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FISH MEAT CONTAMINATION WITH HEAVY METALS – A REAL CONCERN FOR THE FOOD CONSUMPTION

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Abstract

As a result of the increasingly concern of people regarding the food safety, different public media focused on the data collected from reference laboratories inducing a false perception regarding the threat of sea food consumption. Starting with the Minamata disaster more and more people became worried about the sea water pollution with heavy metals. Using the reference data of Commission Regulation (EC) No 1881/2006 setting maximum levels for certain contaminants in foodstuffs and the Codex Alimentarius, 21 fish species from sea water or freshwater were studied for Cd, Mg and Pb. The data were collected from specialized laboratories during the following years: 2016 and 2017. The obtained results were in the acceptable limits for European standards. For 67 of 110 samples the value of LOD/LOQ could not be determined. These data are correlated with the water tests made in the last years, which shows a general decrease of the heavy metal pollution in sea waters.

Key words*: food safety, pollution, water.*

INTRODUCTION

Metals such as Fe, Zn, Cu, Mn are chemical elements indispensable to aquatic organisms acting as essential catalysts in metabolic processes (Canli and Atli, 2003). On the other hand, non-essential metals such as Pb, Hg and Cd, which have no vital role for these organisms, in excessive levels, in ecotrophic systems can lead to serious health problems in humans.

Extractive activities, coal-fired power plants, deforestation, animal farming are sewage are sources of release of these metals into the environment (WHO, 1996).If heavy metals enter in the food chain, especially in the aquatic species, they can be harmful to human health (Goyer, 1997; Copat, 2012; Weiss, 2014). Most vulnerable are the young organisms: fetuses, babies, preschoolers (Giles, 1988; Waalkes, 2000; El-Moselhy, 2014).

The levels of heavy metal accumulation in fish depend on the growth rate, metabolism, feeding pattern and ecological requirements of a given fish species (Uluozlu, 2007; Tüzen, 2003). Taking into account the increasing demand for fish meat-imports in Romania have increased tenfold in the last fifteen years-large quantities of fish and seafood have been brought from the waters adjacent to Asian countries with urban and industrial explosion (Noor, 2014; Rohasliney, 2014).

These are the geographical areas most exposed to heavy metal contamination. As a consequence, the attention of consumers to food safety increases as the information media are presented on this topic.

MATERIALS AND METHODS

Muscle tissue of fish (dorsal muscle) was used in this study because it is the major target tissue for metal storage and is the most edible part of the fish. The study was followed in two years, 2016 and 2017, on 14 species of fish from sea waters or inland waters. For year 2016 the included species are listed in the table: *Acipenser gueldenstaedtii, Cyprinus carpio,*

Gadus macrocephalus, Hypophthalmichthys molitrix, Liza aurata, Merluccius merluccius, Pangasius pangasius, Salmo salar, Salmo trutta, Sander lucioperca, Sardina pilchardus, Scomber scomber, Sparus aurata. For year 2017 the included species are listed in the table: *Acipenser gueldenstaedtii, Alosa immaculata, Clupea harengus, Cyprinus carpio, Dicentrarchus labrax, Esox lucius, Gadus macrocephalus, Hypophthalmichthys molitrix, Hypophthalmichthys nobilis, Liza aurata, Salmo salar, Salmo trutta, Sander lucioperca, Sardina pilchardus, Scomber scomber, Sparus aurata, Thunnus albacares.*

A total of 72 fish were analized using the Analytical Methods for Atomic Absorption Spectrometry with graphite furnace for Pb and Cd (Kito, 1986; Endo, 1995) and Cold Vapor Atomic Absorption Spectroscopy or CVSAA for Mg. The Thin Layer Chromatography or TLC, which is an old method, was used for economical reasons, as it is known to be a money saving analyses.

The mercury content was also determined by SAACV technique.

The AAS spectrometer measures the absorbance of a component-specific (HCL, EDL) absorber that is directly proportional to the metal concentration in the sample and read on the calibration curve previously plotted. CVSAA - Atomic Absorption Spectrophotometry is based on the determination of the concentration of a chemical element in the sample to be analyzed (Perkin, 1996). The samples subjected to the analysis were properly processed (chopping, crushing, homogenization) so as to achieve a uniform and homogeneous mass according to SR EN 13804/2013. Any kind of impurification were excluded during processing. The sample thus processed was stored in tightly closed plastic bags. The second step was the mineralization of samples. A suitable amount of sample, between 0.2-1.0 grams was placed in the reaction vessel and added 6 ml concentrated nitric acid and 2 ml hydrogen peroxide. A mercury / or MRC test and a blank test of the reagents was performed. After the reaction was stabilized (about 20 minutes), the reaction vessel was closed well and placed in the digester (Wet Mineralization Equipment with Pressure and Controlled Temperature). At the end of the program, the reaction vessels were allowed to cool in the oven for 20 minutes. The extract was carefully filtered into graduated tubes, yielding an appropriate volume depending on the matrix.

The digestion vessels were washed, decontaminated with 10% v / v soil nitric acid and passed through a short digestion program for additional decontamination. From these sample solution thus obtained there were also determined the elements: Cd, Pb.

For determination of mercury with SAACV it was drawn the curve representing absorbance obtained by concentration.

The clean porcelain crucible dried and stored in the desiccator was tilted to the analytical balance, then a suitable amount of sample was placed in it and weighed again. All weightings were performed with an accuracy of 10 mg and noted in the work book. For high-water matrices, pre-drying is at the oven.

The product crucibles or capsules were placed on the asbestos screen or on the lonely triangle and burn to the small flame of the cooker to the charcoal stage. The resulting ash was uniform in color (white or gray) and no more black coal. In parallel, a blank test was prepared, with mineralization reagents and a sample enriched with analytes to be detected.

For determination of mercury with SAACG – SAA the technique with atomization in graphite furnace - SAA-CG was performed.

An optimal graph was established, taking into account, in particular, the temperature and time parameters decomposition, temperature and atomization time. Aspiration according to the working diagram of the blank sample solution, the MRC solution / fortified sample, the sample set from which a double sample and the MRC / fortified sample at the end of the sample set read, the concentration was recorded.

From the same solution of the sample, the other elements were determined under the same conditions, using the standard solutions, the specific cathode lamps and the parameters specific to each lamp (FAO– Faolex, 2003).

RESULTS AND DISCUSSIONS

Table 1. LOD and LOQ values of heavy metal

Year	Matrix denomination	Chemical element	Results	Val. LOD/ LOQ mg/kg
2016	Scomber scomber	Cd	0.018	
2016	Salmo salar	Pb	0.023	
2016	Scomber scomber	Cd	0.029	
2016	Salmo salar	Pb	0.036	
2016	Hypophthalmichthys molitrix	Pb	<LOD	0.002
2016	Merluccius merluccius	Pb	0.012	
2016	Merluccius merluccius	Cd	0.008	
2016	Merluccius merluccius	Hg	0.026	
2016	Scomber scomber	Pb	0.019	
2016	Scomber scomber	Cd	0.024	
2016	Scomber scomber	Hg	0.076	
2016	Merluccius merluccius	Pb	0.016	
2016	Merluccius merluccius	Cd	<LOD	0.001
2016	Merluccius merluccius	Hg	0.015	
2016	Salmo trutta	Pb Cd Hg	<LOD <LOD 0.012	0.002 0.001
2016	Sander lucioperca	Cd	<LOD	0.001
2016	Pangasiuspangasius	Cd	<LOD	0.003
2016	Sparus aurata	Cd	0.003	
2016	Liza aurata	Cd	0.007	
2016	Scomber scomber	Hg	0.026	
2016	Acipenser gueldenstaedtii	Hg	0.020	
2016	Hypophthalmichthys molitrix	Cd	<LOD	0.001
2016	Liza aurata	Cd	<LOD	0.001
2016	Sardina pilchardus	Cd	0.022	
2016	Gadus macrocephalus	Cd	0.018	
2016	Sparus aurata	Cd	0.006	
2016	Salmo trutta	Cd	<LOD	0.003
2016	Cyprinus carpio	Cd	<LOD	0.001
2016	Cyprinus carpio	Pb	<LOD	0.002
2016	Salmo trutta	Pb Cd Hg	<LOD <LOD 0.011	0.002 0.001
2016	Cyprinus carpio	Pb Cd Hg	<LOD <LOD 0.010	0.002 0.001
2017	Alosa immaculata	Hg	0.063	
2017	Salmo trutta	Pb Cd Hg	0.029 <LOD 0.010	0.002 0.003
2017	Sardinapilchardus	Cd	0.024	
2017	Salmo salar	Pb	0.037	
2017	Dicentrarchus labrax	Hg	0.031	
2017	Sparus aurata	Cd	0.006	
2017	Sparus aurata	Hg	0.023	
2017	Hypophthalmichthys nobilis	Pb	0.029	
2017	Salmo salar	Pb	0.03	
2017	Scomber scomber	Hg	0.04	
2017	Hypophthalmichthys nobilis	Cd	<LOD	0.001
2017	Sparus aurata	Cd	<LOD	0.001
2017	Scomber scomber	Cd	<LOD	0.001
2017	Liza aurata	Cd	<LOD	0.001
2017	Salmo trutta	Pb Cd Hg	0.03 0.017 <LOD	 0.010
2017	Cyprinus carpio	Pb Cd Hg	0.01 <LOD 0.01	 0.001
2017	Sander lucioperca	Cd	<LOD	0.003
2017	Thunnus albacares	Pb Cd	<LOD 0.012	0.002
2017	Acipenser gueldenstaedtii	Hg	0.028	
2017	Sparus aurata	Cd	0.02	
2017	Sardina pilchardus	Cd	0.018	
2017	Scomber scomber	Cd	0.011	
2017	Salmo salar	Pb	<LOD	0.002
2017	Dicentrarchus labrax	Hg	0.047	
2017	Salmo trutta	Pb Cd Hg	<LOD <LOD <LOD	0.002 0.001 0.010
2017	Sparus aurata	Cd	0.04	
2017	Sparus aurata	Hg	0.024	
2017	Clupea harengus	Hg	0.02	
2017	Scomber scomber	Hg	0.024	
2017	Salmo salar	Pb	<LOD	0.002
2017	Salmo trutta	Pb Cd Hg	<LOD <LOD <LOD	0.002 0.003 0.010
2017	Cyprinus carpio	Pb Cd Hg	0.007 <LOD <LOD	 0.003 0.010
2017	Esox lucius	Hg	0.1	
2017	Cyprinus carpio	Pb Cd Hg	<LOD <LOD <LOD	0.002 0.001 0.003
2017	Salmo trutta	Pb Cd Hg	0.02 <LOD <LOD	 0.001 0.010
2017	Gadus macrocephalus	Cd	<LOD	0.001
2017	Gadus macrocephalus	Cd	<LOD	0.003
2017	Hypophthalmichthys molitrix	Pb Cd Hg	0.03 <LOD <LOD	 0.001 0.010
2017	Cyprinus carpio	Cd	<LOD	0.001
2017	Sardina pilchardus	Cd	0.01	
2017	Cyprinus carpio	Cd	<LOD	0.001

To evaluate the correct values, any analysis needs to be calibrated. Due to the extremely low working values, the errors due to the devices are frequent, which is why blank samples are essential. Each time the standard deviation was established and the validation criteria were those specified in Regulation (EC) No. 333/2007 establishing the methods of sampling and analysis for the official control of the levels of lead, cadmium, mercury, inorganic tin, 3-MCPD and benzo (a) pyrene in foodstuffs.

In the course of sampling, precautions shall be taken to avoid any changes which would affect the levels of contaminants, adversely affect the analytical determination or make the aggregate samples unrepresentative (Regulation (EC) No 152/2009 of 27 January 2009 laying down the methods of sampling and analysis for the official control of feed).

LOD is the smallest amount of substance that can be distinguished from the absence of that substance with a declared level of confidence (generally 99%).

LOQ is the minimum amount of substance that can be quantitatively determined with appropriate precision: the limit to which the difference between two distinct values can be reasonably discussed.

LOD, Limit of Detection, minimum detectable value, detection limit, CCβ (term used in the EU directives)

Determine the standard deviation (s) of ten independent measurements of a blank sample or of a sample with very low concentrations of the measuring. Limit of detection = LOD = s*3.3

LOQ, quantification limit, limit of quantitation, limit of determination, reporting limit, limit of reporting and application limit.

Determine the standard deviation (s) of ten independent measurements of a blank sample or of a sample with very low concentrations of the measure and Limit of quantitation = LOQ = s*10.

Consider the fitness of purpose of using the concentration at which imprecision (coefficient of variation) of the method is 5%.

Regulation (EU) 333/2007 specifies the LOD as 3 times the standard deviation of the mean of blank determinations and LOQ as six or 10 times the standard deviation of the mean of blank determinations (ISO (2000). ISO 11843-2). Interpretation of analysis results with LOD and LOQ can be noticed in the Figure 1.

The Table 1 shows the LOD and LOQ values for the three elements surveyed.

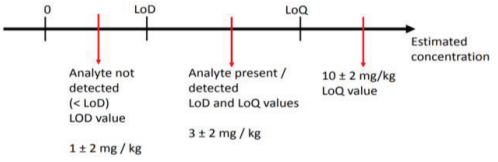

Figure 1. Analysis results with LOD and LOQ

The laboratory test must be homogeneous and representative, with no secondary contamination.

The LOD/LOQ report is interpreted according to the displayed result. If the absolute value displayed has the third decimal (0.00xx), then the result is not taken into account in the contamination of heavy metal fish. For Pb the maximum permissible level in food is 0.30 mg/kg, for Cd is 0.50 mg/kg, for Hg is 0.50 mg/kg.

CONCLUSIONS

Although the principle of biomagnification is demonstrated in many species in the animal world, substances resulting from human activities have different degrees of assimilation and cumulation. The heavy metal toxicity is the result of their binding to the important enzyme systems in the animal cell or certain cell membrane components.

Methylmercury is known to form in aquatic ecosystems via bacterial methylation of inorganic mercury. Methylmercury is excreted slowly over a period of several months, mostly as inorganic mercury in the faeces. It may take 45–70 days for the methylmercury concentrations to fall by a half in a person's blood, and 70–80 days in the entire body, but substantial variations in time-scale can occur (Nielsen and Grandjean, 2000). The need to research the concentration of heavy metals in food was obvious because the accumulation of heavy metals could impact health hazards to human. Following the study above, it can be observed that the concentration of mercury, lead, and cadmium of all analyzed fish types is lower than the maximal allowed concentration (MAC) by the legislation in force, which shows that the presence of heavy metals in the body of the fish (higher concentrations are recorded in the skin and their liver, and smaller in the white muscles) it is more a press topic than a reality.

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