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Determination of heavy metals in different fish species sampled from markets in Croatia and possible health effects

Determiniranje teških metala u različitim vrstama riba uzorkovanih na hrvatskim tržnicama i mogući utjecaj na zdravlje

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Summary

The purpose of this study was to determine and to compare the amount of heavy metals in the meat of white (N = 39) and blue (N = 35) sea fish, considering the spring (N = 38) and fall (N = 36) catch period to study the possible type and seasonal differences especially in Hg concentrations in fish, and to estimate if the amounts registered can have a harmful effect on consumer health. The fish were chosen as an indicator of eco-system contamination with mercury and other heavy metals. The amount of heavy metals, lead (Pb) and cadmium (Cd), in both groups was lower than the Highest Permissible Amounts regulated by law in Croatia and the European Union. Mercury (Hg) exceeded the maximum allowed value in just a number of samples 3/74 (0.04%), while it is relevant for arsenic (As) to be present in almost any sample, although it has simultaneously not been regulated by law. The statistically significant value of arsenic exists in blue fish during the spring period, and the amount of mercury is higher in white fish compared to blue fish. Also a statistically significant difference in mercury levels was recorded during fall compared to spring,. Given the dietary habits and frequent fish consumption in Croatia, there is no risk of harmful health effects.

Key words: heavy metals, sea fish, Croatia, health effects, seasonal variation, mercury, cadmium

Sažetak

Cilj ovoga rada bio je odrediti i usporediti količinu teških metala u 74 uzorka bijele (N = 39) i plave ribe (N = 35), uzorkovanih u proljetnom (N = 38) i jesenskom razdoblju (N = 36), kako bi se istražila moguća razlika u koncentracijama teških metala, posebice žive, ovisno o sezoni ulova ili tipu ribe i procijenilo mogu li determinirane količine imati štetnih utjecaja na zdravlje potrošača. Riba je izabrana, budući da je ona indikator zagađenja teškim metalima u eko sustavu. Količina teških metala, olova (Pb) i kadmija (Cd), u obje grupe uzoraka, bila je niža od maksimalnih dopuštenih koncentracija prema hrvatskih i europskim zakonima. Količina žive (Hg) bila je više od maksimalnih dopuštenih koncentracija u vrlo malom broju uzoraka 3/74 (0,04%). Međutim, u uzrocima su zabilježene relevantne količine arsena (As), iako količine arsena nisu regulirane hrvatskim propisima. Multivarijatna statistička obrada (neparametrijski Mann-Whitney U test) pokazala je statistički značajne razlike u količini arsena u proljetnom razdoblju u odnosu na jesensko razdoblje, kao što je i izmjerena količina žive statistički značajno bila viša u bijeloj u odnosu na plavu ribu, te ona izmjerena u jesenskom razdoblju u odnosu na proljetno. Uzevši, međutim, u obzir potrošačke navike i učestalost konzumacije ribe u Hrvatskoj, izmjerene koncentracije nisu povezane s rizikom štetnih posljedica na zdravlje potrošača.

Ključne riječi: teški metali, morska riba, Hrvatska, zdravstveni učinci, sezonske varijacije, živa, kadmij

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Introduction

Today's nutrition has a tendency to be based on sea fish, as one of the basic foods of a healthy diet. With that in mind, studies of the quantity of heavy metals in fish are of great importance because the assessment of heavy metal intake in humans can be made only based on these studies. In the midst of growing and intensifying use of heavy metals in modern industrial production and urban pollution, which contribute to the contamination of our environment, heavy metals can, apart from meadows and gardens of large cities, contaminate soil, water and air, and enter the food chain.[1,2]

There are over 20 known different heavy metals which can cause a number of harmful effects to human health.[3] With the introduction of fish in daily consumption and heavy metal accumulation in tissues of sea animals, numerous studies follow heavy metal presence in different types of fish for the purpose of assessing their effects on human health.[4-8] Today, the most dangerous heavy metals are thought to be mercury (Hg), arsenic (As), lead (Pb) and cadmium (Cd).

The symptoms of exposure to these metals in high concentrations or in acute poisoning can be various, depending on the type and concentration of heavy metal and also the mode and duration of exposure. Lead (Pb) can lead to a series of health issues including convulsions, coma, kidney failure and even death due to long term exposure.[9]

Mercury (Hg) is one of the most toxic heavy metals in the environment, including lithosphere, hydrosphere and atmosphere. It is known that humans are primarily exposed to mercury or methylmercury by fish and sea mammal consumption.[10] Mercury is five times more dangerous than lead, while methylmercury is 50 times more dangerous compound than elementary mercury, thus being the most important type of mercury in the environment which accumulates in animals and humans. Furthermore, methylmercury is largely responsible for the accumulation of mercury in organisms (bioaccumulation) and the transfer of mercury from one trophic level to another (biomagnification).[11] Out of all types of food, the marine organisms in particular contain the highest levels of methylmercury.[12] Moreover, by frequent consumption of large long-lived fish that are at the top of the food chain (shark, tuna), humans may significantly increase the intake of mercury in the body.[10]

Cadmium (Cd) is an industrial toxin whose toxicology studies began intensively at the half of the last century. In the beginning, it accumulates in the kidneys tied to methalothioneines creating a complex, cadmium-methalothionein, of low molecular mass. The complex is harmless in low concentrations, but it becomes toxic when its concentration in the kidney elevates.[13] Toxic effects of metals are mostly associated with the influence on the brain and the kidneys, but some metals like arsenic (As) can have carcinogenic effects. Acute arsenic poisoning occurs mostly after ingestion of contaminated food, and is manifested by gastrointestinal disorders, stomach convulsions, regurgitation, heart problems, anemia, headaches, dizziness, convulsions, and often coma.[14] Available data on the share of total arsenic in food show that fish and fish products, including algae, store the highest concentrations of arsenic and are the main sources of total arsenic for human population.[15]

Aiming at the furthermost possible protection of consumers' health in the Republic of Croatia, the Act on Contaminants (Official Gazette of the Republic of Croatia no. 39/13) imposed the obligation to implement the Commission Regulation (EC) no. 1881/2006 setting maximum levels for certain contaminants in foodstuffs, including all its amendments and supplements. The aforementioned Regulation and its amendments set the maximum allowed levels of lead (0.3 mg/kg), cadmium (0.05 mg/kg) and mercury (0.5 mg/kg) in fish, crabs and mussels, as well as in cephalopods.[16] Neither the aforementioned Act, nor the Regulation, set the maximum allowed levels of arsenic. However, although there are no legislatively set maximum allowed levels of arsenic in food at the European Union level, certain EU Member States have laid down their highest allowed levels in particular types of food by their national legislation.[17] But just to compare, the national legislation of Bosnia and Herzegovina settled maximum level of arsenic in fish to 2.00 mg/kg.[18]

Suitability for human consumption of different fish species, regarding heavy metal concentration, is assessed according to the Highest Permissible Amounts regulated by the European Union, which include acceptable daily and weekly intake of specific heavy metals.

The aim of this work was to determine, by using approved analytical methods, the amounts of heavy metals (Pb, Cd, As and Hg) in white and blue sea fish sampled at the fish markets during spring and fall of 2012 and 2013, and to establish whether the determined amounts can cause harmful effects to human health, considering the acceptable daily intake of specific metals and fish consumption. Also, the additional aim of the study was to investigate the possible seasonal changes in Hg concentrations in aquatic ecosystems investigated and documented in many studies aiming to describe seasonal patterns[19,20] having in mind that, on the other hand, some studies record no seasonal changes in Hg concentrations.[21] It is necessary to point out that in the marine environment, almost all of the mercury in the muscle of fish is methylated, making it the reason to measure the total amount of mercury presuming that we are measuring almost similar concentration of methyl-mercury that has a significant toxicological impact on human health.[22,23] Fish has been selected as an indicator of eco-system contamination with mercury.[24]

Materials and methods

Fish samples have been divided into blue and white sea fish. Sampled white sea fish included: European hake *(Merluccius merluccius)*, red scorpion fish *(Scorpaena scrofa)*, European sea bass *(Dicentrarchus labrax)*, gilt-head sea bream *(Sparus aurata)*, jack mackerels *(Trachurus trachurus)*, leaping mullet *(Liza saliens)*, conger *(Conger conger)*, bogue *(Boops boops)* and piper gurnard *(Trigla lyra*). Sampled blue sea fish included: Atlantic bluefin tuna *(Thunnus thynnus)*, European pilchard *(Sardina pilchardus)*, Atlantic mackerel *(Scomber scombrus)*, European sprat *(Sprattus sprattus)*, Atlantic bonito *(Sarda sarda)* and chub mackerel *(Scomber japonicus*).

Samples were collected during spring and fall of 2012 and 2013 from registered fish markets by random sampling, aiming to achieve representativeness. All of the samples were placed in cold containers and delivered to a laboratory at a temperature of up to +8ºC. They were stored frozen until analysis. To obtain credibility of the results regarding the amount of heavy metals in blue fish on the territory of Croatia, the amount of the sample was a minimal 1 kg according to the requirements of the Directive on collecting samples for heavy metal analysis.[25]

In the selected period, samples from 74 fish were taken in accordance with the Directive on collecting samples for heavy metal analysis of different areas.

Analyses were performed using suitable analytical techniques described in similar studies, using Direct Mercury Analyzer (DMO), Atomic Absorption Spectrometer (AAS model Perkin Elmer, USA, 2007) and Inductively Coupled Plasma Mass Spectrometer (ICP-MS model Perkin Elmer, USA, 2008).[26-28] Analysis of total mercury was conducted using Direct Mercury Analyzer (DMO).[29,30]

The samples were prepared by filleting fish, with previous removal of the scales, gut and bones. The extracted muscle was homogenized. The sample was not specially prepared for the determination of total mercury. It was, instead, analyzed using Direct Mercury Analyzer. The amount of mercury in the analyzed samples was isolated from the sample in the oxygenated decomposition furnace with controlled heating. The sample was dried and then thermally and chemically decomposed within the decomposition furnace. Flowing oxygen carried the decomposed product into the catalytic part of the furnace, where the oxidation was completed. Halogens and nitrogen/ sulfur oxides were trapped and the remaining decomposition products were then carried to an amalgamator that selectively traps mercury. After the system was flushed with oxygen to remove any remaining gases or decomposition products, the amalgamator was rapidly heated, releasing mercury vapor. Flowing oxygen carried the mercury vapor through absorbance cells positioned in the light path of a single wavelength atomic absorption spectro-photometer. Absorbance (peak value) was measured at 253.7 nm as a function of mercury concentration.

Operating conditions of DMA were: Wavelength: 253.7 nm, slit: 0.7 nm, sample mass: 0.1 – 0.5 g, mode of measuring: peak value.

Sample homogenization and microwave-assisted digestion in a mixture of nitric acid (HNO_3) and hydrogen peroxide (H_2O_2), preceded the quantification of lead, cadmium and arsenic. During the preparation process, 2.0 g of sample had to be weighed and 5.0 ml of nitric acid (65% p.a., Kemika, Croatia) and 1.0 ml of hydrogen peroxide (30% p.a., Kemika, Croatia) had to be added. A prepared sample underwent microwave heating in a closed system. The solution was quantitatively transferred to a volumetric flask of 50 ml and filled to the mark with ultra-clear water (Milli-Q Millipore, 18.2 MWcm-1) from which the identification and quantification of heavy metals was performed using Atomic Absorption Spectrometry (AAS) and Inductively Coupled Plasma Mass Spectrometer (ICP-MS). Operating conditions on AAS were (for lead): Wavelength: 283.3 nm, slit: 0.7 nm, sample volume: 20 μl, mode of measuring: peak surface area. Operating conditions of ICP-MS (for arsenic) were: m/z 74. 9216, argon flow rate: 0.84 l/min, mode of measuring: peak height.

Operating conditions of microwave furnace were: microwave strength: 0-1400 W, 15 min, temperature 20-220ºC, 1.400 W, 15 min, temperature 220ºC, 0 W, 40 min, 20ºC.

Mann-Whitney U test as the non-parametric alternative test to the independent sample t-test and IBM SPSS Statistics software version 21 were used for statistical analysis of heavy metal values in white and blue fish during different season periods.

Results and Discussion

The results of the study are shown in Tables 1-8. Tables 1 and 2 show an overview of the results for all analyzed samples of fish collected during spring and fall of 2012 and 2013.

The amount of lead in all fish samples collected in the spring period was lower than 0.05 mg/kg, cadmium values ranged from <0.01 to 0.02 mg/kg. The values of arsenic ranged from <0.05 to 12.9 mg/kg, which is also the highest determined value for arsenic in this study, and it was determined in the sample of white fish European hake (*Merluccius merluccius*). The amount of mercury in analyzed fish samples, collected in the spring period, ranged from <0.02 mg/kg to 3.40 mg/kg. The highest amount of mercury was determined in the sample of white fish conger (*Conger conger*).

The results of analyzed samples from fall period, when 36 samples were collected (18 samples of blue and 18 samples of white sea fish), showed lower values of heavy metals compared to the samples collected in the spring period. The values of lead ranged from <0.05 to 0.09 mg/kg, while the values of cadmium ranged from <0.01 to 0.03 mg/kg. The values of arsenic ranged from <0.05 to 8.34 mg/kg, and the values of mercury from <0.02 to 1.98 mg/kg.

Table 1 Amounts of heavy metals in samples of sea fish caught during spring 2012 and 2013 (N = 38)
Tablica 1. Iznosi teških metala kod morske ribe ulovljene u proljeće 2012. i 2013. (N = 38)

No *Br.*	Year *God.*	Species *Vrsta*	Amount of lead (mg/kg) *Iznos olova*	Amount of cadmium (mg/kg) *Iznos kadmiuma*	Amount of arsenic (mg/kg) *Iznos arsena*	Amount of mercury (mg/kg) *Iznos žive*
1	2012	European sea bass (*Dicentrarchus labrax*) *Europski lubin*	<0.05*	<0.01*	<0.05*	0.144
2	2012	Gilt-head sea bream (*Sparus aurata*) *Arbun zlatne glave*	<0.05*	<0.01*	<0.05*	0.108
3	2012	European hake (*Merluccius merluccius*) *Europski oslic*	<0.05*	<0.01*	<0.05*	0.118
4	2012	Atlantic mackerel (*Scomber scombrus*) *Atlantska skuša*	<0.05*	<0.01*	0.067	0.064
5	2012	European sea bass (*Dicentrarchus labrax*) *Europski lubin*	<0.05*	<0.01*	<0.05*	0.028
6	2012	Red scorpion fish (*Scorpaena scrofa*) *Crveni škrpun*	<0.05*	<0.01*	<0.05*	0.114
7	2012	Gilt-head sea bream (*Sparus aurata*) *Arbun zlatne glave*	<0.05*	<0.01*	<0.05*	0.085
8	2012	European sea bass (*Dicentrarchus labrax*) *Europski lubin*	<0.05*	<0.01*	<0.05*	0.033
9	2012	European pilchard (*Sardina pilchardus*) *Europska sardela*	<0.05*	<0.01*	0.132	0.067
10	2012	European sprat (*Sprattus sprattus*) *Europska papalina*	<0.05*	<0.01*	0.052	<0.02*

11	2012	European pilchard (*Sardina pilchardus*) *Europska sardela*	<0.05*	<0.01*	0.070	0.057
12	2012	European pilchard (*Sardina pilchardus*) *Europska sardela*	<0.05*	<0.01*	0.097	0.063
13	2012	Bluefin tuna (*Thunnus thynnus*) *Tuna plave peraje*	<0.05*	<0.01*	<0.05*	0.137
14	2012	European pilchard (*Sardina pilchardus*) *Europska sardela*	<0.05*	<0.01*	<0.05*	0.143
15	2012	Atlantic bonito (*Sarda sarda*) *Atlantska palamida*	<0.05*	<0.01*	<0.05*	0.055
16	2012	Jack mackerel (*Trachurus trachurus*) *Skuša Pacifika*	<0.05*	<0.01*	<0.05*	0.194
17	2012	European pilchard (*Sardina pilchardus*) *Europska sardela*	<0.05*	0.02	0.354	0.083
18	2012	Bogue (*Boops boops*) *Atlantski arbun*	<0.05*	<0.01*	<0.05*	0.121
19	2012	European hake (*Merluccius merluccius*) *Europski oslic*	<0.05*	<0.01*	<0.05*	0.988
20	2012	Conger (*Conger conger*) *Ugor*	<0.05*	<0.01*	<0.05*	**3.40****
21	2012	Gilt-head sea bream (*Sparus aurata*) *Arbun zlatne glave*	<0.05*	<0.01*	<0.05*	0.092
22	2012	Leaping mullet (*Liza saliens*) *Cipal*	<0.05*	<0.01*	<0.05*	0.183
23	2013	European pilchard (*Sardina pilchardus*) *Europska sardela*	<0.05*	<0.01*	0.74	0.06
24	2013	Atlantic mackerel (*Scomber scombrus*) *Atlantski škombar*	<0.05*	<0.01*	0.49	0.03
25	2013	European sea bass (*Dicentrarchus labrax*) *Europski lubin*	<0.05*	<0.01*	0.16	0.08
26	2013	European hake (*Merluccius merluccius*) *Europski oslić*	<0.05*	<0.01*	0.97	0.18
27	2013	Gilt-head sea bream (*Sparus aurata*) *Arbun zlatne glave*	<0.05*	<0.01*	0.17	0.12
28	2013	European pilchard (*Sardina pilchardus*)	<0.05*	<0.01*	1.27	0.05

		Europska sardela				
29	2013	European pilchard (*Sardina pilchardus*) *Europska sardela*	<0.05*	<0.01*	1.09	0.04
30	2013	European sea bass (*Dicentrarchus labrax*) *Europski lubin*	<0.05*	<0.01*	0.19	0.22
31	2013	European hake (*Merluccius merluccius*) *Europski oslic*	<0.05*	<0.01*	12.9	0.23
32	2013	Atlantic mackerel (*Scomber scombrus*) *Atlantski škombar*	<0.05*	0.01	0.74	0.04
33	2013	European hake (*Merluccius merluccius*) *Europski oslić*	<0.05*	<0.01*	0.49	<0.02*
34	2013	European hake (*Merluccius merluccius*) *Europski oslić*	<0.05*	<0.01*	9.05	0.24
35	2013	Chub mackerel (*Scomber japonicas*) *Klen*	<0.05*	0.01	1.31	0.17
36	2013	Gilt-head sea bream (*Sparus aurata*) *Arbun zlatne glave*	<0.05*	0.02	0.43	0.10
37	2013	European pilchard (*Sardina pilchardus*) *Europska sardela*	<0.05*	<0.01*	1.07	0.12
38	2013	European pilchard (*Sardina pilchardus*) *Europska sardela*	<0.05*	<0.01*	0.92	0.06
Value range			<0.05	<0.01 to 0.02	<0.05 to 12.9	<0.02 to 3.40

* levels under LOD / **razine ispod LOD*
** Hg values above EU Directive 1881/2006 / *** Hg razine iznad EU directive*
*** As values above Bosnia and Herzegovina national standards (no EU levels) / **** prema razinama iznad standarda Bosne i Hercegovine (bez EU razina)*

Table 2 Amounts of heavy metals in samples of sea fish caught during fall 2012 and 2013 (N = 36)
Tablica 2. Iznosi teških metala kod morske ribe ulovljene u jesen 2012. i 2013. (N = 36)

No *Br.*	Year *God.*	Species/*vrsta*	Amount of lead (mg/kg) *Iznos olova*	Amount of cadmium (mg/kg) *Iznos kadmiuma*	Amount of arsenic (mg/kg) *Iznos arsena*	Amount of mercury (mg/kg) *Iznos žive*	No *Br.*
1	2012	European sea bass (*Dicentrarchus labrax*) *Europski lubin*		<0.05*	<0.01*	<0.05*	0.154
2	2012	Gilt-head sea bream (*Sparus aurata*) *Arbun zlatne glave*		<0.05*	<0.01*	<0.05	0.075

3	2012	European pilchard (*Sardina pilchardus*) *Europska sardela*	<0.05*	0.013	0.100	0.026
4	2012	European sprat (*Sprattus sprattus*) *Europska sardela*	<0.05*	0.024	<0.05*	0.023
5	2012	European pilchard (*Sardina pilchardus*) *Europska sardela*	0.062	<0.01*	0.347	0.072
6	2012	European sprat (*Sprattus sprattus*) Europska sardela	0.07	<0.01*	0.144	0.087
7	2012	European pilchard (*Sardina pilchardus*) *Europska sardela*	0.09	<0.01*	0.377	0.048
8	2012	European hake (*Merluccius merluccius*) *Europski oslic*	<0.05*	<0.01*	0.089	0.140
9	2012	European sea bass (*Dicentrarchus labrax*) *Europski lubin*	<0.05*	<0.01*	0.05	0.041
10	2012	Gilt-head sea bream (*Sparus aurata*) *Arbun zlatne glave*	<0.05*	<0.01*	0.201	0.078
11	2012	Piper gurnard (*Trigla lyra*) *Lastavica*	<0.05*	<0.01*	0.450	0.286
12	2012	European sea bass (*Dicentrarchus labrax*) *Europski lubin*	<0.05*	<0.01*	<0.05*	0.090
13	2012	Gilt-head sea bream (*Sparus aurata*) *Arbun zlatne glave*	<0.05*	<0.01*	<0.05*	0.377
14	2012	European sea bass (*Dicentrarchus labrax*) *Europski lubin*	<0.05*	<0.01*	<0.05*	0.134
15	2012	Gilt-head sea bream (*Sparus aurata*) *Arbun zlatne glave*	<0.05*	<0.01*	<0.05*	0.101
16	2012	European pilchard (*Sardina pilchardus*) *Europska sardela*	<0.05*	0.024	0.097	0.094
17	2012	European pilchard (*Sardina pilchardus*) *Europska sardela*	<0.05*	0.016	0.246	0.080
18	2012	Bluefin tuna (*Thunnus thynnus*) *Tuna plave peraje*	<0.05*	<0.01*	0.296	**1.98** **
19	2012	Atlantic bonito (*Sarda sarda*) *Atlantska palamida*	<0.05*	<0.01*	<0.05*	0.079
20	2012	European pilchard (*Sardina pilchardus*) *Europska sardela*	<0.05*	<0.01*	0.225	0.083

21	2013	European pilchard (*Sardina pilchardus*) *Europska sardela*	0.08	0.02	**2.41*** **	0.07
22	2013	Bluefin tuna (*Thunnus thynnus*) *Tuna plave peraje*	<0.05*	0.01	0.94	**0.72** **
23	2013	European sea bass (*Dicentrarchus labrax*) *Europski lubin*	<0.05*	<0.01*	0.54	0.04
24	2013	European hake (*Merluccius merluccius*) *Europski oslic*	<0.05*	<0.01*	**7.46*** **	0.37
25	2013	European pilchard (*Sardina pilchardus*) *Europska sardela*	<0.05*	<0.01*	**3.03*** **	0.08
26	2013	European pilchard (*Sardina pilchardus*) *Europska sardela*	<0.05*	0.01	**2.46*** **	0.06
27	2013	European sprat (*Sprattus sprattus*) *Europska papalina*	<0.05*	0.02	1.86	<0.02*
28	2013	Gilt-head sea bream (*Sparus aurata*) *Arbun zlatne glave*	<0.05*	0.03	**2.25*** **	0.13
29	2013	Gilt-head sea bream (*Sparus aurata*) *Arbun zlatne glave*	0.05	0.02	**2.18*** **	0.13
30	2013	European sea bass (*Dicentrarchus labrax*) *Europski lubin*	<0.05*	<0.01*	**2.52*** **	0.16
31	2013	European pilchard (*Sardina pilchardus*) *Europska sardela*	<0.05*	<0.01*	**4.69*** **	0.10
32	2013	Leaping mullet (*Liza saliens*) *Cipal*	<0.05*	<0.01*	0.03	0.14
33	2013	Atlantic mackerel (*Scomber scombrus*) *Atlantski škombar*	<0.05*	<0.01*	**3.02*** **	0.04
34	2013	Atlantic mackerel (*Scomber scombrus*) *Atlantski škombar*	<0.05*	<0.01*	**2.81*** **	0.03
35	2013	European sea bass (*Dicentrarchus labrax*) *Europski lubin*	<0.05*	<0.01*	0.92	0.11
36	2013	European hake (*Merluccius merluccius*) *Europski oslic*	<0.05*	<0.01*	**8.34*** **	0.37
Value range			<0.05 to 0.09	<0.01 to 0.03	<0.05 to 8.34	<0.02 to 1.98

* levels under LOD / * *razine pod LOD*
** Hg values above EU Directive 1881/2006 / ** *Hg razine iznad EU directive*
*** As values above Bosnia and Herzegovina national standards (no EU levels) / *** *prema razinama iznad standarda Bosne i Hercegovine (bez EU razina)*

Individual values of determined heavy metals in white and blue sea fish are shown in Table 3 and Table 4, while mean values of lead, cadmium, arsenic and mercury are shown in Table 5. It is clear that there is almost no difference between the amount of lead in blue and white sea fish (≤0.05 mg/kg). The mean value of cadmium is slightly higher in blue fish (0.012 mg/kg) than in white fish (0.011 mg/kg). Mean values of arsenic differ between analyzed samples of blue and white sea fish. Arsenic levels in blue fish were 0.951 mg/kg, while in white fish they amounted to 1.291 mg/kg. Differences are also noticeable in mean values for mercury. In blue fish mercury levels are 0.141 mg/kg, and in white fish 0.249 mg/kg (Table 5).

Table 6 displays daily and weekly exposure to lead, cadmium, arsenic and mercury by consumption of white and blue sea fish, taking into account the records of daily and weekly fish consumption.

Table 3 Amounts of heavy metals in analyzed samples of white fish collected during 2012 and 2013 (N = 39)
Tablica 3. Iznosi teških metala kod analiziranih uzoraka bijele ribe 2012. i 2013. (N = 39)

No *Br.*	Year *God.*	Species/*vrsta*	Amount of lead (mg/kg) *Iznos olova*	Amount of cadmium (mg/kg) *Iznos kadmiuma*	Amount of arsenic (mg/kg) *Iznos arsena*	Amount of mercury (mg/kg) *Iznos žive*	No *Br.*
1	2012	European sea bass (*Dicentrarchus labrax*) *Europski lubin*		<0.05**	<0.01*	<0.05*	0.144
2	2012	Gilt-head sea bream (*Sparus aurata*) *Arbun zlatne glave*		<0.05*	<0.01*	<0.05*	0.108
3	2012	European hake (*Merluccius merluccius*) *Europski oslic*		<0.05*	<0.01*	<0.05*	0.118
4	2012	European sea bass (*Dicentrarchus labrax*) *Europski lubin*		<0.05*	<0.01*	<0.05*	0.028
5	2012	Red scorpion fish (*Scorpaena scrofa*) *Crveni škrpun*		<0.05*	<0.01*	<0.05*	0.114
6	2012	Gilt-head sea bream (*Sparus aurata*) *Arbun zlatne glave*		<0.05*	<0.01*	<0.05*	0.085
7	2012	European sea bass (*Dicentrarchus labrax*) *Europski lubin*		<0.05*	<0.01*	<0.05*	0.033
8	2012	Jack mackerel (*Trachurus trachurus*) *Skuša Pacifika*		<0.05*	<0.01*	<0.05*	0.194
9	2012	Bogue (*Boops boops*) *Atlantski arbun*		<0.05*	<0.01*	<0.05*	0.121
10	2012	European hake (*Merluccius merluccius*) *Europski oslic*		<0.05*	<0.01*	<0.05*	0.988
11	2012	Conger (*Conger conger*) *Ugor*		<0.05*	<0.01*	<0.05*	**3.40****
12	2012	Gilt-head sea bream (*Sparus aurata*) *Arbun zlatne glave*		<0.05*	<0.01*	<0.05*	0.092

13	2012	Leaping mullet (*Liza saliens*) *Cipal*	<0.05*	<0.01*	<0.05*	0.183
14	2013	European sea bass (*Dicentrarchus labrax*) *Europski lubin*	<0.05*	<0.01*	0.16	0.08
15	2013	European hake (*Merluccius merluccius*) *Europski oslic*	<0.05*	<0.01*	0.97	0.18
16	2013	Gilt-head sea bream (*Sparus aurata*) *Arbun zlatne glave*	<0.05*	<0.01*	0.17	0.12
17	2013	European sea bass (*Dicentrarchus labrax*) *Europski lubin*	<0.05*	<0.01*	0.19	0.22
18	2013	European hake (*Merluccius merluccius*) *Europski oslić*	<0.05*	<0.01*	**12.9*** **	0.23
19	2013	European hake (*Merluccius merluccius*) *Europski oslić*	<0.05*	<0.01*	0.49	<0.02*
20	2013	European hake (*Merluccius merluccius*) *Europski oslić*	<0.05*	<0.01*	**9.05*** **	0.24
21	2013	Gilt-head sea bream (*Sparus aurata*) *Arbun zlatne glave*	<0.05*	0.02	0.43	0.10
22	2012	European sea bass (*Dicentrarchus labrax*) *Europski lubin*	<0.05*	<0.01*	<0.05*	0.154
23	2012	Gilt-head sea bream (*Sparus aurata*) *Arbun zlatne glave*	<0.05*	<0.01*	<0.05*	0.075
24	2012	European hake (*Merluccius merluccius*) *Europski oslic*	<0.05*	<0.01*	0.089	0.140
25	2012	European sea bass (*Dicentrarchus labrax*) *Europski lubin*	<0.05*	<0.01*	0.05	0.041
26	2012	Gilt-head sea bream (*Sparus aurata*) *Arbun zlatne glave*	<0.05*	<0.01*	0.201	0.078
27	2012	Piper gurnard (*Trigla lyra*) *Lastavica*	<0.05*	<0.01*	0.450	0.286
28	2012	European sea bass (*Dicentrarchus labrax*) *Europski lubin*	<0.05*	<0.01*	<0.05*	0.090
29	2012	Gilt-head sea bream (*Sparus aurata*) *Arbun zlatne glave*	<0.05*	<0.01*	<0.05*	0.377
30	2012	European sea bass (*Dicentrarchus labrax*) *Europski lubin*	<0.05*	<0.01*	<0.05*	0.134

31	2012	Gilt-head sea bream (*Sparus aurata*) *Arbun zlatne glave*	<0.05*	<0.01*	<0.05*	0.101
32	2013	European sea bass (*Dicentrarchus labrax*) *Europski lubin*	<0.05*	<0.01*	0.54	0.04
33	2013	European hake (*Merluccius merluccius*) *Europski oslić*	<0.05*	<0.01*	**7.46*** **	0.37
34	2013	Gilt-head sea bream (*Sparus aurata*) *Arbun zlatne glave*	<0.05*	0.03	**2.25*** **	0.13
35	2013	Gilt-head sea bream (*Sparus aurata*) *Arbun zlatne glave*	0.05	0.02	**2.18*** **	0.13
36	2013	European sea bass (*Dicentrarchus labrax*) *Europski lubin*	<0.05*	<0.01*	**2.52*** **	0.16
37	2013	Leaping mullet (*Liza saliens*) *Cipal*	<0.05*	<0.01*	0.03	0.14
38	2013	European sea bass (*Dicentrarchus labrax*) *Europski lubin*	<0.05*	<0.01*	0.92	0.11
39	2013	European hake (*Merluccius merluccius*) *Europski oslić*	<0.05*	<0.01*	**8.34*** **	0.37
Value range			<0.05 to 0.05	<0,01 to 0.03	<0,05 to 12.9	<0.02 to 3.40

* levels under LOD / * *razine ispod LOD*
** Hg values above EU Directive 1881/2006 / ** *Hg razine iznad EU directive*
*** As values above Bosnia and Herzegovina national standards (no EU levels) / *** *prema razinama iznad standarda Bosne i Hercegovine (bez EU razina)*

Table 4 Amounts of heavy metals in analyzed samples of blue fish collected during 2012 and 2013 (N = 35)
Tablica 4. Iznosi teških metala kod analiziranih uzoraka plave ribe 2012. i 2013. (N = 35)

No *Br.*	Year *God.*	Sampling location *Mjesto uzimanja uzoraka*	Species *Vrsta*	Amount of lead (mg/kg) *Iznos olova*	Amount of cadmium (mg/kg) *Iznos kadmiuma*	Amount of arsenic (mg/kg) *Iznos arsena*	Amount of mercury (mg/kg) *Iznos žive*
1	2012	Atlantic mackerel (*Scomber scombrus*) *Atlantski škombar*		<0.05*	<0.01*	0.067	0.064
2	2012	European pilchard (*Sardina pilchardus*) *Europska sardela*		<0.05*	<0.01*	0.132	0.067
3	2012	European sprat (*Sprattus sprattus*) *Europska papalina*		<0.05*	<0.01*	0.052	<0.02*

4	2012	European pilchard (*Sardina pilchardus*) *Europska sardela*	<0.05*	<0.01*	0.070	0.057
5	2012	European pilchard (*Sardina pilchardus*) *Europska sardela*	<0.05*	<0.01*	0.097	0.063
6	2012	Bluefin tuna (*Thunnus thynnus*) *Tuna plave peraje*	<0.05*	<0.01*	<0.05*	0.137
7	2012	European pilchard (*Sardina pilchardus*) *Europska sardela*	<0.05*	<0.01*	<0.05*	0.143
8	2012	Atlantic bonito (*Sarda sarda*) *Atlantska palamida*	<0.05*	<0.01*	<0.05*	0.055
9	2012	European pilchard (*Sardina pilchardus*) *Europska sardela*	<0.05*	0.02	0.354	0.083
10	2013	European pilchard (*Sardina pilchardus*) *Europska sardela*	<0.05*	<0.01*	0.74	0.06
11	2013	Atlantic mackerel (*Scomber scombrus*) *Atlantski škombar*	<0.05*	<0.01*	0.49	0.03
12	2013	European pilchard (*Sardina pilchardus*) *Europska sardela*	<0.05*	<0.01*	1.27	0.05
13	2013	European pilchard (*Sardina pilchardus*) *Europska sardela*	<0.05*	<0.01*	1.09	0.04
14	2013	Atlantic mackerel (*Scomber scombrus*) *Atlantski škombar*	<0.05*	0.01	0.74	0.04
15	2013	Chub mackerel (*Scomber japonicus*) *Klen*	<0.05*	0.01	1.31	0.17
16	2013	European pilchard (*Sardina pilchardus*) *Europska sardela*	<0.05*	<0.01*	1.07	0.12
17	2013	European pilchard (*Sardina pilchardus*) *Europska sardela*	<0.05*	<0.01*	0.92	0.06
18	2012	European pilchard (*Sardina pilchardus*) *Europska sardela*	<0.05*	0.013	0.100	0.026
19	2012	European sprat (*Sprattus sprattus*) *Europska papalina*	<0.05*	0.024	<0.05*	0.023
20	2012	European pilchard (*Sardina pilchardus*) *Europska sardela*	0.062	<0.01*	0.347	0.072
21	2012	European sprat (*Sprattus sprattus*) *Europska papalina*	0.07	<0.01*	0.144	0.087

22	2012	European pilchard (*Sardina pilchardus*) *Europska sardela*	0.091	<0.01*	0.377	0.048
23	2012	European pilchard (*Sardina pilchardus*) *Europska sardela*	<0.05*	0.024	0.097	0.094
24	2012	European pilchard (*Sardina pilchardus*) *Europska sardela*	<0.05*	0.016	0.246	0.080
25	2012	Bluefin tuna (*Thunnus thynnus*) *Tuna plave peraje*	<0.05*	<0.01*	0.296	**1.98****
26	2012	Atlantic bonito (*Sarda sarda*) *Atlantska palamida*	<0.05*	<0.01*	<0.05*	0.079
27	2012	European pilchard (*Sardina pilchardus*) *Europska sardela*	<0.05*	<0.01*	0.225	0.083
28	2013	European pilchard (*Sardina pilchardus*) *Europska sardela*	0.08	0.02	**2.41*****	0.07
29	2013	Bluefin tuna (*Thunnus thynnus*) *Tuna plave peraje*	<0.05*	0.01	0.94	0.72
30	2013	European pilchard (*Sardina pilchardus*) *Europska sardela*	<0.05*	<0.01*	**3.03*****	0.08
31	2013	European pilchard (*Sardina pilchardus*) *Europska sardela*	<0.05*	0.01	**2.46*****	0.06
32	2013	European sprat (*Sprattus sprattus*) *Europska papalina*	<0.05*	0.02	1.86	<0.02*
33	2013	European pilchard (*Sardina pilchardus*) *Europska sardela*	<0.05*	<0.01*	**4.69*****	0.10
34	2013	Atlantic mackerel (*Scomber scombrus*) *Atlantski škombar*	<0.05*	<0.01*	**3.02*****	0.04
35	2013	Atlantic mackerel (*Scomber scombrus*) *Atlantski škombar*	<0.05*	<0.01*	**2.81*****	0.03
Value range			<0.05 to 0.091	<0.01 to 0.024	<0.05 to **4.69**	<0.02 to **1.98**

* levels under LOD / * *razine ispod LOD*
** Hg values above EU Directive 1881/2006 / ** *Hg razine iznad EU directive*
*** As values above Bosnia and Herzegovina national standards (no EU levels) / *** *prema razinama iznad standarda Bosne i Hercegovine (bez EU razina)*

Table 5 Mean values of lead, cadmium, arsenic and mercury in analyzed samples according to type of fish (expressed in mg/kg)
Tablica 5. Prosječne vrijednosti kadminuma, arsena i žive kod analiziranih primjeraka prema vrsti ribe (izraženi u mg/kg)

Type of sample *Vrsta uzorka*	Number of samples (N) *Broj uzoraka*	Mean value Pb (mg/kg) *Srednja vrijednost Pb*	Mean value Cd (mg/kg) *Srednja vrijednost Cd*	Mean value As (mg/kg) *Srednja vrijednost As*	Mean value Hg (mg/kg) *Srednja vrijednost Hg*
Blue fish *Plava riba*	35	<0.05*	0.012	0.951	0.141
White fish *Bijela riba*	39	0.05	0.011	1.291	0.249

* levels under LOD / * *razine ispod LOD*

Table 6 Daily and weekly exposures depending on fish species and intake of specific metals
Tablica 6. Dnevna i tjedan izloženost ovisno o vrsti ribe i ulaska specifičnih metala

Type of sample *Vrsta uzorka*	Fish consumption *Potrošnja ribe*	Mean value of Pb intake (μg) *Srednja vrijednostt Pb unosa*	Mean value of Cd intake (μg) *Srednja vrijednsot Cd unosa*	Mean value of As intake (μg) *Srednja vrijednost As unosa*	Mean value of Hg intake (μg) *Srednja vrijednost Hg unosa*
Blue fish *Plava riba*	g/day *Dan*	1	0.2	20	3
	g/week *Tjedan*	7	1.4	140	21
Type of sample *Vrsta uzorka*	g/day *Dan*	1	0.2	30	5
	g/week *Tjedan*	7	1.4	210	35

By statistical evaluation of data, using Mann-Whitney U test and IBM SPSS Statistics software, version 21, it was determined that the statistically significant value of arsenic exists in blue fish during the spring period and the amount of mercury is higher in white fish compared to blue fish, as seen in Table 7. During fall, a statistically significant difference in mercury levels was recorded and the results are displayed in Table 8.

According to the existing regulations, based on which the health suitability of fish is determined, the Highest Permissible Amounts exist for lead, cadmium and mercury,[17,18,25] while they were not determined for arsenic. The results of former studies point to the apparent presence of arsenic and lead in different fish species and their possible health effect on humans.[24-26] For that reasons, it is important to monitor the amount of heavy metals in fish so daily intake of heavy metals, based on fish consumption, could be determined and the health effects assessed.

Significant differences in heavy metal amounts (arsenic, lead, zinc and mercury) were also determined in other fish species like the Japanese threadfin bream (*Nemipterus japonicus*), dorab wolf-herring (*Chirocentrus dorab*), Emperor red snapper (*Lutjanus sebae*), tigertooth croaker (*Otolithes ruber*) and silver pomfret (*Pampus* argenteus).[3]

Higher levels of heavy metals were generally found in predatory species, which may be explained by their position in the food chain.

Low concentrations of lead (0.004 mg/kg) in marine fish (mackerel, anchovy, and tuna) were recorded by Olmedo et al.,[34] as well as Vulić et al. in sea bass and gilt poll, where the mean measured values ranged from 0.039-0.065 mg/kg, i.e. 0.090 to 0.134 mg/kg[35].

Table 7 Mann-Whitney U test for samples of fish collected during the spring period
Tablica 7. Mann-Whitney U test na uzorke ribe ulovljene u proljetnom razdoblju

	Mann-Whitney U	Z	P
Lead (mg/kg) *Olovo*	178.50	0.00	1.000
Cadmium(mg/kg) *Kadmium*	156.50	-1.21	0.523
Arsenic (mg/kg) *Arsen*	109.00	-2.12	0.042
Mercury (mg/kg) *Živa*	84.00	-2.77	0.005

Table 8 Mann-Whitney U test for samples of fish collected during the fall period
Tablica 8. Mann-Whitney U test na uzorke ribe ulovljene u jesenskom razdoblju

	Mann-Whitney U	Z	P
Lead (mg/kg) *Olovo*	133.000	-1.526	0.372
Cadmium(mg/kg) *Kadmium*	113.000	-1.964	0.126
Arsenic (mg/kg) *Arsen*	121.000	-1.304	0.203
Mercury (mg/kg) *Živa*	74.500	-2.769	0.005

EFSA specifies that the average lead concentration for all fish and food originating from the sea amounts to 0.0543 mg/kg, which is in compliance with the results of our research.[36]

Low concentrations of cadmium were established also in other researchers conducted on marine fish (0.001-0.009 mg/kg).[34,35]

Mason et al. established in their research there was no difference in the levels of arsenic between herbivore and carnivore types of fish, however they established huge differences in the concentration of arsenic in the fillets of fatter fish (herring) and less fat fish (codfish). They did not establish evidence in the same study on biomagnification since the arsenic concentrations in organisms showed a tendency of decline with the increase of trophic level.[37] The average concentration of total arsenic in the mixed sample of maritime and freshwater fish and other food of marine origin ranges from 0.100-1.8 mg/kg (SCOOP, 2004).[38] A relatively high range of arsenic (0.298-6.849 mg/kg) in marine fish sampled in the Adriatic was established by Vulić et al.[35] Multiple lower values and lesser range (0.025-0.561 mg/kg) in marine fish sampled in Spain were established by Olmedo et al.[34]

Higher concentrations of mercury, some of which exceed the maximum allowed concentrations, were recorded in predatory species, such as the tuna, shark, mackerel and conger,[16] whereas somewhat lesser values were reported by Martorell et al.[39]

This is also in compliance with the results of this research, in which the highest recorded levels of mercury were established precisely in predatory species conger and tuna.

Considering the daily consumption of fish in Croatia, which is in average 19.54 g per day,[40] or 7.13 kg per year, and the permissible weakly intake of heavy metals regulated by the European Union[25], we can estimate if the obtained results have a substantial contribution to the heavy metal intake. According to the results of this study, we can determine that by consuming fish, we take in arsenic the most from all the studied heavy metals. The amount of daily arsenic intake is 176.0 µg for white fish and 130 µg for blue fish, which is substantially lower than 223.6 µg determined in Spain.[41] The intake of mercury follows with 33.8 µg per day for white fish, and 19.2 µg per day for blue fish. The intake of cadmium is relatively low and amounts to 1.50 µg per day, for blue and white fish. The amounts of heavy metals recorded in this study do not contribute substantially to heavy metal intake and the assessed intakes of heavy metals do not exceed permissible weakly intakes. The potential problem exists only with arsenic intake, whose permissible weakly intake is not regulated. Whereas it is total arsenic and not anorganic arsenic, the obtained amounts will not have a negative effect on human health, other studies pointing to the same conclusions, obtained values being significantly higher.[42-44]

Conclusion

Considering the results, we can conclude that the presence of lead and cadmium was determined in the least amount of analyzed samples, whereas almost all samples contained arsenic and mercury. It is important to state that the number of samples exceeding the highest permissible amounts of heavy metals is insignificant in relation to the number of analyzed samples. It is also important to note and warn about the presence of arsenic and mercury in this particular food group described as extremely healthy and desirable in daily consumption. According to the fact that seasonal changes in the Hg concentrations in aquatic ecosystems have been investigated and documented in many studies aiming to describe seasonal patterns, by using the Mann-Whitney U test. This study has also confirmed seasonal differences as well as the differences that can be observed between blue and white sea fish. The statistically significant value of arsenic exists in blue fish during the spring period, and the amount of mercury is higher in white fish compared to blue fish. Also during the fall, a statistically significant difference in mercury levels was recorded.

Today's nutrition has a tendency to be based on sea fish, as one of the basic foods of a healthy diet. With that in mind, studies of the quantity of heavy metals in fish are of great importance for the purpose of assessing heavy metal daily intake and its possible health effects, which can be made based on the obtained results. According to this study, there is no excessive intake of heavy metals, and therefore there are no harmful health effects. Given that the study showed the presence of arsenic in fish, determination of maximum permissible amount of arsenic in this food group is recommended, as well as further studies of heavy metal presence in fish.

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