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Role of fluoranthene and pyrene associated with suspended particles in their bioaccumulation by zebrafish (*Danio rerio*)

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ABSTRACT

Hydrophobic organic compounds (HOCs) tend to be associated with suspended particles in surface aquatic systems, however, the bioavailability of HOCs on suspended particles to fish is not well understood. In this study, a passive dosing device was used to control the freely dissolved concentrations (C_{free}) of polycyclic aromatic hydrocarbons (PAHs) including fluoranthene and pyrene, and the influence of particle-associated PAHs on their bioaccumulation by zebrafish was investigated. The results showed that, when the C_{free} of PAHs were kept constant, the presence of suspended particles did not significantly affect the steady state of PAH bioaccumulation in zebrafish tissues excluding head and digestive tracts, suggesting that the bioaccumulation steady state was controlled by the freely dissolved concentrations of PAHs. However, suspended particles promoted the uptake and elimination rate constants of PAHs in zebrafish body excluding head and digestive tracts. The uptake rate constants with 0.5 g/L suspended particles were approximately twice of those without suspended particles, and the body burden in zebrafish increased by 16.4% − 109.3% for pyrene and 21.8% − 490.4% for fluoranthene during the first 8-d exposure. This was due to the reasons that suspended particles could be ingested, and part of PAHs associated with them could be desorbed in digestive tract and absorbed by the zebfrafish, leading to the enhancement of uptake rates of PAHs in zebfrafish. The findings obtained from this study indicate that PAHs on suspended particles are partly bioavailable to zebrafish and particle ingestion is an important route in PAH bioaccumulation. Therefore, it is important to consider the bioavailability of HOCs on suspended particles to improve ecological risk assessment.

1. Introduction

Many hydrophobic organic compounds (HOCs) enter aquatic systems through natural and human activities. HOCs are preferentially adsorbed to sediment and suspended particles, and this causes sediment and suspended particles to be carriers to transfer HOCs in the environment. Suspended particles are an important component in rivers, and the concentrations of suspended particles vary temporally and spatially. For example, the concentration of suspended particles was as high as 54.8 g/L in the Yellow River (Xia et al., 2016) and 11.7 g/L in the Delaware River (Putnam and Pope, 2003), while it was only 368 mg/L in the Mississippi River (Rosen and Xu, 2014). In rivers, the suspended particles and sediment may be converted to each other by the effect of natural and human activities such as water conservancy projects (Dong et al., 2015), and such activities can affect the partition of HOCs between suspended particles and sediment. In addition, suspended particles may also be affected by bioturbation (Ciarelli et al., 1999). Previous studies showed that HOCs on suspended particles were partly bioavailable and caused toxic effects to organisms (Zhang et al., 2015b). However, the effect of suspended particles on HOC bioaccumulation in aquatic organisms such as fish is not clearly understood.

Contaminants may be accumulated by aquatic organisms through different routes (Belfroid et al., 1996), which is in turn related to the forms of HOCs. The dissolved HOC in water may be accumulated via passive diffusion (Krauss et al., 2000), while HOCs on suspended particles or sediment can be accumulated through ingestion (Jantunen et al., 2008; Maenpaa et al., 2003; Menon and Menon, 1999; Sormunen et al., 2008; Zhai et al., 2016). Moreover, HOCs can also be accumulated by food ingestion (Djomo et al., 1996; Sijm et al., 2000; Thomann and Komlos, 1999; Xia et al., 2015) leading to HOC transfer in the food web, which may ultimately pose risks to human health. Research showed that highly hydrophobic HOCs bound to particles were accumulated by deposit feeders mainly through ingestion (Leppanen and Kukkonen, 1998; Lu et al., 2004; Weston et al., 2000; Zhai et al., 2016). For example, sediment ingestion accounted for more than 95% of benzo [*a*]pyrene accumulated by oligochaete (*Ilyodrilus templetoni*) (Lu et al.,

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2004), and 61% of pyrene in oligochaete (*Lumbriculus variegatus*) (Leppanen and Kukkonen, 1998). However, the effect of suspended-particle-borne HOCs on their bioaccumulation rate and steady state is not fully understood. Therefore, this research aimed to study the role of HOCs associated with suspended particles in their bioaccumulation kinetics and steady state.

Zebrafish is a model organism that has been widely adopted in bioaccumulation studies (Berends et al., 1997; Djomo et al., 1996; Fang et al., 2016; Xia et al., 2015). Polycyclic aromatic hydrocarbons (PAHs) are a group of HOCs ubiquitously found in the sediments and overlying waters throughout the world (Burgess et al., 2009; Tuncel and Topal, 2015; Xia et al., 2013; Zhang et al., 2015a), with the concentrations of PAHs ranging from 22.56 to 1011.94 μg/g in surface sediments (Kilunga et al., 2017) and from 11.84 to 393.12 ng/L in surface water (Li et al., 2017). Passive dosing with poly dimethyl siloxane (PDMS) elastomer has been used in recent studies (Gouliarmou et al., 2012; Smith et al., 2010a, 2010b; Zhang et al., 2015b) to regulate freely dissolved concentrations (C_{free}) of HOCs while avoiding the use of co-solvents in exposure assays. In addition, the PDMS silicon itself has biocompatibility (Smith et al., 2010a). In this study, passive dosing was used to control C_{free} of fluoranthene and pyrene; the accumulation of PAHs by zebrafish (*Danio rerio*) in the presence or absence of suspended particles were investigated. The effect of suspended particles on the bioaccumulation kinetics and steady state of PAHs in zebrafish was studied, and the effect mechanisms of suspended particles were analyzed.

2. Materials and methods

2.1. Chemicals and materials

Fluoranthene and pyrene with purity > 98% were obtained from Johnson Matthey Company (Alfa Aesar, Heysham, Lancashire, United Kingdom). The internal standard *m*-terphenyl with purity > 98% was purchased from AccuStandard (New Haven, CT, U.S.A.). PDMS elastomer and catalyst were supplied by Baili Medicinal Materials (Shanghai, China). Methanol, dichloromethane and hexane were all high-performance liquid chromatography grade and obtained from J.T. Baker (Center Valley, PA, U.S.A.). All other reagents and chemicals were analytical grade. Pure water used in this study was produced by a Milli-Q water purification system (Super Q-treated, Millipore, Molsheim, Alsace, France).

2.2. Preparation and characterization of suspended particles

The suspended particles were prepared from sediments obtained from Yangtze River at a location in the Wanzhou District, Chongqing, China. Briefly, the sediments were air-dried in the laboratory at room temperature, and aliquots of the sediments were weighed and added in a glass beaker with pure water (w / v = 1: 10). The sediment and water mixtures were stirred thoroughly for about 10 min, and allowed to settle for 4 h to obtain small size suspended particles. After that the overlying water in the glass beaker were filtered through a 0.22-μm polyether sulfone membrane, and the derived suspended particles were freeze-dried and stored in a brown glass bottle before use.

The total organic carbon (TOC) and black carbon (BC) content of the suspended particles were determined using the same method as in our previous research (Liu et al., 2011), and the details are shown in supplementary material. The size distribution of the suspended particles was analyzed using Microtrac S3500 laser particle size analyzer (Microtrac, Florida, U.S.A.), briefly, about 0.5 mL of suspended particle solution (1 g/L) was added and dispersed in pure water in the reservoir of the machine at room temperature, and the particle size range which detected by the machine was from 0.02 μm to 2800 μm. The background values of fluoranthene and pyrene in the suspended particles were analyzed, and the method are shown in Section 2.5.

2.3. Preparation of passive dosing device

The passive dosing device was prepared according to the procedures given previously (Xia et al., 2015). Briefly, the poly dimethyl-siloxane and catalyst (w / w = 10: 1) were mixed thoroughly, then some aliquots of the mixtures were weighed (12.5 g ± 0.2 g) and loaded into glass culture dishes. The dishes were put into the freeze dryer to get rid of the bubbles in the PDMS; then stayed under room temperature for 72 h and solidified at 110 °C for 48 h subsequently. Before preload with PAHs, the PDMS dishes were immersed with methanol for 72 h, and then rinsed with pure water three times. The freely dissolved concentrations of fluoranthene and pyrene were designed at 0.1 μg/L and 0.2 μg/L in the cultured water. To obtain this concentration in our experiment, the concentrations of fluoranthene and pyrene were prepared at 9.1 mg/L and 25.72 mg/L in the methanol according to their methanol-water partition coefficients in our previous work (Xia et al., 2015). The actual freely dissolved concentrations of PAHs were also measured during the experiment. The PDMS dishes were immersed in the PAH methanol solution for more than 96 h before use. According to the values of partition coefficients of fluoranthene and pyrene between PDMS and methanol solution (Smith et al., 2010a), the mass of PDMS to the volume of the methanol solution was set to be 1: 20, and such a ratio could make the change of PAH concentrations in the methanol to be less than 2%.

2.4. Bioaccumulation experiments of PAHs

Cultured water was prepared according to OECD (OECD, 1992), and the concentrations were 294.0 mg/L, 123.3 mg/L, 64.7 mg/L and 5.7 mg/L for $CaCl_2{\cdot}2H_2O$, $MgSO_4{\cdot}7H_2O$, $NaHCO_3$ and KCl, respectively. A series of glass fish tanks were added with 2 L cultured water, and then some aliquots of suspended particles were added. The particle concentrations included 0 g/L, 0.1 g/L and 0.5 g/L, respectively, and they were in the range of concentrations in the river of the world (Xia et al., 2017). PDMS dishes preloaded in the PAH methanol were rinsed with pure water three times, and then added into the tanks. Each tank contained two PDMS dishes. Finally, a submerged pump were added in each tank, and they worked twice per day (each time lasted about 10 min) to suspend the particles.

Zebrafish obtained from Hua-Niao-Chong-Yu market (Beijing, China) were about five-month old. The zebrafish were cultured according to Westerfield (Westerfield, 1993) with a minor adjustment in the laboratory. The zebrafish were cultured in tap water (without chlorine) at 23 ± 2 ℃ for about 10 days before they were used. Our previous work showed that steady state would be reached in eight days between PAHs and sediments (Xia et al., 2013), therefore, 8 d later, 14 zebrafish were added in each tank. There was no food provided for the zebrafish during the exposure process. At time of 0 d, 1 d, 2 d, 4 d, 8 d and 12 d, two fish were sampled from each tank. Each treatment with suspended particle concentration of 0 g/L and 0.5 g/L included three replicates, and each treatment with suspended particle concentration of 0.1 g/L contained six replicates.

To study the effect of suspended particles on body burden of PAHs in zebrafish excluding digestive tract, the fish obtained from the three replicates with suspended particle concentrations of 0 g/L, 0.1 g/L, and 0.5 g/L were treated as follows: the head and digestive tract of these fish were discarded and the remaining of the fish was freeze-dried before the extraction of PAHs. In addition, the fish obtained from the other three replicates with suspended particle concentrations of 0.1 g/L were treated as follows: only the head of the fish was discarded to analyze the effect of suspended particle ingestion through digestive tract on PAH bioaccumulation by zebrafish.

The freely dissolved concentrations of PAHs were determined at time of 0 d, 8 d and 12 d with solid micro-extraction method (details are shown in Section 2.5.2). In addition, at day 12th, the cultured water was filtered through a glass fiber membrane (GF/F, Whatman), and the