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Heavy metal accumulation in a bioindicator species, Limpet *Patella caerulea*, in Yalova (İzmit Bay): Risk assessment for human health

by

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Abstract

In this study, monthly heavy metal concentrations in the whole-body tissue of *Patella caerulea* (Mediterranean limpets), a bioindicator species living in the coastal zone of the Gulf of Izmit (Marmara Sea), were examined for the first time. The mean metal concentrations in *Patella caerulea* (mg kg^{-1} dw) were 2.01–5.74 Cd, 2.45–12.90 Cu, 0.74–1.95 Pb, 21.12–109.57 Zn, 16.31–154.67 Ni, and 1120.67–3086.00 Fe. Cd levels in all months and Pb levels in October and November were found to be above the safe limits set by international organizations. The estimated daily intakes and estimated weekly intakes determined for each heavy metal were below the acceptable daily intakes and provisional tolerable weekly intakes. However, the target hazard quotient and total target hazard quotient values calculated for Cd, Ni, and Fe were found to be higher than 1. The carcinogenic risk value was also found to be high.

Key words: bioaccumulation, limpet, target hazard quotient (THQ), carcinogenic risk (CR), Marmara Sea

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1. Introduction

Increasing environmental pollution in the 21st century threatens not only domestic (land and air) areas, but also aquatic environments. Pollutant levels in aquatic environments, particularly in the form of heavy metals, and their negative effects on aquatic organisms have been the research topic of numerous analyses across many living species. To identify and demonstrate the pollutants in the aquatic environment, bioindicators are used; these are organisms with qualities that allow the quality of a given environment to be determined. For instance, bivalves and gastropods from the mollusk group are frequently used to biologically monitor heavy metal pollution in the marine environment (Conti & Cecchetti 2003; Boudouresque 2004). Gastropods can absorb metals by swallowing, feeding, or absorbing water, and they can accumulate heavy metals in their tissues at a very high rate (Phillips 1977; Koide et al. 1982; Szefer 1986). *Patella caerulea* is one such species as well as an ideal bioindicator that is widely used in heavy metal pollution studies and marine pollution monitoring programs (Bu-Olayan & Thomas 2001; Campanella et al. 2001; Cravo et al. 2002; Storelli & Marcotrigiano 2005; Nakhle et al. 2006; Reguera et al. 2018; Pérez et al. 2019). It is also called the Chinese hat shell, a herbivorous and sedentary organism that lives by fixing itself to the hard ground in the mediolittoral region. Gastropods such as marine snails or patellid limpets are usually preserved fresh or in vinegar and can be eaten without any purification (Ramirez 2013). Mollusks can be consumed as part of a daily diet and have proven health benefits, since they are rich in vital nutrients and active secondary metabolites that can boost the immune response (Benkendorff 2010). It is worth mentioning that *P. caerulea* – like *Mytilus galloprovincialis* – is one of the seafood products which are widely consumed in many Mediterranean countries (Cubadda et al. 2001; Copat et al. 2013). However, since gastropods such as echinoderms and tunicates are not consumed on the same scale as mollusks, much less attention has been paid to their nutritional values (Zlatanos et al. 2009).

Limpets are used in a traditional shellfish dish that is still widely consumed in some regions, such as the Azores (Portugal) or the Canary Islands (Spain) (Pérez et al. 2019). In Turkey, *Patella* spp. inhabits the large intertidal rocky shores of the entire Mediterranean basin, including the Black Sea, and is mostly used as fish feed. However, a study has shown that the populations of certain species are declining as a result of polluted surface waters, the subsequent limitation of habitat, and possible negative effects of human activities (Çulha & Bat 2010). As a result, they are among the most endangered marine invertebrates on the shores of the western Mediterranean (Ramos 1998).

Therefore, limpets are one of the most important species to be investigated in Turkey. While the studies on the meat yield, distribution, and morphometric, taxonomic, and biological parameters of *P. caerulea* have been carried out in Turkey (Öztürk & Ergen 1996; Ayas 2010; Çulha & Bat 2010; Akşit & Falakalı-Mutaf 2007; Akşit & Falakalı Mutaf 2011; Küçükdermenci et al. 2017; Aydın et al. 2021; Mutlu et al. 2021), studies on *Patella* spp. are still very scarce. To the best of our knowledge, there is no national or international literature on *P. caerulea*, especially regarding the detection of regional heavy metal pollution in the Sea of Marmara, although a limited number of studies have been carried out in the Mediterranean (Ramelow 1985; Türkmen et al. 2005; Ayas et al. 2009; Yüzereroğlu et al. 2010; Duysak & Azdural 2017), the Aegean (Uysal et al. 1989; Aydın-Önen & Öztürk 2017), and the Black Seas (Öztürk 1994; Bat et al. 2015). This study is the first to measure the heavy metal concentration in the whole-body tissue of *P. caerulea*, sampled from the Yalova Samanlıdere fishermen's shelter region (selected from the Gulf of Izmit, where intensive industrial production takes place in the Marmara Sea).

Provisional tolerable weekly intake (PTWI) defines the amount of a substance that can be ingested each week without the risk of any adverse health effects during a person's lifetime (FAO/WHO 2004). The daily and weekly consumption rates and carcinogenic risk values were also calculated to determine the possible effects on human health. The data set was compared with the international consumable limits (THQ, TTHQ, and CR values) (TFC 2008; EU 2008; FDA 2001). In addition, the results were evaluated by comparing them with previous studies conducted in other coastal environments.

2. Materials and methods

2.1. Collection of samples

Izmit Bay (on the Marmara Sea) was the study area selected from Yalova Province. It is a fisherman's shelter located in the city center by fishing vessels, particularly in the first months of the fishing season (September, October, and November). The Samanlı Stream, along which tourism, irrigated agriculture, and industry are important elements of the local economy, also connects to the sea. Besides thermal resources, industrial plants producing chemicals, energy, acrylic fiber, textiles, ships, paper cleaning products, packaging, plastic, automotive parts, marble, and frozen food are also part of the local economy. These areas are exposed to different sources of pollution, such as domestic drainage, tourism, and agricultural wastewater (YESR 2018). To examine the spatial trends of the region and to compare the different heavy metal concentrations caused by pollution, monthly samples were taken between April and November 2009 from the zone of Samanlıdere fishermen's shelter, shown in Figure 1. In the coastal rocky zone, 20 specimens of *P. caerulea* were collected each month 15 cm below the water. The samples were transferred to a laboratory via cold chain in sterile polyethylene bags, then stored at -21°C until analysis. In addition, while sampling from the station, the temperature, salinity, dissolved oxygen (DO), and pH of the surface water were measured on-site by using a portable WTW Multi 340i Set Model Multiparameter.

2.2 Analytical methods

Digestion processes for heavy metal analysis were performed according to Bernhard (1976) and Yap et al. (2004). The *P. caerulea* samples were washed before analysis, first with tap water and then with double distilled water. After drying on filter paper, the samples were dissected using stainless-steel scissors and a lancet. Whole-body tissue was homogenized for use in the analysis and dried in an oven at 105°C for 24 hours until it had a constant weight. 10 ml of nitric acid (HNO_3) per gram dry weight (dw) was added to the Erlenmeyer flask; the mixture was left at room temperature overnight closing by a watch glass. The solutions were subsequently heated, first at low temperature (40°C) for 1 hour and then at slowly increasing temperatures (140°C) for 3 hours in a fume hood on a hot plate. One milliliter of HNO_3 was added to the digested solution, which was then diluted to a final volume of 25 ml. Metal concentrations (Fe, Zn, Ni, Cu, Pb, and Cd) of the samples were measured using inductively coupled plasma optical emission spectrometry (ICP-OES, Perkin Elmer). Three replicates of each sample were analyzed with a standard reference material (Oyster tissue NIST® SRM® 1566b) in order to test the accuracy and precision of the instrument. The average fit of all analyzed metals to

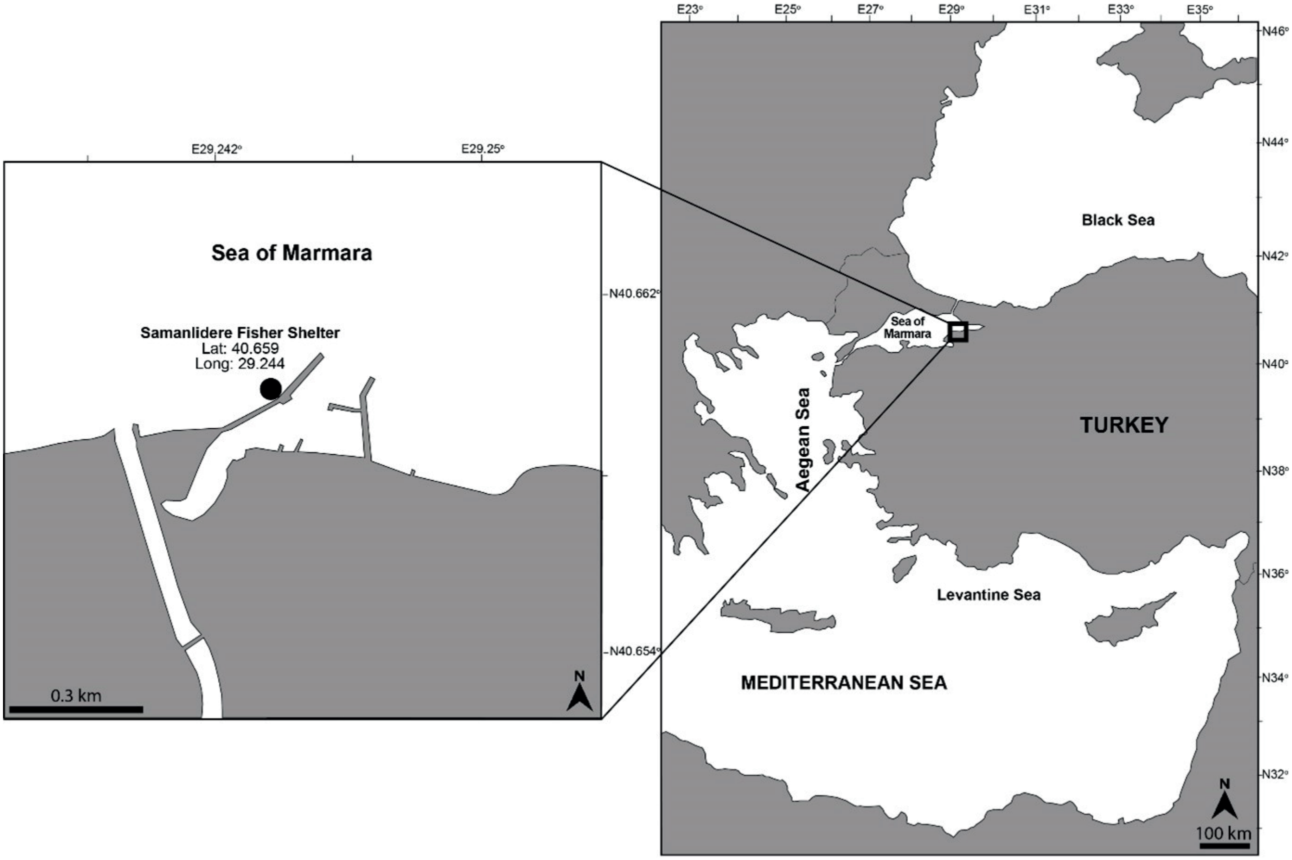

Figure 1

Study area

the reference material was 98.6%. All chemicals used in the tests were of ultrapure quality (Merck Suprapur). Ultrapure water (Milli-Q System, Millipore) was used for the standard solutions. All glassware was cleaned by soaking in 10% v/v HNO_3 for 24 hours before use and rinsing with Milli-Q water.

2.3. Statistical analysis

All the statistical analysis was performed using the statistical package SPSS (version 24; SPSS, Chicago, IL); the statistical significance was set at a p-level of < 0.05. The normality of all the data was tested using the Kolmogorov–Smirnov and Shapiro–Wilk tests; the homogeneity of variance was examined using Levene's test. Where the data were normally distributed ($p > 0.05$), a one-way analysis of variance (ANOVA; a parametric test), was preferred to compare changes in metal concentrations between months. Otherwise, the Kruskal–Wallis test, one of the non-parametric alternatives, was used to find any significant differences in metal concentrations between months. Since ANOVA and the Kruskal–Wallis test can only determine whether there is a significant difference (if $p < 0.05$), the Tukey (parametric data) and Mann–Whitney U tests (non-parametric data) were employed to determine which months were statistically significantly different from other months. In addition, Spearman correlation analysis was utilized to clarify existing relations (between metal concentrations and physical parameters of the seawater). When the p-value was less than 0.05, the correlation between the two parameters was accepted as statistically significant.

In this study, estimated daily intake (EDI) values for each element under study were calculated according to Copat et al. (2013) in order to assess the human health risks from carcinogenic and non-carcinogenic metals associated with mollusk consumption. Estimated weekly intake (EWI) values were also found by multiplying EDI by 7. No data are available on the daily consumption of mollusks. A normal portion of seafood is 0.160 kg (Afonso et al. 2015). Assuming that only one portion is eaten per week, the calculated daily consumption is 0.0228 kg (Gedik & Eryaşar 2020); the EDI values were calculated assuming a 70-kg adult consumes 0.0228 kg of *P. caerulea* per day. The maximum metal levels determined in October were used for the calculations. Based on the weekly consumption, the recommended acceptable daily intake (ADI) and provisional tolerable weekly intake (PTWI) (mg kg^{-1}) values for metals (Cd, Cu, Zn, Fe, Pb, and Ni) were determined by the Joint FAO/WHO Expert Committee on Food Additives (2004; 2007; 2010) and Nasreddin et al. (2010). These were compared with the EDI and EWI values calculated as part of the study. Moreover, THQ, TTHQ, and CR values were calculated by Copat et al. (2013). THQ is the expression of the ratio between the reference dose (RfD) of a metal and the exposure to that metal. A THQ value of >1 indicates a potential risk to the population exposed to the metal in question. The sum of the THQ values of all the elements is determined as TTHQ. According to USEPA (2020) data, the RfD values are 1.10^{-3}, 4.10^{-2}, 4.10^{-3}, 3.10^{-1}, 2.10^{-2}, and 7.10^{-1} for Cd, Cu, Pb, Zn, Ni, and Fe, respectively. CR is used to indicate the carcinogenic risk and has four categories: $\leq 10^{-6}$ = low; 10^{-4} to 10^{-3} = medium; 10^{-3} to 10^{-1} = high; and $\geq 10^{-1}$ = very high (Javed & Usmani 2016). Values of cancer slope factor (CPSo) are used in CR calculations (USEPA 2020). As the CPSo values for Cd, Ni, and Pb are already known, only the CR values of these metals have been calculated.

3. Results and discussion

3.1. Heavy metal concentration in *P. caerulea*

The physicochemical parameters measured in the seawater and the average values of heavy metals determined in *P. caerulea* are shown in Tables 1 and 2. The accumulation of heavy metals is listed as Fe > Ni > Zn > Cu > Cd > Pb. According to the results, it was determined that Fe, Ni, and Zn were the most dominant among the six metals selected for this study. Excesses of Fe, Zn, and Cu were predominantly discovered in the soft tissues of all *Patella* spp. (Brown & Depledge 1998). These metals have been reported to accumulate in soft tissues at a high rate in gastropods other than *Mytilus* spp. and *Ostrea* spp. (Szefer & Szefer 1985; De Wolf et al. 2001; Cravo et al. 2004). The high levels of Fe, Cu, Ni, and Cd in this study indicates that the selected area is affected by pollution. Spearman's correlation analysis revealed statistically significant relationships in *P. caerulea*: between Cd and Fe (r = 0.714, $p < 0.05$), between Cd and Ni (r = 0.905, $p < 0.01$), between Cd and Cu (r = 0.857, $p < 0.01$) between Ni and Fe (r = 0.830, $p < 0.05$) and between Cu and Ni (r = 0.905, $p < 0.01$), showing that these metals have the same origin or result from synergistic interactions among themselves. Türk-Çulha et al. (2017) also reported a similar result to this relationship. In the correlation analysis of the physicochemical parameters, only one negative relationship was found: between saturated oxygen and salinity (r = 0.933, $p < 0.01$). Since heavy metal pollution in gulfs and inland seas is more potent than pollution offshore, it is more likely to negatively affect the lives of coastal organisms.

Table 1

The physicochemical variables at the sampling stations from the Samanlıdere Fisher Shelter, Yalova

Months	Temperature (°C)	DO (mg l^{-1})	pH	Salinity (PSU)
April	15	11.21	8.31	22
May	21.1	**11.22**	8.33	22
June	23.8	9.85	8.33	22
July	23.1	8.69	8.27	24
August	**24.8**	8.97	8.34	23
September	22.5	9.32	**8.42**	24
October	19.2	8.42	8.41	26
November	18.7	8.32	8.33	**27**

There has been a significant increase in the regional population in Yalova Province due to increasing industrialization and immigration. According to the YESR (2018) report, the sources of pollution in Yalova are missing or insufficient sewerage, domestic waste water in settlements, waste water from large industrial plants, pesticides, and chemical fertilizers. The region's Samanlı Stream is also one of the main factors in transferring land-based inputs to the sea. In addition, due to heavy ship traffic, it is thought that the shipyards located east and north of the sampling area, maintenance and repair work, and discharged bilge waters may increase heavy metal exposure. Moreover, Yalova Province, located in the Gulf of Izmit in the Sea of Marmara, may also be affected by the existing sources of pollution in the Sea of Marmara. Likewise, there are various reasons for heavy metal pollution in the Sea of Marmara. The main ones are industrial pollution, solid waste, and port activities. The province with the most industrial activity is Kocaeli, which has more than 1,000 industrial sites representing various sectors. Sectors such as oil refining contribute more than 30% of the fuel usage in Turkey through petrochemical complexes, hazardous and medical waste incineration plants, LPG filling facilities, textile production, tire production, machinery, mining, metals, food, automotive production and services, paper, chemistry, wood, tanning, coal, etc. (Pekey et al. 2004; Pekey et al. 2010; Ergul & Karademir 2020). In addition, Istanbul, a metropolitan city, is a very prominent source of anthropogenic pollution (Altuğ et al. 2009; Mol & Üçok Alakavuk 2011). Another cause of concern in regards to pollution is road and sea traffic. Since the Sea of Marmara is located between the Black Sea and the Aegean Sea, it is a region where maritime traffic is intense (Türk-Çulha et al. 2016). More than 42,553 total ships pass through the Turkish Straits annually (Kutluk 2018). The Pb in the fuel and bilge water that is discharged from ships increases the metal pollution in the Straits (Türk-Çulha et al. 2016). In particular, the heavy metal pollution carried to the Mediterranean by upper currents from the Dardanelles Strait is greater than the pollution carried to the Black Sea by lower currents. It is stated that rising heavy metal pollution in the Dardanelles Strait is caused by wastewater disrupting the stability of the aquatic environment (Süren et al. 2007; Altuğ et al. 2009). The variable water structure in the Sea of Marmara may have contributed to the accumulation of heavy metal pollution in the gulf and coastal areas. In the statistical analysis, significant differences were found in the heavy metal concentration values in all months ($p < 0.05$), most notably in October. Seasonally, the highest metal concentrations were found in autumn and spring, and the lowest in summer, which may be explained by periodic rains and terrestrial inputs (runoff). Similar findings were also reported by Fowler and Oregioni (1976), Bordbar et al. (2015), and Aydın-Önen and Öztürk (2017). The seasonal metal concentration changes are affected by several internal factors, such as the size, weight, age, sex, feeding behavior, metabolism, reproductive period, and growth rate of the organisms, as well as external factors, such as metal concentrations in the environment (terrestrial inputs) and the physicochemical parameters of the water (salinity, hardness, and temperature) (Powell

Table 2

Heavy metal concentrations in *Patella caerulea* samples (mg kg^{-1} dw; mean ± SE)

Months	Cd	Cu	Pb	Ni	Zn	Fe
April	2.60 ± 0.008^{a}	9.36 ± 0.09^{ae}	1.02 ± 0.016^{a}	102.0 ± 0.37^{a}	58.25 ± 0.59^{a}	1611.00 ± 19.86^{a}
May	2.31 ± 0.026^{b}	9.03 ± 0.04^{b}	0.86 ± 0.016^{bce}	74.75 ± 0.32^{bf}	60.76 ± 0.43^{b}	1415.00 ± 3.61^{bd}
June	3.27 ± 0.028^{c}	11.44 ± 0.42^{cd}	0.83 ± 0.005^{c}	83.65 ± 0.20^{cd}	66.22 ± 0.84^{cd}	1337.33 ± 9.53^{c}
July	2.55 ± 0.009^{d}	10.87 ± 0.49^{d}	0.74 ± 0.009^{d}	82.81 ± 0.66^{d}	67.33 ± 0.29^{d}	1436.33 ± 22.73^{d}
August	2.20 ± 0.008^{eh}	9.22 ± 0.01^{e}	0.88 ± 0.013^{ef}	47.59 ± 0.29^{ef}	80.86 ± 0.56^{e}	1365.33 ± 4.18^{e}
September	2.01 ± 0.005^{f}	6.33 ± 0.05^{f}	0.93 ± 0.019^{f}	46.63 ± 0.29^{f}	64.35 ± 0.26^{f}	1120.67 ± 4.26^{fh}
October	**5.74 ± 0.044^{g}**	**12.90 ± 0.13^{g}**	**1.95 ± 0.021^{g}**	**154.67 ± 0.24^{g}**	**109.57 ± 1.07^{g}**	**3086.00 ± 46.13^{g}**
November	2.21 ± 0.006^{h}	2.45 ± 0.02^{h}	1.59 ± 0.035^{h}	16.31 ± 0.02^{h}	21.12 ± 0.09^{h}	1134.00 ± 14.00^{h}

^{a,b,c,d,e,f,g,h} $p < 0.05$

& White 1990; Hernandez et al. 1992; Türk-Çulha et al. 2017). Heavy metal concentrations in *P. caerulea* have been studied in the Mediterranean, Aegean, and Black Seas, but no studies have been carried out on the species in the Marmara Sea. The heavy metal concentrations in *P. caerulea* and *Patella* spp. are presented in Table 3, with the results broken down for Turkish seas and different countries. When the studies from the marine coasts of Turkey are examined, it can be observed that the heavy metal concentrations detected in the *P. caerulea* samples taken from the Aegean (Aydın-Önen & Öztürk 2017), Black (Bat et al. 2015), and Mediterranean Seas (Yüzereroğlu et al. 2010) are considerably lower than those detected in the Sea of Marmara in this study. The concentration of Fe in *P. caerulea* was higher than that in the studies carried out in the Mediterranean Sea (Ramelow 1985; Türkmen et al. 2005; Yüzereroğlu et al. 2010; Duysak & Azdural 2017). The Ni and Cd concentrations measured in *P. caerulea* in the Sea of Marmara are similar to those found in the literature (Ramelow 1985; Türkmen et al. 2005), whereas the Pb concentrations were lower than in the reports of Türkmen et al. (2005), Ayas et al. (2009), and Duysak and Azdural (2017). However, the Cd concentration in the Mediterranean Sea were higher than those in this study (Duysak & Azdural 2017). In view of the results, it can be stated that the Samanlıdere Fisher Shelter (Marmara Sea) has a higher level of heavy metal accumulation than other Turkish coasts.

The heavy metal concentrations in the *Patella* spp. sampled from the Sea of Marmara were also compared to those sampled from different seas (Table 3). The studies which reported higher heavy metal concentrations in *P. caerulea* and *Patella* spp. than those detected in this study are as follows: concentrations of Cd, Cu, Pb, and Zn (Cubadda et al. 2001); Cd (Campanella et al. 2001) in *P. caerulea* samples collected from five stations along the coast of Favignana Island, Sicily, Italy; Cu and Zn (Conti & Cecchetti 2003) in samples taken from six shore stations in the Gulf of Gaeta, Tyrrhenian Sea, Italy; Cu, Ni, and Zn (Kontopoulos et al. 2003) in *P. caerulea* samples collected from 11 stations on the Saronic Gulf coast; Pb and Zn (Hamed & Emera 2006) in *P. caerulea* samples obtained from the west coast of the Gulf of Suez; Pb (Belkhodja et al. 2013) in samples from the northern Tunisian coast; Cu, Fe, and Zn (Bordbar et al. 2015) in samples taken from stations on the coast of Larymna Bay, Greece; and Cu, Pb, and Zn (Kelepertzis 2013) in samples taken from stations in Stratoni and Artemida coastal waters in northeast Greece. The first study regarding heavy metal accumulation in *Patella spp.* in the Marmara Sea showed that *P. careulea* is a good Ni bioindicator, since the Ni concentration was around threefold higher than in other countries. In particular, the studies on coastal regions showed very high heavy metal concentrations. The sources of heavy metal pollution detected in these studies from different parts of the world were collected under the following headings: industrial and urban waste discharge, transportation of geological metals by chemical industries, metal, refinery, rivers, and maritime traffic emissions, port and coastal activities, and discharge of ship waste. It is important to note that all of these factors are anthropogenic. The negative effects of anthropogenic pollution factors on living organisms are also revealed in this study.

Table 3

Heavy metal levels in *P. caerulea* and *Patella* spp. reported in previous studies (mg kg^{-1} dw; Nd – not detectable)

Site	Cd	Cu	Pb	Ni	Zn	Fe	Reference
Aegean Sea	0.004 - 0.065	Nd - 0.249	0.010 - 0.191	0.021 - 0.339	0.26 - 1.66	1.85 - 76.0	Aydın - Önen & Öztürk (2017)
Mediterranean Sea	3.9 - 80.0	1.6 - 25.0	4.9 - 26.1	2.0 - 54.2	22.7 - 756.8	121.3 - 1274	Duysak & Azdural (2017)
Black Sea	0.02 - 0.04	0.61 - 0.85	0.05 - 0.19	- -	12 - 23	21 - 38	Bat et al. (2015)
Mediterranean Sea	0.24 - 0.68	1.09 - 5.58	0.05 - 0.70	0.39 - 1.60	3.70 - 13.71	36.56 - 212.9	Yüzereroğlu et al. (2010)
Mediterranean Sea	0.11 - 2.04	- -	0.00 - 3.74	- -	- -	- -	Ayas et al. (2009)
Mediterranean Sea	2.39 - 4.97	1.58 - 4.02	4.28 - 14.53	3.60 - 12.21	23.13 - 46.59	15.34 - 41.20	Türkmen et al. (2005)
Mediterranean Sea	2.5 - 30.3	1.4 - 13.7	0.3 - 3.2	2.5 - 14.6	44.8 - 96	891 - 1512	Ramelov (1985)
Favignana Island (Italy)	3.30 - 6.30	1.21 - 2.35	0.14 - 1.52	- -	3.5 - 14.6	- -	Campanella et al. (2001)
Saronic Gulf (Greece)	- -	5.0 - 77.4	- -	6.0 - 31.6	43 - 367	96 - 3045	Kontopoulos et al. (2003)
Nord Coasts of Tunisia	0.43 - 1.50	5.59 - 9.29	2.73 - 3.61	3 - 4.14	- -	1.86 - 2.59	Belkhodja et al. (2010)
Tunisian Coastal	0.78 - 1.63	5.59 - 9.29	3.51 - 3.61	3 - 3.43	- -	1.86 - 2.59	Belkhodja & Romdhane (2013)
Larymna Bay (Greece)		7.2 - 95.8	- -	- -	38.1 - 126	843 - 1623	Bordbar et al. (2015)
Favignana Island (Italy)	1.7 - 11.8	0.47 - 3.79	0.06 - 2.18	- -	2.2 - 19.1	- -	Cubbada et al. (2001)
Tyrrhenian Sea (Italy)	2.89 - 4.06	10.2 - 19.2	0.51 - 1.50	- -	87.4 - 117.1	- -	Conti and Cecchetti (2003)
Red Sea	0.63 - 2.13	1.61 - 12.17	6.23 - 70.91	3.06 - 9.88	56.47 - 191.42	1.24 - 2.94	Hamed & Emara (2006)
North East Greece	- -	6 - 28	8 - 96	9	196 - 948	- -	Kelepertsiz (2013)
Marmara Sea	**2.84 ± 0.43 (2.01 - 5.74)**	**8.95 ± 0.16 (2.45 - 12.90)**	**1.10 ± 0.15 (0.74 - 1.95)**	**76.07 ± 14.77 (16.31 - 154.67)**	**66.06 ± 8.69 (21.12 - 109.57)**	**1563.21 ± 224.78 (1120.67 - 3086.00)**	**This study**

3.2. Assessment of health risks

Many bioindicators used to determine the degree of pollution in the environment can have economic characteristics and can make a great contribution to the country's economy through export, though some limitations should be considered in the context of quality control of exported products and safe food consumption. Limiting values may vary depending on the country. Accordingly, the maximum allowable limits for mollusks were compared with those specified by the FDA (2001), the EU (2008), and the TFC (2008). For Cd, the FDA level is 0.2 mg kg^{-1} (2001), the EU limit is 0.05–1 mg/kg^{-1} (2008), and the TFC level is 1 mg kg^{-1} (2008). The Cd values obtained for this study in all months were higher than those limits. The value for Pb is 1.5 mg kg^{-1} according to all three regulatory bodies. The legal consumable limits specified by the FDA (2001) for Zn and Cu are 150 and 100 mg kg^{-1}, respectively. The measured concentrations of Zn and Cu in all months were lower than these values. Since no limit was specified for Ni and Fe, a comparison could not be made.

The seafood consumption value for an adult was used to calculate the EDI and EWI values for *P. caerulea* sampled from Izmit Bay (Table 4), which proved to be lower than the ADI and PTWI values (FAO/WHO [2004; 2007; 2010]). Heavy metals can cause carcinogenic and mutagenic effects in humans over time (Goyer et al. 2003). The International Agency for Research on Cancer (IARC 2014) have reported that there is sufficient evidence that Ni, Cd, and Pb all have carcinogenic effects on humans. RfDs based on rates of seafood consumption are used to estimate the daily exposure of the human population to these metals because people can be exposed at these levels continuously throughout their lives without a significant risk of harmful effects (Saha & Zaman 2013).

THQ is used to express the risk of non-carcinogenic effects of metals and is a useful parameter for assessing the health risks associated with foods contaminated by heavy metals (Jezierska & Witeska 2006; Abdallah 2013). For this study, RfDs were also used to calculate the THQ values for local residents living in coastal areas, taking into account the maximum heavy metal concentrations in *P. caerulea*. The acceptable limit for THQ is 1 (USEPA 2020); values below 1 indicate no potential effect of exposure, while values above 1 are a potential risk to the population if exposed. As shown in Table 4, the THQ values for Cd, Ni, and Fe are greater than 1, indicating a serious health risk. To the best of our knowledge, no previous studies have performed a THQ risk assessment in *P. caerulea*; however, some recent studies on *Mytilus galloprovincialis* (Guendouzi et al. 2020; Jović & Stanković 2014) have reported a THQ value below 1.

Another parameter is CR, which is used to calculate the cancer risk in people exposed to heavy metal pollution through consumption. CR values less than 10^{-6} indicate a negligible carcinogenic risk, while those above 10^{-4} are unacceptable, according to the USEPA (2010), and those between 10^{-6} and 10^{-4} are generally considered acceptable (Fryer et al. 2006). The CR values calculated in the study are given in Table 4. The CPSo values given by the USEPA (2020) for Cd, Pb, and Ni are 6.30, 0.0085, and 1.7, respectively. The CR value for Pb is lower than the limit specified as carcinogenic. Nonetheless, the CR values for Cd and Ni were above the acceptable limits, indicating a potential health hazard for humans consuming *P. caerulea*. CR assessment was performed for the first time in *P. caerulea* and no similar study was found.

4. Conclusion

This is the first study on *P. caerulea* in the Sea of Marmara, and these results are important for monitoring and predicting the future impact of heavy metal concentrations in the region. Despite national and international policies to protect the environment and public health, a high rate of heavy metal pollution was detected in the coastal area of Marmara Sea, İzmit Bay, and Samanlıdere Fisher Shelter. In particular, the

Table 4

ADI, PTWI, EDI, EWI, THQ, THQ, and CR estimates for individual heavy metals caused by the consumption of *P. caerulea* for inhabitants of Samanlıdere Fisher Shelter, Yalova

Heavy Metals	Max. (mg kg^{-1})	ADI (mg kg^{-1})	PTWI (mg kg^{-1})	EDI (mg kg^{-1})	EWI (mg kg^{-1})	THQ	TTHQ	CR (mg kg^{-1})
Cd	5.74	0.06	0.44	0.002	0.014	**1.87**	**6.21**	**1.2 10^{-2}**
Cu	12.90	35	245	0.004	0.025	0.11		
Pb	1.95	0.25	1.75	0.001	0.007	0.16		5.4 10^{-6}
Ni	154.67	0.35	2.45	0.050	0.350	**2.52**		**8.6 10^{-2}**
Zn	109.57	70	490	0.036	0.252	0.12		
Fe	3086	56	392	1.005	7.035	**1.44**		

THQ, TTHQ, and CR values for *P. caerulea* were found to be higher than 1, and it was determined that the levels of Cd and Pb, which were higher than the national and international legal limits, may pose a carcinogenic risk to human health. Furthermore, the high concentration of Ni in *P. caerulea*'s tissue demonstrates that it is a good bioindicator for determining the Ni concentration in the environment. The uncontrolled discharge of industrial, domestic, and agricultural wastes in the Marmara Sea and the recent increase in mucilage is the strongest evidence of pollution in the sea. In light of these findings, we recommend that solid waste disposal methods and wastewater treatment systems be established to protect the ecology of the Marmara Sea and its aquatic life.

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