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Bioavailability and chronic toxicity of bismuth citrate to earthworm *Eisenia andrei* exposed to natural sandy soil

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ABSTRACT

The present study describes bioavailability and chronic effects of bismuth to earthworms *Eisenia andrei* using OECD reproduction test. Adult earthworms were exposed to natural sandy soil contaminated artificially by bismuth citrate. Average total concentrations of bismuth in soil recovered by HNO_3 digestion ranged from 75 to 289 mg/kg. Results indicate that bismuth decreased significantly all reproduction parameters of *Eisenia andrei* at concentrations ≥ 116 mg/kg. However, number of hatched cocoons and number of juveniles seem to be more sensitive than total number of cocoons, as determined by IC_{50}; i.e., 182, 123 and > 289 mg/kg, respectively. Bismuth did not affect *Eisenia andrei* growth and survival, and had little effect on phagocytic efficiency of coelomocytes. The low immunotoxicity effect might be explained by the involvement of other mechanisms i.e. bismuth sequestered by metal-binding compounds. After 28 days of exposure bismuth concentrations in earthworms tissue increased with increasing bismuth concentrations in soil reaching a stationary state of 21.37 mg/kg dry tissue for 243 mg Bi/kg dry soil total content. Data indicate also that after 56 days of incubation the average fractions of bismuth available extracted by KNO_3 aqueous solution in soil without earthworms varied from 0.0051 to 0.0229 mg/kg, while in soil with earthworms bismuth concentration ranged between 0.310–1.347 mg/kg dry soil. We presume that mucus and chelating agents produced by earthworms and by soil or/and earthworm gut microorganisms could explain this enhancement, as well as the role of dermal and ingestion routes of earthworms uptake to soil contaminant.

1. Introduction

Earthworms are the most abundant soil organisms, they represent around 60–80% of the soil biomass (Rida, 1994). They are able to modify physical, chemical and biological properties of soil, as a result of their activities and their interactions with soil organic and inorganic constituents (Edwards, 2004; Lemtiri et al., 2014). Earthworm activities in soil enhance organic matter decomposition and nutriment cycling and influence soil structure and porosity. Thus by improving soil aeration, soil fertility and food availability earthworms affect the distribution and activity of soil biota (Cao et al., 2015; Salmon, 2001). The role of earthworms in metal pollution monitoring is widely recognized in terrestrial ecosystems. Earthworms are one of the best bioindicators of trace metals, because they can biotransform and bioaccumulate metal ions in their body (Usmani and Kumar, 2015).

Bismuth (Bi) is a rare metal in soil, its average background concentration in uncontaminated soil is 0.2 µg/g (Bowen, 1979). Bismuth compounds are widely used in various industrial applications including manufacturing of drugs, cosmetics and fusible alloys. For some years, Bi has been increasingly used as non-toxic alternative to lead (Pb) in various other industrial applications such as ammunition formulations, hunting shot, fishing sinkers and plumbing fixtures (Fahey and Tsuji, 2006; Urgast et al., 2012), water pipes (Nagata, 2015). Such increased industrial and medicinal use of Bi could increase considerably the concentration of this element in soils and aquatic environment. For example, some studies showed that samples of soil collected from military training areas contain high concentrations of Bi, up to 187 times higher than concentrations measured in background samples (Marois et al., 2004), reaching 184.8 mg/kg dry soil (Berthelot et al., 2008) and ranging between 8 and 5140 mg/kg (Johnson et al., 2005). Despite its increased presence in the environment, no data are currently available on the chronic toxic effects of Bi on terrestrial invertebrates such as earthworms. A recent study on the acute toxicity of Bi to the earthworm reported that the 14 d LC_{50} was 416 mg Bi/kg dry soil, and

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no effect observed at 298 mg/kg dry soil (Omouri et al., 2016). In general, little is known about the fate, behavior, transport and ecotoxicological effects of Bi in terrestrial ecosystems. However, a survey of literature reports studying the toxic effect of Bi based compounds on human and wild animal health showed contradictory opinions. Some studies reported that Bi is a harmless metal to humans and wild animal health (Sano et al., 2005; Tillman et al., 1996; Yadav et al., 2012). While others showed that Bi may reduce sperm metabolism and contribute to infertility in men (Ghaffari and Motlagh, 2011) and may accumulate in mouse neuronal tissue (Larsen et al., 2005).

Heavy metals are the most common soil contaminants. Currently, it is widely recognized that measuring metal bioavailability provides a better indicator of metal toxicity than using total metal concentrations in contaminated soil (Lanno et al., 2004; Mahbub et al., 2017b, 2017c). However bioavailability is a very complex concept, different definitions for bioavailability exist across many disciplines including pharmacology and toxicology, thus creating ambiguity and confusion for environmental scientists (Semple et al., 2004). In the present study, we considered the concept of bioavailability as defined by Peijnenburg et al. (1997) and ISO (2008). According to these authors there are three phases to assess bioavailability of metals in soil. In the first, environmental availability (also referred to as bioaccessibility) is considered to designate the fraction of metal in soil resulting from a physicochemically driven desorption process; in the second, environmental bioavailability represents the fraction of metal taken up by the organism following the uptake processes, and thirdly, toxicological bioavailability represents the internal concentration of the contaminant related to toxic effects. Although the three fraction of availability are widely recognized, structural relationship between them has been less investigated. This is probably due to the absence of a single, standardized and direct method to actually measure each fraction and the limits or inability of traditional statistical approaches to explicitly address structural assumptions (Beaumelle et al., 2016). Earthworms are exposed to soil metals by two uptake routes, the first via the dermal surface of the dissolved fraction in pore water and the second by direct ingestion of the fraction adsorbed/desorbed and sequestrated in soil particles (Le Roux et al., 2016; Ma et al., 2009). Current methods to evaluate environmental availability are not suitable to assess the portion of metal absorbed by the earthworms via the ingestion route. This lack of assessment method indicates the obstacles encountered to measure and to predict the actual fraction of bioaccessible metal to earthworms. Furthermore, it is known that once the chemical crosses the cellular membrane, others processes like storage, transformation and elimination take place. Ultimately, toxicological bioavailability represents the internal concentration of metal causing the biological responses and the toxic effects. Therefore in the absence of direct methods to evaluate toxicological bioavailability, biological measurement as biomarkers and toxicological endpoints (e.g. reproduction, growth) continue to be widely used practices. Reproduction parameters have been recommended by some authors as more suitable to evaluate chemicals effects on earthworm's populations (Mahbub et al., 2017a; Žaltauskaitė and Sodienė, 2010). In addition, the use of biomarker approach to estimate the sublethal effects of contaminants such as metals has increased sharply in recent years. Several studies have reported that various metals (e.g. mercury, cadmium, zinc, lead, copper) affected the viability and phagocytic activity of earthworm coelomocytes (Fugère et al., 1996; Fuller-Espie et al., 2011; Sauvé and Fournier, 2005).

The aims of the present study were to investigate bioavailability and sublethal toxic effects of bismuth on the earthworm *Eisenia andrei*, in terms of reproduction parameters, growth and phagocytosis activity of immune cells.

2. Materials and methods

2.1. Chemicals and reagents

Bismuth (III) citrate ($C_6H_5BiO_7$), formaldehyde 37%, and phosphate buffer solution (PBS) were purchased from Sigma–Aldrich. The HNO_3 and HCl were of analytical grade. The standard reference soils TILL-2 and TILL-4 used in this study were provided by Mining and Mineral Sciences Laboratories, Ottawa, Canada. Information on physical and chemical characteristics of the two reference soils TILL-2 and TILL-4 are available on the Natural Resources Canada website (http://www.nrcan.gc.ca/mining-materials/certified-reference-materials/certificate-price-list/8137). Deionized water (ASTM Type II) was obtained using a Millipore Super-Q water purification system or Zenopure Mega-90. Glassware and polyethylene containers were washed with acetone, soaked in nitric acid solution (10%, v/v), and rinsed with deionized water.

2.2. Soil characteristics and samples preparation

The natural sandy soil used in this study was obtained from Canadian Forces Base Valcartier (Qc, CAN) located approximately at 25 km north of Quebec city (latitude: 46.90, longitude: 71.49 and altitude: 170 m). The soil was collected from a site located in a non-contaminated area at 0–15 cm deep. This soil is representative of soil from Canadian range training area and similar soils were used in earlier toxicological studies to consider higher bioavailability of explosive and metals (Dodard et al., 2013; Robidoux et al., 2004; Savard et al., 2007). Physical and chemical characteristics of this soil are shown in Table 1. Soil was spiked by adding bismuth citrate ($C_6H_5BiO_7$) in the powder form to soil samples separately to obtain the following nominal concentrations: 100, 150, 200, 250, 300 and 350 mg Bi/kg dry soil. The nominal concentrations selected were based on the results of preliminary lethality tests (data not shown). A negative control (without Bi citrate added) was prepared by adding deionized water only to the soil. Four replicates for each Bi concentration tested were prepared. Spiked soils were mixed for 20 ± 2 h in a rotary mixer to obtain a homogeneous distribution of Bi citrate. The soil was then rehydrated with deionized water to 75% of its water holding capacity (WHC) by adding 46 mL/500 g dry soil. WHC was determined by saturating the soil with deionized water and measuring the water content (Robidoux et al., 2000). Water content was determined in separate studies, by measuring the loss of soil weight after drying for 18 h at 105 °C in an oven. After hydration, the soil samples were mixed overnight in a rotary mixer, and

Table 1
Summary of physical and chemical characteristics of natural soil collected at the non-contaminated area.

Parameter	Measurement
Ph	5.96
Humidity (%)	7.5
Total organic carbon (%)	2
Sand (%)	97.6
Silt (%)	1.6
Clay (%)	0.7
Argent (ppm)	< 2
Arsenic (ppm)	< 5
Barium (ppm)	11
Cadmium (ppm)	< 0.5
Cobalt (ppm)	2
Magnesium (ppm)	180
Mercury (ppm)	< 0.02
Molybdenum (ppm)	< 1
Nickel (ppm)	1
Lead (ppm)	< 5
Zinc (ppm)	31
Bismuth (ppm)	< 0.05