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Research Article

## Not all that glitters is gold: Glitter causes acute toxicity to nauplii of *Artemia* sp.

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### Abstract

Glitter has been reported as a relevant pollutant, as it is widely used in cosmetic and textile products and craftwork, and often associated with domestic sewage. The particular glitter is composed of thin layers of plastic and metal. This study assessed the acute toxicity of glitter dispersions in the brine shrimp *Artemia* sp. Nauplii of *Artemia* sp. that were exposed to glitter dispersions (0.01, 0.1, 1, 10, and 100 mg/L), obtained by diluting a stock solution in seawater; the control consisted of filtered seawater only. Three replicates were used per treatment and consisted of glass tubes filled with 10 mL of the test solution, and ten nauplii aging over 48 h. After 48 h, the survivors were counted and examined under a microscope. The mean size of glitter particles was 3.94 ( $\pm 0.98$ )  $\mu\text{m}$ ; approximately 44 % of particles were in the range of very fine sand, and 27 % coarse silt. Significant lethal effects ( $p < 0.05$ ) occurred from 0.1 mg/L (Lowest Observed Effect Concentration - LOEC); the lethal concentration to 50 % organisms (LC50-48h) was 0.350 (0.348 - 0.351) mg/L. The exposed organisms also exhibited patches in their digestive tracts, and particles were stranded in their appendices. The results indicate the toxic potential of glitter to brine shrimp. This investigation indicates the need for further studies on the toxicity of glitter to marine invertebrates.

## 1. Introduction

Plastic pollution has become a global problem because of increasing production and inappropriate release into the environment (Geyer et al., 2017; Xu et al. 2019). Plastic residues may have a wide variation in their composition and size; however, smaller particles are of greater concern because of their large quantity and capacity to be absorbed by biota (Catarino et al., 2018; Li et al., 2018; Qu et al., 2020). In particular, microplastics (MPs), i.e., plastic particles ranging from 1  $\mu\text{m}$  to 5 mm (Frias & Nash 2019; Bhardwaj et al., 2024), represent the dominant forms of plastic litter in aquatic environments (Galloway et al., 2017), and have been found to be omnipresent in both terrestrial and aquatic environments (Yurtsever, 2019a), including remote regions such as islands, polar zones, and deep oceans (Lusher et al., 2017a; Bhardwaj, 2024). A large percentage of plastics

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released into the environment tends to be transported to the sea and accumulated in large quantities in coastal regions (Li et al., 2018).

Microplastics discharged into the oceans include a myriad of types and forms of particles but, recently, glitter has been identified as a potential contaminant for coastal and marine waters (Tagg & Ivar do Sul, 2019; Yurtsever, 2019b). The glitter particles are formed by a set of plastic layers covered by thin metallic layers, similar to a sandwich, as shown by Tagg and Ivar do Sul (2019). They include a range of small, plain, and reflective particles, and are used in craftwork, textiles, and cosmetic products and applications (Yurtsever, 2019a; Guerranti et al., 2019). Glitter particles have large similarities to microbeads (i.e., small plastic spheres ranging between 5 µm to 1 mm), composed of various plastic polymers and included in several personal care and housing products (Rochman et al., 2015; Anbumani & Kakkar, 2018). However, glitter has not received much attention from the scientific community as a potential environmental contaminant (Tagg & Ivar do Sul, 2019; Provenza et al., 2022).

Glitter is widely used in makeup, clothes and fancies, carnival floats, and other materials (Albanit et al., 2023). It easily adheres to human skin and is washed off into domestic wastewater, reaching natural aquatic environments (Tagg & Ivar do Sul 2019). Glitter particles that fall from the skin and other surfaces are deposited on the ground, from where they can be washed by stormwater runoff toward water bodies (Provenza et al., 2022). In this sense, glitter particles have already been found in the effluents of a wastewater plant in Norway (Lusher et al., 2017b) and in sediments from UK rivers at high concentrations (Hurley et al., 2018). Yurtsever (2019a) reviewed studies reporting the presence of glitter in natural waters, and addressed the potential environmental problems associated with its trade and use.

However, Tagg & Ivar do Sul (2019) reported a lack of studies on glitter sources, input rates, distribution across the environment, and potential effects on marine biota. Once glitter is released into the environment, it becomes a contaminant that can interfere with aquatic biota, similar to other microplastics. Thus, ingestion of particles can cause physical damage to biota by clogging the digestive tubes, gills, and filtering structures of aquatic organisms (Costa et al., 2023), while the chemicals associated with the particles may leach and intoxicate the organisms. Green et al. (2021) reported that both conventional polyethylene terephthalate (PET) glitter (not biodegradable) and biodegradable glitter (made of alternative materials, such as regenerated cellulose and natural and synthetic mica, which consist of a group of 37 phyllosilicate minerals with a layered or plate-like texture) have adverse effects on freshwater ecosystems. Provenza et al. (2022) demonstrated that exposure to glitter dispersions caused bioaccumulation and oxidative stress in the blue mussel, *Mytilus galloprovincialis*.

Because of the limited information available on the ecological effects of glitter, further studies are required regarding the adverse effects of glitter on marine organisms. This study aimed to investigate the toxicity of glitter to brine shrimp *Artemia* sp. To the best of the authors' knowledge, this is one of the first studies to investigate the toxicity of glitter in marine microcrustaceans.

## 2. Methodology

### 2.1 Test-organism- *Artemia* sp.

The brine shrimp *Artemia* sp. is a cosmopolitan filter-feeding organism that plays an important role in the trophic chain, transferring biomass and energy from plankton to higher trophic levels. This organism is widely used in laboratory bioassays because of their sensitivity, low cost, and ease of maintenance (Veiga & Vital, 2002). They are frequently used in ecotoxicological studies and environmental risk assessments (Nunes et al., 2006; Lu & Yu, 2019).

### 2.2 Glitter characterization

A glitter of white color was purchased from the market. The polymeric composition of this glitter was previously characterized by Albanit et al. (2023) using pyrolysis coupled with gas

chromatography-mass spectrometry (Py-GC-MS); the particles were composed of methyl-acrylate vinyl chloride (MA-VC). They also identified the presence of other chemicals associated with poly (vinyl chloride) PVC, such as hydrogen chloride (HCl), benzene (B), toluene (T), and anthracene (AN). According to information displayed by the manufacturer, its composition included substances such as BHT, propylparaben, talc, liquid paraffin, methylparaben, caprylic/capric triglyceride, hydrolyzed collagen, magnesium carbonate, cyclomethicone, serica powder, zinc stearate, and dimethicone crosspolymer.

Wet laser diffraction is recognized as an efficient method of characterizing particle size distribution (ISO, 2020). However, there is still no standard protocol for analyzing the sizes of anthropogenic particles, such as glitter, using laser diffraction methods. Thus, in this study, to avoid particle aggregation in water, glitter samples were subjected to ultrasonic and pre-measurement dispersion. To estimate particle size distribution, a fraction of the original glitter was examined using a laser diffraction (Mastersizer 2000, Malvern Instruments Ltd, Worcestershire, UK) through the HydroEV dispersion unit (module Hydro 2000MU). To perform glitter measurements, 1,000 mL of water mixture with 10 % sodium pyrophosphate solution was placed in the sample beaker, the pump speed was set to 2,500 rpm, and the ultrasonic probe (power: 20 W) was turned on for 30 s to fully homogenize the dispersants. The glitter sample was slowly added to a beaker until obscuration reached 10 % and stabilized. All samples were measured five times, sequentially, to confirm there was complete dispersion of the sample. Particle sizes were measured by the angular variation in the light intensity after the laser interacted with the particles (ISO, 2020). Numerical values associated with the scattering patterns were recorded, allowing characterization of glitter particle sizes.

### 2.3 Dispersion preparation

Glitter dispersions were prepared by adding 200 mg of glitter particles into 2 L of autoclaved filtered seawater (salinity = 34) to obtain 2 L of stock dispersion. The resulting mixture (100 mg/L) was agitated until the test dispersions were prepared. The stock dispersion was sonicated for 8 min at 40 kHz. Then, this stock dispersion was diluted in filtered seawater to produce five test dispersions: 0.01, 0.1, 1, 10, and 100 mg/L. A negative control was prepared consisting of dilution water without glitter.

### 2.4 Toxicity test

Dehydrated *Artemia* sp. cists were obtained commercially. Approximately 72 h before the experiment, the cists were introduced in a glass beaker containing filtered seawater, and after approximately 36-48h, most of the nauplii had hatched and could be used the next day, according to the test protocol (ABNT, 2021; Veiga & Vital, 2002).

The acute toxicity test with *Artemia* sp. nauplii was performed following the protocol described by the Brazilian standard ABNT NBR 16530, that is published by Associação Brasileira de Normas Técnicas (ABNT, 2021). Glass test tubes were used as replicates, each containing 10 mL of the test solution at the respective concentrations (control, 0.01, 0.1, 1, 10, and 100 mg/L); four replicates were prepared for each test-dispersion. The control group consisted of organisms exposed to autoclaved filtered seawater without glitter, in such a way that no toxicity would be expected. The experimental setup was planned so that the organisms would be expected to be exposed to the particles themselves, and the substances leached from the glitter, as would occur in the natural environment. Then, 10 nauplii were added to each replicate using a droplet, totalizing 30 organisms per treatment, and 180 nauplii in the whole experiment. The organisms were exposed to glitter dispersions for 48 h under controlled laboratory conditions (temperature  $25 \pm 2$  °C, photoperiod 16:8 clear:dark, absence of food, and aeration). After 48 h, the organisms in each replicate were analyzed, and the living and dead organisms were counted. The survivors were fixed in tamponed formaldehyde and analyzed under a microscope (Leica® - model M2005C) to analyze any morphological deformities or aspects, according to Ekonomou et al. (2019) and Abessa et al. (2021). At the beginning and end of the toxicity

tests, the pH and dissolved oxygen (DO) were measured in each test-dispersion and in the control. The pH was measured with a pH meter Lutron 260 coupled with an electrode, while DO levels were measured with an oximeter Hanna coupled with an optical electrode. The temperature was kept constant using an incubator, while salinity was measured only at the beginning of the experiment (salinity = 34 in all treatments), by using a hand refractometer.

## 2.5 Statistical analyses

The results were first checked for normality and variance homogeneity using the Shapiro-Wilk and Levene tests, respectively. The results were analyzed using one-way analysis of variance (ANOVA), followed by Dunnett's test, to compare the treatments with the control (Zar, 1996), using a significance level of 95 % ( $p = 0.05$ ). This statistical test was performed using the software GraphPad Prism version 4.02 (GraphPad Software, Inc.), allowing the determination of the Low Observed Effect Concentration (LOEC) and the No Observed Effect Concentration (NOEC). The lethal concentration to 50 % of organisms after 48 h ( $LC_{50-48h}$ ) was calculated using the Trimmed Spearman-Kärber method.

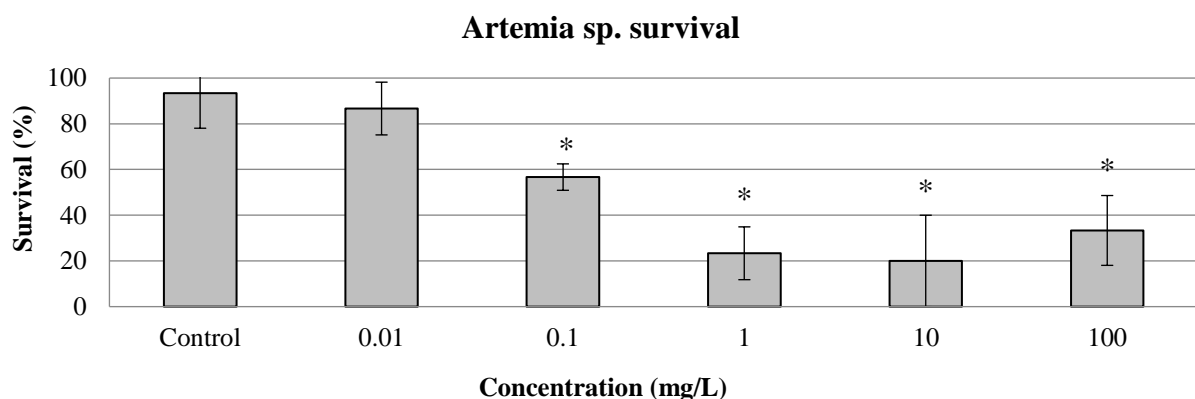
## 3. Results

### 3.1 Glitter particle sizes

Particle size characterization showed that the tested glitter was composed of particles with sizes ranging from 0.06  $\mu\text{m}$  to 0.50 mm. Thus, the glitter samples could be classified as presenting ~62 % of the particle size distribution to the sand class (0.50 to 0.063 mm), and approximately 38 % corresponding to the mud class ( $< 0.063$  mm). The mean diameter of the white glitter was characterized by very fine-like particles which were moderately sorted. Many glitter particles ( $>36$  %) were similar in size to dietary items consumed by *Artemia* sp., such as coccoid bacteria *Acinetobacter* spp. (0.0025 to 0.001 mm, Jung & Park, 2015) and the green microalgae *Dunaliella viridis* (0.015 to 0.005 mm).

### 3.2 Glitter dispersion toxicity

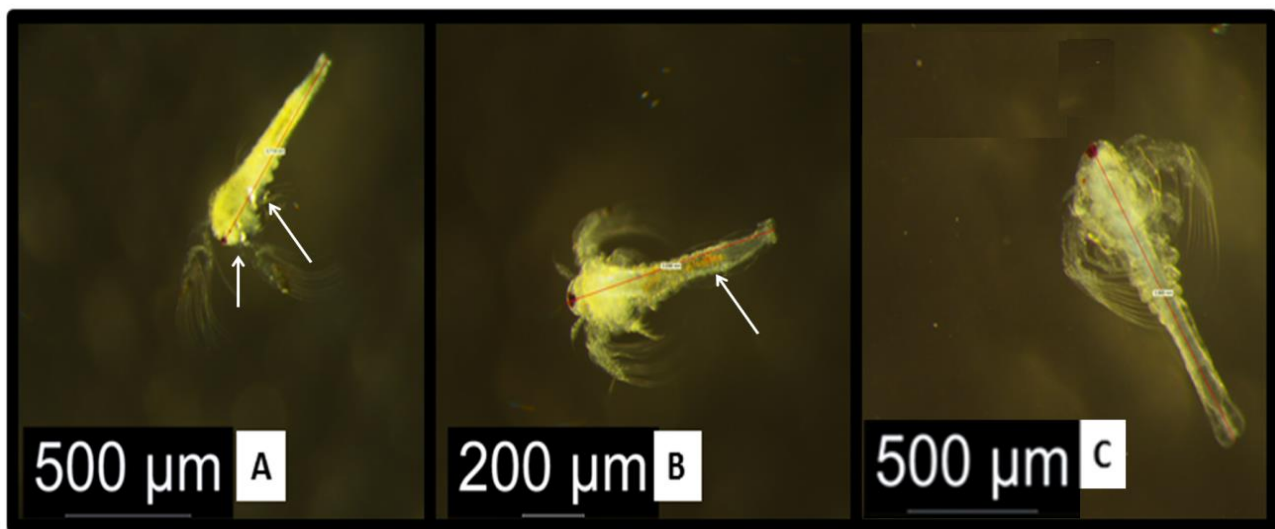
The results of toxicity tests are presented in **Figure 1**. The mean survival rate of *Artemia* sp. nauplii was 93.3 ( $\pm 15.3$  %). The LOEC was estimated as 0.1 mg/L, while the NOEC was 0.01 mg/L, indicating that significant mortality occurred at relatively low concentrations. Mortalities tended to be higher with the increase in glitter concentrations, and the  $LC_{50-48h}$  was estimated as 0.350 (0.348 - 0.351) mg/L. Organisms exposed to higher glitter concentrations had glitter agglomerates trapped in their appendices (**Figure 2A**) and colored patches in their digestive tubes (**Figure 2B**), suggesting that nauplii ingested the metallic layer of the glitter. Control organisms did not exhibit such responses (**Figure 2C**). Physicochemical variables (pH, dissolved oxygen, and salinity) of the glitter suspensions did not change during the experiment (**Table 1**).



**Figure 1** Mean survival rates of *Artemia* sp. nauplii exposed to suspensions of white glitter for 48 h. Asterisks indicate significant difference with the control ( $p < 0.05$ ).

**Table 1** Physicochemical parameters of the tested glitter suspensions along the toxicity test with nauplii of *Artemia* sp.

Concentration (mg/L)	Parameters			
	pH		DO (mg/L)	
	Initial	Final	Initial	Final
Control	7.65	6.77	6.79	7.00
0.01	7.27	7.94	6.66	8.03
0.1	7.09	7.52	6.86	7.48
1	7.86	7.87	6.82	7.99
10	7.78	7.62	7.21	8.22
100	7.46	7.39	7.11	8.05



**Figure 2** Nauplii of *Artemia* sp. exposed to glitter for 48 h, showing organisms with their appendices agglomerated with glitter particles (A), exhibiting colored patches in their digestive tubes (B), and the control group; (C). The gray horizontal bar in the bottom of photos shows the size scale.

#### 4. Discussion

The potential toxic effects of glitter particles on aquatic organisms has started to be investigated in recent years (Green et al., 2021; Piccardo et al., 2022; Pramanik et al., 2023; Provenza et al., 2022; Abessa et al., 2023; Albanit et al., 2023). In this study, white glitter caused significant toxicity to nauplii of *Artemia* sp. from concentrations of 0.1 mg/L after 48 h exposure. Moreover, these effects tended to worsen with increasing concentrations.

The results corroborate with the literature, demonstrating that glitter can cause adverse effects on marine invertebrates. However, glitter is much more toxic to *Artemia* sp. nauplii than to echinoderms and the mussel *Perna perna* (Abessa et al., 2023; Albanit et al., 2023). A possible reason for the worse effects on brine shrimp is the ingestion of particles or their fragments, particularly the metallic parts, as shown in **Figure 2**. In turn, embryos of *P. perna* and echinoderms were exposed only to chemical substances leached from the glitter particles, whereas nauplii of *Artemia* sp. were exposed not only to leached chemicals, but also to ingested particles and through dermal contact.

Abessa et al. (2023) showed that glitter may release metals into the overlying water, and the literature has shown that microplastics may leach different types of chemicals into water (Brede et al., 2003; Kim et al., 2006; Mutsuga et al., 2006; Campanale et al., 2020; Capolupo et al., 2023).

Albanit et al. (2023) and Abessa et al. (2023) discussed the presence of a set of chemicals in the same white glitter studied herein, identifying several compounds and addressing their potential toxicity to marine organisms. Bhardwaj & Sharma (2021) and Bhardwaj (2022) also indicated plastics as carriers of chemical additives, such as phthalates and metals. Other studies also evidenced that glitter leachates could be toxic to embryos of the sea urchin *Paracentrotus lividus* (Piccardo et al., 2022) and the aquatic plant *Lemma minor* (Green et al., 2021), and that glitter dispersions could cause oxidative stress in adults of *Mytilus galloprovincialis* (Provenza et al., 2022) and *Artemia salina* (Pramanik et al., 2023).

The ingestion of particles may be an important exposure route for microplastics, and evidence was obtained that the nauplii of brine shrimps can ingest glitter particles (**Figure 2**). Some glitter particles can be of sizes in the same range as microalgae and bacteria ( $\leq 0.063$  mm), which consist of food for *Artemia* nauplii, such as the green microalgae *Chlamydomonas* spp. and *Dunaliella viridis* (Oren, 2014), the bacteria *Acinetobacter* spp. (Jung & Park, 2005), and the yeast *Saccharomyces cerevisiae* (Coutteau et al., 1990). The glitter particles with mud-like sizes ( $< 0.063$  mm) tend to be dispersed in the water column, or float on the water surface, thereby allowing their ingestion by organisms and consequent trophic transfer through the food web. Piccardo et al. (2022) reported that *M. galloprovincialis* adults exposed to glitter were capable of retaining particles in their digestive tract. Moreover, Gonçalves et al. (2020) and Marinsek et al. (2018) stated that the digestive tract is the main target of contaminants in aquatic organisms. In fact, there are many studies reporting the ingestion of microplastics by crustaceans (e.g. Powell & Berry, 1990; Cole et al., 2013; Lee et al., 2013; Setälä et al., 2014; Batel et al., 2016; Bergami et al., 2016; Gambardella et al., 2017). The presence of metallic particles and microplastics may cause a range of alterations that affect the structure and function of the digestive tract, such as reduction of microvilli, increased numbers of mitochondria, presence of autophagosomes, and cell death (Wang et al., 2019). Ingestion of glitter particles may also reduce nutrient absorption, affecting the ability of organisms to survive (Ziajahromi et al., 2017).

## 5. Conclusions

This study provides evidence that glitter particles can cause adverse effects in *Artemia* sp. nauplii at the tested concentrations. *Artemia* sp. nauplii can be exposed through different ways to glitter particles, exhibiting high mortality rates at relatively low concentrations, above the toxic thresholds previously observed for sensitive organisms such as embryos of bivalves and echinoderms. Thus, this complex form of exposure explains the higher sensitivity to glitter exhibited by the brine shrimps. The results suggest that there is much to be explored regarding the toxicity of glitter on marine organisms, including topics such as the sensitivity of different taxa, the long-term effects, and interactions with other contaminants. The study also reinforces that glitter should be considered as a potential emerging contaminant. Because glitter may be potentially toxic, actions to reduce or forbid its use should be taken, following the decisions recently made by European Union. Recently the EU banned the sale of loose plastic glitter. Actions aimed to increase public awareness are also required, in order to educate citizens to the environmental risks involving glitter and other microplastics.

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