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The levels of heavy metals in the Mediterranean mussel (*Mytilus Galloprovincialis* Lamarck, 1819); Example of Giresun coasts of the Black Sea, Turkey

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Heavy metal accumulation levels in the soft tissue of mussel (*Mytilus galloprovincialis* Lamarck, 1819) individuals, collected from eight stations along the 122 km long Giresun coast, were investigated. Heavy metal levels (Co, Cr, Cu, Fe, Mn, Ni, Pb, Zn, As, and Se) were determined by using Inductively Coupled Plasma – Mass Spectrometer (ICP MS). Results were given as µg/g in wet weight mean ± SE; Cr; 0.56 ± 0.03, Mn; 6.23 ± 0.38, Ni; 12.70 ± 1.01, Zn; 69.06 ± 4.20, Co; 1.97 ± 0.07, Cu; 2.65 ± 0.18, Fe; 161.08 ± 15.89, Pb; 3.16 ± 0.08, As; 3.16 ± 0.11, and Se; 0.62 ± 0.05. Metal concentrations in descending order were as follow; Fe > Zn > Ni > Mn> As > Pb > Cu > Co > Se> Cr, respectively. Length and weight distribution of mussel samples were significantly different between some stations ($p<0.05$). The mean Pb and Zn concentrations were higher than the acceptable limit proposed by International Turkish standards.

[Keywords: Mussel, Public health, Pollution, Metal accumulation, Bio-indicator]

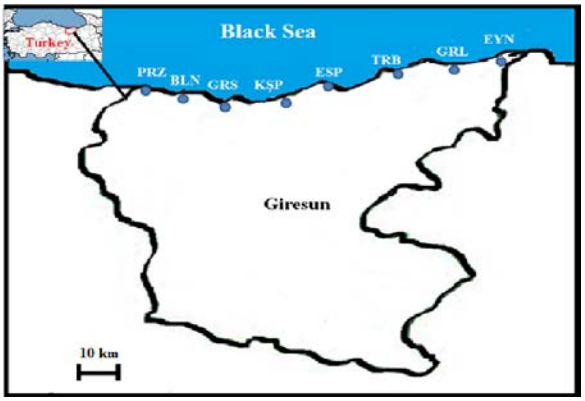
Introduction

The Black Sea, the most isolated from the oceans, is an inland marine basin located along the north of Turkey. The Black Sea, with a surface area of 461,000 km^2, has a total volume of 547,000 km^3 and a maximum depth of around 2210 m. Mussels are considered one of the most sensitive organisms to heavy metals some of which are lethal even at low levels. Due to these properties, mussels have been used as biological indicator organisms to monitor and assess marine pollution [1-2-3-4&5]. The mussel *Mytilus galloprovincialis,* is common along the Black Sea Coast of Turkey due to the low sea water temperature and salinity which are the optimum conditions for life and productivity of *M. galloprovincialis*[6]. A few papers have been published concerning heavy metal concentrations observer in mussel samples at the Black Sea[7-4&5.] However, heavy metal contamination of mussel from Giresun Coast of Black Sea has never been reported earlier.

Present study is to monitor and assess the heavy metal contamination levels of heavy metals in a mussel species along Giresun coasts at the Black Sea. For this purpose, Mediterranean Mussel (*Mytilus Galloprovincialis* Lamarck, 1819) has been sampled and analyzed for their Co, Cr, Cu, Fe, Mn, Ni, Pb, Zn, As, and Se contents. The results of the present study would also be a reference to assess and compare current contamination levels before the recent change in Turkish Mining Waste Directive which allows the discharge of mining waste into seas.

Materials and Methods

Mussels were collected from eight stations, different town each, along 122 km Black Sea Coastline of Giresun in October 2011 (Fig. 1). Thus, name of each sampling town were given as stations name as follows; Piraziz (PRZ), Bulancak (BLN), Giresun (GRS), Keşap (KŞP), Espiye (ESP), Tirebolu (TRB), Görele (GRL), Eynesil (EYN).



Fig—1 Location of sampling stations along the Giresun Black Sea Coasts.

Mussel samples were collected by the aid of a professional diver from 2-4 meter water depth. Investigated mussel species is very common in the Black Sea coastal regions of Turkey, and also have commercial importance. Representative mussels were in proper dimensions for public consumption. The collected samples were brought to the laboratory and freshly dissected according to UNEP, 1982[8]. Approximately 0.5 gram sample of muscle from each mussel were dissected, washed with distilled water, weighed, packed in polyethylene bags, labeled and stored at -18 ^{o}C until the performance of chemical analysis. Tissues were homogenized in a blender and digested with 10 ml of HNO_3 (analytical grade) in Teflon vessels in microwave oven (CEM MARS-5 Closed Vessel Microwave Digestion System). Some of the advantages of microwave digestion process over classical method are the shorter time, less acid consumption, keeps volatile compounds in the solution, and easy to proceed. The following microwave digestion program were used; pressure 200 psi, ramp time 25 min., temperature 210 ^{o}C, maximum power 300 W, hold time 10 min. After cooling, residue was transferred to 25 ml volumetric flasks and diluted to level with deionized water. Before analysis, the samples were filtered through a 0.45 µm membrane filter. Sample blanks were prepared in the laboratory in a similar manner to the field samples and analyzed three blanks for each run to check the purity of reagents and any possible contamination. All samples were analyzed three times for Co, Cr, Cu, Fe, Mn, Ni, Pb, As, Se and Zn by inductively coupled plasma optical emission spectrometer (ICP-OES, VARIAN VISTA-MPX OES). Calibration standards were prepared from multi element standard (Merck). A Dorm-4 certified dogfish tissue (Ontario, Canada) was used as the calibration verification standard. Recoveries between 90% and 115% were accepted to validate the calibration. The results showed good agreement between the certified and the analytical values, the recovery of elements being partially complete for most of them (Table 1). Metal analyses in tissues were performed in the Research and Application Laboratory of Giresun University. One-way analysis of variance (ANOVA) test was conducted on each metal to test for significant differences between sites. All statistical analyses were computed using SigmaStat 3.0, Chicago, USA.

Table 1— Certified and observed values of metals in CRM, Dorm-4 (dogfish muscle) from the NRC (means ± SE, mg kg^{-1} dry wt)

Metals	Certified ± SE	Observed ± SE[a]	Recovery (%)
Cadmium	0.306 ± 0.015	0.278 ± 0.024	91
Cobalt	0.182 ± 0.031	0.177 ± 0.037	97
Chromium	1.87 ± 0.16	2.11 ± 0.17	112
Copper	15.9 ± 0.9	16,6 ± 0.75	104
Iron	142 ± 10	138 ± 12.5	97
Manganese	3.66 ± 0.34	3.49 ± 0.49	95
Nickel	1,36±0,22	1,26±0,11	93
Lead	0,416±0,053	0,479±0,08	115
Zinc	52,2±3,2	53,3±2,29	102

Results and Discussion

Mussel samples examined in this study were summarized with their length (cm) and weight (g) in Table 2. From two neighbor stations, PRZ had the
smallest and BLN had the largest size mussels.

Average length and weight of collected mussels were 5.33 ± 0.19 cm and 11.66 ± 0.83 g, respectively. There was a linear regression between the lengths and weights of mussel (r^2=0.877).

Mean concentrations of chromium, manganese, nickel, zinc, cobalt, copper, iron, and lead in the tissues of mussel from Giresun Coasts by stations
were presented in Table 3. Cadmium was unable to determine except one station. Ranges for Cr, Mn, Ni, Zn, Co, Cu, Fe, As, Se, and Pb as µg/g in wet weight were between 0.37 – 0.80, 4.68 – 8.93, 7.37 – 21.04, 52.16 – 85.56, 1.74 – 2.37, 2.12 – 3.40, 76.86 – 292.59, 2.64 – 3.57, 0.31 – 1.07, and 2.69 – 3.85 µg g^{-1}, respectively. Iron had the highest concentrations in all stations. The second highest metal was zinc after iron. In general, the pattern of heavy metals' occurrence in soft part of mussel samples of decreasing contents was Fe > Zn > Ni > Mn > As > Pb > Cu > Co > Se > Cr.

Table 2— Mean length (cm) and weight (g) of the mussel examined in the present study

Stations	Length Mean ± SE	Weight Mean ± SE
1. PRZ	3,52 ± 0,18a	6,46 ± 0,63a
2. BLN	6,54 ± 0,59b	17,27 ± 2,71b
3.GRS	6,40 ± 0,63b	16,81 ± 3,48b
4. KŞP	5,57 ± 0,33b	12,97 ± 1,43ab
5. ESP	4,67 ± 0,26a	10,00 ± 1,38ab
6. TRB	5,21 ± 0,25ab	9,87 ± 1,13ab
7. GRL	5,73 ± 0,22b	11,10 ± 1,37ab
8. EYN	5,01 ± 0,39ab	8,82 ± 1,26ab
Mean:	5,33 ± 0,19	11,66 ± 0,83

As can be seen from Table 3, metal levels show differences in the accumulation in tissues of the mussel by stations. Highest concentrations occurred at PRZ station for Mn, Co, and Pb, at EYN station for Cr, Ni, and Fe and at ESP station for Zn and As. The highest concentrations of the Cu and Se determined at KŞP and GRL stations, respectively. Minimum metal levels determined at GRL station for Cr, Ni, Zn, Co, Cu, and Fe, and at KŞP station for Mn, Se, and Pb. Minimum concentration of the As determined at EYN station.

There were no relation found between the size and the metal accumulation of mussel samples. There were statistically significant differences between the concentrations of the metals among eight stations. These variations between stations may be due to their different geographic locations.

The comparison of the some heavy metal concentrations obtained in the mussel samples collected from the Giresun shore with the data from different sites and with the tolerance levels in mussels given by the international and Turkish standards was given in Table 4.

The results showed that Pb and Zn levels in the present study were higher than the acceptable values for human consumption set by Turkish regulation. In general, metal levels of the present study were in agreement with the results of previous mussel studies in the different sites of Black Sea.

Iron levels obtained in this study were lower than those reported for Çamburnu, Rize[4] and

Gemlik[9], but were higher than those reported for Marmara Sea[10]. On the other hand, our findings for Fe levels were in agreement with ones reported for Yalova[5]. Levels of chromium from this study were lower than those reported for Mid Black Sea Coast[7], Çamburnu, Rize[4] and Gemlik[9]. Although zinc levels of this study were lower than all other studies presented in Table 4, it was still higher than the acceptable values for human consumption set by Turkish regulation. Nickel levels of the present study were greater than all those studies reported in Table 4. Manganese levels of the present study were agreed well with the findings presented by Ünlü et al. 2008[9]. On the other hand, our levels of Mn were lower than those reported for Çamburnu, Rize[4] and Mid Black Sea Coast[7]. Our Copper findings were lower than all those studies presented in Table 4.

Copper levels of the present study were also lower than national and international guidelines. Levels of lead from this study were higher than those reported for Yalova[5], and Gemlik[9], but were lower than those reported for Çamburnu, Rize[4], and Mid Black Sea Coast[7]. Cobalt levels of the present study were agreed well with the findings presented by Ünlü et al. 2008[9], and Topçuoğlu et al. 2004[10], but were lower than those reported by Çulha et al. 2011[5].

There is no information about maximum arsenic levels in fish samples in Turkish standards[11]. Maximum arsenic level permitted for fishes is 1.0 mg/kg according to Australia standards (Australia New Zealand Food Authority 1998)[12]. Mean As concentrations in mussel was 3.16 μg g^{-1} with the minimum value of 2.64 and the maximum value of 3.57 μg g^{-1}. There is also no tolerance level for

Table 3— Mean metal contents in the muscles of the examined mussel species by stations in this study (mg kg^{-1} wet wt)

Stations	Cr	Mn	Ni	Zn	Co	Cu	Fe	As	Se	Pb
1.PRZ	0,64±0,08^{a}	8,93±2,01^{a}	19,53±4,90^{ac}	61,19±8,59^{a}	2,37±0,41^{a}	2,66±0,21^{a}	266,58±78,53^{ac}	3,42±0,15^{a}	0,47±0,13^{a}	3,85±0,31^{a}
2.BLN	0,58±0,06^{a}	6,37±0,55^{a}	10,48±1,13^{a}	69,52±13,01^{a}	1,80±0,02^{a}	2,33±0,28^{a}	126,57±17,65^{a}	3,48±0,33^{a}	0,57±0,03^{a}	3,20±0,05^{ab}
3.GRS	0,56±0,03^{ab}	5,35±0,86^{a}	8,04±0,69^{b}	66,31±12,41^{a}	1,77±0,07^{a}	2,81±0,38^{a}	90,63±10,46^{b}	3,04±0,45^{a}	0,50±0,13^{a}	3,18±0,19^{ab}
4.KŞP	0,65±0,08^{ac}	4,68±0,26^{ba}	13,56±1,44^{a}	64,88±12,35^{a}	2,00±0,13^{a}	3,40±1,13^{a}	173,50±21,56^{a}	3,00±0,26^{a}	0,31±0,05^{a}	2,69±0,11^{b}
5.ESP	0,49±0,01^{ab}	5,63±0,45^{a}	10,44±1,00^{a}	85,56±20,32^{a}	1,97±0,06^{a}	2,63±0,59^{a}	125,59±15,75^{a}	3,57±0,35^{a}	0,65±0,08^{ab}	3,48±0,12^{ab}
6.TRB	0,39±0,01^{b}	4,98±0,36^{a}	11,17±0,92^{a}	83,93±6,86^{a}	1,82±0,07^{a}	2,50±0,44^{a}	136,32±15,45^{a}	3,39±0,40^{a}	0,74±0,17^{ab}	2,99±0,25^{b}
7.GRL	0,37±0,02^{b}	5,22±0,61^{a}	7,37±0,57^{b}	52,16±7,95^{a}	1,74±0,06^{a}	2,12±0,19^{a}	76,86±9,71^{b}	2,82±0,23^{a}	1,07±0,15^{b}	2,89±0,19^{b}
8.EYN	0,80±0,02^{ac}	8,66±0,66^{a}	21,04±2,10^{c}	68,93±9,29^{a}	2,32±0,23^{a}	2,78±0,40^{a}	292,59±33,75^{c}	2,64±0,26^{a}	0,57±0,08^{ab}	2,97±0,09^{b}
Average:	0,56±0,03	6,23±0,38	12,70±1,01	69,06±4,20	1,97±0,07	2,65±0,18	161,08±15,89	3,16±0,11	0,62±0,05	3,16±0,08

Vertically, letters a, b and c show statistically significant differences ($p<0.05$)

Table 4— Comparison of heavy metal concentrations as mg kg-1 wet wt in mussel samples collected from the different sites with the tolerance levels

Region	Fe	Cr	Zn	Ni	Mn	Cu	Pb	Co	References
Giresun	161,08	0,56	69,06	12,70	6,23	2,65	3,16	1,97	Present Study
Yalova	156,72	-	106,23	3,71	-	5,54	2,92	4,08	[5]
Gemlik	205,4	2,3	196	1,3	5,8	5,5	0,5	2,0	[9]
Marmara Sea	120–415	-	208–319	0.1–13.96		6.7–9.5	0.1–5.2	0.05–1.89	[10]
Çamburnu,Rize	3340	3,0	630	6,0	59	190	21,0	-	[4]
Mid Black Sea Coast	-	7,65–11,3	312,2-396,5	-	46,9-73,05	11,75-23,5	0,05-108,6	-	[7]
Guideline	-	-	50	-	-	20	1,0	-	[11]
Guideline	-	1,0	30-100	-	-	10-100	0,5-6	-	[17]

selenium in fish samples in international or Turkish standards. Selenium contents in the literature have been reported in the range of 1.71 μg g^{-1} in fish feed13, 0.73–2.34 μg g^{-1} in fish and shellfish samples14, 0.19-0.85 μg g^{-1} fish samples[15]. Nickel levels of the present study, with the mean of 12.70 μg g^{-1}, were higher than those of compared literatures. Nickel is present in tobacco at average contents of 2.2 and 2.3 mg per cigarette and estimated daily diet nickel intake is 150 μg day^{-1} which is 500 fold less than the dose reported to cause adverse effects in rats[16].

The results of the present study supply valuable information about metal contents in tissues of the examined mussel species along Giresun coast which indicate the environmental contamination of the area and also evaluate the possible risk associated with their consumption. A comparison of the present study with the previous studies for mussel samples from the Marmara and Black Sea coast shows that heavy metal levels in the present study were in agreement with the results reported for the Yalova[5] ,Gemlik[9], and Marmara Sea[10]. Nickel levels of the present study, however, were significantly higher than those of compared studies. On the other hand, heavy metal levels in mussel were significantly lower in the present study than those in Çamburnu, Rize coast[4].

The results presented above clearly demonstrated that the Giresun coastal water face metal pollution for Zn and Pb since their concentrations in all eight stations were higher than Turkish permissible limits[11].

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