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Evaluation of Metal Concentrations in Mussel *M. galloprovincialis* in the Dardanelles Strait, Turkey in Regard of Safe Human Consumption

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Abstract Concentrations of the elements were evaluated for the first time in *Mytilus galloprovincialis* in the Dardanelles (Canakkale Strait-Turkey). The concentration of elements were measured in samples collected in 2007, 2008 and 2009, while the concentrations of Fe and Ni were evaluated in samples taken in 2009. The maximum concentrations of Cd, Cr, Cu, Pb, Zn, Fe, and Ni were found to be 1.59, 6.04, 12.01, 6.03, 319.6, 402.79, and 3.52 mg/kg, respectively. In terms of the nutritional aspect, taking into account the values recommended by world health authorities, the concentration of elements can generally be considered not to be at levels posing a health risk.

Keywords Mussel · Dardanelles · Heavy metals · *Mytilus galloprovincialis*

Mussels have been widely used as bioindicators of heavy metal pollution in coastal areas due to their accumulative ability to retain various elements and it is known that they can accumulate a wide range of pollutants (Cevik et al. 2008). Metal concentrations found in the soft tissue of mussels reflect the concentrations of the same metals in the aquatic environment in which they are found (Joksimovic et al. 2011). Due to their high consumption rate, the amount of pollutants in the soft tissue of mussels should therefore be evaluated with respect to human health. Possible health risks resulting from high metal content in mussels have been evaluated in many studies (Cardellicchio et al. 2008; Cevik et al. 2008; Joksimovic et al. 2011). Obtained results in the mentioned studies showed that the metal content of mussels in particular areas exceeded the limits of regulatory bodies such as the FAO and WHO (World Health Organisation). In this respect, the heavy metal content of mussels needs to be assessed due to their active human consumption.

The Dardanelles is a narrow strait connecting the Marmara and Aegean Sea. The Dardanelles (Canakkale Strait), together with the Bosporus (Istanbul Strait), are busy international waterways that connect the Black Sea and the Mediterranean. Some of the most industrialized cities in Turkey are located along the coastal areas of the Sea of Marmara, an inner sea that is affected by chemical pollution due to the area being heavily populated with respect to residential housing, industrial activity, and marine transportation. Due to salinity differences, the waters of the Black Sea reach the Mediterranean via an upper downstream current and the waters of the Mediterranean reach the Black sea via an upstream undercurrent in the Dardanelles and Bosphorus (Altug et al. 2009). Because of its transitional position and the two currents, the quality of the water in the Dardanelles is directly affected.

In the present study, concentrations of some metals were determined in the soft tissue of *Mytilus galloprovincialis* collected at six different sampling sites concentrated close to the narrowest part of the Dardanelles. The measured metal concentrations were evaluated for possible human health risks and compared with other results. No scientific data concerning mussels in the Dardanelles have been reported until now, hence the importance of the present study.

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Materials and Methods

In order to determine the concentration of heavy metals in the marine environment of the Dardanelles, samples of the mussel *M. galloprovincialis* were taken at six different localities (Fig. 1). Samples with similar shell length (5–7.5 cm) from each station were collected in the month of February in 2007, 2008 and 2009. The locations, within a radius of approx 25 km along the Dardanelles shoreline, were: Iskele (Canakkale city harbor), the mouth of Saricay Creek, Liman (Kepez harbor), Dardanos holiday beach facilities, Eceabat harbor, and close to Kilitbahir. The first four sampling sites are on the Anatolian side of the strait while the last two are on the European side of the strait.

The collected samples were put into polyethylene bags filled with sea water. All samples were depurated in seawater taken from their collecting site for 24 h after transfer. The mussels were washed with deionized water (18.2 MΩ cm^{-1}) and then the whole soft tissues of 35–40 mussels from each sampling site were separated from their shells and rinsed with deionized water to remove extraneous materials. Subsequently, all the soft tissues of samples were dried in an oven at 60°C until they had achieved constant weight and were homogenized. Acid digestion of the six mussel samples was carried out using a microwave digestion system (CEM Mars X-press). Applied digestion conditions for the microwave digestion system were 15 and 20 min for ramp and hold time at 1,200 W, respectively. For the digestion, 0.5 g of dried mussel tissue was put into a Teflon vessel, 10 mL of concentrated nitric acid (Merck, 65 %) was added, and the mixture was digested. After cooling, the digest was filtered and diluted with deionized water up to 25 mL. In parallel, a blank was digested in the same way. The concentrations of Cd, Cr, Cu, Pb, Zn, Fe and Ni were determined by using inductively-coupled plasma atomic emission spectrometry (ICP-AES). The concentrations of metals in each sample were measured in triplicate and all relative standard deviations (RSD) for replicates were less than 5 %. SPSS 10.0 for Windows was used for the data analysis. Accuracy of measurements was checked by analysis of the Certified Reference Material (BCR-185R) of the European Commission's Joint Research Centre at Brussels for Bovine Liver-Trace Elements.

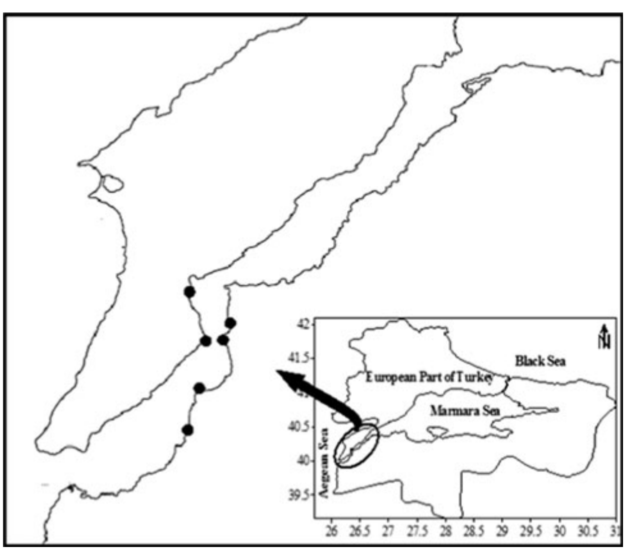

Fig. 1 Mussel *M. galloprovincialis* sampling sites in the Dardanelles Strait, Turkey

Results and Discussion

The obtained result of Certified Reference Material (CRM) is presented (Table 1) in mg/kg of the samples at dry weight (d.w.). Concentrations of the evaluated metals by year and sampling site are presented in Table 2. In this study, the highest mean concentration of Cd was obtained in mussels collected in 2009. However, considering the mean values of all six sampling sites, there were no statistically significant differences between the years for Cd accumulation. Similarly, over the 3 years there were no statistically significant differences between sampling sites. In general, an increasing trend of Cd mean concentration was observed from 2007 to 2009. Comparisons with previous studies revealed Cd concentrations similar to those found on some parts of the coast of Turkey and in other countries (Ergul et al. 2007; Romeo et al. 2005; Sunlu 2006). Additionally, they are significantly lower than those found on the coast of Turkey and Morocco (Cevik et al. 2008; Maanan 2007; Kavun et al. 2002; Sunlu 2006; Topcuoglu 2000; Topcuoğlu et al. 2004). For Cd the average weekly intake for adults (60 kg body weight) was estimated to be 0.42 mg, so the PTWI Cd was 0.007 mg/kg body weight/week (WHO/FAO 1999). When the most contaminated mussel samples from the present study are considered, the required amount to reach the PTWI of Cd for adults (of average weight 60 kg) would be about 266.1 g of dried mussels.

Table 1 Results for certified reference material (mg/kg in dry weight)

	Certified values	Measured values	Recoveries (%)
Cd	0.544	0.577	1.06
Cu	277	256.56	0.93
Pb	0.172	0.148	0.87
Zn	138.6	126.54	0.91

Table 2 Mean concentrations of elements in mussels (mg/kg in d.w.)

	Year	Cd	Cr	Cu	Fe	Ni	Pb	Zn
Dardanos	2007	0.49 ± 0.03	3.82 ± 0.02	8.76 ± 0.32	–	–	2.19 ± 0.02	233.71 ± 12.6
Eceabat	2007	1.00 ± 0.017	2.20 ± 0.06	7.41 ± 0.09	–	–	2.16 ± 0.39	316.93 ± 0.00
İskele	2007	1.12 ± 0.03	0.45 ± 0.01	8.35 ± 0.08	–	–	5.48 ± 0.09	245.39 ± 1.97
Kilitbahir	2007	0.31 ± 0.01	1.67 ± 0.02	9.35 ± 0.08	–	–	2.93 ± 0.06	319.60 ± 2.56
Liman	2007	0.20 ± 0.01	1.40 ± 0.01	7.47 ± 0.12	–	–	3.06 ± 0.04	229.53 ± 7.60
Sarıcay	2007	0.40 ± 0.01	1.09 ± 0.02	8.45 ± 0.02	–	–	3.02 ± 0.04	206.52 ± 1.04
Dardanos	2008	0.58 ± 0.02	6.04 ± 0.01	9.15 ± 0.02	–	–	1.84 ± 0.02	247.90 ± 1.74
Eceabat	2008	1.10 ± 0.02	1.01 ± 0.03	4.37 ± 0.04	–	–	2.92 ± 0.05	260.72 ± 0.26
İskele	2008	0.94 ± 0.02	0.92 ± 0.03	6.07 ± 0.01	–	–	4.80 ± 0.01	297.26 ± 10.1
Kilitbahir	2008	1.45 ± 0.03	1.04 ± 0.04	4.23 ± 0.03	–	–	2.65 ± 0.03	269.17 ± 5.93
Liman	2008	0.47 ± 0.02	3.26 ± 0.04	8.24 ± 0.08	–	–	4.49 ± 0.05	235.86 ± 0.71
Sarıcay	2008	0.32 ± 0.01	0.93 ± 0.01	10.58 ± 0.08	–	–	2.78 ± 0.06	178.78 ± 0.72
Dardanos	2009	0.62 ± 0.01	2.07 ± 0.01	5.75 ± 0.02	260.39 ± 0.09	3.52 ± 0.003	1.84 ± 0.01	170.65 ± 0.03
Eceabat	2009	1.59 ± 0.001	1.05 ± 0.001	5.79 ± 0.01	167.87 ± 0.03	1.70 ± 0.001	1.86 ± 0.01	139.03 ± 0.05
İskele	2009	0.75 ± 0.0001	0.94 ± 0.001	12.01 ± 0.004	289.61 ± 0.10	1.96 ± 0.001	6.03 ± 0.01	153.94 ± 0.02
Kilitbahir	2009	0.75 ± 0.0002	1.01 ± 0.001	4.84 ± 0.0001	134.49 ± 0.06	2.28 ± 0.0001	2.53 ± 0.009	158.62 ± 0.009
Liman	2009	0.98 ± 0.001	2.38 ± 0.001	8.30 ± 0.003	402.79 ± 0.05	3.27 ± 0.002	4.83 ± 0.008	212.45 ± 0.02
Sarıcay	2009	0.89 ± 0.001	1.32 ± 0.001	7.10 ± 0.003	393.53 ± 0.198	3.26 ± 0.04	2.04 ± 0.003	250.23 ± 0.07

Concentrations of Cr were detected in all mussel samples ranging from 0.45 to 6.04 mg/kg d.w. The mean concentration of Cr in mussels collected in 2008 was higher than for those collected in 2007 and 2009. However, calculated one-way ANOVA values showed that there were no statistically significant differences between years concerning Cr accumulation. After the highest mean values occurred in 2008, a decreasing trend was observed. The Cr concentrations are similar to those of previous studies (Cevik et al. 2008; Ergul et al. 2007; Joksimovic et al. 2011; Sunlu 2006; Topcuoğlu et al. 2004) while they are lower than those found on the Safi coast (Maanan 2007). In general, the Cr values were not uniform among the sampling sites. The highest Cr values were obtained at Dardanos in 2007 and 2008, at Liman in 2009. On the other hand, considering all sampling years, the Cr concentration was lowest in the mussels collected at Iskele. Possible differences between sampling sites were investigated by one-way ANOVA. In the case of the Cr-dependent variable, statistically significant differences were found between Dardanos and Iskele, Dardanos and Kilitbahir, Dardanos and Saricay ($p < 0.05$). In general, the mean concentrations of Cu obtained at sampling sites were similar. The mean Cu concentration was in the range of 4.23–12.01 mg/kg. The mean concentrations over the three sampling years were also similar. There were no higher values in any year or at any sampling site. One-way ANOVA results showed no statistically significant differences between years and sampling locations. Obtained results are in good agreement with findings reported previously (Ergul et al. 2007; Joksimovic et al. 2011; Romeo et al. 2005; Sunlu 2006; Kavun et al. 2002; Topcuoglu 2000; Topcuoğlu et al. 2004), while they are lower than those reported by Unlu et al. (2008) and Cardellicchio et al. (2008). On the other hand, heavy metal concentrations reported in some studies were higher than those found in this study (Cevik et al. 2008; Maanan 2007). A joint FAO/WHO/IAEA (International Atomic Energy Agency) report indicated that an upper limit for the safe range of population mean exposures of Cu for adults was of 0.2 mg/kg body weight/day (WHO 1996). In this context, the concentrations of Cu in the sampled mussels are below the corresponding value of 12 mg for a person with a bodyweight of 60 kg on a daily basis.

The highest mean concentration of Pb was obtained at Iskele which was two–three times higher than the other sampling sites. Following this, the mean concentration at Liman was the second highest Pb value. In contrast, there is uniform distribution between years. One-way ANOVA results showed that there were no statistically significant differences over the 3 years. However, the Pb accumulation was different at different sampling sites; this showed up in one-way ANOVA. According to this, apart from Liman, accumulated Pb concentration in mussels collected at Iskele was statistically significant compared to the other sampling sites. There were also statistically significant differences between Liman-Eceabat and Liman-Dardanos. While the findings in other reported studies were lower than the present study (Cardellicchio et al. 2008; Ergul et al. 2007; Sunlu 2006; Unlu et al. 2008), others were higher (Cevik et al. 2008; Maanan 2007). From the point of view of human health, for Pb the average weekly intake for adults (of 60 kg) was estimated to be 1.5 mg, so the PTWI Pb was 0.025 mg/kg body weight/week (FAO/WHO 2004). To reach the maximum Pb value, the required daily amount to reach the provisional tolerable weekly intake would be about 248.3 g of dried mussels.

The zinc accumulation in mussels changed over the 3 years, decreasing from 2007 to 2009. This implication was also supported by one-way ANOVA which showed that the mean Zn concentration in 2009 was statistically significant compared to other years. On the other hand, there were no differences between Zn accumulation in mussels collected in 2007 and 2008. In general, considering the mean concentration of Zn at the sampling sites, it was observed that there were uniform accumulations, also seen in ANOVA results. Zn concentrations in mussels are lower than those presented in this study (Cardellicchio et al. 2008; Ergul et al. 2007; Unlu et al. 2008). On the other hand, Zn determined in mussels collected in the Black Sea (Cevik et al. 2008) was higher than that presented here. However, obtained results are in an agreement with findings by Sunlu (2006)· Maanan (2007), Topcuoğlu et al. (2004). According to WHO (1996), For Zn there is not PTWI value and the adult population mean intake should not exceed 45 mg/day in order to avoid Zn-related interactions. Concerning the maximum value of Zn, the required amount to reach 45 mg/day would be about 140 g of dried mussels.

Fe and Ni were investigated only in mussels collected in 2009. The minimum and maximum Fe concentrations in mussels were found to be in a range of 134.5–402.8 mg/kg. The highest value of Fe was detected in mussels collected at Liman, a site that is used as a harbor. According to the joint FAO/WHO (1999) report, for Fe the provisional tolerable weekly intake for humans is 48 mg/day. So the PTWI for Fe of a person with a bodyweight of 60 kg should be 5.6 mg/kg body weight/week. Therefore, concerning the maximum value, 119 g of dried mussels would be enough to reach the required amount. The obtained mean concentrations of Fe are in good agreement with several studies (Cardellicchio et al. 2008; Joksimovic et al. 2011; Kavun et al. 2002; Topcuoğlu et al. 2004) while lower than reported values measured in mussels collected in the Black Sea (Cevik et al. 2008; Topcuoglu 2000). Ni accumulation in mussels was similar between the sampling sites. The minimum and maximum concentrations were found to be in the range of 1.7–3.52 mg/kg. For Ni there is not PTWI value. Comparison of mean Ni values shows that obtained results in the present study are generally lower than those in other studies (Ergul et al. 2007; Joksimovic et al. 2011; Topcuoglu 2000; Topcuoğlu et al. 2004). Among similar studies using other mussel species as the biomonitor organism, the levels of Ni in this study are similar to those reported by Kavun et al. (2002) in *Mytilus trossulus*. The pooled data for the 3 years were investigated for possible correlations showing common sources of metals. The results showed no positive correlation between metals. On the other hand, negative and statistically significant correlations were determined between Cu and Cd. Overall; correlation results showed that in the study region, there is no common source for the evaluated elements.

Seafood from the Dardanelles is exported in considerable quantities. Concentrations of the evaluated elements in the mussel *M. galloprovincialis* obtained over the 3 year period 2007–2009 are within the permissible limits (for adults with one average weight of 60 kg) established for safe human consumption and are mostly similar to or lower than those reported in previous studies. Therefore, from the nutritional perspective, mussels from the Dardanelles can be safely consumed. However, due to rapid industrialization and urbanization in the area, the level of toxic elements should be monitored periodically in order to prevent possible future health hazards.

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