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Research Article

Distribution of Heavy Metals (Ni, Co, Pb, Cd, Hg) in Tissues of European Chub (*Squalius cephalus* L.) from the Middle Course of the Nitra River, Slovakia

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Abstract
The aim of this study was to determine the levels of Ni, Co, Pb, Cd and Hg in the muscle, hepatopankreas, kidney and gonads of European chub (Squalius cephalus) from the middle course of the Nitra River, during spring and autumn seasons by AAS method. The concentrations of metals (mg.kg-1 wet weight) in the muscle ranged as follows: Ni 0.15–1.18, Co 0.09–0.58, Pb 0.39–1.66, Cd 0.04–0.29, Hg 0.85–2.71. Statistically significant differences among individual tissues, as well as between seasons have been recorded. Higher metals concentrations were detected in inner organs than in muscle. Permissible limits for safe consumption in the case of Pb, Cd and Hg have been exceeded in 100%, 95% and 100%, respectively. Currently, for the Co and Ni are not set any permissible limits.

Key words: accumulation, seasonal variation, permissible limit, health risk, AAS.

Introduction

The Nitra River is the flow of 3rd order with length over 168 km. Flows through 5 districts (Prievidza, Partizánske, Topoľčany, Nitra and Nové Zámky), and inflows into the Váh River on its 30th river kilometer near the Komoča village.
The Nitra River is one of the most contaminated rivers by heavy metals in Slovakia [1]. This situation had been known from the second half of 20th century, mainly from the May of 1965th, when it came to the rupture of fly ash deposition, above the Zemianske Kostoľany village. During this catastrophe, 1.5 million m^3 of fly ash with high levels of toxic elements had escaped into the riverbed over, which contaminated the Nitra River downstream, approximately from the 130th r. km.
Although this situation has been known for a long time, the first information about fish contamination is dated to the end of 20th century [2]. More informations have been published at the start of 21st century [1, 3–6].
The Nitra River has been strongly affected by industrial and mining activities in the upper and middle part, till the end of 20th century [1]. Occurrence of contaminants, their frequency and concentration mainly depends on human activities, although sometimes impurities may be of geological origin [7–10].
Some heavy metals accumulate in tissue and may pose a health risk for those who frequently consume fish [3, 11]. Currently, the Nitra River represents the fishing territory administrated by 6 local organizations of the Slovak Angler´s Union, covering about 11.000 members.
Due to, the purpose of this study was to determine the concentrations of Ni, Co, Pb, Cd, Hg, which

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had been monitored also in previous studies. The metals have been analysed in the muscle, hepatopankreas, kidneys and gonads of chub (*Squalius cephalus*) from the middle part of the Nitra River (Slovak Republic) which may pose risk to recreational and visiting anglers. Furthermore, orders of their accumulation in the fish tissues were analysed.

Material and Methods

For this study, the European chub was chosen (*Squalius cephalus*) as a referent species due to its common occurrence. Specimens of chub were collected by electrofishing below the town of Partizánske, between 111th–112th river kilometer, in spring (May) and autumn (October) season in the year 2015.

Fish (n=20, 10 for each season) were evaluated by standard methods used in ichthyology (standard length – SL, total length – TL and weight – W measurements). After the biometric data recording, the 3 – 5 g samples of fish tissues were obtained – muscle from the dorsal part of fish body, without skin and bones; hepatopankreas, kidneys and gonads from the abdominal cavity. After collection, the tissue samples were kept at –18°C.

For analysis were used blanks samples. Two grams of each tissue sample were mineralised by microwave digestion (MARS X-press, CEM USA) according to EN 13804 and EN 13805. Digested samples were analysed for the presence of Ni, Co, Pb and Cd by fast sequential flame atomic absorption spectrophotometer (FSF–AAS) Varian AA 240FS (Agilent, USA). The total mercury content was determined directly in the sample units by the selective mercury analyser (Advanced mercury analyser, AMA-254, Altec, Czech Republic) based on atomic absorption spectroscopy (wavelength 253.65 nm; limit of quantity 0.002 mg.kg^{-1}). To validate the method and equipment were used certified reference materials AAS/ICP Multi-element Standard Solution CERTIPUR (Merck, USA) and CMR-Hg with concentration of 1.0004 g.l^{-1} (Slovak metrology institute). Values of analysed heavy metals are presented on a wet weight (w.w.) basis in mg.kg^{-1} and compared with the Slovak hygienic limits presented in Commission Regulation (EC) No. 1881/2006, as amended by Commission Regulation (EC) No. 629/2008.

For statistical analysis, the Anova one-way test, Multiple Range test (LSD method), Kruskal-Wallis test, t-test, and Kolmogorov-Smirnov test were used together with the computer program Statgraphics Centurion 18 Professional (Statgraphics Technologies Inc., USA).

Results and Discussions

Base biometric data of analysed fish are given in Table 1. Concentrations of analysed metals for individual tissues are presented in Tables 2 – 6. For comparison with some other authors, the values are transferred from dry to wet ratio of 1:5 expressed as μg.g^{-1} and/or mg.kg^{-1} wet weight [12].

Table 1.

Characteristics of analysed European chub specimens

Season	N	SL (mm)		TL (mm)		W (g)	
		mean ± SD	min–max	mean ± SD	min–max	mean ± SD	min–max
spring	10	250 ± 32.9	190–285	297 ± 40.2	225–340	310.6 ± 121.7	120–459
autumn	10	269 ± 33.2	210–305	322 ± 38.0	250–365	374.8 ± 114.6	196–547

Nickel content varied from 0.82 to 2.35 and from 0.15 to 1.49 mg.kg^{-1} w.w. in spring and autumn season, respectively. Lowest mean concentration in muscle and highest mean concentration in kidneys were recorded (Table 2). Statistically higher significant differences (P<0.001) for nickel accumulation in tissues were detected in spring than autumn season. Higher nickel concentrations in muscle of European chub are presented from the upper Nitra River [2] and polluted site of the River Yeşilırmak in Tokat, Turkey, also with higher value in spring than in autumn season [13]. Opposite situation was noted in muscle of related Aegean chub (Squalius fellowesii), from Tersakan and Sarıçay streams, Turkey, where were detected lower nickel concentrations, than in our findings [14]. Autumn lower nickel concentrations for muscle and gonads of European chub are given also from the upper Jihlava River, Czech Republic [15], as well as from the middle and upper course of the Nitra River [3–4]. The general order of nickel accumulation (data from both seasons) in analysed tissues was: kidneys > gonads > hepatopancreas > muscle. Hygienic level for this element is not defined.

Table 2.

Content of Ni (mg.kg^{-1} w.w.) in tissues of European chub

tissue	spring		autumn	
	mean ± SD	min - max	mean ± SD	min - max
muscle	1.03^{a} ± 0.12	0.82 – 1.18	0.32^{a} ± 0.12	0.15–0.53
hepatopancreas	1.68^{b} ± 0.36	1.14 – 2.25	0.49^{a} ± 0.07	0.41–0.60
kidneys	2.12^{c} ± 0.19	1.78–2.35	1.08^{b} ± 0.39	0.36–1.37
gonads	1.74^{b} ± 0.25	1.14–2.03	0.88^{b} ± 0.27	0.60–1.49

The values with identical superscript in the column are not significant at the p<0.05 level

Level of cobalt copied the trend of nickel. Its values ranged from 0.35 – 1.91 and from 0.09 – 1.51 mg.kg^{-1} w.w., for spring and autumn season, respectively, with lowest mean concentrations in muscle and highest mean concentrations in kidneys (Table 3). Statistically significant higher levels (P<0.05) of cobalt were detected in spring than in the autumn season. Lower mean concentrations in muscle of European chub are known from the middle and lower parts of the Nitra River [3–4], originated from autumn season, from the Kızılırmak River basin, Turkey [16], as well as from the Tersakan and Sarıçay streams, Turkey, in the muscle of related Aegean chub [14]. Tissues order of cobalt accumulation (data from both seasons) was: kidneys > gonads > hepatopancreas > muscle. Hygienic limit for Co is not defined.

Table 3

Content of Co (mg.kg^{-1} w.w.) in tissues of European chub

tissue	spring		autumn	
	mean ± SD	min – max	mean ± SD	min – max
muscle	0.45^{a} ± 0.07	0.35–0.58	0.18^{a} ± 0.08	0.09–0.35
hepatopancreas	0.60^{b} ± 0.10	0.40–0.73	0.32^{ab} ± 0.05	0.22–0.38
kidneys	1.61^{c} ± 0.24	1.25–1.91	1.01^{c} ± 0.40	0.46–1.51
gonads	0.71^{b} ± 0.06	0.59–0.81	0.46^{b} ± 0.14	0.33–0.80

The values with identical superscript in the column are not significant at the p<0.05 level.

Lead concentrations fluctuated between 0.39 – 2.94 and 0.13 – 2.30 mg.kg^{-1} w.w., for spring and autumn season, respectively. In spring season, the lowest mean concentration in muscle and highest mean concentration in hepatopankreas were noted; in autumn season, the lowest mean concentration in kidneys and highest in hepatopankreas were noted (Table 4). Lower mean lead muscle concentrations than in our results are listen from the upper Nitra River [1], from the upper Jihlava River [15], Morava River and Morava River basin (Czech Republic) [17–18], as well as in related species *Squalius svallize* form the lower Neretva River, Croatia. In this same fish species the lower lead levels also in hepatopancreas, kidneys and gonads were detected [7]. Lower lead concentrations, in comparison to our results, have been reported for gonads and hepatopancreas from the upper Jihlava River (Czech Republic) [15] and River Mureş (Romania) [19]. On the other hand, higher lead values in the European chub muscle have been detected from the middle and lower part of the Nitra River [3–4]. Seasonal differences were also confirmed. In the case of muscle, higher concentration in autumn than spring was detected (P<0.05). Opposite results are presented from the River Yeşilırmak in Tokat, Turkey, where have been observed higher lead concentrations in spring than in autumn [13].

In inner organs the higher concentrations were detected for spring season, in the case of kidneys also with statistical significance (P<0.001). Hygienic limit defined in Commission Regulations is set only for muscle and its value is 0.3 mg.kg^{-1} w.w. From this aspect, all analysed muscle samples (100 %) exceeded this limit. Order of lead accumulation in tissues was (data for both seasons): hepatopancreas > kidneys > muscle > gonads.

Table 4

Content of Pb (mg.kg^{-1} w.w.) in tissues of European chub

tissue	spring		autumn	
	mean ± SD	min – max	mean ± SD	min – max
muscle	0.86^{a} ± 0.40	0.39–1.66	1.36^{c} ± 0.37	0.48–1.66
hepatopancreas	2.30^{c} ± 0.28	1.95–2.94	1.78^{d} ± 0.44	1.17–2.30
kidneys	1.90^{b} ± 0.16	1.63–2.12	0.54^{a} ± 0.21	0.13–0.73
gonads	1.16^{a} ± 0.54	0.54–2.16	0.98^{b} ± 0.45	0.20–1.57

The values with identical superscript in the column are not significant at the p<0.05 level.

Cadmium accumulated in analysed tissues from 0.05 to 0.56 and from 0.04 to 0.56 mg.kg^{-1} w.w., for spring and autumn season, respectively, with spring lowest mean concentration in gonads and highest in kidneys (Table 5). The autumn lowest mean concentration for muscle and highest mean concentration for kidneys were detected. Comparable Cd values to our results are given for European chub muscle from the middle part of the Nitra River [3]. Higher muscle concentrations are known from the lower course of the Nitra River [4]. Lower muscle Cd levels have been presented in this same fish species from the upper Nitra River [1], Jihlava and Morava Rivers, Morava River basin (Czech Republic) [15, 17–18], as well as in the muscle of related Aegean chub from Tersakan and Sarıçay streams (Turkey) [14] and *Squalius svallize* form the lower Neretva River (Croatia) [7]. Lower gonads levels of Cd than our results have been published for European chub from the upper Jihlava River (Czech Republic) [15], as well as for related *Squalius svallize* from the lower Neretva River (Croatia) [7]. In this same fish species also lower Cd concentrations in kidneys and hepatopancreas have been recorded. On the other hand, higher levels of cadmium in hepatopancreas have been reported for European chub from the River Mureş (Romania) [19].

Table 5

Content of Cd (mg.kg^{-1} w.w.) in tissues of European chub

tissue	spring		autumn	
	mean ± SD	min – max	mean ± SD	min – max
muscle	0.20^{ab} ± 0.05	0.15–0.29	0.12^{a} ± 0.05	0.04–0.17
hepatopancreas	0.22^{b} ± 0.09	0.11–0.32	0.25^{b} ± 0.05	0.18–0.37
kidneys	0.43^{c} ± 0.11	0.21–0.56	0.44^{c} ± 0.09	0.35–0.56
gonads	0.13^{a} ± 0.07	0.05–0.26	0.21^{b} ± 0.11	0.06–0.48

The values with identical superscript in the column are not significant at the p<0.05 level.

Seasonal differences among analysed tissues were noted. Statistically significant (P<0.05) higher muscle concentrations in spring than in autumn were observed, in comparison to inner organs, where higher concentrations in autumn season were noted, but without statistical significance (P>0.05). Higher muscle values of Cd in spring than autumn season have been detected in European chub from the Yeşilırmak River (Turkey) [13]. Also for Cd, like for Pb, is hygienic limit set for muscle only, and its value is 0.05 mg.kg^{-1} w.w. Analysed muscle samples exceeded this limit in 19 cases from 20 (95 %). Accumulation order for individual analysed tissues was (data for both seasons): kidneys > hepatopancreas > gonads > muscle.

Concentration of mercury in analysed fish tissues ranged in spring season from 0.14 to 8.58 mg.kg^{-1} w.w.; in autumn season from 0.45 to 11.94 mg.kg^{-1} w.w. The lowest mean level in gonads and highest level in hepatopankreas were recorded, for both seasons (Table 6).

Table 6

Content of Hg (mg.kg^{-1} w.w.) in tissues of European chub

tissue	spring		autumn	
	mean ± SD	min – max	mean ± SD	min – max
muscle	1.37^{a} ± 0.31	0.85–1.77	1.59^{a} ± 0.51	1.10–2.71
hepatopancreas	4.93^{b} ± 2.38	1.25–8.58	7.03^{c} ± 2.69	4.34–11.94
kidneys	3.84^{b} ± 1.29	2.21–6.79	3.65^{b} ± 1.62	0.91–5.48
gonads	0.66^{a} ± 0.55	0.14–1.96	0.89^{a} ± 0.41	0.45–1.90

The values with identical superscript in the column are not significant at the p<0.05 level.

For comparison, the higher muscle values are published for the European chub from the middle part of the Nitra River [3], but lower concentrations in muscle, as well as in inner organs are presented in many studies. From Czech Republic are published these data from major rivers, such as Elbe, Vltava, Morava, Jihlava, etc. and their tributaries and/or basins [15, 17–18, 20–23]; from the Croatia for related *Squalius svallize* from the lower Neretva River [7]. In all tissues have been noted highest concentrations in autumn than spring, but without statistically significant differences (P>0.05). Opposite results had been presented from the Elbe River (Czech Republic), where higher Hg concentrations in spring than autumn season were detected [22]. This difference is caused probably by different sampling year. Hygienic limit defined in Commission Regulations is set only for muscle, like in the cases of Pb and Cd. Its value is set on 0.5 mg.kg^{-1} w.w. From this aspect, all analysed muscle samples (100 %) exceeded this limit. General order of mercury accumulation in tissues was (data for both seasons): hepatopancreas > kidneys > muscle > gonads.

Conclusions

In our study is presented the actual situation about heavy metal levels in selected tissues of European Chub from the middle course of the Nitra River. Distribution order of selected metals in analysed tissues was as follow: Ni – kidneys > gonads > hepatopancreas > muscle; Co – kidneys > gonads > hepatopancreas > muscle; Pb – hepatopancreas > kidneys > muscle > gonads; Cd – kidneys > hepatopancreas > gonads > muscle; Hg – hepatopancreas > kidneys > muscle > gonads.

Even after more than 50 years, analysed tissues samples show high concentrations of selected heavy metals. In the case of analysed samples of muscle, these exceed permissible limits defined in Commission Regulations No. 1881/2006 and 629/2008 for Pb, Cd and Hg in 100 %, 95 % and 100 %, respectively. From this point of view, the fish from the middle course of the Nitra River are not safe for direct human consumption and should be fished by the system "Catch and Release" only.

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