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Prediction of mercury bioavailability to common carp (*Cyprinus carpio* L.) using the diffusive gradient in thin film technique

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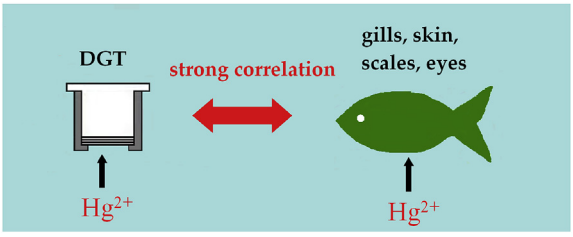
HIGHLIGHTS

- The mercury bioavailability to common carp was observed.
- The diffusive gradient in thin film (DGT) technique was used.
- Correlations between DGT and mercury input in fish tissues were observed.
- The DGT can be used for the assessment of mercury bioavailability in water.

GRAPHICAL ABSTRACT



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ABSTRACT

The mercury bioaccumulation by common carp (*Cyprinus carpio* L.) tissues (gills, skin, eyes, scales, muscle, brain, kidneys, liver, and spleen) and the capability of the diffusive gradient in thin film (DGT) technique to predict bioavailability of mercury for individual carp's tissues were evaluated. Carp and DGT units were exposed to increasing concentrations of mercury (Hg^{2+}: 0 μg L^{-1}, 0.5 μg L^{-1}, 1.5 μg L^{-1} and 3.0 μg L^{-1}) in fish tanks for 14 days. In the uncontaminated fish group, the highest mercury concentration was determined in the muscle tissues and, in fish groups exposed to mercury, the highest mercury concentration was determined in the detoxification (kidneys) and input (gills) organs. A strong and positive correlation between the rate of mercury uptake by the DGT technique and the rate of mercury accumulation by fish tissues (gills, skin, scales, and eyes) was observed.

1. Introduction

Mercury and its organomercury compounds are global contaminants with neurotoxic and accumulative effects (Winship, 1985; Kim et al., 2016). Mercury concentrations in uncontaminated water are often between 1 and 50 ng L^{-1} (Leopold et al., 2010; Mousavi et al., 2011), but mercury concentrations in water located near industrial facilities and mines are many times higher (from

Abbreviations: A, Surface area of the diffusive gel layer; AMA254, Advanced Mercury Analyser; $ANC_{4.5}$, Acid neutralisation capacity; COD_{Cr}, Chemical oxygen demand; D, Diffusion coefficient; DGT, Diffusive gradients in thin film; Δg, Thickness of the diffusive gel layer; (N-NH_4 + NH_3), Ammonium nitrogen; (N-NO_3), Nitrate nitrogen; (N-NO_2), Nitrite nitrogen; (P-PO_4), Orthophosphate; N_T, Total nitrogen; P_T, Total phosphorus.

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hundreds to several thousands of ng L^{-1}) (Fernandez-Martinez et al., 2006; Qiu et al., 2006). Mercury enters into the water ecosystem mostly in the mercuric (Hg^{2+}) inorganic cationic form, but the inorganic mercuric (Hg^{2+}) form is converted into organo-mercury compounds by anaerobic sulphate-reducing bacteria in water ecosystems (Jensen and Jernelov, 1969; Imura et al., 1971; Kim et al., 2016).

The bioavailability of mercury in water ecosystems is influenced by a number of exogenous and endogenous factors, which include mercury speciation and concentration, the physico-chemical properties of water, interaction with other substances, types of aquatic organisms, input into aquatic organisms, and the condition of biological membranes (Erickson et al., 2008).

Direct methods measuring the uptake of toxic substances by aquatic organisms, or analytical techniques (e.g., the diffusive gradient in thin film technique (DGT), ion-selective electrodes) have been used to determine the bioavailability of metals in an aquatic environment (Luider et al., 2004; Royset et al., 2005; Balistrieri et al., 2012; Clarisse et al., 2012; Gillan et al., 2012; Yin et al., 2014; Fernandez-Gomez et al., 2015; Cerveny et al., 2016). Relatively long experimental periods and high cost, however, sometimes limit the use of fish species in biomonitoring procedures (Zhou et al., 2008). In the case of aquatic organisms, metal uptake is controlled by biological membranes (e.g., gills) followed by metal accumulation in fish tissues (Erickson et al., 2008). The DGT technique simulates this process by a diffusive gel membrane followed by metal accumulation in a resin gel (Zhang and Davison, 1995). Compared to the measuring of metal uptake by aquatic organisms, the DGT technique does not imply the sacrifice of living organisms and is more reproducible (Clarisse et al., 2012).

The potential of the DGT technique as a predictor for the biological uptake of some metals (e.g., aluminium, copper) by fish (rainbow trout (*Oncorhynchus mykiss*) and brown trout (*Salmo trutta* L.)) has been published (Luider et al., 2004; Royset et al., 2005). The ability of the DGT technique to mimic mercury bioaccumulation was observed only for a clam (*Macoma balthica,* Baltic clam) (Clarisse et al., 2012). In this publication, a significant linear relationship between methylmercury accumulation by DGT and clams was observed (Clarisse et al., 2012). To date, the ability of the DGT technique to mimic mercury accumulation in fish tissues has not yet been discussed in the literature.

Our objective was the assessment of different mercury distribution in selected tissues of common carp (*Cyprinus carpio* L.) exposed to high concentrations of inorganic mercury (Hg^{2+}) in water and testing of the capability of the diffusive gradient in thin film (DGT) technique to predict the bioavailability of mercury for individual carp tissues. Because no similar studies have been published, the ability of the DGT technique to mimic mercury accumulation by common carp (*C. carpio*) tissues was, at first, observed in the laboratory aquaculture system under controlled conditions. Carp was chosen because it is an omnivorous fish with worldwide incidence and is of great economic importance for the Czech Republic. The correlations between mercury accumulation by DGT devices and mercury distribution in carp tissues were monitored.

2. Experiment

2.1. Chemicals

All reagents were of analytical grade. High-purity demineralised water produced by the Millipore Milli Q system (Millipore, Bedford, MA, USA) was used for dilutions. An inorganic mercury calibration standard of concentration 1.000 ± 0.002 g L^{-1} Hg in 12% w/w HNO_3 (Fluka, Czech Republic) was used for the spiked water environment.

2.2. DGT units

The DGT units (piston type, 3.14 cm^2 exposure area, supplied by DGT Research, Ltd.) were used. The DGT units consist of 0.45 μm pore size membrane filter (hydrophilic polyethersulfone, diameter 25 mm, thickness 140 μm, Pall Corporation, Port Washington, NY, USA), agarose diffusive gel, and resin gel (cation-exchange resin containing thiol functional groups - Ambersep GT74 in a polyacrylamide gel). The DGT gels (diffusive as well as resin) were manufactured in-house. The preparation of ion-permeable diffusive gel and resin gel followed the procedure stated in (Zhang and Davison, 1999; Pelcová et al., 2014).

The mass (M) of mercury accumulated by the resin in the DGT unit is given by Fick's first law of diffusion according to Eq. (1), where D is diffusion coefficient, c is mercury concentration in solution, Δg is thickness of the diffusive gel layer plus the thickness of the filter, A is surface area, and t is deployment time of the DGT device in solution (Davison and Zhang, 1994; Zhang and Davison, 1995).

$$c = \frac{\Delta g M}{DAt} \quad (1)$$

2.3. Fish - carp (C. carpio)

Yearling fish of the common carp (*C. carpio*), body weight 47.67 ± 4.61 g, obtained from a single artificial stripping (Fishpond Management Vodnany, Czech Republic) were used for the study. Fish were relocated into a recirculating aquaculture system with controlled conditions and were fed by granules of SCREETING F1 PB 40 2.5 mm. The feeding finished 10 days before the start of the experiment. The feed contained 0.017 mg kg^{-1} of mercury. Carp were acclimated for at least 1 week in aerated tap water. The water in the tanks was changed twice a day and the fish were exposed to a 12 h light/12 h dark photoperiod. The fish were divided into four groups, each with 15 specimens (1 control group and 3 experimental groups), and placed into glass tanks with a volume of 90 L (85 L of water). The experiment was performed in compliance with the laws for the protection of animals against cruelty as approved by the Ethical Committee of the Mendel University in Brno, Czech Republic.

2.4. Experimental design

Mercury accumulation on DGT units and carp (*C. carpio*) was investigated in the aqueous phase under batch conditions. The glass tanks were filled with 85 L tap water and spiked with mercury standard (0.5 μg L^{-1}, 1.5 μg L^{-1}, and 3.0 μg L^{-1}). One glass tank was chosen as the control. Only tap water without mercury added was in the control tank. The mercury concentration in the control tank was under the limit of detection (0.1 μg L^{-1}). The water in all the tanks was aerated.

Because mercury is well adsorbed on container surfaces (Yu and Yan, 2003), the stability of the mercury solutions in the tanks was checked before fish and DGT unit deployment. The mercury concentrations in the tanks were checked twice daily. Maximum decrease of 8% in mercury concentration was observed over five days. After saturation of the walls, the loss of Hg from the dissolved phase was minimal. Additional spikes of mercury standard realised if necessary twice a day ensured a constant concentration of mercury for the full length of the experiment.

After 7 days of equilibration, 9 DGT units and 15 carp were deployed in each tank. After 4 d, 9 d, and 14 d, three DGT units and

five carp were removed from each tank. The DGT units were immediately dismantled. The fish were killed and muscle (7.86 ± 0.58 g), skin (2.21 ± 0.27 g), scales (0.23 ± 0.18 g), brain (0.20 ± 0.06 g), eyes (0.37 ± 0.11 g), kidneys (0.14 ± 0.04 g), spleen (0.14 ± 0.03 g), gills (0.59 ± 0.32 g) and liver (0.44 ± 0.10 g) were excised and weighed. The weights of individual fish tissues are shown in brackets. The fish tissues were frozen at −80 °C before analysis. The mercury concentration was measured in wet weight of fish tissues.

2.5. Total mercury determination

The total mercury contents in the water, fish tissues, and resin gels were determined by atomic absorption spectrometry (AMA254 - Advanced Mercury Analyser, Altec, Prague, Czech Republic). The fish tissues (100 ± 0.1 mg), water (100 μL), or resin gels were inserted directly into the AMA254 analyser. The elution of mercury from resin gels before AAS determination was not necessary. The samples were thermally decomposed at 550 °C for 180 s under oxygen flow. The exact conditions for the mercury determination are stated in (Pelcová et al., 2014). The reference material - fish protein homogenate, DORM-4 (National Research Council Canada) was used for method validation. The limit of detection (3S/N) of AAS determination was 0.1 μg L^{-1} (for sample weight 100 ± 0.1 mg). The relative standard deviation (RSD) of AAS determination was 3.06% (at 0.410 mg kg^{-1}, n = 10). The limit of detection (3S/N) of DGT-AAS determination was 4.7 ng L^{-1} (for 14 days accumulation at 22 °C, A = 3.14 cm^2, Δg = 0.063 cm). The relative standard deviation (RSD) of DGT-AAS determination was 3.85% (at 3.0 μg L^{-1}, n = 10).

2.6. Water quality analyses

A series of physicochemical parameters (pH, temperature, conductivity, dissolved oxygen, and ammonia nitrogen) were determined four times a day (N = 55). Values of nitrite nitrogen, nitrate nitrogen, phosphate phosphorus, total nitrogen, total phosphorus, calcium, acid neutralisation capacity, chloride, and chemical oxygen demand were determined once every five days (N = 4).

Water temperature, pH, and saturation by oxygen were measured using the portable Hach HQ40D (Hach-Lange, Colorado, USA). Conductivity was measured by conductivity meter HI 98129 (HANNA Instruments, USA).

Ammonium nitrogen (N-NH_4 + NH_3) were determined by the indophenols method, nitrite nitrogen (N-NO_2) by the method using N-(1-naphthyl)-ethylenediamine, and nitrate nitrogen (N-NO_3) by the method using sodium salicylate. Total nitrogen (N_T) was measured with dimethylphenol after transformation of all nitrogen compounds into nitrate by the Koroleff method. Total phosphorus (P_T) and orthophosphate (P-PO_4) were measured by a method using ascorbic acid and ammonium molybdate. Chemical oxygen demand (COD_{Cr}) was measured by using potassium dichromate and calcium (Ca) levels were measured by EDTA titration. The acid neutralisation capacity ($ANC_{4.5}$) was measured by a method using hydrochloric acid and chloride ions (Cl^-) were measured by reaction with mercury thiocyanate to form mercury chloride. The exact conditions of water quality analyses are stated in (Clesceri et al., 1998).

The basic parameters of chemical analysis are summarised in Table S1. The statistically insignificant differences of physicochemical parameters of water were observed between control and experimental tanks. The observed physicochemical parameters did not exceed the optimal values for yearling fish of the common carp *(C. carpio)*.

2.7. Statistical analyses

The Excel software (Microsoft Office Professional Plus 2010) was used for statistical analyses. Statistically significant differences were analysed using analysis of variance (ANOVA). Significance was declared when the *P*-value was equal to or less than 0.05. The Pearson correlation analysis was performed to identify significant relationships between the rate of mercury accumulation determined by the DGT technique and the rate of mercury accumulation determined by fish tissues.

3. Results and discussion

3.1. Distribution of mercury in selected tissues of common carp (C. carpio)

The distribution of mercury in fish tissues is strongly dependent on the exposure pathway (Pickhardt et al., 2006). The main source of mercury is through the food chain. The uptake of mercury through water by gills, skin, and scales in fish habitat is minor, but not insignificant (Pickhardt et al., 2006). The uptake of mercury through water by input organs of fish is rarely discussed in existing literature (Rouleau et al., 1999; Monteiro et al., 2010; Wang et al., 2010; Pereira et al., 2015). The distribution of mercury was evaluated in 9 fish tissues: gills, skin, eyes, scales, muscle, brain, kidneys, liver, and spleen. The tissues were divided into detoxification organs (kidneys, spleen, and liver), mercury input organs (gills, skin, scales, and eyes), and others (muscle and brain). During the experiment, fish were kept without any food supply so mercury was absorbed into fish tissues only from the water environment. The mercury distribution in the tissue was observed in the fish control group (mercury concentration in the water was below the limit of detection (0.1 μg L^{-1})) as well as in three fish groups exposed to mercury (mercury concentration 0.5 μg L^{-1}, 1.5 μg L^{-1} and 3.0 μg L^{-1}).

In the control group, which simulates an uncontaminated water ecosystem, the highest mercury concentration was determined in the muscle tissue (0.051 ± 0.005 mg kg^{-1}) and the lowest concentration was found in the eyes (0.012 ± 0.003 mg kg^{-1}). The mercury concentration monitored in the control group represents natural background originating from their previous exposure (food and water) before the experiment. In this control group, the total mercury content in the tested tissues of common carp decreased in the order of muscle > kidneys > skin = gills > liver > spleen > fish scales > brain > eyes. Statistically non-significant differences ($p > 0.05$) in mercury concentration were found only between the skin and gill tissues; otherwise, statistically significant differences ($p < 0.05$) in mercury concentration were found among the other tested tissues. The total mercury concentrations in the tested tissues are presented in Fig. 1.

Regardless of the mercury exposure route, similar results of mercury distribution for uncontaminated water ecosystems have also been reported by other authors (Celechovska et al., 2007; Has-Schon et al., 2008; Kensova et al., 2010). Also, in these publications, a significantly high mercury concentration was observed in muscle tissue compared to other tested tissues for different fish species (chub, asp, pike, carp, bream, and pikeperch). The statistically significant increase of mercury concentration in tested carp tissues was not found in this group during the 14-day of the experiment (see Fig. 1).

The distribution of mercury in the fish groups exposed to mercury, which simulates a contaminated water ecosystem, was different from the mercury distribution in an uncontaminated water ecosystem. The total mercury content in the tested tissues of common carp decreased in the order of kidneys = gills >

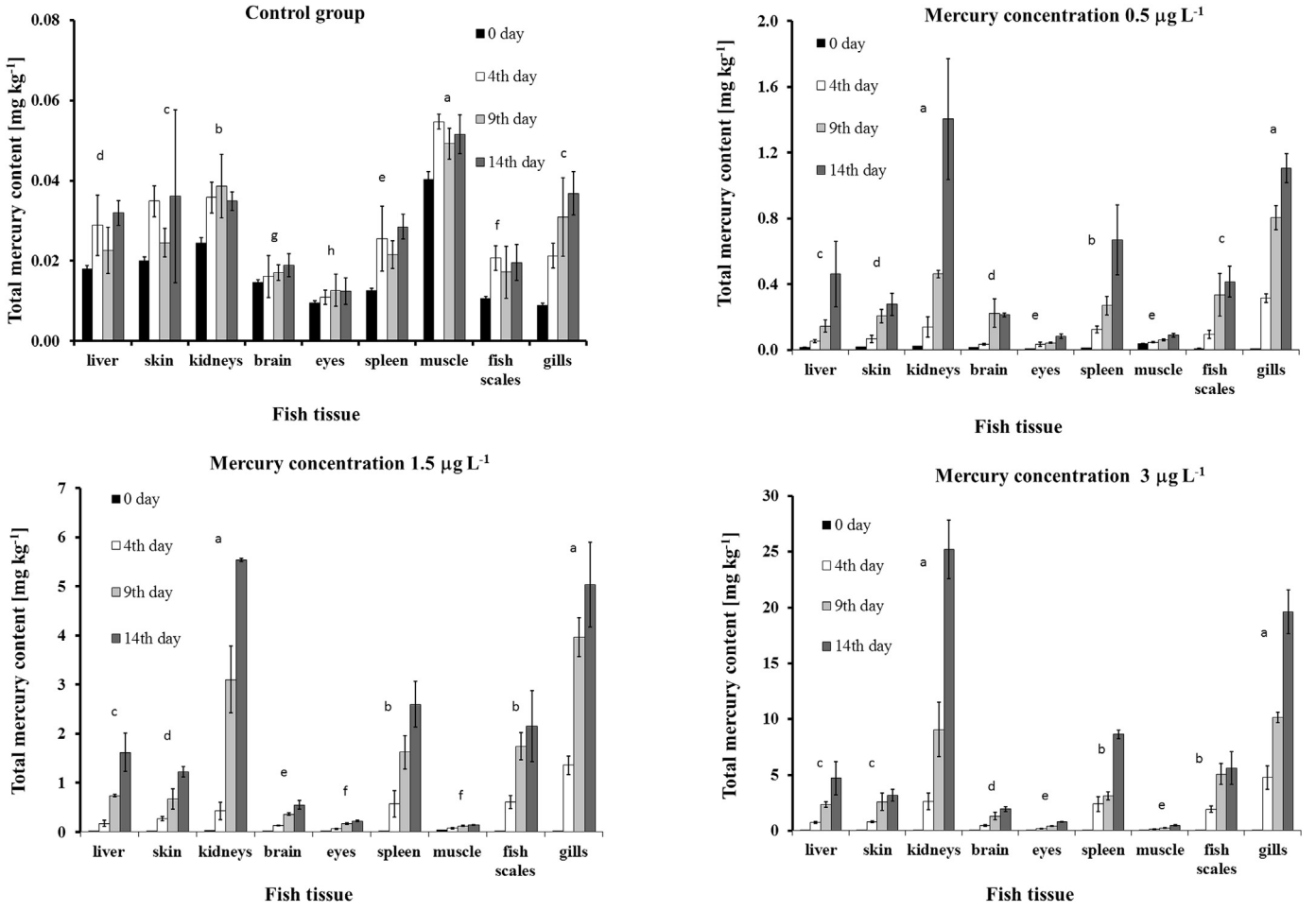

Fig. 1. The total mercury concentrations in the tested fish tissues for the control group (mercury concentration in a water environment below LOD (0.1 μg L^{-1})) and three groups exposed to mercury (concentrations of 0.5 μg L^{-1}, 1.5 μg L^{-1}, and 3.0 μg L^{-1}). Error bars for n = 5. Values with the same letters are not significantly different according to the Tukey test ($p < 0.05$).

scales ≥ spleen > liver > skin > brain > muscle = eyes. The total mercury concentrations in the tested tissues are presented in Fig. 1. Statistically non-significant differences ($p > 0.05$) in mercury concentration were found between kidney and gill tissues, and between eye and muscle tissues. The highest mercury concentration was determined in the detoxification (kidneys) and input (gills) organs; the lowest concentration was found in the muscle tissue and eyes. These results were expected, since the gills are in direct contact with water and suspended particles, and the kidneys are one of the major organs for the detoxification and elimination of metallic pollutants (Pereira et al., 2015; Kim et al., 2016). Although fish eyes are also in direct contact with the water medium, the lowest mercury concentration was determined in the eye samples. We assume that this organ is physiologically protected from mercury absorption. The low mercury concentration in white sea bream eye samples has also been reported by (Pereira et al., 2015). In addition, some authors (Havelkova et al., 2008; Kruzikova et al., 2013) demonstrated that in contaminated locations, the total mercury contents in the fish muscle tissues are significantly lower compared to the liver.

The mercury concentrations in the tissues of common carp increased with mercury concentration in the water environment as well as with time of accumulation. For mercury exposed groups, the statistically significant increase of mercury in tested carp tissues was observed during a 14-day experiment (see Fig. 2A and B). A non-linear (see Fig. 2A) increase in mercury concentration with accumulation time was observed for the detoxification organs (kidneys, liver, and spleen); a linear increase (Fig. 2B, $r = 0.8204–1.0000$) was observed for other monitored tissues.

The rate of mercury accumulation in the selected tissues of common carp was calculated and evaluated for a more comprehensive assessment of the mercury distribution. The mercury accumulation rate (mg Hg kg^{-1} tissue day^{-1}) was calculated from the dependence of mercury accumulated in the fish body (mg Hg kg^{-1} tissue) and the exposure time. It was found that inorganic mercury accumulated the fastest in gill tissue. Apart from muscle tissue, the rate of mercury accumulation significantly increased with increasing mercury concentrations in the tanks for all the tested tissues (Fig. 3). The highest increase in mercury accumulation rate with increasing mercury concentrations was observed for the detoxification and input organs (gills, scales, and spleen), and the lowest increase in mercury accumulation rate was observed in the eyes.

3.2. Mercury accumulation by diffusive gradient in thin film technique (DGT)

As well as the fish, the DGT units were exposed in tanks of mercury concentrations: 0.5 μg L^{-1}, 1.5 μg L^{-1}, and 3.0 μg L^{-1} for 4 d, 9 d, and 14 d.

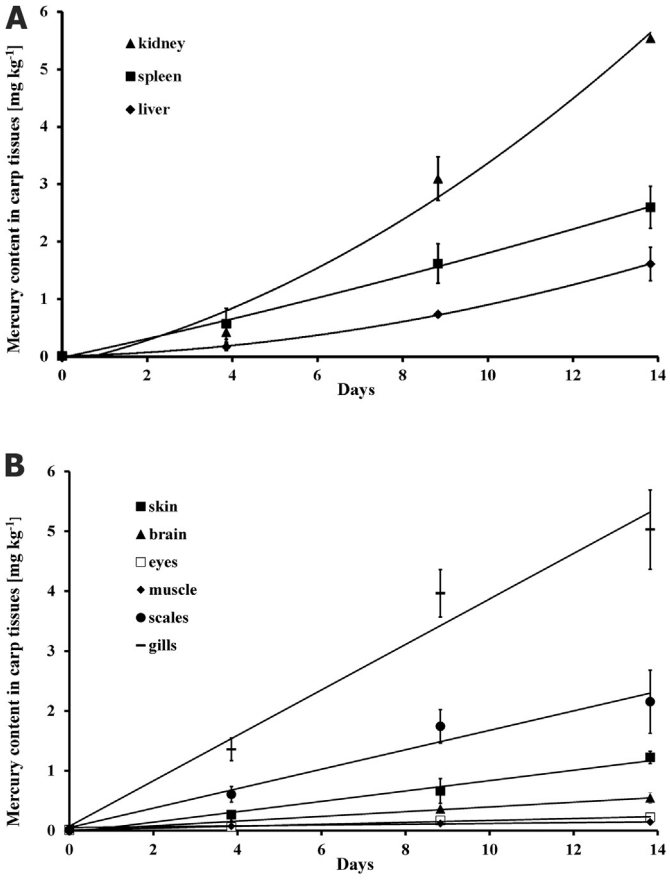

Fig. 2. The non-linear (A) and the linear (B) increase of mercury concentration in carp organs with exposure duration (for carp group exposed to mercury concentration of 1.5 μg L^{-1}). Error bars for n = 5.

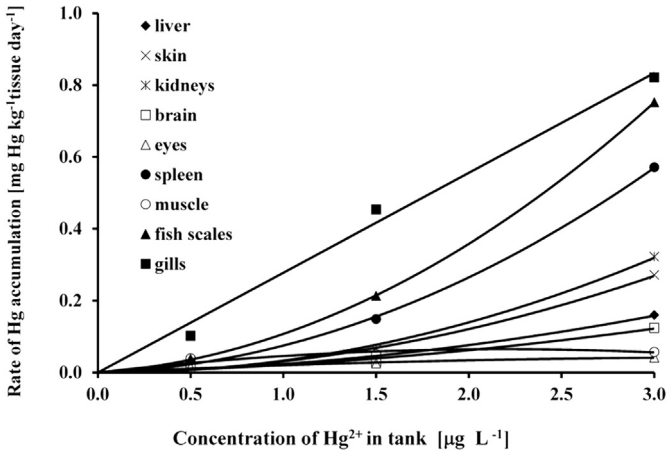

Fig. 3. The increase of mercury rate accumulation with increasing mercury concentrations in tanks for all tested tissues. Error bars are not shown to achieve better clarity (n = 5).

The mass of accumulated mercury in the resin gel (M) increased linearly (r^2 = 0.8612–0.9865) for all three observed concentration levels (0.5, 1.5, and 3.0 μg L^{-1}). The linearity of this aforementioned dependence increased with increasing concentration of mercury in the tank (Fig. 4).

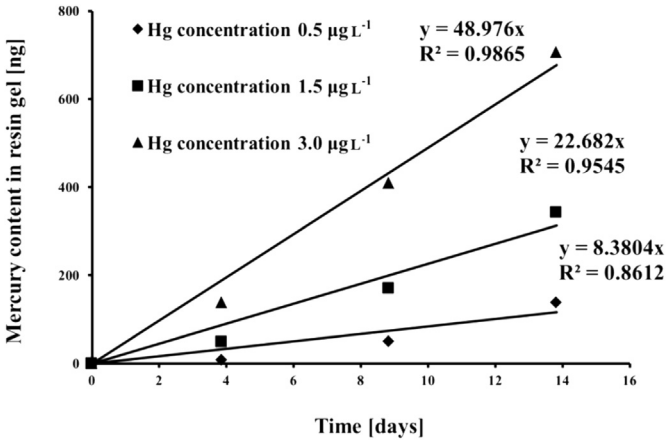

Fig. 4. The DGT time-series experiment. Average mass (M) of Hg^{2+} in resin gel for various periods of time (A = 3.14 cm^2, Δg = 0.063 cm).

3.3. Relationship between mercury accumulation by DGT and by carp tissues

The main objective of this study was to determine whether there are significant relationships between the mercury content in individual fish tissues and the mercury accumulated by DGT units, as well as whether the DGT is able to mimic mercury bioaccumulation by carp tissues. To investigate the potential of the DGT technique to provide information on the process of mercury accumulation by carp tissues, mercury accumulation by carp tissues was correlated with the mercury accumulation by DGT units (Fig. 5). The experimental design was restricted to an area where a steady Hg concentration was monitored and the influence of a depuration process limited. Because the mass of accumulated mercury in the resin gel (M) increased linearly with time of exposure, only carp tissues (muscle, skin, scales, brain, eyes, and gills) with linear increases of mercury concentration were correlated with the mercury accumulation by DGT units. For correlations were not used detoxification organs (kidneys, liver, and spleen), because the non-linear increase in mercury concentration with accumulation time was observed for these organs.

Significant and positive correlations (Pearson correlation coefficients 0.95–1.00) were found between the rate of mercury

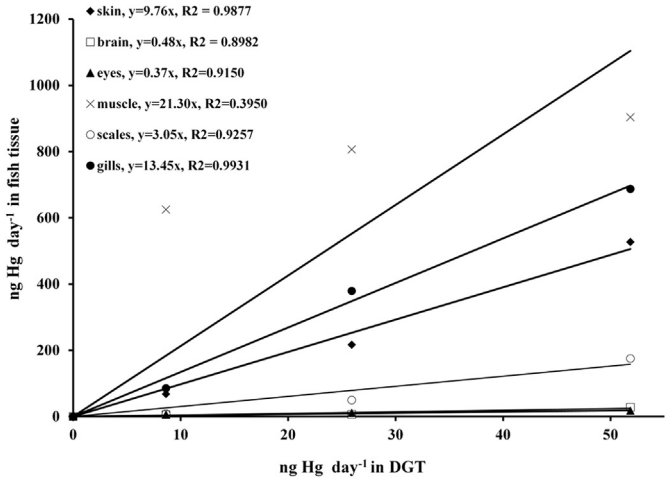

Fig. 5. Relation between the rate of mercury accumulation determined by the DGT technique and the rate of mercury accumulation determined by carp tissues.

accumulation determined by the DGT technique and the rate of mercury accumulation determined by input fish tissues (gills, skin, scales, and eyes) as well as the brain, which is the target organ of mercury. An insignificant correlation was observed between the rate of mercury accumulation determined by the DGT and the rate of mercury accumulation determined by fish muscle. The results showed that in laboratory controlled conditions, DGT offers the possibility of simple prediction of inorganic mercury bioavailability in water and can be used for the assessment of inorganic mercury bioavailability in water instead of commonly-used fish organs (gills, skin, and scales).

4. Conclusions

The tissue distribution of mercury in fish groups exposed to mercury, which simulated a contaminated water ecosystem, was different from the mercury distribution in the control group, which simulated an uncontaminated water ecosystem. In the control group, the highest mercury concentration was determined in the muscle tissues, and in fish groups exposed to mercury, the highest mercury concentration was determined in the detoxification organs (kidneys) and input organs (gills). The mercury concentrations in the tissues of common carp increased with mercury concentration in the water environment, as well as with the time accumulating the mercury.

Laboratory experiments indicated a strong and positive correlation between the mercury measured by the DGT technique and determined by input fish tissues (gills, skin, scales, and eyes) as well as in the brain. So, the DGT technique is simple to use and is able to predict the inorganic mercury accumulation in input fish organs (gills, skin, scales and eyes) as well as in the brain in laboratory controlled conditions. Because the bioavailability of mercury species is greatly affected by the presence of ligands capable of forming extremely strong complexes with ionic mercury, further studies will be designed with conditions more closely imitating real-aquatic ecosystem. The bioavailability of mercury species and the capability of the DGT technique to predict the bioavailability of mercury in the presence of naturally occurring ligands (e.g., humic acid, sodium chloride, etc.) and in a real contaminated mesocosm will be observed. Compared to biological organisms, the DGT technique does not imply the sacrifice of living organisms and is a low-cost tool to assess mercury accumulation in fish tissues.

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Appendix A. Supplementary data

Supplementary data related to this article can be found at http://dx.doi.org/10.1016/j.chemosphere.2017.08.097.

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