Micro- and nano- plastics in the Mediterranean Sea: A preliminary investigation in Mytilene, Greece.

C. Lambrinos, M. Kostopoulou, D. Kitsiou, A. Nikolaou*

University of the Aegean, School of the Environment, Lab. of Environmental Quality & Geospatial Applications, Department of Marine Sciences, University Hill, 81100 Mytilene, Lesvos, Greece

*Corresponding author, email: nnikol@aegean.gr

Abstract

During the latest years an increasing number of papers in the literature report on the abundance and effects of marine plastic litter mainly with emphasis on microplastic particles smaller than 5 mm. Plastic particles in the <100 nm size range (nanoplastics), can occur in considerable amounts in the aquatic environment. Nanoplastics is probably the least known area of marine litter but potentially also the most hazardous. As detection methods are in an early stage of development, to date no nanoplastics have actually been detected in natural aquatic systems. The high surface area of nanoplastics may imply that toxic chemicals are retained by them, possibly increasing overall hazard. In the present study, recent information on micro- and nano- plastics in the area of the Mediterranean Sea is reviewed, while as a case study was performed in coastal areas in Mytilene in Lesvos Island, Greece. A first attempt of measurements of marine litter of various categories in this area was made, and the dominance of plastics was documented.

Keywords: Marine litter, microplastics, nanoplastics, nanomaterials, Mediterranean, pollution

Introduction

During the latest years an increasing number of papers in the literature report on the abundance and effects of marine plastic litter mainly with emphasis on microplastic particles smaller than 5 mm. It has often been suggested that plastic particles in the <100 nm size range (nanoplastics), can occur in considerable amounts in the aquatic environment. Nanoplastics is probably the least known area of marine litter but potentially also the most hazardous. Nanoplastics can occur in the marine environment via release from human activities or via fragmentation of larger particles. However, as detection methods are in an early stage of development, to date no nanoplastics have actually been detected in natural aquatic systems. The high surface area of nanoplastics may imply that toxic chemicals are retained by them, possibly increasing overall hazard (Brown 2001; Browne 2013). Release of additives from small product fragments may add to the hazard of nanoplastics.

Several recent reviews have been published regarding the sources, abundance and negative effects of plastic litter (Andrady 2011; Hammer et al. 2012; Koelmans et al. 2013, 2014). Initial studies paid emphasis on detection and abundance of >5 mm macroplastic in marine ecosystems and biota, followed by an increasing focus on <5 mm microplastics ranging down to the μm-scale. Research on nanoplastics is a very recent area of the environmental sciences. Nanoplastics are of specific interest because of their nano-specific properties, which fundamentally differ from those of the same polymer type in bulk form. Earlier, microplastics has been defined as all particles <5 mm, thus including nanoplastics as well particles. Another recent definition uses <20 μm as a criterion to classify nanoplastics, similar to the cut off used by plankton ecologists for nanoplankton. However this definition includes micrometre-sized particles (microplastics) as well (Klaine et al. 2012).

Various sources of NPs have been suggested such as release from products or formation from larger particles (‘nanofragmentation’) (Andrady 2011; Cózar et al. 2014). Detection methods are in an early stage of development and to date no nanoplastics have actually been detected in natural aquatic systems. Nanoplastic fate studies for rivers show an important role for sedimentation of heteroaggregates, similar to that for non-polymer nanomaterials. Some prognostic effect studies have been performed but effect thresholds seem higher than nanoplastic concentrations expected in the environment. The high surface area of nanoplastics may imply that toxic chemicals are retained by nanoplastics, possibly increasing overall hazard.

Release of non-polymer nanomaterial additives from small product fragments may add to the hazard of nanoplastics. Because of the presence of such co-contaminants, effect studies with nanoplastics pose some specific practical challenges. Some first prognostic bioaccumulation and effect studies have been performed (Brown et al. 2001; Lee et al. 2013; Casado et al. 2013; Besseling et al. 2014) but there is no systematic effect assessment for relevant aquatic species let alone for the community or ecosystem level.

The high surface area of NPs may cause exceptionally strong sorption affinities for toxic compounds, potentially leading to cumulative particle and chemical toxicity effects once NPs have passed cell membranes. Furthermore, if nanofragmentation is a relevant process, release of non-polymer nanoscale additives from the product fragments may further add to the overall hazard (Nowack et al. 2012).

Various recent reviews address the sources, abundance and negative effects of plastic litter (Derraik 2002; Andrady 2011; Hammer et al. 2012; Koelmans et al. 2014, Browne 2015). Science in this field is evolving rapidly, with initial studies mainly focusing on detection and abundance of >5 mm macroplastic in marine ecosystems and biota, followed by an increasing focus on <5 mm microplastics ranging down to the μm-scale. Implications of nanometre-sized plastic particles ('nanoplastics'), constitute a very recent area of the environmental sciences.

The aim of this paper is to provide a brief overview of sources, occurrence and effects of NPs in the marine environment as well as to review papers reporting their presence in the Mediterranean Sea. Furthermore, the results of a case study, regarding the occurrence and categories of marine litter including micro- and nano-plastics in the coastal area of Mytilene, Greece are presented and discussed, using GIS methodology.

Sources and occurrence of nanoplastics in the environment

Primary sources of NPs may relate to release from products and applications, in which nanoplastics are used or formed and that result in emissions to the environment during the product life cycle. Product categories may include waterborne paints, adhesives, coatings, redispersible lattices, biomedical products, drug delivery, medical diagnostics, electronics, magnetics and optoelectronics. Recently, thermal cutting of polystyrene foam has been shown to emit nanometre-sized polymer particles, in the range of ~22–220 nm. Many polymers undergo similar thermal treatments during their life cycle. 3-D printing has been shown to emit nanometre-sized polymer particles, in the range of ~11–116 nm, at considerable rates. Polystyrene and polyethylene nanoparticles are easy to synthesize, are used for research and other applications and thus will find their way into the environment (Lu et al. 2008). Several medical applications include polymeric nanoparticles, nanospheres and nanocapsules, used for drug delivery (Guterres et al. 2007), which are, however, biodegradable solid lipids. Although formally within scope, we argue that such nanoplastics are not likely to be hazardous because of their low persistence in the environment. Cosmetic products are often mentioned in the context of nanoplastics. However, recent product inventories show lowest sizes of ~4 μm present in exfoliating scrubs or skin cleansers (Fendall and Sewell 2009), rendering these products as an unlikely primary source of NPs.

A second speculated source is fragmentation of microplastic to smaller-sized particles eventually reaching the nanoscale (Andrady 2011). Electrospinning of engineered plastics is used to produce mats with nanoscale fibres, which when applied in products might degrade further to the nanoscale (Lu et al. 2008). Polymers consist of a mixture of polymer chains of various lengths. The chains are chemically linked by weak secondary bonds (i.e. hydrogen or Van der Waals bonding) or by physical interaction through entanglement of chains, whereas there is void space in between the chains. The weak interactions are susceptible to breakage at a low energy level. This breakage brings embrittlement, which in combination with other external forces such as friction may cause formation of small particles in the nano-, micro- and millimetre size range, at the surface of the plastics. Shim et al. (2014) were the first to actually report fragmentation of expanded polystyrene (EPS) beads to micro- and nano-sized EPS in experiments involving a month of accelerated mechanical abrasion with glass beads and sand. Formation of nanometre-sized EPS was confirmed with scanning electron microscopy (SEM) and energy-dispersive X-ray spectroscopy (EDS).

Without yet even taking UV exposure into account, these experimental conditions may already mimic conditions at beaches or river banks where prolonged abrasion of macro- and microplastics by sand particles possibly leads to the formation of NPs. The combination of photo-oxidation by UV exposure, high temperature and high humidity at beaches probably enhances fragmentation rates and reduces the size of the plastic particles. However, the occurrence and relative importance of this process still has to be validated in the field. Still, given the available information, we suspect that physical abrasion is a relevant source of NPs.

Although not proven, degradation of microplastics down to the <100 nanometre-scale may constitute a third source of NPs. Slow weathering by photodegradation is well known for all kinds of polymers (Sivan 2011), which is the reason that nanocomposites use manufactured nano-particles (nanofillers) to increase the resistance to oxidation (e.g. Grigoriadou et al. 2011; Bussière et al. 2013). UV-B irradiation aided photo-oxidation of LDPE has been shown to lead to the formation of extractable oxygenated compounds as well as non-oxidised low-molecular weight hydrocarbons, which were utilized by bacteria leading to an LDPE mass loss of 8.4 % in 14 days (Roy et al. 2008). The LDPE films subsequently were too fragile to handle. In a recent environmental study, degradation of 1–1.75 % of PE mass was observed in the laboratory in 30 days, by micro-organisms isolated from marine waters present at high densities (Harshvardhan and Jha 2013).

Effects of nanoplastics on aquatic organisms

The limited literature provides some evidence of effects of NPs to marine and freshwater organisms, yet at relatively high concentrations, i.e. higher than ~0.5 mg/L NPs. There are currently no NP environmental concentrations to which this value can be compared, but the lowest NP effect concentration of 0.54 mg/L (Casado et al. 2013) is about four to six orders of magnitude higher than the 0.4–34 ng/L microplastic concentrations found in freshwaters in the USA (Eriksen et al. 2013) and Europe (Besseling et al. 2014), but almost similar to the highest concentration estimated for marine water (i.e. 0.51 mg/L, Besseling et al. 2014; Lopez Lozano and Mouat 2009). However, because of the limited data, the uncertainties in these numbers and the absence of actual NP exposure data, these comparisons should be interpreted with caution.

Various kinds of additives are added during the manufacturing of plastics to increase its durability. Furthermore, residual monomers may remain in the plastic. For NPs in particular, the high surface area may cause exceptionally strong sorption affinities for ‘external’ toxic compounds which implies that they will always be loaded with hydrophobic toxicants or trace metals (Rochman 2013a, 2014; Holmes et al. 2014). It can be hypothesized that the presence of such additives and absorbed chemicals might lead to increased exposure to these toxicants. In the laboratory, transfer and negative effects of such co-contaminants have indeed been shown upon ingestion of microplastic particles, but only in scenarios where clean organisms were exposed to plastics with rather high concentrations (Rochman et al. 2013b; Browne et al. 2013; Chua et al. 2014), thus forcing a maximum fugacity gradient upon the organism.

Under more realistic natural exposure scenarios where organisms as well as the media water, sediment and plastic were brought at or close to equal chemical fugacity, no or limited (i.e. within a factor of two) increases or decreases in chemical transfer of toxicants were found (Besseling et al. 2013). Several studies even showed beneficial effects of microplastic ingestion by reducing bioaccumulation due to sorption of chemicals to the plastic (Teuten et al.

2007; Gouin et al. 2011; Koelmans et al. 2013; Chua et al. 2014). These different outcomes illustrate how the ‘carrier effects’ of microplastic depend on the initial boundary conditions of the test, which determine the direction of mass transfer between ingested or bioaccumulated plastic and tissue. This is consistent with recent model analyses that systematically explored these exposure scenarios (Gouin et al. 2011; Koelmans et al. 2013, 2014, 2015).

While the actual risk caused by chemical transfer due to microplastic ingestion may thus be of limited importance, exposure to NPs may still constitute a real hazard. Because of the surface effect, it may be possible that NPs retain organic toxic chemicals or heavy metals at higher concentrations than microplastics, thus leading to a fugacity gradient to organism tissue once ingested. If NPs are capable of permeating membranes, passing cell walls, translocate and/or reside in epithelial tissues for prolonged times (Kashiwada 2006; Cedervall et al. 2012; Rossi et al. 2014), the combination of particle and chemical toxicity may yield unforeseen risks. These hypotheses need to be experimentally validated, while also accounting for the possibly low bioavailability of NPs due to aggregation. During nanofragmentation, release of non-polymer nanoscale additives from the polymer nanocomposite product fragments may further add to the overall hazard (Nowack et al. 2012; Schlagenhauf et al. 2014). The smaller the additives, the better the improvement of polymer durability, which explains the addition of engineered nanoparticles such as carbon nanotubes (Grigoriadou et al. 2011; Bussière et al. 2013; Schlagenhauf et al. 2014). Although beneficial for their application, these additives increase the persistence of plastics in the environment and once degraded, may increase the overall risk due to an additional emission of nanomaterials.

Studies in micro- and nano-plastics in the Mediterranean

A limited number of studies, most of them very recent, regard the presence of micro and nano-plastics in the Mediterranean Sea. Avio et al. (2017) recently performed analysis of microplastics in different benthic fish sampled after 2.5 years of huge engineering operations for the parbuckling project on the Costa Concordia wreck at Giglio Island. Fish collected in proximity of the wreck showed a high ingestion of microplastics compared to both fish from a control area and values reported worldwide. Elevated percentage of nylon, polypropylene lines as well as the presence of polystyrene were reported. Vianello et al. (2013) investigated microplastic particles of 1 mm or less through a preliminary monitoring survey in the Lagoon of Venice. Total abundances varied from 2175 to 672 microplastics kg^{-1} d.w., higher concentrations generally being observed in landward sites. Of the ten polymer types identified, the most abundant, accounting for more than 82% of total microplastics, were polyethylene and polypropylene. The most frequent size (93% of observed microplastics) was in the range 30–500 μm. Total values were significantly correlated with the finer sediment fraction and with the metal pollution index.

Fossi et al. (2016) investigated the occurrence of microplastics in fin whales In particular they examined the interaction between free-ranging fin whales (Balaenoptera physalus) and microplastics by comparing populations living in two semi-enclosed basins, the Mediterranean Sea and the Sea of Cortez (Gulf of California, Mexico). The results indicate that a considerable abundance of microplastics and plastic additives exists in the neustonic samples from Pelagos Sanctuary of the Mediterranean Sea. Given the abundance of microplastics in the Mediterranean environment, along with the high concentrations of Persistent Bioaccumulative and Toxic (PBT) chemicals, plastic additives and biomarker responses detected in the biopsies of Mediterranean whales as compared to those in whales inhabiting the Sea of Cortez, the authors believe that exposure to microplastics because of direct ingestion and consumption of contaminated prey poses a major threat to the health of fin whales in the Mediterranean Sea.

De Lucia et al. (2014) reported a quite high average plastic abundance value (0.15 items/m^3) in the region of Sardinia, comparable to the levels detected in other areas of the Mediterranean, with PCBs showing also high levels. Gündoğdu and Çevik (2017) determined the level of micro- and mesoplastic pollution in Iskenderun and Mersin Bays, located in the Northeastern Levantine coast of Turkey. The average level of both micro- and mesoplastic was determined to be 0.376 item/m^2 at seven stations. The highest level was determined in Mersin Bay at the mouth of the Seyhan river (Station no. 7, with 906 items), and the lowest level was found in Station no. 4 in Iskenderun Bay (78 items). Collignon et al. (2014) analyzed the annual variation in neustonic plastic particles and zooplankton in the Bay of Calvi (Corsica) between 30 August 2011 and 7 August 2012. Plastic particles were classified into three size classes, small microplastics (0.2–2 mm), large microplastics (2–5 mm) and mesoplastics (5–10 mm). 74% of the 38 samples contained plastic particles of varying composition: e.g. filaments, polystyrene, thin plastic films. An average concentration of 6.2 particles/100 m^2 was observed. The highest abundance values (69 particles/100 m^2) observed occurred during periods of low offshore wind conditions.

Nadal et al. (2016) reported, for the first time, the presence of microplastics (1 nm to <5 mm) in the gastrointestinal tracts of small semipelagic fish (Boops boops) in the Balearic Islands (Mediterranean Sea) from March to May 2014. The results show microplastic ingestion in 68% of full stomach samples with an average of 3.75 items per fish. Only filament type microplastics were observed in B. boops full gastrointestinal tracts. The frequency of occurrence of microplastics was high, with values ranging from 42% to 80%, in comparison to the other ingested items. Spatial variability among locations is high, which suggests that this type of contamination is ubiquitously distributed and originates from multiple sources.

Van der Hal et al. (2017) investigated the seasonal sea surface microplastic distribution at 17 sites along the Israeli Mediterranean coast. Microplastics (0.3–5 mm) were found in all samples, with a mean abundance of 7.68 ± 2.38 particles/m^3 or 1,518,340 particles/km^2. Some areas had higher abundances of microplastics than others, although differences were neither consistent nor statistically significant. In some cases microplastic particles were found floating in large patches. One of these patches contained an extraordinary number of plastic particles; 324 particles/m^3 or 64,812,600 particles/km^2. Microplastic abundances in Israeli coastal waters are disturbingly high; mean values were 1–2 orders of magnitude higher than abundances reported in other parts of the world. Light-colored (white or transparent) fragments were by far more abundant than all other microplastic colors and types.

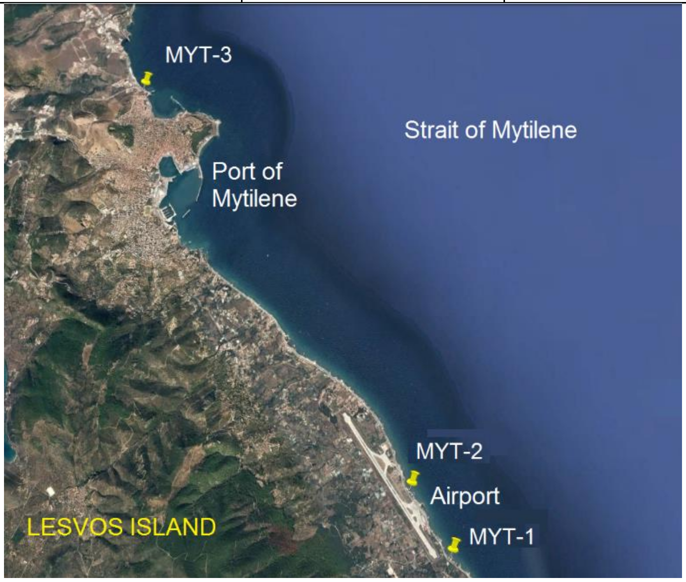
Alomar et al. (2016) reported that sediments from Marine Protected Areas (MPAs) contained the highest concentrations of microplastics (MPs): up to 0.90 ± 0.10 MPs/g suggesting the transfer of microplastics from source areas to endpoint areas. In addition, a high proportion of microplastic filaments were found close to populated areas whereas fragment type microplastics were more common in MPAs. There was no clear trend between sediment grain size and microplastic deposition in sediments, although microplastics were always present in two grain size fractions: 2 mm > x > 1 mm and 1 mm > x 0.5 mm.

Case study: Mytilene Strait, Lesvos, Greece

Two sampling campaigns per beach area (Table 1) located in the coastal area of Mytilene Strait were carried out: One at the end of November 2013 (MYT1 & MYT2, 30/11/2013) and mid-December 2013 (MYT3, 17/12/2013), and the second at the end of January (28 January 2014). Initially, the beach was scanned for any sources that could potentially contribute with litter (e.g. litter or recycle bins etc.). Sampling included manual collection of visible litter and storage in appropriate portable containers depending on the size. The collected litter was transported to the laboratory and put on a substrate for > 3 days to remove moisture in order to weight them. The mass was recorded (accuracy ± 0,02g), while exceptionally small similar items were grouped and weighed together. Litter was classified in groups according to its composition (plastic, paper/cardboard, cloth, glass, metal, organic matter, wood and other) (Silva et al., 2003).

Table 1. Location and sampling areas of the selected beaches in Mytilene Strait.

Code	**MYT1**	**MYT2**	**MYT3**
Coordinates (X, Y)	26.609°, 39.048°	26.602°, 39.057°	26.552°, 39.117°
Sampling area (m^2)	1,200	930	760



Map 1. Beach areas (MYT-1, MYT-2, MYT-3) of Mytilene Strait (Lesvos, Greece).

Results and discussion

The aim of the present study was initially to investigate the existence of information on micro- and nano- plastics in the area of the Mediterranean Sea. Therefore a literature review was performed. As a case study, a coastal area in Mytilene, Greece was selected and a first attempt of measurements of marine litter of various categories including micro- and nano- plastics was made.

According to the results of this preliminary investigation, the occurrence of these categories of marine litter was documented in our case study, whereas limited number of recent papers deal with it in the area of Mediterranean, a marine environment with special features that needs monitoring and protection. Further research on the subject is necessary in order to identify the actual risks. The necessity for method development is also highlighted.

Two sampling campaigns were carried out: in November, December 2013 and in January 2014. It should be noticed that the first sampling resulted to the beach cleaning, while the second one exhibited mostly the contribution of land based sources, and that of the marine environment. For both samplings abundance showed to be the higher for MYT3 (12.77g/m^2 και 4.34g/m^2), indicating the substantial contribution of the urban area. MYT2 abundance, showed to be substantially lower in January 2014, (0.57g/m^2) than in November (1.69g/m^2) which could be attributed mainly to recreational activities lasting till mid-October. For MYT1, abundance showed to be the same for both samplings, being close to 1.33g/m^2 which could be explained by the difference of items size (Table 2).

Table 2. Abundance and density of the marine litter groups on three beaches (MYT1, MYT2, MYT3) located in the coastal area of Mytilene Strait (Lesvos, Greece).

Abundance	**MYT1**				**MYT2**				**MYT3**			
	(30/11/2013)		**(28/01/2014)**		**(30/11/2013)**		**(28/01/2014)**		**(17/12/2013)**		**(28/01/2014)**	
Group	**Weight (g)**	**%**	**Weight (g)**	**%**	**Weight (g)**	**%**	**Weight (g)**	**%**	**Weight (g)**	**%**	**Weight (g)**	**%**
Plastic	1,172	73	449	28	953	61	202	39	4,256	44	825	25
Paper/cardboard	168	11	100	6	325	21	0.70	0	260	2.68	2.38	0.07
Metal	157	9.82	254	16	61	3.93	53	10	119	1.23	128	3.87
Organic matter	9.24	0.58	174	11	18	1.17	93	18	142	1.46	148	4.48
Cloth	12	0.74	60	3.73	8.81	0.56	--	--	19	0.20	1.72	0.05
Wood	30	1.88	314	20	38	2.41	--	--	774	7.96	1,083	33
Glass	12	0.73	9.43	0.59	11	0.69	5.50	1.05	410	4.21	21.68	0.66
Rubber	2.35	0.15	--	--	--	--	--	--	309	3.17	--	--
Other	34	2.12	238	15	149	10	169	32	3,439	35	1,098	33
Total	1,595		1,598		1,564		523		9,727		3,308	
Density (g/m^2)	1.33		1.33		1.69		0.57		12.8		4.34	

The litter on the beaches under investigation did not show any clear spatial difference being mostly spread all over each defined area. ‘Plastic’ was the dominant category followed by ‘Other’, while both ‘Paper’ and ‘Metal’ showed to be quite similar. In this study, abundance of plastics showed to vary from close to 25% to up to 73%, with the highest values being recorded during the first sampling. This is in accordance with other studies carried out in other coastal areas where abundance of plastic litter accounted more than 50% (Kordella et al. 2013). A total of thirteen plastic sub-groups were defined based on their use i.e. fishing lines and nets, plastic bottles (PET), sanitary objects, household, cosmetic and medical bottles, caps, bags, Styrofoam, etc., while a subgroup of plastic included objects which could not be defined due to their degradation and/or small size (<10mm). The highest relative abundance (%) was attributed to the plastic water bottles (PET) which ranged from 18% to 60% with the highest values being recorded during the second sampling (28/1/2014) which could be mostly attributed to land activities. The relative abundance of microplastics and other plastic, which could not be defined, was quite low, however concerns rise about their behavior and fate in the environment. According to the literature, small sized plastics can, under the current environmental conditions, be further degraded, while they can be a source of xenobiotics causing adverse effects to marine organisms (Cole et al., 2011). The litter abundance and composition throughout the study area seems to be influenced by recreational and fishing activities and environmental conditions i.e. winds while sea transport should not be neglected. This study as a first attempt showed the wide variety and the potential uses of litter found on beach areas of a Mediterranean island and the difficulties to appoint their sources. Furthermore, and to prevent potential impacts, among others, population must be informed and stimulated to participate in the maintenance and cleanliness of beach areas.

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