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Does ecotype matter? The influence of ecophysiology on benzo[a]pyrene and cadmium accumulation and distribution in earthworms

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ABSTRACT

Benzo[a]pyrene (BaP) and cadmium (Cd) are soil pollutants that persist in the environment and impacting soil health. Earthworms are selective consumers, yet little is known about how their feeding and burrowing habits translate into species-specific differences in pollution accumulation and distribution. Here, we exposed three ecophysiologically distinct earthworm species, *Eisenia fetida* (epigeic), *Pheretima guillelmi* (endogeic), and *Metaphire guillelmi* (anecic) to different concentrations (0, 1, 10, 30, 60, 100, 300, 500 mg/kg) of BaP or Cd in natural fluvo–aquic soil for 14 days. BaP and Cd accumulation and distribution patterns were analyzed at the individual and organ levels. The results showed that the adsorption behavior of BaP or Cd in earthworms and the organs fit the Langmuir adsorption model well ($R^2 > 0.8$, $p < 0.001$). *E. fetida* and *M. guillelmi* accumulated more BaP than Cd, with the respectively higher predicted maximum internal concentration (C_{max}) of BaP (1946.27 ± 306.29 mg/kg, 2046.61 ± 90.64 mg/kg) and the lower C_{max} of Cd (279.75 ± 49.57 mg/kg, 318.11 ± 60.64 mg/kg), while *P. guillelmi* showed the opposite trend with 927.10 mg/kg of Cd C_{max} and 358.82 ± 68.35 mg/kg of BaP C_{max}. The low mobility of endogeic worms may reduce their BaP accumulation, and lead to a lower BaP bioaccumulation factor (BAF) than that observed for the other two earthworms; *P. guillelmi* had a BAF of 8.64 ± 1.79, which was far less than that of *E. fetida* (106.93 ± 11.84) and *M. guillelmi* (350.16 ± 67.15). Whereas the higher Cd accumulation in *P. guillelmi* may be due to their highly efficient geophagous feeding strategy, at the same time the highest Cd BAF achieved (e.g. 203.54 ± 19.96 under 1 mg/kg Cd exposure). BaP distributed mainly in the body walls of all three earthworms (average 60.78%), as its high hydrophobicity increased its dermal uptake. More BaP than Cd accumulated in the reproductive organs, and C_{max} of BaP in *E. fetida*, *P. guillelmi,* and *M. guillelmi* in the reproductive organs was 4031.08 ± 1237.38 mg/kg, 490.76 ± 79.88 mg/kg, and 3675.24 ± 794.68 mg/kg, respectively. However, Cd was more abundant in the gizzard and gut, as its hydrophilic nature meant Cd was mainly ingested orally, and the C_{max} of Cd in *E. fetida*, *P. guillelmi,* and *M. guillelmi* in the gizzard and gut, respectively, was 452.43 ± 48.33 and 360.83 ± 44.36 mg/kg, 996.03 and 809.11 mg/kg, and 93.37 and 109.19 mg/kg. By contrast, for *E. fetida*, more Cd was distributed in the body wall (average 50.16%), possibly due to its high affinity for this organ (the average $\log K_L$ for the body wall was −3.60) predicted by the Langmuir adsorption model, also the worm ingested less soil. These data suggest that ecotype influences the accumulation and distribution of pollutants in earthworms, as their eco-physiological properties (e.g., motility, food choices, and feeding efficiency) affect their exposure to and ingestion of pollutants. The distinct chemical properties of BaP and Cd also appear to affect their accumulation and distribution in earthworms. These factors should be considered when using earthworms as bioindicators in environmental risk assessments.

1. Introduction

A recent national survey of soil contaminants in China revealed its principal pollutants to be organic polycyclic aromatic hydrocarbons (PAHs) and the inorganic cadmium (Cd; Ministry of Environmental Protection of the People's Republic of China, 2014). Benzo[a]pyrene

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(BaP) has the highest toxicity equivalent factor of all the PAHs, and is of particular environmental concern (Duan et al., 2015). BaP and Cd are both persistent in the environment, highly carcinogenic, and possess a high bioaccumulation potential, thus posing serious ecological and toxicological risks.

Earthworms (phylum Annelida, class Oligochaeta) are important components of the terrestrial ecosystem, playing key roles in maintaining soil structure and food webs. Their sensitivity to soil contaminants suggests that they might also serve as bioindicators in soil risk assessments (Kaschak et al., 2014). *Eisenia fetida* was recommended as the standard test species for soil risk assessments by the Organization for Economic Co-Operation and Development (OECD, 1984), due to its sensitivity to contaminants and the simplicity of culturing it. Recently, indigenous earthworm species have also been used as a more environmentally relevant for evaluating soil toxicity (Chen et al., 2017a; Huang et al., 2017).

Numerous studies have assessed the effects of toxic chemicals on earthworms, with many focusing on growth, cocoon production, and enzymatic responses (Spurgeon et al., 1994; Spurgeon and Hopkin, 1995; Davies et al., 2003; Nahmani et al., 2007a; Xie et al., 2013; Duan et al., 2015; Zhang et al., 2017; Wang et al., 2018). However, toxicological effects will not appear if there is no uptake of the chemicals and, therefore, the accumulation and distribution patterns of chemicals in earthworms have also been documented to better understand the immobilization/detoxification strategies used by these organisms (Bengtsson et al., 1983; Morgan and Morgan, 1990; Shi et al., 2014; Beaumelle et al., 2015, 2016; Chen et al., 2017a).

Earthworms are classified into three functional groups based on their biological habitat: epigeic, endogeic, and anecic (Bouché, 1972, 1977). Epigeic earthworms mainly feed on leaf litter and live at the soil surface, endogeic earthworms are mostly geophagous and rarely venture to the soil surface, and anecic earthworms mainly live in deep burrows, venturing to the soil surface for feeding on leaf litters or mixtures of litters and soil particles. A number of studies have been looking at differences in contaminant accumulation between earthworm ecotypes (Spurgeon et al., 2000; Nahmani et al., 2007b; Ernst et al., 2008; Huang et al., 2017), and some variations were found (Langdon et al., 2005; Nannoni et al., 2011; Qiu et al., 2014). The properties of a particular chemical might influence its accumulation pattern or uptake route in earthworms (Nannoni et al., 2011; Chen et al., 2017a). In general, pollutants are taken up by earthworms either dermally or orally; due to their hydrophobic nature, the hydrophobicity of organic chemicals means they are ingested dermally and tend to accumulate in the body wall, while soft metals are ingested orally and typically accumulate in the gut (Duffus, 2002; Jager et al., 2003; Shi et al., 2014; Chen et al., 2017a).

Analyzing the accumulation of pollutants in earthworms at the individual species level and examining their distribution at the organ level will enhance our understanding of their toxic effects and the detoxification mechanisms utilized by earthworms (Bengtsson et al., 1983; Morgan and Morgan, 1990; Shi et al., 2014). The accumulation of pollutants in the reproductive organs has previously been shown to directly inhibit cocoon production and lead to reproductive toxicity (Ricketts et al., 2004; Hernández-Castellanos et al., 2013; Li et al., 2017a). Chloragogenous tissues were found to store and detoxify xenobiotics, including some soft metals and organic pollutants (Sforzini et al., 2014; Shi et al., 2014); however, few studies have explored pollutant distributions at the organ level.

Here, we compared three ecophysiologically distinct earthworm species for their BaP and Cd sensitivities and their individual and organ–level accumulation patterns of these pollutants. We analyzed one artificially cultured earthworm, *E. fetida* (epigeic), and two indigenous geophagous earthworms from China, *P. guillelmi* (endogeic) and *M. guillelmi* (anecic), in natural fluvo–aquic soil, which is widely distributed in China and has high levels of contamination, making the environmental risk assessments relevant (Wang et al., 2010; Duan et al., 2015). Six earthworm organs (three fractions of the body wall taken from the anterior, clitellum, and posterior regions, as well as three fractions of viscera, including the entire gut, gizzard, and reproductive organs) were analyzed separately to determine their pollutant accumulation. The aims of this study were to: (i) examine the distribution and partitioning of different chemicals (BaP and Cd) in earthworms with distinct ecophysiological characteristics, (ii) compare the relative BaP or Cd sensitivities among the different earthworm species, (iii) compare the accumulation of BaP and Cd both at the individual level and the organ level of three ecophysiologically distinct earthworms.

2. Materials and methods

2.1. Soil sampling and exposure concentrations

Surface (0–20 cm) fluvo–aquic soil was collected from an unpolluted site in Tianjin, North China (N: 28° 12′, E: 16° 55′). The soil properties were analyzed as described by Bao (2000). The soil was air dried and passed through a 2–mm sieve before use. Soil pH was measured at a 1:2.5 ratio of soil to water, using a pH meter. Organic matter (OM) was determined using the $K_2Cr_2O_7$ oxidation method, and O–phenanthroline was used as an indicator and 0.2 mol/L $FeSO_4$ standard solution was used for titration. Total nitrogen (TN) was extracted by the Kjeldahl method, and measured by the flow analyzer. Available phosphorus (Av–P) was analyzed by the molybdenum antimony colorimetric method at 880 nm. Available potassium (Av–K) was measured by the flame photometer method. Cation exchange capacity (CEC) was detected by flame photometer method. To determine clay content, 50 g soil was boiled with 20 mL 0.5 mol/L $Na_2C_2O_4$, and then evaluated using a hydrometer at 25 °C. The results were as follows: pH 7.71 ± 0.04, OM: 34.53 ± 1.37 g/kg, TN: 1.35 ± 0.04 g/kg, Av-P: 96.75 ± 4.42 mg/kg, Av-K: 440.75 ± 26.51 mg/kg, clay content: 39 ± 2.12%, C/N ratio: 10.02 ± 0.50, and CEC: 20.94 ± 1.83 cmol/kg. Total Cd in the soil was 0.17 ± 0.03 mg/kg, and the BaP in the soil was below the detection limit (0.04 μg/kg). The methods used for Cd and BaP analysis are supplied in 2.5 and 2.6.

The soil was artificially polluted by sprinkling it evenly with either BaP dissolved in acetone or an aqueous Cd ($CdCl_2{\cdot}2.5H_2O$) solution. The acetone was allowed to evaporate in a fume hood overnight. The target BaP and Cd exposure concentrations were: 0 mg/kg (control), 1 mg/kg, 10 mg/kg, 30 mg/kg, 60 mg/kg, 100 mg/kg, 300 mg/kg, and 500 mg/kg dry soil. All soils were equilibrated for seven days. The actual BaP and Cd concentrations in soil were listed in Table S1. Three replicates of 100 g soil (*E. fetida*) or 300 g soil (*P. guillelmi* and *M. guillelmi*) were used at each contaminant level.

2.2. Earthworm collection and incubation

Mature earthworms with a clearly developed clitellum were used for this study. The *E. fetida* individuals were purchased from an earthworm farm in Nanjing, China, and weighed 0.5–0.7 g each. *P. guillelmi* were collected from agricultural sites in Nanjing, China, and weighed 4–6 g each. *M. guillelmi* were collected from agricultural sites in Nantong, China, and weighed 3–5 g each. Prior to the experiment, the earthworms were acclimatized for two weeks in uncontaminated soil with a water content of 60% of the maximum water holding capacity, under a natural photoperiod and at 25 ± 1 °C.

2.3. Experimental design and sample collection

Ten *E. fetida* (0.5–0.7 g each) worms were added to 100 g fluvo-aquic soil (Duan et al., 2015; Zhang et al., 2017). Considering the morphological differences among the earthworms, an equal biomass density (average 6%; earthworm weight/dry soil weight) was used for *P. guillelmi* and *M. guillelmi*, which was equivalent to four *P. guillelmi* (4.5–5.0 g each) or four *M. guillelmi* (4.5–5.0 g each) added to 300 g soil.