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Toxicity of sediment-associated unresolved complex mixture and its impact on bioavailability of polycyclic aromatic hydrocarbons

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ABSTRACT

Unresolved complex mixtures (UCMs) and polycyclic aromatic hydrocarbons (PAHs) are ubiquitous in sediment originating from oil leaks, shipping, and other human activities and thus it is necessary to understand the role of UCM on sediment toxicity and PAH bioaccumulation. In the current study, lethal and sublethal effects of sediment-associated UCM were examined in two benthic invertebrates (*Chironomus dilutus* and *Lumbriculus variegatus*) using two spiked sediments. Results showed that UCM alone was toxic to the organisms and its toxicity was species-dependent. Approximately 1% of UCM in sediment caused 50% mortality in *C. dilutus*, which indicated UCM at environmentally relevant concentrations can directly cause sub-lethal and lethal effects to benthic invertebrates. Moreover, bioaccumulation testing of sediment-associated PAHs to *L. variegatus* showed that the addition of UCM to sediment at low concentration (0.01%) increased PAH bioavailability. These findings were further confirmed by assessing bioavailability using Tenax extraction. In contrast, high concentrations of UCM in sediment (0.5%) may have formed non-aqueous phase liquids, which served as an alternative sorption phase for PAHs and reduced PAH bioavailability. Understanding the role of UCM in the overall oil toxicity and its impact on other contaminants would improve risk assessment of sediments impacted by petroleum products in the future.

1. Introduction

The recent tragedy in the Gulf of Mexico has brought attention to the environmental fate of oil (petroleum hydrocarbons) and its impacts on aquatic systems [1]. Most studies on crude oil spills have focused on polycyclic aromatic hydrocarbons (PAHs), and have shown that these contaminants caused detrimental effects on various aquatic organisms [2–6]. Conversely, other constituents in the oil matrix, which were commonly referred to as an unresolved complex mixture (UCM) were less studied [7–10]. The UCM was composed of weathered oils, which originated from natural and/or anthropogenic sources, and was first recognized as the "hump" in the quantification of petroleum-contaminated sediment samples using gas chromatography (GC) [11]. Oil matrices typically have complex compositions and UCM is comprised of thousands of hydrocarbons, which usually remain "unresolved" [11–13].

Although the UCM or "humps" of petroleum hydrocarbons were commonly encountered during GC analysis, little consideration has been given to their toxicological effects [14,15]. Recent studies, however, have showed that the UCM constituents in sediment not only accumulated in benthic organisms, but also caused sub-lethal effects to these organisms [7–11,15]. For example, UCM contributed to the overall toxic effects of oils to the marine amphipod *Corophium volutator* and its contribution was separate from the PAHs [7].

In addition to its own bioaccumulation potential and toxicity, UCM may also alter the bioavailability and toxicity of other contaminants in sediment [16–20]. Mehler et al. [16] noted possible changes in sorptive ability of activated carbon to PAHs during the course of conducting a whole-sediment toxicity identification evaluation test, and speculated that the ineffectiveness of the carbon treatment was caused by the co-existence of sediment-associated UCM, with similar reports found by the U.S. Environmental Protection Agency (USEPA) [17]. Moreover, UCM was also reported to be persistent in sediment for decades [21] and may act as a sedimentary super-sorbent for PAHs and polychlorinated biphenyls [18–20].

In the current study, lethal (*e.g.* mortality) and sub-lethal (*e.g.* growth) effects of sediment-associated UCM to two benthic invertebrates (*Chironomus dilutus* and *Lumbriculus variegatus*) were examined. Additionally, the impact of UCM on the bioavailability of PAHs in sediment was also evaluated using *L. variegatus* bioaccumulation testing and Tenax extraction. By evaluating the

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UCM contribution to the overall oil toxicity and its impact on PAH bioavailability, a better understanding of the role of UCM for oil-based sediment contaminants can be derived, and in turn more accurate risk assessments of oil-contaminated sediments can be conducted in the future.

2. Experimental

2.1. Chemicals and organisms

Because mineral oil (Acros Organics, Geel, Belgium) contained a variety of alkane hydrocarbons that were commonly found in sediment but not PAHs, it was selected as a simplified type of surrogate for UCM, although the UCM may contain more compounds than those present in the mineral oil. In addition, mineral oil was used by the USEPA as the UCM surrogate to evaluate the impact of UCM on toxicity identification evaluation procedures [17]. Three PAHs including phenanthrene (Phe), fluoranthene (Flu), and benzo[a]pyrene (BaP), which were purchased from SpexCertiprep Incorporated (Metu, NJ, USA) were selected as the representative PAHs and their physicochemical properties were listed in Table 1. None of the three PAHs were detected in mineral oil. The internal standard, d_{14}-*p*-terphenyl (Dr. Ehrenstorfer, Germany) was added to the extracts before GC-mass spectrometry (MS) quantification, while Phe-d_{10} and chrysene-d_{12} (Thermo Fisher Scientific, Waltham, MA, USA) were used as the surrogates and added to all samples before extraction.

Hexane (HPLC grade) was purchased from Burdick and Jackson (Ulsan, Korea). Dichloromethane and acetone (analytical grade) were purchased from Chemical Reagent Factory (Tianjin, China) and redistilled before use. Silica gel (80–200 mesh) and neutral alumina (80–200 mesh) were activated at 180 and 250 °C for 6 h, respectively. Anhydrous Na_2SO_4 was used to remove residual water from the extracts and was baked at 450 °C for 4 h before use. Tenax TA sorbents (60–80 mesh) were purchased from Scientific Instrument Service Incorporated (Ringoes, NJ, USA). Reconstituted water was prepared by dissolving various salts to de-ionized water and then aerated overnight prior to use [22].

Two benthic invertebrates, *C. dilutus* and *L. variegatus*, were chosen as the test organisms for the bioassays. Organisms were raised at the Guangzhou Institute of Geochemistry, Chinese Academy of Sciences (GIGCAS) in accordance with the USEPA protocols [22], and 3rd instar midge larvae were selected for the bioassay with instar determined using length and head capsule estimates [22].

2.2. Sediment preparation

Two sediments were used in the current study. One was collected from a drinking water reservoir in Conghua, China (CH) and the other was from a lake in Guangzhou, China (GZ). The sediments were sieved through a 2 mm sieve to remove large debris on site and transported back to the GIGCAS, where they were homogenized and stored at 4 °C. Preliminary studies showed CH sediment had limited contamination and did not exhibit toxicity to benthic organisms, thus it was used as the control sediment. On the other hand, GZ sediment, showed no lethality to *C. dilutus*, however trace PAHs and pyrethroid insecticides were detected. Total organic carbon (TOC) content of the sediments was analyzed using an Elementar Vario ELIII (Hanau, Germany) after removing inorganic carbon by 1 mol/L HCl, and TOC content was $2.75 \pm 0.07\%$ and $0.714 \pm 0.005\%$ for CH and GZ sediments, respectively.

Only mineral oil was spiked into sediment used in acute toxicity testing, whereas both PAHs and mineral oil were added to sediment for bioaccumulation testing. The three PAHs, using acetone as a carrier, were spiked into the sediment and concentrations of PAHs were similar among treatments. Sediment was mixed for 4 h using a drill with a rotating stainless-steel blade, and then mineral oil was spiked into the sediment. The spiked sediment was then thoroughly mixed for an additional 2 h before being stored at 4 °C in the dark for 14 d. Sediment samples were homogenized again prior to use in the bioassays.

2.3. Acute toxicity of UCM to C. dilutus and L. variegatus

To evaluate acute toxicity of sediment-associated UCM alone to *C. dilutus* and *L. variegatus*, both CH and GZ sediments were spiked with mineral oil at nominal concentrations ranging from 0.1 to 5% dry weight (dw), which was equivalent to 1–50 mg/g dw. The UCM was spiked to CH sediment at concentrations of 0.1, 1, 2 and 5%, and 0.1, 0.5, 2, 2.5 and 5% for *C. dilutus* and *L. variegatus* bioassays, respectively. The spiking levels were 0.1, 0.5, 1 and 2.5% and 0.1, 0.5, 2, 2.5 and 5% for *C. dilutus* and *L. variegatus* bioassays in GZ sediment, respectively. The testing was conducted using five replicates with 60 g of wet sediment and 250 ml overlying reconstituted water being used for each replicate. Separate bioassays were performed for each organism, and ten 3rd instar midges or 15 worms were placed in each 500 ml beaker after sediment was settled overnight. The bioassays were terminated at 10 d for *C. dilutus* following the USEPA protocols [22], whereas 14 d tests were conducted for *L. variegatus*, and this length of testing was chosen because it was commonly used for bioaccumulation tests. The testing was conducted at 23 ± 1 °C with a 16:8 light:dark photo-period and pH, conductivity, and dissolved oxygen were monitored daily. The overlying water was renewed twice daily with 100 ml reconstituted water each time. Midge larvae were fed once every day with 1 ml of 6 g/L ground fish food, while no feeding was included for *L. variegatus*.

Lethality and growth impairment were chosen as the toxicity endpoints. At the end of the bioassays, the organisms were sieved from the sediment and mortality was assessed. Additionally, growth of the midges and worms was determined by ash free dry mass (AFDM) measurements and followed the method outlined by Maul et al. [23]. Briefly, the surviving organisms were placed in pre-weighed aluminum pans and dried at 60 °C for 3 d to obtain mean mass per surviving organism per replicate. Next, the organisms and pans were heated at 550 °C for 3 h and reweighed on a Sartorius AgPro 11 microbalance (Gottingen, Germany) to calculate the AFDM.

2.4. Impact of UCM on bioaccumulation of PAHs in sediment

Because GZ sediment showed sublethal toxicity to *C. dilutus*, only CH sediment was used in the bioaccumulation testing. The CH sediment was spiked with 1 μg/g dw of each PAH and various levels of mineral oil (0, 0.01, 0.1 and 0.5% dw) in order to investigate the impact of UCM on bioaccumulation of sediment-associated PAHs to *L. variegatus*. The three PAHs were spiked as a mixture for these tests and the concentrations of PAHs and mineral oil were selected because they were environmental relevant [16,20]. The spiked sediments were aged at 4 °C for 14 d before conducting the bioaccumulation testing in triplicate following USEPA protocols [22]. In short, each 500 ml beaker contained 60 g wet sediment and 250 ml overlying water. After sediment settled overnight, 20 worms were placed into each beaker after recording their wet weights at the beginning of testing. Tests were conducted at 23 ± 1 °C and a 16:8 light:dark photo-period with overlying water being renewed twice a day. Worms were not fed during testing (*e.g.* sediment served as their food source). Tests were terminated by sieving organisms from the sediment at 7 and 14 d, and the organisms were rinsed with reconstituted water and transferred into a beaker containing 200 ml of reconstituted water. After a 3 h gut depuration

Table 1
Physico-chemical properties of the selected polycyclic aromatic hydrocarbons (PAHs) including phenanthrene (Phe), fluoranthene (Flu), and benzo[a]pyrene (BaP).[a]

PAH	Rings	CAS No.	Molecular formula	Molecular weight	Solubility (μg/L) @ 25 °C	Log K_{ow}	Half-life in sediment (d)
Phe	3	85-01-8	$C_{14}H_{10}$	178.2	1600	4.57	29
Flu	4	206-44-0	$C_{16}H_{10}$	202.3	265	5.16	na[b]
BaP	5	50-32-8	$C_{20}H_{12}$	252.3	110	6.2	>1448

[a] Data were from Chemspider (http://www.chemspider.com/Search.aspx).
[b] na: not available.

period, worms were removed from the water, dried, weighed using a Sartorius AgPro 11 microbalance, and frozen. A worm from each replicate was used for lipid analysis using a spectrophotometric method after acid digestion following the method by van Handel [24], and the remaining organisms were used for analyzing PAHs using GC-MS following the analytical methods detailed in Section 2.6.

2.5. Impact of UCM on Tenax extractable PAHs in sediment

In addition to bioaccumulation testing, bioavailability of PAHs was also evaluated in triplicate using a single point 24 h Tenax extraction [25]. Approximately 4 g of wet sediment was mixed with 0.5 g of Tenax, 0.1 g of activated copper, 5 mg of NaN_3, and 45 ml of reconstituted water in a 50 ml centrifuge tube. The tubes were rotated at 20 rpm for 24 h using a QB-228 Rolling Incubator (Kylin-Bell Lab Instruments Company, Haimen, China). Upon completion, Tenax beads were separated from the sediment and sonicated sequentially with 5 ml of acetone for 5 min, and 5 ml of a mixture of acetone:hexane (1:1, v/v) twice for 10 min each time. All extracts were combined, surrogates were added, and the extract concentrated and solvent exchanged to 1 ml of hexane. The Tenax extracts were further cleaned with an alumina/silica gel column and analyzed on GC-MS for the PAHs as described below.

2.6. Chemical analysis

Sediment samples were extracted using a CW-2000 ultrasound-assisted microwave extractor (UAME) (Xintuo Company, Shanghai, China) [26]. Briefly, about 3 g of freeze-dried and ground sediment was extracted with 100 ml of a hexane and acetone (1:1, v/v) solution for 360 s using UAME after adding the surrogates. Activated copper powder was added to the sediment to remove sulfur, and ultrasound and microwave power on the UAME was set at 50 and 100 W, respectively. The extraction was repeated with an additional 50 ml of extraction solution, and the extracts were combined, filtered, evaporated, and solvent exchanged to approximately 1 ml of hexane with a XT-NS-1 Turbovap (Xintuo). Columns with an internal diameter of 1 cm were packed with 12 cm of silica gel, 6 cm of alumina, and 1 cm of anhydrous Na_2SO_4 from the bottom to the top and used to clean the extracts, and 70 ml of a 30% dichloromethane in hexane solution was used as the eluting solvent. The cleaned extracts were concentrated to near dryness and solvent exchanged to 1 ml of hexane, and analyzed on GC-MS after adding 50 ng of the internal standard d_{14}-*p*-terphenyl.

The thawed worms were extracted with 20 ml of acetone in five cycles of 10 s each at 600 W using an ultrasonic processor (Scientz Biotechnology, Ningbo, China) after adding 50 ng of each surrogate. The tissue extracts were then filtered and solvent-exchanged to dichloromethane for further cleanup using gel permeation chromatography (LabTech, Beijing, China). Separation was conducted with a 20 mm × 300 mm column packed with SX-3 Bio-beads with dichloromethane as the mobile phase at a flow rate of 5 ml/min. The collected fractions containing PAHs were concentrated to near dryness and solvent-exchanged to hexane prior to GC-MS analysis.

The three PAHs were analyzed in the cleaned extracts using an Agilent 7890-5975 GC-MS in selected ion monitoring mode (SIM) after separation with a HP-5 MS column (30 m × 0.25 mm i.d. × 0.25 μm film thicknesses). Helium was used as the carrier gas at a flow rate of 1.2 ml/min. The injection was conducted in splitless mode at 280 °C with an injection volume of 1 μl. Temperature of the transfer line, ion source, and quadrupole was set at 260, 230 and 150 °C, respectively. Column temperature was ramped from 60 °C to 200 °C at 10 °C/min, then to 214 °C at 2 °C/min, to 255 °C at 5 °C/min, held for 2 min, and finally to 290 °C at 20 °C/min, and held for 12 min. Identification of analytes was based on detecting the target and qualifier ions within a retention time window of 1%, and the target ions for Phe, Flu, and BaP were with *m*/*z* of 178, 202, and 252, respectively. Five-point internal standard (d_{14}-*p*-terphenyl) calibration curves were used to quantify the analytes.

A calibration standard was analyzed after every 10 samples on GC-MS and the differences between the calibration curve and the daily calibrations were within 20% for all analytes. A method blank (solvent), matrix blank (control sediment), matrix spike, and matrix spike duplicate were included for every 20 samples. No target PAHs were detected in the blanks. Additionally, two surrogates (Phe-d_{10} and chrysene-d_{12}) were added to all samples before extraction to evaluate the efficiency of the sample preparation process. In the bioaccumulation experiments, average recoveries of Phe-d_{10} and chrysene-d_{12} from tissue samples were 71.7 ± 16.7% and 69.5 ± 15.2%, respectively, while average recoveries were 87.2 ± 21.1% and 97.1 ± 20.8% for the two surrogates from sediments, respectively. Acceptable recoveries of Phe-d_{10} and chrysene-d_{12} were also achieved in the Tenax extraction experiments, with recoveries of 85.9 ± 4.9% and 120 ± 3.4% for sediment samples, and 68.4 ± 15.9% and 106 ± 6.6% from Tenax samples, respectively.

2.7. Data analysis and statistical comparison

Bioaccumulation potential of sediment-associated PAHs to *L. variegatus* was assessed using biota-sediment accumulation factors (BSAF). Assuming equilibrium was reached among sediment OC, porewater, and organism lipids, the BSAF was calculated using Eq. (1).

$$\mathrm{BSAF} = \frac{C_b(\mathrm{lipid-normalized})}{C_s(\mathrm{TOC-normalized})} \quad (1)$$

where, C_b was the lipid-normalized PAH concentration in the worm, while C_s was the TOC-normalized PAH concentration in the sediment. Because sediment concentrations were not significantly different before and after the bioassays, their average concentrations were used for calculations.

Previous studies [25] have shown that Tenax extractable concentration at a single time point well represented bioavailability of hydrophobic organic contaminants in sediment. The fraction of chemical in sediment extracted by Tenax within 24 h ($F_{24\,h}$) was calculated by dividing the mass of chemical extracted by Tenax adsorbent by the total mass of chemical in sediment.

Toxicity was evaluated by probit analysis, whereas significant differences among treatments were determined using a Turkey's

Honestly Significant Difference method ($\alpha = 0.05$) (SAS version 9.1; SAS [Cary, NC, USA]).

3. Results and discussion

3.1. UCM toxicity to C. dilutus and L. variegatus

Mineral oil, which was a mixture of a variety of petroleum hydrocarbons, was used in the current study to represent UCM. Toxicity of the mineral oil-spiked sediment was evaluated using two benthic organisms, *C. dilutus* and *L. variegatus* (Fig. 1). In all bioassays, dissolved oxygen (5.9 ± 0.2 mg/L), pH (7.49 ± 0.23), temperature (22.9 ± 0.6 °C), conductivity (314 ± 18 μs/cm), and ammonia (0.50 ± 0.14 mg/L) of the overlying water were monitored, and all water parameters were within the acceptable range required by the USEPA [22].

As shown in Fig. 1a, less than 10% mortality was observed for midges exposed to un-spiked CH and GZ sediments with survival of $94 \pm 6.5\%$ and $96 \pm 5.4\%$, respectively. However, toxicity of UCM alone to midges was evident, with approximately 1% of oil in dry sediment causing 50% mortality to *C. dilutus*. The median lethal concentrations (LC50) were similar for oil in CH and GZ sediments and were 0.92% (0.57–1.4%) and 1.54% (not available), respectively. These values were 3–5-fold higher than the literature LC50 values of sediment-associated gasoline oil to another midge species, *Chironomus riparius* (3200 mg/kg, 0.32% dw) [9]. While the difference in test species and oil type may partially explain the difference in toxicity, the use of nominal oil concentrations versus measured values may also be a factor. Concentrations of the oil in the sediment were measured in the previous study [9], whereas the LC50 values in the current study were estimated from the nominal concentrations of mineral oil. Without quantification of the degradation of the oil during the 14 d aging period, overestimation of the LC50 measurement may have occurred. However, the degradation of oil in 2 weeks may have been limited due to the persistence of UCM in the sediment [21]. In addition, possible loss of oil due to water renewal was not quantified in the current study, and this may have introduced some potential error to the LC50 estimation.

In addition to lethality, growth impairment was also measured by using AFDM for both species. As shown in Fig. 1a, AFDW per organism was less for the midges in un-spiked GZ sediment (0.15 ± 0.09 mg) than those in CH sediment (0.34 ± 0.08 mg), which suggested that sublethal effects were occurring in GZ sediment to *C. dilutus*, even without addition of oil. A previous study [27] detected trace amount of cypermethrin in this GZ sediment, and toxicity of this sediment was enhanced by adding a synergistic agent for pyrethroid insecticides, piperonyl butoxide [28]. Hence, growth impairment of GZ sediment to midges in the current study may be the result of trace levels of cypermethrin in that sediment. Regardless of the sublethal toxicity noted in the GZ sediment, the addition of 0.1% mineral oil significantly reduced growth of midges in both sediments (Fig. 1a).

Hydrophobic organic contaminants tend to bind to sediment OC and their toxicity was negatively related to the TOC [29]. Surprisingly, UCM toxicity to *C. dilutus* was similar for CH and GZ sediments, although the TOC content of the CH sediment was four times greater than the GZ sediment. High concentrations of oil may have overwhelmed the sediment OC binding sites and/or UCM may exist in sediment as a separate phase in the form of non-aqueous phase liquids (NAPLs) [30], which is possibly why sediment OC was not a contributing factor in predicting toxicity. Jonker et al. [18] reported that sediment-associated oil tended to form a significant amount of NAPLs when the oil concentration was over a critical separate phase concentration (CSPC) and CSPC was OC-dependent with values being approximately 15% of sedimentary OC. With TOC contents of 2.75 and 0.714%, the CSPC was 0.4 and 0.1% for CH and GZ sediments, respectively. The CSPCs were lower than the LC50s for the oil in both sediments. This suggested that UCM may not only play a role as a narcotic toxicant, but also may have directly induced physical toxicity to midges by the formation of NAPLs, which would have reduced midge cuticle permeability, and caused a smothering effect in the midges [9].

Mehler et al. [16] detected 0.15–1.2% UCM-like contaminants (*e.g.* measured as total organic matter) in sediments collected from the Illinois River, USA and the UCM content in three-fourths of that batch of sediments was greater than 0.6%. Therefore, UCM in the majority of the Illinois River sediments may have directly caused sublethal and perhaps lethal effects in *C. dilutus*. There is no reason to believe these findings are unique to the Illinois River though, as there has been very little monitoring for these compounds elsewhere.

However, toxicity caused by sediment-associated UCM was species-dependent. Although 0.1% UCM in sediment impaired midge growth, up to 5% UCM showed no growth impairment to *L. variegatus* (Fig. 1b). Additionally, the differences in sub-lethal toxicity between the two species tested could be observed *via* their behavioral activities. Common behavioral mechanisms for *C. dilutus* is the building of burrows, this was not common with the presence of oil, while *L. variegatus* feeding behavior and sediment penetration was not affected. Significant differences in UCM toxicity to the two organisms may be due to these different types of behavioral characteristics or possibly the presence of different physiological structures and metabolic pathways of the organisms [31]. Compared to the midges, *L. variegatus* were generally less sensitive to many toxicants, and had lower biotransformation capacity [31]. Due to their low sensitivity to many toxicants, *L. variegatus* was recommended by the USEPA as the chosen species for sediment bioaccumulation tests [22]. In the current study, bioaccumulation testing was conducted using *L. variegatus* to evaluate the impact of various levels of UCM on bioavailability of PAHs in sediment.

3.2. Impact of UCM on bioavailability of PAHs in sediment

The representative PAHs (Phe, Flu and BaP) were spiked together into CH sediment at a nominal concentration of 36.3 μg/g OC, and the sediment concentration was measured at the beginning of the bioassays (*e.g.* the end of 14 d aging period) and the end of the bioassays (7 and 14 d). No significant difference was observed for the three measurements for any of the compounds, thus the average sediment concentrations were used to calculate BSAFs (Table 2). The measured sediment concentrations ranged from 33 to 38%, 92 to 108%, and 68 to 79% of the spiking concentrations for Phe, Flu, and BaP, respectively. As shown in Table 1, Phe had the shortest half-life in sediment (29 d) and the highest water solubility (1.6 mg/L) among the three PAHs tested, thus losses of Phe by degradation and water renewal were reasonable.

No overt avoidance of the sediment was observed for the worms during bioaccumulation testing, and there were no significant differences in mortality among the treatments and the controls, with percent survival ranging from 90 to 115% (*e.g.* reproduction was noted). No significant differences were noted for lipid content among treatments with the same exposure time. Lipid content slightly decreased with the increase in exposure time although the alteration was not significant. Average lipid content was $2.10 \pm 0.79\%$ and $1.20 \pm 0.32\%$ after 7- and 14-d bioaccumulation tests, respectively. As up to 5% UCM had no toxic effect on the worms in the acute toxicity testing, PAHs may be the main cause of the sublethal effects that were noted in the lipid analysis.

The BSAF values were used to compare the bioaccumulation potential of PAHs to *L. variegatus* exposed to sediments spiked at different UCM concentrations. As shown in Table 2, the BSAF

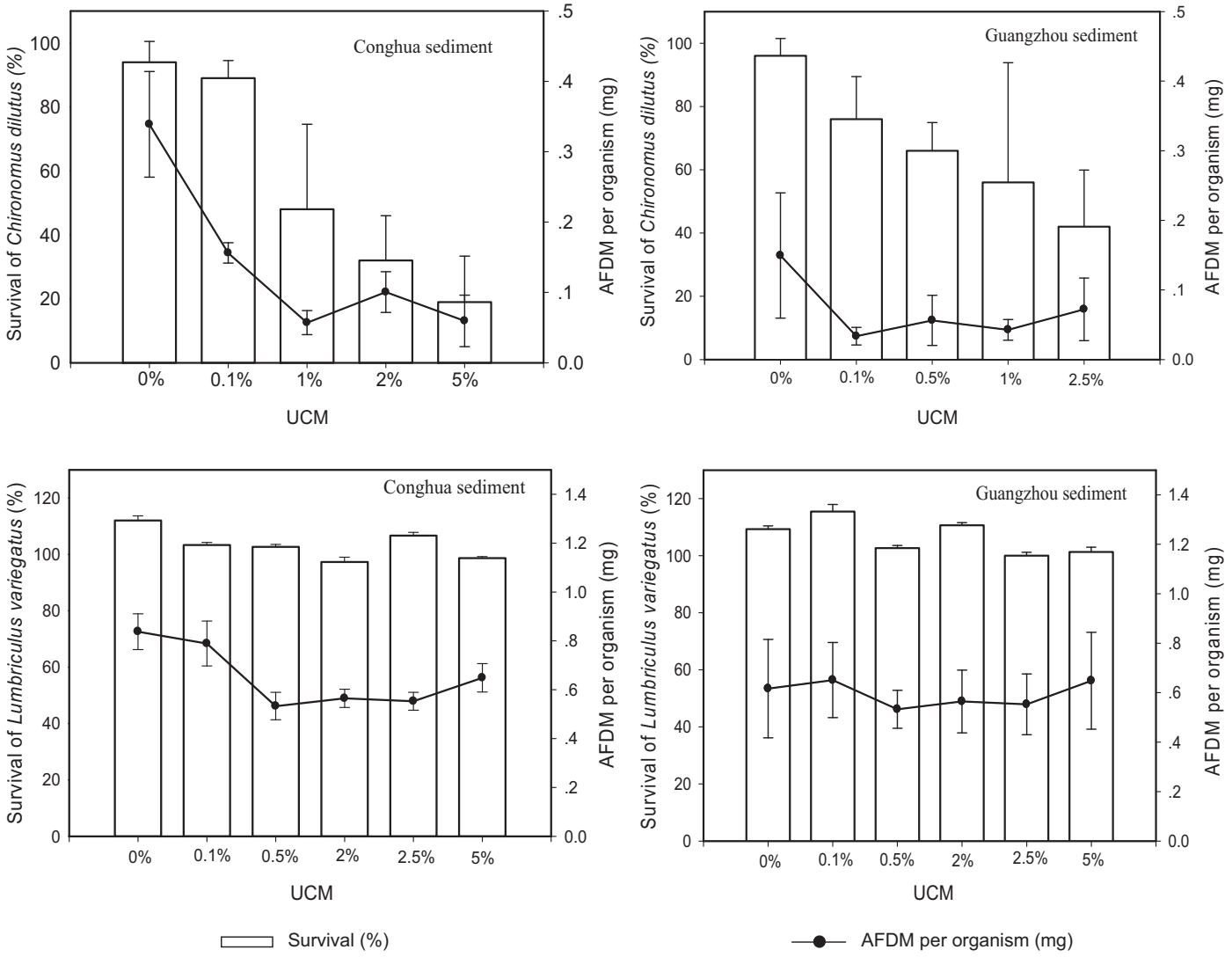

Fig. 1. Survival (%) and ash free dry mass (AFDM) per organism (mg) for *Chironomus dilutus* (a) and *Lumbriculus variegatus* (b) exposed to Conghua and Guangzhou sediments spiked at different levels of unresolved complex mixture (UCM).

Table 2
Evaluating bioavailability of PAHs as influenced by the addition of unresolved complex mixture (UCM) in bioaccumulation testing with *Lumbriculus variegatus*. Sediment concentrations (μg/g organic carbon, OC), organism concentrations (μg/g lipid), and biota-sediment accumulation factors (BSAF) of phenanthrene (Phe), fluoranthene (Flu), and benzo[a]pyrene (BaP) after 7- and 14 d testing. The bioassays were conducted in triplicate and data were presented as mean ± standard deviation. Different letters indicated significant differences among the percent UCM treatments.

UCM in dry sediment (%)	0	0.01	0.1	0.5
Average sediment concentrations (μg/g OC)				
Phe	12.1 ± 2.6[a]	13.3 ± 0.77[a]	13.8 ± 2.7[a]	12.0 ± 4.5[a]
Flu	36.3 ± 5.7[ab]	33.5 ± 2.0[a]	39.2 ± 3.5[b]	38.7 ± 2.0[b]
BaP	24.8 ± 3.8[ab]	24.8 ± 2.3[a]	28.9 ± 3.1[b]	26.3 ± 3.24[ab]
Tissue concentrations after 7 d exposure (μg/g lipid)				
Phe	4.65 ± 1.80[a]	8.29 ± 2.20[a]	5.97 ± 0.52[a]	5.10 ± 1.23[a]
Flu	7.01 ± 0.27[a]	17.7 ± 2.13[b]	9.19 ± 2.33[a]	8.92 ± 5.14[a]
BaP	7.36 ± 0.91[a]	16.8 ± 1.93[b]	9.65 ± 0.99[a]	9.94 ± 4.79[ab]
Tissue concentrations after 14 d exposure (μg/g lipid)				
Phe	6.20 ± 0.89[a]	8.74 ± 0.75[a]	8.42 ± 2.11[a]	8.17 ± 0.87[a]
Flu	12.9 ± 3.81[a]	27.1 ± 4.60[a]	17.2 ± 8.07[a]	16.0 ± 8.38[a]
BaP	14.1 ± 3.54[a]	27.9 ± 3.84[a]	16.2 ± 6.02[a]	17.6 ± 7.75[a]
BSAF after 7 d exposure (g OC /g lipid)				
Phe	0.39 ± 0.17[a]	0.62 ± 0.17[a]	0.43 ± 0.09[a]	0.36 ± 0.19[a]
Flu	0.19 ± 0.03[a]	0.53 ± 0.07[b]	0.23 ± 0.06[a]	0.23 ± 0.13[a]
BaP	0.30 ± 0.06[a]	0.68 ± 0.10[b]	0.33 ± 0.05[a]	0.38 ± 0.19[a]
BSAF after 14 d exposure (g OC /g lipid)				
Phe	0.51 ± 0.13[a]	0.66 ± 0.07[a]	0.61 ± 0.19[a]	0.68 ± 0.26[a]
Flu	0.36 ± 0.12[a]	0.81 ± 0.15[b]	0.44 ± 0.21[ab]	0.41 ± 0.22[ab]
BaP	0.57 ± 0.17[a]	1.13 ± 0.19[b]	0.56 ± 0.22[a]	0.67 ± 0.31[ab]

Table 3
Evaluating bioavailability of PAHs as influenced by the addition of unresolved complex mixture (UCM) using Tenax extraction. The fractions of contaminants (phenanthrene (Phe), fluoranthene (Flu), and benzo[a]pyrene (BaP)) extracted by Tenax absorbent within 24 h ($F_{24\,h}$) are shown below. Tenax extractions were conducted in triplicate and data were presented as mean ± standard deviation. There was a significant difference among treatments of differing UCM levels with p values of 0.185, 0.973 and 0.801 for Phe, Flu and BaP, respectively.

UCM in dry sediment (%)	$F_{24\,h}$		
	Phe	Flu	BaP
0	0.442 ± 0.018	0.632 ± 0.102	0.611 ± 0.141
0.01	0.567 ± 0.031	0.638 ± 0.012	0.668 ± 0.088
0.1	0.513 ± 0.101	0.613 ± 0.147	0.608 ± 0.157
0.5	0.620 ± 0.090	0.654 ± 0.099	0.709 ± 0.141

values were all <1 with the exception of BaP in 0.01% UCM-spiked sediment in the 14 d bioaccumulation test. Di Toro et al. [29] suggested the affinity of hydrophobic contaminants to organism lipids were about twice that of sediment OC, which resulted in BSAF values being approximately two if no biotransformation was involved. Although having weak biotransformation potential [32], recent studies have shown that *L. variegatus* were capable of biotransforming PAHs [33–35], pyrethroids [36], and pentachlorophenol [37]. Therefore, biotransformation of PAHs in organisms may have contributed to their low BSAF values in the current study. Leppanen and Kukkonen [34] suggested that the capability of *L. variegatus* to biotransform PAHs was compound-dependent. In their study [34], *L. variegatus* biotransformed 40% of pyrene, while only 10% of BaP was biotransformed after 14 d. Lyytikainen et al. [35] reported a decrease in PAH body residues with an increase in exposure time for low- and moderate-molecular-weight PAHs, but not for high-molecular-weight PAHs such as BaP. Hence, biotransformation may not play a significant role in the bioaccumulation of BaP in *L. variegatus*. However, BSAF values for Phe and BaP were similar in sediment without addition of UCM in the current study, indicating no preference in biotransformation of the two PAHs in the current test organisms.

On the other hand, BSAF values of Flu and BaP in sediment mixed with 0.01% UCM were significantly higher than those in other treatments (0, 0.1 and 0.5% UCM addition). Similar trends were noted for the two PAHs in both 7- and 14-d tests. Although not statistically significant, the increase in BSAF values after adding 0.01% UCM did occur for the Phe in 7- and 14-d tests. Conversely, addition of 0.1 and 0.5% UCM to sediment showed no impact on bioavailability of the PAHs.

In addition to bioaccumulation testing, bioavailability of sediment-associated PAHs was also estimated using a 24 h Tenax extraction and these results were listed in Table 3. Previous studies showed that Tenax extractable concentrations of sediment-associated HOCs effectively represented bioaccumulation in benthic organisms [25]. Table 3 showed a slight increase in PAH $F_{24\,h}$ values with the addition of UCM to sediment, but the difference in $F_{24\,h}$ for an individual PAH was not significant among treatments.

Overall, both bioaccumulation testing and Tenax extraction demonstrated that the addition of 0.01% UCM to sediment elevated bioavailability of PAHs although results of Tenax extraction were not significant. This is consistent with the results by Jonker et al. [18,19], who reported that sediment-water distribution coefficients (K_d) decreased when oil was added to sediment at relatively low concentrations (300 mg/kg, equaling to 0.03%) [18,19], and that the reduction in K_d suggested more PAHs entered the water phase and were more bioavailable. Competition for sorption sites in sedimentary OC by UCM and PAHs may explain the increase in PAH bioavailability. With oil concentrations lower than their respected CSPC, oil behaved as hydrophobic contaminants and probably occupied sorption sites in the sedimentary OC. As a consequence, sorption of PAHs to the sediment was reduced and BSAFs and $F_{24\,h}$ of PAHs in sediment amended with 0.01% UCM increased.

In addition to changes in sorption of PAHs in sediment, alteration in biological function may have also contributed to the increase in accumulation of PAHs. The uptake, biotransformation, and elimination rates may be altered by exposing the organisms to UCM. Alteration in enzyme activity (metabolic rates) has been widely reported for organisms under stress [32]. However, assessing these factors independently is difficult and beyond the scope of the current study.

Nevertheless, Jonker et al. [18,19] reported that K_d values were dramatically increased when oil concentrations increased and oil was considered as a sedimentary super-sorbent for hydrophobic contaminants. Jonker et al. [18,19] explained the role of the super-sorbent oil as what are commonly referred to as NAPLs. Oil started to form separate phases at high concentrations, which supplied additional sorption pools for PAHs. In the current study, the super-sorptive ability of UCM was not clearly addressed as the greatest oil concentration used in the bioaccumulation test (0.5%) was only slightly higher than the CSPC for this sediment (0.4%). Thus, it is believed that the amount of NAPLs was also low, and had low capability to retain PAHs. It is for this reason that although BSAF values of PAHs in 0.1 and 0.5% UCM-amended sediments were lower than those in 0.01% UCM-added sediment, they were not significantly different from those sediments which did not receive UCM. Further work should be conducted to confirm this hypothesis to better understand the super-sorbent capabilities of the UCM.

4. Conclusions

The UCM, which was composed of weathered oils and originated from natural and/or anthropogenic sources, were ubiquitous in sediment and may play an important role in sediment risk to aquatic organisms. The current study showed that UCM was toxic to sediment-dwelling organisms at environmentally relevant concentrations. Additionally, UCM can alter bioavailability of sediment-associated PAHs, and these changes were concentration-dependent. At relatively low concentrations, UCM increased PAH accumulation by competing for sorption sites in the sediment OC. In contrast, high concentrations of UCM in sediment may have formed NAPLs, which served as an alternative sorption phase for PAHs and reduced PAH bioavailability. Further studies into the issues of persistence, effects on bioavailability of other contaminants, and overall toxicity of UCM alone and in combination with other oil-related contaminants are needed to better assess the risk of sediments contaminated with PAHs.

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