Environmental Pollution 215 (2016) 331–339

Contents lists available at ScienceDirect

Environmental Pollution

journal homepage: www.elsevier.com/locate/envpol

Microplastic interactions with freshwater microalgae: Hetero-aggregation and changes in plastic density appear strongly dependent on polymer type☆

Fabienne Lagarde [a, *], Ophélie Olivier [a, b], Marie Zanella [b], Philippe Daniel [a], Sophie Hiard [b], Aurore Caruso [b]

[a] Institut des Molécules et des Matériaux du Mans, UMR CNRS 6283, Université du Maine, Avenue Olivier Messiaen, 72085 Le Mans Cedex, France
[b] Laboratoire Mer, Molécules, Santé (EA 2160), Université du Maine, Avenue Olivier Messiaen, 72085 Le Mans Cedex, France

ARTICLE INFO

Article history:
Received 23 February 2016
Received in revised form
2 May 2016
Accepted 3 May 2016
Available online 26 May 2016

Keywords:
Hetero-aggregates
Microplastics
Microalgae
Polypropylene
Polyethylene
Plastic pollution

ABSTRACT

In this study, the interactions between microplastics, chosen among the most widely used in industry such as polypropylene (PP) and high-density polyethylene (HDPE), and a model freshwater microalgae, *Chlamydomas reinhardtii*, were investigated. It was shown that the presence of high concentrations of microplastics with size >400 μm did not directly impact the growth of microalgae in the first days of contact and that the expression of three genes involved in the stress response was not modified after 78 days. In parallel, a similar colonization was observed for the two polymers. However, after 20 days of contact, in the case of PP only, hetero-aggregates constituted of microalgae, microplastics and exopolysaccharides were formed. An estimation of the hetero-aggregates composition was approximately 50% of PP fragments and 50% of microalgae, which led to a final density close to 1.2. Such hetero-aggregates appear as an important pathway for the vertical transport of PP microplastics from the water surface to sediment. Moreover, after more than 70 days of contact with microplastics, the microalgae genes involved in the sugar biosynthesis pathways were strongly over-expressed compared to control conditions. The levels of over-expression were higher in the case of HDPE than in PP condition. This work presents the first evidence that depending on their chemical nature, microplastics will follow different fates in the environment.

© 2016 Elsevier Ltd. All rights reserved.

1. Introduction

After less than a century of existence, plastic debris currently represents from 60 to 80% of all marine litter (Derraik, 2002). Plastic is a generic name for a very wide range of synthetic materials essentially constituted by organic polymeric chains. Nowadays, the most commonly used plastic materials are polyethylene (PE), polypropylene (PP), polyvinyl chloride (PVC), polystyrene (PS) and polyethylene terephthalate (PET). They represent approximately 90% of total world production (Andrady and Neal, 2009). They are elements of high molecular weight, non-biodegradable and therefore extremely persistent in the environment. After long residence times in the environment, plastic items undergo fragmentation processes (Andrady, 2011). The fragmentation of macro-debris constitutes the secondary source of plastic particles arriving in the environment. Plastic pellets, fibers and fragments originating directly from industry and domestic sewers are classified as the primary source. Among the fragments constituting aquatic litter, microplastics are defined as particles of plastic whose dimensions are less than 5 mm (Imhof et al., 2013). As plastic items have been accumulating in the environment for the last four decades, the presence of plastic particles has recently been reported in all waters around the world from the Arctic (Lusher et al., 2015) to the Mediterranean Sea (Alomar et al., 2016) and the Pacific Ocean (Benton, 1995; Gregory, 1999) and also in the continental rivers and lakes (Imhof et al., 2013; Mc Cormick et al., 2014; Wagner et al., 2014; Klein et al., 2015). Despite this worldwide dissemination of plastic fragments, the global load of plastic on the open ocean surface has been estimated to be far less than expected, leading to the conclusion that microplastics are still far from being well-understood (Cozar et al., 2014). While the long-term behavior of

☆ This paper has been recommended for acceptance by Maria Cristina Fossi.

* Corresponding author.
E-mail address: fabienne.lagarde@univ-lemans.fr (F. Lagarde).

http://dx.doi.org/10.1016/j.envpol.2016.05.006
0269-7491/© 2016 Elsevier Ltd. All rights reserved.

these particles is still unclear, laboratory experiments can help to elucidate the fate of plastic pollution. In recent works (Andrady, 2011; Law et al., 2010; Cozar et al., 2014), several possible sinks of microplastics have been proposed: shore deposition, nano-fragmentation, biofouling and ingestion. Microplastics have also been found in sediments including deep-sea sediments (Thompson et al., 2004; Van Cauwenberghe et al., 2015a) and their presence may represent a major sink for plastic debris (Woodall et al., 2014). Similarly, in freshwater, microplastics have been found in sediments, such as those of an alpine lake beach (Imhof et al., 2013). However, the dynamics through which polymer fragments less dense than water (polyethylene, polypropylene …) descend to the bottom of the water column are still unclear. It is well-known that all types of solid materials can be rapidly colonized in an aqueous environment by macro and microorganisms (bacteria, microalgae, macroalgae, fungi). As an example of solid substrate, in a natural environment plastic debris have been shown to undergo rapid biofouling (Ye and Andrady, 1991; Lobelle and Cunliffe, 2011). In addition, recent studies have demonstrated that milli- and microplastic fragments sampled from the environment constitute a new habitat for highly diversified organisms both in oceanic waters (Zettler et al., 2013; Reisser et al., 2014) and in freshwaters (Mc Cormick et al., 2014). Large-scale colonization of plastics can play a significant role in their buoyancy as biofouling can lead to increased polymer fragment density (Ye and Andrady, 1991; Moret-Ferguson et al., 2010). However, the changes in polymer densities appear highly variable depending upon seasons, colonizing species and polymer type (Artham et al., 2009). Better understanding of the processes of microplastic biofouling responsible for their negative buoyancy in environmental waters should help to determine the long-term behavior of plastic debris and its distribution in aquatic compartments. In this work, the colonization of microplastics by freshwater microalgae and their aggregation were investigated for two types of polymers. The consequences of the interactions between microplastics and microalgae were studied. Freshwater and freshwater organisms are not often studied in the context of plastic pollution even though it is likely that some plastic garbage will first enter the environment through rivers. *Chlamydomonas reinhardtii* is a common freshwater microalga that is known to acclimate itself to environmental changes including pollution (Zhou et al., 2014; Korkaric et al., 2015). The impact of microplastic presence on these microalgae has been investigated. We studied microalgae growth via measurement of cellular density. The expression of their genes linked to the stress response and apoptosis pathway was also evaluated by molecular biology techniques. High-density polyethylene (HDPE) and polypropylene (PP) were chosen as model polymers as they are two of the most widely produced polymers every year. Changes in their surface chemistry were monitored during their contact with microalgae via contact angle measurement. Colonization of both polymers by microalgae was followed by microscopy.

2. Material and methods

2.1. Microplastic preparation

A disposable PP cup lid and a HDPE container for liquid were washed with demineralized water and cut into 1 cm squares using scissors. The polymer squares were then separately fragmented by high-energy milling (2 cycles of 2 min at 15 strikes per second) in order to generate fragments. The milling was processed at liquid nitrogen temperature to decrease polymer elasticity. Generated fragments were sieved on stainless steel screens to isolate those ranging in size from 400 μm to 1000 μm, the size range that is the most widely sampled in the environment (Masura et al., 2015). The fragments were observed by conventional microscopy to determine their shapes and their chemical nature was verified by infrared spectroscopy using a Vertex 70 (Bruker®). Infrared spectra were also produced to verify the presence of polymers in the hetero-aggregates. The spectra were recorded in Attenuated Total Reflection (ATR) mode with an average of 4 scans.

2.2. Culture conditions and algal growth

Chlamydomonas reinhardtii CC-2935 wild type mt- [Quebec] (*Chlamydomonas* Resource Center; Sack et al., 1994; Pröschold et al., 2005) was grown in 250-mL Erlenmeyer flasks containing 100 mL of TAP medium (Gorman and Levine, 1965) at 24 ± 1 °C. The flasks were placed under an irradiance of 140 μmol photons m^{-2} s^{-1} (Li-Cor quantum meter Li-189) supplied by cool-white fluorescent tubes (Philips TLD 36 W) in a 14 − 10 h light-dark cycle. The Erlenmeyer flasks were randomized each day throughout the experiment in order to receive the same irradiance. The cultures were maintained in suspension using an orbital shaker at 100 rpm (VWR, Model 5000). The initial cell concentration was 6×10^6 cells mL^{-1}. After 7 days, concentration had decreased to 4×10^6 cells mL^{-1}. After that, 100 mg of HDPE or PP fragments were added to the cultures, always at the same irradiance and under agitation. Growth was monitored using a Malassez type hemocytometer every 15 days for two and a half months. Cultures were maintained in the exponential growth phase by diluting with 50 mL of fresh medium after 48 days. Each condition (Control, HDPE and PP) was performed in triplicate (details in Supplementary data – Fig. S1). Because the number of repetitions was low, a Student's statistical *t*-test was performed ($P < 0.05$). At the end of the experiment, 5 mL of each of the 9 cultures was sampled by filtration, frozen in liquid nitrogen and stored at −80 °C until DNA and RNA extraction.

2.3. Genomic DNA extraction, amplification and sequencing

To verify the primer pairs, which were to be used later in real time PCR, DNA was extracted from 5 mL of one of the control cultures as described by Doyle and Doyle (1990). The DNA sample was dissolved in 80 μL water and a 100 ng/μL solution was used as a matrix for PCR. The partial genomic DNA sequences of 13 genes (six chloroplastic genes, two genes related to oxidative stress, one gene involved in apoptosis and four genes involved in extracellular polysaccharide biosynthesis; (Supplementary data - Table S2)) were obtained by the PCR procedure. Primer sequences were designed with the Primer3-Plus software (http://www.bioinformatics.nl/cgi-bin/primer3plus/primer3plus.cgi) to be used in a second part in real-time PCR to control gene expression. Thirty cycles consisting of denaturing for 30 s at 94 °C, annealing for 1 min at 60 °C, and extension for 40 s at 72 °C were performed. The reaction was completed by an extension step at 72 °C for 10 min. The PCR was performed with 0.2 μM of each primer, 100 ng of gDNA and 2.5 units (U) of *Thermus aquaticus* (*Taq*) DNA polymerase (Promega). PCR products were cloned into pGEM-T vector (Promega, Madison WI). The ligation productions were transformed into *Escherichia coli* DH5α and sequenced by the Cogenics-Genome Express Company (England, http://www.cogenics.com).

2.4. RNA extraction, RT-PCR and real-time PCR amplification

Total RNAs were extracted from the 5 mL filtered culture for each condition and triplicate samples. All RNA extractions were treated with 2 U of DNase (Promega) to avoid any gDNA contamination and then resuspended in 50 μL of DEPC-treated water. To avoid differences between the samples in quantities of RNA, the

Download English Version:

https://daneshyari.com/en/article/6316110

Download Persian Version:

https://daneshyari.com/article/6316110

Daneshyari.com