



# Influence of contaminant-spiked polyethylene-type microplastics on the growth and primary production of the freshwater phytoplankton species *Scenedesmus armatus* and *Microcystis aeruginosa*

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## ABSTRACT

Microplastic pollution and its ecological impact on the aquatic environment are a current focus of research in the scientific community. These microplastics may adsorb contaminants discharged into the aquatic environment, thereby serving as a sink and source for the dissemination of these associated chemical contaminants. However, knowledge about the potential risks of microplastics and associated chemical contaminants on aquatic biota, especially on primary freshwater producers, remains to be explored. In this study, the impact of a polyethylene microplastic type (MP) associated with amoxicillin, ibuprofen, sertraline and simazine (OCs) on the cell growth and photosynthetic activity of the green algae *Scenedesmus armatus* and the cyanobacteria *Microcystis aeruginosa* was evaluated after 28 days of exposure. The results show that all the organic contaminants and their respective MP-OC complexes induced stress on cell growth after 28 days of exposure, except when the cyanobacterial strain was exposed to amoxicillin and ibuprofen. Similarly, photosynthetic activity was affected by exposure to MP-OC complexes, with the most evident effect on cellular respiration in the cyanobacterial strain and on net photosynthesis in the green algae strain. Additionally, the ability of the *M. aeruginosa* strain to synthesize microcystin was significantly reduced. These results show that the formation of MP-OC complexes could reduce their adverse effects, although there is wide variability depending on both the type of organic contaminant and the photosynthetic organisms involved, so further studies are needed to better understand the interactions between these aquatic contaminants.

## 1. Introduction

The accumulation of plastic residues in aquatic ecosystems is one of the current main anthropocentric pressures on the environment. Their durability coupled with inadequate management leads to excessive accumulation in natural habitats (Barnes et al., 2009). Under environmental conditions, plastics degrade into smaller particles with a diameter of less than 5 mm and are referred to as microplastics (MPs). However, in addition to these naturally degradation products, there are also MPs that are intentionally incorporated as ingredients in many manufactured products for human use (resin pellets, peels, shower gels,

etc.), and their final destination is often also the environment (Wright et al., 2013).

The size of these plastic particles is important because their bio-accumulation potential increases with decreasing size, with consequent risk throughout the aquatic food web. Additionally, plastics are capable of adsorbing organic pollutants from the surrounding environment (Dekiff et al., 2014; Martín et al., 2021; Fajardo et al., 2022), as well as containing chemical additives (Beiras et al., 2021), making them a source chemical exposure to wildlife.

Although the main social and scientific concern focuses almost exclusively on marine plastic debris, microplastics are also present in

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freshwater ecosystems, where they can also adsorb microcontaminants and pose a risk to freshwater species. For this reason, concerns about the impact of MPs on freshwater ecosystems are legitimate and require more scientific attention. The polymers most frequently detected in freshwater samples include polyethylene followed by polystyrene, with microplastic particles at concentrations of ten orders of magnitude (Koelmans et al., 2019).

The chemical composition of MPs, together with their large surface-to-volume ratio, facilitates the accumulation of water pollutants (Ashton et al., 2010; Koelmans et al., 2013). Among these water pollutants, pharmaceuticals and agrochemicals have emerged as a new class of environmental contaminants, which can have both acute and chronic harmful effects on natural aquatic biota. However, there is little information on the relationship between plastic residues and pollutants, such as pharmaceuticals, phytosanitary compounds, and endocrine compounds.

Some authors have focused on the interaction of MPs with chemicals in adsorption-desorption processes (Llorca et al., 2020; Fan et al., 2021), and although this interaction has demonstrated to be very complex, experiments have shown that MPs can act as vectors for the transfer of pollutants from water to biota (Hartmann et al., 2017; Atugoda et al., 2021). The hydrophobic properties and high surface area-to-volume ratio of microplastics facilitate the accumulation of organic contaminants (Mato et al., 2001; Teuten et al., 2009; Bakir et al., 2012; Li et al., 2018), providing a large solid surface, especially within aqueous environments, and the amounts of organic contaminants accumulated on the plastic surface can be several orders of magnitude higher than those in the surrounding waters.

Currently, most studies have focused on the impact of MPs on aquatic food chain consumers, so information on primary producers to date is quite limited. However, there are already some indications that MPs can damage phytoplankton depending on the concentration, size, and type of polymer to which they are exposed (Wagner and Lambert, 2017; Sánchez-Fortún et al., 2021). The key role played by phytoplankton in freshwater ecosystems justifies the relevance of further studies on these organisms regarding their variation in responses when the culture is exposed to different pollutants present in the aquatic environment (Almeida et al., 2019), including microplastics.

The aim of this study was to evaluate the potential risk of a polyethylene microplastic type associated with amoxicillin, ibuprofen, sertraline and simazine, organic contaminants in urban and industrial discharges into freshwater ecosystems, on the cell growth and photosynthetic activity of the green algae *Scenedesmus armatus* and the cyanobacteria *Microcystis aeruginosa* after 28 days of exposure. The results obtained could clarify the environmental relevance of these MP-OCs complexes on the structure of these freshwater phytoplanktonic communities.

## 2. Material and methods

### 2.1. Microplastic characterization

The plastic material used in these experiments was purchased from Cospheric LLC (Santa Barbara, CA, USA). It is composed of monodisperse, microspherical polyethylene particles with a size of 250–300  $\mu\text{m}$  and a density of 1.35  $\text{g mL}^{-1}$  and contains titanium dioxide ( $\text{TiO}_2$ ). Due to the hydrophobic character of polyethylene microspheres, they were coated with Tween 80 (Sigma, Aldrich Chemie, Taufkirchen, Germany) prior to suspension in BG-11 culture medium.

### 2.2. Organic compound-spiked microplastic complexes

Four aquatic organic contaminants (OCs) were used in this study: amoxicillin (antibiotic), ibuprofen (nonsteroidal anti-inflammatory drug), sertraline (antidepressant) and simazine (herbicide). All were supplied by Sigma-Aldrich (Sigma Aldrich Chemie, Taufkirchen,

Germany).

In this experiment, virgin polyethylene particles were exposed to the selected OCs according to batch adsorption experiments previously reported (Martín et al., 2021). Specifically, 0.4  $\text{mg mL}^{-1}$  polyethylene particles were artificially spiked into glass tubes along with the corresponding contaminant at a concentration of 15  $\mu\text{M}$  (MP-OCs). The tubes were capped and equilibrated in the dark for 72 h at 25 °C with continuous horizontal, rotary agitation (120 rpm).

### 2.3. Freshwater phytoplankton strains and culture conditions

The green microalgae *Scenedesmus armatus* (BEA 1402B) and the cyanobacteria *Microcystis aeruginosa* (BEA 1835B) from the Spanish Bank of Algae (BEA, Gran Canaria, Spain) were selected as model phytoplanktonic organisms to explore the toxic effects.

Cells of both phytoplankton species were grown axenically under laboratory conditions in culture flasks (Thermo Fisher Scientific Inc., MA, USA) with 20 mL of BG-11 culture medium (Sigma Aldrich Chemie, Taufkirchen, Germany). Under these conditions, cells were maintained at 21 °C with continuous light exposure (60  $\mu\text{mol m}^{-2} \text{s}^{-1}$  over the 400–700 nm waveband) in mid-log exponential growth by serial transfers of one-cell inoculums to fresh medium once a fortnight.

To unify the experimentation criteria, all tests were performed while both strains were in the exponential growth phase. Cellular quantification was indirectly estimated from the fluorescence emitted by the photosynthetic pigments of each of the strains using a Tecan Genios plate reader (Tecan Group Ltd., Switzerland), with excitation-emission filters of 485–670 and 590–670 nm for the green algae *S. armatus* and the cyanobacteria *M. aeruginosa*, respectively. This estimate was obtained by measuring the in vivo fluorescence for a phytoplankton dilution series after cell counting on a Neubauer chamber. The relationship between fluorescence and cell density was obtained by linear regression analysis using the computer software package GraphPad v.6. R-squared ( $r^2$ ) values of 0.85 and 0.98 ( $n = 4$ ) were obtained for *S. armatus* and *M. aeruginosa*, respectively.

### 2.4. Stress on freshwater phytoplanktonic cell growth

To determine the stress on cell growth induced by the assessed complexes, *S. armatus* and *M. aeruginosa* strains were tested in 25 mL culture flasks filled with 20 mL of BG-11 medium, with initial cell densities adjusted to  $10^4$  cells  $\text{mL}^{-1}$ . The cultures were shaken by hand twice daily, once in the morning and once in the evening, for 28 days of exposure. Both strains were exposed to 125  $\mu\text{g mL}^{-1}$  of each OC alone or in combination with microplastic (MP-OCs) added to the culture medium for 28 days. This concentration range is compatible with the results compiled in the bibliographic review published by Bellasi et al. (2020), although one of the main problems affecting this research area is that authors use different units to express MP densities and that each set of measurements represents a “snapshot”.

To eliminate a possible shading effect, the selected MP concentration was included in 25 mL BG-11 medium, and light absorption ( $\text{OD}_{627}$  and  $\text{OD}_{720}$ ) was determined in triplicate in a 1 cm path-length cuvette by measuring light absorption relative to a BG-11 medio blank. The results showed that the ODs obtained at the two selected wavelengths did not exceed 0.009, and therefore, shadowing effects in the experiments are not expected.

At 3, 7, 14, 21 and 28 days of exposure, the maximum cell growth rate was estimated with the Malthusian parameter ( $m$ ), which was calculated as:

$$m = \log_e (N_t / N_0) / t$$

where  $N_t$  and  $N_0$  are cell concentrations at time  $t$  and  $t = 0$  (both estimated by fluorescence). The  $m$ -values are expressed as doublings  $\text{d}^{-1}$ .

## 2.5. Photosynthetic activity assessment

Photosynthetic activity was assessed from the production/consumption  $O_2$  balance under light-dark conditions. The light-dark  $O_2$  balance was analysed using a Clark-type  $O_2$  electrode. Dissolved  $O_2$  was measured in a 1 mL reaction chamber with a Chlorolab 2 system (Hansatech, Norfolk, UK). This system enables the study of respiration and photosynthesis processes in liquid samples under automated illumination from red (660 nm) LED light and in darkness. In these photosynthetic activity assays, measurements were taken at 21 °C and 375  $\mu\text{mol m}^{-2} \text{s}^{-1}$  irradiance. The light-dark oxygen balance, or gross photosynthesis rate (Pg), was estimated from the formula:

$$P_g = P_n + R$$

where Pg corresponds to the oxygen production rate under illuminated conditions, R (respiration) corresponds to the process by which phytoplankton consume oxygen and release carbon dioxide in darkness, and Pn (net photosynthesis rate) is defined as the difference between Pg and R.

Under these conditions and after 28 days of exposure, a volume that contained the same cellular concentration ( $5 \times 10^5$  cells) was obtained from each exposure test and diluted in a final volume of 1 mL. Control and exposed samples were included in the reaction chamber, and the light-dark oxygen balance was estimated. Records of each sample were obtained after exposure to 5 min of darkness followed by 5 min of illumination. Four replicates of each experiment were performed ( $n = 4$ ).

## 2.6. Analysis of microcystis (MCs)

After 28 days of exposure of cells to the tested complexes at 125  $\mu\text{g mL}^{-1}$ , 10 mL of each sample of *M. aeruginosa* was centrifuged at 10000 g

and 4 °C for 5 min. The supernatant was used to analyse the extracellular MCs. The residues were resuspended in the original volume (10 mL) of ultrapure water, frozen at  $-80$  °C and quickly thawed at room temperature thrice. The resulting solutions were centrifuged at 10000 g and 4 °C for 5 min, and the supernatants were filtered through 0.22  $\mu\text{m}$  acetate cellulose membranes for analysis of intracellular MCs.

Extracellular and intracellular MC concentrations were detected using a commercial microcystin detection kit based on phosphatase activity inhibition by MCs (MicroCistest, ZEU-IMMUNOTEC S.L., Zaragoza, Spain). Samples containing MCs inhibit the enzyme activity proportionally to the amount of toxin contained in the sample that can be detected at 405 nm.

## 2.7. Data analysis

Statistical analysis was performed using the computer software package GraphPad Prism v6.0 (Graph-Pad Software Inc., USA). Data are expressed as the mean  $\pm$  sd of four experiments ( $n = 4$ ). Student's t test and one-way analysis of variance (ANOVA) were used to check if there were significant differences ( $p < 0.05$ ) among treatments, and a post hoc analysis (Tukey test) was used to check differences among groups.

## 3. Results

### 3.1. Cell growth rate

The results corresponding to the specific growth rates ( $m$ ) of *S. armatus* and *M. aeruginosa* strains exposed to selected OCs, both alone and forming complexes with the polyethylene microspheres and after 28 days of exposure, are plotted in Fig. 1.

The OC exposures on both phytoplankton strains to selected concentrations showed a decrease in the time-dependent cell growth ratio

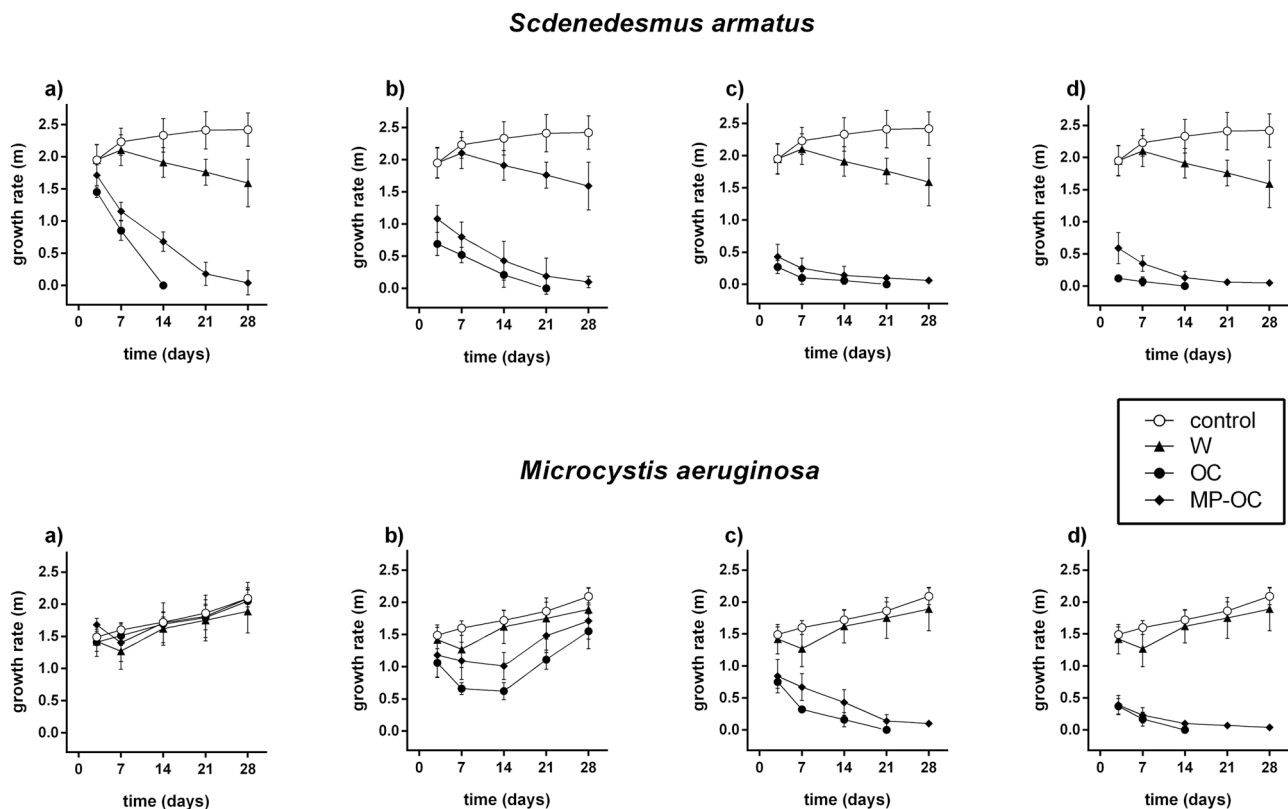


Fig. 1. Cell growth rate exhibited by the green algae *S. armatus* and the cyanobacterium *M. aeruginosa* exposed to white polyethylene microspheres associated with amoxicillin (a), ibuprofen (b), sertraline (c) and simazine (d) during a 28-day exposure period. Points represent the mean  $\pm$  sd of the  $m$  value ( $n = 4$ ) obtained in both the control (○) and microplastic (W, ▲) or organic compound (OC, ●) single assays and MP-OC (◆) complexes.

such that after 28 days, the  $m$  value was negligible. Differences appeared in the exposures to amoxicillin and ibuprofen on the *M. aeruginosa* strain, whose  $m$  values at 28 days of exposure were  $2.05 \pm 0.21$  and  $1.55 \pm 0.27$ , respectively.

In all cases, the cell growth rate exhibited by *S. armatus* populations shows a time-dependent decrease with respect to the control curves, with cell growth being completely inhibited ( $m=0$ ) at 14 days for amoxicillin and simazine exposures or at 21 days for ibuprofen and sertraline exposures.

The cell growth rate exhibited by *M. aeruginosa* populations exposed to the selected OCs showed a time-dependent decrease only in the sertraline and simazine assays, being completely inhibited ( $m=0$ ) at 21 and 14 days, respectively. The effect of ibuprofen on the cell growth ratio induced an initial reduction, obtaining its maximum value at 14 days of exposure ( $m=0.62$ ), which represents an inhibition of 35% with respect to the control value, to subsequently increase until reaching an  $m$  value similar to control assays at 28 days of exposure. There were no significant differences in the cell growth of this cyanobacterial population exposed to amoxicillin with respect to control values.

Comparison between the growth curves of the selected OCs and their corresponding MP-OC complexes showed that the growth response exhibited by the green algae *S. armatus* was variable depending on the OC analysed, and thus, while there were no significant differences in growth rate curves for ibuprofen and sertraline, the analyses on amoxicillin and simazine showed statistically significant differences on the order of  $< 0.0001$  and  $0.0005$ , respectively. However, the growth responses exhibited by the cyanobacteria *M. aeruginosa* showed no significant differences for amoxicillin and ibuprofen and mildly significant differences in the order of  $p = 0.048$  and  $p = 0.012$  for sertraline and simazine, respectively (Table 1).

### 3.2. Photosynthetic activity

Photosynthetic activity analysis exhibited by both phytoplanktonic strains exposed to the selected OCs forming complexes with the polyethylene material (MP) are plotted in Fig. 2.

The results referring to the gross photosynthesis (Pg) parameter showed a significant inhibition for all selected MP-OCs, both on the green algae *S. armatus* and on the cyanobacteria *M. aeruginosa*. Inhibition percentages about  $38.8 \pm 0.66\%$ ,  $43.28 \pm 1.11\%$ ,  $55.69 \pm 2.73\%$  and  $52.2 \pm 3.85\%$  were obtained in *S. armatus* for amoxicillin, ibuprofen, sertraline and simazine, respectively. One-way ANOVA showed that the inhibitory potential of the selected OCs on Pg capacity of *S. armatus* is conformed as amoxicillin  $>$  ibuprofen  $>$  simazine  $>$  sertraline and thus, except for sertraline-related assays, Pg inhibition observed in the assays on *M. aeruginosa* was very significantly lower than that obtained on *S. armatus*. Inhibition percentages of  $15.49 \pm 2.34\%$ ,  $20.70 \pm 1.78\%$ ,  $49.57 \pm 1.85\%$  and  $29.66 \pm 1.34\%$  were obtained for amoxicillin, ibuprofen, sertraline and simazine, respectively. One-way ANOVA showed that the inhibitory potential of the selected OCs on the Pg capacity of *M. aeruginosa* was in the order of amoxicillin  $<$  ibuprofen  $<$  simazine  $<$  sertraline.

Analysis of the results obtained from the breakdown of the

**Table 1**

Statistically significant two-way interactions between the effects of OC and MP-OC exposures on cell growth after 28 days of exposure ( $n = 4$ ).

OCs	<i>Scenedesmus armatus</i>		<i>Microcystis aeruginosa</i>	
	% total variation	P value	% total variation	P value
Amoxicillin	2.85	$< 0.0001^{(****)}$	4.33	$0.5564^{(ns)}$
Ibuprofen	1.57	$0.629^{(ns)}$	2.62	$0.583^{(ns)}$
Sertraline	1.51	$0.847^{(ns)}$	3.13	$0.048^{(*)}$
Simazine	16.04	$0.0005^{(****)}$	1.98	$0.012^{(*)}$

(\*), (\*\*\*) and (\*\*\*\*): significant differences at  $p < 0.05$ ,  $p < 0.001$  and  $p < 0.0001$ , respectively, between both exposures. (ns): not significant

parameters that configure Pg showed very different effects for both phytoplankton strains (Fig. 2). The *Scenedesmus armatus* strain exposed to sertraline and simazine MP complexes did not show significant differences between the percentages of inhibition induced on Pn and R, while amoxicillin and ibuprofen MP complexes induced lower significant differences ( $0.05 < P > 0.01$ ). In *M. aeruginosa* assays, the probability of observing significant differences between Pn and R was also small ( $0.05 < P > 0.01$ ) for all selected MP complexes.

Comparative analysis between both phytoplanktonic strains on the effect induced by the selected MP-OC complexes on Pg indicates that the cyanobacteria *M. aeruginosa* are less sensitive than the green algae *S. armatus*. This lower sensitivity also occurs when Pn is analysed. However, the comparative analysis on R shows differences according to the MP-OC complex involved, and thus, while these are statistically very significant for amoxicillin and ibuprofen, they are not significant when the complexes are constituted by sertraline or simazine (Table 2).

### 3.3. MC synthesis and release from *M. aeruginosa*

Measurement of MC concentration in the culture media showed that, after 28 days of exposure, all MP-OC complexes studied were able to significantly reduce the concentration of MC released with respect to control values for the same cellular concentrations (Fig. 3). While control *M. aeruginosa* cultures released microcystin to  $2.89 \pm 0.03 \mu\text{g L}^{-1}$  to the culture medium, concentrations of  $2.54 \pm 0.02$ ,  $2.47 \pm 0.01$ ,  $2.79 \pm 0.01$  and  $2.67 \pm 0.01 \mu\text{g L}^{-1}$  were released when cyanobacteria were exposed to MP-OC complexes formed with amoxicillin, ibuprofen, sertraline and simazine, respectively.

In a similar order of magnitude to that of MC release for each of the OCs, the intracellular concentration of MC decreased significantly in all cases with respect to the value of  $11.50 \pm 0.11 \text{ fg cell}^{-1}$  obtained in the control assays.

## 4. DISCUSSION

### 4.1. Toxicity Analysis Based on Cell Growth

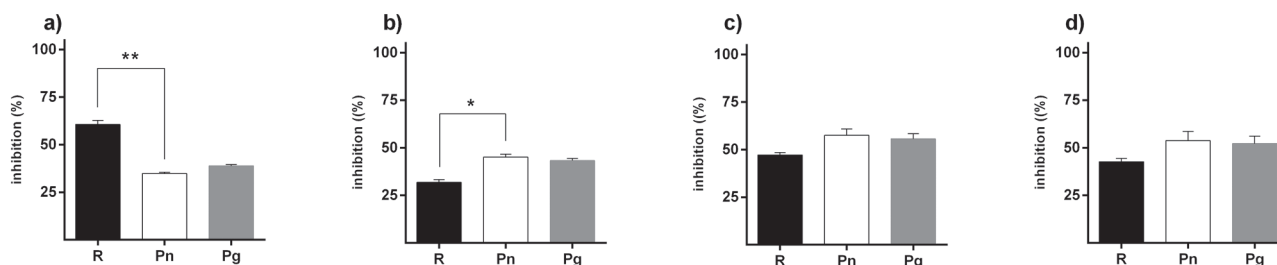
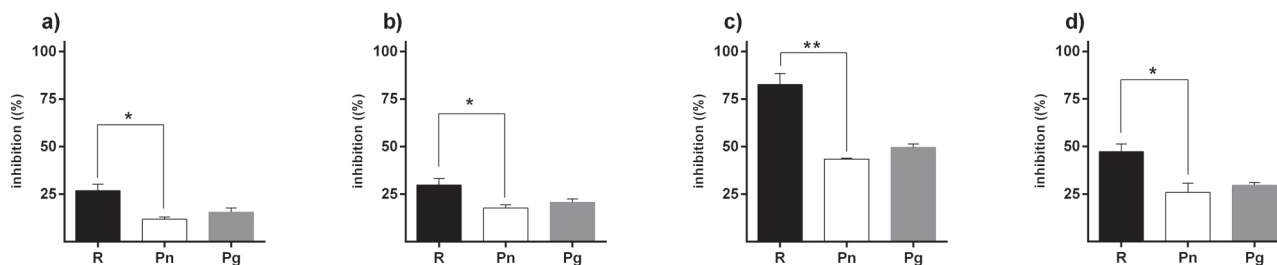
In this study, the green algae *S. armatus* and the toxic *M. aeruginosa* strain had different sensitivities to MP-OCs when the growth or photosynthesis were evaluated. Previous studies conducted in our laboratory showed a scarce toxic impact induced by white polyethylene microspheres on the photosynthetic activity and growth rate of both *S. armatus* and *M. aeruginosa* strains, with green algae being more sensitive than cyanobacteria (Sánchez-Fortín et al., 2021). In addition, our previous studies have demonstrated the capacity of this type of polyethylene microplastic to act as a carrier of contaminants and affect biota (Fajardo et al., 2022). As a consequence of both works, additional research on the bioavailability of these contaminants adsorbed by microplastics and their effects on freshwater ecosystems has been performed.

It is widely recognized that one potential hazard of MP pollution stems from the fact that these plastic particles contain or efficiently adsorb organic chemicals. The ability of MPs to act as a source of organic contaminants to aquatic organisms has been recognized for a long time, as it is the essence of the passive dosing approaches used in ecotoxicology (Claessens et al., 2015). In this context, some recent papers argue that MPs are an important exposure route because the affinity of organic contaminants for them is high.

The results obtained in the present study showed that both green algae and cyanobacteria exhibit different degrees of impairment when exposed to the MP-OC complexes. White microspheres spiked with amoxicillin or simazine induced lower significant effects on the cell growth rate of the *S. armatus* strain than those produced when exposed to the organic contaminant alone; however, this effect was similar when the MP-OC complexes were formed by ibuprofen or sertraline.

It is widely known that in the external environment of aquatic



*Scenedesmus armatus**Microcystis aeruginosa*

**Fig. 2.** Oxygen evolution measured using a Clark-type electrode for 1 mL samples from *S. armatus* and *M. aeruginosa* cultures after 28 days of exposure to white polyethylene microspheres associated with amoxicillin (a), ibuprofen (b), sertraline (c) and simazine (d). Bars represent the mean ± sd (n = 4) of the inhibition effect obtained in gross photosynthesis (gray), net photosynthesis (white) and respiration (black) rate. (\*) and (\*\*): significant differences at p < 0.05 and p < 0.001, respectively, between net photosynthesis (Pn) and respiration (R) values.

**Table 2**

A two-sample t test was performed to compare the photosynthetic activity (Pg, Pn and R) exhibited by *S. armatus* and *M. OC aeruginosa* strains exposed to MP-OCs after 28 days of exposure.

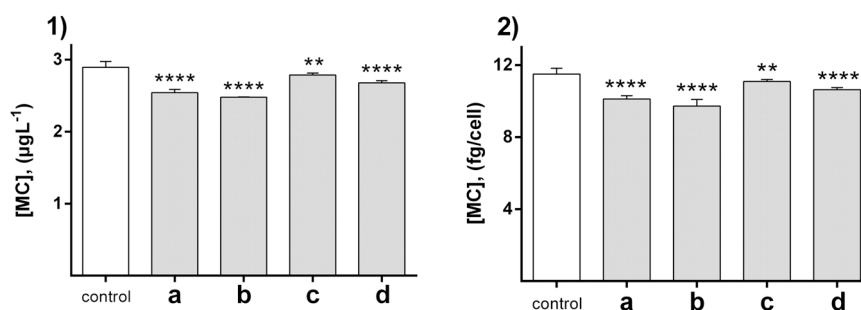
	Amoxicillin	Ibuprofen	Sertraline	Simazine
	Gross Photosynthesis (Pg)			
t	19.14	21.51	3.71	11.07
df	6	6	6	6
P value	< 0.0001(****)	< 0.0001(****)	< 0.0001(****)	0.0099(**)
	Net Photosynthesis (Pn)			
t	34.22	24.71	8.14	8.19
df	6	6	6	6
P value	< 0.0001(****)	< 0.0001(****)	0.0002(***)	0.0002(***)
	Respiration (R)			
t	16.61	1.07	11.91	2.11
df	6	6	6	6
P value	< 0.0001(****)	0.3240(ns)	0.0796(ns)	< 0.0001(****)

(\*\*), (\*\*\*), and (\*\*\*\*): significant differences at p < 0.01, p < 0.001 and p < 0.0001, respectively, between both strains. (ns): not significant.

organisms, organic contaminants can be transferred from microplastics to biota through the aqueous phase or by direct contact exposure to MP-OC complexes (Mayer et al., 2011). Either way, the uptake of organic contaminants would require their prior desorption from the microplastic, a phenomenon largely governed by diffusion and partitioning processes. Considering that these described desorption and transfer processes are valid, it must be considered that desorption processes can significantly vary between the MP and the different organic contaminants considered (Koelmans et al., 2016), a fact that could explain the differences observed in *S. armatus* exposures to selected OCs.

These OC-dependent differences were also evident in exposure assays on the *M. aeruginosa* strain. The absence of a significant effect on the *M. aeruginosa* cell growth rate after exposure to the MP-amoxicillin complex and its subsequent recovery agrees with the results obtained by Liu et al. (2015), which showed that amoxicillin could stimulate algal growth at environmentally relevant concentrations. This effect could be regarded as a hormesis effect of toxicants associated with amoxicillin degradation products, which may promote algal growth.

Ibuprofen is a nonsteroidal anti-inflammatory drug that has been shown to significantly affect cyanobacterial cell growth in the first days



**Fig. 3.** Effects of polyethylene particles spiked with amoxicillin (a), ibuprofen (b), sertraline (c) and simazine (d) on the release (1) and synthesis (2) of microcystin. Bars represent the mean ± sd of 4 experiments (n = 4). (\*\*) and (\*\*\*\*): significant differences at p < 0.005 and p < 0.0001, respectively, compared to the control group.

of exposure (Sanyal et al., 1993; Chowdhury et al., 1996) and may subsequently induce a stimulatory effect on cell growth (Pomati et al., 2004). These data agree with those obtained in our assays and would explain the initial inhibition of cell growth and the consequent increase after 7 days of exposure. Ibuprofen is an unstable chemical degraded in aquatic environments with a  $t_{50} < 1$  day (Richardson and Bowron, 1985). Our evidence therefore suggests that ibuprofen metabolites are nontoxic to the *M. aeruginosa* strain, and they may also have growth-stimulating properties.

The cell growth inhibitory effect exhibited by the *M. aeruginosa* strain exposed to sertraline and simazine agrees with previous results obtained by others. Chalifour et al. (2016) observed that *M. aeruginosa* cell growth was reduced for 22 days following simazine exposure and did not recover until day 30 postexposure. Yang et al. (2019) reported cell growth inhibition percentages above 70% at day 7 of sertraline exposure on both *Chlorella vulgaris* and *M. aeruginosa* strains. The moderate statistical variations obtained between the tests with OCs alone and MP-OCs indicate that the complexes formed did not significantly reduce the adverse effects on cell growth.

#### 4.2. Toxicity analysis based on photosynthetic activity

All selected MP-OCs significantly inhibited photosynthetic activity in both green algae and cyanobacteria. In all cases, the oxygen evolution rate of Pg decreased with respect to the control, and consequently Pn and R rates also decreased. These results agreed with the literature.

Guo et al. (2016) observed that antibiotics significantly inhibited the oxygen evolution rate of gross photosynthesis in chlorophytes and cyanobacteria. Macrolide antibiotics could inhibit the growth of eukaryotic species by interfering with the protein and enzyme synthesis involved in the photosynthesis process (Liu et al., 2011), and the low inhibition of *M. aeruginosa* photosynthetic activity exposed to amoxicillin suggests its involvement in the abovementioned hormesis effect. This low involvement could be related to the responses of two photosynthesis-related genes, *psbA* and *rbcL*, and previous studies have verified the positive correlation between *psbA* and *rbcL* via electron transport in cyanobacteria under various environmental stresses (Qian et al., 2012).

Similar results have been obtained by other authors for the other selected OCs. Wang et al. (2020) suggested that nonsteroidal anti-inflammatory drugs affected chloroplast and mitochondrial functioning, consequently reducing O<sub>2</sub> consumption. Furthermore, the functioning of the photosynthetic electron transport chain from PSI (photosystem I) to PSII, carbon assimilation, and photorespiration were affected. Yang et al. (2019) reported that sertraline reduces photosynthetic efficiency, and Petsas and Vagi (2017) highlighted the effect on photosynthetic activity after exposure to simazine.

#### 4.3. Analysis of microcystin (MC)

Our findings showed that 28 days of exposure to the selected MP-OCs decreased MC release from *M. aeruginosa* at the selected concentration of 15 µM. This effect, in conjunction with the decrease in intracellular MC obtained, suggests that the selected MP-OC complexes can decrease the MC generated by the *M. aeruginosa* strain.

To our knowledge, there have been no studies on this topic for MP-OCs, and the existing studies on the effect induced by individual OC exposures are very heterogeneous. Additionally, previous studies developed in our laboratory showed that this strain exposed to the same type of polyethylene spheres individually exhibited an equal decrease in both intracellular and extracellular MCs (Sánchez-Fortún et al., 2021). Ceballos-Laita et al. (2015) reported that ibuprofen does not have a significant effect on MC production; however, Liu et al. (2016) observed a stimulation in the production and release of MC after 30 days of exposure to amoxicillin. In view of the differences in the results cited in the literature, further research on the interactions between MP-OC

complexes and biota is needed to better understand the responses of aquatic organisms.

## 5. Conclusions

The present study investigated the effect of four MP-OC complexes at a concentration of 15 µM on the maximum exponential growth rate and photosynthetic activity of the freshwater phytoplankton *S. armatus* and *M. aeruginosa*. Although all OC and MP-OC complexes induced time-dependent stress on *S. armatus* cell growth after 28 days of exposure, a significant decrease in the inhibitory effect with respect to individual OC exposure was observed when organisms were exposed to MP-amoxicillin and MP-simazine complexes. In exposures of the *M. aeruginosa* strain, similar effects were observed with sertraline and simazine, both individually and forming MP-OC complexes, but no stressor effect was observed when amoxicillin and ibuprofen were involved, achieving a similar cell growth ratio with respect to the control after 28 days of exposure.

In both phytoplanktonic strains, exposure to the selected MP-OC complexes decreased the photosynthetic activity. After 28 days of exposure, the *M. aeruginosa* strain exhibited a cellular respiration parameter that was significantly more affected than net photosynthesis, while the effect was the opposite in *S. armatus*, except for amoxicillin exposure.

In all exposures to MP-OC complexes, decreased release of microcystin from *M. aeruginosa* into the culture medium, together with its intracellular decrease, demonstrates that MP-OC exposure significantly reduces the ability of the *M. aeruginosa* strain to synthesize microcystin.

In summary, our findings show that although the formation of MP-OCs between polyethylene microplastics and organic compounds generally reduced their adverse effects, the desorption effect together with the wide variability depended on both the type of organic compound and phytoplankton strain involved.

Consequently, further studies are needed to better understand the interactions between microplastics, organic compounds and freshwater biota.

## CRedit authorship contribution statement

**Ana Sánchez-Fortún:** Investigation, Writing – original draft. **Carmen Fajardo:** Investigation, Writing – original draft. **Carmen Martín:** Investigation, Writing – original draft. **Ana Dórs:** Investigation. **Mar Nande:** Investigation. **Gerardo Mengs:** Investigation. **Gonzalo Costa:** Investigation, Writing – original draft. **Margarita Martín:** Investigation, Supervision, Writing – original draft. **Sebastián Sánchez-Fortún:** Conceptualization, Investigation, Supervision, Writing – original draft.

## Declaration of Competing Interest

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

## Data Availability

No data was used for the research described in the article.

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## References

- Almeida, A.C., Gomes, T., Habuda-Stanic, M., Lomba, J.A.B., Romic, Z., Turkalj, J.V., Lillicrap, A., 2019. Characterization of multiple biomarker responses using flow cytometry to improve environmental hazard assessment with the green microalgae *Raphidocelis subcapitata*. *Sci. Total Environ.* 687, 827–838. <https://doi.org/10.1016/j.scitotenv.2019.06.124>.
- Ashton, K., Holmes, L., Turner, A., 2010. Association of metals with plastic production pellets in the marine environment. *Mar. Pollut. Bull.* 60, 2050–2055. <https://doi.org/10.1016/j.marpolbul.2010.07.014>.
- Atugoda, T., Vithanage, M., Wijesekara, H., Bolan, N., Sarmah, A.K., Bank, M.S., You, S., Ok, Y.S., 2021. Interactions between microplastics, pharmaceuticals and personal care products: implications for vector transport. *Environ. Int.* 49, 106367. <https://doi.org/10.1016/j.envint.2020.106367>.
- Bakir, A., Rowland, S.J., Thompson, R.C., 2012. Competitive sorption of persistent organic pollutants onto microplastics in the marine environment. *Mar. Pollut. Bull.* 64, 2782–2789. <https://doi.org/10.1016/j.marpolbul.2012.09.010>.
- Barnes, D.K., Galgani, F., Thompson, R.C., Barlaz, M., 2009. Accumulation and fragmentation of plastic debris in global environments. *Philos. Trans. R. Soc. Lond. B Biol. Sci.* 364, 1985–1998. <https://doi.org/10.1098/rstb.2008.0205>.
- Beiras, R., Verdejo, E., Campoy-López, P., Vidal-Liñán, L., 2021. Aquatic toxicity of chemically defined microplastics can be explained by functional additives. *J. Hazard. Mater.* 406, 124338. <https://doi.org/10.1016/j.jhazmat.2020.124338>.
- Bellasi, A., Binda, G., Pozzi, A., Galafassi, S., Volta, P., Bettinetti, R., 2020. Microplastic contamination in freshwater environments: a review, focusing on interactions with sediments and benthic organisms. *Environments* 7, 30. <https://doi.org/10.3390/environments7040030>.
- Ceballos-Laita, L., Calvo, L., Bes, M.T., Fillat, M.F., Peleato, M.L., 2015. Effects of benzene and several pharmaceuticals on the growth and microcystin production in *Microcystis aeruginosa* PCC 7806. *Limnologia* 34, 237–246. <https://doi.org/10.23818/limn.34.19>.
- Chalifour, A., LeBlanc, A., Sleno, L., Juneau, P., 2016. Sensitivity of *Scenedesmus obliquus* and *Microcystis aeruginosa* to atrazine: effects of acclimation and mixed cultures, and their removal ability. *Ecotoxicology* 25, 1822–1831. <https://doi.org/10.1007/s10646-016-1728-5>.
- Chowdhury, B., Roy, D., Chavan, U., Mukhopadhyay, S., 1996. The anti-inflammatory, antipyretic, analgesic compound ibuprofen also has antibacterial activity against Gram positive bacteria. *Med. Sci. Res.* 24, 801–802.
- Claessens, M., Monteyne, E., Wille, K., Vanhaecke, L., Roose, P., Janssen, C.R., 2015. Passive sampling reversed: coupling passive field sampling with passive lab dosing to assess the ecotoxicity of mixtures present in the marine environment. *Mar. Pollut. Bull.* 93, 9–19. <https://doi.org/10.1016/j.marpolbul.2015.02.028>.
- Dekiff, J.H., Remy, D., Klasmeyer, J., Fries, E., 2014. Occurrence and spatial distribution of microplastics in sediments from Norderney. *Environ. Pollut.* 186, 248–256. <https://doi.org/10.1016/j.envpol.2013.11.019>.
- Fajardo, C., Martín, C., Costa, G., Sánchez-Fortín, S., Rodríguez, C., De Lucas, J.J., Nande, M., Mengs, G., Martín, M., 2022. Assessing the role of polyethylene microplastics as a vector for organic pollutants in soil: ecotoxicological and molecular approaches. *Chemosphere* 288, 132460. <https://doi.org/10.1016/j.chemosphere.2021.132460>.
- Fan, X., Gan, R., Liu, J., Xie, Y., Xu, D., Xiang, Y., Su, J., Teng, Z., Hou, J., 2021. Adsorption and desorption behaviors of antibiotics by tire wear particles and polyethylene microplastics with or without aging processes. *Sci. Total Environ.* 2021 (771), 145451. <https://doi.org/10.1016/j.scitotenv.2021.145451>.
- Guo, J., Selby, K., Boxall, A.B.A., 2016. Effects of antibiotics on the growth and physiology of Chlorophytes, Cyanobacteria, and a diatom. *Arch. Environ. Contam. Toxicol.* 71, 589–602. <https://doi.org/10.1007/s00244-016-0305-5>.
- Hartmann, N.B., Rist, S., Bodin, J., Jensen, L.H.S., Schmidt, S.N., Mayer, P., Meibom, A., Baun, A., 2017. Microplastics as vectors for environmental contaminants: exploring sorption, desorption, and transfer to biota. *Integr. Environ. Assess. Manag.* 13, 488–493. <https://doi.org/10.1002/ieam.1904>.
- Koelmans, A.A., Besseling, E., Wegner, A., Foekema, E.M., 2013. Plastic as a carrier of POPs to aquatic organisms: a model analysis. *Environ. Sci. Technol.* 47, 7812–7820. <https://doi.org/10.1021/es401169n>.
- Koelmans, A.A., Bakir, A., Burton, G.A., Janssen, C.R., 2016. Microplastic as a vector for chemicals in the aquatic environment: critical review and model supported reinterpretation of empirical studies. *Environ. Sci. Technol.* 50, 3315–3326. <https://doi.org/10.1021/acs.est.5b06069>.
- Koelmans, A.A., Nor, N.H.M., Hermens, E., Kooi, M., Mintenig, S.M., De France, J., 2019. Microplastics in freshwaters and drinking water: critical review and assessment of data quality. *Water Res.* 155, 410–422. <https://doi.org/10.1016/j.watres.2019.02.054>.
- Li, J., Zhang, K., Zhang, H., 2018. Adsorption of antibiotics on microplastics. *Environ. Pollut.* 237, 460–467. <https://doi.org/10.1016/j.envpol.2018.02.050>.
- Liu, B.Y., Nie, X.P., Liu, W.Q., Snoeijs, P., Guan, C., Tsui, M.T.K., 2011. Toxic effects of erythromycin, ciprofloxacin and sulfamethoxazole on photosynthetic apparatus in *Selenastrum capricornutum*. *Ecotoxicol. Environ. Safe* 74, 1027–1035. <https://doi.org/10.1016/j.ecoenv.2011.01.022>.
- Liu, Y., Chen, X., Zhang, J., Gao, B., 2015. Hormesis effects of amoxicillin on growth and cellular biosynthesis of *Microcystis aeruginosa* at different nitrogen levels. *Microb. Ecol.* 69, 608–617. <https://doi.org/10.1007/s00248-014-0528-9>.
- Liu, Y., Chen, S., Zhang, J., Gao, B., 2016. Growth, microcystin-production and proteomic responses of *Microcystis aeruginosa* under long-term exposure to amoxicillin. *Water Res.* 93, 141–152. <https://doi.org/10.1016/j.watres.2016.01.060>.
- Llorca, M., Ábalos, M., Vega-Herrera, A., Adrados, M.A., Abad, E., Farré, M., 2020. Adsorption and desorption behaviour of polychlorinated biphenyls onto microplastics' surfaces in water/sediment systems. *Toxics* 8, 59. <https://doi.org/10.3390/toxics8030059>.
- Martín, C., Fajardo, C., Costa, G., Sánchez-Fortín, S., San Andrés, M.D., González, F., Nande, M., Mengs, G., Martín, M., 2021. Bioassays to assess the ecotoxicological impact of polyethylene microplastics and two organic pollutants, simazine and ibuprofen. *Chemosphere* 274, 129704. <https://doi.org/10.1016/j.chemosphere.2021.129704>.
- Mato, Y., Isobe, T., Takada, H., Kanehiro, H., Ohtake, C., Kaminuma, T., 2001. Plastic resin pellets as a transport medium for toxic chemicals in the marine environment. *Environ. Sci. Technol.* 35, 318–324. <https://doi.org/10.1021/es0010498>.
- Mayer, P., Olsen, J.L., Gouliarmou, V., Hasinger, M., Kendler, R., Loibner, A.P., 2011. A contaminant trap as a tool for isolating and measuring the desorption resistant fraction of soil pollutants. *Environ. Sci. Technol.* 45, 2932–2937. <https://doi.org/10.1021/es1033124>.
- Petsas, A.S., Vagi, M.C., 2017. Effects on the photosynthetic activity of algae after exposure to various organic and inorganic pollutants: review. In: Jacob-Lopes, E., Zepka, L.Q. (Eds.), *QueirozMI. Chlorophyll*, London, UK. <https://doi.org/10.5772/67991>.
- Pomati, F., Netting, A.G., Calamari, D., Neilan BA, B.A., 2004. Effects of erythromycin, tetracycline and ibuprofen on the growth of *Synechocystis* sp. and *Lemma minor*. *Aquat. Toxicol.* 67, 387–396. <https://doi.org/10.1016/j.aquatox.2004.02.001>.
- Qian, H.F., Pan, X.J., Chen, J., Zhou, D.M., Chen, Z.G., Zhang, L., Fu, Z.W., 2012. Analyses of gene expression and physiological changes in *Microcystis aeruginosa* reveal the phytotoxicities of three environmental pollutants. *Ecotoxicology* 21, 847–859. <https://doi.org/10.1007/s10646-011-0845-4>.
- Richardson, M.L., Bowron, J.M., 1985. The fate of pharmaceuticals in the aquatic environment. *J. Pharm. Pharm.* 37, 1–12. <https://doi.org/10.1111/j.2042-7158.1985.tb04922.x>.
- Sánchez-Fortín, A., Fajardo, C., Martín, C., D'ors, A., Nande, M., Mengs, G., Costa, G., Martín, M., Sánchez-Fortín, S., 2021. Effects of polyethylene-type microplastics on the growth and primary production of the freshwater phytoplankton species *Scenedesmus armatus* and *Microcystis aeruginosa*. *Environ. Exp. Bot.* 88, 104510. <https://doi.org/10.1016/j.envexpbot.2021.104510>.
- Sanyal, A.K., Roy, D., Chowdhury, B., Banerjee, A.B., 1993. Ibuprofen, a unique anti-inflammatory compound with antifungal activity against dermatophytes. *Lett. Appl. Microbiol.* 17, 109–111. <https://doi.org/10.1111/j.1472-765X.1993.tb01436.x>.
- Teuten, E.L., Saquing, J.M., Knappe, D.R.U., Barlaz, M.A., Jonsson, S., Björn, A., Rowland, S.J., Thompson, R.C., Galloway, T.S., Yamashita, R., Ochi, D., Watanuki, Y., Moore, C., Viet, P.H., Tana, T.S., Prudente, M., Boonyatumanond, R., Zakaria, M.P., Akkavong, K., Ogata, Y., Hirai, H., Iwasa, S., Mizukawa, K., Hagino, Y., Imamura, A., Saha, M., Takada, H., 2009. Transport and release of chemicals from plastics to the environment and to wildlife. *Philos. Trans. R. Soc. B Biol. Sci.* 364, 2027–2045. <https://doi.org/10.1098/rstb.2008.0284>.
- Wagner, M., Lambert, S., 2017. Freshwater Microplastics. *Emerging Environmental Contaminants?* In: Barceló, D., Kostianoy, A. (Eds.), *The Handbook of Environmental Chemistry*, 58 Springer Nature, Switzerland AG, p. 303. <https://doi.org/10.1007/978-3-319-61615-5>.
- Wang, H., Jin, M., Mao, W., Chen, C., Fu, L., Li, Z., Du, S., Liu, H., 2020. Photosynthetic toxicity of non-steroidal anti-inflammatory drugs (NSAIDs) on green algae *Scenedesmus obliquus*. In: *Sci. Total Environ.* 707, 136176. <https://doi.org/10.1016/j.scitotenv.2019.136176>.
- Wright, S.L., Thompson, R.C., Galloway, T.S., 2013. The physical impacts of microplastics on marine organisms: a review. *Environ. Pollut.* 178, 483–492. <https://doi.org/10.1016/j.envpol.2013.02.031>.
- Yang, Z., Lu, T., Zhu, Y., Zhang, Q., Zhou, Z., Pan, X., Qian, H., 2019. Aquatic ecotoxicity of an antidepressant, sertraline hydrochloride, on microbial communities. *Sci. Total Environ.* 654, 129–134. <https://doi.org/10.1016/j.scitotenv.2018.11.164>.