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Research article

Harmful algae and pathogens on plastics in three mediterranean coastal lagoons

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ABSTRACT

Plastic is now a pervasive pollutant in all marine ecosystems. The microplastics and macroplastic debris were studied in three French Mediterranean coastal lagoons (Prevost, Biguglia and Diana lagoons), displaying different environmental characteristics. In addition, biofilm samples were analyzed over the seasons to quantify and identify microalgae communities colonizing macroplastics, and determine potentially harmful microorganisms. Results indicate low but highly variable concentrations of microplastics, in relation to the period and location of sampling. Micro-Raman spectroscopy analyses revealed that the majority of macroplastic debris corresponded to polyethylene (PE) and low-density polyethylene (LDPE), and to a far lesser extent to polypropylene (PP). The observations by Scanning Electron Microscopy of microalgae communities colonizing macroplastic debris demonstrated differences depending on the seasons, with higher amounts in spring and summer, but without any variation between lagoons and polymers. Among the Diatomophyceae, the most dominant genera were *Amphora* spp., *Cocconeis* spp., and *Navicula* spp.. Cyanobacteria and Dinophyceae such as *Prorocentrum cordatum,* a potentially toxic species, were also found sporadically. The use of Primer specific DNA amplification tools enabled us to detect potentially harmful microorganisms colonizing plastics, such as *Alexandrium minutum* or *Vibrio* spp. An additional *in situ* experiment performed over one year revealed an increase in the diversity of colonizing microalgae in relation to the duration of immersion for the three tested

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polymers PE, LDPE and polyethylene terephthalates (PET). *Vibrio* settled durably after two weeks of immersion, whatever the polymer. This study confirms that Mediterranean coastal lagoons are vulnerable to the presence of macroplastic debris that may passively host and transport various species, including some potentially harmful algal and bacterial microorganisms.

1. Introduction

Plastic production is constantly on the increase and reached 368 million metric tons worldwide in 2019, i.e. 200 times more than in 1950 [1]. Nearby 80% of plastics are land-sourced, while the remainder is associated with marine activities [2]. The fate of plastics within marine systems remains largely unknown [3] and it is assumed that the seafloor functions as the ultimate sink [4]. Adverse effects of plastic debris on aquatic wildlife (due to entanglement or ingestion) have been reported on over 1400 species worldwide [5,6] and on 156 species in the Mediterranean region alone [7]. Plastic debris of whatever shape and size undoubtedly has an ecological impact across all levels of biological organization [8]. Plastics may act also as passive samplers in the environment, accumulating hydrophobic organic contaminants [9,10], in addition to their specific additives and their own residual primary monomers, which can be leached and released later within marine systems [11], see [12] for a Mediterranean example]. The direct toxicity of leachates on early life forms of certain aquatic organisms is beginning to be documented [13,14], while metabolism disturbances have been demonstrated for an increasing list of taxa belonging to various families [15].

Marine biofouling has been defined as the growth of marine organisms on submerged supports [16]. Hard surfaces introduced into the marine environment are promptly covered by extracellular polymeric substances produced by bacteria [17], allowing other organisms such as viruses, fungi, algae and eukaryotic taxa to colonize the surface, which can further support the development of a macroscopic community called the Plastisphere [18–20]. This matrix retains particulate substances from the environment, providing nutrients for biofilm organisms. Colonizing microalgae (e.g. Diatomophyceae and Dinophyceae), often phytobenthic or epiphytic, but also some planktonic forms, constitute an important part of biofilm communities [21–25]. They may further alter the physical properties of the polymer particles and then influence the fate and impacts of MP [26]. Potential harmful Dinophyceae such as *Ostreopsis* spp., *Alexandrium* spp. or *Coolia* spp. have also been identified in the form of vegetative cells or temporary cysts from the plastic debris [27]. A very recent study pointed out the regular occurrence of potentially harmful microalgal taxa within the plastisphere of a large number of Mediterranean plastic samples, but without ascertaining their actual toxicity and role in HAB dynamics [28]. Another recent study has assessed the potential role of floating plastics as vectors of toxin transfer from three widespread benthic dinoflagellates, *Gambierdiscus* spp., *Ostreopsis* cf. *ovata* and *Prorocentrum lima*, and specifies that the related risks of colonization of plastics will be significant in the Mediterranean Sea [29]. Some biofilm organisms from plastic debris may be opportunistic pathogens such as specific members of the genus *Vibrio* [18,30,31] whose presence is however not systematically detected within the plastisphere [32]. The composition of bacterial communities associated with plastic biofilms could differ between different polymers, seasons and stages of biofilm succession [22,33–36], while providing a pathway for the spread of potentially antibiotic resistant bacteria [37]. Dussud et al. [38] found in the Mediterranean Sea that several putative pathogens of fish and of invertebrates were more common on plastics compared to surrounding seawater. This echoes observations made by Foulon et al. [39] and Virsek et al. [40] regarding, respectively, some oyster and fish pathogens associated with polymers in marine water. The potential large-scale spread of marine plastic debris, carrying a community of colonizing organisms including pathogens, provides a vector for the transport of alien species and potentially of diseases [41–44].

Most of the previously cited references relate to open marine systems. Through runoff, winds, and gravity [45] and via rivers [46], most plastic debris first enters the sea from the coastal environment [47] where it can remain more or less durably before entering the ocean. Transitional areas such as estuaries may furthermore function as a “microplastic factory” [48] resulting from the fragmentation of macroplastics into microplastics well before they reach the ocean. In the Mediterranean, global models indicate that the immense majority of the plastic debris released in coastal environments do rapidly beach near their emission source [49], supporting the hypothesis that the Mediterranean Sea is primarily an accumulation zone [50]. As everywhere else [36], single-use plastics composed mainly of polypropylene (PP) and polyethylene (PE) constitute the main fraction of floating litter, and therefore also that most frequently found in the marine environment [51–58]. In the Mediterranean Sea, the level of plastic pollution is very high [59], owing to intensive maritime activity, tourism and to the human demography with its ever-growing urban, commercial and industrial needs [60]. Coastal lagoons are particularly exposed to the presence and impact of plastics because of their transitional location between continental and marine biomes, but also because they are confined ecosystems [61–66]. Lagoons with restricted exchange with the adjacent ocean and relatively long water residence times, which are already particularly sensitive to nutrient enrichments from surface runoff and groundwater, will be particularly vulnerable [67].

Coastal lagoons provide essential ecosystem services and have a high economic potential (e.g. aquaculture, tourism), but they have been among the most disturbed coastal ecosystems worldwide since the mid-20th century [68]. The emergence of the plastics issue is obviously of great concern in such ecosystems, although information remains scarce [69]. It may further more echo regular health issues that affect these highly pressured ecosystems. In particular, while coastal lagoons have variable environmental conditions and numerous periodic episodes of HAB [70–72], to our knowledge few studies have been carried out on microalgal communities and potentially harmful microorganisms (in particular HAB species) retrieved from floating plastic debris. The aim of our work was thus to study plastics colonizing communities of microalgae and potentially harmful microorganisms in three Mediterranean coastal lagoons with different environmental characteristics. We focused on two particular aims. (i) Identifying colonizing microalgae communities

and potentially harmful microorganisms and determining their occurrence on macroplastic debris collected in the same three coastal lagoons. (ii) Describing the colonization over time of colonizing microalgae communities and of harmful microorganisms from an *in situ* experiment at one site. We hypothesized that the composition of microalgal communities and harmful microorganisms associated with plastic biofilms might differ between coastal lagoons, seasons, polymer types and/or stages of biofilm succession. The range of microplastic debris (<5 mm) floating at the surface of lagoons will be registered and compared to other environments to give a broad perspective of the degree of plastic pollution in the studied lagoons.

2. Material and methods

2.1. Study area and sampling campaign

In this study, the composition of microbial communities colonizing macroplastic debris is characterized in three French Mediterranean coastal lagoons with different environmental characteristics (Fig. 1). The study area comprises three major coastal lagoons close to urban and sub-urban centers, *i.e.* Prevost lagoon in the south of France close to Montpellier (260,000 inhabitants), Biguglia lagoon on the north-east coast of Corsica close to Bastia (40,000 inhabitants) and Diana lagoon on the east coast of Corsica close to Aleria (2300 inhabitants; Fig. 1). Prevost lagoon is part of a large lagoon complex (Palavas lagoon complex) with a catchment area of 60,000 ha. This lagoon is relatively shallow (mean depth: 0.6 m) covering an area of 380 ha. It receives both freshwater polluted by urban sewage from the river Lez, and seawater through the connecting channel [73]. This lagoon is the site of traditional fishing and shellfish farming (mussel and oyster farming). Biguglia lagoon is a confined and shallow coastal lagoon (mean depth: 1.5 m) covering 1450 ha. The lagoon is linked to the Mediterranean Sea through a long narrow natural channel to the north and receives freshwater from different rivers draining its watershed (18,000 ha) [74]. It was classified as Natural Reserve in 1994. Since then, the whole lagoon surface is a no-go zone with the exception of a small number of professional fishermen allowed to practice this traditional activity. Its

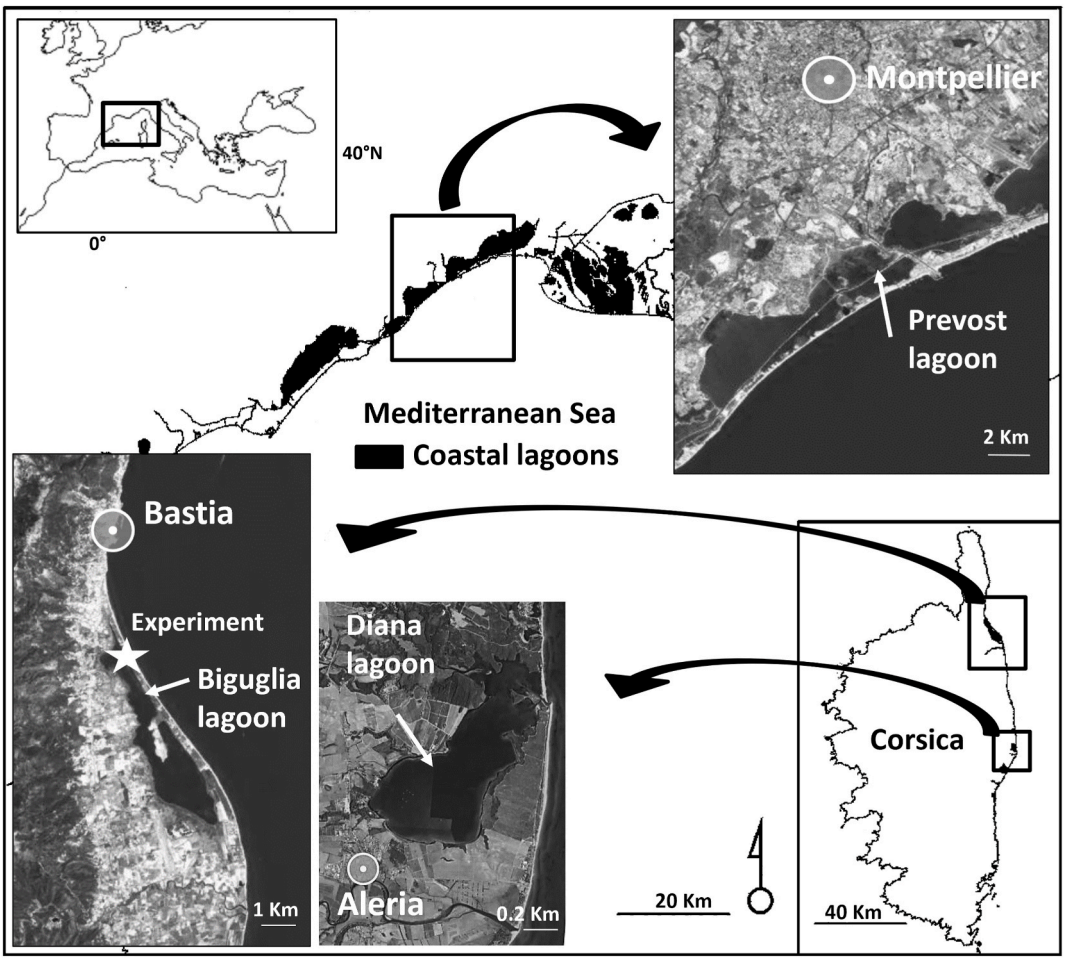


Fig. 1. Location of Prevost (43°31′ N, 03°54′ E), Biguglia (42°36′ N, 09°29′ E) and Diana (42°08′ N, 09°32 E) lagoons in the Western Mediterranean Sea, with location of the experimental site in Biguglia lagoon (satellite images obtained from IGN-Géoportail).

sensitivity to invasive allochtonous species has been investigated [75]. Diana lagoon is relatively deep (mean depth: 6.0 m) covering an area of 570 ha. A regularly maintained channel connects the lagoon to the sea in the northeast and it receives freshwater principally in the north from the rivers draining its watershed (6200 ha). Salinity is reported to be the main controller of the Diana lagoon phytoplankton community [76]. The lagoon is mainly used for aquaculture and traditional fishing. The three coastal lagoons have contrasting trophic status: eutrophic for Prevost lagoon, mesotrophic for Biguglia lagoon and oligotrophic for Diana lagoon [77].

Microplastics and macroplastic debris were collected at three different periods in the three different lagoons for the purpose of investigating seasonal variability: spring (mid-April 2016), summer (early September 2016) and autumn (early December 2016). Sampling was performed when weather conditions were favorable (no wind, no waves) to avoid mixing. Each lagoon was sectorized into three zones: North, Center and South, each sampled once per period. For each sampling lagoon, each period and each zone, subsurface salinity, temperature, dissolved oxygen and turbidity were measured *in situ* with a multiparameter water quality probe (YSI 6600 V2). Monthly local rainfall data in 2016 were obtained by season from Météo-France® for the weather stations closest to the three lagoons: spring (March, April, May), summer (June, July, August) and autumn (September, October, November).

For a better understanding of the colonization processes of colonizing microalgae and potentially harmful microorganisms, an experiment has been conducted over a period of 10 months, from February to December 2018 in the north of Biguglia lagoon (Fig. 1). Pre-cut 1 cm^2 sterile squares of virgin polymers (polypropylene/PP, low-density polyethylene/LDPE, and polyethylene terephthalate/PET) were immersed at 1 m depth. The plastics were collected at the very beginning of the experiment (t0: February 12th), after 2 weeks (t0.5: February 28th), 2 months (t2: April 25th), 7 months (t7: September 18th), and 10 months (t10: December 20th). At each sampling date, subsurface salinity, temperature, dissolved oxygen and turbidity measurements were performed *in situ* with a multiparameter water quality probe (YSI 6600 V2).

2.2. Collection and analyses of microplastic debris

A manta net with a WP2 standard ring net (200 μm mesh size, 57 cm mouth diameter) towed on the water surface at 2–3 knots for 20 min was used to collect microplastics samples. The net was maintained outside of the wake of the boat to avoid turbulence. At the end of sampling, the net was rinsed thoroughly from the outside to ensure that plankton and debris were washed into the collector. 27 surface tows were carried out for quantitative analysis of microplastics (3 lagoons x 3 periods x 3 zones). On the basis of the definition of microplastics [78], samples were sieved through 5 mm and 330 μm mesh grids, without taking into account mesoplastics, as recommended by EU guidelines [60], an approach avoiding possible contamination by smaller plastic particles and fibers, enabling direct measurements.

After gentle mixing, samples were first transferred into a 2 L glass jar; then floating plastic debris were collected from the surface. This process was repeated 3–6 times to ensure that all the smaller floating plastic particles were recovered. Plastic items were counted and quantified under a binocular Olympus SZ × 7 and characterized and classified by shape (spherical, filament, fragment, sheet) according to the MSFD guidelines [60], while possible organic particles were tested with a metal tip. The items were dried at room temperature and weighed with a Mettler AE 240 analytical balance (error ± 0.05 mg). Blanks are regurlarly tested in the laboratory, and the procedure has been intercalibrated with other Mediterranean laboratories [79], while an EU scale Interlaboratory Study on the Analysis of Microplastics in environmental matrices, in collaboration with Quasimeme/Wepal and Norman, is under way (https://www.euroqcharm.eu/en/news/analysis-of-microplastics-in-environmental-matrices-results-of-the-interlaboratory-comparison-study).

Finally, for each haul, data was normalized in relation to the total surface sampled, calculated from the following formula: S = horizontal opening × distance covered; results were expressed in terms of abundance (items/km^2) and mean weight (g/item).

2.3. Collection and analyses of macroplastic debris

Illustrative samples of floating macroplastics were collected to determine the polymers type of the macroplastic debris, and to characterize surface fouling organisms (colonizing microalgae and potentially harmful microorganisms) by Scanning Electron Microscopy (SEM) and molecular analyses (PCR). One item of floating macroplastic debris (the first observed) was sampled by period in each of the three zones of the lagoons (3 lagoons x 3 periods × 3 zones); in total, 27 1-cm squares were cut and collected and then placed in glass vials (1 1-cm square per microplastic debris item). For the colonizing experiment, one sample of each of the three plastics tested was collected at each date for characterizing surface fouling organisms by SEM and PCR.

The identification of macroplastic polymers was done using micro-Raman spectroscopy. Macroplastic debris were analyzed with a LabRam HR800 (HORIBA Scientific, Villeneuve d'Ascq, France) using laser wavelength set at 514 nm (Ar Laser, Melles Griot, Bensheim, Germany). Particle identification was performed by comparing acquired spectra to reference spectra in a home-made database. The home-made spectra database has been built using analysis of reference polymers acquired from GoodFellow (Lille, France).

For SEM studies, all the collected samples were fixed in formaldehyde (2.5%). Plastic items were first rinsed in Milli-Q water and then dehydrated through immersion in each of the following grades of ethanol for 30 min: 30%, 50%, 70%, 90% and 100%. The last immersion in ethanol 100% was repeated twice. After dehydration, samples were immersed 30 min in a solution of ethanol 100%/hexamethyldisilazane (HMDS) (1:2) and 30 min in pure HMDS. Samples were then allowed to air-dry overnight under a hood. Each 1-cm plastic piece was mounted on aluminium studs using double-sided tape and coated with gold/palladium in a Quorum Technologies SC7640 sputter coater. The surface of each sample was examined in its entirety under a Hitachi S-3400-N scanning electron microscope operated at an accelerating voltage of 5 and 10 kV, in the Service d'Etude et de Recherche en Microscopie Electronique at the University of Corsica (Corte, France). In order to compare the biofilm surface coverage of plastics and evaluate the concentration of

colonizing microalgae species, 2 sets of micrographs were taken on the surface of each sample under identical acquisition conditions: 3 micrographs (magnification ×30 with a working distance of 20 mm) allowing observation of an area of 12.6 mm^2, and 5 micrographs (magnification ×100 with a working distance of 8 mm) allowing observation of an area of 1.1 mm^2. Micrographs of each species of the colonizing microalgae were taken for identification with higher magnification adapted to the size of each species (up to ×15,000). Identification was performed at lowest possible taxonomic level with verification based on several books [80–82] and online databases such as the World Register of Marine Species or AlgaeBase.

For molecular analyses, the strategy was aimed at detecting marine microorganisms that are blooming and/or potentially harmful to molluscs and mollusc consumers, depending on environmental conditions. We also considered the presence of *Vibrio* that are associated with the mortality of oysters after OsHV-1 viral infection [83]. The collected samples were fixed in 70% ethanol solution. DNA was extracted from macroplastic debris collected in the three coastal lagoons (2016) and the three polymers immersed in Biguglia lagoon (2018). Based on a previous study where six DNA extraction methods were tested to evaluate microbial colonization of three polymers (LDPE, PP, PA/polyamide) collected after 15 days of immersion in coastal seawater (Bay of Brest), the CTAB/PCI method was selected for extraction of DNA from the polymers immersed in Biguglia lagoon [84]. Amplification of ribosomal RNA (5.8S, ITS, 16S and/or 18S) regions and of DNA polymerase was used to detect potentially harmful marine microorganisms including Dinophyceae, *Alexandrium minutum*, *Ostreopsis* spp., protozoan parasites *Bonamia* sp., *Marteilia refringens,* total *Vibrio* spp., *Vibrio parahaemolyticus*, and the herpes virus, OsHV-1, respectively (Table S1, Supplementary material). The polymers immersed in Biguglia lagoon during the experiment performed in 2018 were examined for all the marine microorganisms except Dinophyceae and *Alexandrium minutum*.

For each target species, a standard curve was prepared with 10-fold serial dilutions of genomic DNA (Total Dinophyceae, *Ostreopsis* spp., *Bonamia* sp., *Marteilia refringens)* or of the linearized plasmid containing the target region or gene (*Alexandrium minutum*, *Vibrio* spp., *Vibrio parahaemolyticus* and OsHV-1) of the species. The composition of the reaction mixture and the PCR cycling conditions varied according to the target microorganisms (Tables S2 and S3, Supplementary material). One to five μL of template DNA (or sterile water for negative controls) were added to the PCR mixture in 96-well plates. The target samples and negative controls were analyzed in duplicate. The plates were loaded onto a BIORAD S1000TM Thermal Cycler (Biorad, France) for conventional PCR, and onto a Stratagene MxPro3000P thermal cycler (Agilent Technologies, Santa Clara, CA, USA) for real-time PCR assays.

2.4. Statistical analysis

All analyses were carried out with XLStat® V2011 5.01 statistics software. We used nonparametric Kruskal-Wallis one-way analysis of variance on ranks to assess the significance of differences between coastal lagoons, between seasons and between polymer types, when possible, given the reduced number of samples collected. Tests were considered significant at $p < 0.05$.

3. Results

3.1. Environmental conditions, microplastic debris and polymer type of macroplastic debris

The temperature, dissolved oxygen concentration and rainfall data followed roughly identical seasonal trends over the three lagoons (Fig. S1, Supplementary material). Turbidity was more variable in Prevost and Diana lagoons over the seasons, while it appeared more stable in Biguglia. On the other hand, Diana lagoon was quite different from other lagoons with regard to the salinity. This deeper lagoon presented higher and less variable salinity over the three seasons studied (Fig. S1, Supplementary material). The environmental variables observed during the Biguglia experiment in 2018 were of the same order of magnitude as those observed in 2016 in Biguglia lagoon, with the exception of turbidity that exhibited higher values in 2018 (Table S4, Supplementary material).

522 microplastics were isolated from all 27 superficial samples, with a minimum of zero and a maximum of 162 particles per sample (maximum abundance: 113,200±88,800 items/km^2), and an average concentration per lagoon and per year in the range of 23,800–40,600 items/km^2 (Table 1). Only one pellet (< one per thousand), 6 pieces of Styrofoam (1.1%) and 15 fibers (2.8%) were

Table 1
Microplastic abundance (items/km^2; mean values ± s.d.) and percentage of fragments in water surface samples collected over three seasons (spring, summer and autumn) in the three coastal lagoons (Prevost, Biguglia and Diana; 380 ha, 1450 ha and 570 ha respectively). The mean annual abundance (items/km^2) and the fragment contribution (%) are provided for each lagoon. The size of the sampled areas and the number of tows are indicated.

Lagoon	Microplastics	Period			
		Spring (n = 8)	Summer (n = 9)	Autumn (n = 9)	Mean Value
Prevost	Microplastics (items/km^2)	71200 ± 49200	9900 ± 6200	0	27033 ± 32000
	% fragments	97	81	–	
	Sampled surface (nb of tows)	1501 m^2 (3)	1344 m^2 (3)	1405 m^2 (3)	Total: 4250 m^2 (9)
Biguglia	Microplastics (items/km^2)	0	8600 ± 5100	113200 ± 88800	40600 ± 25150
	% fragments	–	71	86	
	Sampled surface (nb of tows)	1310 m^2 (2)	815 m^2 (3)	2538 m^2 (3)	Total: 4663 m^2 (8)
Diana	Microplastics (items/km^2)	6700 ± 3200	4600 ± 1300	60300 ± 16800	23866 ± 70200
	% fragments	100	100	98	
	Sampled surface (nb of tows)	1540 m^2 (3)	1540 m^2 (3)	1756 m^2 (3)	Total: 4836 m^2 (9)

collected. Thirty-one films (5.9%) were observed, with 29 of them (93.5% of total film particles) collected in Biguglia lagoon during winter sampling. In addition, six mesoplastics (5–25 mm) were observed in Prevost lagoon in April and September. Evaluation of weighing was performed on 511 samples. For all lagoons and periods, the mean weight value was 0.023 mg per item. Finally, a very high number of plastic fragments (n = 469; 89%) and sheets (n = 31; 5.9%) were found, in comparison with fibers. Bearing in mind that the limited sampling does not enable any in-depth statistical analysis, there was no clear seasonal trend although microplastics abundances seemed lower during summer.

Micro-Raman spectroscopy analyses revealed that four types of polymers were found: LDPE (Low Density Polyethylene), HDPE (High Density Polyethylene), PE + TiO_2 (Polyethylene incrusted by titanium dioxide), and PP (Polypropylene) (Fig. 2). The majority (93%) of the macroplastic debris sampled was identified as Polyethylene (PE), including 59% of the total made of low-density PE (LDPE), and the remaining (7%) identified as Polypropylene (PP) (Table 2). For 22% of the macroplastics spectra, we also observed two peaks (at 448 and 612 cm^{-1}) that do not characterize the PE (Fig. 2), but TiO_2 used as white pigment.

3.2. *Microalgae and potentially harmful microorganisms colonizing macroplastic debris*

Colonizing microalgae were observed by SEM on macroplastic fragments collected in the three coastal lagoons (Prevost, Biguglia and Diana). SEM revealed diverse and mixed communities of colonizers that included both eukaryotic and prokaryotic microorganism assemblages. The number of Taxonomic Units (TU) for all seasons and all lagoons was 40 TU belonging to six taxonomical classes (Fig. 3A and H): Diatomophyceae (78%), Cyanobacteria (10%), Dinophyceae (7%), Cryptophyceae (2%), Chlorophyta (2%) and Chrysophyceae (1%). The average occurrence of the TU by sample was of 8.1 ± 0.6 TU (Fig. 3A). Although the statistical analyses must be treated with caution given the reduced number of samples, the number of retrieved TU varied significantly across seasons: spring and autumn ($p < 0.0001$), summer and autumn ($p = 0.002$), and spring and summer ($p = 0.034$) (Fig. 3B, C, 3D). The highest mean occurrence of TU was found in Biguglia lagoon, followed by Diana and Prevost lagoons, but there was no significant statistical difference between lagoons ($p > 0.05$) (Fig. 3E, F, 3G). Given the small number of plastics sampled by polymer type (Table 2), it was not possible to compare occurrence of the TU by polymer type. Diatomophyceae were dominant regardless of the season or the coastal lagoon (TU>75%) (Fig. 3B, C, 3D, 3E, 3F, 3G). Among the Diatomophyceae class, the most dominant genera were *Amphora* spp., *Cocconeis* spp., and *Navicula* spp., systematically encountered in all macroplastics samples, whatever the season or the lagoon (Fig. 3H). Other dominant TU were also present such as *Diploneis* spp., *Licmophora* spp., *Melosira* spp., *Nitzschia closterium* (Fig. 4A), *Synedra* spp., or *Prorocentrum balticum* (Fig. 3H). Other non-dominant TU were also observed in the samples (Fig. 3H) such as for example *Chaetoceros* spp. (Fig. 4B), *Pleurosigma* spp, (Fig. 4C), *Lauderia* spp. (Fig. 4D), *Amphipora* spp. (Fig. 4E), *Cymbella* spp. (Fig. 4F), *Prorocentrum cordatum* (Fig. 4G) and Prasynophyceae (Fig. 4H). Some colonizing microalgae used stalks to attach to the substrate (Fig. 4H).

The molecular analysis showed that potentially harmful microorganisms have colonized the macroplastic debris collected seasonally in the three coastal lagoons (Table 3A). *Ostreopsis* spp., *Bonamia* spp. and OsHV-1 were not detected. Conversely, 'Total' Dinophyceae were regularly detected in the three lagoons (n = 15/27 samples) and *Alexandrium minutum* in five samples (Table 3A). 'Total' *Vibrio* spp. were found in 19 of the macroplastic debris samples collected in the three lagoons regardless of the season. *Vibrio parahaemolyticus* was detected once in each lagoon. *Marteilia refringens* was observed in only one sample from Prevost lagoon. Overall, no spatial or temporal pattern was noticeable.

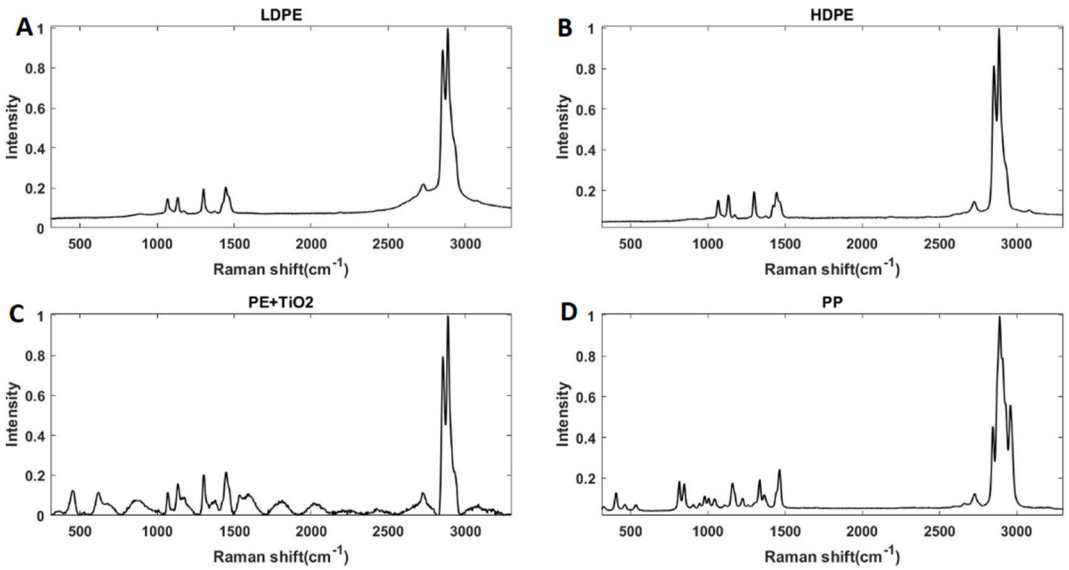


Fig. 2. Micro-Raman spectra of the four types of polymers (A: LDPE/Low Density Polyethylene; B: HDPE/High Density Polyethylene; C: PE = TIO2/Polyethylene incrusted by TI02; D: PP/Polypropylene) identified in the 27 samples collected in three different seasons in the three lagoons (Biguglia, Diana and Prevost). Sample identification was performed by comparing acquired spectra to reference spectra (see Materials and Methods).

Table 2
Dominant polymer of the floating macroplastics sampled in the three coastal lagoons (Prevost, Biguglia and Diana), over three seasons (spring, summer and autumn).

Lagoon	Period		
	Spring	Summer	Autumn
Prevost	LDPE	HDPE+TiO2	LDPE
	LDPE	HDPE+TiO2	HDPE+TiO2
	LDPE	HDPE+TiO2	LDPE
Biguglia	LDPE	LDPE	HDPE+TiO2
	PP	LDPE	HDPE
	LDPE	HDPE	HDPE
Diana	LDPE	LDPE	HDPE+TiO2
	LDPE	LDPE	PP
	LDPE	LDPE	LDPE

The colonization experiment performed in Biguglia lagoon in 2018 revealed that the thickness and coverage of the biofilm observed by MES increased over time for the three tested plastics (LDPE, PET and PP) (Fig. 5). The colonization was high from 15 days of immersion (t0,5 > 50%) and the biofilm coverage was total after 2 months of immersion (t2 = 100%), whatever the plastic type (Fig. 5). The biofilm is already visible in some pictures. Molluscs, crustaceans and other wildlife can be attached and visible from t7 in all the observed images. The number of TU per sample was on average of 12.5 ± 1.6 TU (Fig. 6A). Given the small number of samples taken by polymer type and by sampling time, it was not possible to compare the results statistically. Diatomophyceae were dominant regardless of the polymer type or the immersion duration (TU>40%) (Fig. 6B, C, 6D). The diversity of identified classes did increase with the immersion duration for the three polymer types (Fig. 6B, C, 6D). The following Diatomophyceae were identified in almost all macroplastics samples from t0,5: *Amphora* spp., *Cocconeis* spp., *Navicula* spp., *Achnanthes* spp., *Synedra* spp., *Nitzschia* spp., and *Fragilaria* spp. (Fig. 6E). Other dominant TU were also present including potentially toxic species such as Cyanobacteria and *Prorocentrum cordatum*. Concerning potentially harmful microorganisms identified by molecular analysis, *Vibrio* were detected at all exposure times and as soon as 15 days after immersion of the three polymers in seawater (Table 3B). *Vibrio parahaemolyticus* on LDPE and OsHV-1 on PET were detected once each after two months of exposure but they seemingly did not persist.

4. Discussion

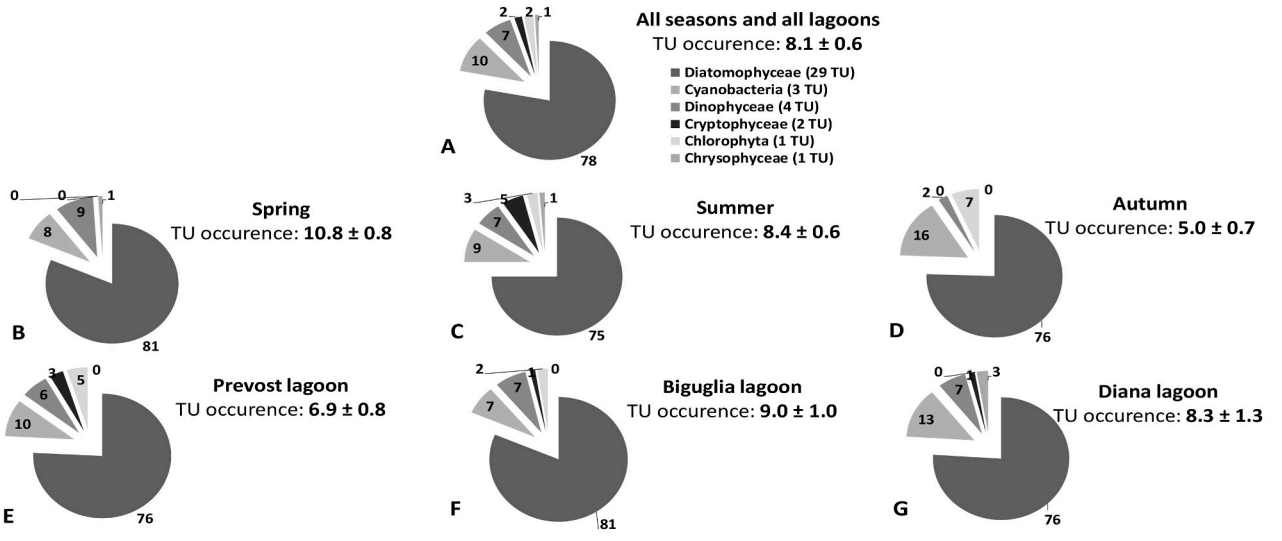
Plastic debris input in coastal lagoons could be driven by a wide range of human activities such as waste disposal, wastewater effluents, agriculture, aquaculture, fishing and tourism activities [85]. The present study investigated macroplastic debris collected from three coastal Mediterranean lagoons (Prevost, Biguglia and Diana), and studied colonizing microalgae communities and potentially harmful microorganisms on macroplastic debris. Such information on plastic debris in coastal lagoons [85], and transitional water masses more broadly [15], remains scarce.

Following the exploratory papers of Shiber [86] and Morris [87] in Mediterranean waters, the presence of floating plastic debris was later thoroughly documented for macroplastics [88,89] and for microplastics as well [90]. Only few data have reported floating microplastics in Mediterranean coastal lagoons [63,66,91], when their presence was shown in sediments [62,92–94] and aquatic biota [61,64,65]. Given the small number of samples taken in the three studied lagoons, it is difficult to precisely quantify the microplastics. Nevertheless, they provide information and allow comparison with other lagoon environments. Our results indicate low concentrations but with a wide variability and which are in the same range as those found in Bizerte lagoon (453 items/m^{-3}) [63] or at the surface of Küçükçekmece lagoon in Sea of Marmara (33,000 items/m^{-3}) [66]. Because of a high variability in the geographical distribution of microplastics, and as found by other authors, it is suggested that patchiness is a common pattern in lagoons with various sources and coastal uses such as urban rivers (Prevost and Biguglia) and shellfish farming (Prevost and Diana) in addition to highly urbanized areas (Prevost and Biguglia).

Despite the small number of samples taken, the majority of macroplastic debris items sampled in the three lagoons were identified as Polyethylene (PE). Actually, PE is one of the most common polymers found in marine environment and it is the main polymer product worldwide [1,95]. PE, and to a much lesser extent PP, have been already reported as dominant in different studies in the Mediterranean area [28,96,97]. Peaks at 448 and 612 cm^{-1} are attributed to titanium dioxide [98]. This result is in accordance with the fact that Titanium dioxide is the mainstay white pigment for the plastics industry [99] that is highly developed in the region around Prevost lagoon.

With the proven presence of plastic debris in aquatic ecosystems, research is now focusing on assessing their impact on ecosystem functions. Plastic debris surfaces are favorable substrates for biofilm formation [16,20,26,100]. These surfaces are gradually coated with extracellular polymeric substances produced by archaea, bacteria and eukaryotic microbes [17]. A matrix is thus formed which retains particulate matter from the environment and provides nutrients to the biofilm organisms. The studies on biofilm organisms rely primarily on microscopy and the application of molecular methods to extend knowledge on the various microorganisms inhabiting the plastisphere [101]. The field of research focusing on the colonization of plastic debris by microorganisms is growing rapidly, but many questions remain, especially regarding the colonization by microalgae communities and potentially harmful microorganisms in the coastal lagoon environment.

In Mediterranean coastal lagoons, the results of our study showed that Diatomophyceae were the most abundant organisms on



H

	Diatomophyceae																															Cyanobacteria			Dinophyceae				Cryptophyceae		Chlorophyta	Chrysophyceae
	Achnanthes spp.	Amphiprora spp.	Amphora spp.	Auricula spp.	Bacterastrum spp.	Campylonels spp.	Chaetoceros spp.	Climacosphenia spp.	Cocconeis spp.	Cylindrotheca spp.	Cymbella spp.	Diatoma spp.	Diploneis spp.	Fragilaria spp.	Lauderia annulata	Licmophora spp.	Lyrella spp.	Mastogloia spp.	Melosira spp.	Navicula spp.	Navicula aboensis	Nitzschia spp.	Nitzschia closterium	Phaeodactylum spp.	Pinnularia spp.	Pleurosigma spp.	Surirella spp.	Synedra spp.	Unidentified diatomophyceae	Anabaena spp.	Spirulina spp.	unidentified cyanobacteria	Prorocentrum cordatum	Prorocentrum balticum	Pentapharsodinium spp.	Unidentified Dinophyceae	Cryptomonas spp.	Unidentified cryptophyceae	Chlorophyta	Paraphysomonas spp.
Occurrence %	19	15	85	7	11	7	26	7	89	4	7	11	15	15	11	22	4	7	19	74	15	7	37	7	11	15	4	33	44	7	7	67	7	15	4	30	7	7	19	7
Spring	11	22	100	22	22	11	33	22	89	11	0	33	44	11	11	56	0	22	44	89	33	22	33	0	0	22	11	78	22	11	11	67	11	44	11	33	0	0	0	11
Summer	33	22	89	0	11	11	33	0	89	0	22	0	0	22	22	11	11	0	11	67	11	0	67	11	11	22	0	11	44	0	11	67	11	0	0	44	22	22	22	11
Autumn	11	0	67	0	0	0	11	0	89	0	0	0	0	11	0	0	0	0	0	67	0	0	11	11	22	0	0	11	67	11	0	67	0	0	0	11	0	0	33	0
Prevost	33	22	67	0	11	0	33	0	78	0	11	0	11	11	11	0	0	0	22	67	0	11	11	22	0	0	0	11	89	0	0	67	11	0	0	33	0	22	33	0
Biguglia	22	11	100	0	11	0	11	22	100	0	0	22	22	33	0	44	11	22	11	78	33	0	33	0	22	22	11	56	33	0	11	56	11	22	0	33	11	0	22	0
Diana	0	11	89	22	11	22	33	0	89	11	11	11	11	0	22	22	0	0	22	78	11	11	67	0	11	22	0	33	11	22	11	78	0	22	11	22	11	0	0	22

Fig. 3. TU occurrence by sample (mean ± standard deviation; A,B,C,D,E,F,G), percentage of TU/classes (A,B,C,D,E,F,G), and TU occurrence (H) of colonizing microalgae on macroplastic debris by season in the three coastal lagoons (Prevost, Biguglia, Diana).

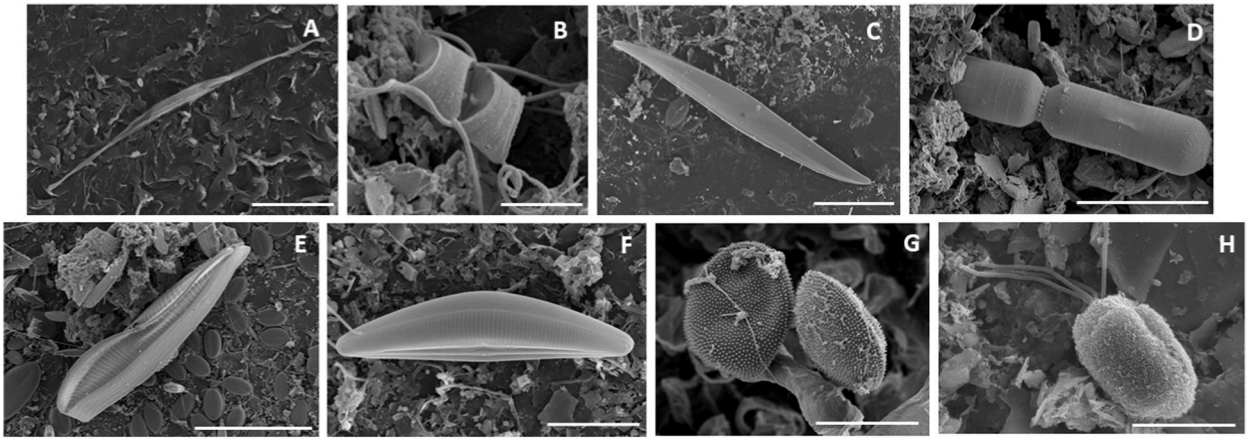


Fig. 4. MES views of microalgae colonizing macroplastic debris in the three coastal lagoons (Prevost, Biguglia, Diana). A) *Nitzschia closterium,* B) *Chaetoceros* sp., C) *Pleurosigma* sp, D) *Lauderia* sp., E) *Amphipora* sp., F) *Cymbella* sp., G) *Prorocentrum cordatum*, H) Prasynophyceae (Chlorophyta). Scale bars: 5 μm (G), 10 μm (A,B,F), 20 μm (G), 30 μm (D,E), 50 μm (C).

macroplastic debris, in accordance with the observations of Briand et al. [102] in small sheltered Mediterranean embayments. Diatoms are ubiquitous residents of the plastisphere, as most studies show [20], and are known to maintain and increase the biofilm layer [18]. The most dominant genera are *Amphora* spp., *Cocconeis* spp., and *Navicula* spp., but also *Nitzschia* spp. or *Achnanthes* spp., as has been observed in marine and freshwater systems [18,21,25,33,103]. Apart from Diatomophyceae, other taxa are also present in coastal lagoons, which can also be found in other environments [18,21,22,33,103,104]. Most of these taxa are not potentially harmful microorganisms, but many studies have shown the occurrence of Dinophyceae, such as *Alexandrium* spp. or *Prorocentrum* sp., with the presence of harmful species [18,21]. As plastics pollution and harmful benthic algal blooms have both increased over the recent decades, do Prado Leite et al. [29] suggest that their interactive effects can become a major threat to marine ecosystems and human health. In our study, *Alexandrium minutum* was found in the three lagoons, while *Prorocentrum cordatum* was found in particular in Prevost and Biguglia lagoons. *Alexandrium minutum* is found throughout the world and affects many bivalves, such as the Pacific oyster of commercial and ecological interest, *Crassostrea gigas*. This toxic bloom-forming species is widespread throughout the Mediterranean Sea [105], as was detected in our study in Prevost and Diana lagoons, which can have a negative impact on aquaculture. *Prorocentrum cordatum* is described as eurytherm and euryhaline, and blooms of this species linked to low salinity values have been reported worldwide in coastal systems [106], and were also identified in our study in Biguglia lagoon, as already described by Garrido et al. [107]. Blooms of this species can have lethal impacts on shellfish and this has been observed in many countries around the world [106]. Although toxicity in this species seems to be strain-dependent [106], most evidence for the harmful effects of *Prorocentrum cordatum* on molluscs has so far been based on observations of shellfish mortalities coinciding with blooms in coastal environment or in laboratory experiments [108]. Several studies have provided evidence that variation in toxicity of *Prorocentrum cordatum* is dependent on environmental conditions and their effects on the physiology of this Dinophyceae. The expression of the toxicity of this Dinophyceae has been reported in relation to feeding molluscs [109]. In effect, HAB exposure affect the haemocytes, the immune defense cells in bivalves and conversely haemocytes can have measurable effects on algal cells, including changes in shape, chlorophyll fluorescence and mortality. Recently, Li et al. [110] demonstrate that senescent cultures of a strain are more bioactive against bivalves than growing cultures. Wikfors and Fernandez [111] also suggest a possible association between bacteriovory and toxicity. The presence of Cryptophyceae, Chlorophyta, and Chrysophyceae have also been observed in our study, but the majority of these taxa are not potentially harmful microorganisms. Conversely, Cyanobacteria have been widely reported as a group easily colonizing plastic debris [33] and many species are potentially able to produce cyanotoxins, responsible for harmful algal blooms (HAB). Among the taxa most regularly detected on plastics, the genus *Phormidium* is often identified and studies attest to its abundant presence in the marine environment [18,112] and in our study in Biguglia lagoon. Other genera are also found in our coastal lagoons, such as Spirulina and Anabaena, able to produce cyanotoxins.

Microorganisms living in a biofilm have advantages compared to the free-living state, as the biofilm protects them from UV radiation, predation and environmental stress, which increases their survival rate [113]. These authors mentioned that the bacterial communities on plastics consist mainly of the orders Alteromonadales, Chitinophagales, Cytophagales, Flavobacteriales, Oceanospirillales Rhodobacterales, and Rickettsiales. Other authors found that the microbial communities on PET fragments varied both with season and location and comprised bacteria belonging to Bacteroidetes, Cyanobacteria, Proteobacteria and members of the Bacillariophyceae and Phaeophyceae. These communities in marine plastic-associated biofilms remain to be investigated in future studies, bearing in mind that the presence of plastics may alter their composition [18,114]. The plastisphere can enhance the dissemination of human pathogenic viruses and lead to more effective transfer and transmission of viral diseases within the environment [115]. Marine viruses are also settling on plastics, including some pathogens, such as the Ostreid herpesvirus 1 (OsHV-1) that is associated with mass mortality events of Pacific oysters [116]. This virus has not been detected in the three lagoons sampled in our experiments but may represent a major threat for oyster production in the French Mediterranean lagoons, possibly favored by plastics

Table 3
Occurrence of potentially harmful microorganisms detected by PCR on macroplastic debris: (A) by seasons and in the three lagoons (Prevost, Biguglia, Diana) with replicas (R1, R2 and R3); (B) depending on time (t0/February, t0.5/February, t2/April, t7/September, t10/December) and macroplastic types (LDPE, PET and PP) for the experiment in the Biguglia lagoon (Dinophyceae, including *A. minutum,* have not been searched during this experiment and are notified 'nd' in the table 5B).

		Dinophyceae	*Alexandrium minutum*	*Ostreopsis* spp.	*Bonamia* spp.	*Martellia refringens*	*Vibrio* spp.	*Vibrio parahaemolyticus*	OsHV-1
(A)	Total	15	5	0	0	1	19	3	0
	Occurrence %	43	14	0	0	3	54	9	0
	Spring occurrence	89	22	0	0	11	67	0	0
	Summer occurrence	33	22	0	0	0	78	11	0
	Autumn occurrence	44	11	0	0	0	67	22	0
	Prevost occurrence	33	22	0	0	11	89	11	0
	Biguglia occurrence	78	11	0	0	0	67	11	0
	Diana occurrence	56	22	0	0	0	56	11	0
Spring	Prevost R1	1	0	0	0	1	1	0	0
	Prevost R2	1	0	0	0	0	1	0	0
	Prevost R3	1	0	0	0	0	1	0	0
	Biguglia R1	1	0	0	0	0	0	0	0
	Biguglia R2	0	0	0	0	0	0	0	0
	Biguglia R3	1	0	0	0	0	1	0	0
	Diana R1	1	1	0	0	0	1	0	0
	Diana R2	1	1	0	0	0	0	0	0
	Diana R3	1	0	0	0	0	1	0	0
Summer	Prevost R1	0	1	0	0	0	1	0	0
	Prevost R2	0	0	0	0	0	1	0	0
	Prevost R3	0	0	0	0	0	0	0	0
	Biguglia R1	1	0	0	0	0	1	0	0
	Biguglia R2	1	1	0	0	0	1	0	0
	Biguglia R3	0	0	0	0	0	1	1	0
	Diana R1	0	0	0	0	0	1	0	0
	Diana R2	0	0	0	0	0	1	0	0
	Diana R3	1	0	0	0	0	0	0	0
Autumn	Prevost R1	0	1	0	0	0	1	0	0
	Prevost R2	0	0	0	0	0	1	0	0
	Prevost R3	0	0	0	0	0	1	1	0
	Biguglia R1	1	0	0	0	0	0	0	0
	Biguglia R2	1	0	0	0	0	1	0	0
	Biguglia R3	1	0	0	0	0	1	0	0
	Diana R1	0	0	0	0	0	0	0	0
	Diana R2	1	0	0	0	0	0	0	0
	Diana R3	0	0	0	0	0	1	1	0
(B)	Total			0	0	0	12	1	1
	Occurence %			0	0	0	86	7	7
t0	LDPE	nd	nd	0	0	0	0	0	0
	PET	nd	nd	0	0	0	0	0	0
	PP	nd	nd	0	0	0	0	0	0
t0.5	LDPE	nd	nd	0	0	0	1	0	0
	PET	nd	nd	0	0	0	1	0	0
	PP	nd	nd	0	0	0	1	0	0
t2	LDPE	nd	nd	0	0	0	1	1	0
	PET	nd	nd	0	0	0	1	0	1
	PP	nd	nd	0	0	0	1	0	0
t7	LDPE	nd	nd	0	0	0	1	0	0
	PET	nd	nd	0	0	0	1	0	0
	PP	nd	nd	0	0	0	1	0	0
t10	LDPE	nd	nd	0	0	0	1	0	0
	PET	nd	nd	0	0	0	1	0	0
	PP	nd	nd	0	0	0	1	0	0

and microplastics dispersion.

As the development of microorganisms is highly dependent on environmental parameters [25], it is necessary to study which parameters exert the most selective pressures. High temperatures lead to rapid development of attached organisms, especially in the establishment and early growth phases of the community [20]. Seasonal differences have often been observed in colonization processes, which are induced by temperature variations [117]. Our study shows the differences between the seasons (higher in spring and

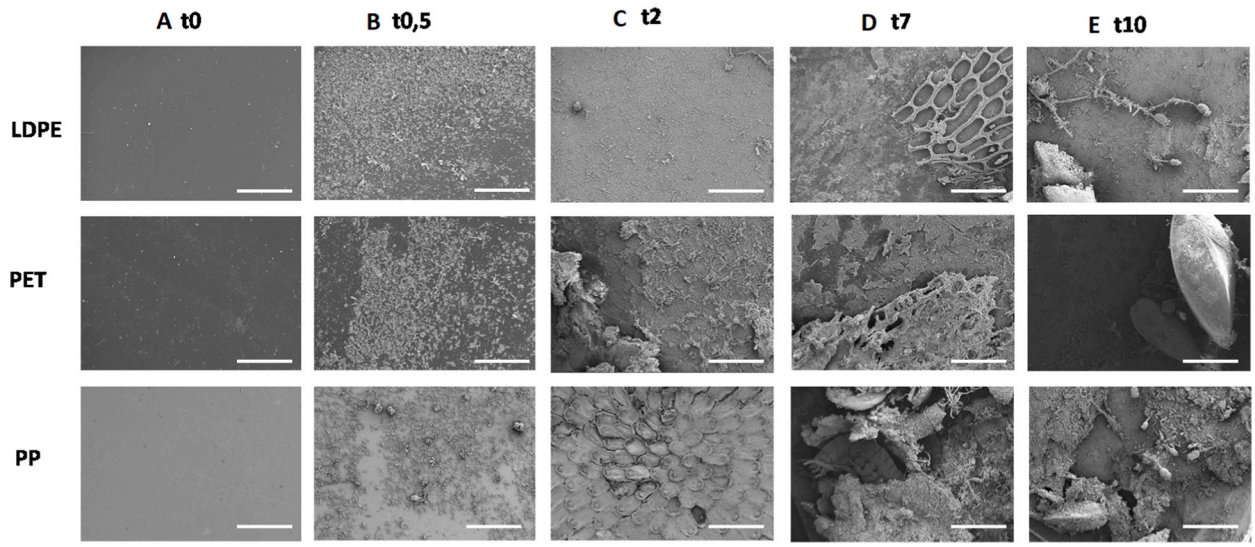
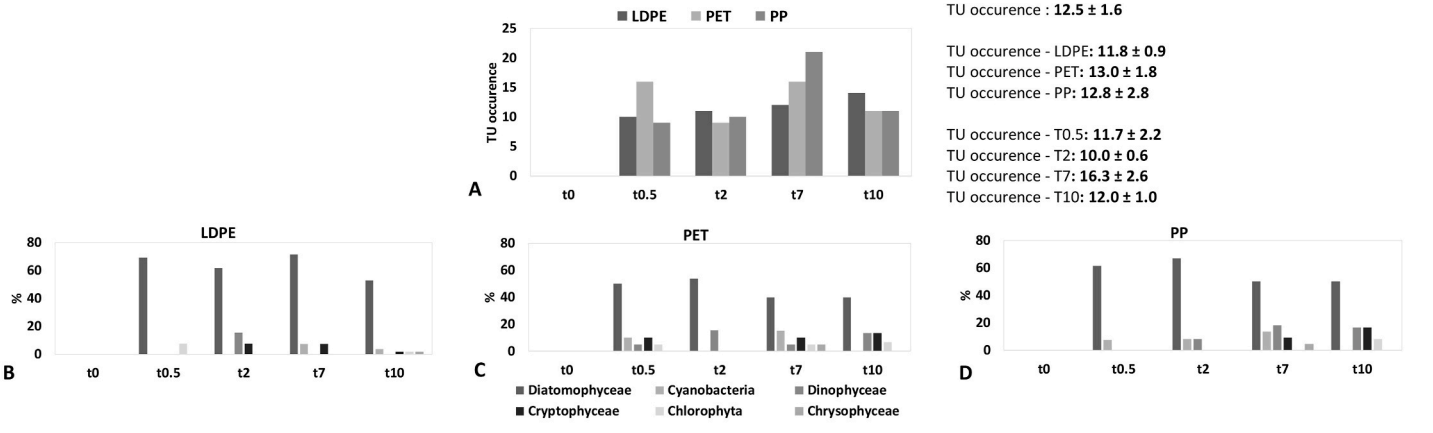


Fig. 5. MES views of colonizing microalgae dynamics over time (A: t0/February; B: t0.5/February; C: t2/April; D: t7/September; E: t10/December) on different macroplastic types (LDPE, PET and PP) during the 2018 experiment in Biguglia lagoon. Scale bars: 1 mm.

summer), both during seasonal sampling in the three sampled lagoons and in the experiment in Biguglia lagoon. A higher diversity of colonizing microorganisms in summer has also been demonstrated in the marine environment [33]. Pinnell and Tuner [118] clearly demonstrated the role of increased water temperature in the formation of the microorganism community on plastics. More generally, species that bloom or spread more often in relation to specific environmental conditions are not regularly found in lagoons, possibly explaining the absence of Dinophyceae, *Ostreopsis* spp., *Bonamia* spp. and OsHV-1 associated with oyster mortality in our recolonization study. A crucial role of salinity has been documented [34,118], which is recognized as shaping communities in coastal lagoons, but no difference has been observed in our study between the sampled lagoons, despite the differences in terms of salinity and others environmental variables. For *Ostreopsis* spp., the species has already been detected as epibenthic to *Zostera noltei* and *Cymodocea nodosa* in a Mediterranean Coastal lagoon [119]. In general, warmer periods marked by high temperature, salinity and water column stability record the highest abundances of *Ostreopsis* spp [120]. The low salinity observed in our colonization study (<25 PSU) may explain its absence. There are still uncertainties as to whether environmental parameters prevail in the biofilm formation of microorganisms on plastics. Can the presence of a plastic surface exert sufficient intrinsic selection to drive species selection and overcome other niche-defining parameters based on seasonal and spatial patterns [20]? The investigations carried out within the framework of this study did not allow the identification of specific taxa by type of plastic debris. Many studies agree that the composition of microorganisms associated with plastic biofilms differs sometimes between different polymers [18,22,33–35,121]. Others have suggested, conversely, that the chemical composition of the polymer did not systematically affect the structure of the microbial communities that developed within biofilms, but rather influenced the functions expressed by these communities [122]. With the exception of Amaral-Zettler et al. [28], the literature is consistent in concluding that there is no effect of environmental parameters or geographical location on the communities developed on plastics in the western Mediterranean basin [123]. These contradictory observations can probably be linked to the origin of the diversity of the systems studied, the different experimental designs or the differences in spatial and temporal scale taken into account [20].

Regarding the colonization time of microorganisms on macroplastic debris, several observations have been made. Studies reveal the presence of diatoms as pioneer colonizers, as well as the presence of dinoflagellates and different microalgal species [18,22,101,112]. Diatoms function as a habitat for hydrocarbon-degrading bacteria [124]. This association stems from the ability of diatoms to accumulate Polycyclic Aromatic Hydrocarbons on the surface of their cells, in order to create a PAH-enriched zone around the phycosphere, which would then attract PAH-degrading bacteria to colonize this area. Our study in coastal lagoons has indeed demonstrated the dominance of Diatomophyceae (e.g. *Amphora* spp., *Cocconeis* spp., *Navicula* spp., *Achnanthes* spp., *Synedra* spp., *Nitzschia* spp., and *Fragilaria* spp.), but also the presence of Cyanobacteria (*Phormidium* spp.), Dinophyceae (*Prorocentrum* spp.) and viruses (*Vibrio* spp.), from the first 15 days and an increase in the community diversity over time, as was also well observed in Amaral-Zettler et al. [101] and in Dudek et al. [22] from the first week. However, the exact sanitary influence of the development of potentially pathogenic microorganisms on plastic surfaces remains to be thoroughly assessed [125,126]. This type of study is particularly essential for environmental biomonitoring. This research makes it possible to specify the risks of the establishment of toxic and/or pathogenic species and to offer guidance for the associated management measures. The transfer of knowledge and decision support to lagoon managers is crucial in this context. Regular observation of macroplastics is required in coastal lagoons, with the aim of depolluting them regularly when possible.



E

	Diatomophyceae																																	Cyanobacteria			Dinophyceae				Cryptophyceae		Chlorophyta	Chrysophyceae
	Achnanthes spp.	Amphiprora spp.	Amphora spp.	Auricula spp.	Campyloneis spp.	Climacosphenia spp.	Cocconeis spp.	Cylindrotheca spp.	Cymbella spp.	Diatoma spp.	Diploneis spp.	Fragilaria spp.	Lauderia spp.	Licmophora spp.	Lyrella spp.	Mastogloia spp.	Melosira spp.	Navicula spp.	Nitzschia spp.	Nitzschia closterium	Pinnularia spp.	Pleurosigma spp.	Sellaphora spp.	Stauroneis spp.	Surirella spp.	Synedra spp.	Unidentified diatomophyceae				Phormidium spp.	Spirulina spp.	Unidentified cyanobacteria		Prorocentrum cordatum	Prorocentrum balticum	Prorocentrum micans	Unidentified Dinophyceae		Cryptomonas spp.	Unidentified cryptophyceae	Chlorophyta	Paraphysomonas spp.
Occurrence %	53	7	67	20	0	13	67	7	0	7	13	73	20	7	0	0	33	73	40	7	7	7	13	0	0	53	80	27	13	47	40	7	7	47	40	47	40	20
LDPE	60	0	60	20	0	20	80	0	0	20	40	80	20	0	0	0	40	80	0	0	20	20	20	0	0	60	80	20	0	40	20	0	0	20	40	40	20	20
PET	40	0	80	20	0	0	80	20	0	0	0	60	0	20	0	0	40	60	40	20	0	0	20	0	0	40	80	40	20	40	40	0	0	80	60	60	60	20
PP	60	20	60	20	0	20	40	0	0	0	0	80	40	0	0	0	20	80	80	0	0	0	0	0	0	60	80	20	20	60	60	20	20	40	40	40	20	20
t0.5	67	0	100	33	0	0	100	0	0	0	33	100	0	33	0	0	33	100	33	0	0	0	67	0	0	100	100	33	0	67	0	0	0	33	33	33	67	0
t2	100	0	67	0	0	0	100	0	0	33	0	100	0	0	0	0	33	100	33	0	0	0	0	0	0	100	100	0	0	33	100	0	0	67	0	33	0	0
t7	100	33	100	67	0	33	67	33	0	0	33	100	33	0	0	0	0	67	67	33	0	33	0	0	0	67	100	67	67	100	33	33	33	67	100	67	33	67
t10	0	0	67	0	0	33	67	0	0	0	0	67	67	0	0	0	100	100	67	0	33	0	0	0	0	0	100	33	0	33	67	0	0	67	67	100	100	33

Fig. 6. TU occurrence by sample (mean ± standard deviation (A); percentage of TU/classes (B,C,D); and TU occurrence of colonizing microalgae over time (t0/February, t0.5/February, t2/April, t7/September, t10/December) on macroplastic types (LDPE, PET and PP) in the Biguglia lagoon experiment (E).

5. Conclusion

This study investigates three Mediterranean coastal lagoons to study harmful algae and pathogens on plastic debris. New information was obtained regarding of the communities of microalgae colonizing macroplastic debris, which revealed changes according to seasons, with higher amounts in spring and summer, but without differences according to lagoons or polymers. Although Diatomophyceae were the most abundant colonizing organisms on macroplastic debris, they were associated with Dinophyceae with the occurrence of potentially harmful species (such as *Alexandrium* spp. or *Prorocentrum* sp.), and with the presence of Cyanobacteria and hazardous bacterial strains (*Vibrio* sp.). An additional *in situ* experiment demonstrated the presence of colonizing microalgae from the first 15 days and an increase in community diversity over time for the three polymers tested. In short, Mediterranean coastal lagoons are vulnerable to the presence of macroplastic debris that may passively host and transport various species, including some potentially harmful algal and bacterial microorganisms.

Coastal lagoons are recognized as being among the ecosystems most vulnerable to global changes that affect aquatic environments, due to their location at the border of terrestrial and marine biomes and the multiple threats and pressures that affect them. Biodiversity hotspots, highly sought-after and with diversified levels of development, Mediterranean coastal lagoons remain paradoxically neglected with regard to the risks that the proliferation of plastic debris represent. Our study seeks to draw attention to and stimulate further study of this issue of great concern.

Author contribution statement

Vanina Pasqualini; Marie Garrido; François Galgani; Conceived and designed the experiments; Performed the experiments; Analyzed and interpreted the data; Contributed reagents, materials, analysis tools or data; Wrote the paper.

Philippe Cecchi: Analyzed and interpreted the data; Wrote the paper.

Coralie Connes: Performed the experiments.

Alain Couté; Maryvonne Henry; Yann Quilichini; Jérémy Simmonet; Emmanuel Rinnert; Thomas Vitre: Analyzed and interpreted the data; Contributed reagents, materials, analysis tools or data.

Maria El Rakwe; Dominique Hervio Heath: Analyzed and interpreted the data; Contributed reagents, materials, analysis tools or data; Wrote the paper.

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Data availability statement

Data will be made available on request.

Additional information

Supplementary content related to this article has been published online at [URL].

Declaration of interest’s statement

The authors declare no conflict of interest.

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Appendix A. Supplementary data

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