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DETERMINATION OF HEAVY METALS IN FRESH FISH

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Abstract: Consumption of fish has increased in last 50 years. Fish as a food is changing red meat because it has unsaturated fat and it is the best source of omega 3 fatty acids. Beside it is full of minerals, vitamins and it has high biological value of proteins.The content of heavy metals in the muscle tissue of fish is directly related to the pollution of the water they come from

The analysis of the content of heavy metals was done by the Institute of Public Health of the Federation of Bosnia and Herzegovina.The content of lead (Pb) in the tested samples of fresh fish ranged from 0.0015 to 0.0381 mg/kg. The measured content of cadmium (Cd) in the examined samples was in the range of $3.3*10^{-5}$ to 0.0053 mg/kg. The content of arsenic (As) in the tested samples ranged from 0.0085 to 1.1668 mg/kg. The mercury (Hg) content in the tested samples of fresh fish ranged from 0.0033 to 0.0991 mg/kg, which is within the allowed values prescribed by the Rulebook. It has been statistically proven that there is a significant difference in the measured values of lead, arsenic and cadmium in the samples of sea and freshwater fish.

Aim of this work was to establish do the samples of fresh fish contain concentration of heavy metals more than concentrations prescribed in Rule book about allowed amounts of certain contaminants in food. Thereby ten samples of fresh fish were tested, five samples of marine fish and five samples of freshwater fish. Results showed that all samples of fish satisfy allowed concentration of heavy metals according to the Rule book.

Keywords: Fresh fish (freshwater and marine fish), heavy metals, contaminants

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Introduction

Modern science has clearly defined the mutual conditioning of water contamination and the content of heavy metals in the organisms of its inhabitants, as well as the ways and routes of intake. It is known from the literature that aquatic organisms accumulate elements from water, even those found in traces. There are different mechanisms of accumulation, which depend both on the element that accumulates and on the organism that accumulates them from water, sediment, through food or contact with dispersed particles(Karahmet et al., 2020., Alić et al., 2004).

Fish as a food can be source of heavy metals such as: Cu, Zn, Cd, Hg and Pb. Contaminants in fish are coming uncontrollably from enviroment or they can be rests of testings which people were using in different phases of producing (Isaković et al., 2021). The intake of heavy metals is not always the result of human activity, but is also the cause of adsorption processes that occur due to the presence of natural soil ingredients (Šarkanj et al., 2010).

Intake of heavy metals, such as lead, copper, cadmium can be potentially toxic, and regular monitoring and monitoring of the factors that determine that intake is necessary. The intake of heavy metals is not always the result of human activity, but is also the cause of adsorption processes that occur due to the presence of natural soil ingredients, (Ashraf, 2006, Korjenić, 2008).

It is known from the literature that aquatic organisms accumulate elements from water, even those found in traces. There are different accumulation mechanisms, depending on the elementthat accumulates, as well as from the organism that accumulates them from water, sediment, through food or contact with dispersedparticles (Tomašević et al., 1988).

Also statistic analysis was done with aim of establishing of eventual differences of heavy metals concentrations between individual samples. Analysis showed existence statisticly important differences in content of heavy metals depends on fish species and their origin.

Material and methods

Ten samples were used to test the content of heavy metals in fresh fish. Five samples of marine fish and five samples of freshwater fish. From saltwater fish: blue hake (*Merluccius merluccius*), sardine (*Sardina pilchardus*), mackerel (*Scomber scombrus*), tuna (*Thunnus*) and Gilt-head seabream (*Sparus aurata*) were used, while samples of freshwater fish consisted of brown trout (*Salmo trutta* —

morpha fario), rainbow trout (*Oncorhynchus Mikiss, Linnaeus*), Carp (*Cyprinus Carpio*), Chub (*Leuciscus cephalus, Linnaeus 1758*) and Gibel carp (*Carassius gibelio*). All fish were delivered to the Institute of Public Health of the Federation of Bosnia and Herzegovina, where the analysis of heavy metals in fish was done.

Two methods were used to determine the content of heavy metals in fresh fish. For the determination of mercury, the Method for determining the content of mercury in fish and fish products was used on the direct mercury analyzer AMA 254, while the determination of the content of other metals (Pb, Cd, As) was done according to the Method for determining heavy metals in food by analysis on an atomic absorption spectrometer.

The prepared sample is transported to the weighing room. After weighing, the sample is transferred to the desiccator to the direct mercury analyzer AMA 254. After starting the program, it is necessary to wait 20 minutes for the device to stabilize. Before starting to analyze the sample, it is necessary to delete the previous blank values.

The analysis is divided into four parts (cleaning, blank test, analysis and final cleaning).

Initially, it is necessary to clean the vessel, which later serves to place the sample. As with any analysis, a blank reading is performed first. This procedure is performed a total of three times. After that, the container is removed with tweezers, placed on a scale, and 10 to 50 mg of the sample is weighed. The tray is returned to the apparatus and the reading follows.

This analytical method has been verified for a range of analyte concentrations: first concentration level: 0.10 ng/g to 27 ng/g, second concentration level: 100 ng/g 400.00 ng/g. For concentrations that are above the measurement range, sample dilutions are made to concentrations within the measurement range.

After preparing samples of a known concentration, they were analyzed by the method of atomic absorption spectroscopy on an atomic absorption spectrometer, SHIMADZU AA-6650.

Statistical data processing was performed with the aim of determining the existence of a statistical difference between the samples, and with the aim of determining the level of significance if the difference exists. A one-way analysis of variance (ANOVA) was used for the analysis, in order to determine statistical significance. When a statistically significant influence of the type/origin of fish on the content of heavy metals was determined, the LSD test was applied to

determine between which modalities there is a statistically significant difference. Student's test was used for statistical analysis of mercury.

Results

The highest content of lead (Pb) in freshwater fish, but also in comparison with sea water fish, had 0.0382 (mg/kg), while the lowest content was recorded in brown trout (0.0015 mg/kg). If sea water fish are observed individually, it can be seen that Gilt head sea bream had the lowest lead content of sea fish (0.0024 mg/kg), while mackerel had the highest lead content (0.0088 mg/kg). The following tables 1 and 2 shows the values of lead (Pb) content in the tested fish samples. By reviewing the average values of the content of lead in the tested samples of fresh fish, it can be concluded that all the tested samples meet the requirements prescribed by the Ordinance, given that the amount of lead in none of the tested samples is higher than the maximum allowed value of 0.3 mg/kg.

Table 1. The content of lead in marine fish

Content Pb	I control (mg/kg)	II control (mg/kg)	III control (mg/kg)	Mean value
Blue hake	0,0037	0,0017	0,0026	0,0027
Gilt head seabream	0,0035	0,0013	0,0024	0,0024
Sardines	0,0036	0,0016	0,0024	0,0025
Mackerel	0,0109	0,0067	0,0088	0,0088
Tuna	0,0044	0,0016	0,003	0,0030

Table 2. The content of lead in freshwater fish

Content Pb	I control (mg/kg)	II control (mg/kg)	III control (mg/kg)	Mean value
Rainbow trout	0,0060	0,0043	0,0051	0,0051
Brown trout	0,0004	0,0029	0,0013	0,0015
Carp	0,006	0,0016	0,004	0,0039
Chub	0,0403	0,0359	0,0383	0,0382
Gibel carp	0,0118	0,0098	0,0106	0,0107

In the research conducted by Alić et al., (20024), the lead content in trout is equal to the results obtained in this research. That is, the content of lead (Pb) in the muscle tissue of fish (mg/kg) from the Una, Vrbas and Drina basins was 0.32 mg/kg, 0.35 mg/kg, 0.57 mg/kg, with the fact that 20 .0 g of sample, while in this research 0.5-1.5 g of sample was used.

Tables 3 and 4 show that mackerel had the highest cadmium content, while carp had the lowest content. If we look only at marine fish, mackerel had the highest cadmium content, while Gilt head sea bream had the lowest cadmium content. Of the freshwater fish, the highest content had Chub, while the lowest content was carp. By reviewing the average values of the cadmium content in the tested samples of fresh fish, it can be concluded that all the tested samples meet the requirements prescribed by the Ordinance, given that the amount of cadmium in none of the tested samples is higher than the maximum allowed value of 0.05 mg/kg, i.e. 0 ,10 mg/kg for mackerel and tuna, and 0.30 mg/kg for sardine.

Table 3. The content of cadmium in marine fish

Content Cd	I control (mg/kg)	II control (mg/kg)	III control (mg/kg)	Mean value
Blue hake	0,0048	0,0048	0,0048	0,0048
Gilt head seabream	0,0002	0,0001	0,0002	0,0002
Sardines	0,0033	0,0036	0,0034	0,0034
Mackerel	0,0051	0,0055	0,0053	0,0053
Tuna	0,0026	0,0027	0,0027	0,0027

Table 4. The content of cadmium in freshwater fish

Content Cd	I control (mg/kg)	II control (mg/kg)	III control (mg/kg)	Mean value
Rainbow trout	0,0002	0,0001	0,0001	0,00013
Brown trout	0,0000	0,0001	0,0001	0,00006
Carp	0,0000	0,0001	0,0000	0,00004
Chub	0,0009	0,0009	0,0009	0,00091
Gibel carp	0,0003	0,0005	0,0004	0,00038

Sardine had the highest arsenic content (1,1668 mg/kg), while carp had the lowest content (0,008519 mg/kg). If we look only at marine fish, sardines had the highest arsenic content (1,1668 mg/kg), while tuna had the lowest arsenic content (0,0197 mg/kg). Of the freshwater fish, brown trout had the highest content (0,066375 mg/kg), while carp had the lowest content (0,008519 mg/kg).

Table 5. The content of Arsenic in marine fish

Content Cd	I control (mg/kg)	II control (mg/kg)	III control (mg/kg)	Mean value
Blue hake	0,4998	0,4985	0,4992	0,4992
Gilt head seabream	0,1177	0,1218	0,1198	0,1198
Sardines	1,1991	1,1345	1,1668	1,1668
Mackerel	0,3032	0,2885	0,2958	0,2958
Tuna	0,0176	0,0220	0,0195	0,0197

Table 6. The content of Arsenic in freshwater fish

Content Cd	I control (mg/kg)	II control (mg/kg)	III control (mg/kg)	Mean value
Rainbow trout	0,0095	0,0210	0,0156	0,015352
Brown trout	0,0589	0,0740	0,0662	0,066375
Carp	0,0052	0,0118	0,0085	0,008519
Chub	0,0253	0,0405	0,0329	0,032893
Gibel carp	0,0200	0,0574	0,0387	0,038681

Rulebook, given that the amount of arsenic in none of the tested samples is higher than the maximum allowed value of 2.0 mg/kg, i.e. 4.0 mg/kg for white sea fish, and 8.0 mg/kg for tuna. Therefore, all tested samples can be used in food without fear of arsenic poisoning.

Table 7. The content of Mercury in all samples

Content Hg	Measured value (mg/kg)
Blue hake	0,09919
Gilt head seabream	0,06033
Sardines	0,05009
Mackerel	0,07886
Tuna	0,09221
Rainbow trout	0,01509
Brown trout	0,02717
Carp	0,09881
Chub	0,00334
Gibel carp	0,08776

By reviewing the mercury content values obtained in the tested samples of fresh fish, it can be concluded that all the tested samples meet the requirements prescribed by the Ordinance, given that the amount of mercury in none of the tested samples is higher than the maximum allowed value of 0.5 mg/kg for muscle meat fish, i.e. 1.0 mg/kg for tuna.

Conclusion

The obtained results, on the basis of which conclusions can be drawn, showed that the lead(Pb) content in the tested samples of fresh fish ranged from 0.0015 to 0.0381 mg/kg, which is within the allowed values prescribed by the Rulebook.The content of cadmium (Cd), arsenic (As) and mercury (Hg) was within the limits allowed by the regulations.Since the statistical analysis of the experimental data showed the existence of statistically significant differences in the content of heavy metals depending on the habitat of the fish, it can be concluded that the habitat of the fish affects the content of heavy metals in the fish. Therefore, in addition to differences between marine and freshwater fish in the content of heavy metals, it has been noted that there are also differences within "one" habitat. This may be due to the proximity of roads, industry, etc.

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