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Toxicity of three pesticides commonly used in Brazil to *Pontoscolex corethrurus* (Müller, 1857) and *Eisenia andrei* (Bouché, 1972)

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ABSTRACT

The indiscriminate and excessive use of pesticides poses serious risks to humans and the environment, including soil biota. Ecotoxicological tests are useful to indicate the extent to which these chemicals are harmful and how and where their effects occur. Some of these tests were standardized by ISO (International Organization for Standartization) using the earthworm species *Eisenia fetida* and *Eisenia andrei*, both native to temperate climates. However, these species may be of lower relevance for soil ecotoxicological studies since they live in the litter and feed on fresh organic matter. The species *Pontoscolex corethrurus*, native to tropical regions, may be an alternative for more relevant ecotoxicological tests as it is an endogeic geophagous species. However, little is known of its sensitivity to pesticides. Therefore, avoidance and mortality tests were performed using *E. andrei* and *P. corethrurus* and three pesticides commonly used in Brazilian agriculture: carbendazim, carbofuran and glyphosate. The tests were conducted in tropical artificial soil (TAS). For carbendazim, the median avoidance concentration (AC_{50}) was 76.1 and 65.8 mg a.i. kg^{-1} and the median lethal concentration (LC_{50}) 19.7 and 15.3 mg a.i. kg^{-1} for *E. andrei* and *P. corethrurus*, respectively. For carbofuran, the AC_{50} was 9.7 and 7.3 mg a.i. kg^{-1} and LC_{50} 13.5 and 9.3 mg a.i. kg^{-1} for *E. andrei* and *P. corethrurus*, respectively. Concentrations applied in the field of these two pesticides have toxic effects on both species. Glyphosate showed no toxic effects for either species even at the highest concentration tested (47 mg a.i. kg^{-1}), although they displayed avoidance behavior at this concentration. The sensitivity of *P. corethrurus* appears to be similar to the standard species for the pesticides evaluated reinforcing the notion that *E. andrei* is a good test species. Nevertheless, further studies should be undertaken using other contaminants to confirm the similar sensitivity of both species and the relevance of *E. andrei* in ecotoxicological tests.

1. Introduction

Brazil is currently the largest consumer of pesticides worldwide and the eighth per cultivated area (Anvisa, 2006; Rebelo et al., 2010). Nevertheless, the side-effects of this high pesticide usage on the soil ecosystem have been little studied in relation to non-target organisms.

Pesticides can affect the soil and its biota by direct contact, or indirectly, by volatilization, leaching and dispersion (Andréa, 2010). The toxicity of a chemical depends on the exposure time, the susceptibility of the organism, concentration, characteristics of the chemical or its applied combinations and environmental factors (Fent, 2004). The toxicity to a test organism of different chemicals may differ between temperate and tropical regions (Laabs et al., 2002; Garcia et al., 2004, 2011; De Silva and van Gestel, 2009b), so that results from the Northern hemisphere may not be directly applicable to tropical ecosystems (Garcia et al., 2011).

The ISO protocols for ecotoxicological tests using earthworms and contaminated soils recommend *Eisenia fetida* (Savigny, 1826) and *Eisenia andrei* Bouché, 1972 native to temperate regions (ISO, 1993, 2007). These epigeic species feed on fresh organic matter on the soil surface and do not ingest soil (Lavelle, 1988), and are recognized as having sub-optimal ecological relevance for assessing exposure effects in natural soils. However, for practical reasons both species are still widely used for these purposes due to their short reproduction time, easy handling in laboratory and the wealth

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of information on their sensitivity to various types of pollutants (Udovic and Lestan, 2010; Gomez-Eyles et al., 2011; Kinney et al., 2012; Wang et al., 2012). Nevertheless, the testing of natural soil or tropical artificial soil (TAS; Garcia et al., 2004) and earthworm species found in tropical areas (particularly geophagous endogeics that are dominant in tropical soils; Lavelle, 1983) in toxicity tests could contribute to a more relevant and reliable risk assessment of chemicals in the tropics (Kuperman et al., 2009).

Pontoscolex corethrurus (Müller, 1857) is a native Brazilian species commonly found in disturbed agricultural and peri-urban soils of tropical regions both in Brazil and across the globe (Brown et al., 2006). It is an endogeic geophagous species that might be a good alternative for more relevant ecotoxicological tests in the tropics, although little is known of the toxicity of pesticides to this earthworm species. Therefore, the present experiment was performed to evaluate the sensitivities of *E. andrei* and *P. corethrurus* to three frequently used pesticides in Brazil – carbendazim, carbofuran and glyphosate.

2. Materials and methods

2.1. Test substrate

The test substrate used was TAS, developed by Garcia et al. (2004) and based on the formulation of the OECD artificial soil (OECD, 1984). In TAS, the organic matter used is powdered coconut fiber, replacing the OECD sphagnum peat (Garcia et al., 2004; De Silva and van Gestel, 2009a). TAS consists of 70% fine sand, 20% kaolinite clay and 10% powdered coconut fiber, with pH adjusted to 6.0 ± 0.5 with calcium carbonate when necessary. The water holding capacity was adjusted to 60% of the TAS water holding capacity (i.e., 54% H_2O on TAS dry matter basis).

2.2. Test species

E. andrei, the species recommended by OECD (1984), ISO (2007) and the Brazilian authorities (IBAMA, 1990; ABNT, 2007), were obtained from vermicompost at the Centro Paranaense de Referência em Agroecologia (CPRA) in Pinhais, Brazil (25°18′47″S; 49°09′28″W), and kept in plastic boxes of 50 L filled with cow manure (from organically raised cows) at the Ecology Laboratory at Embrapa Forestry from March to December 2009. Food (cow manure) was offered weekly. *P. corethrurus* was collected from March to June and September to December 2009 at a rural household garden near Embrapa Forestry in Colombo, Brazil (25°23′30″S; 49°07′30″ W). Prior to the test, the earthworms were kept in plastic boxes of 2 L filled with their natural soil (Cambisol) and fed horse manure (from one organically raised animal) weekly. Previous attempts to keep this species in the laboratory with cow manure were unsuccessful (Buch, personal observation), so horse manure was used instead. Only adults with biomass of 0.7–1.0 g (*P. corethrurus*) and 0.3–0.6 g (*E. andrei*) were used.

2.3. Pesticides and their concentrations

Carbendazim is a highly persistent, systemic carbamate fungicide used to control diseases in fruit, vegetable and grain production and for seed treatment. At present, it is registered for four crops in Brazil and the 10th most widely used pesticide in Brazil (Rebelo et al., 2010) as well as the reference substance for ecotoxicological tests established by the OECD (1984). Carbofuran is a carbamate systemic insecticide and nematicide used in several crops, and has recently been used for earthworm control in rice fields in Southern Brazil, although it is not registered for this crop (Gassen, 2006; Bartz et al., 2009). Farmers in the State of Rio Grande do Sul have complained that earthworms (*Eukerria* spp.) were causing lodging of rice plants, reducing harvests, so they have been applying carbofuran, a known vermicide (Barrion and Litsinger, 1996; De Silva et al., 2010) to try and reduce earthworm populations. Glyphosate is a systemic herbicide recommended for control of monocot and dicot weeds, and is registered for 26 different crops. It is the most widely used active ingredient in Brazil, accounting for 75% of total herbicide consumption nationwide (Rebelo et al., 2010). According to the Brazilian Federal Law No. 7802/89 (Brazil, 1989), carbofuran is extremely toxic (Class I), while glyphosate and carbendazim are moderately toxic (Class III) (Rebelo et al., 2010).

The nominal concentrations of carbendazim – Derosal® 500 SC (500 g L^{-1} a.i.) – used for the avoidance test were 0, 1, 3.2, 10, 31.6, 100, 316 and 1000 mg a.i. kg^{-1} TAS, and for the mortality test 0, 1, 3.16, 10, 31.6 and 100 mg a.i. kg^{-1} TAS, based on application rates used for soybean.

The nominal concentrations of carbofuran – Furadan® 350 SC (350 g L^{-1}a.i.) – used for the avoidance tests were 0, 0.5, 1, 2.5, 5, 10, 20 and 40 mg a.i. kg^{-1} TAS, based on application rates used for cotton, rice and soybean production. For the mortality test, concentrations of 0, 2.5, 5, 10, 16 and 32 mg a.i. kg^{-1} TAS were used.

The nominal concentrations of glyphosate – Pica-Pau® 480 SC (480 g L^{-1} a.i.) – used for both the avoidance and mortality tests were 0, 7, 14, 21, 30 and 47 mg a.i. kg^{-1} TAS, based on application rates used in citrus, cotton, rice and maize production.

Separate soil batches were spiked independently with individual pesticides to prepare the required nominal concentrations, mixed thoroughly, and then weighed out for the ecotoxicological tests with each species.

2.4. Ecotoxicological tests

2.4.1. Avoidance test

This experiment was carried out according to ISO 17512-1 (ISO, 2007). Acclimatization of the animals was performed in TAS for 24 h in plastic boxes of 2 L. Separate tests were performed for each pesticide using each earthworm species. Each pesticide concentration had five replicates. Transparent plastic boxes (26.2 cm × 17.7 cm × 8.5 cm) were filled with TAS up to 4–5 cm height (ca. 500 g dry weight). Half of the box was filled with untreated TAS (control) and the other half with the treated TAS (pesticide-contaminated) with a cardboard divider between them. The cardboard separator was removed and ten *E. andrei* or six *P. corethrurus* were placed on the separating line in each test box. The number of individuals was lower for the latter species due to their larger biomass and body size (*E. andrei* measures 4–5 cm, while *P. corethrurus*, 8–10 cm). The boxes were closed and kept in the dark at 20 ± 4 °C. After 48 h the cardboard divider was reintroduced between the treated and untreated soils in the box and the number of worms on each side counted. The individuals found at the border between the two soil treatments in each box were assigned to the treatment where the anterior part of the body was located.

2.4.2. Acute test

This experiment was carried out according to ISO 11268-1 (ISO, 1993). Acclimatization of the animals was performed in TAS for 24 h in plastic boxes of 2 L. Separate tests were performed for each pesticide using each earthworm species and five replicates for each concentration. Ten *E. andrei* or six *P. corethrurus* were weighed and placed in 500 mL glass jars filled with ca. 500 g dry weight control and contaminated TAS. Each jar received 20 g defaunated (three 3-d freezing cycles) manure (from cows for *E. andrei* and from a horse for *P. corethrurus*), dried and sieved (4 mm) at the beginning of the experiment and on the seventh day, on the surface of the TAS. The jars were closed and kept in the dark at 20 ± 4 °C.

Table 1
Summary of ecotoxicological benchmarks (mg a.i. kg^{-1}) for *Pontoscolex corethrurus* and *Eisenia andrei* determined in the avoidance and mortality tests using tropical artificial soil contaminated with carbendazim, carbofuran, and glyphosate.

Concentrations (mg a.i. kg^{-1} TAS)										
Pesticides/species	AC_{50}	95% confidence limits		LOEC	NOEC	LC_{50}	95% confidence limits		LOEC	NOEC
		Low	High	$p < 0.05$			Low	High	$p < 0.05$	
Carbendazim										
P. corethrurus	66	37	119	31.6	10.0	15	9	25	3.16	1.0
E. andrei	76	51	113	31.6	10.0	20	12	33	3.16	1.0
Carbofuran										
P. corethrurus	7	5	11	2.5	1.0	9	8	11	5.0	2.5
E. andrei	10	7	13	5.0	2.5	13	12	15	10.0	5.0
Glyphosate										
P. corethrurus	45	36	56	47.0	47.0	–	–	–	–	47.0
E. andrei	46	37	56	30.0	47.0	–	–	–	–	47.0

TAS, tropical artificial soil; AC_{50}, median avoidance concentration; LC_{50}, median lethal concentration; NOEC, no observed effect concentration; and LOEC, lowest observed effect concentration.

After 7 days the animals were weighed, dead animals were counted and removed and the remaining individuals re-incubated in their respective glass jars. After 14 days the number of dead animals was determined and the live ones weighed. Morphological and behavioral alterations were recorded, when observed.

2.5. Data analysis

The percentage of avoidance (A) was calculated using the equation:

$$A = \left(C - \frac{T}{N}\right) \times 100$$

where C is the number of animals found in the control soil, T is the number of animals found in the test soil and N is the total number of animals used per treatment. A positive result indicates avoidance and a negative result, attractance to the tested chemical. The attractance was treated as 0% avoidance as recommended in the ISO (2007) norm. NOEC (no observed effect concentration) and LOEC (lowest observed effect concentration) values for the avoidance tests were estimated by Fisher's Exact test (software 50-50 MANOVA). The values for median avoidance concentration (AC_{50}) and the median lethal concentration (LC_{50}), with the respective 95% confidence intervals, were determined by the method of trimmed Spearman–Kärber (Hamilton et al., 1977). The variance of biomass in the acute test was calculated using ANOVA for estimated NOEC and LOEC values. Differences between treatments were assessed by ANOVA and differences between control and each concentration were evaluated by Dunnett's test, at $p < 0.05$ level (software Minitab® 15.1.0.0).

3. Results

3.1. Avoidance test

At the end of the 48 h tests, no earthworm mortality was observed in any of the treatments for any of the three pesticides. For *E. andrei* and *P. corethrurus*, significant avoidance behavior ($p < 0.05$

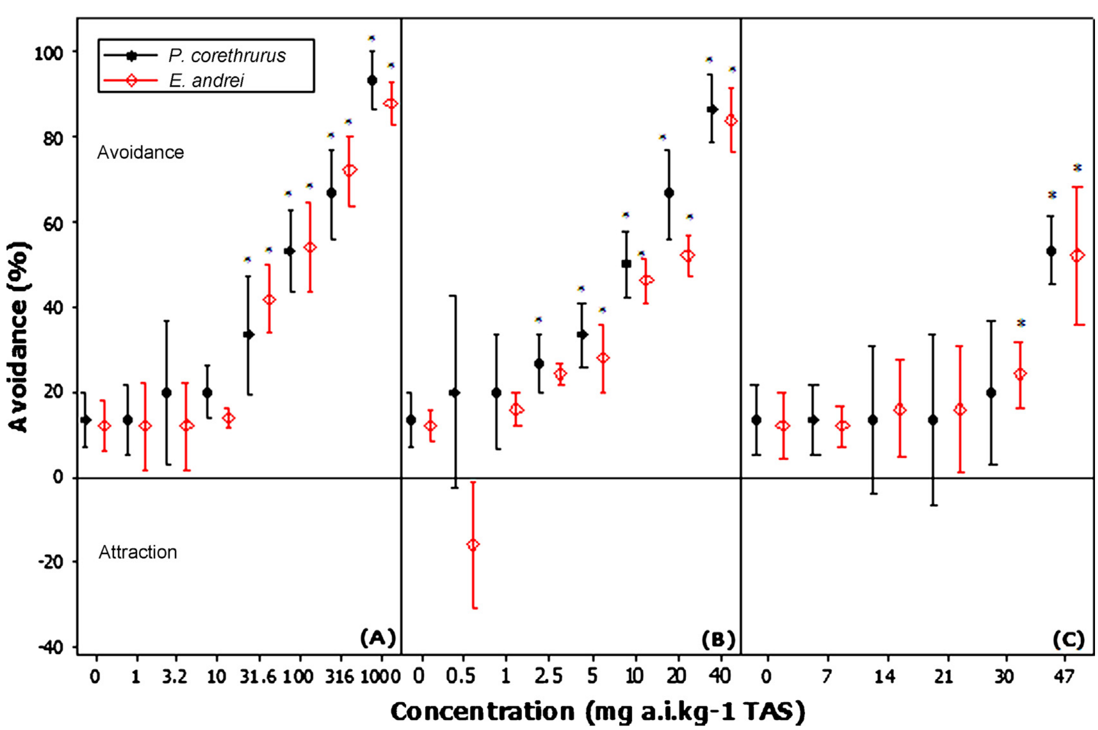

Fig. 1. Avoidance behavior of *Pontoscolex corethrurus* and *Eisenia andrei* in tropical artificial soil contaminated with (A) carbendazim, (B) carbofuran, and (C) glyphosate. Values are means and standard deviations ($n = 5$); *statistically significant effect ($p < 0.05$, Fisher's test).

level) to carbendazim began at concentration 31.6 mg a.i. kg^{-1} or higher (Fig. 1). Based on the AC_{50} values, avoidance reactions revealed similar sensitivity of both species to carbendazim (Table 1).

Carbofuran concentrations of 2.5 mg a.i. kg^{-1} or higher resulted in significant avoidance behaviour in *P. corethrurus*. For *E. andrei* avoidance was significant at 5 mg a.i. kg^{-1} or higher (Fig. 1). The AC_{50} values showed similar sensitivity of both species to this pesticide (Table 1).

Avoidance behavior to glyphosate was significant only at the highest dose, i.e., 47 mg a.i. kg^{-1} ($p < 0.05$ level) for *P. corethrurus* and at 30 mg a.i. kg^{-1} or higher for *E. andrei* (Fig. 1). Nevertheless, the sensitivity of both species was not different (similar AC_{50}; Table 1).

3.2. Acute test

Carbendazim concentrations of 3.16 mg a.i. kg^{-1} or higher caused significant mortality to both *P. corethrurus* and *E. andrei* (Fig. 2). Mortality was similar for both species, as the LC_{50} values were not different (Table 1). At 7 days no differences in earthworm biomass from the controls were observed for *E. andrei*, but *P. corethrurus* biomass was significantly lower above 31.6 mg a.i. kg^{-1} (Fig. 3). After 14 days, above 10 mg a.i. kg^{-1} both species had lower biomass than the controls. Nevertheless, overall sensitivity to carbendazim and biomass loss of both species was similar.

Carbofuran concentrations of 5 mg a.i. kg^{-1} or above had significant lethal effects on *P. corethrurus*, while for *E. andrei* these were observed at concentrations of 10 mg a.i. kg^{-1} and higher (Fig. 2). The LC_{50} was smaller for *P. corethrurus* compared to *E. andrei* (Table 1). After 7 days, significant biomass reduction was observed only with *E. andrei* at 10 and 16 mg a.i. kg^{-1} (Fig. 3). After 14 days, concentrations of 5 and 10 mg a.i. kg^{-1} or higher significantly reduced *P. corethrurus* and *E. andrei* biomass, respectively.

Even the highest concentrations of glyphosate (47 mg a.i. kg^{-1}) did not lead to significant mortality (3–4%) of either *E. andrei* or *P. corethrurus* (Fig. 2). Therefore, no LC_{50} estimates were possible (Table 1). Biomass losses of both species were not significantly different from controls at any concentration after 7 or 14 days (Fig. 3).

Several behavioral and morphological alterations were observed after acute exposure to carbendazim and carbofuran. In TAS contaminated with glyphosate no alterations were observed in the few dead worms encountered. Beginning at 7 days, most (86%) of the *P. corethrurus* individuals rolled into balls at carbendazim concentrations of 10 mg i.a. kg^{-1} or higher and 60% of the *E. andrei* individuals excreted a yellow fluid (probably from the coelom) and glued to the side of the glass jars. At the highest concentration, 25% of the *P. corethrurus* individuals began to show decomposition of the posterior body after the first week. With carbofuran after 1 week at concentrations of 5 mg i.a. kg^{-1} or higher, 63% of the *P. corethrurus* showed autotomy in several body segments. In 4% of the animals, exposed lesions and decomposition began from the mid-to-hind part of the body. In *E. andrei* segment bloating and intestinal rupture was observed in more than 50% of the worms.

4. Discussion

The present experiment confirmed the toxicity class of the insecticide carbofuran as the most toxic to both earthworm species, although the fungicide carbendazim had toxicities similar to carbofuran. On the other hand, the herbicide glyphosate was found to be non-toxic for both species at the concentrations used (Table 1). The concentrations recommended for field applications of carbendazim (6.31 mg a.i. kg^{-1}) and carbofuran (5 mg a.i. kg^{-1}) caused avoidance and mortality of both *P. corethrurus* and *E. andrei*, while only the highest concentrations of glyphosate led to avoidance behavior of these species.

Carbendazim and carbofuran have well known toxicity to native and exotic species of several earthworm families (Haque and Ebing, 1983; Gough, 1986; Martin, 1986; Panda and Sahu, 2004; Ellis et al., 2010; Tripathi et al., 2010; De Silva et al., 2010), while glyphosate generally has non-toxic or less-toxic effects (Dalby et al., 1995; Yasmin and D'Souza, 2007). Most herbicides are less toxic to earthworms than insecticides and fungicides, although some can be lethal, depending on the active ingredient (Edwards and Bohlen, 1992). Furthermore, long-term exposure to herbicides (e.g., from repeated applications or soil contamination) such as glyphosate

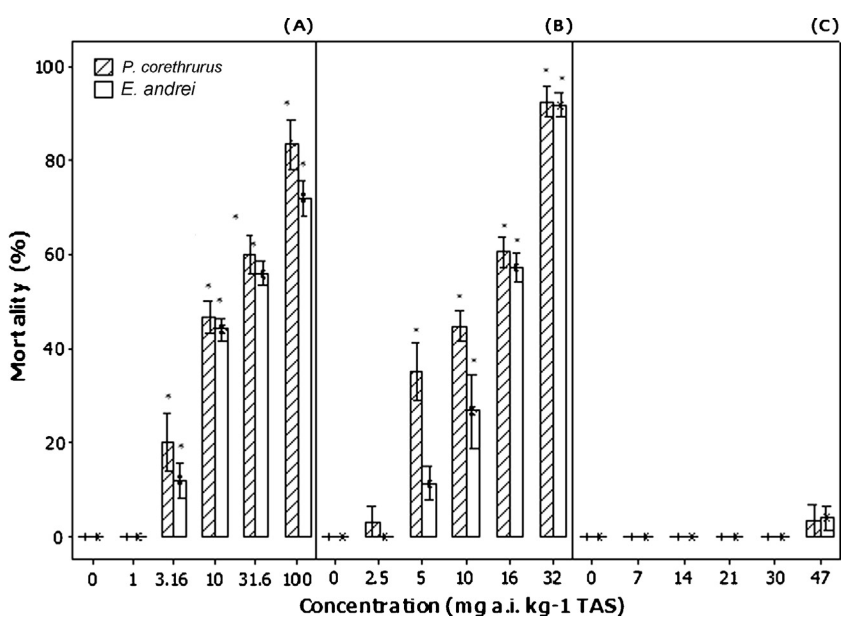

Fig. 2. Mortality of *Pontoscolex corethrurus* and *Eisenia andrei* in Tropical Artificial Soil contaminated with (A) carbendazim, (B) carbofuran, and (C) glyphosate. Values are means and standard deviations ($n = 5$); *statistically significant difference between concentrations used for the same species ($p < 0.05$, Dunnett's test).

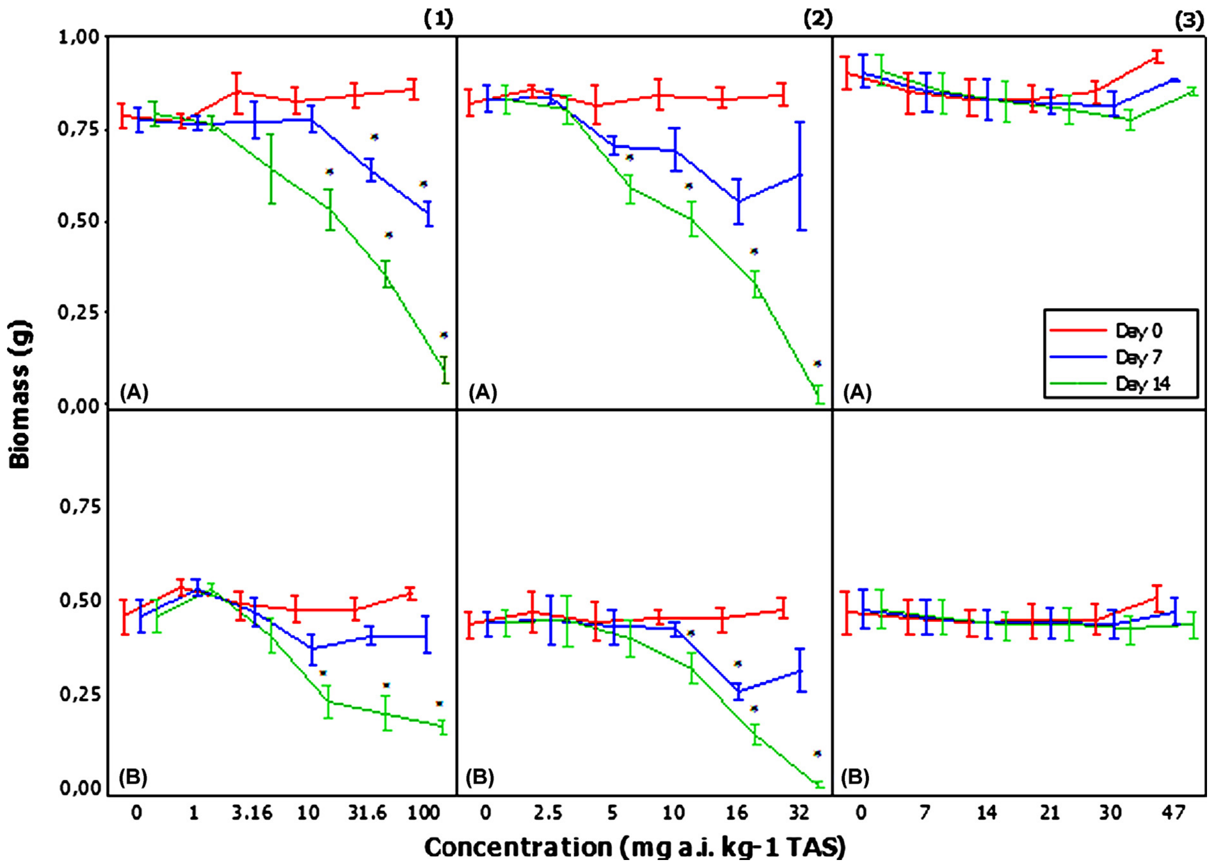

Fig. 3. Biomass of *Pontoscolex corethrurus* (A) and *Eisenia andrei* (B) on day 0, day 7, and day 14, determined in the acute tests with Tropical Artificial Soil contaminated with carbendazim (1), carbofuran (2), and glyphosate (3). Values are means and standard deviations ($n = 5$); *statistically significant difference between concentrations used for the same species ($p < 0.05$, Dunnett's test).

can lead to bio-accumulation in their tissues (Andréa et al., 2004), as well as sub-lethal effects that may alter their reproduction and negatively affect field populations (Springett and Gray, 1992; Avilés and Ordaz, 1998; Casabé et al., 2007). This is particularly concerning given the large amount of glyphosate used annually in Brazil and other countries for weed control, especially in conservation tillage agroecosystems.

Being epigeic and a litter feeder, *E. andrei* is more likely to come into direct contact with pesticides applied on the soil surface and those that have been absorbed by or adsorbed to plant litter. Therefore, in principle, *E. andrei* could be considered less effective for natural soil-based tests, as it is not found in field soils and not ingesting soil, it might be less affected by soil contamination as would an endogeic geophagous species. Standard toxicity tests with earthworms do not involve surface litter. If they did, *E. andrei* might have less contact with the toxicant, since it is added to soil and the worms would stay mostly in the litter. Because no litter is added to the tests, *E. andrei* behaves as an epi-endogeic, burrowing through the mixture, although it is probably feeding on the organic fractions (mainly manure and possibly the coconut fiber). In standard tests, pesticides are added to the water used to hydrate the artificial soil and incorporated as evenly as possible to the substrate. Therefore, it is simulating more the behavior of the contaminant once it has entered the soil matrix. In this scenario, it is mainly the endogeic behavior of earthworms (such as with *P. corethrurus*) that will cause the earthworms to enter into contact with the contaminant.

Under standard test conditions, both *E. andrei* and *P. corethrurus* may be affected by soil contamination by epidermal contact and by passage of the substrate through the gut, where the contaminant may affect internal organs such as the esophagus, crop, gizzard and intestine. In the latter case, higher ingestion rates of substrate may involve greater potential toxicity of the contaminants, though little is known of the ingestion rates of *E. andrei* and *P. corethrurus* in TAS. Alterations in the behavior and morphology of earthworms were observed for both species, and included rolling into balls, autotomy, segment bloating, coelomic fluid secretion, intestinal rupture and body decomposition. Some of these alterations have been reported by other authors, and are typical responses to chemical, environmental and biological stresses. For instance, earthworms frequently roll into a ball in response to extremes in temperature, moisture, pH, and soil contamination (Langdon et al., 1999). Carbofuran is known to induce segment bloating and death of *E. andrei* (Sileo and Gilman, 1975; Roberts and Dorough, 1984) and Nunes (2010) observed autotomy of *E. andrei* with the pesticide Vertimec®. Nevertheless, the mechanisms of toxicological action of pesticides, especially carbamates in earthworms are still poorly known, and further research is necessary, in particular using biomarkers that can reveal sensitive changes at the biochemical, molecular and behavioral levels (Castellanos and Hernandez, 2007).

Different earthworm species often have different sensitivities to the same pesticide (e.g., Haque and Ebing, 1983; De Silva et al., 2009). Garcia (2004) showed higher sensitivity of *P. corethrurus* to carbendazim in relation to *E. fetida*. Higher LC_{50} (>1000 mg i.a. kg^{-1}), NOEC (100 mg i.a. kg^{-1}) and LOEC (316 mg i.a. kg^{-1}) were found for *E. fetida* than for *P. corethrurus* (LC_{50} = 45 mg i.a. kg^{-1}, NOEC = 31.6 mg i.a. kg^{-1} and LOEC = 100 mg i.a. kg^{-1}). Nonetheless, for the pesticides tested in the present experiment there were no important and very few significant differences between both species in their sensitivity to the three pesticides used. The AC_{50} for carbofuran did not differ between species, but the LC_{50} was lower in *P. corethrurus*, indicating a higher sensitivity of this species. In contrast to the findings of Garcia (2004), in our study the toxicity of carbendazim expressed by LC_{50} and AC_{50} in the mortality and avoidance tests were similar for both species. This may be due to the higher temperature used by Garcia (2004) during his tests ($28 \pm 2\,^{\circ}C$), that could have caused a higher toxicity of carbendazim to the tropical species in comparison to the lower temperature ($20 \pm 4\,^{\circ}C$) used in the present experiment (De Silva et al., 2009).

P. corethrurus has been proposed as a good indicator for evaluating soil quality and the disturbance of natural ecosystems (Brown et al., 2006; Römbke et al., 2009), contamination with heavy metals (Ling, 2008; Duarte et al., 2012) and pesticides (Garcia, 2004). In fact its widespread occurrence throughout the tropical world means that it can be readily found and used for ecotoxicological tests. Nevertheless, there are several limitations associated with the widespread use of this species and other tropical earthworm species in ecotoxicological tests. These are mainly related to their culturability, fecundity and life cycle duration: the species must be easily cultured, have high fecundity and a short life cycle to produce a large number of offspring for the tests and facilitate observation of sub-lethal effects in chronic ecotoxicological tests.

P. corethrurus has a relatively long life cycle (1 year; Hamoui, 1991; Buch et al., 2011) and low fecundity (17–118 cocoons individual^{-1} and only one hatchling cocoon^{-1}; Pineda and Hernández, 1983; Bhattacharjee and Chaudhuri, 2002) compared with *E. andrei*, that completes its life cycle in 45–51 days and produces >700 cocoons individual^{-1} and on average 2–3 hatchlings cocoon^{-1} (Domínguez et al., 2005; Reinecke and Viljoen, 1991). These characteristics mean that mass-rearing, although possible is more time-consuming (Pashanasi, 2007) and will yield only with great effort the quantities necessary for routine ecotoxicological tests. Alternatively, adults can be collected with relative ease in the field during most of the year, as long as soil conditions remain adequate for their activities (Lavelle et al., 1987). However, given the parthenogenetic nature of this species and the existence of many different clones and look-alike species (Moreno, 2004), special attention should be taken to the identification of the sources and their possible genetic diversity. Another disadvantage of using field-collected worms is the uncertain age of the adult earthworms and the unknown history of exposure to soil contaminants, including agricultural chemicals, which can alter toxicological responses to test chemicals, compared with responses of unexposed cultured test species. Further research in this topic will certainly yield many interesting results and may turn up a good candidate *P. corethrurus* clone for ecotoxicological tests, particularly acute and avoidance tests (Garcia, 2004). Finally, a wider gamut of contaminants should also be tested to verify the sensitivity and usefulness of *P. corethrurus* as an ecotoxicological test species.

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