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Total mercury content in selected tissues of common carp (*Cyprinus carpio*) pond farmed in the Czech Republic

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Abstract

The aim of this study was to evaluate the total mercury content in selected tissues (muscle, spleen, kidney, liver, scales, brain and gonads) of common carp (*Cyprinus carpio*). Thirty individuals of market carp caught in three ponds (Jaroslavicky, Strachotin, Vrkoc) located in the Morava River basin in the Czech Republic were used for this study. Total mercury was determined by atomic absorption spectrometry on AMA 254 device. The highest total mercury content was found in the muscle of carp (7.6 ± 2.8 $\mu g \cdot kg^{-1}$ in Jaroslavicky, 6.8 ± 2.3 $\mu g \cdot kg^{-1}$ in Vrkoc, and 12.9 ± 4.3 $\mu g \cdot kg^{-1}$ in Strachotin), followed by levels in caudal kidney, liver, spleen, and brain; the lowest mercury content was determined in gonads and scales. The comparison revealed that individual localities significantly differ in the mercury content in muscle, caudal kidney, liver and spleen samples. However, in all cases the values of the mercury content in muscle complied with the hygienic limit of up to 0.3 $mg \cdot kg^{-1}$, which means that mercury does not pose a risk to the consumers of these pond farmed fish and that the benefits of eating fish meat outweigh the potential risk in terms of mercury content.

Market fish, muscle, kidney, liver, spleen

Mercury is a naturally occurring element (EEA report 2018) and an environmental toxicant which has a toxic effect on aquatic organisms (Baldissera et al. 2020). Has-Schon et al. (2015) showed that mercury could lead to injury of liver tissues and brain tissues in common carp. Mercury is released to the environment due to human activities (such as artisanal and small-scale gold mining, coal burning, vinyl chloride, chlor-alkali or acetaldehyde production, etc.) (Pacyna and Pacyna 2005) as well as by natural processes such as volcanic eruptions and forest fires. Elemental mercury and its inorganic compounds used in industry get into the aquatic environment where they can be transformed microbially into the highly toxic organic compound methylmercury which may be deposited in sediments and accumulated in the food chain (Klapstein and O'Driscoll 2018). Mercury concentrations are closely monitored in both the biotic and abiotic part of the environment (Kim et al. 2016) and also in food products of animal origin (Svoboda et al. 2021) to evaluate possible risks arising from their increase.

Fish bioaccumulate or even biomagnify organic mercury (methylmercury) in their muscle tissues, primarily through consumption of lower organisms that contain methylmercury (Burger and Gochfeld 2011). This is why fish are considered suitable bioindicators of contamination of the aquatic environment by mercury and other heavy metals (Wang and Wang 2019). The mercury content is highly variable in fish bodies, depending on many factors such as the fish species, its position in the food chain, contamination of the sampling site (Marsalek et al. 2005; Novotna et al. 2020; Novotna Kruzikova et al. 2022) or the form of mercury. Total mercury (THg) content differs in individual tissues (Sehonova et al. 2022) and the THg liver/muscle index can be used for the assessment of mercury contamination of the aquatic environment (Havelkova et al. 2008;

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Kružíková et al. 2013). The consumption of fish is the most common route of mercury exposure for most of the world's population. The European Union sets maximum limits for the mercury content in fish for consumption at 0.3 (or 0.5) mg·kg^{-1} in most fish species and 1 mg·kg^{-1} in some predatory fish (EU Commission Regulation 2023/915).

The aim of this study was to evaluate the THg content in selected tissues of common carp (*Cyprinus carpio*) from three ponds located in the Morava River basin in the Czech Republic, and based on the THg liver/muscle index, to find out whether the studied ponds are loaded by mercury.

Materials and Methods

Study area

The monitored sites in this study were three ponds: Jaroslavicky, Strachotin, and Vrkoc pond (Fig. 1).

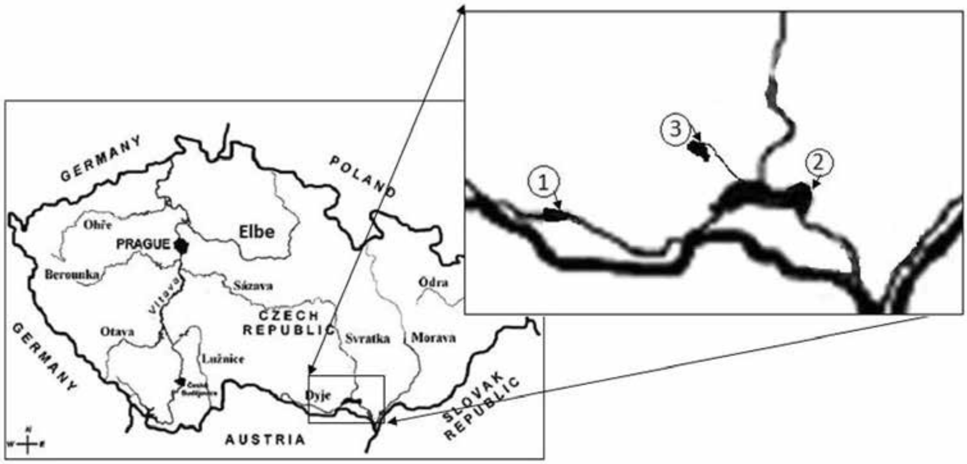

Fig. 1. Map of the Czech Republic with the monitored sites: 1 - Jaroslavicky pond; 2 - Strachotin pond; 3 - Vrkoc pond

Jaroslavicky pond is located about 17 km southeast of the town of Znojmo, near the small village of Jaroslavice bordering with Austria. It is operated by a commercial fish farm and is used for the breeding of carp and other fish. Water supply and outflow is provided by the Dyje-Mill race, a side channel of the Dyje River. It has an area of 245 ha.

Strachotin pond is a pond located next to the village of Strachotin in the immediate vicinity of the Nove Mlyny reservoir. The pond has an area of 54.5 ha and is used for commercial fish farming, i.e. breeding of carp and other fish. Its water source is a nameless left tributary of the Dyje River.

Vrkoc is located between the villages of Ivan and Nova Ves. The water area is 156 ha. The pond is fed from the Cvrcovice Mill race which receives water from the Jihlava River. The pond is also owned by a commercial fish farm and is intended for carp breeding.

Fish sampling

The common carp (*Cyprius carpio* L.) used in this study were collected during the fish harvest at three ponds in the Morava River basin in October 2020 as killed market carp. The carp (*Cyprinus carpio* L.) is an omnivorous fish species from the *Cyprinidae* family that is very common in Czech rivers and reservoirs.

Ten samples of carp, approximately four years of age, were obtained from each pond. All killed fish were transported to the laboratory in ice. Their sex (three females and seven males from the Jaroslavicky and Strachotin ponds and five females and five males from the Vrkoc pond), body and total length and weight were recorded. The main characteristics of the sampled fish are summarised in Table 1. Samples of muscle, liver, kidney, spleen, brain, scales, and gonads from 30 individual fish were collected during the autopsy. Samples of muscle tissues were taken from the cranial parts of the fish above the lateral line. All tissues were sampled using stainless steel scalpels and tweezers and put into polyethylene bags and stored in a freezer at −18 °C until total mercury analysis.

Table 1. The main characteristics of the sampled carp (weight, body length, and total length; mean ± SD).

Pond	Weight (kg)	Body length (cm)	Total length (cm)
Jaroslavicky	2.53 ± 0.24	42.88 ± 2.27	50.48 ± 2.58
Strachotin	2.40 ± 0.27	42.42 ± 1.54	49.69 ± 1.33
Vrkoc	2.81 ± 0.24	46.24 ± 0.95	54.6 ± 1.78

Mercury determination

The THg content in tissues was determined by cold vapour atomic absorption spectrometry in AMA 254 analyser (Altec Ltd., Praha, Czech Republic). The samples were thawed, weighed (analytical scales Precisa 125A, Dialab Praha, Czech Republic) - approximately 50 mg, put into combustion boats and inserted into the AMA 254 without any further sample preparation. The samples were dried at 120 °C for 60 s and thermally decomposed at 550 °C for 150 s under oxygen flow. AMA 254 analyser works in two ranges, these are automatic switch-over for lower and higher mercury concentration (0.05 to 40 and 40 to 500 ng Hg). Mercury standard AA standard 1000 $\mu g \cdot ml^{-1}$ Hg in 5% HNO_3 (Agilent technologies, USA) was used. The detection limit is 0.18 $\mu g \cdot kg^{-1}$ and LOQ 0.59 $\mu g \cdot kg^{-1}$ (LOD is triple SD of blank and LOQ is × 10 SD of blank). Each sample was measured at least twice. If relative standard deviation of the measurement of the duplicate samples was higher than 10%, more parts of the samples were measured till the RSD was less than 10% as required by the manufacturer's instructions. The standard reference material NIST–2976 (muscle tissues, National Institute of Standards and Technology) was used for validation. The measured value was 55.30 ± 0.67 $\mu g \cdot kg^{-1}$ and the certified value was 61.0 ± 3.1 $\mu g \cdot kg^{-1}$. The results were not corrected for the actual value of recovery. The accuracy was 90.6%.

Statistical analyses

Data of 30 samples were statistically analysed. Data manipulation and statistical analyses were performed in Unistat for Excel 6.5. First, data on fish weight were tested for differences between males and females from individual ponds. Since no difference was observed, the data were analysed together. The data from individual ponds and tissues were evaluated from the point of view of mercury content using normality test (Shapiro-Wilk test). Because both normal and non-normal distributions were found, non-parametric tests were used. Differences in the THg content among tissues in one pond and also among ponds were analysed using Kruskal-Wallis one-way ANOVA (Tukey HSD). Difference in the THg content between testes and ovaria was analysed using Mann Whitney test. The level of significance P was determined to be 0.05.

Results

Weight values are given in Table 1. Concentrations and differences in the THg among individual tissues of carp for all thee sampled ponds are shown in Figs 2–4.

The highest average content of THg was found in the muscle of carp from all of the studies ponds (Jaroslavicky 7.6 ± 2.8 $\mu g \cdot kg^{-1}$, Vrkoc 6.8 ± 2.3 $\mu g \cdot kg^{-1}$ and Strachotin 12.9 ± 4.3 $\mu g \cdot kg^{-1}$). This was followed by the levels found in caudal kidney, liver, spleen and brain. On the other hand, the lowest THg content was found in scales (Jaroslavicky 0.8 ± 0.3 $\mu g \cdot kg^{-1}$), gonads (in Strachotin 0.8 ± 0.3 $\mu g \cdot kg^{-1}$ and Vrkoc 0.7 ± 0.5 $\mu g \cdot kg^{-1}$).

A detailed analysis of THg content between the individual sites showed no significant differences in scales, gonads or brain ($P > 0.05$). However, differences were found for muscle, caudal kidney, liver and spleen (Table 2).

Table 2. The differences in total mercury content (THg) in selected tissues of market carp between studied ponds (Jaroslavicky, Strachotin, Vrkoc).

	Jaroslavicky	Strachotin	Vrkoc
Tissue		Mean ± SD THg ($\mu g \cdot kg^{-1}$)	
Muscle	7.6 ± 2.8 ^{ab}	12.9 ± 4.3^{b}	6.8 ± 2.3^{a}
Caudal kidney	3.1 ± 0.6^{ab}	4.6 ± 1.0^{b}	2.8 ± 1.4^{a}
Liver	1.6 ± 0.3^{a}	2.4 ± 0.4^{b}	1.8 ± 0.5^{a}
Spleen	1.1 ± 0.2^{a}	2.0 ± 0.5^{b}	2.3 ± 2.2^{b}

^{a,b} - Different superscripts show significant differences. The THg concentration in fish tissues is given in $\mu g \cdot kg^{-1}$ wet weight.

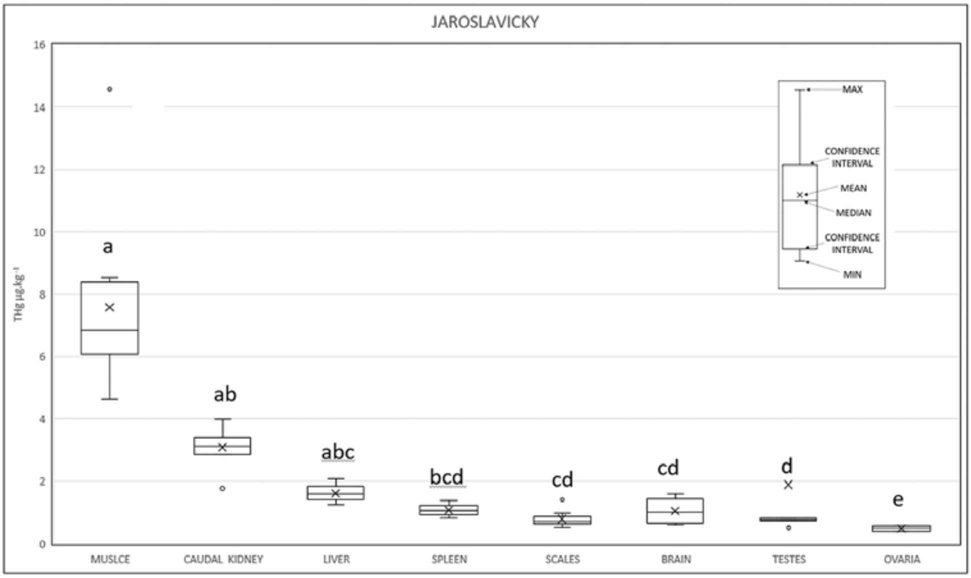

Fig. 2. Comparison of the total mercury content (THg) between the individual tissues of common carp from the Jaroslavicky pond, including significant differences. The THg concentration in fish tissues is given in $\mu g \cdot kg^{-1}$ wet weight. Groups with different superscripts ($^{a, b, c, d}$) differ significantly. The level of significance *P* was determined to be 0.05.

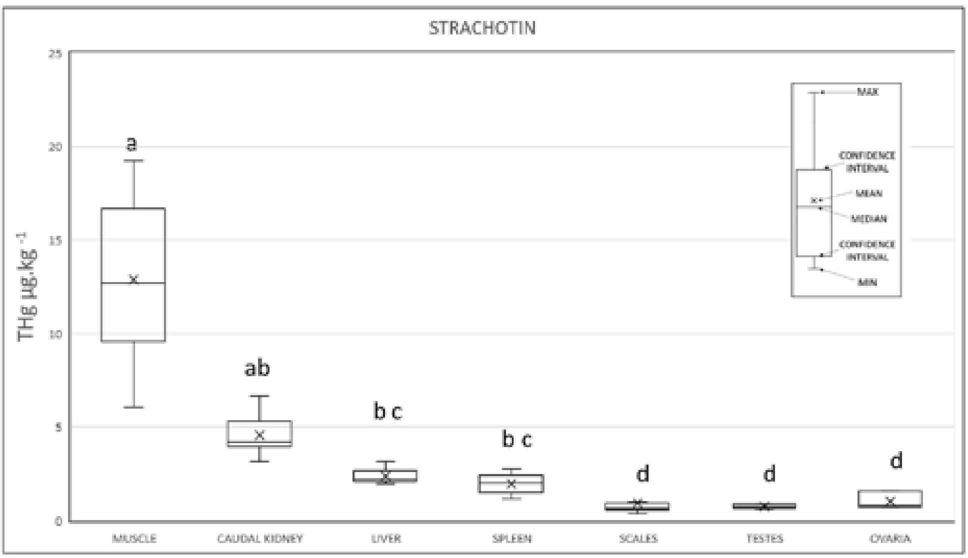

Fig. 3. Comparison of the total mercury content (THg) between the individual tissues of common carp from the Strachotin pond, including significant differences. The THg concentration in fish tissues is given in $\mu g \cdot kg^{-1}$ wet weight. Groups with different superscripts ($^{a, b, c, d}$) differ significantly. The level of significance *P* was determined to be 0.05.

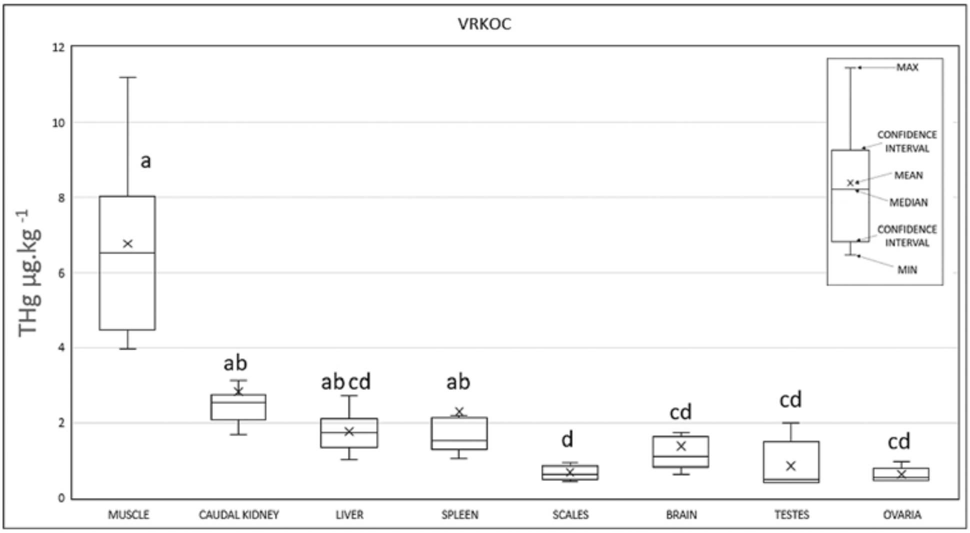

Fig. 4. Comparison of the total mercury content (THg) between the individual tissues of common carp from the Vrkoc pond, including significant differences. The THg concentration in fish tissues is given in $\mu g \cdot kg^{-1}$ wet weight. Groups with different superscripts ($^{a, b, c, d}$) differ significantly. The level of significance *P* was determined to be 0.05.

Discussion

Assessment of the relationship between THg content in muscle and parenchymatous tissue is important for the appraisal of the degree of mercury contamination in the environment. Havelkova et al. (2008) stated that mercury content in uncontaminated localities is higher in muscle than in parenchymous issue. The data of the study are consistent with the findings of Havelkova et al. (2008) and Kruzikova et al. (2011), where they compared THg concentrations in fish from heavily and slightly contaminated areas. Havelkova et al. (2008) used the THg liver/muscle index for the assessment of mercury contamination using the formula THg liver/THg muscle (both in $\mu g \cdot kg^{-1}$). An index higher than 1 means a highly mercury contaminated location, while an index lower than 1 means negligible contamination (Kružíková et al. 2013). The liver has the ability to accumulate pollutants from the environment and plays an important role in detoxification. In case of the uncontaminated/slightly contaminated sites, the THg in muscles is usually higher than in liver. However, where sites are burdened with contamination, the ratio is reversed. When the mercury content in the aquatic environment is high, mercury tends to accumulate more in the liver. From the results found in this study, the THg liver/muscle index was calculated to be 0.222, 0.201, and 0.265, respectively, for the Jaroslavicky, Strachotin, and Vrkoc ponds. This indicates that the studied ponds can be considered pollution-free localities.

At the studied localities (Jaroslavicky, Strachotin and Vrkoc ponds), mercury contents in the muscles were found to be many times lower than the limit for mercury content in the muscle of carp set by the Commission Regulation (EU) 2023/915. The maximum level set for *Cyprinidae* fish is 0.3 $mg \cdot kg^{-1}$. The THg levels in the muscle from the studied ponds correspond with the unloaded localities as well as the position of carp in the food chain. The results found suggest that mercury does not pose a risk to consumers of these commercially farmed fish. Mercury contents in market carp are found to be below the limit in the long term in the Czech ponds in South Bohemia (Král and Svobodová 2020), in West and South of Bohemia (Sehonova et al. 2022), in reservoirs from Bohemian-Moravian Highlands (Vičarová et al. 2016) and also in carp from Slovak surface waters (Kimáková et al. 2018). The results are in accordance with the findings from the studies by Sehonova et al. (2022) and Svobodová et al. (1999) reporting that the mercury content in carp decreases in the following order: muscles > caudal kidney > liver > spleen > brain/gonads > scales.

In conclusion, the results of analyses of market carp show that fish from the studied Jaroslavicky, Strachotin, and Vrkoc ponds are not loaded with mercury. Higher mercury concentration was found in the muscle. According to the THg liver/muscle index, the Jaroslavicky, Strachotin and Vrkoc ponds are considered to be uncontaminated sites. Likewise, the detected values in the muscles of carp do not exceed the established maximum limit for contaminating substances (set at 0.3 $mg \cdot kg^{-1}$ for carp), therefore, the carp are suitable for human consumption.

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