1. Introduction

Microplastics (plastic particles smaller than 5 mm) pollution is a growing concern shared by scientists and many individuals across the world. Human consumerism gave rise to aquatic systems filled with microplastics that can enter into the food chain. Recent studies show that these debris are in aquatic and terrestrial ecosystems and have a worldwide geographic distribution, and can be found even in the most remote locations, such as the Antarctic (Waller et al., 2017) and Artic (Peeken et al., 2018) oceans. Microplastics may come from general consumer household and cosmetic products but can also derive from macromastics that suffer from mechanical action and degradation, largely driven by UV-radiation-induced-photooxidation, releasing increasingly smaller sized low-molecular-weight polymer fragments (Galloway et al., 2017). Two of the industrial most commonly employed plastics, that are found in aquatic environments, are poly(methyl methacrylate) (PMMA), known as acrylic (Browne et al., 2011) and polystyrene (PS) (Alimi et al., 2017). PMMA is a lightweight resistant synthetic fiber (Zeng et al., 2002), that is widely used in the building industry, as a substitute for glass due to its high light transmission and resistance (Harper, 2000). PS is also very versatile and widely used in packaging, household and consumer goods (Maul et al., 2007), as this polymer is usually found in food containers (GESAMP, 2015). Microplastics have a strong influence in freshwater and marine ecosystems, as they are ingested and transferred through the planktonic web (Setälä et al., 2013). Furthermore, they affect higher trophic levels, (Farrel & Nelson, 2013; Cauwenberghe & Janssen, 2014; Besseling et al., 2015); modify genetic expression (Lagarde et al., 2016) and exhibit toxicity and mortality in marine plankton (Bergami et al., 2013). For these reasons, microplastics elimination/removal from
the environment is important before they can enter the trophic chain.

Microalgae are unicellular species that have been recognized as a bioalternative of different stages and functions of wastewater treatment (Abdel-Raouf et al., 2012). Despite their actual usage in water treatment, there is still an immense potential of using microalgae for microparticles treatment. This is because microalgae have the potential to produce exopolymers substances (EPS), which are viscous gel-like structures that are species specific. EPS are long-chain polysaccharides, composed of repeating units of sugar derivatives, with a structural diversity arisen from a broad range of non-carbohydrate substituents and linkage types (Whitfield, 1988). EPS have been characterized, revealing prominent functional reactive groups, such as sulphate, hydroxyl or carboxylic (Shah et al., 2000; Castellane et al., 2015). In this regard, freshwater and marine microalgae can have the potential to affect microparticles bioavailability, via the secretion of EPS, with consequent formation of hetero-aggregates (composed by EPS, microalgae and microparticles) to microparticles removal from aquatic ecosystems. However, this potential is still untapped as very few studies have analysed the formation and composition of EPS and hetero-aggregates, as well as the interactions between EPS and microparticles.

This work aims to evaluate the potential of four phytoplanktonic species as compelling biocompatible solutions to marine, freshwater or urban water treatment, focusing on the hetero-aggregation potential in the presence of microplastics. Two freshwater microalgae, Microcystis panniformis and Scenedesmus sp., as well as two marine microalgae, Tetraselmis sp. and Gloeocapsa sp., all known to be EPS producers were used in the trials. Up until now, Microcystis sp. hepatotoxins (microcystins) quantitative analysis has been used as a reliable method for hazard control in water (Vio-Ordorika et al., 2004) and Scenedesmus obliquus has proven to be an efficient microalgae in coliform bacteria removal from domestic sewage (Sebastian & Nair, 1984). The marine Tetraselmis suecica also has the potential to inhibit pathogenic vibrios (Austin & Day, 1990) and bacterial fish pathogens (Austin et al., 1992). The removal of heavy metals such as Pb2+ has been performed with Gloeocapsa sp., with the results showing up to a 100% removal capacity (Raungsomboon et al., 2008).

The influence of microplastics type (PMMA and PS), size (<106 μm; 106–250 μm), density (high and low) and concentration (12.5 mg L−1 and 125 mg L−1) on the formation of hetero-aggregates and consequent deposition, was studied, in light of the different EPS yields of each species.

2. Materials and methods

2.1. Microalgae and culture conditions

In this study two freshwater species: Microcystis panniformis (Class Cyanobacteria; 3–5 μm; Fig. 1a) and Scenedesmus sp. (Class Chlorophyceae; 5–13 μm; Fig. 1b) were selected, as well as two marine microalgae: Tetraselmis sp. (Class Chlorodendrophyceae; 7–16 μm; Fig. 1c) and Gloeocapsa sp. (Class Cyanobacteria; 38–42 μm; Fig. 1d). Gloeocapsa sp., Scenedesmus sp. and Microcystis panniformis were obtained from the Spanish Algae Bank (BEA) and Tetraselmis sp. was obtained from a private collection of Mariculture Center of Calheta (Madeira).

The freshwater microalgae Microcystis panniformis (axenic) and Scenedesmus sp. (axenic) were grown in commercial BG-11 medium (Sigma-Aldrich), while the marine microalgae, Gloeocapsa sp. (axenic) and Tetraselmis sp. (non-axenic) were grown in commercial f/2 medium (Sigma-Aldrich).

The cultures used in the experiments (see 2.3.) were maintained for 21 days at 20 ± 1 °C, under the irradiance of 1738 lux (HOBO® Pendant® MX Temp MX2201) supplied by a cool white Osram L18W 840 Lunilux lamp, with a 10/14 h (light/dark) photoperiod (Aralab CP500 growth chamber).

The initial cell concentration was 1.9 × 106 cells mL−1 for Microcystis panniformis; 4.7 × 107 cells mL−1 for Scenedesmus sp. and 3.3 × 106 cells mL−1 for Tetraselmis sp. Gloeocapsa sp. could not be counted due to its non-dissociable aggregates, as well as the impossibility to obtain homogeneous preparations. Microalgae cell growth was monitored using a Neubauer chamber (x10), sub-sampling every 2/4 days, for 3 weeks.

2.2. Microplastics

Two types of microplastics were used: fluorescent PMMA (green and purple) and fluorescent PS (yellow and blue). PMMA is a high-density plastic, whereas PS is a low-density plastic. The two types of irregular shaped microplastics were obtained by fragmentation using a milling machine (230 V ~ 50 Hz, 120 W). After this, all microplastics were mesh sieved (Analyssensieb–Retsch), collected and separated according to their size. The green particles of PMMA and the yellow PS were separated into a 106–250 μm fraction and the purple particles of PMMA and blue PS were collected into a lower sized <106 μm fraction. The microplastics were washed with dichloromethane, on a magnetic stirrer for 2 h, at room temperature. Then, the microplastics were filtered, oven-dried at 40 °C overnight and kept in a disector until analysis.

Two distinct stock solutions at two concentrations of microplastics were prepared for this study: high concentration was 250 mg L−1 and low concentration was 25 mg L−1. In both concentrations, two types/densities and sizes of microplastics were used. As they are fluorescent with distinct colours, they are easily distinguishable. The solutions were prepared in f/2 and BG-11 medium, in glass flasks in order to minimize losses of microplastics due to the possible establishment of electrostatic bonds to the flask walls. The solutions were then kept at 4 °C during the experimental period. And finally, Tween 20 (0.1%, v/v) was added to each solution to guarantee homogeneity.

2.3. Exposure conditions of microalgae to microplastics

Three experimental groups were used in triplicate: a control group, using the selected microalgae grown in their correspondent medium (Fig. 2a) and two other experimental groups in which the microalgae were subjected to the two distinct concentrations of microplastics (Fig. 2): 12.5 mg L−1 (low; Fig. 2b) and 125 mg L−1 (high; Fig. 2c). Each concentration contained purple and green PMMA (high density; <106 μm and 106–250 μm size fractions), as well as blue and yellow PS (low density; <106 μm and 106–250 μm size fractions).

The cultures were not renewed during the experimental period and were manually stirred 3 times a day. Nutrients were verified every other day to ensure that culture were nutrient sufficient (Nitrate and Phosphate test kits from Sigma Aldrich). Microalgal cell abundance was determined and used to distinguish the potential effects of the microplastics exposure to the countable species.

2.4. EPS determination

Modifications of Mota et al. (2013) method was used to determine the microalgae EPS production in the control treatments. EPS extraction was not performed in the culture with microplastics due to the fact that these cultures presented aggregates, that include EPS, that were further collected for analysis.

After 21 experimental days, the control cultures were
centrifuged at 6000 rpm for 30 min, at 25 °C to remove microalgae and other debris. The supernatant was concentrated on a magnetic stirrer at 60 °C for 12 h. For the precipitation of the EPS, methanol was gradually added to the concentrated supernatant, kept at 4 °C for 12 h and after centrifuged at 6000 rpm for 20 min. The precipitate was then washed with ethanol and re-dissolved in Milli-Q water. The dissolved EPS were dialysed against distilled water for 2 days to remove ions and salt. The dialysed EPS were frozen and freeze dried. The EPS yield was determined gravimetrically as EPS mg per mL of medium.

2.5. Characterization of hetero-aggregates

2.5.1. Fluorescent microscopy

Hetero-aggregates were collected from the bottom of the experimental flasks using a pipette. Each microplastic and different hetero-aggregates were individually analysed, under different microscopic filters. DAPI filter (excitation 340/80 nm, emission 425 nm) and I3 filter (excitation 450/90 nm, emission 515 nm). Observations were performed with a Leica DM2700P coupled with a CoolLED’s pE-300lite LED fluorescent illumination system.

2.5.2. Scanning electron microscope (SEM)

Before SEM analysis, the formed hetero-aggregates were washed three times with water and centrifuged at 2000 rpm, for 10 min, to remove any floating cells and culture medium compounds. Then, the hetero-aggregates were fixed using 2.5% (w/v) glutaraldehyde in 0.1 M sodium phosphate buffer, at pH 7.2, and stored overnight at 4 °C. After, the samples were washed two times with 0.1 M sodium phosphate buffer, at pH 7.2, and then serially dehydrated with ethanol (30%, 50%, 70%, 90% and dry 100%, v/v, ethanol/water) before freeze-drying. Samples were then preserved in a desiccator until analysis. Scanning electron micrographs of the surface samples were obtained by Scanning Electron Microscopy (SEM), with an HR-FESEM SU-70 Hitachi equipment operating at 4 kV, in the field emission mode. Samples were deposited on a steel plate and coated with carbon before analysis (EMITECH K950X Turbo Evaporator).
2.6. Statistics

Statistical analysis of microalgal growth rates were performed using IBM SPSS statistics software (V.25). The differences in growth rates between different experimental conditions were assessed by one-way analyses of variance (ANOVA), with a level of statistical significance of p-value <0.05.

3. Results

3.1. Microalgae growth

The microalgae growth for control conditions are presented in Fig. 3. Results show that after 21 days of growth, without renewal, *Tetraselmis* sp. was at stationary phase, while *Microcystis panniformis* and *Scenedesmus* sp. were still at exponential phase. For *Gloeocapsa* sp., the initial dilution performed ensured the exponential phase at the end of the experimental period.

At the end of the experimental period, cell abundance of each microalga, in both high and low concentrations of microplastics was also determined and compared with the control groups (Table 1). Both freshwater microalgae species displayed a cell abundance decrease in the microplastics condition cultures when compared with the control groups (Table 1). *Microcystis panniformis* exhibited a considerable and significant decrease (p < 0.05) in both microplastic concentrations; in the high microplastics concentration was 1.9 \times 10^6 cells mL\(^{-1}\) for *Microcystis panniformis* and 4.7 \times 10^6 cells mL\(^{-1}\) for *Scenedesmus* sp. and 3.3 \times 10^6 cells mL\(^{-1}\) for *Tetraselmis* sp.

\[ \frac{C_0}{C_2} = \frac{1}{1+K_1 + K_2} \]

*Microcystis panniformis* showed a considerable decrease (42%) was observed in the low microplastics concentration was 1.9 \times 10^6 cells mL\(^{-1}\) and compared with the control groups (Table 1). *Scenedesmus* sp. did not present a significant difference in cell abundance among all experimental conditions. For *Gloeocapsa* sp., the initial dilution performed ensured the exponential phase at the end of the experimental period.

Table 2 presents the results of EPS and hetero-aggregates formation. After 21 experimental days, EPS production was higher for the marine *Gloeocapsa* sp. (2.146 \pm 0.093 mg mL\(^{-1}\)), followed by *Tetraselmis* sp. (1.287 \pm 0.090 mg mL\(^{-1}\)). *Microcystis panniformis* (0.047 \pm 0.019 mg mL\(^{-1}\)) and *Scenedesmus* sp. (0.012 \pm 0.002 mg mL\(^{-1}\)).

Fig. 3 presents macroscopic photographs of the aggregates formed at the bottom of the culture flasks, after 21 days of culture. The freshwater microalga *Microcystis panniformis* presented no distinguishable hetero-aggregates (Fig. 4a), unlike *Scenedesmus* sp. (Fig. 4b), that presented a clear and visible formation of hetero-aggregates. For *Tetraselmis* sp. (Fig. 4c) there were no visible hetero-aggregates, whilst *Gloeocapsa* sp. (Fig. 4d) flasks displayed identifiable hetero-aggregates.

Macroscopically, it was also possible to observe that at the bottom of the flasks these hetero-aggregates incorporated the microplastics. The low density fluorescent yellow PS microplastics were the most identifiable ones at the bottom of the flasks in these aggregation conditions, as pointed by the arrows in Fig. 4, due to its more intense and vivid colour. The other microplastics exhibit a paler colour that does not make them so easily distinguishable. In the case of *Gloeocapsa* sp. cultures, the microplastics were trapped within its viscous EPS. As observed in Fig. 4b and d, the low-density yellow PS sunk to the bottom of the flask, confirming its aggregation and the microplastics vertical transport. Since not all the different microplastics could be identified at the bottom of the flasks, to confirm if the aggregates were composed of microalgae EPS alone (homo-aggregates) or microalgae/EPS and microplastics (hetero-aggregates), they were analysed by fluorescence microscopy.

3.2. Formation of EPS and hetero-aggregates

3.2.1. Detection of hetero-aggregates by fluorescence microscopy

The micrographs revealed aggregation across all microalgae. *Microcystis panniformis* (Fig. 5a and b) exhibited a bright field observation of the EPS, as depicted by the arrows, confirming the aggregation of the microplastics (formation of hetero-aggregates). The EPS produced by this smaller sized *Microcystis panniformis* were abundant, but small (between 10 and 110 \(\mu\)m) and easily disaggregated (shaking of the culture flasks).

In the case of *Scenedesmus* sp., the micrographs taken under I3 filters (Fig. 5c) revealed a considerable amount of EPS, ranging between 40 and 200 \(\mu\)m in size. The EPS produced by this microalga is comparable to the one produced by *Microcystis panniformis*, in the perspective of its overall size and easiness to disaggregate. The aggregation of high density purple PMMA (1) was observed in Fig. 5c. A second micrograph taken under DAPI filters (Fig. 5c1) exhibited considerable low-density blue (2) PS aggregation and minor low-density yellow PS (3) aggregation. The results showed that *Scenedesmus* sp. exhibited a higher amount of aggregates when compared to *Microcystis panniformis*.

The micrographs of *Tetraselmis* sp. showed a high amount of EPS (Fig. 5d), ranging from 50 to 300 \(\mu\)m in size. When observed under DAPI filters (Fig. 5e), these aggregates revealed a high amount of low density yellow (1) and blue (2) PS microplastic aggregation. A second micrograph (Fig. 5e1) taken under I3 filters, showed aggregation of high density green (3) and purple (4) PMMA, although in much lower quantities. *Tetraselmis* sp. also displayed the ability to colonize and aggregate microplastic much larger than its size (Fig. 5f).

*Gloeocapsa* sp. was the most distinct microalga, due to its abundant, dense, thick and viscous EPS mesh. The micrographs taken under DAPI filters (Fig. 5g and g1) showed abundant aggregation for every type of microplastic studied. From the same photograph (Fig. 5g), it is possible to infer that for this microalga the two types of microplastic did not only adhere to the surface of the exopolymer but were also incorporated.

3.2.2. Scanning electron microscope images

The SEM images (Fig. 6a) confirmed the smaller size of the EPS produced by *Microcystis panniformis*. These micrographs also showed that the size and type of exopolymer produced by
Table 1
Cell abundance (cells ml\(^{-1}\)) for each microalga studied, at the end of the experimental period. Cell abundance variation was calculated for the microplastic conditions against the control, for each microalga.

| Microalgae            | Microplastic concentration | Cell abundance (x10\(^4\) cells ml\(^{-1}\)) | Cell abundance variation |
|-----------------------|----------------------------|-----------------------------------------------|--------------------------|
| Microcystis panniformis | control                   | 6030 ± 102 \(a\)                              | -                        |
|                       | high                      | 4135 ± 182 \(b\)                              | -31%                     |
|                       | low                       | 3497 ± 200 \(c\)                              | -42%                     |
| Scenedesmus sp.       | control                   | 1641 ± 72 \(a\)                               | -                        |
|                       | high                      | 1298 ± 123 \(b\)                              | -21%                     |
|                       | low                       | 1430 ± 84 \(c\)                               | -13%                     |
| Tetraselmis sp.       | control                   | 368 ± 24 \(a\)                                | -                        |
|                       | high                      | 375 ± 7 \(a\)                                 | +1%                      |
|                       | low                       | 365 ± 44 \(a\)                                | -1%                      |

The values are mean of three replicates ± SD; for each microalga species. Different letters indicate significant differences in cell concentration/abundance \((p < 0.05)\).

Table 2
Summary table of the observations, including the EPS yield, size and stability and the main microplastics aggregated as well as the main type of aggregation.

| Microalgae          | EPS | Yield (mg ml\(^{-1}\)) | Size (\(\mu\)m) | Stability | Main Aggregation                        | Type                        |
|---------------------|-----|------------------------|------------------|-----------|-----------------------------------------|-----------------------------|
| Microcystis panniformis |     | 0.047 ± 0.019          | 10–110           | disaggregated | <106 \(\mu\)m PMMA                     | colonized                   |
| Scenedesmus sp.     |     | 0.012 ± 0.002          | 40–200           | disaggregated | <106 \(\mu\)m PMMA and PS             | adhered                     |
| Tetraselmis sp.     |     | 1.287 ± 0.090          | 50–300           | stable     | <106 \(\mu\)m and 106–250 \(\mu\)m PS | colonized and incorporated  |
| Gloecapsa sp.       |     | 2.146 ± 0.093          | –                 | stable     | all                                     | adhered, incorporated and colonized |

Fig. 4. Photographs of each microalga at high microplastics concentration condition culture (the high concentration was used for the photographs for better clarity), as observed from below the culture flasks. Each culture was photographed at the end of the experiment, using a digital camera. (a) Microcystis panniformis (b) Scenedesmus sp. (c) Tetraselmis sp. (d) Gloecapsa sp.
Microcystis panniformis does not allow for a proper aggregation of microplastics larger than 25 μm but revealed its microplastics colonization potential.

The SEM images of Scenedesmus sp. showed that the EPS tend to form a network, benefitting the captation, aggregation and adhesion of microplastics to the Scenedesmus sp. EPS (Fig. 6b). The SEM images (Fig. 6c) showed the microalga and EPS agglomerates (homo-aggregates), with EPS creating a viscous mesh. As previously indicated by fluorescence microscopy, Tetraselmis sp. tends to colonize and coat the microplastics, with no microplastics being visibly aggregated on the surface.

In the case of Gloeocapsa sp., the SEM images (Fig. 6d) exhibited a fibrous, dense and viscous nature of its EPS. The SEM micrographs also showed that this microalga has the potential to colonize, aggregate and adhere microplastics to its surface, independent of its fraction size. In detail, Fig. 6d1 reveals the colonization potential of Gloeocapsa sp., as well as the ability to form hetero-aggregates, using microplastics as support.

4. Discussion

Following on the hypothesis that hetero-aggregation could be linked to cell physiology and EPS production (Lagarde et al., 2016), this study characterized and differentiated the interactions between different freshwater/marine microalgae and two types of microplastics. It aimed to assess the potential of microalgae to be used in water treatment plants based on their capability to produce EPS with their sticky properties. The hetero-aggregation observed in the present study between microplastics, microalgae and EPS is in line with recent field observations that suggest that hetero-aggregation is species specific (Long et al., 2017) and dependent on the polymer type (Lagarde et al., 2016).

4.1. Microplastics effect on microalgal growth

The results of this study showed that under our experimental conditions (12.5 mg L⁻¹ for the lower concentration and 125 mg L⁻¹...
for the higher concentration), both Microcystis panniformis and Scenedesmus sp. cultures with microplastics included in culture medium, presented lower cell abundance when compared with the controls. These results could be explained by (i) the shading effect of the microplastics (reducing the access of microalgae to light), affecting the photosynthesis negatively (Sjollema et al., 2016), and/or (ii) the adsorption effect of the microplastics to the microalgae cells, which cause mobility reduction and consequently the decrease in microalgae growth (Davarpahan & Guilhermino, 2015). However, for Microcystis panniformis the higher depression in cell abundance was observed in the cultures with low microplastic concentration. The formation of micro-scale microplastic aggregates is an important factor when determining if the interactions and growth inhibition takes place (Bergami et al., 2017). Long et al. (2017) showed that microplastics tend to form micro-aggregates, which are larger than the microplastic alone. In this present work, the original size of the microplastics tested were 0–250 μm, but it was observed by microscopic analysis that larger particles were formed due to micro-aggregation, with smaller particles being more likely to interact and affect microalgae mechanisms. And therefore, these particles were more unlikely to adsorb onto smaller microalgae cells. Considering that microplastics in low concentrations tend to aggregate less, the adsorption to microalgae cells will be greater, reducing the mobility and consequently the microalgae growth. In the case of Scenedesmus sp., this would not affect its processes, since it is a non-moving microalga. For the marine Tetraselmis sp., no significant effect was observed for both the microplastics concentration condition. This resistance may be partially explained by the fact that Tetraselmis sp. presents a thick, rigid and complex cell wall (Domozych et al., 1981), that acts as an important barrier to microplastic interactions. Moreover, Tetraselmis sp. is a resistant species that exhibits an ease to grow even in unfavourable conditions (Fabregas et al., 1984; Michels et al., 2014).

Considering the obtained results, it can be inferred that the interactions between microplastics and microalgae varies with microalga size and species characteristics, namely mobility and cell wall composition.

4.2. EPS influence and hetero-aggregates formation

All genus of microalgae used in this study are known to be EPS producers (Zhu et al., 2014; Li et al., 2015; Passow, 2002; Sharma et al., 2008). The freshwater microalga Microcystis panniformis and Scenedesmus sp. produced a smaller quantity of EPS compared to the marine microalgae Tetraselmis sp. and Gloeocapsa sp.

Although for Microcystis panniformis there was a considerable production of EPS (0.047 ± 0.019 mg mL⁻¹), EPS were small, easily disaggregable and the abundance of hetero-aggregates was low. The smaller microplastics sized fraction (<106 μm) and the high density purple PMMA were the most commonly found, agreeing with the small size of the EPS observed. The apparent low EPS viscosity observed while handling Microcystis panniformis cultures may play a crucial role in explaining not only the low rate of microplastics aggregation but also why this microalga could not aggregate the low density small sized blue PS. SEM images of Microcystis panniformis (Fig. 6a) support the fact that this microalga is too small to aggregate microplastics much bigger than 25 μm, but still retains the ability to heavily colonize the microplastics, for this reason microplastics could not be observed in Fig. 4a. Colonization of microplastics by microalgae leads to an increased plastic density (Lagardé et al., 2016). Scenedesmus sp. showed a good amount of EPS production (0.012 ± 0.002 mg mL⁻¹), with satisfactory characteristics for aggregation of smaller sized microplastics. Although the production of EPS from Scenedesmus sp. was lower than Microcystis panniformis EPS production, Scenedesmus sp. exhibited a greater abundance of overall aggregates. The fact that only the smaller sized fractions (<106 μm) of both types of microplastics were aggregated reinforces the above referred, about the
EPS produced by *Scenedesmus* sp., not being able to aggregate microplastics bigger than the size of the exopolymer produced. For this microalga, the type of microplastic is not a determining factor for aggregation, but rather for its size. SEM images of *Scenedesmus* sp. (Fig. 6b) confirmed the aggregation and adhesion of microplastics (arrows). This corroborates the macroscopic observations made in Fig. 4b, with visible hetero-aggregates at the bottom of the flasks. And therefore, indicates the higher stability of the EPS produced by *Scenedesmus* sp. in comparison with the EPS produced by *Microcystis panniformis*, evidenced by the abundance and size of its EPS. This aspect benefits the capture and consequent microplastics aggregation.

In the case of *Tetraselmis* sp., the production of EPS was higher than any of the freshwater microalgae (1.287 ± 0.090 mg mL⁻¹). *Tetraselmis* sp. exhibited a different type of EPS, with more favourable characteristics for the aggregation of smaller sized microplastics.

Consequently, this greater production embodies a higher microplastics aggregation, when compared with *Microcystis pan-

niformis* and *Scenedesmus* sp. The way the microplastics are aggregated reveal a tendency for *Tetraselmis* sp. to aggregate, colonize and completely coat the microplastics that it cannot fully aggregate, contributing to its increase in density and consequent sinking, as shown in Figs. 5f and 6e. These observations also justify those made in Fig. 4c, with the lack of visible hetero-aggregates in the bottom of the flask.

*Gloeocapsa* sp. is a microalgae different from the others under observation, due to its non-dissociable, abundant, dense, thick and viscous EPS. Of all microalgae studied, *Gloeocapsa* sp. proved to be the best microalgae for both EPS production (2.146 ± 0.093 mg mL⁻¹) and microplastics aggregation. The results provided by the fluorescence (Fig. 5g and g1) and SEM (Fig. 6d and d1) micrographs reinforce the observations made in the culture flasks (Fig. 4d), that *Gloeocapsa* sp. has the capability to aggregate both high and low concentration and density microplastics. Furthermore, both the size fractions, contribute to an observable and pronounced vertical deposition of every type and size of microplastics that were studied.

According to the aggregation observed in the present study between microplastics and microalgae, we may advocate that this hetero-aggregation is species specific, depending on the size, strength, viscosity of the microalgal EPS produced. Since the EPS structural and chemical properties were not measured in this work, further studies are required to test the hypothesis that EPS structure and chemical properties (namely viscosity) can be correlated with hetero-aggregation. Results of the present study also showed that the concentrations of microplastics used did not influence the amount of hetero-aggregates formed. However, depending on the microalga species, microplastics concentration may affect growth as shown previously by other authors (see also Sjollema et al., 2016; Long et al., 2017; Lagarde et al., 2016), alongside the EPS production. Furthermore, the results highlight the potential for microalgae to be explored as biosolutions for the removal of microplastics in marine, freshwater or urban waters, due to its aggregation capabilities.

5. Conclusions

The aim of this study was to evaluate the relation between EPS and hetero-aggregation, and also characterize and differentiate the interactions between marine/freshwater microalgae and microplastics, with distinct sizes, shapes, densities and concentrations. And to explore them as possible biosolutions for the elimination/removal of microplastics from aquatic systems. The obtained results showed that:

- the effect of microplastics in microalgae growth varies with the microplastic size and microalgal specie characteristics (cell wall and mobility);
- freshwater microalgae *Microcystis panniformis* and *Scenedesmus* sp., and marine microalgae *Tetraselmis* sp. and *Gloeocapsa* sp. have the potential to colonize microplastics and produce EPS capable of aggregating (adhering and incorporating) microplastics;
- there is a species-specific correlation between EPS yield, size and stability and the type/size of the microplastic when defining aggregation.
- in all microalgae studied, *Gloeocapsa* sp. proves to be the most suitable microalgae regarding EPS production and consequent microplastics (of different types, densities and size fractions) aggregation;
- the microplastics aggregation may contribute to the vertical transport from the water surface to the sediment of microplastics with different characteristics.

The results of this work anticipate the application of EPS, produced by microalgae, in microplastic aggregation to water treatment.

Acknowledgements

This work could not have been possible without the support of project REBECA (MAC/1.1a/060) in correlation with the Spanish Algane Bank (BEA). Observatory of Madeira (OOM) project (M1420-01-0145-FEDER-000001) is also acknowledged for the grant to Marisa Faria.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.envpol.2019.03.046.

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