



**ECOTOXICOLOGICAL ASSESSMENT OF GASOLINE AND DIESEL OIL IN SOIL
USING THE BIOINDICATORS *FOLSOMIA CANDIDA* AND *ENCHYTRAEUS
CRYPTICUS***

**AVALIAÇÃO ECOTOXICOLÓGICA DE GASOLINA E ÓLEO DIESEL EM SOLO
UTILIZANDO OS BIOINDICADORES *FOLSOMIA CANDIDA* E *ENCHYTRAEUS
CRYPTICUS***

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Abstract: Soil contamination by petroleum-derived fuels represents a significant environmental concern, particularly in regions with high densities of fuel stations. This study evaluated the chronic ecotoxicological effects of gasoline and diesel oil on the soil invertebrates *Folsomia candida* and *Enchytraeus crypticus* using Tropical Artificial Soil (TAS). Reproduction was used as the primary endpoint to assess sublethal toxicity across a range of concentrations. Both fuels caused significant dose-dependent reductions in reproduction for the two species, while survival remained unaffected. Effects were observed at the lowest tested concentration (0.00625 mL kg⁻¹), indicating high sensitivity of soil invertebrates to petroleum-derived contaminants. *Enchytraeus crypticus* exhibited greater sensitivity than *Folsomia candida*, and diesel oil showed higher toxicity at lower concentrations, whereas gasoline produced more pronounced effects at higher exposure levels. These findings highlight the ecological relevance of chronic ecotoxicological assays and demonstrate the importance of using multiple bioindicator species to improve environmental risk assessments in petroleum-contaminated tropical soils.

Keywords: Soil ecotoxicology. Soil invertebrates. *Folsomia candida*. *Enchytraeus crypticus*. Petroleum-derived contaminants.

Resumo: A contaminação do solo por combustíveis derivados de petróleo representa uma preocupação ambiental significativa, especialmente em regiões com alta densidade de postos de combustíveis. Este estudo avaliou os efeitos ecotoxicológicos crônicos da gasolina e do óleo diesel sobre os invertebrados do solo *Folsomia candida* e *Enchytraeus crypticus*, utilizando Solo Artificial Tropical (SAT). A reprodução foi utilizada como principal parâmetro para avaliar a toxicidade subletal em uma faixa de concentrações. Ambos os combustíveis causaram reduções significativas e dependentes da dose na reprodução das duas espécies, enquanto a sobrevivência não foi afetada. Foram observados efeitos já na menor concentração testada (0,00625 mL kg⁻¹), indicando alta sensibilidade dos invertebrados do solo aos contaminantes derivados de petróleo. *Enchytraeus crypticus* apresentou maior sensibilidade que *Folsomia candida*, e o óleo diesel demonstrou maior toxicidade em baixas concentrações, enquanto a gasolina produziu efeitos mais pronunciados em níveis mais elevados de exposição. Esses resultados destacam a relevância ecológica dos ensaios ecotoxicológicos crônicos e demonstram a importância do uso de múltiplas espécies bioindicadoras para aprimorar as avaliações de risco ambiental em solos tropicais contaminados por derivados de petróleo.

Palavras-chave: Ecotoxicologia do solo. Invertebrados do solo. *Folsomia candida*. *Enchytraeus crypticus*. Contaminantes derivados de petróleo.

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1 INTRODUCTION

The continuous expansion of the motor vehicle fleet has led to an increase in fuel stations dedicated to the commercialization of petroleum-derived fuels. However, many of these facilities were historically established without strict compliance with environmental regulations, resulting in potential risks of soil and groundwater contamination (Brito et al., 2018; Lima et al., 2017). The petrochemical sector is recognized as a major source of hydrocarbon release into the environment due to the extensive production, processing, and widespread use of petroleum-derived products (Eneh, 2011). In addition, leaks and spills frequently occur during transportation, storage, and commercial operations, often resulting from structural failures, operational errors, or inadequate maintenance of pipelines, tanker trucks, vessels, and especially fuel stations (Biermann, 2018; Cardoso et al., 2017).

In the state of São Paulo, Brazil, a survey conducted by the Environmental Company of the State of São Paulo (CETESB, 2020) reported 6,434 contaminated areas, approximately 70% of which are associated with fuel stations. This scenario highlights the importance of environmental regulations, such as Resolution No. 273/2000 of the Brazilian National Council for the Environment (CONAMA), which requires the investigation of environmental liabilities during licensing procedures to prevent environmental impacts and guide corrective actions, reinforcing the need for reliable tools to assess contaminated areas. This issue is not restricted to Brazil, as petroleum-derived contamination is a widespread environmental problem affecting soils and groundwater worldwide.

Petroleum-derived contaminants pose significant environmental and human health concerns due to the presence of toxic compounds such as benzene, toluene, ethylbenzene, xylenes (BTEX), polycyclic aromatic hydrocarbons (PAHs), polychlorinated biphenyls (PCBs), heavy metals, and volatile organic compounds (VOCs) (Cardoso et al., 2017). To assess soil quality and identify contamination, various physical, chemical, and biological approaches have been adopted. Among these, ecotoxicological assays using soil invertebrates stand out due to their high sensitivity to xenobiotic substances and their ability to provide a more comprehensive evaluation of soil ecosystem functioning. Previous studies have shown that these bioassays are sensitive tools for detecting changes in survival, reproduction, and behavior of soil organisms under contaminant exposure, highlighting their relevance for environmental monitoring and risk assessment (González et al., 2011; Van Loon et al., 2025).

Moreover, soil fauna play key roles in ecosystem services, including organic matter decomposition, nutrient cycling, and soil structure regulation, thereby contributing to overall soil functioning (Briones, 2018). Soil invertebrates are widely used as bioindicators of

contamination (Sisinno et al., 2019; Crouau et al., 1999; Lopes et al., 2024; Calisi et al., 2019). Among the most commonly used organisms, the collembolan *Folsomia candida* and the enchytraeid *Enchytraeus crypticus* are particularly relevant due to their ease of cultivation, consistent biological responses, and international recognition as standard species in soil ecotoxicological assays (Fountain and Hopkin, 2005; Liu et al., 2018; Niemeyer et al., 2017).

Folsomia candida is widely used in standardized reproduction-based toxicity tests due to its high sensitivity to environmental stressors and its suitability as a bioindicator species. In contrast, *Enchytraeus crypticus*, which inhabits deeper soil layers, contributes to organic matter degradation and soil structure formation, also exhibiting high sensitivity to contaminants and being widely applied as a model species in soil ecotoxicology (Ferreira et al., 2022; Castro-Ferreira et al., 2012). Both species respond to a wide range of chemical and environmental stressors, enabling comparative assessments of sensitivity and ecological impact (Bicho et al., 2025), and are therefore particularly suitable for evaluating the effects of complex contaminant mixtures such as petroleum-derived fuels.

Despite advances in the understanding of petroleum-derived contamination, comparative studies assessing the ecotoxicological effects of gasoline and diesel remain scarce, particularly under tropical soil conditions, which present specific physicochemical and biological characteristics that may influence contaminant bioavailability and effects. Considering that these fuels have distinct chemical compositions and, consequently, different environmental behaviors and toxicity profiles, their simultaneous evaluation is essential for a more realistic assessment of the ecological risks associated with this type of contamination (da Cruz et al., 2026)

Therefore, the present study aimed to evaluate and compare the chronic ecotoxicological effects of gasoline and diesel oil on the soil invertebrates *Folsomia candida* and *Enchytraeus crypticus*, using Tropical Artificial Soil (TAS). Survival and reproduction were used as biological endpoints to investigate species sensitivity and to discuss the ecological implications of fuel contamination in tropical soils.

2 MATERIAL AND METHODS

2.1 Test organisms and culture conditions

The soil invertebrates *Folsomia candida* and *Enchytraeus crypticus* were cultured at the Soil Ecotoxicology Laboratory (LAECOS), Faculty of Technology, University of Campinas (UNICAMP), Limeira, São Paulo, Brazil.

Folsomia candida cultures were maintained in polypropylene containers (approximately 220 mL capacity) containing a substrate composed of 400 g gypsum, 50 g activated charcoal, and 300 mL ultrapure water, following ABNT NBR ISO 11267 (ABNT, 2019). Cultures were kept at 20 ± 2 °C under a 12:12 h light:dark photoperiod in a temperature-controlled incubator, ensuring stable and consistent environmental conditions throughout the experimental period. Organisms were fed twice weekly with baker's yeast. Synchronized adults aged 10–12 days, obtained from the same cohort, were used in the toxicity tests to ensure uniform physiological conditions.

Enchytraeus crypticus was cultured in Petri dishes (90x15mm) containing nutritive agar prepared according to ABNT NBR ISO 16387 (2012) and ISO 16387 (2004). Cultures were maintained at 20 ± 2 °C under a 12:12 h light:dark photoperiod in a temperature-controlled incubator, ensuring stable and consistent environmental conditions throughout the experimental period. Organisms were fed twice weekly with finely ground oatmeal, and moisture levels were regularly adjusted with distilled water to maintain optimal conditions.

All cultures were maintained in a climate-controlled chamber to ensure stability of temperature and photoperiod throughout the experimental period.

2.2 Soil used in the toxicity tests

Tropical Artificial Soil (TAS) was prepared in accordance with ABNT NBR 15537 (2014), adapted from OECD artificial soil guidelines (OECD, 2013). The soil composition consisted of 75% fine sand, 20% kaolin clay, and 5% coconut fiber (dry weight basis), ensuring a standardized substrate suitable for ecotoxicological assays.

The use of TAS was adopted to ensure consistency, reproducibility, and comparability among treatments, minimizing variability associated with natural soils, such as differences in organic matter content, texture, and microbial activity.

Prior to contamination, the soil moisture content was adjusted to approximately 60% of its water holding capacity using distilled water, as recommended by standardized guidelines. The soil was then homogenized to ensure uniform distribution of moisture before the application of test substances.

2.3 Sensitivity test of the organisms

Sensitivity tests were conducted to validate the responsiveness of the test organisms and to ensure compliance with standardized ecotoxicological protocols. The assays were performed using boric acid as a reference substance, following Niemeyer et al. (2018). Test concentrations were 25, 50, 100, 200, and 400 mg kg⁻¹ (dry weight basis). For each

concentration, 150 g of contaminated soil were prepared and subsequently divided into five replicates of 30 g each, placed in plastic containers (approximately 150 mL capacity). Ten synchronized adult organisms from the same cohort were introduced into each replicate. Control treatments consisted of uncontaminated soil maintained under identical experimental conditions. Exposure duration was 21 days for *Enchytraeus crypticus* and 28 days for *Folsomia candida*. Throughout the exposure period, soil moisture content and food supply were monitored and adjusted weekly to maintain optimal conditions for the organisms. At the end of the exposure period, organisms were extracted and counted using standard methods: ethanol and rose bengal staining for *E. crypticus*, and water plus ink contrast for *F. candida*, with counting performed from captured images. Reproduction was defined as the primary endpoint, expressed as the number of juveniles produced per replicate, while adult survival was recorded as a secondary endpoint.

2.4 Soil contamination and toxicity assays

Gasoline and diesel fuel used in this study were obtained from a licensed commercial fuel station in Limeira, São Paulo, Brazil. Detailed physicochemical characterization of the fuels was not performed. However, according to the Safety Data Sheets (SDS) and literature data, gasoline is a complex mixture of paraffinic, cycloparaffinic, aromatic, and olefinic hydrocarbons, predominantly in the C4–C12 carbon range and may contain oxygenated compounds such as ethanol (Murphy Oil USA, 2019; USEPA, 2002). It typically contains significant proportions of monoaromatic hydrocarbons, including benzene, toluene, ethylbenzene, and xylenes (BTEX), which are widely recognized as key constituents of gasoline and indicators of petroleum contamination (Cruz & Andrade, 2020).

Diesel fuel is also composed of a complex mixture of paraffinic, cycloparaffinic, aromatic, and olefinic hydrocarbons, generally in the C10–C28 carbon range (USEPA, 2002). In contrast to gasoline, diesel is characterized by a lower proportion of volatile aromatic compounds such as BTEX and a higher content of heavier hydrocarbons, including polycyclic aromatic hydrocarbons (PAHs), which are associated with greater persistence and chronic toxicity in the environment (Liang et al., 2005). These compositional differences directly influence their environmental behavior, persistence, and potential ecotoxicological effects in soil ecosystems.

The fuels were applied at concentrations of 0.00625, 0.0125, 0.025, 0.05, and 0.1 mL kg⁻¹, which were defined based on preliminary laboratory tests. Initially, higher concentrations (0.0625, 0.125, 0.25, 0.5, and 1 mL kg⁻¹) were evaluated and resulted in more evident toxic effects. Based on these observations, the concentration range was

adjusted to include lower levels, allowing the assessment of sublethal effects and the establishment of dose–response relationships.

Contamination was performed by spiking the fuels directly into pre-moistened soil, followed by thorough manual homogenization to ensure uniform distribution of the contaminants throughout the soil matrix. Control treatments consisted of uncontaminated soil maintained under identical experimental conditions.

Five replicates were selected based on OECD/ABNT recommendations to ensure statistical robustness and adequate power for ANOVA analyses. Soil pH was measured at the beginning and at the end of the assays to monitor possible changes during exposure.

Exposure conditions, including temperature, photoperiod, feeding, and moisture adjustment, followed those described for the sensitivity tests. Reproduction was selected as the primary endpoint, expressed as the number of juveniles produced per replicate, while adult survival was recorded as a secondary endpoint. Thus, organism sensitivity was assessed primarily through reproduction and secondarily by survival.

2.5 Data analysis

Statistical analyses were performed using STATISTICA 7.0 (StatSoft Inc., Tulsa, OK, USA). EC_{50} and EC_{20} values were estimated by nonlinear regression using a four-parameter log-logistic model, based on reproduction data. Homogeneity of variances was assessed using Levene's test. Differences between treatments and the control were evaluated using one-way analysis of variance (ANOVA), followed by Dunnett's post hoc test for multiple comparisons ($p \leq 0.05$). Graphs were generated using GraphPad Prism 7.0 software (GraphPad Software Inc., San Diego, CA, USA).

3 RESULTS AND DISCUSSION

All datasets met the assumptions for parametric analysis, with homogeneity of variances confirmed by Levene's test ($p > 0.05$). One-way ANOVA revealed significant effects of treatments on reproduction for all combinations of species and fuels ($p < 0.001$), and Dunnett's test indicated significant differences between all contaminated treatments and the control ($p \leq 0.05$). No mortality was observed in any treatment, indicating that survival was not a sensitive endpoint under the experimental conditions.

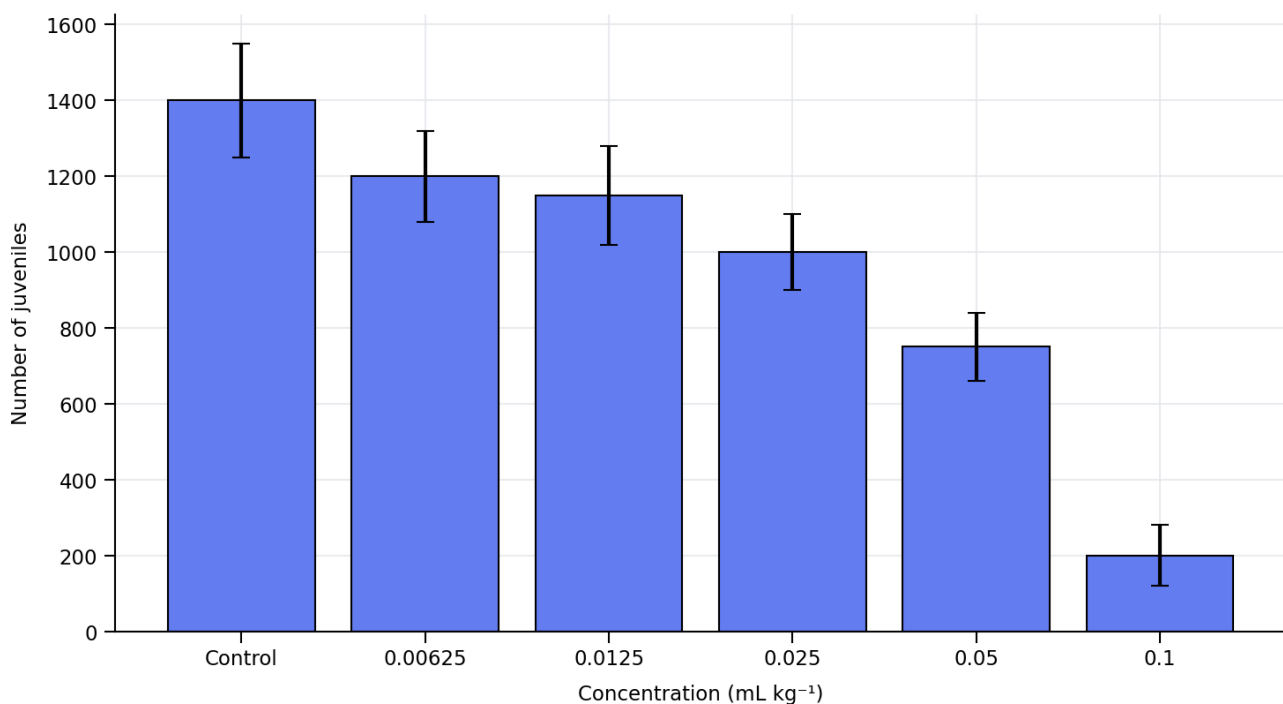
3.1 Toxicity of gasoline in TAS to *Folsomia candida*

Gasoline exposure resulted in a significant reduction in reproduction of *Folsomia candida* at all tested concentrations. A clear dose-dependent response was observed, with

a progressive decrease in the number of juveniles as contamination levels increased. At the highest concentration (0.1 mL kg⁻¹), reproduction was completely inhibited, indicating a strong toxic effect at elevated exposure levels. Survival remained unaffected throughout all treatments. As shown in Figure 1.

The EC₂₀ and EC₅₀ values were 0.032 and 0.050 mL kg⁻¹, respectively (Table 1). No no-observed-effect concentration (NOEC) could be established, as all treatments differed significantly from the control. The lowest observed effect concentration (LOEC) corresponded to 0.00625 mL kg⁻¹.

Figure 1 - Reproduction of *Folsomia candida* exposed to increasing concentrations of gasoline in Tropical Artificial Soil (TAS) after 28 days. Bars represent the mean number of juveniles $\pm$ standard deviation (SD) (n = 5). Significant differences compared to the control are indicated ($p \leq 0.05$; Dunnett's test)

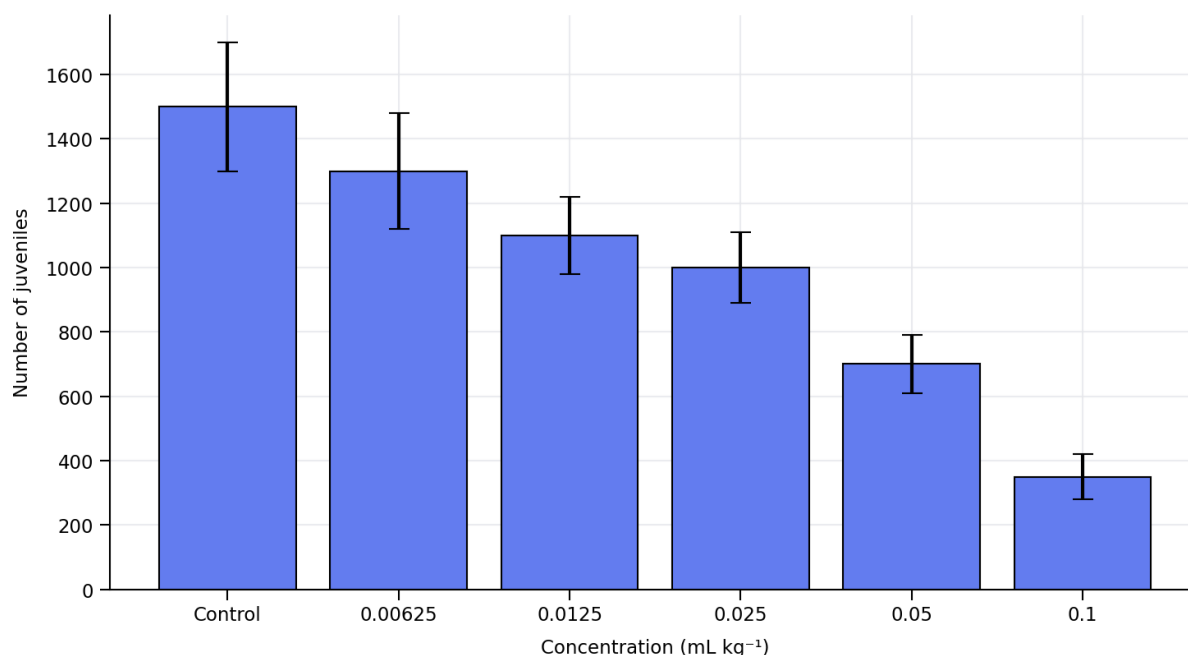


3.2 Toxicity of diesel oil in TAS to *Folsomia candida*

Exposure to diesel oil also caused a significant reduction in reproduction of *F. candida* across all tested concentrations, following a consistent dose-dependent trend. In contrast to gasoline, reproduction decreased progressively but was not completely inhibited within the tested range, suggesting a less abrupt effect at higher concentrations. Survival was not affected. As shown in Figure 2.

The EC₂₀ and EC₅₀ values were 0.012 and 0.042 mL kg⁻¹, respectively (Table 1), indicating higher toxicity of diesel oil at lower concentrations. Similarly, no NOEC could be determined, and the LOEC was 0.00625 mL kg⁻¹.

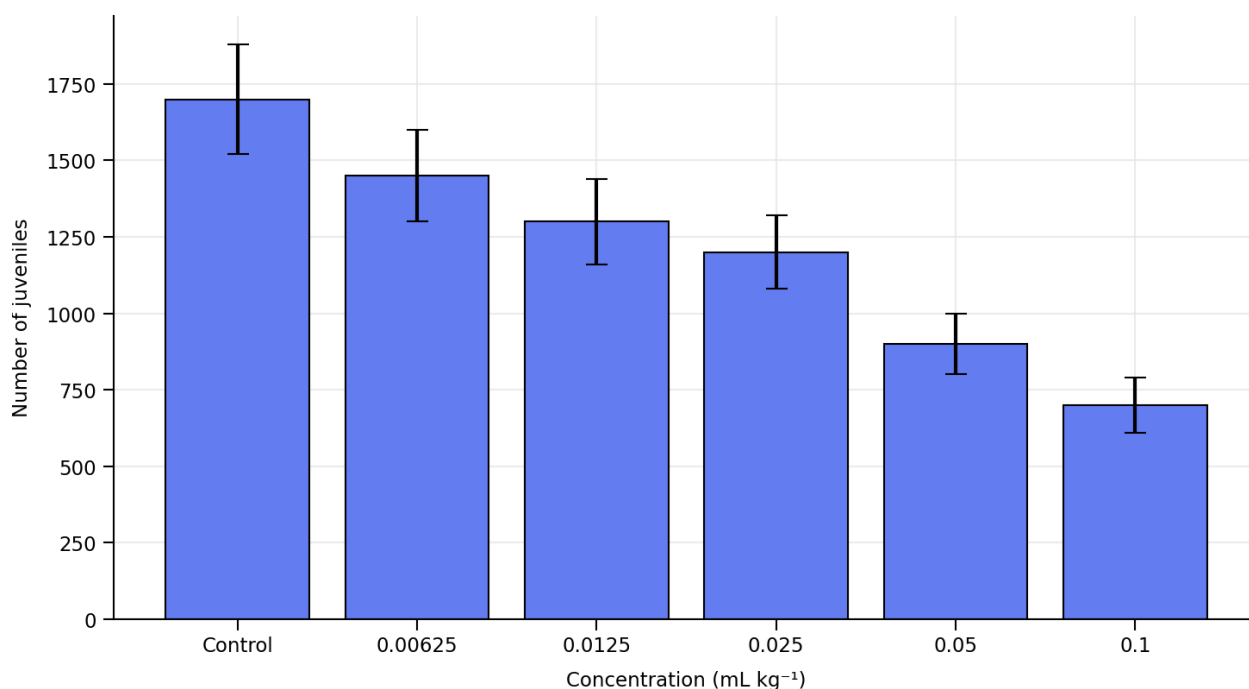
Figure 2 – Reproduction of *Folsomia candida* exposed to increasing concentrations of diesel oil in Tropical Artificial Soil (TAS) after 28 days. Bars represent the mean number of juveniles $\pm$ standard deviation (SD) (n = 5). Significant differences compared to the control are indicated ($p \leq 0.05$; Dunnett's test)



3.3 Toxicity of gasoline in TAS to *Enchytraeus crypticus*

Gasoline exposure led to a significant reduction in reproduction of *Enchytraeus crypticus* at all concentrations tested, with a pronounced dose-dependent pattern. Adverse effects were observed even at the lowest concentration, demonstrating high sensitivity of this species to gasoline contamination. No mortality was recorded. As shown in Figure 3.

Figure 3 - Reproduction of *Enchytraeus crypticus* exposed to increasing concentrations of gasoline in Tropical Artificial Soil (TAS) after 21 days. Bars represent the mean number of juveniles $\pm$ standard deviation (SD) (n = 5). Significant differences compared to the control are indicated ($p \leq 0.05$; Dunnett's test)



The EC₂₀ and EC₅₀ values were 0.0062 and 0.027 mL kg⁻¹, respectively (Table 1), which are lower than those observed for *F. candida*, confirming greater sensitivity of *E. crypticus*. As in the previous tests, no NOEC was identified, and the LOEC was 0.00625 mL kg⁻¹.

3.4 Toxicity of diesel oil in TAS to *Enchytraeus crypticus*

Diesel oil exposure caused significant reductions in reproduction of *E. crypticus* across all concentrations, with marked effects already at low exposure levels. The decrease followed a consistent dose-dependent pattern and was more pronounced at lower concentrations compared to gasoline. As shown in Figure 4.

The EC₂₀ and EC₅₀ values were 0.0027 and 0.0206 mL kg⁻¹, respectively (Table 1), representing the lowest values among all treatments. These results indicate that *E. crypticus* exposed to diesel oil was the most sensitive condition evaluated in this study. No NOEC could be established, and the LOEC was 0.00625 mL kg⁻¹.

Figure 4 – Reproduction of *Enchytraeus crypticus* exposed to increasing concentrations of diesel oil in Tropical Artificial Soil (TAS) after 21 days. Bars represent the mean number of juveniles ± standard deviation (SD) (n = 5). Significant differences compared to the control are indicated (p ≤ 0.05; Dunnett's test).

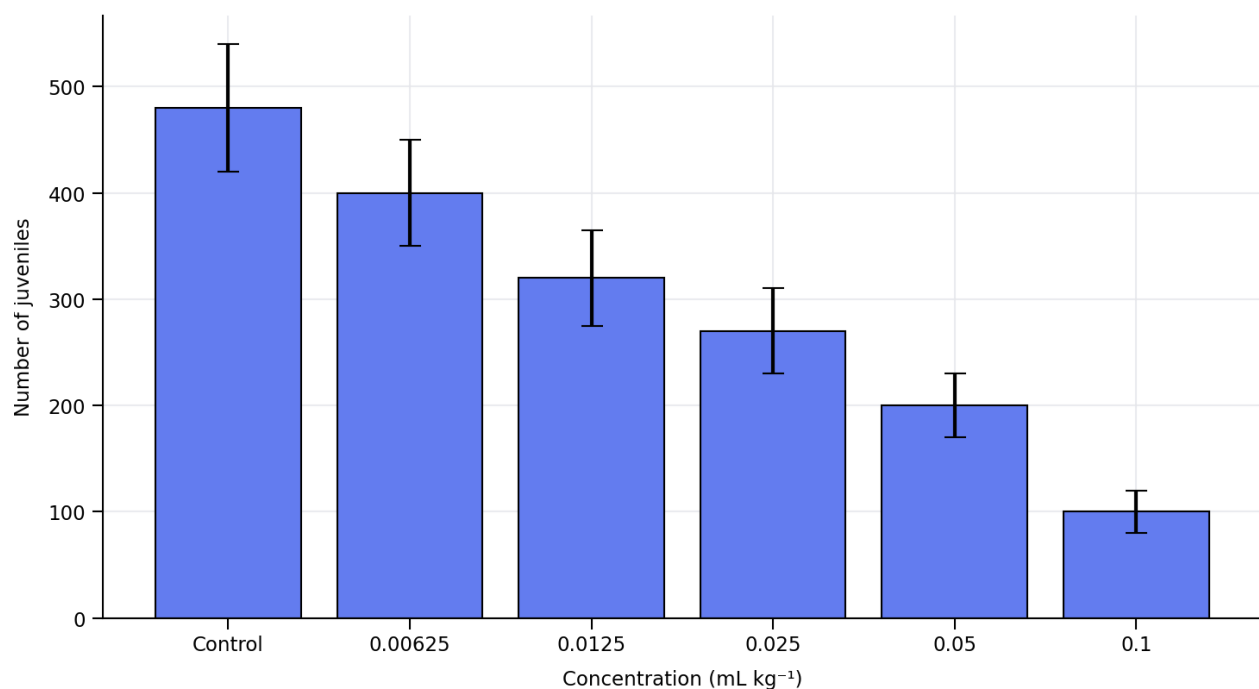


Table 1 shows the EC₂₀ and EC₅₀ values obtained for both species exposed to gasoline and diesel oil.

The results demonstrate that both gasoline and diesel oil induced significant chronic effects on soil invertebrates, primarily affecting reproduction, which is recognized as one of the most sensitive endpoints in soil ecotoxicology. The occurrence of significant effects at

the lowest tested concentration (0.00625 mL kg⁻¹) for all treatments indicates high sensitivity of both species to petroleum-derived contaminants.

Table 1 - Effective concentrations causing 20% (EC₂₀) and 50% (EC₅₀) reduction in reproduction for *Folsomia candida* and *Enchytraeus crypticus* exposed to gasoline and diesel oil in Tropical Artificial Soil (TAS). Values were obtained by nonlinear regression using a four-parameter log-logistic model. R² indicates goodness of fit of the model

Species	Fuel	EC20 (mL kg ⁻¹)	EC50 (mL kg ⁻¹)	R2
<i>Folsomia candida</i>	Gasoline	0.0320	0.0500	0.94
<i>Folsomia candida</i>	Diesel oil	0.0120	0.0420	0.93
<i>Enchytraeus crypticus</i>	Gasoline	0.0062	0.0270	0.96
<i>Enchytraeus crypticus</i>	Diesel oil	0.0027	0.0206	0.94

A consistent pattern was observed, with diesel oil presenting higher toxicity at lower concentrations, as evidenced by lower EC₂₀ and EC₅₀ values (Table 1), indicating that adverse effects occurred at lower exposure levels. This trend was particularly evident for *Enchytraeus crypticus*, which exhibited the highest sensitivity among the tested organisms.

When compared with values reported in the literature, the EC₅₀ values obtained in this study indicate a relatively high sensitivity of both species under tropical conditions. Previous studies on hydrocarbon-contaminated soils or petroleum-derived compounds have generally reported EC₅₀ values for *Folsomia candida* in the range of tens of mg kg⁻¹, such as approximately 45.8 mg kg⁻¹ for phenanthrene (Nota et al., 2009). Similarly, for *Enchytraeus crypticus*, EC₅₀ values for phenanthrene and other PAHs have been reported in higher concentration ranges (Roelofs et al., 2016).

Although reported in different units, these comparisons clearly indicate differences in toxicity magnitude. In contrast, the EC₅₀ values obtained in the present study, particularly for *Enchytraeus crypticus* exposed to diesel oil (0.0206 mL kg⁻¹), are substantially lower, indicating increased toxicity and bioavailability under Tropical Artificial Soil conditions. This difference may be attributed to factors such as soil composition, lower organic matter content, and reduced sorption capacity, which can enhance contaminant exposure to soil organisms. This effect has been previously demonstrated, with toxicity increasing as soil organic matter decreases (van Hall et al., 2025).

Moreover, bioavailability is recognized as a key factor controlling toxicity in soil ecotoxicology (van Gestel, 2012), reinforcing the importance of considering soil type when interpreting ecotoxicological thresholds.

The higher sensitivity of *E. crypticus* may be associated with its ecological and physiological characteristics, such as continuous interaction with the soil matrix through

ingestion and burrowing activity, which increases exposure to contaminants. In contrast, differences in feeding behavior, metabolism, and detoxification processes may explain the comparatively lower sensitivity observed in *F. candida*.

Although diesel oil showed higher toxicity at low concentrations, gasoline produced more severe effects at higher exposure levels, including complete inhibition of reproduction in *F. candida*. This indicates that diesel may pose greater risks under chronic low-level contamination, while gasoline may lead to more acute impacts under higher exposure conditions.

These differences are likely related to fuel composition. Diesel oil contains heavier and more persistent hydrocarbons, including polycyclic aromatic hydrocarbons (PAHs), which are associated with chronic toxicity. Conversely, gasoline is composed mainly of lighter and more volatile compounds, such as BTEX, which may exhibit higher acute toxicity but dissipate more rapidly in the environment.

From an ecological perspective, reductions in reproduction can compromise essential ecosystem functions, including organic matter decomposition, nutrient cycling, and soil structure maintenance. Given the ecological roles of both species, the observed effects suggest potential long-term impacts on soil ecosystem functioning.

Recent studies also demonstrate that, even after remediation processes or partial degradation, soils contaminated with hydrocarbons may retain considerable levels of toxicity, indicating persistent ecological risks (Chai et al., 2023). Therefore, the effects observed in the present study reinforce the importance of considering the chronic impacts of petroleum-derived contaminants, even under scenarios of low-level exposure.

Overall, the absence of NOEC values and the occurrence of effects at the lowest tested concentration highlight the environmental risk associated with petroleum-derived contaminants, even at low levels. These findings reinforce the importance of chronic ecotoxicological assays in environmental risk assessment, particularly under tropical conditions.

These results are also relevant in a regulatory context. In Brazil, standards such as CONAMA Resolution No. 273/2000, as well as contaminated site management frameworks adopted by agencies such as CETESB, establish guidelines for preventing, monitoring, and managing soil contamination by fuel-derived compounds. In this context, the use of standardized bioassays with species such as *Folsomia candida* and *Enchytraeus crypticus* can provide valuable biological evidence to complement physicochemical analyses, supporting more robust environmental risk assessments.

Despite the relevance of the results, some limitations should be considered. The use of Tropical Artificial Soil (TAS), although ensuring standardization, does not fully represent

natural soil complexity. In addition, no detailed physicochemical characterization of the fuels was performed, and interpretations were based on information from Safety Data Sheets (SDS). Future studies integrating chemical analyses, degradation processes, and natural soil conditions are recommended to improve ecological relevance.

4 CONCLUSIONS

This study demonstrated that gasoline and diesel oil induce significant chronic effects on soil invertebrates, primarily through reductions in reproductive performance, while survival remained unaffected. *Enchytraeus crypticus* was more sensitive than *Folsomia candida*, and diesel oil showed higher toxicity at lower concentrations, whereas gasoline caused more pronounced effects at higher exposure levels.

The absence of a NOEC and the identification of effects at the lowest tested concentration indicate that even low levels of petroleum-derived contamination may pose ecological risks. These findings highlight the importance of using sensitive endpoints, such as reproduction, and multiple bioindicator species in ecotoxicological assessments.

From a practical perspective, the results support the integration of soil ecotoxicological assays into environmental monitoring programmes and regulatory frameworks. The use of standardized organisms such as *Folsomia candida* and *Enchytraeus crypticus* can contribute to improving the assessment and management of contaminated areas, particularly in tropical regions. These approaches may strengthen decision-making processes related to soil quality, remediation strategies, and environmental licensing, including applications in regulatory contexts such as those adopted by CETESB.

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