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Research Article

The assessment of the bioaccumulation of microplastics in key fish species from the Bulgarian aquatory of the Black Sea

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Abstract

One of the main problems of the world's oceans, reported by many scientific studies, is the microplastic pollution. Within the Black Sea, one of the main sources of pollution is the same, which is caused by the diverse anthropogenic activities. The present study demonstrated detailed microplastics contamination of in five fish species important for the commercial fishing (Garfish, Mullet, Knout goby, Pontic shad and Mediterranean horse mackerel). They were collected from the Sozopol area on the Bulgarian Black Sea coast. Within each microplastic morphological group, three size classes were recognised: 100–200 μm, 25–100 μm and ≤ 25 μm. Microplastics were found in all studied tissues of the fish, but in varying proportions of pellets, fibres and fragments. Pellets were most frequently isolated, followed by irregularly-shaped fragments and fibres were the least numerous. The bulk of insulated 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 fish population and also human health.

Key words: Anthropogenic pressure, bivalves, food resources, ocean, pollution, sea water

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Introduction

The pollution of the world oceans by plastic waste has been reported in many scientific papers in recent years. There is scientific evidence of plastic particles detected even in the polar regions (Corsi et al. 2021). Plastics, as water-insoluble solid polymers, are widely distributed and used in a wide range of industries due to many advantages – for example, flexibility, rigidity, temperature resistance and chemical stability (Herbort et al. 2018). It is reported that the annual share of plastic production has reached 280 million tonnes. A number of scientific papers have reported the amount of microplastic particles (< 5 mm) in seawater and sediments to be around 4.8 and 12.7 million tonnes (Kumar et al. 2021). These particles impact life in the seas and oceans. Microplastics (MPs) have recently been defined as "a synthetic solid particle or polymer matrix of industrial origin, not soluble in water, of regular or irregular shape and measuring between 1 μm and 5 mm " (Frias and Nash 2019). Plastics are categorised into three groups (macro

(> 100 mm), meso (1–10 mm) and micro (1–1000 mm). MPs can be produced as microparticles for personal care and cleaning products or occur due to the fragmentation of macroplastics in aquatic ecosystems by environmental factors, such as UV radiation and wave abrasion (Zhang et al. 2021). The main sources of microplastic particles are the plastic bags, bottles and straws that are widely used. They are not biodegradable and remain in the environment for hundreds of years (Plastics Europe 2020). For this reason, the fight against microplastic pollution is one of the main environmental priorities of the United Nations Environment Programme (UNEP 2016). Microplastic particles have been reported in the bodies of mammals, shellfish, fish, birds and decapods, particularly in the Northern Hemisphere. Entering the organisms of bivalves and fish through the food, microplastics cause disturbances and damage - from oxidative stress to behavioural disturbances. Other negative impacts, such as decline in the swimming capacity in fish, reduction of reproduction, inhibition of growth and others were reported (De S´a et al. 2015; Wang et al. 2016; Anbumani and Kakkar 2018). Once inside living organisms, microplastic particles have the ability to absorb and release toxic chemicals/organic or inorganic additives, such as bisphenol A, PCBs and DDT, which creates additional potential risks to human health. On the other hand, microbial biofilms can form on microplastic particles, which contain microbial pathogens and have an adverse effect on animals and humans (Leslie et al. 2013; Eerkes-Medrano et al. 2015; Koelmans et al. 2019). The pollution of the Black Sea, known as one of the most degraded marine ecosystems according to BSC (2007), makes this semi-enclosed sea more vulnerable to microplastic pollution. On the other hand, the estuaries of the Danube, Dnieper, Dniester and Don Rivers further pollute the Black Sea with plastic waste (BSC 2009). In their work, Lechner et al. (2014) report that 4.2 tonnes of plastic/day reach the Black Sea via the Danube River. The Black Sea is surrounded by several industrialised countries and is an important place for both small-scale and large-scale fisheries (BSC 2007). There are several studies in the region on macroscale plastics and they showed a large amount of macroplastic pollution along the Black Sea coasts (Simeonova et al. 2017; Terzi and Seyhan 2017; Oztekin et al. 2020; Terzi et al. 2020; Erùz et al. 2022). So far, more than 890 fish species have been found to ingest MPs and the abundance of MPs has been reported to be highly variable amongst different fish species (Bowen et al. 2021). In addition, out of a total of 323 fish species found to ingest MPs in different regions of the world, 262 were reported as commercial species. In the modern world, people are increasingly interested in a healthy lifestyle and healthy eating. Fish has been one of the staple foods for humans for decades. In many cases, it is recommended as a healthy food and source of very useful omega fatty acids, vitamins and minerals. On the other hand, for many people living on the Black Sea coast, fishing is the main way of livelihood. There are data on the impact that microplastic particles have on the health of marine life, but there are no data on what microplastics would do to the human body and how this would affect human health and the health of our children in the future. Popular science films have recently emerged that clearly show that people who eat seafood have circulating microplastics in their blood. There are no data on studies from the Bulgarian Black Sea water area regarding the condition and amount of microplastic particles in fish. This is the first pilot study on the composition of microplastic particles in different parts of key fish species from the Bulgarian Black Sea water area.

Materials and methods

Sampling location and duration of the study

The study was conducted at the Department of Biology, University of Shumen, Bulgaria. Probes from different anatomical structures were sampled in Garfish (*Belone belone*), Mullet (*Mugil cephalus*), Knout goby (*Mesogobius batrachocephalus*), Pontic shad (*Alosa immaculata*) and Mediterranean horse mackerel (*Trachurus mediterraneus*) caught in the region of Sozopol in February 2022.

Collection of samples

After catching, the fish was immediately transported to the laboratory at 4 °C, where it was dissected. Samples were taken from the skin, musculature, gills, intestinal tract and caviar and these probes were subjected to analysis. The probes were obtained from three specimens of each fish species, which were similar in size.

Tissue digestion and microscopic inspection

Tissues obtained from fish were minced according to Roch and Brinker (2017). To prevent contamination with microplastics, work was undertaken only with glass and metal tools. All tools used were rinsed with bidistilled water before use. Similarly, the reagents used in the analysis were tested for the presence of microplastics using the black sample method. After cutting each sample, 5 ml of 1M sodium hydroxide (NaOH) were added to it and heated to 50 °C for 15 minutes. The temperature was controlled at all times. A total of 17.5 ml of nitric acid (HNO_3) (49%) and 2.5 ml of ultrapure water were added to the NaOH-treated sample. This was followed by reheating to 50 °C for 15 minutes, with the temperature again controlled. The whole sample was heated for 15 minutes, but at the temperature up to 80 °C, which aimed to remove the remaining suspended solids from the sample. The next step was filtration, after which the samples were diluted 1:2 (v:v) with ultrapure water heated to 80 °C. Filtration was performed through a cellulose nitrate filter (Ø 47 mm, pore size 8 μm, Sartorius Stedim Biotech, Goettingen, Germany). The glassware used in the experiment was also filtered and washed. Nitrocellulose filters were dried for 24 h at 37 °C and then analysed for MPs. For microplastics identification, the filters were visually observed under a stereomicroscope SZM-D (OPTIKA Italy, 1000× magnification) coupled with Dino-Eye AM4023X 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 ìm), based on their largest cross section and shape (pellets, fibres and fragments).

Polymer identification

To identify the polymer, we used FTIR spectroscopy according to Ibryamova et al. (2022). All results were done in triplicate.

Results

A total of three specimens from each species was analysed for the presence of microplastics. In all tables, averaged results from the three samples were plotted. The presence of microplastics was detected in 100% of the specimens. The microplastic particles ranged from 10 to 40 particles per individual (Fig. 1, Tables 1–4).

The microscopic pictures in Fig. 1 demonstrate the types of MP particles which were isolated - irregularly shaped (Fig. 1A, pellets (Fig. 1B and fibres (Figs. 1C, D) and also different colours, which are probably due of the various impurities and trace elements used by the manufacturer for the specific type of microplastic.

The data in Table 1 showed that, in the Garfish, the pellets with sizes ≤ 25 µm isolated from the gills, muscles and skin of the fish were predominant. Fibres of the same size ≤ 25 µm were isolated from the gills only. Irregularly- formed MPs were isolated in all parts of the fish, most notably from the skin and gastrointestinal tract. As in the skin, particles with larger sizes (25–100 µm) were also found (Fig. 1A). MPs with sizes of 100–200 µm were not isolated.

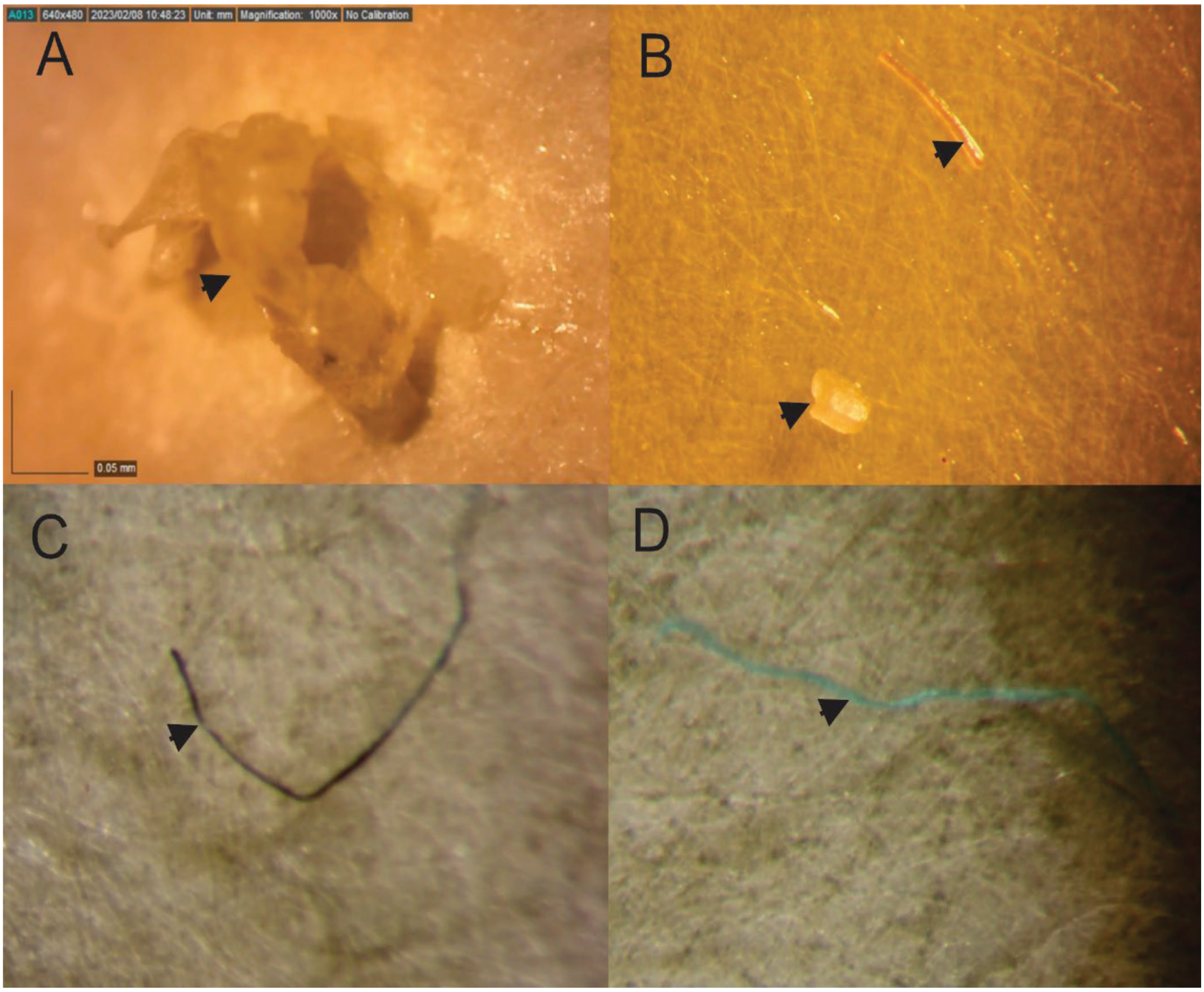

Figure 1. Stereomicroscope picture of morphological types of microplastics (arrowheads) recognised in the studied species from: A) *B. belone*; B) *M. batrachocephalus* C) *A. immaculata* and D) *T. m. ponticus*.

Table 1. Number of microplastic particles in samples from *B. belone*.

Species	*B. belone*								
Form of MPs	Pellets			Fibres			Irregular form		
	≤ 25 µm	25–100 µm	100–200 µm	≤ 25 µm	25–100 µm	100–200 µm	≤ 25 µm	25–100 µm	100–200 µm
skin 1	0	0	0	0	0	0	4	2	0
skin 2	0	0	0	0	0	0	1	0	0
skin 3	0	0	0	0	0	0	4	0	0
meat 1	1	0	0	0	0	0	0	0	0
meat 2	0	0	0	0	0	0	1	0	0
meat 3	2	0	0	0	0	0	1	0	0
gills 1	10	0	0	6	0	0	0	0	0
gills 2	7	0	0	6	0	0	0	0	0
gills 3	6	0	0	9	0	0	2	0	0
gl tract 1	0	0	0	0	0	0	2	0	0
gl tract 2	0	0	0	0	0	0	3	0	0
gl tract 3	0	0	0	0	0	0	1	0	0
caviar 1	3	0	0	0	0	0	0	0	0
caviar 2	3	0	0	0	0	0	0	0	0
caviar 3	4	0	0	0	0	0	2	0	0

Table 2. Number of microplastic particles in samples from *M. cephalus*.

Species	*M. cephalus*								
Form of MPs	Pellets			Fibres			Irregular form		
	≤ 25 µm	25–100 µm	100–200 µm	≤ 25 µm	25–100 µm	100–200 µm	≤ 25 µm	25–100 µm	100–200 µm
skin 1	2	0	0	0	1	0	1	0	0
skin 2	3	0	0	0	0	0	0	0	0
skin 3	0	0	0	0	0	0	0	0	0
meat 1	0	0	0	0	0	0	3	1	0
meat 2	0	0	0	0	0	0	2	1	0
meat 3	0	0	0	1	1	0	4	0	0
gills 1	3	0	0	0	0	0	1	0	0
gills 2	3	0	0	0	0	0	0	0	0
gills 3	2	0	0	2	0	0	1	0	0
gl tract 1	11	0	0	0	0	0	1	0	0
gl tract 2	8	0	0	1	1	0	1	0	0
gl tract 3	11	0	0	1	1	0	1	0	0
caviar 1	0	0	0	0	0	0	0	0	0
caviar 2	0	0	0	0	0	0	0	0	0
caviar 3	0	0	0	0	0	0	0	0	0

In the Mullet (Table 2), the pellets with sizes ≤ 25 µm prevailed. They were isolated mostly from in the gastrointestinal tract, gills and skin. Pellets 100–200 µm in size were detected in the muscles, gills and the gastrointestinal tract. Fibres ≤ 25 µm in size were isolated from skin, the muscles and the gastrointestinal tract. Irregularly-formed MPs were isolated from all parts of the fish, except the chaviar, but were most abundant in the muscles and the gastrointestinal tract. As in the muscles, particles with size of 25–100 µm were detected.

Table 3. Number of microplastic particles in samples from *M. batrachocephalus*.

Species	*M. batrachocephalus*								
Form of MPs	Pellets			Fibres			Irregular form		
	≤ 25 µm	25–100 µm	100–200 µm	≤ 25 µm	25–100 µm	100–200 µm	≤ 25 µm	25–100 µm	100–200 µm
skin 1	2	0	0	1	0	0	2	1	0
skin 2	5	0	0	0	0	0	2	0	0
skin 3	2	0	0	0	0	0	1	0	0
meat 1	0	0	0	1	0	0	3	0	0
meat 2	0	0	0	1	0	0	1	0	0
meat 3	2	0	0	1	0	0	1	0	0
gills 1	1	0	0	0	0	0	0	0	0
gills 2	0	0	0	0	1	0	0	0	0
gills 3	2	0	0	0	0	0	2	1	0
gl tract 1	1	0	0	0	0	0	0	0	0
gl tract 2	1	0	0	0	0	0	0	0	0
gl tract 3	2	0	0	0	0	0	1	0	0
caviar 1	0	0	0	0	0	0	0	0	0
caviar 2	0	0	0	0	0	0	0	0	0
caviar 3	0	0	0	0	0	0	0	0	0

Table 4. Number of microplastic particles in samples from *A. immaculata*.

Species	*A. immaculata*								
Form of MPs	Pellets			Fibres			Irregular form		
	≤ 25 µm	25–100 µm	100–200 µm	≤ 25 µm	25–100 µm	100–200 µm	≤ 25 µm	25–100 µm	100–200 µm
skin 1	1	0	0	0	3	0	0	0	0
skin 2	0	0	0	0	0	0	0	0	0
skin 3	1	0	0	0	0	0	0	0	0
meat 1	3	0	0	0	0	0	1	0	0
meat 2	3	0	0	0	0	0	1	0	0
meat 3	3	0	0	0	0	0	3	1	0
gills 1	1	0	0	7	0	0	0	0	0
gills 2	1	0	0	6	0	0	0	0	0
gills 3	0	0	0	7	1	0	0	0	0
gl tract 1	0	0	0	0	0	0	0	0	0
gl tract 2	0	0	0	0	0	0	0	0	0
gl tract 3	0	0	0	0	0	0	0	0	0
caviar 1	0	0	0	0	0	0	0	0	0
caviar 2	0	0	0	0	0	0	0	0	0
caviar 3	0	0	0	0	0	0	0	0	0

From *M. batrachocephalus*, pellets with sizes ≤ 25 µm were isolated from the skin, gastrointestinal tract, gills and flesh of the fish prevailed (Table 3). Fibres ≤ 25 µm in size were isolated from muscles and the skin. Fibres 100–200 µm in size were also detected in the gills. Irregularly-formed MPs were isolated in all parts of the fish except the caviar, most abundantly in the muscles and the skin. In the skin and gills, larger particles of 25–100 µm were also found (Fig. 1C).

In *A. immaculata*, the pellets with sizes ≤ 25 μm predominated and they were isolated from the skin and the gills of the fish. Fibres ≤ 25 μm in size were isolated from the gills (Fig. 1C). Fibres 25–100 μm in size were also isolated from the skin and gills. Irregularly-shaped MPs were isolated from the muscles. Particles of larger size (25–100 μm) were also found.

In *T. m. ponticus*, pellets with sizes ≤ 25 μm prevailed. They were isolated from the gastrointestinal tract, the gills, the muscles and the skin of the fish. Fibres ≤ 25 μm and 25–100 μm in size were isolated from the gastrointestinal tract (Fig. 1). In the gills and in the skin, larger particles of 25–100 μm were also found.

FTIR spectral analysis was performed to determine the nature of the isolated MPs. The obtained results are represented in Appendix 1.

From the isolated microplastic particles, the following types were identified: LDPE – low-density polyethylene used in the production of plastic cups; PA – polyamide used in the production of cords; PET – polyethylene terephthalate used in the production of bottles for soft drinks; PP – polypropylene used in the production of shampoo bottles; PC – polystyrene/polystyrene used in the production of CD cases; EPS - expanded polystyrene used in the production of packaging and PVC - plasticised polyvinyl chloride.

Some of the isolated microplastic particles were shown to be a polyamide (nylon fibres) (Appendix 1: Fig. A1B). They are specific by spectra that are characterised by strong amide I-amide II spectral bands at 1650 and 1543 cm^{-1} and polyethylene terephthalate (polyester, PET) (Appendix 1: Fig. A1C) showing C=O with bands at 1725 cm^{-1}, C–O with bands at 1250 and 1100 cm^{-1}. Two of the analysed fibres have a spectrum with a peak at 1729 cm^{-1} (Appendix 1: Figs A1E, G). This peak is consistent with the C=O stretching mode of the acrylates. The absence of a nitrile band (2237 cm^{-1}) allowed us to reject polyacrylonitrile as a component of the polymer matrix. The plain translucent fibre presents a spectrum similar to that of siloxanes, characterised by peaks at 1030–1065 cm^{-1} (Si-O-Si stretch) and 1280 cm^{-1} (Si-CH3 strain) (Appendix 1: Fig. A1C).

Discussion

This study presents a detailed assessment of MPs contamination in commercially important fish species, caught in the south of the Bulgarian Black Sea aquatory. All investigated species were contaminated with MPs. A study by (Eryașar et al. 2022) reports on contamination with 95 MPs in three commercial fish species - *Engraulis encrasicolus*, *Merlangius merlangus* and *Mullus barbatus* from the Turkish Black Sea coast. The most contaminated with MPs was the red mullet and the MPs were mainly in the form of fibres. However, these authors did not dissect the fish into separate parts and it is not clear exactly in which part of the fish the respective microplastics were isolated.

However, they found that polyethylene and polypropylene were the most dominant type of polymers. This is in line with our results, as we also isolated mostly polyethylene. In our study, pellets were the most abundant, followed by fibres.

Neves et al. (2015) found, that, along the Portuguese coast, the number of MPs per fish was higher, compared to the study of Eryaşar et al. (2022). Compared to our results, however, these are relatively low numbers. Additionally, a lower number of MPs was reported in the analysis of the gastrointestinal tract in fish from the Turkish Marmara, Aegean and Mediterranean coasts (Gǜven et al. 2017; Gündo˘gdu et al. 2020), the Spanish coast (Bellas et al. 2016) and Portuguese coasts (Bessa et al. 2018).

In our study, the most MPs were reported in the gastrointestinal tract in one the species - 36 MPs particles. Differences in the number of MPs can be a consequence from the differing methodologies in the papers and from the different degrees of pollution (Neves et al. 2015; Bellas et al. 2016; Peters et al. 2017; Wang et al. 2021). In general, benthic fish species reported higher MPs content, which could be related to differences in fish feeding and behaviour and due to the sedimentation and deposition processes of the particles (Woodall et al. 2014; Jabeen et al. 2017; Wootton et al. 2021). There is evidence that bottom-dwelling fish species can ingest sediment in the process of feeding (Lusher et al. 2013), which may lead to higher levels of MPs uptake, through one of the main pathways for MPs ingestion, via the gastrointestinal tract (McGoran et. al. 2017; Wang et al. 2021).

Nevertheless, a higher percentage of MPs was reported in pelagic fish species – Garfish and Mediterranean horse mackerel (Tables 1, 5).

This type of material is commonly used to make domestic and marine sealants. The majority of isolated microplastic particles mainly contain polyethylene (PE) and polyethylene terephthalate. Polyethylene is used and is included in the composition of plastic bottles, cups, stirrers and plastic bags. This polymer is very light and floats on the surface of the sea because its density is lower than that of water. Polyethylene terephthalate, on the other hand, is denser than water and is more likely to sink and accumulate in the sea bed and benthic organisms. These polymers are widely used in fabrics, in nets, ropes and strings used for fishing - one of the main economic activities of the Black Sea. The predominant types of polymers - the PE, corresponds to the content

Table 5. Number of microplastic particles in samples from *T. m. ponticus*.

Species	*T. m. ponticus*								
Form of MPs	Pellets			Fibres			Irregular form		
	≤ 25 µm	25–100 µm	100–200 µm	≤ 25 µm	25–100 µm	100–200 µm	≤ 25 µm	25–100 µm	100–200 µm
skin 1	1	0	0	0	0	0	1	1	0
skin 2	0	0	0	0	0	0	1	0	0
skin 3	0	0	0	0	0	0	2	0	0
meat 1	2	0	0	0	0	0	1	0	0
meat 2	4	0	0	0	0	0	1	0	0
meat 3	2	0	0	0	0	0	1	0	0
gills 1	4	0	0	0	0	0	3	1	0
gills 2	7	0	0	0	0	0	1	1	0
gills 3	4	0	0	0	0	0	6	0	0
gl tract 1	5	0	0	6	0	0	1	0	0
gl tract 2	8	0	0	3	2	0	1	0	0
gl tract 3	7	0	0	5	0	0	3	0	0
caviar 1	0	0	0	0	0	0	0	0	0
caviar 2	0	0	0	0	0	0	0	0	0
caviar 3	0	0	0	0	0	0	0	0	0

of manufactured plastics all around Europe, as almost half of the plastics produced in Europe are reported as PE (Plastics Europe 2020). The sinking of plastics and sedimentation is related to the fact that the upper layer of the Black Sea is less dense than other seas. On the other hand, the weight of these particles increases due to the accumulation of marine plants and nutrients in them and this can affect the distribution of plastics and their sedimentation on the sea bed. In studies on the black mussel, it was reported that these polymers are dominant in the mussels (Ibryamova et al. 2022).

Regarding fish health, it has been reported that plastics < 1000 μm in size can reach the digestive tract or the gills and, in turn, can cause adverse effects, such as a weak immune response and reduced fertility (Browne et al. 2008; Prokic et al. 2019; Jaafar et al. 2021). No particles larger than 100 μm were found in our study. Considering the wide variety of types of MPs detected in the digestive tract, we assumed that the fish regularly ingest the MPs during feeding. Some researchers reported that the main mode of MPs ingestion is not by misidentifying these particles as prey, but fish passively consume MPs during the feeding process (Sun et al. 2019; Wang et al. 2021). Many nutrients are also adsorbed on the plastic particles which deceives the fish that this is food. It can be assumed that the fish do not recognise the microplastics, but identify it as a food. Bowen et al. (2021) showed that fish ingest MPs inadvertently rather than intentionally. The authors found that fish did not actively ingest microfibres and, instead, the MPs were passively absorbed by breathing. Fish have also been reported to exhibit fibre rejection behaviour by expelling them out when mixed with mucus (Bowen et al. 2021). MPs can accumulate in the predatory fish species; unfortunately, very limited research has been performed on bioaccumulation and biomagnification in food webs (Wootton et al. 2021); therefore, more studies are needed to reach this conclusion.

MPs enter seawater food chains in different pathways and threaten entire ecosystems through their ability to transport pollutants, pathogenic microorganisms and alien species. Having in mind the intensifying economic activity in the Black Sea coast and the consequent influence on the riverine water quality (Gartsiyanova et al. 2024), river mouths can be considered as a potential source of MPs. This is especially so near the Kamchia River mouth, which is the largest intra-territorial river in Bulgaria, entering directly into the Black Sea, with a cathment area above 5300 km^2 (Doychev 2023).

This cathment and the entire Black Sea coast, where agriculture is well developed (Gartsiyanova et al. 2024) is a potential source of MPs, which have the ability to absorb and release toxic chemicals of organic and inorganic origin, such as Bisphenol A (BPA), PCBs and DDT, creating an additional potential risk to human health. Humans are exposed to BPA in the environment they live in from the air we breathe, the food and drinks we consume etc. Therefore, even if BPA intake is below some accepted limits, this does not guarantee that the additive will not accumulate and cause more pronounced effects and chronic toxicity in the food chain, given the tendency to accumulate.

As a consequence of the obtained results and the amount and type of polymer found in the study and literature, the source of contamination, in our opinion, can be mainly attributed to domestic wastewater discharges coming from the washing of synthetic fabrics. However, detailed studies are needed to prove this. In Bulgaria, wastewater is discharged directly or after purification into marine and freshwater ecosystems, as is the case in other neighbouring countries along the Black Sea coast-Our results show a wide variety of micropollutants originating from the commonly used plastic cups, stirrers, bags, soft drink bottles, fishing nets, packaging of hygiene and personal hygiene preparations and others that have systematically entered the Black Sea and are degraded into microplastic particles. The present study demonstrated the MPs contamination of five commercial fish species from the Black Sea with higher abundance of MPs in pelagic species. It is important future research to determine the toxicological side effects of plastic ingestion for fish communities in both benthic and pelagic habitats.

However, even if introducing plastics into the water system is stopped, both groups of fish will continue to be impacted, since the number of microplastics can increase due to the breakdown of larger plastics in the environment.

This study shows the need to carry out further studies and characterisation of microplastics using different types of microscopic and spectral analysis. Even though microplastics may not pose a risk to humans who consume fish, these contaminants pose a potential risk to marine food webs and endangered species. We found particles of different sizes, types and colours in different fish species. We believe that the variability of polymer species in fish can reveal/indicate the polymer species in water to some extent. Our results show that fish are important as ecological bioindicators and serve as a basis for future studies on microplastic pollution in tourist sandy beaches.

Additional information

Conflict of interest

The authors have declared that no competing interests exist.

Ethical statement

No ethical statement was reported.

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Author contributions

Tsveteslava V. Ignatova-Ivanova and Nikolay D. Natchev conceived and designed the study. Nikolay D. Natchev and Radoslav Ivanov obtained the samples. Teodora Koynova performed the fish dissection. Tsveteslava V. Ignatova-Ivanova and Nikolay D. Natchev supervised the data analysis and wrote the manuscript. Sevginar F. Ibryamova, Stephany Toschkova, Darina Ch. Bachvarova and Elitca Stanachkova performed the testing and contributed to data analyses and summaries. All authors have read and agreed to the published version of the manuscript.

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Appendix 1

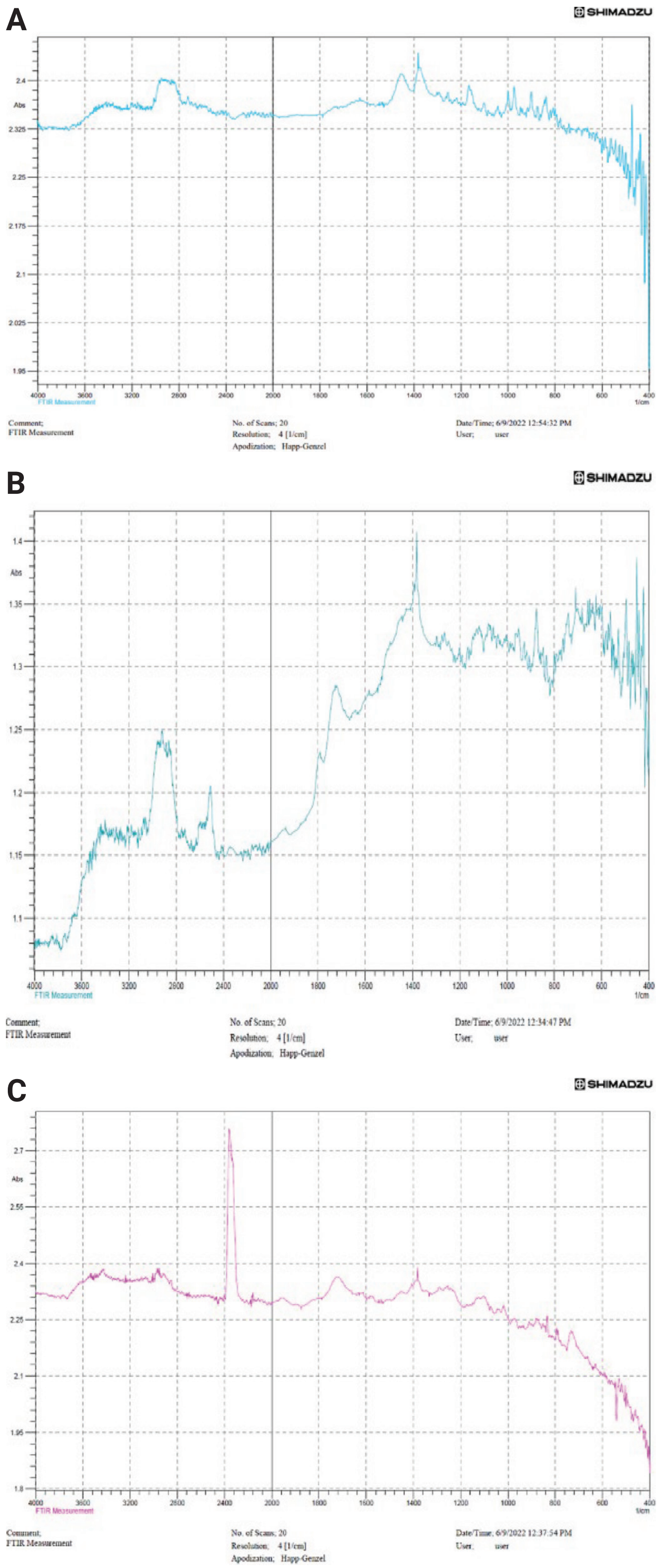

Figure A1. FTIR spectrum of the microplastic particles isolated from the fish **A** LDPE – low density polyethylene **B** PA – polyamide **C** PET – polyethylene terephthalate **D** PP – polypropylene **E** PC – polystyrene/polystyrene **F** EPS - expanded polystyrene **G** PVC – plasticised polyvinyl chloride spectrum.

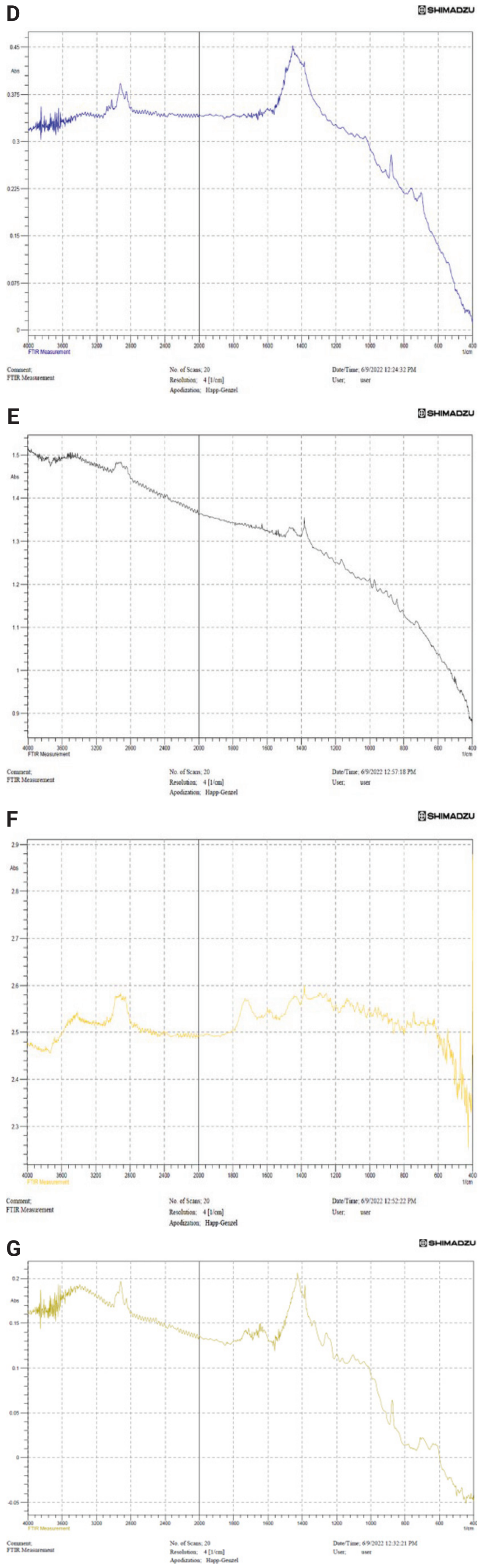

Figure A1. Continued.