Environ Geochem Health
https://doi.org/10.1007/s10653-021-00861-x

ORIGINAL PAPER

Mercury in fish tissues in the area of Malachov Hg-ore deposit (Slovakia)

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Received: 22 January 2020 / Accepted: 14 February 2021

Abstract The abandoned Malachov deposit belongs among the most important historic Hg deposits in the world. The soil, groundwater, surface water, plants, and animals are still heavily contaminated by potentially toxic elements such as As and Cd, but mainly by Hg. This article is focused on the Hg contamination of aquatic plants and animals in the Malachov creek. Mercury concentrations were measured in fresh water (on average 3 μg L^{-1}), in the zoobenthos (on average 362.47 μg kg^{-1}), and in the phytobenthos (on average 578.36 μg kg^{-1}). Higher Hg concentrations were determined in the muscles of *Salmo trutta* morpha *fario* (on average 362.47 μg kg^{-1}) and lower in *Cottus poecilopus* (on average 352.75 μg kg^{-1}). The Hg concentrations were higher in the internal tissues (578.36–1185.75 μg kg^{-1}) than in the muscles of the fish. The Hg content in fresh water exceeded Regulation of the Slovak Government No. 269/2010, which stipulates the criteria for achieving a proper water balance, but the Hg content in the fish muscles of both fish species was below the specified limit of the Food Code of Governmental Regulation of the Slovak Republic No. 608/3/2004–100. The Hg contamination in fishes is controlled by their weight and age. The presented data may be used as the base information for future studies in order to be able to estimate consumption recommendations and warnings.

Keywords Mining country · Mercury · Contamination · Fishes · Malachov · Water

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Introduction

Mercury is a well-known toxic contaminant. In the environment, Hg is usually present in Hg^{2+} form (Bjørklund et al., 2017; Navarro, 2008). The main primary source of Hg is volcanic activity and the mineral cinnabar—HgS (Dadová et al., 2016). The environment is usually more substantially contaminated by human activities such as the processing of Hg-ore, burning of fossil fuels, various types of waste, as well as a consequence of industrial and agricultural activities (Barbieri et al., 2018; Bjørklund et al., 2017).

Published online: 28 February 2021

Bacteria can modify less toxic inorganic Hg compounds (mainly in anaerobic conditions) into their substantially more toxic organic forms (diethylmercury—$C_4H_{10}Hg$, dimethylmercury—$C_2H_6Hg^+$ and mercury metylhydrargyriumhydroxide—CH_3HgOH; Cappon & Smith, 1995; Kafka & Punčochářová, 2002). The toxicity of Hg is controlled by its solubility, pH, and Eh. The toxicity at high pH values (> 10.65) markedly decreases (Randall, 2004). Inorganic forms such as Hg^0 and Hg^+ are less soluble than organic Hg^{2+} compounds (Langford & Ferner, 1999), which are highly soluble in both water and grease (Clifton, 2007). Since Hg is also known for its high affinity to biogenic sulfur compounds, it binds to proteins and enzymes and thus causes a harmful effect on the organism (Zmetáková & Šalgovičová, 2006) because of the high transference potential (Barbieri et al., 2014, 2018). Aquatic organisms demonstrate high sensitivity to Hg intoxication (Kuwabara et al., 2007; Sunderland, 2007). The order of individual components in relation to Hg content is usually as follows: water < zooplankton < zoobenthos < fish (Kafka & Punčochářová, 2002). Predatory fish usually have a higher Hg content in comparison with fish that feed on phytobenthos and plants (Beltran-Pedreros et al., 2011). Hg from the benthos enters the food chain through aquatic organisms (mainly fish) (Cappon and Smith 1995).

The Malachov region is situated in a mild climate zone. The average monthly temperature ranges from − 2.1 °C (January) to 18.9 °C (July). The total precipitation averages from 850 to 1250 mm (Bárdiová, 2019).

The first records about the exploitation of Hg-ores in the surroundings of Malachov date from 1390 (Jeleň & Galvánek, 2015). The mining activity culminated in the sixteenth century, with several interruptions occurring between 1580 and 1780 and subsequently between 1839 and 1950, continuing up to 1990 (Ferenc et al., 2013). In this year, the economical value of the ore resources was considered as exhausted, and the mine was closed. The mine belonged together with Almaden (Spain) and Idria (Slavonia) among the most important Hg mines in Europe. As an example, even in 1535, 20 kg of mercury per week was extracted here. It is estimated that about 5700 kg of mercury in total was extracted during this period. The present resources are estimated to be approximately 282 kt of ore with about 0.22% Hg (Knésl & Linkešová, 1971; SMY, 2009).

The disseminated and lens-shaped bodies of Hg-As mineralization are hosted by the conglomerates and dolomitic sandstones of the Paleogene age, in the Middle Triassic dolomites, and in the Rhaetic limestones in the underlying bed from the Paleogene series, as well as locally in Miocene volcanites. In most of these occurrences, cinnabar ore is hosted largely by faults and fractures of a N–S direction, which are collectors of mineralization due to the fact that these structures control the pathways for ore-forming fluids (Dadová et al., 2016; Ferenc et al., 2013). The other ore minerals are pyrite, marcasite, and metacinnabar (Koděra et al., 1990).

The mining area is drained by the Malachov creek. The area of the river basin is 16.35 km^2, and the density of water stream system is 1.135 km per km^2. The main water stream is the 9.9 km-long Malachov creek. The average flow is 0.3 $m^3\ s^{-1}$ per year (Bárdiová, 2019).

The long-term cinnabar extraction activity in the Malachov mine influenced the environment by introducing several toxic metals, mainly Hg, As, and Cd (Dadová et al., 2016). The aim of the article is to test the Hg accumulation in the two most common fish species, river trout (*Salmo trutta* morpha *fario*) and Siberian bullhead (*Cottus poecilopus*), in the studied area downstream of the Malachov mine district, expecting higher Hg content in their viscera than in the muscles (as presented in many works e. g. Luczyńska et al., 2016; Zmetáková & Šalgovičová, 2006).

Material and methods

Study area

The Malachov creek belongs to the Hron River Basin. The creek drains the area of Vel'ká Studňa Hg-ore field and consequently flows through several occurrences of Hg mineralization at the Ortúty ore field and through the nearby village of Malachov. In the stream sediments at the bottom of the creek, a great accumulation of cinnabar can be observed. Locally on the surface of larger rocks, it is possible to see captured vegetation (mosses, water plants, and phytobenthos).

Sampling

Samples were taken near the Ortúty ore field. The water from the creek was taken about 500 m upstream of the village in May and June (during sunny and dry weather period, temp.—up to 27 °C), October, and November (sporadic rains, temp.—up to 10 °C) 2018 and stabilized with 10 ml of concentrated HCl per 1000 ml of surface water.

The fishes were caught mainly in shady places at the beginning of August using electric aggregate.

Seven pieces of river trout (*Salmo trutta* morpha *fario*) and 6 pieces of Siberian bullhead (*Cottus poecilopus*) were caught. The length of the river trout varied from 12 to 26 cm, and their weight ranged from 168 to 360 g; for the Siberian bullhead, from 11 to 13 cm, whereas its weight fluctuated from 153 to 180 g. The caught Siberian bullheads were smaller than the river trout, which were caught closer to the Ortúty ore field.

The river trout (*Salmo trutta* morpha *fario*) is a freshwater predatory fish of the Salmonidae family. It feeds on aquatic insect larvae, predominantly on *Trichoptera, Ephemeroptera*, *Chironomidae,* and zooplankton but also on terrestrial insects. Larger individuals feed on smaller fishes, amphibians, and even on small mammals (Gerstmeier & Romig, 1998). The alpine bullhead (*Cottus poecilopus*) is also a nocturnal freshwater predatory fish and belongs to the family *Cottidae* of *sculpins*. It lives nearby the bottom of creeks. It feeds mainly on insects, various crustaceans, and some small invertebrate prey that it finds on the bed of a stream (Janiga, 2018). There were no omnivorous, herbivorous, and parasitic fishes caught in the creek for comparison because they are very rare in the aforementioned creek.

The phytobenthos was obtained from the surface of creek boulders and branches lying on the bottom of a creek bed, and the zoobenthos was sampled by means of a sifter.

Analyses

The pH of the water was measured in situ using the Multi 340 instrument (WTW, Germany) equipped with SenTix_41 electrodes. The pH-meter was adjusted to pH 7.0 with the pH 7.00 buffer using the calibration knob.

The Hg concentration in the water was analyzed after filtration by hydride generation atomic absorption spectroscopy (standard error: 10,002 ± 5 μg mL^{-1}) in the laboratories of the Regional Authority of Public Health in Banská Bystrica. As a reference material Mercury AA Standard (Ultra Scientific, 99.999) was used.

The zoobenthos and phytobenthos samples were dried at laboratory temperature while the fish tissues were analyzed directly without drying. The total Hg content both in the zoobenthos, phytobenthos, and the fish were determined by thermal decomposition-gold amalgamation atomic absorption spectroscopy (AMA-254 Advanced Mercury Analyzer, Altec ltd.). Two types of fish tissues were analyzed: muscles and internal organs (mixed samples of livers and kidneys). One sample of river trout (*Salmo trutta* morpha *fario*) spawn was analyzed separately. The weight of the samples varied from 1 to 65 mg according to the character of the sample. After drying, the sample was burned in an oxygen stream in a furnace at atmospheric pressure. The residues were taken through the amalgamator by the oxygen stream. The absorption of Hg radiation at 254 nm was measured using a method validated and certified under the ISO/IEC 17025: 2005 standard by the Slovak National Entity for Accreditation. The method had limit of detection of 5 μg kg^{-1} and limit of quantification of 1.6 μg g^{-1}.

The correlations between the selected Hg content and body parameters were described by Spearman's rank correlation coefficient (r) with the significance level $p \leq 0.05$. The statistical analyses were performed by means of the IBM SPSS Statistics 19 software application.

Results

The results of the pH and Hg concentration analyses in the surface water of the Malachov creek are presented in Table 1. The pH was, in all cases, close to the neutral value but slightly alkaline. The pH values fluctuated from 7.04 in November to 7.83 in October 2018. The average pH value was 7.6 (Table 1). The Hg concentration in water is relatively high. The mean Hg concentration calculated from four measurements was 3 μg L^{-1}, which exceeded the permissible limit (0.05–1 μg L^{-1}) given by The Regulation of the Slovak Government No. 269/2010.

Table 1 Mercury concentrations in the surface water of the Malachov creek (St. dev.—standard deviation)

Sample	Date of sampling	pH	Hg μg L^{-1}	Law limit
1	May 5th 2018	7.76	0.3	0.05–1
2	July 4th 2018	7.82	9	
3	October 19th 2018	7.83	2	
4	November 22nd 2018	7.04	2	
Average		7.60	3	
St. dev.		0.38	3.87	

Hg content in benthos

The majority of the zoobenthos belongs to the species *Trichoptera*, (a good indicator of the water quality), *Gammaruspulex*, and *Hirudo* sp. (Bárdiová, 2019). The average Hg concentration in the zoobenthos is 255.65 μg kg^{-1}.

The phytobenthos consisted of three main aquatic plants: common liverwort (*Marchantia polymorpha),* moss (*Brachythecium rivulare*), and cress (*Cardamine amara*). The average Hg concentration in the phytobenthos is similar to the Hg concentration in the zoobenthos, 250.55 μg kg ^{1}.

Hg content in fishes

The Hg concentration in the muscles of *Salmo trutta* morpha *fario* varied within the range of 252.0–481.9 μg kg^{-1}. The average Hg concentration in the muscles of this fish species is 362.47 μg kg^{-1}, whereas the Hg concentration in the muscles of *Cottus poecilopus* varied within the range from 288.5 to 406.4 μg kg^{-1}. The average Hg concentration in the muscles of this species was slightly lower (352.75 μg kg^{-1}) as in *Salmo trutta* morpha *fario* (Table 2).

The Hg concentration in the fish viscera (in livers and kidneys) was substantially higher in the Siberian bullhead (*Cottus poecilopus*), where it ranged from 673.3 to 2510.1 μg $kg^{-\ 1}$ (the average Hg concentration is 1185.75), while in the river trout (*Salmo trutta* morpha *fario*) the average Hg concentration is 1185.75 μg kg^{-1} (Table 2).

The Hg concentration in the viscera of the river trout (*Salmo trutta* morpha *fario*) was very variable. It varied in a wide range from 402.0 to 1085.7 μg kg^{-1}. The average Hg concentration was 578.36 μg kg^{-1}

Table 2 Hg concentrations in the muscles and viscera of fishes from the Malachov creek (St. dev.—standard deviation; significant values are in bold). One measurement was conducted for each sample

Fish species	Sample	The length of the fish (cm)	The weight of the fish (g)	Muscles Hg (μg kg^{-1})	Viscera
Salmo trutta morpha *fario*	1	18	252	420.0	**1085.7**
	2	26	360	481.9	**830.0**
	3	16	224	283.7	**471.9**
	4	15	210	264.5	**405.4**
	5	12	168	252.0	**402.0**
	6	13	182	447.9	**444.8**
	7	12	205	387.3	**408.7**
	Average	16	229	362.5	578.4
	St. dev.	4.93	64.00	94.42	270.70
Cottus poecilopus	8	12	165	288.5	**830.0**
	9	11	153	365.0	**673.3**
	10	13	185	344.6	**2510.1**
	11	12	172	406.4	**1302.4**
	12	11	180	312.8	**918.6**
	13	12	179	399.2	**880.1**
	Average	12	171	352.8	1185.8
	St. dev.	0.75	11.76	46.85	681.39
Law limit				500.0	100.0

(Table 2). In a single sample of river trout spawn, the Hg concentration was determined to be 79 μg kg^{-1}.

Discussion

Mining activity may cause the heavy contamination of country's components, which is noticeable even after several hundred (or thousand) years. The Malachov ore deposit can be considered as this type of locality. The impact of cinnabar extraction from the Hg mine has been described by numerous authors (Andráš et al., 2014; Dadová et al., 2016; Midula et al., 2017) with regards to the occurrence of Hg both in surface and groundwater, soil, plants, and animals, including aquatic animals which are potential food for fish (Bajčan et al., 2013; Budtz-Jørgensen et al., 2007; Sunderland, 2007). The Malachov creek represents an active source of Hg which appears to be bound to the suspended particles of cinnabar in the stream's sediments. The fish are also generally able to accumulate Hg from non-polluted sediments (Hošek et al. 2019). Similarly, as at Idrija (Cerovac et al., 2017), at Malachov cinnabar is also the most prevalent form followed by matrix-bound Hg (Dadová et al., 2014).

Trichoptera larvae and *Gammar usfossarum* are usually considered to be sensitive bioindicators of clean water, because they need extraordinarily high oxygen and calcium amounts (Barber-James et al., 2008; Pereira et al., 2012). However, Hg concentration in the phytobenthos and in the larvae of *Trichoptera* and *Ephemeroptera*, following the other organisms of zoobenthos from the Malachov creek, were relatively high (255.65 and 250.55 μg kg^{-1}, respectively).

The high Hg concentration measured in the water during the summer period (July) may be caused by high atmospheric temperatures and subsequently the low aquosity of the creek. The other considered cause originates from the groundwater drainages (Andráš et al., 2014) including the ones directly streaming from the mines.

The highest Hg concentration in fish muscles from Malachov creek (481 μg kg^{-1}) was determined in the largest sample of *Salmo trutta* morpha *fario.* In the viscera, the highest Hg concentration (2510.1 μg kg^{-1}) was determined in *Cottus poecilopus.* However, it should be noted with respect to the fact that the viscera of freshwater fish are not usually consumed in Slovakia. Within the limits set, the Hg concentration in the representative fish muscle samples selected by the competent authority shall not exceed 0.5 mg kg^{-1} of fresh weight.

Similar research was undertaken by Maršálek et al., (2005) at the Skalka water reservoir (Czechia), which was polluted by Hg from a chemical factory. They analyzed several species of fish: *Rutilus rutilus*, *Abramis brama*, *Aristichthis mobilis*, *Aspius aspius*, *Anguilla anguilla,* and *Silurus glanis* (altogether 30 fish individuals). Their investigation determined higher Hg content in the liver, less in the muscles, and the least amount of Hg in the gonads, whereas a higher Hg content was found in predatory fish.

Cappon and Smith (1995), Toman et al. (2001), Együdová and Šturdík (2004), Kimáková & Bernasovská (2005, 2007a, 2007b), Zmetáková and Šalgovičová (2006) as well as Luczyńska et al. (2016) reported higher Hg concentration (both inorganic Hg and MeHg forms up to 5 mg kg^{-1}) in the muscles than in the internal organs (viscera: liver and kidneys). They explain it by Hg affinity to –SH groups in the protein. Dadová et al. (2016) published the first data from the Malachov region about the Hg contamination of *Salmo trutta* morpha *fario* and proved that the Hg content was higher in the viscera than in the muscles. Similar results were also published by Brázová (s.a.) and Vulterin & Vasileská (1996).

Our study showed that the Hg content was higher in the viscera in comparison with those in the muscles of both studied fish species. In *Salmo trutta* morpha *fario*, the Hg content in the viscera was approximately two times higher than in the muscles and in *Cottus poecilopus* nearly four times higher than in the muscles. In one sample of *Cottus poecilopus* (sample 10), the Hg content in the viscera was even nearly eight times higher than in the muscles.

The muscles of both investigated fish species contained comparable Hg concentration (Siberian bullhead—*Cottus poecilopus* 352.75 μg kg^{-1} and river trout—*Salmo trutta* morpha *fario* 362.47 μg kg^{-1}).

The Hg content in the same organs of different fish species and aquatic animals differs and usually correlates with the weight and age of the fish (Luczyńska et al., 2016). This correlation was not clearly proven by our study. Significant correlation was described for the weight of the fish and the Hg content in their viscera (for *Salmo trutta* morpha *fario* $r = 0.82$ and for *Cottus poecilopus* $r = 0.83$). These findings correspond with those published by Sackett et al., (2013). The other significant correlation is described for *Salmo trutta* morpha *fario*, specifically between body lengths and Hg content in the viscera ($r = 0.83$). This is very reasonable, since the weights and lengths of these species undoubtedly correlate ($r = 0.94$).

Kafka and Punčochářová (2002) highlight that Hg is highly soluble in grease. The bodies of older fish generally contain a higher grease content, so there are more suitable conditions for Hg uptake to the tissues of their bodies. However, a correlation degree of the Hg contents in the muscles and in the viscera vs. the weight (body mass) of the fish (and thus their age) was not detected.

Conclusions

Hg content in fish tissues is the subject of permanent monitoring, especially in areas where a source is well-known. In the Malachov mine district, the lack of relevant data is obvious.

The surface (creek) water, zoobenthos, and phytobenthos were contaminated by Hg. The average Hg concentration in the water, phytobenthos, and zoobenthos was 3 µg L^{-1}, 250.55 µg kg^{-1}, and 255.65 µg kg^{-1}, respectively.

Higher Hg concentration was detected in the muscles of river trout (*Salmo trutta* morpha *fario*—362.5 µg kg^{-1}) than in the muscles of Siberian bullhead (*Cottus poecilopus*—352.8 µg kg^{-1}). The average Hg concentration in the viscera of the river trout (*Salmo trutta* morpha *fario*) was 578.4 µg kg^{-1}, whereas in the Siberian bullhead (*Cottus poecilopus*) it was 1185.8 µg kg^{-1}. Thus, the Hg content in the viscera can be considered significantly higher than in the muscles.

In a single sample of river trout spawn, the Hg concentration was determined to be 79 µg kg^{-1}.

The most likely source of Hg contamination in the organs of the fish seems to be the phytobenthos and zoobenthos.

The high Hg content in fish which is commonly used as a food source can be generally considered as a threat for public health. Both the Hg concentrations in the muscles and in the viscera of the fish (apart from the fact that the viscera of freshwater fish in Slovak conditions are not usually consumed) did not exceed the limits given by the Food Code of Governmental Regulation of Slovak Republic No. 608/3/2004–100: 0.5 µg kg^{-1} for the muscles, and 100 µg kg^{-1} for the viscera. In order to prevent human harm, permanent monitoring should be established and information on local Hg contamination should be available to the public.

Authors' contribution PA—composed the main chapters of the manuscript and supervised the whole research, JD—performed the detailed research of mining activity in Malachov, RR, DB—performed the sampling and analytical works, PM, VD—performed statistical evaluation, contributed with several paragraphs in chapters Results and Discussion, prepared the formal shape of the manuscript.

Funding This research was funded by grant scheme No. 1/0291/19 of the Grant Agency VEGA (Vedecká Grantová Agentúra MŠVVaŠ SR a SAV).

Code availability The authors confirm that the data supporting the findings of this study are available within the article.

Compliance with ethical standards

Conflict of interest The authors declare that there is no conflict of interests.

Availability of data and material The authors confirm that the data supporting the findings of this study are available within the article.

Ethical approval All procedures performed in this study involving living animals were in accordance with ethical standards and were supervised by Slovak Fishermen's Association.

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