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Original Research Paper

THE CONTENTS OF Cu, Zn, Fe AND Mn IN SLOVENIAN FRESHWATER FISH

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Summary: From 1999 to 2003, 141 samples of freshwater fish from various Slovenian rivers and brooks were analysed for their content of Cu, Zn, Fe and Mn. The concentrations of elements were determined in the muscle/skin (in natural proportion), head, liver and kidney. Brown trout *(Salmo trutta m. fario)*, marble trout *(Salmo marmoratus)*, brook trout *(Salevelinus fontinalis)*, rainbow trout *(Oncorhynchus mykiss)*, grayling *(Thymallus thymallus)*, chub *(Leuciscus c. cephalus)*, nase *(Chondrostoma nasus)*, Danube roach *(Rutilus pigus virgo)* and barbel *(Barbus barbus)* were examined. Flame atomic absorption spectrometry was used. The distribution of trace elements in various tissues was studied. It was found that the distribution is specific for each element and also varied by fish species. The contents of elements were the lowest in fish muscle for all species. The target tissues for the elements examined are the metabolically active ones, such as the liver and kidney.

Key words: food contamination; food analysis; copper; zinc; iron; manganese; spectrometry; fishes

Introduction

Fish represent a high quality source of dietary protein, but could lose these properties due to environmental contamination. Contaminants can be introduced into the environment through natural sources, industry, urbanisation and intensive agriculture. In aquatic ecosystems, heavy metals have received considerable attention due to their toxicity and accumulation. Some metals are toxic for living organisms even at low concentrations. Others are essential and necessary in trace amounts for the functioning of biological systems but can also be toxic at higher concentrations (1, 2). Aquatic organisms, especially fish located at the end of the aquatic food chain, are widely used for biological monitoring variation of environmental levels of anthropogenic pollutants (1, 2, 3, 4, 5). Fish receive trace elements, either directly from the water through their grills, or indirectly from food through the alimentary tract (2). Together with biological factors such as feeding behaviour and interactions between elements, chemical factors such as acidity, buffer capacity, and the presence of calcium and organic compounds in water may influence the bioavailability and accumulation of heavy metals in fish (2, 6, 7).

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The aim of this study was to determine the content of Cu, Zn, Fe and Mn in the muscle/skin in natural proportion, heads, livers and kidneys of freshwater fish caught in Slovenian rivers and brooks. The distribution of elements in the fish body and the differences in level of accumulation in various fish species were investigated.

Material and methods

Samples

From 1999 to 2003, 141 freshwater fish samples caught in various Slovenian rivers and brooks were analysed. The fish were sampled by local fisherman and sent to the laboratory either fresh or frozen. Samples were sorted by date and location of catch, fish species, length and weight. 74 samples of various species of salmonids such as brown trout (*Salmo trutta m. fario*), marble trout (*Salmo marmoratus*), brook trout (*Salevelinus fontinalis*) and rainbow trout (*Oncorhynchus mykiss*), 23 samples of chub (*Leuciscus c. cephalus*), 12 samples of nase (*Chondrostoma nasus*), 9 samples of Danube roach (*Rutilus pigus virgo*), 12 samples of grayling

Table 1: Fish species, rivers and number of samples analysed

River \ Species		Salmonids	Chub	Nase	Danube roach	Grayling	Barbel	Total
Sava	I	13	3	1		3	3	**23**
Javornik		3						**3**
Tržiška Bistrica		4						**4**
Kokra		1						**1**
Sora		6	2	2		2	2	**14**
Kamniška Bistrica[a]		2	1					**3**
Ljubljanica		2	12	2	5	7		**28**
Obrh		1						**1**
Bober (brook)		7						**7**
Savinja		2	3	3			3	**11**
Sopota (brook)		2						**2**
Krka					2			**2**
Lahinja and Krupa		1	1	1	1			**4**
Drava	II		1	3	1		2	**7**
Bistra		1						**1**
Meža		6						**6**
Šentanelska reka		2						**2**
Soča[b]	III	11						**11**
Nadiža		2						**2**
Idrijca		6					1	**7**
Hubelj		1						**1**
Rižana		1						**1**
Total		**74**	**23**	**12**	**9**	**12**	**11**	***141***

[a] including the Debenski potok and Titanova mlinščica brooks
[b] including the Lepena and Doblarec brooks

(*Thymallus thymallus*) and 11 samples of barbel (*Barbus barbus*) were examined in the study. The number of samples, fish species and location of catch (name of open water) are listed in Table 1.

The rivers and brooks in Table 1 are sorted in three groups (I, II, III). Group I includes the Sava River with subsidiary streams and Group II includes the Drava River with subsidiary streams. The rivers and brooks in the first two groups flow to the Black Sea. In Group III are rivers and brooks that flow to the Adriatic Sea. From 1 to 3 fish were prepared per sample, according to the procedure described in the Association of Official Analytical Chemists (8). Before preparation the fish were washed, dried and scaled. For each fish, the head, liver and kidney were sampled and fillets in natural proportion were prepared from muscle and skin. The fillets were then minced with a Büchi-400 homogenizer. Different tissues were packed separately and kept below –18° C until the day of analysis. Some of the fish samples received were already dissected and without viscera and/or heads, and in those cases only the muscle/skin was prepared. In some cases only the liver and kidney were received, but for all of them the data regarding species, weight, length, date and location of catch were available.

Reagents

Standard solutions of Cu, Zn, Fe and Mn were prepared from commercial stock standard solutions (Merck) at concentrations of 1000 mg/L. Double deionised water was used throughout. Working standard solutions were prepared by dilution of stock standard solution with the addition of hydrochloric acid, so that the acid concentration in working standard solutions matched the acid concentration in digested solutions. All reagents used were an analytical-reagent grade.

Sample preparation

10 g of muscle/skin or whole head, liver or kidney were weighed in quartz-glass crucibles, dried

Table 2: Results of analyses of certified reference materials. Mean and standard deviation in mg/kg. Number of analysis in parentheses

Metal		BCR N°185R	BCR N°422	BCR N°186
Cu	Found	0.533 ± 0.021 (6)	0.84 ± 0.06 (4)	28.8 ± 1.2 (5)
	Certified	0.544 ± 0.017	1.05 ± 0.07	31.9 ± 0.4
Zn	Found	128.6 ± 2.6 (6)	19.2 ± 0.4 (6)	119 ± 7 (6)
	Certified	138.6 ± 2.1	19.6 ± 0.5	128 ± 6
Fe	Found	/	4.66 ± 0.59 (5)	278 ± 17 (6)
	Certified	/	5.46 ± 0.30	299 ± 10
Mn	Found	10.66 ± 0.24 (6)	0.512 ± 0.023 (6)	8.0 ± 0.5 (6)
	Certified	11.07 ± 0.29	0.543 ± 0.028	8.5 ± 0.3

in a drying oven at 105° C and ashed overnight at 450° C ± 25° C in a programmable furnace. The ash was dissolved with diluted hydrochloric acid (1/1, v/v). The solution was evaporated to dryness. The remains were redissolved with diluted hydrochloric acid (1/10, v/v), filtered into a 50 mL volumetric flask and diluted with water to the mark.

Sample analysis

The Cu, Zn, Fe and Mn concentrations in the samples were determined by flame atomic absorption spectrophotometry (FAAS), using a Varian SpectrAA 220 instrument with deuterium background correction. The determinations were preformed by aspirating the final solutions into an AA spectrophotometer using an air-acetylene flame. Absorption wavelengths were as follows: 324.8 nm for Cu, 213.9 nm for Zn, 248.3 nm for Fe and 279.5 nm for Mn. Concentrations of metals in samples were evaluated using an external standard method.

The accuracy of the analytical procedure was checked by analyzing three different standard reference materials: cod muscle BCR 422, bovine liver BCR 185R and pig kidney BCR 186. The agreement for all the elements investigated was 80–98%. The results of analysis of standard reference material are shown in Table 2.

A *t-test* was used to statistically evaluate the sample data. A *p* value lower than 0.05 was considered to be significant. If the concentration of a metal was below the detection limit, a value of half of the detection limits was used in order to facilitate statistical comparisons

Results

The contents of the metals examined in various tissues of various fish species are given in Table 3.

The data in Table 3 reveal low levels of Cu and Mn in tissues and higher levels of Zn and Fe. The lowest concentrations of all elements were found in muscle/skin. The average concentrations of Cu in tissues of various fish species are shown in Fig. 1.

The highest concentrations of Cu were always found in the liver, followed by the kidney, head and muscle/skin. The ratio between the level of Cu in the liver and muscle/skin was particularly high in salmonids. The concentration of Cu in the liver of other fish species was approximately ten times lover than in salmonids and we did not observe any significant differences between them. The kidney, head and muscle/skin of barbel contained significantly higher levels of Cu than chub, nase, Danube roach and grayling. The kidney and muscle/skin, but not the head, of salmonids also contained significantly higher concentrations of Cu than the species mentioned above.

The average concentrations of Zn in the tissues of various fish species are shown in Figure 2.

Chub and Danube roach contained the highest amount of Zn in the kidney, and nase, grayling and barbel in the head. Salmonids contained an equal amount, and the highest amount of Zn in the head and liver. The kidney of barbel, nase, salmonids and grayling contained on average less than 25 mg Zn/kg. Higher concentrations were detected in the kidney of chub and the highest in the kidney of Danube roach. In the liver, concentrations of Zn were significantly higher in Danuble roach and chub than in barbel, nase and grayling. In the muscle/skin, the highest concentrations were also detected in Danube roach and the lowest in barbel and grayling. In the head, higher concentrations of Zn were detected in chub, nase and Danube roach than in salmonids, grayling and barbel.

Figure 3 presents the average concentrations of Fe in the tissues of various fish species, from

Table 3: Cu, Zn, Fe and Mn contents in tissues of various fish species, expressed in mg/kg wet weight

		Muscle and skin			Head			Liver			Kidney		
		N	min. - max.	average ± SD	N	min. - max.	average ± SD	N	min. - max.	average ± SD	N	min. - max.	average ± SD
Cu	Salmonids	60	<0.05 - 1.38	**0.36** ± 0.19	48	<0.05 - 0.82	**0.32** ± 0.17	42	3.43 – 324	**75** ± 69	38	0.29 – 4.02	**1.67** ± 0.87
	Grayling	12	<0.05 - 0.26	**0.17** ± 0.07	12	0.14 – 0.35	**0.23** ± 0.08	10	0.48 – 9.77	**2.63** ± 2.62	9	0.30 – 0.80	**0.57** ± 0.16
	Chub	15	<0.05 - 0.43	**0.22** ± 0.12	16	0.10 – 0.55	**0.32** ± 0.15	16	<0.06 – 8.39	**2.31** ± 2.36	14	<0.10 – 1.17	**0.50** ± 0.34
	Nase	12	<0.05 - 0.62	**0.30** ± 0.17	10	0.28 – 0.58	**0.40** ± 0.11	3	2.67 – 6.28	**4.09** ± 1.93	4	0.19 – 0.63	**0.44** ± 0.21
	Danube roach	8	0.11 - 0.30	**0.21** ± 0.08	5	0.18 – 0.45	**0.30** ± 0.13	5	0.37 – 2.32	**1.35** ± 0.87	4	0.21 – 0.78	**0.43** ± 0.25
	Barbel	10	<0.05 - 0.54	**0.37** ± 0.18	10	0.22 – 1.01	**0.53** ± 0.22	7	0.40 – 7.03	**2.31** ± 2.20	6	0.44 – 1.63	**0.94** ± 0.50
Zn	Salmonids	60	5.2 - 17.6	**9.7** ± 2.5	48	19.5 - 62.1	**33.9** ± 10.9	42	10.6 – 87.2	**35.3** ± 13.5	38	14.8 – 46.9	**23.1** ± 5.8
	Grayling	12	5.3 - 12.2	**8.5** ± 2.5	12	22.6 – 44.1	**33.5** ± 7.0	10	15.7 – 27.7	**22.4** ± 3.9	9	12.6 – 20.0	**15.4** ± 2.2
	Chub	15	4.4 - 13.3	**9.3** ± 2.3	16	12.8 – 80.8	**50.0** ± 17.4	16	13.6 – 81.0	**43.1** ± 18.7	14	50.2 – 404	**112** ± 93
	Nase	12	6.2 - 12.0	**9.4** ± 1.7	10	26.8 – 58.4	**42.7** ± 9.2	3	19.1 – 23.9	**21. 5** ± 2.4	4	17.8 – 22.5	**20.3** ± 2.2
	Danube roach	8	6.2 - 18.5	**12.8** ± 4.5	5	26.8 – 49.1	**41.7** ± 9.5	5	25.2 – 78.3	**54.1** ± 2.3	4	115 – 387	**270** ± 118
	Barbel	10	5.8 - 10.2	**7.8** ± 1.5	10	22.4 – 34.9	**30.1** ± 3.8	7	16.7 – 30.9	**20.4** ± 4.9	6	10.6 – 25.5	**16.1** ± 5.2
Fe	Salmonids	39	2.6 - 7.7	**4.8** ± 1.5	48	5.0 - 70.2	**15.4** ± 9.6	42	54 – 501	**178** ± 104	38	61– 325	**144** ± 54
	Grayling	12	2.9 - 5.8	**4.0** ± 0.8	12	8.9 – 22.5	**15.4** ± 4.6	10	36 – 175	**95** ± 48	9	110 – 205	**165** ± 31
	Chub	14	1.9 - 6.8	**4.1** ± 1.3	16	4.5 – 96.4	**18.3** ± 21.2	16	12 – 378	**72** ± 91	14	24 – 115	**71** ± 30
	Nase	11	4.1 - 9.2	**6.2** ± 1.9	10	10.9 – 40.8	**18.2** ± 9.2	3	65 – 193	**113** ± 70	4	100 – 179	**132** ± 35
	Danube roach	8	2.9 - 9.2	**4.8** ± 2.0	5	7.4 – 36.1	**17.2** ± 11.1	5	20 – 378	**172** ± 153	4	35 – 195	**111** ± 70
	Barbel	10	3.9 - 14.4	**6.7** ± 3.4	10	6.1 – 49.2	**19.1** ± 13.7	7	67 – 235	**144** ± 65	6	35 – 422	**166** ± 135
Mn	Salmonids	42	<0.07 - 0.35	**0.17** ± 0.08	48	0.21 - 3.89	**1.11** ± 0.73	42	0.40 – 2.61	**1.01** ± 0.45	37	0.16 – 1.25	**0.42** ± 0.24
	Grayling	12	0.14 - 0.37	**0.26** ± 0.08	12	1.21 – 8.03	**4.17** ± 2.36	10	1.04 – 3.13	**1.56** ± 0.59	9	0.21 – 4.00	**1.15** ± 1.42
	Chub	15	0.12 - 0.47	**0.28** ± 0.11	16	2.02 – 6.86	**4.08** ± 1.38	15	0.40 – 5.74	**2.18** ± 1.85	14	<0.14 – 0.91	**0.60** ± 0.21
	Nase	12	0.24 - 0.86	**0.47** ± 0.18	10	5.37 – 16.30	**9.95** ± 3.44	3	1.51 –2.66	**2.19** ± 0.60	4	0.85 – 1.09	**0.95** ± 0.11
	Danube roach	8	<0.07 - 1.43	**0.34** ± 0.45	5	1.52 – 6.72	**3.84** ± 2.18	4	0.82 – 1.42	**1.15** ± 0.31	4	0.42 – 0.77	**0.61** ± 0.14
	Barbel	10	0.26 - 1.20	**0.62** ± 0.31	10	2.72 – 12.94	**6.34** ± 2.96	7	0.46 – 2.45	**1.12** ± 0.71	6	0.29 – 8.09	**1.95** ± 3.03

N - number of samples

which it is evident that Fe accumulates in the liver and kidney. Salmonids and Danube roach contained higher concentrations of Fe in the liver than in the kidney, but barbel, nase and grayling had higher concentrations in the kidney than in the liver. Chub contained on average an equal amount of Fe in the liver and kidney and significantly lower concentrations than in other fish species. In the muscle/skin, the concentrations of Fe were significantly higher in nase and barbel than in chub, Danube roach, salmonids and grayling. No significant differences were observed in Fe concentrations in the head.

The average concentration of Mn in tissues of various fish species is presented in Figure 4. The highest concentrations of Mn were observed in the head of all fish species, followed by the liver, kidney and muscle/skin. All tissues of salmonids contained significantly lower levels of Mn than other fish species examined. Between chub, Danube roach and grayling no differences were observed in the content of Mn in the head, but significantly higher concentrations were detected in the head of nase and barbel. Significant differences between fish species in Mn contents were also observed for the muscle/skin, liver and kidney.

Discussion

The distributions of trace elements in tissues were specific for each element. The content of Cu, Zn, Fe and Mn in all fish species were the lowest in the muscle/skin. Similar results were also reported for a number of other fish species. Muscle is not an active tissue regarding the accumulation of metals (2, 3, 9). The contents of Fe and Zn in tissues were higher than the contents of Cu and Mn. These results were expected because Zn is present in many enzymes in the fish's organism and Fe is used to transport oxygen throughout the fish's body. The elements accumulate mainly in metabolically active tissues such as the liver and kidney, with the exception of Mn, which accumulates in the head. In the liver and kidney, metals are bound to metallothioneins, low molecular weight proteins with a high cysteine content. Metallothioneins are synthesized in the liver and kidney, and also in the gills after waterborne and dietary exposure and are considered to be involved in regulation of essential metals as well as Cd and Hg (1, 4, 5, 9). For all the elements studied, significant differences in concentration levels were found between various fish species, demonstrating that the concentration levels attained are species-related. The highest differences between fish species were observed in Cu content in the liver. Salmonids had approx-

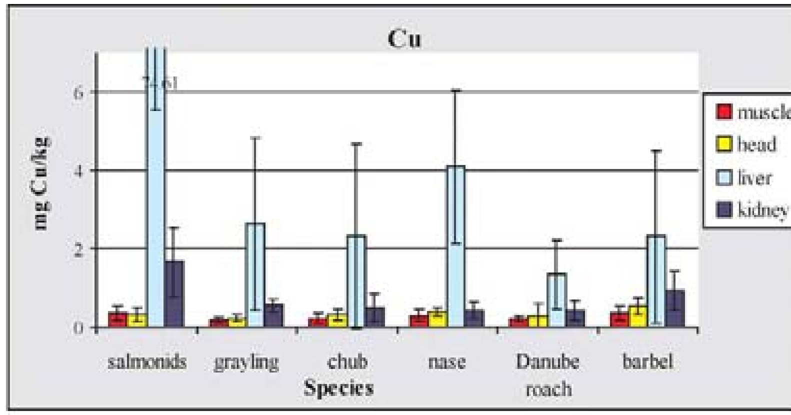

Figure 1: Average concentrations of Cu in tissues of various fish species (mg/kg wet weight)

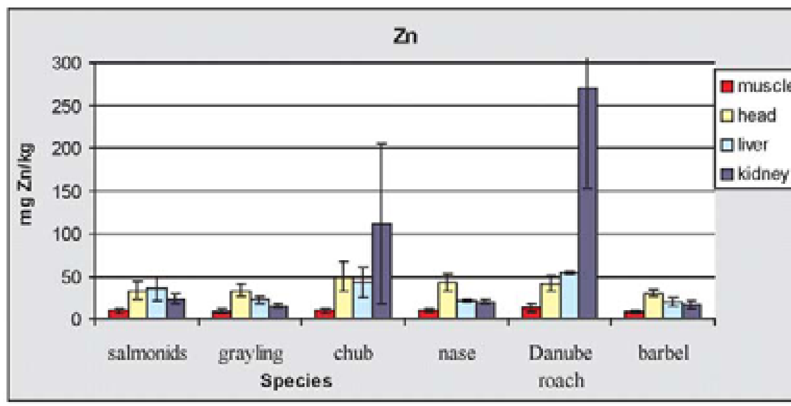

Figure 2: Average concentrations of Zn in tissues of various fish species (mg/kg wet weight)

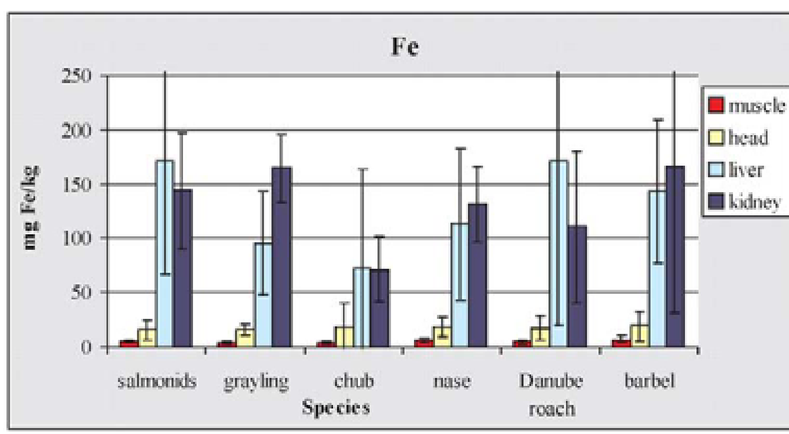

Figure 3: Average concentrations of Fe in tissues of various fish species (mg/kg wet weight)

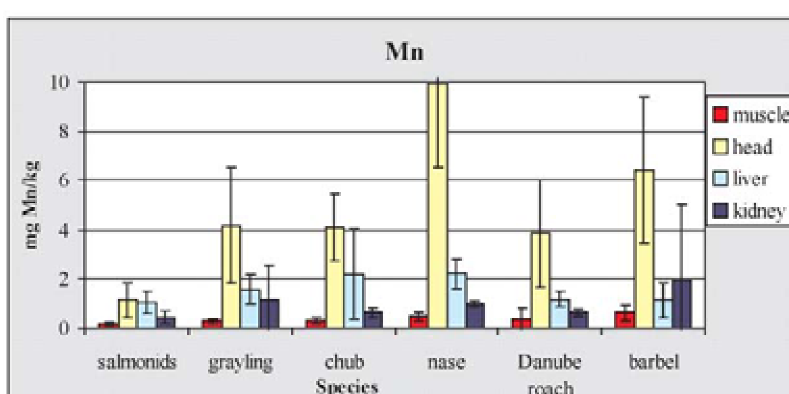

Figure 4: Average concentrations of Mn in tissues of various fish species (mg/kg wet weight)

imately an order of magnitude higher concentration than other fish species, which was also reported by Amundsen et al. (2). Various data in the literature indicate that the liver of brown trout from contaminated rivers contained around 200 to 300 mg Cu/kg wet weight (2, 6, 7). In our research only two samples of salmonids that were analysed contained more than 200 mg Cu/kg in the liver. Those samples were from a stream in the Soča Valley. However, Farkas et al. (11) reported that Cu concentration in the liver also increases with increasing length and weight of fish. The fish from the Soča Valley were much larger (1.2 kg and 3 kg) than the fish from contaminated water analysed by Olsvink et al. (6, 7), which weighed from 13-91 g. It could be concluded that the cause of the high amount of Cu in our samples is the weight of fish. Fish feeding on invertebrates have been found to have higher concentrations of Zn than piscivorous species (2). Our results largely support these observations, because Danube roach and chub had higher levels of Zn, especially in the kidney, than barbel and nase. All these fish species belong to family *Cyprinidae.* The Danube roach feeds on worms, molluscs, crustaceans and considerable amounts of plant matter, the chub feeds on algae and also on worms, molluscs, crustaceans as well as on small fish, but the nase feeds mostly on algal growth on stones (12). The highest concentrations of Zn in the kidney of Danube roach and chub were detected in fish from the Ljubljanica. These chubs were around 25 cm long, but larger chubs from the Ljubljanica contained lower values of Zn. Because the Zn concentrations in tissue decreased significantly with increasing length of the fish (2, 11), high levels of Zn in small chub and Danube roach are probably not a consequence of contamination. In all salmonid tissue, the levels of Mn were lower than in tissues of other fish species. Karadede and Ünü (9) also reported that Mn was not accumulated in salmonids. Cu, Zn, Fe and Mn are essential elements and are carefully regulated by physiological mechanisms in most organs. The differences observed between fish species can be explained by varying abilities for homeostatic control, detoxification and rejection, and varying fish feeding behaviour (9, 10).

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VSEBNOST BAKRA, CINKA, ŽELEZA IN MANGANA V SLADKOVODNIH RIBAH V SLOVENIJI

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Povzetek: V obdobju od leta 1999 do 2003 smo v 141 vzorcih sladkovodnih rib iz različnih slovenskih rek in potokov določili vsebnost Cu, Zn, Fe in Mn. Določili smo jo v mesu s kožo (v naravnem razmerju), v glavah, jetrih in ledvicah rib. Analizirali smo tkiva potočne postrvi *(Salmo trutta m. fario)*, soške postrvi *(Salmo marmoratus)*, potočne zlatovčice *(Salevelinus fontinalis)*, šarenke *(Oncorhynchus mykiss)*, lipana *(Thymallus thymallus)*, klena *(Leuciscus c. cephalus)*, podusti *(Chondrostoma nasus)*, platnice *(Rutilus pigus virgo)* in mrene *(Barbus barbus)*. Vsebnost elementov v tkivih smo določili z metodo plamenske atomske absorpcijske spektrometrije. Na podlagi rezultatov analiz smo proučili porazdelitev elementov po posameznih tkivih rib in ugotovili, da je le-ta specifična za vsak element in odvisna od vrste ribe. Največje razlike med vrstami rib smo opazili v vsebnosti Cu v jetrih in Zn v levicah. Koncentracija Cu v jetrih salmonidov je bila približno desetkrat višja (povprečje 74,61 mg/kg) kot v jetrih ostalih vrst rib. Ledvice salmonidov, lipanov, podusti in mren so vsebovale v povprečju manj kot 25 mg Zn/kg, ledvice klena povprečno 112 mg Zn/kg in platnice 271 mg Zn/kg. Razlike v vsebnosti Zn v ledvicah omenjenih vrst rib so najverjetneje posledica različnega načina prehrane. Ugotovili smo, da je bila vsebnost vseh preiskovanih elementov najnižja v mesu rib. Elementi se akumulirajo v metabolično aktivnih tkivih, kot so jetra in ledvice, z izjemo Mn, katerega najvišje koncentracije smo ugotovili v glavah rib (območje 0,21 - 16,30 mg/kg).

Ključne besede: hrana, kontaminacija; hrana, analize; baker; cink; železo; mangan; spektrometrija; ribe