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Original Research

Age-Dependent Heavy Metal Content in Muscle Tissue of Brown Bullhead (*Ictalurus nebulosus*, Le Suerur, 1819)

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Abstract

We used brown bullheads (*Ictalurus nebulosus*) from Milicz Ponds in our study. The authors examined the impacts of age, body length, body weight, and the condition of the fish on concentrations of metals (Cd, Pb, Ni, Cu, and Zn) in their muscle tissue (n=62). The metal contents in muscle tissue increased in the order from Cd < Ni < Pb < Cu < Zn and amounted to 0.0177, 0.0754, 0.1121, 0.1979, 4.7358 mg/kg of wet weight respectively. Also, the bioconcentration factors (BCF) were calculated based on the metal contents in muscle tissue, water and bottom sediment.

The obtained results allow us to conclude that the metal concentrations in muscle tissue decrease with age (for Pb and Zn, the correlation is highly statistically significant, with the p value ≤ 0.01). A relationship between body length and weight and Zn content ($p \leq 0.01$) was confirmed, as well as between the condition of the fish (expressed by Fulton's condition factor) and Cd and Cu concentrations ($p \leq 0.05$).

Keywords: age, heavy metals, bioconcentration, brown bullhead

Introduction

Metal concentrations in fish tissues have most often been investigated in commercial fish species that are important in economic terms. Other species have been examined only occasionally. The brown bullhead, *Ictalurus nebulosus*, is native to North America and it is an alien and invasive species among Polish fish fauna [1].

In recent years we have observed an expansion of this species in Polish waters. A large population of brown bullhead has been present in the Milicz Ponds area, a nearly 10-century-old group of carp ponds protected under the Ramsar Convention on Wetlands and Living Lakes Global Nature Fund, as well as being included in the European Natura 2000 Network [2].

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According to Polish law, it is forbidden to introduce brown bullheads into unenclosed waters (also after capture) [3, 4]. This positively contributes to the use of this species as an indicator in environmental pollution testing [5].

The brown bullhead's muscle tissue was investigated for contents of the heavy metals Cd, Hg, Ni, Cu, and Zn, and the determination of the bioconcentration factor (BCF); however, researchers have usually failed to consider the age of the fish. These elements play various roles: Hg and Cd are toxic even in small concentrations (their indispensability in living organisms has not been confirmed); Cu and Zn are essential for living organisms' vital functions, but they are toxic at high concentrations; and Ni has a non-established status, although it has been identified (as have Cd and Pb) as a priority substance in water environment by the European Union [6, 7].

The metal concentrations in fish tissues depend on numerous parameters, including metal content in water and food, and on environmental parameters. Many authors have demonstrated the relationship between the metal contents in tissues and fish age and size (body length, weight) [5, 8-12].

The aim of this paper was to determine the relationship between the metal concentration in the muscle tissue of the brown bullhead and their age and size (body length, body weight, Fulton's condition factor). At the same time, an attempt was made to determine the relationship between concentrations of specific metals.

Materials and Methods

Brown bullheads were obtained from Bolko Pond, a component of Stawno Fishing Farm in the Milicz Ponds area during the commercial autumn capture of carp. A total of 62 fish were acquired, which were later measured and weighed, their ages were determined on the basis of vertebrate growth rings [13, 14], and their muscle tissue was collected for further investigation.

During the summer production season, water and bottom sediment samples were collected from the pond. Select water parameters were analysed: alkalinity, total hardness, electrolytic conductivity, pH, dry residue, dissolved substance, and suspension. Next, water samples were thickened by adding HNO_3. Sediment samples were air-dried and subjected to wet mineralisation using mixed nitric acid (HNO_3) and perchloric acid ($HClO_4$) (3:1) in a Mars 5 microwave digestion system (CEM Corporation, USA).

The muscle tissue was subjected to wet mineralisation using nitric acid (HNO_3) in a Mars 5 microwave digestion system (CEM Corporation, USA).

In the obtained mineralisation (of water, sediments, muscle tissue), Cd, Pb, Ni, Cu, and Zn concentrations were determined using an atomic absorption spectrophotometer (SpectrAA FS220, Varian, Australia).

The results of the analyses were verified using the certified reference material DORM-3 (fish protein from the National Research Council Canada).

The concentrations of the examined elements in the water were given in $mg \cdot dm^{-3}$, in the sediment in $mg \cdot kg^{-1}$ of dry weight, and in muscles in $mg \cdot kg^{-1}$ of wet weight; fish weight was given in g and length in cm.

Based on the obtained fish measurements, Fulton's condition factor (K) was calculated.

$$F = \frac{W \times 100}{TL^3}$$

...where W means the fish weight (in g), and TL is total length (in cm) [15].

Bioconcentration factors were calculated using the formula:

$$BCF_e = \frac{C_f}{C_e}$$

...where C_f means the metal content in muscle tissue ($mg \cdot kg^{-1}$) and C_e means the metal concentration in the environment (water in $mg \cdot dm^{-3}$ or bottom sediment in $mg \cdot kg^{-1}$) [6, 16].

The coefficient of variation (V) was calculated using the formula:

$$V = \frac{SD}{x} \times 100\%$$

...where SD is the standard deviation, and x the arithmetic mean.

Metal pollution index (MPI) was calculated using the formula:

$$MPI = (C_{f1} \times C_{f2} \dots C_{fn})^{1/n}$$

...where C_{fi} is concentration for metal i in the sample [17].

Statistical calculations were performed using Statistica 10.0 (StatSoft).

Significant differences in metal concentrations across specific age groups were determined based on the Kruskal-Wallis one-way analysis of variance (ANOVA), and relationships between determined parameters were determined based on Spearman's rank correlation coefficient.

Results

Water and bottom sediment samples were collected from the pond in summer. The results of physical and chemical investigations can be typical for water of carp ponds in our climate zone (Table 1). The metal content of water and bottom sediments are summarized in Table 2.

The ages of the examined fish were determined to range from 1-4 years (average of 2.7 years). The mean body length was 22 cm (8.5-30.5 cm), mean body weight 141.5 g (6.2-378.8 g). Fulton's condition factor (K) ranged from 0.89 to 1.44 and its mean value was 1.13 (Table 3).

Table 1. Some physical and chemical parameters of fishpond water in summer.

Parameter	Unit	Value
Alkalinity	$mg\ CaCO_3 \cdot dm^{-3}$	80.82
Total hardness		135.66
Electrolytic conductivity	$\mu S \cdot cm^{-1}$	360
pH	-	7.63
Dry residue	$mg \cdot dm^{-3}$	247
Dissolved substance		198
Suspension		49

Table 2. Metal concentrations in fishpond water (mg·dm^{-3}) and bottom sediment (mg·kg^{-1} dry weight) in summer.

Material	Unit	Metal concentration					
		Cd	Pb	Ni	Cu	Zn	MPI
Water	mg dm^{-3}	0.0007	0.0038	0.0273	0.0077	0.0336	0.0072
Bottom sediment	mg·kg^{-1} (d.wt.)	0.519	5.391	2.565	0.597	4.875	1.836

MPI – metal pollution index

Table 3. Fish age, length, weight, Fulton's condition factor (K), and the mean, standard deviation, ranges of concentration, and variability of investigated elements.

	Age (year)	Length (cm)	Weight (g)	K	Metal concentration (mg·kg^{-1} w.wt)					
					Cd	Pb	Ni	Cu	Zn	MPI
Mean	2.68	22.05	141.47	1.1266	0.0177	0.1121	0.0754	0.1979	4.7358	0.1254
SD	0.86	5.12	84.13	0.1127	0.0115	0.0395	0.1357	0.1387	1.2209	0.0785
Min	1	8.50	6.20	0.8893	0.0005	0.0628	0.0020	0.0030	3.1525	0.0248
Max	4	30.50	378.80	1.4399	0.0547	0.3394	0.7824	0.5996	9.2744	0.3730
V	32.01	23.20	59.47	10.00	65.28	35.24	180.05	70.11	25.78	62.60

SD – standard deviation, V – coefficient of variability (%), MPI – metal pollution index, K – Fulton's condition factor

Table 4. Metal concentrations in muscle of brown bullhead as dependence on age (mg·kg^{-1} w.wt).

Metal		Age			
		1 (n=5)	2 (n=21)	3 (n=24)	4 (n=11)
Cd	Mean±SD	0.0435 ^{Aa}±0.0095	0.0125 ^{Bb}±0.0086	0.0174 ^{ABb}±0.0091	0.0173 ^{ABab}±0.0074
	Range	0.0287-0.0547	0.0005-0.0322	0.0050-0.0322	0.0022-0.0292
	V	21.9303	68.9765	51.9993	42.6042
Pb	Mean±SD	0.2001 ^{Aa}±0.0825	0.1105 ^{ABb}±0.0221	0.1000 ^{Bb}±0.0221	0.1024 ^{Bb}±0.0245
	Range	0.1370-0.3394	0.0818-0.1738	0.0628-0.1488	0.0656-0.1460
	V	41.2112	20.0319	22.1344	23.9152
Ni	Mean±SD	0.0926±0.0960	0.0560±0.0854	0.0598±0.1215	0.1383±0.2376
	Range	0.0020-0.2443	0.0020-0.2751	0.0020-0.4408	0.0020-0.7824
	V	103.7168	152.4550	203.1447	171.8018
Cu	Mean±SD	0.3646±0.1875	0.1764±0.1312	0.1852±0.1286	0.1991±0.1308
	Range	0.1007-0.5996	0.0030-0.5162	0.0030-0.4066	0.0160-0.5085
	V	51.4268	74.4025	69.4433	65.7037
Zn	Mean±SD	7.6197 ^{A}±0.6878	4.7834 ^{AB}±1.1570	4.3107 ^{B}±0.5514	4.3248 ^{B}±0.9932
	Range	6.5902-8.4450	3.6651-9.2744	3.5870-5.4654	3.1525-6.9712
	V	9.0260	24.1870	12.7922	22.9655
MPI	Mean±SD	0.3344 ^{a}±0.2068	0.1045 ^{b}±0.06248	0.1144 ^{b}±0.0718	0.1299 ^{ab}±0.0626
	Range	0.1114 – 0.7201	0.0551-0.2693	0.0248-0.2724	0.0403-0.2660
	V	61.8266	59.7552	62.7387	48.2079

Different letters (vertical) indicated statistically significant differences: ^{AB} – $p \leq 0.01$, ^{ab} – $p \leq 0.05$;
SD – standard deviation; V – coefficient of variability (%), MPI – metal pollution index

Table 5. The factors of select metals bioconcentration from water (BCF_w) to the muscle of brown bullhead as dependent on age.

Metal		Age			
		1 (n=5)	2 (n=21)	3 (n=24)	4 (n=11)
Cd	Mean±SD	62.20±13.64	17.83±12.30	24.89±12.94	24.66±10.51
	Range	41.00-78.14	0.71-46.00	7.14-46.00	3.14-41.71
Pb	Mean±SD	52.67±21.70	29.07±5.82	26.32±5.83	26.94±6.44
	Range	36.04-89.30	21.52-45.73	16.52-39.17	17.27-38.43
Ni	Mean±SD	3.39±3.52	2.05±3.13	2.19±4.45	5.07±8.70
	Range	0.07-8.95	0.07-10.08	0.07-16.15	0.07-28.66
Cu	Mean±SD	47.35±24.35	22.90±17.04	24.06±16.71	25.86±16.99
	Range	13.08-77.87	0.39-67.04	0.39-52.81	2.08-66.04
Zn	Mean±SD	226.78±20.47	142.36±34.43	128.29±16.41	128.71±29.56
	Range	196.14-251.34	109.08-276.02	106.76-162.66	93.82-207.48

SD – standard deviation

Table 6. The factors of select metals bioconcentrations from bottom sediment (BCF_s) to the muscle of brown bullhead as dependent on age.

Metal		Age			
		1 (n=5)	2 (n=21)	3 (n=24)	4 (n=11)
Cd	Mean±SD	0.0839±0.0184	0.0240±0.0166	0.0336±0.0175	0.0333±0.0142
	Range	0.0553-0.1054	0.0010-0.0620	0.0096-0.0620	0.0042-0.0563
Pb	Mean±SD	0.0371±0.0153	0.0205±0.0041	0.0186±0.0041	0.0190±0.0045
	Range	0.0254-0.0629	0.0152-0.0322	0.0116-0.0276	0.0122-0.0271
Ni	Mean±SD	0.0361±0.0374	0.0218±0.0333	0.0233±0.0474	0.0539±0.0926
	Range	0.0008-0.0952	0.0008-0.1073	0.0008-0.1719	0.0008-0.3050
Cu	Mean±SD	0.6107±0.3141	0.2954±0.2198	0.3103±0.2155	0.3335±0.2191
	Range	0.1687-1.0044	0.0050-0.8647	0.0050-0.6811	0.0268-0.8518
Zn	Mean±SD	1.5630±0.1411	0.9812±0.2373	0.8842±0.1131	0.8871±0.2037
	Range	1.3518-1.7323	0.7518-1.9024	0.7358-1.1211	0.6467-1.4300

SD – standard deviation

The obtained results (Tables 2, 3) allowed putting the examined elements in order from the lowest to highest concentration.

Water: Cd < Pb < Cu < Ni < Zn
Sediment: Cd < Cu < Ni < Zn < Pb
Muscle: Cd < Ni < Pb < Cu < Zn

The highest metal concentration (except for Ni) was found in muscle tissue of the youngest fish (1 year-old). The lowest metal concentrations were observed in 2- (Cd, Ni, Cu) or 3-year-old fish (Pb, Zn) (Table 4). Significant differences in muscle tissue metal concentrations (Cd, Pb, Zn, and MPI) at different ages were statistically confirmed.

Irrespective of a fish's age, Cd muscle tissue concentration was the lowest, and Zn concentration was the highest. In 1-3-year old fish, metals ordered according to growing concentrations, formed a rank identical to the rank with no division into age groups. In the fourth year, Ni switched with Pb (Cd<Pb<Ni<Cu<Zn). This was caused by considerable growth of Ni concentration (more than double the growth in relation to the third year).

Based on the metal content in brown bullhead muscle tissue, in water, and in the sediment, the BCF were calculated. Respectively, BCF_w for accumulation in relation to water concentration (Table 5) and BCF_s in relation to metal concentration in sediments (Table 6).

Correlations between age, body length, body weight, Fulton's condition factor and metal content in the brown bullheads' muscle tissue were determined (Table 7). Correlations were different depending on the metal.

Correlations between concentrations of individual metals in the muscle tissue were also determined (Table 8).

Table 7. Correlations between age, length, weight, Fulton's condition factor (K), and metal concentration in muscle of brown bullhead.

	Spearman's correlation coefficient					
	Cd	Pb	Ni	Cu	Zn	MPI
Age	0.0140	-0.3418**	-0.0226	-0.0771	-0.4381**	-0.0628
Length (cm)	-0.0079	-0.2250	0.0156	-0.0967	-0.3912**	-0.0192
Weight (g)	0.0502	-0.2152	0.0142	-0.0453	-0.3918**	-0.0016
K	0.2628*	-0.0376	-0.0127	0.2731*	-0.0430	0.1179

*$p \leq 0.05$; **$p \leq 0.01$;
K – Fulton's condition factor; MPI – metal pollution index

Table 8. Correlations between concentrations of investigated metals.

	Spearman's correlation coefficient			
	Cd	Pb	Ni	Cu
Pb	0.0890			
Ni	0.2541*	0.0451		
Cu	0.6024*	0.2254	0.2725*	
Zn	-0.0122	0.6268*	0.1062	0.3246*

*$p \leq 0.05$

Discussion

Metal concentrations in muscle tissue of fish reported in the literature differ significantly (Table 9).

Metal concentrations in the tissues of fish depend on the position of a given species in the trophic level [32-34]. And the highest metal concentrations have been reported in ground fish species. The brown bullhead is omnivorous; it mainly eats bottom-dwelling invertebrates, but it becomes a predator with age and may feed on smaller fish species. In general, it may be concluded that metals accumulate in muscle tissue in the following order: Cd<Pb<Cu<Zn (according to increasing concentrations), which was confirmed herein. Ni concentration is usually lower or comparable to Pb concentration.

Metal concentrations were changing with the fish's age. Except for Ni, metal concentrations in the muscle tissue of 4-year-old fish were lower than in the muscle tissue of 12-month-old fish. This difference ranged from 43% (Zn) to 60% (Cd), with statistically significance differences confirmed in the cases of Pb, Zn ($p \leq 0.01$), and Cd ($p \leq 0.05$). These changes, however, were not identical in subsequent years. The largest fall in metal concentrations was observed between the first and second years of life and it concerned all examined elements. The differences ranged from 37% (Zn) to 71% (Cd). Statistically significance differences between metal content in muscle tissue of 1- and 2-year-old fish for Cd ($p \leq 0.01$), Pb, and Zn ($p \leq 0.05$) were confirmed. Also, between the first and second years, the concentrations of all metals expressed by MPI (by 69%, $p \leq 0.05$) decreased statistically.

The correlations between age, body weight, body length, and Fulton's condition factor were low (Table 7). Relationships between Cu, Pb, and Zn concentrations and MPI, age, body weight, and body length were negative. Statistically significant negative correlations ($p \leq 0.01$) between Pb and age, Zn and age, Zn and body length, and Zn and body weight were reported. Changes in metal concentration in muscle tissue were observed along with fish age and have been confirmed by many researchers [9, 20, 25, 38]. However, the reported coefficients of correlations between metal content in the muscle tissue and the fish's age vary. Dobicki and Polechoński [9] also found negative correlations between Pb and age (pike perch, bream), and Zn and age (roach, bream), but at the same time they observed positive correlations between Pb and age in perch, and Zn and age in pike perch. In Nile tilapia, negative correlation between age and Zn was found [38]. Age-dependant changes in metal concentrations in the muscle tissue may be caused by differences in metabolism (faster in growing fish), defence mechanisms developed with age and detoxication ability, and changes in their position in the food chain (changes in the type of consumed food) [9, 20, 39].

Negative correlation between Zn concentration and body length is reported, among others, in brown bullhead [23], perch [39], white sucker [23], bream [9, 25], and roach [9]. In pike perch [9, 21] and carp [30], on the other hand, positive correlation was observed. Similarly, in the case of the correlation between Zn and body weight, some authors have reported a negative correlation in bream, roach [9], and goldfish [30], whereas others have reported a positive correlation in pike perch [9, 21] and carp [30].

Correlations ($p \leq 0.05$) between concentrations of the following metals: Cd-Ni, Cd-Cu, Pb-Zn, Ni-Cu, and Cu-Zn were confirmed to be statistically significant. All correlations were positive. In the muscle tissue of various species, the following correlations were also reported: Cd-Ni, Cd-Cu [31], and Pb-Zn [30]. But correlations between metal concentrations are species-specific and they depend on environmental parameters. One such parameter may be environmental pollution leading to increased metal concentration in the water. This may be confirmed by results of studies conducted on tilapias. They prove that in the case of fish exposed to metal pairs (Cu and Cd [40] as well as Zn and Cd [41]), accumulation of each pair is lower than in the

Table 9. Mean metal concentrations in fish muscle (mg·kg^{-1} w. wt.).

Type of food	Species	Average metal concentration in fish muscle (mg·kg^{-1} w.wt.)					References
		Cd	Pb	Ni	Cu	Zn	
Piscivorous	Pike *Esox lucius*	0.0030	0.0200	0.0300	0.130	6.80	[18]
					0.140	9.40	[19]
					0.190	6.20	
	Pikeperch *Sander lucioperca*	0.0067	0.2761	0.2062	0.173	16.51	[20]
		0.0100		0.1800	0.378	5.53	[21] [a]
	Perch *Perca fluviatilis*		0.0600	0.0430	0.200	4.80	[22] [a]
			0.0600	0.0360	0.940	2.70	
			0.0740	0.0360	0.560	4.40	
		0.0030	0.0100	0.1300	0.160	5.20	[18]
Omnivorous	Roach *Rutilus rutilus*		0.0480	0.0200	0.260	5.80	[22] [a]
			0.1220	0.0360	0.580	2.80	
			0.0820	0.0200	0.440	5.60	
	Brown bullhead *Ictalurus nebulosus*	0.0177	0.1121	0.0754	0.198	4.74	this study
		0.0060	0.1440	0.0800	0.240	6.00	[23] [a]
		0.0220	0.1380	0.1880	0.484	4.94	[24] [a]
	Bream *Abramis brama*	0.0114	0.3084	0.0869	1.332	17.58	[20]
		0.1220	0.3260		0.444	2.90	[25] [a]
		0.0840	0.0880		0.354	2.18	
		0.0020	0.0100	0.0500	0.170	2.80	[18]
					0.180	3.20	[19]
					0.240	3.00	
	Common carp *Cyprinus carpio*	0.0800	0.0440		0.517	4.60	[26]
					0.530	4.98	[27]
		0.0759	0.1359	0.0393			[28]
		0.0042	0.0354			5.08	[29] [a]
		0.0160	0.0480	0.2800	0.514		[24] [a]
		0.0010	0.0100	0.1000	0.080	3.30	[18]
		0.0129	0.0217		1.130	9.34	[30]
		0.0580	1.2780	0.3000	0.994	5.71	[31] [a]
		0.0500	1.9900		0.180	4.32	[12]
	Goldfish *Carassius auratus*	0.0026	0.0574		0.378	26.00	[29] [a]
		0.0170	0.0849		0.920	12.83	[30]
		0.1000	0.2900		0.610	13.50	[32]
Herbivorous	Grass carp *Ctenopharyngodon idella*	0.0834	0.2640	0.1340	0.118	0.38	[33] [a]

[a] – in the original article in dry weight – recalculated on wet weight according formula w.wt. = d.wt./5 [22]

case of exposure to single metals. Moreover, this relationship is more discernible in the case of lower water metal concentrations. Furthermore, this phenomenon makes it more difficult to analyse the results of metal accumulation in the muscle tissue of fish.

The BCF value of metal concentration in muscle tissue, calculated in relation to metal concentration in water (BCF_w) or sediment (BCF_s), was changing along subsequent life years (Tables 5 and 6). The highest BCF_w and BCF_s values were reported for Zn. The lowest BCF_w value was reported for Ni, and the lowest BCF_s in 1-year-old fish for Ni, whereas in older fish (2-4 years old) for Pb. The literature offers scarce data for comparing the obtained BCF values. The BCF_w for Zn reported for pike perch [39], pike, and bream [19] approximates the value obtained in our research. The BCF_w for Cu reported for pike and bream [19], on the other hand, is 10-fold higher than that reported for the brown bullhead. These differences do not stem from different metal concentrations in the muscle tissue (lower metal concentration in lake water). The 10-fold higher BCF_w value for Cd, Pb, Ni, Cu, and Zn in the muscle tissue of eight species from Taihu Lake was also reported by Tao et al. [42]. In this case, apart from significantly lower metal concentrations in water, their higher concentrations in muscle tissue were observed.

The calculated BCF_s values approximate those reported for roach, perch pike, silver bream, and rudd [43]. Considerably lower BCF_s values are reported for crucian [32].

However, the calculated BCF and values taken from literature suggest that the accumulation of metals in the muscle tissue of fish not only depends on their water or sediment concentrations, but also on their concentration in food.

Difficulties with confirming statistically significance differences between groups or correlations between analysed factors arose from differentiation of results within specific groups. This is confirmed by a high coefficient of variation (V), which may be caused by individual idiosyncrasies (other than age, body length, body weight). The physiological condition of the fish was considered in the study during the calculation of Fulton's condition factor (K). Correlations between Cu and the condition factor, as well as Cd and the condition factor ($p \leq 0.05$), were statistically confirmed with both correlations being positive. Farkas et al. [25] confirmed correlations between the condition factor and Hg and Zn (negative), and Pb (negative in September and positive in May).

It appears, however, that this factor has a minor impact on the accumulation of metals in the muscle tissue of fish, as usually the relationship between the condition factor and the metal concentration or BCF in fish muscle tissue cannot be statistically confirmed.

Conclusions

1. Metal concentrations in brown bullhead muscle tissue change with age.
2. Negative correlations between Pb and Zn concentrations in the muscle tissue of brown bullhead and the fish's age, as well as between Zn concentration and body length and weight, were confirmed.
3. Positive correlations between Cd and Cu concentrations and the condition of the brown bullhead expressed *via* Fulton's condition factor were confirmed.

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