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Original Scientific Article

THE HEAVY METAL CONTENTS (Cd, Pb, Cu, Zn, Fe AND Mn) AND ITS RELATIONSHIPS WITH THE SIZE OF THE RUDD (*Scardinius erythrophthalmus*) FROM LAKE CERKNICA, SLOVENIA

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Summary: The concentrations of Pb, Cd, Cu, Zn, Fe and Mn were measured in different tissues of rudd (*Scardinius erythrophthalmus*) from Lake Cerknica, Slovenia. The content of heavy metals in rudd muscle/skin was low; therefore, rudd from Lake Cerknica are suitable for human consumption. Among the heavy metals studied, Pb was not detected in rudd tissues and Cd was undetectable in meat samples. The content of other elements was also the lowest in the meat samples. The highest concentrations of Cd and Zn were found in the kidneys, and those of Cu, Mn and Fe in the livers. The relationships between fish weight or length and metal concentrations were investigated. In the meat and liver of the rudd, the Pearson correlation analysis for Zn and Mn revealed a negative association related to size (length, weight). However, the concentrations of Fe in kidney and Cd in liver and kidney increased with the size of rudd.

Key words: lead; cadmium; copper; zinc; iron; manganese; rudd; size

Introduction

In aquatic ecosystems, heavy metals merit considerable attention due to their toxicity and accumulation in biota. High levels of heavy metals in freshwater environments may occur due to the natural weathering of minerals in sediment and bedrock, or as a result of anthropogenic activities, such as mining. Some of these elements are toxic to living organisms even at low concentrations; others are biologically essential and only become toxic at high concentrations. Fish are at the top of the aquatic food chain and, therefore, are often used as bio-indicators of water pollution with heavy metals (1-6).

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However, when fish are used as bio-indicators for water pollution, the obtained results must be correctly interpreted, taking into consideration several factors that may affect the accumulation of different pollutants in the body. For example, the bioaccumulation of heavy metals in fish is related to the physical and chemical properties of water, such as salinity, pH value, hardness, temperature and concentration of organic material in water. The eating behavioural patterns, age, sex, spawning of the fish, as well as the season of fish capture were also found to be essential for metal accumulation in fish tissues (1-3, 6).

The aim of our study was to determine the amount of lead (Pb), cadmium (Cd), copper (Cu), zinc (Zn), manganese (Mn) and iron (Fe) in different tissues of the rudd (*Scardinius erythrophthalmus*) from Lake Cerknica. Concentrations of the above-mentioned elements were measured in livers, kidneys and muscle tissue with skin. In this research, the relationship between fish size and metal concentrations in different tissues were also studied.

Materials and methods

In this study, 30 rudd (*Scardinius erythrophthalmus*) caught in the Lake Cerknica were analysed. Rudd is a benthic cyprinid fish with omnivorous feeding habits. The rudd diet primarily contains various macrophytes, bryophytes, and filamentous algae along with some animal material and detritus. Rudd show a size-dependent diet shift from microcrustaceans while small, to macro-invertebrates at larger sizes. The lipid content in fish affects metal concentration in the tissues (7). In order to avoid this effect, the rudd were sampled in a one-month period in September 2004. Lake Cerknica is a large intermitted lake situated in the southwest of Slovenia. Its area usually reaches 28 km^2 but can also reach up to 38 km^2. According to the literature (8), water in the Lake Cerknica had in year 2003 pH between 7.9 and 9.1. The content of metals in water was mostly below the limit of quantification. The sediment in the lake contained measurable quantities of metals. However, the levels were low except for a slightly higher content of Cd. Nevertheless, the sediment of the tributary named Cerkniščica contained slightly higher levels of Cu, Zn and Pb.

The concentration of Pb, Cd, Zn, Cu, Mn and Fe content in fish tissues were determined with a Varian SpectrAA 220 flame atomic absorption spectrophotometer, using deuterium background correction. The collected fish were rinsed with water. The total body length (cm) and body weight (g) of each fish were recorded. The age of fish was determined from scale samples according to the annual ring structure (Table 1).

The scales, head with gills and abdominal contents were removed. From the abdominal contents, the liver and kidney were separated. Muscle with skin was homogenized without bones. Until the analysis, the samples were stored separately in tightly closed containers at -18°C. Each of studied tissues (whole liver, whole kidney and 10 g of homogenized muscle) was weighed in a quartz crucible. Samples were dried in a drying oven at 103°C and then put into a cold programmable furnace. The temperature in the furnace was increased slowly at a maximum rate 50°C/h to 450°C and left at this temperature overnight. Ash was wetted with 5 mL of water and 5 mL concentrated hydrochloric acid, and the solution was evaporated on a hot plate. The residue was dissolved in 50 mL of diluted hydrochloric acid (0.25 M). The contents of Zn, Cu, Mn and Fe were measured directly from the acidic solution of ash. An external calibration curve was used for the evaluation of the results. For Pb and Cd, a pre-concentration step was used. They were determined as diethylammonium diethyldithiocarbamate complexes extracted into methylisobutylketone (9). For the evaluation of the results, the method of standard addition calibration was used. The accuracy of the methods used was checked via analysing the standard reference materials (BCR 185R Bovine liver, BCR 422 Cod muscle, BCR 184 Bovine muscle and BCR 186 Pig kidney). The recovery rates ranged from 90% to 110% for all investigated elements. The limits of quantification (LOQ) expressed as mg of metal per liter of solution obtained after digestion were 0.01 (Pb), 0.0006 (Cd), 0.006 (Zn), 0.015 (Cu), 0.02 (Mn) and 0.03 (Fe).

The Pearson correlation test was used to check for significant relationships between individual heavy metal concentration and length or weight. The level of significance was set at a probability lower than 0.05. Samples with a detectable amount of metal were used for calculations. All calculations were carried out using the Microsoft Excel program for Windows XP.

Results and discussion

The Cd, Zn, Cu, Mn and Fe contents in fish tissues are presented in Table 2. The level of Pb was below LOQ in all analysed samples. Cd was also undetectable in muscle/skin samples; therefore, all analysed samples of rudd were suitable for human consumption. According to the Commission Regulation no. 1881/2006 the maximum acceptance level of Pb and Cd in the meat of the fish is 0.30 and 0.050 mg/kg ww (wet weight), respectively (10).

The results of this study are in accordance with previous studies done in Slovenia, which showed that Pb contents in fish tissues from

Table 1: Age, weight, length and number of analysed rudd (*Scardinius erythrophthalmus)*

n	Age (years)	Weight - W (g)	Length – L (cm)
30	1–6	25.7–603.6	13.0–32.0

Table 2: Concentrations of Cd, Zn, Cu, Mn and Fe in rudd tissues

	Element	n_0	n	Concentration (mg/kg wet weight)			
				min	max	me	av
Muscle with skin	Cd	30	30	all < 0.003			
	Zn	30	30	6.8	26.0	16.0	16.4
	Cu	30	30	< 0.08	0.29	0.14	0.14
	Mn	30	30	0.11	0.32	0.20	0.20
	Fe	30	30	2.3	4.9	3.2	3.1
Liver	Cd	30	23	0.046	0.359	0.115	0.151
	Zn	29	29	34.7	366	62.8	94.0
	Cu	30	30	6.56	85.0	17.8	27.7
	Mn	30	28	0.66	5.10	1.17	1.61
	Fe	30	30	90.2	519	168	199
Kidney	Cd	29	24	0.138	1.850	0.573	0.706
	Zn	29	29	68.0	373	165	174
	Cu	29	20	0.33	1.08	0.70	0.70
	Mn	29	18	0.33	0.80	0.54	0.53
	Fe	29	29	32.2	179	104	108

n_0 – number of samples; n – number of samples were, based on the LOQ concentration of element could be determined; me – median; av – average

Slovenian rivers are generally low. The meat of rudd from the Šalek lakes, situated in the close vicinity of the Šoštanj Thermal Plant, a great source of anthropogenic emission of metals into the environment, also contained very low levels of Pb (in average 0.02 mg/kg ww) (11). In our previous study, higher levels of Pb were found only in the muscle/skin of some fish from the lower flows of the Drava (max. 1.21 mg/kg ww) and Sava rivers (max. 0.32 mg/kg ww) and in all analysed muscle/skin samples of fish from the Mežica valley (0.39–0.77 mg/kg ww), where a Pb smelter used to operate (12).

The highest concentrations of Cd were found in the kidneys, with a median level 0.573 mg/kg ww (Table 2), while the content in the livers was 0.115 mg/kg ww. The level of Cd in kidneys and livers is an important indicator of environmental pollution. In tissues, Cd binds to metalothionein, which is synthesized in the liver and then transferred to the kidneys. Higher Cd content in the kidneys than in the liver is considered to be a consequence of long-term exposure, but in the case of acute poisoning more Cd is found in the liver than in the kidneys (2). Taking into account this fact, we can conclude that Cd content is a consequence of long-term exposure. In the study on freshwater fish from Slovenian rivers, sampled in the period from 1999 to 2003 (12), only 28% of the kidneys contained more than 0.5 mg/kg ww of Cd. Higher amounts of Cd were found in fish kidneys from the lower flows of the Drava, Meža and Ljubljanica rivers. According to the data of the Agencije republike Slovenije za okolje (Slovenian

Table 3: Pearson correlation coefficient (r) and level of significance (p) of correlation between element content and mass, and element content and length of the rudd

	Weight	Length	Cu	Zn	Mn	Fe
Muscle/skin						
Cu	0.29	0.33	1.00			
Zn	–0.79***	–0.76***	–0.39*	1.00		
Mn	–0.43*	–0.48**	–0.19	0.21	1.00	
Fe	–0.18	–0.09	0.14	0.25	–0.33	1.00
Kidney						
Cu	–0.27	–0.32	1.00			
Zn	0.38*	0.28	–0.05	1.00		
Mn	0.20	0.21	–0.18	0.17	1.00	
Fe	0.46*	0.47*	0.09	0.38*	0.52*	1.00
Cd	0.66***	0.67***	–0.16	0.07	0.68**	0.56**
Liver						
Cu	–0.18	–0.19	1.00			
Zn	–0.46*	–0.50**	0.18	1.00		
Mn	–0.54**	–0.63***	0.05	0.06	1.00	
Fe	0.30	0.30	0.10	0.04	–0.30	1.00
Cd	0.57**	0.59**	–0.01	–0.33	–0.25	0.23

*** $p < 0.001$; ** $p < 0.01$; * $p < 0.05$; in cases without mark, r is not statistically different from 0.
Number of analysed samples is in Table 2.

Table 4: Regression equations relating the element content in different tissues to the weight and length of the rudd (equation is given only in cases in which the correlation is statistically significant)

	Muscle/skin	Kidney	Liver
Zn	X = –0.02 w + 22.61	X = 0.11 w + 137	X = –0.18 w + 153
	X = –0.65 l + 32.21		X = –6.65 l + 256.6
Mn	X = –0.0001 w + 0.23		X = –0.003 w + 2.6
	X = –0.004 l + 0.03		X = –0.12 l + 4.54
Fe		X = 0.06 w + 88.5	
		X = 2.08 l + 57.0	
Cd		X = 0.002 w + 0.057	X = 3x10^{-4} w + 0.02
		X = 0.068 l – 1.084	X = 0.014 l – 0.231

w – weight, l – length

Environment Agency), the Drava and Meža rivers in 2002 and 2003 contained high levels of Cd in the sediment (Drava at Ormož 1.8 mg/kg and at Maribor Island 9.7 mg/kg; Meža at Otiški Vrh 7.9 mg/kg and at Podklanc 34 mg/kg) (13, 14), so the high Cd contents can be attributed to the environmental pollution. The Cd content in the sediment of Lake Cerknica was found to be rather low (Gorenje Jezero 1.1 mg/kg, Dolenje Jezero 0.37 mg/kg and Cerkniščica 0.63 mg/kg) (8); therefore, the high content of Cd in the kidneys of the rudd in our study cannot be explained only by pollution of water environment but also by the feeding habits of the rudd. It is an omnivorous fish, feeding on zooplankton, aquatic insects, filamentous algae, higher aquatic plants, and occasionally on fish eggs or small fish. Amundsen et al. (1) found that fish feeding with invertebrates contained more Cd in the tissues than piscivorous fish. Fish that eat zooplankton, aquatic insects, fingerlings and shrimp also contained higher levels of Cd than herbivorous fish did (6). Guerrin et al. (15) analysed rudd caught in a fishpond in France, and found less Cd (0.134–0.190 mg/kg ww) in kidneys than we did, although the sediment of the French fishpond contained similar amount of Cd as Lake Cerknica did (< 1 mg/kg). However, in the study, only four samples of rudd were analysed, and they were also smaller (average weight 220 g) than the rudd in our study (average weight 381 g).

The lowest concentrations of Zn, Cu, Mn and Fe were found in muscle/skin. The highest amounts of Zn were found in the kidneys, while the highest amounts of Cu, Fe and Mn were found in the liver. Levels of Zn, Fe and Mn in rudd tissues were found to be similar to the contents found in our previous study (16) considering similar fish species, such as Danube roach (*Rutilus pigus virgo*) and chub (*Leuciscus c. cephalus*), which belongs to the carp family (Cyprinidae) and are also omnivorous the same as rudd. A somewhat different situation was found regarding Cu contents. In our study, we found higher concentrations of Cu in the rudd liver (average 27.8 mg/kg ww) than we did in the liver of the Danube roach (1.35 mg/kg ww) and chub (2.31 mg/kg ww) analysed in a previous study (16). The content of Cu in the sediment of Lake Cerknica in 2003 was low (11 mg/kg Dolenje jezero) (8) compared to the sediment of Slovenian rivers (75 mg/kg Drava – Ormož, 350 mg/kg Ljubljanica – Zalog, 45 mg/kg Soča – Solkan) (14). Therefore, the high content of Cu in the liver of rudd was probably due to the higher accumulation of this element in the rudd's liver than in the Danube roach and chub liver. However, rudd in livers accumulates lower levels of Cu than salmonids. According to literature data (1) and the findings in our study (16), salmonids accumulate very high amounts of Cu in their livers (average 75 mg/kg).

The correlation analysis between the element concentrations in the fish tissues and their size (length or weight) revealed significant relationships. In Table 3, the Pearson correlation coefficient (r) and the level of significance (p) of the relationships between the tissue metal concentrations of the rudd and their weight or length are shown. A regression equation for the tissue metal concentrations and weight or length was calculated only for the elements for which the correlation is statistically characteristic (at a 95% confidence level). Equations are represented in Table 4. Lipid content in fish decreases during winter and spring and reaches its peak at the end of main feeding period – autumn. The metal concentration in tissues is affected by lipid content (7). In order to avoid this effect, the rudd were sampled in a one-month period in September. The lipid content in fish with the same size was similar, which was confirmed by good length-weight relationship that could be described with the formula $W = 0.0046L^{3.42}$ where W is weight and L is length (r = 0.99).

The content of Zn and Mn in the muscle/skin and liver tissue decreased significantly with fish size (weight and length). However, in the kidneys, the content of Zn increased with the body weight of the fish. The content of Fe in the kidneys and the content of Cd in the kidneys and liver also significantly increased with fish size.

In the tissues of rudd, significant correlation between some elements, such as the correlation between Mn-Cd, Fe-Cd, Fe-Zn and Fe-Mn in the kidneys and between Zn-Cu in the meat of the rudd, were observed.

For the essential elements, we assume that the content in tissues is homeostatically controlled, resulting in positive or negative correlation. Accumulation of the elements in the bodies is controlled by absorption, elimination and detoxification, which are highly dependent on the rate of metabolism. The rate of fish metabolism varies with age. The ratio between the surface area and the volume of fish, fish diet and the concentration of certain biologically important compounds involved in the process of accumulation changes with the age of fish. All these factors affect the concentration of the metals in the fish tissues (18). Cu is an essential element, and most authors observed positive or negative correlation between its content in fish tissues and the size of fish (7, 19). However, in our study, the correlation was not confirmed. The negative correlation between Zn content in the muscle/skin or liver tissue and body size was also observed in other studies on other fish species (1, 7, 19). The negative correlation between the element content and the size of fish does not mean that the element is no longer absorbed by fish during growth. A negative correlation is linked with a different rate of absorption through the intestine and with the more efficient secretion of older fish. The fact that the main route of absorption is through the gills, and that gill size, relative to body size, diminishes with the size of the fish, could also be the reason for negative correlation between the element concentration and fish size.

The content of heavy metals in rudd muscle/skin was low; therefore, rudd from Lake Cerknica are suitable for human consumption. The element content in rudd tissues depends on the length and weight of fish. The concentration of elements in fish also varies among different fish species, and these two facts should be considered in comparative biomonitoring studies.

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VSEBNOST TEŽKIH KOVIN IN NJIHOVA POVEZAVA Z VELIKOSTJO RDEČEPERKE (*Scardinius erythrophthalmus*) IZ CERKNIŠKEGA JEZERA, SLOVENIJA

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Povzetek: V tkivih rdečeperke (*Scardinius erythrophthalmus*) iz Cerkniškega jezera (Slovenija) smo proučevali vsebnosti Pb, Cd, Cu, Zn, Fe in Mn. Rdečeperke iz Cerkniškega jezera so primerne za prehrano ljudi glede vsebnosti težkih kovin, saj je bila njihova vsebnost v mišičnem tkivu nizka. Ugotovili smo, da je bila vsebnost Pb v vseh tkivih rdečeperke pod mejo zaznavnosti, Cd pa nismo zaznali v mišičnem tkivu rdečeperk (< 0,003 mg/kg). Tudi koncentracije Cu, Zn, Fe in Mn so bile najnižje v mišičnem tkivu. Najvišjo koncentracijo Cd in Zn smo ugotovili v ledvicah, najvišjo koncentracijo Cu, Mn in Fe pa v jetrih. Proučili smo tudi povezavo med maso oziroma dolžino rib z vsebnostjo kovin v tkivih. V ta namen smo izračunali Pearsonov korelacijski koeficient in ugotovili, da je ta za Zn in Mn v mišičnem tkivu in jetrih negativen, kar pomeni, da se koncentracija manjša z velikostjo rib. Za Fe v ledvicah ter za Cd v ledvicah in jetrih pa je Pearsonov koeficient pozitiven, kar pomeni, da koncentracija z velikostjo rdečeperke narašča.

Ključne besede: svinec; kadmij; baker; cink; železo; mangan; rdečeperka; velikost