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Trace elements concentrations (Zn, Cu, Pb, Cd, As and Hg) in the Mediterranean mussel (*Mytilus galloprovincialis*) and evaluation of mussel quality and possible human health risk from cultivated and wild sites of the southeastern Adriatic Sea, Montenegro

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Abstract: The Mediterranean mussel *Mytilus galloprovincialis* (L.) was collected from the fall 2005 to the winter 2009 from the six sites on the Montenegrin coastline. Two wild samples were collected from the open sea coastline, and two cultivated and two wild were from the Boka Kotorska Bay. The mussels soft tissue was analyzed for zinc, copper, lead, cadmium, arsenic and total mercury. Concentrations of these metals, in mg kg^{-1} dry weight, ranged from 135–210 for Zn, 6.2–14.5 for Cu, 4.0–11.5 for Pb, 1.7–2.1 for Cd, 5.8–12.4 for As and 0.1–0.5 for Hg. The metals were found to be present in the samples at different levels, but not in concentrations higher than maximum residual levels prescribed by the European Union (EU) and US Food and Drug Administration (USFDA) regulations for seafood. This indicates that the consumption of wild or cultivated mussels from the studied area is safe in moderate quantities.

Keywords: *M. galloprovincialis*; trace elements; mussel quality; health risks; Montenegro; Adriatic.

INTRODUCTION

With the increase in consumption of seafood in recent years,[1,2] marine mussels have become commercially more important seafood species worldwide.[3] Among these, the Mediterranean mussel *Mytilus galloprovincialis* (L.) is widely

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distributed in the coastal waters of the eastern Atlantic–Mediterranean region and is mainly cultivated in the northwestern coastal waters of Spain and the northern shores of the Mediterranean Sea.[4] More recently, production of this mussel has been reported on the shores of the southern Mediterranean countries and on the Adriatic coast. Until recently, the main seafood product on the Montenegrin market was fish, but currently, the demand for nutrition sources such as mussels and other shellfish, has increased.[5]

The mussel *M. galloprovincialis* is a native species of this area and there are some indications that this bivalve was cultivated a hundred years ago. Cultivation of mussels in Montenegro is a family-run business and all farms are located in the Boka Kotorska Bay where the hydrological, physical and biological conditions are suitable for the type of rearing used on these farms. There is no modern mechanization on the farms and almost all the procedures are performed manually, which results in a long production cycle and low productivity. During the period 2003–2007, the average annual production of mussels in Montenegro was 150 t,[5] which is very small in comparison with the largest producers China (600,000 t year^{-1}) and Spain (200,000 t year^{-1}).[4] The farmed mussels are sold mostly to restaurants, hotels and on the local market stalls and small quantities of mussels are exported to neighboring countries, primarily to Serbia. Although the economic value of the aquaculture sector is currently very low, significant potential for the production and export of mussels, *M. galloprovincialis*, has been identified.[5]

Mussels are an important source of Ca and Fe, some vitamins such as niacin and thiamine, and are a good source of protein for humans.[6] Even though mussels are an excellent source of nutrients, they can potentially be toxic because certain metals, such as Pb, Cd, As and Hg, can accumulate in their soft tissue over time making them detrimental to human health.[7–10] As the natural habitats of *M. galloprovincialis* (L.) are usually close to estuaries, they are exposed to contaminants from land-based activities, as well as to sea based ones, making them an excellent heavy metal biomonitoring agent.[11] Conveniently, *M. galloprovincialis* (L.) are sedentary, long-lived, and easily identifiable and sampled organisms. They are fairly abundant and available throughout the year, reasonably tolerant to environmental change and pollution, and they have good net accumulation capacities.[4,11] Due to the propensity of *M. galloprovincialis* (L.) to accumulate metals and other contaminants in their soft tissue[12–16] and shells,[17] mussels have actively been used as a biomonitoring agent, as the determination of contaminant levels in mussel species provides a means of assessing the possible toxicant risk to public health.[4,8,18,19] Regular consumers of bivalve mollusks in Europe were found to have higher dietary exposures to Cd, up to 4.6 μg kg^{-1} body weight (b.w.).[20] Human exposure to Pb is mainly *via* food and water. On average, Pb dietary exposure ranges from 0.36 to 1.24, up to 2.43 μg kg^{-1}

b.w. per day for large mussels consumers in Europe.[21] The accepted tolerable weekly intakes of trace elements *via* the food change over time; concentrations of trace elements which were thought to be safe were later found to be harmful.[22,23]

Since trace elements are non-biodegradable chemicals which cannot be metabolized and do not break down into harmless forms,[24] the measurement of their concentration in mussel soft tissue intended for human consumption has become increasingly significant. Accumulation of toxic metals to above permissible limits in *M. galloprovincialis* (L.) would certainly create a notorious food image from the public health point of view, as it is well known that chronic exposure to trace elements is associated with disease,[25] as well as other health problems.[26]

All elements are toxic above some threshold bioavailable level, but Hg, Cd, As and Pb are particularly toxic[4,20–23] for animals and humans. Zn and Cu are two of the essential trace elements for both and zinc toxicity is rare and in addition, this element appears to have a protective effect against toxicities of cadmium and lead.[8]

In this study, the concentration levels of essential elements, Zn and Cu, and toxic ones, *i.e.*, Pb, Cd, As and total Hg, were determined in the soft tissue of *M. galloprovincialis* collected from natural (wild) and cultivation farms of the south-eastern Adriatic – Montenegrin coast. The aim was to investigate whether the concentrations of these metals were within the permissible limits and the mussels thereby acceptable for human consumption.

EXPERIMENTAL

Sampling, storage and sample preparation

The sampling sites are shown in Fig. 1. Sampling was conducted between the fall 2005 and winter 2009. Wild mussels were collected from four locations (two from the open sea and two in the enclosed Boka Kotorska Bay) and cultivated mussels from two farms in the Bay. Mussels of similar shell length (the approximate age of the cultivated mussels was one and half years and the wild ones having the same shell length were assumed to be of the same age) were collected at each sampling site (Table I), placed in plastic bags with sea water and transported to the laboratory. After washing with distilled water, the size and weight of the samples were determined before dissection making sure that there were no significant differences regarding the size and weight of the samples among sites and within seasons ($p < 0.05$). The average water content in the soft tissues varied between 80.3 % (spring–fall) and 87.7 % (winter). Mussels were dissected by removing the byssus and shell and composite samples of mussel tissue were rinsed with de-ionized water, freeze-dried and homogenized using a mill. About 25–30 mussels from each sampling site were selected, pulverized and analyzed for the trace elements.

Analysis of trace elements

Preparation of mussel soft tissue (approximately 0.5 g) for trace metal analysis was performed as follows: the soft tissue was digested with a mixture of concentrated HNO_3 (65 % Merck, Suprapur) and H_2O_2 (30 % Merck, Suprapur) in a Microwave Digestion System (CEM. CORPORATION, MDS-2100). Prior to microwave decomposition, samples with added reagents were allowed to digest at room temperature in loosely capped Teflon beakers for at least 1 h. Digested samples were diluted to 25 ml with deionized water containing 1.0 % HNO_3.

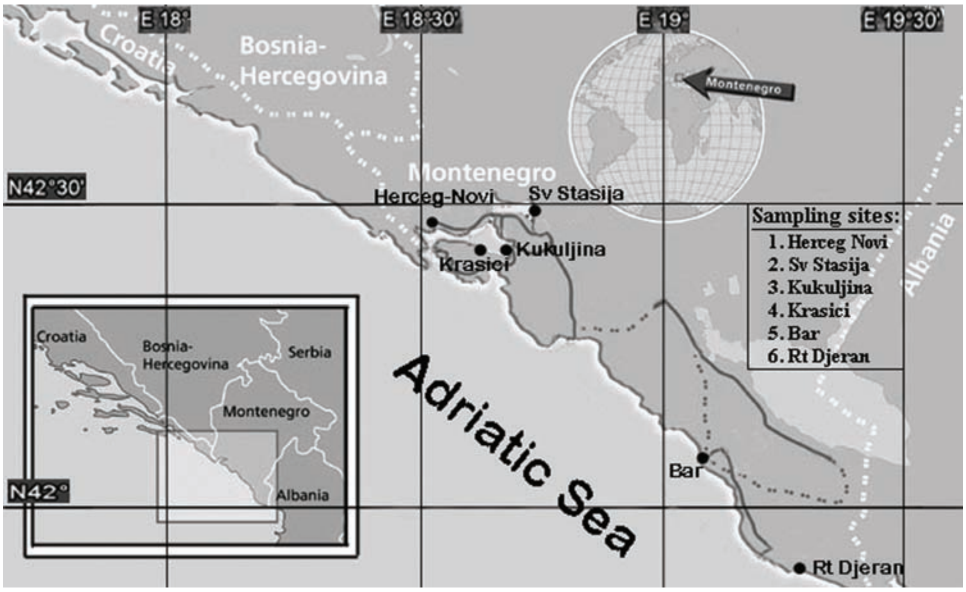

Fig. 1. Sampling sites of the Mediterranean mussel *M. galloprovincialis* from the southeastern Adriatic Sea, Montenegro.

TABLE I. Sampling locations and shell length of the Mediterranean mussel *M. galloprovincialis* collected from the SE Adriatic coast, Montenegro (Nos. follow those indicated in Fig. 1)

No.	Location	Sampling date	Sample nature	*N*	Shell length, mm
1	Herceg Novi, in	19 Sep. 2005	Wild	25	71.3 (68.6–78.8)
	Boka Kotorska	23 May 2006			70.3 (65.3–74.6)
	Bay	24 Sep.2006			74.8 (62.3–79.7)
		16 May 2007			69.4 (63.9–74.5)
		21 Sep. 2007			72.3 (66.8–76.3)
		04 Feb. 2008			68.7 (64.0–72.2)
		23 Jun 2008			70.9 (68.0–75.6)
		06 Oct. 2008			74.5 (67.8–79.2)
		09 Feb. 2009			70.2 (67.4–73.9)
2	Sveta Stasija, in	19 Sep. 2005	Wild	25	73.6 (68.9–80.3)
	Boka Kotorska	23 May 2006			72.0 (67.5–79.5)
	Bay	24 Sep.2006			72.5 (66.8–78.0)
		16 May 2007			70.8 (63.9–75.4)
		21 Sep. 2007			71.9 (65.6–76.9)
		04 Feb. 2008			67.1 (60.4–72.5)
		23 Jun 2008			68.5 (64.3–73.7)
		06 Oct. 2008			72.6 (61.0–81.9)
		09 Feb. 2009			68.0 (56.9–71.3)

TABLE I. Continued

No.	Location	Sampling date	Sample nature	*N*	Shell length, mm
3	Kukuljina, in Boka Kotorska Bay	21 Sep.2007	Cultured	27	64.8 (56.9–71.6)
		04 Feb.2008			62.4 (53.4–69.8)
		23 Jun 2008			63.7 (54.6–70.1)
		06 Oct.2008			67.1 (59.5–72.5)
		09 Feb. 2009			62.9 (52.1–71.4)
4	Krašići, in Boka Kotorska Bay	21 Sep. 2007	Cultured	25	66.9 (59.1–72.0)
		04 Feb. 2008			63.6 (55.9–70.7)
		23 Jun 2008			64.9 (56.0–71.9)
		06 Oct. 2008			68.0 (61.3–73.4)
		09 Feb. 2009			63.1 (56.8–68.9)
5	Bar, open coastal area	19 Sep. 2005	Wild	30	62.8 (53.8–69.6)
		23 May 2006			61.7 (53.0–66.9)
		24 Sep. 2006			63.1 (55.3–70.2)
		16 May 2007			61.4 (52.7–68.2)
6	Rt. Djeran, open coastal area	19 Sep. 2005	Wild	30	63.5 (54.4–72.5)
		23 May 2006			61.7 (53.4–70.3)
		24 Sep. 2006			63.1 (56.2–71.8)
		16 May 2007			60.9 (54.5–69.1)

The Zn and Cu concentrations were determined using flame atomic absorption spectrometry (Perkin-Elmer, AAnalyst 200) with an air–acetylene flame. Analyses of 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 the analyses of As and Hg (Perkin-Elmer, AAnalyst 200). Each reported value is the average of five determinations. All results were measured in mg kg^{-1} of sample dry weight (dw). The accuracy of the applied analytical procedure for the determination of trace elements in mussels was tested using SRM 2976 (Mussel homogenate, NIST) certified reference material. To avoid possible contamination, all employed glassware and equipment were acid-washed. To check for contamination, procedural blanks were analyzed after every five samples. Quality control samples, made from standard solutions of Zn, Cu, Pb, Cd, As and total Hg, were analyzed after every five samples to check for element recovery, and these 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 total Hg. The limits of detection (LOD / mg kg^{-1}) and standard errors of the mean for each element were: Zn, 0.09 and 11.4; Cu, 0.05 and 1.32; Pb, 0.02 and 1.06; Cd, 0.02 and 0.07, As, 0.05 and 1.01 and Hg, 0.05 and 0.06, respectively.

The concentrations of the investigated elements according to dry weight were converted into those based on wet weight by calculating the ratio between the dry and wet weight for each sample in each season. The mean and range of the minimal and maximal values of the studied elements related to dry and wet weight (mg kg^{-1}) are presented in Table II.

In this study, the provisional tolerable weekly intake (*PTWI*) values were used for the calculation of the metal concentration levels of concern associated with the consumption of wild and cultivated mussel from the study area. As there is no data on the average national rate of shellfish consumption (RSC) in Montenegro,[27] the *PTWI* values were employed as standards for calculating the metal concentration levels of concern associated with possible amounts of mussel consumed. According to the FAO/WHO,[28] *PTWI* (μg kg^{-1} of body weight per 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, whereby the assumed weight of an adult is 60 kg, of an accumulative contaminant which can be ingested without appreciable health risk over a lifetime. The average weekly intake per person for adults (in mg, 60 kg body weight) was estimated to be: 420 for Zn, 30 for Cu, 1.5 for Pb, 0.42 for Cd, 0.9 for As and 0.3 for Hg, according to the FAO/WHO.[28]

TABLE II. Concentrations (mg kg^{-1}) of Hg, Cd, Pb, As, Cu and Zn in the whole soft tissues of *M. galloprovincialis* collected from the Montenegrin coastal area, southeastern Adriatic Sea (Nos. follow those indicated in Fig. 1)

No.	Nature	Weight basis	Hg	Cd	Pb	As	Cu	Zn
1	Wild	Dry	0.51	2.14	6.40	12.4	6.54	205.8
			(0.1–1.0)	(1.0–3.2)	(3.0–10.0)	(5.6–17.8)	(3.7–11.5)	(103–433)
		Wet	0.10	0.350	1.06	2.00	1.08	33.90
			(0.02–0.17)	(0.19–0.59)	(0.5–1.7)	(0.9–2.9)	(0.6–1.9)	(17.0–71.5)
2	Wild	Dry	0.30	1.90	6.50	7.80	6.20	135.5
			(0.07–0.95)	(1.20–3.20)	(1.5–12.2)	(1.9–24.8)	(3.7–8.9)	(60–255)
		Wet	0.050	0.310	1.08	1.30	1.02	22.40
			(0.01–0.15)	(0.20–0.60)	(0.25–1.73)	(0.3–4.1)	(0.6–1.5)	(9.9–42.0)
3	Cultured	Dry	0.39	1.94	5.20	5.97	7.17	186.2
			(0.18–0.79)	(1.2–3.22)	(2.0–7.5)	(2.6–10.0)	(4.5–13.7)	(126–325)
		Wet	0.060	0.320	0.860	0.990	1.18	30.70
			(0.03–0.13)	(0.20–0.53)	(0.33–1.24)	(0.4–1.7)	(0.7–2.3)	(20.8–54.0)
4	Cultured	Dry	0.44	1.83	4.00	7.97	7.35	180.8
			(0.25–0.76)	(1.2–2.8)	(1.5–7.5)	(2.5–13.0)	(3.7–13.2)	(101–309)
		Wet	0.080	0.300	0.660	1.30	1.21	29.80
			(0.04–0.12)	(0.20–0.46)	(0.24–1.24)	(0.4–2.1)	(0.6–2.2)	(16.7–51.0)
5	Wild	Dry	0.49	2.13	11.5	5.80	14.5	205.9
			(0.25–1.06)	(1.0–3.5)	(8.5–15.8)	(2.7–8.0)	(12.6–17.0)	(101–300)
		Wet	0.090	0.350	1.70	0.960	2.40	34.00
			(0.04–0.17)	(0.17–0.58)	(1.4–2.4)	(0.45–1.30)	(2.1–2.9)	(17.0–49.5)
6	Wild	Dry	0.11	1.70	5.78	9.67	10.5	210.0
			(0.08–0.14)	(1.0–2.3)	(1.30–7.86)	(4.2–20.5)	(7.5–12.4)	(118–345)
		Wet	0.020	0.290	0.950	1.60	1.80	34.60
			(0.01–0.02)	(0.16–0.38)	(0.21–1.30)	(0.7–3.4)	(1.3–2.0)	(19.5–57.0)

RESULTS AND DISCUSSION

In the present study, six trace elements were analyzed in wild and cultivated mussels: zinc, copper, total arsenic, lead, cadmium and total mercury. The mean, and minimal and maximal range concentrations of the investigated metals of five replicates found in the soft tissues of *M. galloprovincialis* from six sites in the reported period are given in Table II. In general, the concentrations of the investigated elements found in the soft tissues of *M. galloprovincialis* sampled from different locations and periods from the Montenegrin coastal area showed that Zn was the element present in the highest mean levels 135 to 210 mg kg^{-1} dw (22.4–

–34.6 mg kg^{-1} ww), followed by Cu in the range from 6.2 to 14.5 mg kg^{-1} dw (1.0–2.4 mg kg^{-1} ww), total As from 5.8 to 12.4 mg kg^{-1} dw (1.0–2.0 mg kg^{-1} ww), Pb from 4.0 to 11.5 mg kg^{-1} dw (0.7–1.7 mg kg^{-1} ww), Cd from 1.7 to 2.1 mg kg^{-1} dw (0.30–0.35 mg kg^{-1} ww) and total Hg from 0.11 to 0.51 mg kg^{-1} dw (0.02–0.1 mg kg^{-1} ww) (Table II).

In order to compare the heavy metal concentrations with the legal limits regulated by law, the dry weight concentrations of the metals were converted into wet weight concentrations. As in other countries, Montenegrin law[29] defines legal limits for the permissible concentrations of trace elements in shellfish (Table III). In comparison with the permissible limits set by the Montenegrin Food Regulation[29] for total Hg (1.0 mg kg^{-1} ww), Cd (1.0 mg kg^{-1} ww), Pb (1.0 mg kg^{-1} ww), total As (4.0 mg kg^{-1} ww), Cu (30.0 mg kg^{-1} ww) and Zn (100 mg kg^{-1} ww), all the mean and range values (mg kg^{-1} ww) of these metals from all locations were lower than the limits, except for Pb (Table III). In the present study, the Pb mean concentrations in wild mussels (*M. galloprovincialis*) from locations Herceg Novi and Sveta Stasija (1.1 mg kg^{-1} ww) were the same as the Montenegrin limit, but from the location Bar (1.7 mg kg^{-1} ww), they were higher than the Montenegrin limit for Pb. At the locations where mussels were cultivated, Krasici (0.7 mg kg^{-1} ww) and Kukuljina (0.9 mg kg^{-1} ww), the Pb mean values in the investigated mussels were below the Pb limit set by the Montenegrin Food Regulation.[29] The EC[30] and USFDA[31] have permissible limits for Pb set at higher values than the Montenegrin Food Regulation[29] law, *i.e*. at 1.5 mg kg^{-1} ww and 1.7 mg kg^{-1} ww, respectively. The mean Pb concentration in the investigated samples from the Bar location was above the EC limit,[30] but the same as the USFDA limit,[31] Table III. In this study mercury was not detected in 10 % of the investigated samples and total mercury concentrations below the regulatory limits were found in the remaining samples. Total Hg concentrations in the edible mussel soft tissues ranged from 0.02 to 0.10 mg kg^{-1} ww, as given in Table II. The total Hg limit allowed in the European Union, regulated by the European Commission, is 0.5 mg kg^{-1} ww, except for some species for which it is 1.0 mg kg^{-1} ww.[30] In Montenegro, the total Hg limit, regulated by the Montenegrin Food Regulation law, is 1.0 mg kg^{-1} ww as given in Table III. The mean concentrations of Hg (0.02–0.10 mg kg^{-1} ww) and Cd (0.3–0.4 mg kg^{-1} ww) were below the legal limits regulated by the presented regulations,[29–31] and the mean As concentrations (1.0–2.0 mg kg^{-1} ww) were also below the legal limits[29,31] in the all investigated samples, Tables II and III.

Zn is one of the essential trace elements for both animals and humans. Nevertheless, the consumption of too much Zn is harmful. Zn is required for the synthesis of enzymes and proteins.[26,32] Zn toxicity is rare and Zn appears to have a protective effect against the toxicities of cadmium and lead.[8] The Zn–Cd ratio is very important as the toxicity and storage of Cd are greatly elevated with Zn deficiency.[8]

TABLE III. Guidelines for the concentrations of trace elements (mg kg^{-1}) based on wet weight for food safety set by different countries

Origin	Hg	Cd	Pb	As	Cu	Zn
Permissible limits by Montenegrin Food regulation (2002)[29]	1.0	1.0	1.0	4.0	30	100
EU (2006)[30], Comm. Regulation (EC), No. 188/2006	0.5	1.0	1.50	–	–	–
USFDA (2007)[31], Food and Drug Administration of the United States	1.0	4.0	1.70	86	–	–

The Zn content in the collected samples ranged from 135–210 mg kg^{-1} dw or 22.4–34.6 mg kg^{-1} ww, with lower mean levels in the cultivated samples. The Joint Expert Committee of the FAO/WHO[28] established a provisional tolerable weekly intake (*PTWI*) for Zn of 7 mg kg^{-1} per week, *i.e.*, 420 mg per person per week. According to the present study, the concentrations of Zn in the wild and cultivated mussels in the investigated period were more then ten times below the toxic limits of 420 mg per person per week.[28]

Cu is another essential metal which is contained in several enzymes[32] and is necessary for the synthesis of hemoglobin.[10] Cu-containing proteins play an important role in animal biology, including dioxygen transport or activation, electron transfer and reduction of nitrogen oxides.[32] The mean concentrations of Cu found in the collected samples ranged from 6.2–14.5 mg kg^{-1} dw (1.0–2.4 mg kg^{-1} ww), with the highest concentrations found in the wild *M. galloprovincialis* from the Bar site, 12.6–17.2 mg kg^{-1} dw (2.1–2.9 mg kg^{-1} ww). The concentrations of Cu in the wild and cultivated mussels in the investigated period were more than one order of magnitude below the toxic limits of 30 mg per person per week.[28]

Some types of seafood contain up to 10 times of more As than other foods and people who consume large amounts of seafood may, therefore, ingest significant amounts of As.[4,23] As occurs naturally in the environment, in both organic and inorganic forms.[23,33] Inorganic As is more toxic than organic As.[33] The symptoms and signs of chronic As poisoning include pigmented skin, anemia and paralysis.[8] As in seafood is primarily present in organic forms.[8] This study gives the results for the total (inorganic and organic) concentrations of As. The JECFA recently re-evaluated As and the Committee withdrew the previous *PTWI* value of 15 μg kg^{-1} body weight set for inorganic As,[23,34] since a lower value of 0.3 μg kg^{-1} body weight (bw) per day was found to increase lung cancer.[23,34] The JECFA noted that more accurate information on the content of inorganic As in foods is required to improve assessments of dietary exposures of inorganic As species.[34] However, the majority of As in seafood is present in the organic arsenobetaine (AB), less toxic, water-soluble form,[33,34] which is considered non-hazardous and totally safe for human consumption.[34] The percentages of inorganic As in seafood are 1–5 %, while in mussels,[4] they are 1.9–6.5 %, but the toxic inorganic fraction of As increased with increasing content of total As.[34] Nevertheless, seafood also contributes substantially to dietary As, which is one of the trace elements of concern in relation to food safety.[34] No value of As intake is reported in this study because the As determined refers to the total As (Table II).

Pb is known to cause both acute and chronic adverse effects in the hematopoietic, nervous, gastrointestinal and renal systems.[4] Absorbed Pb is bound to erythrocytes in the blood and initially distributed to the liver, kidney and heart, where it preferentially binds to cell membranes and mitochondria, causes anemia due to a decrease in the hemoglobin levels leading to organ damage, but Pb is not carcinogenic.[4,21] The *PTWI* value for Pb is 1.5 mg person^{-1} week^{-1}.[28] The mean Pb content, in the all the collected mussel samples ranged from 0.7–1.7 mg kg^{-1} ww. Calculating the *PTWI* value for Pb in relation to the highest Pb level at the Harbor Bar, 1.7 mg kg^{-1} ww, the consumption of 880 g of fresh mussels on a weekly basis would reaching 1.5 mg person^{-1} week^{-1} for Pb, which is definitely feasible for large mussel consumers.[35] The absorption of Pb from ingested food greatly depends on the levels of other elements present in the diet, such as Ca, Fe and Zn. It was shown that dietary deficiencies in these essential elements enhance Pb absorption.[35]

Cd is an element that occurs naturally at low levels in the environment, and food represents the major source of Cd exposure.[4,20] Cd is found in marine waters mostly in the dissolved form, distributed in the marine environment at low concentrations and mussels accumulate Cd effectively[4,20] and may act as a poison to humans.[36] Under chronic Cd exposure of humans, Cd can inhibit the development of bone softening due to decalcification, a characteristic of ltai-itai disease.[36] The mean concentrations of Cd found in the analyzed mussel samples ranged from 0.3–0.4 mg kg^{-1} ww in the investigated period at all locations. With regards to the mean Cd concentrations, large mussel consumers would reach the permitted level of 0.42 mg per person per week for Cd by eating 1.2–1.4 kg per week of fresh mussels. In cultivated mussel samples from the Boka Kotorska Bay, from the farms Krasici and Kukuljina, the maximum Cd content was found to be 0.50 mg kg^{-1} ww (Table II), and according to the *PTWI* value, the safe weekly intake of cultivated mussels from these farms is estimated to be below 790–910 g of fresh mussels per week for a 60-kg adult.

Hg is one of the most closely scrutinized pollutants because of its effect on marine organisms and its potential hazard to human health. Methyl-mercury, formed in aquatic sediments through bacterial methylation of organic mercury, is a toxic compound which affects the kidneys and the central nervous system.[4,7] The joint FAO/WHO Expert Committee on Food Additives[28] established a provisional tolerable weekly intake of 0.3 mg of total Hg, of which no more than 200 µg should be present as methyl mercury.[28] This amount equates weekly to 5 µg of total Hg per kg body weight and 3.3 µg of methyl-mercury per kg body weight.[28] However, as there is insufficient data on mussel consumption for Montenegro,[27] consumer exposure to Hg was assessed based on the highest mean value of Hg concentration found in mussels from two of the investigated samples, 0.20 mg kg^{-1} ww at the wild sites Herceg Novi in the Bay and Bar (a sea port in the open coastal area of Montenegro), which were compared with the *PTWI* value for Hg. Based on this data, a weekly consumption of 2.0 kg ww of mussel is sufficient to reach the *PTWI* limit for Hg (5 µg kg^{-1} body weight per day), which is an amount that may result in a risky daily intake of Hg if the exposure is long-term. In all others samples, including the cultivated ones from Krasici and Kukuljina, a weekly consumption of more than 2.5 kg ww of fresh mussels would be necessary to attain the *PTWI* limit for Hg.

In view of the *PTWI* limits estimated for the consumption of Mediterranean mussel from the Montenegrin coastline, in terms of Zn, Cu, Pb, Cd, As and Hg, consumption based on Cd would be the limiting factor for mussels as a food from the Bay farms, such as Krašići and Kukuljina, in terms of large consumers and coastal residents. A weekly consumption of 800–900 g of fresh cultivated mussels, or wild mussels from the location Bar, is sufficient to reach the Cd and Pb *PTWI* limits, which is the amount that may result in a risky weekly intake of Cd and Pb if the exposure is long-term.

A comparison of the data obtained in this study with those previously reported indicates that the levels of trace elements found in *M. galloprovincialis* are comparable to those reported for other areas of the Adriatic and Mediterranean regions[11] (Table IV).

TABLE IV. A comparison of reported concentrations (mg kg^{-1}) of Hg, Cd, Pb, As, Cu and Zn in the whole soft tissue of *M. galloprovincialis* from regional studies with the present results (WB: weight basis)

Location	WB	Hg	Cd	Pb	As	Cu	Zn	Ref.
NW Mediterranean	Dry	0.87	0.4–5.9	2.7–117	11.9–39.3	0.5–28.8	97–644	37
The Bay of Piran, N. Adriatic	Dry	0.28–1.3	2.9–8.3	–	11.9–39.3	6.5–7.6	102–108	38
Lim Chanel, Croatia	Dry	–	1.0	1.37	–	11.5	149	39
Eastern Adriatic coast, Croatia	Dry	–	0.7–1.7	1.2–8.3	–	4.2–17.7	109–189	40
Krka river estuary, Adriatic	Dry	–	0.8–2.3	0.5–4.1	–	4.6–7.7	124–269	41
Portugal southern coast	Dry		1.2–3.8	–	–	4.3–7.2	165–545	42

TABLE IV. Continued

Location	WB	Hg	Cd	Pb	As	Cu	Zn	Ref.
Black Sea, Romania	Dry	0.026–0.03	0.96–1.74	–	–	6.6–8.3	108–190	16
NW Mediterranean	Dry	0.18–0.96	1.13–1.82	1.07–1.43	–	–	–	43
E Adriatic, in Mali Ston Bay Croatia	Dry	0.15	0.4–2.4	0.24–3.7	–	2.0–11	49–418	18
Gulf of Gemlik, SE Marmara Sea, Turkey	Dry	–	2.4	0.5	–	5.5	196	44
Montenegro, SE Adriatic (6 sites)	Dry	0.10–0.50	1.7–2.1	4.0–11.5	5.8–12.4	6.2–14.5	135–210	This study

However, the values obtained for trace elements, *i.e.*, Hg, Cd, Pb and As, in the mussels from the enclosed Boka Kotorska Bay are similar to the average values found in more urbanized areas of the Adriatic and Mediterranean coast. Only the wild samples collected from the site close to the Port Bar had higher levels of the investigated trace elements. The concentrations of trace elements in mussels from the southeastern Adriatic coast of Montenegro could be attributed to anthropogenic and/or natural metal sources affecting their habitats.

CONCLUSIONS

All concentrations of the investigated element (Zn, Cu, Pb, Cd, As and Hg) found in the edible tissue of cultivated mussels from farms in the Boka Kotorska Bay, expressed on a wet weight basis, where lower than the maximum permissible levels for fresh mussels regulated by EU and USFDA laws, implying that their consumption is not harmful for humans. In addition, the results of an evaluation of the risks to human health associated with the consumption of mussels containing trace elements suggest that there is no health risk for people who consume mussels from this part of the Adriatic in moderation, *i.e.*, the consumption of 500 g of fresh mussels on a weekly basis or approximately 26 kg per person per year would not be risky in the long term.

A comparison of the results obtained in this work with already published values for this kind of mussel on the Adriatic and Mediterranean coasts shows that the values obtained herein fall in the range of values most commonly found in low to moderately polluted areas of the Mediterranean and Adriatic Sea. This means that the ecosystem of the Montenegrin coastal area and its Boka Kotorska Bay are not seriously polluted with the investigated trace elements.

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ИЗВОД

КОНЦЕНТРАЦИЈЕ ТРАГОВА ЕЛЕМЕНАТА (Zn, Cu, Pb, Cd, As И Hg) У УЗГАЈАНОЈ И ДИВЉОЈ МЕДИТЕРАНСКОЈ ШКОЉКИ (*M. Galloprovincialis)* УЗОРКОВАНОЈ НА ЛОКАЦИЈАМА ЈУГОИСТОЧНОГ ЈАДРАНА, ЦРНА ГОРА: ЕВАЛУАЦИЈА КВАЛИТЕТА И УТИЦАЈ НА ЧОВЕКОВО ЗДРАВЉЕ

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Медитеранска школька *Mytilus galloprovincialis* (L), или дагња, узоркована је од јесени 2005. до зиме 2009. године са шест локација црногорског приобаља. Узорковање је вршено на четири локације у Бококоторском заливу, два узорка са узгајалишта школьки и два са дивљих станишта, и два преостала узорка дивљих школьки била су са обале на отвореном мору. Мека ткива дагњи анализирана су на цинк, бакар, олово, кадмијум, арсен и укупну живу. Концентрације ових метала кретале су се у опсегу од 135,5–210,0 за Zn; 6,2–14,5 за Cu; 4,0–11,5 за Pb; 1,7–2,1 за Cd; 5,8–12.4 за As и 0,11–0,51 за Hg, изражено у mg kg^{-1} суве масе. Утврђено је да метали у испитиваним узорцима школьки нису били изнад максимално дозвољених концентрација које су прописане законима Европске Уније и америчке агенције за храну и лекове (USFDA) за школьке које се користе у исхрани. Ово указује на то да је конзумирање гајених или дивљих дагњи са црногорског приобаља безбедно у умереним количинама.

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