Assessment of Heavy Metals in Wild Mussels *Mytilus Galloprovincialis* From the Marmara Sea Coast of Tekirdag (Turkey)

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Abstract

Concentrations of eight heavy metals (Cd, Cr, Cu, Hg, Ni, As, Pb, and Zn) were identified in tissues of the mussel *Mytilus galloprovincialis* that are seasonally collected along the Marmara coast of Tekirdag. The concentrations (mg/kg d.w.) of these metals ranged from 1.20 to 2.79 for arsenic (As), 0.13 to 0.75for cadmium (Cd), 0.42 to 2.46 for chromium (Cr), 1.55 to 3.5 for copper (Cu), 1.01 to 2.46 for nickel (Ni), 76.8 to 88.98 for zinc (Zn), and 2.67 to 9.2 for lead (Pb). These levels were lower than the permissible limits set by the European Commission and Food and Drug Organisation Permissible limits for Pb, As, and Zn were exceeded during some periods. Evaluation of the public health risk associated with consumption of mollusks indicates that there is no evidence of risk to the *Mytilus Galloprovincialis* consumer. However, because chronic exposure of to trace metals can cause health problems for humans, toxic chemicals must be periodically and carefully monitored.

Keywords: Mussel (*Mytilus galloprovincialis*), Heavy metals, Marmara Sea, Coastal pollution

1.Introduction

Concentrations of heavy metals in coastal areas and inland seas are higher than those in open seas [1]. Heavy metals represent a considerable share of the pollutants causing marine pollution. Although some metals are important for living creatures, they accumulate in the body until a certain concentration is reached and eventually result in toxic effects. Particular sea creatures, such as mussels, lobsters, oysters, or shrimp, are of importance as bio-indicators in the determination of heavy metal pollution. In aquatic systems, heavy metals accumulate in their bodies [2-3].

The coasts of Tekirdag are the regions where agricultural, domestic, and industrial waste is discharged and anthropogenic and tourism activities are intensive, especially during the summer months. This represents a significant problem for public health as well. It is suggested that for any physiological response to be used in the determination of pollution, the reproductive period of the specified organisms must be considered [4]. Further, these studies should be conducted on a seasonal basis over periods of two or three months.

In consideration of the growing concern for marine coastal environmental quality, the goal of this study was to determine the seasonal levels of Cu, Cd, Ni, Cr, Pb, Hg, As (total), and Zn in the soft tissue of mussels *Mytilus Galloprovincialis* along the Tekirdag. The study concluded that the levels of toxic metals in the mussels were within the maximum residual levels prescribed by the International Turkish Standards, the European Union, and the United States Food and Drug Adminstration.

2. Materials and methods

2.1. Sampling Area

As there are no domestic and industrial waste water treatment facilities, wastewater is discharged into Ergene River and the sea. The Marmara Sea is directly influenced by sewage outfall from Tekirdag, summer resorts (untreated effluent discharged into the sea), industrial discharges, agricultural runoff, a sunflower oil factory, fishing, and shipping activities. In this study, mussels *Mytilus galloprovincialis* (35–55 mm) were seasonally collected from twenty different stations in a coastal area of 35 kilometers between Marmara Ereglisi and Tekirdag (Fig. 1). According to one study, “The water temparature and salinity range from 6.09 to 14.20 and 22.36 to 37.95‰, respectively, in winter and from 15.01 to 26.30 and 214 to 36.0‰, respectively, in summer of 2012 [5].

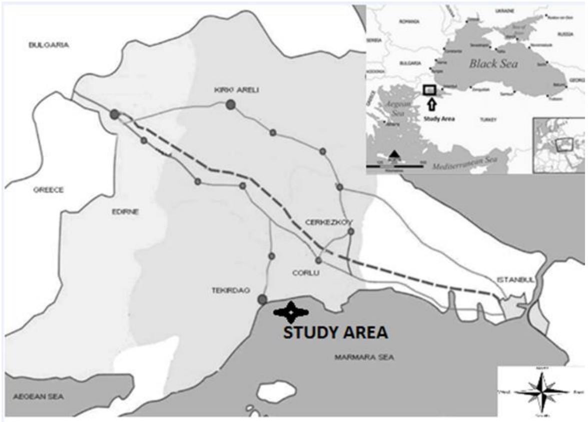

Fig. 1. Marmara Sea showing the sampling locations

2.2. Collection of samples and preparation of mussels

In our study, sampling was performed along the coastline of Tekirdağ during the winter, spring, and summer of 2012. The mussel samples were prepared in accordance with UNEP/FAO/IOC/IAEA, 1993 [6]. Approximate 50 mussel samples were collected in each location (a total of 200 samples) along the coast of Tekirdağ, stored in bags, kept in a cooler box with ice, and transported to the laboratory. For a 24-hour period, the mussel samples were kept in filtered seawater to allow depuration of particulate matter residues present in the mantle cavity and digestive tract. For the analysis of mussels, soft tissue from a minimum of 75 specimens was pooled as a composite sample, homogenized, and freeze-dried [7]. To avoid contamination, all parts of the homogenizer that came into contact with a sample were covered with Teflon adaptors. The homogenates were then lyophilized and stored in polyethylene bottles. An aliquot of the homogenized sample of mussel tissue was used to calculate dry weight using oven drying at 105°C until a constant weight was achieved.

For metal analysis, a 0.25 g lyophilized sample was mineralized with 9 ml of concentrated HNO_3 (70% v/v) and 1 ml of H_2O_2 (30%v/v) (USEPA Method 3052) in a closed Teflon vessel using a microwave digestion system (heating to 175°C for 5.5 min and holding at 175°C for 4.5 min) (MARS-X CEM Corporation, Matthews, NC). After mineralization, digests were cooled and the resulting solutions were diluted to a known volume (50 ml) with Milli-Q® water and stored in polyethylene bottles until analysis. A blank digest was performed in the same way.

All the chemicals used in sample treatments were of ultrapure grade (Merck Suprapur, Darmstadt, Germany), and all glassware was cleaned prior to use by soaking in 10% v/v HNO_3 for 24 h and rinsed with Milli-Q® water. All solutions were daily prepared using ultra-pure deionized water (<0.1 μs at 25°C) obtained by treating double distilled water in a Milli-Q® system (Millipore, Milford, Ma., USA).

2.3.Analytical measurements

All digested solutions were analyzed by the AEM Environment Analysis and Laboratory (an accredited laboratory) using Shimadzu AA-6300 Full atomic absorption spectrophotometry (Cu, Zn, Ni by flame AAS; Cd, Pb, As and Cr by electrothermal AAS; flow injection-cold vapor–AAS: total Hg). For calibration, all standards were prepared in the same matrix as the one used for mussel analyses. The detection limits (LOD) expressed in mg/kg dry weight (mg/kg d.w.) were: As: 0.01; Cd: 0.002; Cr: 0.02; Cu: 0.003; Hg: 0.004; Pb: 0.02; Zn: 0.02; and Ni: 0.03. All elements of heavy metals were expressed in mg kg^{-1} dry weight. Data were expressed as mean ± standard deviation, each performed in triplicate. Tests were performed at a 95% confidence level.

3.Results And Discussion

Hg, Cd, Pb, As, Ni, Fe, Cu, and Zn were analyzed in the soft tissues of the mussels *M. Galloprovincialis* from the North Marmara sea. The primary concentrations of the investigated trace metals (mg/kg d.w) in the mussels and standard deviations in the sampling seasons of 2012 are given in Table 1. Considering all metals and sampling periods, the mean values obtained decreased in the following order: Zn > Pb > Cu > As > Cr ≥ Ni > Cd > Hg. Based on a comparison of the permissible limits set for Zn (50.0 mg/kg w.w.-TFC, 2005), Pb (0.5 mg/kg w.w.-TFC, 2005), Cu (20.0 mg/kg w.w.- TFC, 2005), As (1.0 mg/kg -TFC, 2005), Cr (1.0 mg/kg w.w.-FAO, 1983), Ni (70.0 mg/kg w.w.-USFDA, 1993), Cd (0.5 mg/kg w.w.-TFC, 2005), and Hg (0.5 mg/kg w.w.-TFC, 2005), all the mean values of analyzed metals were lower than EC regulations and FAO limits for fishery products with the exception of Pb, As, and Zn [8-10]. The levels for Pb, As, and Zn in mussels were probably related to discharge from urban areas and industries flowing into the Marmara sea.

Table 1. Mean values for heavy metals (mg kg^{-1} d.w.), standard deviation (mean ± SD, mgkg−1), and dry ratio in soft tissues of *Mytilus galloprovincialis* collected from Tekirdağ coast (n = 3)

Measured Parameter	Winter Period 2012 (n:75)	Spring Period 2012 (n:75)	Summer Period 2012 (n:75)	TFC,2005 [8]
Zn	77.12±2.5	76.8±5.66	88.98±3.4	50
Cd	0.13±02	<0,1	0.75±0.04	0.5
Cr	2.46±0.4	0.42±0.02	<0.33	12[1]
Hg	<0.1	<0.24	<0.33	0.5
As	<0.33	1.20±0.14	2.79±0.02	1.0
Cu	1.55±0.01	2.89±0.45	3.5±0.17	20
Pb	2.67±1.06	4,88±1.21	9.2±1.74	0.5
Ni	2.46±0.3	1.01±0.02	<0.33	70[1]

[1]US FDA 1993

Pb concentrations in mussels sampled during all periods (2.67; 4.88; 9.2 mg kg-1, respectively) were above the limits set by the TFC. The presence of lead during the summer could be related to an increase of urban populations during this period, anthropogenic sources, or shipping, where other metals are contained in Pb protective paints [11]. This increase in the bioaccumulation of Pb in mussels during the summer depends not only on environmental concentrations but also on the chemistry of metals in seawater and the physiology of mussels [12]. Further, the values exceeding the limits are probably due to high lead concentrations in area sediments [13,14]. Pb is a toxic, bioaccumulative heavy metal with no known biological function. Its absorption may constitute a serious risk to public health [15-17]. The international Agency for Research on Cancer (IARC) classified inorganic Pb as being likely carcinogenic to humans. During all sampling periods in this study, Zn concentrations (77.12–88.98 mg kg^{-1}) were found to be higher than the recommended limits established by the TFC (Table 1). Seafood is a major source of zinc and an important of human diets. Zinc is an essential trace element. However, in excess quantities, essential elements can also be poisonous and cause serious threats to human health [18-19]. In this study we analyzed total (inorganic and organic) As concentrations (1.2 and 2.79 mg kg^{-1}, respectively) were found to be higher than the permissible limits for TFC. The fact that the arsenic content of mussels, especially in the spring and summer periods, was higher than the limiting values may indicate anthropogenic (agricultural and industrial) activities in the Thrace region. Factories there allow uncontrolled discharges of pollutants into ground and surface water. The toxicity of organic arsenic compounds is relatively low, and they are eliminated faster from the organism [16]. A wide range of arsenic compounds, including inorganic arsenic, has been reported in marine organisms. The percentage of inorganic As in seafood is 1–5%; while in bivalve mollusks, they are 1.9–6.5%, and mussels contain approximately 1–2% of inorganic As compounds; the great majority of seafood arsenic consists of complex organic arsenical compounds [20-22]. Values exceeding the limits are probably due to natural sediment enrichment. However, no published data are available about the metal content of sediment or background levels in these areas. There is no data in the Turkish Seafood Standards about Ni (1.01–2.46 mg kg^{-1}) and Cr (0.42–2.46 mg kg^{-1}) in mussel species. Mercury was not detected in any of the samples studied and does not pose a risk to public health. Water contamination varies from season to season and between sites. Depending on contaminant type and concentration, this variation could be one possible cause of the seasonal variation of metal bioavailability and bioaccumulation in mussel [12,23].

5. Conclusions

The toxicity and accumulation of heavy metals in the environment presents serious problems for human and ecosystem health. Industrial and agricultural activities are highly intensive in the Thrace region. As a result, water resources are continuously subject to pollution. Concentrations of Pb, As, and Zn higher than permissible limits during the summer period may result from the following factors: intensive agricultural activities in the region, decreasing rainfall, and wastewater discharges into surface water that reach the sea without being diluted. To prevent pollution, adequate treatment systems must be implemented immediately. An inventory of pollutant discharges relating to the sea and inland waters should be conducted immediately and its results disclosed to the public and relevant organizations. Financial support should also be provided to projects and solutions that focus on this issue. In particular, the continued monitoring of heavy metal concentrations in the sea is necessary to control the amount of heavy metals in mussels. Moreover, heavy metal concentrations must be monitored with respect to human and environmental health.

References

[1] O. Egemen, Environment and Water Pollution. Ege Universitesi. Su Ürünleri Fakültesi Yayınları. No:42, Bornova, İzmir (2000) (in Turkish).

[2] P.S. Rainbow, Biomonitoring of Heavy Metal Availability in the Marine Environment. Marine Pollution Bulletin 31 (1995) 83-192

[3] A.H. Dokmeci, T. Yildiz, A. Ongen, N. Sivri, Heavy metal concentration in deepwater rose shrimp species (Parapenaeus longirostris, Lucas, 1846) collected from the Marmara Sea

Coast in Tekirdağ. Environmental Monitoring Assessment 186 (2014) 2449–2454.

[4] J. Widdows, Physiological responses to pollution. Marine Pollution Bulletin 16: (1985)129-134.

[7] MAREM, Marmara denizi'nin değişen oşinografik şartlarının izlenmesi projesi 2012 senesi çalışma verileri (Ön Raporlar) 2012 Kış ve Yaz Dönemi Oşinografik-Hidrografik-Biyolojik-Kimyasal-Sedimantolojik Klimatolojik-Mikrobiyolojik-Antropojenik İstasyon Çalışmaları (2013) (in Turkish).

[8] UNEP/FAO/IOC/IAEA, Guidelines for monitoring chemical contaminants in the sea using marine organisms. Reference Method No 6, UNEP (1993).

[9] OSPAR Commission: Coordinated environmental monitoring programme (CEMP) (Reference number: 2009-1). OSPAR Commission, London (2009).

[10] TURKISH FOOD CODES (TFC): Official Gazette. 23 September 2002, No:24885 (2005). (in Turkish).

[11] FAO (Food and Agriculture Organization). Compilation of Legal Limits for Hazardous Substances in Fish and Fishery Products. FAO Fisheries Circular No. 464 (1983) 5-100.

[12]United States Food And Drug Adminstration (USFDA) Guideline for toxic elements. Chapter 23: Environmental chemical contaminants and pesticides. In Seafood Network Information Center, Sea Grant Extension Program. Sea Grant, Carlifornia (1993).

[13]C.D. Klassen, M.O. Amdur, J. Doull, Toxicology. 3th Ed. Macmillan Publishing Company, Newyork, USA (1986).

[14] O. Rouane-Hacene, Z. Boutiba, B.Belhaouaria, M.E. Guibbolini-Sabatierb, P.Francourb, C.Risso-De Faverneyb, "Seasonal assessment of biological indices, bioaccumulation and bioavailability of heavy metals in mussels *Mytilus galloprovincialis* from Algerian west coast, applied to environmental monitoring." Oceanologia 57:4 (2015) 362-374.

[15] S. Topcuoğlu, H.A. Ergül, A. Baysal, E. Ölmez, D. Kut, Determination of radionuclide and heavy metal concentrations in Biota and Sediment Samples from Pazar and Rize Stations in The Eastern Black Sea. Fresenius Environmental Bulletin 12 (2003) 695– 699.

[16] S. Topçuoğlu, Ç. Kirbaşoğlu, N.Balkis, Heavy metal contents of algae of Turkish coast in the Black Sea (1979-2001). Journal of the Black Sea / Mediterranean Environment 10 (2004) 21-44 (in Turkish).

[17] S. Stankovic, M. Jovic, A.R Stankovic, L. Katsikas, Heavy metals in seafood mussels. Risk for human health. p. 311-373. In: Environmental Chemistry for a Sustainable World. Volume 1: Nanotechnology and Health Risk. Lichtfouse, E., Schwarzbauer, J., Robert, D. (Eds). Springer, Netherlands (2012).

[18] I. Dokmeci, A.H. Dokmeci, Diagnosis and Treatment of Poisoning, 4th Edition, Nobel Tıp Kitabevi, p.449-96. ISBN: 978-9944-211-43-7 (2009) (in Turkish).

[19]Commission Regulation (EC) No 1881/2006 of 19 December 2006 setting maximum levels for certain contaminants in foodstuffs (OJ L364, 20.12.2006, page 5).

[20] D. Joksimovic, I. Tomic, R.A. Stankovic, M. Jovic, S.Stankovic, Trace metal concentrations in Mediterranean blue mussel and surface sediments and evaluation of the musselquality and possible risks of high human consumption. Food Chem. 127 (2011) 632 –637

[21] L.M. Plum, L. Rink, H. Haase, The Essential Toxin: Impact of Zinc on Human Health. International Journal of Environmental Research and Public Health 7:1342-1365. DOI 10.3390/ijerph7041342 (2010).

[22] J.J. Sloth, Survey of inorganic arsenic in marine animals and marine certified reference materials by anion exchange high-performance liquid chromatography-inductively coupled plasma mass spectrometry. Journal of Agricultural and Food Chemistry 53:60 (2005) 11-8.

[23] J. Borak, H.D. Hosgood, Seafood arsenic: Implications for human risk assessment. Regul Toxicol Pharmacol. 47:2 (2007) 204-212

[24] L. Dahl, M. Molin, H. Amlund, M.H. Meltzer, K. Julshamn, J. Alexander, J.J. Sloth, Stability of arsenic compounds in seafood samples during processing and storage by freezing, Food Chem. 123:3 (2010) 720-727

[25] K.M. Kasiotis, C. Emmanouil, P. Anastasiadou, A. Papadopoulos, O. Okay, K. Machera, Organic pollution and its effects in the marine mussel Mytilus galloprovincialis in Eastern Mediterranean coasts, Chemosphere, 119 (2015) 145-152.