Heavy Metals in *Mytilus galloprovincialis*, *Rapana venosa* and *Eriphia verrucosa* from the Black Sea Coasts of Turkey as Bioindicators of Pollution

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Abstract

In the present study, the Mediterranean mussel (*Mytilus galloprovincialis*), the veined Rapa whelk (*Rapana venosa*), and the warty crab (*Eriphia verrucosa*) were collected from the Black Sea coast of Turkey in order to obtain data on the bioavailability of aluminium, arsenic, copper, zinc, mercury, iron, cadmium, and lead off the Sinop coast of the Black Sea in 2013. The aims of the study were to investigate the levels of heavy metals in benthic macroorganisms, in order to compare the results with the maximum permissible limits of these heavy metals proposed by the Ministry of Agriculture, Fisheries and Food, the European Union, the World Health Organization, and the Turkish Food Codex. Hg showed the least concentrations in all organisms, and was not detected in both *M. galloprovincialis* and *R. venosa*. Pb was also not detected in organisms, except in *M. galloprovincialis*. The highest values of Cu, Zn, and Fe were determined in *E. verrucosa*. Al was at the limit of detection in *R. venosa*, but was measurable in *E. verrucosa* (1.4 µg g⁻¹ dry wt.) and *M. galloprovincialis* (1.4 µg g⁻¹ dry wt.). As, Cu, Zn, Fe, and Cd levels in macrobenthic organisms ranged from 2.3 µg g⁻¹ dry wt. in *M. galloprovincialis* to 6.7 µg g⁻¹ dry wt. in *R. venosa*, 0.8 µg g⁻¹ dry wt. in *M. galloprovincialis* to 53.9 µg g⁻¹ dry wt. in *E. verrucosa*, 21.6 µg g⁻¹ dry wt. in *R. venosa* to 54.8 µg g⁻¹ dry wt. in *E. verrucosa*, 29.3 µg g⁻¹ dry wt. in *M. galloprovincialis* to 86.5 µg g⁻¹ dry wt. in *E. verrucosa*, and 0.32 µg g⁻¹ dry wt. in *E. verrucosa* to 4.4 µg g⁻¹ dry wt. in *R. venosa*, respectively. The weekly intakes of the studied metals per kg of body values did not exceed the Provisional Tolerable Weekly Intake established, with the exception of the highest Cd level in *R. venosa*. The obtained data, compared with the guidelines, showed that the metal concentration in *M. galloprovincialis*, *R. venosa*, and *E. verrucosa* are below the permissible level defined by international organizations, with no presentable danger to human consumption. These species could be a very good biomonitor for heavy metals.

Keywords: Heavy metals, *Mytilus galloprovincialis*, *Rapana venosa*, *Eriphia verrucosa*, Black Sea, Provisional Tolerable Weekly Intake (PTWI)

Introduction

The Black Sea is a unique marine environment, representing the largest landlocked anoxic basin in the world, with a maximum depth of 2200 m, a surface area of 4.2×10⁵ km², and a volume of 5.3×10⁵ km³ [1]. The basin is completely anoxic, containing an oxygenated upper layer (10 - 15 % of total sea volume) and anoxic deep water with hydrogen sulphide [2]. A permanent halocline separates the oxic and anoxic waters [3]. Its waters are almost completely isolated from the world’s ocean. There is a restricted exchange with the Mediterranean Sea through the Turkish Straits System: the Bosphorus, Dardanelles Straits, and the Sea of Marmara. The basin-wide distribution of oxygen-carrying cold intermediate layer waters has important implications for the health and ecology of the Black Sea [4,5]. The aerobicotic waters...
of the Black Sea are biologically productive [6] because of high run-off from many rivers, including the Danube, Dnepr, Dnestr, Kizilirmak, and Yesilirmak around the basin. The Black Sea is being threatened by the discharge of untreated sewage wastes and industrial effluents through the rivers flowing into the sea [7-9] which affects the sustainability of living resources and public health [10]. One of the most important pollutants in the Black Sea is heavy metals. Metal enrichments in the Black Sea were observed close to the major urban areas of coastal waters, mostly associated with large scale industrialization [7,8].

Many aquatic organisms have been known to accumulate significant quantities of contaminants in their tissues. Phillips [11] draws attention to the fact that for, an organism to be a useful biomonitor of heavy metal pollution, there should be a simple relationship between heavy metal levels in the environment and in the organism. Phillips and Rainbow [12] suggested the use of the term bioindicator for such species as the most accurate to describe organisms which accumulate contaminants in their tissues, and therefore can be analysed to identify the abundance and bioavailable of such contaminants in aquatic environments. The use of aquatic organisms, like bioindicators for heavy metal pollution, is common in actual studies, molluscs being the most used indicators. These are biofilter organisms that retain small particles from the water, so that the presence of pollutants in mussel tissues indicates a contamination of the marine environment. This study provides information about heavy metals pollution and their bioaccumulation mechanisms in the aquatic organisms living in the Black Sea.

The bioindicators chosen are the Mediterranean mussel, *Mytilus galloprovincialis*, the veined Rapa whelk, *Rapana venosa* and the warty crab (known in the Black Sea as rocky crab), *Eriphia verrucosa*. The Mediterranean mussel, *M. galloprovincialis*, is commonly employed in the monitoring of metal pollution [13-16] because they are consumed by humans and have a broad geographical range [17,18]. They also fulfil many of the criteria listed by [11,12]. Similarly, *R. venosa* is a useful heavy metal biomonitor [19]. It is one of the most important species in the Black Sea, coming from the Sea of Japan [20] after its introduction by a ship carrying its eggs attached to its hull in 1946 [21]. They mainly feed on mussels, oysters, and other bivalves [22], being strong accumulators of metals from food; its damage to the benthic ecosystem of the Black Sea is devastating [23]. Bondarev [24] pointed out that, in the Black Sea, only one crab, *E. verrucosa*, could be potentially dangerous to *R. venosa*, which lives in shallow waters, using as a refuge coastal rocks and rock scatterings, also inhabited by the favourite prey item of *R. venosa* - the mussel *M. galloprovincialis*. *E. verrucosa*, called pavurya in Turkish and küflü by the local people, is found in shallow waters and lives among stones and seaweeds down to depths of 5 - 15 m. They feed on molluscs and polychaetes [25]. Not much has been done in terms of the heavy metal accumulation in the edible tissues of *E. verrucosa* from the Turkish Black Sea coast, except the study of [26]. However comparative databases are available for all selected species as biomonitor. Determination of heavy metals from the tissues of macro benthic organisms from the Black Sea is very important, because the seawater contains discharged effluents, with various concentrations of pollutants. The flesh of *M. galloprovincialis*, *R. venosa*, and *E. verrucosa* are considered as suitable for human consumption. *R. venosa* meat has been exported from Turkey to Japan for many years.

The present study assessed heavy metal concentrations, measured by Inductively Coupled Plasma - Mass Spectrometer (ICP-MS), in the Mediterranean mussel, *M. galloprovincialis*, the veined Rapa whelk, *R. venosa*, and the warty crab, *E. verrucosa*, collected from the Black Sea coast, and evaluated the utility of these species as bioindicators. The aims of the current study are (1) to investigate the levels of Al, As, Cu, Zn, Hg, Fe, Cd and Pb in *M. galloprovincialis*, *R. venosa*, and *E. verrucosa* collected from the Black Sea coast of Turkey; (2) to compare the results of the present study with the maximum permissible limits of these heavy metals proposed by the Ministry of Agriculture, Fisheries and Food (MAFF, England), the European Union (EU), the World Health Organization (WHO), and the Turkish Food Codex; and (3) to compare the results with previous studies related to same species.

**Materials and methods**

**Study area**

Macrobenthic organisms were collected by scuba divers at depths of 1 - 10 m monthly between May and August in 2013 from the upper-infra littoral zone of Hamsilos (42°03´37´´ N and 35°02´36´´ E) and
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the port-side (42°01´18´´ N and 35°09´10´´ E) of the Sinop Peninsula of the Black Sea coast of Turkey (Figure 1). The station of the port-side was chosen in a way to include hotspots of pollution around Sinop City, such as domestic wastewater discharge points in the coastal zone of Sinop [27], and harbour activities, such as dredging, the dumping of ship wastes, and other coastal activities [15,28]. Hamsilos is located near the mouths of rivers which carry industrial discharges of pollutants to the offshore waters [28]. It is also common for fishing activities to be conducted at both stations.

Living specimens of molluscs were transported immediately from the sampling sites to the Fisheries Faculty Laboratory of Sinop University; subsequently, they were kept separately in clean seawater in tanks (20×20×25 cm) for 24 h for the contents of alimentary canals to be defecated [15,16,19]. Following elimination of the gut contents, the specimens were sorted with respect to their sizes and were separated into soft parts. Five analytical groups were prepared, in which the soft tissues of 25 individual specimens from each station were pooled for each group in order to obtain means of the samples.

Analytical procedure

All samples were stored in plastic bags in a deep freezer at −21 °C until their analysis. Metal analysis in macrobenthic organisms was performed using the m-AOAC 999.10- ICP/MS (Inductively Coupled Plasma - Mass Spectrometer) method accredited by the ÇEVRE Industrial Analysis Laboratory Services Trade Company (TÜRKAK Test TS EN ISO IEC 17025 AB-0364-T). EN 15763 European Standard methods were applied. The limits of detection used for the analysis of aluminium, arsenic, copper, zinc, mercury, iron, cadmium, and lead were 0.5, 0.05, 0.5, 0.5, 0.05, 0.5, 0.02, and 0.05 ppm, respectively.

Statistical analysis

For metal concentrations in organisms, the differences between organisms and between locations were determined by analysis of variance (ANOVA), followed by the post-hoc test of Tukey [29]. Possibilities of less than 0.05 (p<0.05) were considered statistically significant. Statistical calculations were performed with the Statistica 7.0 statistical package program for Windows. Microsoft Excel (2010) was used to calculate the mean and standard deviation and to plot graphs. All values were expressed on an mg/kg dry wt. basis. In addition, the weekly intake levels set by international organizations for health safety were estimated using the minimum and maximum metal levels in macrobenthic organisms EWI (Estimated Weekly Intakes) = maximum level of Hg (mg/kg), multiplied by seafood consumption (kg/70 kg body weight/week).
Results and discussion

Figures 2 - 9 show the variations in heavy metals content in organisms from Hamsilos and the port-side of the Sinop Peninsula of the Black Sea. Hg showed the least concentrations in all organisms, and was not detected in both M. galloprovincialis and R. venosa. Pb was also not detected in macrobenthic organisms, except in M. galloprovincialis. The highest values of Cu, Zn, and Fe were determined in E. verrucosa. Cu and Fe showed a significantly low accumulation in mussels compared to sea snail and crab. Al was at the limit of detection (<0.5) in all organisms, except in both E. verrucosa (1.4 µg g⁻¹ dry wt.) and M. galloprovincialis (1.4 µg g⁻¹ dry wt.) samples taken from the port-side station. The concentrations of other heavy metals in macrobenthic organisms ranged from 2.3 µg g⁻¹ dry wt. in M. galloprovincialis of the port-side station to 6.7 µg g⁻¹ dry wt. in R. venosa of Hamsilos, 0.8 µg g⁻¹ dry wt. in M. galloprovincialis to 53.9 µg g⁻¹ dry wt. in E. verrucosa of the port-side station, 21.6 µg g⁻¹ dry wt. in R. venosa of the port-side station to 54.8 µg g⁻¹ dry wt. in E. verrucosa of Hamsilos, 29.3 µg g⁻¹ dry wt. in M. galloprovincialis of Hamsilos to 86.5 µg g⁻¹ dry wt. in E. verrucosa of the port-side station and 0.32 µg g⁻¹ dry wt. in E. verrucosa to 4.4 µg g⁻¹ dry wt. in R. venosa of Hamsilos for As, Cu, Zn, Fe, and Cd, respectively.

![Figure 2](image_url)

**Figure 2** Difference of mean Al levels (µg/g dry wt.) among the species and stations. The different letters beside the vertical bars indicate that the values are significantly different (p < 0.05).
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Figure 3 Difference of mean As levels (µg/g dry wt.) among the species and stations. The different letters beside the vertical bars indicate that the values are significantly different (p < 0.05).

Figure 4 Difference of mean Cu levels (µg/g dry wt.) among the species and stations. The different letters beside the vertical bars indicate that the values are significantly different (p < 0.05).
Figure 5  Difference of mean Hg levels (µg/g dry wt.) among the species and stations. The different letters beside the vertical bars indicate that the values are significantly different (p < 0.05).

Figure 6  Difference of mean Fe levels (µg/g dry wt.) among the species and stations. The different letters beside the vertical bars indicate that the values are significantly different (p < 0.05).
**Figure 7** Difference of mean Cd levels (µg/g dry wt.) among the species and stations. The different letters beside the vertical bars indicate that the values are significantly different (p < 0.05).

**Figure 8** Difference of mean Pb levels (µg/g dry wt.) among the species and stations. The different letters beside the vertical bars indicate that the values are significantly different (p < 0.05).
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Figure 9: Difference of mean Zn levels (µg/g dry wt.) among the species and stations. The different letters beside the vertical bars indicate that the values are significantly different (p < 0.05).

These variations between stations may be due to differences in geographical locations. The highest concentrations of Al, Cu, Hg, Fe, and Pb were observed in the samples from the port-side station; this may be due to the discharge of untreated domestic wastes, harbour activities, fishery activities, the dumping of ship wastes, and other coastal activities. However, the highest concentrations of As, Zn, and Cd were observed in those from Hamsilos. This may be due to increasing metal input in the coastal zone from both rivers and non-point sources coming from many diffuse sources. In the area’s summer months, tourism activities and local fishing activities also may be affected. Al was only detected in *E. verrucosa* and *M. galloprovincialis* at the port-side station. However, Al has no known biological role, and its classification into toxic metals is controversial. Similarly, in this study, Hg was only detected in *E. verrucosa* (maximum 0.11 µg g⁻¹ dry wt.) at both stations. The maximum allowable level of Hg is 0.5 µg g⁻¹ wet wt. in crustaceans in the Turkish Food Codex [30] and the Commission Regulation [31]. Clarkson and Magos [32] pointed out that Hg vapor is emitted into the atmosphere from anthropogenic and natural sources. Bellinger and Benham [33] and Young *et al.* [34] emphasized that human anthropogenic activity input into the marine environment include contamination from ships in docks and harbor activities from the use of some heavy metals in antifouling paints and preservative paints. Moreover, industrial effluents containing metals used in processes such as electroplating, galvanizing, the production of alloys, pigments, electrical wiring, batteries, fertilizers and fungicides, and atmospheric input from the use of leaded petrol in motor vehicles are the source of metals in the coastal marine environment [35]. Pb, for example, is extremely insoluble and is readily absorbed by organic matter, especially under reducing conditions, and is highest in the immediate vicinity of industrial activities and river inputs [36]. The maximum Pb (0.7 µg g⁻¹ dry wt.) was found in *M. galloprovincialis* of the port-side station. Another non-essential metal, Cd, was found at the maximum (4.4 µg g⁻¹ dry wt.) in *R. venosa* from Hamsilos. The Turkish Food Codex [30,37] and the Commission Regulation [31] indicate that the maximum levels for mollusca are 1.5 mg kg⁻¹ wet wt. for Pb and 1.0 mg kg⁻¹ wet wt. for Cd. However, it is better to bear in mind that, in the present study, the results of the metal levels are given as dry wt. basis. The maximum levels of Zn and Cu were found in *E. verrucosa* at both stations at 54.8 and 53.9 µg g⁻¹ dry wt., respectively. Legal thresholds are not available for essential elements for the Commission Regulation. Tolerable values of Zn and Cu are 50 and 20 mg kg⁻¹ wet wt. for crustacea [30,38]. It is highlighted that higher Zn levels are permitted in foods which naturally contain more than 50 mg.kg⁻¹ wt., and higher levelsof copper in food are permitted if the copper is of natural occurrence [38]. Moreover, Zn and Cu
levels in *M. galloprovincialis* and *R. venosa* at both stations were quite below the the maximum tolerance levels for human consumption established by comparing the Turkish Food Codex (TGK) [30] and the Ministry of Agriculture, Forestry and Fisheries (MAFF) [38].

Recently, Bat [10] reviewed the heavy metals in biota from the Black Sea coast and suggested that among the molluscs, the mussel *M. galloprovincialis* is commonly used as a biomonitor of heavy metal pollution in coastal waters, followed by *R. venosa*. Mussels are used as filtering organisms; large volumes of water enter in contact with their body surface, and they are well known to accumulate heavy metals in their soft tissues. They are easy for the identification and collection of organisms, as they are abundant in ecosystems. Further, by consuming mussels, humans are exposed to metals with a potential danger to human health [10]. The highest Fe, Zn, Ni, Cu, Mn, Pb, Cd, and Co concentrations in *M. galloprovincialis* were 4030±121 µg/g dry wt. at Çayeli [43], 630±32 µg/g dry wt. at Çamburnu [39], 43.8 µg/g dry wt. at Samsun [40], 260±8 µg/g dry wt. at Rize [39], 73.05 µg/g dry wt. at Samsun [40], 108.6 µg/g dry wt. at Samsun [40], 6.44±0.01 µg/g dry wt. at Amasra [41], and 5.36±0.33 µg/g dry wt. at Rize [41], respectively. Heavy metals do not show clear trends of evolution in one specific direction, as large fluctuations in concentrations have been observed over years and between stations [10]. The predator gastropod, *R. venosa*, prefer mussels, especially *M. galloprovincialis*, as food. Thus, it is suggested that there are differences in inherent response to heavy metals among molluscan species [10]. *R. venosa* was also studied for heavy metal pollution, collected from Fatsa, Perşembe, Rize, and the Sinop coast [19,41-46]. Heavy metals except Co reviewed by Bat [10] in *M. galloprovincialis* were higher than those in *R. venosa*. In general, heavy metal levels were found to be lower than in those other studies. It can be said that the Sinop coast is a relatively unpolluted marine environment, since no industry, and only small settlements, exist in the surrounding region [47].

Crustaceans have also been used as bioindicators in the Black Sea. One reason is that they are a very successful group of animals, distributed in a number of different habitats, and are thus interesting candidates for comparative investigations [10]. Very little information is available on the bioaccumulation of metals in crustaceans [26,28,48-50]. Only one study [26] is available on heavy metal levels in *E. verrucosa* from the Turkish Black Sea coast. When compared with the current study, Fe, Zn, Cu, and Cd levels in *E. verrucosa* were lower than those in this study, whereas Pb was higher than those in this study.

The results revealed that Fe concentrations were the highest, followed by Zn and Cu. Essential metals, such as Fe, Zn, and Cu, play an important role in biological systems for metabolism, while non-essential metals, such as Hg, Pb, and Cd, are toxic, even in trace amounts [51]. Essential metals may produce toxic effects at high concentrations, but their concentrations in aquatic organisms tend to be highly regulated compared to non-essential metals [52,53].

Among the different metals analysed, Hg, Cd, and Pb are classified in seafood as chemical hazards, and maximum residual levels have been prescribed for humans by various organizations [31,38,54]. In the current study, Hg, Pb, and Al levels in most of the samples were not detected.

Under regular consumption habits, it is important to assess daily intake of the metals from seafood and compare it with the total acceptable daily intake values set by international organizations for health safety. The average daily mollusca and other seafood consumption in Turkey is 1 g per person, which is equivalent to 7 g/week for Turkey [55]. The Provisional Tolerable Weekly Intake (PTWI) value is an estimate of the amount of a contaminant that can be consumed by a human over a lifetime without appreciable risk. PTWI was established by the Joint Food and Agricultural Organization for the United Nations (FAO) / World Health Organization (WHO) Expert Committee on Food Additives (JECFA) [56]. PTWI values were used in the present study to serve as reference values for safe levels of these metals. *Table 1* shows accepted safe levels of the Joint FAO/WHO Expert Committee on Food Additives (JECFA) and the Council of Europe for the metals. PTWI values are for an adult person with a 70 kg body weight.
Table 1 Internationally accepted safe levels for the studied metals.

<table>
<thead>
<tr>
<th>Metals</th>
<th>Standard</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>Al</td>
<td>PTWI of 1 mg per kg body wt. per week</td>
<td>[57]</td>
</tr>
<tr>
<td>As</td>
<td>Withdrawn</td>
<td>[57]</td>
</tr>
<tr>
<td>Cu</td>
<td>PTWI of 3.5 mg per kg body wt. per week</td>
<td>[58,59]</td>
</tr>
<tr>
<td>Zn</td>
<td>PTWI of 7 mg per kg body wt. per week</td>
<td>[58,59]</td>
</tr>
<tr>
<td>Hg</td>
<td>PTWI of 0.004 mg per kg body wt. per week</td>
<td>[57]</td>
</tr>
<tr>
<td>Fe</td>
<td>PTWI of 5.6 mg per kg body wt. per week</td>
<td>[57]</td>
</tr>
<tr>
<td>Cd</td>
<td>PTWI of 0.007 mg per kg body wt. per week</td>
<td>[60-62]</td>
</tr>
<tr>
<td>Pb</td>
<td>PTWI of 0.025 mg per kg body wt. per week</td>
<td>[56,61]</td>
</tr>
</tbody>
</table>

Estimated Weekly Intake (EWI) and Estimated Daily Intake (EDI) for an adult person with a 70 kg body weight on the basis of the present study results, using minimum and maximum metal levels, are presented in Table 2, and were uses the standards in Table 1 for assessment of these metals in macroorganisms. As can be seen from Table 2, the estimated EWIs of the metals in the current study are quite below the established accepted safe levels, except for the maximum Cd level in R. venosa from Hamsilos.

Table 2 Estimated Weekly Intakes (EWI) and Estimated Daily Intakes (EDI) of the metals in tissue of M. galloprovincialis, R. venosa, and E. verrucosa from the Black Sea coast of Turkey. (LOD = Limits of detection).

<table>
<thead>
<tr>
<th>Metals</th>
<th>PTWI (mg/week/70 kg body wt.)</th>
<th>PTDI (mg/day/70 kg body wt.)</th>
<th>EWI (min.-max.)</th>
<th>EDI (min.-max.)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Al</td>
<td>70</td>
<td>10</td>
<td>LOD-0.048</td>
<td>LOD-0.0069</td>
</tr>
<tr>
<td>As</td>
<td>-</td>
<td>-</td>
<td>0.0161-0.046</td>
<td>0.0023-0.0067</td>
</tr>
<tr>
<td>Cu</td>
<td>245</td>
<td>35</td>
<td>0.0056-0.377</td>
<td>0.0008-0.0539</td>
</tr>
<tr>
<td>Zn</td>
<td>490</td>
<td>70</td>
<td>0.1512-0.3836</td>
<td>0.0216-0.0548</td>
</tr>
<tr>
<td>Hg</td>
<td>0.28</td>
<td>0.04</td>
<td>LOD-0.00077</td>
<td>LOD-0.00011</td>
</tr>
<tr>
<td>Fe</td>
<td>392</td>
<td>56</td>
<td>0.2051-0.6055</td>
<td>0.0293-0.0865</td>
</tr>
<tr>
<td>Cd</td>
<td>0.49</td>
<td>0.07</td>
<td>0.0024-0.0308</td>
<td>0.00032-0.0044</td>
</tr>
<tr>
<td>Pb</td>
<td>1.75</td>
<td>0.25</td>
<td>LOD-0.0049</td>
<td>LOD-0.0007</td>
</tr>
</tbody>
</table>
Conclusions

In many studies of the Black Sea, *M. galloprovincialis* [14-16,39-41] have been used as a biomonitor; very little information about such programmes using marine crustaceans can be found, at least not those dealing specifically with heavy metals [10]. However, due to their great importance in the food webs of the intertidal zone in the Black Sea, crustaceans, such as *E. verrucosa*, merit further consideration. The results of the current study supply valuable information on the heavy metals concentrations in *M. galloprovincialis*, *R. venosa*, and *E. verrucosa* from the southern Black Sea, and indicate that these species could be very good biomonitors for heavy metals in their habitat.

The weekly intakes of the studied metals per kg of body values did not exceed the Provisional Tolerable Weekly Intake (PTWI) established, with the exception of the highest Cd level in *R. venosa* in Hamsilos. It may be suggested that continuous care must be taken to biomonitor heavy metal levels in *R. venosa*, especially if they exceed international and local permissible limits for human consumption. However the obtained data, compared with the guidelines, showed that the metal concentrations in *M. galloprovincialis*, *R. venosa*, and *E. verrucosa* are below the permissible levels defined by the European Commission Regulation, MAFF, and the Turkish Food Codex, with no present danger to human consumption.

Acknowledgments

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