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TRACE ELEMENT CONCENTRATIONS IN WILD MUSSELS FROM THE COASTAL AREA OF THE SOUTHEASTERN ADRIATIC, MONTENEGRO

JELENA MARKOVIC[1], DANIJELA JOKSIMOVIC[2] and SLAVKA STANKOVIC[3]

[1] *Institute of Nuclear Science Vinča, University of Belgrade,* 11000 Belgrade, Serbia
[2] *IBM, University of Montenegro,* 85330 Kotor, Montenegro
[3] *Faculty of Technology and Metallurgy, University of Belgrade,* 11 000 Belgrade, Serbia

Abstract - The aim of the present investigation was to quantify the levels of trace elements (Zn, Cu, As, Pb, Cd and total Hg) in the Mediterranean mussel, *Mytilus galloprovincialis* (L.). Based on their levels, the quality of Montenegro seawater for future mussel farming was estimated. The mussel *M. galloprovincialis* (L.) was collected from four sites in the Montenegrin costal area in the period of two years to determine trace element concentrations and to classify the quality of the coastal water and possible health risks from its consumption. The mean metal concentrations in the mussels ranged from 133.5-205.9 for Zn, 7.50-14.5 for Cu, 4.42-13.3 for As, 4.70-12.9 for Pb, 1.73-2.41 for Cd and 0.07-0.59 for total Hg in mg/kg dry weight. The levels of toxic metals (except for Pb) in the mussels were within the maximum residual levels prescribed by the laws of Montenegro, the EU and the USFDA. In addition, the trace metal concentrations found in the mussels in this study were similar to regional data using this mussel as a biomonitoring agent of seawater quality.

Key words: Trace elements, wild mussels, southeastern Adriatic, seawater quality, health risk

INTRODUCTION

With the increase in the consumption of seafood in recent years, marine mussels have become a commercially more important seafood species worldwide (Stankovic et al., 2011). Among these, the Mediterranean mussel *Mytilus galloprovincialis* (L.) is widely distributed in the coastal waters of the eastern Atlantic and the Mediterranean region, and it is cultivated mainly in the coastal waters of Spain and France, in southeastern Mediterranean countries such as Greece (FAO, 2007a), and more recently in Turkey (Karayucel et al., 2010). This type of mussel is distributed, cultured and investigated for different metals in the Mediterranean countries of the coastal area of the Adriatic Sea, especially in Italy and Croatia (Joksimovic et al., 2011). Small mussel farms are to be found in Montenegro since the year 2000 (FAO, 2007b). The investigation of mussels is more marked along the Italian coast than along the eastern Adriatic coast (Joksimovic et al., 2011). Consumption patterns for seafood and aquaculture products have increased in recent years in the Mediterranean region. In Montenegro, seafood consumption is relatively low in rural areas compared to the coastline, (FAO, 2010).

Mussels are an important species, both ecologically and commercially. They are available throughout the year; they are reasonably tolerant to environmental change and pollution, and they have good net accumulation capacities. They are exposed to contaminants from land-based activities, as well as to sea-based ones, making them an excellent metal biomonitoring agent (Jovic et al., 2011; Stankovic et al., 2011). Also, this type of shellfish is a commercially important seafood since it provides a good source of proteins, Ca and Fe, some vitamins, omega-3 fatty acids, selenium and iodine for human consumption (Dahl et al. 2010). However, they are potentially toxic because of the accumulation of metals in their soft tissue over time, making them detrimental to human health (Joksimovic et al. 2011; Stankovic et al. 2011). Due to the propensity of *M. galloprovincialis* (L.) to accumulate metals, the consumption of metal-contaminated seafood can cause outbreaks in humans of metal poisoning, such as happened in Japan with mercury and cadmium in 1956 and 1965, respectively (Eto, 2000). Chronic exposure of humans to metals such as Cu, Pb, Zn, As, Cd and Hg is associated with human health problems (Eto, 2000; Crinnion, 2000; Dahl et al., 2010; Stankovic et al., 2011).

The contribution of aquaculture in Montenegro is insignificant, but there is potential for its development. In light of this, the cultivation of mussels along the Montenegrin coast has been increasing (150 tons/per year in last decade (FAO, 2007b)), particularly in the Boka Kotorska Bay due to the good natural conditions; but it is still underdeveloped. The mussel *M. galloprovincialis* (L.) is widely distributed in the coastal waters of Montenegro, where mussels are cultivated on farms for the market, while wild ones are still hand-collected for personal consumption. This study, initiated from fall 2005 to spring 2007, and is related to wild mussels because of their hand-collection for human consumption and the expansion of mussel aquaculture in Montenegro, especially in area of the Bay.

The aims of this study were: (1) to determine the levels of Zn, Cu, Pb, Cd, As and Hg in the soft tissue of wild *M. galloprovincialis* from fall 2005 to spring 2007; (2) to evaluate their levels in relation to the maximum limits prescribed by national/international regulations, i.e., to investigate whether the concentrations of these metals were within the permissible limits, thereby rendering them acceptable for human consumption; (3) to compare data of this study with regional data, and (4) to evaluate the environmental quality of the coastal waters of Montenegro for future mussel farming.

MATERIALS AND METHODS

Sampling, storage and sample preparation

Wild mussel samples were seasonally collected from four sites: Herceg Novi, Sveta Stasija, Rt Djeran and Bar between the fall 2005 and spring 2007. The first two sites are in the Boka Kotorska Bay, a semi-enclosed bay located in the southeastern part of the Adriatic Sea which is naturally divided into four smaller bays: the Herceg Novi, Tivat, Risan and the Kotor bays. The other two sites are in an open coastal area south of the Bay. Herceg Novi is a popular tourist town with a small marina located at the entrance to the Bay. Sveta Stasija is in the small Kotor bay, a suburb of the town of Kotor located close to the river Ljuta. Bar is the biggest port of the southeastern Adriatic Sea. Rt Djeran was chosen because it is located on the open sea, near to the river Buna. The sampling sites are shown in Fig. 1. At each site and in each season more than 2 kg of mussels were randomly collected by hand, placed in plastic bags with seawater and transported to the laboratory. From each site in each season, pooled samples of 25-30 mussels, similar in length (Table 1) were selected, cleaned and rinsed with deionized water, dissected fresh and the soft tissue was rinsed with Milli Q water. The mussels were not depurated after collection and the gut content was included in the analyses. All samples were measured before freezing at −10°C. The samples were freeze-dried at −40°C for 48 h, weighed, homogenized to a fine powder and stored in plastic bags until analyses.

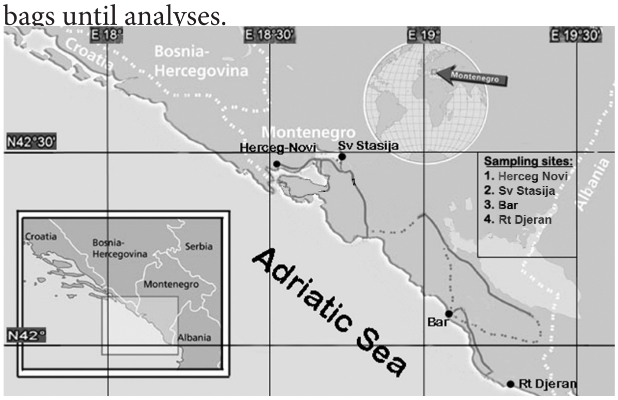

Fig. 1. Sampling sites of Mediterranean blue mussel *M. galloprovincialis* from the southeastern Adriatic Sea, Montenegro.

Table 1. Sampling data of the Mediterranean blue mussel *M. galloprovincialis* collected from the four locations of the SE Adriatic coast, Montenegro

No.	Location	Sampling Date	N	Shell length (mm)	Water %	Sea water temperature (° C)	Sea water salinity (‰)
1.	Herceg Novi,	fall 2005	25	71.3 (68.6-78.8)	78.2	17.0	29.6
	in Boka	spring 2006	26	70.3 (65.3- 74.6)	82.9	24.5	26.8
	Kotor Bay	fall 2006	25	74.8 (62.3-79.7)	79.8	20.6	36.3
		spring 2007	27	69.4 (63.9 -74.5)	80.2	22.5	32.6
2.	Sveta Stasija,	fall 2005	25	73.6 (68.9-80.3)	78.9	16.8	33.3
	in Boka	spring 2006	25	72.0 (67.5- 79.5)	81.0	18.3	12.9
	Kotor Bay	fall 2006	25	72.5 (66.8-78.0)	78.6	18.8	32.8
		spring 2007	26	70.8 (63.9- 75.4)	82.9	16.1	13.2
3.	Bar,	fall 2005	28	62.8 (53.8-69.6)	79.1	19.8	35.5
	at the open	spring 2006	30	61.7 (53.0-66.9)	82.0	24.0	29.3
	coastal area	fall 2006	29	63.1 (55.3- 70.2)	79.9	20.9	36.7
		spring 2007	30	61.4 (52.7- 68.2)	81.4	20.5	35.2
4.	Rt Djeran,	fall 2005	26	63.5 (54.4-72.5)	79.7	19.0	18.6
	at the open	spring 2006	29	61.7 (53.4- 70.3)	81.3	24.8	28.6
	coastal area	fall 2006	27	63.1 (56.2- 71.8)	78.0	18.4	36.2
		spring 2007	30	60.9 (54.5- 69.1)	80.7	20.2	30.5

Nos. follow those indicated in Fig.1.

The size and weight of the samples were determined before dissection to ensure that there were no significant differences regarding the size and weight of the samples among the sites and within the seasons ($p > 0.05$), while the average water content of the soft tissues varied between 80.3 % (spring and fall) and 87.7 % (winter) (Table 1). The results of Zn, Cu, Pb, Cd, As and Hg are presented as mean values of one pooled sample with 3 measured replicates of 0.5 g from each station in each season.

Means and standard deviations were calculated using Excel program. The Kruskal-Wallis test was applied to test the differences between the metal concentrations in the mussels related to all the locations from 2005-2007. The metal concentrations in the combination of four locations and four seasons (fall 05 – fall 06, spring 06 – spring 07, fall 05 – spring 06 and fall 06 – spring 07) were analyzed by the Mann-Whitney U test (SPSS).

Analysis of heavy metals

Five replicates of dried pooled mussel homogenate sample per site were analyzed for trace metals: approximately 0.5 g of the soft tissue was digested with a mixture of concentrated HNO3 (65% Merck, Suprapur) and H2O2 (30 % Merck, Suprapur) in a high microwave digestion system (CEM CORPORATION, MDS-2100). Prior to microwave decomposition, the samples with added reagents were allowed to digest at room temperature in loosely capped Teflon beakers for at least l h. The digested samples were diluted to 25 ml with Milli Q water containing 1.0% HNO_3 and stored in polyethylene bottles until analysis.

The zinc and cooper concentrations were determined using flame atomic absorption spectrometry (PerkinElmer, AAnalyst 200) with an air-acetylene flame. The analyses for Pb and Cd were performed using graphite furnace atomic absorption spectrometry (Perkin-Elmer, 4100ZL, with Zeeman background correction). Hydride generation and cold vapor techniques were used for analyses of As and Hg (PerkinElmer, AAnalyst 200). The accuracy of the applied analytical procedure for the determination of heavy metals in the mussels was tested using SRM 2976 (mussel homogenate; NIST) certified reference material. To check for contamination, procedural blanks were analyzed after every fifth sample. Quality control samples made from standard solutions of Zn, Cu, Pb, Cd, As and Hg were analyzed after every fifth sample to check for metal recovery, and the found percentages were 98.5% for Zn, 106% for Cu, 94.3% for Pb, 110.9% for Cd, 92.1% for As and 103.8% for Hg.

RESULTS AND DISCUSSION

Wild mussels are usually collected for human consumption on the coastal area of Montenegro, especially in the Boka Kotorska Bay. The mussel soft tissues are typically eaten whole and after harvesting for human consumption, they were not depurated. In addition, some researchers have concluded that a mussel digestive gland must be included in the analyses since it often serves as storage for biological metals, and depuration of mussels for 2-3 days might cause the loss of some metals from the mussels (Airas, 2003). Because of these reasons, the mussel analyses for heavy metals were done without their prior purification. In order to monitor food safety, the gut content was included in the analysis.

The condition and characteristics of the seawater physical parameters in the coastal area of Montenegro were examined from 2005-2007 (Joksimovic, 2010), including the levels of some metals in wild mussels and sediments (Joksimovic et al., 2011). The information related to the collected samples and seawater physical parameters at the investigated sites are given in Table 1. The Harbor Bar had the highest water salinity (29.3-36.7‰) and Sveta Stasija had the lowest water temperature (16.1-18.8°C) as a consequence of the Ljuta freshwater inflows during the investigated period.

In the present study, six trace metals were analyzed in wild mussels: zinc (Zn), copper (Cu), total arsenic (As), lead (Pb), cadmium (Cd) and total mercury (THg). The mean, minimal and maximal range concentrations of the investigated metals of five replicates found in the soft tissues of *M. galloprovincialis* from four sites in the reported period are given in Table 2. In general, the concentrations obtained in this study for the different mussel samples analyzed from different locations and periods showed that Zn is the element present at the highest concentration (133.5-205.9 mg/kg), followed by Cu (5.5-14.5 mg/kg), total As (4.42-13.3 mg/kg), Pb (4.7- 12.9 mg/kg), Cd and total Hg (0.07-0.59 mg/kg). The order of the investigated mean metal concentrations in the wild mussels from the Montenegrin coastline in the period 2005-2007 is in the opposite order of these metals' toxicity, persistence and bioaccumulation characteristics, since the most dangerous heavy metals for the marine ecosystem and human health in trace are Hg, Cd and Pb, in that order, followed at a distance by Cu, Zn and etc. (Türkmen et al., 2009).

Trace metal concentrations in the investigated wild mussels from fall 2005 to fall 2007 were subjected to statistical analysis as well. The level of significance in all statistical tests was 0.05. Using the Kruskal-Wallis and Mann-Whitney U test, the significant differences in Zn concentrations were found related to all the locations ($p < 0.05$) and between spring and fall ($p < 0.05$). Zn concentrations were high compared with the other metals (Table 2), and the significant differences in Zn concentrations between spring and fall (Mann-Whitney U test, $p < 0.05$) indicated that the growth and reproduction of the mussel *M. galloprovincialis* showed a change in metabolic activity, occuring mostly in spring and early summer, changing the level of Zn (Aral, 1999). The zinc concentrations in mussels depend on the pollution condition of the investigated locations (Jovic et al., 2011) and chemical speciation of zinc in water (Joksimovic et al., 2011). Using both tests on Cu, Pb, Cd, As and Hg concentrations, there were no significant differences in these metal concentrations related to the locations and different seasons ($p > 0.05$).

Table 2. The mean concentrations of heavy metals (mg/kg d.w.) in the soft tissue of wild mussels collected from four locations in the period from fall 2005 to spring 2007

		Seasons			
Locations	Metals (mg/kg dw)	Fall 05	Spring 06	Fall 06	Spring 07
1. H. Novi					
	Zn	190.0 ± 13.6	170.8 ± 18.1	190.0 ± 12.2	151.9 ± 14.3
	Cu	4.6 ± 0.31	11.5 ± 1.1	5.8 ± 0.5	10.2 ± 0.9
	As	17.8 ± 1.92	13.0 ± 1.2	13.9 ± 1.4	8.5 ± 1.0
	Pb	3.5 ± 0.33	6.6 ± 0.7	7.8 ± 1.3	10.1 ± 1.3
	Cd	2.1 ± 0.21	2.9 ± 0.3	1.7 ± 0.5	2.9 ± 0.8
	Hg	0.2 ± 0.08	1.0 ± 0.3	1.0 ± 0.3	0.1 ± 0.02
2. Sv. Stasija					
	Zn	132.0 ± 12.4	82.0 ± 6.7	205.0 ± 14.1	115.0 ± 11.1
	Cu	7.0 ± 0.63	8.2 ± 0.51	5.9 ± 0.52	8.9 ± 0.76
	As	7.4 ± 0.55	2.9 ± 0.22	5.5 ± 0.64	1.9 ± 0.23
	Pb	9.1 ± 0.69	11.4 ± 0.75	8.1 ± 0.56	9.8 ± 0.86
	Cd	2.1 ± 0.20	2.9 ± 0.33	1.7 ± 0.21	1.4 ± 0.24
	Hg	0.9 ± 0.04	0.06 ± 0.005	0.03 ± 0.01	0.07 ± 0.01
3. Bar					
	Zn	280 ± 30.2	101.5 ± 5.2	300.0 ± 26.7	142.2 ± 12.4
	Cu	15.1 ± 1.2	12.6 ± 1.5	17.2 ± 1.5	13.2 ± 1.2
	As	7.5 ± 0.5	8.0 ± 0.20	4.9 ± 0.5	2.7 ± 0.1
	Pb	11.1 ± 1.0	8.5 ± 0.42	15.8 ± 0.75	16.3 ± 0.7
	Cd	3.53 ± 0.5	2.2 ± 0.40	1.8 ± 0.3	1.0 ± 0.12
	Hg	0.3 ± 0.04	0.3 ± 0.02	1.06 ± 0.01	0.25 ± 0.06
4. Rt Djeran					
	Zn	167.0 ± 15.3	118.1 ±10.8	345.0 ± 32.9	123.1 ± 11.5
	Cu	11.7 ± 0.8	12.4 ± 1.0	7.4 ± 0.60	7.60 ± 0.45
	As	20.5 ± 2.37	4.4 ± 0.5	6.6 ± 0.35	4.16 ± 0.35
	Pb	7.9 ± 0.36	1.30 ± 0.07	7.2 ± 0.61	2.34 ± 0.01
	Cd	1.0 ± 0.02	2.30 ± 0.06	1.9 ± 0.01	1.70 ± 0.04
	Hg	0.14 ± 0.01	0.08 ± 0.01	0.03 ± 0.01	0.03 ± 0.01

The levels of As in most foods are very low, with the exception of seafood. A wide range of arsenic compounds, including inorganic arsenic, has been reported in marine organisms (Dahl et al., 2010). Seafood is known to be the most significant source of arsenic in the diet and, consequently, the total human intake of arsenic depends on the quantity of seafood consumed (Stankovic et al., 2011). Arsenic is found in seafood in different chemical forms, differing in their degree of toxicity and the pathologies that they generate (Dahl et al., 2010). Essential elements such as Cu and Zn occur naturally in all organisms and are essential for many enzymatic and protective functions, but in high doses essential elements can also be poisonous and cause hazardous effects on organisms (Joksimovic et al., 2011). Non-essential elements, such as As, Pb, Cd and Hg, do not have any positive effects on organisms and are already harmful at low doses (Dahl et al., 2010; Stankovic et al., 2011). In order to compare the heavy metal concentrations in mussels with the legal limits regulated by law, the dry weight concentrations of the metals (Table 2) were converted into wet weight by calculating the ratio between the dry and wet weights for each sample in each season: the mean and the range of minimum and maximum values of the studied el-

Table 3. The concentrations (mean, range min–max, mg/kg) of mercury (Hg), cadmium (Cd), lead (Pb), arsenic (As), copper (Cu) and zinc (Zn) in the soft tissues of wild mussels collected in the period from fall 2005 to spring 2007

No	Location	N	WB[1]	Hg (mg/kg)	Cd (mg/kg)	Pb (mg/kg)	As (mg/kg)	Cu (mg/kg)	Zn (mg/kg)
1	**H. Novi,**	5		**0.59**	**2.41**	**7.0**	**13.3**	**8.0**	**175.5**
	B. Kotor		Dry	(0.15-1.04)	(1.7-2.9)	(3.5 – 10.1)	(8.5-17.8)	(4.6-11.5)	(151.9-190)
	Bay			***0.11***	***0.47***	*1.38*	*2.62*	*1.57*	*34.6*
			Wet	(0.03-0.23)	(0.34-0.53)	(0.76– 2.0)	(1.7-3.9)	(1.0-1.96)	(30- 39.5)
2	**Sv. Stasija** B.			**0.27**	**2.02**	**9.8**	**4.42**	**7.5**	**133.5**
	Kotor	5	Dry	(0.03-0.9)	(1.4 -2.9)	(8.1 -11.4)	(1.9-7.4)	(5.9-8.9)	(82.0-205)
	Bay			*0.06*	*0.40*	*1.93*	*0.87*	*1.5*	*26.3*
			Wet	(0.006 -0.17)	(0.24 -0.55)	(1.54 -2.1)	(0.32-1.6)	(1.2-1.52)	(15.6-43.8)
3	**Bar**								
	at the open	5		**0.49**	**2.13**	**12.9**	**5.82**	**14.5**	**205.9**
	coastal area		Dry	(0.25-1.06)	(1.0-3.53)	(8.5- 16.3)	(2.75-8.05)	(12.6-17.2)	(101.5-300)
				0.10	*0.42*	*2.67*	*1.15*	*2.8*	*40.5*
			Wet	(0.05-0.2)	(0.18-0.74)	(1.58 - 3.3)	(0.51-1.6)	(2.4 -3.45)	(18.3-60.3)
4	**Rt Djeran**								
	at the open	5		**0.07**	**1.73**	**4.7**	**8.9**	**9.8**	**188.3**
	coastal area		Dry	(0.03.-0.14)	(1.0-2.3)	(1.30- 7.86)	(4.16-20.5)	(7.45-12.4)	(118.1-345)
				0.01	*0.34*	*0.97*	*1.84*	*2.02*	*39.0*
			Wet	(0.005-0.03)	(0.2- 0.43)	(0.24 -1.6)	(0.84 - 4.1)	(1.64 -2.3)	(22.1 -75.9)

Nos. correspond to locations indicated in Fig.1.
N – Number of observations

1 WB – weight basis

Table 4. Guidelines on heavy metals for food safety set by different countries

Location	WB	Hg (mg/kg)	Cd (mg/kg)	Pb (mg/kg)	As (mg/kg)	Cu (mg/kg)	Zn (mg/kg)
Permissible limits by Montenegrin Food regulation (2002)	Wet	1.0	1.0	1.0	4.0	30	100
EU (2006) Comm. Regulation (EC) No. 188/2006	Wet	0.5	1.0	1.50	-	-	-
USFDA (2007) Food and Drag Administration of the United States	Wet	1.0	4.0	1.70	86	- -	- -

ements related to the dry and wet weights (mg/kg) are presented in Table 3. Similar to European and other countries, Montenegrin law defines the legal limits of permissible concentrations of heavy metals in shellfish (Table 4). In comparison with the permissible limits set by the Montenegrin Food Regulation (2002) for total Hg (1.0 mg/kg ww), Cd (1.0 mg/kg ww), Pb (1.0 mg/kg ww), total As (4.0 mg/kg ww), Cu (30.0 mg/kg ww) and Zn (100 mg/kg ww), all the mean and range values (mg/kg ww) of these metals from all the studied locations were lower, except for Pb (Table 3). In the present study, the Pb mean concentrations in wild mussels (*M. galloprovincialis*) were lower than the Montenegrin limit only at the site Rt Djeran (0.97 mg/kg ww). At all the other locations, the Pb mean values in the investigated mussels were above the Pb limit set by the Montenegrin Food Regulation (2002). The EC (2006) and USFDA (2007) have higher permissible limits for Pb than those set by Montenegrin law i.e. 1.5 mg/kg ww and 1.7 mg/kg ww, respectively. The Pb mean concentrations in the investigated samples from the Bar location (2.67 mg/kg ww) and Sv. Stasija (1.93 mg/kg ww) were above the EC (2006) and USFDA (2007) limits (Table 3). The mean concentrations of Hg (0.015-0.11 mg/kg ww) and Cd (0.34 - 0.47 mg/kg ww) were below the legal limits regulated by the EC (2006) and the USFDA (2007) at all locations, and As concentrations were below the legal limits regulated by the USFDA (2007) in the all investigated samples (Tables 3 and 4).

The quality of Montenegrin seawater is generally similar to that of the rest of the Adriatic when the toxic metal concentrations in mussels (Table 3) are compared with the legal limits regulated by law (Table 4) and compared with the Airas (2003) seawater classification, which is based on of the trace metal concentration levels found in mussels: from class I, unpolluted, to class V, highly polluted.

The temperature and salinity of the Montenegrin coastal water is optimal for mussel cultivation (Joksimovic, 2010), since *M. galloprovincialis* grows to an optimum size in seawater temperatures of 15°C-25°C and salinity between 20‰-35‰ (Braby and Somero, 2006). In view of the main sources of contamination of the Montenegrin coastal water (Jovic et al., 2011), wild mussels have to be depurated before consumption.

With regard to the trace element level determined in wild mussels, the seawater in the areas of Rt Djeran and Sveta Stasija is unpolluted or slightly polluted by Hg, Cd, As, Cu and Zn and related to their concentrations in investigated mussels, belongs to the class I (unpolluted). However, the concentration of Pb in the seawater belongs to class II (moderately polluted). The seawater in Herceg Novi and Bar Harbor is polluted by Hg, belonging to class III, or/and class II, compared to the other elements measured in wild mussels from these two areas.

As there are no data on the average national rate of shellfish consumption (*RSC*) in Montenegro (FAO, 2010), the average weekly serving size for typical local diet containing mussels is proposed to be 350 g and this data is related to fresh mussels. The mean and min-max range in mg/kg values of Hg, Cd, Pb, Cu and Zn concentrations in the dry and wet weight of mussels in the investigated period are given in Table 3. In this study, the provisional tolerable weekly intake (PTWI) was used for the calculation of the metal concentration levels of concern associated with wild mussel consumption in the study area.

According to the FAO/WHO (2004), PTWI (μg/kg of body weight/week) is the term used by the JECFA (Joint FAO/WHO Expert Committee on Food Additives) to define the level of intake by an adult of an accumulative contaminant without appreciable health risk over a lifetime, where an adult is assumed to weigh 60 kg. According to the FAO/WHO (2004), the average weekly intake for adults was estimated to be 420 mg for Zn ($PTWI_{Zn}$ = 7 mg/kg body weight/week), 210 mg for Cu ($PTWI_{Cu}$ = 3.5 mg/kg body weight/week) 1.5 mg for Pb ($PTWI_{Pb}$ = 25 μg/kg body weight/week), 0.42 mg for Cd ($PTWI_{Cd}$ = 7 μg/kg body weight/week), 0.9 mg for As ($PTWI_{As}$ = 15 μg/kg body weight/week) and 0.3 mg for THg ($PTWI_{Hg}$ = 5 μg/kg body weight/week). The PTWI for methyl-mercury was set at 96 μg (1.6 μg/kg of body weight/week).

The JECFA has recently re-evaluated As and the Committee withdrew the previous PTWI of 15 μg/kg bodyweight set for inorganic arsenic (Dahl et al., 2010), since the lowest value of 0.3 μg/kg bodyweight (bw) per day was linked to increased lung cancer (Dahl et al., 2010). The JECFA noted that more accurate information on the inorganic As content in foods is needed to improve assessments of dietary exposures to inorganic arsenic species (Dahl et al., 2010). However, the majority of arsenic in seafood is present in organic arsenobetaine (AB), a less toxic, water-soluble form (Stankovic et al., 2011), which is considered non-hazardous and safe for human consumption (Sloth, 2003). According to Munoz et al. (1999), the percentages of inorganic arsenic in sea-

Table 5. A comparison of reported concentrations (mg/kg) of mercury (Hg), cadmium (Cd), lead (Pb), arsenic (As), copper (Cu) and zinc (Zn) in the whole soft tissue of the mussel (*Mytilus galloprovincialis)* from regional studies with the present results

Location	WB[1]	Hg (mg/kg)	Cd (mg/kg)	Pb (mg/kg)	As (mg/kg)	Cu (mg/kg)	Zn (mg/kg)	References
Ionian Sea, Southern Italy	Dry	-	0.2-0.8 0.3-0.9	1.3-3.1 1.2-4.3	0.2-3.4 0.8-5.3	6.9-12.8 6.3-15.7	68-107 73-109	Cardellicchio et al., (2008)
Adriatic Sea, Slovenia	Dry	-	0.43-1.12	0.76-11.8	-	-	-	Scancar et al., (2007)
Eastern Adriatic coast, Croatia	Dry	0.08-0.28	0.2-2.0	0.9-15.1	-	3.1- 60	44 -387	Kljakovic-Gaspic et al., (2006)
E Adriatic, in Mali Ston Bay Croatia	Dry	0.15	0.4-2.4	0.24-3.7	-	1.98 -11	49-418	Kljakovic-Gaspic et al., (2007)
Montenegro, SE Adriatic (4 sites)	Dry Wet	0.07 -0.59 0.015-0.11	1.73 -2.41 0.34 - 047	4.7 - 12.9 0.97-2.67	4.42-13.3 1.15-2.62	7.5 -14.5 1.5 - 2.8	135.5- 205.9 26.3 - 40.5	This study

WB – weight basis

food are 1-5%, while in mussels, they are 1.9-6.5 %, but the inorganic toxic arsenic fraction increased with increasing contents of total arsenic (Sloth and Julshamn, 2008). Nevertheless, seafood also contributes substantially to dietary arsenic, which is one of the trace elements of concern in relation to food safety (Dahl et al., 2010). No value of As intake is reported in this study because the As determined refers to total arsenic (Table 2 and 3).

Hg accumulates in mussels and can be highly toxic to human health (Eto, 2000; Stankovic et al., 2011). Mercury and methyl mercury may accumulate over time in the human body Fleisher (2001), and both forms are immunotoxic, although they differ quantitatively and qualitatively in their effects on the human immune system (Crinnion, 2000; Clarkson 2002). Based on the average of total Hg concentrations (0.01-0.11 mg/kg ww) obtained from the wild mussels (Table 3), the concentrations of THg consumed by populations residing in the coastal area of Montenegro in a typical diet containing 350 g ww of mussels were below the PTWI, i.e. between 3.8-38.5 μg person^{-1} week^{-1}. Using the maximum concentration of 0.23 mg/kg ww in wild mussels from the site Herceg Novi, 80.5 μg of THg person^{-1} week^{-1} was still below the PTWI value of 0.3 mg of THg person^{-1} week^{-1} established by the joint FAO/WHO Expert Committee on Food Additive (2004).

Cd is found in marine waters mostly in the dissolved form, distributed in the marine environment at low concentrations. Mussels accumulate Cd effectively (Cardellicchio, 2008; Stankovic et al., 2011) and it may act as a poison to humans (Eto, 2000). Under chronic Cd exposure, Cd can inhibit the development of bone softening due to decalcification, a characteristic of itai-itai disease (Han et al., 2000; Eto, 2000). The mean concentrations of Cd found in the analyzed mussel samples ranged from 0.34-0.47 mg/kg ww in the investigated period. As regards Cd, none of the mussel samples analyzed in this study presented levels above those permitted, i.e. 0.42 mg person^{-1} week^{-1}. The concentrations of Cd in a typical diet containing mussels were between 0.12-0.16 mg person^{-1} week^{-1}, below the PTWI for Cd. The highest Cd value measured was in mussels from Bar Harbor, 0.74 mg/kg ww. Based on this data, the consumption of 350 g ww of mussels on a weekly basis from this location is also below the PTWI for Cd, 0.26 mg person^{-1} week^{-1}.

The PTWI value for Pb is 1.5 mg person^{-1} week^{-1}. The mean Pb content in all wild samples collected ranged from 0.97-2.67 mg/kg ww. The concentration of Pb in a typical diet containing 350 g ww mussels was between 0.34-0.93 mg person^{-1} week^{-1}, which is below the PTWI for Pb. Calculating the PTWI value for Pb in relation to the highest Pb level at Bar Harbor (3.3 mg/kg ww), the consumption of 350 g ww mussels on a weekly basis is reaching 1.15 mg person^{-1} week^{-1} for Pb, which is still below the PTWI value for Pb. The absorption of lead from ingested food greatly depends on the levels of other elements present in the diet, such as calcium, iron and zinc, i.e. dietary deficiencies in these essential elements enhance lead absorption, (Goyer, 1995).

Cu is an essential element that is an integral part of several enzymes and it is necessary for hemoglobin synthesis (Sivaperumal et al. 2007; Joksimovic et al., 2011). The mean concentrations of Cu found in the analyzed mussel samples ranged from 1.5-2.8 mg/kg ww, with the highest concentration found in the mussels from the Bar Harbor location. These concentrations of Cu were more than five to ten times below the Cu toxic limit of 6.3 mg person^{-1} week^{-1}. The consumption of 350 g ww of mussels on a weekly basis is reaching 0.53-0.98 mg person^{-1} week^{-1} for Cu.

Zn is one of the essential trace elements for animals and humans. Zinc is important for the synthesis of DNA, RNA and proteins, it appears to have a protective effect against the toxicities of cadmium and lead (Sivaperumal et al., 2007) and its toxicity is rare. The mean concentrations of Zn found in the analyzed mussel samples ranged from 26.3-40.5 mg/kg ww, with the highest Zn concentration found in the mussels from the Bar Harbor location, 60.3 mg/kg ww. With the consumption of 350 g ww of mussels on a weekly basis (the highest Zn concentration is in the Bar Harbor), Zn intake is reaching 21.1 mg person^{-1} week^{-1}. The PTWI value for Zn is 420 mg person^{-1} week^{-1}.

The concentrations of Hg, Cd, Pb, Cu and Zn consumed in a typical diet containing 350 g ww of wild mussels on a weekly basis were below the PTWIs. The contribution of mussels to diet is highly variable, but may be very high in certain groups, since individuals regularly hand-collect wild mussels for their personal consumption. In general, large amounts of mussel would need to be consumed each week in order to exceed the prescribed PTWIs.

Comparing the levels of elements from different Adriatic and Mediterranean areas (Kljakovic-Gaspic et al., 2006 and 2007; Scancar et al. 2007; Cardellicchio et al., 2008; Stankovic et al., 2011), the obtained data in this study indicates that the metal levels found in wild *M. galloprovincialis* from the Montenegrin coastal area are similar to the metal concentrations reported for other areas of the Adriatic (Table 5).

CONCLUSIONS

The metal concentrations found in the edible tissue of wild mussels expressed in wet weight were lower than the maximum permissible levels for fresh mussels set by various authorities, except for Pb. Based on the weekly consumption of mussels (350 g ww), there is no risk to human health in relation to all investigated elements. The PTWI values appear to support the conclusion that the risk to human health from typical local dietary exposure to heavy metals from wild mussels is relatively low in the case of Hg, Cd, Pb, Cu and Zn concentrations. No value of As intake is reported in this study because the As determined refers to total arsenic.

The comparison of the results obtained in this work with already published values for the Adriatic and the Mediterranean shows that the values of trace element concentrations in mussels obtained herein fall in the range of values most commonly found in moderately polluted areas of the Mediterranean and Adriatic Seas. The investigated areas of Rt Djeran and Sveta Stasija belong to unpolluted or slightly polluted areas as regards Cd, As, Cu and Zn concentrations, but related to the Pb concentration in mussels from these two areas, the seawater is moderately polluted.

Herceg Novi and Harbor Bar are polluted by Hg, but only moderately polluted by concentrations of the other elements.

As this was a pilot study to evaluate the environmental quality of the coastal waters of Montenegro for future mussel farming and possible health risks for the residents on the shore who hand-collect wild mussels for consumption, the continued monitoring of seawater quality is necessary in order to control the amount of trace elements in mussels from this part of Adriatic.

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