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Distribution of Heavy Metals in Muscles of Fish: Concentrations and Change Tendencies

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Concentrations of six heavy metals (Pb, Cd, Cr, Ni, Cu, V) in muscles of freshwater fish have been determined in this work. Samples are taken from 20 freshwater reservoirs in Lithuania. The concentrations of HMs have been determined using the ICP-MS model "Element" (Finnigan MAT). It is found that in 20 % of samples the concentration of Pb exceeds the Maximum Tolerable Limit (MTL). The highest Cd concentration – 0.14 mg/kg - almost 1.5 times higher than the MTL is found in the sample from the Elektrėnai Pond which collects cooling water from the thermal electric power station. In other samples of fish muscle the Cd concentration does not exceed the MTL and ranges from 0.042 mg/kg to 0.058 mg/kg.

When comparing the results of the present research with the data from 1998, it is found that the concentrations of HMs are increasing. Increase in the concentrations of Pb and Cd is particularly significant. As an example, an increase in the amount of Pb in 3 samples ranges between 11.3 % and 14 %, in one sample – almost 27 %, in 4 samples – from 50 % to 66 %, in one sample – 89 %, and in one – almost 18 times. The concentration of Cd in 2 samples has increased almost by 20 %, in 5 samples, on average - by 40–70 %, in one sample – 86 %, and in one sample – 3 times. The concentration of other HMs in fish muscles has increased in the following way: the arithmetic average of Cr concentration increase is 82. 4 %, Cu and V increase, on average - 28 %, Ni – 17 times. In order to develop the fishing industry in freshwater reservoirs it is important to maintain the water quality standards, and to regularly monitor the levels of accumulation of HMs in water, sediments and fish.

Key words: *heavy metals, freshwater fish, fish muscles, spectroscopy.*

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1. Introduction

Heavy metals are the most hazardous pollutants due to the speed of their dissemination in biosphere and their accumulative concentration. They permeate the environment by various means, penetrate the circle of metabolism, become toxic and disturb physiological functions of organism [1]. While regulating constituents of food products, the World Health Organization (WHO) as well as the Food and Agriculture Organization (FAO) suggest monitoring the concentrations of heavy metals (HMs).

There are a lot of lakes, rivers, ponds and other water reservoirs in Lithuania. One of the most precious water treasures is fish. Intensification of agricultural and industrial activities affects the quality of water and directly contributes to contamination of water reservoirs. Water reservoirs are collectors of nearly all the materials distributed by or left as a result of human industrial or agricultural activities and production. HMs penetrate into water reservoirs via atmosphere, drainage, soil waters and soil erosion. As the concentration of HMs in the environment increases, chemicals inevitably enter the biochemical cycle [2]. Having contaminated water, HMs accumulates in organisms, which are consumed by fish or permeate the fish directly through skin and gill. HMs cause mutation of fish inner organs, disturb

reactions of immune system, change blood parameters, reduce adaptation qualities, vitality, and resistance to diseases of an organism resulting in the loss of fry as well as degeneration and diminution of valuable fish species [3]. Usually, several or dozens of toxic compounds affect organisms in nature at the same time, each of them having a specific effect on physical and chemical processes that influence condition and reaction of an organism. Therefore, in order to maintain the quality of all human nutrition, including fish products, it is important to regularly monitor and evaluate the pollution levels in fish as well as in water reservoirs.

Both the World Health Organization and the Food and Agriculture Organization of the United Nations state that monitoring of eight elements in fish – Hg, Cd, Pb, As, Cu, Zn, Fe, Sn – is obligatory and monitoring of the others (Sb, Ni, Cr, Al, F, J) is suggested [4]. Lithuanian Standards of Hygiene regulate the maximum tolerable limits (MTL) of Pb, Cd in fish meat [5].

2. Materials and Methods

Thirty samples of fish from different freshwater sample points in Lithuania were analyzed during the research (Table 1). The concentrations of HMs were determined using the ICP-MS model "Element" (Finnigan MAT). Samples were prepared according to the reported Lithuanian Standard technique [6]. Each sample was analyzed at least twice.

Before the investigation the fresh fish (0.1–0.2 kg) was placed in a polyethylene bag and frozen to - 20º C for 4–6 hours. Preparing the samples for analysis included cleaning the fish, freeing them of mechanical additives, and warming them to - 1º C. Fish heads, fins, and inner organs together with hard roe were removed. Fish flesh was separated from spinal column and ribs [6].

Table 1. Fish species and the sampling points

Sample No.	Sampling locations	Fish
1.	Elektrėnai Pond	Perch (*Perca fluvatilis*)
2*.	Elektrėnai Pond	Roach (*Rutilus rutilus*)
3.	Elektrėnai Pond	Silver bream (*Rabdosargus sarba*)
4*.	Angininkai Lake, Alytus region	Roach (*Rutilus rutilus*)
5.	Nemunas River near Vėžininkai	Silver bream (*Rabdosargus sarba*)
6*.	Nemunas River near Vėžininkai	Semi-bream
7.	Kuršiai Sea, Nemunas delta	Perch (*Perca fluvatilis*)
8*.	Kuršiai Sea, Nemunas delta	Roach (*Rutilus rutilus*)
9.	Nevėžis (delta)	Silver bream (*Rabdosargus sarba)*
10*.	Nevėžis (delta)	Roach (*Rutilus rutilus*)
11*.	Nevėžis (delta)	Chub (*Leuciscus cephalus*)
12.	Nemunas at sea, Šilutė region	Smelt (*Osmerus eperlanus*)
13.	Totoriškiai 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*.	Vilkvė Lake, Dzūkija	Pike (*Esox Lucius*)
20*.	Nevėžis River near Kaunas	Roach (*Rutilus rutilus*)

** Sampling locations, where the samples where taken in 1998.*

Preparation of fish samples. At least two laboratory samples of fish muscles were prepared from each fish specimen. The samples of 0.3000–0.5000 g (±0.0001 g) were weighed in plastic test-tube (accuracy of 0.0001 g), poured with 2.5 ml of concentrated HNO_3 and stirred in the room temperature for 0.5 hour. Then 5 ml of bidistilled H_20 and 1 ml of 35% H_20_2 solution were added and the samples were placed into a microwave bath for dissolving. Afterwards, the samples were quantitatively poured from a plastic test-tube into a 50 ml volumetric flask, diluted with bidistilled H_20 up to 50 ml while stirring.

Calibration of ICP-MS. The device was calibrated everyday. During the process of analysis of fish samples, radon gas was fumed regularly. After two empty samples, 6–7 standard solutions were analyzed, starting from the smallest concentration and increasing the concentrations in the process. Before the analysis of each sample bidistilled H_20 was poured for 2 min, then – 1% HNO_3 for another 2 min. In the process of calibration, both the natural concentration of metal isotopes (evaluated in μg/kg) and the subordination of relative intensity were determined. The calibration graphs were formed for each analyzed metal isotope: ^{110}Cd, ^{111}Cd, ^{112}Cd, ^{114}Cd ^{204}Pb, ^{206}Pb, ^{207}Pb, ^{208}Pb, etc. A correlation coefficient for calibration data is 0.9995 [7].

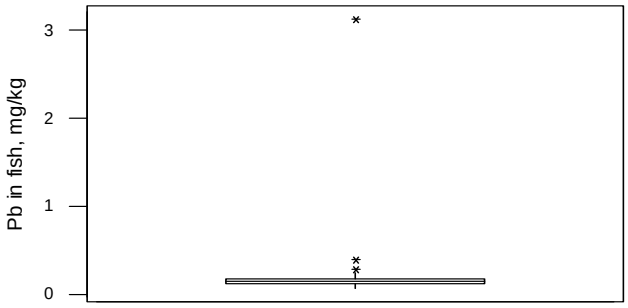
Analysis of fish samples with ICP-MS. A portion of 5 ml of each sample was poured into special plastic test-tubes. The samples were analyzed automatically. The measuring procedure was performed in the radon gas atmosphere. After each portion of samples the system was washed first with

bidistilled H_20, then – with 1% HNO_3 solution. The results of measurement were presented as mass spectra that allow to trace interdependence of relative intensity and molecular mass of metal isotopes, as well as the relation between relative intensity of metal isotopes and the quantity of heavy metals found in fish in μg/l ($1 \cdot 10^{-6}$ g/l). The determined quantity of HM in μg/l was revaluated into mg/kg. The results of analysis were processed using the statistic program "Minitab" (version 13) [8].

3. Results and Discussion

Accumulation of Pb in fish tissues depends on its amount in water and sediments. According to the materials of the Lithuanian Ministry of Environment, the concentration of Pb has recently increased on average 3–5 times and varies in the range of 0.75÷53.7 mg/kg [9]. Accumulation of Pb can be also strongly influenced by fish age, its species and pH and temperature of water [10–13].

The concentration of Pb in fish muscles is presented in Figure 1. The average concentration of Pb does not exceed the MTL of the HM concentration. The maximum concentration of Pb – 3.125 mg/kg – almost 16 times above the MTL was found in fish from the Obelija Lake. Obviously, this can be interpreted as an exceptional case, but it confirms the necessity to monitor the concentration of HMs in fish flesh. The concentration of Pb in chub from the Nevėžis Delta almost twice exceeds the MTL, and in two samples (No. 1, 5) the amount of Pb is slightly bigger than the MTL. The minimal amount of Pb – 0.059 mg/kg was found in roach from the Nemunas River near Alytus city. The obtained results indicate that in 20 % of the samples the amounts of Pb exceed the MTL (0.2 mg/kg). In comparison to the results obtained by other researchers, the concentration of Pb was close to that found during our analysis and basically varied within 0.02–0.1 mg/kg scale, although one example had 0.96 mg/kg of Pb concentration [14–18]. In "The Overview of the Research Results on Accumulation of HMs in Fish and Sediments" publicized by the Lithuanian Ministry of Environment (2007-07-12) the concentration of Pb in fish muscles varies from 0.07 to 0.72 mg/kg [9].

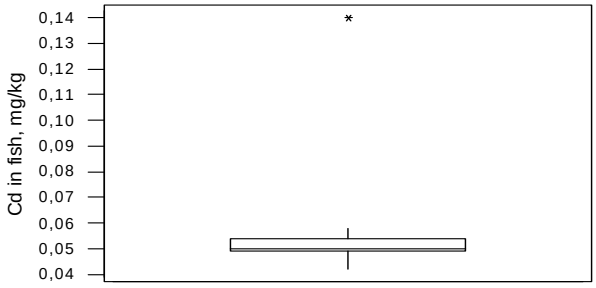


	Concentration of Pb, mg/kg
Min.	0,059
25 %	0,112
Md.	0,145
75 %	0,171
Max.	3,125

Fig. 1. Box-Whisker diagram dissemination of Pb concentration in fish muscles

The maximum concentration of Cd – 0.14 mg/kg (Fig. 2) – 1.5 times exceeding the MTL was found in silver bream from the Elektrėnai Pond, which is located in the vicinity of a thermal electric power station and collects cooling water from the power plant. The concentration of Cd in other fish samples varies from 0.042 mg/kg to 0.058 mg/kg (Fig. 2) Most of the results from similar researches show lower amounts of Cd concentration in fish muscles – from 0.03 mg/kg to 0.06 mg/kg [9, 11, 16].

The amount of Cr in fish muscles ranges from 0.526 mg/kg to 1.426 mg/kg (Table 2). The concentration of Ni in fish muscles varies in the range of 0.12÷0.2 mg/kg, except for one sample from Elektrėnai pond perch, where the concentration of Ni is 10.66 mg/kg. The amounts of Cu in fish muscles vary from the lowest of 0.125 mg/kg to almost five times higher concentration of 0.564 mg/kg (Table 2).The maximum concentration of V – 0.289 mg/kg was found in silver bream from the Nemunas River near Vėžininkai. In other samples the amounts vary in the range of 0.058÷0.081 mg/kg (Table 2). Lithuanian State Food and Veterinary Service monitors the amounts of Pb, Cd, Hg in fish muscles, the amounts of other HMs are evaluated on commission.



	Concentration of Cd, mg/kg
Min.	0,042
25 %	0,049
Md.	0,05
75 %	0,054
Max.	0,14

Fig. 2. Box-Whisker dissemination diagram of Cd concentration in fish muscles

Table 2. Box-Whisker dissemination of HMs concentrations [mg/kg] in fish samples

	Cu	Zn	Fe	Ni	Cr	Mn	V
Min.	0,125	10,03	0,211	0,12	0,526	0,01	0,058
25 %	0,131	10,47	0,74	0,13	0,766	0,025	0,0615
Md.	0,289	14,84	2,059	0,15	0,932	0,07	0,069
75 %	0,417	20,99	6,108	0,18	1,03	0,215	0,0815
Max.	0,564	22,0	7,947	10,66	1,426	0,64	0,289

In this research the concentration of HMs in fish muscles was compared with the results of the fish samples analyzed in 1998. The samples were taken from the areas marked by * in Table 1. The results of comparative analysis are presented in Figure 3. The concentration of Pb in the majority of samples analyzed in 1998 does not exceed the MTL, except for sample No. 11 from the Nevėžis Delta, where the maximum concentration of Pb was found – 0.261 mg/kg. The lowest levels of Pb were found in a sample from the Nemunas River near Alytus – 0.053 mg/kg (Fig. 3). The exceptionality of sample No. 17 is confirmed, because in 1998 the concentration determined in sample from the Obelija Lake in Lazdijai was 0.174 mg/kg, which does not exceed the MTL. An increase in Pb concentration (%) is demonstrated in Figure 5, where augmentation is presented as follows: in 3 samples – from 11.3% to almost 14%, in 1 sample – almost by 27%, in 4 samples – from 50 % to almost 66 %, in 1 – by 89 %, and in one – almost 18 times.

The variation of the concentration of Cd in fish muscles is presented in Figure 4. In all the samples from the research of 1998 the concentration of Cd does not exceed the MTL. From the data presented in Figure 5 one can determine that the Cd concentration increases in the following way: in 2 samples ~ by 20 %, in 5 samples – on average by 40–70 %, in one sample – by 86 %, and in the Angininkai Lake in Alytus region – 3 times.

As for comparative analysis of the concentration of other HMs in fish muscles we have only 4 samples available, therefore no wider generalization can be drawn, however, we can trace the tendencies. From the data presented in Table 3, it is obvious that the concentration of Cr is especially high in samples 2, 4, 6, and arithmetical average is up by 82.4 %. Particularly, the concentration of Ni has increased in all samples of fish muscles, and the arithmetical average is up 17 times. The concentrations of Cu and V have increased in comparison with the research data from 1998 on average by 28 %.

The level of contamination by HMs in fish, however, does not represent the real extent of their toxic impact on ichthyofauna or the whole hydrosphere. Elements from water are taken by fish through gills and inner organs, and through the system of metabolism they permeate into muscles, bones and accumulate there [14]. Due to an increase in the quantity of HMs in fish, their respiratory disorders, coagulation of gill mucus, deformation of spine, breakage of nervous system, digression of immune system, growth and reproduction, disorders in ferment system, functioning of DNR, metabolism can occur leading to genetic mutations [3].

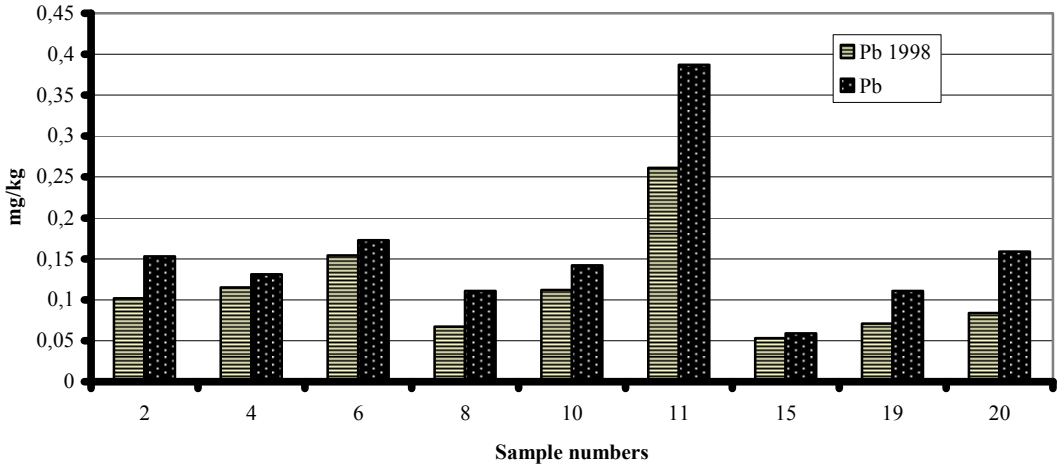


Fig. 3. Comparative data of Pb concentration in fish muscles

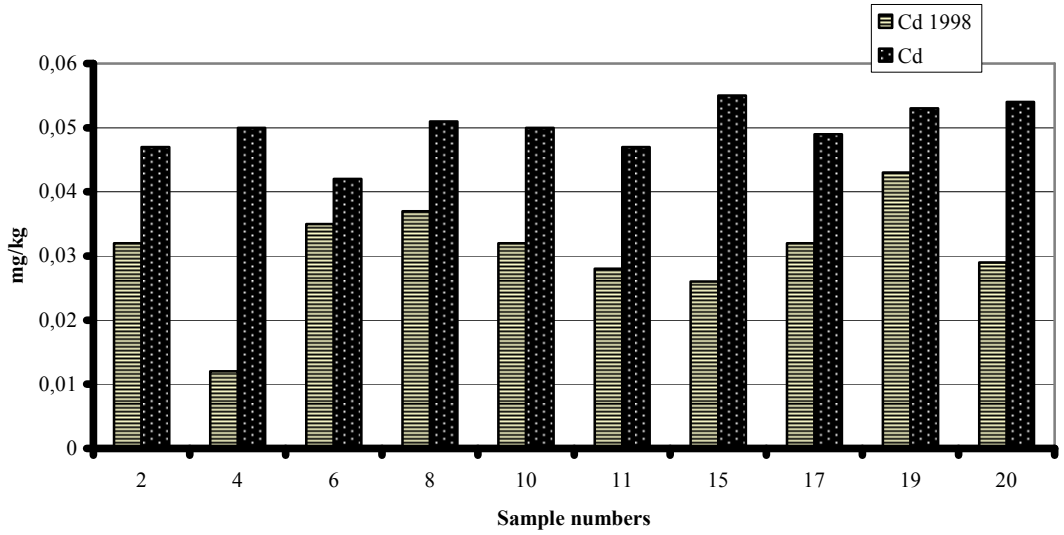


Fig. 4. Comparative data of Cd concentration in fish muscles

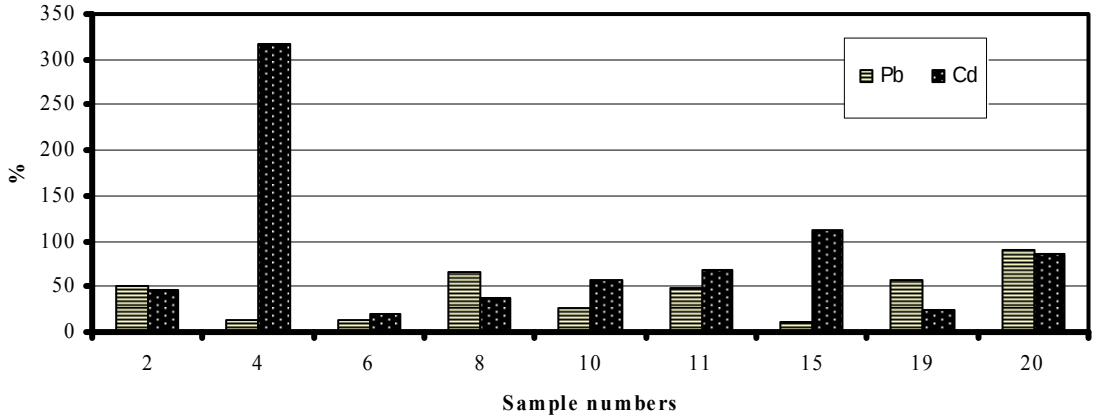


Fig. 5. Increase of Pb and Cd concentration in fish muscles as compared with 1998 data (percentage)

Table 3. Cr, Ni, Cu, V concentrations in fish muscles [mg/kg]

Sample No	Cr		Ni		Cu		V	
2	0,895*	1,673	0,084*	0,911	0,943*	1,234	0,073*	0,062
4	0,314*	0,726	0,079*	0,756	0,634*	0,862	0,042*	0,058
6	0,342*	0,572	0,013*	2,342	0,342*	0,385	0,06*	0,061
8	0,289*	0,384	0,091*	0,536	0,358*	0,432	0,04*	0,078
Md,mg/kg	0,46*	0,839	0,067*	1,136	0,569*	0,728	0,054*	0,065

** Analyzed in1998*

In conclusion, it may be stated that in order to evaluate the ecological condition of freshwater fish, not only fish muscles but also inner organs are to be monitored regularly. In order to develop the fishing industry in freshwater reservoirs, it is important to maintain water quality standards, and to monitor regularly the levels of contamination of water reservoirs and fish.

4. Conclusions

1. The concentration of Pb in fish muscles varies from a minimal amount of 0.059 mg/kg to the maximum of 3.125 mg/kg. It is determined that in 20 % of samples the concentration of Pb exceeds the MTL.
2. The highest Cd concentration – 0.14 mg/kg – almost 1.5 times higher than the MTL is found in the sample from the Elektrėnai Pond. In other fish muscle samples the concentration of Cd does not exceed the MTL and ranges from 0.042 mg/kg to 0.058 mg/kg.
3. When comparing the results of the present research with the data of that performed in 1998, we can state that the concentrations of HMs are increasing. Increase in the concentrations of Pb and Cd is particularly significant. As an example, increase in the amount of Pb in 3 samples ranges between 11.3 % and 13.9 %, in one sample – 26.8 %, in 4 samples – from 50 % to 65.7 %, in one sample – 89.3 %, and in one – almost 18 times. The concentration of Cd in 2 samples has increased almost by 20 %, in 5 samples - on average by 40–70 %, in one sample – by 86 %, and in one sample – 3 times.
4. The concentration of other HMs in fish muscles has increased in the following way: the arithmetic average of Cr concentration increase is 82. 4 %, Cu and V – on average by 28 %, Ni – 17 times.
5. In order to develop the fishing industry in freshwater reservoirs it is important to maintain water quality standards, and to monitor the levels of accumulation of HMs in water, sediments and fish regularly.

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Sunkiųjų metalų sklaida žuvų raumenyse: koncentracijos ir kaitos tendencijos

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(gauta 2008 m. gegužės mėn.; atiduota spaudai 2009 m. birželio mėn.)

Sunkieji metalai (SM) pagal plitimo biosferoje greitį ir didėjančią koncentraciją yra pavojingiausi teršalai, kurie, įvairiais keliais patekę į aplinką, įsitraukia į medžiagų apykaitos ratą, tampa toksiški ir sutrikdo organizmų fiziologines funkcijas Intensyvėjanti ūkinė žmogaus veikla kenkia vandens kokybei ir tiesiogiai prisideda prie vandens telkinių teršimo. Maisto ir žemės ūkio organizacijos (FAO/WHO), reglamentuodamos maisto produktų komponentus, siūlo tikrinti ir SM koncentraciją.

Šio darbo tikslas yra nustatyti šešių sunkiųjų metalų (Pb, Cd, Cr, Ni, Cu, V) koncentracijas žuvų raumenyse. Žuvų mėginiai paimti iš 20 gėlo vandens telkinių Lietuvoje. Tyrimas buvo atliktas firmos „Finnigan Mat“ ICP masių spektrometru. Tiriant nustatyta, kad 20 % bandinių švino (Pb) koncentracija žuvų raumenyse viršija didžiausią leistiną koncentraciją (DLK). Didžiausia kadmio (Cd) koncentracija (0,14 mg/kg), kuri beveik 1,5 karto viršija DLK, nustatyta bandinyje iš Elektrėnų tvenkinio (į jį leidžiamas šaldomasis šiluminės elektrinės vanduo). Kituose žuvų mėginiuose nustatyta Cd koncentracija neviršija DLK ir įvairuoja nuo 0,042 mg/kg iki 0,058 mg/kg.

Palyginti su 1998 m. tyrimo rezultatais, matoma, kad visų tirtų SM koncentracija žuvų raumenyse didėja. Ypač padidėja Pb ir Cd koncentracijos. Pvz., Pb kiekis 3 bandiniuose padidėja 11,3–14 %, viename bandinyje – beveik 27 %, 4 bandiniuose 50–66 %, viename bandinyje – 89 %, viename bandinyje – beveik 18 kartų; Cd koncentracija 2 bandiniuose padidėja ~ 20 %, 5 bandiniuose – vidutiniškai 40–70 %, viename bandinyje – 86 %, o bandinyje iš Naginiņkų ežero (Alytaus regione) – 3 kartus. Kitų SM koncentracija žuvų raumenyse padidėja taip: chromo (Cr) koncentracijų aritmetinis vidurkis – 82,4 %, vario (Cu) ir vanadžio (V) – vidutiniškai 28 %, nikelio (Ni) – 17 kartų. Norint vystyti konkurencingą žuvininkystę gėlo vandens telkiniuose, būtina laikytis vandens kokybės normų, nuolatos kontroliuoti vandens telkinių taršą ir stebėti žuvų taršą SM.