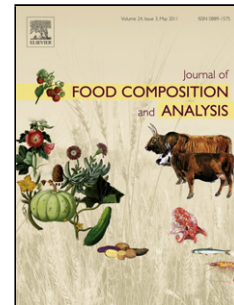


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**Levels of toxic elements in canned fish from the Serbian markets and their health risks
assessment**

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Original Research Article

Highlights

- Levels of As, Cd, Pb and Hg were evaluated in three canned fish species
- Fish were canned tuna, sardine and mackerel purchased on the Serbian retail market
- Analysis was performed using ICP–MS
- Human health risk assessment of As, Cd, Pb and Hg in canned fish were investigated
- There should be no significant adverse health effects from canned fish consumption

ABSTRACT

A total of 138 samples of canned tuna, sardines and mackerel from the Serbian market were analyzed for toxic (As, Cd, Pb, Hg) element levels using ICP–MS. The highest average contents of toxic elements were found to be 1.70 mg kg⁻¹ for arsenic and 0.026 mg kg⁻¹ for cadmium in sardine and 0.068 mg kg⁻¹ for mercury in tuna. The lead content was very similar (~0.048 mg kg⁻¹) of the three types of canned fish. None of the fish analyzed contained toxic elements in levels exceeding the maximum allowable levels currently in force. Human health risk assessment of As, Cd, Pb and Hg in canned fish were investigated. A risk assessment based on the measured levels of examined elements indicated that consumption of canned fish according to current eating habits in Serbia presents little risk to human health since the carcinogenic risk is within the acceptable range, 10⁻⁴-10⁻⁶.

Keywords: food analysis; food composition; toxic elements; canned fish; Serbian market; ICP-MS; health risks assessment

1. Introduction

Seafood including molluscs, crustaceans, cephalopods, fish and others is an important part of the human diet across the world that can be naturally rich or contaminated with some heavy metals (Falandysz, 1991; García, et al., 2016; Yi et al., 2017). Fish consumption is associated not only with proteins, vitamins D and A, or minerals but also with omega-3-polyunsaturated fatty acids (PUFA) that play an important role in the prevention of most cardiovascular pathologies (Simopoulos, 1999). However, the indisputable benefits deriving from fish consumption may be offset by the presence of environmental contaminants at elevated level, and example can be Hg because of a continuing emissions and ongoing pollution of the seas with Hg and risk of methylmercury accumulation (Storelli et al., 2010; Adel et al., 2016). Also, the presence of As, Cd and Pb can be of a concern, which compounds in typically small concentration are present in many fish species (Subotić et al., 2013a). Fish and fish products can be to some degree useful as an integrating medium in a study of environmental pollutants in aquatic ecosystems (Usero et al., 2003).

Canned fish is the most common fish product in Serbia (SORS, 2013). Consequently, data and publications on different aspects of fish and fish products are increasing (Janković et al., 2012; Subotić et al., 2013a, 2013b; Marković et al., 2015; Kartalović et al., 2016; Petrovic et al., 2016). Quantitative evaluation of the intake of mercury, and assessment of potential health risks related to fish consumption among the Serbian population was completed by Janković et al. (2012). Subotić et al. (2013b) investigated the distribution and accumulation of As, Cu, Fe, Hg, Mn, and Zn in tissues of fish species from different trophic levels in the Danube River at the confluence with the Sava River (Serbia).

However, to the best of our knowledge, number of data concerning the occurrence of essential and toxic elements in canned fish available in the Serbian market is highly limited (Janković et al., 2012; Škrbić et al., 2013), in contrast to numerous studies from different countries (Ashraf et al., 2006; Tuzen and Soylak, 2007; Usydus et al., 2008; Shiber 2011; Hosseini et al., 2015; Okyere et al., 2015). Moreover, periodic surveillance and studies on toxic elements in canned fish are needed to assess the

safety of such products with respect to human health. The main objectives of this study were to: (1) analyze and compare the concentrations of four toxic (As, Cd, Pb, Hg) elements in three different canned fish species from the Serbian retail market; (2) determine which canned fish species contained the highest concentration of each element; (3) ascertain whether concentrations of elements in consumable canned fish exceed the maximum levels currently in force; (4) estimate the potential human health risks derived from oral consumption of canned fish species (tuna, sardine and mackerel) by provisional tolerable weekly intake (PTWI); and (5) determine non-carcinogenic and carcinogenic risks.

2. Materials and methods

2.1. Sample collection

In a total of 138 samples of canned tuna (n=72), sardine (n=45) and mackerel (n=21) in oil were purchased from Serbian retail markets during 2015. Cans were transported to the laboratory, labelled accordingly for easy identification and stored in a clean dry place until preparation. Fish products were then homogenized subsequently and a portion was taken for analysis.

2.2. Sample preparation and reagents

An amount, $0.5 \text{ g} \pm 0.1 \text{ mg}$, of homogenized canned fish, was transferred into a teflon vessel and mineralized by adding 5 mL of nitric acid (69.5% Fluka Chemica, Buchs, Switzerland) and 1.5 mL of hydrogen peroxide (30% analytical grade Hydrogen peroxide 30%, Perhydrol stabilized for higher storage temperature, Merck, Darmstadt, Germany).

Microwave digestion was then performed in a Multiwave 3000 SOLVE Microwave Digestion System (Anton Paar, Graz, Austria). Microwave digestion conditions were: max power (1000W); ramp

to 180°C in 5 min; hold at 180°C 15 min; cool 20 min in the oven and a further 15 min at room temperature. After cooling to room temperature, the digests were quantitatively transferred into polypropylene volumetric flasks and diluted to 100 mL with deionized water produced by a water purification system (MILLI – Q Reagent Water System, MILLI – RO 60, Billerica, Massachusetts, USA). Blank (solvents) and spiked samples were included in each batch of digestion and analysis. All solutions (standards, internal standards and samples) were prepared in 2% nitric acid. Preparation and analytical procedures were performed under clean conditions as it is necessary for trace element determination.

Analysis of the following four elements, As, Cd, Pb and Hg, was performed by inductively coupled plasma mass spectrometry (ICP-MS), (iCap Q mass spectrometer, Thermo Scientific, Bremen, Germany). The most abundant isotopes were used for quantification. Operating conditions of the ICP-MS system were: RF power (1550 W); cooling gas flow (14 L min⁻¹); nebulizer flow (1 L min⁻¹); collision gas flow (1 mL min⁻¹); operating mode (Kinetic Energy Discrimination-KED); dwell time (10 ms).

2.3. Standards

Standard stock solutions containing 1000 mg L⁻¹ of each element (As, Cd, Pb and Hg) were obtained from SOLUTIONS Plus. Inc. (Fenton, Missouri, USA). The purity of the starting material in standards was 99.999% for each element. For qualitative analysis of the samples, a five-point calibration curve (including zero) was constructed for the ⁷⁵As, ¹¹¹Cd, ²⁰⁸Pb and ²⁰²Hg isotopes. Concentrations of elements were measured using external calibration solutions and were corrected for response factors of internal standards. Linear ranges used for calibration and the coefficients of determination (R) used to assess the linearity were >0.99 for all elements. Before external calibration, instrument autotuning was performed in order to optimize the instrument for the highest sensitivity. The analytical method was

validated by Guidelines for Single Laboratory Validation (SLV) of Chemical Methods for Metals in Food (AOAC).

2.4. Quality assurance

The accuracy of the analysis was verified by analyzing the certified reference material BCR-185R bovine liver (bovine liver, IRMM¹, Geel, Belgium). Reference material (n=10) was prepared in the same manner as canned fish samples, using microwave digestion as described. Measured concentrations were corrected for response factors of internal standards using the interpolation method and were within the range of the certified values for all isotopes (Table 1). No information was given regarding Hg in the reference material and, therefore, analytical recoveries of 89-100% were determined using spiked samples (Hg c=10 µg kg⁻¹, n=10).

Table 1

2.5. Provisional tolerable weekly intake (PTWI)

The Provisional tolerable weekly intake (PTWI) of toxic elements through consumption of analyzed canned fish was calculated using the following equation (Pinto et al., 2016):

$$PTWI = (C_{\text{elements}} \times WC_{\text{canned fish}}) / bw$$

where C_{elements} is the concentration of element (mg kg⁻¹) detected in canned fish, $WC_{\text{canned fish}}$ is the average Serbian per capita weekly consumption of canned fish (3.04 g × 7 = 21.3 g). According to statistical office of the Republic of Serbia (SORS, 2013), the average amount of canned fish consumed per capita per day in Serbia is 3.04 g. Bw is the individual's body weight (bw; assumed to be 70 kg).

¹ Institute for Reference Materials and Measurements

2.6. Non-carcinogenic and carcinogenic risks

The non-carcinogenic and carcinogenic risks have been calculated according to the equations presented to on the RAIS webpage (Risk Assessment Information System, <https://www.rais.ornl.gov>) for ingestion of the canned fish based on the measured concentrations of toxic elements and by usage the average amount of canned fish consumed daily in Serbia and an average body weight. The other parameters were those presented within the equations.

The equation for non-carcinogenic risk is:

$$\text{HQ (noncarcinogenic risk)} = ((C_{\text{element}} \times \text{EF} \times \text{ED} \times \text{IR} \times \text{CF}) / (\text{AT} \times \text{BW})) / \text{RfD}$$

where C_{element} is the detected concentration of element in canned fish (mg kg^{-1}), EF is exposure frequency ($350 \text{ days year}^{-1}$), ED is exposure duration (26 years), IR is ingestion rate (3.04 g day^{-1}), CF is conversion factor ($10^{-3} \text{ kg g}^{-1}$), AT is an average time ($350 \text{ days year}^{-1} \times 26 \text{ years}$) and BW is body weight (70 kg), and RfD is reference dose, element-specific toxicological value (Table 2).

Carcinogenic risk has been calculated according to the following equation:

$$\text{CR (carcinogenic risk)} = ((C_{\text{element}} \times \text{EF} \times \text{ED} \times \text{IR} \times \text{CF}) / (\text{AT} \times \text{BW})) \times \text{CSF}$$

where the differences are within the AT, this is an average lifetime ($350 \text{ days year}^{-1} \times 70 \text{ years}$) and CSF is a carcinogenic slope factor, element-specific value (Table 2).

Table 2

2.7. Statistical analysis

Statistical analysis of experimental data was performed using software Statistica 10.0 (StatSoft Inc., Tulsa, OK, USA). Analysis of variance (ANOVA) and Tukey's HSD comparison of the means of results were used for analyzing variations. Principal component analysis (PCA) was used to group the observed results and to discover the possible correlations among measured parameters.

3. Results and Discussion

Levels of toxic elements determined in 138 fish (tuna, sardine and mackerel) products are shown in Table 3. Significant differences in mean levels of elements between the three types of canned fish are presented in Figure 1. The results are discussed in comparison to those reported by available literature data.

Table 3

Figure 1

The three types of canned showed the highest levels of As and the lowest levels of Cd (Table 3). Canned tuna and mackerel showed significantly lower levels of As compared to sardine (Fig 1A). Also, canned tuna had significantly lower Cd levels compared to sardine, but this was not significantly different to that established in mackerel, while Cd levels in sardine and mackerel also did not differ (Fig 1B). The levels of Pb and Hg did not statistically differ between the types of canned fish analyzed (Fig 1C, 1D).

3.1. Arsenic

Arsenic can be toxic to humans, animals and plants, and its toxicity varies with its different chemical forms and fueled by the wide recognition of toxic effects on humans, even at low concentrations (Llorente-Mirandes et al., 2017). The International Agency for Research on Cancer (IARC) classified arsenic as an element which is carcinogenic to humans (Group I) (IARC, 2016). Data on As occurrence in food shows that fish and seafood account about 90% of total exposure to As in

food (Adams et al., 1994). The data obtained from this study showed that As was the most abundant toxic element in the analyzed canned fish (Table 3), and the level was significantly higher in sardine (1.70 mg kg^{-1}) than in tuna (0.620 mg kg^{-1}) or mackerel (0.837 mg kg^{-1}) (Fig 1A). A large variation in As levels was observed in canned fish according to the literature data (Table 3). Among published data, Usydus et al. (2008) established the highest As content in sardine (1.93 mg kg^{-1}) in comparison with tuna (1.05 mg kg^{-1}) and mackerel (1.22 mg kg^{-1}). The As content in canned fish from the current study were lower than levels published by Usydus et al. (2008). However, the As content we determined in canned tuna was well within the As levels in different brands of canned tuna from local markets in Tehran (Iran) ($0.51\text{-}0.79 \text{ mg kg}^{-1}$) (Andayesh et al., 2015). Besides Usydus et al. (2008), to the best of our knowledge, only Ikem and Egiebor (2005) investigated the As content in canned mackerel (0.01 mg kg^{-1}), tuna (0.31 mg kg^{-1}) and sardine (0.22 mg kg^{-1}) originating from local markets; their results for As levels in canned fish were much lower than results from this study.

3.2. Cadmium

Cadmium is one of the most toxic heavy metals and poses a significant health risk to humans (Järup et al., 1998). IARC classified Cd as carcinogenic to humans (Group 1) (IARC, 2016). Cadmium is poorly absorbed into the body, but once absorbed, is slowly excreted and accumulates in the kidney causing renal damage. Kidneys from animals are a major source of Cd in the human diet, although lower levels are found in other foods (Okoye et al., 2015; Škrbić et al., 2013). Fish products, especially canned fish, have been found to contain increasingly high amounts of Cd (Ashraf et al., 2006; Iwegbue, 2015). The data obtained in this study showed that the average concentration of Cd was the highest in canned sardine (0.026 mg kg^{-1}), which is in accordance with recently determined Cd levels in sardine from Serbian markets (0.029 mg kg^{-1} ; Škrbić et al., 2013). The concentration of Cd in canned sardine was significantly higher than in canned tuna (0.014 mg kg^{-1}) (Fig 1B), while significant differences

were not established between the Cd levels in sardine and mackerel or between tuna and mackerel (Fig 1B). However, much higher levels of this toxic element were found in other countries: 0.83 mg kg⁻¹, 0.44 mg kg⁻¹ and 0.58 mg kg⁻¹ in canned tuna, sardine and mackerel, respectively, from Nigerian markets (Iwegbue, 2015), as well as 0.64 mg kg⁻¹ and 0.69 mg kg⁻¹ in canned tuna and sardine, respectively, consumed in Saudi Arabia (Ashraf et al., 2006).

3.3. Lead

Lead is a toxic element mainly present in an inorganic form in food products but organic forms have also been identified. The IARC classified Pb as possibly carcinogenic to humans (Group 2B) (IARC, 2016). The toxic effects of Pb have been well documented (EFSA 2010). The European Food Safety Authority (EFSA) summarized analytical data for Pb in food, collected during a nine-year period (EFSA, 2010). Game meat (3.1 mg kg⁻¹) showed the highest level of Pb followed by edible offal from game animals (1.25 mg kg⁻¹) and algae-based supplements (1.02 mg kg⁻¹). Abi-Ghanem et al. (2014) established high levels of Pb in seafood products (6 mg kg⁻¹ in mussels, crustaceans and molluscs) while Olmedo et al. (2013) found up to 0.5 mg kg⁻¹ in fish and fish products. The results from this study showed that the average Pb contents of the three analyzed canned fish species were not significantly different (Table 3, Fig 1C). Our data (0.048 mg kg⁻¹ in canned tuna and sardine; 0.042 mg kg⁻¹ in mackerel) were within the range of most previous studies that have provided a large variation in the Pb levels in canned fish (Table 3). However, in Iwegbue's study (2015), Pb was detected in unusually high maximum concentrations in canned fish species (tuna: 2.56 mg kg⁻¹; sardine: 2.98 mg kg⁻¹; mackerel: 2.82 mg kg⁻¹), exceeding EU and Serbian maximum levels of 0.3 mg kg⁻¹ (European Commission, 2006; Serbian regulation, 2014), currently in force. In the current study, Pb levels in all canned fish examined were below the maximum legally allowed level.

3.4. Mercury

Mercury occurs in various chemical forms: metallic-elemental, inorganic and organic compounds. Mercury is widely distributed in food at very low levels mainly as the divalent inorganic form (Hg^{2+}) and methylmercury (CH_3Hg) as the most common form of organic Hg in the food chain. Primarily, Hg in food is present in the less toxic inorganic form, but the most toxic form of Hg, methylmercury, is found at significant levels only in fish and seafood (Storelli, et al. 2002). Elemental Hg and inorganic Hg compounds are not classifiable as to their carcinogenicity to humans (Group 3) while methylmercury compounds (Group 2B) classified as possibly carcinogenic to humans (IARC 2016). Exposure to high levels of Hg can cause serious health problems (Byeong-Jin et al., 2016). In this study, similar total Hg levels were established in the three different canned fish species (Fig 1D). However, it can be highlighted that tuna showed a mean Hg level (0.068 mg kg^{-1}) almost 1.5- and 2-fold higher than sardine and mackerel, respectively (Table 3). Average Hg levels in published studies were 0.067 mg kg^{-1} in canned tuna from Polish market (Usydus et al., 2008), 0.04 mg kg^{-1} in canned sardine from local markets in Ghana (Okyere et al., 2015) and 0.036 mg kg^{-1} in canned mackerel from USA (Ikem and Egiebor 2005). These levels were similar to the Hg levels determined in canned fish in Serbia.

3.5. Intake of toxic elements by consumers through canned fish

As dietary intakes of heavy elements through food consumption are of high public concern, regular monitoring and updates of dietary intakes of heavy elements are required. Concerning the health risks derived from in taking the toxic elements Cd, Pb, Hg and As, the results obtained were compared with the available toxicological values for these elements. The Joint FAO/WHO¹ Expert Committee on Food Additives (JECFA) established the PTWI, (JECFA, 2014) while EFSA²

¹ Food and Agriculture Organization of the United Nations/World Health Organization

² [European Food Safety Authority](#)

established a tolerable weekly intake (TWI). The EFSA Panel on Contaminants in the Food Chain (CONTAM), (EFSA, 2009), regarding Cd, established a TWI of $2.5 \mu\text{g kg}^{-1} \text{bw}^{-1}$, which replaced the previous PTWI of $7 \mu\text{g kg}^{-1} \text{bw}^{-1}$. Afterwards, in 2010, JECFA set a provisional tolerable monthly intake (PTMI) of $25 \mu\text{g kg}^{-1} \text{bw}^{-1}$ (JECFA, 2014). In the case of Hg, the PTWI for methylmercury of $1.6 \mu\text{g kg}^{-1} \text{bw}^{-1}$ and of $4 \mu\text{g kg}^{-1} \text{bw}^{-1}$ for inorganic mercury, established by JECFA (2014), are still appropriate. In this study, Hg was analyzed as total Hg. Considering the fact that Hg in fish and seafood are present almost completely in the methylated form (more than 90%, Storelli et al., 2002), for calculation in this study, we used the CONTAM Panel established TWI value for methylmercury of $1.3 \mu\text{g kg}^{-1} \text{bw}$, expressed as mercury (EFSA, 2012). Table 4 shows the PTWI of analyzed toxic elements considering the average Serbian per capita consumption of canned fish, expressed as % of the established PTWI or TWI values.

Table 4

Canned fish does not importantly contribute to the PWTI of Cd and Pb (Table 4). Canned fish consumption represents less than 0.35% of the TWI for Cd and 0.07% of the PTWI for Pb. The estimated contribution of As and Hg is higher (Table 4). For Hg, canned tuna represents 1.59 % of the TWI, and for As, canned sardine showed the highest value, representing 3.44% of the PTWI. Overall, the health risk assessment demonstrated the estimated PTWIs of toxic elements due to canned fish consumption were significantly lower than the established PTWI and TWI values, indicating that the consumption of these canned fish presents little risk to human health.

To ensure food safety, the European Commission has established maximum levels for some contaminants in human foods, including the toxic elements analyzed in this study, whereas for As, no limits have yet been published. Maximum levels for Cd, Pb and Hg in fish and/or fish products have

been set by Commission Regulation No 1881/2006 (European Commission, 2006) and they are in line with Serbian regulation (2014), (Table 3). However, Serbian regulation (2014) also defined the maximum residue level in fish and/or fish products for As (12 mg kg^{-1}). The obtained levels of toxic elements in analyzed canned fish from the Serbian markets were below both legal national and EU limits (Serbian regulation, 2014; European Commission, 2006).

The calculated non-carcinogenic risk of analyzed elements indicates that there is a slight risk for human health from eating this food since for the majority of analysed samples the total HQ (as a sum of elements HQ for each sample) falls within the range of 0.1-1. The dominating element, the one that has the greatest HQ values in the majority of samples, was As (Fig. 2a) followed by Hg in canned tuna. The Hg HQ values in some canned tuna samples were the greatest compared with its values in the other two groups of canned fish. The highest values of As non-carcinogenic risks were observed in sardine samples, as it was for determined Cd concentrations although the Cd HQ values were the lowest ones and negligibly contributes to the total HQ value for each sample.

Arsenic shows the highest carcinogenic risks in all samples compared with those calculated from the Pb concentrations (Fig. 2b). The As carcinogenic risks in all canned fish samples are above $1 \cdot 10^{-6}$ which is considered as the lowest acceptable level of risk, and for the majority of samples the calculated values are within the 10^{-5} - 10^{-4} range. These results should not be neglected because the eating these canned fish may not be the major cause of illness, but people are exposed to multiple sources of pollution on a daily basis, which can contribute to the development of various diseases and the possibility for development of cancer.

Figure 2

3.6. PCA analysis

Principal component analysis was applied in order to establish possible correlations between the measured parameters and group the experimental data (Brllek et al., 2013). For visualizing the data trends and for the discriminating the efficiency of the used descriptors, a scatter plot of analysis results using the first two principal components (PCs) from PCA of the data matrix for the canned fish was obtained (Figure 3). As can be seen, there is a neat separation of the three types of fish products, according to the eight observed elements. The quality results showed that first two principal components explained 100.00% of the total variance.

The contents of As (which contributed 40.4% of the total variance, calculated based on the correlation) and Cd (40.2%) were the most negatively influential factors for the first principal component evaluation, while the content of and Hg exerted a positive influence for the first principal component (18.7% of the total variance). The most negatively influential parameters for second principal component were the contents of Pb (60.8% of the total variance) and Hg (33.3%).

Figure 3

The influence of different parameters that describes the observed samples could be evaluated from the scatter plot (Figure 3), in which sardines, with higher As and Cd content, are located at the left side of the graph, while tuna is located at the bottom side of the graphic (with increased Pb and Hg content). Mackerel is located in the upper part of Figure 3, distanced from the origin and with the lowest element concentrations, compared to the other fish types.

4. Conclusions

This study aimed to provide information on toxic (As, Cd, Pb, Hg) element levels in three different canned fish species (tuna, sardine and mackerel) from the Serbian market. The results showed

that significant differences exist only in the levels of As and Cd among the analyzed canned fish.

Arsenic was the most abundant toxic element in all canned fish and the highest level was established in canned sardine. The levels of analyzed elements did not exceed maximum residue levels according both to Serbian and EU regulations. The calculated Provisional tolerable weekly intake, non-carcinogenic and carcinogenic risks on the measured levels of toxic elements suggest that consumption of canned fish from the local market does represent a slight health risk for consumers, mainly because of As content in all three canned fish, and in some tuna samples, Hg has a notable contribution. Since production practice as well as the quality of fish, due to deterioration of environmental conditions, change with time, comprehensive and periodic control of foodstuffs is needed to ensure the quality and safety of food with respect to human health as well as widen the knowledge of toxic elements in food from local markets.

Declarations of interest

The authors declare they do not have any actual or potential conflicts of interest. The authors alone are responsible for the content and writing of the paper.

Disclaimers

This manuscript presents the opinions of the authors and any data included in articles on commercial foods are reported solely as factual information and are limited to the samples analyzed. No warranty or guarantee is made or implied that other samples of these products will have the same or similar composition. The inclusion of such articles or data does not imply endorsement of any product.

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Figure caption

Figure 1. Levels of eight elements in canned tuna, sardine and mackerel. Data are presented as mean \pm SD. Different letters in the bars of each element indicate significant differences of means between types of canned fish, according to Tukey's HSD test ($p < 0.05$).

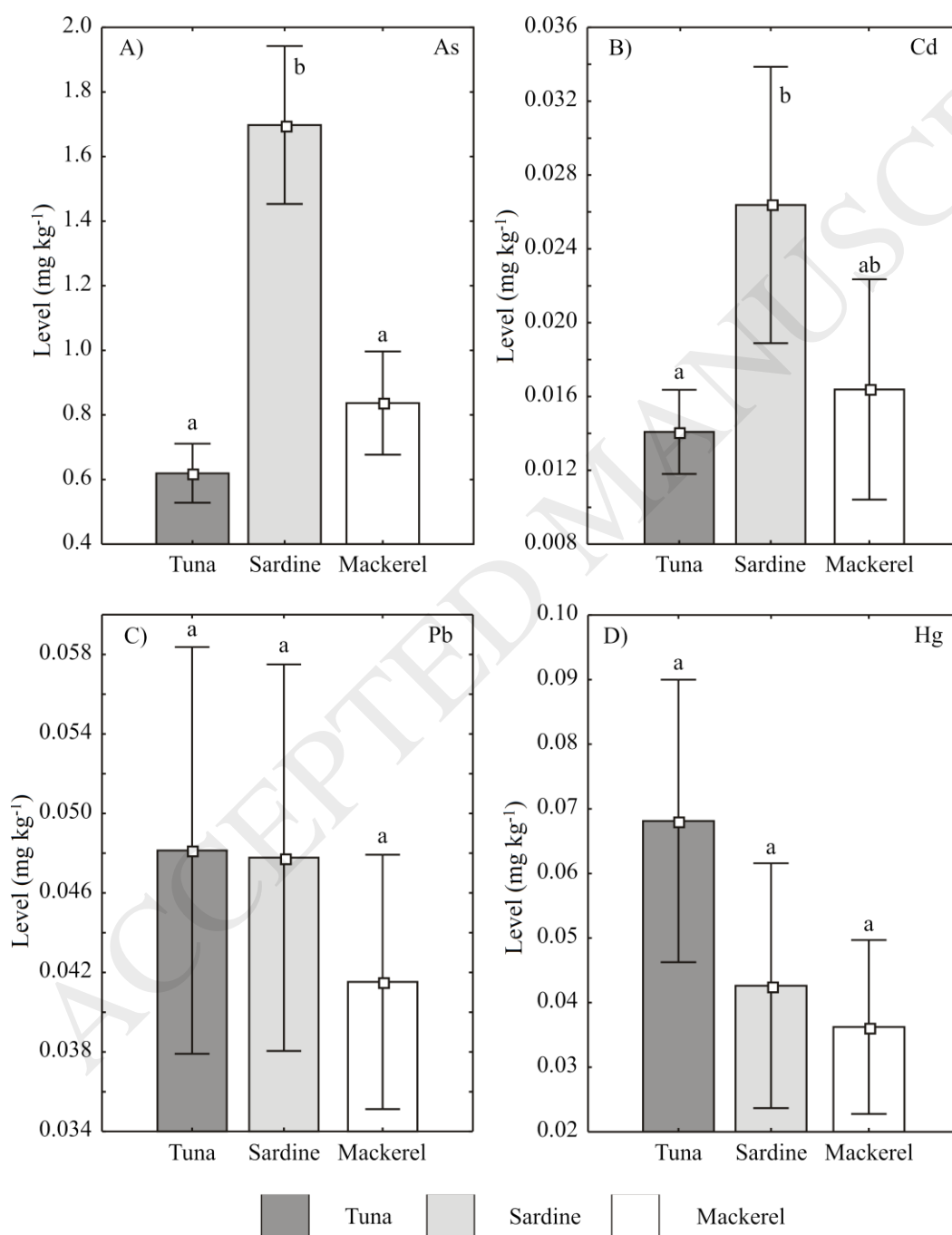


Figure 2. Box-plots of a) non-carcinogenic risks calculated from As, Hg and Cd concentrations in canned tuna (HQ-T), canned sardine (HQ-S) and canned mackerel (HQ-M); and b) carcinogenic risks calculated from As and Pb concentrations in canned fish (CR-T; CR-S; CR-M).

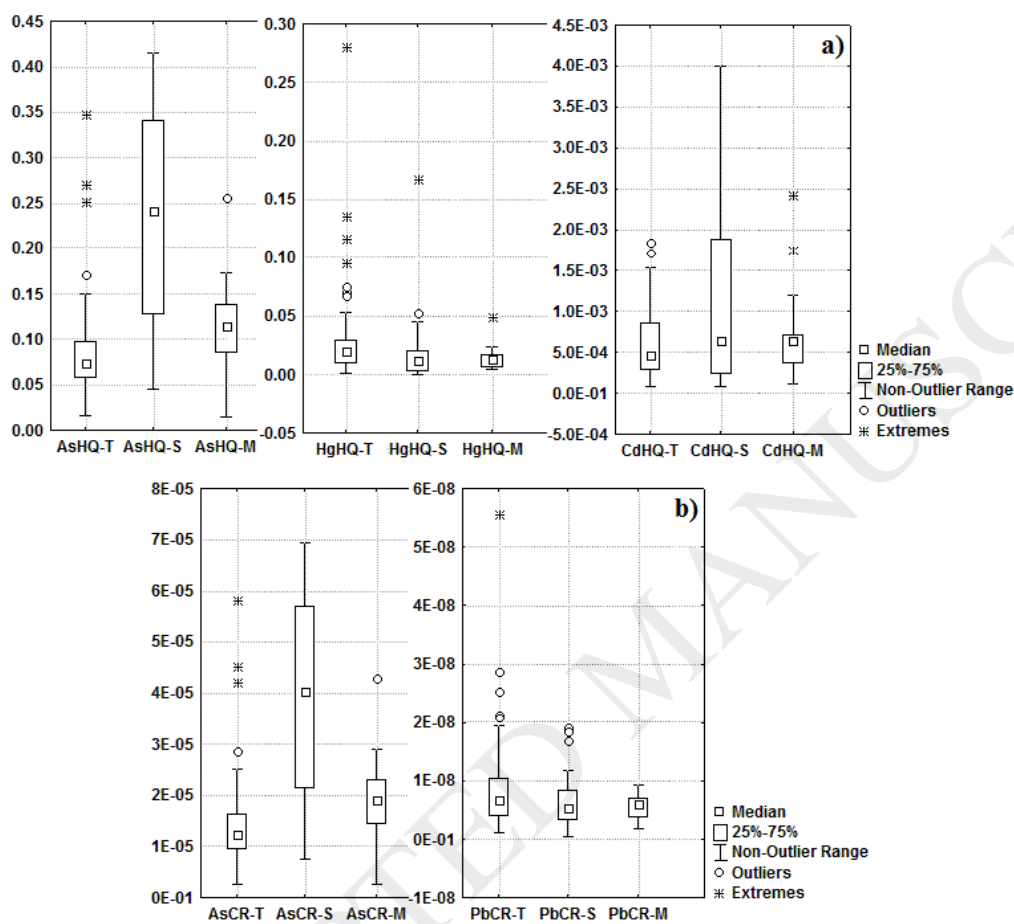
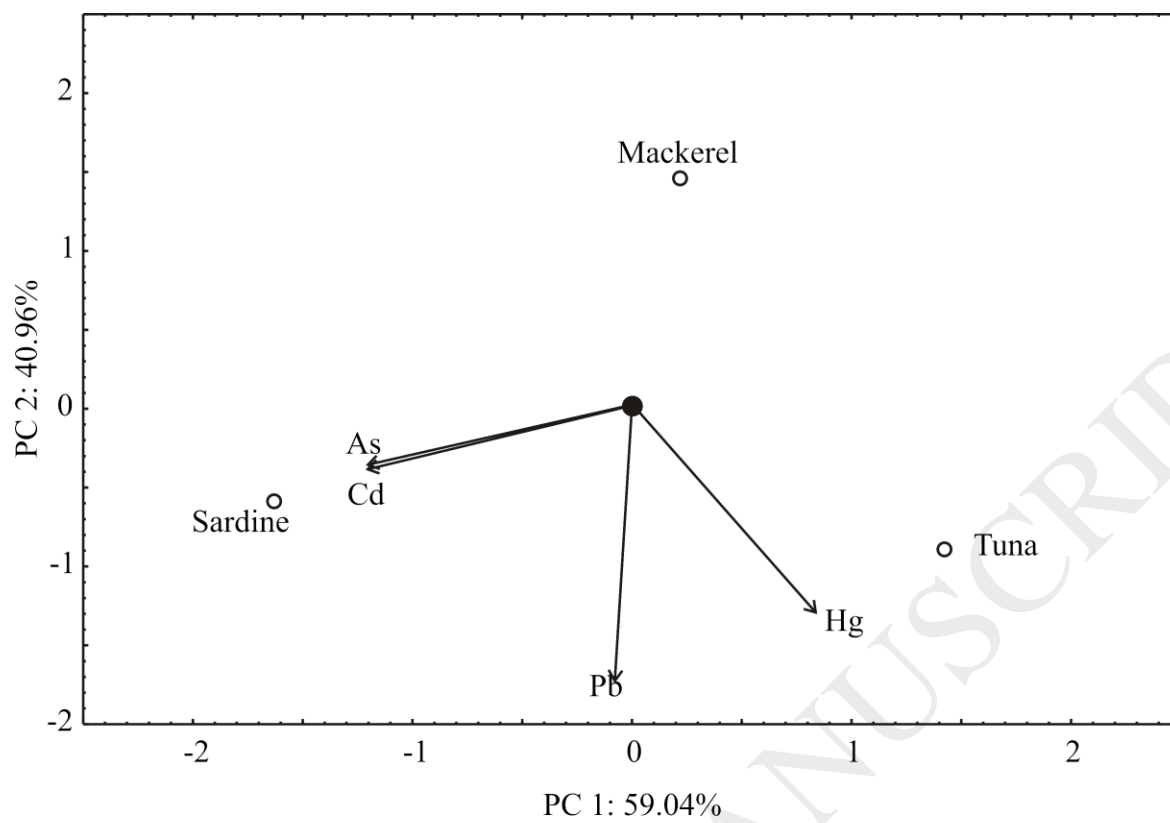


Figure 3. Biplot graphic for As, Cd, Pb and Hg levels in samples of canned tuna, sardine and mackerel.



Table

Table 1

Limit of detection (LOD), limit of quantification (LOQ), repeatability/precision and assigned and measured concentrations of the BCR-185R (bovine liver, IRMM³, Geel, Belgium) reference material used for quality control (n=10).

Elements	LOD	LOQ	Method repeatability/precision	Certified value ^a	Analysed value	Recovery
	($\mu\text{g kg}^{-1}$)	($\mu\text{g kg}^{-1}$)	(%)	($\mu\text{g kg}^{-1}$)	($\mu\text{g kg}^{-1}$)	(%)
As	1.4	4.2	4.12	33.0±2.9	31.0±3.0	93.9
Cd	1.2	3.6	9.57	544±17	548±26	101
Pb	3	9	4.33	172±9	175±11	102
Hg	0.5	1.5	8.66	-	-	-

The data are presented as means \pm standard deviation.

^aCertified value as given by the manufacturer.

³ Institute for Reference Materials and Measurements

Table 2

Toxicological reference values of analysed toxic elements (taken from RAIS website), (Risk Assessment Information System, www.rais.ornl.gov).

	RfD ^a (mg kg ⁻¹ day ⁻¹)	CSF ^b (mg kg ⁻¹ day ⁻¹)
As	0.0003 ^c	1.5 ^c
Cd	0.001 ^c	
Pb		0.0085 ^d
Hg	0.0001 ^c	

^aRfD - reference dose, element-specific toxicological value; ^bCSF - carcinogenic slope factor, element-specific value; ^c IRIS (U.S. EPA Integrated Risk Information System); ^d CALEPA (The California Environmental Protection Agency) and OEHHA (Office of Environmental Health Hazard Assessment)

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Table 3

Levels (Mean \pm SD, Median, mg kg⁻¹) of toxic elements in canned fish in oil purchased in Serbian markets and ranges of these elements published in the literature.

		Tuna (n = 72)			Sardine (n = 45)			Mackerel (n = 21)		
		Our data		LD ^b	Our data		LD	Our data		LD
	MRL ^a	Mean \pm SD	Median		Mean \pm SD	Median		Mean \pm SD	Median	
As	12.0	0.620 \pm 0.389	0.526	0.201-1.05	1.70 \pm 0.813	1.73	0.22-1.93	0.837 \pm 0.351	0.818	0.01-1.22
Cd	0.1	0.014 \pm 0.010	0.011	<0.001-0.83	0.026 \pm 0.025	0.015	<0.001-0.69	0.016 \pm 0.013	0.015	<0.001-0.58
Pb	0.3	0.048 \pm 0.044	0.034	0.001-2.56	0.048 \pm 0.032	0.039	0.0002-2.98	0.042 \pm 0.014	0.044	0.0-2.82
Hg	1.0	0.068 \pm 0.093	0.045	0.03-0.285	0.043 \pm 0.063	0.027	0.01-0.107	0.036 \pm 0.030	0.028	<0.01-0.096

^aMRL – maximum residue level (mg kg⁻¹) for element in fish and other seafood products according to Serbian regulation (2014); ^bLiterature data, LD, (mg kg⁻¹).

Table 4

The provisional tolerable weekly intake (PTWI) of toxic elements considering the average Serbian per capita canned fish consumption (21.3 g person⁻¹ week⁻¹).

Toxic elements	PTWI (expressed as % of the established PTWI/TWI)			Established PTWI ^a	Established TWI ^b
	Tuna	Sardine	Mackerel	($\mu\text{g kg}^{-1}$ bw)	($\mu\text{g kg}^{-1}$ bw)
As	1.26	3.44	1.70	15 ^c	
Cd	0.17	0.32	0.20		2.5
Pb	0.06	0.06	0.05	25 ^c	
Hg	1.59	1.00	0.85		1.3

^a Established PTWI by JECFA (Joint FAO/WHO¹ Expert Committee on Food Additives) (2014).

^b Established TWI by CONTAM (Contaminants in the Food Chain) Panel (EFSA, 2009, 2012).

^c Former values (PTWI withdrawn. Not possible to establish a new PTWI that would be considered health protective), (JECFA, 2014).

¹ Food and Agriculture Organization of the United Nations/World Health Organization