

Arsenic, cadmium, mercury, and lead in date mussels from the Sarajevo fish market (Bosnia and Herzegovina): a preliminary study on the health risks

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Abstract: The main objective of this study was to determine the content of heavy metals (Cd, Pb, Hg) and metalloid (As) in samples of date mussels (*Lithophaga lithophaga* L., 1758) in order to evaluate the health risks for mussel consumers. Samples of shellfish were obtained from commercial sources and fishery in the area of Sarajevo. The content of heavy metals and arsenic is determined in a total of 46 samples. Samples were prepared by microwave digestion. For the analysis of total As, cadmium and lead, an atomic absorption spectrophotometer with graphite furnace was used, while mercury content was determined using a direct Hg analyser. In all samples, the content of heavy metals and arsenic was above the quantification limit of the given methods. The content of all analysed metals and metalloid was below the limits of their maximum allowable concentration in food, according to the current regulations in Bosnia and Herzegovina and the European Union. Evaluation of the public health risks associated with date mussels' consumption indicates that there is no evident risk for a moderate adult consumer.

Key words: Heavy metals, metalloid, date mussel, health risk

1. Introduction

Consumption of fish and seafood are important elements of a balanced diet in human. The potential health benefits of fish and seafood for humans are related to the presence of high-quality proteins, minerals, vitamins, and essential unsaturated fatty acids, especially polyunsaturated fatty acids (PUFAs) such as omega-3-PUFAs [1].

On the other hand, fish and seafood are in constant contact with the aquatic environment and are susceptible to its changes due to natural and anthropogenic activity. In the marine environment, toxic substances and even heavy metals come from different sources, among which the most significant are the industrial, agricultural, and urban wastes. Aquatic organisms have the ability to bioconcentrate and bioaccumulate, from a contaminated aquatic ecosystem (water and sediment), toxic substances that are recognized as ubiquitous environmental pollutants such as heavy metals and polychlorinated organic components [2]. For this reason, fish and seafood can represent a significant source of human exposure to the specified contaminants, and their consumption becomes more and more important.

Because metals are very common in food and the environment as contaminants and pollutants, food control and the assessment of the input of certain metals in the human body are important in terms of estimating and determining the exposure of people to the harmful effects of metals.

Recommendations on maximum allowable concentration (MAC) of certain toxicants, their acceptable daily intake (ADI), and provisional tolerable weekly intake (PTWI) for food suppliers are provided by the World Health Organization (WHO) and the Food and Agriculture Organization (FAO). Furthermore, according to their studies, and recommendations of WHO and FAO, each country has adopted national rules which prescribe the MAC of certain contaminants in food.

The recommended PTWI values of WHO for methyl Hg are 1.6 µg/kg body weight (b.w.) [3] and inorganic Hg 4 µg/kg b.w. [4], which are based on the assumption that a prevalent form of Hg in food that is not fish and seafood, is just inorganic Hg. Consequently, a concentration of 4 µg/kg b. w. is used for exposure to total Hg from food that does not include fish and seafood.

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In 2012, based on new scientific findings related to the toxicity of inorganic and methyl Hg; European Food Safety Authority (EFSA) found that the value of tolerable weekly intake (TWI) for inorganic Hg of 4 µg/kg b. w. is acceptable. However methyl Hg has a new TWI value of 1.3 µg/kg b. w. [5].

The previously recommended WHO's PTWI of 2.5 µg/kg b. w. for the Pb was discarded in 2011 [4]. Meta-analysis of epidemiological studies found that at the chronic children exposure to Pb at a dose of 0.6 µg/kg b. w. leads to a reduction of intelligence coefficient for 1 point of total IQ score scale (taken as a response to the reference dose in children). In adults, exposure at a dose of 1.3 µg/kg b. w. leads to an increase of systolic pressure of 1 mmHg (taken as a response to adult reference dose). Based on this, it was found that the previously recommended PTWI for Pb resulted in a reduction of the intelligence coefficient for at least 3 points in children, or the increase of a systolic pressure for at least 3 mmHg in adults, and it was discarded.

The recommended WHO's PTWI values for Cd is 2.5 µg/kg b. w. EFSA also recommends that PTWI for Cd should be 2.5 µg/kg b. w. [5].

The previously recommended WHO's PTWI value of 2.1 µg/kg b.w. per day for As was also withdrawn [4]. Based on the meta-analysis of epidemiological studies, WHO/FAO identified a benchmark dose lower (BMDL_{0.5}) confidence limit for a 0.5% increased incidence of lung cancer (BMDL_{0.5}) as 3.0 µg/kg b.w. per day [5]. In that sense, the previously established PTWI could be considered less protective as it was the same level as the BMDL_{0.5}.

The MAC of metals (Pb, Cd, and Hg) in the Republic of Bosnia and Herzegovina are set out in the ordinance on the MAC of certain contaminants in foods [6]. For bivalve molluscs, the MAC according to this act is 0.5 mg/kg w.w., 1 mg/kg w.w., and 1.5 mg/kg for total mercury, cadmium and lead, respectively [6].

Currently, there are no maximum levels (MLs) established for arsenic in food at EU level, although MLs are laid down in national legislation in some member states. Commission Regulation (EC) No: 1881/2006 of 19 December 2006 set maximum levels for certain contaminants in foodstuffs [7]. This regulation lays down the MLs of Pb, Hg, and Cd in fish, crabs, shellfish, and cephalopods and these values are the same as those laid down in the ordinance on the MAC of certain contaminants in foodstuffs of BiH [6].

Bosnia and Herzegovina is a Mediterranean country and has 1400 ha of seacoast (24 km long). Bosnia and Herzegovina also have a very long and rich tradition in aquaculture. According to FAO, data fish and seafood consumption in Bosnia and Herzegovina has increased in the past 10 years [8]. Although essential nutritional and

health benefits from eating fish and seafood are publicized by different organizations, public campaigns, and media [1], consumption of these contaminated fish and seafood pose a risk to human.

In this paper, the presence and the content of total As, Cd, Pb, and total Hg in the samples of date mussels (*Lithophaga lithophaga* L., 1758), originated from the area of Neum bay and purchased from commercial sources were examined. The results of the heavy metal content were compared with the limits on their maximum allowable concentrations in food, in accordance with the relevant regulations in Bosnia and Herzegovina and the European Union. Also, the risk due to As, Cd, Hg, and Pb intakes associated with shellfish consumption in Sarajevo market were assessed.

2. Material and methods

2.1. Reagents preparation

Standards solutions for Pb, Hg, Cd, and As (1000 mg/L ± 4 mg/L, Sigma-Aldrich, Seelze, Germany) were used to build up calibration diagrams. Standard dilutions were prepared from a stock solution of 1 g/L by successive dilution for each metal. Aqueous solutions of reagents and standard solutions were prepared using ultra-pure water (18,2 MΩ/cm, Arium611, Sartorius Mechatronics, Hamburg, Germany).

To prevent the cross-contamination, all glassware and utensils used in the work were soaking in 10% HNO₃ v/v for 24 h, after which they were washed with ultra-pure water. Quartz vessels for microwave digestion were also cleaned: firstly, washed with detergent, thoroughly rinsed with ultra-pure water, and dried at 105 °C for 2 h. Thereafter, the so-called UV-test was made with a special program defined in the microwave oven software, which additionally provides the purity of the vessels. Quartz vessels that were heated after the UV test were repurified.

2.2. Sampling collection

Mussels- *Lithophaga lithophaga* L. 1758 (n = 46) were collected from commercial sources during March 2016. The origin of the samples was local fisheries in the area of Sarajevo. The shellfish samples were transferred to the laboratory where the shells were open, and the soft tissues were removed for further analysis. Before opening, each sample has been weighed and recorded, together with the mass and the length. In order to avoid cross-contamination, the desk was covered with aluminium foil that was replaced with each sample, and the knife was cleaned with acetone.

The shellfish soft tissues were wrapped in aluminium foil [9], were paced in a separated plastic bag, were labelled and stored in the freezer (-20°C) before analysis.

2.3. Sample preparation

Sample preparation was done with standard method of microwave digestion of Institute for Standardization of Bosnia and Herzegovina (BAS EN ISO 13804:2015).

Approximately 0.5 g of homogenised samples was microwave-digested in a *Microwave Ethos D* (Milestone, Sorisole, Italy) oven for 30 min in a closed quartz vessel with 7 mL of 65% nitric acid (Sigma-Aldrich, Seelze, Germany) and 1 mL of 30% hydrogen peroxide (Sigma-Aldrich, Seelze, Germany). The microwave oven was programmed at 1500 W and 45 bar as maximum power and pressure limit (ramp time 15 min, hold time 15 min, and cooling time 20 min). An appropriate blank (7 mL of 65% nitric acid and 1 mL of 30% hydrogen peroxide) was prepared in the same way.

After mineralization, the quartz vessels were opened and the resulting solutions were transferred to volumetric flasks, diluted to a known volume (50 mL) with ultrapure water and stored until analysis.

2.4. Analysis of total As, Cd, Pb, and total Hg

Analyses of total As, Cd, and Pb were performed on the AA-7000F Dual Atomizer System (Shimadzu, Columbia, US) atomic absorption spectrometer, equipped with self-reversal (SR) method background correction, autosampler, and graphite furnace. The argon (Ar) flow is used to remove residues from the graphite tube during pyrolysis. Analyses were done using standard method for determination of trace elements in foodstuffs of Institute for Standardization of Bosnia and Herzegovina (BAS EN ISO14084:2003).

Total Hg concentration in homogenized samples was measured directly in triplicate with a direct Hg analyser AMA 254 Mercury Analyzer (Curtage Analyzes Service, Italy), which uses the principle of thermal decomposition, amalgamation and atomic absorption.

Samples of date mussels were analysed in triplicate. The blanks and calibration standards were analysed using the same methods. Quality control was performed using standard referent material (SRM), standard Hg 0.5 mg/L made from RM (referent material, label RM-H-1) 1.000 ± 0.002 g/L.

The concentrations of heavy metals were expressed in milligrams per kilogram of wet weight sample. The limits of detection and quantification (LoD and LoQ), expressed in mg/kg wet weight, were: As: 0.0007 and 0.0021; Cd: 0.0266 and 0.0798; Pb: 0.0003 and 0.0011; Hg: 0.004; for LoD and LoQ, respectively.

2.5. Dietary exposure assessment

The weekly dietary heavy metals exposure (WDE) through date mussels' consumption was assessed by combining consumption data of date mussels with contamination data for As, Cd, Hg, and Pb and divided by the individual b.w. (Eq. 1).

$$WDE = \frac{\text{Weekly consumption} \left[\frac{\text{g}}{\text{week}} \right] \times \text{Concentration of contaminant} \left[\frac{\mu\text{g}}{\text{g}} \right]}{\text{Body weight [kg]}} \quad (1)$$

The total diet study has not been undertaken in Bosnia and Herzegovina, so far. For that reason, for intake assessment, we used the data available in the GEMS/Food Consumption Cluster Diet database. According to this database, the estimated average weekly consumption of total fish related to the general population in Bosnia and Herzegovina is 156.8 g/week of which 2.8 g/week belongs to molluscs and cephalopods. The b.w. of 70 kg was used as the average b.w. for the European adult population (aged above 18 years) [10].

As, Cd, Hg, and Pb intake was calculated using 4 concentration levels of certain contaminants: mean, maximal, 50th and 95th percentile; 2 fish consumption levels: the first one was obtained from GEMS/Food Consumption Cluster Diet database [10]-2.8 g/week and the second one was obtained from the recommendations of fish and seafood intake from Food-Based Dietary Guidelines (FBDG) of 19 European countries-300 g total fish per week [11] and body mass value of 70 kg (8 different exposure scenarios).

For each scenario hazard index (HI) for Cd and Hg and margin of exposure (MOE) for As and Pb were calculated by Eq. (2) and (3):

$$HI = \frac{\text{Calculated weekly intake for certain contaminant}}{\text{Provisional tolerable weekly intake}} \quad (2)$$

$$MOE = \frac{\text{Benchmark Dose Level (BMDL)}}{\text{Calculated weekly intake for certain contaminant}} \quad (3)$$

Any HI value greater than value 1 indicates a potential health risk. If the HI value is less than 1, there is no obvious risk. Although HI does not quantify the likelihood of adverse health effects, it does provide an indication of health risk.

According to the view of EFSA's Scientific Committee in general a MOE of 10,000 or higher is considered as a low concern from a public health point of view with respect to the carcinogenic effect and MOE only indicates a level of concern and does not quantify risk [12]

3. Results and discussion

The heavy metal concentrations in date mussel samples are summarised in Table 1. The concentration ranged from 0.118 to 0.822 mg/kg, from 0.003 to 0.309 mg/kg, from 0.082 to 0.372 mg/kg, and from 0.0055 to 0.4330 mg/

Table 1. Summary of As, Cd, Pb, and Hg concentration (mg/kg) in date mussel samples.

[µg/g]	Total As	Cd	Pb	Total Hg
Mean	0.226	0.156	0.186	0.126
SD	0.196	0.073	0.077	0.106
Min	0.118	0.003	0.082	0.006
Max	0.822	0.309	0.372	0.433
Median	0.155	0.143	0.171	0.097
5th	0.006	0.0002	0.004	0.0003
95th	0.666	0.209	0.258	0.398

kg, for total As, Cd, Pb, and total Hg, respectively. Metal contents in date mussel samples follow the decreasing order: As>Cd>Pb>Hg.

Comparing total As concentrations with the literature data (Table 2), it is possible to show that As levels in this study were much lower than the other Mediterranean regions.

As shown in Table 2, Cd levels were much lower than those reported for the Adriatic Sea [13,14] and Malesia market [15] but higher than Cd levels reported by Olmedo et al. [16], for the Spain market.

By comparing the data from different Mediterranean regions (Table 2) it is possible to note that Hg concentrations observed in this study in the Adriatic Sea were higher than those observed by Markovic et al. [13], along the south-eastern Adriatic Coast Montenegro, and Gavrilovic et al. [17] in Mali Ston (Croatia).

Also, Pb concentration in mussel of this study region was relatively lower than that found along the Croatian coast [17], and in Malesia market [15], but relatively higher than the Pb level observed by Olmedo et al. [16] in Spain market (Table 2).

The determined contents of As, Cd, Pb, and Hg in the samples of *L. lithophaga* collected from the Sarajevo fish market are within the range acceptable for the use in human nutrition according to the current regulations in

Bosnia and Herzegovina and the European Union [6,18].

According to the EFSA data, fish and seafood belong to the category of food that contributes most to the dietary exposure of the population to methyl Hg. The average exposure of adult population to methyl Hg via diet is 0.24 µg/kg per week [5]. Considering the fact that the contribution of molluscs in the total exposure of methyl Hg is 0%–7.2% [5] it can be calculated that exposure of adult European population to methyl Hg, due to the consumption of molluscs is 0–0.017 µg/kg per week. Using the same calculation gives the data on the average weekly exposure of adult European population to inorganic Hg due to the consumption of molluscs of 0.03 µg/kg per week.

In samples analysed in this paper, the total Hg was determined. In order to make the difference between exposure to methyl and inorganic Hg, the total Hg was recalculated to methyl and inorganic Hg using a conversion factor. For crabs, shells, and amphibians for methyl Hg, the conversion factor of 0.8 is used, while for inorganic Hg a conversion factor of 0.5 is used [5]. Weekly intake of methyl and inorganic Hg have been calculated by the deterministic model using fixed mean, maximum, 50th or 95th percentile of contaminants concentration (Table 3).

The data presented in Table 3 indicate that the average weekly exposure of the adult population of Bosnia and Herzegovina to methyl Hg through consumption of date mussels is lower than both the weekly methyl and inorganic Hg intake by adult population in a different European country due to molluscs' consumption. In the present study, calculated weekly intake for methyl and inorganic Hg did not exceed the value of PTWI of 1.6 µg metHg/kg day and 4 µg metHg/kg day. Calculated HIs have shown that there is no risk of exposure to Hg due to date mussels' consumption (Table 4).

According to the data reported by EFSA, the average exposure of the adult population of Europe to Cd is 1.77 µg/kg b.w. per week (range from 1.50 to 2.23 µg/kg). The contribution of food category fish and seafood in this exposure is 1.2%–31.2%. Within this total contribution, the specific contribution of shellfish is in average 44.1%

Table 2. Selected literature data on heavy metals in shellfish (wet weight) along with residues in this study.

References	Shellfish origin	Total As[mg/kg]	Cd [mg/kg]	Total Hg [mg/kg]	Pb [mg/kg]
This study	Neum bay, Bosnia and Herzegovina	0.118–0.822	0.003–0.309	0.006–0.433	0.082–0.372
Markovic et al., 2012 [13]	Southeastern Adriatic Coast, Montenegro	--	0.18–0.74	0.05–0.23	0.24–3.3
Bogdanovic et al., 2014. [14]	Eastern Adriatic Coast, Croatia	1.420–6410	0.097–1.270	0.032–0.680	0.284–1.063
Sharif et al., 2016 [15]	Malesia market	0.95–22.10	0.08–5.45	-	0.18–0.88
Olmedo et al., 2013 [16]	Spain market	0.131–0.381	0.079–0.251	-	0.004–0.025
Gavrilovic et al, 2004 [17]	Mali Ston, Croatia	0.938–2.06	0.054–0.082	0.24–0.413	<0.01–0.93

Table 3. Weekly intakes of As, Cd, Pb, and Hg via date mussels consumption.

Weekly intake ($\mu\text{g}/\text{kg b.w.}$)				
Date mussels intake	FAO/WHO	EFSA	FAO/WHO	EFSA
Concentration	Mean/Median	Mean/Median	Max/95th	Max/95th
As	0.009/0.006	0.017/0.012	0.033/0.027	0.063/0.051
Cd	0.006/0.006	0.012/0.011	0.012/0.008	0.024/0.016
Pb	0.007/0.007	0.014/0.013	0.015/0.010	0.028/0.020
metHg	0.004/0.003	0.008/0.006	0.014/0.0001	0.027/0.00034
inorgHg	0.002/0.004	0.005/0.004	0.009/0.0001	0.0166/0.0002

FAO/WHO-intake [10] of molluscs and cephalopods proposed by FAO/WHO (2.8 g/week). EFSA-intake (2010) [5] of fish and seafood proposed by FBDG of 19 European countries-300 g total fish per week.

Table 4. Hazard indexes for Hg and Cd due to date mussels' consumption among the adult population in Bosnia and Herzegovina.

Scenario	Body weight (kg)	HI			
		Concentration	metHg	iHg	Cd
FAO/WHO	70	Mean	0.0040	0.0025	0.0025
FAO/WHO	70	Median	0.0024	0.0005	0.0023
EFSA	70	Mean	0.0059	0.0012	0.0048
EFSA	70	Median	0.0045	0.0009	0.0044
FAO/WHO	70	Max	0.0107	0.0022	0.0049
FAO/WHO	70	95th	0.0001	0.0000	0.0033
EFSA	70	Max	0.0204	0.0041	0.0095
EFSA	70	95th	0.0003	0.0001	0.0064

FAO/WHO-intake [10] of molluscs and cephalopods proposed by FAO/WHO (2.8 g/week). EFSA-intake [5] of fish and seafood proposed by FBDG of 19 European countries-300 g total fish per week. HI>1, unacceptable risk.

[19]. Taking into account this shellfish contribution in the overall average food exposure of the adult European population to Cd, estimated Cd exposure by shellfish consumption is ranging from 0.009 to 0.244 $\mu\text{g}/\text{kg b.w.}$ per week.

Table 3 shows the weekly intake of Cd of the adult population of Bosnia and Herzegovina due to date mussels' consumption. The calculated weekly intake for Cd did not exceed the value of PTWI of 2.5 $\mu\text{g}/\text{kg day}$. Also, calculated HIs have shown that there is no risk of exposure to Cd due to date mussels' consumption (Table 4).

According to the EFSA report [20], the average exposure of the adult European population to Pb is 0.50 $\mu\text{g}/\text{kg b.w.}$ per day. The contribution of food category fish

and seafood to overall Pb exposure is 1.8% (0.009 $\mu\text{g}/\text{kg}$ body mass per day).

EFSA has identified a number of possible adverse effects of Pb on human health taking into account the experimental and epidemiological evidences [21]. However, these data do not give or only give poor evidence for a threshold for a number of critical endpoints including developmental neurotoxicity and adult nephrotoxicity. That is why EFSA recommends to calculate the approximate margin of exposure (MOE). The EFSA's [5] opinion identified a 95th percentile lower confidence limit of the benchmark dose of 1 % extra risk (BMDL_{01}) of 0.50 $\mu\text{g}/\text{kg b.w.}$ per day for developmental neurotoxicity in young children. It also lists cardiovascular effects and nephrotoxicity in adults

as potential critical adverse health effects of the Pb with respective $BMDL_{01}$ and $BMDL_{10}$ of 1.50 and 0.63 $\mu\text{g}/\text{kg}$ b.w. per day [20].

Table 3 shows the values for the estimated exposure to Pb of the adult population of Bosnia and Herzegovina due to consumption of date mussels. These values are lower than the estimated average exposure of adult European populations [20] and are also lower than the established BMDL values for harmful cardiovascular and renal effects of Pb [21].

Table 5 shows the values for the estimated margin of exposure to Pb. As the margin of exposure is closer to the estimated contaminant exposure, the potential adverse effects on consumers cannot be excluded. In this study, calculated MOEs were below the lower limit value of 10,000.

One of the most important sources of uncertainty in assessing As exposure is the determination of the relationship between total As and inorganic As (iAs), diversity of data on fish and seafood consumption, and interpretation and processing of content data below the LoQ. The literature data show that there is no clear correlation between the content of total As and iAs in aquatic organisms [22]. One of the possible reasons is that

the relative contribution of iAs has a tendency to decrease, consequently, the amount of total As increases, while the ratio of iAs and total As may vary depending on the species of aquatic organisms [23]. Lebnak [24] states that the contribution of iAs in total As present in aquatic organisms is only 0.4%–5.3% [23]. According to the EFSA (2014) data, crab and shellfish samples have higher iAs than fish [10].

In the largest number of studies, including this, the total As was determined. In the estimation of the content of iAs, different conversion factors can be used. However, EFSA in its scientific opinion has determined that in risk assessment of dietary exposure to iAs, a more practical approach is to assume a constant contribution of iAs from fish of 0.03 mg/kg and from seafood of 0.1 mg/kg. On the basis of available scientific information, EFSA also concluded that the risk characterization for iAs should use BMDL values of 0.3 to 8 $\mu\text{g}/\text{kg}$ body mass per day [10].

Using the constant value of contribution of iAs, the estimated daily exposure to iAs by the shellfish consumption analysed in this paper was 0.004 $\mu\text{g}/\text{kg}$ b.w. per day (intake of molluscs and cephalopods proposed by FAO/WHO, 2.8 g/week) and 0.008 $\mu\text{g}/\text{kg}$ body mass per day (intake of fish and seafood proposed by FBDG of 19 European countries-300 g total fish per week).

Table 5. Margin of exposure to Pb for selected BMDL values.

Scenario	Effect	BMLD ($\mu\text{g}/\text{kg}$ b.w. per day)	Concentration	MOE
FAO/WHO	Cardiovascular effects	1.5	Mean	1411.3
FAO/WHO	Cardiovascular effects	1.5	Median	1535.1
EFSA	Cardiovascular effects	1.5	Mean	737.2
EFSA	Cardiovascular effects	1.5	Median	801.9
FAO/WHO	Cardiovascular effects	1.5	Max	705.6
FAO/WHO	Cardiovascular effects	1.5	95th	1019.6
EFSA	Cardiovascular effects	1.5	Max	368.6
EFSA	Cardiovascular effects	1.5	95th	532.6
FAO/WHO	Renal effects	0.63	Mean	592.7
FAO/WHO	Renal effects	0.63	Median	644.7
EFSA	Renal effects	0.63	Mean	309.6
EFSA	Renal effects	0.63	Median	336.8
FAO/WHO	Renal effects	0.63	Max	296.4
FAO/WHO	Renal effects	0.63	95th	428.2
EFSA	Renal effects	0.63	Max	154.8
EFSA	Renal effects	0.63	95th	223.7

FAO/WHO-intake (2017) of molluscs and cephalopods proposed by the FAO/WHO (2.8 g/week). EFSA-intake (2010) of fish and seafood proposed by FBDG of 19 European countries-300 g total fish per week. Cardiovascular effects ($BMDL_{01} = 1.5 \mu\text{g}/\text{kg}$ b.w. per day). Renal effects ($BMDL_{01} = 0.63 \mu\text{g}/\text{kg}$ b.w. per day) [21]. $MOE > 10,000$, unacceptable risk.

Table 6. Margin of exposure to inorganic As for selected BMDL values.

Scenario	Effect	BMDL ($\mu\text{g}/\text{kg}$ b.w. per day)	Concentration	MOE
FAO/WHO	Skin lesions	0.93-3.7	0.1	1627.5–6475
EFSA	Skin lesions	0.93-3.7	0.1	813.75–3237.5
FAO/WHO	Lung cancer	0.34-0.69	0.1	595–1207.5
EFSA	Lung cancer	0.34-0.69	0.1	297.5–603.8
FAO/WHO	Bladder cancer	3.2-7.5	0.1	5600–13125
EFSA	Bladder cancer	3.2-7.5	0.1	2800–6562.5

FAO/WHO-intake (2017) of molluscs and cephalopods proposed by the FAO/WHO (2.8 g/week). EFSA-intake (2010) of fish and seafood proposed by FBDG of 19 European countries-300 g total fish per week. Lung carcinoma (BMDL₀₁ = 0.34–0.69 $\mu\text{g}/\text{kg}$ b.w. per day) (Francesconi, 2010) [25]. Skin lesion (BMDL₀₁ = 0.93–3.7 $\mu\text{g}/\text{kg}$ b.w. per day) (Xia et al., 2009) [26]. Bladder carcinoma (BMDL₀₁ = 3.2 Bladder carcinoma 7.5 $\mu\text{g}/\text{kg}$ b.w. per day) (Chiou et al., 2001) [27]. MOE>10,000, unacceptable risk.

Mean dietary exposure to inorganic As among the adult population in Europe ranged from 0.09 to 0.38 $\mu\text{g}/\text{kg}$ b.w. per day [10].

The overall contribution of fish and seafood to iAs exposure was very small in most of the countries. However, this contribution can be significant in the regions where fish and seafood are dominated food category in the dietary habits of the population.

Table 6 shows the values for the estimated margin of exposure to iAs for the selected values of BMDL [25,26,27] because the calculated MOEs were <10,000 potential for risk is possible.

3.1. Limitations of the study

The results presented herein are the first results for the content of As, Cd, Hg, and Pb in date mussel and for the risk characterization of those heavy metals associated with date mussel consumption in Bosnia and Herzegovina. The shellfish sample is obviously small and this does not allow to provide a scientifically sound conclusion that the risk to public health seems to be negligible for the general Bosnia

and Herzegovina population but still, this first set of data represent a very valuable starting point for the future research.

4. Conclusion

The data presented in this study indicate that the average weekly exposure of the adult population of Bosnia and Herzegovina to heavy metals through consumption of date mussels is lower than the weekly intake of this contaminants by adult population in different European countries due to molluscs' consumption.

HIs calculated for Cd, methyl and inorganic Hg was below 1, indicating that risk due to exposure seems to be insignificant. The margin of exposure for inorganic As and Pb were in all scenarios below 10,000 which indicates that potential risk is possible.

The data of this first investigation indicate that there is a need for more precise research on consumption frequency of date mussels, especially among the local population, in order to evaluate a reliable risk assessment per population.

References

1. Antonijevic B, Jankovic S, Curcic M, Durgo K, Stokic E et al. Risk characterization for mercury, dichlorodiphenyltrichloroethane and polychlorinated biphenyls associated with fish consumption in Serbia. *Food and Chemical Toxicology* 2011; 49 (10): 2586-2593. doi: 10.1016/j.fct.2011.06.078
2. Storelli MM. Potential human health risks from metals (Hg, Cd, and Pb) and polychlorinated biphenyls (PCBs) via seafood consumption: Estimation of target hazard quotients (THQs) and toxic equivalents (TEQs). *Food and Chemical Toxicology* 2008; 46: 2782-2788. doi: 10.1016/j.fct.2008.05.011
3. Joint FAO/WHO Expert Committee on Food Additives (JECFA). Safety evaluation of certain food additives and contaminants. *WHO Food Additives* 2007; 58: 269-317.
4. Joint FAO/WHO Expert Committee on Food Additives (JECFA). Safety evaluation of certain food additives and contaminants. *WHO Food Additives* 2011; 63: 605-685.
5. EFSA Scientific Committee. Scientific panels and units in the absence of actual measured data. *EFSA Journal* 2012; 10: 2579. doi: 10.2903/j.efsa.2012.2579

6. Ured za Zakonodavstvo. Pravilnik o izmjenama pravilnika o najvećim dozvoljenim količinama određenih kontaminanata u hrani. Službeni glasnik BiH 2018; 84: 15-18 (in Bosnian).
7. The Commission of the European Communities. Commission Regulation (EC) No. 1881/2006 of 19 December 2006 setting maximum levels for certain contaminants in foodstuffs. Official Journal 2016; L 364/5: 1-39.
8. Food and Agriculture Organization. Food Supply-Livestock and Fish Primary Equivalent. Rome, Italy: FAO;2018.
9. Djedjibegovic J, Marjanovic A, Burnic S, Omeragic E, Dobraca A et al. Polychlorinated biphenyls (PCBs) in fish from the Sana River (Bosnia and Herzegovina): a preliminary study on the health risk in sportfishermen. *Journal of Environmental Science and Health, Part B* 2015; 50: 638-644.
10. World Health Organization. GEMS/Food Consumption Database. Geneva, Switzerland: WHO; 2012.
11. European Food Safety Authority. Dietary exposure to inorganic arsenic in the European population. *EFSA Journal* 2014; 12 (3): 3597. doi: 10.2903/j.efsa.2014.3597
12. European Food Safety Authority. Statement on the applicability of the Margin of Exposure approach for the safety assessment of impurities which are both genotoxic and carcinogenic in substances added to food/feed. *EFSA Journal* 2012; 10 (3): 2578. doi: 10.2903/j.efsa.2012.2578
13. Markovic J, Joksimovic D, Stankovic S. Trace element concentrations in wild mussels from the coastal area of the southeastern Adriatic, Montenegro. *Archives of Biological Science Belgrade* 2012; 64: 265-275. doi: 10.2298/ABS1201265M
14. Bogdanovic T, Ujevic I, Sedak M, Listeš E, Simat V et al. As, Cd, Hg and Pb in four edible shellfish species from breeding and harvesting areas along the eastern Adriatic Coast, Croatia. *Analytical Methods* 2014; 146: 197-203. doi: 10.1016/j.foodchem.2013.09.045
15. Sharif, Chong E, Meng KC. Human Health Risk Assessment of Heavy Metals in Shellfish from Kudat, Sabah. *Malaysian Journal of Nutrition* 2016; 22 (2): 301-305.
16. Olmedo P, Pla A, Hernández AF, Barbier F, Ayouni L et al. Determination of toxic elements (mercury, cadmium, lead, tin and arsenic) in fish and shellfish samples. Risk assessment for the consumers. *Environment International* 2013; 59: 63-72. doi: 10.1016/j.envint.2013.05.005
17. Gavrilovic M, Srebocan E, Petrinc Z, Pompe-Gotal J, Prevedar-Crnica A. Teški metali u kamenicama i dagnjama molostonskog zaljeva. *Naše More* 2004; 51: 50-58 (in Croatian).
18. European Food Safety Authority. Scientific opinion on arsenic in food. *EFSA Journal* 2009; 7: 1351. doi: 10.2903/j.efsa.2009.1351
19. European Food Safety Authority. Cadmium dietary exposure in the European population. *EFSA Journal* 2012; 10: 2551. doi: 10.2903/j.efsa.2012.2551
20. European Food Safety Authority. Lead dietary exposure in the European population. *EFSA Journal* 2012; 10: 2831. doi: 10.2903/j.efsa.2012.2831
21. European Food Safety Authority. Scientific opinion on lead in food. *EFSA Journal* 2010; 8: 1570. doi: 10.2903/j.efsa.2010.1570
22. Sirot V, Guérin T, Volatier JL, Leblanc JC. Dietary exposure and biomarkers of arsenic in consumers of fish and shellfish from France. *Science of the Total Environment* 2009; 407: 1875-1885. doi: 10.1016/j.scitotenv.2008.11.050
23. Ferreccio C, Gonzalez C, Milosavljevic V, Marshall G, Sancha AM et al. Lung cancer and arsenic concentrations in drinking water in Chile. *Epidemiology* 2000; 11: 673-679. doi: 10.1097/00001648-200011000-00010
24. Leblanc JC. CALIPSO Study: Fish and Seafood Consumption Study and Biomarker of Exposure to Trace Elements, Pollutants and Omega 3. Paris, France: AFSSA; 2006.
25. Francesconi KA. Arsenic species in seafood: origin and human health implications. *Pure Applied Chemistry* 2010; 82: 373-381. doi: 10.1351/PAC-CON-09-07-01
26. Xia Y, Wade TJ, Wu K, Li Y, Ning Z et al. Well water arsenic exposure, arsenic induced skin-lesions and self-reported morbidity in Inner Mongolia. *International Journal of Environmental Research and Public Health* 2009; 6: 1010-1025. doi: 10.3390/ijerph6031010
27. Chiou HY, Chiou ST, Hsu YH, Chou YL, Tseng CH et al. Incidence of transitional cell carcinoma and arsenic in drinking water: a follow-up study of 8,102 residents in an arseniasis endemic area in northeastern Taiwan. *American Journal of Epidemiology* 2001; 153: 411-418. doi: 10.1093/aje/153.5.411