



Prevention values for copper (low tier approach) in subtropical acidic soils

Daniela Aparecida de Oliveira¹ · Thiago Ramos Freitas¹ · Vanessa Mignon Dalla Rosa¹ ·
Luís Carlos Iuñes Oliveira Filho¹ · Milton da Veiga² · Mari Lucia Campos¹ · David José Miquelluti¹ ·
Osmar Klauberg-Filho¹

Received: 3 June 2024 / Accepted: 6 October 2024

© The Author(s), under exclusive licence to Springer-Verlag GmbH Germany, part of Springer Nature 2024

Abstract

Copper is a trace element in plants and animals whose importance can be understood due to its role in different essential metabolic processes. Anthropogenic activities such as agriculture and mining are potential sources of pollution due to the emission of copper into the environment. Brazilian legislation ties soil quality to guideline values, among which the Prevention Value indicates the critical environmental limit for trace elements. The aim of this study was to obtain PVs for copper for two subtropical soils (Cambisol and Nitisol), given that the pedological richness was not considered when deriving the PVs contained in the federal normative. Reproduction assays followed ISO guidelines with the earthworm species *Eisenia andrei* and *Perionyx excavatus*, the enchytraeids *Enchytraeus crypticus* and *Enchytraeus bigeminus* and the springtails *Folsomia candida* and *Proisotoma minuta*. Results showed that the sensitivity of the organisms was greater in Cambisol. The most sensitive species were the earthworms, especially *Perionyx excavatus* ($EC_{50} = 67.83$ in Cambisol; $EC_{50} = 264.96$ in Nitisol). The springtails, on the other hand, were the least sensitive to contamination. These findings reinforce the need to include organisms from different ecological groups in ecotoxicological assessments. It was also observed that the PV adopted in federal legislation ($= 60 \text{ mg kg}^{-1}$) is in fact protective for the species and soils we evaluated, since the PVs we obtained based on the EC_{50} were $346.74 \text{ mg kg}^{-1}$ in Nitisol and $134.05 \text{ mg kg}^{-1}$ in Cambisol. It is important to note that our results do not exclude the need for evaluations with other subtropical soils, given the influence of their properties on the toxicity and bioavailability of copper to soil organisms.

Keywords Ecotoxicology · Soil screening values · Trace metals · Soil contamination · Soil bioindicators

Introduction

Among the main contaminants of environmental interest, metals require some attention due to their metabolic involvement, because, although essential, in excess they can result in damage to organisms (Witkowska et al. 2021). Copper

(Cu) is a micronutrient for plants and animals that is naturally present in the soil due to the weathering processes taking place on the parent rock (Kabata-Pendias, 2010). However, the development of human activities, such as mining, agricultural practices and industrial activities (Rehman et al. 2019), has contributed to an increase in Cu concentrations in the environment, which has the potential to undermine the supply of environmental services because of contamination on key species' ecological dynamics.

The group of invertebrates performs ecosystem functions that are extremely important for maintaining life in the soil, with their activities linked to the processes of organic matter and nutrient cycling, soil structuring and bioregulation (Decaëns et al. 2006; Briones 2018; Heděnc et al. 2022). Therefore, it is essential to pay particular attention to the effects of Cu contamination on these organisms to preserve environmental quality. According to Sereni et al. (2021), soil

Responsible Editor: Kitae Baek

✉ Osmar Klauberg-Filho
osmar.klauberg@udesc.br

¹ Center for Agroveterinary Sciences, Department of Soils and Natural Resources, Laboratory of Soil Ecology and Terrestrial Ecotoxicology, Santa Catarina State University, Lages, Santa Catarina, Brazil

² Department of Agronomy, Santa Catarina West University, Campos Novos, Santa Catarina, Brasil

fauna in Cu-contaminated sites exhibit morphological and behavioral alterations that can lead to trophic imbalance. Understanding the effects of this contamination has become essential for protecting biodiversity and ecosystem services connected with soil. Although the topic has been extensively researched in northern hemisphere soils (Spurgeon & Hopkin 1996; Lukkari et al. 2005; Kuperman et al. 2006; Maraldo et al. 2006; Amorim & Scott-Fordsmand 2012; Renaud et al. 2020), there are few reports on the effects of Cu in tropical and subtropical soils.

The Brazilian federal legislation, in the form of Resolution 420/2009 of the National Environment Council (CONAMA), ties soil and groundwater quality to guideline values. Among these, the prevention value (PV) refers to the concentrations above which it can be inferred that the soil is polluted by a certain element or substance (Brasil 2009). Due to the great pedological diversity, it is necessary to determine specific PVs to the broadest possible range of tropical and subtropical soil types. This is attributable to the fact that the PVs currently adopted at the national level are those obtained only from soil analysis in the state of São Paulo, making it difficult to identify and manage contaminated areas in other states.

The obtaining procedure of PV is closely linked to analyzing the effects of the exposure on key organisms such as arthropods and oligochaetes. These assessments are fundamental to understanding the effects of contamination and, as a result, to establishing effective prevention and management guidelines. The European Chemicals Agency (ECHA) recommends that such evaluations must be carried out with species representative of the different trophic levels (ECHA 2008). The main methods commonly used for this purpose are described by the International Organization for Standardization (ISO) guidelines and aim to measure the dose–response relationship in parameters such as organism survival (lethality) and reproduction capacities when exposed to contaminated soils (Van Gestel 2012).

Considering this scenario, the aim of the present research was to derive PVs for Cu for two subtropical representative soils of South Brazil through reproduction assessments with the earthworm species *Eisenia andrei* and *Perionyx excavates*, the enchytraeids *Enchytraeus crypticus* and *Enchytraeus bigeminus*, and the springtails *Folsomia candida* and *Proisotoma minuta*. Soil fauna species were chosen due to their well-documented sensitivity to inorganic contaminants and their use in standardized ecotoxicological assessments (Nursita et al. 2005; Kwak et al. 2014; Madani et al. 2015; Simões et al. 2020; Assis et al. 2023). While ecosystems encompass a diverse array of organisms, employing native species requires prior evaluations of their ecological characteristics. Therefore, findings from standardized species are often utilized to elucidate potential impacts on the dynamics of native species. To achieve these objectives, we

hypothesized that (i) the effects of contamination measured on the reproduction of the organisms evaluated vary from soil type, and (ii) the PVs adopted at the national level are not fitting for the subtropical soils tested. The methodology employed was to produce species sensitivity distribution curves (SSDs) and estimate risk concentrations (HC_5 and HC_{50}) based on the effect concentrations (EC_{50}) obtained in the exposure tests.

Materials and methods

Soils sampling and characterization

Two natural soils representative of the subtropical region of Brazil and a tropical artificial soil (TAS) were used in the ecotoxicological assessments: an Cambisol (Cambisol Húmico (Santos et al. 2018)) was collected in Lages, SC [27°48'57"S; 50°21'45"W] and a Nitisol (Nitossolo Vermelho (Santos et al. 2018)) was collected in Concórdia, SC [27°48'71"S; 51°59'34"W]. The soil samples were collected in the surface layer (0–20 cm) in areas under natural, unmanaged vegetation, free from fertilization and the use of agrochemicals. The TAS was made following the proportions proposed by Garcia et al. (2004), i.e. 75% fine sand, 20% kaolinitic clay and 5% dried and sieved coconut fiber. The TAS' pH was corrected to 6.0 ± 0.5 by adding calcium carbonate ($CaCO_3$) (ISO 2012). The characteristics of the soils were determined according to the procedures suggested by EMBRAPA (Teixeira et al. 2017) and are shown in Table 1.

Table 1 Chemical and physical properties of Nitisol, Cambisol, and TAS

Soil properties	Nitisol	Cambisol	TAS
Organic matter (g kg ⁻¹)	42	52	22
CEC ¹	12.5	6.5	1.2
pH ²	5.8	4.7	6.2
Ca (cmol _c dm ⁻³)	11.7	2.6	2.0
Mg (cmol _c dm ⁻³)	2.5	1.4	0.9
P (mg dm ⁻³)	47.1	6.9	11.1
K (mg dm ⁻³)	162	52	262
Al (cmol _c dm ⁻³)	0.2	3.0	0.0
Cu (mg dm ⁻³)	10.6	2.8	0.2
Zn (mg dm ⁻³)	29.0	3.8	0.7
B (mg dm ⁻³)	0.65	0.67	1.38
Mn (mg dm ⁻³)	159	21.1	2.2
Clay (g kg ⁻¹)	543.7	133.7	94.9
Silt (g kg ⁻¹)	272.7	115.7	108.7
Sand (g kg ⁻¹)	183.5	750.5	796.4
Texture	Clay	Sandy Loam	Sandy Loam

¹Cation Exchange Capacity at pH 7.0 (cmol_c dm⁻³). ²pH in water

Copper spiking procedure

The soils were spiked with a $\text{Cu}(\text{NO}_3)_2$ solution (Table 2), following a three weeks incubation (Natal-da-Luz et al. 2011). The nominal tested concentrations were based on toxicity data reported in the literature (Spurgeon et al. 1994; Sandifer & Hopkin 1997; Amorin et al. 2005; Lukkari et al. 2005; Domínguez-Crespo et al. 2012; Gomes et al. 2014). Prior to the testing procedure, the moisture content was corrected to 50% of the soil's water holding capacity (WHC) in accordance with the ISO 11268–2 protocol (ISO 2012). After the spiking procedure, soil samples were used in ecotoxicity assays. Soil Cu contents were evaluated through X-ray fluorescence spectrometry (Nascimento Filho 1999), as required by CONAMA Resolution n. 420/2009 (Brasil 2009). The Standard Reference Material was a 2709a San Joaquin Soil.

Test organisms

The earthworms were cultivated in a substrate consisting of equine manure (chemical-free), coconut husk fiber and fine sand (in the proportion of 7:2:1). The pH of the mixture was corrected to 5.7 ± 0.3 range with CaCO_3 . The organisms were fed weekly with oat flakes. The species *Eisenia andrei* was kept at 20 ± 2.0 °C room and the *Perionyx excavatus* species at a 25 ± 2.0 °C. The photoperiod for both was kept in 16 h light. The enchytraeids were grown in TAS (Garcia et al. 2004) according to the ISO 16387 protocol (ISO 2014a). The springtails were cultivated in a substrate composed of plaster, distilled water and activated charcoal (in a ratio of 11:7:1) maintained at a temperature of 20 ± 2.0 °C and at a photoperiod of 16 h light.

Experimental procedures

The assays with the earthworm species *Eisenia andrei* and *Perionyx excavatus* followed the guidelines established in the ISO 11268–2 protocol (ISO 2012). The experimental units ($n=5$) consisted of containers filled with around 500 g

of dry soil and in the presence of ten clitellate individuals weighing between 250 and 600 mg. The organisms were fed weekly with approximately 15 g of horse manure (free from pollutants) and the moisture was kept at 50% of WHC. After 28 days, the adults were removed, and the cocoons and juveniles were incubated for a further 28 days. At the end of the 56 days, the organisms were extracted using a temperature gradient in a laboratory water bath (60°C). The evaluations with enchytraeids were carried out according to the guidelines established in ISO 16387 (ISO 2014a) with adjustments proposed by (Kuperman et al. 2006), and the assay was conducted for four weeks (28 days) for *Enchytraeus crypticus* and three weeks (21 days) for *Enchytraeus bigeminus* (Bandow et al. 2013). The criterion for selecting adult individuals for *Enchytraeus crypticus* was the presence of a clitellum; for *Enchytraeus bigeminus*, organisms were chosen according to their body size (8 to 12 mm), since this species is a fragmented reproducer (Bandow et al. 2013). Ten organisms were placed in containers with 30 g of soil ($n=4$) and fed weekly with around 20 mg of rolled oats and the moisture content was kept at 50% of WHC. At the end of the test, 5 mL of pure alcohol (96% ethanol), 1 mL of rose bengal dye solution (1% w/v) and 80 mL of distilled water were added to each experimental unit for subsequent counting of the organisms. The springtails' assessments lasted 28 days and were carried out according to the procedures listed under ISO standard 11267 (ISO 2014b). Ten organisms with 12 days old were kept in containers with 30 g of soil ($n=5$). Food was added once a week and consisted of around 20 mg of dry biological yeast and the moisture content was kept at 50% of the WHC. At the end of the evaluation period, for the *Folsomia candida* juveniles, each experimental unit was filled with about 80 mL of water and a few drops of black stamp ink (to increase the contrast between the organisms and the surface of the water). After gentle stirring, the number of adult and juvenile individuals was determined using ImageJ 1.54d software (Schneider et al. 2012). To determine the reproduction rate of the *Proisotoma minuta* species, the method used was dry extraction in Berlese-Tüllgren funnels for a period of 24 h with subsequent addition of water and red dye. This adaptation was necessary due to the smaller

Table 2 Tested nominal concentrations of copper for each ecotoxicological assay depending on the soil type

Soil	Organisms and copper nominal concentrations (mg kg^{-1})
Nitisol and TAS Cambisol	Earthworms (<i>Eisenia andrei</i> and <i>Perionyx excavatus</i>) 0, 100, 200, 400, 800, 1200, 1600 and 2000
	0, 50, 100, 200, 300, 500, 700 and 1000
Nitisol Cambisol and TAS	Enchytraeids (<i>Enchytraeus crypticus</i> and <i>Enchytraeus bigeminus</i>) 0, 75, 150, 300, 450, 600, 750 and 1000
	0, 25, 50, 75, 150, 250, 500 and 750
Nitisol, Cambisol and TAS	Springtails (<i>Folsomia candida</i> and <i>Proisotoma minuta</i>) 0, 75, 150, 300, 450, 600, 750 and 1000
	0, 75, 150, 300, 450, 600, 750 and 1000

size and color of the species' exoskeleton (Hopkin 1997; Mendonça et al. 2015). Afterwards, the samples containing the organisms were carefully shaken and the number of individuals determined using ImageJ 1.54d software (Schneider et al. 2012).

Data analysis

The ecotoxicological data were subjected to the Shapiro–Wilk normality test ($p > 0.05$) and Bartlett's homogeneity test ($p > 0.05$). The means were then compared using the Dunnett test ($p \leq 0.05$) in order to obtain the No Effect Concentration (NOEC) and the Lowest Effect Concentration (LOEC). The data was then submitted to a non-linear regression analysis to determine the dose of copper corresponding to a 50% effect on the population of organisms (EC_{50}). The EC_{50} values were determined using the model that best fitted the data (Environment Canada 2005). All analyses were carried out using STATISTICA 10.0 software (Statsoft Inc. 2011).

Using ETX 2.0 software (Van Vlaardingen et al. 2004), sensitivity curves were developed for the species tested (SSDs). These distributions illustrate the variability in sensitivity among species to a specific toxicant, allowing us to identify the most and least sensitive organisms. This information is essential for assessing ecological risks and determining safe exposure levels for ecosystems. SSDs are constructed through a cumulative plot of logarithmically transformed NOECs or EC_x against rank-assigned percentiles, to which a statistical distribution is fitted (Wheeler et al. 2002; Fox et al. 2020). To this end, the EC_{50} data were cumulatively grouped into a log-normal distribution. The SSDs were employed to estimate the HC_5 and HC_{50} , with a 95% confidence interval. Since the model assumes a log-normal distribution of the data, log-normality was tested using the Anderson–Darling, Kolmogorov–Smirnov and Cramer von Mises tests ($p < 0.01$). The PVs were obtained after the HC_5 had been calculated considering the Quality Reference Values ($QRV = 93.84 \text{ mg kg}^{-1}$) for soils in the state of Santa Catarina (IMA 2021), according to the equation $PV = QRV + HC_5$.

Results

Ecotoxicological assays

The validation criteria were all met for the species *Eisenia andrei*, *Perionyx excavatus*, *Enchytraeus crypticus*, *Folsomia candida* and *Proisotoma minuta* (ISO 2012, 2014a, b). For the assays with the species *Enchytraeus bigeminus*, the criterion adopted was the occurrence of 25 juveniles in the control groups with a coefficient of variation lower than 50%

(Bandow et al. 2013). The choice of a specific criterion is justified due to the impossibility of identifying clitellate adult individuals as it is a fragmenting species.

The reproduction of the earthworm species *Eisenia andrei* and *Perionyx excavatus* was affected ($p \leq 0.05$) in all the soils that were evaluated (Figure S1). Reductions in the number of *Eisenia andrei* juveniles were observed starting at doses of 50 mg kg^{-1} in Cambisol, 100 mg kg^{-1} in TAS and 200 mg kg^{-1} in Nitisol. For the species *Perionyx excavatus*, the effects were observed at the lowest concentrations evaluated (50 mg kg^{-1} in Cambisol and 100 mg kg^{-1} in TAS and Nitisol). The most significant effect for both species was found in the Cambisol, since the EC_{50} values for this soil (77.52 mg kg^{-1} for *Eisenia andrei* and 67.83 mg kg^{-1} for *Perionyx excavatus*) were lower than those found in Nitisol ($428.01 \text{ mg kg}^{-1}$ for *Eisenia andrei* and $264.96 \text{ mg kg}^{-1}$ for *Perionyx excavatus*) and TAS ($173.81 \text{ mg kg}^{-1}$ for *Eisenia andrei* and $110.46 \text{ mg kg}^{-1}$ for *Perionyx excavatus*). All the enchytraeid species evaluated were also affected ($p \leq 0.05$) by soil contamination with Cu (Figure S2). The reduction in the number of juveniles of *Enchytraeus crypticus* occurred from the concentration of 25 mg kg^{-1} in TAS, 50 mg kg^{-1} in Cambisol and 150 mg kg^{-1} in Nitisol. For the species *Enchytraeus bigeminus*, the effects were verified starting at a concentration of 250 mg kg^{-1} in the TAS, 50 mg kg^{-1} in the Cambisol and 75 mg kg^{-1} in the Nitisol. As observed for the earthworms, the most significant effects for the enchytraeids were also observed in the Cambisol, since the EC_{50} values obtained were lower than those found in the Nitisol and TAS. The springtails *Folsomia candida* and *Proisotoma minuta* also had their reproduction affected ($p \leq 0.05$) in all the soils evaluated (Figure S3). The reduction in the number of juveniles of the species *Folsomia candida* was observed from the concentration of 75 mg kg^{-1} in Nitisol and 300 mg kg^{-1} in Cambisol and TAS. For the *Proisotoma minuta* species evaluations, the significant effects on reproduction occurred from the concentrations of 75 mg kg^{-1} in Cambisol, 150 mg kg^{-1} in Nitisol and 450 mg kg^{-1} in TAS. The NOEC, LOEC and EC_{50} for each assessment are presented in Table 3.

In general, it can also be said that the earthworm species *Perionyx excavatus* is the most sensitive of all the species evaluated, whereas the springtail *Proisotoma minuta* was the least affected by the contamination, independent of soil type.

Chemical evaluations

The data from the analysis of the Cu levels in the natural soils (Table 4) indicated an average percentage recovery (in relation to the nominal dose) of 83.8% in Nitisol and 86% in Cambisol, while in TAS the recovery was around 92%. For this reason, it was decided to employ the nominal doses in the construction of the SSD and the subsequent derivation of the PV.

Table 3 Ecotoxicological endpoints (NOEC, LOEC and EC₅₀) for each evaluated species in Nitisol, Cambisol e TAS. In parenthesis the 95% confidence limits (lower and upper) are presented

	NOEC (mg kg ⁻¹)	LOEC (mg kg ⁻¹)	EC ₅₀ (mg kg ⁻¹)
Nitisol			
<i>Eisenia andrei</i>	100	200	428.01 (300.58–555.44)
<i>Perionyx excavatus</i>	< 100	100	264.96 (184.08–345.15)
<i>Enchytraeus crypticus</i>	75	150	366.61 (328.56–404.67)
<i>Enchytraeus bigeminus</i>	< 75	75	444.71 (303.99–585.44)
<i>Folsomia candida</i>	< 75	75	456.22 (305.94–606.51)
<i>Proisotoma minuta</i>	75	150	636.00 (597.15–674.86)
Cambisol			
<i>Eisenia andrei</i>	< 50	50	77.52 (66.16–88.87)
<i>Perionyx excavatus</i>	< 50	50	67.83 (54.95–80.72)
<i>Enchytraeus crypticus</i>	25	50	142.25 (109.50–175.00)
<i>Enchytraeus bigeminus</i>	25	50	126.77 (56.30–197.24)
<i>Folsomia candida</i>	150	300	255.03 (223.52–286.53)
<i>Proisotoma minuta</i>	< 75	75	513.17 (453.78–572.56)
TAS			
<i>Eisenia andrei</i>	< 100	100	173.81 (148.99–198.63)
<i>Perionyx excavatus</i>	< 100	100	110.46 (73.82–137.10)
<i>Enchytraeus crypticus</i>	< 25	25	171.71 (124.79–218.63)
<i>Enchytraeus bigeminus</i>	150	250	213.70 (181.36–246.03)
<i>Folsomia candida</i>	150	300	333.38 (297.35–369.42)
<i>Proisotoma minuta</i>	300	450	578.74 (512.03–645.45)

Table 4 Nominal concentration (mg kg⁻¹) and chemical measured concentration (mg kg⁻¹) of copper in subtropical natural soils and TAS

Nominal	Real		
	Nitisol	Cambisol	TAS
25		16.7	20.9
50		33.8	41.9
75	48.9	58.3	63.4
100	65.05	77.8	84.5
150	122.8	129.0	136.4
200	170.5	172.0	188.2
250			290.1
300	260.9	261.0	
400	326.5		384.0
450	375.2	429.1	
500		477.5	422.6
600	539.4	551.0	
700		668.5	
750	647.3	716.3	743.4
800	719.2		792.8
1000	889.0	955.0	
1200	1078.8		1185.6
1600	1438.5		1586.1
2000	1798.1		1982.4

Species Sensibility Distribution and prevention values

Once it was possible to assess the EC₅₀ for each of the tested organisms, the SSD were generated and the HC₅ and HC₅₀ were obtained for all the tested soils (Fig. 1). The highest HC₅ values were recorded in Nitisol (252.9 mg kg⁻¹), followed by TAS (76.12 mg kg⁻¹) and Cambisol (40.21 mg kg⁻¹). It can therefore be inferred that Cu contamination is more problematic in Cambisol, since the concentration capable of endangering the organisms is approximately six times lower than in Nitisol.

Finally, based on the Cu QRV (93.84 mg kg⁻¹) published for Santa Catarina state soils (IMA 2021), it was possible to estimate the PV via the HC₅ data (Table 5). We chose not to utilize HC₅₀ for this purpose because we intended to propose PVs that are ecologically safer.

Discussions

The ecotoxicological data gathered from studies conducted in temperate climate soils are extrapolated to tropical and subtropical climate zones. Nevertheless, this decision sometimes proves to be inappropriate since the differences

Fig. 1 Species sensitivity distribution based on the EC_{50} data for the evaluated soils

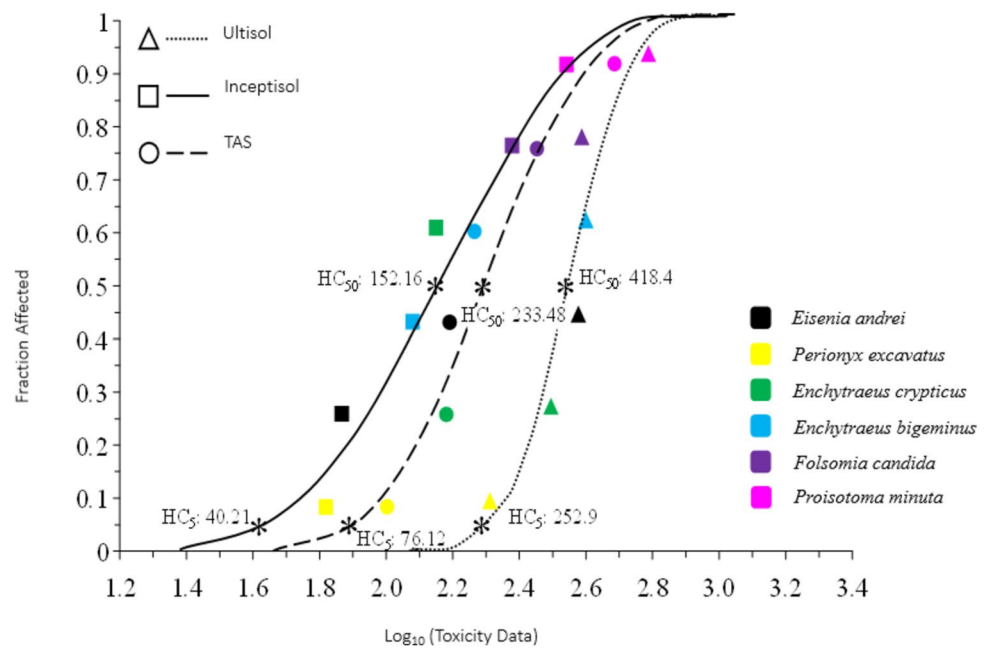


Table 5 Hazardous concentration for 5% of the species and prevention values for Cu based on EC_{50} data for each evaluated soil. LL HC_5 : lowest limit for the calculated HC_5 ($p \leq 0.05$); HC_5 : hazardous concentration for 5% of the species ($p \leq 0.05$) based on the EC_{50} values; UL HC_5 : upper limit for the calculated HC_5 ($p \leq 0.05$); PV: prevention values ($PV = QRV + HC_5$)

Soil	LL HC_5	HC_5	UL HC_5	PV
Nitisol	144.1	252.9	325.40	346.74
Cambisol	9.08	40.21	78.24	134.05
TAS	26.17	81.82	136.24	175.66

in climate conditions originate soils with contrasting characteristics. In this sense, the results presented here tend to overcome this constraint and, in an original way, clarify the Cu dose–effect relationship in natural subtropical Brazilian soils.

The assays carried out on natural soils with different chemical properties, as well as on artificial soil, indicated that the organisms were able to respond to the contamination in different ways, with the adverse effects being associated with greater bioavailability of Cu. According to Kabata-Pendias (2010), the bioavailability of trace elements is a result of the soil's adsorptive capacity, which is directly related to CEC, pH and clay content. In fact, Cambisol had lower clay content, CEC, and pH levels when compared to Nitisol. This finding is in line with the results of Duan et al. (2016), who reported that Cu toxicity in *E. fetida* is inversely associated with CEC and pH level. These attributes also tend to result in greater damage to other faunal species, given the dynamics of the contaminant (Natal-da-Luz et al. 2008). Furthermore, despite Cambisol having a lower CEC and clay content, the

organisms were more susceptible to Cu contamination in TAS than in Cambisol. This observation suggests that other factors, such as the presence of Al (which is a toxic element to soil organisms) may influence the organisms vulnerability. These findings confirm the first hypothesis upon which this evaluation was built.

The results of the ecotoxicological assessments showed that Cu was able to reduce the reproduction of all organisms at distinct levels. The species most sensitive to the contaminant was the earthworm *Perionyx excavatus*, followed by the enchytraeid *Enchytraeus crypticus* (for the evaluations in TAS and Nitisol) and the earthworm *Eisenia andrei* (in Cambisol), all oligochaetes with similar trophic habits and forms of exposure to the contaminant. On the other hand, the springtails were less susceptible to Cu exposure. These differences illustrate a scenario that emphasizes the importance of carrying out assessments with organisms from different taxonomic groups, especially when determining critical concentration limits for one or more contaminants in the soil.

In accordance with our findings, Mirmonsef et al. (2017), when assessing the effects of Cu on the reproduction of eight earthworm species collected in a contaminated area, found that the abundance of cocoons decreased as the concentration of the metal increased, thereby indicating a harmful effect on the reproduction of the organisms. Similarly, Maboeta and Fouché (2014), in a study aiming to determine the Cu toxicity in the species *Eisenia andrei*, found a significant reduction in oviposition and cocoon hatching rates. Kwak and An (2021), in a 7-day assessment, were able to identify physiological abnormalities, such as mucous secretion, bleeding and swelling, for doses above 400 and 600 mg of Cu kg^{-1} of dry soil in adults of *Perionyx excavatus* and

Eisenia andrei respectively. Regarding the variation in LOEC which we have verified here between the two earthworm species evaluated, the results suggest that the *Perionyx excavatus* species is more sensitive. This observation corroborates the findings of Spurgeon and Hopkin (1996), who, in an evaluation of ecologically relevant species, concluded that the *Eisenia* genus is the one with the greatest resistance to soil contamination by trace elements. Given the different responses, the importance of using native species in ecotoxicological assessments is therefore emphasized. This is because the use of tropical/subtropical species, such as *Perionyx excavatus* (Silva & van Gestel 2009), can better represent ecological risk analysis in Brazilian soils.

In terms of the damaging effects of Cu on enchytraeids, and in accordance with the results of this evaluation, Maraldo et al. (2006), when assessing the survival of *Enchytraeus crypticus* in Danish soils contaminated with Cu, found a reduction in the survival of this organism at concentrations of 600 mg kg⁻¹. However, there have been few publications to this date on the effect of Cu on the reproduction of enchytraeids in tropical and subtropical soils. Nevertheless, Konečný et al. (2014), in a study assessing the influence of trace elements on *Enchytraeus crypticus*, observed that the reproduction of the animals shows a negative correlation with the concentrations of Co and Cu, the latter being the most environmentally relevant contaminant, with its EC₅₀ determined for the species at 351 mg kg⁻¹. These findings support the need to determine PV for Cu in different soils, given its effects on the reproductive capacity of organisms and the subsequent impact on the ecological functions of important biological groups.

The Cu exposure also had a negative effect on the springtails, although these were the organisms whose results indicate greater resistance to the effects of the metal. According to Renaud et al. (2020), the toxicity of trace elements in *Folsomia candida* tends to be directly related to the solubility of the cations in the soil. In this sense, it should be noted that the changes followed the same pattern as for earthworms and enchytraeids, i.e. the effects in Cambisol were more significant than in the TAS and Nitisol. In contrast to the possibilities here suggested to justify the greater sensibility of the organisms in Cambisol, Sandifer and Hopkin (1996) concluded that there was no clear correlation between the increase in toxicity as a function of the decrease in pH when evaluations were carried out on *Folsomia candida*. However, it should be considered that the assays conducted by the authors were carried out on OECD artificial soil (OECD 1984), where the organic matter content (in the order of 10%) is too high, given that the great majority of tropical soils rarely exceed 5% (Amorim et al. 2005). According to Fountain and Hopkin (2001), although the species feeds on organic matter and is an important fragmenting agent, whose relevance to the provision of ecosystem services is of the

utmost importance, it is highly tolerant to food contamination. Therefore, considering that the Cambisol had the highest levels of organic matter and that the most significant effects were recorded in this soil, it can be assumed that the factor with the greatest impact on Cu toxicity for these species was likely to have been CEC and pH.

Another outstanding aspect of this investigation, and in line with what was observed by Buch et al. (2016) when conducting assessments on the toxicity of Hg in tropical soils, was the greater sensitivity recorded for *Folsomia candida* when compared to *Proisotoma minuta*. In this sense, it should be noted that this difference in responses to environmental stress justifies the need to include more than one species into the same group of organisms to ecotoxicological assessments; this is because, as pointed out by Salmon et al. (2014), springtails tend to show different interspecific responses due to the different habitats and trophic niches they occupy. Moreover, the greater tolerance of certain species may be related to their morphological characteristics, which reflect their ability to absorb, eliminate and immobilize metals (Janssens et al. 2009). Finally, it should be added that the summary choice of bioindicators should be based on their sensitivity to the stressor, considering the mechanisms of toxic action and exposure to the contaminant. Thus, to endorse the use of *Proisotoma minuta* in the current evaluation, it is worth noting that the species shows reasonable sensitivity to Cu (Nursita et al. 2005), despite its lower sensitivity when compared to *Folsomia candida*.

The process of drawing up the SSD made it possible to assess the organisms' sensitivity to Cu by determining risk concentrations (i.e. HC₅). In short, the graphical observation of adverse effects provides a better understanding of the results as it also represents a versatile approach to the development of practices and policies for the prevention, monitoring and mitigation of environmental degradation (Posthuma et al. 2019). Several studies have aimed to develop retrospective and predictive analysis of the effect of contaminants in Ecological Risk Assessment (ERA) and have employed the modeling of such curves, arguing positively for the use of the method (Princz et al. 2017; Posthuma et al. 2019; Bandeira et al. 2021). As stated before, the organisms were more susceptible to contamination in Cambisol, TAS and Nitisol, respectively, implying that the PV differed depending on the soil type. The decision to obtain PV for the TAS was taken in order to clarify which chemical characteristics of the soils, other than their clay content, have the greatest influence on copper toxicity, since Cambisol and TAS have similar textural compositions. The AI content and the greater amount of nutrients seemed to attenuate the effects of contamination in TAS.

The results presented here are pioneering in terms of the construction of the SSD and the concomitant derivation of PVs for subtropical soils. In this sense, it should be noted

that in 2021, the State of Santa Catarina published its first quality reference values (QRV) in compliance with the Brazilian legislation. However, the QRV for Cu in Santa Catarina went beyond the PV present in the federal regulatory framework (Brasil 2009; IMA 2021); therefore, the results presented here tend to improve the ERA and make a unique contribution to the conservation of ecosystems in the Atlantic Forest Biome. The decision to base the PV estimation on the EC_{50} was driven by the need to safeguard ecosystems without being strictly restrictive, since the PV were estimated according to the HC_5 . However, if the amount of protection has to be greater, further assessments should be conducted so that the SSDs are generated with data from higher protective effect concentrations (EC_x), such as EC_{20} .

That said, it is worth noting that the results showed that the PV based on the EC_{50} data for Cu were higher than the stipulated in the CONAMA Resolution (Brasil 2009), thus indicating the effectiveness of the legislation in protecting soil fauna organisms and refuting the second hypothesis here suggested. However, further assessments are required before assuming the efficacy of the national legislation since soils tend to have different capacities for retaining and making Cu bioavailable. The recently published work by Messias et al. (2023) reinforce the discussion of the need to update the PVs for Cu, since the authors found adverse effects at concentrations below the limit established in the federal regulations. In this way, although it can be inferred that the PV set out in the CONAMA Resolution is protective for the soils evaluated, it cannot be concluded beyond the results presented here, given the influence of the chemical characteristics of the soils on the environmental dynamics of Cu.

Conclusions

The results demonstrate that the reproduction of soil organisms, including earthworms, enchytraeids, and springtails, was significantly affected by copper contamination across the different soils evaluated. Responses varied notably among soil types, with Cambisol being the most sensitive, presenting lower EC_{50} values compared to Nitisol and TAS. This variation in organism responses is likely attributable to soil chemical properties, such as pH and clay content. Additionally, *Perionyx excavatus* proved to be the most sensitive species, while the springtail *Proisotoma minuta* was the least affected, regardless of soil type.

Based on the comparison between the results and current Brazilian legislation, it can be inferred that the preventive guideline values (i.e. PV) for copper are protective for the bioindicators assessed in the soils studied. However, further assessments using additional soil types representative of subtropical climates are needed before it can be conclusively

determined that the PVs established in federal regulations are protective of subtropical terrestrial ecosystems.

It is noteworthy that the data presented here tend to support similar investigations aimed at building an ecotoxicological database, which will enhance our understanding of the effects of trace element pollution on the biotic constituents of various Brazilian ecosystems. This represents a significant step forward in developing public policies focused on environmental conservation, as the evaluated groups of organisms play essential roles in providing the ecosystem services that soil offers.

Supplementary Information The online version contains supplementary material available at <https://doi.org/10.1007/s11356-024-35271-w>.

Authors contribution Daniela Aparecida de Oliveira: Investigation, Formal analysis, Writing – Original Draft, Validation, Visualization. Thiago Ramos Freitas: Formal analysis, Writing – Original Draft, Validation, Visualization. Vanessa Mignon Dalla Rosa: Formal analysis, Writing – Original Draft, Validation, Visualization. Luís Carlos Iuñes Oliveira Filho: Supervision, Visualization, Validation, Writing – Review & Editing. Mari Lucia Campos: Conceptualization, Supervision, Writing – Review & Editing, Validation. Milton da Veiga: Conceptualization, Writing – Review & Editing. David José Miquelluti: Conceptualization, Supervision, Writing – Review & Editing, Validation, Project administration, Funding acquisition. Osmar Klauberg-Filho: Conceptualization, Supervision, Writing – Review & Editing, Validation, Project administration, Funding acquisition.

Funding This study was funded by the financial support from Santa Catarina State Research and Innovation Support Foundation (FAPESC, Grant Number: 2023TR000733). V.M.D.R. and T.R.F. are grateful for the scholarship granted by the Brazilian Coordination for the Improvement of Higher Education Personnel (CAPES).

Data availability Datasets related to this article can be made available upon request to the corresponding author Osmar Klauberg-Filho (osmar.klauberg@udesc.br).

Declarations

Ethical approval Not applicable.

Consent to participate Not applicable.

Consent to publish Not applicable.

Competing interest The authors declare that they have no competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

References

- Amorim MJB, Scott-Fordsmand JJ (2012) Toxicity of copper nanoparticles and $CuCl_2$ salt to *Enchytraeus albidus* worms: survival, reproduction and avoidance responses. Environ Pollut. <https://doi.org/10.1016/j.enCEol.2012.01.015>
- Amorim MJB, Römbke J, Schallnaß HJ, Soares AMVM (2005) Effect of soil properties and aging on the toxicity of copper for *Enchytraeus albidus*, *Enchytraeus luxuriosus*, and

- Folsomia candida*. Environ Toxicol Chem. <https://doi.org/10.1897/04-505r.1>
- Assis O, Niva CC, Brown GG (2023) Using fragmenting enchytraeid species in ecotoxicological tests: regeneration as a new endpoint. Ecotoxicology 16387:32. <https://doi.org/10.21203/rs.3.rs-3453327/v1>
- Bandeira FO, Alves PRL, Hennig TB, Brancalione J, Nogueira DJ, Matias WG (2021) Chronic effects of clothianidin to non-target soil invertebrates: Ecological risk assessment using the species sensitivity distribution (SSD) approach. J Hazard Mater 419:126491. <https://doi.org/10.1016/j.jhazmat.2021.126491>
- Bandow C, Coors A, Römbke J (2013) Enchytraeus bigeminus (Enchytraeidae, Oligochaeta) as a new candidate for ecotoxicological laboratory tests. Soil Org. <https://doaj.org/article/d703aa72e046470dbb6def2bd87ab6af>. Accessed 15 Feb 2024
- Brasil (2009) Resolução nº 420, de 28 de dezembro de 2009
- Briones MJJ (2018) The serendipitous value of soil fauna in ecosystem functioning: the unexplained explained. Front Environ Sci. <https://doi.org/10.3389/fenvs.2018.00149>
- Buch AC, Niemeyer JC, Correia MEF, Silva-Filho EV (2016) Ecotoxicity of mercury to *Folsomia candida* and *Proisotoma minuta* (Collembola: Isotomidae) in tropical soils: Baseline for ecological risk assessment. Ecotoxicol Environ Saf. <https://doi.org/10.1016/j.ecoenv.2016.01.0>
- Decaëns T, Jiménez JJ, Gioia C, Measey GJ, Lavelle P (2006) The values of soil animals for conservation biology. Eur J Soil Biol. <https://doi.org/10.1016/j.ejsobi.2006.07.001>
- Domínguez-Crespo MA, Sánchez-Hernández ZE, Torres-Huerta AM, Negrete-Rodríguez MDLLX, Conde-Barajas E, Flores-Vela A (2012) Effect of the heavy metals Cu, Ni, Cd and Zn on the growth and reproduction of epigeic earthworms (*E. fetida*) during the vermistabilization of municipal sewage sludge. Water Air Soil Pollut 223(2):915–931. <https://doi.org/10.1007/s11270-011-0913-7>
- Duan X, Xu M, Zhou Y, Yan Z, Du Y, Zhang L, Zhang C, Bai L, Nie J, Chen G, Li F (2016) Effects of soil properties on copper toxicity to earthworm *Eisenia fetida* in 15 Chinese soils. Chemosphere. <https://doi.org/10.1016/j.chemosphere.2015.11.099>
- ECHA (2008) Scientific Report SC070009/SR1. Environment Agency. https://assets.publishing.service.gov.uk/government/uploads/system/uploads/attachment_data/file/964467/An-Ecological-Risk-Assessment-Framework-for-contaminants-in-soil.pdf. Accessed 28 Dec 2023
- Environment Canada (2005) Guidance document on statistical methods for environmental toxicity tests / [issued by] method development and applications section, environmental technology centre, environment Canada: En49–7/1–46E-PDF
- Fountain MT, Hopkin SP (2001) Continuous Monitoring of *Folsomia candida* (Insecta: Collembola) in a Metal Exposure Test. Ecotoxicol Environ Saf. <https://doi.org/10.1006/eesa.2000.2007>
- Fox DR, Van Dam RA, Fisher R, Batley GE, Tillmanns AR, Thorley J, Schwarz CJ, Spry DJ, McTavish K (2020) Recent Developments in Species Sensitivity Distribution Modeling. Environ Toxicol Chem. <https://doi.org/10.1002/etc.4925>
- García MVB, Roembke J, Martius C (2004) Proposal for an artificial soil substrate for toxicity tests in tropical regions. In: 25th Annual Meeting of Society of Environmental Toxicology and Chemistry (SETAC). <http://abstracts.co.allenpress.com/pweb/setac2004/document/?id=41943>. Accessed 22 Jan 2024
- Gomes SIL, Scott-Fordsmand JJ, Amorim MJB (2014) Profiling transcriptomic response of *Enchytraeus albidus* to Cu and Ni: Comparison with Cd and Zn. Environ Pollut. <https://doi.org/10.1016/j.envpol.2013.11.031>
- Hedéne P, Jiménez JJ, Moradi J, Domene X, Hackenberger DK, Barot S, Frossard A, Oktaba L, Filser J, Kindlmann P, Frouz J (2022) Global distribution of soil fauna functional groups and their estimated litter consumption across biomes. Sci Rep. <https://doi.org/10.1038/s41598-022-21563-z>
- Hopkin SP (1997) Biology of the Springtails. Oxford University Press EBooks, Oxford. <https://doi.org/10.1093/oso/9780198540847.001.0001>
- IMA (2021) Instrução Normativa nº 45: valores orientadores de qualidade dos solos e águas subterrâneas de Santa Catarina. DOE nº 21485 em 24/03/2021. <https://consultas.ima.sc.gov.br/portarias/pdf/2802>. Accessed 20 May 2024
- ISO (2012) Soil quality - Effects of pollutants on earthworms - Part 2: Determination of effects on reproduction of *Eisenia fetida*/*Eisenia andrei*. ISO, guideline 11268-2. International Organization for Standardization, Geneva
- ISO (2014a) Soil quality — Effects of contaminants on Enchytraeidae (*Enchytraeus* sp.) — Determination of effects on reproduction. ISO, guideline 16387. International Organization for Standardization, Geneva
- ISO (2014b) Soil quality — Inhibition of reproduction of *Collembola* (*Folsomia candida*) by soil contaminants. ISO, guideline 11267. International Organization for Standardization, Geneva
- Janssens TKS, Roelofs D, Van Straalen NM (2009) Molecular mechanisms of heavy metal tolerance and evolution in invertebrates. Insect Sci 16:3–18. <https://doi.org/10.1111/j.1744-7917.2009.00249.x>
- Kabata-Pendias A (2010) Trace elements in soil and plants, 4th edn. CRC Press, Boca Raton
- Konečný L, Ettler V, Kristiansen SM, Amorim MJB, Křibek B, Mihaljevič M, Šebek O, Nyambe I, Scott-Fordsmand JJ (2014) Response of *Enchytraeus crypticus* worms to high metal levels in tropical soils polluted by copper smelting. J Geochem Explor. <https://doi.org/10.1016/j.gexplo.2013.10.004>
- Kuperman RG, Amorim MJB, Römbke J, Lanno R, Checkai RT, Dodard SG, Sunahara GI, Scheffczyk A (2006) Adaptation of the enchytraeid toxicity test for use with natural soil types. Eur J Soil Biol. <https://doi.org/10.1016/j.ejsobi.2006.07.028>
- Kwak JI, An YJ (2021) Microplastic digestion generates fragmented nanoplastics in soils and damages earthworm spermatogenesis and coelomocyte viability. J Hazard Mater. <https://doi.org/10.1016/j.jhazmat.2020.124034>
- Kwak JI, Kim SW, An YJ (2014) A new and sensitive method for measuring in vivo and in vitro cytotoxicity in earthworm coelomocytes by flow cytometry. Environ Res. <https://doi.org/10.1016/j.envres.2014.07.014>
- Lukkari T, Aatsinki M, Väisänen A, Haimi J (2005) Toxicity of copper and zinc assessed with three different earthworm tests. Appl Soil Ecol. <https://doi.org/10.1016/j.apsoil.2005.02.001>
- Maboeta M, Fouché T (2014) Utilizing an earthworm bioassay (*Eisenia andrei*) to assess a south african soil screening value with regards to effects from a copper manufacturing industry. Bull Environ Contam Toxicol. <https://doi.org/10.1007/s00128-014-1302-x>
- Madani S, Coors A, Haddioui A, Ksibi M, Pereira R, Sousa JP, Römbke J (2015) Effects of contaminated soils from a former iron mine (Ait Amar, Morocco) on enchytraeids (*Enchytraeus bigeminus*) and predatory mites (*Hypoaspis aculeifer*) in standard laboratory tests. Ecotoxicol Environ Safe. <https://doi.org/10.1016/j.ecoenv.2015.04.040>
- Maraldo K, Christensen B, Strandberg B, Holmstrup M (2006) Effects of copper on enchytraeids in the field under differing soil moisture regimes. Environ Toxicol Chem. <https://doi.org/10.1897/05-076r.1>
- Mendonça MC, Queiroz GC, Silveira TC (2015) Two new species of *Proisotoma* Börner, 1901 from Southeastern Brazil (Collembola: Isotomidae). Soil Org. <https://soil-organisms.org/index.php/SO/article/view/407>. Accessed 16 Mar 2024
- Messias TG, Alves PRL, Cardoso EJBN (2023) Are the Brazilian prevention values for copper and zinc in soils suitable for protecting earthworms against metal toxicity? Environ Sci Pollut Res Int. <https://doi.org/10.1007/s11356-022-25106-x>

- Mirmonsef H, Hornum HD, Jensen J, Holmstrup M (2017) Effects of an aged copper contamination on distribution of earthworms, reproduction and cocoon hatchability. *Ecotoxicol Environ Saf*. <https://doi.org/10.1016/j.ecoenv.2016.10.012>
- Nascimento Filho VF (1999) Técnicas Analíticas Nucleares de Fluorescência de Raios X por Dispersão de Energia (EDXRF) e por Reflexão Total (TXRF). http://www.raiosx.ufc.br/site/wp-content/uploads/downloads/2012/10/EDXRF_TXRF.pdf. Accessed 23 Feb 2024
- Natal-da-Luz T, Römbke J, Sousa JP (2008) Avoidance tests in site-specific risk assessment—Influence of soil properties on the avoidance response of collembola and earthworms. *Environ Toxicol Chem*. <https://doi.org/10.1897/07-386.1>
- Natal-da-Luz T, Ojeda G, Pratas J, van Gestel CAM, Sousa JP (2011) Toxicity to *Eisenia andrei* and *Folsomia candida* of a metal mixture applied to soil directly or via an organic matrix. *Ecotoxicol Environ Saf*. <https://doi.org/10.1016/j.ecoenv.2011.05.017>
- Nursita AI, Singh B, Lees E (2005) The effects of cadmium, copper, lead, and zinc on the growth and reproduction of *Proisotoma minuta* Tullberg (Collembola). *Ecotoxicol Environ Saf*. <https://doi.org/10.1016/j.ecoenv.2004.05.001>
- OECD (1984) Test no. 207: Earthworm, acute toxicity tests, OECD guidelines for the testing of chemicals, section 2. OECD Publishing, Paris. <https://doi.org/10.1787/9789264070042-en>
- Posthuma L, van Gils J, Zijp MC, van de Meent D, Zwart D (2019) Species sensitivity distributions for use in environmental protection, assessment, and management of aquatic ecosystems for 12386 chemicals. *Environ Toxicol Chem*. <https://doi.org/10.1002/etc.4373>
- Princz J, Becker L, Scheffczyk A, Stephenson GL, Scroggins RP, Moser T, Römbke J (2017) Ecotoxicity of boric acid in standard laboratory tests with plants and soil organisms. *Ecotoxicology*. <https://doi.org/10.1007/s10646-017-1789-0>
- Rehman M, Liu L, Wang Q, Saleem MH, Bashir S, Ullah S, Peng D (2019) Copper environmental toxicology, recent advances, and future outlook: a review. *Environ Sci Pollut Res*. <https://doi.org/10.1007/s11356-019-05073-6>
- Renaud M, Cousins M, Awuah KF, Jegede O, Sousa JP, Siciliano SD (2020) The effects of complex metal oxide mixtures on three soil invertebrates with contrasting biological traits. *Sci Total Environ*. <https://doi.org/10.1016/j.scitotenv.2020.139921>
- Salmon S, Ponge JF, Gachet S, Deharveng L, Lefebvre N, Delabrosse F (2014) Linking species, traits and habitat characteristics of Collembola at European scale. *Soil Biol Biochem*. <https://doi.org/10.1016/j.soilbio.2014.04.002>
- Sandifer RD, Hopkin SP (1996) Effects of pH on the toxicity of cadmium, copper, lead and zinc to *Folsomia candida* Willem, 1902 (Collembola) in a standard laboratory test system. *Chemosphere*. [https://doi.org/10.1016/s0045-6535\(96\)00348-7](https://doi.org/10.1016/s0045-6535(96)00348-7)
- Sandifer RD, Hopkin SP (1997) Effects of temperature on the relative toxicities of Cd, Cu, Pb, and Zn to *Folsomia candida* (Collembola). *Ecotoxicol Environ Saf*. <https://doi.org/10.1006/eesa.1997.1536>
- Santos HG, Jacomine PKT, Anjos LHC, Oliveira VA, Lumberras JF, Coelho MR, Almeida JA, Araújo Filho JC, Oliveira JB, Cunha TJF (2018) Sistema Brasileiro de Classificação de Solos, 5th edn. Embrapa, Brasília
- Schneider CA, Rasband WS, Eliceiri KW (2012) NIH Image to ImageJ: 25 years of image analysis. *Nat Methods* 9:671–675. <https://doi.org/10.1038/nmeth.2089>
- Sereni L, Guenet B, Lamy I (2021) Does copper contamination affect soil CO₂ emissions? A literature review. *Front Environ Sci*. <https://doi.org/10.3389/fenvs.2021.585677>
- Silva PMCS, van Gestel CAM (2009) Comparative sensitivity of *Eisenia andrei* and *Perionyx excavatus* in earthworm avoidance tests using two soil types in the tropics. *Chemosphere*. <https://doi.org/10.1016/j.chemosphere.2009.09.034>
- Simões BF, Mazur N, Correia MEF, Niemeyer JC, Matos TS (2020) Ecotoxicity test as an aid in the determination of copper guideline values in soils. *Ciência Rural*. <https://doi.org/10.1590/0103-8478r20180961>
- Spurgeon DJ, Hopkin SP (1996) The effects of metal contamination on earthworm populations around a smelting works: quantifying species effects. *Appl Soil Ecol*. [https://doi.org/10.1016/0929-1393\(96\)00109-6](https://doi.org/10.1016/0929-1393(96)00109-6)
- Spurgeon DJ, Hopkin SP, Jones DT (1994) Effects of cadmium, copper, lead and zinc on growth, reproduction and survival of the earthworm *Eisenia fetida* (Savigny): assessing the environmental impact of point-source metal contamination in terrestrial ecosystems. *Environ Pollut*. [https://doi.org/10.1016/0269-7491\(94\)90094-9](https://doi.org/10.1016/0269-7491(94)90094-9)
- Statsoft Inc. (2011) Statistica (Data Analysis Software System). Version 10. <https://www.Statsoft.com>
- Teixeira PC, Donagemma GK, Fontana A, Teixeira WG (2017) Manual de Métodos de Análise de Solo, 3rd edn. Brasília, Embrapa
- Van Gestel CAM (2012) Soil ecotoxicology: state of the art and future directions. *ZooKeys*. <https://doi.org/10.3897/zookeys.176.2275>
- Van Vlaardingen PLA, Traas TP, Wintersen AM, Aldenberg T (2004) ETX 2.0. A Program to calculate hazardous concentrations and fraction affected, based on normally distributed toxicity data. <http://hdl.handle.net/10029/9005>. Accessed 4 Apr 2024
- Wheeler JR, Grist EPM, Leung KMY, Morritt D, Crane M (2002) Species sensitivity distributions: data and model choice. *Mar Pollut Bull*. [https://doi.org/10.1016/S0025-326X\(01\)00327-7](https://doi.org/10.1016/S0025-326X(01)00327-7)
- Witkowska D, Słowik J, Chilicka K (2021) Heavy metals and human health: Possible exposure pathways and the competition for protein binding sites. *Molecules*. <https://doi.org/10.3390/molecules26196060>

Publisher's Note Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.

Springer Nature or its licensor (e.g. a society or other partner) holds exclusive rights to this article under a publishing agreement with the author(s) or other rightsholder(s); author self-archiving of the accepted manuscript version of this article is solely governed by the terms of such publishing agreement and applicable law.



Ecotoxicity of alkaline residue from the pulp and paper industry to soil faunal organisms

Joseli Pietro Biasi^a, Thiago Ramos Freitas^a, Eduardo Oliveira da Silva Lunardi^a,
Luís Carlos Iuñes de Oliveira Filho^a, Mari Lucia Campos^a, Osmar Klauber-Filho^a,
Dilmar Baretta^{b,*}

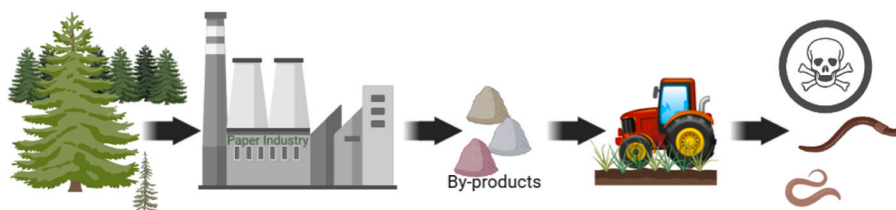
^a Santa Catarina State University, Center for Agroveterinary Sciences, Department of Soils and Natural Resources, Lages, Santa Catarina, Brazil

^b Santa Catarina State University, Center for Higher Education of the West, Department of Animal Science, Chapecó, Santa Catarina, Brazil

HIGHLIGHTS

- Dregs are a by-product from the pulp and paper industry that is suggest to be a soil pH corrective.
- Soil organisms are vital to the maintenance of the soil health.
- Dregs are capable of affect the reproduction rates of soils organisms.
- The toxicity of dregs varies according to the soil chemical characteristics.
- Metal levels in the dregs matrix are less of concern than the increases in Na content.

GRAPHICAL ABSTRACT



ARTICLE INFO

Handling editor: Prof. X. Cao

Keywords:

Dregs
Contamination
Earthworm
Potworm
Springtail

ABSTRACT

The increased production and expanding demand for cellulosic products have required companies to provide solutions for the proper disposal of generated residues without compromising soil quality. In this context, the current research aimed at evaluating the ecotoxicological impacts of dregs application in subtropical soils. The experiments were carried out with the earthworm *Eisenia andrei*, the springtail *Folsomia candida*, and the potworm *Enchytraeus crypticus* in two subtropical soils (Neosol and Cambisol) collected in the southern region of Brazil. The considerable differences in texture and organic matter content motivated the choosing of these soils. The reproductive rate of all organisms was influenced, with varying EC_{50} values depending on the soil type. However, more pronounced effects were observed for Neosol. The springtail *F. candida* proved to be the most sensitive to contamination ($EC_{50} = 5.8 \text{ g kg}^{-1}$ for Neosol and 48.5 g kg^{-1} for Cambisol), followed by the earthworm *E. andrei* ($EC_{50} = 62.4 \text{ g kg}^{-1}$ for Neosol and 129.5 g kg^{-1} for Cambisol) and the potworm *E. crypticus* ($EC_{50} = 67.2 \text{ g kg}^{-1}$ for Neosol and 230.4 g kg^{-1} for Cambisol). As a result, while dregs can be used to correct soil acidity, they have been shown to have negative effects on important organisms responsible for functions critical to maintaining ecosystem quality.

* Corresponding author. Department of Soils and Natural Resources, Santa Catarina State University, Lages, SC 88520-000, Brazil.

E-mail address: dilmar.baretta@udesc.br (D. Baretta).

<https://doi.org/10.1016/j.chemosphere.2024.143448>

Received 6 March 2024; Received in revised form 14 September 2024; Accepted 1 October 2024

Available online 2 October 2024

0045-6535/© 2024 Elsevier Ltd. All rights are reserved, including those for text and data mining, AI training, and similar technologies.

Environmental implication

Paper and cellulose production processes generate a large number of byproducts with various chemical characteristics that can be used to fertilize the soil. Dregs are made by chemically digesting fibrous material in the presence of sodium hydroxide and sodium sulfide. This research looks at how Dregs pollution affects reproduction, which advances the creation of measures to protect soil health. By studying the effects of Dregs pollution on reproduction, researchers can better understand the potential harm these byproducts may pose to soil health. Using this information, measures to reduce the adverse effects of Dregs pollution can then be developed.

1. Introduction

Brazil is the world's second-largest producer of pulp and hardwood pulp (FAO, 2023). Large-scale waste is produced throughout the pulp and paper industry's manufacturing process since, for every 100 t of product, around 50 t of waste is produced (Bellote et al., 1998). In this sense, as the demand for cellulosic products grows, the amount of waste generated has drawn attention in an effort to reduce environmental risks while meeting circular economy demands (Toledo et al., 2015).

Among the main residues from the large-scale production of pulp and paper, green liquor, lime mud, grits, and dregs stand out, the latter being produced in greater amounts than the others (Maciel et al., 2015; Kristanto and Asaloei, 2017). The dregs originate from the chemical digestion of the fibrous material in the presence of sodium hydroxide (NaOH) and sodium sulfide (Na_2S), whose purpose is to promote the delignification of cellulose (Bajpai, 2018). As the dregs are essentially composed of calcium carbonate (CaCO_3) and magnesium (MgCO_3) precipitates, several studies have suggested their use as a soil conditioner (Nurmesniemi et al., 2005; Medeiros et al., 2009; Kristanto and Asaloei, 2017).

However, dregs have metallic elements with high deleterious potential in their constitution, such as lead (Pb), chromium (Cr), and nickel (Ni) (Almeida et al., 2007). Therefore, despite the potential use for the chemical correction of the soil, the application of dregs can increase not only the concentration of essential nutrients (such as Ca, Mg, K, P, and S) but also of metals in concentrations capable of bioaccumulating along the food chain (Nurmesniemi et al., 2005). In addition, the high amounts of sodium (Na) in the dreg's matrix composition can trigger an osmotic imbalance in soil, leading to severe toxic effects on soil organisms (Sá et al., 2021).

Aiming for the protection of ecosystems and the maintenance of soil and water quality, in terms of their ability to support life, the Resolution n° 420/2009 of the Brazilian National Council for the Environment (CONAMA) establishes guidelines for soil preservation according to trigger values (Brasil, 2009). However, there is no specific and detailed legislation regarding the application of residues from the cellulosic industry for agronomic purposes considering the protection of important groups of soil organisms, such as oligochaetes and microarthropods, which perform relevant functions in the soil (Alves et al., 2016; Segat et al., 2020).

Earthworms (*Eisenia andrei*), potworms (*Enchytraeus crypticus*) and springtails (*Folsomia candida*) are excellent bioindicators since they play a key role in providing regulatory ecosystem services and are also highly sensitive to the presence of certain pollutants, such as metals and complex organic matrices (van Gestel et al., 1993; Natal-da-Luz et al., 2011; Maccari et al., 2016; Segat et al., 2020). Although the ecosystem is made up of a wide range of organisms, results with standardized species are widely used as a way to elucidate potential effects on the dynamics of native species. The species *E. andrei* is among the most studied organisms in ecotoxicological assessments, as well as *E. crypticus* and *F. candida* (Natal-da-Luz et al., 2011; Maccari et al., 2016; Segat et al., 2020). Therefore, it is crucial to understand the impending threat to soil health posed by the loss of these organisms capacity for reproduction as

a result of the application of dregs. Nonetheless, few studies have investigated the biological effects of pulp and paper industry by-products on these organisms (Fraser et al., 2009; Song et al., 2018).

An environmental risk assessment with dregs is necessary to evaluate the potential risks associated with its disposal in various environmental scenarios, since soil chemical properties play a critical role in governing the dynamics of heavy metals, which constitutes dreg's matrix in abundance (Almeida et al., 2007). Recent studies have shown that pH significantly influences metal solubility and mobility. Acidic soils, for example, increase the mobility and bioavailability of metal ions, posing higher risks to water contamination (Hou et al., 2020). On the other hand, organic matter contributes to the adsorption and immobilization of metals through complexation, reducing their availability (Zhou et al., 2023). It is essential to emphasize that soils with lower organic matter content are highly susceptible to contamination by several cationic metals due to their strong affinity for the organic fraction (Kabata-Pendias, 2010). This suggests that in regions where soils have this characteristic, extra caution should be taken with organic fertilization and the prolonged use of metal-based pesticides.

In this paper, the ecotoxicological effect of dregs on the reproduction of soil invertebrates (earthworms, enchytraeids, and springtails) was evaluated and effective concentrations determined. The dregs' toxicity and their chemical properties were discussed along with the difference in sensitivity of the test organisms and soil properties. In this sense, this study aimed to assess the potential ecological risk of land application of dregs as soil amendments, using two subtropical soils with contrasting textural and chemical properties. This approach tends to significantly enhance the understanding of contamination processes in subtropical regions, where soils exhibit considerable chemical and physical variability. Thus, considering the above, we hypothesized that (i) dregs have negative effects on the reproduction of studied soil invertebrates; and (ii) the effects vary according to the soil type due to their properties influencing the availability of potentially hazardous chemical elements present on the dregs. Stating that, it is worth noting that this is the first study documenting the effects of dregs on soil organisms in subtropical soils.

2. Material and methods

2.1. Soils and test substance

Two Brazilian subtropical soils were evaluated, a Neosol collected in Lages (29° 00' 19.98" S e 49° 31' 02.84" W) and a Cambisol collected in Araranguá, both municipalities belonging to the Santa Catarina State (Brazil), known to host several subsidiary branches of a pulp and paper industry, (27° 53' 06" S e 50° 24' 57" W). According to the Brazilian Soil Classification, the Neosol is classified as a Neossolo Quartzarênico Órtico típico, and the Cambisol as a Cambissolo Háplico distrófico (Santos et al., 2018). The soils were chosen due to their contrasting chemical properties (such as pH and organic matter content) and texture. The samples were collected at a 0-0,2 m depth. Both soils were air dried sieved ($\phi \leq 2$ mm and manually homogenized. In addition to the clay, silt and sand contents, according to the method proposed by Gee and Bauder (1986), the following chemical parameters (Table 1) were also analyzed: pH (ISO, 2005); electrical conductivity (EC) (ISO, 1994); soil organic matter content (SOM); cation exchange capacity (CEC); and the contents of Al^{3+} , H + Al (Tedesco et al., 1995), Al, Ca, Cr, Cu, Fe, Mg, Ni, Pb, and Zn (USEPA, 1999).

Four subsamples were collected at random points from a batch of dregs from the kraft pulping process, supplied by a pulp and paper industry. The subsamples were air-dried, sieved ($\phi \leq 2$ mm) and homogenized so that they could be used in the tests. The chemical properties of the dregs are shown in Table S1.

Table 1
Chemical and physical characteristics of Neosol and Cambisol.

Attribute	Neosol	Cambisol
pH (KCl)	6.4	5.1
EC (H ₂ O) (dS m ⁻¹) ^a	6.1	4.5
SOM (%) ^b	1.3	3.1
CEC pH 7.0 (cmol _c dm ⁻³) ^c	4.9	20.9
Al ³⁺ (cmol _c dm ⁻³)	0.00	0.22
H + Al (cmol _c dm ⁻³)	1.55	13.75
Al (g kg ⁻¹)	3.44 ± 0.11	37.92 ± 7.75
Ca (g kg ⁻¹)	0.69 ± 0.19	0.53 ± 0.04
Cr (mg kg ⁻¹)	8.53 ± 0.13	32.84 ± 0.13
Cu (mg kg ⁻¹)	nd ^d	88.11 ± 0.65
Fe (g kg ⁻¹)	1.99 ± 0.09	23.24 ± 0.25
K (g kg ⁻¹)	0.18 ± 0.01	0.31 ± 0.04
Mg (g kg ⁻¹)	0.24 ± 0.02	0.65 ± 0.08
Mn (g kg ⁻¹)	117.44 ± 0.18	783.55 ± 0.19
Na (g kg ⁻¹)	2450.01 ± 2.47	349.49 ± 3.33
Ni (mg kg ⁻¹)	nd ^d	22.63 ± 0.47
Pb (mg kg ⁻¹)	7.67 ± 0.28	11.63 ± 0.26
Zn (mg kg ⁻¹)	4.16 ± 0.08	21.18 ± 0.55
Clay (g kg ⁻¹)	40	330
Silt (g kg ⁻¹)	590	230
Sand (g kg ⁻¹)	370	440
Texture	Sandy loam	Clay loam

^a EC: Electrical conductivity in H₂O.

^b SOM: soil organic matter.

^c CEC: cation exchange capacity.

^d nd: not determined.

2.2. Test organisms

The earthworm *E. andrei* was cultivated in a substrate consisting of equine manure, coconut husk fiber and fine sand (in the proportion 70:20:10). The organisms were fed weekly with oat flakes and kept at a temperature of 23 ± 2 °C at a photoperiod of 12 h light and 12 h dark. The potworm *E. crypticus* was cultivated according to the ISO 16387 protocol (ISO, 2014). The springtail *F. candida* was cultivated in a substrate composed of plaster, distilled water and activated charcoal (in the proportion 11:7:1), kept in a controlled environment at a temperature of 20 ± 2 °C and a photoperiod of 12 h light and 12 h dark. All cultures were fed three times a week.

2.3. Experimental procedures

The assays to measure the effects of dregs on the reproduction rates followed the guidelines of the specific protocols for earthworms (ISO, 2012a), enchytraeids (ISO, 2014) and springtails (ISO, 2012b). Assays were performed for both soils Neosol and Cambisol, independently, following a completely randomized design with at least four replications per tested dose.

Earthworms were selected according to their body mass (between 300 and 500 mg) and the presence of a well-developed clitellum. Before the beginning of the tests, the organisms were acclimatized for a period of 24 h in uncontaminated soil, with moisture maintained at about 60% of the water holding capacity (WHC). After this period, 10 adult earthworms were placed in 1 L plastic containers (height: 9 cm; diameter: 14.5 cm), filled with 500 g of soil (n = 4). The organisms were fed weekly with 5 g of uncontaminated dry horse manure. The enchytraeids were also selected according to the presence of clitellum and remained acclimatized for 24 h. The tests were carried out in plastic containers with 120 mL capacity (height: 4.5 cm; diameter: 7.6 cm), filled with 30 g of soil (n = 5) at a moisture of 60% of the WHC. The treatments consisted of the presence of 10 organisms, which were fed weekly with approximately 2 mg of finely ground oats. Assays with springtails consisted of the presence of 10 organisms aged between 10 and 12 days, kept in plastic containers with 120 mL capacity (height: 4.5 cm; diameter: 7.6 cm), filled with 30 g of soil (n = 5) at a moisture of 70% of the WHC. The organisms were fed weekly with approximately 2 mg of dry yeast.

The dregs tested concentrations for all the organisms are presented in Table 2.

2.4. Chemical analysis

For all doses and species evaluated, the EC in H₂O (ISO, 1994) and pH in KCl (ISO, 2005) were measured before and after the tests (Tables S2 and S3). Following soil contamination, three samples of each tested dose were chosen to create a composite sample on which the contents of Al, Ca, Co, Cr, Cu, Mg, Mn, Ni, Pb, Sr, and Zn were determined using the ICP-OES method according to the USEPA 3050B method (USEPA, 1996), and the contents of Na and K were determined using a flame photometer (Tables S4 and S5). The Standard Reference Material (SRM) used was a 2709a San Joaquin Soil.

2.5. Data analysis

The normality and homoscedasticity of variances were evaluated using the Shapiro-Wilk and Bartlett tests, respectively. After confirming the assumptions, the reproduction data were submitted to a variance analysis test (one-way ANOVA) and then compared by Dunnett's test (M < control, p ≤ 0.05) using the Statistica 7.0 software (StatSoft, 2004). The estimated concentration capable of reducing the reproduction of organisms by 50% (EC₅₀) was calculated through non-linear regression using pre-defined mathematical models that best fit (R²) the data (Environment Canada, 2005). The Behrens-Fisher significance test (p ≤ 0.05) was performed to determine whether there is a difference between the two evaluated soils.

3. Results

3.1. Chronic toxicity assay

The average mortality in the control vessels was below 10%, 20%, and 20% for *E. andrei*, *E. crypticus*, and *F. candida*, respectively. The mean number of juveniles was above 30, 25, and 100 individuals to *E. andrei*, *E. crypticus*, and *F. candida*, respectively, with coefficients of variation (CV) below 30% in all the assays. Therefore, the results meet the criteria recommended by ISO 11268-2 (ISO, 2012a), ISO 16387 (ISO, 2014), and ISO 11267 (ISO, 2012b).

For Neosol (p ≤ 0.05), the dregs affected the reproduction of *E. andrei* from the dose of 25 g kg⁻¹ (Fig. 1a) while for Cambisol the effects were observed from the 90 g kg⁻¹ dose (Fig. 1b). The EC₅₀ was 62.4 g kg⁻¹ (43.6–81.3) to Neosol and 129.5 g kg⁻¹ (103.1–155.8) to Cambisol (Table 3).

In the *E. crypticus* assays a Hormesis effect (p ≤ 0.05) was verified for both evaluated soils (Fig. 1c and d). In Neosol, an increase in the number of juveniles was observed at doses of 10 and 20 g kg⁻¹ (compared to the control treatment) with a subsequent decrease from the dose of 40 g kg⁻¹ (Fig. 1c). In Cambisol, there was an increase in the number of juveniles for the 10, 20, 40, and 100 g kg⁻¹ doses when compared to the control treatment, with a negative effect recorded at doses of 200 and 400 g kg⁻¹. The EC₅₀ determined for Neosol was 67.2 g kg⁻¹ (62.1–72.3) and 230.4 g kg⁻¹ (196.9–263.8) for Cambisol (Table 3).

The results obtained for *F. candida* in the Neosol showed a negative

Table 2
Tested concentrations of dregs mixed in each soil.

Soil	Organisms	Dregs tested concentrations (g kg ⁻¹)
Neosol	<i>E. andrei</i>	0; 10; 15; 25; 40; 65; 100
	<i>E. crypticus</i>	0; 10; 20; 40; 60; 100; 200
	<i>F. candida</i>	0; 2.5; 5; 10; 20; 30; 50; 90
Cambisol	<i>E. andrei</i>	0; 10; 20; 30; 50; 90; 150
	<i>E. crypticus</i>	0; 10; 20; 100; 200; 400
	<i>F. candida</i>	0; 10; 20; 40; 80; 150; 300

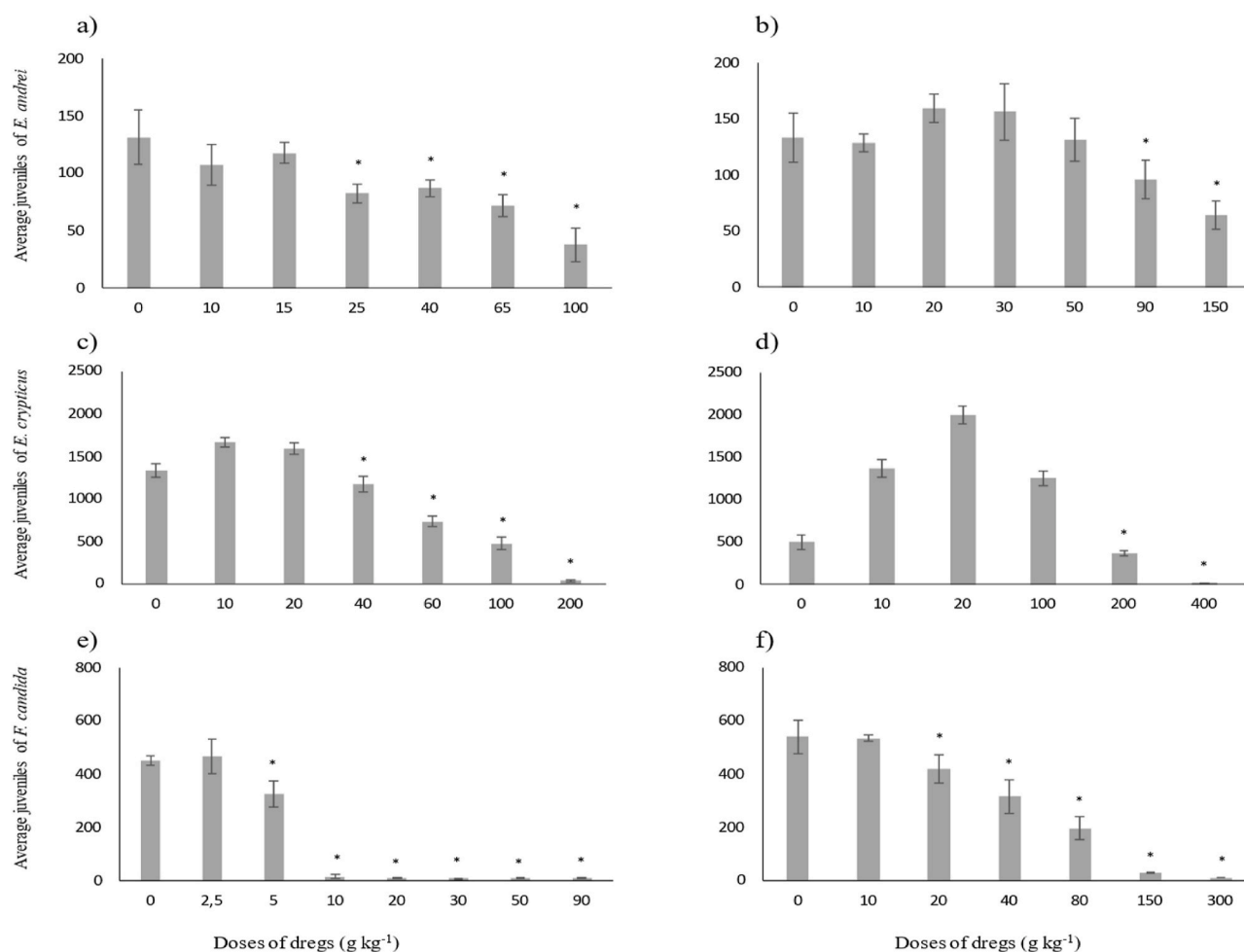


Fig. 1. Mean number of juveniles of *E. andrei* (a), *E. crypticus* (c), and *F. candida* (e) in Neosol and *E. andrei* (b), *E. crypticus* (d), and *F. candida* (f) in Cambisol. (*) indicates a significant difference ($p \leq 0.05$). (–) Standard deviation ($n = 4$). Concentration of 0 represents the control treatment (free from contamination).

Table 3

Ecotoxicological parameters (NOEC, LOEC and EC_{50}) obtained in the acute and chronic toxicity tests in subtropical soils (Neosol and Cambisol).

Test species	Endpoint	Parameter	Concentration of dregs (g kg^{-1})	
			Neosol	Cambisol
<i>E. andrei</i>	56-d reproduction	NOEC	15	50
		LOEC	25	90
		EC_{50}	62.4 (43.6–81.3)	129.5 (103.1–155.8)
<i>E. crypticus</i>	21-d reproduction	NOEC	20	100
		LOEC	40	200
		EC_{50}	67.2 (62.1–72.3)	230.4 (196.9–263.8)
<i>F. candida</i>	28-d reproduction	NOEC	2.5	10
		LOEC	5	20
		EC_{50}	5.8 (5.4–6.2)	48.5 (40.3–56.7)

NOEC – No Effect Concentration; LOEC – Lowest Effect Concentration; EC_{50} – Effect Concentration which reduces by 50% the reproduction of organisms.

effect ($p \leq 0.05$) on reproduction from the 5 g kg^{-1} dose (Fig. 1e). For Cambisol, a decrease in the number of juveniles was observed ($p \leq 0.05$) from the 20 g kg^{-1} dose (Fig. 1f). The EC_{50} was 5.8 g kg^{-1} (5.4–6.2) for Neosol and 48.5 g kg^{-1} (40.3–56.7) for Cambisol (Table 3).

The dregs' toxicity varies ($p \leq 0.05$) between the two evaluated soils

for all the assays performed.

3.2. Chemical evaluations

The addition of increasing concentrations of dregs resulted in an elevation of pH and EC in both soils (Tables S2 and S3). These findings suggest that the metals present in the matrix composition of the dregs (Tables S4 and S5) are possibly found precipitated in the soil.

In the Cambisol there was an evident increase in Na, K, Ca, Cr, Cu, Mg, Mn, Ni, Sr, and Zn contents as the dreg's doses increased. On the other hand, there was a progressive reduction in the Al, Co, and Pb contents (Table S4). For the Neosol, although less evident due to the lower applied concentrations of dregs, there was a progressive increase of most elements as the doses increased, except for Pb and Ba, which remained below the detection limit of the equipment (LDI) for almost all concentrations evaluated (Table S5).

4. Discussions

It is observed that soil contamination by dregs could inhibit the reproduction of all organisms evaluated at low percentages of application, confirming the first. We emphasize the similarity in toxicity levels between *E. andrei* and *E. crypticus*, both oligochaetes with scavenging habits, and the greater susceptibility of *F. candida* to contamination (Table 3). In fact, different responses are expected among different faunal groups (Natal-da-Luz et al., 2004). An example of these variations in sensitivity is that in a study conducted by Fraser, O'halloran and van

den Heuvel (2009), no acute effects (mortality) or reduction in the biomass of *E. fetida* exposed to organic residues from the cellulosic industry were observed; nevertheless, the authors verified a significant reduction in the reproduction of *Enchytraeus albidus*. Moreover, Renaud et al. (2017) found that *F. candida* is a more sensitive bioindicator to organic contamination than *E. crypticus* and *Eisenia* spp., which corroborates the results observed in our evaluations with dregs, despite its inorganic nature. Concerning the implications of the contamination, negative effects on the activity of enzymes involved in the biotransformation process as well as damage to the genetic structure were found for *E. fetida* when exposed to green liquor. (Song et al., 2018). These comparisons shows how by-products from the pulp and paper industry can significantly and negatively impact soil organisms.

The addition of the dregs increased the amount of Na in the soils (Tables S4 and S5), which may have implied harmful consequences on the organisms. This is particularly true given the possibility of an osmotic imbalance in the epithelial cells. Owojori and Reinecke (2009) assessing the effects of NaCl found significant avoidance behavior for the species *E. fetida* at a concentration of 500 mg kg⁻¹, which may suggest a possible saline effect on our results, since the Na concentrations (Tables S4 and S5) in the LOEC determined by us were all higher than the LOEC found by the authors. In our study, we verified that the estimated contents of Na at the lowest doses of dregs with a significant effect for all the evaluated organisms were higher in Neosol than in Cambisol. This is probably why prominent effects were observed in Neosol (Table 3), even though it was not possible to confirm a correlation between the increase in Na content in the soil and the organisms' ability to reproduce. Having stated that we can confirm the second hypothesis on which our study was based.

The greater sensitivity of organisms to the dregs contamination in the Neosol can be attributed to the lower organic matter and clay contents, which are essential for metal retention in soils by enhancing the adsorption and immobilization of contaminants. In the Neosol, this deficiency increases the mobility of metals, raising their bioavailability and toxicity. As Amorim et al. (2005) suggest, soils with low levels of these constituents tend to create more toxic environments for soil organisms when exposed to cationic contaminants. In contrast, soils like Cambisols, richer in clay and organic matter, retain metals more efficiently, restricting their mobility and minimizing harmful effects (Hernandez-Soriano and Jimenez-Lopez, 2015; Hou et al., 2019; Ma et al., 2020). Similarly to what we observed, Maccari et al. (2016) reported pronounced effects in soil with lower levels of CEC and contents of organic matter and clay when analyzing the influence of liquid pig manure on *F. candida* reproduction in subtropical soils, linking the presence of metallic cations to the reported toxicity. Nevertheless, because the highest levels of Co, Cr, Cu, Ni, Pb, Sr, and Zn were found in Cambisol, we might deduce that the metals present in the dregs' matrix (Table S1) are not as concerning as the saline effect. Furthermore, it should be noted that the addition of dregs increased the pH of the soil to levels where metal cations are more likely to precipitate as oxides and hydroxides, which are not bioavailable to the soil organisms (Wan et al., 2024). However, it is crucial to exercise caution in assuming that the metals found in the dregs' matrix are of no concern at all, as some of them have no biological function and are toxic to soil organisms and may pollute water at underground levels. For example, Pb has already been linked to a decrease in *E. crypticus* reproduction (Zhang et al., 2019). Consequently, it is not possible to assume that the metal input via dregs has no negative effect on the environment.

We highlight that due to particularly the difference we found in dregs toxicity between the two evaluated soils, our findings suggest that the chemical and physical characteristics of the soils should be previously considered before proposing the use of dregs for agronomic purposes. It is important to note that the protection of the functions performed by soil fauna organisms is vital to guaranteeing the environmental services provided by the soil, therefore, we emphasize that the lowest of the EC₅₀ verified represents an indication of the maximum capacity of the dregs

that the soil can receive. These EC₅₀ values (5.8 g kg⁻¹ for *F. candida* in Neosol and 48.5 g kg⁻¹ in Cambisol) correspond to agronomic doses of 5.8 and 48.5 t of dregs per hectare (considering only the first 10 cm layer) which are higher than the doses evaluated in a study by Medeiros et al. (2009) that indicates that dregs improved the chemical attributes of an Cambisol. Based on the data presented, we can infer that the agronomic doses being explored are likely significantly lower than the effect concentrations found in this study. However, evaluations with other types of soil are required before it can be stated that dregs do not pose a risk to the environment. Another reason to be cautious is that the input of Na via dregs can also act as a clay dispersant, resulting in soil architecture damage that may affect the organism's activity. However, physical assays were not performed, exposing a gap on which to focus future evaluations. Furthermore, despite not assessing the effects of dregs on plant endpoints, high doses of elements such as Mg, Na, and Ca may trigger nutritional imbalances in crops and eventually impair crop yield in the long term (Sundari and Kanakarni, 2001).

Regarding the ecological risk analysis, the pioneering of the present research, concerning ecotoxicological assessments in subtropical soils with alkaline residue from the pulp and paper industry, tends to help understand the implications of using dregs as soil corrective and fertilizer. Finally, we would like to draw attention to the fact that, whereas the world is moving toward a fully circular economy, it is not always possible to apply industrial by-products into the soil for agricultural uses without previous chemical treatment. This means that although dregs have improved soil pH and are capable of providing certain important nutrients to plant crops, their effects on soil organisms should not be neglected since the ecosystem operates with interdependencies and interferences in key-species dynamics tend to be harmful to soil health.

5. Conclusions

The application of dregs in subtropical soils was able to reduce the reproductive rate of all evaluated soil invertebrates. The highest sensitivity was recorded for the springtail *F. candida*, followed by *E. andrei* and *E. crypticus*. Different effects regarding the toxicity of dregs were also observed depending on the soil evaluated, leading us to infer that soil properties can attenuate the effects of contamination. The most pronounced effects were observed in the Neosol, possibly due to its lower organic matter and clay content. However, although metal concentrations were higher in the Cambisol, the Na content appeared to be the main factor responsible for the ecotoxicological effects evaluated.

To summarize, despite its use as a soil pH corrective, dregs may pose a risk to soil ecosystems, as important species could potentially become endangered due to its application, particularly as a result of increased Na concentration. However, further evaluations with other agriculturally relevant soil types are necessary to improve our understanding of dregs toxicity in soils.

CRedit authorship contribution statement

Josieli Pietro Biasi: Writing – original draft, Investigation, Formal analysis. **Thiago Ramos Freitas:** Writing – original draft, Visualization, Validation, Formal analysis. **Eduardo Oliveira da Silva Lunardi:** Writing – original draft, Visualization. **Luís Carlos Iúñez de Oliveira Filho:** Writing – review & editing, Visualization, Validation, Supervision. **Mari Lucia Campos:** Writing – review & editing, Supervision, Funding acquisition. **Osmar Klauber-Filho:** Writing – review & editing, Supervision, Funding acquisition, Conceptualization. **Dilmar Baretta:** Writing – review & editing, Supervision, Project administration, Funding acquisition, Conceptualization.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence

the work reported in this paper.

Data availability

Data will be made available on request.

Acknowledgement

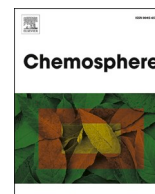
This research was supported by the Brazilian Coordination for the Improvement of Higher Education Personnel (CAPES), The Brazilian National Council for Scientific and Technological Development (CNPq) (Grant Number: D.B.: 308189/2022-1) and the Santa Catarina State Research and Innovation Support Foundation (FAPESC) (Grant Number: 2023TR000733).

Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.chemosphere.2024.143448>.

References

- Almeida, H.C., Silveira, C.B., Ernani, P.R., Campos, M.L., Almeida, D., 2007. Composição química de um resíduo alcalino da indústria de papel e celulose (DREGS). Quím. Nova 30 (7), 1669–1672.
- Alves, P.R.L., Silva, E.B., Cardoso, E.J.B.N., Alleoni, R.R.F., 2016. Ecotoxicological impact of arsenic on earthworms and collembolans as affected by attributes of a highly weathered tropical soil. Environ. Sci. Pollut. Control Ser. 25 (14), 13217–13225.
- Amorim, M.J.B., et al., 2005. Effect of soil properties and aging on the toxicity of copper for *Enchytraeus albidus*, *Enchytraeus luxuriosus*, and *Folsomia Candida*. Environ. Toxicol. Chem., [S.L.] 24 (8), 1875.
- Bajpai, P., 2018. Biermann's Handbook of Pulp and Paper, vol. 3. Elsevier, p. 647, 2018.
- Bellote, A.F.J., Silva, H.D., Andrade, G.C., Moro, L., 1998. Resíduos da indústria de celulose em plantios florestais. Boletim de Pesquisa Florestal 37, 99–106, 1998.
- Brasil, 2009. Resolução nº 420, de 28 de dezembro de 2009.
- Environment Canada, 2005. Guidance Document on Statistical Methods for Environmental Toxicity Tests: Method Development and Applications section. Environmental Technology Centre, Ottawa.
- FAO, 2023. Online data FAOSTAT. <https://www.fao.org/faostat/en/#data/FO>.
- Fraser, D.S., O'Halloran, K., van den Heuvel, M.R., 2009. Toxicity of pulp and paper solid organic waste constituents to soil organisms. Chemosphere 74 (5), 660–668.
- Gee, G.W., Bauder, J.W., 1986. Particle size analysis. In: Klute, A. (Ed.), Methods of Soil Analysis. Part 1. Physical and Mineralogical Methods. SSSA Book Series, pp. 383–411.
- Hernandez-Soriano, M.C., Jimenez-Lopez, J.C., 2015. Linking dissolved organic matter composition to metal bioavailability in agricultural soils: effect of anionic surfactants. Biogeosci. Discuss., [S.L.] 12, 5697–5723.
- Hou, D., O'Connor, D., Igalavithana, A.D., Alessi, D.S., Luo, J., Tsang, D.C.W., Sparks, D. L., Yamauchi, Y., Rinklebe, J., Ok, Y.S., 2020. Metal contamination and bioremediation of agricultural soils for food safety and sustainability. Nat. Rev. Earth Environ., [S.L.] 1 (7), 366–381.
- Hou, S., Zheng, N., Tang, L., Ji, X., Li, Y., 2019. Effect of soil pH and organic matter content on heavy metals availability in maize (*Zea mays* L.) rhizospheric soil of non-ferrous metals smelting area. Environ. Monit. Assess., [S.L.] 191 (10), 634.
- ISO 10390:2005. Soil quality - Determination of pH.
- ISO 11268-2:2012a. Soil quality - Effects of pollutants on earthworms (*Eisenia fetida*) - Part 2: Determination of effects on reproduction.
- ISO 11268-2:2012b. Soil quality - Inhibition of reproduction of collembola (*Folsomia candida*) by soil pollutants.
- ISO 16387:2014. Soil quality - Effects of pollutants on Enchytraeidae (*Enchytraeus* sp.) - Determination of effects on reproduction and survival.
- ISO 11265, 1994. Soil quality - Determination of the specific electrical conductivity.
- Kabata-Pendias, A., 2010. Trace Elements in Soil and Plant, vol. 4. CRC Press, Boca Raton, p. 548.
- Kristanto, G.A., Asaloei, H., 2017. Assessment of anaerobic biodegradability of five different solid organic wastes. AIP Conf. Proc., 020029.
- Maccari, A.P., Baretta, D., Paiano, D., Leston, S., Freitas, A., Ramos, F., Sousa, J.P., Klauber-Filho, O., 2016. Ecotoxicological effects of pig manure on *Folsomia candida* in subtropical Brazilian soils. J. Hazard Mater. 314, 113–120.
- Ma, H., Li, X., Wei, M., Zeng, G., Hou, S., Li, D., Xu, H., 2020. Elucidation of the mechanisms into effects of organic acids on soil fertility, cadmium speciation and ecotoxicity in contaminated soil. Chemosphere 239, 124706. S.L.J.
- Maciel, T.M.S., Alves, M.C., Silva, F.C., 2015. Atributos químicos da solução e do solo após aplicação de resíduo da extração de celulose. Rev. Bras. Eng. Agrícola Ambient. 19 (1), 84–90.
- Medeiros, J.C., Albuquerque, J.A., Mafra, A.L., Batistella, F., Grah, J., 2009. Calagem superficial com resíduo alcalino da indústria de papel e celulose em um solo altamente tamponado. Rev. Bras. Ciência do Solo 33 (6), 1657–1665.
- Natal-da-Luz, T., Ojeda, G., Pratas, J., VAN Gestel, C.A.M., Sousa, J.P., 2011. Toxicity to *Eisenia andrei* and *Folsomia candida* of a metal mixture applied to soil directly or via an organic matrix. Ecotoxicol. Environ. Saf. 74 (6), 1715–1720.
- Natal-da-Luz, T., Ribeiro, R., Sousa, J.P., 2004. Avoidance tests with Collembola and earthworms as early screening tools for site-specific assessment of polluted soils. Environ. Toxicol. Chem. 23 (9), 2188.
- Nurmesniemi, H., Pöytkö, R., Perämäki, P., Kuokkanen, T., 2005. The use of a sequential leaching procedure for heavy metal fractionation in green liquor dregs from a causticizing process at a pulp mill. Chemosphere 61 (10), 1475–1484.
- Owojori, O.J., Reinecke, A.J., 2009. Avoidance behaviour of two eco-physiologically different earthworms (*Eisenia fetida* and *Aporrectodea caliginosa*) in natural and artificial saline soils. Chemosphere, [S.L.] 75 (3), 279–283.
- Renaud, M., Chelinho, S., Alvarenga, P., Mourinha, C., Palma, P., Sousa, J.P., Natal-da-Luz, T., 2017. Organic wastes as soil amendments – effects assessment towards soil invertebrates. J. Hazard Mater. 330, 149–156.
- Sá, C.S.B., Shiosaki, R.K., Santos, A.M., Campos, M.A.S., 2021. Salinization causes abrupt reduction in soil nematode abundance in the Caatinga area of the Submedio San Francisco Valley, Brazilian semiarid region. Pedobiologia 85–86, 150729.
- Santos, H.G., Jacomine, P.K.T., Anjos, L.H.C., Oliveira, V.A., Lumberas, J.F., Coelho, M. R., Almeida, J.A., Araujo Filho, J.C., Oliveira, J.B., Cunha, T.J.F., 2018. Sistema Brasileiro de Classificação de Solos. Embrapa, Brasília.
- Segat, J.C., Sousa, J.P., Baretta, D., Klauber-Filho, O., 2020. Increasing level of liquid pig manure reduces *Eisenia andrei* and *Enchytraeus crypticus* reproduction in subtropical soils. Sci. Rep. 10 (1).
- Song, P., Ping, L., Gao, J., Li, X., Zhu, M., Wang, J., 2018. Ecotoxicological effects of fertilizers made from pulping waste liquor on earthworm *Eisenia fetida*. Ecotoxicol. Environ. Saf. 166, 237–241.
- StatSoft, I., 2004. STATISTICA (Data Analysis Software System).
- Sundari, S., Kanakarni, M.S.P., 2001. The effect of pulp unit effluent on agriculture. J. Ind. Pollut. Control 17 (1), 83–97.
- Tedesco, M.J., Gianello, C., Bissani, C.A., Bohnen, H., Volkweiss, S.J., 1995. Análise de Solo, Plantas e Outros Materiais. 2. Porto Alegre: UFRGS, p. 174.
- Toledo, F.H.S.F., Venturin, N., Carlos, L., Dias, B.A.S., Venturin, R.P., Macedo, R.L.G., 2015. Composto de resíduos da fabricação de papel e celulose na produção de mudas de eucalipto. Rev. Bras. Eng. Agrícola Ambient. 19 (7), 711–716.
- USEPA, 1999. Background report on fertilizer use, contaminants and regulations.
- USEPA 3050B, 1996. Acid Digestion of Sludges Solids and Soil.
- van Gestel, C.A.M., Breemen, E.M. Dirven-Van, Baerselman, R., 1993. Accumulation and elimination of cadmium, chromium and zinc and effects on growth and reproduction in *Eisenia andrei* (Oligochaeta, Annelida). Sci. Total Environ. 134, 585–597.
- Wan, Y., Liu, J., Zhuang, Z., Wang, Q., Li, H., 2024. Heavy Metals in Agricultural Soils: sources, influencing factors, and remediation strategies. Toxics, [S.L.] 12 (1), 63.
- Zhang, L., Verweij, R.A., van Gestel, C.A.M., 2019. Effect of soil properties on Pb bioavailability and toxicity to the soil invertebrate *Enchytraeus crypticus*. Chemosphere 217, 9–17.
- Zhou, Z., Zhang, C., Xi, M., Ma, H., Jia, H., 2023. Multi-scale modeling of natural organic matter-heavy metal cations interactions: aggregation and stabilization mechanisms. Water Res. 238, 120007. S.L.J.



Copper prevention values in Brazilian subtropical soils

Thiago Ramos Freitas^a, Rafaela Alves dos Santos Peron^a, Luis Carlos Iuñes de Oliveira Filho^a,
Mari Lucia Campos^a, Osmar Klauberg-Filho^a, Dilmar Baretta^{b,*}

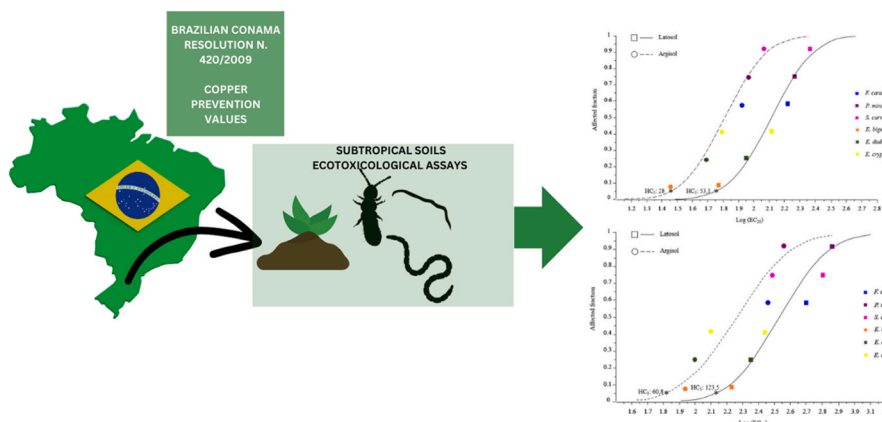
^a Santa Catarina State University, Center for Agroveterinary Sciences, Department of Soils and Natural Resources, Lages, Santa Catarina, Brazil

^b Santa Catarina State University, Center for Higher Education of the West, Department of Animal Science, Chapecó, Santa Catarina, Brazil

HIGHLIGHTS

- The Brazilian National Environmental Council Resolution 420/2009 establishes soil quality guideline values for copper.
- Soil organisms are vital to the maintenance of the soil health.
- Copper contamination was capable of affect the reproduction rates of soils organisms.
- The toxicity of copper varies depending on the chemical characteristics of the soil.
- The Brazilian Prevention Value adopted for copper is suitable for protecting the organisms we evaluated.

GRAPHICAL ABSTRACT



ARTICLE INFO

Handling editor: Keith Maruya

Keywords:

Soil ecotoxicology
Soil screening values
Enchytraeids
Springtails
Effect concentrations

ABSTRACT

The Brazilian National Environmental Council (CONAMA) Resolution 420/2009 establishes soil quality guideline values for copper, as the prevention value (PV). It defines the maximum concentrations of chemical substances permitted in the soil. Thus, the aim of the present research was to evaluate the effectiveness of the copper PV adopted at the national level by conducting exposure assays with enchytraeids (*Enchytraeus crypticus*, *Enchytraeus bigeminus* and *Enchytraeus dudichi*) and springtails (*Folsomia candida*, *Proisotoma minuta* and *Sinella curviseta*) in two Brazilian subtropical soils: a Latosol and an Argisol. Results revealed that copper reduces the reproductive capacity of all organisms as its concentration increases in the evaluated soils. Argisol presented the lowest effective concentration of 20% (EC₂₀) and 50% (EC₅₀) in species reproduction for all organisms evaluated. The most sensitive organism was the enchytraeid *E. bigeminus*, with EC₂₀ values of 58 mg kg⁻¹ in Latosol and 30 mg kg⁻¹ in Argisol, and EC₅₀ values of 155 mg kg⁻¹ in Latosol and 91 mg kg⁻¹ in Argisol. Among the springtails, *S. curviseta* exhibited the highest EC₂₀, with values of 230 mg kg⁻¹ in Latosol and 136 mg kg⁻¹ in Argisol. Conversely, the highest values of EC₅₀ were recorded for the species *P. minuta* (526 mg kg⁻¹ in Latosol and 415 mg kg⁻¹ in Argisol). Based on the species sensitivity distribution (SSD) approach, the PVs calculated were 172 mg kg⁻¹ for Latosol and 106 mg kg⁻¹ for Argisol, derived from EC₅₀ data. For EC₂₀ data, the PV values calculated

* Corresponding author. Department of Animal Science, Santa Catarina State University, Chapecó, SC, 89815-630, Brazil.

E-mail address: dilmar.baretta@udesc.br (D. Baretta).

<https://doi.org/10.1016/j.chemosphere.2025.144079>

Received 18 October 2024; Received in revised form 30 December 2024; Accepted 3 January 2025

Available online 10 January 2025

0045-6535/© 2025 Elsevier Ltd. All rights are reserved, including those for text and data mining, AI training, and similar technologies.

were 158 mg kg⁻¹ in Latosol and 100 mg kg⁻¹ in Argisol. By comparing the PV with the Brazilian adopted PV for copper (60 mg kg⁻¹), it was observed the efficacy of the CONAMA Resolution.

1. Introduction

The accumulation of metals in soil due to anthropogenic activities poses significant risks to both human health and the environment. These risks arise via exposure through food consumption, skin contact, and inhalation of contaminated particles (Nriagu and Pacyna, 1988; Hooda, 2010). While some metals are essential for biological processes, their excessive concentration can cause harmful effects, disrupting ecosystems and increasing health and environmental risks (Cannas et al., 2020; Poggere et al., 2023). Recognizing these risks and understanding the complex interactions of metals in soil systems is essential for implementing more effective environmental management strategies (Ahmad et al., 2021).

Copper is of particular concern due to its widespread use in agriculture and other human activities, such as the application of animal waste as fertilizer, pesticide application, mining, and fossil fuel combustion (Xiong et al., 2010; Segat et al., 2019; Pesce et al., 2024; Cassella et al., 2007). Its low mobility in soil results in accumulation in surface layers, particularly in organic matter, severely impacting soil biodiversity and disrupting ecosystem services (Strawn and Baker, 2007; Kabata-Pendias, 2010; Sereni et al., 2022).

In Brazil, the National Environmental Council (CONAMA) Resolution 420/2009 sets Prevention Values (PVs) for metals in soil, aimed at protecting soil-dwelling organisms and identifying areas at risk of contamination. The concept of pollution aligns closely with the PV definition, which refers to the introduction of substances at levels capable of harming soil health and ecosystem integrity (Brasil, 2009). This overlap underscores the importance of PVs as preventive tools to mitigate the impacts of contamination. However, the PVs established in CONAMA Resolution 420/2009 are based solely on phytotoxicity tests and fail to account for the diversity of soil types in Brazil, which vary in factors such as texture, composition, and pH – each of which influences the behavior and toxicity of copper (Alloway, 1995; Messias et al., 2023).

As environmental services also result from the functions performed by soil fauna, the PVs should consider the toxic effects of copper on soil animals, rather than relying solely on phytotoxicity data. It is important to note that soil fauna plays a crucial role in maintaining ecosystem functionality, with activities linked to nutrient cycling and organic matter decomposition (Decaëns et al., 2006; Briones, 2018; Hedénec et al., 2022). These functions are vital for sustaining agricultural productivity and ensuring soil health. Thus, by protecting soil biodiversity, we also safeguard human food security.

In this context, several soil fauna bioindicators have been extensively utilized in ecotoxicological assessments, particularly in countries with well-established frameworks to the establishment of soil guiding values, such as those within the European Union (ECHA, 2008), the United Kingdom (Environment Agency, 2022), and the United States (US EPA, 2005). The species recommended for the ecotoxicological assessment are described in standards from the International Organization for Standardization (ISO), which include oligochaetes and arthropods, such as enchytraeids and springtails (ISO, 2014a, 2014b). Additionally, the European Food Safety Authority (EFSA, 2017) recommends the use of alternative non-standardized species to enhance the accuracy of toxicity result interpretations.

Although ecotoxicological assays allow for the establishment of metrics to assess the effects of environmental contamination on several bioindicators, the PVs can only be obtained by considering ecological aspects and employing statistical validation and extrapolation procedures (ECHA, 2008). One plausible approach in this case is the construction of species sensitivity distributions (SSDs) by considering the set

of responses to contamination and the subsequent calculation of hazardous concentrations (HC_x). These HC_x represent a percentage level of protection for the evaluated species, generally relative to the lower fifth percentile (HC₅).

Therefore, the aim of the present research was to evaluate the effectiveness of the copper PV adopted at Brazilian national level and to propose a more suitable PV by conducting exposure assays with soil fauna bioindicators in two Brazilian subtropical soils: a Latosol and an Argisol. To this end, the effects of contamination were assessed on the reproduction of the enchytraeid species *Enchytraeus crypticus*, *Enchytraeus bigeminus*, and *Enchytraeus dudichi*, as well as on the springtail species *Folsomia candida*, *Proisotoma minuta*, and *Sinella curviseta*. The hypotheses evaluated were that (i) the effects of contamination vary according to the chemical characteristics of the evaluated soils, and (ii) the copper PV established in CONAMA Resolution 420/2009 does not provide adequate protection for the assessed organisms.

2. Materials and methods

2.1. Soils sampling and characterization

Two natural soils were used in the ecotoxicological assessments: a dystrophic Red-Yellow Argisol (native forest: 28°23'24.0"S, 49°22'6.9"W) developed from the weathering of sedimentary rocks (sandstone and siltstone), and a dystrophic Red Latossol (native forest: 27°22'33.6"S, 51°05'27.6"W) derived from basaltic rocks. The soils were selected due to their contrasting clay contents. The samples were collected in the surface layer (0–0.2 m) in areas under natural vegetation, air-dried, sieved through a 2 mm mesh, manually homogenized, and maintained at room temperature until the assays were conducted.

The chemical and textural attributes of the soils (Table 1) were obtained from the assessment of a composite sample (n = 3) using the methods described in the Soil Analysis Methods Manual of the Brazilian Agricultural Research Corporation (Teixeira et al., 2017), while the total concentrations of copper were determined using method 3050B (US EPA, 2007).

2.2. Test organisms and experimental procedures

The springtail and enchytraeid cultures were maintained at a temperature of 20 ± 2 °C with a photoperiod of 16 h of light, in accordance with the recommendations of ISO standards for chronic assays (ISO,

Table 1
Chemical and physical properties of the Argisol and Latosol.

Soil properties	Latosol	Argisol
pH in water	5.53	5.32
pH in KCl	4.34	4.07
Ca (cmol _c kg ⁻¹)	9.16	0.15
Mg (cmol _c kg ⁻¹)	2.44	0.29
K (cmol _c kg ⁻¹)	1.04	0.09
Na (cmol _c kg ⁻¹)	0.23	0.25
Al (cmol _c kg ⁻¹)	4.69	8.84
H + Al (cmol _c kg ⁻¹)	9.97	5.55
CEC ^a (cmol _c kg ⁻¹)	22.84	6.33
P (mg kg ⁻¹)	6.14	3.56
TOC (g kg ⁻¹)	18.9	9.05
Cu (mg kg ⁻¹)	155	8
Sand (g kg ⁻¹)	39	649
Clay (g kg ⁻¹)	621	201
Silt (g kg ⁻¹)	340	150

^a Cation Exchange Capacity at pH 7.

2014a; ISO, 2014b). The substrate for culturing the enchytraeids was a tropical artificial soil (TAS), consisting of a mixture of 75% fine industrial sand, 20% kaolinitic clay, and 5% coconut coir dust (Garcia et al., 2004). The pH of the TAS was adjusted to 6.0 ± 0.5 by adding CaCO_3 . The springtails were cultured in a substrate composed of a mixture of plaster, distilled water and activated charcoal in a ratio of 11:7:1. The enchytraeids were fed twice a week with properly autoclaved fine oat flakes to prevent contamination of the cultures by mites or other external agents. Meanwhile, the springtails received about 20 mg of dry yeast as food. Only healthy adult individuals, exhibiting normal behavior and no visible signs of damage to their morphological structures, were selected for the ecotoxicological assays.

Reproduction assessments were conducted following the methodologies outlined in ISO standards for chronic testing with springtails and enchytraeids (ISO, 2014a; ISO, 2014b). Soils were contaminated with increasing concentrations of copper, distinct for each evaluated faunal group (Table 2), through the addition of saline solutions prepared with deionized water and CuCl_2 . The doses were chosen based on the PV (60 mg kg^{-1} ; Brasil, 2009) and literature data (Posthuma et al., 1997; Lock and Janssen, 2002; Amorim and Scott-Fordsmand, 2012; Messias et al., 2023). Dilutions for each dose were prepared using volumetric flasks, corresponding to approximately 50% (g/g dry soil) of the water holding capacity (WHC) of each soil, and the mixtures (soil + solution) were manually homogenized in plastic bags. Control treatments included the addition of only deionized water until 50% of the WHC of the soils was reached. The contamination procedure was conducted individually for each assessment based on the bioindicator species and soil type.

The plastic bags containing the contaminated soils and controls were sealed and kept closed for 7 days to allow chemical equilibrium of the contaminant with the solution. This adaptation of the methodology was proposed by Fendorf et al. (2004).

The assays with enchytraeids involved adding approximately 20 g of dry soil weight into cylindrical glass containers (5 cm in diameter and 8 cm in height) with perforated lids, to which approximately 10 mg of fine oats were also added weekly to ensure that the organisms' feeding was not interrupted. Ten individuals were selected according to specific criteria for each species, namely, only adults of *E. crypticus* with a well-defined clitellum (ISO, 2014b); and adults individuals of the species *E. dudichi* and *E. bigeminus*, which are non-clitellated, with a length ranging from 0.8 to 12 mm (Bandow et al., 2013). To meet the validation criteria for the assays, all treatments included five replicates ($n = 5$). Soil moisture was adjusted weekly by adding deionized water, while the photoperiod and temperature were maintained under the same cultivation conditions (16 h of light; $20^\circ\text{C} \pm 2$). At the end of the 21-day experimental period, 5 mL of absolute alcohol (96% ethanol) was added to preserve the samples, along with 1 mL of rose bengal dye solution (1% w/v) and approximately 50 mL of distilled water. After a period of 72 h the samples were washed on a 125 μm mesh sieve and placed in trays for the subsequent counting of the organisms.

The assays with springtails were conducted by adding 30 g of dry soil mass into cylindrical plastic containers (7.5 cm in diameter and 5 cm in height) with properly perforated lids to allow air exchange with the external environment. The organisms were fed weekly with approximately 10 mg of granulated yeast, and moisture was maintained at about 50% of the WHC. For the evaluations with the species *F. candida* and

P. minuta, 10 individuals aged between 10 and 12 days were added to each experimental unit. For the species *S. curviseta*, 20 individuals were inoculated into the experimental units, following the adaptation proposed by Bandow et al. (2013). The assays lasted for 28 days, and at the end of the experimental period, distilled water and dark stamp ink were added to the experimental units for the assays with *F. candida*. Although the same procedure was followed for *P. minuta* and *S. curviseta*, no dye was added at the end of the assays, as the exoskeleton of these species has some coloration. Subsequently, photographs of the experimental units were taken, and the organisms (adults and juveniles) were identified using ImageJ 1.54d software (Schneider et al., 2012). All treatments included five replicates ($n = 5$).

2.3. Data analysis

The ecotoxicological data were subjected to the Shapiro-Wilk normality test ($p > 0.05$) and Bartlett's homogeneity test ($p > 0.05$). Means were compared using Dunnett's test ($p \leq 0.05$), and outliers were identified and removed from the statistical evaluation using Grubbs' test ($p \leq 0.05$). Data was then submitted to a non-linear regression analysis, employing exponential, logistic or Gompertz models (Environment Canada, 2007), to determine the copper doses corresponding to a 20% and 50% effect on species reproduction (EC_{20} and EC_{50}). The EC values were determined using the model that best fitted the data. The results of EC_{20} and EC_{50} were subjected to the Behrens-Fisher significance test ($p \leq 0.05$) (generalized likelihood ratio test) to determine whether there were differences in EC values among the evaluated soils. All analyses were carried out using STATISTICA 10.0 (Statsoft Inc., 2011).

2.4. SSDs and copper prevention values

Using ETX 2.0 software (Van Vlaardingen et al., 2004) the EC_{20} and EC_{50} were grouped, individually, into a log-normal distribution to construct the SSD with a 95% confidence interval. The log-normality of the data was evaluated using the Anderson-Darling ($p > 0.01$) and the Kolmogorov-Smirnov ($p > 0.01$) tests.

It is important to note that the SSD are mathematical models grounded in statistical distributions, and as such, the quantity and the quality of data is a significant variable in establishing more feasible protection levels (Messias et al., 2023). According to Del Signore et al. (2016), the European Union recommends using a minimum of five species for the construction of an SSD.

As a result, the predicted no-effect concentrations (PNECs) were determined using a restrictive assessment factor (AF) of 5 (Environment Canada, 2007). The following equation describe how the PNEC were calculated.

$$\text{PNEC} = \text{HC}_5/\text{AF}$$

Finally, the Quality Reference Values (QRVs), corresponding to the natural copper contents in the soils, were added to the PNEC to obtain the PVs. This procedure is crucial, as the calculation of PVs is based on the added risk concept, which refers to the concentration of the element that exceeds the natural background level and poses a potential risk to the dynamics of the assessed species.

QRVs were established by the local environmental agency (IMA, 2021) through normalization of chemical data across a wide range of soils. The concentrations established are $146.92 \text{ mg kg}^{-1}$ for soils with clay content equal to or greater than 59%, and 93.84 mg kg^{-1} for soils with clay content below 59%. The following equation outlines how we calculated the PVs for copper.

$$\text{PV} = \text{QRV} + \text{PNEC}$$

Table 2

Tested nominal concentrations of copper for each evaluated species.

Test species	Copper nominal concentrations (mg kg^{-1})
<i>E. crypticus</i>	0, 25, 50, 75, 150, 300 and 600
<i>E. bigeminus</i>	
<i>E. dudichi</i>	
<i>F. candida</i>	0, 60, 120, 240, 480, 720 and 1080
<i>P. minuta</i>	
<i>S. curviseta</i>	

3. Results

3.1. Ecotoxicological assays

The average mortality in the control treatments was below 20% for all assessments performed. The average number of juveniles exceeded 25 and 100 individuals for the tests with enchytraeids and springtails, respectively, with a coefficient of variation (CV) not exceeding 30% (Table S1). Thus, all validation criteria outlined in the ecotoxicological test standardization guidelines for springtails and enchytraeids were met (ISO, 2014a; ISO, 2014b).

For tests with the fragmenting species (*E. bigeminus* and *E. dudichi*), where adults could not be identified at the end of the experimental period, the validation criteria were consistent with those reported by Bandow et al. (2013).

Springtail reproduction was affected by copper ($p \leq 0.05$) in both evaluated soils (Fig. 1). A reduction in juveniles of *F. candida* was observed at doses of 120 mg kg⁻¹ in the Argisol and 60 mg kg⁻¹ in the Latosol. For the species *P. minuta* and *S. curviseta*, the observed effects occurred at a dose of 120 mg kg⁻¹ in both soils. Overall, the most pronounced contamination effects for all evaluated springtail species were observed in the Argisol, where the EC₂₀ and EC₅₀ values were lower than those observed in the Latosol (Table 3).

Regarding the sensitivity of springtails, based on the EC₂₀ results presented here for both evaluated soils, it can be inferred that the species most affected by contamination was *F. candida*, followed by *P. minuta* and *curviseta*, respectively. However, when determining the EC₅₀, a different outcome emerged; that is, the organisms showed a reduction in

Table 3

Ecotoxicological endpoints (mg kg⁻¹) for each evaluated species in Latosol and Argisol. Parentheses indicate the confidence interval (lower and upper limits) ($p \leq 0.05$).

Test Species	Latosol	Argisol
EC₂₀		
<i>F. candida</i>	163 (114–212) ^e	95 (54–137) ^{lo}
<i>P. minuta</i>	180 (123–237) ^{lo}	107 (42–171) ^{lo}
<i>S. curviseta</i>	230 (127–334) ^g	136 (87–187) ^g
<i>E. bigeminus</i> ^a	58 (36–80) ^g	30 (16–44) ^g
<i>E. dudichi</i> ^a	89 (62–116) ^g	53 (42–65) ^{lo}
<i>E. crypticus</i> ^a	127 (93–161) ^g	67 (47–87) ^g
EC₅₀		
<i>F. candida</i>	385 (304–466) ^e	333 (255–410) ^{lo}
<i>P. minuta</i>	526 (445–606) ^{lo}	415 (288–542) ^{lo}
<i>S. curviseta</i>	470 (371–569) ^g	350 (289–410) ^g
<i>E. bigeminus</i> ^a	155 (126–184) ^g	91 (70–112) ^g
<i>E. dudichi</i> ^a	198 (169–227) ^g	105 (91–119) ^{lo}
<i>E. crypticus</i> ^a	232 (203–261) ^g	136 (116–157) ^g

^a Reflect significant differences (calculated using the Behrens-Fisher generalized likelihood ratio test ($p \leq 0.05$)) between the EC obtained in different soils for the same species. Superscript letters indicate the regression model used: e for exponential; lo for logistic; and g for Gompertz.

reproduction in the following order: *F. candida* > *S. curviseta* > *P. minuta*.

All species of enchytraeids were also affected ($p \leq 0.05$) by soil contamination with copper (Fig. 2). The recorded effects began at a dose of 75 mg kg⁻¹ for the species *E. crypticus* in both soils. For the species *E. bigeminus*, a significant reduction was observed at doses of 50 mg kg⁻¹ in the Argisol and 75 mg kg⁻¹ in the Latosol. The impacts on the

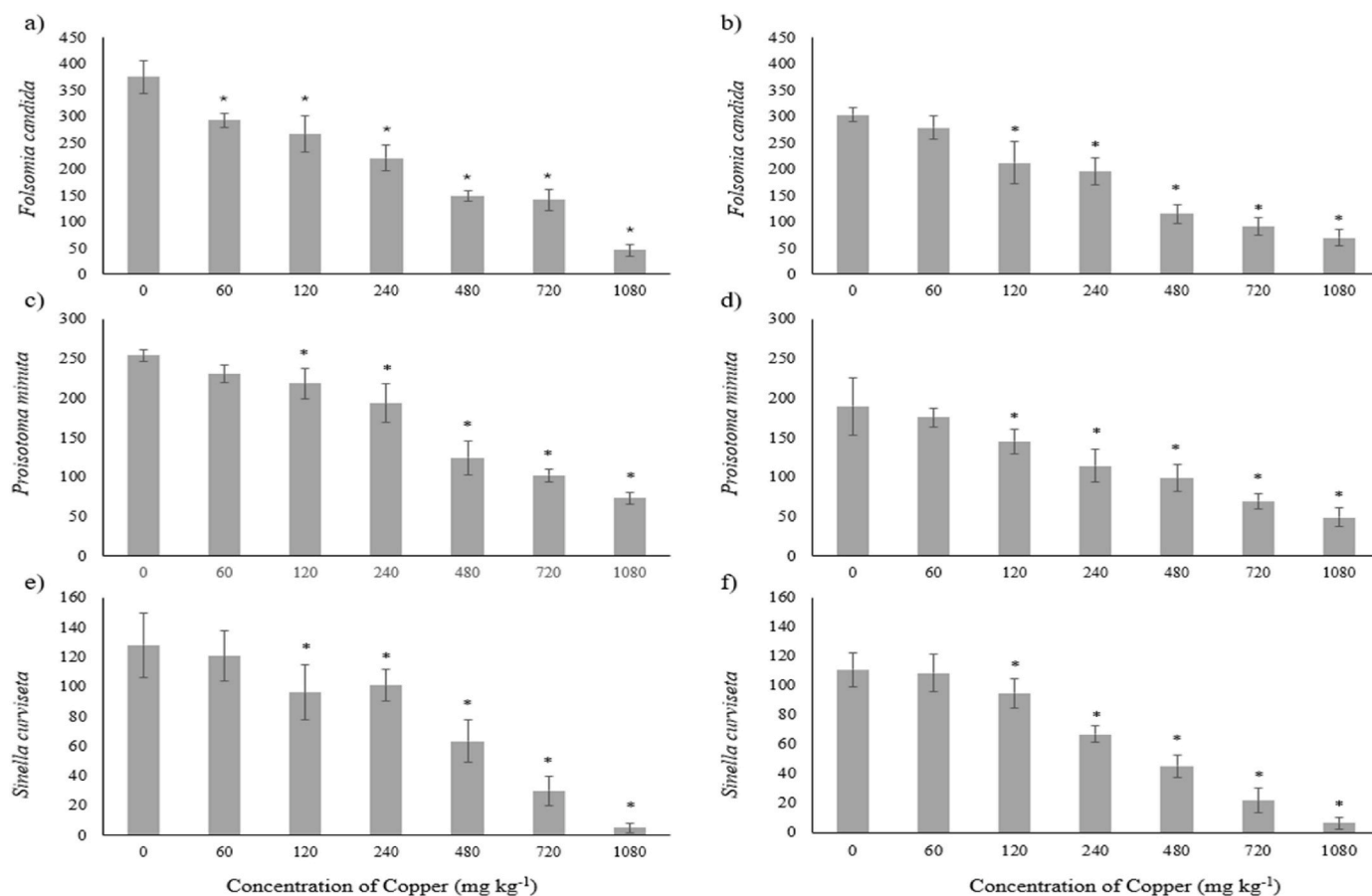


Fig. 1. Average number of juvenile of *Folsomia candida*, *Proisotoma minuta*, and *Sinella curviseta* in Latosol (a, c, e) and in Argisol (b, d, f) contaminated with different copper concentrations.

* Indicate statistically significant difference ($p \leq 0.05$) by the Dunnett test. (&boxhd) Indicates the standard deviation. Concentration 0 represents control treatment (free from contamination).

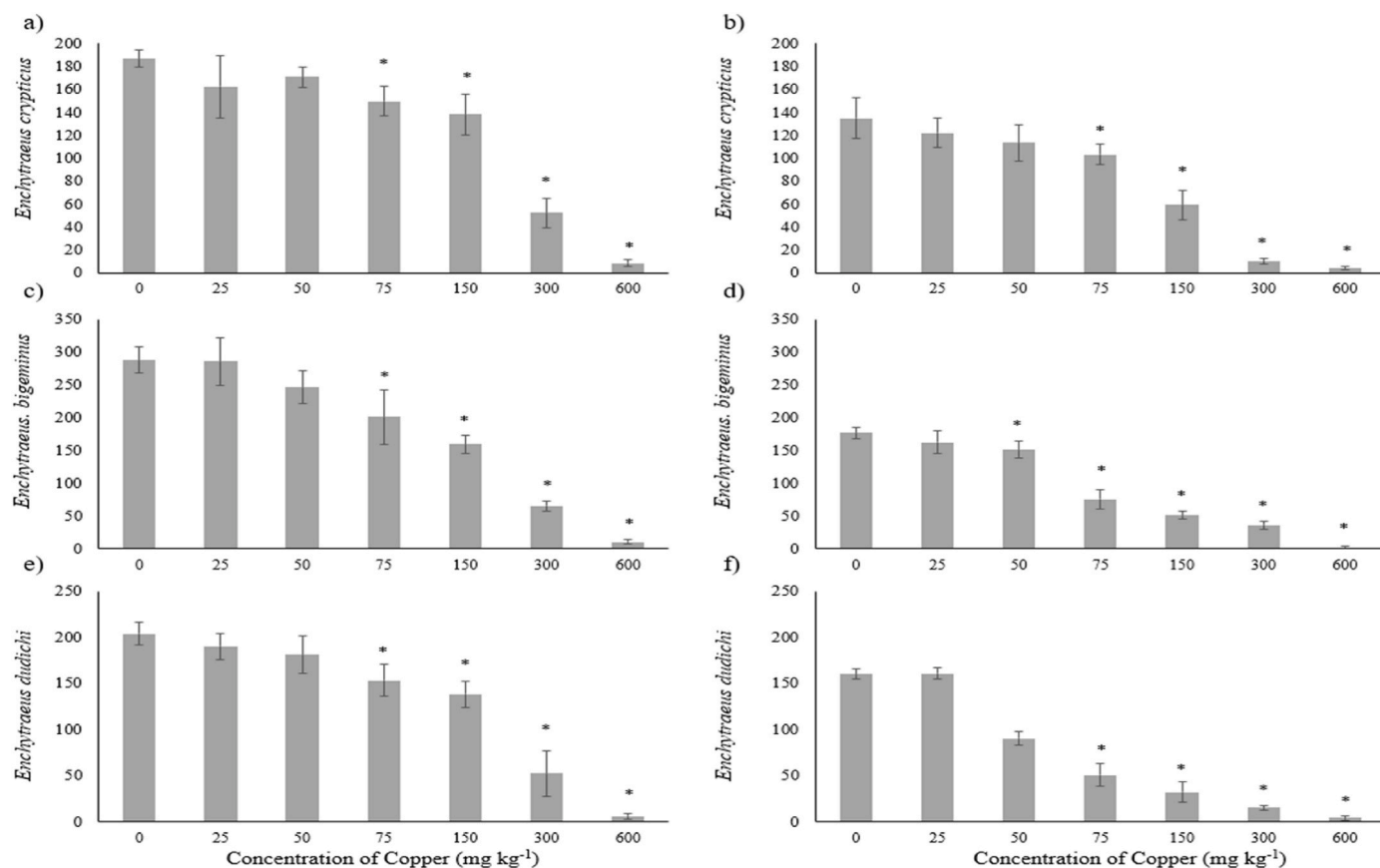


Fig. 2. Average number of juvenile of *Enchytraeus crypticus*, *Enchytraeus bigeminus*, and *Enchytraeus dudichi* in Latosol (a, c, e) and in Argisol (b, d, f) contaminated with different copper concentrations.

* Indicate statistically significant difference ($p \leq 0.05$) by the Dunnett test. (—) Indicates the standard deviation. Concentration 0 represents control treatment (free from contamination).

reproduction of the species *E. dudichi* were observed, for both evaluated soils, starting at a dose of 75 mg kg⁻¹. As with the springtails, the most pronounced effects for the enchytraeids were also observed in the Argisol, since the EC₂₀ and EC₅₀ values were lower than those found in the Latosol. Regarding the enchytraeids sensitivity, the fragmenting species *E. bigeminus* was the most severely impacted by contamination in both evaluated soils, followed by *E. dudichi* and *E. crypticus*. This trend was observed in both the EC₂₀ and EC₅₀ results.

It was found through the Behrens-Fisher statistical test ($p \leq 0.05$) that the enchytraeids were more sensitive to contamination in the Argisol. Springtails exhibited a similar pattern to enchytraeids, however, no significant differences in sensitivity were observed between the assessed soils (Table 3).

The SSDs derived based on the EC₂₀ and EC₅₀ for all evaluated species in both Latosol and Argisol are presented in fFigures S1 and S2, respectively. The PV are presented in Table 4.

Table 4

HC₅, PNEC and PV (mg kg⁻¹) as function of the AF (=5) and the ecotoxicological endpoint for Latosol and Argisol.

Soil	Ecotoxicological endpoint	HC ₅	PNEC	PV
Latosol	EC ₂₀	53	11 (4–17)	158
	EC ₅₀	123	25 (9–38)	172
Argisol	EC ₂₀	28	6 (2–9)	100
	EC ₅₀	61	12 (3–22)	106

QRV for copper is 146.9 mg kg⁻¹ for soils with clay content equal to or greater than 59% (such as the Latosol) and 93.8 mg kg⁻¹ for soils with clay content less than 59% (such as the Argisol) (IMA, 2021). In parentheses, the lower and upper limits of the confidence interval for the PNEC value.

4. Discussion

The assessments revealed a significant reduction in the reproductive capacity of all organisms evaluated due to copper contamination. Among them, enchytraeids, particularly the fragmenting species *E. bigeminus* and *E. dudichi*, proved to be the most sensitive. Although these species are not standardized, ISO guidelines (ISO, 2014b) recommend the genus *Enchytraeus* for ecotoxicological evaluations. This recommendation is supported by the fact that organisms capable of asexual reproduction, like some species of the genus *Enchytraeus*, offer an advantage in ecotoxicological studies due to their genetic uniformity, which leads to more consistent and precise contamination responses (Weltje, 2003; Bandow et al., 2013). However, it is important to note that while genetic uniformity can reduce variability within laboratory strains, the sensitivities of asexually reproducing species may vary significantly in the field and across geographic regions (Fisker et al., 2011).

In addition to the higher sensitivity of fragmenting species, it is noteworthy that the effect concentrations estimated in this study (i.e., EC₅₀ and EC₂₀) were significantly lower than those reported by other researchers in evaluations conducted with temperate climate soils. For instance, Amorim and Scott-Fordsmand (2012) determined an EC₅₀ value of 251 mg kg⁻¹ and an EC₂₀ value of 137 mg kg⁻¹ while studying the effects of CuCl₂ contamination on the reproductive capacity of *E. albidus*. Similarly, Maraldo et al. (2009) reported an EC₅₀ value of 341 mg kg⁻¹ in their study on copper contamination in Danish soils and its impact on the reproductive capacity of *E. crypticus*. These discrepancies between the results presented here and those found in the literature can be attributed to differences in soil properties (Kabata-Pendias, 2010). In

this context, Bandow et al. (2013) suggest that the fragmenting species *E. bigeminus* is more suitable for evaluations in warm-climate soils, thus justifying its use as a bioindicator in ecotoxicological testing.

Enchytraeids exhibited greater sensitivity in assessments conducted in Argisol, likely due to the higher bioavailability of copper. According to Amorim et al. (2005), soils with low organic matter and clay content tend to create a more toxic environment for enchytraeids when exposed to cationic contaminants. Supporting this observation, Oliveira et al., (2024) identified more pronounced effects in subtropical soils with lower pH and organic matter contents. Similarly, although the evaluated Latosol exhibited a natural copper content eighteen times higher than that in Argisol, this characteristic does not appear to have directly influenced the toxicity to enchytraeids when comparing the effects between the soils. This may be attributed to the stability of copper within the structure of the clay minerals and the greater adsorptive capacity of Latosol (Tiecher et al., 2013).

The sensitivity of springtail species to contamination effects on reproduction was consistent in both Latosol and Argisol assessments. *F. candida* had the lowest EC₅₀ value, followed by *S. curviseta* and *P. minuta*. Although only *F. candida* is recommended for ecotoxicological assessments according to ISO 11267 (ISO, 2014a), the Organisation for Economic Co-operation and Development (OECD) advocates for the inclusion of *P. minuta* and *S. curviseta* in ecotoxicological evaluations (OECD, 2016).

Therefore, it was observed that springtails were the organisms exhibiting greater resistance to the harmful effects of the contaminant. Renaud et al. (2020) found that the toxicity of trace elements in *F. candida* is directly related to the solubility of cations in the soil. However, although the bioavailability of copper may be higher in Argisol, no significant differences were observed between the soils when comparing the EC₂₀ and EC₅₀ values. Sandifer and Hopkin (1996) argue that there is no clear correlation between increased toxicity and lower pH values when assessments are conducted with this species. As a result, by comparing the effects between the two soil types, we can partially confirm the first hypothesis, as enchytraeids exhibited distinct and significant effects between the evaluated soils, while springtails did not.

In comparison to the results presented here for *F. candida*, Sandifer and Hopkin (1996) reported an EC₅₀ value of 700 mg kg⁻¹ in artificial soil. Similarly, Amorim et al. (2005) determined an EC₅₀ of 794 mg kg⁻¹ in assessments involving European soils. For the species *P. minuta*, Nursita et al. (2005) obtained an EC₅₀ of 696 mg kg⁻¹ in evaluations conducted with Australian soils. Lastly, concerning the results for the springtail *S. curviseta*, Xu et al. (2009) identified an EC₅₀ value of 442 mg kg⁻¹ in Chinese soil. All these findings diverge from the results presented here for subtropical soil assessments. Variations in effect concentrations recorded among organisms of the same species arise from the influence of soil chemical attributes on contaminant dynamics. Consequently, it is not reasonable to compare the effects between soils from different countries for legal purposes. According to Marques et al. (2022), the primary factor influencing copper bioavailability in soil is the parent material. Soils derived from basalt, such as the Latosol evaluated in this study, exhibit a greater capacity to retain cationic elements compared to soils formed from sandstones.

Although no significant differences were observed in the reproductive rates of springtails among the evaluated soils, it is essential to highlight that soils with lower organic matter content are highly susceptible to copper contamination due to the element's strong affinity for the organic fraction (Kabata-Pendias, 2010). This suggests that in regions where such soils predominate, extra care should be taken regarding organic fertilization and the prolonged use of copper-based pesticides. Frequent applications may lead to the saturation of functional groups and subsequent interactions with lower-energy adsorption sites, thereby increasing the concentration of this element in the soil solution (Couto et al., 2014; Biasi et al., 2024). Furthermore, the formation of chelates with soluble organic ligands tends to enhance copper mobility within the soil profile, resulting in a higher likelihood of

groundwater contamination, particularly in sandy and well-drained soils (Marques et al., 2022).

When determining the PNEC and the corresponding PV, it is important to highlight two possible approaches: a deterministic, which derives the guiding value based on effect concentrations or on the no observed effect concentrations of the most sensitive organism to the contaminant; and a stochastic, which aims to determine a probabilistic concentration of concern, typically associated with the fifth percentile of the ecotoxicological response, as we have implemented (ECHA, 2008). Among the various factors influencing the choice between these two approaches, the quantity and quality of data play a crucial role. For example, the probabilistic approach requires data from at least five species, although a larger dataset is preferable to reduce uncertainties in data extrapolation (Posthuma et al., 2001; Del Signore et al., 2016; Environment Canada, 2007).

Regardless of the chosen approach, both methods link the PNEC to an AF. The selection of this factor should specifically consider (i) the quality of the data and the endpoints addressed, (ii) the diversity and representativeness of the evaluated taxonomic groups, and (iii) the availability of field assessment data or studies conducted in simulated ecosystems (ECHA, 2008; Environment Agency, 2022). However, guidance on selecting the most appropriate AF is limited to these general points. It is noteworthy that in the risk assessment of trace elements, employing an AF greater than 10 often yields values that fall below the geochemical background, as reported by Scott-Fordsmand et al. (1996). In such instances, the data become practically meaningless and cannot be utilized for legal purposes. This is one of several reasons why the Canadian Environmental Agency recommends that the AF should not exceed a value of 5 (Environment Canada, 2007). Chapman et al. (1998) emphasize that the application and selection of these AFs are matters of policy rather than purely scientific considerations, stemming from the need to establish a safety threshold, particularly for human health.

For these reasons, we suggest adopting PVs based on a more restrictive AF, as illustrated by the results (Table 4). The justification for this choice is that, while the ecotoxicological assessment has produced results capable of generating a realistic PV, it is essential to evaluate other taxonomic groups at different trophic levels that fulfill various ecosystem functions. It is important to note that a permissive PV may not accurately reflect the vulnerabilities of the ecosystem or support intervention actions according to the soil classification criteria established in Articles 13 and 20 of the CONAMA Resolution 420/2009 (Brasil, 2009). On the other hand, adopting an excessively restrictive PV, while protective, could adversely affect significant economic activities that do not cause environmental harm. Therefore, the solution lies not only in enhancing the robustness of the ecotoxicological assessment but also in fostering dialogue between regulatory policymakers and civil society.

Comparing the PVs for copper, specifically 172 mg kg⁻¹ in Latosol and 106 mg kg⁻¹ in Argisol, with the data from Oliveira et al., (2024) reveals a lower tolerance to contamination in the soils evaluated in this study. This is because the authors determined PVs for copper of 363.9 mg kg⁻¹ for a Nitisol and 151.2 mg kg⁻¹ for a Cambisol. It is important to highlight that the proposed PVs in the study were based on an AF of 1. In contrast, in our study we adopted an AF of 5; however, with a larger dataset covering additional trophic levels and ecosystem functions this assessment factor could potentially be reduced.

These differences, along with the limited number of assessments involving other soil types, pose challenges in determining a common PV for different soil types, as published for soils in the Brazilian State of São Paulo (Cetesb, 2021). Nevertheless, the United Kingdom's updated guidelines for Ecological Risk Assessment propose a common HC₅ for the same trace element, regardless of soil type. In this case, the prevailing concept of added risk suggests summing the contaminant's HC₅ with its respective geochemical background to obtain the PV (Crommentuijn et al., 2000), as we have done. However, the guideline precludes extrapolating the PV to an entire set of soils, as the method does not account for pedological influences on the contaminant's ecotoxicity

(Environment Agency, 2022).

Therefore, it is argued that it is not possible to determine a single PV that is common to the evaluated soils. To calculate a common PV, greater statistical robustness would be necessary, achievable only with a substantial amount of toxicity data on the contaminant. Furthermore, it is essential to emphasize that the inability to generate a common PV for both soils is also justified by differences in their clay content.

In regard to the comparison between the results of this study and the PVs outlined in CONAMA Resolution 420/2009, several observations are necessary. First, we decided not to suggest a PV based on EC₂₀ data due to the inability to calculate the no observed effect concentration for *F. candida* in the Latosol evaluation. Second, the aforementioned resolution does not clarify the procedure used for determining the PVs, specifically which approach was chosen. For this reason questions arise regarding the statistical methods used to validate the data. Although Article 9 of the aforementioned Resolution states that the PV was established based on phytotoxicity assays or ecological risk assessment, the data from the ecotoxicological evaluation is not published, nor the AF employed (Brasil, 2009). However, by comparison and considering the publication dates of the resolutions, it can be assumed that the national PVs were based solely on the analysis of soils from the Brazilian State of São Paulo (Cetesb, 2001).

For copper, the nationally adopted PV (60 mg kg⁻¹) is lower than the results presented here for both Latosol (172 mg kg⁻¹) and Argisol (106 mg kg⁻¹). Therefore, it can be concluded that the second hypothesis is not valid. This is because there was no evidence suggesting the need to update the PV for copper based on the findings from our ecotoxicological assessment. This same conclusion can be verified in the update of soil guidance values published by the Environmental Company of the State of São Paulo, as the regulation maintains the copper PV at 60 mg kg⁻¹ (Brasil, 2009; Cetesb, 2001). This consistency highlights the importance of ongoing assessments to ensure regulatory values align with the latest scientific findings while safeguarding environmental integrity.

5. Conclusions

Copper contamination in soil reduced the reproductive capacity of enchytraeids and springtails, with the extent of this effect varying according to soil type. The fragmenting species *E. bigeminus* was the most sensitive to the deleterious effects of copper. Among the springtails, *S. curviseta* showed the highest EC₂₀, while the highest EC₅₀ values were verified for *P. minuta*. These observations highlight the necessity of including a wide range of organisms in ecotoxicological assessments, as we observed that the effects vary even within the same biological group.

It is also important to note that all enchytraeid species were more sensitive to contamination when exposed to copper in Argisol. This sensitivity may be attributed to the lower organic matter content in this soil type. However, no differences in the sensitivity of springtails between the soils were observed. In this context, it is noteworthy that Latosol has a significantly higher copper geochemical background than Argisol; however, this characteristic does not appear to have directly influenced the measured effects.

The ecotoxicological assessment data involving six different species of soil fauna allowed for the construction of SSDs and the subsequent determination of HC₅ for both Latosol and Argisol. However, due to statistical limitations, primarily associated with uncertainties in the extrapolation of the results, the application of a restrictive AF (=5) was necessary for the PVs calculation. Consequently, when comparing the results with those outlined in CONAMA Resolution 420/2009, it was observed that the Brazilian adopted PV is sufficiently protective, as it is lower than the PV we calculated.

Finally, it is important to emphasize that a common PV for soils was not proposed due to the contrasting clay content of the soils evaluated. Therefore, it was deemed imprudent to combine the ecotoxicological assessment results for the soils into a single SSD. Moreover, assessments involving other taxonomic groups are necessary to reduce the statistical

uncertainties arising from the extrapolation of the data to the entire soil ecological community.

CRediT authorship contribution statement

Thiago Ramos Freitas: Writing – original draft, Visualization, Validation, Investigation, Formal analysis. **Rafaela Alves dos Santos Peron:** Investigation, Formal analysis. **Luís Carlos Iuñes de Oliveira Filho:** Writing – review & editing, Validation, Supervision. **Mari Lucia Campos:** Writing – review & editing, Supervision, Funding acquisition. **Osmar Klauber-Filho:** Writing – review & editing, Supervision, Funding acquisition, Conceptualization. **Dilmar Baretta:** Writing – review & editing, Supervision, Project administration, Funding acquisition, Conceptualization.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgement

This research was supported by the Brazilian Coordination for the Improvement of Higher Education Personnel (CAPES), The Brazilian National Council for Scientific and Technological Development (CNPq) (Grant Number: D.B.: 308189/2022-1) and the Santa Catarina State Research and Innovation Support Foundation (FAPESC, Grant Number: 2023TR000733).

Appendix ASupplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.chemosphere.2025.144079>.

Data availability

Data will be made available on request.

References

- Ahmad, W., Alharthy, R.D., Zubair, M., Ahmed, M., Hameed, A., Rafique, S., 2021. Toxic and heavy metals contamination assessment in soil and water to evaluate human health risk. *Sci. Rep.* 20, 17006.
- Alloway, B.J., 1995. *Heavy Metals in Soils*, 2 ed. John Wiley & Sons, New York.
- Amorim, M.J.B., Römbke, J., Schallnass, H.J., Soares, A.M., 2005. Effect of soil properties and aging on the toxicity of copper for *Enchytraeus albidus*, *Enchytraeus luxuriosus*, and *Folsomia candida*. *Environ. Toxicol. Chem.* 24, 1875–1885.
- Amorim, M.J.B., Scott-Fordsmand, J.J., 2012. Toxicity of copper nanoparticles and CuCl₂ salt to *Enchytraeus albidus* worms: survival, reproduction and avoidance responses. *Environ. Pollut.* 164, 164–168.
- Bandow, C., Coors, A., Römbke, J., 2013. *Enchytraeus bigeminus* (Enchytraeidae, Oligochaeta) as a new candidate for ecotoxicological laboratory tests. *Soil Org* 85, 103–112.
- Biasi, J.P., Freitas, T.R., Lunardi, E.O.S., Oliveira Filho, L.C.L., Campos, M.L., Klauber-Filho, O., Baretta, D., 2024. Ecotoxicity of alkaline residue from the pulp and paper industry to soil faunal organisms. *Chemosphere* 366, 143448.
- Brasil, 2009. Resolução nº 420 do Conselho Nacional de Meio Ambiente.
- Briones, M.J.I., 2018. The serendipitous value of soil fauna in ecosystem functioning: the unexplained explained. *Front. Environ. Sci.* 6.
- Cannas, D., Loi, E., Serra, M., Firinu, D., Valera, P., Zavattari, P., 2020. Relevance of Essential Trace Elements in Nutrition and Drinking Water for Human Health and Autoimmune Disease Risk Nutrients, vol. 12, p. 2074.
- Cassella, R.J., Wagener, A.L., Santelli, R.E., Wagener, K., Tavares, L.Y., 2007. Distribution of copper in the vicinity of a deactivated mining site at Carajás in the Amazon region of Brazil. *J. Hazard Mater.* 142, 543–549.
- CETESB, 2021. Decisão de Diretoria nº 125-2021-E.
- CETESB, 2001. Relatório de Estabelecimento de Valores Orientadores para Solos e Águas Subterrâneas. Estado de São Paulo.
- Chapman, P.M., Fairbrother, A., Brown, D., 1998. A critical evaluation of safety (uncertainty) factors for ecological risk assessment. *Environ. Toxicol. Chem.* 17, 99–108.

- Couto, R.R., Benedet, L., Comin, J.J., Belli Filho, P., Martins, S.R., Gatiboni, L.C., Radetski, M., Valois, C.M., Ambrosini, V.G., Brunetto, G., 2014. Accumulation of copper and zinc fractions in vineyard soil in the mid-western region of Santa Catarina, Brazil. *Environ. Earth Sci.* 73, 6379–6386.
- Crommentuijn, T., Polder, M., Sijm, D., Bruijn, J., Plassche, E., 2000. Evaluation of the Dutch environmental risk limits for metals by application of the added risk approach. *Environ. Toxicol. Chem.* 19 (6), 1692–1701.
- Decaëns, T., Jiménez, J.J., Gioia, C., Measey, G.J., Lavelle, P., 2006. The values of soil animals for conservation biology. *Eur. J. Soil Biol.* 42, 23–38.
- Del Signore, A., Hendriks, A.J., Lenders, H.J.R., Leuven, R.S.E.W., Breure, A.M., 2016. Development and application of the SSD approach in scientific case studies for ecological risk assessment. *Environ. Toxicol. Chem.* 35 (9), 2149–2161.
- ECHA, 2008. Scientific Report – SC070009/SR1. An Ecological Risk Assessment Framework for Contaminants in Soil.
- EFSA, 2017. Scientific Opinion Addressing the State of the Science on Risk Assessment of Plant Protection Products for In-Soil Organisms.
- Environment Canada, 2007. Guidance Document on Statistical Methods for Environmental Toxicity Tests: Method Development and Applications Section. Environmental Technology Centre, Ottawa.
- Environment Agency, 2022. Scientific report – ShARE id 26 (revised). Derivation and Use of Soil Screening Values for Assessing Ecological Risks.
- Fendorf, S., Laforce, M.J., Li, G., 2004. Temporal changes in soil partitioning and bioaccessibility of arsenic, chromium, and lead. *J. Environ. Qual.* 33, 2049–2055.
- Fisker, K.V., Sørensen, J.G., Damgaard, C., Pedersen, K.L., Holmstrup, M., 2011. Genetic adaptation of earthworms to copper pollution: is adaptation associated with fitness costs in *Dendrobaena octaedra*? *Ecotoxicology* 20, 563–573.
- Garcia, M.V.B., Röembke, J., Martius, C., 2004. Proposal for an artificial soil substrate for toxicity tests in tropical regions. In: 25th Annual Meeting of Society of Environmental Toxicology and Chemistry (SETAC), Portland. 25th Annual Meeting of SETAC.
- Hedénec, P., Jiménez, J.J., Moradi, J., Domene, X., Hackenberger, D.K., Barot, S., Frossard, A., Oktaba, L., Filsler, J., Kindlmann, P., Frouz, J., 2022. Global distribution of soil fauna functional groups and their estimated litter consumption across biomes. *Sci. Rep.* 12, 17362.
- Hooda, P.S. (Ed.), 2010. Trace Elements in Soils. Wiley-Blackwell, Chichester.
- IMA, 2021. Instrução Normativa nº 45: valores orientadores de qualidade dos solos e águas subterrâneas de Santa Catarina.
- ISO, 2014a. Guideline 11267. Soil Quality: Inhibition of Reproduction of Collembola (*Folsomia candida*) by Soil Pollutants.
- ISO, 2014b. Guideline 16387. Soil Quality: Effects of Contaminants on Enchytraeidae (*Enchytraeus* sp.) – Determination of Effects on Reproduction.
- Kabata-Pendias, A., 2010. Trace Elements in Soil and Plants, vol. 4. CRC Press, Boca Raton.
- Lock, K., Janssen, C.R., 2002. Mixture toxicity of zinc, cadmium, copper, and lead to the potworm *Enchytraeus albidus*. *Ecotoxicol. Environ. Saf.* 52, 1–7.
- Maraldo, K., Christensen, B., Strandberg, B., Holmstrup, M., 2009. Effects of copper on enchytraeids in the field under differing soil moisture regimes. *Environ. Toxicol. Chem.* 25, 604–612.
- Marques, A.C.R., Trapp, T., Almeida, H.S., Morais, G.P., Bueno, J.M.M., Giombelli, L.D., Tiecher, T.L., Silva, R.F., Tiecher, T., Dall’Orsoletta, D.J., Brunetto, G., 2022. Valores de Referência de Toxicidez e Contaminação Ambiental de Cobre e Zinco em Solos e Plantas. In: BRUNETTO, G., et al. (Eds.), Contaminação em Solos de Pomares e Vinhedos: Causas, Efeitos e Estratégias de Manejo. SBCE - Núcleo Regional Sul, pp. 105–126 (Org.).
- Messias, T.G., Alves, P.R.L., Cardoso, E.J.B.N., 2023. Are the Brazilian prevention values for copper and zinc in soils suitable for protecting earthworms against metal toxicity? *Environ. Sci. Pollut. Res.* 14, 40641–40653.
- Nriagu, J.O., Pacyna, J.M., 1988. Quantitative assessment of worldwide contamination of air, water and soils by trace metals. *Nature* 333, 134–139.
- Nursita, A.I., Singh, B., Lees, E., 2005. The effects of cadmium, copper, lead, and zinc on the growth and reproduction of *Proisotoma minuta* Tullberg (Collembola). *Ecotoxicol. Environ. Saf.* 60, 306–314.
- OECD, 2016. Test No. 232: Collembolan Reproduction Test in Soil.
- Oliveira, D.A., Freitas, T.R., Dalla Rosa, V.M., Oliveira Filho, L.C.I., Veiga, M., Campos, M.L., Miquelluti, D.J., Klauber-Filho, O., 2024. Prevention values for copper (low tier approach) in subtropical acidic soils. *Environ. Sci. Pollut. Res.* 31, 60895–60904.
- Pesce, S., Marny, L., Sanchez, W., Artigas, J., Bérard, A., Betoulle, S., Chaumot, A., Coutellec, M.A., Crouzet, O., Faburé, J., Hedde, M., Leboulanger, C., Margoum, C., Martin-Laurent, F., Morin, S., Mougin, C., Munaron, D., Nélieu, S., Pelosi, C., Leenhardt, S., 2024. The use of copper as plant protection product contributes to environmental contamination and resulting impacts on terrestrial and aquatic biodiversity and ecosystem functions. *Environ. Sci. Pollut. Res.* <https://doi.org/10.1007/s11356-024-32145-z>.
- Poggere, G., Gasparin, A., Barbosa, J.Z., Melo, G.W., Corrêa, R.S., Motta, A.C.V., 2023. Soil contamination by copper: sources, ecological risks, and mitigation strategies in Brazil. *J. Trace Elem. Min.* 4, 100059.
- Posthuma, L., Baerselman, R., Van Veen, R.P., Van Breemen, E.M., 1997. Single and joint toxic effects of copper and zinc on reproduction of *Enchytraeus crypticus* in relation to sorption of metals in soils. *Ecotoxicol. Environ. Saf.* 38, 108–121.
- Posthuma, L., Suter, I.I.G.W., Traas, T.P. (Eds.), 2001. Species Sensitivity Distributions in Ecotoxicology. CRC press, New York.
- Renaud, M., Cousins, M., Awuah, K.F., Jegede, O., Sousa, J.P., Siciliano, S.D., 2020. The effects of complex metal oxide mixtures on three soil invertebrates with contrasting biological traits. *Sci. Total Environ.* 738, 139921.
- Sandifer, R.D., Hopkin, S.P., 1996. Effects of pH on the toxicity of cadmium, copper, lead and zinc to *Folsomia candida* Willem, 1902 (Collembola) in a standard laboratory test system. *Chemosphere* 12, 2475–2486.
- Schneider, C.A., Rasband, W.S., Eliceiri, K.W., 2012. NIH Image to ImageJ: 25 years of image analysis. *Nat. Methods* 9, 671–675.
- Scott-Fordsmand, J.J., Pedersen, M.B., Jensen, J., 1996. Setting a soil quality criterion. *Toxicol. & Ecotoxicol. News.* 3, 20–24.
- Sereni, L., Guenet, B., Lamy, I., 2022. Mapping risks associated with soil copper contamination using availability and bio-availability proxies at the European scale. *Environ. Sci. Pollut. Res.* 30, 19828–19844.
- Strawn, D.G., Baker, L.L., 2007. Speciation of Cu in a contaminated agricultural soil measured by XAFS, μ -XAFS, and μ -XRF. *Environ. Sci. Technol.* 42, 37–42.
- Segat, J.C., Baretta, D., Oliveira Filho, L.C.I., Sousa, J.P., Klauber-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. *Ecotoxicol. Environ. Saf.* 168, 457–465.
- Statsoft Inc., 2011. STATISTICA (data analysis software system). <https://www.statsoft.com>.
- Teixeira, P.C., Donagemma, G.K., Fontana, A., Teixeira, W.G. (Eds.), 2017. Manual de Métodos de Análise de Solo, vol. 3. EMBRAPA, Brasília.
- Tiecher, T.L., Ceretta, C.A., Comin, J.J., Girotto, E., Miotto, A., Benedet, L., Ferreira, P.A., Lorenzi, C.R., Couto, R.R., 2013. Forms and accumulation of copper and zinc in a sandy typic hapludalf soil after long-term application of pig slurry and deep litter. *Rev. Bras. Ciênc.* 37, 812–824.
- US EPA, 2005. Guidance for Developing Ecological Soil Screening Levels.
- US EPA, 2007. Method 3050B: acid digestion of sediments. Sludges and Soils.
- Van Vlaardingen, P.L.A., Traas, T.P., Wintersen, A.M., Aldenberg, T., 2004. ETX 2.0. A Program to calculate hazardous concentrations and fraction affected, based on normally distributed toxicity data. <http://hdl.handle.net/10029/9005>.
- Weltje, L., 2003. Integrating Evolutionary Genetics and Ecotoxicology: on the correspondence between reaction norms and concentration response curves. *Ecotoxicology* 12, 523–528.
- Xiong, X., Yanxia, L., Wei, L., Chunye, L., Wei, H., Ming, Y., 2010. Copper content in animal manures and potential risk of soil copper pollution with animal manure use in agriculture. *Resour. Conserv. Recycl.* 54, 985–990.
- Xu, J., Krogh, P.H., Wang, Y., Luo, Y., Song, J., 2009. Evaluation of growth and reproduction as indicators of soil metal toxicity to the Collembolan, *Sinella curviseta*. *Insect Sci.* 16, 57–63.

Daniela Tomazelli | Dennis Góss-Souza | Luís Carlos Luñes de Oliveira Filho |
Osmar Klauberg-Filho

BIOLOGIA DO SOLO

Conceitos e Aplicações nas Ciências Agrárias



Freitas Bastos Editora

Copyright © 2024 by Aline de Liz Ronsani Malfatti, Daniela Tomazelli,
Dennis Góss-Souza, Douglas Alexandre, Luís Carlos Iuñes de Oliveira Filho, Nathalia Turkot
Candiago, Osmar Klauberg-Filho, Pâmela Niederauer Pompeo, Rafaela Alves dos Santos
Peron e Thiago Ramos Freitas

Todos os direitos reservados e protegidos pela Lei 9.610, de 19.2.1998.
É proibida a reprodução total ou parcial, por quaisquer meios, bem como a produção de
apostilas, sem autorização prévia, por escrito, da Editora.
Direitos exclusivos da edição e distribuição em língua portuguesa:
Maria Augusta Delgado Livraria, Distribuidora e Editora

Direção Editorial: Isaac D. Abulafia
Gerência Editorial: Marisol Soto
Revisão: Tatiana Lopes de Paiva
Diagramação e Capa: Madalena Araújo

Dados Internacionais de Catalogação na Publicação (CIP) de acordo com ISBD

B615	Biologia do Solo: conceitos básicos e aplicações nas ciências agrícolas / Aline de Liz Ronsani Malfatti ... [et al.] ; organizado por Daniela Tomazelli ... [et al.]. - Rio de Janeiro, RJ : Freitas Bastos, 2024. 196 p. ; 15,5cm x 23cm. ISBN: 978-65-5675-397-3
2024-1275	1. Biologia. 2. Solo. 3. Ciência Agrícolas. I. Malfatti, Aline de Liz Ronsani. II. Tomazelli, Daniela. III. Góss-Souza, Dennis. IV. Alexandre, Douglas. V. Oliveira Filho, Luís Carlos Iuñes de. VI. Candiago, Nathalia Turkot. VII. Klauberg-Filho, Osmar. VIII. Pompeo, Pâmela Niederauer. IX. Peron, Rafaela Alves dos Santos. X. Freitas, Thiago Ramos. XI. Título. CDD 570 CDU 57

Elaborado por Vagner Rodolfo da Silva - CRB-8/9410

Índice para catálogo sistemático:

1. Biologia 570
2. Biologia 57



Freitas Bastos Editora

atendimento@freitasbastos.com

www.freitasbastos.com

9. TRANSFORMAÇÕES MICROBIANAS DO CARBONO NO SOLO

Daniela Tomazelli, Thiago Ramos Freitas, Osmar Klauberg-Filho e Dennis Góss-Souza

9.1 Introdução

O carbono é a estrutura básica de qualquer molécula orgânica, tanto fazendo parte da estrutura da célula tanto como componente das membranas e paredes celulares, ou em compostos relacionados ao metabolismo (enzimas). A matriz energética ou conjunto de energia disponível evolui junto com a humanidade. Nesse contexto fica exposta a importância do carbono, que é componente das principais bases energéticas, assim como os combustíveis fósseis formados por hidrocarbonetos (CH_4 , C_4H_{10} , C_8H_{18}), madeira e carvão.

A relevância do carbono no solo está intrinsecamente ligada às características que esse elemento confere, envolvendo aspectos da estrutura física e sua associação com minerais do solo. Além disso, certos compostos de carbono, produzidos por fungos, atuam como uma espécie de cola, promovendo a agregação das partículas do solo e, conseqüentemente, aprimorando sua porosidade. As transformações relacionadas ao carbono no solo não apenas otimizam a infiltração e o armazenamento de água, mas também aumentam a disponibilidade de nutrientes, contribuindo para uma maior qualidade biológica. Em termos práticos, essa dinâmica será abordada no contexto da matéria orgânica do solo (MOS).

10. ECOTOXICOLOGIA TERRESTRE

Luís Carlos Iuñes de Oliveira Filho, Thiago Ramos Freitas

10.1 Introdução

O solo, como discutido nos capítulos anteriores, desempenha diversos serviços ecossistêmicos e serve de hábitat e fonte de alimento para vários organismos. No entanto, está suscetível à contaminação por diversos tipos de poluentes, o que pode acarretar impactos negativos no ecossistema. A contaminação do solo por agrotóxicos, fármacos, dejetos, resíduos e outros produtos resultantes da atividade humana é uma realidade preocupante. Essa contaminação pode levar à perda de serviços ecossistêmicos e afetar toda a cadeia alimentar. A avaliação de áreas contaminadas geralmente se baseia em parâmetros químicos, mas essas análises, muitas vezes, não são suficientes para prever os efeitos nos organismos do solo, mesmo em concentrações muito baixas. Por esse motivo, para uma avaliação mais precisa, é recomendável que as análises químicas sejam complementadas por testes ecotoxicológicos.

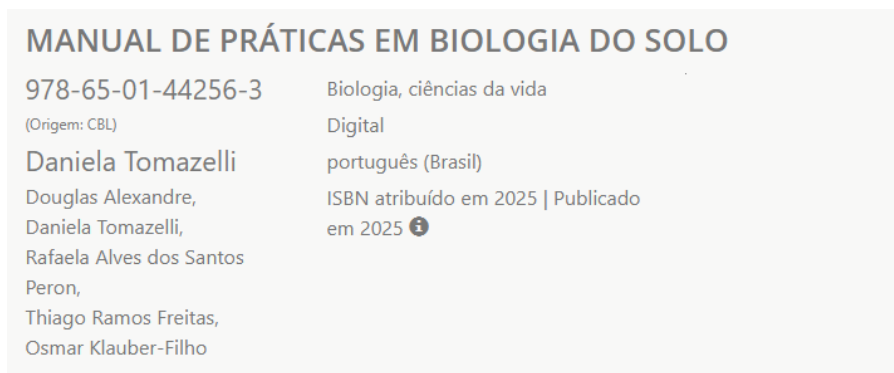
A **ecotoxicologia** é a ciência que busca associar conceitos em ecologia e toxicologia, de modo que tem por intuito estudar os efeitos tóxicos de contaminantes dentro do contexto dos ecossistemas. O ramo da ecotoxicologia terrestre tem avaliado o efeito potencial de contaminantes no solo, objetivando determinar quais são os limites críticos das substâncias, isto é, a concentração capaz de resultar em efeitos deletérios aos organismos

LISTA ISBN LIVROS

Este documento tem por intuito atestar a veracidade das informações fornecidas pelo candidato. Todos os livros e seus respectivos ISBNs podem ser consultados tanto no site da Amazon, como também no site da Câmara Brasileira do Livro (www.cblservicos.org.br).

1. Manual de Práticas em Biologia do Solo

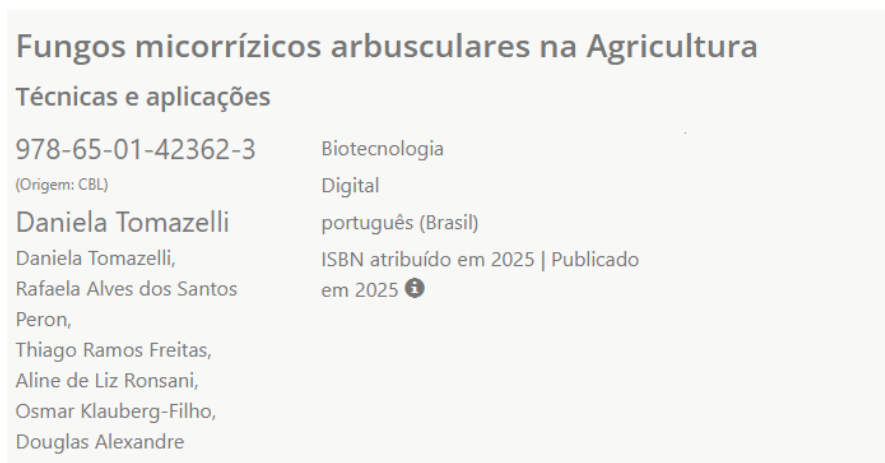
- a. Link Amazon (com ISBN): [MANUAL DE PRATICAS EM BIOLOGIA DO SOLO eBook : Alexandre, Douglas, Tomazelli, Daniela, Alves dos Santos Peron, Rafaela, Ramos Freitas, Thiago , Klauberg-Filho, Osmar: Amazon.com.br: Livros](#)
- b. ISBN: 978-65-01-44256-3



c.

2. Fungos Micorrízicos Arbusculares na Agricultura: Técnicas e Aplicações

- a. Link Amazon (com ISBN): [Fungos micorrízicos arbusculares na Agricultura: Técnicas e aplicações eBook : Tomazelli, Daniela, Peron, Rafaela Alves dos Santos, Freitas, Thiago Ramos, Alexandre, Douglas, Ronsani, Aline de Liz, Klauberg-Filho, Osmar: Amazon.com.br: Livros](#)
- b. ISBN: 978-65-01-42362-3



c.

3. Aprendendo com Minhocas: Guia Prático de Compostagem

- a. Link Amazon (com ISBN): [Aprendendo com minhocas: Guia prático de compostagem eBook : Alves dos Santos Peron, Rafaela, Tomazelli, Daniela, Alexandre, Douglas , Ramos Freitas, Thiago: Amazon.com.br: Livros](#)
- b. ISBN: 978-65-01-45011-7

Aprendendo com Minhocas

Guia prático sobre compostagem

978-65-01-45011-7

(Origem: CBL)

Daniela Tomazelli

Rafaela Alves dos Santos
Peron,
Daniela Tomazelli,
Douglas Alexandre,
Thiago Ramos Freitas

Meio ambiente; Conservação e
Proteção

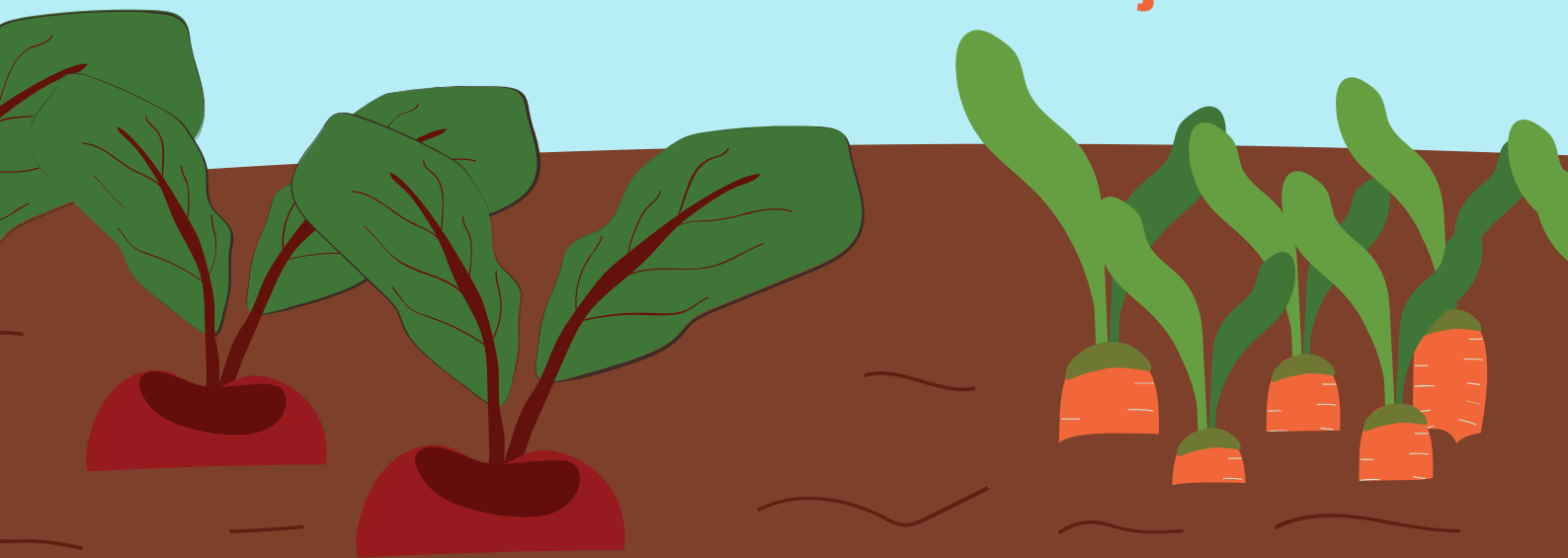
Digital

português (Brasil)

ISBN atribuído em 2025 | Publicado
em 2025 ⓘ

MANUAL DE PRÁTICAS EM BIOLOGIA DO SOLO

1º WORKSHOP CATARINENSE DE EDUCAÇÃO EM SOLOS



DOUGLAS ALEXANDRE
DANIELA TOMAZELLI
RAFAELA ALVES DOS SANTOS PERON
THIAGO RAMOS FREITAS
OSMAR KLAUBERG FILHO

ATIVIDADE 1 – ANÁLISE DA FERTILIDADE DO SOLO

OBJETIVO

Permitir que os alunos analisem características químicas básicas do solo (como pH e nutrientes) para compreender a relação entre composição química e fertilidade.

MATERIAIS NECESSÁRIOS:

- Solo coletado: Amostras de diferentes locais (ex: jardim da escola, terrenos baldios, áreas agrícolas).
- Recipientes plásticos ou copos descartáveis (Para armazenar o solo).
- Vinagre branco (Para testar pH ácido).
- Bicarbonato de sódio (Para testar pH básico).
- Água filtrada (Para preparar misturas).
- Colheres (Para manuseio do solo).
- Etiquetas ou marcadores (Para identificar as amostras).
- Fichas de registro de dados (Para que os alunos anotem os resultados).

PROCEDIMENTOS

Parte 1: Coleta do Solo

- Identificação dos locais: Divida os alunos em grupos e escolha diferentes locais para coletar amostras (ex.: áreas sombreadas, áreas expostas ao sol, terrenos arenosos ou argilosos).
- Coleta: Oriente os alunos a coletar cerca de 200g de solo por local.
- Armazenamento: Identifique cada amostra com etiquetas, incluindo data e local de coleta.

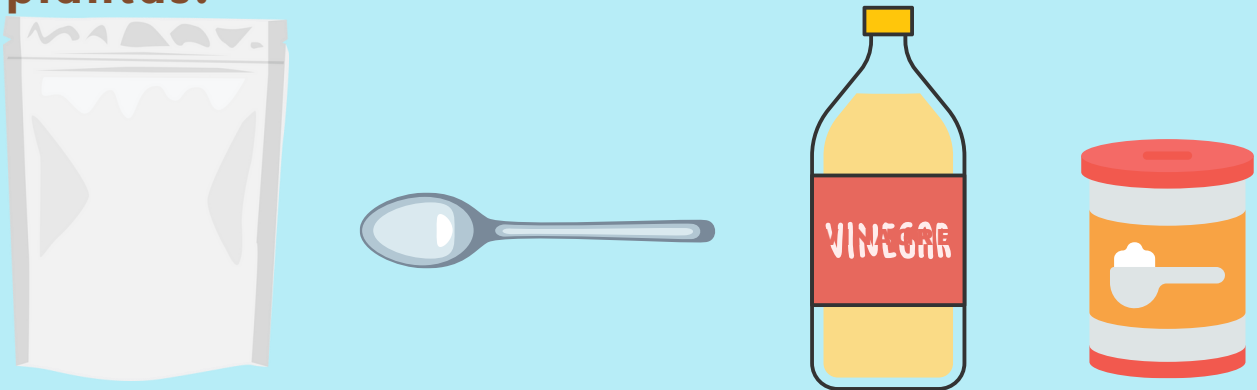


Parte 2: Teste de pH

- **Separação do solo:** Separe uma pequena quantidade de solo (cerca de 2 colheres de chá) para os testes de pH.
- **Teste com vinagre (ácido):**
 - Coloque o solo em um recipiente.
 - Adicione vinagre (2 colheres de sopa).
 - Observe a reação: borbulhas indicam solo alcalino.
- **Teste com bicarbonato (básico):**
 - Em outro recipiente, misture o solo com água (1:1) para formar uma pasta.
 - Adicione bicarbonato (1 colher de chá).
 - Observe a reação: borbulhas indicam solo ácido.
- **Registro dos resultados:** crie uma ficha com as seguintes colunas: local de coleta, cor do solo, reação do vinagre, reação do bicarbonato.

Parte 3: Discussão e Interpretação

- Peça aos alunos que comparem os resultados das diferentes amostras e correlacionem os dados de pH com a fertilidade do solo.
- Oriente uma discussão sobre a importância de corrigir o solo em práticas agrícolas e a influência das características químicas no crescimento das plantas.



ATIVIDADE 2 – OBSERVAÇÃO DE ORGANISMOS DO SOLO COM FUNIL DE BERLESE

OBJETIVO

Permitir que os alunos visualizem a fauna do solo aprendendo sobre sua biodiversidade e a importância ecológica desses organismos.

MATERIAIS NECESSÁRIOS:

- Solo coletado: Amostras de solo de diferentes ambientes (ex.: jardim, áreas agrícolas, mata, solos compactados).
- Funil de Berlese artesanal (Feito com garrafas PET cortadas ou funis plásticos)
- Recipientes coletoras: (Potes ou copo pequenos para capturar os organismos.)
- Álcool 70% (Para preservar os organismos coletados).
- Luminária ou lâmpada (Fonte de calor para forçar a migração dos organismos do solo).
- Gaze ou pano fino (Para evitar que o solo caia no recipiente coletor).
- Pinças e lupas (Para observar os organismos coletados).
- Etiquetas e fichas de registro (Para identificar e catalogar os organismos).

OPCIONAIS

- Microscópio ou lupa binocular: Para observação detalhada dos organismos menores.
- Câmera de celular ou microscópio digital portátil: Para fotografar os organismos.



PROCEDIMENTOS

Parte 1: Coleta do Solo

- **Identificação do local:** Oriente os alunos a coletar amostras de solo de diferentes ambientes.
- **Armazenamento:** Coloque cada amostra em um saco plástico identificado com o local de coleta, data e características do solo (ex.: úmido, seco, arenoso).

Parte 2: Montagem do Funil de Berlese

- **Montagem do funil:**
 - Use uma garrafa PET cortada ou um funil plástico.
 - Coloque uma camada fina de gaze ou pano na base do funil para evitar que o solo caia no recipiente coletor.
 - Encha o funil com uma amostra de solo (cerca de 200g).
 - Posicione o funil sobre um pote coletor contendo uma pequena quantidade de álcool 70%.
- **Adição da fonte de calor:**
 - Coloque uma lâmpada acesa a cerca de 15 cm acima do funil.
 - Oriente os alunos a observar por 24-48 horas, conforme os organismos migram para longe da luz e do calor, caindo no recipiente coletor.

Parte 3: Observação dos Organismos

- **Exame inicial:** Use lupas ou pinças para observar os organismos no recipiente coletor.
- **Análise detalhada:**
 - Se disponível, use microscópios para examinar as características dos organismos.
- **Incentive os alunos a desenhar ou fotografar o que observaram.**



FUNIL DE BERLESE ARTESANAL



**LÂMPADA SERVINDO COMO
FONTE DE CALOR PARA
FORÇAR A MIGRAÇÃO DOS
ORGANISMOS DO SOLO**



**FUNIL DE BERLRSE
ARTESANAL - FEITO DE
GARRAFA PET CONTENDO
A AMOSTRA DE SOLO**



**RECIPIENTE COLETOR
CONTENDO ÁLCOOL 70%
PARA PRESERVAR OS
ORGANISMOS**



DISCUSSÃO E INTERPRETAÇÃO

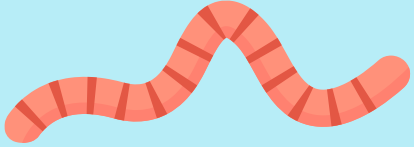
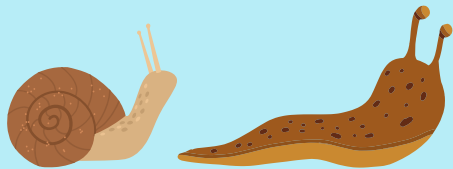
- Compare a diversidade de organismos entre as diferentes amostras de solo.
- Discuta a importância desses organismos para o ecossistema do solo, como decomposição de matéria orgânica e formação de húmus.
- Relacione a presença de fauna com a qualidade do solo e possíveis impactos da poluição ou compactação.

EXPANSÃO DA ATIVIDADE

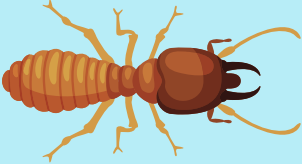
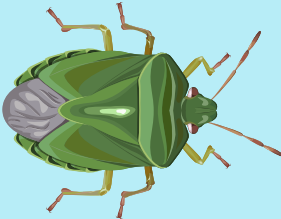
- Recursos adicionais: Inclua amostras de solos tratados com fertilizantes ou pesticidas para observar o impacto na fauna.
- Interdisciplinaridade: Relacione a atividade com tópicos de química (pH do solo) ou geografia (formação do solo).
- Projeto prático: Proponha a criação de um relatório sobre a biodiversidade do solo em diferentes áreas da escola ou comunidade.



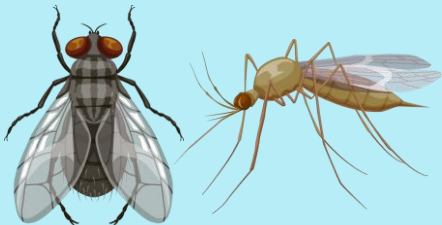
IDENTIFICAÇÃO DOS ORGANISMOS

ORGANISMO	CARACTERÍSTICAS	FUNÇÕES
MINHOCA 	Corpo cilíndrico e alongado, segmentado. Cor marrom ou rosada e pele úmida e sem olhos.	A sua locomoção forma tuneis que promovem a aeração e drenagem do solo; decomposição de matéria orgânica; formação de húmus.
LESMA E CARACOL 	Corpo não segmentado, mole e úmido; possuem cabeça, caracóis têm concha; possuem rádula para raspar alimentos.	Alimentam-se de restos vegetais em vários estágios de decomposição; ajudam na ciclagem de nutrientes.
BESOURO 	Corpo duro com élitros (asas endurecidas); tamanhos variados; antenas curtas.	Decomposição de matéria orgânica (alguns são detritívoros); controle de pragas (besouros predadores);
FORMIGA 	Corpo segmentado (cabeça, tórax, abdome); antenas dobradas; pernas adaptadas para escavação.	Escavação que melhora a estrutura do solo; transporte de sementes (mirmecocoria); controle de pragas.

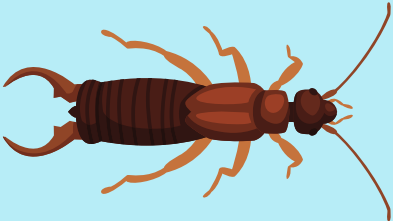
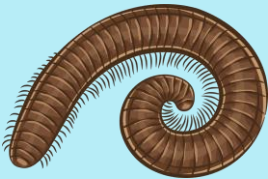
IDENTIFICAÇÃO DOS ORGANISMOS

ORGANISMO	CARACTERÍSTICAS	FUNÇÕES
CUPIM 	Corpo pequeno, mole e de cor clara; vivem em colônias organizadas; mandíbulas fortes.	Decomposição de matéria lignocelulósica; formação de galerias que melhoram a infiltração de água.
GRILLOS E GAFANHOTOS 	Três pares de pernas; pernas traseiras longas para salto; mandíbulas robustas.	Consumo de matéria vegetal, influenciando a ciclagem de nutrientes; alimento para predadores.
BARATA 	Corpo achatado e oval; antenas longas; aparelho bucal mastigador; asas podem estar ausentes ou reduzidas.	Consumo de folhas e matéria vegetal; influenciam a ciclagem de nutrientes e servem de alimento para predadores no ecossistema.
PERCEVEJO 	Corpo achatado e oval; aparelho bucal sugador; asas parcialmente endurecidas.	Alguns são pragas que sugam seiva; outros predadores ajudam no controle de pragas.

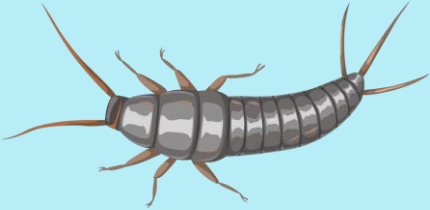
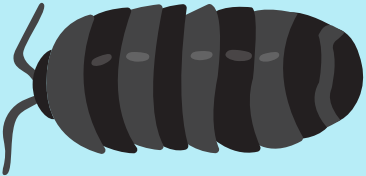
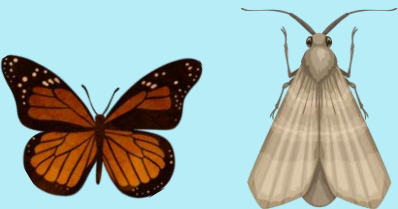
IDENTIFICAÇÃO DOS ORGANISMOS

ORGANISMO	CARACTERÍSTICAS	FUNÇÕES
MOSCA E MOSQUITO 	Corpo dividido em cabeça, tórax e abdome; asas membranosas; aparelho bucal sugador ou lambedor.	Consumo de matéria orgânica em decomposição; vetor de poluentes e organismos patogênicos.
LAGARTA 	Corpo alongado, segmentado; aparelho bucal mastigador; presença de pernas torácicas e falsas pernas.	Consumo de folhas e matéria vegetal; influenciam a ciclagem de nutrientes e servem de alimento para predadores no ecossistema.
ARANHA 	Corpo dividido em cefalotórax e abdome; 4 pares de pernas; fiandeiras para teias.	Predadoras que controlam populações de insetos e outros pequenos artrópodes.
ÁCARO 	Corpo pequeno e redondo; 4 pares de pernas; tamanhos microscópicos ou pequenos.	Decomposição de matéria orgânica; controle de fungos; alguns são pragas de plantas.

IDENTIFICAÇÃO DOS ORGANISMOS

ORGANISMO	CARACTERÍSTICAS	FUNÇÕES
ESGOPRIÃO 	Corpo segmentado com pedipalpos (pinças); cauda com ferrão venenoso.	Controle de populações de insetos no solo; contribuem para o equilíbrio ecológico.
TESOURINHA 	Corpo alongado; pinças (cerci) na extremidade do abdome; asas curtas ou ausentes; aparelho bucal mastigador.	Consumo de matéria orgânica e pequenos insetos; ajudam na decomposição e no controle de populações de outros artrópodes.
LACRAIA 	Corpo alongado e segmentado; + 15 pares de pernas; predadores rápidos.	Controle de populações de insetos e outros pequenos artrópodes no solo.
PIOLHO DE COBRA 	Corpo cilíndrico e segmentado; + 100 pares de pernas; enrola-se em espiral para proteção.	Decomposição de matéria vegetal e ciclagem de nutrientes.

IDENTIFICAÇÃO DOS ORGANISMOS

ORGANISMO	CARACTERÍSTICAS	FUNÇÕES
TRAÇA 	Corpo pequeno; asas escamadas; aparelho bucal mastigador ou sugador, dependendo da fase.	Consumo de matéria vegetal e alimentos armazenados; algumas contribuem para a decomposição de matéria orgânica.
TATU-DE-JARDIM 	Corpo oval, rígido e segmentado; 7 pares pernas; capacidade de enrolar-se em bola.	Decomposição de matéria orgânica; ajudam na ciclagem de nutrientes.
BORBOLETA E MARIPOSA 	Corpo coberto por escamas coloridas; aparelho bucal sugador (adultos) e mastigador (larvas).	Polinização (adultos); larvas consomem folhas, contribuindo para a ciclagem de matéria orgânica.
LARVA DE BESOURO 	Corpo mole, cilíndrico ou em forma de C; aparelho bucal mastigador.	Decomposição de matéria orgânica (algumas); alimentação de predadores e contribuição para o ciclo do carbono no solo.

ATIVIDADE 3- COMPOSTAGEM

OBJETIVO

Demonstrar o processo de decomposição e como a matéria orgânica é transformada em nutrientes disponíveis para as plantas, destacando o papel dos micro-organismos e da fauna do solo.

MATERIAIS NECESSÁRIOS:

- **Resíduos orgânicos:** Cascas de frutas, legumes e folhas secas.
- **Recipientes transparentes:** Potes plásticos ou garrafas PET cortadas
- **Solo:** Uma camada fina para iniciar o processo de decomposição.
- **Gaze ou pano fino:** Para cobrir os recipientes e evitar entrada de insetos indesejados.
- **Fichas de registro:** Para acompanhar a evolução do processo.
- **Pinças ou luvas:** Para manuseio dos materiais.

PROCEDIMENTOS

Parte 1: Montagem do Sistema de Compostagem

- **Em um recipiente transparente, crie camadas alternadas de resíduos orgânicos e solo.**
- **Cubra com gaze para permitir ventilação.**
- **Armazene o recipiente em local sombreado.**



MATERIAIS NECESSÁRIOS:

- **Resíduos orgânicos:** Cascas de frutas, legumes e folhas secas.
- **Recipientes transparentes:** Potes plásticos ou garrafas PET cortadas
- **Solo:** Uma camada fina para iniciar o processo de decomposição.
- **Gaze ou pano fino:** Para cobrir os recipientes e evitar entrada de insetos indesejados.
- **Fichas de registro:** Para acompanhar a evolução do processo.
- **Pinças ou luvas:** Para manuseio dos materiais.

PROCEDIMENTOS

Parte 1: Montagem do Sistema de Compostagem

- Em um recipiente transparente, crie camadas alternadas de resíduos orgânicos e solo.
- Cubra com gaze para permitir ventilação.
- Armazene o recipiente em local sombreado.



ATIVIDADE 4 - POLUIÇÃO DO SOLO COM PLANTAS INDICADORAS

OBJETIVO

Explorar o impacto de poluentes no solo, utilizando o crescimento de plantas como indicador de qualidade.

MATERIAIS NECESSÁRIOS:

- Vasos pequenos ou copos descartáveis.
- Solo de diferentes áreas: Natural, contaminado (ex.: óleo, sabão em pó) e misturas.
- Sementes de crescimento rápido: Feijão ou alpiste.
- Fichas de registro: Para anotar altura e aparência das plantas

PROCEDIMENTOS

Parte 1: Plantio

- Distribua diferentes tipos de solo em vasos.
- Plante 2-3 sementes em cada vaso e regue com quantidades iguais de água.

Parte 2: Monitoramento

- Oriente os alunos a medir a altura das plantas e registrar mudanças na aparência (ex.: folhas amarelas ou crescimento atrofiado).
- Discuta as diferenças de crescimento entre os solos contaminados e os naturais.

Discussão e Interpretação:

- Relacione os efeitos observados com tipos de poluição no solo.
- Discuta métodos para remediação, como biorremediação ou fitorremediação.



ATIVIDADE 5 - CULTIVO DE MICORGANISMOS EM MEIO DE CULTURA CASEIRO

OBJETIVO

Investigar a presença de micro-organismos no solo e seu papel na decomposição.

MATERIAIS NECESSÁRIOS:

- Solo coletado de diferentes áreas.
- Ágar caseiro: Gelatina sem sabor misturada com caldo de feijão ou batata.
- Placas de Petri ou pratos descartáveis com tampa.
- Cotonetes esterilizados.

Opcionais:

- Microscópio ou lupa binocular.
- Corantes simples: Para destacar colônias.

PROCEDIMENTOS

Parte 1: Preparação do Ágar e Coleta de Amostras

- Prepare o ágar caseiro e despeje em pratos de Petri.
- Usando cotonetes, colete amostras de solo e passe levemente sobre o ágar.

Parte 2: Incubação e Observação

- Armazene os pratos em local quente (30°C) por 2-3 dias.
- Oriente os alunos a observar o crescimento de colônias e registrar as diferenças entre amostras.

Discussão e Interpretação:

- Relacione o crescimento das colônias com a função dos micro-organismos no solo.
- Discuta os impactos de práticas agrícolas, como uso de fertilizantes e pesticidas, na microbiologia do solo.



ATIVIDADE 6 - EPLORANDO O JARDIM: OBSERVAÇÃO E REGISTRO DA BIODIVERSIDADE DO SOLO

Parte 1: Registro e Desenho

- Cada aluno deve escolher um organismo observado (minhoca, formiga, larva, etc.) para desenhar com detalhes.
- Peça que anotem informações como:
- Onde o organismo foi encontrado (ex.: úmido, próximo a plantas).
- Como ele se movia ou agia.

Parte 3: Discussão em Grupo

- Reúnam-se e compartilhem os desenhos e observações.
- Pergunte:
 - Qual foi o organismo mais comum?
 - Qual a importância dele no solo?
 - Como podemos proteger esses organismos?

Expansão da Atividade

- Relacione com o Cotidiano: Discuta como práticas agrícolas ou poluentes podem afetar esses organismos.
- Projeto Coletivo: Proponha criar um "mapa da biodiversidade do jardim" com as observações de todos.
- Desafios: Inclua perguntas investigativas, como: "Por que encontramos mais organismos em áreas úmidas?"



ATIVIDADE 7 - CICLOS BIOGEOQUÍMICOS: O PAPEL DOS MICRORGANISMOS

OBJETIVO

Ajudar os alunos a compreenderem os ciclos biogeoquímicos (carbono, nitrogênio e fósforo) e a importância dos microrganismos nesses processos, utilizando uma abordagem interativa e visual.

MATERIAIS NECESSÁRIOS:

Essenciais (baixo custo):

- Cartolina ou folhas grandes de papel kraft.
- Canetas, lápis de cor ou marcadores.
- Fichas ou pedaços de papel para representar os microrganismos (ex.: bactérias, fungos) e os compostos químicos (ex.: CO_2 , NH_4^+ , NO_3^- , PO_4^{3-}).
- Fita adesiva ou cola.
- Exemplos reais (se possível):
- Solo rico em matéria orgânica (para ilustrar a decomposição).
- Folhas secas, esterco ou outros resíduos orgânicos.
- Opcionais (médio custo):
- Modelos plásticos ou impressos de microrganismos.
- Vídeos curtos sobre os ciclos (projektor ou celular com acesso à internet).

PROCEDIMENTO DA ATIVIDADE:

Parte 1: Introdução e Contextualização

1. Explique brevemente os ciclos biogeoquímicos principais: carbono, nitrogênio e fósforo.
2. Destaque o papel dos microrganismos, como:
 - o Bactérias fixadoras de nitrogênio no ciclo do nitrogênio (*Rhizobium*, *Nitrosomonas*).
 - o Fungos decompositores no ciclo do carbono.
 - o Microrganismos no ciclo do fósforo, liberando fósforo de compostos orgânicos.



Parte 2: Montagem do Ciclo em Grupo

1. Distribuição dos Materiais:

Cada grupo receberá:

- Um ciclo biogeoquímico específico (carbono, nitrogênio ou fósforo).
- Materiais para desenhar ou construir o ciclo.

2. Criação do Ciclo:

- Os alunos desenharão um esquema do ciclo escolhido na cartolina.
- Utilizarão fichas para adicionar os microrganismos e seus papéis em etapas específicas.

Exemplos:

Ciclo do Carbono: Adicionar fungos como decompositores, liberando CO_2 .

Ciclo do Nitrogênio: Mostrar a conversão de N_2 em amônia por bactérias fixadoras.

Ciclo do Fósforo: Mostrar a mineralização do fósforo orgânico por microrganismos.

Parte 3: Apresentação e Discussão

1. Apresentação:

Cada grupo apresenta seu ciclo para a turma, explicando o papel dos microrganismos.

2. Discussão:

Oriente perguntas como:

- O que aconteceria sem os microrganismos?
- Como as ações humanas (ex.: fertilizantes, poluição) afetam esses ciclos?
- Como podemos proteger esses processos naturais?



ATIVIDADE 8 - COLETA DE MONÓLITOS DE SOLO E CATAÇÃO MANUAL DE FAUNA EDÁFICA

OBJETIVO

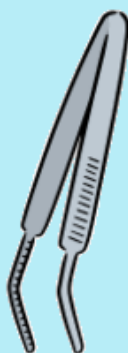
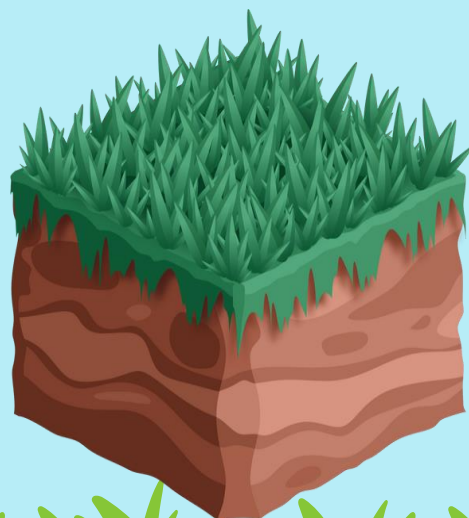
Ensinar aos alunos a realizar a coleta de monólitos de solo e a identificar organismos da fauna do solo manualmente, promovendo a compreensão sobre biodiversidade edáfica e a função desses organismos nos ecossistemas.

MATERIAIS NECESSÁRIOS:

- Pás ou cavadeiras pequenas.
- Recipientes rígidos para armazenar o solo coletado (ex.: caixas plásticas ou baldes).
- Peneiras (malha fina e média) para separar o solo.
- Lonas ou folhas de papel kraft para espalhar o solo.
- Lupas simples para observar organismos.
- Pinças ou colheres pequenas para manipular organismos.
- Etiquetas e canetas para identificação das amostras (local e horário).
- Fichas de registro para anotar as observações.

Opcionais (médio custo):

- Kits de identificação de fauna do solo (guias ilustrados ou aplicativos).
- Lupa binocular ou microscópio portátil para detalhamento dos organismos menores.
- Bandejas brancas para facilitar a visualização da fauna.



PROCEDIMENTOS

Parte 1: Coleta de Monólitos de Solo

1. Escolha do Local:

Divida os alunos em grupos e escolha diferentes locais de coleta, como:

- Jardim escolar.
- Áreas sombreadas e ensolaradas.
- Áreas compactadas ou com resíduos orgânicos.

2. Instruções de Coleta:

- Delimite um bloco de solo com dimensões de 10 x 20 x 20 cm usando a pá.
- Retire cuidadosamente o monólito e coloque-o em um recipiente identificado (local, data, características).
- Evite quebrar ou compactar o bloco durante a coleta.

Parte 2: Separação e Catatação Manual

1. Montagem do Ambiente:

- Espalhe o monólito sobre uma lona ou papel kraft.
- Divida o solo em camadas (superficial, intermediária e profunda) para análise mais detalhada.

2. Catatação Manual:

Oriente os alunos a procurar organismos manualmente, utilizando lupas e pinças para organismos menores.

- Separem os organismos encontrados em recipientes específicos, como potes com álcool 70% para preservação.



3. Classificação Inicial:

Os alunos podem identificar visualmente os organismos (ex.: minhocas, formigas, besouros, ácaros, colêmbolos) usando guias simples.

Anotem o número de indivíduos e suas características (ex.: cor, tamanho, comportamento).

Parte 3: Discussão e Interpretação

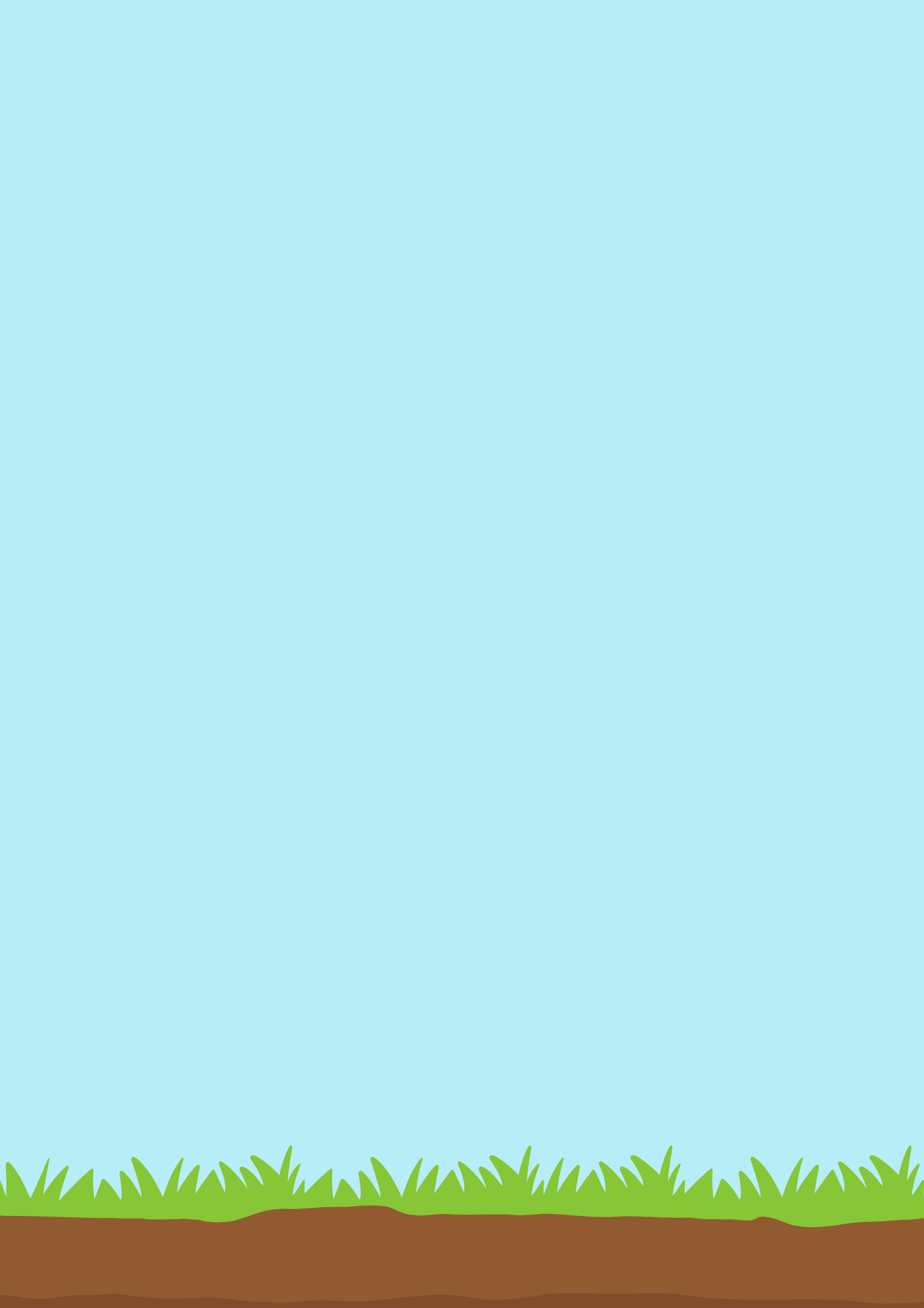
1. Comparação entre Locais:

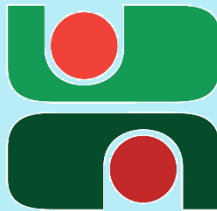
- Qual local apresentou maior diversidade de organismos?
- Há diferenças no tipo de organismos encontrados em diferentes profundidades do solo?

2. Discussão sobre a Fauna:

- Importância ecológica dos organismos encontrados (ex.: decomposição, aeração do solo, ciclos de nutrientes).
- Impactos de práticas humanas como compactação, poluição e manejo agrícola.







UDESC
LAGES · CAV



Fungos micorrízicos arbusculares na Agricultura



**Daniela Tomazelli
Rafaela Alves dos Santos Peron
Aline de Liz Ronsani
Douglas Alexandre
Thiago Ramos Freitas
Osmar Klauberg Filho**

Autores



Daniela Tomazelli
Eng. Agrônoma
Dra Ciência do solo
Pesquisadora
(FAPESC/UDESC)



Rafaela A.S. Peron
Eng. Agrônoma
Msc. Ciência do solo
Pesquisadora
(FAPESC/UDESC)



Aline L. Ronsani
Eng. Agrônoma
Msc. Ciência do solo
Pesquisadora
(UDESC)



Thiago R. Freitas
Eng. Ambiental
Dr. Ciência do solo
Pesquisador (FAPESC)
Analista Ambiental
(ECx soluções em saúde do solo)



Douglas Alexandre
Eng. Agrônomo
Dr. Ciência do solo
Professor (UDESC)
Pesquisador (FAPESC)



Osmar Klauberg-Filho
Eng. Agrônomo
Dr. Ciência do solo
Professor (UDESC)

Sumário

Tópico 1: Importância, conceitos e aplicação dos fungos micorrízicos arbusculares

Tópico 2: Inoculantes comerciais de fungos micorrízicos arbusculares e Produção de inoculo On-farm

Tópico 3: Extração de esporos de FMAs e espécies de Fungos micorrízicos arbusculares: ocorrência e potencial de uso

Tópico 4: Potencial de inóculo em sistemas agrícolas e colonização micorrízica em plantas de interesse agrícola

Tópico 1: Importância, conceitos e aplicação dos fungos micorrízicos arbusculares

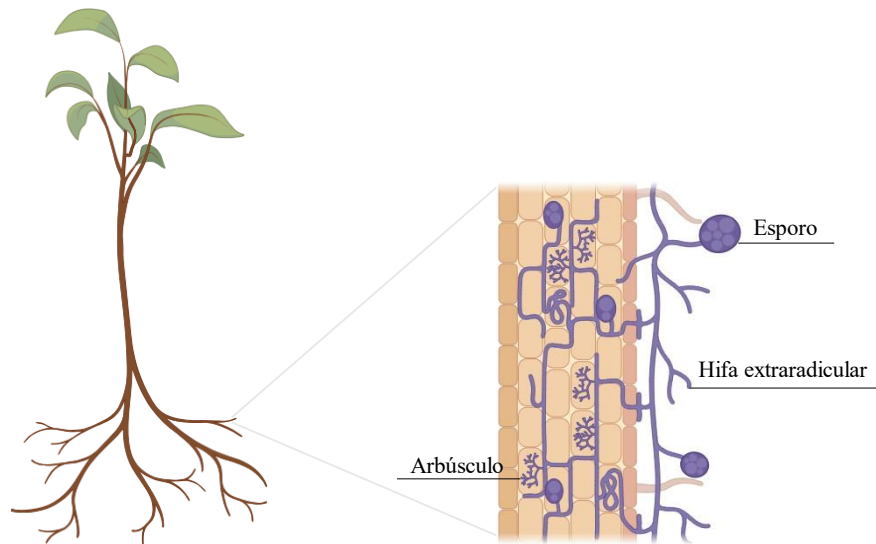
Os Fungos Micorrízicos Arbusculares (FMAs) são microrganismos pertencentes ao filo Glomeromycota, estes são simbioses obrigatórios que estabelecem uma relação simbiótica com plantas com cerca de 80% das plantas terrestres, formando as micorrizas. Essa relação beneficia tanto o fungo quanto a planta, contribuindo para a absorção de nutrientes essenciais, como o fósforo e o nitrogênio, e expandindo a área de abrangência do sistema radicular, melhorando a tolerância das plantas a condições de estresse, como seca e solos pobres.

Quadro 1. Benefícios da associação micorrízica para o fungo e para a planta

Fungo micorrízico	Plantas
-Abrigo e proteção nas células das raízes; -Hospedeiro para completar o ciclo; -Recebe açúcares provenientes da fotossíntese.	-Maior absorção de água; -Maior absorção de nutrientes, principalmente os pouco móveis como o P; -Aumento da taxa fotossintética; -Estimula o crescimento vegetal; -Proteção contra patógenos radiculares.

Estão presentes naturalmente nos solos, mas a quantidade de propágulos (esporos e hifas) pode variar significativamente. Dentre as estruturas encontradas, estão os **esporos**, que são estruturas reprodutivas, as quais são dispersas no solo e posteriormente germinam e estabelecem simbiose com novas plantas. As **hifas extraradicular** são consideradas propágulos, contudo a principal função é absorver água e nutrientes (interceptação radicular) quando estão em simbiose. Já os **arbúsculos** são estruturas que indicam a simbiose efetiva, esses são o ponto de troca entre a planta e o fungo, onde a planta disponibiliza açúcares fotoassimilado da fotossíntese para o fungo, e a planta recebe água e nutriente absorvidos com o auxílio do fungo. Algumas espécies de FMAs, possuem uma estrutura denominada vesícula, que são estruturas de reserva de lipídios. Na Figura 1, pode ser observado as estruturas dos FMAs mencionadas no texto acima.

Figura 1. Estruturas dos fungos micorrízicos arbusculares (FMAs).



1.1 Importância agrícola

Os fungos micorrízicos arbusculares (FMAs) desempenham um papel essencial na agricultura, oferecendo uma ampla gama de benefícios que impactam diretamente a produtividade. Estudos confirmam que a inoculação com FMAs pode levar ao aumento significativo da produtividade em cereais como trigo (*Triticum aestivum*), arroz (*Oryza sativa*), cevada (*Hordeum vulgare*), sorgo (*Sorghum bicolor*) e milho (*Zea mays*) (Zhang et al., 2019). Esses ganhos produtivos estão associados a capacidade dos FMAs de formar associações simbióticas com as raízes das plantas, otimizando a absorção de nutrientes, dentre eles o fósforo sendo esse um nutriente frequentemente limitado nos solos, e de extrema importância para plantas. Promovem a biodiversidade do solo e reduzem impactos ambientais.

1.2 Influência do manejo do solo e práticas culturais

Diversos fatores podem impactar as qualidades dos fungos micorrízicos arbusculares (FMA), práticas agrícolas intensivas, aplicação excessiva de fertilizantes e implementação prolongada de monoculturas, causando uma diminuição na densidade de esporos e diversidade de espécies de FMAs (SÄLE et al., 2015).

A simbiose micorrízica é estabelecida pelas plantas, em situações de necessidade, devido a isso o excesso de adubação fosfatada pode reduzir o potencial de estabelecimento da simbiose (QIN et al 2015). Para contornar esse problema, sugere a adoção de práticas de manejo conservacionistas, que visam preservar e promover a função dos FMA no solo agrícola (SILVA, et al., 2020). Estudos apontam que cultivos com consorcio de plantas ocorre o aumento da riqueza e diversidade de espécies de FMAs (GUZMAN et al., 2021).

Referências

GUZMAN, A.; MONTES, M.; HUTCHINS, L.; DELACERDA, G.; YANG, P; KAKOURIDIS, A.; RUTH M; DAHLQUIST-WILLARD, M. K.; FIRESTONE, T. B.; KREMEN, C. Crop diversity enriches arbuscular mycorrhizal fungal communities in an intensive agricultural landscape. **New Phytologist**, v. 231, n. 1, p. 447-459, 2021.

QIN, H.; LU, K.; STRONG, P. J.; XU, Q.; WU, Q.; XU, Z.; XU, J.; WANG, H. Long-term fertilizer application affects the soil, root arbuscular mycorrhizal fungi and community composition in rotation agriculture. **Applied Soil Ecology**, v. 89, p. 35-43, 2015.

SÄLE, V.; AGUILERA, P.; LACZKO, E.; MÄDER, P.; BERNER, A.; ZIHLMANN, U.; Van Der Heijden, M. G. A.; OEHL, F. Impact of conservation tillage and organic farming on the diversity of arbuscular mycorrhizal fungi. **Soil Biology and Biochemistry**, v. 84, p. 38-52, 2015.

SILVA JÚNIOR, M. L.; DE MELO, V. S. Atributos biológicos em diferentes sistemas de manejo do solo no município de Paragominas, Pará. *Brazilian Journal of Development*, 6(9), 72858-72870, 2020. <https://doi.org/10.34117/bjdv6n9-655>.

ZHANG, S.; LEHMANN, A.; ZHENG, W.; YOU, Z.; RILLING, M. C. Arbuscular mycorrhizal fungi increase grain yields: a meta-analysis. **New Phytologist**, v. 222, n. 1, p. 543-555, 2019.

Tópico 2: Inoculantes comerciais de fungos micorrízicos arbusculares e Produção de inóculo *On-farm*

O uso de produtos à base de microrganismos, tem tido um crescimento significativo em todo o mundo, e no Brasil esse crescimento é ainda mais expressivo. Existem muitas empresas que ofertam esses produtos, no entanto é possível uma produção dentro da própria propriedade, esse sistema é denominado *on farm*. Esse sistema, facilita a vida de muitos produtores rurais, onde eles fazem suas próprias multiplicações. Entretanto esses sistemas de multiplicações vêm enfrentando um grave problema de contaminantes, onde raramente se encontra os microrganismos de interesse, e quando encontrado vem em baixa concentrações.

2.1 Programa Nacional de Bioinsumos

O Programa Nacional de Bioinsumos (PNB), instituído pelo Decreto nº 10.375/2020, representa um marco significativo para o desenvolvimento sustentável da agropecuária brasileira. Seu principal objetivo é promover a adoção e o fortalecimento do uso de bioinsumos como uma alternativa estratégica para aumentar a eficiência produtiva, preservar os recursos naturais e reduzir os impactos ambientais associados à agricultura convencional.

De acordo com o decreto, bioinsumos são produtos, processos ou tecnologias de origem vegetal, animal ou microbiana. Eles se destinam ao uso na produção, armazenamento e beneficiamento de produtos agropecuários, em sistemas de produção aquáticos ou de florestas plantadas. Esses insumos interferem positivamente no crescimento, desenvolvimento e mecanismos de resposta de animais, plantas e microrganismos, além de interagirem com produtos e processos físico-químicos e biológicos.

Os bioinsumos englobam uma ampla gama de produtos e tecnologias baseados em organismos vivos ou em compostos naturais, como microrganismos benéficos – incluindo fungos micorrízicos arbusculares –, biofertilizantes, biodefensivos e promotores de crescimento vegetal. O uso desses insumos contribui diretamente para a saúde do solo, melhora a eficiência no uso de nutrientes e promove o equilíbrio ecológico nas áreas de cultivo.

2.1.1 Legislação de Bioinsumos e o Desafio da Produção On-Farm

Os bioinsumos abrangem uma variedade de produtos destinados ao manejo e à promoção do crescimento vegetal, incluindo biofertilizantes, inoculantes e pesticidas biológicos. Cada uma dessas categorias segue legislações específicas que regulamentam seu controle, registro, classificação e fiscalização no Brasil. Entre as principais legislações estão a Lei nº 14.785/2023, que trata do controle, registro, fiscalização e classificação de agrotóxicos, e a Lei nº 6.894/1980, regulamentada pelo Decreto nº 4.954/2004, que estabelece normas para fertilizantes, inoculantes e corretivos do solo. A Instrução Normativa nº 13 de 2011 do Ministério da Agricultura, Pecuária e Abastecimento (MAPA) define inoculantes como substâncias contendo microrganismos com atuação favorável ao desenvolvimento vegetal, regulamentando sua produção, comercialização e aplicação.

O Decreto nº 10.375/2020 também define uma categoria específica de bioinsumos denominada bioestimulantes, descritos como produtos que contêm substâncias naturais, com diferentes composições e proporções, capazes de atuar direta ou indiretamente sobre plantas cultivadas. A aplicação de bioestimulantes tem como objetivo incrementar a produção, melhorar a qualidade das sementes, estimular o desenvolvimento radicular e favorecer o equilíbrio hormonal da planta. Além disso, eles podem acelerar a germinação, estimular a divisão celular, a diferenciação e o alongamento celular, promovendo uma germinação mais rápida e uniforme.

. No entanto, apesar dessa definição oficial presente no decreto, não existe uma definição de bioestimulante que tenha efeito legal reconhecido de forma unificada pelos reguladores internacionais, o que gera desafios para sua classificação e regulamentação em nível global.

A ausência de um consenso internacional sobre a definição de bioestimulantes reflete a diversidade de produtos e processos associados a essa categoria, dificultando a criação de uma legislação harmonizada entre diferentes países. No Brasil, enquanto os bioestimulantes não possuem uma regulamentação específica detalhada, sua definição no âmbito do Programa Nacional de Bioinsumos reforça seu papel como uma ferramenta importante para aumentar a produtividade agrícola de forma sustentável, sem depender de substâncias agrotóxicas.

2.1.2 Produção e Comercialização de Bioinsumos: Requisitos Legais

Para garantir a qualidade e a segurança dos bioinsumos produzidos e comercializados, a legislação brasileira estabelece uma série de exigências. De acordo com o Decreto nº 4.954 de 2004, todos os estabelecimentos que produzem, comercializam, exportam ou importam fertilizantes, inoculantes, biofertilizantes e corretivos devem se registrar junto ao MAPA. Além disso, esses estabelecimentos precisam contar com um laboratório habilitado – próprio ou terceirizado – para realizar análises de controle de qualidade.

No caso específico dos inoculantes, a Instrução Normativa nº 13 de 2011 determina que todos os produtos fabricados, importados ou comercializados no território nacional devem ser registrados no MAPA. Os produtores e importadores de inoculantes devem adquirir, anualmente, os microrganismos utilizados na produção de uma instituição responsável pela manutenção de bancos de germoplasma, assegurando a identidade e a pureza do material biológico.

O decreto também estabelece critérios rigorosos para a produção, como a necessidade de suporte esterilizado, estrutura física adequada, meio de cultura apropriado, equipamentos de qualidade e metodologias padronizadas. Essas exigências visam garantir a eficácia dos bioinsumos, evitando riscos ambientais e sanitários.

2.2 A Brecha Legal na Produção *On-Farm*

Antes da publicação da Lei nº 15.070/2024 a produção *on-farm* de bioinsumos – caracterizada pela multiplicação caseira de microrganismos na própria propriedade rural era uma prática comum e incentivada, mas enfrentava um vácuo regulatório. O Decreto nº 4.954 de 2004 isentava do registro os bioinsumos destinados ao uso próprio, desde que não utilizassem aditivos químicos. Esse cenário abriu brechas legais que permitiam a produção e a comercialização informal de bioinsumos, muitas vezes sem a devida padronização e controle de qualidade.

Embora essa prática tenha oferecido uma alternativa econômica para pequenos e médios agricultores, também gerou riscos legais, ambientais e à saúde humana, devido à ausência de fiscalização adequada. A produção *on-farm* em pequena escala, frequentemente realizada com infraestrutura limitada, contrastava com a produção industrial, que segue rígidos padrões de qualidade.

2.2.1 Riscos Associados à Produção *On-Farm* de Bioinsumos

Apesar das vantagens econômicas e ambientais a produção *on-farm* de bioinsumos apresenta riscos significativos relacionados à qualidade, segurança e eficácia dos produtos. Estudos realizados em diferentes regiões do Brasil demonstram que a ausência de controle rigoroso no processo produtivo pode levar à contaminação por microrganismos patogênicos. Por exemplo, Valicente et al. (2018) avaliaram a qualidade de biopesticidas de *Bacillus thuringiensis* produzidos por três agricultores no Mato Grosso e encontraram contaminação em todas as amostras, incluindo a presença de microrganismos potencialmente patogênicos, como *Bacillus cereus*, *Microbacterium sp.* e *Enterococcus casseliflavus*.

Outro estudo relevante, realizado por Santos et al. (2020) no Vale do Rio São Francisco, avaliou amostras de bioinsumos de cinco produtores on-farm e detectou contaminação em 100% das amostras. Dentre os contaminantes, 84% das amostras continham coliformes fecais, 75% microrganismos termotolerantes e 75% *Salmonella* sp.. Esses resultados evidenciam o potencial risco para a saúde dos manipuladores e consumidores finais, além de comprometer a eficiência agrônômica dos bioinsumos utilizados.

Esses dados evidenciam que, embora a produção *on-farm* seja uma alternativa viável e de baixo custo, a falta de padronização, infraestrutura adequada e controle de qualidade representa um risco potencial à saúde humana, animal e ambiental. A multiplicação de microrganismos em condições inadequadas pode resultar em produtos de baixa qualidade, comprometendo a eficiência agrônômica e a segurança do uso agrícola. Portanto, o desenvolvimento de boas práticas de fabricação, a assistência técnica e o acompanhamento regulatório são essenciais para minimizar esses riscos e garantir a sustentabilidade dessa prática.

2.3 Inoculantes Comerciais de Fungos Micorrízicos Arbusculares e Desafios na Produção

Os fungos micorrízicos arbusculares (FMAs) desempenham um papel fundamental na sustentabilidade dos sistemas agrícolas, contribuindo para o aumento da eficiência no uso de nutrientes, a saúde do solo e a resiliência das plantas frente a estresses abióticos. A simbiose micorrízica é amplamente reconhecida por melhorar a absorção de fósforo e outros nutrientes pouco móveis no solo, além de favorecer a estruturação do solo e a proteção das plantas contra patógenos do sistema radicular. Esses benefícios têm impulsionado o interesse no desenvolvimento e uso de inoculantes comerciais de FMAs como uma alternativa sustentável aos fertilizantes químicos. No entanto, a produção de inoculantes comerciais de FMAs enfrenta desafios únicos, principalmente devido às características biológicas desses fungos.

Diferentemente de outros microrganismos utilizados como bioinsumos, os FMAs são obrigatoriamente simbióticos, ou seja, dependem de uma planta hospedeira para completar seu ciclo de vida. Isso torna a produção em larga escala mais complexa e onerosa em comparação com a produção de inoculantes bacterianos, como os de *Bradyrhizobium* e *Azospirillum*.

Os principais desafios na produção de inoculantes de FMAs:

- **Produção em sistemas simbióticos:** Os FMAs não podem ser cultivados em meios de cultura sintéticos, exigindo sistemas simbióticos que envolvem plantas hospedeiras. Os métodos mais comuns de produção incluem o cultivo em substrato sólido (em viveiros ou bandejas) exigindo infraestrutura e condições controladas de temperatura, umidade e luz. Esse processo é lento e requer espaço físico considerável, além de demandar um tempo prolongado para o desenvolvimento dos propágulos viáveis (esporos, fragmentos de hifas e raízes colonizadas).
- **Padronização e viabilidade dos propágulos:** A variabilidade biológica dos FMAs dificulta a padronização dos inoculantes comerciais. Diferentes espécies de FMAs possuem taxas distintas de colonização e eficácia em diferentes condições edafoclimáticas. Além disso, garantir a viabilidade dos propágulos durante o armazenamento e o transporte é um desafio significativo, já que os esporos precisam manter sua capacidade de germinação e colonização ao longo do tempo.
- **Controle de qualidade:** A produção de inoculantes de qualidade requer controle rigoroso em todas as etapas, desde a seleção das espécies de FMAs até a comercialização. É necessário assegurar que os produtos contenham uma quantidade mínima de propágulos viáveis e livres de contaminantes. Contaminações por outros fungos ou bactérias durante a produção podem comprometer a eficácia do inoculante e a segurança de seu uso no campo.

- **Especificidade entre fungo e planta hospedeira:** A eficácia dos FMAs depende da compatibilidade entre o fungo e a planta hospedeira. Diferentes espécies de FMAs apresentam graus variados de simbiose com plantas de diferentes famílias botânicas. Assim, a seleção de espécies eficientes para cada tipo de cultura é um desafio técnico que exige pesquisa e desenvolvimento contínuos.
- **Baixa competitividade com fungos nativos:** No campo, os FMAs presentes no inoculante precisam competir com a microbiota nativa do solo para colonizar as raízes das plantas. A eficácia do inoculante pode ser reduzida em solos já ricos em fungos micorrízicos nativos ou em condições adversas que favoreçam a microbiota local.
- **Custo de produção e escalabilidade:** O custo elevado de produção é outro fator limitante para a adoção em larga escala dos inoculantes de FMAs. A necessidade de sistemas complexos de cultivo simbiótico e de mão de obra especializada encarece o processo produtivo. Além disso, a escalabilidade da produção enfrenta barreiras logísticas relacionadas ao manejo e à distribuição de grandes volumes de substrato colonizado.
- **Regulamentação e registro:** Assim como outros bioinsumos, os inoculantes de FMAs estão sujeitos à regulamentação pelo Ministério da Agricultura, Pecuária e Abastecimento (MAPA). O registro exige comprovação de eficácia agrônômica, estabilidade do produto e ausência de contaminantes. O processo de registro pode ser demorado e oneroso, dificultando a entrada de novos produtos no mercado.

Os inoculantes comerciais de fungos micorrízicos arbusculares representam uma ferramenta promissora para a agricultura sustentável, contribuindo para a redução do uso de fertilizantes químicos e para a melhoria da qualidade do solo. No entanto, os desafios associados à produção, padronização e registro ainda limitam a expansão desse mercado. O desenvolvimento de tecnologias inovadoras, associado a políticas públicas que incentivem a pesquisa e a adoção desses produtos, é essencial para superar essas barreiras e ampliar o uso de inoculantes micorrízicos na agricultura.

No Brasil, o único produto comercial disponível atualmente é o **Rootella®**, um inoculante micorrízico baseado em esporos viáveis de *Rhizophagus irregularis*, amplamente utilizado em culturas como soja, milho e hortaliças. O Rootella® é produzido utilizando tecnologia de cultivo simbiótico em condições controladas, garantindo alta pureza e concentração de propágulos viáveis, o que assegura sua eficácia no campo. No entanto, devido ao processo produtivo complexo e ao tempo necessário para a multiplicação dos FMAs, o custo do produto comercial ainda é elevado, o que pode limitar sua adoção por pequenos e médios produtores.

2.3.1 Desafios da produção *on-farm* de FMAs

Embora a produção *on-farm* de bioinsumos, como inoculantes bacterianos, tenha se tornado uma prática viável em algumas regiões, a produção *on-farm* de FMAs enfrenta obstáculos que dificultam sua disseminação. Os FMAs exigem plantas hospedeiras para completar seu ciclo de vida, tornando a produção mais complexa e demorada. Além disso, a necessidade de infraestrutura adequada, como viveiros e substratos esterilizados, eleva os custos iniciais e dificulta a implantação por pequenos agricultores. A baixa padronização dos produtos resultantes e o alto risco de contaminação por microrganismos oportunistas comprometem a eficácia agrônômica e a segurança do uso. Ainda, a competição com fungos nativos no solo reduz a eficiência dos inoculantes *on-farm*, enquanto restrições legais impostas pela Lei nº 15.070/2024 proíbem sua comercialização sem registro, restringindo essa prática ao uso próprio em pequenas propriedades.

Nos últimos anos, tem crescido a oferta de inoculantes micorrízicos vendidos pela internet, especialmente voltados para fins de jardinagem e pequenos cultivos. No entanto, muitos desses produtos não atendem aos critérios mínimos de qualidade e produção exigidos pela legislação, apresentando graves problemas, como ausência de registro no Ministério da Agricultura, Pecuária e Abastecimento (MAPA) e falta de controle de qualidade.

Em diversos casos, análises laboratoriais apontam que os produtos comercializados não contêm a espécie de fungo micorrízico alegada, ou apresentam concentrações extremamente baixas de propágulos viáveis, o que compromete completamente a eficácia agrônômica. Além disso, a ausência de garantias quanto à pureza e à ausência de contaminantes microbiológicos representa um risco potencial ao solo e às plantas. Dessa forma, a aquisição de produtos não regulamentados pode resultar em perdas econômicas e agrônômicas, reforçando a necessidade de maior fiscalização e conscientização sobre a importância de utilizar inoculantes devidamente registrados e certificados.

Como um simbiote obrigatório os FMAs demandam de seus hospedeiros carbono para completar seu ciclo de vida. Nesta simbiose, os FMAs podem promover o crescimento vegetal, maior sanidade e tolerância a agentes estressores como déficit hídrico, ataque de patógenos entre outros (St-Arnaud and Elsen 2005; Subramanian and Charest 2009). Ademais, sua função de aporte nutricional é de extrema relevância para sustentabilidade na produção agrícola (Gemma et al. 1997).

A produção industrial de inoculantes a base de FMAs é relativamente nova e mesmo após com a comprovação da sua eficiência agrônômica a adoção destes inoculantes ainda tem sido baixa. Já a alguns anos tem sido produzido a nível mundial inoculantes a base de FMAs, sendo comumente encontrado inoculante a base de uma espécie isolada. Entretanto, espécies nativas mostram-se mais efetivas no estabelecimento da simbiose micorrízica do que espécies introduzidas ao sistema, como evidenciado pelos autores Lambert et al. (1980); Henkel et al, (1989); Calvente et al, (2004) and Rowe et al, (2007).

Alternativas têm sido exploradas para aumentar a eficiência e viabilizar o uso desses fungos no campo. Entre essas alternativas, destacam-se os indutores de colonização, substâncias que estimulam a formação e o desenvolvimento da simbiose micorrízica, mesmo em solos onde os fungos micorrízicos arbusculares já estão presentes. Esses produtos podem atuar como catalisadores, promovendo a micorrização de forma mais eficiente e rápida, reduzindo a necessidade de aplicação direta de esporos ou propágulos viáveis.

Myconate® e Mycoform® são produtos derivados sintéticos de um isoflavonóide chamado formononetina, comercializados internacionalmente pela empresa Pant Health Care. A formononetina é um composto natural encontrado em plantas leguminosas, que desempenha um papel importante na sinalização química entre plantas e fungos micorrízicos, estimulando a formação de hifas e a colonização radicular. Embora esses produtos tenham sido testados no Brasil com resultados promissores, não há comercialização regular no país devido à ausência de registro e regulamentação.

O uso de indutores de colonização apresenta diversas vantagens, como a redução da necessidade de altos volumes de inoculantes, a possibilidade de aplicação em solos já colonizados por fungos nativos e a facilitação do manejo micorrízico em grandes áreas. No entanto, a ausência de produtos regulamentados e disponíveis comercialmente limitam o uso dos FMAs na agricultura atualmente.

A produção de inoculantes em larga escala sempre foi um desafio e o grande obstáculo está na dificuldade de geração de propágulos de maneira uniforme. Além disso, o tempo de produção do inoculante por cultura armadilha é extenso podendo levar até 4 meses. A característica de biotrófico obrigatório é um fator do qual a multiplicação dos FMAs torna-se limitante pela necessidade da associação com a planta viva para completar seu ciclo de vida. A transformação genética de raízes com *Agrobacterium rhizogenes* aplicada em cultura com esporos de FMAs é uma possibilidade de produzir inóculo em condições axénicas. A técnica conhecida como Técnica de cultura de raízes orgânicas ou transformadas (CRO) é uma técnica promissora, limpa, viável, e livre de contaminação da qual possibilita estudos para avaliar vários aspectos da simbiose.

Entretanto esta técnica não tem sido aplicada para produção de inóculo, a nível comercial a produção de culturas puras de FMAs é mais recorrente. Esta multiplicação normalmente ocorre com o uso de substrato estéril, um hospedeiro e diferentes fontes de propágulos de uma espécie de FMA (Morton et al. 1995). Entretanto, há inúmeras problemáticas a respeito dos inóculos comerciais, onde o potencial destes produtos como a concentração dos esporos, infectividade dos propágulos não são conhecidas.

Corkidi et al, (2004) realizou um estudo onde avaliou-se 10 inóculo comerciais de FMAs, destes, metade dos inóculos testados reprovaram nos testes com plantas. Ademais, outros estudos relataram os mesmos problemas com inoculantes comerciais (Tarbell & Koske, 2007; Gaur et al. (1998) and Rowe et al. (2007). Uma das justificativas seria da baixa adaptação das espécies comercializadas as condições edáficas locais. Ademais a introdução de espécies exóticas tem potencial risco de afetar a estrutura e composição de comunidades nativas (Mummey et al, 2009).

Uma alternativa sustentável e viável seria a multiplicação On farm de espécies de FMAs nativa. Em um estudo realizado os autores propuseram método de multiplicação de espécies nativas adaptadas ao clima temperado possível de ser realizado por agricultores. O substrato composto por vermiculita foi a alternativa que se obteve maior quantidade de propágulos fúngicos e mostrou-se uma alternativa sustentável de ser reproduzida por agricultores para produção On-farm de inoculantes micorrízicos (Douds-Jr et al, 2010). Além da produção industrial e *on-farm* de inoculantes micorrízicos, Brasil limita sua adoção. A pesquisa científica e a inovação tecnológica na área de bioinsumos podem contribuir para a criação de novos indutores de colonização viáveis e acessíveis ao mercado nacional, ampliando o uso da simbiose micorrízica em sistemas agrícolas.

Referências

BRASIL. Decreto no 10.375, de 26 de maio de 2020. Programa Nacional de Bioinsumos. Diário Oficial da União, seção 1, edição 100, p. 105, 27 maio 2020.

CORKIDI, L.; EDITH, B. A.; DONALD, M.; ALLEN, M.F.; DOWNER, J.; BOHN, J.; EVANS, M. Assessing the infectivity of commercial mycorrhizal inoculants in plant nursery conditions. **Journal of Environmental Horticulture**, v. 22, n. 3, p. 149-154, 2004.

DOUDS JR, D. D.; NAGAHASHI, G.; PFEFFER, P.E.; KAYSER, W. M.; REIDER, C. On-farm production and utilization of arbuscular mycorrhizal fungus inoculum. **Canadian Journal of Plant Science**, v. 85, n. 1, p. 15-21, 2005.

GAUR, A.; ADHOLEYA, A.; MUKERJI, K.G. A comparison of AM fungi inoculants using *Capsicum* and *Polianthes* in marginal soil amended with organic matter. **Mycorrhiza** 7:307–312, 1998.

SOUZA, F.A; MACENA, L.H.S.; GOMES-JÚNIOR, C.C.; MEDEIROS, K.M.R.; CARNEIRO, A.A. Fungo micorrízico arbuscular com potencial para o desenvolvimento de inoculante para a cultura do milho e para a produção de mudas florestais. EMBRAPA, Circular técnica, 2021.

MORTON, J.B.; BENTIVENGA, S.P.; BEVER, J.D. Discovery measurement and interpretation of diversity in arbuscular endomycorrhizal fungi (Glomales, Zygomycetes). **Can J Bot** 73:25–32, 1995.

MUMMEY, D. L.; ANTUNES, P. M.; RILLIG, M. C. Arbuscular mycorrhizal fungi pre-inoculant identity determines community composition in roots. **Soil Biology and Biochemistry**, v. 41, n. 6, p. 1173-1179, 2009.

ROWE, H.I.; BROWN, C.S.; CLAASSEN, V.P. Comparison of mycorrhizal responsiveness with field soil and commercial inoculum for six native Montane species and *Bromus tectorum*. **Restor Ecol** 15:44–5, 2007.

TARBELL, T. J.; KOSKE, R. E. Evaluation of commercial arbuscular mycorrhizal inocula in a sand/peat medium. **Mycorrhiza**, v. 18, p. 51-56, 2007.

Tópico 3: Extração de esporos e espécies de Fungos micorrízicos arbusculares: ocorrência e potencial de uso

Os fungos micorrízicos arbusculares (FMAs) são onipresentes na maioria dos ambientes terrestres, e há evidências de que eles contribuem para a diversificação das plantas e a produtividade do ecossistema (VAN DER HEIDJEN et al., 1998; FERNANDES et al., 2016).

Os FMAs são encontrados em muitos tipos diferentes de habitats, incluindo desertos, florestas tropicais e florestas temperadas (BRUNDRETT, 1991; CEOLA et al., 2021). Ocorrendo naturalmente nos solos que contêm uma variedade de fontes de propagação, incluindo esporos, hifas extra-radiculares e raízes colonizadas. Os métodos aplicados para avaliar a ocorrência e potencial de inóculo dos FMAs serão descritos na sequência desse tópico.

3.1 Técnica Extração de esporos

Para avaliar a ocorrência de FMAs é realizada a extração de esporos, os quais são pequenas estruturas que variam de 39 μm (*Paraglomus occultum*) a 1050 μm (.e. *Dentiscutata nigra*), variando de acordo com a espécie. Devido ao tamanho, não é possível enxergar grande maioria a olho nu, além dos esporos estarem distribuídos no perfil do solo de forma não esperada, o que gera a necessidade de realizar a extração dos esporos. Uma das técnicas mais antigas utilizadas para extração foi descrita por Gerdemann e Nicolson (1963) onde a partir de um volume conhecido de solo, cujo volume é suspenso mais de 10 vezes em água de torneira, os esporos do solo são retidos em peneiras após decantação e avaliados através de microscopia. A união deste método com Daniel e Skipper (1982), que propõem a centrifugação com gradiente de sacarose, tornou-se o método mais comum, conforme indicado pelo INVAM (*The International Collection of Arbuscular Mycorrhizal Fungi*).

3.3.1 Equipamentos, matérias e soluções necessários

- Centrífuga;
- Microscópio ótico;
- Tubos de plástico de 50mL;
- Grades para tubos;
- Espátulas;
- Peneiras de 45 μ m (A depender da espécie a ser isolada);
- Peneiras de 300 μ m (A depender da espécie a ser isolada);
- Placa de Petri;
- Papel toalha;
- Becker de plástico (100ml);
- Becker de plástico (1000ml);
- Becker de plástico (2000ml);
- 2 Frasco de borossilicato (1000ml);
- 2 Bastão de vidro;
- Lâminas de vidro para microscopia;
- Lamínulas (20mm);
- Reagentes (Solução sacarose ou açúcar 20 e 60%).

Solução sacarose 20%: Pesar 200g de sacarose/açúcar em um Becker em uma balança semi-analitica após tarar o becker e pesar 800g de água destilada. Homogeneizar bem a solução e transferir para um frasco de borossilicato.

Solução sacarose/açúcar 60%: Pesar 600g de sacarose/açúcar em um Becker em uma balança semi-analitica após tarar o Becker e pesar 400g de água destilada. Homogeneizar bem a solução e transferir para um frasco de borossilicato.

3.3.2 Procedimentos laboratoriais

É pesado uma alíquota de solo de 50g, sendo importante que as amostras incluam raízes visto que algumas espécies de FMAs produzem esporos intraradiculares. A amostra é colocada em um becker plástico com capacidade de 2 L, suspendida com água de torneira (até a marca de 1,7 L) e agitada vigorosamente com um bastão de vidro. Após, a suspensão é passada através de duas peneiras sobrepostas, tomando-se o cuidado para que a maioria das partículas de solo fique retida no balde plástico. A peneira de baixo possui abertura de 45 μm onde a maioria dos esporos ficará retido. A peneira de cima possui abertura de 350 μm . Nesta peneira ficará retido as raízes de maior calibre, detritos orgânicos e partículas maiores do solo (areia) e esporos grandes e esporocarpos.

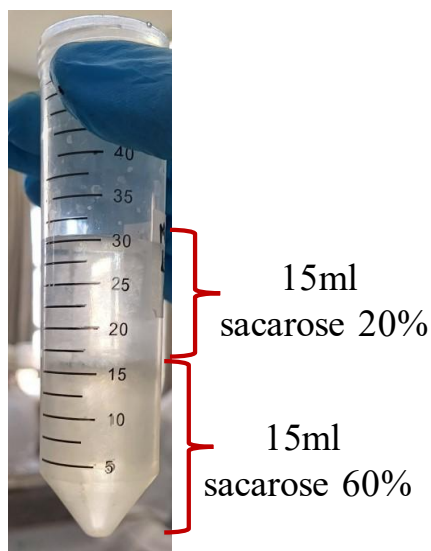
A escolha do diâmetro das peneiras deverá ser conforme o objetivo, para extração de esporos de uma população nativa, importante utilizar até três peneiras para facilitar a visualização. Entretanto, havendo o objetivo de extrair um inóculo puro de uma espécie conhecida a escolha das peneiras irá variar pelo diâmetro dos esporos da espécie que se irá trabalhar. Por exemplo: Esporos da espécie *Rhizoglyphus clarum* apresentam diâmetro de 100-260. Neste caso, havendo a disponibilidade de peneiras, usaria a de menor abertura como sendo 80 μm e a de maior abertura 300 μm .

O material retido na peneira de 350 μm é transferido para uma placa de Petri grande e inspecionada na lupa para observação e coletada de esporos grandes e esporocarpos. O material retido na peneira de 45 μm é inicialmente coletado em becker com ajuda de um “rubber policeman” preso a um bastão de vidro ou uma espátula e então transferido para tubos Falcon de 50 ml contendo o gradiente de 20% e 60% de sacarose (15 ml de cada uma das soluções). Os tubos são então centrifugados por 1 minuto a 2000 rpm. Neste processo, o solo e outras partículas sedimentam e os esporos e partículas orgânicas finas ficam suspensos na sacarose (Figura 2).

O sobrenadante de cada tubo é então rapidamente decantado em uma peneira de 45 μm feita de inox. Esta peneira tem a mesma abertura que a peneira utilizada na peneiragem úmida, porém tem um tamanho menor. O material coletado nesta peneira é lavada por 1 minuto com água de torneira para remover o excesso de sacarose e transferido para placas de Petri. Os esporos são observados na lupa e coletados manualmente com uma pipeta de Pasteur com a ponta afinada no fogo.

Figura 2. Resumo do procedimento de extração de esporos

1) Preparo do gradiente sacarose



2) Peneiragem úmida

50g solo



↓ + 1,7L água



3) Inserção no gradiente sacarose

Passar o solo retido na peneira de 45 μm no gradiente



4) Centrifugação

Programa

Rotação: 2000 rpm

Tempo: 2:30 minutos



5) Limpeza dos esporos

Despejar o conteúdo do tubo centrifugado na peneira de 45 μm



3.3.2 Contagem de esporos

A contagem de esporos é feita utilizando a uma placa de Petri marcadas com grade de 1cm², para facilitar a localização dos esporos na placa.

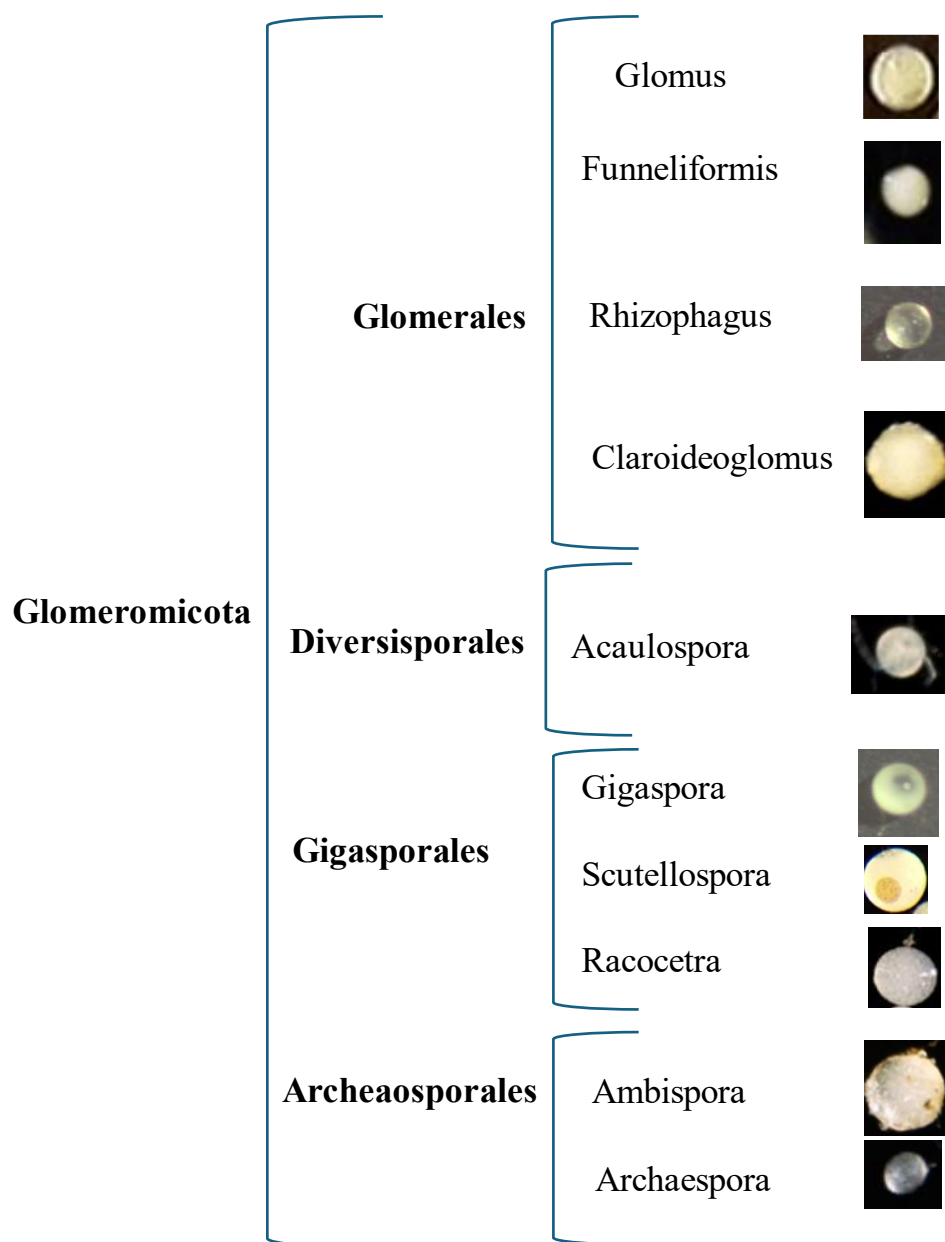
3.3.2 Identificação taxonômica de FMAs

Toda a base taxonômica empregada neste grupo específico de fungos está relacionada à estrutura reprodutiva, denominada glomerosporo, sendo que a delimitação de taxa representa um desafio significativo. Nesse contexto, é de grande importância para a taxonomia do grupo a divulgação dos fundamentos (caracteres morfológicos associados à classificação e identificação de famílias, gêneros e espécies) da sistemática, a fim de que novas evidências possam ser rapidamente integradas e propiciar a expansão do conhecimento científico.

Walker (1983) propôs uma sistematização para a descrição de espécies de fungos micorrízicos arbusculares (FMA), introduzindo uma terminologia específica para classificar os tipos de “paredes” (ou camadas) dos glomerosporos: evanescente, laminada, unitária e membranosa. Além dessa classificação, o autor desenvolveu um esquema representativo, denominado murografia, para ilustrar a disposição dessas paredes no esporo. Esse conceito foi amplamente adotado pelos taxonomistas na descrição de novas espécies e, ao longo do tempo, novas categorias de “paredes” características foram sugeridas dentro deste grupo.

Os principais gêneros que são encontrados no Brasil são ilustrados na figura abaixo (Figura 3).

Figura 3. Principais de Gêneros de FMAs que ocorrem em solos Brasileiros



3.4 Atividade prática

- Extrair esporos de diferentes amostras de solos agrícolas;
- Comparar o número e esporos e as características dos esporos encontrados;

Tabela 1. Número de esporos.

Amostra	R1	R2	R3
Solo 1-			
Solo 2-			
Solo 3-			

- Selecione os diferentes tipos de esporos quanto ao formato.

Referências

BRUNDRETT, M. Mycorrhizas in natural ecosystems. In *Advances in ecological research* (Vol. 21, pp. 1 71-313). Academic Press, 1991.

CEOLA, G.; GOSS-SOUZA, D., ALVES, J., ALVES DA SILVA, A., STÜRMER, S. L., BARETTA, D., KLAUBERG-FILHO, O. (2021). Biogeographic patterns of arbuscular mycorrhizal fungal communities along a land-use intensification gradient in the subtropical atlantic forest biome. **Microbial ecology**, 1-19.

DANIELS, B.A.; SKIPPER, H.A. Methods for the recovery and quantitative estimation of propagules from soil. In: Schenck, N.C., Ed., *Methods & Principles of Mycorrhizal Research*, **American Phytopathological Society**, St. Paul, 29-35, 1982.

FERNANDES, R. A.; FERREIRA, D. A.; SAGGIN-JUNIOR, O. J.; STÜRMER, S. L.; PAULINO, H. B.; SIQUEIRA, J. O.; CARNEIRO, M. A. C. Occurrence and species richness of mycorrhizal fungi in soil under different land use. **Canadian Journal of Soil Science**, v. 96, n. 3, p. 271-280, 2016.

GERDEMANN, J.W.; NICOLSON, T.H. Spores of Mycorrhizal Endogone Species Extracted from Soil by Wet Sieving and Decanting. **Transactions of the British Mycological Society**, 46, 235-244, 1963.

VAN DER HEIDJEN, M.G.A.; BOLLER, T.; WIENKEN, A.; SANDERS, I.R.. Different arbuscular mycorrhizal fungal species are potential determinants of plant community structure. **Ecology**, 79(6): 2082–2091, 1998.

Tópico 4: Potencial de inóculo em sistemas agrícolas e colonização micorrízica em plantas de interesse agrícola

4.1 Técnica do Potencial de Inóculo

O potencial de inóculo micorrízico (PIM) refere-se à quantidade e viabilidade de esporos, hifas e raízes colonizadas por FMAs presentes no solo ou substratos, que podem potencialmente colonizar as raízes das plantas e formar relações simbióticas com elas. O potencial de inóculo dos FMAs pode influenciar o estabelecimento, o crescimento e a produtividade das plantas, bem como a saúde do solo e a ciclagem de nutrientes. O PIM é avaliado para determinar porcentagem de colonização no sistema radicular de plantas agrícolas (alho, *Brachiaria*) cultivadas por 30 dias em solos, resultando assim na capacidade de um inóculo em estabelecer simbiose com plantas.

4.2 Técnica da Extração de hifas

As hifas dos fungos micorrízicos arbusculares (FMA) funcionam como extensão das raízes das plantas, uma vez que, devido à sua grande capacidade de ramificação, exploram o solo, realizando absorção de água e nutrientes minerais, que são transferidos para as plantas por meio de estruturas intracelulares (SOUZA et al., 2017), além disso essa rede que as hifas criam vão atuar na estruturação do solo, formando uma extensa malha que contribui significativamente para a biomassa fúngica.

Considerando que uma parcela significativa do comprimento total de hifas no solo é atribuída aos FMA, estima-se que sua biomassa possa alcançar até 1 tonelada por hectare nos 20 cm superficiais do perfil do solo. Além disso, as hifas dos FMA representam cerca de 20 a 30% da biomassa microbiana total do solo, desempenhando um papel crucial na agregação do solo e na formação de macroagregados. Portanto, o comprimento das hifas dos FMA é um parâmetro vital na quantificação da biomassa fúngica, influenciando diretamente a saúde e a estrutura do solo. A quantificação do comprimento de micélio extraradicular total no solo é realizada conforme descrito por Melloni, (1996).

4.2.1 Equipamentos e matérias

- Liquidificador (Capacidade para 1.5 L);
- Bomba de vácuo
- Microscópio com aumento de 400x (ocular 10x e objetiva 40x);
- Porta filtro;
- Becker de plástico (1000ml; 2000ml);
- Peneiras (150 μm , 0,63 μm e 0,45 μm);
- Membranas de nitrato de celulose (4,7 μm de diâmetro, 0,45 μm de porosidade);
- Grade ocular – 10 x 10 (1mm²);
- Água destilada;
- Lâmina de vidro;
- Bastão de vidro;
- Espátulas;
- Óleo vegetal de amêndoa.

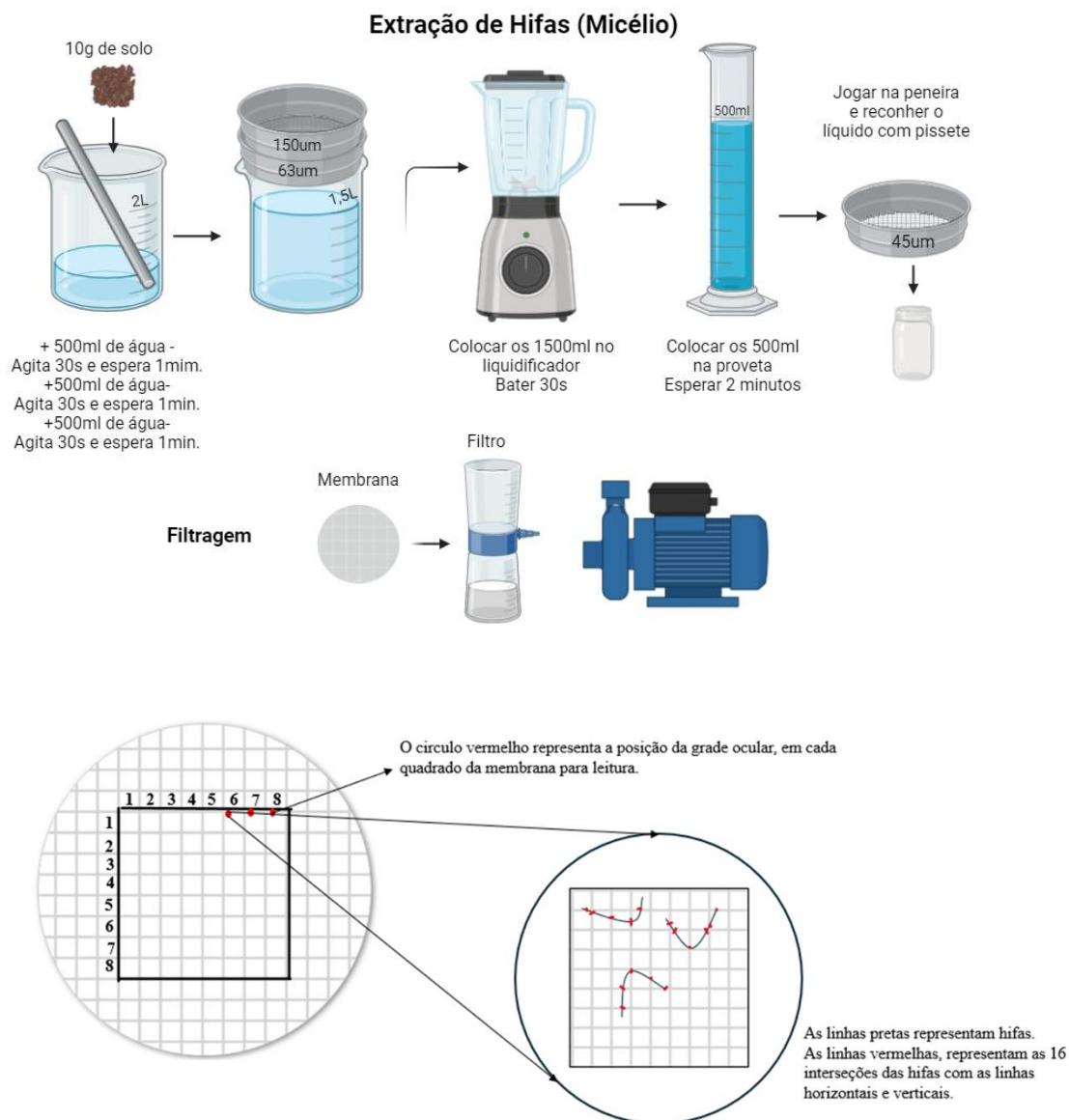
4.2.2 Procedimentos laboratoriais

Colocar uma amostra de 10g de solo, em um Becker de 100ml, colocar 500ml de água da torneira, agitar com bastão de vidro por 30 segundo deixar repousar por 1 minutos, sobrepor as peneiras de 150 μm em cima da e 0,63 μm , em cima de um Becker de 2000ml, esta operação deverá ser realizada por três vezes e o filtrado será submetido à agitação em liquidificador durante 30 segundos na menor velocidade. Após um período de repouso de 2 minutos, retirar 500 mL de sobrenadante passando por uma peneira de 0,045 μm .

O material retido na peneira e filtrado a vácuo em membrana quadriculada de celulose nitrato com diâmetro de 4,7 cm e porosidade de 0,47 μm . Em seguida, a membrana e colocada sob lâmina de vidro de 5 x 5 cm, lubrificada com uma gota de óleo de amêndoas para facilitar a visualização no microscópio óptico. São avaliados 64 campos em cada membrana, determinando-se o número de intersecções de hifas com as linhas horizontais de uma grade (8 x 8 quadrículos de 1 mm) na ocular do microscópio no aumento de 40 vezes.

Em uma das oculares foi acoplada uma lente com grade de 20 x 20 quadrículos. Para determinar o número de interseções das hifas com a linhas horizontais da grade da ocular. O comprimento do micélio extrarradicular total, expresso em centímetros de hifa por grama de solo seco, e obtido pela seguinte relação: $C = [(0,0347 \cdot N) / (10 - U)] \cdot 100$, onde: C = comprimento de micélio extrarradicular total, em centímetros de hifa por grama de solo seco; N = soma do número de interseções entre as hifas e linhas horizontais e verticais do gride; 110 U = umidade da amostra de solo, expressa em gramas de água. O processo pode ser visualizado na Figura 4.

Figura 4. Resumo do procedimento de extração e leituras de hifas



4.3 Técnica da Colonização micorrízica

A medida de colonização micorrízica é um indicativo da presença de FMAs e a da simbiose efetiva. A simbiose se inicia através de sinalização bioquímica, as plantas exsudam as estrigolactonas que são sinais positivos para os FMAs que liberam oligossacarídeos para sinalizar o início do estabelecimento da simbiose (CHO; SUMMERS; PASZKOWSKI, 2018).

A partir disso o fungo inicia a colonização nas células radiculares, penetrando nas células com as hifas e posteriormente formando os arbúsculos, que são as estruturas de troca em planta e fungo. Outras estruturas como hifas extraradicular, vesículas e esporos podem ser observados na microscopia.

Para a visualização das estruturas é necessário realizar a descoloração das raízes, utilizando uma solução básica (Hidróxido de potássio - KOH) aquecidos em banho maria a 90°C, essa metodologia foi proposta por Koske e Gemma em 1989. Em caso de raízes muito velhas e escuras pode ser necessário adicionar uma etapa utilizando peróxido de hidrogênio (H_2O_2). Posterior a descoloração, é realizado a acidificação que aumenta a afinidade do corante com as paredes fúngica. O corante utilizado por muito tempo foi azul de tripam, mas devido a periculosidade dessa substância foi substituído por tinta de caneta preta, como proposto por Vierheilig et al (1998).

Após o preparo das raízes, é a realizado a microscopia, para essa etapa é importante ter um treinamento prévio com um profissional que conheça e consiga diferenciar as estruturas fúngicas. Ainda para cada planta e fungo, podem existir particulares quanto ao padrão de estruturas na colonização, por isso é importante fazer algumas leituras prévias para definir o que será considerado presença de FMAs.

4.3.1 Equipamentos, matérias e soluções necessários

- Banho maria;
- Microscópio ótico;

- Tubos de plástico ou tubos de ensaio de 15mL;
- Grades para tubos (Resistentes ao calor);
- Pinças;
- Peneiras;
- Bisturi + lâmina;
- Placa de Petri;
- Papel toalha;
- Becker de plástico (500ml);
- Lâminas de vidro para microscopia;
- Lamínulas (20mm);
- Reagentes (Hidróxido de potássio; Ácido acético glacial; peróxido de hidrogênio; tinta de caneta preta).

Solução 1: Hidróxido de potássio (KOH) 10% - A solução de KOH 10% é preparada adicionando 100g de KOH e aferindo para 1000ml de água destilada;

Solução 2: Solução ácido acético glacial (AAG) 5% - A solução de AAG 5% é preparada utilizando 50ml de AGG e em um balão volumétrico aferindo para 1000mL com água destilada;

Solução 3: Tinta de caneta preta 5% - São utilizados 50mL de tinta de caneta preta (marcas: Sheaffer® e Perklin), e aferindo para 1000mL utilizando a solução 2 como diluente;

Solução 4: Solução de Peróxido de hidrogênio a 15%- Essa solução é utilizada em caso de raízes muito pigmentadas, como raízes provenientes de coleta de campo, ou de plantas com mais de 60 dias.

4.3.2 Procedimentos laboratoriais

As raízes coletadas devem ser higienizadas em água corrente, selecionadas os fragmentos mais finos, e cortadas. Após é realizado a descoloração mediante imersão em KOH 10 %, por 5 a 40 minutos (depende do tipo de raiz) em banho-maria a 90 °C (KOSKE e GEMMA, 1989). A duração do período de cozimento em KOH deve ser previamente testada, esse tempo é variável de acordo com a idade, espessura e pigmentação das raízes.

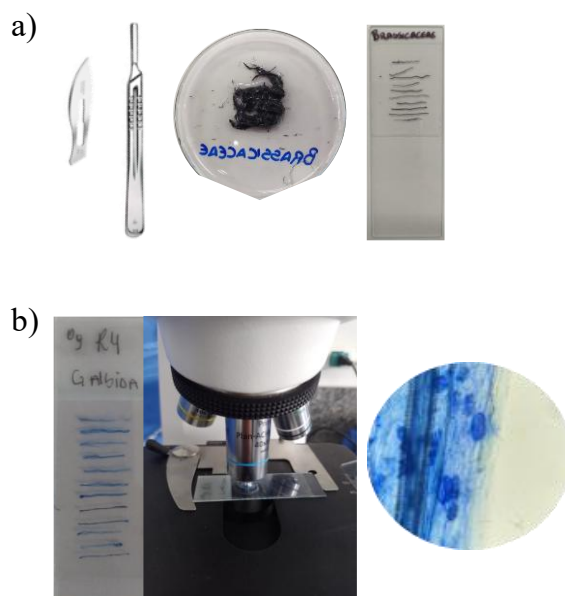
Após o cozimento em KOH, as raízes devem passar por água corrente, e posteriormente acidificadas por 50 min em ácido acético glacial (AAG) a 5 %. Com as raízes descoloridas e acidificadas, é realizada a coloração com tinta de caneta tinteiro preta 5 % por cerca de 8 a 20 minutos (o melhor período deve ser definido por teste prévio) em banho maria a 90 °C (VIERHEILIG et al., 1998). Posterior a coloração, as raízes são lavadas em água corrente e armazenadas em tubos de ensaio com água destilada (Figura 5).

Figura 5. Processo de clarificação e coloração das raízes.



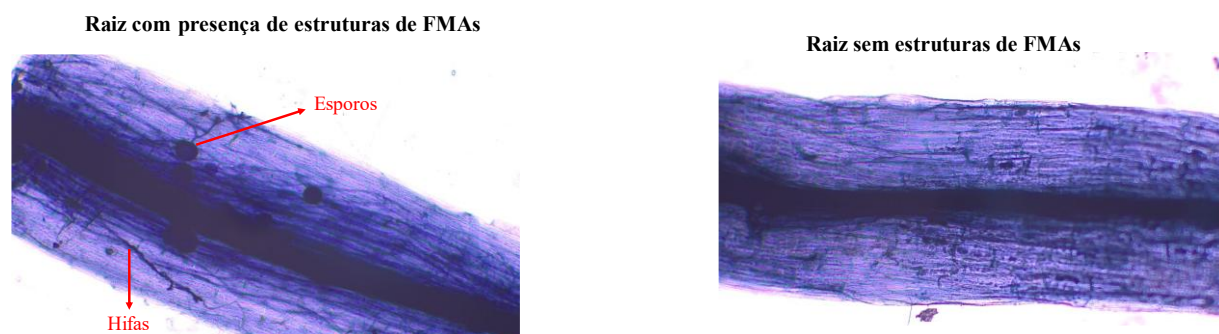
Com as raízes coloridas, é feito o preparo das lâminas para microscopia para isso são alocados 10 fragmentos de raízes em uma lâmina de vidro e na sequência os fragmentos são selados com uma lamínula (Figura 6).

Figura 6. Etapa de preparo de lâminas (a) e microscopia de fragmentos de raízes para avaliar a colonização micorrízica (b).



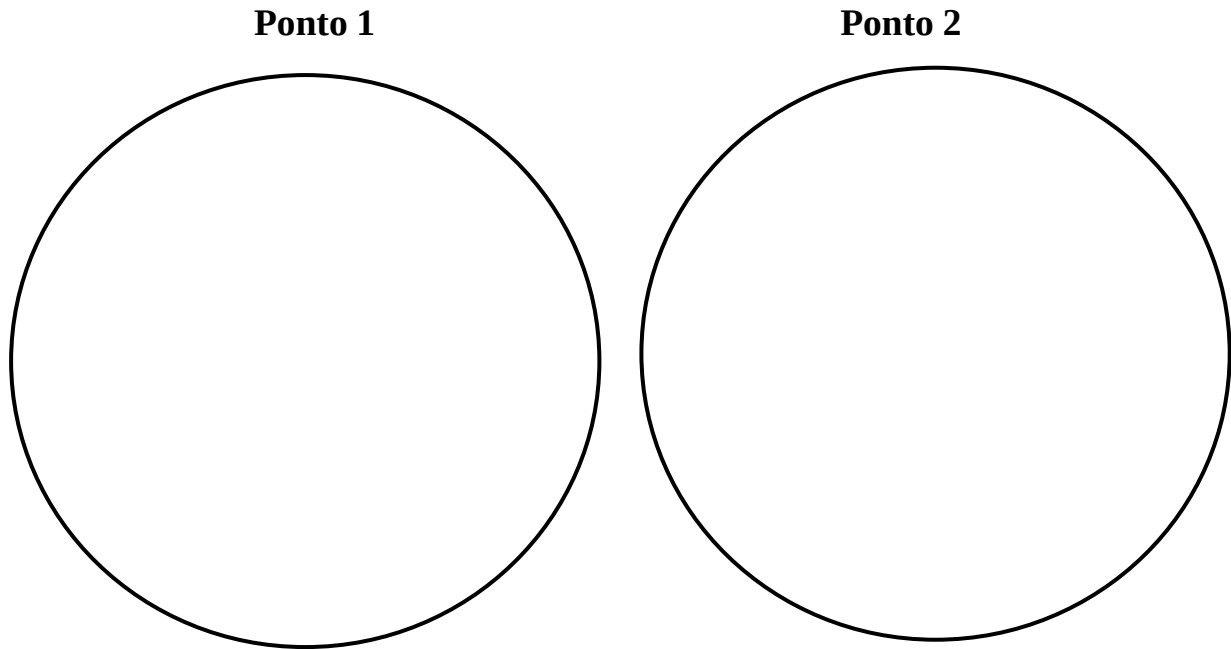
Na microscopia são observadas a presença e ausência de estruturas em 10 pontos por fragmento, totalizando 100 pontos por lâmina. Sendo as estruturas: hifas extra radiculares, vesículas, esporos e arbúsculos (Figura 7).

Figura 7. Microscopia de raízes de alho inoculados com *Rhizophagus irregularis*, exemplo da presença e ausência de estruturas de FMAs.



4.4 Atividade prática

- Montar de 1 lâmina com 10 fragmentos de raízes;
- Desenhe as estruturas presentes em dois pontos observados na microscopia e denomine o que foi encontrado:



Referências

CHOI, J; SUMMERS, W; PASZKOWSKI, U. Mechanisms underlying establishment of arbuscular mycorrhizal symbioses. **Annual Review of Phytopathology**, v. 56, n. 1, p. 135-160, 2018.

KOSKE, R. E.; GEMMA, J. N. A modified procedure for staining roots to detect arbuscular mycorrhizas. **Mycological Research**, New York, v. 92, parte 4, p. 486-505, 1989.

VIERHEILIG, H. Ink and vinegar, a simple staining technique for arbuscular mycorrhizal fungi. **Applied and Environmental Microbiology**, v. 64, n. 12, p. 5004-5007, 1998.

MELLONI, R. Quantificação de micélio extrarradicular de fungos micorrízicos arbusculares em plantas cítricas. Piracicaba, SP, ESALQ, Dissertação (Curso de Mestrado), 1996.



Apoio



UDESC
UNIVERSIDADE
DO ESTADO DE
SANTA CATARINA

LAGES · CAV
CENTRO DE CIÊNCIAS
AGROVETERINÁRIAS



fapesc

Fundação de Amparo à
Pesquisa e Inovação do
Estado de Santa Catarina

2024TR001181
2023TR000306