

TEMPORAL - SPATIAL DISTRIBUTIONS AND DEGRADATION OF
BISPHENOL A IN MARINE AND FRESHWATERS IN TURKEY

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BISPHENOL A IN MARINE AND FRESHWATERS IN TURKEY

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ABSTRACT

TEMPORAL - SPATIAL DISTRIBUTIONS AND DEGRADATION OF BISPHENOL A IN MARINE AND FRESHWATERS IN TURKEY

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Bisphenol A (BPA) is an endocrine disrupting chemical compound that mimics estrogen and is linked to developmental disorders. It is commonly detected in the marine and freshwater environments. However, lack of data from the developing countries prevent the comprehensive global assessment of this contaminant. This study was conducted to understand temporal and spatial distributions and degradation of BPA in marine and freshwaters in Turkey. The first chapter's aim was to report BPA concentrations in a time series at coasts of Erdemli, located in the Northeastern Mediterranean Sea, and regional rivers. Furthermore, seawater samples from the Black Sea, Bosphorus, the Sea of Marmara and the Mediterranean Sea were investigated to better understand regional and seasonal variations. The second chapter focused on the degradation processes of BPA in natural and artificial river and seawater at different temperatures. Concentrations of BPA in river and seawater ranged from 4.16 to 29.92 µg/L. Temporal variation of BPA was more conspicuous than its spatial variation in the northeastern Mediterranean Sea. Similar levels of BPA concentrations were found in all seas investigated. BPA concentration found in deep waters (500-1000 m depth) were very similar to surface water BPA concentrations indicating this compound had physically mixed into the whole water column. Samples prepared from the artificial waters did not show any BPA degradation in 150 days, however, natural

river water started to degrade after day 50. The degradation rate of the samples from natural river water at 25 °C was faster than samples at 4 °C. In natural seawater samples, there were no significant degradation in 150 days at 4 °C and 25 °C, however, samples prepared natural seawater kept outside and exposed over 40 °C showed degradation after day 50. Slow degradation process in the region compared to other studies could be caused from the lack of BPA degrading microorganisms in the river and the seawater. This study shows that BPA contamination reach to serious levels in aquatic environments in Turkey. It also suggests that due to their distinct degradation rates, BPA contamination on a marine organisms and environment could be more persistent than on freshwaters.

Keywords: Bisphenol A; The Mediterranean Sea; The Black Sea; The Sea of Marmara; Time series; Degradation

ÖZ

BİSFENOL A' NIN TÜRKİYE' NİN DENİZ VE NEHİRLERİNDE ZAMANSAL – MEKANSAL DAĞILIMI VE BOZUNMASI

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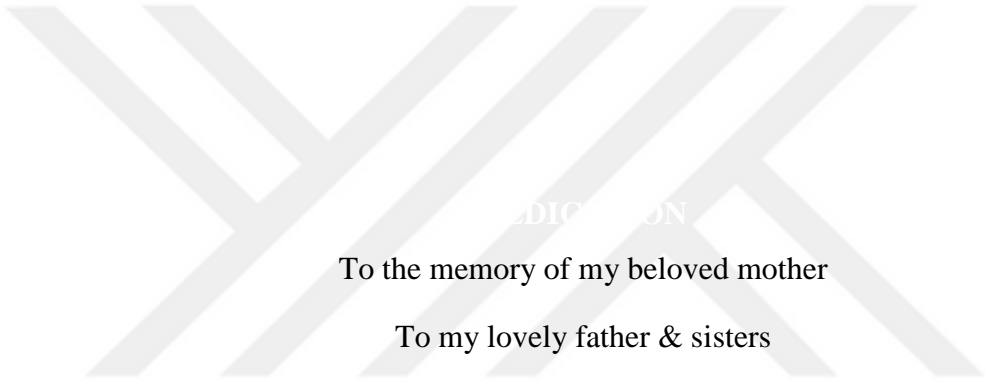
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Bisfenol A (BPA), bir endokrin sistem bozucu kimyasal bileşik olup östrojeni taklit eder ve gelişimsel bozukluklarla bağlantılı olduğu bulunmuştur. Deniz ve tatlı su ortamlarında yaygın olarak tespit edilmektedir. Gelişmekte olan ülkelerdeki veri eksikliğinden dolayı dünya genelinde tam anlamıyla bir BPA değerlendirmesi yapılamamaktadır. Bu tez çalışması BPA' nın Türkiye'deki deniz ve tatlı sulardaki zamansal ve alansal dağılımını ve bozunmasını anlamak için yapılmıştır. Tezin ilk bölümünün amacı BPA konsantrasyonlarını Erdemli zaman serisinde ve bölgesel nehirlerde belirlemektir. Ayrıca, bu çalışmada Karadeniz, İstanbul Boğazı, Marmara Denizi ve Akdeniz'den deniz suyu örnekleri alınarak bölgesel ve mevsimsel değişimleri daha iyi anlamak amaçlanmıştır. Tezin ikinci bölümü BPA' nın, farklı sıcaklıklarda doğal ve yapay, -nehir ve deniz sularında-, bozunma süreçlerine odaklanmıştır. BPA' nın nehir ve deniz suyu konsantrasyonları 4.16 ve 29.92 µg/L arasında değişim göstermiştir. Kuzeydoğu Akdeniz' de BPA konsantrasyon değişimindeki zamansal varyasyon, alansal varyasyona göre çok daha belirgin bulunmuştur.

Çalışma yapılan Türkiye' nin tüm denizlerinde benzer konsantrasyonlarda BPA tespit edilmiştir. Akdeniz'de derin deniz suyundaki (500-1000 m derinlik) BPA miktarı

yüzeysel sularındaki ile benzerlik göstererek BPA' nın su kolonunda tamamen yayıldığı görülmüştür. Yapay sulardan hazırlanan numuneler 150 gün içinde herhangi bir BPA bozunması göstermemiştir, ancak doğal nehir suyu 50 günden sonra bozunmaya başlamıştır. Doğal nehir suyu içeren örnekler 25 °C' de 4 °C' dekinden daha hızlı bir biçimde bozunmaya uğramıştır. Doğal deniz suyu örneklerinde, 4 °C ve 25 °C' de 150 gün içinde önemli bir bozulma görülmemiştir, ancak, doğal deniz suyu numunelerinde dışarıda tutulan ve 40 °C' nin üzerinde sıcaklığa maruz kalanlar 50. günden sonra bozunmaya başlamışlardır. Diğer çalışmalarla kıyaslandığında bu çalışmada tespit edilen yavaş bozunma hızları belki de BPA bozunmasından sorumlu mikro organizmalarının bu bölgedeki azlığı ile açıklanabilir. Bu çalışma, BPA kirliliğinin Türkiye'deki sucul ortamlarda ciddi seviyeye ulaştığını göstermektedir. Ayrıca bozunma hızları kıyaslandığında, deniz ortamı ve organizmalarında BPA kontaminasyonun tatlı sulardakinden daha kalıcı olabileceğini de öne çıkarmaktadır.

Anahtar Kelimeler: Bisfenol A; Akdeniz; Karadeniz; Marmara Denizi; Zaman Serisi; Bozunma



To the memory of my beloved mother

To my lovely father & sisters

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CHAPTER 1

INTRODUCTION

Marine Pollution and Plastics

A contamination emerges when a certain chemical compound is present in a certain habitat and/or the organisms living there, at concentrations higher than usual or the background values due to non-natural causes (Clark, et al., 1989). When the contamination results in adverse biological effects to a habitat, it is defined as pollution, therefore all pollutants are contaminants, but all contaminants are not pollutants (Chapman, 2007). Marine Pollution is defined by GESAMP (Joint Group of Experts on the Scientific Aspects of Marine Pollution) as; "Pollution means the introduction by man, directly or indirectly, of substances or energy into the marine environment (including estuaries) resulting in such deleterious effects as harm to living resources, hazards to human health, hindrance to marine activities including fishing, impairment of quality for use of sea water and reduction of amenities" (GESAMP, 1991).

The polymers used in plastics are generally harmless when they are used in pure form, however, in almost all commercial plastics, they are "compounded" with monomeric ingredients to improve their processing and end-use performance. The monomeric additives (in order of total volume used) can be classified as; reinforcing fibers, fillers, and coupling agents; plasticizers; colorants; stabilizers (halogen stabilizers, antioxidants, ultraviolet absorbers, and biological preservatives); processing aids (lubricants, others, and flow controls); flame retardants, peroxides; and antistats (Deanin, 1975). Potential toxicity and safe handling of these additives during processing, manufacture and fate of plastics products are obligatory.

There has been substantial research on the impacts of plastic marine debris, such as entanglement and ingestion, while there is significant uncertainty and complexity in the kinetics and thermodynamics of the interaction, plastic debris appears to act as a vector transferring persistent, bioaccumulative and toxic substances (PBTs) from the water to the food web, increasing risk throughout the marine food web, including

humans. Because of the extremely long lifetime of plastic and PBTs in the ocean, prevention strategies are vital to minimizing these risks (Engler, 2012).

What is Bisphenol A (BPA)?

Bisphenol A (BPA) is only one of many monomers, plasticizers, flame retardants, antimicrobials, and other chemicals used in plastics manufacturing that are able to migrate into the environment. BPA, a synthetic chemical compound, is ubiquitous all over the world as it has been produced in high volumes since 1960s (Staples et al., 1998). BPA, $C_{15}H_{16}O_2$, 4,4'-(propane-2,2-diyl) diphenol (molecular structure is shown in Figure 1.1). It is a solid and in the form of crystal, prill or flake under the ambient conditions. The water solubility of BPA is 120-300 mg/L (Staples et al., 1998).

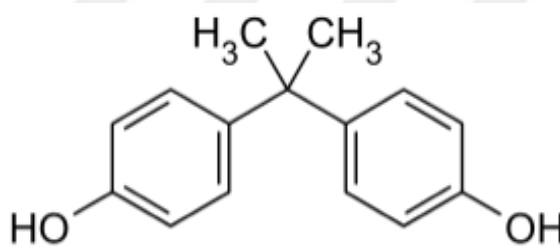


Figure 1.1. Molecular structure of Bisphenol A (BPA)

BPA has been widely used commercially in plastic industry, epoxy resins, for the production of flame retardants and other industrial fields since the first synthesized by Russian chemist Alexander P. Dianin in 1891. The global production of BPA was around 5.5 million tons in 2015 and the biggest consumption field was polycarbonate as 67% of total consumption; the second biggest consumption field was epoxy resin as about 30% of total consumption; moreover, BPA has been used in producing new synthetic resins such as PEI, polysulfone resin, polyarylester, phenoxy resin, etc. (CNCIC, 2015). In polycarbonates, due to its advantageous properties, BPA is used in optical media, construction materials, and the electrical and electronics industries and also a small fraction for bottles and other food-contact applications and for medical and healthcare applications (Zielińska et al., 2019). In epoxy resins, due to its desirable properties, it is used as coatings for consumer and industrial applications, protective coatings for automotive and marine uses, electrical and

electronic laminates, adhesives, paving applications and also internal coatings of coils and of food and beverage cans (Zielińska et al., 2019). In daily application of BPA is an ingredient used to make adhesives, containers, electronic coatings, boat hulls, reusable drink containers, food storage containers, canned foods, children's toys, and even receipts from stores. Consequently, all of these commonly used products are introduced as the potential sources of BPA contamination.

Effects and Risks Assessments of Bisphenol A on Human Health

BPA has been shown to interact with estrogen receptors and to act as agonist or antagonist via endocrine receptor (ER) dependent signaling pathways, and play a role in the pathogenesis of several endocrine disorders including female and male infertility, precocious puberty, hormone dependent tumours such as breast and prostate cancer and several metabolic disorders including polycystic ovary syndrome (PCOS) (Diamanti-Kandarakis et al., 2009, Konieczna et al., 2015).

According to the European Food Safety Authority (EFSA, the agency of the European Union), BPA is an endocrine disrupting chemical one of the most extensively studied (EFSA, 2010). In the last few decades, due to its adverse health effects like cancer and infertility; BPA usage was banned or restricted in some products (especially in baby bottles) (Tsai, 2006). BPA have been detected in human urine samples at high frequency (up to 99%) (Ye et al., 2015) indicating extensive contamination of it. The European Food Safety Authority (EFSA) had a first full risk assessment of BPA in 2006 and revisited in 2008, 2009, 2010, 2011 and 2016 with new scientific information on BPA considered hundreds of scientific publications. In January 2011, the European Commission accepted, Directive 2011/8/EU, prohibiting the use of BPA for the manufacture of polycarbonate infant feeding bottles. EFSA published a comprehensive re-evaluation of BPA exposure and toxicity, and reduced the TDI (dose/tolerable daily intake) for BPA from 50 to 4 µg/kg bw/day (bodyweight) in January 2015 (Authority EFS, 2015).

Considering potentials of BPA and its impact as an endocrine disruptor, BPA is included in the MSFD (Marine Strategy Framework Directive), OSPAR commission and WFD (Water Framework Directive) list of priority substances.

Bisphenol A Exposure on Environment

As the high production volumes and disposal of products made from BPA, polycarbonate plastic and epoxy resins, BPA has entered terrestrial and aquatic environments. It is introduced into the sea mainly from rivers, wastewater treatment plants (WWTPs), landfill leachates, and leaching from discarded BPA-based materials (e.g., hydrolysis of polycarbonate, recycled paper) (reviewed by Im and Löffler 2016). Major BPA sinks and attenuation processes in the environment are including degradation via AMO: ammonia monooxygenase, HCA: 4-hydroxycumyl alcohol, ROS: reactive oxygen species (Figure 1.2) (Im and Löffler 2016).

According to a study that examined the biodegradation potential of wastewater micropollutants, including BPA, by *Nitrosomonas europaea* and mixed ammonia-oxidizing (AMO) bacteria in nitrifying activated sludge, regardless of the presence of the AMO inhibitor, BPA degraded by two different nitrified activated sludge samples suggested that both ammonia-oxidizing bacteria and heterotrophic microorganisms in the activated sludge can degrade BPA (Roh et al., 2009).

Another study about the transformation of BPA by ammonia-oxidizing bacteria (AOB) *Nitrosomonas europaea* was investigated and transformation of BPA into nitro- and dinitro-BPA was found, suggesting that abiotic nitration between the biogenic nitrite and BPA played a major role in the transformation of BPA in the batch AOB system; the results from the local WWTP showed the occurrence of nitro-BPA and dinitro-BPA during the biological treatment process and in the effluent, indicating that nitration of BPA is also a pathway for removal of BPA and also implicated that AOB in the WWTPs might contribute to removal of selected endocrine-disrupting compounds (EDCs) through abiotic nitritation (Sun et al., 2012).

Manganese and iron form reactive minerals that affect the fate of organic contaminants by sorption, hydrolysis and/or oxidative transformation, in particular, manganese oxides are strong, naturally occurring oxidants, recent studies indicates that manganese dioxide (MnO_2) mediates BPA transformation (Im and Löffler 2016). The study which investigated the abiotic transformation of BPA in the presence of MnO_2 , indicated that oxidative reactions with MnO_2 may play an important role in the fate of BPA in soil systems. The results of the study indicated that, at a fixed MnO_2 concentration, the reaction rate decreased as a function of initial BPA concentration, and at a fixed initial BPA concentration, the rate increased as a function of MnO_2 concentration, and also the overall reaction rates decreased rapidly as the solution pH increased, whereas a higher ionic strength of the background solution resulted in slower reaction rates (Gao et al., 2011).

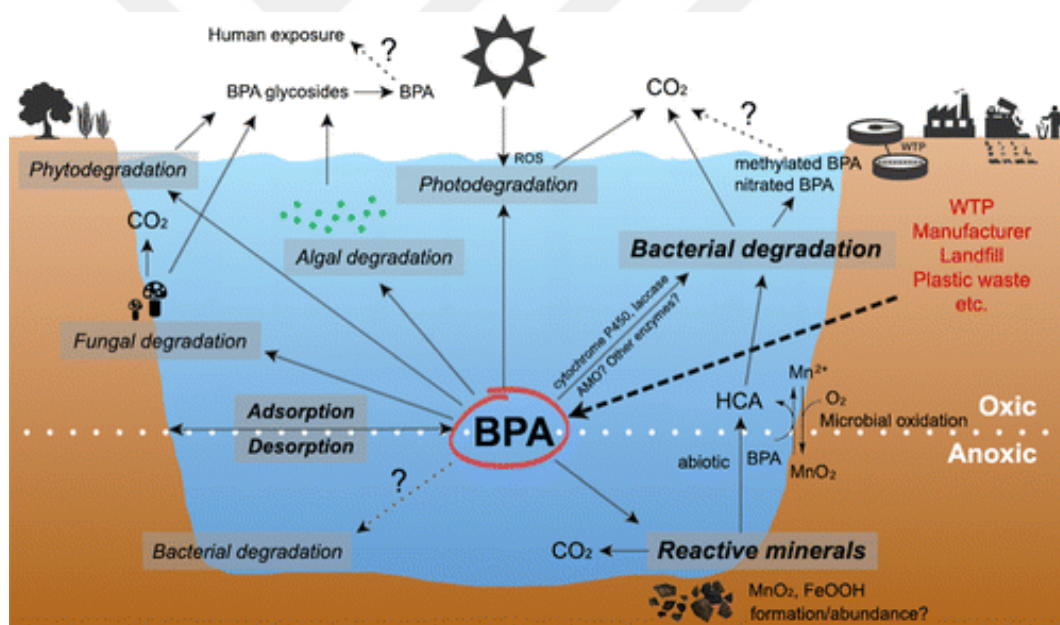


Figure 1.2. Major BPA sources (red), sinks and natural attenuation processes in the environment. AMO: ammonia monooxygenase, HCA: 4-hydroxycumyl alcohol, ROS: reactive oxygen species, WTP: wastewater treatment plant (Im and Löffler, 2016)

Laboratory studies, which is focus on aquatic species, indicate that in non-mammalian vertebrates, in particular aquatic species, BPA causes developmental and reproductive

effects, including reduction of male hormones, death of testicular cells, decreased sperm density and motility, inhibition of spermatogenesis and egg production, along with delayed or absent ovulation, and impairment of sex ratio (Canesi and Fabri, 2015).

Detection of BPA in water began in the late 1990s since then many studies have been conducted on BPA in aquatic environments and there is an accelerating increase of the number of publications per year all over the world reporting detection of bisphenol A (BPA) in surface water and effluent (Figure 1.3) (Corrales et al. 2015).

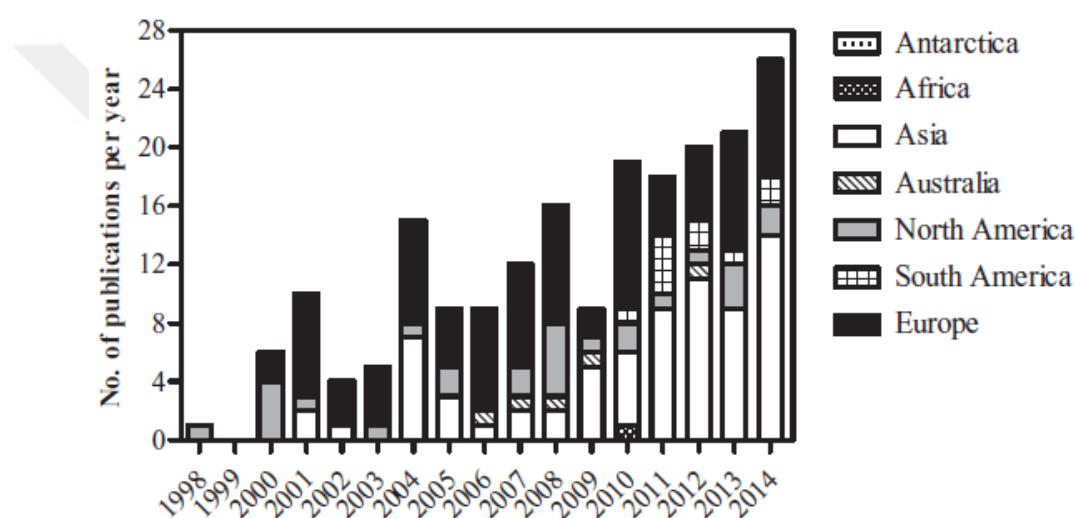


Figure 1.3. Historic overview of the number of publications per year among geographic locations reporting detection of bisphenol A (BPA) in surface water and effluent (Corrales et al., 2015)

Concentrations of BPA in Seawater

As mentioned before by the high production volumes and disposal of products made from BPA has entered marine environments. Most environmental monitoring results show that the concentrations of BPA in water bodies are lower than 1 $\mu\text{g/L}$, however, some of the landfill leachates and effluents from paper manufacturing plants, contained more than 50 $\mu\text{g/L}$ of BPA (Tsai, 2006). Water column suspended particles also contained high concentrations of BPA. In one of the study conducted in Adriatic

Sea found that suspended matter in water column had 103.06 µg/kg BPA (Andelic et al. 2015).

The U.S. Environmental Protection Agency (EPA) released a “Bisphenol A Action Plan” in March 2010, to list BPA as a chemical of concern present an unreasonable risk to certain aquatic species at concentrations similar to those found in the environment, to consider rulemaking to gather additional data relevant to environmental effects, and to initiate collaborative alternatives assessment activities and reductions in BPA releases and exposures (EPA, 2010). EPA, approve Kolpin et al. (2002) research that indicates BPA concentration was detected at a median concentration of 0.14 µg/L and a maximum concentration of 12 µg/L in 41.2% of 85 samples collected from U.S. streams in 1999 and 2000 (Kolpin et al., 2002; EPA, 2010).

Because of the high production volume of bisphenol A (BPA), Corrales et al. (2015) reviewed and examined literature results over 500 peer-reviewed studies to understand its global distribution regarding BPA concentrations in effluent discharges, surface waters, sewage sludge, biosolids, sediments, soils, air, wildlife, and humans. In surface water, BPA concentrations ranged from nondetect to 56 µg/L. For BPA Predicted No Effect Concentrations (PNECs) in surface waters were reported for the different regions, as in Canada (750 ng/L), the European Union (1500 ng/L), and Japan (1600 ng/L) (Corrales et al., 2015). In addition to PNEC values, countries try to establish threshold values for industrial discharges such as Environment Canada's proposed water pollution limit for industry is 1.7 µg/L (ECCC, 2013).

Objectives of the Thesis

Although BPA is a widely studied compound, the lack of data from the majority of developing countries around the globe make the understanding of global patterns challenging. As the first BPA study in Turkey, this study attempts to understand temporal and spatial distributions of BPA in Turkish seas and rivers, and also to understand its fate in rivers and seawater regarding its degradation processes.

This thesis study is divided into two chapters. In the first chapter, concentrations of BPA in Turkish seas and rivers were determined. There were three distinct goals for this chapter: 1) to understand temporal and spatial distribution of BPA in the NE Mediterranean Sea by using a time series, Erdemli time series, and regional rivers in one year period, 2) to assess the spatial distribution of BPA throughout the Northeastern Mediterranean Sea, and 3) to evaluate the impact of a megacity, Istanbul, on regional seawater BPA concentrations in the Black Sea, Bosphorus, and the Sea of Marmara. The sampling locations during the study of three distinct regions is shown in Figure 1.4.

In the second chapter, it was aimed to investigate the degradation process of BPA in marine and freshwaters under different conditions; microbial degradation, photodegradation, and temperature effect to understand fate and the persistence of BPA in aquatic environments.

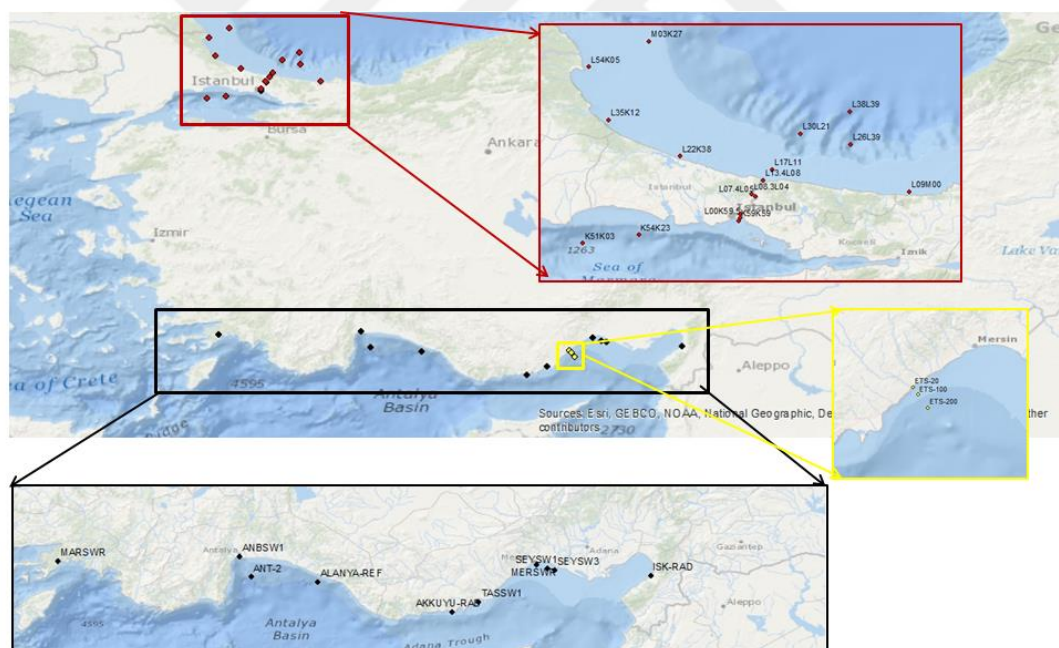


Figure 1.4. Bisphenol A sampling stations along the coasts of Turkey, three distinct samplings during this study

CHAPTER 2

TEMPORAL - SPATIAL DISTRIBUTIONS OF BISPHENOL A IN MARINE AND FRESHWATERS IN TURKEY

2.1. Abstract

Bisphenol A (BPA), is one of the important synthetic chemicals, as it is produced at high volumes. Since 1960s it has been used commercially widely in plastic industry, epoxy resins and other fields and it has been shown its adverse health effects such as cancer, infertility. They are commonly detected in the marine environment. This study was conducted to understand the distribution of BPA in marine and freshwaters of Turkey. The aim was to report monthly BPA concentrations on the coast of Erdemli (ETS-Erdemli Time Series) and in regional rivers, which are located at the Northeast Mediterranean. Furthermore, seawater at other coasts of Turkey –The Black Sea, Bosphorus, the Sea of Marmara and the Mediterranean Sea- were analyzed to have a better understanding of regional variations of BPA concentrations in the Turkish Seas. Concentrations of BPA in seawater ranged from 4.16 to 16.92 $\mu\text{g/L}$ and in rivers ranged from 4.62 to 29.92 $\mu\text{g/L}$. While the spatial variation was very low in the NE Mediterranean Sea, the temporal variation was high. It is also shown that BPA can reach to the deep sea and there was no significant difference between deep sea and surface seawater concentrations of it. This study shows that BPA contamination reach to serious levels in aquatic environments in Turkey. This study is also the first report regarding BPA concentrations in Turkish seas that can guide lawmakers in Turkey regarding marine protection regulations.

2.2. Introduction

Bisphenol A (BPA) is one of the endocrine disrupting chemicals (EDCs) and ubiquitous all over the world and become one of the important synthetic chemicals as it has been produced at high volumes since 1960s (Staples et al, 1998). BPA, $C_{15}H_{16}O_2$, 4,4'-(propane-2,2-diyl) diphenol, is first synthesized by Russian chemist Alexander P. Dianin in 1891 (Rubin, 2011).

BPA has been widely used commercially in plastic industry, epoxy resins, for the production of flame retardants and other industrial fields since 1960s. The BPA usage was banned in some products (e.g. baby bottles) or restricted due to adverse health effects like cancer, infertility (Tsai, 2006). The monomer is also found weakly estrogenic and therefore of the interest to investigate its effects on environment and human health. (Staples et al, 1998). Due to the high production volumes and disposal of products made from BPA, polycarbonate plastic and epoxy resins, BPA has entered terrestrial and aquatic environments. It is introduced to the sea mainly from rivers and WWTPs. Considering potentials of BPA and its impact as an endocrine disruptor, BPA is included in the list of priority substances of MSFD (Marine Strategy Framework Directive), OSPAR commission and WFD (Water Framework Directive). Detection of BPA in water began in the late 1990s and since then many studies have been conducted on BPA in aquatic environments (reviewed by Corrales et al. 2015). Although BPA is a widely studied compound, lack of data from the majority of developing countries around the globe make the understanding of global patterns challenging.

Because there is no information about BPA concentrations in the river and marine environment in Turkey, it is the first data set present BPA investigations obtained in rivers, surface and deep seawater including the Mediterranean Sea, the Black Sea, and the Sea of Marmara. Our objectives in this study were (1) to indicate temporal and spatial distribution of BPA in rivers and on the coast of Erdemli with a monthly time series study (ETS) in the NE Mediterranean Sea in one year period, (2) to show spatial distribution throughout the Mediterranean Sea, (3) to determine BPA concentrations in the close vicinity to the mega city, Istanbul.

2.3. Materials and Methods

2.3.1. Sampling and Sampling Stations

For the investigation of temporal and spatial distribution the seawater sampling locations, they were selected to have advantages of ongoing monthly time series study on the coast of Erdemli (ETS). The selected rivers were the regional largest rivers. Along the coasts of Turkey for the investigation spatial distribution sampling locations, the stations were selected as many as possible to reflect the regional changes. Particularly, the Bosphorus stations were selected in a transect and some stations in close vicinity to the transect were selected to compare the concentrations that were impacted by Istanbul.

The samplings were performed from the sea by using a CTD rosette sampler with Niskin bottles. Triplicates of non-filtered 500 ml samples were taken during research cruises of Institute of Marine Sciences METU (by R/V Bilim 2). All non-filtered waters were collected into 500 mL polypropylene (BPA free) plastic bottles and taken into the laboratory. All the analysis were performed within a week after samples reached to the laboratory.

There were three distinct sampling stations (Figure 2.1);

(1) Surface seawater samples were collected monthly Erdemli Time Series programme (ETS) from the transect (ETS20, ETS100 and ETS200) and four regional rivers (Göksu, Lamas, Berdan and Seyhan) between August 2016 and October 2017 (Figure 2.2). The latitude and longitude coordinates of Erdemli Time Series sample locations are given in the appendices Table A.1.

(2) Surface seawater samples were taken from the Black Sea, Bosphorus, the Sea of Marmara in December 2016 (Figure 2.3). The latitude and longitude coordinates of the Black Sea, Bosphorus, the Sea of Marmara sample locations are given in the appendices Table A.2.

(3) Samples along the coastal Mediterranean Sea in August 2017 (Figure 2.4). The latitude and longitude coordinates of the Mediterranean Sea sample locations are given in the appendices Table A.3.

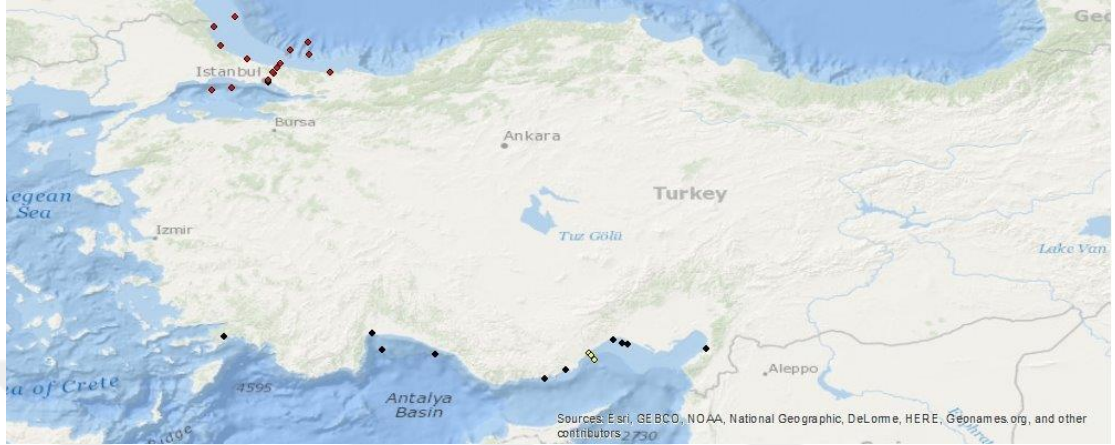


Figure 2.1. Bisphenol A sampling stations on the coasts of Turkey

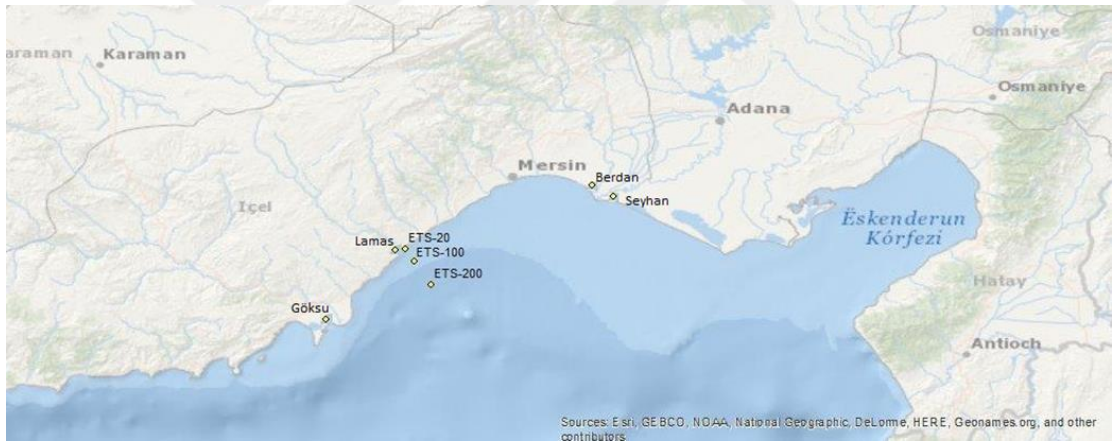


Figure 2.2. Erdemli time series (ETS) stations and locations of the rivers on the NE Mediterranean coast; Göksu, Lamas, Berdan, Seyhan



Figure 2.3. The Black Sea, Bosphorus, the Sea of Marmara stations

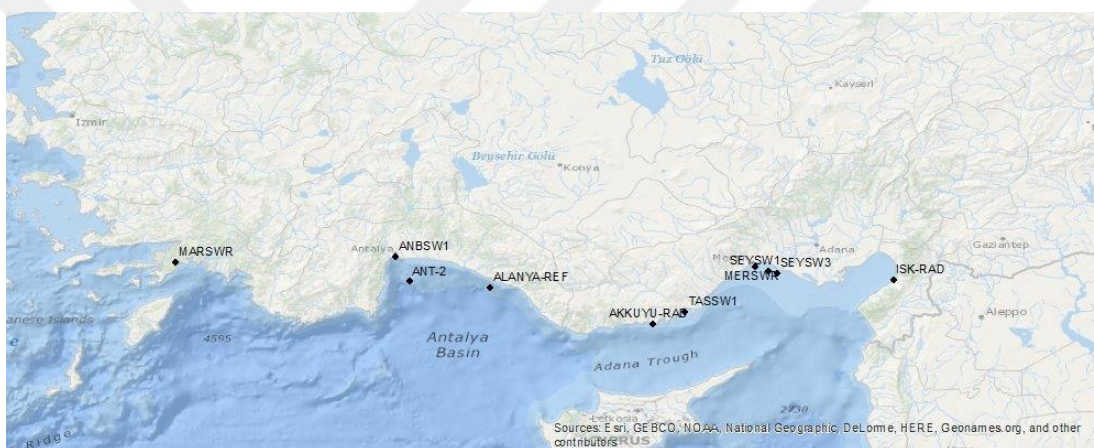


Figure 2.4. The Mediterranean Sea stations

2.3.2. Extraction of Water Samples

Initially, extraction was achieved by purification and concentration of the 500 mL portion of the sample through *ThermoFisher Scientific Dionex SolEx C18 Silica-Based solid phase extraction (SPE) cartridges*, which provides extraction of organic compounds from a liquid sample off-line using a solid-phase adsorbent under vacuum. Samples passed through the cartridge at a speed of 7-10 mL/min. At the end, after reach complete dryness under vacuum, the analyte was collected in 5 mL methanol (analytical grade) in this manner samples were concentrated 100 times. To assess the efficiency and reproducibility of the analytical procedures, spiked seawater and distilled water samples were recurrently analyzed.

2.3.3. HPLC Analysis

High performance liquid chromatography (HPLC) equipped with FLC Cell Agilent 1100 series fluorescence detector was used for BPA analysis. The HPLC unit had reverse phase C18 column (Vydac, Model: 201TP52, S/N: NE981208-3-1) kept at 25 °C during the analysis. Analytical grade methanol at a constant flow rate of 1 mL/min was used as the mobile phase.

The HPLC unit consisted of a pump (Agilent 1100 series), an auto-sampler (Agilent 1260 Infinity), a fraction collector (Agilent 1290 Infinity), a fluorescence detector (FLC Cell Agilent 1100 series) and a column oven (Agilent 1100 series).

The standard of bisphenol A (2,2-Bis(4-hydroxyphenyl)propane; 4,4'-Isopropylidenediphenol) was identified as $\geq 99\%$; Sigma-Aldrich®; CAS 80-05-7; lot number MKBS0991V. The detection limit of the analysis were 1 $\mu\text{g/L}$. Standard BPA concentrations were at 10, 50, 100, 500, 1000, 5000 $\mu\text{g/L}$, a linear slope was calculated from the peak areas of the standards (Figure 2.5).

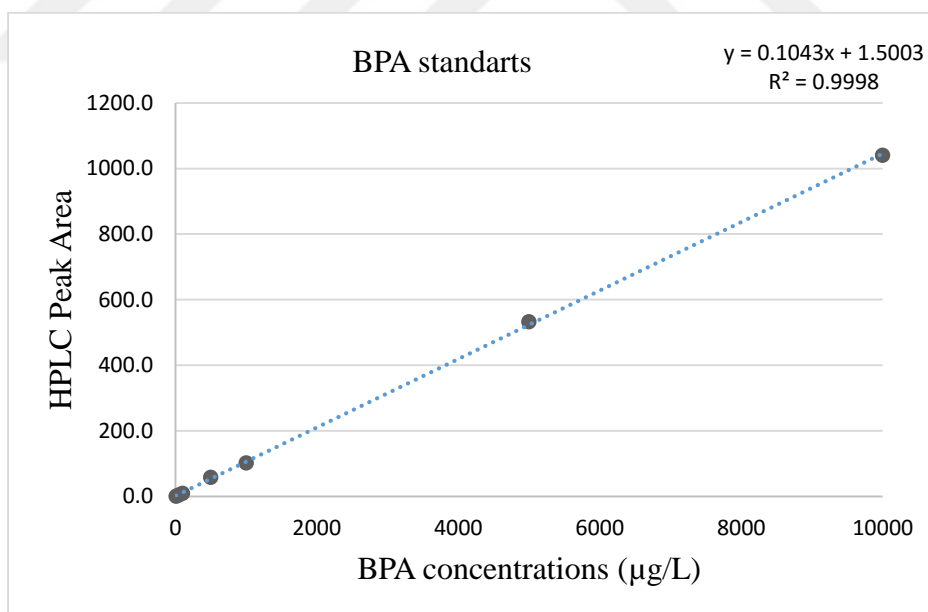


Figure 2.5. A Bisphenol A (BPA) standard curve of the peak areas of the standards

2.3.4. Statistical Analysis

All statistical analyses requiring a comparison of treatments were carried out using SigmaStat 12.3 software (Systat Software, Inc., San Jose, CA, USA). ANOVA and *t*-tests were performed to evaluate significance of individual differences with a probability threshold of 0.05, followed by a Holm-Sidak test.

2.4. Results and Discussions

HPLC extraction efficiency was calculated by repetitive analysis of spiked seawater and distilled water samples. The extraction efficiency was found 99 ± 1.8 %. It indicates that the extraction method and solvents worked well for the BPA analysis.

According to the results of the time series stations, the samples taken in the same period were very close to each other, there was no significant spatial variation observed (Figure 2.6). It indicates that BPA can impact not only the coastal site but also offshore stations (13 km away from the coast). However, the seasonal variations in BPA concentrations were observed on ETS stations. The concentrations of BPA on ETS stations in one year period were between 4.16 and 16.92 $\mu\text{g/L}$. BPA concentrations in seawater were at lowest levels in October and November 2016, and September 2017. Highest BPA concentrations were detected in January and February 2017.

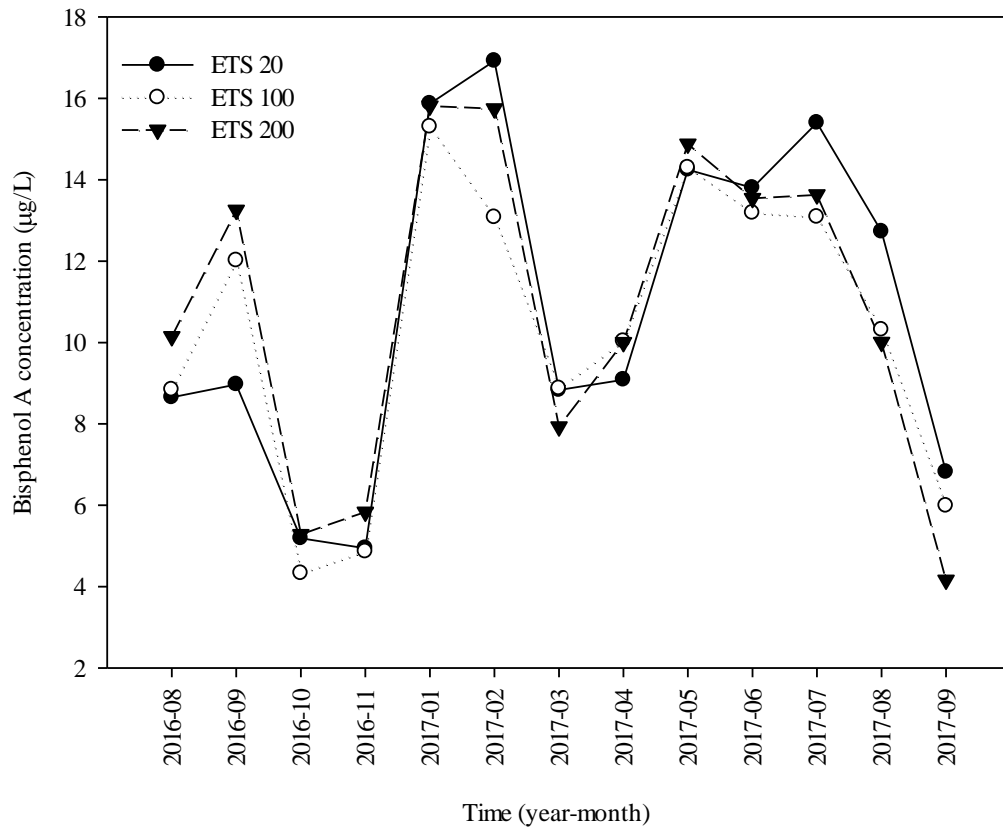


Figure 2.6. Bisphenol A concentrations at Erdemli Time Series (ETS) stations

In a similar pattern, concentrations of BPA in the rivers were between 4.62 and 29.92 µg/L, changing temporarily (Figure 2.7). In the same sampling periods, regardless of river discharge rates, BPA concentrations in all the rivers were very close to each other. Spring season river and seawater BPA concentrations were the closest to each other, but in summer, there was a difference in pattern and concentrations of BPA (Figure 2.8). Apparently, high river discharge seasons (e.g. winter and spring) greatly contributed BPA levels in seawater and distributed more homogeneously and made seawater reach its highest concentrations on the following seasons. Due to the lack of data on WWTP discharge and its BPA contents, it did not allow us to evaluate its contribution to the seawater BPA levels.

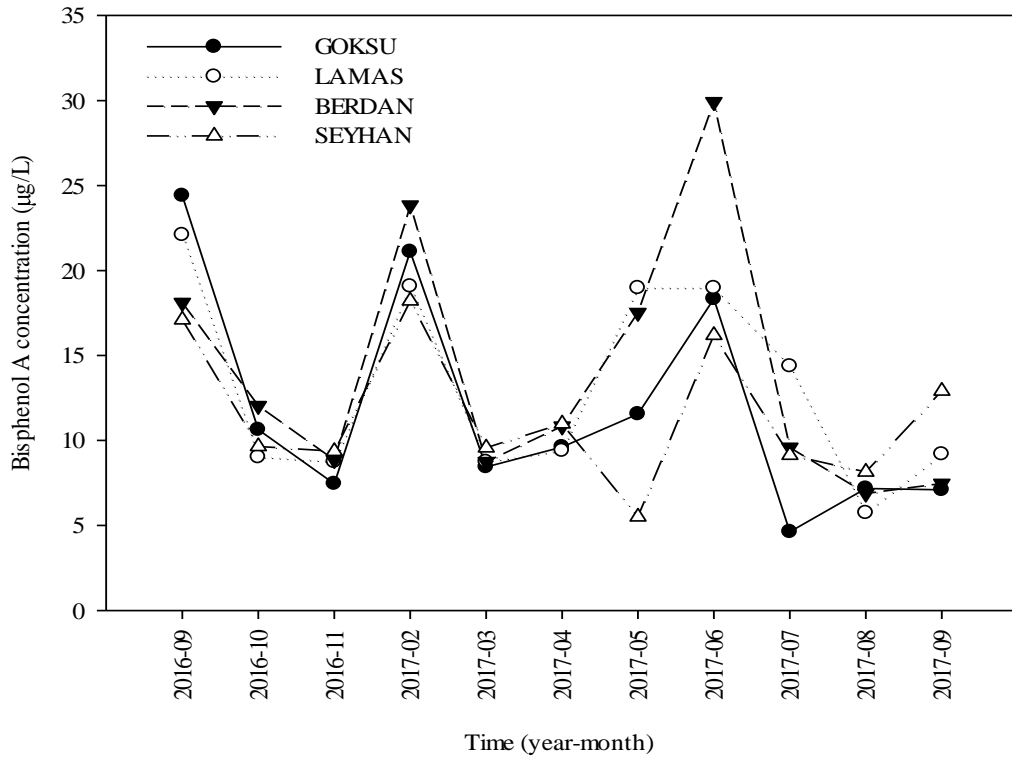


Figure 2.7. Bisphenol A concentrations in regional rivers on the NE Mediterranean Sea region

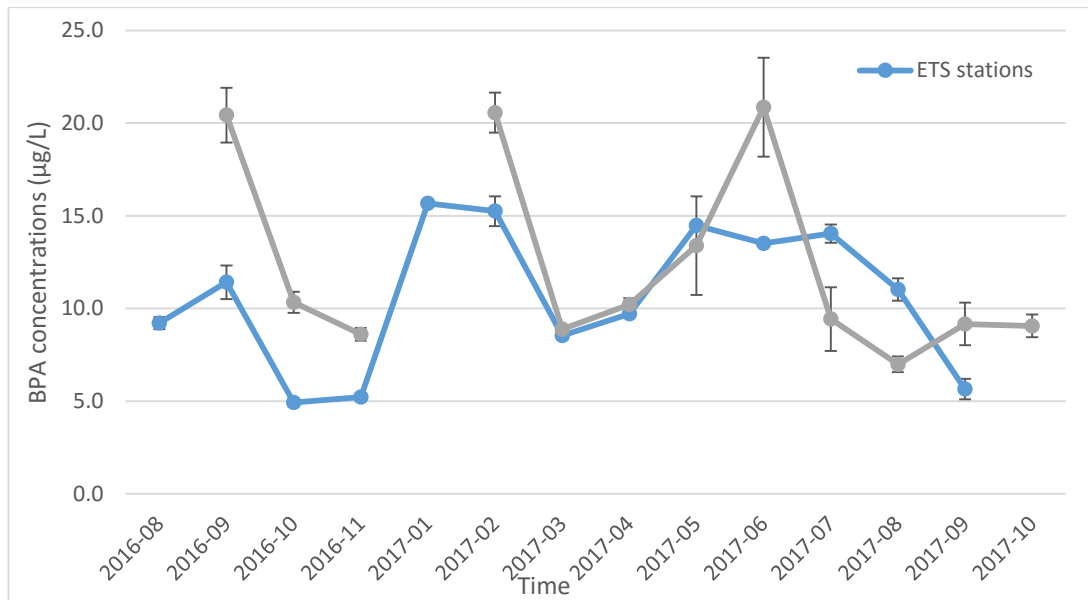


Figure 2.8. Average BPA concentrations at ETS stations and regional rivers on the NE Mediterranean Sea

The impact of megacities onto the pollution of coastal areas is well known. In the study, BPA concentrations in the surrounding water bodies of Istanbul (the population is 15M), the biggest city in Turkey, the Black Sea, Bosphorus, and the Sea of Marmara was investigated. Concentrations of BPA in the Black Sea, Bosphorus, and the Sea of Marmara has changed in a narrow range between 8.85 and 14.76 $\mu\text{g/L}$ (Figure 2.9). The highest concentrations were observed in a river mouth, in Bosphorus where wastewater discharge of Istanbul occur. It is an indication of direct impact of river and wastewater discharge in this region. Bosphorus reached its peak BPA concentrations in the middle part of the transect (Figure 2.9). The Black Sea surface water enters from the north of the transect on the surface and moves downward on the surface to the Marmara Sea. The offshore station on the Black Sea had a low concentration of BPA, 9.7 $\mu\text{g/L}$. Similarly, stations on the Sea of Marmara had the lowest concentration found in the region. This shows that BPA concentrations were enriched thru the transect by the impact of Istanbul.

Similarly, the Mediterranean Sea survey showed that BPA concentrations in surface seawater are in a narrow range of 13.80-15.34 $\mu\text{g/L}$ (Figure 2.10). The spatial variation on BPA concentrations among stations was very low. To investigate the depth profile of BPA concentrations in the Mediterranean Sea, two stations were chosen and samples were taken from different depths (Figure 2.11). The results were not significantly different from each other (Figure 2.11). It indicates that BPA was physically mixed in the vertical water column almost homogeneously.

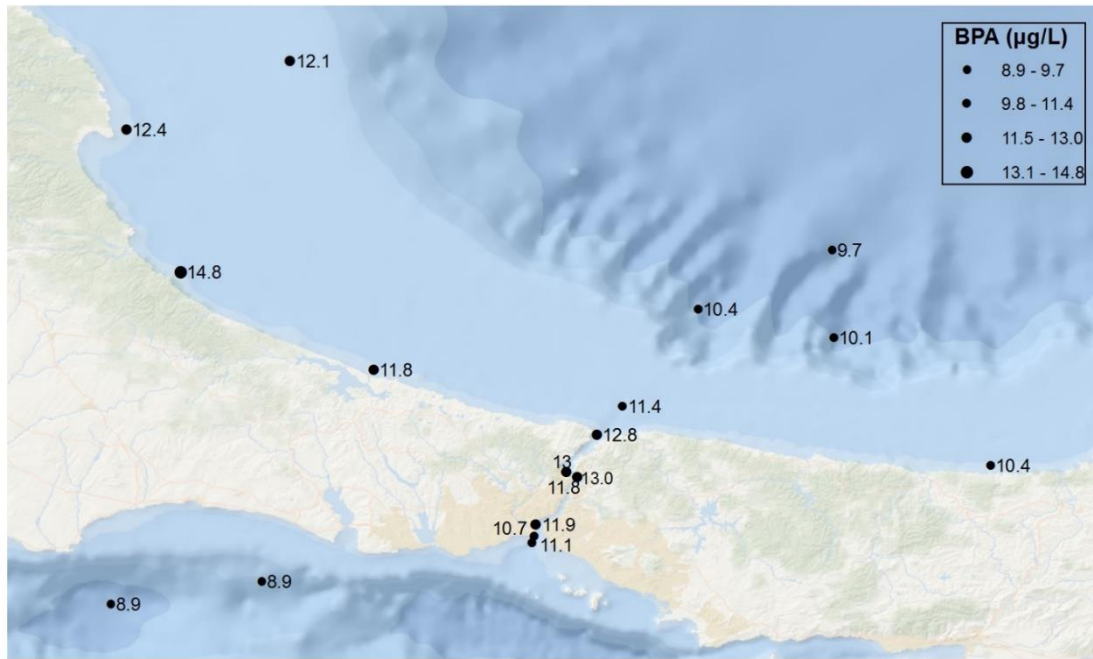


Figure 2.9. Bisphenol A concentrations on the Black Sea, Bosphorus, the Sea of Marmara

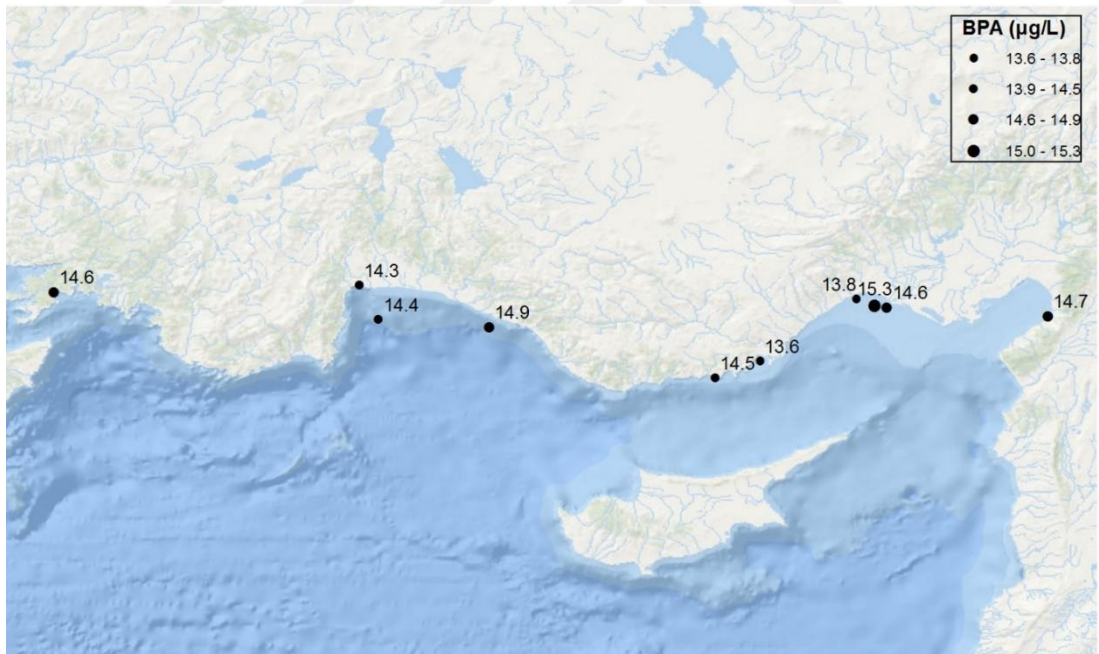


Figure 2.10. Surface seawaters Bisphenol A concentrations in the Mediterranean Sea

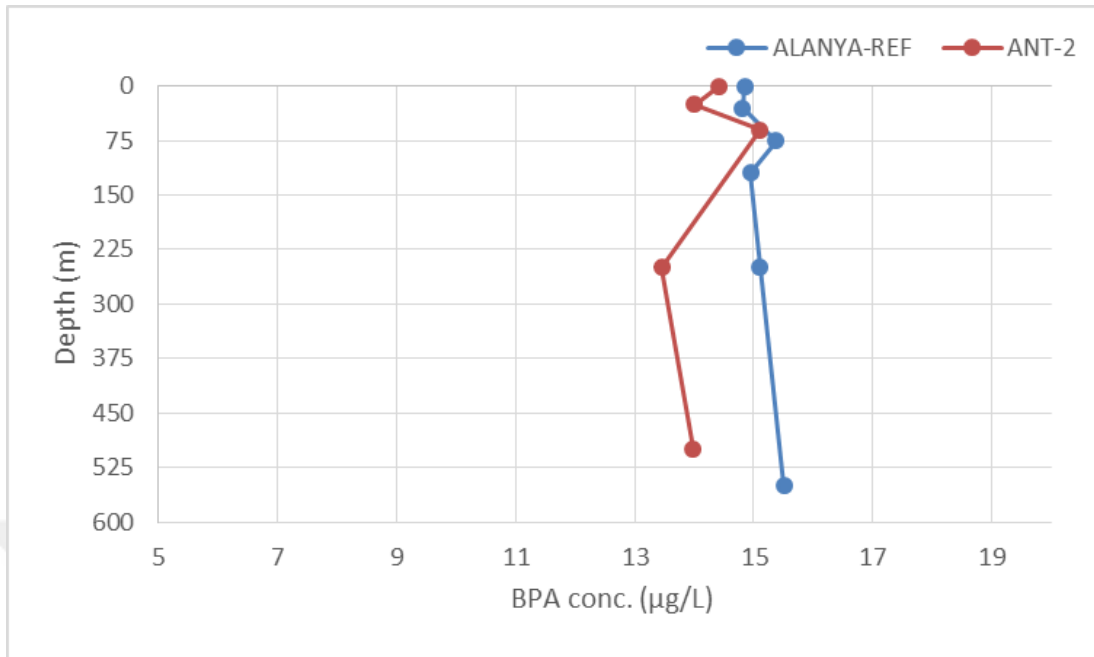


Figure 2.11 Concentration profiles of Bisphenol A in the Mediterranean Sea at different depths

The collected physicochemical (temperature, salinity, nutrients, dissolved oxygen, chl-*a*, and pH) data was gathered from the same cruises that BPA samples were taken. BPA concentration's variations with these physicochemical parameters were analyzed. The only strong correlation ($R^2= 0.908$) was found between BPA concentration and salinity in the Bosphorus transect (stations start from K51K03 to L13.4L08 shown in Figure 2.3). Along with this transect from north to south, salinity values changed from 17.66 to 27.60 (Figure 2.12) due to mixing of the Black Sea water and the Mediterranean Sea (Ozsoy et al., 1993). Reduction of BPA concentrations from north to south with greater physical mixing, indicating relatively more contaminated the Black Sea water is diluted with the Mediterranean Sea water.

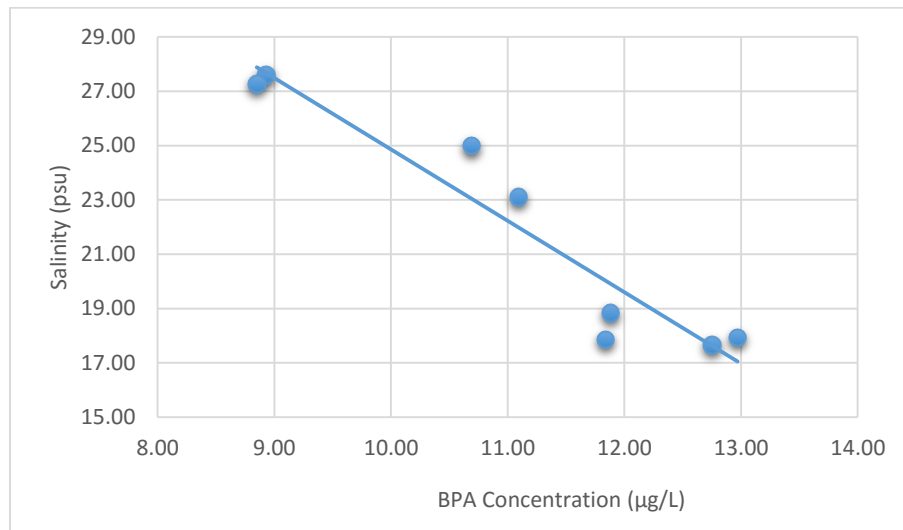


Figure 2.12. The correlation between Bisphenol A (BPA) concentrations and salinity changes in the Bosphorus transect

In the literature, surface water BPA concentrations ranged from nondetect to 56 µg/L (Corrales et al., 2015). Andelic et al. (2015) investigated the occurrence, seasonal and vertical distribution of BPA in the sediments and suspended matter sampled in Kaštela Bay, Adriatic Sea, Croatia during almost 3 years, finally BPA was detected in all samples of sediments and suspended matter at concentrations ranged from 5.32 µg/kg to 15.52 µg/kg in sediment, while 4.66 µg/kg to 103.06 µg/kg in suspended matter. The seawater along the Slovenian coast, which is part of the North Adriatic Sea, in the shellfish farms and the open sea was contaminated with an average BPA concentration of 0.007 µg/L (Cerkvenik-Flajs et al., 2018).

Corrales et al. (2015) analyzed all peer reviewed literature results regarding BPA concentrations in seawater and ranked them using the Weibull formula. According to this ranking our results are higher than 80% of the literature values. For BPA Predicted No Effect Concentrations (PNECs) in surface waters were reported for the different regions such as in Canada (750 ng/L), the European Union (1500 ng/L), and Japan (1600 ng/L) (Corrales et al., 2015). All of our results are higher than PNECs values already determined by different regions. It shows that the BPA pollution in Turkish seas and rivers is in serious condition.

It is widely believed that hard plastics are the main source of BPA in oceans (ACS, 2010). The team analyzed sand and seawater from more than 200 sites in 20 countries, mainly in Southeast Asia and North America. All contained a "significant" amount of BPA, ranging from 0.01 µg/L to 50 µg/L. They concluded that polycarbonates and epoxy resin coatings and paints were the main source (ACS, 2010) of BPA. The high concentrations of BPA found in this study potentially indicates serious plastic pollution the Mediterranean confronted.

One of the important point in this study was very low spatial variation regardless of different seas in Turkey studied. In WWTPs there is no prevention of BPA and many of WWTP are lack tertiary/advanced treatment systems in Turkey. So, their contribution to BPA marine pollution is unavoidable. This study also shows that the rivers contain high levels of BPA and their continuous discharge is a considerable contributor to the marine BPA pollution.

The most of the aquatic toxicity data of BPA from various sources shows that EC₁₀ (EC₁₀-the concentration in a laboratory or field toxicity test at which it is expected 10% of the test organisms would show an adverse effect), EC₅₀ (EC₅₀-the concentration in a laboratory or field toxicity test at which it is expected 50% of the test organisms would show an adverse effect), and LC₅₀ (LC₅₀-the median lethal concentration or the concentration of a substance at which 50% of the test population are killed) values for microorganisms, invertebrates and fishes are higher than 1000 µg/L (e.g., Alexander et al., 1988, Hendriks et al., 1994). However, a recent review (Kang et al., 2007) shows more recent studies found BPAs' adverse endocrine-disruptive effects on aquatic organisms as low as 1.75 µg/L on Brown trout (Lahnsteiner et al., 2005), 1–25 µg/L on mud snail (Jobling et al., 2003), 50 µg/L on mussels (Ortiz-Zarragoitia and Cajaraville, 2006). Thus, the values detected in our seas apparently exceed the values that can potentially show adverse endocrine-disruptive effects for the aquatic organisms.

2.5. Conclusions

The occurrence of BPA was investigated in the marine environment and rivers of Turkey. Concentrations of BPA in seawater ranged from 4.16 to 16.92 $\mu\text{g/L}$, in rivers 4.62 to 29.92 $\mu\text{g/L}$. The spatial distribution was investigated in different seas but the variation was low. In the NE Mediterranean Sea, the temporal variation was high but there was no clear pattern regarding BPA concentration in surface seawater. It is also shown that BPA can reach to the deep sea and there was no significant difference between deep sea and surface seawater concentrations of BPA. This is the first study showing BPA's temporal and spatial distributions in the Turkish Seas and will guide lawmakers in Turkey in terms of precaution and prevention of BPA in freshwater and marine environments.

CHAPTER 3

DEGRADATION OF BISPHENOL A IN MARINE AND FRESHWATERS

3.1. Abstract

Bisphenol A (BPA), is one of the important synthetic chemicals, as it is produced at high volumes. They are commonly detected in the marine and freshwater environment. This study aims to investigate the degradation of BPA in marine and freshwaters under different conditions; microbial degradation, photo degradation, and temperature effect. The results showed that BPA content in samples prepared from the artificial waters did not degrade in 150 days. BPA concentrations in natural river water started to degrade after day 50 and the degradation rate was faster for the samples at 25 °C than ones at 4 °C. In natural seawater samples, there were no degradation detected in 150 days at 4 °C and 25 °C, however, samples prepared natural seawater kept outside and exposed over 40 °C temperature showed degradation after day 50. A treatment exposed to the sunlight showed higher rate of the degradation indicating the additive/synergistic role of the photodegradation. Our study suggest that high temperatures (> 25 °C) are required for BPA degradation in seawater. River water was more potent than seawater regarding BPA degradation. It suggest that BPA contamination on a marine environment could be more persistent than the one on freshwater environment.

3.2. Introduction

Bisphenol A (BPA) is one of the endocrine disrupting chemicals (EDCs) and ubiquitous all over the world and become one of the important synthetic chemicals as it has been produced at high volumes since 1960s (Staples et al, 1998). Due to the high production volumes and disposal of products made from BPA, polycarbonate plastic and epoxy resins, BPA has entered terrestrial and aquatic environments. Due to the widespread use and inefficient disposal of these products, BPA is released into the environment, mainly through processing of BPA in manufacturing, inefficient/no removal during wastewater treatment, landfill leachates, and leaching from discarded BPA-based materials (e.g., hydrolysis of polycarbonate, recycled paper). When BPA reach to aquatic environments, it doesn't stay as BPA (Im and Löffler 2016) long time; transform through phytodegradation, photodegradation, bacterial degradation, fungal degradation and react with reactive minerals. The biotransformation of BPA is performed by vertebrates, invertebrates and plants (Michałowicz, 2014). A variety of biotic and abiotic processes that contribute to BPA transformation, degradation have been documented in the laboratory (e.g., Roh et al., 2009, McCormick et al., 2011, Sun et al., 2012).

The literature indicates that no microbial BPA degradation was observed in anoxic conditions in freshwater sediment, marine sediments, and soil, leading to the conclusion that BPA is persistent and undergoes little or no biodegradation in the absence of oxygen (Im and Löffler, 2016). The study of the biotransformation of the flame retardants including BPA, was examined in anoxic estuarine sediments, indicates that no further degradation of BPA was observed in separate incubations under conditions promoting either methanogenesis, sulfate-reduction, iron (III)-reduction, or nitrate-reduction; indicating that the potential for accumulation of BPA in anoxic sediments is significant given the widespread use of these chemicals (Voordeckers et al., 2002).

In establishing chemical environmental safety, a hazard assessment using environmental exposure and effects information is required. Many bacteria are capable of degrading BPA in aquatic environments but differ in their potential for

biodegradation (reviewed by Im and Löffler 2016). Considering distinct microbial communities and other physicochemical properties of waters, BPA degradation should be investigated in different regions to better understand global fate of the compound. It may not be possible to determine chemical fate unless site-specific conditions are present in the experimental test environment. For this purpose, this study aims to determine BPA degradation under different abiotic and physicochemical conditions in the North Eastern Mediterranean Sea.

3.3. Materials and Methods

3.3.1. Sampling, Samples Preparations and Storage Conditions

To monitor degradation amount of BPA, natural and artificial seawater and river water were used (Table 3.1). Natural seawater and river water collected from the Mediterranean Sea and Göksu River. Collected non-filtered sea and river waters were brought to the laboratory in ambient temperature and the experiments were immediately started. Artificial seawater and river water were directly prepared from Milli-Q water. For the artificial seawater preparation, there was an addition of 35 g/L sodium chloride (NaCl - for analysis EMSURE® ACS, Reag. Ph Eur; Merck® 106404) and 200 mg/L sodium hydrogen carbonate (NaHCO₃ - for analysis EMSURE® ACS, Reag. Ph Eur; Merck® 106329) into the water. All waters were aliquoted into 500 mL polypropylene plastic bottles and spiked with 100 µg/L BPA. They were kept at 4 °C and 25 °C in dark boxes except one natural seawater treatment; those bottles were kept outside – half in a dark box the other half under the direct sunlight. BPA degradation experiment started on April 12, 2017 and continued until September 11, 2017. Samples were taken at day 0, 6, 14, 21, 50 and 150 for the BPA analysis with HPLC system. In each sampling days, two replicates of two bottles were used.

Table 3.1. Experimental conditions

Seawater					River water				
Natural			Artificial		Natural		Artificial		
25 °C	4 °C	Outside		25 °C	4 °C	25 °C	4 °C	25 °C	4 °C
		Dark	Sunlight						

3.3.2. Extraction of Water Samples

Initially, extraction was achieved, the 500 ml portion of the sample was concentrated and purified through *ThermoFisher Scientific Dionex SolEx C18 Silica-Based solid phase extraction (SPE) cartridges*, which provides extraction of organic compounds from a liquid sample off-line using a solid-phase adsorbent under vacuum. Samples passed through the cartridge at a speed of 7-10 ml/min. At the end, after reach complete dryness under vacuum, the analyte was collected in 5 mL methanol (analytical grade) in this manner samples were concentrated 100 times. To assess the efficiency and reproducibility of the analytical procedures, spiked seawater and distilled water samples were recurrently analyzed.

3.3.3. HPLC Analysis

High performance liquid chromatography (HPLC) equipped with FLC Cell Agilent 1100 series fluorescence detector was used for BPA analysis. The HPLC unit had reverse phase C18 column (Vydac, Model: 201TP52, S/N: NE981208-3-1) kept at 25 °C during the analysis. Analytical grade methanol at a constant flow rate of 1 mL/min was used as the mobile phase.

The HPLC unit consisted of a pump (Agilent 1100 series), an auto-sampler (Agilent 1260 Infinity), a fraction collector (Agilent 1290 Infinity), a fluorescence detector (FLC Cell Agilent 1100 series) and a column oven (Agilent 1100 series).

The standart of bisphenol A (2,2-Bis(4-hydroxyphenyl)propane; 4,4'-Isopropylidenediphenol) was identified as $\geq 99\%$; Sigma-Aldrich®; CAS 80-05-7; lot number MKBS0991V. The detection limit of the analysis were 1 µg/L. Standard BPA concentrations were at 10, 50, 100, 500, 1000, 5000 µg/L, a linear slope was calculated from the peak areas of the standards (Figure 3.1).

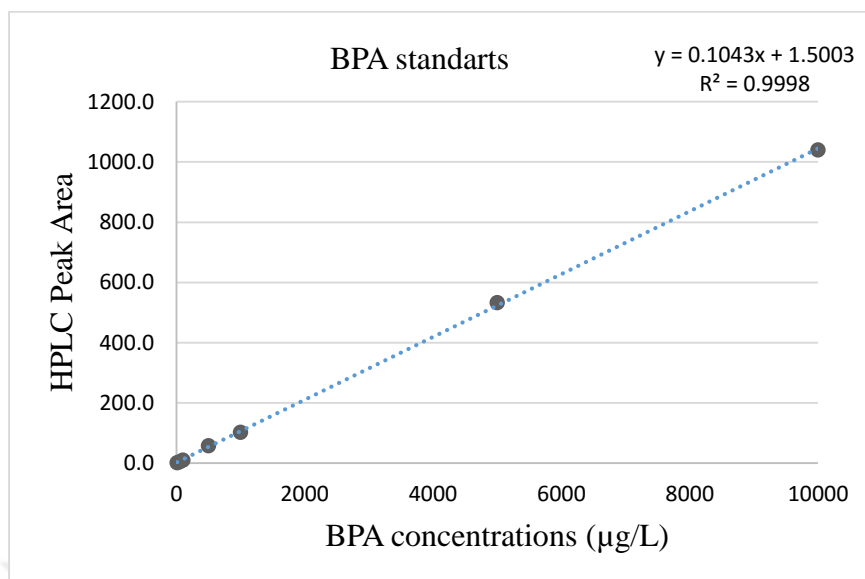


Figure 3.1. A Bisphenol A (BPA) standard curve

3.3.4. Statistical Analysis

All statistical analyses requiring a comparison of treatments were carried out using SigmaStat 12.3 software (Systat Software, Inc., San Jose, CA, USA). ANOVA and *t*-tests were performed to evaluate significance of individual differences with a probability threshold of 0.05, followed by a Holm-Sidak test.

3.4. Results

HPLC extraction efficiency was calculated by repetitive analysis of BPA spiked seawater and distilled water samples. The extraction efficiency was found $99 \pm 1.8 \%$. It indicates that the extraction method and solvents worked well for the BPA analysis.

Seawater samples were kept at 4 °C and 25 °C and directly sampled for BPA concentrations at day 0, 6, 14, 21, 50 and 150. The analysis showed that neither artificial nor natural seawater samples BPA concentrations changed significantly throughout the experiment in 150 days (Figure 3.2).

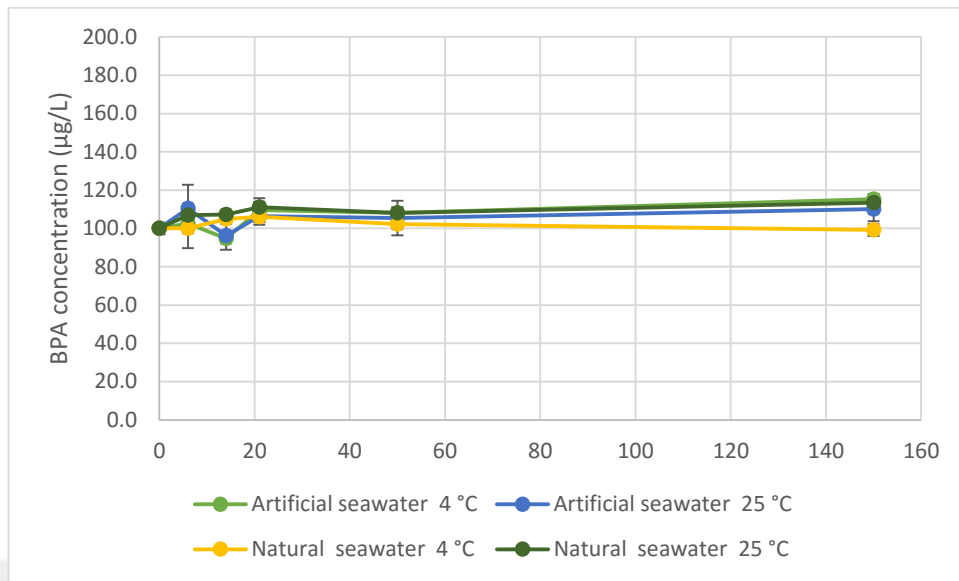


Figure 3.2. Bisphenol A concentrations of seawater samples that were kept at 4 °C and 25 °C

River water samples prepared from the natural and artificial waters were kept at 4 °C and 25 °C. In 150 days, BPA in artificial river waters did not show any significant degradation, but BPA in natural river water samples started to degrade after day 50 (Figure 3.3). Natural river water sample kept at 25 °C started to degrade quickly after day 50 and reached to the half-life at day 90.8. The concentration of BPA in natural river water samples at 25 °C at day 150 BPA was significantly different than the other treatments (ANOVA; $P < 0.05$). BPA in natural river water kept at 4 °C started to degrade relatively slow after day 50 until day 150. The concentration difference of this treatment compared to other treatments was not significant at day 150 (ANOVA; natural river water 4 °C vs. artificial river water 4 °C $p = 0.082$; vs. artificial river water 25 °C $p = 0.086$).

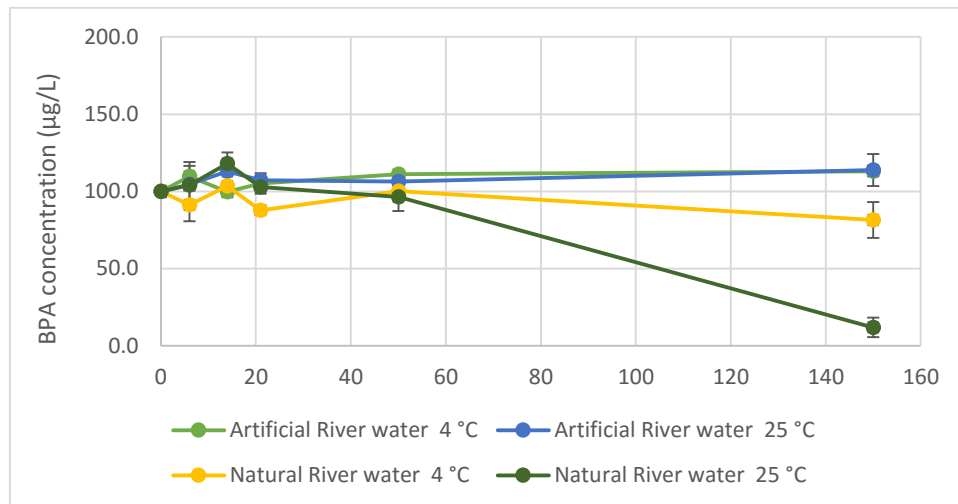


Figure 3.3. Bisphenol A concentrations of river water samples that were kept at 4 °C and 25 °C

The other treatments prepared from natural seawater were kept outside: one treatment were under the sunlight and the other one was kept in a dark box. The results showed that both treatments started to degrade after day 50 and reached significant BPA degradation ($p < 0.05$) at day 150 (Figure 3.4). The treatment kept in the dark box showed a slow degradation after day 50, however, the treatment kept under the direct sunlight degraded at faster rates. The half-lives are >150 and 97.8 days for the treatment kept at dark and sunlight, respectively.

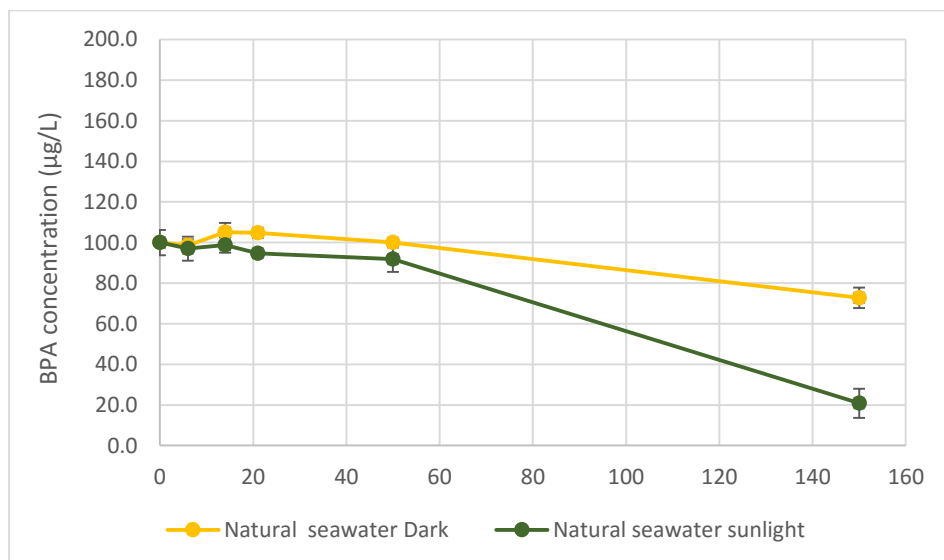


Figure 3.4. Bisphenol A concentrations of seawater samples that were kept outside

3.5. Discussions

There are controversial studies in the literature about BPA degradation in aquatic systems, publications are listed and their conditions are summarized in Table 3.2. While some of the studies show rapid biodegradation and very short half-lives of BPA in river and seawater (Klecka et al., 2001; Robinson and Hellou 2009; Im and Löffler, 2016), the others claimed that there was no degradation or very long half-lives of BPA observed during the course of the evaluations (Howard, 1989, Ying et al., 2003). The studies of the fate of BPA in aquatic systems are very controversial because of the variety of biotic and abiotic processes under environmentally relevant conditions that contribute to BPA transformation, degradation and decomposition (e.g., Roh et al., 2009, McCormick et al., 2011, Sun et al., 2012). The variations on the BPA degradation rates can be due to the results of biological processes, presence of oxygen, bacteria, fungi, algae and even higher plants metabolize BPA, and the nonbiological processes that lead to BPA transformation and degradation.

Table 3.2. Summary of the literature about BPA degradation in aquatic systems

References	Degradation Speed / Half-life	Degradation Medium/ Conditions	Comment on Degradation Process
Dorn et al., 1987	Rapid, short half-life / 2 days (on the 4. day, BPA was reduced below detection limit)	A spike of 3 mg/L BPA was added to the samples and analysed during the 8-day at 22-25 °C. Four water samples containing fresh water (control), Houston Ship Channel water, Patricks Bayou water (downstream from a BPA chemical plant discharge), and the chemical plant treated process effluent.	The control unit shows no change of BPA concentration within experimental error during the 8-day test, Sharp reduction of BPA occurred in all three waters in the following two days. On the fourth day, BPA was reduced below detection limit (0.1 mg/l).
Howard 1989	No degradation, long half-life / Extended until 160 days	In surface waters	Very long half-life of BPA degradation was observed
Ike et al., 2000	Rapid, short half-life / 2 to 5 days	Biodegradation of BPA in the aquatic environments was investigated with 3 activated sludge and 44 river water microcosms.	The experimental results suggested that BPA-degrading bacteria ubiquitously exist in the aquatic environments, but that they cannot completely degrade BPA, leading to the accumulation. For complete degradation, the presence of microbes which can degrade metabolites is necessary together with BPA-degrading bacteria.
Klecka et al., 2001	Rapid, short half-life / 2 to 4 days	Rapid biodegradation of BPA was observed in all rivers	BPA degrading microorganisms are widely distributed in nature. These observations provide clear evidence that BPA is not persistent in the aquatic environment.

References	Degradation Speed	Degradation Medium/ Conditions	Comment on Degradation Process
West et al., 2001	Rapidly biodegradable / <10 days	Seven different fresh and estuarine water samples with inoculated mineral medium, benzoate and different BPA concentrations analysed during the 28-day test period.	Tests of ready for biodegradability, eventually BPA was rapidly biodegraded in the environment
Kang and Kondo, 2002	Rapidly under aerobic conditions; hardly under anaerobic conditions / 2-3 days (aerobic); no degradation for 10 days (anaerobic)	BPA biodegradability in river water and to identify the difference of BPA degradation under aerobic and anaerobic conditions	Three river water samples spiked with BPA were rapidly degraded under aerobic conditions, the half-life for BPA degradation ranged from 2 to 3 days, and BPA was below detection limit on the 10th day. But a decrease of BPA under anaerobic conditions was hardly identified at 30°C for 10 days.
Ying and Kookana, 2003	Long half-life and no degradation / 42 days (seawater), 14.5 days (aerobic, sediment), and remained unchanged after 56 days (aerobic, sediment)	The seawater and marine sediment were collected from the coastal area around South Australia.	In Seawater under aerobic degradation within 35 day after treatment, little degradation, but there was rapid degradation in the following week; 42 days. In Marine Sediment; under aerobic degradation half-life of 14.5 days; under anaerobic degradation no degradation was observed.
Ying et al., 2003	No degradation; long half-life / Remained unchanged after 70 days	In the aquifer media under the aerobic and anaerobic conditions, BPA remained unchanged over 70 days.	Sorption and degradation of BPA, has been investigated in the laboratory using sediment and groundwater from an aquifer in Bolivar, South Australia.

References	Degradation Speed	Degradation Medium/ Conditions	Comment on Degradation Process
Kang and Kondo, 2005	Rapidly, 3-5 days (non-autoclaved river water) / slow degradation, decreased after 40 days (non-autoclaved seawater) / no degradation in 60 days (autoclaved seawater)	Sea and river water samples spiked with BPA to identify a relationship between changes of bacterial counts and BPA degradation under aerobic or anaerobic conditions, and at temperatures of 4, 25, and 35 °C for 60 days.	This study showed non-autoclaved river water samples degraded rapidly at 25 °C and 35 °C with a half life of 3-5 days; also for seawater samples non-autoclaved seawater BPA content decreased after 40 days with the increasing temperature; while autoclaved seawater remain unchanged at 4 °C, 25 °C, and 35 °C for 60 days.
Sakai et al., 2007	Rapid, short half-life / 20 - 40 days	The degradation of BPA by <i>Sphingomonas</i> sp. strain BP-7 and several strains	A mixed culture of strains showed complete degradation of BPA within 7 d, while <i>Sphingomonas</i> strain7 alone took about 40 d for complete consumption of bisphenol A. On a nutritional supplementary medium, <i>Sphingomonas</i> strain7 completely degraded bisphenol A within 20 h.
Danzl et al., 2009	Rapid, short half-life / 12 to 21 days	Artificial seawaters and seawater, 6 samples, was collected at two sites in the bay of Osaka, Japan	Degradation of BPA and its metabolites were measured by the TOC potential test and the Sea die-away (SDA) methods, which are both a kind of river-die-away test

References	Degradation Speed	Degradation Medium/ Conditions	Comment on Degradation Process
Robinson and Hellou 2009	Rapid, short half-life / 4 to 14 days	Seawater and sediment were collected on three occasions from Halifax Harbour, Canada at a location near a major sewage effluent. Biodegradation by microbial communities had compered with the control samples which were sterilized by autoclave for 1 h at 121 °C.	The results from this study indicate that native microbial communities can degrade endocrine disrupting chemicals. The sterilized control samples were degraded at a slower rate than the non-autoclaved samples.
Gatidou et al., 2010	Slow degradation / 26 days	The tissue concentrations of BPA in the Mediterranean mussel were investigated at the field survey, in the Aegean Sea (Greece) unfiltered seawater and clean water.	Mussels were relocated to unfiltered seawater for 28 days of BPA exposure, the tissue concentrations of BPA were constantly increased. Then they were relocated to clean water, observed in next 28 days at the end of depuration phase, the residual concentrations were higher than the initial concentrations.
Eio et al., 2015	Rapid, short half-life / 7 days	The algae, <i>C. sorokiniana</i> , were cultivated with 10, 20, and 50 mg/L BPA, in mineral salts medium (MSM), for 7 days.	This study demonstrated the potential removal of BPA, in biodegradation intermediates through the symbiotic relationship between <i>C. sorokiniana</i> and the BPA-degrading bacterial consortium.

Natural seawater and river water samples contain native microbial communities that are potentially able to degrade BPA. Biodegradation is a significant way to remove or detoxify BPA from the aquatic environment or aquatic organisms. Bacteria capable of biodegrading BPA are distributed in aquatic environments. Many different organisms; not only diverse taxa of bacteria but also fungal, algae and even higher plants metabolize BPA (Im and Löffler, 2016). One of the earliest study regarding BPA degradation showed that BPA concentration within experimental research, about 15-25% of BPA disappeared during the first 48 hours than sharp reduction of BPA occurred in the following two days therefore, on the fourth day, BPA was reduced below detection limit (Dorn et al., 1987). BPA was also assessed in surface waters from seven different rivers across the United States and Europe (Klecka et al., 2001). Rapid biodegradation of BPA was observed in all rivers, regardless of geographic location, following lag phases ranging from 2 to 4 days (Klecka et al., 2001). Other studies also indicated the rapid BPA degradations; <10 days (West et al. 2001), 4 to 14 days (Robinson and Hellou 2009). The most of the study reports average half-life for BPA biodegradation is less than 10 days.

Even though some studies showed the fast degradation of BPA in natural waters, some studies reported much higher half-lives of BPA in natural waters. For example, BPA concentrations in groundwater remained unchanged after 70 days (Ying and Kookana, 2003; Ying et al., 2003) and half-lives of BPA in surface waters extended until 160 days (Howard 1989).

Biodegradability of BPA in seawater is varied as microbial communities altered by environmental factors such as nutrients, temperature and water flow, the results indicates that availability of some microorganisms in the seawater degraded BPA (Sakai et al., 2007). And the efficiency of photolytic degradation depended on pH, turbidity, water turbulence, and other factors (Howard 1989). So, it is likely to observe very distinct half-lives of BPA depending on locations where biological and physicochemical parameters vary. In this study, half-lives of BPA in natural river waters were found > 90 days and > 150 days at 4 °C and 25 °C, respectively (Figure 3.3). It is a good example for the location and temperature dependency of the BPA

degradation. However, it is shown that the temperature is only effective on the degradation only if the microorganisms are present in the medium.

In 150 days, the unchanged BPA concentration in seawater at 4 °C and 25 °C indicates that either lacking of organisms capable of biodegrading BPA in bottles or they were not activated at those temperatures. However, the treatments prepared from the same seawater kept outside were significantly degraded in 150 days (Figure 3.4). The only differences between the treatments were temperature and sunlight effects. The samples kept outside were exposed temperature higher than 40 °C in summer (Figure 3.5). While outside temperature reach to 40 °C on July and August, the temperature inside the bottles under direct sunlight can reach to the much higher temperatures. It is expected to observe higher temperatures in bottles under the sunlight than the bottles kept in dark box. Another expectation is that the transparent bottles kept under the sunlight exposed to photodegradation. The higher rate of degradation of BPA in the bottles kept under the sunlight compared to bottles kept in the box apparently caused from joint effect of the high temperatures and the photodegradation. Unchanged BPA concentrations in the same seawater kept at 4 °C and 25 °C indicates that microorganisms were present in the bottles but they were activated when they reached to the higher temperatures.

In this study, unchanged BPA concentrations in artificial waters, lacking of native microbial communities, shows that microorganisms are responsible from the BPA degradation by means of elevated temperatures and photodegradation. Similar results from a study by Kang and Kondo (2005) showed that non-autoclaved river water samples degraded rapidly at 25 °C and 35 °C; also for seawater samples non-autoclaved seawater BPA content decreased after 40 days with the increasing temperature while autoclaved seawater remain unchanged at 4 °C, 25 °C, and 35 °C for 60 days (Kang and Kondo 2005).

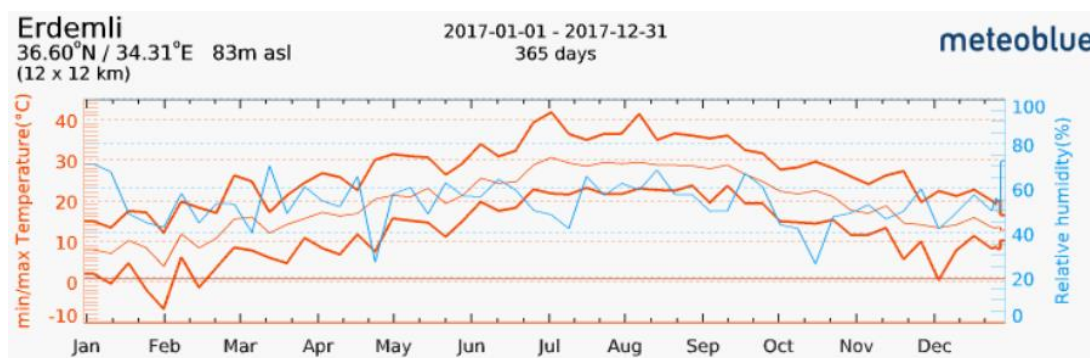


Figure 3.5. One year temperature variation of the region where experiments taken place (Meteoblue, “Weather archive Erdemli, 2017”)

Our results shows that the temperature can play an important role on BPA degradation. Seawater temperatures can reach around 33 °C in summer in the Northeastern Mediterranean Sea that makes summer compared to other seasons the most potent season regarding the BPA degradation. Other studies also agree with our findings, higher temperature in summer accelerate the degradation of BPA (Nie et al., 2012).

According to the survey results of Nie et al., (2012), about the fate and seasonal variation of several typical endocrine-disrupting chemicals (EDCs), including BPA, in a municipal sewage treatment plant (STP) with an anaerobic/anoxic/oxic (A/A/O) process located in China were investigated. The primary removal mechanism was the biodegradation in the anaerobic/anoxic/oxic (A/A/O) process. The BPA concentrations in the effluent and return sludge were much higher in winter and spring than in summer and autumn, which was related to the microbial activity and the concentration of mixed liquor suspended solids (Nie et al., 2012).

The locations, which have rapid BPA degradation, has been expected to have a low potential to bioaccumulation of BPA in organisms. The Northeastern Mediterranean Sea apparently the region has high potential for BPA bioaccumulation in organism. It is suggested that BPA in seawater compared to river water can be much more persistent due to its very slow or no degradation processes in this region. It propose the possibility of BPA contamination in a marine organism could be higher than that in freshwater organisms.

To the best of our knowledge, this is one of the first studies showing BPA degradation in the NE Mediterranean Sea, it is still unknown what really cause very slow degradation of BPA in the NE Mediterranean Sea. Is it just physicochemical properties of the NE Mediterranean Sea? Or, the bacterial community structure (amount of present BPA degrading marine organisms such as phytoplankton, zooplankton or bacteria), their long acclimation time requirements, or their BPA degradation abilities among different strains could be one of the reason. So, microbial community analysis on BPA degradation could be the next step to better understand fate of BPA in the NE Mediterranean Sea.

3.6. Conclusions

In this study, artificial river and surface seawater samples were evaluated in terms of BPA degradation at different temperatures for 150 days. In seawater samples, there were no degradation observed at 4 °C and 25 °C in 150 days regardless they are prepared from natural or artificial seawaters. The seawater sample treatments that were kept outside (prepared from natural seawater) started to degrade after day 50. The samples kept outside in dark degraded at lower rates than the ones kept under direct sunlight. In river waters, the artificial ones did not degrade at all in 150 days but natural river water samples started to degrade after day 50. This study shows that the impact of the temperature since different degradation rates were observed at different temperatures. The higher the temperature, the faster BPA degradation. In additionally, photodegradation contributed to the BPA degradation either additively or synergistically. Bacterial communities' effect was observed on BPA degradation since the artificial water samples were not degraded at all in 150 days. Different half-lives of BPA depending on the location is an indication of the effect of different biological composition and physicochemical properties of the aquatic systems. More regional data are required around the globe for the global assessment about the fate of BPA in aquatic systems.

CHAPTER 4

4. 1. OVERALL CONCLUSIONS

In this thesis work, it is the first time the Turkish seas were investigated regarding BPA concentrations and the degradation of BPA was evaluated under different conditions. In the first chapter, seawater samples from Turkish seas including the Mediterranean Sea, the Black Sea, the Sea of Marmara and river water samples from Göksu, Lamas, Berdan, and Seyhan were collected and analyzed for BPA concentrations. Concentrations of BPA in seawater ranged from 4.16 to 16.92 µg/L, in rivers 4.62 to 29.92 µg/L. In the Mediterranean Sea, BPA concentrations were evaluated in the time series and was found that the temporal variation was high but the spatial distribution was very low. Similarly, samples from the Sea of Marmara and the Black Sea showed very low spatial variations. It is an indication of BPA distribution in Turkish Sea are widely dispersed from the coast to offshore (approximately 13 km). It is also shown that BPA can reach to the deep sea and there was no significant difference between deep sea and surface seawater concentrations of BPA. BPA concentrations are in the high end compared to other studies around the globe showing Turkish Seas are under the serious threat in terms of BPA contamination. Outcome of this study is hoped to guide lawmakers in Turkey in terms of precaution and prevention of BPA in marine environments.

In the second chapter, the fate of BPA in seawater and river water was evaluated under different conditions for 150 days. Treatments were separated as natural vs. artificial water to understand native microbial biodegradation effect as well as different temperatures exposed to understand the impact of the temperature on BPA degradation. In seawater samples, there were no degradation observed at 4 °C and 25 °C in 150 days regardless they are prepared from natural or artificial seawaters. Seawater samples that were kept outside started to degrade after day 50. The samples kept outside in dark degraded at lower rates than the ones kept under direct sunlight. In river waters, the artificial river water samples did not degrade at all in 150 days but natural river water samples started to degrade after day 50 indicating bacterial communities' effect on BPA degradation. Additionally, photodegradation contributed

to the BPA degradation. Collectively, BPA's very low degradation character amplify the threat, which is caused by the high concentration of BPA in river and seawater in Turkish Seas.

Long term continuous discharge of BPA by rivers and waste water treatment plants and very low degradation character of BPA led to accumulation of this endocrine disruptor compound in the marine environment in Turkey. All of those collectively might well explain the low spatial distribution of BPA in Turkish seas.



4.2. RECOMMENDATIONS

This study's first objective was to investigate the spatial distribution of BPA in marine and freshwaters in Turkey. In accordance with this objective, the Mediterranean Sea, Bosphorus, and some stations from the Black Sea, and the Sea of Marmara which are close to Bosphorus were sampled; however from other locations such as the Aegean Sea, middle and west of the Black Sea coast and also the most important rivers are needed to be investigated to have more comprehensive outcomes. In the Turkish Seas, more offshore stations could be sampled to show the range of the impact of rivers and WWTPs can reach. Additionally for a better understanding of temporal distributions more marine and freshwaters in Turkey would be analyzed seasonally.

During the literature survey it is indicated that one of the biggest source of BPA contamination is the WWTPs, in which the knowledge of amount of BPA exposure is scarce. The BPA concentrations released from WWTPs might be the first step to investigate the contribution of WWTPs weight on BPA contamination. Moreover, the microorganisms can be responsible for the biodegradation to design an effective waste water treatment plants since the primary removal mechanism in the anaerobic/ anoxic/ oxic (A/A/O) process of the biodegradation of BPA; therefore WWTPs have to be investigated in order to figure out whether a mitigation is necessary. In order to prevent accumulation of BPA contamination in aquatic environment as a precaution WWTPs may be structured in terms of biodegradable organisms.

The degradation chapter of this study is missing the part of the relationship between BPA degradation process and presence of microorganisms, which are responsible from the degradation of BPA. It is highly suggested that it is required to analyze microbial communities in different regions to better understand the fate of BPA in aquatic environments. In additionally, these micbobial communities can play an important role on all the mitigation strategies.

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APPENDICES

Table A.1. The latitude and longitude coordinates of Erdemli Time Series sample locations

Sampling Stations	Latitude	Longitude
ETS20	36.559	34.260
ETS100	36.518	34.288
ETS200	36.438	34.347

Table A.2. The latitude and longitude coordinates of The Black Sea, Bosphorus, The Sea of Marmara sample locations

Sampling Stations	Latitude	Longitude
K51K03	40.850	28.051
K54K23	40.900	28.385
K59K59	40.985	28.983
L00K59.5	41.000	28.989
L01.3K59.5	41.025	28.992
L07.4L05	41.130	29.084
L08.3L04	41.142	29.059
L09M00	41.156	29.998
L13.4L08	41.224	29.127
L17L11	41.287	29.184
L22K38	41.368	28.633
L26L39	41.438	29.651
L30L21	41.502	29.351
L35K12	41.584	28.205
L38L39	41.633	29.647
L54K05	41.899	28.086
M03K27	42.050	28.448

Table A.3. The latitude and longitude coordinates of The Mediterranean Sea sample locations

Sampling Stations	Latitude	Longitude
AKKUYU-RAD	36.136	33.525
ANBSW1	36.872	30.692
ISK-RAD	36.622	36.172
MARSWR	36.817	28.264
MERSWR	36.760	34.648
SEYSW1	36.712	34.792
SEYSW3	36.691	34.892
TASSW1	36.270	33.880
ALANYA-REF	36.538	31.726
ANT-2	36.600	30.847