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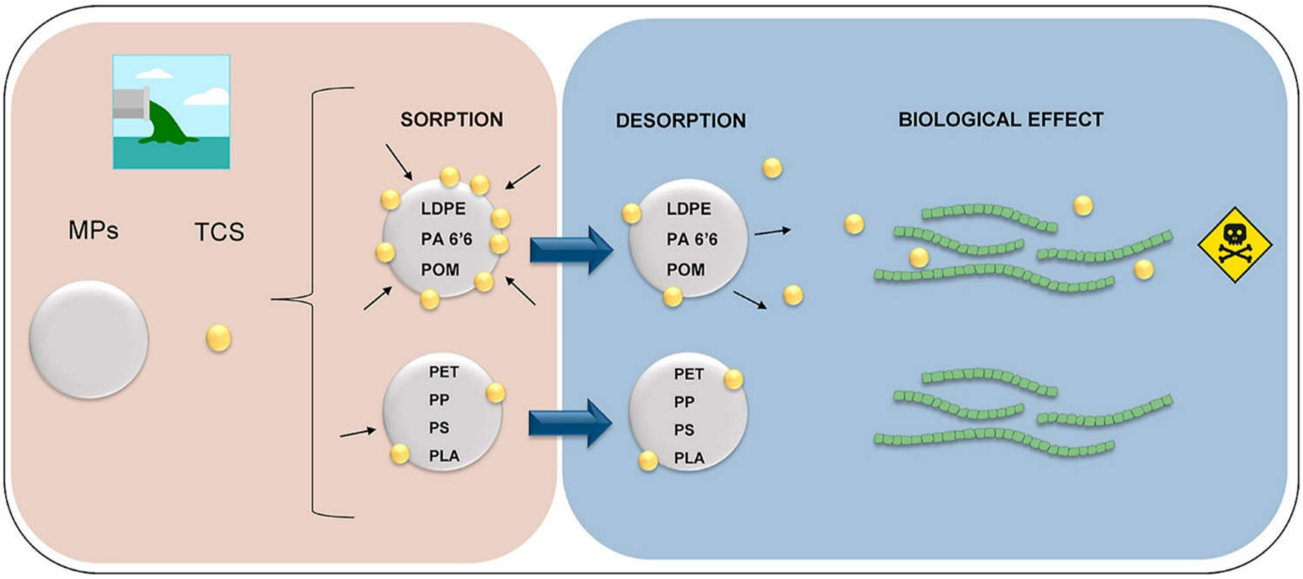

Microplastics can act as vector of the biocide triclosan exerting damage to freshwater microalgae

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Abstract

Despite the large number of recent studies on microplastics (MPs) and their ability to act as carriers of pollutants, the knowledge about the biological effects of MPs loaded with chemicals is scarce. The aim of this study was to evaluate the potential of MPs as vectors for the antimicrobial triclosan (TCS). For it, we tested low-density polyethylene (LDPE), polyamide (PA), polyethylene terephthalate (PET), polyoxymethylene (POM), polypropylene (PP), polystyrene (PS) and the biodegradable polylactic acid (PLA). Thus, chemical analysis of sorption and desorption of TCS by these MPs was evaluated. The effect of TCS-loaded MPs to *Anabaena* sp. PCC7120, a cyanobacterium model of primary producers in freshwater ecosystems, was investigated. Chemical analyses showed different capacity of sorption depending on the MP type, which was related to some of their physicochemical properties. PA (104.7 μg/g), POM (57.4 μg/g) and LDPE (18.3 μg/g) were the polymers that sorbed the highest amounts of TCS. Glass transition temperature of polymers and their physicochemical interaction with TCS explained the extent of sorption. Significant decreases were found in growth, 22.3%, 94.6% and 81.0%, and chlorophyll a content, 58.4%, 95.0% and 89.6%, of Anabaena when exposed to TCS-loaded LDPE, PA and POM beads, respectively, which were the only MPs displaying significant sorption-desorption of TCS, implying that these MPs could act as vectors of TCS towards freshwater microalgae. This finding is of fundamental relevance as microalgae are at the base of the aquatic trophic chain and support growth of upper organisms.

Keywords: Triclosan; Microplastics; Sorption; Desorption; Cyanobacterium; Toxicity

1. Introduction

The widespread use and distribution of plastic particles is an emerging worldwide concern. They can be found in every ecosystem, from densely populated cities to remote areas such as the Poles (Bergmann et al., 2019; Zhang et al., 2019; González-Pleiter et al., 2020). According to PlasticsEurope (2019), the worldwide production of plastics reached 359 million tonnes in 2018, a tendency that if not enough measures are taken will double in the next 20 years (MacArthur et al., 2016). Smaller plastic particles, known as microplastics (MPs; between 1 μm and 5 mm along their largest dimension) (GESAMP, 2020) are included intentionally in household items or personal care products (PCPs; such as glitter and facial cleaners, where microbeads replace natural scrubs) (Cole et al., 2011; Alimi et al., 2018). All these particles are known as primary MPs because they are purposely manufactured in that size (Browne et al., 2011; Cole et al., 2011). Small plastic particles produced by weathering of larger materials, are known as secondary MPs (Andrady, 2011). Plastic debris has been studied at a larger extent in marine environments than in freshwater ecosystems (Prata, 2018; Wagner and Lambert, 2018). However, there is evidence of their occurrence in freshwater ecosystems with concentrations between 3×10^{-4} MPs per m^3 in estuaries until 1.87×10^5 MPs per m^3 in waters surrounding big cities and up to about 10^6 MPs per m^3 in wastewater treatment plant (WWTPs) influents (Li et al., 2018a). It has been shown that the smallest plastic fractions are not totally retained by the conventional wastewater treatment systems (Wagner et al., 2014).

MPs *per se* can induce a wide range of effects to organisms including oxidative stress, inflammatory responses, impairment of energy allocation functions and false sensation of satiation, among others (Browne et al., 2013; Naik et al., 2019). Smaller fragments, produced from the fragmentation of larger ones, may

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become more bioavailable and affect microorganisms such as microalgae, which are at the base of the trophic chain. Therefore, any negative effect on them might impair the upper organisms in the food web of a particular environment. In addition, physicochemical wearing may accelerate the release of the additives included in their composition. These chemicals, some of unknown composition and/or concentration due to the secrecy associated to proprietary formulations, may also negatively affect co-occurring organisms (Hahladakis et al., 2018; Munier and Bendell, 2018). Besides, it has been recognized that MPs are a new niche for microorganisms including pathogens and antibiotic resistance bacteria and cognate antibiotic resistance genes (Munier and Bendell, 2018; Naik et al., 2019).

WWTPs are a significant point of entry for many environmental pollutants. Recently, it has been recognized that many pollutants can interact with plastic particles. Thereby, plastics may act as vectors of pollutants altering their environmental fate and opening a new route for the entry of pollutants into food webs (Cole et al., 2011; Turner and Holmes, 2015; Hüffer and Hofmann, 2016; Alimi et al., 2018; Li et al., 2018b, 2019; Munier and Bendell, 2018; Wagner and Lambert, 2018; Liu et al., 2019a). Previous studies showed that MPs may act as carriers for heavy metals (Rochman et al., 2013; Velzeboer et al., 2014; Alimi et al., 2018), hydrophobic organic chemicals (Mato et al., 2001; Bakir et al., 2014a; Hartmann et al., 2017) and pharmaceuticals (Razanajatovo et al., 2018). There is intense debate as to whether this might represent an important pathway for the transfer of sorbed chemicals from MPs to co-occurring organisms in a given environment. Modelling studies suggested that plastics might not be a relevant pathway for the transfer of pollutants to biota in marine environments (Gouin et al., 2011). However, it is a fact that plastics can sorb pollutants from the environment relatively quickly and it has been established that their concentration on them can become orders of magnitude higher than in the surrounding aquatic environment (Mato et al., 2001). More work is needed to assess this fact across, as well as its effect to a wider range of organisms from different environments such as freshwaters, soils and sediments (Bakir et al., 2016). Overall, the question of whether MPs act as driver for hazardous chemicals to organisms is still unanswered and requires further evidences (Wagner et al., 2014; Hong et al., 2017).

Triclosan (TCS) is a common antimicrobial compound used in consumer products for decades (Wang and Farrell, 2004; Lehutso et al., 2017). It is one of the best-studied substances from a toxicological point of view. It has been concluded that it is a persistent endocrine disruptor and bioaccumulative in different aquatic and non-aquatic organisms (Lindström et al., 2002; Halden et al., 2017). Although this compound is in wide use, some restrictions have been applied recently. In EU the maximum legal concentration is 0.3% in toothpastes and other products such as soaps, shower gel, make up or deodorants, and 0.2% in mouthwashes (Decision, 2016/110/EU; Commission Regulation EU 358/2014). Furthermore, TCS is not completely removed in WWTPs and appears both in their effluent and in the biosolids used as fertilizer (Cho et al., 2011; Fekadu et al., 2019). In this context, TCS occurrence in freshwater (surface water and wastewater) was reported in concentrations ranging from 0.5 ng/L to 14 mg/L in different regions in the world (Mezcua et al., 2004; Wang and Farrell, 2004; Aranami and Readman, 2007; Cho et al., 2011; Fekadu et al., 2019; Li et al., 2019). Its continuous discharge and high persistence in the environment make it detectable even in concentrations toxic to the biota (Fekadu et al., 2019; Li et al., 2019).

So far, the study of the interaction between MPs and triclosan is limited, with a reduced number of studies on this topic (Li et al., 2019; Ma et al., 2019). The toxicological effects of co-exposure of TCS and MPs have been evaluated in several aquatic organisms (Syberg et al., 2017; Zhu et al., 2019) showing that MPs may sorb TCS, increasing the toxicity when ingested by marine copepods (Syberg et al., 2017) but decreasing the toxicity by antagonistic mechanisms when marine microalgae were co-exposed to both (Zhu et al., 2019). Browne et al. (2013), and Nobre et al. (2020), evaluated the capacity of pre-exposed MPs to transfer TCS to marine invertebrates, indicating that MPs could act as carriers, and increasing the toxicity as measured by different biological parameters. In this paper, we studied the sorption and desorption of TCS from seven different types of MPs including the non-biodegradable low-density polyethylene, the polyamide nylon 6′6, polyethylene terephthalate, polyoxymethylene, polypropylene and polystyrene as well as the biodegradable polylactic acid. The biological effect of TCS transferred from MPs to aquatic biota was evaluated using a strain of the commonly occurring and ecologically relevant cyanobacterial genus *Anabaena*. The relevance of cyanobacteria lies on the fact that they are primary producers and essential components of phytoplankton communities.

2. Materials and Methods

2.1. Chemicals

Triclosan (TCS; 5-chloro-2-(2,4-dichlorophenoxy)-phenol, $C_{12}H_7Cl_3O_2$, CAS: 3380-34-5, purity ≥ 97,0%) was purchased from Sigma-Aldrich. The physicochemical properties of TCS are shown in Table S1 (Supplementary Materials, SM). Stock solutions (10,000 μg/mL) were prepared in MeOH 100%; filtered by 0.22 μm and stored in the dark at 4 °C in glass amber flasks. The content of MeOH in working TCS solutions was <0.05% (v/v) to avoid co-solvent toxic effects (Pinal et al., 1990).

The sorbents chosen for this study represent a wide range of plastic polymers commonly used and found in environmental samples. Low-density polyethylene (LDPE), polystyrene (PS), polyoxymethylene (POM, acetal copolymer), polypropylene (PP) and polylactic acid (PLA) were purchased as MP beads from Goodfellow (Huntingdon, England). Polyethylene terephthalate (PET) and the polyamide nylon 6′6 (PA) were purchased as MP beads from Sigma-Aldrich. The physicochemical properties of MPs are shown in Table S2. Glass transition temperature (T_g), melting point and crystallinity were determined using differential scanning calorimetric analysis (DSC) (DSC apparatus SDT Q600 from TA Instruments). Water contact angle (WCA) was measured with a DSA25 Krüss Analyzer. Glass beads were used as control sorbent representing an inorganic substrate.

2.2. Abiotic studies

Virgin MPs and glass (150 beads/L) were put into contact with TCS (0.7 μg/mL) in 20 mL of Milli-Q water (18.2 MΩ cm at 25 °C) using 50 mL glass Erlenmeyer flasks. Leslie et al. (2017) indicated the presence of up 300 particles/L between 0.3 and 5 mm in influents and up to 14 μg/mL of TCS was found in sewage water as Wang and Farrell (2004) pointed out. Thus, the concentrations of sorbents and sorbate used in this study correspond to those of environmental contaminated areas. MPs and glass beads were exposed to TCS for 4 h, which was selected as representative of the time MPs and pollutants could be in contact in the aeration tanks in activated sludge WWTPs (Pan et al., 2004; Kumar et al., 2015; Noguera-Oviedo and Aga, 2016).

2.2.1. Sorption experiment

MPs and glass beads in contact with TCS were incubated in a rotary shaker (140 rpm) at 25 °C for 4 h in darkness and wrapped with aluminium foil to prevent photodegradation during exposure (Behera et al., 2010). Immediately after incubation, MPs and glass beads were separated from the liquid phase, and subsequently centrifuged (4700 rpm, 30 s) to remove the liquid adhered to the beads. This process rendered TCS-loaded MPs and glass beads. Each exposure was performed in triplicate.

Methanol (100%) was chosen to extract the TCS sorbed into the MPs and glass beads (León et al., 2018). The extraction was performed in darkness for 24 h because longer times did not result in further extraction. Methanol extracts were stored (−20 °C) in amber glass flasks until chemical analysis by high-performance liquid chromatography (HPLC).

2.2.2. Desorption experiment

To determine potential desorption of TCS from MPs and glass beads, beads separated from water containing TCS as explained before were added to Erlenmeyer flasks with 20 mL of cyanobacterial culture medium (see *section 2.3* below). Samples were incubated in a rotary shaker (140 rpm) at 25 °C under light (50 μmol photon m^{-2} s^{-1}), simulating the conditions of the toxicological bioassay (see below). Samples were collected at prescribed times, namely 2, 4, 24, 48 and 72 h. Each treatment was conducted by triplicate. All samples were stored at −20 °C in amber glass flasks until analysis. To quantify the desorbed TCS, preconcentration using solid phase extraction (SPE) was performed prior to HPLC analyses method which was performed using Strata-X 33u Polymeric Reversed Phase cartridges (Phenomenex). Additional details are given elsewhere (Amariei et al., 2020).

2.2.3. HPLC determination of TCS

The concentration of TCS was measured by HPLC using a 1200 Series Agilent apparatus with

Promosil C18 (4.6 × 150 mm, 60 mm) column. The mobile phase was 70:30 acetonitrile-Milli-Q water adjusted to pH 2 with phosphoric acid (H_3PO_4). The injection volume was 20 µL at a flow rate of 1 mL/min. TCS was detected at 210 nm (Amariei et al., 2020). The amount of TCS sorbed per mass unit of sorbent (µg/g), *q*, was calculated according to Eq. (1). Eq. (2) indicates the percentage of sorption (% sorbed). In both, C_f is the final concentration of TCS (µg/mL) in solution at a given time, C_o is the initial TCS concentration (µg/mL), *V* is the volume of the solution (mL) and *m* is the mass of sorbent used (g):

$$q = \frac{\left(C_f \times V\right)}{m} \qquad [1]$$

The percentage of desorbed TCS (% desorbed) was determined by Eq. (3), where C_D is the measured TCS concentration in solution (µg/mL) at a given time, *V* is the volume of the culture medium (mL), *q* is the sorbed concentration of TCS (µg/g) according to Eq. 1 and *m* is the mass of sorbent (g):

$$\left(\%\, sorbed\right) = \frac{\left(C_f \times 100\right)}{C_o} \qquad [2]$$

2.3. Biological assays

The filamentous cyanobacterium *Anabaena* sp. PCC7120 (hereinafter, *Anabaena*) was routinely cultured in sterilized conditions in Allen & Arnon medium diluted eight-fold (Leganes et al., 2014) supplemented with nitrate (5 mM), buffered with 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid (HEPES) (20 mM) and adjusted to pH 7.5 (hereinafter AA/8+N) in 100 mL in a 250 mL Erlenmeyer flasks. A rotary shaker set at 140 rpm, 25 °C, and 50 µmol photon $m^{-2}\,s^{-1}$ was used for cultures. Stock cyanobacterial cultures were kept in exponential growth by adding fresh culture medium at regular intervals.

Cyanobacterial cells in exponential growth phase were used as inoculum for ecotoxicity assays. 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) virgin MPs (3 beads in 20 mL); (2) TCS at the initial concentration (0.7 µg/mL); (3) TCS-loaded MPs (3 beads in 20 mL) as described above in *section 2.2* and (4) TCS applied individually, at the maximum desorbed concentration measured by HPLC in abiotic studies as indicated in *section 2.2.3.* For each sorbent. All exposure experiments were conducted for every type of MP: LDPE, PA, PET, POM, PP, PS, PLA, and for glass beads. Thus, five exposure treatments (control, virgin MPs, TCS at initial concentration, TCS-loaded MPs, desorbed TCS) x seven MP types (LDPE, PA, PET, POM, PP, PS, PLA) x three replicates resulted in 105 experimental units. Non-treated cells (not exposed to sorbents or TCS) were used as control. After 72 h, culture density was determined at 750 nm, and dry weight was calculated (Tamayo-Belda et al., 2019). Chlorophyll *a* content was determined according to Marker (1972). For it, the photosynthetic pigment was extracted in methanol (100%) at 4 °C for 24 h in darkness and absorbance at 665 nm was measured in a Hitachi U-2000 spectrophotometer.

2.4. Statistical analysis

Results were analyzed by one-way analysis of variance (ANOVA) to determine significant differences between treatments. Previously parametric assumptions were checked. A *p*-value of $p < 0.05$ was used to show significant differences. When significant differences were observed, the post-hoc multiple-range Tukey's HSD test was run. IBM SPSS Statistics 26 and Graph Pad Prism 8.0 software were used for statistical analyses and graphical representations, respectively.

3. Results

3.1. Abiotic studies

3.1.1. Sorption experiment

Fig. 1 shows the amount of TCS sorbed (µg) per unit mass of sorbent (g) after 4 h in contact with a solution containing 0.7 µg/mL TCS. PA reached the highest sorbed amount, 104.7 ± 7.0 µg/g, followed by POM, 57.4 ± 5.0 µg/g; and LDPE, 18.3 ± 0.9 µg/g. The rest of the MPs also sorbed TCS in the following decreasing order, PS > PET > PP > PLA, although to a lesser extent. Glass beads was the material displaying lower sorption. Statistically significant differences ($p < 0.05$) were only found between LPDE, PA and POM with the rest of tested sorbents. Table S3 shows the statistical parameters in detail (F-statistics, degrees of freedom and *p*-values). The percentage of TCS sorbed for each type of MPs and glass beads with respect to the initial amount in solution is shown in Fig. S1.

3.1.2. Desorption experiment

The concentrations of TCS in culture medium after 2, 4, 24, 48 and 72 h desorbed from TCS-loaded MPs are shown in Fig. 2. No TCS was detected in the desorption media from glass beads (G), PET, PP, PS, PLA at any time. Concentrations of TCS above the limit of quantification were measured only in culture medium in contact with loaded LDPE, PA and POM. The maximum concentration of TCS in culture medium after release from TCS-loaded LPDE reached 0.03 ± 0.01 μg/mL after 48 h, while the maximum concentration of desorbed TCS from TCS-loaded PA and POM beads was 0.07 ± 0.01 μg/mL and 0.04 ± 0.01 μg/mL, respectively after 4 h. These concentrations represented desorption percentages with respect to the sorbed amount of 38.9 ± 6.1% for TCS-loaded LDPE, 26.8 ± 4.5% for TCS-loaded PA and 29.1 ± 4.6% for TCS-loaded POM. Table S4 shows the statistical parameters in detail (F-statistics, degrees of freedom and *p*-values).

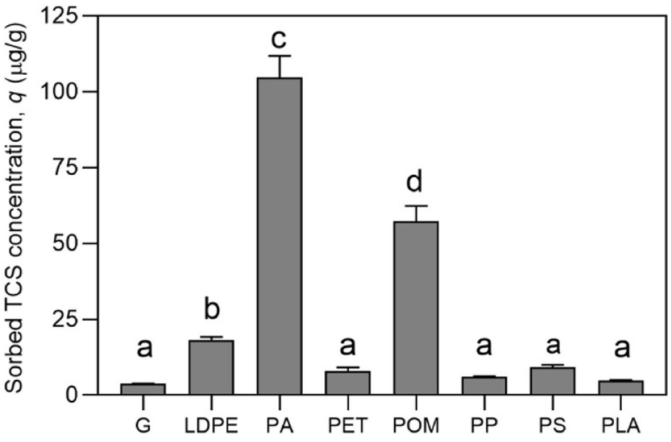

Figure 1. Amount of sorbed TCS per unit mass of sorbent (μg/g) (q) after 4 h of exposure to the MPs (LPDE, PA, PET, POM, PP, PS and PLA) and glass beads (G) as control. Initial concentration of TCS in solution, 0.7 μg/mL. Different letters indicate significant differences between treatments ($p < 0.05$). Results are expressed as mean values ± SD in error bars (n = 3).

3.2. Biological assays

With the aim of assessing the potential vector effect of TCS by MPs, the biological effect of TCS-loaded MPs was assessed in the cyanobacterium *Anabaena* sp. PCC7120 by measuring growth and chlorophyll *a* content as endpoints.

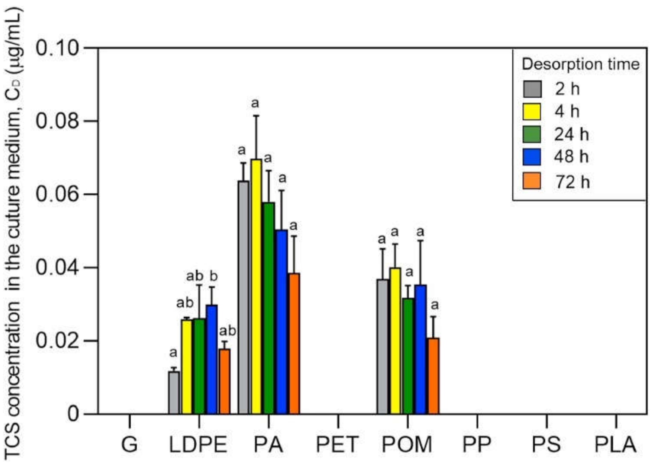

Figure 2. Desorbed TCS concentration in the culture medium (μg/mL) from TCS-loaded beads at 2, 4, 24, 48 and 72 h. Different letters indicate significant differences between treatments ($p < 0.05$) in each sorbent beads. Results are shown as mean ± SD (n = 3).

In Fig. 3, corresponding to the effect on growth of *Anabaena,* it can be observed that virgin MPs did not cause any growth inhibition. Regarding TCS-loaded MPs, only three of them, namely LDPE, PA and POM, caused significant effect on cyanobacterial growth. Growth decrease represented 22.3 ± 1.8% of growth inhibition for TCS-loaded LDPE; 94.6 ± 0.5% for TCS-loaded PA and 81.0 ± 6.7% for TCS-loaded POM. It should be noticed that significant amounts of TCS were desorbed only from these three MPs (see Fig. 2). For these MPs, the maximum desorbed TCS concentration (d-TCS) was also tested: 0.03 μg/mL from LDPE; 0.07 μg/mL from PA and 0.04 μg/mL from POM. As shown in Fig. 3, the same amount of TCS, namely 0.03, 0.07 and 0.04 μg/mL caused the same inhibitory effect when applied directly to cyanobacterial cultures, which was consistent with the role of MPs as TCS drivers. As shown in the figure, the initial concentration of TCS (0.7 μg/mL) caused 94.9 ± 1.8% of growth inhibition. Table S5A shows the statistical parameters in detail (F-statistics, degrees of freedom and *p*-values).

Fig. 4 shows the effect on chlorophyll *a* content. As in the case of growth inhibition, only TCS-loaded LDPE, PA and POM induced significant decrease in chlorophyll *a* content. The observed inhibition percentage was 58.4 ± 2.1%, 95.0 ± 1.4%, and 89.6 ± 3.6% for TCS-loaded LDPE, PA and POM, respectively. TCS desorbed from LDPE, PA and POM caused the same decrease in this parameter as TCS-loaded MPs. As shown in

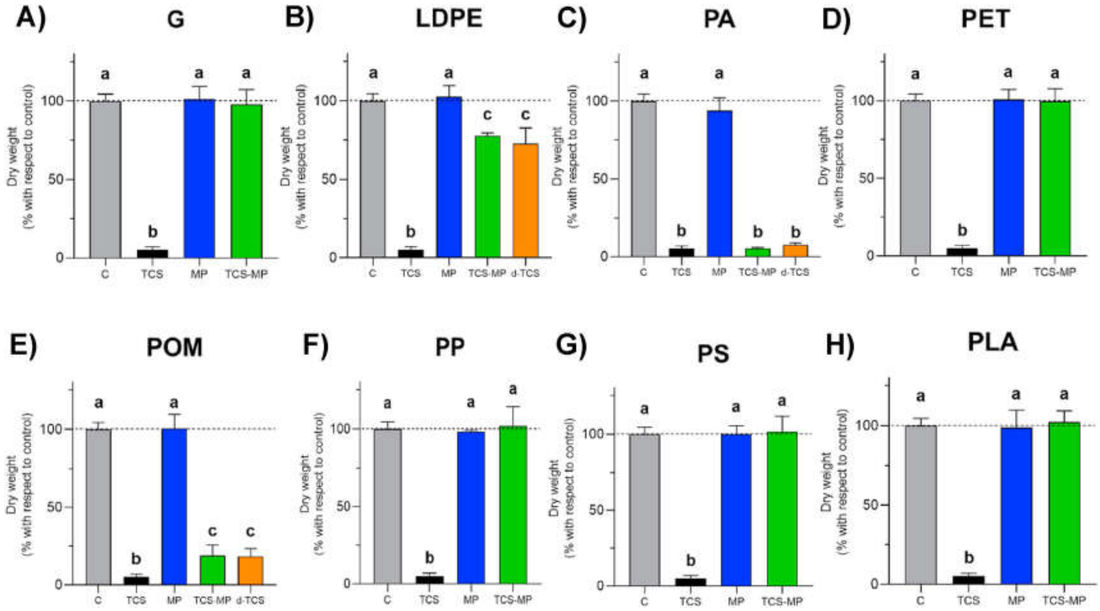

Figure 3. Effect of virgin MPs or glass beads (MP), TCS-loaded MPs or glass beads (TCS-MP) and TCS desorbed (d-TCS) (corresponding to the maximum concentration of TCS desorbed during exposure of *Anabaena* to TCS-loaded MPs: 0.03 µg/mL from LDPE; 0.07 µg/mL from PA and 0.04 µg/mL from POM) on growth (dry weight, mg/L) of the cyanobacterium after 72 h of exposure to different sorbents: Glass (G) (A), LDPE (B), PA (C), PET (D), POM (E), PP (F), PS (G), and PLA (H). Control (c) represents non-exposed cyanobacterial cells and TCS_0 corresponds to TCS alone at the initial concentration (0.7 µg/mL). Results are expressed as percentage ± SD with respect to control (100% is indicated by the dashed line). Different letters indicate significant differences between treatments (Tukey's HSD, $p < 0.05$).

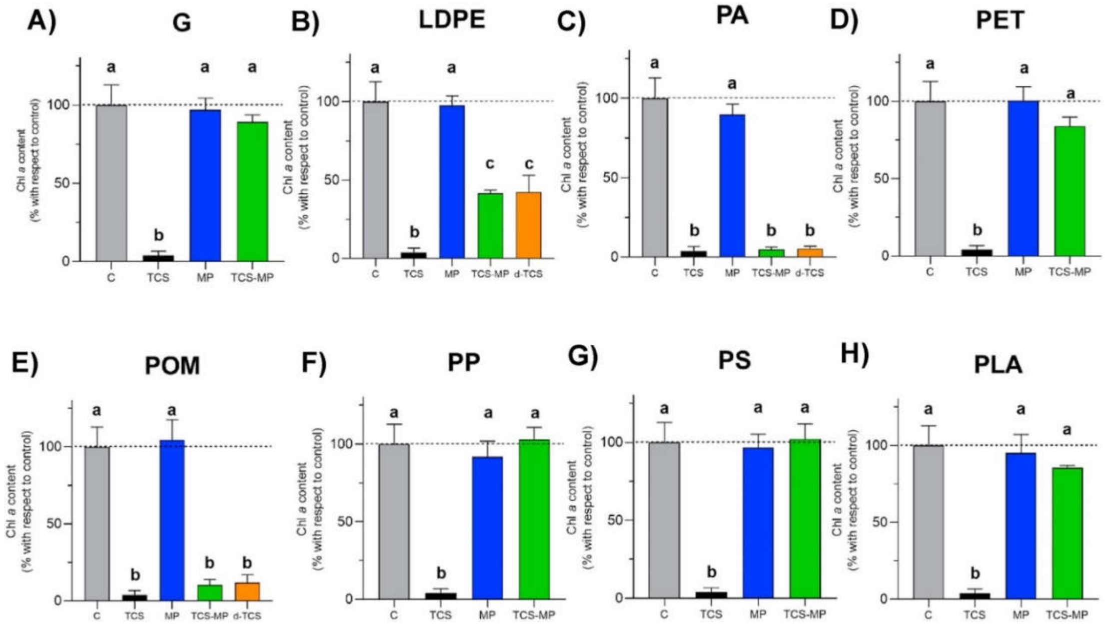

Figure 4. Effect of virgin MP (MP), TCS-loaded MP beads (TCS-MP) and TCS desorbed (d-TCS) (corresponding to the maximum concentration of TCS desorbed during exposure of *Anabaena* to TCS-loaded MPs: 0.03 µg/mL from LDPE; 0.07 µg/mL from PA and 0.04 µg/mL from POM) on chlorophyll *a* content (mg/L) of the cyanobacterium after 72 h of exposure to different sorbents: Glass (G) (A), LDPE (B), PA (C), PET (D), POM (E), PP (F), PS (G), and PLA (H). Control (c) represents non-exposed cyanobacterial cells and TCS_0 corresponds to TCS alone at the initial concentration (0.7 µg/mL). Results are expressed as percentage ± SD with respect to control (100% is indicated by the dashed line). Different letters indicate significant differences between treatments (Tukey's HSD, $p < 0.05$).

Fig. 4 the initial concentration of TCS (0.7µg/mL) caused 96.0 ± 2.6% of decrease in chlorophyll *a* concentration. Table S5B shows the statistical parameters in detail (F-statistics, degrees of freedom and *p*-values).

4. Discussion

The interaction between plastics and pollutants is a complex process influenced by different factors (Ma et al., 2019). In this paper, we analyzed the potential of different types of MPs to sorb and desorb TCS thereby acting as carrier of TCS able to impair freshwater organisms such as the primary producer *Anabaena* sp. PCC7120. To date, a considerable research effort has been dedicated to study the capacity of MPs for the sorption of pollutants, both organic and inorganic (Yu et al., 2019). However, desorption studies are scarce and even less studies report on the vector effect of pollutant-loaded MPs to ecologically relevant aquatic biota. Some of them include the lungworm, *Arenicola marina*; the Japanese medaka, *Oryzias latipes;* the branquiopod *Artemia nauplii* and zebrafish; the African catfish, *Clarias gariepinus*; the mussel *Mytilus galloprovincialis*, the copepod *Acantia tonsa* and the oyster, *Crassostrea brasiliana* (Browne et al., 2013; Rochman et al., 2013; Batel et al., 2016; Karami et al., 2016; Pittura et al., 2018; Bellas and Gil, 2020; Nobre et al., 2020). Up to our knowledge, the effect of MPs as vectors of TCS on freshwater photosynthetic microorganisms has not been studied yet.

The degree of crystallinity and glass transition temperature (Tg) have been recognized as the main drivers to explain the sorption properties of plastics (Hartmann et al., 2017; Endo and Koelmans, 2019; Fred-Ahmadu et al., 2020). A polymer generally consists of a well-ordered crystalline phase and an amorphous region. The lower the crystallinity, the higher is the diffusion rate of hydrophobic molecules within the plastic matrix (Sun et al., 2019). For polymers at a temperature above Tg, the amorphous phase turns into a viscous or rubbery liquid due to the higher mobility of their molecules. Polymers with lower Tg display higher chain mobility, allowing free molecules to diffuse more easily into their matrix (Cheng et al., 2020). The molecular properties of polymers are also important in explaining the interaction with organic compounds. A measure of the affinity of a polymer to interact with a given solute is the Hildebrand solubility parameter (δ), which is defined as the square root of the cohesive energy density consequence of the intermolecular interactions between polymer and solute. The closer δ of solute and polymer, the better the solubility of the solute into the polymer matrix. The values of δ for TCS and the polymers used in this work are given in Table S2. Surface interaction is another relevant factor for the sorption of chemicals on polymers. Surface physicochemistry can be modified by the degree of polymer weathering, usually revealed by the formation of hydroxyl, carbonyl, carboxyl or other polar surface moieties, which are more prone to interact with polar or semipolar compounds, and an increase of external surface due to cracking (Hüffer et al., 2018). Surface hydrophobicity, measured as water contact angle, is also shown in Table S2 for the polymers tested in this work.

In general, the kinetics and extent of the sorption/desorption of pollutants on surfaces, are expected to be influenced by environmental variables like salinity, pH and the presence of organic matter, due to competing sorption or to the modification of surface properties. However, several studies reported limited influence of such variables in the case of microplastics. Bakir et al. (2014b) studied the effect of salinity on the sorption and desorption of non-polar pollutants onto polyvinyl chloride (PVC) and PE microplastics and showed negligible effect upon kinetics and limited effect on sorption capacity. Liu et al. (2019a) showed that the sorption of phthalate esters onto PVC, PE and PS microplastics was not significantly affected by changes in pH or by the presence of natural organic matter, although high concentrations of salts slightly enhanced sorption because of the salting-out effect. Specifically concerning TCS, its sorption on PS microplastics was not influenced by ionic strength and the influence of pH was explained by its speciation. The sorption of the dissociated form ($pH > pKa = 8.14$) was preferred in agreement with sorption proceeding through hydrophobic interaction (Li et al., 2019). In our case, the ionic strength of the desorption medium was within the usual range of wastewater. In some cases, however, the presence of dissolved organic matter could reduce the amount sorbed when its interaction with the solute is stronger than that of the solute with microplastics (Xu et al., 2018). Overall, the physical characteristics of the polymer and local conditions like the relative amount of plastic and residence time would be the main drivers for the physical transport of contaminated plastics (Bakir et al., 2014b).

This study showed that the sorption capacity for TCS increased in the order PA > POM > LDPE. The rest of MPs, namely PET, PP, PS and PLA, sorbed considerably lower amounts. TCS could interact with MPs through hydrophobic, electrostatic interaction, hydrogen and halogen bonding (Cho et al., 2011; Li et al., 2019; Ma et al., 2019). The pK_a value of TCS (8.14) indicated that at neutral pH, the molecule was not ionized. Besides, the hydrophobicity of TCS ($\log K_{ow}$ of 4.76) would suggest a preferable interaction with non-polar surfaces, but the values among all the polymers tested in this work were not very different (Table S2). The capacity of PA to interact with solutes by hydrogen bonding was already proposed to explain the high sorption capacity of 17β-estradiol to PA in comparison with other polymers, notably polyolefins (Liu et al., 2019b). A similar explanation was proposed for the sorption of TCS onto PA-12 microspheres (Han et al., 2013). Accordingly, the most probable explanation for the observed higher amount of TCS sorbed by PA compared to the other MPs is the formation of hydrogen bonds between amide groups and the proton on the C(1) hydroxyl moiety of TCS. Despite their relatively high degree of crystallinity, the low T_g of POM could explain its sorption capacity, higher than that of the other polymers. In this case, the amorphous phase would be in a viscous rubbery state, thereby facilitating the diffusion of higher amounts of TCS into their mobile polymer chains (Ahn et al., 2005). The sorption capacity of LDPE was compatible with its low crystallinity (25%) and low T_g value (−90 °C). The enhanced sorption of non-polar compounds into less crystalline polymers has been shown before for different pollutants and plastics (Guo et al., 2012, 2019; Li et al., 2018b). Furthermore, according with our results, Endo et al. (2011) indicated that POM presents a higher affinity to sorption than LDPE because of the presence of repeating polar groups ($-CH_2-O-CH_2-$). A less mobile rubbery phase due the higher glass transition temperature compared to PE and POM and the lack of polar groups in the molecule could explain the low sorption capacity of PP (Guo et al., 2012; Wang et al., 2015). PS and PLA are glassy polymers because their T_g is above room temperature for our experimental conditions (25 °C), which may explain the low amount of TCS retained. Glass beads, as negative controls, displayed the lower sorption capacity (Fig. 1). For further clarification, the amount of TCS sorbed by each MP plotted in relation to the determining parameters of sorption by polymers in this study (T_g and δ) is shown in Fig. S2. According to Endo and Koelmans (2019), the transference of a chemical tends to reach an equilibrium between the concentration of the substance in the solid and the liquid phase. So, in this study a spontaneous release is being facilitated. The desorption experiments did not detect TCS from TCS-exposed beads of PET, PP, PS and PLA, probably as a result of the low sorbed amount. The same for glass beads. TCS desorption was detected in the liquid samples from TCS-loaded LDPE, PA and POM as shown in Fig. 2. The decrease of TCS in the liquid culture medium for higher desorption times suggested a possible photodegradation of TCS. It has already been shown that TCS photodegrades in freshwater and seawater with half-lives of a few days, yielding 2,8-dichlorodibenzo-p-dioxin as main by-product (Aranami and Readman, 2007; Yuval et al., 2017).

The tested virgin MPs did not cause any effect on growth or chlorophyll *a* content of the cyanobacterium. However, three TCS-loaded MPs, namely LDPE, PA and POM caused significant effects to both endpoints. TCS was found in the cyanobacterial growth medium only desorbed from these three MP types (Fig. 2). In fact, there was a complete agreement between the observed growth inhibition and chlorophyll *a* decrease when TCS-loaded LDPE, PA and POM and d-TCS were individually applied, meaning that the driver of the observed toxicity of TCS-loaded MPs is the release of TCS from them. This implies that LDPE, PA and POM, being able to both adsorb and desorb TCS, may act as vector of this biocide to co-occurring microorganisms in aquatic environments. Syberg et al. (2017) showed that the toxicity of TCS to the copepod *Acantia tonsa* increased in the presence of polyethylene particles (in non-toxic MPs concentrations) and was attributed to the ingestion of TCS sorbed onto PE which could be released in the digestive tract. Similarly, a toxic effect upon ingestion of TCS-loaded MPs was reported for the lugworm *Arenicola marina* (Browne et al., 2013). The experiments with

TCS-loaded MPs are conceptually different from those that involve co-exposure (joint exposure, no previous loading of MPs with the pollutant so that no desorption or release is contemplated) of TCS and MPs where synergism or antagonism might be found. In this context, Zhu et al. (2019) reported that the joint exposure to TCS and several types of MPs resulted in reduced toxicity to the marine microalga *Skeletonema costatum*, which could also be explained by the sorption capacity of MPs for TCS. Similarly, Zhang et al. (2018) reported a decrease of the toxicity of poly(vinyl chloride) and glyphosate in presence of nanoplastics in the cyanobacterium *Microcystis aeruginosa*. Cyanobacteria and eukaryotic microalgae (phytoplankton) are organisms that sustain the trophic webs in aquatic environment. These microorganisms might not be able to internalize larger-size pollutant-loaded MPs as fish, mussels or copepods do (Karami et al., 2016; Syberg et al., 2017; Pittura et al., 2018). However, this study shows that MPs may act as carriers of pollutants such as the biocide TCS. Eventually, sorbed pollutants may be released from the MPs when the conditions of the external medium changes, particularly regarding their concentration, but also environmental variables like salinity, pH and the presence of organic matter (as already discussed above). The release of pollutants would eventually affect ecologically relevant microorganisms, which are present in aquatic environments. This study also indicates that not all MP types are able to sorb and eventually desorb pollutants to the same extent and that the physicochemical characteristics of both sorbate and sorbent play a key role. Further studies are necessary to elucidate if the transport of pollutant by plastic particles is relevant in comparison to natural pathways (such as sediments or suspended organic matter. Our results showed that plastics in contact with wastewater discharges can be exposed to high concentration of toxics and, therefore, might act as carrier for them.

5. Conclusions

Our results revealed that there is an interaction between TCS and MP at environmental concentrations. Polymer characteristics such as glass transition temperature and molecular structure (presence of polar groups) were responsible for the sorption of TCS to the tested MPs. All the studied polymers sorbed TCS to a certain extent, but only LDPE, PA and POM retained in sufficient amount to desorbing it in concentrations high enough to induce toxic effects on the cyanobacterium *Anabaena*, an ecologically relevant organism that is a key component of phytoplankton. The same effect was found when exposed to the same amount of TCS as measured desorbed from MPs, implying that the driver of the toxicity found was the release of TCS from the MPs. This study provides novel data on the role of MPs as vectors of pollutants towards primary producers at the base of the aquatic food chain.

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SUPPLEMENTARY MATERIAL

Microplastics can act as vector of the biocide triclosan exerting damage to freshwater microalgae

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Contents

Supplementary Table S1. Main physicochemical properties of triclosan.

Supplementary Table S2. Main physicochemical properties of the materials used in this work.

Supplementary Table S3. Statistical parameters of ANOVA and Tukey's HSD test from the sorption experiment. F-statistics, degrees of freedom (DF) and *p*-values are shown.

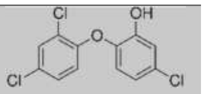
Supplementary Table S4. Statistical parameters of ANOVA and Tukey's HSD test from the desorption experiment. F-statistics, degrees of freedom (DF) and *p*-values are shown.

Supplementary Table S5. Statistical parameters of ANOVA and Tukey's HSD test from the biological experiment, for dry weight (mg/L) (A) and chlorophyll *a* content (mg/L) (B). F-statistics, degrees of freedom (DF) and *p*-values are shown.

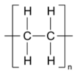
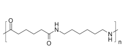
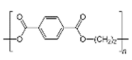
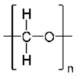
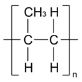
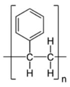
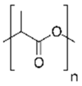
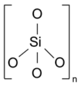
Supplementary Figure S1. Percentage of TCS (%) sorbed onto Glass (G), LDPE, PA, PET, POM, PP, PS and PLA beads after 4 h of exposure. Results are expressed as mean values ± SD in error bars (n=3).

Supplementary Figure S2. Amount of sorbed TCS per unit mass of MP (µg/g) (q) after 4 h of exposure to the MPs (LPDE, PA, PET, POM, PP, PS and PLA) *vs.* parameters of polymers, glass transition temperature (T_g, Cº) (A) and Hildebrand solubility parameter (δ, MPa$^{1/2}$) (B).

Supplementary Table S1. Main physicochemical properties of triclosan.

Compound	Molecular Weight (g/mol)	pKa	Log Kow	Water Solubility (mg/L)	Hildebrand solubility parameter ($MPa^{1/2}$)	Structure
Triclosan	289.54	8.14	4.76	10	29.4	

Supplementary Table S2. Main physicochemical properties of the materials used in this work.

	LDPE	PA 6/6	PET	POM	PP	PS	PLA	Glass
Molecular formula	$(C_2H_4)_n$	$(C_6H_{11}NO)_n$	$(C_{10}H_8O_4)_n$	$(CH_2O)_n$	$(C_3H_6)_n$	$(C_8H_8)_n$	$(C_3H_4O_2)_n$	$(SiO_2)_n$
Mean Diameter (mm)	5[a]	3[a]	4 [a]	3 [a]	5 [a]	3.5 [a]	3 [a]	3
Mean Mass (mg/bead)	28.1	16.6	22.8	16.0	25.3	15.6	37.9	37.2
Density (g/cm^3)	0.9[a]	1.2 [a]	1.7 [a]	1.4 [a]	0.9 [a]	1.1 [a]	1.2 [a]	2.5
Crystallinity	25	17	11	47	41	-	36	100
Glass Transition Temperature. T_g (ºC)	< -90	57	78.5	< -90	-12	107	60.6	-
Melting point (ºC)	110.6	267.5	258.4	167.8	166.8	-	150.6	1500
Water contact angle. WCA (º)	95	86	84	82	91	77	87	74
Hildebrand solubility parameter. d ($MPa^{1/2}$)	16.7	26.1	21.2	21.1	16.2	18.3	20.2	-
Structure								

[a] data from supplier's information.

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Supplementary Table S3. Statistical parameters of ANOVA and Tukey's HSD test from the sorption experiment. F-statistics, degrees of freedom (DF) and *p*-values are shown.

ANOVA test				**TUKEY´s HSD test**
F	**DF**	***p*-value**		***p*-value**
403.8	7	<0.0001	G vs LDPE	0.0007
			G vs PA	<0.0001
			G vs PET	0.6849
			G vs POM	<0.0001
			G vs PP	0.9803
			G vs PS	0.4323
			G vs PLA	0.9996
			LDPE vs PA	<0.0001
			LDPE vs PET	0.0181
			LDPE vs POM	<0.0001
			LDPE vs PP	0.0039
			LDPE vs PS	0.0417
			LDPE vs PLA	0.0017
			PA vs PET	<0.0001
			PA vs POM	<0.0001
			PA vs PP	<0.0001
			PA vs PS	<0.0001
			PA vs PLA	<0.0001
			PET vs POM	<0.0001
			PET vs PP	0.9912
			PET vs PS	0.9998
			PET vs PLA	0.9122
			POM vs PP	<0.0001
			POM vs PS	<0.0001
			POM vs PLA	<0.0001
			PP vs PS	0.9120
			PP vs PLA	0.9998
			PS vs PLA	0.7097

Supplementary Table S4. Statistical parameters of ANOVA and Tukey's HSD test from the desorption experiment. F-statistics, degrees of freedom (DF) and *p*-values are shown.

	ANOVA test				**TUKEY´s HSD test**
	F	**DF**	***p*-value**		***p*-value**
LDPE	4.845	4	0.0279	2 h vs 4 h	0.1337
				2 h vs 24 h	0.1337
				2 h vs 48 h	0.0302
				2 h vs 72 h	0.6969
				4 h vs 24 h	>0.9999
				4 h vs 48 h	0.9124
				4 h vs 72 h	0.4877
				24 h vs 48 h	0.9042
				24 h vs 72 h	0.3592
				48 h vs 72 h	0.1183
PA	3.343	4	0.0784	-	-
POM	2.436	4	0.1321	-	-

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Supplementary Table S5. Statistical parameters of ANOVA and Tukey's HSD test from the biological experiment, for dry weight (mg/L) (A) and chlorophyll *a* content (mg/L) (B). F-statistics, degrees of freedom (DF) and *p*-values are shown.

A	**ANOVA test**			**TUKEY´s HSD test**	
	F	**DF**	***p*-value**		***p*-value**
G	693.8	3	<0.0001	C vs TCS	<0.0001
				C vs MP	0.9634
				C vs TCS-MP	0.9280
				TCS vs MP	<0.0001
				TCS vs TCS-MP	<0.0001
				MP vs TCS-MP	0.8119
LDPE	537.5	4	<0.0001	C vs TCS	<0.0001
				C vs MP	0.9203
				C vs TCS-MP	<0.0001
				C vs d-TCS	<0.0001
				TCS vs MP	<0.0001
				TCS vs TCS-MP	<0.0001
				TCS vs d-TCS	<0.0001
				MP vs TCS-MP	<0.0001
				MP vs d-TCS	<0.0001
				TCS-MP vs d-TCS	0.6971
PA	976.9	4	<0.0001	C vs TCS	<0.0001
				C vs MP	0.1642
				C vs TCS-MP	<0.0001
				C vs d-TCS	<0.0001
				TCS vs MP	<0.0001
				TCS vs TCS-MP	>0.9999
				TCS vs d-TCS	0.8504
				MP vs TCS-MP	<0.0001
				MP vs d-TCS	<0.0001
				TCS-MP vs d-TCS	0.9433
PET	865.7	3	<0.0001	C vs TCS	<0.0001
				C vs MP	0.9887
				C vs TCS-MP	0.9999
				TCS vs MP	<0.0001
				TCS vs TCS-MP	<0.0001
				MP vs TCS-MP	0.9890
POM	604.4	4	<0.0001	C vs TCS	<0.0001
				C vs MP	0.9998
				C vs TCS-MP	<0.0001
				C vs d-TCS	<0.0001
				TCS vs MP	<0.0001
				TCS vs TCS-MP	0.0019
				TCS vs d-TCS	0.0028
				MP vs TCS-MP	<0.0001
				MP vs d-TCS	<0.0001
				TCS-MP vs d-TCS	>0.9999
PP	708.8	3	<0.0001	C vs TCS	<0.0001
				C vs MP	0.9501
				C vs TCS-MP	0.9126
				TCS vs MP	<0.0001
				TCS vs TCS-MP	<0.0001
				MP vs TCS-MP	0.7680
PS	762.4	3	<0.0001	C vs TCS	<0.0001
				C vs MP	>0.9999
				C vs TCS-MP	0.9510
				TCS vs MP	<0.0001
				TCS vs TCS-MP	<0.0001
				MP vs TCS-MP	0.9738
PLA	653.7	3	<0.0001	C vs TCS	<0.0001
				C vs MP	0.9868
				C vs TCS-MP	0.9152
				TCS vs MP	<0.0001
				TCS vs TCS-MP	<0.0001
				MP vs TCS-MP	0.8529

Supplementary Table S5. (Cont.)

B	ANOVA test				TUKEY´s HSD test
	F	**DF**	***p*-value**		***p*-value**
G	214.5	3	<0.0001	C vs TCS	<0.0001
				C vs MP	0.9573
				C vs TCS-MP	0.2766
				TCS vs MP	<0.0001
				TCS vs TCS-MP	<0.0001
				MP vs TCS-MP	0.6896
LDPE	156.0	4	<0.0001	C vs TCS	<0.0001
				C vs MP	0.9925
				C vs TCS-MP	<0.0001
				C vs d-TCS	<0.0001
				TCS vs MP	<0.0001
				TCS vs TCS-MP	<0.0001
				TCS vs d-TCS	<0.0001
				MP vs TCS-MP	<0.0001
				MP vs d-TCS	<0.0001
				TCS-MP vs d-TCS	>0.9999
PA	215.5	4	<0.0001	C vs TCS	<0.0001
				C vs MP	0.3662
				C vs TCS-MP	<0.0001
				C vs d-TCS	<0.0001
				TCS vs MP	<0.0001
				TCS vs TCS-MP	0.9998
				TCS vs d-TCS	0.9988
				MP vs TCS-MP	<0.0001
				MP vs d-TCS	<0.0001
				TCS-MP vs d-TCS	>0.9999
PET	204.4	3	<0.0001	C vs TCS	<0.0001
				C vs MP	0.9997
				C vs TCS-MP	0.0637
				TCS vs MP	<0.0001
				TCS vs TCS-MP	<0.0001
				MP vs TCS-MP	0.1401
POM	171.5	4	<0.0001	C vs TCS	<0.0001
				C vs MP	0.9514
				C vs TCS-MP	<0.0001
				C vs d-TCS	<0.0001
				TCS vs MP	<0.0001
				TCS vs TCS-MP	0.8345
				TCS vs d-TCS	0.8050
				MP vs TCS-MP	<0.0001
				MP vs d-TCS	<0.0001
				TCS-MP vs d-TCS	0.9997
PP	199.1	3	<0.0001	C vs TCS	<0.0001
				C vs MP	0.5574
				C vs TCS-MP	0.9657
				TCS vs MP	<0.0001
				TCS vs TCS-MP	<0.0001
				MP vs TCS-MP	0.4768
PS	200.6	3	<0.0001	C vs TCS	<0.0001
				C vs MP	0.9468
				C vs TCS-MP	0.9840
				TCS vs MP	<0.0001
				TCS vs TCS-MP	<0.0001
				MP vs TCS-MP	0.8814
PLA	191.2	3	<0.0001	C vs TCS	<0.0001
				C vs MP	0.8478
				C vs TCS-MP	0.1187
				TCS vs MP	<0.0001
				TCS vs TCS-MP	<0.0001
				MP vs TCS-MP	0.5975

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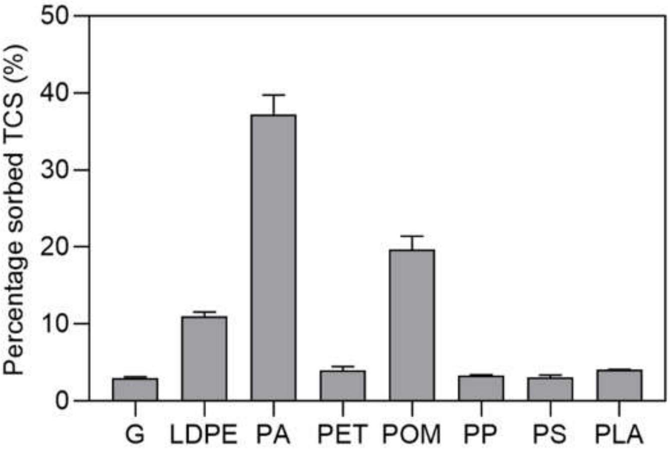
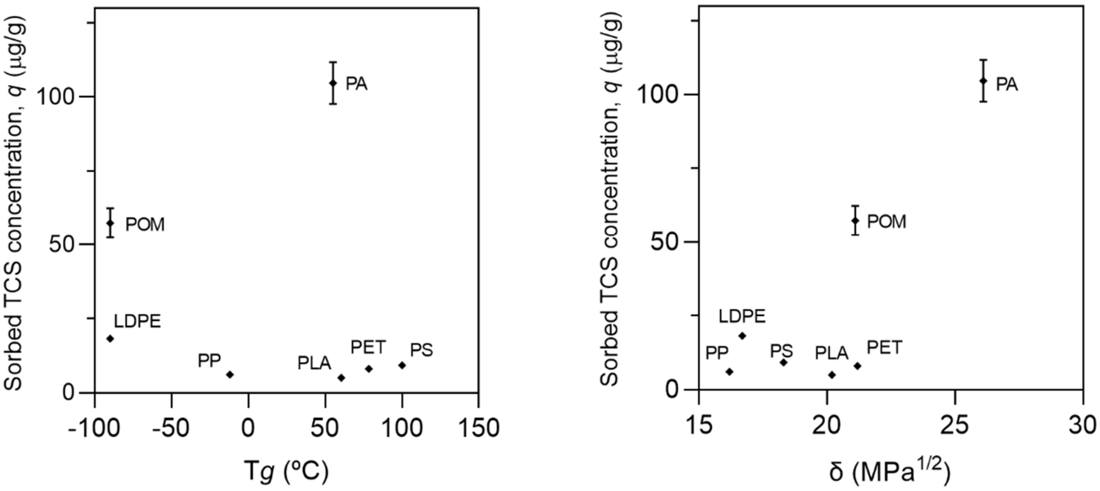


Supplementary Figure S1. Percentage of TCS (%) sorbed onto Glass (G), LDPE, PA, PET, POM, PP, PS and PLA beads after 4 h of exposure. Results are expressed as mean values ± SD in error bars (n=3).



Supplementary Figure S2. Amount of sorbed TCS per unit mass of MP (μg/g) (*q*) after 4 h of exposure to the MPs (LPDE, PA, PET, POM, PP, PS and PLA) *vs.* parameters of polymers, glass transition temperature (T_g, Cº) (A) and Hildebrand solubility parameter (δ, MPa$^{1/2}$) (B).

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