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Assessment of contamination with heavy metals in food of animal origin

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Abstract

Heavy metals such as arsenic, cadmium, lead, and mercury are natural chemical compounds. They are present in water, soil, and the atmosphere in different amounts, but their presence has also been found in food because of contamination during production and storage, which is not desirable due to the bioaccumulation of heavy metals in the human body and its harmful effects on human health. The aim of the work is to determine the amount of heavy metals in certain types of food of animal origin that are available on the market in Republic of Srpska and Bosnia and Herzegovina in order to assess the real risks to human health based on compliance with the maximum allowed amount according to the Ordinance on the maximum allowed amounts of individual contaminants in food. The amount of total arsenic was determined by the hydride formation method of atomic absorption spectrometry according to *BAS EN 14546*, mercury by the spectrophotometric method on the Advanced Mercury Analyzer AMA-254, and cadmium and lead by the graphite technique AAS according to *BAS EN 14084*. Based on the test results, it was established that there were no heavy metals in the analyzed food samples that exceed the maximum allowed amounts, so based on this fact it can be concluded that the contamination in products of animal origin on the market of Republic of Srpska and Bosnia and Herzegovina with heavy metals does not pose a risk to human health. However, given the number of tests and increasing environmental contamination and heavy metal toxicity, monitoring of lead, cadmium, mercury, and arsenic must be carried out continuously to protect human health.

Key words: heavy metal, contamination of food, human health

Introduction

Heavy metals such as arsenic, cadmium, lead, and mercury are present in natural chemical compounds. They are present in water, soil, and the atmosphere in different amounts, but their presence has also been found in food because of contamination during production and storage, which is not desirable due to the bioaccumulation of heavy metals in the human body and its harmful effects on human health (Mitra et al., 2022; Tchounwou et al., 2012). Where heavy metals levels are higher, there is greater absorption of arsenic by animals, plants, and generally there is more of it in the food of that region (Tchounwou, et al., 2012).

Due to the toxic effects of heavy metals, in order to protect human health, the highest permitted amounts of contaminants in human food are prescribed, taking into account the most sensitive population groups, such as children, the elderly and pregnant women (Regulation, 2021). Arsenic is a metalloid that reacts chemically to produce both metallic and non-metallic characteristics. Unlike inorganic trivalent arsenic (As^{3+}), arsenic bound to organic molecules (As^{5+}) and elemental arsenic are not toxic (Mudhoo et al., 2011). Inorganic arsenic compounds have been linked to various types of cancer, according to refer to literature data (NRC, 1999). Due to its presence in the environment, arsenic can be found in food. Meat, dairy products and cereals are the main sources of inorganic arsenic, while seafood, fruits and vegetables are the main sources of organic arsenic (arsenobetaine). Organic compounds of arsenic, e.g. methylarsenic and dimethylarsenic acid are found in much smaller quantities compared to inorganic compounds such as arsenite and arsenate (Emsley, 2005). Levels of arsenic found naturally in soil, air and water are usually low, but can vary depending on the area, developed industry, arable agricultural land and other environmental pollutants. Past use of arsenic-containing pesticides on food crops, as well as pollution from mining, fracking, and coal-fired power plants, may contribute to higher levels of arsenic in the environment. Fish and shellfish are the main sources of organic As compounds, making them the main dietary sources of total As (EFSA, 2014, Vasi-Raki et al., 2010). Cadmium pollution of the not only aquatic environment occurs due to soil erosion into sediments, toxic pollution, and absorption. Cadmium can make people sick if they consume toxic food, breathe toxic air or drink water containing the metal. Cadmium does not possess properties that are beneficial for plants, animals, and humans' growth and metabolic activity (Hayat et al., 2018). The number of servings that can be eaten, before the Tolerable Weekly Intake (TWI) for methylmercury is reached, depends on the methylmercury content of the fish. For toddler children and women of childbearing age, the benefits of eating fish should be achieved by increasing consumption of species low in methylmercury. Apart from limiting the intake of high-mercury fish/seafood species in the daily diet, in order to avoid regular exposure above the TWI, it is not possible to give general recommendations on fish consumption throughout Europe. To achieve the health benefits derived from fish/seafood consumption, each country should consider its own fish consumption pattern and carefully assess the risk of exceeding the TWI for methylmercury (EFSA, 2015).

Environmental scientists have been interested in the toxicity of lead (Pb) because of its harmful effects on humans, animals, and plants. Lead can exist in both organic and inorganic forms (Kumur et al., 2020). Both forms of Pb are toxic, however organic Pb-complexes are excessively toxic to biological systems compared to inorganic Pb (WHO, 2010).

With canned food, there is a potential risk of lead migrating from the canning material into the food. The simplest transfer of lead to humans is through dust from the soil that enters people's living spaces, but also into drinking water. Lead exposure is of concern because lead is carcinogenic (group "B") to humans (IARC, 2019).

Due to the possible presence of heavy metals in meat and meat products, as a result of intentional or accidental exposure of domestic animals during fattening, and contamination of raw materials and finished products during processing, distribution and storage, it is necessary to continuously monitor the occurrence of these substances (Pleadin and Kovačević, 2016).

The aim of the work is to determine the amount of heavy metals in certain types of food of animal origin that are available on the market in Republic of Srpska and Bosnia and Herzegovina in order to assess the real risks to human health based on compliance with the maximum allowed amount according to the Ordinance on the maximum allowed amounts of individual contaminants in food. The assessment of the conformity of the test results of heavy metals is carried out according to the Ordinance on the Maximum allowed amounts for Certain Contaminants in Food (Official gazette of the Republic of Srpska, no 32/21).

Materials and Methods

Material

The total number of official food samples received in the laboratory for testing heavy metals was as follows: 181 samples of meat and meat products, 13 samples of milk and milk products, 38 samples of fish and meat products. The laboratory conducted a total of 661 tests of heavy metals, of which: 181 analyzes of arsenic, 177 analyzes of lead, and 176 analyzes of cadmium in meat and meat products; in milk and milk products 5 lead analyses, 13 arsenic analyses were done, and in fish and fish products 38 lead analyses, 38 mercury analyzes and 33 cadmium analyses were done. All samples that were delivered fresh or frozen until analysis was stored at refrigerator temperature or at -20 °C. Before analysis, meat and fish samples were chopped into 2-3 cm thick pieces and homogenized, and the products were tempered to room temperature and homogenized after opening. The samples were collected from April 2022 to March 2023.

Methods

For the determination of arsenic (As), the HGAAS atomic absorption spectrophotometric method was applied according to BAS EN 14546 Food products - Determination of total arsenic by hydride generation atomic absorption (HGAAS) after dry ashing. An Agilent instrument (Model AA 240) with a steam generator was used in these studies. The amount of mercury (Hg) was determined by the spectrophotometric method on the Advanced Mercury Analyzer AMA-254, and the test results were expressed in mg/kg of wet weight. The amount of lead (Pb) was determined at on atomic absorption spectrophotometer Agilent Technologies 240Z AA with Zeeman correction (GFAAS), and the measured quantities are expressed in mg / kg after microwave digestion according to BAS EN 14084 Food products - Determination of trace elements - Determination of lead, cadmium, zinc, copper, and iron by atomic absorption spectrometry (AAS).

The assessment of the conformity of the test results of heavy metals was carried out according to the Ordinance on the Maximum allowed amounts for Certain Contaminants in Food (Official gazette of the Republic of Srpska, no 32/21). Categories of tested samples and maximum allowed amounts (Regulation, 2021) are shown in Table 1, Table 2, and Table 3.

Table 1. Maximum permitted amounts of arsenic and lead in milk and milk products

Heavy metals	Type of food	Maximum limit*(mg/kg)
Pb	Raw milk, heat-treated milk and milk for production of dairy products	0.020
As	Milk and milk products	0.1

*Maximum allowed amounts

Table 2. Maximum allowed amounts for arsenic, lead and cadmium in meat and meat products

Heavy metals	Type of food	Maximum limit (mg/kg)
Pb	Meat (except offal) of cattle, sheep, pigs and poultry	0.10
Cd	1. Meat (except offal) of cattle, sheep, pigs and poultry 2. Liver of cattle, sheep, pigs, poultry and horses Kidneys of cattle, sheep, pigs, poultry and horses	0.050 0.50 1.0
As	1.Meat of cattle, sheep, pigs and poultry, except offal 2.Beef, sheep, pig and poultry meat products 3. Offal of cattle, sheep, pigs and poultry and their products	0.1 0.3 0.5

Table 3. Maximum allowed amounts for mercury, lead and cadmium in fish and fish product

Heavy metals	Type of food	Maximum limit (mg/kg)
Pb	Fish muscle Cephalopods Shellfish Shells	0.3 0.3 0.5 1.5
Cd	1.Muscle meat of fish, except for the species specifically mentioned in 2.Tuna 3.Muscle meat of the following fish: anchovy (Engraulis species) swordfish (Xiphias gladius) sardines (Sardina pilchardus) 4.crustaceans 5.shellfish 6.cephalopods	0.050 0.10 0.25 0.50 1.0 1.0
Hg	1. Fish products and fish muscle	0.5 or 1.0 **

** according to the types of fish and fish products that are defined according to the Regulation (2021).

Results and Discussion

Table 4 shows the average test results, as well as the minimum and maximum values of heavy metals expressed in mg/kg in meat and products.

Table 4. Test results of total number of analysis for heavy metals in meat and products

Heavy metals	Total analysis	Number of samples with quantity below <LOQ ***	****Mean ±SD mg/kg	Min (mg/kg)	Max (mg/kg)
Arsenic	181	37	0.011±0.0044	0.005	0.030
Lead	177	74	0.018±0.0092	0.010	0.059
Cadmium	176	68	0.002±0.0031	0.001	0.030

***LOQ for As: <0.005 mg/kg; Pb: <0.010 mg/kg; Cd: <0.001 mg/kg
****mean heavy metals content in the total number of analysed samples ± standard deviation of the mean value

Average values for lead. cadmium and arsenic in meat and products were respectively 0.018±0.0092 mg/kg. 0.002±0.0031 mg/kg and 0.011±0.0044 mg/kg. In the case of meat and meat products, the highest proportion of samples (42%) below the LOQ was recorded for Pb. Table 5 shows the average test results. as well as the minimum and maximum values of arsenic (As). expressed in mg/kg in different types of meat and products and offal.

Table 5. Results of testing the amount of As in meat, meat products and offal

Type of food- Meat	Mean ±SD (mg/kg)	Min-Max (mg/kg)
Beef meat Pork meat Poultry meat	0.011±0.0045 0.010±0.0042 0.011±0.0032	0.005-0.030 0.005-0.022 0.006-0.015
Meat products	0.014±0.0047	0.010-0.022
Offal of cattle. sheep. pigs and poultry and their products	0.014±0.0050	0.010-0.017
Mechanically deboned meat	0.009±0.039	0.005-0.016

In 20% of the tested meat samples, the amount of arsenic was not quantified (< LOQ). The highest average value of arsenic was determined in meat products 0.014±0.0047 mg/kg and offal 0.014±0.0050 mg/kg, and the lowest in mechanically deboned meat 0.009±0.039 mg/kg. The highest amount of arsenic was found in imported beef (0.030 mg/kg). In a study conducted from October 2015 to April 2022, Pećanac et al. (2022) determined the highest average value of arsenic in meat products of 0.023±0.0457 mg/kg, which is higher than the amount determined in this paper. Also, the mentioned authors recorded average values of arsenic in pork of 0.014±0.0078 mg/kg, in beef 0.013±0.0073 mg/kg, in poultry 0.012±0.00761 mg/kg and in mechanically deboned meat 0.012±0 .0054 mg/kg, which is in accordance with the results obtained in this paper. Pećanac et al. (2022) found that the highest amount of arsenic in meat products was 0.205 mg/kg.

In broiler meat tissues (wet weight), Ghosh et al (2012) determined the highest concentration of arsenic (µg/kg) in skin (218.8 ± 11.7), followed by liver (102.1 ± 8.0), lung (96.3 ± 5.6), kidney (88.2 ± 7.5) and thigh muscle (67.8 ± 5.1). Arsenic in broiler meat was below the maximum tolerable limits for humans.

Table 6 shows the average test results, as well as the minimum and maximum values of cadmium (Cd), expressed in mg/kg in different types of meat, meat products, and offal.

Table 6. Overview of results for Cd in meat and offal

Type of food	Mean ± SD (mg/kg)	Min-Max (mg/kg)
Beef meat	0.002±0.0008	0.001-0.012
Poultry meat	0.001	0.001
Pork meat	0.001±0.0011	0.001-0.007
Offal - chicken liver	0.030	0.030
Mechanically deboned meat	0.001±0.0006	0.001-0.002

In 74 samples (38.43%), out of a total of 177 samples, the amount of cadmium was below the limit of quantification (≤0.001 mg/kg). The highest amount of cadmium was determined in the liver sample of 0.030 mg/kg, and the highest average value of cadmium determined in beef meat was 0.002±0.0008 mg/kg. The average value in pork was 0.001±0.0011, with a maximum recorded amount of 0.007 mg/kg. Amount of 0.001±0.0006 mg/kg was determined in mechanically deboned meat, and 0.001 mg/kg in poultry meat. Pećanac et al. (2016) determined the amount of cadmium below the limit of quantification (56.12%) in 133 samples out of a total of 237. The same authors recorded the highest amount of cadmium in pork meat (0.025 mg/kg), and in beef meat a lower amount of 0.010 mg/kg. In poultry meat, the highest determined amount of Cd was 0.008 mg/kg. The results obtained in this paper are not in accordance with the results of Paćanac et al. (2016). Mračević et al. (2017) determined the amount of cadmium in pork at 0.03 mg/kg, and in beef at 0.04 mg/kg, which is significantly higher than the results obtained in this paper. In pig liver, the mentioned authors determined 0.18 mg/kg of cadmium. Table 7 shows the average test results, as well as the minimum and maximum lead content values expressed in mg/kg in different types of meat, products and offal.

Table 7. Test results for Pb in meat, meat products and offal

Type of food	Mean ± SD (mg/kg)	Min-max (mg/kg)
Beef meat poultry meat pork meat	0.018±0.0107 0.015±0.007 0.018±0.0079	0.011-0.059 0.011-0.031 0.010-0.040
Offal (pork liver)	0.012	0.012
Mechanically deboned meat	0.022±0.0008	0.018-0.031

The highest average value of lead was determined in mechanically deboned meat (0.022±0.0008 mg/kg). and the lowest in poultry meat (0.015±0.007 mg/kg). The highest amount of lead was recorded in beef meat (0.059 mg/kg), and the lowest in pork meat (0.010 mg/kg) (Table 6). The most important heavy metals as pollutants of meat products are cadmium (Cd), lead (Pb), arsenic (As) and mercury (Hg), especially Pb and Cd, since their presence in meat products has been confirmed by numerous studies (Hoha et al., 2014). Hana et al. (2022) determined the following average levels of As, Cd, Cr, Cu, Hg, Ni and Pb respectively: 0.018, 0.002, 0.061, 0.801, 0.0038, 0.055 and 0.029 mg/kg wet weight respectively in fresh meat. The average values for As and Cd determined in this paper agree with the results of the mentioned authors, while regarding Pb our results are somewhat lower.

Table 8 shows the total number of analyzes for heavy metals in milk and milk products, as well as the minimum and maximum values expressed in mg/kg. An overview of the results for As and Pb in milk and products is presented in Table 9.

Table 8. Overview of the number of tests and the average value of As in milk and milk products

Heavy metals	Total analysis	Number of samples with quantity below <LOQ***	Mean ± SD mg/kg	Min (mg/kg)	Max (mg/kg)
Arsenic in milk	5	4	-	0.011	0.011
Arsenic in milk product	8	-	0.019±0.0095	0.012	0.038
Lead	5	5	-	-	-

***LOQ for As: <0.010 mg/kg; ***LOQ for Pb: <0.008 mg/kg

Raw milk can be contaminated with heavy metals during its production (Ziarati et al., 2018). In the analyzed samples of milk and milk products, lead was quantified in only one out of five milk samples, while the amount of lead was above the LOQ in milk products in all tested samples. In all analised milk samples, lead was not quantified, the amount was below the LOQ.

Table 9. Test results for arsenic in milk and products of milk

Milk and Dairy Products	Amount (mg/kg)	Min-Max (mg/kg)
Raw milk, heat-treated milk, and milk for production of dairy products	<LOQ***	-
Cream with a minimum of 10% milk fat	0.019	-
Cream (crust) that it contains at least 70% milk fat in dry matter	0.029	-
milk spread	0.038	-
Cheese	0.014±0.0015	min 0.012 – max 0.015

***LOQ for As <0.010 mg/kg

The average value of the amount of arsenic obtained by testing five cheeses was 0.014±0.0015 mg/kg, and the highest determined amount was 0.015 mg/kg (table 8). Tajkarimi et al. (2008) determined mean concentrations of lead in fresh milk varying from 1.5 to 23.4 ng/L, in regions of Iran. Sola-Larrañaga and Navarro-Blasco (2009) determined the amount of lead in fresh milk which ranged from 0.55 to 18.7 μg/L, thus confirming the quantification of lead in milk. Mendil (2006) determined the amount of lead in different types of cheese in Turkey, whose values varied from 0.140 to 1.1 mg/kg.

Table 10 shows the total number of analysis for heavy metals in fish and fish products, as well as the minimum and maximum values expressed in mg/kg. An overview of the results for Hg in fish and fish products is presented in Table 11. Our analyzes of fish and fish products have shown that the content of Pb, Cd and Hg was significantly below the maximum allowed limits.

Table 10. Test results of total number of analysis for heavy metals in fish and products

Heavy metals	Total analysis	Number of samples with quantity below <LOQ***	Mean ± SD mg/kg	Min	Max
Pb	31	5	0.042±0.0221	0.010	0.313
Cd	31	-	0.028±0.0769	0.001	0.433
Hg	36	-	0.036±0.033	0.004	0.176

***LOQ for Pb <0.010 mg/kg

Table 11. Test results for Hg in the fish and fish product

Type of food	Mean ± SD (mg/kg)	Min-Max (mg/kg)
Hake	0.028±0.0298	0.020-0.061
Tuna	0.065±0.0581	0.009-0.176
Mackerel	0.031±0.0117	0.020-0.045
Sardine	0.035±0.0334	0.006-0.069
Herring	0.029±0.0255	0.004-0.052
Carp	0.025±0.0224	0.005-0.059
Mussels	0.007	0.007
Squids	0.015±0.009	0.006-0.024

In this paper, the lowest amount of mercury was recorded in herring (0.004 mg/kg). The average amount of mercury in fish and fish products was 0.036±0.033 mg/kg, and the highest amount of mercury 0.176 m/kg was determined in tuna meat, which is in accordance with the results of Pećanac et al. (2017), who examined 79 fish samples in their research, and the highest amounts of mercury were found in sea bass (0.230 mg/kg), tuna and monkfish (0.160 mg/kg) and sea bream (0.138 mg/kg). Pećanac et al. (2014) determined higher amounts of mercury, but still less than the maximum allowed amount in tuna in vegetable oil 0.256 mg/kg and canned tuna in pieces 0.229 mg/kg, which is higher than the amounts determined in tuna in this paper. The mentioned authors found the lowest amounts of mercury in fresh trout meat and frozen pangasius (0.005 mg/kg), and slightly more in fresh carp (0.006 mg/kg), which is lower than the amount of mercury determined in this work for carp.

Out of a total of 38 fish and product samples tested, in five samples the amount of lead was below the limit of quantification (<0.010 mg/kg). The average amount of lead in fish and fish products was 0.042±0.0221 mg/kg, and the highest amount of lead, according to the types of samples tested for lead, was recorded in sardines in tomato sauce 0.313 mg/kg. The highest amount of Cd was recorded in mussels (0.433 mg/kg), and the average amount of Cd in fish and products was 0.028±0.0769 mg/kg. Vitošević et al. (2007) examined the amounts of Cd, Pb and As in fish and products. The average amount of lead (0.0373±0.0176 mg/kg) agrees with our results, while the average amount of cadmium is significantly higher and does not agree with our results. Malavolti et al. (2022) examined the amount of lead in food and among other foods recorded the most lead in crustaceans and molluscs (0.039 mg/kg), which is not in agreement with our results.

Conclusion

Based on the obtained results, it was determined that the amounts of analyzed heavy metals in the food samples of animal origin (meat, milk, fish, and products), from the market of Republic of Srpska and Bosnia and Herzegovina, that was the subject of the testing in this work was not higher than the maximum allowed amount, thus complying with the requirements of the regulation. Based on this fact it can be concluded that the contamination of food with heavy metals does not pose a risk to human health in this region. However, given the number of tests and increasing environmental contamination and heavy metal toxicity, monitoring of lead, cadmium, mercury, and arsenic must be carried out continuously to protect human health.

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