MICROPLASTICS ASSESSMENT IN THE KRKA RIVER ESTUARY SURFACE WATER

Marija Parać [1*], Marin Lovrić [1], Nuša Cukrov [1], Tomislav Bulat [1], Neven Cukrov [1]
[1] Ruđer Bošković Institute, Bijenička cesta 54, 10 000 Zagreb, Croatia
*E-mail of corresponding author: mparac@irb.hr

Abstract: *Microplastics (MPs), commonly defined as particles less than 5 mm, are a persistent ubiquitous anthropogenic contaminant that can be found in every environment, making it a global environmental, health, and socioeconomic problem. Due to their high surface area, MPs adsorb toxic pollutants that become bioavailable to organisms upon ingestion as they are often mistaken for food leading to biomagnification (Bule et al., 2020). The sampling area represents the lower part of the Krka River Estuary and is under direct anthropogenic influence from the city of Šibenik runoff waters, nautical and communal ports, city harbor, tourism, mariculture, and fishing. Estuaries and harbors have been recognized as hotspots and transfer pathways for MPs primarily because of the vicinity of the urban environment that emits contaminants from various sources (Miller et al., 2021). The main focus of this research was to determine MPs size, shape, color, surface area, and abundance in surface water using volume-reduced samples collected by a net. Laboratory protocol included sieving, wet peroxidation (H2O2), density separation (saturated NaCl solution), sonication, and filtration. Filter papers were then visually inspected for MPs. Image processing and measurements were carried out with ImageJ/Fiji open-source software.*
Keywords: *microplastics (MPs), Krka River estuary, surface water, ImageJ/Fiji*

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1. INTRODUCTION

Microplastics (MPs) are plastic particles (synthetic polymers) smaller than 5.0 mm in size. The lower limit is still not specified, but often the mesh size (300 µm) of neuston or manta nets is considered, which are used for sampling (Masura et al., 2015). MPs come from a variety of sources, which can be classified into two groups: primary and secondary MPs (Miller et al., 2021). Primary MPs are purposefully manufactured as pellets for plastic production, abrasive blasting (sandblasting), paints, adhesives, detergents, and microbeads incorporated into personal care products (face scrubs, toothpaste, bath products, etc.) (Miller et al., 2021). They can be released into the environment intentionally as part of regular usage of the product, via spillage, sewage discharge, runoff, or by domestic and industrial effluents (Razeghi et al., 2021). Whereas secondary MPs occur because of the fragmentation of larger plastic particles or synthetic fibers already found in the environment caused by UV photodegradation, mechanical abrasion, chemical breakdown, or biodegradation. They are more abundant in the marine environment than primary MPs (Miller et al., 2021; Razeghi et al., 2021). Several pathways of secondary MPs entering the marine environment have been proposed (Bailey et al., 2021; Freeman et al., 2020): washed off the land by rain or translocated by wind (atmospheric deposition), via treated or untreated wastewater discharged into waterways, via effluent from municipal wastewater treatment plants (WWTPs), via sewage sludge used in agriculture as fertilizer, via tearing of plastic components used in WWTPs, boating and fishing activities, etc.
MPs have a high affinity for toxic pollutants, such as heavy metals, persistent organic pollutants (POPs), polycyclic aromatic hydrocarbons (PAHs), polychlorinated biphenyls (PBCs), chemical additives, and plasticizers (phthalates), due to their large surface area to volume ratio (He et al., 2021; Razeghi et al., 2021). In addition, MPs can easily be ingested by marine biota, as they are often mistaken for food (Bule et al., 2020). Thus, organisms that ingest such MPs are a threat to the whole food web, including humans (Defontaine et al., 2020).
Estuaries worldwide have been suggested as hotspots for MPs contamination and dispersion (Piehl et al., 2021), although MPs movement in estuarine environments is not yet fully understood, despite growing research. It is suggested that the MPs distribution highly depends on complex estuarine hydrodynamics, bathymetry, intense currents, and harbor activities (Defontaine et al., 2020; Miller et al., 2021). The sampling area represents the lower part of the Krka River Estuary, with limited water exchange and is directly subjected to anthropogenic influence from the city of Šibenik. This urbanized estuary receives MPs from various sources and activities, including urban runoff waters, nautical and communal ports, city harbor, tourism, mariculture, and fishing. No previous study regarding MPs abundance, size, shape, and color was done in this area prior to this research. Therefore, the main

goal of this research is to quantify and characterize the types of plastics affecting the area. It is crucial to assess the abundance of MPs particles to implement prevention measures against MPs contamination.

2. MATERIALS AND METHODS

All methods and protocols used were adapted from the National Oceanic and Atmospheric Administration (NOAA) (Masura et al., 2015), and from the multinational BASEMAN project, which is funded under the EU Joint Programme Initiative (JPI) Ocean (Gago et al., 2019).

2.1. Quality assurance and quality control (QA/QC)

Due to their ubiquitous nature, MP particles could easily contaminate samples that we are working with, which could lead to overestimations of the abundance of MPs in the sample. Given that, it is crucial to avoid any cross-contamination by implementing several contamination minimization procedures, as we did, such as: always wearing a clean 100% cotton laboratory coat and nitrile gloves; avoiding wearing synthetic clothing even underneath the coat, especially fleece; closing doors and windows to minimize air movement in the laboratory; cleaning all equipment and working stations with 70% ethanol and rinsing it 3 times with Mili-Q water; washing glassware with 10% HNO3 and rinsing it 3 times with Mili-Q water; covering all equipment and samples with aluminum foil; inspect all Petri dishes, filters, and forceps under stereomicroscope; using non-plastic material (steel, glass, aluminum); work in a fume hood; pre-filtering all working solutions and reagents with LLG Syringe Filters SPHEROS, PTFE (pore size: 0.22 µm); running field, laboratory and procedural blanks.

2.2. Microplastics sampling

Surface water samples were taken from the lower part of the Krka River estuary, mainly in Šibenik bay (43°43.245'N, 15°54.144'E), in March 2022. The average vessel speed was 1.7 knots and the duration time for towing was 20 minutes. Volume-reduced samples were obtained from the first 40 cm of water surface with a "Net for Microplastic Sampling" (Hydro-Bios, Apparatebau GmbH, Germany; mesh size: 300 µm; net aperture: 0.28 m^2 [width 70 cm, height 40 cm, length 260 cm]) deployed behind the vessel. The volume of water that passed through the net was calculated with a flow meter (Mechanical Flow Meter, Hydro-Bios, Apparatebau GmbH, Germany) positioned at the net rim, according to the given manual by the manufacturer. After towing, the net was rinsed from the outside with Mili-Q water from a pressure container to avoid sample contamination. All sampled MP particles were gathered in the collecting glass jar with a lid from the cod end. The samples were refrigerated at +4°C until further laboratory processing.

2.3. Sieving

Obtained samples from glass jars were wet sieved through a series of stacked sieves (4 mm, 2 mm, 1 mm, 250 µm, 125 µm, 63 µm), thoroughly rinsed with Mili-Q water to collect all MP particles and transferred to a new clean and marked glass jar. Multiple metal sieves were used for easier visual inspection.

2.4. Organic matter removal

For organic matter removal, Fenton's reagent (a mixture of 0.05 M Fe (II) sulfate (7.5 g of FeSO4*7H2O (from Gram-Mol d.o.o.) in 500 ml of Mili-Q water and 3 ml of concentrated sulfuric acid (from Acros Organics) with a 30% H2O2 solution (from Gram-Mol d.o.o.)) was used. 40 ml of Fenton's reagent was added to each beaker and heated on a "hot plate" at 75°C for 2h. Some samples had higher amounts of organic matter, so for complete removal, the second addition of 40 ml of Fenton's reagent was necessary.

2.5. Density separation

A saturated salt solution (1.2 g/cm^3) was prepared by dissolving 360 g of NaCl in 1000 ml Mili-Q water and placing it on a heated magnetic stirrer for 30 minutes to fully dissolve. The solution was filtered over glass microfibre filters (LGG Labware; pore size 1.6 µm; filter diameter Ø 47 mm) placed on a filtration system (MF31, Rocker Scientific) connected to a vacuum pump (Büchi® V-500). 100 ml of saline solution and the sample were poured into a clean beaker, put on a magnetic stirrer for 2 minutes and sonicated for 15 minutes (Sonorex Super RK 255 H, Bandelin). The solution was left to sediment for at least 2h whilst covered with aluminum foil to avoid airborne contamination. The supernatant containing MPs was then transferred to a clean beaker. The walls of a density separator were thoroughly rinsed with Mili-Q water to transfer all MP particles. This step was repeated 3 times to increase the recovery rate of plastic particles.

2.6. Filtration

All samples and solutions were filtered over glass microfibre filters (LGG Labware; pore size 1.6 µm; filter diameter Ø 47 mm) placed on a filtration system (MF31, Rocker Scientific) connected to a vacuum pump (Büchi® V-500). The funnel walls were thoroughly rinsed with Mili-Q water to transfer all MP particles to filters.

2.7. Visual inspection & quantification of microplastics

Each fragment was visually inspected and photographed through a stereomicroscope (Nikon SMZ745T) equipped with Bresser MikroCam PRO HDMI 5 MP using multiple magnifications ranging from 10-50x (eyepiece: Nikon C-W10xB/22). For the acquisition of images, MikroCamLabII version 4.7.15283 (Bresser, GmbH) software was used. Using ImageJ/Fiji (Schindelin et al., 2012), images containing MPs were measured, determining Maximum Feret's diameter (mm) and surface area (mm^2). For each image, a calibration scale was set. Total plastic abundance (items/m^3; items/m^2), plastic surface area (mm^2), sampling area (m^2), sampling volume (m^3) were calculated, alongside the number of particles per sample.
Furthermore, MP particles were classified based on type (fiber, filament, fragment, pellet, microbead, film, foam), color (white, black, transparent, blue, red, green, pink, violet, yellow, orange, brown, grey, or multicolor) and size (> 5 mm; 2-5 mm; 1-2 mm; 0.5-1 mm; 0.3-0.5 mm; <0.3 mm).
Certain criteria for MP recognition were followed to discern them from organic matter: MP particles must not have visible cellular or organic structures; fibers should be equally thick throughout their entire length; and particles should exhibit clear and homogeneous color throughout (Cutroneo et al., 2020). Only such particles were considered for further processing.

3. RESULTS AND DISCUSSION

Total of 507 particles were visually detected and considered as MPs from the sampled surface waters with a mean abundance of 0.730 items/m3 and 0.292 items/m2, respectively. The maximal abundance (1.364 items/m3, 0.545 items/m2) was recorded in the proximity of the Mandalina marina, gradually decreasing as the sampling proceeded further from the marina, with a minimal abundance of 0.310 items/m3 and 0.124 items/m2. This indicates that the highest concentrations of MPs were directly linked with anthropogenic influences associated with activities from the nearby marina.
MPs were found in different shapes, sizes, and colors. Several images of collected MPs in surface water from the Krka River Estuary are shown in Figure 1. Fragments (58.38%) were the dominant shape, followed by foams (14.40%), filaments (10.06%), fibers (8.28%), microbeads (3.94%), films (3.55%) and pellets (1.38%) (Figure 2.). Fragments usually originate from the breakdown of larger plastic particles (Bošković et al., 2022), fibers and filaments are usually derived from clothes, fabrics and fishing gear, microbeads from cosmetic products (Fan et al., 2021), films from packaging, bags or wrapping material and foams are probably due to the degradation of domestic packaging and pristine polystyrene (PS) foam (Fiore et al., 2022).

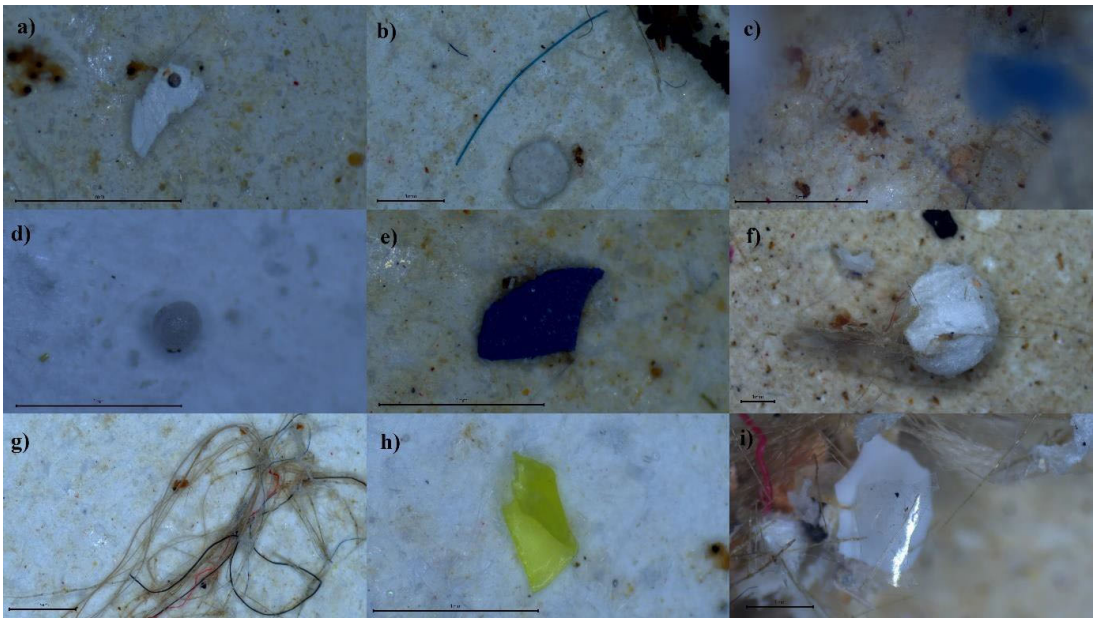

Figure 1. Images of MPs visually identified by Nikon SMZ745T stereomicroscope: a), e), h) fragments; b) fiber; c) microbeads; d) pellet; f) foam; g) filaments; i) film.

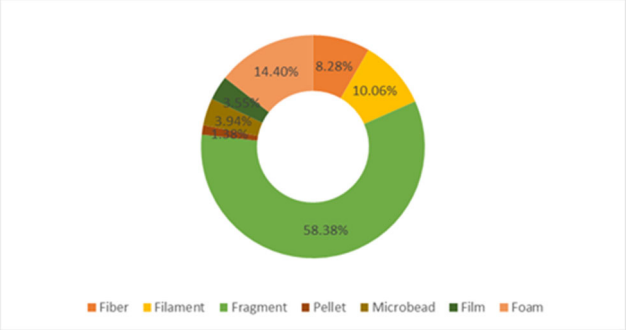

Figure 2. Distribution of surface water MPs by shape.

Based on color (**Figure 3**.), the most abundant were blue (26.43%) > transparent (20.91%) > white (20.71%) > red (18.74%) > black (3.35%) > orange and multiple colors (2.76%) > yellow (1.58%) > green (1.18%) > brown (0.79%) > gray (0.59%) > pink (0.20%) and no purple MPs. Most fibers were blue (52.38%), followed by black (14.29%) and red (14.29%). Filaments were mostly transparent (49.02%), blue (21.57%) and white (7.84%); fragments blue (33.78%), red (29.05%), transparent (13.85%); pellets white (71.43%) and transparent (28.57%); microbeads white (85%) and transparent (15%); films transparent (94.44%) and black (5.56%); foam white (56.16%), transparent (17.81%) and orange (15.07%) as can be seen in **Table 1**.

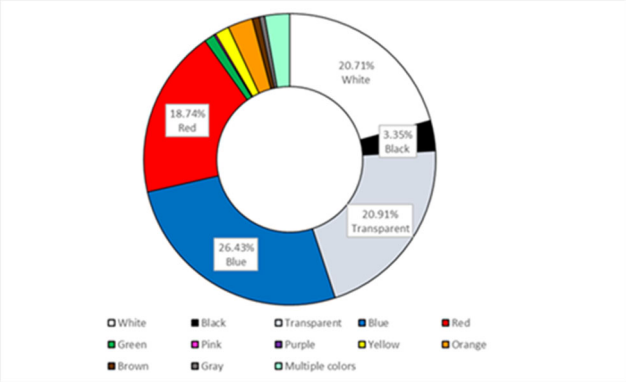

Figure 3. Distribution of surface water MPs by colour.

Table 1. Distribution of MPs by shape and colour.

		Fiber	Filament	Fragment	Pellet	Microbead	Film	Foam
	Total (n)	(%)						
White	105	2.38	7.84	12.50	71.43	85.00	0.00	56.16
Black	17	14.29	3.92	2.36	0.00	0.00	5.56	1.37
Transparent	106	11.90	49.02	13.85	28.57	15.00	94.44	17.81
Blue	134	52.38	21.57	33.78	0.00	0.00	0.00	1.37
Red	95	14.29	3.92	29.05	0.00	0.00	0.00	1.37
Green	6	2.38	3.92	0.68	0.00	0.00	0.00	1.37
Pink	1	2.38	0.00	0.00	0.00	0.00	0.00	0.00
Purple	0	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Yellow	8	0.00	1.96	2.36	0.00	0.00	0.00	0.00
Orange	14	0.00	0.00	1.01	0.00	0.00	0.00	15.07
Brown	4	0.00	5.88	0.34	0.00	0.00	0.00	0.00
Gray	3	0.00	0.00	1.01	0.00	0.00	0.00	0.00
Multiple colours	14	0.00	1.96	3.04	0.00	0.00	0.00	5.48
	507	42	51	296	7	20	18	73

The size distribution among sampled MPs is shown in Figure 4. MPs were divided into 6 size categories: > 5 mm, 2-5 mm, 1-2 mm, 0.5-1 mm, 0.3-0.5 mm and <0.3 mm according to BASEMAN Project standardized

protocol for monitoring microplastics in seawater (Gago et al., 2019). Maximum Feret's diameter was measured for size determination. MPs in the size category 0.5-1 mm (30.18%) were the most abundant across all samples, following by sizes 0.3-0.5 mm (21.89%), 1-2 mm (20.71%), 2-5 mm (13.61%), <0.3 mm (11.44%) and >5 mm (2.37%). Used mesh size (300 µm) often causes underestimation of the real abundance of MPs because of the loss of smaller particles (Cutroneo et al., 2020). Different sizes, shapes and colors of MPs could indicate different sources and degradation rates of plastic particles (Hidalgo-Ruz et al., 2012). Even though all samples were sieved through a 4 mm sieve, the reason why we got particles greater than that lies in the elongated shape of particles that got through perpendicular with regards to the sieve.

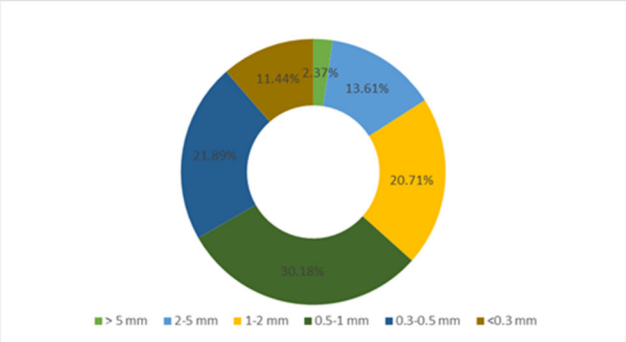

Figure 4. Distribution of MPs by size.

4. CONCLUSION

A total of 507 plastic particles were found in the lower part of Krka River estuary's surface water. All particles were classified into 7 shape categories. The most abundant shape were fragments, followed by foams, filaments, fibers and to a less extent microbeads, films, and pellets. Blue, transparent, white, and red were the most dominant colors. According to maximum Feret's diameter used in size determination, MPs in the size category 0.5-1 mm (30.18%) were the most abundant. Various shapes, colors and sizes could indicate various sources and different degradation rates of plastic particles. Our results showed the highest abundance of MPs in the vicinity of marina Mandalina, which was expected because of high anthropogenic pressure. We have provided useful basis for further research to improve sampling and processing techniques, considering the whole water column, not only the surface water. Given that, an overall picture of MPs abundance in certain area could be assessed.

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