Influence of bioturbation on bioavailability and toxicity of PAHs in sediment from an electronic waste recycling site in South China

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\section*{1. Introduction}

The hydrophobicity and tendency of hydrophobic organic contaminants (HOCs) to bind to sediment organic carbon (OC) result in sediment becoming the sink of these types of compounds. Sediment is also an important secondary contamination source in aquatic ecosystems by releasing the historically accumulated HOCs to water column (Joseffson et al., 2010; Latimer et al., 1999; Yang et al., 2008). The release of HOCs into the water column is caused mainly by two processes including physical disturbance and bioturbation. Physical disturbance can be caused by natural processes (e.g. wave and current actions) or human activities (e.g. dredging and trawling). Bioturbation refers to the disturbance caused by the activities of sediment-dwelling macro-invertebrates, and includes activities such as burrowing, feeding, defecating, and tube-building (Ciarelli et al., 1999). An example of the importance of bioturbation activities is evident in a study by Hedman et al. (2009), where researchers found greater amounts of polychlorinated biphenyl (PCBs) were emitted to water by bioturbation of the amphipod \textit{Monoporeia affinis} than that by physical resuspension.

Bioturbation alters geochemical properties of sediment, such as particle size, sediment porosity, dissolved oxygen, nutrients, and redox potentials (Ko et al., 2003; Pelegri et al., 1994). Additionally, the activities of organisms also affect the fate, transport, and bioavailability of sediment-associated contaminants (Green and Chandler, 1994; Reible et al., 1996; Thibodeaux and Bierman, 2003). Oligochaetes have been frequently used as the model organisms to study bioturbation, as they have been noted as organisms that highly contribute to the bioturbation in freshwater systems (Bosworth and Thibodeaux, 1990; Thibodeaux and Bierman, 2003). Their use as test organisms also benefits from those organisms possessing a high tolerance to a wide range of contaminants, conveyor-belt feeding behaviors, widespread distribution, and high population densities in environment.

Bioturbation by conveyor-belt feeders is achieved by the continuous movement of buried sediment particles to the surface through ingestion and defecation processes, resulting in considerable amounts of contaminants being transported to the sediment–water interface (Green and Chandler, 1994; Karickhoff and Morris, 1985; Koelmans and Jonker, 2011; Reible et al., 1996). For instance, the presence of Tubificid oligochaetes in sediment increased...
HOC concentrations in overlying water 4–6 folds (Karickhoff and Morris, 1985). If more HOCs are released into the water phase, bioturbation may substantially enhance HOC bioavailability (Thibodeaux and Bierman, 2003). Although previous studies (Ciarelli et al., 1999; Friedman et al., 2009; Schuler et al., 2002) have shown that bioturbation significantly changed HOC distribution between sediment and water, limited studies have been conducted to evaluate the effects of bioturbation by freshwater oligochaetes on bioavailability and toxicity of sediment-associated HOCs to other benthic and epibenthic organisms.

The present study consisted of two objectives to evaluate the influence of bioturbation on alteration of bioavailability and toxicity of sediment-associated contaminants. The first objective was to evaluate the effects of bioturbation of the oligochaete Lumbricus variegatus on the bioavailability of polycyclic aromatic hydrocarbons (PAHs) in a field-contaminated sediment. This was achieved by using various densities of L. variegatus to represent different levels of bioturbation. The bioavailability of PAHs was then quantified by the use of body burden evaluations (with bioaccumulation testing) and the use of two biomimetic extraction techniques including matrix-solid phase microextraction (matrix-SPME) and Tenax extraction. The second objective was to determine the potential increase in toxicity to other organisms that reside in the water column. This was accomplished by performing the above mentioned bioassays simultaneously with the epi-benthic amphipod Hyalella azteca.

2. Material and methods

2.1. Chemicals and reagents

A standard solution containing 28 PAH compounds (Table S1 in the Supplementary data) was purchased from Thermo Fisher Scientific (Pittsburgh, PA, USA). Five surrogates including naphthalene-d8, acenaphthene-d10, phenanthrene-d10, chrysene-d12, and perylene-d12 were bought from Acros Organics (Morris Plains, NJ, USA) and Dr. Ehrenstorfer GmbH (Augsburg, Germany). The surrogates were added to sediment and tissue samples prior to extraction to verify the performance of the analytical processes. Meanwhile, three internal standards (2-fluoro-1,1-biphenyl, p-terphenyl-d14, and dibenzo[a.h]anthracene-d14) were used for gas chromatography/mass spectrometry (GC/MS) quantification and were acquired from Dr. Ehrenstorfer GmbH.

Hexane (HPLC-grade) was bought from Burdick and Jackson (Ulsan, South Korea). Analytical grade aceton, methanol, and dichloromethane were obtained from Chemical Reagent Factory (Tianjin, China) and were redistilled before use. The absorbents silica gel and alumina were washed with acetone, dried, and baked at 180 and 250 °C, respectively. Anhydrous Na2SO4 was used to remove the residue waters from the extracts and was baked at 450 °C for 4 h before use. Copper powder which was used to remove sulfur interference in sediment was activated using concentrated HCl and washed with distilled water and acetone sequentially. Sodium azide (NaN3, analytical grade) was used to inhibit microbial growth during Tenax extraction and was purchased from Lianhe Chemical Company (Chengdu, China).

2.2. Sediment and organisms

Field-contaminated sediment was collected from a creek near an electronic waste (e-waste) recycling site in Longtang, Guangdong in South China. The top 5 cm of sediment was collected using a shovel and sieved through a 2-mm sieve to remove rocks and other large debris. Sediment was stored at 4 °C and immediately transported back to the laboratory. The sediment was then homogenized, passed through a 500-µm sieve to remove macrofauna, and stored in the dark at 4 °C prior to use. Another sediment sample collected from a drinking water reservoir in Conghua, Guangdong was used as a control sediment. A previous study (Mehler et al., 2011a) showed this control sediment was non-toxic to benthic organisms and contained limited contamination. Total sediment OC was measured using an Elementar Vario ELIII (Hanau, Germany) after removing the inorganic carbonates with 1 mol/L HCl. The TOC contents were 1.92 ± 0.07 percent and 2.75 ± 0.07 percent for the PAH-contaminated and control sediments, respectively.

The benthic oligochaete L. variegatus and the epi-benthic amphipod H. azteca were used as test organisms in the present study. The organisms were cultured in accordance with US Environmental Protection Agency (USEPA) protocols (2000) at Guangzhou Institute of Geochemistry, Chinese Academy of Sciences (GIGCAS). The oligochaetes are subsurface conveyor-belt feeders, and generally burrow into sediment with their heads buried into the sediment and délicate on the surface as their tails break the sediment–water interface. Their burrowing, feeding, and respiratory activities strongly affect sediment conditions, thus L. variegatus were used as the bioturbators in the present study. The amphipod lives on the sediment surface, and resides for a large portion of its life in the overlying water, and H. azteca was used in the present study to evaluate the toxicity of the contaminants released from the sediment. Before bioassays began, L. variegatus with similar body lengths of approximate 5 cm were isolated from the culture, whereas juvenile H. azteca (14–21 d old) were obtained by passing the organisms through 500 and 1000 µm mesh sieves (Schuler et al., 2002).

2.3. Bioturbation testing

Bioturbation testing was conducted by following the standardized 28 d L. variegatus bioaccumulation test protocols (USEPA, 2000) with few modifications. The experiment design was depicted in Fig. 1. As shown in Fig. 1, four treatments were included to evaluate the effects of bioturbation of L. variegatus on the bioavailability and toxicity of PAHs to both species. No organisms were added to the beakers in treatment A, and this was used as a reference to determine the bioavailability of PAHs without the presence of any organisms. In treatment D, ten H. azteca were exposed to determine the
toxicity of the sediment without the presence of L. variegatus. Treatments B and C consisted of three sub-treatments each, namely B27, B54, and B108 as well as C27, C54, and C108, in which different numbers of L. variegatus (27, 54 or 108 individuals) were added to the sediment, respectively. Ten H. azteca were added to each replicate in treatment C, while there was no H. azteca in treatment B. The numbers of L. variegatus in each replicate (27, 54 and 108 individuals per beaker) were equivalent to 2500, 5000 and 10,000 individuals/m² animal densities, and were selected as these were within the density range observed in biological survey studies in China (Liu, 2007).

In another study, a control treatment was also conducted using the control sediment collected from the drinking water reservoir, with the similar experiment design (Fig. 1) being used. The control tests were used to assess the possible stressors to test organisms other than chemical contamination (e.g. organism density and food scarcity).

The sediment was allowed to settle overnight before the addition of test organisms. Each replicate in each treatment was performed at a 1:8 light:dark cycle. All tests were static with overlying water being renewed once at 14 d with the beakers being continuously aerated throughout the testing. Overlying water quality parameters (temperature, dissolved oxygen, pH, and conductivity) were monitored daily. Ammonia was measured at the beginning and the end of each experiment.

At the conclusion of a bioassay, both species were collected by sieving sediment through a 500 µm sieve. Mortality of H. azteca was assessed and the survived amphipods were collected, while L. variegatus were transferred into clean reconstituted water for 6 h gut-purging process as dictated by the USEPA protocol (2000). After that, the worms were blotted dry and weighed using a Sartorius AG Pro 11 microbalance (Göttingen, Germany). One worm from each replicate was used for lipid analysis using a spectrometry method and after acid digestion (van Handel, 1985), whereas the remaining organisms were frozen at −20°C for body residue analysis.

2.4. Biomimetic extraction

Matrix-SPME was performed simultaneously with the bioassays following previously published procedures (You et al., 2006). The disposable SPME fibers (Fiberguide, Stirling, NJ, USA) which were coated with 10 µm of polydimethyilsiloxane (equivalent to 0.069 µl/cm² phase volume) were placed into the sediments of all treatments to measure the freely dissolved PAHs in sediment pore water. The fragile fibers were protected by envelopes made from copper mesh. Before use, the envelopes with fibers were sonicated with methanol for 10 min in an ultrasonic bath (Hechuang Sonication Instrument, Kunshan, China) and then washed with deionized water. The SPME packages were inserted into sediment before the addition of test organisms. At the end of the bioassay, the fibers were retrieved from the sediment, washed with de-ionized water, dried, extracted with 1 ml of hexane by sonication for 5 min in the ultrasonic bath, and then washed with de-ionized water. The absorbed PAHs were desorbed from the fibers at 214°C for 2 min, heat to 255°C at 5°C/min and held 2 min, then heat to 300°C at 15°C/min, and finally held at 300°C for 12 min. The injector temperature was set at 280°C, and 1 µl of extract was injected in splitless mode. The analytes were ionized in electron impact mode. Selective ion monitoring was used, and the most abundant ion in the scan mode was selected as the target ion for each analyze. Identification of analytes was based on the detection of target ions and qualifier ions within 1 percent of the retention time window, while chemical quantification was based on internal standard calibration. Six calibration standards were used to construct the calibration curves ranging from 20–2000 ng/ml, while concentrations of the internal standards (2-ethynyl-1,1-biphenyl, p-terphenyl-d14, dibenz[a,j]anthracene-d14) were kept constant at 1000 ng/ml.

A calibration standard was analyzed after every 10 samples on GC/MS and the relative difference between the calibration curve and the daily calibrations was within 20 percent for all analytes. A solvent blank, matrix blank, matrix spike, and matrix spike duplicate were included with every two samples as part of the quality control plan. Additionally, five surrogates were added to each sample before extraction to quantify efficiency of the sample preparation procedures. No target PAHs were detected in blanks. The recoveries of the five surrogates (naphthalene-d8, ace-naphthene-d10, phenanthrene-d10, chrysene-d12, and perylene-d12) for sediment samples were 67.2 ± 18.8 or 107 ± 17.5, 93.6 ± 21.6, 87.8 ± 14.4, and 94.3 ± 12.1 percent, respectively, and were 61.6 ± 10.4, 107 ± 12.5, 105 ± 9.3, 93.5 ± 7.4, and 82.1 ± 8.5 percent for tissue samples, respectively. Reporting limits (RL) were used in the present study to define the lowest concentrations that could be accurately quantified, and were calculated by multiplying the lowest concentrations of the calibration standards to the final volume of extracts, and dividing by the sediment or organism masses.

2.7. Data analysis

As shown in Eq. (1), biota-sediment accumulation factor (BSAF) was used to describe the bioaccumulation potential of sediment-associated PAHs to L. variegatus, and was calculated by dividing the lipid-normalized PAH concentration in organism (Corg) by the TOC-normalized PAH concentration in sediment (Csed).

\[
\text{BSAF} = \frac{C_{\text{org}}}{C_{\text{sed}}}
\]

The freely dissolved PAH concentration in sediment pore water (Cspw) was the ratio of PAH concentration in SPME fiber (Cspme) and the partition coefficient between the SPME fiber and the water (Kspme) (Eq. (2)), and Kspme was calculated using the equation logKspme = 0.83 log Knorg + 0.07 by Erical and Robert (2010) and the Knorg values from de Maagd et al. (1998).

\[
C_{\text{spw}} = C_{\text{spme}} / K_{\text{spme}}
\]

Bioavailability of PAHs was also assessed by the fraction of chemical extractable by Tenax absorbents within 24 h (F24h) using the following equation:

\[
F_{24h} = \frac{C_{\text{exc}}}{C_{\text{org}}}
\]

where Cexc and Corg stand for the Tenax extractable sediment concentrations in 24 h and sediment concentrations of PAHs, respectively.

The measured PAH concentrations in all matrices were expressed as the mean ± standard deviation (of the replicates). The comparison among treatments was conducted using one-way analysis of variance (ANOVA, α = 0.05) and Dunnett’s multiple comparison test using SAS 9.1.3 (SAS Institute Inc., Cary, NC, USA).

3. Results and discussion

3.1. Sediment analysis

In the present study 28 PAHs were analyzed in the contaminated sediment samples (Tables 1 and S1). The sum PAH concentrations...
were 472 ± 38.6 ng/g dry weight (dw) in the PAH-contaminated sediment, and no target PAHs were detected above the RLs in the control sediment. The high PAH concentrations detected in the field-contaminated sediment were not surprising. The sediment was collected from a creek in an e-waste recycling site. As one of the largest e-waste recycling sites in South China, Longtang has a history of over 30 years in recycling the wastes by the primitive dismantling activities, such as open burning, plastics peeling and melting, and acid leaching. As a result, metals and HOCs were ubiquitous in the waterways in this area. Previous study showed the toxicity contributions to benthic organisms from metals, PCBs and polybrominated diphenyl ethers were low (Mehler et al., 2011b), thus PAHs were chosen as the target contaminants in the present study.

As shown in Table S1, low-ring PAHs were the most dominant PAHs. The five PAHs having the highest concentrations were 2-methylnaphthalene, naphthalene, fluoranthene, phenanthrene, and 2,6-dimethylnaphthalene. These five compounds contributed to nearly half of the sum PAH concentrations (12.0, 11.0, 10.9, 9.1 and 5.2 percent each, respectively). The sum PAH concentrations and composition profiles in this contaminated sediment from Longtang were consistent with previous studies at the same location (Mehler et al., 2011b) and studies at Guiyu and Taizhou, the two most studied e-waste recycling sites in China (Leung et al., 2006, Ma et al., 2009).

In addition to analyzing PAH concentrations in the sediment before bioassays, sediment was also analyzed for contaminants after the 28 d bioturbation testing for all treatments. As shown in Tables 1 and S1, PAH concentrations before and after the bioturbation in treatment A were similar, indicating that no significant degradation of PAHs. This was also evident in treatments D, B27, and C27 with low organism density. Conversely, PAH concentrations significantly declined in treatments B54, B108, C54, and C108 (Table 1). The losses of PAHs in sediment increased when worm density increased suggesting that the two factors may be related. One possibility was that an increase in PAH uptake occurred due to increasing organism density, another possibility was that release and degradation of PAHs in these sediment were enhanced due to elevated bioturbation activity. Negligible amounts of PAHs (0.04 percent) were accumulated by the worms compared to the total amounts of PAHs in sediment and no significant differences were noted among the treatments. Therefore, the release and degradation of PAHs induced by bioturbation were the main reasons for the decline in PAH concentrations in sediments with high worm density. This suggested that worm densities (2500–10,000 organisms/m²) which are commonly found in the field (Liu, 2007) may play an important role in the fate of HOCs in sediment.

3.2. Influence of bioturbation on PAH bioavailability

3.2.1. Bioaccumulation in L. variegatus

Overlying water quality parameters were monitored throughout the bioassays, and all the parameters met the USEPA guidelines (USEPA, 2000) with dissolved oxygen, pH, conductivity, temperature and ammonia being 4.94 ± 0.27 mg/L, 7.42 ± 0.36, 441 ± 39.9 μS/cm, 22.9 ± 0.91 °C and < 0.4 mg/L, respectively.

No overt avoidance of the sediment by L. variegatus was observed for all the bioassays using both sediments. The average lipid content of L. variegatus was 1.4 ± 0.1 percent and the lipid contents of the organisms in control testing did not show significant differences when compared to those in test treatments with PAH-contaminated sediment (F<sub>3,17</sub> = 2.14, p = 0.36). Therefore, this average lipid content (1.4 ± 0.1 percent) was used in calculating lipid-normalized body residues.

PAH residues in L. variegatus, however, were significantly different among test treatments. As shown in Table 2, the sum PAH concentrations in L. variegatus were 5.79–21.7 μg/g lipid and

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### Table 1

Concentrations of polycyclic aromatic hydrocarbons (PAHs) in sediment collected from an electronic waste site in Longtang, South China before and after 28 d bioturbation tests with different *Lumbricus variegatus* densities. PAH concentrations in sediment (C<sub>n</sub>, ng/g dry weight) are presented as the sum concentration of PAHs by ring count as well as the total of 28 PAHs. Data are represented as mean ± standard deviation (n = 4).

<table>
<thead>
<tr>
<th>Treatment&lt;sup&gt;a&lt;/sup&gt;</th>
<th>C&lt;sub&gt;n&lt;/sub&gt; (ng/g dry weight)</th>
<th>&lt;div style=&quot;text-align: center; width: 100%;&quot;&gt;Two-ring&lt;sup&gt;b&lt;/sup&gt;</th>
<th>Three-ring</th>
<th>Four-ring</th>
<th>Five-ring</th>
<th>Sum of 28 PAHs</th>
</tr>
</thead>
<tbody>
<tr>
<td>Before test</td>
<td>159 ± 12.1&lt;sup&gt;a&lt;/sup&gt;</td>
<td>132 ± 8.5&lt;sup&gt;a&lt;/sup&gt;</td>
<td>115 ± 6.2&lt;sup&gt;a&lt;/sup&gt;</td>
<td>66.7 ± 3.4&lt;sup&gt;a&lt;/sup&gt;</td>
<td>472 ± 38.6&lt;sup&gt;a&lt;/sup&gt;</td>
<td></td>
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<tr>
<td>A</td>
<td>160 ± 7.6&lt;sup&gt;a&lt;/sup&gt;</td>
<td>117 ± 12.9&lt;sup&gt;a&lt;/sup&gt;</td>
<td>116 ± 9.7&lt;sup&gt;a&lt;/sup&gt;</td>
<td>67.3 ± 3.6&lt;sup&gt;a&lt;/sup&gt;</td>
<td>461 ± 33.1&lt;sup&gt;a&lt;/sup&gt;</td>
<td></td>
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<tr>
<td>D</td>
<td>140 ± 9.5&lt;sup&gt;a&lt;/sup&gt;</td>
<td>116 ± 7.1&lt;sup&gt;a&lt;/sup&gt;</td>
<td>98.6 ± 3.5&lt;sup&gt;a&lt;/sup&gt;</td>
<td>59.2 ± 2.7&lt;sup&gt;a&lt;/sup&gt;</td>
<td>414 ± 28.3&lt;sup&gt;a&lt;/sup&gt;</td>
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<tr>
<td>B27</td>
<td>155 ± 11.3&lt;sup&gt;a&lt;/sup&gt;</td>
<td>120 ± 7.5&lt;sup&gt;a&lt;/sup&gt;</td>
<td>117 ± 2.5&lt;sup&gt;a&lt;/sup&gt;</td>
<td>55.6 ± 3.2&lt;sup&gt;a&lt;/sup&gt;</td>
<td>447 ± 25.5&lt;sup&gt;a&lt;/sup&gt;</td>
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<tr>
<td>B54</td>
<td>121 ± 9.5&lt;sup&gt;a&lt;/sup&gt;</td>
<td>85.8 ± 8.0&lt;sup&gt;a&lt;/sup&gt;</td>
<td>84.3 ± 4.5&lt;sup&gt;a&lt;/sup&gt;</td>
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<td>342 ± 16.7&lt;sup&gt;a&lt;/sup&gt;</td>
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<tr>
<td>B108</td>
<td>108 ± 13.9&lt;sup&gt;a&lt;/sup&gt;</td>
<td>78.7 ± 8.7&lt;sup&gt;a&lt;/sup&gt;</td>
<td>74.1 ± 8.0&lt;sup&gt;a&lt;/sup&gt;</td>
<td>38.9 ± 2.8&lt;sup&gt;a&lt;/sup&gt;</td>
<td>300 ± 54.6&lt;sup&gt;a&lt;/sup&gt;</td>
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<tr>
<td>C27</td>
<td>180 ± 20.9&lt;sup&gt;a&lt;/sup&gt;</td>
<td>127 ± 11.4&lt;sup&gt;a&lt;/sup&gt;</td>
<td>132 ± 7.6&lt;sup&gt;a&lt;/sup&gt;</td>
<td>55.0 ± 4.0&lt;sup&gt;a&lt;/sup&gt;</td>
<td>494 ± 80.5&lt;sup&gt;a&lt;/sup&gt;</td>
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<tr>
<td>C54</td>
<td>113 ± 11.5&lt;sup&gt;a&lt;/sup&gt;</td>
<td>82.7 ± 8.8&lt;sup&gt;a&lt;/sup&gt;</td>
<td>83.6 ± 4.0&lt;sup&gt;a&lt;/sup&gt;</td>
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<td>326 ± 34.9&lt;sup&gt;a&lt;/sup&gt;</td>
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<tr>
<td>C108</td>
<td>102 ± 8.1&lt;sup&gt;a&lt;/sup&gt;</td>
<td>75.8 ± 6.4&lt;sup&gt;a&lt;/sup&gt;</td>
<td>79.3 ± 4.6&lt;sup&gt;a&lt;/sup&gt;</td>
<td>31.7 ± 3.1&lt;sup&gt;a&lt;/sup&gt;</td>
<td>289 ± 35.8&lt;sup&gt;a&lt;/sup&gt;</td>
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Different superscript capitalized letters indicate significant differences among treatments (p < 0.05).

*<sup>a</sup> Treatment A: no organisms; B27: 27 L. variegatus only; B54: 54 L. variegatus only; B108: 108 L. variegatus only; C27: 27 L. variegatus and ten H. azteca; C54: 54 L. variegatus and ten H. azteca; C108: 108 L. variegatus and ten H. azteca; D: ten H. azteca only.

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5.11–22.5 μg/g lipid in the treatments B and C, respectively. The PAH concentrations were significantly different when the densities of L. variegatus varied, with body residues dramatically decreasing when the worm density increased. Similar trends were also noted for the sum PAH concentration when grouped by compound structure (i.e. number of rings) (Table 2). As stated previously, decreases in PAH concentrations in sediment were noted with increasing worm density, this in turn may account for a similar decline in PAH residues in test organisms. It should also be noted that higher worm densities may affect food availability and produce stress to the organisms, which in turn would affect the bioaccumulation process. Similarly, BSAFs showed a similar trend (Tables 3 and S2). As shown in Table 3, BSAF values in the treatments with differing densities were significantly different (Treatment B: 0.37 ± 0.11–0.93 ± 0.08 g OC/g lipid; Treatment C: 0.34 ± 0.06–0.87 ± 0.23 g OC/g lipid, respectively).

As shown in Table S2, the majority of BSAF values were less than 1, with BSAFs for the worms exposed at the highest density being all < 0.5. Equilibrium partitioning theory suggested that the affinity of organism lipid to HOCs was about twice that of sediment OC, and BSAF values for HOCs, without extensive biotransformation, were close to 1.7 (Di Toro et al., 1991). Thus, rapid biotransformation and limited uptake may contribute to the low BSAFs in the present study. Although L. variegatus is regarded as an organism with limited biotransformation capacity (USEPA, 2000), it has been reported to be capable of biotransforming certain PAHs (Leppänen and Kukkonen, 2000; Lytytäinen et al., 2007; Mäenpää et al., 2008; You et al., 2009). The biotransformation of PAHs has been shown as compound-dependent. Forbes et al. (1996) found Capitella sp. can metabolize fluoranthene but not high-molecular-weight PAHs. Lytytäinen et al. (2007) reported that low-ring PAHs were relatively more unstable, and thus they were biotransformed more quickly in organisms and eliminated from the body more rapidly. Although not significant, BSAF values of the target PAHs slightly increased when the numbers of benzene rings increased (Table S2), and compound-dependent biotransformation may be one of the reasons for the low BSAF values of low-ring PAHs. Additionally, accumulation of PAHs by the organisms was reduced at higher worm density in the present study. The reduction in exposure to contaminants may be caused by food limitations (especially in treatments with higher worm densities) and the architometric reproduction process which has been reported to influence the feeding behavior of L. variegatus since the tail parts ceased to eat while they were growing the anterior part (Leppänen and Kukkonen, 2000).

### 3.2.2. Biomimetic extraction

Various biomimetic extraction techniques have been developed to assess bioavailability of HOCs in sediment (Reichenberg and Mayer, 2006). The influences of bioturbation on PAH bioavailability were also evaluated using two biomimetic extraction techniques, SPME and Tenax extraction. While SPME measures chemical activity using the freely dissolved chemical concentrations in sediment pore water (Cpw), Tenax extraction estimates bioaccessibility using the rapidly desorbing fraction of chemicals from sediment (Reichenberg and Mayer, 2006; You et al., 2011).

Similar to PAH concentrations in sediment and organism, Cw and C24 increased when organism density increased. As shown in Table S3, the sum of PAH concentrations in SPME fibers significantly dropped from 45.1 ± 0.21 to 27.9 ± 0.58 μg/ml and 43.5 ± 0.57 to 32.9 ± 0.23 μg/ml for treatments B and C, respectively when L. variegatus density increased four times (i.e. from 27 to 108 worms per beaker). Similar reductions in C24 were also noted as a 30 percent decrease in C24 was observed when worm density increased four times (Table S4).

Furthermore, Cpw of individual PAH was calculated using Cw and the Kow values from the literature (de Maagd et al., 1998; Eriical and Robert, 2010). Low-ring PAHs generally had greater Cpw values compared to the high-ring PAHs (Table S5). Because of their low sediment concentrations and high hydrophobicity, Cpw of the five-ring PAHs were all below the RLs. The sum of PAH Cpw in treatment A, in which no organisms were present, was 42.2 μg/L, and this value declined with the addition of organisms to test replicates. The Cpw was significantly different when comparing treatment A to the treatments at the highest density (e.g. 108 worms per beaker; 10,000 individuals/m2) by nearly 60 percent. While declines were noted when comparing treatment A to treatments D, B27, B54, C27 and C54 these reductions were not significant. The decrease in PAH concentration in sediment (Cw) is believed to be the main reason for the decrease in Cpw when the worm densities increased.

Reduction in Cw affected SPME and Tenax extraction measurements, hence, Cw/Cs and F24/h (e.g. C24/h/Cs) were used to alleviate the deviations among sediments (Table 3). Although it was not significant, a slight increase in Cw/Cs was evident when the numbers of L. variegatus increased from 7 to 28 in treatment B where the values increased from 1.69 to 2.18. Meanwhile, C24/h/Cs values among all treatments were similar (~0.48), indicating no significant change in PAH desorption from sediment (Table 3). Enhanced desorption of sediment-associated HOCs in the presence of benthic organisms has been reported in previous field studies (Joseffsson et al., 2010; Qin et al., 2010), suggesting that bioturbation not only promoted the transport of HOCs to overlying water by resuspension of sediment particles, but also elevated freely dissolved HOCs in pore water. However, no significant change in bioavailability, estimated by biomimetic techniques, was observed in the present study, which may be an artifact of conducting laboratory-based testing (limited sediment volume and testing time differences).

The BSAFs significantly decreased when organism density increased (Table 3). Because Cw/Cs and F24/h were similar among the treatments, reductions in BSAFs were possibly due to organism stress induced by overcrowding, although it should be noted that the worm densities used in the present study are environmentally relevant.

### 3.3. Influence of bioturbation on PAH toxicity

As stated in Section 3.2.1, overlying water quality parameters were within the USEPA guidelines (USEPA, 2000). Interestingly, the water was more turbid in beakers with the presence of L. variegatus compared to those containing no organisms or only amphipods.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>BSAF</th>
<th>Cw/Cs</th>
<th>C24/h/Cs</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td></td>
<td>2.04 ± 0.22</td>
<td>0.48 ± 0.06</td>
</tr>
<tr>
<td>D</td>
<td></td>
<td>2.00 ± 0.21</td>
<td>0.49 ± 0.06</td>
</tr>
<tr>
<td>B27</td>
<td>0.93 ± 0.08</td>
<td>1.94 ± 0.16</td>
<td>0.47 ± 0.04</td>
</tr>
<tr>
<td>B54</td>
<td>0.67 ± 0.12</td>
<td>2.07 ± 0.15</td>
<td>0.49 ± 0.04</td>
</tr>
<tr>
<td>B108</td>
<td>0.37 ± 0.11</td>
<td>1.79 ± 0.58</td>
<td>0.48 ± 0.16</td>
</tr>
<tr>
<td>C27</td>
<td>0.87 ± 0.23</td>
<td>1.69 ± 0.45</td>
<td>0.44 ± 0.13</td>
</tr>
<tr>
<td>C54</td>
<td>0.87 ± 0.09</td>
<td>1.98 ± 0.36</td>
<td>0.49 ± 0.09</td>
</tr>
<tr>
<td>C108</td>
<td>0.34 ± 0.06</td>
<td>2.18 ± 0.43</td>
<td>0.51 ± 0.11</td>
</tr>
</tbody>
</table>

Different capitalized superscript letters indicate significant differences among treatments (p = 0.05).

* The definition of treatments and PAH compounds were shown in Table 1.
The increased turbidity of the overlying water was believed to be due to the \textit{L. variegatus} burrowing and feeding activities. These activities caused a greater degree of resuspension of sediment particles and hence contaminants associated with those particles into the overlying water column. Therefore, bioturbation activities by benthic organisms might potentially cause adverse effects to pelagic and/or epi-benthic organisms which utilize both strata.

Sediment toxicity to \textit{L. variegatus} and \textit{H. azteca} was also assessed at the end of 28 d bioassay. The survival of both organisms was greater than 90 percent in all tests with control sediment (Fig. 2a). In PAH-contaminated sediment, \textit{L. variegatus} had nearly 100 percent survival (98.6 ± 10.6 to 104.6 ± 11.5 percent) in all treatments, whereas survival of \textit{H. azteca} was more variable, as treatments D, C27, C54 and C108 had survival of 77.5 ± 5.0, 75.0 ± 5.8, 65.0 ± 17.3 and 37.5 ± 5.0 percent, respectively. A significant mortality of \textit{H. azteca} was observed for C108 compared to the other treatments (Fig. 2b). Concentrations of PAHs were analyzed in the survived amphipods and they were 38.9, 38.1, 112 and 33.7 μg/g lipid in treatments C27, C54, C108, and D, respectively.

Although no mortality and changes in lipid contents were observed for \textit{L. variegatus}, the growth of the worms (expressed by wet weight per worm) was greatly slowed when the worms were exposed at the highest density. The worm weights decreased in treatment B from 6.163 ± 0.061 to 3.932 ± 0.035 mg and in treatment C from 5.477 ± 0.250 to 4.181 ± 0.010 mg when the organism densities increased from 27 to 108 worms per beaker, respectively (Fig. 2b). The results revealed that sublethal effects to the worms occurred due to the high worm densities, which was consistent with previous studies (Lotufo et al., 2000; Millward et al., 2001; Mäenpää et al., 2009). Similar to the present study, sublethal toxicity was believed to be the reason for the decrease in bioaccumulation of pyrene in the oligochaetes \textit{Limnodrilus hoffmeisteri} and \textit{L. variegatus} (Millward et al., 2001; Mäenpää et al., 2009). Lotufo et al. (2000) reported worms at high density were more susceptible to toxicant effects on their growth, and they suggested competition for food sources may be one of the reasons. The present study also found slower growth in companion with smaller BSAF values for organisms exposed at higher density. Additionally, the worms may cease feeding during architometric reproduction, resulting in low bioaccumulation of contaminants (Leppänen and Kukkonen, 1998).

Although reproduction was noted in some cases, survival of the worms was all close to 100 percent, thus reproduction was not the reason for the decline in worm weights in the present study. These results, which are consistent with the literature (Lotufo et al., 2000; Millward et al., 2001; Mäenpää et al., 2009), indicated that overcrowding may change organism susceptibility to contaminants, which in turn may alter sediment toxicity. Thus, future studies should take this into consideration when analyzing the effects of bioturbation on bioavailability of contaminants in sediments.

Dissimilar to \textit{L. variegatus}, \textit{H. azteca} exposed to PAH-contaminated sediment tried to avoid the sediment. As shown in Fig. 2b, significant greater mortality occurred for \textit{H. azteca} co-exposed with \textit{L. variegatus} at the highest density (C108) when compared to the treatment without \textit{L. variegatus} (D). The two organisms reside in different niches. While the oligochaetes burrows into sediment, the amphipod mainly resides at sediment–water interface and in the overlying water column. The additional toxicity to \textit{H. azteca} in treatment C108 may be caused by the bioturbation by the oligochaetes as their activities remobilized particle-associated contaminants to sediment surface and promoted chemical release to the overlying water. Significantly greater PAH concentrations in the amphipods in treatment C108 compared to those in other treatments suggested more PAHs were released to the overlying water due to elevated bioturbation activities. The increased turbidity of overlying water in the presence of the worms also supported these conclusions. Additionally, this remobilization activity became stronger at higher animal density which has been previously reported as well (Karickhoff and Morris, 1985; Thibodeaux and Bierman, 2003). Therefore, it is believed that exposure of contaminants, and hence toxicity, to the amphipod increased when the worm density increased. Although the present study only focused on PAHs, other contaminants in sediment from the e-waste recycling practices, such as metals, PCBs, and polybrominated diphenyl ethers (Mehler et al., 2011b), may be affected by bioturbation and contributed to the toxicity to the amphipods as well. It should also be noted that overcrowding and food competition may have also contributed to mortality of the amphipod in the treatment with highest worm density. However, the contribution is believed to be insignificant since no amphipod mortality was observed in the control study at the same density of organisms (Fig. 2a).

4. Conclusions

Bioavailability and toxicity of PAHs in sediment from an e-waste recycling site in South China was assessed using 28 d bioassays and two biomimetic extraction techniques to elucidate the effects of bioturbation by the freshwater oligochaetes. When density of \textit{L. variegatus} increased, PAH concentrations in
sediment, L. variegatus, SMPE fibers, and Tenax absorbents all decreased. This may be in part due to the decline of PAH concentrations in sediment, as no significant differences were noted in bioavailability measured by the biomimetic techniques when sediment concentration was taken into account.

Significant mortality and elevated PAH residues were observed to H. azteca which were co-exposed in PAH-contaminated sediment with the highest density of L. variegatus, whereas no alteration of toxicity was observed for H. azteca exposed to the control sediment with various densities of L. variegatus. The increased mortality of the amphipod in the treatments at the highest organism density was probably because of the transport of sediment-associated contaminants to sediment surface and water column by the bioturbation activities of L. variegatus. Future studies should determine if the decrease in BSAF values in the worms exposed at higher density may be the combined results of sublethal toxicity, overcrowding, and food competition. As density and bioturbation are interrelated, future work which can separate those components will help elucidate the relevance of bioturbation on assessing toxicity in aquatic ecosystems. Overall, the present study suggests that bioturbation can alter the toxicity of contaminants, thus providing the foundation for future studies to examine how benthic invertebrates and their interactions with the sediment should be considered in sediment risk assessment.

Acknowledgments

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Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at http://dx.doi.org/10.1016/j.ecoenv.2012.07.007.

References