Microplastics presence in aquatic environments in Montenegro: A review on methods, occurrence and sources

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Abstract. Microplastic (MPs) pollution represents a scientific topic that has received increasing attention over the last decade, due to the constant increase in plastic production and its subsequent disposal and accumulation in the aquatic environment. Worldwide reports of MPs in aquatic environment remarking the large spatial distribution of this contaminant and therefore the possible implications which MPs might have in aquatic habitat and food webs. This literature review is based on presence of MPs in the Montenegrin aquatic environment with an emphasis on sustainable water resources.

Keywords: microplastic, sustainable development, freshwater

1 Introduction

Plastic products are used by most societies worldwide and their production has increased dramatically since their initial commercial development in the 1950s [1]. Currently, there are no signs indicating any future decrease in the production of these synthetic polymers that are derived from crude oil [1]. Plastic debris pollution is distributed from pole to pole, the Arctic to the Antarctic, and it is of great concern to social and scientific communities [2, 3]. With the increasing world population, the usage of plastic has increased, meanwhile the waste management of plastics is still concern for researchers [4]. Due to its characteristics such as chemical inertness and slow degradation rates, most plastic debris results in an accumulation in the environment [5].

There are more than 5000 types of synthetic polymers used in plastic items, but 80 % of the total plastic polymers are polypropylene (PP), polyethylene (PE), polyvinyl chloride (PVC), polyethylene terephthalate (PET) and polystyrene (PS) [6]. As plastic debris is found in smaller micro to nano-particle sizes, it becomes more challenging to identification the plastic particles in environment. The term microplastics (MPs) refers to ubiquitous plastic particles smaller than five millimeters (5 mm) in size [7]. MP have attracted public attention for their ubiquity and persistence in the aquatic environment, and the potential risk to the health of ecosystems [8]. Furthermore, due to its minute size, MP can be taken up by organisms from different trophic levels and with different feeding strategies, thus they can

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enter the food chain and accumulate at higher trophic levels [9, 10]. The negative effects of MP include physical damage to the gastrointestinal tract of ingesting organisms and toxicological effects caused by toxic chemicals and additives adsorbed in the MPs, which can be carcinogenic, and endocrine-disrupted [11].

The objectives of this review were divided to several parts as follows: (1) MPs pollution in Montenegrian aquatic environments; (2) Evaluation of MPs pollution in biota and (3) Suggestion for future research related to the monitoring and expansion of the analysis of the presence of MPs in the freshwater ecosystems of Montenegro in order to find adequate solutions for the improvement and preservation of the environment, as well as the implementation of the principles of sustainable development.

The literature review showed that there are currently only three studies on the MPs presence in the surface sediment of the Montenegrin coast and one study on the MPs presence in commercially important fish species from the Montenegrin coast [12-14], while there are no studies on the MPs presence in the freshwater ecosystems of Montenegro.

2 MPs analysis methodology

Details on the methodology used for the analysis and calculation of MP abundance are critical when comparing data between studies. It is also important for authors to provide enough raw data in their reports or appendixes so that others can calculate MP densities in different ways and enable more comparisons. Details on the methodology used in review of the literature [12-14] for the analysis of MPs in marine environments studies from Montenegro is presented in Fig. 1.

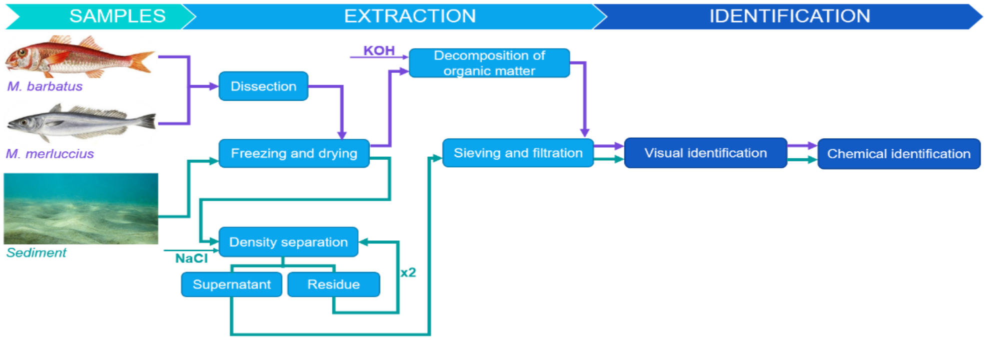

Fig. 1. Methodology of MPs analysies

Sediment samples were collected for analysis using Van Veen grab, stored in the aluminum container, frozen at -18 °C and freeze-dried at -40 °C for 48 h (CHRIST, Alpha 2-4 LD plus, Germany). For MPs density separation, was used a method proposed by Thompson et al. [7]. The method is based on the usage of the concentrated NaCl solution (1.2 g/dm^3). After sedimentation, the solution was decanted. The supernatant was sieved through the 63 μm steel sieve. The material retained on the sieve was rinsed with Mili-Q water to a glass Petri dish. Then, the sediment samples were filtered using a vacuum pump on to fiberglass filters (Whatman GF-F, diam. 47 mm, nominal porosity 0.7 mm) [12-14].

Fish was carried out with triple standing nets and trawls, i.e. demersal nets. Subsequently, fish were transported to the laboratory where they were dissected. The GITs of fish were analyzed for MP ingestion. The GIT of each fish examined was removed. Samples were stored in a freezer at −18 °C until further analysis. Frozen fish stomachs samples were subjected to a cold-drying procedure under vacuum at −40 °C for 48 h (CHRIST, Alpha 2-4 LD plus, Germany). Potassium hydroxide (KOH, 10 %) was used to digest organic matter in the fish to reduce the possibility of confounding small plastic particles. KOH efficiently degrades organic material with minimal degradation of plastic polymers and facilitates the comparison and sharing of data by the scientific community [15-17]. After decomposition of the organic matter, the thick liquid was filtered through a steel wire sieve with a pore size of 63 μm. After the organic matter digestion in the fish samples were completed, they were vacuum filtered on GF/C grade Whatman glass fiber filters with a pore size of 45 μm. Subsequently, each filter paper was kept in covered Petri dishes, which were placed in an oven at 40 °C for 12 h to dry before determination of MPs [14].

To identify MPs, filters were visually observed under an Olympus SZX16 imaging microscope (DP-Soft software). Identified MPs were counted and categorized by shape, size, and color according to the Marine Strategy Framework Directive protocol [18]. MPs were assigned to four categories of particle shapes: fragments, filaments, granules, and films and four size classes: 0.063–0.1 mm, 0.1–0.5 mm, 0.5–1.0 mm and 1.0–5.0 mm [19]. Visual identification of MPs is based on the physical and morphological characteristics of the particles [20].

Chemical composition was determined by Fourier transform infrared spectroscopy (FTIR) (Perkin Elmer Spotlight 200i, ATR FTIR Spectrum Two) to confirm the origin of the synthetic polymers. Fourier Transform Infrared (FT-IR) spectroscopy is an excellent technique used to positively identify the synthetic polymers in a sample, improves the spatial resolution with the combination of microscopy [1].

In all analyses (sampling, dissection, digestion, density separation, filtration, visual inspection under microscope), blank samples were included for quality control to exclude air contamination in the laboratory. Blank tests were conducted at the same time as sample analyses. Analyses were performed expeditiously so as to expose samples to air for as little time as possible. All procedures were performed in a clean fume hood, and work areas were cleaned with alcohol. Glass and metal dishes were used for each analytical step; before each procedure they were washed with ultra-pure MilliQ water [12-14].

3 Current knowledge of MPs in marine environment

3.1 Occurrence and distribution

3.1.1 Sediment

The MP presence has been recorded in surface sediment worldwide. More recently, MPs have been found in surface sediment of Montenegro [12-14]. This literature review provide basic information on MPs types, occurrence, and distribution in surface sediments along Montenegrin coastline. The average abundance and overview distribution of MPs in marine sediment studies are shown in Fig. 2, summarizing the new developments in this research focus.

Authors indicated that MPs were found in all examined sediment samples [12-14]. The MP abundance in marine sediment from Montenegrin coast varies widely among different study areas, location, season and year of sampling.

Comparing the areas, Boka Kotorska Bay (Dobrota, Orahovac, Sveta Nedjelja, Tivat, Bijela, Herceg Novi) and the coastal part of the open sea (Žanjice, Budva, Bar, Ada Bojana), it is concluded that the average presence of MPs was usually higher in surface sediments at the locations from the Bay, which are characterized by reduced contact with the open sea, than at the locations on the coastal part of the open sea, Fig. 2.

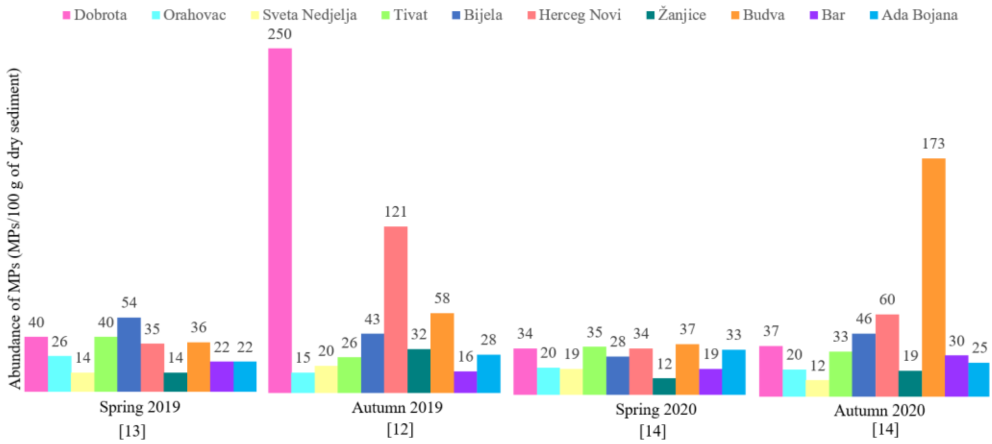

Fig. 2. The average abundance and overview distribution of MPs in marine sediment

Authors indicated that MPs abundance greatly varied with sampling location [12-14]. 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, Fig. 2. 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, Fig. 2. Lower prevalence of MPs were recorded at Bar and Ada Bojana, Fig. 2. Authors 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 [12-14]. Similar observations were made previously [21-23].

The average number of MPs found in all sediment samples collected in the spring was lower than that reported for the Montenegrin coast at the same locations during the autumn period [12-14]. The largest difference in the number of MPs in the sediment from the Montenegrin coast sampled during the autumn period compared to the spring period may be a consequence of anthropogenic impact due to increased tourist activity and accumulation of MPs during summer [22, 24, 25]. Present studies confirms the influence of anthropogenic factors, which is enhanced by tourism [12-14]. Piazzolla et al. [26] indicated that repeated long-term investigations and seasonal surveys of MPs pollution in sediments give more precise information important for further investigations and monitoring.

In present studies was recorded a higher prevalence of MPs in sediments in 2019 compared to 2020, Fig. 2 [12-14]. Authors indicate that one of the possible reasons for the higher prevalence of MPs in the sediments sampled in 2019 is the greater anthropogenic impact during the summer tourist season in 2019 compared to 2020 [12-14]. The best tourist season in Montenegro was recorded in 2019, and one of the worse seasons in 2020 [27]. The impact of the epidemiological measures caused by the COVID-19 virus in 2020 had a noticeable effect. During this period, activities such as tourism and fishing have decreased, which is why it is considered that the representation of MPs in the sediment sampled in 2020 is lower compared to the representation of MPs in the sediment sampled in 2019.

Compared with literature data for the Adriatic Sea and around the world, the average abundance of MPs found in sediment from three studies in Montenegrin coast [12-14] were medium to moderately contaminate with MPs.

3.1.2 Fish

Many organisms swallow pieces of plastic, which can accumulate in their digestive system of biota [4]. Table 1 presents the results of the MP abundance in GIT of fish [14]. Of the 120 fish sampled (70 *M. barbatus* and 50 *M. merluccius*), 68 (56.7 %) contained MPs in their GIT.

Table 1. MP abundance in GIT of fish

Species	Sampling area	Sampling period	Average number of MPs per positive fish
M. barbatus	***2019***		
	Boka Kotorska Bay	Spring	2.0 ± 0.6
	Coastal part of the open sea		3.6 ± 1.2
	Boka Kotorska Bay	Autumn	3.0 ± 2.0
	Coastal part of the open sea		3.2 ± 1.8
	2020		
	Boka Kotorska Bay	Spring	3.4 ± 1.9
	Coastal part of the open sea		3.0 ± 1.7
	Coastal part of the open sea	Autumn	2.7 ± 1.8
M. merluccius	***2019***		
	Coastal part of the open sea	Spring	3.2 ± 2.7
	Coastal part of the open sea	Autumn	4.7 ± 3.1
	2020		
	Boka Kotorska Bay	Spring	2.0 ± 0.8
	Coastal part of the open sea		2.6 ± 1.5
	Coastal part of the open sea	Autumn	3.6 ± 1.0

The frequency of MP ingestion was 58.6 % for *M. barbatus* and 54 % for *M. merluccius* [14]. The average concentration of ingested MPs in all positive fish was 3.1 ± 0.7 items/individual [14]. The average number of MPs by fish species was 2.9 ± 0.5 items/individual for *M. barbatus* and 3.2 ± 1.0 items/individual for *M. merluccius* [14]. Compared to the literature, the frequency of MPs ingestion and average number of ingested MPs in the GITs of *M. barbatus* and *M. merluccius* were similar to that observed in literature from the same fish species.

The difference in the frequency of ingested MPs and the average number of MPs per positive individual in *M. barbatus* samples were not consistent with respect to year and sampling period [14]. For *M. merluccius*, the frequency of MP ingestion and the average number of MPs per positive individual were highest in the 2019 sampling year and in the autumn sampling period [14]. A higher average number of ingested MPs per individual was observed in both fish species sampled in the coastal part of the open sea compared with fish from Boka Kotorska Bay, except for the samples of *M. barbatus* sampled in 2020 (Table 1) [14].

Authors suggest that relationship between the MP accumulation in fish and sediment from the Montenegrin coast indicate that the higher MP accumulation in fish from the Montenegrin coast may be closely related to their habitat and feeding habits [14]. *M. merluccius*, a benthopelagic species that moves and lives in two habitats, has a greater possibility of interacting and thus ingesting MPs than *M. barbatus*, a benthic species that moves and lives in a single habitat [20]. The present study showed that these two fish species are suitable for monitoring MPs in the study area [14]. Sediments are considered MP sinks, increasing the exposure and risk of MP ingestion by benthic organisms [28]. The increased MP accumulation in sediments and fish detected in present studies indicated that the

Montenegrin Adriatic region is highly affected by MP pollution, which requires further monitoring and research [14].

3.2 Visual and chemical MPs identification in marine environment

Visual identification, using an optical microscope, determined the shape, color, and size of MPs in fish and sediments samples. Size, shape, color and polymer type of MPs could indicate the different origin of the plastics but also the different degrees of accumulation and degradation [32, 33].

The filaments were the most common MPs shape in surface sediments and fish samples from Montenegrin coast. There are significant correlations between fish and sediment samples with respect to the shape category of MPs (PERMANOVA, Monte Carlo test, $p < 0.05$) [14]. Previous studies have also reported that filaments were the dominant type of MP in marine environment [7, 24].

The most common MPs color throughout the studies in samples of fish and sediments from Montenegrin coast was blue [12-14]. Significant correlations were observed in the MP abundance color in fish and sediment samples (PERMANOVA, Monte Carlo, $p < 0.05$) [14]. Several studies have reported that blue MPs were dominant color in fish and sediment samples [34-37, 38].

There are no significant correlations between fish and sediment samples in terms of MPs size ($p > 0.05$) [14]. The most abundant MPs size category in surface sediment were in range of 1.0–5.0 mm, while in fish species it was MPs between 0.1 and 0.5 mm [12-14].

Chemical composition is the most basic criterion for defining MP pollution [30]. Polypropylene (PP) and polyethylene (PE) were the most common types of polymers in this studies [12-14]. Significant correlations were found between the fish and sediment samples in terms of the abundance of polymer type of MPs (PERMANOVA, Monte Carlo test, $p < 0.05$) [14]. PP and PE are two polymers with very high annual demand; hence, it is not surprising that they are the most common polymers found in marine environments around the world, as well as in the Adriatic Sea [22, 39-41].

The accumulation and characteristics of MPs (shape, color, and polymer types) in fish correlated with the results obtained in sediments in present studies, suggesting that MP pollution in the study area is reflected in the accumulation in sediment and fish.

4 MPs sources in Montenegro

In present studies, several factors were observed that can be related to the occurrence and distribution of the MPs contamination in the marine environment: (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 many authors [25, 42-44].

MPs can be discharged into the sea indirectly via wastewater [25, 45]. We emphasize that the issue of wastewater treatment has not been completely solved on the Montenegrin coast. Furthermore, Montenegro has a problem with the management and storage of municipal waste, which can significantly affect the quality of marine sediment and contribute to pollution. In addition to major sea outfalls, there are many uncontrolled local discharges. More of the outfalls in the coastal region of Montenegro are old and in poor operational condition, deficient, and have been earmarked for replacement or termination [46].

A higher MP concentration in sediment and fish samples from Montenegrin coast could be conditioned by a higher inflow of fresh water. The mouth of the Bojana River flows through Montenegro and Albania carrying various pollutants with it [47]. The mouth of the

Bojana River, which carries plastics from the territories of Montenegro and Albania, is the second largest land-based source of plastics in the whole Adriatic, exceeded by only the Po River [48-50]. In addition, Montenegro is the most affected country exposed to seaborne plastic pollution from other Mediterranean countries, receiving 33 % of plastics from their neighbors, followed by Slovenia (18 %), Croatia and Bosnia and Herzegovina (12 %), Cyprus (12 %), and Greece (10 %), with the remaining countries receiving < 10 % [50]. 20–30 % of the floating debris from northern Albania goes to Montenegro [48].

Many authors indicated that the high percentage of filaments in this studies suggests that the high MP abundance is due to sewage wastewater discharges (the main source of filaments in the marine environment) followed by fragmentation of fishing gear (e.g. ropes and nets) and from fabric and textile industrial production [23, 25, 36].

PE and PP are the most abundant polymers detected by many authors in fish [39, 40] and sediments samples [22, 41]. PE and PP are the most abundant plastics in the world and the dominant plastic waste, coming mainly from wide range of applications (domestic and industrial), most commonly used for packaging such as plastic bags and bottles [8, 51].

5 Further studies

Further studies are needed to better evaluate risks for marine biota associated with MPs pollution with an emphasis on sustainable water resources. The MP presence has been a recognized problem in the aquatic environment for the last 15 years [7, 52, 53]. MPs are present in all aquatic ecosystems (oceans, seas, rivers and lakes) [1]. However, most studies report the MP presence in marine ecosystems, while knowledge about the MP presence and impact in freshwater ecosystems, which represent important natural resources and support human life, economic development, and are closely related to human well-being and aquatic organisms, is limited [54-56].

The MP accumulation in freshwater ecosystems highlights the ubiquity of this form of pollution [43, 54, 57]. Studies indicate that freshwater ecosystems play an important role in the MP transport. Freshwater is considered one of the main sources of MPs in the sea and the main transport vector of plastic waste from terrestrial sources [58], so the study of freshwater ecosystems is of great importance for identifying the sources of pollution, dynamics, dispersion, accumulation and fate of MPs [59]. The widespread MP presence in freshwater ecosystems threatens the environment, as MPs can be distributed and affect aquatic organisms by disrupting their food chain and affecting biodiversity [60].

The presence and distribution of MPs in lakes and rivers is influenced by several forces that modify their presence and transport, among them are: climatic variables (wind-driven surface currents, storms, floods, runoff), geomorphological characteristics (water depth, coastal area development), anthropogenic activities (lease of dams, tourism, fishing) and trophic state (degree of pollution) [61].

Montenegro has a specific hydrology. The territory of Montenegro is divided into two basins: the Black Sea basin and the Adriatic Sea basin. The Black Sea basin covers 52% of the territory, and the Adriatic basin covers 48% of the territory. The largest part of the Adriatic basin belongs to the Skadar Lake basin, which includes the Morača, Zeta and Cijevna rivers, and the Skadar Lake is drained by the Bojana River, which flows into the Adriatic Sea. The main rivers of the Black Sea are Piva, Tara, Lim, Ibar and Ćehotina [62]. Apart from rivers, Montenegro is also rich in lakes, both natural and artificial.

Bearing in mind the above, future research will aim to comprehensively, for the first time, evaluate the level of representation, dynamics of movement, characterization and sources of MPs in the freshwater ecosystems of Montenegro, as well as to assess the pollution of the investigated localities from the point of view of the presence of MPs in sediments. The subject analysis will represent the basis for the creators of legislation in Montenegro in order to find adequate solutions for the improvement and preservation of the environment, as well as the implementation of the principles of sustainable development.

6 Conclusion

MP have become one of the emerging pollutants in the aquatic environment. This review of the literature complied the comprehensive information about importance of study on MPs pollution as a critical environmentally issue. In this regard, the following topics were discussed: a) MPs analysis methodology; b) Current knowledge of MPs in marine environment; c) Source of MPs and d) Existing gaps and recommendation for future works. This review of the literature indicate a relationship between the MP accumulation in fish and sediment samples. The presence of MPs in fish and sediment samples indicates the wide distribution of MPs on the Montenegrin coast due to various anthropogenic activities. Also, results showed that MP abundance in fish and sediment samples mostly depended on MP pollution of the surrounding environment and that the Montenegrin coast is moderately polluted by MPs compared to the surrounding areas. This review of the literature will help to increase the knowledge about the presence, distribution and typology of MPs in aquatic environments. The occurrence of MPs in rivers, lakes has attracted widespread attention from scientists, policymakers, and the public. However, there is still insufficient knowledge about the monitoring, distribution, and influencing factors of MP in freshwater environments. These review provide the basis for future research and monitoring activities and implementation of the principles and objectives of sustainable development.

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