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INTERNATIONAL SYMPOSIUM ON INNOVATION AND TECHNOLOGY
QUANTUM TECHNOLOGIES: The information revolution that will change the future
International Year of Quantum Science and Technology
SENAI CIMATEC UNIVERSIDADE

ECOTOXICOLOGICAL EVALUATION OF AQUATIC ENVIRONMENT CONTAMINATED BY DIESEL AND BIODIESEL USING *Daphnia similis* AS TEST ORGANISM

Clara Rodrigues Pereira[1], Adriano Carvalho Simões Guimarães[2], Edna dos Santos Almeida[3], Lilian Lefol Nani Guarieiro[4]
[1,2,3,4] *Universidade Senai Cimatec, Salvador, Bahia, Brazil*

Abstract: This study aimed to evaluate the ecotoxicity of an aquatic environment contaminated with Diesel S10 and Biodiesel B100, using *Daphnia similis* as the test organism. For this purpose, ecotoxicological tests were performed based on the ABNT NBR 12713 standard, during which the *Daphnia* were exposed to different test solutions of the fuels for 24 and 48 hours. The results demonstrated that exposure to Diesel S10 caused significantly more severe effects than exposure to Biodiesel B100. These data highlighted that Diesel S10 has a higher toxic potential in aquatic environments than Biodiesel B100.

Keywords: Ecotoxicity; Diesel; Biodiesel; Daphnia similis.

1. Introduction

Due to the growing energy demand, the use of fossil fuels, such as diesel, has intensified. However, the high cost of these fuels and their significant environmental impacts have driven the search for cleaner and more sustainable alternatives [1]. The continued use of these fuels raises concerns about their environmental impact, affecting both the atmosphere and the flora and fauna. In this context, aquatic ecosystems are particularly vulnerable to contamination, whether from pollutants emitted through engine exhausts or from fuel spills during maritime transport [2,3].

Fossil fuels, particularly diesel, which is widely used in maritime transport, can have serious environmental impacts on aquatic ecosystems. Diesel contains chemicals that, when released into the environment, can degrade water quality, compromising not only the ecological balance of bodies of water, but also the health of the species that inhabit these environments [4]. The impact on water quality after a diesel spill may be related to the presence of polycyclic aromatic hydrocarbons (PAHs), known for their high toxic and carcinogenic potential. The severity of this impact varies according to the solubility of PAHs in water: low molecular weight PAHs are more soluble and volatile, while high molecular weight PAHs are more persistent in the marine environment [4,5].

In this context, fuels derived from renewable sources are gaining prominence due to their lower environmental impact. Biodiesel has emerged as a more strategic alternative to minimize the impacts caused by the use of fossil fuels, highlighting a possible rapid degradation of its components in an aqueous medium and has become an increasingly adopted option [1,6]. It’s crucial to assess the impact of fuels of this nature to mitigate the possible adverse effects they may cause in aquatic environments.

Thus, ecotoxicological tests have proven to be extremely useful for investigating spills in aquatic environments. They allow for a more detailed analysis of the impacts, with emphasis on the use of *Daphnia* as an effective bioindicator [2,7].

Therefore, ecotoxicological tests with *Daphnia* are highly significant, since these organisms are widely used to assess toxicity and water quality. *Daphnia* are microcrustaceans of the order Cladocera, with sizes ranging from 0.5 to 5.0 mm, and are widely used in ecotoxicological tests due to their ease of cultivation and accessibility. Among the species used, *Daphnia magna* and *Daphnia similis* are the most common. It should be noted that *Daphnia similis* is particularly suitable for tropical climates, where it adapts well to environmental conditions [8].

In this context, this study sought to evaluate the toxicity of an aquatic environment contaminated by diesel and biodiesel, using *Daphnia similis* as a test organism.

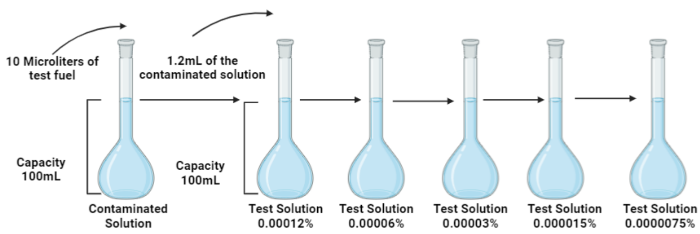
2. Methodology

The methodology employed in this study involved conducting acute ecotoxicological tests with *Daphnia similis*, adhering to the guidelines outlined in standard NBR 12713 (Aquatic Ecotoxicology — Acute Toxicity — Test Method with *Daphnia* spp) [9]. The test fuels comprised pure Diesel S10 and Biodiesel B100. For each fuel, a contaminated solution (effluent) was prepared by mixing 10 microliters of the fuel with 100 mL of the organisms' culture medium.

The purpose of preparing this effluent was to simulate a fuel spill scenario in an aquatic environment. Following the standard's guidelines, five test solutions (doses) were created, each with a specific concentration of the contaminated solution, to assess the toxicity of S10 and B100 on *Daphnia*. These concentrations were determined based on preliminary analyses involving the exposure of *Daphnia* to various fuel levels.

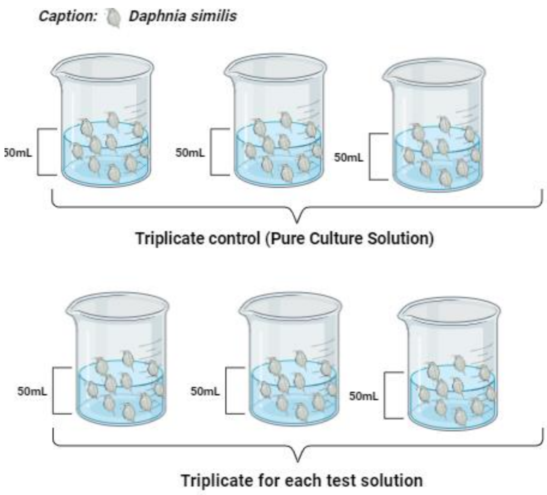
The test doses were prepared using the successive dilution method (Figure 1), which involves diluting the most concentrated solution by 50% in the culture medium, starting from the previous dose to create progressively lower levels of contamination. Consequently, the test solutions for Diesel S10 ranged from 0.00024% to 0.000015%, while those for Biodiesel B100 ranged from 0.00012% to 0.0000075%.

Figure 1. Preparation of test solutions.



Thus, the test with *Daphnia similis* was conducted with 24-hour and 48-hour exposure periods, under the temperature (18 to 22°C), pH (7 to 7.6), and photoperiod (16 hours light/8 hours dark) conditions specified by the standard. In this setup, the test was performed in triplicate, with each replicate containing 10 organisms and 50 mL of the test solution (Figure 2), along with triplicate controls using pure culture solution.

Figure 2. Acute Ecotoxicological Test Design.



As a supplementary analysis of the ecotoxicological test involving *Daphnia similis*, the calculation of the EC50 (Median Effective Concentration) was performed. EC50 estimates the concentration required to cause an adverse effect (immobility and mortality) in 50% of the exposed organisms. This determination was made using the Trimmed Spearman-Karber (TSK) statistical model, which generates a dose-response curve based on empirical values within the range of the tested solutions. The model assesses the extreme high and low effects, disregards these points, and estimates the EC50 based on the intersection of the resulting curve. Notably, in this study, the model was implemented using the RStudio software, using the *drc* package, specifically developed for non-linear regression and dose-response analyses in ecotoxicological data.

3. Results and Discussions

The results presented in this section detail the effects observed in *Daphnia similis* after 24 and 48 hours of exposure (Figures 3 and 4). These findings illustrate the behavior of the organisms during the experiment with different concentrations of the contaminated solutions of Diesel S10 and Biodiesel B100.

Figure 3. Results for Diesel S10 Test Solutions in *Daphnia similis*: a) 24h, b) 48h.

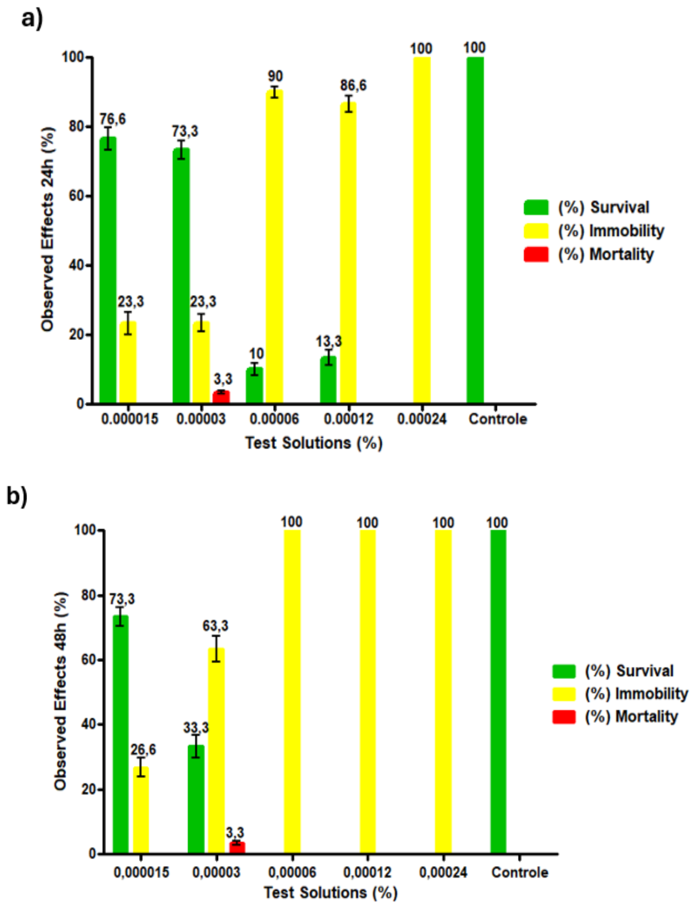
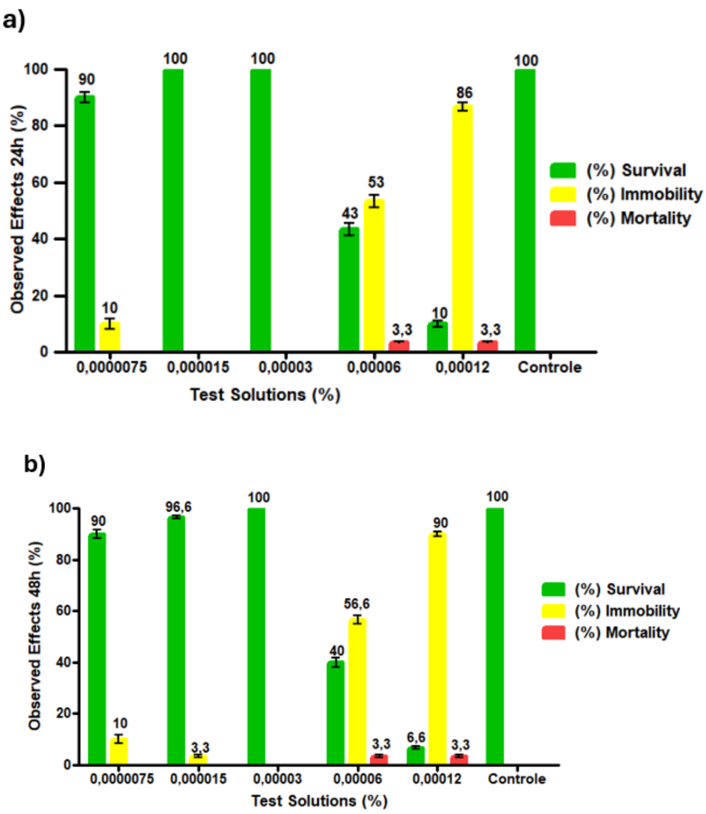


Figure 4. Results for Biodiesel B100 Test Solutions in *Daphnia similis*: a) 24h, b) 48h

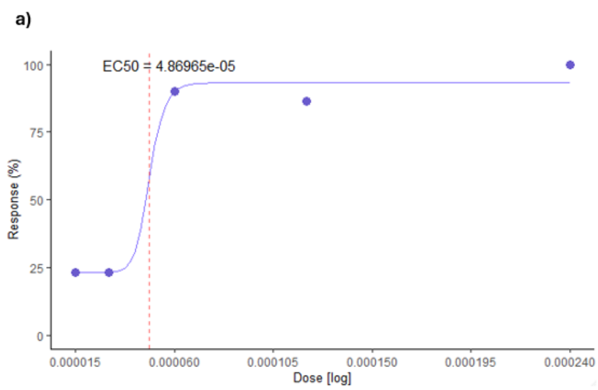


The results highlight that the effects observed in organisms exposed to Diesel S10 were more severe compared to those exposed to Biodiesel B100. According to Müller et al. (2019) [10], the water-soluble fraction of diesel was found to be more toxic than that of biodiesel when assessed with *Daphnia magna*, leading to marked reductions in reproduction, longevity, and growth. These findings align with the present results, where Biodiesel B100 showed a lower toxic effect compared to Diesel S10, reinforcing its reduced ecotoxicological potential.

Specifically, *Daphnia* exposed to Diesel S10 exhibited 100% immobility within 48 hours at concentrations ranging from 0.00006% to 0.00024%. In contrast, exposure to Biodiesel B100 resulted in immobility only at higher concentrations, without reaching 100% immobility even at the highest concentration of 0.00012%. This indicates that Diesel S10 has a higher toxic potential compared to Biodiesel B100, impacting the organisms more significantly and rapidly. However, it is crucial to evaluate the toxicity of these substances at various dilutions to understand their contamination profile in aquatic environments in spill scenarios.

In this context, the immobility data from the assay were analyzed using RStudio software to calculate the EC50 for each fuel, as shown in Figures 5 and 6, for the established exposure periods. This process was essential for assessing and confirming the toxicity of these substances based on the observed effects in the experiment.

Figure 5. EC50 Results for Diesel S10 in *Daphnia similis*: a) 24h, b) 48h



ISSN: 2357-7592

b)

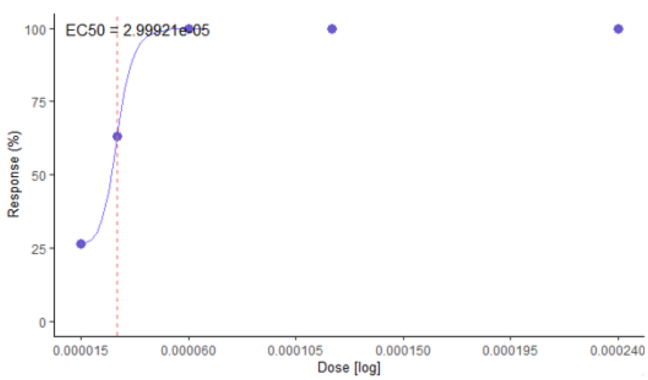
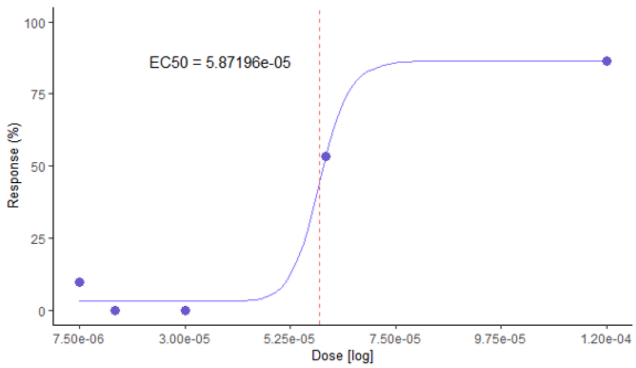


Figure 6. EC50 Results for Biodiesel B100 in *Daphnia similis*: a) 24h, b) 48h

a)



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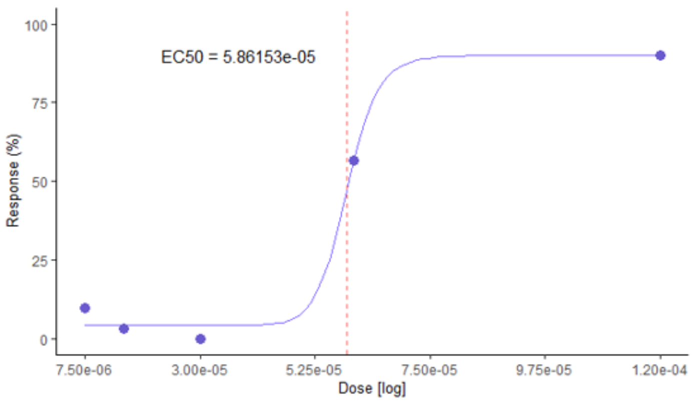

Based on the results, the EC50 (48 hours) for Diesel S10 was identified to be between 0.000015% and 0.00006%, while for Biodiesel B100, it ranged from 0.00003% to 0.00006%. These findings align with the observed effects in the experiments, showing that the lower EC50 for Diesel S10 is associated with its higher toxicity in aquatic environments, as it impacts organism health at lower concentrations. This analysis is illustrated by the dose-response curves, where the curve for Diesel S10 is steeper, indicating a faster effect on *Daphnia*. In contrast, Biodiesel B100 takes longer to affect the organisms, suggesting lower toxicity in aquatic settings.

This work emphasized the calculation of EC_{50}, a parameter that reflects the concentration required to impair mobility in half of the exposed *Daphnia similis*. However, determining LC_{50} in parallel could provide complementary insights, as it specifically quantifies lethality. Including both endpoints in future assessments would strengthen the reliability of ecotoxicological studies and enable a broader understanding of acute fuel toxicity.

4. Conclusion

The results of this study revealed significant differences in the toxicity of the culture medium solution for *Daphnia similis* contaminated with Diesel S10 and Biodiesel B100. The analyses demonstrated that Diesel S10 has a considerably higher toxic potential, leading to complete immobility of the organisms within the first 48 hours of exposure to test solutions ranging from 0.00006% to 0.00024%. In contrast, Biodiesel B100 showed a less pronounced toxic effect, requiring higher concentrations to induce immobility.

These findings are supported by the EC50 calculations, which indicated a significantly lower EC50 value for Diesel S10 compared to Biodiesel B100. This reinforces the conclusion that Diesel S10 may pose a higher toxicity risk in aquatic spill scenarios, consistent with the existing literature. The study also highlighted the need for further research with various dilutions and experimental conditions to gain a deeper understanding of the contamination profiles and the potential ecotoxicological risks associated with these fuels.

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