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ORIGINAL RESEARCH

The effect of composted and non-composted poultry litter on survival and reproduction of *Folsomia candida*

Ana Paula Maccari[1], Julia Corá Segat[2*], Manuela Testa[3], Carolina Riviera Duarte Maluche Baretta[4], Dilmar Baretta[2]

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Abstract

Purpose The objective was to evaluate the effect of the application of composted and non-composted poultry litter, as fertilizer in agricultural areas, on the survival and reproduction of the springtails (*Folsomia candida*) through standardized ecotoxicological tests.

Methods The treatments included in the application of composted and non-composted poultry litter on Entisol were in the following doses 0, 10, 20, 40, 60, 80, 100 t ha^{-1}, for the lethality test, and 0, 5, 7.5, 10, 15, 30, 40, 60 t ha^{-1} for the reproduction test.

Results For the composted litter, the LC_{50} value was 76.45 t ha^{-1} and for $EC_{50,}$ it was 17.96 t ha^{-1}. For the non-composted poultry litter, the values of LC_{50} was 43.12 t ha^{-1} and EC_{50} value was 19.36 t ha^{-1}.

Conclusion The highest toxicity was observed in the non-composted litter, emphasizing the importance of stabilization of the organic compound before its use as fertilizer.

Keywords Animal production, Environmental evaluation, Organic fertilizer, Soil mesofauna

Introduction

The continuous and accelerated growth of poultry production in Brazil and world causes the production of large amounts of waste (Mierzwa-Hersztek et al. 2016). This waste, consists of shavings, excreta, feathers and other compounds that accumulate during the production process (Terzich et al. 2000; Gilmour et al. 2004). The main destination of the poultry litter is its application as organic fertilizer to the soil (Arthur et al. 2015; Mierzwa-Hersztek et al. 2016), as well as their use to produce organo-mineral fertilizers. This practice is economically desirable as it represents an internal resource of rural property and is a residue containing a high concentration of nutrients (Oviedo-Rondón 2008; Agyarko-Mintah et al. 2016a). However, from an environmental point of view, there are great restrictions on its use, because if it is poorly managed, it can pollute air, soil and water, making it mandatory to include the concept of sustainability and environmental management in this sector (Marín et al. 2015; Agyarko-Mintah et al. 2016b; Dunlop et al. 2016; Mierzwa-Hersztek et al. 2016).

In the last decades, studies have been developed with the purpose of knowing the dynamics of decomposition and the release of nutrients from the poultry litter, and from this, to establish those recommendations for the application of the same fertilizer for the crops, so as not to offer environmental risk (Pitta et al. 2012). However, studies to evaluate the effects of poultry litter on biological soil parameters are still scarce in the literature and there are no information on the influence of composting treatment of poultry litter

✉ Julia Corá Segat
juliasegat@yahoo.com

1 Professora do Instituto Federal de Santa Catarina - IFSC. São Miguel do Oeste, SC, Brazil
2 Professor do Departamento de Zootecnia, UDESC, Chapecó, SC, Brazil
3 Mestre em Zootecnia, UDESC, Chapecó, SC, Brazil
4 Professora Área de Ciências Exatas e Ambientais, UNOCHAPECÓ, Chapecó, SC, Brazil

prior to its disposal in the soil, regarding the toxicity of this residue to the edaphic communities.

The lack of information on edaphic organisms in regions with high animal production causes great concern, and improper disposal of manure from the poultry production system can modify soil properties and promote changes in community structure, changes in wildlife, activity and diversity of edaphic fauna (Baretta et al. 2003; Alves et al. 2008; Maccari et al. 2016).

Studies using soil organisms as soil quality bioindicators to assess the toxicity of organic wastes warn the potential short and long-term risks of excessive and/or continued use of waste in agricultural areas (Domene et al. 2007; Martin and Stanislav 2010; Segat et al. 2015; Renaud et al. 2017). Among the organisms used, *Folsomia candida* springtails have shown an important response to the use of organic waste (Maccari et al. 2016), since these organisms are associated with the dynamics of transformation of organic matter in the soil. Therefore, the objective of this study was to evaluate the effect of the application of increasing doses of composted and non-composted poultry litter on the survival and reproduction rate of *Folsomia candida* by means of standardized ecotoxicological tests (ISO 1998).

Material and Methods

Sampling and characterization of the poultry litter

The poultry litter tested came from a farm with commercial production of broiler chickens that used wood shavings (*Pinus elliottii*) as an absorbent substrate for poultry excreta, with eight lots each consisting of 45 days of production. After the collection, the poultry litter was separated into two parts, one destined to the composting. To compost the material, it was placed in a pile and covered with polyethylene for 60 days, handling this very common in the properties and well representative of the region, for the use in the treatment of composted poultry litter (C) and the other part was packed in plastic bags and kept at a low temperature (-20° C) for the maintenance of its characteristics for later use in the treatment of non-composted poultry litter (NC). The chemical characterization of the poultry litter was performed according to the method proposed by Tedesco et al. (1995), and the percentage of carbon and total nitrogen analyzed by total combustion of the sample in elemental analyzer (CHNS). Chemical parameters of the composted (C) and non-composted poultry litter (NC) are shown in Table 1.

Table 1 Chemical parameters of the composted (C) and non-composted poultry litter (NC)

	pH (H_2O)	Moisture (%)	N total (%)	C total (%)	Ratio C/N	P	K	Mn	Cu	Mg	Ca	Fe	Zn
						....	....	....	.... %	....	....	....	....
C	8.5	24.23	3.13	41.0	13.1	3.3	4.2	0.06	0.05	1.72	3.38	0.27	0.04
NC	9.0	30.85	2.51	39.1	15.6	2.7	4.3	0.04	0.04	1.57	2.59	0.09	0.03

Soil test

In this study, samples of the superficial layer (0-0.20 m depth) of the Entisol (Embrapa 2006) colected in Araranguá, SC (29º 00' 19.98" S e 49º 31' 03.84" W) were used. The soil was oven dried (65 ° C) and sieved in a 2 mm mesh sieve for homogenization. The characterization of soil chemical and physical parameters were carried out according to the methodology proposed by Tedesco et al. (1995) and are presented in Table 2.

Table 2 Chemical and physical parameters of Entisol evaluated at depth 0-0.20 m

OM[a]	pH	P	K	CEC[b]	Ca	Mg	Al	H+Al	Cu	Zn	Fe	Mn	Sand	Silte	Clay
%	H_2O	mg dm^{-3}		..........$cmol_c$ dm^{-3}............					.........mg kg^{-1}.........				.............%............		
0.9	5.8	6.7	34	4.92	2.0	0.83	0.0	2.0	1.5	1.0	72.5	2.1	37.0	59.0	4.0

[a] Organic matter

[b] Cations Exchange Capacity

As reference, we used Tropical Artificial Soil (TAS) (Garcia 2004). This soil consists of a mixture of 70% industrial sand (fine), 20% kaolinite clay and 10% dry and sifted coconut fiber (2 mm). In the present study, the TAS was used only as a control for the validation of the tests. For the tests, the soil moisture content was adjusted to 65% of the maximum water retention capacity (ISO 11465 1993). Soil pH was measured at the beginning and at the end of the test.

Test organisms

The tests were conducted with *Folsomia candida*, the organisms were created according to (ISO 11268-2 1998) on gypsum and activated charcoal substrates (11:1 – v:v), with controlled photoperiod of 12:12 h (light:dark), and kept in a controlled temperature environment (20°C ± 2). Weekly the organisms were fed with 2 mg of Saccharomyces cerevisiae (Maccari et al. 2016) and the humidity of the breeding medium corrected by the addition of distilled water.

Treatments

The treatments consisted of increasing doses of poultry litter, in the form of composted litter (C) and non-composted litter (NC). For the lethality test, the doses used were 0; 10; 20; 40; 60; 80 and 100 t ha^{-1}. Based on the results obtained in the lethality tests, doses were defined for the reproduction test: 0; 5; 7.5; 10; 15; 30; 40 and 60 t ha^{-1}, for both evaluated litters. The tests were conducted in a completely randomized experimental design, with four replicates for the lethality test and five replicates for the reproduction test.

Lethality and reproduction tests

The evaluation of lethality and reproduction of *F. candida* followed the recommendations of the ISO 11267 (1999) and ISO 11268-2 (1998), respectively. For both tests, each experimental unit consisted of a plastic container (height: 6.0 cm; diameter: 6.5 cm) filled with 30 g of soil with the respective dose of poultry litter. Each experimental unit received 10 adult individuals (10-12 days of life).

To evaluate the lethality of individuals, at 14 days (ISO 11267 1999; Maccari et al. 2016), water and a few drops of black paint were added to the contents of each container. After slight stirring, the surviving organisms floated and the color contrast with the ink allowed their counting. The reproduction test had duration of 28 days (ISO 11268-2 1998; Maccari et al. 2016) and after this period, the subjects were evaluated. As for the lethality test, 150 mL of water and five drops of black paint were added to the contents of the containers. The living individuals found on the surface were photographed and counted using ImageTool software.

Statistical analysis

The data obtained in the lethality test were tested for normality and homogeneity by the Kolmogorov-Smirnov and Cochran Bartlett tests, and then submitted to analysis of variance (ANOVA One-way) and the means compared by the Dunnett test ($p \leq 0.05$) using the Software Statistica 7.0. LC_{50} values (50% lethal concentration) were calculated using PriProbit® Software 1.63.

For the reproduction test, normality and homogeneity were evaluated by the Kolmogorov-Smirnov and Cochran Bartlett tests. The data were transformed into square root ($^2\sqrt{}$). A variance analysis (ANOVA One-way) was performed and the means were compared by the Dunnett test ($p \leq 0.05$) using Software Statistica 7.0. EC_{50} values (50% effective concentration) were calculated by logistic nonlinear regression using Software Statistica 7.0.

Results and Discussion

Both tests were valid according to the respective standards (ISO 11267 1999). In the acute toxicity test, the adult springtails lethality rate mean was 96% survival. In the reproduction test, the mean number of juveniles in the control was 222 and the coefficient of variation of the test was 26%.

The results obtained in the *F. candida* lethality and reproduction tests in Entisol with increasing doses of composted and non-composted poultry litter are presented in Figures 1 and 2, respectively. The calculated toxicity values (LC_{50} and EC_{50}) for the same tests are shown in Table 3.

The survival was significantly affected ($p \leq 0.05$) by the 60 t ha^{-1}, regardless of composted or no-composted poultry litter. When the results obtained were individually analyzed, it was found that the poultry litter non-composted showed higher toxicity to the springtails, causing 100% of lethality at the doses of 80 and 100 t ha^{-1}. This condition can also be seen in the estimated LC_{50} and values were 76.45 t ha^{-1} for composted litter and 43.12 t ha^{-1} for non-composted litter (Table 3).

The higher toxicity found for non-composted poultry litter is directly related to the presence of toxic compounds that are reduced in the composting process. Among the toxic compounds that are reduced by the stabilization process, we have the ammonium that is already reported as toxic to organisms in other studies in the literature indicating that the application of high doses of organic waste can negatively impact the *F. candida* population and the population of soil organisms (Domene et al. 2007; Maccari et al. 2016; Renaud et al. 2017). Composting of organic wastes prior to their application to the soil is an important process to decrease toxicity to soil fauna (Domene et al. 2008).

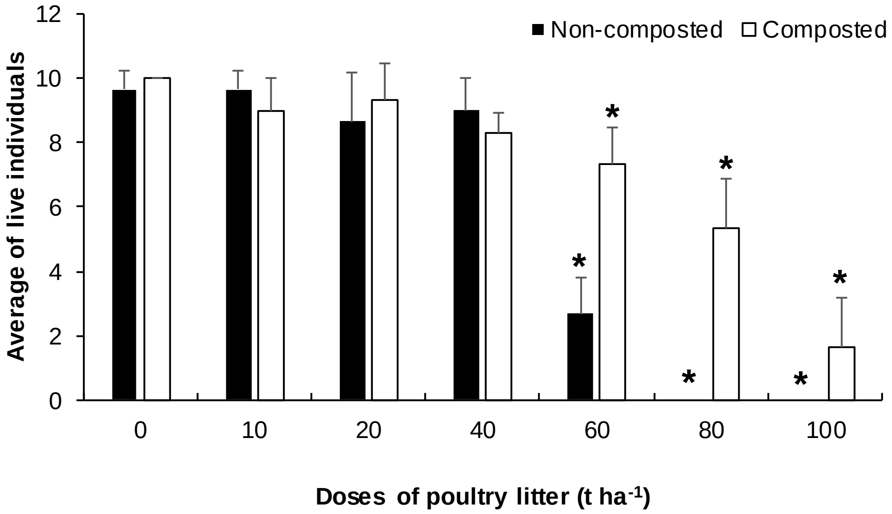

Fig. 1 Average of live individuals (mean + SD) of *Folsomia candida* in Entisol with doses of composted and non-composted poultry litter. *Statistical difference in relation to control (Dose 0 t ha^{-1}) by the Dunnett test ($p \leq 0.05$)

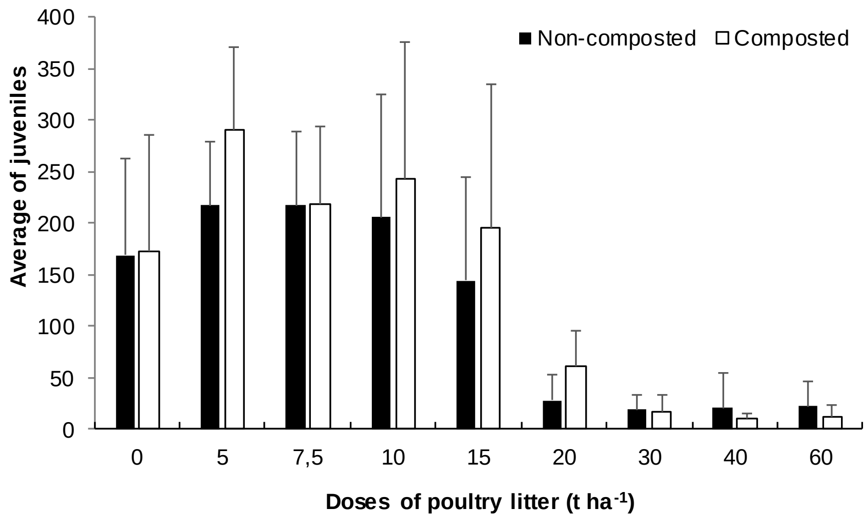

Fig. 2 Average of juveniles (mean + SD) of *Folsomia candida* in a Entisol contaminated with doses of composted and non-composted poultry litter. * Statistical difference by the Dunnett test ($p \leq 0.05$)

Reproduction of the organisms was reduced ($p \leq 0.05$) by the application of 20 t ha^{-1} of composted poultry litter and 30 t ha^{-1} of non-composted poultry litter (Fig. 2). The estimated EC_{50} values were 17.96 t ha^{-1} for composted poultry litter and 19.36 t ha^{-1} for non-composted poultry litter (Table 3). In the reproduction tests, it was not possible to verify differences between composted (EC_{50} of 17.96 t ha^{-1}) and non-composted poultry litter (EC_{50} of 19.36 t ha^{-1}) that could be associated to the treatment and stability of the waste. The effects on reproduction are possibly derived from a combination of toxic compounds presented in the poultry litter and the physicochemical properties of the studied soil.

Table 3 Lethal concentration (LC_{50}) and effective concentration EC_{50} (t ha^{-1}) for *Folsomia candida* in Entisol with composted (C) and non-composted (NC) poultry litter

	LC_{50}	EC_{50}
C	76.45 (55.83 133.80)	17.96 (15.04 20.87)
NC	43.12*	19.36 (13.76 24.95)

*Confidence interval not calculated.

The Entisols are poorly developed soils and their texture is basically sandy, with low nutrient adsorption capacity, which implies high losses due to leaching, low cohesion between the particles due to the low content of cementing agents and the low levels of organic matter (Oliveira 2008; Sales et al. 2010). Due to the predominantly sandy constitution and low water retention, the Entisols present low available water capacity, high macroporosity, reduced microporosity and higher soil density (Spera et al. 1999), with a close relationship between macroporosity and soil permeability (Reichert et al. 2009). Due to these characteristics, this soil presents low support capacity compared to other more clayey soils, and greater availability of some nutrients to springtails.

Soil characteristics interfere with the availability and mobility of contaminants and may alter toxicity (Domene et al. 2010) for edaphic organisms (Natal-da-Luz et al. 2008). Therefore, the greater availability of nutrients presents in the litter of poultry, such as nitrogen, may be one of the factors that affected the reproduction of the springtails.

The results of the present study resemble those found by Maccari et al. (2016), when studying the use of swine manure as an organic fertilizer, the organic matrix provided nutrients to the organisms, reaching a level of toxicity for the *F. candida*. The authors found more significant effects for the lethality test, where the soil contaminated with swine manure caused mortality of the individuals already at the lowest applied dose (25 m^3 ha^{-1}) and caused 100% mortality in the other doses tested (50, 75 and 100 m^3 ha^{-1}), and in the reproduction test, a significant reduction in the number of juveniles due to dose increase of swine manure.

In contrast, (Domene et al. 2007; Martin and Stanislav 2010) found smaller sensitivity in reproduction than in *F. candida* survival when tested with organic material. The organic matter content can have a positive effect, serving as a source of nutrients for organisms, however, in very high amounts the pollutant load present in the organic materials can cause toxic effects to the organisms, which can make the interpretation of the results of toxicity more difficult (Andrés and Domene 2005), as in the results found in the present study.

The reduction in springtails survival and reproduction observed at the highest doses tested may be associated with an increase in pH values of the soil promoted by the addition of the poultry litter. According to (Jänsch et al. 2005), *F. candida* species are tolerant to a range of environments and can withstand the pH range between 3.2 and 7.6 but prefer soils with pH between 5.5 and 6.0 and organic matter between 1.0 and 11.5%. The pH of the Entisol used in this study was 5.8, considered within the zone of preference of the springtails. With the addition of poultry litter doses, there was an increase in pH, where the reading reached values between 7 and 8 at the highest doses (Tables 4 and 5).

Table 4 Initial and final (at 14 days) pH values for the lethality test with *Folsomia candida* in Entisol with increasing doses of composted (C) and non-composted (NC) poultry litter

	Test start		Final of 14 days	
Doses (t ha^{-1})	C	NC	C	NC
0	4.44	4.89	4.71	4.55
10	6.43	6.60	6.07	5.96
20	7.03	6.39	7.12	6.43
40	7.35	7.50	7.89	7.27
60	7.44	8.03	8.06	7.46
80	7.55	8.23	7.99	8.02
100	8.02	8.28	8.21	7.93

Table 5 Initial and final (at 28 days) pH values for the *Folsomia candida* reproduction test in Entisol with increasing doses of composted (C) and non-composted (NC) poultry litter

	Test start		Final of 28 days	
Doses (t ha^{-1})	C	NC	C	NC
0	4.32	4.24	4.75	4.55
5	5.18	4.95	5.35	4.77
7.5	5.10	5.47	5.40	5.44
10	4.44	5.20	5.88	5.74
15	5.54	5.52	7.12	6.64
20	6.13	6.55	6.64	6.52
30	6.16	6.77	6.78	6.77
40	6.86	6.81	6.86	7.00
60	7.53	7.36	7.17	8.41

The highest pH changes were found in the tests with the non-composted litter, in which the material did not undergo a stabilization process. For the non-composted poultry litter test, at 100 t ha^{-1} dose, there was 100% mortality from adult springtails and at this dose, the final pH was 7.93. This value is above the tolerance range of organisms, according to (Jänsch et al. 2005). In the reproduction test, at the 60 t ha^{-1} dose, the final test pH for the non-composted bed was 8.41 and at this dose, the reproduction of the springtails was significantly affected (Table 5).

It is of great importance that the establishment of a strategy for the application of poultry litter as fertilizer for the different soils should occur, which does not compromise the presence of functionally stable communities of soil fauna. The activity of soil invertebrates directly affects characteristics such as water infiltration, soil structure, fragmentation and decomposition of organic matter and regulation of microbial activity (Cardoso et al. 2013), due to the role of these organisms in the realization of ecosystem services. In the present study, the doses of poultry litter that presented toxicity to the springtails were superior to those used by producers in the organic fertilization of agricultural areas. However, it is important to emphasize that field practice is the frequent reapplication of this material in different types of soils.

Conclusion

It can be observed that the *F. candida* species had their survival and reproduction affected when exposed to the Entisol with high doses of composted and non-composted poultry litter.

The highest toxicity was observed in the non-composted poultry litter emphasizing the importance of the stabilization of the organic compound before its use as fertilizer. The results presented are of great importance as they seek to improve and make production systems increasingly efficient and sustainable.

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Compliance with ethical standards

Conflict of interest The authors declare that there are no conflicts of interest associated with this study.

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