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Evaluation of abundance of microplastics in the Bulgarian coastal waters

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Abstract

Plastic pollution in seawaters is ubiquitous, but quantitative estimates on the floating microplastics in the Black Sea are still limited. Plastics may adsorb persistent environmental contaminants, thus representing a potential risk for marine organisms.

Aim: The aim of the study was evaluation of the presence and characteristics of microplastic particles (MPs) in waters from the Black Sea coast of Bulgaria.

Materials and methods: Samples of coastal waters were collected from March 2021 to April 2022 from different stations on the Black Sea coast, including protected, aquaculture and industrial areas. In order to determine the number of plastic particles, 23 samples were collected from the surface waters at depth of 1–3 m close to the Bulgarian shore. Samples were treated with H_2O_2, plastic particles were isolated by density separation and filtered over a membrane filter. Identification analysis of micro particles (< 5 mm) was performed visually by microscopy.

Main results: Results indicated widespread presence of microplastics in coastal waters. Mean MPs concentration was calculated 7.3 ± 4.9 pt/l. The comparison of the North, Varna and South sampling area showed that there is no significant difference in the abundance of plastic particles. The most dominant type forms were fibres followed by fragments. The most abundant size class of fragments was 101–500 μm Ferret diameter.

Conclusion: Further studies are needed in order to fill knowledge gap and to evaluate distribution of plastic particles in the Black Sea and their potential ecological risk.

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Keywords
microplastics, sea waters, the Black Sea, Bulgaria

Introduction

Marine debris has been of concern to the scientific community for decades. The last report of EC 2020 on the implementation of the Marine Strategy Framework Directive (EC 2008) highlighted the main pressures affecting marine ecosystems: fishing, contaminants and marine litter (EC 2020). It was indicated that there are sizeable gaps in the data on litter on the seabed, in the surface layer and micro-litter and their effects on marine species (EC 2020). Plastic waste in the marine environment is classified into macro-, micro- and nanoplastics depending on their sizes. In recent years, microplastics (MPs; < 5 mm) have occurred in all geographical regions of the Oceans (Desforges et al. 2014), including the Arctic (Obbard et al. 2014; O'Donovan et al. 2018; Kanhai et al. 2020) and Antarctica (Lusher 2015). The occurrence of plastic particles was mostly determined in the last decade in marine water, sediment or biota samples all over the world (Cincinelli et al. 2017; Baini et al. 2018; Rios-Fuster et al. 2023).

The Black Sea is a semi-enclosed area and its coasts are subjected to high levels of marine litter pollution from rivers discharges (Danube, Dnieper etc.), harbours, industrial cities, fishery, tourism and agriculture in the region (Simeonova et al. 2010; Pojar et al. 2021; González-Fernández et al. 2022). Recent studies of microplastics in surface waters have been carried out along the Black Sea coast (Aytan et al 2016; Oztekin and Bat 2017; Berov and Klayn 2020; Pojar et al. 2021). Microplastics and nanoplastics (< 0.1 μm) pose a danger to marine organisms that can ingest them, causing physiological disorders and even death. Microplastics have the potential to adsorb persistent organic pollutants (POPs) from the marine environment, which may increase their detrimental impact once assimilated by the organisms (O'Donovan et al. 2018). Several studies identified that POPs residues (such as polychlorinated biphenyls (PCBs), PAHs and organochlorine pesticides (e.g. DDT, DDE) are present in the marine environment and biota of the Black Sea (Stancheva et al. 2017; Georgieva et al. 2016; Georgieva et al. 2022). These contaminants are lipophillic and could accumulate on the surface of plastics (Barnes et al. 2009; Cole et al. 2011). Ingestion of microplastics by marine organisms is a potential route for bioaccumulation of toxic chemicals in the food chain (Teuten et al. 2009).

Plastic pollution in the marine environment is ubiquitous, but the scientific data about the presence and characterisation of microplastics in surface waters from the Bulgarian coast of the Black Sea are still insufficient (Berov and Klayn 2020). The aim of the study was evaluation of the presence and characteristics of microplastic particles (MPs) in waters from the Black Sea coast of Bulgaria.

Methods

Sampling and sample preparation

The investigation of plastic particles in the coastal waters of the Bulgarian Black Sea targeted sampling areas with a high ecological importance, including protected, aquaculture and industrial areas such as the cities of Varna and Burgas (main harbours) and tourism resorts.

Samples of coastal waters (n = 23) were collected from March 2021 to April 2022 from different stations near the Black Sea coast. Surface waters at depth of 1 to 3 m were sampled aboard a vessel by a manta net. After sampling, the net haul concentrates were adjusted to water volume of 1.5 l using seawater, filtered by 20 μm mesh. In the laboratory, each sample was transferred into glass jars, using Milli-Q water for rinsing. A total of 23 samples were stored at 4 °C in darkness to minimise algal growth until analysis. The sampling plan, sampling preparation and microplastics' characterisation were conducted in accordance with Guidelines for the monitoring and assessment of plastic litter and microplastics in the ocean (GESAMP 2019).

The samples were transferred into individual pre-cleaned glass beakers and organic matter was digested using hydrogen peroxide. A total of 20 ml 30% hydrogen peroxide and 20 ml of 0.05 M ferrous sulphate ($FeSO_4$), were carefully added to 250 ml of water sample. The mixture was placed on a hot plate set to 60 °C (30 min) and the reaction was allowed to continue until all organic material disappeared (about 24 h). Plastic particles were isolated by density separation and filtered over a membrane filter (sterile MCE, 0.45 μm pore size, 47 mm diameter, FiltraTECH). Identification analysis of microparticles (< 5 mm) was performed visually by a technique under stereomicroscopy Primo Star (Zeiss, Jena).

Visual identification

For identification and characterisation of microplastics, membrane filters were examined using a Zeiss Primo Star microscope at 4× and 10× magnifications. All of the images were captured with a Zeiss Axiocam ERc 5 s microscope. All plastic items with a size range from 20 μm to 5 mm were taken into account and measured with micrometer ocular lens. Plastic particles were numbered and classified by size, characterised by colour (white, blue, red, yellow, black, transparent, green, other colours) and shape (spherical, fibre, filament, fragment, sheet, films) according to the MSFD guidelines (Hanke et al. 2013; GESAMP 2019). The criteria for visual identification, classification and characterisation by morphological types, size classes (Ferret diam.) and colour of plastics were based on the protocol by Hidalgo-Ruz et al. (2012) and Kovač Viršek et al. (2016).

Quality assurance

In order to prevent sample contamination, glass and stainless-steel materials were used for the laboratory work. During sample preparation and storage, all samples were covered with aluminium foil. Each series of samples included procedure blank, using same the amounts of reagents and ultrapure Milli-Q water, which was digested in the same way as the other samples. All results were corrected with data from a parallel blank (Milli-Q water). Statistical significance of the differences in the mean values of particles in samples from the three regions were tested at $\alpha = 0.05$ with Student's t-Test, Excel for MS Office Professional Plus (Microsoft Corporation. 2018).

Results

Microplastic particles were identified by means of visual identification. The filters were visually examined and all potential microplastic particles ranging from 20 µm to 5 mm were registered and counted. The plastic particles were observed in 22 from a total of 23 samples (Table 1). Particle abundance was based on filtered water and the amount of identified plastic particles was present as particles per litre (pt/l). Mean concentrations of microplastics ranged from 1.3 pt/l (ME7) to 16.3 pt/l (ME60) (Table 1).

Table 1. Sampling sites and total concentrations of microplastics (mean and standard deviation), particles per litre (pt/l) in coastal waters of the Bulgarian Black Sea coast.

Sample No	Sampling season	Sampling site	GPS coordinates	Total concentration, pt/l
ME7	Winter 2021	Cape Kaliakra	43°19'24.5"N, 29°10'56.1"E	1.3 ± 0.9
ME10	Winter 2021	Varna Bay	43°12'45.9",N 28°16'13.9"E	2.3 ± 2.3
ME16	Spring 2021	Kavarna	43°23'36.9"N, 28°22'52.0"E	14.0 ± 2.0
ME22	Spring 2021	Burgas Bay	42°17'36.0"N, 27°17'41.2"E	2.3 ± 0.6
ME38	Summer 2021	Kamchia estuary region	43°01'23.5"N, 27°53'22.0"E	10.7 ± 1.5
ME49	Summer 2021	Ravda	42°38'14.3"N, 27°40'26.5"E	12.3 ± 1.5
ME60	Autumn 2021	Pasha dere, near Varna	43°07'09.8"N, 28°02'52.1"E	16.3 ± 1.2
ME61	Autumn 2021	Sts. Constantine and Elena	43°13'31.9"N, 28°02'12.3"E	4.3 ± 0.6
ME62	Autumn 2021	Golden Sands	43°16'52.5"N, 28°07'03.9"E	12.7 ± 0.3
ME65	Autumn 2021	Pomorie	42°33'19.4"N, 27°38'19.4"E	2.3 ± 0.6
ME67	Autumn 2021	Nesebar	42°39'58.6"N, 27°43'06.5"E	3.3 ± 0.6
ME77	Autumn 2021	Cape Kaliakra	43°21'01.7"N, 28°28'49.8"E	3.3 ± 0.6
ME78	Autumn 2021	Kavarna	43°23'49.9"N, 28°19'36.1"E	3.7 ± 0.6
ME79	Autumn 2021	Cape Emine	42°43'15.0"N, 27°55'26.1"E	4.3 ± 0.6
ME84	Winter 2021	Kamchia estuary region	43°01'23.5"N, 27°53'22.0"E	6.3 ± 0.6
ME87	Winter 2021	Varna Bay – Karantinata	43°10'28.3"N, 27°54'60.0"E	4.7 ± 0.6
ME90	Winter 2021	Varna Bay – 4th buna beach	43°12'42.2"N, 27°57'30.1"E	14.3 ± 0.6
ME93	Winter 2021	Lake Varna	43°11'36.8"N, 27°51'46.5"E	5.7 ± 0.6
ME102	Winter 2022	Durankulak	43°43'08.2"N, 28°35'09.8"E	12.0 ± 1.0
ME103	Winter 2022	Krapets	43°39'12.5"N, 28°36'10.1"E	12.7 ± 0.6
ME104	Winter 2022	Shabla	43°34'34.4"N, 28°38'33.8"E	nd
ME112	Spring 2022	Pomorie	42°33'19.4"N, 27°38'19.4"E	8.3 ± 1.2
ME113	Spring 2022	Nesebar	42°39'58.6"N, 27°43'06.5"E	10.0 ± 1.0

nd – not detected.

Discussion

Distribution of microplastics along the Bulgarian Black Sea coast

The results of our study indicated widespread presence of microplastics in coastal waters of the Bulgarian part of the Black Sea. The mean MPs concentration in seawater was calculated 7.3 ± 4.9 pt/l. A total of 23 samples of sea water were studied (Table 1) from the three samples regions North, Varna and South. The highest concentrations were found in samples ME60 (Pasha Dere, near Varna region) – 16.3 ± 1.2 pt/l, ME90 (Varna Bay) – 14.3 ± 0.6 pt/l and ME16 (Kavarna, North region, mussel farm) – 14.0 ± 2.0 pt/l. The lowest number of plastic particles was recorded at sampling points ME7, ME77 – 3.8 pt/l (Cape Kaliakra), ME65 and ME67 (Nesebar and Pomorie) – 2.3 and 3.3 pt/l (Autumn 2021).

Comparison of the North, Varna and South sampling areas showed that there is no significant difference in the abundance of plastic particles (7.8, 8.7 and 6.4 pt/l, respectively) – Fig.1. The large variation in results for each region is likely due to different sources of land-based plastic waste pollution (Simeonova et al. 2010; Simeonova et al. 2017).

Our findings showed the highest number of plastic particles in the surface water samples from the northern coast of the Bulgarian Black Sea (near Kavarna). Pojar et al. (2021) reported the highest plastic abundances occurred close to the mouth of the Danube River. The authors assumed that the plastic pollution might occur via the Danube River flows into the Black Sea. Close to Varna, the mean amount of microplastics in seawater samples was 8.6 ± 5.0 pt/l and several kilometers to the north in resort Sts. Constantine and Elena, the concentration decreased to 4.3 pt/l (ME61). The harbour city of Varna is one of the major ports in Bulgaria with industrial activity and high frequency of ships traffic. Surprisingly, in the South sampling area, the amount of

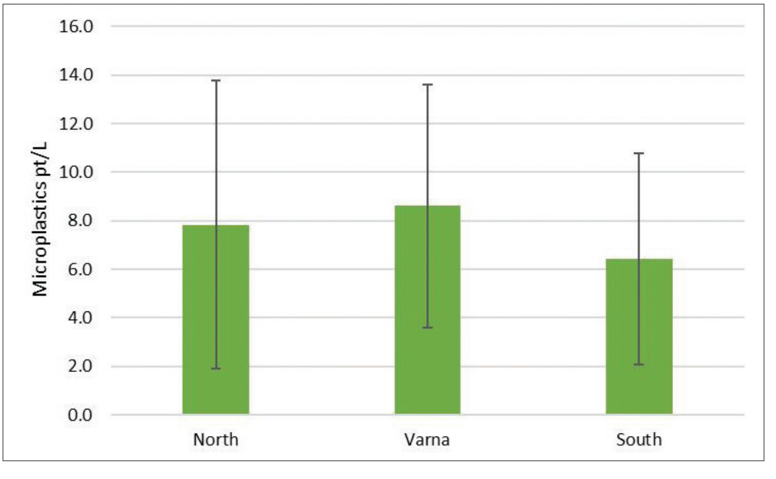

Figure 1. Distribution of microplastics along the Bulgarian Black Sea coast by sampling area.

plastics detected nearby urbanised areas Burgas Bay (2.3 ± 0.6 pt/l –ME22) was lower than in the environs of the protected areas (Ravda, Pomorie, Nesebar). The reason was the limited number of samples in this area and, thus, the results gives only first insights into the plastic pollution of south part of the Bulgarian coast of the Black Sea.

In a recent study, Berov and Klayn (2020) found that microplastic pollution was highest in the area of Cape Kaliakra. Our results showed relatively lower levels of abundance of microplastics in samples from Cape Kaliakra compared to other protected areas (ME102 and ME103) in the Northern region. Relatively high concentrations of macroplastics were found in front of the protected area in the Kamchia River Estuary (ME38 and ME84). The river is characterised with the largest catchment area and local sources are the possible emitters of plastic particles in this sampling site of the Black Sea coast. Similar findings were reported in a recent study by Berov and Klayn (2020). The MPs abundance in samples from protected areas along the Bulgarian coast was in the same ranges compared to nearshore regions close to the industrial cities of Varna and Burgas. This might be explained by the sea flows, seasonal influence, wind regime, sea surface currents and sea tides (Simeonova et al. 2017; Shapiro 2019; Pojar et al. 2021).

Higher concentrations of microplastic particles could be expected in the Northern region, due to the influence of freshwater inflows from the Danube River in the western Black Sea. The data showed variations in the amount of floating particles due to surface currents along the coast from north to south (Pojar et al. 2021) and the current regime of the Black Sea, generally described as counter-clockwise (Oguz et al. 1993).

Characterisation of plastics in coastal waters of the Black Sea, Bulgaria

A difference in plastic morphology is observed: in all samples, only fragments, fibres and films (secondary microplastics as a product of the degradation of macroplastics) were identified. Microparticles such as spherules, microbeads and granules (primary plastics) were not found in examined filtered samples.

The most dominant forms were fibres (73.3%), followed by fragments (23.4%) and other forms (3.2%) – Fig. 2. Fibres predominated in areas around ports and populated areas, because they originate mainly from wastewater, clothing and ropes, as well as shipping activities (Hidalgo-Ruz et al. 2012; Gewert et al. 2017).

Amongst the different colours found, transparent pieces were the most abundant (38%), followed by blue (29%) and black (26%) – Fig. 3. Other colours (red, yellow and others) were found in residual amounts and combined made up 7% of the entire sample. Several scientific studies reported a prevalence of black, grey and more brightly coloured particles (Cole et al. 2011, 2014; Kanhai et al. 2020). Further investigation of the possible sources of contamination could explain this higher concentration of the colourless and blue particles in the water samples from the Bulgarian Black Sea coast.

Analysis of the size distribution of microplastics in surface samples showed that the most abundant size class of fragments was 101–500 μm Ferret diameter in the whole dataset (Fig. 4). The small size of the particles poses a high risk to marine organisms

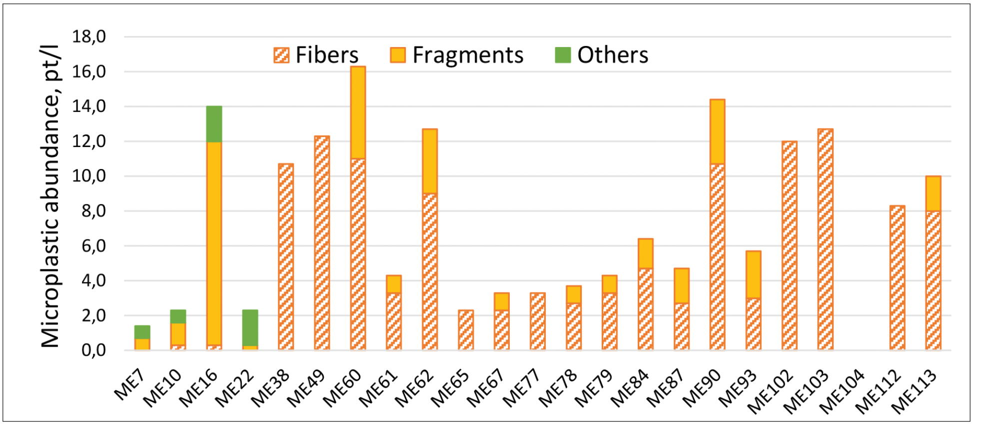

Figure 2. Distribution of MP particles by form types, particles per litre (pt/l) in coastal waters from different stations along the Bulgarian Black Sea coastline for the whole monitoring period.

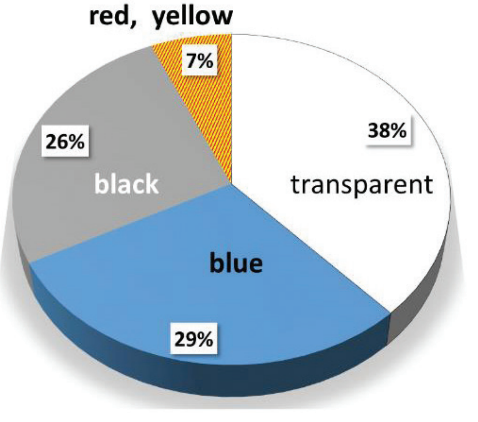

Figure 3. Composition of microplastic particles in seawater samples according to their colour classes.

which could accidentally ingest them during feeding (Teuten et al. 2009; O'Donovan et al. 2018). The items with sizes below 100 μm represented the minor portion of the total microplastics. A similar pattern was described in a recent study conducted in the Mediterranean Sea, Tuscany, Italy (Baini et al. 2018).

The mean concentration of microplastic particles (mean value 7.3 ± 4.9 pt/l) in coastal waters of Bulgaria was found comparable with data from other studies conducted in the Black Sea (Aytan et al. 2016; Berov and Klayn 2020), as well as with a previous study the Mediterranean Sea (Gündoğdu 2017). The results from the present study showed significantly higher abundance of microplastics than the microparticles contamination reported by Pojar et al. 2021 (average 7 pt/m^3) from the western Black Sea, Romania and by Scott et al. (2019) in UK coastal waters – 1.97 to 3.38 pt/m^3.

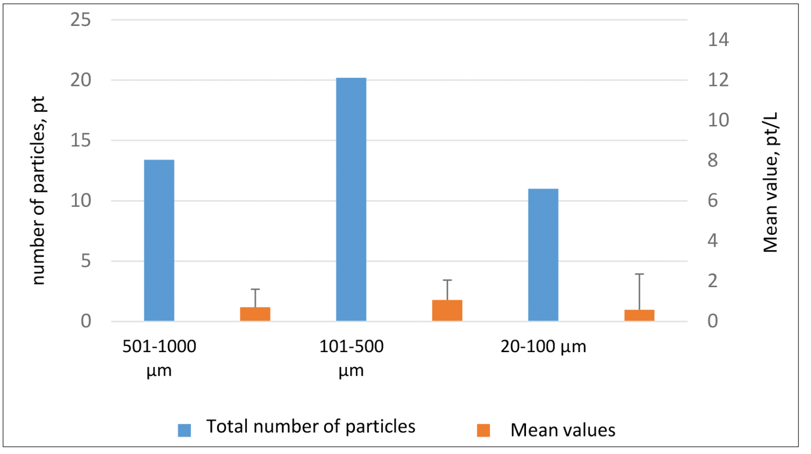

Figure 4. Size classes of MP particles (fragments and others*) in coastal waters (*fibres is not included).

Conclusion

With the aim of filling the gap and increasing the knowledge about plastic pollution, the present study provides data on the abundance and characteristics of microplastics in the surface waters along the Bulgarian Black Sea coast. The results showed that plastic pollution is ubiquitous as more plastic particles are present in the water samples from the Varna Bay and the northern Black Sea coast. The confirmation of the possible sources of pollution requires further analyses, for example, polymer composition by Pyrolysis GC/MS or FTIR spectrometry. Considering the limited number of samples and variation in results, we suggest to develop more a complex programme and continue monitoring the studied regions. There is an urgent need to coordinate monitoring methodologies regarding plastic pollution at national, regional and EU levels (EC 2020). Further studies are needed in order to evaluate the distribution of plastic particles in the Black Sea and their potential ecological risk.

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