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Species-dependent effects of biochar amendment on bioaccumulation of atrazine in earthworms

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ABSTRACT

We observed that at a contamination level of 4.25 mg-atrazine/kg-soil, the biota–soil accumulation factor (BSAF) for the anecic *M. guillelmi* is approximately 5 times that for the epigeic *E. foetida*. This is attributable to the fact that bio-uptake by *E. foetida* is mainly through dermal absorption, whereas bio-uptake by *M. guillelmi* is largely affected by the gut processes, through which the physical grinding and surfactant-like materials facilitate the desorption of atrazine from soil. Strikingly, biochar amendment resulted in much greater reduction in BSAF for *M. guillelmi* than for *E. foetida*. At a biochar dose of 0.5% (wt:wt) the difference in BSAF between the two species became much smaller, and at a dose of 2% no statistical difference was observed. A likely explanation is that gut processes by *M. guillelmi* were much less effective in extracting atrazine from the biochar (the predominant phase wherein atrazine resided) than from soil particles.

1. Introduction

Amendment of contaminated soils and sediments with carbonaceous materials (e.g., activated carbons and black carbons) is one of the most commonly adopted approaches for the risk reduction of soil and sediment contamination (Beckingham and Ghosh, 2011; Beesley et al., 2010; Hale and Werner, 2010; Hilber and Bucheli, 2010; Josefsson et al., 2012; Millward et al., 2005; Rakowska et al., 2012). Owing to their strong adsorption affinities, high surface area, and large pore volume, carbonaceous materials are superb adsorbents for a range of contaminants. Thus, a common presumption is that once mixed with contaminated soil/sediment, carbonaceous materials can result in the redistribution of contaminants among different phases (in particular, significant reduction of contaminant concentrations in porewater), leading to the reduction of the bioavailable fraction of contaminants (Fagervold et al., 2010; Gomez-Eyles et al., 2011; Millward et al., 2005; Song et al., 2012; Sun and Ghosh, 2007; Tang et al., 2007; Wen et al., 2009; Yang et al., 2009).

Different biological species may uptake contaminants from soil/sediment via different mechanisms. For many species uptake from porewater, including porewater ingestion and dermal absorption, is the predominant route (e.g., Belfroid et al., 1995); for certain other species, however, ingestion of soil/sediment particles can be a major or even predominant route (Leppänen and Kukkonen, 1998; McLeod et al., 2007). While it is relatively straightforward to understand how carbonaceous materials can reduce the concentrations of contaminants in porewater (or overlaying water) and consequently hinder the bioaccumulation of contaminants in porewater-route-dominated species, the specific mechanisms via which carbonaceous materials affect the bioaccumulation in soil/sediment-particle-ingesting species are not well understood. Thus far, only a very limited number of studies have been conducted to understand the effects of carbonaceous material amendment on the bioaccumulation of different benthic species, and the findings are not always consistent. For example, Cornelissen et al. (2006) found that while adding 2% activated carbon to PAH (polycyclic aromatic hydrocarbons)-contaminated sediment samples from three Norwegian harbors reduced the bioaccumulation in polychaete (*Nereis diversicolor*) by 6–7 fold for two of three samples, it had little effect on the bioaccumulation in polychaete for the third sediment sample and the bioaccumulation in gastropod (*Hinia reticulate*) for all three samples. They proposed that the differences in digestive biology between polychaetes and gastropods could have been the cause. Ferguson et al. (2008) found that adding 1.93% single-walled carbon nanotubes to sediments resulted in significant reduction in the bioaccumulation of PAHs, polychlorinated biphenyls, and polybrominated diphenyl ethers in a deposit/

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suspension-feeding polychaete (*Streblospio benedicti*), but no noticeable effects on the bioaccumulation in a deposit-feeding meiobenthic copepod (*Amphiascus tenuiremis*). The authors argued that the different effects seen between *S. benedicti* and *A. tenuiremis* can be explained by the differences in their gut surfactant activities.

Only in a few recent papers have the effects of carbonaceous material amendment on the bioaccumulation in earthworms been examined, using *Eisenia foetida* as the test species (Cao et al., 2011; Chai et al., 2011; Denyes et al., 2012; Fagervold et al., 2010; Gomez-Eyles et al., 2011; Petersen et al., 2009; Song et al., 2012; Wen et al., 2009). In general, carbonaceous materials (e.g., activated carbons and carbon nanotubes) can effectively decrease the bioaccumulation of hydrophobic contaminants (e.g., PAHs) and the reduction in porewater concentrations has been attributed as the main mechanism (Cao et al., 2011; Petersen et al., 2009; Song et al., 2012). It is proposed that compared with contaminants sorbed in natural organic matter, contaminants adsorbed to black carbons would desorb in much slower rates (Wen et al., 2009) or cannot desorb even in the presence of the digestive enzymes and microbial community of the intestinal tracts of earthworms (Langlois et al., 2011; Denyes et al., 2012). Nonetheless, it can be anticipated that carbonaceous material amendment might exert significantly different effects on different types of earthworms, particularly among species of different feeding ecology and bio-uptake routes. To date, there are no published data that compare the effects of carbonaceous material amendment on the bioaccumulation among different earthworm species.

The primary objective of this study was to understand the effects of carbonaceous material amendment on the bioaccumulation of soil-bound contaminants by earthworm species of different feeding ecology. Two typical earthworm species, including *E. foetida*, an epigeic species, and *Metaphire guillelmi*, an anecic species, were selected as the test species. A pine-tree-derived biochar was used as the model carbonaceous material. Biochar is derived from organic wastes and is considered a more cost-effective material than activated carbons for soil/sediment remediation (Chen and Chen, 2009; Manyà, 2012; Xu et al., 2012). Atrazine, a common herbicide, was chosen as the model contaminant. The sorption properties of atrazine to a typical agricultural soil and to the biochar were examined, the bioaccumulation capabilities of the two earthworm species for soil-bound atrazine were compared, the effects of biochar amendment on bioaccumulation reduction were examined and the mechanisms controlling the different effects between the two species were illustrated using a mathematical approach.

2. Materials and methods

2.1. Materials

E. foetida were obtained from Tianjin Biological Supply Co. (Tianjin, China) and *M. guillelmi* from Jurong Biological Supply Co. (Jiangsu Province, China); both species were cultured in the laboratory. The average wet weight of *E. foetida* used in the bioaccumulation experiments was 0.38 ± 0.04 g/worm, the average wet weight of *M. guillelmi* was 2.00 ± 0.06 g/worm. The lipid contents of the worms were determined using the method of Lu et al. (2003). The measured average lipid contents of *E. foetida* and *M. guillelmi* were 11.4% and 7.0% (dry-weight based), respectively.

A typical Chinese agricultural soil, black soil, was used in this study. Soil collected from the surface layer (20 cm) was air-dried at room temperature, ground, and passed through a 2-mm sieve. The soil contained 52% clay, 42% silt, and 6% sand. The fractional organic carbon content of the soil, f_{OC}, was 0.0249. The soil did not contain detectable quantity of atrazine.

A pine-wood-derived biochar was prepared by atmospheric pyrolysis using the method of Braida et al. (2003). Briefly, pine-wood shavings were heated at 400 °C in a beaker covered with a watch glass in a muffle furnace for 2 h. The pyrolyzed product was washed by shaking in 0.01 M $CaCl_2$ for 1 d (Jonker and Koelmans, 2002). Then, the suspension was centrifuged at 2600 *g* for 20 min, and the supernatant was discarded. The washing–centrifuging cycle was repeated 14 times. The washed fractions were then collected and dried at 80 °C for 48 h, and finally pulverized gently in a mortar and sieved through a 0.15-mm sieve. The Brunauer–Emmett–Teller (BET) surface area (120 m^2/g) of the biochar was determined by multipoint N_2 adsorption–desorption using a Micromeritics ASAP2010 accelerated surface area and porosimetry system (Micromeritics Co., USA). To measure the organic carbon content of the biochar (63.2%), 0.5 g biochar was first treated with 30 ml 3 M HCl for 24 h to remove the inorganic carbon, and then dried at 105 °C overnight. Afterward, 10 mg of the treated sample was analyzed with an analytikjena multi N/C 3100 (Germany) to measure the C content.

Atrazine was purchased from Sigma–Aldrich (St. Louis, MO, USA). Stock solutions of atrazine were prepared in methanol. All organic solvents were analytical grade or higher.

2.2. Sorption experiments

The sorption isotherms of atrazine to the soil and biochar were obtained using a batch sorption approach developed in our previous study (Yang et al., 2008). Approximately 12 g soil or 10 mg biochar was added to each of a series 40-ml amber glass vials and pre-wetted for 24 h with approximately 40 ml of an electrolyte solution containing 0.01 M NaCl, 0.01 M $CaCl_2$, and 0.01 M NaN_3 (as the bio-inhibitor). Then, a stock solution of atrazine (in methanol) was spiked to the vials, and the volume percentage of methanol was kept below 0.1% (v/v) to minimize the co-solvent effects. The vials were then filled with the electrolyte solution to leave minimal head space and was mixed end-over-end at 3 rpm for 7 d (the time required to reach sorption equilibrium was predetermined). Afterward, the vials were centrifuged at 3000 *g* for 30 min, and the supernatant was withdrawn to analyze the concentrations of atrazine in the aqueous solution. The pH of all the samples at equilibrium was near neutral. To account for the losses from processes other than sorbent sorption (i.e., adsorption to glass vials and other possible interactions in solution), calibration curves were conducted separately from controls receiving the same treatment as the sorption samples but without the sorbent (Wang et al., 2012). Calibration curves included at least ten standards over the test concentration range. Based on the obtained calibration curves, the sorbed mass of atrazine was calculated by subtracting the mass in aqueous solution from the mass added. Each isotherm contains six individual data points and each data point was run in duplicate.

2.3. Preparation of contaminated and biochar amended soil samples

Three contaminated soil samples were prepared; one of the samples was unamended and is denoted as sample #1, and the other two were amended with 0.5% (wt:wt) and 2.0% biochar and denoted as sample #2 and sample #3, respectively. (The doses of biochar used were chosen based on the results of single-point sorption experiments; the low dose was chosen so that approximately 50% of atrazine in the soil samples would be associated with the biochar, and the high dose was chosen so that most of the atrazine would be with the biochar.) Each sample was run in quadruplicates (two of the quadruplicates would later be used in the bioaccumulation experiment of *E. foetida* and the other two in the experiment of *M. guillelmi*). To prepare the soil samples, 80 g of the soil was added to each of 12 brown glass bottles of 250-ml capacity, and 0.4 g or 1.6 g of biochar was added to each of the eight bottles used to prepare soil samples #2 and #3. Then, approximately 200 ml of an electrolyte solution containing 0.01 M NaCl and 0.01 M $CaCl_2$ was added to each of the bottles, and the bottles were sealed with Teflon-lined screw caps and mixed end-over-end at 3 rpm for more than 14 d to ensure the thorough mixing of the soil and the biochar. Afterward, a stock solution of atrazine (in methanol) was spiked to the bottles, and the volume percentage of the methanol was kept below 0.1% (v/v). The bottles was then filled with the background electrolyte solution to leave minimal head space and was mixed end-over-end at 3 rpm for 7 d. Then, the bottles were centrifuged at 2500 *g* for 30 min, and the supernatant was withdrawn to analyze the equilibrium concentration of atrazine in the aqueous solution. The concentrations of atrazine in the sorbed phase were calculated using the same approach described in Section 2.2. The soil (or biochar-amended soil) settled in the bottom of the bottles (with a water content of approximately 39%) was used immediately in bioaccumulation experiments.

2.4. Bioaccumulation experiments

Bioaccumulation experiments were conducted using a method similar to that reported in the literature (Kreitinger et al., 2007; White et al., 1998). One day before initiating the experiments, worms of similar size were gently removed from the culture and held in Petri dishes. In a typical experiment, 10 *E. foetida* or three *M. guillelmi* worms were placed in one of the 250-ml glass bottles in which the contaminated soil samples were prepared. Each of the bioaccumulation experiments was run in duplicates. The bottles were kept in an incubator with a constant temperature of 22 °C for 14 d to ensure that steady state was reached (the lids of the containers were opened once every 12 h to refresh the headspace). Preliminary tests showed that accumulation of atrazine by *E. foetida* and *M. guillelmi* reached an apparent steady state within 6–7 d. At the end of a bioaccumulation experiment, the worms were carefully removed, rinsed, and allowed to purge their gut contents for 24 h on moistened filter paper. All worms were active after the 14-d period. Then, the worms from each individual sample were weighed. The body mass loss at the end of the bioaccumulation experiments was approximately 18% for *E. foetida* and