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Dissolved organic matter affects both bioconcentration kinetics and steady-state concentrations of polycyclic aromatic hydrocarbons in zebrafish (*Danio rerio*)

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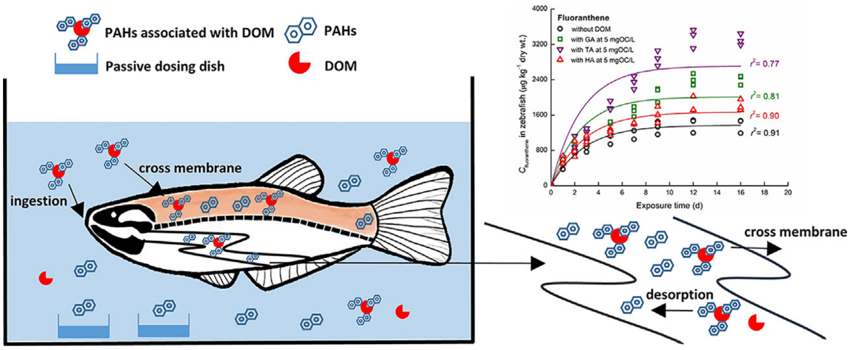
HIGHLIGHTS

- DOM increased the steady-state concentrations of PAHs in zebrafish ($C_{\text{b-ss}}$).
- The increase of $C_{\text{b-ss}}$ caused by 1–15 mgOC L^{-1} HA ranged from 28.1% to 139.8%.
- The increase of $C_{\text{b-ss}}$ caused by TA with middle molecular weight (1700 Da) was the highest.
- The effects of DOM concentration on $C_{\text{b-ss}}$ varied with DOM molecular weight.
- PAHs associated with DOM may directly pass through membrane of zebrafish.

GRAPHICAL ABSTRACT



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ABSTRACT

Dissolved organic matter (DOM) is ubiquitous in natural aquatic ecosystems. The association of hydrophobic organic compounds (HOCs), such as polycyclic aromatic hydrocarbons (PAHs), with DOM may have a large impact on HOC fractions in water and their bioconcentration in fish. However, the effects of DOM on HOC bioconcentration in fish are not well understood, especially whether DOM will affect the bioconcentration steady-state concentrations of HOCs in fish is still confusing. Thus, this study investigated the effects of three DOM including gallic acid (GA), tannic acid (TA), and humic acid (HA) with molecular weights ranging from 170 Da to about 10 kDa at different concentrations (1, 5, and 15 mgOC L^{-1}) on the bioconcentration of PAHs including phenanthrene, anthracene, fluoranthene, and pyrene in zebrafish (*Danio rerio*), with the PAH freely dissolved concentrations maintained constant by passive dosing systems. The results revealed that the presence of DOM generally increased the bioconcentration steady-state concentrations of the PAHs in zebrafish ($C_{\text{b-ss}}$), with the increase ranging from 28.1% to 204.0%, and the increase of $C_{\text{b-ss}}$ promoted by TA with middle molecular weight (1700 Da) was the highest among the studied DOM. Moreover, the $C_{\text{b-ss}}$ increased with the concentrations of GA with low molecular weight and TA with middle molecular weight in water, whereas decreased with increasing concentrations of HA with high molecular weight. The uptake rate constants of the PAHs in zebrafish with DOM were higher than that without DOM. Ingestion of DOM and direct accumulation of PAHs associated with DOM might be primary influencing mechanisms of DOM on the $C_{\text{b-ss}}$, and whether the facilitated diffusive mass transfer by DOM will affect the $C_{\text{b-ss}}$ needs to be further studied. This study suggested that DOM-associated HOCs should be considered in future HOC risk assessment in addition to the freely dissolved HOCs.

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1. Introduction

The bioavailability of hydrophobic organic compounds (HOCs) is important for us to understand their environmental behaviors and assess their environmental risks (Ehlers and Luthy, 2003; Semple et al., 2004; Qi et al., 2017; Lin et al., 2018). It was considered that the bioavailability of HOCs is only controlled by their freely dissolved concentrations (Escher and Hermens, 2004; Smith et al., 2009; Xia et al., 2012; Xia et al., 2013a). However, the natural aquatic environment is a complex matrix. Dissolved organic matter (DOM), which can reach up to mgOC L^{-1} levels on the basis of organic carbon in natural aquatic environments (Haitzer et al., 2001; Hestir et al., 2015), can associate with organic pollutants due to its complex binding actions or adsorption behaviors (Pan et al., 2007, 2008), making HOCs associated with DOM cannot be neglected when it comes to discuss the bioavailability of HOCs.

Bioconcentration is a good way to study bioavailability (Escher and Hermens, 2004), and by doing so the bioavailability of HOCs was assessed in many studies (Ke et al., 2007; Chen et al., 2008; ter Laak et al., 2009a; Xia et al., 2013a). Some studies proposed that the presence of DOM would decrease the bioavailability of HOCs (Landrum et al., 1987; Haitzer et al., 2001; Lee et al., 2003). However, some found that DOM would increase the bioconcentration factor of HOCs in aquatic organisms, and hence inferred that the bioavailablity of HOCs increased resulting from DOM (Chiou et al., 1986; Haitzer et al., 1998; Matsuo et al., 2006). These studies indicate that the effects of DOM on bioavailability of HOCs are still confusing. One of the reasons is the difficulty to measure and control the freely dissolved concentrations of HOCs in water in the presence of DOM (Durjava et al., 2007; Ke et al., 2007). The developed solid phase microextraction and passive dosing methods largely solve the dilemma (Birch et al., 2010; Smith et al., 2010; Gouliarmou et al., 2012; Lai et al., 2015). With these techniques, several studies proposed that the presence of DOM could enhance mass transfer of HOCs between mediums and aquatic organisms when freely dissolved HOC concentrations were kept constant (Mayer et al., 2007; ter Laak et al., 2009a, 2009b). Moreover, freely dissolved concentrations of polycyclic aromatic hydrocarbons (PAHs) were maintained successfully during a 16-day exposure for zebrafish by applying passive dosing methods (Xia et al., 2015), which demonstrated the possibility extending this technique in longer exposure period for higher trophic level animals. However, it is still lack of studies investigating the effects of DOM on bioconcentration of HOCs in aquatic organisms in higher trophic levels, such like fish.

Additionally, it is not clear if the presence of DOM will affect the steady-state concentrations in aquatic organisms when freely dissolved concentrations were maintained constant. The key influencing factor on this issue is whether HOCs associated with DOM are bioavailable. Some studies proposed that pollutants associated with DOM might be bioavailable to aquatic organisms (Haitzer et al., 1998; Voets et al., 2004; Zindler et al., 2016). For example, Lin et al. (2018) recently observed the elevated toxicity of pyrene on *Daphnia magna* in the presence of DOM when its freely dissolved concentration was kept constant, indicating that pyrene associated with DOM was partly bioavailable to organisms. Therefore, we hypothesized that HOCs associated with DOM might be bioavailable to higher trophic level aquatic organisms, thus the steady-state concentrations of HOCs would increase in the presence of DOM.

To test this hypothesis, we investigated the effects of DOM with various molecular weights at different concentrations on the bioconcentration kinetics and steady-state concentrations of HOCs in zebrafish, a higher trophic level aquatic organism which has been widely applied in studying adverse effects of pollutants on aquatic organism and human health (Howe et al., 2013; Wen et al., 2017). PAHs, which are ubiquitous in natural aquatic environments with concentrations ranging from ng L^{-1} to μg L^{-1} levels (Emelogu et al., 2013), were chosen as typical HOCs in the present study. In detail, phenanthrene, anthracene, fluoranthene, and pyrene representing 3 or 4 rings with logarithmic octanol−water partition coefficients ($\log K_{\mathrm{ow}}$) ranging from 4.54–5.98 were studied, which do not exhibit hydrophobic cutoff phenomenon in bioconcentration (Jonker and van der Heijden, 2007). The commercial Aldrich humic acid (~10 kDa) is always employed as DOM in many studies. Gallic acid (170 Da) and tannic acid (1700 Da) are two of the major components of phytic substances, which are ubiquitous in the environment as a result of decomposition of plant biomass and often used as a surrogate of low molecular weight and middle molecular weight DOM, respectively (Dalton et al., 2005; Chow et al., 2008; Toth et al., 2014; Wang et al., 2016). Therefore, gallic acid (GA), tannic acid (TA), and humic acid (HA) from commercial sources were selected as typical DOM. The bioconcentration kinetics of the PAHs in the presence of three DOM with different molecular weights at different concentrations was determined with the PAH freely dissolved concentrations maintained constant by passive dosing systems, and the influencing mechanisms of the DOM on bioconcentration steady-state of the PAHs in zebrafish were investigated.

2. Materials and methods

2.1. Chemicals and materials

The GA, TA, and HA in solid phase (purity >98%) were purchased from Sigma-Aldrich (Germany). Though HA is a mixture of DOM with molecular weight at a wide range, the vast majority of HA presented in high molecular weight (~10 kDa, >97%) in the current study. Phenanthrene, anthracene, fluoranthene, and pyrene in solid phase (purity >98%) were purchased from Johnson Matthey Company (Alfa Aesar). The standard solution mixture of 16 kinds of PAHs at a certified concentration of 200.0 μg mL^{-1} for each PAH in dichloromethane and methanol solution (v:v, 1:1) was purchased from AccuStandard. Surrogate standard 2-fluorobiphenyl was obtained from J&K Chemical Ltd. with purity >97%. The internal standard substance *m*-terphenyl was purchased from AccuStandard with purity >98%. The poly(dimethylsiloxane) (PDMS) elastomer was prepared from a Silastic MDX4-4210 BioMedical grade Elastomer kit (Dow Corning) purchased from Baili (Shanghai) Medicinal materials trade Inc. of China. High-performance chromatography grade methanol, *n*-hexane, dichloromethane, and acetone were purchased from J.T. Baker. All other analytical-grade reagents were from Xilong Chemical Co., Ltd. Milli-Q water was used in the present study, and the artificial water (AFW) was made from Milli-Q water with the composition of 294 mg L^{-1} $CaCl_2 \cdot 2H_2O$, 123 mg L^{-1} $MgSO_4 \cdot 7H_2O$, 64.8 mg L^{-1} $NaHCO_3$, and 6.25 mg L^{-1} KCl (pH 7.8 ± 0.2, hardness 250 ± 25 mg L^{-1} as $CaCO_3$, <0.2 mg L^{-1} dissolved organic carbon).

2.2. Preparation of DOM solutions

A stock solution was prepared for three kinds of DOM, respectively. For HA stock solution (200 mgOC L^{-1}), an aliquot of HA was first dissolved in small amount of 1 M NaOH solution, and then the liquid phase was transferred into bulk water. This procedure was repeated for three times. Finally, all the mixture was transferred including the solid phase. Then the mixture was shaken by hand and placed for >2 days for stabilization. After, the mixture was filtered through 0.45 μm pre-heated glass-fiber filter (Whatman, GF/F). The pH value of the final solution was adjusted to about 7 (1 M HCl). The GA and TA stock solutions were prepared by directly dissolving 250 mgOC L^{-1} GA and TA in Milli-Q water, respectively. The stock solutions were placed for >2 days for stabilization, and then filtered through 0.45 μm pre-heated glass-fiber filter (Whatman, GF/F). The final exposure mediums were obtained by diluting corresponding stock solutions in AFW in terms of the concentrations of DOM on the basis of organic carbon set in the present study. Total organic carbon (TOC) of each solution was measured by a Shimadzu TOC-L CPH/CPN total organic carbon analyzer.