Taylor & Francis
Taylor & Francis Group

Food Additives & Contaminants: Part B
Surveillance

ISSN: (Print) (Online) Journal homepage: https://www.tandfonline.com/loi/tfab20

Heavy metals and PAHs in mussels on the Serbian market and consumer exposure

Nikolina J. Novakov, Brankica D. Kartalović, Željko A. Mihaljev, Krešimir M. Mastanjević, Nenad S. Stojanac & Kristina J. Habschied

To cite this article: Nikolina J. Novakov, Brankica D. Kartalović, Željko A. Mihaljev, Krešimir M. Mastanjević, Nenad S. Stojanac & Kristina J. Habschied (2021): Heavy metals and PAHs in mussels on the Serbian market and consumer exposure, Food Additives & Contaminants: Part B, DOI: 10.1080/19393210.2021.1931475

To link to this article: https://doi.org/10.1080/19393210.2021.1931475

Published online: 02 Jun 2021.

FOOD ADDITIVES & CONTAMINANTS: PART B
https://doi.org/10.1080/19393210.2021.1931475

Heavy metals and PAHs in mussels on the Serbian market and consumer exposure

Nikolina J. Novakov[a], Brankica D. Kartalović[b], Željko A. Mihaljev[b], Krešimir M. Mastanjević[c], Nenad S. Stojanac[a], and Kristina J. Habschied[c]

[a]Associate Professor in Fish Diseases and Fishery, Department of Veterinary Medicine, Univesity of Novi Sad, Novi Sad, Serbia; [b]Research Associate in Analytical Chemistry, Department for Food and Feed Quality, Bureau for Food Safety and Drug Analysis, Research Veterinary Institute Novi Sad, Novi Sad, Serbia; [c]Faculty of Food Technology, Josip Juraj Strossmayer University of Osijek, Osijek, Croata

ABSTRACT

The goal of the study was to investigate the concentration of heavy metals and polycyclic aromatic hydrocarbons (PAHs) in 84 samples of mussels, collected from supermarkets and fish markets in Serbia. Lead, cadmium, mercury and arsenic concentrations were determined using an inductive coupled plasma mass spectrometry method. Sixteen PAHs were determined using a gas chromatography-mass spectrometry method. Heavy metals in the mussels were in the range (mg/kg) of 0.01–0.74 for lead, 0.01–0.38 for cadmium, 0.01–0.15 for mercury and 1.12–5.87 for arsenic. Metals and PAHs levels in all analysed samples were under the legal European and Serbian legislation limits. The provisional tolerable intake values were calculated on the base of the obtainable values of heavy metals. Mussels are considered to be safe for human consumption. However, one should take care of the amount and frequency of mussel consumption, primarily due to consumer's cadmium and mercury burden.

ARTICLE HISTORY
Received 21 January 2021
Accepted 11 May 2021

KEYWORDS
Mussels; heavy metals; PAHs; serbian market

Introduction

Shellfish, together with marine fish, crustaceans and cephalopods, make the basis of human nutrition obtained from the sea (Grienke et al. 2014). Bivalve molluscs are considered to be highly nutritive, as they are a good source of proteins, carbohydrates, minerals and provide fatty acids, primarily highly valuable polyunsaturated fatty acids (Bongiorno et al. 2015). There are many species of shellfish in the world's seas and oceans. Serbia as a continental country has not got shellfish production but only import from other countries. Mussels, usually the Mediterranean mussel (*Mytilus galloprovincialis*) are the most important and most frequently consumed shellfish, usually due to their lower price comparing the other shellfish and habits of consumers. These shellfish can be found in the market as fresh and frozen. Shellfish have been shown to contain varying amounts of heavy metals, what resulted in growing concern about the health benefits and risks of their consumption (Olmedo et al. 2013).

Mercury, cadmium, lead and arsenic are toxic even in the trace amounts, causing different effects on human health, including toxic effects on the nervous, renal, endocrine and cardiovascular systems, as well as carcinogenic effects ([ATSDR] Agency for Toxic Substances and Disease Registry 2003, 2007, 2012, 2019). Heavy metals are accumulated in the marine environment and transfer to the shellfish by different ways. Heavy influx of sewage, industrial effluents and agricultural wastewater can increase their concentrations in the environment (Jovic et al. 2011; Andayesh et al. 2015). Due to their filter-feeding activity, shellfish can accumulate different contaminants and contain 100 to 1000 times higher concentrations compared to water where they are cultivated (Avelar et al. 2000; Lehel et al. 2018).

Polycyclic aromatic hydrocarbons (PAHs) are ubiquitous environmental pollutants produced by incomplete combustion of organic matter ([WHO] World Health Organization 2006). Over 100 PAHs have been found, but the US Environmental Protection Agency (US EPA) identified a total of 16 polycyclic aromatic hydrocarbons [naphthalene (Nap), acenaphthylene (Anl), acenaphthene (Ane), fluorene (Flu), anthracene (Ant), phenanthrene (Phen), fluoranthene (Flt), benz(a) anthracene (BaA), pyrene (Pyr), chrysene (Chry), benzo(b)fluoranthene (BbF), benzo(k)fluoranthene (BkF), benzo(a)pyrene (BaP), dibenz(a,h)anthracene (DahA), benzo(g,h,i)perylene (BghiP) and indeno (1,2,3-cd)pyrene (InP)] (PAH16) as priority environmental pollutants ([US EPA] US Environmental Protection Agency 2008). In food, PAHs can be present

CONTACT Nikolina J. Novakov milosevicnina@gmail.com

due to thermal treatment, such as smoking, roasting, grilling and cooking (Falco et al. 2003; Ledesma et al. 2016). They affect the human's health by interfering the normal cell function, disrupting cell membrane and enzyme system (Bogdanović et al. 2019). European Commission (Commission 2006) and Serbian legislation (Serbia 2019) established maximum levels permitted in food and set benzo(a)pyrene and sum of benzo(a)pyrene, benzo(a)anthracene, benzo(b)fluoranthene and chrysene as priority PAH contaminants.

Although many studies have been conducted to determine trace elements and PAHs levels in shellfish such as in Croatia (Bilandžić et al. 2018; Bogdanović et al. 2019), Hungary (Lehel et al. 2018), Norway (Næss et al. 2020), Itally (Storelli and Marcotrigiano 2001; Spada et al. 2013; Di Lena et al. 2017), Spain (Franco et al. 2002; Usero et al. 2005; Olmedo et al. 2013), France (Guéguen et al. 2011), Turkey (Turk Culha et al. 2007; Bat 2012; Yigit et al. 2018), Montenegro (Joksimović et al. 2011; Jović and Stanković 2014), there is no much information regarding heavy metals and PAHs concentrations in mussels marketed in Serbia.

The aim of the present study was to determine the degree of contamination by mercury (Hg), lead (Pb), cadmium (Cd) and arsenic (As), as well as 16 PAHs in mussels consumed by the general population in Serbia. Since heavy metals may have negative effects on human health in high concentrations, it is necessary to monitor food and assess the potential risks of its consumption.

Materials and methods

Sample collection

Samples containing 50 g of mussel meat were collected randomly from supermarkets and fish markets in Serbia during the period from January 2019 to March 2020. The study included 84 samples of mussels from different geographical regions (Slovenia, Croatia, Spain and Chile), in their original packaging, as frozen and fresh ones. Twenty-one samples of imported fresh mussels were collected from Slovenia, the same as from Croatia. Also, 21 samples of imported frozen mussels were collected from Spain, as well as from Chile. Fresh mussels were stored at 4°C and frozen mussels at −18°C, before analysis.

Chemicals and standards

All chemicals and reagents used in this study were of analytical grade with high purity. Nitric acid (HNO_3) in 2:1 ratio, (Fisher Scientific, Loughborough, UK), 30% hydrogen peroxide (H_2O_2) (AppliChem, Darmstadt, Germany) and deionised water with resistivity of 18.0 MΏ obtained from Smeg SpA (Guastala, Italy) deioniser, were used for heavy metal analysis. Chemicals used for PAH identification were: acetonitrile (ACN), purchased from Sigma-Aldrich (St. Louis, MO, USA), anhydrous magnesium sulphate ($MgSO_4$), anhydrous sodium acetate (CH_3COONa), primary and secondary amine (PSA) and C18, purchased from Merk (Darmstadt, Germany).

To construct calibration curves for lead, cadmium, mercury and arsenic, certified standards (Accutrace, New Haven, USA) with a concentration of 1000 μg/mL were used. The efficiency of the microwave digestion and method recovery was determined with the certified reference material obtained from MPZ ÙKZÙZ (Central Institute for Supervising and Testing in Agriculture, Brno, Czech Republic). Standard solutions of PAHs were prepared using a mixture of 16 polycyclic aromatic hydrocarbons (lot. CL-6064, Ultra Scientific, North Kingstown, USA) in concentration of 500 ± 0.2 μg/mL. For internal control, a PAH mixture (lot CH-0209, Ultra Scientific, North Kingstown, USA) was used.

Sample preparation

Heavy metal samples were prepared with the microwave digestion system using an Ethos, Microwave lab station (Milestone s.r.i., Sorisole, Italy). In a polytetrafluo roethylene microwave vessel, 0.2 g of homogenised sample, 8 mL of diluted HNO_3 and 2 ml of H_2O_2 were added. Samples were digested on a maximum temperature of 180°C during 30 min, with a maximum power of 1000 W and left for an hour to cool down to room temperature. The obtained solutions were diluted to a final volume of 100 mL using deionised water.

Sample preparation of PAHs was based on the extraction with ACN, in the presence of anhydrous magnesium sulphate and anhydrous sodium acetate, using multiresidue preparations that ensure quick, easy, cheap, effective, rugged and safe preparation (QuEChERS, Anastassiades et al. 2003) as adapted from the Association of Analytical Communities (AOAC), official method 2007.01 for extraction and clean-up, described and adjusted by Novakov et al. (2017) and Mastanjević et al. (2019). Three grams of the sample was transferred into centrifuge tube, with 3 ml of water and 3 ml of ACN. After intensive stirring on a vortex for 1 min, 3 g of anhydrous magnesium sulphate and 1 g of anhydrous sodium acetate were added. The sample was then centrifuged until 5 min at 3000 rpm. 1 ml of upper (ACN) layer of the extract was transferred into the 5 ml tube, which contained 150 mg of anhydrous magnesium sulphate, 100 mg of PSA and 50 mg of C18. The tube content was centrifuged again for 5 min at 3000 rpm, obtaining a clear and pure extract.

Then, 0.5 ml of the extract was evaporated under nitrogen gas and reconstituted with hexane, resulting in a sample ready for GC-MS analysis.

Instrumentation and analysis

Heavy metals were determined using inducted coupled plasma mass spectrometry (ICP-MS). An Agilent ICP-MS 7700 (Agilent Technologies, Santa Clara, CA, USA) was used with assessment of the isotopes ^{208}Pb, ^{111}Cd, ^{201}Hg and ^{75}As, analysed under the following operating conditions: lead (NoG-M (no gas mode), IT (intergation time) 0.1 s/P), cadmium (NoG-M, IT 1 s/P), mercury (NoG-M, IT 1 s/P) and arsenic (He-M (helium mode), IT 1 s/P).

An Agilent GC-MS 7890B/5977A (Agilent Technologies, Santa Clara, CA, USA) system was used for the PAHs analysis. GC operating conditions were: a fused silica column (30 m x 0.25 µm film of HP (Hewlett Packard) – 5 M – thickness); and injection temperature of 280°C, using splitless injector volume of 4 µL. The column temperature programme was: 50°C for 0.4 min; 50–195°C at 25°C/min, hold for 1.5 min; 195–265 at 8°C/min and continued to 315°C for 1.25 min at 20°C/min. The MSD was set at 280°C. Peak verification was based on retention times and target ions of external PAHs. Procedural and solvent blanks were analysed, but no PAHs were found.

Method validation

Precision, accuracy, linearity, limit of detection (LOD) and limit of quantification (LOD) for heavy metals were determined by spiking six samples for each analyte to blank samples. Spiked samples were prepared with various concentrations for each analysed element (Table 1). The LOD was expressed as the analyte concentration corresponding to 3 times the standard deviation (SD). The LOQ was calculated as 10 times the standard deviation. Calculations were performed by MassHunter Software (Agilent Technologies, Santa Clara, CA, USA). The method was used in Proficiency Test MPZ ÙKZÙZ, 2018), Analysis of Feedstuffs, where the z-scores for lead, cadmium and arsenic ranged from − 0.40 to +1.66.

A validation plan of the modified accredited method for PAHs, was prepared in accordance with ISO 17025, including determination of precision, reproducibility, accuracy, linearity, LOQ and LOD (Table 2). The method precision was evaluated by repeatability using the olive oil fortified with PAH concentrations injected in triplicate (50.0 µg/kg, n = 20). Accuracy was calculated by recovery. Linearity was tested in range from 5 to 500 µg/kg and proven to be satisfactory in all ranges. LOD and LOQ were defined as signal-to-noise ratios of 3 and 10, respectively. The method used in this study was applied in the FAPAS-PAHs PT in smoked fish products (September–November 2018, Round 0677), where the z-score for sum ΣPAH4 (BaP, BaA, Chry and BbF) was 0.4, indicating good performance (FAPAS, 2018).

Statistical analysis and exposure evaluation

Data analysis was carried out using Statistica 12.7 (StatSoft Inc. Tulsa, 2015, OK, USA). to determine the descriptive statistic parameters (mean, standard deviation, range) and one-way analysis of variance (ANOVA). ANOVA was used for the assessment of variation in metal and PAHs concentrations among fresh and frozen mussels. Post-hoc Tukey's test was used for statistical analysis of differences, with significance defined at $p \leq 0.01$.

Provisional tolerable weekly intake (PTWI) of Hg, Pb, Cd and As, through consumption of the analysed mussels was calculated based on the concentration of heavy metals detected in the tested samples and the standard portion of seafood for adults which is 200 g (JECFA-776 1989; JECFA-959 2011; JECFA-960 2011). This amount was then divided by the average adult weight (70 kg) and multiplied by 7 days, i.e. by 4 weeks, thus obtaining the dietary weekly or monthly intake of the given heavy metal. The values obtained were then compared with the recommended provisional tolerable weekly (PTWI) or monthly (PTMI) intakes set by the

Table 1. Precision, accuracy, linearity, LOD and LOQ for heavy metals.

Element	Pb	Cd	Hg	As
Spiked blank sample (µg/L)	0.95 ± 0.01	0.051 ± 0.001	0.35 ± 0.05	3.43 ± 0.16
Added standard (µg/L)	8	10	8	6
Measured value (µg/L)	9.23 ± 0.03	9.53 ± 0.02	7.98 ± 0.79	9.21 ± 0.06
Precision (%)	0.7	5.1	12.1	2.7
Accuracy (%)	103.1	94.8	95.6	97.6
Linearity (r^2)	0.9981	1.0000	0.9994	0.9992
LOD (µg kg^{-1})	3.01	1.12	2.04	1.06
LOQ (µg kg)	10.37	3.24	7.12	3.14
Sensitivity Δc = 1 µg/L (Cps)	70936	12744	9530	2858
Measurement uncertainty (%)	4.2	5.3	5.1	3.8
Assigned PT value (µg/L)	2.23	0.74	n.a.	0.96
Measured value (µg/L)	3.78	0.61	n.a.	0.76

n.a. = not available.

Table 2. Precision, reproducibility, accuracy, linearity, LOD and LOQ for PAHs.

PAH	Precision (%)	Reproducibility (%)	Accuracy (%)	Linearity (r^2)	LOQ (μg kg^{-1})	LOD (μg kg^{-1})
Nap	11.3	6.3	95.0	0.99853	1.21	0.36
Anl	7.9	7.8	99.0	0.99672	1.33	0.39
Ane	8.5	8.3	99.3	0.99768	1.05	0.32
Flu	2.8	10.2	100	0.99792	1.11	0.30
Ant	3.5	3.7	98.6	0.99847	1.10	0.30
Phen	4.3	11.4	85.9	0.99603	1.18	0.35
Flt	3.6	3.7	95.3	0.99787	1.13	0.30
BaA	9.4	8.6	89.7	0.99792	1.30	0.37
Pyr	4.7	6.9	91.1	0.99825	1.21	0.32
Chry	5.3	8.2	92.5	0.99810	1.13	0.34
BbF	8.5	14.3	86.4	0.99408	1.30	0.36
BkF	3.5	3.3	94.3	0.99796	1.21	0.32
BaP	1.9	3.8	96.8	0.99871	2.00	0.53
DahA	8.7	11.2	91.2	0.99781	1.99	0.51
BghiP	9.7	11.3	81.5	0.99524	1.90	0.45
InP	9.5	10.3	85.3	0.99534	1.91	0.53

World Health Organization (WHO) (JECFA-776 1989; JECFA-940 2007; JECFA-959 2011; JECFA-960 2011). In this way, it was calculated how much one average portion of mussels contributes to PTWI/PTMI.

Results and discussion

Heavy metal concentrations and exposure analysis

Concentrations of lead, cadmium, mercury and arsenic (total) are presented in Table 3. The study results show the levels of investigated metals in fresh anf frozen mussles including minimal, maximum, arithmetic means and standard deviation values. In order to show frequencies, the percent of quantified samples for each analysed element is also presented. The results of calculated weekly and monthly Pb, Cd, Hg and As intakes are presented in Table 4.

Lead is a highly toxic and carcinogenic metal. European (EC 2015) and Serbian (Serbia 2019) legislation set a maximum limit of 1.5 mg/kg for Pb, in the meat of bivalve molluscs. Lead content in fresh anf frozen mussles were below the prescribed limit, with a mean of 0.32 mg/kg and 0.21 mg/kg, respectively. Maximum concentration reached 0.74 mg/kg. Lead levels in 84 (100%) samples were above the LOQ (0.01 mg/kg). Arıcı and Bat (2016) reported similar levels of lead in Mediterranean mussels (*Mytilus galloprovincialis*) from Turkey, which ranged from 0.05–0.7 mg/kg, as well as Guéguen et al. (2011), who reported 0.49 mg/kg ww for Pb in shellfish of the main marketed species at the bay of the Seine. Lehel et al. (2018) investigated heavy metal concentrations in seafood from a fish market in Hungary and reported a mean value of 0.95 mg/kg in shellfish. On the other hand, there are numerous studies in which the high concentrations of lead have been reported, such as study of Franco et al. (2002), which measured very high Pb levels in *Mytilus* spp. (5.68 mg/kg d.w.) and in *C. angulata* (3.25 mg/kg d.w.) from Spain. The Joint FAO/WHO Expert Committee on Food Additives (JECFA) established the PTWI while European Food Safety Authority (EFSA) established a tolerable weekly intake (TWI). The calculated average PTWI values for Pb were 6.39 μg/kg and 4.2 μg/kg for fresh and frozen mussels. PTWI values were below the recommended tolerable limit of 25 μg/kg. Eating one portion of 200 g mussels, contributed with 25.6% and 16.8% to the PTWI, respectively. However, it should be mentioned that the previous PTWI value for lead was retracted in 2010 by EFSA, since a PTWI value was no longer considered to be health protective. Comparing the data from different studies and calculated PTWI values in this study, mussels present in the Serbian market are safe to consumers regarding lead content.

Cadmium is highly toxic to kidney and may accumulate in the human body and lead to kidney dysfunction,

Table 3. Heavy metal levels (mg/kg) in fresh and frozen mussels.

Element	Fresh mussels n = 42		Frozen mussels n = 42	
	Ranges	Mean ± sd	Ranges	Mean ± sd
Pb	0.12–0.74	0.32[a] ± 0.18	0.01–0.54	0.21[a] ± 0.14
Cd	0.11–0.28	0.16[a] ± 0.05	0.01–0.38	0.17[a] ± 0.08
Hg	0.03–0.15	0.06[a] ± 0.03	0.01–0.09	0.04[a] ± 0.02
As	1.45–5.87	3.97[a] ± 0.87	1.12–2.87	1.56[b] ± 0.36

a,b Means with equal indexes in the same row are not significantly different.

Table 4. Calculated weekly and monthly Pb, Cd, Hg and As intake (μg/kg).

Element	Fresh mussels		Frozen mussels	
	Average	Ranges	Average	Ranges
Pb	6.39	2.39–14.8	4.2	0.19–10.8
Cd	12.8	8.79–22.4	13.6	0.79–30.4
Hg	1.19	0.59–2.99	0.79	0.19–1.80
As (total)	79.4	29.0–117	31.2	22.4–56.0
As (5% of total)	3.97	1.45–5.87	1.56	1.12–2.80

skeletal damage and reproductive deficiencies (Bernard 2008). Food is the main source of human intake of cadmium. European (EC 2014) and Serbian legislation (Serbia 2019) set a maximum level of 1 mg/kg for bivalve molluscs. The maximum level of Cd obtained in the present study was 0.38 mg/kg, so below the regulated level. Mean concentrations of cadmium were 0.16 mg/kg in fresh and 0.17 mg/kg in frozen mussels. Frequencies of the concentrations above LOQ were 100%. Compared with other studies, Guéguen et al. (2011) found the similar mean level of cadmium (0.15 mg/kg) in mussels from France as in the present investigation. Lehel et al. (2018) determined slightly higher mean values in shellfish marketed in Hungary, up to 0.26 mg/kg. Joksimovic and Stankovic (2011) reported a higher mean concentration of Cd (0.84 mg/kg) in mussel's tissue from Montenegro. Franco et al. (2002) found Cd concentrations above the regulated maximum level in *Mytilus* spp. (3.11 mg/kg d.w.) from the coast of northern Spain. Calculated average PTMI values (Cd) for fresh and frozen mussels were 12.80 μg/kg and 13.60 μg/kg, respectively. Obtained values contributed to recommended PTMI values (25 μg/kg) with 51.2% and 54.4%, respectively. In one sample, the value exceeds 100% of the PTMI, so the amount and frequency of consumption of this type of seafood should be taken into account. Although the Cd levels obtained in present study are not high when compared to other investigations, taking into account the PTWI values and the cumulative effect of this toxic metal, care should be taken about mussel's consumption frequency, especially for sensitive consumers.

Mercury is considered to be the most toxic heavy metal in the environment. In water, microorganisms convert mercury into methylmercury (MeHg). Consumption of fish and fishery products is the major route of human exposure to methyl mercury (Trasande et al. 2005). Maximum limit for mercury in bivalve molluscs was set at 0.5 mg/kg in European (Commission 2006) and Serbian (Serbia 2019) legislation. All samples contained Hg below this limit, with a mean of 0.06 mg/kg and 0.04 mg kg in fresh and frozen mussels, respectively. Compared with other studies, similar results are reported by Joksimovic and Stankovic (2011), where Hg mean concentration in mussels originating from Montenegro was 0.05 mg/kg. Also, Lehel et al. (2018) reported low levels of Hg in shellfish marketed in Hungary. The levels of Hg in mussels from the French and Spanish Mediterranean coast were in the range of 0.02–1.24 and 0.03–2.21 mg/kg d.w., respectively, while in the Adriatic Sea they were 0.02–0.50 mg/kg d.w. (Jovic et al. 2011; Stanković and Jovic 2012). There is no recommended PTWI value for Hg, but the one for methylmercury is 1.6 μg/kg. The main source of mercury in fish and other seafood is in the form of MeHg, the most toxic chemical form of mercury. Mercury exposure is of particular concern to pregnant women and young children because of the neurodevelopment toxicity of MeHg (Karagas et al. 2012; Sheehan et al. 2014). The EFSA Panel on Contaminants in the Food Chain (CONTAM 2012), reported that in general 80–100% of the total mercury in fish muscle is MeHg, so this percentage will be used to calculate mercury values. The calculated average PTWI of Hg in fresh and frozen mussels was 1.19 and 0.79 μg/kg, respectively. The calculated PTWI values contribute to the recommended PTWI value of 1.6 μg/kg with 74.3% and 49.4%, respectively. The present results show that mercury levels in investigated mussels marketed in Serbia are safe, when the recommendations regarding the amount and frequency of mussel consumption are followed.

The International Agency for Research on Cancer classifies As as a carcinogenic agent for humans in category 1 (Sirot et al. 2009). All analyses in this study were carried out for total (organic and inorganic) arsenic. Most arsenic found in fish and seafood is in the form of organic arsenic, which is less toxic (Francesconi 2010; Julshamn et al. 2012). No maximum level in EU legislation has been established for total arsenic in foods. However, previous Serbian law (Serbia 2014) set a limit for total arsenic of 2 mg/kg for freshwater and saltwater fish, of 3 mg/kg for products from marine fish and of 12 mg/kg for products of tuna fish. Maximum levels of As in the analysed samples reached 5.87 mg/kg. Arsenic was found in all samples, a with mean concentration in fresh mussels of 3.97 mg/kg and in frozen mussels of 1.56 mg/kg. Lehel et al. (2018) found a mean total arsenic concentration of 3.01 mg/kg in shellfish. Joksimovic and Stankovic (2011), reported high mean levels of As in mussels from Montenegro (14.7 mg/kg d.w.). In this investigation, analysis of variance shows significant variations ($p \leq 0.01$) in the concentration of arsenic between fresh and frozen mussels. Fresh mussels used in this study originate from Croatia and Slovenia, which have access to the Adriatic Sea, while frozen mussels originate from Spain and Chile. The results obtained in the investigation of Stankovic et al. (2011) generally indicated higher values of As in Adriatic mussels, than in mussels originating from the Spanish Mediterranean cost. The high level of As in the mussels of the Adriatic region could be explained by the exhibited high concentrations of As (1–19 mg/kg) found in the sediments of the southern Adriatic (Dolenec et al. 1998). Calculating exposure analysis for As, a PTWI of 15 μg/kg set by JECFA (JECFA-776 1989; JECFA-959 2011) was used.

Table 5. PAHs (μg/kg) in fresh and frozen mussels; Ane, DahA, BghiP and InP concentrations were below LOD.

	Fresh mussels n = 42			Frozen mussels n = 42		
PAH	Frequency[a] (%)	Range	Mean ± sd	Frequency[a] (%)	Range	Mean ± sd
Nap	91.5	0.36–3.45	1.03[b] ± 1.79	85.7	0.39–3.89	1.37[b] ± 0.90
Anl	88.1	0.39–1.75	0.72 [b] ± 0.37	92.8	0.39–1.47	0.65[b] ± 0.28
Flu	35.7	0.32–1.23	0.66[b] ± 0.25	50	0.25–1.14	0.56[b] ± 0.21
Ant	83.3	0.32–2.87	0.78[b] ± 0.59	88.1	0.35–2.45	0.82[b] ± 0.57
Phen	42.8	0.39–1.41	0.69[b] ± 0.27	28.6	0.36–1.38	0.67[b] ± 0.28
Flt	23.8	0.36–1.20	0.69[b] ± 0.27	26.2	0.38–1.42	0.57[b] ± 0.30
BaA	21.4	0.38–0.98	0.67[b] ± 0.22	19	0.38–0.85	0.59[b] ± 0.17
Pyr	61.9	0.34–1.33	0.63[b] ± 0.28	54.8	0.32–1.23	0.64[b] ± 0.23
Chry	21.4	0.36–0.88	0.51[b] ± 0.22	23.8	0.35–0.98	0.53[b] ± 0.21
BbF	19	0.38–0.86	0.59[b] ± 0.19	16.6	0.38–0.86	0.58[b] ± 0.23
BkF	16.6	0.37–0.78	0.52[b] ± 0.17	16.6	0.54–0.91	0.56[b] ± 0.18
BaP	11.9	0.58–0.93	0.75[b] ± 0.15	14.3	0.62–1.02	0.77[b] ± 0.16
∑ BaP, BaA, Chry and BbF	18.5		2.52[b] 0.10	18.5		2.47[b] ± 0.11
Total PAHs			9.27			8.31

[a]Frequency = [(the number of quantified samples)/(number of total samples)] x 100.
[b]Means with equal indexes in the same row are not significantly different.

Recently, the WHO has withdrawn this PTWI value and has not established a new one (Lehel et al. 2018; [WHO] World Health Organization 2021). The calculated value for As is the value of the total metal because it is not possible to calculate the value of inorganic and organic based on the method used in this study. According to the average ratio of inorganic As substances of 5% in aquatic organisms (Lehel et al. 2018), the calculated PTWI values (5%) of 3.97 μg/kg and 1.56 μg/kg are far below the recommended limit. Also, considering the average annual Serbian per capita consumption of mussels of 2.74 g (SORS 2019), the population is generally safe regarding heavy metal content in mussels.

PAH concentrations

Concentrations of 16 PAHs are presented in Table 5, showing levels of investigated PAHs, including frequencies, minimal, maximum, mean and standard deviation values. Total PAH content in fresh and frozen mussels was 9.27 μg/kg and 8.31 μg/kg, respectivelly. The European Commission (Commission 2006) set a maximum limit for benzo(a)pyrene at 10 μg/kg and for the sum of benzo(a)pyrene, benzo(a)anthracene, benzo(b)fluoranthene and chrysene at 30 μg/kg, comparable to the Serbian national legislation (Serbia 2019) of 5 μg/kg and 30 μg/kg in shellfish. Content of those PAHs in all investigated samples were below this limit. Benzo(a)pyrene reached a maximum level of 1.02 μg/kg and the sum of benzo(a)pyrene, benzo(a)anthracene, benzo(b)fluoranthene and chrysene had a mean level of 2.52 μg/kg. Compared with the results of other studies, Bogdanović et al. (2019) reported similar values for 16 PAHs in fresh mussels from the Croatian market. Galgania et al. (2011) found higher concentrations of total PAHs in mussels (*Mytilus galloprovincialis*) from the western basin of the Mediterranean Sea. Based on the study results, mussels marketed in Serbia are safe for human consumption regarding PAHs, with levels obtaind far below the legal limits.

Conclusion

Although analysed mussel samples do not exceed the legal limits, it can be calculated that consumption of a portion of 200 g of mussels in a 70 kg adult in a high percentage contributes to the PTWI/PTMI for mercury and cadmium. Moreover, because heavy metals are potentially harmful for human health, their presence can limit the quantity of mussels humans can consume. Therefore it is necessary to continue monitoring, because mussels are known to accumulate heavy metals, as well as to increase the attention to reduction of environmental pollution. According to the measured PAH concentrations in mussels marketed in Serbia, their consumption does not involve any danger to public health.

Disclosure of potential conflicts of interest

The authors report no conflict of interest. The authors alone are responsible for the content and writing of the manuscript.

Funding

This study was supported by the Ministry of Education, Science and Technological Development, Republic of Serbia (grant number TR 31011/2011-2020).

ORCID

Nikolina J. Novakov http://orcid.org/0000-0002-3951-4282
Brankica D. Kartalović http://orcid.org/0000-0002-2798-8958
Krešimir M. Mastanjević http://orcid.org/0000-0002-2110-6141

Nenad S. Stojanac http://orcid.org/0000-0002-4519-2777
Kristina J. Habschied http://orcid.org/0000-0001-9404-5688

References

Anastassiades M, Lehotay SJ, Stajnbaher D, Schenck FJ. 2003. Fast and easy multiresidue method employing acetonitrile extraction/partitioning and "dispersive solid-phase extraction" for the determination of pesticide residues in produce. J AOAC Int. 86(2):412–431. doi:10.1093/jaoac/86.2.412.

Andayesh S, Rasoul Hadiani M, Mousavi Z, Shahram Shoeibi S. 2015. Lead, cadmium, arsenic and mercury in canned tuna fish marketed in Tehran, Iran. Food Addit Contam B. 8(2):93–98. doi:10.1080/19393210.2014.993430.

Arıcı E, Bat L 2016. Heavy metal levels in Mediterranean mussel Mytilus galloprovincialis from the Turkish Black Sea coast as biomonitor and potential risk of human health. 2nd International Conference on One Medicine One Secience, April 24-27, 2016;Minneapolis (USA): University of Minnesota.

[ATSDR] Agency for Toxic Substances and Disease Registry. 2003. Toxicological profile for mercury. Atlanta(GA):U.S. Department of Health and Human Services, Centers for Diseases Control and Prevention.

[ATSDR] Agency for Toxic Substances and Disease Registry. 2007. Toxicological profile for arsenic. Atlanta (GA):U.S. Department of Health and Human Services, Centers for Diseases Control and Prevention.

[ATSDR] Agency for Toxic Substances and Disease Registry. 2012. Toxicological profile for cadmium. Atlanta (GA):U.S. Department of Health and Human Services, Centers for Diseases Control and Prevention.

[ATSDR] Agency for Toxic Substances and Disease Registry. 2019. Toxicological profile for lead. (draft for public comment). Atlanta (GA):U.S. Department of Health and Human Services, Centers for Diseases Control and Prevention.

Avelar WEP, Mantelatto FLM, Tomazelli AC, Silva DML, Shuhama T, Lopes JLC. 2000. The marine mussel Perna perna as an indicator of contamination by heavy metals in the Ubatuba bay, Sao Paulo, Brazil. Water Air Soil Pollut. 118(1/2):65–72. doi:10.1023/A:1005109801683.

Bat L. 2012. Trace element concentrations in the Mediterranean mussel Mytilus galloprovincialis Lamarck, 1819 caught from sinop coast of the Black Sea, Turkey. The Open Marine Biology Journal. 6(1):1–5. doi:10.2174/1874450801206010001.

Bernard A. 2008. Cadmium & its adverse effects on human health. Indian J Med Res. 128(4):557–564.

Bilandžić N, Sedak M, Čalopek B, Đokić M, Varenina I, Kolanović BS, Luburić ĐB, Varga I, Benić M, Roncarati A. 2018. Element contents in commercial fish species from the croatian market. J Food Compos Anal. 71:77–86. doi:10.1016/j.jfca.2018.02.014

Bogdanović T, Petričević S, Listeš E, Pladin J. 2019. Polycyclic aromatic hydrocarbons in selected food items coming from the Croatian market. Ital J Food Sci. 31:661–680.

Bongiorno T, Iacumin L, Tubaro F, Marcuzzo E, Sensidoni A, Tulli F. 2015. Seasonal changes in technological and nutritional quality of Mytilus galloprovincialis from suspended culture in the Gulf of Trieste (North Adriatic Sea). Food Chem. 173:355–362. doi:10.1016/j.foodchem.2014.10.029.

Di Lena G, Casini I, Caproni R, Fusari A, Orban E. 2017. Total mercury levels in commercial fish species from Italian fishery and aquaculture. Food Addit Contam B. 10(2):118–127. doi:10.1080/19393210.2017.1281353.

Dolenec T, Faganeli J, Pirc S. 1998. Major, minor and trace elements in surficial sediments from the open Adriatic Sea: a regional geochemical study. Geol Croat. 51:47–58.

EFSA Panel on Contaminants in the Food Chain (CONTAM). 2012. Scientific Opinion on the welfare of cattle kept for beef production and the welfare in intensive calf farming systems. EFSA Journal. European Food Safety Authority. 10(5):2985. Available online www.efsa.europa.eu/efsajournal .

European Commission Regulation . 2006. Regulation No 1881/2006 setting maximum levels for certain contaminants in foodstuffs. consolidated version, last amendment.

Falco G, Domingo JL, Llobet JM, Teixido A, Casas C, Muller L. 2003. Polycyclic aromatic hydrocarbons in foods: human exposure through the diet in Catalonia, Spain. J Food Prot. 66(12):2325–2331. doi:10.4315/0362-028X-66.12.2325.

FAPAS. 2018. Food analysis performance assessment scheme. Proficiency Testing Report 0677, PAHs in smoked fish products. September-November 2018. York (UK): The Food and Environment Research Agency.

Francesconi KA. 2010. Arsenic species in seafood: origin and human health implications. Pure Appl Chem. 82 (2):373–381. doi:10.1351/PAC-CON-09-07-01.

Franco J, Á B, Solaun O, Perez V. 2002. Heavy metals in molluscs from the Basque Coast (Northern Spain): results from an 11-year monitoring programme. Baseline/Marine Pollut Bull. 44:956–976.

Galgania F, Martínez-Gómezb C, Giovanardic F, Romanellic G, Caixachd J, Centoe A, Scarpatoc A, BenBrahimf S, Messaoudig S, Deuderoh S, et al. 2011. Assessment of polycyclic aromatic hydrocarbon concentrations in mussels (Mytilus galloprovincialis) from the Western basin of the Mediterranean Sea. Environ Monit Assess. 172(1–4):301–307. doi:10.1007/s10661-010-1335-5.

Grienke U, Silke J, Tasdemir D. 2014. Bioactive compounds from marine mussels and their effects on human health. Food Chem. 142:48–60. doi:10.1016/j.foodchem.2013.07.027.

Guéguen M, Amiard J-C, Arnich N, Badot P-M, Claisse D, Guérin T, Vernoux J-P. 2011. Shellfish and residual chemical contaminants: hazards, monitoring, and health risk assessment along French coasts. Rev Environ Contam Toxicol. 213:55–111. doi:10.1007/978-1-4419-9860-6_3.

JECFA-776. 1989. Evaluation of certain food additives and contaminants. 33rd report of joint FAO/WHO expert committee on food additives, Technical report series 776. Geneva

JECFA-940 2007. Evaluation of certain food additives and contaminants. 67rd Report of Joint FAO/WHO Expert Committee on Food Additives, Technical report series 940. Geneva

JECFA-959 2011. Evaluation of certain food additives and contaminants. 72nd Report of Joint FAO/WHO Expert Committee on Food Additives, Technical report series 959. Geneva

JECFA-960 2011. Evaluation of certain food additives and contaminants. 73rd Report of Joint FAO/WHO Expert Committee on Food Additives, Technical report series 960. Geneva

Joksimović D, Tomić I, Stanković AR, Jović M, Stanković S. 2011. Trace metal concentrations in Mediterranean blue mussel and surface sediments and evaluation of the mussels quality and possible risks of high human consumption. Food Chem. 127(2):632–637. doi:10.1016/j.foodchem.2011.01.057.

Joksimovic D, Stankovic S. 2011. The trace metal accumulation in marine organisms of the southeastern Adriatic coast, Montenegro. J Serb Chem Soc. doi:10.2298/JSC110323159J

Jović M, Stanković S. 2014. Human exposure to trace metals and possible public health risks via consumption of mussels Mytilus galloprovincialis from the Adriatic coastal area. Food Chem Toxicol. 70:241–251. doi:10.1016/j.fct.2014.05.012.

Jovic M, Stanković A, Slavković-Beskoski L, Tomić I, Degetto S, Stanković S. 2011. Mussels as a bio-indicator of the environmental quality of the coastal water of the Boka Kotorska Bay (Montenegro). J Serb Chem Soc. 76 (6):933–946. doi:10.2298/JSC101007075J.

Julshamn K, Nilsen BM, Frantzen S, Valdersnes S, Maage A, Nedreaas K, Sloth JJ. 2012. Total and inorganic arsenic in fish samples from Norwegian waters. Food Addit Contam B. 5(4):229–235. doi:10.1080/19393210.2012.698312.

Karagas MR, Choi AL, Oken E, Horvat M, Schoeny R, Kamai E, Cowell W, Grandjean P, Korrick S. 2012. Evidence on the human health effects of low-level methylmercury exposure. Environ Health Perspect. 120 (6):799–806. doi:10.1289/ehp.1104494.

Ledesma E, Rendueles M, Díaz M. 2016. Contamination of meat products during smoking by polycyclic aromatic hydrocarbons: processes and prevention. Food Control. 60:64–87. doi:10.1016/j.foodcont.2015.07.016

Lehel J, Bartha A, Dankó D, Lányi K, Laczay P. 2018. Heavy metals in seafood purchased from a fishery market in Hungary. Food Addit Contam B. 11(4):302–308. doi:10.1080/19393210.2018.1505781.

Mastanjević K, Kartalović B, Petrović J, Novakov N, Puljić L, Kovačević D, Jukić M, Lukina J, Mastanjević K. 2019. Polycyclic aromatic hydrocarbons in the traditional smoked sausage Slavonska kobasica. J Food Compos Anal. 83:103282. doi:10.1016/j.jfca.2019.103282

MPZ ÙKZÙZ. Central Institute for Supervising and Testing in Agriculture. 2018-2/Analysis of Feedstuffs. KZ-Basic Parameters in Feedstuffs. Detrmination of Se and Heavy Metals. Brno (Czech Republic).

Næss S, Aakre I, Lundebye A-K, Ørnsrud R, Kjellevold M, Wik Markhus M, Dahl L. 2020. Mercury, lead, arsenic, and cadmium in Norwegian seafood products and consumer exposure. Food Addit Contam B. 13(2):99–106. doi:10.1080/19393210.2020.1735533.

Novakov N, Mihaljev ŽA, Kartalović B, Blagojević B, Petrović J, Ćirković M, Rogan D. 2017. Heavy metals and PAHs in canned fish supplies on the Serbian market. Food Addit Contam B. 86 (3):208–215. doi:10.1093/jaoac/86.2.412.

Olmedo P, Pla A, Hernández AF, Barbier F, Ayouni L, Gil F. 2013. Determination of toxic elements (mercury, cadmium, lead, tin and arsenic) in fish and shellfish samples. Risk Assessment for the Consumers Environ Int. 59:63–72.

Serbia. 2014. Serbian regulation, maximum allowable contaminants in Food and Feed Act. 2014. Ministry of Agriculture. The official gazette of the Republic of Serbia. No 29/2014.

Serbia 2019. Serbian regulation, maximum allowable contaminants in Food and Feed Act. 2019. Ministry of Agriculture. The official gazette of the Republic of Serbia. No 81/2019.

Sheehan MC, Burke TA, Navas-Acien A, Breysse PN, McGready J, Fox MA. 2014. Global methylmercury exposure from seafood consumption and risk of developmental neurotoxicity: a systematic review. Bull World Health Organ. 92(4):254–269. doi:10.2471/blt.12.116152.

Sirot V, Guerin T, Volatier JL, Leblanc JC. 2009. Dietary exposure and biomarkers of arsenic in consumers of fish and shellfish from France. Sci Total Environ. 407:1875–1885.

SORS. 2019. Statistical Office of the Republic of Serbia. Accessed 2020 Dec 21. Available at: http://opendata.stat.gov.rs/odata/

Spada L, Annicchiarico C, Cardellicchio N, Giandomenico S, Di Leo A. 2013. Heavy metals monitoring in mussels Mytilus galloprovincialis from the Apulian coasts (Southern Italy). Mediterr Mar Sci. 14(1):99–108. doi:10.12681/mms.323.

Stanković S, Jovic M. 2012. Health risks of heavy metals in the mediterranean mussels as seafood. Environ Chem Lett. 10:119–130. doi:10.1007/s10311-011-0343-1

Stankovic S, Jovic M, Milanov R, Joksimovic D. 2011. Heavy metals concentrations (Zn, Cu, Pb, Cd, As and Hg) in the Mediterranean mussel M. galloprovincialis collected from cultivated and wild sites of the southeastern Adriatic, Montenegro. J Serb Chem Soc. doi:10.2298/JSC110420095S

[US EPA] US Environmental Protection Agency. 2008. EPA's Report on the Enviroment. Washington (DC): National Center for Environmental Assessment; EPA/600/R-07/045F. [accessed 2020 December 28]. Available from the National Technical Information Service, Springfield, VA, and online at http://www.epa.gov/roe

Storelli MM, Marcotrigiano GO. 2001. Polycyclic Aromatic Hydrocarbons in Mussels (Mytilus galloprovincialis) from the Ionian Sea, Italy. J Food Prot. 64(3):405–409.

[WHO] World Health Organization. 2006. Polycyclic Aromatic Hydrocarbons. WHO Food Additives Series 55: safety evaluation of certain contaminants in food. International Programme of Chemical Safety (IPCS). Geneva (Switzerland): World Health Organization; p. 563–743.

[WHO] World Health Organization. 2021. Evaluations of the Joint FAO/WHO Expert Committee on Food Additives (JECFA). Accessed 2021 Mar 29. Available at: https://apps.who.int/food-additives-contaminants-jecfa-database/chemical.aspx?chemID=1863

Trasande L, Landrigan PJ, Schechter C. 2005. Public health and economic consequences of methyl mercury toxicity to the developing brain. Environ Health Perspect. 113 (5):590–596.

Turk Culha S, Bat L, Çulha M, Efendioglu A, Andac MB, Bati B. 2007. Heavy metals levels in some fishes and molluscs from Sinop peninsula of the Southern Black sea, Turkey. 38th CIESM Congress. 38: 323. [2007 April 09. Rapp Comm Int Mer Medit. :.

Usero J, Morillo J, Gracia I. 2005. Heavy metal concentrations in molluscs from the Atlantic coast of southern Spain. Chemosphere. 59:1175–1181.

Yigit M, Celikkol B, Yilmaz S, Bulut M, Ozalp B, Dwyer RL, Maita M, Kizilkaya B, Yigit U, Ergün S, et al. 2018. Bioaccumulation of trace metals in Mediterranean mussels (Mytilus galloprovincialis) from a fish farm with copper-alloy mesh pens and potential risk assessment. Hum Ecol Risk Assess. 24(2):465–481. doi:10.1080/10807039.2017.1387476.