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Evaluation of Heavy Metal Levels in Mediterranean mussels from Dardanelles Strait Ports, Türkiye

Rüştü Ilgar[1*], Selma Nur Avci[2], Serkan Yavuz[3]

[1]Çanakkale Onsekiz Mart University, Faculty of Education, Department of Geography Education, Campus of Anafartalar, 17100 Çanakkale, TURKİYE
[2]Ms Çanakkale Onsekiz Mart University, Institute of Graduate Studies- Department of Turkish and Social Sciences Education- Geography Graduate Education, Campus of Anafartalar, 17100 Çanakkale, TURKİYE
[3]Çanakkale Onsekiz Mart University, Faculty of Education, Department of Geography Education, Campus of Anafartalar, 17100 Çanakkale, TURKİYE
*Corresponding author: ilgar@comu.edu.tr

Abstract. This study was carried out to determine the heavy metal accumulation levels in the Dardanelles, to evaluate the usability of mussels *Mytilus galloprovincialis* (Lamarck, 1819) as a bioindicator and to reveal the potential effects of this pollution on the aquatic ecosystem. A total of 125 samples were collected from various ports in Çanakkale Strait and the concentrations of As, Cd, Cu, Fe Hg, Pb and Zn were analysed using ICP-MS technique. The results showed that arsenic (As) levels reached dangerous levels at all stations and the highest concentration was observed at Lapseki station (1.4 mg/kg dry weight), while all other heavy metals (Cu, Cd, Hg, Pb, and Zn) were below the limit values. Health risk assessments were based on Estimated Daily Intake (EDI), Temporary Tolerable Daily Intake (PTDI) and Target Hazard Quotient (THQ) calculations. The results showed that the THQ value for arsenic exceeded 1, posing the highest health risk, the total HI value was 1.687, and the other metals posed negligible health risks. Statistical analyses revealed that there was no significant difference in heavy metal accumulation between stations, but there were significant differences among heavy metals. *Mytilus galloprovincialis* was evaluated as an effective bioindicator for monitoring marine pollution in Çanakkale Strait and it was concluded that arsenic levels in particular should be monitored regularly.

Key words: *Mytilus galloprovincialis*, marine pollution, heavy metal, Dardanelles, biomonitoring.

Introduction

The Dardanelles is a strategically important waterway located in the Marmara Region of Turkey, connecting the Marmara Sea and the Aegean Sea. This waterway, which is approximately 61 kilometers long, starts from Gelibolu in the north and ends at Kumburnu in the south. In 2022, the Dardanelles was the waterway hosting the highest number of ship traffic in Turkey with 42,340 ship passages (Ministry of Transport and Infrastructure, 2022).

Wastes such as bilge water, sludge, waste oil, ballast water releases, oil and chemical spills left by ships cause significant environmental degradation in marine ecosystems (Walker et al., 2019). Aquatic ecosystems, which are highly sensitive to heavy metal accumulation (Crain et al., 2009; Halpern et al., 2008), are under ecological risk due to increasing concentrations of heavy metals, which are trace amounts in natural conditions, due to intensive marine traffic (Masindi & Muedi, 2018).

Due to their toxicity, cumulative and non-biodegradable nature, heavy metals are potentially hazardous to terrestrial and aquatic ecosystems and thus to human and animal life (Nagajyoti et al., 2010; Tchounwou et al., 2012). Humans, as an example of organisms that feed at the highest level, are more prone to serious health problems due to increased concentrations of heavy metals in the food chain (Lee et al., 2002).

The Dardanelles, a critical part of the ecosystems of the Marmara and Aegean Seas, is under intense pollution pressure due to various human activities. The main pollutants reaching the strait include heavy metals from pesticide and fertilizer use (Ministry of Environment, Urbanization and Climate Change, 2022d; Top & Tiryaki, 2022; Polat & Tiryaki, 2019), wastewater discharged from industrial facilities (Akbulut et al., 2006; Kelkit, 2003; Üstünada et al, 2011), domestic sewage wastes (Ministry of Environment, Urbanization and Climate Change, 2022d), pollution carried by rivers such as Sarıçay (Akbulut et al., 2010; Akarsu et al., 2022; Kaya et al., 2014) and multidirectional pollutants resulting from ship traffic and river transport (Güven & Ilgar, 2002).

These pollutants disrupt the chemical and biological balance of the strait ecosystem and threaten the ecological and economic sustainability of the region. Biomonitoring studies offer an alternative way to determine the geographical distribution of pollution and to monitor its effects. In short, biomonitoring is the use of organisms to obtain information about environmental quality (Yenisoy-Karakaş & Tuncel, 2004; Wolterbeek, 2002). In biomonitoring studies, biomonitors can be used to determine geographical and/or temporal changes in the bioavailability of heavy metals in the marine environment (Rainbow, 1995). Especially *Mytilus galloprovincialis* (Lamarck, 1819) is one of the most preferred biomonitors in toxicological studies (Maanan, 2007; Rayment & Barry, 2000; Rittschof & McClellan-Green, 2005; Uysal, 1970). Mussels are preferred as biomonitors due to their benthic lifestyle, wide geographical distribution, easy sampling and capacity to accumulate environmental pollutants (Chase et al., 2001; O'Connor, 1996; Vlahogianni et al., 2007; Widdows et al., 1995). Thanks to their suspension-feeding properties, mussels provide an effective tool for the assessment of heavy metal pollution by interacting with dissolved and suspended metals (Rainbow, 1995).

The Dardanelles Strait is an important source of food with its underwater richness, as well as a recreational area/strait with high tourism potential with its natural and historical beauties. Increasing anthropogenic activities in the last few decades have become a global environmental problem by significantly increasing heavy metal inputs in air, soil and water ecosystems as well as their concentrations in marine environments (Ansari et al., 2004; Charlesworth et al., 2011). In this context, the assessment of heavy metal concentrations in the coastal areas of Çanakkale Strait is of critical importance for monitoring ecosystem quality.

The main objective of this study was to determine the heavy metal accumulation levels in Çanakkale Strait and to evaluate the use of mussels *M. galloprovincialis* as a biomonitor of heavy metal pollution. The research aims to identify the pollution sources by analyzing the regional distribution of heavy metals accumulated in mussels and to reveal the potential effects of this pollution on the aquatic ecosystem. The data to be obtained in this direction is expected to provide a scientific basis for aquatic ecosystem management, development of sustainable environmental policies and combating pollution.

Materials and methods

This research is an experimental study based on the biomonitoring method. The study was carried out to evaluate heavy metal concentrations in Çanakkale Strait based on the bioaccumulation mechanism of *M. galloprovincialis* species. The findings obtained for this purpose were compared with previous studies conducted at the sampling points.

The universe of the study is the Dardanelles. The length of the strait is approximately 70 km. The maximum width is 8275 meters between Intepe coast and Domuz Creek (Ilgar, 2015). Between Çimenlik Castle and Kilitbahir Castle, which is the narrowest part of the strait, the width decreases to approximately 1300 meters and the Çanakkale city center is located at the narrowest point. At the narrowest point of the strait, the depth reaches the highest level. Here the depth reaches 104 meters, while the average depth of the strait is 55 meters. Surface currents in the strait can reach 6-7 knots.

Within the scope of the study, *M. galloprovincialis* samples obtained from the sampling area were used to collect data to represent the general characteristics of the population. The samples were obtained from five different stations on 12 December 2022, with 50 individuals collected from each station (Fig. 1 & Table 1).

The sample size in the study is limited to the environmental effects of mucilage and anthropogenic activities in the Marmara Sea. From the obtained samples, 25 individuals of homogeneous size were selected from each station to be used in the analyses. Data obtained from previous studies (Besada et al., 2011; Özden, 2013) were taken as reference in determining the number of samples to represent the *M. galloprovincialis* population.

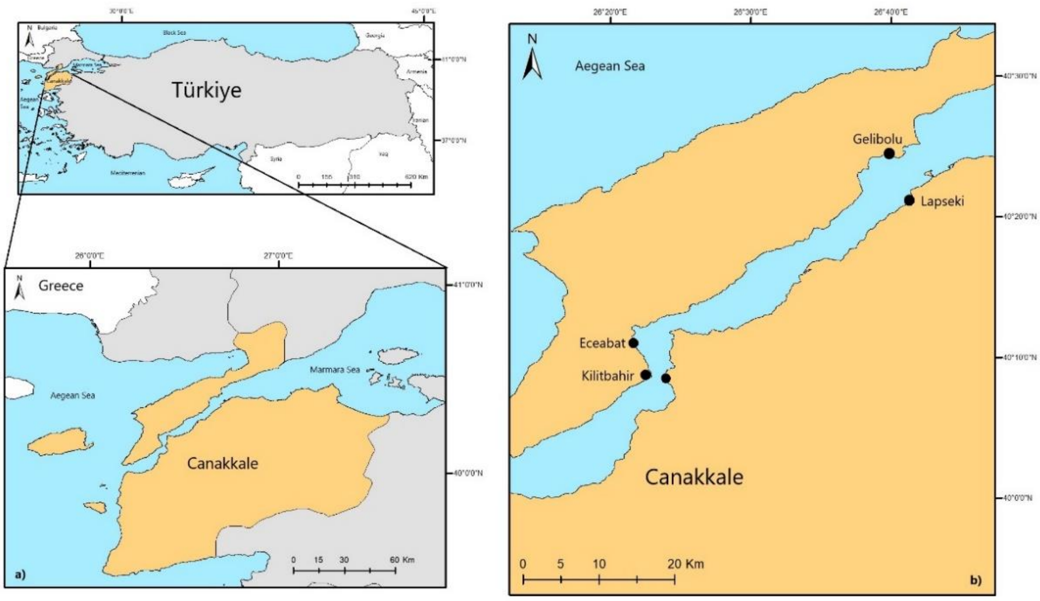

Fig. 1. Çanakkale province location (a), sampling area (b)

Table 1. Samples name, location, geographic coordinates of *M. galloprovincialis* sampled along the Dardanelles areas.

Code	Sampling Site	Geographic Coordinate
R1	Lapseki ferry pier	40.34765° N, 26.68374° E
R2	Fishing harbour near the Gallipoli pier	40.40512° N, 26.67108° E
R3	Fishing harbour near Eceabat pier	40.18527° N, 26.35961° E
R4	Canakkale ferry pier	40.15094° N, 26.40289° E
R5	Kilitbahir ferry pier	40.15342° N, 26.37884° E

The collected samples were placed in clean polythene bags and each station was marked with an acetate pen. Samples were stored at -25°C (Tüzen, 2003) to prevent deterioration until analysis. Since shell size and shape is an important criterion for species identification (Innes & Bates, 1999), individuals with similar length, width and weight were selected to minimize species differ-rences. The average length of the mussel samples used in the study was 4.4 cm, the average width was 3.1 cm and the average weight was 20.30 grams.

In the literature, it is stated that the optimum temperature and salinity conditions for the growth processes of mussels are in the range of 5-25°C temperature and 15-40‰ salinity (Lök, 2014; Besada et al., 2011). This study was carried out in the Marmara Sea and the average seawater temperature data for December was recorded as 13.7°C (Ministry of Environment, Urbanization and Climate Change, 2022a). Sea water salinity was measured as 22‰ at the southern end of the Bosphorus and 29‰ at the entrance of the Dardanelles (Öztürk, 2021). In addition, the pH value of the water was reported to vary between 7.8-8.4 (Ministry of Environment, Urbanization and Climate Change, 2022b). These physicochemical parameters indicate that the optimum conditions mentioned in the literature for the growth of mussels are provided. For the preparation of mussel samples, the shells were opened with the help of sterile pliers and the soft tissues were homogenized using a homogenizer. From the homogenized samples, 0.5±0.2 g were weighed in microwave digestion dishes and 8 mL of 65% HNO_3 and 2 mL of 30% H_2O_2 were added to each. The deconvolution process was carried out at 250 W power, 250 PSI pressure and 180°C temperature, after which the vessels were cooled to room temperature. The samples were filtered through ashless filter paper by opening the lids in a fume hood and transferred to 25 mL balloon jugs. A blind sample was prepared by the same procedure to check the method.

Heavy metal concentrations of the samples transferred to centrifuge tubes were analyzed using ICP-MS device (Agilent 7700). Within the scope of the analysis, the concentrations of As, Cd, Cu, Fe, Hg, Pb and Zn were determined. Heavy metal levels of these elements were expressed in mg/kg dry weight to ensure comparability of the results. The analyses were carried out by an accredited laboratory (Çanakkale Food Control Laboratory Directorate of the Ministry of Agriculture and Forestry).

The data obtained were analyzed to determine the enrichment levels and heavy metal differences between the stations. Data analyses were performed using Scikit-posthocs Python software. For the enrichment level analysis, Kruskal-Wallis test was applied to determine whether there were significant differences between the stations. The Kruskal-Wallis test was also used to determine the differences between heavy metals. Then, Post Hoc Dunn's test was applied to determine between which two groups there were significant differences. A measure of the central spread of the data was used to determine the center of the data distribution and how spread out the data points were. These measures include statistics such as mean, median, mode and quartiles. These measures were used to summarize and analyze the data.

Estimated Daily Intake (EDI), Target Hazard Quotient (THQ), and Hazard Index (HI) were calculated to evaluate heavy metal intake through mussel consumption. EDI is used to assess the potential risk of heavy metals to humans and is compared to specific guideline values for risk assessment (Katsikantami et al., 2019). THQ estimates the amount of heavy metal intake compared to a standard reference dose, while HI represents the sum of different THQs (Mok et al., 2014). However, one of the main challenges in risk assessment is determining the average mussel consumption of the local population. This is due to the lack of statistical data on the consumption of various seafood groups, except for specific studies (Nekhoroshkov et al., 2024). To address this issue, reference data from the literature were utilized. For instance, Stankovic et al. (2012) calculated the Tolerable Daily Intake (mg/person/day) for each element based on a weekly intake of 200 g/person (a single mussel meal) and used this amount as a reference for an adult weighing 70 kg. These calculations facilitate the evaluation of cumulative health risks using EDI, along with THQ and HI values. EDI is expressed by the following formula:

$$EDI = \frac{\textbf{\textit{Heavy metal concentration}} \times \textbf{\textit{Daily food consumption}}}{\textbf{\textit{average body weight}}}$$

The data obtained within the scope of the research were compared with the maximum acceptable limits determined by various international organizations for bivalve mollusks (Table 2).

Table 2. Maximum acceptable heavy metal limits in bivalve mollusks (mg/kg dry weight)

Organizations	As	Cd	Cu	Fe	Hg	Pb	Zn
FAO 1983	1	2	70	-	1	2.5	150
FAO 2003	-	1	-	-	0.5	1	-
EC 2006	-	1	-	-	0.5	1.5	-
TGK 2002	-	1	20	-	0.5	1.5	50
TGK 2011	-	1	-	-	0.5	1.5	-
SCA 2017	0.5	2	-	-	0.5	1.5	-
EC 2022	-	1	-	-	0.5	1.5	-

*FAO: Farmer and Agriculture Organization *EC: European Commission *TGK: Turkish Food Codex *SCA: Standardization Administration of China

Results and Discussion

The average values obtained from the samples collected simultaneously from the harbors of the settlements in Çanakkale Strait during the research period are presented in Table 3.

The results presented in Table 4 were derived by evaluating all data points from the datasets of the mentioned organizations based on their acceptable average values (mean), which were used as reference points (Table 2 & Table 3).

Table 3. *M. galloprovincialis* heavy metal concentration (mg/kg dry weight)

Station	As	Cd	Cu	Fe	Hg	Pb	Zn
R1	1.4	0.0013	0.947	11.043	0.3	0.06	11.043
R2	1.18	0.0006	0.47	10.263	0.225	0.04	10.263
R3	0.93	0.0007	0.77	12.541	0.229	0.04	12.541
R4	0.98	0.0012	0.6	12.171	0.28	0.09	12.171
R5	0.94	0.0003	0.942	11.471	0.221	0.02	11.471

Table 4. Central propagation measure of stations

Stations	R1	R2	R3	R4	R5
count	25.000	25.000	25.000	25.000	25.000
mean	0.541	0.383	0.3939	0.390	0.424
std	0.608	0.482	0.4289	0.401	0.479
min	0.001	0.0006	0.0007	0.001	0.000
25%	0.060	0.040	0.040	0.090	0.020
50%	0.300	0.225	0.229	0.280	0.221
75%	0.947	0.470	0.770	0.600	0.940
max	1.400	1.180	0.930	0.980	0.942

Central tendency measures such as mean, median, and quartiles indicate a similar dispersion across stations, except for R1, where elevated arsenic and copper levels increased the standard deviation. The Kruskal-Wallis test showed no significant differences between stations ($p \geq 0.05$). However, significant differences were observed for specific heavy metals ($p<0.05$). The Post Hoc Dunn's Test results in Table 5 show statistically significant differences ($p<0.05$) between heavy metals in mussels collected from the Dardanelles. Especially the differences bet-ween metal pairs such as As-Cd, Cd-Fe, Hg-Fe and Pb-Fe are remarkable.

Table 5. Post Hoc Dunn test results

	As	Cd	Hg	Pb	Cu	Fe
As	1.000	**0.0005**	0.072	**0.016**	0.541	0.297
Cd	**0.0005**	1.000	0.098	0.297	**0.004**	**0.000007**
Hg	0.072	0.098	1.000	0.541	0.235	**0.004**
Pb	**0.016**	0.297	0.541	1.000	0.072	**0.0005**
Cu	0.541	**0.0045**	0.235	0.072	1.000	0.098
Fe	0.297	**0.000007**	**0.004**	**0.0005**	0.098	1.000

In this study, risk assessment was carried out based on heavy metal (As, Cd, Cu, Hg, Pb, Zn) concentrations in mussels collected from Çanakkale Strait. The data obtained were analyzed using daily intake (EDI), tolerable daily intake (PTDI) and target hazard quotient (THQ).

In terms of human health, the results showed that the THQ value for arsenic (As) exceeded the PTDI limit and posed the highest health risk (Table 6). The THQ values of other metals (Hg, Cd, Pb, Cu, Zn) were below the limits and were found to have negligible health risks. The total HI value was calculated as 1.67. Based on the arsenic levels, it was calculated that an individual would need to consume approximately 140 grams of mussels per day to pose a health risk. However, reaching risk levels for other metals, such as Hg, requires much greater amounts of consumption. These results show the importance of strict monitoring and control of arsenic contamination.

Table 6. Risk Assessment Based on EDI, PTDI, THQ and HI (Mok et al., 2014)

Metal	Average Concentration (mg/kg)	EDI (µg/kg/day)	PTDI* (µg/kg/day)	THQ (Target Hazard Quotient)
As	1.086	0.443265	0.3	1.477551
Cd	0.00082	0.000335	0.83	0.000403
Cu	0.7458	0.304408	40.0	0.007610
Hg	0.251	0.102449	0.57	0.179735
Pb	0.05	0.020408	3.57	0.005717
Zn	12.1498	4.948041	300.0	0.016493

Statistical analyses indicate that the accumulation levels of metals can be influenced by different pollution sources and vary depending on environmental conditions. This can be interpreted as being related to environmental pollution sources, industrial effects or ecological factors in the region (Table 5). Mandal & Suzuki (2002) stated that pesticide use and mining activities cause arsenic formation. In the study, As levels were determined as 1.4 mg/kg and 1.1 mg/kg in Lapseki and Gelibolu stations, respectively, and these values exceeded the limit of 1 mg/kg set by FAO and 0.5 mg/kg by SCA. The levels in Çanakkale, Kilitbahir and Eceabat stations were below the limit value, but close to the limit value. The As levels obtained in this study are higher than the values reported in previous studies in and around Çanakkale Strait (Kayhan et al., 2006; Lök et al., 2010), indica-ting a significant increase in As accumulation over time.

Muntau & Baudo (1992) stated that cadmium (Cd) enters the aquatic environment through wastewater discharges due to Cd contained in fossil fuel combustion, fertilizer use, mining residues, solid wastes and zinc reserves. In the study, Cd levels in all sampling stations were found to be below the maximum acceptable limit values. This result indicates that Cd levels in mussels do not pose a threat in terms of coastal pollution. In addition, compared to Cd levels reported in other studies (Çayır et al., 2012; Egemen et al., 1997; İrkin et al., 2021; Karafistan & Ormancı, 2010; Lök et al., 2010), Cd levels were lower in all stations.

UNEP (2018) identified activities such as mining, coal and fossil fuel combustion, non-ferrous metal and cement production, and oil refining as anthropogenic sources of mercury (Hg). In the study, Hg levels at all sampling stations were found to be below acceptable limit values. However, levels close to the limit values were observed in Lâpseki and Çanakkale stations with 0.3 mg/kg and 0.28 mg/kg dry weight, respectively. This indicates that these areas have a potential risk for Hg pollution. Higher concentrations were obtained compared to Hg levels reported in previous studies (Lök et al., 2010).

Ilgar & Sarı (2008) indicated Mn, Pb, Zn pollution in same land. Özmert et al. (2003) stated that lead (Pb) originates from human activities and its main sources are paint, old water pipes and vehicle use. In the study, Pb concentrations were found to be well below the reference values in all stations. Çanakkale station had the highest Pb concentration compared to the other stations with 0.09 mg/kg dry weight, which was attributed to the intensity of shipping activities. The Pb concentration data obtained were lower in all stations compared to the values reported in previous studies (Çayır et al., 2012; Egemen et al., 1997; İrkin et al., 2021; Karafistan & Ormancı, 2010; Lök et al., 2010; Özden, 2015).

Liu et al. (2010) stated that copper (Cu) originates from mining, agriculture and industrial activities. In the study, Cu levels in all stations were found to be below acceptable limits, but the highest concentration was reached in Lapseki with 0.94 mg/kg. This value was higher than the levels reported by Çayır et al. (2008), Karafistan & Ormancı (2010) and İrkin et al. (2021), but showed a concentration above the value reported by Egemen et al. (1997) - 0.21 mg/kg.

Pertsemli & Voutsa (2007) stated that the main sources of zinc (Zn) pollution are municipal wastewater discharges, thermal power plants, metal production processes and atmospheric fallout. In this study, it was found that Zn levels in all sampling sites were below the permissible limit value (FAO, 1983; TGK 2002) and were similar among the stations. The Zn levels obtained were higher than the values reported by Lök et al. (2010), Karafistan & Ormancı (2010), Çayır et al. (2012) and İrkin et al. (2021), but lower than the values of 8.26 mg/kg reported for Çanakkale by Egemen et al. (1997), 11 mg/kg reported for Eceabat and 9 mg/kg reported for Kilitbahir by Özden (2015).

The average Fe concentration obtained was calculated as 11.498 mg/kg dry weight. This value was higher than previously reported Fe levels at Kilitbahir, Gelibolu and Eceabat stations (Özden, 2015). However, some Fe levels previously reported in Eceabat, Çanakkale and Kilitbahir stations were higher than the values in our study (Çayır, 2012).

In petroleum derivative transportation, some of the transported cargo remains as residue on the inner walls and bottom of the tanks. While 80% of tankers in the world discharge their ballast into waste tanks in refineries, 20% cause pollution by discharging it into the sea at the port (Aktoprak et al., 2015). Oil and its derivatives contain many heavy metals such as lead, cadmium, copper and iron (Acar, 2019; Alloway, 2013; Başgel, 2012; Hodgson, 1954). In Turkey, oil and its derivatives constitute 70% of ship-borne wastes with 416.37 m^3 per year (Ministry of Environment, Urbanization and Climate Change, 2022c).

In 2022, 25% of the ships passing through the Dardanelles were tankers carrying oil and derivatives. The fact that the strait is an important sea route increases the risks of marine pollution. Activities such as heavy maritime traffic, ship maintenance and repair, refueling and waste disposal lead to the accumulation of toxic elements. Filling operations in port areas, especially oil and leaks from ferries are among the possible sources of pollution in the region (Oran & Erginal, 2023).

There are many sources of anthropogenic heavy metal deposition in the Dardanelles. Çanakkale hosts industrial facilities such as iron and steel factories, fertilizer factories, liquid gas and coal transportation piers, as well as various production facilities along its coastline. Industrial wastes from these facilities and domestic wastewater from settlements are discharged directly into the Dardanelles (Akbulut et al., 2006; Kelkit, 2003; Üstünada et al., 2011). Most of the wastewater is directed to the Marmara Sea or its tributaries and the same points are also used for the discharge of recreational waters (Ministry of Environment, Urbanization and Climate Change, 2022d).

Another study is focused on Atikhisar Dam and Sarıçay River from Dardanelles. Sarıçay is affected by domestic, industrial and agricultural pollution and flows into the Dardanelles (Akbulut et al., 2010). Studies on Sarıçay and Atikhisar show that heavy metal pollution is present in these areas (Akarsu et al., 2022; Kaya et al., 2014). This situation brings to the agenda the negative effects of river-based pollution on the marine ecosystem.

The agricultural sector in Çanakkale province is also an important source of pollution. Intensive use of pesticides and fertilizers in the region causes heavy metals to enter soil and groundwater. Umurbey River has extremely heavy metal concentration (Ilgar, 2015). In 2021, it was reported that 896,673 kg and 692,897 liters of pesticides were used in agricultural control. Studies show that the pesticides and herbicides used are below acceptable limit values (Top & Tiryaki, 2022; Polat & Tiryaki, 2019). However, heavy metals such as cadmium, mercury, arsenic and lead in phosphate fertilizers reach the seas through soil and water and have negative impacts on the marine ecosystem (Yılmaz & Alagöz, 2008).

Conclusions

A study was carried out to assess heavy metal pollution in the harbors of the Dardanelles and to examine its potential effects on the aquatic ecosystem. Analyses according to FAO, EC, TGK and SCA standards showed that arsenic (As) concentrations reached hazardous levels in all stations, especially in Lapseki and Gelibolu stations. These findings emphasize the alarming level of arsenic pollution and the necessity of regular monitoring systems. Mercury (Hg) concentrations were found close to the limit values and increased compared to previous studies. Concentrations of other heavy metals (Cd, Cu, Pb, Zn,) were below the limit values, but Cu and Zn showed higher values in some stations compared to previous studies. Statistical analyses showed that there were no significant differences between stations, but there were significant differences between heavy metals. Health risk assessments showed that the THQ value for arsenic exceeded 1 and posed the highest health risk, the total HI value was 1.687 and the other metals posed low health risks. These results indicate that arsenic and mercury pollution should be addressed as a priority and that *Mytilus galloprovincialis* can be effectively used as bioindicator.

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