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Toxicological and biochemical responses of the earthworm *Eisenia fetida* exposed to contaminated soil: Effects of arsenic species

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HIGHLIGHTS

- We conducted a toxicology research for four different arsenic species.
- Dose-response relationship between inorganic arsenics and biomarkers were found.
- As(III) species has more toxicity than As(V) at molecular and subcellular level.
- Demethylation process of organic arsenics induces adverse effects on organisms.
- Arsenic in earthworm could be detoxified by a biotransformation pathway.

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ABSTRACT

Arsenic is a pollutant that can be detected in different chemical forms in soil. However, the toxicological effects of different arsenic species on organisms have received little attention. In this study, we exposed earthworms *Eisenia fetida* to artificial soils contaminated by arsenite [As(III)], arsenate [As(V)], monomethylarsonate (MMA) and dimethylarsinate (DMA) for 28 and 56 days. Three biomarkers including lipid peroxidation (LPO), metallothioneins (MTs) and lysosomal membrane stability (LMS) were analyzed in the organisms. In addition, the contents of total arsenic and arsenic species in earthworms were also determined to investigate the effects of bioaccumulation and biotransformation of arsenic on biomarkers and to evaluate the dose-response relationships. The results showed that the relationship between the three biomarkers and the two inorganic arsenic species were dose dependent, and the correlation levels between the biomarkers and As(III) were higher than that between the biomarkers and As(V). Trivalent arsenic species shows more toxicity than pentavalent arsenic on the earthworms at molecular and subcellular level, including oxidative damage, MTs induction and lysosomal membrane damage. The toxicity of MMA and DMA was lower than inorganic arsenic species. However, the occurrence of demethylation of organic arsenics could lead to the generation of highly toxic inorganic arsenics and induce adverse effects on organisms. The biotransformation of highly toxic inorganic arsenics to the less toxic organic species in the earthworms was also validated in this study. The biomarker responses of the earthworm to different arsenic species found in this study could be helpful in future environment monitoring programs.

1. Introduction

Arsenic (As) is a widely occurring environmental contaminant with inorganic arsenic recognized as a class-one carcinogen (Hartley et al., 2013). Arsenic in the soil is derived from both the parent materials and anthropogenic sources such as industrial wastes, mining activities, the use of arsenic-containing biocides/pesticides, irrigation of arsenic-contaminated water, etc. (Meharg et al., 2009). Arsenic contamination in soil may cause biotoxicity in plants and animals. Its entry into the food chain via bio-accumulation and bioconcentration can also pose a significant risk to human health. Therefore, large numbers of studies have been conducted for arsenic analysis in soil environment, and arsenic element has been listed in many quality standards and safety

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standards in many countries (Smith et al., 2002; Kim et al., 2005; Kapaj et al., 2006; Chakraborti et al., 2013; Hartley et al., 2013). However, most of these researches and standard documents only focused on total arsenic or one arsenic species (e.g., pentavalent arsenic), while different arsenic species were seldom considered and differentiated.

The toxicity, biological availability and transport mechanisms of arsenic depend upon its chemical forms (Ruiz-Chancho et al., 2007). Previous studies have proved that the inorganic arsenic including arsenite [As(III)] and arsenate [As(V)] are more toxic than organic arsenic species for most organisms, and As(III) is considered to be more toxic than As(V) (Langdon et al., 2003). Among the organic arsenic species, monomethylarsonate (MMA) and dimethylarsinate (DMA) are thought to be less toxic, while arsenobetaine (AsB) and arsenosugars are considered to be of non-toxicity (Liu et al., 2013). Although arsenic is mainly present in inorganic forms (trivalent and pentavalent arsenic) in soils, the presence of organic compounds MMA and DMA has also been reported (Takamatsu et al., 1982; Chappell et al., 1995; Chatterjee and Mukherjee, 1999; Yehl et al., 2001; Márquez-García et al., 2012). Therefore, the toxicity of different arsenic species should be taken into consideration when activities including risk assessment and toxicity test were conducted for arsenic element in soils.

Earthworms are very important species for soil formation and organic matter breakdown in terrestrial soil environments, thus having been considered to be indicators of land use and soil fertility (Paoletti, 1999). Due to their strong interaction with soil, earthworms can be profoundly affected by soil pollution and accumulate contaminants in the body. These features, among others, have led to the use of earthworms as bioindicator organisms of soil contamination (Lanno et al., 2004; Xiao et al., 2006). In this study, *Eisenia fetida* was chosen because of the standardization of acute and chronic ecotoxicological assays. It has been widely used as a model species in standard tests for evaluating the adverse effects of chemicals on soil organisms (OECD, 1984; Calisi et al., 2011; Chen et al., 2012).

The use of biomarkers in earthworms has received increased attention over the past few years. Compared with traditional approach to soil quality assessment based on the analysis of the concentrations of pollutants in soil, the biomarker approach can offer more information about the stress response of organism to toxicants (Svendsen et al., 2004; Sanchez-Hernandez, 2006; Calisi et al., 2011; Muangphra et al., 2013). Thus, there is an increasing interest for investigating biochemical responses of earthworms to contaminates in order to develop sensitive biomarkers in response to soil chemical contamination (Beliaeff and Burgeot, 2002). However, only a few studies aimed at the evaluation of the impact of arsenic stress on earthworms have been performed, and there has been no literature regarding the toxicity of different arsenic species on earthworms and the dose-response relationship between biochemical markers and arsenic species (Lee and Kim, 2009; Button et al., 2010).

In this study, earthworm *E. fetida* were exposed to artificial soils contaminated by four common arsenic species in real environment including As(III), As(V), MMA and DMA, over a period of 28 and 56 days. Three biomarkers (lipid peroxidation, metallothioneins and lysosomal membrane stability) were selected for toxicity assessment of the four arsenic species. Lipid peroxidation (LPO) is a biomarker for oxidative damage. It has been used as an integrated biochemical response in earthworms associated with the disruption in the lipid component of cellular membranes, which reflects exposure and toxicity to pollutants, e.g. metals/metalloids (Colacevich et al., 2011; Markad et al., 2015; Zhang et al., 2015). Metallothioneins (MTs) have been widely used as specific biomarkers for metal and metalloid contamination, as several studies have found clear time- and dose-response relationships between metal exposure and MTs concentration or expression (Gruber et al., 2000; Brulle et al., 2006; Demuynck et al., 2007). Lysosomes are among the subcellular systems shown to be directly affected by pollution, leading to the development of lysosomal membrane stability as a subcellular biomarker of contamination stress (Svendsen et al., 2004; Rocco et al., 2011; Chen et al., 2012). The method for lysosomal membrane stability assessment is the neutral-red retention time (NRRT) assay, which has been proved to be reliable and dose-related to be used in terrestrial systems (Weeks and Svendsen, 1996).

The aim of the present study was, therefore, to evaluate the biomarker responses of the earthworm, *E. fetida*, to the four different arsenic species in artificial soils. Furthermore, the contents of total arsenic and arsenic species in earthworms were also determined to investigate the effects of bioaccumulation and biotransformation of arsenic on the biomarkers and to evaluate the dose-response relationships.

2. Materials and methods

2.1. Chemicals and earthworms

Standard solutions of As(III) (1.011 μmol mL^{-1}), As(V) (0.233 μmol mL^{-1}), MMA (0.335 μmol mL^{-1}) and DMA (0.706 μmol mL^{-1}) were purchased from the China Standard Certification Center. Ultrapure water (18 MΩ) was obtained by using a Milli-Q water purification system (Millipore, USA). Chemicals used for biomarker analysis were obtained from Sigma-Aldrich China Co. (Shanghai, China). All other reagents were of analytical grade and obtained from Beijing Chemical Co. (Beijing, China).

Adult *Eisenia fetida* earthworms sexually mature with well-developed clitella were purchased from a local commercial earthworm farm company in Jinan, China. The earthworms were selected from a synchronized culture with the same age for each exposure group and control group. The weight of the selected earthworms ranged from 350 to 450 mg. They were acclimated for 7 d to the artificial soil under controlled conditions (20 °C, 12 h light/12 h dark cycle, 80% ambient humidity) prior to test.

2.2. Experimental setup

The artificial soil was prepared as described in the OECD guideline 207 (OECD, 1984). The composition of the OECD soil (dry weight) was a mixture of 70% quartz sand, 20% kaolinite, and 10% finely ground sphagnum peat, with pH adjusted to 6.5 by addition of calcium carbonate.

As the four arsenic species showed different degrees of toxicity, in order to compare biomarker responses of the earthworm to different arsenic species, the exposure concentrations were designed according to the 14 d median lethal concentration (14 d LC_{50}) of the four arsenic species obtained in an acute toxicity test for artificial soil following the OECD guideline 207. The results of our pre-experiment following the standard test showed that the 14 d LC_{50} for As(III), As(V), MMA and DMA were 293 ± 9, 352 ± 14, 3425 ± 35, 3730 ± 26 mg kg^{-1}, respectively. Therefore, based on the limit value of arsenic in Chinese Environmental Quality Standard for Soils (GB 15618-1995) and the arsenic concentration in typical contaminated soils in China (Zhao et al., 2014), the maximum concentrations of spiked soils were set at one-tenth of the 14 d LC_{50}, namely 29.3, 35.2, 342.5, 373.0 mg kg^{-1} for the four arsenic species. These concentration values were represented by letter D, and the corresponding concentration series for each arsenic species were set as 1/4D, 1/2D, 3/4D and D, which are shown in Table 1.

Spiking solutions were prepared using standard solutions and