aeet
ASOCIACIÓN ESPAÑOLA DE ECOLOGÍA TERRESTRE

Ecosistemas 29(3): 2069 [Septiembre-Diciembre 2020]
https://doi.org/10.7818/ECOS.2069

MONOGRÁFICO:
Plásticos en ecosistemas acuáticos: presencia, transporte y efectos
Editores: Gissell Lacerot, Juan Pablo Lozoya y Franco Teixeira de Mello

ecosistemas
REVISTA CIENTÍFICA DE ECOLOGÍA Y MEDIO AMBIENTE

ISSN 1697-2473
Open access / CC BY-NC 3.0
disponible en www.revistaecosistemas.net

Study of the plastisphere: biofilm development and presence of faecal indicator bacteria on microplastics from the Río de la Plata estuary

R. S. Pazos[1,2,*], J. C. Suárez[1,2], N. Gómez[1,2]

(1) Instituto de Limnología "Dr. Raúl A. Ringuelet", UNLP-CONICET (CCT La Plata), CC 712, 1900 La Plata, Argentina.
(2) Consejo Nacional de Investigaciones Científicas y Técnicas (CONICET), Argentina.

* Autor de correspondencia: R. Soledad Pazos [rpazos@ilpla.edu.ar]

> Recibido el 10 de agosto de 2020 - Aceptado el 16 de septiembre de 2020

Pazos, R.S., Suárez, J.C., Gómez, N. 2020. Study of the plastisphere: biofilm development and presence of faecal indicator bacteria on microplastics from the Río de la Plata estuary. *Ecosistemas* 29(3): 2069. https://doi.org/10.7818/ECOS.2069
Microplastics (MPs) are considered emerging pollutants and often enter aquatic ecosystems carried by currents and tides until they accumulate on the shorelines. In many cases they may be colonized by diverse microorganisms forming a community called plastisphere, which can even act as a reservoir for pathogenic microorganisms. This study carried out in the Río de la Plata estuary (southern coastal fringe, Argentina) focused on two main objectives, the analysis of the biofilm colonizing MPs under laboratory conditions, and the detection of bacteria indicating faecal contamination (*Escherichia coli* and Enterococci), in MPs from the intertidal sediment at coastal sites with different land uses, in the freshwater sector of the Río de la Plata estuary. The colonization experiment was carried out in the laboratory with water from the estuary for a period of 35 days (residence time of the water in the freshwater sector of the estuary). The results revealed a remarkable development and diversity of biofilm organisms from the second week of colonization on, covering the surface of the microplastic and thus masking this pollutant. On the other hand, the presence of faecal indicator bacteria in the MPs of the intertidal sediment was confirmed in all the studied sites, being proportionally higher on MPs found in areas influenced by sewage discharges

Keywords: contaminants; plastics; colonization; *Escherichia coli*

Pazos, R.S., Suárez, J.C., Gómez, N. 2020. Estudio de la plastisfera: desarrollo del biofilm y presencia de bacterias indicadoras fecales en microplásticos del estuario del Río de la Plata. *Ecosistemas* 29(3): 2069. https://doi.org/10.7818/ECOS.2069
Los microplásticos (MPs) son considerados contaminantes emergentes y suelen ingresar en los ecosistemas acuáticos donde son transportados por las corrientes y mareas hasta acumularse en las costas. En muchos casos éstos pueden ser colonizados por diversos microorganismos conformando una comunidad denominada plastisfera y hasta pueden actuar como reservorio de microorganismos patógenos. En el estuario del Río de la Plata (Franja Costera Sur, Argentina), se realizó un estudio cuyo objetivo fue analizar el biofilm que se desarrolla sobre MPs en condiciones de laboratorio y analizar la presencia de bacterias indicadoras de contaminación fecal (E*scherichia coli* y Enterococos) en MPs hallados en el sedimento intermareal en sitios costeros con diferentes usos del suelo, en el sector de agua dulce del estuario del Río de la Plata. La experiencia de colonización se realizó en laboratorio con agua procedente del estuario durante un período de 35 días (tiempo de residencia del agua en el sector de agua dulce del estuario). Los resultados revelaron un notable desarrollo y diversidad de organismos del biofilm a partir de la segunda semana de colonización, recubriendo la superficie del microplástico y enmascarando así a este contaminante. Por otra parte, se confirmó la presencia de bacterias indicadoras fecales en los MPs del sedimento intermareal en todos los sitios estudiados, siendo proporcionalmente mayor en los MPs hallados en áreas influenciadas por descargas cloacales.

Palabras clave: contaminantes; plásticos; colonización; *Escherichia coli*

Introduction

Currently, plastic contamination is a matter of great concern since they have reached a volume of approximately 8 million tons per year in the oceans (Rodrigues, Duarte, et al. 2019). A particular type of contaminant within plastic litter is microplastics (MPs), which are pieces smaller than 5 mm (Arthur et al. 2009), defined as a heterogeneous mixture of differently shaped materials (EFSA 2016), and considered by the United Nations Environment Program as one of the 10 emerging problems, because they are widely distributed in many ecosystems around the world (Lithner 2011).

Once inside ecosystems, organisms interact with MPs, being able to ingest them or colonize their surfaces. In the latter case, MPs in aquatic environments can serve as substrates for various microorganisms, that is, in addition to accumulating organic pollutants, microplastic surfaces can be colonized by microbial communities, that form a biofilm (Zettler et al. 2013; McCormick et al. 2014; Oberbeckmann et al. 2014; De Tender et al. 2015; Hoellein et al. 2017; Dussud et al. 2018). The biofilm developed on plastic is called "plastisphere" (Zettler et al. 2013) and that surface represent a different habitat for the development of the microbial community.

The biofilms that colonize plastic exposed to the marine environment are mainly modulated by biogeographic and environmental factors, such as salinity and the concentration of nutrients in the water (Amaral-Zettler et al. 2015; Oberbeckmann et al. 2018). Microplastic surfaces themselves also influence colonization processes, since some organisms in the plastisphere could use plastic as an energy source due to their ability to degrade highly complex biopolymers such as lignin and petroleum derivatives (Zettler et al. 2013; Oberbeckmann et al. 2016; Ogonowski et al. 2018).

Recently, the potential danger of microbial communities associated with MPs has begun to be studied, since the role of MPs as carriers of antibiotic resistance genes has been discussed (Arias-Andres et al. 2018). These authors analysed the permissiveness of aquatic bacteria towards a model antibiotic resistance plasmid, comparing the organisms of biofilms on MPs *vs.* those that are free-living. They observed that a horizontal gene transfer in this habitat could distinctly affect the ecology of aquatic microbial communities on a global scale. In addition to the role of MPs as carriers of antibiotic resistance genes, they could act as vectors that favour the distribution of possible pathogen agents from areas of wastewater discharge to aquatic ecosystems not affected by such contamination (Oberbeckmann et al. 2015). Pathogens such as members of the genus *Vibrio*, have been reported as abundant on MPs (Zettler et al. 2013; Frère et al. 2018). Likewise, the biofilm that colonizes the surface of the MPs could act as a reservoir for indicators of faecal contamination, such as *Escherichia coli*. Therefore, it is essential to better understand the potential of MPs to facilitate the survival of these organisms and, therefore, to increase exposure routes in humans by providing a vehicle for dispersal in coastal waters (Rodrigues, Oliver, et al. 2019).

Most papers on plastisphere are focused on marine environments, but it is important to study them in estuarine ecosystems due to the services provided by these environments as well as to the large load of contaminants that reach their coasts. Specifically, in South America, most of them suffer the consequences of urban and industrial centres settled on their margins, the expansion of agriculture and aquaculture, water extraction, wastewater discharge, and the entry of various contaminants, among which are the MPs (Barletta et al. 2019). In Argentina, the Río de la Plata estuary is part of Del Plata basin, which is the second largest in South America (Mianzan et al. 2001), and is an important water resource that provides different ecosystem services for the region. This resource is the main source of drinking water and provides services such as fishing, recreational and navigation activities, but it also receives agricultural runoff, industrial discharges and sewage (Gómez et al. 2012; Gómez and Cochero 2013).

Microplastics have been recorded in the water column of the Río de la Plata estuary integrating the plankton community, in fish assemblages and in mussels of the species *Limnoperna fortunei* (Pazos et al. 2017, 2018, 2020). However, the colonization dynamics of the biofilm on MPs is still unknown, as well as whether they can act as a substrate for organisms that indicate faecal contamination. In this sense, the goals of this study are to analyse the colonization dynamics of the microbial biofilm that develops on the surface of the MPs under laboratory conditions, and to analyse the presence of bacteria indicating faecal contamination (*Escherichia coli* and Enterococci) on MPs found in the intertidal sediment at coastal sites with different land uses in the freshwater sector of the Río de la Plata estuary.

Materials and methods

Study area

The Río de la Plata receives the discharge from the Paraná and Uruguay rivers, which with an average annual flow of 22 000 m^3 s^{-1} provide more than 97% of the inland water intake. Its circulation pattern is modulated by ocean, river and atmosphere forcings (Fossati and Piedra Cueva 2013). According to its geomorphology and dynamics, the estuary is divided into two regions: interior (freshwater) and exterior (mixohaline). These regions are separated by a geomorphological barrier named Barra del Indio (which extends along a line from Punta Piedras (Argentina) to Montevideo (Uruguay), 6.5-7 m deep (FREPLATA 2005). This barrier, together with the isohaline of 0.5 UPS (1000 µS cm^{-1}) form the boundary between freshwater (37% of the surface of the estuary) and the brackish zone (Urien 1972).

To analyse the presence of bacteria indicating faecal contamination, seven sampling sites were selected in the freshwater sector of the estuary covering 120 km of the Argentine coast (34° 42' 24" S, 58° 13' 48" W and 35° 16´ 37" S, 57° 13´ 26" W), exposed to different land uses (Gómez and Cochero 2013). In Quilmes site (QUI), recreational and fishing activities are carried out. It is exposed to the impact of the city of Buenos Aires, and downstream the discharge from a highly polluted basin such as the Matanza-Riachuelo River. Berazategui site (BE) is located near the sewage effluent of the city of Buenos Aires, and Punta Colorada site (PC) is located downstream. In Punta Lara site (PL) mostly recreational and fishing activities are carried out. Bagliardi site (BAG) is located in the area surrounding the sewage effluent of the city of La Plata, and Balandra site (BAL) is located downstream. The southernmost site of the study area is Punta Indio (PI), which is the closest to the Maximum Turbidity Front of the estuary with salinity close to 10 PSU (Licursi et al. 2010).

Laboratory experiment

To analyse the colonization of the biofilm on MPs under laboratory conditions, water was extracted from the PL site, and refrigerated during its transport to the laboratory. Before starting the bioassay, all the materials were autoclaved. Eighteen glass jars with 60 ml of water from the sampling site were used, adding 20 spherical MPs (size: 3 mm, polymer: PE (polyethylene), color: pink and white) to each of them. The jars were placed in a shaker with a rotary movement at 150 mot min^{-1} under laboratory conditions (mean temperature 20º C, average light intensity of 790 µM m^2s^{-1}). The water was partially renewed once a week and the duration of the bioassay was 35 days, considering the residence time of the water in the freshwater sector of the estuary (FREPLATA 2005). For biofilm analysis, samples were extracted in triplicate on days 2, 7, 14, 21, 28 and 35 after the start of the test. The MPs were collected with entomological tweezers and placed in glass jars containing 5 ml of distilled water, which were sonicated in an ultrasound bath (Cleanson), for three periods of 30 seconds in order to release the biofilm.

Microorganisms analysis

In order to analyse the viable and non-viable bacteria contained in the biofilm, 0.5 ml of the sonication of MPs was used (in triplicate). For the analysis of bacterial viability, the kit LIVE/DEAD® *Bac*Light™ was used, which stains the nucleic acids of bacteria in fluorescent green (SYTO®9), both those with complete membranes and those with damaged membranes. In addition, the kit has another stain called propidium iodide (fluoresces in red) that penetrates only bacteria with damaged membranes, generating a reduction in the fluorescence of SYTO®9 when both stains are present. As a consequence, bacteria with intact membranes fluoresce in green (considered live) and bacteria with damaged membranes fluoresce in red (considered dead) (Sathicq and Gómez 2018).

The stain was prepared by dissolving in equal parts each of the mentioned stains in 5 ml of sterile milliQ water. Then the sample (0.5 ml) and the prepared staining (0.5 ml) were combined. Subsequently, the samples were incubated in the dark and at room temperature for a period of 15 minutes, before being filtered through black polycarbonate Gamafil filters (25 mm diameter and 0.2 µm pore). Filters were placed on slides with *Bac*Light mounting oil, and viewed under a direct microscope (Olympus BX50) at 1000x with eplifluorescence and an Olympus filter U-MWB2 (excitation filter BP 460-490; emission filter BA 520 IF; dichromatic filter DM 500) (Boulos et al. 1999). The count of bacteria in each filter was made from photos captured in 20 random fields, with an Olympus camera Q-Color 5 (Romaní and Sabater 2001). The count was carried out using the Image J program and the results are expressed in ind mm^{-2}.

The sample (in triplicate) for the count of microalgae, protozoans, and invertebrates was fixed with formalin (final concentration 4% [v/v]). For the count we used 1 ml obtained from the sonication of the MPs, which was analysed under an inverted microscope (Olympus IX51) with magnifications of 400x and 600x, in a 5 ml Utermöl sedimentation chamber. Lugol was added to the sample, once it was placed in the chamber and allowed to settle for twelve hours.

The individuals were counted by cells. In the case of the filamentous algae, the cells were measured, and the number of total cells was calculated according to the length of the filament. Taxonomic identification was carried out at the level of large groups: diatoms, chlorophytes, cyanobacteria, euglenophytes, chrysophyceaes, ciliates, rotifers and nematodes (Desikachary 1959; Olivier 1965; Bourrelly 1972; Streble and Krauter 1987; Tell and Conforti 1986; Krammer and Lange-Bertalot 1986, 1988, 1991a, 1991b; Komárek and Anagnostidis 1999, 2005; Coelho-Botelho 2003). Results were expressed as ind mm^{-2}.

Analysis of bacteria indicating faecal contamination on MPs

The sampling was carried out between November and December 2018. In each sampling site, 32 MPs were collected from the sediment along a transect parallel to the coastline, located in the area of maximum accumulation caused by high tide in the intertidal zone. All samplings were performed at low tide.

Materials and solutions were previously autoclaved. Each MP was collected with tweezers, gently washed with distilled water in order to remove the adhering sediment and placed in an Eppendorf tube with 500 µl of sodium pyrophosphate (dispersing solution). Tweezers were sterilized with alcohol 70% between each sample. MPs were refrigerated until their analysis which was carried out within 24 hours. The Eppendorf tubes containing the MPs as brought from the field were placed in a mechanical rotator for half an hour to detach the bacteria from the MPs. Each tube was then vorterized to homogenize the sample, prior to pouring it into the microtubes of the microtiter plates. Later, 200 µl of each the sample was pipetted into a well with differential culture medium for the detection of *E. coli* and another 200 µl of the same sample in a well with specific culture to detect Enterococci. This procedure was performed with each of the MPs extracted in the field, so 32 well were incubated for each sampling site. After incubation of the plates at 44° C for 48 to 72 hours, the plates were read under UV light of 266 nm wavelength. In this way, it was recorded which well showed fluorescence, meaning a positive result with bacterial growth (presence) and which ones did not fluoresce, being a negative result, without bacterial growth (absence). Results were calculated as number of MPs with bacteria (positive well) / number of total MPs (total well = 32) for each site, expressed as percentages. Regarding the characteristics of the MPs found, colour, size and shape were recorded in order to classify them following the categories most commonly used: fragment, film, pellet and foam (Rezania et al. 2018).

Statistical analysis

The statistical analysis was performed in R version 3.5.1. One-way ANOVA analysis were performed to explore the differences in the density of microalgae, protozoans, invertebrates and bacteria between the different dates during the colonization experience in laboratory. The differences (significance level $p \leq 0.05$) were analysed post-hoc using the Fisher test.

Results

Laboratory experiment

The results revealed a remarkable development and diversity of biofilm organisms on the MPs throughout the 35 days of the test. By the second week, the density of organisms had increased one order of magnitude. In a first stage, bacteria, cyanobacteria and ciliates dominated, and towards the end, diatoms and rotifers together with a higher proportion of viable bacteria.

Within the heterotrophic component, the bacteria colonization at the beginning of the test (**Fig. 1.c**), was only 25% viable, but increased to 70% by day 35 (**Fig. 2.a**, **2.b** and **2.c**). No significant differences were observed in bacteria density ($p= 0.23$; $F= 1.59$; $df= 5$).

However, significant differences in the density of the autotrophic component were observed between the start and end of the experiment ($p<0.001$; $F= 12.01$; $df= 5$) (**Fig.1.a**), attributable to the greater development of diatoms (**Fig. 1.b**). In a more detailed analysis, diatoms, chlorophytes, euglenophytes and cyanobacteria were observed from day 2, the latter being dominant, while on day 7 the chrysophyceae were also observed and diatoms were the dominant group (represented mainly by *Cyclotella meneghiniana*, *Gomphonema parvulum*, *Nitzschia frustulum*, *Nitzschia levidensis* and *Nitzschia palea*) which continued to predominate until the end of the experiment. Towards the end of the test, the number of chlorophytes increased.

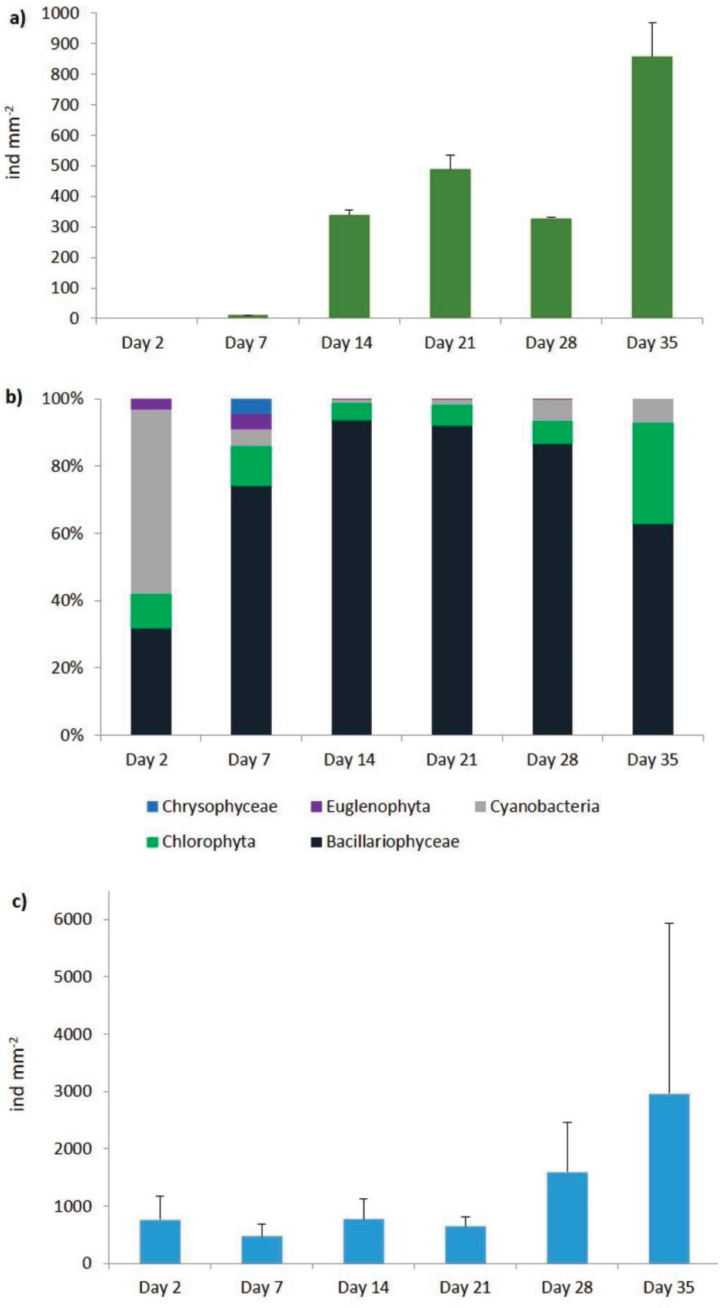

Fig. 1 (a) *Total density (and standard deviation) of the autotrophic component observed in the colonization experiment.* ***(b)*** *Percentage of the groups forming the autotrophic component (Chrysophyceae, Euglenophyta, Cyanobacteria, Chlorophyta and Bacillariophyceae) observed in the colonization experiment.* ***(c)*** *Density of total heterotrophic bacteria (and standard deviation) during the colonization experiment.*

Fig. 1 (a) *Densidad total (y desviación estándar) del componente autotrófico observado en la experiencia de colonización.* ***(b)*** *Porcentaje de los diferentes grupos del componente autotrófico (Chrysophyceae, Euglenophyta, Cyanobacteria, Chlorophyta and Bacillariophyceae) observado en la experiencia de colonización.* ***(c)*** *Densidad de bacterias heterotróficas totales (y desviación estándar) durante la experiencia de colonización.*

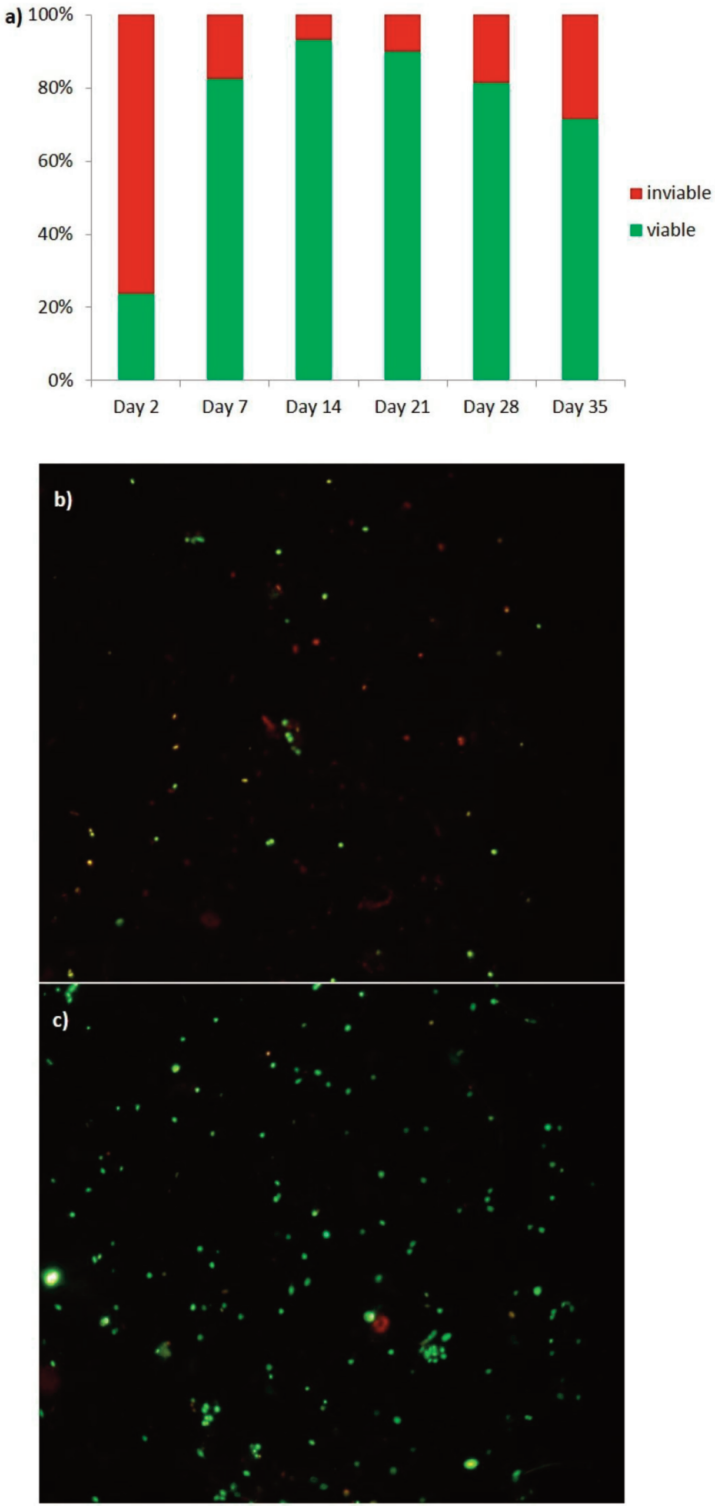

Fig. 2 (a) *Relative abundance of viable and inviable bacteria during the colonization experiment. Bacteria on day 2* ***(b)*** *and day 35* ***(c)*** *of the colonization experiment, observed with epifluorescence (1000X). Viable bacteria fluoresce in green and inviable bacteria fluoresce in red.*

Fig. 2 (a) *Abundancia relativa de bacterias viables e inviables durante la experiencia de colonización. Bacterias en el día 2* ***(b)*** *y en el día 35* ***(c)*** *de la experiencia de colonización observadas con eplifluorescencia (1000X). Las bacterias viables fluorescen en verde y las inviables en rojo.*

With regard to the protozoans and invertebrates observed, the density was lower than bacteria and the autotrophic component. The colonization was alternately by ciliates and rotifers, and from day 28 on, nematodes were observed. (**Fig. 3.a** and **3.b**). There were no significant differences in their densities during the experiment ($p= 0.24$; $F= 1.57$; $df= 5$).

Analysis of bacteria indicating faecal contamination on MPs

The analysis of bacteria indicative of faecal contamination revealed their presence in the MPs of the intertidal sediment of the seven sites. *E. coli* was recorded in all the sites, whereas Enterococci were found only in three of them (QUI, BE and BAG). The highest proportion of bacteria recorded in the MPs was found in site BAG, followed by QUI and BE. The frequency of *E. coli* on the MPs varied between 3.1% (PC and PL) and 50% (BAG). Whereas the frequency of Enterococci was much lower, varying from 6.2% (BE) to 15.6% (BAG) (**Fig. 4**). In addition, in those sites where the proportion of *E. coli* was lower than 6% (PC, PL, BAL and PI), the Enterococci were not recorded.

The MPs on which faecal indicators were analysed corresponded to four categories (**Fig. 5.a**): fragment (69.6%), film (20.5%), pellet (9.4%) and foam (0.4%). The size of the MPs was greater than 1000 µm, being the most frequent category >2500 ≤ 3000 µm (**Fig. 5.b**). Also, blue-coloured MPs were dominant (29%) followed by red (22%), followed by other colours in a smaller proportion (**Fig. 5.c**). Of the 224 MPs analysed, *E. coli* was recorded on 48 (21.4%) and Enterococci on 10 (4.5%). Considering only the MPs in which bacteria was found, *E. coli* was present on fragments, pellets and films, while Enterococci were observed on fragments and film. Neither of the two faecal contamination indicators were observed in foam. *E. coli* was present on MPs of different colours, unlike Enterococci were recorded only on blue, red and green MPs. Regarding the size of the MPs, both faecal indicators were present in various size categories (**Table 1**).

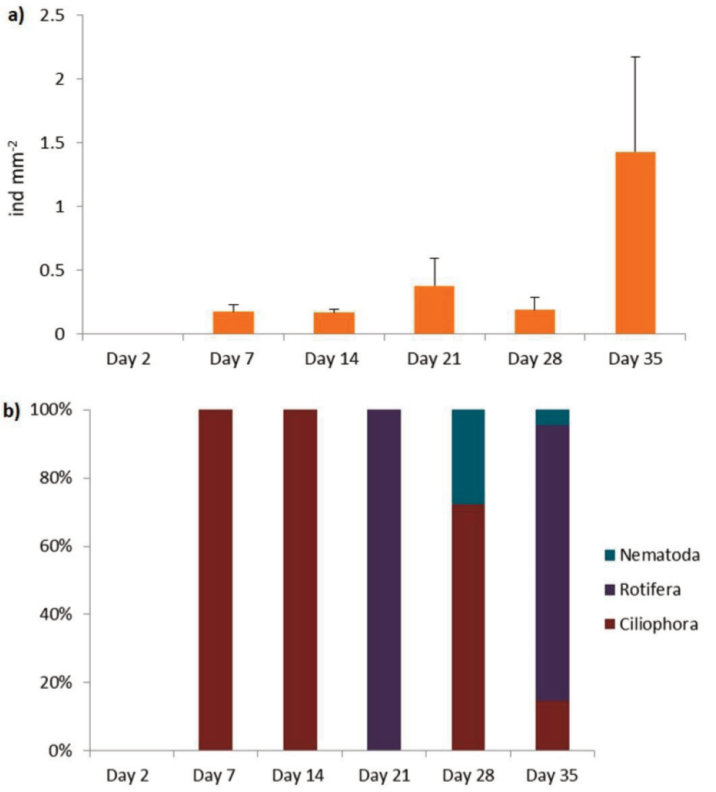

Fig. 3 (a) *Total density of ciliates, rotifers and nematodes (and standard deviation) during the colonization experiment.* ***(b)*** *Relative abundance of ciliates, rotifers and nematodes of the colonization experiment.*

Fig. 3 (a) *Densidad total de ciliados, rotíferos y nematodes (y desviación estándar) durante la experiencia de colonización.* ***(b)*** *Abundancia relativa de ciliados, rotíferos y nematodes de la experiencia de colonización.*

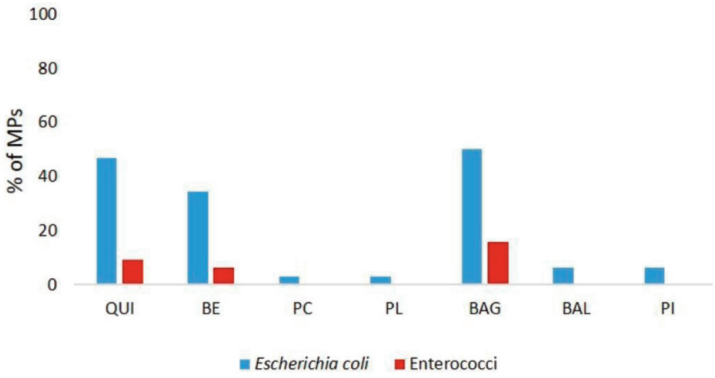

Fig. 4 *Percentage of MPs bearing Escherichia coli and Enterococci in the studied sites (QUI: Quilmes; BE: Berazategui; PC: Punta Colorada; PL: Punta Lara; BAG: Bagliardi; BAL: Balandra; PI: Punta Indio).*

Fig. 4 *Porcentaje de MPs con presencia de Escherichia coli y Enterococos en los sitios analizados (QUI: Quilmes; BE: Berazategui; PC: Punta Colorada; PL: Punta Lara; BAG: Bagliardi; BAL: Balandra; PI: Punta Indio).*

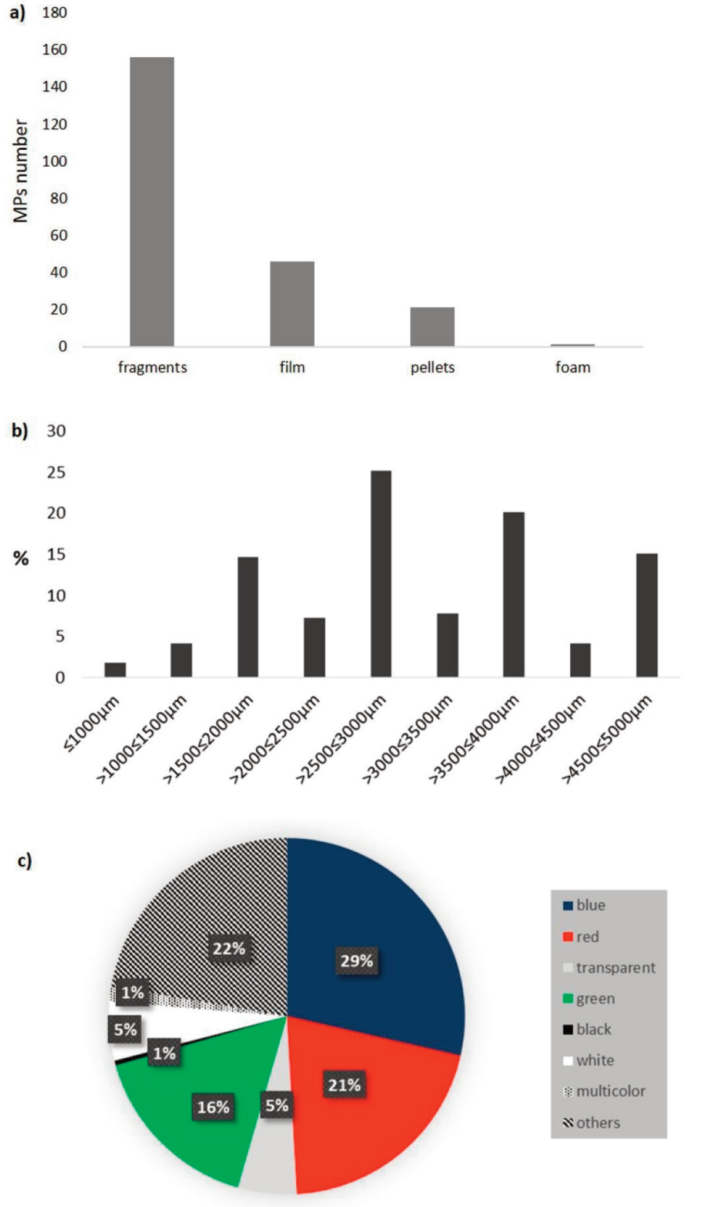

Fig. 5 (a) *Amounts of microplastics (MPs) types (fragments, film, pellets and foam) found in the intertidal sediments of the seven sites analysed.* ***(b)*** *Percentage of microplastics sizes found in the study area.* ***(c)*** *Percentage of microplastics colours found in the study area.*

Fig. 5 (a) *Cantidad de tipos de microplásticos (MPs) (fragmentos, film, pellets y foam) hallados en el sedimento intermareal de los siete sitios analizados.* ***(b)*** *Porcentaje de tamaños de microplásticos hallados en el área de estudio.* ***(c)*** *Porcentaje de colores de microplásticos hallados en el área de estudio.*

Table 1. *Characteristics (types, colours and sizes) and amount of microplastics (MPs) with Escherichia coli and Enterococci.*

Tabla 1. *Características (tipos, colores y tamaños) y cantidad de los microplásticos (MPs) en los que se registró presencia de Escherichia coli y Enterococos.*

Characteristics of MPs	MPs with *E. coli*	MPs with Enterococci
Types		
fragments	38	8
pellets	3	0
film	7	2
Colours		
blue	14	3
red	12	4
transparent	1	
green	11	3
white	2	
multicolor	1	
others	7	
Sizes		
>1000≤1500µm	2	
>1500≤2000µm	6	2
>2000≤2500µm	1	1
>2500≤3000µm	16	3
>3000≤3500µm	2	
>3500≤4000µm	11	3
>4000≤4500µm	4	
>4500≤5000µm	6	1

Discussion

Most published studies on plastisphere have examined community composition through colonization experiments (Amaral Zettler et al. 2020). The experimental designs in the literature have included suspension within the natural water column, in sediments, static laboratory systems in containers of various sizes with water collected once from the aquatic system of interest; different MPs types, sizes and polymers and various exposition times. The differences in laboratory conditions make it difficult to compare studies, but some specific examples provide evidence of common members of the plastisphere (Amaral Zettler et al. 2020). Despite the fact that experiments under laboratory conditions can only provide an abstraction of the complex ecology of a natural environmental system (Harrison et al. 2014), they provide an opportunity to explore the characteristics of the biofilm coating the MPs. In this sense, this study showed that the surface of the MPs was favourable for the attachment of biofilm organisms from the water from Río de la Plata estuary, in a short period of time. According to Amaral Zettler et al. (2020) the coverage increased rapidly for the first weeks and then stabilized (15–25% of the plastic surface for live cells).

The results demonstrated the fast colonization of microorganisms on MPs, as two days after the beginning of the experience a diversified community of organisms, belonging to different taxonomic groups of autotrophs and heterotrophs, was observed. Moreover, 35 days later the MPs had changed its original appearance by being covered by biofilm, similar to what is observed in natural substrates of the coast of the Río de la Plata estuary (Gómez et al. 2003; Bauer et al. 2007).

The heterotrophic bacteria were early colonizers, and the most abundant group of the plastisphere during the experiment. Bacterial adhesion is a highly controlled and regulated process by which adhering cells produce extracellular polymers to form structured and complex matrices (Costerton et al. 1995). Microbial biofilms can subsequently trigger the attachment of specific invertebrates and algae, which increases the degree of biofouling (Zardus et al. 2008).

According to studies by Foulon et al. (2016) and Harrison et al. (2014) microorganisms colonize plastic substrates within hours of their immersion in water, and after a week the biofilm is dominated by individual pennate and filamentous diatoms. In our study, diatoms were observed from the second day of the experiment on, and during the first week this taxonomic group came to represent more than 60% of the autotrophs of the plastisphere community, particularly pennate species tolerant to contamination and eutrophication in freshwater ecosystems (Licursi and Gómez 2004; Licursi et al. 2010). The diatoms are early and sometimes dominant colonizers in plastic debris (Costerton et al.1995; Kettner et al. 2019) and most studies have shown that are common and omnipresent residents of the plastisphere (Amaral Zettler et al. 2020). Cyanobacteria often join diatoms among the autotrophs that contribute to making net primary production positive on plastic substrates (Bryant et al. 2016). In our study, colonization and subsequent predominance of cyanobacteria was observed from day two. They were also present during the whole experiment, but in lower abundances.

Colonization by protozoans and invertebrates it was alternated between ciliates and rotifers, being the ciliates the early and dominant colonizers. These are also common taxa in the plastisphere, since ciliates are observed on MPs from marine and freshwater and/or brackish samples (Amaral Zettler et al. 2020).

Bacteria indicative of faecal contamination was found in MPs of the intertidal sediment, being their frequency higher in the sites where the sewage discharges were located or in the sites influenced by an intense urban activity, which demonstrates the sanitary risk of this contaminant. According to the data reported by Suárez and Mariñelarena (2019), the superficial sediments of the intertidal zone of the estuary retain and concentrate bacteria indicators of faecal contamination, particularly in areas affected by sewage discharges. Therefore, the results of the current study are in concordance with the ones provided by such authors, who warned that the BAG site presented the highest counts of bacteria indicating faecal contamination. As stated by Rodrigues, Oliver, et al. 2019, MPs deposited in the intertidal sediment can be easily colonized by *E. coli* during their time in the water column or by direct contact on the beach, either by bird or dog faeces. Furthermore, it is also recognized that plastic waste can be quickly colonized by biofilm in water (Amaral-Zettler et al. 2015). In the Río de la Plata estuary, with a semi-day tidal regime, the MPs deposited on the coast are submerged twice a day. Rodrigues, Oliver, et al. (2019) reported that in marine environments, the effect of the tide would favour the *E. coli* biofilm formation on the MPs, as these bacteria can remain on the beach or be dragged into the water with the ebb of tide.

Microbial colonization is proved to be influenced by the time that plastic has been in the environment, being those MPs that have remained for a longer time more likely to be in contact with microorganisms (Kirstein et al. 2018). In addition, older and more degraded plastics generally have grooves and tears in the surface, increasing the surface area for microbial colonization (Fotopoulou and Karapanagioti 2012). The types of MPs that usually have these characteristics in particular are fragments, since they frequently acquire irregular shapes as they weather in the environment. According to Puglisi et al. (2019) because of their higher level of wear, fragments favour colonization by bacteria. In our study, fragments were the most abundant among all the MPs, and this was the type of MPs in which the presence of *E. coli* and Enterococci was frequently recorded.

Regarding the potential of MPs to act as a substrate for various microorganisms, it is known that bacteria form biofilms on different substrates as a survival strategy against environmental stressors. This is because in biofilms they can use nutrients that have been trapped, resist antibiotics and establish associations with other bacteria (Thompson et al. 2004), being also a favourable habitat for pathogenic organisms (Kirstein et al. 2016; Rodrigues, Oliver, et al. 2019).

In relation to the size of the MPs, the most frequently colonized by both indicators of faecal contamination were between >2500 ≤ 3000 µm, and regarding colours, blue, red and green MPs. Among the latter, the blue colour has been reported as most abundant in water, the intestinal content of fish and the soft tissue of mussels in the coastal sector of the Río de la Plata estuary (Pazos et al. 2017, 2018, 2020). Although in this study it was not explored whether colour affects the degree of adherence of bacteria, there is evidence in the literature that dyes influence the type of bacterial assemblages found in MPs (Puglisi et al. 2019).

Recent studies by Miao et al. (2019) recognize that MPs act as a particular habitat for biofilm, since they can change the structure of this community by modifying its functionality and consequently, the ecological functions that microbial communities fulfil in aquatic ecosystems. In turn, it is recognized that biofilms developed on the MPs can produce significant changes in the physicochemical properties of the plastic (e.g. surface hydrophobia and buoyancy), which in turn are influenced by environmental characteristics (Lobelle and Cunliffe 2011).

Colonization on plastic polymers has advantages, such as an increased access to limited nutrients (Zobell 1943), but also challenges, like more susceptibility to grazing pressure. But still according to Amaral Zettler et al. (2020), some questions remain, like if the succession of species presence and dominance on a polymer is predictable, and if it can help us determine how long a microplastic has been in the environment. Therefore, deepening the knowledge of the relationship between the plastisphere and the MPs (results obtained under laboratory conditions in this study) along the environmental gradient generated by salinity in an estuary, such as the Río de la Plata, is a challenge to better understand the implications of the interaction of the biofilms with the pollutants of this ecosystem, as well as of the capacity to harbour pathogens.

Conclusions

The results revealed a remarkable development and diversity of biofilm organisms from the second week of colonization on, covering the surface of the microplastic and thus masking this pollutant. On the other hand, the presence of faecal indicator bacteria in the MPs of the intertidal sediment was confirmed in all the studied sites, being proportionally higher on MPs found in areas influenced by sewage discharges, positioning them as potential dispersal vectors. These results highlight the risks of plastic waste on the coast of the Río de la Plata estuary and the need of implementing management measures that regulate this contaminant.

Acknowledgements

Financial support for this study was provided by the Grant: 22920160100049CO (CONICET, Argentina). The authors would like to thank María Belén Sathicq and Joaquín Cochero for their contributions in the methodology of this study and Roberto Jensen for his assistance in the field. Finally, the authors would like to express their thanks to the editor and the anonymous reviewers for improvements in this manuscript. This paper is Scientific Contribution No 1186 of the Institute of Limnology "Dr. Raúl A. Ringuelet" (ILPLA)-CCT-LaPlata CONICET, UNLP.

References

Amaral-Zettler, L.A., Zettler, E.R., Slikas, B., Boyd, G.D., Melvin, D.W., Morrall, C.E., et al. 2015. The biogeography of the Plastisphere: implications for policy. *Frontiers in Ecology and the Environment* 13(10):541-546.

Amaral-Zettler, L.A., Zettler, E.R., Mincer, T.J. 2020. Ecology of the plastisphere. *Nature Reviews Microbiology* 1-13.

Arias-Andres, M., Klümper, U., Rojas-Jimenez, K., Grossart, H.P. 2018. Microplastic pollution increases gene exchange in aquatic ecosystems. *Environmental Pollution* 237:253-261.

Arthur, C., Baker, J., Bamford, H. 2009. *Proceedings of the International Research Workshop on the Occurrence, Effects and Fate of Microplastic Marine Debris*. NOAA Technical Memorandum NOS-OR & R-30. Disponible en: https://marinedebris.noaa.gov/proceedings-international-research-workshop-microplastic-marine-debris

Barletta, M., Lima, A.R., Costa, M.F. 2019. Distribution, sources and consequences of nutrients, persistent organic pollutants, metals and microplastics in South American estuaries. *Science of the Total Environment* 651:1199-1218.

Bauer, D.E., Gómez, N., Hualde, P.R. 2007. Biofilms coating *Schoenoplectus californicus* as indicators of water quality in the Río de la Plata Estuary (Argentina). *Environmental Monitoring and Assessment* 133:309–320.

Boulos, L., Prevost, M., Barbeau, B., Coallier, J., Desjardins, R. 1999. LIVE/DEAD® BacLight™: application of a new rapid staining method for direct enumeration of viable and total bacteria in drinking water. *Journal of microbiological Methods* 37(1):77-86.

Bourrelly, P. 1972. *Les algues d eau douce. Initiation à la systématique. I. Les algues vertes*. Boubée and Cie. Paris, France.

Bryant, J.A., Clemente, T.M., Viviani, D.A., Fong, A.A., Thomas, K.A., Kemp, P., et al. 2016. Diversity and activity of communities inhabiting plastic debris in the North Pacific Gyre. *MSystems* 1(3):e00024-16.

Coelho-Botelho, M.J. 2003. Dinâmica da Comunidade Zooplanctônica e sua Relação com o Grau de Trofia em Reservatórios. *V Simpósio e IV Reunião e Avaliação do Programa Biota/Fapesp. CETESB*. Disponible en: https://www.researchgate.net/publication/314279710_Dinamica_da_Comunidade_Zooplanctonica_e_sua_Relacao_com_o_Grau_de_Trofia_em_Reservatorios

Costerton, J.W., Lewandowski, Z., Caldwell, D.E., Korber, D.R., Lappin-Scott, H.M. 1995. Microbial biofilms. *Annual Review of Microbiology* 49:711–745.

Desikachary, T.V. 1959. Cyanophyta. Indian Council of Agricultural Research, New Delhi, India. 686 pp.

De Tender, C.A., Devriese, L.I., Haegeman, A., Maes, S., Ruttink, T., Dawyndt, P. 2015. Bacterial community profiling of plastic litter in the Belgian part of the North Sea. *Environmental science and technology* 49(16):9629-9638.

Dussud, C., Meistertzheim, A.L., Conan, P., Pujo-Pay, M., George, M., Fabre, P., Coudane, J., et al., 2018. Evidence of niche partitioning among bacteria living on plastics, organic particles and surrounding seawaters. *Environmental Pollution* 236:807-816.

EFSA 2016. Panel on Contaminants in the Food Chain. Presence of microplastics and nanoplastics in food, with particular focus on seafood. *EFSA J* 14(6):4501.

Fossati, M., Piedra-Cueva, I. 2013. A 3D hydrodynamic numerical model of the Río de la Plata and Montevideo's coastal zone. *Applied Mathematical Modelling* 37(3):1310-1332.

Foulon, V., Le Roux, F., Lambert, C., Huvet, A., Soudant, P., Paul-Pont, I. 2016. Colonization of polystyrene microparticles by Vibrio crassostreae: light and electron microscopic investigation. *Environmental science and technology* 50(20):10988-10996.

FREPLATA 2005. *Análisis Diagnóstico Transfronterizo del Río de la Plata y su Frente Marítimo. Protección Ambiental del Río de la Plata y su Frente Marítimo: Prevención y Control de la Contaminación y Restauración de Hábitats*. Documento Técnico. Proyecto PNUD/GEF RLA/99/G31. Montevideo, Uruguay.

Frère, L., Maignien, L., Chalopin, M., Huvet, A., Rinnert, E., Morrison, H., Kerninon, S., et al. 2018. Microplastic bacterial communities in the Bay of Brest: Influence of polymer type and size. *Environmental Pollution* 242:614-625.

Gómez, N., Licursi, M., Hualde, R.R. 2003. Epiphytic algae on the bulrush (*Scirpus californicus* (Mey) Steud) in the Río de la Plata (Argentina): structure and architecture. *Archiv für Hydrobiologie Supplementband* 147(3-4):231-247.

Gómez, N., Licursi, M., Bauer, D.E., Ambrosio, E.S., Capítulo, A.R. 2012. Assessment of biotic integrity of the coastal freshwater tidal zone of a temperate estuary of South America through multiple indicators. *Estuaries and coasts* 35(5):1328-1339.

Gómez, N., Cochero, J. 2013. Un índice para evaluar la calidad del hábitat en la Franja Costera Sur del Río de la Plata y su vinculación con otros indicadores ambientales. *Ecología Austral* 23:18-27.

Harrison, J.P., Schratzberger, M., Sapp, M., Osborn, A.M. 2014. Rapid bacterial colonization of low-density polyethylene microplastics in coastal sediment microcosms. *BMC microbiology* 14(1):1-15.

Hoellein, T.J., McCormick, A.R., Hittie, J., London, M.G., Scott, J.W., Kelly, J.J. 2017. Longitudinal patterns of microplastic concentration and bacterial assemblages in surface and benthic habitats of an urban river. *Freshwater Science* 36(3):491-507.

Fotopoulou, K.N., Karapanagioti, H.K. 2012. Surface properties of beached plastic pellets. *Marine environmental research* 81:70-77.

Kettner, M.T., Oberbeckmann, S., Labrenz, M., Grossart, H.P. 2019. The eukaryotic life on microplastics in brackish ecosystems. *Frontiers in microbiology* 10:538.

Kirstein, I.V., Kirmizi, S., Wichels, A., Garin-Fernandez, A., Erler, R., Löder, M., Gerdts, G. 2016. Dangerous hitchhikers? Evidence for potentially pathogenic Vibrio spp. on microplastic particles. *Marine environmental research* 120:1-8.

Kirstein, I.V., Wichels, A., Krohne, G., Gerdts, G. 2018. Mature biofilm communities on synthetic polymers in seawater-Specific or general? *Marine Environmental Research* 142:147-154.

Komárek, J., Anagnostidis, K. 1999. Cyanoprokaryota 1. Teil: Chroococcales. In: Ettl, H., Gärtner, G., Heyning, H., Möllenhauer, D. (eds.), *Süßwasserflora von Mitteleuropa* 19(3):1-548.

Komárek, J., Anagnostidis, K. 2005. Bd. 19/2. *Cyanoprokaryota: teil 2: Oscillatoriales*. Elsevier. München, Germany.

Krammer, K., Lange-Bertalot, H. 1986. Bacillariophyceae. 1. Teil: Naviculaceae. In: Ettl, H., Gerloff, J., Heynig, H., Mollenhauer, D. (eds.), *Süßwasserflora von Mitteleuropa*, Vol. 2/1. Gustav Fisher Verlag, Jena, Germany.

Krammer, K., Lange-Bertalot, H. 1988. Bacillariophyceae. 2. Teil: Bacillariaceae, Epithemiaceae, Surirellaceae. In: Ettl, H., Gerloff, J., Heynig, H. Mollenhauer, D. (eds.), *Süßwasserflora von Mitteleuropa,* Vol. 2/2. VEB Gustav Fischer Verlag, Jena, Germany.

Krammer, K., Lange-Bertalot, H. 1991a. Bacillariophyceae. 3. Teil: Centrales, Fragilariaceae, Eunotiaceae. In: Ettl, H., Gerloff, J., Heynig, H., Mollenhauer, D. (eds.), *Süßwasserflora von Mitteleuropa,* Vol. 2(3): 1-576*,* Gustav Fisher Verlag, Jena, Germany.

Krammer, K., Lange-Bertalot, H. 1991b. Bacillariophyceae. 4. Teil: Achnanthaceae. Kritische Ergänzungen zu Navicula (Lineolatae) und Gomphonema. Gesamtliteraturverzeichnis. Teil 1-4. In: Ettl, H., Gärtner, G., Gerloff, J., Heynig, H., Mollenhauer, D. (eds.), *Süßwasserflora von Mitteleuropa.* 2(4): 1-437. Gustav Fisher Verlag, Jena, Germany.

Licursi M., Gómez N. 2004. Aplicación de Indices bióticos en la evaluación de la calidad del agua en sistemas lóticos de la llanura pampeana a partir del empleo de diatomeas. *Biología Acuática* 21:31-49.

Licursi, M., Gómez, N., Donadelli, J. 2010. Ecological optima and tolerances of coastal benthic diatoms in the freshwater-mixohaline zone of the Río de la Plata estuary. *Marine Ecology Progress Series* 418:105-117.

Lithner, D. 2011. Environmental and health hazards of chemicals in plastic polymers and products.

Lobelle, D., Cunliffe, M. 2011. Early microbial biofilm formation on marine plastic debris. *Marine pollution bulletin* 62(1):197-200.

McCormick, A., Hoellein, T.J., Mason, S.A., Schluep, J., Kelly, J.J. 2014. Microplastic is an abundant and distinct microbial habitat in an urban river. *Environmental science and technology* 48(20):11863-11871.

Mianzan, H., Lasta, C., Acha E., Guerrero, R., Macchi, G., Bremec, C. 2001. The Río de la Plata Estuary, Argentina-Uruguay. In: Seeliger U., Kjerfve B. (eds.), *Coastal Marine Ecosystems of Latin America. Ecological Studies (Analysis and Synthesis)*, vol 144. Springer, Berlin, Heidelberg, Germany.

Miao, L., Wang, P., Hou, J., Yao, Y., Liu, Z., Liu, S., Li, T. 2019. Distinct community structure and microbial functions of biofilms colonizing microplastics. *Science of the Total Environment* 650:2395-2402.

Oberbeckmann, S., Loeder, M.G., Gerdts, G., Osborn, A.M. 2014. Spatial and seasonal variation in diversity and structure of microbial biofilms on marine plastics in Northern European waters. *FEMS microbiology ecology* 90(2):478-492.

Oberbeckmann, S., Löder, M.G., Labrenz, M. 2015. Marine microplastic-associated biofilms–a review. *Environmental Chemistry* 12(5):551-562.

Oberbeckmann, S., Osborn, A.M., Duhaime, M.B. 2016. Microbes on a bottle: substrate, season and geography influence community composition of microbes colonizing marine plastic debris. *PLoS One* 11(8):e0159289.

Oberbeckmann, S., Kreikemeyer, B., Labrenz, M. 2018. Environmental factors support the formation of specific bacterial assemblages on microplastics. *Frontiers in Microbiology* 8:2709.

Ogonowski, M., Motiei, A., Ininbergs, K., Hell, E., Gerdes, Z., Udekwu, K.I., et al. 2018. Evidence for selective bacterial community structuring on microplastics. *Environmental microbiology* 20(8):2796-2808.

Olivier, S.R. 1965. Rotíferos Planctónicos de Argentina. *Revista del Museo de la Plata* (n.s.) 8 (63).

Pazos, R.S., Maiztegui, T., Colautti, D.C., Paracampo, A.H., Gómez, N. 2017. Microplastics in gut contents of coastal freshwater fish from Río de la Plata estuary. *Marine pollution bulletin* 122(1-2):85-90.

Pazos, R.S., Bauer, D.E., Gómez, N. 2018. Microplastics integrating the coastal planktonic community in the inner zone of the Río de la Plata estuary (South America). *Environmental Pollution* 243:134-142.

Pazos, R.S., Spaccesi, F., Gómez, N. 2020. First record of microplastics in the mussel *Limnoperna fortunei*. *Regional Studies in Marine Science* 101360.

Puglisi, E., Romaniello, F., Galletti, S., Boccaleri, E., Frache, A., Cocconcelli, P.S. 2019. Selective bacterial colonization processes on polyethylene waste samples in an abandoned landfill site. *Scientific reports* 9(1):1-13.

Rezania, S., Park, J., Din, M.F.M., Taib, S.M., Talaiekhozani, A., Yadav, K.K., Kamyab, H. 2018. Microplastics pollution in different aquatic environments and biota: A review of recent studies. *Marine pollution bulletin* 133:191-208.

Rodrigues, J.P., Duarte, A.C., Santos-Echeandía, J., Rocha-Santos, T. 2019. Significance of interactions between microplastics and POPs in the marine environment: a critical overview. *TrAC Trends in Analytical Chemistry* 111:252-260.

Rodrigues, A., Oliver, D.M., McCarron, A., Quilliam, R.S. 2019. Colonisation of plastic pellets (nurdles) by E. coli at public bathing beaches. *Marine pollution bulletin* 139:376-380.

Romaní, A.M., Sabater, S. 2001. Structure and activity of rock and sand biofilms in a Mediterranean stream. *Ecology* 82(11):3232-3245.

Sathicq, M.B., Gómez, N. 2018. Effects of hexavalent chromium on phytoplankton and bacterioplankton of the Río de la Plata estuary: an ex-situ assay. *Environmental Monitoring and Assessment* 190:229.

Streble, H., Krauter, D. 1987. *Atlas de los Microorganismos de Agua Dulce. La vida en una gota de agua*, Ediciones Omega, Barcelona, España. 336 pp.

Suarez, J.C., Mariñelarena, A. 2019. La arena como reservorio de la contaminación fecal en playas de la ribera sur del Río de La Plata. In: Cortelezzi, A., I. Entraigas, F. Grosman e I. Masson (eds.), *Encuentro de saberes para la gestión responsable de ecosistemas acuáticos pampeanos. X Congreso EMEAP, Azul, Buenos Aires*, pp. 237-239. Universidad Nacional del Centro de la Provincia de Buenos Aires, Buenos Aires, Argentina. Disponible en: http://digital.cic.gba.gob.ar/handle/ 11746/10584

Tell, G., Conforti, V. 1986. Euglenophyta pigmentadas de la Argentina. Biblioteca. *J. Cramer. Berlin/Stuttgart. Corporation, New York/London*, USA/UK.

Thompson, F.L., Iida, T., Swings, J. 2004. Biodiversity of vibrios. *Microbiology and molecular biology reviews* 68(3):403-431.

Urien, C.M. 1972. Río de la Plata estuary environments. *Geological Society of America Memoirs* 133: 213-234.

Zardus, J.D., Nedved, B.T., Huang, Y., Tran, C., Hadfield, M.G. 2008. Microbial biofilms facilitate adhesion in biofouling invertebrates. *The Biological Bulletin* 214(1): 91–98.

Zettler, E.R., Mincer, T.J., Amaral-Zettler, L.A. 2013. Life in the “plastisphere”: microbial communities on plastic marine debris. *Environmental science and technology* 47(13):7137-7146.

Zobell, C.E. 1943. The effect of solid surfaces upon bacterial activity. *Journal of bacteriology* 46(1):39.

ECOSISTEMAS es una revista editada por: aeet ASOCIACIÓN ESPAÑOLA DE ECOLOGIA TERRESTRE con la colaboración de: GOBIERNO DE ESPAÑA VICEPRESIDENCIA CUARTA DEL GOBIERNO MINISTERIO PARA LA TRANSICIÓN ECOLÓGICA Y EL RETO DEMOGRÁFICO Fundación Biodiversidad