## The fate of Microplastics in the Aquatic Environment: The case of the Thermaic Gulf

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**Introduction:** Plastic is a general term that refers to a family of organic polymers derived from petroleum sources, including polyvinylchloride (PVC), nylon, polyethylene (PE), polystyrene (PS), and polypropylene (PP). Common plastic polymers include PP, PE, low-density polyethylene (LDPE), and polyacrylates (Frias et al., 2014). Microplastics are now found worldwide in all aquatic compartments such as surface water, water column, and sediments (Ivar do Sul and Costa, 2014), as well as in many aquatic animals, from invertebrates (Wright et al., 2013) to whales (Baulch and Perry, 2014). Plastic production and use has increased steadily over the past 50 years, with global production reaching 322 million tonnes in 2015 and 335 million tonnes in 2016 (Plastics Europe, 2015). These usage patterns suggest that plastic production and quantities of plastics (including microplastics) in aquatic environments will likely continue to increase over time. Recently, it has been estimated that 5 trillion microparticles are floating globally, while up to 890,000 plastic particles/km<sup>2</sup> were predicted in the Mediterranean surface waters (Eriksen et al., 2014). The density of microplastics on the surface of Greek waters were estimated up to 344 090 particles/km<sup>2</sup>.

The defined size of a particle constituting a "microplastic" varies, but an upper limit of 5 mm is generally agreed upon in the literature, and many researchers use 0.5 or 1 mm as the cut-off between macro or mesoplastic and microplastic (Cole et al., 2011). Some of the earliest studies has reported microplastics (fibers and fragments) in various marine organisms such as zooplankton, fish and mussels raising concerns on their potential effects on physiology and welfare of marine biota. These organisms are also used for human consumption and this may rise up a concern about the impact of marine litter ingestion on fishery resources and the potential risk human consumption. Most studies on microplastic ingestion in Mediterranean waters have been carried out in the Western Mediterranean Sea (Digka et al., 2018; Fossi et al., 2018).

The aim of this study is to quantify the abundance of floating microplastics in sediments and surface water in the Thermaic Gulf and to identify the spatial distribution of the microplastic particles and their polymeric characterization. Additionally, in order to simulate the bioaccumulation of microplastics in marine biota, a mesocosm was designed and constructed at AUTH and the integration of plastic particles within most abundant fish species (sea bass) of the Thermaic Gulf was explored.

**Material and Methods:** Thermaic Gulf constitutes the northwest corner of the Aegean Sea. The city of Thessaloniki is at its northeastern tip, and it is bounded by Pieria and Imathia on the west and the Chalkidiki peninsula on the east, with Cape Kassandra at the southeasternmost corner. It is about 100 km long.

Water surface samples were collected during the winter (February-March 2017). A total of 25 samples were collected at 500 m, 5 km and 10 km distance from the coast. Surface samples were collected using neuston net with cod end (100 µm mesh size, 40 × 70 cm mouth opening) equipped with a flowmeter and towed on the water surface at 2-3 knots for 20 min, kept at a distance of about 70 m from the boat to avoid the turbulence induced by the wake of the ship. At the end of samplings, the nets were rinsed thoroughly from the outside to ensure that both plankton and debris were washed into the end of the net and to prevent any contamination by rinsing water. Organic matter attached to the surface of microplastics was removed to allow clear identification of the type of plastics, using a solution of 30% H<sub>2</sub>O<sub>2</sub>. For sediment sampling, boxcorer grabs were used and to ensure representativeness of the samples, at least five sample replicates (5 m between replicates) were collected. Density separation of microplastics was carried out with a Munich Plastic Sediment Separator. After separation and purification, target microplastics were sorted from the remaining matrix. Large plastics were sorted out directly, while smaller-sized ones needed further observation under a dissection microscope. For plastic particle analysis, samples were observed under a stereo-microscope with a LED light and measured with micrometer ocular lens. Plastic particles were characterized and classified by colour, size and shape. The particles were dried at room temperature and weighted in analytical balance. Additionally, the polymer fingerprint was detected using the Fourier Transform Infrared (FT-IR) spectroscopy technique. Time of-flight secondary ion mass spectrometry (TOF-SIMS)-based analysis and imaging was also used for microplastic detection, so as to directly detect plastic particles smaller than 10 µm sand with a fragment ion of m/z 113, which was showed sufficient power to discriminate polyethylene from environmental matrices. When identified, plastic particles were counted manually with the assistance of microscope or weighted on a scale. Non-plastic sampling tools, latex gloves, and cotton clothes were used during sampling. During the extraction process in the laboratory, recovery rates of standard MPs (with the similar sizes and classifications of field samples) using the applied extraction method were provided. Since there were some synthetic fibers in the atmospheric fallout blanks were performed to minimize the effect of the experimental environment.

The experimental system for the mesocosm consisted of an indoor rectangle tank with 1 m height and  $\sim 200$  l capacity provided with a flow-through supply of sea water, collected directly from the Thermaic Gulf. To avoid the entrance and/or exit of microplastics each tank was provided with filters. A total of 15 sea bass were used for the experimentations. Fish were fed daily for 90 days with different experimental treatments; the control food contained no added microplastics, while the food experimental treatments contained microplastics that have previously sampled from Thermaic Gulf. Every 15 days after the beginning of the experiment, water samples were collected from 30 cm below the surface of the tank for the measurements of water chemistry (pH, temperature, BOD<sub>5</sub>). Also, the tissues from sea bass were analyzed every 30 days from the beginning of the experiments.

**Results and Discussion:** A total of 1200 microplastics were isolated from all 25 surface water samples, with a range of 13 to 520 total particles per sample. The minimum concentration was 13752 particles/km<sup>2</sup> and the maximum was 420513 particles/km<sup>2</sup>. Regarding sediment, the minimum concentration was 0.02 MPs / g dry sediment and the maximum was 0.1 MPs / g dry sediment. Analysis of the size distribution of microplastics in surface samples showed that the most abundant size class is 1–2.5 mm both in the whole dataset. This finding suggests a potential higher hazard for zooplankton organisms. Plastic fragments were found to be the most characteristic plastic particles isolated. Polyethylene (PE) and Polypropylene (PP) were the most abundant, a result that is in good agreement with previous studies, where these polymers account for the majority of the plastic particles floating in the Mediterranean Sea. The results showed a higher abundance of microplastics in the waters located at 5-10 km distance to the coast. Microplastics were also detected in the guts, skin and muscle of the sea bass and ranged from 0.08 g<sup>-1</sup> to 1.1 g<sup>-1</sup>.

**Conclusions:** This study reports the results of a monitoring campaign of microplastics in three different media. These data represent the first attempt to present the current state of microplastics in the Thermaic Gulf and to establish the baseline level for a future environmental and health impact study. Thermaic Gulf is an important port for European countries and because of its morphological features, generate important quantities of waste originating from the South European counties so the evaluation of the presence of plastics is mandatory to apply strategies to reduce the quantity of microplastics in the region.

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