



ASSESSMENT OF THE BIOACCUMULATION OF MICROPLASTICS IN THE BLACK SEA MUSSEL *MYTILUS GALLOPROVINCIALIS* L., 1819

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ABSTRACT:

The accumulation of microplastics in the world oceans is one of the main global problems of the modern world. The Black Sea is the main source of seafood for people from several countries, including Bulgaria. The pollution of the Black sea is a result of various factors, especially anthropogenic. The aim of this study was to determine the presence and accumulation of microplastics in the Black Sea mussel *Mytilus galloprovincialis* L., 1819, harvested from different locations along the northern Bulgarian Black Sea coast. Within each microplastic morphological group, three size classes were recognized: 100-200 µm, 25-100 µm, and ≤ 25 µm. Microplastics were found in the soft tissues of all studied specimens but in a different ratio of pellets, fibers and fragments. Generally, the pellets were the most often registered particles, followed by irregularly-shaped fragments, whereas fibers were less numerous. The dominant part of the isolated plastics are made of polyethylene (PE), and polyethylene terephthalate (PET). Our results pointed out serious pollution with plastic particles in the Bulgarian Black Sea aquatory, which in the future may seriously affect the health of the mussel population and also human health.

Keywords: ocean, pollution, anthropogenic pressure, bivalves, food resources, sea water,

INTRODUCTION

One growing threat affecting aquatic ecosystems around the world is the pollution with plastics. This kind of pollution has led to intensive research in this direction in recent years. It has been estimated that between 4.8 and 12.7 million metric tons of land-based plastic waste entered the oceans in 2010 [1]. Larger particles came mostly from various land depoes of wastes. These large particles may often break down into smaller fragments under the impact of various physical and chemical factors such as mechanical decomposition, UV light or biological processes [2]. These small plastic fragments are called microplastic particles and include all fibers and polymers with dimensions smaller than 5 mm [3]. Microplastics can

also have other origins, for example, toothpaste, detergents, paper bleaches, waste disposal sites, tourism activities, fishing nets, etc. There is a wide range of plastics including smaller sizes less than 5 mm with various applications that include pellets, plastic fragments, scrubbers, films, flakes, and fishing gear [4]. According to some scientists, rivers and lakes are the main routes through which microplastic enters the seas and oceans and leading to a significant increase in their concentrations [5, 6]. Risks for aquatic microorganisms are related to the direct ingestion of microplastic pellets, fibers and particles and their additives as persistent organic pollutants. The problem of microplastics already affects zooplankton, invertebrates, shellfish, fish and birds [7]. The most numerous studies that have been carried out so far on the presence of microplastics are from the contents of the gastrointestinal tract of fish [8, 9]. Microplastic pollution in the Black Sea has also been reported [10-13]. The northwestern part of the Black Sea is the most polluted section according to Cincinelli A. et al. [14]. The authors found that microplastic particles were 10 times more abundant in surface waters compared to the depth of the sea. The detection of microplastics in the Black mussel (*M. Galloprovincialis*) from the Mediterranean region was first reported by Gedik K. et al. [15]. There is evidence that microplastics can affect the digestion of mussels and also affect sex hormones [16]. Through seafood consumption, microplastics can also reach humans and lead to serious problems to the human body [17]. At the moment, there are no data on the accumulation of microplastics in the body of the Black mussel from the Bulgarian aquatory of the Black Sea. The aim of this paper was to determine the presence and accumulation of microplastics in *M. galloprovincialis* harvested from different locations along the Bulgarian Black Sea coast.

MATERIALS AND METHODS

Sampling

Place and duration of the study: The study was conducted at the Department of Biology, University of Shumen, Bulgaria, from January 2021 until March 2021. The sam-

ples were collected from the regions of Tylenovo (43.4947° N, 28.5844°E), Kavarna mussel farm (43.4036°N, 28.3886°E), and Bolata (43.3817°N, 28.4709°E) -Datum WGS 84 (fig. 1).

Fig. 1. Sampling locations at the Black sea coast: grey represents Kavarna; black represents Bolata; white represents Tylenovo



Collection of the samples

After collection, the samples (about 10 kg) were immediately refrigerated (4°C) and transported to the laboratory for analysis.

Tissue digestion and microscopic inspection

In order to avoid MPs cross-contamination during analysis, only glass vessels and metal tools were used, and before the procedure, all laboratory glassware and tools were rinsed three times with filtered ultrapure water. All used reagents were preliminarily tested for the presence of possible MPs pollution using the blank sample method. Tissue digestion was performed after [18]. The shell of each clam was removed, and the soft tissue was finely chopped and placed in an Erlenmeyer flask, which was covered by watch glass to prevent contamination. Five ml of 1M NaOH was added, and samples were heated to 50°C on a tempera-

ture-controlled shaker for 15 min. Then 17.5 ml HNO₃ (65 %) and 2.5 ml ultrapure water was added (final concentration of HNO₃: 10 M; ~49 %), and the samples were heated at 50°C for another 15 min. Thereafter the temperature was increased to 80°C for 15 min for removing the resistant suspended solids. Before filtration, the samples were diluted at a 1:2 ratio (v:v) with ultrapure water heated to 80°C. Filtration was performed on a vacuum pump through a cellulose nitrate filter (Ø 47 mm, 8 µm pore size, Sartorius Stedim Biotech, Goettingen, Germany). The flasks and filtration devices were rinsed with warm ultrapure water to ensure no loss of material. The filters were dried for 24 h at 37°C and subsequently analyzed.

For microplastics identification, the filters were visually observed under a stereomicroscope SZM-D (OPTIKA Italy, 1000x magnification) coupled with Dino-Eye AM-4023X eyepiece camera (Dino-Lite ANMO Electronics, Taiwan). The digital images were examined by using "DinoCapture 2.0" software and the plastic particles were quantified by size (≤ 25 µm, 25-100 µm, and 100-200 µm) based on their largest cross section and shape (pellets, fibers and fragments).

Polymer identification

FTIR spectroscopy was used to explore the chemical characteristics of microplastics in order to identify the polymers. When infrared radiation passes through the sample, some rays passed through the sample while the rest was transmitted; as a result, the generated spectrum characterized the molecular structure of the samples. It is very beneficial to determine the polymer composition because different samples produce different spectra due to various chemical structures. The samples were prepared for FTIR spectroscopy using potassium bromide (KBr) as the medium to identify the chemical composition of microplastics. The potassium bromide was mixed and meshed with microplastic particles using a proportion of 99% KBr and 1% of sample considering all precautionary measures during the sample preparation. Potassium bromide is an appropriate medium because it lacks absorption or spectral peaks from 250 to 4000 cm⁻¹ in FTIR spectroscopic analysis. Varian 2000 FTIR spectrometer (FT-IR Afinity-1 Shimadzu) model instrument was used for the microplastic polymer identification in the present study. The spectra were acquired across the range from 400 to 4000 cm⁻¹ at a resolution of 4 cm⁻¹ to enhance the accuracy of the results. The microplastic particles were extracted from the filter paper using tweezers. The obtained spectra were correlated with the reference spectra of the database from the literature and matched to identify the types of polymers. All results were performed with three replicates.

RESULTS

A total of 50 mussels from each site were analyzed for the presence of microplastics. The presence of microplastics was detected in 100% of the specimens from each point. The microplastic particles ranged from 10 to 40 particles per individual (fig. 2 and tables 1-3).

Fig. 2. Stereomicroscope picture of morphological types of microplastics recognized in the studied mussels from: **A)** region of Tylenovo; **B)** region of Kavarna; **C)** region of Bolata. The pictures were performed by the use of stereomicroscope OPTIKA (Italy) and DinoEye, Eyepiece camera, USB, 1.3 megapixels, up to 5 megapixels.

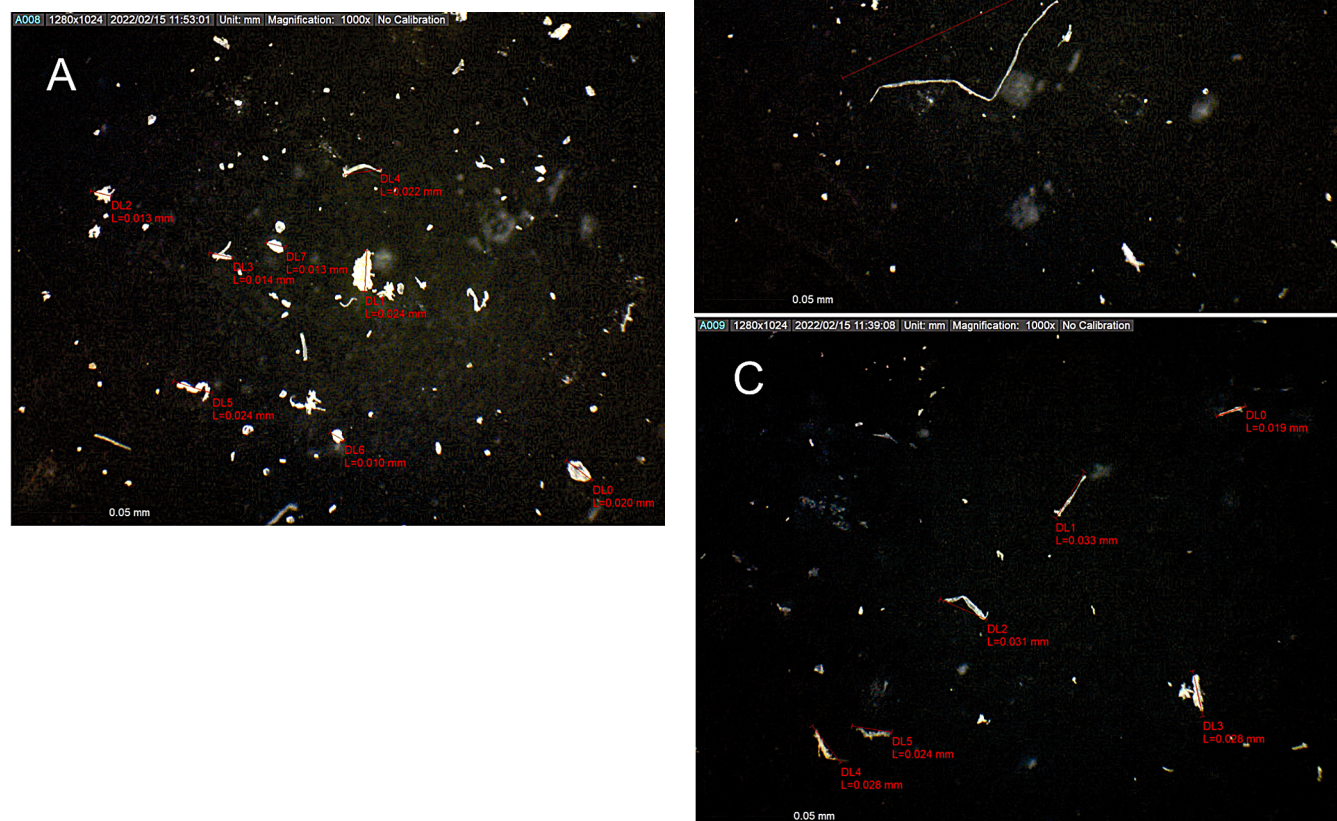


Table 1. Number of microplastic particles in samples from the Tylenovo region

location	Tylenovo - 21. 01 – 20. 03. 2021								
	pellets			fibers			Irregular form		
Specimen/datum	≤ 25 μm	25-100 μm	100-200 μm	≤ 25 μm	25-100 μm	100-200 μm	≤ 25 μm	25-100 μm	100-200 μm
1 /21.01.21	230	0	0	4	0	0	6	1	0
2/21.01.21	129	0	0	3	0	0	5	1	0
3/21.01.21	130	0	0	1	0	0	6	0	0
1/ 20.03.21	234	0	0	4	0	0	7	1	0
2/20.03.21	133	0	0	2	0	0	5	1	0
3/20.03.21	134	0	0	3	0	0	6	0	0

The largest number of pellets (with sizes $\leq 25 \mu\text{m}$) was detected in the samples from the Tylenovo area (table 1, fig. 2 A). Pellets of larger sizes were not found in these specimens. The filament particles were less abundant and they were $\leq 25 \mu\text{m}$ in size. The number of irregularly shaped

microplastic particles was mainly $\leq 25 \mu\text{m}$, but 1 or 2 particles per specimen were with sizes between 25-100 μm . In terms of seasons, the number of microplastic particles increased in the start of the spring compared to that detected in January.

Table 2. Number of microplastic particles in samples from the Kavarna farm

location	Kavarna - 21. 01 – 20. 03. 2021								
	pellets			fibers			Irregular form		
Specimen/datum	≤ 25 μm	25-100 μm	100-200 μm	≤ 25 μm	25-100 μm	100-200 μm	≤ 25 μm	25-100 μm	100-200 μm
1 /21.01.21	210	0	0	0	0	0	5	0	0
2/21.01.21	111	0	0	0	0	0	5	0	0
3/21.01.21	19	0	0	0	0	0	6	0	0
1 /20.03.21	213	0	0	0	0	2	7	0	0
2/20.03.21	114	0	0	0	0	2	7	0	0
3/20.03.21	114	0	0	0	0	3	6	0	0

The largest number of pellets isolated from in the samples from the Kavarna farm were with sizes ≤ 25 μm (table 2, fig. 2 B). Pellets of larger sizes were not found. The number of filaments was the least and they were between

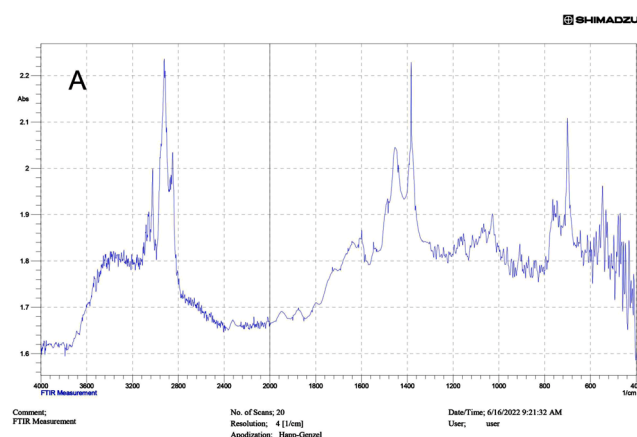
100 and 200 μm in size. The biggest number of irregularly shaped microplastic particles was of those ≤ 25 μm in size. The seasonal display was similar of that detected in the region of Tylenovo.

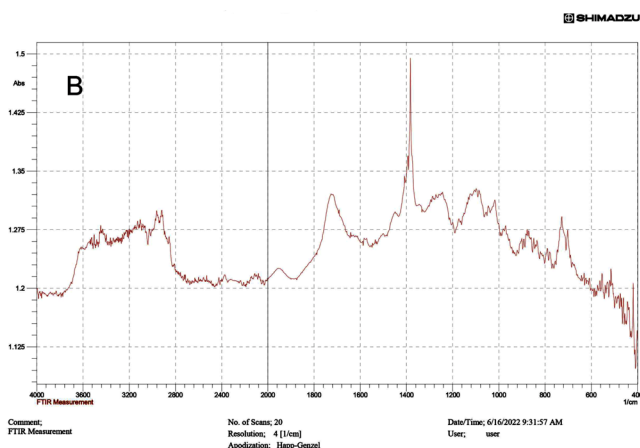
Table 3. Number of microplastic particles in samples from Bolata region

location	Bolata - 21. 01 – 20. 03. 2021								
	pellets			fibers			Irregular form		
Specimen/datum	≤ 25 μm	25-100 μm	100-200 μm	≤ 25 μm	25-100 μm	100-200 μm	≤ 25 μm	25-100 μm	100-200 μm
1/21.01.21	27	0	0	0	0	0	1	0	0
2/21.01.21	15	0	0	0	0	0	1	0	0
3/21.01.21	15	0	0	0	0	0	1	0	0
1/20.03.21	27	0	0	0	2	0	2	0	0
2/20.03.21	16	0	0	0	2	0	2	0	0
3/20.03.21	17	0	0	0	3	0	2	0	0

The largest number of pellets in the specimens from Bolata area were of these with sizes ≤ 25 μm (table 3, fig. 2 C). Pellets of larger sizes were not detected. The main number of filaments was between 25-100 μm in size. The number of irregularly shaped microplastic particles was the smallest and these were mainly ≤ 25 μm in size. The seasonal trend in detection of particles detected for Tylenovo and Kavarna was confirmed also for Bolata region. From the obtained results, it could be concluded, that with the warming of the sea water, the number of microplastic particles increases. Generally, the most particles were found in the mussels from Tylenovo region and the least in the mussels from Bolata region.

Our FTIR analysis indicated clearly, that the types of polymers that were identified in the Black mussel were polyethylene and polyethylene terephthalate (fig. 3). More specifically, in the investigated mussels, 75% of the microplastics were defined as PE, and 15% as PET. The rest of the microplastics were not categorically identified.

Fig. 3. FTIR spectrum of the microplastic particles isolated from the Black mussel: **A)** polyethylene (PE) spectrum; **B)** polyethylene terephthalate (PET) spectrum.



DISCUSSION

This study demonstrated the presence of microplastics in the Black mussel *M. galloprovincialis* in the waters of the northern section of the Bulgarian Black Sea aquatory. The highest percentage of microplastic particles was found in the Tylenovo region and the lowest in the Bolata region. As a commercial product, the black mussel is grown and used for a food resource by the Bulgarian population. The Black mussel showed a relatively high capacity of accumulation of microplastics in its edible parts. In correlation with our data, according to [19] Black mussels and sardines accumulate the highest percentage of microplastics. The same authors showed that in a study of microplastic particles in the Black mussel from the Ionian Sea region, the most abundant category of microplastic particles were found to be fragments, followed by filaments. The number of isolated fragments was 77.8% of the total number of microplastic particles. Besides the optical detection, the authors also confirmed the presence of plastics by FTIR analysis. Similar increases in the amounts of microplastic particles were reported by other authors for several habitats: Giglio Island, Italy [20], the French Atlantic coast [21], and UK [22]. On the basis of published data and on the basis of our results, we propose that in the world's oceans there is a predominance of microplastic objects with small sizes $\leq 25 \mu\text{m}$. Regarding the size of microplastic particles in the Black mussel, most isolated plastics were exactly those with sizes $\leq 25 \mu\text{m}$. The feeding strategies of different sea organisms also impact the potential for accumulation of microplastics [23]. We propose that this is related to the filter feeding mode of the bivalves. There is evidence that mussels can ingest microplastic particles of smaller sizes than benthic fish species [19].

Digka N. et al. [19] demonstrated that the number of microplastic parts was higher in farmed than in wild mussels, despite the fact that farmed specimens were larger in size. There is data in the literature that show the opposite - lower amounts of microplastics in farmed mussels than in wild mussels [24]. In our case, no correlation between the amount of microplastic particles in mussels harvested from

the farm in Kavarna and mussels collected from wild habitats, such as Tylenovo and Bolata, was found. The mussels that we examined were of similar size and weight. We found no relationship between size and the amount of microplastic particles. It is possible that the habitat characteristics are crucially important for the aggregation of microplastics in the mussels, but also the proximity to the more polluted area (in our case the Danube River). Research data indicated that mussels are considered a very good indicator of microplastics in coastal marine areas. A recent study showed that the Chinese coast is abundant with microplastics, which have been found in large quantities in shellfish [25].

In our study, the type of detected microplastic particles was mainly fragments (pellets) and to a lesser extent fibers. Similar data was provided by other authors [19, 26-27]. However, most studies on mussels show opposite results - some authors [28-30] report on the presence of more fibers than pellets and fragments. A possible explanation for these differences may be related to the different waste sources in different parts of the world. It is well known fact, that the Black Sea is subject to intensive pollution, and the territory around the Romanian coast is seriously affected by wastes transported by the Danube River. Since we noticed that with distance from the Danube, the number of microplastic particles decreases, we assume that the pollution comes, to a large extent, from the Danube delta, although we do not rule out anthropogenic pollution from tourism. Tourism pressure increased seriously in recent years. The pollution may be related to the tourism with used and discarded plastic bottles, cups, stirrers and plastic bags, which easily break into fragments in seawater. The dominant part of the isolated plastic particles consists of polyethylene (PE), and polyethylene terephthalate (PET). Polyethylene is the basis of plastic bottles, cups, stirrers, and plastic bags. This polymer is light and floats on the surface because its density is lower than that of water. Conversely, polyethylene terephthalate is denser than water and is more likely to sink and accumulate in benthic organisms. Nevertheless, our results indicate that the fragments (the pellets and the irregularly shaped particles) were of polyethylene, and the fibers were made of PET. Probably due to sedimentation of PET at the bottom, the amount of such filaments in the Black mussels was found to be of lesser volume. A study from this year [31] on the Black mussel from the Sea of Marmara, reported similar to our result concerning PET. According to the authors, the main microplastic pollutant of the Sea of Marmara is the Black sea. The main microplastic pollution can be attributed to industrial factors. Mussels rely on seawater filtration and possess a high capacity to absorb microplastics. A new study provided another hypothesis, according to which adherence and not ingestion, led to the accumulation of microplastics in such organs of the mussels that are

not involved in the feeding process [32]. Having in mind the specific anatomy and physiology of the Black mussel, we propose that both, ingestions, as well as adherence, may be involved in the accumulation of microplastics in the tissue of these bivalves.

CONCLUSION

This study is the first investigation on the presence of microplastic particles in *M. galloprovincialis* from the Bulgarian Black Sea aquatory. The results indicated high amounts of microplastic particles in the mussel, which is consistent with filter-feeding and attached lifestyle. The presence of microplastics indicates serious pollution of

the Bulgarian Black Sea and also indicates the possible decrease in the quality of the Black mussels as a food product. Regular monitoring is needed in the future for improving our knowledge of the accumulation of microplastics in the Black sea inhabitants.

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