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Assessment of microplastics distribution and stratification in the shallow marine sediments of Samos island, Eastern Mediterranean sea, Greece

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Abstract

Microplastics (MPs) are becoming ubiquitous in the marine environment and shallow marine sediments are one of the major sinks for MPs. In order to improve the knowledge about the environmental fate of MPs in the Aegean Sea shallow sediments, the occurrence and spatial distribution of MPs in Samos Island (Greece) was investigated for the first time, along a land-sea transect. The highest concentration of MPs was recorded at the *Beach* (37.2 ± 6.9 MPs/Kg DW), while the lowest concentration was recorded in the *Intertidal* zone (1.1 ± 0.9 MPs/Kg DW). In relation to the different shapes, fragments and pellets were more abundant on the *Beach*, while fibers were more abundant in the *Intertidal* zone and barely any sheets were found along the transect, suggesting a distribution probably governed not only by size but also morphology and perhaps chemical composition. A higher accumulation of particles was recorded in the first 10 cm of sediments, in comparison to the deepest 5 cm, indicating an increase of microplastics in the shallow marine sediments.

Keywords: Microplastics; shallow marine sediment; Samos Island; Eastern Mediterranean Sea; Greece.

Introduction

Microplastics (MPs) are ubiquitous in the environment and are a growing concern, as the production of plastic has increased yearly since the 1950s (Wagner *et al.,* 2014; Plastics Europe, 2016). Different size based-definitions of MPs have been proposed by several authors over the past few years (see references in da Costa *et al.,* 2016). For the purpose of this study, we use the definition of MPs proposed by Arthur *et al.* (2009), considering MPs as particles that are <5 mm in at least one of their dimensions.

MPs and plastics, in general, originate from the terrestrial environment, but eventually enter the aquatic and marine environment (Boucher & Friot, 2017). Insufficient waste management has led to a release of an estimated 4.8 to 12.7 Mtons per year (Jambeck *et al*., 2015). However, also through sewage sludge or treated effluent MPs can reach the environment (Leslie *et al*., 2017). A significant amount of 1.5 Mton of MPs is thought to be released every year via effluent and sewage sludge (Boucher & Friot, 2017). A distinction can be made between primary and secondary MPs. Primary MPs are plastics directly released into the environment in the form of small particulates (Sundt *et al.*, 2014), mostly as synthetic fibers through the washing of textiles (34.8%) and car tire particles through the erosion of car tyres (28.3%) (Boucher & Friot, 2017). Additional primary sources, such as resin pellets and microbeads in cosmetics are estimated to contribute less than 3% of MPs to the world oceans (Boucher & Friot, 2017). Secondary MPs sources entail the degradation of larger plastics, to eventually, the micro-sized scale (Mathalon & Hill, 2014). The mechanical degradation by wave action and abrasion by sand has experimentally been shown to be an important mechanism for the generation of smaller particles (Shim *et al.*, 2014; Lambert & Wagner, 2016) and is possibly the most important process explaining the formation of secondary MPs (Koelmans *et al.,* 2015). Additional processes that can enhance the breakdown of MPs are chemical and biological degradation through UV radiation and microbial activity (Lambert *et al.,* 2014).

Macroplastic environmental debris with a higher density than seawater (1.02 g cm^{-3}) may descend and accumulate in the sediments, while particles with a lower density, tend to be buoyant. However, through biofouling and the formation of aggregates with suspended organic matter, low-density plastics may eventually accumulate in the seafloor sediments and organic wrack (Barnes *et al*., 2009; Alomar *et al.*, 2016; Pietrelli *et al*., 2017), becoming available to suspension and filter feeders (Alomar *et al*., 2016).

Shallow marine sediments are a potential sink for plastic debris and MPs due to high biological activities and the anthropogenic impact related to populated and polluted areas (Clark *et al.*, 2016). However, local wind directions, main oceanic currents, tidal strength and also the geography of the coastline, can affect the spatial and temporal distribution of MPs (Barnes *et al*., 2009). In general, the proximity of anthropogenic activities such as sewage systems, trade routes and fisheries are correlated to an increase in MPs. A strong correlation between human population and the MPs density in sediment has previously been reported by Barnes (2005) and Barnes *et al*. (2009).

Previous studies showed that MPs could potentially be transported up the food chain (Farrell & Nelson, 2013; Setälä *et al*., 2014). MPs have been reported to be ingested, but also egested by estuarine and marine organisms such as, zooplankton (Cole *et al.*, 2013), holothurians (Graham & Thompson, 2009), mussels (von Moos *et al*., 2012; Browne *et al*, 2008), barnacles, amphipods lugworms (Thompson *et al*., 2004; Iannilli *et al.,* 2018), marine reptiles (Staffieri *et al*., 2019), birds (Battisti *et al*., 2019) and mammals (Poeta *et al*., 2017). Little is known about the negative effects of the ingestion of MPs. Cole *et al*. (2013) showed that microplastics negatively affected the tissues and cells of *Mytilus edulis*. Additionally, Wright *et al*. (2013) showed that microplastics cause adverse effects on the energy reserves of *Arenicola marina* and von Moos *et al.* (2012) showed that *Mytilus edulis* had an inflammatory response to high-density polystyrene. However, eco-toxicological experiments usually test concentrations that are not naturally realistic (Van Cauwenberghe *et al*., 2015). This highlights the importance of studies investigating the distribution and abundance of MPs, especially in marine sediments (Graham & Thompson, 2009), as this is poorly understood. It is crucial to assess particles concentration in sediments to accurately estimate the potential risk that MPs could have on the environment in general and on marine organisms in particular (Van Cauwenberghe *et al*., 2015). Moreover, MPs are expected to increase in marine sediments over time (Claessens *et al.*, 2011; Everaert *et al*., 2018). However, through bioturbation (Näkki *et al.*, 2017), human activity and storms the top layers of sediment could be partly or completely homogenized (Claessens *et al.*, 2011).

The Mediterranean Sea has one of the highest concentrations of marine plastic debris with 72-16,198 items per km^2 (Barnes *et al.*, 2009). As it is a semi-enclosed sea, dispersion of plastics by water circulation and tidal flow is limited. Additionally, it has a densely populated coastline with intensive marine traffic (Katsanevakis & Katsarou, 2004; Barnes *et al.*, 2009; Lebreton *et al*., 2012). Only a few studies investigated the presence of MPs in the Mediterranean Sea in shallow coastal sediments in the Western (Balearic Islands, Alomar *et al*., 2016) and central part (Malta Island, Turner & Holmes, 2011; Venice Lagoon, Vianello *et al*., 2013) of the basin. Hence, only limited information on the occurrence and abundance of MPs in the shallow sediments of the Aegean Sea is available at this time. The aim of this study was to make an assessment of the occurrence and the distribution (lateral and vertical) of MPs along a land-sea transect in the shallow marine sediments of Samos Island in Greece (Aegean Sea, Eastern Mediterranean Sea).

Methods

Sampling location

This study was conducted on Samos Island (Greece) which lies in the Aegean Sea (Fig. 1). A beach located in the southeast area of the island was chosen (Psili Ammos 37.707948 N, 27.017611 E). This sandy beach is frequently visited by tourists during the summer months. The tid-

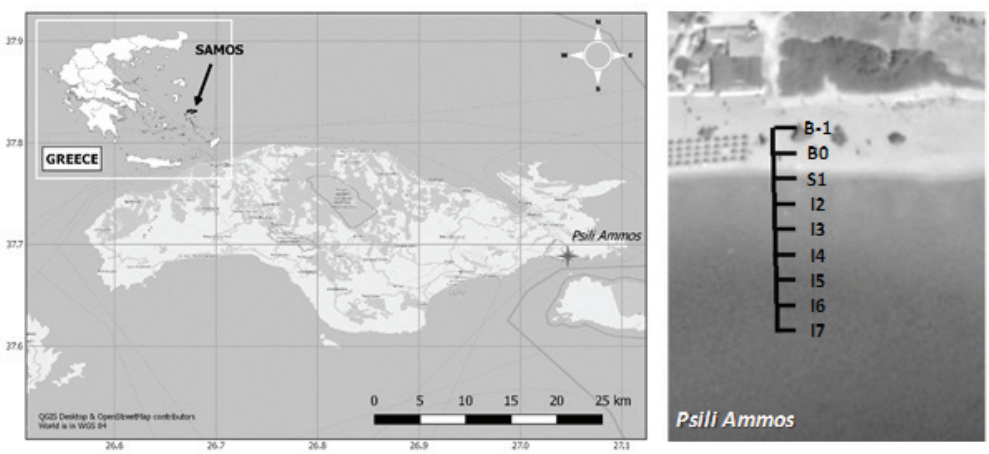

Fig. 1: Map indicating the location of the study area: Psili Ammos beach located SE in Samos Island (Greece) and land-sea transect with the 9 sites identified as described in the text.

al range of the sampling location is 0.11-0.85 m (www.tideschart.com). In 2017, the clean-coast index (CCI) was applied to evaluate the coast cleanliness (Alkalay *et al.*, 2007). Five transects, covering a total area of 54 m^2, were analysed (4 hours, 5 replicates). The beach was categorized as "extremely dirty" with an average of 26.86 plastic particles/m^2. The particles found consisted mainly of hard fragments but also fisherman's twine, cigarettes, styrofoam, resin pellets, straws, Q-tips and bottle tops.

Sample collection

Sample collection took place during September 2017. The land-sea transect (length 80 m) was perpendicular to the shoreline in the central part of the bay (Fig. 1). Along the transect, 9 sampling points were identified: Point B-1 and B0 are located at the *Beach*, while Point S1 is located at the *Shoreline*. Points I2-I7 are located in the shallow sea water (*Intertidal*), with an interval of approximately 10 meters. To avoid possible plastic contamination during the sampling procedures, sediment samples were collected using stainless steel hand cores with cork lids (length: 36 cm; diameter: 76 mm). At each sampling point three sediment replicates, separated by a distance of about 1-1.5 m, were taken by scientific divers. Sediment cores were held vertically and carefully transported to the laboratory.

Laboratory analysis

The methodological approach used in this study was based on Masura *et al*. (2015) with some modifications. Once in the laboratory, sediment core samples were extruded using a wooden plunger and subsampled using a metal knife into 5 cm sections (i.e. 0- 5 cm 5-10 cm and 10-15 cm) and placed in aluminium containers. Samples were then dried at 50 °C for 48 hours or until dry. Samples were homogenized and sieved with a stainless-steel sieve (mesh size: 3 mm) and the retained particles were sorted (i.e. particles >3mm). MPs <3 mm were then extracted by means of a slightly modified version of Thompson *et al*. (2004) density separation method. From each sample 200 g of sieved sediment was placed in a glass jar to which 150 ml H_20_2 (30%) was added for organic matter oxidation. To avoid overflow of sediment from jars, samples were placed in a water bath to cool down overnight. Samples were swirled to enhance organic matter oxidation. Once the reaction had ceased, 250 ml of saturated NaCl solution (1.2 g/cm^3) was added. Jars were shaken vigorously and left to settle for at least 4 hours. The resulting supernatant, with floating particles, was filtered using a vacuum pump and Whatman GF/F filter (diam. 47 mm, nominal porosity: 1.2 μm). To ensure that all particles were extracted, the density separation step was repeated twice.

Particles retained in the sieves (>3mm) and in the filter (<3mm) were analysed following the method described by Hildalgo-Ruz *et al.* (2012) and Marine & Environmental Research Institute (2012) with minor alterations. In short, all the particles classified as Large MPs (LMPs 3-5mm) and Small MPs (SMPs <3mm) were identified as MPs when (i) no cellular or organic structures are visible, (ii) a clear homogenous colour throughout is exhibited, (iii) an equal thickness throughout the entire length is visible (generally valid for fibers). MPs were counted using a stereoscope with a 10x and 20x magnification and classified as fragment, fiber, pellet or sheet (Fig. 2). In cases of difficult identification between organic matter and plastic, particles were exposed to the hot needle test based on De Witte *et al.* (2014), since in the presence of a hot needle, plastic will melt or curl. If after this test a conclusion still remained uncertain, particles were removed

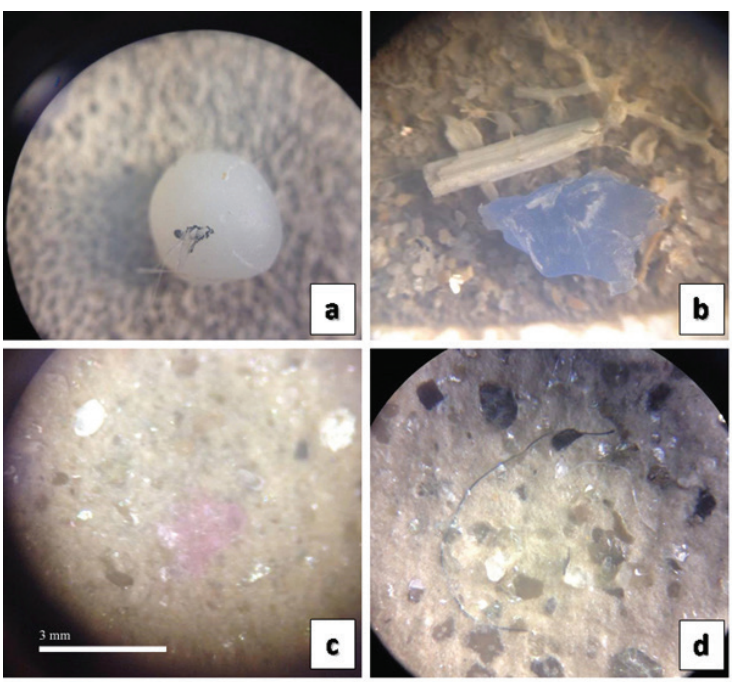

Fig. 2: MPs shapes under stereoscope with 10x magnification: (a) white pellet, (b) blue fragment, (c) pink sheet and (d) black fiber.

and placed under a microscope with 40x magnification for further analysis.

Measures to avoid contamination were adopted. All material used during the analysis was rinsed with distilled water and alcohol. Glass Petri dishes were left open in the laboratory as *control* and checked for any MPs contamination. A subsample (500 g) of the NaCl used during the analysis was filtered to check for possible MPs contamination. Latex gloves and 100% cotton lab coats were worn while handling samples.

Data Analysis

Results of the shape and size of MPs were expressed as mean ± standard deviation of MPs/Kg dry weight of sediment (DW). For analysis of the lateral distribution points B-1 and B0 were categorized as Beach, point S1 as Shoreline and points I2-I7 as Intertidal. Permutation Multivariate Analysis of Variance (PERMANOVA) (Anderson et al., 2008) was used to test for the lateral distribution of the different shapes of MPs (Fragment, Fiber, Pellet and Sheet) using the factor Position (Beach, Shoreline and Intertidal) as fixed factor. The vertical stratification in the three sections of the cores (0-5, 5-10 and 10-15 cm) was tested using PERMANOVA and the same statistical design described above.

The vertical stratification and the lateral distribution of MPs in the different sampling points were analysed using the non-parametric Kruskal-Wallis test due to the non-normal distribution of the data (Quinn & Keough, 2002). In case of significant results, data were further analysed using the Dunn test for multiple comparisons with a Bonferroni correction method to identify the significantly differing groups (Quinn & Keough, 2002). Statistical analysis were performed using the R environment (version 3.5.1) in RStudio (Version 1.1.463).

Results

In the *control* Petri dishes (n. 9) a total of 4 MPs, all fibers, were recorded: one blue, one yellow, one blue/transparent striped and one black. In the NaCl *control*, only one blue/transparent striped fiber was found. The fibers, probably originating from clothing, from the *control* were easily distinguishable from the environment samples. To avoid overestimation of MPs, similar fibers were excluded from the count of MPs (<1%).

Lateral distribution

The mean concentration of MPs in the sediment cores was 11.5 ± 10.5 MPs/Kg DW (Fig. 3). The highest concentration was recorded at the *Beach* (point B0, 37.2 ± 6.9 MPs/Kg DW), while the lowest MP concentration was recorded in the *Intertidal* zone (point I5, 1.1 ± 0.9 MPs/Kg DW) with significant differences between the sampling points (Kruskal-Wallis, H = 22.324, $p<0.01$) (Fig. 3). In general, a significantly higher quantity of MPs was found on the *Beach* compared to the *Intertidal* zone which accounted to 20.7 ± 12.6 and 6.9 ± 4.7 MPs/Kg DW, respectively (Kruskal-Wallis, H = 11.341, $p<0.005$) (Fig. 3).

Looking at the two MPs size classes identified, LMPs were mostly found only at the *Beach* and in the *Shoreline* zones with concentrations respectively being 3.2 ± 4.6 and 0.6 ± 1.2 MPs/Kg DW. LMPs were almost absent in the *Intertidal* zone 0.0 ± 0.2 MPs/Kg DW (Kruskal-Wallis, H = 15.192, $p<0.001$).

The distribution of the four different shapes was significantly affected by the position along the transect (PERMANOVA, Pseudo-f = 9.888, p<0.001) with significantly differences between the *Intertidal* and the two other zones (Pair-wise tests: *Beach* vs *Intertidal* *P(MC)*<0.001; *Shoreline* vs *Intertidal* *P(MC)*<0.05). In particular, the sediment cores on the *Beach* mainly con-

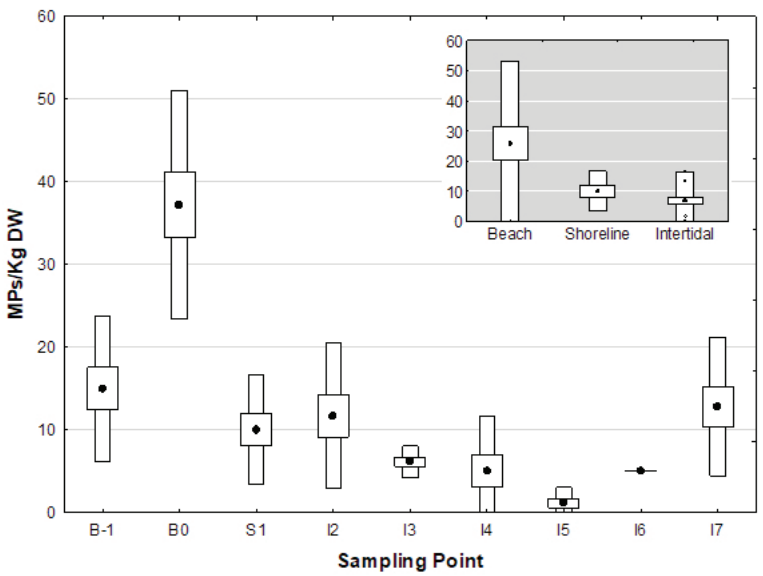

Fig. 3: Box-plot of the mean abundance of MPs (MPs/Kg DW) recorded in the sampling points and along the three zone of the transect.

tained fragments (21.4 ± 12.6 MPs/Kg DW), whereas in the sediment cores of the *Intertidal* zone a very low concentration of fragments was found (0.1 ± 0.4 MPs/Kg DW) (Kruskal-Wallis, H = 21.257, p <0.001) (Fig. 4). Fibers were more numerous in the *Intertidal* zone than on the *Beach* with 6.4 ± 4.7 and 3.0 ± 3.7 MPs/Kg DW, respectively (Kruskal-Wallis, H = 11.355, p <0.01) (Fig. 4). Pellets were mainly found on the *Beach* (2.8 ± 2.4 MPs/Kg DW) and to a lesser degree in the *intertidal* zone, (0.1 ± 0.4 MPs/Kg DW) (Kruskal-Wallis, H = 15.964, p <0.001) (Fig. 4). Barely any MPs in the form of sheets were found along the transect (0.1 ± 0.4 MPs/Kg DW) with no significant differences related to the position (Kruskal-Wallis, H = 4.940, p >0.05) (Fig. 4).

Vertical distribution

The vertical distribution of MPs in the sediment cores was significantly affected by the position along the transect (PERMANOVA, Pseudo-f = 6.204, p<0.01) with significant differences between the *Beach* and the *Intertidal* zones (Pair-wise tests, *P(MC)*<0.001). Significant differences were found for the first two layers (p <0.05) with significantly higher values at the *Beach (*0-5 cm: 14.7 ± 10.3; 5-10 cm: 6.1 ± 4.6) compared to the *Intertidal* zone (0-5 cm: 3.0 ± 2.5 MPs/Kg DW; 5-10 cm: 2.0 ± 1.9 MPs/Kg DW) (Fig. 5).

Discussion

This study represents an assessment of the occurrence and distribution of MPs along a land-sea transect in the marine shallow sediment of Samos Island in the Aegean Sea. While only in few regions the abundance of MPs has been recorded in shallow marine environments (e.g. Vianello *et al.*, 2013), the abundance of MPs on beaches has been reported throughout the world (Van Cauwenberghe *et al.*, 2015). As a result of different size-based definition (da Costa *et al.*, 2016), sampling approaches, extraction procedures and identification methods (Van Cauwenberghe *et al.*, 2015) comparison of worldwide MPs concentrations is difficult. For example, Song *et al.* (2015) pointed out how the use of microscope overestimates the number of fibers and underestimates the number of fragments compared to the Fourier Transform Infrared Spectroscopy (FT-IR), with identification of fragments being up to 7.9 fold higher using the FT-IR (Song *et al*., 2015). Moreover, the use of NaCl (ρ = 1.2 g/cm^3) in the density separation procedure reduces the identification of higher density particles such as PVC (ρ = 1.14 – 1.56 g/cm^3) and PET (ρ = 1.32 – 1.41 g/cm^3). We are aware of the limitations and of the possible underestimation and/or overestimation of MPs in this study. However, our main purpose was to give a first insight into the qualitative distributions of MPs along a land-sea transect in the Aegean Sea.

In this study, the total concentration of MPs was higher at the beach compared to the intertidal zone, indicating a possible accumulation on the shoreline instead of an accumulation in deeper sediment. The concentrations measured were lower than those found by Claessens *et al*. (2011) along the Belgian coast and by Vianello *et al.* (2013) in the Venice Lagoon, Italy. As mentioned before, this may be due to differences in the extraction methods and analysis and/or environmental characteristics of the area, but also to the different water pollution levels of the different study sites. However, MPs tend to accumulate in sites with low hydrodynamics as reported for the Venice Lagoon (Vianello *et al.*, 2013). The abundance of SMPs reported in our study was always higher than LMPs, suggesting how the abundance of MPs increased exponentially with decreasing particle size (Song *et al.*, 2014). This is probably due to fragmentation and degradation mechanisms which lead to the formation of MPs (Barnes *et al.*, 2009). Moreover, the most common shape of plas-

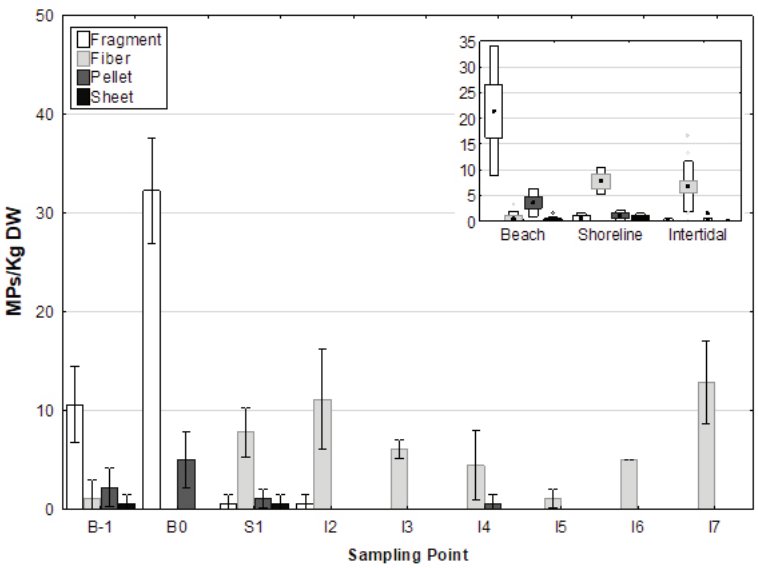

Fig. 4: Mean concentration of the different MPs (MPs/Kg DW) shapes recorded in the sampling points and box-plot of the mean concentration of MPs (MPs/Kg DW) along the three zone of the transect.

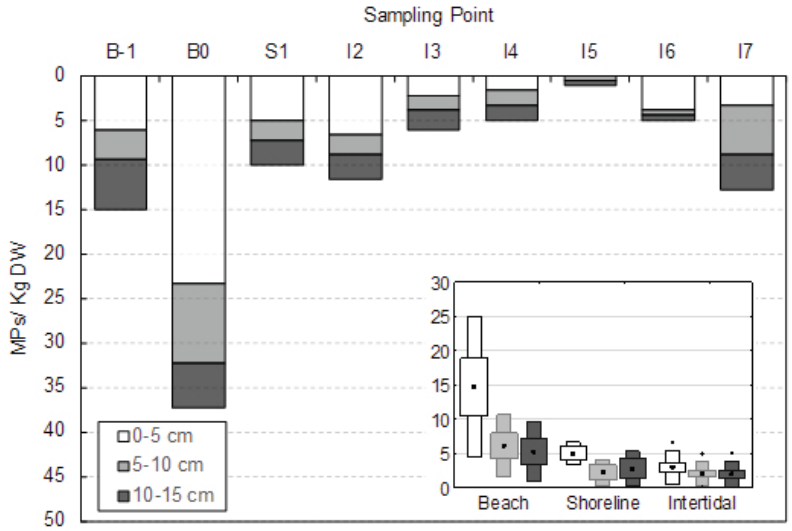

Fig. 5: Vertical stratification of MPs (MPs/Kg DW) per 5 cm section of sediment core in the sampling points and box-plot of the mean concentration of MPs (MPs/Kg DW) along the three zones of the transect.

tic found on the beach were fragments, which could originate from the degradation of larger plastic objects (Alomar *et al.*, 2016) left behind by tourists. However, as the most important mechanism explaining the fragmentation of macroplastics to MPs is the mechanical abrasion by sand through wave action (Koelmans *et al.,* 2015), it could also be that these fragments were washed up on shore. Hardly any fibers were recorded at the beach, most of them were located in the intertidal zone. These fibers probably originate from sewage treatment plants (Browne *et al*., 2011) and/or the wear and tear of fishing gear (da Costa *et al.*, 2016). Our findings are in accordance with Thompson *et al.* (2004) who reported a lower concentration of fibers at beaches (0.4 fibers/50 mL) than in estuarine (2.4 fibers/50 ml) and in subtidal areas (5.6 fibers/50 ml) around Plymouth, UK. Opposite results were reported by Claessens *et al*. (2011) in Belgian marine sediments, where almost the same concentration of fibers were reported for the beach (82.1 ± 32.6 MPs/Kg DW) and the intertidal zone (83.7 ± 25.5 MPs/Kg DW).

In Psili Ammos, fragments and fibers showed an inverse spatial distribution with fragments being more abundant at the beach and fibers more abundant in shoreline and intertidal zones. Notwithstanding that this distribution is probably largely related to different sources of these differently shaped particles, it also suggests that environmental drivers such as wind, tides and hydrodynamics can transport particles especially the more buoyant ones, far away from the source (Oliveira *et al.*, 2015; Ryan, 2015). Moreover, as particles become more elongated their surface area to volume ratio increases; this makes them more suitable for fouling by algae and epibionts (Ryan, 2015). Consequently, elongate plastic items such as fibers should sink more rapidly than fragments (Ryan, 2015), resulting in an accumulation of fibers in deeper waters (e.g. intertidal zones).

Furthermore, the fate of MPs is also dependent on their chemical composition. Lagarde *et al.* (2016) compared the long-term colonization of the microalgae *Chlamydomas reinhardtii* on Polypropylene (PP) and high-density polyethylene (HDPE) particles. While on both types of plastic microalgae colonize quickly, only in the case of PP, eventually heteroaggregates were formed increasing their density significantly. These authors also hypothesized that different Extracellular Polymeric Substances are produced by the microalgae depending on the chemical composition of the MPs, leading to differences in the buoyancy of aggregates (Lagarde *et al.,* 2016). In this study we did not analyse the chemical composition of the MPs found along the transect, indicating that further investigations are necessary.

Even though they were found in minor concentrations compared to the fragment shape, we also found pellets in our samples. This particular plastic shape can be identified as "suspension beads", i.e. the raw plastic material shipped to fabricators (Claessens *et al.*, 2011). Therefore, particles of this type originate either from the spillage of ships or through insufficient waste management from plastic-producing factories (Claessens *et al.*, 2011), suggesting that recreational activities could not be the sole source of plastic pollution on Psili Ammos beach. A very low number of sheets was recorded in this study. Sheets or film were occasionally identified in the Lagoon of Venice by Vianello *et al.* (2013) and low concentrations were reported at the Belgian coast by Claessens *et al*. (2011).

Despite the estimated abundances for car tire particles being relatively high (Boucher & Friot, 2017), these particles were not recorded in this study. It might be that with our methods we have simply overlooked them, as they do not react to hot needle as other plastics would do. Additionally, as they resemble organic matter, they might have been disregarded (Boucher & Friot, 2017). An alternative cause maybe that Samos island is not a highly populated island which means the output of car tire particles is probably low.

As the production of plastic has increased every year since the 1950's, one would expect increasing concentration of MP's in the sediment cores from bottom to top. This is reflected by the data in this study; a higher concentration of particles was recorded in the first 10 cm of

the sediment cores, in comparison to the deepest 5 cm, indicating increase of microplastics over time. The vertical accumulation of MPs is more apparent at the beach than at the shoreline or intertidal sites. It should be noted however that no data of sedimentation rates or hydrodynamics regime were available for the study area, hence it is difficult to determine time trends of particles recirculation.

Conclusion

This study is the first to investigate the occurrence and the spatial distribution of MPs along a land-sea transect from a beach to the shallow marine sediments in the Aegean Sea. The results show higher concentrations of MPs at the *Beach* compared to the *Intertidal* zone, indicating that transport by hydrodynamics and *in situ* deposition by humans may be important processes explaining the lateral distribution of MPs. Fragments and pellets were more abundant on the *Beach*, while fibers were more abundant in the *Intertidal* zone. The distribution of MPs could indicate that spatial distribution is not only governed by size, but perhaps also morphology and chemical composition. Finally, although the methodology investigating the vertical distribution of MPs needs further improvement, this study indicates that concentrations of MPs are increasing in marine sediments.

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