

Ecotoxicological effects of pre-salt and post-salt drilling cuttings waste on soil invertebrates and plants: Implications for terrestrial ecosystem risk and land management

Dara C. M. V. P. Cruz

Federal University of Santa Catarina

Higor E. F. Lorin

Federal University of Santa Catarina

Fabrielle P. Reis

Federal University of Santa Catarina

Cibele A. Costa

Federal University of Santa Catarina

Simone B. Fontoura

Federal University of Santa Catarina

Heraldo N. Souza

Cenpes

Everaldo Zonta

Federal Rural University of Rio de Janeiro (UFRRJ)

Júlia Carina Niemeyer

`julia.carina@ufsc.br`

Federal University of Santa Catarina

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Abstract

This study assessed the ecotoxicological effects of drilling cuttings waste (DCW) generated from offshore oil exploration, focusing on samples derived from both pre-salt and post-salt geological layers. A multi-species approach was employed, including avoidance and reproduction tests with four soil invertebrates (*Proisotoma minuta*, *Folsomia candida*, *Enchytraeus crypticus*, and *Sinella curviseta*), and growth and germination tests with two plant species (*Lactuca sativa* and *Mimosa scabrella*). Species-specific sensitivity patterns were identified, with *P. minuta* showing the highest sensitivity to pre-salt DCW (EC50: 0.13%) and *S. curviseta* the greatest tolerance (EC50: 6.89%). Notably, *E. crypticus* exhibited higher tolerance to pre-salt than to post-salt residues, indicating that compositional differences influence ecotoxicity. In contrast, plant responses showed no significant effects on germination or biomass development at DCW concentrations up to 5%, suggesting lower susceptibility under short-term exposure. These findings underscore the importance of incorporating ecologically relevant species in ecotoxicity testing to encompass the full range of biological responses and potential risks associated with DCW application in terrestrial ecosystems. Furthermore, the absence of phytotoxicity under tested conditions supports the feasibility of controlled DCW reuse in land management strategies, such as soil restoration and formulation of organomineral fertilisers. However, long-term monitoring and site-specific evaluations are recommended to mitigate potential cumulative impacts.

1. Introduction

Since 1970, global energy consumption has more than doubled, with fossil fuels remaining the dominant energy source, accounting for over 50% of the total supply. Projections indicate no substantial decline in their share in the near future (de Almeida et al., 2017), where oil and gas exploration play a key role in energy supply. However, these operations generate large volumes of waste, particularly during well drilling, producing drilling fluids and drilling cuttings wastes (DCW) (Costa et al., 2023; de Almeida et al., 2017; IOGP, 2016).

DCW are generated from well-drilling activities across diverse regions worldwide, including North America, East Asia, the Middle East, Europe, Africa, and Latin America (Costa et al., 2023). The disposal of this waste presents significant environmental concerns due to the large volumes produced and the presence of potentially toxic components (Costa et al., 2023; de Almeida et al., 2017). The three primary disposal methods are a) offshore discharge, b) offshore reinjection, and c) transportation for onshore treatment, disposal, or recycling (IOGP, 2016). Nevertheless, offshore discharge remains the primary destination for DCW (de Almeida et al., 2017; IOGP, 2016).

DCW consists of drilling rock fragments (Costa et al., 2023; IOGP, 2016) that may contain drilling fluids and contaminants, such as salts, hydrocarbons, and metals (de Almeida et al., 2017), though its composition can vary significantly (Kujawska and Pawłowska, 2022). Consequently, the variability of DCW means there is no standardized composition, posing significant challenges in generalizing its ecotoxicity and environmental impacts (Costa et al., 2023; Kujawska and Pawłowska, 2022).

In Brazil, more than 97% of oil production comes from offshore operations (ANP, 2023). Generally, DCW in this region originates from geological formations composed of carbonates and sandstones, located both in the post-salt layer (3,000 to 5,000 m) and the pre-salt layer (below 5,000 m) (Costa et al., 2023). Where offshore disposal is not viable, due to limited infrastructure or environmental regulations, DCW may be disposed in landfills designed for industrial waste (de Almeida et al., 2017).

To minimize environmental impacts and reduce landfill disposal costs, reuse strategies should be prioritized, as DCW has potential for repurposing (Aslan et al., 2019; Costa et al., 2023).

In this sense, DCW reuse as a component in organomineral fertilizer formulations presents a promising alternative. However, caution is warranted due to the possible presence of contaminants (Costa et al., 2023; de Almeida et al., 2017; Kujawska and Pawłowska, 2022; Soares et al., 2022). Furthermore, the high salinity of DCW could compromise ecological processes in the soil, especially in regions where these wastes might be applied for degraded soil recovery (Niemeyer et al., 2020). Salinity affects ecological functions, such as weathering, nutrient cycling, and primary production, directly impacting soil quality and associated ecosystem services (Makovníková et al., 2017; Yan et al., 2015).

Ecotoxicological studies with soil mesofauna have shown that invertebrates such as earthworms, enchytraeids and springtails are used as bioindicators because they are sensitive to contaminants, salinity and even fertilizers, and can provide insights into potential soil ecosystem risks (Bicho et al., 2024; Buch et al., 2016; Niemeyer et al., 2020; Owojori et al., 2009; Owojori and Reinecke, 2009; Pelosi and Römbke, 2016; Pereira et al., 2015; Serbource et al., 2024). Among ecotoxicological assessments, avoidance and reproduction tests with model organisms are widely used in ecological risk assessment, identifying concentrations that induce stress in soil organisms and potentially compromise their ecological functions (Niemeyer et al., 2020; Owojori et al., 2009).

Nevertheless, in this context, little is known about the sensitivity of native and/or alternative species from tropical regions compared to the standard species (*Folsomia candida*) used in ISO tests (ISO, 2011). In this regard, tests with local species, such as *Proisotoma minuta*, and alternatives (*Sinella curviseta*) may offer additional insights, given the variability in contaminant responses among organisms from different regions (Buch et al., 2016). Therefore, assessing these organisms' responses to DCW is essential to understanding the ecological impact of adding such waste to soil systems.

Additionally, given that soil amendment with DCW may provide a potential nutrient source, its use in organomineral fertilizer formulations could hold promise. Ensuring safe concentrations for soil invertebrate communities and cultivated plants is necessary. Fast-growing plant species with low nutritional requirements, such as *Mimosa scabrella*, are employed in degraded area recovery, contributing to improved soil fertility conditions and facilitating ecological succession (Pille da Silva et al., 2019).

Given the relevance of DCW in oil exploration and the need for sustainable alternatives for reuse and/or disposal, this study aims to evaluate the ecotoxicity of drilling cuttings waste (DCW) from pre-salt and

post-salt layers, determining safe concentrations for soil invertebrates and plants and identifying the test organisms most sensitive to DCW. This study contributes to understanding the effects of DCW on soil, presenting data that may inform safe dosage recommendations for agricultural and forestry use.

2. Materials and methods

2.1. Drilling cutting waste

The drilling cutting wastes (DCW) samples were of marine origin (offshore), collected from different depths and geological formations, presenting distinct chemical characteristics (Table 1). The sample collected from the pre-salt layer, originating from well 9-MLL-83-RJS at a depth of 4,450–4,520 m, was designated as "pre-salt DCW." The sample collected from the post-salt layer, originating from well 7-MLL-82HA-RJS at a depth of 3,400 m, was designated as "post-salt DCW."

Table 1
Chemical characterization (pseudo-total concentrations) of the samples of Drilling Cuttings Wastes (DCW) from pre-salt and post-salt layers.

Elements	Pre-salt DCW	Post-salt DCW
	mg kg ⁻¹	
Al	1,078	336.7
Ba	3,640	345.0
Ca	490,775	63,757
Cd	0.34	0.24
Cr	29.99	122.07
Cu	24.54	4.01
Fe	11,158	3,218
K	219.68	9,303
Mg	5,7042	23,232
Mn	183.32	38.59
Na	2,1560	19,669
Ni	10.38	94.24
P	5,521	4,126
Pb	13.57	1.57
Zn	160.16	20.42

Table 2 shows the average pH and electrical conductivity (EC) values of the soil mixtures, determined at the beginning of the tests, along with the proportions of drilling cuttings wastes and the corresponding Na concentrations based on the chemical analysis of the DCW samples.

Table 2

Average pH values ($\pm$ SD) (n = 6; KCl 1 mol) and initial electrical conductivity (deionized water) with sodium (Na) concentrations in mg kg⁻¹ corresponding to the proportions of pre-salt and post-salt drilling cuttings (1 mm particle size) in Tropical Artificial Soil (TAS)

DCW layer	DCW concentration in TAS (%)	pH	Electrical conductivity (dS.m ⁻¹)	Na (mg.kg ⁻¹)
	0.00	6.39	0.35	0.0
	0.25	7.65 $\pm$ 0.30	0.32	53.9
	0.50	8.05 $\pm$ 0.22	0.43	107.8
	1.00	8.34 $\pm$ 0.56	0.53	215.6
Pre-salt	2.50	8.48 $\pm$ 0.71	0.83	539.0
	4.00	8.61 $\pm$ 0.76	1.22	862.4
	5.00	9.01 $\pm$ 0.34	1.28	1078.0
	7.50	9.12 $\pm$ 0.35	1.79	1617.0
	10.0	9.26 $\pm$ 0.28	2.19	2156.0
	0.00	6.29	0.27	0.0
	0.25	6.99 $\pm$ 0.25	0.30	49.2
	0.50	7.16 $\pm$ 0.32	0.40	98.3
	1.00	7.53 $\pm$ 0.26	0.54	196.7
Post-salt	2.50	8.07 $\pm$ 0.28	0.78	491.7
	4.00	8.34 $\pm$ 0.32	0.93	786.7
	5.00	8.34 $\pm$ 0.27	1.17	983.4
	7.50	8.54 $\pm$ 0.19	1.67	1475.1

DCW layer	DCW concentration in TAS (%)	pH	Electrical conductivity (dS.m ⁻¹)	Na (mg.kg ⁻¹)
	10.0	8.58 ± 0.24	2.19	1966.9

2.2. Avoidance tests

Avoidance tests with earthworms of the species *Eisenia andrei* Bouché 1972 (Oligochaeta:Lumbricidae) were conducted according to ISO 17512-1 guideline (ISO, 2008), using five replicates for treatment. The plastic test boxes (20 x 13 x 6 cm) were divided into two sections by a plastic divider, adding 300 g of control soil in one section (A) and 300 g of test soil in the other section (B). The divider was removed and ten earthworms were placed in the middle at the surface.. After a 48 h incubation period at 20 ± 2°C in the dark, the divider was reinserted and the number of earthworms in each section was recorded. Five control replicates, conducted under the same conditions but with control soil in both sections, confirmed the random distribution of the organisms in the absence of contamination.

Avoidance tests with collembolans of the species *Folsomia candida* Willem 1902 (Collembola:Isotomidae) were conducted following ISO 17512-2 guideline (ISO, 2011). The drilling cuttings waste (DCW) concentrations tested ranged from 0.25–5% in Tropical Artificial Soil (TAS). Each treatment included five replicates using plastic recipients (7 cm Ø, 4.8 cm height, 125 ml capacity). Each recipient was divided into two sections by a divider: one section was filled with 30 g of control soil and the other with 30 g of test soil. Twenty collembolans of 10–12 d old were introduced along the central line of each replicate. The test was incubated at 20 ± 2°C with a 12 h light/12 h dark photoperiod. After 48 h, the divider was reinserted to separate the control soil from the test soil, which were transfer for another recipients. Tap water was added to make the organisms float and some drops of stamp ink were added to facilitate the visualization of them. The number of organisms in each section was recorded. Five control replicates, conducted under the same conditions but with control soil in both sections, confirmed the random distribution of the organisms in the absence of contamination.

2.3. Reproduction tests

Reproduction tests with collembolans of the species *F. candida*, *Proisotoma minuta* Tullberg 1871 (Collembola: Isotomidae), and *Sinella curviseta* Brook 1882 (Collembola: Entomobryidae) were conducted according to the ISO 11267 (ISO, 2011). For each treatment, five replicates (7 cm Ø, 4.8 cm height, 125 ml capacity) were prepared, containing 30 g of soil and 10 organisms with 10–12 d old for *F. candida* and *P. minuta*, and 20–22 d old for *S. curviseta* (DE LIMA E SILVA et al., 2021). The moisture lost by water evaporation was replenished weekly based on the initial weight in all replicates. The organisms were fed on days 1 and 14 with 2 mg of granular dry yeast. The tests were maintained in an incubator at 20 ± 2°C with a 16:8 h (light:dark) photoperiod for 28 d. To evaluate the test, water and stamp ink were added to each replicate, allowing photographic recording and subsequent counting of juveniles using Image J software (SCHNEIDER, RASBAND, ELICEIRI, 2012).

Reproduction tests with enchytraeids of the species *Enchytraeus crypticus* (Oligochaeta: Enchytraeidae) were conducted following the ISO 16387 guideline (ISO, 2014). For each treatment, five replicates (7 cm Ø, 4.8 cm height, 125 ml capacity) were prepared, containing 30 g of soil and 10 clitellate enchytraeids were introduced. The organisms were fed once a week with portions of oat flour. The test vessels were opened twice a week for aeration. Distilled water was added weekly to maintain initial moisture levels. On day 28, alcohol (70%) was added to the vessels until the soil was fully saturated, and a few drops of Bengal rose were applied to preserve and color the organisms. After 48 h, the enchytraeids were counted using a stereomicroscope at 60 x magnification.

2.4. Growth and biomass test with *Mimosa scabrella*

This experiment was conducted in accordance with the guidelines outlined in ABNT NBR/ISO 11269-2 (ABNT, 2014), with specific adaptations for using the arboreal species *Mimosa scabrella* Benth (Fabaceae) as the test organism. Each treatment consisted of six replicates, using tubes containing 140 g of soil and three seeds per replicate. To overcome seed dormancy, the technique of immersing the seeds in water at 80°C for 18 hours was employed. The experiment was carried out in a greenhouse under controlled temperature conditions of $20 \pm 2^\circ\text{C}$. The germination period was restricted to 10 days, after which thinning was performed to maintain a single seedling per replicate, in accordance with the Rules for Seed Analysis (BRAZIL, 2009). After thinning, the test continued for an additional 35 days. The plants were manually irrigated at least once per day. At the end of the experiment, the plants were removed from the tubes for measuring the shoot height and root length, and then placed in paper bags for the determination of dry mass of the shoots and roots. The plants were sectioned at the hypocotyl region and weighed after drying in a forced-air oven at 60°C until a constant weight. The evaluated endpoints included root and shoot length, and the shoots and roots dry mass.

2.5. Root elongation toxicity test

This test was conducted in accordance with EPA 712-C-96-154 (US-EPA, 1995) using *Lactuca sativa* L. seeds (without chemical treatment). Four replicates were prepared for each treatment, with 10 seeds placed in Petri dishes (15 cm Ø). Each replicate contained seed germination paper and 5 ml of eluate (1:10; residue: distilled water) prepared according to ABNT NBR/ISO 15469 (2015). The experiment was carried out in an incubator at a controlled temperature of $20 \pm 2^\circ\text{C}$. The initial phase lasted 48 hours in complete darkness, allowing for the germination of at least 65% of the seeds in the control group. The second phase extended for 7 days, during which a photoperiod of 16:8 h (light:dark), enabling the roots in the control to reach a length > 2 cm. At the end of the test, the number of germinated seeds in each replicate was counted, and root length was measured.

2.6. Data analysis

For the avoidance behavior tests, Fisher's exact test ($p < 0.05$) was applied. To verify the random distribution of organisms in the test container, five double-control replicates (TAS on both sides of the box) were performed in parallel with the contaminated soil tests.

The data from invertebrate reproduction tests and plant tests were checked for normality and homogeneity of variances using the Shapiro-Wilk and Bartlett tests ($p < 0.05$). When data met these assumptions, they were analyzed via analysis of variance (ANOVA) followed by Dunnett's multiple comparisons test ($p < 0.05$), comparing treatment results with those from control soil. Non-parametric data were evaluated with the Kruskal-Wallis's test followed by Dunn's multiple comparisons test ($p < 0.05$). Based on significant differences, the No Observed Effect Concentration (NOEC) and Lowest Observed Effect Concentration (LOEC) were determined. All statistical analyses were conducted using R software (R Development Core Team, 2020).

To determine the Effective Concentration (EC_x) values for 20% and 50% of the population (EC₂₀ and EC₅₀, respectively), non-linear regression models, Logistic Exponential and Gompertz, were applied using Statistica 7.0 software (StatSoft, 2004).

3. Results and discussion

3.1. Avoidance tests

The avoidance tests with *E. andrei* revealed significant effects ($p < 0.05$) of DCW residues starting at concentrations of 2.5% (EC ≥ 0.78 dS/m) for both residues tested. At this concentration, over 80% of the earthworms migrated to the control soil (TAS), indicating a loss of soil habitat function (Hund-Rinke and Wiechering, 2001) following the application of $\geq 2.5\%$ of pre-salt or post-salt DCW (see the red line, Figs. 1A and B).

Similar results has been observed with earthworms in soils with EC levels comparable to those in this study (≥ 0.77 dS/m). Soils irrigated with wastewater from onshore drilling cuttings (EC 0.26 to 0.98 dS/m) also resulted in habitat function loss for *E. andrei* (Niemeyer et al., 2020).

The significant avoidance behavior ($p < 0.05$) of *F. candida* varied between the two DCW. Pre-salt DCW induced a dose-response effect with significant avoidance from 0.5% onward (Fig. 1C). In contrast, for post-salt DCW, avoidance was observed only at higher concentrations (4% and 5%) (Fig. 1D). Although a significant response was noted at 0.25%, it was not sustained at subsequent concentrations (0.5%, 1.0%, and 2.5%), suggesting an atypical response (Fig. 1D).

Loss of habitat function, according to ISO 17512-2 (ISO, 2011), was evident for pre-salt DCW at concentrations $\geq 0.5\%$, with over 70% of individuals migrating to the control soil (TAS) (see the red line, Fig. 1C). However, this loss of habitat function only occurred at concentrations equal to or above 4% for post-salt DCW (Fig. 1D).

Our results indicate that EC alone cannot predict the loss of habitat function in soils exposed to different saline wastes (pre-salt and post-salt DCW) and does not appear to be the primary factor influencing the avoidance behavior of collembolans. Avoidance of pre-salt DCW was observed at EC values of $0.43 \text{ dS}\cdot\text{m}^{-1}$ (0.5%), whereas for post-salt DCW concentrations with $\text{EC} \leq 0.78 \text{ dS}\cdot\text{m}^{-1}$ did not result in

significant avoidance (Fig. 1D). Such results support the findings that EC alone may not be an optimal predictor of ecotoxicity for earthworms, as seen for *E. fetida* in Owojori and Reinecke (2014).

Comparisons between earthworms (*Aporrectodea caliginosa* and *E. fetida*) and collembolans (*F. candida*) revealed a higher sensitivity of earthworms to saline stress than collembolans (Owojori et al., 2009). In our study, this pattern was more evident for post-salt DCW, where collembolan avoidance occurred only at $EC \geq 0.93 \text{ dS}\cdot\text{m}^{-1}$, while *E. andrei* exhibited avoidance at EC levels of 0.83 and $0.78 \text{ dS}\cdot\text{m}^{-1}$ for 2.5% pre-salt and post-salt DCW, respectively (Table 2).

The avoidance of collembolans in pre-salt DCW, even at relatively low EC levels ($0.43 \text{ dS}\cdot\text{m}^{-1}$), suggests that factors other than salinity influence their behavior.

Considering that DCW represent a complex matrix, it may contain several potential contaminants (de Almeida et al., 2017). The difference in organism sensitivity between pre-salt and post-salt DCW may be attributed to higher concentrations of toxic elements in pre-salt residues, such as zinc (Zn), lead (Pb), and aluminum (Al), which are present at higher levels in this residue than in the post-salt DCW (Table 1). Additionally, other unanalyzed contaminants, including hydrocarbons and other metals, may have also contributed to the avoidance behavior observed in collembolans.

Avoidance tests indicated that the impact on soil invertebrate communities varies according to both the origin (pre-salt or post-salt) and concentration of DCW. These tests can serve as an initial tool in ecological risk assessment of contaminants (Niemeyer et al., 2020), and their results may be as sensitive to salinity stress as reproduction tests (Owojori and Reinecke, 2009).

3.2. Reproduction tests with soil invertebrates

After 28 days of exposure, significant chronic effects on the reproduction of *F. candida* were observed at concentrations of 2.5% or higher for both pre-salt and post-salt DCW residues (Fig. 2A). DCW concentrations near this level also caused a 50% effect on the population of this species (EC_{50} : 2.82% and 2.51% for pre-salt and post-salt, respectively) (Table 3).

Although avoidance behavior was recorded at lower concentrations for pre-salt DCW (0.5% and 1%) (Fig. 1C), organisms maintained normal reproduction at these levels (Fig. 2A). This phenomenon suggests that avoidance may occur at salinity levels lower than those that induce reproductive damage. These findings indicate that avoidance tests may be more sensitive or within the same sensitivity range as reproduction tests for saline substances (Owojori and Reinecke, 2014, 2009). However, this was not observed with post-salt DCW. *F. candida* did not avoid the 2.5% post-salt DCW concentration within 48 h (Fig. 1D) but exhibited significant chronic reproductive effects under prolonged exposure (28 d) (Fig. 2A).

A concentration of 2.5% DCW resulted in EC : $0.83 \text{ dS}\cdot\text{m}^{-1}$ for the pre-salt and $0.78 \text{ dS}\cdot\text{m}^{-1}$ for the post-salt DCW (Table 2). These values suggest a potential chronic effect on the reproduction of *F. candida*. Previous studies with soils exposed to NaCl, showing EC values of $0.7 \text{ dS}\cdot\text{m}^{-1}$ (LUFA 2.2 natural soil) (Bicho et al., 2024), $1.03 \text{ dS}\cdot\text{m}^{-1}$ (natural soil from South Africa) (Owojori et al., 2009) and $1.09 \text{ dS}\cdot\text{m}^{-1}$

(OECD artificial soil) (Pereira et al., 2015), also reported suppression of juvenile reproduction in *F. candida*. The higher EC values, approximately 30% higher than those found in this study, support the hypothesis that EC alone may not be a reliable predictor of the ecotoxicity of saline substances (Owojori and Reinecke, 2014).

The standard species *F. candida* (ISO 11267, 2011) may be less protective for native species. This is due to our observation of lower sensitivity in *F. candida* compared to *P. minuta* (Fig. 2A and B). For pre-salt DCW, the standard species showed a LOEC of 2.5% and an EC₅₀ of 2.82%, while the alternative species *P. minuta* exhibited a LOEC ten times lower, at 0.25%, and an EC₅₀ of 0.13% (Table 3). A similar difference in sensitivity was observed for post-salt residues (EC₅₀: 2.51% versus EC₅₀: 0.80%) (Table 3). These results highlight the importance of using alternative and native species in ecotoxicological testing of substances prior to environmental exposure.

Different collembolas species can exhibit varying sensitivities to contaminants (Buch et al., 2016). Our results demonstrate this difference in sensitivity among the three collembolas species evaluated when exposed to DCW. Based on the EC₅₀ values (Table 3), we established a sensitivity hierarchy as follows: *P. minuta* > *F. candida* > *S. curviseta*. Due to the low sensitivity of *S. curviseta*, this species was used only for evaluate the pre-salt DCW. *S. curviseta* exhibited the lowest sensitivity, with no significant reduction in reproduction observed (Fig. 2C) and a NOEC ≥ 5% (Table 3) when exposed to the tested DCW concentrations (0.25–5%). This low sensitivity underscores the variability in ecotoxicological responses depending on the species (Buch et al., 2016) and the specific type of residue or contaminant involved (Peijnenburg et al., 2012).

In addition to inter-species differences, there are also variations in sensitivity to different contaminants within the same species. For instance, *P. minuta* shows lower sensitivity to mercury contamination compared to *F. candida* (Buch et al., 2016). However, our results show that *P. minuta* is much more sensitive than *F. candida* to residues similar in nature to pre-salt and post-salt DCW.

Furthermore, the standard species *F. candida* may have limited ecological relevance in natural and agricultural environments (Krogh, 2009). In contrast, *P. minuta* holds ecological significance due to its presence in tropical forests in countries such as Brazil, Australia, and New Zealand (Buch et al., 2016), and it has also been reported in other regions, including Canada (Princz et al., 2012).

Reproduction tests for *E. crypticus* revealed a higher sensitivity to post-salt DCW in comparison to pre-salt DCW (Fig. 2B and D). The lower LOEC (2.5%) and EC₅₀ values (2.42%) for post-salt DCW exposure, compared to pre-salt DCW (LOEC: 4%, EC₅₀: 4.32%) (Table 3), suggest that *E. crypticus* is more tolerant to pre-salt DCW despite its higher electrical conductivity (1.22 dS.m⁻¹ for pre-salt versus 0.78 dS.m⁻¹ for post-salt). Additionally, Bicho et al. (2024) documented hormesis effects in *E. crypticus* with gradually increased salinity. Our findings similarly indicate this hormesis phenomenon at concentrations around 1% DCW, where juvenile production exceeded that observed in the control (TAS) (Fig. 1D).

Moreover, according to the literature, *E. crypticus* may exhibit higher tolerance in environments with elevated EC levels compared to collembolas (Bicho et al., 2024; Owojori et al., 2009; Pereira et al., 2015). In this sense, we observe that the most restrictive EC₅₀ value (indicating the most sensitive species) was found in reproduction tests with *P. minuta* (EC₅₀: 0.13%) for pre-salt DCW, while an intermediate sensitivity was observed for *F. candida* (EC₅₀: 2.82%), and the highest tolerance to this residue was recorded for *S. curviseta* (EC₅₀: 6.89%) (Table 3).

Differences in sensitivity Among collembolans species may be related to different ecological traits, meaning different exposure to contaminants. The species *F. candida* and *P. minuta* belong to Order Poduromorpha, characterized by short appendices and usually soil dwellers, while *S. curviseta* belongs to Order Entomobryomorpha, with long antennae and legs, and elongated bodies, being common inhabitants of the litter surface. Surface-dwelling species (as *S. curviseta*) usually have lower body exposure to soil contaminants in comparison to edaphic species.

Concerning the high tolerance of *E. crypticus*, it may be attributed to physiological mechanisms that help annelids adapt to saline stress (Pereira et al., 2015). Despite having a soft body, which theoretically makes them more susceptible to salts than the exoskeleton of springtails (Peijnenburg et al., 2012), enchytraeids have been shown to possess two primary mechanisms: the ability to excrete hypotonic urine and membrane impermeabilization (Pereira et al., 2015). These features likely contribute to the higher tolerance of enchytraeids to the DCW in comparison to *F. candida* and *P. minuta*.

Different levels of sensitivity to contaminants among soil mesofauna species underscores the importance of including multiple soil organism groups in ecotoxicity testing. Since different groups respond differently to contaminants, relying on a single taxon, whether microarthropods or annelids, may lead to an incomplete perspective when assessing the environmental safety of substances used in ecosystems.

Table 3

Summary of the No Observed Effect Concentration (NOEC), Lowest Observed Effect Concentration (LOEC) and Effect Concentrations (ECx) for species when exposed to pre-salt and post-salt DCW

Species	Test	Pre-salt DCW (%)	Lower - upper limits (%)	Model	Post-salt DCW (%)	Lower - upper limits (%)	Model
<i>F. candida</i>	NOEC	1.00			1.00		
	LOEC	2.50			2.50		
	EC20	1.87	1.20–2.53	Logistic	1.98	0.86–3.11	Gompertz
	EC50	2.82	2.28–3.36	Logistic	2.51	1.40–3.62	Logistic
<i>P. minuta</i>	NOEC	< 0.25			0.50		
	LOEC	0.25			1.00		
	EC20	0.06	0.01–0.12	Exponential	0.33	0.07–0.58	Logistic
	EC50	0.13	0.02–0.23	Exponential	0.80	0.40–1.19	Logistic
<i>S. curviseta</i>	NOEC	5.0					
	LOEC	> 5.0					
	EC20	2.76	0.97–4.54	Linear	-	-	-
	EC50	6.89	2.43–11.36	Linear	-	-	-
<i>E. crypticus</i>	NOEC	2.50			1.00		
	LOEC	4.00			2.50		
	EC20	3.80	3.40–4.20	Logistic	1.45	0.96–1.93	Logistic
	EC50	4.32	4.03–4.61	Logistic	2.42	1.98–2.86	Logistic

3.3. Germination and Root elongation toxicity test

Germination and root elongation tests for *Lactuca sativa* were conducted using elutriates from pre-salt and post-salt DCW at concentrations of 0.25–5%. Germination rates and root elongation (cm) showed no significant inhibition compared to the control (0% DCW), indicating that these DCW concentrations do not adversely impact early growth stages in *L. sativa* ($p < 0.05$). Detailed root elongation data can be found in the supplementary materials.

The tolerance of *L. sativa*, known for its sensitivity to high salinity and frequent use as a bioindicator in soil contamination studies (Fasciglione et al., 2015), suggests that both pre-salt and post-salt DCW at concentrations up to 5% do not induce phytotoxic effects. These results align with previous findings on the species' responses to moderate salinity levels, despite its documented sensitivity to high

concentrations of Na^+ and Cl^- (Rosseto, 2021). Therefore, this study supports the environmental feasibility of using DCW in controlled soil applications without phytotoxic risks to sensitive plant bioindicators.

3.4. Growth and biomass test with *M. scabrella*

The growth test with *M. scabrella*, a pioneer native tree species commonly used in reforestation and ecosystem restoration, revealed no significant effects ($p < 0.05$) on key growth parameters, including shoot and root length and shoot and root dry biomass, when exposed to pre-salt and post-salt DCW at concentrations up to 5%. EC and sodium (Na) levels associated with the 5% pre-salt DCW treatment were measured at 1.28 dS.m^{-1} and $1,078 \text{ mg Na.kg}^{-1}$, respectively, while the post-salt DCW exhibited values of 1.17 dS.m^{-1} and $983 \text{ mg Na.kg}^{-1}$. According to EMBRAPA's soil classification (EMBRAPA, 2018), these values do not indicate salt levels toxic to most crops (EC threshold: $\geq 7 \text{ dS.m}^{-1}$).

Exceptions were noted in the pre-salt DCW treatments at 1% and 4%, and in the post-salt DCW at 2.5%, where slight reductions in root length and biomass were observed. However, these reductions were not consistent with a dose-response trend, indicating a lack of direct correlation with DCW exposure. Detailed shoot and root length and shoot and root dry biomass data can be found in the supplementary materials.

The lack of observed adverse effects on *M. scabrella* aligns with previous research suggesting that this species can tolerate moderate salinity levels (Avrella et al., 2019), further supporting its suitability for reforestation efforts in soils affected by saline or DCW residues. The maximum tested concentration, 5% DCW, corresponds to an estimated field application of approximately 130 tons.ha^{-1} , reflecting a potential use for controlled land applications. Nevertheless, repeated applications could lead to the gradual accumulation of salts in the soil, potentially impacting soil health and other plant species sensitive to salinity (Fan et al., 2021; Princz et al., 2012).

Studies indicate that salt stress in plants can reduce growth through ionic toxicity, ionic imbalance, and effects on cell wall integrity, with high salt concentrations often resulting in the accumulation of potentially toxic ions, such as Na^+ and Cl^- (Munns, 2005; Munns & Tester, 2008; Álvarez et al., 2018). In our study, however, these adverse effects were not observed in either the sensitive species *L. sativa* or the pioneer tree species *M. scabrella*. Nevertheless, under scenarios involving repeated DCW applications, soil monitoring is recommended to prevent salt accumulation and mitigate potential long-term impacts (Hussain et al., 2017).

4. Conclusions

This study demonstrates notable variability in species-specific sensitivity to drilling cuttings waste (DCW), underscoring the need for ecotoxicity assessments that include a range of organisms. A hierarchy of sensitivity to DCW was established: *P. minuta* > *F. candida* > *E. crypticus* > *S. curviseta*. These

results highlight the importance of selecting ecologically relevant species to capture a more complete range of potential ecological impacts of DCWs.

The origin of DCW also affected ecotoxicity: *E. crypticus* displayed greater tolerance to pre-salt DCW compared to post-salt DCW, which had lower LOEC and EC₅₀ values. This suggests that DCW from different geological layers may exhibit varying toxic effects, likely due to differences in chemical composition and salinity.

In contrast, the tested plants (*L. sativa* and *M. scabrella*) showed no significant effects in germination and growth across the DCW concentration range of 0.5–5%, suggesting that plants may be less sensitive to DCW under the laboratory conditions used.

These findings support a multi-species approach for ecotoxicological evaluations of DCWs, incorporating both standard model organisms and alternative species with ecological relevance. Relying solely on standard species such as *F. candida* may overlook the broader spectrum of sensitivity in soil ecosystems, particularly for residues with complex and variable composition like DCWs. Furthermore, the results of this study highlight the potential use of DCW in controlled land applications as a soil restoration strategy, as well as its possible incorporation into organomineral fertilizer formulations.

Declarations

Conflict of interest

Non-financial interests: The author JCN is Associated Editor of the Ecotoxicology Journal.

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Supplementary Material

The Supplementary Material file is not available with this version.

Figures

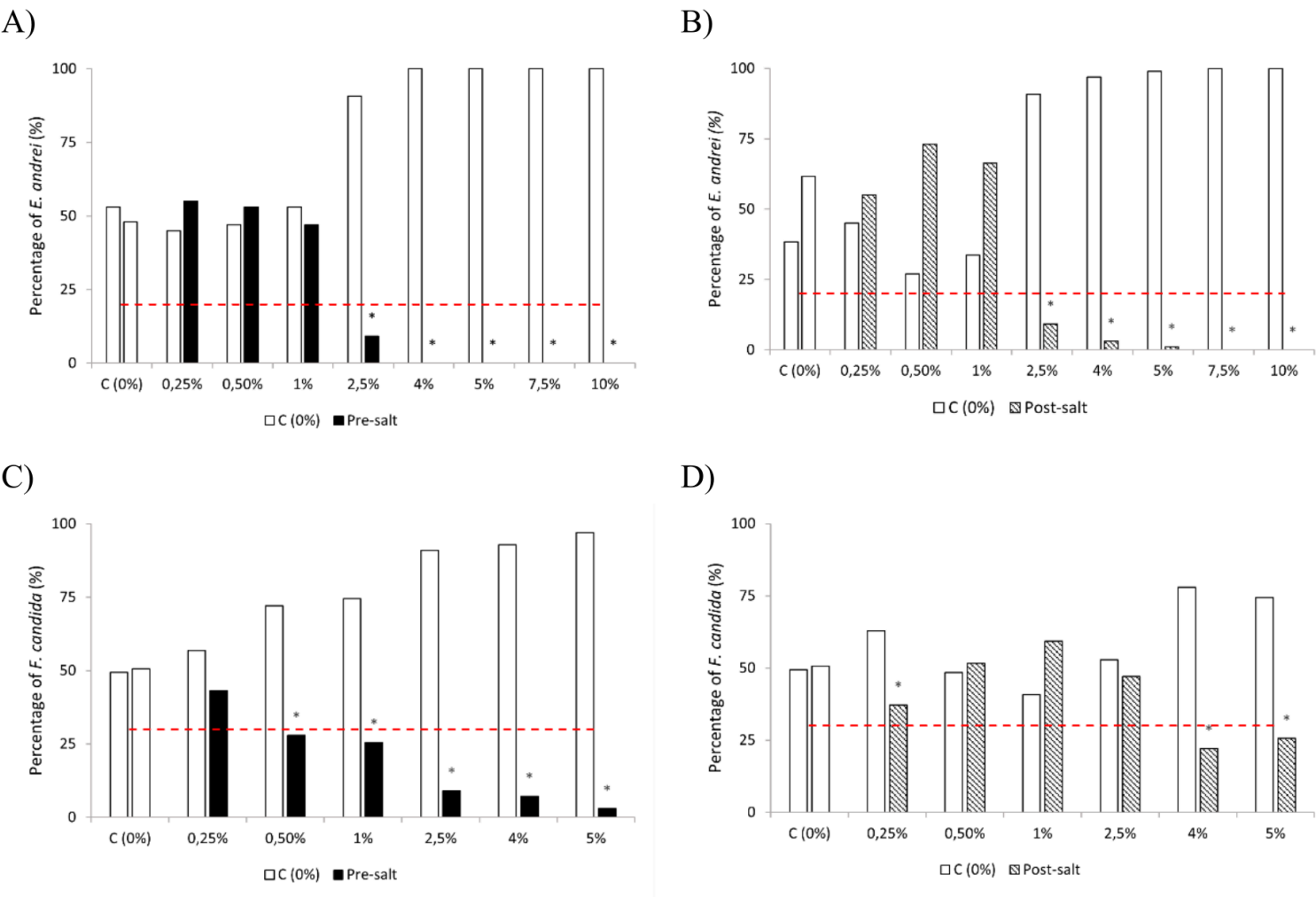


Figure 1

Avoidance behavior of earthworms *E. andrei* (A, B) and collembolans *F. candida* (C, D) shown as a percentage of organisms after 48 h of exposure to test soils with concentrations of drilling cutting wastes (DCW) from oil wells. A and C: pre-salt DCW layer (solid black bars); B and D: post-salt DCW layer

(hatched bars). (*) Asterisks denote significant differences between the control soil (TAS: 0% DCW) and test soils (Fisher's Exact Test, $p < 0.05$).

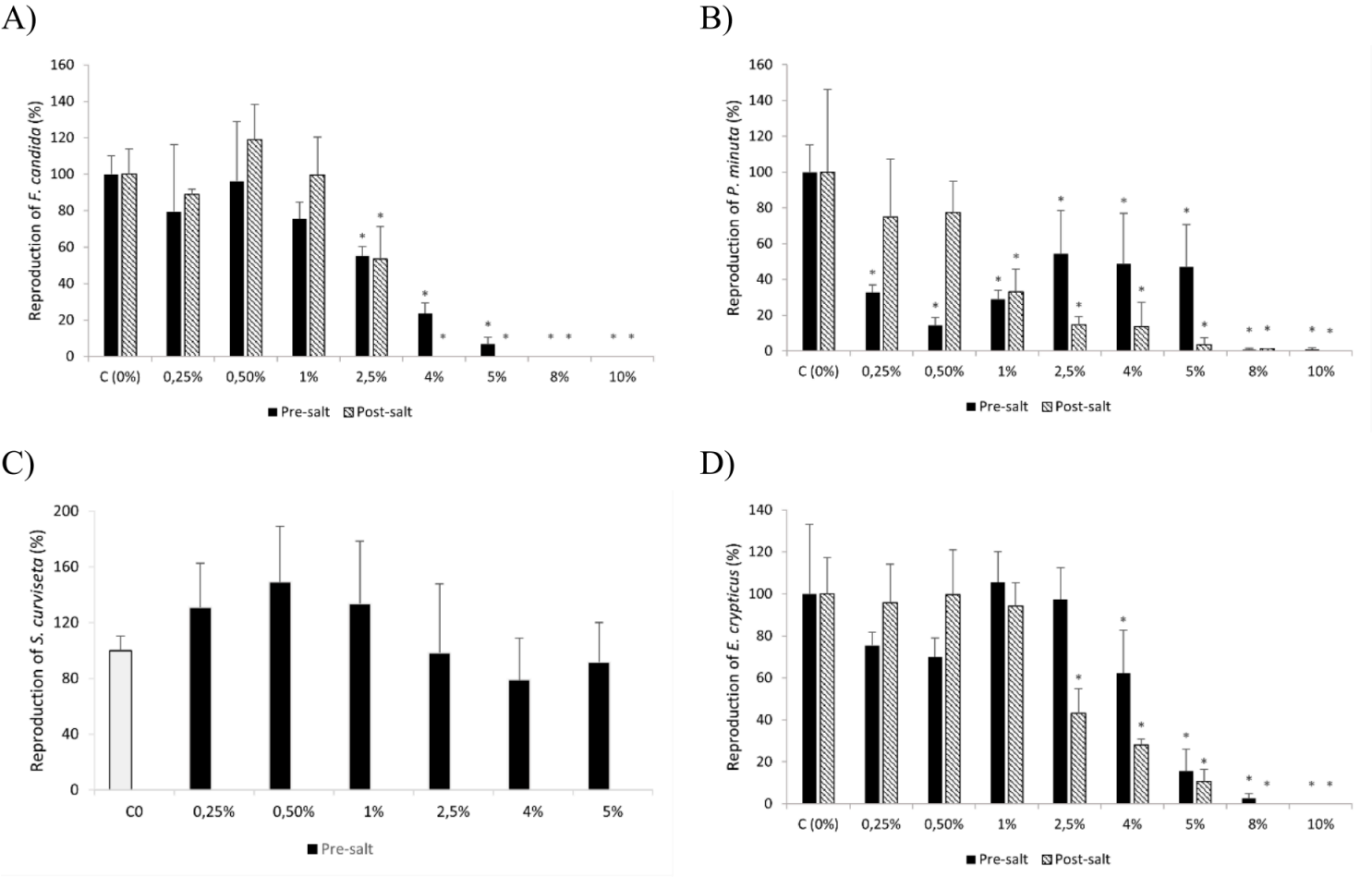


Figure 2

Reproduction tests with the collembolan species *Folsomia candida* (A), *Proisotoma minuta* (B), *Sinella curviseta* (C), and the enchytraeid species *Enchytraeus crypticus*(D) under different concentrations of drilling cutting wastes (DCW) from oil wells: pre-salt layer (solid black bars) and post-salt layer (hatched bars). Reproduction (mean ± SD) is represented as percentage of the reproduction in control soil (TAS – Tropical Artificial Soil) for collembolans, and number of juveniles for enchytraeids. (*) Asterisks denote significant differences between the control soil (TAS: 0% DCW) and treated soils.