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Original Research

Distribution of Heavy Metals in Tissues of Freshwater Fish in Lithuania

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Abstract

Concentrations of Pb, Cd, Cu, Zn, Fe, Ni, Cr, Mn and V (HMs) were determined in flesh, fishbone, liver, gill and intestine of 20 freshwater fish (perch, roach, silver bream, semi-bream, chub, smelt, tench and pike) netted in various Lithuania fresh waters. 90% of fish flesh samples were contaminated with Pb at the concentration below the maximum tolerable level of Lithuanian Standards of Hygiene, although one sample was contaminated with 3.125 mg/kg Pb, which is 8 times above the normal. 40% of fish flesh samples were contaminated with the concentration of Cd exceeding the Maximum Tolerable Limit (MTL) value of the European Union. However, only in one fish flesh sample did Cd concentration exceed 1.5 times the Lithuanian MTL value. The concentration of HM in fishbone was higher than in fish flesh, except for Cr and V, where the concentration in bone and flesh was similar. The concentration of Pb and Cd in fishbone corresponded to the allowable standards for fodder additives, processed from fish products and other sea organisms. The highest amounts of Fe, Zn and Mn were found in fishbone and inner organs: liver, gill and intestine. The concentration of HM in inner organs of fish was from several to twelve times higher than that in flesh. The largest amount of HM was found in liver. In order to develop the fishing industry in freshwater reservoirs it is necessary to maintain water quality standards, to monitor water and fish flesh pollution regularly as well as to control the concentration of HM in fish.

Keywords: heavy metal, pollution, fish tissues, freshwater fish, toxic metals

Introduction

Lithuanians annually consume approximately 17 kg fish per person [1]. Although it constitutes a small part of food intake in comparison to other products, fish contains polyunsaturated fats, which are essential for human nutrition and their consumption should increase. Contamination of freshwater fish with heavy metals (HMs) is a recognised environmental problem. The World Health Organization as well as the Food and Agriculture Organization of the United Nations state that monitoring eight elements in fish – Hg, Cd, Pb, As, Cu, Zn, Fe, Sn – is obligatory and monitoring of others is suggested.

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Increases in agricultural and industrial activities in an area directly influences the quality of water. In other words, water reservoirs are collectors of all materials spread by human industrial and agricultural activities. HMs penetrate into water reservoirs via atmosphere, drainage, soil waters and soil erosion. As the concentration of HMs in the environment increases, the metals inevitably enter the biogeochemical cycle [2-4]. Having contaminated water, HMs accumulate in organisms, which are consumed by fish or penetrate into fish directly through skin and gill later [5, 6]. HMs cause the mutation of fish inner organs, disturb immune reactions, change blood parameters, reduce an organism's adaptation qualities, vitality, resistance to diseases. Loss of fry and degeneration and diminution of valuable varieties

of fish are observed as a result of HM pollution [7-10]. Usually, many toxic compounds affect organisms in nature at the same time, each of them having a specific effect on physical and chemical processes that influence an organism's condition and reactions. Therefore, in order to maintain the quality of food it is important to regularly monitor and evaluate the pollution levels in fish as well as in water reservoirs.

The aim of this work was to determine the concentrations of Pb, Cd, Cu, Zn, Fe, Ni, Cr, Mn and V in flesh, bones, liver, gill and intestines of freshwater fish collected in throughout of Lithuania.

Materials and Methods

Twenty samples of fish from different freshwater sampling points in Lithuania were analyzed (Fig. 1 and Table 1.). The concentrations of Pb, Cd, Cu, Zn, Fe, Ni, Cr, Mn and V in flesh, fishbone, liver, gill and intestines were determined using ICP-MS model "Element" (Finnigan MAT, Bremen) in the Federal Institute of Consumer Health Protection and Veterinary Medicine in Berlin, Germany. Samples were prepared according to the reported Lithuanian Standard technique [11]. The analysis was regulated following the Commission's Decisions [12, 13]. Each sample was analyzed in at least two repetitions.

Before the investigation the fresh fish (0.1-0.2 kg) was placed in a polyethylene bag and frozen to -20°C for 4-6 hours. Preparing samples for analysis included cleaning the fish, freeing them of mechanical additives, and warming them to -2°C. Fish heads, fins, and inner organs together with hard roe were removed. Fish flesh was separated from the spinal column and ribs. Flesh, bones, liver, gill and intestine were analyzed separately.

At least three laboratory samples of fish flesh, bones, liver, gill and intestines were prepared from each fish specimen. A sample of 0.3000-0.5000 g was weighed in a plastic test-tube, 2.5 ml of concentrated HNO_3 (Suprapure, Merck) was added and the sample was stirred at room temperature for 30 minutes. Then 5 ml of bidistilled H_2O and 1 ml of 35% H_2O_2 solution (35%, pure for analysis, Merck) were added and the sample was placed into

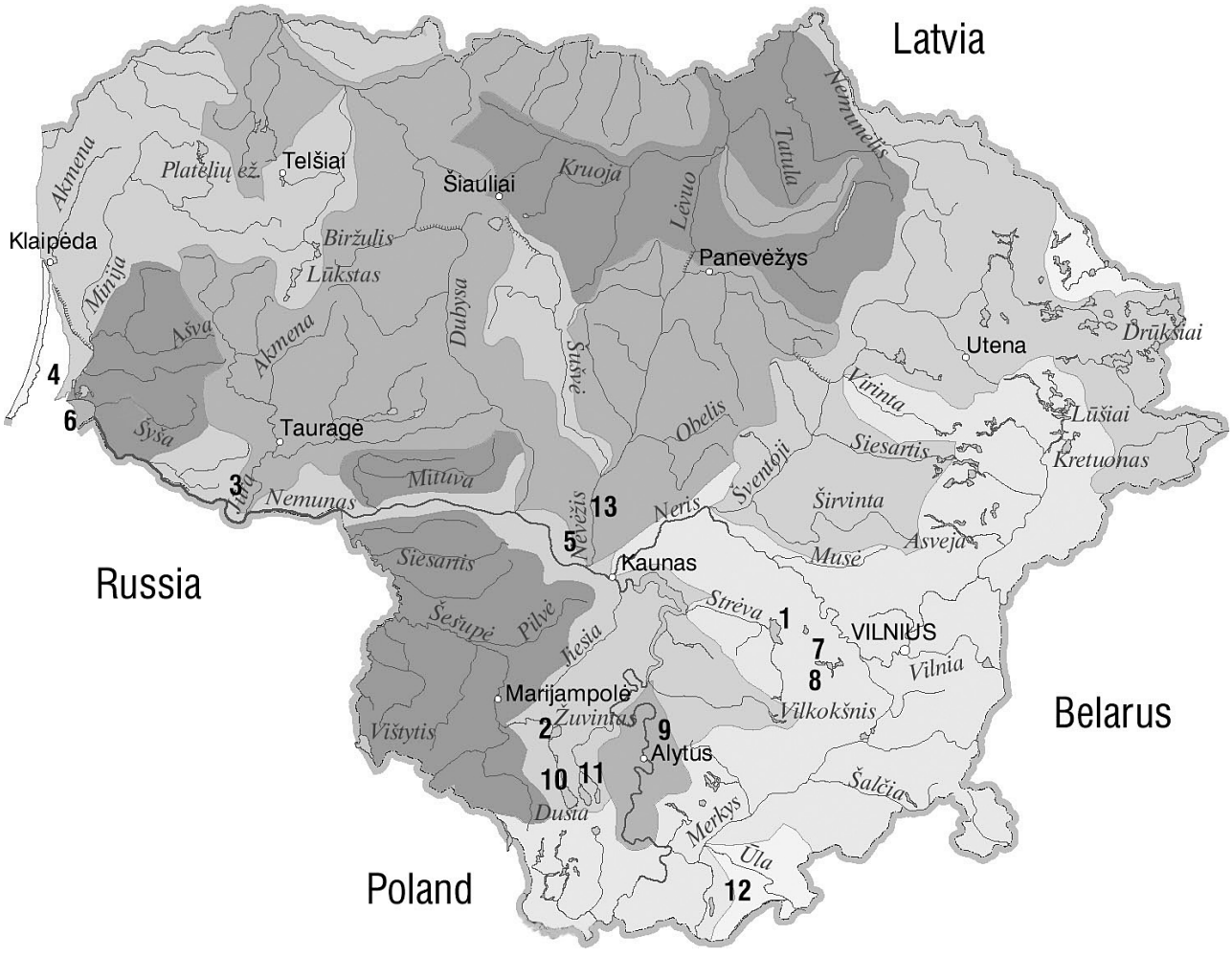


Fig. 1. Different freshwater sampling points in Lithuania: 1-Elektrenai Pond, 2-Angininkai Lake, 3-Nemunas River (Vezininkai), 4-Kursiai Sea, 5-Nevezis River (delta), 6-Nemunas at sea, 7-Totoriskiai Lake, 8-Babrukas Lake, 9-Nemunas River (Alytus), 10-Dusia Lake, 11-Obelija Lake, 12-Vilkve Lake, and 13-Nevezis River (Kaunas).

a microwave bath for dissolving. Afterwards, the sample was made up to 50 ml with bidistilled H_2O while stirring. A portion of 5 ml of each sample was poured into the plastic test-tube and the measuring procedure was performed in radon gas atmosphere [14]. The results of analysis were processed using the "Minitab" statistic program, Version 13 [15].

Results

The concentration of Pb in fish flesh is presented in Fig. 2. The maximum concentration of Pb (3.125 mg/kg) was found in fish from Obelija Lake. The minimum amount of Pb: 0.059 mg/kg, was found in fish from the Nemunas River near Alytus town.

The concentration of Cd in fish flesh is presented in Fig. 3. The maximum concentration of Cd – 0.140 mg/kg – was found in the fish flesh from Elektrenai Pond. The dissemination of Cu, Zn, Fe, Ni, Cr, Mn and V concentration in fish flesh is presented in Table 2.

The dispersion of Pb in fishbone is presented in Fig. 4. The maximum amount of Pb in fishbone is 3.30mg/kg. The concentration of Cd in fishbone is presented in Fig. 5. This data varies similarly to that of the fish flesh (0.040÷0.053 mg/kg). The dissemination of the concentration of Cu, Zn, Fe, Ni, Cr, Mn and V in fishbone is presented in Table 2.

In order to determine the level of contamination of the fish inner organs, the concentration of HM in fish liver, gill and intestines of examined fish was analyzed. The dissemination of the concentrations of Pb and Cd in inner organs of fish is presented in Table 3. The highest concentration of Cd – 8.250 mg/kg was determined in intestine, of Pb – 3.500 mg/kg in gill. The dissemination of Cu, Zn, Fe, Ni, Cr, Mn and V concentration in liver, gill and intestines is presented in Table 2.

Discussion

In food, the allowed amount of HMs is defined by norms, which are based both on the WHO recommendations and local requirements. Therefore, norms for HMs vary in each country. For example, according to Lithuanian Standards of Hygiene [16] the maximum tolerable limit (MTL) of Pb in fish meat is 0.4 mg/kg, whereas in the European Union it is 0.2 mg/kg [17].

Table 1. Fish species and the sampling points.

Sample No.	Sampling locations	Fish
1.	Elektrenai Pond	Perch *(Perca fluvatilis)*
2.	Elektrenai Pond	Roach *(Rutilus rutilus)*
3.	Elektrenai Pond	Silver bream *(*Abramis bjoerkna*)*
4.	Angininkai Lake, Alytus region	Roach *(Rutilus rutilus)*
5.	Nemunas River near Vezininkai	Silver bream *(Rabdosargus sarba)*
6.	Nemunas River near Vezininkai	Bream *(Abramis brama)*
7.	Kursiai Sea, Nemunas delta	Perch *(Perca fluvatilis)*
8.	Kursiai Sea, Nemunas delta	Roach *(Rutilus rutilus)*
9.	Nevezis (delta)	Silver bream *(*Abramis bjoerkna*)*
10.	Nevezis (delta)	Roach *(Rutilus rutilus)*
11.	Nevezis (delta)	Chub *(Leuciscus cephalus)*
12.	Nemunas at sea, Silutë region	Smelt *(Osmerus eperlanus)*
13.	Totoriskiai Lake, Trakai	Roach *(Rutilus rutilus)*
14.	Babrukas Lake, Trakai	Roach *(Rutilus rutilus)*
15.	Nemunas River near Alytus	Roach *(Rutilus rutilus)*
16.	Dusia Lake, Lazdijai region	Perch *(Perca fluvatilis)*
17.	Obelija Lake, Lazdijai	Roach *(Rutilus rutilus)*
18.	Obelija Lake, Lazdijai	Tench *(Tinca tinca)*
19.	Vilkve Lake, Varena region	Pike *(Esox Lucius)*
20.	Nevezis River near Kaunas	Roach *(Rutilus rutilus)*

The maximum concentration of Pb (3.125 mg/kg). Exceeding the Lithuanian MTL value 8 times, was found in fish from Obelija Lake. Obviously it is an exceptional case, but it confirms the necessity to monitor the concentration of HMs in fish flesh. The minimum amount of Pb, 0.059 mg/kg, was found in fish from the Nemunas River near Alytus town. The amounts of Pb meet the LSH norms in 90% of fish flesh samples (except one). In 20% of samples the concentration of Pb exceeded the MTL of the European Union, however. In comparison to results obtained during a similar study, the concentration of Pb was close to the one found during our analysis and varied within 0.020 mg/kg – 0.100 mg/kg range, with one sample of 0.960 mg/kg of Pb [18-20].

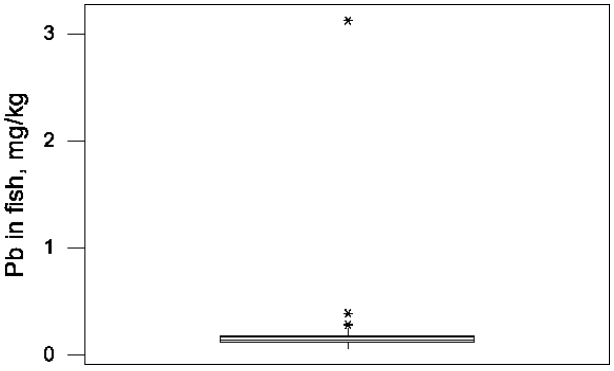
The maximum concentration of Cd equal to 0.140 mg/kg was found in the fish flesh from Elektrenai Pond, which is located in the vicinity of a thermal electric power station. This amount exceeded the Lithuanian MTL value equal to 0.100 mg/kg almost 1.5 times and it is almost 3 times higher than the EU norm equal to 0.050 mg/kg. The rest of the samples contained Cd in concentrations from 0.042 to 0.054 mg/kg, whereas in eight samples the concentration was slightly higher than the EU MTL. In a similar study Gerulaitis and Valiusiene [18] found lower Cd levels in fish flesh: from 0.030 to 0.060 mg/kg.

Concentrations of Cu, Zn, Fe, Ni, Cr, Mn and V in fish flesh are presented in Table 2. The maximum concentration of Cu (0.564 mg/kg) exceeded the Lithuanian MTL value by almost 5 times. The determined amount of Fe in fish flesh is 4-10 times lower than in LSH. Ni concentration in fish flesh samples is within the limits of LSH, except for one sample, where the concentration of Ni amounted to 10.660 mg/kg. The average concentration of Mn in fish flesh samples was relatively low with the exception of two samples (0.301 and 0.641 mg/kg respectively). The maximum concentration of V in fish flesh was 0.289 mg/kg and in the rest of samples varied from 0.058 to 0.081 mg/kg.

Fishbone is used in production of food additives, i.e. gelatine. Therefore, its health quality is also very important. The maximum amount of Pb found in fishbone (3.301 mg/kg) is higher than that determined in fish flesh (Fig. 4.), but stays within the limits of allowable amount of Pb (MTL is equal to 10.0 mg/kg) in food additives produced from processed fish products and other sea organisms [21]. The concentration of Cd in fishbone (Figure 5.) varied similarly to that of fish flesh and was slightly lower, except for one sample.

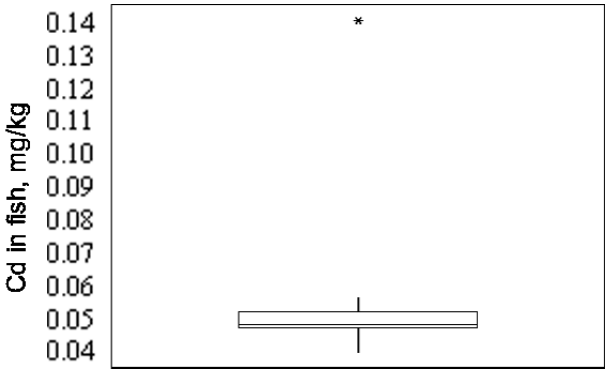
Cu level in fishbone was 2-6 times higher than in fish flesh. The amount of Zn in fishbone was much higher than in flesh. Also Fe concentration in fishbone was considerably higher than that in fish flesh. The amount of Ni in fishbone is 5-8 times larger than that of flesh, although the fishbone from the delta of the Nemunas River contained a more than 100 times higher amount of this element. The concentration of Cr in fish flesh and bones was similar: it varied from 0.526 to 1.426 mg/kg, on average, except for one sample, where the concentration of Cr reached the value of 8.524 mg/kg. The amount of Mn in fishbone was 10 times higher than in flesh, whereas the concentration of V was similar to its amount in flesh.

It should be stressed that the level of HM in fish flesh may not necessarily represent the real extent of HM's impact on ichthyofauna or the whole hydrosphere [22]. Elements from water are taken by fish through gills and a gastrointestinal tract, where they can be accumulated in



	Pb [mg/kg]
Min.	0.059
25 %	0.112
Md.	0.145
75 %	0.171
Max.	3.125

Fig. 2. Box-Whisker diagram dissemination of Pb concentration in flesh.



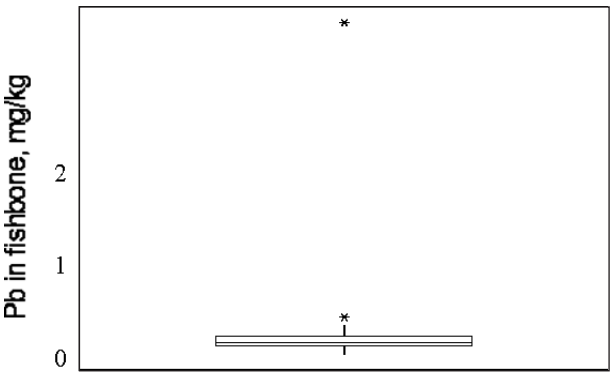
	Cd [mg/kg]
Min.	0.042
25 %	0.049
Md.	0.051
75 %	0.054
Max.	0.140

Fig. 3. Box-Whisker dissemination diagram of Cd concentration in flesh.

inner organs, leading to pathological changes [7-10]. In order to determine the level of contamination of fish inner organs, concentrations of HMs were analyzed in fish liver, gill and intestines. The highest concentration of HMs was determined as follows: Pb, Ni, Cr and Mn in liver, Cd in liver and intestine, Cu in intestine and a slightly lower concentration in gill and liver, Zn in liver and fishbone, Fe in fishbone, gill and liver, and Ni in liver. Through the small number of samples and considerable dispersion of the results one can observe that Fe and Zn were present in fish in the highest concentrations. Mn, Cr, Cu, Ni were determined in slightly lower amounts. In fish liver the concentrations of Pb, Cu, and Cr are 4 times, concentrations of Cd, Zn, and Ni are 9 times, concentrations of Fe are 80 times, and concentrations of Mn are more than 100 times higher than in flesh. The lowest concentration of HMs was found in fish flesh, whereas the highest was determined in fish liver (Table 2.). These results do not contradict other researchers' information and differences are statistically insignificant [14, 18, 23-28].

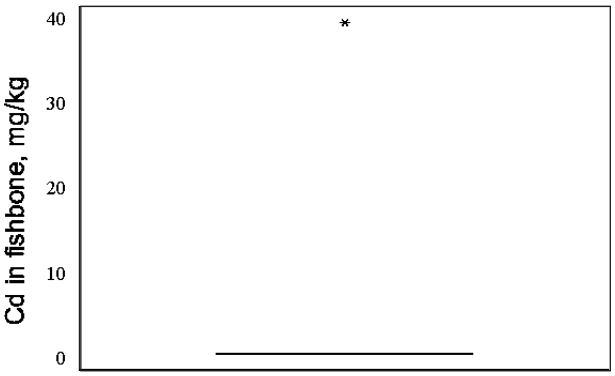
Table 2. Box-Whisker dissemination of HM concentrations [mg/kg] in fish flesh, fishbone, liver, gill and intestines.

	Cu	Zn	Fe	Ni	Cr	Mn	V
Box-Whisker dissemination of HM concentrations [mg/kg] in fish flesh							
Min.	0.125	10.034	0.211	0.125	0.526	0.011	0.058
25%	0.131	10.471	0.743	0.133	0.766	0.025	0.061
Md.	0.289	14.842	2.059	0.152	0.932	0.072	0.069
75%	0.417	20.991	6.108	0.184	1.033	0.215	0.081
Max.	0.564	22.011	7.947	10.660	1.426	0.641	0.289
Box-Whisker dissemination of HM concentrations [mg/kg] in fishbone							
Min.	0.061	46.432	61.214	0.234	0.083	1.179	0.001
25%	0.216	91.514	148.101	0.374	0.277	2.861	0.019
Md.	0.376	122.311	174.012	0.713	0.544	7.060	0.042
75%	0.651	174.301	204.621	1.979	0.819	9.780	0.064
Max.	1.234	530.886	397.534	20.114	8.524	16.012	0.229
Box-Whisker dissemination of HM concentrations [mg/kg] in fish liver							
Min.	0.711	61.011	79.098	0.298	0.871	2.691	0.002
25%	0.905	74.498	123.198	0.730	1.611	4.953	0.027
Md.	1.139	120.496	162.099	1.311	3.859	9.253	0.081
75%	1.648	290.201	178.010	2.161	5.762	10.661	0.265
Max.	2.851	389.699	198.597	26.759	15.991	15.120	0.281
Box-Whisker dissemination of HM concentrations [mg/kg] in fish gill							
Min.	0.641	74.396	105.090	0.341	0.529	0.149	0.011
25%	0.841	78.201	113.793	0.472	1.490	4.050	0.005
Md.	1.209	97.311	172.601	0.669	2.481	7.579	0.109
75%	1.499	250.498	183.095	1.125	3.885	9.489	0.178
Max.	1.989	259.299	204.699	1.719	5.139	11.541	1.361
Box–Whisker dissemination of HM concentrations [mg/kg] in fish intestines							
Min.	0.809	36.797	16.398	0.119	0.179	0.529	0.019
25%	1.075	42.091	22.201	0.119	0.515	0.891	0.025
Md.	1.389	76.398	33.191	0.248	1.151	1.949	0.061
75%	3.811	109.199	68.102	0.581	3.665	3.481	0.075
Max.	4.489	181.701	106.899	3.410	4.831	4.861	0.089



	Pb [mg/kg]
Min.	0.061
25 %	0.138
Md.	0.168
75 %	0.235
Max.	3.301

Fig. 4. Box-Whisker dissemination diagram of Pb concentrations in fishbone.



	Cd [mg/kg]
Min.	0.041
25 %	0.043
Md.	0.045
75 %	0.048
Max.	0.053

Fig. 5. Box-Whisker dissemination diagram of Cd concentrations in fishbone.

Table 3. Box-Whisker dissemination of Pb and Cd concentrations in inner organs of fish.

	Pb [mg/kg]			Cd [mg/kg]		
	liver	gill	intestine	liver	gill	intestine
Min.	0.210	0.191	0.110	0.411	0.169	0.349
25%	0.435	0.269	0.251	0.415	0.231	0.415
Md.	0.589	0.368	0.151	0.441	0.251	0.451
75%	0.739	0.975	0.169	0.485	0.275	0.481
Max.	0.941	3.498	0.219	3.029	0.289	8.250

In conclusion it may be stated that in order to evaluate the ecological condition of freshwater fish, both fish flesh and inner organs must be monitored regularly. To develop the fishing industry in freshwater reservoirs it is necessary to maintain water quality standards, and to monitor contamination levels of water reservoirs and fish regularly.

Conclusions

1. 90% of fish flesh samples were contaminated with concentrations of Pb, which laid within the limits of maximum tolerable levels of Lithuanian Standards of Hygiene, although one sample was contaminated with 3.125 mg/kg of Pb, which is 8 times above the norm. An enlarged concentration of Cd was determined for 40% of fish flesh samples, which exceeded the MTL value of the European Union. However, only in one fish flesh sample did Cd concentration exceed the Lithuanian MTL value 1.5 times.
2. Concentrations of HMs in fishbone are higher than in fish flesh, except from Cr and V, as their concentrations in bones and flesh is similar. The concentrations of Pb and Cd in fishbone correspond to the allowable standards for food additives produced from the processed fish products and other sea organisms.
3. Concentrations of HMs in inner organs of fish are from several to twelve times higher than in flesh. The largest amount of HMs was found in liver, where the concentrations of Pb, Cu, Cr are 4 times, concentrations of Cd, Zn, Ni are 9 times, and concentrations of Mn are more than 100 times higher than in flesh.

References

1. PECIUKYNAS A. Carp.; Vilnius: Mokslas. Lithuania, pp. 125, **1996.**

2. RIGET F., DIETZ R., VORKAMP K., JOHANSEN P., MUIR D. Levels of spatial and temporal contaminants in Greenland biota: an updated review. Science of the Total Environment. **331,** 29, **2004.**
3. KENDRICK MH, MAY MT, PLISHKA MI, ROBINSON KD. Metals in biological systems. Ellis Horwood Ltd, pp. 34-82, **1992.**
4. MANSOUR S.A., SIDKY M.M. Heavy metals contaminating water and fish from Fayoum Governorate, Egypt. Food Chemistry. **78,** 15, **2002.**
5. SINHA A.K, DASGUPTA P., CHAKRABARTY S, BHATTACHARYYA G., BHATTACHARJEE S. Bio – accumulation of heavy metals in different organs of some of the common edible fishes of Kharkai River, Jamshed pur. Indian. J Environ Health. **46,** 102, **2002.**
6. SUREC B. Accumulation of heavy metals by intestinal helmints in fish: an overview and perspective. Parasitology, **126**, 53, **2003.**
7. BLASCO J., ARIAS A.M., SAENZ V. Heavy metals in organisms of the River Guadalquivir estuary: possible incidents of the Aznalcollar disaster. The Science of the Total Environment. **242,** 249, **1999.**
8. KIME D.E. A strategy for assessing the effects of xenobiotics on fish reproduction. The Science of the Total Environment. **225,** 3, **1999.**
9. BIRD G.A., HESSLEIN R.H., MILLS K.H., SCHWARTZ W.J., TURNER M.A. Bioaccumulation of radionuclides in fertilized Canadian Schield lake basins. The Science of the Total Environment. **218,** 67, **1998.**
10. ALABASTER J. S., LLOYD R. Water quality criteria for freshwater fish. Moscow, pp. 25, **1994.**
11. Lithuanian Standards: Fishes and fish products. Admission, Formation of specimens and preparation for analysis. LST 1613: **2000**.
12. Commission Decision 90/15/EC of September 1990 laying down the reference methods for detecting residues of heavy metals and arsenic.
13. Commission Decision 2001/22/EC the sampling methods and the methods of analysis for the official control of the levels of lead, cadmium, mercury and 3-MCPD in foodstuffs. 2001/873/EC.
14. ONYENEKWE P. C., STANISKIENE B., PALAVINSKAS R., BOESS C. Concentration of heavy metals in fish from Berlin, Lithuanian and Nigerian inland waters. Toxicology Letters, **2**, 57, **1999.**
15. Minitab Statistical Software. Minitab Inc. 120, **1999.**
16. Standards of Hygiene of Ministry of HealthCare of the Republic of Lithuania: Food products. Maximum tolerable limit of residue of contaminants and pesticides. LR HN 54: **2001.**
17. European Commission. Amending Commission Regulation (EC) No 194/97. Brussels, pp.2–28, **2000.**
18. GERULAITIS A., VALIUSIENE V. Heavy metals in fishes and their effect on ichtyofauna of the lower Nemunas and the Kursiu bay, Conference Proceedings of LSA, 73, **1994.**
19. AMUNDSEN P.A., STALDVIK F.J., LUKIN A.A., KUSHULEN N.A., POPOVA O.A., RESHETNIKOV Y.S. Heavy metal contamination in freshwater fish from the border region between Norway and Russia. The Science of the Total Environment **201,** 211, **1997.**
20. ZAUKE G.P., SAVINOV V.M., RITTERHOFF J., SAVINOVA T. Heavy metals in fish from the Barents Sea. The Science of the Total Environment. **227,** 161, **1999.**
21. Produktu, skirtu mitybai, privalomuju saugos reikalavimu techninis reglamentas. Valstybes zinios. **56**, 69, **2003.**
22. JEZIERSKA B., WITESKA M. Metal toxicity to fish.; Academia Podlaska, Siedlce, pp. 51, **2001.**
23. KIM S.G., JEE I.H., KANG J.C. Cadmium accumulation and elimination in tissues of juvenile olive flounder, *Paralichthys olivaceus,* after sub – chronic cadmium exposure. Environ Pollut, **127**, 117, **2004.**
24. SKIBNIEWSKA K.A. Estimation of iron, copper, zinc and manganese intake from duplicate diets provided by hospitals in Poland, 1993–96. Food additives and Contaminants. **19,** 969, **2002.**
25. KHANSARI F.E., KHANSARI M.G., ABDOLLAHI M.A. Heavy metals content of canned tuna fish. Food Chemistry. **30,** 1, **2004.**
26. TURKMEN A., TURKMEN M., TEPE Y., AKYURT I. Heavy metals in three commercially valuable fish species from Turkey. Food Chemistry. **91,** 15, **2005.**
27. TUZEN H. Determination of heavy metals in fish samples of the middle Black Sea by graphite furnace atomic absorption spectrometry. Food Chemistry. **27,** 521, **2002.**
28. BOSNIR J., PUNTARIC D. Toxic metals in freshwater fish from the Zagreb area as indicators of environmental pollution. Coll Antropol, 9, **2003.**