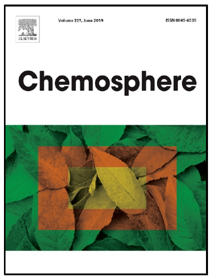
Accepted Manuscript



EVALUATION OF HEAVY METALS AND RADIONUCLIDES IN FISH AND SEAFOOD PRODUCTS

Biljana Milenkovic, Jelena M. Stajic, Natasa Stojic, Mira Pucarevic, Snezana Strbac

PII:	S0045-6535(19)30852-5
DOI:	10.1016/j.chemosphere.2019.04.189
Reference:	CHEM 23697
To appear in:	*Chemosphere*
Received Date:	18 February 2019
Accepted Date:	24 April 2019

Please cite this article as: Biljana Milenkovic, Jelena M. Stajic, Natasa Stojic, Mira Pucarevic, Snezana Strbac, EVALUATION OF HEAVY METALS AND RADIONUCLIDES IN FISH AND SEAFOOD PRODUCTS, *Chemosphere* (2019), doi: 10.1016/j.chemosphere.2019.04.189

EVALUATION OF HEAVY METALS AND RADIONUCLIDES IN FISH AND SEAFOOD PRODUCTS

Biljana Milenkovic[a], Jelena M. Stajic[a], Natasa Stojic[b], Mira Pucarevic[b], Snezana Strbac[c]

[a] *University of Kragujevac, Faculty of Science, Radoja Domanovica 12, 34000 Kragujevac, Serbia*

[b] *Educons University, Faculty of Environmental Protection, Vojvode Putnika 87, Sremska Kamenica, Serbia*

[c] *University of Belgrade, Institute of Chemistry, Technology and Metallurgy, Centre of Chemistry, Studentski Trg 12-16, 11000 Belgrade, Serbia*

Corresponding author: Jelena M. Stajic

Tel.: +381 34336223; fax: +381 34335040

E-mail address: stajicjelena11052012@gmail.com

ABSTRACT

Despite the existence of a legislation regarding food contaminants, food safety control in Serbia is a matter of great concern. This study investigates the radioactivity levels and heavy metal concentrations in fish and seafood commercially available in Serbian markets. Domestic fish species (caught in the Danube River) and fishery products imported from Europe, Asia and America were analyzed. The content of natural radionuclides and ^{137}Cs were investigated by gamma spectrometry. Activity concentration of ^{40}K was measured in the range of 44-165 Bq kg^{-1}; low levels of ^{137}Cs were detected in two samples (2.8 and 3.0 Bq kg^{-1}), while concentrations of ^{226}Ra and ^{232}Th were below minimal detectable values. Concentrations of heavy metals (Cd, Hg and Pb) were determined using ICP-OES method. Cd concentration ranged from 0.01 to 0.81 mg kg^{-1} in sea fish and from 0.01 to 0.03 mg kg^{-1} in freshwater fish. Hg concentrations were in the range of 0.01-1.47 mg kg^{-1}; the highest value was measured in the predator fish - shark. The highest level of Pb (6.56 mg kg^{-1}) was detected in a blue sea fish (Atlantic mackerel). The health risks associated with the intake of heavy metals and radionuclides via fish consumption were evaluated. The results indicate that fish and seafood consumption do not pose a significant health concern in the case of the usual consumption rate which is typical for the population of Serbia. However, a highly frequent consumption of fishery products can have adverse health effects, especially due to Hg and Pb contamination.

Keywords: fish; seafood; heavy metal; radionuclide; health risk

1. INTRODUCTION

Fish plays a key-role in human diet. Consuming fish provides an important source of high-quality protein, selenium (Duran et al., 2014), polyunsaturated fatty acids (Olmedo et al., 2013), liposoluble vitamins (Storelli et al., 2010) and essential minerals, which are associated with health benefits and normal growth (Elnabris et al., 2013). Omega-3 polyunsaturated fatty acids (PUFAs) in fish protect people against coronary heart disease and contribute to satisfactory neurodevelopment in children (Ruelas-Inzunza et al., 2012). Also, fish and seafood have been known as the products with the highest contribution to the total dietary uptake of chemical contaminants (Bae et al., 2017). Chemical compounds produced by human activities are released into the environment, transferred by the food chain to human (Duran et al., 2014). Thus, fish consumption represents the most important contributor of human exposure to heavy metals, and several persistent organic pollutants (POPs) (Storelli et al., 2003).

Natural and artificial radionuclide and heavy metal pollutants in the aquatic environment have been known as a serious environmental concern (Pappa et al., 2016). There has been current worldwide concern about the detection of radionuclides and heavy metals in fish (Görür et al., 2012; Galimberti et al., 2016; Chen et al., 2016; Baltas et al., 2017; Fathabadi et al., 2017; Yi et al., 2017; Fasae and Isinkaye, 2018; Núñez et al., 2018; Liu et al., 2018).

Heavy metals are considered the most marked forms of pollution in aquatic environments (Núñez et al. 2018). Heavy metals are discharged into aquatic environment through agriculture, combustion, mining, urban and industrial discharge. They can remain in solution or in suspension and precipitate to the bottom, or be taken up by organisms, thus forming a potential source of heavy metal pollution in the aquatic environment (Bilandzic et al., 2011). Heavy metal concentrations in fish depends on the distribution, habitat preferences, location, feeding habits, age, trophic level, size, duration of exposure to metals, homeostatic regulation activity (Sankar et al., 2006) and metabolic activity (Langston, 1990). Adverse health effects are related to the type of heavy metal and its chemical form, and are time- and dose-dependent (Tchounwou et al., 2012).

Cadmium in the environment is mainly derived from anthropogenic emissions of fuel combustion and its subsequent atmospheric deposition (Núñez et al., 2018). Mercury is emitted from both, natural and anthropogenic sources. Application of agricultural fertilizers and industrial wastewater disposal releases Hg directly into soil or water. Through the food chain, Hg has the capacity to biomagnify and bioaccumulate (Adel et al., 2018). Pb contamination of the environment significantly increased during the industrial age when Pb was added to the fuel oil. Regulations adopted to reduce the permissible gasoline Pb content have significantly contributed to a reduction in environmental Pb concentrations (Núñez et al., 2018).

The Earth's crust contains primordial ^{238}U and ^{232}Th radionuclides. These primordial radionuclides including isotopes of thorium, radium, radon, lead, polonium, etc. Another commonly occurring primordial radionuclide is ^{40}K. These radionuclides are distributed throughout the environment (sediment, seafood, air, soil, foodstuff, surface and groundwater) in trace amounts (Dinh Chau et al., 2011). Their concentration primarily depends on the geology of a given area. However, geochemistry of each element also plays a role in its migration (Bolaji et al., 2015). Due to mineral leaching, naturally occurring radionuclides could contaminate the environment. Pathways that could supply significant quantities of natural radionuclides in the aquatic environment are: direct groundwater discharge, river runoff, and wind-blown particles (Linsley et al., 2004). Artificial radionuclides were released into environment as the result of anthropogenic activities i.e. atmospheric nuclear weapon tests and accidents. The most important artificial radionuclide is a fission product ^{137}Cs which is recognized as a persistent environmental pollutant due to its long half-life ($T_{1/2}$ = 30.1 y). The primary pathway leading to human exposure from the occurrence of radionuclides in the aquatic environment (river and marine) is consumption of fish and seafood (Görür et al., 2012).

Serbia is a developing country which has adopted legislation setting maximum levels of certain contaminants in foodstuffs (Serbian Regulation 2011; 2013). However, food safety control is still a matter of great concern for the population, particularly regarding imported food products. Analyses of contaminants content in fishery products are one of the most important activities when controlling food safety (Galimberti et al., 2016). The aim of this study is to determine the radioactivity levels and heavy metal concentrations in the muscles of commercial fish and seafood available in Serbian markets.

2. MATERIALS AND METHODS

2.1 Sampling and preparation

A total of 25 samples of technologically processed (packaged) fish and seafood products, and 5 fresh fish from river were collected and analyzed.

Homogenized fish samples (0.4 g each) were transferred into a teflon vessel and mineralized by adding 7 mL of nitric acid (69% PanReac, AppliChem, cat. no. 721037.0012) and 2 mL of hydrogen peroxide (30% analytical grade Hydrogen peroxide 30% PanReac, AppliChem, cat. no. 121076.1211). Microwave digestion performed by Berghof MSW 3+ Microwave Digestion System. Conditions for microwave digestion were: max power (1000 W); ramp to 230

°C in 3 min; hold at 230 °C 30 min; cool for 20 min in the oven and a further 15 min at room temperature. After cooling, digests were quantitatively transferred into volumetric flasks and diluted with 25 mL ultrapure water produced by a water purification system (EasyPure system). Analysis of the elements was performed by inductively coupled plasma optical emission spectrometry (ICP-OES) (Thermo iCAP 6500 Duo), method EPA 6010C. Conditions for the ICP-OES system were: RF power (1250 W); cooling gas flow (12 L min^{-1}); nebulizer flow (0.4 L min^{-1}); collision gas flow (0.5 mL min^{-1}); purge gas flow: normal; pump rate 50 rpm. Standard stock solutions containing 1000 mg L^{-1} of each element (Cd, Hg and Pb) were obtained from J. T. Baker, USA, INSTRA. Elements concentrations were measured using external calibration solutions and were corrected for response factors of internal standards. The accuracy of the analysis was verified by analyzing the certified reference material ERM- BB422, fish muscle, LGC Germany. Reference material was prepared in the same manner as fish samples, using microwave digestion as described.

Gamma counting was used to determine radioactivity levels in the samples. Homogenized fish samples were hermetically sealed in 450 ml Marinelli beakers and left for more than 4 weeks to achieve secular equilibrium between ^{226}Ra and its progeny. Activity concentrations of ^{226}Ra, ^{232}Th, ^{40}K and ^{137}Cs were determined using coaxial HPGe detector (GEM30-70, ORTEC). The detector had relative efficiency of 30% and energy resolution (FWHM) of 1.85 keV at 1.33 MeV (^{60}Co). The detector was calibrated using standardized solution of common mixture of gamma-emitting radionuclides (MBSS 2) provided by the Czech Metrological Institute. It was shielded with 10 cm lead in order to reduce the background. The real time of each gamma-activity measurement was set to 172 800 s (dead time was 0.01%). The gamma-ray lines at 1460.7 keV and 661.6 keV were used for estimating activity concentrations of ^{40}K and ^{137}Cs, respectively. The presence of ^{226}Ra and ^{232}Th in samples were examined by observing the counts at the energies related to their progeny: ^{214}Pb (351.9 keV), ^{214}Bi (609.3 keV and 1764.5 keV), ^{228}Ac (338.3 keV, 911.1 keV and 968.9 keV), and ^{208}Tl (583.0 keV and 860.6 keV).

To perform human health risk assessment the quality of the fish for human consumption was analyzed. Content of radionuclides and heavy metals were compared with certified human consumption safety guidelines recommended for fish and seafood in Serbia (Serbian Regulation 2011; 2013) and European Union Commission Regulation (EC) No 1881/2006. To estimate the potential risk for human health derived from ingesting contaminated seafood we have evaluated: radionuclides ingestion dose, maximum tolerable weekly intake of heavy metals (MTWI), estimation of the daily intake of heavy metals (EDI) and target hazard quotients of heavy metals (THQ-TTHQ).

According to the ICRP (1995), the ingestion dose from radionuclides is given by:

$$H_{T,r} = \sum U_i \cdot C^r \cdot g_{T,r} \tag{1}$$

The subscript *i* represents a food group, the coefficient U_i represents the consumption rate (kg year^{-1}); C^r is activity concentration of the radionuclide *r* (Bq kg^{-1}), and $g_{T,r}$ is the dose conversion coefficient for the ingestion of the radionuclide (Sv Bq^{-1}) in tissue T. For adults, the recommended dose conversion coefficients $g_{T,r}$ for ^{40}K and ^{137}Cs are 6.2×10^{-9} Sv Bq^{-1} and 1.3×10^{-8} Sv Bq^{-1}, respectively (ICRP, 2012).

Maximum tolerable weekly intake (in grams) of each category of fish that does not compromise human health, concerning heavy metals (Galimberti et al., 2016) can be calculated as:

$$MWI = \frac{PTWI \cdot BW}{MHM} \quad (2)$$

where *PTWI* is the Provisional Tolerable Weekly Intake set by Joint FAO/WHO Expert Committee for Cd, Hg and Pb (5 μg kg^{-1} b.w. for total Hg, 2.5 μg kg^{-1} b.w. for Cd and 25 μg kg^{-1} b.w. for Pb) (Joint FAO/WHO, 2011). *BW* is the body weight of a generic adult (in this case 70 kg) and *MHM* is the median concentration of the heavy metal.

Estimated daily intake (mg kg^{-1} b.w. day^{-1}) of heavy metals was calculated according to the equation reported by Łuczyńska et al. (2018):

$$EDI = \frac{C \cdot IR}{BW} \quad (3)$$

where *C* is the concentration of heavy metals in fish and seafood (mg kg^{-1} w.w.), *IR* is daily ingestion rate (g $person^{-1}$ day^{-1}), *BW* is the mean body weight. All consumption limits and risk factors were calculated assuming a meal size for adults of 227 g and a body weight (*BW*) of 70 kg (Adel et al., 2018).

THQ was calculated according to the equation reported by Liang et al. (2018).

$$THQ = \frac{EFr \cdot ED \cdot FiR \cdot C}{RfD \cdot BW \cdot TA} \cdot 10^{-3} \quad (4)$$

where *EFr* is the exposure frequency (365 days $year^{-1}$), *ED* is the exposure duration (70 years), *FiR* is the fish ingestion rate (g^{-1} $person^{-1}$ day^{-1}), *C* is the mean concentration of heavy metals in food stuffs (μg g^{-1} w.w.), *RfD* is the oral reference dose (mg kg^{1} day^{-1}), *BW* is the mean body weight (70 kg), *TA* is the mean exposure time (365 days $year^{-1}$ x ED).

THQ<1 means that there are predominant health benefits of fish consumption and that the consumers are safe, whereas THQ >1 suggested high adverse health effects Łuczyńska et al. (2018). The total THQ (TTHQ) was calculated as sums of individual THQs obtained for each metal:

$$TTHQ = THQ_{Cd} + THQ_{Hg} + THQ_{Pb} \quad (5)$$

Statistical analysis of experimental data was performed using software MiniTab 17. To group the observed results and to determine the possible correlations between measured parameters, principal component analysis (PCA) and cluster analysis were used.

3. RESULTS

Activity concentrations of ^{40}K and ^{137}Cs in fish samples are given in Table 1. Natural radionuclide ^{40}K was detected in all samples. The highest average values of ^{40}K activity concentrations were observed in white and blue sea fish (141 and 143 Bq kg^{-1}, respectively) while the lowest values were measured in shrimps and mussels (48 Bq kg^{-1}). Artificial radionuclide ^{137}Cs was detected in two samples (2.8 and 3.0 Bq kg^{-1}) which belonged to the same species (European sprat) imported from two different countries (Estonia and Poland). According to Currie's method, minimum detectable activities (MDAs) of ^{226}Ra, ^{232}Th, ^{40}K and ^{137}Cs were 0.26, 0.34, 2.10 and

0.15 Bq kg^{-1}, respectively (Currie, 1968; Done and Ioan, 2016). Activity concentrations of ^{226}Ra and ^{232}Th were below MDAs in all samples.

Radionuclide ingestion doses are also presented in Table 1. According to Faostat, the consumption rate of 5.4 kg y^{-1} per capita was used for calculation (HelgiLibrary). The values were obtained by summing the doses for K and Cs (where applicable).

Table 1. The concentrations of Cd, Hg, Pb (mg kg^{-1} w.w.) and activity concentrations of ^{40}K and ^{137}Cs (Bq kg^{-1} w.w.) in the edible part of the aquatic organisms. Ingestion doses, H_T (for ^{40}K and ^{137}Cs) are expressed in μSv y^{-1}

			Cd	**Hg**	**Pb**	**^{40}K**	**^{137}Cs**	**H_T**
White sea fish								
Merluccius merluccius	European hake	Spain	0.03	0.04	0.20	143	-	4.8
Merluccius merluccius	European hake	Argentina	0.05	0.04	0.44	138	-	4.6
Merluccius merluccius	European hake	Argentina	0.02	0.07	0.45	133	-	4.4
Merluccius merluccius	European hake	Spain	0.03	0.04	1.61	149	-	5.0
Scorpaena scrofa	Red scorpionfish	Iceland	0.04	0.07	0.12	144	-	4.8
Scorpaena scrofa	Red scorpionfish	Iceland	0.02	0.18	0.14	133	-	4.5
Scorpaena scrofa	Red scorpionfish	Norway	0.02	0.17	0.15	125	-	4.2
Sparus aurata	Sea bream	Croatia	0.02	0.12	0.94	147	-	4.9
Sparus aurata	Sea bream	Greek	0.01	0.17	0.19	132	-	4.4
Dicentrarchus labrax	European seabass	Greek	0.01	0.11	0.13	150	-	5.0
Dicentrarchus labrax	European seabass	Croatia	0.01	0.14	0.63	157	-	5.3
		min	*0.01*	*0.04*	*0.10*	*125*	-	*4.2*
		max	*0.05*	*0.18*	*1.61*	*157*	-	*5.3*
		average	*0.02*	*0.10*	*0.45*	*141*	-	*4.7*
		stdev	*0.01*	*0.06*	*0.46*	*10*	-	*0.3*
Blue sea fish								
Scomber scombrus	Atlantic mackerel	Northern Ireland	0.81	0.08	0.57	142	-	4.7
Scomber scombrus	Atlantic mackerel	United States	0.07	0.02	0.22	165	-	5.5
Scomber scombrus	Atlantic mackerel	Spain	0.03	0.05	6.56	164	-	5.5
Scomber scombrus	Atlantic mackerel	Norway	0.04	0.17	0.15	122	-	4.1
Thunnus thynnus	Atlantic bluefin tuna	Spain	0.02	0.52	0.30	149	-	5.0
Sprattus sprattus	European spratt	Estonia	0.01	0.02	0.25	118	2.8	4.1
Sprattus sprattus	European spratt	Poland	0.05	0.03	0.66	144	3.0	5.0
		min	*0.01*	*0.02*	*0.15*	*118*	*2.8*	*4.1*
		max	*0.81*	*0.52*	*6.56*	*165*	*3.0*	*5.5*
		average	*0.15*	*0.13*	*1.24*	*143*	*2.9*	*4.8*
		stdev	*0.29*	*0.18*	*2.35*	*18*	*0.1*	*0.6*
Landings								
	Shark	Spain	0.01	1.47	0.17	144	-	4.8

Cephalopod								
	Teuthida	New Zealand	0.16	0.04	0.71	92	-	3.1
	Teuthida	New Zealand	0.60	0.02	0.13	82	-	2.7
		min	*0.16*	*0.02*	*0.13*	*82*	-	*2.7*
		max	*0.60*	*0.04*	*0.71*	*92*	-	*3.1*
		average	*0.38*	*0.03*	*0.42*	*87*	-	*2.9*
		stdev	*0.31*	*0.02*	*0.41*	*7*	-	*0.2*
Shrimps and mussels								
	Seafood	China	0.32	0.02	0.50	57	-	1.9
	Seafood	Croatia	0.18	0.02	1.13	44	-	1.5
	Seafood	Spain	0.15	0.03	0.25	45	-	1.5
		min	*0.15*	*0.02*	*0.25*	*44*	-	*1.5*
		max	*0.32*	*0.03*	*1.13*	*57*	-	*1.9*
		average	*0.22*	*0.02*	*0.63*	*48*	-	*1.6*
		stdev	*0.09*	*0.00*	*0.45*	*8*	-	*0.3*
Freshwater fish								
Pangasius sanitwongsei	Giant pangasius	Vietnam	0.01	0.01	0.83	77	-	2.6
Acipenser ruthenus	Sterlet	Serbia	0.03	0.10	0.21	82	-	2.8
Barbus barbus	Barbel	Serbia	0.01	0.09	0.15	114	-	3.8
Abramis brama	Common bream	Serbia	0.01	0.17	0.08	105	-	3.5
Zingel balcanicus		Serbia	0.02	0.22	0.02	138	-	4.6
Cyprinus carpio	Common carp	Serbia	0.01	0.50	0.16	116	-	3.9
		min	*0.01*	*0.01*	*0.02*	*77*	-	*2.6*
		max	*0.03*	*0.50*	*0.83*	*138*	-	*4.6*
		average	*0.02*	*0.18*	*0.24*	*105*	-	*3.5*
		stdev	*0.01*	*0.17*	*0.30*	*23*	-	*0.8*

The ranges, average values, and standard deviations of Cd, Hg, and Pb concentrations in fish samples are given in Table 1. Samples of sea fish contain Cd in the concentration from 0.01 to 0.81 mg kg^{-1}, and seafood from 0.15 to 0.32 mg kg^{-1}. In the group of cephalopods, Cd concentrations ranged from 0.16 to 0.60 mg kg^{-1}. Concentration of Cd in freshwater fish ranged from 0.01 to 0.03 mg kg^{-1}.

The measured Hg values ranged from 0.01 to 1.47 mg kg^{-1} (Table 1). In the group of cephalopods, Hg ranged from 0.02 to 0.04 mg kg^{-1}. The highest concentration of Hg was determined in the predator fish - shark (Spain) (1.47 mg kg^{-1}) (Table 1).

Pb values in sea fish ranged from 0.10 to 6.56 mg kg^{-1}. In seafood, Pb concentrations ranged from 0.25 to 1.13 mg kg^{-1} (Table 1), and in the group of cephalopods from 0.13 to 0.71 mg kg^{-1}.

Freshwater fish samples contain Pb concentrations in the range from 0.02 to 0.83 mg kg^{-1} (Table 1).

Table 2 presents Spearman correlation matrix for heavy metals. Moderate negative correlation was found between Hg and Cd, and between Hg and Pb.

Table 2. The Spearman correlation matrix for heavy metals content

	Cd	Hg	Pb
Cd	1	-0.527**	0.278
Hg		1	-0.466**
Pb			1

**Correlation is significant at the 0.01 level

PCA analysis provides a direct insight into the relationships of variables and provides empirical support for solving conceptual issues related to the basic data structure. When determining the number of components for the analysis of the main components, latent root criterion is considered, according to which only those factors with an eigenvalue greater than 1 are taken into account. Based on this criterion, two components that account for 64.9% of the total variance should be taken into account. The Scree test (Figure 1) searches for the place at which the line changes rapidly, and to this point counts the components to be included in the analysis. Based on the latent root criteria, it can be seen that the first two components are optimal for defining a sample.

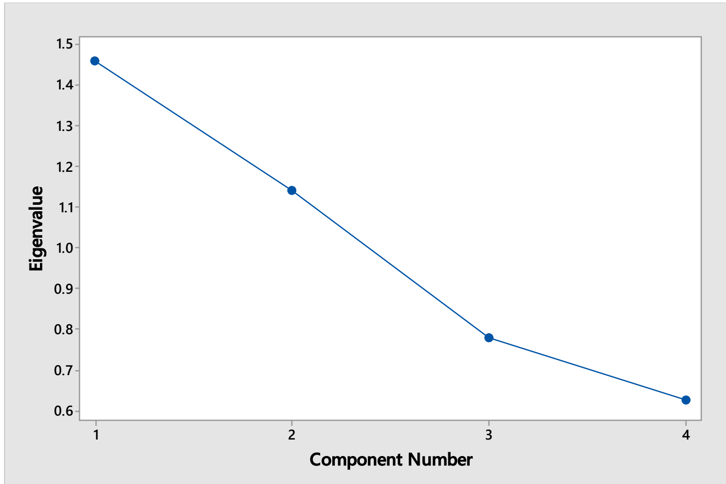


Figure 1. Scree plot of the eigenvalues or the percentages of total variation for each principal component for the PCA

The share of the first major component in the total variance is 36.4% and this percentage defines the variation of the data resulting from the first major component (Table 3). The second component has a share of 28.5%, respectively

Table 3. Eigen analysis of the Correlation Matrix

Eigenvalue	1.457	1.140	0.777	0.625
Proportion	0.364	0.285	0.194	0.156
Cumulative	0.364	0.649	0.844	1.000

From the geometric component matrix (Table 4) and biplot analysis of the main components (Figure 2) the highest positive loads for the first component in terms of parameters are ^{40}K and Hg (0.639 and 0.478), and the negative load Cd (-0.584). In the second component, the maximum load is for Pb (0.820).

Table 4. Analysis of the main components

Variable	PC1	PC2

Cd	-0.584	0.041
Hg	0.478	-0.523
Pb	0.155	0.820
^{40}K	0.638	0.229

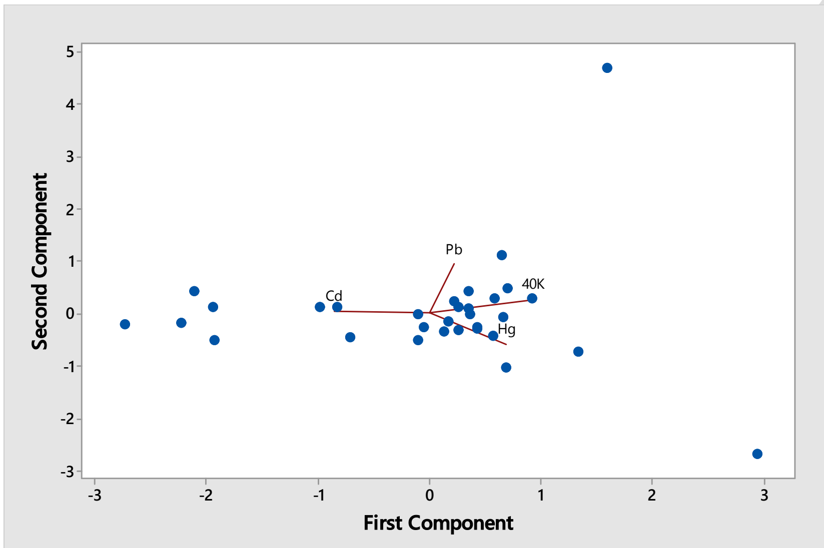


Figure 2. Biplot analysis of the main components

The results of the cluster analysis are illustrated by a dendrogram in Figure 3. A smaller distance between the clusters indicates a stronger connection between the variables. This result is consistent with the results of the analysis of the main components. The second cluster consists of concentrations of Hg and ^{40}K. The third cluster is separated by Pb concentrations as well as in PCA analysis. The fourth cluster identified the concentration of Cd.

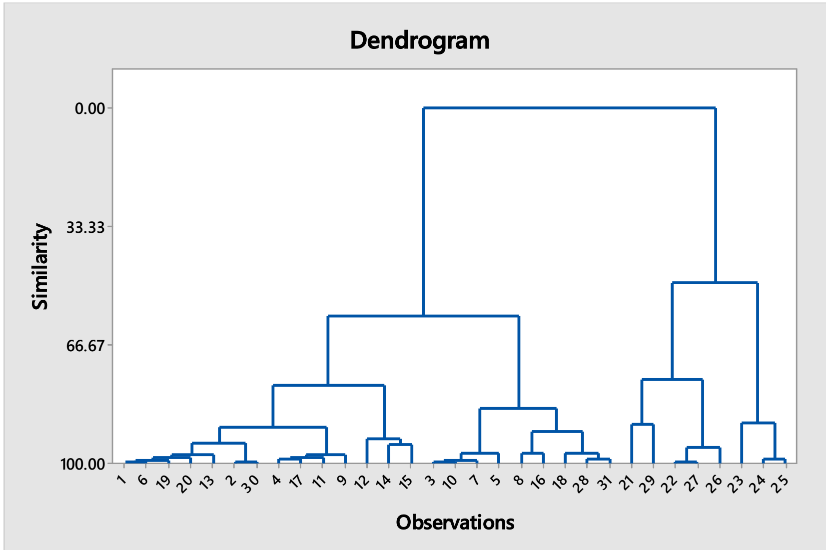


Figure 3. Dendrogram of geometric parameters tested

Observing the PCA results together with the analysis of the grouping, it can be concluded that the concentrations of ^{40}K and Hg were singled out as the first parameter. PCA analysis showed the highest load on this parameter, as well as in clustering. It independently separated in the concentration analysis Cd.

4. DISCUSSION

The activity concentrations of ^{40}K in fish samples are from 44 Bq kg^{-1} in seafood to 165 Bq kg^{-1} in a sample of Atlantic mackerel. The activity concentrations of ^{40}K were higher than the values measured in fish samples from the Black Sea (Görür et al., 2012; Baltas et al., 2017). However, activity concentrations of ^{137}Cs were far below the limit of 150 Bq kg^{-1} recommended for fish and seafood in Serbia (Serbian Regulation, 2013). Baltas et al. (2017) have also reported no detection of ^{137}Cs in anchovy samples from the Black Sea in Rize, Turkey. Chen et al. (2016) have observed

^{137}Cs level of 6.1 Bq kg^{-1} in freshwater fish from the experimental lakes area in Ontario, Canada. For *E. encrasicholus* in Trabzon and *T. mediterranus* and in Rize, activity concentration of ^{137}Cs ranged from 0.06 to 1.53 Bq kg^{-1} (Görür et al., 2012).

The concentrations of the analyzed heavy metals are relatively low in the freshwater fish species, compared to other investigated species. In marine fishes the dietary uptake is the dominant path of metal accumulation, and in freshwater fish the intake of heavy metals is primarily due to the accumulation of dissolved metals from the environment (Liu et al. 2015).

Atlantic mackerel form Northern Ireland and cephalopods had the highest concentration of Cd compared to other samples. The sample of Atlantic mackerel contains Cd concentration above the maximum levels recommended for fish and seafood by the EU Commission Regulation (EC) No. 1881/2006 and Serbian regulation (Serbian Regulation, 2011). In the studies conducted on freshwater and sea fish, low Cd concentrations (<0.005 - 0.023 mg kg^{-1}, 0.001 - 0.009 mg kg^{-1}) were found (Đeđibegović et al., 2012; Noël et al., 2013; Olmeda et al., 2013). In cephalopods, Cd accumulates primarily in digestive gland organ involved in storage and metal detoxification (Pastorelli et al., 2012). EU Commission Regulation (EC No 1881/2006) and Serbian regulation (Serbian Regulation, 2011) established limits for the edible part of cephalopods without internal organs (1.0 mg kg^{-1}). Literature reported differences in Cd concentration in cephalopod species (Galimberti et al., 2016). Cd concentration is higher in deeper waters, and decreases closer to the water surface (Storelli and Marcotrigiano, 1999). Cephalopods sampled from Turkey contain higher Cd concentrations (0.12 to 34.7 mg kg^{-1}) (Duysak et al., 2013). Consequently, Cd was present also in crustacean which also accumulates heavy metals such as Cd, Cu and Zn in the digestive gland (Engel and Brower, 1986). The investigation of Marković et al. (2012) on shellfish from the Adriatic Sea showed similar Cd concentrations (0.18 - 0.74 mg kg^{-1}). Olmedo et al. (2013) found lower Cd concentrations in crayfish sampled in Spain (0.01 to 0.07 mg kg^{-1}).

The highest Hg concentrations were measured in predatory fish; tuna fish and shark contained Hg concentration of 0.52 mg kg^{-1} and 1.47 mg kg^{-1}, respectively. Our results are similar to numerous studies of other authors. Martorell et al. (2011) and Storelli et al. (2012) found similar values of Hg concentration in tuna (~ 0.50 mg kg^{-1}). In Persian bamboo shark (*Chiloscyllium arabicum*) from the Persian Gulf, Adel et al. (2018) found Hg concentrations from 0.01 to 0.09 mg kg^{-1}. For swordfish, tuna fish and sharks (and for some other species) in the European Union legislation (Regulation (EC) No 1881/2006 and its modifications) 1 mg kg^{-1} was established as the maximum level of Hg, while for other fishery products the limit is 0.50 mg kg^{-1}. Concentration of Hg in shark sample is above maximum levels recommended for fish and seafood by Commission Regulation (EC) No 1881/2006 and Serbian regulation (Serbian Regulation, 2011). Olmedo et al. (2013) found similar values in crustacean and mussels, but Hg was not detected in the Teuthida. Marković et al. (2012) found a higher range of Hg concentration in mammals sampled in Montenegro (0.05-0.23 mg kg^{-1}). In our research, sample of common carp contained concentrations approximate to the maximum levels (0.5 mg kg^{-1}). In marine pelagic ecosystems, predatory fish are at the top of the food chain and tend to accumulate great amounts of Hg (Galimberti et al., 2016). Furthermore, pelagic fish are characterized by digestion and growth rates two to five times higher than other species (Storelli et al., 2012).

In our study, the obtained values of Pb concentrations in marine fish were higher in comparison with the research of Olmedo et al. (2013). The concentrations above the maximum levels recommended for fish and seafood by EU Commission Regulation (EC) No 1881/2006 and Serbian regulation (Serbian Regulation, 2011) were found in 8 samples (Table 1): two samples of

European hake from Argentina, and one sample from Spain (0.44 mg kg^{-1}, 0.45 mg kg^{-1} and 1.61 mg kg^{-1}, respectively), Atlantic mackerel from Northern Ireland and Spain (0.57 mg kg^{-1} and 6.56 mg kg^{-1}), Sea bream and European seabass from Croatia (0.94 mg kg^{-1} and 0.63 mg kg^{-1}, respectively), and European spratt from Poland (0.66 mg kg^{-1}). Marković et al. (2012) and Bogdanović et al. (2014) found that shellfish from the Adriatic Sea contained high values of Pb (from 0.24 to 3.3 mg kg^{-1} and from 0.14 to 2.072 mg kg^{-1}, respectively). In our research, Pb concentrations in seafood were below the maximum levels defined by the above regulations. Concentrations of Pb in freshwater fish species (Table 1) were similar to those measured in other studies (Matašin et al. 2011; Gül et al., 2011; Noël et al., 2013). Sample of Giant Pangasius from Vietnam contain Pb concentration above maximum levels.

4.1 Health risk assessment through fish consumption

Total quantities of each category of fish and seafood that correspond to maximum weekly intake for adult person of 70 kg are about:

- 7.6 kg of frozen white sea fish (European hake, Red scorpionfish, Sea bream and European seabass)
- 6.4 kg of frozen blue sea fish (Atlantic mackerel, Atlantic bluefin tuna and European Spratt);
- 7.7 kg of frozen shark;
- 7.5 kg of teuthida;
- 6.5 kg of seafood (shrimps and mussels);
-14.1 kg of freshwater fish (Giant pangasius, Sterlet, Barbel, Common bream and Common Carp).

According to European Commission (2012), usual fish intake for a person in European Union is 23.3 kg per year. Since the average annual intake in Serbia is even lower, the consumption of frozen fish and seafood from markets could be considered safe. Besides, THQ values of individual metals and TTHQ in this study are less than 1. Exposure level less than 1 indicates that daily exposure is implausible to cause serious adverse effects during the lifetime of a person (Yi et al., 2017). Therefore, no significant health risks should be associated with fish consumption in Serbia. Total metal THQ showed that Hg and Pb are two major risk contributors of the TTHQ and accounted with 52.53 % and 28.93 %, respectively.

On the other hand, US EPA (USEPA, 2000) has proposed oral reference doses (RfDo) for Cd, Hg and Pb in fish (0.001 mg kg^{-1} day^{-1} b.w.; 0.00016 mg kg^{-1} day^{-1} b.w.; and 0.004 mg kg^{-1} day^{-1} b.w.). Calculation results in this study showed that:

- EDI for Cd in Atlantic mackerel from Northern Ireland was higher than the RfDo;
- EDI for Hg in European hake from Argentina; Red scorpionfish from Iceland and Norway; Sea bream from Croatia, Greek; European seabass from Greek, Croatia; Atlantic mackerel from Northern Ireland, Norway; Atlantic bluefin tuna from Spain; Shark from Spain; Sterlet, Barbel, Zingel balcanicus, Common bream, and Common carp from Serbia were higher than the RfDo;
- EDI for Pb in Atlantic mackerel from Spain was higher than the RfDo.

These results indicate that frequent consumption of fish from markets in Serbia might still have an adverse effect on human health, especial from Hg contamination.

5. CONCLUSION

This study investigates heavy metal (Cd, Hg and Pb) and radionuclide (^{226}Ra, ^{232}Th, ^{40}K and ^{137}Cs) concentrations in fish muscles. Although it is well known that fish muscle is not active tissue in accumulating heavy metals, it is the most consumed part of fish. The results indicate that fish and seafood consumption in Serbia do not pose a significant radiological health concern. Considering the usual intake, fish and seafood products can also be considered safe regarding heavy metal content. However, a highly frequent consumption of fishery products can have an adverse effect on human health, especially due to Hg and Pb contamination.

Acknowledgement

The present work was supported by the Ministry of Education, Science and Technology Development of the Republic of Serbia, under the Projects No. 171021, 176006, 176019, III 43004.

References

Adel, M., Copat, C., Saeidi, M.R., Conti, G.O., Babazadeh, M., Ferrante, M., 2018. Bioaccumulation of trace metals in banded Persian bamboo shark (Chiloscyllium arabicum) from the Persian Gulf: A food safety issue. Food and Chemical Toxicology 113, 198–203.

Bae, H.S., Kang, I.G., Lee, S.G., Eom, S.Y., Kim, Y.D., Oh, S.Y., Kwon, H.J., Park, K.S., Kim, H., Choi, B.S., Yu, I.J., Park, J.D., 2017. Arsenic exposure and seafood intake in Korean adults. Human & Experimental Toxicology 36, 451–460.

Baltas, H., Kiris, E., Sirin M., 2017. Determination of radioactivity levels and heavy metal concentrations in seawater, sediment and anchovy (Engraulis encrasicolus) from the Black Sea in Rize, Turkey. Marine Pollution Bulletin 116, 528–533.

Bilandzic, N., Dokic, M., Sedak, M., 2011. Metal content determination in four fish species from the Adriatic Sea. Food Chemistry 124, 1005–1010.

Bolaji, B.B., Francis, D.S., Ibitoruh, H., 2015. Human health impact of natural and artificial radioactivity levels in the sediments and fish of Bonny estuary, Niger Delta, Nigeria. Challenges 6, 244–257.

Bogdanović, T., Ujević, I., Sedak, M., Listeš, E., Šimat, V., Petrićević, S., Poljak, V., 2014. As, Cd, Hg and Pb in four edible shellfish species from breeding and harvesting areas along the eastern Adriatic Coast, Croatia. Food Chemistry 146, 197–203.

Chen, J., Rennie, M.D., Sadi, B., Zhang, W., St-Amant, N., 2016. A study on the levels of radioactivity in fish samples from the experimental lakes area in Ontario, Canada. Journal of Environmental Radioactivity 153, 222–230.

Currie, L.A., 1968. Limits for qualitative detection and quantitative determination. Application to radiochemistry. Analytical Chemistry 40, 586–593.

Done, L., Ioan, M-R., 2016. Minimum Detectable Activity in gamma spectrometry and its use in low level activity measurements. Applied Radiation and Isotopes 114, 28–32.

Dinh Chau, N., Dulinski, M., Jodlowski, P., Nowak, J., Rozanski, K., Sleziak, M., et al., 2011. Natural radioactivity in groundwater--a review. Isotopes in Environmental and Health Studies 47, 415–437.

Duran, A., Tuzen, M., Soylak, M., 2014. Assessment of trace metal concentrations in muscle tissue of certain commercially available fish species from Kayseri, Turkey. Environmental Monitoring and Assessment 186, 4619–4628.

Duysak, Ö., Ersoy, B., Dural, M., 2013. Metal Concentrations in Different Tissues of Cuttlefish (Sepia officinalis) in İskenderun Bay, Northeastern Mediterranean. Turkish Journal of Fisheries and Aquatic Sciences 13 205–210.

Đeđibegović, J., Larssen, T., Skrbo, A., Marjanović, A., Sober, M., 2012. Contents of cadmium, copper, mercury and lead in fish from the Neretva river (Bosnia and Herzegovina) determined by inductively coupled plasma mass spectrometry (ICP-MS). Food Chemistry 131, 469–476.

Elnabris, K.J., Musyed, S.K., El-Asar, N.M., 2013. Heavy metal concentrations in some commercially important fishes and their contribution to heavy metals exposure in Palestinian people of Gaza strip (Palestine). Journal of the Association of Arab Universities for Basic and Applied Sciences 13, 44–51.

Engel, D.W., Brouwert, M., 1986. Cadmium and copper metallothioneins in the American Lobster, Homarus americanus. Environmental Health Perspectives 65, 87–92.

European Union Commission Regulation (EC) No 1881/2006. Maximum levels for certain contaminants in foodstuffs. Official Journal of the European Union L 364, 5–24, 2006.

Fathabadi, N., Salehi, A.A., Naddafi, K., Kardan, M.R., Yunesian, M, Nodehi, R.N., et al., 2017. Radioactivity levels in the mostly local foodstuff consumed by residents of the high level natural radiation areas of Ramsar, Iran. Journal of Environmental Radioactivity 169-170, 209–213.

Galimberti, C., Corti, I., Cressoni, M., Moretti, V.M., Menotta, S., Galli, U., Cambiaghi, D., 2016. Evaluation of mercury, cadmium and lead levels in fish and fishery products imported by air in North Italy from extra-European Union Countries. Food Control 60, 329–337.

Görür, K.F. Keser, R., Akcay, N., Dizman, S., 2012. Radioactivity and heavy metal concentrations of some commercial fish species consumed in the Black Sea Region of Turkey. Chemosphere 87, 356–361.

Gül, A., Yilmaz, M., Benzer, S., Taşdemir, L., 2011. Investigation of zinc, copper, lead, and cadmium accumulation in the tissues of *Sander lucioperca* (L., 1758) living in Hirfanlı Dam Lake. The Bulletin of Environmental Contamination and Toxicology 87, 264–266.

HelgiLibrary. Available: <https://www.helgilibrary.com/indicators/fish-consumption-per-capita/serbia/> (Accessed 10 January 2019)

ICRP, 1995. Age-dependent Doses to the Members of the Public from Intake of Radionuclides - Part 5 Compilation of Ingestion and Inhalation Coefficients. ICRP Publication 72. Ann. ICRP 26 (1)

ICRP, 2012. Compendium of Dose Coefficients based on ICRP Publication 60. ICRP Publication 119. Ann. ICRP 41(Suppl.)

Joint FAO/WHO Food Standards Programme Codex Committee on Contaminants in Foods Fifth Session the Hague, the Netherlands, 21 - 25 March 2011. Working document for information and use in discussions related to contaminants and toxins in the GSCTFF

Langston, W.I., 1990. Toxic effects of metals and the incidence of marine ecosystems. In: Furness, R.W, Rainbow, P.S.(Eds.), Heavy Metals in the Marine Environment. CRC Press, New York, pp. 256.

Liang, H., Wu, W.-L., Zhang, Y.-H., Zhou, S.-J., Long, C.-Y., Wen, J., et al., 2018. Levels, temporal trend and health risk assessment of five heavy metals in fresh vegetables marketed in Guangdong Province of China during 2014–2017. Food Control 92,107–120.

Linsley, G., Sjöblom, K.-L., Cabianca, T., 2004. Overview of the point sources of anthropogenic radionuclides in the oceans. In: Livingston, H.D. (Ed.), Marine radioactivity. Radioactiv. Environm Vol, pp. 6.

Liu, J.L., Xu, X.R., Ding, Z.H., Peng, J.X., Jin, M.H., Wang, Y.S., et al., 2015. Heavy metals in wild marine fish from South China Sea: levels, tissue- and species-specific accumulation and potential risk to humans. Ecotoxicology 24, 1583–1592.

Liu, Y., Liu, G., Yuan, Z., Liua, H., Lam P.K.S., 2018. Heavy metals (As, Hg and V) and stable isotope ratios ($\delta^{13}C$ and $\delta^{15}N$) in fish from Yellow River Estuary, China. Science of the Total Environment 613-614, 462–471.

Łuczyńskaa, J, Paszczyk, B., Łuczyński M.J., 2018. Fish as a bioindicator of heavy metals pollution in aquatic ecosystem of Pluszne Lake, Poland, and risk assessment for consumer's health. Ecotoxicology and Environmental Safety 153, 60–67.

Marković, J., Joksimović, D., Stanković, S., 2012. Trace concentrations in wild mussels from the coastal area of the southeastern Adriatic, Montenegro. Archives of Biological Science Belgrade 64, 265–275.

Martorell, I., Perello, G., Martì-Cid, R., Llobet, J. M., Castell, V., Domingo, J. L., 2011. Human exposure to arsenic, cadmium, mercury, and lead from foods in Catalonia, Spain: temporal trend. Biological Trace Element Research 14, 309–322.

Mataš in, Ž ., Ivanuš ić , M., Oreš ć anin, V., Nejedli, S., Talk Gajger, I., 2011. Heavy metal concentrations in predator fish. Journal of Animal and Veterinary Advances 10, 1214–1218.

Noël, L., Chekri, R., Millour, S., Merlo, M., Leblanc, J.L., 2013. Distribution and relationships of As, Cd, Pb and Hg in freshwater fish from five French fishing areas. Chemosphere 90, 1900–1910.

Núñez, R., García, M.Á., Alonso, J., Melgar, M.J., 2018. Arsenic, cadmium and lead in fresh and processed tuna marketed in Galicia (NW Spain): Risk assessment of dietary exposure. Science of the Total Environment 627, 322–331.

Olmedo, P., Pla, A., Hernández, A.F., 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. Environment International 59, 63–72.

Pappa, F.K., Tsabaris, C., Loannidou, A., Patiris, D.L., Kaberi, H., Pashalidis, I., et al., 2016. Radioactivity and metal concentrations in marine sediments associated with mining activities in Ierissos Gulf, north Aegean Sea, Greece. Applied Radiation and Isotopes 116, 22–33.

Pastorelli, A.A., Morelli, S., Baldini, M., Stacchini, P., Baldini, G., Morelli, S., et al., 2012. Human exposure to Pb, Cd and Hg through fish and seafood product consumption in Italy: a pilot evaluation. Food Additives & Contaminants: Part A 29, 1913–1921.

Ruelas-Inzunza, J., Soto-Jiménez, M., Ruiz-Fernández, A., Bojórquez-Leyva, H., Pérez-Bernal, H., Páez-Osuna, F., 2012. ^{210}Po activity and concentrations of selected trace elements (As, Cd, Cu, Hg, Pb, Zn) in the muscle tissue of tunas Thunnus albacares and Katsuwonus pelamis from the Eastern Pacific Ocean. Biological Trace Element Research 149, 371–376.

Sankar, T.V., Zynudheen, A.A., Anandan, R., Viswanathan Nair, P.G., 2006. Distribution of organochlorine pesticides and heavy metal residues in fish and shellfish from Calicut region, Kerala, India. Chemosphere 65, 583–590.

Serbian Regulation, 2011. The rules on the quantities of pesticides, metals and metaloids and other substances, chemiotherapeutics, anabolic and other substances that can be determined in foodstuffs. Off. Gazette of RS, 5/92, 11/92, 32/2002, 25/2010, and 28/2011

Serbian Regulation, 2013. The rules of granting of radionuclide content in water for drinks, living facilities, foodstuffs, medicines, general uses, construction materials and other goods to be marketed. Off. Gazette of RS 86/2011 and 97/2013.

Storelli, M.M., Marcotrigiano, G.O., 1999. Cd and total Hg in some cephalopods from the south Adriatic Sea (Italy). Food Additives and Contaminants 16, 262–265.

Storelli, M.M., Stuffler, R.G., Marcotrigianoi, G.O., 2003. Polycyclic aromatic hydrocarbons, polychlorinated biphenyls, chlorinated pesticides (DDTs), hexachlorocyclohexane, and hexachlorobenzene residues in smoked seafood. Journal of Food Protection 66, 1095–1099.

Storelli, M.M., Barone, G., Cuttone, G., Giungato, D., Garofalo, R., 2010. Occurrence of toxic metals (Hg, Cd and Pb) in fresh and canned tuna: public health implications. Food and Chemical Toxicology 48, 3167–3170.

Storelli, M.M., Normanno, G., Barone, G., Dambrosio, A., Errico, L., Garofalo, L., 2012. Toxic metals (Hg, Cd, and Pb) in fishery products imported into Italy: suitability for human consumption. Journal of Food Protection 75, 189–194.

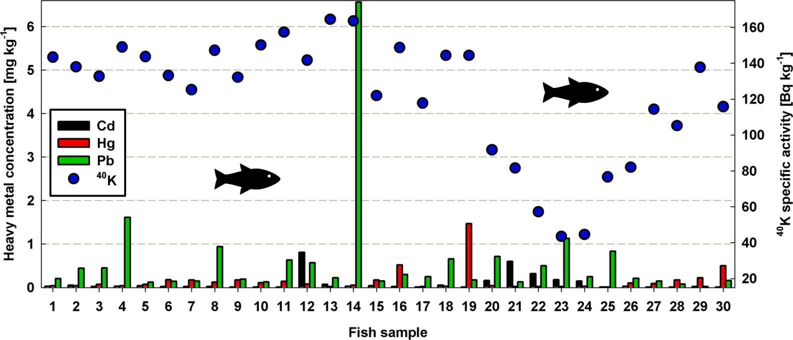
Tchounwou, P.B., Yedjou, C.G., Patlolla, A.K., Sutton, D.J., 2012. Heavy metal toxicity and the environment. EXS. 101, 133–164.

USEPA, 2000. Agency, U.S.E.P. Supplementary guidance for conducting health risk assessment of chemical mixtures.

Yi, Y., Tang, C., Yi, T., Yang, Z., Zhang, S., 2017. Health risk assessment of heavy metals in fish and accumulation patterns in food web in the upper Yangtze River, China. Ecotoxicology and Environmental Safety 145, 295–302.

Highlights

- Radioactivity and heavy metal concentrations in fish and seafood were investigated.
- The health risks were evaluated via ingestion dose, MTWI, EDI, and THQs.
- The results indicate no significant radiological health hazards.
- Frequent intake of fish may pose health risks due to Hg and Pb contamination.



Heavy metal concentration [mg kg^{-1}]
^{40}K specific activity [Bq kg^{-1}]
Fish sample
Cd
Hg
Pb
^{40}K
0
1
2
3
4
5
6
20
40
60
80
100
120
140
160
1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20 21 22 23 24 25 26 27 28 29 30