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Mechanisms of cadmium accumulation (adsorption and absorption) by the freshwater bivalve *Corbicula fluminea* under hydrodynamic conditions☆

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ABSTRACT

Many heavy metals in sediments and water have potential adverse effects on aquatic organisms such as *Corbicula fluminea* (O.F. Müller, 1774), a bivalve species frequently used as a biomonitor for metal pollution. Studies over the past decades examining the heavy metal uptake by *C. fluminea*, very few has investigated the effect of hydrodynamic conditions on accumulation of heavy metal by *C. fluminea*. Therefore, in this study, to investigate the mechanism of intracellular and extracellular accumulation of metal, individuals of *C. fluminea* were exposed to cadmium (Cd)-treated water under three different hydrodynamic conditions. These included exposures in two set ups: three rates of rotation (500, 350, 200 r/min) in beakers for 10 days, and then exposure to Cd-treated sediment under two naturally turbulent water conditions (14 cm/s and 3.2 cm/s) in experimental flumes for 23 days. Hydrodynamic force increased the burrowing rate but decreased the activity of *C. fluminea*. After 10 days of exposure, the extracellular concentrations of Cd in the tissues of *C. fluminea* in the sand group were significantly higher than that in the gravel groups. The intracellular and extracellular concentrations of Cd in the tissues of *C. fluminea* dramatically increased in the Cd-treated sediment test. Moreover, the concentration of the extracellular Cd adsorbed on the tissues of *C. fluminea* in the 14 cm/s and 3.2 cm/s groups was significantly higher than that in the control group, whereas the effect of hydrodynamic force on absorption of Cd by *C. fluminea* was not obvious. These results suggest that hydrodynamic condition plays an important role in extracellular accumulation of Cd by *C. fluminea*. In future study, when using *C. fluminea* to assess Cd pollution in aquatic environment, extracellular Cd adsorbed on the tissue should be removed to avoid the influence of hydrodynamics.

1. Introduction

Heavy metal pollution in aquatic environments has become a global issue (Roberts, 2012; Wang et al., 2014). The presence of heavy metals in sediments and the overlying water column has potentially adverse effects on aquatic organisms (Chao et al., 2015; De Jonge et al., 2012; Liu and Wang, 2013). Cadmium (Cd) is one of the widespread toxic contaminants (Bigot et al., 2011; Hare et al., 2001; Villar et al., 1999). Freshwater bivalves such as *Corbicula fluminea* (O.F. Müller, 1774) are widely distributed in water–sediment interface in the freshwater environments (Shoults-Wilson et al., 2010). They usually have high growth rate and a short life span (3 years or less) (Stites et al., 1995). A growing number of studies have been performed to investigate the accumulation of heavy metals by bivalves in aquatic environment, and it has been demonstrated that bivalves accumulate heavy metals through three pathways: contact with suspended solids, via the water column, and/or through diet (Hare et al., 2001; Rand, 1995; Villar et al., 1999). Ingestion of sediment and particulate matter is considered a major route of metal accumulation (Wu et al., 2012).

Chaharlang et al. (2012) found that various port activities (e.g., shipyard and fishing boat activities) could increase the concentrations of metals in the water, and positive correlations were

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observed between the levels of heavy metals in sediment and in the soft tissues of bivalves. Bioturbation and dredging of the sediment increased metal concentration of the particles suspended in the water in this situation, the metal concentration in the freshwater mussels remain unchanged (Ciutat and Boudou, 2003; Ciutat et al., 2007; Otter et al., 2015). Schmitz et al. (2015) suggested that the metals bound to the suspended solids may be less bioavailable. The concentrations of metals concentrated by bivalves varied by species, and by tissue type (Brooks et al., 2015; Rzymski et al., 2014). However, so far, there is little information about the influence of environmental conditions, such as hydrodynamic conditions on heavy metal bioavailability and uptake by bivalves.

The water flow in many shallow lakes follows a particular pattern, with stable hydrodynamic conditions in most areas and turbulent conditions in others (Zou et al., 1996). The breathing and feeding activities of *C. fluminea* proceed in dynamic water. Hydrodynamic forces increase the oxidation-reduction potential (Eh), and then lead to acid volatile sulfide (AVS) and total organic carbon (TOC) breakdown in the surface sediment (De Jonge et al., 2012; Geng et al., 2015). The force can also increase the amount of solids suspended in the overlying water by causing resuspension of the sediment (Huang et al., 2012; Zheng et al., 2013). Thus, these changes can increase the content and bioavailability of metals in the aquatic environment (Zhang et al., 2014). Such metals in suspended sediment are toxic to filter-feeding aquatic animals (Hill et al., 2009; Schmitz et al., 2015). The effect of suspended solids on the metal accumulation by bivalves have been investigated (Fraysse et al., 2000; Legeay et al., 2005; Pynnonen, 1995), but hydrodynamic conditions and the responses of the clam have been poorly studied. Building further understanding of how hydrodynamic conditions affect the accumulation of heavy metals by *C. fluminea* is key to accurate assess the metal pollution of the aquatic environment.

In the present study, to investigate the behavior response of the *C. fluminea* to the hydrodynamic forces/suspended sand and the Cd accumulation of the tissues from the water under these conditions, *C. fluminea* individuals were exposed to three different hydrodynamic conditions caused by using three rotation speeds (500, 350, 200 r/min) in beakers for 10 days (Cd-treated water test). And in order to simulate the Cd accumulation (adsorption and absorption) by the *C. fluminea* under the natural hydrodynamic conditions, the clams were exposed under two naturally turbulent water conditions (14 cm/s and 3.2 cm/s) in experimental flumes for 23 days (Cd-treated sediment test). We confirmed the effect of hydrodynamic conditions on soft-tissue absorption and absorption of Cd by individuals of this species.

2. Materials and methods

2.1. Gravel, sand, sediment and C. fluminea

Gravel, sand, and *C. fluminea* samples were provided by the fishery management office at Hongze Lake (Jiangsu, China). The average diameter of the gravel was 10 mm and the median diameter of the sand was 0.4 mm. *C. fluminea* specimens were cultured for 30 days prior to beginning the experiment. Cd-treated sediment was prepared by adding $CdCl_2$ to the natural sediment collected from Zhushan Bay in Taihu Lake, China. A nominal concentration of 45 μg/g (dw) of Cd was chosen for the Cd-treated sediment (Ciutat and Boudou, 2003). A calculated concentration of aqueous solution of $CdCl_2$ (18 g Cd^{2+}) was added to the sediment (400 kg, dw) and the sediment was stirred once a day for 30 days to make it homogenous. The process of preparing the Cd-treated sediment was described in a previous study (Geng et al., 2015).

2.2. Cd-treated water test

A laboratory experiment was conducted using gravel or sand as the substrate in 24 beakers (diameter: 150 mm; height: 200 mm) with an electric stirrers (Fig. S1). A solid polyethylene cylinder (diameter: 70 mm; height: 80 mm) was placed in the center of each beaker, and was then surrounded by gravel or sand. This provided an open space in the center through which a vertical flow of water was directed by the stirrer. There were one gravel group (4 beakers) and one sand group (4 breakers) in one treatment. Gravels were used as substrate (7 cm thick) in the beakers in the gravel group. Fifteen *C. fluminea* individuals (2.0 ± 0.3 cm in length) were placed on the substrate. Prior to the experiment, 1500 mL of ultrapure water (Milli-Q; Millipore, MA, USA) with Cd at a concentration of 30 μg/L was added to each beaker. Three rotation speeds (500, 350, and 200 r/min) were used to indicate different levels of water turbulence. According to our preliminary tests, the sand started to suspend under the rotation of 200 r/min and was fully suspended under the rotation of 500 r/min in the experimental conditions of this study. A similar beaker, but without rotation of the water, served as the hydrostatic control. In the sand group, sand was used as the substrate instead of gravel. One treatment (rotated water: 3 beakers with gravel, 3 beakers with sand; still water: 1 beaker with gravel, 1 beaker with sand) had three replicates.

Water quality parameters were measured in situ before (as background values) and after the experiment: pH (HACH pH Sension2), dissolved oxygen (DO) (HACH Dissolved Oxygen Sension6), electrical conductivity (EC) (HACH Conductivity Sension5) and temperature. The response of *C. fluminea* to the flow and the suspended sands was assessed and recorded by camera every four hours during the experiment. And the response was quantified by visually counting the number of clams that opened shells and burrowed into the sand. Samples of the water, sand, and *C. fluminea* specimens were collected after 10 days. Water samples were drawn from each beaker and stored in 500-mL polypropylene bottles for granulometric analysis with the Mastersizer 2000, determination of suspended solids, and Cd analysis in filtered water. The gut contents of *C. fluminea* were subsequently depurated by placing them in reconstituted freshwater for 6 h. 50% of the organisms were used for the total Cd concentration analysis. The soft tissues were only rinsed with ultrapure water (Milli-Q; Millipore, MA, USA) after the shells were opened with a tweezer and wiped. The remaining 50%, to be used for analysis of intracellular Cd concentration, were washed with EDTA (4 mM, pH 8) for 10 min to remove Cd adsorbed on the tissue surfaces (Bere and Tundisi, 2012), and then rinsed with ultrapure water and wiped. Each soft tissue of clam was dissected into four tissue samples: gills, mantle, foot with adductor muscles, and visceral mass (Baudrimont et al., 1997). Four kinds of tissue samples were frozen at −80 °C separately and then freeze-dried with lyophilizer (Labconco FreeZone 18, USA) for Cd analysis.

2.3. Cd-treated sediment test

Details of the experimental setup and design for the Cd-treated sediment test are described in a previous paper (Geng et al., 2015). Cd-treated sediment was added to two hydrodynamic flumes, which connected to a circulating water system. The rates of flow (Group 1:14 ± 1.3 cm/s; Group 2: 3.2 ± 0.8 cm/s) were monitored using an Acoustic Doppler Velocimeter (ADV, Son Tek). A sink served as the hydrostatic control. In each group, 600 *C. fluminea* individuals (2.0 ± 0.3 cm in length) were scattered on the substrate.

Samples of the overlying water (500 mL) and *C. fluminea* specimens (20 individuals) in each parallel container in the group were collected seven times (i.e., at 0, 1, 3, 6, 10, 16, and 23 days). The specimens were prepared according to the methods in the "Cd-