1 **Importance of Suspended Sediment (SPS) Composition and Grain Size**
2 **in the Bioavailability of SPS-associated Pyrene to *Daphnia magna***

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Abstract

Hydrophobic organic compounds (HOCs) tend to associate with suspended sediment (SPS) in aquatic environments; the composition and grain size of SPS will affect the bioavailability of SPS-associated HOCs. However, the bioavailability of HOCs sorbed on SPS with different compositions and grain sizes is not well understood. In this work, passive dosing devices were made to control the freely dissolved concentration of pyrene, a typical HOC, in the exposure systems. The effect of pyrene associated with amorphous organic carbon (AOC), black carbon (BC), and minerals of SPS with grain sizes of 0-50 μm and 50-100 μm on the immobilization and enzymatic activities of *Daphnia magna* was investigated to quantify the bioavailability of pyrene sorbed on SPS with different grain sizes and compositions. The results showed that the contribution of AOC-, BC-, and mineral-associated pyrene to the total bioavailability of SPS-associated pyrene was approximately 50%-60%, 10%-29%, and 20%-30%, respectively. The bioavailable fraction of pyrene sorbed on the three components of SPS was ordered as AOC (22.4%-67.3%) > minerals (20.1%-46.0%) > BC (9.11%-16.8%), and the bioavailable fraction sorbed on SPS of 50-100 μm grain size was higher than those of 0-50 μm grain size. This is because the SPS grain size will affect the ingestion of SPS and the SPS composition will affect the desorption of SPS-associated pyrene in *Daphnia magna*. According to the results obtained in this study, a model has been developed to calculate the bioavailability of HOCs to aquatic organisms in natural waters considering both SPS grain size and composition.

Keyword: Suspended sediment; BC; Organic carbon; Bioavailability; Passive dosing device; freely dissolved concentration

Capsule: The bioavailability of SPS-associated pyrene to *D. magna* depends on both particulate grain size affecting SPS ingestion, and particulate composition affecting desorption of pyrene.

1 Introduction

In natural waters, most hydrophobic organic compounds (HOCs) are mainly associated with particulates because of their low solubility in water, especially for rivers with high suspended sediment (SPS) concentrations (Li et al., 2006; Dong et al., 2015). SPS is an important carrier of pollutants and the sorption of HOCs on SPS is the key process controlling the transport, distribution, and bioavailability of HOCs, such as polycyclic aromatic hydrocarbons (PAHs) with high octanol-water partition coefficients, in waters (Feng et al., 2007; Wang and Keller, 2009; Dong et al., 2013; Zhang et al., 2015).

Some researchers have found that the particle-associated HOCs are partly bioavailable to organisms (Verriest et al., 2001; Gillis et al., 2005). Our previous research revealed that the SPS enhanced the toxicity of phenanthrene and pyrene to filter-feeding invertebrate *Daphnia magna* (*D. magna*) compared to that in the absence of SPS with identical freely dissolved phenanthrene or pyrene concentration in water (Zhang et al., 2014; Zhang et al., 2015). This infers that the HOCs associated with SPS should not be ignored in the risk assessment of HOCs in natural waters.

SPS consists of organic materials and minerals. Sediment organic carbon (SOC) has been recognized as the key parameter governing the distribution of HOCs in the aquatic environment (Lohmann et al., 2005; Wang et al., 2009; Birch et al., 2012; Gebremariam et al., 2012). Not only the content but also the composition of SOC has a strong influence on the sorption and behavior of HOCs in SPS. Organic matter in SPS consists of heterogeneous mix of organic materials often classified as an amorphous, gel-like "soft carbon" matrix and a condensed, glasslike "hard carbon" matrix (Weber et al. 1992). The former often refers to the amorphous organic carbon (AOC), and the latter includes lignin and "black carbon" which are highly carbonaceous organic matters from partial combustion processes such as chars, soots etc. (Gelinis et al., 2001; Accardi-Dey and Gschwend, 2003). Usually, the black carbon (BC) and AOC in soils and sediments could be separated under 375 °C combustion, and BC is defined as the organic carbon which could be retained under 375 °C combustion while AOC is decomposed (Caroline et al., 2005; Edmondson et al., 2015).

BC fraction in sediments can be as high as 45~55% of total organic carbon (Allen-King et al.

2002; Schmidt et al. 2002; Huang et al., 2015). It is increasingly being recognized that BC is a key vector for the transport and partitioning of HOCs in aquatic environments (Lohmann et al. 2005). Armitage et al. (2008) found that for the estimation of *K_{oc}* or dissolved concentrations of HOCs, the sorption models inclusive of black carbon provided greatly improved estimates compared to the amorphous carbon only model. In addition, many researchers reported that sorption of HOCs on BC is generally highly non-linear and irreversible, leading to a strong sorption-desorption hysteresis and reduced HOC bioavailability (Braida et al. 2002; Yu et al. 2006; Xia et al., 2011). Therefore, not only the organic carbon content but also the organic carbon types of SPS might influence the bioavailability of SPS-associated HOCs. In addition, the SPS size also has critical influences on the sorption of HOCs (Xia and Wang, 2008; Luo et al., 2015), and the ingestion of SPS by organisms also depends on SPS grain size (Zhang et al., 2015).

The above mentioned results suggest that the bioavailability of HOCs sorbed on SPS to aquatic organisms might vary with the SPS composition and grain size. However, the related information is very limited. Especially, no research has been carried out to quantify the bioavailable fraction of HOCs sorbed on different compositions of SPS with various grain sizes. The main reason for this gap is the lack of well-defined and stable freely dissolved HOCs for bioavailability tests (Rufli et al., 1998). In recent years, the passive dosing system (Kramer et al., 2010; Smith et al., 2010) has enabled defined and constant freely dissolved HOC exposure in a simpler and less costly way than traditional methods, which is suitable for aquatic toxicity tests or bioconcentration tests.

In this study, we chose pyrene as a typical HOC to study the bioavailability of HOCs associated with SPS of various compositions and grain sizes to *D. magna*. The passive dosing devices were made to control the freely dissolved concentration of pyrene in the exposure systems. The effect of pyrene associated with SPS of different compositions and grain sizes on the immobilization and enzymatic activity of *D. magna* was investigated to quantify the bioavailability of SPS-associated pyrene. The bioavailable fraction of pyrene sorbed on each component (including AOC, BC, and minerals) of SPS was studied, and a model was developed to calculate the bioavailability of HOCs in natural waters considering both the composition and grain size of SPS.

2 Materials and Methods

2.1 Chemicals and materials

Pyrene (solid powder) was purchased from AccuStandard Inc. (New Haven, USA). *Meta*-terphenyl used as internal standard and Di-fluoro-biphenyl used as recovery standard were obtained from J&K Scientific Ltd. (Beijing, China). Hexane (HPLC grade), methanol (HPLC grade), dichloromethane (GC-MS grade), and acetone (HPLC grade) were purchased from J&K Scientific Ltd. (Beijing, China). Ethanol, NaHCO₃, KCl, CaCl₂, and MgSO₄ (analytical grade) were purchased from Beijing Chemical Reagents Company (Beijing, China). Polydimethylsiloxane (PDMS) pre-polymer and catalyst used to make passive dosing devices were purchased from Dow Corning Co. Ltd. (Shanghai, China). All glassware was soaked in a 10% HNO₃ solution (analytical grade, Beijing Chemical Reagents) for 24 h followed by a washing with tap water and ultrapure water (each beaker for three times), afterwards dried in an oven (DHG-9070A, Shanghai, China) at 105°C and heated in a muffle furnace (KSW-5-12A, Tianjin, China) at 350-400°C for 5 h.

2.2 Sediment sampling and characterization

Sediment samples were collected from the Xiaolangdi Hydrological station in the middle reach of the Yellow River and the Datong Hydrological station in the lower reach of the Yangtze River in September of 2013. The samples were placed in coolers, transported to the laboratory and dried in freeze-driers followed by sieving through a 100 mesh sieve. Then two size fractions, 0~50 µm and 50~100 µm, were used to study the effect of SPS composition and size on the bioavailability of SPS-associated pyrene. This is because these two size fractions are the main components of SPS in natural rivers (Dong et al., 2015; Lai et al., 2015), and our previous study showed that the bioavailability of PAH associated with them was much higher than the SPS with particle size larger than 100 µm (Zhang et al., 2015). Then the samples were washed twice using pure water to remove the dissolved organic carbon (DOC) from sediment particles according to the method described in a previous study (Zhang et al., 2015). The result showed that the DOC was lower than the detection limit after double washing. After that, a part of each sediment sample containing AOC+BC+minerals was heated at 375°C for 24 h to obtain the sample containing BC+minerals; another part of each sediment sample was combusted at 700°C for 3 h

to obtain the sample containing minerals. Further experimental analysis showed that the combustion did not affect the SPS size significantly. Total organic carbon (TOC) of each sample was measured using an elemental analyzer (Vario El, Elementar Analysensysteme GmbH, Germany), and the total concentrations of 16 priority PAHs identified by the USEPA sorbed on each treated SPS sample was determined (Data are available in [Table S1](#)). The physio-chemical properties of sediment samples are listed in [Table 1](#).

2.3 Preparation of passive dosing dishes and exposure systems

The passive dosing dishes, which were used to control the freely dissolved concentration of pyrene, were prepared according to our previous research ([Xia et al., 2015](#)). In brief, passive dosing dishes were prepared by casting PDMS into 40 mm-diameter glass culture dishes. A total of 12 ± 0.01 g mixture of PDMS pre-polymer and the matched catalyst (10:1, weight) were added to each culture dish, which was considered to be sufficient to hold 300 mL water ([Mayer et al., 2000](#)). These dishes were then vacuumed to eliminate trapped air and subsequently placed in an oven at 110°C for 48 h to complete the curing. Cured dishes were soaked in methanol for 72 h to remove impurities and oligomers and were then rinsed with Milli-Q water three times before pyrene loading. The loading solution was made by dissolving 0, 0.3, 2.0, 3.0, 4.0, 6.0 g pyrene in glass bottles containing 1 L methanol respectively, and 0.25 mL acetone was added as the co-solvent to the 4.0 and 6.0 g pyrene systems, respectively. This amount would not cause confusing effect on pyrene partition between methanol and PDMS. Then the bottles were ultra-sonicated in an ultrasonic apparatus (KQ5200, Kunshan, China) for 6 h to facilitate the dissolution of pyrene in methanol. Dishes cured in triplicate were immersed in these solutions for at least 72 h (100 ml solution per dish) with loading solutions refreshed every 24 h. After that, loaded dishes were rinsed with Milli-Q water three times, and then they were placed in artificial freshwater (AFW) for at least 48 h to complete the dosing procedure, which was used to prepare the biotest solutions. AFW was prepared in lab according to the guideline of Organization for Economic Cooperation and Development for the testing of chemical ([OECD, 2008](#)). The details about the calculation of partition coefficient of pyrene between PDMS and methanol as well as between AFW and PDMS have been described in our previous study ([Zhang et al., 2015](#)). The

partition coefficient of pyrene between methanol and AFW was calculated as 100875 in this study, and the concentration of pyrene in AFW can be deduced from that in methanol and vice versa.

For the establishment of passive dosing exposure systems, the loading solution was prepared by dissolving 2.5218 g pyrene in one liter methanol to obtain $25.0 \mu\text{g L}^{-1}$ freely dissolved pyrene according to the partition coefficient of pyrene between methanol and AFW. After the loading process as mentioned above, these dishes were rinsed with ultrapure water and then used for the following biological experiments.

2.4 Biological experiments

The tests were conducted in 500 mL glass beakers containing 300 mL exposure solution. PDMS dish spiked with pyrene was placed upside down on a V-shaped glass rod to prevent the suspended sediments from depositing on the surface of PDMS (Figure S1). Then sediment samples with various compositions and grain sizes were added to a series of beakers, respectively to obtain 1 g L^{-1} SPS in each test water system. Then the beakers were covered by parafilm and equilibrated at room temperature on magnetic stirrers for 48 h. Although the passive dosing kinetics for pyrene might be slower in water with SPS than in the absence of SPS (Gouliarmou et al., 2012), our experiment results showed that 48 h was long enough for pyrene to reach equilibrium in the water-SPS systems under stir conditions. After that, the stir bars were taken out and 30 *D. magna*, at the age of 6~24 h old, were placed into each beaker. They were maintained in a temperature-monitored artificial climate incubator (RXZ-500B, China) at $20 \pm 1^\circ\text{C}$ for 48 h with a 16:8 (light: dark) photoperiod (light intensity 12,000 lux, wavelength 400-700 nm), during which the solution was gently stirred with the V-shaped glass rod repeatedly to keep the sediments in suspension. In addition, the control experiments were conducted without spiked pyrene in the presence of 1 g L^{-1} SPS of various compositions and grain sizes, and the blank experiments were conducted with neither SPS nor spiked pyrene. Each experimental treatment was conducted in triplicate.

The immobilization (%) of *D. magna* was monitored every 12 h during the 48 h exposure and the immobility was considered to have happened when *D. magna* did not swim within 15 seconds after shaking the glass rod in the beaker. At the end of the test, living *D. magna* in each beaker were collected and the total wet weight of all the collected daphnids was obtained using a

balance with an accuracy of 0.00001 g; then the protein contents and enzymatic activities of *D. magna* were determined. The SPS in each beaker was collected by filtering the water through a 0.45 μm membrane, then freeze-drying the membrane for 48 h to measure the pyrene associated with SPS. To avoid the effects of filter membrane on the freely dissolved concentration of pyrene (C_{free}), the middle part (120 mL) of the filtrate was used for the determination of C_{free} .

2.5 Biochemical and chemical analysis

2.5.1 Biochemical analysis of enzymatic activity

The total superoxide dismutase (T-SOD) and protein content of *D. magna* were analyzed in this study. The collected alive *D. magna* were placed in a 2 mL centrifuge tube which contained 1.5 mL 0.05 M Tris-HCl buffer on ice (pH = 8.2). Then the organisms were disrupted with an ultrasonic tissue destructor (Sonics Uibra Cell VCX 105) in the ice bath to obtain homogenates, which was then centrifuged at 11739 $\times$ g at 4 $^{\circ}\text{C}$ for 20 min. The supernatant was used for the determination of the protein content and T-SOD; they were measured using the reagent kit purchased from Nanjing Jiancheng Bioengineering Institute. The results of the enzymatic assay are given in units of enzymatic activity per milligram of protein (U mg^{-1}) (Cana-Europa et al., 2008).

2.5.2 Analysis of freely dissolved and SPS-associated pyrene

The freely dissolved concentration of pyrene in water was measured using solid-phase microextraction (SPME) (Heringa and Hermens, 2003), a passive sampling technique. The detailed preparation and usage of polydimethylsiloxane-coated glass fibers can be found in our previous research (Lai et al., 2015). The pyrene sorbed on sediment and SPS samples were extracted using elution of hexane-dichloromethane (1:1 volume) with accelerated solvent extractor (Dionex ASE300) (Shen et al., 2012). Pyrene was analyzed with a Varian 3800 gas chromatography-4000 ion trap mass spectrometry (GC/MS) system equipped with a Varian Factor FourTM highly inert capillary column VF-5ms (30 m $\times$ 0.25 mm diameter, and 0.25 μm film thickness). Details for the above procedures are shown in supporting information (SI).

2.6 Calculation of bioavailable fraction of pyrene sorbed on SPS

The theoretical dissolved concentration of pyrene ($C_{\text{effective}}$, $\mu\text{g L}^{-1}$) required to produce the observed immobilization of *D. magna* in systems with SPS could be calculated according to the linear relationship between *D. magna* immobilization and pyrene concentration in the absence of SPS shown in Figure S2. There were three types of SPS samples, including S1 containing AOC+BC+minerals, S2 containing BC+minerals, and S3 containing minerals. The theoretical dissolved pyrene concentrations required to produce the observed immobilization of *D. magna* in the presence of the three types of SPS ($C_{\text{effective-S1}}$, $C_{\text{effective-S2}}$, $C_{\text{effective-S3}}$, $\mu\text{g L}^{-1}$) were calculated accordingly. Then the bioavailable fraction of pyrene sorbed on the original SPS containing AOC+BC+minerals, AOC, BC, and minerals (F_{SPS} , F_{AOC} , F_{BC} , F_{mineral} , %) could be calculated as follows:

$$F_{\text{SPS}} = \frac{C_{\text{effective-S1}} - C_{\text{free}}}{0.001 \times C_{\text{SPS}}} \times 100\% \quad (1)$$

$$F_{\text{AOC}} = \frac{C_{\text{effective-S1}} - C_{\text{effective-S2}}}{0.001 \times C_{\text{AOC}}} \times 100\% \quad (2)$$

$$F_{\text{BC}} = \frac{C_{\text{effective-S2}} - C_{\text{effective-S3}}}{0.001 \times C_{\text{BC}}} \times 100\% \quad (3)$$

$$F_{\text{mineral}} = \frac{C_{\text{effective-S3}} - C_{\text{free}}}{0.001 \times C_{\text{mineral}}} \times 100\% \quad (4)$$

where C_{SPS} is the pyrene concentration associated with original SPS ($\mu\text{g kg}^{-1}$); C_{AOC} , C_{BC} , and C_{mineral} are the pyrene concentrations associated with AOC, BC, and mineral phases in SPS, respectively ($\mu\text{g kg}^{-1}$); 0.001 is the SPS concentration in the exposure systems (kg L^{-1}). Detailed information for calculation of C_{AOC} , C_{BC} , and C_{mineral} is described in SI.

2.7 Data analysis

All statistical analyses were performed using SPSS 18.0 for Windows (SPSS Inc., Chicago IL, USA). Analysis of variance (ANOVA, one factor) was carried out to test differences between each two compared groups. Difference was considered significant when the significance level was smaller than 0.05. The Pearson correlation coefficient was calculated and used to test the significance of correlation between each two variables.

2.8 Quality assurance and quality control

The detection limit of pyrene was $0.10 \mu\text{g L}^{-1}$ for GC/MS and the correlation coefficient for pyrene calibration curve of GC/MS was higher than 0.99. The recovery of pyrene on SPS was $83.3 \pm 4.5\%$ (detailed processes for the determination are described in [SI](#)). The PDMS coat without spiked pyrene did not show influence on the survival and growth of *D. magna* either in the absence or presence of SPS. It indicates that the cured polydimethylsiloxane material in PDMS dishes was biocompatible. The measured C_{free} of pyrene in AFW maintained by the PDMS dishes ranged from 22.3 to $25.1 \mu\text{g L}^{-1}$ (Data shown in Table S2), which was consistent with the calculated value ($25.0 \mu\text{g L}^{-1}$) based on the partition coefficient of pyrene between methanol and AFW. The variation of pyrene in the water phase was within 1.02% and the water environmental condition was stable during the exposure experiments: the pH was 7.04 ± 0.05 and dissolved oxygen was $8.02 \pm 0.11 \text{ mg L}^{-1}$. The sensitivity of *D. magna* to the standard toxicant, potassium dichromate, was determined and the EC_{50} (24 h) was 0.95 mg L^{-1} , which was within the normal range of 0.50~1.2 mg L^{-1} reported by OECD ([2008](#)).

3 Results and Discussion

3.1 Effect of SPS itself without spiked pyrene on the immobilization of *D. magna*

According to the results shown in [Table S1](#), the concentrations of native pyrene and sixteen kinds of PAHs sorbed on the SPS with various grain sizes and compositions were less than 12.7 ng g^{-1} and 99.1 ng g^{-1} , which accounted for less than 0.11% and 0.86% of the spiked pyrene associated with SPS in the exposure tests, respectively. Therefore, the background PAHs would not exert significant influence on the sorption/desorption of pyrene in the exposure systems.

According to our previous research, the SPS themselves and the native pollutants sorbed on SPS might cause mixture toxicity to *D. magna* ([Zhang et al., 2015](#)). However, the immobilization of *D. magna* in the presence of 1 g L^{-1} SPS without spiked pyrene was less than 4.4% after exposure for 48 h, which was significantly lower than that in the test groups with spiked pyrene ($p < 0.05$). To reflect the effect of spiked pyrene associated with SPS on the immobilization of *D. magna*, the results of test groups with SPS of various grain sizes and compositions have been corrected by respectively subtracting the immobilization caused by the SPS itself without spiked pyrene.

3.2 Effect of pyrene sorbed on SPS with various compositions and grain sizes on the immobilization of *D. magna*

As shown in [Figure 1](#), the immobilization of *D. magna* caused by pyrene increased with time during a 48 h exposure for each system. Compared to the systems in the absence of SPS, the presence of 1 g L⁻¹ SPS significantly elevated the immobilization of *D. magna* when the C_{free} of pyrene was kept constant. The immobilization of *D. magna* caused by pyrene in the systems with SPS was 1.5-3.2 and 1.3-3.6 times higher than that without SPS after exposure for 36 h and 48 h, respectively. This indicates that the pyrene associated with SPS exerted toxic effects to *D. magna*. Because the C_{free} of pyrene was kept constant in the presence or absence of SPS, and DOC was not detected in these systems, the difference in immobilization of *D. magna* between with and without SPS was caused by the SPS-associated pyrene. As shown in [Table 2](#), the contribution of pyrene sorbed on 1 g L⁻¹ SPS to the immobilization of *D. magna* caused by total pyrene in the exposure system ranged from 49.4% to 72.1%.

As shown in [Figure 2](#), the toxicity of SPS-associated pyrene to *D. magna* varied with SPS composition. Regardless of the SPS grain size and source, the immobilization caused by pyrene sorbed on SPS followed a decreasing order of SPS containing AOC+BC+minerals > SPS containing BC+minerals > SPS containing minerals. For example, after exposure for 48 h, the immobilization of *D. magna* caused by pyrene sorbed on SPS containing AOC+BC+minerals was 2.0 and 4.8 times that on SPS containing BC+minerals and on SPS containing minerals, respectively for the 0-50 μm SPS samples from the Yangtze River.

In addition, the immobilization of *D. magna* caused by pyrene sorbed on SPS from the Yangtze River was larger than that from the Yellow River. For instance, according to the results shown in [Figure S3](#), after exposure for 48 h, the immobilization of *D. magna* caused by pyrene associated with 50-100 μm SPS containing AOC+BC+minerals, containing BC+minerals, and containing minerals from the Yangtze River was 1.6, 1.6, and 1.5 times that from the Yellow River, respectively. This means that, in addition to the composition of SPS, the origin of SPS will also exert influence on the bioavailability of SPS-associated pyrene.

According to the results shown in [Figure 2](#) and [Figure S4](#), the immobilization of *D. magna* caused by pyrene sorbed on the SPS of 50-100 μm grain size was higher than those of 0-50 μm

grain size. For the SPS containing AOC+BC+minerals from the Yangtze River, the immobilization caused by pyrene sorbed on 0-50 μm and 50-100 μm SPS was 30.9% and 46.4% after exposure for 48 h, respectively; the latter was approximately 1.5 times of the former. For the SPS containing AOC+BC+minerals from the Yellow River, the immobilization caused by pyrene sorbed on 50-100 μm SPS was 1.6 times that sorbed on 0-50 μm SPS after exposure for 48 h.

3.3 Effect of SPS-associated pyrene on protein content and T-SOD activity of *D. magna*

The protein content of aquatic organisms is important for their well-being, and it is related not only to the level of organic matters but also to the toxicant stresses in their habitat (Chen et al., 2005). In the present research, *Daphnia* had an average protein content of around 1.9% wet weight, and the protein content varied with the SPS composition and grain size. As shown in Figure 3, compared to the blank systems with neither SPS nor pyrene, the protein content increased in the systems with pyrene or with SPS containing BC+minerals and pyrene except for the 50-100 μm SPS from the Yangtze River, while the protein content decreased in the systems with SPS containing AOC+BC+minerals and pyrene except for the 50-100 μm SPS from the Yellow River. The order of the theoretical dissolved concentration of pyrene ($C_{\text{effective}}$) among different treatments was: with neither SPS nor pyrene < with pyrene < with pyrene and minerals < with pyrene and BC+minerals < with pyrene and AOC+BC+minerals (Figure 3). This means that a low $C_{\text{effective}}$ of pyrene can stimulate the protein synthesis in *D. magna* while a high $C_{\text{effective}}$ of pyrene could inhibit the protein synthesis. An increase in protein content is beneficial for *Daphnia* to resist the adverse environmental conditions (De Coen and Janssen, 1997). For the systems with pyrene and SPS composed of minerals, the protein content of *D. magna* was decreased compared to other systems. This might be due to the fact that the contents of organic carbon and other nutrient substances sorbed on minerals, which are used for the synthesis of protein and growth of organisms (Taipale et al., 2014), were the lowest among the systems with SPS. In addition, the presence of minerals increased the bioavailable concentration of pyrene in systems compared to the system without SPS. Therefore, the relatively lower organic carbon and higher bioavailable concentration of pyrene led to the lowest protein content in the system with

minerals.

Superoxide dismutase (T-SOD) is an important antioxidant defense enzyme in nearly all living cells exposed to oxygen, especially when environmental stresses including chemical stress induce enhanced production of reactive oxygen species (ROS) (Amirjani and Mahdizadeh, 2013). Superoxide is produced as a by-product of oxygen metabolism and, if not regulated, causes many types of cell damage (Peters and Livingstone, 1996). T-SOD often protects organisms against ROS toxicity (Di Giulio et al., 1989). In the present study, compared to the blank systems with neither pyrene nor SPS, T-SOD activity was elevated in the systems with pyrene or with pyrene and SPS composed of minerals (Figure 4), suggesting that a low pyrene concentration can stimulate the production of T-SOD in *D. magna*. However, T-SOD activity was decreased in the systems with 50-100 μm SPS containing BC+minerals or containing AOC+BC+minerals in the presence of pyrene. It means that a relatively high concentration of pyrene will inhibit the T-SOD activity, which is in agreement with other researches (De Coen and Janssen, 1997; Fan et al., 2009). Besides, for both SPS containing AOC+BC+minerals and containing BC+minerals, the pyrene associated with 50-100 μm SPS exerted more inhibition to T-SOD activity than 0-50 μm SPS. For example, the T-SOD activity of *D. magna* in the systems with 50-100 μm SPS decreased by 16.7-17.5% and 11.7-23.5% compared to that with 0-50 μm SPS from the Yangtze River and the Yellow River, respectively; this is because the $C_{\text{effective}}$ of pyrene in the former system was higher than the latter (Figure 4).

3.4 Bioavailability of pyrene sorbed on various components of SPS

The bioavailable fraction of SPS-associated pyrene was related to SPS composition. As shown in Table 2, among the three components of SPS, the bioavailable fraction of pyrene sorbed on AOC was the highest, followed by that sorbed on minerals, and that sorbed on BC was the lowest. The lowest bioavailable fraction of pyrene sorbed on BC is due to the fact that the sorption of HOCs on BC is generally highly non-linear and irreversible (Yu et al. 2006; Xia et al., 2011), and it might also be hard for pyrene to desorb from BC in *D. magna*. Research has shown that the BC has stronger binding to HOCs relative to other SOM so that the bioavailability of HOCs to organisms would be affected by the presence and quantity of sediment BC (Jia and Gan, 2014; Wang et al., 2011). Besides, we found that pyrene can also be sorbed on minerals in the

present research, and this is in agreement with previous research results that contribution of mineral surface to the sorption of HOCs may not be ignored, especially for the sorbents with low content of SOC (Schoonheydt and Johnston, 2006; Meleshyn et al., 2011). Furthermore, the bioavailable fraction of pyrene sorbed on minerals was lower than that on AOC, suggesting that it is more difficult for pyrene to desorb from the minerals than from the AOC in *D. magna*.

According to the immobilization caused by pyrene sorbed on each component of SPS as shown in Table 2, the contribution of AOC-associated pyrene to the total bioavailability of pyrene sorbed on the original SPS containing AOC+BC+minerals was approximately 50%-60%; the contribution of BC-associated pyrene ranged from 10% to 29% and the contribution of mineral-associated pyrene ranged from 20% to 30%. It suggests that AOC in SPS plays a crucial role in controlling the bioavailability of SPS-associated HOCs. For example, the bioavailable fraction of pyrene sorbed on the original SPS containing AOC+BC+minerals from the Yangtze River was higher than that from the Yellow River (Table 2). This is due to the fact that the AOC content in the SPS from the Yangtze was higher than the Yellow river.

In addition, the bioavailable fraction of SPS-associated pyrene was dependent on SPS grain size. The bioavailable fractions of pyrene sorbed on 50-100 μm size SPS were higher than those on 0-50 μm size SPS (Table 2). For example, the bioavailable fractions were 45.2% and 19.9% respectively for the 50-100 μm and 0-50 μm grain size SPS containing AOC+BC+minerals from the Yangtze River. The bioavailable fraction of pyrene associated with 0-50 μm grain size SPS was comparable to the result reported by Weston et al. (2000) that an average of 5-21% of the ingested benzo[a]pyrene was absorbed from gut contents of the deposit-feeding polychaete.

3.5 Importance of both composition and grain size of SPS in the bioavailability of SPS-associated pyrene

According to the results obtained in the present study, the immobilization of *D. magna* caused by pyrene sorbed on SPS varied with SPS composition. The compositions of SPS exert influences on the distribution of pyrene between SPS and water. As shown in Table 3, the AOC-water and BC-water distribution coefficients of pyrene were approximately 2-3 orders of magnitude higher than the minerals-water one. More than 72% of pyrene sorbed on the original

375 SPS containing AOC+BC+minerals was associated with AOC and BC. This infers that the
376 concentration of pyrene sorbed on SPS depend on the contents of AOC and BC as well as the
377 freely dissolved concentration of pyrene. In addition, the bioavailable fraction of pyrene
378 associated with the three components of SPS was ordered as AOC > minerals > BC. Therefore,
379 the bioavailability of SPS-associated pyrene will depend on the composition of SPS and the
380 freely dissolved concentration of pyrene in natural waters.

381 In addition, the SPS grain size was another important factor affecting the bioavailability of
382 SPS-associated pyrene. For example, for the SPS from the Yangtze River, although both the
383 AOC and BC contents in the 50-100 μm grain size fraction were lower than those in the 0-50 μm
384 grain size fraction (Table 1), the immobilization of *D. magna* caused by pyrene sorbed on the
385 former was higher than the latter (Figure 2). This is because the SPS grain size will affect the
386 ingestion and retention of SPS in *D. magna* (Wilber et al., 2001). According to our previous
387 research (Zhang et al., 2015), as a result of the difference in intake and retention of SPS in the
388 alimentary canal of *D. magna* among various SPS grain size fractions, the amount of 50-100 μm
389 SPS in the alimentary canal of *D. magna* was higher than 0-50 μm grain size fractions after
390 exposure for 48 h (Table S3). Only the pyrene sorbed on SPS particles, which can be ingested by
391 *D. magna* and stay in *D. magna* for a long enough period of time, can be desorbed in and exert
392 toxicity to *D. magna*. Therefore, the ingestion of SPS by aquatic organisms is of crucial
393 importance for the bioavailability of SPS-associated HOCs to aquatic organisms. Additionally,
394 the desorption of SPS-associated HOCs in and their absorption by aquatic organisms depend on
395 both SPS composition and HOC characteristics. The fact that sediment ingestion is an important
396 route in HOC bioaccumulation by benthic organisms has been proved by other researchers
397 (Leppänen and Kukkonen, 1998; Lu et al., 2004). Weston et al. (2000) reported that through the
398 selective ingestion of fine-grained material (<63 μm), the deposit-feeding polychaete was able to
399 increase the benzo[a] pyrene content of ingested sediment by 10-35% above that of the bulk
400 material. Our previous study found that carbonaceous materials (CMs) could be ingested by
401 *Chironomus plumosus* larvae, and perfluoroalkyl substances (PFASs) associated with CMs could
402 be partly desorbed by the larvae digestive juice, leading to the accumulation of CM-associated
403 PFASs by larvae (Zhai et al., 2016). Moreover, it is generally accepted that the importance of

sediment ingestion increases with the hydrophobicity of the organic compounds (Leppänen and Kukkonen, 1998; Sormunen et al., 2008).

In natural waters, HOCs mainly exist in three forms, including freely dissolved, SPS associated, and DOC associated. Many researchers have reported that dissolved organic carbon is another important factor for HOC bioavailability besides SPS (Akkanen et al., 2003; Xia et al., 2009; Xia et al., 2015). Therefore, considering the SPS composition and grain size as well as dissolved organic carbon, the bioavailability of HOCs to aquatic organisms can be expressed with the following formula:

$$C_{\text{bio}} = C_{\text{free}} + \sum_{i=1}^n (C_{i\text{-AOC}} \cdot f_{i\text{-AOC}} + C_{i\text{-BC}} \cdot f_{i\text{-BC}} + C_{i\text{-mineral}} \cdot f_{i\text{-mineral}}) + C_{\text{DOC}} \cdot f_{\text{DOC}} \quad (5)$$

where C_{free} is the freely dissolved concentration of HOC ($\mu\text{g L}^{-1}$); i is the fraction of SPS with grain size of i , $C_{i\text{-AOC}}$, $C_{i\text{-BC}}$, and $C_{i\text{-mineral}}$ are the HOC concentrations associated with AOC, BC, and mineral phases in SPS with grain size of i ($\mu\text{g L}^{-1}$), respectively; $f_{i\text{-AOC}}$, $f_{i\text{-BC}}$, and $f_{i\text{-mineral}}$ are the bioavailable fractions of HOC sorbed on AOC, BC, and minerals in SPS with grain size of i (%), respectively; C_{DOC} is the HOC concentration associated with DOC ($\mu\text{g L}^{-1}$); f_{DOC} is the bioavailable fraction of HOC associated with DOC (%).

Native HOCs in the environment might be present at lower concentrations compared to the present experiments. Also, they will have longer contact time with SPS and sediments in natural waters compared to the present study, which will lead to higher sorption, especially stronger irreversible sorption in black carbon, in the environment compared to the experiments. Therefore, more research should be conducted to extrapolate the model we put forward to the field. Furthermore, the availability from different particles can also be partly organism dependent because target size can differ among different filter feeding groups (Weston et al., 2000; Wilber et al., 2001). For the non filter feeders, the bioavailability of particle-associated HOCs might not be important while the DOC-associated might be of importance. However, if we evaluate the eco-environment risk of HOCs in aquatic environments, all the aquatic organisms including filter and non-filter feeders should be taken into consideration.

4 Conclusions

In this work, the passive dosing devices were made to control the freely dissolved concentration of pyrene in the exposure systems. The effect of pyrene associated with SPS of

different compositions and grain sizes on the immobilization and enzymatic activity of *D. magna* was investigated to quantify the bioavailability of SPS-associated pyrene. The results showed that the contribution of pyrene sorbed on 1 g L^{-1} SPS to the immobilization of *D. magna* caused by total pyrene in the exposure system ranged from 49.4% to 72.1%. The contribution of AOC-, BC-, and mineral-associated pyrene to the total bioavailability of SPS-associated pyrene was approximately 50%-60%, 10%-29%, and 20%-30%, respectively. The sequence of bioavailable fraction of pyrene sorbed on the three components of SPS was AOC (22.4%-67.3%) > minerals (20.1%-46.0%) > BC (9.11%-16.8%), and the bioavailable fraction of pyrene sorbed on SPS of 50-100 μm grain size was higher than those of 0-50 μm grain size. This is due to the fact that the SPS grain size will affect the ingestion of SPS and the SPS composition will affect the desorption of SPS-associated pyrene in *D. magna*. This study suggests that both the composition and grain size of SPS should be considered for the accurate estimation of HOC toxicity and establishment of water quality criteria for HOCs in natural waters, especially for the rivers with high SPS concentrations.

Acknowledgements

This study was supported by the National Science Foundation for Distinguished Young Scholars (No. 51325902), National Science Foundation for Innovative Research Group (No. 51421065), and the National Natural Science Foundation of China (No. 91547207).

References

- Accardi-Dey, A. and Gschwend P., 2003. Reinterpreting literature sorption data considering both absorption into organic carbon and adsorption onto black carbon. *Environ. Sci. Technol.* 37 (1), 99-106.
- Akkanen, J. and Kukkonen, J., 2003. Measuring the bioavailability of two hydrophobic organic compounds in the presence of dissolved organic matter. *Environ. Toxicol. Chem.* 22(3), 518-524.
- Allen-King, R., Grathwohl, P., Ball, W., 2002. New modeling paradigms for the sorption of hydrophobic organic chemicals to heterogeneous carbonaceous matter in soils, sediments, and rocks. *Adv. Water Resour.* 25(8-12), 985-1016.
- Amirjani, M. and Mahdihyeh, M., 2013. Antioxidative and biochemical responses of wheat to drought stress. *ARPJ. Agr. Biol. Sci.* 8(4), 292-301.
- Armitage, J., Cousins, I., Persson, N., Gustafsson, O., Cornelissen, G., Saloranta, T., Broman, D., Npes, K., 2008. Black carbon-Inclusive modeling approaches for estimating the aquatic fate of Dibenzo-p-dioxins and dibenzofurans. *Environ. Sci. Technol.* 42(10), 3697-3703.
- Birch, H., Mayer, P., Lutzhoft, H., Mikkelsen, P., 2012. Partitioning of fluoranthene between free and bound forms in stormwater runoff and other urban discharges using passive dosing. *Water Res.* 46(18), 6002-6012.
- Braida, W., White, J., Zhao, D., Ferrandino, F., Pignatello, J., 2002. Concentration-dependent kinetics of pollutant desorption from soils. *Environ. Toxicol. Chem.*, 21(12), 2573-2580.
- Cana-Europa, E., López- Galindo, G., Hernández-García, A., Blas-Valdivia, V., Gallardo-Casas, C., Vargas-Lascari, M., Ortiz-Butrón, R., 2008. Lidocaine affects the redox environment and the antioxidant enzymatic system causing oxidative stress in the hippocampus and amygdale of adult rats. *Life SCI.* 83(19-20), 681-685.
- Caroline, T., John, J., Albert, A., 2005. Black carbon and ecological factors affect in situ biota to sediment accumulation factors for hydrophobic organic compounds in flood plain lakes. *Environ. Sci. Technol.* 39(9), 3101-3109.
- Chen, W., Song, L., Ou, D., Gan, N., 2005. Chronic toxicity and responses of several important enzymes in *Daphnia magna* on exposure to sublethal microcystin-LR. *Environ. Toxicol.*

480 20(3), 323-330.

481 De Coen, W. and Janssen, C., 1997. The use of biomarkers in *Daphnia magna* toxicity testing.

482 Cellular Energy Allocation: a new methodology to assess the energy budget of

483 toxicant-stressed *Daphnia* populations. J. Aquatic Ecosyst. Stress Recovery. 6(1), 43-55.

484 Di Giulio, R., Washburn, P., Wenning, R., Winston, G., Jewell, C., 1989. Biochemical responses

485 in aquatic animals: A review of determinants of oxidative stress. Environ. Toxicol. Chem.

486 8(12), 1103-1123.

487 Dong, J., Xia, X., Wang, M., Lai, Y., Zhao, P., Dong, H., Zhao, Y., Wen, J., 2015. Effect of

488 water-sediment regulation of the Xiaolangdi reservoir on the concentrations, bioavailability,

489 and fluxes of PAHs in the middle and lower reaches of the Yellow River. J. Hydrol. 527,

490 101-112.

491 Dong, J., Xia, X., Zhai, Y., 2013. Investigating particle concentration effects of polycyclic

492 aromatic hydrocarbon (PAH) sorption on sediment considering the freely dissolved

493 concentrations of PAHs. J. Soil. Sediment. 13(8), 1469-1477.

494 Edmondson, J., Stott, I., Potter, J., Lopez-Capel, E., Manning, D., Gaston, K., Leake, J., 2015.

495 Black carbon contribution to organic carbon stocks in urban soil. Environ. Sci. Technol. 49(14),

496 8339-8346.

497 Fan, W., Tang, G., Zhao, C., Duan, Y., Zhang, R., 2009. Metal accumulation and biomarker

498 responses in *Daphnia Magna* following cadmium and zinc exposure. Environ. Toxicol. Chem.

499 28(2), 305-310.

500 Feng, C., Xia, X., Shen, Z., Zhou, Z., 2007. Distribution and sources of polycyclic aromatic

501 hydrocarbons in Wuhan section of the Yangtze River, China. Environ. Monit. Assess.

502 133(1-3), 447-458.

503 Gebremariam, S., Beutel, M., Yonge, D., Flury, M., Harsh., 2011. Adsorption and desorption of

504 chlorpyrifos to soils and sediments. Rev. Environ. Contam. T. 215, 123-175.

505 Gelinas, Y., Prentice, K., Baldock, J., Hedges, J., 2001. An improved thermal oxidation method

506 for the quantification of soot/graphitic black carbon in sediments and soils. Environ. Sci.

507 Technol. 35(17), 3519-3525.

508 Gillis, P., Chou-Fraser, P., Ranville, J., Ross, P., Wood, C., 2005. *Daphnia* need to be gut-cleared

509 too: the effect of exposure to and ingestion of metal-contaminated sediment on the
510 gut-clearance patterns of *D. magna*. *Aquat. Toxicol.* 71, 143-154.

511 Gouliarmou, V., Smith, K., de Jonge, L., Mayer, P., 2012. Measuring Binding and Speciation of
512 Hydrophobic Organic Chemicals at Controlled Freely Dissolved Concentrations and without
513 Phase Separation. *Anal. Chem.* 84(3), 1601-1608.

514 Heringa, M. and Hermens, J., 2003. Measurement of free concentrations using negligible
515 depletion-solid phase microextraction (nd-SPME). *Trac-Trends Anal. Chem.* 22(3), 575-587.

516 Huang, L., Zhang, J., Wu, Y., Wang, J., 2015. Distribution and preservation of black carbon in
517 the East China Sea sediments: Perspectives on carbon cycling at continental margins. *Deep Sea*
518 *Research Part II: Topical Studies in Oceanography*. doi:10.1016/j.dsr2.2015.04.029.

519 Jia, F., Gan, J., 2014. Comparing black carbon types in sequestering polybrominated diphenyl
520 ethers (PBDEs) in sediments. *Environ. Pollut.* 184, 131-137.

521 Kramer, N., Busser, F., Oosterwijk, M., Schirmer, K., Escher, B., Hermens, J., 2010.
522 Development of a partition-controlled dosing system for cell assays. *Chem. Res. Toxicol.*
523 23(11), 1806–1814.

524 Lai, Y., Xia, X., Dong, J., Lin, W., Mou, X., Zhao, P., Jiang, X., Li, Z., Tong, Y., Zhao, Y., 2015.
525 Equilibrium state of PAHs in bottom sediment-water-suspended sediment system of a large
526 river considering freely dissolved concentrations. *J. Environ. Qual.* 44(3), 823-832.

527 Leppänen, M. and Kukkonen, J., 1998. Relative importance of ingested sediment and pore water
528 as bioaccumulation routes for pyrene to oligochaete (*Lumbriculus variegatus*, Muller). *Environ.*
529 *Sci. Technol.* 32(10), 1503-1508.

530 Li, G., Xia, X., Yang, Z., Wang, R., Voulvoulis, N., 2006. Distribution and sources of polycyclic
531 aromatic hydrocarbons in the middle and lower reaches of the Yellow River, China. *Environ.*
532 *Pollut.* 144(3), 985-993.

533 Lohmann, R., Macfarlane, J., Gschwend, P., 2005. Importance of black carbon to sorption of
534 native PAHs, PCBs, and PCDDs in Boston and New York Harbor sediments. *Environ. Sci.*
535 *Technol.* 39(1), 141-148.

536 Lu, X., Reible, D. and Fleeger, J., 2004. Relative importance of ingested sediment versus pore
537 water as uptake routes for PAHs to the deposit-feeding oligochaete *Ilyodrilus templetoni*. *Arch.*

538 Environ. Contam. Toxicol. 47(2), 207-214.

539 Luo, P., Bao, L., Li, S., Zeng, E., 2015. Size-dependent distribution and inhalation cancer risk of
 540 particle-bound polycyclic aromatic hydrocarbons at a typical e-waste recycling and an urban
 541 site. Environ. Pollut. 200, 10-15.

542 Mayer, P., Vaes, W., Hermens, J., 2000. Absorption of hydrophobic compounds into the
 543 poly(dimethylsiloxane) coating of solid- phase microextraction fibers: high partition
 544 coefficients and fluorescence microscopy images. Anal. Chem. 72(3), 459-464.

545 Meleshyn, A. and Tunega, D., 2011. Adsorption of phenanthrene on Na-montmorillonite: A
 546 model study. Geoderma. 169(6), 41-46.

547 OECD. 2008. OECD Guidlines for the testing of chemicals. Organization for Economic
 548 Co-operation and Development, Paris.

549 Peters, L. and Livingstone, D., 1996. Antioxidant enzyme activities in embryologic and early
 550 larval stages of turbot. J. Fish Biol. 49(5), 986-997.

551 Rufli, H., Fisk, P., Girling, A.E., King, J., Länge, R., Lejeune, X., Stelter, N., Stevens, C., Suteau,
 552 P., Tapp, J., 1998. Aquatic toxicity testing of sparingly soluble, volatile, and unstable
 553 substances and interpretation and use of data. Ecotox. Environ. Safe. 39(2), 72-77.

554 Schmidt, M., 2002. Carbon isotope geochemistry and nanomorphology of soil black carbon:
 555 Black chernozemic soils in central Europe originate from ancient biomass burning. Global
 556 Biogeochem. Cy. 16(4), 701-708.

557 Schoonheydt, R. and Johnston, C., 2006. Chapter 3 Surface and interface chemistry of clay
 558 minerals. Developments in Clay Science. 1, 87-113.

559 Shen, M., Xia, X., Wang, F., Zhang, P., Zhao, X., 2012. Influences of multiwalled carbon
 560 nanotubes and plant residue chars on bioaccumulation of polycyclic aromatic hydrocarbons by
 561 *Chironomus plumosus* larvae in sediment. Environ. Toxicol. Chem., 31(1), 202-209.

562 Smith, K., Blust, R., Mayer, P., 2010. Controlling and maintaining exposure of hydrophobic
 563 organic compounds in a quatic toxicity tests by passive dosing. Aquat. Toxicol. 98(1), 15-24.

564 Sormunen, A., Leppanen, M. and Kukkonen, J., 2008. Influence of sediment ingestion and
 565 exposure concentration on the bioavailable fraction of sediment-associated tetrachlorobiphenyl
 566 in oligochaetes. Environ. Toxicol. Chem. 27(4), 854-863.

567 Taipale, S., Brett, M., Hahn, M., Martin-Creuzburg, D., Yeung, S., Hitunen, M., Strandberg, U.,
568 Kankaala, P., 2014. Differing *Daphnia magna* assimilation efficiencies for terrestrial, bacterial,
569 and algal carbon and fatty acids. *Ecology*. 95(2), 563-576.

570 Verrhiest, G., Clément, B., Blake, G., 2001. Single and combined effects of sediment- associated
571 PAHs on three species of freshwater macroinvertebrates. *Ecotoxicology*. 10(6), 363-372.

572 Wang, F., Bu, Q., Xia, X., Shen, M., 2011. Contrasting effects of black carbon amendments on
573 PAH bioaccumulation by *Chironomus plumosus* larvae in two distinct sediments: role of water
574 absorption and particle ingestion. *Environ. Pollut.*, 159(7), 1905-1913

575 Wang, P. and Keller, A. A., 2009. Partitioning of hydrophobic pesticides within a
576 soil-water-anionic surfactant system. *Water Res.* 43(3), 706-714.

577 Weber, W., McGinley, P., Katz, L., 1992. A distributed reactivity model for sorption by soils and
578 sediments. 1. Conceptual basis and equilibrium assessments. 26: 1955-1962.

579 Weston, D., Penry, D. and Gulmann, L., 2000. The role of ingestion as a route of contaminant
580 bioaccumulation in a deposit-feeding polychaete. *Arch. Environ. Contam. Toxicol.* 38(4),
581 446-454.

582 Wilber, D., Clarke, D., 2001. Biological effects of suspended sediments: A review of suspended
583 sediment impacts on fish and shellfish with relation to dredging activities in estuaries. *N. Am. J.*
584 *Fish. Manage.* 21(4), 855-875.

585 Xia, X., Dai, Z., Rabearisoa, A., Zhao, P., Jiang, X., 2015. Comparing humic substance and
586 protein compound effects on the bioaccumulation of perfluoroalkyl substances by *Daphnia*
587 *magna* in water. *Chemosphere* 119, 978-986.

588 Xia, X., Dai, Z., Zhang, J., 2011. Sorption of phthalate acid esters on black carbon from different
589 sources. *J. Environ. Monitor.* 13(10), 2858-2864.

590 Xia, X., Li, G., Yang, Z., Chen, Y., Huang, G., 2009. Effects of fulvic acid concentration and
591 origin on photodegradation of polycyclic aromatic hydrocarbons in aqueous solution:
592 Importance of active oxygen. *Environ. Pollution*. 157(4), 1352-1359.

593 Xia, X., Li, H., Yang, Z., Zhang, X., Wang, H., 2015. How does predation affect the
594 bioaccumulation of hydrophobic organic compounds in aquatic organisms? *Environ. Sci.*
595 *Technol.* 49(8), 4911-4920.

596 Xia, X., Wang, Ran., 2008. Effect of sediment particle size on PAH biodegradation: importance
 597 of the sediment-water interface. *Environ. Toxicol. Chem.*, 27(1), 119-125.

598 Xia, X., Xia, N., Lai, Y., Dong, J., Zhao, P., Zhu, B., Li, Z., Ye, W., Yuan, Y., Huang, J., 2015.
 599 Response of PAH-degrading genes to PAH bioavailability in the overlying water, suspended
 600 sediment, and deposited sediment of the Yangtze River. *Chemosphere* 128, 236-244.

601 Yu, X., Ying, G. and Kookana, R., 2006. Sorption and desorption behaviors of diuron in soils
 602 amended with charcoal. *J. Agric. Food Chem.* 54(22), 8545-8550.

603 Zhai, Y., Xia, X., Zhao, X., Dong, H., Zhu, B., Xia, N. and Dong, J., 2016. Role of ingestion
 604 route in the perfluoroalkyl substance bioaccumulation by *Chironomus plumosus* larvae in
 605 sediments amended with carbonaceous materials. *J. Hazard. Mater.* 302, 404-414.

606 Zhang, X., Xia, X., Dong, J., Bao, Y., Li, H., 2014. Enhancement of Toxic Effects of
 607 Phenanthrene to *Daphnia Magna* due to the Presence of Suspended Sediment. *Chemosphere*
 608 104(4), 162-169.

609 Zhang, X., Xia, X., Li, H., Zhu, B., Dong, J., 2015. Bioavailability of Pyrene Associated with
 610 Suspended Sediment of Different Grain Sizes to *Daphnia magna* as Investigated by Passive
 611 Dosing Devices. *Environ. Sci. Technol.* 49(16), 10127-10135.

612 Zhang, Y., Pignatello, J., Tao, S., Xing, B., 2015. Bioaccessibility of PAHs in Fuel Soot Assessed
 613 by an in Vitro Digestive Model: Effect of Including an Absorptive Sink. *Environ. Sci. Technol.*
 614 49(6), 3905-3912.

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Table 1 Physio-chemical properties of the suspended sediment samples
(Mean $\pm$ standard deviation, n=3)

		Yangtze river		Yellow river	
		0-50 μm	50-100 μm	0-50 μm	50-100 μm
SPS containing AOC+BC+minerals	AOC(mg g ⁻¹)	6.59 $\pm$ 0.07	5.19 $\pm$ 0.05	0.87 $\pm$ 0.05	0.95 $\pm$ 0.06
	BC (mg g ⁻¹)	3.78 $\pm$ 0.04	3.11 $\pm$ 0.06	1.87 $\pm$ 0.05	1.02 $\pm$ 0.03
	TOC(mg g ⁻¹)	10.4 $\pm$ 0.11	8.30 $\pm$ 0.08	2.74 $\pm$ 0.07	1.97 $\pm$ 0.08
SPS containing BC+minerals	AOC(mg g ⁻¹)	0	0	0	0
	BC (mg g ⁻¹)	3.78 $\pm$ 0.04	3.11 $\pm$ 0.06	1.87 $\pm$ 0.05	1.02 $\pm$ 0.03
	TOC(mg g ⁻¹)	10.4 $\pm$ 0.11	8.30 $\pm$ 0.08	2.74 $\pm$ 0.07	1.97 $\pm$ 0.08
SPS containing minerals	TOC(mg g ⁻¹)	0	0	0	0

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Table 2 Bioavailability of pyrene sorbed on various components of SPS to *D. magna* after exposure for 48 h ((Mean $\pm$ standard deviation, n=3)

	Yangtze River		Yellow River	
	0-50 μm	50-100 μm	0-50 μm	50-100 μm
Total immobilization caused by total pyrene in water-original SPS (AOC+BC+minerals) system (%)	48.9 $\pm$ 1.92	64.4 $\pm$ 2.82	35.6 $\pm$ 1.69	46.7 $\pm$ 3.02
Immobilization caused by pyrene sorbed on the original SPS (%)	30.9 $\pm$ 1.77	46.4 $\pm$ 1.08	17.6 $\pm$ 2.12	28.7 $\pm$ 2.46
Contribution of pyrene sorbed on the original SPS to the total immobilization (%)	63.2 $\pm$ 3.62	72.1 $\pm$ 1.68	49.4 $\pm$ 5.96	61.4 $\pm$ 5.27
Bioavailable fraction of pyrene sorbed on the original SPS (%)	19.9 $\pm$ 3.69	45.2 $\pm$ 4.67	17.0 $\pm$ 3.09	33.2 $\pm$ 2.09
Immobilization caused by pyrene sorbed on AOC (%)	15.6 $\pm$ 1.02	27.8 $\pm$ 1.68	8.89 $\pm$ 1.58	17.2 $\pm$ 1.82
Contribution of pyrene sorbed on AOC to the total immobilization (%)	31.8 $\pm$ 2.09	43.1 $\pm$ 2.61	25.0 $\pm$ 4.44	36.9 $\pm$ 3.90
Bioavailable fraction of pyrene sorbed on AOC (%)	32.5 $\pm$ 2.57	67.3 $\pm$ 1.69	22.4 $\pm$ 4.70	49.6 $\pm$ 3.88
Immobilization caused by pyrene sorbed on BC (%)	8.89 $\pm$ 1.02	5.56 $\pm$ 0.36	4.44 $\pm$ 0.42	2.78 $\pm$ 1.01
Contribution of pyrene sorbed on BC to the total immobilization (%)	18.2 $\pm$ 2.09	8.62 $\pm$ 0.56	12.5 $\pm$ 1.18	5.95 $\pm$ 2.16
Bioavailable fraction of pyrene sorbed on BC (%)	11.8 $\pm$ 3.67	16.8 $\pm$ 4.22	10.2 $\pm$ 4.70	9.11 $\pm$ 2.79
Immobilization caused by pyrene sorbed on minerals (%)	6.44 $\pm$ 0.86	13.1 $\pm$ 1.03	4.22 $\pm$ 0.42	8.67 $\pm$ 0.08
Contribution of pyrene sorbed on minerals to the total immobilization (%)	13.2 $\pm$ 1.76	20.3 $\pm$ 1.60	11.9 $\pm$ 1.18	18.6 $\pm$ 0.17
Bioavailable fraction of pyrene sorbed on minerals (%)	20.1 $\pm$ 1.12	46.0 $\pm$ 2.98	21.0 $\pm$ 1.60	41.3 $\pm$ 1.78

622 Table 3 Pyrene concentration sorbed on various components of SPS and
623 its distribution coefficients between each SPS component and water

	Yangtze river		Yellow river	
	0-50 μm	50-100 μm	0-50 μm	50-100 μm
$C_{\text{SPS-AOC}} (\mu\text{g g}^{-1})$	5.23 ± 0.21	4.50 ± 0.15	4.34 ± 0.09	3.79 ± 0.13
$C_{\text{SPS-BC}} (\mu\text{g g}^{-1})$	8.25 ± 0.48	3.61 ± 0.52	4.77 ± 0.27	3.33 ± 0.38
$C_{\text{SPS-mineral}} (\mu\text{g g}^{-1})$	3.49 ± 0.14	3.11 ± 0.10	2.20 ± 0.18	2.29 ± 0.24
$C_{\text{SPS}} (\mu\text{g g}^{-1})$	17.0 ± 1.75	11.2 ± 0.42	11.3 ± 1.55	9.41 ± 1.11
$\log K_{\text{AOC}} (\text{L Kg}^{-1})$	4.54	4.58	5.34	5.35
$\log K_{\text{BC}} (\text{L Kg}^{-1})$	4.97	4.70	5.04	5.19
$\log K_{\text{mineral}} (\text{L Kg}^{-1})$	2.18	2.13	1.98	1.63

624 K_{AOC} means AOC-water distribution coefficient values; K_{BC} means BC-water distribution coefficient
625 values; K_{mineral} means mineral-water distribution coefficient values.
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Figure captions

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628 Figure 1 Effect of pyrene sorbed on SPS with various compositions on the immobilization of
629 *Daphnia magna* during a 48-h exposure. Data are mean $\pm$ standard deviation (n=3)

630 Figure 2 Immobilization of *Daphnia magna* caused by pyrene sorbed on SPS with various
631 compositions after exposure for 48 h. Data are mean $\pm$ standard deviation (n=3).

632 Figure 3 Total protein content of *Daphnia magna* in the absence or presence of 1 g L⁻¹ SPS with
633 various compositions after exposure for 48 h. Data are mean $\pm$ standard deviation (n=3).

634 Figure 4 Effect of SPS composition and grain size on T-SOD activities of *Daphnia magna* after
635 exposure for 48 h. Data are mean $\pm$ standard deviation (n=3).

636 Figure 1 Effect of pyrene sorbed on SPS with various compositions on the immobilization of
 637 *Daphnia magna* during a 48-h exposure. Data are mean $\pm$ standard deviation (n=3).

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Figure 2 Immobilization of *Daphnia magna* caused by pyrene sorbed on SPS with various compositions after exposure for 48 h. Data are mean $\pm$ standard deviation (n=3).

Figure 3 Total protein content of *Daphnia magna* in the absence or presence of 1 g L⁻¹ SPS with various compositions after exposure for 48 h. Data are mean ± standard deviation (n=3).

Figure 4 Effect of SPS composition and grain size on T-SOD activities of *Daphnia magna* after exposure for 48 h. Data are mean $\pm$ standard deviation (n=3).