Geoderma 335 (2019) 88–93

Contents lists available at ScienceDirect

Geoderma

journal homepage: www.elsevier.com/locate/geoderma

Sodium arsenite modified burrowing behavior of earthworm species *Metaphire californica* and *Eisenia fetida* in a farm soil

Yali Wang[a], Hao Tang[b], Cory Matthew[c], Jiangping Qiu[a], Yinsheng Li[a,*]

[a] School of Agriculture and Biology, Shanghai Jiao Tong University, Shanghai 200240, China
[b] Shanghai Academy of Environmental Sciences, Shanghai 200233, China
[c] School of Agriculture & Environment, Massey University, Private Bag 11-222, Palmerston North, New Zealand

ARTICLE INFO

Keywords:
Arsenite
Burrow
Behavior
Earthworm
Metaphire californica
Eisenia fetida

ABSTRACT

Arsenic (As) is a widely distributed contaminant in soil and is toxic to plants, animals and humans. Many studies have explored the effects of As on various physiological and biochemical processes in soil fauna. However, there is no report on the effect of sodium arsenite on the behavior of soil fauna such as earthworms. In this study, two earthworm species, *Metaphire californica* and *Eisenia fetida*, were exposed to sodium arsenite in farm soils at concentrations of 5, 10, 20, 40 and 80 mg As kg^{-1} soil for 7 days to investigate the effect on burrowing behavior. Earthworm burrowing behavior responded significantly to both arsenite concentration and exposure time. Both species displayed an initially similar burrowing rate in farm soil with no added As. With added As, *M. californica* displayed an 'escape response' of increased burrowing activity while *E. fetida* displayed an 'exposure avoidance' response of decreased burrowing activity and increased burrow reuse. Data are consistent with an early, concentration dependent sensory response, followed by addition of a physiological response component as As bioaccumulates in earthworm tissues with time. Any bioassay using earthworms to detect As contamination would need to be designed with awareness of the complex behavioral response.

1. Introduction

Arsenic (As) is widely distributed in the environment with elevated concentrations in some soils attributable to both natural factors and anthropogenic contamination (Wang and Cui, 2016). In China, As is the third most important soil inorganic pollutant (Resources and Protection, 2014). Most reported values range from < 10 mg As·kg^{-1} soil, to values approaching 100 mg As·kg^{-1} soil (Huang et al., 2013; Liu et al., 2010). However, values can be higher in areas with heavy As pollution, with one report of 935 mg As·kg^{-1} soil (Liu et al., 2010). As can cause toxicity symptoms in plants or may accumulate in plants and thereby enter animal or human food chains (Wang and Mulligan, 2006). Although arsenic has four oxidation states (V, III, 0, –III), inorganic arsenite [As(III)] and arsenate [As(V)] predominate in aquatic and soil environments (Oremland and Stolz, 2003). As(III) has more severe effects on flora and fauna than As(V). Earthworms make an important contribution to soil health and organic matter cycling, and are often regarded as indicators of land productive capacity and soil fertility status (Pelosi et al., 2017; Blouin et al., 2013; Sun et al., 2017; Brussaard et al., 2007). Earthworms are also acknowledged as bio-indicators of heavy metals or other pollutants (Calisi et al., 2013; Lin et al., 2012; Wang et al., 2017; Mo et al., 2012). It is known that earthworms concentrate As in their body tissues, and are adversely affected by As-contaminated soils (Lee et al., 2013). In addition, As(III) exposure can lead to lipid peroxidation, increased ROS and DNA damage, and to a decrease in antioxidant enzyme activities and lysosomal membrane stability (Wang and Cui, 2016; Wang et al., 2016; Wang et al., 2018).

The adverse effects of sub-lethal doses of contaminants on soil fauna can, in the case of earthworms, lead to behavior changes, including changes in burrowing behavior (Langdon et al., 2001; Langdon et al., 2003b; Li et al., 2016). Such effects of chemical exposure are much less studied than the physiological effects of As noted above. Multiple studies of the reaction of earthworms to arsenate have been published by Langdon et al. (1999, 2002, 2003a, 2003b), but there has been little research on earthworm response to arsenite.

Bouché (1977) defined three ecologically different behavior patterns in earthworm species: epigeic, anecic and endogeic, according to their burrowing habits, among other characteristics. The two species used in this study were *Eisenia fetida* (Savigny, 1826) and *Metaphire californica* (Kinberg, 1867). Both are generally considered to epigeic, or surface-dwelling, and a photo of each is provided in Supplementary

* Corresponding author.
E-mail address: yinshengli@sjtu.edu.cn (Y. Li).

https://doi.org/10.1016/j.geoderma.2018.08.015
Received 19 January 2018; Received in revised form 6 August 2018; Accepted 8 August 2018
0016-7061/

Fig. 1a and b. *E. fetida*, however, is known to display facultative endogeic behavior when confined to mineral soils (Tang et al., 2016), and the same would be expected for *M. californica*. *E. fetida* is normally found at sites rich in leaf litter or compost. *M. californica* (Supplementary Fig. 1a) is widely distributed globally (Chang et al., 2009), and is common and at times the most abundant earthworm species present in farm soils in the Shanghai area, especially at sites with higher soil organic matter content. However, there have been few previous studies of *M. californica*. We found only brief comments on its distribution and taxonomic status in two papers (Chang et al., 2009; Zhang et al., 2015). For both species the maximum burrow depth (MBD) is typically < 10 cm below the soil surface, but can extend to 30 cm depth (Li et al., 2016; Tang et al., 2016).

The presence of earthworms in agroecosystems can lead to a 25% increase in crop yield and a 23% increase in aboveground biomass (van Groenigen et al., 2014). Therefore, it is important both from an ecological and agricultural perspective to have information on *M. californica* behavior in soils and an understanding of the response of this species to various contaminants.

E. fetida (Supplementary Fig. 1b) is often used as a test species in ecotoxicology research, and a number of studies have reported burrowing behavior changes of *E. fetida* in response to a range of soil pollutants. The burrow length of *E. fetida* in soil exposed to Hg (Tang et al., 2016), the herbicide imidacloprid (Capowiez et al., 2003), and the organic compounds18-crown-6 (Du et al., 2014) and enrofloxacin (Li et al., 2016), was reduced compared with that of control groups. Toxic effects of cadmium on *E. fetida* were enhanced in the presence of residues of the antibiotic enrofloxacin (Li et al., 2016). Thus, the prior research makes *E. fetida* a useful benchmark species for this study.

We were interested to study the behavioral component of earthworm response to As because the roles of organisms in an ecosystem are determined both by physiology and behavior, and in the case of earthworms, the behavioral component is up to now less studied. Such information should facilitate synthesis of knowledge about potential ecological risks of arsenite in soil. In this study, we monitored burrowing of the two earthworm test species in 2-dimensional terraria. The terraria contained farm soil with no contaminant added (control) or spiked with sodium arsenite at various concentrations. An avoidance test of earthworms to arsenite was also conducted to aid interpretation of data. A further aim was to explore if there is potential for earthworm behavior data to be used as a bioindicator for As contamination in ecotoxicology and environmental safety applications.

2. Materials and methods

2.1. Reagents, earthworms and soil

Adult *M. californica* earthworms were obtained from 3 sites within the campus of Shanghai Jiao Tong Univerisity, Shanghai, China, which were originally farmed but have now been fallow for 20 years. Specimens of *E. fetida* were purchased from a local commercial supplier. Healthy adult *E. fetida* earthworms (2 months old, with a weight of 300–500 mg, and with well-developed clitellae) were used for all experiments. Adult *M. californica* specimens were collected from the field and had similar weight and a well developed clitellum.

To provide soil for laboratory terraria, surface soil (0–20 cm) was collected from one of the sites at Shanghai Jiao Tong University used to obtain specimens of *M. californica*. The natural soil bulk density was measured before soil collection, and the soil was then air-dried, mixed, and passed through a 2 mm sieve, and stored for packing into terraria as described below. Key physical and chemical properties of the soil, analyzed using methods described by Bao (2000) were: pH 7.75; electrical conductivity 129.5 $\mu S \cdot cm^{-1}$ at 20% gravimetric soil moisture; bulk density 1.09 $g \cdot cm^{-3}$, porosity 59.3%, organic matter content 18.6 $g\ kg^{-1}$; total phosphorus 0.958 $g \cdot kg^{-1}$; total nitrogen 1.85 $g \cdot kg^{-1}$; total potassium 2.53 $g \cdot kg^{-1}$. Total arsenic content was $7.54 \times 10^{-3}\ g \cdot kg^{-1}$, which complies with the China Quality Standard for GradeIsoil (< 15×10^{-3} g total arsenic$\cdot kg^{-1}$ soil).

2.2. Test of contaminant effects on burrowing behavior

The burrowing activity of *E. fetida* and *M. californica* was observed in 2D terraria, built to specifications adapted from those described elsewhere (Du et al., 2014; Li et al., 2016; Tang et al., 2016). Briefly, each terrarium consisted of two glass sheets (width 30 cm, height 42 cm) held 3 mm apart by spacer strips, and filled with soil prepared as above. Prior to the experiment we performed tests to check the LC_{50} of arsenite to *E. fetida* in the soil used, and obtained a value of 122 $mg.kg^{-1}$. Based on these data, we set five arsenic concentrations (5, 10, 20, 40, 80 mg As kg^{-1} dry soil) in this study. Sodium arsenite ($NaAsO_2 \geq 99.0\%$) was obtained from Merck KGaA, Germany, and dissolved in ultrapure water (resistivity 18 MΩ.cm at 25 °C), and diluted to the desired concentrations. Appropriate aliquots were then added to batches of farm soil as above. To fill terraria, the dry soil quantity required to achieve the same bulk density in terraria as in the natural field soil (1.09 $g \cdot cm^{-3}$) was first weighed, then arsenite solution, and water added to bring soil moisture content (mass water:mass dry soil) to 25%. The control soil was mixed with the same volume of distilled water. Filled terraria were fitted with a gauze cover to allow gas exchange at the soil surface. The experiment had 4 replicates (24 terraria); each terrarium was stocked with one adult earthworm (weight 400–600 mg; length 5.0–6.5 cm) with a well-developed clitellum, after allowing time for depuration. Terraria were kept in a dark, climate-controlled room at 20 ± 1 °C. After placing earthworms in terraria, burrowing behavior was observed six times per day at 4 h intervals for the next 7 days, using a tracing method (Du et al., 2014; Tang et al., 2016). Earthworm movement and behavior was observed easily in these terraria. Tracing of burrows was carried out under a very low intensity white light placed at a distance from the terraria, to minimize disturbance to the earthworms. From daily burrowing data recorded in this way, the cumulative burrow length, MBD (the greatest depth reached during the experimental period) and rate of burrow reuse were determined from analysis of digital images of the burrow tracks using ArcGIS10.0 (ESRI, 2011). Sometimes, earthworms were observed to return to the same channel between the two recording time points. We termed this category of burrows ‘reused’. Earthworm biomass was recorded before and after the burrowing behavior test.

2.3. Avoidance tests

The avoidance tests were conducted in plastic containers based on ISO, 2008 17512-1 (2008). One half of the container was filled with 300 g dry arsenite contaminated soil with the same concentration range as the above burrowing test, and the other was filled with the same amount of control soil. The soil was weighed in two 300 g lots (control and contaminated) for each test box, and 75 mL water added to each soil lot and mixed through. When preparing the As contaminated soils, water was added in two steps: first the arsenite was dissolve into 30 mL water and added to the soil, then the additional water needed to reach the target soil moisture 25% was added, and the soil placed into the plastic containers. Finally, the test earthworms were placed in the pots. Pots were stored in the dark, and left undisturbed for 48 h, at which point the earthworm distribution between the control and contaminated sections of containers was counted. There were 5 replicates and 10 adult earthworms per replicate. Both earthworm species were tested.

2.4. Statistical analyses

Statistical analysis was carried out using the repeated measures option of the GLM Procedure in SAS (SAS Institute Inc., Cary, NC). Data on burrowing length per 4 h period in each terrarium were aggregated