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HEAVY METALS RISK ASSESSMENT FOR CONSUMPTION OF WILD MEDITERRANEAN MUSSELS *MYTILUS GALLOPROVINCIALIS* LAMARCK, 1819 ALONG SAMSUN COASTS OF THE BLACK SEA

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ABSTRACT: The present study aimed to determine the concentration of metals in the soft tissue of wild mussels in coastal of Samsun and to assess human consumption. Using inductively coupled plasma mass spectrometry, the quantities of cadmium, mercury, lead, copper, iron, and zinc in *Mytilus galloprovincialis* were measured. The heavy metals found in Mediterranean mussels are arranged in the following ascending order: Cd < Hg < Pb < Cu < Fe < Zn. Concentrations of toxic metals were safe according to European Commission guidelines and the Ministry of Agriculture, Forestry and Fisheries. According to the estimated daily intake (EDI), eating Mediterranean mussels poses no risks. The target hazard quotients (THQs) in metals are also found <1, which implies no threat to consumers. In conclusion, the current study confirmed that the concentration of heavy metals in the Mediterranean mussels is safe for people intake in terms of their toxicity.

KEYWORDS: Estimated daily intake, target hazard quotients, risk index

INTRODUCTION

The Black Sea is a semi closed sea. Its maximum depth is 2212 m with mean depth 1300 m. When it comes to biodiversity, the Black Sea is the most significant sea (Bat *et al.*, 2011). Marine resources are of vital importance for the Black Sea coastal cities of Türkiye. Fishing, especially along the Black Sea coast of Türkiye, is extremely important (Bat *et al.*, 2013, 2018a).

Over the course of several decades, there have been concerns expressed by the scientific community, the political establishment, and the public regarding the degradation of Black Sea ecosystems due to the introduction of various pollutants into the coastal regions by uncontrolled human activities such as dredging, dumping, industrial emissions, domestic discharges, and agricultural waste (Bat *et al.*, 2009, 2018b). Coastal ecosystems can be impacted by a multitude of human-caused factors, such as the enormous amounts of environmental pollutants that are brought into coastal areas (Bakan and Büyükgüngör, 2000). The land-based pollutants enter the Black Sea through rivers (Bat *et al.*, 2009). As a result of natural causes and anthropogenic activities, pollutants transported from industrialized countries through large rivers are carried into the Black Sea in large proportions and reach high concentrations (Bat *et al.*, 2018b).

Among these pollutants, especially heavy metals persist in the marine environment for a long time, are non-biodegradable and accumulate in the tissues of living organisms, making them important. Metals repeatedly bond to the surfaces of particles after being dissolved in water columns and settle into the sediment (Bat and Özkan, 2019).Metals from the water, sediment and suspended particle elements can accumulate in the organs of marine organisms especially benthic and sessile ones like mussels. Biomagnification occurs when metals are transferred from microscopic marine organisms to small fish, which are then consumed by larger fish. Because they are in close contact with their environment and have accumulated more metals than the water and sediment, mussels are among the most useful bioindicators for heavy metal contamination of marine ecosystems. This is concerning for human health because eating seafood may be consuming contaminated water. These accumulations become even more important, especially for the seafood consumed. Mussels are known to be ideal indicator species (Bat *et al.*, 1999, 2012, 2018c,d,e, 2021; Bat and Öztekin, 2016)because they are sessile organisms, they cannot escape from pollutants, they filter water and take pollutants into their bodies. *M. galloprovincialis* is one of the most studied species as a biomonitoring organisms (Bat and Arici, 2018).In addition, the intensive consumption of mussels brings health risks to the forefront (Bat *et al.*, 2018c).

Revealing the metal pollution of the Black Sea is important because it provides insight into the health of the marine ecosystem, the impacts of human activities on the environment, and potential risks to human health.

Samsun is a large city between the two most important basins of Türkiye, Kızılırmak and Yeşilırmak rivers, and many streams flow to its shores. Although there has been treatment or disposal of domestic wastes in Samsun city recently, heavy metals reach the coasts because of many activities. With the increase in touristic and industrial activities in Samsun (Bakan and Böke Özkoç, 2007), accumulation of heavy metals from industrial discharges and other pollutants from agricultural activities have been detected in Samsun coasts (Üstün Odabaşı *et al.*, 2022).

Samsun is one of the most important fishing cities of the Black Sea and its coasts have an important place not only in fishing but also in mariculture. In addition to fish, mussels are also consumed by both local people and tourists in Samsun. To being one of the biggest cities in the Southern Black Sea, it has a high population density, as well as intensive agricultural, industrial and harbour activities. Heavy metals, especially Cd, Hg and Pb can accumulate in the food chain and have toxic effects on marine organisms, including shellfish that are consumed by humans.

Metal analysis is important in assessing the safety of mussels consumed in coastal cities, especially during periods of increased consumption by tourists. In Samsun, both tourists and local people eat mussels. This consumption is higher in July and August. In this context, in our study, the concentration of heavy metals in *M. galloprovincialis* collected from the coast of Samsun was determined and the health risk for consumers of the Mediterranean mussel was evaluated.

MATERIALS AND METHODS

Sample collection: The Mediterranean mussels (*M. galloprovincialis*) were collected in 2022 from Samsun coasts of the Black Sea (Fig. 1) and they were quickly moved to the laboratory using an ice box to be examined further. A total of 50 mussels were collected for each month. The average total length of *M. galloprovincialis* was 6.5±1 cm based on biometric measurements. Tap water was used to clean the specimen in order to get rid of surface adherents. After that, the mussels were kept in clean sea water for two days to empty the digestive system. After being taken out of their shells, they were put in zip-lock nylon bags and frozen at -21 °C till their metal testing.

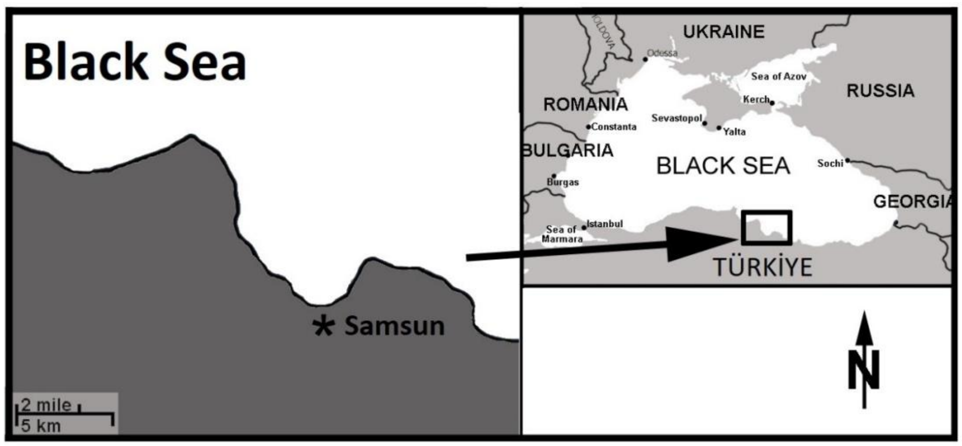

Fig. 1. Samsun coasts.

The Mediterranean mussel's analysis: To obtain three homogeneous samples of each specimen for metal analysis, a portion of edible tissue of the Mediterranean mussels was taken. Then they were washed with nitric acid(HNO_3) and rinsed in ionized water. For metal analysis, the samples were treated using Suprapur® HNO_3 in the milestone microwave elimination system, Start D 260. To confirm the accuracy and precision of the procedure, certified reference material samples and blanks underwent comparable processing in parallel. Using the Standard Reference Material (SRM) 2976 from the National Institute of Standards and Technology (NIST), the accuracy of the analytical method in the current study was assessed. The results agreed between the certified and the analytical values, and the difference did not exceed 10%.An Inductively Coupled Plasma – Mass Spectrometer (ICP-MS) has been employed to analyze the samples. The method's sensitivity was assessed based on the spectrometer's set limit of detection (LOD), which was less than 0.001 µg/L for Fe, Pb, and Cd, less than 0.01 µg/L for Zn and Hg, and less than 0.0001 µg/L for Cu. The limit of quantification (LOQ) was 3.1×LOD.

All of the plastic containers and glassware used in the analysis were carefully cleaned with tap water, left overnight in a solution of nitric acid, rinsed with deionized water, and dried before being used to avoid contamination. The results of the triplicate analyses of each sample were expressed as milligrams per kilogram of wet weight (wt.).

Evaluation of health risks

Estimated daily intake (EDI): The formula below was used to calculate the EDI.

$$\mathrm{EDI} = \frac{\mathrm{C} \times \mathrm{FIR}}{\mathrm{BW}}$$

Where C is the concentration of heavy metal in the Mediterranean mussels (mg/kg wet weight), FIR is the food ingestion rate (g/day) and BW is the mean body weight. FAO (2010) reported that the average mollusk consumption in Türkiye is 1 g per day. Republic of Türkiye Ministry of Agriculture and Forestry, Directorate General of Fisheries and Aquaculture stated that the daily consumption of seafood including fish is 18.36 g/person/day (BSGM, 2023). However, seafood consumption is higher especially in coastal cities of Türkiye. Therefore, in this study, two groups, 30 kg children and 70 kg adults, were considered and 0.027, and 0.041 kg consumption per day, respectively (UNSCEAR, 2010).

Non-carcinogenic risk to health: The target hazard quotient (THQ) was used to assess the noncarcinogenic risk associated with eating Mediterranean mussels and the heavy metals they relate to:

$$\mathrm{THQ} = \frac{\mathrm{EDI}}{\mathrm{RfD}}$$

RfD values are Cd (0.0001), Hg (0.0003), Cu (0.04), Zn (0.3) and Fe (0.7) mg/kg/day (RAIS, 2023; USEPA, 2023).

The TTHQ calculates the cumulative risk of several heavy metal exposures.

$$\mathrm{TTHQ} = \sum_{\mathrm{i}=1}^{\mathrm{n}} \mathrm{THQ_i}$$

There is no lifetime risk to one's health if the TTHQ is less than 1. There may be a health danger if the THQ is higher than 1.

Carcinogenic Risk Index: For Pb, there is no Rf.D. value. Conversely, oral slope factor (SF) is limited to lead and compounds and is expressed as 0.0085 mg/kg-day (RAIS, 2023; USEPA, 2023). The risk index (RI) was calculated using the following formula:

$$\mathrm{RI} = \mathrm{EDI} \times \mathrm{SF}$$

If the RI is less than 10^{-6}, it is deemed inconsequential; if the RI is between $10^{-6}$and 10^{-4}, it is acceptable or bearable; and if the RI is greater than 10^{-4}, it is deemed significant.

RESULTS AND DISCUSSION

The coasts of the Black Sea are susceptible to a range of environmental contaminants, including heavy metals, organic and oil compounds, pesticides, and herbicides, that are released by both natural and man-made sources. Heavy metals are one of the most important human-caused sources because of their toxicity, ability to linger in the environment, ability to contaminate food chains, and range of health risks (Bat *et al.*, 2018b).Figure 2 displays the accumulation of Cd, Hg, Pb, Cu, Fe and Zn in the

Mediterranean mussels from Samsun coasts of the Black Sea. The increasing order of heavy metals in the Mediterranean mussels is as follows: Cd < Hg < Pb < Cu < Fe < Zn. The Zn level was notably higher in *M. galloprovincialis* when compared to other metals. Cd was significantly low concentration followed by Hg and Pb. Cd, Hg and Pb are known toxic heavy metals to people via consumption polluted seafood especially mussels (Bat and Arici, 2018).However, the values of the same metals measured in July and August were not statistically different (P>0.05).

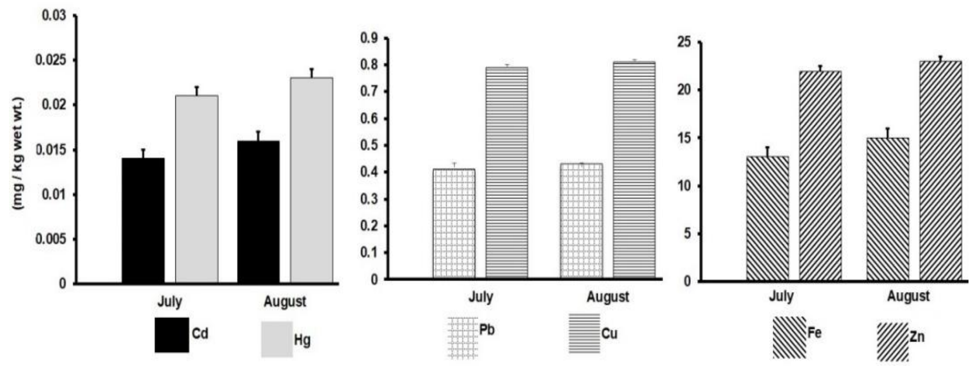

Fig. 2. The mean heavy metal concentrations in the edible tissues of *M. Galloprovincialis* from Samsun coasts of the Black Sea.

In the present study, the heavy metal values measured in the Mediterranean mussels were found to be quite low compared to the values permitted by international institutions and organisations for consumed molluscs. These values are 1, 0.5, 1.5(Commission Regulation, 2006), 20 and 50 (MAFF, 1995) mg/kg wet weight for Cd, Hg, Pb, Cu and Zn, respectively.

The EDIs for all heavy metals in two groups consuming the Mediterranean mussels from Samsun coasts of the Black Sea for this study and is displayed in Table 1.In light of the results, the EDI rate for Cd, Hg, Pb, Cu, Fe and Zn in the Mediterranean mussels are lower than the Rf. D. in both children and adult. Table 1 also presents the THQs and TTHQ of Cd, Hg, Cu, Fe and Zn in the Mediterranean mussels for children and adults' consumers. Metals in Mediterranean mussels are available because of the presence of multiple industries along the Black Sea coast of Samsun, as well as the discharge of industrial and urban wastewater that contains a variety of metals and raises the concentration of those metals in this area. However, the Mediterranean mussel's consumption has no risks to consumers, according to the outputs of the current study into THQs levels in both children and adults. The TTHQ values for the metals in July and August are discovered to be lower than 1 in the Mediterranean mussels from Samsun coasts of the Black Sea, which means there are no negative effects for the consumers. Additionally, it is shown that there is an acceptable or bearable risk of carcinogenesis associated with the amount of CR in metal exposure (Table 1). Therefore, the consumption of the Mediterranean mussels containing studied heavy metals would not cause any health risks to people.

Table 1. The EDIs for all heavy metals, THQs and TTHQ for Cd, Hg, Cu, Zn and Fe, and carcinogenic risk index (RI) for Pb in two groups consuming the Mediterranean musselsfrom Samsun coasts of the Black Sea.

Metal	Months	EDIs		THQs		RI	
		children	adults	children	adults	children	adults
Cd	July	$1.3x10^{-5}$	$8.2x10^{-6}$	$1.3x10^{-2}$	$8.2x10^{-3}$		
	August	$1.4x10^{-5}$	$9.4x10^{-6}$	$1.4x10^{-2}$	$9.4x10^{-3}$		
Hg	July	$1.9x10^{-5}$	$1.2x10^{-5}$	$6.3x10^{-2}$	$4.1x10^{-2}$		
	August	$2.1x10^{-5}$	$1.3x10^{-5}$	$6.9x10^{-2}$	$4.5x10^{-2}$		
Pb	July	$3.7x10^{-4}$	$2.4x10^{-4}$			$3.14x10^{-6}$	$2.04x10^{-6}$
	August	$3.9x10^{-4}$	$2.5x10^{-4}$			$3.29x10^{-6}$	$2.14x10^{-6}$
Cu	July	$7.1x10^{-4}$	$4.6x10^{-4}$	$1.8x10^{-2}$	$1.16x10^{-2}$		
	August	$7.3x10^{-4}$	$4.7x10^{-4}$	$1.8x10^{-2}$	$1.19x10^{-2}$		
Zn	July	$2.0x10^{-2}$	$1.3x10^{-2}$	$6.6x10^{-2}$	$4.3x10^{-2}$		
	August	$2.1x10^{-2}$	$1.3x10^{-2}$	$6.9x10^{-2}$	$4.5x10^{-2}$		
Fe	July	$1.2x10^{-2}$	$7.6x10^{-3}$	$1.7x10^{-2}$	$1.1x10^{-2}$		
	August	$1.4x10^{-2}$	$8.8x10^{-3}$	$1.9x10^{-2}$	$1.3x10^{-2}$		
TTHQ	July			$1.7x10^{-1}$	$1.15x10^{-1}$		
	August			$1.9x10^{-1}$	$1.24x10^{-1}$		

Table 2 lists the previous studies in which heavy metal levels were determined in mussels in the Black Sea according to years. As can be seen, studies have increased since the 1990s and are continuing. Mussels are immobile, which means that they are likely to be affected by local conditions compared to other organisms. This makes them particularly useful for assessing long-term trends in environmental pollution and good bioindicator species. Table 2 shows us that metal accumulations in mussels vary according to different times and regions. It is understood from the studies that the metal values detected in mussels show fluctuations. Since metals are detected at high values in some regions, it is recommended to know where they are collected before consumption and not to eat too much. Toxic metals can be hazard even if consumed at very low levels. However, essential metals can be harmful to human health if consumed in large quantities, causing health problems ranging from gastrointestinal discomfort to serious neurological damage. Therefore, it is important to regularly study mussels for metal contamination and ensure that they meet regulatory safety standards before they are consumed.

Table 2. Comparison of heavy metals in *Mytilus galloprovincilis* with values got from the literature as mg/kg wet wt. (*dry wt.).

Area	Cd	Hg	Pb	Cu	Zn	Fe	REFERENCES
Sinop	0.075-0.86		0.32-3.65	0.075-0.86	0.82-8.95		Öztürk 1991
Southwestern Black Sea*				15.88	184	228	Andreev and Simeonov, 1992
Southeastern Black Sea			0.05-2.94	0.29-6.03			Ünsal *et al.*,1992
Bulgaria*			3.6	2.41	12	149	Andreev *et al.*, 1994
Trabzon*			0.48-0.63	1.89-8.94	10.90-18.33		Boran and Karacam, 1997
Romania	10.03-30.76		4.44-9.36	16.66-22.45		118.43-176.4	Bologa *et al.*, 1998
Black Sea	0.33-1.05		5-10.5	1.2-1.9			Ünsal *et al.*, 1998
Sinop			0.11-1.18	0.10-1.89	1.58-7.28		Bat *et al.*, 1999
Romania	0.087-0.82		0.005-0.32	1.49-4.69			Oros *et al.*, 2002
Black Sea*			<0.05	7.21-11.52	78.12-256.4	151-598	Topcuoglu *et al.*, 2002
Romania	1.5-3.3		1.2-11.02	3.6-6.5	35.24	38.78	Oros *et al.*, 2003
Western Black Sea	0.05-0.08	0.07-0.09	0.04-0.08				Altug and Güler, 2004
Bulgaria*	0.005-0.008		0.0452-0.0591				Gorinstein*et al.*, 2005
Romania	0.96-1.74	0.026-0.033		6.64-8.34	108-190	95-106	Roméo *et al.*, 2005
Romania	1.71		8.95	5.88-9.87			Stanciu *et al.*, 2005
Crimean		0.003-0.083					Egorov *et al.*, 2006
Bulgaria*				12.9-24.2	112-143		Gorinstein *et al.*, 2006
Romania				12.38-20.69			Petisleam *et al.*, 2006
Sinop*	0.305-4.878			4.301-10.96	24.86-519.7		Turk Culha *et al.*, 2007
Eastern Black Sea*	0.6-6.2		0.59-4.59	0.60-3.62	1.204-680	1.140-870	Cevik *et al.*, 2008

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Area	Cd	Hg	Pb	Cu	Zn	Fe	REFERENCES
Romania	0.11-0.98		0.16-0.55	1.46-8.61			INCDM, 2008
Sinop and Samsun*	0.41-0.49	<0.05	1.09-1.87				Das *et al.*, 2009
Romania	0.32-0.83		0.27-0.75	1.49-2.88			INCDM, 2009
Romania	0.08-2.58		0.01-3.44	0.90-4.67	8.29-44.13		Oros *et al.*, 2009
Romania	0.32		1.73	2.09			INCDM, 2010
Romania	0.08-2.58		0.01-3.44	0.90-4.67			Oros and Gomoiu, 2010
Sinop*	0.27-0.98		2.10-4.10	2.41-4.82	79-163		Bat *et al.*, 2012
Romania	0.1-4.69		0.01-11.02	0.10-10.77			Oros and Gomoiu, 2012
Bulgaria	0.044-0.090	0.08-0.32	0.11-0.18				Stancheva *et al.*, 2012
Bulgaria	0.98-2.24				104.4-239.2		Simeonova *et al.*, 2013
Romania	0.13		3.07	8.83	124	730	Demina and Budko, 2014
Romania	0.20-0.42		0.07-1.36	2.71-4.60			Jitar *et al.*, 2015
Black Sea	<0.02-0.11	<0.05	<0.05-0.45	<0.5-2.1	5-46	16-55	Arıcı and Bat, 2016
Black Sea			0.7	0.8	29.3		Bat and Öztekin, 2016
Samsun	0.08		0.541	9	16	64	Bat *et al.*, 2016
Black Sea	0.23-1.94		0.51-2.71	8.2-80.1	16-163	38-289	Belivermis *et al.*, 2016
Romania	0.67-0.73		0.33-0.88	1.36-2.49			Catherine *et al.*, 2016
Romania	1.85-2.15		0.01-0.27	27.49-29.57			Rosioru *et al.*, 2016
Giresun			3.16	2.65	69.06	161.08	Tepe and Süer 2016
Western Black Sea	0.67		0.104	2.04			Coatu*et al.*, 2016

Continued………

Area	Cd	Hg	Pb	Cu	Zn	Fe	REFERENCES
Eastern Black Sea*			1.92-17.92	12.69-65.41	134.14-174.25		Baltas *et al*., 2017
Tekirdağ*	<0.1-0.75	<0.1-<0.33	2.67-9.2	1.55-3.5	77.12-88.98		Dökmeci, 2017
Romania-Ukraine cross border		0.07-0.23	0.07-0.16	0.57-1.31			Strungaru *et al*., 2017
Russia*	1.9-3.4	<0.05	0.39-0.53	4.5-4.7	106-196	57-206	Temerdashev *et al*., 2017
Sinop	0.03	0.02	0.08	1.12			Bat *et al*., 2017
Sinop	0.04-0.10	0.03-0.07	0.06-0.31	0.5-1.8	8-27	18-35	Bat *et al*., 2018c
Sinop	0.03	0.02	0.08				Bat *et al*., 2018d
İğneada	0.07-0.11	0.03-0.06	0.14-0.21				Bat *et al*., 2018e
Black Sea*	0.097	0.405	0.375				Kupluluet *al*., 2018
Sinop	0.05-0.08	0.007-0.10	0.15-0.23				Bat, 2019
Black Sea*	0.005-0.640	0-0.121	0-0.332	0.86-7.70	7.5-38.2	4.2-112.9	Manev *et al*., 2020
Sinop	0.006-0.011	0.015-0.019	0.13-0.45	0.52-0.93	16.4-21.8		Bat *et al*., 2021
Romania*	1.69-5.37	0.085-0.114					Bucşe*et al*., 2021
Crimean*	<0.05-7.7	0.06-2.2	0.16-4.8	0.67-14.9	12.5-636	48-305	Kapranov *et al*., 2021
Bulgaria	0.14-0.64		0.03-0.19				Peycheva *et al*., 2021
Romania*		0.02		11.19	179.53		Bucşe *et al*., 2022
Romania	0.143-2.915		0.010-0.373	0.930-12.86			Damir *et al*., 2022

CONCLUSION

The unique characteristics of the Black Sea make it an important and fascinating ecosystem, but also make it vulnerable to environmental threats. Revealing the metal pollution of the Black Sea is important because it provides insight into the health of the marine ecosystem, the impacts of human activities on the environment, and potential risks to human health. Heavy metals, such as Cd, Hg and Pb, can accumulate in the food chain and have toxic effects on marine organisms, including shellfish that are consumed by humans. Additionally, metal pollution can lead to long-term environmental degradation, such as the destruction of habitats and loss of biodiversity. Metal analysis is important in assessing the safety of mussels consumed in coastal cities like Samsun, especially during periods of increased consumption by tourists. Mussels are filter feeders, which mean that they accumulate heavy metals from the water they live in. This makes them effective indicators of environmental pollution and a useful tool for monitoring the health of marine ecosystems. In general, regulatory agencies set limits on metal levels in mussels based on the tolerable intake of these metals for humans, which is the amount that can be safely consumed over a lifetime without causing harm to health. These limits are based on extensive scientific studies and consider factors such as body weight and any possible dangers connected to each metal. In this study, the amounts of heavy metals measured in mussels were below the international permissible limits. The fact that TTHQ was below 1 did not create a health problem for consumers in terms of health.

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