As a Useful Biomonitor of Toxic Trace Element Contamination in the Highly Urbanised Istanbul, Turkey; Mytilus Galloprovincialis

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Research Article

Keywords: Mytilus galloprovincialis, Toxic trace element, Bioindicator

DOI: https://doi.org/10.21203/rs.3.rs-365809/v1

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Abstract

In this study, concentrations of toxic trace elements were determined by an ICP-MS device in the edible tissues of *Mytilus galloprovincialis* from the Marmara Sea, İstanbul-Turkey. Concentrations of As, Pb, Cd, Hg, Ni, and Zn were investigated in digestive gland, gills, and muscles tissues of the mussel. According to the results obtained in the study, As, Pb, Cd, Ni, and Zn in the digestive gland of *M. galloprovincialis* were above the permissible concentrations (mg kg\(^{-1}\)), which were 0.672 for Cd, 6.870 for As, 0.788 for Pb, 1.990 for Ni and 42.6 for Zn. The results show that Cd and As mean concentrations in muscle of *M. galloprovincialis* were above the permissible legal limits. Furthermore, Cd and Ni mean concentrations in gills of *M. galloprovincialis* were above the permissible levels. Furthermore, it was observed that the highest value belonged to As in the digestive gland of mussels with 65.42% of Provisional Tolerable Weekly Intake. At the same time, the lowest percentage belonged to Zn with 0.22% of PTWI in muscles and 0.313% of PTWI in gills of the mussels. Thus, it is recommended that As, Pb, Cd, Hg, Ni, and Zn concentrations in seafood samples, particularly in mussels, should be monitored periodically.

Introduction

Toxic trace elements are among the pollutants that cause significant problems. These elements can be transmitted through the respiratory and digestive systems of aquatic species or skin and mucosa. Aquatic organisms retrieve metals with particle shapes, readily solved metals, and suspended materials in water via their gills with wide and thin surfaces. As inorganic pollutants, heavy metals, which are taken into the body directly or indirectly through water and food, are transferred to tissues through binding to transport proteins (Olsson et al., 1998). Heavy metals are accumulated at higher levels in organs that are related to metal metabolism and detoxification (Kayhan et al., 2016). The Mediterranean mussel (*Mytilus galloprovincialis*), which is a significant aquatic food, can easily grow in regions with heavy pollution as well as clean and semi-clean waters. Just as other mussels, this species retrieves their food from water by filtering and they are regarded as an important bioindicator in investigations on sea pollution (Uysal, 1970; Rayment & Barry, 2000).

The creatures in aquatic environments are the first creatures to be affected by the pollution in waters. Bivalves, which feed by filtering water, are especially among the invertebrates that are exposed to pollution the most. Mussels are creatures that survive by feeding on organic materials and planktons in waters by locating themselves in firm areas in deep seas. Heavy metals, which alter the chemical structure of waters, are accumulated in the tissues and shells of mussels. Mussel species are extremely sensitive to the chemical structure of waters (Varlık, 1991). Among the invertebrate species, *Mytilus galloprovincialis* is the most commonly chosen species in studies on determining pollution elements. The creatures that reflect the level of pollution in living environments are called “bioindicators”. Certain basic functions are required for species to be used as bioindicators in determining heavy metal pollution in addition to the function of collecting elements. These include an extended presence in collection areas,
presence in a wide geographical region, convenient sampling, and absence of identity problems. *Mytilus galloprovincialis* is a bioindicator species with qualities that meet all these criteria and thus, they were chosen as the target species of this study (Aksoy et al., 1999). Conducting studies that investigate the accumulation and damages of heavy metals in aquatic organisms is of importance in determining biochemical, physiological, structural, and functional defects to occur in organisms in addition to determining species that possess high sensitivity to these metals. Metallic pollutants that are investigated in creatures as indicators of environmental pollution can frequently reach high levels, especially in aquatic products. Thus, metal traces, such as mercury, cadmium, and lead, are frequently, despite at low levels, are retrieved and significantly affect environmental and human health (Kayhan et al., 2009).

Mussels have open circulatory systems that are exposed to changing environmental conditions including contaminants. Hemocytes in the immune defense of bivalves have an ability of amoeboid locomotion and play key roles in the immune defense (Cheng, 1981). Briefly, heavy metals, whether required or not for organisms, cause toxic effects at high concentrations. Heavy metals demonstrate their effects by reacting with enzymes or binding to membrane structures. These interactions between metals and cell structures result in structural and functional changes. Consequently, muscle, circulatory, respiratory, hormonal, and immune systems are damaged and significant changes occur in populations.

Therefore, determining the heavy metal concentrations in mussel tissues is of great importance in terms of determining the toxic effects of concentrations higher than certain levels on human health. In circumstances where the detected concentrations are above the designated limits, the consumption of these products should be restricted and the consumers should be informed about not consuming fisheries hunted in regions with excessive pollution. In our study, in the light of this information, whether *Mytilus galloprovincialis* species mussels were within toxic limits was investigated due to toxicity for aquatic creatures and possible negative effects on non-targeted creatures. In this study, the main reasons for choosing the Mediterranean mussel (*Mytilus galloprovincialis*) as the research material were their abundance in seas and being prominent biological indicators that pollution in waters, which is related to their collection of metals at high intensities and storage of these in their bodies for a long time.

### Materials And Methods

**Instrumentation**

The Perkin-Elmer Elan 9000 ICP-MS (Perkin-Elmer SCIEX, Woodbridge, ON, Canada) was used for multi-element detection of As, Pb, Cd, Hg, Ni, and Zn, simultaneously. The operating conditions of the ICP-MS were presented in Table 1.
Table 1
Operating conditions of Perkin-Elmer ELAN9000 inductively coupled plasma mass spectrometer (ICP-MS)
(Perkin-Elmer SCIEX, Woodbridge, ON, Canada)

| ICP-MS conditions                  |   |
|-----------------------------------|---|
| Plasma gas flow rate L min⁻¹      | 15|
| Carrier gas flow rate L min⁻¹      | 0.9|
| Sample uptake rate L min⁻¹        | 1.0|
| Nebulizer                         | Crossflow|
| Spray chamber                     | Ryton®, Double pass|
| Sample uptake rate                | 1.0 mL min⁻¹|
| Plasma power (RF power)            | 1000 W|

Analysis of Metals

*Mytilus galloprovincialis*, which is a commonly consumed species, was caught weekly from Istanbul, Turkey (Fig. 1). In total, approximately 3 tissues of this species (*Mytilus galloprovincialis*) were collected from January to February 2016 (mean value of three subsamples). *M. galloprovincialis* species were purchased eight times from local anglers and the sampling was conducted weekly. The sampling was conducted in winter months (January to February) because of the periodical convenience of providing all the seafood species in these months. Furthermore, there was more pollution during the winter months in this region.

Chemical Analysis of Heavy Metals

Following the collection of *Mytilus galloprovincialis*, the samples were quickly transferred to the laboratory within the ice. Various muscles of the mussels, which are the edible parts of every mussel, were extracted by cutting with a stainless steel scalpel. Then, the samples were washed and placed in polyethylene bags to be stored at -4 °C until the analyses. For the microwave solubilization process, 0.4 g fresh samples were transferred to a solubilization container. Then, 8 mL of concentrated nitric acid was added and Teflon bombs were placed into microwave ovens. The samples were digested at 800 watts and 180 °C for 15 minutes. Following the solubilization process, the Teflon bombs were left for cooling for some time without removing their covers. Then, the solved sample was transferred to a 25-ml volumetric flask and diluted to 25 mL by pure water (Millipore pure water). The Teflon bombs were cleaned after each application. Analyses were conducted at least three times for each sample. Then, the samples were placed in previously numbered flasks and sent to Maxxam Analytics Laboratory in Canada for the investigation of As, Pb, Cd, Hg, Ni, and Zn via ICP-MS.

Analytical Performance
To check the validity of the measurements, a standard reference material (NCS ZC73016 chicken trace elements) was used. For most of the analyzed metals, the recoveries were determined to be 92–97%. The related results were presented in Table 2. In all the analyzed samples, the accuracy of the method indicated that the relative standard deviation (RSD, %), was commonly < 10%. Limit of detection (LOD) values, which were calculated as three times the standard deviation of a blank, and limit of quantification (LOQ) values were presented in Table 2. The Student’s t-test was also conducted in the study (p < 0.05). All the metal concentrations were determined on a wet weight basis.

| Elements | LOD (µg kg\(^{-1}\)) | LOQ (µg kg\(^{-1}\)) | Certified values (mg kg\(^{-1}\)) | Measured values (mg kg\(^{-1}\)) | Recovery (%) |
|----------|----------------------|-----------------------|-----------------------------------|----------------------------------|--------------|
| Cd       | 0.004                | 0.01                  | -                                 | -                                | -            |
| Hg       | 0.006                | 0.02                  | 0.0036 ± 0.0009                   | 0.0033 ± 0.0012                  | 92           |
| As       | 0.035                | 0.10                  | 0.117 ± 0.013                     | 0.109 ± 0.009                    | 93           |
| Pb       | 0.07                 | 0.20                  | 0.114 ± 0.014                     | 0.108 ± 0.011                    | 95           |
| Ni       | 0.35                 | 0.95                  | 0.165 ± 0.020                     | 0.156 ± 0.013                    | 95           |
| Zn       | 1.6                  | 5.0                   | 26.0 ± 1.0                        | 25.1 ± 1                         | 97           |

- LOD: Limit of Detection, LOQ: Limit of Quantification
- The Certified Reference Material (CRM) that was used in the study was chicken muscle NCS ZC73016 chicken trace element.

### Statistical Analyses

Statistical analyses were performed by using Statistica 8.0 (2007) for Windows (StatSoft, Inc., Tulsa, USA). Data were log-transformed when necessary for meeting the normal distribution assumptions of ANOVA and Pearson’s product-moment correlation tests. Pearson’s product-moment correlation test was used to check for significant relationships between metal concentrations and sample lengths. A two-way ANOVA test was used for investigating statistical differences between seasons and tissue types for each species (p < 0.05). Additionally, a three-way ANOVA test was performed for significant differences among seasons, species, and tissue types (p < 0.05).

### Human Health Risk Assessment

The risk assessment for human health was conducted by using provisional tolerable weekly intake (PTWI) and reference dose (RfD) estimates (FAO, 1993/WHO, 1985).
Results

The mean concentrations of arsenic, lead, cadmium, mercury, nickel, and zinc, which were obtained from \textit{Mytilus galloprovincialis}, were calculated with relative standard deviations. In the study, the comparisons of concentrations of the elements were conducted by considering the legal limits of Turkey and other countries, which were presented in Table 4.

In the linearity test conducted according to the ICP-MS method, it was determined that As, Pb, Cd, Hg, Ni, and Zn provided acceptable results. Additionally, it was determined that the LOD and LOQ values obtained in this study met the Commission Regulation (EC) No 333/2007, which was demonstrated in Table 2. The recovery analyses provided 92% and 97% values. According to the limit of repeatability (the metrological approach), all the results that were obtained in the study were acceptable. Furthermore, the Horrat values of repeatability and reproducibility in all the concentration levels were below 2, which is in accord with the Commission Regulation No 836/2011. In the study, the DMA80 test was conducted by adopting the US EPA method 7473 (Di Bella et al., 2018). Accordingly, the determination method used for Hg was presented with validation results in Table 2.

In the analyses, the cadmium concentrations in the muscles, gills, and digestive gland of the mussels (\textit{M. galloprovincialis}) were determined as 0.143 mg kg$^{-1}$, 0.180 mg kg$^{-1}$, and 0.672 mg kg$^{-1}$, respectively, while mercury concentrations were determined as 0.012 mg kg$^{-1}$, 0.072 mg kg$^{-1}$, 0.040 mg kg$^{-1}$, respectively (Table 4, Fig. 2). The maximum cadmium level permitted for sea products is 0.10 mgkg$^{-1}$ according to the Turkish Food Codex. The maximum cadmium level permitted is 0.5 mgkg$^{-1}$ for FAO (1983), 0.2 mgkg$^{-1}$ for MAFF (1995) and 0.05 mgkg$^{-1}$ for EU (2001, 2008). The maximum cadmium level muscle and gills of mussel were found to be lower than legal limits of Turkish standards (2011) and MAFF (1995) but cadmium level in muscle and gills of mussel were found to be higher than legal limits of EU (2001,2008) and Turkish Standards (2011). Cadmium level in mussel's digestive gland was higher than 0.05 mgkg$^{-1}$ for EU (2001, 2008), 0.1 mgkg$^{-1}$ for Turkish standards and 0.2 mgkg$^{-1}$ MAFF (1995). According to these results, the concentrations of mercury in all tissues were below all legal limit values which were 0.5 mgkg$^{-1}$ for FAO (1983), FEPA (2003), MAFF (1995), Turkish Standards and 1.0 mgkg$^{-1}$ for EU (2001).

In our study, the concentrations of lead in muscles, gills, and digestive gland of mussels (\textit{M. galloprovincialis}) were determined as 0.235 mg kg$^{-1}$, 0.248 mg kg$^{-1}$, 0.788 mg kg$^{-1}$, respectively (Table 4, Fig. 2). The maximum lead level permitted is 2.0 mgkg$^{-1}$ for WHO (1996) and FEPA (2003) MAFF (1995) and 0.5 mgkg$^{-1}$ for FAO (1983) and 0.30 mgkg$^{-1}$ for Turkish standards.

In the current study, the concentrations of heavy metals in mussel tissues were examined. In the analyses, it was determined that the concentrations of arsenic were 1.080 mg kg$^{-1}$ in muscles, 0.656 mg kg$^{-1}$ in gills, and 6.870 mg kg$^{-1}$ in the digestive gland of the mussels (\textit{M. galloprovincialis}), which were presented in Table 4 and Fig. 2. It was determined that the arsenic concentrations in muscle tissues and digestive glands of the mussels in question exceeded the daily limit for acceptable arsenic intake, which
was 1.0 mg kg\(^{-1}\). According to the Australia standard and EU (2001), the maximum level permitted for arsenic in fish is 1.0 mg kg\(^{-1}\). In the examination of the heavy metal concentrations in mussel tissues within the framework of the study, it was determined that nickel concentrations were 0.233 mg kg\(^{-1}\) in muscles, 0.813 mg kg\(^{-1}\) in gills, and 1.990 mg kg\(^{-1}\) in digestive glands of the mussels (\textit{M. galloprovincialis}), which were demonstrated in Table 4 and Fig. 2. However, Turkish standards do not include any information about the acceptable nickel levels in fish samples. In Table 4, FEPA determines the allowed maximum nickel level as 0.5 mg kg\(^{-1}\) while EU states the limit to be 1.5 mg kg\(^{-1}\). In our study, it was determined that the nickel levels of the muscle samples were below limits indicated by WHO (2001), FEPA (2003), and EU (2001). However, the nickel levels in the digestive glands exceed all the limits.

In the analyses, it was determined that the zinc concentrations of the mussels were 11.109 mg kg\(^{-1}\) in muscles, 15.337 mg kg\(^{-1}\) in gills, and 42.60 mg kg\(^{-1}\) in digestive glands (\textit{M. galloprovincialis}). The Turkish Food Codex (Anonymous, 2002) states that the maximum zinc level in sea products is permissible up to 50 mg kg\(^{-1}\) while this limit is 30 mg kg\(^{-1}\) in FAO (1983) and 50 mg kg\(^{-1}\) in MAFF (1995). In our study, it was determined that the nickel levels in the muscle tissues and gills of the mussels were below all the limits. On the other hand, the zinc concentrations in the digestive glands of mussels were determined to be higher than the limits of FAO (1983)'s, which is 30 mg kg\(^{-1}\).

These results prove useful in terms of investigating the chemical quality of certain aquatic products, determining possible risks related to their consumption, and realizing that the accumulation of metals depends on the species, distinct tissues, and aquatic environments. In the current study, significant differences were observed in terms of metal accumulation in various tissues and various species. In the results, it was generally observed that heavy metals existed at low concentrations in the muscle tissues of the species while higher concentrations were observed in gill and digestive gland tissues. In conclusion, the digestive systems of mussels are useful bioindicators that demonstrate the metals present in water systems.

In our study, it was determined that the digestive gland tissues of the mussels were above the limit values for Cd according to the Turkish Standards, MAFF (1995), EU (2001, 2008), and FAO (1983). Also, the digestive gland tissues of the mussels were above the limit values for Pb according to FAO (1983) and Turkish standards. Additionally, the digestive gland tissues of the mussels were above the limit values for Ni according to the WHO (1985, 1996), FEPA (2003), and EU (2001). Additionally, the amount of As in the muscle of the mussels was above the limit of the European Union (2001) and Australia Standard (1998) and the As concentrations in digestive gland tissues of mussels were above the limit of European Union (2001), Australia Standard (1998) and Eisler (1994). The Hg concentrations in the muscle, gills, and digestive glands of the mussels were below the limits determined by the Turkish Standards (2011), FEPA (2003), EU (2001) and FAO (1983). The Zinc levels in the digestive glands of mussels were above the limit value according to FAO (1983). The concentration of the heavy metals observed in various tissues of the mussels in the study could be ranked as muscles < gills < digestive gland.
Table 3
PTWI values of trace elements in the organs of mussels

| Element | PTWI = Cx0.1 kg per week: as mg/adult | Muscle  | Gills  | Digestive gland | The reference value for PTWI |
|---------|--------------------------------------|---------|-------|-----------------|-----------------------------|
| Pb      |                                      | 0.0235  | 0.0248| 0.0788          | 1.75<sup>a</sup>           |
| As      |                                      | 0.1080  | 0.0656| 0.687           | 1.05<sup>e</sup>           |
| Cd      |                                      | 0.0143  | 0.018 | 0.0672          | 0.40<sup>b</sup>           |
| Hg      |                                      | 0.0012  | 0.0072| 0.0040          | 0.35<sup>a</sup>           |
| Ni      |                                      | 0.0233  | 0.0813| 0.199           | 2.45<sup>d</sup>           |
| Zn      |                                      | 1.1109  | 1.5337| 4.26            | 490<sup>c</sup>            |

<sup>a</sup> PTWI values retrieved from FAO/WHO (2004)
<sup>b</sup> PTWI values retrieved from FAO/WHO (2010)
<sup>c</sup> PTWI values retrieved from FAO/WHO (2007)
<sup>d</sup> PTWI values retrieved from WHO (1993)
<sup>e</sup> PTWI values retrieved from WHO (1998) for inorganic Arsenic

The results were considered that one person (70 kg) consumed 100 g of mussels in one week (Bilgin and Uluturhan−Suzer, 2017).

The concentration intervals and distributions of the six elements determined by using ICP-MS in mussels were presented in Table 3. The element with the highest mean concentration in the digestive gland of mussels was determined as Zn (42.6 mgkg<sup>-1</sup>) while the element with the lowest mean concentration in the muscle of mussels was Hg (0.012 mgkg<sup>-1</sup>). The concentration intervals of the six elements analyzed in mussels were as the following (presented in mgkg<sup>-1</sup>): Pb (0.235−0.788), As (0.656−6.87), Cd (0.143-0.672), Hg (0,012−0,072), Ni (0.233−1.990), Zn (11.109−42.6).

The PTWI values were calculated according to an adult individual with 70 kg of weight. According to the results, Pb in muscles and gills of mussels were 1.3% and 1.41% of PTWI, respectively while Pb in the digestive gland were approximately 5% of PTWI. The concentration of As in the muscle of mussels was approximately 10.28% of PTWI while these values were 65.42% and 6.25% of PTWI in the digestive gland and gills of the mussels, respectively. When the concentrations of Cd in mussels were investigated, it was determined that this value was 3.58% of PTWI in muscles, 4.5% of PTWI in gills, and 16.80% of PTWI in the digestive gland. Additionally, the values calculated according to Hg concentration were 0.34% of PTWI in muscles, 2.05% of PTWI in gills, and 1.14% of PTWI in the digestive gland. The values of Ni in mussels
were 0.95% of PTWI in muscles, approximately 3.32% of PTWI in gills, and approximately 8.12% of PTWI in the digestive gland. Furthermore, the concentrations of Zn in mussels were 0.22% of PTWI in muscles, 0.86% of PTWI in the digestive gland, and 0.313% of PTWI in gills (Table 3).

Considering these results, it was observed that the highest value belonged to As in the digestive gland of mussels with 65.42% of PTWI. On the other hand, the lowest percentage belonged to Zn with 0.22% of PTWI in muscles and 0.313% of PTWI in gills of the mussels (Table 3).
Table 4
Hg, Cd, As, Pb, Ni and Zn concentrations (mg kg\(^{-1}\)) of the *M. galloprovincialis* obtained from Istanbul, Turkey, and the comparisons with permissible limits in fisheries in different countries (the results are presented in mean values ± standard deviation on wet weight, n = 3)

|                  | Hg     | Cd      | As      | Pb      | Ni      | Zn      |
|------------------|--------|---------|---------|---------|---------|---------|
| Muscle           | 0.012 ± 0.001 | 0.143 ± 0.007 | 1.080 ± 0.085 | 0.235 ± 0.018 | 0.233 ± 0.018 | 11.109 ± 0.856 |
| Gills            | 0.072 ± 0.006 | 0.180 ± 0.014 | 0.656 ± 0.038 | 0.248 ± 0.012 | 0.813 ± 0.048 | 15.337 ± 0.997 |
| Digestive gland  | 0.040 ± 0.001 | 0.672 ± 0.041 | 6.870 ± 0.345 | 0.788 ± 0.045 | 1.990 ± 0.137 | 42.60 ± 1.543 |

Permissible limits of fisheries

|                   | Hg | Cd | As | Pb | Ni | Zn |
|-------------------|----|----|----|----|----|----|
| FAO(1983)\(^a\)   | 0.5 | 0.5 | -  | 0.5 | -  | 30 |
| WHO (1985, 1996)\(^b\) | -  | -  | -  | 2.0 | 0.6 | -  |
| FEPA (2003)\(^c\)  | 0.5 | -  | -  | 2.0 | 0.5 | -  |
| EU (2001)\(^d\)    | 1.0 | 0.05 | 1.0, 1.0\(^e\), 1.3\(^f\) | -  | 1.5 | -  |
| EU (2008)\(^d\)    | -  | 0.05–0.1 | -  | -  | -  | -  |
| MAFF(1995)\(^h\)   | 0.5 | 0.2 | -  | 2.0 | -  | 50 |
| Turkish Standards\(^g\) | 0.5 | 0.10 | -  | 0.30 | -  | 50 |

\(^a\) Food and Agriculture Organization of the United Nations  
\(^b\) World Health Organization  
\(^c\) Federal Environmental Protection Agency  
\(^d\) European Union  
\(^e\) Australia Standard (Australia New Zealand Food Authority, 1998)  
\(^f\) Eisler(1994)  
\(^g\) Turkish Standards (Anonymous, 2008; TFC, 2011)  
\(^h\) Ministry of Agriculture, Fisheries and Food; Turkish Standards, 1995.
Discussion

Arsenic, lead, cadmium, mercury, and nickel are among the 129 priority pollutants and the 14 most toxic elements, which were listed by the Environmental Protection Agency (IARC). Commonly, these elements enter cells by anion transporters and attack DNA. The IARC reported that there was considerable evidence in experimental animals in terms of the carcinogenicity of the elements in question and/or compounds of them (IARC). Currently, EU legislation and EPA include maximum levels for these elements in seafood. The results in the current study indicated that As, Pb, Cd, Hg, Ni, and Zn in the gills and digestive gland of *M. galloprovincialis* exceeded the permissible concentrations levels (mg kg$^{-1}$), which were 0.072 for Hg, 0.672 for Cd, 6.870 for As, 0.788 for Pb, 1.990 for Ni, and 42.6 for Zn. Furthermore, metal concentrations in muscles were below the permissible levels. Therefore, it is suggested that As, Pb, Cd, Hg, Ni, and Zn concentrations in seafood samples (particularly in mussels) should be monitored periodically. In general, if the PTWI is above the permissible levels, the exposure to the contaminant element is a potential health concern. Within this framework, the amount of a contaminant that enters the body via food requires a separate evaluation for each kind of food/or food group that is consumed. Finally, the Estimated Daily Intakes of all kinds of food should be included in the evaluations. Considering the totality of diets, mussels contribute variably, but potentially by significant amounts of the toxic elements. In the current study, when the data presented in Table 3 were considered, element concentrations of the samples varied from 0.012 mg kg$^{-1}$ to 0.072 mg kg$^{-1}$ Hg, from 0.143 mg kg$^{-1}$ to 0.672 mg kg$^{-1}$ Cd, from 0.656 mg kg$^{-1}$ to 6.870 mg kg$^{-1}$ As, and from 0.235 mg kg$^{-1}$ to 0.788 mg kg$^{-1}$ Pb. When the heavy metal concentrations of mussels (*M. galloprovincialis*) in the study were analyzed, the concentrations of arsenic in the muscles, gills, and digestive gland of mussels were 1.080 mg kg$^{-1}$, 0.656 mg kg$^{-1}$, and 6.870 mg kg$^{-1}$, respectively. The Turkish Standards do not include any information for the maximum level of arsenic in fisheries (Anonymous, 2002 and 2008). On the other hand, the European Union (EU) reported permissible limits for arsenic in fisheries, which was 1 mg kg$^{-1}$ (European Union, 2001). Furthermore, Food Standards Australia New Zealand authority stated that the permissible value for arsenic in fisheries consumed by people was 1 mg kg$^{-1}$ according to age intervals. The US Environmental Protection Agency (USEPA) stated that the maximum value for arsenic was 1.3 mg kg$^{-1}$ according to age intervals (Eisler, 1994; Burger & Gochfeld, 2005).

When the literature was reviewed, a study conducted in the Bartin and Inebolu Ports, Turkey, compared *M. galloprovincialis* samples between the two ports. In this study, the researchers reported that the amounts of As (9.89 mgkg$^{-1}$, dry weight) and Pb (0.88 mgkg$^{-1}$, dry weight) from Bartin port were significantly higher than the samples collected from Inebolu port (6.38 and 0.83 mgkg$^{-1}$, dry weight, respectively). Furthermore, the study reported that the concentrations of Co (0.64 mgkg$^{-1}$, dry weight), Cu (39.50 mgkg$^{-1}$, dry weight), and Fe (458.66 mgkg$^{-1}$, dry weight) were significantly higher than the samples collected from the Bartin port (0.16, 7.05, and 409.84 mgkg$^{-1}$, dry weight, respectively) (p < 0.05). In the comparison of the heavy metal concentrations with the limit values, which were determined by the EU Commission (EC, No. 1881/2006), it was determined that As, Cd, Cu and Zn of *M. galloprovincialis* in
Inebolu port and As, Cd and Zn concentrations in Bartin port exceeded the limit values. This indicated the presence of pollution in terms of heavy metals (Gökkuş & Berber, 2019).

The World Health Organization stated that for all groups of humans, the provisional tolerable weekly intake (PTWI) of Cd and Pb were 5 and 25 µg kg\(^{-1}\) of body weight, respectively. These are equivalent to 350 and 1,750 µg/week, for an adult human weighing 70 kg, respectively (WHO/FAO/IAEA, 1996). The maximum allowance concentrations (MAC) for each food are reported by considering domestic food consumption data. Accordingly, the total amounts of the metal of interest from those food species should not exceed the amounts mentioned above (weekly, 350 µg Cd, 1,750 µg Pb, for an adult human weighing 70 kg). On the other hand, the data for food consumption depends on variables such as income, household consumption, age groups, and socioeconomic factors. Furthermore, it is obvious that diets significantly vary depending on countries. Therefore, the MAC values reported by authorities in developing countries do not indicate the actual intake values in underdeveloped countries (Yaman et al., 2014).

Furthermore, these results can be related to certain bioaccumulation trends, which are based on different habitats, lifestyles, and diet regimes of crustaceans. Certain studies reported that metal accumulation resulted in capacities that were unique to species for bivalves (Usero et al., 1997; Irato et al., 2003). Additionally, certain researchers reported that these differences were associated with the metabolic rates of bivalves (Najdek & Sapunar, 1987). In the current study, the concentrations of As, Pb, Cd, Ni, and Zn in the digestive gland of the mussels were compared to the concentrations in other tissues. Within this framework, the results pointed out that the toxic materials were accumulated in the digestive glands for storage or detoxification. In terms of bivalves, gut content includes a mixture of biogenic and lithogenic materials. The filter-feeding bivalves uptake metals from specific sources by ingesting suspended particulate matter, which includes planktonic organisms, bacteria, and inorganic particulate matter from the water column. In previous studies, it was reported that the availability of terrigenous inorganic materials, such as Cr, Cd, Al, and Fe, were discovered in foods (Szefer et al. 2004; Giarratano and Amin, 2010). Additionally, variations of metal concentrations based on seasons in bivalves are due to numerous factors such as considerable differences in water temperatures, the stream of particulate metals to coastal waters, food availability due to the transfer of metals from water to filter-feeding organisms, and changes in weight during gonadal development, and release of biomass related to sexual production (Szefer et al., 2004; Giarratano & Amin, 2010).

According to the study conducted by Bilgin and Ulutarhan in Homa Lagoon, *M. galloprovincialis* had Hg concentrations between 0.095 µg g\(^{-1}\) and 0.154 µg g\(^{-1}\) (dry weight) in soft tissues while having Cd concentrations between 0.22 µg g\(^{-1}\) and 0.51 µg g\(^{-1}\), Pb concentrations between 0.81 µg g\(^{-1}\) and 2.47 µg g\(^{-1}\), and Zn concentrations between 79.8 µg g\(^{-1}\) and 232 µg g\(^{-1}\). The digestive gland of *M. galloprovincialis* had Hg concentrations between 0.139 µg g\(^{-1}\) and 0.251 µg g\(^{-1}\) (dry weight), Cd concentrations between 0.29 µg g\(^{-1}\) and 0.48 µg g\(^{-1}\), Pb concentrations between 0.99 µg g\(^{-1}\) and 2.77 µg g\(^{-1}\), and Zn concentrations between 61.0 µg g\(^{-1}\) and 147 µg g\(^{-1}\). The maximum Hg concentrations of *M. galloprovincialis* were respectively discovered in February and May, which are the pre-spawning
periods. It was reported that due to the increased feeding and meat weight during gonadal development, higher accumulations of Hg and other chemicals occurred in the pre-spawning periods (Najdek & Sapunar, 1987; Vlahogianni et al., 2007).

In a study by Camilleri et al. (2020), the toxic mineral elements of M. galloprovincialis taken from different regions were investigated. The mean values of heavy metals obtained in this study were below the limits imposed by the EC Reg. 1881/2006, which suggested the absence of important risks in the sampling areas. In this study, the values obtained were also significantly lower than the values found by Maanan (2007) in mussel samples obtained from Morocco, which had mean concentrations of Cd, Pb, Hg, Cr, and Mn from 7 to 10 times higher than the values obtained in our study. Additionally, in the current study, the Cd, Pb, Hg, Cr, and Mn values were 3 to 10 times lower than the values reported by the study of Orescanin et al. (2006), which evaluated samples obtained from the Adriatic coasts of Croatia. The highest mercury concentrations were discovered in mussels samples from the Catania coast and these values could be associated with the Etna volcanic activity, which is considered to be one of the major natural sources of mercury release into the environment (Martin et al., 2012). These matters were also confirmed by the highest concentrations of mercury in samples of mussels, which were obtained from the eastern coast of Sicily (Messina, Milazzo, and Syracuse). In the current study, the mean mercury concentrations in all the evaluated tissues of M. galloprovincialis were calculated to be 0.012–0.072 mg kg\(^{-1}\). The tolerable mercury concentration level was determined to be 0.5 mg kg\(^{-1}\) by the Turkish Standards, FEPA, and FAO (EU, 2001; Anonymous, 2008; TFC, 2011; FAO, 1983). In the current study, the concentrations of mercury in the gills of mussels were calculated to be exceeding these legal limits. Mussels and other marine invertebrates are inclined to accumulate pollutants in their tissues (Odžak et al., 1994; Salvo et al., 2016) while not demonstrating any apparent harmful effects. Probably, this is because these invertebrates respond to the presence of high concentrations of xenobiotic substances. The results of this study indicated an overall absence of risks in the sampling areas in question. This indicated that there were reports of the presence of heavy metals in the M. galloprovincialis specimens on the Marmara coast and further studies are needed on the use of stress indicator markers to conduct a comprehensive assessment of the presence of inorganic pollutants around Istanbul.

**Conclusion**

This study provides valuable information regarding the trace metal concentrations in M. galloprovincialis obtained from the Marmara Sea, Istanbul-Turkey. It is known that M. galloprovincialis is commonly used for biomonitoring studies. In this study, M. galloprovincialis is a useful bioindicator for investigating metal pollution around seas. Due to the intensity of the population and industrialization around Istanbul, organic and inorganic pollutants strongly affect seawater. The concentrations of trace metals in both bivalves and predictions of international indices (PTWI) pointed out that metal accumulation for all the metals in the digestive gland was higher compared to those in gills and muscles. The reason for that was probably the metabolic activity of the aquatic creatures. Organs with higher metabolic activities, such as
the digestive gland and gills, accumulate higher metals compared to those with lower metabolic activities.

Considering these legal limits, the results of this study demonstrate that total arsenic concentrations in mussel muscle and digestive gland exceeded the permitted limit of arsenic. The other investigated metal levels in muscles were below the limits designated by WHO (1989) and FAO (1983). However, it was determined that the metal concentrations in the digestive gland were above the limits suggested by organizations such as FAO, WHO, and EU. Furthermore, to determine the health risks, investigating the heavy metals in various species of marine animals from these region seas will be beneficial.

Declarations

Funding: Finance of this research has been provided by the Firat University Scientific Research Project Council of Turkey (Project number: SYO.15.03).

Conflict of Interest: The authors declare that they have no conflict of interest.

Availability of data and material: All data generated or analyzed during this study are included in this published article.

Ethics approval: Ethical approval is not required.

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Figures
Figure 1

The diffusion region and the concentration area of *Mytilus galloprovincialis* in Turkey (Google Maps)

Note: The designations employed and the presentation of the material on this map do not imply the expression of any opinion whatsoever on the part of Research Square concerning the legal status of any country, territory, city or area or of its authorities, or concerning the delimitation of its frontiers or boundaries. This map has been provided by the authors.
Figure 2

The concentrations of heavy metals in M. galloprovincialis muscles (the results were given in mg kg$^{-1}$, n = 3)