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FULL PAPER TAM MAKALE

HEAVY METALS IN EDIBLE TISSUES OF BENTHIC ORGANISMS FROM SAMSUN COASTS, SOUTH BLACK SEA, TURKEY AND THEIR POTENTIAL RISK TO HUMAN HEALTH

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Abstract:

The present study was aim to determine the concentrations of copper, cobalt, lead, zinc, cadmium, manganese, nickel and iron in the edible tissues of *Liocarcinus depurator* (decapoda), *Rapana venosa* (gastropoda) and *Mytilus galloprovincilais* (mollusca) collected from Samsun coasts of the Black Sea, Turkey in 2010. These biomonitor species are major food sources of benthic ecosystem. Exposing to heavy metals at higher concentrations might be toxic to demersal fish species and also humans. The concentrations of the metals were carried out using Flame Atomic Absorption Spectrophotometer (UNICAM 929). Metal concentrations in *L. depurator*, *R. venosa* and *M. galloprovincilais* decrease in the order: Fe > Zn > Mn > Cu > Ni > Pb > Co > Cd; Fe > Zn > Cu > Pb > Mn > Cd > Ni and Fe > Zn > Cu > Pb > Mn > Co > Ni > Cd, respectively. The results showed that the Fe and Zn concentrations were the highest in edible tissues of the three benthic organisms, respectively. Mn was higher in the edible tissues of *L. depurator* than those in other species, while *R. venosa* and *M. galloprovincilais* shows more of Cu and Pb levels. The estimates of EWI and EDI indicated no health risk as values are lower than the allowed tolerable levels cited by internationals committees. Based on the above results of this study, metal accumulation in the biomonitor demersal species study did not exceed the permissible limits set for heavy metals by FAO/WHO.

Keywords: *Liocarcinus depurator*, *Rapana venosa*, *Mytilus galloprovincialis*, South Black Sea of Turkey, Heavy metals, Seafood, EWI.

Introduction

Heavy metals occur naturally; on the other hand their levels have increased with domestic, industrial, mining and agricultural activities. Especially discharge of heavy metals into the marine environment may cause extensive ecological differences, due to their toxicity, persistence and accumulative behaviour in the organisms (Dural *et al.* 2006, Bat *et al.* 2009).

The Black Sea had been exposed to pollution pressure that derives both from human activities and natural sources. Samsun Province is situated in the middle of the Black Sea in Turkey which extends along the coast between two major rivers, Kızılırmak and Yeşilırmak (Figure 1). The city also encounters rapid population growth, urbanization without conforming to city plans and intense agricultural activity involving making use of fertilizers, insecticides, and herbicides etc. (Pınarlı *et al.* 1991). Fertilizers and pesticides are seriously used and increased in agricultural fields in this region and all of them create local problems of heavy metal pollution along the Samsun coasts (Kurt and Özkoç 2004, Bakan *et al.* 2010). Besides small or full scale industrial activities such as food, cement, resin, plastic and textile are carried out in this region (Altaş and Büyükgüngör 2007, Bakan *et al.* 2010). Moreover, the province has the largest Black Sea commercial port which covers an area of 9579 km^2.

The decapod crustacean, *Liocarcinus depurator*, called harbour crab, is a species of crab found in the Black Sea and also North Sea, Atlantic Ocean and Mediterranean Sea. There is no available data on heavy metal levels of *L. depurator* in the Black Sea, while some studies are available on taxonomy, length-weight relationship and condition factor of this species (Ateş 1999, Aydın *et al.* 2013). It is not commercially important species for human consumption in Turkey, but it is a common prey and predator of many marine commercial and non-commercial species that is the main food source for crustaceans, molluscs, polychaetes and fishes (Freire 1996). Gastropods are useful and generally used in the monitoring of metal pollution (Bat *et al.* 2000). Veined Rapa whelk, *Rapana venosa*, is a species of large predatory gastropod whose main diet consists of mollusc species and mussels. Filter feeding organisms, bivalves have a sedentary life, so they are directly influenced by environmental conditions. *Mytilus galloprovincialis*, the Mediterranean mussel, is a species of bivalve have a broad geographical range. It is consumed by humans, thus it is used in monitoring metal pollution (Bat *et al.* 1999; Özden *et al.* 2010). These demersal species are important for transferring energy between trophic levels and to represent coastal environmental conditions.

The aim of this study was aim to determine the concentrations of essential (Cu, Co, Fe, Zn, Mn, Ni) and non-essential (Pb, Cd) heavy metals in the edible tissues of *L. depurator*, *R. venosa* and *M. galloprovincilais* collected from Samsun coasts, Turkey in 2010 and to compare the international and national permissible limits set for heavy metals by FAO/WHO.

Materials and Methods

Study area

The study area, Samsun, is located in the middle of the Southern Black Sea of Turkey (Figure 1) that was selected as sampling location because it is in the way of various industrial and domestic effluent discharges, harbour and agricultural activities.

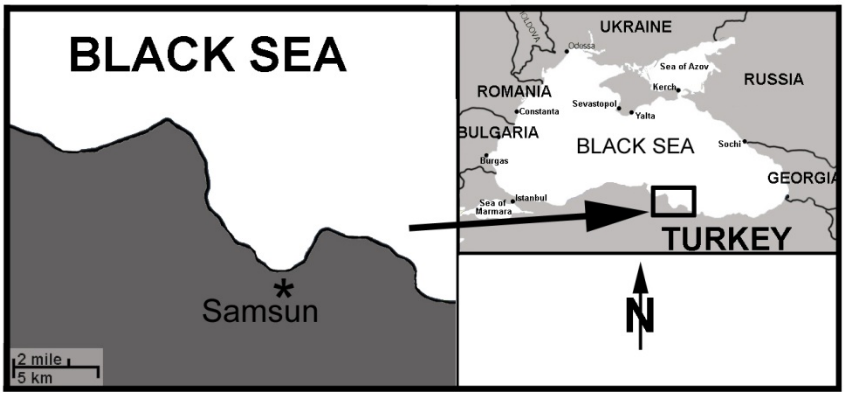


Figure 1. Sampling area from Samsun coasts of the Black Sea, Turkey

Materials

Samples of the harbour crab or sandy swimming crab (*Liocarcinus depurator*), veined Rapa whelk (*Rapana venosa*) and the Mediterranean mussel (*Mytilus galloprovincialis*) were collected by scuba divers from the upper-infra littoral zone of Samsun coasts of the Black Sea, Turkey, at a water depth of 0-5 m, between July and September in 2010.

The size of mussels for marketing changes between 50-80 mm in Europe while the minimum size of the mussels for consumption is approximately 70 mm in length in Turkey (Aral 1999). In this study the size of mussels (6-8 cm) (Bat et al. 2012), snails (8-12 cm) (Bat et al. 2000) and the carapace length (2.5-3 cm) and width (3-3.5 cm) of crabs were chosen for metal analysis.

Methods

The samples were brought to the laboratory and washed thoroughly with double distilled water then were rinsed with clean seawater to remove sand particles and then placed in about 20 litres of constantly aerated clean seawater for 24 hours to allow depuration (Bat *et al*. 1999). Following elimination of the gut contents the shell of the samples were removed and discarded. Subsequently, whole edible bodies of 25 individuals for each species blotted, homogenized and stored at -21°C until required for analysis. Only edible parts of all the samples were analysed. Upon thawing, the whole soft parts were removed using stainless steel instruments. The samples were analysed with 3 replicates for each measurements. Aliquots of 10 g fresh weight were digested with 20 ml HNO_3 at 30-40°C. After a clear yellow solution was formed and this was diluted with double distilled water and filtered before heavy metal (Cu, Co, Pb, Zn, Cd, Mn, Ni and Fe) analysis (modified from Bernhard 1976, UNEP 1984 and 1985).

The heavy metal concentrations were determined by the Flame Atomic Absorption Spectrophotometer (UNICAM 929). All reagents used in the analysis were of reagent grade. Double-deionized water was used for all dilutions. Chemicals were of high pure quality (Merck). The element standard solutions from (Merck) that were used for the calibrations were prepared by diluting stock solutions of 1000 mg L^{-1}.The working standard values were used to plot a standard curve. The standards and blank were treated in the same way as the real samples to minimize matrix interferences during analysis. Laboratory glassware was kept overnight in a 10% v/v HNO_3 solution and then rinsed with deionised double distilled water (UNEP 1984 and 1985). The measured concentrations are expressed in mg/kg wet wt. with the mean ± standard deviation.

Intake Levels Calculation

The average heavy metal weekly intake was calculated according to the following formula:

Heavy metals intake level = average heavy metal content X consumption of seafood per person/ body weight

Statistical analysis

The data collected from heavy metals analysis was statistical analysed by analysis of variance (ANOVA) and with the multiple comparisons Tukey's test to find out significant level among organisms using Statistica 7.0 statistical package program. In all cases, the estimation was carried out at significant level of 0.05 (Zar 1984).

Results and Discussion

The concentrations of the metals in edible tissues of the samples are given in Figure 2 (A, B, C, D, E, F, G and H) along with the statistical parameters. Statistical analysis of the data showed significant differences among all of the samples. The mean heavy metal levels varied among considerably depending on the type of species.

The present study was performed for assessment of eight heavy metals (Cu, Co, Pb, Zn, Cd, Mn, Ni and Fe) in the edible tissues of *L. depurator, R. venosa* and *M. galloprovincilais* collected from Samsun coasts of the Black Sea, Turkey in 2010. These heavy metals were chosen in this study. They are not only toxic to marine organisms, but transfer through the food chain means that they are also potentially harmful to humans (Underwood 1977, Förstner and Wittmann 1983). The bioaccumulation degree depends on the type of food chain and also bioavailability and persistence of the heavy metals in the water.

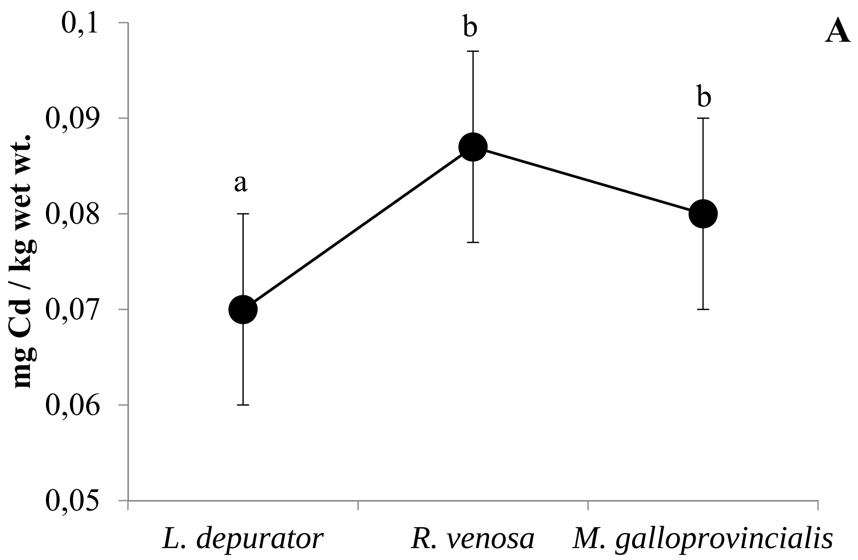
Cu, Co, Zn, Mn, Ni and Fe are essential elements for organisms whereas Pb and Cd are non-essential metals and have no role in biological processes in living organisms and are toxic at low concentrations. Legal thresholds are not available for essential metals in European Commission Regulation. However, in the edible tissues of the average Zn and Cu concentrations were on average below the maximum tolerance levels for human consumption established by the Turkish and international legislations (Anonymous 1995, MAFF 1995). The highest concentration of Zn (19 ±3 mg/kg wet wt.) found in *L. depurator* is less than 50 mg/kg wet wt. in Crustaceans, well below the guideline level (Anonymous 1995, MAFF 1995). The highest Cu concentration (9 ±3 mg/kg wet wt.) was found in *M. galloprovincialis* and this level is also quite below the guideline level of 20 mg/kg wet wt. for Mollusca (Anonymous 1995; MAFF 1995).

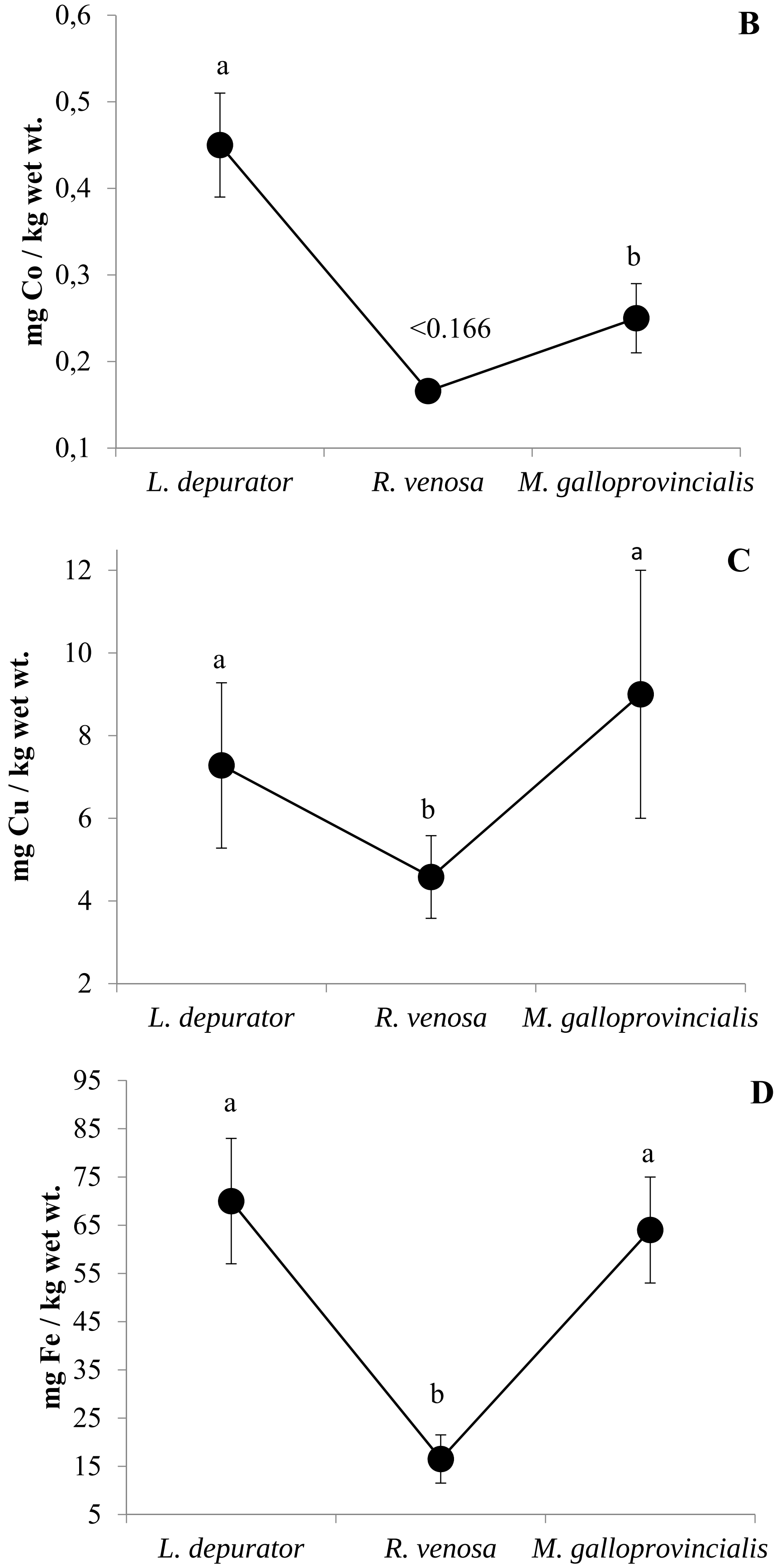
The Cu level (9±3 mg/kg wet wt.) in the mussels of this study higher than those (0.850-3.473 mg/kg wet wt.) in the Sea of Marmara (Mol and Üçok Alakavuk 2011), it is probably related to discharge form urban areas and small industries flowing into the coast of Samsun. It might be said that examined mussels are safe for human consumption regarding Cu and Zn. Cd concentrations in *M. galloprovincialis* (0.008±0.001 mg/kg wet wt.) in this study, were lower than those indicated by Mol and Üçok Alakavuk (2011) for the Sea of Marmara while they were between 0.296 and 0.740 mg/kg wet wt. where Pb levels were similar.

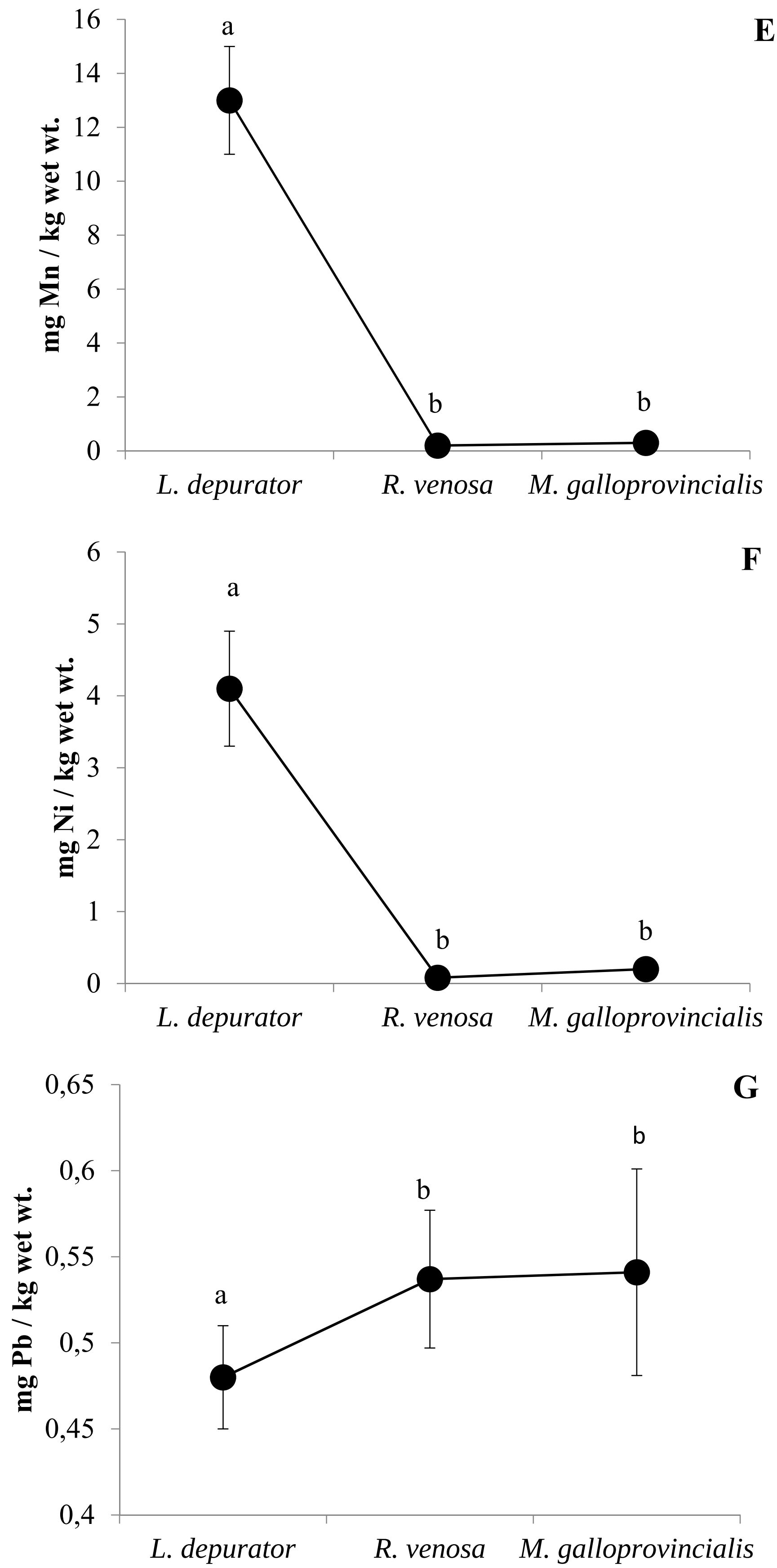
Cd and Pb are toxic and bio accumulative these metals with no known biological function and its absorption may constitute a serious risk to public health. By comparing the data from different the Southern West Black Sea regions (Mülayim and Balkıs 2015) it is possible to note that Cd concentrations (0.087 ±0.01 mg/kg) in *R. venosa* observed in this study in the Samsun coast of the Black Sea were lower than those observed (0.1-1.6 mg/kg) by Mülayim and Balkıs (2015). However Pb levels were similar in both studies.

However comparing data obtained in this study, Cd concentrations in crabs were much lower than those reported in other studies (Ayas 2013; Mülayim and Balkıs 2015), where Pb values were similar in all studies. Moreover Cu and Zn levels in *E. verrucosa* were lower than those in blue swimmer crabs (*Portunus pelagicus*) from the North-eastern Mediterranean Sea (Ayas 2013).

Pb and Cd are extremely toxic to humans through food chains. Commission Regulation (EC) (2006) and Turkish Food Codex (TFC) (2008) indicate that maximum level of Pb in Crustacea and Mollusca are 0.5 and 1.5 mg/kg wet wt., respectively. In case of the maximum level of Cd in Crustacea and Mollusca are 0.5 and 1.0 mg/kg wet wt., respectively.




B
mg Co / kg wet wt.
0,6
0,5
0,4
0,3
0,2
0,1
a
b
<0.166
L. depurator
R. venosa
M. galloprovincialis
C
mg Cu / kg wet wt.
12
10
8
6
4
2
a
b
a
L. depurator
R. venosa
M. galloprovincialis
D
mg Fe / kg wet wt.
95
85
75
65
55
45
35
25
15
5
a
b
a
L. depurator
R. venosa
M. galloprovincialis


E
mg Mn / kg wet wt.
16
14
12
10
8
6
4
2
0
a
b
b
L. depurator
R. venosa
M. galloprovincialis
F
mg Ni / kg wet wt.
6
5
4
3
2
1
0
a
b
b
L. depurator
R. venosa
M. galloprovincialis
G
mg Pb / kg wet wt.
0,65
0,6
0,55
0,5
0,45
0,4
a
b
b
L. depurator
R. venosa
M. galloprovincialis

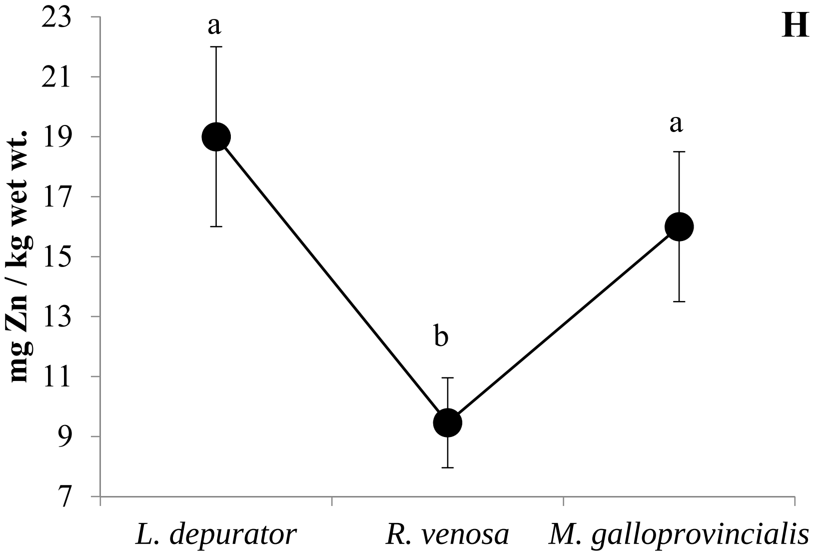


Figure 2 (A,B,C,D,E,F,G,H). The means with standard deviations (vertical line) of Cd (A), Co (B), Cu (C), Fe (D), Mn (E), Ni (F),Pb (G) and Zn (H) concentrations (mg/kg wet wt.) in the edible tissues of *Liocarcinus depurator* (decapoda), *Rapana venosa* (gastropoda) and *Mytilus galloprovincilais* (mollusca) from Samsun coasts of the Black Sea Turkey in 2010. a, b = The same letters beside the vertical bars in each graph indicate the values are not significantly different (P>0.05).

Overall, the findings from the present study revealed that Cu, Co, Pb, Zn, Cd, Mn, Ni and Fe concentrations in the edible tissues of *L. depurator*, *R. venosa* and *M. galloprovincilais* were lower than the maximum permissible limit as recommended by the Commission Regulation (EC, 2006) and Turkish Food Codex (TFC, 2008). Aquatic organisms having toxic metals could present a toxic threat for the consumer which is dependent on the metal concentration and amount of their consumed. Therefore, the tolerable weekly intakes were estimated by means of references for edible tissues of aquatic organisms consumed by people. According to FAO estimates of Molluscs and other (except fish) daily consumption in Turkey is 1 g per person (FAO 2010). This is also equivalent to 7 g/week. However, the annual quantity of seafood (including fish) consumed is 6,918 kg / person in 2010 (TUIK 2014), which is equivalent to 18.95 g/day for Turkey. The tolerable weekly intake of heavy metals as PTWI (Provisional Tolerable Weekly Intake), are set by the Food and Agriculture Organization/World Health Organization (FAO/WHO) Joint Expert Committee on Food Additives (JECFA). PTWI is the maximum amount of a contaminant to which a person can be exposed per week over a lifetime without an unacceptable risk of health effects. The estimated daily intake (EDI) and estimated weekly intake (EWI) in this study were calculated and presented in Table 1. Intake estimates were expressed as per unit body weight (mg/kg body wt. /weekly and daily).

Although the estimated Co intake is 0.2 to 1.8 mg/day (Codex, 1995), JECFA has not evaluated cobalt. There is no evidence that the intake of cobalt is ever limiting in the human diet, and no Recommended Dietary Allowances (RDA) is necessary (National Academy of Sciences 1989). It seems that the use of cobalt in general causes no problems (Council of Europe 2001). The Joint FAO/WHO Expert Committee on Food Additives established a PTWI for Cu, Pb, Zn, Cd, Mn, Ni and Fe of 3.5, 0.025, 7, 0.007, 2-5, 0.035 and 5.6 mg/kg body weight/week which was equivalent to 245, 1.75, 490, 0.49, 140-350, 2.45 and 392 mg/week for a 70 kg adult person, respectively (National Academy of Sciences, 1989; FAO/WHO, 1996; Council of Europe, 2001; WHO, 2011). In the present study the EWIs and EDIs for Cu, Co, Pb, Zn, Cd, Mn, Ni and Fe from consumption of seafood from Samsun coasts of the Blacks Sea were estimated (see Table 1).

Table 1. Estimated Weekly Intakes (EWI) and Estimated Daily Intakes (EDI) of heavy metals in edible tissues of seafood from Samsun Coastal waters of the Black Sea, Turkey.

Metals	PTWI[a]	PTDI[b]	EWI[c]		EDI[d]	
			Minimum	Maximum	Minimum	Maximum
Cu	245	35	0.608	1.194	0.087	0.171
Co	-	-	<0.022	0.060	<0.003	0.008
Pb	1.75	0.25	0.064	0.072	0.009	0.010
Zn	490	70	1.255	2.520	0.179	0.360
Cd	0.49	0.07	0.009	0.012	0.001	0.002
Mn	140-350	20-50	0.022	1.724	0.003	0.246
Ni	2.45	0.35	0.010	0.544	0.001	0.078
Fe	392	56	2.191	9.286	0.313	1.327

[a]PTWI (Provisional Tolerable Weekly Intake) (mg/week/70 kg body wt.)
[b]PTDI (Permissible Tolerable Daily Intake) (mg/day/70 kg body wt.)
[c]EWI (Estimated Weekly Intake) (mg/week/ kg body wt.)
[d]EDI (Estimated Daily Intake) (mg/day/ kg body wt.)

Conclusion

The obtained data with guidelines showed that the metal levels in *L. depurator*, *R. venosa* and *M. galloprovincilais* are below the permissible level defined by Commission Regulation (EC), MAFF and Turkish Food Codex. On the other hand these data provided that the heavy metal concentrations in these organisms do not present any danger to human health. Consequently, adverse human health effects may occur if contaminated seafood is consumed too much. However it can be seen that the estimated EWIs and EDIs of heavy metals in this study are far below the recommended PTWIs and/or PTDIs and indicated no adverse effects to the consumers. It may be suggested that continuous care must be taken to biomonitor the heavy metal levels in the harbour crab or sandy swimming crab, veined Rapa whelk and the Mediterranean mussel especially if they exceed international and local permissible limits for human consumption.

It was concluded that benthic organisms from the Samsun coast of the Black Sea, Turkey is safe as regards the heavy metals studied. Thus, heavy metal levels in seafood must be monitored periodically with respect to consumer health. On the other hand, these data could allow people to make informed decisions about which product to eat in order to reduce the risks of contaminants.

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