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SEDIMENT CHALLENGES AND OPPORTUNITIES

Distribution and characterization of microplastics in marine sediments from the Montenegrin coast

Neda Bošković[1] · Danijela Joksimović[1] · Ana Perošević-Bajčeta[1] · Milica Peković[1] · Oliver Bajt[2,3]

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Abstract

Purpose Plastic pollution in the world has led to an abundance of microplastics (MPs) and has been identified as a potential factor that can lead to serious environmental problems, especially in oceans and seas. Information on the current status of MPs pollution along the Montenegrin coast is insufficiently investigated. This study monitors the abundance, distribution, and sources of MPs, and identifies present polymers in the surface sediment of the Montenegrin coast, as well as comparison with previous research.

Materials and methods Ten sampling sites along the Montenegrin coast were selected to collect surface sediment samples. The upper layer of sediment (0–5 cm) was collected by a Petite ponar grab. The samples were dried, and density separation was performed using a NaCl solution. The abundance and morphological characteristics of MPs were determined using an optical microscope (DP-Soft software), while FT-IR analysis was done to identify the polymer type.

Results and discussion Microplastics were identified in all sediment samples with an average abundance of 307 ± 133 (SD) MPs/kg in dry sediment. The highest abundance of MPs was found in locations in the vicinity of highly populated areas, near wastewater discharges, and areas with high fishing and tourist activities. The most dominant shape types of MPs in all samples were filaments and fragments. The most common colors of MPs were blue and red, while the dominant MPs sizes were 0.1–0.5 mm and 0.5–1.0 mm. Of the eight identified polymers, PP, PE, and PET were the most common.

Conclusion This study reveals MPs characteristics (abundance, distribution, shape type, colors, size, polymers type) in surface sediment along the Montenegrin coast, as well as the most significant sources of MPs pollution, and provides important data for further research on MPs to identify the effects of MPs pollution on the quality, health, and functionality of the marine environment.

Keywords Microplastics · Marine sediment · FT-IR · Montenegro · Adriatic Sea

1 Introduction

The term microplastics (MPs) describes any synthetic solid particle or polymeric matrix, with regular or irregular shape, with size ranging from 1 µm to 5 mm, primary or secondary manufacturing origin and which are insoluble in water (Frias and Nash 2019). Primary MPs are intentionally produced MPs of synthetic polymers that have a wide range of applications including micro-beads incorporated into cosmetic products, resin pellets, and beads used for abrasive blasting (Ryan et al. 2009; Hintersteiner et al. 2015; Wang et al. 2020). Also, primary MPs can originate from the abrasion of synthetic textiles during washing or abrasion of large plastic objects during manufacturing, use, or maintenance such as the erosion of tyres (Sundt et al. 2014). Secondary MPs are formed by the fragmentation of larger pieces of plastic due to the action of various environmental factors (physical, chemical, and biological), which results in the decomposition of plastic into smaller fragments, meaning that macroplastics will fragment into microplastics (Thompson et al. 2004; Arthur et al. 2009; Cole et al. 2011; Yu et al. 2020).

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✉ Neda Bošković
nedaboskovic93@gmail.com

1 Institute of Marine Biology, University of Montenegro, Put I Bokeljške brigade 68, Kotor 85330, Montenegro

2 Marine Biology Station, National Institute of Biology, Fornače 41, Piran 6330, Slovenia

3 Faculty of Maritime Studies and Transport, University of Ljubljana, Pot pomorscakov 4, Portoroz 6320, Slovenia

Side effects of MPs on marine organisms can be physical and chemical. Physical effects are most often related to the size and shape of MPs, while the chemical effects are related to the fact that plastic carries a "cocktail of chemicals" with it (Browne et al. 2011). Among the chemicals present in MPs are those incorporated into plastic polymers during their production (various additives) and those present in water that are adsorbed on the surface of MPs, such as various organic and inorganic pollutants (Godoy et al. 2019).

A large number of studies indicate the presence of plastic as a pollutant in the Adriatic Sea and predict that the Adriatic region will be one of the main areas of plastic accumulation in the Mediterranean, both due to its oceanographic conditions and the high degree of different anthropogenic pressures present in the small area (Liubartseva et al. 2016; Carlson et al. 2017). In the Adriatic Sea, MPs have been found in abiotic and biotic areas, including beaches (Munari et al. 2017), surface waters (Gajšt et al. 2016; Suaria et al. 2016; Vianello et al. 2018), sediment (Vianello et al. 2013; Laglbauer et al. 2014; Renzi and Blašković 2020; Bošković et al. 2021), fish (Avio et al. 2015; Anastasopoulou et al. 2018; Giani et al. 2019), and shellfish (Gomiero et al. 2019; De Simone et al. 2021).

Increased awareness of the growing production and subsequent accumulation of plastic pollution in the environments worldwide has identified MPs as a potential factor contributing to the biodiversity loss in the oceans and seas (Gall and Thompson 2015), which has encouraged the inclusion of various international legislation and projects in the field of marine environment protection. The Marine Strategy Framework Directive (MSFD) states that member states are obliged to take action to achieve and maintain good environmental status and emphasizes the need to obtain as accurate data as possible on the identification, quantification, distribution, and monitoring of environmental MPs, as defined in priority descriptor 10.1.3 (MSFD 2008/56/EC 2008).

The aim of this study is to give additional and more precise information on sources, abundance, and distribution of MPs in surface sediment on the Montenegrin coast. This is important for undertaking available measures to reduce MPs levels in the marine environment, as well as further investigations and monitoring in this field contributing to the efforts of the MSFD.

2 Materials and methods

Sediment sampling was performed during the spring of 2021. The study areas for sediment analysis included six locations in Boka Kotorska Bay (Dobrota, Orahovac, Sveta Nedjelja, Tivat, Bijela, and Herceg Novi) and four locations on the coastal area of the open sea (Žanjice, Budva, Bar, and Ada Bojana) (Fig. 1). Sampling locations selected for the research had different geographical positions, morphological and hydrological characteristics, and were influenced by different anthropogenic factors. In Table 1, the basic sampling data are presented.

Surface sediment (upper 5 cm) was sampled using a Petite ponar grab, Wildco (composite sample of two samples from one location). Sediment samples after the homogenization which was carried out by conning and quartering (about 500 g) were then frozen at −18 °C and subjected to a cold drying procedure in a freeze-dryer (CHRIST, Alpha 2–4 LD plus) under a vacuum at −40 °C for 48 h. In order to

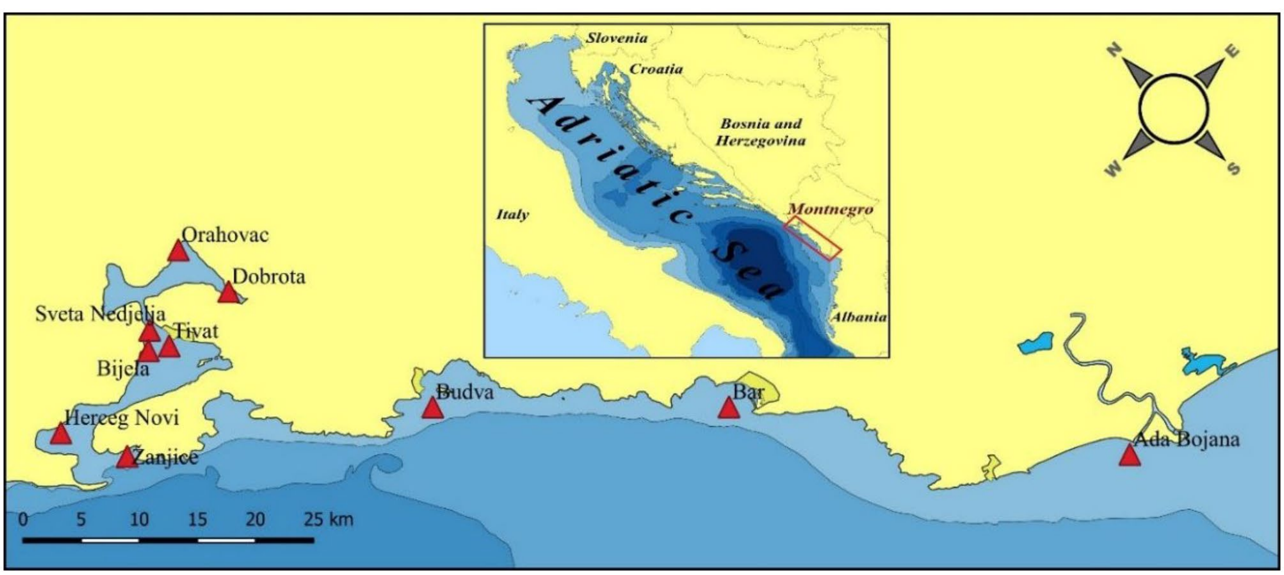

Fig. 1 Sampling locations of surface sediments along the Montenegrin coast

Table 1 The basic sampling data

Sampling locations	Coordinates		Depth of sampling (m)	Date of sampling	Type of sediment*
Dobrota	42.436738	18.762041	10	12.04.2021	Slightly gravelly muddy sand
Orahovac	42.486974	18.753844	20	12.04.2021	Slightly gravelly muddy sand
Sveta Nedjelja	42.457092	18.674193	19	12.04.2021	Gravelly muddy sand
Tivat	42.437744	18.677641	38	12.04.2021	Slightly gravelly muddy sand
Bijela	42.446168	18.658379	24	12.04.2021	Slightly gravelly muddy sand
Herceg Novi	42.446485	18.532894	42	12.04.2021	Slightly gravelly muddy sand
Žanjice	42.397888	18.566368	9	12.04.2021	Slightly gravelly muddy sand
Budva	42.262911	18.833523	31	16.04.2021	Slightly gravelly sand
Bar	42.104562	19.057053	32	16.04.2021	Slightly gravelly muddy sand
Ada Bojana	41.863054	19.323559	12	16.04.2021	Slightly gravelly sand

*According to classification by Folk (1954)

extract MPs from the sediment, a density separation process was applied according to the method proposed by Thompson et al. (2004), using supersaturated NaCl solution (1.202 g cm^{-3}). In a glass jar (1 L), 100 g of dry sediment and 0.5 L of concentrated NaCl solution were added. The sample was manually vigorously shaken for 2 min. After 24 h, the supernatant was decanted through a 63 µm steel sieve. The residue (precipitate) for each sample was again subjected to a density separation process. After sieving, the samples were filtered on glass fiber filters of Grade C using a vacuum pump, and then transferred to glass Petri dishes.

In order to visually identify and count the number, determine the shape, color, size, and texture of MPs present in the samples, the samples were analyzed under a microscope. Microplastics are usually divided into four size categories: < 0.1 mm, 0.1–0.5 mm, 0.5–1.0 mm, and 1.0–5.0 mm and four types of shapes: fragments, filaments, films, and granules (Galgani et al. 2013). Fragments represent irregularly shaped particles, such as crystals, powder and flakes, rigid, thick, with sharp curved edges. The filaments or fibres are thread-shaped, oblong, may look like strips or have a cylindrical shape. Films are irregularly shaped, thin, flexible and usually transparent compared to fragments. Granules are spherical particles, such as pellets of common resins, spherical microbeads and microspheres (Claessens et al. 2011; Frias and Nash 2019). Even though color is not considered to be crucial to defining MPs, because color differentiation is subjective (Frias and Nash 2019), categorizing MPs according to color is useful to identify potential sources as well as potential contaminations (Hartmann et al. 2019). Visual analysis of MPs was performed using an Olympus SZX16 optical microscope (DP-Soft software). During visual identification, we followed the guidelines proposed by Hidalgo-Ruz et al. (2012) to reduce errors. The MPs on the filters were counted three times, with a discrepancy that did not exceed 5%. Chemical identification of MPs was performed using FT-IR microspectroscopy (Perkin Elmer Spotlight 200i FT-IR spectroscopy), which allows accurate identification of polymer particles according to their IR spectrum (Thompson et al. 2004; Ng and Obbard 2006; Reddy et al. 2006; Frias et al. 2010; Harrison et al. 2012; Löder and Gerdts 2015). Special care was taken to analyze all types of particles (different colors, shapes, sizes, and structures) using FT−IR spectroscopy. Approximately 30% of the particles were recorded on FT-IR in each sample individually. Each MPs particle was recorded on FT-IR which was previously photographed and their spectra were preserved. Procedural blanks were performed and collected during all analyses. All results were corrected according to the level of contamination measured during sample processing and analysis, to compensate for external contamination. Abundances of MPs were calculated as the total number of MPs/kg of dry sediment.

2.1 Quality assurance and quality control

As contamination in the work can cause significant overestimation of quantitative results (Foekema et al. 2013), in all phases (sampling, transport, drying, density separation, visual, and chemical identification), special care was taken to prevent contamination or cross-contamination of samples. In other words, plastic accessories were avoided during the analysis. Glass and metal utensils/glasswear, washed and rinsed with Milli-Q water, were used during each analysis. We paid special attention to ensure the cleanliness of the laboratory space, especially in regards to dust or other particles. The samples were exposed to air for a minimum time and the analysis procedures were performed in a clean laboratory (fume hood). Work surfaces were cleaned with high-quality ethanol before each process/activity. After filtration, the filters were stored in glass Petri dishes. Pure cotton lab coats were used all the time and synthetic clothing was limited.

2.2 Statistical analyses

Statistical analysis was carried out using principal coordinate analysis (PCO) and cluster analyses with the Premanova Monte Carlo test to verify the significant difference between MPs abundance at different sampling locations ($p < 0.05$). Data were square-root transformed before analysis based on the Bray–Curtis similarity matrices. All data analyses were carried out in PRIMER v7 with PERMANOVA+ software.

3 Results and discussion

From the total 348 particles of MPs visually detected in the surface sediments at all locations, 29.31% of them were analyzed for chemical identification of polymer types using FT−IR spectroscopy. Polymer identification by FT-IR spectroscopy identified eight polymer types: polypropylene (PP, 33.3%), polyethylene (PE, 15.7%), polyethylene terephthalate (PET, 14.7%), polyamide (PA, 4.9%), polystyrene (PS, 3.9%), and acrylate copolymer (AC cop., 2.9%). Some MPs particles (12.7%) were identified as polymers, but due to their decomposition during years (aged plastic), it was difficult to determine which polymer category it fell into (because of the high number of different copolymers, which have emerged during years), so we marked them as unidentified polymers (Unid. poly.). The remaining 11.8% of MPs were non-synthetic materials, cellulose. Cellulose was identified in the surface sediments at 6 of the 10 sampling locations, and these were usually filaments.

Results of chemical identification positively identified 88.2% of the analyzed MPs as plastic, so the corrected average abundance of MPs in surface sediment from 10 locations along the Montenegrin coast sampled during the spring of 2021 was 307 ± 133 (SD) MPs/kg of dry sediment. Figure 2a shows the percentage of polymers in the sediments samples from the Montenegrin coast, and Fig. 2b shows examples of the identified spectra by FT-IR of the most common polymers in the analyzed sediments All sediment samples contained a minimum of three and a maximum of seven different polymer types. Polypropylene and PE were detected in surface sediments at all 10 sampling locations.

Polypropylene and PE were the most common types of polymers in the study done by Bošković et al. (2021). However, in this study, polymers such as PET, PS, and PA were not identified in sediment sampled during the autumn of 2019 (Bošković et al. 2021). Statistical significance ($p < 0.05$) was observed in the presence of different polymers in this study from spring 2021 and the study Bošković et al. (2021) from autumn 2019 (Permanova, Monte Carlo test). Polypropylene and PE are two polymers with very high annual demand and many authors revealed that these polymers are the most frequently found polymers in marine environments around the world (Vianello et al. 2013; Frère et al. 2017; Abidli et al. 2017, 2018; Bošković et al. 2021). They are widely distributed in household appliances, such as packaging, durable textiles, pipes, but are also used for fishing nets, strapping ropes, bottles, packaging bags, etc. (Mistri et al. 2017; Vianello et al. 2018; Fan et al. 2021). Polystyrene, in addition to PP and PE, is one of the most

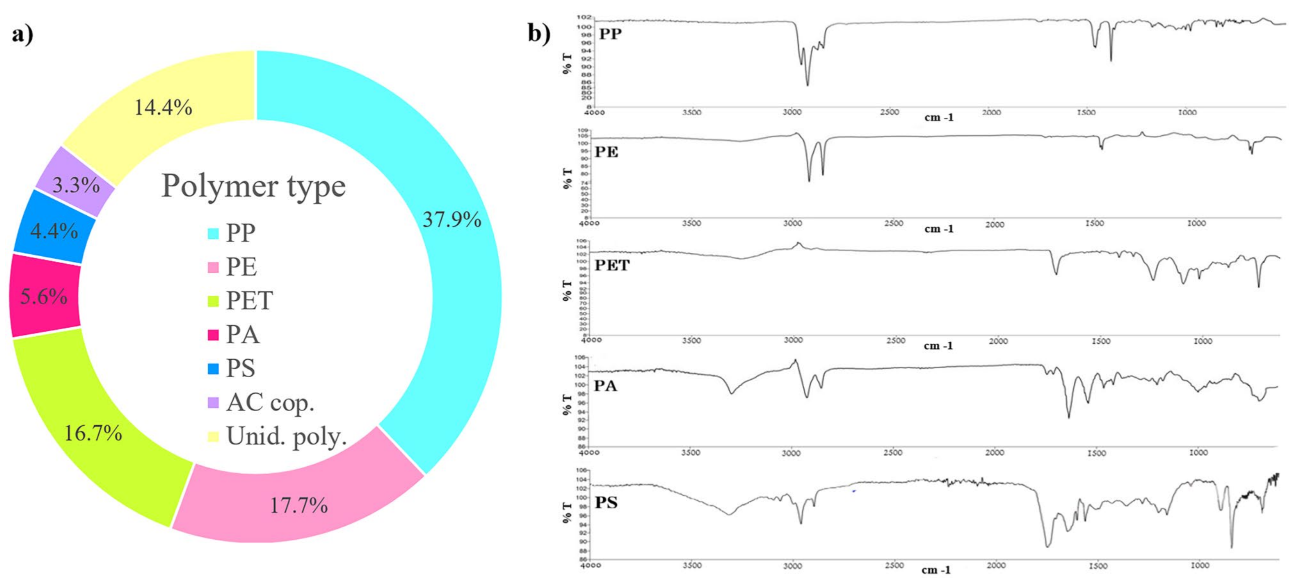

Fig. 2 **a** Distribution of polymers in surface sediments at all sampling locations and **b** FT-IR spectroscopy spectra of the most common polymers collected in this study

commonly used plastics. The use of PS includes protective packaging, containers, lids, bottles, trays, baking cups, and disposable utensils (Maul et al. 2007). Polyethylene terephthalate is used in clothing fibers, for the production of bags, sacks and wrappers, packaging, containers, and also in combination with glass fibers for engineering resins (Oliveira et al. 2020; Fan et al. 2021). Polyamide has commercial application in the production of fabrics, fibers, nets, and films (mainly for food packaging) (Ndiaye and Forster 2007), while the AC cop. is widely used in the cosmetics industry for the production of sunscreens, skin and hair care products, shaving creams, body washes, and moisturizers (Yayayürük 2017).

The mutual PCO and cluster analysis of the distribution of identified polymers with respect to sampling sites and sampling zones are shown in Fig. 3. The results of the PCO show that factors 1 and 2 explain 85.8% of the total variance in the data matrix, where factor 1 explains 54.3% of the total variance, and factor 2 explains 31.4% of the total variance. PCO showed significant correlations between different sampling zones in relation to the polymer distribution ($p < 0.05$). The cluster analysis showed two separate clusters whose mutual similarity and connection is 40%, while within the clusters, individually, it is from 60 to 80%. The first cluster includes sediment samples from the locations Sveta Nedjelja and Žanjice which are connected by a similar presence of the PE as the dominant polymer, followed by AC cop., Unid. poly., and PP. The second cluster includes sediment samples from the locations Ada Bojana, Budva, Herceg Novi, Bijela, Tivat, Orahovac, Dobrota, and Bar and reveals several different types of polymers. Only at the locations of Bijela and Tivat, had PS identified in addition to all other polymers, which is why they are in the subcluster, while Orahovac, Bar, and Dobrota in the subcluster are linked by a similar presence of PA and Unid. poly. in addition to other present being polymers (Fig. 3).

Microplastics were identified at all 10 locations. The average concentrations of MPs in the surface sediments of the Montenegrin coast were in the descending order Bijela > Dobrota > Tivat > Budva > Herceg Novi > Orahovac > Bar > Ada Bojana > Sveta Nedjelja > Žanjice. The overall abundance of MPs at all sampling locations of the Montenegrin coast is shown in Fig. 4.

The abundance of MPs greatly varied with sampling location. The locations characterized by the highest population density, and therefore the greatest anthropogenic influences, the highest concentrations of MPs (Dobrota, Tivat, Bijela, Herceg Novi, and Budva) were recorded. As expected, locations Orahovac, Sveta Nedjelja, and Žanjice, had lower concentrations of MPs, since these locations are not densely populated, except during the summer months when they are tourist hotspots. Lower prevalence of MPs were recorded at Bar and Ada Bojana. This can be explained by the greater scattering of MPs in the areas influenced by the open sea due to greater and stronger actions of currents and waves in comparison to the Bay. Similar observations were made previously by Alomar et al. (2016), Abidli et al. (2018), Korez et al. (2019), Palatinus et al. (2019), and Bošković et al. (2021). Comparing the zones, Boka Kotorska Bay and the coastal part of the open sea, it is concluded that the average

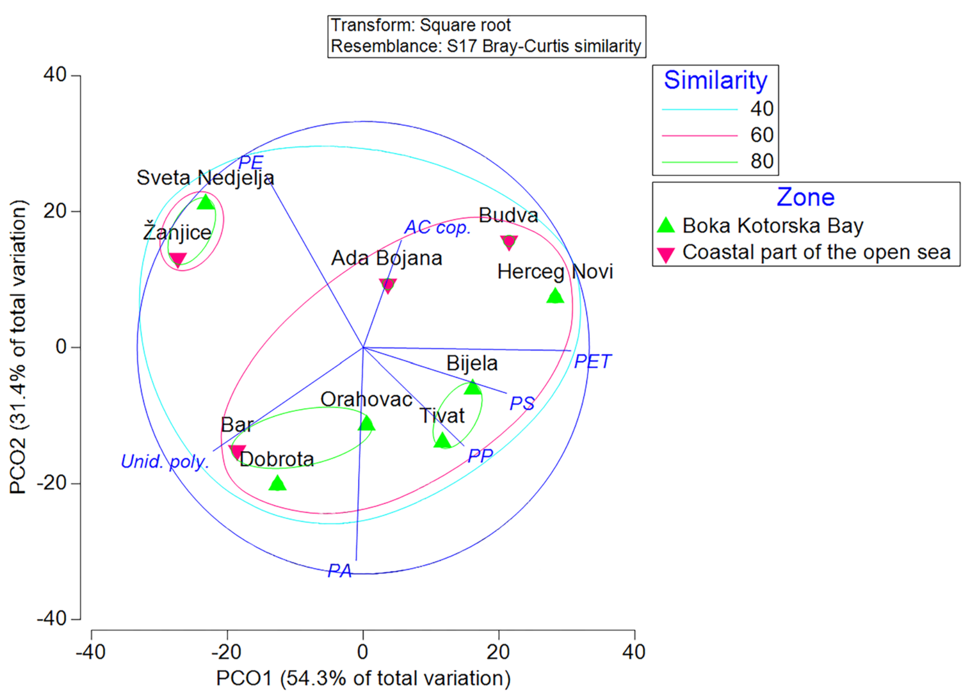

Fig. 3 Graphical representation of the distribution of polymers in the sampled sediments in relation to the locations and sampling zones, PCO + cluster analysis (PRIMER v7 with PERMANOVA+)

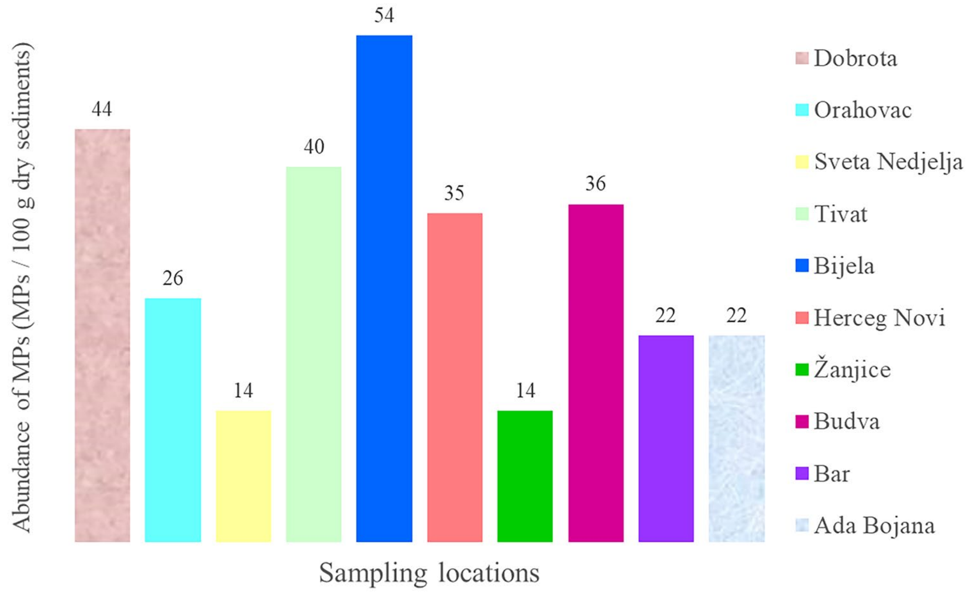

Fig. 4 The abundance of microplastics in surface sediments at 10 sampling locations along the Montenegrin coast

presence of MPs was significantly higher in surface sediments at the locations from the Bay than at the locations on the coastal part of the open sea.

The average number of MPs found in all sediment samples collected in the spring of 2021 was twice as low than that reported for the Montenegrin coast at the same locations during the autumn period of 2019 (Bošković et al. 2021). More precisely, at the locations of Dobrota, Sveta Nedjelja, Herceg Novi, Žanjice, Budva, and Ada Bojana, there were significantly higher concentrations of MPs in the sediment sampled during autumn 2019 compared to sediment sampled in this study (Bošković et al. 2021). It is important to note that in 2019, it was recorded as the best tourist season in Montenegro (Government of Montenegro 2019). The impact of epidemiological measures caused by COVID-19 in 2020 had a noticable effect. During this time, activities such as tourism and fishing lessened. Locations representing port centers such as Tivat, Bijela, and Bar carried out all their usual activities during the pandemic caused by COVID-19. At these locations, higher concentrations of MPs were recorded in this study compared to the study by Bošković et al. (2021). The largest difference in the number of MPs in the sediment from the Montenegrin coast sampled during the autumn period 2019 compared to the spring period 2021 may be a consequence of anthropogenic impact due to increased tourist activity and accumulation of MPs during summer (Claessens et al. 2011; Browne et al. 2011; Abidli et al. 2018). Compared with literature data from the Adriatic and the Mediterranean Sea, the average abundance of MPs found in all sediment samples of this study was lower than that reported for Croatia, Italy, and Spain (Vianello et al. 2013; Alomar et al. 2016; Palatinus et al. 2019; Renzi et al. 2019), and higher than MPs abundance found for sediment samples from Slovenia, Croatia, Italy, and Tunisia (Laglbauer et al. 2014; Blašković et al. 2017; Abidli et al. 2018; Renzi et al. 2018, 2019; Renzi and Blašković 2020). In this study, several factors were observed that can be related to the occurrence and distribution of the MPs contamination in the surface sediments: (1) natural factors, such as plastic properties, meteorological, and hydrodynamic conditions, and (2) anthropogenic factors such as dense populations, tourist, fishing activities, wastewater discharges, solid waste, passenger ships, and harbors. Similar observations were made by Barnes et al. (2009), Browne et al. (2011), Wagner et al. (2014), Abidli et al. (2017), Naji et al. (2017), and Fan et al. (2021).

Microplastics appear in different shape, size, and color. The images of collected MPs in surface sediments from the Montenegrin coast are shown in Fig. 5. The highest proportion of shapes was recorded for filaments (52.8%), followed by fragments (35.5%), films (6.5%), and granules (5.2%) (Fig. 6a). Filaments and fragments were found at all examined locations, while films and granules were identified at five sampling locations (Dobrota, Sveta Nedjelja, Bijela, Žanjice, and Ada Bojana for films, and Dobrota, Žanjice, Budva, Bar, and Ada Bojana for granules). Sediments from Orahovac, Tivat, and Herceg Novi had all four shape types. Filaments accounted for over 50% of the total MPs at seven of the 10 sampling locations. Filaments in surface sediments can originate from a wide range of sources, such as peeling of plastic fishing gear, domestic sewage (laundry wastewater), and the industrial production of fabrics and textiles (Mistri et al. 2018; Fan et al. 2021). Abundance of filaments was similar in sediment samples in this study and at the same locations sampled during 2019 (Bošković et al. 2021). Abundance of fragments and films was twice as high in sediment samples in this study compared to the study conducted in 2019, while the abundance of granules was four times higher in the

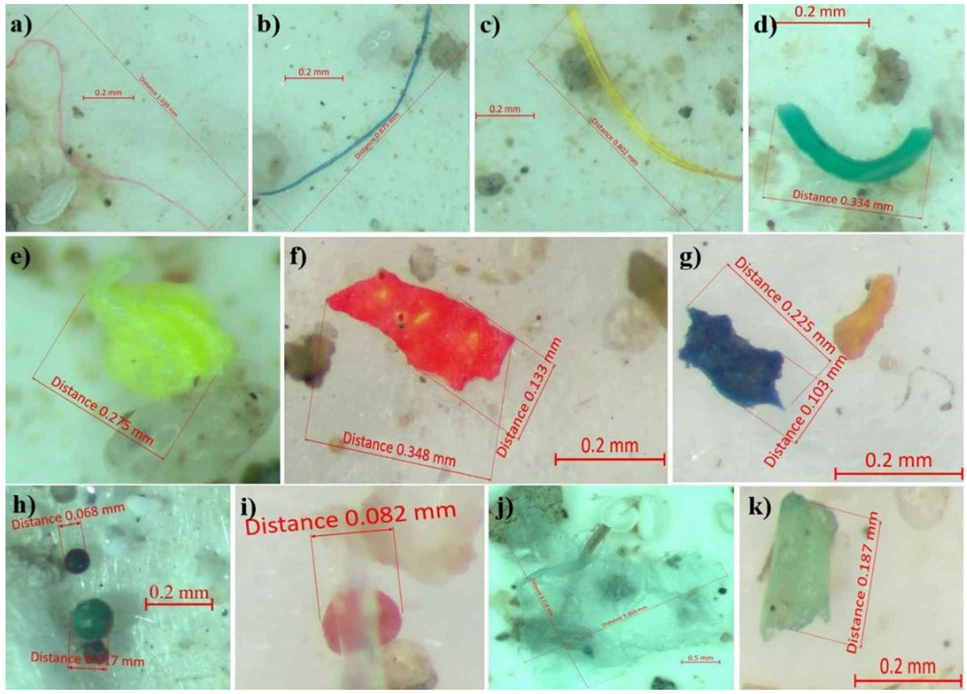

Fig. 5 Images of microplastic particles identified by using an Olympus SZX16 optical microscope: filaments (**a**–**d**), fragments (**e**–**g**), granules (**h**, **i**), and films (**j**, **k**)

study from 2019 compared to this study (Bošković et al. 2021). Statistical significance ($p < 0.05$) was observed between different sampling years (2021 and 2019) and different zones (Boka Kototska Bay and coastal part of the open sea) in relation to the presence of different shape types of MPs (Permanova, Monte Carlo test). Previous studies reported that filaments were the dominant shape type of MPs in sediments (Thompson et al. 2004; Vianello et al. 2013; Blašković et al. 2017; Mistri et al. 2017, 2018; Bošković et al. 2021). The source of fragments is related to the breakdown of larger plastic debris, films mainly originate from the weathering and cracking of packaging/bags or plastic wrappers, while granules could originate from various cosmetic products (Claessens et al. 2011; Abidli et al. 2017, 2018; Fan et al. 2021).

In terms of color, there were clear differences in abundance: blue (37.8%) > red (25.1%) > green (11.1%) > black (10.1%) > yellow (7.8%) > clear (6.14%) (Fig. 6b). The majority of filaments were blue (46.9%), followed by red (16%), black (15.4%), clear (14.8%), yellow (4.94%), and green (1.85%). Fragments were dominated by red (39.4%), blue (33.9%), green (21.1%), yellow (4.6%), and black (0.9%) color. Films by green (40%), yellow (30%), red (20%), black (5%), and clear (5%). Lastly, granules by black (31.3%), yellow (31.3%), red (25%), and blue (12.5%) color.

The size distribution of MPs in the studied samples is presented in Fig. 6c. Microplastics were divided into four size categories: < 0.1 mm, 0.1–0.5 mm, 0.5–1.0 mm, and 1.0–5.0 mm. Small-sized MPs usually have a high abundance because large particles can be split into small ones

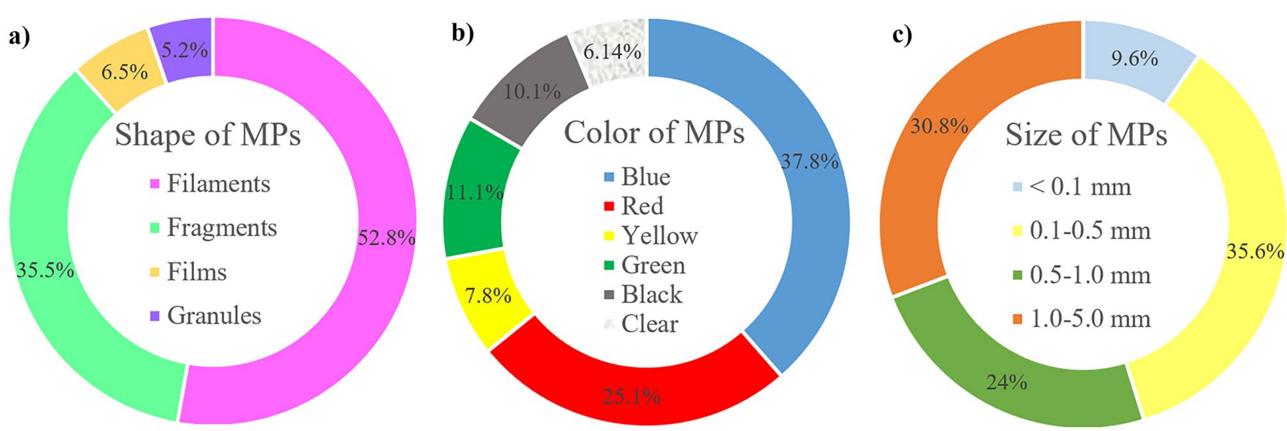

Fig. 6 Distribution of microplastics in surface sediments regarding **a** shape, **b** color, and **c** size at all sampling locations

(Browne et al. 2010; Zhang et al. 2020; Fan et al. 2021). Microplastics in the size category of 0.1–0.5 mm (35.6%) were the most abundant in the sediment samples at all sampling locations, following by sizes 1.0–5.0 mm (30.8%), 0.5–1.0 mm (24%), and < 0.1 mm (9.6%). Differences in the size, shape, and color of MPs could indicate the different origin of the plastics but also the different degrees of accumulation and degradation (Hidalgo-Ruz et al. 2012; Choi et al. 2021).

This study confirms the influence of anthropogenic factors, which is enhanced by tourism. This statement can be approved by the fact that the presence of MPs decreased twice compared to the previous measurement period, during the autumn of 2019, after the best summer tourist season (Bošković et al. 2021). Similarly, Piazzolla et al. (2020) indicated that repeated long-term investigations and seasonal surveys of MPs pollution in sediments give more precise information important for further investigations and monitoring. Additionally, in this study, approximately 30% of MPs particles were analyzed on FT-IR and compared with the study from autumn of 2019, in which 15% of MPs particles were analyzed of the total number of identified MPs (Bošković et al. 2021). Therefore, the results from this study give a more precise insight into the presence of different polymer types in the analyzed sediments and are crucial for undertaking prevention measures to reduce MPs levels in the marine environment. Nevertheless, further studies are needed to better evaluate risks for marine biota associated with MPs pollution.

4 Conclusions

Microplastics were detected in the surface sediments at all sampling locations along the Montenegrin coast. The average abundance of the MPs was 307 ± 133 (SD) MPs/kg of dry sediment. The highest abundance of MPs in surface sediments was detected at the locations in the vicinity of highly populated centers. This result indicates that different human activities might play an important role in MPs pollution around the study area. Additionally, the distribution of MPs depends on meteorological and hydrological factors that can lead to the dispersal or accumulation of MPs in sediments. Filaments and fragments were the dominant shape type of MPs, blue and red were the most common colors, while dominated MPs sizes in all the samples were 0.1–0.5 mm and 0.5–1.0 mm. Eight different polymers were identified in sediments from the examined locations, the most dominant of which were PP, PE, and PET. Polypropylene and PE were present at all sampling locations. In the future, in order to prevent and control plastic pollution, additional studies should be conducted on the analysis of pollution sources as well as on environmental risks arising from the increased presence of MPs in the marine environment.

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Declarations

Conflict of interest The authors declare no competing interests.

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