 ppm with additional requirements. PCBs may be present in natural gas compressors, scrubbers, filters, and in condensate
Research & Development	Authorized primarily for purposes relating to environmental analysis, management, and disposal of PCBs. R&D for PCB products is prohibited.

Table 2.3 continues

Scientific Instruments	Examples include oscillatory flow birefringence & viscoelasticity instruments for the study of the physical properties of polymers, microscopy mounting fluids, microscopy immersion oil, and optical liquids.
Carbonless copy paper	Use of existing carbonless copy paper is permitted; manufacturing of new carbonless copy paper is not authorized.
Electromagnets, switches, voltage regulators, circuit breakers, reclosers, cable	No restrictions on existing use; restrictions on PCB concentrations if serviced and oil is removed or replaced.
Porous surfaces	EPA considers building materials, such as concrete, porous with respect to PCB leaks and spills. Porous building materials may be left in place following spills provided various conditions are met. Older industrial machinery often was designed to slowly leak (PCB-containing) hydraulic oil as a lubricant.

Source: EPA (2002)

2.6 Human Health Effects of PCBs

2.6.1 Health Effects

PCBs have been shown to cause a variety of adverse health effects. They have been demonstrated to cause both cancer and non-cancer health effects in animals, containing: effects on the immune, reproductive, nervous, endocrine systems. Several studies were performed in humans for potential carcinogenic and non-carcinogenic effects of PCBs (EPA, 2017).

They can covalently bind to DNA, RNA and induce DNA strand breaks and DNA repair, which can cause the toxic effect of PCBs. Arene oxide intermediates can also be conjugated with glutathione and further metabolized to form methylsulfonyl metabolites, which have been found in human serum and tissue samples. Binding of methylsulfonyl metabolites cause the toxic effects of PCBs (ATSDR 2000; Safe, 2007; ATSDR 2016).

Occupational exposure to PCBs can result in a broad spectrum of effects that includes;

- Increased levels of some liver enzymes, with possible hepatic damage,

- Chloracne and related dermal lesions, and
- Respiratory problems (ATSDR, 2016)

Potential adverse human health effects of low-level environmental exposure to PCBs are complex and still need further validation (Safe, 2007). In animal studies, commercial PCBs elicit a broad range of toxic responses including:

- Acute lethality,
- Body weight loss,
- Carcinogenesis,
- Dermal toxicity,
- Fatty liver,
- Genotoxicity,
- Hepatomegaly,
- Immunosuppressive effects,
- Neurotoxicity,
- Porphyria,
- Reproductive and developmental toxicity,
- Thymic atrophy, and
- Thyroid hormone-level alterations.

2.6.2 Public Health Standards

However PCBs are no longer used in the United States, people can still be exposed to PCBs. Many old transformers, capacitors, fluorescent lighting fixtures, electrical devices (such as television and refrigerators) may still contain PCBs. Small amounts of PCBs may get into the air, while these electrical instruments get hot during operation, and increase the level of PCBs in indoor atmosphere. EU regulation limits for non-dioxin like PCBs in food stuff were given in Table 2.4

Small amounts of PCBs can be observed in all outdoor and indoor air, soil, sediments, surface water and animals. Nevertheless, PCB levels have generally decreased since PCB production stopped in 1977. People are exposed to PCBs primarily from contaminated food (such as fish, meat) and contaminated air. The estimated daily

intake of PCBs in adults from foods decreased from about 1.9 nanograms to less than 0.7 nanograms. Recent studies showed that PCB levels in fish are lower than bottom-feeders such as crap. Meat and milk products are other important sources of PCBs in food, with PCB levels ranging from less than 1 to a few ppb.

Table 2.4 Public health limits for EU countries and Turkey

	Maximum Levels
Foodstuffs (Fish or Derived Products)	Sum of PCB28, PCB52, PCB101, PCB138, PCB153 and PCB180
Muscle meat of fish and fishery products and products thereof, with the exemption of:—wild caught eel—wild caught fresh water fish, with the exception of diadromous fish species caught in fresh water — fish liver and derived products—marine oils The maximum level for crustaceans applies to muscle meat from appendages and abdomen. In case of crabs and crab-like crustaceans (Brachyura and Anomura) it applies to muscle meat from appendages	75 ng/g wet weight
Muscle meat of wild caught fresh water fish, with the exception of diadromous fish species caught in fresh water, and products thereof	125 ng/g wet weight
Muscle meat of wild caught eel (Anguilla anguilla) and products there of	300 ng/g wet weight
Fish liver and derived products thereof with the exception of marine oils	200 ng/g wet weight
Marine oils (fish body oil, fish liver oil and oils of other marine organisms intended for human consumption)	200 ng/g fat

Official Journal of the European Union (2006)

People who live near hazardous waste sites may be exposed to PCBs by consuming PCB-contaminated foods, by breathing PCBs in air, or by drinking PCB-contaminated water. Due to industrial activities, PCBs can occur during repair and maintenance of PCB transformers; accidents, fires, or spills involving PCB transformers and older computers and instruments; and disposal of PCB materials. (ATSDR, 2000).

CHAPTER THREE
MATERIAL AND METHODS

3.1 Characteristics of İzmir Bay

İzmir Bay (western Turkey) is one of the great natural bays of the Eastern Mediterranean Sea. The Bay is composed of three sections: Inner, Middle and Outer Bays according to the topographical, hydrological and ecological characteristics of the bay (Figure 3.1). The İzmir Metropolitan Municipality, covering 88000 hectares and population of almost 4.5 million residents is settled around the İzmir Bay. İzmir is one of the most important commercial, industrial and rich in cultural heritage center. Industrial activities contain a wide range of industries including food processing, tanneries, paint, chemicals, textile and petroleum refining. More than 6.000 industrial establishments were registered with the Chamber of Industry in İzmir. However, many establishments are not registered. Various pollution sources and pathways of pollutants are given in Table 3.1 Accordingly, the main sources of pollution in the bay are domestic and industrial inputs, which account for 50 % of the observed organic pollution.

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

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 %

The total surface area of bay is more than 500 km^2, water capacity is 11.5 billion m^3, a total length is 64 km and bay opens in the Aegean Sea. The hinterland is relatively fertile agricultural area irrigated by several rivers the longest of which is the Gediz which flows into the Outer Bay. The climate of the Bay is relatively mild with a mean annual temperature of 17°C. A 13 m deep sill, the Yenikale Strait, divides the Middle Bay from the Inner Bay.

The inner part of the Bay extends from the head of the Bay to the Yenikale lighthouse. The water capacity of the Inner Bay is $6x10^8$ m^3, mean 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 (Kucuksezgin, 2011). The principal source of pollution is several creeks that flow to the bay and high levels of organic material were detected in the Inner Bay coastal zones that are situated near the discharge points. The alluvion of Gediz River just flowed in the west of Karsıyaka until the second half of nineteen centuries filled the north part of the Inner Bay. Because of that, the İzmir Harbour had met a getting shallow problem and the delta of Gediz River had been moved to the Outer Bay. The water depth increase steeply from the inshore to the offshore that the route of ships must be through the shallow channel with a depth of 10 m between the delta of old Gediz River and Narlıdere (UNEP, 1993).

The Middle Bay extends from Yenikale lighthouse to the Kokola point. The water capacity of the Middle Bay $9x10^8$ m^3, mean water depth is about 16 m. Surface area of the Middle Bay is relatively 57 km^2. The Middle Bay is a corridor zone with pollutant levels intermediate between Outer and Inner Bays, which are a clear indication of distributing pollution in the bay. Due to the less water depth of the Inner and Middle Bays, water exchange and self-purification capacities are very limited.

The Outer Bay extends from Kokola point to the mouth of the bay. The water volume of the Outer Bay $1x10^{10}$ m^3, mean 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 unpolluted according to the most of the pollution indicators. Gediz River is the second biggest river along the Aegean coast and main fresh

water source of the İzmir Bay. The area of Gediz River is about 18000 km^2 and annual mean flow is estimated to be $2.33x10^9$ m^3.

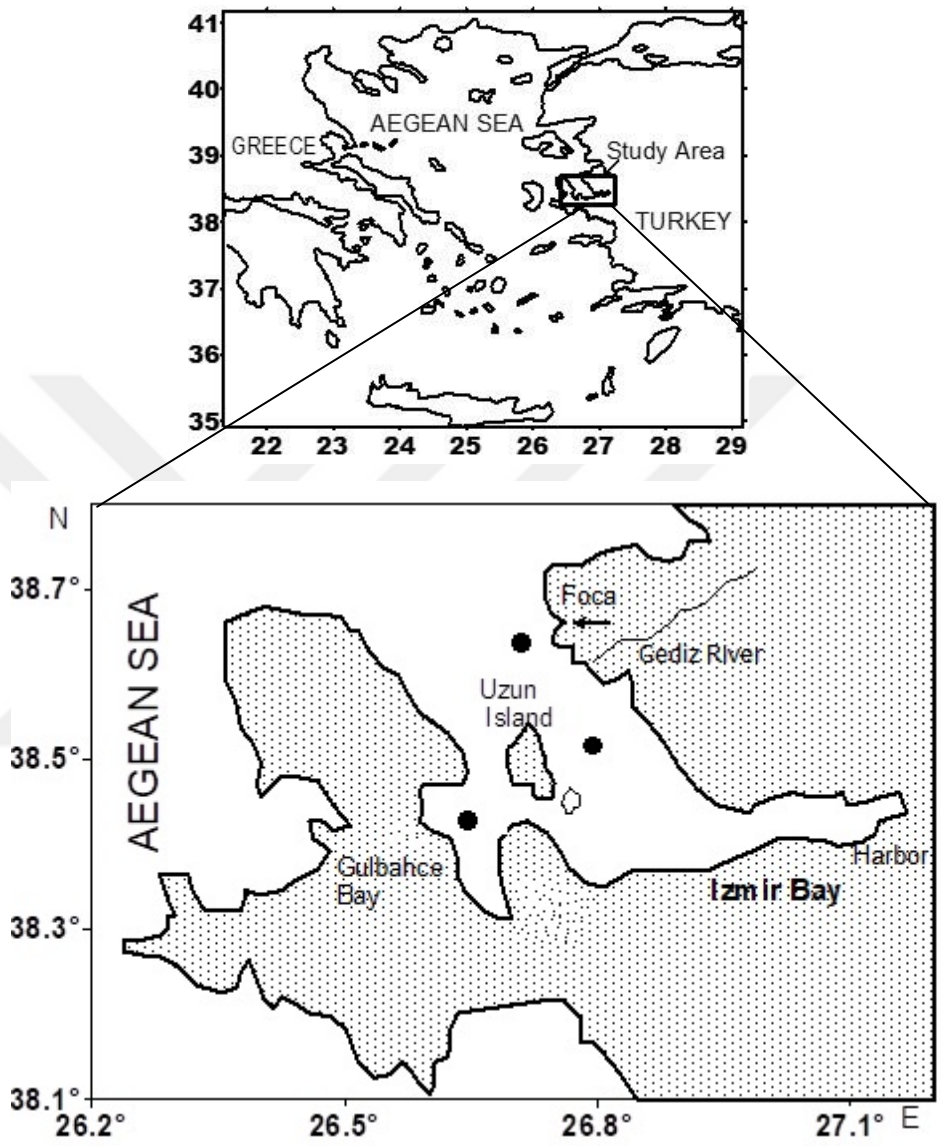


Figure 3.1 Biota sampling sites in İzmir Bay

3.2 Sampling

Fish species were collected from three regions in İzmir Bay (Foca-Gediz, Uzunada, Gulbahce) in 2010, 2011, 2012, 2013 during cruises of *R.V.* K. Piri Reis. A total of 624 individuals of *Mullus barbatus* (Red mullet) (N=298), *Diplodus annularis* (Annular seabream) (N=326) were taken by trawling from İzmir Bay.

Table 3.2 Sampling locations, date, species, numbers, weight and length

Region	Sampling Date	Species	N	Weight (gr)	Length (mm)
Foça-Gediz	1_{st} April 2010	*M. barbatus*	26	16 - 88	90 - 150
Foça-Gediz	1_{st} April 2010	*D. annularis*	18	18 - 188	105 - 220
Gülbahçe	2_{nd} April 2010	*M. barbatus*	15	15 - 25	110 - 130
Gülbahçe	2_{nd} April 2010	*D. annularis*	19	14 66	90 - 160
Foça-Gediz	29_{th} April 2011	*D. annularis*	10	62 - 86	140 - 160
Foça-Gediz	29_{th} April 2011	*D. annularis*	20	32 - 54	110 - 135
Gülbahçe	28_{th} April 2011	*M. barbatus*	20	24 - 32	110 - 122
Gülbahçe	28_{th} April 2011	*M. barbatus*	20	32 - 46	120 - 130
Gülbahçe	28_{th} April 2011	*M. barbatus*	20	40 - 74	129 - 155
Gülbahçe	28_{th} April 2011	*D. annularis*	12	56 - 88	140 - 160
Gülbahçe	28_{th} April 2011	*D. annularis*	20	22 - 42	101 - 128
Uzunada	28_{th} April 2011	*M. barbatus*	16	18 - 56	100 - 130
Uzunada	28_{th} April 2011	*D. annularis*	15	68 - 144	140 - 188
Uzunada	28_{th} April 2011	*D. annularis*	20	40 - 76	115 - 140
Foça-Gediz	11_{th} April 2012	*M. barbatus*	12	22 - 46	115 - 135
Foça-Gediz	11_{th} April 2012	*D. annularis*	12	42 - 88	129 - 235
Gülbahçe	11_{th} April 2012	*M. barbatus*	20	40 – 74	136 - 162
Gülbahçe	11_{th} April 2012	*M. barbatus*	20	16 - 28	100 - 130
Gülbahçe	11_{th} April 2012	*M. barbatus*	20	28 - 44	128 - 142
Gülbahçe	11_{th} April 2012	*D. annularis*	20	60 - 110	140 - 170
Gülbahçe	11_{th} April 2012	*D. annularis*	20	46 - 68	130 - 145
Uzunada	10_{th} April 2012	*M. barbatus*	18	30 - 52	120 - 150
Uzunada	10_{th} April 2012	*M. barbatus*	20	20 - 30	105 - 125
Uzunada	10_{th} April 2012	*D. annularis*	10	58 - 104	140 - 170
Uzunada	10_{th} April 2012	*D. annularis*	15	48 - 72	130 - 155
Foça-Gediz	30_{th} April 2013	*M. barbatus*	8	10 - 38	85 - 135
Foça-Gediz	30_{th} April 2013	*D. annularis*	5	68 - 152	135 - 175
Foça-Gediz	30_{th} April 2013	*D. annularis*	17	34 - 60	115 - 135
Foça-Gediz	30_{th} April 2013	*D. annularis*	13	22 - 38	95 - 125
Gülbahçe	1_{st} May 2013	*M. barbatus*	21	30 - 44	115 - 135
Gülbahçe	1_{st} May 2013	*M. barbatus*	24	14 - 28	100 - 120
Gülbahçe	1_{st} May 2013	*D. annularis*	19	36 - 66	120 - 145
Gülbahçe	1_{st} May 2013	*D. annularis*	20	62 - 100	145 - 165
Uzunada	29_{th} April 2013	*M. barbatus*	2	174 - 194	230 - 235
Uzunada	29_{th} April 2013	*M. barbatus*	16	12 - 44	95 - 130
Uzunada	29_{th} April 2013	*D. annularis*	20	88 - 132	155 - 175
Uzunada	29_{th} April 2013	*D. annularis*	21	46 - 90	125 - 150

Details of individual species were given in Table 3.2 These fish species are characteristic of İzmir Bay and were chosen as monitoring species due to commercially important and mostly eaten by humans. Immediately, muscle tissues were dissected in the field and preserved at -20º until carrying to labs. Muscle tissues were freeze-dried and homogenized, then kept at 20˚C until analysis. Dry weight/wet weight was calculated as 0.28. Extractable organic matter (EOM) with hexan was measured. (UNEP, 1996).

The EOM is detected in the following method; a known volume of the extract is evaporated (up to 100 µl) in the weighing pan of an electrobalance, and the residue is weighted with a precision of about ± 1 µg. The quantity of EOM is calculated as:

$$\text{EOM}(\mu g/g) = \frac{\textit{Weight}\ \text{of residue (}\ \mu g)\ \text{x volume of the extract}\left(\text{ml}\right)\ \text{x } 1000}{\textit{Volume evaporated}\ \left(\mu l\right)\ \text{x quantity of sample extracted}\ \left(g\right)} \qquad (3.1)$$

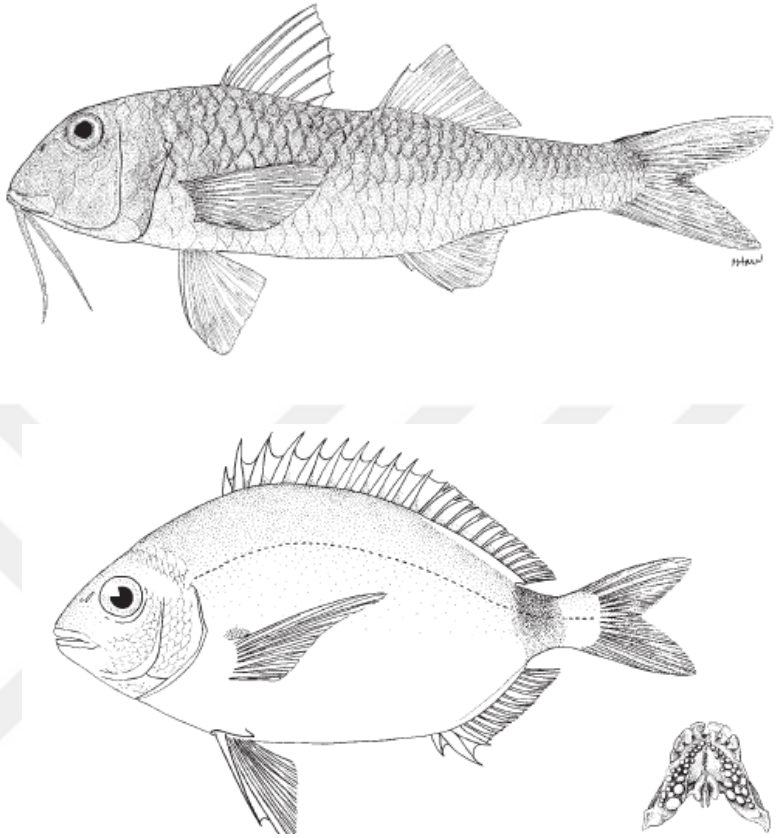
When the lipid content of the extract exceeded 100-150 g, sulphuric acid was used for the removal of lipids (UNEP, 1996).

3.3 Fish Species

Mullus barbatus (Red mullet)

Mullus barbatus is a demersal species which is typically found on sandy, muddy and hard substrates (Figure 3.2a). It can also be found in coral. It feeds on benthic and sub-benthic invertebrates. The spawning seasons are in spring and in summer. This species is sympatric with *M. surmuletus*, however there is partial differentiation in habitat use between these two species, with *M. barbatus* being more abundant in waters between 51 and 200 metres on muddy bottoms, and between 51 and 100 metres on rough bottoms. (Lombarte et al., 2000). *Mullus barbatus* is distributed along the Mediterranean coasts. All year classes and nursery and spawning areas are well distributed and this species reaches sexual maturity at one year old (EC, 2013). It can

reach a maximum size of 30 cm, but is more commonly seen from 10 to 22 cm (Golani, 2016).



a

b

Figure 3.2 Fish species in the sampling area

Mullus barbatus is a commercially important species which is caught by trawl (Golani, 2016*). Mullus barbatus* has been utilized in a number of studies to evaluate the biological effects of chemical pollutants in marine organisms in the Mediterranean, and was chosen as a pilot species for the MED POL II Pilot Program (Mathieu et al., 1991; Lionetto et al., 2003).

Diplodus annularis **(Annular seabream)**

Diplodus annularis is a demersal species found in groups above sandy bottoms and seagrass bed habitats, at depths ranging from surface to 100 m (Figure 3.2b). It lives mainly Zostera seagrass beds but is also found on rocky bottoms from the co-

astline to about 20 m depth. The genders are separated, although these fish are potential hermaphrodites; certain individuals are protandric hermaphrodites. *Diplodus annularis* is a commercial species. It is caught with trawl nets, beach seines, hand lines, bottom long lines, and gill and trammel nets. (Carpenter, 2016).

3.4 Analytical Method

The fish samples were extracted using analytical procedure which is called "Modular Multiple Analytical Method for the Determination of Pesticide Residues in Foodstuffs" included in the Official German Manual as Method L 00.00-34 (DFG Method S 19, 1999). MODULE E 7 extraction was used for the removal of extractable organic matter (lipids) in the samples.

MODULE E 7

Dried samples with lipid content was mixed intensely with the suspension of a synthetic calcium silicate (trade name: Calflo E) in acetonitrile. The suspension solution was filtered and the volume of extract was measured. Then the extracted sample was evaporated to dryness and the residue was solved in GPC eluting mixture. For cleaning up procedure, gel permeation chromatography (module GPC) was used in the procedure. Reagents and apparatus were given below during extraction procedure in this method:

Reagents: Calflo E, filter aid, Celite 545 (heated at 550 °C for at least 2 h), acetone, acetonitrile, cyclohexane, ethyl acetate, isooctane (for residue analysis), GPC eluting mixture (cyclohexane + ethyl acetate 1:1 (v/v)) and if necessary, redistilled as an azeotropic mixture.

Apparatus: High-speed homogenizer

Weigh 5-10 g of freeze dried samples into a glass beaker including 20 g Calflo E and 10 g Celite 545. The mixture of acetonitrile (240 ml) and internal standard (Al-

drin, 10 ml) was added to the sample then the mixture was homogenized for 2 min and then was gently filtered to prevent the evaporation of the filtrate (100-150 ml) using a filter paper in a porcelain or glass filter funnel. Then filtrate through a dry fluted filter paper, covered with 0.5 g Calflo E, into a 250-ml graduated cylinder and measure the volume obtained. Transfer the filtrate quantitatively, rinsing with acetone, into a weighed round-bottomed flask, add 10 ml isooctane and evaporate the solution to 0.5 to 1 ml using a rotary evaporator. Evaporate the remaining traces of solvent with a gentle stream of air. Weigh the flask again and calculate the mass of the residue. If less than 2 g of fat remain, dissolve it in approx. 10 ml GPC eluting mixture. Transfer the solution into a 25-ml graduated cylinder, make up to 15 ml (VEnd) with GPC eluting mixture and filter through a membrane filter, if necessary. If more than 2 g of fat remain, dissolve it in GPC eluting mixture and make up to an appropriate volume (VEnd). With the solution, proceed as described in the module GPC.

GPC (Gel permeation chromatography)

The extract obtained from module E7 is cleaned up by GPC using the polystyrene gel Bio-Beads S-X3 and elution is performed with a mixture of cyclohexane and ethyl acetate.

Reagents: Bio-Beads S-X3, 200-400 mesh (Bio-Rad Laboratories GmbH, München), Cyclohexane, for residue analysis, Ethyl acetate, for residue analysis, GPC eluting mixture: cyclohexane + ethyl acetate 1:1 (v/v); GPC test solution, e.g. approx. ρ= 0.035 µg/ml HCB, 0.13 µg/ml trifluralin, 0.2 µg/ml flucythrinate and 0.25 µg/ml chinomethionat in GPC eluting mixture

Apparatus: Automated equipment for gel permeation chromatography

Packing gel permeation column: Suspend 50 g of Bio-Beads in the GPC eluting mixture and allow them to swell overnight. Pour the suspension all at once into the chromatographic column (capacity approx. 180 ml). Once the gel has settled to a

height of approx. 32 cm and is free from air bubbles, insert the end adapter and lower it down to the bed level. If the gel bed is compressed further after prolonged use, adjust the adapter accordingly. For further details refer to manufacturer's instructions.

Checking elution volumes: Check the elution conditions for every new gel permeation column and for a column whose separation efficiency has diminished. To do so, set the following parameters:
Dump 0 min to discard 0 ml
Collect 2 min to collect 10 ml
Inject 5.0 ml of the GPC test solution and elute with the GPC eluting mixture at 5.0 ml/min. Discard fractions 1 to 7. Collect the fractions 8 to 23 separately and analyze them by gas chromatography mass spectrometry. From the recoveries obtained for the four analytes determine the appropriate range of elution volumes.

Clean up of crude extracts: Inject approx. 10 ml of the filtered extract solution derived from one of the modules E into the 5-ml sample loop (5.0 ml = V_{GA}: is the aliquot portion of the extract volume injected onto the GPC column). Elute the gel permeation column with the GPC eluting mixture at 5.0 ml/min. To do so, set the determined parameters beforehand, e.g. Discard ("Dump") 17 min corresponding to 85 ml Collect ("Collect") 22 min corresponding to 110 ml. The "Dump"-phase is discarded. Collect the "Collect"-fraction in a 150-mL long-neck round-bottomed flask or in a 250-ml pear-shaped flask. Concentrate it to approx. 1 ml in a rotary evaporator (rotate slowly, immerse the flask only slightly in the water bath). Pipet the remaining solution quantitatively into a ground-stoppered graduated test tube, rinse the flask with acetone, and make up to 1.0 ml (V_{GE}:is the final volume of GPC eluate after concentration with acetone.)

3.5 Gas Chromotography Conditions for PCBs

Quantitative analysis was performed with Shimadzu GC-MS.2010 Plus. An analytical column RTX-CL-Pesticide2 20m×0.18mm×0.14μm was used. To analyze

PCB congeners, the temperature program of GC/MS was as follows: initially 115°C, then increase to 200°C with a velocity of 45°C/min then increase to 230°C with a velocity of 15°C/min and up to 300°C with a velocity of 30°C/min held for 5 min. The six PCBs (PCB 28, PCB 52, PCB 101, PCB 138, PCB 153, PCB 180) were analyzed in the biota samples collected from İzmir Bay.

3.6 Quality Assurance for PCBs

The analytical data quality was certified using the reference material of IAEA-435 the biota sample (from IAEA). The whole methodology was verified on this reference sample, obtaining results in good agreement with the certified values (Table 3.3).

Table 3.3 Certified and observed values for PCB congeners in reference material (IAEA 435)

Analyte	Certified values ($ng\ g^{-1}$)	Observed values ($ng\ g^{-1}$)
PCB 28	1.4±0.55	bdl*
PCB 52	4.4±2.5	5.11
PCB 101	23±10	23.33
PCB 138	70±32	71.19
PCB 153	81±37	102
PCB 180	32±18	30.31

*bdl: below detection limit

Blanks were run periodically during the analysis to confirm the absence of contaminants. The blank values of the analytical procedure remained always below the detection limits. The recoveries for sediments fell with a fairly narrow range between 88.0 and 119.0 %. LOQ and LOD values were found as 0.20 ngg^{-1}, 0.07 ngg^{-1} (dry weight), respectively. Chromatograms of PCBs for selected sample and reference standard (100 ng/g) were given in Figure 3.3 and Figure 3.4.

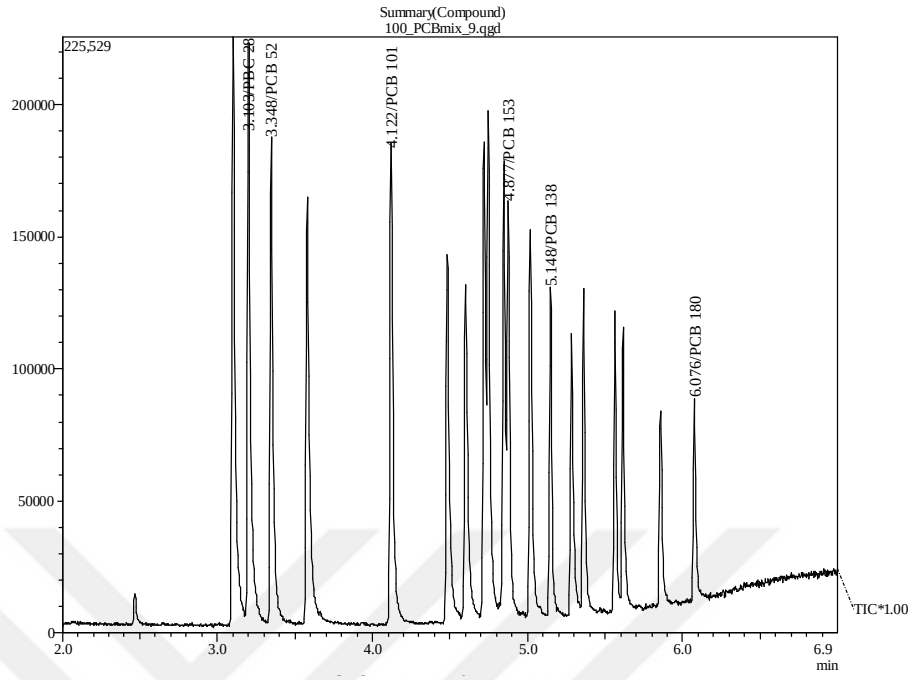


Figure 3.3 Chromatogram for PCB mix (100ng g^{-1})

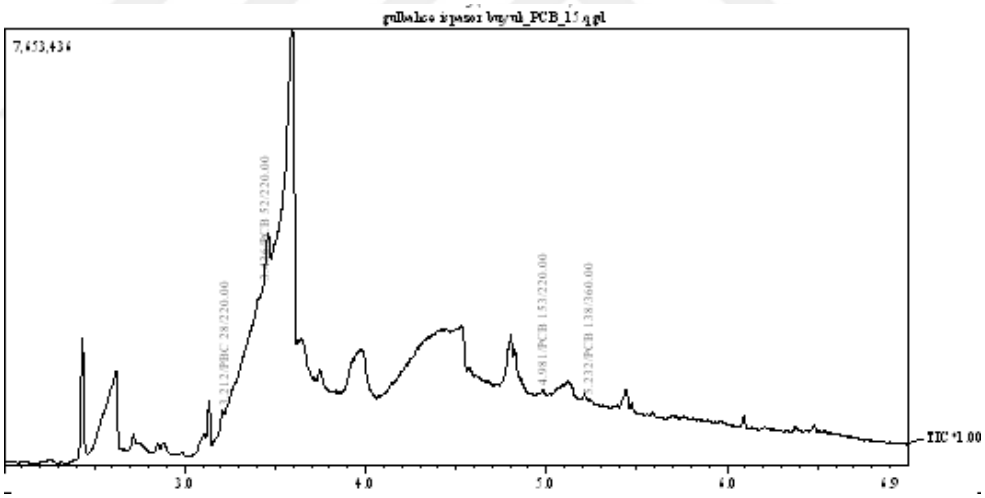


Figure 3.4 Chromatogram for *D. annularis* in Gulbahçe Region

3.7 Statistical Data Analyses

Statistical analysis was performed using STATISTICA for Windows, Release 12, Copyright Stat Soft, Inc.1998-2014. The distribution of data was controlled using Kolmogorov-Smirnov test and homogeneity of the variances was checked by Levene test. Then, transformation of data was carried out if required. Spearman's Rank Or-

der correlation test was used to check significant relationships between PCB congeners and concentration of extractable organic matter. In all case, the level of significant was set at $p< 0.05$. One-way ANOVA was used to find effect of sampling sites and period on variations in PCBs levels in different fish species from İzmir Bay. Post hoc Tukey HSD test was utilized to determine statistically significant differences ($p< 0.05$) following ANOVA. A principal component analysis (PCA), based on a Pearson's correlation matrix, was performed to determine the relationship between the measured variables and to assess contamination effects in İzmir Bay. In the following, a varimax rotation of the normalized factor loadings was performed in this study.

CHAPTER FOUR
RESULTS AND DISCUSSION

4.1 Concentrations of PCBs

The concentrations of PCBs (PCB 28, PCB 52, PCB 101, PCB 138, PCB 153, PCB 180) were measured in two fish species collected from different sites of İzmir Bay during 2010-2013. The obtained results were presented in Table 4.1, 4.2.and 4.3 as dry weight (dwt), wet weight (wwt) and lipid weight (lwt) basis, respectively. The six PCBs were analysed in *Mullus barbatus* and *Diplodus annularis.* The concentrations of PCBs in all samples ranged between bdl-34.3 ng g^{-1} (dry weight) from all sampling sites during sampling periods. The highest concentrations of PCBs were found in Gülbahçe region for *Diplodus annularis* and *Mullus barbatus* 2010 and 2011, respectively.

The Stockholm Convention on Persistent Organic Pollutants (POPs) recommends measurement of six indicator PCBs (PCB-28, PCB-52, PCB-101, PCB-138, PCB-153, and PCB-180) to characterize contamination by PCBs. For monitoring of PCB levels, fish samples are analysed for six indicator PCB congeners in the sampling regions. These congeners were chosen because they are found at higher concentrations in the environment. PCB 101 and PCB 180 were found below detection limit in both studied fish species during all sampling periods. The levels of PCBs in two species between 2010-2013 were given in Figure 4.1 and 4.2 as lipid weight basis.

PCB153 was predominant congener in Gülbahçe region, located in the outer part of the bay, followed by PCB 28 in both species. In addition there is no industrial activity in this area. However, the source of PCB 153 may be due to sewage inputs or from atmospheric inputs. Similar result was found in Marmara Sea by Okay et al. (2009) as well. PCB138 and PCB52 were measured for both species in 2012-2013.

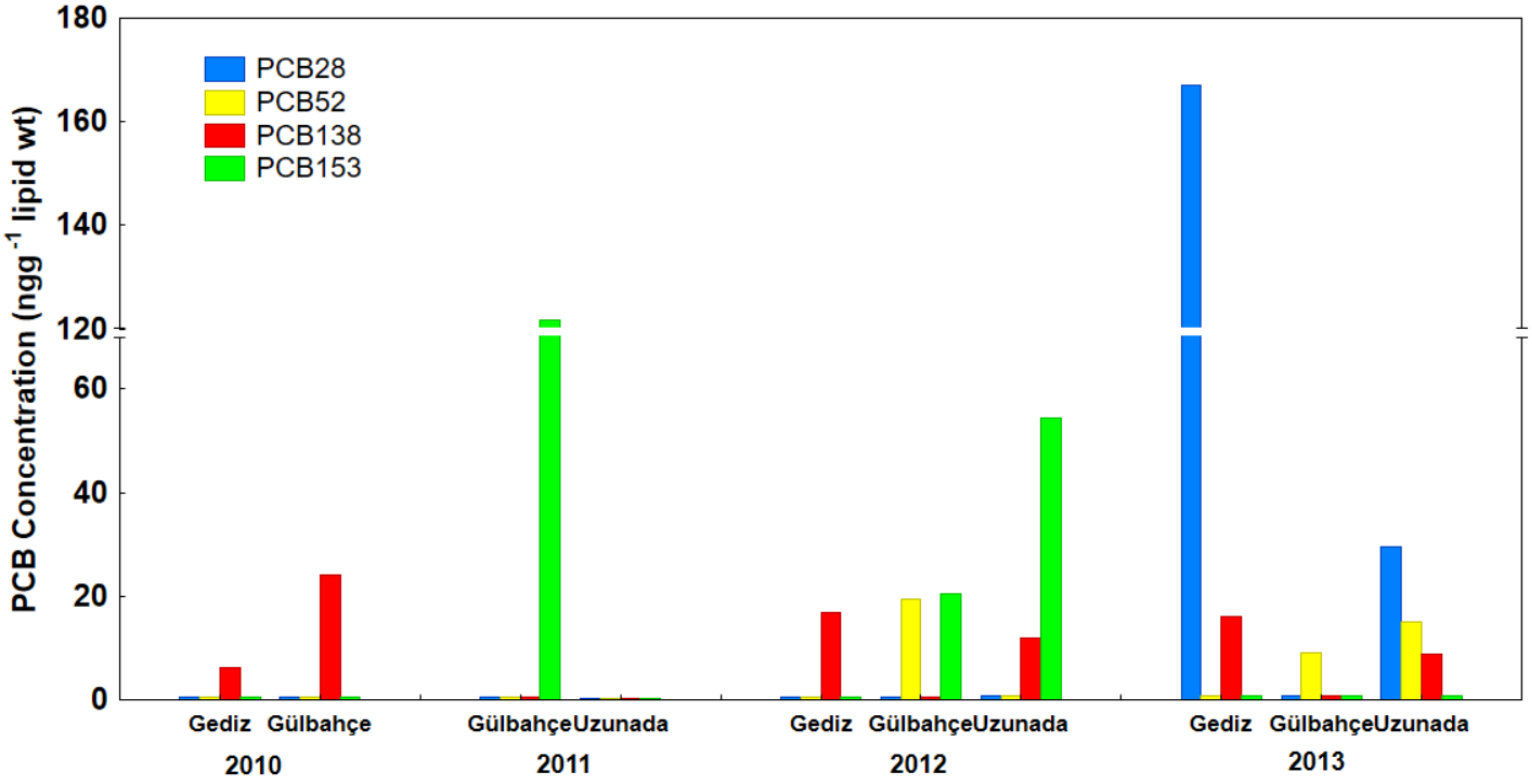


Figure 4.1 Distribution of PCB congeners in *M. barbatus* during sampling periods

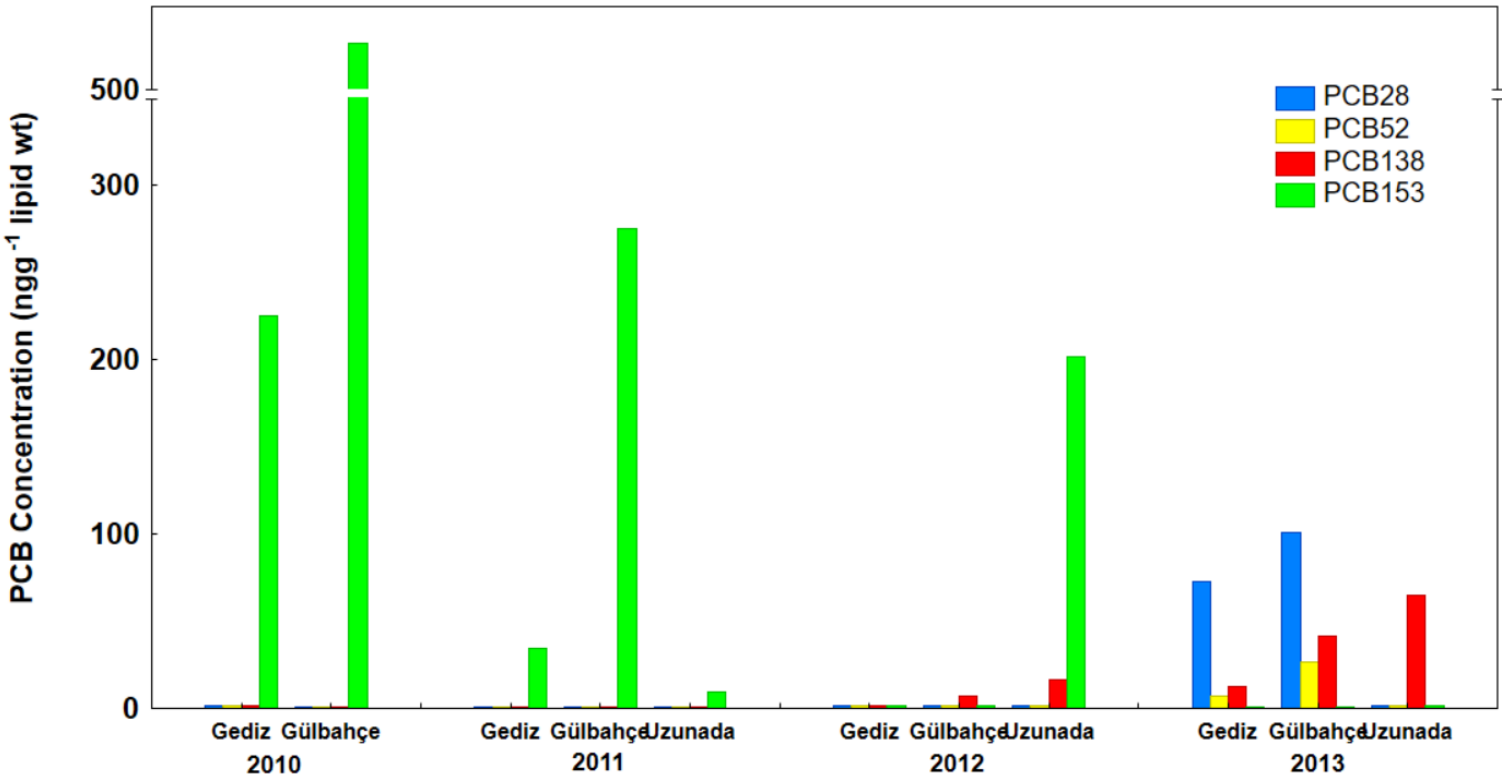


Figure 4.2 Distribution of PCB congeners in *D. annularis* during sampling periods

PCB28 was predominant congener in Foça-Gediz region located between Gediz River estuary and Foça in the outer bay for *Mullus barbatus* and *Diplodus annularis*. PCB153 was only found in *Diplodus annularis* during 2010-2011. PCB28 is a tri-chlorinted congener typical of an Aroclor 1248 source, while PCB153 is prominent in both Aroclors 1254 and 1260. PCB153 was dominant congener in Uzunada region in both species followed by PCB138 in the eastern part of Gulbahçe region. PCB28 was observed in 2013, while PCB153 was not measured in that period for both species. PCB52 was only measured in Gülbahçe and Uzunada during 2012 and 2013.

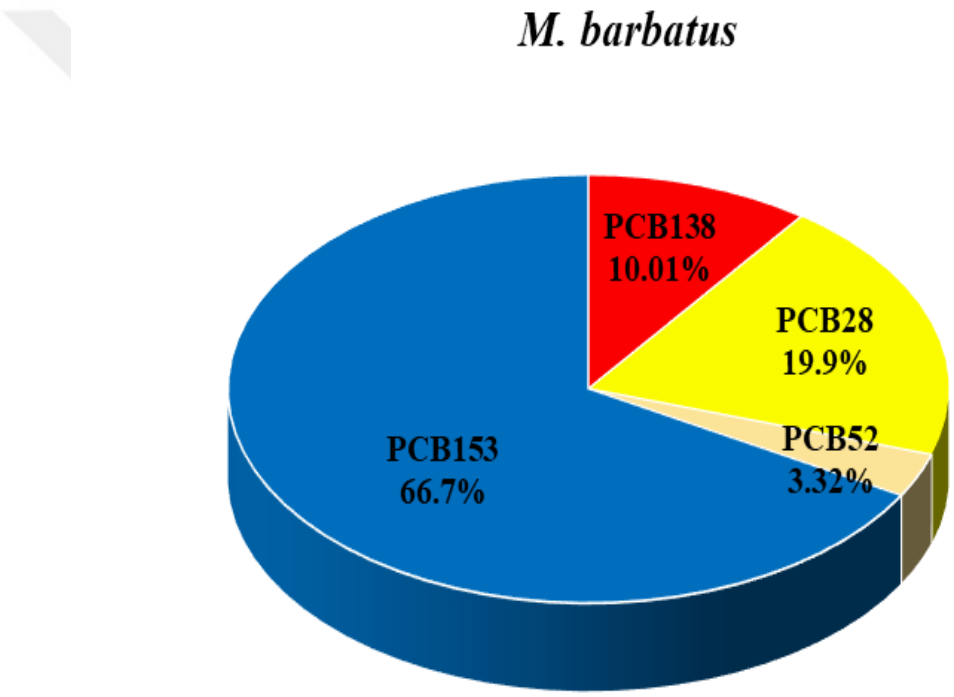


Figure 4.3 Percantage of PCB congeners in *M. barbatus* in İzmir Bay

With regard to 6 PCB congeners, which measured in the study area, the most frequently observed compounds were PCB153, PCB28, PCB52, PCB138 and PCB153, PCB28, PCB138, PCB52 in *Mullus barbatus* and *Diplodus annularis*, respectively (Figure 4.3 and 4.4).

Table 4.1 Concentrations of PCBs (ng g^{-1} dry weight) and HEOM (mg g^{-1}) in fish samples collected from İzmir Bay

Site/Year	Species	PCB 28	PCB 52	PCB 101	PCB 138	PCB 153	PCB 180	HEOM
2010								
Foça-Gediz	*M. barbatus*	bdl*	bdl	bdl	0.84	bdl	bdl	134
Foça-Gediz	*D. annularis*	bdl	bdl	bdl	bdl	12.4	bdl	55
Gülbahçe	*M. barbatus*	bdl	bdl	bdl	2.68	bdl	bdl	111
Gülbahçe	*D. annularis*	bdl	bdl	bdl	bdl	34.3	bdl	65
2011								
Foça-Gediz	*D. annularis*	bdl	bdl	bdl	bdl	bdl	bdl	137
Foça-Gediz	*D. annularis*	bdl	bdl	bdl	bdl	7.19	bdl	104
Gülbahçe	*M. barbatus*	bdl	bdl	bdl	bdl	2.36	bdl	107
Gülbahçe	*M. barbatus*	bdl	bdl	bdl	bdl	24.3	bdl	111
Gülbahçe	*M. barbatus*	bdl	bdl	bdl	bdl	22.6	bdl	182
Gülbahçe	*D. annularis*	bdl	bdl	bdl	bdl	20.4	bdl	79
Gülbahçe	*D. annularis*	bdl	bdl	bdl	bdl	17.5	bdl	60
Uzunada	*M. barbatus*	bdl	bdl	bdl	bdl	bdl	bdl	170
Uzunada	*D. annularis*	bdl	bdl	bdl	bdl	3.00	bdl	157
Uzunada	*D. annularis*	bdl	bdl	bdl	bdl	bdl	bdl	95
2012								
Foça-Gediz	*M. barbatus*	bdl	bdl	bdl	1.81	bdl	bdl	107
Foça-Gediz	*D. annularis*	bdl	bdl	bdl	bdl	bdl	bdl	56
Gülbahçe	*M. barbatus*	bdl	7.41	bdl	bdl	bdl	bdl	130
Gülbahçe	*M. barbatus*	bdl	bdl	bdl	bdl	bdl	bdl	102
Gülbahçe	*M. barbatus*	bdl	bdl	bdl	bdl	6.89	bdl	115
Gülbahçe	*D. annularis*	bdl	bdl	bdl	0.50	bdl	bdl	39
Gülbahçe	*D. annularis*	bdl	bdl	bdl	bdl	bdl	bdl	35
Uzunada	*M. barbatus*	bdl	bdl	bdl	1.92	10.6	bdl	98
Uzunada	*M. barbatus*	bdl	bdl	bdl	0.30	bdl	bdl	75
Uzunada	*D. annularis*	bdl	bdl	bdl	bdl	9.89	bdl	59
Uzunada	*D. annularis*	bdl	bdl	bdl	1.38	10.6	bdl	45
2013								
Foça-Gediz	*M. barbatus*	14.0	bdl	bdl	1.36	bdl	bdl	84
Foça-Gediz	*D. annularis*	12.2	bdl	bdl	2.88	bdl	bdl	110
Foça-Gediz	*D. annularis*	4.52	1.98	bdl	0.55	bdl	bdl	101
Foça-Gediz	*D. annularis*	4.81	bdl	bdl	0.47	bdl	bdl	78
Gülbahçe	*M. barbatus*	bdl	0.49	bdl	bdl	bdl	bdl	106
Gülbahçe	*M. barbatus*	bdl	1.27	bdl	bdl	bdl	bdl	93
Gülbahçe	*D. annularis*	4.94	bdl	bdl	0.91	bdl	bdl	55
Gülbahçe	*D. annularis*	7.94	3.75	bdl	4.71	bdl	bdl	71
Uzunada	*M. barbatus*	5.59	bdl	bdl	1.55	bdl	bdl	108
Uzunada	*M. barbatus*	0.68	2.84	bdl	0.31	bdl	bdl	97
Uzunada	*D. annularis*	bdl	bdl	bdl	6.01	bdl	bdl	47
Uzunada	*D. annularis*	bdl	bdl	bdl	bdl	bdl	bdl	45

*bdl: below detection limit

Table 4.2 Concentrations of PCBs (ng g^{-1} wet weight) in fish samples collected from İzmir Bay

Site/Year	Species	PCB 28	PCB 52	PCB 101	PCB 138	PCB 153	PCB 180
2010							
Foça-Gediz	*M. barbatus*	bdl	bdl	bdl	0.24.	bdl	bdl
Foça-Gediz	*D. annularis*	bdl	bdl	bdl	bdl	3.47	bdl
Gülbahçe	*M. barbatus*	bdl	bdl	bdl	0.75	bdl	bdl
Gülbahçe	*D. annularis*	bdl	bdl	bdl	bdl	9.60	bdl
2011							
Foça-Gediz	*D. annularis*	bdl	bdl	bdl	bdl	bdl	bdl
Foça-Gediz	*D. annularis*	bdl	bdl	bdl	bdl	2.01	bdl
Gülbahçe	*M. barbatus*	bdl	bdl	bdl	bdl	0.66	bdl
Gülbahçe	*M. barbatus*	bdl	bdl	bdl	bdl	6.80	bdl
Gülbahçe	*M. barbatus*	bdl	bdl	bdl	bdl	6.33	bdl
Gülbahçe	*D. annularis*	bdl	bdl	bdl	bdl	5.71	bdl
Gülbahçe	*D. annularis*	bdl	bdl	bdl	bdl	4.90	bdl
Uzunada	*M. barbatus*	bdl	bdl	bdl	bdl	bdl	bdl
Uzunada	*D. annularis*	bdl	bdl	bdl	bdl	0.84	bdl
Uzunada	*D. annularis*	bdl	bdl	bdl	bdl	bdl	bdl
2012							
Foça-Gediz	*M. barbatus*	bdl	bdl	bdl	0.51	bdl	bdl
Foça-Gediz	*D. annularis*	bdl	bdl	bdl	bdl	bdl	bdl
Gülbahçe	*M. barbatus*	bdl	2.07	bdl	bdl	bdl	bdl
Gülbahçe	*M. barbatus*	bdl	bdl	bdl	bdl	bdl	bdl
Gülbahçe	*M. barbatus*	bdl	bdl	bdl	bdl	1.93	bdl
Gülbahçe	*D. annularis*	bdl	bdl	bdl	0.14	bdl	bdl
Gülbahçe	*D. annularis*	bdl	bdl	bdl	bdl	bdl	bdl
Uzunada	*M. barbatus*	bdl	bdl	bdl	0.54	2.97	bdl
Uzunada	*M. barbatus*	bdl	bdl	bdl	0.08	bdl	bdl
Uzunada	*D. annularis*	bdl	bdl	bdl	bdl	2.77	bdl
Uzunada	*D. annularis*	bdl	bdl	bdl	0.39	2.97	bdl
2013							
Foça-Gediz	*M. barbatus*	3.92	bdl	bdl	0.38	bdl	bdl
Foça-Gediz	*D. annularis*	3.42	bdl	bdl	0.81	bdl	bdl
Foça-Gediz	*D. annularis*	1.27	0.55	bdl	0.15	bdl	bdl
Foça-Gediz	*D. annularis*	1.35	bdl	bdl	0.13	bdl	bdl
Gülbahçe	*M. barbatus*	bdl	0.14	bdl	bdl	bdl	bdl
Gülbahçe	*M. barbatus*	bdl	0.36	bdl	bdl	bdl	bdl
Gülbahçe	*D. annularis*	1.38	bdl	bdl	0.25	bdl	bdl
Gülbahçe	*D. annularis*	2.22	1.05	bdl	1.32	bdl	bdl
Uzunada	*M. barbatus*	1.57	bdl	bdl	0.43	bdl	bdl
Uzunada	*M. barbatus*	0.19	0.80	bdl	0.09	bdl	bdl
Uzunada	*D. annularis*	bdl	bdl	bdl	1.68	bdl	bdl
Uzunada	*D. annularis*	bdl	bdl	bdl	bdl	bdl	bdl

Table 4.3 Concentrations of PCBs (ng g^{-1} lipid weight) in fish samples collected from İzmir Bay

Site/Year	Species	PCB 28	PCB 52	PCB 101	PCB 138	PCB 153	PCB 180
2010							
Foça-Gediz	*M. barbatus*	bdl	bdl	bdl	6.3	bdl	bdl
Foça-Gediz	*D. annularis*	bdl	bdl	bdl	bdl	225	bdl
Gülbahçe	*M. barbatus*	bdl	bdl	bdl	24	bdl	bdl
Gülbahçe	*D. annularis*	bdl	bdl	bdl	bdl	528	bdl
2011							
Foça-Gediz	*D. annularis*	bdl	bdl	bdl	bdl	bdl	bdl
Foça-Gediz	*D. annularis*	bdl	bdl	bdl	bdl	69	bdl
Gülbahçe	*M. barbatus*	bdl	bdl	bdl	bdl	22	bdl
Gülbahçe	*M. barbatus*	bdl	bdl	bdl	bdl	219	bdl
Gülbahçe	*M. barbatus*	bdl	bdl	bdl	bdl	124	bdl
Gülbahçe	*D. annularis*	bdl	bdl	bdl	bdl	258	bdl
Gülbahçe	*D. annularis*	bdl	bdl	bdl	bdl	292	bdl
Uzunada	*M. barbatus*	bdl	bdl	bdl	bdl	bdl	bdl
Uzunada	*D. annularis*	bdl	bdl	bdl	bdl	19	bdl
Uzunada	*D. annularis*	bdl	bdl	bdl	bdl	bdl	bdl
2012							
Foça-Gediz	*M. barbatus*	bdl	bdl	bdl	17	bdl	bdl
Foça-Gediz	*D. annularis*	bdl	bdl	bdl	bdl	bdl	bdl
Gülbahçe	*M. barbatus*	bdl	57	bdl	bdl	bdl	bdl
Gülbahçe	*M. barbatus*	bdl	bdl	bdl	bdl	bdl	bdl
Gülbahçe	*M. barbatus*	bdl	bdl	bdl	bdl	60	bdl
Gülbahçe	*D. annularis*	bdl	bdl	bdl	13	bdl	bdl
Gülbahçe	*D. annularis*	bdl	bdl	bdl	bdl	bdl	bdl
Uzunada	*M. barbatus*	bdl	bdl	bdl	20	108	bdl
Uzunada	*M. barbatus*	bdl	bdl	bdl	4.0	bdl	bdl
Uzunada	*D. annularis*	bdl	bdl	bdl	bdl	168	bdl
Uzunada	*D. annularis*	bdl	bdl	bdl	31	236	bdl
2013							
Foça-Gediz	*M. barbatus*	167	bdl	bdl	16	bdl	bdl
Foça-Gediz	*D. annularis*	111	bdl	bdl	26	bdl	bdl
Foça-Gediz	*D. annularis*	45	20	bdl	5.4	bdl	bdl
Foça-Gediz	*D. annularis*	62	bdl	bdl	6.0	bdl	bdl
Gülbahçe	*M. barbatus*	bdl	4.6	bdl	bdl	bdl	bdl
Gülbahçe	*M. barbatus*	bdl	14	bdl	bdl	bdl	bdl
Gülbahçe	*D. annularis*	90	bdl	bdl	17	bdl	bdl
Gülbahçe	*D. annularis*	112	53	bdl	66	bdl	bdl
Uzunada	*M. barbatus*	52	bdl	bdl	14	bdl	bdl
Uzunada	*M. barbatus*	7.0	29	bdl	3.2	bdl	bdl
Uzunada	*D. annularis*	bdl	bdl	bdl	128	bdl	bdl
Uzunada	*D. annularis*	bdl	bdl	bdl	bdl	bdl	bdl

PCB153 was the major contributed congener (60.78 to 66.70 %), followed by PCB 28 (18.48 to 19.91 %) in all sampling areas and all periods. Also, PCB52 and PCB138 contributed to the sum of PCBs in low percentages (3.32 to 10.93 %) at all sampling periods and sites (Figure 4.3 and 4.4).

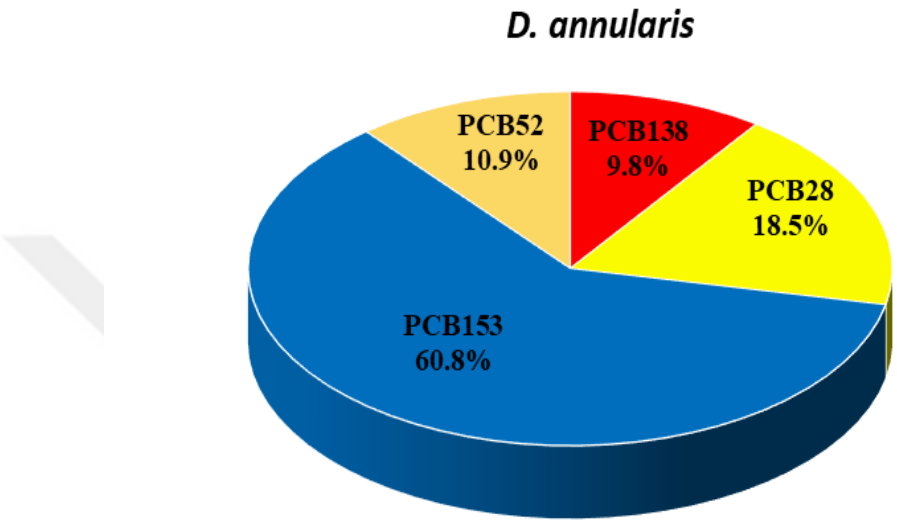


Figure 4.4 Percantage of PCB congeners in *D. annularis* in İzmir Bay

PCBs levels in biota samples from various areas around the world are given in Table 4.4. The contamination levels of PCBs in M. *barbatus* from Izmir Bay (∑PCB 64.5 ng g^{-1} lwt), (∑PCB 2.1 ng g^{-1} wwt) are lower than *M. barbatus* samples collected from Adriatic Sea (∑PCB 395 ng g^{-1} lwt) (Di Muccio et al., 2002), Black Sea (∑PCB 28.6 ng g^{-1}wwt) (Georgieva et al., 2012). Although PCBs in *M. barbatus* (∑PCB 7.32 ng g^{-1} dwt) is lower than *Octopus Sp* (∑PCB 10.3 ng g^{-1} dwt), besides, *D. annularis* (∑PCB 12.6 ng g^{-1} dwt) is higher than *Octopus Sp* (∑PCB 10.3 ng g^{-1} dwt) from Southern Indian Ocean (Monod et al., 1995). In addition, PCB levels in our study are (∑PCB 118 ng g^{-1} lwt) are lower than Marmara Sea (∑PCB 219.1 ng g^{-1} lwt) (Coelhan et al., 2006) and lower from Istanbul Strait (Okay et al., 2009), North-East Atlantic Ocean (Strid et al., 2007), Adriatic Sea (Di Muccio et al., 2002).

Table 4.4 The mean concentration of PCB congeners and the mean and range of ∑PCB in biota samples from different areas in the world (as ng g^{-1})

Location	**Species**	**PCB28**	**PCB52**	**PCB101**	**PCB138**	**PCB153**	**PCB180**	**∑PCB**	**References**
Southern Indian Ocean, Amstermdam Island (dwt)	Octopus *sp.*	0.60	1.4	bdl*	2.7	1.4	1.3	7.4	Monod et al., 1995
Southern Indian Ocean, Saint Paul Island (dwt)	Octopus *sp.*	1.8	2.9	bdl	1.7	4.2	2.6	13	Monod et al., 1995
Lagos, Nigeria, gulf of Guinea (wwt)	Tilapia *guineensis*	0.40	1.4	0.60	0.15	0.39	0.10	3.0	Igbo et al., 2018
North-East Atlantic (lwt)	Somniosus *microcephalus*	24	210	340	1100	1200	550	3424	Strid et al., 2007
Adriatic Sea (lwt)	*M. barbatus*	bdl	8.9	bdl	28	24	12	395	Di Muccio et al., 2002
Black Sea (wwt)	*M. barbatus*	3.3	0.80	bdl	6.3	12	6.0	29	Georgieva et al. 2012
Adriatic Sea (wwt)	Eel	bdl	2.8	14	18	19	12	65	Storelli et al., 2005
Istanbul Strait (wwt)	Mussel	155	287	687	542	932	87	2689	Okay et al., 2009
Marmara Sea (lwt)	Fish	12	12	26	73	66	29	219	Coelhan et al., 2006
Eastern Aegean, İzmir Bay	*M. barbatus*	75	26	bdl	13	107	bdl	4.0- 219 Mean:65	This study (lwt)
Eastern Aegean, İzmir Bay	*M. barbatus*	6.8	3.0	bdl	1.4	13	bdl	0.30- 24 Mean:7.3	This study (dwt)
Eastern Aegean, İzmir Bay	*M. barbatus*	1.9	0.84	bdl	0.38	3.7	bdl	0.08-6.8 Mean:2.1	This study (wwt)
Eastern Aegean, İzmir Bay	*D. annularis*	84	36	bdl	37	224	bdl	13- 528 Mean:172	This study (lwt)
Eastern Aegean, İzmir Bay	*D. annularis*	6.9	2.9	bdl	2.2	14	bdl	3.4- 35 Mean:13	This study (dwt)
Eastern Aegean, İzmir Bay	*D. annularis*	1.9	0.80	bdl	0.61	4.0	bdl	0.84- 9.6 Mean:3.4	This study (wwt)

*bdl: below detection limit

4.2 Public Health Risk Assessment

Polychlorinated biphenyls (PCBs) are persistent organic pollutants comprising a group of 209 congeners, which can be divided into two different groups according to their toxicological properties. The twelve congeners (dioxin-like) that display all descriptors are referred to as "dioxin-like". These twelve PCBs (PCB-77, PCB-81, PCB-105, PCB-114, PCB-118, PCB-123, PCB-126, PCB-156, PCB-157, PCB-167, PCB-169, and PCB-189) have been assigned toxicity equivalency factors (TEFs, ascribed by WHO in 1998 and revised in 2005) (Van den Berg et al., 2006).

Depending on the content of the research, specific PCB congeners may be examined. For instance, the Stockholm Convention on Persistent Organic Pollutants (2009) suggests measurement of six indicator PCBs (PCB-28, PCB-52, PCB-101, PCB-138, PCB-153 and PCB-180) to describe contamination by PCBs. These congeners are called "non-dioxin like" and were selected because they are found at higher levels in the environment. According to country and the aim of study, different congeners may be used, e.g. 6 indicator PCB congeners for this study was chosen. Additionally, these PCB congeners are used widespread in the world, especially in Europe, and is based on PCB congeners from different levels of chlorination and their greater abundance.

According to EC regulation 1259/2011, the limit for the sum of the six indicator PCB congeners (PCB 28, 52, 101, 138, 153 and 180) is established to 75 ng/g ww. In the present study, these ∑PCB6 congeners (0.59-6.64 ngg^{-1} wwt, Table 4.5) represent 0.78-8.85 % of limit of 75 ng/g ww set by the Environmental Food Safety Authority (EFSA) in fish regardless of species. For this reason, that value is considered as an appropriate marker of environmental contamination in particular for occurrence and human exposure (European Food Safety Authority, 2005). In this study, determined indicator ndl-PCB6 values were compared with iPCB6 limits of 75 ng/g ww set by the EFSA and two fish species did not exceed the safe limits of iPCB6 set by EU.

Table 4.5 Mean levels of ∑PCB6 (ng g^{-1} ww), estimated daily intake levels (ng kg^{-1} body wt d^{-1}) in different fish species by human in İzmir Bay during sampling periods and acceptable daily intake (ADI) value (ng kg^{-1} body wt d^{-1})

	∑PCB6	EDI	TDI[a]
M. barbatus			
2010	0.59	0.58	
2011	3.55	3.47	
2012	1.45	1.42	
2013	1.66	1.62	
D. annularis			10
2010	6.64	6.50	
2011	2.35	2.30	
2012	1.35	1.32	
2013	2.31	2.26	

[a]WHO (2003)

The estimated daily intake (EDI) of PCB congeners via fish consumption for the local inhabitants was calculated by the equation as follows,

EDI = C (concentration of indicator of PCBs) × CR / BW

where, EDI is the estimated daily intake (ng kg^{-1} bw d^{-1}); C represents mean concentration of indicator PCB congeners (iPCB6) in each species (ng g^{-1} ww); CR represents fish consumption per day (g day^{-1}), BW represents consumer body weight (assuming a typical weight of 70 kg). Köprücü (2007) and Şahin (2011) explained that annual per capita fish products in Turkey has showed difference between the regions and it is calculated to be about 25 kg per year in the coastal regions. Consequently, fish consumption per adult person is 68.5 g daily in the wester coast of Turkey.

However, for all 209 congeners a tolerable daily intake (TDI) of 20 ng kg^{-1} bw d^{-1} has been derived for mixtures of PCBs (WHO, 2003). Assuming that the sum of the so called indicator PCBs ∑6 PCBs; PCB 28, 52, 101, 138, 153, and 180) represents about 50% of all PCB congeners in food, a TDI of 10 ng kg^{-1} bw d^{-1} was initially proposed by the WHO at the ‘‘2nd PCB workshop’’ in Brno (Czech Republic, May

2002), but has already been used as a reference value by others (Netherlands National Institute for Public Health and the Environment (RIVM), 2001; French Food Safety Agency(AFFSA), 2007; VKM, 2007). In Europe, more than 90% of PCB exposure in the general population is through food consumption (EFSA, 2005). Mean daily dietary intakes of the sum of PCB6 (non-dioxin like) range between 10 and 45 ng/kg bw for adults and two and a half times higher in children.

The EDI values of total indicator ndl-PCB congeners are given in Table 4.5 through the observed fish species consumption in the population of İzmir. Mean EDI values were different in *M. barbatus* (0.58-3.47 ng kg^{-1} bw d^{-1}) and *D. annularis* (1.32-6.50 ng kg^{-1} bw d^{-1}) in the four year period from 2010 to 2013. Average EDI value for *D. annularis* in 2010 was the highest at all sampling periods. The EDI of 6 PCB congeners by the people were below the tolerable daily intake (TDI, 10 ng kg^{-1} bw d^{-1}) recommended by WHO (2003) suggesting this intake would not pose adverse effect to inhabitants of İzmir at the present. Considering the European population EFSA has estimated an average exposure to ndl-PCB indicators ranging from 4.3 to 25.7 ng kg^{-1} bw d^{-1} (EFSA, 2012).

4.3 Statistical Analyses

4.3.1 Statistical Summary

A general description of the statistical summary (Figure 4.5-4.8) shows distribution of PCB congeners in *Mullus barbatus* and *Diplodus annularis* within sampling periods (as ng g^{-1} wet weight); lower and upper box edges indicate mean±SE; outlying bars are mean±1.96 SE. According to results *Mullus barbatus* accumulated higher level of PCB52 in comparison with other PCB congeners during all sampling periods. Interestingly, the standard error for PCB52 concentrations were very large in *M. Barbatus*. In contrast, the higher concentrations of PCB28, PCB138 and PCB153 were measured in *D. annularis.*

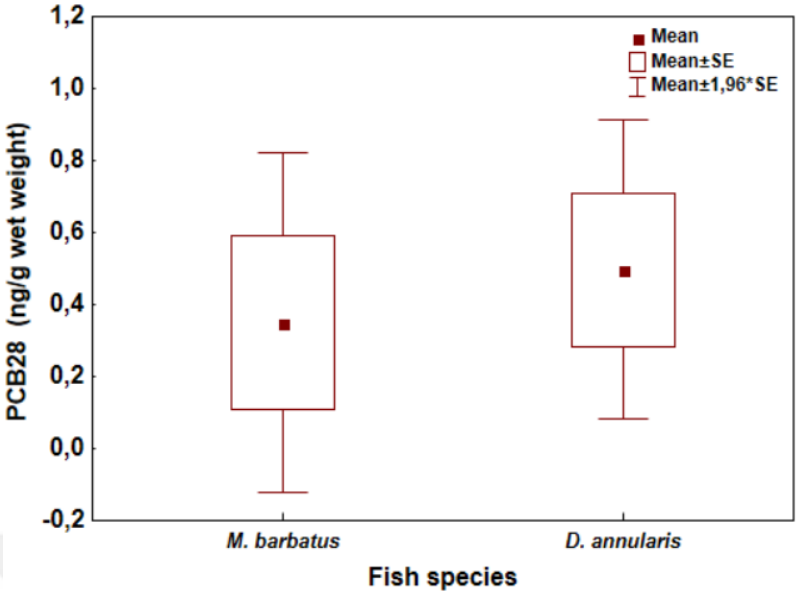


Figure 4.5 Means, mean±standard error and confidence intervals (1.96σ) of PCB28 levels in different species

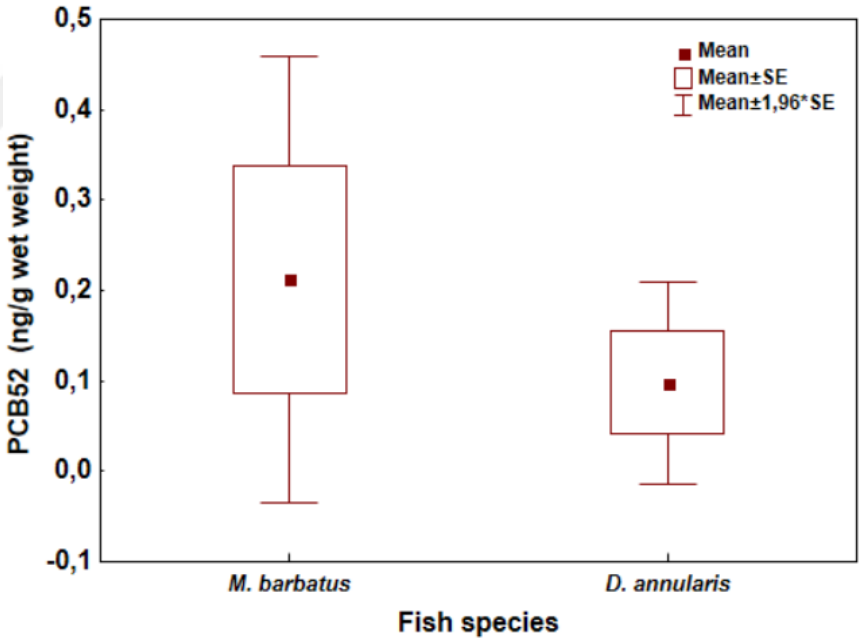


Figure 4.6 Means, mean±standard error and confidence intervals (1.96σ) of PCB52 levels in different species

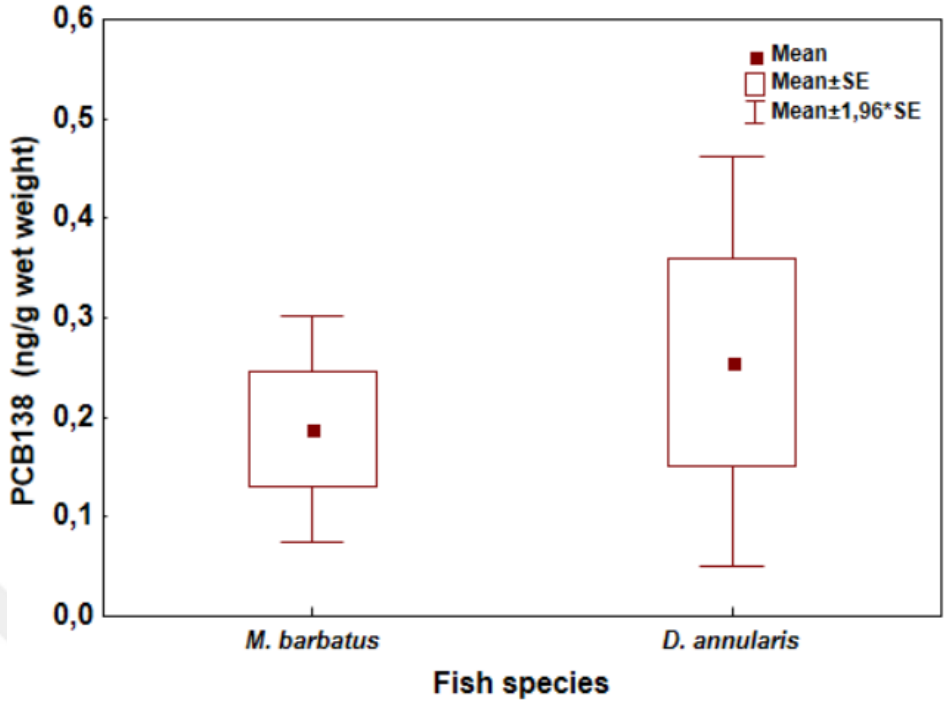


Figure 4.7 Means, mean±standard error and confidence intervals (1.96σ) of PCB138 levels in different species

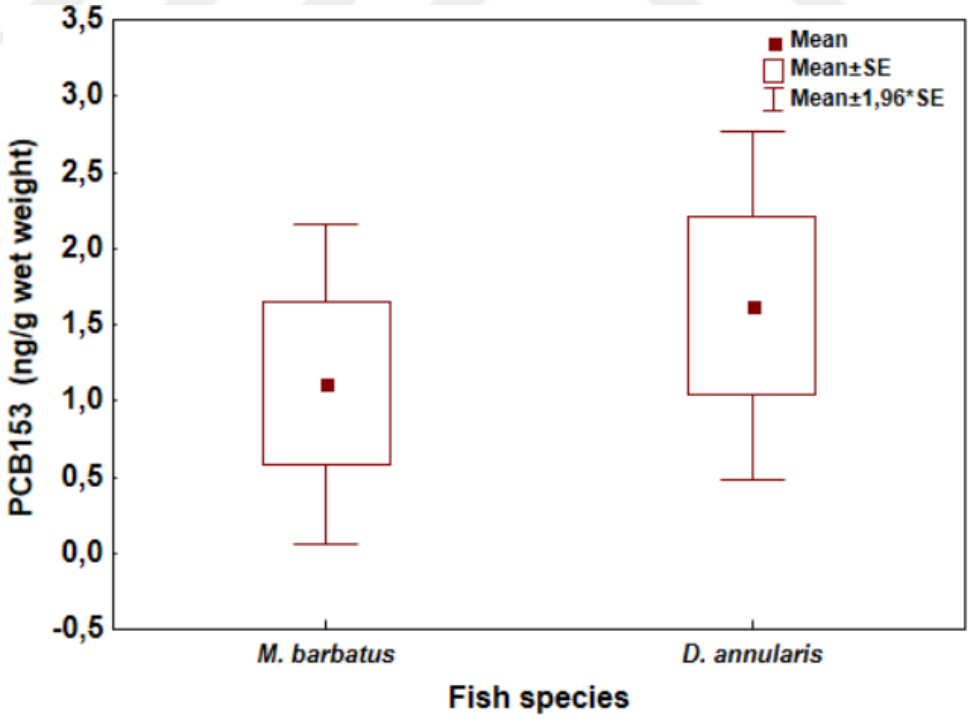


Figure 4.8 Means, mean±standard error and confidence intervals (1.96σ) of PCB153 levels in different species

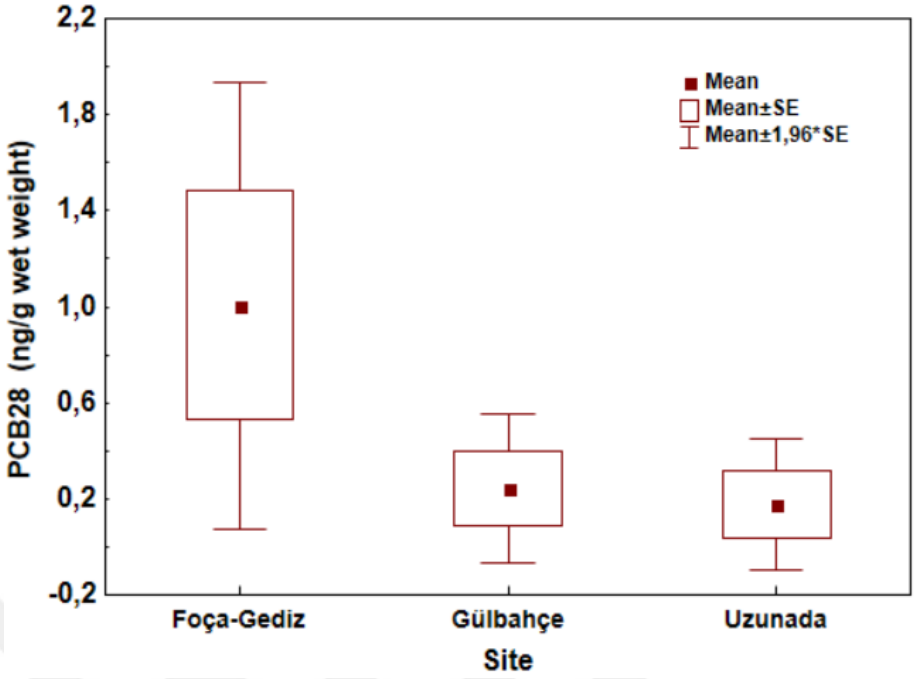


Figure 4.9 Means, mean±standard error and confidence intervals (1.96σ) of PCB28 levels in sampling sites for different species

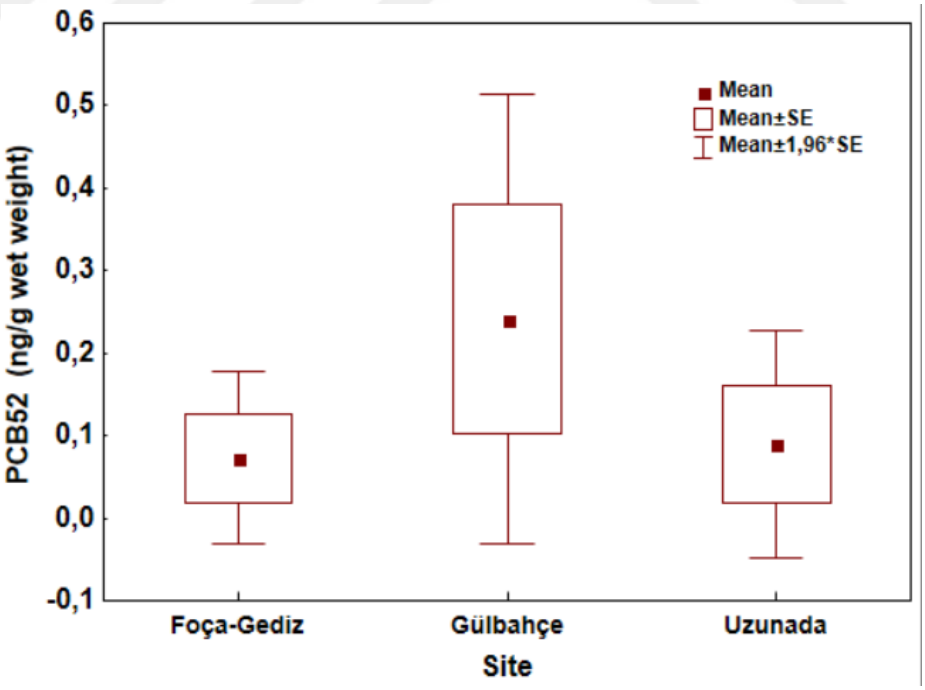


Figure 4.10 Means, mean±standard error and confidence intervals (1.96σ) of PCB52 levels in sampling sites for different species

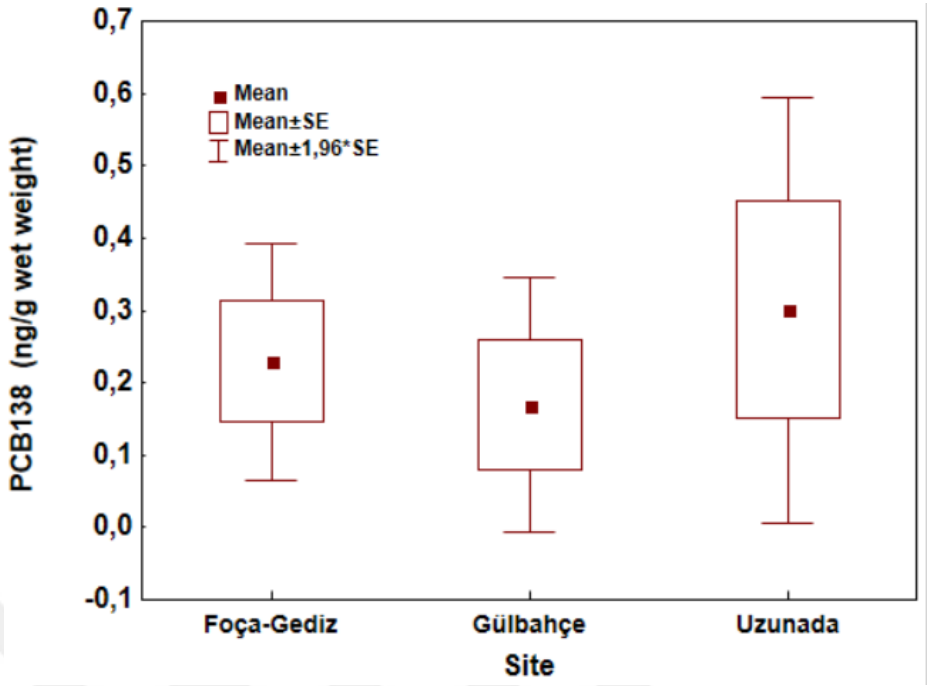


Figure 4.11 Means, mean±standard error and confidence intervals (1.96σ) of PCB138 levels in sampling sites for different species

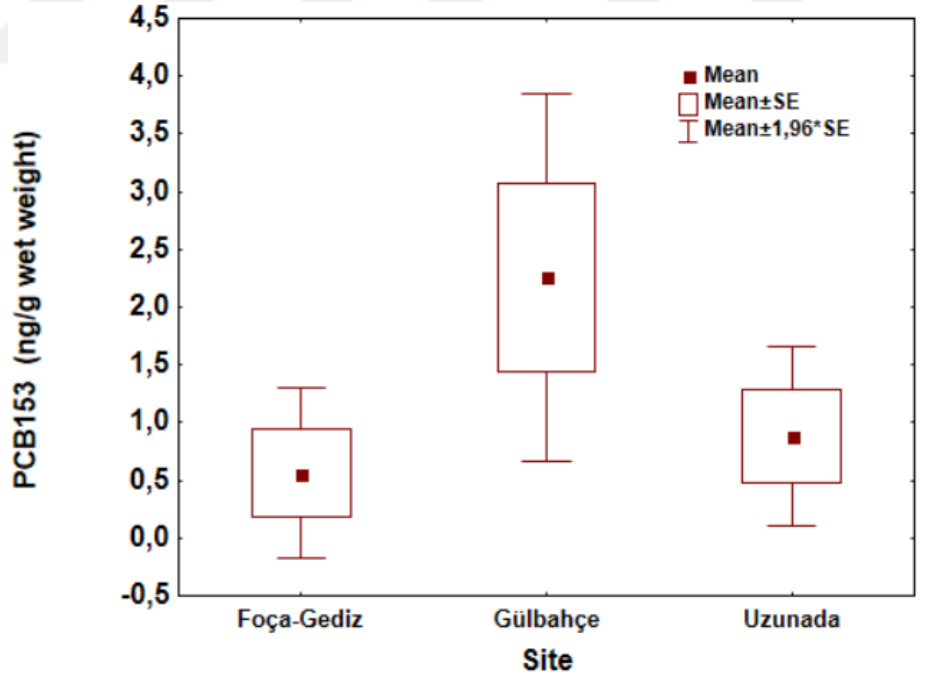


Figure 4.12 Means, mean±standard error and confidence intervals (1.96σ) of PCB153 levels in sampling sites for different species

Figures 4.9 and 4.12 indicate that the means, related standard errors and approximate confidence intervals of PCB28, PCB52, PCB138 and PCB153 levels for different sampling sites in İzmir Bay. PCB28 concentrations in fish species increased at Foça-Gediz site while the higher PCB52 and PCB153 levels were observed at Gülbahçe region. On the other hand, maximum PCB138 concentration was measured at Uzunada site.

4.3.2 Relationships Between Variables

The significance of correlations between PCB congeners, extractable organic matter and fish length were investigated with Spearman Rank Order Correlations test for two fish species. Correlation coefficients were presented in Figure 4.6 and 4.7.

Table 4.6 Spearman Rank Order correlation coefficients for PCBs (ng g^{-1} dry wt), EOM (mg g^{-1}) and mean length (mm) in *M.barbatus* ($p<0.05$)

Variable	Fish Length	PCB28	PCB52	PCB138	PCB153	EOM
Fish Length	1.000000	-0.223488	-0.089135	-0.010642	0.422244	**0.507995**
PCB28	-0.223488	1.000000	0.081694	0.381044	-0.291487	-0.352345
PCB52	-0.089135	0.081694	1.000000	-0.292395	-0.347330	-0.187942
PCB138	-0.010642	0.381044	-0.292395	1.000000	-0.251912	-0.249770
PCB153	0.422244	-0.291487	-0.347330	-0.251912	1.000000	0.289270
EOM	**0.507995**	-0.352345	-0.187942	-0.249770	0.289270	1.000000

Significant correlation was found between extractable organic matter and fish length (r = 0.51) for *M. barbatus* while relationships were observed significantly between PCB28 and PCB52 (r = 0.54), PCB138 (r = 0.66), PCB153 (r = -0.45) for *D. annularis*, respectively. Positive relationships were calculated between fish length and PCB153 (r = 0.42), PCB28 and PCB138 (r = 0.38), PCB153 and EOM (r = 0.29) for *M. barbatus* and additionally PCB28 and EOM (r = 0.26), PCB52 and PCB138 (r = 0.43), PCB52 and EOM (r = 0.20) for *D. annularis*.

Table 4.7 Spearman Rank Order correlation coefficients for PCBs (ng g^{-1} dry wt), EOM (mg g^{-1}) and mean length (mm) in *D. annularis* ($p<0.05$)

Variable	Fish Length	PCB28	PCB52	PCB138	PCB153	EOM
Fish Length	1.000000	-0.193641	0.017403	0.170137	-0.067373	-0.142912
PCB28	-0.193641	1.000000	**0.536769**	**0.662508**	**-0.446133**	0.263049
PCB52	0.017403	**0.536769**	1.000000	0.430031	-0.260625	0.196420
PCB138	0.170137	**0.662508**	0.430031	1.000000	-0.421935	-0.138433
PCB153	-0.067373	**-0.446133**	-0.260625	-0.421935	1.000000	0.057751
EOM	-0.142912	0.263049	0.196420	-0.138433	0.057751	1.000000

PCB28 and PCB153, EOM; PCB52 and PCB138, PCB153; PCB138 and PCB153, EOM were negatively correlated in *M. barbatus*. Nagative relationships were found between PCB153 and PCB52, PCB138 for *D. annularis*. There is no significant correlations between PCB congeners and fish length and EOM in *D. annularis*.

4.3.3 One-way ANOVA Analysis

One-way ANOVA test was performed to compare indicator non-dioxin like PCB congener concentrations between sampling areas and years. Post hoc Tukey HSD test was applied to obtain statistically significant differences ($p<0.05$) after ANOVA. This test presented non significant spatial variations among sampling sites (Table 4.8).

Significant temporal variations were found for PCB28, PCB153 and PCB28, PCB138, PCB153 in *Mullus barbatus* and *Diplodus annularis*, respectively. According to post hoc Tukey HSD analysis, PCB28 levels in 2012 differed significantly from 2013 ($p<0.04119$) and PCB153 was significantly different in 2010 from 2011 ($p<0.02358$) for *M. barbatus* (Table 4.8).

Table 4.8 The results of one-way ANOVA for sampling sites and periods (p<0.05) in İzmir Bay

Variable	Sampling period			Sampling site		
	df	F	p	df	F	p
Mullus barbatus						
PCB28	3	3.6633	*	2	2.0067	ns
PCB52	3	1.1746	ns	2	0.5061	ns
PCB138	3	2.1074	ns	2	1.7394	ns
PCB153	3	4.4305	*	2	1.2021	ns
Diplodus annularis						
PCB28	3	14.78	**	2	1.6297	ns
PCB52	3	4.28	ns	2	0.4325	ns
PCB138	3	1.85	*	2	0.0167	ns
PCB153	3	4.85	**	2	0.3138	ns

*p<0.05;**p<0.01; ***<0.001, ns: non significant.

Post hoc HSD analysis indicated that PCB28 concentrations differed significantly in 2013 (p<0.00136) from other sampling periods for *Diplodus annularis* in the study area. PCB138 showed significantly difference between 2011 and 2013 periods (0.00441) in *D. annularis*. PCB153 is different in 2010 (p<0.01349) and 2011 (p<0.04487) from 2013 in the sampling area for *D. annularis* (Table 4.8). Similar results were obtained for PCB153 during 2010 and 2011 periods for two different species.

The significant differences for PCBs among sampling sites were not found in muscle tissue of *Mullus barbatus* and *Diplodus annularis*. The knowledge of the physiology and ecology of the marine organisms are necessary in order to understand the meaning of the data on contaminant levels. Generally, the differences in contaminant burdens between the different species are related to the physiological differences between different species or the quantity and type of food taken by different populations of the same species. Variations in concentration within species may come about through the migration of fish species from unpolluted areas to relatively more polluted areas.

4.3.4 Principal Component Analysis

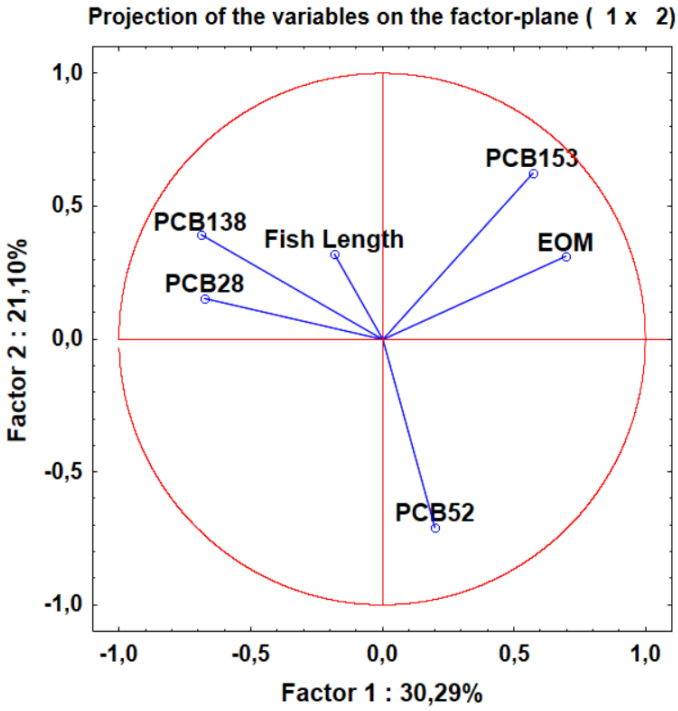
In the PCA, 82.3 % of the total variability was presented by components 1, 2, 3 and 4 for *M. barbatus* (Fig. 4.13a and b). Hovewer, the first factorial plane, Fig. 4.3.4.1a, demonstrates almost the half of the variance, 52% with a first axes value of 30.3%. It is on the base of these five factors that the subsequent analysis of the groups will be performed. Factor coordinates of the variables and Eigen values of the correlation matrics for *M. barbatus* were given in Table 4.9 and 4.10.

Table 4.9 Factor coordinates of the variables based on correlations for *M. barbatus*

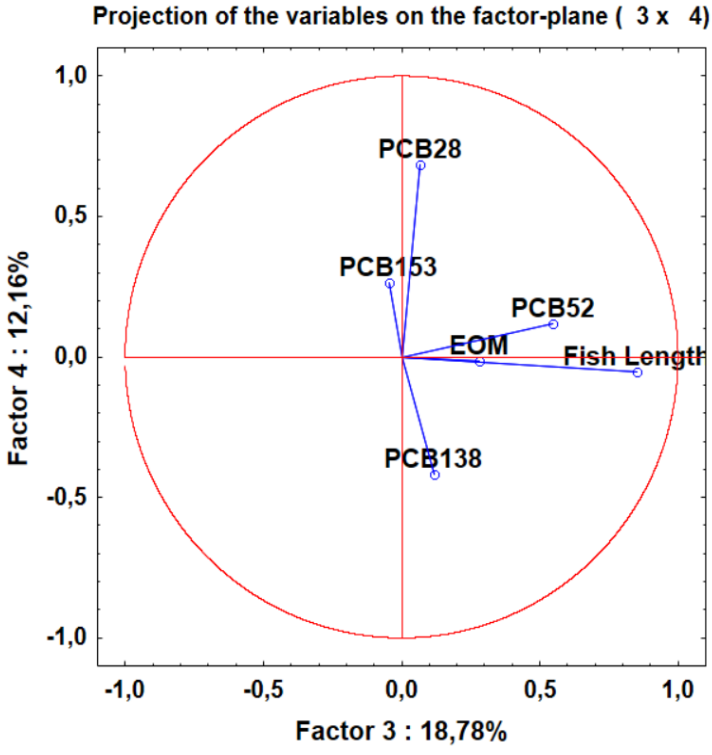
Variable	Factor 1	Factor 2	Factor 3	Factor 4	Factor 5
PCB28	**-0.674063**	0.151245	0.065583	**0.685179**	-0.221341
PCB52	0.199093	**-0.709657**	0.548066	0.119169	-0.036620
PCB138	**-0.689867**	0.390603	0.116761	-0.415831	-0.209479
PCB153	**0.570575**	0.622794	-0.045269	0.264811	0.285961
EOM	**0.698720**	0.309418	0.282595	-0.013832	-0.571691
Fish length	-0.182978	0.321610	**0.852477**	-0.053725	0.273964

Table 4.10 Eigen values of correlation matrix and related statistics for *M. barbatus*

Value number	Eigen value	% Total variance	Cumulative Eigen value	Cumulative %
1	1.817161	30.28602	1.817161	30.2860
2	1.266103	21.10172	3.083264	51.3877
3	1.126937	18.78228	4.210201	70.1700
4	0.729789	12.16315	4.939990	82.3332
5	0.577875	9.63125	5.517865	91.9644
6	0.482135	8.03558	6.000000	100.0000



a



b

Figure 4.13 Output of PCA by correlation circles for factors 1, 2 (a) and 3, 4 (b) for *M. barbatus*

The interpretation of the factors depends on the coordinates of the variables. These coordinates explain the linear coefficient factors of the variable with the factor. The typical representation of these relations is so called correlation circle, with a ray of 1. In the correlation circle concerning the first two components, Fig. 4.13a, PCB153, EOM in the positive part and PCB28, PCB138 in the negative part of component 1 with the highest correlation coefficient $r > 0.57$ and $r > -0.67$, respectively. The variable with the highest correlation coefficient for factor 2, $r > -0.70$ in the negative part, was described to be PCB52.

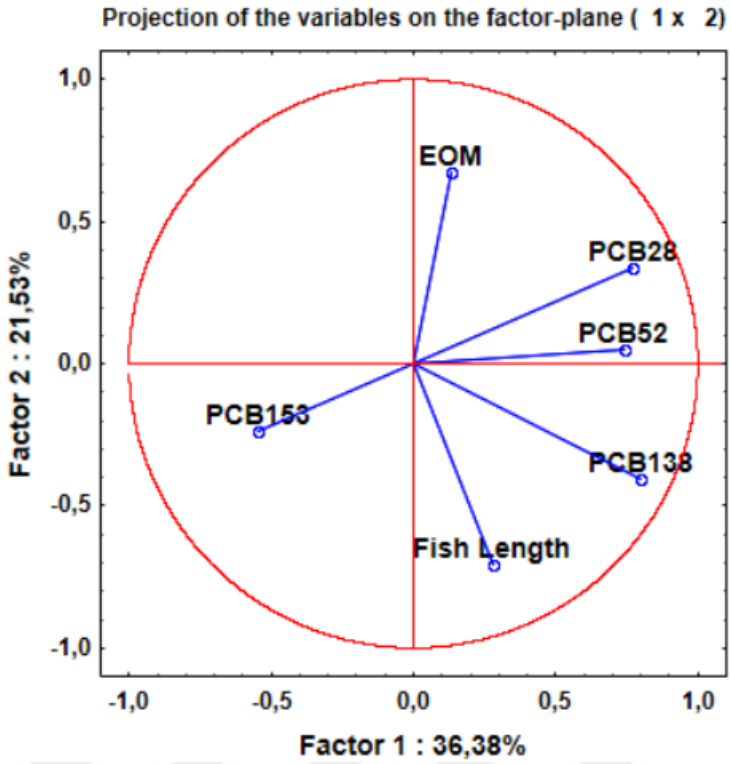
According to PCA results, 18.8 % and 12.26 % of the total variability was presented by components 3 and 4, respectively for *M. barbatus*. Furthermore, the only variable relatively to factor 3 was negative and with a high linear correlation coefficient, $r > 0.85$, found for fish length. Relatively to factor 4, the unique variable with the highest positive value was PCB28, with $r > 0.68$ (Fig. 4.13b).

Table 4.11 Factor coordinates of the variables based on correlations for *D. annularis*

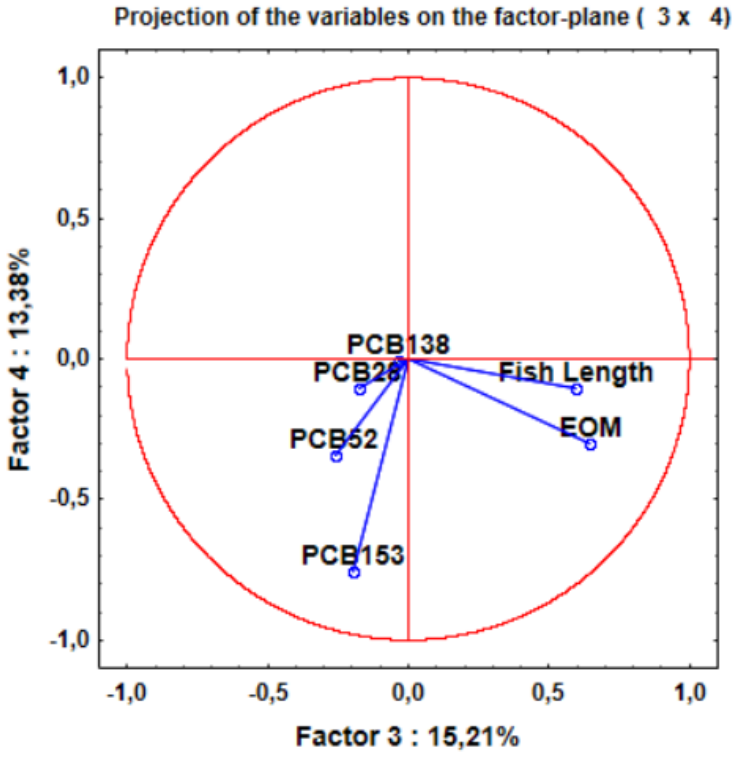
Variable	Factor 1	Factor 2	Factor 3	Factor 4	Factor 5
PCB28	**0.773451**	0.338505	-0.177486	-0.106155	0.404002
PCB52	**0.741271**	0.051677	-0.260567	-0.344379	-0.509506
PCB138	**0.798747**	-0.404994	-0.038162	-0.009817	0.213719
PCB153	-0.549468	-0.239453	-0.198112	**-0.754541**	0.177448
EOM	0.132611	**0.672261**	0.644894	-0.304339	-0.001679
Fish length	0.278782	-0.707923	**0.597361**	-0.104069	-0.027894

Table 4.12 Eigen values of correlation matrix and related statistics for *D. annularis*

Value number	Eigen value	% Total variance	Cumulative Eigen value	Cumulative %
1	2.182925	36.38208	2.182925	36.3821
2	1.291705	21.52842	3.474630	57.9105
3	0.912830	15.21384	4.387460	73.1243
4	0.802747	13.37912	5.190207	86.5034
5	0.500759	8.34598	5.690965	94.8494
6	0.309035	5.15058	6.000000	100.0000

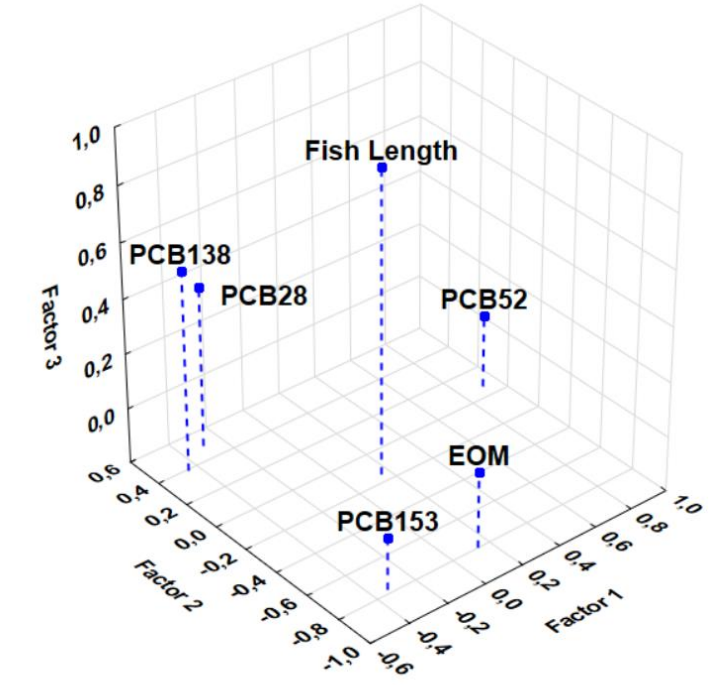


a

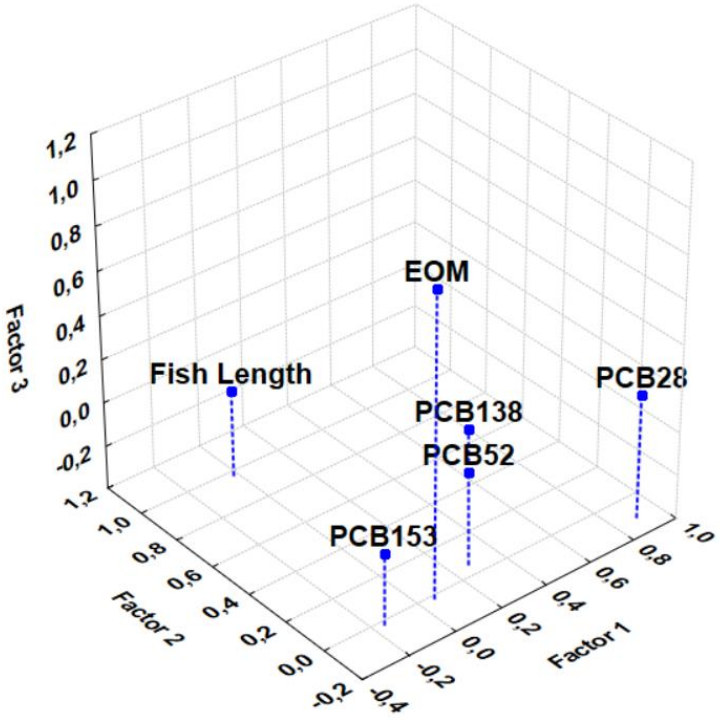


b

Figure 4.14 Output of PCA by correlation circles for factors 1, 2 (a) and 3, 4 (b) for *D. annularis*



a



b

Figure 4.15 Crossplots resulting from PCA of *M. barbatus* (a) and *D. annularis* (b) samples

Factor coordinates of the variables and Eigen values of the correlation matrix and related statistics for *D. annularis* were given in Table 4.11 and 4.12.For *D. annularis* samples, the first four principal components accounted for 86.5 % of the total variation. Hovewer, the first factorial plane, Fig. 4.14a, shows almost the half of the variance, 57.9 % with a first axes value of 36.4 %. PCB28, PCB52 and PCB138 in the positive scores ($r > 0.70$) were connected on factor 1 with the highest correlation coefficients (Figure 4.14a). Extractable organic matter contents was related with factor 2 ($r = 0.67$).

PCA results showed that, 15.2 % and 13.4 % of the total variability was presented by components 3 and 4, respectively for *D. annularis* (Figure 4.14b). PCB138, PCB28, PCB52 and PCB153 displayed a similar distribution pattern concerning factors 15.2 % of the total variance. Fish length was characterized by high correlation ($r > 0.59$) on factor 3. Two more principal component was included into the analysis (94.8 % of the total variation was explained) and it was shown that only PCB153 was related with factor 4 with high negative score ($r > -0.75$).

The PCA crossplots were presented in Figure 4.15a-b. These figures show that not only certain groups of PCBs are characterized by different bioaccumulation and biotransformation features but some differences among fish species can be also observed.

CHAPTER FIVE
CONCLUSIONS

This study first documented investigation of PCB congeners in two fish species biota from İzmir Bay. Biota samples were collected during 2010, 2011, 2012 and 2013. Results obtained with the present study provided useful knowledge in order to assess PCB levels and health risks in selected organisms of the İzmir Bay.

According to 6 PCB congeners, PCB153 was the most frequently compound in two fish species, followed by PCB28 in the sampling area. PCB153 was dominant congener in Gülbahçe and Uzunada regions, while PCB28 was predominant compound in Foça-Gediz region.

PCA analysis was performed to evaluate the correlation of the determined variables in two different fish species. The PCA results showed that in *M. barbatus*;

- the concentrations of PCBs are not correlated with fish length,
- bioaccumulation of PCB28, PCB138, PCB153 show difference with PCB52 and PCB congeners except PCB52 are related with EOM.

These PCA results suggested that in *D. annularis*;

- EOM levels are not correlated with fish length and PCB congeners,
- the accumulation features of PCB28, PCB52, PCB138 are similar. Hovewer, these congeners are different from PCB153 in terms of accumulation features.
- in *D. annularis* samples fish length are not related with PCB congeners.

One-way ANOVA test was applied to compare indicator non-dioxin like PCB congeners between sampling regions and years. This test exhibited non significant spatial variations among sampling sites. Significant temporal variations were observed for PCB28, PCB153 and PCB28, PCB138, PCB153 in *Mullus barbatus* and *Diplodus annularis*, respectively.

As stated by EC regulation 1259/2011, the limit for the six indicator PCB congeners (PCB 28, 52, 101, 138, 153 and 180) is given to 75 ngg^{-1} ww. In this study, these ∑PCB6 congeners were found as 0.59-6.64 ngg^{-1} ww and compared with iPCB6 limits of 75 ngg^{-1} ww set by the Environmental Food Safety Authority (EFSA). Fish samples analysed did not exceed the safe limits of iPCB6 set by EU.

The EDI values of total indicator non dioxin like PCB congeners were calculated for two fish species in this study. EDI values were different in *M. barbatus* (0.58-3.47 ng kg^{-1} bw d^{-1}) and *D. annularis* (1.32-6.50 ng kg^{-1} bw d^{-1}) in the sampling periods between 2010 and 2013. The EDI of 6 PCB congeners by the people were below the tolerable daily intake (TDI, 10 ng kg^{-1} bw d^{-1}) recommended by WHO (2003) suggesting this intake would not pose adverse effect to inhabitants of İzmir at the present.

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