



The prediction of mercury bioavailability for common carp (*Cyprinus carpio* L.) using the DGT technique in the presence of chloride ions and humic acid

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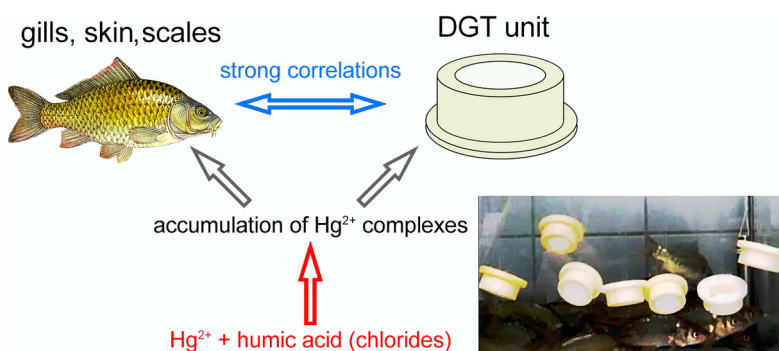
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HIGHLIGHTS

- The Hg^{2+} bioavailability was observed in the presence of humic acid and chlorides.
- Chlorides and humic acids play an important role in the bioavailability of mercury.
- The DGT technique was tested for predicting mercury bioaccumulation.
- A correlation between DGT and mercury in input tissues of common carp was observed.
- The DGT can be used for the assessment of mercury bioavailability in natural water.

GRAPHICAL ABSTRACT



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ABSTRACT

The ability of the DGT technique to predict Hg^{2+} bioavailability for input tissues (skin, gills, and scales) of common carp in the presence of chloride ions and humic acid (HA) was evaluated. The mercury accumulation by the DGT units and input tissues of carp decreased with an increasing concentration of chloride ions (29–180 mg L^{-1}) and HA (0–5 mg L^{-1}). In the presence of chloride ions and HA, statistically significant correlations (Pearson's correlation coefficients 0.731–0.954) were determined between the rate of mercury accumulation by input tissues of carp and the rate of mercury accumulation by the DGT units. The laboratory experiments suggest the possibility of using the DGT technique for predicting the mercury bioaccumulation in natural aquatic ecosystems instead of commonly used input tissues of fish.

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Abbreviations: AMA 254, Advanced Mercury Analyser; DGT, diffusive gradient in thin film; HA, humic acid.

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1. Introduction

In our previous work (Pelcova et al., 2017), we found significant correlations between mercury accumulated by input tissues of

common carp (*Cyprinus carpio* L.) and by the DGT units. However, further experiments were necessary because mercury bioavailability is affected by the presence of ligands ordinarily present in natural aquatic ecosystems (Erickson et al., 2008; Sari and Tuzen, 2009; Tuzen et al., 2009a; b). The most important ligands affecting the bioavailability of mercury in aquatic ecosystems include chloride ions and HA (Erickson et al., 2008). To date, the ability of the DGT technique to predict the mercury bioavailability for fish in the presence of naturally occurring ligands has not been discussed in literature.

The aim of this work was to test the ability of the DGT technique to predict the mercury bioavailability for input tissues of carp in the presence of chloride ions and HA and to evaluate the ability of the DGT technique to replace the commonly used bio-indicators: input tissues of fish.

2. Experiment

The chemicals used, the preparation of the DGT units and the determination of mercury by atomic absorption spectrometry-AMA 254 (Altec Praha, Czech Republic) are described in (Pelcová et al., 2014). The water analyses are described in (Pelcova et al., 2017). The determined physicochemical parameters of water were not significantly different from those in (Pelcova et al., 2017). NaCl (Penta, Czech Republic) and HA (Sigma-Aldrich, Switzerland) were used for this experiment.

2.1. Common carp (*Cyprinus carpio* L.)

The carp were acclimatised for two weeks in the recirculating aquaculture system. The carp feed (SCREETING F1 PB 40, 2.5 mm) contained 0.018 mg kg^{-1} of mercury. Fish feeding was stopped five days prior to the experiment. The fish body weight was $57.6 \pm 0.7 \text{ g}$.

2.2. Experimental design

Mercury accumulation by the DGT units and selected input

tissues (skin, gills, and scales) of carp were investigated under batch conditions. After eliminating the chlorine that is used for bacteriological protection of water, tap water (85 L) was filled into seven glass experimental tanks and spiked with Hg^{2+} standard ($1.5 \mu\text{g L}^{-1}$). The mercury concentration in non-spiked tap water was under limit of detection ($0.1 \mu\text{g L}^{-1}$). After seven days saturation of the tank walls by mercury, ten carp and ten DGT units were deployed in each experimental tank. The water in the experimental tanks was aerated. In all experimental tanks, the mercury concentration ($1.5 \mu\text{g L}^{-1}$) was determined by AMA 254 and maintained twice daily.

The influence of chloride ions ($29\text{--}600 \text{ mg L}^{-1}$) and HA ($0\text{--}10 \text{ mg L}^{-1}$) on mercury uptake by the DGT units and selected carp tissues was studied. Chloride ions were applied in the form of NaCl. The tap water contained 29 mg L^{-1} of Cl^- . After 72 h, the carp and the DGT units were removed from the experimental tanks.

The mercury contents in sorption gels and in carp tissues (wet weight) were determined by AMA 254 (Pelcová et al., 2014).

2.3. Statistical analyses

The STATISTICA 12 software (StatSoft, Inc., USA) was used for data processing. Data were tested for normal distribution by Shapiro-Wilk's test and were evaluated by analysis of variance (ANOVA) and Tukey's post hoc test. Significance levels were declared when the p -value was less than 0.05. The significant relationships were identified by Pearson's correlation analysis.

3. Results and discussion

3.1. Distribution diagrams

The distribution diagrams for Hg^{2+} in the presence of increasing concentration of chloride ions were created using the freely available Medusa software (Royal Institute of Technology, Stockholm, Sweden) for better understanding of mercury accumulation by the DGT units and selected tissues of carp (Fig. 1A–C). $\text{Hg}(\text{OH})_2$ is the

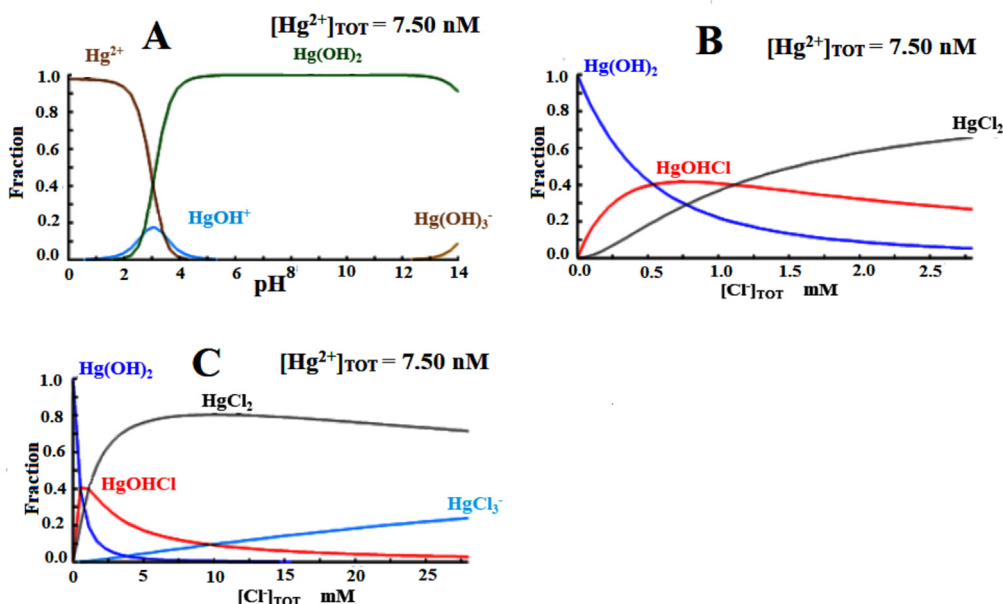


Fig. 1(A, B, C). The distribution diagrams for Hg^{2+} ($1.5 \mu\text{g L}^{-1}$) in the presence of increasing concentration of chloride ions.

main species of mercury when the chloride concentration is close to 0 mg L⁻¹ and the pH levels range from 4 to 14. When the chloride concentration is increased from 0 to 29 mg L⁻¹, the fraction of Hg(OH)₂ is decreased, and HgOHCl and HgCl₂ species become dominant. At chloride concentrations between 29 mg L⁻¹ to 180 mg L⁻¹, the HgOHCl species decreases significantly, and HgCl₂ becomes the dominant species of mercury. HgCl₂ is the major mercury species at chloride concentration from 44 mg L⁻¹ to 600 mg L⁻¹.

3.2. Mercury accumulation by DGT

A statistically significant decrease (about 15%, $p = 0.002$) of mercury accumulation by DGT units was observed when the concentration of chloride ions increased from 29 mg L⁻¹ to 180 mg L⁻¹. However, further increase of the chloride concentration from 180 mg L⁻¹ to 600 mg L⁻¹ did not cause a statistically significant decrease of mercury accumulation. We assume that since the increase of chloride concentration from 180 mg L⁻¹ to 600 mg L⁻¹ does not cause a change in the dominant mercury species (HgCl₂), no statistically significant reduction of mercury accumulation by the DGT units has been observed (Fig. 2A).

In the presence of HA, the most significant decrease of mercury accumulation (about 81.5%, $p = 2.5 \times 10^{-7}$) was observed when the HA concentration was increased from 0 mg L⁻¹ to 1 mg L⁻¹. The statistically significant decrease (about 63%, $p = 3.6 \times 10^{-5}$) of mercury accumulation was also observed when the HA concentration was increased from 1 mg L⁻¹ to 5 mg L⁻¹ (Fig. 2B). However, further increase of HA concentration from 5 mg L⁻¹ to 10 mg L⁻¹ did not cause a statistically significant decrease of mercury accumulation by the DGT units. We assume that Hg²⁺ (conc. 1.5 µg L⁻¹) in the experimental tank should be quantitatively bound to the Hg-HA complex at concentration 5 mg L⁻¹ of HA. Therefore, the increase

of HA concentration from 5 mg L⁻¹ to 10 mg L⁻¹ did not cause further statistically significant decrease of the accumulation of mercury by the DGT units.

3.3. Mercury accumulation by input tissues of carp

In the presence of chloride ions and HA, the mercury content in the analysed tissues of carp decreased in order of gills > scales > skin (Fig. 2C and D).

Statistically significant decreases of mercury in all analysed input tissues of carp were observed when the chloride concentration was increased from 29 mg L⁻¹ to 180 mg L⁻¹ (about 30–45%, $p < 0.001$) or HA concentration was increased from 0 mg L⁻¹ to 5 mg L⁻¹ (about 86–93%, $p < 0.03$). However, further increase of the chloride concentration from 180 mg L⁻¹ to 600 mg L⁻¹ and HA concentration from 5 mg L⁻¹ to 10 mg L⁻¹ did not cause a statistically significant decrease of mercury in the monitored tissues.

The HA had significantly higher ability to reduce mercury accumulation by input tissues of carp than chloride ions (Fig. 2C and D).

3.4. Correlations

The rates of mercury accumulation by carp tissues (expressed as mg of Hg intake into fish tissue per hour) were correlated with the rates of mercury accumulation determined by the DGT (expressed as ng of Hg intake into DGT unit per hour). In the presence of chloride ions and HA, statistically significant correlations (Pearson's correlation coefficients 0.731–0.954) were determined between the rate of mercury accumulation by input tissues of carp (skin, gills, and scales) and the rate of mercury accumulation by the DGT units. Our laboratory experiments show that the DGT technique can imitate mercury accumulation by input tissues of carp in the presence of chloride ions and HA.

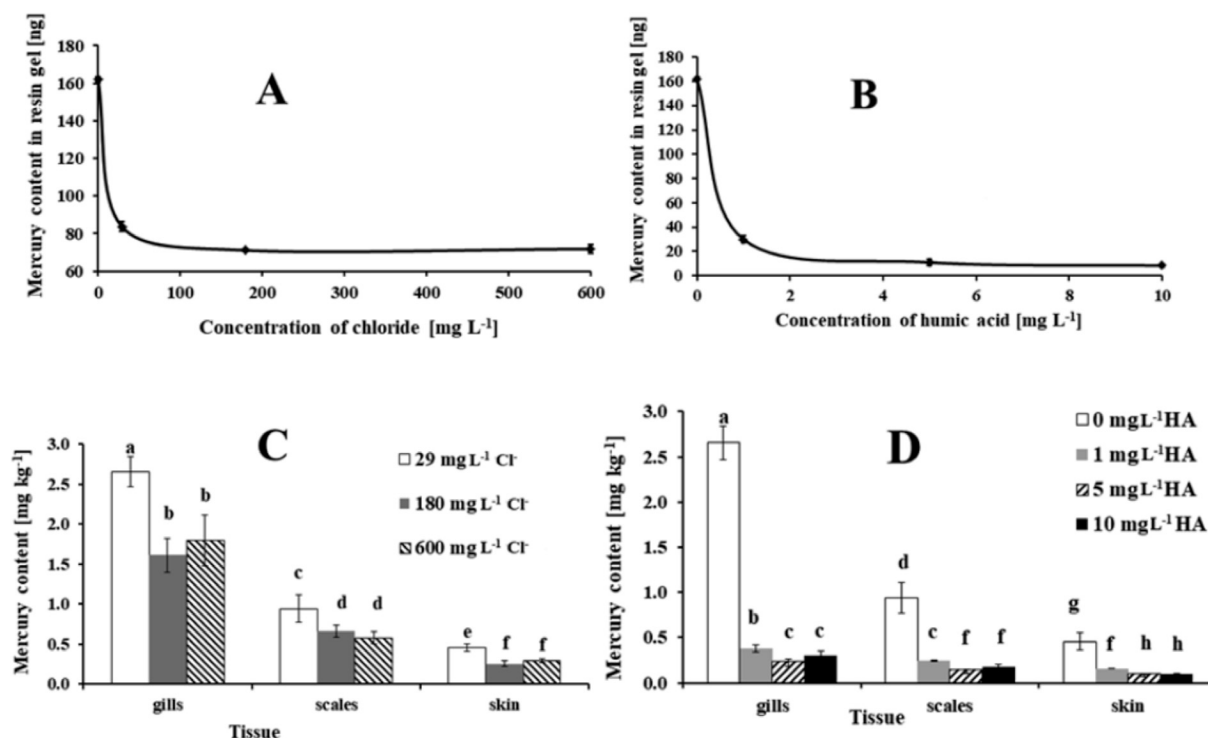


Fig. 2. The effect of concentration of chlorides and HA on mercury accumulation by the DGT units (A, B) and fish tissues (C, D) (Hg²⁺ concentration 1.5 µg L⁻¹, accumulation time 72 h). The error bars for $n = 10$. Values with the same letters are not significantly different according to the Tukey's post hoc test ($p < 0.05$).

4. Conclusion

The results indicate that although a number of parameters can control the bioavailability of mercury in water ecosystems, chloride ions and HA occurring in natural waters play a significant role in the mercury bioavailability. Laboratory experiments indicate strong and positive correlations between the mercury determined in input tissues (skin, gills, and scales) of common carp and measured by the DGT technique and suggest the possibility of using the DGT technique for predicting the mercury bioaccumulation in natural aquatic ecosystems instead of commonly used inputs tissues of fish.

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