UNIVERSITY OF THE AEGEAN – SCHOOL OF THE ENVIRONMENT – DEPARTMENT OF MARINE SCIENCES

FLOATING MICROPLASTICS ABUNDANCE AND COMPOSITION OFF THE EASTERN COAST OF LESVOS ISLAND (NE AEGEAN) IN AUTUMN 2017

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BACHELOR'S THESIS

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BACHELOR'S THESIS

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THESIS TOPIC:

Floating microplastics abundance and composition off the eastern coast of Lesvos island (NE Aegean) in autumn 2017

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Abstract

Microplastic pollution is a major environmental issue nowadays. Despite the increasing microplastic awareness globally, over the last decade, it still threatens the marine environment. The Aegean Sea (Northeast Mediterranean Sea) where the present study refers to has not been previously examined thoroughly for macro- and microplastic abundance and composition. In this study, seven sampling transects were conducted at Mytilene Strait off the Eastern coast of Lesvos island, in which floating plastic debris were collected with a Manta net. Overall, thirteen samples were collected at three different coastal areas close to the urban area of Mytilene; namely offshore the Waste Water Treatment Plant, the Power Plant area and the Varia suburb where a small fishing port operates. The samples were analysed and the distribution, the size, the abundance and the shape of microplastic items was determined. Results revealed a high concentration of 75,218 items km^{-2} nearshore (<2km). Fragments were found in higher abundance (586 items) in all samples, followed by filaments (148 items), foams (60 items) and films (44 items), while pellets were present in only two samples. Sizes of small-sized particles were varying from <1mm, 1-5mm and 5-15mm. Polymer identification by ATR-FTIR spectroscopy revealed that dominant polymers were polyethylene and polypropylene and in some areas polyvinyl acetate.

Περίληψη

Η ρύπανση των μικροπλαστικών αποτελεί ένα μείζον περιβαλλοντικό πρόβλημα στις μέρες μας. Παρά την αυξανόμενη ευαισθητοποίηση παγκοσμίως, την τελευταία δεκαετία, σχετικά με το συγκεκριμένο ζήτημα το θαλάσσιο περιβάλλον εξακολουθεί να απειλείται. Το Αιγαίο πέλαγος (Βορειοανατολική Μεσόγειος) όπου και η παρούσα μελέτη έλαβε χώρα, δεν έχει προηγουμένως ερευνηθεί για τις συγκεντρώσεις και αφθονίες μακρο- και μικροπλαστικών. Στη συγκεκριμένη έρευνα, εφτά δειγματοληψίες πραγματοποιήθηκαν στο στενό της Μυτιλήνης, στην Ανατολική πλευρά της Λέσβου, κατά τις οποίες συλλέχθηκαν επιπλέοντα πλαστικά με τη χρήση διχτυού Manta. Συνολικά, δεκατρία δείγματα συλλέχθηκαν σε τρείς διαφορετικές παράκτιες περιοχές κοντά στην αστική περιοχή της Μυτιλήνης: στην υπεράκτια περιοχή της Μονάδας Επεξεργασίας Λυμάτων, του εργοστασίου παραγωγής ηλεκτρικής ενέργειας και το προαστίου της Βαριάς όπου και υπάρχει ένα μικρό αλιευτικό λιμάνι. Τα δείγματα αναλύθηκαν και προσδιορίστηκε η κατανομή, το μέγεθος, η αφθονία και το σχήμα των μικροπλαστικών. Τα αποτελέσματα φανερώνουν αυξημένη συγκέντρωση της τάξης των 75,218 items km^{-2} κοντά στην ακτή (<2km). Σε μεγαλύτερες αφθονίες βρέθηκαν τα θραύσματα πλαστικών (586 αντικείμενα) σε όλα τα δείγματα, ακολουθούν τα νημάτια (148 αντικείμενα), τα αφρώδη (60 αντικείμενα), τα φιλμ (44 αντικείμενα), ενώ βρέθηκαν συνολικά μόνο δύο σφαιρίδια. Τα μεγέθη των μικροπλαστικών που βρέθηκαν κυμαίνονται μεταξύ 1-5mm και 5-15mm. Η ταυτοποίηση των πολυμερών με τη χρήση φασματοσκοπίας ATR-FTIR αποκάλυψε ότι τα κυρίαρχα πολυμερή που ανιχνεύτηκαν ήταν το πολυαιθυλένιο και το πολυπροπυλένιο και σε ορισμένες περιοχές το οξικό πολυβινύλιο.

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1. Introduction

1.1. Overview

Plastic pollution is a global issue nowadays as plastic items are present everywhere in the land and the water. The annual plastic production worldwide has increased from 1.5 million tonnes in the 1950s to 322 million tonnes in 2015. Since the development of the first real synthetic plastic, Bakelite in 1907, numerous innovative manufacturing techniques have been optimised and had led to a massive production of lightweight and durable plastics for diverse applications (PlasticEurope, 2018). Due to their low cost and the versatility of their properties, plastics are used in large scale of products that benefit the global economy (Andrady & Neal, 2009).

Of particular concern nowadays are plastics that through irrational use and disposal, are entering the marine environment (Gregory, 2009). Records of marine plastic pollution date back to the early 1970s (Fowler, 1987; Carpenter and Smith, 1972; Colton and Knapp, 1974) but the following decades the topic has received increasing research interest (Andrady, 2011). Marine debris is characterized as any manufactured solid material, diserted, and abandoned in the coastal or marine environment (Coe and Rogers, 1997; Galgani et al., 2010). The durable nature of plastics results in debris persisting and accumulating in the environment for many years (Derraik, 2002; Lusher et al., 2013). The resistance to degradation in the marine environment and the buoyancy of debris enables them to travel for long distances before settlement which characterizes them as marine pollutants (Thompson, 2006).

The environmental impact that large plastic debris, known as “macroplastics" cause in the marine environment has been of increasing concern for a number of reasons such as entanglement, injury, ingestion and death of animals (Derraik, 2002; Lozano and Mouat, 2009). However, in recent years, there has been an increasing environmental interest about “microplastics”: smaller pieces of plastic debris, including those not visible to the naked eye, which derive from the breakdown of macroplastics or are being used as scrubbers in cosmetics and air-blasting (Derraik, 2002; Ryan et al., 2009; Thompson et al., 2004). Additionally, microplastics derive from photo-degradation caused by the ultraviolet (UV) rays of the sun, mechanical forces, waves and weather (Thompson et al., 2004; Cole et al., 2011). Due to their small size, microplastics are considered bioavailable to organisms throughout the food chain (Cole et al., 2011). The composition and density of microplastics determine their position in the water column and eventually their environmental effects (Browne et al, 2010b).

1.2. Plastic Production

Plastics are synthetic organic polymers deriving from natural, organic materials such as cellulose, natural gas, salt, coal and crude oil. Polymerisation and polycondensation are the two principle processes used to produce plastics (PlasticEurope, 2018). Virgin plastic polymers are not commonly used by themselves and normally the polymer resins are mixed with different additives to improve their quality in various applications (Meeker et al.,

2009). There are two main polymer groups, the thermoplastics and the thermosets which are differentiated based on their behavior in the presence of heat, having thus very different properties and applications.

Thermoplastics are soft and flexible at high temperatures but when they allowed to cool, they solidified again (Crawford, 1998). Examples of thermoplastics are polyethylene (PE), polyethylene tetraphthalate (PET), polyvinyl chloride (PVC), polystyrene (PS), polycarbonate (PC), polymethyl methacrylate (PMMA), polypropylene (PP), polyamides (nylon), cellulose acetate, etc. On the contrary, thermosets are characteristically quite rigid materials and they can't soften once they have been moulded. The highly cross-linked structure of thermosetting plastics is only fully formed during the manufacturing process (Mills, 1993). The most common thermosets are epoxide (EP), phenol-formaldehyde (PF), polyurethane (PUR), polytetrafluoroethylene (PTFE), unsaturated polyester resins (UP), etc. (PlasticEurope, 2018)

1.2.1. Common types of plastics & their properties

A) Polypropylene (PP)

The versatility of Polypropylene and its structure makes it the plastic with the higher converter demand (19.3%). Its properties are similar to polyethylene, but it is more heat resistant, stiff and slightly harder. The density of PP is between 895-920 kg/m^3 which is the lowest of all thermoplastics. Compared to polyethylene (PE) it has higher mechanical properties and thermal resistance, but less chemical resistance. It is used in a wide variety of applications as food packaging, microwave containers, pipes, sweet and snack wrappers, etc. (PlasticEurope, 2018; Figure 1).

B) Low Density Polyethylene (LDPE)

The second most widely used plastics (17.5%); Low Density Polyethylene is very tough and flexible with a density range of 917–930 kg/m^3. It can withstand temperatures of 80°C - 90 °C for a short time (Crawford, 1998). Its major application is in manufacturing various containers, in packaging, in reusable bags, agricultural films, trays, electrical cable coating, milk carton coating, etc. (PlasticEurope, 2018; Figure 1).

C) High Density Polyethylene (HDPE)

The density of High Density Polyethylene can range from 935-965 kg/m^3 and is more crystalline and has stronger tensile strength than LDPE. The temperature that withstands is 120°C for short time and can be used in numerous applications such as pipes, housewares, food containers, industrial wrapping and film, cables, etc. (Crawford, 1998; Figure 1).

D) Polyvinyl chloride (PVC)

Polyvinyl chloride is the world's third-most widely produced synthetic plastic polymer. It is available in two forms: rigid and flexible. Lightness, strength, durability and combination of properties that enables it to be used in many applications. Some of those applications are building products (windows, roof sheets etc.), pipes, packaging, cable insulations, medical products etc. (PlasticEurope, 2018; Figure 1).

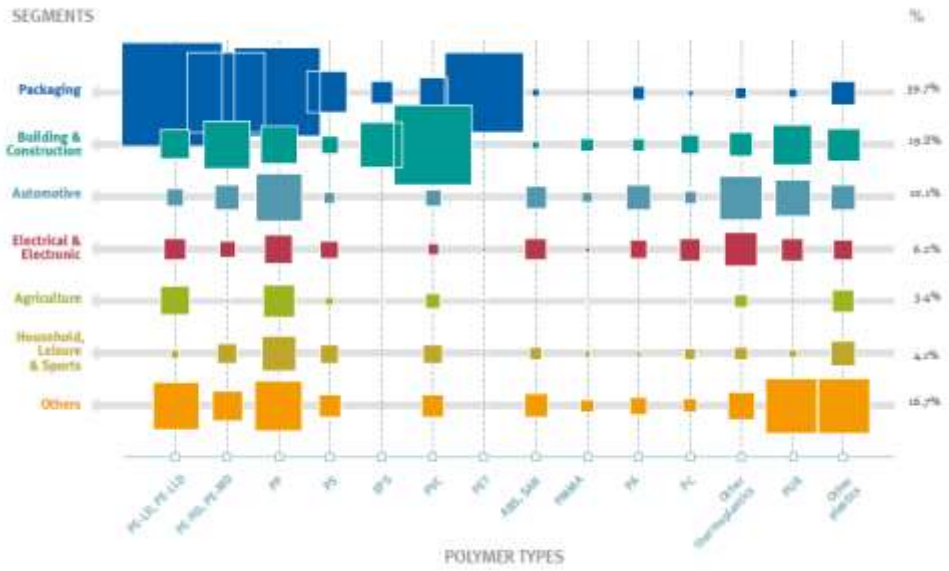

Figure 1. *European plastic converter demand by segments and polymer types in 2017 (*PlasticEurope, 2018).

E) Polyurethane (PUR)

Polyurethane is a flexible, rigid and durable manufactured material. It is characterised by high strength and good chemical and abrasion resistance. PUR can be used in a very wide range of products as high-resilience foam seating, rigid foam insulation panels, coatings, adhesives, furniture, etc. (PlasticEurope, 2018; Figure 1).

F) Polyethylene terephthalate (PET)

Polyethylene terephthalate is the most common thermoplastic polymer resin of the polyester family. It's strength, exhibit toughness, low friction and chemical resistance are some of its characteristics (Crawford, 1998). The applications of PET can be divided into three main categories: fibers, bottles and industrial use. The majority of the world's PET production is focused on bottle production accounting for about 30% of global demand. The two widely used applications are PET fabrics and PET bottles. Other functions where PET can be used are in synthetic fibers; food and other liquid containers; thermoforming applications; 3D printing filament, as well as in the 3D printing plastic PETG (Li-na, 2013; Figure 1).

G) Polystyrene (PS)

Polystyrene is a synthetic aromatic polymere made from monomer styrene. It can be rigid or foamed and generally is clear, hard and brittle. At room temperature polystyrene is in a solid state whereas it flows if heated above 100^{o}C. Major applications that PS can be used are in packaging, household appliances, consumer electronic products, food cartons, etc. (PlasticEurope, 2018; Figure 1).

1.3. Microplastic Pollution

Nowadays the problem of marine pollution due to microplastics has been expanded. Over the last four decades microplastics are accumulating in the oceans causing highly environmental problems (Thompson et al., 2004,

2005). Microplastics, smaller than 5mm, represent an increasing proportion of plastic debris, in oceans worldwide (Barnes et al., 2009). Numerous size-ranges of microplastics have been attributed, varying from study to study, with diameters of <10mm (Graham and Thompson, 2009), <5mm (Barnes et al., 2009; Betts, 2008; Fendall and Sewell, 2009; Moore, 2008), 2- 6mm (Derraik, 2002), <2mm (Ryan et al., 2009) and <1mm (Browne et al., 2007, 2010; Claessens et al., 2011).

Many researchers have used the term “microplastics” and “microlitter” differently to describe small plastic debris. Gregory and Andrady (2003) have defined “microlitter” as particles barely visible that can pass through a 500μm sieve but retained by a 67μm sieve and they have a diameter of approximately 0.06 to 0.5 mm, while particles larger than this size were called “mesolitters”. Larger particles as virgin resin pellets are commonly known as “mesoplastics”.

However, the most widely used definition is that microplastics are particles less than 5 mm in their longest dimension and this definition has been accepted by the National Oceanographic and Atmospheric Administration (NOAA) of the United States of America and the Marine Strategy Framework Directive (MSFD) of the European Union. Thus, in this study the definition that microplastics are particles consisting of a heterogeneous mixture of differently shaped materials in the range from 0.1 μm to 5,000 μm in their longest dimensions will be adopted (Lusher et al., 2017). Plastic particles with size ranging from 0.001 μm to 0.1 μm are defined as nanoplastics having the potential to be more harmful compared to MPs, as they are small enough to permeate membranes and are thus capable of entering cells (Klaine et al., 2012).

Plastic materials that have dimensions ranging from a few μm to 500μm (5mm) are commonly found in the aquatic environment (Ng and Obbard, 2006; Barnes et al., 2009). Those are mainly fragments, fibers, films, foams and spheres and due to the harmful effects that can cause on various aquatic organisms, they have attracted particular attention (Wright et al., 2013; Rochman et al., 2013; Brown et al., 2011; McCormick et al. 2014). Some microplastics, for instance microbeads, are typically polyethylene or polypropylene and are constructed to be of a microscopic size and are used in skin exfoliators and cosmetics and in air-blasting technology (Derraik, 2002; Gregory, 2009; Fendall and Sewell, 2009; Eriksen et al., 2013). Moreover, microplastics are derived from macroplastic fragmenting and disintegrating into smaller particles through the photo-degradation process caused by the ultraviolet rays of the sun, mechanical forces, and also local weather conditions (Derraik, 2002; Thompson et al., 2004; Cole et al., 2011). Those macroplastics are usually polyolefins (polyethylene and polypropylene) primarily used for single-use packaging (Browne et al., 2010b). Furthermore, another source of microplastic in the marine environment seems to be acrylic, polyester, and polyamide fibers from textile (Browne et al., 2011). The size and the density of plastics are various and their position in the water column and in long term their environmental effects including interaction with marine organisms, is mainly determined by the density of the polymer (Browne et al., 2010b; Table 1).

Polymers denser than seawater (> 1.027 g/cm^3, e.g. PVC) will sink, while those with lower density (e.g. PE and PP) will tend to float and can be detected on the ocean surface. Processes like biofouling and the colonization of organisms onto the plastic surface increase the weight of particles, thus accelerating their sinking to bottom sediments (Lobelle and Cunliffe, 2011); the weathering of polymers and the leaching of additives can also change the density of objects and their distribution within the water column.

Table 1. *Classes of plastics that are commonly encountered in the marine environment, -their specific gravity and their use (Andrady et al., 2011; GESAMP, 2015).*

Plastic Class	Specific Gravity	Products and typical origin
Low-density polyethylene; LDPE-LLDPE	0.91 - 0.93	Plastic bags, six-pack rings, bottles, netting, drinking straws
High-density polyethylene; HDPE	0.94	Milk and juice jugs
Polypropylene; PP	0.85 - 0.83	Rope, bottle caps, netting
Polystyrene; PS	1.05	Plastic utensils, food containers
Polystyrene (expanded)	0.01 - 1.05	Floats, bait boxes, foam cups
Nylon or Polyamide; PA	1.15	Netting and traps
Thermoplastic Polyester; PET	1.37	Plastic beverage bottles
Poly(vinyl chloride); PVC	1.38	Plastic film, bottles, cups
Cellulose Acetate; CA	1.22 - 1.24	Cigarette filters
Polyester resin, glass fibre	>1.35	Textiles, boats

Microplastics are also categorized as “primary” or “secondary” based on their origin (GESAMP, 2015). More specific, “primary microplastics” are deliberately manufactured as small-sized particles for industrial purposes and include preproduction nibs, resin pellets, air-/media-blasting particles and microspheres - a substance of many skin cleansers (Zitko and Hanlon, 1991), and blasting micro-sized powders for textile coatings (Gregory, 1996). In “secondary microplastics” are included fragmented particles deriving from any organic synthetic polymer products as microfibers from fabric and rope, coatings that have peeled off, debris from tire wear (Gregory, 1996) or are formed by the breakdown of macroplastics (Arthur et al., 2009).

Over time a combination of physical, chemical and biological processes can reduce the structural integrity of plastic debris, resulting in their fragmentation (Browne et al., 2007). Figure 2 presents a collection of the diverse anthropogenic primary and secondary microplastics that can be found in the marine environment (Esiukova, 2017). Many microplastics that are usually released in the aquatic environment, are by-products of human activity, for example in regions close to large boat breaking yards (Reddy et al., 2006; Srinivasa Reddy et al., 2003) or numerous times are been discarded from ships along with permitted wastes (Franeker et al., 2004).

Figure 2. *Different types of microplastics (Esiukova, 2017).*

1.4. Plastics degradation under marine conditions

Plastic items entering the marine environment from terrestrial and/or maritime sources experience dynamic changes of their properties. Degradation is a chemical change that reduces rapidly the average molecular weight of polymers and oxygen-rich functional groups are generated (Andrady, 2011) resulting to a decline of the main polymer properties. Alterations induced by degradation affect polymer composition, and modify the physical integrity of the plastic particles and their surface properties. When such changes occur after a long time in the natural environment, the term used is "natural" aging or "weathering" (Paul-Pont et al., 2018). During weathering, the plastic particles typically discolour, develop surface features, and consequently become weak and brittle over time. Any mechanical force (e.g. wind, wave, animal bite and human activity) can break the highly degraded, embrittled plastics into fragments (secondary plastics).

Degradation of plastic polymers can proceed by either abiotic or biotic pathways. Generally abiotic degradation precedes biodegradation, and is initiated thermally, hydrolytically, or by UV-light in the environment. Photo-oxidative degradation of plastics is a result of their exposure to sunlight. More specifically, UV radiation causes oxidation of the polymer matrix, although is much slower in the water than in the air (Andrady, 2011; Barnes et al., 2009; Browne et al., 2007; Moore, 2008; Rios et al., 2007). However, UV and heat stabilizers and antioxidants used as additives often markedly retard light-induced degradation of the plastic material. Thermooxidative degradation is caused by the slow oxidative breakdown at moderate temperatures, whereas high temperatures are responsible for thermal degradation of plastics but is not a degradation mechanism that takes place in environmentally relevant conditions. Finally, hydrolysis is the result of the polymer reaction with water; however, is not a usual mechanism in seawater (Andrady, 2011). Numerous factors are responsible for the rate of degradation (Figure 3). Some of them, apart from the physical, chemical and biological conditions to which the plastics are subjected, are: the properties of plastics as some polymers have weak bonds, the introduction of additives such as pro-oxidants which can increase the thermal degradation (Khabbaz et al., 1999) and the colour of plastics can also affect heat accumulation and eventually enhance thermal degradation (Massey, 2006).

Biodegradation is the action of living organisms usually microbes (Andrady, 2011), in which the polymer is being completely or partially converted to CO_2, water, methane, hydrogen, ammonia and other simple inorganic compounds, and new biomass by microorganisms. Thermosetting plastics, such as polyester, bakelite, and silicone, are more susceptible to biodegradation (Zheng et al., 2005) due to their hydrolysable ester bonds (Howard, 2002).

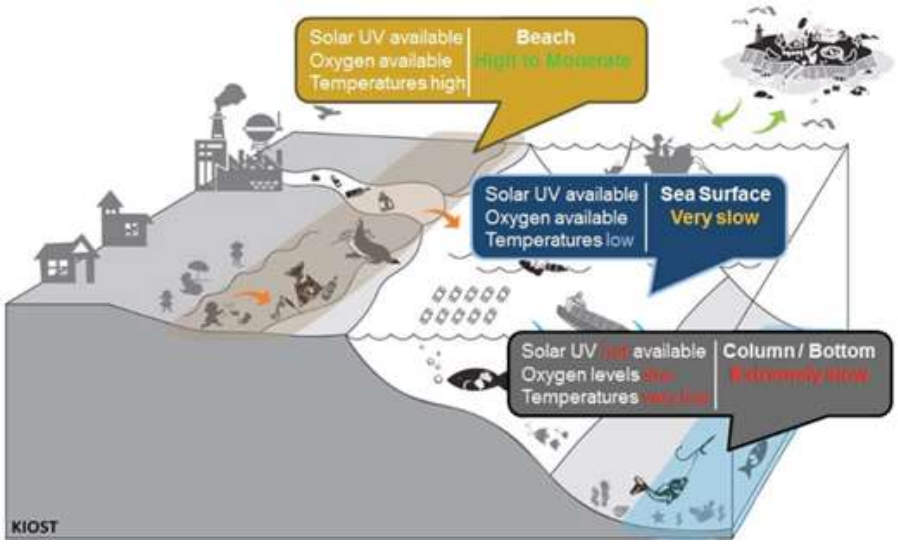

Figure 3. *Factors affecting the degradation and fragmentation of plastic in different ocean compartments (UNEP, 2016).*

Plastic floating on the ocean surface is exposed to moderate temperatures, solar radiation at wavelengths of 300 nm and longer and oxidizing conditions. Since temperatures are moderate, the most important factors initiating abiotic degradation are oxygen and sunlight (Figure 3), while weathering is accelerated by physical abrasion due to wave activity. Furthermore, some plastic polymers can be hydrolyzed (Gewert et al., 2015). The surface coverage of microplastics by a complex mixture of organic and inorganic molecules and the water itself might shield them from UV radiation, which would lead to a reduction of the rate of photo-initiated degradation. The state of the weathered plastic also plays a role, as smaller particles and plastics with a high surface cracking are more susceptible towards degradation. Once plastic becomes buried and enters the water column the rate of degradation becomes extremely slow. This is due to decreased UV exposure, lower temperature and lower oxygen levels. Objects such as PET bottles and fishing gear observed on the seafloor often do not appear to be degraded (UNEP, 2016).

1.5. Methods of sampling, detection and analysis

Microplastics and fibers are being found in huge concentrations in the marine environment. Although, the small size range of microplastics is a major challenge and problem in the analysis of environmental matrices. Visual observation or hardness tests that are usually being used, are not reliable in distinguish microplastics from other inorganic particles and organic fibers such as debris from coatings cellulose or paints. Inaccurate methods of

identification can lead to inaccurate results about the problem that microplastic debris cause to the marine environment. Thus, determining distribution characteristics and the occurrence of microplastics in a qualitative and quantitative manner is essential for further risk assessment of microplastics and microplastic-derived pollutants. However, no global consensus has been agreed upon the cut-off size and sampling methods used for investigating ‘‘microplastics’’ (Hidalgo-Ruz et al., 2012). Recently, dramatic discrepancies have been observed in the recorded abundance and composition pattern of microplastics caused by the different sampling methods used in analyzing seawater (Chae et al., 2014; Song et al., 2014). Therefore, it is considered that a standard sampling protocol is required to make an accurate representation of the distribution of microplastics (Kim et al., 2015). Some of the methods that are being followed for microplastic research are being further analysed (Huppertsberg et al., 2018).

1.5.1. Collection of floating microplastics

The collection of floating microplastics from surface water though is been conducted by towed net sampling techniques inherited from plankton research (Lenz et al., 2018). A variety of filtering techniques that are been used, includes on-site filtration via Manta or Neuston nets, Bongo nets, WP-2 nets, cascade filtration and other special techniques (Cincinelli et al., 2017; Enders et al., 2015; Figure 4). Moreover, in other studies filtration devices with encapsulated flow have been used for microplastic samplings (Enders et al., 2015; Desforges et al., 2014). Due to the clogging of the nets, there is a limitation on the use of nets; as smaller mesh size leads to faster clogging of nets and as a result filter cakes can formed rapidly, retaining particles smaller that the chosen mesh size.

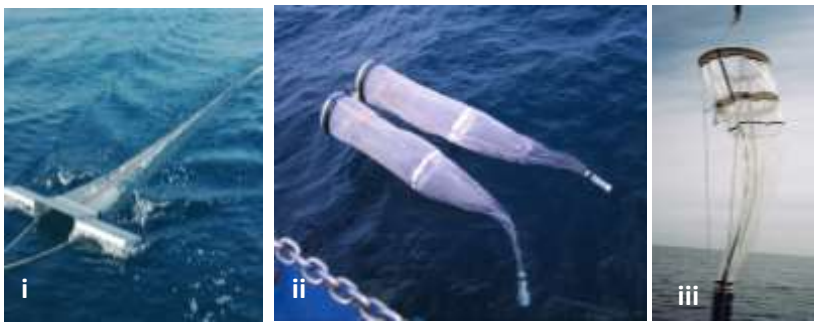

Figure 4. *Microplastic nets; (i) manta net (Photo: Myrsini Lymperaki); (ii) bongo net (Photo: California Current Ecosystem Long Term Ecological Research (CCE LTER) Teacher at Sea); (iii) WP-2 net (Photo: Department of Marine Ecology, IO PAS, Sopot, Poland).*

Cascade filtration is another filtering technique that enables the fragmentation of particles of various size classes and limits the clogging of the finely pored filters (Huppertsberg et al., 2018). However, in most of the studies, samples are collected with towed nets of mesh sizes between 300 and 390μm (Hidalgo et al., 2012) and often are exposed to airborne contamination while handling and transfer time on board. Also, the material of equipment that is used on samplings is another important factor while usually it may consist of a variety of plastic polymers, such as synthetic textiles like ropes or monophilament polyamide meshes are made of micro-fibrous polyethylene

terephthalate (PET). In addition, polyethylene (LDPE or HDPE), polypropylene (PP), or polyvinylchloride (PVC) are commonly used in cod-ends and connectors (Lenz et al., 2018).

1.5.2. Separation of microplastics

Matrix components are being separated from plastic particles by different sample preparations depending on the type of samples and the analytical approach. For sediment samples, density separation is widely used (Klein et al., 2015). In this method, multiple minerals with different densities are placed in an intermediate density liquid and grains with densities less than that of the liquid float while others sink (Figure 5). Density separation is often referred to as heavy liquids separation, because of the use of high density liquids. Typical mineral densities can be range from 2.2 g/ cm^3 to as much as 8 g/ cm^3. Although, different density separation solutions have been used by many researchers, resulting in different density cut-offs for the separation. Imhof et al. (2012) have developed the semi-automated Munich Microplastic Sediment Separator (MPSS), which enables an improved separation of microplastic particles from sediments (Figure 5).

Figure 5. *Microplastic separation methods; (i) density separation (Photo: Myrsini Lymperaki); (ii) MPSS separation (Photo: Imhof et al., 2012).*

Another separation method for microplastic samples is the Field Flow Fractionation (FFF), which is categorized in: additional flow (flow FFF), centrifugal forces (sedimentation FFF), temperature gradients (thermal FFF), and gravitation (gravitational FFF), depending on the nature of separation force.

Sedimentation FFF could be the most suitable for MPs because it is based on the different densities of MP and natural particles like in density separation. Sedimentation FFF is an alternative technique to density separation that does not require saturated salt solutions, but only an advanced instrumental setup. Both water and sediment samples contain natural organic matter (NOM) which is removed by oxidation or enzymatic digestion. In order to eliminate NOM strong oxidizing agents like a mixture of 3:1 H_2SO4 conc.:H_2O_2 and Fenton's reagent (H_2O_2 conc. + Fe^{2+}) are used. However, due to their high reactivity, side reactions with polymer substance may occur leading to the alteration of MP. Alternatively ozonation can be applied since ozone is less reactive to the polymers, but the

reaction times increases to several days. Enzymatic removal of the organic matrices is also possible (Huppertsberg et al., 2018).

In order to avoid any possible contamination from fibers and particles, sample preparation and analysis should be carried out in clean air devices instead of fume hoods or laboratory benches (Wesch et al., 2017). Moreover, blank samples are highly important when carry out MP analysis, to control the laboratory environment (Wiesheu et al., 2016).

1.5.3. Detection of microplastics

The detection of microplastics is been succeeding with a variety of methods. Raman microscopy is a method which enables collecting spectra from distinct points of an image generated by the microscope. With Raman, morphological and chemical properties of every microplastic particles can be determined as well as it enables the detection and location of (polymer) particles in organic tissues (Ivleva et al., 2017), which helps to understand the effects that microplastics have on organisms (Huppertsberg et al., 2018).

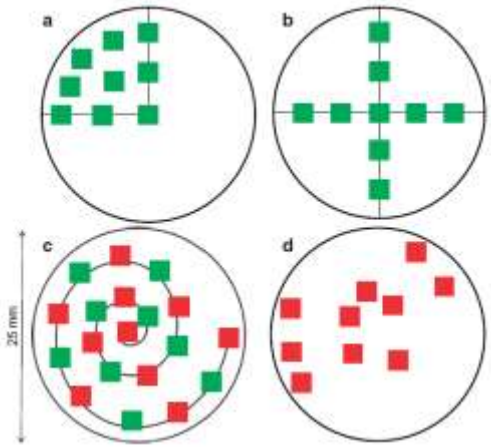

Figure 6. *Different approaches for representative measurement areas on 25-mm filters. The squares represent measurement areas of 2 × 2 mm. The green squares cover 7.3% of the entire filter area and the red squares represent 8.2% of the filter; the combined area is 15.5%. (a) Represents a quarter of the filter; (b) cross section of both axis; (c) helical assembly; (d) randomized or distribution-based assembly (Huppertsberg et al., 2018).*

Another method of microplastic detection is by Fourier Transform Infrared Spectroscopy (FT-IR) spectra. Attenuated total reflectance (ATR) is a special mode of reflectance measurements in which the sample is placed onto a diamond cell manually and compressed between two plates and an infrared beam is passing through the sample resulting in a better quality spectrum. However, this method is limited on the minimal size of particles determination (about 500μm) due to a minimal required contact area, as well as the airborne contamination of the sample and the crystal surface. Despite these limitations, the particular method is suitable for the identification of larger MP particles (mm range). Combing Raman and FT-IR methods single particles or entire filters can be analysed. Usually, depending on the number of detected and identified plastic particles on the sampled area, extrapolation of the total number of particles varies remarkably. In order to avoid false validation of microplastics, representative areas for measurement are required (Huppertsberg et al., 2018; Figure 6).

Focal plane array (FPA) detectors have a grid of detectors (e.g., 64 × 64) and also are capable of recording several thousand spectra simultaneously (Löder et al., 2015; Harisson et al., 2012). With this method more samples can be measured at the same time compared to single-particle FT-IR. Both Raman and FTIR are suitable non-destructive methods used for the detection of MP particles in environmental samples and also for the morphological characterization and the chemical composition of detected particles. However, both methods have similar limitations such as complex sample preparation and the presence of interferences caused by surface modifications of particles. Current databases lack modified or weathered polymer reference spectra, which need to be included to further study surface modifications, in order to understand the extent of error to MP identification as caused by spectral alterations. The detectable particle size is one major difference between Raman and FTIR microscopy. More specifically, Raman microscopy can detect particles with diameters of 1 µm, whereas detection via single-particle FTIR is limited to particle sizes of 10 µm. Raman spectroscopy shows interventions with fluorescent samples, but neither requires contact with samples as ATR-FTIR nor IR-transparent filters as FTIR in transmission mode (Huppertsberg et al., 2018; Figure 7).

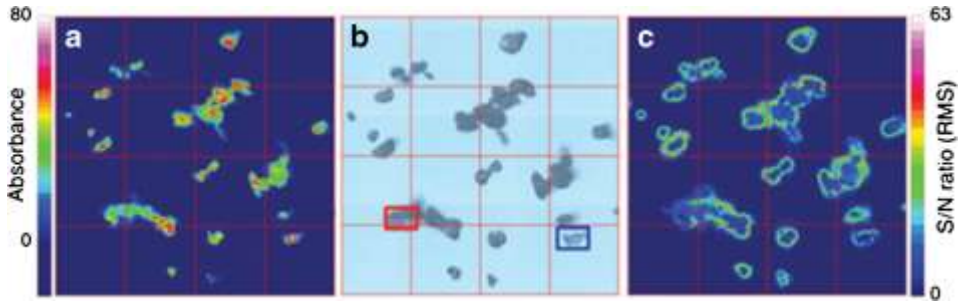

Figure 7. *Imaging of transmittance focal plane array detector of PE powder; (a) Imaging by absorbance of bands 2980–2780 cm^{-1}; (b) Visual picture of PE sample; (c) S/ N ratio of generated image (Huppertsberg et al., 2018).*

Thermo-analytical methods are also used in synthetic polymer analytics. Methodologies for the detection of MPs were adapted based on known thermo-analytical methods (thermogravimetry, differential scanning calorimetry, etc.). Promising results were obtained when thermogravimetry was coupled to differential scanning calorimetry (TGA-DSC) (Majewsky et al., 2016), pyrolysis gas chromatography-mass spectrometry (Py-GC-MS) (Fries et al., 2013) and thermal extraction desorption gas chromatography-mass spectrometry (TED-GC-MS) which is a modification thereof (Dümichen et al., 2017). TGA-DSC is based on changing heat capacities during the solid-liquid phase transition of a polymer, whereas Py-GC-MS and TED-GC-MS employ pyrolyzation to identify and quantify polymers (Huppertsberg et al., 2018).

1.6. Effects of microplastics in the marine environment

Microplastic debris can enter the marine environment from numerous transport pathways, including rivers, drainage or sewerage systems (Barnes et al., 2009), transport via the wind and currents, accidental loss of fishing gear and overboard disposal (Lattin et al., 2004). Heterogeneity in the distribution of microplastics may vary with local environmental and weather conditions (Cunningham and Wilson, 2003). Due to their composition and

buoyancy may be dispersed over vast distances transported by ocean currents, waves and winds. Except of the aesthetic problem that floating debris create, additionally constitute an environmental and economic issue which threatens the biodiversity of marine ecosystems (Sutherland et al., 2010; CBD, 2012). Negative effects between marine plastic debris and marine organisms have been reported for 663 different species (CBD, 2012). A growing number of studies illustrate that MPs are easily accessible to a broad range of marine biota because of their small sizes, posing potential risks to numerous marine species worldwide from subcellular to population levels (e.g., Galloway et al., 2017).

Microplastics have the potential to be ingested by a wide range of marine biota, independently of their feeding mechanism, and may enter their circulatory system and accumulate in different types of tissues, as has been proven in laboratory experiments (e.g., Browne et al., 2008) and studies based on specimens collected from the field (e.g. Digka et al., 2018). For the latter, microplastics have been found on mussel's gills and digestive glands, collected from a port and from a long line-type mussel culture farm (Digka et al., 2018). Furthermore, nanoparticles have been also found in hemocyte layers obtained from a fish market (Canesi et al., 2015). Ingestion has already been demonstrated for organisms at the base of the food chain. For instance, a large variety of planktonic organisms, such as copepods, euphausiacea and larval stages of molluscs, decapods, and echinoderms take up microplastics while feeding (Figure 8; Cole et al., 2013).

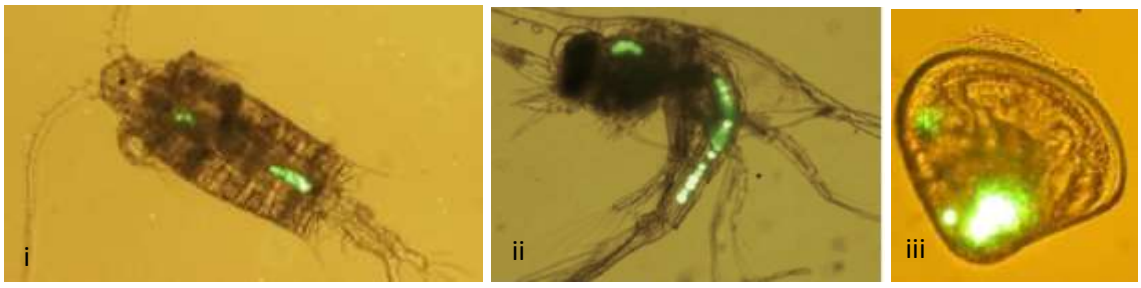

***Figure 8**. Microplastics of different sizes ingested by zooplankton, as visualized using fluorescence microscopy: (i) the copepod Centropages typicus containing 7.3 µm polystyrene (PS) beads (dorsal view); (ii) a D-stage bivalve larvae containing 7.3 µm PS beads (dorsal view); (iii) a Porcellanid (decapod) larvae, containing 30.6 µm PS beads (lateral view) (Cole et al., 2013).*

Other invertebrates, such as polychaetes, bivalves, echinoderms and decapods can also consume small-sized particles (Graham and Thompson, 2009; Murray and Cowie, 2011; Thompson et al., 2004). For higher trophic level organisms, microplastics can either be ingested directly or indirectly through the consumption of lower trophic level prey (Farrell and Nelson, 2013). Microplastics ingestion may result in a limited food uptake through the blockage and injury of the digestive tract of fish (Cannon et al., 2016; Nadal et al., 2016), which can lead to starvation or malnutrition (Gregory, 2009).

In addition to potential adverse effects from ingesting the microplastics themselves, toxic responses could also result from (a) inherent contaminants leaching from the microplastics, and (b) waterborne pollutants, adhered to the microplastics and again released with time.

Plastic additives, often termed ‘‘plasticisers’’, are incorporated into plastics during manufacture to change their properties or extend the life of the plastic. The additives are usually not covalently bonded to the polymer and therefore they can leach out from the plastic as it degrades and enter the marine environment introducing potentially hazardous chemicals to biota (Barnes et al., 2009; Lithner et al., 2011; Talsness et al., 2009). Due to the large surface-area-to-volume ratio of microplastics, marine biota may be directly exposed to leached additives after microplastics are ingested. Such additives and monomers may interfere with biologically important processes, potentially resulting in endocrine disruption, which in turn can impact upon mobility, reproduction and development, and carcinogenesis (Barnes et al., 2009; Lithner et al., 2009, 2011). Commonly used additives, including polybrominated diphenyl ethers, phthalates and the constituent monomer bisphenol A, are well recognized to be endocrine-disrupting chemicals as they can mimic, compete with or disrupt the synthesis of endogenous hormones (Talsness et al., 2009).

Persistent organic pollutants (POPs) that are present in the marine environment at low concentrations derive from meso-/microplastics via partitioning. Ingestion of these contaminated microplastics by marine species works as a pathway where POPs can enter the marine food web (Teuten et al., 2009). The potential bio-magnification of POPs in the food web (Teuten et al., 2007), as well as the extend of bioavailability to the biota (Moore, 2008) has not been studied in detail. Microplastics have also the ability to accumulate toxins on their surface and due to the ingestion by fish, birds and other marine animals, those toxins are passing through the food chain to human bodies (Esiukova et al., 2017 and references within). The absorbed pollutants such as levels and types of incorporated additives differ with the size, polymer type, and weathering extent of microplastics (Endo et al., 2005; Teuten et al., 2007). Besides POPs, microplastics are a transport vector of heavy metals as well as a source of chemical pollutants as phthalate plasticisers, polybrominated diphenyl ether flame retardants (PBDE) and bisphenols (Rochman, 2013).

1.7. Floating microplastics in the Mediterranean Sea

Although the accumulation zones of floating debris have been identified in the world’s ocean, recent numerical models found that the Mediterranean Sea has one of the highest concentrations of marine litter in the world (Lebreton et al., 2012). The Mediterranean Sea is a very large enclosed basin that is surrounded by developed countries, with 466 million inhabitants settled on its coasts (UNEP/MAP, 2012). The densely populated coastline, the intense navigation, the highly developed tourism, the important industrial and fishing activities along the Mediterranean coast and the lack of proper waste management in some coastal countries are the major causes of the high amounts of litter recorded. Furthermore, the structure of its circulation, as well as its being embedded in the global ocean conveyor belt, promotes retention of floating material inside the basin (Zambianchi et al., 2017).

The abundance of floating plastic debris in the Mediterranean was first reported in 1980 (Moris, 1980), using quantitative visual survey, in which around 1,300 plastic items/km^2 were reported in a central region of the basin. In recent years several studies using surface net tows have been performed in order to detect the levels and

properties of microplastics that are floating in the surface of the Mediterranean basin. This awareness is linked with the implementation of the Marine Strategy Framework Directive 2008/56/EC (MSFD). MSFD is a European Union directive committing EU Member States to take action aimed at achieving the "Good Environmental status" (GES) of the EU's marine environment by 2020, according to 11 key descriptors of environmental status. Among these, the Descriptor 10 focuses the attention on the emerging problem of marine litter and its effects on the marine environment and biota according to the Commission Decision on criteria and methodological standards 2010/477/EU. To this end several studies have been conducted aiming to provide background information on the occurrence of floating microplastics in European waters, as part of the implementation data of the Marine Strategy Framework Directive requested by the criterion 10.1.3 "Trends in the amount, distribution and, where possible, composition of micro-particles (in particular microplastics)". Although, the number of published articles on the topic has been steadily increasing in the last decade, shows a heterogeneous spatial distribution of the sampling efforts between the eastern and western Mediterranean basins, being much less intense in the eastern basin (Cincinelli et al., 2019; Figure 9).

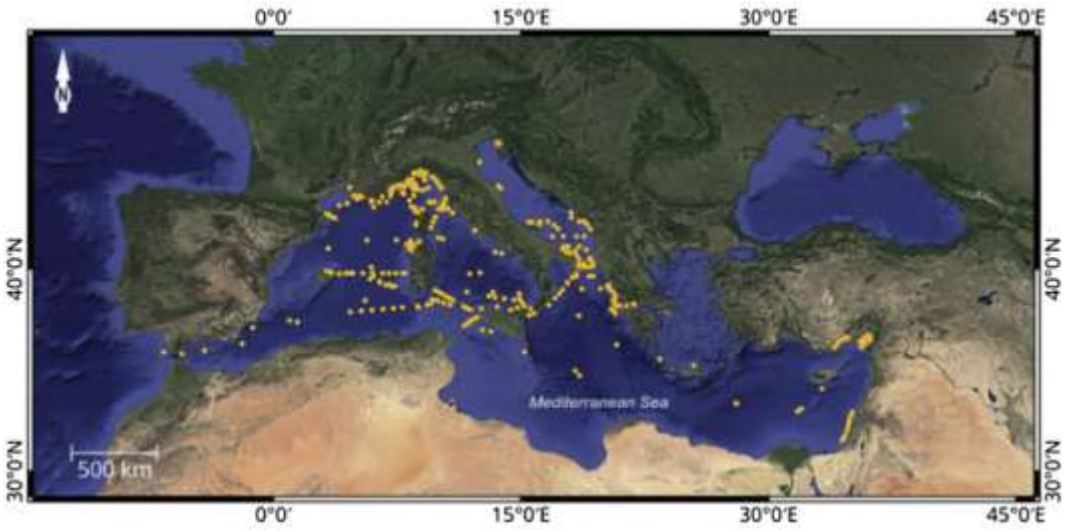

Figure 9. *Location of the stations where floating plastic debris have been collected in the Mediterranean Sea (Cincinelli et al., 2019).*

Observation of Figure 9 reveals that among the different sub-basins of the Eastern Mediterranean, the Adriatic and Ionian Seas are the more extensively studied areas due to the concerted activities in the frame of various projects of scientific groups from Albania, Bosnia and Herzegovina, Italy, Slovenia, Croatia, Montenegro and Greece (e.g., Gajšt et al., 2016; Palatinus et al., 2017; Zeri et al., 2018). Israeli and Turkish scientists have published data on the abundance and other characteristics of microplastic particles collected in surveys along the coast of the easternmost part of the Mediterranean basin (Gündoğdu, 2017; Gündoğdu and Cevik, 2017; van der Hal et al., 2017). To our knowledge there are no published data thus far on floating microplastics in the Aegean Sea except of a general sampling of floating marine litter performed in July 1997 in the southwestern Cretan Sea (Kornilios et al., 1998), a very general survey of floating marine debris along the Eastern Aegean (Topcu et al.,

2010) and one station sampled for floating plastic debris during a cruise across the Mediterranean basin in May 2013 (Cózar et al., 2015).

However, a circulation model linked with a particle-tracking model to simulate the transport of floating litter particles in the Aegean Sea, has shown a tendency of the Aegean Sea to act as a source rather than receptor of floating litter pollution in the Eastern Mediterranean Sea (Politikos et al., 2017). Furthermore, the same modelling approach depicted major tendency of floating litter particles to accumulate over the North Aegean Sea plateau, in the Saronikos Gulf and along the Evia and Crete islands, while the eastern part of the Aegean was the least affected. The use of models provides information on the pathways of floating litter transport and distribution, however the Aegean Sea is an archipelago with complicated coastline and numerous islands, so more in situ data are needed to assess the status of floating litter pollution and to verify the modelling results. Sometimes specific local conditions regarding the sources of marine debris and/or the regional small scale circulation may obscure the results of the simulation studies.

The current study is aiming to contribute to fill this knowledge gap and provide for the first time crucial information about the distribution, abundance, sizes and composition of floating microplastics in the Northeastern Aegean Sea.

2. Methodology

2.1. Study Area

The Strait of Mytilene is the marine region between the island of Lesvos and Asia Minor at the Northeast Aegean Sea. It is a relatively narrow strait having a width that ranges between 2.5 to 8 nautical miles (nm), its length is ~30 nm, and the maximum depth is about 50-60 m. The city of Mytilene is located at the east coast of Lesvos Island; the city and its suburbs have a population of about 38,000 (ELSTAT, 2011) that increases during summer due to tourism. Since 2015 about 10000 refugees and migrants are hosted in two major camps in the vicinity of the city. At the northern edge of the city, the Waste Water Treatment Plant operates and the treated effluents are discharged through a 450m long submersible pipe at 15m depth at Mytilene Strait. The southern (and more recent) part of the town is not connected to the central sewerage network, has sinks and sewers built of stone or plastic and the domestic effluents are partially discharged untreated to the sea through outfalls and ephemeral streams along the coastline.

The port of Mytilene is relatively small and is regularly connected by ferries with the ports of Piraeus, Kavala and the nearby islands of Lemnos and, Chios. In addition, small ferries are crossing the Mytilene Strait connecting Lesvos island with the ports of Ayvalik and Dikili in Turkey. Furthermore, several vessels of various types (cargo, tanker etc.) and capacities are travelling along the Mytilene Strait in their route to or from the Dardanelles and the Maramara and Black Sea ports. Several point sources of MPs are spread along the coastline of Mytilene Strait and include touristic and recreational activities as well as two small fishing ports one at Varia area and the other one close to the Waste Water Treatment Plant area.

2.2. Samplings of microplastics on the sea surface

Floating plastic debris were sampled at Mytilene's strait, in Lesvos island, from October 6th to November 6th 2017 (Figure 10). Seven samplings were carried out at three different sites along the eastern coastline of Lesvos island, influenced by different types of land-based activities, namely the Waste Water Treatment Plant (WWTP) where only one sampling was performed, the Power Plant and the suburb of Varia where a small fishing port operates. In each one of the last two locations three consecutive transects were performed with distances to land ranging from 0.2 to 1.8 km (Table 1). The samplings were conducted with the research boat "Amphitriti" of the University of the Aegean.

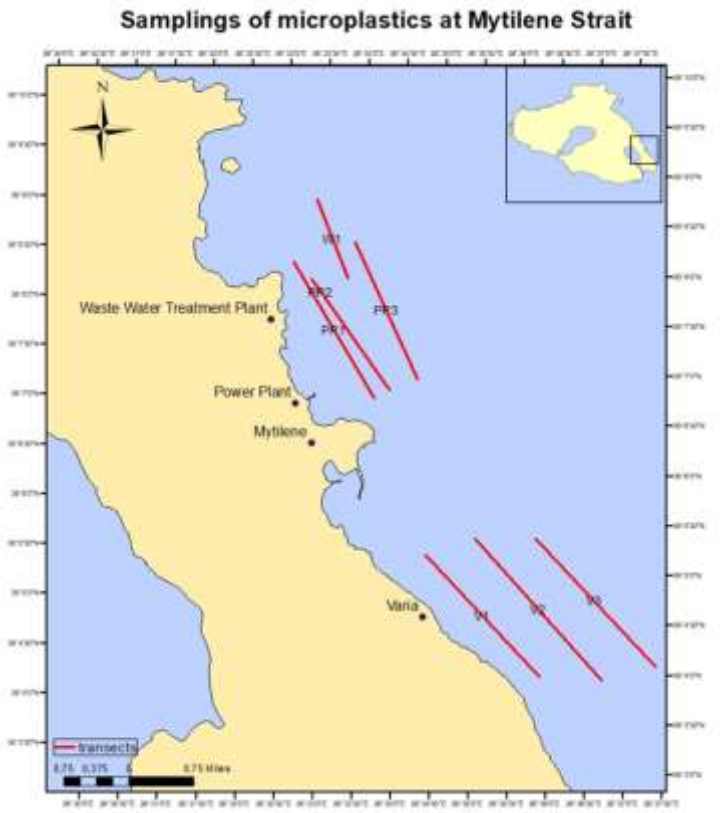

Figure 10. *Map of manta net sampling transects for microplastics collection at Mytilene Strait (Lesvos island).*

The methodology that was followed was based on the protocol developed in the frame of the DeFishGear project (http://www.defishgear.net/; Palatinus et al., 2015; Kovač Viršek et al., 2016; Figure 12). Samples were collected using Manta net with 335 μm mesh size with a rectangular net opening of 60 x 20 cm (Figure 11). The net was towed, at a distance of ~4 m from the boat, at the top ~10 cm of the sea surface with an average speed of 2.5 knots during c.a. 30 min. Sea conditions during all samplings were calm, there were small swell waves, while the average wind speed was ~3.5 knots in order to minimize the effect of mixing floating plastic debris from the wind.

Figure 11. *Manta net.*

The covered area of each tow was approximately 1600 m^2 and the average filtered volume was ~190 m^3. The filtered seawater volume was roughly estimated by multiplying the rectangular net opening (1200 cm^2) with the

length of each transect. The length of the route and GPS coordinates were written down after each tow into a data sheet. During the trawls a steady linear course was maintained at a constant speed (Table 2). More details are presented in Annex (Table I).

Table 2. *Data sheet with coordinates and the distance from the coast of each sampling.*

Location	Transect		Coordinates of samplings				Distance from the coast (m)
		Date (mm/dd/yy)	Start Lat	Start Long	End Lat	End Long	
WWTP	W1	6/10/2017	39° 8'52.28"N	26°33'16.64"E	39° 8'4.52"N	26°33'38.50"E	737
Varia	V1	10/10/2017	39° 5'24.60"N	26°35'57.60"E	39° 4'10.36"N	26°36'35.35"E	356
	V2	10/10/2017	39° 5'16.61"N	26°34'32.35"E	39° 4'1.22"N	26°35'57.30"E	939
	V3	10/10/2017	39° 5'25.55"N	26°35'10.82"E	39° 4'2.39"N	26°36'16.62"E	1,619
Power Plant	PP1	6/11/2017	39° 6'51.77"N	26°33'56.23"E	39° 8'15.51"N	26°32'56.91"E	889
	PP2	6/11/2017	39° 8'4.99"N	26°33'10.68"E	39° 6'54.24"N	26°34'2.95"E	987
	PP3	6/11/2017	39° 8'26.23"N	26°33'44.86"E	39° 7'2.61"N	26°34'30.31"E	1,649

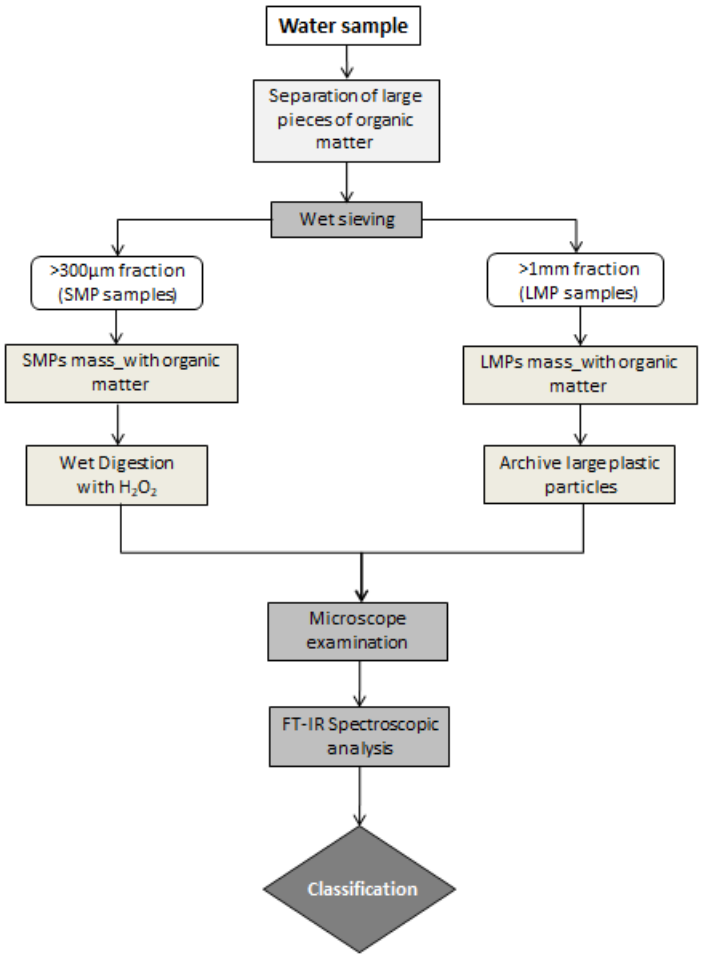

Figure 12. *Flow diagram for the analysis of floating microplastics on sea surface samples.*

2.3. Transfer of collected items & Wet sieving

At the end of each tow, on the boat, the manta net was rinsed meticulously from the outside with seawater using submersible pump, in order to collect all the particles and the organic matter that were stuck on the sides of the net. The direction of rinsed water was from the manta mouth to the cod end, so all particles were concentrated into the cod end. The cod end was safely removed and the sample was sieved using stacked stainless steel sieves. This step was been repeated until there were no longer any particles inside the cod end. Sieves used were of 1 mm and 300 μm mesh size so samples were separated in large plastic particles (>1mm) including LMPs (1-5 mm) and larger plastic objects (macro- and meso-plastics) and in small microplastic particles-SMPs (1 mm-300 μm). Large pieces of organic matter as seagrasses of *Posidonia oceanica* retained at the sieve of 1mm were separated from samples into petri dishes, using tweezers (Figure 13, 14; Figure 36); afterwards they were carefully rinsed with deionized water in order to acquire any microplastic particles attached on their surface.

Figure 13. *Large Microplastic particles-LMPs (1-5 mm) and larger plastic objects (macro- and meso-plastics) from different samplings in stainless steel sieves.*

Figure 14. *Small Microplastic samples-SMPs (300μm-1mm) from different samplings in stainless steel sieves.*

After sieving, each sample was collected into glass jars using stainless steel spatula and/or tweezers. Then jars were covered with aluminium foil and labelled from the outside. From each sampling two different jars were obtained, the first of >300μm mesh size and the second >1mm. Plastic tools, containers and synthetic clothes were been avoided during samplings. A total of 14 samples were preserved into the refrigerator, until they were analysed in the laboratory (Figure 15).

Figure 15. *Freezed samples were preserved in the refrigerator until their laboratory analysis.*

2.4. Lab analysis

2.4.1. Determination of LMPs mass

In the lab, samples were transferred into glass beakers covered with aluminium foil and dried at the oven at 70-90 ^{o}C for more than 24 hours (Figure 16). The dry samples were transferred in pre-weighed petri-dishes in order to determine the total mass of samples, in which organic matter and plastics were included. Large plastic litter objects of a size >5 mm (macro- and meso-litter) were carefully extracted from LMP samples with a tweezer and were rinsed with deionized water to obtain any microplastic particles possibly attached on their surface.

Figure 16. *Sieved samples are dried in the oven to determine dry weight.*

After plastics were dried at room temperature they were placed into empty pre-weighed petri-dishes in order to determine their weight. Plastic items were counted and examined visually using a stereo dissecting microscope (OLYMPUS SZX10) and transferred with a tweezer into another pre-weighed petri-dish, in order to determine the mass of Large Microplastic Particles (LMPs mass) (Figure 17). Microplastics were categorized into five type categories: fragments, thin films, filaments, foam and industrial pellets.

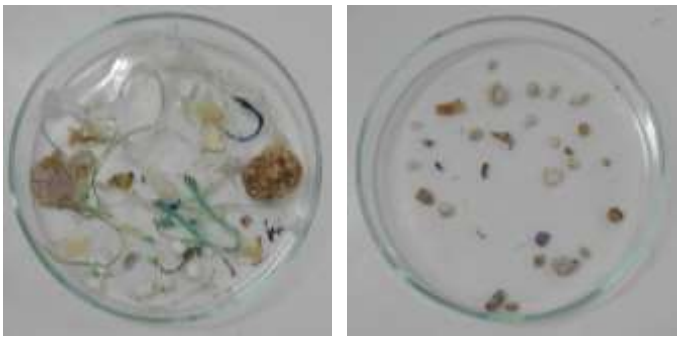

Figure 17. *Large plastic litter objects (>5mm) were extracted from LMP samples to determine the LMPs mass.*

2.4.2. Wet Digestion with H_2O_2

Regarding the determination of SMP samples, after determining the total mass with organic matter, samples were digested with 40 ml of 30% hydrogen peroxide (H_2O_2) per 3 g of dry sample and heated at a hotplate at 60-70 ^{o}C until the digestion was completed. Then samples were filtered into pre-weighted filters (GF/C; 1,2μm particle retention) using plenty of deionized water. After the filtration all filters were dried in the oven at 60^{o}C and weighted again, in order to obtain the mass of Small Microplastic Particles (SMPs mass) (Figure 18).

Figure 18. *Wet digestion of samples with H_2O_2 on a hotplate (left), dried sample after the filtration (right).*

2.4.3. Microscope examination

Microplastics were counted, measured and categorized into five types (fragments, filaments, films, foam and pellets), by imaging analysis using a stereo dissecting microscope (OLYMPUS SZX10). Plastic particles from each filtered sample were carefully transferred to a new clean and pre-weighted filter. The length and the area of each plastic particle were calculated under the stereomicroscope by using imaging analysis (x40 or x80 magnification). All plastic particles from SMP (>300μm) and LMP (>1mm) samples were counted and classified (Figure 19). At the laboratory, three “blank samples” with pre-weighted filters were placed next to the bench during all stages of the analyses and cross examined, in order to identify any possible contamination. Any fibers suspected of being of a textile origin were excluded from the analysis because they could be airborne contamination from clothing during the sampling or processing. During the microscopic analysis of samples the lab conditions were clean.

Figure 19. *Large Microplastic sample- LMPs (left) and Small Microplastics sample-SMPs (right) analysing in stereo dissecting microscope.*

2.5. Spectroscopic analysis

A total of 837 microplastic items retained on the filters from both the LMP and SMP samples, were counted and then were examined by Fourier Transform Infrared Spectroscopy (FTIR) using FTIR spectrometer (Figure 20). The FTIR spectroscopic analysis was performed at the premises of the Institute of Oceanography of HCMR in Anavyssos. In ATR-FTIR, infrared radiation is passed through the sample for the generation of the absorbance or transmittance spectrum. ATR- FTIR is preferred because it can measure the whole wavelength range at once. The spectrum that is created when infrared frequency identical to the vibrational frequency of a bond is absorbed, it is known as the molecular "fingerprint" of the sample (Kalogerakis et al., 2017). Because each different material is a unique combination of atoms, no two compounds produce exact the same infrared spectrum. Therefore, infrared spectroscopy can result in a positive identification (qualitative analysis) of every different kind of material. The shape, the position, and the intensity of peaks in this spectrum reveal details about the molecular structure of the sample.

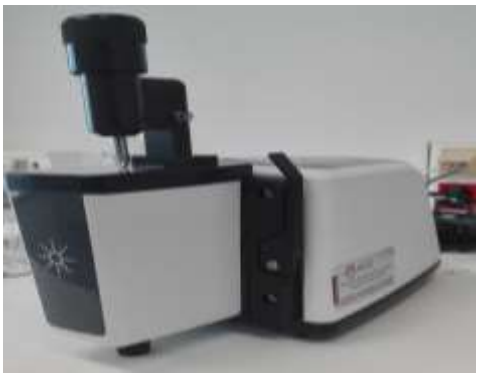

Figure 20. *Fourier Transform Infrared (FTIR) spectrometer.*

All spectra were recorded in the spectral range of 4000–600cm^{-1}. Each plastic item was placed with a tweezer onto the diamond cell and compressed between two plates into a thin uniform thickness enough to allow for adequate transmission of infrared beam through the sample to the detector and resulting in a better quality spectrum. The spectral resolution was set at 4.0 cm^{-1} with a step of 2.0 cm^{-1} every 20 ns and the measured signal was digitized and sent to the computer where the Fourier transformation was taken place. The final infrared spectrum was then presented and saved in a dataset (Figure 21).

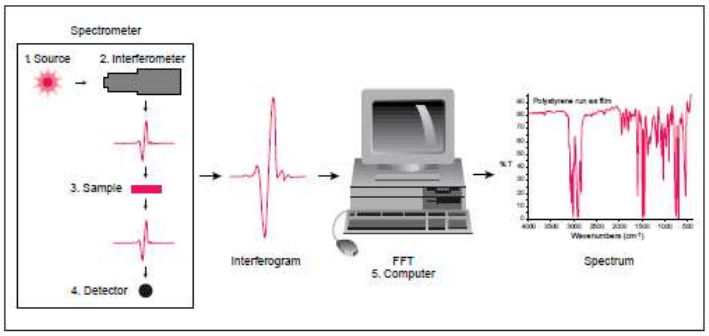

Figure 21. *Steps of FTIR analysis* (Thermo Nicolet, 2001).

FTIR analysis was carried out on an Agilent Cary 630 FT-IR spectrometer and the obtained spectra of all the plastic items was compared with spectra of known plastic polymers to identify styrenics (homopolystyrene (PS), PS-based copolymers or PS foams) and polymers belonging to the family of the polyolefins (polyethylene (PE), propylene (PP) and polyethylene vinyl acetate (PVA)) (Figure 22).

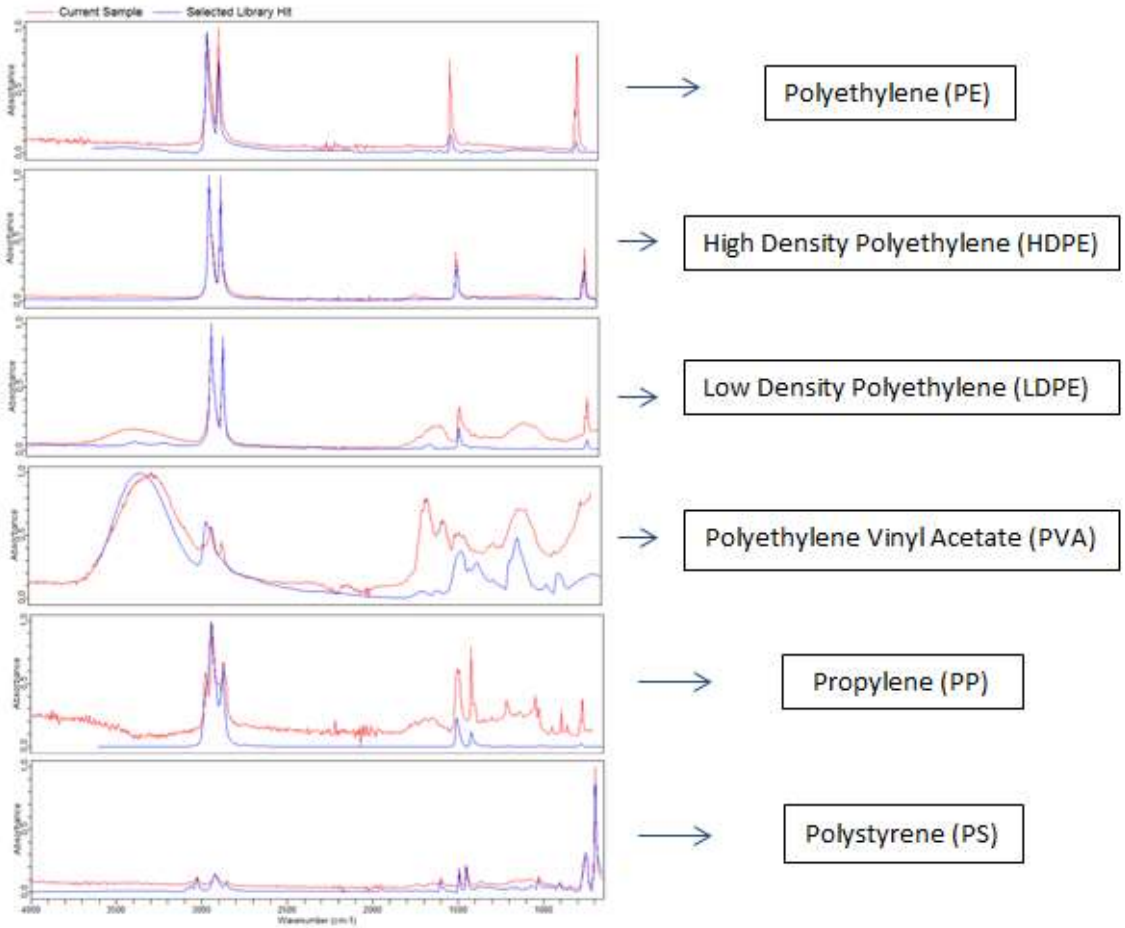

Figure 22. *FTIR spectra of samples (red) compared with spectra of known plastics (blue).*

3. Results

In the present study, 7 samplings were conducted at Mytilene Strait during October-November of 2017 and 13 samples were collected using Manta net with 335 μm mesh size, from three different locations: Waste Water Treatment Plant area (W1), Varia area (V1, V2, V3) and Power Plant area (PP1, PP2, PP3) (Figure 43; Annex). A total of 837 floating microplastic items were collected and examined in the laboratory and the total mass with organic matter was determined. After digestion with 30% H_2O_2, the total mass without organic matter was calculated. Then plastic items were counted, the length and the area of each one were measured and categorized into five types (fragments, filaments, films, foam and pellets), by imaging analysis using stereomicroscope (Figure 46; Annex). The abundance of microplastics (items/m^2) was calculated. Then, microplastic items were analysed by Fourier Transform Infrared Spectroscopy in order to identify their polymeric composition. The presence of airborne fibres on samples similar to those on "blank" samples was much less than <10% of the average values determined on the samples themselves and was not taking into account (TSG-ML, 2013).

The length of each transect was ranged between ~1.5 to ~3.1 km while the average distance from the coast was ~1.3 km. The highest sampled surface was at Varia area with an average of 1.7 m^2 in which the total sampled mass with and without the organic matter was the highest of all being 42.21 g and 0.1556 g, respectively. Following the Power Plant area had the second highest mass of plastic debris (0.0322 g), while Waste Water Treatment Plant had the lowest concentration on plastics (0.0027 g) (Table 3). The box plot on Figure 23 is presenting the total number of microplastic items found in the three different areas. It is quite obvious that the larger number of items was recorded at Varia as the 75% of the data lies above the median value.

Table 3. *Length and distance from the coast of each transect and total sample mass with and without organic matter in the total area.*

Location	Sampling	Length of the transect (m)	Distance from the coast (m)	Sampled area (m^2)	Total sample mass_with organic matter (g)	Total sample mass_without organic matter (g) (only plastics)
WWTP	W1	1,580.28	737	948.17	14.04	0.0027
Varia	V1	3,107	356	1,864.20	42.21	0.1556
	V2	3031.2	939	1,818.72		
	V3	2,518.50	1,619	1,511.10		
Power Plant	PP1	2,944.91	889	1,766.95	40.33	0.0322
	PP2	2,526.89	987	1,516.13		
	PP3	2,837.17	1,649	1,702.30		

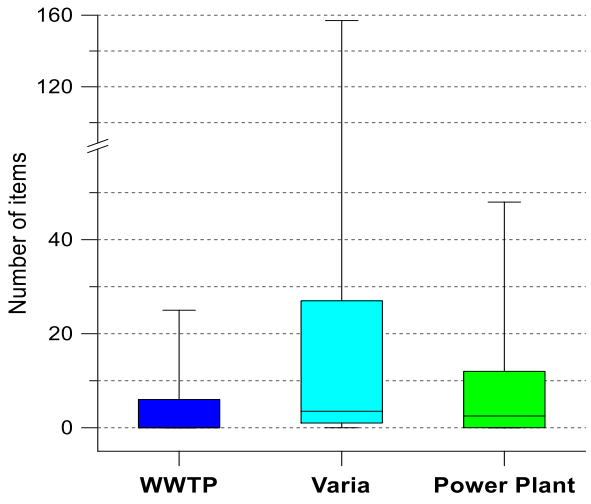

Figure 23. *Box-Whisker plot of the number of items found in the three different areas. The boundaries of the boxes indicate the 25th and 75th percentiles, the whiskers above and below the boxes the 95th and 5th percentiles. The horizontal line denotes the median value.*

3.1. Number of items and abundance of SMPs and LMPs in different transects

3.1.1. Number of items

The number of plastic items varies between different transects. The following Figures are presenting the number of Small Microplastic particles (SMPs) and Large Microplastic particles (LMPs) that were found at the different areas. For the transect in Waste Water Treatment Plant area the number of fragments was higher (76 items) in SMP samples, whereas in LMP samples only 2 items were found. Regarding filaments and films, they were found at the same quantity (8 items) in SMP samples, while there was only a small amount of films (3 items) in LMP samples. One item of pellet was found only in samples having size >1 mm (Figure 24).

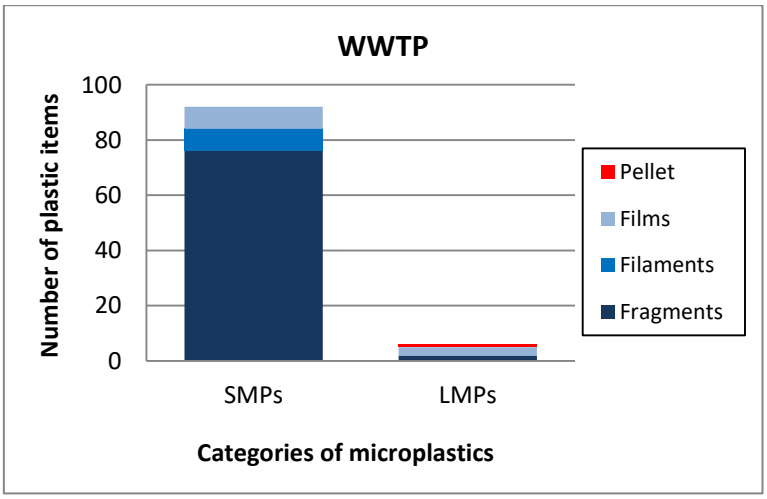

Figure 24. *Number of microplastic items at WWTP transect.*

At the area of Varia the total number of fragments was higher in SMPs with 360 items, than in LMPs (40 items). The number of filaments was bigger in SMPs (99 items) as well as the number of films (15 items) and foams (31 items) than in LMPs. One item of pellet was found again in LMPs (Figure 25_left). At the Power Plant area, the number of fragments was higher in SMP samples (105 items), as well as the number of filaments (22 items) (Figure 25_right).

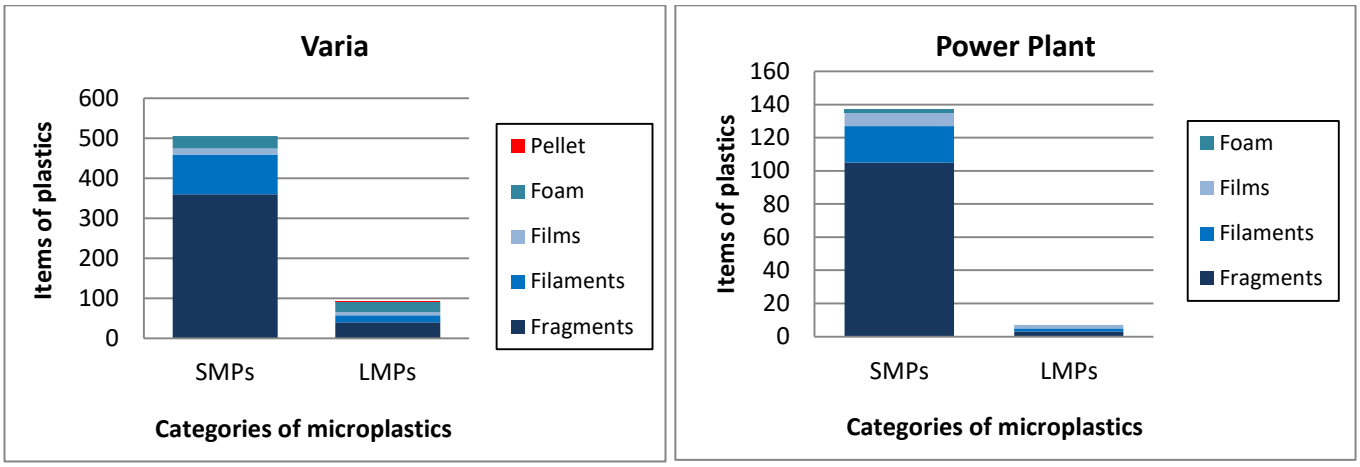

Figure 25. *Number of microplastic items at Varia (left) and Power Plant areas (right).*

Overall, Varia area had the highest numbers of both small and large microplastics (SMP, LMP) in comparison with the other two areas. As far as LMPs concerns, fragments were the most numerous of all microplastics (40 items), followed by foams (27 items), filaments (17 items) and films (8 items). The only items of pellets were found at Waste Water Treatment Plant and Varia transects. For SMPs Power Plant transects was characterised by higher number of microplastics, especially fragments, than offshore the Waste Water Treatment Plant (Figure 26_left). Comparing the LMPs and SMPs for Varia area is obvious that the amount of small microplastic debris was about five times higher than the amount of large particles (Figure 26_right). Detailed information about the number of items is presented at Table II (Annex).

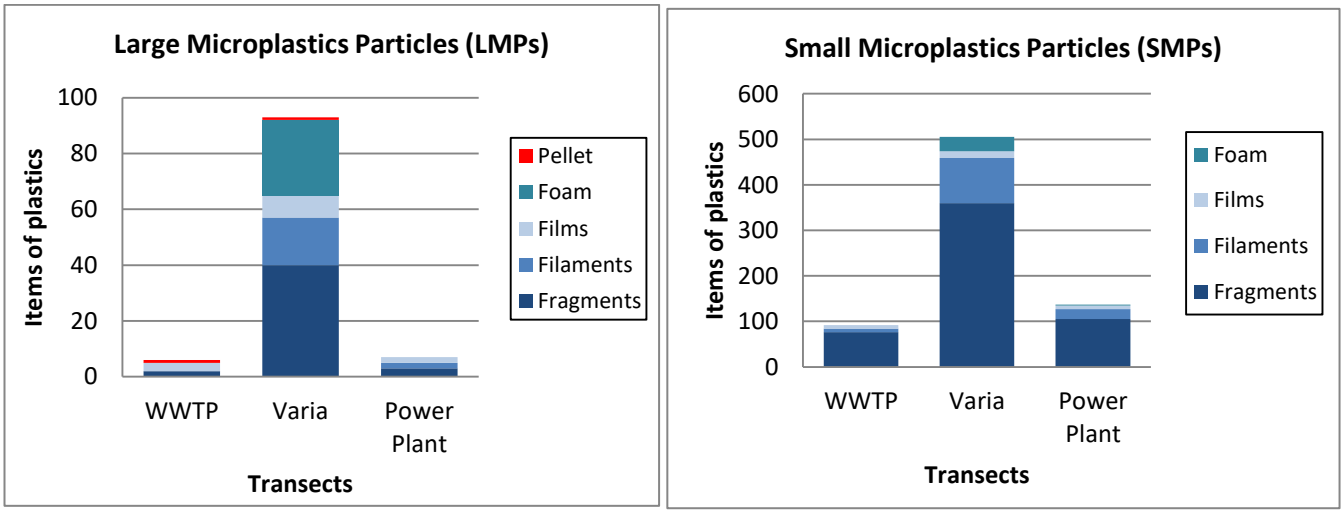

Figure 26. *Comparison between the number of microplastic items of different sizes (LMPs and SMPs) in the three areas.*

3.1.2. Abundance

The abundance of microplastic debris showed similar pattern to the number of microplastic items. At the Waste Water Treatment Plant area, SMPs were in higher abundance than LMPs with fragments being found 0.080 items/m^2 (Figure 27_left). The abundance of microplastics at Varia area was higher in SMPs with fragments being found at larger amount (0.208 items/m^2), followed by filaments (0.064 items/m^2), whereas in LMPs fragment's abundance was only 0.022 items/m^2. Foamed particles' abundance was the same on both types of microplastics (0.017 items/m^2). Films and pellets were present in negligible abundance (Figure 27_right).

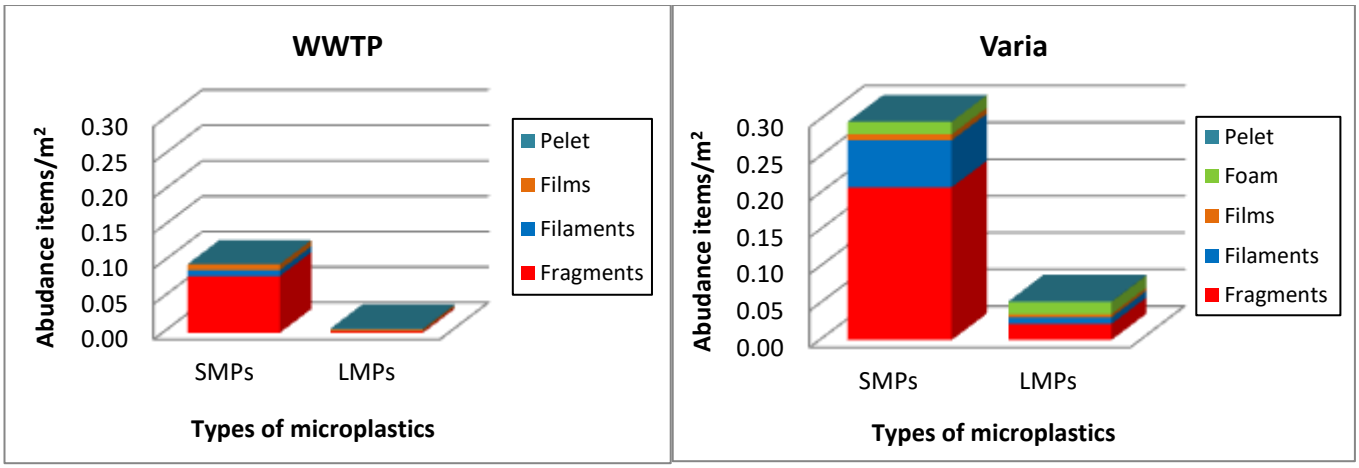

Figure 27. *Abundance of microplastics at Waste Water Treatment Plant and Varia areas.*

At Power Plant transects the abundance of fragments in SMPs was found 0.064 items/m^2, while in LMPs the fragment's abundance was very low (0.002 items/m^2). Moreover, filament's abundance was 0.013 items/m^2 (Figure 28).

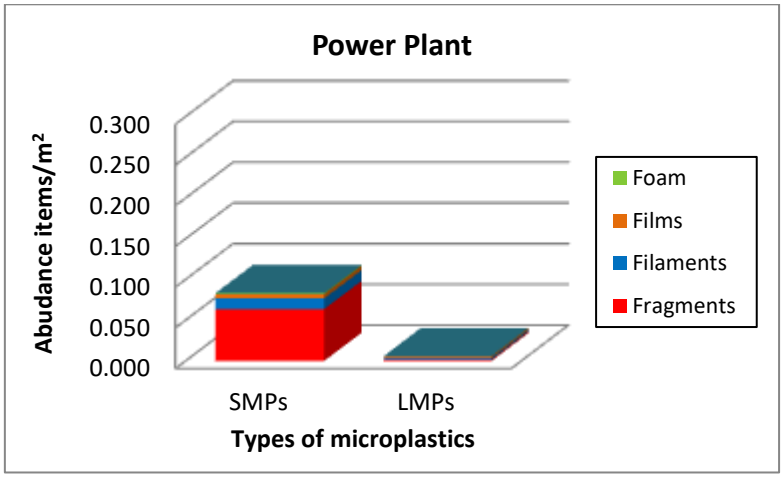

Figure 28. *Abundance of microplastics at Power Plant area.*

Comparing the results from all three sampling sites, the highest abundance of microplastics was observed at Varia area. For LMPs, fragments were found in higher abundance at Varia's transects (0.022 items/m^2). Foams also, were in high level in comparison to the other types of plastics (0.017 items/m^2) (Figure 29).

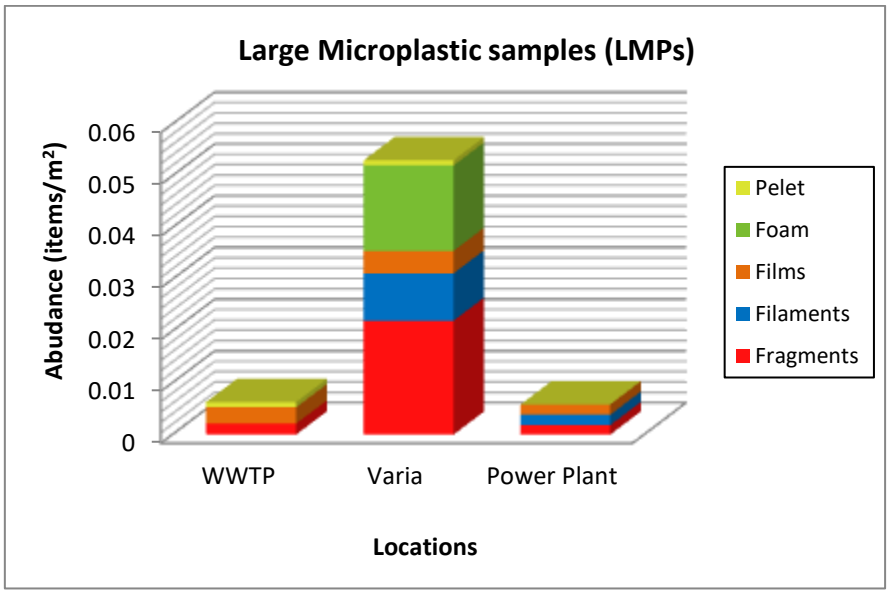

Figure 29. *Comparison of the abundance of large microplastics between the three sampling sites.*

As far SMPs concerns, the abundance of fragments was higher at all transects, but especially at Varia's transects (0.208 items/m^2). Filaments were also highly abundant, followed by foams (Figure 30). Comparing the results from both Figures 29 and 30 it becomes clear that the abundance of small microplastic debris was higher than of large ones. Remarkable difference between the two Figures is the abundance of foams and filaments, as the first were mainly found in LMP samples than in SMP samples, whereas filaments were in higher amount on SMP samples. Additional information about the abundance of MPs is presented at Table III (Annex).

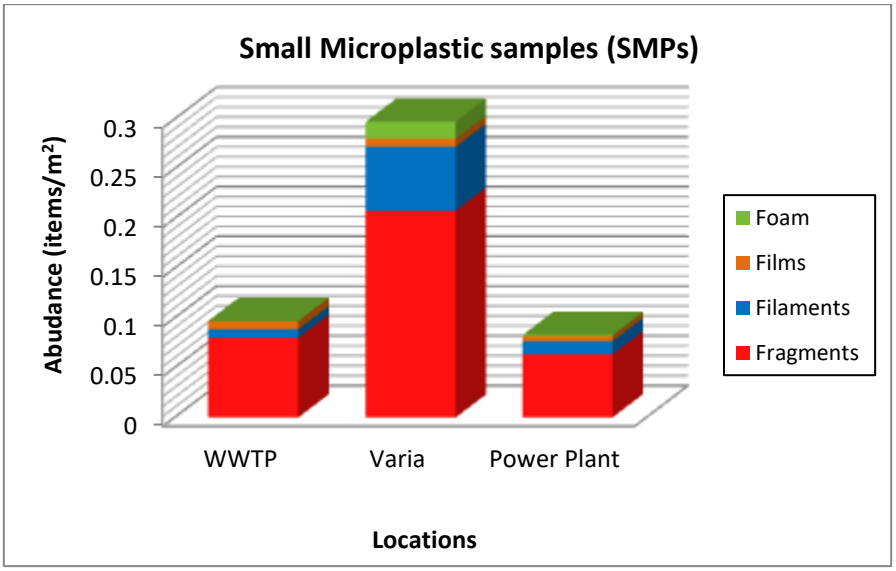

Figure 30. *Comparison of the abundance of small microplastics between the three sampling sites.*

3.2. Size of microplastics

Results from all transects showed that the microplastics of the three size classes, >300μm (SMPs), 1mm- ≤ 5mm (LMPs) and >5mm meso-plastics, that have been found, had an average length and area of 20.21 mm and 23.97 mm^2, respectively. More specifically, bigger microplastics were found at Varia's transects especially at transect V2 (35.413 mm) but also at PP2 (26.249 mm) and PP3 (22.063 mm) of Power Plant's transects. The smallest length of all samples was found at transect PP1 (4.742 mm). As for the average area, microplastics that were collected at transect PP3 (59.941 mm^2) had the biggest area of all the samples followed by transects V3 (29.452 mm^2) and V1 (27.687 mm^2) (Table 4).

Table 4. *Average length and area of microplastics in SMP and LMP samples related to the distance from the coast.*

Location	Transect	Distance from the coast (m)	SMPs		LMPs		Total	
			Average length (mm)	Average area (mm^2)	Average length (mm)	Average area (mm^2)	Total length (mm)	Total area (mm^2)
WWTP	W1	737	8.132	12.710	6.895	12.166	15.027	24.876
Varia	V1	356	4.243	4.645	14.29	23.042	18.533	27.687
	V2	939	4.391	3.901	31.022	8.267	35.413	12.168
	V3	1,619	4.743	2.759	14.718	26.693	19.461	29.452
Power Plant	PP1	889	4.742	3.141	0	0	4.742	3.141
	PP2	987	5.000	3.638	21.249	6.907	26.249	10.545
	PP3	1,649	3.562	2.412	18.501	57.529	22.063	59.941

Comparing the size of microplastics that were found in every transect, it is obvious from the following Figure that microplastics of size 1-5mm were the dominant size class in all transects (595 items), while particles with sizes 0.5-1mm and 5-15mm were found in the same number (101 items) (Figure 44; Annex).

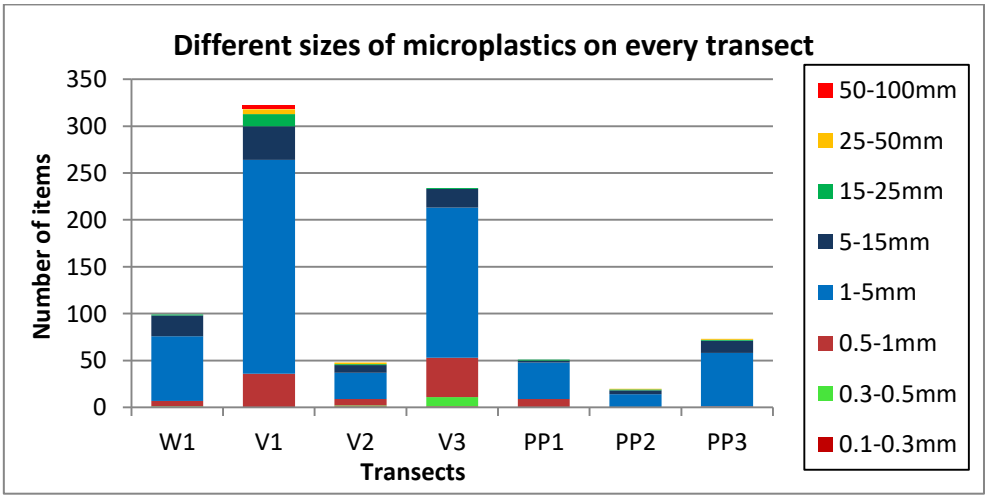

Figure 31. *Different sizes of microplastic debris on every transect.*

The largest number of microplastics was found at Varia transect (V1) where most of microplastic particles were at the size class of 1-5mm followed by the 0.5-1mm and the 5-15mm size classes. Moreover, from all samplings, only at V1 transect were found 13 particles of size 15-25mm and 4 particles of 50-100mm. Comparing the three sampling areas we can conclude that at Varia's transects (V1, V2, V3) the plastic items had greater size variability

ranging between 0.1 and 100 mm, whereas at the transects of the other areas plastic debris were at smaller sizes (Figure 31; Figure 45; Annex).

3.3. Type of microplastics and distance from the coast

Generally the type of microplastics of both SMPs and LMPs that were found at transects were mainly fragments, filaments, foams and films, while pellets were in negligible amount. Results in comparison to the distance from the coast revealed that most of microplastic particles that were found at distance less than 1km were fragments (245 items) and foams (36 items). At 1km from the coast microplastics debris were mostly fragments (192 items), while filaments and films had approximately the same quantity of 33 and 25 items respectively. At distances close to 2 km the larger amount of MPs was fragments (149 items) and filaments (93 items) (Table 5).

Table 5. *Type of plastics and distance of each transect from the coast.*

Locations	Transects	Distance from the coast (m)	Fragments	Filaments	Films	Foam	Pellet
WWTP	W1	737	78	8	11	0	0
Varia	V1	356	245	21	18	36	0
	V2	939	35	7	5	1	0
	V3	1,619	120	86	0	21	1
Power Plant	PP1	889	39	11	0	0	0
	PP2	987	40	6	9	0	0
	PP3	1,649	29	6	1	2	1

Comparing the size of the collected floating plastic items and the distance from the coast it is obvious that particles of sizes ranging from 0.5- 1mm to 5-15mm were more numerous irrespectively to the distance from the coast. More specifically, at distances of 1-2 km from the coast, the MPs size class of 1-5 mm was clearly the dominant one, whereas closer to the coast (<1 km) only a few number of plastic particles were at the same size range (Figure 32).

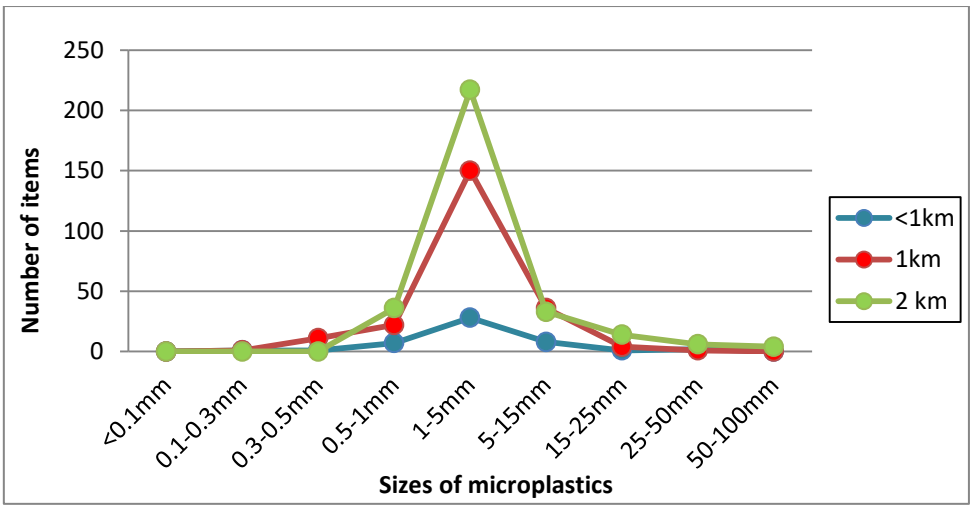

Figure 32. *Size of floating plastic items in relation to the distance from the coast.*

3.4. Polymeric composition in different samplings

Microplastic samples that were collected from all three areas were analysed using FT-IR spectroscopy. Polymer identification was performed using a combination of instrument's and in-house libraries. Few characteristic bands of some of most well-known type of plastics are being presented on Table 6 (Bessa et al., 2018; Hummel, 2002).

Table 6 *Infrared characteristic bands of selected particles representing the most abundant polymer types. Assignments used in this table have been adapted from Hummel (2002) and Bessa et al. (2018)*

ID	Compound	Characteristic band (cm^{-1})	Spectral assignment
1	Polyethylene	2918 vs, 2850s	$\nu_{as}(CH_2)$
		1472 w	$\nu_{s}(CH_2)$
2	Polypropylene	2960 vs, 2877 m	$\nu_{as}(CH_3)$,
		2918 s, 2838m	$\nu_{s}(CH_3)$
		1460m	$\nu_{as}(CH_2)$
		1377m	$\nu_{s}(CH_2)$, $\delta_{as}(CH_3)$, $\delta_{s}(CH_3)$
3	Rayon	3650-3000 s	ν(OH)
		2990-2820 w	ν(CH)
		1425, 1372, 1320m	δ(CH)
		1200-1000 vs	δ(C-OH)+δ(CC) + ν(C-OC)+ ν(C-OH)
4	Polyester	2966 w	ν(CH)
		1720 vs	ν(C=O)
		1408m	δ(ring)
		1340m	δ(CH)
		1261/1246 s, 1117/	ν(COC)
		1102 s	δ(CH ring)
		1018 m, 728m	
5	Polyacrylonitrile	2935 m, 2870m	$\nu_{as}(CH_2)$,
		2243 vs	$\nu_{s}(CH_2)$
		1731 s	ν(CN)
		1451 s	ν(C=O), $\delta(CH_2)$
6	Polyamide 6	3300 s, 3082 w	ν(NH)
7	Nylon	2934 m, 2863m	ν_{as}(CH2),
		1642 vs	ν_{s}(CH2)
		1544 vs	ν(C=O)
		1463 m, 1371m	δ(NH)
		1263m	δ(CH), δ(CN)

Figure 33 presents the indicative spectrum of Polystyrene and peaks of absorbance in a certain wavenumber.

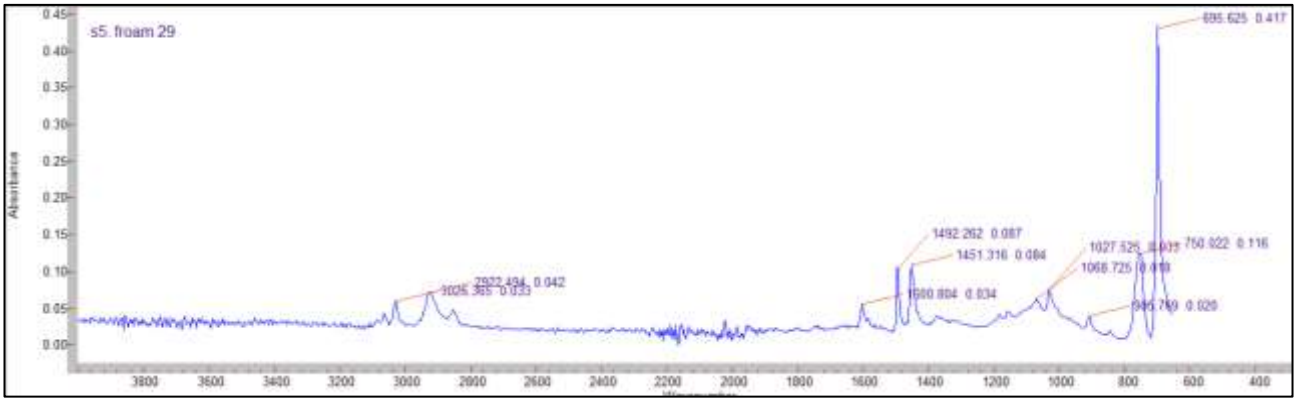

Figure 33. *Infrared spectra of microplastic sample (foam - Polystyrene).*

Indicative infrared spectra obtained from extracted microplastics are being represented at the following figure, as well as smaller figures of each type of plastic polymer have been inserted (Figure 34).

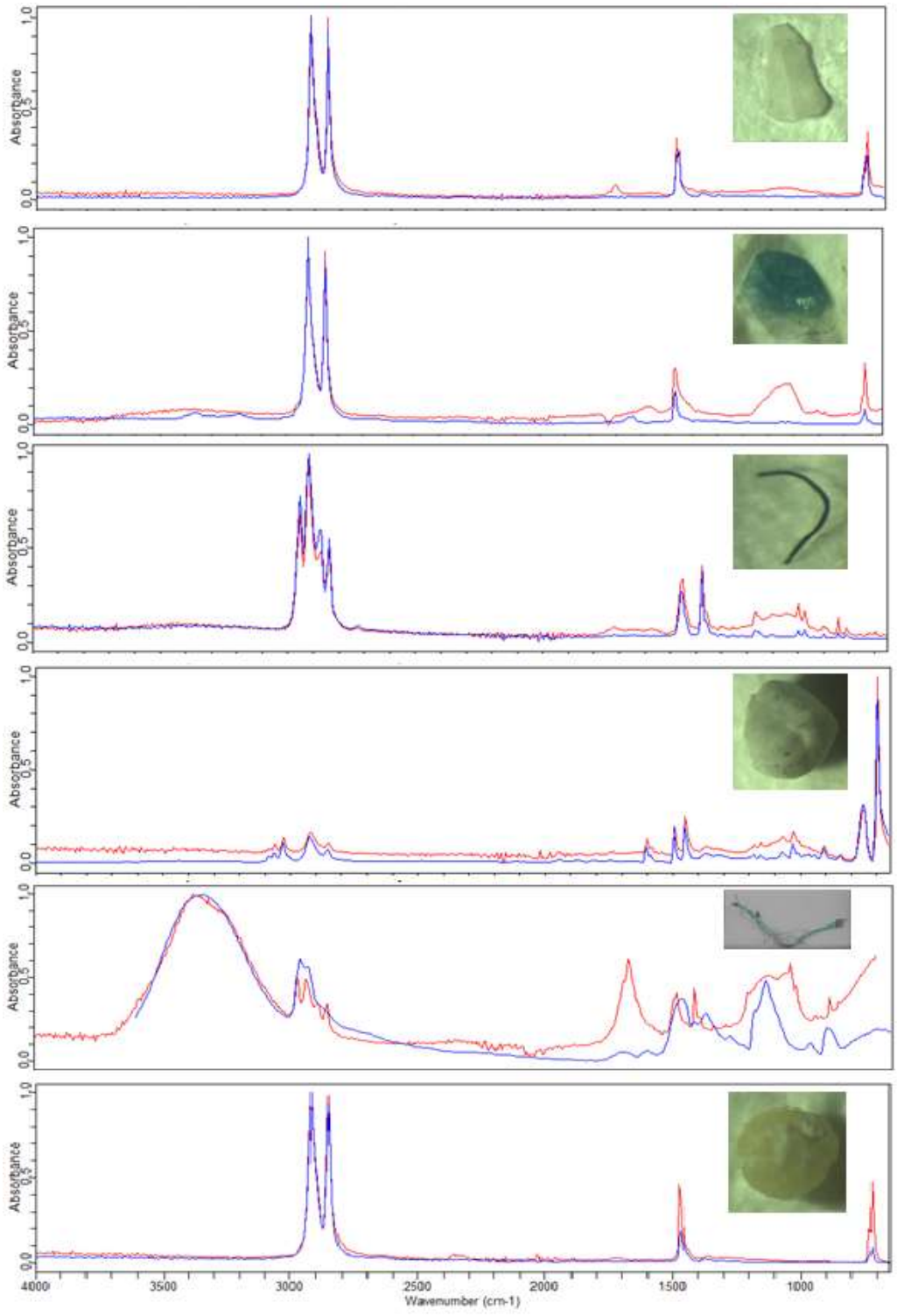

Figure 34. *Infrared spectra of representative samples identified as (from top to bottom): high density polyethylene-HDPE (fragment white); low density polyethylene-LDPE (blue fragment); polypropylene-PP (black fibre); polystyrene-PS (foam); polyvinyl acetate-PVA (blue filament); PE (pellet).*

The FTIR analysis of 837 microplastic items of both SMP and LMP samples, showed that most of microplastics that were found at Varia's transects were LDPE (157 items). Moreover, at a large quantity were PP microplastics (61 items) and Polyvinyl Acetate (PVA) microplastics (46 items). At Power Plant's transects LDPE were in higher abundance (48 items), as well as PE (23 items). PS and PP were at approximately the same number. At Waste Water Treatment Plant's transect more abundant were LDPE microplastics (25 items), while PE and PET where found at the same quantities (10 items). HDPE was found in highest amount at Varia's samplings (13 items) and at Power Plant area (9 items). Other types of plastics such as PU, PVC, SPE, Polymethylpentene (PMP) and Hydroxylpropyl Methylcelluloce (HPMC) were found at very small quantities. Beside plastic items pieces of card board were present in all samples in small number, mostly at Power Plant transects. Finally one item of glass filter was identified in one of Varia's samplings (Table 7). More details about the polymeric composition are presented in Table IV (Annex).

Table 7. *Polymeric composition in different transects.*

Quality-Transects	**WWTP**	**Varia**	**Power Plant**
PS	0	14	15
PE	10	27	24
PP	6	61	12
SPE	0	3	0
PET	10	1	0
LDPE	25	157	48
HDPE	0	13	9
PVA	3	46	4
PMP	0	4	0
PU	0	1	0
PVC	0	1	0
HPMC	0	0	1
CARD BOARD	1	3	4
GLASS FILTER	0	1	0

3.4.1. Percentages of polymeric composition

Results from both SMPs and LMPs collected at the Waste Water Treatment Plant transects showed that LDPE microplastics corresponded to 45%, followed by PE and PET with 18%, while PP contributed only 11% (Figure 35).

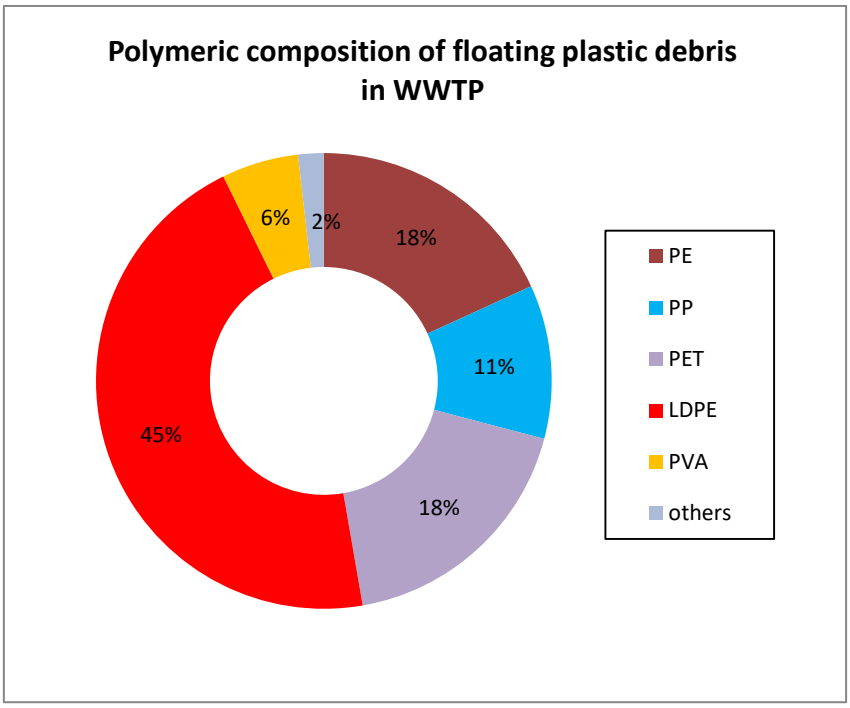

Figure 35. *Percent contribution of the different polymers of MPs at WWTP area.*

Floating microplastics that were found at Varia's transects were LDPE (47%), PP (19%) and PVA (14%). The rest types of plastics were found at small percentage (Figure 36).

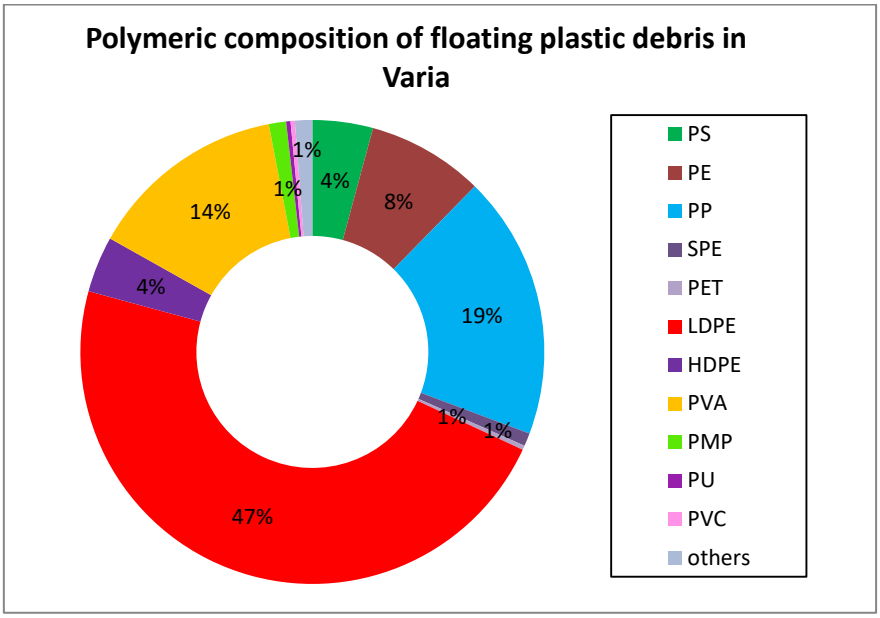

Figure 36. *Percent contribution of the different polymers of MPs at Varia area .*

Results from the Power Plant area represent that LDPE was at higher percentage (41%), and PE constitute only 21% of the total MPs. The average percentage of PS and PP was 11% and the other types of polymers were found at smaller percent (Figure 37).

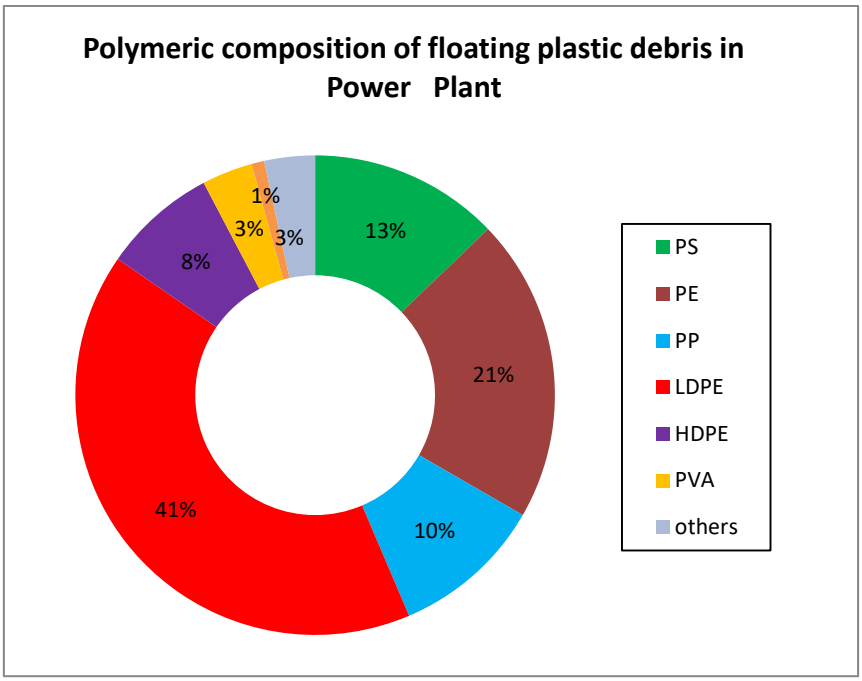

Figure 37. *Percent contribution of the different polymers of MPs at Power Plant area.*

As far as large microplastic items larger than 5mm concerns, the analysis of polymeric composition showed that PVA was at highest amount (47%) in comparison with LDPE that was found at 22%. PP accounted only for 10%. All the other types of plastics were at lower percentage (Figure 38).

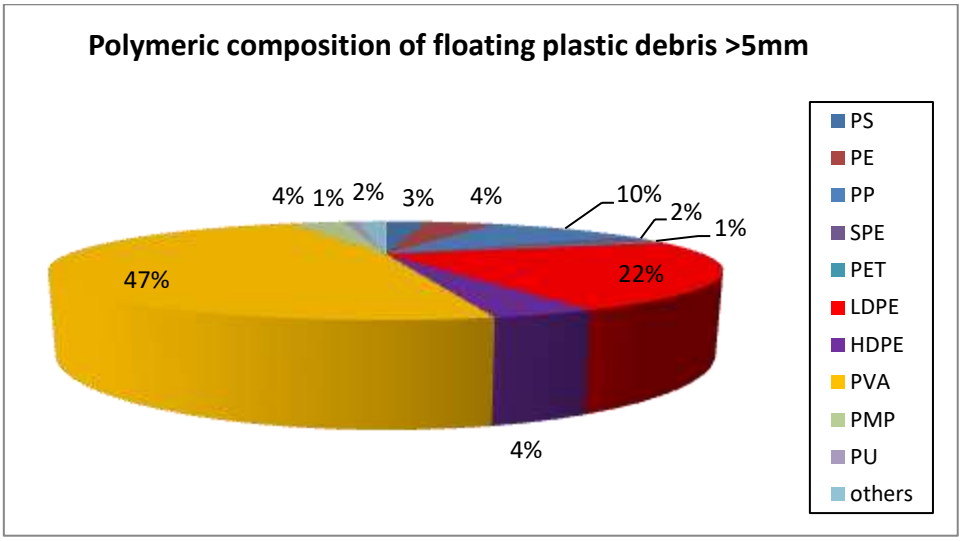

Figure 38. *Pie chart of polymeric composition of large microplastics.*

3.5. Climate data

Climate data of September 2017 until November 2017 were collected from the meteorological station of the Institute of Environmental Research of the National Observatory of Athens, at Thermi (near Mytilene) http://penteli.meteo.gr/stations/lesvos-thermi/. One month before samplings, at September of 2017, wind

direction was most of the time west while some other days there were northeast winds with an average wind speed of 9km/h and the highest of 62.8km/h. Total precipitation was 19.8mm with a maximum daily value at 19.6mm at 26th of September.

The period from September until November wind directions were varying from west-southwest (WSW) to north-northeast (NNE) with speeds of 9.5km/h and sometimes bigger than 60 km/h (Figure 39). During September until October westerlies were dominating with frequency approximately 30% with speeds reaching 10 km/h. As for N-NNE winds speeds higher than 15km/h were the most frequent. At the sampling period from October until November the average wind speed was approximately 11km/h and the highest was 59km/h on the 24th of October, few days before transects at Power Plant areas. Moreover, high values of wind speed ranging from 29.0km/h to 62.8km/h had been recorded the last week before Waste Water Treatment Plant's and Varia's transect. As for precipitation during the sampling period, a total of 92mm was recorded, with the highest value of 43.8 on the 24th of October (Tables V-VI; Annex). From all the above, weather conditions were strong during the sampling period with mostly high wind speed and low precipitation.

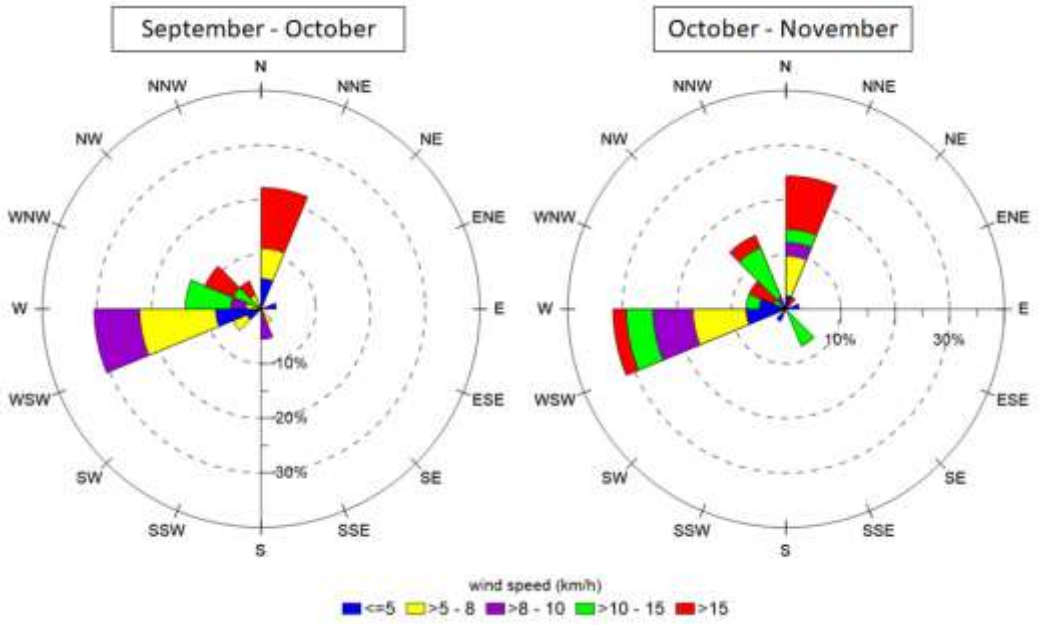

Figure 39. *Wind speed rose diagrams for September until 6th of October; the day of the sampling (left) and for October until the end of sampling period (right).*

3.6. Comparison with other studies

A summary of floating MPs abundances (items km^{-2}) found in different areas of the Mediterranean Sea accompanied by informations regarding the number of samples, year of sampling, sampling net and mesh size, and instrumental methods used for detection of MPs is reported in Table 8. Although the first study was conducted in 1997 (Kornilios et al., 1998), it is obvious that the number of publications regarding MPs have been intensified over the latest years as the accumulation of MPs in the marine environment has been recognised to be

a global issue. Most of researches have been reported on the Western Mediterranean Sea (Fossi et al., 2012; 2016; 2017; Collignon et al., 2014; Faure et al., 2015; Panti et al., 2015; Pedrotti et al., 2016; Fossi et al., 2017; Baini et al., 2018; Schmidt et al., 2018) and on the Adriatic Sea (Suaria et al., 2016; Gajšt et al., 2016; Zeri et al., 2018) during 2011 until 2015. Only few studies have been conducted in the Northeastern Mediterranean Sea and the Levantine Sea (van der Hal et al., 2017; Güven et al., 2017; Gündoğdu, 2017; Gündoğdu et al., 2017). The data presented at Table 8 reveal high variability ranging between 37,600 items km^{-2} (Gündoğdu et al., 2017) to 1,067,120 items km^{-2} (Gündoğdu et al., 2017).

High concentration of microplastics close to the coast has been previously observed in the Mediterranean (Gündoğdu, 2017; van der Hal et al., 2017). The abundances recorded in the current study (75,218 items km^{-2}) are lower than the majority of the studies conducted at the Mediterranean sea and of similar magnitude to those recorded off the Tyrrhenian Sea (69,161± 83,244 km^{-2}; Baini et al., 2018) and at the Western Mediterranean Sea (82,000 ± 79,000 km^{-2}; Fossi et al., 2017). As for different sizes of sampling nets that have been used before, 300-330μm size of Manta trawl nets were most dominant.

Table 8. *Microplastic abundance in Mediterranean surface waters and instrumental analysis methods used for detection of MPs, in different studies*

Region	Year	Number of samples	Sampling nets	Net mesh (μm)	Items km^{-2} ± SD	Instrumental Analysis Method	Source
Cretan Sea	1997	25	net Mesh	500			Kornilios et al., 1998
NW Mediterranean Sea	2010	40	net Mesh	333	116,000	Binocular microscope	Collignon et al., 2012
Western Mediterranean (Ligurian Sea and Sardinian Sea)	2011	23	WP2	200	310,000 ± 100,000	Stereomicroscope	Fossi et al., 2012
Western Mediterranean Sea (Bay of Calvi-Corsica)	2011–2012	38	WP2	200	62,000	Binocular microscope	Collignon et al., 2014
Western Mediterranean Sea	2011–2012	41		333	135,000		Faure et al., 2015
Western Mediterranean Sea (Ligurian Sea)	2012	70	WP2	200	310,000 ± 117,000	Stereomicroscope	Fossi et al., 2016
Western Mediterranean Sea (Ligurian Sea)	2013	33	Neuston net	333	125,930 ± 132,485	Zooscan digital scanner/FTIR	Pedrotti et al., 2016
Whole Mediterranean Sea	2013	39	Neuston net	200	243,853	Stereomicroscope	Cózar et al., 2015
Western & Central Mediterranean Sea	2011–2013	71	Manta trawl	333	147,500 ± 25,051	Optical microscope	Ruiz-Orejón et al., 2016
Western Mediterranean & Adriatic Sea	2013			200	400,000 ± 740,000		Suaria et al., 2016
Western Mediterranean Sea (Tyrrhenian Sea)	2013–2014	24	Manta trawl	330	69,161± 83,244	Stereomicroscope/FTIR	Baini et al., 2018
Levantine Sea (Israel coastal waters)	2013–2015	108	Manta trawl	333	1,518,340	Stereomicroscope/FTIR	van der Hal et al., 2017
Western Mediterranean Sea	2014	21	High Speed Manta Trawl	330	82,000 ± 79,000	Stereomicroscope/FTIR	Fossi et al., 2017
Western Mediterranean Sea	2014	6	Manta trawl	780	112,000	Zooscan digital scanner	Schmidt et al., 2018
Adriatic Sea	2014	17	Epineuston net	300	472,000 ± 201,000	Near Infrared Spectroscopy	Gajšt et al., 2016
Adriatic Sea	2014–2015	65	Manta trawl	330	315,009 ± 568,578	Stereomicroscope/FTIR	Zeri et al., 2018
Levantine Sea	2015	17	Manta trawl	330	16,339 - 520,213	Stereomicroscope/FTIR	Güven et al., 2017
Levantine Sea	2016	7	Manta trawl	330	37,600	Microscope	Gündoğdu et al., 2017
Northeastern Levantine coast of Turkey	2017	14	Manta trawl	330	1,067,120	Microscope/FTIR	Gündoğdu , 2017
Northeastern Mediterranean Sea (Northeast Aegean Sea)	2017	7	Manta trawl	335	75,218	Stereomicroscope/FTIR	Present study

4. Discussion & Conclusions

The purpose of the present study was to evaluate for the first time the abundance and composition of floating microplastic particles in the marine environment of Mytilene Strait in the vicinity of the urban area of Mytilene city. More specifically, floating plastics are being distributed in the aquatic environment from various sources such as fishing areas, urban and touristic activities, shipping lanes etc., wastewater treatment plants – WWTPs, rivers (FAO, 2016; Zeri et al., 2018). Results from our research at Mytilene Strait reveal that some of the potential plastic sources could be (i) the waste water treatment plant (ii) the fishing and (iii) the shipping activities, as well as (iv) the urbanized coastal zone.

Overall, a large number of microplastics was collected from all transects at WWTP, Power Plant and Varia areas. In order to better visualize our results, ESRI ArcGIS 10.1 has been used. The concentration of microplastic debris was higher at Varia's transects especially at V1 transect (321 items) closest to the coast (<1km) and at V3 transect (229 items) further than 1km. Out of the five types of particles identified, fragment had the highest share (586 items), followed by filaments (148 items), foams (60 items) and films (44 items), while pellets were present in only two samples (Figure 40).

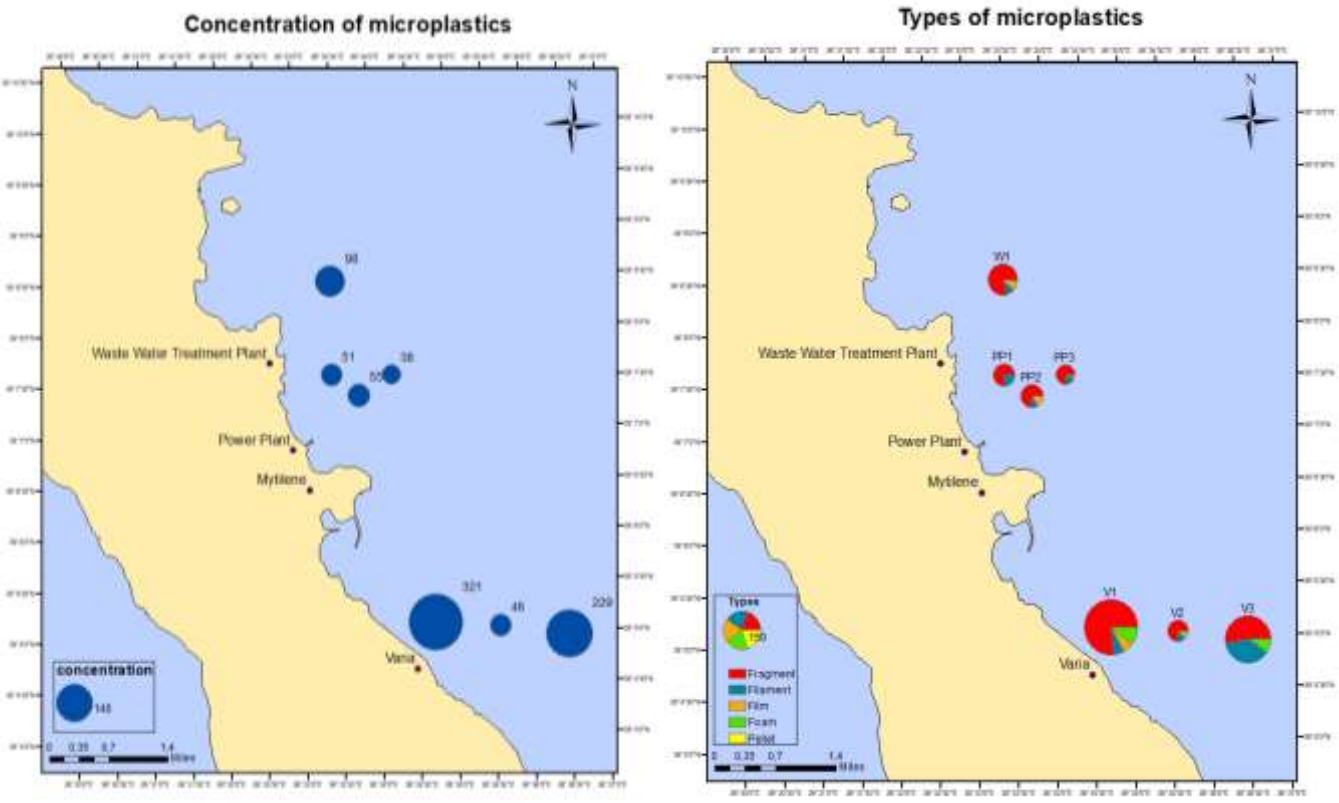

Figure 40. *Microplastic concentration (left_map) and different types of particles (right_map) at each transect at Mytilene Strait.*

Floating plastic particles were found in three major size classes: SMP (300μm - 1mm); LMP (1mm- ≤ 5mm) and meso-plastics (>5mm). At highest number were particles of size 1-5mm followed by particles of 0.5-1mm and 5-15mm sizes. Comparing sizes of plastic particles to the distance from the coast at the following graph, it is obvious that most common size class of microplastics at all distances was the 0.5-15mm. Varia's transects had also the highest variation of sizes (Figure 40). However, few particles of size bigger than 15 mm were also found at distance further than 1km from the coast (Figure 41).

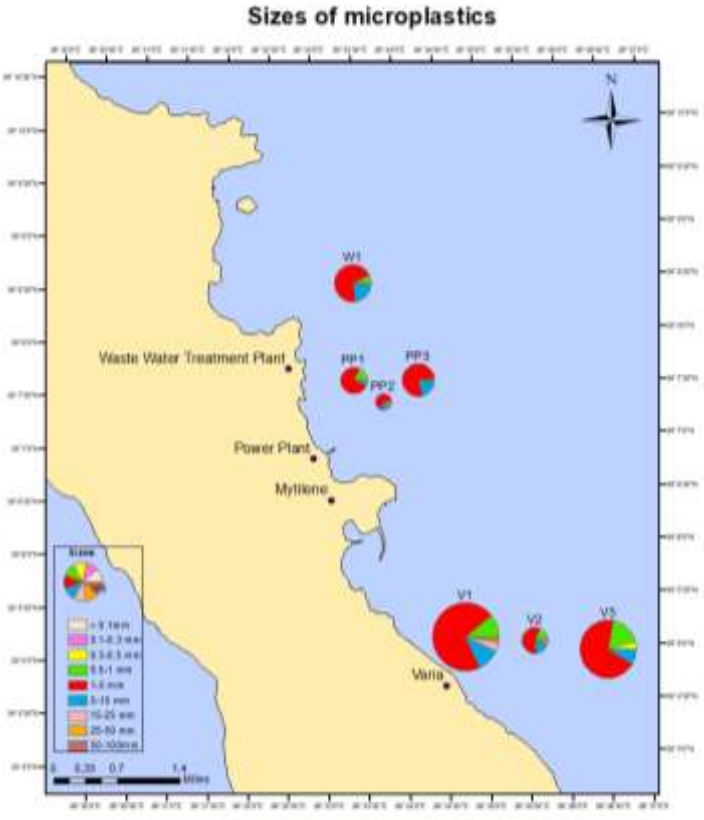

Figure 41. *Size distribution of microplastics at Mytilene Strait.*

Most common polymers found in the marine environment are polyethylene (PE), high density polyethylene (HDPE), low density polyethylene (LDPE), polystyrene (PS), polyvinyl chloride (PVC), polyvinyl acetate (PVA) polyvinyl terephthalate (PET). Polymer identification by ATR-FTIR spectroscopy revealed that most of the analysed particles were polyethylene PE (LDPE and HDPE), followed by polypropylene (PP). At Varia's transects where most of plastic particles were collected, in higher occurrence were LDPE, PP and PVA particles.

Results from the present study demonstrate that the amount of small-sized meso-plastics (1-10mm) is significant in the Northeastern Aegean Sea, at Mytilene Strait. This seems of a particular concern with regard to the environmental status of the coastal waters, as those microplastic particles are being a great threat for marine animals and humans. Their accumulation on coastal waters of the Eastern part of Lesvos island is expected to be increased within time, as these small-sized items cannot be controlled and removed totally from the marine environment.

The abundance of microplastics found is highly related to the density population of the coastal zone at Mytilene Strait. Sea-based sources of macro- and microplastic items, as well as their different buoyancy features are some potential explanations of the presence of microplastics in inshore waters. Irregular shapes among microplastic particles indicate the alteration at the hydrodynamic characteristics. Thin films and fibers for example may have higher buoyancy and lower settling velocity than spheres with the same density and volume (Filella, 2015). This explains the reason why pellets were less abundant at surface waters off the eastern coast of Lesvos island, than films or fibers. Moreover, particle shape and specific surface area (surface area to volume ratio) have been proved to be important for determining the aggregation and biofouling processes, which increases the particle density leading to their faster sinking (Chubarenko et al., 2016).

Coastal seas are highly energetic regions with continuous influence from strong hydrodynamic factors such as wind, tides, waves and thermohaline gradients. Consequently the microplastic distribution, temporally and spatially, indicates that climatic forces as well as coastal transport processes form the dynamic patterns (e.g. Isobe et al., 2014). Inputs of plastics from land, rivers, fishing activities and marine sources go through beaching, drifting, and settling pathways until they reach temporary reservoirs on beaches, estuaries, and marine sediments (Zhang, 2017).

Taking into account the climatic data from the meteorological station of Thermi along with results from the analysis of microplastic items we came to a conclusion that most possible paths of accumulation of plastics were human activities, fisheries and shipping. The distribution of microplastics through inshore and offshore waters is achieved through current circulation, waves and microplastic composition itself. Differences between the properties of the plastic debris i.e. polymer type, shape, size and weight determine the position of those items in the marine environment; floating on the surface, suspending on water column or sinking at the bottom.

Our findings indicate relatively high microplastic abundance at the coastal area of the North Eastern Aegean Sea. Better knowledge about the extent of microplastic contamination of the Aegean waters in now a research imperative. For a more comprehensive assessment of this threat, the adoption of a monitoring plan, based on fixed transects along and across the Mytilene Strait that will allow to better evaluate the occurrence of microplastics and its variations in relation to the distance from land and to the seasonality, is necessary.

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Annex

Table I. *Data sheet with coordinates and information about transects.*

Locations	Transects	Date (mm/dd/yy)	Coordinates of samplings				Distance from the coast (m)	Sea conditions	Wind speed	Sky conditions
			Start Lat	Start Long	End Lat	End Long				
WWTP	W1	6/10/2017	39° 8'52.28"N	26°33'16.64"E	39° 8'4.52"N	26°33'38.50"E	737	Swel SE	2 kn W	visibility
Varia	V1	10/10/2017	39° 5'24.60"N	26°35'57.60"E	39° 4'10.36"N	26°36'35.35"E	356	Swell E	2,88 kn W	
	V2	10/10/2017	39° 5'16.61"N	26°34'32.35"E	39° 4'1.22"N	26°35'57.30"E	939	Swell E	3 kn W	
	V3	10/10/2017	39° 5'25.55"N	26°35'10.82"E	39° 4'2.39"N	26°36'16.62"E	1,619	Swell E	4 kn W	
Power Plant	PP1	6/11/2017	39° 6'51.77"N	26°33'56.23"E	39° 8'15.51"N	26°32'56.91"E	889	Calm	2 kn N	
	PP2	6/11/2017	39° 8'4.99"N	26°33'10.68"E	39° 6'54.24"N	26°34'2.95"E	987	Calm	2 kn N	
	PP3	6/11/2017	39° 8'26.23"N	26°33'44.86"E	39° 7'2.61"N	26°34'30.31"E	1,649	Calm	2 kn N	

Table II. *Number of different types of LMPs and SMPs in the different areas.*

Transects / Type of plastics	WWTP		Varia		Power Plant	
	LMPs	**SMPs**	**LMPs**	**SMPs**	**LMPs**	**SMPs**
Fragments	2	76	40	360	3	105
Filaments	0	8	17	99	2	22
Films	3	8	8	15	2	8
Foam	0	0	27	31	0	2
Pellet	1	0	1	0	0	0

Table III. *Abundance (items m^{-2}) of different types of LMPs and SMPs in the different areas.*

Transects / Types of plastics	WWTP		Varia		Power Plant	
	LMPs	**SMPs**	**LMPs**	**SMPs**	**LMPs**	**SMPs**
Fragments	0.002	0.080	0.022	0.208	0.002	0.064
Filaments	0.000	0.008	0.009	0.064	0.002	0.013
Films	0.003	0.008	0.004	0.008	0.002	0.005
Foam	0.000	0.000	0.017	0.017	0.000	0.001
Pellet	0.001	0.000	0.001	0.000	0.000	0.000

Table IV. *Composition of microplastics shape of samples at different transects.*

Transects / Shape of plastics	WWTP		VARIA						POWER PLANT				
	sample1	**sample2**	**sample3**	**sample4**	**sample5**	**sample6**	**sample7**	**sample8**	**sample9**	**sample10**	**sample11**	**sample12**	**sample13**
PS	0	0	0	0	2	0	4	4	4	4	0	4	0
PE	0	9	2	2	2	0	13	5	1	6	9	5	2
PP	3	2	0	0	3	9	27	16	0	1	0	5	4
SPE	0	0	0	0	1	0	0	0	0	0	0	0	0
PET	10	0	0	0	0	0	0	0	0	0	0	0	0
LDPE	0	23	8	1	17	13	65	31	3	10	15	12	8
HDPE	0	0	0	0	1	0	6	2	2	2	1	2	2
PVA	0	0	0	0	2	0	0	0	0	0	0	0	0
PMP	0	0	0	0	0	0	0	0	0	0	0	0	0
PU	0	0	0	0	0	0	0	0	0	0	1	0	0
PVC	0	0	0	0	1	0	0	0	0	0	0	0	0
HPMC	0	0	0	0	0	0	0	0	0	0	0	0	1
CARD BOARD	0	0	0	1	0	2	0	0	2	0	0	2	0

Table V. *Monthly climatological summary for September 2017.*

Day	Mean Temp	High Temp	Time	Low Temp	Time	Rain	AVG Wind Speed	High Speed	Time	Direction
1	23.0	28.6	19:10	18.3	7:00	0	12.2	29.0	8:50	WNW
2	23.4	31.3	18:10	16	7:10	0	8.0	24.1	2:00	NNW
3	22.6	27.3	17:10	17.5	6:30	0	3.7	22.5	12:30	N
4	24.8	30.8	17:00	21.4	3:40	0	6.8	32.2	18:00	W
5	24.1	26.7	19:40	21.4	6:10	0	11.1	35.4	22:50	WNW
6	22.6	26.1	15:40	19.2	23:50	0	13.5	38.6	14:30	NW
7	22.1	27.7	17:40	15.7	6:30	0	9.8	30.6	16:30	W
8	22.8	29.3	17:40	17.4	7:10	0	4.8	20.9	18:40	W
9	22.0	26.9	18:20	16.7	6:50	0	3.2	19.3	12:10	W
10	23.3	29.3	14:10	19.3	7:00	0	4.7	32.2	13:50	WSW
11	23.9	32.3	16:30	18.1	6:50	0	6.0	32.2	17:20	SSE
12	26.9	32.3	14:20	21.2	23:50	0	9.2	43.5	10:30	S
13	23.2	27.4	19:00	19	7:20	0	4.7	24.1	11:40	E
14	26.2	31.6	16:00	21.3	4:10	0	10.8	30.6	14:00	WNW
15	24.7	30.1	17:40	19.8	4:10	0	6.0	20.9	11:20	W
16	24.6	28.7	15:30	20.9	6:00	0	6.3	22.5	9:50	WNW
17	24.3	29.6	17:30	21.5	5:10	0	6.0	27.4	10:30	N
18	24.4	30.3	16:30	19.4	7:20	0	1.0	14.5	20:50	W
19	23.8	28.9	18:30	19	7:40	0	2.7	19.3	10:10	N
20	26.4	34.0	14:30	18.9	6:00	0	8.4	41.8	10:20	S
21	24.4	27.7	14:40	18.9	0:00	0	8.2	46.7	15:20	W
22	20.4	25.6	15:50	16.1	6:30	0	8.0	43.5	4:30	W
23	19.4	23.3	17:50	14.9	6:40	0	7.9	33.8	9:40	WSW
24	22.1	26.1	17:20	16.8	5:40	0	12.4	37.0	21:00	NW
25	21.8	25.5	16:40	18.2	23:50	0	5.3	30.6	0:30	W
26	20.5	23.8	18:10	16.6	6:00	19.6	5.3	29.0	12:30	W
27	20.9	25.6	17:10	17.8	3:30	0	9.7	33.8	19:40	WNW
28	22.0	23.9	14:40	20.4	23:50	0	23.7	62.8	18:20	NNE
29	19.8	20.8	14:20	18.8	6:20	0.2	29.8	59.5	18:50	NNE
30	18.9	20.2	22:20	17.7	19:30	0	21.7	46.7	1:10	NW
TOTAL	**23.0**	**34.0**	**20**	**14.9**	**23**	**19.8**	**9.0**	**62.8**	**22**	**W**

Table VI. *Monthly climatological summary for October 2017.*

Day	Mean Temp	High Temp	Time	Low Temp	Time	Rain	AVG Wind Speed	High Speed	Time	Direction
1	19.1	20.8	15:20	17.7	8:50	0	17.4	33.8	4:20	NW
2	19.1	21.2	13:50	17.9	6:20	0	21.9	49.9	21:20	NNE
3	18.9	21.6	16:40	17.3	6:20	0	19.5	40.2	0:10	NNE
4	19.1	23.3	16:50	14.7	5:00	0	16.1	35.4	19:40	NNW
5	18.9	23.1	18:20	14.1	7:50	0	6.9	29.0	0:10	N
6	19.6	26	15:40	13.7	7:40	0	8.7	41.8	16:00	W
7	22.1	25.8	15:20	17.3	2:30	0	12.4	45.1	14:40	SSE
8	16.8	22.9	0:10	14.2	0:00	14.8	11.3	35.4	0:10	WNW
9	16.3	20.3	17:20	10.4	7:30	0	7.7	29.0	21:50	W
10	17.1	21.1	17:00	11.8	3:00	0	9.5	32.2	23:00	W
11	19.3	22.6	15:40	14.9	6:40	0	14.3	30.6	11:00	NNW
12	19.1	22.9	17:10	13.2	7:50	0	11.7	29.0	1:50	NNW
13	19.2	22.7	18:10	14.8	6:20	0	11.3	33.8	19:40	NNW
14	19.2	22.9	15:30	16.3	2:30	0	15.3	46.7	20:00	W
15	19.3	21.7	16:30	17	7:40	0	18.3	43.5	3:40	NNE
16	20.5	24.2	15:00	16.7	5:20	0	15.0	41.8	22:50	NNW
17	20.4	25.3	16:30	18.2	8:00	0	16.9	38.6	7:10	NW
18	18.6	22.6	14:50	14.1	7:50	0	3.7	22.5	0:40	WNW
19	17.1	22.3	16:40	13.3	8:00	0	3.4	19.3	11:40	SW
20	16.9	22.2	16:10	13.1	6:00	0	2.4	11.3	10:50	E
21	17	22.4	17:30	12.9	7:00	0	2.7	14.5	10:10	N
22	16.8	21.9	18:00	13.1	7:10	0	1.6	11.3	11:30	WNW
23	18.7	23.8	13:50	12.6	7:00	0	11.6	53.1	23:10	SSE
24	17.9	20.1	0:10	14.8	23:40	43.8	10.1	59.5	3:20	SSE
25	16.4	19.8	14:10	13.8	8:30	10.2	5.0	29.0	4:20	W
26	16	18.8	15:10	12.7	6:50	0	9.8	30.6	19:10	NNE
27	15.1	18.8	16:00	12.3	0:00	0	6.6	29.0	3:30	N
28	15.3	18.7	15:20	12.1	1:30	0.4	7.1	40.2	21:00	W
29	14.4	16.8	0:00	12	0:20	0	12.7	53.1	4:00	NW
30	16.3	19.9	10:30	11.9	19:50	0	10.6	40.2	10:50	W
31	12.7	15.6	14:50	8.8	22:50	0	10.0	32.2	4:50	NNW
TOTAL	**17.8**	**26**	**0:00**	**8.8**	**0:00**	**69.9**	**10.7**	**59.5**	**24.00**	**W**

Table VII. *Climatological summary for November 2017 (ten first days).*

Day	Mean Temp	High Temp	Time	Low Temp	Time	Rain	AVG Wind Speed	High Speed	Time	Direction
1	13.1	14.9	15:10	9.2	0:40	0	18.0	45.1	23:10	NE
2	12.4	15.4	15:50	9.8	23:50	0	12.9	37.0	1:50	NNE
3	12.1	16.3	15:30	7.6	6:50	0	8.2	29.0	12:20	W
4	14.6	18.8	15:00	10.1	7:00	0	12.9	54.7	21:40	W
5	15.8	17.4	14:00	13.9	23:50	0	18.2	43.5	2:30	NNE
6	13.3	17.3	14:30	9.8	6:10	0	6.3	19.3	0:20	N
7	13.2	17.9	14:30	9.8	6:00	0.2	5.1	17.7	10:40	W
8	14.6	19.8	15:20	9.7	6:20	0	4.8	30.6	11:00	W
9	15.2	18.9	16:10	11.9	23:50	0	3.1	14.5	4:00	W
10	13.7	17.8	15:40	10.2	5:40	2.6	6.9	17.7	10:30	W
TOTAL	**13.8**	**19.8**	**14**	**7.6**	**20**	**2.8**	**9.6**	**54.7**	**10**	**W**

***Figure 42**. Collection of samples onboard the research boat "Amphitriti".*

***Figure 43.** Large plastic debris from different transects.*

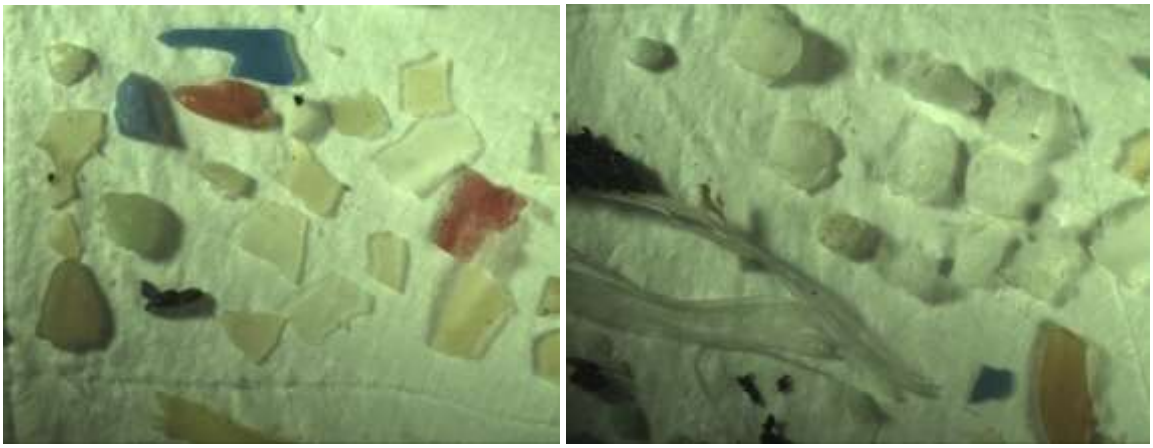

***Figure 44**. Small microplastic particles from Varia transect (V3).*

***Figure 45.** Different types of small microplastics; (i) fragment, (ii) foam, (iii) pellet, (iv) filaments, (v) fragment, (vi) fragment, (vii) filament, (viii) films.*