



## BIOLOGICAL SCIENCES

# Ecotoxicological effects of swine manure on *Folsomia candida* in subtropical soils

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**Abstract:** The production model used in Brazilian pig farms promotes a concentration of animals in small territorial extensions, causing difficulty in disposing of the manure generated, since the economically viable alternative is to use this as an agricultural fertilizer. The objective was to evaluate the effect of swine manure doses on the biological and behavioral parameters in *Folsomia candida* by ecotoxicological tests. An Ultisol, Oxisol and Tropical Artificial Soil (TAS) were contaminated with 0, 25, 50, 75 and 100 m<sup>3</sup> ha<sup>-1</sup> of swine manure, to which springtails were exposed for evaluation of mortality, and the doses 0, 10, 15, 20 and 25 m<sup>3</sup> ha<sup>-1</sup> of swine manure to evaluate reproduction and avoidance. Lethality was observed in all doses as well as all soils, indicating toxicity of the manure. In the reproduction tests dose since 10 m<sup>3</sup> ha<sup>-1</sup> caused a reduction of juveniles in all soils. Avoidance behavior was observed in all doses of the Oxisol treatments and at 20 and 25 m<sup>3</sup> ha<sup>-1</sup> in Ultisol treatments. However, in TAS occurred not avoidance response of *F. candida*. We conclude that the swine manure was toxic to *F. candida* and the toxicity is dependent on the soil characteristics and the manure concentrations applied.

**Key words:** Collembolans, ecotoxicology, liquid pig manure, soil fauna, waste management.

## INTRODUCTION

The outstanding position of Brazil in the world scenario of the swine chain is the result of technological advances and strategies of the breeding system that allows for a high concentration of animals per unit area (Lima & Santos 2015), becoming a possibility of financial gains even in properties with small territorial extensions. This increasing expansion and intensification of swine production generates a large amount of manure, increasing the difficulty of handling and finding the best destination of this residue by the producers.

Currently the disposal of manure in soil because of its nutrient composition has been the most usual practice presenting the highest

economic viability. When properly used, this material can provide a reduction of the amount of chemical fertilizers needed by crops, reducing crop production costs (Congreves et al. 2016, Oliveira et al. 2016). On the other hand, inappropriate uses of this residue may result in environmental problems, due to accumulation of metals and of veterinary products used in animal husbandry as growth promoters and for disease prevention and treatment (Scherer et al. 2010, Maccari et al. 2016) and the intensity of this impact is directly dependent on the doses and the type of soil onto which this waste is applied (Segat et al. 2015, Maccari et al. 2016). The environmental risks associated with excessive use of swine manure as a soil fertilizer, being hazardous to faunal biodiversity, have been

recognized internationally (Alves et al. 2008, Segat et al. 2015), although in Brazil this type of study is still incipient (Scherer et al. 2010).

Once the toxic potential of swine manure in the environment is recognized, it is necessary to measure or estimate the damage that its use in agriculture can cause to terrestrial ecosystems. The magnitude of the effects of swine manure application on soil biota can be measured through terrestrial ecotoxicology using toxicity tests, which has proved to be an adequate tool for the monitoring of pollutants in ecosystems. The use of terrestrial invertebrate species that have high sensitivity and good response capacity when exposed to the adversities of contaminated environments (Alves & Cardoso 2016) is based on internationally standardized methodologies. Collembola are known for their important role in the dynamics of organic material in the soil, acting as decomposers and organic waste cyclers (Maccari et al. 2016, Segat et al. 2018). Among them, the species *Folsomia candida*, considered representative of the other species of springtails in the soil for its sensitivity, has helped to identify the danger of the application of animal residues in agricultural soils (Maccari et al. 2016).

The objective of the present study was to evaluate, through terrestrial ecotoxicological tests, the impacts caused by the addition of doses of liquid swine manure in natural soils and an artificial soil by testing the survival, reproduction and avoidance of *Folsomia candida*.

## MATERIALS AND METHODS

### Soils

Two different natural soils were used, an Ultisol (27°02'13.1"S 52°37'42.1"W) and an Oxisol (27°05'27.6"S 52°38'11.5"W) collected in the 0 - 0.20 m layer in forest areas and without a history of agricultural use. The soils were oven-dried at 65 °C and sieved at 2 mm. An artificial soil was also used, Tropical Artificial Soil (TAS), composed of a mixture of 70% fine industrial sand, 20% kaolinite clay and 10% ground and dried coconut fiber (Garcia 2004). The pH of the TAS and the natural soils was corrected to  $6.0 \pm 0.5$  with the addition of  $\text{CaCO}_3$  and the moisture to 60% of the water retention capacity of each soil. For each of the evaluated soils, the cation exchange capacity (CEC), clay and organic matter (OM) contents, as well as the available amounts of mineral nutrients, obtained according to Tedesco et al. (1995), are presented in Table I.

### Swine manure

The swine manure was collected directly from the installation of the animals and underwent a stabilization process for 120 days (CQFSRS/SC 2016). We tested swine in the growth phase of animals with approximately 8 to 25 kg of body weight, characterized as a phase with a high supply of Zn and Cu in the diet of the animals. During this time, there is the highest incidence of gastrointestinal disorders, caused by the change from liquid to solid diet, which favors the loss of nutrients from food through excreta. The

**Table I. Physical and chemical parameters of the soils used in the ecotoxicology tests.**

	OM	CEC	Clay	K	Ca	Mg	H+Al	P	Cu	Zn	Fe
	(g dm <sup>-3</sup> )		(%)	.....mmol <sub>c</sub> dm <sup>-3</sup> .....				.....mg kg <sup>-1</sup> .....			
<b>Ultisol</b>	27	92.8	52	3.9	24	7	58	26	9.6	3.2	31
<b>Oxisol</b>	53	144.4	50	1.8	17	4	121	12	8.2	2.1	88
<b>TAS</b>	49	6.8	20	25.5	14	9	22	39	0.2	0.7	3

animals from which the manure was collected were fed corn and soybean meal, and none of the animals had been medicated for illness for a month before the collection of the material. For the tests the swine manure was used in natural moisture. The manure was collected only once and homogenized for all tests. Moisture and chemical parameters of the manure, analyzed following the methods preconized by Van Raij et al. (2001), are in Table II.

**Acute toxicity test (lethality)**

The treatments consisted of combinations of the doses 0, 25, 50, 75 and 100 m<sup>3</sup> ha<sup>-1</sup> of swine manure added to three soils. To evaluate the effects of the manure on the survival of the springtails, according to ISO 11267 (1999). In glass containers, with a height of 11.5 cm and a diameter of 3.5 cm, we added 30 g soil contaminated with manure with increasing concentrations or with control soil (without application of swine manure). Each experimental unit received 10 individuals, aged between 10 and 12 days. After 14 days of the onset, the number of surviving individuals was assessed. Therefore, the contents of one experimental unit were transferred to another recipient containing water and black ink, where the surviving organisms floated and their color contrast with the ink allowed the counting of these individuals.

**Chronic toxicity test (reproduction)**

For the chronic toxicity test, treatments consisted of combinations of doses 0, 10, 15, 20 and 25 m<sup>3</sup> ha<sup>-1</sup> of swine manure added to three soils studied, which were based on the results of the

acute toxicity test. The test methodology was by following the ISO 11267 (1999) and executed in the same glass containers described above and filled with the different soils with swine manure doses. Each experimental unit received ten juvenile collembolans of 10-12 day old. Weekly, the containers were opened to promote aeration and to correct soil moisture by weight difference as time passed by. At the beginning of the test and after 14 days, the organisms were fed with biological yeast. After 28 days the number of juveniles were counted, as in the survival test, by floating in water and black ink.

**Avoidance test (behavioral)**

The avoidance test was conducted according to ISO 17512-2 (2008). The treatments used were combinations of doses 0, 10, 15, 20 and 25 m<sup>3</sup> ha<sup>-1</sup> of swine manure added to three soils studied conducted in five replicates (dose x soil). Circular plastic containers (6.5 cm diameter) were used, which were divided in half with the aid of a vertically inserted plastic separator, where each side received 30 g of soil. One side of the vessel was filled with soil without swine manure addition (control) and the other side with soil containing the respective doses of swine manure and 20 individuals were placed on the central separation line. The different doses of swine manure were mixed before placing the soil in the containers for each of the doses and for control the soil moisture was corrected to 65% of the water holding capacity. At the end of the test, the plastic separator was removed.

The containers remained closed, in the dark, to avoid influence of luminosity on the faunal

**Table II. Moisture and chemical parameters of the swine manure.**

Moisture	Total C	Org. C	Total N	Total P	Total K	pH	Cu	Zn	Fe
..... % .....						(KCl)	...mg kg <sup>-1</sup> ...		
74.7	9.48	7.95	0.73	1.26	0.42	7.3	164	367	4809

behavior, and they were kept in an environment with controlled temperature of  $23\text{ }^{\circ}\text{C} \pm 2$ . The test lasted for 48 hours and the organisms received no food. After 48 h, the partition was inserted vertically in the middle of the container, dividing it into two sections (contaminated soil and non-contaminated soil), and we counted the number of individuals in each section of the box. As for the other tests performed, it was necessary to add water and black ink to account for the individuals. The soil was transferred to a 300 mL capacity vessel, where 200 mL of water and 3 drops of ink were added. The contents were stirred in circular motions for approximately 30 s. The values were expressed as percentage of avoidance, i.e., individuals who avoided contaminated soil.

In addition to the treatments, “double controls” were set up, which consisted of five containers (6.5 cm diameter) that received uncontaminated control soil on both sides, to verify the reliability of the test results from the validation criteria described by the protocol ISO 17512-2 (2008).

### Data analyses

Reproduction and lethality data were submitted to analysis of variance (ANOVA) and the means were compared by the Dunnett test ( $p \leq 0.05$ ) using Software SAS 9.2. The values of LOEC (lowest concentration tested with an observed effect) and NOEC (highest concentration tested without any observed effect) were obtained from the mean comparison test.  $LC_{50}$  values (lethal concentration at 50% of the population) were obtained using PriProbit® Software 1.63.  $EC_{50}$  values (effect concentration in 50% of the population) were obtained from the reproduction tests, using non-linear regression analyses that were performed with the STATISTICA® 7.0 program,

The number of individuals showing avoidance behavior were analyzed using the Fisher Exact Test according to Agresti (1992).  $AC_{50}$  values (concentration causing avoidance in 50% of the population) were also obtained with PriProbit® 1.63 Software.

## RESULTS

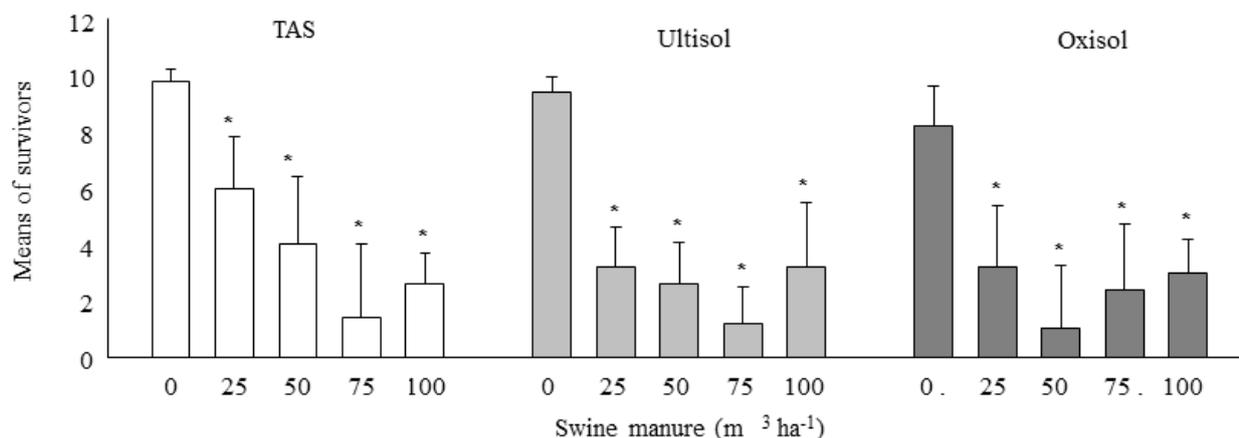
### Validation of the experiments

The validation criteria of the tests were attended for all tests performed. For the acute toxicity test, the percentage of dead individuals in the control was 2% with a coefficient of variation (CV) of 4.5%. For the reproduction tests, the average number of juveniles generated was 509 with a CV of 21%. In the avoidance test, the distribution of organisms in the double control was 50%/50%, and no significant differences were found ( $p > 0.05$ , Fischer Exact Test). Therefore, there was homogeneous distribution of the individuals in the two sections, which proves that there was no preference for one side of the container when both sides contained only control soil.

### Ecotoxicology tests

All doses of swine manure applied to the three different soils caused a significant reduction in the survival of *F. candida* (Figure 1). The values estimated for the  $LC_{50}$  were different among the different soil types, varying between 2.9 and 33.1  $\text{m}^3 \text{ha}^{-1}$  (Table III).

Reproduction of *F. candida* was reduced in most of the doses applied in the three soils, and the reduction increased with increasing doses. For TAS and Oxisol, there was a reduction in the number of juveniles already in the first dose of swine manure, equivalent to an evaluated LOEC of 10  $\text{m}^3 \text{ha}^{-1}$ , however, for Ultisol, the LOEC value was equal to 15  $\text{m}^3 \text{ha}^{-1}$  (Figure 2). But the concentrations estimated to cause 50%



**Figure 1.** Means of *Folsomia candida* individuals surviving in Tropical Artificial Soil (TAS), Ultisol and Oxisol with increasing concentrations of swine manure. \*Significant statistical difference ( $p < 0.05$ ) by the Dunnett test. (—) Standard deviation ( $n = 5$ ).

reduction in springtail reproduction did not vary among the different soil types (Table III).

Regarding the behavioral tests, for TAS, there was no avoidance response of the contaminated soil, since more organisms were present in the contaminated soil at all doses (Figure 3). In Ultisol, the lowest doses (10 and 15 m<sup>3</sup> ha<sup>-1</sup>) did not show avoidance, but this behavior was found for doses of 20 and 25 m<sup>3</sup> ha<sup>-1</sup> (Figure 3) and in Oxisol all doses resulted in significant avoidance to the uncontaminated soil (Figure 3). The calculated AC<sub>50</sub> values for the three soils are shown in Table III.

## DISCUSSION

The results obtained in the acute toxicity tests showed that all the doses evaluated caused significant lethality of the individuals, which probably can be explained by high values of Cu and Zn in the manure. The estimated values of Cu and Zn added to the soil at LC<sub>50</sub>, were 6.6, 2.2 and 2.2 mg Cu kg<sup>-1</sup> dry soil and for Zn 11.1, 4.9 and 4.6 mg kg<sup>-1</sup> dry soil for TAS, Ultisol and Oxisol, respectively.

Domene et al. (2007) evaluated the effect of different organic residues added to the

artificial soil on the survival of springtails and found lethality effects when swine manure was applied (24 g kg<sup>-1</sup> soil) and suggested that the cause might be the high values of Zn in the substrate. Sandifer & Hopkin (1996) evaluating soils contaminated with Zn, also observed lethality of *F. candida* with 1000 µg kg<sup>-1</sup> dry soil. Scott-Fordsmand et al. (2000), when evaluating a Cu contamination gradient, found a correlation of the lethality of *F. fimetaria* with the increasing concentration of the metal in the soil. Crouau & Pinelli (2008) confirmed that metal toxicity may be due to the presence of Zn (2700 µg g<sup>-1</sup> dry soil) and Van Gestel & Hesenbergen (1997) report an additivity in the toxic effect of Cd when in conjunction with Zn (1600 µg Cd g<sup>-1</sup> and 3200 µg Zn g<sup>-1</sup> dry soil). All the values cited in the other studies were less than the values estimated in the present study. Finally, Crouau et al. (1999) associated collinear lethality with variations in soil pH. In our present study, pH variation occurred after 14 days, from 5.7 in the Ultisol without addition of manure to 6.2 in the same soil with the highest applied manure dose. For Oxisol, this increase was from 5.8 to 6.4, and in TAS, the increase was from 6.2 to 6.5. These variations may have influenced the survival

**Table III. Calculated values of LC<sub>50</sub>, EC<sub>50</sub> and AC<sub>50</sub> and confidence interval for *F. candida* toxicity tests in Tropical Artificial Soil (TAS), Ultisol and Oxisol with swine manure.**

Soil	LC <sub>50</sub> (m <sup>3</sup> ha <sup>-1</sup> )	EC <sub>50</sub> (m <sup>3</sup> ha <sup>-1</sup> )	r <sup>2</sup> *	AC <sub>50</sub> (m <sup>3</sup> ha <sup>-1</sup> )
TAS	33.1	10.6 (9.9 - 11,4)	0.9817	>25
Ultisol	12.0	11.9 (10.6 - 13,1)	0.9843	28.8
Oxisol	2.9	11.1 (10 - 12)	0.9837	26.0

\* r<sup>2</sup> for non-linear regression analysis for EC<sub>50</sub> values.

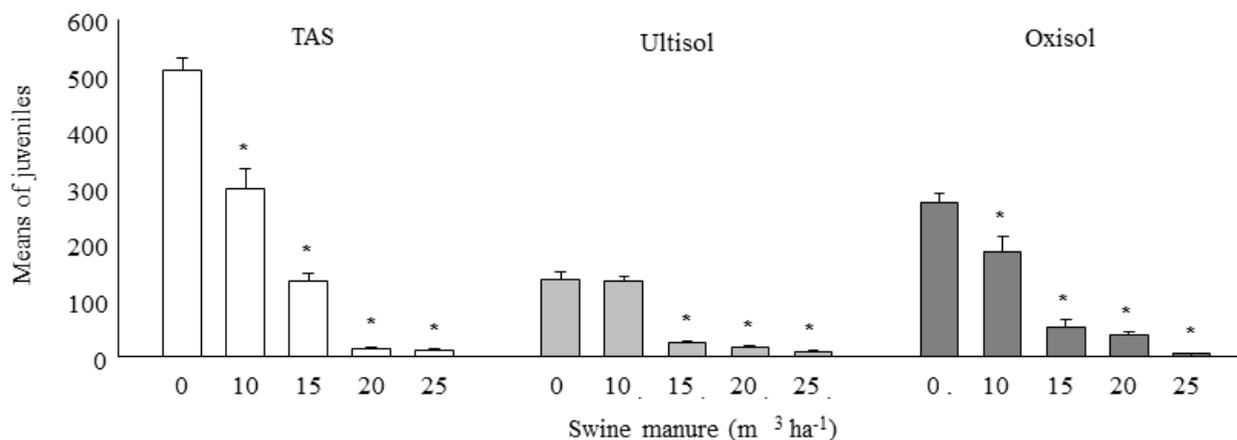
of the springtails, since pH is one of the main factors regulating the bioavailability of metals in the soil. However, according to Crouau et al. (2002), it is difficult to evaluate the toxicity of organic compounds to springtails, since in addition to the influence of pH there may be influence of the moisture content and presence of high amounts of organic matter.

In the springtail's reproduction test in TAS and Oxisol, all doses tested caused a reduction in the number of juveniles generated. Ultisol however did not present a significant reduction in the reproduction rate in the dose of 10 m<sup>3</sup> ha<sup>-1</sup>. Domene et al. (2007) evaluated the toxicity of seven different organic residues added to the soil and verified that swine manure had the greatest negative effect on the reproduction of *F. candida*. The EC<sub>50</sub> value found by the authors was 24 g of swine manure per kg of soil. In the present study the calculated value of EC<sub>50</sub> was 10.6; 11.9 and 11.1 m<sup>3</sup> ha<sup>-1</sup> for TAS, Ultisol and Oxisol, respectively, values that are equivalent to 5.3; 6 and 5.5 g swine manure kg<sup>-1</sup> of soil. The difference in the toxicity values found in our study (on average twice as low) may be mainly due to the soil types used. The soils used by the authors cited (Domene et al. 2007) were from a region with temperate climate, with greater predominance of negative loads and, consequently, higher CEC, about to the tropical soils used in our study. The higher toxicity of organic wastes, drugs and metals in soils with

lower CEC and Organic Matter has already been reported in other papers (Segat et al. 2015, 2019, Maccari et al. 2016, Zortéa et al. 2018). This conclusion is related to the ability of different soils to retain the possible soil contaminants.

Although in our study we inferred the possibility of the negative effects of swine manure being mostly due to Cu and Zn, the effects of Zn toxicity on springtails are still quite discrepant. Smit & Van Gestel (1995), evaluating the toxicity of Cl<sub>2</sub>Zn on *F. candida*, found EC<sub>50</sub> values of 1240 to 1705 µg Zn g<sup>-1</sup> of dry soil in samples one year after the addition of Zn. For soils that were contaminated close to the faunal exposure, these values change to 185 to 348 µg Zn g<sup>-1</sup> in temperate soil (Smit & Van Gestel 1996). Van Gestel & Hensber (1997) found values of 626 µg of Zn g<sup>-1</sup> of artificial soil, values higher than the Zn equivalent values found in the present study 4.7; 4.9 and 4.9 g kg<sup>-1</sup> of dry natural soil for TAS, Ultisol and Oxisol, respectively.

Domene et al. (2011), evaluating 19 different natural soils, found lower reproductive performances in the soils with higher concentrations of total N. For Domene et al. (2007), N-derived compounds are toxic to *F. candida*, especially ammonium, which during the conduction of the test, can accumulate in the recipient. In our present study, the containers were opened only once a week to allow the exchange of air.



**Figure 2.** Mean of juveniles of *Folsomia candida* in Tropical Artificial Soil (TAS), Ultisol and Oxisol with increasing concentrations of swine manure. \*Significant statistical difference ( $p < 0.05$ ) by the Dunnett test. (┐). Standard deviation ( $n = 5$ ).

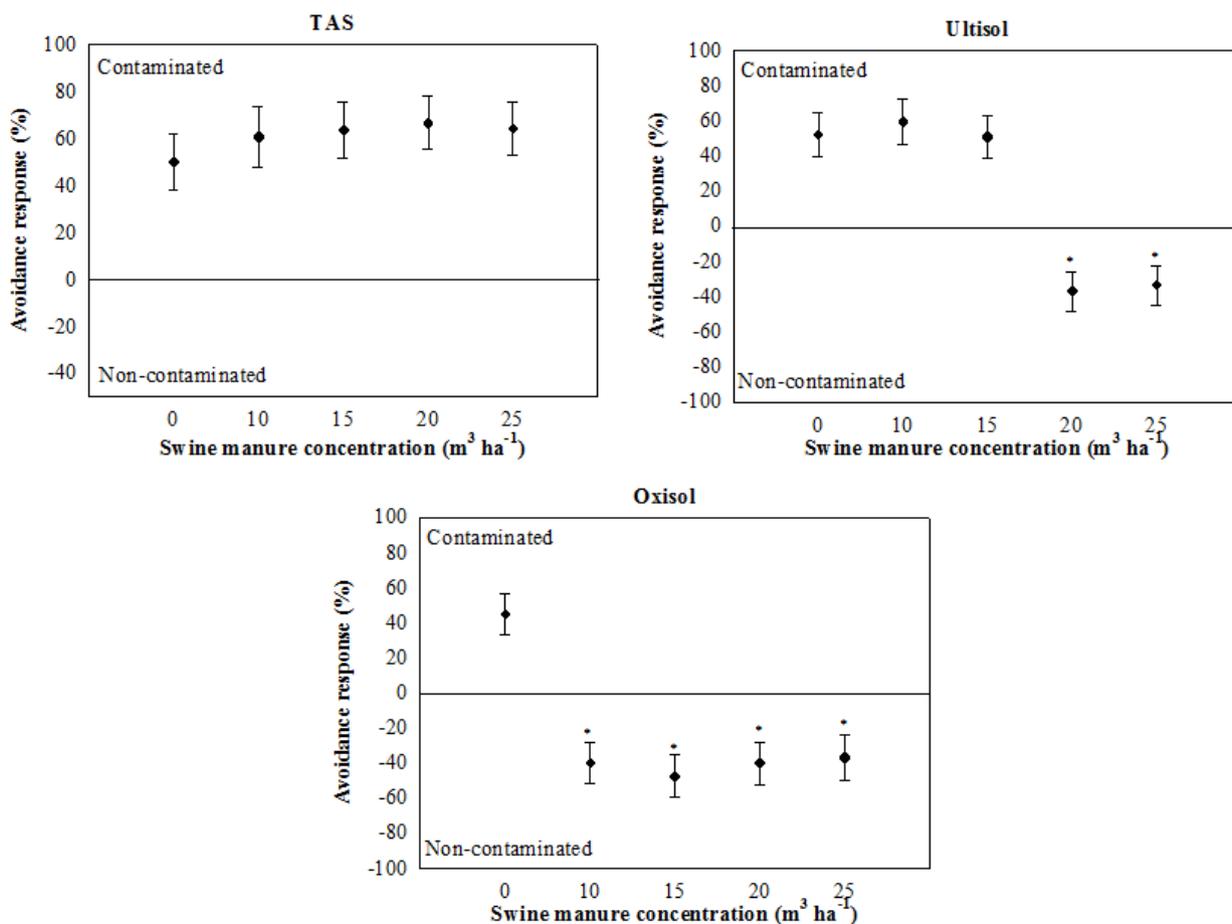
Another possible cause for the reduction of reproduction and for increases in mortality may be the reduction in feeding of the animals. Results of similar studies, evaluating the inhibition of food consumption by individuals, showed a reduction of 72% in food consumption with the addition of 1 g kg<sup>-1</sup> of thermally treated swine manure, and this inhibition still increased with increasing doses of applied animal waste (Domene et al. 2007). These authors also correlate the lethality and loss of reproductive potential with the feeding restriction caused by this residue.

Variations in the number of juveniles found in treatments with addition of swine manure could be associated with changes in soil pH, as well as suggested for effects on survival. Some authors affirm that pH has a negative influence on the reproduction of springtails (Van Gestel & Van Diepen 1997, Crouau et al. 1999, Greenslade & Vaughan 2003) and that maximum reproduction occurs in values close to 5.5, being the number of juveniles generated reduced in values above and below this value. However, in the present study, pH values in natural soils do not confirm the hypothesis that pH influences reproduction rate.

In the TAS, the evaluated doses did not cause avoidance of the organisms to the soil

without addition of manure. This behavior may have occurred due to differences in microbial populations. According to Domene et al. (2011), microbial populations are important to determine the behavior of springtails, since these organisms feed on microorganisms. Increase in the microbial population may have been caused by the addition of swine manure, however these parameters were not evaluated in this work.

The significant avoidance behavior of *F. candida* caused in Ultisol with the two highest manure doses (20 and 25 m<sup>3</sup> ha<sup>-1</sup>) (Figure 3), while the lower doses did not cause avoidance, show that in small doses swine manure is not toxic or even unpleasant to collembolans however, with increasing doses it can affect their behavior. According to Andrés & Domene (2005), organic residues may themselves represent both, a food source and toxic material. We know that the difference between these two responses may be only a matter of the material's concentration. In the Oxisol, however, all doses of swine manure caused significant avoidance of the organisms. The differences between the physical and chemical constitution of the three soils tested in our study is the main reason for metals being much more available in the Oxisol, followed by



**Figure 3.** Avoidance response of *Folsomia candida* in the behavioral test with tropical artificial soil (TAS), Ultisol and Oxisol, all treated with increasing concentrations of swine manure (n = 5). \*Significant statistical difference (p<0.05). Fischer exact test (n = 5).

the Ultisol and perhaps almost non-available in TAS. Greenslade & Vaughan (2003) state that springtails can avoid concentrations of Cu below the levels that cause some toxic effect and, according to Natal-da-Luz et al. (2004) this same behavior can be expected for Zn.

Based on our results and data from published scientific literature, it is possible to use swine manure in the soil as organic fertilizers, provided that the retention capacity of the soils that will receive the material is respected. When this condition is respected the toxic effects can be minimized, favoring the practice and maintaining the sustainability of the productive system. In this way, studying soils with different retention

capacities and effects on other soil organisms is fundamental to the accomplishment of this important agricultural practice.

### CONCLUSIONS

A gradient of increasing swine manure doses applied to natural soils can cause lethality, reduction in the reproduction rate and avoidance behavior of collembolans of the species *F. candida*.

Even the lowest dose of swine manure evaluated in the acute toxicity tests (25 m³ ha⁻¹) caused significant mortality on the springtails in all soils tested.

The application of  $10 \text{ m}^3 \text{ ha}^{-1}$  of manure caused a decline in the reproduction of collembolans in the Tropical Artificial Soil and in the Oxisol, and the dose of  $15 \text{ m}^3 \text{ ha}^{-1}$  caused the same effect when applied in Ultisol.

The effects on *Folsomia candida* are associated with the Cu and Zn concentrations the swine manure.

In the Tropical Artificial Soil, there was no avoidance response of the organisms. For the natural soils, this behavior was found at 10 and  $15 \text{ m}^3 \text{ ha}^{-1}$  for Oxisol and Ultisol, respectively.

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#### Author contributions

J.C.S and E.J.B.N.C motivated and designed the study. J.C.S., D.B and P.R.L.A. analyzed the data and prepared the figures. J.C.S., E.J.B.N., D.B. and P.R.L.A discussed the results and wrote the paper.

