Uptake of Hg(II) by the Clam *Ruditapes philippinarum* under Different Organic Carbon Conditions

Ting-wan ZHANG¹,²*, Xue-jiao ZHANG¹,² and Romana MANZOOR¹,²

¹Key Laboratory of Marine Environment and Ecology, Ministry of Education, Ocean University of China, Qingdao, 266100, P. R. China

²College of Environmental Science and Engineering, Ocean University of China, Qingdao 266100, P. R. China

*Corresponding author

**Keywords:** *Ruditapes philippinarum*, Mercury, Uptake rate, Humic acid.

**Abstract.** The presence of dissolved organic matter in natural waters is assumed to decrease dissolved metal bioavailability by binding metal ions. In this study, the role of humic acids, with a concentration range of 0 to 8 mg L⁻¹, was investigated in the uptake of mercury by the clam *Ruditapes philippinarum*. The metal uptake rates increased linearly with the mercury concentrations, but the variation of mercury concentrations didn’t influence the dry weight concentration factors determined at the end of exposure experiments. Experimental results showed that in the presence of humic acid, the predominant way of mercury uptake varies from passive diffusion to ingestion process, and the mercury concentration in the digestive gland increased, the distribution in gill declined. And the absorbing capacity of mercury by biological tissue was different, order was the digestive gland > the other soft tissues > the gill.

**Introduction**

Mercury (Hg) is a high toxic and persistent trace metal, which originates from both natural and anthropogenic processes into environment in elemental, organic and inorganic forms [1]. Due to its high versatility, Hg is used intensively in a number of human activities such as industry, pharmacology, gold mining and agriculture. However, its high toxicity to both humankind and ecosystems was not recognized until the breakthrough of the sensational and notorious Minamata Disease. Rise in mercury concentration is of great concern over past few decades, especially its bioaccumulation and trophic transfer along the aquatic food chain. This can lead to a significant risk to human health through seafood consumption.

Natural organic matter (NOM) is ubiquitous on Earth, including approximately 662 Gt of dissolved organic carbon (DOM) stored in the ocean [2]. DOM is an assemblage of heterogeneous organic molecules with diverse biogeochemical and ecological roles and is ubiquitous in aquatic ecosystems. It is sourced from both allochthonous (e.g., soil runoff) and autochthonous (e.g., microbial exudates) biomass [3]. DOM often represented as “dissolved organic carbon” (DOC), plays an important role in controlling Hg bioavailability to aquatic organisms [4].

There are conflicting results regarding the effect of DOC on the bioavailability of metals to bivalves. Most studies suggest a decrease in uptake, as expected on the basis of the free-ion activity model (FIAM) [5]. The unexpected results indicate an increase in uptake with humic substances were obtained [6]. Additionally, data of the influence of DOC on mercury uptake are scarce. Further studies are still necessary to well put forward the correct interpretation of the mechanism by which DOC influences metal uptake.

The aim of this study was to investigate the effects of DOC on the uptake of Hg by *Ruditapes philippinarum*. In this study, we utilized HA as models for studying the effect of NOM on Hg uptake, which includes the effect of metal concentration on metal uptake and the different uptake rates and dry weight concentration factors under different HA concentrations. In addition, we examined the body distribution of Hg to extend our understanding of the effect of HA on the uptake of Hg.
Materials and Methods

Clams *Ruditapes philippinarum* were collected from the tidal flats around Shazikou, Qingdao. The healthy individuals with a shell length of 2 - 3 cm were chosen and cleaned. The clams were acclimated in the laboratory at 23 °C for 1 - 2 weeks in acryl tanks filled with chemically defined artificial seawater (ASW) prior to the experiments. ASW was according to the formulation described in Lorenzo et al. (2002) [7]. They were continuously fed with the diatom *Thalassiosira pseudonana* during the acclimation period. Before the uptake experiments, the clams were not fed with the diatom food overnight and were placed in artificial seawater to avoid any production of feces during the exposure period. Before each experiment, all water parameters (pH, salinity, temperature) remained constant. HA used in this study was purchased from Jufeng Chemical Technology Company Limited, Shanghai.

To minimize the influence of the volatility of mercury, we chose to measure mercury uptake in a short-term exposure. Seven individual clams were used in each treatment as replicates. Clams are transferred to polypropylene beakers containing 200 mL ASW. A total of four treatments with different Hg concentrations (5, 10, 100, 200 ng L$^{-1}$) were set up. The experiments started when the clams opened their shell valves. After 0.5 h exposure, the clams were washed with ASW to remove the exposure solution from the clam cavity and the weakly adsorbed metal, respectively. Subsequently, the soft tissues were dissected with fresh blades. Then, the soft tissues were transferred into the oven and dried for 48 h at 80 °C, the weight variations were recorded. Finally, atomic fluorescence spectrometry was used to analyze the mercury concentration in the soft tissues of clams and the solution after exposure. The dry weight concentration factor (DCF) was calculated as follows:

$$\text{DCF (L Kg}^{-1}) = \frac{A_{ST}}{A_W}$$  \hspace{1cm} (1)

where $A_{ST}$ is the mercury concentration in the soft tissues of clams (mg/Kg), and $A_W$ is the mercury concentration in the solution after exposure (mg/L).

The second uptake experiment was conducted to determine the influence of HA on the metal uptake. Clams were placed to polypropylene beakers containing five different HA concentrations (0, 1, 2, 4, 8 mg L$^{-1}$) with a mercury concentration of 60 ng L$^{-1}$. Five parallel groups were set up for each concentration. Experimental conditions and protocol were the same as described for the first experiment. After 0.5 h exposure, the soft tissues we acquired were dissected into gill, digestive gland and other soft tissues, and the mercury concentrations in the three parts were determined to assess the tissue distribution of mercury.

Uptake bioassay data which include uptake rate, DCF and tissue distribution of mercury were expressed as mean ± standard deviation (SD). Linear regression analysis was performed using Microsoft Office Excel 2007. A one-way analysis of variance (ANOVA) was performed to assess the significance of differences observed between the situations in the absence and presence of HA. A value of $p < 0.05$ was considered significant.

Results and Discussion

The effects of mercury concentration on uptake rates were expressed as a power function (Figure. 1):

$$I_w = K_u C_w^b$$  \hspace{1cm} (2)

where $I_w$ is metal uptake rate in clam tissue (ng·g$^{-1}$.h$^{-1}$), $K_u$ is the uptake rate constant, $C_w$ is metal concentration in water and $b$ (power coefficient) is the slope of the log-log relationship between $I_w$ and $C_w$. The metal uptake rate increased linearly with the mercury concentration, which was explained by the fact that mercury uptake by clams is governed by passive or facilitated transport and don’t need ATP to power the transport. The rate constant ($K_u$) was metal - and species - specific and can be used as an indicator of bioavailability of dissolved metals, the uptake rate constant is 0.35 in our work. Fig. 1 also shows the measured DCF in clams under different mercury
concentrations which indicated that mercury concentrations have little influence on DCF value ($p > 0.05$). However, the large value of measured DCF suggests that *Ruditapes philippinarum* has a strong capacity to absorb mercury.

The DCF in clams for mercury as a function of HA concentrations after 0.5 h of exposure is shown in Fig.2. The value of DCF had increased 2.3-fold as the humic acid concentration increased from 0 to 1 mg L$^{-1}$. With the continual increase of humic acid concentrations, the uptake of mercury started to decrease. As HA concentration reached to 8 mg L$^{-1}$, DCF was 1.9 times lower than that at 1 mg L$^{-1}$. There was a significant difference ($p < 0.05$) when the four treatments compared with the control.

Results of uptake rates are shown in Fig.2. The uptake rate for Hg in treatments with elevated HA concentrations got higher than that in the control, even though Hg showed a decrease in uptake rate with increasing HA concentration after the initial increase. As it is illustrated in Fig. 2, a positive effect of HA on Hg adsorption to clams is observed. The uptake rate increased about 2-fold as the HA concentration increased from 0 to 1 mg L$^{-1}$. Then, the uptake rate showed a consistent drop extending to a HA concentration of 8 mg L$^{-1}$, which had decreased 1.7-fold. There was a significant difference ($p < 0.05$) when compared with the control except the last treatment.

A number of previous studies have shown that metal bioavailability to aquatic organisms can be reduced as DOC concentration increases [8, 9], a trend that can be predicted on the basis of FIAM which is widely applied to aquatic organisms. Nevertheless, our observations seem to conflict with
Some authors have shown that the metal-HA complexes are labile or quasi-labile [10]. Dissociation of complexes in the diffusion layer surrounding the surface of living cells is a usual explanation for deviations from FIAM predictions. Recently, it has also proposed that ternary metal complexes with HA at the surface of aquatic organisms may lead to the enhanced uptake of metals [11].

Table 1. Tissue distribution of mercury in clams at the end of 0.5 h of aqueous exposure.

<table>
<thead>
<tr>
<th>Compartment</th>
<th>HA concentration (mg L⁻¹)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0</td>
</tr>
<tr>
<td>gill</td>
<td></td>
</tr>
<tr>
<td>SD</td>
<td>3.2</td>
</tr>
<tr>
<td>digestive gland</td>
<td>47.1</td>
</tr>
<tr>
<td>SD</td>
<td>3.1</td>
</tr>
<tr>
<td>other soft tissue</td>
<td>15.8</td>
</tr>
<tr>
<td>SD</td>
<td>3.4</td>
</tr>
</tbody>
</table>

*Values represent the percentage of metal associated with each tissue compartment (gill, digestive gland, other soft tissue). SD represent the standard deviation; n = 5 per humic acid (HA) concentration.

Table 1 shows the tissue distribution of mercury at the end of 0.5 h of exposure for each HA concentration. Our experimental results clearly indicate that Hg uptake sites by clams predominantly are gill and digestive glands in the absence of HA. However, with the high HA concentration, the percentage of mercury associated with gill decreased, whereas it increased in digestive glands. There are several possible pathways of metal transport in organisms: passive diffusion, active transport, facilitated transport, channel transport, lipid permeation and pinocytosis. The enhanced metal uptake in the digestive gland observed in our study can be explained by the changed transport pathways that HA can form stable complexes with mercury which can be directly absorbed into the digestive glands by clams. It is also a part of reasons for the enhanced mercury uptake. Recently, it is also confirmed that Cu-HA aggregates can be ingested by mussels leading to Cu absorption in the digestive system [12].

Conclusions

The main component of organic matter is humic acid (HA) which had influence on bioavailability of mercury in our study. Several factors may contribute to the increased bioavailability in the presence of HA. Our results can improve a better understanding of the role DOC played in metal bioavailability. It is important to consider environmental factors for evaluating metal bioaccumulation and risk assessment in aquatic environments.

Acknowledgement

This study was supported by the National Natural Science Foundation of China (Grant No. 41276024), Natural Science Foundation of Shandong Province, China (Grant No. ZR2011DM013) and Major International Joint Research Project of Natural Science Foundation of China (Grant no. 41320104008).

References


