Ambiente & Água - An Interdisciplinary Journal of Applied Science
ISSN 1980-993X – doi:10.4136/1980-993X
www.ambi-agua.net
E-mail: ambi.agua@gmail.com

Morphochemical characterization and interactions of secondary microplastics with paracetamol and microalgae

ARTICLES doi:10.4136/ambi-agua.3017

Received: 20 Sep. 2024; Accepted: 11 Feb. 2025

Diego Caetano Campos de Lelis[1]; Rafaela Luiza Dias da Cunha[1]; Lycia de Brito-Gitirana[2*]

[1]Programa de Pós-Graduação em Ciências Morfológicas. Instituto de Ciências Biomédicas. Universidade Federal do Rio de Janeiro (UFRJ), Avenida Carlos Chagas Filho, n° 373, CEP: 21941-902, Cidade Universitária, RJ, Brazil. E-mail: lelis.dcc@gmail.com, rafaelaluizadias@gmail.com
[2]Laboratório de Histologia Integrativa. Instituto de Ciências Biomédicas. Universidade Federal do Rio de Janeiro (UFRJ), Avenida Carlos Chagas Filho, n° 373, CEP: 21941-902, Cidade Universitária, RJ, Brazil.
[*]Corresponding author. E-mail: lyciabg@histo.ufrj.br

ABSTRACT

The morphochemical properties of microplastics influence their interactions with pollutants, biota, and toxicity. This study focused on the effects of microalgae exposure to secondary microplastics (SMP) derived from different plastic materials, often associated with pharmaceutical residues. *Tetraselmis sp*. were exposed to SMP alone and in combination with paracetamol. The SMP exhibited irregular morphology and sizes ranging from 8 to 1749 µm. morphochemical properties were analyzed using FTIR, SEM-EDS, and zeta potential. FTIR analysis identified three types of SMP (linear low-density polyethylene, polypropylene, and polystyrene), all showing a negative surface charge. Carbon, oxygen, calcium, chlorine, aluminum, bromine, and titanium were detected in all samples. Microalgae growth remained unaffected by SMP exposure, except at paracetamol concentrations of 400 and 500 $mg.L^{-1}$. The study suggests that the surface charge of SMPs significantly influences their toxicological effects. Furthermore, the resilience of microalgae to paracetamol and microplastics underscores their potential as bioindicators in microplastic-contaminated environments.

Keywords: morphochemical properties, paracetamol, secondary microplastic, *Tetraselmis sp*., toxicity interactions.

Caracterização morfoquímica e interações de microplásticos secundários com paracetamol e microalgas

RESUMO

As propriedades morfoquímicas dos microplásticos influenciam suas interações com poluentes, biota e toxicidade. Este estudo focou nos efeitos da exposição de microalgas a microplásticos secundários (MPS) derivados de diferentes materiais plásticos, frequentemente associados a resíduos farmacêuticos. *Tetraselmis sp.* foram expostas aos MPS isoladamente e em combinação com paracetamol. Os MPS apresentaram morfologia irregular e tamanhos variando de 8 a 1749 µm. As propriedades morfoquímicas foram analisadas usando FTIR, SEM-EDS e potencial zeta. A análise de FTIR identificou três tipos de MPS (polietileno linear de baixa densidade, polipropileno e poliestireno), todos com carga superficial negativa.

Carbono, oxigênio, cálcio, cloro, alumínio, bromo e titânio foram detectados em todas as amostras. O crescimento das microalgas não foi afetado pela exposição aos MPS, exceto em concentrações de paracetamol de 400 e 500 mg.L^{-1}. O estudo sugere que a carga superficial dos MPS influencia significativamente seus efeitos toxicológicos. Além disso, a resiliência das microalgas ao paracetamol e aos microplásticos destaca seu potencial como bioindicadores em ambientes contaminados por microplásticos.

Palavras-chave: interações tóxicas, microplástico secundário, paracetamol, propriedades morfoquímicas, *Tetraselmis sp*.

1. INTRODUCTION

The term “Anthropocene” describes a geological epoch during which human actions have had a significant impact on the planet, resulting in changes to the quality of the atmosphere, soil availability, forest health, climate, and species diversity (Porta, 2021). There is significant apprehension regarding the ecological consequences caused by inadequate disposal of plastic-based products because of human activities. Worldwide plastic production has significantly increased over the past seven decades, rising from approximately 1.5 million tons in 1950 to 367 million tons in 2020 (Plastics Europe, 2008). If plastic production, consumption, and management persist, the situation may worsen. According to projections, the annual amount of waste released into the oceans will reach 29 million tons by 2040 (Lau *et al.*, 2020). Inappropriate disposal of plastic waste may result in the generation of progressively smaller particles that may reach micrometric and nanometric dimensions. The natural process of plastic fragmentation facilitates the dispersion of particles in the marine environment (Cole *et al.*, 2011).

Microplastics are plastic fragments that measure less than 5 mm in size (Thompson, 2004). These particles exhibit diverse physicochemical properties, such as size, shape, surface area, composition, and surface charge, which influence their behavior and interaction with the environment (Zimmermann *et al.*, 2020), that enable them to attract, repel, adsorb, and accumulate an extensive variety of organic and inorganic pollutants, including pesticides, dioxins, heavy metals, and pharmaceutical residues, among others. The presence of microplastics in the food chain may result in incorporation of toxins, which can accumulate across different trophic levels, causing adverse effects (Teuten *et al.*, 2009). Based on their method of production, microplastics can be categorized as either primary or secondary plastics (Cole *et al.*, 2011). Primary microplastics are often manufactured in specific sizes for various industrial and household applications (Betts, 2008; Moore, 2008), such as blast media (Gregory, 1996), facial cleansers and cosmetics (Zitko and Hanlon, 1991), and as drug carriers in medicine (Patel *et al.*, 2009).

The fragmentation of pre-existing plastic waste in the environment leads to the formation of secondary microplastics. This fragmentation can result from a range of degradation processes, including physical degradation caused by abrasive forces, heating, freezing, drying, and photodegradation. The fragmentation may occur as a result of various factors, including exposure to UV radiation, chemical degradation through oxidation or hydrolysis, and biodegradation by microorganisms such as bacteria, fungi, and algae (Klein *et al.*, 2018). Despite these various degradation processes, the chemical structure of microplastics remains largely unchanged, allowing for the identification of their polymeric group. Among various types of plastic, polyethylene, polystyrene, and polypropylene are the most commonly used and abundant polymers found in the environment (Andrady and Neal, 2009; Plastics Europe, 2021).

In addition to microplastics, various emerging contaminants such as pesticides, steroids, hormones, surfactants, and pharmaceutical residues contribute to pollution in the aquatic

ecosystem. In this sense, there is growing global concern about the presence of pharmaceutical residues in the environment (Bottoni *et al.*, 2010; WHO, 2012; Rehman *et al.*, 2015; aus der Beek *et al.*, 2016). Another significant concern is the increase of pharmaceutical residues in the environment as a result of their widespread production and the lack of regulatory measures governing their appropriate disposal (Kasprzyk-Hordern, 2010). Improper disposal of pharmaceuticals has been noticed in various studies as a significant factor contributing to their presence in wastewater (Nguyen *et al.*, 2020), drinking water (WHO, 2012), surface water (Bottoni *et al.*, 2010; aus der Beek *et al.*, 2016), as well as lakes and oceans (Mezzelani *et al.*, 2018). Paracetamol, also known as acetaminophen, is a widely used analgesic and antipyretic, ranking among the most commonly used medicines (Xu *et al.*, 2008; Wu *et al.*, 2012; Rhee *et al.*, 2013). Its uncontrolled use and the release of its byproducts contribute to its presence in aquatic environments, thereby exposing marine organisms to its effects. Due to its propensity for accumulation, paracetamol is considered a hazardous drug because of its toxicity, solubility, hydrophilia, and persistence in the aquatic environment (De Voogt *et al.*, 2009). Paracetamol has been detected in various aquatic environments in several countries, with concentrations found in surface waters (0.1–5.71 $\mu g.L^{-1}$), groundwater (0.01–1.89 $\mu g.L^{-1}$), and wastewater (0.1–300 $\mu g.L^{-1}$) (Al-Kaf *et al.*, 2017; Phong Vo *et al.*, 2019; Agarwal, 2022). Even at low concentrations, paracetamol and its metabolites exhibit toxic effects on aquatic organisms across different trophic levels, including algae, microcrustaceans, mollusks, and fish (Nunes *et al.*, 2014). Its adverse effects include disruptions in the hypothalamus-pituitary-gonadal axis, DNA alterations, and changes in blood and liver functions due to the induction of oxidative stress and lipid peroxidation (Żur *et al.*, 2018).

The interactions between microplastics (MPs) and pharmaceutical residues in aquatic environments are complex, governed by various physicochemical processes. Adsorption is the primary mechanism driving these interactions, influenced by the aging of MPs and the specific physicochemical properties of both the MPs and pharmaceuticals (Moura *et al.*, 2023; Guo *et al.*, 2024). Aging processes, such as photo-oxidation, increase the surface area and adsorption capacity of MPs, enhancing hydrophobic interactions. Electrostatic interactions are also significant, particularly for charged pharmaceutical compounds (Moura *et al.*, 2023; Guo *et al.*, 2024). Additionally, other intermolecular forces, such as hydrogen bonding, cation bonding, and CH/π interactions, play crucial roles in adsorption. These interactions depend on the chemical structure of the pharmaceuticals and the surface characteristics of MPs (Guo *et al.*, 2024; Sun *et al.*, 2023). The combined effects of these mechanisms make MPs effective vectors for the transport of pharmaceuticals, potentially amplifying their distribution and ecological impact in aquatic ecosystems. However, there is a significant lack of information about the interactions between secondary microplastics and paracetamol, highlighting the need for further investigation.

The objective of this study was to investigate the physicochemical properties of secondary microplastics (SMP) and assess the effect of SMP, both alone and in combination with paracetamol, on the growth of microalgae *Tetraselmis* sp. This is particularly relevant due to increasing amounts of pollutants and the co-occurrence of microplastics with other substances in the environment.

2. MATERIAL AND METHODS

2.1. Secondary microplastic

To obtain secondary microplastics (SMP), plastic waste was collected from a beach in Guanabara Bay (-22.863757217895728, -43.215141072914776), an area known for its pollution and large accumulation of trash. The collected items included black plastic bags, colored plastic cups, and colored plastic bags.

After collection, the samples were washed first in running water and then in distilled water.

Subsequently, they were dried in an oven at 50ºC. The plastic samples were then cut into pieces smaller than 2 cm using sterile scissors. These fragments were frozen in liquid nitrogen and milled into smaller pieces using a microknife mill (SL-30, SOLAB) equipped with a 0.1 mm steel sieve. This fragmentation process was conducted at the Real Time Process and Chemical Analysis Development Center of the Federal University of Rio de Janeiro (UFRJ).

2.2. Microplastic size characterization

The size of the plastic particles was characterized using two techniques. Firstly, visual morphometry was performed using stereomicroscopic (Model Axio Zoom V.16, Zeiss) and scanning electron microscope images (Model VEGA3 LMU, Tescan). For this method, measurements of one hundred particles were made using the ImageJ software.

Secondly, a laser diffraction particle size analyzer (Model Shimadzu Sald-2201) was employed to measure fragments ranging from 0.01 µm to 10 µm, which are too small to be analyzed effectively by stereomicroscopy.

2.3. Polymeric group identification

Fourier transformation infrared spectroscopy (FTIR) using a Shimadzu IR Prestige-21 was employed to identify the polymeric group of microplastics. The analysis was conducted at the Brazilian Center for Physical Research (CBPF) using a Shimadzu IR Prestige-21.

For this analysis, the previously milled sample was macerated in 110 mg of potassium bromide (KBr) pellet disk from each sample (1% in KBr). The mixture was then transferred to the granule-forming matrix and pressed with a force of 8 tons for 2 to 3 minutes to form a translucent pellet suitable for spectroscopic measurements.

The FTIR spectra range for measurement was 4000-400 cm^{-1} with a resolution of 4.0 cm^{-1} and 256 scans. To determine the polymer type, all spectra were compared with the polymer library (Noda *et al.*, 2007; Domenighini and Giordano, 2009; Jung *et al.*, 2018).

2.4. Surface morphology and microanalysis of secondary microplastics

To characterize the surface morphology and identify the chemical composition, the microplastics were prepared as follows: they were mounted on a metallic support using carbon adhesive tape and then sputtered with carbon (Desk V Sample Preparation Model, DentonVacuun).

Subsequently, the samples were examined using a scanning electron microscope (SEM) (Model VEGA3 LMU, Tescan) coupled with an energy dispersion X-ray spectroscopy (EDS) detector (Model X-Max 20, Oxford Instruments). The SEM was operated at 5 kV for surface analysis and at 20-30 kV for EDS analysis.

These analyses were conducted at the Multidisciplinary Research Center (Numpex-Bio) at the Federal University of Rio de Janeiro (UFRJ).

2.5. Surface charge (zeta potential)

The determination of the surface charge of secondary microplastic, paracetamol, and microalgae was carried out at the Brazilian Center for Physical Research (CBPF) using a particle analyzer equipped with a dynamic light scatterer (Model Brookhaven 90Plus Nanoparticle Size Analyzer, Brookhaven Instruments Corporation).

For this analysis, each sample was diluted in a Guillard (F/2) nutrient solution (pH 8.2 and 30% salinity) and placed in a 1.5 mL cuvette. The average value of each sample was calculated using ten repetitions.

2.6. Microalgae cultivation

Tetraselmis sp. cultures were established using algae samples collected in 2016 from the seawater of Guanabara Bay, Rio de Janeiro, Brazil. Individual cells were isolated using a

capillary glass tube under an inverted microscope and transferred to sterile f/2 medium (Guillard, 1975) prepared with water from Guanabara Bay. The salinity was adjusted to 20, and cultures were maintained at 26°C under a photon flux of 50 µmol photons m−2 s−1 (cool white fluorescent tubes) with a 14:10 light:dark cycle. After approximately one month, clonal cultures were established using fluorescence-activated cell sorting (FACS) with a MoFlo® (Beckman-Coulter) flow cytometer, as described by Fistarol *et al.* (2018).

For the isolation of single microalgal cells, the MoFlo® flow cytometer was equipped with an electrostatic droplet deflection device and a 100-µm nozzle. The blue laser line (488 nm, 100 mW) was used for excitation. Sheath pressure was maintained between 10 and 12 psi, with a drive frequency of 10–12 kHz. Drop charge was minimized, ensuring clearance of the side stream from the waste drain. Drop-delay optimization was performed using fluorescent microspheres, achieving an efficiency of over 99.5% as per the manufacturer's instructions (Fistarol *et al.*, 2018). Microalgal cells were detected using forward-scattered light and chlorophyll fluorescence, collected through a 630/30-nm band-pass filter. Samples were processed at a flow rate of approximately 100 µL min−1 for 5–15 minutes until microalgal populations were distinguishable in the scatter plots. Logical gates were applied to select populations with high chlorophyll fluorescence, and the sorting mode was set to maximize purity. Single cells within the gated regions were sorted into 96-well polypropylene plates containing 100 µL of culture medium. Sorting event rates were maintained below 100 s−1 to ensure accuracy (Fistarol *et al.*, 2018). These microalgae cultures, established through this rigorous methodology, were subsequently added to the Culture Collection of Microorganisms (CCMR) at the Universidade Federal do Rio de Janeiro. Tetraselmis sp. CCMR165 was cultured in flasks containing Guillard´s (F/2) medium (30% salinity) prepared using filtered natural seawater that had been autoclaved before incubation. The microalgae were maintained in a growth chamber (BOD incubator) under a photoperiod with a light intensity of 100 µmol.m^{-2} s^{-1}, at a temperature of 22°C, and with a photoperiod of 16 h of light followed by 8 h darkness.

2.7. Toxicity assays of secondary microplastics and microplastics associated with paracetamol

The toxicity of secondary microplastics (SMP) was assessed by exposing Tetraselmis sp. to three types of SMP: linear low-density polyethylene (LLDPE), polypropylene (PP), and polystyrene (PS), at concentrations of 100, 200, and 300 mg.L^{-1}. To evaluate the effects of paracetamol alone and in combination with SMP on microalgae growth, a stepwise experimental approach was used.

First, a series of tests were conducted to determine the range of paracetamol concentrations that significantly impacted Tetraselmis sp. growth. Environmentally relevant concentrations of paracetamol (0.01, 0.1, and 0.5 mg.L^{-1}) were initially tested, but no significant reduction in microalgae growth or EC_{50} (half-maximal effective concentration) was observed (unpublished data). Higher concentrations of paracetamol (10, 50, 100, 200, 300, 400, and 500 mg.L^{-1}) were then tested, with the EC_{50} determined to fall between 100 and 500 mg.L^{-1}. Despite exceeding typical environmental levels, these concentrations were used as they allowed microalgae growth and were relevant for understanding *Tetraselmis sp.*'s resilience under these conditions.

Using the established range of 100–500 mg.L^{-1}, paracetamol was tested both independently and in combination with SMP to assess potential synergistic or antagonistic effects. The following experimental conditions were tested:

- Blank 01– only F/2 medium
- Blank 02 – F/2 medium with paracetamol
- Control – only *Tetraselmis spp*

- Paracetamol alone (no SMP)
- Linear low-density polyethylene with paracetamol (+LLDPE)
- Linear low-density polyethylene without paracetamol (–LLDPE)
- Polypropylene with paracetamol (+PP)
- Polypropylene without paracetamol (–PP)
- Polystyrene with paracetamol (+PS)
- Polystyrene without paracetamol (–PS)

All experiments were conducted in triplicate using 6-well sterile culture plates, with a total volume of 10 mL per well. Each well was inoculated with 6.0×10^4 cells of Tetraselmis sp. and exposed to the test conditions for 96 hours. Cell counts were performed post-exposure using a Neubauer chamber. The pH of the culture medium, with and without paracetamol, was maintained at 8.2 ± 0.1 throughout the experiment. The paracetamol used in the study (CAS Registry No. 103-90-2) was sourced from Sigma-Aldrich®. While the study evaluated the effects of paracetamol and secondary microplastics on microalgae growth, potential changes in paracetamol concentration in the fluid phase during exposure to microplastics were not measured.

2.8. Statistical analyses

The statistical analyses were performed using GraphPad Prism (Version 7) to determine whether there were statistically significant differences in microalgae growth due to varying microplastic concentrations. A one-way ANOVA was used for datasets involving a single factor, while a two-way ANOVA was applied for datasets with two factors. Groups with significantly different mean values were identified using Tukey's multiple comparison test ($p \leq 0.05$). In addition, inhibitory dose-response curves were generated using non-linear regression analysis to model the relationship between inhibitor concentration and the normalized response (number of cells.mL^{-1}) with a 95% confidence level.

3. RESULTS

3.1. Polymeric group

The polymeric groups of secondary microplastics were identified using FTIR (fourier-transform infrared), which analyzes the vibrational movements and absorption pattern of infrared light to identify the natural frequency of each type of chemical bond. The spectra of the SMP were compared to two distinct databases (Noda *et al.*, 2007; Jung *et al.*, 2018).

The infrared absorption pattern of the black plastic bag displayed the most intense peaks at wave numbers (cm^{-1}) 2916, 2848, 1467, 1375, and 717 (Figure 1A). Using the methodology proposed by Jung *et al.* (2018), the presence of the 1377 cm^{-1} band was used to differentiate the types of polyethylene (Figure 1B), confirming that the black plastic bag was composed of linear low-density polyethylene (LLDPE).

The spectra of the colored rigid plastic cup exhibited intense peaks at wave numbers (cm^{-1}) 2960, 2920, 2837, 1458, 1377, 1166, 997, 972, 840, and 808 (Figure 1C), identifying it as polypropylene (PP).

Similarly, the spectra of the soft-white plastic showed intense peaks at wave numbers (cm^{-1}) 3024, 2924, 2848, 1602, 1492, 1452, 1028, 756, 698, and 540 (Figure 1D), indicating it was composed of polystyrene (PS).

The combination of specific wave numbers and vibrational patterns revealed that the black plastic bag (Figures 1A–B) was composed of linear low-density polyethylene (LLDPE). The colorful rigid plastic cup (Figure 1C) was composed of polypropylene (PP), whereas the soft white plastic cup (Figure 1D) was made of polystyrene (PS).

3.2. Zeta potential

The zeta potential, which measures the electrostatic attraction between adjacent particles in a dispersion, plays a crucial role in determining colloidal stability. High zeta potential, whether positive or negative charged, indicates stable particles that avoid agglomeration. In contrast, when the potential is low, attractive forces may prevail over repulsive forces, leading to fragmentation and flocculation of the dispersion.

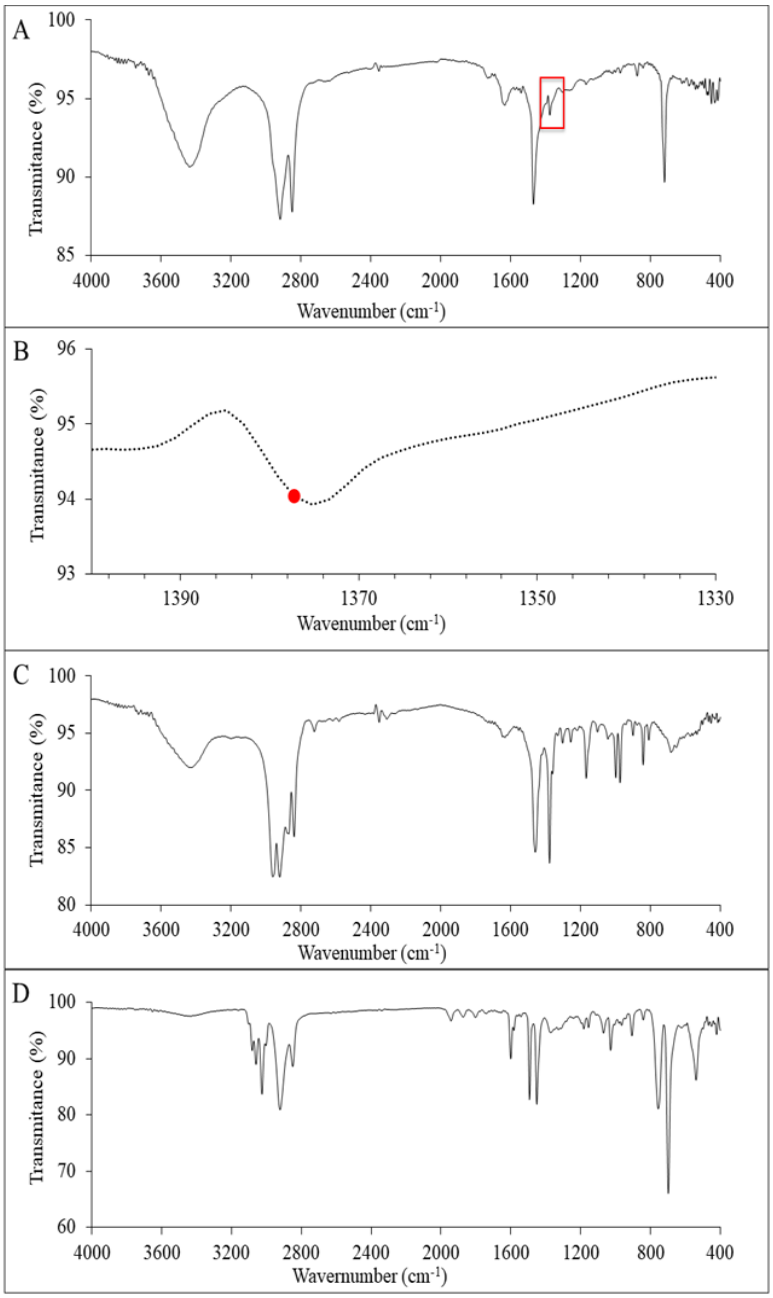

Figure 1. Identification of the polymeric group of microplastics. (A) FTIR spectrum showing characteristic peaks for polyethylene. The red box indicates the region of the spectrum for analysis according to Jung *et al.* (2018). (B) Presence of the band at 1377 cm−1 (red dotted line) characteristic of linear low-density polyethylene polymers. Characteristic spectra of polypropylene (C) and polystyrene (D). (A-B) Sample of a black plastic bag. (C) Sample of a colorful, rigid plastic cup. (D) Sample of a soft-white plastic cup.

The zeta potential value of -23.37 mV obtained for *Tetraselmis spp*. indicates that the system is close to instability. Additionally, the zeta potentials of paracetamol, LLDPE, PP, and PS were found to be -2.49 mV, -6.0 mV, -7.63 mV, and -9.16 mV, respectively. This indicated that these particles are susceptible to rapid coagulation or flocculation (Figure 2).

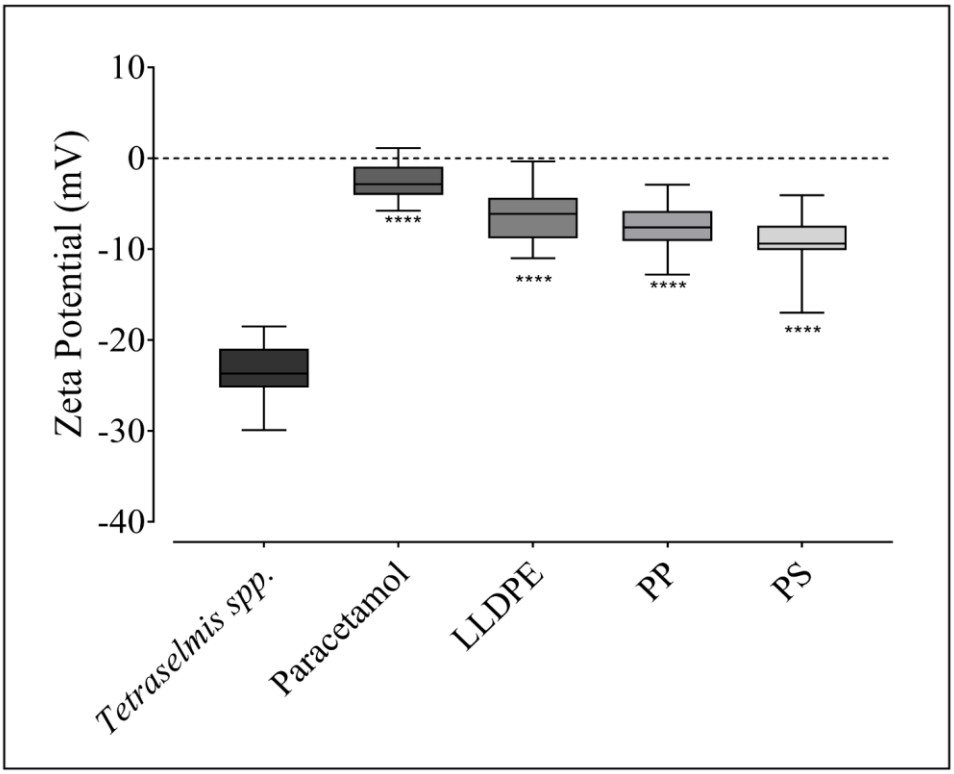

Figure 2. Zeta potential. Characterization of the surface charge of *Tetraselmis* sp. exposed to paracetamol and different types of microplastic: linear low-density polyethylene (LDPE), polypropylene (PP) and polystyrene (PS). Statistically significant values are indicated by asterisks (****) after conducting the Tukey test. $p < 0.0001$.

3.3. Morphological analyses

The morphological analysis revealed that the SMP of LLDPE exhibits an elongated morphology with contorted features and pointed branches (Figures 3A–B), and the surface of LLDPE exhibited folds and indentations because of its distorted morphology (Figure 3C).

The secondary microplastics of PP (Figures 3D–E) and PS (Figures 3G–H) exhibited surfaces that tend to be flat, misshapen, and flake-like, showing a rough texture with small protrusions (Figure 3F). In addition, PP particles displayed a rough surface with depressions similar cracks (Figure 3I).

After size measurement, LLDPE microplastics have an average size of 523 µm with a range of 0.046–1749 µm, while PP microplastics showed an average size of 410 µm, with a range of 1.57–1136 µm. For PS microplastics, the average size was 298 µm, with a range of 0.241–1007 µm (Figure 4).

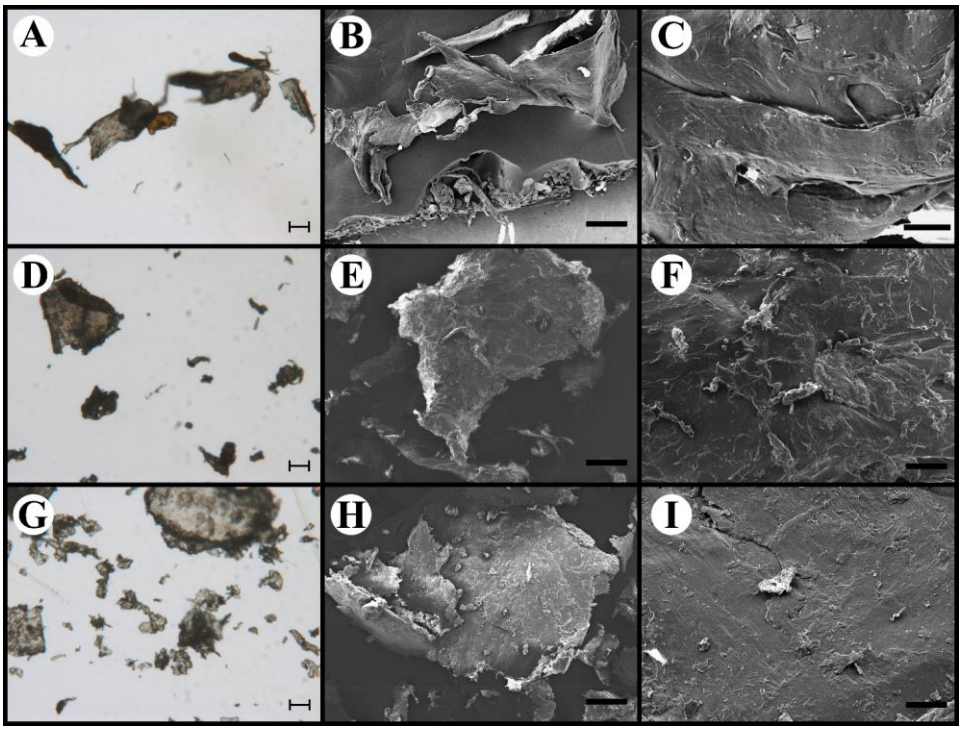

Figure 3. Morphological analysis of secondary microplastics using stereoscopic light microscopy (A, D, and G) and scanning electron microscopy (B, C, E, F, H, and I). Linear low-density polyethylene (A–C), polypropylene (D–F), and polystyrene (G–I). Scale bars: 50 µm (A, D, G), 100 µm (B, E, H), and 20 µm (C, F).

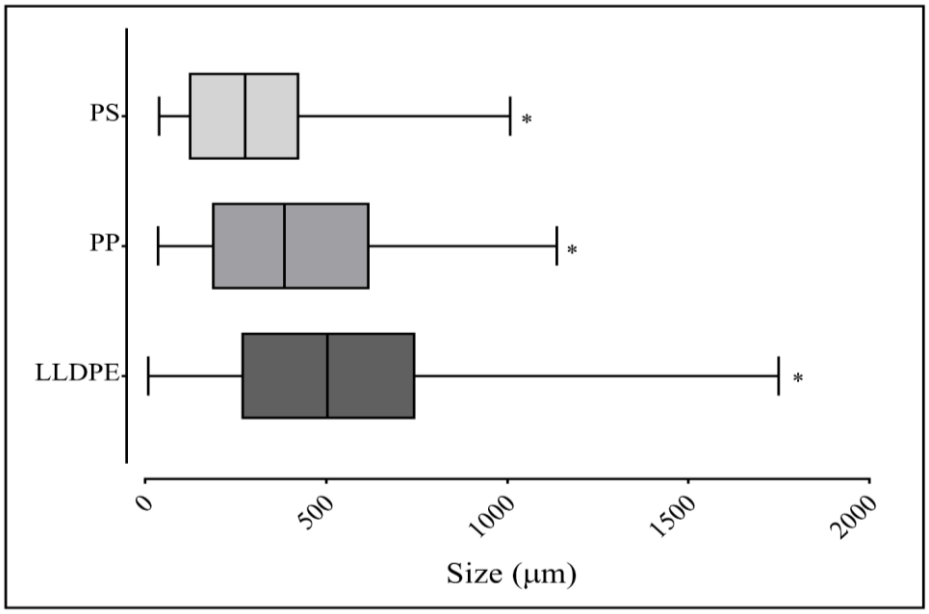

Figure 4. Secondary microplastic size measurement of linear low-density polyethylene (LDPE), polypropylene (PP) and polystyrene (PS). Statistically significant values are indicated by asterisks (*) after applying the Tukey test. $p < 0.01$.

3.4. Microanalysis of chemical elements

Based on the SEM-EDS spectrum, seven distinct chemical elements were identified, namely carbon (C), oxygen (O), calcium (Ca), chlorine (Cl), aluminum (Al), bromine (Br), and titanium (Ti). Both carbon and oxygen are components of all microplastics. The LLDPE-type

polyethylene revealed Ti, Cl, and Ca (Figure 5A), while Ti, Br, and Ca have been identified in PP (Figure 5B). In PS, only Al was found (Figure 5C).

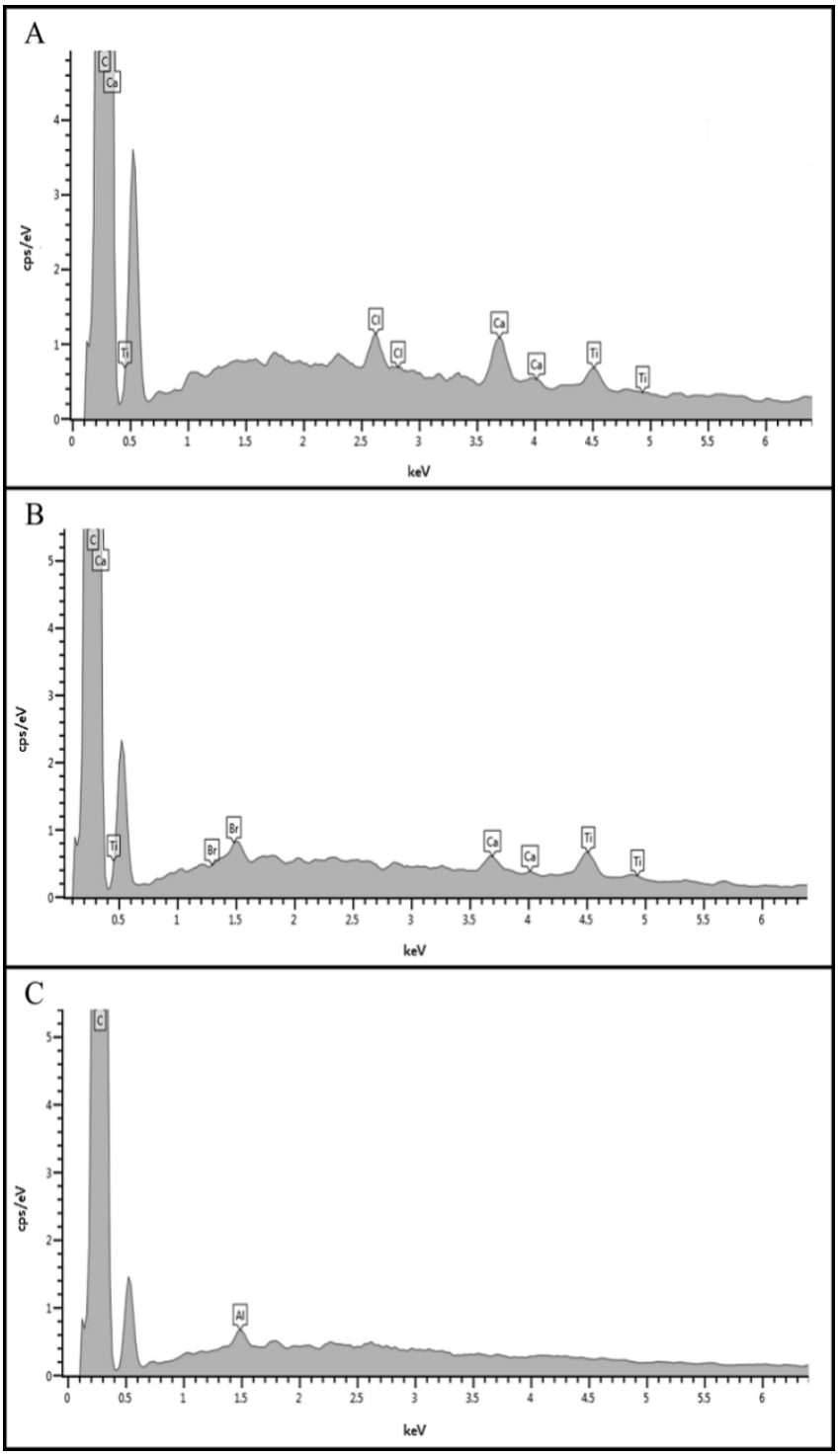

Figure 5. Elements in secondary microplastics identified through microanalysis by SEM-EDS. The energy-dispersive X-ray spectroscopy indicates the presence of carbon (C), oxygen (O), calcium (Ca), chlorine (Cl), and titanium (Ti) in LLDPE (A). In PP (B), carbon (C), oxygen (O), calcium (Ca), bromine (Br), and titanium (Ti) were found, and in PS (C), carbon (C), oxygen (O), and aluminum (Al) were detected.

3.5. Microplastic and paracetamol toxicity assay

Tetraselmis spp. were cultivated for 96 hours in the presence of secondary microplastics at three different paracetamol concentrations (100, 200, and 300 mg.L^{-1}) to evaluate the toxicity of microplastics on the growth of microalgae.

The results of the cell counting indicated that LLDPE, PP, or PS types of microplastics did not significantly affect *Tetraselmis* sp. (Figure 6A). Nonetheless, it was noted that paracetamol, both combined with (+LLDPE, +PP) secondary microplastic and without (-LLDPE, -PP) secondary microplastic, caused a statistically significant decrease only at the 500 mg.L^{-1} concentration (Figs. 6B–C). In addition, paracetamol, combined with PS and without PS, revealed significant reductions in cell growth at concentrations of 200, 300, and 500 mg.L^{-1} (Figure 6D).

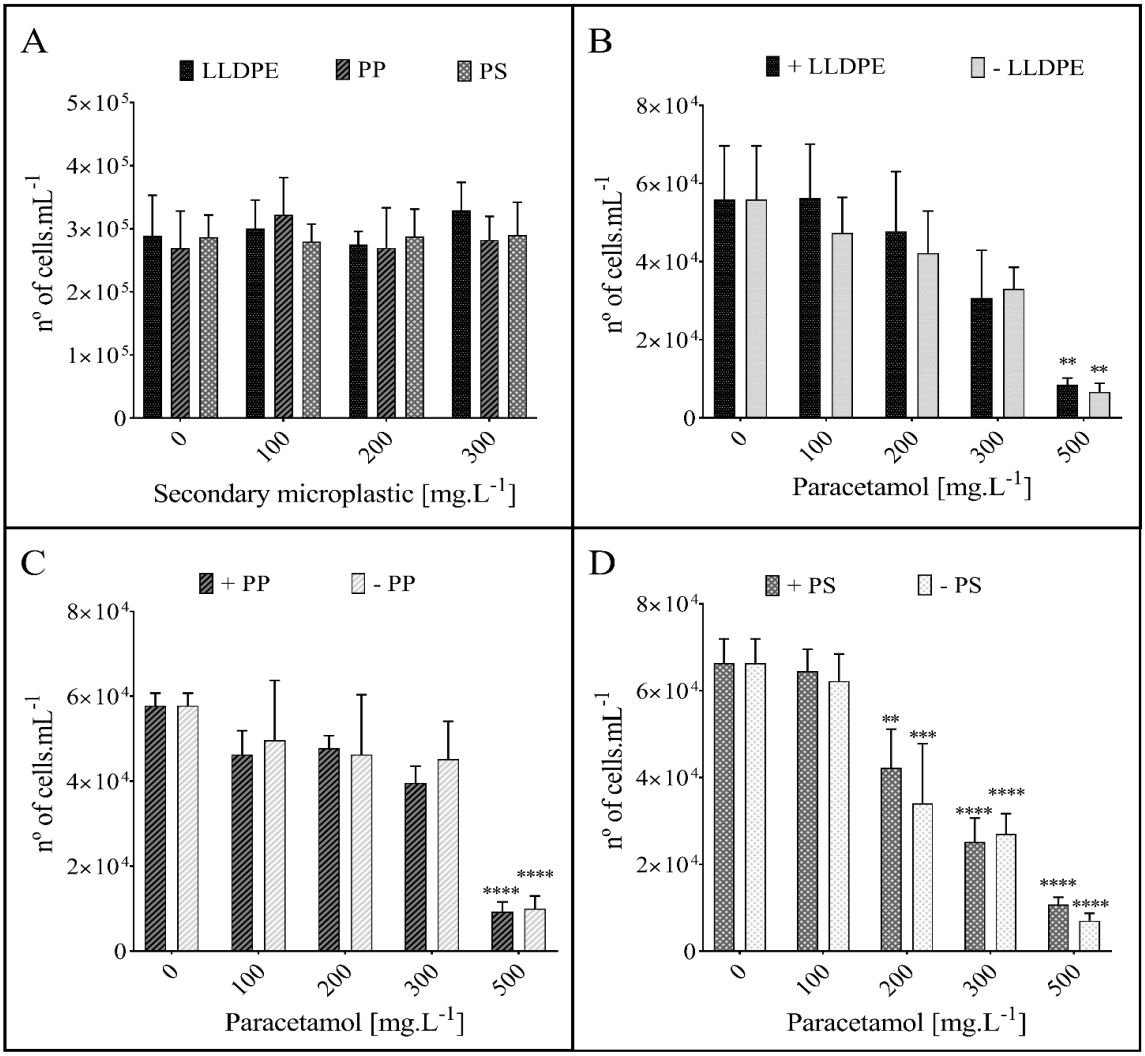

Figure 6. The effect of secondary microplastics, both isolation and in combination with paracetamol, on the growth of *Tetraselmis* sp. (A) The number of microalgae after a 96-hour exposure to secondary microplastics at various concentrations. Microalgae were exposed to different concentrations of paracetamol in combination with LLDPE (B), PP (C), and PS (D), each at a concentration of 300 mg.L^{-1}. The asterisks indicate significantly different values as determined by the Tukey test. (**) $p < 0.01$, (***) $p < 0.0005$ and (****) $p < 0.0001$.

The dose-response curve for microalgae exposed to paracetamol showed EC_{50} values of 278.5 mg.L^{-1} for LLDPE (Figure 7A), 397 mg.L^{-1} for -PP (Figure 7B), and 205.6 mg.L^{-1} for -PS (Figure 7C). When microalgae were exposed to paracetamol in combination with SMP, the

EC_{50} values were 285 mg.L^{-1} for PELBD (Figure 7A), 311 mg.L^{-1} for PP (Figure 7B), and 218.6 mg.L^{-1} for PS (Figure 7C).

By comparing the EC_{50} values in different experimental conditions, specifically when microalgae were exposed only to paracetamol (256.7 + 41/-45) versus in the presence of microplastics such as LLDPE (285 + 70/-61), PP (311.7 + 48/-52), and PS (218 + 23/-22), revealed that the different types of microplastics did not affect microalgae growth when combined with paracetamol (Figure 7D). There were no significant differences in the EC_{50} values when paracetamol was combined with different types of microplastics (Figs. 7A–D), indicating that microplastics neither increase nor reduce the growth of microalgae under these experimental conditions.

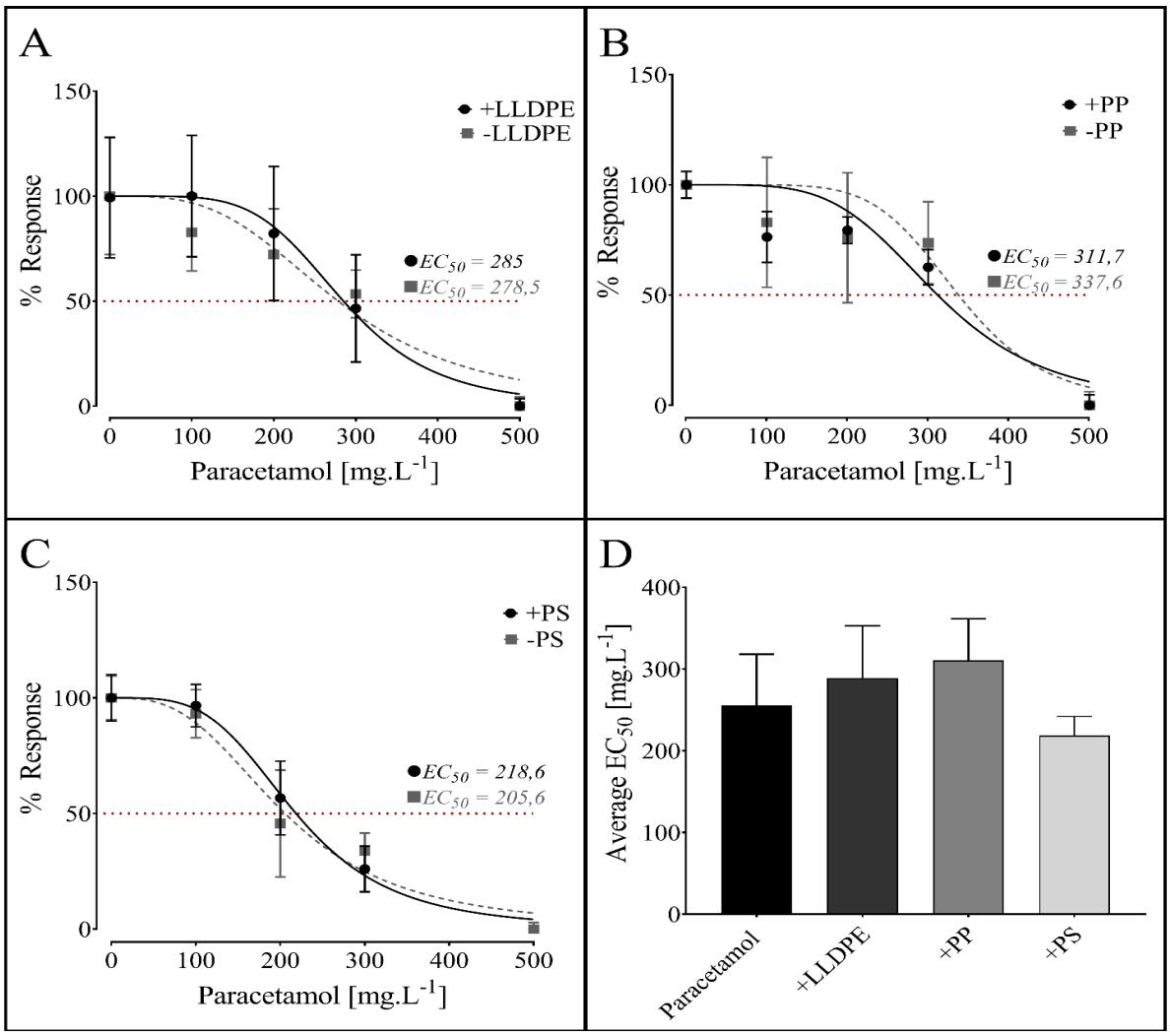

Figure 7. The values of the EC of microalgae exposed to different types of SMP in combination with paracetamol. The growth of Tetraselmis spp. populations is reduced by 50% upon exposure to paracetamol in the presence of SMP (+) and in the absence of SMP (–). Panels represent different types of SMP: (A) +LLDPE, –LLDPE; (B) +PP, –PP; (C) +PS, –PS. A comparative analysis revealed that there were no statistically significant differences ($p > 0.05$) (D).

4. DISCUSSION

The microplastic surface was analyzed using SEM-EDS, revealing the presence of seven chemical elements, namely carbon (C), oxygen (O), calcium (Ca), chlorine (Cl), aluminum (Al), bromine (Br), and titanium (Ti). These elements may be incorporated during the plastic

manufacturing process. Given that plastics are combustible materials, it is possible that flame-retardant additives are added as an alternative way of mitigating the propagation of flames during plastic production (Mensah *et al.,* 2022).

Bromine-containing compounds act as flame retardants that are integrated into plastics to mitigate burning intensity by causing an interruption in the combustion process. Additionally, mineral additives, such as calcium carbonate ($CaCO_3$), aluminum trihydrate (ATH), and titanium dioxide (TiO_2), are commonly used in plastic manufacturing. These additives modify the physical properties of plastics, influencing their texture, surface quality, color, and appearance (Harper and Petrie, 2004).

Certain additives, including bromate flame retardants, phthalates, nonylphenols, bisphenol A, and antioxidants, are categorized as persistent organic pollutants (POPs). These substances are subject to restrictions in some countries due to their bioaccumulative, toxic, and/or endocrine disrupting properties (Barrick *et al.,* 2021).

In Brazil, Resolution No. 17, dated March 17, 2008, establishes the technical norms regarding the use of additives in plastic materials used for food packaging and equipment in contact with food. Nevertheless, this resolution ignores any restrictions or limitations on the use of various additives containing Ca, Cl, Al, Cu, or Ti (Anvisa, 2008).

It is important to note that many studies fail to characterize microplastics based on their chemical composition, often overlooking the presence of additives. This omission may lead to the misattribution of adverse effects caused by microplastics solely to their physical characteristics, such as shape, color, or morphology, while ignoring the potential influence of their chemical composition. Additives and their degradation products can significantly alter the physicochemical interactions between microplastics and other substances, such as pharmaceutical or non-pharmaceutical residues, potentially amplifying their impact on aquatic environments. Thus, understanding the combined effects of microplastic composition and additives on marine organisms is essential. Developing new methodologies to evaluate the toxicity of these complex mixtures remains a critical challenge (Zimmermann *et al.*, 2020).

According to several authors, the morphological structure, size, and surface load of microplastics are factors that influence the adsorption and transport of substances (Alimi *et al.,* 2018; Carbery *et al.*, 2018; Prata *et al.*, 2019). Tsiaka *et al.* (2013) noted an inverse correlation between the toxicity of microplastics and their size. The authors noticed that small fragments measuring 0.05 μm caused a notable decrease in density of *Dunaliella tertiolecta* compared to large fragments ranging from 0.5 to 6 μm.

Studies on *Skeletonema costatum* have demonstrated that the size of microplastics composed of polyvinyl chloride (PVC) affects the growth of this diatomaceous. Particles measuring 1 μm in size have been shown to inhibit growth, decrease chlorophyll levels, and diminish photosynthetic activity. Conversely, microplastics of PVC measuring 1000 μm in size exhibited no apparent toxicological effects (Zhang *et al.*, 2017).

The study conducted by Prata *et al.* (2019) showed a decrease in photosynthetic activity in *Chlorella* sp. when exposed to PS fragments measuring 0.02 μm. Conversely, no significant reduction in activity was observed when microalgae were exposed to fragments of 0.5 μm.

Microplastics of different types reveal different effects on aquatic microorganisms. The growth of *Chlamydomonas reinhardtii* was inhibited when these microalgae were exposed to PP fragments ranging from 400 to 1000 μm in size at a concentration of 400 $mg.L^{-1}$. Nonetheless, the microalgae growth did not exhibit a decrease when exposed to HDPE under the same conditions (Lagarde *et al.,* 2016). Studies with *Tetraselmis chuii* exposed for 96 hours to fragments of PE of 1 and 5 μm at a concentration of 0.046 -- 0.048 mg L^{-1} caused a reduction in its growth (Lagarde *et al.*, 2016; Zimmermann *et al.*, 2020). In the microcrustacean *Daphnia magna*, exposure to varying concentrations of PVC, polyurethane (PUR), and polylactic acid (PLA) at levels of 10, 50, 100, and 500 mg L^{-1} showed a reduction and slowdown of its growth

(Zimmermann *et al.*, 2020). In contrast, when *D. magna* were exposed to microplastics (ranging from 10 to 100 μm) at a concentration of 50 mg L^{-1} no effects on mobility or survival were observed (Jemec Kokalj *et al.*, 2022).

Although the type and size of a polymer are important aspects that can influence the toxicity of microplastics, the present study showed that the polymer type (LLDPE, PP, and PS), chemical composition (Ca, Ti, Cl, Br, Al), size (8-1749 μm) and concentration (100, 200, and 300 $mg.L^{-1}$) of SMP did not have a significant effect on *Tetraselmis spp*. Moreover, this study demonstrated that microalgae exhibited notable resistance to PELBD, PP, and PS, surpassing the concentrations usually found in aquatic ecosystems.

The resistance of *Tetrasselmis* sp. to SMP can be attributed to their surface charge. After analyzing the zeta potential, it was observed that both microplastics and microalgae possess negatively charged surfaces. The carboxyl groups and sulfates on the cellulose wall could be responsible for the negative charge on the surface of microalgae. The negative charge on the surface of microplastics can cause electrostatic repulsion with other negatively charged microalgae. Nonetheless, the electrostatic interaction between negatively charged microalgae and positively charged microplastics may facilitate their attraction. This phenomenon is attributed to various mechanisms, which include electrostatic interactions, hydrogen bonds, and hydrophobic interactions (Bhattacharya *et al.*, 2010; Thiagarajan *et al.*, 2021). Larue *et al.* (2021) have reported that the toxicity of cationic microplastics is comparatively higher in phytoplankton than anionic microplastics. Another factor that can contribute to electrostatic repulsion is particle size. Although not well understood, it is believed that particle size and zeta potential are directly proportional properties (Yuan *et al.*, 2022).

It is essential to consider that microplastics are not present independently in aquatic ecosystems, as pharmaceutical waste and drugs may also occur in the same environment. In the present study, paracetamol caused a reduction in the growth of microalgae, both isolated and in combination with secondary microplastics.

Despite the toxicity of paracetamol to microalgae, experimental results revealed an EC_{50} value of 256 ± 53 $mg.L^{-1}$, which is notably greater than the usual concentrations observed in aquatic ecosystems (ranging from $ng.L^{-1}$ to $g.L^{-1}$) (Noda *et al.*, 2007). In this study, the simultaneous exposure of paracetamol with SMP resulted in no statistically significant alterations compared to when microalgae were exposed only to paracetamol. The widespread availability of paracetamol on the planet leads to its occurrence in aquatic ecosystems with unique attributes, resulting in adverse effects on various organisms (Xu *et al.*, 2008; Wu *et al.*, 2012; Rhee *et al.*, 2013). Additionally, the co-occurrence of these pollutants in the environment could produce distinct results compared to their individual effects (Fonte *et al.*, 2016; Wu *et al.*, 2016; Ziajahromi *et al.*, 2016).

The morphology, dimensions, size, and surface charge of microplastics are significant physical characteristics that might influence the adsorption and transport of substances (Davarpanah and Guilhermino, 2015; 2019; Fonte *et al.*, 2016; Wu *et al.*, 2016; Ziajahromi *et al.*, 2016; Yuan *et al.*, 2022)

According to reports, microplastics have the capacity to adsorb and accumulate copper, especially in pellets that have aged compared to those that are in their original state. The presence or absence of microplastics does not seem to have a discernible impact on the growth rate of *Tetraselmis chuii* in the presence of copper (Davarpanah and Guilhermino, 2015). However, when microplastics were combined with gold nanoparticles, an increase in copper toxicity in microalgae growth was observed (Davarpanah and Guilhermino, 2019). In *T. chuii*, the presence of microplastics affects the toxicity of pharmaceuticals (Wu *et al.*, 2016).

The findings indicated that the growth of microalgae was not inhibited by concentrations ranging from 100 to 200 $mg.L^{-1}$. Despite the toxicity of paracetamol for microalgae, the half-maximal effective concentration (EC_{50}) was found to be 256±53 mg. This concentration

exceeded the average levels noticed for microalgae in the aquatic environment (Al-Kaf *et al.*, 2017). The metabolic processes of mixotrophic microalgae may alternate between autotrophy and heterotrophy depending on the accessibility of carbon sources and nutrients (Borowitzka, 2018). Some species of microalgae have been described as capable of metabolizing pharmaceutical pollutants (Xiong *et al.*, 2018). *Tetraselmis* sp. is a species of mixotrophic microalgae, and our results indicate that the resistance of the microalgae to different concentrations of paracetamol may suggest that this species can biodegrade this emerging pollutant. However, future studies that aim to understand the mechanisms that confer resistance to paracetamol in *Tetraselmis* sp. and whether this resistance applies to other pharmaceutical residues are necessary.

5. CONCLUSION

The investigation revealed that the toxicity of secondary microplastics is not solely dependent on factors such as the type of polymer, concentration, or additives, as previously noted in the literature. Instead, the main physical-chemical characteristics that influence their toxicity are the surface charge and size of microplastics. Furthermore, the study emphasizes the potential use of *Tetraselmis* sp. as a valuable model for investigating the impact of pharmaceutical residues on aquatic microorganisms in their natural habitat. The aim of this study was also to encourage further research to clarify both acute and long-term ecotoxicological effects of microplastics and other commonly used pharmaceuticals in aquatic environments.

6. DECLARATION OF COMPETING INTEREST

The authors declare that they have no known competing financial interests or personal relationships that could have influenced the work reported in this paper.

7. REFERENCES

AGARWAL, N. Paracetamol - A Contaminant of High Concern: Existence in Environment and Adverse Effect. **Pharmaceutical Drug Regulatory Affairs Journal**, v. 5, n. 1, 2022. https://doi.org/10.23880/pdraj-16000128

ALIMI, O. S.; HERNANDEZ, L. M.; TUFENKJI, N. Microplastics and nanoplastics in aquatic environments: Aggregation, deposition, and enhanced contaminant transport. **Environmental Science & Technology**, v. 52, p. 1704 – 1724, 2018. Https://doi.org/10.1021/acs.est.7b05559

AL-KAF, A. G.; NAJI, K. M.; ABDULLAH, Q. Y.; EDREES, W. H. A. Occurrence of paracetamol in aquatic environments and transformation by microorganisms: A review. **Chronicles of Pharmaceutical Science**, v. 1, p. 341–355, 2017.

ANDRADY, A. L.; NEAL, M. A. Applications and societal benefits of plastics. **Philosophical Transactions of the Royal Society B: Biological Sciences**, v. 364, p. 1977–1984, 2009. Https://doi.org/10.1098/rstb.2008.0304

ANVISA (Brasil). Resolução nº 17, de 17 de março de 2008. Dispõe sobre Regulamento Técnico sobre Lista Positiva de Aditivos para Materiais Plásticos destinados à Elaboração de Embalagens e Equipamentos em Contato com Alimentos. **Diário Oficial [da] União**: seção 1, Brasília, DF, p. 43-51, 18 mar. 2008.

AUS DER BEEK, T.; WEBER, F.-A.; BERGMANN, A. *et al.* Pharmaceuticals in the environment: Global occurrences and perspectives. **Environmental Toxicology and Chemistr**y, v. 35, p. 823–835, 2016. Https://doi.org/10.1002/etc.3339

BARRICK, A.; CHAMPEAU, O.; CHATEL, A. *et al.* Plastic additives: challenges in ecotox hazard assessment. **PeerJ**, v. 9, p. e11300, 2021. Https://doi.org/10.7717/peerj.11300

BETTS, K. Why small plastic particles may pose a big problem in the oceans. **Environmental Science & Technology**, v. 42, p. 8996, 2008. Https://doi.org/10.1021/es802970v

BHATTACHARYA, P.; LIN, S.; TURNER, J. P.; KE, P. C. Physical adsorption of charged plastic nanoparticles affects algal photosynthesis. **The Journal of Physical Chemistry C**, v. 114, p. 16556–16561, 2010. Https://doi.org/10.1021/jp1054759

BOROWITZKA, M. A. Chapter 3 - Biology of microalgae. *In*: LEVINE, I. A.; FLEURENCE, J. B. T. **Microalgae in health and disease prevention**. Academic Press, 2018. p. 23–72.

BOTTONI, P.; CAROLI, S.; CARACCIOLO, A. B. Pharmaceuticals as priority water contaminants. **Toxicological and Environmental Chemistry**, v. 92, p. 549–565, 2010. Https://doi.org/10.1080/02772241003614320

CARBERY, M.; O'CONNOR, W.; PALANISAMI, T. Trophic transfer of microplastics and mixed contaminants in the marine food web and implications for human health. **Environment International**, v. 115, p. 400–409, 2018. Https://doi.org/10.1016/j.envint.2018.03.007

COLE, M.; LINDEQUE, P.; HALSBAND, C.; GALLOWAY, T. S. Microplastics as contaminants in the marine environment: A review. **Marine Pollution Bulletin**, v. 62, p. 2588–2597, 2011. Https://doi.org/10.1016/j.marpolbul.2011.09.025

DAVARPANAH, E.; GUILHERMINO, L. Single and combined effects of microplastics and copper on the population growth of the marine microalgae *Tetraselmis chuii*. Estuarine, **Coastal and Shelf Science**, v. 167, p. 269–275, 2015. Https://doi.org/10.1016/j.ecss.2015.07.023

DAVARPANAH, E.; GUILHERMINO, L. Are gold nanoparticles and microplastics mixtures more toxic to the marine microalgae *Tetraselmis chuii* than the substances individually? **Ecotoxicology and Environmental Safety**, v. 181, p. 60–68, 2019. Https://doi.org/10.1016/j.ecoenv.2019.05.078

DE VOOGT, P.; JANEX-HABIBI, M. L.; SACHER, F. *et al.* Development of a common priority list of pharmaceuticals relevant for the water cycle. **Water Science and Technology**, v. 59, p. 39–46, 2009. Https://doi.org/10.2166/wst.2009.764

DOMENIGHINI, A.; GIORDANO, M. Fourier transform infrared spectroscopy of microalgae as a novel tool for biodiversity studies, species identification, and the assessment of water quality. **Journal of Phycology**, v. 45, p. 522–531, 2009. Https://doi.org/10.1111/j.1529-8817.2009.00662.x

FISTAROL, G. O.; HARGREAVES, P. I.; WALTER, J. M.; VIANA, T. V.; GOMES, P. D. F.; LOURENÇO, C. B. *et al.* Rapid isolation of culturable microalgae from a tropical shallow lake system. **Journal of Applied Phycology**, v. 30, n. 3, p. 1807–1819, 2018. https://doi.org/10.1007/s10811-018-1404-7

FONTE, E.; FERREIRA, P.; GUILHERMINO, L. Temperature rise and microplastics interact with the toxicity of the antibiotic cefalexin to juveniles of the common goby (*Pomatoschistus microps*): Post-exposure predatory behaviour, acetylcholinesterase activity and lipid peroxidation. **Aquatic Toxicology**, v. 180, p. 173–185, 2016. Https://doi.org/10.1016/j.aquatox.2016.09.015

GREGORY, M. R. Plastic 'scrubbers' in hand cleansers: a further (and minor) source for marine pollution identified. **Marine Pollution Bulletin**, v. 32, p. 867–871, 1996. Https://doi.org/10.1016/S0025-326X(96)00047-1

GUILLARD, R. R. L. Culture of Phytoplankton for Feeding Marine Invertebrates. *In*: SMITH, W. L.; CHANLEY, M. H. (eds.). **Culture of Marine Invertebrate Animals**: Proceedings — 1st Conference on Culture of Marine Invertebrate Animals Greenport. Springer US, 1975. p. 29–60. https://doi.org/10.1007/978-1-4615-8714-9_3

GUO, M.; NOORI, R.; ABOLFATHI, S. Microplastics in freshwater systems: Dynamic behaviour and transport processes. **Resources, Conservation and Recycling**, v. 205, p. 107578, 2024. https://doi.org/10.1016/j.resconrec.2024.107578

HARPER, C. A.; PETRIE, E. M. Plastics materials and processes - a concise encyclopedia - Book Review. **IEEE Electrical Insulation Magazine**, v. 20, p. 47–48, 2004. Https://doi.org/10.1109/MEI.2004.1318854

JEMEC KOKALJ, A.; DOLAR, A.; DROBNE, D. *et al.* Environmental hazard of polypropylene microplastics from disposable medical masks: acute toxicity towards *Daphnia magna* and current knowledge on other polypropylene microplastics. **Microplastics and Nanoplastics**, v. 2, p. 1–15, 2022. Https://doi.org/10.1186/s43591-021-00020-0

JUNG, M. R.; HORGEN, F. D.; ORSKI, S. V. *et al.* Validation of ATR FT-IR to identify polymers of plastic marine debris, including those ingested by marine organisms. **Marine Pollution Bulletin**, v. 127, p. 704–716, 2018. Https://doi.org/10.1016/j.marpolbul.2017.12.061

KASPRZYK-HORDERN, B. Pharmacologically active compounds in the environment and their chirality. **Chemical Society Reviews**, v. 39, p. 4466–4503, 2010. Https://doi.org/10.1039/c000408c

KLEIN, S.; DIMZON, I. K.; EUBELER, J.; KNEPPER, T. P. Analysis, occurrence, and degradation of microplastics in the aqueous environment. *In*: WAGNER, M.; LAMBERT, S. (eds.). Cham: **Springer International Publishing**, 2018. p. 51–67.

LAGARDE, F.; OLIVIER, O.; ZANELLA, M. *et al.* Microplastic interactions with freshwater microalgae: Hetero-aggregation and changes in plastic density appear strongly dependent on polymer type. **Environmental Pollution**, v. 215, p. 331–339, 2016. Https://doi.org/10.1016/j.envpol.2016.05.006

LARUE, C.; SARRET, G.; CASTILLO-MICHEL, H.; PRADAS DEL REAL, A. E. A critical review on the impacts of nanoplastics and microplastics on aquatic and terrestrial photosynthetic organisms. **Small**, v. 17, p. 2005834, 2021. Https://doi.org/10.1002/smll.202005834

LAU, W. W. Y.; SHIRAN, Y.; BAILEY, R. M. *et al.* Evaluating scenarios toward zero plastic pollution. **Science**, v. 369, p. 1455–1461, 2020. Https://doi.org/10.1126/science.aba9475

MENSAH, R. A.; SHANMUGAM, V.; NARAYANAN, S. *et al.* A review of sustainable and environment-friendly flame retardants used in plastics. **Polymer Testing**, v. 108, p. 107511, 2022. Https://doi.org/10.1016/j.polymertesting.2022.107511

MEZZELANI, M.; GORBI, S.; REGOLI, F. Pharmaceuticals in the aquatic environments: Evidence of emerged threat and future challenges for marine organisms. **Marine Environmental Research**, v. 140, p. 41–60, 2018. Https://doi.org/10.1016/j.marenvres.2018.05.001

MOORE, C. J. Synthetic polymers in the marine environment: A rapidly increasing, long-term threat. **Environmental Research**, v. 108, p. 131–139, 2008. Https://doi.org/10.1016/j.envres.2008.07.025

MOURA, D. S.; PESTANA, C. J.; MOFFAT, C. F.; HUI, J.; IRVINE, J. T. S.; LAWTON, L. A. Characterisation of microplastics is key for reliable data interpretation. **Chemosphere**, v. 331, p. 138691, 2023. https://doi.org/10.1016/j.chemosphere.2023.138691

NGUYEN, H. T.; YOON, Y.; NGO, H. H.; JANG, A. The application of microalgae in removing organic micropollutants in wastewater. **Critical Reviews in Environmental Science and Technology**, v. 0, p. 1–34, 2020. Https://doi.org/10.1080/10643389.2020.1753633

NODA, I.; DOWREY, A. E.; HAYNES, J. L.; MARCOTT, C. Group frequency assignments for major infrared bands observed in common synthetic polymers. *In*: MARK, J. E. (ed.). **Physical Properties of Polymers Handbook**. New York: Springer, 2007. p. 395–406.

NUNES, B.; ANTUNES, S. C.; SANTOS, J. *et al.* Toxic potential of paracetamol to freshwater organisms: A headache to environmental regulators? **Ecotoxicology and Environmental Safety**, v. 107, p. 178–185, 2014. Https://doi.org/10.1016/j.ecoenv.2014.05.027

PATEL, M. M.; GOYAL, B. R.; BHADADA, S. V. *et al.* Getting into the brain: Approaches to enhance brain drug delivery. **CNS Drugs**, v. 23, p. 35–58, 2009. Https://doi.org/10.2165/0023210-200923010-00003

PHONG VO, H. N.; LE, G. K.; HONG NGUYEN, T. M.; BUI, X.-T.; NGUYEN, K. H.; RENE, E. R. *et al*. Acetaminophen micropollutant: Historical and current occurrences, toxicity, removal strategies and transformation pathways in different environments. **Chemosphere**, v. 236, p. 124391, 2019. https://doi.org/10.1016/j.chemosphere.2019.124391

PLASTICS EUROPE. **The compelling facts about plastics 2007: An analysis of plastics production, demand and recovery for 2007 in Europe.** 2008. Available in: https://plasticseurope.org/wp-content/uploads/2021/10/2007-Compelling-facts.pdf

PLASTICS EUROPE. **Plastics - The facts 2021**. 2021. Available in: https://plasticseurope.org/knowledge-hub/plastics-the-facts-2021/

PORTA, R. Anthropocene, the plastic age and future perspectives. **FEBS Open Bio**, v. 11, p. 948–953, 2021. Https://doi.org/10.1002/2211-5463.13122

PRATA, J. C.; DA COSTA, J. P.; LOPES, I. *et al.* Effects of microplastics on microalgae populations: A critical review. **Science of the Total Environment**, v. 665, p. 400–405, 2019. Https://doi.org/10.1016/j.scitotenv.2019.02.132

REHMAN, M. S. U.; RASHID, N.; ASHFAQ, M. *et al.* Global risk of pharmaceutical contamination from highly populated developing countries. **Chemosphere**, v. 138, p. 1045–1055, 2015. Https://doi.org/10.1016/j.chemosphere.2013.02.036

RHEE, J.-S.; KIM, B.-M.; JEONG, C.-B. *et al.* Effect of pharmaceuticals exposure on acetylcholinesterase (AchE) activity and on the expression of AchE gene in the monogonont rotifer, *Brachionus koreanus*. **Comparative Biochemistry and Physiology Part C: Toxicology & Pharmacology**, v. 158, p. 216–224, 2013. Https://doi.org/10.1016/j.cbpc.2013.08.005

SUN, S.; WANG, Q.; WANG, N.; YANG, S.; QI, H. High-risk antibiotics positively correlated with antibiotic resistance genes in five typical urban wastewater. **Journal of Environmental Management**, v. 342, p. 118296, 2023. https://doi.org/10.1016/j.jenvman.2023.118296

TEUTEN, E. L.; SAQUING, J. M.; KNAPPE, D. R. U. *et al.* Transport and release of chemicals from plastics to the environment and to wildlife. **Philosophical Transactions of the Royal Society B: Biological Sciences**, v. 364, p. 2027–2045, 2009. Https://doi.org/10.1098/rstb.2008.0284

THIAGARAJAN, V.; ALEX, S. A.; SEENIVASAN, R. *et al.* Interactive effects of micro/nanoplastics and nanomaterials/pharmaceuticals: Their ecotoxicological consequences in the aquatic systems. **Aquatic Toxicology**, v. 232, p. 105747, 2021. Https://doi.org/10.1016/j.aquatox.2021.105747

THOMPSON, R. C. Lost at sea: Where is all the plastic? **Science**, v. 304, p. 838–838, 2004. Https://doi.org/10.1126/science.1094559

TSIAKA, P.; TSARPALI, V.; NTAIKOU, I. *et al.* Carbamazepine-mediated pro-oxidant effects on the unicellular marine algal species *Dunaliella tertiolecta* and the hemocytes of mussel *Mytilus galloprovincialis*. **Ecotoxicology**, v. 22, p. 1208–1220, 2013. Https://doi.org/10.1007/s10646-013-1108-3

WHO. **Pharmaceuticals in drinking-water**, 2012. Available in: https://www.who.int/publications/i/item/9789241502085

WU, C.; ZHANG, K.; HUANG, X.; LIU, J. Sorption of pharmaceuticals and personal care products to polyethylene debris. **Environmental Science and Pollution Research**, v. 23, p. 8819–8826, 2016. Https://doi.org/10.1007/s11356-016-6121-7

WU, S.; ZHANG, L.; CHEN, J. Paracetamol in the environment and its degradation by microorganisms. **Applied Microbiology and Biotechnology**, v. 96, p. 875–884, 2012. Https://doi.org/10.1007/s00253-012-4414-4

XIONG, J. Q.; KURADE, M. B.; JEON, B. H. Can microalgae remove pharmaceutical contaminants from water? **Trends in Biotechnology**, v. 36, p. 30–44, 2018. Https://doi.org/10.1016/j.tibtech.2017.09.003

XU, J. J.; HENDRIKS, B. S.; ZHAO, J.; DE GRAAF, D. Multiple effects of acetaminophen and p38 inhibitors: Towards pathway toxicology. **FEBS Letters**, v. 582, p. 1276–1282, 2008. Https://doi.org/10.1016/j.febslet.2008.01.063

YUAN, T.; GAO, L.; ZHAN, W.; DINI, D. Effect of particle size and surface charge on nanoparticles diffusion in the brain white matter. **Pharmaceutical Research**, v. 39, p. 767–781, 2022. Https://doi.org/10.1007/s11095-022-03222-0

ZHANG, C.; CHEN, X.; WANG, J.; TAN, L. Toxic effects of microplastic on marine microalgae *Skeletonema costatum*: Interactions between microplastic and algae. **Environmental Pollution**, v. 220, p. 1282–1288, 2017. Https://doi.org/10.1016/j.envpol.2016.11.005

ZIAJAHROMI, S.; NEALE, P. A.; LEUSCH, F. D. L. Wastewater treatment plant effluent as a source of microplastics: Review of the fate, chemical interactions and potential risks to aquatic organisms. **Water Science and Technology**, v. 74, p. 2253–2269, 2016. Https://doi.org/10.2166/wst.2016.414

ZIMMERMANN, L.; GÖTTLICH, S.; OEHLMANN, J. *et al.* What are the drivers of microplastic toxicity? Comparing the toxicity of plastic chemicals and particles to *Daphnia magna*. **Environmental Pollution**, v. 267, 2020. Https://doi.org/10.1016/j.envpol.2020.115392

ZITKO, V.; HANLON, M. Another source of pollution by plastics: Skin cleaners with plastic scrubbers. **Marine Pollution Bulletin**, v. 22, p. 41–42, 1991. Https://doi.org/10.1016/0025-326X(91)90444-W

ŻUR, J.; PIŃSKI, A.; MARCHLEWICZ, A. *et al.* Organic micropollutants paracetamol and ibuprofen—toxicity, biodegradation, and genetic background of their utilization by bacteria. **Environmental Science and Pollution Research**, v. 25, p. 21498–21524, 2018. Https://doi.org/10.1007/s11356-018-2517-x