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Influence of suspended sediment characteristics on the bioaccumulation and biological effects of citalopram in *Daphnia magna*

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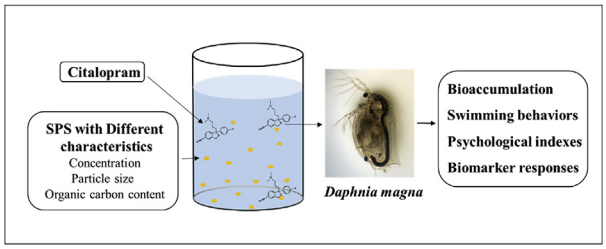
HIGHLIGHTS

- The CIT distribution and bioaccumulation were altered under different SPS characteristics.
- The swimming activities and oxidative stress were significantly enhanced at SPS concentration of 1 g/L.
- The influence of f_{oc} on swimming and physiological activities was more remarkable than particle size.
- The SPS adsorption and desorption capacity and ingestion habits of *D. magna* might be the potential reasons.

GRAPHICAL ABSTRACT



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ABSTRACT

The influence of suspended sediment (SPS) characteristics on the bioavailability of the antidepressant citalopram (CIT) was investigated in the cladoceran *Daphnia magna*. The bioaccumulation, swimming behaviours, psychological indices, and oxidative stress were examined. The CIT bioaccumulation were altered in the presence of SPS, such that the body burden of CIT decreased as the concentration of SPS increased and as the organic carbon content of SPS (f_{oc}) increased; moreover, the body burden of CIT increased as SPS particle size increased. All the biomarker activities of *D. magna* were markedly induced at a CIT exposure concentration of 10 μg/L. However, the biological effects of CIT did not depend on the body burden of CIT as SPS concentration increased, while the swimming activities and oxidative stress were significantly enhanced by SPS concentration at 1 g/L. The influences of SPS particle size and f_{oc} on the activities of swimming and physiological indicators were mainly associated with the CIT bioaccumulation, while f_{oc} in SPS was more substantial than particle size. In addition, the antioxidant activities decreased as f_{oc} increased and were significantly strengthened at particle sizes of 30–60 μm. The impacts of different SPS characteristics on the adsorption and desorption capacity of CIT and the ingestion habits of *D. magna* were the main reasons for the variations in CIT body burden and biological effects. According to the results obtained in this study, the SPS characteristics should be considered in the risk assessment of contaminants in natural aquatic environments.

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1. Introduction

Selective Serotonin Re-uptake Inhibitor (SSRI) antidepressants are a class of mood altering psychotropic drugs, which have been widely marketed since the mid-1980s. Many SSRIs are persistent in the aquatic environment due to the low removal efficiency of sewage treatment plants and have been detected in different environmental matrices such as wastewater, surface and ground water, and sediments (Silva et al., 2015). The primary mode of action (MOA) of SSRIs, which is highly conserved and exists in both vertebrates and invertebrates, is to block serotonin (5-HT) re-uptake into the pre-synaptic nerve ending, thereby generating a raised concentration of 5-HT in the synaptic cleft. Moreover, 5-HT is responsible for regulating many biological functions within individuals, such as reproduction, metabolism and behaviour via the same MOA (Olsén et al., 2014). Thus, a growing number of studies have found that SSRIs can be bioconcentrated and induce a series of behavioural and biochemical responses in aquatic biota, thereby posing potential risks to non-target organisms. For example, oxidative stress of bivalves (Magni et al., 2017), swimming activity and aggression alternation in fish (Xie et al., 2015a), feeding habits and reproductive fitness disturbance in mussel (Gonzalez-Rey and Bebianno, 2013), and phototactic behaviour and reproduction enhanced of daphnids (Rivetti et al., 2016) have all been reported for SSRIs. However, the current understanding of the uptake and toxicity of SSRIs is based almost entirely on studies of organisms exposed to chemicals through the water column; few investigations have considered the factors that influence the bioavailability of SSRIs in the natural aquatic environment.

Suspended sediment (SPS), a natural component of aquatic ecosystems, arises from the resuspension of bottom sediments and the erosion of the surrounding land, which is ubiquitous in the aquatic environment. For SPS-associated contaminants, their fates in the aquatic system are determined by the fates of SPS. Meanwhile, an increasing number of studies have demonstrated that SPS characteristics play an important role in the transport, partitioning and bioavailability of contaminants (Boenigk et al., 2005; Zhang et al., 2015). For example, the partitioning and desorbability of two hydrophobic pesticides (diuron and atrazine) are soil particle-size dependent (Wang and Keller, 2009). The biodegradation rates of polycyclic aromatic hydrocarbons (PAHs) were enhanced due to the presence of sediment, followed by the order of fine silt > clay > coarse silt (Xia and Wang, 2008). The immobilization rates of phenanthrene (PHE) in *Daphnia magna* were increased by 1.6–2.7-fold with SPS concentration ranging from 1 to 5 g/L, and the inhibition of superoxide dismutase of individuals was significantly higher in the presence of SPS than in the absence of SPS (Zhang et al., 2015). However, the current studies have mainly focused on the influence of SPS on bioavailability of metal and PAHs, although similar effects also occur with emerging organic chemicals, especially SSRIs, due to their widespread occurrence in the aquatic environment (Liu et al., 2015, 2018; Xie et al., 2015b). Therefore, a comprehensive study needs to be conducted to clarify the impact of SPS properties on the bioavailability of SSRIs.

Bioaccumulation is a valuable method for evaluating the bioavailability of contaminants, because it can help to clarify the connection between external circumstances and biological effects (Yan et al., 2017). Swimming performance (such as velocity and accelerate velocity) is a sensitive behavioural indicator that is linked to contaminant-induced stress responses at the individual level. An increasing number of studies have found that induced oxidative stress affected the swimming behaviours of aquatic organisms (Xie et al., 2015a; Parolini et al., 2018). Meanwhile, the physiological endpoints (such as the beating rate of the thoracic limbs and heart rates) can link organismal survival and behavioural responses to cellular and molecular endpoints (Lari et al., 2017). Previous studies have reported that SSRIs can alter these biomarker activities of *D. magna*, which is a filter-feeding zooplankton that can ingest microparticles as food in the aquatic environment (Rivetti et al., 2016; Yang et al., 2017). Therefore, connecting biological levels is of particular importance in understanding the mechanism of biological features of individuals in adverse outcome pathways.

In the present study, we chose citalopram (CIT) as a typical SSRI to assess the influence of different SPS properties on the bioaccumulation and biological effects of SSRIs using *D. magna*. The CIT concentrations, both dissolved in the water column and adsorbed in SPS, were determined to clarify the impacts of SPS properties on CIT body burden in *D. magna*. The swimming behaviours and physiological activities along with oxidative stress indicators were determined to comprehensively evaluate the influence of SPS on the biological effects induced by CIT.

2. Materials and methods

2.1. Chemicals and reagents

Citalopram hydrobromide (CAS 59729-32-7, purity > 98%, referred to as CIT herein) and fluorescent 2, 7-dichlorofluorescein diacetate (CAS 2044-85-1, purity > 99%, referred to as H_2DCFDA herein) were purchased from J&K Scientific Co., Ltd. (Shanghai, China). Kits for the assays of total antioxidant capacity (T-AOC), malondialdehyde (MDA) level and protein quantification were obtained from Jiancheng Co., Ltd. (Nanjing, China). Acetonitrile and methanol (HPLC grade) were obtained from Merck Serono Co., Ltd. (Darmstadt, Germany). Chemical stock solutions were prepared in acetonitrile and stored at −20 °C. Deionized water was obtained using a Milli-Q integral water purification system (Millipore, Milford, MA, USA).

2.2. Preparation for SPS with different characteristics

The surface sediments (≤10 cm in depth) were sampled from the Nanjing section of the Yangtze River and from Mochou Lake in Nanjing, China in November of 2016. The samples were then freeze-dried, passed through a 2 mm sieve and stored at 4 °C. To prevent the interference of dissolved organic matter, the sediment was processed before exposure as described by Zhang et al. (2015). First, the sediment samples were placed in centrifuge tubes with 100 ml pure water, shaken for 24 h, and centrifuged at 1000 × g for 5 min. After that, the supernatant was discarded, which was done twice to remove the dissolved organic carbon (DOC) from the sediment. The DOC in the supernatant was measured with a TOC analyser (Model 1030, OI Analytical, USA) until the result showed that its concentrations were below the detection limit after having been washed twice. The organic carbon content of suspended sediments (f_{oc}) was determined by an elemental analyser (muti EA4000, Germany). Then, the samples were further processed to prepare SPS with different particle sizes and f_{oc}. The samples with f_{oc} of 0.78% from the Yangtze River were used for SPS with different particle size, and samples f_{oc} of 2.54% from Mochou Lake were prepared for SPS with different f_{oc}. Based on the results of the size distribution of SPS in the Yangtze River delta of China, the percentage of SPS in the size fraction of 0–32, 32–63 and > 63 μm in the Yangtze River delta were 78.4%, 2.1% and 0.4%, respectively (Yang et al., 2008). Thus, we divided the sediment sample into three sieve fractions (including 0–30, 30–60, 60–90 μm) with 490 mesh (30 μm), 240 mesh (60 μm) and 170 mesh (90 μm) in the test. With regard to the preparation for SPS with different f_{oc}, the methods were those described in our previous study, using hydrogen peroxide to reduce the content of the organic matter (Yang et al., 2017). The f_{oc} values

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