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Ecotoxicity of mercury to *Folsomia candida* and *Proisotoma minuta* (Collembola: Isotomidae) in tropical soils: Baseline for ecological risk assessment

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ABSTRACT

Mercury (Hg) is a highly toxic nonessential trace metal. Despite its natural occurrence in the Earth's Crust, its concentrations have been steadily increasing in the environment due to anthropogenic sources. Recent studies have showed great concern about soil fauna, once the potential adverse effects of mercury concentrations in the environment of these invertebrates are still poorly understood, especially when linked to forest soils and tropical biota. Different collembolan species can show distinct toxicity effects to the contaminants, impairing its developing lifelong and affecting its diversity and abundance in the environment. Laboratory studies were performed to evaluate the ecotoxicity of Hg(II) to collembolan species collected in Brazil, *Proisotoma minuta* (autochthonous) and *Folsomia candida* (allochthonous), as a tool to predict effects in ecological risk assessment of tropical regions. Behavioral, acute and chronic tests were carried under temperatures of 20 °C and 24 °C using two test soils, natural and artificial, spiked with increasing mercury concentrations. *F. candida* was more sensitive to mercury contamination than *P. minuta*, presenting the most restrictive values of EC_{50} and LC_{50}. Reproduction was a considerably more sensitive endpoint than avoidance and mortality. The 28-day lower EC_{50} values were found in chronic tests for *F. candida* in natural soil to 24 °C (3.32 mg Hg kg^{-1}), while for *P. minuta* was in tropical artificial soil to 20 °C (4.43 mg Hg kg^{-1}). There were similarity for each collembolan species to respond at the Hg (II) effects when exposed at 20 °C and 24 °C. *F. candida* can be suitable as a bioindicator species to mercury ecotoxicity tests in tropical forest soils.

1. Introduction

Since the industrial revolution the levels of mercury (Hg) have been increasing in the atmosphere and environment. Anthropogenic sources are responsible for about 30% of annual emissions of mercury to air. Another 10% comes from natural geological sources, and 60% is from "re-emissions" of these sources previously released mercury that has built up over decades and centuries in surface soils and oceans (UNEP, 2013). Mercury is considered a global pollutant with ability to undergo long distance transportation in the atmosphere and to be easily redistributed in terrestrial and aquatic ecosystems (Marumoto et al., 2015; Silva-Filho et al., 2006). Mercury deposition in these ecosystems can be carried out by dry and/or wet processes, via particulate materials, rain and fog, respectively, and also gaseous exchange between the air, water and soil (Juillerat et al., 2012). More than 90% of atmospheric mercury (elemental and reactive) is absorbed by forests being potentially a large mobile reservoir that may Hg provide to terrestrial and aquatic fauna (Grigal, 2003; Richardson et al., 2013). Recent studies have reported the accumulation of atmospheric Hg in tropical forest soils, remaining in greater quantity in litter and soil (Silva-Filho et al., 2006; Teixeira et al., 2012; Buch et al., 2015).

All soils are in some extent contaminated by global mercury pollution, due to the relatively long lifetime of Hg in the atmosphere, which means that the metal can travel long distances before being oxidized and deposited (Schroeder and Munthe, 1998; Liu et al., 2014). The soils have a high ability to retain and store Hg in function of the strong coupling of this with the carbon in the soil, providing favorable conditions for the formation of inorganic mercury compounds, such as mercury (OH) and also

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Table 1
Summary of characteristics of *Folsomia candida* and *Proisotoma minuta*.

Species	*Folsomia candida* (Willem, 1902)	*Proisotoma minuta* (Tullberg, 1871)
Morphology[a,b]		
Body length	≈2.8 mm	≈1.2 mm
Pigment body	white	Greyish brown
PAO	Present	Present
Eye	Ocelli absent	8+8 ocelli
Abdomen	Abd IV/V and VI fused	Abd V and Abd VI separate
Anal spines	Absent	Absent
Furca	Present	Present
Manubrium	Ventral side with numerous setae (at least 8+8 and up to 16+16)	Ventral side with 1+1 apical setae
Dens	Ventral side with about 28 setae	Dorsal side with ridges
Mucro	Two teeth	Three teeth
Life history traits in laboratory cultures (20 ± 2 °C)		
Time until oviposition after egg-hatching	20–25 days	18–20 days
Time of egg development	10 days	8 days
Stage to exposure	Juvenile (10–12 days)	Juvenile (10–12 days)
Optimal growth and reproduction conditions of temperature	20 ± 2 °C	20 ± 2 °C
Reproduction[a,b]	Asexual	Sexual
Environment[a,c]		
Distribution	Cosmopolitan, widely found in temperate regions	Cosmopolitan, widely found in tropical regions
Ecological-group	Hemiedaphic (soil–litter interface)	Hemiedaphic (soil–litter interface)
Soil condition	Decaying organic matter	Decaying organic matter
Land use	Agriculture, forestry and pasture	Agriculture, forestry and pasture

[a] Hopkin (2007).
[b] Fjellberg (2007).
[c] Mendonça et al. (2015).

HgCl, and the mercuric cation coming from of complex compounds with organic anions (Soares et al., 2015). According to Kabata-Pendias (2011) the residence time of Hg in the soil can be estimated between 500 to 1000 years. Approximately three-quarters of biospheric mercury resident in soils is mainly in the species of inorganic mercury (Hg I or II), which tend the bioaccumulate and biomagnificate to methylmercury, posing risk to plants, invertebrates and microorganisms (Tipping et al., 2011).

Soil fauna has fundamental role in the soil structure, decomposition process and enhancement of primary production (Quijas and Balvanera, 2013). The continued exposure of these invertebrates to pollutants may result in morphological, physiological and behavioral changes to species more sensitive, causing an unbalance of populations and consequently impairment of ecosystem functioning (Santorufo et al., 2012).

Collembola is one of the most abundant groups in terrestrial ecosystems. They participate in the microfragmentation of plant detritus and stimulate the activity of bacterial and fungal colonies. In addition, they act in the maintenance of good soil quality, participate in the soil organic matter dynamics and nutrient mineralization, being thus considered excellent bioindicators of forest condition and change (Cassagne et al., 2006; Sterzyńska et al., 2014) and in ecotoxicological studies, once that they are easy to sample and to culture in laboratory conditions (Fountain and Hopkin, 2005).

Approximately 7000 collembolan species have been described in the world (Deharveng, 2004). The tropical forests of countries such as Brazil, Australia and New Zealand are characterized by a high species richness including several endemic species. However, the lack of knowledge about this great biodiversity in species and of its sensibility to environmental contamination may reduce or extinguish species (Lewinsohn and Prado, 2005).

Ecotoxicity of Hg(II) is still poorly understood for soil fauna, especially in tropical regions. This study evaluated behavioral, acute and chronic effects of Hg(II) spiked in soils for Collembola aiming to predict effects of mercury atmospheric deposition in forest tropical soils to order of auxiliary scientifically in decisions of ecological risk assessments (ERA) and of risk management of contaminated sites.

2. Materials and methods

2.1. Sampling of collembolans and culture conditions

Collembolan species were obtained from forest soils of two Forest Conservation Units of Rio de Janeiro state, Brazil, being Três Picos State Park (Latitude: 22°35′52.24″S, Longitude: 43°14′21.15″ O, altitude: 74 m) and Taquara Municipal Natural Park (Latitude: 22°30′8,76″S, Longitude: 42°51′21,95″O, altitude: 72 m). Surface dwelling collembolans were collected in eight uncovered pitfall traps (diameter of 7 cm) for each site. Traps were placed along a 20 m transect with a spacing of 5 m between them and filled with a substrate of plaster of Paris and activated charcoal (10:1) with a water slide surface of 3 cm depth. Sampled invertebrates from the traps were removed every day over a period of seven days. Thereafter in laboratory, only collembolans were isolated in culture boxes, when the more abundant species were determined to species level, following the dichotomous key of Fjellberg (2007) and Hopkin (2007).

Four collembolan species were isolated and maintained in laboratory: *Proisotoma minuta* (Tullberg, 1871) present on 68% of samples, *Entomobrya* sp. – on 17%, *Sminthurides* sp. – on 13% and *Folsomia candida* (Willem, 1902) on 2% of samples. This last specie was selected because its use as a standard test organism to assessment soil quality by ISO guidelines. Collembolans were cultured in plastic containers lined with a mixture of plaster of Paris and activated charcoal 8:1 (ISO, 1999) under temperature of 20 ± 2 °C and with a photoperiod of 12:12 h light:dark. Cultures were kept moistened and a small amount of granulated dry yeast

was added as a food source once a week. A better development of cultures of *Sminthurides* sp. and *Entomobrya* sp. were observed after insertion of moss (approximately 1 g), revealing a habitat and food preference for this material.

Sminthurides sp. did not reproduce in laboratory during eight months of monitoring, possibly because we found few specimens of males. It is known that in the Family Sminthurididae, the males choose the female and attach themselves with antennae modified for clasping (Niklasson et al., 2000).

Entomobrya sp. (Entomobryidae) survived by two months in laboratory, while the culture declined gradually, indicating low adaptation to laboratory conditions.

For *F. candida* and *P. minuta*, the establishment of the cultures occurred after four months and they were monitored during one year. Its biological characteristics and life history can be observed in Table 1.

Aiming to assess the sensitivity of *F. candida* and *P. minuta* to mercury, adults were transferred to new boxes with culture substrate for egg-laying to obtain synchronized juveniles (10–12 days old) for the ecotoxicity tests.

2.2. Test soils

Two soils were used as test substrate for the ecotoxicological tests: a natural and an artificial soil. The natural soil (NS) was a forest soil collected at 20 cm top layer from Forest Conservation Unit, Três Picos State Park (Latitude: 22°35′52.24″ S, Longitude: 43°14′21.15″O, altitude: 74 m), Rio de Janeiro State, Brazil. The soil was defaunated through two freeze-thawing cycles and mechanically ground and sieved to obtain a 2 mm fraction. Physical and chemical characterization of soil were performed by Brazilian Agricultural Research Corporation – EMBRAPA-Agrobiology, following the methods described in Nogueira and Souza (2005), and the water holding capacity (WHC) was determined according to ISO (1999). The natural soil (NS) was classified as Dystrophic Ta Haplic Cambisol, according to EMBRAPA (2013), being predominant in crop fields and forest soils in Brazil. Properties of NS are shown in Table 2. Low mercury concentrations were detected in the soil samples (Table 2), and such natural budget concentrations were considered to spike the tested soils.

The artificial soil used was proposed by OECD (1984) and adapted by (Garcia et al., 2004, 2011) as a Tropical Artificial Soil (TAS), composed by 70% of fine sand, 20% of kaolinite clay and 10% of powdered coconut fiber, this was used as source of organic matter replacing the sphagnum peat. When necessary the pH value was adjusted to 6.0 ± 0.5 with calcium carbonate.

2.3. Ecotoxicity tests

Synchronized collembolans of 10–12 days old were exposed to mercury concentrations added to natural and artificial soil. Hg(II) was added and hand-homogenized to the soil as aqueous solutions of chloride salt ($HgCl_2$, Sigma-Aldrich, St Louis, MO, USA). Mercury nominal concentrations were based on previous studies (e.g., Lock and Janssen, 2001): 0, 0.5, 1, 1.8, 3.2, 5.6, 10 and 16 mg Hg kg^{-1} dry weight. Controls received just deionized water. The moisture of soils was adjusted to 50–60% of the maximum WHC. Tests were performed on cylindrical glass containers (10 cm diameter and 8 cm height). Ecotoxicity tests were conducted with a 12:12 (light/dark) photoperiod.

Table 2
Physicochemical parameters of natural soil (NS) used in this study.

Soil parameters	Value
pH (1 M KCl)	3.98 ± 0.4
Water holding capacity	59 ± 0.8
Mercury concentration (mg kg^{-1})	0.05 ± 0.004
Cation exchange capacity ($cmol_c$ kg^{-1})	9.63 ± 0.2
Electrical conductivity (μS cm^{-1}, 1:5)	70.8 ± 0.3
Organic matter (%)	2.6 ± 0.2
Texture (%)	
Sand	50
Silt	15
Clay	35

± Standard Deviation.

2.3.1. Behavioral tests

Avoidance tests were carried out according to ISO 17512-2 (ISO, 2011) for each tested soil (TAS and NS) at 20 ± 2 °C. Each treatment had 5 replicates.

Test containers were divided in two sides by a lamina glass divider inserted transversally in the middle position. One section was filled with 30 g dry weight of control soil (TAS or NS) and the other side was filled with 30 g of contaminated soil (TAS or NS). After soil addition, the divider was removed and twenty juvenile springtails (10–12 days old juvenile) were carefully placed in the midline of the container. Containers were covered with an opaque plastic film to reduce water loss by evaporation and to prevent springtails from scape. At the end of the test period (48 h), the control and test soils in each container were separated by the lamina glass divider. Both soils were flooded with water in separated containers. After the addition of some drops of ink and gently stirring it with a spatula, the springtails floating on the water surface were counted. The tests would be invalid if the number of dead or missing springtails were $>20\%$ per treatment.

2.3.2. Acute tests

The acute toxicity of Hg(II) to *F. candida* and *P. minuta* was assessed following the ISO 11267 (ISO, 1999). The lethality tests were carried out during 14 days at 20 ± 2 °C and 24 ± 2 °C. Containers were filled with 30 g of TAS and/or NS (fresh weight – FW) containing the mercury concentrations (treatments) or deionized water (controls). Five replicates were prepared per treatment. At the start of the test, 10 collembolans were introduced in each experimental unit and about 2 mg of granulated dry yeast was added as food in each glass container. All the containers were covered with parafilm. Once a week, containers were opened to allow gas exchange. At the 14th day, the content of each container was carefully transferred to a larger container, which was filled with water, such that the surviving individuals floated on the water surface. Drops of black ink were added to increase the visual contrast between collembolans and the liquid. Collembolans were counted and the lethality for each treatment was recorded. Due to the rapid degradation of dead springtails, missing organisms are assumed to have died during the test period. The tests would be invalid if the number of dead or missing springtails were $>20\%$ in controls.

2.3.3. Chronic tests

Reproduction tests were carried out according to ISO 11267 (ISO, 1999), evaluating the responses of *F. candida* and *P. minuta* in TAS and NS, at 20 ± 2 °C and 24 ± 2 °C. One day before starting the tests, the test organisms were acclimatized on TAS or NS. The test design was composed by seven Hg(II) concentrations and the control (distilled water) × two species × two temperatures, using five replicates with collembolans. Three additional test containers for each concentration, but without organisms were used for pH, moisture content and residual Hg(II) determinations at the end of the test (28 days). At the start of the test, 10 juvenile springtails (10–12 days old juvenile) and approximately 2 mg of granulated dry yeast were placed in each test container. All containers were

covered with parafilm and placed within the incubator. Twice a week the test containers were opened briefly to allow the aeration and the moisture was replaced once a week.

2.4. Mercury concentrations and pH analysis at the end of chronic exposures

Measured concentrations of Hg(II) were determined in soil (mg kg^{-1}) at the beginning and at the end of the tests. Soil samples were manually homogenized and analyzed using a Lumex RA-915+ device, an atomic absorption spectrometer with a pyrolysis unit (RA-915M), where samples matrix were destructed and mercury atoms were counted on an atomic absorption spectroscopy (EPA, 2004). Determination of pH values was carried out in a 1:2.5 soil:liquid ratio with 1 M KCl, following ISO guideline (ISO, 1999).

2.5. Data analysis

Results of avoidance and lethality toxicity tests were expressed as percentage of response organisms, while results of chronic tests were expressed as mean number of juveniles. The percentual effect of avoidance (A) for Hg(II) concentrations was calculated using the expression: $A=((C-T)/N)^{*}100$, where C is the number of individuals in the control soil, T is the number of individuals in the test soil and N is the total number of individuals. NOEC (no observed effect concentration) and LOEC (lowest observed effect concentration) for the avoidance tests were estimated by Fisher's Exact test. The concentrations causing 50% effect (EC_{50}) for avoidance and the median lethal concentration (LC_{50}) for lethality tests were calculated using probit analysis with PriProbit Software 1.5 (Sakuma, 1998).

NOEC and LOEC values were determined by ANOVA followed by Dunnett's test, differences between control and each concentration were evaluated at $p<0.05$ level (software Minitab® 15.1.0.0).

The EC_{50}s for reproduction were calculated using non-linear regressions, according to Environmental Canada (EC, 2014). The best fitting model was Gompertz, expressed by the expression (extracted from Chelinho et al. (2014)):

$Y=c^{*}\exp((\log(0.5))^{*}(\log conc/x^{b})$, where: Y is the number of juveniles, c is the control response, logconc is the log-transformed exposure concentration, x is the estimate of 50% effect concentration and b is the scale parameter (estimated between 1 and 4).

Table 3
Summary of the ecotoxicological effects of mercury for collembolan species.

Species	Toxicity test	Soil-Test	Temp. °C	EC_{50} or LC_{50} mg Hg kg^{-1} dry wt	NOEC	LOEC
Behavior	*F. candida*	TAS	20	6.24 (4.66–7.82)	1.8	3.2
		NS		5.44 (4.13–6.75)	1.8	3.2
	P. minuta	TAS	20	7.52 (5.28–9.76)	1.8	3.2
		NS		7.90 (5.78–10.02)	3.2	5.6
Acute	*F. candida*	TAS	20	6.99 (4.71–9.27)	3.2	5.6
		NS		6.12 (3.74–8.50)	1.8	3.2
	P. minuta	TAS	20	7.02 (5.42–8.62)	3.2	5.6
		NS		7.16 (3.88–10.44)	3.2	5.6
	F. candida	TAS	24	6.71 (5.00–8.42)	3.2	5.6
		NS		6.06 (3.62–8.50)	1.8	3.2
	P. minuta	TAS	24	6.87 (6.10–7.64)	3.2	5.6
		NS		7.09 (5.28–8.90)	1.8	3.2
Chronic	*F. candida*	TAS	20	3.81 (3.67–3.95)	1.0	1.8
		NS		3.40 (3.18–3.62)	1.0	1.8
	P. minuta	TAS	20	4.43 (4.28–4.58)	1.8	3.2
		NS		4.47 (4.31–4.64)	1.8	3.2
	F. candida	TAS	24	3.64 (3.48–3.79)	0.5	1.0
		NS		3.32 (3.11–3.54)	1.0	1.8
	P. minuta	TAS	24	4.47 (4.33–4.62)	1.8	3.2
		NS		4.58 (4.43–4.73)	1.8	3.2

TAS – Tropical Artificial Soil; NS – Natural Soil; Temp. – Temperature; EC_{50} – Concentration causing 50% effect; LC_{50} – Concentration causing 50% mortality; Data in brackets correspond to the 95% confidence intervals; NOEC – No observed effect concentration; LOEC – Lowest observed effect concentration.

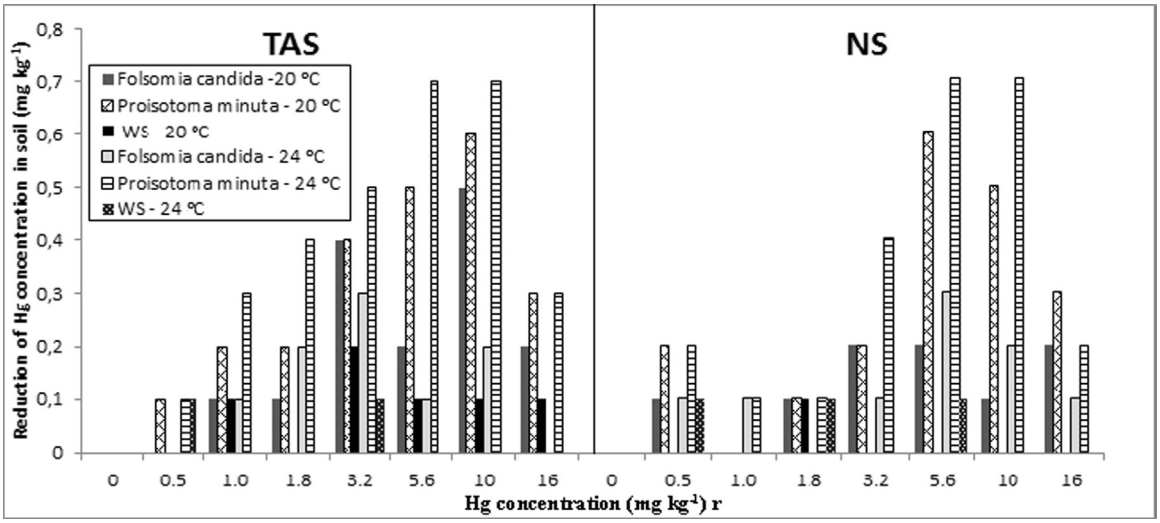

Fig. 1. Mean reduction of mercury concentrations in soils, in chronic reproduction tests with collembolans after 28 d of exposure, determined by Lumex RA-915+. TAS – Tropical Artificial Soil; NS – Natural Soil; WS – Treatments without collembolans.

3. Results

3.1. Avoidance responses

No mortality was observed in all tests. A dose response was observed in the avoidance tests for both tested species towards mercury contamination (showed in Supplementary material, Fig. S1). No avoidance response occurred at 0.5, 1.0 and 1.8 mg Hg kg^{-1} dry wt. EC_{50} values are shown in Table 3. The most restrictive value was found to *F. candida* in NS (EC_{50}=5.44 mg Hg kg^{-1} dry wt) and the less restrictive value was to *P. minuta* in NS (EC_{50} =7.90 mg Hg kg^{-1} dry wt).

The concentration of 16 mg Hg kg^{-1} dry wt showed to be limiting for habitat function of soils for both species, where on average $>$80% of collembolans were found in the control soil.

3.2. Acute responses

No lethality of collembolans was found in controls. A dose response was observed in lethality tests for both tested species towards mercury contamination. NOEC, LOEC and derived LC_{50} are shown in Table 3. The lowest NOEC value (1.8 mg Hg kg^{-1} dry wt) was found in NS for *F. candida* at 20 °C and at 24 °C, and for *P. minuta* at 24 °C. The derived LC_{50} ranged from 6.06 mg Hg kg^{-1} dry wt (*F. candida* in NS at 24 °C) to 7.16 mg Hg kg^{-1} dry wt (*P. minuta* in NS at 24 °C). LC_{50} were similar among species, soils and temperatures.

The concentration of 16 mg Hg kg^{-1} dry wt caused 100% lethality for the tested species in both soils and temperatures (showed in Supplementary material, Fig. S2).

3.3. Chronic responses

Reproduction rate in controls reached a minimum of 100 instars per container, with the coefficient of variation lower than 30%, fulfilling the validity criteria of the tests. No significant differences were found for reproduction of each species between tested soils and temperature in controls (one-way ANOVA, Tukey's test, $p<0.05$). These findings can indicate that the tested temperatures, 20 °C and 24 °C, can be in a range of adequate conditions for reproduction of *F. candida* and *P. minuta* (showed in Supplementary material, Fig. S3).

A dose response was observed in reproduction tests for both tested species towards mercury contamination. NOEC, LOEC and derived EC_{50} are shown in Table 3. Each tested species showed similar response when exposed at 20 °C or 24 °C. The lower EC_{50} was found for *F. candida* in NS at 24 °C (EC_{50}=3.32 mg Hg kg^{-1} dry wt), while for *P. minuta* the lower EC_{50} was found in TAS at 20 °C (EC_{50}=4.43 mg Hg kg^{-1} dry wt). Reproduction of the species was practically ceased at 10 mg Hg kg^{-1} dry wt.

The results evidenced that *F. candida* is more sensitive to mercury contamination than *P. minuta*, and they showed reproduction as the most sensitive endpoint when compared to lethality and avoidance behavior of collembolans to this contaminant.

3.4. Mercury concentrations and pH analysis at the end of chronic exposures

There was no significant variation in pH values for each soil type in the treatments evaluated, when comparing the start (day 0) and the end (28 d) values of chronic tests. TAS pH values ranged from 5.82 ($\pm$0.10) to 5.79 ($\pm$0.11) at 20 °C and from 5.80 ($\pm$0.10) to 5.77 ($\pm$0.14) at 24 °C. NS pH values ranged from 3.82 ($\pm$0.10) to 3.76 ($\pm$0.11) at 20 °C and from 3.80 ($\pm$0.10) to 3.74 ($\pm$0.11) at 24 °C.

At the end of the reproduction test, reduction of Hg concentrations (mg kg^{-1}) was higher in the presence of springtails than in replicates without organisms (Fig. 1). Despite not having been measured mercury concentrations in the tissues in springtails, the reduction of these concentrations leads us to believe that the mercury can have been bioaccumulated by collembolans or volatized in the environment. This reduction occurred in both soils tested and the effect was most notorious for replicates with *P. minuta*. Furthermore, it was possible to perceive that for *P. minuta* and *F. candida* the temperature was a factor that contributed to lower values of Hg in soils at the end of reproduction test (Fig. 1).

4. Discussion

In recent years, the searches by new species more representative (in terms of abundance, wide geographic distribution and sensitivity) have been carried out in different regions. Soil metal contamination has been evaluated using common species of temperate regions, in Europe as *Folsomia fimetaria* (Krogh et al., 2008) and *F. candida* (Crouau et al., 1999), *Orchesella cincta* (Van Straalen et al., 1987), endemic species in Asia as *Lobella sokamensis*

(An et al., 2013), *Paronychiurus kimi* (Son et al., 2007b), *Onychiurus yodai* and *Sinella umesaoi* (Nakamori et al., 2008), and species from tropical regions in Brazil and Australia as *P. minuta* (Nursita et al., 2005b; Greenslade and Vaughan, 2003).

P. minuta has been tested in studies of tropical soil quality assessment spiked with trace metals, being considered an important bioindicator of environmental changes, showing high sensitivity to cadmium, copper and zinc (Nursita et al., 2005b) but tolerating high levels of lead (Nursita et al., 2005a) and arsenic (Greenslade and Vaughan, 2003). In the present study, *P. minuta* was less sensitive than *F. candida* to mercury contamination. These findings could indicate that the ISO standardized tests with *F. candida* are protective of autochthonous species as *P. minuta*. However, Son et al. (2007b) found that *Paronychiurus kimi* (common species in Korea) was ten times more sensitive than *F. candida* (EC_{50} = 0.23 mg Hg kg^{-1} dry wt on reproduction tests; LC_{50} = 2.6 mg Hg kg^{-1} dry wt in lethality tests). As the main characteristic of a good indicator is to be sensitive to contaminants, leading to protect the entire community, we can conclude that more knowledge is necessary about sensitivity of native species to contaminants in the tropics.

The resistance to contaminants can be related to the trophic level of the species, its physiology (metal uptake, elimination and immobilization ability), life stages and morphological traits. Presence or absence of hairs/scales can interfere in the bioaccumulation of pollutants, while the presence of sensory and locomotory organs as ocelli, antenna and furca, can help in perception and avoidance when in presence of stressors (Janssens et al., 2009; Salmon and Ponge, 2012). In the environment, ecological groups can show distinct effect-response because inhabit and feed on particular soil layer or stratification, which results on differences in exposure (Salmon et al., 2014). Some collembolans species can show differences in metal sensitivity, decreasing in abundance in direct proportion to the increased concentration, whereas others species can maintain or even increase their population (Santorufo et al., 2012).

P. minuta and *F. candida* collected from rainforest successful reproduced in laboratory at either 20 or 24 °C. These results are in accordance with Niemeyer et al. (2015) which reported successful reproduction of *F. candida* in natural soils from Brazil, in laboratory tests incubated at 25 °C. However, Sandifer and Hopkin (1997), evaluating the effects of Cd, Cu, Pb and Zn in OECD artificial soil to collembolans, reported almost complete inactivity of *F. candida* in chronic tests at 25 °C even in controls. Other studies demonstrated that the upper limit of tolerance for *F. candida* would be of 26 °C and some alteration in the ambient temperature could affect its reproduction (Marshall and Kevan, 1962; Snider and Butcher, 1973). The combination of sublethal stress factors, as high (20–35.5 °C) or low (0 to −7.5 °C) temperatures with mercury concentrations (0–48 mg Hg L^{-1}) can cause an increase in lethality of *F. candida* (Holmstrup et al., 2008; Slotsbo et al., 2009). Such effects were not observed by the authors when these factors were tested separately, which can indicate a significant potentiation of effects when the temperature is out of the optimum for the species. In the present work, each tested species showed similar response to Hg(II) in lethality and reproduction when exposed at 20 °C or 24 °C. The results suggest that the tested temperatures (20 and 24 °C) are in a range of adequate condition for the tested species.

A dose response towards mercury contamination was observed in all tests performed. *F. candida* was more sensitive to mercury contamination than *P. minuta*, presenting the most restrictive values of EC_{50} and LC_{50} in both soils and temperatures.

The performance of *F. candida* at 20 °C in tropical artificial soil with Hg(II) in chronic and acute tests was similar to that found by Lock and Janssen (2001) in OECD soil. These authors reported EC_{50} value of 3.26 mg Hg kg^{-1} dry wt to the 28-day reproduction test and total mortality at 10 mg Hg kg^{-1}dry wt, whereas this work observed EC_{50} and LC_{50} values of 3.42 and 6.99 mg Hg kg^{-1} dry wt, respectively. However, Liu et al. (2010) found different results in a sandy loam soil from China. The EC_{50} values based on reproduction and avoidance tests with *F. candida* were 9.29 and 3.88 mg Hg kg^{-1} dry wt, respectively. Such values for reproduction were higher than the findings of the present work, while avoidance behavior was the most sensitive endpoint.

These dissimilarities could be related to genetic differences between populations. Diogo et al. (2007) found differences in tolerance between laboratory strains of *F. candida* in avoidance behavior towards pesticide exposure, but no differences were found in reproduction. These authors point that the use of clonal lineages from different origins can influence reproducibility of toxicological tests with collembolans.

Furthermore, differences in toxicity can be related to soil properties. In the present study, the tested soils were different in pH and OM content. High pH values and OM content can increase chemical sorption and consequently decrease bioavailability and toxicity (Sandifer and Hopkin, 1996; Kabata-Pendias, 2011). These parameters can also influence the reproduction of collembolans itself (Son et al., 2007a). The most restrictive value of EC_{50} was found in reproduction tests with *F. candida* in NS at 24 °C. These findings could be expected due to lower pH on NS (3.89) in comparison to TAS (6.0), and the lower OM content in NS (3%) in comparison to TAS (10%). Luo et al. (2014) evidenced that the low pH of forest soils may amplify the ecological risk caused by bioaccumulation of lead. Moreover, the type of OM present has influence in bioavailability of contaminants. According to Da Silva et al. (2013), the coconut peat fibers, OM source in TAS, have high ability to retain trace elements due the presence of functional groups such as hydroxyl, carboxyl, and carbonyl. The results reinforce the importance of using natural soils to assess toxicity of contaminants.

Despite bioaccumulation was not determined in this work, the decrease of measured concentrations of Hg(II) in soil at the end of the chronic test can suggest this occurrence. Results of Nursita et al. (2009) showed a positive correlation between time of exposure and cadmium concentration in tissues of *P. minuta*. Such results may differ among species due to ecological characteristics of the species that modify their exposure, uptake and elimination rates of contaminants. In Bur et al. (2010), *F. candida* showed cadmium concentration in tissues up to 10 times higher than that measured in the soils to which they were exposed. In the present work, the reduction of measured concentrations of Hg(II) was more noticeable in replicates with *P. minuta*. Further studies should be carried out to confirm the fate of mercury in soil contamination.

4.1. Ecological risk assessment (ERA)

Given of the complexity of an Ecological Risk Assessment (ERA) to mercury contamination in tropical forest soils is necessary the understanding of three investigation lines that are complementary (EA, 2008). A multidisciplinary approach with these lines, chemical, ecological and toxicological, will allow that we can propose environmental and ecological measures to mitigate and even prevent the harmful effects of mercury to soil fauna.

Ecological (quali-quantitative of soil fauna) and chemical (Hg levels) information from mercury atmospheric deposition in Forest Conservation Units, of Rio de Janeiro state, Brazil, can be found in Buch et al. (2015). Toxicological data of the most locally representative taxonomic groups are being studied. The present study to Collembola allows us to affirm that not always native species are the most adequate to represent the risks that Hg

contamination in soil would entail in collembolans species. So, even being *F. candida* an exotic species, its higher sensitivity help us the further restrict mercury levels to be present in tropical forest soils. Ensuring this way the diversity of a range of species, be native or exotic, and especially protecting the performance of its functionality in terrestrial ecosystems.

5. Conclusion

This study attempted to assess the sensitivity to mercury of autochthonous collembolan species most representative of tropical forest soils. Among these collected species *Sminthurides* sp., *Entomobrya* sp. and *P. minuta*, just the last was successful reproduced in laboratory. The standard tested species *F. candida* used in this work for comparisons was also collected and isolated from tropical forest soils and satisfyingly well developed in laboratory conditions, well as at either 20 or 24 °C.

Results showed that *F. candida* was more sensitive to mercury than the autochthonous species *P. minuta*. Among the endpoints evaluated, reproduction presented the most restrictive EC_{50} values. Each tested species showed similar response in lethality and reproduction when exposed at 20 °C or 24 °C. The lower EC_{50} values of Hg(II) for *F. candida* were found in reproduction tests with the natural soil at 24 °C, while for *P. minuta* were in reproduction tests with the tropical artificial soil at 20 °C.

The results presented in this manuscript can be used in environmental risk of mercury contamination, leading to a more realistic assessment.

This manuscript presents some effects of mercury accumulations in soil to collembolan populations, indicating that concentrations where decrease in abundance can be expected.

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Appendix A. Supplementary material

Supplementary data associated with this article can be found in the online version at http://dx.doi.org/10.1016/j.ecoenv.2016.01.009.

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