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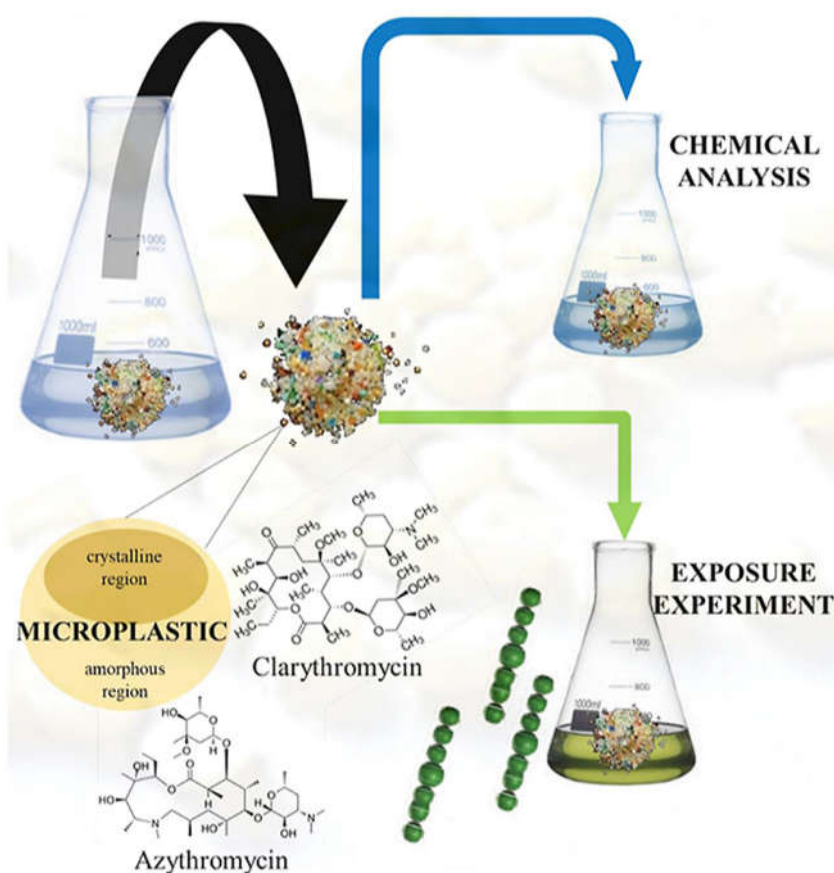
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Microplastics as vectors of the antibiotics azithromycin and clarithromycin Effects towards freshwater microalgae

Miguel González-Pleiter^{1,2,*}, Alicia Pedrouzo-Rodríguez¹, Irene Verdú¹, Francisco Leganés¹, Eduardo Marco¹, Roberto Rosal², Francisca Fernández-Piñas¹

¹ Departamento de Biología, Facultad de Ciencias, Universidad Autónoma de Madrid, Cantoblanco, E-28049 Madrid, Spain

² Departamento de Ingeniería Química, Universidad de Alcalá, Alcalá de Henares, E-28871 Madrid, Spain

* Corresponding author: mig.gonzalez@uam.es

Abstract

Water pollution due to microplastics (MPs) is recognized as a major anthropogenic impact. Once MPs reach the ecosystems, they are exposed to a variety of other pollutants, which can be sorbed on them, transported and eventually desorbed. In this work, we tested the hypothesis that MPs can behave as conveyors for delivering chemicals toxic to aquatic microorganisms by investigating the vector role of MPs of polyethylene terephthalate (PET), polylactic acid (PLA), polyoxymethylene (POM) and polystyrene (PS) to the macrolide antibiotics azithromycin (AZI) and clarithromycin (CLA). AZI and CLA were chosen, as they are included in the Watch List for EU monitoring concerning water policy by Decision (EU) 2018/840. MPs were loaded in contact with 500 µg/L of AZI or 1000 µg/L of CLA. Results showed that both antibiotics were sorbed on all tested MPs. The more hydrophobic AZI was sorbed in higher proportion than CLA. Both antibiotics were desorbed from MPs upon contact with water with percentages between 14.6 ± 2.6 % for AZI and 1.9 ± 1.4 % for CLA of the concentrations to which the MPs were initially exposed. Virgin MPs were not toxic to the cyanobacterium *Anabaena* sp. PCC7120. However, antibiotic-loaded MPs significantly inhibited the growth and chlorophyll content of the cyanobacterium. Most of the sorbed antibiotics became released upon contact with cyanobacterial cultures, which was the cause for the observed toxicity. Therefore, MPs can play a role as vectors of antibiotics in freshwaters systems affecting the basic trophic level of photosynthetic microorganisms.

Keywords: Antibiotics; Cyanobacteria; Macrolides; Microplastics; Toxicity Vectors

1. Introduction

Plastic pollution in ecosystems has attracted increasing public attention. The plastics industry produces around 359 million tonnes of plastic per year (Plastics Europe, 2019), which show the magnitude of the current environmental challenge that arises when an enormous amount of them ends up as debris in the ecosystems every year. In order to address the issue, some states are implementing policies to reduce and prevent the occurrence of plastic in the environment (Directive 2019/904, EU). In fact, some of these policies (such as in the case of the European Union including Norway and Switzerland) have already managed to reduce the production of plastics by almost 2.6 million tonnes last year (Plastics Europe, 2019). Once in the environment, plastics degrade due to different biotic processes (da Costa et al., 2016; Mattsson et al., 2018) and/or abiotic processes such as photo-oxidation, mechanical abrasion, and thermal

oxidation (Alimi et al., 2018). During degradation processes, plastics are converted into smaller particles (Thompson et al., 2004). According to the NOAA definition, the plastic fragments less than 5 mm long are classified as microplastics (Gago et al., 2016).

Microplastics (MPs) have been detected in essentially all compartments. They have been found in surface and subsurface waters in the oceans, shorelines, estuaries, continental waters, soils, sediments, the atmosphere, and Arctic and Antarctic regions (Browne et al., 2011; Law and Thompson, 2014; Lusher et al., 2015; da Costa et al., 2016; Bergmann et al., 2017; Vaughan et al., 2017; Gray et al., 2018; Peeken et al., 2018; Xiong et al., 2018; Allen et al., 2019; Zhang et al., 2019; González-Pleiter et al., 2020). The life cycle of MPs depends on the interconnection of all environmental compartments. For example, it has been estimated that most MP pollution in marine

ecosystems derives from land sources. Consequently, soils and freshwater networks (including probably groundwater and glaciers) play a key role in their fate (Li et al., 2016). In this regard, an important pathway for the entry of MPs in freshwater are wastewater treatment plants (WWTPs) (Lambert and Wagner, 2018; Gatidou et al., 2019; Lv et al., 2019; Sun et al., 2019; Edo et al., 2020). Besides, wastewater sludge used as fertilizer is one of the main routes of entry of MPs into soil environments (Weithmann et al., 2018).

Over the last decade there has been a growing concern on the potential role of MPs as carriers of different kinds of pollutants co-occurring with the MPs in wastewater, oceans, urban runoff, and landfill leachates (Bakir et al., 2014; Ma et al., 2016; Zhan et al., 2016; Talvitie et al., 2017). In this context, it has been pointed out that MPs could sorb pollutants from the environment, and introduce them to organisms (Rochman et al., 2013; Velzeboer et al., 2014; Turner and Holmes, 2015; Guo et al., 2019a; Guo and Wang, 2019b). By sorbing pollutants, MPs may affect their distribution and transport in different environments (Bakir et al., 2014; Hüffer et al., 2019). The sorption behavior of MPs is influenced by their physicochemical properties as well as those of the pollutants and the external medium (Hüffer and Hofmann, 2016; Guo and Wang, 2019a, b). Early studies indicated MPs are probably of limited importance as vectors of chemicals in marine environments because, due to their relatively low concentration, organic chemicals would partition between air and water rather than in plastic phase (Gouin et al., 2011). More recently, it has been recognized that higher partitioning to the plastic fraction could take place in other compartments determining the bioavailability of chemicals and the effects of their combined exposure with MPs (Tourinho et al., 2019). When aquatic organisms get in touch with pollutant-sorbed MPs, they may desorb and translocate, eventually accumulating in tissues (Paul-Pont et al., 2016; Qu et al., 2018). Therefore, the sorption and desorption of pollutants by MPs can play a key role in their environmental fate.

Like MPs, antibiotics are also a cause for global concern. It has been estimated that the total antibiotic use amounted to 92,700 tonnes in 2013 in China and that about half of these antibiotics were released into the environment coming from human and animal consumption (Zhang et al., 2015). Several works have identified urban

wastewater as the main source of antibiotics for the planet's ecosystems (Wang et al., 2019). Antibiotics are only partially removed in conventional WWTPs and, therefore, residual antibiotics are continuously released into the environment reaching surface waters, groundwater or sediments (Kümmerer, 2009a, b). High concentrations of antibiotics have been reported in wastewater, surface water, sediments, groundwater, soil, and drinking water (Wang et al., 2019). They can exert inhibitory effects on microorganisms decreasing the efficiency of the WWTPs (He et al., 2015) and, most importantly, they may trigger the increase and spread of antibiotic resistance bacteria and cognate antibiotic resistance genes (Neu, 1992). Among the different classes of antibiotics, macrolides, whose main representative are azithromycin (AZI), clarithromycin (CLA), and erythromycin have been found in relatively large amounts (reaching tens of µg/L) in wastewater (Kulkarni et al., 2017; Lin et al., 2018; Senta et al., 2019). In fact, they have been included in the EU Watch List for EU monitoring concerning water policy by Decision (EU) 2018/840. Up to our knowledge, the biological effect of macrolide antibiotics sorbed on plastics has not been assayed before. In this work, we tested the hypothesis that polystyrene (PS), polyethylene terephthalate (PET), polylactic acid (PLA) and polyoxymethylene (POM) MPs may behave as conveyors for delivering the macrolide antibiotics AZI and CLA to the freshwater cyanobacterium *Anabaena* sp. PCC 7120, a primary producer which is a key element for the health of aquatic ecosystems.

2. Materials and Methods

2.1. Materials

The chosen MPs as well as the methods to measure their main characteristic are detailed in Supplementary Section 1. PET and PS MPs were chosen because they are some of the MPs more commonly found in the environment. In the case of PLA, it is a biodegradable biopolymer that is considered the best candidate to replace current fossil-derived plastics (The future of plastic, 2018). Regarding POM, it is the most widely used co-polymer, besides, this is the only co-polymer frequently used in rubbery state under environmental conditions, which plays a key role in the sorption and desorption of chemical compounds by plastics (Teuten et al., 2007; Wang et al., 2015). AZI (> 98% purity, CAS 117772-70-0) and CLA (> 98% purity, CAS 81103-11-9) were

purchased from TCI Chemicals Europe. Stocks of AZI and CLA were prepared in methanol (AZI: 10 g/L; CLA: 1 g/L), sterilized by filtration through a 0.22 μm filter, and stored at $-20\text{ }^{\circ}\text{C}$ until use. The dilutions of antibiotics were freshly performed before each experiment. The final concentration of methanol in the assay media did not result in any significant effect on the cyanobacterial growth or chlorophyll *a* content. Macrolide antibiotics have been reported as very stable over time, even up to 72 h (Erah et al., 1997; Saita et al., 2018). These antibiotics were chosen in this study to respond to the growing concern on their risk to the aquatic environment as recently warned by the European Union (Decision (EU) 2018/840 of June 5, 2018).

2.2. Sorption and desorption of antibiotics and analyses

A flow diagram of the experimental design is depicted in Supplementary Figure S1. Each type of virgin MP beads (1 g) was incubated individually in Milli-Q water (20 mL) with AZI (initial concentration: 500 $\mu\text{g/L}$) or CLA (initial concentration: 1000 $\mu\text{g/L}$) using 50 mL Erlenmeyer flasks under constant shaking (135 rpm) at $28\text{ }^{\circ}\text{C}$ and dark conditions for 4 h. Both initial concentrations used were in the upper range of macrolide concentrations found in WWTPs (Kulkarni et al., 2017). The incubation time (4 h) is the average hydraulic retention time of the activated sludge process in Europe (Bolmstedt, 2004), thus, it is a realistic time in which MPs and antibiotics could be in contact -under constant shaking, $28\text{ }^{\circ}\text{C}$ (Tejaswini et al., 2019) and dark conditions-in an activated sludge wastewater treatment process, which is the most widely used treatment system (Noguera-Oviedo et al., 2016). It should be noted that wastewater treatment plants are the major pathway for the entry of microplastics and antibiotics into freshwater (Lambert and Wagner, 2018; Gatidou et al., 2019; Lv et al., 2019; Sun et al., 2019; Edo et al., 2020).

The sorption analyses followed the procedures mentioned above using different initial concentrations of antibiotics (CLA: 100 $\mu\text{g/L}$, 500 $\mu\text{g/L}$, 1000 $\mu\text{g/L}$, and 10,000 $\mu\text{g/L}$; AZI: 100 $\mu\text{g/L}$, 500 $\mu\text{g/L}$, 1000 $\mu\text{g/L}$, 10,000 $\mu\text{g/L}$). Sorption kinetics (0 h, 2 h, 4 h, and 6 h) was studied using 500 $\mu\text{g/L}$ of AZI or 1000 $\mu\text{g/L}$ of CLA.

To quantify the amount of CLA and AZI sorbed by MP beads after incubation, CLA and AZI loaded MP beads were centrifuged for 30 s at 1000 rpm, to remove all the remaining antibiotic solution.

After that, beads were allowed to dry at room temperature in a laminar flow hood. To measure the sorption of antibiotics from MP beads, 1 g of the antibiotic-loaded dried beads was incubated in methanol for 24 h to recover all sorbed antibiotics. Methanol extracts were analysed to determine the concentration of sorbed antibiotics. To measure the desorption of antibiotics from MP beads, 1 g of the loaded dried beads was immersed in 20 mL sterilized Milli-Q water in 50 mL Erlenmeyer flasks and incubated under illumination ca. 65 $\mu\text{mol photons m}^{-2} \text{ s}^{-1}$, on a rotary shaker (135 rpm) at $28 \pm 2\text{ }^{\circ}\text{C}$. After 72 h, MP beads were removed, and the supernatants were analysed.

The quantification of AZI and CLA was performed by liquid chromatography-mass spectrometry (LC-MS) using a TSQ QUANTUM LC-MS Quantum triple-quadrupole mass spectrometer with ESI ion source (positive polarity, 3000 V spray voltage, capillary temperature $350\text{ }^{\circ}\text{C}$). The chromatographic separation was carried out in an ACE EXCEL 3-C18-PFP 150 mm \times 3 mm i. d., 3 μm particle size column operating in isocratic flow of 0.3 mL/min with acetonitrile/methanol/water (40:40:10) and 20 mM ammonium acetate. The analytical method provided linear responses in the 3–500 $\mu\text{g/L}$ range with 15% RSD for both AZI and CLA.

2.3. Exposure experiments

The freshwater cyanobacterium *Anabaena* sp. PCC 7120 was grown in 100 mL of AA/8 + N buffered with HEPES 20 mM, pH 7.5 (Allen and Arnon, 1955) in 250 mL Erlenmeyer flasks under continuous shaking (135 rpm) and 65 $\mu\text{mol photons m}^{-2} \text{ s}^{-1}$ illumination at $28 \pm 2\text{ }^{\circ}\text{C}$. Stock cyanobacterial cultures were kept in exponential growth by adding fresh culture medium at regular intervals. For exposure experiments, stock cyanobacterial cultures were diluted in AA/8 + N to OD_{750nm} 0.2 (dry weight = 75.3 mg/L and chlorophyll *a* content = 1.2 mg/L) and exposed during 72 h to the following treatments: (1) exposure to each type of virgin MP applied individually (1 g in 20 mL), (2) to AZI and CLA at the initial concentrations (AZI: 500 $\mu\text{g/L}$; CLA: 1000 $\mu\text{g/L}$). (3) to AZI or CLA-loaded MP (1 g of the dried antibiotic-loaded MP in 20 mL) and (4) to AZI and CLA applied individually at the concentrations obtained in MP desorption experiments as measured by LC-MS (AZI in the range 65.2–92.4 $\mu\text{g/L}$ range and CLA in the 5.4–39.4 $\mu\text{g/L}$ range; the full set of concentrations is indicated below). The measured endpoints were

cyanobacterial growth in terms of dry weight and chlorophyll *a* content after 72 h of exposure. Chlorophyll *a* content was estimated according to the spectrophotometric method of Marker (1972). The information about the statistical analysis is detailed in Supplementary Section 2.

3. Results

3.1. Concentration of antibiotics desorbed from previously loaded MPs

The results indicated that both antibiotics were significantly sorbed on all MPs. The concentrations of both antibiotics sorbed on all MPs increased with their concentration in solution and reached maximum values for PS (Fig. S2 A and B, in Supplementary material). It should be noted that when AZI at 500 µg/L or CLA at 1000 µg/L was used for the sorption experiments, the amount of sorbed AZI and CLA was similar after 4 and 6 h of incubation (Fig. S2 C and D). Therefore, the sorption reached after 4 h approached equilibrium values. The sorbed concentration was generally higher for AZI than for CLA. AZI sorption concentrations ranged between 0.00178 ± 0.00078 and 0.00270 ± 0.00018 mg/g without significant differences among tested MPs (Fig. 1 A). In the case of CLA, the sorbed concentration is shown in Fig. 1B. PS (0.00487 ± 0.00018 mg/g) significantly ($p < 0.05$) sorbed more CLA than PET (0.00298 ± 0.00027 mg/g), PLA (0.00081 ± 0.00024 mg/g) and POM (0.00094 ± 0.00019 mg/g). In this sense, PET slightly sorbed more

CLA than PLA and POM. See Table S1, supplementary material, for the whole set of data.

3.2. Concentrations of antibiotics desorbed from previously loaded MPs

The amount of AZI and CLA desorbed from all microplastics upon 72 h in Milli-Q water is shown in Fig. 2. The concentration of AZI desorbed from MPs ranged between 65.2 ± 1.6 µg/L (PLA) and 92.4 ± 9.2 µg/L (PS), with the following order: PS > POM ≥ PET ≥ PLA (Fig. 2A). The highest concentration of AZI was desorbed from PS while no statistical differences were found in the desorption of AZI from POM, PET and PLA. The concentration of CLA desorbed from MPs ranged between 5.4 ± 5.0 µg/L (PLA) and 39.4 ± 11.3 µg/L (PS) in the following order: PS > POM ≥ PET ≥ PLA (Fig. 2B). As in the case of AZI, the highest concentration of CLA was that desorbed from PS. This concentration was significantly different to the concentrations desorbed from POM, PET and PLA, which did not show any significant differences among them. The percentage of AZI desorbed from MPs after 72 h represented between 54 and 73% of the amount sorbed (Table S1). In the case of CLA, these percentage ranged from 11% to 34% (Table S1).

It should be noted that the percentage of antibiotics desorbed from MP represented $14.6 \pm 2.6\%$ for AZI and $1.9 \pm 1.4\%$ for CLA of the concentration to which the MPs were initially exposed (Table S1).

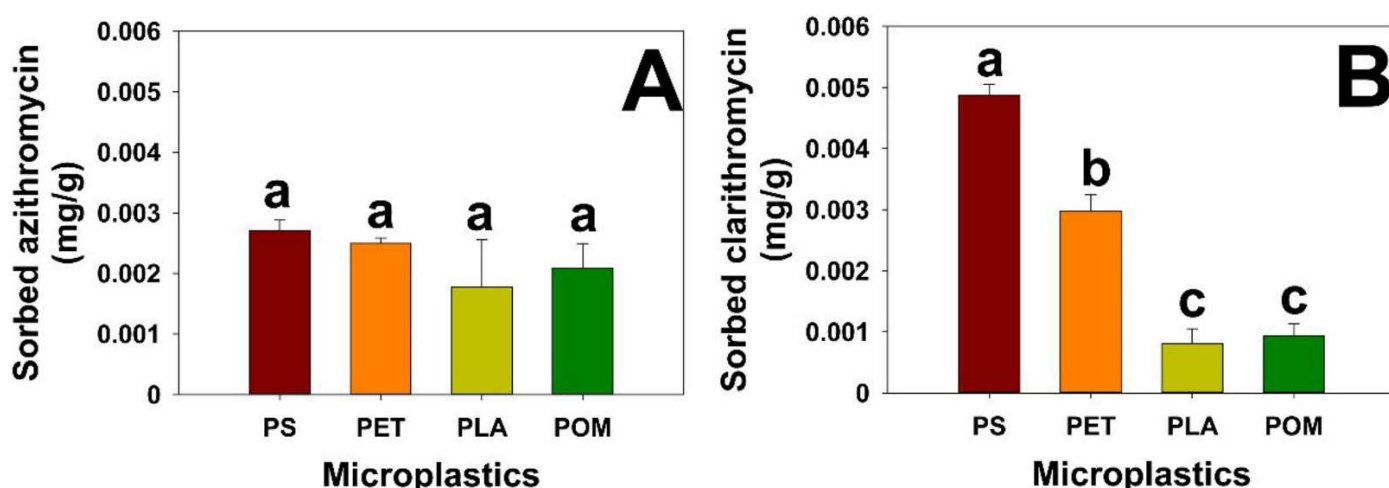


Figure 1. Concentrations (mg/g) of AZI (A) and CLA (B) sorbed on each type of MP (PS, PET, PLA and POM) after 4 h of incubation. Data are expressed as mean values \pm SD. Treatments with different letters are significantly different (Tukey test; $p < 0.05$).

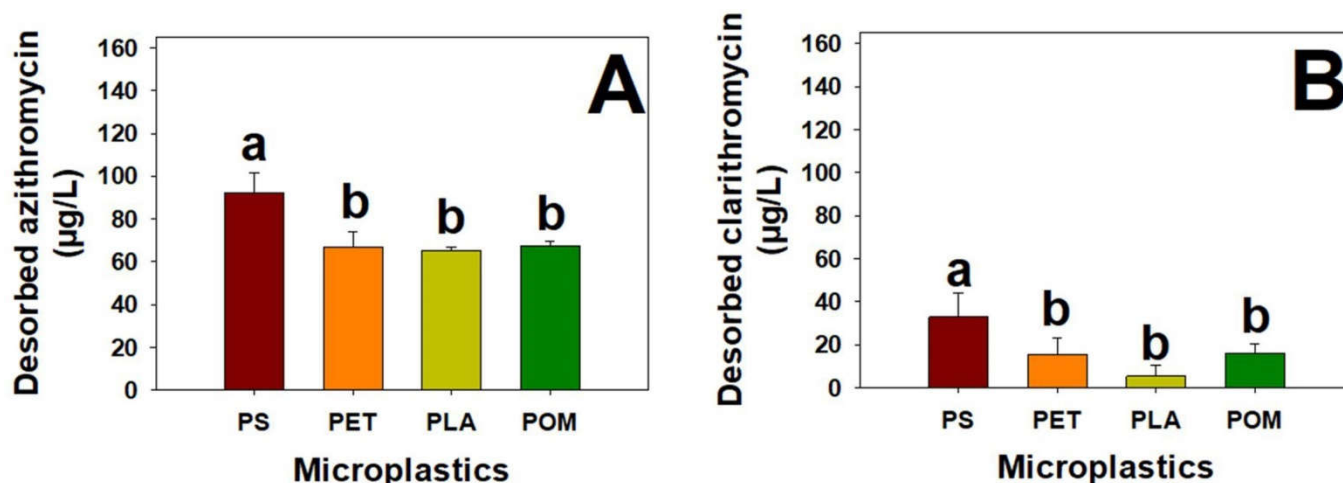


Figure 2. Concentrations (µg/L) of AZI (A) and CLA (B) desorbed from each MP (PS, PET, PLA and POM) after 72 h in distilled water. Data are expressed as mean values \pm SD. Treatments with different letters are significantly different (Tukey test; $p < 0.05$).

3.3. The effects of the different treatments on the cyanobacterium

Fig. 3 shows the effects of virgin MPs, AZI or CLA at the initial concentrations (AZI: 500 µg/L; CLA: 1000 µg/L), AZI or CLA-loaded MPs and AZI or CLA applied individually at the corresponding desorbed concentrations from each MP on cyanobacterial growth and chlorophyll *a* content. All virgin MPs (PS, PET, PLA and POM) (Fig. 3A and B) did not alter any of the two endpoints, meaning that the virgin MPs, under the tested conditions, did not have any negative effect on the cyanobacterium. Both antibiotics at the initial concentrations (AZI 500 µg/L and CLA 1000 µg/L) induced a significant ($p < 0.05$) cyanobacterial growth inhibition with respect to unexposed control (AZI: $63.0 \pm 4.6\%$ and CLA: $45.7 \pm 5.4\%$) and a significant ($p < 0.05$) decrease in chlorophyll *a* content (AZI: $25.4 \pm 5.0\%$; CLA: $19.1 \pm 6.0\%$) with respect to the unexposed control (Fig. 3C, D, E, F).

The treatments with antibiotic-loaded MPs, denoted as MP + AZI (Fig. 3C and D) and MP + CLA (Fig. 3E and F) had, in general, significant ($p < 0.05$) effects on both cyanobacterial growth and chlorophyll *a* content. PLA + AZI ($86.4 \pm 6.1\%$ of growth with respect to the unexposed control), PET + AZI ($85.8 \pm 6.6\%$), POM + AZI ($84.1 \pm 6.6\%$) and PS + AZI ($80.6 \pm 3.4\%$) significantly ($p < 0.05$) decreased cyanobacterial growth. Similarly, PLA + CLA ($85.3 \pm 5.1\%$), POM + CLA ($85.9 \pm 4.4\%$) and PS + CLA ($86.9 \pm 5.4\%$), significantly ($p < 0.05$) decreased cyanobacterial growth, although PET-CLA did not result in any differences with respect to

controls (Fig. 3C, E). Regarding chlorophyll *a* content, PET + AZI ($75.1 \pm 7.1\%$), PLA + AZI ($66.7 \pm 5.4\%$), POM + AZI ($75.3 \pm 6.2\%$), PS + AZI ($69.4 \pm 5.5\%$), PLA + CLA ($69.2 \pm 9.5\%$), POM + CLA ($81.1 \pm 6.1\%$), PET + CLA ($77.8 \pm 6.3\%$) and PS + CLA ($78.1 \pm 8.2\%$) induced a significant ($p < 0.05$) decrease in cyanobacterial chlorophyll *a* content (Fig. 3D, F).

No significant differences ($p < 0.05$) were found between the effects of AZI and CLA loaded MPs with the effects of AZI (Fig. 3C, D) and CLA (Fig. 3 E, F) applied individually at the corresponding desorbed concentrations from each MP (Table S2), implying that the toxicity observed in the cyanobacterium seems to be due to the antibiotic desorbed from MP.

4. Discussion

The main physicochemical properties of MPs that affect their sorption capacity are the plasticity of the amorphous phase, the degree of crystallinity, polarity and surface area (Guo et al., 2012; Wang et al., 2015; Brennecke et al., 2016). Besides, the sorption behaviour of microplastics is influenced by the properties of pollutants (sorbates) and the characteristics of sorption environments (Guo and Wang, 2019b). Previous studies have indicated that the octanol-water partition coefficients ($\log K_{ow}$) of sorbates are essential in determining their sorption extents on MPs (Huffer and Hofmann, 2016; O'Connor et al., 2016; Wu et al., 2016). Our results indicated that MPs could sorb higher amount of AZI than CLA. The difference can be explained in terms of their octanol-water partition coefficients. Although both antibiotics are rather

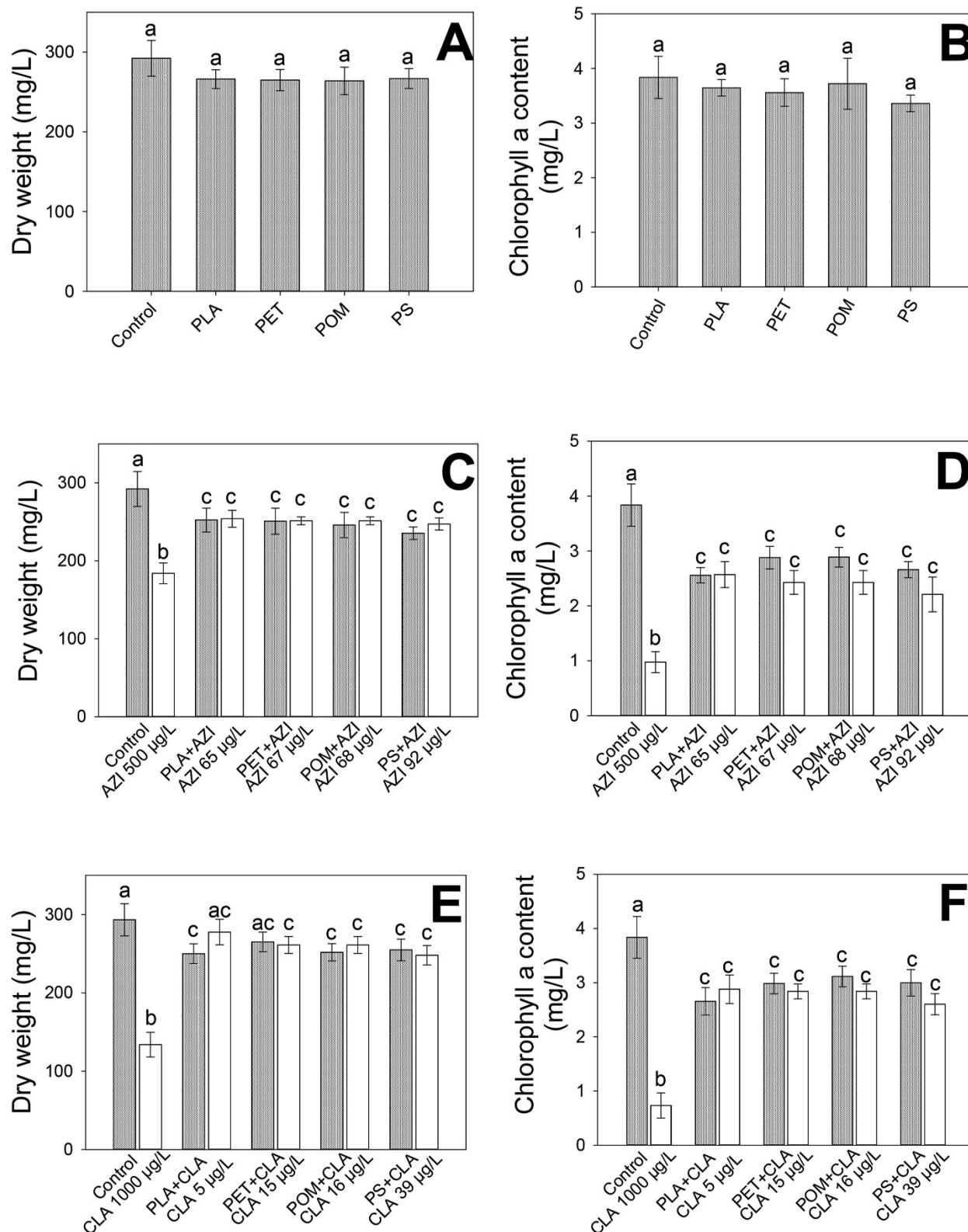


Figure 3. Effects on cyanobacterial growth (dry weight; left column) and chlorophyll a content (right column) in *Anabaena* sp. PCC7120 after 72 h of exposure to virgin MPs (A, B); AZI (C,D) or CLA (E,F) at the initial concentrations (AZI: 500 μ g/L; CLA: 1000 μ g/L), AZI (C, D) and CLA (E, F) loaded MPs and AZI (C, D) and CLA (E, F) applied individually at the corresponding desorbed concentrations from each MP. Data are expressed as mean values \pm SD. Treatments with the same superscript letter were not significantly different (Tukey's Method; $p < 0.05$) within each experiment.

hydrophobic compounds, AZI ($\log K_{ow}$ 4.02) is more hydrophobic than CLA ($\log K_{ow}$ 3.16), which could cause a higher interaction with hydrophobic polymers. As shown in Table S3, all

polymers tested in this work were hydrophobic as revealed by the values of their free energy of interaction ΔG_{sws} , which is negative for hydrophobic surfaces and positive when they are

hydrophilic. The degree of hydrophobicity is given by the negative value of ΔG_{SWS} . Li et al. (2018) also found a correlation between $\log K_{\text{ow}}$ values and the sorption of a series of antibiotics on polypropylene (PP), PS, polyethylene (PE) and polyvinyl chloride (PVC); their results showed that hydrophobic antibiotics (with higher $\log K_{\text{ow}}$ values) had higher affinity to PP, PS, PE, and PVC. However, that correlation did not apply to PA, which had the strongest sorption capacity for antibiotics, suggesting that hydrogen bonding and not hydrophobic interaction was the main driver for their sorption on PA. In this context, Xu et al. (2018) found that the sorption of tetracycline, a polar and ionizable antibiotic, on three types of microplastics (PE, PP and PS) could not be explained just only by hydrophobic interactions but also other interactions (e.g. electrostatic interactions) played important roles in the sorption process. All the MPs tested in this work (PS, POM, PET and PLA), showed higher sorption capacity for AZI than CLA as revealed by the amount of antibiotic sorbed. Consistent with the dominant role played by hydrophobic interactions, AZI was sorbed in higher amount on the more hydrophobic polymers (PS). Polar interactions might play a secondary role in case of the less polar polymers (PLA, PET and POM), although the differences were not high. Guo et al. (2018) found that the sorption of the antibiotic tylosin on MPs followed the order of PVC > PS > PP > PE, which might be related to the surface properties of the MPs. That same group (Guo et al., 2019b) showed that the sorption of sulfamethazine (SMT) on six types of MPs followed the order PA-SMT > PET-SMT > PE-SMT > PVC-SMT > PS-SMT > PP-SMT. Some authors have pointed out some of the physical properties of MPs that might explain the differences in their sorption capacities for different types of pollutants. It has been suggested that semicrystalline polymers may convey pollutants due to their dissolution into their viscous glassy phase (Guo et al., 2012). The reason is that small molecules diffuse into the free-volume holes formed by thermal fluctuations in the amorphous phase of polymers when $T > T_g$ (Lützow et al., 1999). Accordingly, Teuten et al. (2007) and Wang et al. (2015) showed that the rubbery plastic PE showed higher sorption capacity than plastics with high glass transition temperature. Crystallinity increases the tortuosity diffusion paths and reduces the volume fraction accessible for the absorption of pollutants. In our

case, the relatively high sorption obtained for POM, a polymer with a relatively polar backbone might be explained by its low T_g (about 188 K). Li et al. (2018) found that PS had the maximum sorption capacity for several antibiotics, which can be attributed to its amorphous nature with open structure that may facilitate polar interactions and p-p interactions between PS and the antibiotics. In addition, the roughness of the surface of MPs may influence the sorption capacity. For instance, Zhang et al. (2018) reported that sorption of oxytetracycline onto aged PS with rough surfaces were higher than the virgin PS. Regarding the MPs used in this study, measured crystallinity (Table S3) was as follows: PET: 11%; PLA: 36%; POM: 47% (PS is amorphous). No clear effect of the degree of MP crystallinity was observed regarding the sorption of the macrolide antibiotics on the tested MPs, probably because of the limited influence of this factor in case of large molecules like AZI and CLA.

Due to their small size and presence in aquatic systems, many organisms may ingest MPs causing direct damage or accumulating throughout the food chain (Besseling et al., 2013; Wardrop et al., 2016; Zhan et al., 2016; Alomar et al., 2017; Canniff and Hoang, 2018; Digka et al., 2018; Zhu et al., 2019). In addition to these direct effects, ingested MPs might release the additives used in their formulation (plasticizers flame retardants and antioxidants, among many others), which may produce toxic effects to aquatic organisms (Engler, 2012; Koelmans et al., 2013). The fact that they may behave as vectors of other aquatic pollutants has raised concerns because when ingested, sorbed pollutants might be released causing damage. In this context, Rochman et al. (2013a) reported that chemical pollutants sorbed on PE-MPs had the potential to bioaccumulate and exert toxicity to fish by ingestion. It was also reported that organic pollutants and additive chemicals could be transferred by MPs into gut tissues of lugworms, leading to alterations in ecophysiological functions linked to health and biodiversity (Browne et al., 2013). Karami et al. (2016) reported that low density polyethylene (LDPE) modulated the impacts of phenanthrene in African catfish (*Clarias gariepinus*) possibly by changing its bioavailability. Pittura et al. (2018) also worked with LDPE and reported that this type of MP acted as a vehicle of benzo(a)pyrene to the

Mediterranean mussel, *Mytilus galloprovincialis*. They found that the pollutant was bioaccumulated by the mussel with a concomitant modulation of the immune responses; the authors were concerned about the possibility that chronic exposure to MPs loaded with co-occurring pollutants might have long term effects on the health status of aquatic organisms. Despite these evidences, some authors disagree about the role of MPs as vectors for other pollutants. Koelmans et al. (2016) modelled the available data on MPs as vectors of hydrophobic organic chemicals (HOC) to marine animals. They concluded that the flux of HOCs bioaccumulated from natural prey is higher than the flux from ingested MPs for most habitats, implying that MP ingestion is not likely to increase the exposure to and thus risks of HOCs in the marine environment. However, this result comes from the modelling of marine medium and does not necessarily reflect the situation of other compartments (Tourinho et al., 2019).

All the mentioned studies were performed with high trophic biota, paying little attention to primary producers such as microalgae including cyanobacteria (phytoplankton), organisms that sustain the trophic webs in aquatic environment. These organisms are, in general, too small to ingest virgin MPs as well as pollutant-loaded MPs. However, as our study demonstrates, MPs may act as vector of pollutants, even pharmaceuticals as antibiotics, concentrating them in their surface or sorbed in their inner voids, transporting them to other locations, and eventually desorbing them and affecting these small but key organisms that may co-occur in the same aquatic environments. Nevertheless, more empirical data should be gathered both regarding sorption but also desorption of different kind of pollutants on and from MPs and their effect on a range of organisms across the trophic web in order to convincingly demonstrate that the sorption of pollutants onto MPs may really pose a threat to aquatic organisms.

5. Conclusions

In this work, we tested the biological effect of the macrolide antibiotics azithromycin (AZI) and clarithromycin (CLA) sorbed on polystyrene (PS), polyethylene terephthalate (PET), polylactic acid (PLA), and polyoxymethylene (POM) MPs. Both antibiotics became sorbed on MPs particles with loading concentrations reaching the order of

µg/g of plastic. The role of hydrophobic interactions as the main driver of sorption agreed with the fact that the more hydrophobic AZI showed the highest sorption on all MPs. Both antibiotics became desorbed from preloaded MPs once in contact with water. The extent of desorption reached 15% for AZI and 1.9% for CLA after 72 h with respect to the concentration to which the MPs were initially exposed. Virgin MPs did not give rise to any significant toxicity towards the primary producer cyanobacterium *Anabaena* sp. PCC7120. However, antibiotic-loaded MPs were toxic and resulted in significant inhibition of bacterial growth and chlorophyll a content. Desorbed antibiotics explained the observed toxicity. Our results demonstrated the potential of MPs to accumulate pollutants and to transfer them eventually affecting the primary producers of aquatic ecosystems.

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SUPPLEMENTARY MATERIAL

Microplastics as vectors of the antibiotics azithromycin and clarithromycin Effects towards freshwater microalgae

Miguel González-Pleiter^{1,2,*}, Alicia Pedrouzo-Rodríguez¹, Irene Verdú¹, Francisco Leganés¹, Eduardo Marco¹, Roberto Rosal², Francisca Fernández-Piñas¹

Miguel González-Pleiter^{1,2}, Carlos Edo², David Velázquez¹, María Cristina Casero-Chamorro³, Francisco Leganés¹, Antonio Quesada¹, Francisca Fernández-Piñas¹, Roberto Rosal^{2,*}

¹ Departamento de Biología, Facultad de Ciencias, Universidad Autónoma de Madrid, Cantoblanco, E-28049 Madrid, Spain

² Departamento de Ingeniería Química, Universidad de Alcalá, Alcalá de Henares, E-28871 Madrid, Spain

* Corresponding author: mig.gonzalez@uam.es

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Supplementary Section 1. Materials. Microplastics of PS (3.5 mm beads, density 1.05 g/cm³), PET (3-5 mm beads, density 1.4 g/cm³), PLA (3-5 mm, beads, density 1.24 g/cm³) and POM (3 mm beads, density 1.4 g/cm³) were obtained from Goodfellow Cambridge Ltd. (Huntingdon, England). Their crystallinity was measured by differential scanning calorimetry (DSC) in a Q100 apparatus (TA Instruments). Ultrapure water was generated using Direct-Q™ 5 Ultrapure Water Systems from Millipore (Bedford, MA, USA) with a specific resistance of 18.2 MΩ cm at 25 °C. The hydrophobicity of polymers was measured by determining water, glycerol and diiodomethane contact angles according the sessile drop method in a DSA25 Krüss Analyzer as explained elsewhere (Van Oss, 2007). High-resolution 3D microscope with interferometry and profilometry model Leica DCM 8 with the analysis in confocal mode were used to characterize roughness of the MPs.

Supplementary Section 2. Statistical analysis. Means and standard deviation values were calculated for each treatment from three independent replicate experiments. To determine significant differences among test treatments, data were statistically analysed by conducting an overall one-way analysis of variance (ANOVA) using R software. $p < 0.05$ or $p < 0.01$ was considered statistically significant. When significant differences were observed, means were compared using multiple-range Tukey's HSD test and Holm-Sidak method.

Supplementary Table S1. Concentration of antibiotics sorbed on MPs and subsequent desorption in water.

MP	Antibiotic	Amount of the antibiotic sorbed after 4 hours of incubation with MP (mg/g)	Concentration of the antibiotic after immersing antibiotic-loaded MPs for 72 hours in water (µg/L)	Percentage of antibiotics desorbed from MP after 72 hours with respect to the antibiotic initially sorbed	Percentage of antibiotics desorbed from MP with respect to the concentration to which the MPs were initially exposed
PS	AZI*	0.00270 ± 0.00018	92.4 ± 9.2	68.4	18.5
PET	AZI*	0.00249 ± 0.00009	66.9 ± 7.2	53.6	13.4
PLA	AZI*	0.00178 ± 0.00078	65.2 ± 1.6	73.4	13.0
POM	AZI*	0.00208 ± 0.00041	67.5 ± 2.1	64.8	13.5
PS	CLA**	0.00487 ± 0.00018	39.4 ± 11.3	16.2	3.9
PET	CLA**	0.00298 ± 0.00027	15.6 ± 7.6	10.5	1.6
PLA	CLA**	0.00081 ± 0.00024	5.4 ± 5.0	13.3	0.5
POM	CLA**	0.00094 ± 0.00019	16.1 ± 4.2	34.3	1.6

* Initial concentration: 500 µg/L

** Initial concentration: 1000 µg/L

Supplementary Table S2. Tukey test ($p < 0.05$) comparing the effects of AZI and CLA loaded MPs with the effects of AZI and CLA applied individually at the corresponding desorbed concentrations from each MP on growth and chlorophyll *a* content of *Anabaena* sp. PCC 7120.

Antibiotic – loaded MPs	Antibiotic concentration desorbed from each MP ($\mu\text{g/L}$)	Endpoint	Tukey's test ($p < 0.05$) Statistical significance between the effect of Antibiotic-loaded MPs and corresponding desorbed antibiotic concentration
PS + AZI	AZI (92.4)	OD $_{750\text{ nm}}$	No
PS + AZI	AZI (92.4)	[Chl <i>a</i>]	No
PS + CLA	CLA (39.4)	OD $_{750\text{ nm}}$	No
PS + CLA	CLA (39.4)	[Chl <i>a</i>]	No
PET + AZI	AZI (66.9)	OD $_{750\text{ nm}}$	No
PET + AZI	AZI (66.9)	[Chl <i>a</i>]	No
PET + CLA	CLA (15.6)	OD $_{750\text{ nm}}$	No
PET + CLA	CLA (15.6)	[Chl <i>a</i>]	No
PLA + AZI	AZI (65.2)	OD $_{750\text{ nm}}$	No
PLA + AZI	AZI (65.2)	[Chl <i>a</i>]	No
PLA + CLA	CLA (11.1)	OD $_{750\text{ nm}}$	No
PLA + CLA	CLA (11.1)	[Chl <i>a</i>]	No
POM + AZI	AZI (67.5)	OD $_{750\text{ nm}}$	No
POM + AZI	AZI (67.5)	[Chl <i>a</i>]	No
POM + CLA	CLA (16.1)	OD $_{750\text{ nm}}$	No
POM + CLA	CLA (16.1)	[Chl <i>a</i>]	No

Supplementary Table S3. Properties of the polymers used in this work.

Polymer	WCA ($^{\circ}$)	Hydrophobicity*	Crystallinity (%)	T_g ($^{\circ}\text{C}$)**	Sdr (%)***	Sku****
PS	87.4 ± 3.5	-59.2 ± 2.6	amorphous	~ 100	8.2 ± 3.4	2.9 ± 0.4
PET	81.3 ± 1.5	-47.6 ± 2.2	11	80	8.2 ± 1.7	7.4 ± 7.1
PLA	81.1 ± 2.9	-51.5 ± 3.1	36	60-65	24.1 ± 13.4	6.9 ± 2.8
POM	80.4 ± 1.6	-39.6 ± 1.8	47	-60	22.4 ± 41.0	4.1 ± 1.7

* ΔG_{sws} (mJ/m^2) as determined by van Oss (2007). A surface is hydrophobic if $\Delta G_{\text{sws}} < 0$ and hydrophilic if $\Delta G_{\text{sws}} > 0$. The degree of hydrophobicity is given by the negative value of ΔG_{sws} .

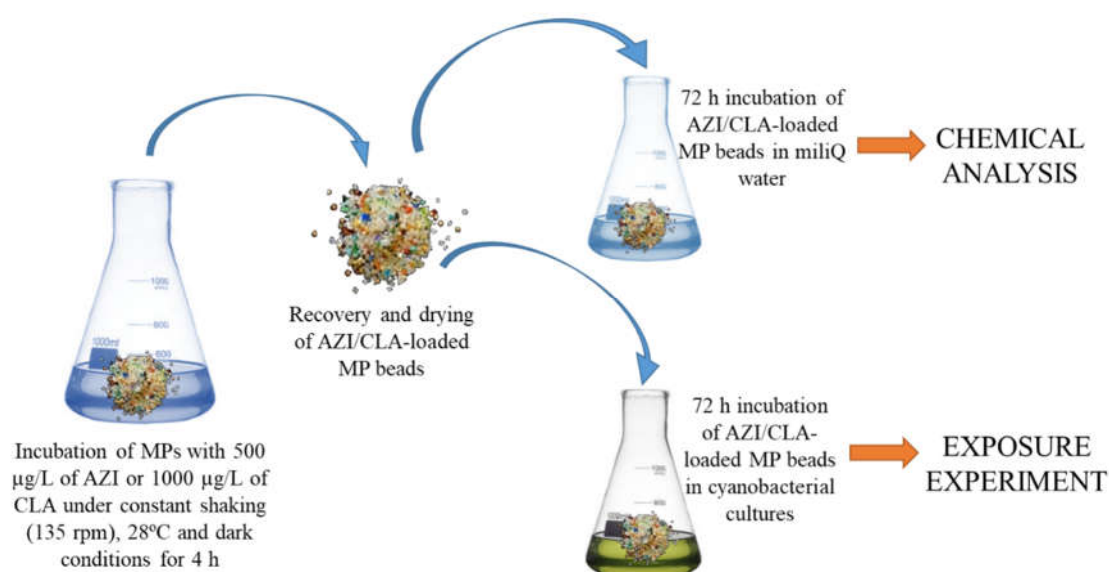
** Glass transition temperature

*** Sdr is the developed interfacial area ratio defined as the percentage of additional area due to texture if compared to planar area (zero represents a flat surface).

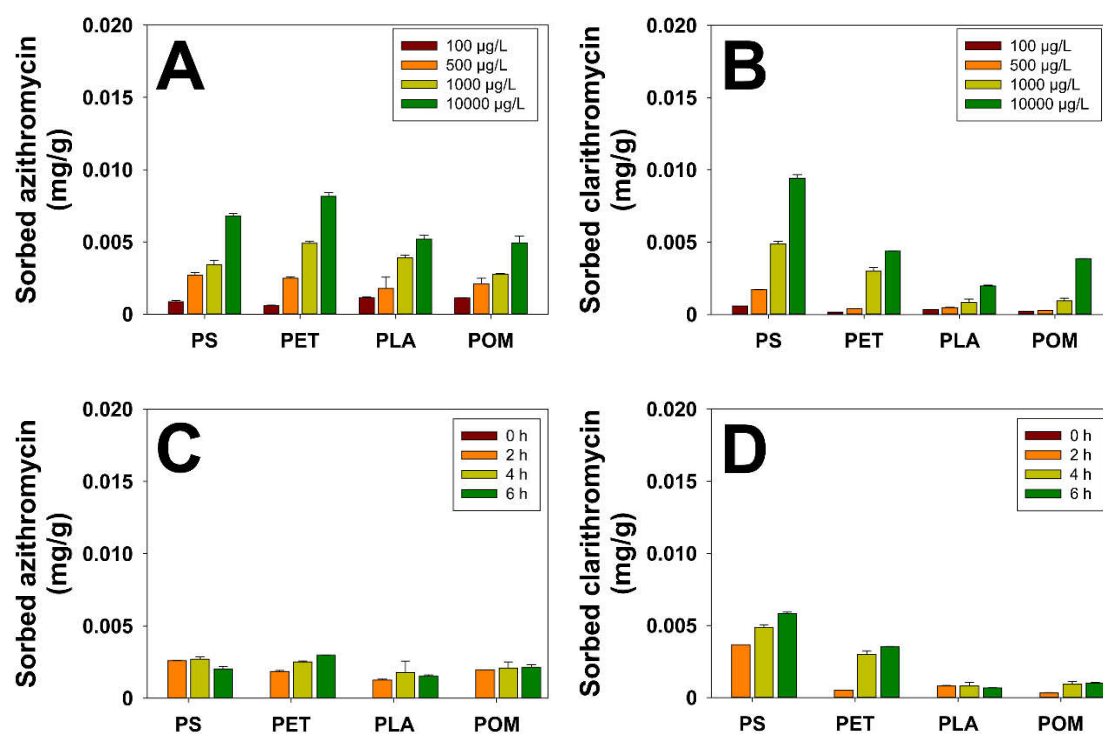
**** Sku: kurtosis of roughness profile; $\text{Sku} > 3$: spiked distribution with numerous high peaks and low valleys; $\text{Sku} < 3$: means few peaks and low valleys.

Reference

Van Oss, C.J., 2007. Development and applications of the interfacial tension between water and organic or biological surfaces. *Colloids and surfaces B: Biointerfaces* 54, 2-9.



Supplementary Figure S1. Flow diagram of the experimental set up.



Supplementary Figure S2. AZI (A) and CLA (B) sorbed on each type of MP (PS, PET, PLA and POM) after 4 h incubated in initial concentration of 100, 500, 1000 and 10000 µg/L of AZI and of 100, 500, 1000 and 10000 µg/L of CLA. AZI (C) and CLA (D) sorbed on each type of MP (PS, PET, PLA and POM) after 0 h, 2 h, 4 h and 6 h incubated in initial concentration of 500 µg/L of AZI and of 1000 µg/L of CLA.