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Abstract

Plastic debris carry fouling a variety of class-size organisms, among them harmful microorganisms that potentially play a role in the dispersal of allochthonous species and toxic compounds with ecological impacts on the marine environment and human health. We analyzed samples of marine plastics floating at the sea surface using a molecular qPCR assay to quantify the attached microalgal taxa, in particular, harmful species. Diatoms were the most abundant group of plastic colonizers with maximum abundance of 8.2 x 104 cells cm-2 of plastics, the maximum abundance of dinoflagellates amounted to 1.1 x 103 cells cm-2 of plastics. The most abundant harmful microalgal taxon was the diatom Pseudo-nitzschia spp., including at least 12 toxic species, and the dinoflagellate Ostreopsis cf. ovata with 6606 and 259 cells cm-2, respectively. The abundance of other harmful microalgal species including the toxic allochthonous dinoflagellate Alexandrium pacificum ranged from 1 to 73 cells cm-2. In the present study, a direct relationship between the abundance of harmful algal species colonizing the plastic substrates and their toxin production was found. The levels of potential toxins on plastic samples ranged from 101 to 102 ng cm-2, considering the various toxin families produced by the colonized harmful microalgal species. We also measured the rate of adhesion by several target microalgal species. It ranged from 1.8 to 0.3 day-1 demonstrating the capacity of plastic substrate colonizing rapidly by microalgae. The present study reports the first estimates of molecular quantification of microorganisms including toxin producing species that can colonize plastics. Such findings provide important insights for improving the monitoring practice of plastics and illustrate how the epi-plastic community can exacerbate the harmful effects of plastics by dispersal, acting as an alien and toxic species carrier and potentially being ingested through the marine trophic web.

Keywords	assemblages; dispersal; harmful algae; biotoxins; marine plastics; qPCR

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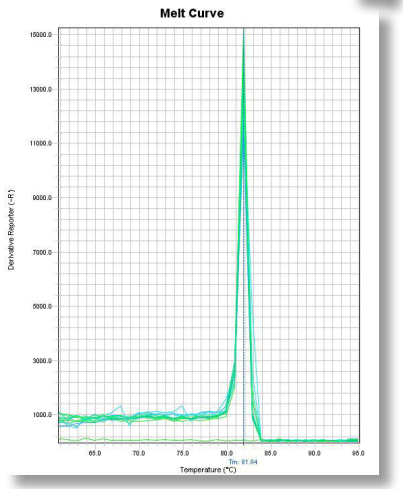
HIGHLIGHTS

- Toxic microalgal species colonized on marine plastic debris.
- Colonized toxic microalgal taxa on marine plastics was quantified by qPCR.
- *Pseudo-nitzschia* spp. were the predominant microalgal taxon on marine plastics.
- Concentrations of toxins on marine plastics ranged from 10^1 to 10^2 ng cm^{-2}.
- Harmful Algal species can be dispersed through plastics in marine environment.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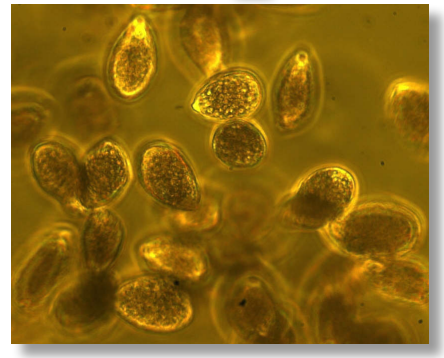

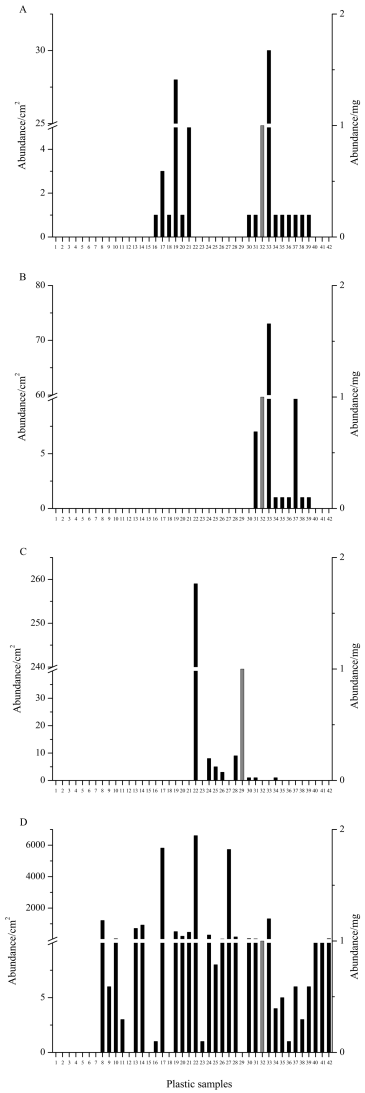
A
B
C
D
Abundance/cm²
Abundance/mg
Plastic samples

Plastic-associated harmful microalgal assemblages in marine environment

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Declaration of interest: none.

Abstract

Plastic debris carry fouling a variety of class-size organisms, among them harmful microorganisms that potentially play a role in the dispersal of allochthonous species and toxic compounds with ecological impacts on the marine environment and human health. We analyzed samples of marine plastics floating at the sea surface using a molecular qPCR assay to quantify the attached microalgal taxa, in particular, harmful species. Diatoms were the most abundant group of plastic colonizers with maximum abundance of 8.2 x 10^4 cells cm^{-2} of plastics, the maximum abundance of dinoflagellates amounted to 1.1 x 10^3 cells cm^{-2} of plastics. The most abundant harmful microalgal taxon was the diatom *Pseudo-nitzschia* spp., including at least 12 toxic species, and the dinoflagellate *Ostreopsis* cf. *ovata* with 6606 and 259 cells cm^{-2}, respectively. The abundance of other harmful microalgal species including the toxic allochthonous dinoflagellate *Alexandrium pacificum* ranged from 1 to 73 cells cm^{-2}. In the present study, a direct relationship between the abundance of harmful algal species colonizing the plastic substrates and their toxin production was found. The levels of potential toxins on plastic samples ranged from 10^1 to 10^2 ng cm^{-2}, considering the various toxin families produced by the colonized harmful microalgal species. We also measured the rate of adhesion by several target microalgal species. It ranged from 1.8 to 0.3 day^{-1} demonstrating the capacity of plastic substrate colonizing rapidly by microalgae. The present study reports the first estimates of molecular quantification of microorganisms including toxin producing species that can colonize plastics. Such findings provide important insights for improving the monitoring practice of plastics and illustrate how the epi-plastic community can exacerbate the harmful effects of plastics by dispersal, acting as an alien and toxic species carrier and potentially being ingested through the marine trophic web.

Plastics contribute to disperse species-specific toxic and allochthonous microalgae and biotoxins in the marine environment

Keywords: assemblages, dispersal, harmful algae, biotoxins, marine plastics, qPCR

1. Introduction

It has been estimated that 23,150 of the 268,940 tons of plastic particles floating on the surface of the world's oceans are found on the Mediterranean Sea surface (Lebreton et al., 2012; Eriksen et al, 2014). This global issue is coming to the fore, with mounting evidence of how plastic pollution affects the marine ecosystem and the food web. The growing production of plastics is undoubtedly related to the expanding human consumption (Derraik, 2002; Brown et al. 2015; Jambeck et al., 2015).

Plastics accumulate along the coasts and in open oceans through currents and gyres (Law et al., 2010; Andrady, 2011) and their distribution is influenced by wind mixing, affecting their vertical movement (Kukulka et al., 2012). The densities of microplastics also affect their distribution as floating plastics remain on the surface, whereas their denser counterparts fall to the sea floor (Engler, 2012). Further, it is known that plastic debris can persist at the sea surface for years or even decades due to the stability of plastics and nature of the material that was composed by polymers having less density than seawater (Andrady et al., 2011; Cózar et al., 2014).

Floating plastics are a durable and persistent substratum for the bio-adhesion of micro- and macro-organisms enabling the dispersal from native to new habitats (Reisser et al., 2014; Fazey et al., 2016). Consequently, the settlement of non-indigenous organisms can potentially lead to the establishment of alien communities, which may alter existing communities, introduce disease vectors and facilitate the dispersal of organic pollutants accumulated from surrounding waters (Zettler et al., 2013; Goldstein et al., 2014). This has already been shown to occur for bacteria, plants, harmful microalgae of dinoflagellates and animals (Masó et al., 2003; De Tender et al., 2015; Oberbeckmann et al., 2015).

In particular, microbial adhesion and colonization on plastic particles in marine environment occur quickly (Bakker et al., 2003; Zettler et al., 2013; Oberbeckmann et al., 2015). Indeed, microbial biofilm formation on bags can occur after a week (Lobelle and Cunliffe, 2011). The surface – attached bacterial communities are able to colonize on various types of plastic materials, such as PET (polyethylene terephthalate), PVC (polyvinyl chloride) and PS (polystyrene), being subjected to seasonal conditions and habitat variation (Webb et al., 2009). It has also been shown that biofilms on the floating plastics contain mostly diatoms, "cyanobacteria" and "coccolithophores" (Briand et al., 2012; Oberbeckmann et al., 2014; Reisser et al., 2014). The biological adhesion of plastic debris is mediated by their hydrophobic surface, and the microbial biofilms on microplastics can affect the physical properties of plastic particles (e.g. size, weight, buoyancy) by accelerating their sedimentation on the sea bottom (Salta et al., 2013; Zettler et al., 2013). The plastics may be a vector for dispersal by colonized harmful microorganisms. In fact, in addition to high abundance of *Vibrio* spp., *Campylobacter* spp., *Pseudomonas* spp., which have been detected, the harmful dinoflagellates *Alexandrium*, *Coolia* and *Ostreopsis* have been retrieved on plastics floating in coastal waters of the Mediterranean Sea (Masò et al., 2003; Zettler et al., 2013; Masò et al., 2016). The relationship between an *A. taylorii* bloom event and the detection of *A. taylorii* cells attached to plastic fragments has been suggested (Masó et al., 2003). Analyses of pelagic and benthic marine plastic debris by scanning electron microscopy (SEM), has confirmed the presence of diatoms (100 - 94% of the pelagic and benthic plastics sampled, respectively), dinoflagellates (58% and 13% of the pelagic and benthic plastics sampled, respectively) (Masò et al., 2016).

In the Mediterranean Sea, the HAB events are mostly caused by dinoflagellate species. Their blooms appear to be influenced and determined by the hydrodynamic regimes of coastal areas and life-history of strategies, and plastics can be subjected to the same physical processes favouring their accumulation and providing substrates for microalgae proliferation (Basterretxea et al., 2007). Moreover, the increasing anthropic pressures and global climate change are intensifying the HAB phenomenon facilitating noxious and allochthonous species dispersal in the seas through ballast

99 waters, shellfish translocation or plastic vectors (Hamer et al., 2001; Hallegraeff, 2010; Masó et al.,
100 2016).
101 Therefore, macroplastics and microplastics pose risk to zooplankton, marine mammals, birds and
102 fishes, as well as a potential vector for the dispersal of allochthonous and harmful microrganisms
103 (Andrady, 2011; Masò et al., 2016). In particular, the harmful algal (HA) species produce a variety
104 of marine biotoxins that are always implicated in episodes of filter or grazer-feeding animal
105 contamination and human poisoning sometimes being lethal worldwide (Anderson et al., 2012;
106 Parsons et al., 2012; Trainer et al., 2012). If ingested, plastics may act as a vehicle for toxins to
107 enter the food chain. Indeed, in the micrometer range, plastics have been found to be ingested and
108 egested by the marine biota, whereas nonoplastics can pass through cell membranes, potentially
109 causing harm to organisms (Cole et al., 2013).
110 This study aims to characterize and quantify target harmful microalgal taxa, diatoms (class
111 Bacillariophyceae), and dinoflagellates (class Dinophyceae) potentially attached to micro and
112 macro plastics collected in the coastal waters and off-shore of the Mediterranean Sea using
113 molecular qPCR based assay. The investigated harmful algal taxon was the diatom *Pseudo-*
114 *nitzschia*. Genus *Pseudo-nitzschia* include at least 12 toxic species (e.g. *P. multiseries, P. australis,*
115 *P. seriata* and *P. multistriata*). These species produce domoic acid (DA), a neurotoxin that cause
116 amnesic shellfish poisoning (ASP) (Trainer et al., 2012). The other studied species were the toxic
117 dinoflagellates *Alexandrium pacifium, A. minutum* and *Ostreopsis* cf. *ovata*. Both *Alexandrium*
118 species produce the PSTs (paralytic shellfish toxins), which are neurotoxins causing paralytic
119 shellfish poisoning (PSP) (Franco et al., 1994; Krock et al., 2007; Penna et al., 2015). The toxic
120 epiphytic *O.* cf. *ovata* produces ovatoxins (OVTXs) and palytoxin (PLTX), one of the most potent
121 non-protein marine toxins so far known (Ciminiello et al., 2012; Tartaglione et al., 2017). All these
122 harmful microalgal taxa are abundantly distributed along the coastal waters of the Mediterranean
123 Sea.

In the present study, all plastic samples were characterized by Fourier-transform infrared spectroscopy (FTIR).

Furthermore, since the plastics are considered potentially vehicles for the transport of toxic microrganisms, toxin levels of harmful algae collected from plastic surfaces during a HAB (harmful algal bloom) event were measured by liquid chromatography coupled with high resolution mass spectrometry (LC-HRMS) to determine if marine plastic debris could directly constitute a toxin vector and the potential risk of the dispersion of such vectors in the marine environment.

In addition to these investigations in the field, *in vitro* experiments using plastic sheets and target cultured harmful microalgal species were carried out to estimate the capacity and rate of adhesion of toxic algal species to plastic surfaces.

2. Materials and methods

2.1 Microalgal cultures

The strains of harmful dinoflagellate *Alexandrium pacificum* (CNR-ACAT5D1, CNR-ACAT6A2, CNR-ACAT6FA, CNR-ACAT6D4, CNR-ACAT7A2, CNR-ACAT6D5, CNR-ACAT15P) were obtained from various plastic samples collected at the sea surface during a monospecific bloom at Syracuse Bay, Ionian Sea (Italy) in the same survey of plastic debris in 2016-2017 as described below. The microalgal cells were removed from plastics by gently scraping in 5 mL sterile seawater. Single cells were isolated by micropipetting and clonal cultures were established and maintained in F/2 (Guillard, 1975). These strains together with *A. pacificum* CNR-ACAT15, CNR-ACATA1, CNR-ACAT02 strains, which were isolated from Syracuse harbour, were further analysed for the PSTs (paralytic shellfish toxins) content by LC-HRMS.

Further, a total of 22 microalgal strains were used in the present study for plastic adhesion experiments and molecular standard curves used in the qPCR quantification assay (Table S1).

All strains were maintained in different media at 23 ± 1°C and 16 ± 1°C for species belonging to the class Dinophyceae and Bacillariophyceae, respectively. Light was provided by cool-white

150 fluorescent bulbs (photon flux of 100 μE m^{-2} s^{-1}) on a standard 14:10 h light-dark cycle. Culture
151 subsamples, each containing 1.0 x 10^5 cells were harvested by centrifugation (4000 rpm for 10 min)
152 during the exponential growth phase. Microalgal pellets were stored at -80 °C for subsequent
153 molecular analyses for standard curves mixtures with the exception of *Pseudo-nitzschia*
154 *multistriata, A. pacificum* strains (CNR-ACAT5D1, CNR-ACAT6A2, CNR-ACAT6FA, CNR-
155 ACAT6D4, CNR-ACAT7A2, CNR-ACAT6D5, CNR-ACAT15P) and *Protoceratium reticulatum*.
156
157 *2.2 Environmental plastic sampling and processing*
158 A total of 42 plastic samples were collected in different areas of the Mediterranean Sea from March
159 2016 to May 2017 (Table S2). Sampling was done by the use of a manta net of 330 μm mesh size,
160 which is composed of (i) a steel mouth of about 0.50 x 0.25 m of opening, (ii) two buoyant wings,
161 (iii) a conic net about 3 m long, (iv) a collector at the code end. Manta net was towed for about 20
162 min at a speed of approximately 1.5 - 2 knots: this procedure allows manta net to float on the sea
163 with the lower half-mount under the surface. In addition, macroplastic samples, i.e. floating pieces
164 of plastic on the sea surface, were also collected manually. After the collection, micro- and
165 macroplastic samples were preserved immediately at -20°C. These micro and macroplastic samples
166 were analyzed by qPCR using length (cm^2) or weight (mg) for molecular abundance quantification
167 of attached microalgal taxa.
168 Laboratory processing and subsequent sorting of microplastics collected by the manta net was
169 carried out as follows: once placed on board, the net was rinsed with seawater from the outside
170 inwards to collect all the material captured by the net in the collector container. The container was
171 then detached from the net and the sample filtered using a sieve for discharging particles greater
172 than 5 mm size. Then, the sample was poured into a 1000 mL, 500 mL or 250 mL glass jars. Plastic
173 samples were rinsed with distilled water several times. They were then dried off at room
174 temperature for 48 hours, and stored in the dark at room temperature for subsequent chemical
175 analyses.

176

177 *2.3 FTIR spectroscopy*

178 A Nicolet 6700 FT-IR spectrometer (Thermo Fisher, USA) fitted with a single bounce attenuated
179 total reflectance (ATR) accessory was used to characterize the plastic samples. The ATR accessory
180 was equipped with zinc selenide (ZnSe) reflection crystal. ATR-FTIR spectra was collected by
181 coaddition of 32 scans at a resolution of 4 cm^{-1} against a background spectrum from the clean ATR
182 crystal. ATR-FTIR measurements were conducted at room temperature (20°C). Spectra were
183 recorded in absorbance mode in the spectral region 4000–600 cm^{-1}. Plastic samples were analyzed
184 directly on the ZnSe ATR crystal. The sample spectra were submitted to automated library search
185 and finally the spectrum of the candidate polymer was overlaid and compared with the sample
186 spectrum to check the result.

187

188 *2.4 DNA extraction and qPCR analyses on environmental marine plastic samples*

189 Macroplastic samples were sized and bigger pieces of macroplastics were cut by sterile scissors for
190 subsequent analyses. Microplastics (< 5 mm in size) obtained from manta net sampling were used
191 directly. Genomic DNA was extracted from microalgal cultures and from marine macro and
192 microplastic samples with size range from 1.7 to 80 cm^2 and weight from 95 to 400 mg,
193 respectively. The genomic DNA was extracted using the DNeasy PowerSoil Kit (Qiagen, Hilden,
194 Germany), according to the manufacturer's instructions with DNA being eluted in a final volume of
195 100 μL elution buffer. DNA was quantified using a Qubit fluorometer with a Quant-iT dsDNA HS
196 assay Kit (Invitrogen, Carlsbard, CA) and stored at -20°C until PCR analysis.
197 Class-specific primers for the detection of Dinophyceae and Bacillariophyceae (Godhe et al., 2008)
198 were used for the amplification and quantification of SSU rDNA fragments obtained from several
199 different species of cultured algal strains and microalgal assemblages attached to the plastic field
200 samples. Positive plastic samples were analysed for the quantification of LSU or 5.8S-ITS rDNA

fragments of several target harmful dinoflagellate and diatom genera and species by qPCR based assay (Table S3).

The qPCR assay was performed as described in Perini et al. (2018). The standard curves for the copy number quantification of Dinophyceae and Bacillariophyceae classes were constructed using a six point ten-fold dilution series of purified SSU rDNA PCR products (from 2 to 1.0 x 10^6 copies) generated from DNA of *A. pacificum* CNR-SRA4 and *Chaetoceros* sp. CBA22 (Perini et al., 2018). On the other hand, the species-specific standard curves of *L. polyedrum, A. minutum, A. pacificum* and *O.* cf. *ovata* were obtained using plasmids (Perini et al., 2011; 2018), as well as those for the quantification of the genus *Pseudo-nitzschia* (Penna et al., 2013).

Two different cellular standard curves, namely DinoMix and BaciMix, of ten-fold serial dilution in the 1.0 x 10^{-2} - 1.0 x 10^3 and 1.0 x 10^0 - 1.0 x 10^3 cellular range were constructed to quantify Dinophyceae and Bacillariophyceae classes in plastic field samples, respectively. Because class primers were able to amplify many dinoflagellate and diatom species, the cellular standard curves were obtained by mixing purified DNAs obtained from all 10 different monoclonal cultures of Dinophyceae and Bacillariophyceae, respectively (Table S4). To evaluate the potential variability of SSU rDNA copy number, diverse mixtures were made of the final volume of 10 µL containing different mixed amounts of genomic DNAs that were extracted from 1.0 x 10^5 cultured cells. The mixtures were performed using various percentages of DNA ranging from 10% to 40%, corresponding to 1.0 x 10^3 to 4.0 x 10^3 cells.

Acquisition of qPCR data and subsequent analyses were carried out using StepOne software ver. 2.3. A dissociation curve was generated after each amplification run to check for amplicon specificity and primer dimers. The automatically generated standard curves were accepted when the slope was between - 3.32 and - 3.55 (100 – 91% efficiency) and the correlation coefficient (R^2) was at least 0.99. An estimation of the SSU rDNA copies cm^{-2} and copies mg^{-1} was provided for Dinophyceae and Bacillariophyceae classes. The quantification of phytoplankton species by class primers was performed using DinoMix and BaciMix cellular curves. Dinophyceae and

227 Bacillariophyceae abundance was calculated by plotting the Ct (threshold cycle) values of
228 environmental plastic samples on the cellular standard curves, taking into account the DNA elution
229 volume and dilution factors. The previously described standard curves were used for the
230 quantification of the copy number of both cultured strains and purified cells from environmental
231 plastic samples by qPCR. Taking into account the dilution factor, the total copy number in each
232 sample was obtained. This value was finally divided by the copy number per cell, derived from
233 cultured strains of the different species. The abundance of Dinophyceae, Bacillariophyceae and the
234 other harmful algal species attached to plastic samples was normalized to cells cm^{-2} and cells mg^{-1}
235 for macroplastic or microplastic samples. Statistical analyses were performed with non parametric
236 Mann–Whitney, Kruskal Wallis and Spearman correlation tests using PAST ver. 3.14 with a p-
237 value < 0.05 determining significance.
238
239 *2.5 Extraction of algal pellet and Liquid Chromatography-High Resolution Multiple Stage Mass*
240 *Spectrometry (LC-HRMS^n, n=2)*
241 A total of 10 *A. pacificum* cultured strains (Table S1) at a cellular concentration from 1.5 x 10^6 to
242 1.0 x 10^7, was collected during the exponential growth phase, centrifuged at 4000 rpm for 15 min
243 and the pellets were then frozen at -80°C until chemical analyses. Water, acetonitrile and methanol
244 (HPLC grade), glacial acetic acid and formic acid (Laboratory grade) were purchased from Sigma-
245 Aldrich Corporation (St. Louis, MO). A 25% ammonia solution (LC-MS grade) was obtained from
246 Merck Millipore (Germany). Standard solutions of PST (paralytic shellfish toxin) were obtained
247 from the NRC Certified Reference Materials (CRM) Program (Halifax, NS, Canada).
248 The 10 pellets of *A. pacificum* were separately extracted with 0.5 mL of 0.1 M acetic acid in water,
249 while being sonicated for 10 min in pulse mode in an ice bath. The mixture was centrifuged at 8000
250 rpm for 10 min and the supernatant was decanted. Each residue was extracted once again with 0.5
251 mL of the extraction solvent, the supernatant was decanted and the two extracts were combined (1

mL total). A 50 µL of each extract was added to 150 µL of acetonitrile and subsequentially analyzed by HILIC-HRMS^n (5 µL injected).

A Dionex Ultimate 3000 quaternary system coupled with a hybrid linear ion trap LTQ Orbitrap XLTM Fourier Transform MS (FTMS) equipped with an ESI ION MAX^{TM} source (Thermo-Fisher, San Jose, CA, USA) was used. For PST HILIC – HRMS^n separations were performed according to Turner et al. (2017) with slight modifications. A Water Acquity UPLC BEH Glycan 1.7 µm, 2.1 x 150 mm column (Batch 0161) was used in conjunction with a Waters VanGuard BEH Amide cartridge 1.7 µm, 2.1 x 5 mm (Waters, Massachusetts, USA) maintained at 60°C. The column was eluted with water + 0.015% formic acid + 0.06% ammonia 25% (eluent A) and 70% acetonitrile/water + 0.01% formic acid (eluent B) according to the following gradient: time (t)= 0 min, 99% B; t= 10 min, 99% B; t= 15 min, 50% B; t= 18 min, 50% B; t=19 min, 99% B; t=20 min, 99% B. A flow rate of 0.20 mL/min was used for the first 15 min and of 0.25 mL/min for the rest of the acquisition time. Re-equilibration time at the initial conditions was 12 min.

In all cases, HRMS^n analyses (n = 1-3) were performed in positive ion mode at a resolving power of 60,000 (FWHM at *m/z* 400) and a spray voltage of 4.8 kV. PST detection was accomplished in collision induced dissociation (CID) MS^2 mode using the following source settings: capillary temperature = 220 °C, capillary voltage = 49 V, sheath gas = 62 and auxiliary gas= 16.5 (arbitrary units), tube lens voltage= 250 V; an activation Q of 0.250, and an activation time of 30 ms using collision energies in the range 22−28%. The tube lens voltage was set at 120 V. Extracted ion chromatograms (XIC) of the fragment ions of PST (Perini et al., 2014) were obtained at a 5 ppm mass tolerance and used for quantification. Quantification of the toxins was accomplished by direct comparison of XIC areas of individual toxins to relevant PST CRM solutions injected at similar concentrations injected under the same experimental conditions. The results were expressed on a per cell basis (fg cell^{-1}).

2.6 Adhesion rate of harmful algal species to the plastic substrate

Each strain of the diatoms *P. multistriata* CBA174, *Skeletonema marinoi* CBA4, and the dinoflagellates *A. minutum* CBA57, *A. pacificum* CNR-SRA4, *L. polyedrum* LPA0510, *P. reticulatum* PRA0311, *G. spinifera* CBA5 and *O.* cf. *ovata* CBA1291 were grown in 1 L glass bottles containing 0.4 L of sterilized medium together with sterilized PE plastic sheets (3 cm x 3 cm), which were cut from bigger fragments collected previously along the shoreline. The initial cell number was the same for all the selected strains (1.0×10^3 cells mL^{-1}). Culture media conditions were those described above. A plastic sheet was harvested every four days from the inoculum and gently scraped in 10 mL of sterile seawater 0.45 μm filtered using a spatula in order to recover the cells adhering to the plastic sheets. Subsamples of 1 mL were collected for cell density estimation using Sedgewick-Rafter counting chamber. Cell amount was estimated using an inverted microscope (ZEISS Axiovert 40CFL) at 400X magnification. The adhesion rates, defined as the rate of increased abundance on the plastic sheets, were calculated on the basis of the longest possible period of exponential growth using the equation: $\mu = \ln (Nt/N0)/\Delta t$, where N is the number of the plastic adherent cells expressed as cells cm^{-2} and Δt is the time interval expressed in days (Wood et al., 2005).

3. Results

3.1 Plastic type and structure analysis

The amount of sampled microplastics corresponding to a 64% was non-polar (polyethylene and polypropylene) with a typical crystalline structure, the remaining 36% had a certain level of polarity with a typical amorphous structure (Fig. 1). Detailed ATR-FTIR spectra of analysed plastic samples were shown in Figs. S1-S6.

3.2 Molecular quantification of microalgal assemblages and harmful algal taxa on marine plastics

3.2.1 Dinophyceae and Bacillariophyceae standard curve characterization

304 The mean standard curves of Dinophyceae and Bacillariophyceae showed a PCR efficiency of 93
305 and 99% respectively (y = -3.49x + 35.21; y = -3.36x + 38.86 for Dinophyceae and
306 Bacillariophyceae, respectively), a linear correlation of 6 $\log_{10}$ (R^2 = 0.99) and a sensitivity of 2
307 copies/reaction (Ct mean = 33.03 ± 0.88 and Ct mean = 36.76 ± 0.34 for Dinophyceae and
308 Bacillariophyceae, respectively). The reproducibility was also assayed and the CV_{Ct} mean values
309 were 1% and 11% (1.0 x 10^6 – 2.0 x 10^0 molecules), for Dinophyceae and Bacillariophyceae
310 respectively, while, the CV_{Cn} mean values were 18% and 9%, for Dinophyceae and
311 Bacillariophyceae, respectively. Species-specific standard curve characterization of *A. pacificum, A.*
312 *minutum*, *L. polyedrum, P. reticulatum* and *G. spinifera* are reported in Perini et al. (2018), while
313 the standard curve of *Pseudo-nitzschia* spp. and *O.* cf. *ovata* are described in Penna et al. (2013) and
314 Perini et al. (2011), respectively.
315
316 *3.2.2 The qPCR assay of DNA mixtures of Dinophyceae and Bacillariophyceae*
317 The different mixtures assembled using variable percentage of DNA from a known number of
318 cultured cells of various species of Dinophyceae and Bacillariophyceae were analyzed by qPCR
319 assay, and the obtained Ct values showed no significant differences (Hc = 2.554, p = 0.278 and Hc
320 = 2.333, p = 0.312 for Dinophyceae and Bacillariophyceae, respectively) allowing the use of these
321 mixtures for the construction of DinoMix and BaciMix standard curves.
322 The cellular standard curves used for the quantification of Dinophyceae and Bacillariophyceae
323 classes showed a PCR efficiency of 100 – 91% (y = -3.322x + 28.60 and y = -3.549x + 33.84,
324 respectively), a linear correlation of 5 or 4 log10 (R^2 = 0.99) and a quantification limit of 0.01 (Ct
325 mean = 34.49 ± 0.48) and one cell (Ct mean = 33.66 ± 0.22) per PCR reaction for Dinophyceae and
326 Bacillariophyceae, respectively. The reproducibility, analyzed as CV_{Ct} mean inter-assay variability
327 of different cellular standard curves was 0.6% (range 0.01 - 1000 cells) for Dinophyceae and 0.4%
328 (range 1 - 1000 cells) for Bacillariophyceae.
329

330 *3.2.3 The qPCR assay for harmful microalgal species quantification on plastics*

331 All the 42 plastic samples tested positive for the presence of Dinophyceae and Bacillariophyceae
332 (Fig. 2). The total SSU rDNA copy number and abundance of both dinoflagellates and diatoms
333 attached to the plastics determined by qPCR assay were plotted. The major axis regression between
334 cell concentration and rDNA copy number assessed by qPCR showed a highly significant linear
335 correlation: n = 33, Spearman rs = 0.9983, $p << 0.001$ for dinoflagellate abundance determined per
336 cm^{-2}; n = 9, Spearman rs = 0.9832, $p << 0.001$ for dinoflagellate abundance determined per mg^{-1}; n
337 = 33 Spearman rs = 0.9996, $p << 0.001$ for diatom abundance per cm^{-2}; n = 9, Spearman rs =
338 0.9748, $p < 0.001$ for diatom abundance determined per mg^{-1}.
339 All the plastic samples were quantified for the presence of Dinophyceae. They showed variable
340 amount values that were expressed both as cellular abundance or copy number. These values ranged
341 from 1 to 1.1 x 10^3 cells cm^{-2} and 1 to 61 cells mg^{-1} or from 38 to 8.3 x 10^4 SSU rDNA copy
342 number cm^{-2} and 8 to 4.0 x 10^3 LSU rDNA copy number mg^{-1}. Six samples showed very low
343 Dinophyceae abundance, as one cell cm^{-2} or 73 ± 30 copy number cm^{-2} (samples 9, 16, 23) and
344 one cell mg^{-1} or 14 ± 6 copy number mg^{-1} (samples 1, 2, 15). Indeed, the Bacillariophyceae showed
345 a higher number of cells attached to the plastics than did Dinophyceae with values ranging from 1
346 to 8.2 x 10^4 cells cm^{-2} and 1 to 2.1 x 10^2 cells mg^{-1} or of 43 to 3.6 x10^6 SSU rDNA copy number
347 cm^{-2} and 10 to 8.6 x 10^3 SSU rDNA copy number mg^{-1}. Using this molecular assay, the
348 quantification of a single cell was feasible. One cell cm^{-2} (43 ± 8 copy number cm^{-2}) and one
349 cell/mg (20 ± 14 copy number mg^{-1}) was found in the analysed environmental samples (36 and 2,
350 and 29, respectively). Furthermore, all plastic samples were analyzed for the quantification of
351 harmful target microalgal taxa, namely *A. pacificum*, *A. minutum*, *O.* cf. *ovata,* and *Pseudo-*
352 *nitzschia* spp. (Fig. 3). Sixteen plastic samples showed the presence of the toxic and allochthonous
353 *A. pacificum* and higher amounts were found in sample 33 from Syracuse (Ionian Sea) with 30 cells
354 cm^{-2}. Samples 17, 19 and 21 contained an abundance of *A. pacificum* in the range of 3 - 28 cells cm
355 $^{-2}$, while the remaining samples showed amount of one cell cm^{-2}. Sample 32 showed one cell mg^{-1}

356 of *A. pacificum*. Nine samples tested positive for the presence of *A. minutum* with the highest
357 abundance found in sample 33 from the Syracuse site showing 73 cells cm $^{-2}$. Samples 31 and 37
358 contained 7 and 13 cells cm $^{-2}$ of *A. minutum*, respectively. Sample 32 showed one cell mg^{-1} of *A.*
359 *minutum*. The highest abundance of *O.* cf. *ovata* was found in sample 22 from the Taormina site
360 (Ionian Sea) with 259 cells cm $^{-2}$, the remaining samples from the same site showed a cell range of
361 1 - 9 cells cm $^{-2}$. Sample 29 showed one cell/mg of *O.* cf. *ovata*. *Pseudo-nitzschia* spp. was present
362 in almost all plastic samples resulted positive for Bacillariophyceae (n = 31) with cellular values
363 ranging from 1 to 6.6 x 10^3 cells cm $^{-2}$. Three samples showed one cell cm $^{-2}$ (samples 16, 23, 36).
364 Sample 32 showed one cell mg^{-1} of *Pseudo-nitzschia* spp. Moreover, *L. polyedrum, P. reticulatum*
365 and *G. spinifera* were also investigated and showed scarce or low abundance. One cell cm $^{-2}$ of *L.*
366 *polyedrum* was found in samples from the Syracuse site (samples 31 - 39), with the exception of
367 sample 32, which showed one cell mg^{-1}. One cell cm $^{-2}$ of *G. spinifera* was found at the Taormina
368 (sample 25) and Bari sites (sample 16), and one cell mg^{-1} was found at Genoa site (sample 4). No
369 amplification was obtained for *P. reticulatum* (data not shown).
370
371 *3.3 Toxin content of cultured* A. pacificum *strains isolated from plastic debris*
372 All the 10 strains of *A. pacificum* produced PST (Table 1), with a total toxin content in the range
373 35.14-6032 fg cell^{-1}; CNR-ACAT6FA was the most productive strain, while CNR-ACAT5D1 was
374 the least productive one. A rather high variability in toxin profiles and the relative abundance of
375 individual analogues was observed. Most of the strains produced GTX5, C1/2, GTX1/4, and
376 GTX2/3. STX was produced by 5 out of 10 strains, while dcGTX2 and NEO were found in just one
377 strain; dcGTX3 and dcNEO were absent in all the strains. As for the relative abundance of
378 individual toxins, C2 accounted for 10-56% of the total toxin content, GTX1 for 14-79%, and
379 GTX5 for 1-56%.
380
381 *3.4 Adhesion rate of harmful algal species on plastic surface*

382 The Kruskall Wallis test showed that the adhesion rates of target harmful algal target species varied
383 significantly (Hc = 17.73 $p<0.05$). Moreover, the *a posteriori* pairwise Mann-Whitney test showed
384 that the differences between the algal species were significant ($p<0.05$) with the exception of two
385 species as *A. minutum* and *O.* cf. *ovata*. The dinoflagellate *A. pacificum* appeared to be the species
386 that adhered to the plastics the most rapidly, followed by the diatoms *S. marinoi* and *P. multistriata.*
387 The species that adhered at the slowest rate were *A. minutum* and *O.* cf. *ovata* (Fig. 4). The other
388 investigated harmful species, namely *L. polyedrum, G. spinifera* and *P. reticulatum* showed
389 adhesion rates much lower than 0.1 day^{-1}(data not shown). Moreover, the maximum abundance of
390 attached cells to the plastics determined by microscopy were $3.1 \times 10^5 \pm 1.5 \times 10^4$, $2.8 \times 10^4 \pm 1.6 \times 10^3$, $6.0 \times 10^3 \pm 3.4 \times 10^2$, $2.5 \times 10^3 \pm 1.5 \times 10^2$, $5.6\ 10^2 \pm 3.3 \times 10^1$ cells cm^{-2} for *P. multistriata, S.*
391 *marinoi, A. pacificum*, *O.* cf. *ovata* and *A. minutum*, respectively.
392
393

394 **4. Discussion**

395 In the present study, the molecular quantification by qPCR analysis of the microalgal assemblages
396 on micro and macroplastic debris across different areas within the Mediterranean Sea provided for
397 the first time the evidence that also toxic microalgal species can colonize plastics in high amount
398 and can be dispersed through an alternative substrate. The dispersal of these associated HAB
399 microorganisms across the ocean by currents and winds can be facilitated by floating plastics,
400 because they are persistent, widespread and have various shape and size (Kiessling et al., 2015).
401 In the present study, we showed that the communities of Bacillariophyceae and Dinophyceae were
402 attached to all the plastic samples that were analyzed using the molecular qPCR assay. The
403 abundance of the communities was highly variable with Bacillariophyceae showing up to 10^4 cells
404 cm^{-2} or 10^2 cells mg^{-1} and Dinophyceae showing 10^3 cells cm^{-2} or 61 cells mg^{-1}. The numerical
405 variability and the observed composition of microalgal assemblages were probably related to the
406 dispersion rate and period of time the plastics were floating in the areas where the plastics were
407 collected. In the present study, Bacillariophyceae showed the highest abundance of cells attached to

408 plastics in the areas in the Mediterranean Sea where samples were collected. This is consistent with
409 recent findings regarding the diatom microbiota found attached to plastic debris and analysed using
410 other techniques, such as SEM or metabarcoding qualitative analysis (Farrell et al., 2013; Zettler et
411 al., 2013). Diatoms are probably more able to form biofilms on plastics by producing extracellular
412 substances, which can further facilitate their colonization and the colonization or grazing of other
413 organisms (Reisser et al., 2014). Thus, diatom fouling seems to retain high diversity and to be
414 widespread on marine plastics. However, it is not yet known if the relationship between grazers and
415 plastics has a positive or negative impact on the new marine food web considering that plastics can
416 retain toxic compounds (Cole et al., 2013). Within the Bacillariophyceae, the potentially toxic
417 complex of the *Pseudo-nitzschia* spp. showed the highest abundance of cells attached to the plastic
418 sheets. Indeed, also from the *in vitro* experiments of plastic adhesion rate, the algal species that
419 showed the highest rate of adhesion were the diatoms *S. marinoi,* which is known to form high
420 density blooms and producing noxious effect on copepods by the production of polyunsaturated
421 aldehydes (Ianora et al., 2004; Lauritano et al., 2016), and the potentially toxic *P. multistriata* (data
422 not shown) with the highest maximum abundance of 10^4 and 10^5 cells cm^{-2}, respectively.
423 On the other hand, Dinophyceae showed lower abundance than Bacillariophyceae on field marine
424 plastics, but the harmful dinoflagellate investigated species such as *A. pacificum, A. minutum* and *O.*
425 cf. *ovata* were able to attach to the environmental plastics. In the present study, the *in vitro*
426 experiments showed that the potentially allochthonous and PSP producing dinoflagellate species *A.*
427 *pacificum* attached (with maximum abundance of 6.0 x 10^3 cells cm^{-2}) to the surface of the plastic
428 sheets more quickly than other microalgal species. The potentially allochthonous and toxic *A.*
429 *pacificum* blooms regularly in semi closed coastal areas of the Mediterranean Sea (Penna et al.,
430 2005; 2015) characterized by high surface temperature and the trophic input of nutrients (Bravo et
431 al., 2010). Its blooms are always correlated with the potential of PSP toxin contamination of
432 shellfish (Farrell et al., 2013). The recent dispersal of *A. pacificum* from the western Mediterranean
433 to the central areas (Penna et al., 2015) may have occurred naturally or through anthropically

mediated factors, such as ballast waters, aquaculture activities and plastic pollution (Masó et al., 2016). Indeed, the other toxic dinoflagellate *O.* cf. *ovata* and *A. minutum,* which are responsible for large toxic blooms along the Mediterranean coasts (Vila et al., 2005; Tartaglione et al., 2017), appeared to have a lower rate of adhesion to the plastics, but still showed a valid adhesion potential of 10^3 and 10^2 cells cm^{-2} maximum abundance, respectively. *Ostreopsis* cf. *ovata* is already known to grow on different substrates, such as macrophytes and hard substrates (e.g., rocks, sand and mollusc shells) (Totti et al., 2010) and the biofilm formation observed in our experiments confirmed its ability to grow also on plastic sheet substrates and hence its potential dispersal through benthic and floating plastic sheets. Furthermore, *Alexandrium* species and dinoflagellate resting cysts have already been found on floating plastic marine debris and accumulating along shorelines of the Mediterranean Sea (Masó et al., 2003). Therefore, it is likely that *A. minutum* also has the ability to adhere to plastics. Furthermore, it has already been shown that the phyto - benthic and -plankton communities can attach to both micro and macro plastics (Masó et al., 2016). The specific properties of marine plastics seem to influence colonization and succession processes, and thus the composition of associated rafting community (Kiessling et al., 2015). This could explain the species-specific adhesion of harmful microalgal species shown in the present study. In fact, the qPCR assay was also applied for the specific quantification of other harmful microalgal taxa on surface marine plastics; these species, on the contrary, were quantified in few amounts or were not detectable, such as *L. polyedrum*, *G. spinifera* or *P. reticulatum*, respectively.

Thus, using a qPCR approach we were able to show that some target HA species adhere tightly to floating plastic debris, which suggests that the selected harmful microalgal species could potentially spread through marine ecosystems and the trophic chain negatively impacting human health and environment.

In fact, if we consider the total toxin concentration ranges of any of the toxic compounds produced by the various harmful species, significant amounts of biotoxin can be dispersed in the ocean by the microorganism producers attached to the plastics. In the present study, we found that the genus

460 *Pseudo-nitzschia*, which includes toxic species (i.e. *P. multiseries, P. australis, P. seriata* and *P.*
461 *multistriata*) were the taxon attached to the plastics showing the highest cellular values, and we
462 hypothesized that *Pseudo-nitzschia* can potentially contribute to domoic acid transport with values
463 in the range of 5 - 443 ng cm^{-2} on floating plastics based on the literature values (Trainer et al.,
464 2012). Indeed, based on the maximum abundance of 259 cells cm^{-2} of *O.* cf. *ovata* found on the
465 plastics, we can hypothesize that at least 1 - 62 ng PLTX compounds cm^{-2} must be accumulated in
466 floating plastics debris as established by the recent data of the Mediterranean PLTX production by
467 *O.* cf. *ovata* (Accoroni et al., 2011; Ciminiello et al. 2012; Brissard et al., 2014; Tartaglione et al.,
468 2017). From this study, we can assume that the PSP producing species such as *A. minutum* and *A.*
469 *pacificum*, could transport 1 – 72 pg cm^{-2} of PST, respectively (see also Touzet et al., 2007;
470 Anderson et al., 2012; Pistocchi et al., 2012).
471 It is known that chemical hazards are associated with plastic debris in the marine environment
472 (Rochman, 2015). Marine plastic debris can be linked to a mixture of chemicals that can include
473 marine biotoxins. These chemical compounds produced by microalgae attached to the plastic
474 surface can be transferred throughout lower trophic level organisms as zooplankton by grazing to
475 higher trophic level organisms, which represent contaminated seafood for humans. Also filter
476 feeding animals (i.e. shellfish) can accumulate microplastics that can be colonized by toxic
477 microalgae and thus, they can represent potentially contaminating seafood to humans (Kiessling et
478 al., 2015).
479 Here, there may be a potential risk of indirect toxin accumulation on plastics in the investigated
480 areas of Mediterranean Sea. Obviously, this is a prudent estimate because it does not take into
481 account the length of time that plastics are in the water. But, it is already known that plastic
482 substrata can persist in seawater for years or decades. The plastic properties of buoyancy and
483 resistance of decay make these abiotic substrata more resistant than other biotic substrata (wood,
484 macroalgae) allowing the adhesion of microorganism communities, including allochthonous
485 species, and transport for long distance of organisms that can survive for prolonged time (Pegram

and Andrady, 1989; Thiel and Gutow, 2005). Therefore, marine plastics may act as both sink and source for chemical contaminants including their transfer into marine foodwebs (Rochman, 2015). Hence, it needs urgently to acquire information on the environmental fate of biotoxins associated to plastic debris in the sea.

In the present study, this qPCR based assay of plastics has the advantage of being able to quantify the main phytoplankton groups and harmful taxa on any plastic surface debris floating on the surface of the seawater. The qPCR assay can support biological monitoring of plastic debris using taxon specific primers or probes for microorganisms attached to the plastics achieving high detection sensitivity and specificity (up to one cell cm^{-2} or mg^{-1}).

5. Conclusions

New information was obtained regarding the quantities of noxious microbiota attached to the plastics floating in the Mediterranean Sea based on taxa specific rDNA gene. These findings illustrate the potential risk of truly harmful microorganism dispersal associated with plastic pollution. HA dinoflagellate species abundance was found to be lower than that of diatom communities; however, these diatom communities include potentially toxic *Pseudo-nitzschia* spp. Moreover, in high biomass blooming events, when plastic debris remains longer in the water column, the potential risk of the transport of large amounts of toxins is likely.

In short, in view of recent findings pointing to the association of toxic biofilms with marine plastics, their ingestion through the trophic web and the consequent implications for human health, measures for preventing and managing plastic pollution are urgently needed.

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Table 1. Individual and total toxin content on a per cell basis (fg cell^{-1}) of the Mediterranean *Alexandrium pacificum* strains.

Strains	C1	C2	dcGTX2	dcGTX3	GTX2	GTX3	GTX1	GTX4	GTX5	STX	NEO	dc-NEO	Total
CNR-ACAT 5D1	ND	ND	ND	ND	1.63	ND	19.93	ND	13.28	0.29	ND	ND	35.14
CNR-ACAT6A2	ND	18.97	ND	ND	ND	ND	13.01	4.78	1.70	5.12	ND	ND	43.58
CNR-ACAT6FA	26.62	1413.22	ND	ND	10.11	ND	422.68	181.11	3978.50	ND	ND	ND	6032.25
CNR-ACAT15	3.84	117.76	ND	ND	13.11	70.11	217.57	66.80	320.68	ND	ND	ND	809.87
CNR-ACAT6D4	4.38	432.41	ND	ND	15.23	342.02	247.95	79.97	444.13	18.04	ND	ND	1584.12
CNR-ACATA1	1.77	160.00	ND	ND	10.08	2.45	227.03	64.99	139.56	3.21	ND	ND	609.09
CNR-ACAT7A2	0.74	114.74	ND	ND	1.13	ND	45.39	23.23	71.81	ND	ND	ND	257.04
CNR-ACAT02	52.97	388.20	ND	ND	1.13	3.62	119.66	112.31	ND	1.11	10.263	ND	689.26
CNR-ACAT6D5	32.31	887.33	1.15	ND	35.55	ND	ND	ND	844.77	ND	ND	ND	1801.11
CNR-ACAT15P	6.52	148.76	ND	ND	4.62	ND	103.19	57.90	250.08	ND	ND	ND	571.08

ND, not detectable.

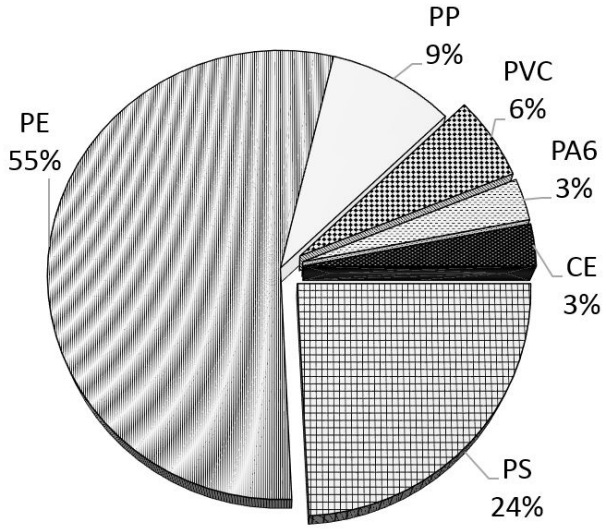
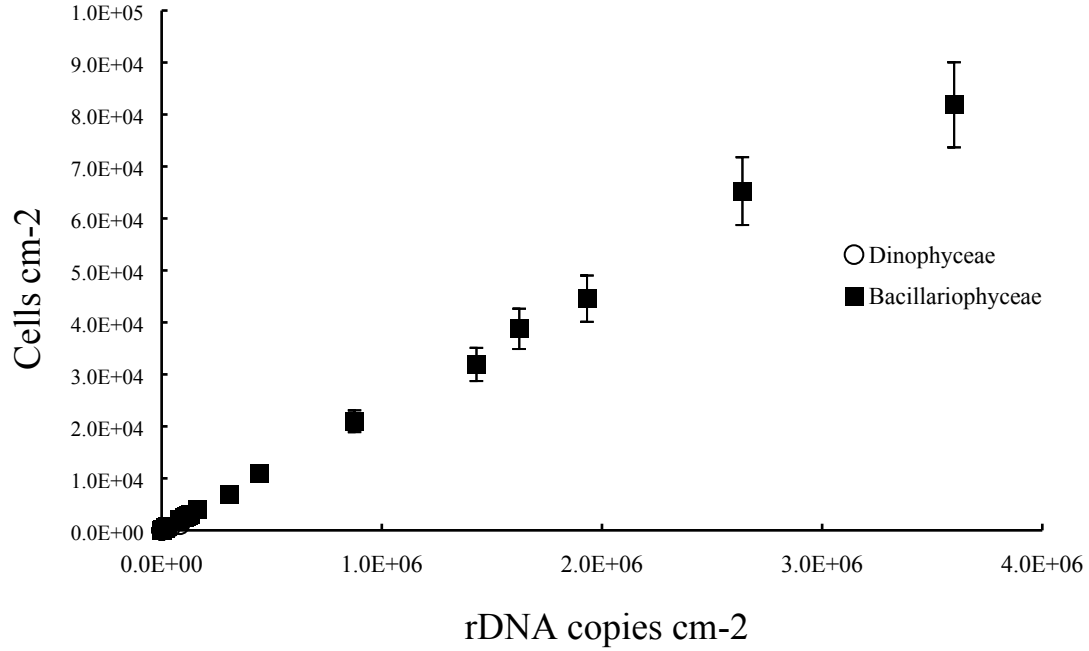
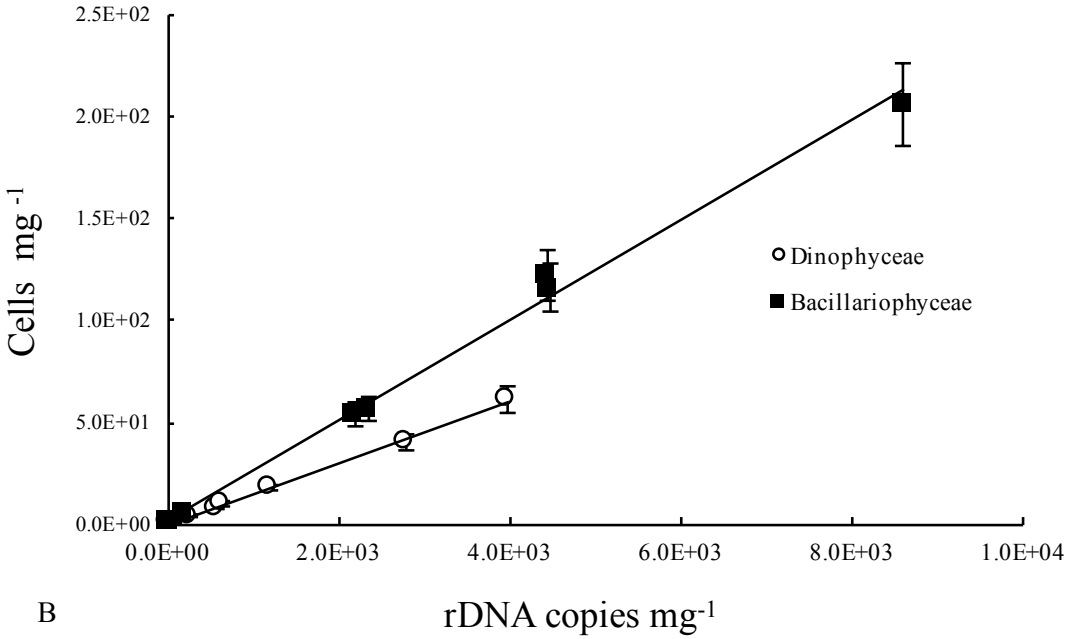


Figure 1. Polymer identification by ATR-FTIR. Identification analysis of plastic samples were expressed as percentage amount. PE: polyethylene; PP: polypropylene; PVC: polyvinylchloride; PA 6: polyamide 6; CE: cellulose; PS: polystyrene. Sectors with grid indicated polar and amorphous polymers. Chemical structures of the polymers were reported in Table S5 of the supplementary material.



A



B

Figure 2. Linear relationship between Bacillariophyceae and Dinophyceae abundance expressed as (A) cells cm^{-2} and rDNA gene content (copies cm^{-2}) or (B) cells mg^{-1} and rDNA gene content (copies mg^{-1}) determined on various plastic substrates using the qPCR assay. Two different cellular standard curves were used for diatom and dinoflagellate cellular estimation. Bacillariophyceae and Dinophyceae rDNA gene copy number were determined using standard curves from purified SSU rDNA PCR products. Linear correlations between Bacillariophyceae and Dinophyceae abundance and rDNA gene content were high and significant ($p << 0.01$).

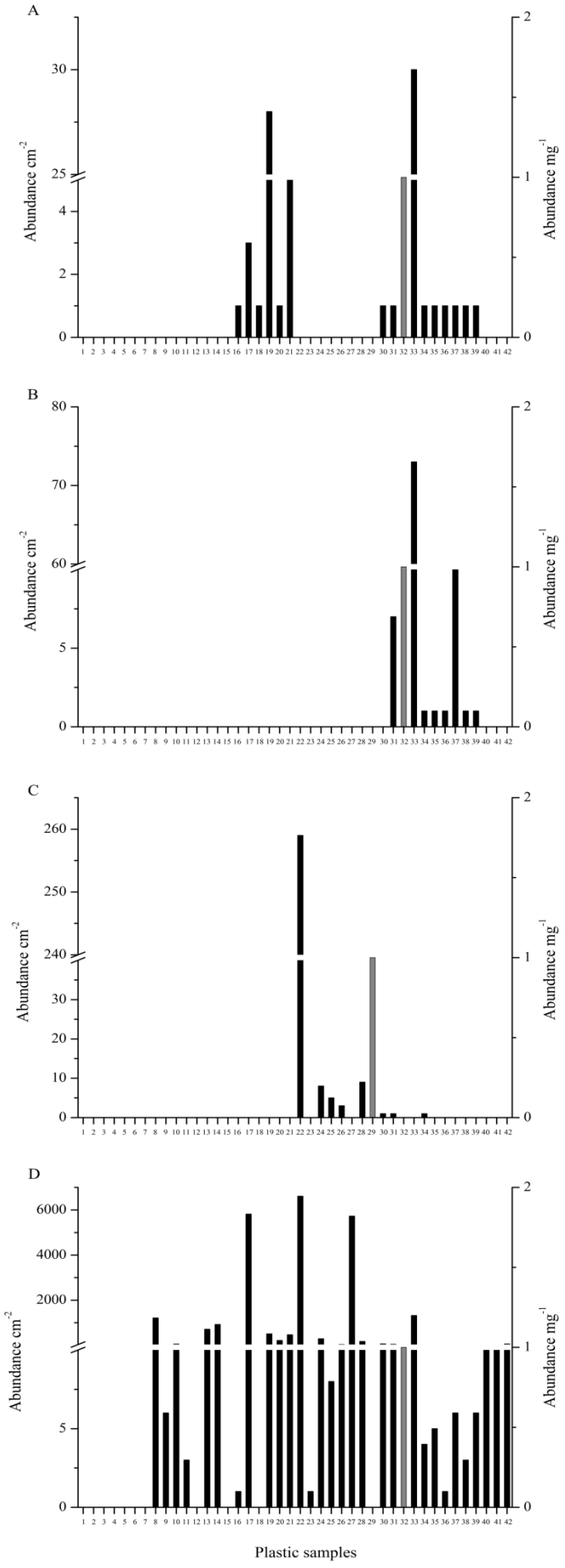


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Figure 3. Molecular quantification of target harmful microalgal species abundance on plastic samples collected in the Mediterranean Sea in 2016-2017 period by qPCR assay. (A) *Alexandrium pacificum,* (B) *A. minutum,* (C) O*streopsis* cf. *ovata*, (D) *Pseudo-nitzschia* spp. Black columns indicate abundance cm^{-2}; grey columns indicate abundance mg^{-1}. One cell mg^{-1} was detected in samples n. 29 for *O.* cf. *ovata* and n. 32 for *A. pacificum, A. minutum* and *Pseudo-nitzschia* spp. Note the y axis break in all graphs.

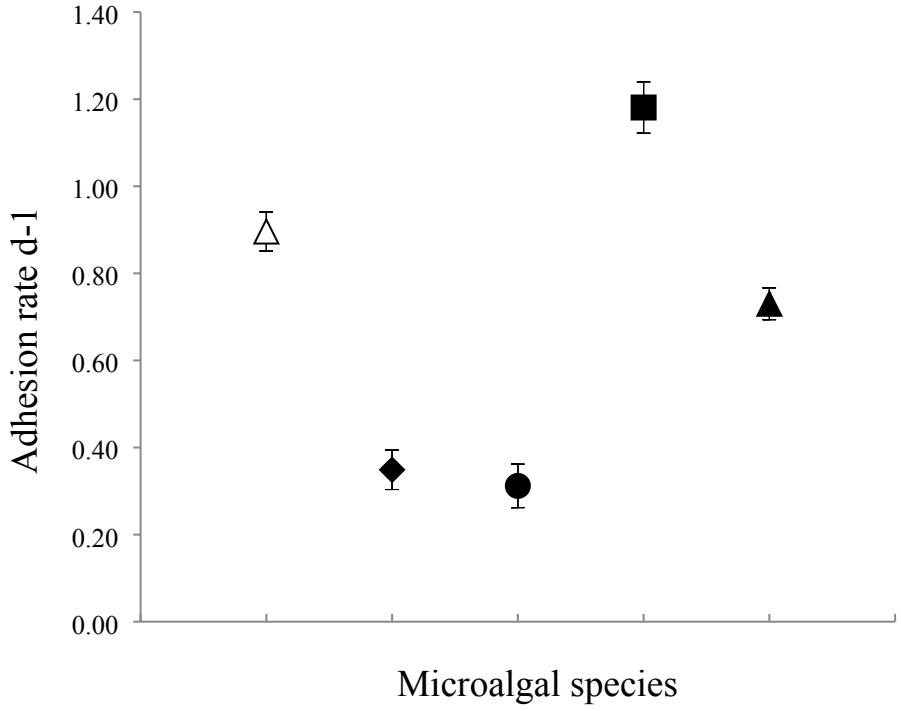


Figure 4. Adhesion rates of different cultured harmful microalgal strains to the plastic surfaces (means ± SD, n = 3). *Alexandrium pacificum* (■), *Skeletonema marinoi* (△), *Pseudo-nitzschia multistriata* (▲), *Alexandrium minutum* (◆) and *Ostreopsis* cf. *ovata* (●).

Supporting Information

Plastics as Vectors for Potentially Harmful Algal Species Dispersal in marine Environment

by Silvia Casabianca, Samuela Capellacci, Maria Grazia Giacobbe, Carmela Dell'Aversano, Fabio Varrale, Luciana Tartaglione, Riccardo Narizzano, Fulvia Risso, Paolo Moretto, Alessandro Dagnino, Rosella Bertolotto, Enrico Barbone, Nicola Ungaro, Antonella Penna

Table S1. Microalgal strains used for plastic adhesion experiments, molecular standard curves in the qPCR assay or chemical analysis of LC-HRMS.

Species	Strain	Class	Medium	Analysis
Alexandrium andersoni	VGO664	Dinophyceae	f/2	molecular
Alexandrium minutum	CBA57	Dinophyceae	f/2	adhesion/molecular
Alexandrium pacificum	CNR-SRA4	Dinophyceae	f/2	adhesion/molecular
Alexandrium pacificum	CNR-ACAT5D1	Dinophyceae	f/2	chemical
Alexandrium pacificum	CNR-ACAT6A2	Dinophyceae	f/2	chemical
Alexandrium pacificum	CNR-ACAT6FA	Dinophyceae	f/2	chemical
Alexandrium pacificum	CNR-ACAT15	Dinophyceae	f/2	chemical
Alexandrium pacificum	CNR-ACAT6D4	Dinophyceae	f/2	chemical
Alexandrium pacificum	CNR-ACATA1	Dinophyceae	f/2	chemical
Alexandrium pacificum	CNR-ACAT7A2	Dinophyceae	f/2	chemical
Alexandrium pacificum	CNR-ACAT02	Dinophyceae	f/2	chemical
Alexandrium pacificum	CNR-ACAT6D5	Dinophyceae	f/2	chemical
Alexandrium pacificum	CNR-ACAT15P	Dinophyceae	f/2	chemical
Coolia monotis	CBA1	Dinophyceae	f/4	molecular
Gonyaulax spinifera	CBA5	Dinophyceae	f/4	adhesion/molecular
Heterocapsa sp.	CBAB/D5	Dinophyceae	f/2	molecular
Lingulodinium polyedrum	LPA0510	Dinophyceae	f/10	adhesion/molecular
Ostreopsis cf. *ovata*	CBA1291	Dinophyceae	f/4	adhesion/molecular
Prorocentrum lima	CBA3	Dinophyceae	f/4	molecular
Protoceratium reticulatum	PRA0311	Dinophyceae	f/4	adhesion
Scrippsiella sp.	CBA1	Dinophyceae	f/2	molecular
Chaetoceros sp.	CBA22	Bacillariophyceae	f/2 with Si	molecular
Cylindroteca sp.	CBA7	Bacillariophyceae	f/2 with Si	molecular
Coscinodiscus sp.	CBA1	Bacillariophyceae	f/2 with Si	molecular
Guinardia flaccida	CBA1	Bacillariophyceae	f/2 with Si	molecular
Pseudo-nitzschia cf. *arenysensis*	CBA163	Bacillariophyceae	f/2 with Si	molecular
Pseudo-nitzschia calliantha	CBA189	Bacillariophyceae	f/2 with Si	molecular
Pseudo-nitzschia multistriata	CBA174	Bacillariophyceae	f/2 with Si	adhesion
Pseudo-nitzschia pungens	CBA180	Bacillariophyceae	f/2 with Si	molecular
Skeletonema marinoi	CBA4	Bacillariophyceae	f/2 with Si	adhesion/molecular
Thalassionema sp.	CBA10	Bacillariophyceae	f/2 with Si	molecular
Thalassiosira sp.	CBA1	Bacillariophyceae	f/2 with Si	molecular

Table S2. List and properties of environmental plastic samples collected in the Mediterranean Sea.

Sample ID	Sampling date	Regional Sea	Location	Geographic Coordinates	Polymer type	Sample size
1	15th March 2016	Ligurian Sea	Genova - Voltri	44°25'4.15"N; 8°45'59.03"E	PE	220 mg
2	15th March 2016		Genova - Voltri	44°25'4.15"N; 8°45'59.03"E	PE	260 mg
3	26th September 2016		Genova - Voltri	44°24'54.17"N; 8°46'0.03"E	PE	400 mg
4	26th September 2016		Genova - Voltri	44°24'54.17"N; 8°46'0.03"E	PE	350 mg
5	26th September 2016		Genova - Voltri	44°24'54.17"N; 8°46'0.03"E	PE	95 mg
6	17th May 2016	Adriatic Sea	Fano	43°49'59.55"N; 13°03'38.23"E	PE	2.3 cm^2
7	17th May 2016		Fano	43°49'59.55"N; 13°03'38.23"E	PE	6.3 cm^2
8	22th May 2016		Pesaro	43°56'32.31"N; 13°58'18.04"E	PS	5 cm^2
9	1st July 2016		Pesaro harbour	43°55'41.78"N; 12°53'09.77"E	PE	24 cm^2
10	1st July 2016		Pesaro harbour	43°55'41.78"N; 12°53'09.77"E	PE	40 cm^2
11	1st July 2016		Croce Pesaro	43°55'41.78"N; 12°53'09.77"E	PP	70 cm^2
12	1st July 2016		Croce Pesaro	43°55'41.78"N; 12°53'09.77"E	PE	12 cm^2
13	4th August 2016		Fano	43°52'05.21"N; 13°00'56.61"E	PS	4 cm^2
14	4th August 2016		Fosso Sejore	43°53'05.79"N; 12°58'18.04"E	PS	4.5 cm^2
15	19th October 2016		Bari	41°7'44.36"N; 16°57'10.73"E	PE	188 mg
16	19th October 2016		Bari	41°7'44.36"N; 16°57'10.73"E	PE	60 cm^2

17	15th May 2016	Ionian Sea	Siracusa	37°03'50.21"N; 15°17'4.53"E	PS	4.5 cm^2
18	15th May 2016		Siracusa	37°03'50.21"N; 15°17'4.53"E	PS	4 cm^2
19	26th May 2016		Siracusa	37°03'52.32"N; 15°16'47.52"E	PE	5 cm^2
20	26th May 2016		Siracusa	37°03'51.71"N; 15°16'48.71"E	n.a.	8 cm^2
21	26th May 2016		Siracusa	37°03'51.71"N; 15°16'48.71"E	PS	6 cm^2
22	4th September 2016		Isolabella	37°51’0.99"N; 15°18’2.93"E	CE	1.7 cm^2
23	4th September 2016		Lido Mendolia	37°51'8.94"N; 15°18'0.52"E	PE	15 cm^2
24	4th September 2016		Isolabella Diving	37°51'10.40"N; 15°18'6.28"E	PVC	24 cm^2
25	4th September 2016		Isolabella Diving	37°51'10.20"N; 15°18'7.17"E	PE	16 cm^2
26	4th September 2016		Isolabella Diving	37°51'09.55"N; 15°18'7.13"E	PE	12 cm^2
27	4th September 2016		Isolabella	37°51'0.99"N; 15°18'2.93"E	PA	7.5 cm^2
28	4th September 2016		Isolabella	37°51’0.99"N; 15°18'2.93"E	PA	12 cm^2
29	26th October 2016		Porto Cesareo	40°13'41.99"N; 17°51'54.07"E	PE	210 mg
30	26th October 2016		Porto Cesareo	40°13'41.99"N; 17°51'54.07"E	PE	42 cm^2
31	19th April 2017		SR small Port	37°04'2.29"N; 15°17'19.63"E	PS	4.3 cm^2
32	19th April 2017		SR big Port	37°03'48.94"N; 15°16'52.46"E	PE	250 mg
33	19th April 2017		SR small Port	37°04'2.29"N; 15°17'19.63"E	PS	3.9 cm^2
34	19th April 2017		SR small Port	37°04'2.29"N; 15°17'19.63"E	PVC	36 cm^2
35	7th May 2017		Circolo Nautico	37°04'2.29"N; 15°17'19.63"E	PS	22 cm^2

36	7th May 2017		Cantiere nautico	37°03'54.18"N; 15°16'48.94"E	PE	80 cm^2
37	7th May 2017		Cantiere nautico	37°03'51.71"N; 15°16'48.71'E	PS	13 cm^2
38	7th May 2017		SR small Port	37°04'8.14"N; 15°17'28.24"E	PE	18 cm^2
39	7th May 2017		SR small Port	37°04'8.14"N; 15°17'28.24"E	PS	14.5 cm^2
		Tyrrhenian Sea				
40	18th August 2016		Lipari	38°27'18.47"N; 14°55'58.80"E	PE	60 cm^2
41	18th August 2016		Lipari	38°27'34.85"N; 14°55'57.25"E	PE	54 cm^2
42	18th August 2016		Lipari	38°27'39.81"N; 14°55'20.71"E	PE	2.5 cm^2

PE, Polyethylene; PS, Polystyrene; CE, Cellulose; PVC, Polyvinylchloride; PA, Polyamides
na, not analyzed

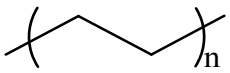
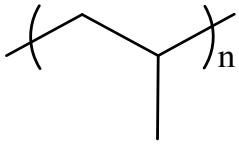
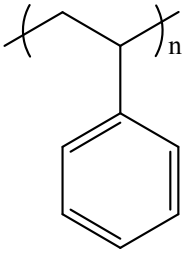
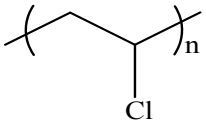
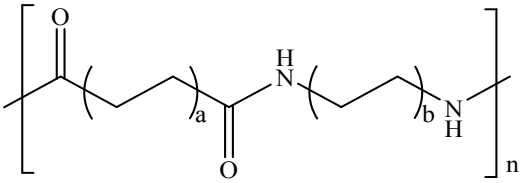
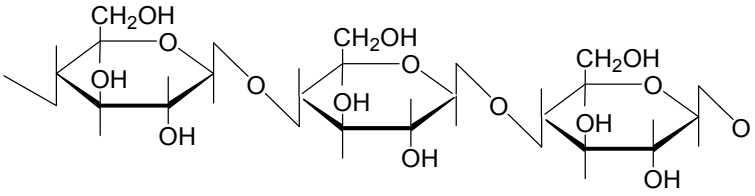
Table S3. Primers used for the amplification of SSU, 5.8S-ITS and LSU rDNA of microalgal taxa.

Target	Primer	Sequence	Primer location	Primer concentration [nM]	Reference
Dinophyceae	EUK528f	Forward 5'-CCGCGGTAATTCCAGCTC-3'	SSU	300	Godhe et al., 2008
	Dino18SR1	Reverse 5'-GAGCCAGATRCDCACCCA-3'	SSU	300	
Bacillariophyceae	1209f	Forward 5'-CAGGTCTGTGATGCCCTT-3'	SSU	300	Godhe et al., 2008
	Diatom18SR1	Reverse 5'-CAATGCAGWTTGATGAWCTG-3'	SSU	300	
Alexandrium minutum	ITS1m	Forward 5'-CATGCTGCTGTGTTGATGACC-3'	ITS1	200	Penna et al., 2007
	5.8S-3'	Reverse 5'-GCAMACCTTCAAGMATATCCC-3'	5.8S	200	
Alexandrium pacificum	ITS1p	Forward 5'-AGCATGATTTGTTTTTCAAGC-3'	ITS1	200	Penna et al., 2007
	5.8S-3'	Reverse 5'-GCAMACCTTCAAGMATATCCC-3'	5.8S	200	
Gonyaulax spinifera	GspinF_for	Forward 5'-GAAACTCCTTCTGTGGATGC-3'	LSU	200	Perini et al., 2018
	GspinR_rev	Reverse 5'-TCACAGTTCCCTCATGGTACT-3'	LSU	200	
Lingulodinium polyedrum	Lpoly RT-for	Forward 5'-AACTCGTTGGCGAGCATTTT-3'	ITS2	400	Perini et al., 2018
	Lpoly RT-rev	Reverse 5'-CGCTAGCAAAGCACTCGCTTA-3'	ITS2	400	
Ostreopsis cf. *ovata*	Ovata rt F	Forward 5'-TTTGATCACTTTGGCAATCT-3'	LSU	300	Perini et al., 2011
	Ovata rt R	Reverse 5'-TGAACTTTACCATGCCATTAG-3'	LSU	300	
Protoceratium reticulatum	Pret RT-for	Forward 5'-GGTGCAGTGAAATGTATTAGGCATT-3'	5.8S	200	Perini et al., 2018
	Pret RT-rev	Reverse 5'-TCCCAAAAACATAGAATACGTTCAAT-3'	5.8S	200	
Pseudo-nitzschia spp.	Pseudo 5'	Forward 5'-CGATACGTAATGCGAATTGCAA-3'	5.8S	300	Penna et al., 2007
	Pseudo 3'	Reverse 5'-GTGGGATCCRCAGACACTCAGA-3'	5.8S	300	

Table S4. Percentage of various Dinophyceae and Bacillariophyceae DNAs mixtures set up (see also Materials and Methods). Each mixture was a combination of various amounts of DNA for building up the mixed standard curves to quantify the abundance of Dinophyceae and Bacillariophyceae.

	Percentage DNA of *Alexandrium andersonii*	Percentage DNA of *Coolia monotis*	Percentage DNA of *Scrippsiella* sp.	Percentage DNA of *Ostreopsis* cf. *ovata*	Percentage DNA of *Gonyaulax spinifera*
Mixture					
A	20	20	20	20	20
B	20	10	10	20	40
C	10	20	20	40	10
D	20	10	40	10	20
E	40	20	10	20	10
F	10	40	20	10	20
	Heterocapsa sp.	*Lingulodinium polyedrum*	*Prorocentrum lima*	*Alexandrium minutum*	*Alexandrium pacificum*
Mixture					
G	20	20	20	20	20
H	20	10	10	20	40
I	10	20	20	40	10
L	20	10	40	10	20
M	40	20	10	20	10
N	10	40	20	10	20
	Pseudo-nitzschia pungens	*Chaetoceros* sp.	*Thalassionema* sp.	*Skeletonema marinoi*	*Cylindroteca* sp.
Mixture					
A’	20	20	20	20	20
B’	40	20	10	20	10
C’	20	10	10	20	40
D’	20	10	40	10	20
E’	40	20	10	20	10
F’	10	40	20	10	20
	Guinardia flaccida	*Pseudo-nitzschia calliantha*	*Coscinodiscus* sp.	*Pseudo-nitzschia* cf. *arenysensis*	*Thalassiosira* sp.
Mixture					
G’	20	20	20	20	20
H’	20	10	10	20	40
I’	10	20	20	40	10
L’	20	10	40	10	20
M’	40	20	10	20	10
N’	10	40	20	10	20

Table S5. Chemical structure of the identified polymers in plastic samples.

Polymer	Chemical structure
Polyethylene (PE)	
Polypropylene (PE)	
Polystyrene (PS)	
Polyvinylchloride (PVC)	
Polyamides (PA) All polyamides; a, b vary from 0 to 12 based on monomer type.	
Cellulose (CE)	

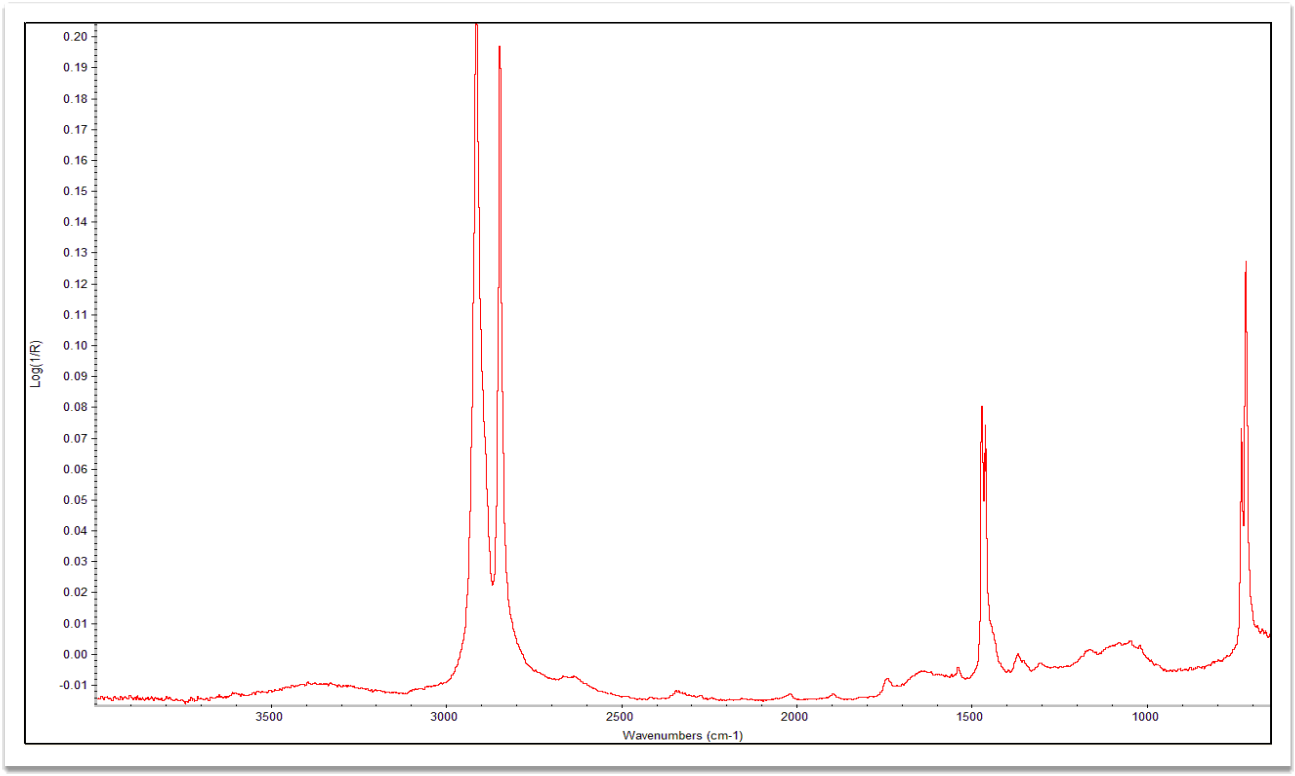


Fig. S1. ATR-FTIR spectrum of Polyethylene of analyzed plastic samples.

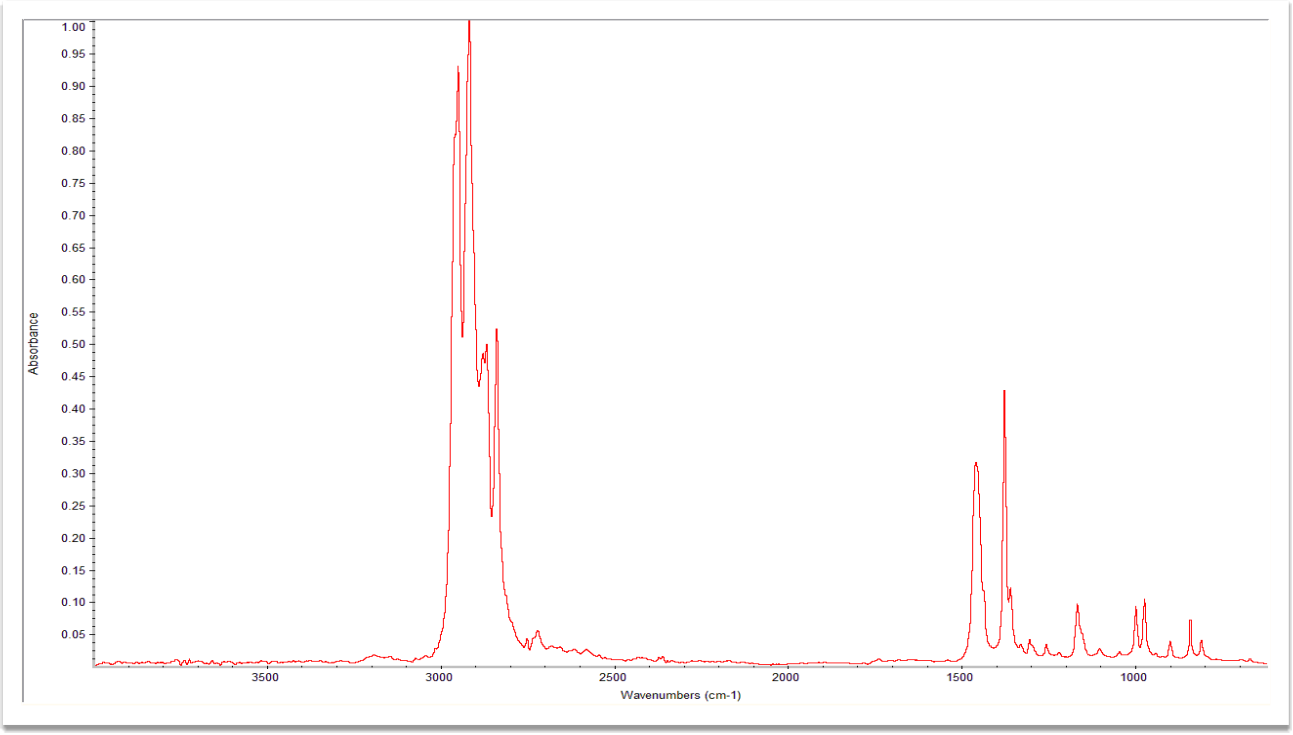


Fig. S2. ATR-FTIR spectrum of Polypropylene of analyzed plastic samples.

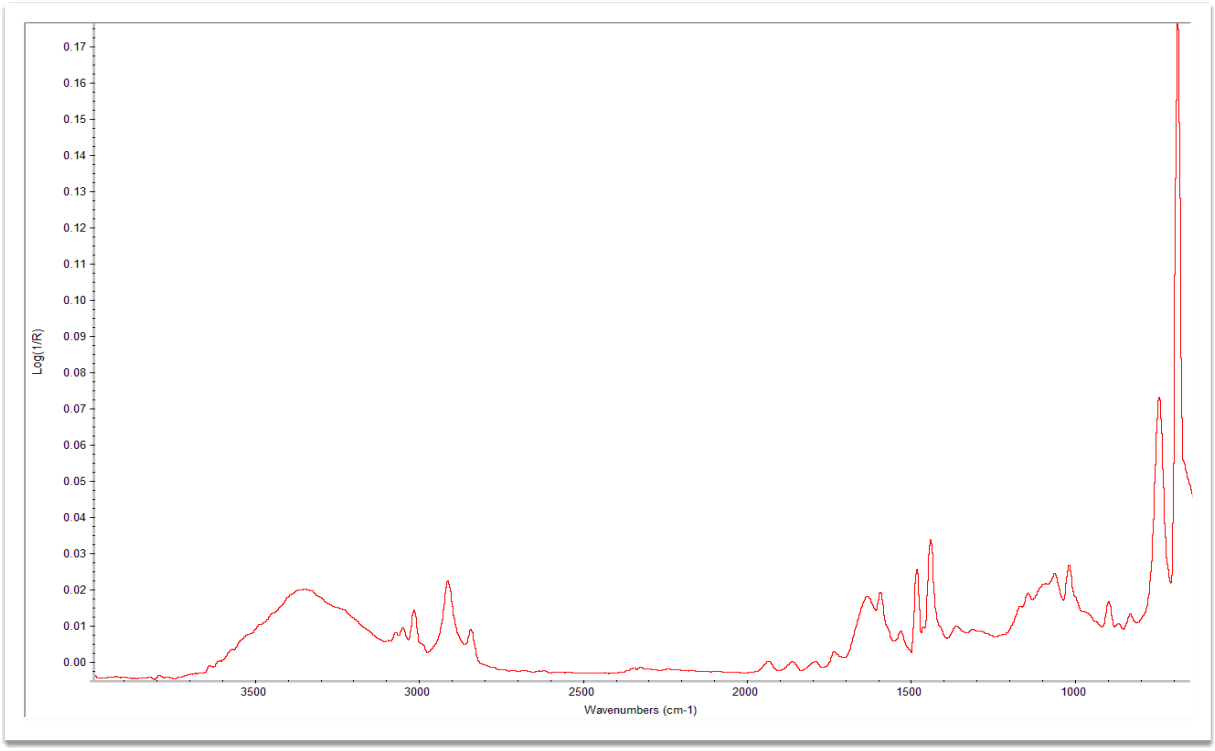


Fig. S3. ATR-FTIR spectrum of Polystyrene of analyzed plastic samples.

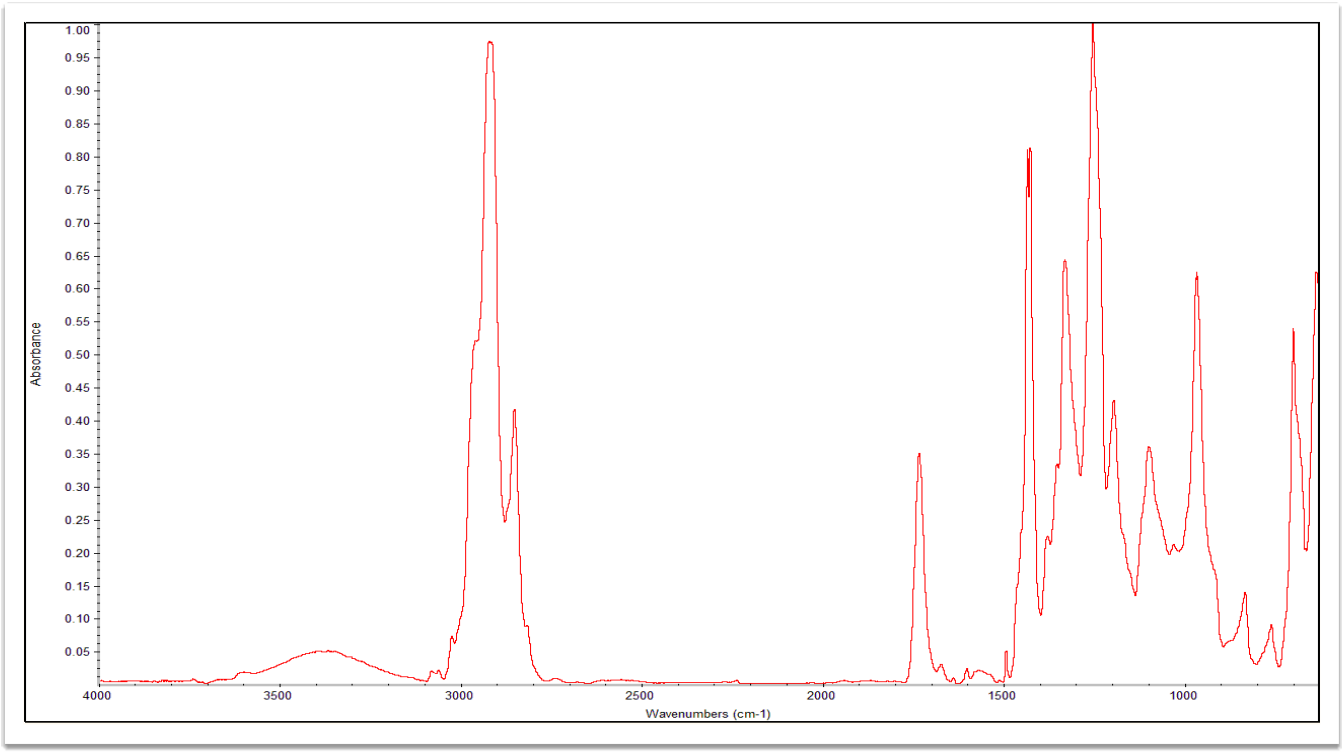


Fig. S4. ATR-FTIR spectrum of Polyvinylchloride of analyzed plastic samples.

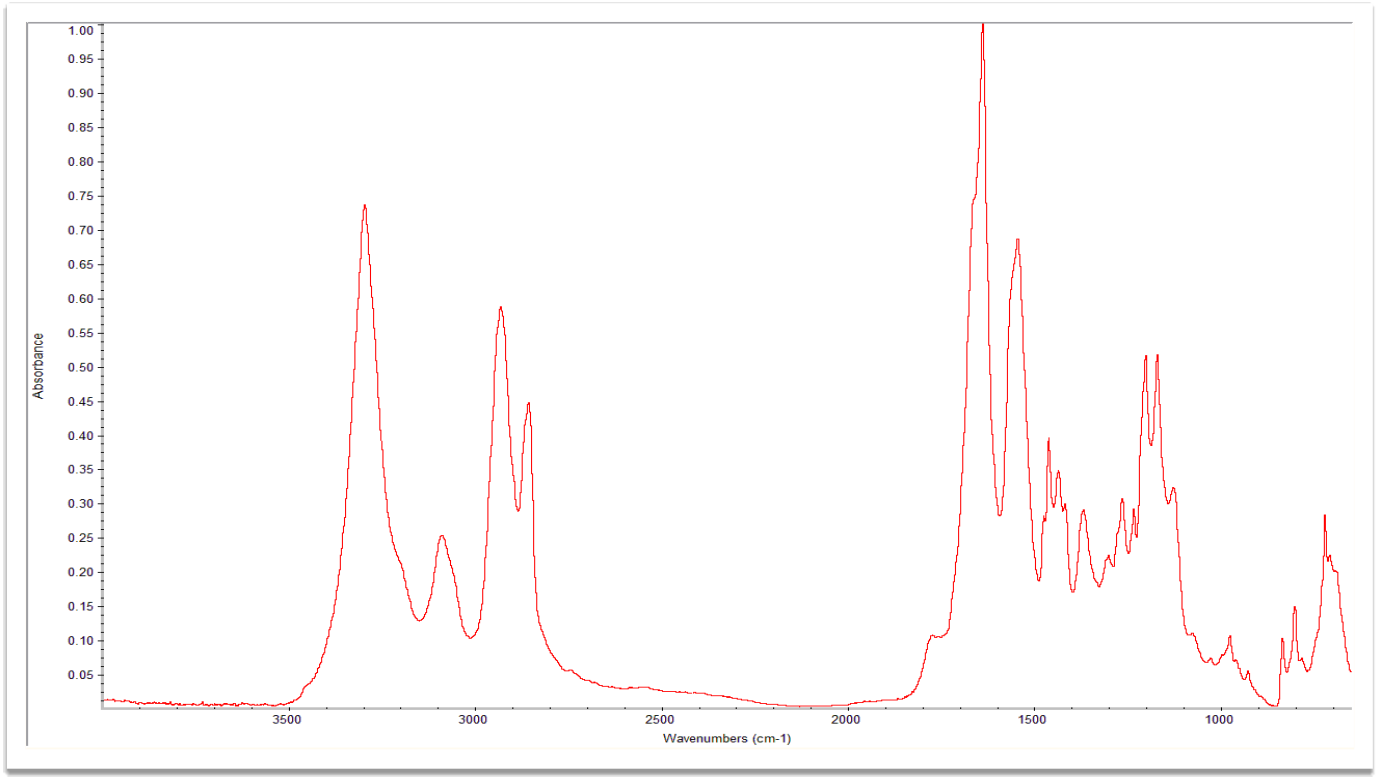


Fig. S5. ATR-FTIR spectrum of Polyamide 6 of analyzed plastic samples.

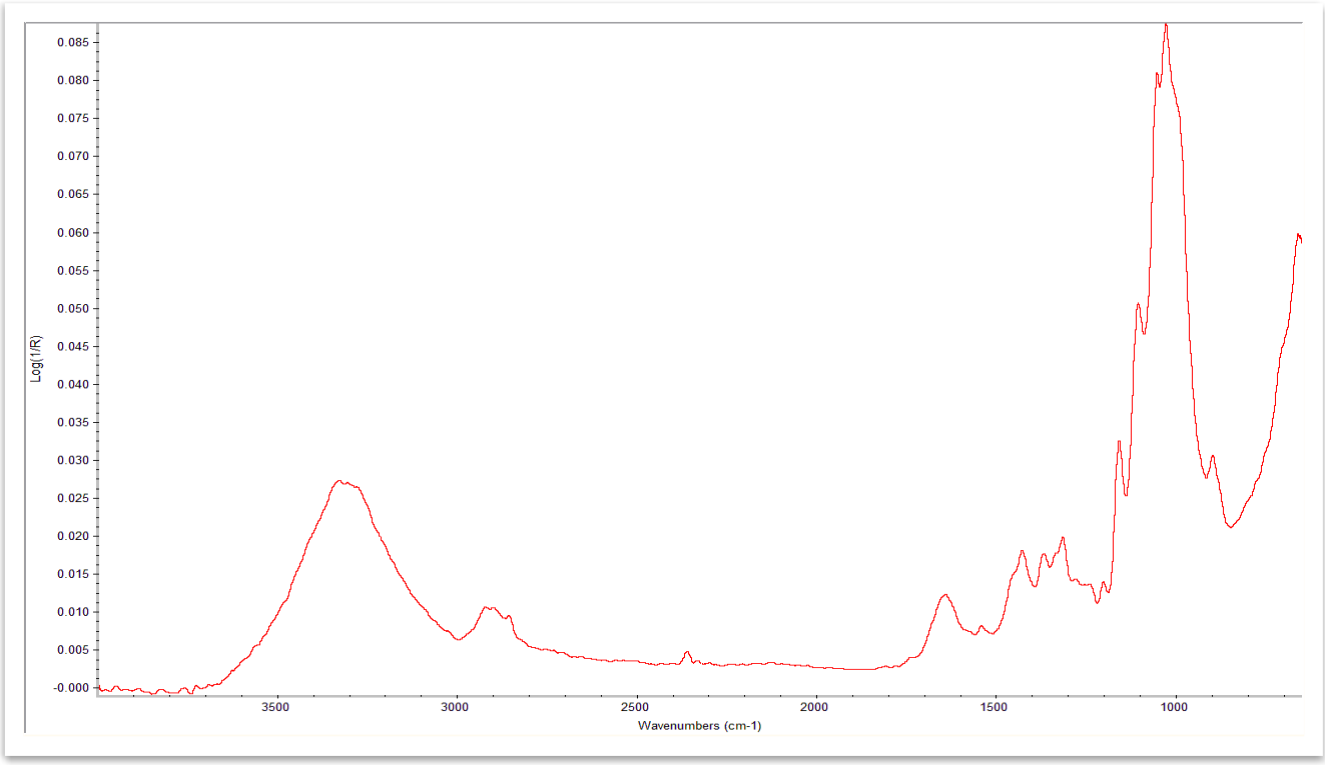


Fig. S6. ATR-FTIR spectrum of Cellulose of analyzed plastic samples.