First evaluation of floating microplastics in the North-Western Adriatic Sea

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Abstract

Plastic pollution in the marine environment is becoming a problem of global concern, and the Mediterranean is believed to be one of the worst affected regional seas. The present study presents data on floating microplastics in the North-Western Adriatic Sea in order to evaluate the possible contribution of two significant potential sources: the lagoon of Venice and the Po River. Samples were collected in March and April 2014 along two transects located off Pellestrina Island (Venice) and the Po Delta, each consisting of four sampling stations at 0.5, 3, 10 and 20 km from the shoreline. Microplastics were quantified and classified according to their colors and shapes, and analyzed by µATR-FT-IR. Microplastics were found in all samples, albeit with high spatial and temporal variability. The highest concentrations were observed in March at the offshore station of the Pellestrina transect (10.4 particles m^{-2}) and the two landward stations off the Po Delta (2.1 and 4.3 particles m^{-2}), highlighting the influence of various factors, such as surface circulation and river discharges, in determining specific accumulation patterns. The most common polymers were polyethylene and polypropylene, and most of the particles were secondary microplastics (83.5%). The patchy distribution of microplastics observed in the study area is driven by hydrodynamic and meteorological factors acting on short time-scales.

Key-words: Floating microplastics, coastal pollution, µATR-FT-IR analysis, North Western Adriatic Sea

Introduction

Plastic pollution in the aquatic environment is now recognized as a problem of critical concern, involving the whole world and affecting both marine and freshwater ecosystems. The constant growth of worldwide production, estimated to be 335 million tons in 2016 (Plastics Europe, 2017), combined with the increased use of disposable goods and the low degradability of polymers, have contributed to pollution by debris originating from environmental plastic weathering and fragmentation. Plastic litter is now accumulating at growing rates in the environment (Barnes et al. 2009). Recent estimates indicate that about 10% of plastic waste ends up in the oceans, where it is transported by the currents to the remotest areas, such as the polar zones (Barnes et al. 2009). Large plastic items undergo fragmentation processes mainly as a consequence of mechanical breakdown caused by physical phenomena (sand abrasion, wave action), accelerated by photochemical processes triggered by UV light (Corcoran et al. 2009; Cooper and Corcoran 2010; Andrady 2011; Song et al. 2017). The smaller plastic fragments (basically ≤5 mm) have been categorized as microplastics (Thompson et al. 2004; Moore 2008). In addition to the microparticles resulting from fragmentation of larger items (secondary microplastics), microplastics can also be manufactured *ex novo* for industrial, cosmetic and pharmaceutical purposes (primary microplastics).

The widespread occurrence of microplastics in various marine habitats worldwide is now well documented, and has been investigated on sandy beaches (Frias et al. 2010; Laglbauer et al. 2014; Munari et al. 2017), in coastal/transitional sediments (Blaskovic et al. 2017: Browne et al. 2010; Claessens et al. 2011; Vianello et al. 2013) and in the open and deep sea (Claessens et al. 2011; Pham et al. 2014; Zarfl et al. 2011), as well as in various marine species (Lusher 2015). Ingestion, which is the most likely interaction between marine organisms and microplastics, has been demonstrated in almost 60 fish species, as well as invertebrate species with a range of feeding behaviors (Galgani 2015; Lusher 2015). Microplastic particles are ingested intentionally by fish, which confuse them for food as they are similar in size and appearance to plankton (Ory et al.

2017), and accidentally by filter feeders (Lusher 2015; Wesch et al. 2016; Ory et al. 2018). This may negatively influence both feeding activity and the nutritional value of a plankton-based diet, particularly in those species which cannot discriminate the food source (Moore et al. 2001; Browne et al. 2008). When ingested by marine organisms, microplastics can cause chemical and physical damage, such as inflammation, hepatic stress and decreased growth, as well as attachment of the polymer to external surfaces, consequently impeding mobility and clogging the digestive tract (Setälä et al. 2016). Moreover, microplastics contain organic pollutants, either added during plastic production or absorbed from seawater, which can become bioavailable to organisms after the ingestion of plastic particles (Auta et al. 2017; Avio et al. 2015).

Elevated concentrations of plastic waste are predicted to occur in highly populated, shallow and enclosed waters, such as the Mediterranean basin, which has recently been proposed as the sixth great accumulation zone for marine litter, along with the five ocean gyres, i.e. North and South Pacific, North and South Atlantic and Indian (Cozar et al. 2015). The Mediterranean Sea is strongly influenced by human activities. It houses around 10% of the global coastal population (CIESIN 2012), represents one of the world´s busiest crossroads for maritime navigation (UNEP/MAP-Plan Bleu 2009), and receives waters from rivers characterized by densely populated drainage basins (e.g., the Nile, Ebro, Rhone and Po). Moreover, the Mediterranean Sea has a water residence time of up to a century, being connected to the Atlantic Ocean only by the narrow Strait of Gibraltar (Lacombe et al. 1981). Lebreton et al. (2012) performed a modeling study concerning the transport and distribution of floating debris across the oceans which identified the Mediterranean Sea as a potentially important accumulation zone. The calibration of the model estimated the weight of plastic floating in the Mediterranean Sea at 23,150 tons (Eriksen et al. 2014).

Concerning more specifically the Adriatic Sea, the modeling study performed by Liubartseva et al. (2016) showed that the area of highest plastic concentrations corresponded to an elongated band off the Italian coastline narrowing from northwest to southeast. Moreover, the same authors showed that the distributions and concentrations of floating debris are clearly related to the spatial distributions of plastic debris inputs (rivers, cities and shipping lanes), as well as being connected with general Adriatic circulation patterns.

The main aim of this work is to provide qualitative and quantitative data about the presence of floating microplastics along the coast of the Veneto Region in the North-Western Adriatic Sea. Data were obtained from two sampling campaigns performed in 2014 as part of activities to implement the Marine Strategy Framework Directive (Directive 2008/56/EC of the European Parliament and of the Council establishing a framework for community action in the field of marine environmental policy) in the Veneto region. To our knowledge, this study represents the first attempt to evaluate sea surface microplastics along the western coast of the northern Adriatic Sea. At present, data are available on the occurrence of microplastic particles on beaches along the North-Western Adriatic coast (Munari et al. 2016, 2017) and in sediments collected along a coast-open sea transect in the Central Adriatic Sea (Mistri et al., 2017). Floating microplastic concentrations have been investigated in the Slovenian coastal areas of the northern Adriatic (Gajšt et al. 2016) and in the southern part of the basin (Cozar et al. 2015; Suaria et al. 2016). Although these studies demonstrate the pervasiveness of microplastic pollution in the Adriatic Sea and highlight possible harmful effects on marine and human life, direct investigations of the occurrence and distribution of floating microplastics on the Italian side of the northern Adriatic Sea are still lacking, despite the recognized importance of the Po River and the cities of Venice and Chioggia as potential contributors of floating plastic debris (Liubartseva et al. 2016).

Materials and methods

Study area

The northern Adriatic Sea is the shallower part of the Adriatic epi-continental shelf, with maximum depths of less than 70 m and a mean depth of 30 m. Its oceanographic conditions are mainly influenced by the basin morphology and meteorological regimes, with large seasonal variations in thermal and salinity conditions (Franco and Michelato 1992; Boldrin et al. 2005). The northern Adriatic Sea receives significant freshwater input from several rivers along the north-eastern coast of Italy, the most important being the Po, the country's largest river with a length of 673 km and a drainage basin of 71,000 km^2. It flows through one of the most productive agricultural and industrial areas in the country, entering the northern Adriatic Sea through a large delta with five distributaries (Maistra, Pila, Tolle, Gnocca, and Goro), differing in terms of their water discharge and solid loads (ARPAV, 2012). The northern Adriatic Sea is also subject to heavy marine traffic from merchant ships, supplier vessels for offshore activities (e.g. gas extraction), ferries, fishing vessels and recreational craft.

Samplings were performed along two transects located respectively off the Lagoon of Venice, i.e. the Pellestrina transect, and off the Po delta (Fig. 1). These two coastal areas were chosen because they are affected by different anthropogenic inputs. The former, located between two of the Lagoon's seaward inlets, is characterized by the presence of anthropic coastal infrastructures along more than 60% of its coastline and is mainly affected by maritime traffic (both commercial cargo and passenger vessels) passing through the two inlets. Moreover, it is subject to tourism-related impacts, particularly in the summer period, and to intense fishing and mussel farming. The Po delta is strongly affected by the Po River plume. Specifically, the transect is located off the Po della Pila, the river's main distributary, which accounts for more than 40% of total water discharge (ARPAV 2012) and 74% of the discharged solid load (Nelson 1970).

Four sampling stations were located along each transect at 0.5, 3, 10 and 20 km from the shoreline (Fig. 1), in order to obtain a characterization from the coastal to the offshore areas of the Gulf of Venice.

Sample collection

Floating microplastics were collected during two surveys in late March and late April 2014. The two periods were decided on the basis of the meteorological and Po discharge conditions just before samplings. Data for wind, waves, rainfall and surface current were measured at the "Acqua Alta" oceanographic platform, located in the northern Adriatic about 15 km off the Venetian coast (Fig. 1). Po River discharge data were obtained from ARPA-ER (https://www.arpae.it). In March, before the first sampling survey, high instability was observed, with strong Bora winds (i.e. the cold north-easterly wind, occurring frequently in fall and winter), waves often > 1.5 m, heavy rainfall and Po river discharge of 2615 $m^3 s^{-1}$, much higher than the mean flow rate (1502 $m^3 s^{-1}$ for the period 1918–2016). In April, meteorological conditions were less intense, with dominant Scirocco winds (i.e. the warm and humid wind from the southeast), mean waves of 0.6 m, lower rainfall and Po river discharge of 1786 $m^3 s^{-1}$ (Fig. 2).

Samples were collected using a Manta trawl with a 330 µm mesh net and a 0.6 x 0.3 m rectangular net opening. Samplings were always performed in calm conditions, with wind speed not exceeding 2 on the Beaufort wind force scale, since with stronger winds the rough conditions prevent reliable sample collection on the sea surface.

During sampling, the Manta trawl was lowered laterally from the vessel, taking care to position it outside the boat's wake, and was maintained in flotation for 20 minutes with a boat speed of 1 - 3 knots. The sampling position was determined by GPS before and after each trawl. The sampled area was calculated by multiplying the length of sea surface trawled, determined with the GPS, by the width of the Manta trawl, and particle abundance per square meter was consequently calculated.

Water temperature and salinity were measured at the beginning of each sampling using a CTD probe (IDRONAUT Ocean Seven mod. 316).

After each sampling, the Manta net was thoroughly washed to collect all the debris particles stuck to the mesh and samples were transferred into 1 L glass jars. Samples were then stored in the dark at 4°C until analysis. Fixatives were not used for sample conservation, in order to avoid procedural interference in the subsequent chemical analyses conducted using infrared spectroscopy.

Sample analysis

Each sample was processed according to the Standard Operation Protocol proposed by the Italian Ministry of the Environment and Protection of Land and Sea (Ministero dell'Ambiente e della Tutela del Territorio e del Mare, also known as MATTM), slightly modified. Precautions were taken to avoid sample contamination during both transport and processing; only glass and metal (preferably stainless steel) tools, cotton coats and nitrile gloves were used.

Samples were thoroughly shaken to facilitate detachment of the microplastics from the walls of the 1l glass container and then filtered through two metal sieves, 5 mm and 300 µm mesh-size, rinsing the glass container several times with filtered sea water (1 µm). The fraction retained by the 5 mm sieve, mainly consisting of plant debris and large planktonic organisms, was thoroughly washed with filtered seawater to remove any adhering microplastics. The obtained fraction was further washed with a saturated solution of NaCl (density 1.2 g cm^{-3}). This cleaning step made it possible to clearly separate buoyant plastics from other materials. The floating plastic fragments and the few that precipitated (with a density higher than 1.2 g cm^{-3}), were then sorted by microscopic inspection**.**

The microscopic analysis was performed in accordance with the "Reference analytical methods" described in the "Memorandum of Understanding for analytical methods in the analysis of microplastics CdR 25/09/2013-1”. All plastic fragments were transferred to vials containing distilled water, filtered by gravity through a 100 µm nylon filter, previously dried at 50°C for 24 h and weighed. The microplastics deposited on the filter were weighed and then transferred into a glass Petri dish to facilitate microscopic observation (Stereoscope Leica). Microplastic particles were then counted and classified according to color (white, black, red, blue, clear, green and other colors), and shape (films, fibers, flat fragments, irregular fragments, granules, pellets and spheres). The results were expressed as the number of particles and weight in mg per m^{-2}.

Polymer identification: µFT-IR analysis

The microplastic particles were qualitatively analyzed to allow polymer identification by micro-Fourier transform-infrared spectroscopy (µFT-IR), which couples an infrared spectrometer to a microscope, making it possible to determine the chemical composition of substances and materials, both natural and synthetic, including synthetic polymers. Infrared spectroscopy is one of the most widely used techniques for identifying polymer types (Vianello et al. 2013; Rocha-Santos and Duarte 2014).

The instrument used for polymer assessment was a Nicolet iN10 infrared microscope (Thermo Fisher Scientific, Madison, WI, USA) with a liquid nitrogen-cooled MCT detector and motorized stage. The analyses were carried out by acquiring the signal in SR-ATR (Single Reflection - Attenuated Total Reflectance) using a slide-in Germanium crystal (refractive index n = 4) with a micro-tip of 350 μm diameter (Micro-Tip ATR). The crystal, placed in "optical contact" with the sample with the application of sufficient pressure, allowed clear spectrum acquisition, directing the IR beam from the crystal to the sample's surface (about 1 μm in depth) and back to the detector; the ATR-FT-IR spectra were obtained in the spectral range of 4000 - 673 cm^{-1}, acquiring 64 co-scans at 4 cm^{-1} using custom aperture depending on the particle's shape and surface roughness. The spectra thus obtained were processed by removing the contribution of CO_2, changing the units from Transmittance % to Absorbance [$A = 2 – \log_{10} \%T$ or $A = \log_{10} (1/T)$] and performing an automatic baseline correction. μATR-FT-IR was chosen as the most suitable analytical method because it does not require complex sample preparation and is not influenced by sample thickness (Zbyszewski and Corcoran 2011; Rocha-Santos and Duarte 2014; Löder et al. 2015; Käppler et al. 2015).

The percentages of microplastic fragments analyzed varied according to the total number counted in each sample as follows: 10% when there were at least 500 particles, 50% when the number of particles ranged from 500 to 100, and 100% when there were less than 100 particles.

As well as for polymer identification, μATR-FT-IR spectroscopy was used to assess the relative aging of polyethylene (PE) and polypropylene (PP), the most common polyolefins. Each PE and PP spectrum was checked for the presence of the carbonyl band occurring around 1712-15 cm^{-1}. Relative polymer aging was determined by calculating the Carbonyl index (Nagle et al. 2010; Veerasingam et al. 2016), in accordance with the following equations:

a) Carbonyl Index (CI_{PE}) = I_{1712}/I_{1468} (for PE particles)

b) Carbonyl Index (CI_{PP}) = I_{1712}/I_{1458} (for PP particles)

where I is the intensity (peak height) at 1712-15 cm^{-1} (carbonyl band), 1465-69 cm^{-1} (PE methylene deformation band) and 1453-58 cm^{-1} (PP methyl+methylene deformation band). Since the degree of photo-degradation of the polymers is related to exposure time, the CI is used as a proxy for the aging of polymers (Endo et al. 2005; Veerasingam et al. 2016; Matsuguma et al., 2017).

Data analysis

The spatial and temporal variability of microplastic samples, expressed as the mean number of particles m^{-2} and mg m^{-2} detected at the four stations of each of the two transects, was analyzed by the Kruskall-Wallis test, as the data did not show normal distribution (Shapiro-Wilk's test). Principal Component Analysis (PCA) was carried out to elucidate the spatial variability of samples using a data matrix composed of temperature, salinity, number of particles m^{-2} and mg m^{-2}. The general surface circulation pattern of the two study areas during the March and April surveys was inferred from satellite-derived turbidity maps. Landsat 8 satellite images were processed in accordance with the method described in Braga et al. (2017).

Results

The main field parameters detected during each survey are shown in Table 1. Salinity was the most variable parameter, with lower values detected in the inshore stations of the Po Delta. The concentration of microplastics observed in March on the Pellestrina transect at the first three stations from the coast (Pe 1, Pe 2 and Pe 3) ranged from 0.1 to 0.3 particles m^{-2}, whereas a higher concentration was detected in the most offshore station (Pe 4), amounting to 10.4 particles m^{-2}. During the same period, on the Po Delta transect, higher concentrations were found at the two landward stations (2.1 and 4.3 particles m^{-2} at stations Po 1 and Po 2, respectively), whereas at stations Po 3 and Po 4, the concentrations were 0.3 and 0.2 particles m^{-2}. In April, the total amount of microplastics collected along the two transects was generally lower and the distribution more homogeneous than what was observed during the first survey, with concentrations ranging from 0.1 to 0.3 particles m^{-2} off Pellestrina and from 0.03 to 0.6 particles m^{-2} off the Po Delta (Table 2). During both sampling surveys, microplastic dry weight values varied in a similar way to the number of particles, with a particularly high value detected at Pe 4 in March (Table 2). The mean values of particles m^{-2} calculated with values detected at the four stations of each transect showed no significant differences between transects (Fig. 3A), whereas a significant difference was observed when comparing the March and April means for mg m^{-2} off the Po Delta (Fig. 3B).

The results of the multivariate analysis (PCA) of microplastic number and weight and the main environmental parameters showed that Factors 1 and 2 explained 83% of total variance in the data matrix (Figure 4). Factor 1 explained 53% of total variance, with strong negative loadings for the number of particles m^{-2} and particle weight m^{-2} (-0.96 and -0.95, respectively). Factor 2 explained 30% of total variance, with strong positive loadings for salinity (0.84). The spatial distribution of samples along Factor 2 reflects the influence of salinity on particle number and weight. A general relationship was also observed between microplastic abundance and turbidity, particularly in the Po Delta area (Fig. 5). A notable exception was the high concentration observed in March at Pe 4.

The classification of microplastics by color showed that the most abundant particles in all samples were transparent, except at Pe 3 in March, where white particles accounted for 63% (Figure 6). Flat and irregular fragments were the most abundant off Pellestrina in March, with similar percentages along the transect. Off the Po Delta, irregular fragments were the most frequent particles in the three onshore stations, whereas the offshore station (Po 4) showed higher variability, with 28% flat fragments, 47% irregular fragments and 18% fibers (Figure 7A). In April off Pellestrina, flat fragments made up 16%-42% of the samples and irregular fragments 33%-46%, whereas the shape of particles on the Po Delta transect was more variable (Figure 7B).

μATR-FT-IR analyses were performed on a total of 2829 items. The isolated and identified polymeric species and their percentages in each sample are shown in Figure 8, while images of the most common fragment types, obtained with a stereo-microscope and relative μATR-FT-IR spectra, are shown in Figure 9. The most abundant polymer was PE, which varied in March from 63% to 74% off Pellestrina and from 51% to 77% off the Po Delta, while in April it varied from 34% to 51% off Pellestrina and from 41% to 79% off the Po Delta. The other most frequent polymers were PP (ranging from 5 to 30%), ethylene vinyl acetate (EVA, ranging from 1 to 32%) and polystyrene (PS, ranging from 0 to 9%). Most of the PE was in the form of flat and irregular transparent fragments and irregular white fragments, whereas PP was mainly transparent and colored irregular fragments. EVA was predominantly flat and irregular transparent fragments (data not shown).

As for the relative oxidation calculated as CI, the site-averaged CI_{PE} ranged from 0.34 to 0.75 (Station Po 2 in April and Station Po 4 in March respectively), while CI_{PP} ranged from 0.28 to 0.63 (Station Po 3 in March and Station Pe 2 in April respectively) (Fig. 10). Considering all the analyzed samples, the total percentage of oxidized PE and PP was 23% and 24% respectively.

Discussion

Distribution and behavior of microplastics in the North-Western Adriatic

Microplastic particles were found in all the collected samples, albeit with high spatial and temporal variability. In March, the average concentration of microplastics observed in the study area was 2.2 ± 3.6 particles m^{-2}, whereas in April it was 0.2 ± 0.1 particles m^{-2} (total number of microplastic particles: 15282 and 1078 items respectively). The concentration recorded in March was substantially higher than what was reported by Avio et al. (2017) and Suaria et al. (2016) for floating microplastics in various areas of the Mediterranean Sea (mean values ranging from 0.062 to 0.4 particles m^{-2}). The values observed off the Po Delta (on average 3 particles m^{-2}) were comparable to those reported in the study by Gajšt et al. (2016), performed off the Slovenian coast in the northern Adriatic, which observed maximum concentrations of 2-3 particles m^{-2}. Similarly, Suaria et al. (2016) found concentrations of up to 2.3 particles m^{-2} in the southern Adriatic The value observed in March at the offshore Pellestrina station (10.4 particles m^{-2}) was higher than any other previously reported for the Adriatic basin.

The patchy distribution of microplastic particles in the area covered by this study might be related to short time-scale sources of variability, as already suggested for other Mediterranean areas (Cozar et al. 2015). It is well known that local microplastic accumulation patterns depend on complex interaction between plastic sources and prevailing environmental conditions in the area, i.e. marine circulation and weather conditions (Welden and Lusher, 2017). Considering the possible environmental factors that may have led to the different microplastic distribution patterns on the two transects, we made some assumptions on the basis of the main hydrological features of the area. March was characterized by a greater presence of diluted water, and related suspended material, throughout the basin. This was particularly evident off the Po Delta, where the observed microplastic distribution along the transect was positively related to turbidity (Fig. 5) and inversely related to salinity (Fig. 4). These observations confirm the importance of contributions from the mainland via river discharge as the main factor determining microplastic concentrations, at least in this period. The high concentrations observed at the two landward stations may be related to the relatively high input of fresh water, since before the sampling survey the Po River was recorded as being in flood (Braga et al. 2017). Off Pellestrina, the main factor behind the high concentration of anthropogenic material observed in the most seaward station of the transect might be passive transport driven by the cyclonic circulation of the basin, This entails a strong near-surface current (Western Adriatic Coastal Current - WACC), as confirmed by the presence of a mass of water with lower turbidity in the offshore north-east area (Fig. 5). The meteorological conditions recorded a few days before the survey were characterized by a strong NE-bora wind event that lasted more than 24 hrs with wind speed >10 m s^{-1}, (maximum speed 15.2 m s^{-1}). The bora wind induces a counterclockwise gyre in the northern Adriatic basin, increasing the surface currents (Bolaños et al.

2014), as observed during the two days before sampling, when the current speed at the "Acqua Alta" platform reached 0.19 m s^{-1} with a SW direction (Bastianini, pers. comm.). This circulation increases water flushing along the Italian coast (Boldrin et al. 2009; Cozzi and Giani 2011), with the main compensating inflow occurring along the eastern boundary (Eastern Adriatic Current - EAC), characterized by high salinity (Marini et al. 2008). This cyclonic gyre might be responsible for the transport of anthropogenic material from the eastern shore of the Adriatic and/or discharged by the several rivers flowing into the North-Western part of the basin. Moreover, river flow in the study period was certainly affected by the rainfall events recorded in the days immediately preceding the sampling survey, which may have ultimately increased the transport and distribution of plastic particles in the area, as already described for the northernmost Adriatic area (Gajšt et al. 2016). The contribution of microplastics from the Lagoon of Venice, assumed to be about 2.9% (35.8 kg (km day)$^{-1}$) of the total inputs of plastic debris in the Adriatic basin (Liubartseva et al. 2016), should not be excluded, although we would have expected to see a concentration gradient from the landward to the seaward stations.

In April, the hydrological conditions of the study area were less variable. The basin was characterized by lower input of riverine waters as well as less variable salinity values (range 16.66-32.37 psu compared to 11.06-35.08 psu in March). Moreover, the high-turbidity layer was limited to a narrow strip near the coast (Fig. 5). In the days before the April survey, wind speeds were on average below 5 m s^{-1}, precipitation was practically absent, and the Po River flow was lower than in the previous month. Therefore, the lower observed variability of concentration might be partly due to the less pronounced local hydrodynamism (mean current speed in the two days before sampling of 0.08 m s^{-1}), as well as to more stable weather conditions than those observed in March.

Transparent microplastics were the most abundant in almost all the analyzed samples, followed by white ones. Together, these two colors accounted for 60-86% of all samples. Moreover, variation in the percentage of samples accounted for by the various colors was generally narrow, considering both transects and stations within each transect. The higher abundance of flat fragments in the

Pellestrina transect in March is in agreement with what has been reported by other authors, who usually find transparent and white fragments to be the most abundant (see for review Hidalgo-Ruz et al. 2012; Viršek et al. 2016).

Several studies have shown that the potential bioavailability of particles to marine organisms depends not only on abundance but also color, density and shape (Wright et al. 2013). For example, it has been observed that white and transparent plastic and microplastic particles are generally the most frequently ingested (Boerger et al. 2010; Choy and Drazen 2013; Nelms et al. 2016; Ory et al. 2017; Tanaka and Takada 2016). Even though it is not clear if this observation is a result of selectivity or due to the differing proportions of colors in seawater, the concentrations, particularly high in some samples, of transparent and white particles might represent a real threat to the biota in the study area. Specifically, concerns arise when considering that the northern Adriatic Sea is one of the most intensively fished areas in Europe, where several fishing and farming (e.g. mussel farms) activities operate simultaneously. Microplastics have been found in various commercially exploited species worldwide, both invertebrates and fish, such as mussels (De Witte et al. 2014; Van Cauwenberghe et al. 2015), oysters (Van Cauwenberghe and Janssen 2014) and brown shrimps (Devriese et al. 2015), as well as in the livers of European anchovies (Collard et al. 2017) and the stomach contents of large pelagic fish, e.g. *Xiphias gladius*, *Thunnus thynnus* and *T. alalunga* (Romeo et al. 2015). Recently, Karlsson et al. (2017) investigated the presence of microplastics in sediments, waters and mussels collected simultaneously. The measured concentrations in mussels were approximately a thousand-fold higher (per unit weight or volume) than in the surrounding sediment and water, showing that microplastics can accumulate in filter-feeders. Although the possible risk resulting from the consumption of seafood contaminated by microplastics has not been yet clarified, the potential consequences for human health should be considered (Sharma and Chatterjee 2017).

Polymer identification

The most abundant polymers (PE 60%, PP 22%, EVA 5%, PS 4%), in particular PE and PP, are generally the most common among floating microplastic particles. This is not surprising, due to their low densities (low-density PE: 0.91 – 0.925 g cm^{-3}; high-density PE: 0.959 to 0.965 g cm^{-3}; PP: 0.90 g cm^{-3}, from Andrady 2017), lower than that of sea water, and to the fact that they are the most widely used on a global level (Andrady 2015, 2017; Plastic Europe 2016; Suaria et al. 2016; Vianello et al. 2013).

Most of the analyzed microplastics (83.5%, considering irregular fragments, flat fragments, fibers and films) have been tentatively attributed to the category of secondary microplastics, known to originate from the fragmentation of larger plastic objects that have been discarded, disposed of or abandoned in the environment. Micro-particles showing a typical "primary microplastic" morphology, i.e. pellets, spheres and granules, made up a smaller percentage (16.5%). Spectroscopic investigations combined with stereo-microscopy showed that the surface of many of the analyzed fragments was degraded and characterized by pitting (widespread cratering) and cracks.

Thanks to the µATR-FT-IR analyses, oxidation of the floating microplastics was also demonstrated, using the Carbonyl index (Cooper and Corcoran 2010; Veerasingam et al. 2016). The results indicated that a high percentage of the analyzed fragments of PE and PP (23% and 24%, respectively) were degraded, possibly confirming that they originated from the fragmentation of larger plastic debris. It is worth noting that photo-oxidation increases the rate of chemical reaction occurring at the polymer’s surface, leading to further degradation and fragmentation and hence to nano-plastic formation (Acosta-Coley and Olivero-Verbel 2015).

Consistent shape and color patterns were detected for the identified polymers. PE was mostly in the form of flat or irregular transparent fragments, PP in the form of transparent and colored irregular fragments, EVA predominantly in the form of flat or irregular transparent fragments and lastly PS as white irregular fragments and spheres. These patterns reflect their main industrial uses and can help to elucidate their possible origin (land and sea activities). PE, with an annual global production of ~80 million tonnes, is mainly used in packaging film, six-pack rings, juice and milk containers, bottle caps, disposable bags and fishing gear; PP, with an annual global production of ~55 million tonnes, is used in ropes, packing bands, rigid plastic furniture and fishing gear (Andrady 2015; Thompson et al. 2004). EVA is mainly used in toys and athletic goods, flexible packaging, films and floats for fishing gear, whereas PS is used in food service utensils, packaging and Expanded Polystyrene Foam (EPS). The PS spheres found in our samples were probably primary plastic material used for the industrial production of EPS (Andrady 2015). Moreover, a recent study of abandoned, lost or discarded fishing gear along the Veneto coastline, performed in the framework of the Life-GHOST Project (12/BIO/IT/000556; http://www.life-ghost.eu/index.php/it), showed that the plastic parts used to manufacture the fishing gear retrieved from the seabed (essentially, the nets) are generally made of PA, PP and PE (Delaney 2016). These results suggest that at least part of the floating fragments composed of these polymers might originate from discarded or abandoned fishing gear.

Conclusions

This study provided the first evidence of floating microplastics along the coast of the Veneto Region, contributing to knowledge of their distribution and abundance in the northern Adriatic Sea. Specifically, a transient convergence zone off the Lagoon of Venice and the contribution of discharge from the Po River were highlighted, showing occasional peaks in microplastic concentrations higher than what has been reported by other authors for the Adriatic basin. The patchy distribution of microplastics observed in this area can be related to short time-scale sources of variability linked to hydrodynamic and meteorological drivers. However, the results of this study should be regarded as a preliminary snapshot of a dynamic and complex phenomenon which can only be understood thoroughly by combining field monitoring studies with hydrodynamic models.

This requires specifically designed monitoring surveys in order to better resolve the behavior, transport and fate of floating microplastics in the Adriatic basin.

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Table 1 – Stations and environmental parameters recorded during the 1st (March 2014) and 2nd (April 2014) surveys. For each station the following parameters are given: bottom depth, geographical coordinates at the start and end of sampling, average boat speed, sea state (Douglas scale), wind force (Beaufort scale), wind direction, sea surface temperature (°C), salinity (psu) and density (kg m^{-3}).

Transect	Station	Bottom depth (m)	Starting coord. (Lat. N)	Starting coord. (Long. E)	Ending coord. (Lat. N)	Ending coord. (Long. E)	Trawling Speed	See state	Wind force	Wind direction	Temp.	Salinity	Density
1st survey													
Pellestrina	Pe 1	5.5	45°19.014	12°19.476	45°19.008	12°20.514	2.0	2	2	23	12.9	32.1	1024
	Pe 2	11.0	45°18.750	12°21.456	45°18.852	12°22.440	2.3	2	2	23	13.2	32.4	1024
	Pe 3	16.5	45°18.702	12°26.736	45°18.630	12°27.630	2.1	1	0	nd	13.7	34.4	1026
	Pe 4	22.0	45°18.738	12°34.554	45°18.504	12°35.568	2.4	0	0	nd	13.7	35.1	1026
Po Delta	Po 1	8.6	44°57.336	12°34.062	44°57.120	12°34.926	2.0	1	1	270	12.3	11.1	1008
	Po 2	23.8	44°57.528	12°36.282	44°57.402	12°37.470	2.5	0	0	nd	13.3	20.5	1015
	Po 3	29.0	44°57.612	12°41.676	44°57.576	12°43.002	2.9	1	1	135	14.1	22.4	1016
	Po 4	30.5	44°57.582	12°49.710	44°57.636	12°48.996	1.7	1	1	135	15.5	30.3	1022
2nd survey													
Pellestrina	Pe 1	4.5	45°19.146	12°20.0832	45°18.7842	12°21.132	2.5	1	1	315	17.0	28.2	1020
	Pe 2	11.0	45°18.981	12°21.4908	45°18.558	12°22.5948	2.6	1	1	315	16.7	28.6	1021
	Pe 3	16.5	45°18.7728	12°27.087	45°18.6792	12°28.2822	2.8	1	1	225	17.2	29.9	1022
	Pe 4	22.5	45°18.6912	12°34.731	45°18.6618	12°33.7602	2.1	1	2	315	16.9	32.4	1024
Po Delta	Po 1	8.5	44°57.2988	12°34.3758	44°57.1938	12°35.535	2.5	1	2	338	15.7	17.3	1012
	Po 2	23.4	44°57.2988	12°36.4068	44°57.7992	12°36.522	1.7	1	2	23	15.7	16.7	1012
	Po 3	29.3	44°57.5298	12°41.550	44°57.9678	12°41.7198	1.6	1	2	23	16.8	25.2	1018
	Po 4	30.8	44°57.3522	12°49.4592	44°57.8358	12°49.3728	1.7	2	2	23	16.4	32.4	1024

Table 2 – Total water volume filtered by the Manta trawl, total number of microplastics, number of microplastics m^{-2}, total weight of microplastics (mg) and mg of particles m^{-2} collected during the two sampling surveys on the Pellestrina and Po Delta transects.

Transect	Station	Surface area (m^2)	N. particles	N. particles m^{-2}	Total weight (mg)	mg m^{-2}
March						
Pellestrina	Pe 1	818	90	0.1	17.6	0.02
	Pe 2	776	218	0.3	33.9	0.04
	Pe 3	700	35	0.1	9.1	0.01
	Pe 4	849	8796	10.4	18695.6	22.02
Po Delta	Po 1	770	1631	2.1	220.6	0.29
	Po 2	953	4095	4.3	914.8	0.96
	Po 3	1046	280	0.3	196.0	0.19
	Po 4	573	137	0.2	80.3	0.14
April						
Pellestrina	Pe 1	940	125	0.1	18.7	0.02
	Pe 2	1000	48	0.1	13.9	0.01
	Pe 3	904	258	0.3	93.8	0.10
	Pe 4	778	57	0.1	17.7	0.02
Po Delta	Po 1	938	202	0.2	29.3	0.03
	Po 2	576	53	0.1	8.7	0.02
	Po 3	519	319	0.6	52.7	0.10
	Po 4	555	16	0.03	47.4	0.09

Figure captions

Figure 1 – Location of the two transects along the northern Adriatic coast. Pellestrina transect: stations Pe 1 – Pe 4; Po Delta transect: stations Po 1 – Po 4. The circle indicates the position of the oceanographic platform "Acqua Alta", where meteorological data, surface currents and waves were measured.

Figure 2 - Meteorological data, i.e. winds (A), waves (B), rainfall (C) and Po River discharge for the period March – April 2014. Red circles indicate sampling days. Wind, wave and rainfall data were measured at the "Acqua Alta" oceanographic platform in the period February - April. Po River discharge data are from ARPA-ER (https://www.arpae.it).

Figure 3 – Mean number of particles m^{-2} (A) and mg m^{-2} (B) detected on the Pellestrina and Po Delta transects in March and April. Mean ± s.e., N = 4. Mann-Whitney test * $p < 0.05$.

Figure 4 - Principal Component Analysis based on temperature, salinity, number of particles m^{-2} and mg m^{-2} detected at each sampling station during the first and second sampling surveys and related station distribution. Pellestrina, March: Pe 1-1 – Pe 4-1; April: Pe 1-2 – Pe 4-2. Po Delta, March: Po 1-1 – Po 4-1; April: Po 1-2 – Po 4-2.

Figure 5 – Landsat 8 turbidity maps of the northern Adriatic Sea acquired on 31 March 2014 (left panel) and 25 April 2014 (right panel), with the related distribution of total microplastic particles (n. particles m^{-2}).

Figure 6 – Classification of microplastic particles (in %) according to color. Samples collected in March (A) and April (B) off Pellestrina (Pe 1 – Pe 4) and the Po Delta (Po 1 – Po 4).

Figure 7 – Classification of microplastic particles (in %) according to shape. Samples collected in March (A) and April (B) off Pellestrina (Pe 1 – Pe 4) and the Po Delta (Po 1 – Po 4).

Figure 8 – Classification of microplastic particles (in %) according to polymer type. Samples collected in March (A) and April (B) off Pellestrina (Pe 1 – Pe 4) and the Po Delta (Po 1 – Po 4). Polyethylene (PE), polypropylene (PP), polystyrene (PS), ethylene vinyl acetate (EVA), polyethylene terephthalate (PET), Others (polyacrylate, polyurethane, poly(2,6-dimethyl-p-

phenylene oxide), nylon, polyisoprene, silicone, polyacrylonitrile, cellulose-rayon, paraffin wax, vinyl chloride-vinyl acetate, styrene-butadiene rubber), and not identified (Not ID).

Figure 9 – Sample spectra of the most abundant polymers (polyethylene, PE; polypropylene, PP; ethylene vinyl acetate, EVA; polystyrene, PS; polyethylene terephthalate, PET) compared to reference spectra and examples of related fragments.

Figure 10 – µATR-FT-IR spectra of two oxidized microplastic particles (PE – red spectrum; PP – green spectrum) (A). In the box, the bands used to measure the Carbonyl Index are highlighted (1850 – 1300 cm^{-1}); Carbonyl Index measured for PE and PP at each sampling site during the March (B) and April (C) sampling surveys.

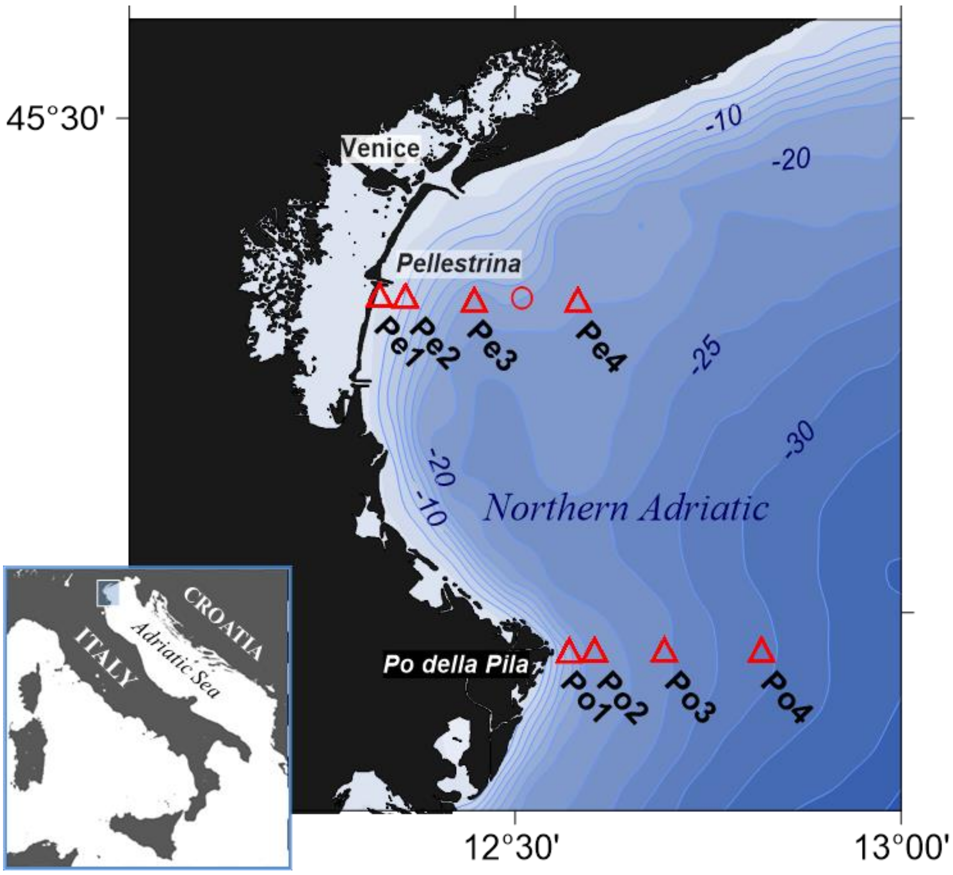
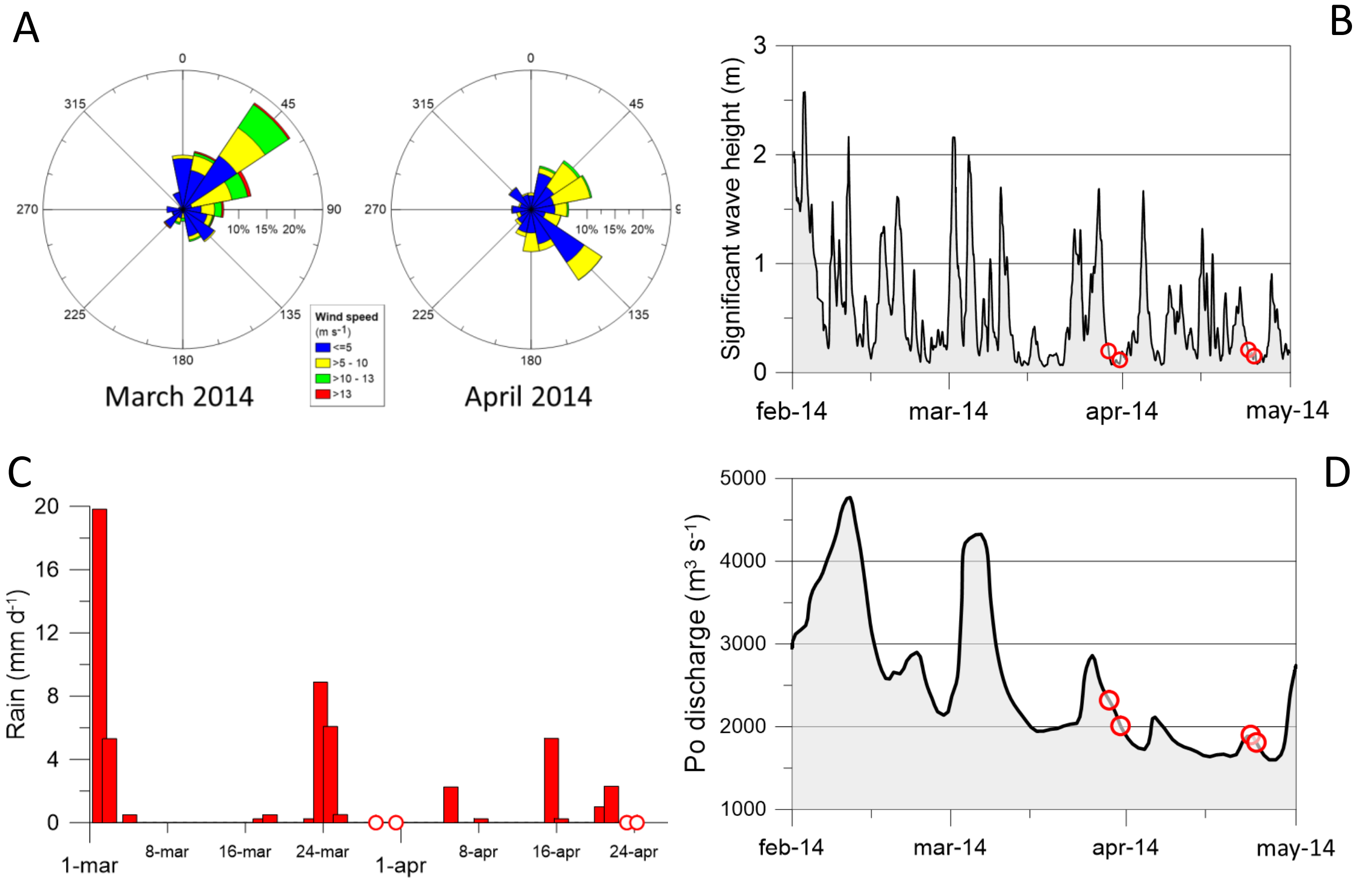

45°30'
Venice
Pellestrina
Pe1
Pe2
Pe3
Pe4
-10
-20
-25
-30
-20
-10
Northern Adriatic
Po della Pila
Po1
Po2
Po3
Po4
12°30'
13°00'
CROATIA
ITALY
Adriatic Sea

Figure 1



A
March 2014
April 2014
Wind speed (m s-1)
<=5
>5 - 10
>10 - 13
>13
B
Significant wave height (m)
feb-14
mar-14
apr-14
may-14
C
Rain (mm d-1)
1-mar
8-mar
16-mar
24-mar
1-apr
8-apr
16-apr
24-apr
D
Po discharge (m3 s-1)
feb-14
mar-14
apr-14
may-14

Figure 2

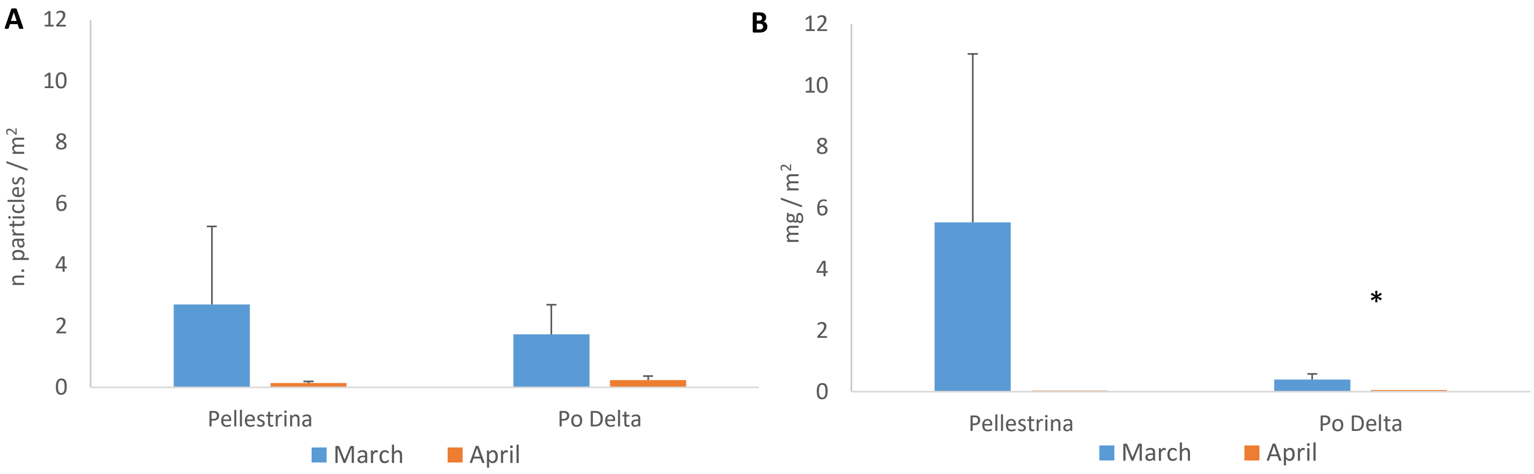
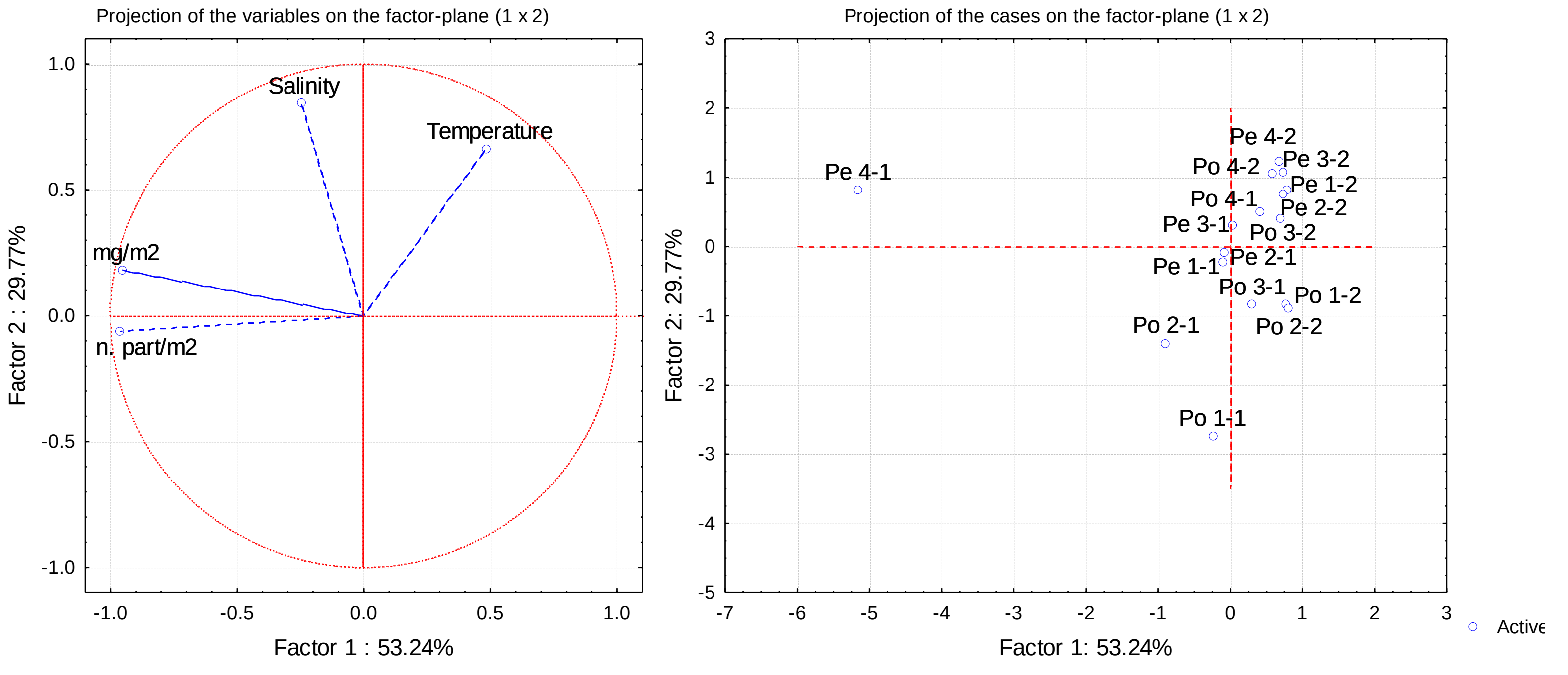

A
B
n. particles / m²
mg / m²
12
10
8
6
4
2
0
*
Pellestrina
Po Delta
March
April

Figure 3



Projection of the variables on the factor-plane (1 x 2)
Salinity
Temperature
mg/m2
n. part/m2
Factor 2 : 29.77%
Factor 1 : 53.24%
Projection of the cases on the factor-plane (1 x 2)
Pe 4-1
Pe 4-2
Pe 3-2
Po 4-2
Pe 1-2
Po 4-1
Pe 2-2
Pe 3-1
Po 3-2
Pe 2-1
Pe 1-1
Po 3-1
Po 1-2
Po 2-1
Po 2-2
Po 1-1
Factor 2: 29.77%
Factor 1: 53.24%
Active

Figure 4

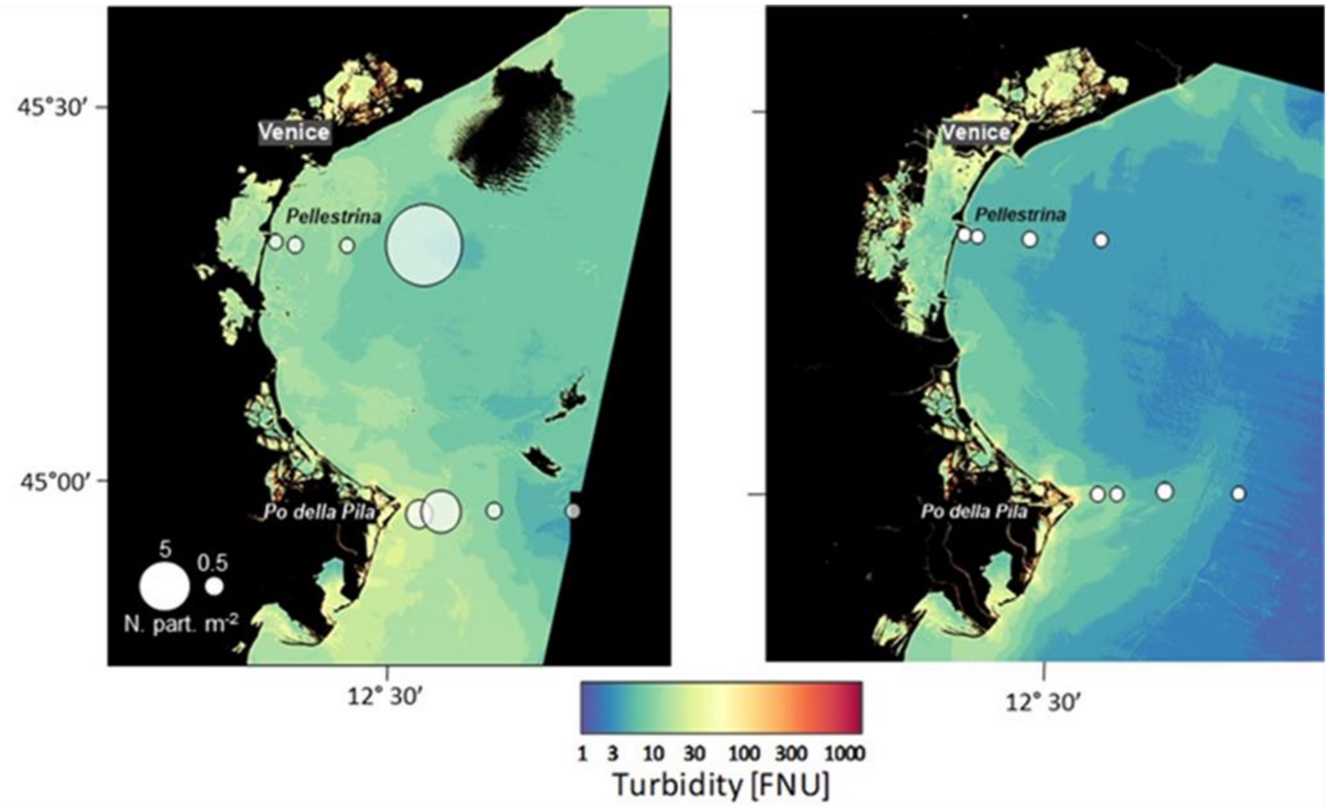

45°30'
45°00'
Venice
Pellestrina
Po della Pila
5
0.5
N. part. m-2
12° 30'
Venice
Pellestrina
Po della Pila
12° 30'
1 3 10 30 100 300 1000
Turbidity [FNU]

Figure 5

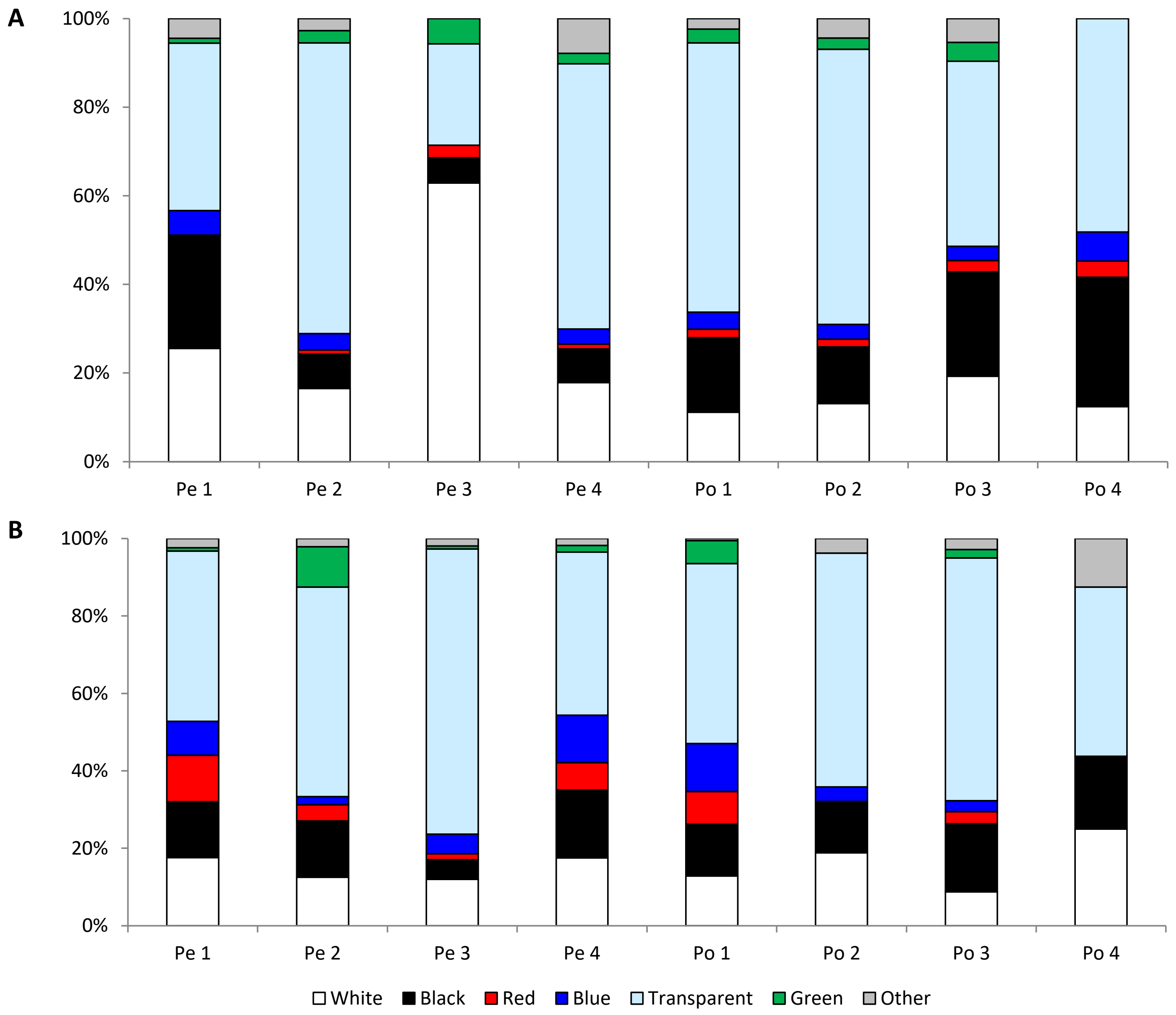

A
100%
80%
60%
40%
20%
0%
Pe 1
Pe 2
Pe 3
Pe 4
Po 1
Po 2
Po 3
Po 4
B
100%
80%
60%
40%
20%
0%
Pe 1
Pe 2
Pe 3
Pe 4
Po 1
Po 2
Po 3
Po 4
White
Black
Red
Blue
Transparent
Green
Other

Figure 6

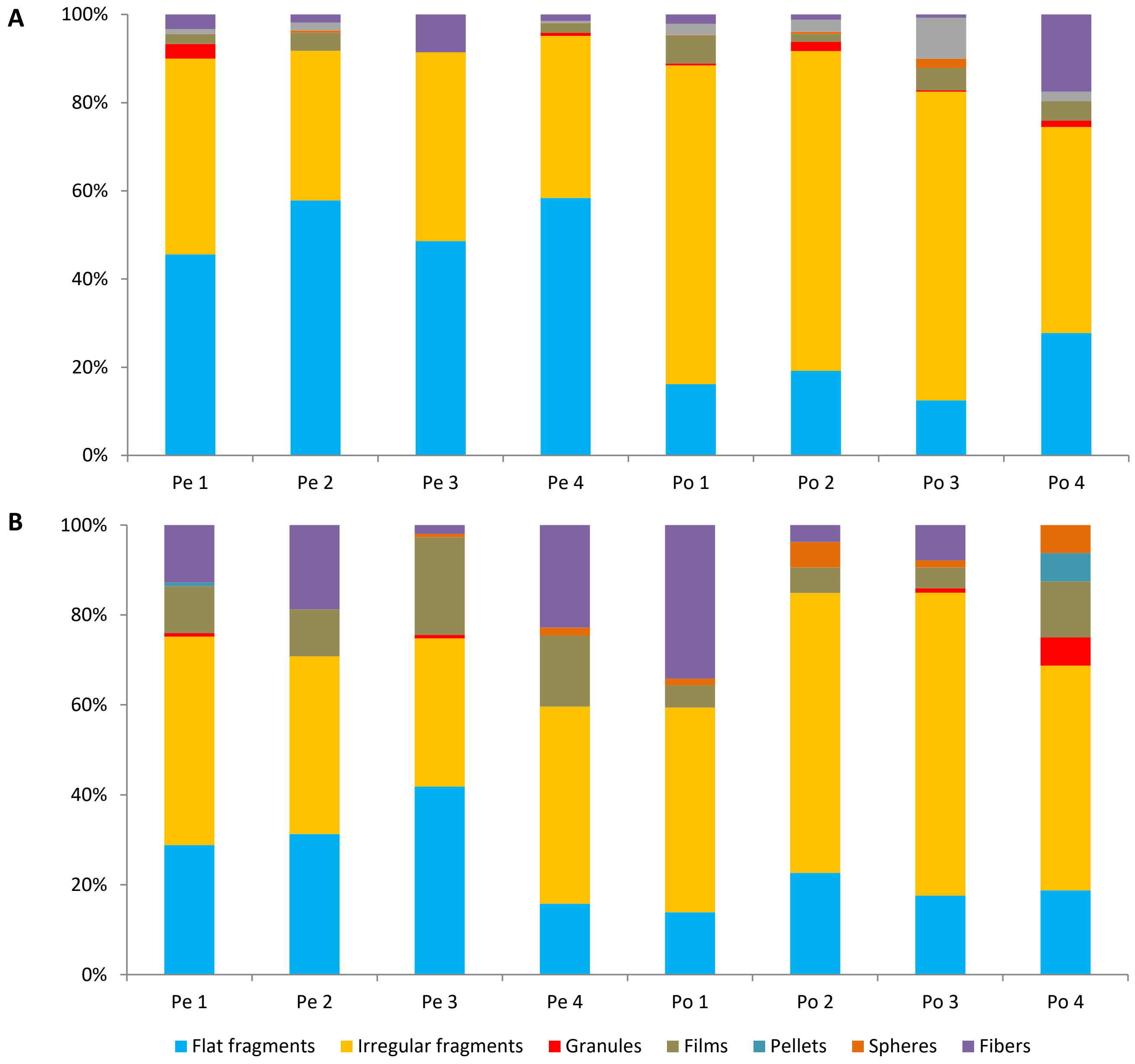

A
100%
80%
60%
40%
20%
0%
Pe 1
Pe 2
Pe 3
Pe 4
Po 1
Po 2
Po 3
Po 4
B
100%
80%
60%
40%
20%
0%
Pe 1
Pe 2
Pe 3
Pe 4
Po 1
Po 2
Po 3
Po 4
Flat fragments
Irregular fragments
Granules
Films
Pellets
Spheres
Fibers

Figure 7

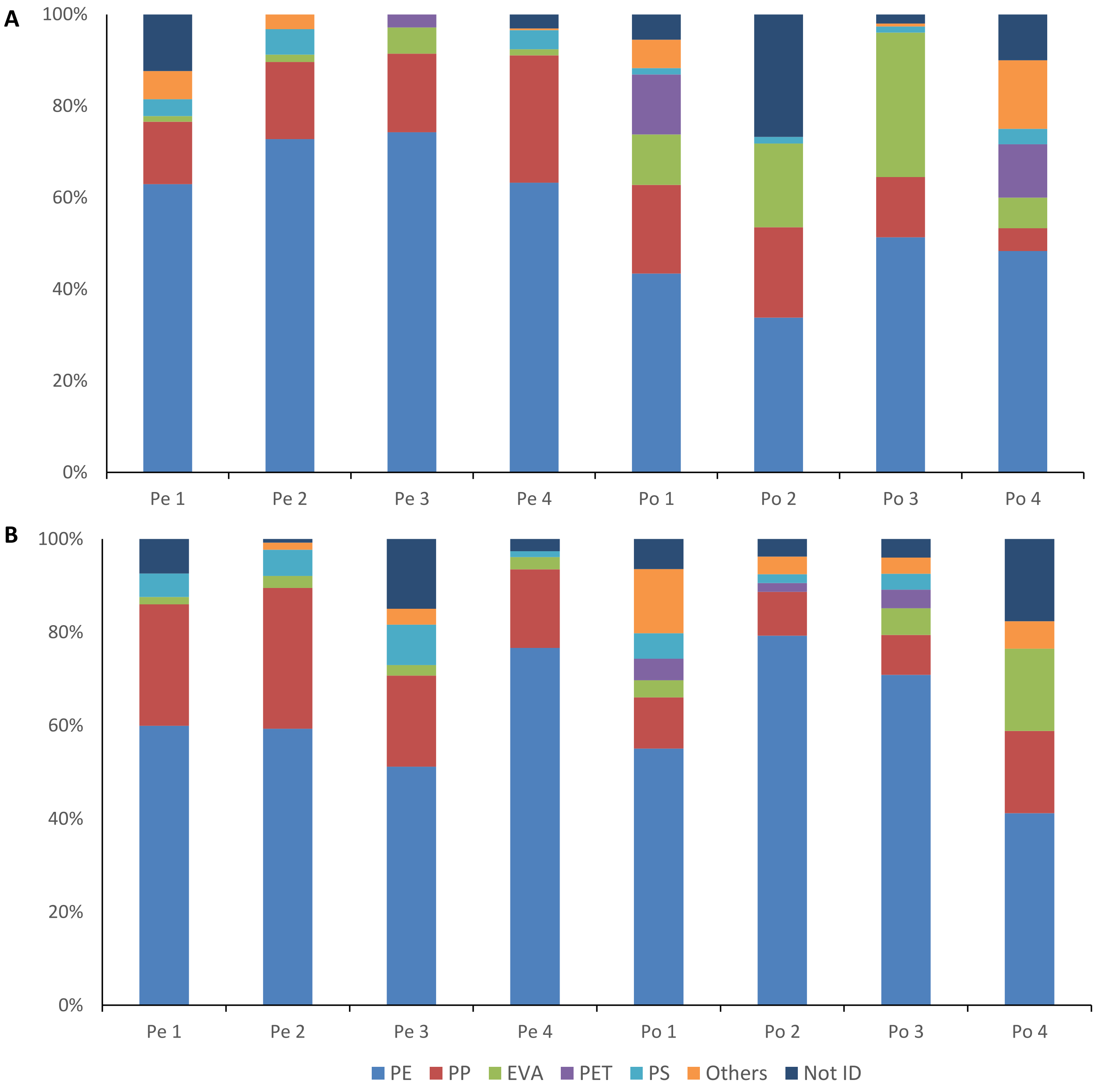
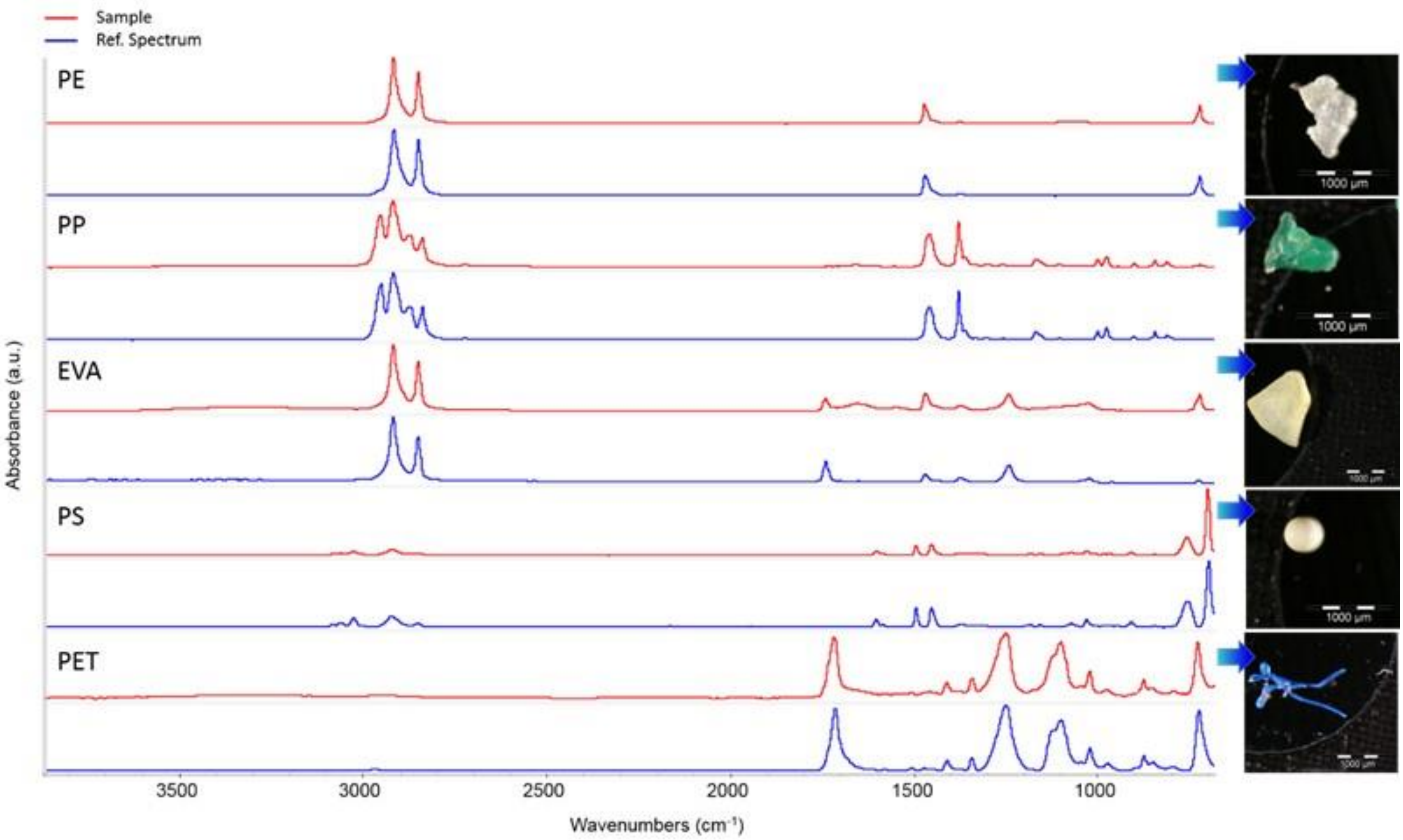

A
B
100%
80%
60%
40%
20%
0%
Pe 1
Pe 2
Pe 3
Pe 4
Po 1
Po 2
Po 3
Po 4
PE
PP
EVA
PET
PS
Others
Not ID

Figure 8



Sample
Ref. Spectrum
PE
PP
EVA
PS
PET
Absorbance (a.u.)
3500
3000
2500
2000
1500
1000
Wavenumbers (cm⁻¹)
1000 µm

Figure 9

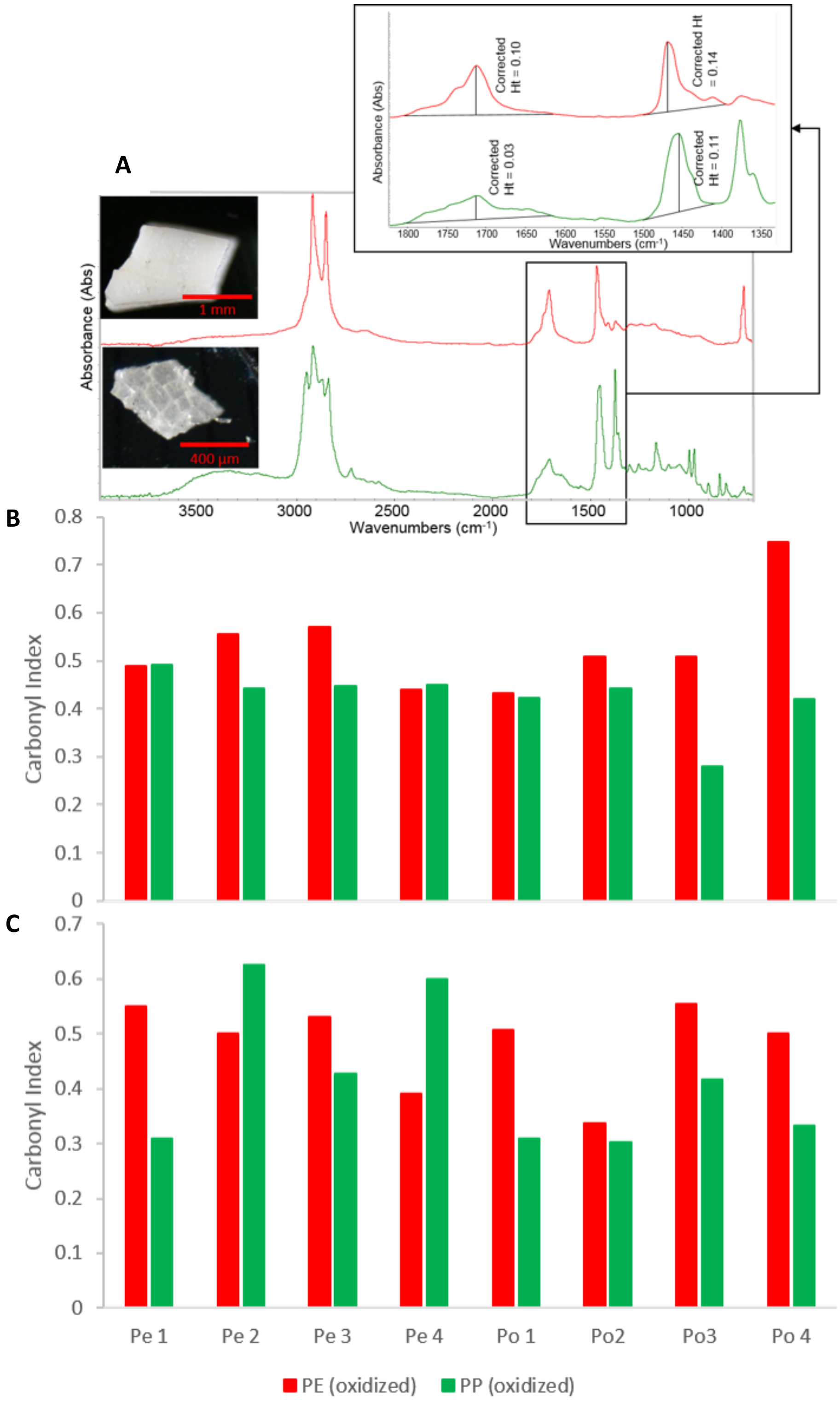

A
1 mm
400 µm
Absorbance (Abs)
Wavenumbers (cm-1)
Corrected Ht = 0.10
Corrected Ht = 0.14
Corrected Ht = 0.03
Corrected Ht = 0.11
B
C
Carbonyl Index
Pe 1
Pe 2
Pe 3
Pe 4
Po 1
Po2
Po3
Po 4
PE (oxidized)
PP (oxidized)

Figure 10