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Ecotoxicology of mercury in tropical forest soils: Impact on earthworms

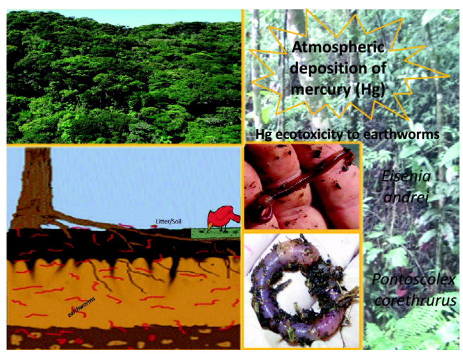
Andressa Cristhy Buch [a,*], George Gardner Brown [b], Maria Elizabeth Fernandes Correia [c], Lúcio Fábio Lourençato [a], Emmanoel Vieira Silva-Filho [a]

[a] Department of Environmental Geochemistry, Fluminense Federal University, Outeiro São João Baptista, s/n., Centro, 24020-007 Niterói, RJ, Brazil
[b] Embrapa Forestry, Estrada da Ribeira km. 111, C.P. 319, 83411-000 Colombo, PR, Brazil
[c] Embrapa Agrobiology, BR 465 km. 7, 23890-000 Seropédica, RJ, Brazil

HIGHLIGHTS

- *E. andrei* showed higher sensitivity to Hg in behavioral, acute and chronic tests.
- *P. corethrurus*, a geophagous endogeic species bioaccumulated more Hg (II).
- Toxicity of mercury was higher in natural than in artificial soils for *E. andrei*.
- Cast production can be used as complementary tool to assess Hg toxicity.

GRAPHICAL ABSTRACT



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ABSTRACT

Mercury (Hg) is one of the most toxic nonessential trace metals in the environment, with high persistence and bioaccumulation potential, and hence of serious concern to environmental quality and public health. Emitted to the atmosphere, this element can travel long distances, far from emission sources. Hg speciation can lead to Hg contamination of different ecosystem components, as well as biomagnification in trophic food webs. To evaluate the effects of atmospheric Hg deposition in tropical forests, we investigated Hg concentrations in earthworm tissues and soils of two Forest Conservation Units in State of Rio de Janeiro, Brazil. Next, we performed a laboratory study of the biological responses (cast analysis and behavioral, acute, chronic and bioaccumulation ecotoxicological tests) of two earthworms species (*Pontoscolex corethrurus* and *Eisenia andrei*) to Hg contamination in tropical artificial soil (TAS) and two natural forest soils (NS) spiked with increasing concentration of $HgCl_2$. Field results showed Hg concentrations up to 13 times higher in earthworm tissues than in forest soils, while in the laboratory Hg accumulation after 91-days of exposure was 25 times greater in spiked-soils with 128 mg Hg kg^{-1} (dry wt) than in control (unspiked) soils. In all the toxicity tests *P. corethrurus* showed a higher adaptability or resistance to mercury than *E. andrei*. The role of earthworms as environmental bioremediators was confirmed in this study, showing their ability to greatly bioaccumulate trace metals while reducing Hg availability in feces.

* Corresponding author.
E-mail address: andressabuch@hotmail.com (A.C. Buch).

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1. Introduction

Mercury (Hg) is a persistent, bioaccumulative and toxic substance (PBTs) widely released into the environment. For over a century, it has been known as an environmental pollutant (Schroeder and Munthe, 1998). Every year, 1010–4070 tons of Hg are emitted into the atmosphere from anthropogenic and natural sources (Li et al., 2016; UNEP, 2013). Atmospheric mercury exists mainly in the form of elemental mercury vapor (Hg^0) (90 to 99%), particle bound mercury (<5%) and gaseous divalent mercury (Hg^{2+}) (<5%) (Fu et al., 2012). The residence time of Hg^0 in the atmosphere is approximately one year (Kim et al., 2016). The Hg released to the atmosphere can be distributed over long distances and deposited far away from its source. It is estimated that approximately 90% of the total mercury arriving from the atmosphere is captured in soils of terrestrial ecosystems (Gustin, 2012; Driscoll et al., 2013).

Forest ecosystems are large sinks of trace metals and metalloids, receiving considerable inputs of these through atmospheric deposition (Luo et al., 2015; Siudek et al., 2016). Recent research reported atmospheric accumulation of mercury in litter and soil of tropical forests (Buch et al., 2015; Da Silva et al., 2016). Dry and wet deposition plays a major role in the transfer of Hg from the atmosphere to soils and leaching into groundwater, or movement to surface waters. Hg^{2+} can be transformed to methyl-Hg by bacterial activity at and below the sediment/water interface or in algal mats (Zhou et al., 2016). Generally, inorganic mercury is relatively immobile in the environment, but the transformation to an organic form, primarily by bacterial action, enhances substantially mercury uptake and mobility (Arenas-lago et al., 2014). According to Rieder et al. (2013) the inorganic Hg is methylated in earthworms and not by bacteria introduced into soils by earthworms.

Trace metals like mercury may be bioaccumulated in earthworms and this is strongly associated to the metal speciation in soils as well as the earthworm species and ecological category (Rieder et al., 2011). Furthermore, mercury is consistently biomagnified along food chains due to its easy sorption in alimentary tracts, penetration into placenta, through the blood-brain barrier and damage to membranes, enzymes and various protein components, as well as damage to nucleic acid chains (Kabata-Pendias, 2011).

Earthworms are an essential component of soil-litter food webs, and a major food source for many small mammals, insects, fish, reptiles and birds. They are also consumed by humans in countries such as Venezuela, Thailand and China, besides being present in the composition of many nutritional supplements and foods distributed worldwide, which are very rich in protein (Paoletti, 2004; Péres et al., 2011). Furthermore, earthworms constitute up to 80% of the total biomass of the soil fauna and spend most of their life in the soil, where they concentrate their feeding, burrowing and casting activities (Sivakumar, 2015). They participate in the mixing of organic and inorganic fractions of soil, the formation of stable aggregates, the dynamic and recycling of nutrients from organic matter decomposition, and their burrows help soil aeration, and water infiltration and drainage in soil (Smagin and Prusak, 2008).

Eisenia fetida (Savigny, 1826) and *Eisenia andrei* Bouché, 1972 are epigeic species of temperate regions that feed on fresh organic matter and normally do not ingest soil (Domínguez et al., 2005). Although they are widely used in ecotoxicological assessments for a range of pollutants in soils (Spurgeon et al., 2003), their use and ecological relevance remains questionable for tropical soils and environments (Buch et al., 2013; Kuperman et al., 2009). *Pontoscolex corethurus* (Müller, 1857) is an endogeic geophagous species native to Northern South America but commonly found throughout tropical and subtropical regions in disturbed agricultural, forest and peri-urban soils (Ortíz-Gamino et al., 2016; Brown et al., 2006). Ecotoxicological tests including native endogeic species widely distributed throughout soils worldwide have greater ecological relevance but few tests have used these species for Hg contamination and bioaccumulation assessments. Therefore, the following study was undertaken to assess the potential impact of mercury contamination in tropical forest soils on earthworms, both in situ (from atmospheric deposition) and ex-situ in the laboratory (spiking), using two earthworm species.

2. Materials and methods

2.1. Study I: in situ

2.1.1. Location of sampling sites

The study was carried out in two Forest Conservation Units (FCU) of the Rio de Janeiro State in Brazil, which are Três Picos State Park (P), Latitude: 22°30′8,76″S, Longitude: 42°51′21.95″W, altitude: 72 m and Taquara Municipal Natural Park (T), Latitude: 22°35′52.24″ S, Longitude: 43°14′21.15″W, altitude: 74 m. The first Park is inserted in a rural area, away from industrial activities and at 18.2 km from the largest petrochemical complex in Brazil, currently under construction, with inauguration scheduled for 2017. The second Park is located in an industrial zone (next to chlor-alkali and paper industries) and 12.4 km distance from a big petroleum refinery, which was activated 55 years ago and is still functioning. Both forest units have the same phytophisiognomy, i.e., Atlantic rainforest - Tropical Lowland Dense Rain Forest. A full description and the choice of these areas is provided in Buch et al. (2015).

2.1.2. Earthworm and soil sampling

In each FCU soil and earthworms were sampled in eight sampling points (within a radius of 20 m) at two depths in the soil profile (0–20 and 20–40 cm). The earthworms were manually removed 50 × 50 cm monoliths following an adaptation of the TSBF (Tropical Soil Biology and Fertility) method developed by Anderson and Ingram (1993). Complete and apparently healthy adult and juvenile earthworms were collected. These specimens were quantified and identified in the laboratory at species level.

Five soil sub-samples taken in each sampling point per FCU for the two depths were air dried, homogenized and sieved (2 mm-mesh) for the determination of mineralogical, physical and chemical parameters, following methods described in EMBRAPA (2011). Mineralogical analyses of the clay fractions were performed using an X-ray diffractometer (Rigaku) and interpreted according to Brown and Brindley (1980). Crystalline iron and aluminum oxides were extracted using dithionite-citrate-bicarbonate (DCB) and amorphous iron and aluminum oxides were extracted using ammonium oxalate, following Mehra and Jackson (1960) and McKeague and Day (1966) respectively. Important properties of these natural soils (NS) are given in Table 1.

2.1.3. Mercury analyses

Earthworms collected in the field were separated in two groups according to their development stages (adult and juvenile). They were weighed and their biomass recorded before and after gut clearance in the laboratory at 22 °C ± 2 °C for 48 h on moist filter paper. Basically, 24 h is normally enough to achieve depuration and maintain worm health (Hinton and Veiga, 2008), but purges within three days are also satisfactory and recommended (OECD, 2010). Thereafter soil and earthworms samples were frozen, freeze-dried and ground to a fine powder and stored until processing. Total mercury analyses were performed using a Lumex RA-915$^+$ anatomic absorption spectrometer with a pyrolysis unit (RA-915M), where sample matrix is incinerate and mercury atoms counted by an atomic absorption spectroscopy (EPA, 2004).

The Hg bioaccumulation factor (BAF) in earthworms was calculated by taking the ratio of total Hg concentration in earthworms to the total soil Hg content, following OECD guidelines (317, 2010).

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