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Toxicity of arsenite to earthworms and subsequent effects on soil properties

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ABSTRACT

Arsenic (As) is widely distributed in soil and is toxic to plants, animals and humans. In this study, earthworms (*Eisenia fetida*) were exposed to five concentrations of sodium arsenite (5, 10, 20, 40, and 80 mg As kg^{-1}) in farm soils for 28 d. With increasing soil As(III) concentrations, As bioaccumulation in earthworms increased (maximum bioaccumulation factor 3.77), and levels of reactive oxygen species (ROS) and malondialdehyde (MDA) were elevated. The expression of nuclear factor erythroid 2- related factor 2 (Nrf2) and heme oxygenase -1 (HO-1) were upregulated by As in a dose-dependent pattern, and reached 5.93 and 2.94 times the control values for Nrf2 and HO-1 respectively, at 28 d in the 80 mg As kg^{-1} soil treatment. Similarly, DNA damage, as measured in earthworm sperm using the comet assay, increased with increasing As(III) concentrations, with 'Olive tail moment' values in the comet assay ranging from c. 0.5 in Control to c. 3.5 at 80 mg As kg^{-1} soil. In contrast, activity of the antioxidant enzymes, superoxide dismutase (SOD) and catalase (CAT), decreased. These results indicate that As(III) caused oxidative stress that resulted in damage to lipids and DNA. Nrf2 and HO-1 protein expression was demonstrated in earthworms for the first time to our knowledge, and found to be a sensitive biomarker of arsenic contamination. The presence of earthworms was also found to change the distribution of As in soil, in particular, reducing the proportion in the residual fraction and increasing the proportion in As bound to Fe-oxides. This may result in increased bioavailability of bound arsenic. Soil organic matter, NH_4^+-N, NO_3^--N and available K were indirectly changed by the As(III) through its toxicity to earthworms. This study helps to inform future assessments and biomonitoring of soil arsenic contamination.

1. Introduction

Arsenic (As) is widely distributed in the environment with elevated concentrations of As in soils attributable to natural elevations or anthropogenic contamination (Kwon et al., 2012; Hartley et al., 2013; Wang and Cui, 2016). Arsenic contamination in soil may cause toxicity in plants and animals (Jack et al., 2003; Pendergrass and Butcher, 2006). Although arsenic has four oxidation states (V, III, 0, –III), inorganic As, mainly arsenate [As(V)] and secondarily arsenite [As(III)], are commonly present in aquatic and soil environments (Oremland and Stolz, 2003). Arsenic content in contaminated soils in China varies in different reports. Most reported values range from several hundred mg As per kg soil, to less than 100 mg As per kg soil (Huang et al., 2006, 2013; Liu et al., 2010). Values can be even higher in some areas with heavy As pollution, with one report of 935 mg As.kg^{-1} soil (Liu et al., 2010).

The inorganic arsenicals, primarily, sodium arsenite, were historically widely used as insecticides and preservatives, and are still extensively used in modern industry (Mandal and Suzuki, 2002). As(III) is 100 times more toxic than As(V) as it is the more soluble and mobile form (Rosen, 2002). Arsenite has high affinity for thiol groups in organisms and results in inhibition of respiration by binding to vicinal thiols in pyruvate dehydrogenase and 2-oxoglutarate dehydrogenase (Oremland and Stolz, 2003).

Earthworms are one of the most important soil ecosystem engineers, affecting soil functioning through their burrowing, feeding and casting activities (Wu et al., 2015). A meta-analysis showed that the presence of earthworms in agroecosystems led to a 25% increase in crop yield and a 23% increase in aboveground biomass (van Groenigen et al., 2014). Indicators based on earthworm data have been used as tools for soil monitoring, characterization and risk assessment (Pérès et al., 2011). Elevated arsenic in soils can result in a number of effects on earthworms

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such as mortality (Langdon et al., 2001a), changes in antioxidase activity (Wang and Cui, 2016), decreased activity (Langdon et al., 1999, 2001b), and avoidance behavior (Langdon et al., 2001b). Reproductive impacts of arsenic toxicity were studied in field soils or As(V) contaminated soils using the cocoon reproduction as the test parameter (Leduc et al., 2008; Lee and Kim, 2008; Langdon et al., 2009; Neaman et al., 2012; Yesudhason et al., 2013), but without exploration of the molecular mechanism. Some routine toxicological parameters such as antioxidase activities, lipid peroxidation, metallothionein induction, and lysosomal membrane damage have been studied in earthworms exposed to arsenic, for the purpose of assessing their application as biomarkers of early arsenic exposure in soil ecotoxicology (Wang et al., 2016b; Rahman et al., 2017).

Cellular oxidative stress is a common response of the exposure of organisms to stress, including pollutant stress (Li et al., 2015). Cellular oxidative stress induces many negative effects, including membrane peroxidation, ionic leakage, protein cleavage, and DNA strand breakages. Oxidative stress arises when there is an imbalance between reactive oxygen species (ROS) and antioxidant activity mainly consisting of antioxidases such as superoxide dismutase (SOD), catalase (CAT) and glutathione peroxidase (GSH-px) (Sanchez-Hernandez, 2006). Arsenic may participate in the cellular oxidation-reduction reactions resulting in the formation of excess ROS such as superoxide anions O_2^- and hydroxyl radicals (·OH) via a chain reaction (Wang and Cui, 2016; Wang et al., 2016b). The activities of antioxidant enzymes can effectively remove ROS and reduce the lipid peroxidation and associated malondialdehyde (MDA) in earthworms exposed to arsenic (Wang and Cui, 2016; Wang et al., 2016b). Antioxidases and MDA have been shown to be important biomarkers of many soil contaminants (Li et al., 2016; Nneji et al., 2016). DNA damage has been reported in earthworms exposed to heavy metals and pesticides (Mateos et al., 2008; Wang et al., 2016a). However no studies were found by the authors on the effect of As(III) on earthworm sperm, which has a direct effect on population stability and development.

The nuclear factor erythroid 2-related factor 2 (Nrf2) is a key transcription factor regulating the expression of a range of antioxidant and detoxification enzymes, including heme oxygenase-1 (HO-1), likely through the effects of catalytic by-products of Nrf2 (Motohashi and Yamamoto, 2004; Music et al., 2014; Zhu et al., 2016). As(III) resulted in up-regulation of Nrf2 and HO-1 protein levels in human hepatocytes, microvascular endothelial and bronchial epithelial cells (Meng et al., 2010; Li et al., 2013), mouse liver, bladder and kidney tissue (Jiang et al., 2009; Miltonprabu et al., 2017), although to our knowledge the presence of Nrf2 and HO-1 has not been reported for earthworms. Inducible HO-1 could exhibit anti-apoptotic, anti-oxidative and anti-inflammatory properties (Dulak et al., 2008). The critical role of HO-1 has also been reported in studies of organ injuries (Billings et al., 2014) and has been identified as a response biomarker for arsenic exposure in various types of cells (Gong et al., 2016).

Soil heavy metal contamination may alter microbial and ion absorption in soils and thus change the soil physical and chemical properties (Pérès et al., 2011; Romero-Freire et al., 2015). On the other hand, heavy metals may have toxic effects on earthworms and thus indirectly affect the soil properties through changed earthworm activity. The activity of the earthworm in the soil can change some of the soil properties, such as the pH, dissolved organic carbon etc, as well as the fraction of the heavy metals such as Zn, Cu, Cr, Cd, Co, Ni, and Pb (Wen et al., 2004). Heavy metals including As have been reported to affect soil enzyme activities, and microbial biomass (Kuperman and Carreiro, 1997). The changes of biological properties can further alter the soil chemical and physical properties. However, we are unaware of any research into the indirect effects of As contamination on soil properties, through As toxicity to earthworms.

For a particular organism, arsenic bioavailability and hence toxicity depends on the soil fractions to which As is bound (Yang et al., 2016; Rahman et al., 2017). Therefore, assessment of the distribution of As between different fractions in soils, which can be accomplished by using sequential extraction procedures, may offer greater insight into the biological response of As than total As concentrations (Wu et al., 2006, 2016). Arsenic in soil may also affect soil P, because similarity of chemical properties of the two elements can cause competition during their adsorption onto soil particles (Zeng et al., 2012).

The overall aim of this study was to evaluate the effects of As(III) at a range of sublethal concentrations on the physiology of the earthworm *Eisenia fetida*. In particular we wished to document the time course and dose thresholds for ROS generation, antioxidase induction, lipid peroxidation and sperm DNA damage to provide a greater understanding of the mechanisms of As(III) toxicity. We also sought to establish the involvement of Nrf2 and HO-1 in As defense in earthworms, and to explore the indirect effect of As contamination on soil properties arising from the toxicity to earthworms. Such information could assist with formulation of strategies to manage As contamination, or lead to identification of biomarkers for As exposure.

2. Materials and methods

2.1. Reagents, soils and animals

Sodium arsenite ($NaAsO_2$, ≥99.0%) was obtained from Merck KGaA, Germany. Primary antibodies of Nrf2 (H-300: sc-13032), HO-1 (H-105: sc-10789), β-actin (I-19: sc-1616) and corresponding secondary antibodies were all purchased from Santa Cruz Biotechnology (Santa Cruz, CA, USA), the BCA Protein Assay Kit, RIPA (Radio Immunoprecipitation Assay) Lysis Buffer and the ROS Assay Kit was purchased from Beyotime Biotechnology, the Bradford Protein Assay Kit was purchased from Sangon Biotech (Shanghai) Co. Ltd. All other chemicals used were of the highest grade commercially available. Water used in all the preparations was ultrapure water with resistivity of 18 MΩ.cm.

Soil from farm land in the Shanghai suburban district, which had not been farmed for more than 20 years, was air-dried and sieved to 2 mm ped size. This rice paddy soil is common in China, and some localities where it occurs have been polluted by arsenic. The soil properties such as pH, electrical conductivity (EC), organic matter content (OM), total phosphorus (TP), Olsen-P, total potassium (TK), available K (Av-K), total nitrogen (TN), NH_4^+-N and NO_3^--N were analyzed by Bao's method (2000) and the results were as follows: pH 7.75; EC 129.5 $\mu S.cm^{-1}$; OM 18.6 g kg^{-1}; TP 0.958 $g.kg^{-1}$; Olsen-P 32.0 $mg.kg^{-1}$; TN 1.85 $g.kg^{-1}$; NH_4^+-N 12.5 $mg.kg^{-1}$; NO_3^--N 11.5 $mg.kg^{-1}$; TK 2.53 $g.kg^{-1}$; Av-K 295 $mg.kg^{-1}$. Total arsenic content in the soil collected from the field was 7.54 $mg.kg^{-1}$.

Earthworms (*E. fetida*) were purchased from a commercial breeder. A batch of cocoons was hatched specifically for the experiment and reared to reproductive maturity so that the earthworms used were of a similar age. They were introduced into the collected soils at least two weeks before starting the experiment, to adapt to the soil environment. Cow dung was dried at 60 °C, ground to pass through a 2 mm sieve, and added as food. Healthy adult earthworms with a well-developed clitellum (0.3–0.5 g) were used for the arsenic exposure experiments. When confined to a mineral soil such as that used in this experiment, *E. fetida* displays a facultative endogeic behavior, burrowing to 20–30 cm soil depth (Li et al., 2016).

2.2. Arsenite exposure experiments

Based on published data for As contamination of soils in China, mentioned above, and preliminary testing of the acute toxicity of arsenite to *E. fetida* (we found an LC_{50} of 122 $mg.kg^{-1}$, data not shown), we chose five concentrations (5, 10, 20, 40, 80 mg added As kg^{-1}dry soil, additional to the background level of 7.54 mg As kg^{-1}soil) to be spiked with the soils in this study. A single stock solution of sodium arsenite in water was prepared, diluted to the required concentrations,