QUANTITATIVE MICROFIBER EVALUATION IN MYTILUS GALLOPROVINCIALIS, WESTERN BLACK SEA, ROMANIA

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Abstract. Plastic particles have entered both aquatic and terrestrial ecosystem since the industrialization era, reaching dangerous levels of pollution throughout the entire food chain. The amount of microfibers quantified from several individuals of *Mytilus galloprovincialis* collected nearby the Constanta harbour was determined by microscopic evaluation, a high variation of the ingested particles being noted. The number of microfibers in the mussels soft tissue ranged from 0 to 14, being negatively correlated with the length and weight of the mussels. With an average concentration of 6.05 MPs per individual, fibers represent over 90% of the identified microplastics. Moreover, the microplastic concentrations reflect a high degree of pollution of the sampling area, previously observed in marine surface water and sediments.

Key words: Microplastics, mussels, Black Sea, Constanta harbour

1. INTRODUCTION

Microplastic particles (MPs) can be defined as plastic fragments or beads, ranging between 1 µm and 5 mm (Thompson *et al.*, 2004), being by far ones of the most threatening environmental modern issues. This complex contaminant is described as abundant, ubiquitous, and extremely environmentally persistent (Ahmad *et al.*, 2020). It is estimated that more than 5 trillion microplastics are floating globally, making those accountable for approximately 92.4% of the total marine plastic debris (Bergmann *et al.*, 2019).

Due to the degradation of macroplastics that will eventually break down into microplastic fragments, these values may increase exponentially over time. MPs are continuously accumulating in the environment, especially in the ocean realm, and even in the most pristine areas, such as the Arctic (Bergmann *et al.*, 2019), Amazon rivers or other remote areas (Gerolin *et al.*, 2020).

The main risk associated with microplastics is their availability to marine organisms (Wright *et al.*, 2013; Desforges *et al.*, 2015). Ingestion of microplastics has been identified and reported in over 220 aquatic, terrestrial and aerial species. More than half of these were recognized as being economically important for the food industry (Lusher *et al.*, 2020). Microplastic ingestion is not only harmful to marine species, but also to the entire food chain, including humans (Galloway, 2015; GESAMP, 2016; Wright *et al.*, 2017, Prata *et al.*, 2020). Moreover, it has been predicted that by the year 2050 most of the seabird species will contain microplastics at least in their digestive tract, if not in their muscle tissues and other organs as well (Ter Halle *et al.*, 2017; Wilcox *et al.*, 2015; Hu *et al.*, 2016).

Mussels are commonly and widely used in the biomonitoring of marine environments, being directly exposed to microplastic in water due their non-selective filter-feeding activity (Moore *et al.*, 2001). Even though they are one of the most common benthic species for MPs investigations, there is still quite a lack of such studies carried out on mussels inhabiting the coastal waters (Li *et al.*, 2016).

Considerable amount of plastic litter is transported daily along with sediments and water via the Danube River (Lechner *et al.*, 2014) into the Black Sea basin. The fate of the litter is determined by natural factors such as the exposure to sunlight, oxidation, currents, waves and wind regime (Wright *et al.*, 2013; Crawford & Quinn, 2016). As plastic litter and MPs are sinking due to the biofouling process (Wang *et al.*, 2016), the benthic fauna can easily ingest the particles and transfer it to the entire food chain.

The objective of this study is to evaluate the concentration of microfibers (MFs) from 17 individuals of *Mytilus galloprovincialis*, collected from an area expected to have a high MP pollution, such as the large marine harbour of Constanța (Fig. 1). This type of pollution in exploited marine species for human consumption is scarcely described in the Western Black Sea, thus the information presented here may be considered as preliminary data for further studies.

2. MATERIALS AND METHODS

In February 2019, 17 individuals of *Mytilus galloprovincialis* were collected from the concrete jetties outside of the Agigea harbour (Fig. 1), located southeast of Constanța city (western coast of the Black Sea). The mussels were transfer to aluminium recipients and frozen immediately for preservation during transport.

2.1. Sample preparation

The following analytical procedures were performed in the microplastic laboratory located in GeoEcoMar headquarter (Bucharest, Romania). The mussel shells were measured, weighted (Fig. 2a), and rinsed with distilled water to avoid any MPs contamination before extraction of the soft tissue. The sample preparation consisted in organic digestion (Fig. 2b) using a mixed solution of potassium hydroxide (KOH, 10 M) and hydrogen peroxide (H_2O_2, 30%), in which the samples were mixed for 5-7 days using an oscillatory shaker (Ehlers *et al.*, 2019; Scherer *et al.*, 2020). The samples pH neutralization was performed using formic acid (HCOOH, 95%) and distilled water to ensure a safe filtration process (Fig. 2c). The contents of each sample were filtrated through 4.7 mm, 1.2 µm fiberglass membranes using a vacuum pump and a three-post stainless-steel Buchner system (Fig. 2d).

2.2. Microscopic evaluation

The MPs visual investigation was performed using a stereomicroscope (Leika EZ4W) and concluded in quantitative evaluation, morphology, colour, and dimensional characterization of MPs, according to Hidalgo-Ruz *et al.* (2012) method. In order to have a realistic evaluation, all particles smaller than 50 µm were discriminated. Moreover, due to the lack of qualitative investigations of the observed particles, only fiber concentrations are presented, as other types of particles require polymer identification.

2.3. Quality assurance

Sample contamination prevention was ensured by cleaning the working space with ethanol prior to sample preparation sessions. Glass and stainless-steel utensils and materials were used for laboratory procedures, while operators were wearing cotton lab coats during both laboratory work and microscopic investigations. Moreover, several blanks were used during laboratory sample preparation and visual inspection of the filters. In addition to these, all sample beacons and recipients used for solution storage were covered with aluminium foil/parafilm throughout the chemical digestion and storage. The same amounts of potassium hydroxide, hydrogen peroxide, formic acid, and distilled water were used for laboratory blanks.

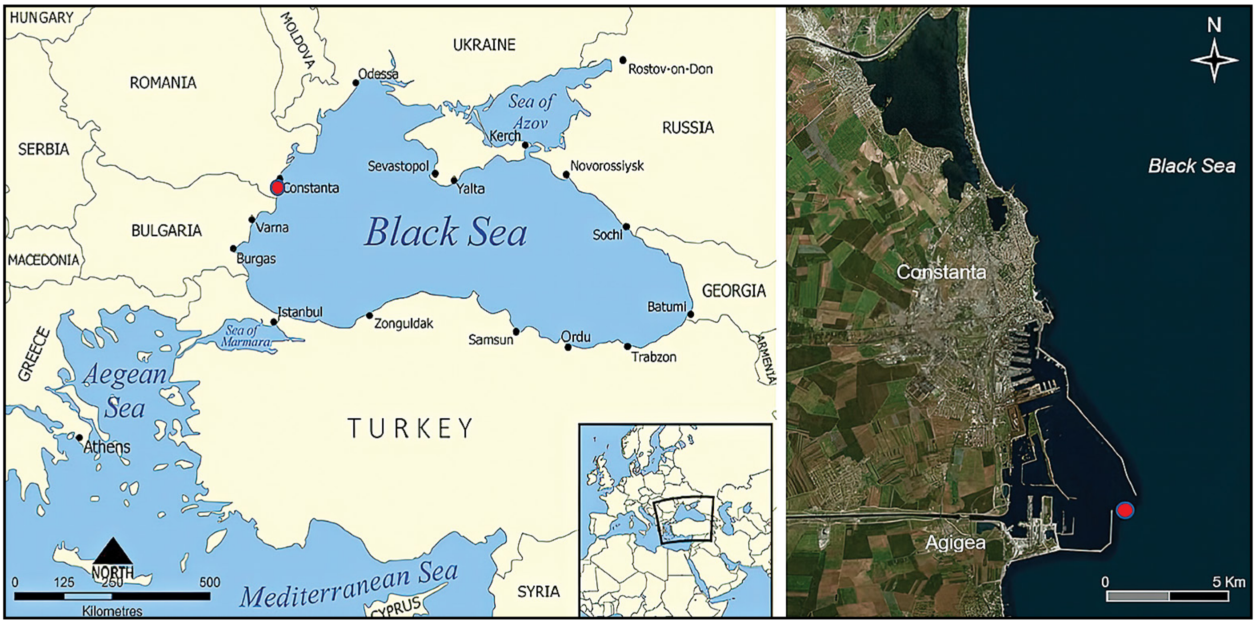

Fig. 1. Sampling location of the *Mytilus galloprovincialis* (map source: maps.nls.uk)

Fig. 2. Sample preparation process including mussel morphological characterization (**a**), digestion (**b**), pH neutralization (**c**), filtration (**d**) and visual inspection of membranes (**e**).

The microscopy blanks consisted of filter membranes left uncovered during the visual inspection of the samples, used for airborne contamination control. Corrections were applied to each sample, considering the morphology and colours of the particles observed in blanks. All of the laboratory procedures described here were conducted inside a chemical hood.

3. RESULTS AND DISCUSSIONS

In all 17 analysed individuals of *M. galloprovincialis* were identified 117 MPs larger than 50 µm. Although only counted fibers were taken into consideration for the present study, other observed and suspected to be plastic-based particles – with a typical morphology, colour, and external aspect - are also described as preliminary data.

The dominant morphology of particles identified in mussels is fiber, including fiber clumps, having an abundance of 89.17%, respectively 91.32% of total (Fig. 3). Minimum concentrations were determined for fragments (3.43%), flakes / films (1.78%) and spherules (0.07%). The sampling area, located in the proximity of the Constanța harbour is characterized by a high vessel traffic that usually releases a considerable amount of MPs as flakes, fibers and fragments. These microparticles are released by the weathering of vessel hull paint coating (up to 35% of the microdebris input), ropes and other items (Turner, 2021; Dibke *et al.*, 2021).

The average concentration of the MFs identified in all 17 individuals is 6.05, although two individuals were identified with 0 MFs in the soft tissue. The maximum number of fibers observed in one mussel is 14. Regarding the colouring, black fibers are the most encountered (39.8%), other colours being rarely encountered (Fig. 4).

Concerning the length and weight of mussels observed in the present study, the concentrations of the MFs does not show any trend correlated to size or mass (Fig. 5).

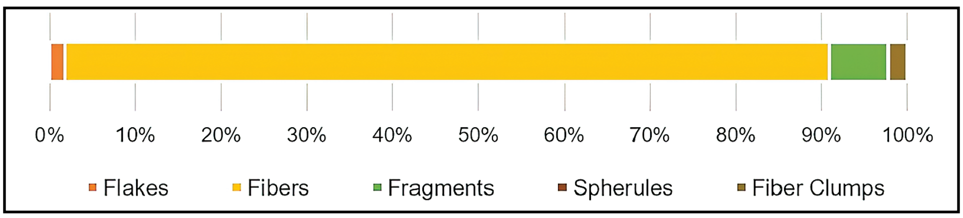

Fig. 3. The abundance of MPs morphology types in *M. galloprovincials* individuals.

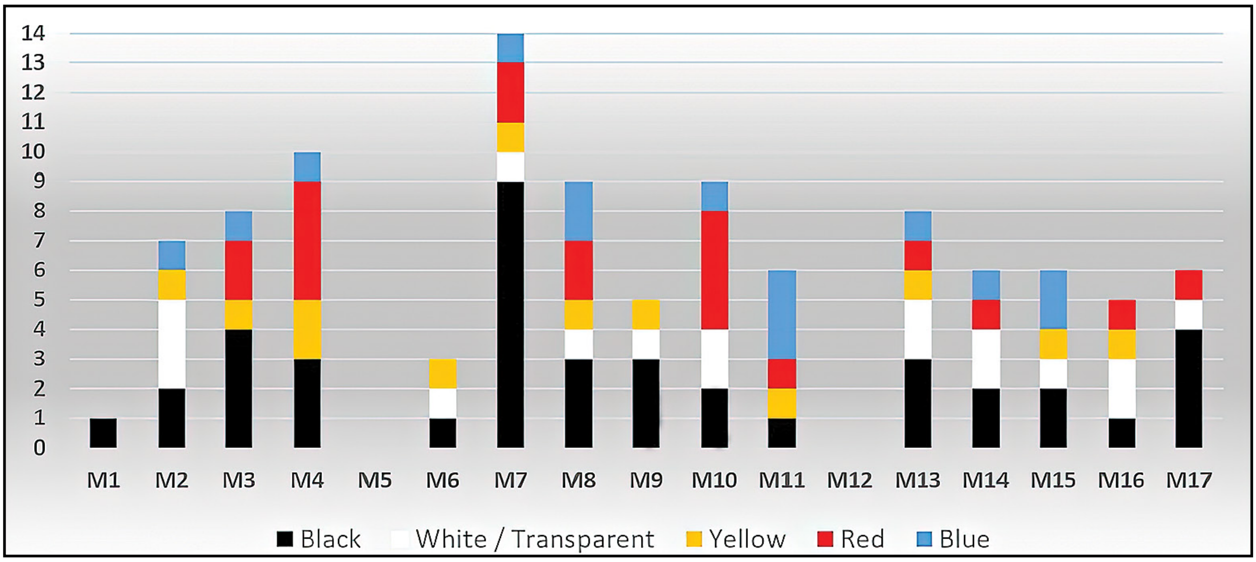

Fig. 4. The number of MFs by colours of each individual of *M. galloprovincialis*.

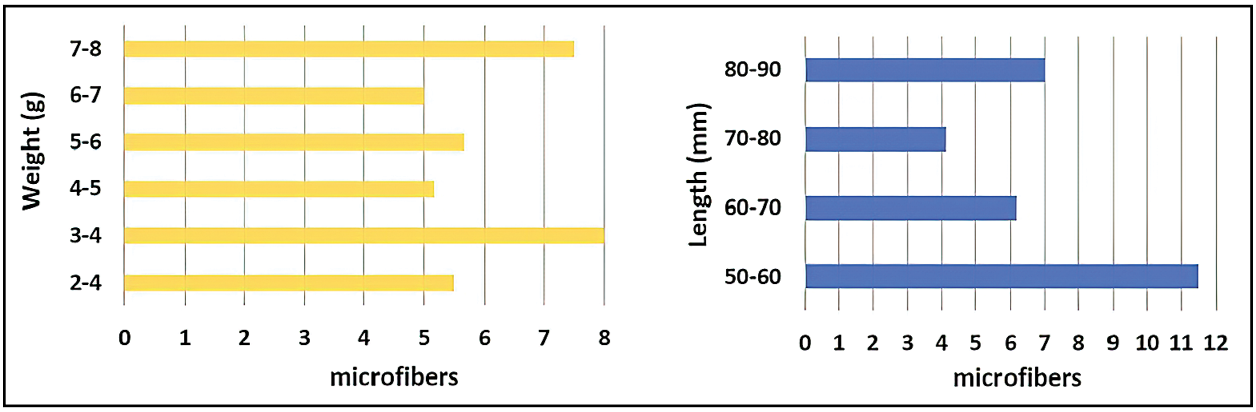

Fig. 5. Average number of MFs ingested by *M. galloprovincialis* per different weight classes (left) and per different size classes (right).

A reasonable argumentation may involve the position of the individuals in the mussel layer, as well as each individual position against the water current. Therefore, the highest concentrations of MPs / MFs may be found in individuals close to the edge of the bed or, presumably if the filtration area of the mussel is orientated against the current. Thus, the smallest individuals of *M. galloprovincialis* (3 - 4 g and 50 – 60 cm) are identified with highest values of MFs concentrations, possibly due to the individual disposition to absorb higher volumes of sediments and MPs than the other individuals.

The identified particles were categorized into two groups (Fig. 6), which can offer a better overview of the different morphological types of the whole MPs concentrate. Although other particles than fibers are not certified as plastic polymers, their occurrence in *M. galloprovincialis* soft tissue vary according to their sizes. Particles smaller than 1 mm were identified with a higher percentage of fragments and flakes than in the MPs category larger than 1 mm. Therefore, it can be assumed that the rate of ingestion of the mussel may be higher for small-sized particles.

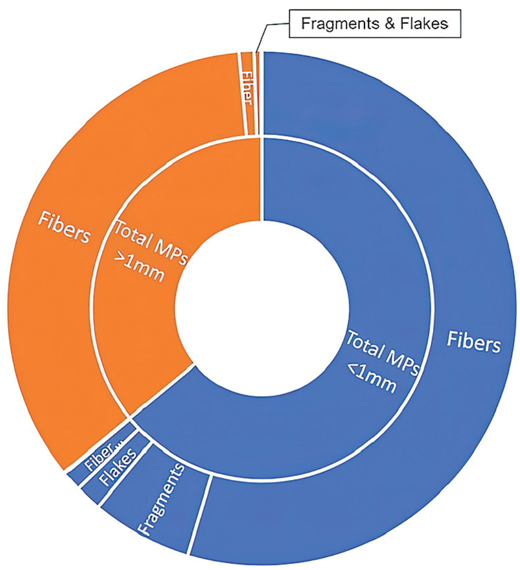

Fig. 6. The occurrence of MPs, smaller and larger than 1 mm, in all analysed mussels, for each identified morphological type.

The interaction between benthic species as *Mytilus galloprovincialis* and seafloor plastic litter was studied recently by Snigirova *et al.* (2020), who analysed the macrozoofouling on plastic bags and bottles. The results show a high ability of *M. galloprovincialis* to develop on these surfaces with an abundance of 838 ± 497 ind.·m^{-2} on plastic bags and 329 ± 152 ind. m^{-2}, on bottles. Therefore, it can be assumed that *M. galloprovincialis* is less affected by an artificial environment than other species of molluscs.

An overview of the literature regarding MPs identified in *M. galloprovincialis* from the Black Sea shows that the quantitative results of the present study were significantly higher than the values of Gedik & Eryaşar (2020). These authors identified an average of 0.69 MPs per individual of *M. galloprovincialis* and the polymeric studies show the presence of polyethylene terephthalate (32.9%), polypropylene (28.4%) and polyethylene (19.4%) (Fig. 7).

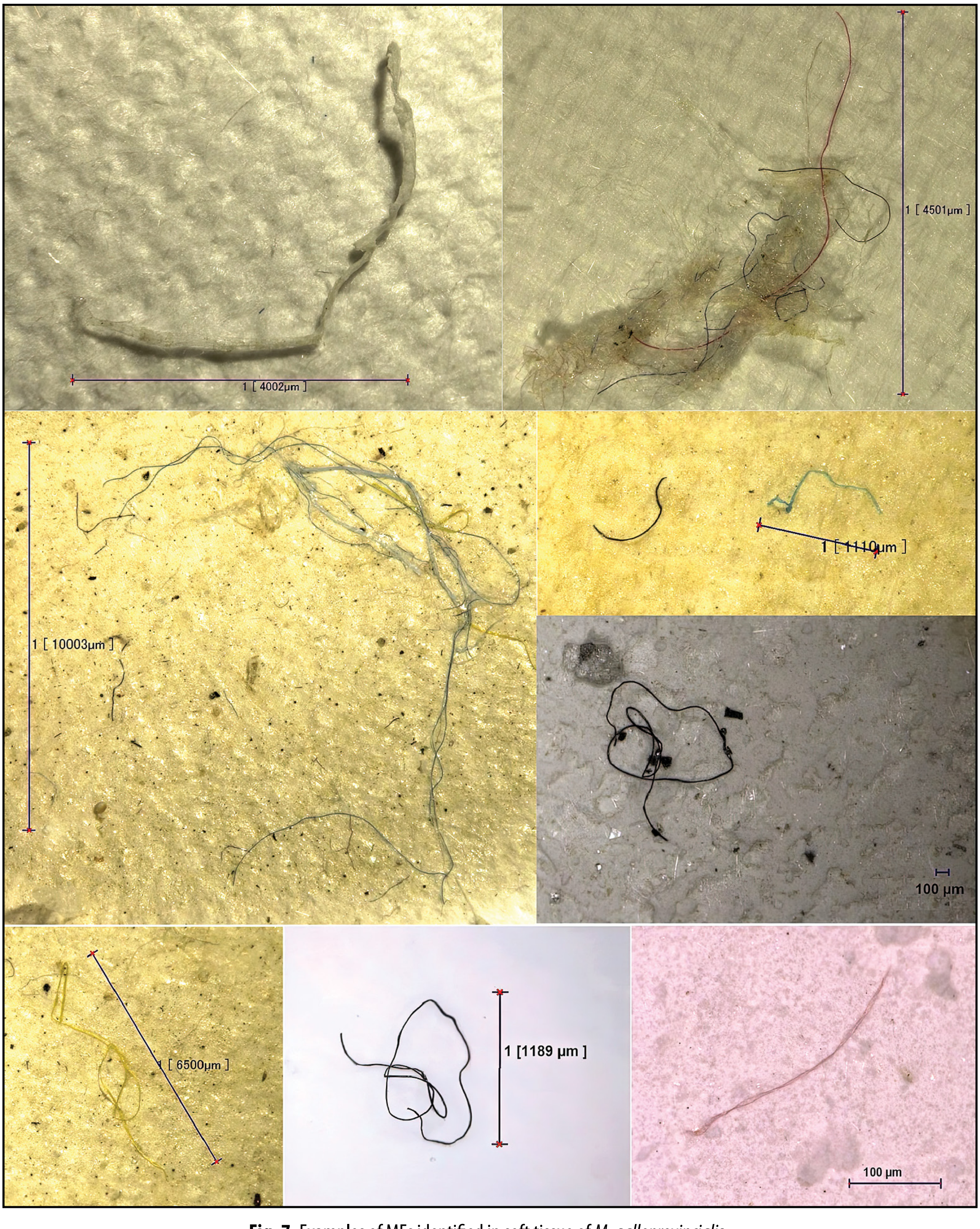

Fig. 7. Examples of MFs identified in soft tissue of *M. galloprovincialis*.

Moreover, the morphological types of the MPs identified in the mussels collected from several sites from the Turkish coast (Southern Black Sea, Marmara Sea and eastern coast of Aegean Sea) were dominantly fragments, while the fibers were observed with a low concentration of 8.4%. The differences of MPs abundance and their morphological characteristics may be explained by the proximal sources of pollution, as well as the present natural factors in the local ecosystem.

As other ecologically and commercially important species, *M. galloprovincialis* takes up large amounts of pollutants that conduct to bioaccumulation. Thus, making it a potential source of MPs in the human alimentation, along with other pollutants absorbed by plastic particles. The fibers observed in the soft tissue of mussels (Fig. 7) may have several premises of provenance: *i)* Danube origin - particles are discharged by the river and are transported to the south of the Black Sea basin, and along the fluvial and marine transport, they absorb high quantities of pollutants (Issac & Kandasubramanian, 2021), due to the long exposure in aquatic environment; *ii)* local origin - particles are discharged by proximal sewers and waste water treatment plants (WWTPs), then the particles absorb limited amounts of other pollutants, and *iii)* other sources represented by MPs transported or originated from marine currents, airborne, or derived from vessel hull paint coating, particles that rarely are isolated for detailed studies.

5. CONCLUSIONS

A significant higher concentration of MPs and, implicitly, MFs were identified in *M. galloprovincialis* in comparison with results from other similar studies from Southern European marine areas. In the average concentration of 6.05 MPs per individual a mean concentration of 89.17% fibers was identified, which reflects the typical pollution forms of the study area.

The increased abundances might be caused by high quantities of litter discharged by the Danube into the Black Sea, the poor plastic waste management in the harbour area, and of the WWTPs of the Constanta city. On the other hand, the studies regarding MPs contamination show that *M. galloprovincialis* and other mussels are the most contaminated in comparison to other benthic species.

This study offers a preliminary analysis of the microfibers ingested by several individuals of *Mytilus galloprovincialis* collected from an expected high polluted area - Constanța harbour. For an accurate overview of the MPs ingestion by mussels and other ecological and economical species key locations need to be monitored and further qualitative and quantitative analysis are required.

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