

Microplastic Contamination in Protected Areas of the Gulf of Venice

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Abstract We investigated the concentration and composition of plastics in 7 Special Areas of Conservation (SACs) from the Gulf of Venice (northern Adriatic Sea). A total of 42 sediment samples were analyzed from 21 sites from 2017 to 2018. All sites except one were found to be polluted by plastics, with density ranging between 2250 and 28.4 items kg⁻¹. Microplastics ranged from 100 to 61.6% of the collected plastics. Fragments were more represented than filaments. The greatest plastic concentrations were generally recorded in western SACs. Identification

through FT-IR spectroscopy evidenced the presence of 8 polymer types: in western SACS, the majority were low-density polymers (PE, PP, PS, and TPU), while in eastern SACs they were high-density polymers (PET, nylon, and PVC). In addition to the role of large rivers (all on the western side of the Gulf) in conveying plastics into the sea, a possible role of the cyclonic water circulation of the northern Adriatic Sea on distribution and composition of plastics along the Gulf coasts is likely.

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1 Introduction

Plastic pollution is increasingly recognized as a major threat to marine biodiversity (Bergmann et al., 2015). In the last 50 years, world plastic production has increased from 1.7 to 348 million tons, with a proportional increase in the production of plastic waste (PlasticsEurope, 2018). Part of this waste is eventually discharged into the environment, a problem exacerbated by the common use and inappropriate disposal of throw-away "user" plastic products that ultimately reach the sea. Approximately 14 million tonnes of plastic litter is dumped in the oceans each year (Jambeck et al., 2015). An estimated 40% of that falls into the "single-use" category, which means it



winds up in the ocean within the same year as it was produced (UNEP, 2018). When at sea, plastic litter degrades slowly through a combination of photo-oxidation and mechanical abrasion, resulting in the production of small fragments and microplastics. Microplastics are considered specifically in descriptor 10 of the Marine Strategy Framework Directive (MSFD, 2008/56/EC), i.e., "Trends in the amount, distribution and, where possible, composition of micro-particles (in particular micro-plastics)," and "The amount of litter and micro-litter ingested by marine animals is at a level that does not adversely affect the health of the species concerned." Indicators for the latter criterion should be developed in the current implementation cycle of MSFD.

The assessment of plastic and microplastic pollution at sea is relatively recent, and extensive areas of the ocean remain poorly explored. This is the case of the Mediterranean Sea (UNEP/MAP/MED-POL, 2009), whose shores house around 10% of the global coastal population. Microplastic pollution in the Mediterranean is a problem whose extent has been only recently recognized (Gago et al., 2015). In their review, Cózar et al. (2015) reported that floating small plastic abundances in the Mediterranean Sea (from 22 to 1934 g km⁻²) were similar to those found in the Pacific Ocean gyres. Among Mediterranean regions, the Adriatic Sea is characterized by one of the greatest seafloor plastic pollutions (from 171 ± 36 to $47.87 \pm 23.38 \text{ kg km}^{-2}$; Strafella et al., 2015; Pasquini et al., 2016; Mistri et al., 2017), and recent studies suggest that the northern Adriatic coast is vulnerable to plastic accumulation on the shores (Laglbauer et al., 2014; Munari et al., 2016, 2017a, b).

Protected areas (e.g., Sites of Community Importance, SCIs, and Special Areas of Conservation, SACs, under the Habitats Directive 92/43/CEE) are highly recommended tools for the conservation of marine habitats and species in the face of the impact of human activities (Lubchenco and Grorud-Colvert, 2015). Many activities are excluded in these areas, such as dumping of dredged materials, spilling of wastewaters, and mining for oil and gas. However, these areas, despite the standards of protection to which they are subjected, can be equally subject to accumulation of waste and pollutants coming from elsewhere. In the Gulf of Venice (the northern part of the Adriatic Sea, limited by an imaginary diagonal line between the Sacca di Goro on the west and Rt

Kamenjak on the east), there is a number of transition protected areas (SACs, under the Habitats Directive) both on eastern and western side. The Gulf of Venice is heavily impacted by many human activities: intense marine traffic, fisheries, coastal and offshore aquaculture, and pollutants and litter discharge through many rivers (dominated by the Po).

Protected areas are also impacted by the arrival of pollutants on their seafloor, especially by microplastics (Vianello et al., 2013). Plastic pollution is particularly problematic in coastal ecosystems subject to riverine inputs (e.g., lagoons, estuaries, and coastal wetlands) that provide essential habitat for many commercially important species, such as fish and shellfish. Ingestion of plastic by organisms may pose a variety of risks including oxidative stress, cell damage, inflammation, and leaching of chemical contaminants (Vethaak & Leslie, 2016). For these reasons, it is important to document the occurrence of microplastics in coastal systems of the northern Adriatic Sea, an area reported as one of the most plastic-polluted seas of the world (Pasquini et al., 2016; Strafella et al., 2015).

To obtain a full picture of the microplastic pollution in protected transition systems of the Gulf of Venice, we conducted an extensive investigation of the abundance and composition of microplastics in the sediments of selected SACs from 2017 to 2018.

2 Materials and Methods

Figure 1 shows a map of the Gulf of Venice with the 7 considered SACs. These were as follows: IT4060005 "Sacca di Goro" (2 sites: GOR1 and GOR2), IT3270017 "Delta del Po: tratto terminale e delta veneto" (4 sites: MAR1, MAR2, CAL1, and CAL2), IT3250030 "Laguna medio-inferiore di Venezia" (2 sites: VE1 and VE2), IT3250031 "Laguna superiore di Venezia" (2 sites: VE3 and VE4), SI3000240 "Sečoveljske Soline in Estuarij Dragonje" (3 sites: JERKA1, JERKA2, JERKA3), SI3000238 "Strunjanske Soline s Stjužo" (3 sites: STJUZA1, STJUZA2, and STJUZA3), and SI3000252 "Škocjanski Zatok" (3 sites: SKOZA1, SKOZA2, and SKOZA3). Coordinates and characteristics of the sampling sites are reported in Table 1.

Surface sediment samples (about 2 kg) were collected in duplicate at each sampling site. Sediment



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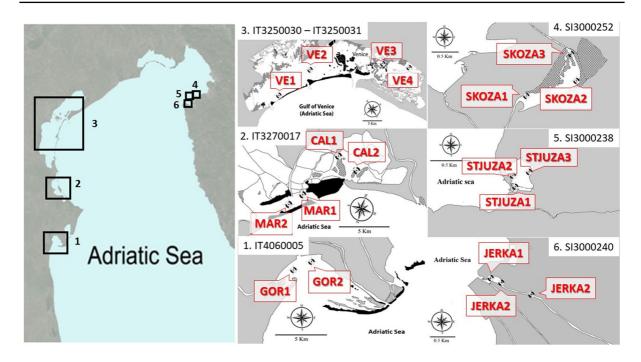


Fig. 1 Gulf of Venice: study site location

Table 1 Sampling site coordinates and characteristics

SAC	Site	Lat	Long	Depth m	Sand %	Silt %	Clay %	Seagrass
				111	70	70	70	
IT4060005	GOR1	44°49.585′N	12°16.602′E	1.2	11.9	37.7	50.5	NO
	GOR2	44°50.155′N	12°17.814′E	0.8	11.2	32.9	55.9	NO
IT3270017	MAR1	45°03.584′N	12°21.614′E	1.0	66.1	22.1	11.8	NO
	MAR2	45°03.144′N	12°22.659′E	1.4	69.0	20.5	10.5	NO
	CAL1	45°04.696′N	12°18.236′E	0.8	19.6	48.6	31.8	NO
	CAL2	45°05.835′N	12°18.809′E	0.8	21.1	54.2	24.7	NO
IT3250030	VE1	45°15.258′N	12°17.566′E	1.0	49.6	40	11	Zostera marina
	VE2	45°21.833′N	12 17.452'E	1.9	61.9	38	0	Cymodocea nodosa
IT3250031	VE3	45°30.924′N	12°26.052′E	0.8	23.2	50	26	NO
	VE4	45°33.983′N	12°29.956′E	0.8	10.2	61	28	Ruppia cirrhosa
SI3000238	STJUZA 1	45°31.709′N	13°36.238′E	0.8	21.6	34.6	43.8	Cymodocea nodosa
	STJUZA 2	45°31.762′N	13°36.215′E	0.8	26.9	46.9	26.2	Cymodocea nodosa
	STJUZA 3	45°31.816′N	13° 36.392′E	0.8				Cymodocea nodosa
SI3000240	JERKA 1	45°29.980'N	13°35.436′E	0.5	1.1	45.5	53.4	Cymodocea nodosa
	JERKA 2	45°29.917′N	13°35.565′E	0.5	1.2	36.1	62.7	Cymodocea nodosa
	JERKA 3	45°29.780'N	13°36.033′E	0.5				Cymodocea nodosa
SI3000252	SKOZA 1	45°32.545′N	13°44.616′E	0.5				NO
	SKOZA 2	45°32.703′N	13 45.268'E	0.5				NO
	SKOZA 3	45°33.010′N	13°45.157′E	0.5				NO

samples were sieved through a 0.5-mm stainless steel sieve. Then the plastic debris was visually identified and removed under a dissection microscope (Nikon SMZ45T, magnification $3.35-300\times$), counted, measured, and classified into three groups (GESAMP, 2019): large microplastics (0.5–5 mm), mesoplastics (>5–25 mm), and macroplastics (>25 mm). Plastic debris was also categorized according to shape, i.e., filaments and fragments. Plastic debris in sediment was reported as density (items kg^{-1}) per unit of dried sediment.

Fourier transform infrared spectroscopy (FT-IR) analysis of plastic debris was carried out with a CARY 600 FT-IR (Agilent Technologies) instrument. Measurements were carried out in attenuated total reflectance (ATR) configuration, with a Pike Miracle diamond cell. Tests were carried out at 25 °C in dry air. Particles were identified by comparing FT-IR absorbance spectra of the microplastics to those in a polymer reference library. A subsample (about 30%) of the collected plastics was analyzed.

All necessary precautions were implemented to minimize microplastic contamination while handling and processing the samples: cotton lab coats and nitrile gloves were worn in the laboratory, and the samples were capped with aluminum foil and glass caps during the identification and analysis to minimize airborne contamination. The fraction of smaller microfibers (<0.5 mm) is reported as the one that can be overestimated due to environmental contamination (Lusher et al., 2017): in our study we believe that this error may be negligible, as 0.5 mm was the minimum size of the particles we have considered.

Table 2 Percent amount per SAC of size type and shape type of collected plastics

SAC	Sites	Size		Shape		
		Micro %	Meso %	Macro %	Filament %	Fragment %
IT4060005	GOR	77.9	20.2	1.9	30.4	69.6
IT3270017	CAL-MAR	74.9	21.8	3.3	22.4	77.6
IT3250030	VE (1-2)	100	0	0	1.9	98.1
IT3250031	VE (3-4)	100	0	0	4.9	95.1
SI3000238	STJUZA	69.8	16.4	13.8	35.8	64.2
SI3000240	JERKA	61.6	19.4	19.0	49.5	50.5
SI3000252	SKOZA	68.8	15.3	15.9	31.5	68.5

3 Results

A total of 42 sediment samples were analyzed from the 21 sites. Granulometric analysis (Folk, 1980) showed sediment consisting of fine sediments, with a texture of silt and clay for the majority of samples, while some samples from IT3270017 and IT3250030 showed sediment composition of sand-silt (Table 1).

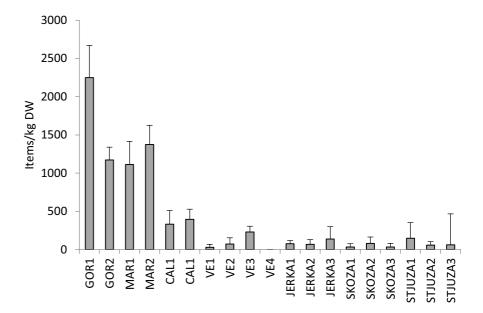
All sites except one (VE4 at IT3250031) were found to be polluted by plastics. Plastic particles ranged from 0.5 to 12 mm in length. The samples contained both filaments and fragments in a range of colors, implying that particles may have originated from multiple sources. In Table 2, the percent amount per SAC of size type and shape type of collected plastics is shown. Considering size, large microplastics (0.5-5 mm) accounted for the vast majority of the collected plastics, ranging from 100% at sites VE1, 2, and 3 (IT3250030 and IT3250031) to 61.6% at sites JERKA (SI3000240). Considering shape, fragments were more represented than filaments. Fragments are likely derived from the breakdown of larger plastics, while filaments may be derived from the breakdown of synthetic fishing lines, nets, and ropes.

The greatest plastic concentration in terms of counted items (Fig. 2) was recorded at site GOR1 (2250±420 items kg⁻¹; IT4060005), while the lowest was at site VE1 (28.4±40.2 items kg⁻¹; IT3250030) with the exception of the clear samples recorded at site VE4 (none; IT3250031). As reported in Fig. 2, the variability in plastic concentrations between stations was relatively high, as shown by the quite large values of SD, and the values recorded at different SACs were significantly different from one another. One-way ANOVA



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Fig. 2 Sediment plastic concentration at the study sites (bars are standard deviation)



on square root-transformed concentration data (Cochran test; Snedecor & Cochran, 1968), followed by LSD pairwise comparison tests, showed that there were significant differences in spatial variation of amount of plastic items, with the Po Delta lagoons (IT4060005 and IT3270017, sites GOR, MAR, CAL) showing higher concentrations of plastics (all p < 0.001).

A subset of plastic particles (n = 70) collected from 6 SACs (all except SI3000238) was analyzed using FT-IR and compared with reference spectra. Identification through FT-IR spectroscopy showed the presence of 8 polymer types: polyethylene (omo and copolymer, PE), polypropylene (omo and copolymer, PP), polyethylene terephthalate (PET), polyvinyl chloride (PVC), nylon 6.6 (nylon), polysiloxanes (PSX), polystyrene (PS), and thermoplastic polyurethane (TPU), plus some fragments which, due to the high level of degradation, we have not been able to identify. Some examples of plastic particles collected during the study are shown in Fig. 3. The composition by abundance of polymer type is shown in Fig. 4. There were differences in the most common polymers found at different SACs: PET was dominant in the eastern areas (49.4% and 36% at JERKA and SKOZA, respectively), while PE was dominant in the western areas (50% at VE; 42.2% and 43.4% at GOR and MAR, respectively).

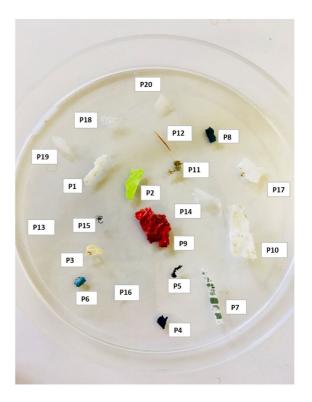
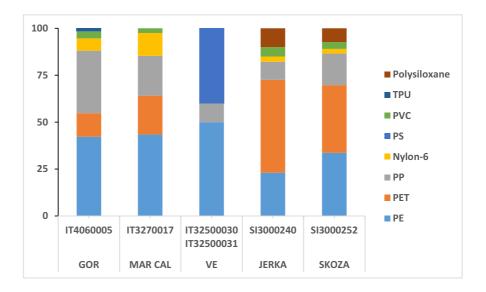


Fig. 3 Examples of plastic particles collected during the study (P1: polysiloxane; P2, P3, and P4: PET; P5: PVC; P6: PET; P7: PE; P8 and P9: PET; P10: PP; P11: nylon-6; P12: PP; P13: unknown; P14: PE; P15: PET; P16: unknown; P17: PE; P18: unknown; P19: polysiloxane; P20: PE)



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Fig. 4 Composition of polymer type at the five analyzed SACs



4 Discussion

Studies concerning contamination by plastics and microplastics of Mediterranean protected Special Areas of Conservation (SACs) are not numerous. Vianello et al. (2013) extensively studied microplastics in the Venice Lagoon, while Blăsković et al. (2017) studied those in the natural park of Telaščica Bay. We gathered seabed small plastic debris (>0.5 mm) while sampling benthic invertebrates from 7 SACs in the Gulf of Venice. Because of the high cost involved with sampling the seabed, no historical data are available from SACs in the Gulf of Venice for assessing trends in plastic and microplastic contamination. Despite some limitations, such as the minimum size of plastics collected, this "by-catch study" is the first extensive study to present an assessment of seabed plastic pollution in lagoon and estuarine protected areas of the Gulf of Venice. "By-catch studies" like this one may help to address the gap in knowledge and to make available otherwise unavailable data for future comparisons for instance in the framework of the MSFD implementation (e.g., Ramirez-Llodra et al., 2013).

Differences in plastic concentrations between the different geographic locations considered in this study can be due to: (i) the riverine contribution (Lebreton et al., 2017), in particular of the Po river (Munari et al., 2021), in transporting plastic debris, and (ii) the type of currents and circulation in the Gulf of Venice. In fact, the general surface circulation of the Gulf may

be described as a large-scale cyclonic meander, with a northerly flow along the eastern coast and a southerly return flow along the western coast (Orlić et al., 1992). This probably causes the plastic debris carried by rivers to settle preferentially in the environments of the western side of the Gulf: the spatial distribution for plastic contamination seems to follow the conceptual model proposed by Liubartseva et al. (2016) for floating debris in the northern Adriatic Sea.

Sediments of transition environments are subjected to the accumulation of plastics (Table 3). The concentrations we found are in the same range with those studies; however, concerning shape types our results are in contrast with the majority of them, where fibers are the most common shape type of plastics found in sediments. Gago et al. (2018), reviewing published data from 1960 to 2017, found that fibers were found in sediments of almost every marine habitat around the world, in a higher number compared to plastic pellets or fragments, even in the most remote areas (González-Pleiter et al., 2020). However, in their pioneering study on microplastic contamination of the Venice Lagoon, Vianello et al. (2013) found that fragments (87%) were more common than filaments (10%) on the lagoon seabed. A similar result was found by Gray et al. (2018) in Carolina estuaries.

A hypothesis which could justify the difference of our results from others could be that the method of collection and identification adopted meant that part of the smaller filaments were lost and thus not counted. However, the same method was used by us in



Table 3 Concentration and most common shape in plastics/microplastics from transition environments

Transition environment	Sediment concentration (items kg ⁻¹)	Dominant shape	Reference
Gulf of Venice (Italy–Slovenia)	28–2250	Fragments	This study
Guanabara Bay (Brazil)	160-1000	Fibers	Alves and Figuereido (2019)
Jagir Estuary (Indonesia)	92-590	Fibers	Firdaus et al. (2020)
Tampa Bay (USA)	30-790	Fibers	McEachern et al. (2019)
Rías Baixas (Spain)	70.2 ± 74.2	Fibers	Carretero et al. (2021)
Venice Lagoon (Italy)	672-2175	Fragments	Vianello et al. (2013)
Charleston Harbor (USA)	0-2524*	Fragments	Gray et al. (2018)
Winyah Bay (USA)	0-796*	Fragments	Gray et al. (2018)

*items m⁻²

other studies in marine environments (central Adriatic: Mistri et al., 2017; Ross Sea: Munari et al., 2017a, b; Pianosa island: Mistri et al., 2018; northern Tyrrhenian: Mistri et al., 2020), and in all those studies we found that the filaments and fibers were significantly more numerous than the fragments. All those studies were carried out on much deeper seabeds (30-120 m depth), differently from the bottoms considered in this study, whose depth was lower (0.5–2 m depth). Harris (2020) analyzed the information reported in 80 papers about different marine environments and reported higher fiber contents for the following: beach (median value 90%), deep sea environments (median value 75%), tide-dominated shelf (median value 63%), and shallow coastal environments (61%). The lowest median values for fibers were reported in tide-dominated estuarine systems (median value 49%), like those examined in this case study. The comparison with other studies, however, is very difficult because the analytical techniques are often very different.

The majority of the polymers identified along the Italian west coast were low-density polymers such as PE, PP, PS, and TPU (which globally composed 64–76% of the polymers) that are expected to float and travel for longer distances before sinking because of fouling or ingestion by marine organisms (Fazey and Ryan, 2016; Harris, 2020). Conversely PET is expected to sink quickly because of its high density (higher than the average seawater density of 1.03 g cm⁻³) and presented higher percentages along the Slovenian coast highlighting the possible role of the cyclonic water circulation of the northern Adriatic Sea on distribution and composition of plastics along the coasts. PS was found in large quantities at VE sites, confirming the observations of Vianello et al.

(2013) about the extensive contamination caused by fragments of this polymer on the seabed of the Venice Lagoon.

The types of polymers found cover virtually any use, and it is therefore extremely difficult to understand where they come from, probably deriving from both land- and sea-based sources. PE, PP, and PET are mainly used for packaging for food (plastic bags, plastic films, containers including bottles), textiles, and stationery; PE, PP, and nylon are used to produce bags and ropes, which are widely used in the local aquaculture and fishing industry; PS is used for producing disposable plastic cutlery and dinnerware; TPU has applications which include sporting goods, footwear, inflatable rafts, and, more recently, in 3D printing; and polysiloxanes are used in many products: electrical (insulation), electronics (coatings), household (cooking utensils), automobile (gaskets), toys, bracelets, personal care (skin and haircare), and even many others.

The seabed is a sink for plastics (Woodall et al., 2014), but, due to its depth, it remains a limited area of study compared to surface water. There are very few studies dealing with plastic contamination of Mediterranean seabed (Llorca et al., 2020), due to the greater technical difficulties in sampling, and the scarcity of resources allocated for these tasks. After that of Vianello et al. (2013), this is the first study evaluating the contamination by plastics and microplastics of the seabed of protected SACs in the Gulf of Venice. We found small plastic particles in almost all the sediment samples collected in transition areas of the Gulf, where plastic contamination appeared to be almost ubiquitous, although the concentration of plastic particles differed among sites. Much higher plastic



concentration was found at sites on the western side of the Gulf, highlighting the primary role that large rivers (all on the western side of the Gulf), together with the particular type of circulation of the northern Adriatic, play in conveying this type of pollutant.

5 Conclusions

This study demonstrates that plastic particles are widely dispersed and abundant in sediments of protected transition areas in the Gulf of Venice, but in higher quantities on the seabed of the western side SACs. As SACs like those considered in this study provide feeding, reproductive, and wintering ground for fish, migratory birds, and living areas for many species with great economic value (e.g., clams, mussels), the accumulation of plastics could pose a threat to the biota and ultimately to human health.

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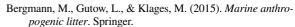
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Data Availability The datasets generated and/or analyzed during the current study are available from the corresponding author on reasonable request.

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