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RESEARCH PAPER



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Reduction of Mercury Accumulation in Carp Tissues (*Cyprinus carpio* L.) by Natural Organic Matter and Chlorides

Petra Vičarová¹ · Hana Dočekalová¹ · Eva Poštulková² · Jan Mareš² · Radovan Kopp² · Pavlína Pelcová^{1,3}

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Abstract

In natural waters and in breeding tanks certain substances are naturally present in the water or deliberately added to it. Some of these substances are characterized by the formation of strong complexes with mercury and thus they can reduce the intake of mercury by aquatic organisms. The accumulation of mercury in six selected tissues (skin, scales, kidneys, muscle, liver and gills) of *Cyprinus carpio* L. in the presence of humic acid and sodium chloride was studied. Carp fingerlings were exposed in fish tanks for 3 days to the bulk solution containing a defined concentration of Hg^{2+} (1.5 $\mu\text{g/l}$) and increasing concentration of humic acid (0–5 mg/l) and chloride ions (18–600 mg/l). The fish were not fed during the experiment and mercury accumulated in the carp tissues from fish tank water only. Total mercury content in water and in selected tissues was determined by the atomic absorption spectrometer AMA 254. Increased concentration of both humic acid and sodium chloride caused reduction of mercury accumulation in carp tissues up to 93 and 45%, respectively.

Keywords Common carp · Humic acid

Introduction

Mercury is well known as one of the most toxic metals in nature. Increasing concentrations of atmospheric mercury from anthropogenic sources have led to intensification of its deposition, which is the main source of mercury in surface waters. All mercury compounds are toxic to humans and animals. Even very low levels of mercury in surface waters (only few ng/l) can bioaccumulate and biomagnify through the food chain to the hazardous levels in the aquatic organisms (concentration increase by a factor of 10^7). The accumulation of mercury in fish tissues comes from water and predominantly from food, which

includes lower aquatic animals and plants. These organisms are then the main source of fish contamination via the food chain. Contaminated fish can pose a serious hazard to fish eating populations (Zhou et al. 2008; Watanabe et al. 2012). The bioavailability and potential toxicity of mercury for humans and other organisms varies widely depending on the pathway of exposure, the amount, the vulnerability of the person exposed and largely upon the chemical form (speciation) of mercury (Syversen and Kaur 2012; Tuzen et al. 2009a, b, c; Sari and Tuzen 2009). The speciation and thus also the bioavailability of mercury is greatly affected by the presence of ligands capable of forming extremely strong complexes with ionic mercury. The formation of these complexes reduces the content of the Hg free-ion form, which has greater chemical reactivity and has been shown to be more bioavailable (Guo et al. 2001).

Extremely important natural ligands in aquatic systems are organic molecules of varied origin and composition which are produced by biodegradation of dead organic matter. Major organic constituents of many natural waters are humic substances. Humic substances (HS), complex and heterogeneous mixtures of polydispersed materials are formed by biochemical and chemical reactions during the

✉ Petra Vičarová
vicarovapetra3@seznam.cz

¹ Department of Chemistry and Biochemistry, Mendel University in Brno, Zemědělská 1, 613 00 Brno, Czech Republic

² Department of Zoology, Fisheries, Hydrobiology and Apiculture, Mendel University in Brno, Zemědělská 1, 613 00 Brno, Czech Republic

³ Central European Institute of Technology, University of Technology, Brno, Czech Republic

decay and transformation of plant and microbial remains. In surface waters and at higher concentrations they can impart a dark colour, especially in brown fresh water ponds, lakes, and streams. In aqueous systems such as rivers and lakes about 50% of the dissolved organic materials are HS. Aquatic HS contain two main fractions: humic acids (HA) and fulvic acids (FA). The precise properties and structure of a given HS sample depend on the water or soil source. Nevertheless, the average properties of HA and FA from different sources are remarkably similar. The chelating characteristics of humic substances with conditional stability constants ($\log K'$) in the range of 21–24 indicate that the vast majority of ionic inorganic mercury dissolved in natural waters is associated with organic complexes (Ravichandran 2004).

The inorganic speciation of Hg(II) in waters is dominated by chloride. The formation of chloride complexes (HgCl^+ , HgCl_2 , HgOHCl) is expected in oxic condition with higher concentration of chlorides in the absence of organic matter. But under low chloride and oxic conditions, the vast majority of ionic inorganic mercury dissolved in freshwater and coastal saltwater is associated with organic complexes (Seline 2009).

The common carp (*Cyprinus carpio* L.) is the most widely distributed freshwater fish worldwide. It represents a major resource for aquaculture production in several countries. The common carp is also the predominant fish farmed in Czech ponds and reservoirs. It provides 87–90% of all fish consumed in the Czech Republic (Celechovska et al. 2007; Kensova et al. 2010; Cervený et al. 2014). Fish from contaminated localities may indirectly influence the harmful effects of mercury to the human beings. Therefore, there have been many studies in recent years, which pay attention to mercury concentration in tissues of common carp fished in Czech lakes and reservoirs. Carp are fished in breeding reservoirs and also by anglers (Spurný et al. 2002; Cervený et al. 2014; Svobodova et al. 2004; Celechovska et al. 2007).

While humic acids are naturally present in all surface waters, sodium chloride is used in fish breeding for antiparasitic treatments from the early stages of fish until their marketable size (Ravichandran 2004; Svobodova et al. 2007). Therefore, in our investigation we chose humic acid and chloride ions as the studied ligands.

The goal of our work was to improve the knowledge on mercury uptake and distribution of mercury in the selected tissues (skin, scales, kidneys, muscle, liver and gills) of common carp (*Cyprinus carpio* L.) in the presence of various concentrations of humic acid and sodium chloride in laboratory conditions and to assess the effect of both these ligands on mercury accumulation in fish.

Materials and Methods

Fish (*Cyprinus carpio* L.)

A group of healthy, disease-free carp fingerlings procured in a fish-pond management in the Vodňany region (Czech Republic) were acclimatized for 2 weeks under laboratory condition with proper aeration. The fish were fed with commercially available feeds (granules SCREETING F1 PB 40 (2.5 mm, 0.018 mg/kg of total mercury) and during all experiments were exposed to a 12-h light/12-h dark photoperiod. 5 days before experiment fish were not fed. The weights of fish taken into study were 57.7 ± 0.8 g.

Chemicals

Mineralized water produced by the Aqua Osmotic 02 system (Aqua Osmotic, Czech Republic) was used for preparation of all solutions. Water in tanks was spiked with inorganic mercury calibration standard for ICP of concentration 1.000 ± 0.002 g/l Hg in 2% v/v HNO_3 (Fluka, Canada). Sodium chloride (Penta, Czech Republic) and humic acid (Sigma–Aldrich, Switzerland) were used in this experiment. All reagents were of analytical grade.

Experiment

Six glass fish tanks were each filled with 85 l of tap water. The water parameters of all tanks were maintained as in the natural habitats of this fish species. The physico-chemical parameters of laboratory water conditions were analysed by the methods of Clesceri et al. (1998). Instruments for analysis of water parameters: portable meter HACH HQ40D (pH, temperature and dissolved oxygen content in the water)—Hach-Lange, USA; and Hanna meter combo (conductivity)—Hanna Instruments, USA, are shown in Table 1.

Table 1 The water parameters

	Hg + Cl	Hg + HA
Conductivity (μS)	511.2–3240.4 ^a	477.8 $\pm$ 7.8
pH	7.3 $\pm$ 0.1	7.0 $\pm$ 0.1
Temperature ($^{\circ}\text{C}$)	19.1 $\pm$ 0.1	20.0 $\pm$ 0.1
Dissolved oxygen (mg/l)	8.2 $\pm$ 0.1	8.3 $\pm$ 0.1

Data are presented as mean $\pm$ SD

^aConductivity was increased with increasing concentration of chloride in the water (for control tank = 511.2 ± 19.8 μS , for concentration 180 mg l^{-1} chloride = 1300 ± 240.8 μS and for concentration 600 mg l^{-1} chloride = 3240.4 ± 32.6 μS)

The concentration of mercury in the used tap water was below the value under Czech Regulation No. 252/2004 Sb of Coll. for the concentration of mercury in drinking water ($< 0.1 \mu\text{g/l}$). After elimination of chlorine used for bacteriological protection of drinking water, the water in tanks was spiked with $1.5 \mu\text{g/l}$ mercury. Three tanks were used for the chloride experiment. Chloride ions were applied in the form of sodium chloride. Water in the first tank was kept as the control unenriched with chloride ions. Natural concentration of chloride ions in used tap water was 18 mg/l . Concentration of chlorides in the second tank was enriched to 180 mg/l and in the third tank to 600 mg/l . The remaining three tanks were used for the experiment with humic acids. The first tank remained without addition of humic acid. Water in the second tank was adjusted to 1 mg/l and in the third tank to 5 mg/l of humic acid.

Sixty carp fingerlings were randomly divided into six groups containing equal numbers (ten fish/group) and every group was exposed into corresponding tank for 3 days with continuous aeration. During the whole experiment, the concentration of mercury, in the fish tanks, was monitored by atomic absorption spectrometer AMA 254 (Altec Praha, Czech Republic) and was adjusted in case of change to the required values. The concentration of humic acid and chlorides was monitored once a day. Argentometric method (Mohr method) was used for determination of chlorides. The concentration of humic acid was monitored as the amount of organic substances in the water system by determination of chemical oxygen demand (COD). During the exposure, fish were kept without any food supply, thus mercury was accumulated to the fish tissues only from water. After 72 h, the carps were removed from tanks, killed (bleeding by syringe) and individual carp tissues (skin, scales, kidneys, muscle, liver and gills) were selected and analysed together with a non-exposed group of ten carps.

Determination of Mercury

Atomic absorption spectrometer AMA 254 (Altec Praha, Czech Republic) was used for determination of total mercury in the tank water and in carp tissues. The

homogenized solid samples ($10\text{--}100 \text{ mg}$) was directly weighed into pre-cleaned combustion boats, and inserted into the AMA 254 analyser. The samples were dried at 120°C for 60 s and thermally decomposed at 550°C for 150 s under oxygen flow. The selectively trapped mercury was released from the amalgamator by a brief heat-up and finally quantified (measuring cycle, 57 s) as Hg^0 by cold-vapour AAS technique at 253.7 nm . The limit of detection for mercury was $0.11 \mu\text{g/kg}$. The results were given as mg/kg wet weight.

Data were presented as mean $\pm$ SD. Statistical analysis for accumulation of mercury in each treated group was carried out by one-way analysis of variance (ANOVA) to determine the significance level and the results were compared to f -table values at the p values < 0.05 of probability level.

Results and Discussion

Method Validation

The reference material DORM-4 (fish protein, Canada) was used for method validation. Concentration of total mercury in reference material measured by AMA 254, $0.409 \pm 0.007 \text{ mg/kg}$, was in very good agreement with the certified value of $0.410 \pm 0.055 \text{ mg/kg}$.

Concentration of Total Mercury in Tissues of *Cyprinus carpio* L.

The concentration of total mercury was determined in six selected tissues (skin, scales, kidneys, liver, gills and muscle) of common carp. The studied tissues were analysed and divided into two groups: tissues commonly used for biomonitoring (skin, scales and gills) and detoxifying organs (kidneys and liver). Muscle, which is the tissue predominantly used for human consumption, formed a separate category. Mercury concentrations accumulated in individual tissues of non-exposed carps and carps exposed to the solution spiked with $1.5 \mu\text{g/l}$ mercury in the absence of chloride ions and humic acid are summarized in Table 2.

Table 2 Concentration of mercury in carp tissues of non-exposed group and exposed group (concentration of mercury in solution $1.5 \mu\text{g/l}$, time of application 72 h, ten carp fingerlings)

Carp tissues	Hg (mg/kg) non-exposed group	Hg (mg/kg) exposed group
Gills	0.009 ± 0.001	2.661 ± 0.150
Scales	0.011 ± 0.001	0.944 ± 0.142
Kidneys	0.025 ± 0.001	0.687 ± 0.131
Skin	0.020 ± 0.003	0.465 ± 0.080
Liver	0.018 ± 0.001	0.278 ± 0.031
Muscle	0.040 ± 0.001	0.102 ± 0.030

Data are presented as mean $\pm$ SD, $n = 10$

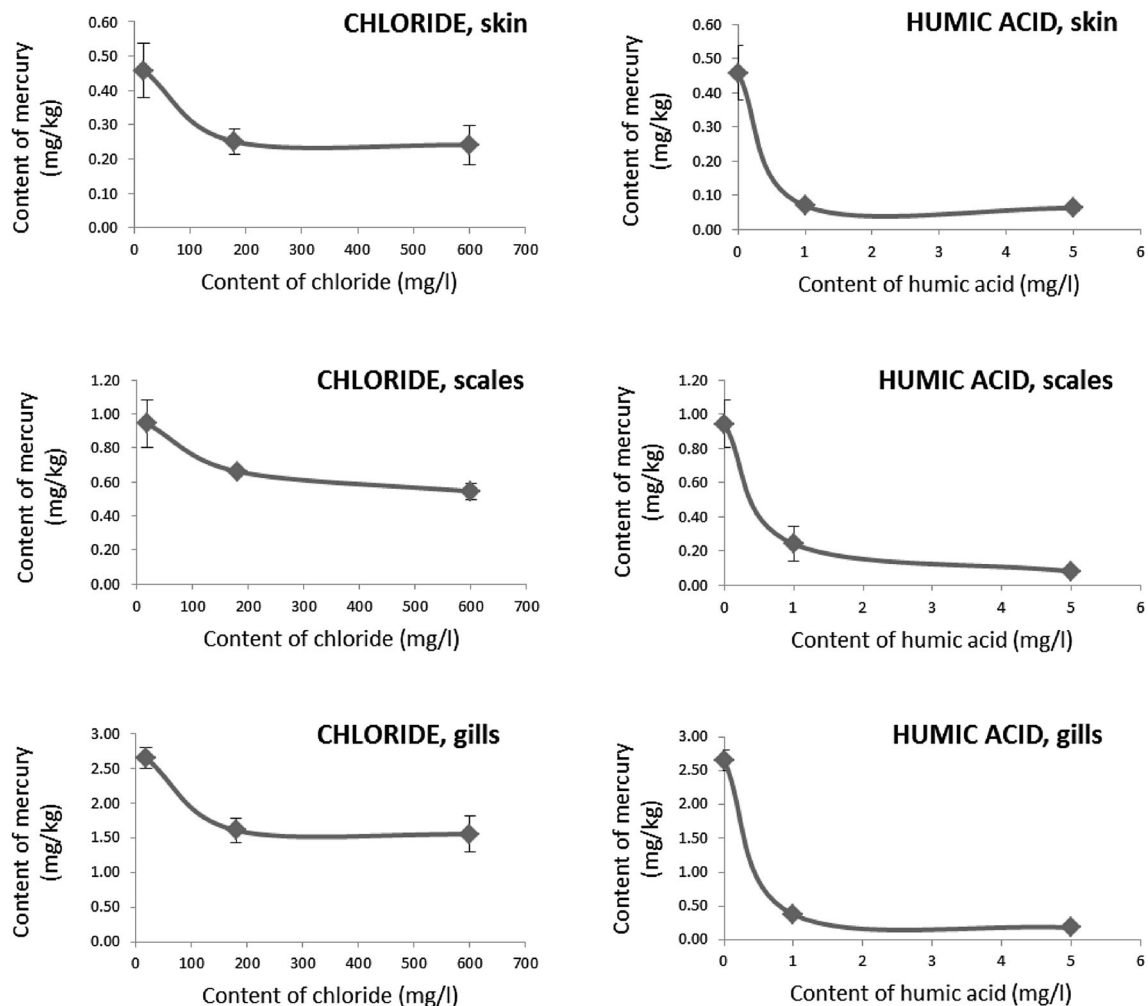


Fig. 1 **a** Concentration of total mercury in carp tissues ($n = 10$)—skin, scales and gills. **b** Concentration of total mercury in carp tissues ($n = 10$)—muscle, kidneys and liver

The highest concentration of mercury in non-exposed group was found in muscle. The liver/muscle ratio was studied for common carp, common goldfish and zebrafish (Svobodova et al. 1993, 1995, 2002). It was described, that in fish from clean localities, mercury was deposited preferentially in the muscle, while in heavily contaminated areas it was deposited preferentially in liver. It infers a buffering action of liver, protecting the other tissues, namely muscle, against mercury accumulation and the subsequent toxicity in highly contaminated localities. The liver/muscle ratio (0.045) found indicated that carp fingerlings used in our experiment were bred in a clean environment. Carp exposure to the solution enriched with mercury (concentration 1.5 $\mu\text{g/l}$) changed liver/muscle ratio to 2.7. In spiked solution clear differences in mercury accumulation were observed resulting in the pattern gills > scales > kidneys > skin > liver > muscle. The highest accumulation was found in gills. The direct mercury uptake from the water via gills was determined as an important

mercury uptake route. Therefore, gills are frequently used in monitoring of metal contaminations in natural waters (Celechovska et al. 2007; Spurny et al. 2002; Taravati et al. 2012; Visnjic-Jeftic et al. 2010).

The mode of mercury distribution and deposition was shown to be dependent on a specific tissue (Mieiro et al. 2009). It is commonly known that toxic metals accumulate in detoxifying organs and organs in direct contact with the contaminated environment. The highest amount of mercury in detoxifying organs was found in kidneys. Kidneys together with liver are two principal target organs for inorganic mercury in fish (Watanabe et al. 2012). Kidneys are one of the major organs for detoxification and elimination of metallic pollutants (Ghosh and Mandal 2012). It serves as a means of water excretion, based primarily on filtration at the glomerulus followed by solute reabsorption. It is the primary site where mercury accumulates after chronic exposure (WHO 1991). The lowest mercury accumulation was found in muscle. The significantly

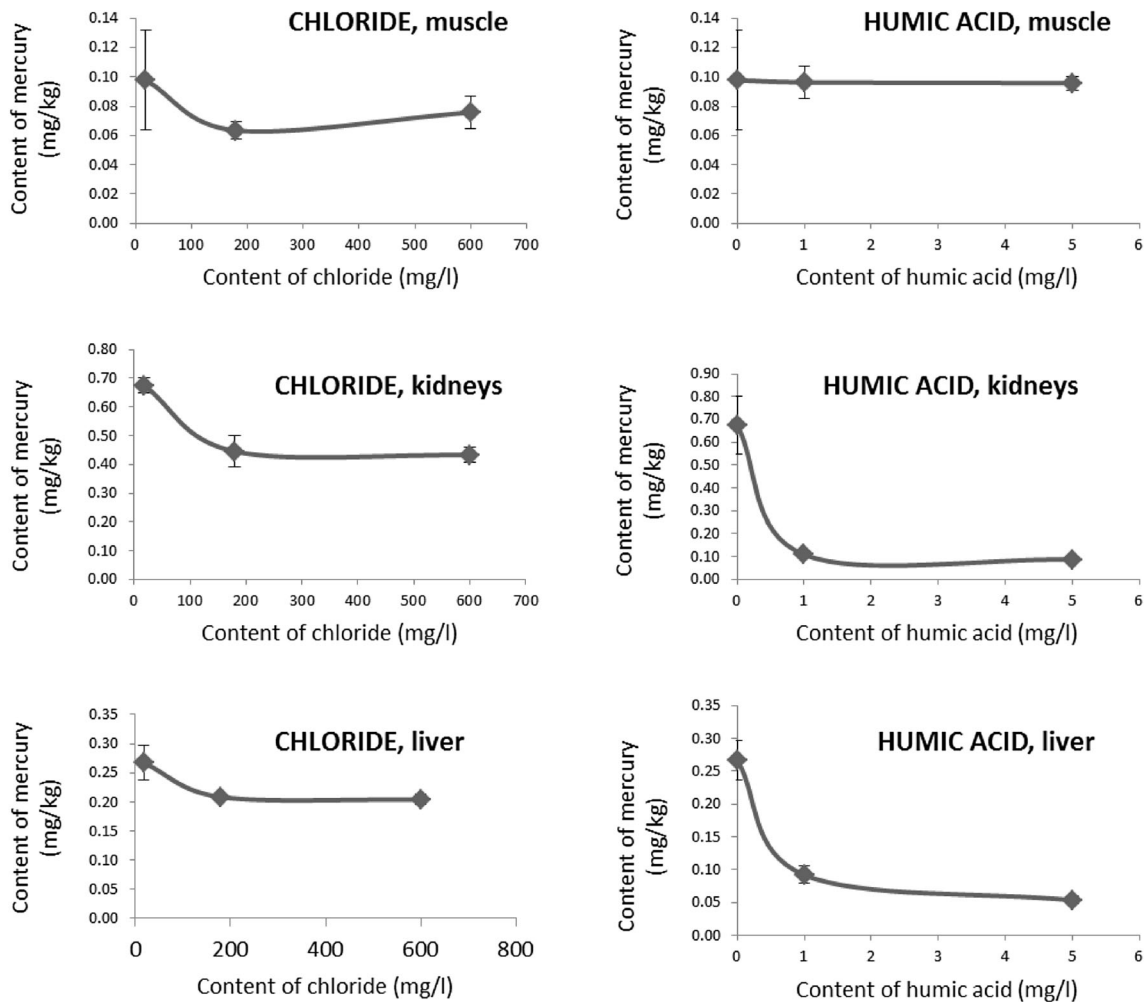


Fig. 1 continued

higher concentrations of mercury in gills, kidneys and liver relative to the muscle tissue are in line with findings from other studies, which indicate that the liver and gills are metabolically active and accumulate metals in higher levels relative to the muscle, not only in *Cyprinus carpio* L. (Velcheva 2006; Alhashemi et al. 2012; Ardakani and Jafari 2014) but more generally in fish (Allen 1994; Spehar et al. 1998). This is because, unlike other tissues, the muscle does not come into direct contact with metals as it is covered externally by the skin and also does not function as an active site for detoxification.

The concentrations of total mercury in tissues in dependence of chloride ions and humic acids concentrations are shown in Fig. 1a, b.

Chloride ions are commonly found in natural waters. Their concentration depends on the composition of the geological bedrock. The highest concentration of chloride ions used in our work (600 mg/l) increased the concentration of chloride ions by a factor of a little more than two in comparison with the Czech Regulation (No. 252/2004

Sb) for surface waters. As shown in Fig. 1, even the lower concentration of chloride ions (180 mg/l) used reduced the absorption of mercury in carp tissues. The highest reduction was observed in the tissues used for monitoring: skin (45%), gills (40%) and scales (30%). The concentration of mercury in detoxifying organs decreased about 34% (kidneys) and 22% (liver). The same decrease (22%) was found for muscle. The increase of chloride ions concentration to 600 mg/l did not cause any significant reduction. Only few papers are focused on the effect of chloride ions on deposition of mercury in fish tissues. The intense reduction of mercury accumulation in gills of *Phoxinus phoxinus* was found (Block et al. 1997). The bioavailability of mercury for aquatic organism in the presence of chloride ions was also described in the study of Fernandez-Gomez et al. (2015). The authors indicated the formation of stable complexes with chloride ions as the reason of this mercury bioavailability.

Sodium chloride is not only naturally presented in waters, but is it also used in fish breeding for antiparasitic

treatments. It is mostly applied in the form of short-time baths when the parasites of the families *Cryptobia*, *Ichthyobodo*, *Chilodonella*, *Trichodina* and *Trichodinella* are found, or at the occurrence of moulds on fish surfaces. Salt bath contains 10–30 g of sodium chloride per litre, and the duration of the bath is 15–60 min. Such salt baths can be repeated in most fish species. Sodium chloride is also used in a long-term occurrence of chilodoneosis in fish kept in fish tanks or little handling ponds during autumn or spring. In such cases, sodium chloride is used in the concentration of 1–2 g/l for 1–2 days (Svobodova et al. 2007).

The obtained results indicate a positive effect of chloride ions on deposition of mercury in fish tissues. Even low concentrations of chlorides often occurred in natural waters are able to decrease bioavailability of mercury significantly.

Stronger effect than chloride ions on mercury bioavailability can be exerted using an organic matter such as humic acid. The formation of strong and stable complexes of mercury with humic acid was described in many papers (Ravichandran 2004; Luider et al. 2004). Two orders lower concentrations of humic acid showed higher reduction of mercury accumulation compared to chloride ions. In the case of the lower concentration of humic acid (1 mg/l) used, the reductions in all tested monitoring and detoxifying organs were 70–90%. Muscle was the only exception. Reduction of mercury absorption was in the range of statistical error. Decrease of mercury accumulation in tissues in the presence of humic acid was described for other fish species, for example for *Fundulus heteroclitus* (Dutton and Fisher 2012).

Increasing concentration of metals in natural waters appears only local and their presence is mostly caused by natural conditions (geological bedrock), therefore the levels given under Czech Regulations (No. 252/2004) have been only sporadically exceeded. Mercury which can be found occasionally in surface waters without any evident reason is an exception.

The added amount of both humic acid and sodium chloride in fish tank solution caused reduction of mercury accumulation in all studied carp tissues. Humic acids form strong and volumetric large complexes with mercury, which are absorbed in living organisms much worse than mercury complexes with chloride ions.

Conclusion

Our results confirmed that humic acid and chlorides play an extremely important role part in bioavailability of mercury in the aquatic environment. Their concentrations in natural waters often far exceed natural ionic mercury concentrations. The majority of ionic mercury is therefore bound in

strong and stable complexes with lower bioavailability and the accumulation of mercury in fish tissues is relevantly reduced.

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