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Evaluation of biotoxins and toxic metal risks in mussels from the Sea of Marmara following marine mucilage

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A R T I C L E I N F O

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A B S T R A C T

The mucilage phenomenon observed in the Sea of Marmara in 2021, has raised public concern about seafood safety. Mediterranean mussels serve as a vehicle in food chain, enabling the transfer of pollutants. Farmed and wild mussels were collected from 4 different stations throughout the fishing season. Biotoxins causing amnesic, paralytic, or diarrhetic shellfish poisonings (ASP, PSP, or DSP) were examined during monthly samplings. Potential health risks posed by cadmium, lead and arsenic were assessed. Health risks were evaluated considering 150 g/week mussel consumption, accounting for the different age groups of consumers (50, 60, 70 kg). Estimated Weekly Intake calculations of metals were determined to be lower than Provisional Tolerable Weekly Intake at all age groups throughout the sampling period in all stations. Target Hazard Quotient$_{Cd}$ of mussels captured from Istanbul Strait was always determined <1, while it was equal to 1 for 50 kg individuals in Gelibolu samples. All THQ$_{As}$ were >1. Target carcinogenic Risk was evaluated for Pb and iAs, which were found to be negligible and acceptable, respectively. No biotoxins responsible for ASP, PSP, or DSP were detected. Hg levels were under detectable limits. Excluding Cd, the results did not reveal any risks associated with mussel consumption during mucilage.

1. Introduction

Food safety is a branch of science that focuses on preventing the risks that threaten health and on warning and informing the consumer. Chemical residues and contaminants are the substances that contaminate food or form in the structure of food as environmental pollution factors and cause acute or chronic health hazards in humans, depending on their chemical structure and exposure status. The widespread presence of toxic chemicals in the environment, their long biological half-life, and their stability and bioaccumulative properties play an important role in people's exposure to foods contaminated with chemicals (Thompson and Darwish, 2019). Most of the chemical residues and contaminants in seafood are considered parameters to be monitored due to their negative effects on health. Toxic metals and algal toxins are among the main residues and contaminants that contaminate seafood products both from environmental sources and due to nutritional and physiological conditions (Farabegoli et al., 2018; Kouali et al., 2020).

The dominant power of anthropological effects in environmental pollution is incontrovertible. Furthermore, intense environmental pollution poses risks in terms of food safety and threatens public health. Marine foods suffer the most from these threats. Marine mucilage, a large-scale environmental disaster, occurred in the Sea of Marmara in 2021 due to the pressure from domestic, industrial, and agricultural resources as well as maritime activities augmented by intense anthropologic pollution. Although many different reasons come to the fore in the formation of marine mucilage, three main factors are taken as the basis (Şeker and Yalçın, 2021). These factors are listed as the sea rising temperatures above annual mean levels, the increasing rate of pollution in the seas, and the stagnation of the sea (Keleş et al., 2020; Tuzcu Kokal et al., 2022). It is known that if the three of these situations occur,

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marine mucilage increases as some plankton species begin to multiply rapidly. While scientific studies focus on the formation mechanism, the composition, and the algae that cause mucilage, there is very little information on the safety of seafood products caught from the seas where mucilage densely occurred.

Mediterranean mussel (*Mytilus galloprovincialis* Lamarck, 1819) is a bivalve species that can thrive in clean waters as well as in heavily polluted seas and is frequently preferred for consumption (Kouali et al., 2020). There are rich mussel beds in the Sea of Marmara, especially in the Turkish straits region (Çinar et al., 2020). Southern Marmara Sea is especially suitable for cultivation of mussels and there are many aquaculture farms in this region (Çolakoğlu et al., 2019). These species, which play an important role in determining the structure and composition of pollution in the seas, are considered biological indicators and model organisms (Kouali et al., 2020). Assessing toxic metals and algae toxins accumulation in wild and cultured mussels captured from the Sea of Marmara will provide important data to determine the environmental pollution extent of mucilage and its impact on seafood safety. The presence of biotoxins was investigated utilizing quick test kits, whereas the toxic metals were detected using instrumental analyses. The potential health risks posed by biotoxins and toxic metals associated with the incidence of mucilage were evaluated considering different age groups of consumers. In order to ascertain whether consuming mussels constitutes a potential hazard to food safety, it is essential to carry out a thorough evaluation of health risks, even when the concentrations of pollutants remain within the thresholds prescribed by regulatory standards.

2. Material and methods

2.1. Sample preparation

Wild mussels were captured in accordance with the "Regulation on Commercial Fishing in the Seas and Inland Waters" implemented by the Ministry of Agriculture of the Republic of Türkiye. Samples were collected from two stations: Istanbul and Çanakkale Straits. Cultured mussels were obtained from two mussel production farms in Bandirma and Gelibolu (Fig. 1). Monthly sampling was conducted from four stations throughout the fishing season from September 2021 to May 2022. Sampling from the farm in Gelibolu continued in June and July 2022. Three bags of mussels (1 kg/bag) were transported to the laboratory in polyurethane (PU) boxes on ice. In each monthly sampling, randomly chosen 33 individual mussels were utilized from four stations. The total length and weight of the shells as well as the weight of mussel meat from each sampled mussel were measured according to their respective stations. The seasonal average measurements of mussels are presented in Table 1. Mussel meat tissues were dissected using a stainless-steel knife and homogenized with a tissue blender (Retsch, GM 200, Germany). Each sample from the respective stations was packed in zip-lock polythene bags and stored at −20 °C until analysis.

2.2. Biotoxin analysis

Mussel samples were analyzed using rapid-resulting toxin kits to investigate the presence of toxins that cause Amnesic, Diarrhetic, or Paralytic Shellfish Poisoning. Qualitative analyzes of toxins were carried out with the Neogen Raptor Solo 9696 device using Reveal® 2.0 rapid test kits. The Neogen kit was reported to be the most appropriate test kit for the determination of PSP toxins among three different manufacturers in terms of sensitivity, performance, and speed (Dorantes-Aranda et al., 2017).

Toxin determinations were performed according to the manufacturer's standard protocol. Only the solutions, consumables, and equipment supplied with the kits were used. DSP-toxin analysis was conducted according to the method described by Jawaid et al. (2015a). Briefly, the test procedure for the DSP analysis (lot no: 9561-56) was as follows: 2 g (±0.1 g) of homogenized mussel sample was mixed with 8 mL of analytical-grade methanol in a plastic extraction bag containing a mesh filter. After homogenization manually with a roller for the 30 s, the filtrate was taken to a sample cup. Eight hundred μL of the filtrate was transferred into a glass vial. NaOH (100 μL, 5 M) was added, shaken for 30 s, and the vial was put into a heater set to 76 °C for 40 min. Then HCl (100 μL) was added and shaken as well. This solution (100 μL) was

Table 1
Length, shelled weight and mussel meat weight parameters of mussels obtained from Istanbul and Çanakkale Straits or farms from Bandirma and Gelibolu stations.

Seasonally mean ± SD	Istanbul St. (Wild)	Çanakkale St. (Wild)	Bandirma (Farmed)	Gelibolu (Farmed)
Length (cm)	6.18 ± 0.50	6.07 ± 0.53	5.82 ± 0.27	6.27 ± 0.21
Weight (g)	28.93 ± 5.76	23.23 ± 5.59	15.26 ± 0.79	18.86 ± 1.84
Mussel meat weight (g)	11.57 ± 3.00	8.76 ± 0.89	6.26 ± 0.29	7.83 ± 0.32

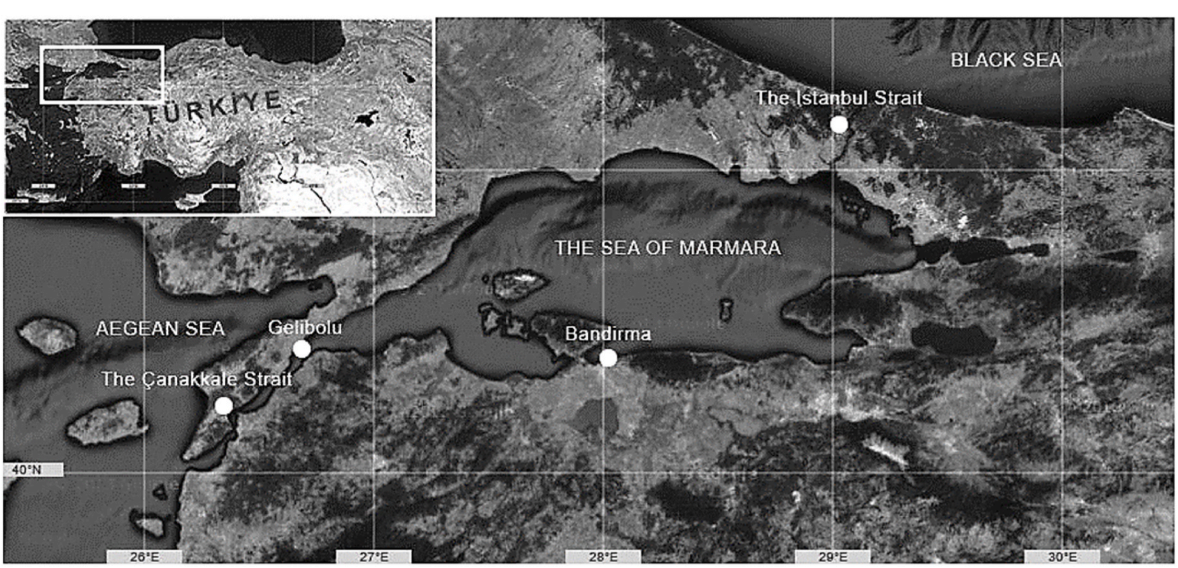

Fig. 1. Sampling stations from the Sea of Marmara: wild mussels from Istanbul and Çanakkale Straits, and cultured mussels from farms Gelibolu and Bandirma.

transferred into buffers A and B, respectively. The assay strip was placed into a Raptor cartridge. The cartridge containing the test strip was inserted into the port within the Raptor device. Then the diluted sample (400 μL) was immediately transferred into the cartridge. The strip was read automatically. DSP results were displayed on the device's screen after the 15 min testing.

Analysis of toxins causing ASP and PSP was carried out using the methods detailed by Jawaid et al. (2013, 2015b), respectively. For the determination of ASP (lot no: 9560-29) and PSP (lot no: 9562-44) toxins, 1 g (±0.05 g) of mussel sample at room temperature was homogenized with 30 mL of purified water manually in an extraction bag containing a mesh filter. The filtrate was poured into a sample cup. One hundred μL sample extracts were transferred into ASP or PSP buffers and shaken for 30 s. Assay strips were placed into one Raptor cartridge at a time. The same protocol was used as above. ASP and PSP results were displayed on the device's screen after the 5 or 10 min testing, respectively. Depending on the density of the test band appearing on the strip, a positive or negative result was expected to be screened. Samples containing the maximum permitted or higher amount of toxin were expected to yield a positive result.

2.3. Toxic metal analysis

For each mussel sample, approximately 0.5 g were weighed into a Teflon digestion vessel. Subsequently, 5 mL of 65% nitric acid (HNO_3) and 3 mL of 30% hydrogen peroxide (H_2O_2) were added. The digestion process occurred in a microwave digestion system (Milestone SK10) for 45 min at 180 °C. After digestion, the samples were cooled, filtered, and diluted to a final volume of 50 mL with ultra-pure water. A blank digest was carried out using the same procedure. The dissolved samples were adjusted to a final volume of 50 mL with deionized water. Toxic metal analyses (Hg, Pb, Cd, and As) of the samples were conducted using an ICP-MS instrument (Model 7700x, Agilent Technologies, USA) following an in-house method, EN15763 DMS19236_12, which was a modification of AOAC 999.10. Calibration curves were established using Multielement Calibration Standard solutions (Agilent Technologies, USA) at a concentration of 10 μg/mL. Prior to the analysis, standard solutions for ICP-MS calibration were freshly prepared using high-purity deionized water. The accuracy and precision of the analytical method were evaluated using certified reference material (ERM-CE278k, Geel, Belgium), specifically, powdered mussel tissue. All analysis results are reported based on wet weight (ww), and the relative standard deviation (RSD) was found to be within the 5.0% limit. Accuracy was further ensured by subjecting a certified reference material to analysis (n = 3). The method demonstrated satisfactory performance, with recoveries ranging from 92 to 99%.

2.4. Health risk assessment

The weight scale of certain age groups was established by US EPA (2011) as 51 kg for 11-14 year-old adolescents, 67 kg for 15-19 year-old adolescents, 72 kg for 20-24 year-old adults, 77 kg for 25-64 year-old adults, and 72 kg for >65 year-old seniors. EFSA (2012a) also uses body weight statistics of specific age categories, namely 43.4 kg for 10-14 year-old adolescents, 61.3 kg for 14-18 year-old adolescents, 73.9 kg for 18-64 year-old adults, 76 kg for 65-75 year-old adults, and 71.2 kg for >75 year-old seniors. It is known that genetic, environmental and growth factors, and cultural nutrition patterns affect the body weight of the consumers. Therefore, all calculations were made considering an average body weight of the age groups (50, 60 or 70 kg), depending on the age of mussel consumption in the general population. Potential health risks of Pb, Cd, and As were evaluated in mussel samples. Since the inorganic arsenic (iAs) is the most toxic form, 10% of the total As was regarded as inorganic in the samples (Qin et al., 2015).

Mussel consumption was estimated as 150 g of mussel meat per week (Alves et al., 2018). The estimated weekly intake (EWI), the provisional tolerable weekly intake (PTWI), the toxic metal target hazard quotient (THQ), and the target carcinogenic risk (TR) were assessed according to Table 2. Considering the PTWI (μg/kg BW) values, estimation was only conducted for Cd which was set at 2.5 μg/kg BW by EFSA (2012b). The percent PTWI (%PTWI) was also calculated for comparison with the EWI results. According to the Joint FAO/WHO Expert Committee on Food Additives (JECFA), the PTWI values of 15.0 μg/kg for As (EFSA, 2009) and 25.0 μg/kg for Pb (EFSA, 2010) were no longer appropriate for health risk assessments, due to the carcinogenic potential of As and the high toxicity of Pb.

In addition to the explanations given in Table 2, the oral reference dose (RfD) for Cd, and iAs were 1×10^{-4}, and 3×10^{-4}, respectively (US EPA, 2023a). Due to the adverse health effects that may occur even at low blood lead levels, the derived RfD for Pb was considered inappropriate by the US EPA (2004). Therefore, THQ was only calculated for Cd and iAs. The cancer slope factors (CSF) utilized for calculating TR of Pb and iAs were 8.5×10^{-3} (mg/kg-day)$^{-1}$ and 1.5 (mg/kg-day)$^{-1}$ respectively (US EPA, 2023a). Due to the lack of persuasive proof demonstrating a direct and proportional relationship between oral cadmium exposure and an increased cancer risk (US EPA, 1989) and no established CSF, TR was not calculated for Cd.

2.5. Statistical analyses

The data for each measured variable were presented as mean ± standard deviation. Statistical analyses were carried out using SPSS V16.0 software package (Chicago, IL, USA). For the comparison of metal concentrations in mussels, one-way analysis of variance (ANOVA) followed by Tukey's multiple comparison tests were used. Statistical significance level was set as 0.05 (Van Emden, 2008).

3. Results and discussion

Mussels are known to be sentinel organisms for biomonitoring of environmental pollution and contaminants (Kouali et al., 2020). Since mussel beds in the Sea of Marmara constitute extensive stocks, the impacts of hypoxia or anoxia in the water body can be tackled by

Table 2
The formulas and abbreviations used in order to calculate health risk assessments for consumers weighing 50, 60, or 70 kg.

Formulas		Abbreviations	
EWI	$= \frac{Cm * IRw}{BW}$	**Cm**	Metal concentration (mg/kg)
		IRw	Weekly ingestion rate (g/week)
		PTWI *m*	Provisional tolerable weekly intake of each metal established by the authorities
PTWI	= PTWI *m* * BW	**BW**	Body weight (kg)
		THQ	Target hazard quotient
		EF	Exposure frequency (350 days/yr)
THQ[a]	$= \frac{EF * ED * IFR * Cm}{BW * ATn * RfD} * 0.001$	**ED**	Exposure duration (26 yrs)
		IFR	Food ingestion rate (g/day)
		RfD	Oral reference dose (mg/kg-day)
TR[a]	$= \frac{EF * ED * IFR * Cm * CSF}{BW * ATc} * 0.001$	**AT*n***	Average exposure time for noncarcinogens (365 days/yr x 26 yrs)
		AT*c*	Average exposure time for carcinogens (365 days/yr x 70 yrs)
		TR	Target carcinogenic risk
		CSF	Oral carcinogenic slope factor

[a] The THQ and TR equations for fish ingestion formulas were derived from US EPA (2023b).

overseeing the condition of mussels periodically (Çinar et al., 2020). Also, mucilage phenomenon affected the mussel populations in the Sea of Marmara as well as the Çanakkale Strait and around the Gelibolu Peninsula. Due to the accumulation of mucilage in the seawater column and the release of toxic substances, mass mussel deaths were observed. Although decreases in the stocks through the strait were reported in 2021, the population has been reported to have revived after the incident (Gezen, 2022).

3.1. Mucilage and toxin interaction

Marine toxins are produced by certain toxic or harmful algae species during harmful algal blooms (HABs). The proliferation and predominance of these species pose a health threat to seafood consumers (Zingone et al., 2021). Biotoxins produced by harmful algae are the precursors of shellfish poisonings. Since mussels are filter-feeding bivalve shellfish, they can accumulate biotoxins. Furthermore, they act as vectors for the trophic transfer of toxins throughout the food chain (Farabegoli et al., 2018). Consumption of contaminated shellfish may lead to poisoning with a variety of symptoms, depending on the toxins ingested. Diarrhetic shellfish poisoning (DSP), paralytic shellfish poisoning (PSP), amnesic shellfish poisoning (ASP), neurotoxic shellfish poisoning (NSP), and azaspiracid poisoning (AZP) are the common HAB-related illnesses caused by mussels (Twiner et al., 2008; Grattan et al., 2016).

Mucilage formations due to harmful algal blooms (HABs) substantially affects the ecosystem. Studies investigating the blooms indicate presence of toxin producing species that may cause shellfish poisonings (Taş et al., 2016). During HABs, it is important to monitor and prevent certain outbreaks by using fast, low-cost, and easy-to-use tools. Validated test kits based on lateral flow assays enable the rapid screening of toxins. Since these kits do not require complicated equipment, they have the advantages of on-site monitoring of marine toxins and is suitable for use by non-specialists (Mills et al., 2022). According to qualitative rapid kit results, none of these biotoxins were determined during the 11-month observation period in this research.

The maximum permitted limit values for biotoxins were established by Food and Agriculture Organization of the United Nations and World Health Organization in Codex Alimentarius Standard for Live and Raw Bivalve Molluscs (CXS 292–2008) (Codex Alimentarius, 2015). Toxins that cause amnesic shellfish poisoning (ASP) are produced by toxigenic diatoms of the genus *Pseudon-nitzschia* and contain a large amount of domoic acid (DA). The maximum permitted limit value was determined as 20 mg DA per kg (20 ppm) of whole shellfish. Despite many researches monitoring the phytoplankton in the Sea of Marmara, studies focusing on the marine toxins formed by harmful algal blooms (HABs) are limited (Taş et al., 2016). The first report of the DA from the Sea of Marmara was in December 2010 and February 2011. The toxin concentrations in seawater from different coastal stations ranged from 0.96 to 5.25 μg/mL DA (Dursun et al., 2016). Similarly, these values were well below the permitted value. Besides, ASP outbreaks have never been encountered in the Sea of Marmara despite the presence of toxic species (Dursun et al., 2016).

Toxin groups okadaic acid (OA) and dinophysistoxins (DTXs), causing diarrheal shellfish poisoning (DSP), are produced by dinoflagellates such as *Dinophysis*. The established maximum level is 160 μg/kg (160 ppb) OA equivalent (OA, DTXs, pectenotoxins) (Codex Alimentarius, 2015). During the mucilage phenomenon in 2007, Tas et al. (2020) detected DSP causing species in the Sea of Marmara, however they stated that the cell density of these species were found to be quite low. According to another research conducted in mussels in the Sea of Marmara and Aegean Seas, it was observed that DTX was never encountered (Küçükgünay, 2000). It was also reported that even in the absence of microalgae, DSP toxins may exist by adhering to mucilage (Jiang et al., 2017).

Another genera of dinoflagellates, including *Alexandrium* and *Gymnodinium*, are responsible for paralytic shellfish poisoning (PSP) by producing saxitoxin (STX). The limit value set for PSP toxins is 80 μg/100 g (800 ppb) STX (Codex Alimentarius, 2015). Küçükgünay (2000) reported that STX was detected in İzmir, Aegean Sea, and Balıkesir, The Sea of Marmara in the late spring and summer. However, STX amount was so low that mussels carried no risks for human health. STX was also reported from the Golden Horn Estuary (Istanbul Strait, Türkiye) (Taş et al., 2016).

Yurga (2022) reported that PSP causing 1 dinoflagellate species, DSP causing 3 dinoflagellate species, and ASP causing 3 diatom species were detected in the sea snot during the mucilage phenomenon in 2021. Balkis-Ozdelice et al. (2020) also stated *Pseudo-nitzschia* sp. (ASP causing), *Alexandrium minutum* (PSP causing), and *Dinophysis acuminata, D. acuta, D. caudata, and D. fortii* (DSP causing) were detected during 2006–2008 period in Gulf of Erdek. However, toxin analyzes were not performed in these studies. Mussel samples collected during the mucilage period showed no indication of health risks related to poisonings, as all samples were toxin-negative.

3.2. Toxic metal concentrations in mussels

Another risk factor for the safe consumption of mussels is toxic metals, which can accumulate to a high degree in algae in polluted regions where the nutrient balance in the ecosystem is disturbed. The overgrowth of algae is considered as a vehicle for the transfer of hazardous substances to other organisms that feed directly or indirectly on seafood. Bioaccumulation of toxic metals in aquatic environments can also be regarded as a stress factor for algae and leads to the formation of toxic secondary metabolites in response. Together with mucilage, algal exudates may provide an indirect mechanism for adsorption or retention of hazardous ions in the water body (Volterra and Conti, 2000). Because mussels are non-selective in filtering phytoplankton and affect phytoplankton abundance (Wilson, 2003) they are considered bioindicators of pollution.

Water quality is of paramount importance in mussel production whether cultured or wild. Only shellfish aquaculture removes contaminants from their production environment rather than releasing them into the environment during farming (Avdelas et al., 2021). Bivalve mollusks are able to take in high quantities of contaminants from sediments, suspended particles, the water column, and food sources because of their physiological structure and immobility (Laffon et al., 2006). The variation between the concentrations of toxic metals also depends on many factors, including seasons (Dokmeci, 2017). In this study, Hg was under the detectable limits in each of the samples regardless of the season. Similarly, Hg was also not found in mussels captured from Yalova region in the Sea of Marmara (Acarlı et al., 2023). In a previous study, no Hg was detected from ten different stations in the Sea of Marmara and wild mussels were regarded to be safe for consumption (Mol and Üçok Alakavuk, 2011).

The mean toxic metal concentrations of Cd and Pb in all samples were lower than the maximum permissible levels (Table 3). The maximum levels of certain metals that can be found in fish and shellfish were set by the European Commission regulations. The limits of Cd and Pb for bivalve mollusks are 1.00 mg/kg and 1.50 mg/kg ww, respectively. The maximum permitted value for Hg in mollusks is 0.50 mg/kg ww (European Commission (EC) No 2023/915) (European Commission, 2023). On the other hand, there is no established limit value for As in seafood by the authorities.

Annual Cd concentrations in the samples from Istanbul Strait were higher in comparison to other sampling sites. Considering seasonal differences, it was also found that the Cd levels of mussels from İstanbul Strait were significantly higher ($P < 0.05$) in winter and spring (Table 3). There was also no statistical difference ($P > 0.05$) between the fall samples of four sites. Unlike our study, Kuplulu et al. (2018) reported relatively lower Cd levels (0.087 mg/kg) in mussels obtained from the Sea of Marmara. In another study, the Cd concentrations in mussels were

Table 3
Toxic metal concentrations of mussels from the Istanbul and Çanakkale Straits, Bandirma, and Gelibolu stations according to seasons.

Station	Season	Pb (mg/kg)	Cd (mg/kg)	As (mg/kg)
Istanbul St.	Fall	$0.300 \pm 0.198^{a,x}$	$0.277 \pm 0.083^{a,x}$	$0.181 \pm 0.105^{a,x}$
	Winter	$0.140 \pm 0.050^{a,x}$	$0.363 \pm 0.003^{a,x}$	$0.115 \pm 0.015^{a,x}$
	Spring	$0.092 \pm 0.021^{a,x}$	$0.386 \pm 0.131^{a,x}$	$0.106 \pm 0.053^{a,x}$
	Summer	n/a	n/a	n/a
	Annual	0.177 ± 0.089	0.342 ± 0.072	0.134 ± 0.058
Çanakkale St.	Fall	$0.209 \pm 0.172^{a,x}$	$0.309 \pm 0.080^{a,x}$	$0.179 \pm 0.065^{a,x}$
	Winter	$0.120 \pm 0.040^{a,x}$	$0.201 \pm 0.039^{ab,y}$	$0.165 \pm 0.015^{a,xy}$
	Spring	$0.046 \pm 0.042^{a,x}$	$0.133 \pm 0.028^{b,yz}$	$0.145 \pm 0.008^{a,x}$
	Summer	n/a	n/a	n/a
	Annual	0.125 ± 0.085	0.214 ± 0.049	0.163 ± 0.029
Bandirma	Fall	$0.113 \pm 0.062^{a,x}$	$0.354 \pm 0.210^{a,x}$	$0.130 \pm 0.037^{a,x}$
	Winter	$0.082 \pm 0.011^{a,x}$	$0.179 \pm 0.058^{a,y}$	$0.221 \pm 0.023^{b,yz}$
	Spring	$0.094 \pm 0.011^{a,x}$	$0.149 \pm 0.012^{a,yz}$	$0.205 \pm 0.016^{b,x}$
	Summer	n/a	n/a	n/a
	Annual	0.096 ± 0.028	0.227 ± 0.093	0.185 ± 0.025
Gelibolu	Fall	$0.158 \pm 0.074^{a,x}$	$0.433 \pm 0.095^{a,x}$	$0.173 \pm 0.045^{a,x}$
	Winter	$0.118 \pm 0.029^{a,x}$	$0.224 \pm 0.038^{b,y}$	$0.240 \pm 0.047^{a,z}$
	Spring	$0.087 \pm 0.025^{a,x}$	$0.210 \pm 0.068^{b,xz}$	$0.203 \pm 0.058^{a,x}$
	Summer	0.083 ± 0.023^{a}	0.138 ± 0.018^{b}	0.149 ± 0.017^{a}
	Annual	0.111 ± 0.038	0.251 ± 0.055	0.191 ± 0.042

n/a: No sample available.
^{a,b}: The letters indicate the statistical difference ($P < 0.05$) between the seasons in a group for each metal.
^{x,y,z}: The letters show the statistical difference ($P < 0.05$) between the groups in a season for each metal.

between 0.11 and 0.19 mg/kg captured from different stations in Yalova (Acarlı et al., 2023). Additionally, the Cd concentration of mussels obtained from Istanbul were reported to be lower (0.14 mg/kg) in the muscle tissue but higher in the digestive gland (0.67 mg/kg) in comparison to our study (Türkoğlu et al., 2023). Belivermiş et al. (2016) detected Cd levels ranging from 0.09 to 1.05 mg/kg on a dry weight (dw) basis at seven different stations.

Regarding the Pb concentrations, no significant difference ($P > 0.05$) was found between the sampling sites, whether in wild or farmed mussels, in any season (Table 3). Various Pb concentrations in mussels were reported from different regions of the Sea of Marmara such as 0.267 mg/kg by Kuplulu et al. (2018), and 0.11–0.72 mg/kg by Acarlı et al. (2023), and 0.24 mg/kg by Türkoğlu et al. (2023), which were below the maximum limit. Conversely, higher Pb concentrations were determined at 2.67–9.2 mg/kg (Dokmeci, 2017), 0.19–22.92 mg/kg (Belivermiş et al., 2016), and 2.54–3.32 mg/kg dw (Çulha et al., 2011) from the Sea of Marmara.

There was no statistical significance ($P > 0.05$) between the sampling stations in the fall and spring regarding As concentrations. However, the winter samples obtained from Gelibolu had significantly higher ($P < 0.05$) As levels than Istanbul and Çanakkale Straits samples (Table 3). The results of this study were lower than the previous studies carried out in the Sea of Marmara, which reported As concentrations in mussels at levels of 0.61–2.59 mg/kg (Acarlı et al., 2023) and 2.85 mg/kg (Kuplulu et al., 2018). Additionally, higher As levels (1.20–2.79 mg/kg dw) were found in the mussels captured from along the coasts of Tekirdag region in the Sea of Marmara (Dokmeci, 2017).

In accordance with the above studies, the Cd, Pb, and As levels detected in both farmed and wild mussels were various. Therefore, it is not possible to completely ascribe the metal concentrations found in mussels to the mucilage that occurred in the Sea of Marmara.

3.3. Human health risk assessment associated with consumption of mussels

The EWI provides a clear assessment of the health risks of seafood for human consumption in children and adults (EFSA, 2015). The toxicity of toxic metals depends on the dose, frequency, and duration of exposure to the metal. These substances differ between individuals and by age. Therefore, calculating the EWI is a commonly employed tool to define safe levels of consumption that highlights the benefits and risks (Bat et al., 2022). The EWI calculations for three toxic metals are listed in Tables 4–6.

The EWI values were calculated based on a weekly seafood consumption of 150 g (Alves et al., 2018). For instance, in the case of fried mussels, 10–12 mussels are served in shish sticks. Also, stuffed mussel portion is 8–10 mussels as popular street foods. Especially consumers who like consume seafood, eat large quantities at once. Likewise, Masiá et al. (2022) reported one serving size of meal as 20 mussels. In this study, the weekly consumption value of mussels was considered to be 150 g on the average mussel consumption of ca. 15 mussels for Turkish individuals weighing 50, 60, and 70 kg, respectively. There are various studies that calculated the weekly consumption value between 125 and 200 g of mussels (Sloth and Julshamn, 2008; Jović et al., 2012; Alves et al., 2018; Nekhoroshkov et al., 2021). In Türkiye, stuffed mussels and fried mussels are the frequently consumed shellfish products, especially in the coastal areas (Kocatepe et al., 2020). This consumption quantity was determined based on mussel and seafood consumption in the Marmara region being higher than the per capita seafood consumption of Turkish consumers. Although the PTWI values for As and Pb are currently withdrawn, the obtained EWI values were compared with PTWI values set forth by EFSA (2009, 2010). The calculated EWI values for Cd, Pb, and As in all mussel samples for all weights and stations during all seasons were considerably lower than the PTWI values (Tables 4–6). The intake of toxic metals through black mussel consumption showed no potential risk in the Sea of Marmara at these stations. Similar to our study, the EDI values for Cd, Pb, and As calculated based on an annual per capita consumption of 0.34 kg in mussels captured in the same stations from the Sea of Marmara, were found to be below the PTWI values (Belivermiş et al., 2016). In another study, EWI values calculated for Cd, Pb, and As in stuffed mussels sold in Istanbul were similarly risk-free for children (32 kg) and adults (70 kg) (Köşker et al., 2022). In our study, the highest annual average EWI values for Cd and Pb were found in mussels caught from the Istanbul Strait, while the lowest EWI values were found in mussel samples caught from the Çanakkale Strait and Bandirma, respectively. One possible explanation is the retention of Cd carried by rainwater in the Istanbul Strait due to mucilage, as well as fishing boats, and industrial and agricultural discharges. The annual EWI value for As was found to be the lowest in Istanbul Strait, while the highest value was determined in the Gelibolu station. This is attributed to the prevailing strong currents within the Istanbul Strait, alongside a decrease in both anthropogenic and natural activities (Atique Ullah et al., 2019; Altıok et al., 2023).

As indicated in the above recent studies, the risks associated with mussels and mussel consumption were evaluated within the limits given in the regulation EC 1907/2006, while health risks due to toxic metals have begun to be evaluated with THQ and TR indexes. THQ is a method used to assess the risk of a population's lifelong exposure to metals. However, this value does not provide a precise measure of health effects. Nevertheless, if the THQ value is 1 or higher, it may indicate non-carcinogenic potential health risks associated with excessive exposure to contaminants (Effiong et al., 2023). THQ values of Cd and As are listed in Tables 4 and 6 The annual THQ value for Cd was above 1 in three different weights in the mussels from the Istanbul Strait, indicating

Table 4
Estimated weekly intake (EWI), the percentage of provisional weekly intake (PTWI), and target hazard quotient (THQ) calculations of Cd determined in mussels captured from different stations (Istanbul and Çanakkale Straits, Bandirma and Gelibolu) seasonally.

		EWI			%PTWI			THQ Cd		
	Season	50	60	70	50	60	70	50	60	70
Istanbul St.	**Fall**	0.831	0.693	0.594	0.66	0.50	0.34	**1.1**	0.9	0.8
	Winter	1.089	0.908	0.778	0.87	0.61	0.44	**1.5**	**1.2**	**1.1**
	Spring	1.158	0.965	0.827	0.93	0.64	0.47	**1.6**	**1.3**	**1.1**
	Annual	1.026	0.855	0.733	0.82	0.58	0.42	**1.4**	**1.2**	**1.0**
Çanakkale St.	**Fall**	0.927	0.773	0.662	0.74	0.52	0.38	**1.3**	**1.1**	0.9
	Winter	0.603	0.503	0.431	0.48	0.34	0.25	0.8	0.7	0.6
	Spring	0.399	0.333	0.285	0.32	0.22	0.16	0.5	0.5	0.4
	Annual	0.643	0.536	0.459	0.51	0.36	0.26	0.9	0.7	0.6
Bandirma	**Fall**	1.062	0.885	0.759	0.85	0.59	0.43	**1.5**	**1.2**	**1.0**
	Winter	0.537	0.448	0.384	0.43	0.30	0.22	0.7	0.6	0.5
	Spring	0.447	0.373	0.319	0.36	0.25	0.18	0.6	0.5	0.4
	Annual	0.682	0.568	0.487	0.55	0.38	0.28	0.9	0.8	0.7
Gelibolu	**Fall**	1.299	1.083	0.928	1.04	0.72	0.53	**1.8**	**1.5**	**1.3**
	Winter	0.672	0.560	0.480	0.54	0.37	0.27	0.9	0.8	0.7
	Spring	0.630	0.525	0.450	0.50	0.35	0.26	0.9	0.7	0.6
	Summer	0.414	0.345	0.296	0.33	0.23	0.17	0.6	0.5	0.4
	Annual	0.754	0.628	0.538	0.60	0.42	0.31	**1.0**	0.9	0.7

Note: The numbers 50, 60 and 70 refer to the consumers' body weight of (kg).
*All calculations were based on $PTWI_m$ for Cd, 2.5 μg/kg BW, as established by EFSA (2012b).

Table 5
Estimated weekly intake (EWI), the percentage of provisional weekly intake (%PTWI), and target carcinogenic risk (TR) calculations of Pb determined in mussels captured from different stations (Istanbul and Çanakkale Straits, Bandirma and Gelibolu) seasonally.

		EWI			%PTWI			TR Pb		
	Season	50	60	70	50	60	70	50	60	70
Istanbul St.	**Fall**	0.900	0.750	0.643	0.07	0.05	0.04	3.89E-07	3.89E-07	3.89E-07
	Winter	0.420	0.350	0.300	0.03	0.02	0.02	1.82E-07	1.51E-07	1.30E-07
	Spring	0.276	0.230	0.197	0.02	0.02	0.01	1.19E-07	9.95E-08	8.53E-08
	Annual	0.532	0.443	0.380	0.04	0.03	0.02	2.30E-07	2.13E-07	2.01E-07
Çanakkale St.	**Fall**	0.627	0.523	0.448	0.05	0.03	0.03	2.71E-07	2.71E-07	2.71E-07
	Winter	0.360	0.300	0.257	0.03	0.02	0.01	1.56E-07	1.30E-07	1.11E-07
	Spring	0.210	0.175	0.150	0.02	0.01	0.01	9.08E-08	7.57E-08	6.49E-08
	Annual	0.399	0.333	0.285	0.03	0.02	0.02	1.73E-07	1.59E-07	1.49E-07
Bandirma	**Fall**	0.339	0.283	0.242	0.03	0.02	0.01	1.47E-07	1.47E-07	1.47E-07
	Winter	0.246	0.205	0.176	0.02	0.01	0.01	1.06E-07	8.87E-08	7.60E-08
	Spring	0.282	0.235	0.201	0.02	0.02	0.01	1.22E-07	1.02E-07	8.71E-08
	Annual	0.289	0.241	0.206	0.02	0.02	0.01	1.25E-07	1.12E-07	1.03E-07
Gelibolu	**Fall**	0.474	0.395	0.339	0.04	0.03	0.02	2.05E-07	2.05E-07	2.05E-07
	Winter	0.354	0.295	0.253	0.03	0.02	0.01	1.53E-07	1.28E-07	1.09E-07
	Spring	0.261	0.218	0.186	0.02	0.01	0.01	1.13E-07	9.41E-08	8.06E-08
	Summer	0.249	0.208	0.178	0.02	0.01	0.01	1.08E-07	8.97E-08	7.69E-08
	Annual	0.335	0.279	0.239	0.03	0.02	0.01	1.45E-07	1.29E-07	1.18E-07

*All calculations were based on $PTWI_m$ for Pb, 25 μg/kg BW, as established by EFSA (2010).
Note: The numbers 50, 60 and 70 refer to the consumers' body weight of (kg).

a risk to consumer health. Moreover, the THQ value of mussel samples obtained from Gelibolu indicated a potential risk for individuals weighing 50 kg when consumed in terms of Cd. For the samples from Çanakkale and Bandirma, it was found to be close to the threshold for the same individuals. The THQ value greater than 1 indicates an exposure above the RfD of that metal, indicating that the consumption of mussels may pose a risk to the consumer in terms of Cd (US EPA, 2023a). The Marmara region, encompassing both urban and rural areas, faces high levels of urbanization and is heavily exposed to abundant municipal, household, agricultural, and animal wastes. Particularly, Istanbul stands out as a major producer of household waste (Ocak and Acar, 2021). Similarly, the THQ for Cd in Mediterranean mussels (*M. galloprovincialis*) caught from Homa Lagoon, Eastern Aegean Sea, was greater than 1 (Bilgin and Uluturhan-Suzer, 2017). A study conducted by Kouali et al. (2022) found that the THQ for Cd in Mediterranean mussels captured from two different stations along the Atlantic coast of northwest Morocco was significantly higher than 1 throughout the season, specifically for individuals weighing 69.3 kg, 72.2 kg, and 15 kg. Different trace elements including Pb, Cd, and As were evaluated in shellfish from four regions in Shenzhen, China. In contrast to our study, the EWI and THQ values of As and Cd indicated significant health risks with regarding consumption of shellfish from two regions for children weighing 16 kg and adults weighing 70 kg. The study highlighted that the increased presence of ecological and human discharges in these areas led to a greater introduction of toxic metals into the delta basin (Liu et al., 2020). In another study, Cd and Pb levels exceeding the recommended level for consumers and the provisional weekly tolerable intake (PTWI) were detected in green mussels collected along the East Java coast of Indonesia. The THQ values of these metals were found to be greater than one and were determined to pose a risk to the non-carcinogenic consumer (Soegianto et al., 2021).

Mussel samples collected from all stations exhibited THQ values for iAs ranging from 0.01 to 0.03 seasonally and annually, all of which were found to be less than 1. A THQ value of less than 1 indicates that the exposure level is lower than the RfD. This suggests that daily exposure from mussel consumption in all stations of consumers of different weights is unlikely to have lifelong adverse health effects on the human population for As. Similar to our study, THQ values for As in stuffed

Table 6
Estimated weekly intake (EWI), the percentage of provisional weekly intake (%PTWI), PTWI, target hazard quotient (THQ), and target carcinogenic risk (TR) calculations of iAs determined in mussels captured from different stations (Istanbul and Çanakkale Straits, Bandirma and Gelibolu) seasonally.

		EWI			%PTWI			THQ iAs			TR iAs		
	Season	50	60	70	50	60	70	50	60	70	50	60	70
Istanbul St.	**Fall**	0.054	0.045	0.039	0.01	0.01	0.00	0.02	0.02	0.02	4.14E-06	3.45E-06	2.96E-06
	Winter	0.035	0.029	0.025	0.00	0.00	0.00	0.02	0.01	0.01	2.63E-06	2.19E-06	1.88E-06
	Spring	0.032	0.027	0.023	0.00	0.00	0.00	0.01	0.01	0.01	2.43E-06	2.02E-06	1.73E-06
	Annual	0.040	0.034	0.029	0.00	0.00	0.00	0.02	0.02	0.01	3.07E-06	2.56E-06	2.19E-06
Çanakkale St.	**Fall**	0.054	0.045	0.038	0.01	0.00	0.00	0.02	0.02	0.02	4.10E-06	3.42E-06	2.93E-06
	Winter	0.050	0.041	0.035	0.01	0.00	0.00	0.02	0.02	0.02	3.78E-06	3.15E-06	2.70E-06
	Spring	0.044	0.036	0.031	0.01	0.00	0.00	0.02	0.02	0.01	3.32E-06	2.77E-06	2.37E-06
	Annual	0.049	0.041	0.035	0.01	0.00	0.00	0.02	0.02	0.02	3.73E-06	3.11E-06	2.67E-06
Bandirma	**Fall**	0.039	0.033	0.028	0.01	0.00	0.00	0.02	0.01	0.01	2.98E-06	2.48E-06	2.13E-06
	Winter	0.066	0.055	0.047	0.01	0.01	0.00	0.03	0.03	0.02	5.06E-06	4.22E-06	3.61E-06
	Spring	0.062	0.051	0.044	0.01	0.01	0.00	0.03	0.02	0.02	4.69E-06	3.91E-06	3.35E-06
	Annual	0.056	0.046	0.040	0.01	0.01	0.00	0.03	0.02	0.02	4.24E-06	3.54E-06	3.03E-06
Gelibolu	**Fall**	0.052	0.043	0.037	0.01	0.00	0.00	0.02	0.02	0.02	3.96E-06	3.30E-06	2.83E-06
	Winter	0.072	0.060	0.051	0.01	0.01	0.00	0.03	0.03	0.02	5.5E-06	4.58E-06	3.93E-06
	Spring	0.061	0.051	0.044	0.01	0.01	0.00	0.03	0.02	0.02	4.65E-06	3.87E-06	3.32E-06
	Summer	0.045	0.037	0.032	0.01	0.00	0.00	0.02	0.02	0.01	3.41E-06	2.84E-06	2.44E-06
	Annual	0.057	0.048	0.041	0.01	0.01	0.00	0.03	0.02	0.02	4.38E-06	3.65E-06	3.13E-06

*All calculations were based on $PTWI_m$ for As, 15.0 μg/kg BW, as established by EFSA (2009).
Note: The numbers 50, 60 and 70 refer to the consumers' body weight of (kg).

mussels sold in Istanbul and Ankara were below 1 (Köşker et al., 2022). In the same study, the THQ value of the As level of stuffed mussels offered for sale in other cities was found to be greater than 1. As levels did not pose a risk to public health at any of the stations. This may be due to mussels structurally accumulating 1–2 percent of iAs in their bodies. The mucilage structure may have affected arsenic absorption or may be due to the lack of sediment in the sea. Furthermore, water contamination can vary from season to season and region to region, and factors such as seasonal absorption cycle, salinity, and temperature affect the bioaccumulation of As (Dokmeci, 2017; Kuplulu et al., 2018). The high levels of As in some regions can be explained by a common source of the elements or synergistic interaction between them as shown in the study (Azizi et al., 2020). The notable variances in metal levels are primarily attributable to anthropogenic influences (Kouali et al., 2022). Since Hg was not detected in our study, it did not pose any health risk. Similarly, no Hg was detected in mussels caught from the Tekirdag Region, the Sea of Marmara, and it was emphasized that this situation may vary seasonally (Dokmeci, 2017). In some studies, correlations between the accumulation of metals in mussels indicated that the inability to detect metal depended on the duration of mussels' exposure to metals and the level of contamination (Çevik et al., 2008, Kouali et al., 2022). Furthermore, mucilage may have impeded this accumulation of Hg from transferring into the soft tissue of the mussel.

Because CSFs were available only for Pb and As, their cancer risk values for mussel samples were determined. The TR values of Pb were less than 1×10^{-6} (Table 5), and the carcinogenic risk was negligible for mussel consumption. The cancer risk is acceptable if the TR value is between 1×10^{-4} and 1×10^{-6}. The carcinogenic risk of iAs exposure due to mussel consumption was acceptable at all weights for all stations. The lack of carcinogenic health effects of mussel consumption was assessed based on the annual and national average consumption of the region. However, this does not rule out the possibility that individuals who consume larger quantities of mussels may be exposed to higher amounts of toxic metals and therefore potentially face a higher risk. Soegianto et al. (2021) found that the TR values of the Cd content of green mussels were higher than 10^{-4} at certain locations, indicating that lifetime exposure to Cd from the consumption of these mussels may develop cancer in humans. However, similar to our findings, they reported that the tolerable intake (TR) value of Pb was safe for human health.

4. Conclusion

Mussel is an important source of seafood for Turkish consumers. Being rich in nutritional value and highly popular among consumers, shellfish consumption has significantly raised the exposure to toxic metals. The changing balance of nutrients in the ecosystem, raises the concern about the toxic metal and mucilage relationship. At a time of increasing public concern, during the mucilage occurrence in the Sea of Marmara, mussel consumption was found to be safe for consumers in terms of the studied biotoxins and toxic metals. Whether farmed or wild, none of the mussel samples were toxin-positive in any month. Although the Cd concentrations in the mussels were below the threshold, the presence of Cd may present health risks, particularly for children, and adults who consume larger quantities of mussels. However, the toxic metal concentrations determined in farmed and wild mussels cannot be solely attributed to the mucilage phenomenon that occurred in the Sea of Marmara in 2021. To address and mitigate these concerns, it is recommended to conduct regular toxic metal accumulation analyses periodically by establishing a consistent monitoring program. Additionally, consumers should be informed accurately regarding consumption levels and associated risks.

CRediT authorship contribution statement

Hande Doğruyol: Writing – original draft, Data curation, Conceptualization. **Şafak Ulusoy:** Writing – original draft, Data curation, Conceptualization. **Nuray Erkan:** Writing – review & editing, Project administration, Funding acquisition. **Sühendan Mol:** Visualization, Validation. **Özkan Özden:** Visualization, Validation. **İdil Can Tunçelli:** Methodology, Investigation. **Şehnaz Yasemin Tosun:** Methodology, Investigation. **Didem Üçok:** Methodology, Investigation. **Eda Dağsuyu:** Visualization, Investigation. **Refiye Yanardağ:** Visualization, Investigation.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

Data will be made available on request.

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