

# Ecotoxicological Evaluation of Forest Biomass Ash on Springtails and Earthworms in Subtropical Soils of Brazil

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## Abstract

The forest biomass ash used in agriculture as fertilizer has characteristics that can negatively affect the terrestrial environment. This study aimed to evaluate the effect of the application of eucalyptus forest biomass ash on springtails and earthworms in subtropical soils through ecotoxicological tests. The subtropical soils Oxisol and Entisol were used for the ecotoxicological tests of survival and reproduction of springtails (*Folsomia candida*) and earthworms (*Eisenia andrei*). The concentrations of forest biomass ash were 0, 5 10 and 20 t ha<sup>-1</sup>, based on the recommendation of the application of plant ash as fertilizer. The ecotoxicological results obtained in Oxisol demonstrate that earthworms and springtails are not affected by the presence of ash. In Entisol, the results of sensitivity of the organisms were more evident, and effects were observed from the dose of 5 t ha<sup>-1</sup> for springtails with estimated LC<sub>50</sub> of 15.68 t ha<sup>-1</sup> (CI: not calculated) and EC<sub>50</sub> of 7.36 t ha<sup>-1</sup> (CI: 7.11-7.62 t ha<sup>-1</sup>). Earthworms were affected from 10 t ha<sup>-1</sup>, with an estimated EC<sub>50</sub> of 11.97 t ha<sup>-1</sup> (CI: 9.49-14.46 t ha<sup>-1</sup>). Application of forest biomass ash in the soil negatively affects springtails and earthworms, but the magnitude of the effects is mainly related to characteristics of the soil, amount applied and sensitivity of the species; negative effects of ash application are directly related to the change in soil pH.

**Keywords:** *Folsomia candida*, *Eisenia andrei*, eucalyptus ash

## 1. Introduction

Currently, the process of converting woody biomass into energy is an alternative in several production systems, including in Brazil. According to James et al. (2012), Brazil can generate approximately  $1.4$  to  $2.7 \times 10^5$  Mg year<sup>-1</sup> of ash using wood as fuel.

As the demand for bioenergy production increases, there is an increase in ash volume and, consequently, the concern with the correct disposal of this residue. The reuse of ash as fertilizer presents itself as an alternative of disposal, allowing the reduction in the use of chemical fertilizers (Bougnom & Insam, 2009; Cruz-Paredes et al., 2017), also functioning as soil corrective (Bonfim-Silva et al., 2018). According to Demirbas (2005) and Monti et al. (2008), ash composition depends on the type of plant used, growth conditions and ash fraction, and the main elements in the composition of its biomass are Ca, Si, Al, Fe, Mg, Na, K, S and P (Cruz-Paredes et al., 2017; Khan et al., 2009; Liiri et al., 2002; Maeda et al., 2008). In addition, it can also contain metals such as As, Cd, Pb and Hg (Liiri et al., 2002; Noyce et al., 2016; Vamvuka, 2009).

European legislations present, as restrictions on the application of woody biomass ash, the risk of metal accumulation (Mortensen et al., 2018), and countries such as Denmark regulate through the Ministry of the Environment (Danish Ministry of the Environment, 2008) the application of ash in agricultural and forest systems based on the Cd contents found in the material. In Brazil, currently there is no legislation regulating the use of woody biomass ash in agricultural and forest systems. Despite being common and increasing, its application in the soil should be evaluated carefully. Thus, the potential use of ash is influenced by its contaminants (e.g. metals), which depend on the source of biomass used (Cruz-Paredes et al., 2017; Khan et al., 2009), and may cause negative environmental impacts when incorrectly disposed (Anderson et al., 2013; Huotari et al., 2015).

Although the impact of different sources of ash and its effects on soil microorganisms (Bougnom & Insam, 2009; Jokinen et al., 2006; Klavina et al., 2016; Perkiömäki & Fritze, 2002) and edaphic fauna (Burton & Eggleton, 2016; Grumiaux et al., 2015; Liiri et al., 2007; Lodenius et al., 2009) have been extensively investigated, little has been studied at the ecotoxicological level. Ecotoxicological tests are conducted using indicator organisms, which according to their characteristics have a very low ecological tolerance limit and, when exposed to certain contaminants, are able to exhibit physiological, morphological or behavioral alterations (Magalhães & Ferrão-Filho, 2008).

Currently, springtails and earthworms stand out among the main organisms used in terrestrial ecotoxicity tests, due to their easy adaptation and reproduction in laboratory with relatively short life cycles (Fountain & Hopkin, 2005), as well as sensitivity to different types of pollutants (Greenslade & Vaughan, 2003). Authors such as Römcke et al. (2009), evaluating the effect of ash from the carbonization of municipal waste, in the artificial soil OECD, on the lethality of earthworms (*E. fetida*), observed that these organisms were sensitive to this type of waste when evaluated through ecotoxicological tests. More recently, Qin et al. (2017), through standardized ecotoxicological tests, found an effect on the survival and reproduction of springtails (*F. candida*) using forest biomass at concentrations equivalent to those used in

field applications, with no response of other mesofauna organisms (*Hypoaspis aculeifer* and *Enchytraeus crypticus*) to the application of the same waste.

The use of ash as a source of nutrients is increasing because it is a great soil corrective, contributing to the elevation of pH and release of essential nutrients to plants. However, its excessive use can cause damage to the soil for having metals in its composition (Oliveira et al., 2006), so it can affect the environment, contaminate the soil (Cassol et al., 2001) and influence the edaphic fauna (Baretta et al., 2003). Similarly, the risk of bioaccumulation of metals from the ash depends on its type and composition, soil conditions and on the organisms themselves (Mortensen et al., 2018), so the evaluation in natural soils is necessary.

This study aimed to evaluate through standardized tests (ISO) the effect of the application of forest biomass ash with fertilizing potential on the rates of survival and reproduction of *F. candida* springtails and *E. andrei* earthworms in subtropical soils.

## 2. Material and Methods

### 2.1 Test Soils

Two subtropical soils were used to conduct the ecotoxicological tests: Rhodic Hapludox (Oxisol), with clayey texture according to the classification of USDA (United States Department of Agriculture) (Soil Survey Staff, 2014), collected in the municipality of Chapecó – SC, Brazil (27°05'274"S; 52°38'085"W); and Typic Quartzipsamment (Entisol), with sandy texture (Soil Survey Staff, 2014), collected in the municipality of Araranguá – SC, Brazil (29°00'19.98"S; 49°31'02.84"W). Both soils were collected from the 0-20 cm layer in non-anthropized areas. Subsequently, they were dried in an oven at 65 °C, sieved through 2-mm-mesh sieves and defaunated in two freeze-thaw cycles (48 h at -20 °C followed by 48 h at 25 °C per cycle). For the tests, soil moisture was adjusted to 60% of the maximum water retention capacity (WRC) (ISO, 1993).

Chemical parameters were determined in both soils and in the forest biomass ash used: pH in H<sub>2</sub>O (1:2 ratio [m:v]), percentage of organic matter (OM), cation exchange capacity at pH 7.0 (CEC) and contents of macronutrients, micronutrients and metals, according to the methodology described by Tedesco et al. (1995) (Table 1). Soil particle-size composition was determined according to the methodology proposed by Embrapa (2011).

Table 1. Chemical parameters and particle-size composition of the subtropical soils and forest biomass ash used in the tests

| <b>Chemical and physical parameters</b>                                 | <b>Oxisol</b> | <b>Entisol</b> | <b>Ash</b> |
|---|---------------|----------------|------------|
| Organic matter (%)  | 2.9           | 1.0            | -          |
| CEC <sub>pH7.0</sub> (cmol <sub>c</sub> dm <sup>-3</sup> ) <sup>1</sup> | 18.30         | 4.92           | -          |
| pH <sub>H2O</sub>   | 6.2           | 5.8            | 12.4       |
| P (mg kg <sup>-1</sup> )  | 16.1          | 6.7            | 2.9        |
| K (mg kg <sup>-1</sup> )  | 236.0         | 34             | 6.0        |
| Ca (cmol <sub>c</sub> kg <sup>-1</sup> )                                | 12.8          | 2.00           | 7.8        |
| Mg (cmol <sub>c</sub> kg <sup>-1</sup> )                                | 3.1           | 0.8            | 5.2        |
| Al (cmol <sub>c</sub> kg <sup>-1</sup> )                                | 0.00          | 0.0            | -          |
| H + Al (cmol <sub>c</sub> kg <sup>-1</sup> )                            | 1.8           | 2.0            | -          |
| Cu (mg kg <sup>-1</sup> )   | 10.6          | 1.5            | 0.01       |
| Zn (mg kg <sup>-1</sup> )   | 0.4           | 1.0            | 0.01       |
| Fe (mg kg <sup>-1</sup> )   | 2.5           | 72.5           | 1.12       |
| Mn (mg kg <sup>-1</sup> )   | 1.8           | 2.1            | 0.43       |
| Pb (mg kg <sup>-1</sup> )   | 0.0           | 0.0            | 0.00       |
| Cd (mg kg <sup>-1</sup> )   | 0.0           | 0.0            | 0.30       |
| Sand (%)  | 23            | 37             | -          |
| Silt (%)  | 22            | 59             | -          |
| Clay (%)  | 55            | 4              | -          |

<sup>1</sup>Cation Exchange Capacity. Dashes (-) represent 'not determined'.

## 2.2 Test Substance and Concentrations Used

The forest biomass ash used came from the burning of eucalyptus in the boilers of the industry of production of ingredients for animal feed. The concentrations were defined based on the usual application of this material by rural producers, used to increase soil fertility levels, and the dose usually applied is 10 t ha<sup>-1</sup>. This value was used to determine the treatments, which consisted of the ash concentrations applied to the Oxisol and Entisol, equivalent to: 0 (control), 5, 10 and 20 t ha<sup>-1</sup>. The experiment was carried out in a completely randomized design with five replicates.

## 2.3 Organisms and Test Conditions

The specimens of *F. candida* (Collembola: Hexapoda) and *E. andrei* (Oligochaeta: Lumbricidae) used in the tests were obtained from cultures kept in the laboratory, and follow the guidelines of ISO (1999) and ISO (1998), respectively. The cultivation of organisms and the tests were conducted in a controlled environment with temperature of 20 ± 2 °C and photoperiod of 12:12 h (light:dark). The cultures of springtails were kept in 200-mL circular containers, with perforated lids (facilitating gas exchanges), on a 1-cm-thick layer of hardened culture medium, composed of gypsum, distilled water and activated charcoal at the proportion of 11:7:1 (m/m/m). Springtails were fed three times a week with dry organic yeast (*Saccharomyces cerevisiae*) and moistened with distilled water. Earthworms were kept in plastic boxes with opening on their surface to allow the organisms to breathe, in culture medium consisting of a mixture of two parts of dry equine manure sieved to 2 mm, one part of coconut fiber powder, and 10% of the total dry weight of the two components (manure and fiber) of fine sand (90/100 granulometry). The pH of the medium was corrected to values of 6 ± 0.5 through the addition of calcium carbonate (CaCO<sub>3</sub>), and the earthworms were fed weekly with oat in fine flakes.

The tests of lethality and reproduction with *F. candida* followed the recommendations of ISO 11267 (ISO, 1999), where each replicate consisted of a plastic container (diameter: 3.5 cm; height: 6.5 cm), filled with 30 g of soil (fresh weight), where 10 synchronized juvenile springtails were allocated (10-12 days old). The springtails were fed at the beginning of the test and after 14 days from the beginning of the test, with approximately 2 mg of dry granular organic yeast. Soil moisture was corrected weekly and the flasks were opened twice a week for aeration. At 28 days, the contents of the flasks were emptied into another container, which received water and black ink. After stirring the contents of the container, digital photos were taken and the number of live adults (survival) and juveniles (reproduction) were recorded using the program ImageTool 3.0 (University of Texas Health Science Center, 2002).

The test with *E. andrei* followed the recommendations of ISO 11268-2 (ISO, 1998). Each replicate consisted of a plastic pot (diameter: 11.5 cm; height: 9 cm), filled with 500 g of soil, and each experimental unit received 10 synchronized and clitellated earthworms (mass between 250 and 600 mg). The earthworms were fed at the beginning of the test and weekly with 5 g of moist manure of equine animals with no history of medication use and diet based on pasture, sieved and defaunated by freeze-thaw cycles. After 28 days, the adult earthworms were removed for the evaluation of lethality, and the cocoons were incubated for more 28

days. At 56 days, reproduction was evaluated by keeping the plastic pots in a water bath at 60 °C for 40-50 minutes, to force the juveniles to move to the surface of the soil, facilitating the count.

The tests were conducted under controlled conditions, with temperature of  $20 \pm 2$  °C and photoperiod 12:12 h (light/dark). The values of soil pH of the treatments were measured at the beginning and at the end of each test.

#### 2.4 Statistical Analysis

Data normality and homogeneity were checked using the Kolmogorov-Smirnov test and, when the assumptions were not met, the data were transformed using Log X. Data of lethality and reproduction of springtails and earthworms were subjected to analysis of variance (One-way ANOVA), followed by Dunnett's test ( $p < 0.05$ ), using the program Statistica 7.0 (StatSoft, 2004). Values of  $LC_{50}$  (lethal concentration causing death in 50% of the organisms) were estimated using the program PriProbit® 1.63 (Sakuma, 1998). For the reproduction tests, the values of  $EC_{50}$  (effective concentration causing 50% of effects on the reproduction of the organisms) were calculated by nonlinear regression analysis, with pre-defined models, Logistic for the data with *F. candida* and Linear for the data with *E. andrei*, using the program Statistica 7.0 (StatSoft, 2004).

Data of pH values were checked for normality and homogeneity, using the Kolmogorov-Smirnov test, and were subjected to One-way ANOVA, followed by Tukey test ( $p < 0.05$ ), using the program Statistica 7.0 (StatSoft, 2004).

### 3. Results

#### 3.1 Validation of the Tests

The lethality test of *F. candida* met the validation criteria according to ISO 11267 (ISO, 1999), and the survival rate of the springtails in the control soil was  $\geq 80\%$  (mean survival of 84% in Oxisol and 94% in Entisol) with coefficient of variation lower than 30% (CV of 1.43% for Oxisol and 0.51% for Entisol). As observed for lethality, the reproduction of *F. candida* met the validation criteria according to ISO 11267 (ISO, 1999), and the number of juvenile springtails in the control soil was  $\geq 200$  individuals (mean of 654 individuals for Oxisol and 541 for Entisol) with coefficient of variation of lower than 30% (CV of 5.75% for Oxisol and 3.30% for Entisol).

The lethality test of *E. andrei* met the validation criteria according to the respective norm OECD 207 (OECD, 1984). The mortality rate did not exceed 10% of the total number of individuals in the control in both soils tested (mean survival of 98% for Oxisol and 100% in Entisol) with coefficient of variation lower than 30% (CV of 1.38% for Oxisol and 0% for Entisol). The reproduction of *E. andrei* also met the validation criteria according to ISO 11268-2 (ISO, 1998), with a number of juveniles  $\geq 30$  in the control soil in both tested soils (mean of 55 individuals in the Oxisol and 46 in the Entisol) with coefficient of variation  $< 30\%$  in the control soil (CV of 14.84% in Oxisol and 12.63% in Entisol).



### 3.2 Survival of Adults and Reproduction of *F. candida*

The results obtained in the test of lethality (28 days) of *F. candida* show that the rates of survival ( $F = 0.57$ ;  $p > 0.05$ ) and reproduction ( $F = 0.68$ ;  $p > 0.05$ ) in the Oxisol were not affected by ash application (Figure 1A). For the Entisol, the survival of the organisms was significantly affected at the highest concentration tested, equivalent to  $20 \text{ t ha}^{-1}$  ( $F = 14.29$ ;  $p < 0.05$ ), as well as their reproduction rates at concentrations equivalent to 10 and  $20 \text{ t ha}^{-1}$  ( $F = 784.63$ ;  $p < 0.05$ ) (Figure 1B). The estimated  $LC_{50}$  was  $15.68 \text{ t ha}^{-1}$  (confidence interval could not be calculated) and the estimated  $EC_{50}$  was  $7.36 \text{ t ha}^{-1}$  ( $7.11\text{-}7.62 \text{ t ha}^{-1}$ ).

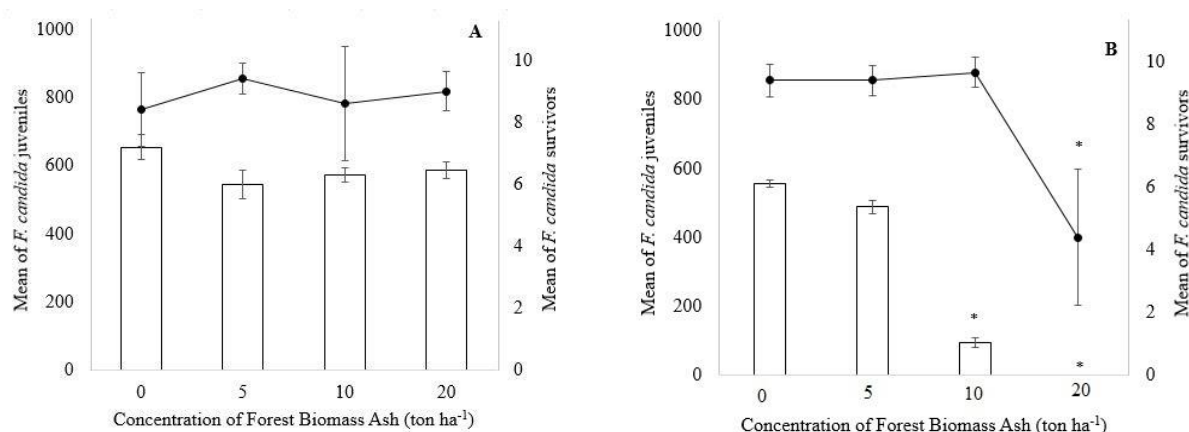


Figure 1. Survival (lines) and reproduction (bars) of *F. candida* springtails in Rhodic Hapludox - Oxisol (A) and Typic Quartzipsamment - Entisol (B) contaminated with concentrations of forest biomass ash. (⊥) standard deviation (n = 5)

Asterisk (\*) indicates a significant difference for the average number of live adults and the average number of juveniles ( $p < 0.05$ ; One-Way ANOVA followed by Dunnett's test).

### 3.3 Survival of Adults and Reproduction of *E. andrei*

The application of ash concentrations in Oxisol did not affect the survival and reproduction of *E. andrei* ( $F = 0.67$ ;  $F = 2.31$ ;  $p > 0.05$ , respectively). For the Entisol, the survival of the earthworms was not affected at any of the concentrations evaluated ( $F = 2.37$ ;  $p > 0.05$ ), but the reproduction rate was significantly affected by ash application of  $20 \text{ t ha}^{-1}$  ( $F = 19.42$ ;  $p < 0.05$ ) (Figure 2A and 2B). The estimated value of  $EC_{50}$  was  $11.97 \text{ t ha}^{-1}$  ( $9.49\text{-}14.46 \text{ t ha}^{-1}$ ).



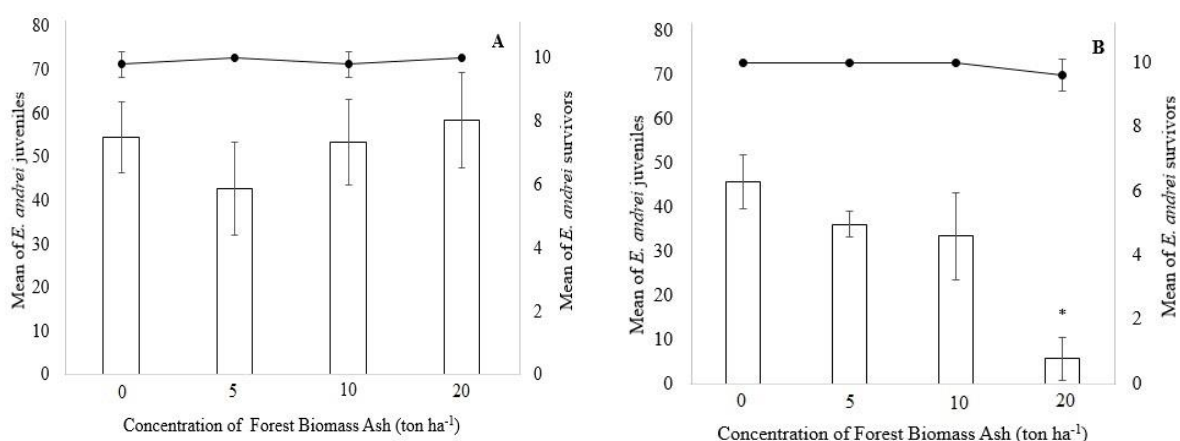


Figure 2. Survival (lines) and reproduction (bars) of earthworms *E. andrei* in Rhodic Hapludox - Oxisol (A) and Typic Quartzipsamment - Entisol (B) contaminated with concentrations of forest biomass ash. (⊥) standard deviation (n = 5)

Asterisk (\*) indicates a significant difference for the average number of live adults and the average number of juveniles ( $p < 0.05$ ; One-Way ANOVA followed by Dunnett's test).

### 3.4 Values of Metals in the Ash and pH Values in the Soil

According to the guiding values of prevention established by the Environmental Company of the São Paulo State (CETESB, 2014), at any of the concentrations the estimated contents of Cu and Zn approached the prevention limit ( $60 \text{ mg kg}^{-1}$  of Cu and  $86 \text{ mg kg}^{-1}$  of Zn) (Table 2). For Fe and Mn, no guiding values were established for the soil. These values determine the limit concentration of a given substance in the soil, so that it is capable of sustaining the ecosystem services. The Cd contents estimated at the concentrations exceeded the reference values of prevention (RVP =  $1.3 \text{ mg kg}^{-1}$  of Cd) and of investigation (RVI =  $3.0 \text{ mg kg}^{-1}$  of Cd) of trace elements in Brazilian soils, determined by the CONAMA Resolution n° 420/2009 (Brasil, 2009) (Table 2).

Table 2. Values of metals estimated for each ash concentration (5, 10 and  $20 \text{ t ha}^{-1}$ ), regardless of soil type

| Metals ( $\text{mg kg}^{-1}$ of soil) | Ash concentrations ( $\text{t ha}^{-1}$ ) |       |        |
|---------------------------------------|---|-------|--------|
|                                       | 5   | 10    | 20     |
| Cu                                    | 0.15                                      | 0.30  | 0.60   |
| Zn                                    | 0.27                                      | 0.54  | 1.08   |
| Fe                                    | 28.00                                     | 56.00 | 112.00 |
| Mn                                    | 10.70                                     | 21.40 | 42.80  |
| Cd                                    | 7.50                                      | 15.00 | 30.00  |

There was a significant difference in the pH values found for the different concentrations tested within the periods of evaluation ( $p < 0.05$ ). As the ash concentration applied to the soil increased, the pH values increased in the tested soils at each time of evaluation. However, over time, there was a reduction of pH between the concentrations in the Oxisol. However, in the Entisol, in addition to a smaller variation in the pH values between the ash concentrations, there was a process of alkalization over time (Table 3). The evaluations indicate that, at the end of the periods of the lethality and reproduction tests of springtails and earthworms, the harmful effects occurring on organisms may be related to changes in soil pH.

Table 3. Mean values of pH in KCl (1M, 1:5 soil:solution) of the Rhodic Hapludox (Oxisol) and Typic Quartzipsamment (Entisol) at the beginning of the test (Day Zero) and at the end of the test (28 and 56 days)

| Concentrations (t ha <sup>-1</sup> ) | Oxisol        |                |               |
|--------------------------------------|---------------|----------------|---------------|
|                                      | Day zero      | 28 days after  | 56 days after |
| Control                              | 5.94 ±0.55 aA | 5.86 ±0.31 aA  | 5.67 ±0.21 aA |
| 5                                    | 6.56 ±0.55 bB | 6.13 ±0.31 abA | 5.97 ±0.24 bA |
| 10                                   | 7.12 ±0.55 cC | 6.34 ±0.30 bB  | 6.00 ±0.21 bA |
| 20                                   | 7.96 ±0.55 dB | 6.43 ±0.30 bA  | 6.23 ±0.21 cA |
|                                      | Entisol       |                |               |
| Control                              | 5.65 ±0.12 aC | 4.90 ±0.14 aA  | 5.14 ±0.13 aB |
| 5                                    | 5.74 ±0.13 aA | 6.69 ±0.14 bB  | 7.26 ±0.12 bC |
| 10                                   | 6.39 ±0.12 bA | 6.80 ±0.14 bB  | 7.41 ±0.12 cC |
| 20                                   | 6.93 ±0.12 cA | 8.07 ±0.14 cB  | 8.34 ±0.13 dC |

\*Means followed by the same lowercase letters in the column and uppercase letters in the row do not differ statistically by Tukey test at 5% probability level.

#### 4. Discussion

The application of forest biomass ash negatively affected the survival and reproductive potential of *F. candida* and *E. andrei*, and the magnitude of these effects depends mainly on the type of soil and the amount of forest biomass ash applied. These results may be associated with increased pH promoted by the application of the highest ash concentrations. The pH for the two soils tested was different between the ash concentrations applied, and all treatments

showed a pH difference at 28 and 56 days (Table 3). Due to its high contents of oxides and hydroxides, wood ash has alkaline properties and is often used as an acidity corrective agent in acidic soils (Hevi ánkov áet al., 2014; Neina & Dowuona, 2013). Thus, the increase of soil pH caused by ash application represents toxicity to soil organisms because, at pH above 7, there is already inhibition in the reproduction of some soil organisms (Greenslade & Vaughan, 2003).

According to J änsch et al. (2005), the species *F. candida* has a great variability of tolerance to pH, with values from 3.2 to 7.2. Thus, the results of pH at the two highest concentrations in the Entisol were respectively 6.8 and 8.07 and were considered high for the organisms. The same authors report that earthworms of the species *E. andrei* can tolerate pH values between 4 and 9, but prefer neutral or slightly acidic pH conditions between 5 and 7 and soils with high contents of organic matter. These conditions did not occur in the Entisol, which in addition to promoting a greater effect of pH elevation due to its lower buffering power, has lower availability of organic matter for these organisms, as shown in its chemical characterization (Table 1).

The sensitivity of the edaphic organisms to pH was also reported by Greenslade & Vaughan (2003), who observed that at pH 8.03 there was no more reproduction of *F. candida*. Likewise, Benazzi (2015) worked with oil drilling residues and observed that the highest concentrations tested exceeded the pH 7.5, affecting the reproduction of *F. candida* springtails. According to these authors, the pH directly influences the reproduction of springtails.

For Antonioli et al. (2013), the pH influences the sorption of the elements in the soil sites, causing greater or lesser toxicity. The same authors worked with springtails in soil with addition of petroleum derivatives and observed that they changed soil pH to values that are not favorable for the reproduction of these organisms, indicating a negative effect on their reproduction.

The present study also verified a greater sensitivity of springtails to the effect of forest biomass ash addition to the soil when compared to earthworms. The earthworm species evaluated (*E. andrei*) was not affected with respect to survival in any of the soils studied, and only the highest dose of ash (20 t ha<sup>-1</sup>) had a significant effect on the reduction of its reproductive rate in Entisol, reducing the number of individuals to approximately 83% of that found in the control treatment (Figure 2). For springtails (*F. candida*), the reduction of survival and reproduction in Entisol was observed from the ash dose of 10 t ha<sup>-1</sup> (Figure 1), the usual dose of ash applied by rural producers for a fertilizing effect.

The toxicity effect of forest biomass ash may vary according to the edaphic organisms, as verified by Qin et al. (2017), who found no effect on the lethality and reproduction rates of mites (*H. aculeifer*) and enchytraeids (*E. crypticus*), but observed a reduction of these parameters in an ecotoxicological study with *F. candida*, at concentrations equivalent to 17.4 t ha<sup>-1</sup>, in a soil of Denmark with predominance of sand and higher content of organic material, compared to that found in the Entisol of the present study. The authors suggest that ash toxicity is mainly caused by osmotic stress, since the increase of soil pH triggered an increase

in the concentrations of  $\text{Ca}(\text{OH})_2$ , which when converted to osmolarity generates  $1565 \text{ mOsm kg}^{-1}$  of water from soil pores, while the osmolarity of the hemolymph of soil arthropods, such as collembolans, is usually around  $300 \text{ mOsm kg}^{-1}$  (Bayley & Holmstrup, 1999).

Collembolans naturally live in water films found inside the soil and, for these organisms, the cuticle and the ventral tube (diameter of approximately 5 mm) are important exposure pathways for substances dissolved in water from soil pores (Fountain & Hopkin, 2005), but also for pH alterations occurring in these sites, affecting the maintenance of the integrity of their tissues due to alterations in the osmolarity of the water contained in these pores.

In this work, the discussions about the data obtained for ash application on terrestrial organisms were also based on studies with other residues already tested, in addition to the ash itself, since there is limited data in the literature with application of ash in terrestrial ecotoxicology, especially for some edaphic organisms. In addition, the possible presence of some metals in the composition of the ashes, already reported in literature, is also considered. Toxicity data available in the literature for Cu, Zn, Fe, Mn and Cd, using reproduction of organisms, vary considerably, mainly due to differences in the forms of the substances used, toxicity parameter evaluated, types of soil, pH variations and organic matter content.

According to Waalewijn-Kool et al. (2013), the bioavailability of metals to soil organisms, in general, is associated with chemical properties such as pH, organic matter content and cation exchange capacity. According to Antonioli et al. (2013), the use of Zn and Cu in their work affected the springtails, due to the reduction of pH, associated with the effect of these metals on the soil. According to these authors, the addition of these metals to the soil reduces pH, affecting the organisms. This effect was not observed in the present study, because the increase in the amount of ash, despite causing a proportional increase in the concentration of metals, did not reduce soil pH, even in the Entisol, with lower buffering power (Tables 2 and 3). These results are similar to those found by Pedersen & van Gestel (2001), who tested concentrations of metals in soil and found no effect on springtails up to high concentrations, because there was no alteration in soil pH.

The values found for Fe, Cu, Zn and Mn at the highest concentration studied (Table 2) were much lower than the guiding values for soil contamination proposed by the CONAMA Resolution 420/209 (Brasil, 2009), which little influenced the responses of these organisms. Greenslade & Vaughan (2003) found  $\text{EC}_{50}$  of  $751 \text{ mg Cu kg}^{-1}$  of soil for *F. candida*. According to Kuperman et al. (2009),  $\text{LC}_{50}$  values of 2,575 and  $927 \text{ mg Mn kg}^{-1}$  of soil reduced the survival and values of 1,663 and  $927 \text{ mg Mn kg}^{-1}$  of soil interfered in the reproduction of *F. candida* and *E. andrei*, respectively. For Cd, the estimated values at the tested concentrations were higher than the guiding values for soil contamination proposed by the CONAMA Resolution 420/209 and may represent a potential risk of contamination with this element in the soil by the ash, affecting organisms of the edaphic fauna.

Factors such as soil pH and organic matter content directly affect Cd availability to the soil (Liu et al., 2018; Mortensen et al., 2018). At higher pH, Cd availability decreases due to the increase in its adsorption to the charges of the soil and less accessible sites of organic matter, reducing its availability in the soil matrix (Mortensen et al., 2018). At pH close to 6.0, only

35% of soil Cd is available, whereas at pH 8.0 the availability of this element increases to 55% (Mortensen et al., 2018; Sauvée et al., 2000). In the present study, for the Oxisol, the pH was close to the range considered adequate (~6) as a limiting agent of Cd availability, whereas for the Entisol, the values exceeded 8.0 (Table 2).

Thus, the results found indicate that ash application in soils such as the Entisol, in addition to directly affecting soil pH, which exceeded the tolerance of the organisms at the highest concentrations tested, affecting their reproduction (Table 3), may also be promoting the availability of elements such as Cd. Authors such as Liu et al. (2018) found  $EC_{50}$  from 41.4 to 146.9 mg Cd kg<sup>-1</sup> of soil for *F. candida* when assessing the effect of Cd toxicity on different soils, and found a high correlation of the toxicity response associated with the values of pH and organic matter of these soils. For earthworms, similarly, studies indicate that soil pH and organic matter content affect the bioaccumulation and toxicity (Irizar et al., 2015). Onuoha & Worgu (2011) found  $EC_{50}$  values from 28.51 to 29.82 mg Cd kg<sup>-1</sup> of soil in artificial soil, whereas Irizar et al. (2015) found  $EC_{50}$  values of 10.45, 15.15 and 51.0 mg Cd kg<sup>-1</sup> of soil in artificial soil with different concentrations of organic material.

As observed in the present study, there were differences between the levels of toxicity in relation to the type of soil tested. These differences are intrinsically related to soil factors such as textural class, organic matter content and dynamic equilibrium between the solid and liquid phases of the soil in terrestrial environments (Smit & Van Gestel, 1996). Hence, all factors together determine the processes of adsorption of contaminants and, consequently, cause ecotoxicological effects of different magnitudes (Zortéa et al., 2018a,b). The Entisol has low contents of organic matter and clay (1% and 4%, respectively), and these elements are the main responsible for the adsorption of contaminants. On the other hand, the Oxisol has high contents of organic matter and clay (2.9% and 55%, respectively), which probably had higher capacity for adsorption and, therefore, led to a reduction of toxicity to earthworms and springtails. Thus, the effect of greater toxicity to the organisms of the fauna with the application of forest biomass ash in Entisol should be considered, especially with respect to promoting greater pH elevation and probably making toxic elements such as Cd available, because the doses of this residue usually used by the producers caused a toxicity effect on the edaphic organisms studied.

Landfill disposal has traditionally been the most widely used method for ash, but the soil is a good alternative for disposal because ash is an effective fertilizer and neutralize soil acidity. In this region, farmers apply ash in large quantities (values not determined) because they do not have technical-scientific knowledge about its effect on the soil and consequently about the optimum quantities for application. A probable dose of 5 ton may be a safe recommendation for the application of this residue in soils such as the Entisol, whereas 15 ton maybe a safe dose for the Oxisol. However, this recommendation depends on the type of ash used, metal composition and frequency of application. Therefore, further studies are needed to determine the appropriate quantity and frequency of application, depending on the effects on soil (mainly chemical and biological properties) and on plant, and thus to determine maximum limits and ensure maximum adsorption of contaminants from ash, without exceeding these limits.

## 5. Conclusion

Application of forest biomass ash in the soil negatively affects earthworms and springtails, and the magnitude of the effects is related to soil characteristics and sensitivity of the species. There was no effect of toxicity for the Oxisol with ash application at any dose evaluated for any of the organisms tested. In the Entisol, the results of sensitivity of the organisms were more evident and effects were observed from the dose of 10 t ha<sup>-1</sup> for springtails, which were more sensitive to it, and from 20 t ha<sup>-1</sup> for the earthworms.

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