FROM CLEANLINESS TO CONTAMINATION: INVESTIGATING THE TOXICITY OF A COMMERCIAL DETERGENT USING *ARTEMIA SALINA* L. AS A MODEL ORGANISM

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ABSTRACT: Detergents are widely used in industries and households for cleaning surfaces and objects. However, after use, the foam from this compound often ends up contaminating aquatic ecosystems, which can trigger problems such as eutrophication and a reduction in available oxygen levels. In this study, the toxic potential of a biodegradable commercial detergent on the microcrustacean *Artemia salina* was evaluated. Six different concentrations of the detergent (2.98, 9.54, 30.52, 97.66, 312.5, and 1000 µg/mL^{-1}) were prepared through dilution in artificial seawater. The toxicity test followed the protocols of the NBR 16530 standard. Newly hatched nauplii of *A. salina* were exposed to the different concentrations for a period of 24 hours. After this period, the mortality rate and the median lethal concentration (LC_{50}) were evaluated. The results showed that the commercial detergent caused significant mortality starting at a concentration of 30.52 µg/mL^{-1}, resulting in 47.50% of deaths. At concentrations of 312.5 and 1000 µg/mL^{-1}, 100% mortality was observed. The LC_{50} after 24 hours of exposure was calculated as 35.04 µg/mL^{-1}, indicating that the detergent has a high degree of toxicity to *A. salina*, despite its biodegradability.
Keywords: Aquatic ecosystems, Environmental risk, Surfactants, Water pollution.

DA LIMPEZA À CONTAMINAÇÃO: INVESTIGANDO A TOXICIDADE DE UM DETERGENTE COMERCIAL UTILIZANDO *ARTEMIA SALINA* L. COMO ORGANISMO MODELO

RESUMO: Detergentes são amplamente utilizados nas indústrias e nas residências para a limpeza de superfícies e objetos. No entanto, após o uso, a espuma desse composto frequentemente acaba contaminando ecossistemas aquáticos, o que pode desencadear problemas como a eutrofização e a redução dos níveis de oxigênio disponível. Neste estudo, foi avaliado o potencial tóxico de um detergente comercial biodegradável sobre o microcrustáceo *Artemia salina*. Seis concentrações diferentes do detergente (2,98; 9,54; 30,52; 97,66; 312,5; e 1000 µg/mL^{-1}) foram preparadas através de diluição em água do mar artificial. O teste de toxicidade seguiu os protocolos da norma NBR 16530. Náuplios de *A. salina* recém-eclodidos foram expostos às diferentes concentrações por um período de 24 horas. Após esse período, foram avaliadas a taxa de mortalidade e a concentração letal média (LC_{50}). Os resultados demonstraram que o detergente comercial causou mortalidade significativa a partir da concentração de 30,52 µg/mL^{-1}, resultando em 47,50% de mortes. Nas concentrações de 312,5 e 1000 µg/mL^{-1}, observou-se 100% de mortalidade. A LC_{50} após 24 horas de exposição foi calculada como 35,04 µg/mL^{-1}, indicando que o detergente possui alto grau de toxicidade para *A. salina*, apesar de sua biodegradabilidade.
Palavras-chave: Ecossistemas aquáticos, Risco Ambiental, Surfactantes, Poluição da água.

DE LA LIMPIEZA A LA CONTAMINACIÓN: INVESTIGANDO LA TOXICIDAD DE UN DETERGENTE COMERCIAL UTILIZANDO *ARTEMIA SALINA* L. COMO ORGANISMO MODELO

RESUMEN: Los detergentes son ampliamente utilizados en industrias y hogares para limpiar superficies y objetos. Sin embargo, después de su uso, la espuma de este compuesto a menudo contamina los ecosistemas acuáticos, lo que puede desencadenar problemas como la eutrofización y la reducción de los niveles de oxígeno disponible. En este estudio, se evaluó el potencial tóxico de un detergente comercial biodegradable sobre el microcrustáceo *Artemia salina*. Se prepararon seis concentraciones diferentes del detergente (2,98; 9,54; 30,52; 97,66; 312,5; y 1000 µg/mL^{-1}) mediante dilución en agua de mar artificial. La prueba de toxicidad siguió los protocolos de la norma NBR 16530. Los nauplios de *A. salina* recién eclosionados fueron expuestos a las diferentes concentraciones durante un período de 24 horas. Después de este período, se evaluaron la tasa de mortalidad y la concentración letal media (LC_{50}). Los resultados demostraron que el detergente comercial causó una mortalidad significativa a partir de una concentración de 30,52 µg/mL^{-1}, lo que resultó en un 47,50% de muertes. En las concentraciones de 312,5 y 1000 µg/mL^{-1}, se observó un 100% de mortalidad. La LC_{50} después de 24 horas de exposición se calculó en 35,04 µg/mL^{-1}, lo que indica que el detergente es altamente tóxico para *A. salina*, a pesar de su biodegradabilidad.
Palavras clave: Ecosistemas acuáticos, Riesgo Ambiental, Surfactantes, Contaminación del agua.

INTRODUCTION

In recent decades, rapid industrialization and urbanization have wrought significant transformations upon aquatic ecosystems, primarily attributed to the introduction of foreign substances known as xenobiotics. Within this category, detergents stand out as high-impact contaminants. These versatile substances find ubiquitous use in both industrial and household settings, representing a substantial global investment, with an annual allocation of approximately 60 billion dollars to their production[1,2].

Detergents are available in various forms on the market, including liquids, powder, paste, or bars. Their mode of action involves the synergistic action of surfactants for the removal of fats and dirt, often combined with alkaline or acidic agents to facilitate residue dissolution[3,4]. However, concerns surrounding water pollution have intensified in recent years, primarily due to the high toxicity and easy absorption of chemical compounds, including detergents[5,6].

One of the concerning consequences of the high concentration of detergents in aquatic environments is the formation of a foam layer on the water's surface, impeding the diffusion of atmospheric oxygen. This leads to a deficit in the absorption of dissolved oxygen by aquatic organisms[7,8]. Notably, detergents often contain significant phosphate content, whose excessive release into water bodies can trigger the eutrophication process and foster uncontrolled algae growth[9]. The proliferation of algae contributes to the accumulation of organic matter, reducing dissolved oxygen levels and impacting biodiversity within the ecosystem[10].

Beyond these environmental challenges, it's essential to recognize the potential harm that phosphate and surfactant-contaminated water can pose to both aquatic organisms and humans[5,9]. Given the complexity of this scenario, there is an urgent need for a comprehensive assessment of the risks and potential impacts associated with detergent use in aquatic ecosystems. This is where ecotoxicology emerges as a valuable ally, utilizing model organisms to assess the potential risks and consequences of these chemical substances in the natural environment.

Among the several model organisms suitable for studying aquatic ecosystems, the microcrustacean *Artemia salina* L. holds a prominent position. The toxicity assays employing this species offer multiple advantages, including the ease of obtaining and low cost of the cysts used in tests. Additionally, the procedure is notably simple and reproducible, without the need for complex equipment. *A. salina* also exhibits remarkable sensitivity, demonstrating reliable correlations with mammalian models. Furthermore, its position in the food chain amplifies its significance as an indicator of environmental effects within aquatic ecosystems[11,12,13].

In this context, the present study aims to assess the toxicity of a commercial detergent concerning the microcrustacean *A. salina*. Through this research, we endeavor to contribute significantly to a deeper understanding of the potential adverse effects of this detergent on the environment. Furthermore, this effort seeks to promote the continuous development of safer and ecologically friendly chemical products.

METHODOLOGY

Preparation of test solutions

The commercial formulation of the biodegradable liquid detergent was locally sourced and had a complex chemical composition, including various components such as surfactants, chelating agents, thickeners, preservatives, coadjuvants, dyes, fragrances, water, and linear alkylbenzene sulfonate[14]. Following the methodology recommended by OECD 202[15], six solutions of the commercial detergent were prepared for toxicity evaluation. To achieve this, a multiplication factor of 3.2 was employed between each dose, aiming to obtain a diversified concentration scale.

These concentrations were reached by diluting the commercial detergent in mineral water, resulting in the following concentrations: 2.98; 9.54; 30.52; 97.66; 312.5; and 1000 $\mu g/mL^{-1}$. For comparison purposes, a control group was also prepared using only mineral water.

Since *A. salina* is a marine microcrustacean, the salinity of both the test solutions and the control group was adjusted to 3.5% using commercial sea salt.

Toxicity test with *A. salina*

Cysts of *A salina* were acquired from specialized aquaculture suppliers and used in obtaining nauplii. For this purpose, 0.1 gram of cysts was immersed in 1 liter of oxygen-saturated artificial seawater. This solution was prepared by adding sea salt to mineral water and adjusting the salinity to 3.5%. The cysts were kept in a container with controlled temperature, maintained at 24 ± 2°C, for a period of 30 hours. After this time interval, the newly hatched nauplii were collected and employed in the toxicity test.

The toxicity test was conducted following the guidelines of standard NBR 16530[16], with some minor modifications. The experimental design adopted was completely randomized, including seven treatments (six detergent concentrations + control) and four replicates for each treatment, as recommended by NBR 16530 (NBR, 2021). For each replicate, 20 mL acrylic containers were used. In each container, 10 mL of the test solutions, which were prepared in advance, were added, and shortly after, 10 newly hatched nauplii were added using a Pasteur pipette. The containers were then sealed with plastic wrap and maintained at a controlled temperature of 24 ± 2°C for a period of 24 hours. After this time interval, the counting of dead organisms was performed.

Data analysis

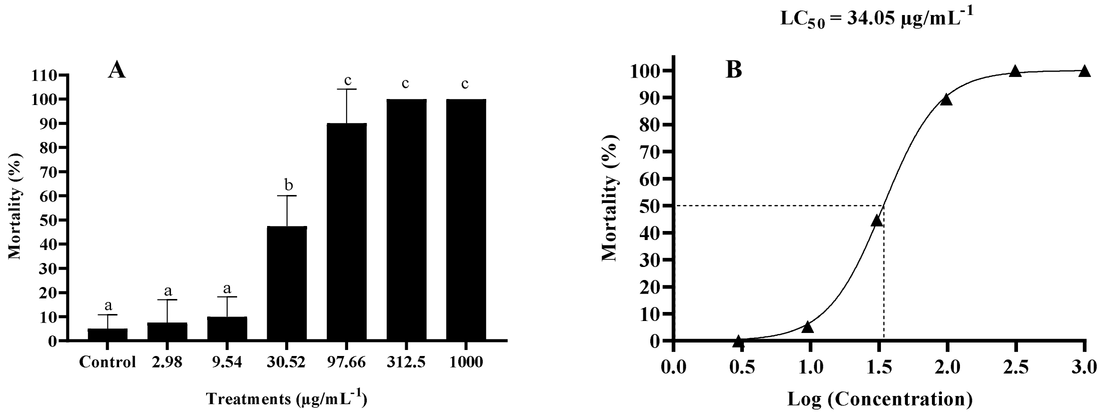
The mortality rate data were subjected to analysis of variance, and the means were compared using the Sisvar software. Additionally, the average lethal concentration, i.e., the concentration that caused 50% mortality of the exposed organisms (LC_{50}), was calculated using the GraphPad Prism software.

RESULTS

The mortality rate in the control group was 5%, which validates the test since it complies with the parameters established by the NBR 16530 standard. At concentrations of 2.98 and 9.54 ug/mL^{-1} of the commercial detergent, mortality did not differ statistically from the control, remaining below 10% (Figure 1A). However, at the concentration of 30.52 ug/mL^{-1}, mortality reached 47.50%, significantly differing from the control group (Figure 1A). In the case of the detergent concentration of 97.66 ug/mL^{-1}, the observed mortality rate was 90%, and at the other concentrations (312.5 and 1000 ug/mL^{-1}), it reached 100% (Figure 1A).

It's worth noting that at the concentration of 1000 ug/mL^{-1}, nauplii mortality occurred during the setup of the experiment, only 10 minutes after exposure to the detergent. The mean lethal concentration (LC_{50}) after 24 hours of exposure was calculated to be 35.04 ug/mL^{-1} (Figure 1B).

Figure 1 - Mortality (%) and Mean Lethal Concentration (LC_{50}) obtained in the acute toxicity test with *A. salina* after 24 hours of exposure to different concentrations of a commercial detergent.



Source: Authors, 2023.

DISCUSSION

The toxicity of detergents towards aquatic organisms has been a subject of research since the late 1960s[17]. However, most studies up to this point have primarily focused on assessing the toxic effects of surfactants[18,19,20] or analyzing some of the components present in detergents[21]. Specific investigation of commercial product toxicity has been limited[22,23,24].

In this particular study, we used a biodegradable commercial detergent and observed its toxicity to the microcrustacean *A. salina*. Previous studies have indicated that despite their biodegradable nature, detergents exhibit toxicity to aquatic species[25,26,27,28]. However, few studies have evaluated the toxicity of these detergents on freshwater or saltwater microcrustaceans, especially using *A. salina*. These organisms play a crucial role in the ecological chain of these environments, serving as a food source for various species. Therefore, it is of utmost importance to assess the effects of chemicals like detergents on these organisms.

Throughout this study, we determined that the median lethal concentration (LC_{50}) for *A. salina* was 34.05 µg/mL^{-1} (Figure 1B). According to Clarkson et al. (2004)[29], substances with LC_{50} values below 100 µg/mL^{-1} are considered extremely toxic. In a previous study conducted by Nunes et al. (2005)[30], the acute toxicity of a detergent to the microcrustacean *Artemia parthenogenetica* Bowen & Sterling, 1978 was evaluated, resulting in an LC_{50} of 12.2 µg/mL^{-1} after 48 hours of exposure, a concentration very close to that observed in our current study.

Furthermore, another study conducted by Liwarska-Bizukojc et al. (2005)[31] assessed the toxic potential of five detergents, three of which were anionic (A1, A2, A3), and two were non-ionic (N1 and N2), using *A. salina* as a model organism. Notably, detergent A2 contained the same chemical constituent present in the detergent evaluated in our study, linear alkylbenzene sulfonate. The LC_{50} values obtained after 24 hours of exposure were as follows: A1 = 41.04 µg/mL^{-1}; A2 = 40.4 µg/mL^{-1}; A3 = 11.97 µg/mL^{-1}; N1 = 0.62 µg/mL^{-1}; and N2 = 4.54 µg/mL^{-1}. Once again, the LC_{50} values obtained were low and quite similar to those observed in the present study, characterizing the evaluated detergents as extremely toxic.

Understanding the toxicity of detergents in aquatic organisms is not yet fully elucidated. Detergents, including biodegradable ones, have been identified as causing toxic effects and osmoregulatory imbalances in aquatic life, especially when present in concentrations exceeding metabolic needs[32].

The toxicity of a detergent is intrinsically related to the presence of surfactants in its chemical composition, influenced by the molecular structure of the surfactant, the type of organism involved[33,34,35], and how the surfactant is ingested or absorbed by cells[18]. The nonspecific interaction between the surfactant and the cell membrane exemplifies the toxicity of this compound in aquatic organisms, resulting in the modification of cell membrane permeability and disruption of respiratory organ function[18].

In aquatic species, detergents also induce changes in liver and kidney function[18], as well as inhibit enzymatic processes[37]. Additionally, effluents containing detergents have been identified as causing severe damage to vital organs such as gills, skin, heart, and brain[1,38,39,40]. Furthermore, the indiscriminate release of detergents into aquatic ecosystems can reduce the concentration of dissolved oxygen, impairing respiration and leading to situations of asphyxiation, unconsciousness, or death due to low blood oxygenation[1].

Specifically concerning microcrustaceans, in addition to lethal effects, detergents also affect biomarkers of oxidative stress. In a study involving *A. parthenogenetica*, a significant decrease in acetylcholinesterase (AChE) activity of *A. parthenogenetica* was observed, indicating neurotoxic effects[41]. In another study conducted with water fleas (*Daphnia magna* Straus, 1820), the authors also observed acetylcholinesterase inhibition under *in vitro* and *in vivo* conditions[42].

CONCLUSION

The commercial detergent, despite being labeled as biodegradable, has proven to be extremely toxic to *A. salina*, an organism that plays a crucial role in the food chain of saline aquatic environments. It is important to note that the toxic effects observed in this study can be extrapolated to freshwater environments where other microcrustaceans are also present. These findings raise serious concerns, as toxicity in key organisms of the food chain can result in significant disruptions in ecosystem balance.

In this context, it is crucial to conduct further research with the aim of developing more biodegradable and environmentally friendly detergent products to preserve the integrity of aquatic ecosystems.

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