Research Article

Bioaccumulation monitoring of chemical contaminants in mussels *Mytilus galloprovincialis* from the southern coast of the Marmara Sea, Turkey

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Abstract: *Mytilus galloprovincialis*, the Mediterranean mussel, is an important shellfish species that constitutes the majority of production and consumption among bivalve mollusks in Turkey. Since shellfish can accumulate toxic chemicals from seawater, it is important to monitor bioaccumulation from their natural beds. For this reason, in this study, the aim was to monitor the levels of dioxins and dioxin-like polychlorinated biphenyls (PCBs), polycyclic aromatic hydrocarbons (PAHs), and toxic metals (Pb, Cd, and Hg) in *M. galloprovincialis* collected from the southern coast of the Marmara Sea from 2014 to 2017. According to the results, the maximum levels of dioxins, PAHs, and toxic metals during the monitoring period were determined as 0.30 pg/g, 2.52 mg/kg, and 0.43–0.34–0.76 (Pb–Cd–Hg) mg/kg, respectively. Dioxin, PAHs, and toxic metal amounts in mussel samples were determined to be below the threshold levels enforced by the European Commission. The highest benzo(a)pyrene and total PAHs were determined in winter 2015, while toxic metals, dioxins, and dioxin-like PCBs (WHO/PCDD/F-PCB-TEQ) were higher in autumn 2014 than the rest of the sampling period. In conclusion, toxic chemicals monitored in *M. galloprovincialis* were found at low concentrations from the point of view of food safety. However, monitoring of these or other toxic chemicals should be repetitively performed in the future to ensure food safety in aquatic animals.

Key words: *Mytilus galloprovincialis*, dioxins, dioxin-like PCBs, PAHs, toxic-metals

1. Introduction

Shellfish are known to be nutritional and well-balanced diet components for humans for many years. They are filter-feeding organisms and can take up all components found in seawater. However, they are not selective at filtering; thus, they can also accumulate toxic chemicals from seawater in their body.

There are numerous chemical contaminants that can cause potential toxicity in consumers, including inorganic chemicals and organic compounds such as PCBs and PAHs. Although these toxic chemicals are generally produced by anthropogenic activities, some of them, such as toxic metals, can be naturally found in the marine environment [1]. Whether natural or anthropological, toxic chemicals accumulate in living organisms and cause biomagnification, which ends in humans having highly elevated levels [1]. Exposure to these chemicals generally leads to some serious impacts in humans such as neurological, carcinogenic, systemic, and immunological effects [2].

Protecting consumers from chemical contaminants is nearly impossible unless there is fully controlled production in natural shellfish beds. These contaminants affect consumers via accumulation in tissues over time, since they cannot be easily detected and they are not effective in the short term on health (other than acute intoxication). Some of these contaminants are well known, such as toxic metals like lead (Pb), cadmium (Cd), and mercury (Hg). These metals are chemicals where their concentrations in the marine environment rise in parallel with industrial development. Their levels can reach harmful thresholds if precautions are not properly designed and applied.

In the last decades, organic contaminants namely dioxins, dioxin-like polychlorinated biphenyls (PCBs), and polycyclic aromatic hydrocarbons (PAHs) have been widely observed in marine animals [3–5]. Dioxins and dioxin-like PCBs are abundant in the industrialized environment and they are highly persistent and hydrophobic in nature [6]. They are also highly resistant to metabolism that results in the accumulation of these chemicals in the food chain

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at very high rates. PAHs, on the other hand, are known to be major ingredients of agricultural pesticides, and are chemically stable, lipophilic compounds make them highly persistent in the environment and cause bioaccumulation in organisms, especially aquatic ones. Due to their natural tendency to intake all substances from water, shellfish can be considered more susceptible to accumulation of PAHs than other marine animals [3].

While limited studies about dioxins in marine animals (bivalves constitute the major parts of investigations) exist in the literature, PAHs were well documented in many types of research from agricultural areas and marine environments [5,7–9]. However, there is always a necessity to monitor these substances continuously in these animals to ensure that no public health issues originate from food or the environment, especially if they are close to agricultural or industrialized areas. In these places, shellfish are becoming more important than other marine animals due to their natural accumulation tendencies. Many bivalves such as mussels and oysters were utilized to determine the coastal contamination rates in different projects and programs [1].

In Turkey, the monitoring of organic and inorganic contaminants has been carried out in accordance with European Union law in bivalve production areas in recent years. In shellfish production, *Mytilus galloprovincialis* is a well-known species that constitutes the major bivalve species harvested in Turkey. According to official data in Turkey, the total production of this species was reported to be approximately 1025 tons in 2017, and 80% of the production took place in this region.[1] Since this species prefers to live close to coastal waters in the marine environment, they are a good risk bioindicator for both human nutrition and environmental issues. At this point, using *M. galloprovincialis* as a biomonitoring species is an important preventative action at the point of food safety and environmental monitoring. For this reason, the present study was designed to investigate toxic chemical contaminants in *M. galloprovincialis* from one of the regions with highest harvest in Turkey.

2. Material and methods

2.1. Chemicals
Internal standards for polycyclic aromatic hydrocarbons were selected as acenaphthene-D_{10}, naphthelene-D_8, phenanthrene-D_{10}, and 1,4-dichlorobenzene-D_4 and purchased from Supelco, Bellefonte, PA, USA. Stock solutions of these PAHs were prepared by dissolving 0.01 g standard in dichloromethane and n-hexane (25:75).

Internal standards of polychlorinated biphenyls and dioxins (EDF-7999, EDF-8999, EC-4986, EC-4987, EC-

4188, EC-4058, ED-911, ED-996, ED-907) used in the analyses for PCBs were obtained from Cambridge Isotope Laboratories, Inc. (Tewksbury, MA, USA). Concentrations of the standards used to determine PCDD/PCDF were prepared in the range of 0.02–20 pg/μL. Concentrations of nonortho, monoortho, and indicator PCBs (28, 52, 101, 153, 138, 180) were prepared in the range of 0.10–50 pg/μL. $^{13}C_{12}$ labelled surrogate internal and recovery standards were added to calibrated standards for dioxins and nonortho PCBs with monoortho, and indicator PCBs.

Chemicals used in the stages of preparation, extraction, clean-up, and instrumental analysis of samples and standards were of analytical grade. Dichloromethane, n-hexane, anhydrous sodium sulphate, and toluene were purchased from Sigma-Aldrich (Germany).

A Pyrex glass chromatography column with PTFE Stopcock (300 mm × 22 mm) was purchased from Fisher Scientific (Bellefonte, PA, USA). Silica gel (70–230 mesh, 63–200 μm) was obtained from Supelco (Bellefonte, PA, USA). Methylene chloride used for the preparation of silica gel was obtained from Sigma-Aldrich (Germany).

2.2. Sample collection and preparation
This study was conducted on two natural beds named Paşalimanı (A) and Ocaklar (B) stations. The stations are located at Bandırma–Erdek on the southern coast of the Marmara Sea, Turkey (Figure 1). The research materials, *M. galloprovincialis*, were collected by SCUBA divers from 5 to 10 m depth in the littoral zones of the mentioned stations in seasons from winter 2014 to summer 2017. Ten kilograms of mussels were collected during each sampling [mean weights and lengths of *M. galloprovincialis* were 16.98 ± 0.36 g and 59.96 ± 0.49 mm] and samples were packaged with rope wire meshes and transported with high humidity to the laboratory in 2 h via ice-cooled box.

2.3. Determination of dioxin levels
Frozen samples of 50 g were placed into a clean jar and homogenized by using Ultra-Turrax (IKA-Yellow Line) 25,000 rpm for 3 min. Homogenized samples were freeze-dried and total moisture was noted by measuring the difference in the weights. An aliquot 10 g of homogenized sample was taken and spiked with $^{13}C_{12}$-labelled surrogate standards. The extraction of spiked samples was performed by using ASE 200 (Thermo Fisher Science, Dionex, Sunnyvale, CA, USA) at 120 °C with a pressure of 1500 PSI. The solvent was n-hexane with a 60% flush rate. Extraction was repeated twice (static time of 5 min × 2 cycles) and lasted for 10 min for each sample. Obtained extracts for each sample (approximately 30 mL) were mixed with 100 μL of the internal standard solutions. Then the extracts were concentrated to 1 mL (near dryness) by using

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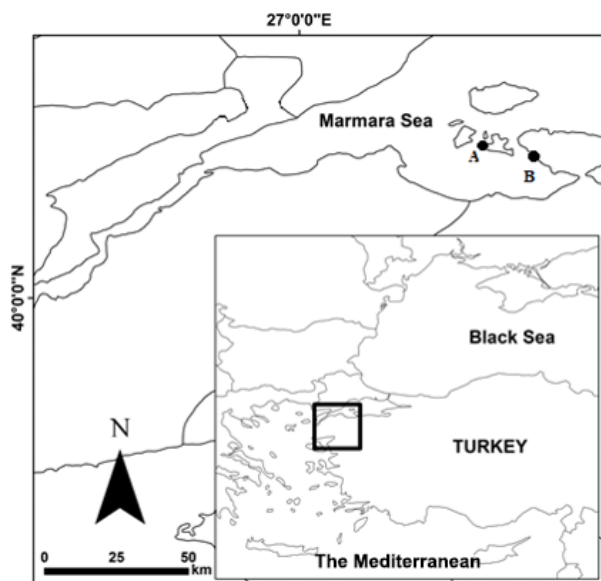
i.d., 0.25 mm film thickness) was used. Helium (>99.99% purity) was used as carrier gas with a constant column flow of 3 mL/min. The injection port was operated in splitless mode. The temperatures of the detector and the injection port were adjusted to 290 °C and 260 °C, respectively. The oven temperature program was 80 °C for 2 min, increased to 170 °C at the rate of 6 °C/min, held for 5 min, increased to 210 °C at the rate of 8 °C/min, after 5 min increased to 250 °C at the rate of 10 °C/min, and then held at 250 °C for 15 min. The identification and quantification of benzo(a) pyrene, benz(a)anthracene, benzo(b)fluoranthene, and chrysene were performed by including the retention times and peak calculations of the internal standards. The detection limit of the device for analyzed PAHs was 0.40 µg. Results are presented as µg/ kg of wet sample.

The recoveries of internal standards and real samples were determined to be in the range of 78.41–93.26% and 76.49–94.11%, respectively. The total contamination rate of the whole analysis (extraction, clean-up, and chromatography) was determined to be in the range of 4.63–8.47%. The linearity of the method (R^2) was evaluated by plotting the instrument response for the chromatographic peak area obtained from running the standards and it was determined to be in the range of 0.9385–0.9641.

2.5. Determination of toxic metal levels
A total of 0.5 g portion of sample meat was put into microwave vessels. They were then turned into soluble forms in a microwave (CEM Mars Xpress) by addition of 8 mL of concentrated HNO_3 (65%, w/v; Merck, Germany) and 2 mL of H_2O_2 (35%, w/v; Merck, Germany). Operating conditions of the microwave oven were as follows; Power: 1000 W, Pressure: 220 PSI, Heat: 200 °C, Ramp time: 20 min, Hold Time: 15 min. A blank sample was prepared using ultra-pure water. After the organic digestion process, the samples were filtered and diluted with deionized water [13]. Following digestion of the samples, the levels of lead, cadmium, and mercury were determined using inductively coupled plasma mass spectrometry (ICP-MS) (Agilent 7700). Calibration of the device and control of matrix effect were performed with prepared standard mixture solutions of Pb, Cd, and Hg. An internal stock solution of Bi, Ge, In, Li, Lu, Rh, Sc, and Tb (250 µg/L) was used in the analysis, and gold (Au) was used to stabilize the Hg and minimize memory effects in the tubing and during nebulization [12,14]. The results obtained from the device were calculated as mg/kg wet weight of the sample.

2.6. Statistical analysis
The mean, standard error, and range of the levels of PCBs, PAHs, and toxic metals along with the one-way variance analysis of the interactions between seasons, chemicals, and stations were calculated using Microsoft Office Excel 2016 software (Seattle, WA, USA), and SPSS (IBM SPSS Statistics 20). Significance was established at $P < 0.05$ [15].

3. Results
3.1. Levels of dioxins
The levels of total dioxins, total dioxins with dioxin-like PCBs, and a total of six important PCBs are summarized in Figure 2 as picogram (1.0×10^{-12}) World Health Organization–Toxic Equivalent/gram of wet weight of samples (pg WHO-TEQ/g of ww). Total dioxin amount ranged between 0.03 and 0.28 pg WHO-TEQ/g ww during the period of 2014 to 2017. Minimum and maximum dioxin levels with dioxin-like PCBs were 0.01 and 0.35 pg WHO-TEQ/g ww, respectively. The total of six PCBs ranged from 0.11 to 0.38 pg WHO-TEQ/g ww during the sampling period. The maximum levels of these three groups of dioxins were determined in 2014 and decreased through the years at both stations (Figure 2). The interaction of stations, seasons and total dioxins, dioxin-like PCBs, and the total for important PCBs (PCB 28, 52, 101, 138, 153, and 180) were found to be significant ($P < 0.05$). Summer and autumn seasons, in general, were found to have higher levels of dioxins than winter and spring seasons during 2014 to 2017 ($P < 0.05$). At both stations, the total dioxin amounts were found to be lower than dioxin-like PCBs and other selected PCBs during the sampling period ($P < 0.05$).

3.2. Levels of polycyclic aromatic hydrocarbons
The levels of benzo(a)pyrene and total benzo(a)pyrene, benz(a)anthracene, benzo(b)fluoranthene, and chrysene are presented in Figure 3 as µg/kg ww of samples. The detection limit for PAHs was 0.40 µg/kg. In general, total levels of monitored PAHs were below the detection limits for both stations. Among seasons in which PAHs were determined, the maximum levels of both benzo(a)pyrene and other totals of PAHs were found to be 0.93 and 2.52 µg/ kg in samples from winter 2015 at station A, respectively (Figure 3). Another maximum level for total PAHs was found to be 1.67 µg/kg in the samples from winter 2016 at station B (Figure 3).

It was found that the levels of benzo(a)pyrene and other PAHs were not affected by season at station A, except for winter 2015 samples ($P < 0.05$). The levels of PAHs in spring seasons of 2015 to 2017 and autumn of 2014 at station B were found to be higher than other seasons at the same station, and for the whole sampling period at Paşalimanı except for winter 2015 ($P < 0.05$).

3.3. Levels of toxic metals
The levels of toxic metals (Pb, Cd, and Hg) found in *M. galloprovincialis* during the sampling period are summarized in Figure 4. The presented values are given as mg/kg of samples on a wet weight basis. The minimum and maximum levels of toxic metals for both stations were determined as 0.05–0.66 (Pb), 0.04–0.42 (Cd), and 0.02–0.76 (Hg) mg/kg, respectively. The maximum Pb level (0.65 mg/kg) was determined in the samples from January
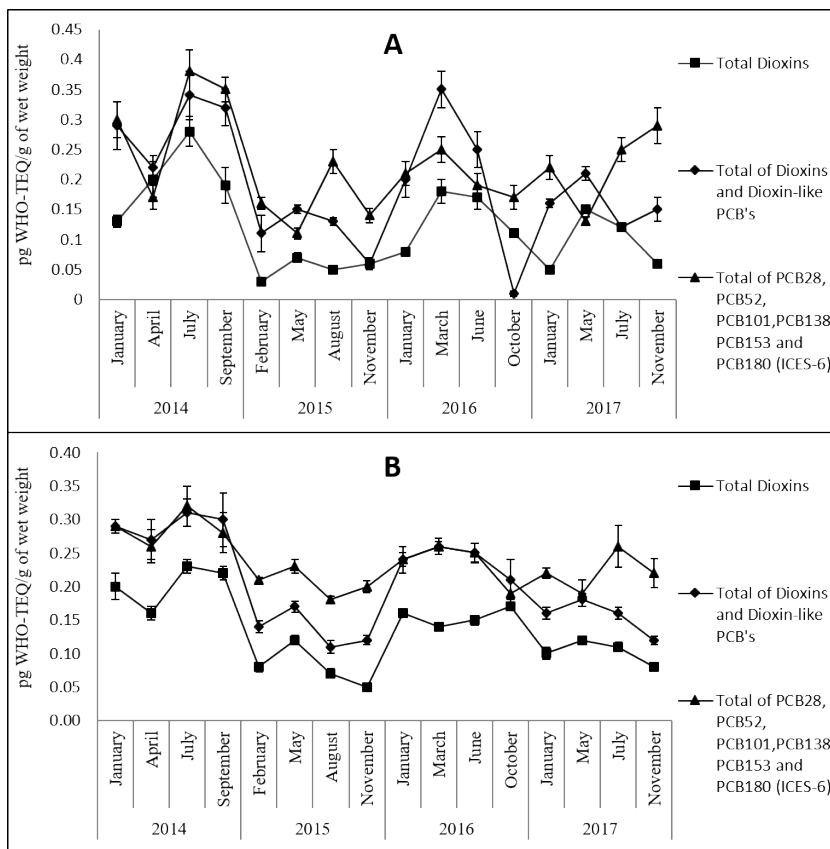
Figure 2. The levels of total dioxins, total dioxins with dioxin-like PCBs, and six important PCBs determined at (A) Paşalimanı and (B) Ocaklar Stations.

2017 at station B. The 0.42 mg/kg value of Cd was also found at the same station in samples from summer 2015. On the other hand, Hg, in general, was not determined much at both sampling stations and in the research period; however, the level for winter 2015 samples at station A was found as 0.76 mg/kg.

The interaction among seasons, sampling stations, and toxic metal type was found to be significant ($P < 0.05$). While Cd and Pb levels differed according to the station during seasons, Hg levels remained similar at both stations, except for the highest level found in the November 2015 samples ($P < 0.05$).

4. Discussion
In this study, the aim was to research the long-term monitoring of toxic chemicals, such as dioxins, dioxin-like substances (PCBs), indicator PAHs, and toxic metals (Pb, Cd, and Hg). The TEQ values of total dioxins, dioxin-like PCBs, and six important PCBs were found in the range of 0.01–0.38 pg WHO-TEQ/g for all samples from both

stations during the period. These levels for PCBs were found to be below the threshold level (4 pg WHO-TEQ for the total of dioxins, dioxin-like PCBs) enforced by the EU Commission [16]. PCB levels found in this study were found to be lower than the results reported in some prior studies conducted about PCBs levels in shellfish. Among these previous studies, the average levels of polychlorinated PCDD/F and dioxin-like DL-PCBs in *M. galloprovincialis* farms in the Ionian Sea of southern Italy were reported in the range of 1.61–5.63 pg WHO 2005 - TEQ/g wet weight [17]. Jaikanlaya et al. [18] reported that total PCB concentrations in mussels, oysters, and shrimp collected from the eastern coast of Thailand ranged between 19 and 1100 ng/g lipid. In that study, the highest concentrations of PCBs in mussels and oysters were reported as 454 ± 125 and 304 ± 65 ng/g lipid weight, respectively [18]. In another study, which reports the situation for dioxin levels in some fish and crustaceans found near industrialized areas, the levels of dioxins and other PCBs were reported to be above their national threshold level (4.0 pg TEQ/g).[2]

[2] NSW (2007) Dioxin in fish and prawns in Homebush Bay and Parramatta River, Australia, New South Wales Office of Environment and Heritage, Environment Protection Science Branch and NSW Department of Primary Industries-Fisheries. Website http://www.rms.nsw.gov.au/maritime/environment/homebush-bay-sediment-management.html [accessed 15.05.2019].
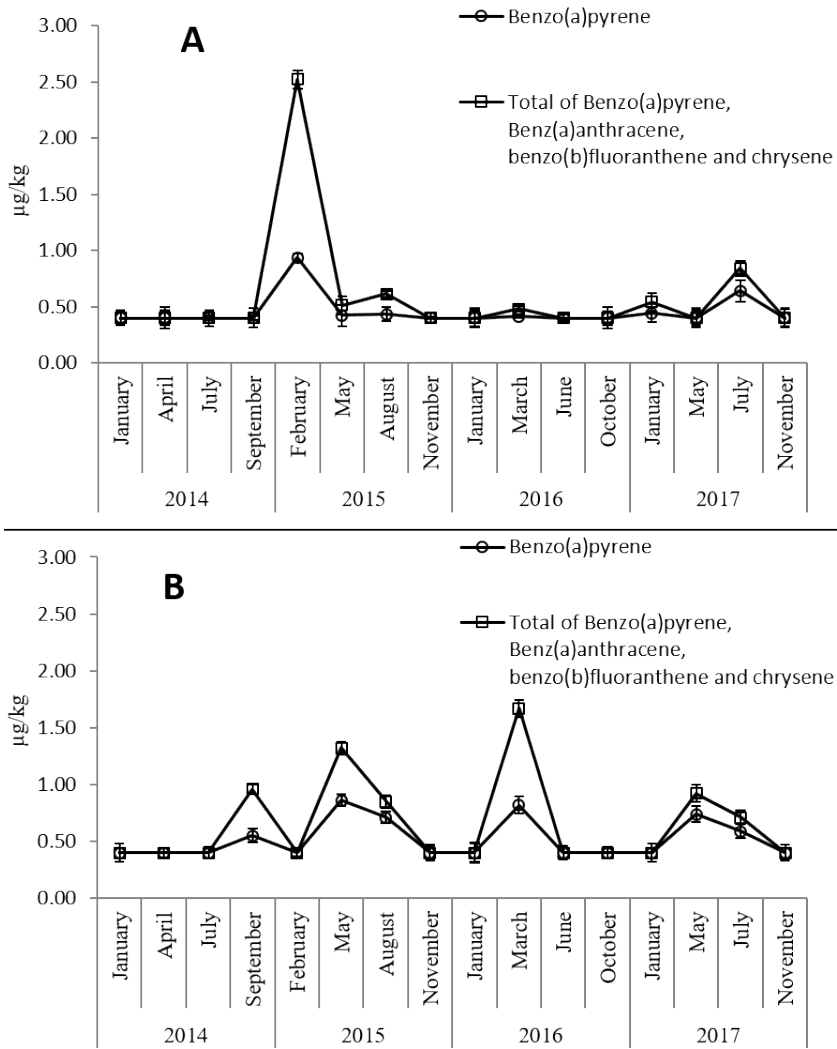
Figure 3. The levels of benzo(a)pyrene and total of benzo(a)pyrene, benz(a)anthracene, benzo(b)fluoranthene, and chrysene determined in *Mytilus galloprovincialis* at (A) Paşalimanı and (B) Ocaklar Stations.

On the other hand, our results show a similarity with some of the previous results like those reported by Llobet et al. [4] and Lee et al. [19], who reported the TEQ of dioxins and dioxin-like PCB levels were under the threshold level in retail seafood and mussels. In the literature, sampling stations are mentioned as the major factor affecting the PCB levels in samples. For example, in *Mytilus edulis* used as a bioindicator of PCBs and PAHs at 17 sampling sites in coastal waters around Europe, the highest concentrations of PCBs and PAHs were observed near estuaries of large rivers flowing through urban areas and industrial regions [20]. In our study, sampling stations are also very close to industrialized areas; however, the results were found to be higher than the results reported by Olenycz et al. [20]. The main reasons for the differences in studies are clearly linked to the different industrialized study areas in addition to the

use of different bivalve species as bioindicators. Different bivalve species (for example, between mussels and oysters) collected from the same locations also have remarkable differences in the concentrations of organic compounds such as total PCB and PAH [21]. This may be due to the differences in water filtration capacity of the bivalves and their biology.

The levels of important PAHs, namely benzo(a)pyrene, benz(a)anthrecene, benzo(b)fluoranthene, and chrysene, were monitored during 2014–2017. A total of 4 years of sampling indicated that there may be potential contamination near coastal waters; however, the amounts were not determined at elevated levels. The highest benzo(a)pyrene levels were recorded in winter 2015 as 0.93 μg/kg for station A and in spring 2015 as 0.86 μg/kg for station B. The highest levels of PAH4, namely total benzo(a)pyrene,
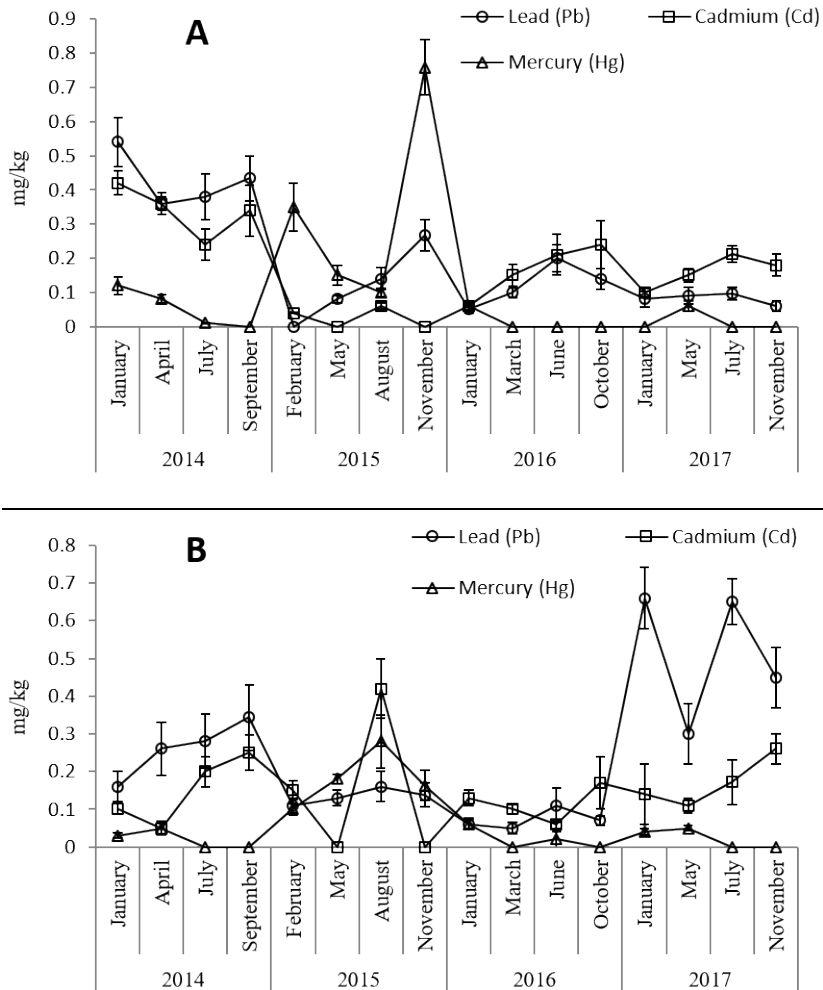
Figure 4. The levels of Lead (Pb), Cadmium (Cd), and Mercury (Hg) determined in *Mytilus galloprovincialis* at (A) Paşalimanı and (B) Ocaklar Stations, during the sampling period.

benz(a)anthrecene, benzo(b) fluoranthene, and chrysene, were also determined as 2.52 μg/kg in winter 2015 for station A, and 1.67 μg/kg in spring 2016 for station B. Even these highest values were found to be far below the threshold level described as 30 μg/kg by the Commission Regulation (EU) No 835/2011 [16]. Among prior studies, Nwaichi and Ntorgbo [5] reported that the levels of benzo(a)pyrene and total benz(a)anthrecene, benzo(b) fluoranthene, and chrysene in three different seafoods (mudskipper, periwinkle, and oyster) were in the range of 0.001–87400 μg/kg. In native mussels (*Brachidontes rodriguezii*) collected from a critical industrialized estuary in Argentina, the total concentrations of PAHs were reported to be under the limit of 482.4 ng/g [8]. In studies related to *M. galloprovincialis*, the total PAHs concentrations were reported in wide ranges from different study areas; 664 to 9083 ng/g from the Prince Islands in the Marmara Sea [22], 25 to 390 ng/g from the western Mediterranean

Sea (French Riviera, Corsica, Sardinia) [23], and 14.8 to 645.3 mg/kg from two inlets found in the Gulf of Taranto (Ionian Sea, Italy) [24]. The common agreement between these studies is the highest total concentrations can most likely be found at stations affected by industrial fallout, urban wastewaters, and contaminants transported via riverine discharge [5,24]. Moreover, the seasons can affect the PAHs levels in *M. galloprovincialis*. It was reported that the maximum levels for benzo(*a*)pyrene in mussels (*M. galloprovincialis*) collected in winter from the Gulf of Naples (Tyrrhenian Sea, Italy) exceeded 71.43%, while the value was considerably lower in the summer [7]. Although these reported values were different from our results, the use of shellfish as bioindicators and finding elevated levels of PAHs in industrial regions are common points.

In this study, the lowest and highest Pb levels were determined as 0.05 mg/kg (in spring 2016 samples) and 0.66 mg/kg (in winter 2017 samples), respectively. The

European Commission set the legal limit for lead in shellfish as 1.50 mg/kg [16]. In the studies conducted on different bivalve species (*Ostrea edulis*, *Donax trunculus*, *Ruditapes philippinarium*, *Chamelea gallina*) collected from different zones of the Marmara Sea, the levels of Pb were reported in the range of 0.05–4.16 mg/kg [25,26,27]. In general, these Pb values reported in prior studies are slightly higher than our results. Differences in both sampling areas and species are thought to be the main reasons for the different results.

Cd level was determined in the range of 0.04–0.42 mg/kg in mussel samples. These values are found far below the threshold level set as 1.00 mg/kg by the European Commission [16]. In prior studies, Cd levels in mussel samples were reported in the range of 0.02–2.80 mg/kg for different bivalve species collected from different coasts on the Marmara Sea [25–27]. Our results display similarities to those previously reported values from the Marmara Sea.

Mercury (Hg) is considered among the top 10 chemicals of "major public health concern" by the World Health Organization [28]. Hg turns into methylmercury (MeHg) in the marine environment and accumulates in food networks. Exposure to MeHg can occur mainly by consumption of seafood, which results in neurotoxicity [28]. In this study, Hg levels in analyzed samples ranged from 0.02 mg/kg to 0.76 mg/kg during the monitoring period. The EU Commission has set the maximum level of Hg in some seafood as 0.5–1.0 mg/kg [15]. However, there is no clear threshold level for bivalve mollusks so they need specific identification of certain contaminants due to their natural accumulation tendency. Considering the 1 mg/kg threshold level, the highest level of Hg (0.76 mg/kg) determined in this study is below this enforced level.

In conclusion, in the present study, long-term monitoring of toxic chemicals in *Mytilus galloprovincialis* was performed. These chemicals are known to be important for both the environment and for food safety issues. Aquatic animals, especially filter-feeding bivalves (such as mussels, oysters) are important organisms as they reflect the chemical quality of water and their safety as food in an easy and reliable way. *M. galloprovincialis* was used as monitoring material due to high consumption and harvesting rate, abundance, and living close to coastal areas. All toxic chemicals monitored in this study were determined to be under the threshold levels enforced by the EU Commission [15]. However, Hg should be included in all environmental or food monitoring investigations due to its presence at maximum levels. Further investigation is required in the near future since the chemical composition and/or pollution of the marine environment can vary rapidly and easily.

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