Revista Brasileira de Ciências Ambientais
Brazilian Journal of Environmental Sciences

Does the compost barn system bedding as a source of organic fertilizer cause toxicity in soil invertebrates?

A cama do sistema *compost barn* como fonte de adubo orgânico causa toxicidade nos invertebrados do solo?

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ABSTRACT

Modernizing dairy cattle farming techniques promotes a gradual shift from traditional pasture-based systems to confinement with higher investments in animal comfort and welfare, such as the compost barn system (CBS). In this method, animals generate composted waste in the barn itself, on the bedding where they are housed, which is of high fertilizing potential, and whose subsequent application to soils can affect the functionality of edaphic organisms. The study aimed to evaluate the toxicity on edaphic organisms (*Folsomia candida* and *Eisenia andrei*) of increasing doses of CB bedding applied to two soils with different textural characteristics. Ecotoxicological tests were standardized following the recommendations of ISO 11268-1 and 11268-2 for earthworms, and ISO 11267 for springtails. The experimental design was completely randomized with five replications, with the treatments being CB bedding doses (0, 6, 12, 18, 24, and 30 t of waste ha^{-1}). Data were subjected to normality (Shapiro-Wilk) and variance homogeneity (Levene) tests, followed by analysis of variance (ANOVA) and *post-hoc* comparisons using the Dunnett test ($p<0.05$). Still, no significant difference was found between treatments. The tested doses of CB bedding as organic fertilizer showed no acute or chronic toxicity in the two organisms studied (*E. andrei* and *F. candida*) in both soils Oxisol and Entisol Quartzipsamments. The proportional increase in dose promoted the growth of earthworm body mass and stimulated reproduction, as found in springtails. Thus, it is concluded that doses of up to 30 t ha^{-1} of CB bedding do not have acute and chronic toxicity effects on

RESUMO

A modernização das técnicas de criação de bovinos leiteiros promove a migração de maneira gradativa do sistema tradicional a pasto para o de confinamento com maiores investimentos em níveis de conforto e bem-estar animal, como o sistema *compost barn* (CB). Neste método, os animais geram um resíduo compostado no próprio galpão, na cama onde ficam alojados, que é de elevado potencial fertilizante, e cuja posterior aplicação nos solos pode afetar a funcionalidade dos organismos edáficos. O estudo objetivou avaliar a toxicidade sobre organismos edáficos (*Folsomia candida* e *Eisenia andrei*) de doses crescentes da cama do CB aplicadas em dois solos de características texturais distintas. Os testes ecotoxicológicos foram padronizados, seguindo as recomendações das normas ISO 11268-1 e 11268-2 para minhocas, e ISO 11267 para colêmbolos. O delineamento experimental foi inteiramente casualizado com cinco repetições, sendo os tratamentos as doses de cama do CB (0, 6, 12, 18, 24 e 30 t de dejeto ha^{-1}). Os dados foram submetidos aos testes de normalidade (Shapiro-Wilk) e homogeneidade de variância (Levene), seguidos de análise de variância (ANOVA) e comparações *post-hoc* pela aplicação do teste de Dunnett ($p<0,05$), porém, sem diferença significativa entre os tratamentos. As doses testadas de cama do CB como fertilizante orgânico não apresentaram toxicidade aguda e crônica nos dois organismos estudados (*E. andrei* e *F. candida*), em ambos os solos Latossolo Vermelho (Oxisol) e Neossolo Quartzarênico (Entisol). O incremento proporcional da dose promoveu nas minhocas um aumento da sua biomassa corporal e um estímulo à sua reprodução,

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Conflicts of interest: the authors declare no conflicts of interest.
Funding: Community University of the Chapecó Region (Unochapecó); Foundation Coordination for the Improvement of Higher Education Personnel (CAPES) (Process Number: 88887.486847/2020-00); National Council for Scientific and Technological Development (CNPq) (Process Number: DB, 3081895939/2022-1; RSR 302044/2022-1; CRDMB: 30249483/2022-0); and the Agricultural Research and Rural Extension Company of Santa Catarina (Epagri).
Received on: 12/04/2023. Accepted on: 06/22/2024.
https://doi.org/10.5327/Z2176-94781854

earthworms and springtails in Oxisol and Entisol Quartzipsamments. The greater sensitivity of chronic assays with these organisms shows a positive impact of the applied doses, promoting increased reproduction of these organisms.

Keywords: animal bedding; *E. andrei*; *F. candida*; terrestrial ecotoxicology.

assim como encontrado nos colêmbolos. Desta forma, conclui-se que para doses de até 30 t ha^{-1} da cama do CB não há efeito de toxicidade aguda e crônica em minhocas e colêmbolos quando em Oxisol e Entisol Quartzarênico. A maior sensibilidade dos ensaios crônicos com estes organismos mostra um efeito positivo das doses aplicadas, promovendo o aumento da reprodução destes organismos.

Palavras-chave: dejeto animal; *E. andrei*; *F. candida*; ecotoxicologia terrestre.

Introduction

The compost barn system (CBS) comprises confinement facilities for dairy cattle housed in extensive barns as resting areas and waste storage. These barns have flooring covered by an absorbent material bed (such as straw or sawdust), providing thermal comfort for the animals (Pilatti et al., 2018; Andrade et al., 2022).

The daily turnover of the CBS bedding fosters suitable temperature and humidity conditions by incorporating the waste from housed animals, thereby enhancing aerobic microbial activity and facilitating the composting process (Leso et al., 2020; Silva et al., 2023). At the end of the animals' housing period, the resulting residue is commonly used as a source of organically derived nutrients for fertilizing agriculturally essential plants (Tomazi and Gai, 2022). This residue exhibits high levels of nitrogen (N), phosphorus (P), and potassium (K), reducing costs associated with mineral fertilizers for food production (Caldato et al., 2020).

Organic fertilizers and crop rotation augment soil organic matter (SOM)'s water retention capacity and increase pH and nutrient cycling within biogeochemical cycles. This promotes ecosystem services and enhances soil life diversity compared to inorganic fertilizers, especially N based ones prone to losses through volatilization and leaching in the soil (Grunennvaldt et al., 2022; Rambaut et al., 2022; Heinen et al., 2023; Luo et al., 2023). However, excessive application of organic fertilizers without adherence to agronomic recommendations and prevailing environmental standards may cause ecological impact and deleterious effects on terrestrial ecosystems, impacting ecosystem functions such as organic matter decomposition and nutrient cycling and even the regulation of biological populations (Ribeiro, 2020), as a reflection of acute and chronic effects on organisms (Maccari et al., 2016, 2020; Segat et al., 2019). This is due to potentially toxic elements and residues of substances, such as pharmaceuticals, originating from the nutritional and sanitary management of these animals (Liu et al., 2020).

In Brazil, the CBS has experienced exponential growth over the past decade due to the extensive migration from pasture-based to confinement management systems (Galama et al., 2020). Consequently, the quantity and volume of waste produced have increased, intensifying the utilization and commercialization of this waste as fertilizers (Black et al., 2013). However, there has been limited consideration regarding the safety of applied waste doses and their environmental implications.

The adverse effects resulting from the improper use of organic and inorganic compounds are manifold, and standardized assays [International Organization for Standardization (ISO) and Organization for Economic Co-operation and Development (OECD)] conducted in laboratories with bioindicator organisms such as earthworms, springtails, and enchytraeids already indicate ecotoxicological effects from the use of pig and poultry waste in natural soils. Segat et al. (2020) identified toxicity effects on reproducing earthworms and enchytraeids at 112 and 150 m^3 ha^{-1} of liquid pig manure, respectively. Maccari et al. (2020), studying chicken bedding differences between composted and non-composted in the survival and reproduction of springtails (*Folsomia candida*), observed higher toxicity in the non-composted bedding, highlighting the importance of stabilizing organic compost before its use as fertilizer.

To date, ecotoxicological studies assessing the effects of CBS bedding on soil fauna are infrequent in the literature, as well as the chemical and biological impact on soils through its application as fertilizer, underscoring the significance of this work. Hence, this work used standardized ecotoxicological tests to evaluate toxicity in soil organisms (*Folsomia candida* and *Eisenia andrei*) from increasing doses of CB bedding applied to two soils with distinct textural characteristics.

Based on ecotoxicological studies evaluating the application of other waste materials, the field application dose might induce deleterious effects on soil organisms, allowing the formulation of the following hypotheses regarding chronic sensitivity after exposure to increasing doses of CB bedding: i.) it differs according to the tested standard species; and ii.) it depends on soil type.

Materials and Methods

Soils tested

Two natural soils of clayey and sandy textures, classified as Oxisol and Entisol Quartzipsamments (EQ), according to the Soil Taxonomy (United States, 1999; Bockheim et al., 2014), were utilized in this work. The Oxisol was collected in Chapecó (SC), Brazil, at geographic coordinates 27°05'274" S and 052°38'085" W. Meanwhile, the EQ was collected in the city of Araranguá (SC), Brazil, at geographic coordinates 29°00'19.98" S and 49°31'03.84" W. Both soils originated from native forest areas without a history of pesticide application and agricultural activities, were collected from the 0–0.20 m depth layer, with the help of a cutting blade, stored in plastic bags and transported to the Soil Laboratory in Chapecó, where they were air-dried and sieved through a 2 mm mesh. Following these procedures, they were stored in a ventilated area and chemically and physically characterized, serving as standard soils for the tests until a new collection was required.

Natural soils provide a more realistic assessment of contaminant impacts on ecosystems due to their texture characteristics, nutrient availability, water retention capacity, and specific contaminant retention capabilities, thereby yielding a better expression of their response (Zortéa et al., 2018). The tested soils were used as a reference for tropical natural soils in studies assessing swine manure application (Segat et al., 2020) and poultry bedding (Maccari et al., 2020). In the laboratory, the soils underwent three cycles of freezing (24h) and drying in an oven at 65°C (24h) to eliminate the existing soil fauna in the samples (Segat et al., 2015). Given the aim to simulate agricultural conditions for crop production when using material from CBS bedding, the soil pH was adjusted to 6.0, standard deviation ±0.5, by adding calcium carbonate — $CaCO_3$ (ISO, 2005). The water retention capacity (CRA) was adjusted to 60% of its maximum retention (ISO 11268/2; ISO, 2012). The physical and chemical characteristics of the soil samples are presented in Table 1.

Treatments – compost barn system bedding

The CB bedding used in this work was collected from a dairy cattle farm in Chapecó, using a tractor within the barn. The aerobic biological process that stabilizes organic compounds, composting, was conducted during the bedding's usage in the barns, following the recommended method (Shane et al., 2010; Bewley et al., 2017). Therefore, no further treatment was needed post-collection, as the producer managed the bedding with three daily turnovers. The characteristics of the material from the CB bedding were pH 8.3, 55.65% humidity, 33.21% organic carbon, 1.64% phosphorus pentoxide (P_2O_5), 1.91% potassium oxide (K_2O), 2.28% nitrogen (N), 0.0012% copper (Cu), 0.0029% zinc (Zn), 1.131% iron (Fe), and 0.0121% magnesium (Mn). Such results were obtained following the methodology proposed by Tedesco et al. (1985).

The treatments comprised increasing doses of CB organic fertilizer: 0, 6, 12, 18, 24, and 30 t of residue ha^{-1}. These same doses were tested following the recommended application for field fertilization with residue from CB bedding, using the recommendation for dairy cattle residue as a reference, as per the Manual of Liming and Fertilization for the States of Rio Grande do Sul and Santa Catarina (Comissão de Química e Fertilidade do Solo, 2016).

Chronic *E. andrei* and *F. candida* tests were conducted in a completely randomized experimental design with five repetitions, according to their respective ISO standards as detailed in ecotoxicological tests.

Test organisms

The indicator organisms *E. andrei* earthworms and *F. candida* springtails, used in the ecotoxicity tests, originated from established laboratory cultures. They were maintained under controlled photoperiod (12:12h light/dark) and temperature conditions (20±2°C) following ISO 11268-2 (ISO, 1998) and 11267 (ISO, 1999) recommendations, respectively.

The earthworms used were raised in a substrate consisting of screened dry equine manure (from animals raised without antibiotics or dewormers in their management), defaunated, along with coconut fiber and sand in a proportion of 75:20:5, respectively, with a pH of 5.7±0.3. They were fed cooked rolled oats. The springtails were cultured in plastic containers filled with a moistened substrate of gypsum and activated charcoal in a ratio of 11:1. Their diet consisted of moistened biological yeast (*Saccharomyces cerevisiae*).

Ecotoxicological tests

The *E. andrei* (Lumbricidae, Oligochaeta) test followed the guidelines established by ISO 11268-1 (ISO, 1993) and 11268-2 (ISO, 1998). Synchronized adult earthworms for reproduction, with a visible clitellum weighing between 250 and 600 mg, were acclimated and subsequently added to cylindrical plastic containers containing 500 g of soil with increasing doses of CB bedding (0, 6, 12, 18, 24, and 30 t of residue ha^{-1}), with ten individuals per repetition. For the calculation of the doses of the waste applied in the field, the reference was its application on 1 hectare (ha) in the arable layer (0–0.10 m depth) for supplying compost waste with equivalent values of 60, 120, 180, 240, and 300 kg N ha^{-1}, respectively adjusted to doses of 6, 12, 18, 24, and 30 tons of waste, plus the control treatment without application. From the waste analysis, values of 2.28% N and 55.65% moisture in its composition were considered. The doses were calculated individually per experimental unit, considering the volume of 500 g of soil (0, 1.5, 3, 4.5, 6, and 7.5 g of waste). The volume provided by the waste and the remaining water was considered for soil moisture correction. After 28 days, the surviving adult earthworms were removed, counted, and assessed for survival. They were weighed to determine organism biomass, leaving only cocoons and newly hatched juvenile organisms. At the end of 56 days from the start of the tests, the juvenile organisms were removed via a water bath and counted.

Table 1 – Oxisol and Entisol Quartzipsamments physicochemical parameters.

	OM[1]	pH	CEC[2]	Clay	Silt	Sand	P	K	Ca	Mg	H+Al	Cu	Zn	Fe
	%	$_{H2O}$		----- % -----			mg dm^{-3}		--- $cmol_c$ dm^{-3} ---			-- mg dm^{-3} --		
O	3.9	4.3	12.3	55.0	41.4	3.6	3.5	124.0	3.2	1.2	7.8	1.4	0.8	79.7
EQ	0.9	4.1	4.9	4.0	35.2	60.8	6.7	34.0	2.0	0.8	2.0	1.5	1.0	72.5

O: oxisol; EQ: entisol quartzipsamments; [1]OM: organic matter; [2]CEC: cation exchange capacity a pH 7.0 ($cmol_c$ dm^{-3}); pH: hydrogen potential; P: phosphorus; K: potassium; Ca: calcium; Mg: magnesium; H+Al: active acidity; Cu: copper; Zn: zinc; Fe: iron.

The test with *F. candida* springtails (Isotomidae, Collembola) followed ISO 11267 (ISO, 1999) guidelines. Springtails aged between 10 and 12 days were synchronized in a laboratory culture medium. For each replica containing ten individuals, a plastic container (diameter: 3.5 cm; height: 11.5 cm) was filled with 30 g of soil with increasing doses of CB bedding (0, 6, 12, 18, 24, and 30 t of fresh residue ha^{-1}). As detailed in *E. andrei*, the waste values applied to the pot were adjusted to the soil volume (30 g), namely 0, 0.18, 0.36, 0.54, 0.72, and 0.9 g of waste. The volume provided by the waste and the remaining water was considered for soil moisture correction.

During the test, springtails were fed biological yeast (*Saccharomyces cerevisiae*) once a week, with moisture adjusted as necessary. After 28 days, surviving organisms (adults and juveniles) were transferred to a container with water and black India ink and photographed for organism count using ImageTool software (University of Texas Health Science Center, 2002).

Data analysis

Data underwent normality (Shapiro-Wilk) and variance homogeneity (Levene) tests, followed by analysis of variance (ANOVA) and *post-hoc* comparisons when significant, using the control sample and Dunnett's test (p<0.05).

Results

Acute and chronic tests with *Eisenia andrei*

The tests conducted with *E. andrei* complied with the validation criteria outlined in ISO 11268-2 (ISO, 1998) guidelines with mortality less than 10% in the control soil, coefficient of variation less than 30% for lethality assays, a minimum of 30 individuals per replication, and coefficient of variation less than 30% for reproduction assays. In the test with Oxisol, the average number of adult individuals in the control group was 10, with 67 juvenile organisms, and a coefficient of variation equal to 5.31%. For the EQ, the average number of adult individuals in the control group was 9.8, with 66.7 juvenile individuals and a coefficient of variation of 3.87 and 8.06%, respectively.

The obtained results indicate no significant difference in acute toxicity (p=0.208; F=1.161) and chronic toxicity (p=0.301; F=1.203) in Oxisol and acute toxicity (p=0.213; F=1.317) and chronic toxicity (p=0.118; F=1.401) in EQ, following exposure of earthworms to increasing doses of CB (Figures 1A and 1B). The values of lethal concentration (LC_{50}) and half maximal effective concentration (EC_{50}) for CB bedding in the two soils studied could not be calculated due to the absence of effects at the doses tested, which were higher than the maximum dose tested in this work (30 t of residue ha^{-1}).

When assessing the biomass of individuals, the data indicate no significant difference for Oxisol (p=0.52; F=4.82) and EQ (p=0.56; F=6.09) at the evaluated doses for the earthworms, as depicted in Figure 2. The average biomass of individuals was approximately 5,846 and 7,009 mg kg^{-1} for Oxisol and EQ, respectively.

Acute and chronic tests with *Folsomia candida*

The tests conducted with *F. candida* springtails met the validation criteria outlined in ISO 11267 (ISO, 1999) guidelines with mortality less than 10% in the control soil, a coefficient of variation less than 30% for lethality assays, a minimum of 30 individuals per replication, and coefficient of variation less than 30% for reproduction assays. In the test with Oxisol, the average number of adult individuals in the control group was

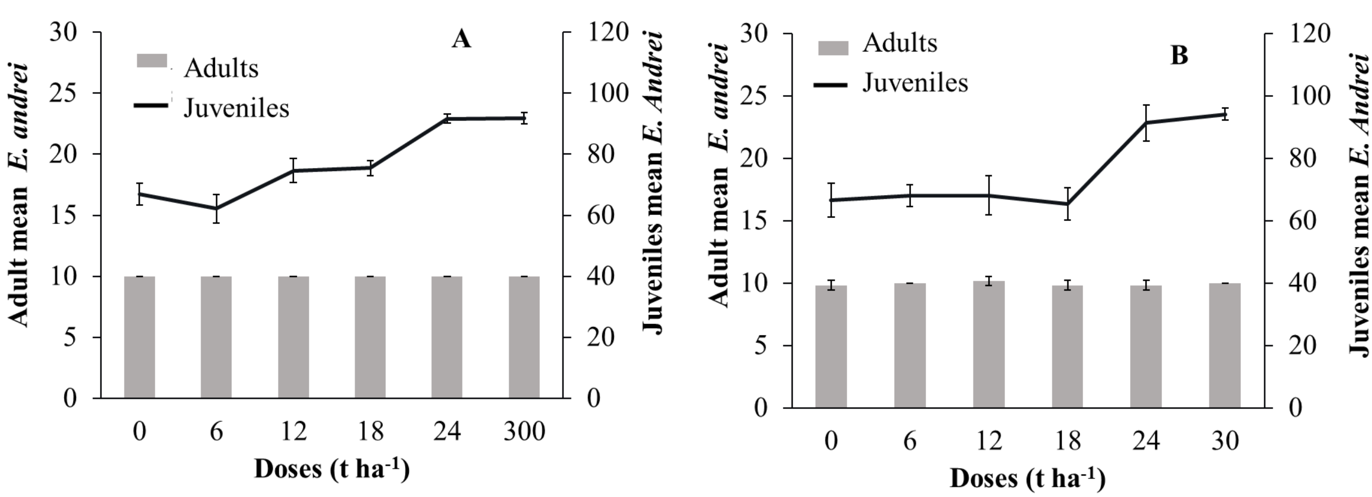

Figure 1 – Number of adult individuals and juveniles of *Eisenia andrei* in lethality and reproduction assays with increasing doses of compost barn in (A) Oxisol and (B) Entisol Quartzipsamments. Means±standard deviation.
The absence of an asterisk indicates no significant difference compared to control (p>0.05).

nine organisms, and the average number of juvenile individuals was 435, with a coefficient of variation of 9.07 and 6.35%, respectively. For the EQ, the average number of adult individuals in the control group was 9.6, with 459.5 juvenile individuals and a coefficient of variation of 7.71 and 8.57%, respectively.

Similar to earthworms, no significant difference was recorded in acute toxicity in tests with *F. candida* (p=0.46; F=5.20) and chronic toxicity (p=0.10; F=66.50) in Oxisol, as well as in acute (p=0.26; F=6.45) and chronic toxicity (p=0.25; F=64.45) in EQ, following exposure to increasing doses of CB (Figures 3A and 3B). The values of LC50 and EC_{50} for CB bedding in the two soils studied could not be calculated due to the absence of effects at the doses tested, which were higher than the maximum dose tested in this work (30 t of residue ha^{-1}).

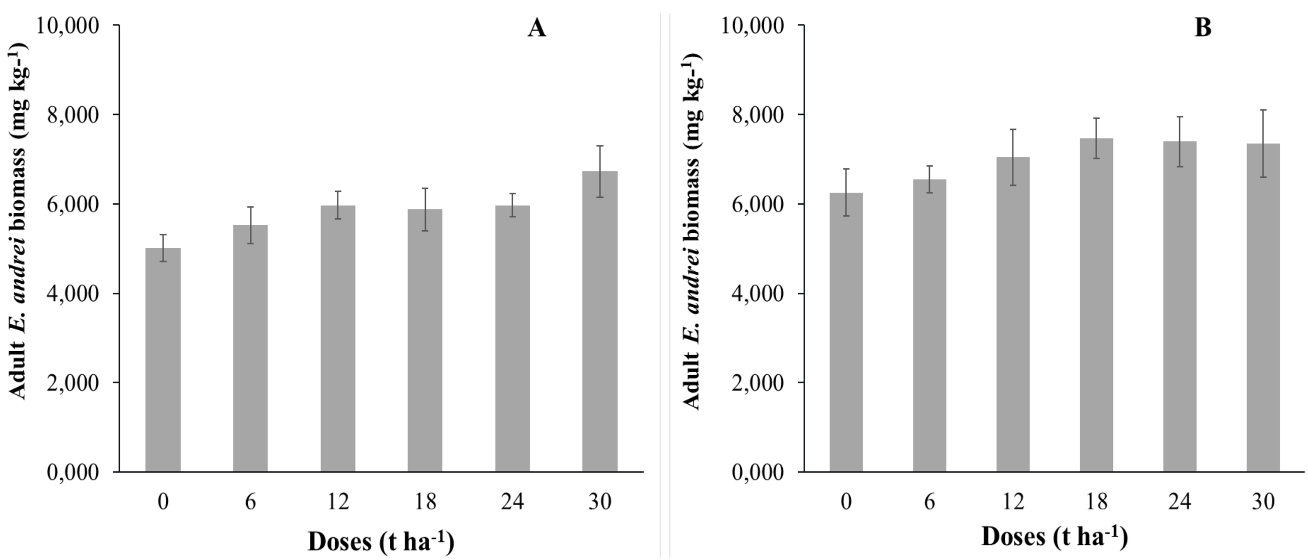

Figure 2 – Biomass of *Eisenia andrei* (mean±standard deviation) when exposed to increasing doses of compost barn in (A) Oxisol and (B) Entisol Quartzipsamments.
The absence of an asterisk indicates no significant difference compared to control (p>0.05).

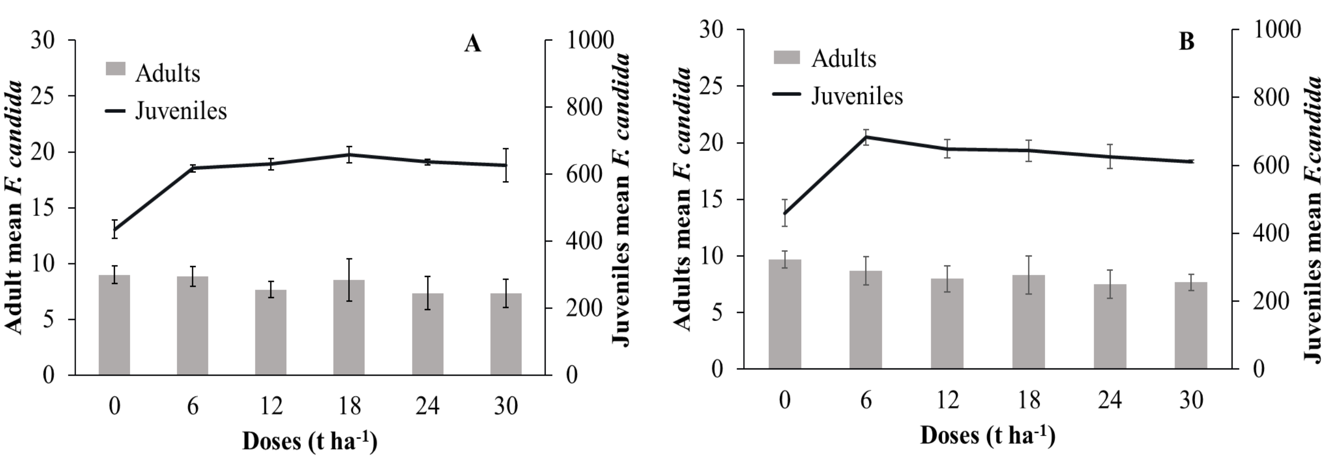

Figure 3 – Number of adult individuals and juveniles of *Folsomia candida* in lethality and reproduction assays with increasing doses of CB in (A) Oxisol and (B) Entisol Quartzipsamments. Means±standard deviation.
The absence of an asterisk indicates no significant difference compared to control (p>0.05).

Discussion

The use of waste for soil fertilization has become increasingly common in agricultural areas (Galloway et al., 2017). Waste from farm activities, such as animal manure or organic residues, has the potential to supply essential nutrients to plants, improve soil fertility, and reduce dependence on chemical fertilizers (Mota et al., 2019; Tomazi and Gai, 2022), directly impacting production costs (Lopes et al., 2019). Therefore, studies related to the potential terrestrial toxicity of animal waste, such as CB bedding for environmentally relevant species, are necessary to determine the possible environmental risks of their use, ensuring that waste fertilization practices are environmentally safe.

While recent studies have focused on assessing the toxicity of dairy cattle waste created in a traditional system (Da Costa Soares et al., 2021; Mousavi et al., 2022; Hagner et al., 2023), there are no ecotoxicological investigations on the use of CB bedding, highlighting potential risks to terrestrial organisms. Studies with other animal wastes such as sheep (Zortéa et al., 2020), liquid pig waste (Segat et al., 2020), and chicken bedding (Maccari et al., 2020) already indicated toxicity to soil organisms at specific doses, with effects dependent on soil type. In the present work, even soils with different textural and chemical characteristics (Table 1) were not sufficient to demonstrate a negative, chronic, or acute effect of the studied waste, showing that the CB at the tested doses stimulates earthworm mass gain and reproduction of earthworms and springtails. Thus, the hypothesis that the response is dependent on the type of soil is refuted in the present work when evaluating the harmful effects of the waste.

The doses tested in this work represent possible field recommendations for future applications of CB waste as a fertilizer source, such as replacing mineral N fertilization. There were no adverse effects on the mortality and reproduction of earthworms and springtails at doses ranging from 6 to 30 tons per hectare of fresh CB residue (60 to 300 kg of N ha^{-1}). This was demonstrated by the average biomass gain of these organisms, indicating that they represent nutrient sources. Comparing the average biomass of earthworms in the control treatment, the doses of 6, 12, 18, 24, and 30 tons of fresh residue per hectare via CB residue promoted gains of 10.30, 19.19, 19.19, 19.19, and 24.35%, respectively, for the doses tested in the Oxisol and 4.71, 12.66, 12.66, 18.26, and 17.57% for the EQ. A study evaluating the ecotoxicity of cattle waste on *Eisenia fetida* earthworms found no mass loss over time, and the reproduction and lethality of these organisms were unaffected (Schubert et al., 2019). However, when cattle waste was compared to plant residues or other food sources for soil fauna, it promoted a higher average body weight and reproduction rate of these individuals (Li et al., 2020).

The chronic test was more sensible than lethal for both tested organisms, mainly due to the absence of lethality. This way, the hypothesis that chronic assays would be more sensitive in detecting dose-related effects is proven, albeit on a positive aspect. The results with springtails indicate that treatments receiving CB bedding showed a higher reproduction rate than those without application. The same effect was found for earthworms when comparing the applied doses to the control treatment with higher doses in both soils, demonstrating a favorable reproduction rate of these organisms as the dose increased. The positive effect on juvenile earthworms was observed at doses higher than 12 e 24 t ha^{-1} for Oxisol and EQ, respectively; for springtails, this positive effect is noted at doses higher than 6 t ha^{-1} for both soils. This behavior may be related to the increase in organic material in the soil due to these organisms' increased dose and food preferences.

According to Da Costa Soares et al. (2021), consecutive annual applications of cattle waste in reduced tillage systems had a positive impact on soil biota, as opposed to the study by van Eekeren et al. (2009), which found no apparent differences in the fauna activity after five consecutive years of cattle waste application. However, the authors suggest that cattle waste provides more excellent bacterial activity in the soil than mineral fertilizer as an N source at studied doses of approximately 150 kg N ha^{-1} for both sources (mineral and organic). Another factor to note is that cattle waste is also positive when analyzing the soil's microbiological aspect, increasing soil microbial biomass carbon (Belinato et al., 2020; Qaswar et al., 2020). This characteristic is essential as it favors microbial populations serving food for soil organisms, such as springtails (Bourgeois et al., 2023).

For springtails, the positive effect on the number of juveniles in applying the studied waste is independent of soil type and begins from the first tested dose. This response may be associated with the habitat of these organisms, which live in the porous spaces of these soils and with the moisture of the material itself, favoring their feeding and reproductive habits. The CBS presents itself as stabilized material with a high moisture content, favoring microbial populations of bacteria, actinomycetes, and primarily fungi involved in the degradation process of cellulose and lignin (Guesine et al., 2023). Therefore, springtails ingest a diverse range of microorganisms, with a feeding preference for fungal mycelium and their spores (Parkinson et al., 1979; Oliveira Filho and Baretta, 2016), which may be favored by the availability of waste. The authors found in their study (data not shown) a linear response of soil microbial biomass carbon (R^2 0.93) to these same applied waste doses. Soil microbial biomass represents the labile carbon of the soil and is mainly composed of fungal biomass (Dulazi et al., 2023).

The average number of earthworms found at the treatment doses in both soils was similar to springtails. However, the positive effect on the reproduction of these organisms was already manifested at doses from 12 t ha^{-1} for the Oxisol and only observed at doses higher than 24 t ha^{-1} in the EQ. This explanation may be associated with their chemical and textural composition. Thus, the hypothesis that the response depends on soil type is accepted for the positive effect of waste on earthworm reproduction. The Oxisol is a clayey soil that favors water retention and presents a greater availability of nutrients and soil organic matter when compared to EQ (Table 1). Earthworms of the *E. andrei* species are epigeic and ingest large amounts of organic material as a food source, being used to indicate soil alteration and contamination (Ribeiro, 2020). Therefore, without a toxic response, Oxisol provides part of the necessary food to these organisms through its composition (3.9% SOM). For the EQ, due to its lower organic matter content (0.9% SOM) and nutrients, a favorable response to reproduction is obtained at higher doses of CB applied to the soil.

The absence of toxic effects in ecotoxicological tests with *E. andrei* earthworms and *F. candida* springtails in soils fertilized with CB waste is a desirable result. The studied doses can be used for field application. However, it is essential to note that results may vary depending on specific experimental conditions, such as the composition of the CB bedding used, the doses tested, and the exposure conditions. It is worth noting that the present study did not seek to identify an effective dose but rather to verify if, at the tested field doses, CB waste was toxic to the organisms tested. Therefore, a more specific evaluation with higher doses applied over time is necessary to investigate the effects of CB bedding on the soil and the organisms within it to ensure the safety and sustainability of this fertilization practice by developing regulations that govern the application of these wastes in the field.

Conclusion

Therefore, without any tested toxic response, compost barn does not appear to be harmful to the studied species of earthworms and collembolans at application rates of up to 30 tons ha^{-1} when applied to Oxisol and Entisol, with no chronic or acute effects. Contrariwise, a positive impact on chronic tests for juvenile earthworms and sprigtails was supported in this research as a nutritional effect of the manure on organisms that gained weight throughout the test, as in the case of earthworms. However, we draw attention to the fact that animal management can affect the composition of compost barn manure and its toxicity potential due to the specificities of the sanitary and nutritional handling of herds in confinement. Thus, how continuous reapplication in different soil types can have cumulative effects is not yet known. The present study did not aim to exhaust the discussion on the topic but to instigate new studies that can deepen the environmental definition of dose limits for the safe application of this waste.

Acknowledgments

To Coordination for the Improvement of Higher Education Personnel (CAPES), and National Council for Scientific and Technological Development (CNPq) for the scientific productivity scholarship to the authors DB (Project number: 3081895939/2022-1), RSR (Project number: 302044/2022-1), and CRDMB (Project number: 30249483/2022-0).

Authors' contributions

Silva, E.R.: data curation, formal analysis, project administration, methodology, resources, software, validation, visualization, writing – original draft, writing – review & editing. **Reis**, T.R.: formal analysis, investigation, methodology, software, validation, visualization, writing – original draft, writing – review & editing. **Schneider**, V.F.M.: methodology. **Baretta**, D.: project administration, resources, supervision, validation, visualization, writing – original draft, writing – review & editing. **Rezende**, R.S.: project administration, resources, supervision, validation, visualization, writing – original draft, writing – review & editing. **Maluche-Baretta**, C.R.D.: conceptualization, data curation, formal analysis, project administration, resources, software, supervision, validation, visualization, writing – original draft, writing – review & editing.

References

Andrade, R.R.; Tinôco I.F.F.; Damasceno, F.A.; Ferraz, G.A.E.S; Freitas, L.C.D.S.R.; Ferreira, C.F.S; Barbari, M.; Baptista, F.J.F.; Coelho, D.J.R., 2022. Spatial distribution of bed variables, animal welfare indicators, and milk production in a closed compost-bedded pack barn with a negative tunnel ventilation system. Journal of Thermal Biology, v. 105, 103111. https://10.1016/j.jtherbio.2021.103111.

Belinato, L.D.C.; Kraft. E.; Solivo, R.; Oliveira, P.A.; Spagnollo, E.; Baretta, D.; Maluche-Baretta, C.R.D., 2020. Multivariate tools for evaluating the use of organic fertilizers on soil microbial properties and maize yield. Journal of Agricultural Studies, v. 8 (3), 257-279. https://doi.org/10.5296/jas.v8i3.16073.

Bewley, J.M.; Robertson, L.M.; Eckelkamp, E.A., 2017. A 100-Year Review: Lactating dairy cattle housing management. Journal of Dairy Science, v. 100 (12), 10418-10431. https://doi.org/10.3168/jds.2017-13251.

Black, R.A.; Taraba, J.L.; Day, G.B.; Damasceno, F.A.; Bewlwy, J.M., 2013 Compost bedded pack dairy barn management, performance, and producer satisfaction. Journal of Dairy Science, v. 96, 8060-8074. https://doi.org/10.3168/jds.2013-6778.

Bockheim, J.G.; Gennadiyev, A.N.; Hartemink, A.E.; Brevik, E.C., 2014. Soil-forming factors and Soil Taxonomy. Geoderma, v. 226, 231-237. https://doi.org/10.1016/j.geoderma.2014.02.016.

Bourgeois, T.P., Suffert, F., Dury, G., Biau, G., Lacoste, S., Prado, S., Dupont, J.; Salmon, S., 2023. Dietary preferences of *Heteromurus nitidus* (Collembola) among wheat fungal communities: Implications for bioregulation of two widespread pathogens. Applied Soil Ecology, v. 188, 104897. https://doi.org/10.1016/j.apsoil.2023.104897.

Caldato, R.M.E.; Caldato. A.; Marcondes, I.M.; Rotta, P.P., 2020. Manual Técnico de Construção e Manejo de *Compost barn* para Vacas Leiteiras. Viçosa: Universidade Federal de Viçosa, Pró- Reitoria de Extensão e Cultura, Divisão de Extensão.

Comissão de Química e Fertilidade do Solo, 2016. Manual de adubação e calagem para os estados do Rio Grande do Sul e Santa Catarina. 11. ed. Sociedade Brasileira de Ciência do Solo - Núcleo Regional Sul, Porto Alegre.

Da Costa Soares, S.; Barizão, C.S.; Vezzani, F.M., Favaretto, N.; Cherobim, V.F.; Dieckow, J., Pauletti, V.; Barth, G., 2021. Biota of subtropical Oxisols under no-tillage with application of liquid cattle manure. Soil Research, v. 60 (4), 423-433. https://doi.org/10.1071/SR21130.

Dulazi, A.A.; Kukal, S.S.; Sharma, S.; Dhaliwal, J., 2023. Soil organic carbon and its labile pools as modulated by soil microbes under different land use practices in Typic Ustochrepts. Tropical Ecology, v. 64 (2), 276-286. https://doi.org/10.1007/s42965-022-00257-7.

Galama, P.J.; Ouweltjes, W.; Endres, M.I.; Sprecher, J.R.; Leso, L., Kuipers, A.; Klopčič, M., 2020. Symposium review: Future of housing for dairy cattle. Journal of Dairy Science, v. 103 (6), 5759-5772. https://doi.org/10.3168/jds.2019-17214.

Galloway, J.N.; Leach, A.M.; Erisman, J.W.; Bleeker, A., 2017. Nitrogen: the historical progression from ignorance to knowledge, with a view to future solutions. Soil Research, v. 55 (6), 417-424. https://doi.org/10.1071/SR16334.

Grunennvaldt, R.L.; Paungfoo-Lonhienne, C.; Trueman, S.J.; Gallart, M., 2022. Effects of organic and inorganic fertilisers and *Paraburkholderia* sp. SOS3 on growth and drought responses of the therapeutic-honey plant *Leptospermum polygalifolium*. Rhizosphere, v. 21, 100471. https://doi.org/10.1016/j.rhisph.2022.100471.

Guesine, G.D.; Silveira, R.M.F.; Da Silva, I.J.O., 2023. Physical and environmental characteristics of the compost barn system and its effects on the physical integrity, reproduction and milk production of dairy cattle: a scoping review. Journal of Animal Behaviour and Biometeorology, v. 11 (2), 2023010. https://doi.org/10.31893/jabb.23010.

Hagner, M.; Pohjanlehto, I.; Nuutinen, V.; Setälä, H.; Velmala, S.; Vesterinen, E.; Pennanen, T.; Lemola, R.; Peltoniemi, K., 2023. Impacts of long-term organic production on soil fauna in boreal dairy and cereal farming. Applied Soil Ecology, v. 189, 104944. https://doi.org/10.1016/j.apsoil.2023.104944.

Heinen, J.; Smith, M.E.; Taylor, A.; Bommarco, R., 2023. Combining organic fertilisation and perennial crops in the rotation enhances arthropod communities. Agriculture, Ecosystems and Environment, v. 349, 108461. https://doi.org/10.1016/j.agee.2023.108461.

International Organization for Standardization (ISO), 1993. ISO 11268-1 – Soil quality – effects of popllutants on earthworms (*Eisenia fetida*) – Part 1: determination of acute toxicity using artificial soil substrate. Geneva, ISO.

International Organization for Standardization (ISO), 1998. ISO-11268-2 – Soil quality–Effects of pollutants on earthworms (*Eisenia fetida*) – Pt 2: Method for the determination of effects on reproduction. Geneva, Switzerland.

International Organization for Standardization (ISO), 1999. ISO 11267. Soil quality – inhibition of reproduction of collembola (*Folsomia candida*) by soil pollutants. Geneva, Switzerland.

International Organization for Standardization (ISO), 2005. ISO 10390. Soil quality - determination of pH. Beuth-Verlag, Berlin.

Leso, L.; Barbari, M.; Lopes, M.A.; Damasceno, F.A.; Galama, P.; Taraba, J.L.; Kuipers, A., 2020. Invited review: compost-bedded pack barns for dairy cows. Journal of Dairy Science, v. 103 (2), 1072-1099. https://doi.org/10.3168/jds.2019-16864.

Li, Y.; Yang, X., Gao, W., Qiu, J.; Li, Y., 2020. Comparative study of vermicomposting of garden waste and cow dung using *Eisenia fetida*. Environmental Science and Pollution Research, v. 27, 9646-9657. https://doi.org/10.1007/s11356-020-07667-x.

Liu, W.R.; Zeng, D.; She, L.;Su,W.X.; He, D.C.; Wu, G. Y.; Ma,X. R.; Jiang, S.; Jiang, C.H.; Ying, G.G., 2020. Comparisons of pollution characteristics, emission situations, and mass loads for heavy metals in the manures of different livestock and poultry in China. Science of the Total Environment, v. 734, 139023. https://doi.org/10.1016/j.scitotenv.2020.139023.

Lopes, H.L.S.; Lima, D.C.; Sampaio, A.S.O.; Souto, L.S.; de Souza Pereira, A.C.; da Silva, A.M.; Maracajá, P.B., 2019. Crescimento inicial da cultura do rabanete (*Raphanus sativus* L.) submetida a níveis e fontes de fertilizantes orgânicos. Revista Brasileira de Gestão Ambiental, v. 13 (1), 19-24. ISSN 2317-3122.

Luo, J.; Liao, G.; Banerjee, S.; Gu, S.; Liang, J.; Guo, X.;Zhao, H.; Liang, Y.; Li, T., 2023. Long-term organic fertilization promotes the resilience of soil multifunctionality driven by bacterial communities. Soil Biology and Biochemistry, v. 177, 108922. https://doi.org/10.1016/j.soilbio.2022.108922.

Maccari, A.P.; Baretta, D.; Paiano, D.; Leston, S.; Freitas. A.; Ramos, F.; Sousa, J.P.; Filho, O.K., 2016. Ecotoxicological effects of pig manure on Folsomia candida in subtropical Brazilian soils. Journal of Hazardous Materials, v. 314, 113-120. https://doi.org/10.1016/j.jhazmat.2016.04.013.

Maccari, A.P.; Segat, J.C.; Testa, M.; Maluche-Baretta, C.R.D.; Baretta, D., 2020. The effect of composted and non-composted poultry litter on survival and reproduction of Folsomia candida. International Journal of Recycling of Organic Waste in Agriculture, v. 9 (1), 99. https://doi.org/10.30486/ijrowa2020.1885804.1012.

Mota, V.C.; Andrade, E.T. de; Leite, D.F., 2019. Bed temperature in compost barns turned with rotary hoe and offset disc harrow. Engenharia Agrícola, v. 39 (3), 280-287. https://doi.org/10.1590/1809-4430-Eng.Agric.v39n3p280-287/2019.

Mousavi, H.; Cottis, T.; Pommeresche, R.; Dörsch, P.; Solberg, S.Ø., 2022. Plasma-treated nitrogen-enriched manure does not impose adverse effects on soil fauna feeding activity or springtails and earthworms abundance. Agronomy, v. 12 (10), 2314. https://doi.org/10.3390/agronomy12102314.

Oliveira Filho, L.C.I.; Baretta, D., 2016. Por que devemos nos importar com os colêmbolos edáficos? Scientia Agraria, v. 17 (2), 21-40. https://doi.org/10.5380/rsa.v17i2.48242.

Parkinson, D.; Visser, S.; Whittaker, J.B., 1979. Effects of collembolan grazing on fungal colonization of leaf litter. Soil Biology and Biochemistry, v. 11 (5), 529-535. https://doi.org/10.1016/0038-0717(79)90013-0.

Pilatti, J.A.; Vieira, F.M.C.; Rankrape, F.; Vismara, E.S., 2019. Diurnal behaviors and herd characteristics of dairy cows housed in a compost-bedded pack barn system under hot and humid conditions. Animal, v. 13 (2), 399-406. https://doi.org/10.1017/S1751731118001088.

Qaswar, M.; Jing, H., Ahmed, W.; Dongchu, L.; Shujun, L.; Lu, Z.; Cai, A.; Lisheng, L.; Yongmei, X.; Jusheng, G.; Huimin, Z., 2020. Yield sustainability, soil organic carbon sequestration and nutrients balance under long-term combined application of manure and inorganic fertilizers in acidic paddy soil. Soil and Tillage Research, v. 198, 104569. https://doi.org/10.1016/j.still.2019.104569.

Rambaut, L.A.E.; Tillard, E.; Vayssières, J.; Lecomte, P.; Salgado, P., 2022. Trade-off between short and long-term effects of mineral, organic or mixed mineral-organic fertilisation on grass yield of tropical permanent grassland. European Journal of Agronomy, v. 141, 126635. https://doi.org/10.1016/j.eja.2022.126635.

Ribeiro, H.M., 2020. Mineralização-imobilização dos nutrientes vegetais presentes nos resíduos. UC Valorização Agronómica e Florestal de Resíduos e Bioprodutos, ISA, Portugal (Accessed March 01, 2023) at:. https://fenix.isa.ulisboa.pt/courses/vaer-846413499990882/programa.

Schubert, R.N.; Morselli; T.B.G.A.; Tonietto, S.M.; Henriquez, J.M.O.; Trecha, R.D.; Eid, R.P.; Lima, A.P.F., 2019. Macrofauna edáfica no processo de vermicompostagem de resíduos animais e vegetais. Brazilian Journal of Biology, v. 79 (4), 589-593. https://doi.org/10.1590/1519-6984.184765.

Segat, J.C.; Alves, P.R.; Baretta, D.; Cardoso, E.J., 2020. Ecotoxicological effects of swine manure on *Folsomia candida* in subtropical soils. Anais da Academia Brasileira de Ciências, v. 92, e20180758. https://doi.org/10.1590/0001-3765202020180758.

Segat, J.C.; Alves, P.R.L.; Baretta, D.; Cardoso, E.J.B.N., 2015. Ecotoxicological evaluation of swine manure disposal on tropical soils in Brazil. Ecotoxicology and Environmental Safety, v. 122, 91-97. https://doi.org/10.1016/j.ecoenv.2015.07.017.

Segat, J.C.; Baretta, D.; Oliveira Filho, L.C.I.; Sousa, J.P.; Klauberg Filho, O., 2019. Disentangling the effects of the aqueous matrix on the potential toxicity of liquid pig manure in sub-tropical soils under semi-field conditions. Ecotoxicology and Environmental Safety, v. 168, 457-465. https://doi.org/10.1016/j.ecoenv.2018.10.085.

Shane, E.M.; Endres, M.I.; Janni, K.A., 2010. Alternative bedding materials for compost bedded pack barns in Minnesota: a descriptive study. Applied Engineering in Agriculture, v. 26 (3), 465-473. https://doi.org/10.13031/2013.29952.

Silva, M.V.; Pandorfi, H.; de Almeida, G.L.P.; da Silva, R.A.B.; Morales, K.R.M.; Guiselini, C.; De Oliveira Júnior, J.F., 2023. Spatial modeling via geostatistics and infrared thermography of the skin temperature of dairy cows in a compost barn system in the Brazilian semiarid region. Smart Agricultural Technology, v. 3, 100078. https://doi.org/10.1016/j.atech.2022.100078.

United States, 1999. Soil Taxonomy. A basic system of soil classification for making and interpreting soil surveys. Agriculture Handbook, v. 436, 96-105.

Tedesco J.M.; Gianello, C.; Bissani, C.A.; Bohnem, H.; Volkweiss, S.J., 1995. Análise de solo, plantas e outros materiais. 2. ed. Universidade Federal do Rio Grande do Sul, Porto Alegre. 174 p. (Boletim Técnico de Solos, 5).

Tomazi, C.V.; Gai, V.F., 2022. Produtividade de milho para silagem com utilização do *Compost barn*. Revista Cultivando o Saber, v. 15, 9-19.

van Eekeren, N.; de Boer, H.; Bloem, J.; Schouten, T.; Rutgers, M.; de Goede, R.; Brussaard, L., 2009. Soil biological quality of grassland fertilized with adjusted cattle manure slurries in comparison with organic and inorganic fertilizers. Biology and Fertility of Soils, v. 45, 595-608. https://doi.org/10.1007/s00374-009-0370-2.

Zortéa, T.; Dos Reis, T.R.; Serafini, S.; de Sousa, J.P.; da Silva, A.S.; Baretta, D., 2018. Ecotoxicological effect of fipronil and its metabolites on *Folsomia candida* in tropical soils. Environmental Toxicology and Pharmacology, v. 62, 203-209. https://doi.org/10.1016/j.etap.2018.07.011.

Zortéa, T.; Segat, J.C.; Conte, R.A.; Barreta, D.A.; Silva, A.W.L.; Paulino, A.T.; Baretta, D., 2020. Effects of sheep manure in agricultural soils on the behavior of Folsomia candida and initial growth and development of *Avena sativa*. Brazilian Journal of Biology, v. 81, 1030-1035. https://doi.org/10.1590/1519-6984.234126.

Wilcox, D.; Dove, B.; McDavid, D.; Greer, D., 2002. Image Toll for Windows. 3.00. Texas: The University of Texas Health Science Center.