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DETERMINATION OF HEAVY METALS IN THE MUSCLES OF SOME FISH SPECIES FROM LAKES OF THE NORTH-EASTERN POLAND

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Lead, cadmium and total mercury concentrations have been determined in the muscle of six fish species: roach (*Rutilus rutilus* L.), bream (*Abramis brama* L.), perch (*Perca fluviatilis* L.), pike (*Esox lucius* L.), vendace (*Coregonus albula* L.) and whitefish (*Coregonus lavaretus* L.) from selected lakes in North-Eastern Poland (Łańskie, Pluszne, Dłużek and Maróz), caught over the period October 1999 to October 2000. Levels of Pb and Cd have not differed in fish of different feeding type, with the exception of cadmium in fish from Lake Łańskie ($p \leq 0.01$). Generally, the higher concentrations of total mercury were found in predatory than non-predatory fish ($p \leq 0.01$). Only some specimens (perch of the Dłużek and Maróz lakes and roach of the Dłużek Lake) had Pb levels exceeding 0.2 mg/kg. The Hg concentration in muscle of some perch (except from Lake Łańskie) exceeded the Polish safety limit of 0.5 mg/kg.

INTRODUCTION

Mercury, lead and cadmium are regarded as elements which do not perform any biological functions, either in human or in animal organisms. Being one of the last elements of both the human trophic and aquatic environment chains, fish can be seen as an effective indicator of food and aquatic environment contamination with some elements and compounds. The limit values which are currently in force in Poland, concern mainly toxic metals and for the three elements – Pb, Cd and Hg – are 0.20 mg/kg, 0.05 mg/kg and 0.50 mg/kg of fresh and processed fish, respectively, with the exception of certain marine species, detailed in subsequent paragraphs of the regulations. Pike (*Esox lucius* L.) is one of the species covered by this study, which is included in one of the groups; the highest acceptable mercury content for this fish is 1.0 mg/kg [Journal of Statues, 2003].

These elements contaminate air, soil, water, sediments and biota including aquatic organisms [Sorensen *et al.*, 1990; Szulkowska-Wojaczek *et al.*, 1992; Karadede & Ünlü, 2000].

Protasowicki & Ociepa [1978] showed that the muscle tissue is a good indicator of food and environmental contamination with mercury, as it contains the highest concentrations of this element among all the organs studied. Similar conclusions were found by Lodenius *et al.* [1983], Barak & Mason [1990a, b] and Voigt [2000]. Barak & Mason [1990 b] found the concentration of lead, mercury and cadmium to be dependent on the species. Håkanson [1984] found that the concentration of toxic heavy metals (Pb, Hg, Cd) in fish is affected by many biological factors, such as species, sex, age, feeding type (planktonovorous, benthosovorous, predators), and environmental factors, such as the season of the year, pH value of water, temperature, dissolved oxygen and salinity.

A literature review shows that numerous factors, both biological (species and feeding type) and the environmental affect the degree of contamination of fish muscle tissue with lead, mercury and cadmium. Consequently, this study aimed at determining the level of heavy metals (Hg, Pb, Cd) in fish muscles (depending on the species). As this region plays a leading role in the national fisheries industry and has high ecological values, a study of the fish from four lakes of the Olsztyn Lake District would seem to be beneficial. Such a study would be all the more important as there is little information concerning the contamination of fish from the lakes of north-eastern Poland.

MATERIAL AND METHODS

MATERIAL STUDIED

Six fish species: roach (*Rutilus rutilus* L.), bream (*Abramis brama* L.), perch (*Perca fluviatilis* L.), pike (*Esox lucius* L.), vendace (*Coregonus albula* L.) and whitefish (*Coregonus lavaretus* L.) caught from October 1999 to October 2000 were examined. The fish were caught in four vendace-type lakes: Łańskie, Pluszne, Dłużek and Maróz, located in the Olsztyn Lake District (Figure 1). The fish were killed, weighed and the total length (l.t.) of each fish was measured (Table 1). Muscles from the dorsal part were mixed and samples were frozen in plastic bags at 378 K (-25°C) until analysis. If the fish were small (body weight < 160 g), each sample was prepared from muscle tissues taken from two to nine fish. Whereas in the case of large fish, each sample was prepared from the tissue taken from one or two fish.

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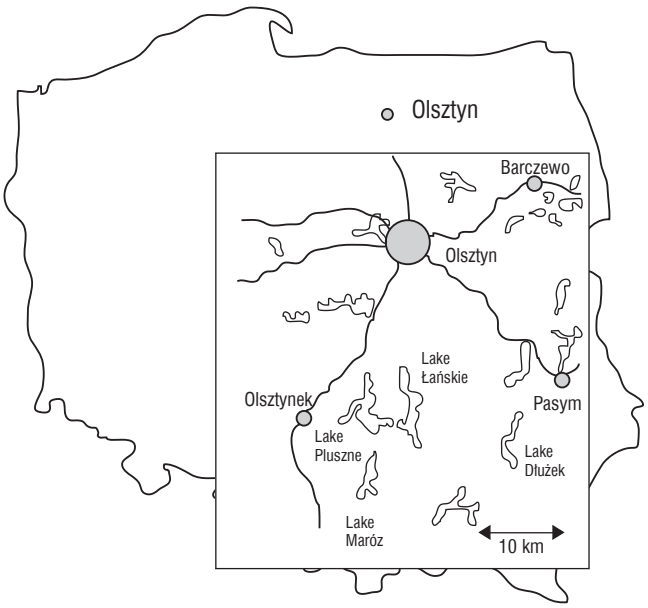

FIGURE 1. Map illustrating location of investigated sites.

METHODS

Lead and cadmium determination. About 10 g samples of muscle tissue were dry-digested at (450°C). The white ash was dissolved in 1 mol/L HNO_3 (Suprapur-Merck) and each of samples was the quantitatively transferred to a 25 mL volumetric flask. Lead and cadmium were measured using flameless atomic absorption spectrometry in graphite cuvette GF AAS (PERKIN ELMER with a ZEEMAN background correction).

Mercury determination. For the determination of total mercury the samples were wet-digested in nitric and sulphuric acid mixture HNO_3:H_2SO_4 (2:1) (v/v) at 100–110°C. Organic compounds were ultimately oxidised by the addition of 6% w/v solution of $KMnO_4$. An excess of $KMnO_4$ was reduced with hydroxylammonium hydrochloride (20% w/v) until the sample was discoloured. The concentration of total mercury was determined using UNICAM 939 SOLAR flameless cold vapour atomic absorption spectrometry [Hatch & Ott, 1968].

All samples were processed in duplicate. Three blanks and four standards were analyzed with each batch of samples. The methods were tested by reference material: CRM 422 – cod muscle tissue with a certified concentration of Pb, Cd and Hg. The percent recovery rate were 103.2% for Pb, 102.9% for Cd and 100.2% for Hg, n=4 (variability coefficients V(%) – 8.02 for Pb, 3.30 for Cd and 3.27 for Hg) [Quevauviller *et al.*, 1993].

Statistical analysis. The concentrations of heavy metals in muscle of fish are expressed in mg/kg wet weight. Bartlett's test showed that the variances were heterogeneous therefore mean values in particular groups were transformed (log $\bar{x}$). The one-way analysis of variance ANOVA (Duncan's test) was used to test significant differences. In all these cases statistical significance was estimated at $p \leq 0.01$ and $p \leq 0.05$ [Babiak, 1998].

TABLE 1. Body weight and total length of the freshwater fish examined.

Species	Lakes	Number of fish	Weight (g) range, mean±SD	Length (cm) range, mean±SD
Roach	Łańskie	48	42 – 342 156.0 ± 117.3	15.8 – 29.0 21.9 ± 5.3
	Pluszne	38	36 – 532 273.6 ± 221.0	15.5 – 33.4 25.3 ± 7.4
	Dłużek	49	26 – 540 236.3 ± 214.1	14.1 – 35.0 24.3 ± 9.2
	Maróz	43	38 - 445 213.2 ± 175.6	15.4 – 32.0 23.6 ± 6.9
Bream	Łańskie	12	370 – 1552 942.9 ± 423.5	31.8 – 47.6 40.4 ± 5.2
	Pluszne	12	502 – 1612 876.5 ± 329.5	35.6 – 50.1 41.3 ± 4.6
	Dłużek	12	284 – 1500 867.4 ± 366.2	29.8 – 48.0 40.2 ± 5.6
	Maróz	12	520 - 1614 1062.2 ± 402.2	34.5 – 48.8 42.5 ± 4.9
Vendace	Łańskie	34	72 – 218 104.4 ± 27.4	21.5 – 30.1 24.2 ± 1.5
	Pluszne	40	46 – 86 57.9 ± 4.7	18.1 – 23.1 20.4 ± 0.5
	Dłużek	46	38 – 78 51.6 ± 2.8	17.0 – 21.6 19.2 ± 0.4
	Maróz	44	52 - 100 69.4 ± 4.2	19.1 – 23.7 21.3 ± 0.3
Whitefish	Łańskie	6	396 – 672 517.7 ± 102.3	36.0 – 40.0 37.7 ± 1.43
	Pluszne	6	564 – 1130 803.7 ± 202.4	38.4 – 46.2 42.5 ± 2.7
	Dłużek	6	384 – 610 536.3 ± 83.4	33.0 – 38.1 36.5 ± 1.9
	Maróz	6	372 - 1130 440.7 ± 57.7	34.0 – 36.6 35.4 ± 0.9
Perch	Łańskie	37	36 – 584 247.0±216.9	14.6 – 33.6 23.3±7.4
	Pluszne	36	40 – 766 383.2±301.9	15.9 – 36.1 27.4±7.7
	Dłużek	52	22 – 862 349.4±331.8	12.6 – 36.8 24.0±9.6
	Maróz	31	46 – 927 329.8±287.1	12.6 – 38.7 25.1±8.3
Pike	Łańskie	12	155 – 2682 1115.5±805.4	28.7 – 71.9 49.8±13.9
	Pluszne	12	744 – 2012 1241.1±449.9	48.0- 63.5 55.5±6.2
	Dłużek	12	684 – 5235 1782.5±1451.7	44.4 – 92.0 57.5±14.0
	Maróz	12	571 – 1854 1035±474.8	43.5 – 65.1 52.3±8.9

RESULTS

Lead

The mean concentration of lead (Figure 2a) in muscles of fish from Lake Łańskie ranged from 0.060 mg/kg (roach) to 0.093 mg/kg (pike). For the fish from Lake Pluszne, the values ranged from 0.047 mg/kg (pike) to 0.092 mg/kg (bream) The contents of lead in fish from Lake Dłużek and Maróz

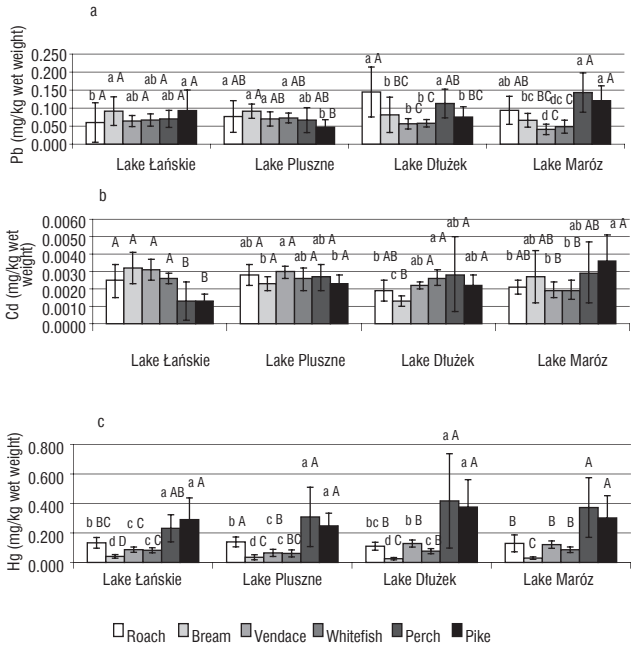

FIGURE 2. Comparison of heavy metals (mean and standard deviation) in the muscle tissue of six examined species from different areas (mg/kg wet weight).
a) lead, b) cadmium, c) total mercury
a, b, c, d – significant difference ($p \leq 0.05$); A, B, C, D – significant difference ($p \leq 0.01$). Means that do not share the same letters are significantly different, (n=12 in the case of roach, bream, perch and pike, n=6 in the case of vendace and whitefish).

were from 0.057 mg/kg (vendace) to 0.145 mg/kg (roach) and from 0.04 mg/kg (vendace) to 0.143 mg/kg (perch), respectively. The interspecific differences in lead levels in muscles were not always statistically significant ($p > 0.05$), as large differences in the concentrations of the element were observed within species.

Cadmium

The concentration of cadmium in the muscles of all the fish was found to be very low (Figure 2b). The lowest mean concentrations of cadmium (0.0013 mg/kg) in the fish caught in Lake Łańskie were in the muscles of pike and perch ($p \leq 0.01$). Whereas the highest values, but not different ($p > 0.05$) from the concentration of cadmium in the muscles of non-predatory fish, were found in the muscles of bream (0.0032 mg/kg). The muscles of bream caught in Lake Dłużek contained significantly the lowest amounts of cadmium ($p \leq 0.05$) – similar to the concentrations in the predatory fish from Lake Łańskie. The highest mean concentration of cadmium in the fish caught in Lake Dłużek was found in the muscles of perch (0.0028 mg/kg). As for the fish caught in the other two lakes, the lowest mean concentrations of cadmium were found in the muscles of pike and bream from Lake Pluszne (0.0023 mg/kg) and vendace from Lake Maróz (0.0019 mg/kg). The highest mean concentrations of cadmium in the muscles of fish from Lake Maróz and Pluszne were found in vendace (0.0030 mg/kg) and pike (0.0036 mg/kg). The interspecific differences of the concentrations of cadmium in the fish from the three lakes were not always statistically significant ($p > 0.05$).

Total mercury

Higher contents of mercury were found in the case of predatory fish than non-predatory fish (Figure 2c). The highest mean concentration of mercury ($p \leq 0.01$) recorded in the muscles of fish was 0.309 mg/kg, 0.418 mg/kg and 0.372 mg/kg (perch from Lakes Pluszne, Dłużek and Maróz, respectively) and 0.291 mg/kg (pike from Lake Łańskie) The lowest mean concentrations of mercury ($p \leq 0.01$) were found in the muscles of bream; *i.e.* 0.041 mg/kg (Łańskie), 0.035 mg/kg (Pluszne), 0.025 mg/kg (Dłużek) and 0.030 mg/kg (Maróz).

DISCUSSION

Lead

The same fish species from lakes of the Olsztyn Lake District contained more lead in their muscles than those caught in Danish lakes. Lead (0.02 and 0.05 mg/kg) in Danish freshwater fish seem not to pose any problems to the consumers [Andersen *et al.*, 1986]. Dobicki [1990] indicated much higher concentrations of lead in the muscles of roach (2.34 mg/kg), bream (4.26 mg/kg) and perch (2.71 mg/kg) caught in the river Oława (Poland), as compared to the same fish species, included in this study (Figure 2a). These concentrations considerably exceeded the acceptable limit (1 mg/kg), which disqualified the fish in terms of their usability for consumption. The mean content of lead in the muscles of bream and perch from the lakes of the Olsztyn Lake District was also much lower than the mean concentration of Pb in the muscles of bream (0.705 mg/kg) and perch (1.375 mg/kg) caught in 5 lakes situated in the Łęczyńsko-Włodawskie Lake District [Litwińczuk *et al.*, 2000]. The same authors observed that the concentration of lead in the muscles of about 16% of the fish, including the muscles of perch, was higher than 2 mg/kg. The mean concentration of lead in the fish covered by this study did not exceed the acceptable values (0.2 mg/kg). In separate cases, these values were only exceeded in the muscles of roach from Lake Dłużek and in the muscles of perch from Lake Dłużek and Lake Maróz (Figure 2a). In the case of muscle tissue of fish caught in carp ponds of the Barycza drainage area, the contents of lead (0.10–3.52 mg/kg) also exceeded permissible limit for consumer fish [Szulkowska-Wojaczek *et al.*, 1992].

The higher levels of Pb (0.22 mg/kg – 0.99 mg/kg) to those reported in the present study were found by Perkowska & Protasowicki [2000]. Muscle tissue of the studied fish (Figure 2a) also contained a lower lead content than in the Lake Dąbie, the Odra mouth and the southern part of the Szczecin Lagoon [Protasowicki, 1991]. High concentrations of lead (0.48 mg/kg – 1.12 mg/kg) in muscles of pike in five ponds in Latvia were reported by Kļaviņš *et al.* [1998]. Anthropogenic pollution of other Latvian lakes (Kisezers, Juglas and Liepajas) was the cause of perch muscle contamination with lead (2.62 mg/kg, 4.12 mg/kg and 3.29 mg/kg). The concentration of lead in the muscles of perch caught in the unpolluted lakes of Latvia, which ranged from 0.28 mg/kg (Dreimanu) to 1.86 mg/kg (Alūksnes). The values are quite different from those found in perch and pike caught in the lakes of the Olsztyn Lake District (Figure 2a).

Cadmium

Significant differences between the predatory and non-predatory fish existed only in the species caught in Lake Łańskie ($p \leq 0.01$). Srebočan *et al.* [1997] did not observe significant differences between the concentration of cadmium in the muscles of predatory and non-predatory fish caught in ponds and rivers. Although the mean concentrations of cadmium found in the muscles of the fish did not exceed the highest acceptable values (0.1 mg/kg), its concentration in 11.3% of samples were higher than this value. Litwińczuk *et al.* [2000] did not find any significant interspecific differences ($p > 0.05$) between roach, bream and perch. Comparing the mean concentrations of cadmium in the muscles of these species from Lakes Pluszne and Maróz (Figure 2b) also confirmed the absence of any significant differences ($p > 0.05$).

Higher cadmium contents (0.032 mg/kg – 0.044 mg/kg and 0.01 mg/kg – 0.04 mg/kg, respectively) as compared to the present study were observed by Protasowicki [1991] and Perkowska & Protasowicki [2000]. The level of contamination of fish with cadmium is largely affected by environmental pollution. An example of such an impact is a high concentration of the element (from 0.38 mg/kg to 0.44 mg/kg) in the muscles of roach, bream and perch from the drainage area of the Oława River [Dobicki, 1990]. The values were higher than the acceptable limit for cadmium in fish (0.05 mg/kg). None of the investigated samples of fish (Figure 2b) exceeded acceptable values for cadmium [Journal of Statues, 2003]. The muscles of roach, bream, whitefish, perch and pike from Danish lakes did not pose any threat to humans in terms of contamination with cadmium (0.0 mg/kg – 0.045 mg/kg) [Andersen *et al.*, 1986]. According to the authors, the mean concentrations of cadmium in the muscles of roach, bream and perch were similar. A lower concentration of cadmium (0.002 mg/kg) in the muscles of whitefish from these lakes was similar to the mean concentration of the element in the muscles of whitefish from Lake Maróz (Figure 2b). Low mean concentrations of cadmium in the muscles of pike from the Vermeulle (0.001 mg/kg) and Dollier reservoirs (0.003 mg/kg) were measured by Belinsky *et al.* [1996]. Polak-Juszczak [2003] observed similar values in the muscle tissue of bream, roach and perch living in Wiślany Bay and Szczeciński Bay. Whereas, the mean contents of Cd in muscles of predatory and non-predatory fish caught in Vistula River near Włocławek were 0.0066 mg/kg and 0.0043 mg/kg respectively [Stężycka *et al.*, 2003].

Total mercury

Predatory fish (Figure 2c) accumulated much higher levels of mercury than the non-predatory fish ($p \leq 0.01$). The only exception were the perch from Lake Łańskie ($p \leq 0.05$). Svobodová *et al.* [1999] postulated that there is a relationship between the accumulation of mercury and the length of the food chain ($p < 0.05$): predatory fish (*Silurus glanis*, *Esox lucius*) > benthophagous with substantial portion of fish in food (*Perca fluviatilis*) > typical benthophagous (*Abramis brama*, *Blicca bjoerkna*, *Carassius auratus*) > planktonophagous fish species (*Rutilus rutilus*, *Scardinius erythrophthalmus*). Other authors also confirm that contamination of the fish muscles largely depends on the feeding manner [Amundsen *et al.*, 1997; Voigt, 2000]. The relationship was not confirmed in the study of Szulkowska-Wojaczek *et al.* [1998], who found the mean concentration of mercury in the muscles of perch, roach and bream to reach respectively: 0.874 mg/kg, 1.132 mg/kg and 0.915 mg/kg. According to the authors, the level of accumulation of mercury in the fish tissues is affected by multiple environmental and biological factors.

Żarski *et al.* [2000] observed not only interspecific differences of mercury in muscles, linked mainly to the manner of feeding, but also environmental variability. According to the authors, the muscles of roach caught in the mid-stream Vistula, between the Narew and the Bzura contained less mercury (0.1694 mg/kg) than roach from lakes (0.2452 mg/kg). The above values are higher than those recorded for the roach from 4 lakes of the Olsztyn Lake District (Figure 2c). The studies conducted by other researchers provide grounds for the conclusion that the concentration of mercury in muscles is affected by the fish's habitat [Cattaneo *et al.*, 1988; Protasowicki, 1991; Srebočan *et al.*, 1993; Voigt, 2000]. Stężycka *et al.* [2003] found respectively the mercury in muscles of predatory and non-predatory fish at the mean level of 0.271 mg/kg and 0.175 mg/kg.

The differences, expressed as the values of standard deviations, show that predatory fish from some lakes (Figure 2c) may contain more mercury than is acceptable – the values currently in force in Poland being 0.5 mg/kg (e.g. perch from Lakes Pluszne, Dłużek and Maróz). The level of mercury, higher than the standard recommended by the World Health Organisation (0.5 mg/kg), was also measured by Belinsky *et al.* [1996] during the study of pike from selected water reservoirs in Quebec. Rehulká [2001] examined the fish caught in three reservoirs (the Czech Republic) and found that the highest acceptable levels of mercury concentration (0.5 mg/kg for predatory fish and 0.1 mg/kg for non-predatory fish) had been exceeded. Values of mercury in the muscle of fish from the water reservoir of Kružberk ranged from 0.289 to 1.560 mg/kg (predatory fish) and from 0.154 to 0.360 mg/kg (non-predatory fish). The concentrations of mercury in the muscles of the predatory fish from reservoir of Šance, were higher than 1 mg/kg (1.018–1.900 mg/kg), whereas the proportion of fish from the reservoir of Morávka in which the highest acceptable levels were exceeded was 50%. In a later study, the same author found the highest acceptable values to be exceeded in the muscles of 78% of the fish from the reservoir of Slezská Harta [Rehulká, 2002].

On the other hand, Perkowska & Protasowicki [1999] received low values of mercury (from 0.006 mg/kg to 0.032 mg/kg) in the fish muscles from Świdwie Lake. Polak-Juszczak [2003] showed low mean mercury contents in muscle of roach and perch caught in Wiślany Bay (0.047 mg/kg and 0.060 mg/kg, respectively) and Szczeciński Bay (0.055 mg/kg and 0.052 mg/kg, respectively).

CONCLUSIONS

The results of this study into the contamination of selected fish species with mercury have confirmed the observations made by previous authors who found that this element accumulates in much higher concentrations in the muscles of predatory fish (perch and pike). However, no significant effect has been found of a varying feeding manner (non-predatory and predatory fish) on the concentration of lead and cadmium in the muscles of fish. In light of the Regulation of the Ministry of Health in force since the previous year, which excludes perch from the group of

fish with elevated concentration of mercury as compared with the non-predatory fish, a problem arises regarding the relatively frequent occurrence of excessive amounts of this element in the muscles of large perch. In terms of lead concentration, the highest acceptable values were exceeded only in single individuals of such species as perch and roach.

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PORÓWNANIE ZAWARTOŚCI METALI CIĘŻKICH W TKANCE MIĘŚNIOWEJ NIEKTÓRYCH GATUNKÓW RYB Z JEZIOR PÓŁNOCNO-WSCHODNIEJ POLSKI

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Zawartość ołowiu, kadmu i rtęci oznaczono w tkance mięśniowej sześciu gatunków ryb takich jak: płoć (*Rutilus rutilus* L.), leszcz (*Abramis brama* L.), okoń (*Perca fluviatilis* L.), szczupak (*Esox lucius* L.), sielawa (*Coregonus albula* L.) i sieja (*Coregonus lavaretus* L.). Ryby odławiano w wybranych jeziorach północno-wschodniej Polski (Łańskie, Pluszne, Dłużek i Maróz), w okresie od października 1999 do października 2000. Nie stwierdzono zależności pomiędzy stężeniem ołowiu i kadmu w badanych rybach a sposobem ich odżywiania się, z wyjątkiem zawartości kadmu w rybach z jeziora Łańskiego ($p \leq 0.01$). W większości przypadków, zawartość rtęci była większa w mięśniach ryb drapieżnych aniżeli w tkance mięśniowej ryb spokojnego żeru ($p \leq 0.01$). W przypadku pojedynczych osobników (okoń z jeziora Dłużek i Maróz oraz płoć z jeziora Dłużek) poziom ołowiu przekraczał dopuszczalny limit 0.2 mg/kg. Zawartość rtęci w mięśniach niektórych okoni (z wyjątkiem okoni z jeziora Łańskiego) przekraczała dopuszczalny w Polsce limit 0.5 mg/kg.