

Short Communication

Sorption and Bioaccessibility of 17 β -Estradiol on Environmental Microplastics: Particle Size, Aging, Competitive Interactions and Co-exposure

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Received March 10, 2025; Accept July 30, 2025

Abstract

Microplastics (MPs) have been extensively studied in aquatic environments due to their potential to act as vectors for emerging contaminants. This study investigated the sorption of the natural hormone 17 β -estradiol (E2) onto polyethylene terephthalate (PET) and polyethylene (PE) MPs, considering particle size and aging effect over sorption capacity and toxicity of co-exposure. MPs were obtained from plastics collected from the Monjolinho River in São Paulo, Brazil, and ground into different size fractions. Additionally, artificial aging was simulated by exposing PET samples to ultraviolet (UV) radiation (254 nm, 16W) for 45 days, and co-exposure with bisphenol A (BPA) was also assessed. To evaluate the potential toxicity of E2 and environmental MPs, a cell viability assay (MTT) was performed using zebrafish (*Danio rerio*) hepatocytes (ZF-L). The results showed that for PET, the highest sorption occurred in particles ranging from 500 to 1000 μ m, while for PE, the highest adsorption was observed in particles between 350 and 500 μ m. Particles smaller than 125 μ m exhibited low sorption, likely due to their tendency to aggregate. Scanning electron microscopy analysis revealed increased surface roughness in artificially aged particles. However, no significant variation in E2 sorption was observed between aged and pristine MPs, indicating that superficial aging did not alter their retention capacity on tested conditions. Furthermore, in the presence of BPA, only this compound was sorbed, suggesting that competitive interactions between contaminants may influence sorption in MPs. The cell viability assay showed no toxicity for E2, MPs, or their co-exposure. These findings highlight the complexity of sorption processes involving microplastic particles and other emerging contaminants, contributing to the knowledge about how environmental factors influence the bioavailability of these compounds and the potential risks for aquatic biota.

Keywords: Microplastics, 17 β -estradiol, Polyethylene terephthalate, Polyethylene, Sorption.

INTRODUCTION

Developed in the 20th century, plastic revolutionized various industries and became essential to daily life due to its versatility. Its widespread use is primarily attributed to its low cost, lightweight nature, malleability, and high durability, making it a fundamental material in modern society (Menéndez-Pedriz & Jaumot, 2020; Zhang *et al.*, 2021). However, mismanaged disposal

allows plastic waste to enter aquatic environments, where it does not readily degrade. Instead, exposure to external factors such as rainfall, wind, and ultraviolet (UV) radiation causes plastic to fragment into microplastics (MPs) and nanoplastics (NPs) (Andrady, 2011).

In recent years, the presence of MPs in aquatic environments has attracted significant global attention and has become a subject of extensive research. The harmful effects of MPs on aquatic organisms are still under

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investigation. Studies indicate that various species, including fish, crustaceans, and mollusks, can ingest MPs, potentially leading to physical damage and chemical toxicity (Sodré *et al.*, 2023; Ribeiro *et al.*, 2024). Another major concern is the role of MPs as carriers of contaminants, including pharmaceuticals, potentially toxic metals, and pesticides, which can accumulate on their surfaces at several orders of magnitude higher than in the surrounding water (Atugoda *et al.*, 2021). Once sorbed, these plastic particles can transport contaminants in aquatic systems through sorption/desorption mechanisms (Liu *et al.*, 2019), affecting bioaccessibility process. The extent of this partitioning process depends on multiple factors, including MP polymer, particle size, aging, salinity, pH, and the physicochemical properties of the contaminants, which influence sorption capacity (Martín *et al.*, 2022).

In aquatic systems, the estrogen 17 β -estradiol (E2) naturally excreted by mammals, act as endocrine disrupting chemical (EDC) for organisms, causing damaging effects such as feminization and infertility (Cislak *et al.*, 2023), even in very low concentrations (ng L⁻¹). These environmental concentrations of E2 have been detected in different aquatic matrices as water, sediment and biota (Wang *et al.*, 2015; Campanha *et al.*, 2015; Pusceddu *et al.*, 2019) and its pseudo-persistence can be attributed to the constant input mainly through raw sewage or effluents from not efficient sewage treatment plants (Grzegorzek *et al.*, 2024). Once in environment, the interaction between E2 and different microplastics polymers can be observed and depends on many physical-chemical processes (Liu *et al.*, 2019).

Given the complex interactions between MPs and contaminants, studies must consider variations in polymer type and particle size to enhance environmental relevance (Moura *et al.*, 2024). In this context, the present study investigates how factors such as particle size, MP composition, particle aging, and the presence of another contaminant —bisphenol A (BPA)— affect the sorption of the natural hormone 17 β -estradiol (E2). The selected polymers, polyethylene terephthalate (PET) and polyethylene (PE), were analyzed across different particle size ranges. The influence of artificial and natural aging on contaminant sorption was assessed exclusively for PET MPs. To ensure the environmental relevance, the naturally aged MPs were derived from plastic debris collected along the banks of the Monjolinho River, a significant water body in São Carlos, São Paulo, Brazil. Additionally, cell viability assays were conducted to evaluate the toxicity of environmentally relevant E2 concentrations and the potential co-exposure effects of MPs using a biological model.

MATERIAL AND METHODS

Microplastic Collection and Preparation

The plastic microparticles used in this study were prepared following the "true-to-life" concept proposed by Ducoli *et al.* (2022). To obtain the materials, plastic objects made of polyethylene terephthalate (PET) and polyethylene (PE) were collected from the banks of the Monjolinho River, located in São Carlos, São Paulo, Brazil (Figure 1A). These materials consisted of plastic bottles and colorful pool balls, respectively. After cleaning, the objects were ground using a Willye-type knife mill (Tecnal TE-650) with liquid nitrogen. The resulting particles were then classified into six granulometric ranges through sieving: 1–5 mm, 500–1000 μ m, 350–500 μ m, 250–350 μ m, 125–250 μ m, and <125 μ m (Figures 1B and 1C). For experiments the 3 most representative sizes: < 125 μ m, 350–500 μ m and 500–1000 μ m were chosen and applied for the sorption tests. For experimental controls, identical bottles to those collected from the environment were purchased from supermarkets and subjected to the same processing.

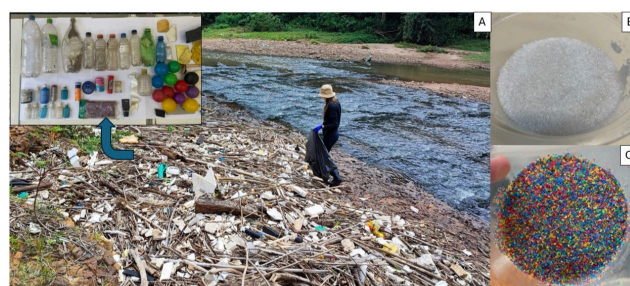


Figure 1. (A) Accumulation of plastic waste along the banks of the Monjolinho River – inset: macroplastics collected for sorption studies; (B) PET and (C) PE microplastics obtained using the “true-to-life” method for sorption experiments.

Considering the factors that influence contaminant sorption and the toxicity of plastic microparticles, the tests were conducted using particles of different sizes and subjected to both natural and artificial aging processes. To simulate artificial aging, PET microparticles (350–500 μ m) were exposed to ultraviolet (UV) radiation (254 nm) in a UV chamber equipped with two 8W lamps for 45 days following the methods adapted from literature (Sutkar *et al.*, 2023; Zjadic *et al.*, 2023). To assess whether UV exposure altered the surface of PET particles, scanning electron microscopy (SEM) was performed under an acceleration voltage of 15 kV, a working distance of 9 mm, a magnification of 5000x, and a secondary electron detector (SE1).

Sorption Studies

The experiments were conducted to separately evaluate the sorption capacity of each polymer concerning the contaminant 17 β -estradiol (100 μ g L⁻¹). Isotopically labeled 17 β -estradiol-d5 (5 μ g L⁻¹) was used as an internal

standard. Sorption tests were performed following OECD Guideline 106 (OECD, 2000). For each assay, 25 mg of plastic microparticles and 5 mL of the exposure solution were added to 40 mL vials and maintained at 22 ± 2 °C for 72 hours under constant agitation in a rotary shaker (160 rpm) and protected from light.

After 72 hours, 950 μ L of the sample was withdrawn and added to a vial containing 50 μ L of the internal standard. The sample was then filtered (PTFE 0.22 μ m) and analyzed by liquid chromatography coupled with mass spectrometry. Each experiment was performed in triplicate and included the following controls: blank (MPs without the analyte), degradation control (analyte without MPs for 72 hours), and quality control of the initial solution (t_0). The sorption percentage was determined based on the difference between the analyte concentration in the aqueous phase of the control group and the analyte concentration in the MPs samples. The granulometric ranges selected for the tests were <125 μ m, 350–500 μ m, and 500–1000 μ m for each polymer. All experiments were conducted in triplicate and expressed as percentage of sorption (%) $\pm$ relative standard deviation (RSD %). Differences between observed sorption's were checked using one-way ANOVA with a confidence level of 95% ($p=0.05$).

Chromatographic Parameters

For the analyses, a liquid chromatography system coupled with tandem mass spectrometry (LC-MS/MS) was used (Waters, TQD model) with electrospray ionization (ESI $^-$), using an ACQUITY BEH C18 chromatographic column (2.1 mm $\times$ 50 mm, 1.7 μ m particle size). The mobile phase consisted of water with 0.1% NH $_4$ OH (A) and methanol (B) in gradient mode. The total run time was 8.9 minutes.

The mass-to-charge ratio (m/z) transitions monitored in Selected Ion Monitoring (SIM) mode were 271.2 to 145.1 and 183.1 for 17 β -estradiol, while for the internal standard 17 β -estradiol-d5, the monitored transition was 276.2 to 147. For both compounds, the dwell time was set to 0.005 s. The cone and collision energies applied for 17 β -estradiol detection were 50 V, whereas for 17 β -estradiol-d5, these values were 60 V and 50 V, respectively. Method calibration demonstrated good linearity for 17 β -estradiol, with a quantification range of 0.05 to 3 μ g L $^{-1}$ and a determination coefficient (R^2) of 0.993. The limits of detection (LOD) and quantification (LOQ) were determined as 0.001 μ g L $^{-1}$ and 0.05 μ g L $^{-1}$, respectively, highlighting the method's high sensitivity. The internal standard 17 β -estradiol-d5 was used to correct analytical variations.

Cell viability

Zebrafish liver (ZFL) cell line was cultured in Leibovitz's L-15 Medium (VtrocCell) Leibovitz's L-15 Medium GlutaMAX $^{\text{TM}}$ (Gibco) #31415029, supplemented with 10% fetal bovine serum (FBS) (v/v) and maintained in culture flasks in an incubator at 29 °C.

For cell viability assays, cells were trypsinized, counted to adjust for cell concentration, and seeded into 96-well flat-bottomed cell culture plates (KASVI) at 3.0×10^4 cells/well density. The plates were incubated at 29 °C for 24 hours for cell attachment. After this period, E2 (ranging from 0.01 to 100 ppb) and MPs (PE and PET $<125\mu$ m, 0.1 mg L $^{-1}$) concentrations were added and then, treated cells were incubated under the same conditions for more 24 hours. The E2 concentrations range were chosen according to environmental relevant concentrations described in literature (Campanha *et al.*, 2015; Wang *et al.*, 2015), while MPs concentration was also based in previous experiments using zebrafish cells (Brandts *et al.*, 2020; Bohacková *et al.*, 2023). After incubation, 50 μ L of MTT solution (1 mg L $^{-1}$ in PBS) was added to each well. The plates were further incubated at 29 °C for 3 hours. During this incubation, mitochondrial dehydrogenase in viable cells reduces the MTT and forms purple formazan crystals. The addition of isopropanol then solubilized these crystals, and the absorbance of the wells was measured using a microplate reader (BioTek $^{\text{TM}}$, Epoch $^{\text{TM}}$) at a wavelength of 540 nm. The absorbance values were then used to determine the cell viability. The data were normalized using GraphPad Prism version 8, with control readings set as 100% viability. Statistical analysis was performed using one-way ANOVA followed by Dunnett's multiple comparison test, comparing the means of treatment groups to the control group mean.

RESULTS AND DISCUSSION

Influence of Particle Size on 17 β -Estradiol Sorption

Recent studies have explored the impact of particle size on contaminant sorption by microplastics (MPs); however, research focusing on MPs sampled directly from the environment remains limited. In this study, the E2 sorption capacity to environmental microplastics followed the order PET > PE, with intensity varying depending on the tested particle sizes (Figure 2). Considering the E2 sorption to different microplastics, Al-Jandal *et al.* (2022) also reported greater E2 sorption onto PET compared to other MPs (including LDPE) under both laboratory and field conditions, indicating the affinity of the natural estrogen to PET particles. Leng *et al.* (2023) observed the following sorption trend: PE > PP > PS, characterizing the process as spontaneous, exothermic, and primarily occurring at the surface. The sorption mechanism of E2 is attributed to hydrogen bonding and π - π interactions (Hu *et al.*, 2020), which are

influenced by polymer structure and hydrophobicity (Liu *et al.*, 2019). Both PET and PE exhibit moderate to high hydrophobicity, with water contact angles ranging from 80° to 94°, which may facilitate significant E2 sorption onto their surfaces. The tested polymers (PET and PE), along with polypropylene (PP), polystyrene (PS), and polyamide (PA), are among the most frequently detected MPs in Brazilian waters and are considered representative of those found in both biotic and abiotic matrices (Amparo *et al.*, 2023).

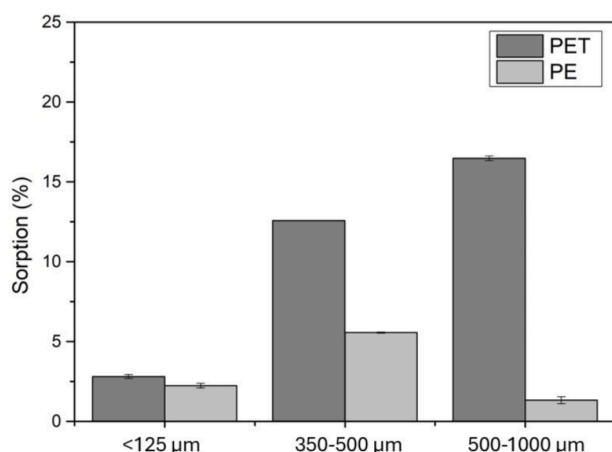


Figure 2. Sorption percentage (% $\pm$ CV) of estrogen E2 onto PET and PE microplastics of different size ranges (<125, 350–500, and 500–1000 μm). The sorption study was conducted over 72 hours at a concentration of 100 $\mu\text{g L}^{-1}$ and analyzed using ESI-LC-MS/MS.

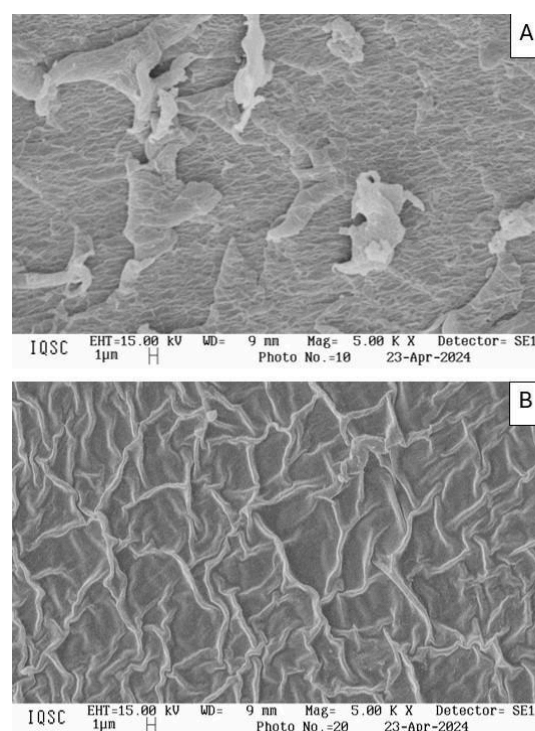
In general, smaller MPs tend to exhibit higher sorption capacities due to their increased surface-area-to-volume ratio (Leng *et al.*, 2023). However, in the case of PET, the highest sorption rate of E2 was observed in particles ranging from 500 to 1000 μm , reaching $16.47 \pm 0.15\%$. For PE, the highest sorption occurred in particles between 300 and 500 μm , with a sorption percentage of $5.53 \pm 0.03\%$ (Figure 2). Considering the highest sorption for PE and medium sorption using PET microplastics, this fraction (300 and 500 μm) was chosen for further experiments involving natural and artificial aging. For particles smaller than 125 μm , E2 sorption remained below 5% for both polymers. Besides its use in toxicity tests, this fraction was not chosen for aging experiments for this reason, and, besides the use of this fraction for toxicity tests. Additionally, the use of this fraction for toxicity tests was not considered for aging experiments for this reason. Besides its use in toxicity tests, this fraction was not chosen for aging experiments due to its low sorption capacity. This reduced sorption efficiency may be attributed to the tendency of smaller particles to interact with each other, potentially forming loose aggregates that reduce the effective surface area available for analyte interaction, consequently decreasing sorption capacity (Fu *et al.*, 2021; Menéndez-Pedriz & Jaumot, 2020). These findings

suggest that factors beyond particle size, such as polymer composition and potential aggregation, may influence the sorption behavior of MPs (Liu *et al.*, 2019). In an aquatic environment, other factors, such as humic substances, can also affect the sorption capacity of organic pollutants to polymers, including PE (Madeira *et al.*, 2025).

Sorption Study After Artificial and Natural Aging and Competitive Interactions

Beyond particle size, the effect of aging on E2 sorption onto PET MPs was assessed through both natural and artificial exposure, given that PET exhibited the highest sorption capacity in this study. Artificial aging was simulated by exposing PET MPs to UV radiation for 45 days. SEM analysis revealed that artificially aged particles exhibited increased surface roughness compared to pristine MPs (Figures 3A and 3B). However, despite these physical changes, no significant difference ($p > 0.05$) in E2 sorption was observed between aged and pristine MPs, with sorption rates remaining around 10% in both cases.

Additionally, experiments using naturally aged PET MPs (derived from bottles collected from the Monjolinho River) revealed no significant differences ($p > 0.05$) in E2 sorption compared to pristine MPs (Figure 3C). Several factors may have contributed to the lack of increased sorption in aged particles, as reported in the literature (Moura *et al.*, 2024). Among them, insufficient exposure of plastic bottles to weathering may have prevented significant surface degradation. For example, Bhagat *et al.* (2022) demonstrated that aging polyethylene (PE) microparticles can increase the sorption of organic contaminants, such as phenanthrene and methylene blue. However, the extent of aging and the specific contaminants involved are key determinants of this effect.



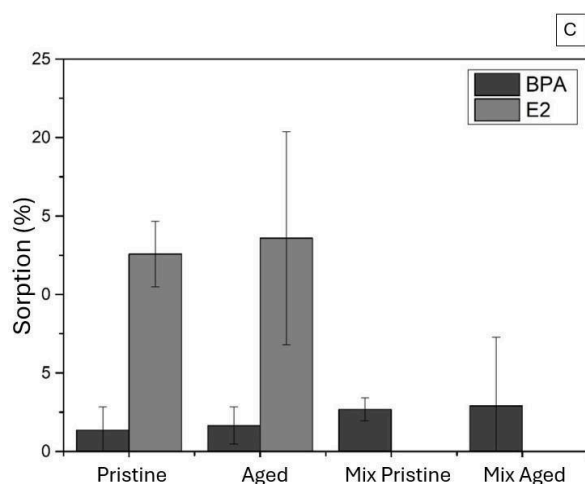


Figure 3. SEM images of samples from the 45-day UV aging experiment: (A) Pristine PET, (B) UV-aged PET, and (C) Sorption percentage (% $\pm$ CV) of BPA and E2 onto pristine PET and naturally aged PET (350–500 μ m) collected from the Monjolinho River in São Carlos. The sorption study was conducted over 72 hours at a concentration of 100 μ g L⁻¹ and analyzed using ESI-LC-MS/MS.

To investigate potential competitive interactions, additional tests were conducted to assess the sorption of BPA onto pristine and naturally aged PET MPs. As observed in Figure 3C, when exposed individually, E2 exhibited significantly higher sorption onto MPs compared to BPA, regardless of the degradation status. This may be attributed to structural differences, as BPA is more water-soluble than E2, while PET and PE exhibit moderate to high hydrophobicity, favoring the sorption of the less soluble compound (E2). However, under simultaneous binary exposure, only BPA was sorbed, suggesting competitive interactions that influence sorption dynamics. This effect and the increased sorption of BPA instead of estrogens were also observed in literature, considering the binary exposure to sediments, based on the distribution coefficient (β) of these compounds (Li *et al.*, 2014), where the possible sorption of other environmental contaminants could have reduced the number of available sites for E2 sorption. These findings align with previous studies indicating that the presence of multiple pollutants can alter the sorption behavior of individual contaminants on MPs (Budhiraja *et al.*, 2022), in addition to BPA.

As observed in our experiments, the sorption mechanisms of emerging contaminants in MPs are highly complex and depend on various factors beyond particle size and aging. Studies have highlighted the role of environmental conditions such as pH, ionic strength, biofilm formation, and dissolved organic matter in influencing sorption (Sodré *et al.*, 2023; Wang *et al.*, 2020; Madeira *et al.*, 2025). Additionally, the environmental implications of E2 sorption onto MPs extend beyond sorption itself, as these interactions may affect the bioaccessibility and desorption potential of contaminants, ultimately influencing their environmental

fate and ecological risk.

Cell viability and Co-exposure test

Zebrafish hepatocytes (ZF-L) were exposed to various concentrations of E2 co-exposed to PE and PET MPs (<125 μ m). The results showed no significant difference in cell viability between the control group (DMSO) and the exposed cells (Figure 4). Even under co-exposure with MPs, no toxic effects were observed in zebrafish cells under acute exposure conditions. These findings suggest that ZF-L cells are not sensitive to environmentally relevant concentrations of the tested contaminants, ranging from 0.01 to 100 ppb for E2 and 0.1 mg mL⁻¹ for MPs (<125 μ m). However, MPs and E2 are contaminants continuously released into aquatic systems, and chronic exposure must be considered when assessing potential long-term effects and chronic exposure. Previous studies have investigated MP toxicity in fish liver, reporting inflammatory responses and oxidative stress (Sun *et al.*, 2024). In zebrafish (ZF-L cell lines), toxicity has been observed for PS nanoplastic (Brandts *et al.*, 2020). For larger MPs particles, even at higher concentrations, no toxic effects were detected for PET MPs (ranging from 25 to 90 μ m) (Boháčková *et al.*, 2023), similar to what was observed in the present exposure. Like sorption behavior, toxicity is influenced by a wide range of exposure conditions and organismal sensitivity, where the presence of other contaminants in the aquatic system can contribute to the increase of this toxicity. The complexity of microplastic interactions in the biological system remains a significant challenge, highlighting the need for further research to understand better to understand their potential risks better.

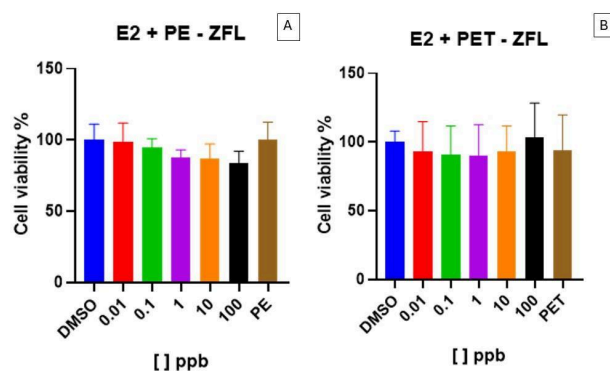


Figure 4. Cell viability of ZFL cell line after 24 h of treatment with E2 combined with various concentrations of PE (A) and PET (B).

CONCLUSION

The results demonstrated that E2 sorption capacity to environmental microplastics varied with particle size, with PET MPs in the 500–1000 μ m range and PE MPs in the 350–500 μ m range exhibiting the

highest sorption. In contrast, particles smaller than 125 μm showed reduced sorption, likely due to their tendency to aggregate, which decreases available surface area. Artificially aged PET MPs exhibited increased surface roughness. Still, no significant difference in E2 sorption was observed compared to pristine MPs, suggesting that the level of aging applied in this study did not substantially alter their retention capacity. Furthermore, in the presence of BPA, only this compound was sorbed, indicating competitive interactions between contaminants that may influence sorption dynamics in real environmental conditions. Cell viability assays revealed that neither E2 nor MPs, alone or in combination, induced toxic effects in ZF-L cells under acute exposure conditions and considering the tested MPs fraction. These findings suggest that at environmentally relevant concentrations, these contaminants do not pose immediate cytotoxicity risks. However, considering that MPs and E2 are continuously introduced into aquatic ecosystems, chronic exposure and long-term effects should be further investigated. Overall, this study highlights the complexity of MP-contaminant interactions and their potential implications for environmental fate and bioavailability.

ACKNOWLEDGMENTS

Dra. Dayana Moscardi dos Santos thanks the FAPESP São Paulo Research Foundation (grant #2023/10312-1) for financial support. Dra. Roberta Cerasi Urban thanks FAPESP (grants #2018/04820-6, #2022/03087-9, #2023/14823-0, #2023/06768-0).

AUTHOR CONTRIBUTIONS

All authors contributed equally to this work.

CREDIT AUTHOR STATEMENT

SLRB: Conceptualization, Experimental. **NSS:** Conceptualization, Data curation, Writing- Original draft preparation. **GMF:** Conceptualization, Data curation, Writing- Original draft preparation. **TT:** Experimental, Data curation. **FVR:** Writing- Reviewing and Editing. **RCU:** Writing- Reviewing and Editing. **PSF:** Writing- Reviewing and Editing. **DMS:** Conceptualization, Experimental, Data curation, Writing- Original draft preparation.

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Editor-in-chief:

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