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Concentration and Characterisation with Spectroscopic Technique of Microplastics in the Surface Sediment and Commercial Fish Species of Gemlik Bay (Marmara Sea)

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Abstract

In this study, we aimed to evaluate and characterise the microplastic pollution in the sediment and commercial fish species in the Gemlik Bay, the Marmara Sea. Our results showed that the highest concentration of microplastics in the sediment was at the station in the Gemport Harbour (9.73 pieces.kg^{-1}) and the lowest concentration was at the Kurşunlu offshore (3.33 pieces.kg^{-1}). The highest microplastic concentration per individual was in the gills of *Synapturichthys kleinii* (Risso, 1827) (14.5 pieces.ind^{-1}) and the lowest in *Diplodus annularis* (Linnaeus, 1758) (0.33 pieces.ind^{-1}). The highest concentration (8.75 pieces.ind^{-1}) was indicated in the gastrointestinal tract of *Chelidonichthys lucerna* (Linnaeus, 1758), but the lowest concentration (0.88 pieces.ind^{-1}) was in that of *D. annularis* species. The fiber-type particles were the most determined microplastics in both the sediment and fish samples. The Micro-Raman Spectrometer revealed that Polyvinyl chloride and polypropylene were dominant in the sediment, and polyoxymethylene and polyphenylene sulfone polymers were dominant in fish species.

Introduction

Plastic usage, which has become widespread in the world, and plastics in the marine environment were first reported in the early 1970s (Carpenter and Smith, 1972; Fowler, 1987). The widespread existence of plastic debris from the equator to the poles and from the coasts to the deepest points of the seas is very well known today, and they accumulate and cause environmental pollution (Barnes et al., 2009). That is why studies on their concentration have become the focus of pollution monitoring studies. According to the results of the modeling studies, the number of plastics in the aquatic environment is thought to be 150 million tons, and considering this situation, it has been predicted to be 1 ton of plastic waste per every 3 tons of fish up to 2025 (Dąbrowska et al., 2021).

Microplastics are defined as plastic particles in the size range 1 µm to <5 mm (GESAMP, 2015). Primary microplastics are heavily used, especially in the cosmetics industry where they are frequently used in facial cleansing gels, creams and masks to remove dead skin due to their small and hard structure (Napper et al., 2015). In addition, decomposition from synthetic textile products, plastic pellets from the industry during production processes, ship paints, plastics worn from car tyres and transported to the seas by wind, worn parts from road markings are also important primary sources of microplastics. (Boucher and Friot, 2017).

Additionally, microplastics have a wide scope of usage in many other cosmetic materials such as shampoos, suntan lotions and toothpaste (UNEP, 2015). Secondary microplastics are formed through the breakdown of microplastics (GESAMP, 2015). Micro and nanosized plastics can penetrate tissues and accumulate in organs by overcoming biological barriers due to their small sizes (Kashiwada, 2006; Von Moos et al., 2012). They accumulate in organisms through the food chain and create a suitable surface area for the adhesion of many chemicals and pollutants, thus their toxic potential is increased when they diffuse into the body of organisms (Cole et al., 2011; Wright et al., 2013; Gallowey et al., 2017; Ogonowski et al., 2018; Hahladakis, 2020).

The number of microplastic studies has recently increased in Turkish coastal waters Aytan et al. (2016, 2022, 2023); Sönmez et al., 2023; Güven et al., 2017; Gündoğdu and Çevik, 2017; Tunçer et al., 2018; Doğruyol et al., 2019; Gündoğdu et al., 2020; Çullu et al., 2021; Sarı Erkan et al., 2021; Yozukmaz, 2021; Belivermiş et al., 2021; Gedik et al., 2022; Gedik and Gozler, 2022; Bat and Öztekin, 2022.

Gemlik Bay is affected by various forms of marine pollution, including harbors, industrial facilities, anthropogenic pressures, tourism, agricultural areas, and river transportation. An extensive investigation was conducted to examine the levels of metal, PAH, and radioactive pollution in the Bay (Ünlü et al., 2006a; Ünlü et al., 2006b; Ünlü et al., 2008; Ünlü et al., 2009; Yümün et al., 2021). Nevertheless, the absence of any previous research on microplastic contamination in the Bay makes this work particularly significant as it provides the first-ever insights into the presence and properties of microplastics in the sediment and several organs of commercially important fish species in Gemlik Bay. The density and dispersion of microplastics are influenced by various environmental conditions, including river inflows, currents, winds, and human activities (Mehra et al., 2020). The Bay reflects both environmental and antropogenic influences. Hence, in this study, our objective was to evaluate the influence of these factors on the content of microplastics (MP) by collecting samples from areas with high concentrations of river inputs, harbors, towns, tourism activities, and industrial facilities.

Materials and Methods

Study Area

The sampling of the study was carried out on the 23rd and 24th June 2021 at 15 stations at different depths of the Gemlik Bay, located in the southeast of the Marmara Sea (Figure 1, Table 1). Marmara Sea is a fairly small inland sea (Beşiktepe et al., 1994). This sea is connected to the Black Sea by the Istanbul Strait in the northeast and the Aegean Sea by the Dardanelles Strait in the southwest. It consists of a two-layered water system due to its connection with seas which have very different salinity (Beşiktepe et al., 1994). The Gemlik Bay, where the study was conducted, is separated from the Marmara Sea by a 50 m deep threshold. The length of the bay is 36 km, and its width is 11 km (Yaltırak and Alpar, 2002). The deepest part of the bay is the Burgaz Trench in the northwest, which has a depth of 110 m (Yaltırak and Alpar, 2002; Kuşçu et al., 2009). The general livelihood of the region is olive cultivation, soap making, oil making and agriculture. Gemlik Bay is the centre of industry and trade because it possesses many ports for the exportation of manufactured products (Ünlü and Alpar, 2006). Commercial ports in the region are concentrated in the southern part of the bay, and Gemport, Borusan and Roda are the most important among them (Koday and Baki, 2014). The Gemlik Bay is considered the second most polluted region of the Marmara Sea following the Izmit Bay (Ünlü and Alpar, 2006).

Sampling

Sampling was carried out at 15 stations at depths ranging from 34 to 108 m in the Gemlik Bay. Sediment samples were taken in triplicate using a Van Veen grab with a sampling area of 0.1 m^2. Nitrile gloves and cotton clothing were used during sampling instead of plastic materials to avoid contamination. Surface sediment samples taken via metal spoons and 400 g sediments were stored in glass containers at -20°C until the analysis period. Fish samples were collected by gill net from a depth of 20 m and sampled at only one station (station 14) in the bay. Fish samples were collected by small-scale fishing out of the bay. These samples were also preserved at -20 °C until analysis (Figure 1). Since we only collected fish from the net, the number of individuals from each species was different. The taxonomic classification of the fish samples obtained in the study was made according to Nelson (2016), and the systematic species determinations were made according to Whitehead et al. (1986). However, Mater et al. (2001) and Şalcıoğlu et al. (2021) were used to determine *Spicara maena* and *Spicara flexuosa* species, which are considered two different species.

Microplastic Analysis

Sediment samples were homogenized and dried at 40°C. We used the density separation method and 100 ml of saturated sodium chloride (NaCl) solution (NaCl ρ=1.20 g/ml) was added the five grams of each sediment (three replicates) and mixed in a magnetic stirrer at a constant speed for five minutes (Zhao et al., 2018; Erni-Cassola et al., 2019; Belivermiş et al., 2021). The mixed samples were kept on a flat surface for five minutes for precipitation, then the liquid part was transferred to another beaker. This process was repeated twice. To decompose the organic material, 10 ml of 30% hydrogen peroxide (H_2O_2) was added to the beakers and left for 24

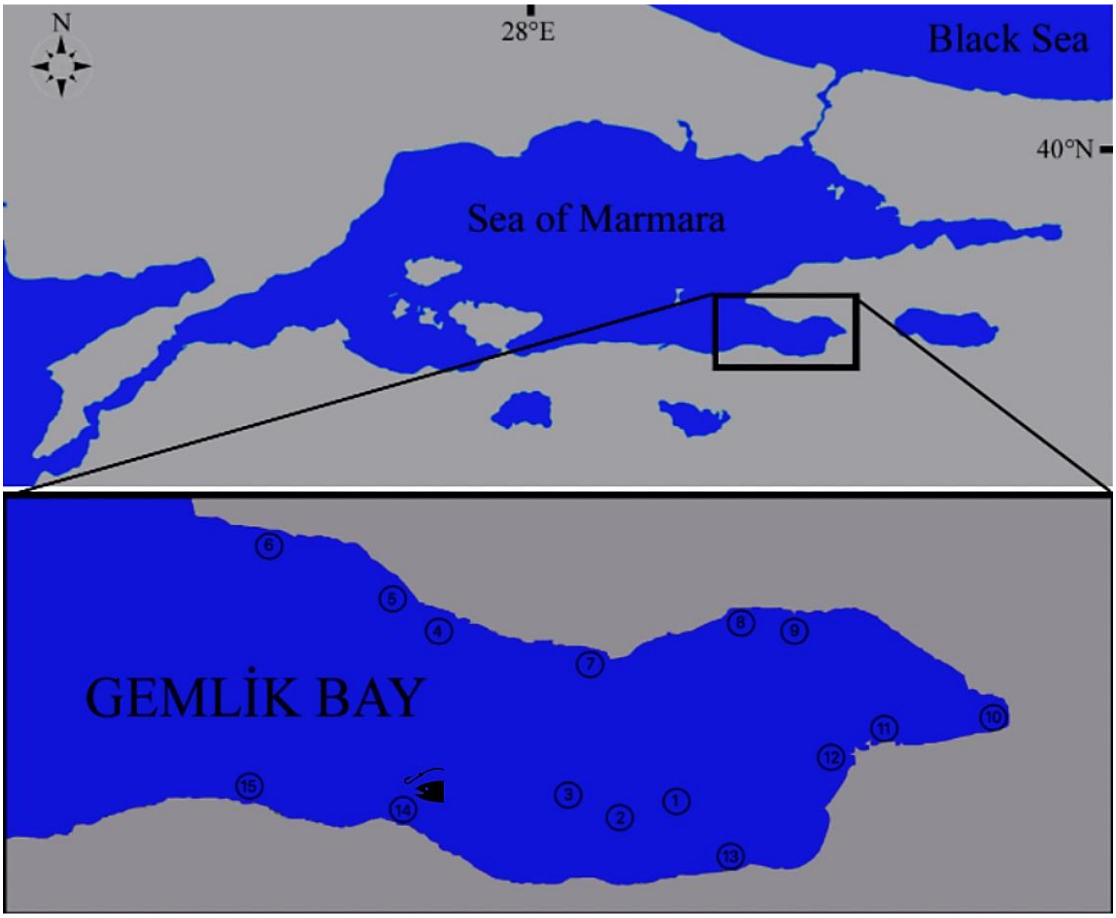

Figure 1. Sampling stations in the Gemlik Bay (Stations 1-13 and 15 Sediment Sampling; 14-Fish Sampling)

Table 1. Depth and coordinates of the of sediment sampling stations and the amount of microplastic in the sediment samples

Stations	Location	Coordinates	Depth (m)	Microplastic amount (pieces.kg^{-1}dry wt.)
1	Kurşunlu Offshore	40° 23' 42.6"- 29° 01' 13.8"	90	3333.3
2	West Kurşunlu	40° 22' 43.3"- 28° 58' 58.9"	99.5	4400
3	Burgaz Trench	40° 23' 34.8"- 28° 57' 03.5"	108	4066.6
4	Fıstıklı Offshore	40° 26' 52.6"- 28° 52' 24"	90	7066.6
5	Fıstıklı	40° 28' 54.2"- 28° 52' 24"	60	6066.6
6	Armutlu	40° 29' 41.2"- 28° 48' 26.6"	36	6266.6
7	Kapaklı	40° 27' 35.4"- 28° 57' 48.2"	36	4466.6
8	Narlı	40° 28' 41.1"- 29° 02' 00.4"	36	3866.6
9	Küçükkumla	40° 28' 31.1"- 29° 03' 40.4"	36	3333.3
10	Gemlik-Karsak	40° 25' 49.4"- 29° 08' 37.6"	36	8000
11	Port of Gemlik (Gemport)	40° 25' 08.5"- 29° 06' 14.6"	34	9733.3
12	Port of Rodaport	40° 25' 05.0"- 29° 04' 30.6"	34	4400
13	Kurşunlu	40° 21' 48.3"- 29° 01' 17.3"	35	9466.6
14	Mudanya	40° 22' 20.1"- 28° 54' 24.4"	45	3600
15	Tirilye-Kumyaka	40° 23' 55.6"- 28° 48' 30.2"	36	3333.3

hours. Then the supernatant was drawn with a glass pipette washed with distilled water and samples were filtered through Whatman GF/0.47 µm filter papers in a vacuum filtration system. Afterwards, the samples were left to dry at room temperature in a sterile Petri dish (Zhao et al., 2018).

The fish samples were measured and weighed, then washed with distilled water prior to dissection. All processes were performed under a fume hood to prevent contamination and all instruments to be used in the dissection were washed with distilled water. Moreover, to eliminate possible microplastic contamination, distilled water as a blank solution was kept in a 500 ml beaker in the place where dissection was carried out, and the plastics and fibers detected within the beaker were then removed from the microplastic counts of samples. The gills and gastrointestinal tract of the fish were carefully removed, and the tissues from each sample were transferred to individual flasks. 50 ml of 10% potassium hydroxide (KOH) was added to each flask and stored in a shaking incubator at 40 °C and 100 rpm for 48 hours. Afterwards, 10 ml of 30% H_2O_2 was slowly added to decompose the remaining organic substances, and all samples were left at room temperature for one more night. 50 ml of distilled water was added to the solution and filtered on Whatman GF/0.45 µm filter papers in a vacuum filtration system and left to dry at room temperature in sterile glass Petri dishes (Yuan et al., 2019).

Counting and Classification of Microplastics

The filters were examined under a Leica M 205C stereo microscope. Detected microplastics were counted and classified according to their colour and shape (Frias et al., 2018). Cotton clothes and nitrile gloves were worn during the counting process. Again, a blank solution was kept in the working place.

Microplastic Characterisation with Spectroscopic Technique

Analysis of the microplastics, which were grouped by counting and have dimensions not visible to the naked eye, was performed using the Jasco NRS 3100 Model Micro Raman Spectrometer. There are two laser sources in the NRS 3100 Dispersive Micro Raman spectrometer. The first of these is green laser with a wavelength of 532 nm, and the second is a red laser with a wavelength of 785 nm. A red laser with a wavelength of 785 nm was preferred for the analyses. Raman spectrometers have a grating system of 1800 lines/mm, 1200 lines/mm and 600 lines/mm. The 1200 lines/mm grating system was preferred for analysis. By using different lenses (5x, 20x and 100x) in the Micro Raman device, it was possible to focus on microplastics, and spectra were obtained in the desired amount and time. Micro-scale samples extracted from sediment and various fish samples were placed in the device. First, their locations were determined using a 5x microscope and then a 20x microscope was selected to target the appropriate region of the sample. To increase the spectrum quality and to minimise the noise ratio, the number of concentrations was increased to 50, 100, 150 and 200 and the exposure time was set to 2 sec. The slit pitch was 0.1 x 6mm, the applied laser power was around 30.6 mW and the spectrum resolution was around 2.90 cm^{-1}. Spectra were obtained for all samples in three different wavenumber regions (centre 1,550 cm^{-1}, centre 950 cm-1, and centre 500 cm^{-1}). No baseline adjustment was made to prevent peak shifts in the spectra, but smoothing correction was applied to reduce noise. The OpenSpecy DATA (Cowger et al., 2021) was used to determine the microplastic type from the obtained spectra, which is an open-source spectral analysis software and a library focused on polymer characterisation.

Statistical Analysis

Pearson correlation analysis was performed to determine the relationship of microplastics in fish with height, weight and trophic level of fish species.

Results

The Distribution of Microplastics in Surface Sediment Samples of Gemlik Bay

In our study the stations that were found to have the highest microplastic occurrences were the 11th station with 9733.3 pieces.kg^{-1} and 13th station with 9466.6 pieces.kg^{-1} (Table 1). The 11th station is located near Gemport Harbour, and the 13th station is located at the deep discharge area in the Kurşunlu region. The 10th station, which is the point where the Karsak Stream reaches the sea, was the third station where the pollution was intense with 8000 pieces.kg^{-1}. The lowest values were recorded at the 1st, 9th, 14th and 15th stations, each of which had 3333.3 pieces.kg^{-1}. These stations are located near Kurşunlu, Küçükkumla, Mudanya and Tirilye, respectively. The distribution of microplastics determined by colour, shape and types at the stations is shown in Table 2 and Figure 2. Accordingly, the most common particle type was fibers (76%). Fibres were followed by fragmet (20%), film (3%) and pellets (1%). Black fibers constituted the largest proportion (45.4%), while blue fibers accounted for 33.8% of all fibers. The fragments consisted mainly of blue (51%) and black (45.2%). The prevailing color in films was black, accounting for 69.2% of the total films. Black pellets accounted for 43.1%, while red pellets accounted for 28.4% of total pellets.

Microplastics Determined in Gills and Gastrointestinal Tract of Fish Specimens

In this study, 123 individuals belonging to 10 different fish species were obtained from a depth of 20 m using a gill net (Table 3). The highest individual numbers (51) had *Trachurus trachurus* (Linnaeus, 1758), while the lowest numbers (1) belonged to the *Spicara maena* (Linnaeus 1758) species. To examine the concentration between species, we performed an analysis even if a single individual emerged. We calculated the concentration per individual and evaluated this way. However, we did not compare species statistically due to the inequality in the number of individuals. Before the fish were dissected, the samples were measured and weighed (Table 3). The shortest average lengths were *Trachurus trachurus* and the lowest average weight values were found in *Engraulis encrasicolus* (Linnaeus 1758), while the longest average length and highest weight values both were found in *Chelidonichthys lucerna* (Linnaeus 1758).

In the result of our studies of fish species living in different habitats and different feeding behaviours, the

Table 2. Details of microplastics distribution in sediment by color, shape and types (pieces.kg^{-1}dry wt.).

	Fiber									Fragment				Film		Pellet			
St	**Blu**	**Bla**	**R**	**P**	**T**	**G**	**Pu**	**O**	**Y**	**Blu**	**Bla**	**R**	**G**	**Blu**	**Bla**	**Blu**	**Bla**	**R**	**O**
1	933.3	1000	200	66.7		66.7				800	66.7	66.7		133.3	66.7				
2	1533.3	1666.7	66.7	66.7	66.7	133.3				600	266.7								
3	1066.7	1533.3	66.7		66.7	200	66.7			533.3	466.7	66.7							
4	2266.7	3666.7	200		400	266.7				400.0	66.7								
5	2333.3	2200	200			333.3		66.7		1000	66.7		66.7	66.7			66.7		
6	2400	2533.3	800			400				200	266.7								
7	1333.3	1933.3	466.7		66.7	466.7				200	333.3	66.7							66.7
8	1000	1200	400		200	533.3				466.7	333.3		66.7		133.3	66.7			
9	933.3	866.7	200		133.3	600				666.7	266.7	66.7			133.3		2.0		
10	2266.7	4000	400		66.7	666.7			66.7	133.3	666.7		66.7		133.3		66.7	66.7	
11	2200	4333.3	466.7		1466.7	733.3	66.7			666.7	400	66.7							
12	1533.3	1800	66.7	66.7	66.7	800				333.3				66.7	133.3				
13	2733.3	3000	200		133.3	866.7				333.3	3000						66.7		
14	1333.3	1533.3	133.3			933.3				400	66.7							66.7	
15	800	1933.3	133.3			1000	66.7			333.3									
Mean	**1644.4**	**2213.3**	**266.7**	**66.7**	**266.7**	**533.3**	**66.7**	**66.7**	**66.7**	**471.1**	**482.1**	**66.7**	**66.7**	**88.9**	**119.9**	**66.7**	**50.5**	**66.7**	**66.7**
%	**33.8**	**45.4**	**5.5**	**0.3**	**3.6**	**10.9**	**0.3**	**0.1**	**0.1**	**51**	**45.2**	**2.4**	**1.4**	**30.8**	**69.2**	**14.2**	**43.1**	**28.4**	**14.2**

St: Stations **Blu:** Blue **Bla:** Black **R:** Red **P:** Pink **T:** Transparent **G:** Green **Pu:** Purple **O:** Orange **Y:** Yellow

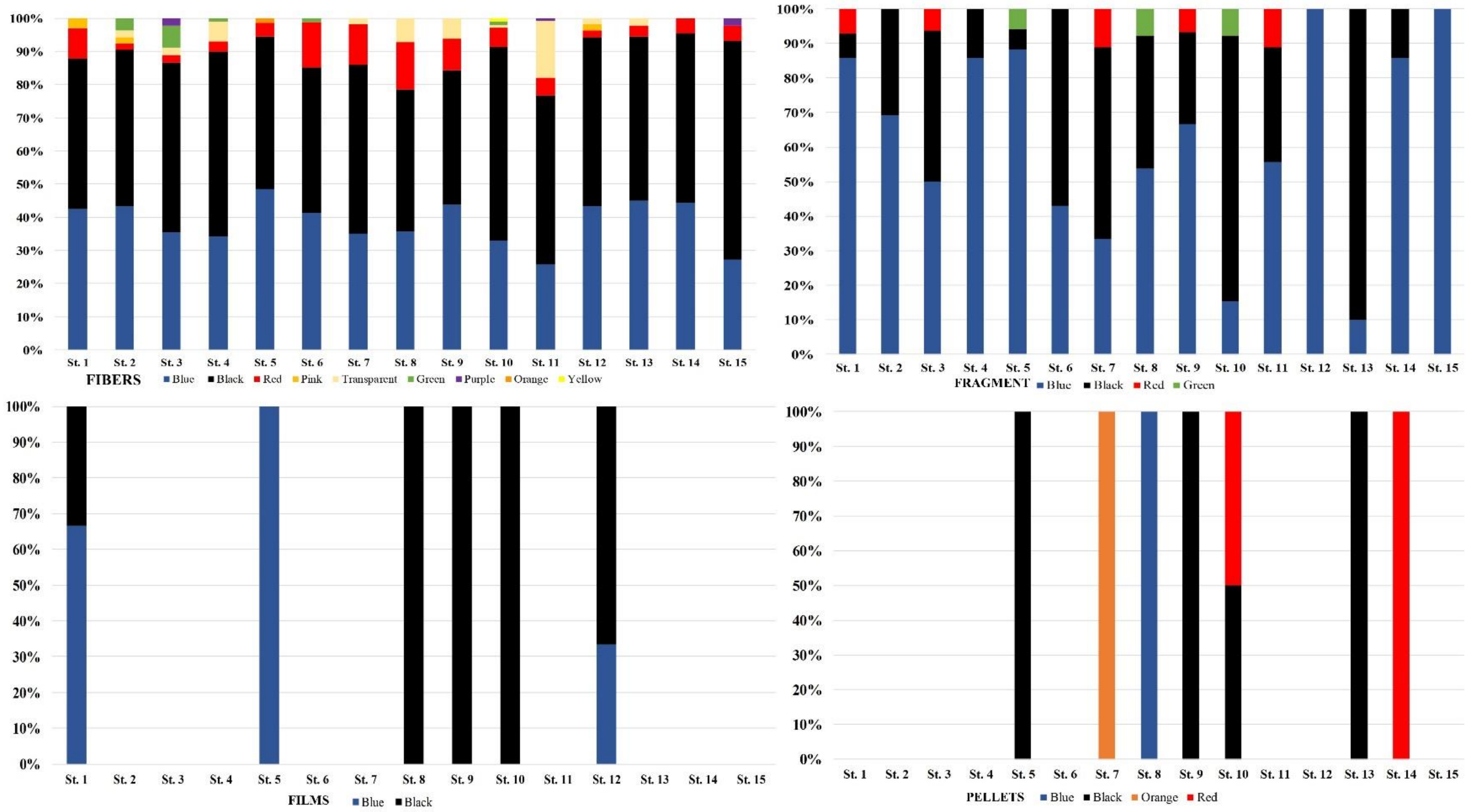

Figure 2. Microplastics distribution in sediment by color, shape and types

Table 3. Microplastic amount in the gill and gastrointestinal track and average length and weight values and trophic levels of fish samples

Species	IN (n)	AL (cm)	AW (g)	TL	The number of microplastics per individual in the gill (Pieces.ind^{-1})	The number of microplastics per individual in Gastrointestinal (Pieces.ind^{-1})
Trachurus trachurus (Linnaeus, 1758)	51	11.19	9.88	3.65	1.19	4.25
Engraulis encrasicolus (Linnaeus, 1758*)*	16	12.14	1.32	3.43	5.25	4.6
Diplodus annularis (Linnaeus, 1758)	9	12.5	15.05	3.36	0.33	0.88
Chelidonichthys lucerna (Linnaeus, 1758)	4	26.62	66.16	3.57	7	8.75
Solea solea (Linnaeus, 1758)	5	22.8	28.9	3.17	2.6	3.8
Synapturichthys kleinii (Risso, 1827)	2	15.5	16.935	3.17	14.5	5.5
Scorpena porcus (Linnaeus, 1758)	11	17.5	68.65	3.77	2.81	4.54
Mullus barbatus (Linnaeus, 1758)	16	15.11	46.66	3.27	3.6	3.31
Spicara maena (Linnaeus, 1758)	1	18	51.04	3.15	11	14
Spicara flexuosa (Rafinesque, 1810)	8	14.31	14.65	3.15	5.5	5.25

IN: Individual Number **AL:** Average Length **AW:** Average Weigth **TL:** Trophic Level

highest concentration of microplastics per individual in the gills was observed in *Synapturichthys kleinii* (14.5 pieces.ind^{-1}) and the lowest concentration was observed in *Diplodus annularis* (0.33 pieces.ind^{-1}). However, in the gastrointestinal tract, the highest concentration was determined in *Chelidonichthys lucerna* (8.75 pieces.ind^{-1}), while the lowest concentration was determined in *Diplodus annularis* (0.88 pieces.ind^{-1}) (Table 3). Microplastics determined in fish samples were classified according to their shape and colour (Figure 3).

The relationship among the types and number of plastics and the number of individuals, trophic level, average height and weight was evaluated by calculating the Spearman correlation coefficient (Tables 4 and 5). According to Table 4, the strong positive correlations between fibers and the total number of individuals (r: 0.8 $P<0.01$), and between fibers and the total amount of plastic (r: 0.89 $P<0.01$), were found in the gills. Table 5 represents the correlations among the types and number of plastics and individual numbers, trophic level, average length and weight in gastrointestinal tracts. A strong positive correlation was detected between fiber amount and fragment (r: 0.93; $P<0.01$); the fiber amount was strongly positively correlated with fragment (r: 0.93; $P<0.01$), film amount (r: 0.97; $P<0.01$), total number of individuals (r: 0.98; $P<0.01$) and total plastic (r: 0.92; $P<0.01$). Likewise, the fragment was strongly positively correlated with film (r: 0.95; $P<0.01$), total number of individuals (r: 0.94; $P<0.01$) and total plastic (r: 0.96; $P<0.01$). A strong positive correlation was also found between the total number of plastics and the total number of individuals (r: 0.92; $P<0.01$).

Analyzing the composition of microplastic samples collected from sediment and fish using spectroscopic techniques

Microplastics determined in the surface sediment samples and gill and gastrointestinal tracts of fish samples were analysed using the Jasco NRS 3100 Model Micro Raman Spectrometer. The sediment samples contained the following polymer distribution: 27% polyvinyl chloride (PVC), 20% polyphenylene sulfone (PPS), 13% polypropylene (PP), 13% polyisoprene, 13% polyvinyl burial (PVB), 7% Poly (2-hydroxyethyl methacrylate) and 7% polyoxymethylene (POM) .

The distribution of polymers in the gills of fish samples was as follows: 25% Polyoxymethylene (POM), 13% polysulfone (PSU), 13% polyphenylene sulfide (PPS), 13% Poly2-hydroxyethyl Methacrylate, 6% Epoxy, Polyisoprene, PP, PTFE, Polyphenylsulfone (PPSU) and PVC.

The distribution in the gastrointestinal system consisted of 17% PP, POM, PPSU, PTFE, and 16% PEG and 16% Polyphenylether Sulfane. Additional polymer varieties were discovered in the gill samples.

Spectra of microplastics are given in Figure 4 and 5. In the sediment samples the peaks observed at 1448 cm-^{1},1452 cm^{-1} and 1339 cm^{-1}, 1341 cm^{-1} in the spectrum correspond to the bending vibrations of the CH_3 group in the PP polymer. Another peak observed around 952 cm^{-1} and 954 cm^{-1} indicated the vibrational motion of the CH_3 group. Many microplastic particles detected in the sediment, gill and gastrointestinal tract of fish were analysed with a Raman Spectrometer. However, during the analysis, it was observed that the most noiseless spectra were taken from the blue coloured samples, so the spectra of the blue samples were generally given in the study. During the Raman analysis, it was observed that the particles determined in the sediment gave less noisy spectra than the particles determined in the gills, while the particles in the gills gave less noisy spectra than the particles determined in the gastrointestinal tract. It was thought that the microplastics determined in the fish were noisier than the microplastics determined in the sediment, since the fish were exposed to chemicals and heat for a long time while the microplastic analysis was performed. The spectra of microplastic particles determined in the gastrointestinal tract were thought to be noisier than the gills, and it was thought to be due to the fish's exposure to digestive enzymes, temperature

GASTROINTESTINAL TRACT

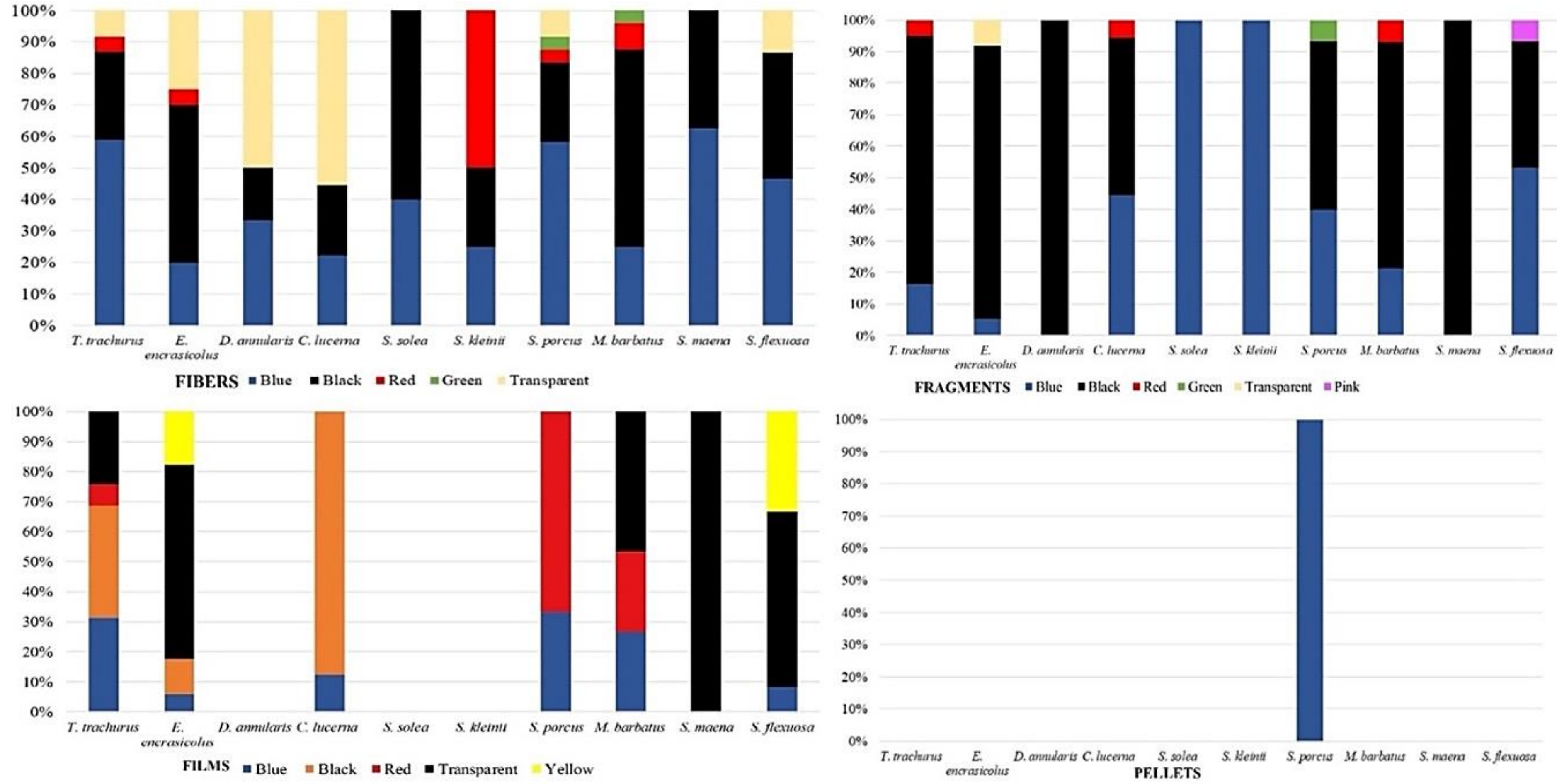

Figure 3. Sampling stations in the Gemlik Bay (Stations 1-13 and 15 Sediment Sampling; 14-Fish Sampling).

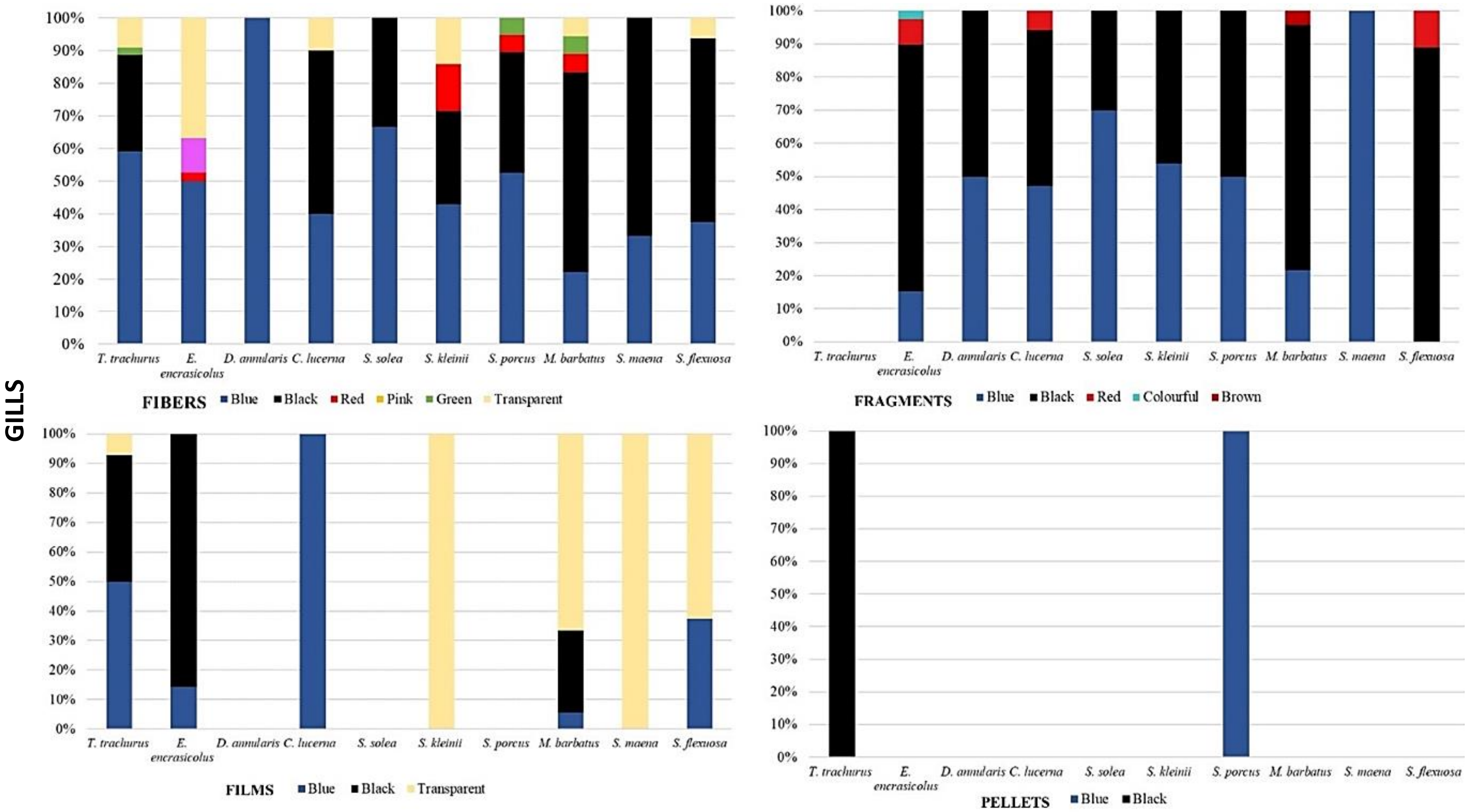

Figure 3 (*Continued*). Sampling stations in the Gemlik Bay (Stations 1-13 and 15 Sediment Sampling; 14-Fish Sampling)

Table 4. Correlation of microplastics in gills between individual number, average length and weight.

	Fr	Fi	IN	AL	AW	TM	AMP
Fi	0.34	0.48	0.80**	-0.49	-0.40	0.89**	-0.17
Fr		0.13	-0.12	0.11	-0.14	0.67*	0.10
Film			0.48	-0.35	-0.29	0.62*	-0.18
IN				-0.47	-0.36	0.57*	-0.52
AL					0.68*	0.27	0.1
AW							0.1
TP							-0.121

Fi: Fiber **Fr:** Fragment **MP:** Microplastic **IN:** Individual Numbers **AL:** Average Length **AW:** Average Weigth **T:** Total Microplastic **AMP:** Average Microplastic

*Correlation is significant at the 0.05 level (1-tailed).

**Correlation is significant at the 0.01 level (1-tailed).

Table 5. Correlation of microplastics in the gastrointestinal system among individual number, trophic level, average length and weight.

	Fr	Fl	IN	AL	AW	TL	TMP	AMP
Fi	0.93**	0.97**	0.98**	-0.41	-0.22	0.56	0.92**	-0.18
Fr		0.95**	0.94**	-0.34	-0.37	0.55	0.96**	-0.19
Fl			0.96**	-0.41	-0.32	0.46	0.96**	-0.12
IN				-0.47	-0.36	0.52	0.92**	-0.33
AL					0.68*	0.03	-0.33	0.27
AW						0.31	-0.29	0.45
TL							0.54	-0.18
TP								-0.16

Fi: Fiber **Fr:** Fragment **Fl:** Film **IN:** Individual Numbers **AL:** Average Length **AW:** Average Weigth **TL:** Trophic-Level **TMP:** Total Microplastic **AMP:** Average Microplastic.

*Correlation is significant at the 0.05 level (1-tailed).

**Correlation is significant at the 0.01 level (1-tailed).

and mechanical breakdown in the digestive system before analysis. Among the sediment samples, the most detected polymers were PVC, PPS and PP. Among the fish samples, the most detected polymer types were PP, PPS-PSU and POM.

Discussion

Microplastics, which are identified in various environments from freshwater to sea and air to land, have become a major global problem and require comprehensive monitoring studies regarding microplastic pollution. Our study focused on Gemlik Bay, a densely populated location in the Marmara Sea known for its extensive commercial and industrial operations. We conducted research to measure the levels of microplastic in both sediment and commercially caught fish species in this region. The results of our study revealed that the highest concentration of microplastic pollution was observed at the 11th station, located in close proximity to Gemport Port, an important port inside the bay. According to a paper by Núñez-Flores et al. (2019), ship traffic and ports are identified as the primary contributors to plastic pollution. This phenomenon was also distinctly observed in our investigation. We found a significant concentration of microplastics in the sediment at the 13th station. This region is densely inhabited and in close proximity to a significant discharge location. Furthermore, it is situated in close proximity to Kurşunlu, where a significant quantity of microplastic pollution was observed, comparable to that found at the 11th station. Research has demonstrated that pollution has a greater impact in areas near human settlements, harbors, or sewage outlets compared to other places (Wang et al., 2021; Murphy et al., 2016). According to Yümün et al. (2021), ship traffic in Gemlik, Narlı, and Kurşunlu is characterized by a high intensity, resulting in elevated pollution index values. The researchers have also noted that pollution in Gemlik and Kurşunlu has primarily stemmed from industrial and agricultural activities, as well as ship traffic. These variables can additionally explain the elevated degree of microplastic contamination in the location where we conducted our research. To determine the effect of industrial rivers on the pollution load in the marine environment, sediment and fish samples were collected from the 10th station located in the Karsak Stream, where the important industrial establishments of the region discharge their waste into the sea. It has been reported that 80% of the microplastics found in the seas are of terrestrial origin and the most important factor to their transport into the seas is rivers (Jambeck et al., 2015; Watkins et al., 2019). Researchers showed that the Karsak River carried many pollutants into the bay. Domestic discharges from the cities and ports that have intense ship transportation

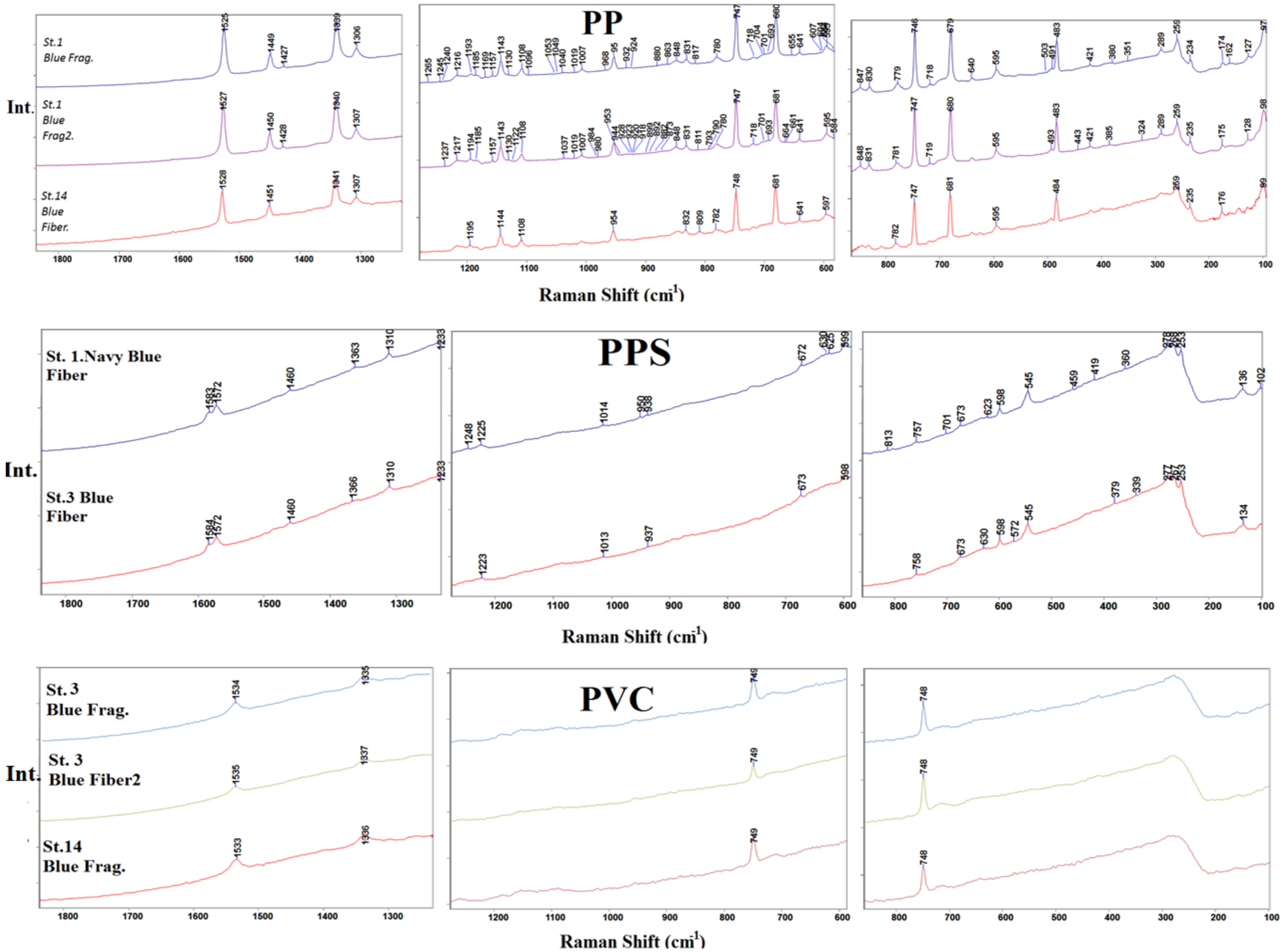

Figure 4. The polymer distributions of the microplastic particles obtained from the sediment

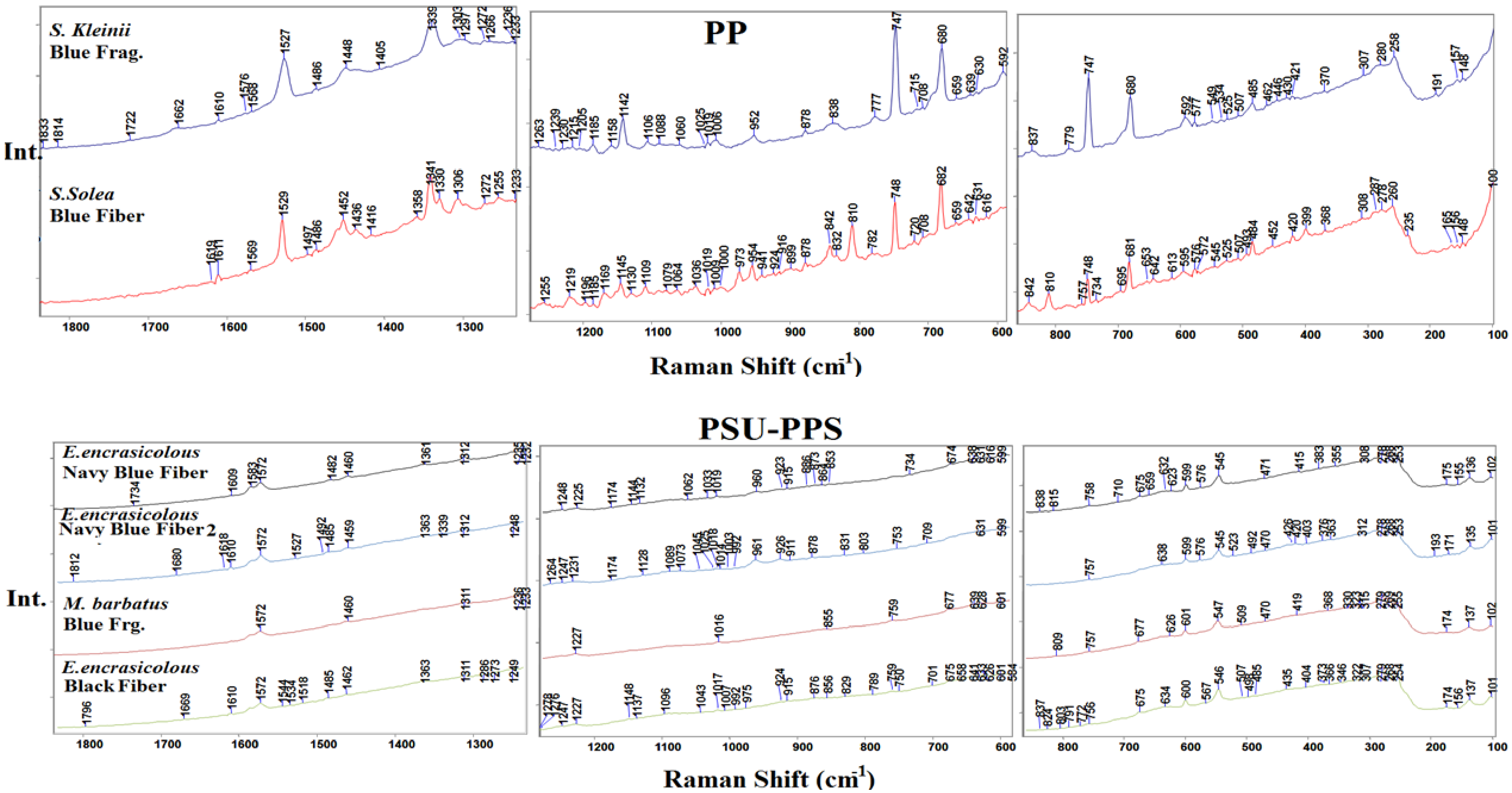

Figure 5. The polymer distributions of the microplastic particles obtained from the gastrointestinal tract and gills of fishes.

have also negatively affected Gemlik Bay. Besides, anthropogenic inputs are also high in these regions (Ünlü and Alpar, 2006; Teksoy et al., 2019). Karsak Stream carries pollutants not only from Gemlik but also from the Iznik region to the gulf (Teksoy et al., 2019). In a study by Cincinelli et al. (2021)

in the Black Sea (22–2,131 m), there was an average of 106.7 particles per kg in the sediment, but this number reached 390 particles in the northwest stations where the Danube and Dnieper rivers were discharged. The data here shows the adverse effects caused by river inputs.

The stations where we detected the least microplastics were in Kurşunlu offshore, Tirilye-Kumyaka and Küçükkumla. Among these regions, Küçükkumla and Tirilye-Kumyaka are regions where tourism activities are intense, and the region between Tirilye and Kumyaka has been declared as a 'Natural Site-Qualified Natural Protection Area' and 'Natural Site-Sustainable Conservation and Controlled Use Area' (Republic of Türkiye Ministry of Environment, Urbanisation and Climate Change, 2020).

The most common type of microplastic in sediment samples was fiber. In our study it was found that 81% of the particles were composed of fiber-shaped plastics. Previous studies also showed that fibers, fragments and films were dominant in the sediment (Table 6). It is known that the most important source of these fibers in marine ecosystems is the wastewater generated because of laundry (Browne et al., 2011). At least 1,900 fibers from clothes are mixed into the wastewater in each wash (Browne et al., 2011; Dodson et al., 2020).

We found that the Gemlik Bay sediment contained a higher rate of microplastics compared to previous studies conducted in the Marmara Sea. Previous microplastic monitoring studies related to Marmara Sea sediment have generally focused on Istanbul and its surroundings, and there has been no studies conducted in the Gemlik Bay. In this present study, microplastic particles values in the surface sediments were considerably higher than the values found in the Golden Horn sediment (0–140 pieces.kg^{-1}), between Pendik and Tuzla (13–5,100 pieces.kg^{-1}) and in the Istanbul coast and the Bosphorus (1,957.37 pieces.kg^{-1}) (Doğruyol et al., 2019; Baysal et al., 2020; Sarı Erkan et al., 2021). When our results were compared with the studies conducted in different seas, our results revealed that the microplastic pollution determined in Gemlik Bay was much higher than in the regions represented in Table 6. The high microplastic concentration in Gemlik Bay may be the result of heavy ship traffic, intense industrial facilities and an increasing population.

The dominant polymer types in the studies of the other seas were PP, PE and PS (Table 6). In our study, PVC (27%) PPS (20%) and PP(13%) polymers were most dominant among the microplastics.

Mullus barbatus has been proposed as a biomonitor demersal fish species for evaluation of microplastic concentration (Bray et al., 2019). We found the lowest microplastic concentration was determined in *Mullus barbatus* (3.31 pieces.ind^{-1}), while Gianni et al. (2019) detected an average of 1.8 pieces.ind^{-1} in *Mullus barbatus* in three different seas (North Tyrrhenian, Adriatic and Ionian) and Gundogdu et al. (2020) detected 0.9 pieces.ind^{-1} in this species from the Istanbul coast of the Marmara Sea. Our results were high when compared to the results o of these researchers.

In our study, *C. lucerna* was the species with the highest number of microplastic particles (8.75 pieces.ind^{-1}) in the gastrointestinal tract. Güven et al. (2017) determined the average microplastic concentration of *C. lucerna* as 1.60 pieces.ind^{-1} in the stomach contents of the individuals sampled in the Mediterranean. Demersal fish species *C. lucerna* is not used as a biomonitor organism. However, Bray et al. (2019) suggested that *C. lucerna* should be considered as a biomonitor species because it has decreased plastic ingestion levels due to a limited home range. Our results were also consistent with this suggestion.

Barboza et al. (2020) determined an average of 0.6±0.8 pieces.ind^{-1} in the gill of *T. trachurus* and 0.7±1.0 pieces.ind^{-1} in its gastrointestinal tract in Northeast Atlantic waters. In our study, 1.19 pieces.ind^{-1} were determined in the gills and 4.25 pieces.ind^{-1} in the gastrointestinal tract of *T. trachurus* individuals. In individuals of the *E. encrasicolus* species obtained from the Gemlik Bay, 4.6 pieces.ind^{-1} were determined in the gastrointestinal tract. A study by Compa et al. (2018) on the coast of Spain found 0.18±0.20 plastic fibers per individual in the gastrointestinal tract of the same species.

Pellini et al. (2018) examined the concentration of microplastics in *S. solea* species in the Adriatic Sea in 2014 and 2015. They found an average of 1.73±0.05 pieces.ind^{-1} in the gastrointestinal tract of the individuals sampled in 2014 and 1.64±0.1 pieces.ind^{-1} in 2015. The number of microplastic particles determined in *S. solea* species sampled in Gemlik Bay was much higher than in individuals found in the Adriatic Sea. We detected 3.8 pieces.ind^{-1} in the gastrointestinal tract and 2.6 pieces.ind^{-1} in the gills. Examples of studies conducted in fish species are given in Table 7. Generally, in the studies examining the gastrointestinal tract, most of the determined amounts were lower than those we detected in the Gemlik Bay.

Considering the colour and shape distribution of microplastics determined in the gill and gastrointestinal tracts of the fish species obtained in our study, it was found that black (43.49%) and blue (35.45%) colours were dominant, but black was more dominant in the gills and gastrointestinal tract. Green, pink, orange and purple were the least common colours in both the gills and the gastrointestinal tract. In the shape classification, microplastics were defined in four different groups as fiber, fragment, granule and film. The most common type of microplastic among the determined groups was fiber. Of the 360 particles detected in the gills, 47.2% were in the form of fibers and 38.1% of 523 microplastic

Table 6. Microplastic pollution studies in sediments in different locations

Location	Microplastik Number pieces.kg^{-1} d.w.	Dominant Microplastic type	Dominant Polimer Type	Reference
Gemlik Bay	3333.3–9733.3	Fiber	PP, PVC,PPS	This Study
Marmara Sea (Golden Horn)	0–140	Film	-	Doğruyol et al., 2019
Marmara Sea (Pendik-Tuzla)	13–5100	Fragment	ABS, EVA, PS	Baysal et al., 2020
Istanbul (Marmara Sea- İstanbul Strait)	1957.37±4079.96	Filament. Fragment	-	Sarı Erkan et al., 2021.
Marmara Sea (Golden Horn)	1545	Fragment	-	Belivermis et al., 2021
Southwestern Black Sea (Türkiye)	28–684	Fiber	PET, PE, PP	Gedik and Gozler, 2022
Belgium	166.7 ± 92.1	Fiber	PP, PE, PS	Claessens et al., 2011
Italy	672–2175	-	PE, PP	Vianello et al., 2013
Hong Kong	49–279	Fragment	PP, LDPE, HDPE	Tsang et al., 2017
China	20–340	Fiber	Rayon	Peng et al., 2017
Spain	88.9–280.3	Fiber	-	Filgueiras et al., 2019
Black Sea	390–0	Fragment	PE, PP	Cincinelli et al., 2021

Table 7. Microplastic studies in fish species

Location	Species Number	Individual Number	Organ	MP (Pieces.ind^{-1})	Dominant Microplastic Type	Dominant Polimer Type	Reference
Gemlik Bay	10	123	Gill	5.38	Fiber	PP, POM, PPS	**This Study**
Gemlik Bay	10	123	Gastrointestinal Tract	5.49	Fiber	PPS	**This Study**
İzmit Bay (Marmara Sea)	12	374	Gastrointestinal Tract	1.14±1.03	Fiber	PET, PP,PA, EVA, PVA	**Aytan et al., 2023**
Türkiye (Black Sea)	7	650	Gastrointestinal System	2.06±1.09	Fiber	PP, PES, Acrylic, PE, PS	**Aytan et al., 2022**
Türkiye (Marmara Sea, Aegean Sea, Mediterranean Sea)	5	243	Gastrointestinal System	1.1	Fiber	PP, PE, PET,PES	**Gündoğdu et al., 2020**
Türkiye (Mediterranean Sea)	28	1337	Gastrointestinal System	2.36	Fiber	LDPE, PP	**Güven et al., 2017**
China	24	738	Gill and Gastrointestinal System	0.027±1.000	-	PES, PP, PE	**Koongolla et al., 2020**
Portugal	3	120	Gastrointestinal System	1.67±0.27	Fiber	PES, PP, Rayon	**Bessa et al., 2018**
North of Atlantic	10	761	Digestive System	1.2	Fiber	-	**Lusher et al., 2016**
Spain	3	212	Stomach	1.56±0.5	Fiber	-	**Bellas et al., 2016**
Portugal	**26**	**263**	**Stomach**	**0.63±0.27**	**Fiber**	**PP, PE**	**Neves et al., 2015**

particles in the gastrointestinal tract were also in this form. Fiber-type plastics were also dominant in the other studies reviewed in Tables 6 and 7.

As stated in the results section, a strong positive correlation was found between the fibers and the total number of individuals (r: 0.8; P<0.01) and the total amount of plastic (r: 0.890; P<0.01) since most of the microplastics were composed of fibers. That is why the correlation with the total amount of plastics was an expected result (Table 7). Güven et al. (2017) examined the concentration of microplastics in fish species at different trophic levels, but no correlation was found. Similarly, we did not observe any correlation between trophic level of the fish species and the microplastic concentration in Gemlik Bay. These results may be due to the close trophic levels of fish species.

Conclusion

This study has demonstrated the significance of the impact of port activities, river discharge, and river transit on microplastic contamination in Gemlik Bay. This outcome demonstrates the influence of maritime traffic, particularly in the port close proximity, and highlights the imperative of monitoring ship discharges. Furthermore, we have noticed that the Karsak Stream, which flows into Gemlik Bay, has a significant impact on the buildup of pollutants. Therefore, we strongly advise monitoring the pollution indicators of the stream. The elevated microplastic (MP) concentration in demersal and benthopelagic fish species is influenced by the substantial MP concentration in the sediment due to their feeding behavior as bottom dwellers. This outcome demonstrates the impact of marine pollution on the food web.

Ethical Statement

All authors followed the ethical responsibilities of this journal.

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Author Contribution

AM acquired the financial support for the project leading to this publication, conceptualization, writing original draft. AM and EY analysed and interpreted the data and did preparation of sediments for analysis. SKG and EY made Raman analysis, figures and visualization. All authors have read and agreed to the published version of the manuscript.

Conflict of Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this article.

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