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Fate of phenanthrene and mineralization of its non-extractable residues in an oxic soil☆

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ABSTRACT

The fate of organic pollutants in the environment, especially the formation and stability of non-extractable (i.e., bound) residues (NERs) determines their environmental risk. Using ^{14}C-tracers, we studied the fate of the carcinogen phenanthrene in active or sterilized oxic loamy soil in the absence and presence of the geophagous earthworm *Metaphire guillelmi* and characterized the NERs derived from phenanthrene. After incubation of ^{14}C-phenanthrene in active soil for 28 days, 40 ± 3.1% of the initial amount was mineralized and 70.1 ± 1.9% was converted to NERs. Most of the NERs (>92%) were bound to soil humin. Silylation of the humin-bound residues released 45.3 ± 5.3% of these residues, which indicated that they were physically entrapped, whereas the remainder of the residues were chemically bound or biogenic. By contrast, in sterilized soil, only 43.4 ± 12.6% of the phenanthrene was converted to NERs and all of these residues were completely released upon silylation, which underlines the essential role of microbial activity in NER formation. The presence of *M. guillelmi* in active soil significantly inhibited phenanthrene mineralization (24.4 ± 2.6% mineralized), but NER formation was not significantly affected. Only a small amount of phenanthrene-derived residues (1.9–5.3% of the initial amount) accumulated in the earthworm body. When humin-bound residues were mixed with fresh soil, 33.9% (humin recovered from active soils) and 12.4% (humin recovered from sterilized soils) of the residues were mineralized after 75 days of incubation, respectively, which indicated a high bioavailability of NERs, albeit lower than the initial addition of phenanthrene. Our results indicated that many phenanthrene-derived NERs, especially those physically entrapped, are still bioavailable and may pose a toxic threat to soil organisms.

1. Introduction

Polycyclic aromatic hydrocarbons (PAHs) are ubiquitous organic pollutants in the environment and originate from both anthropogenic activities and natural processes (Shen et al., 2013). PAHs are of great concern because they are carcinogenic and mutagenic, especially for humans (Perera, 1997; Zhang et al., 2009). One of the most important sinks for PAHs in the environment is soil (Mai et al., 2003; Tang et al., 2005; Wild and Jones, 1995). In agricultural soils in China, PAH concentrations range from 178 ng/g to 3234 ng/g (soil dry weight), with a high spatial heterogeneity (Wang et al., 2010; Xing et al., 2011; Yang et al., 2012; Yin et al., 2008; Zhao et al., 2014).

PAHs in soil are subjected to many processes, e.g., adsorption, desorption, volatilization, biodegradation, uptake by biota, and leaching (Doick et al., 2005; Du et al., 2011). One of the most important processes is the formation of non-extractable (bound) residues (NERs), which leads to the persistence of PAHs in soil (Kästner et al., 1999; Richnow et al., 2000). NERs are classified into three types: residues entrapped in the structural voids of the soil matrix, residues covalently bound to soil organic matter, and biogenic residues (Kalathoor et al., 2015; Kästner et al., 2014; Possberg et al., 2016; Riefer et al., 2013). Reactive metabolites are generated and covalently bound residues are formed through the activity of soil microorganisms (Kästner, 2000). Biogenic residues in soil organic matter are formed after the death of microbes that used the carbon or nitrogen atoms from pollutants for synthesizing cell biomass, e.g., proteins and fatty acids (Nowak et al., 2011, 2013;

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Richnow et al., 2000).

Earthworms are one of the most important ecosystem engineers and have the potential to remove large amounts of PAHs from the soil environment (Contreras-Ramos et al., 2006). Due to their biological, chemical, and physical activities, e.g., burrowing, ingestion, digestion, and excretion, earthworms might promote the biodegradation of organic contaminants. Earthworms can change the microenvironment by aerating soil aggregates, stimulating microbial processes, and making pollutants more accessible for microbes (Hickman and Reid, 2008). On the other hand, earthworms can increase the association of natural organic compounds with soil minerals, increase aggregate formation, and decrease microbial activity in earthworm casts, thereby inhibiting the degradation of the compounds (Ma et al., 2013; Zhang et al., 2000). However, little is known about how earthworms influence the fate of organic pollutants.

NERs can be remobilized due to changes in environmental conditions, agricultural practices, and soil chemistry (Gao et al., 2015; Gevao et al., 2000; Lerch et al., 2009; Liu et al., 2013) or during remediation processes (Gevao et al., 2001; Liu et al., 2015). However, the mechanisms and factors driving the formation of NERs from PAHs and the release of NERs still need to be elucidated. The aim of this study was to investigate the effects of soil microbes and earthworms on the fate of phenanthrene in soil under oxic conditions and on the mineralization of the NERs derived from phenanthrene.

2. Materials and methods

2.1. Chemicals

Radioactively labeled phenanthrene- [9-^{14}C] (^{14}C-phenanthrene; 99% purity) was purchased from Hartmann Analytic GmbH (Braunschweig, Germany). Non-labeled phenanthrene (98% purity) was purchased from Sigma-Aldrich (Shanghai, China). Other chemicals were chromatographic or analytical grade. A 5 mg/mL stock solution of ^{14}C-phenanthrene (92.5 KBq/mL) for addition to soil was prepared in acetone.

2.2. Soils and earthworms

A rice paddy soil typical for the Yangtze River Delta area was collected from an agricultural field in Jurong, Jiangsu Province, China. Topsoil collected at 5–20 cm depth was immediately taken to the laboratory, air-dried, and sieved through a 1-mm mesh. The soil was a claycar-vertic-gleyic-stagnic anthrosol with 3.7 ± 0.5% total organic carbon and 0.63 ± 0.03% total nitrogen. The texture of the soil was loam with 51.2 ± 0.9% sand, 38.0 ± 1.5% silt, and 10.8 ± 0.6% clay content and a pH ($CaCl_2$) of 6.80 ± 0.08.

Anecic earthworms (*Metaphire guillelmi*) were collected from the same field where soil was sampled. The earthworms were raised in containers filled with the test soil under the experimental conditions for 3 weeks before the experiments commenced. Only active individuals with a fresh body weight of 2.0–2.5 g were selected for the experiments.

2.3. Soil incubation experiments

About 50 g of air-dried active or sterilized soil was added to each 250-mL flask. Soil was sterilized by autoclaving at 121 °C for 1 h three times on three consecutive days.

^{14}C-labeled phenanthrene (200 μL of the stock solution) and unlabeled phenanthrene were added to the soil to obtain a final concentration of 20 μg phenanthrene/g soil (dry weight) and a specific radioactivity of 370 Bq/g soil (dry weight). After the solvent of the stock solution evaporated, the water content of the soil was adjusted 60% of the maximum water-holding capacity, and the soil was mixed thoroughly. In experiments testing the effect of earthworms, one individual earthworm was placed into each flask. All flasks were sealed with stoppers. A 6-mL vial containing 1.0 mL of 1 M NaOH was suspended from the bottom of each stopper to adsorb the $^{14}CO_2$ released from the soil (Shan et al., 2011a). The flasks were incubated for 28 days at 20 °C in the dark and were opened for several minutes every 2 days to allow fresh air exchange. All experiments (active soil with and without an earthworm, sterilized soil with and without an earthworm) were carried out in triplicate. Flasks containing the same soil but without earthworms were used as controls.

2.4. Analysis of residues in soil and earthworms

After incubation for 28 days, earthworms were removed from the soil samples, and the soil was freeze-dried. Aliquots of 5 g soil were extracted three times with 20 mL mixture of dichloromethane and acetone (1: 1, by volume) by repeated ultrasonic suspension (0.09 kW, 20 kHz), shaking (220 rpm, 1 h), and centrifugation (10000*g*, 10 min) (Song et al., 2002). The recovery of the extraction method was 97.8 ± 0.1%. Each set of three supernatants were combined, and radioactivity in aliquots were quantified by liquid scintillation counting (LSC, see below).

The NERs of phenanthrene and its metabolites in soil obtained after this exhaustive extraction with organic solvents were fractionated into fulvic acid (FA)-, humic acid (HA)-, and humin-bound residues according to Shan et al. (2011b). Briefly, the soils after organic solvent extraction were further extracted with 0.1 M oxygen-free NaOH. The radioactivity in the alkaline extracts (alkaline-soluble humic substances) was determined by LSC (see below). The alkaline-soluble humic substances were acidified to pH 1 with 6 M HCl to precipitate the HA fraction; the FA fraction remained in solution. The radioactivity in the FA fraction was determined by LSC (see below). The soil pellets after alkaline extraction contained humin-bound residues. The pellets were freeze-dried, and radioactivities in aliquots were determined in a biological oxidizer (see below). Another aliquot of each freeze-dried soil pellet was silylated according to the method described by Shan et al. (2011b) to separate residues physically entrapped in humin from covalently bound residues and residues incorporated into microbial biomass (i.e., biogenic residues; Kästner et al., 2014). Briefly, 0.3 g of a soil pellet was suspended in 663 μL of a mixture of dimethyl sulfoxide: trimethylsilyl chloride: pyridine (200: 20: 1, by volume) and shaken on a rotary shaker at 250 rpm for 12 h at 30 °C. After centrifugation (14,000 g, 15 min, 15 °C), the radioactivity in the supernatant was determined by LSC (see below) and was defined as residues physically entrapped in humin. The radioactivity in the pellet was determined in a biological oxidizer (see below) and represented covalently bound residues and biogenic residues.

The earthworms were killed by freezing at −20 °C for 2 h, freeze-dried, cut into small pieces (<3 mm) with scissors, and ground with a mortar and pestle (Shan et al., 2010a). The residues in each ground earthworm sample (0.5–1 g dry biomass) were extracted three times with 20 mL dichloromethane: acetone (1: 1, by volume) by repeated ultrasonic suspension (0.09 kW, 20 kHz), shaking (220 rpm, 1 h), and centrifugation (10000*g*, 10 min). Each set of three supernatants were combined and concentrated to about 3 mL in a rotary evaporator. Radioactivity in the concentrated extracts was quantified by LSC (see below). Non-extractable radioactivity in earthworm tissue was determined in a biological oxidizer (see below).