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D3.2.1 Report assessing the quantity and composition of marine litter for each indicator in the widening area of ML monitoring

Activity 3.2.1

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1. Introduction

Any solid material that is processed, persistent and disposed or abandoned in the marine and coastal environment, is considered marine litter and is globally acknowledged as a major societal challenge of our times due to its significant environmental, economic, social, political and cultural implications. Although the Mediterranean is the sea most affected by marine litter, information regarding marine litter in the Adriatic remain limited, inconsistent and fragmented which results in lack of reliable scientific data needed to address marine litter more effectively (Vlachogianni et al., 2016).

Specific methodology was used in the MARLESS project modifying and following the methods of monitoring and analysis described in: Galgani et al. (2013): Guidance on Monitoring of Marine Litter in European Seas. MSFD Technical Subgroup on Marine Litter (TSG-ML) European Commission, OSPAR (2010): Guidelines for Monitoring Marine Litter on the Beaches in the OSPAR Maritime area, NOAA (2013): Marine Debris Monitoring and Assessment: Recommendations for Monitoring Debris Trends in the Marine Environment, while considering the UNEP/MAP MEDPOL (2013): Monitoring Guidance Document on Ecological Objective 10: Marine Litter. By implementing the same guidelines, a high level of consistency and data quality was secured.

This document contributes in achieving the objectives of WP3, in particular those defined in the frame of Activity 3.2, which aim is to assess the quantity and composition of marine litter for each indicator in the widening area of Marine Litter monitoring.

The MARLESS project has scheduled and conducted monitoring campaigns to sample and to count the number of macro and micro litter objects collected in each campaign.

It is expected that the results of this data analysis, increases our knowledge of the marine litter present in the Adriatic Sea. That will contribute to the identification of the processes that prevail in the generation of micro plastic in the cooperation area, the potential sources and the impacts that they have in the ecosystem.

2. Beach litter

a. Study area

Beach litter surveys were carried out on beaches located in Croatia and Italy (Figure 1). A total of 11 beaches (locations) (Table 1) were investigated. Sites were selected randomly, taking into consideration following criteria: vicinity of ports or harbors, vicinity of river mouths, vicinity of coastal urban, vicinity of tourist destinations and cities (relatively remote areas). Surveys span from September 2021 to March 2023 in which 41 transects were sampled.

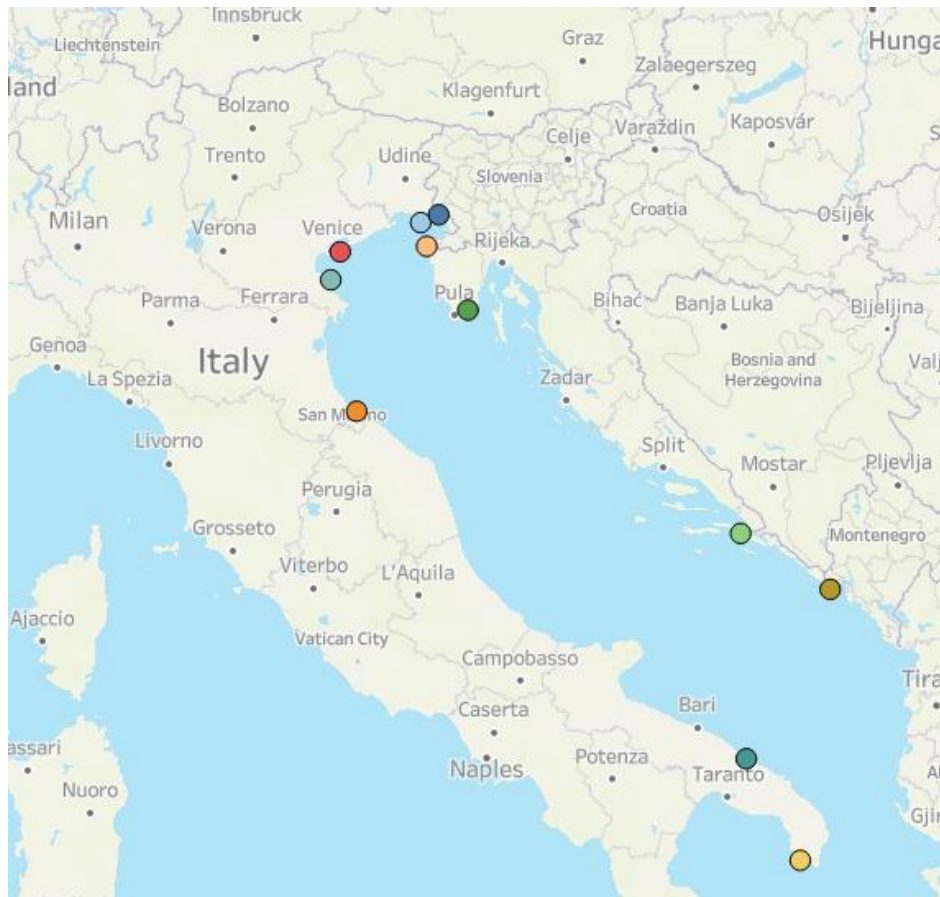


Figure 1- Sampling sites for beach litter

Table 1 - Sampling sites from each country with the location and the surveying organization

COUNTRY	REGION	LOCATION	SURVEYING ORGANISATION
CROATIA	Dubrovnik-Neretva	Prevlaka	University of Dubrovnik Center for Marine Research, Ruđer Bošković Institute
		Trstenik	
	Istria	Vinjole	Center for Marine Research, Ruđer Bošković Institute
		Zambratija	
ITALY	Friuli Venezia Giulia	Canovella degli Zoppolli	ARPA Friuli Venezia Giulia
		Grado Pineta	
	Veneto	Punta	ARPA Veneto
		Verde	
	Emilia Romagna	Riccione	Fondazione Cetacea
	Apulia	Fasano	ARPA Puglia
		Ugento	

The beaches had to be 100 m or longer except in location where the morphology of the coastline didn't permit, characterized by a low to moderate slope (~ 1.5 - 4.5°), which precludes very shallow tidal mud flat areas that might be kilometers long with clear access to sea (not blocked by breakwaters or jetties) such that marine litter is not screened by anthropogenic structures, be accessible to survey teams throughout the year, ideally not be subject to cleaning activities. If the selected beaches were subjected to litter collection activities, the timing of non-survey related beach cleaning had to be known so that the litter flux rates (the amount of litter accumulation per unit time) could be determined. Protected areas were considered excluded although their exclusion depended on local management arrangements. While selecting sampling sites, partners could have selected

beaches that didn't comply with the listed criteria, if by their expert judgment and experience of the coastal area and marine litter situation in their country were considered the best option. Totally 4 beaches in Croatia and 7 beaches in Italy were sampled.

b. Methodology

All surveys performed followed the "Methodology for Monitoring Marine Litter on Beaches (Macro-Debris >2.5 cm)" that was agreed within the framework of the MARLESS project. The methodology was prepared based on the IPA-Adriatic DeFishGear project (2014), the EU MSFD TG10 "Guidance on Monitoring of Marine Litter in European Seas" (Galgani et al., 2013), the OSPAR "Guideline for Monitoring Marine Litter on the Beaches in the OSPAR Maritime Area" (OSPAR, 2010) and the NOAA "Marine Debris Monitoring and Assessment: Recommendations for Monitoring Debris Trends in the Marine Environment (Lippiatt et al., 2013), taking into consideration the draft UNEP/MAP MEDPOL "Monitoring Guidance Document on Ecological Objective 10: Marine Litter (UNEP/MAP MEDPOL, 2014)". Sampling was performed on a fixed section of the beach (spanning from the strand line to the beginning of coastal vegetation, cliff base or anthropogenic structures such as roads or seawalls) which represents a sampling unit. Surveys were conducted pre and post touristic season (before and after summer). Sampling site /shoreline characterization was made (type of substrate, beach topography, beach usage, distances from urban settlements, shipping lanes, river mouths, etc.). Only litter larger than 2.5 cm was collected with the inclusion of caps & lids and cigarette butts. Larger items that couldn't be removed were marked to avoid their reappearance in the next survey. All items found on the sampling unit entered the 'Beach Litter Monitoring Sheet'. The amount of marine litter is expressed as number of items per meter (items/m).

c. Abundance and composition

A total of 36 523 items were recorded and collected in 11 surveyed beaches (Table 2). Most prevalent category of marine litter was plastic while other material were least present. The abundance and type of items differed between sites although Cigarette butts and filters were dominant on most beaches. At the top of the abundance list are Croatian sampling sites. Zambratija beach had the highest abundance of litter with the total number of 8811 items and Trsteno beach had the second highest abundance with the total number of 8222 items. Vinjole and Prevlaka sites followed with 5086 and 4164 number of items. As for Italian sampling sites, highest number of items were found in Verde, 3423 items, followed by Riccione (2108), Fasano (1360), Canovella degli Zoppoli (1279), Grado Pineta (1095), Punta (629) and Ugento, with the lowest total number of collected items, just 346. The highest total number per survey period were in Autumn 2021 (12640) followed by Autumn 2022 (10096). The two Spring survey were significantly lower, in Spring 2022 was 8330 and 5457 in Spring 2023. The average of collected items per survey were 830. The main source of litter on the survey beaches is Land-base activities. The most abundant item collected was Cigarette butts and filters with the 21 % contribution in total, followed by Plastic (8 %) and Polystyrene pieces 2.5cm><50cm (6%). By analyzing the Croatian beaches separately from the Italian ones, it can be seen that cigarette butts are confirmed as the most commonly collected waste on Croatian beaches, while on Italian beaches it is only in third place, preceded by plastic pieces (MA79) and mussel net (MA45).

Table 2- Sampling sites with a total number of items and with the item found in highest abundance

COUNTRY	LOCATION	TOTAL NUMBER OF ITEMS	ITEM FOUND IN HIGHEST ABUNDANCE
CROATIA	Zambratija	8811	46 % Cigarette butts and filters
	Trstenik	8222	27 % Cigarette butts and filters
	Vinjole	5086	15 % Cigarette butts and filters
	Prevlaka	4164	19 % Polystyrene pieces 2.5cm><50cm

ITALY	Verde	3423	15 % Polystyrene pieces 0-2.5 cm
	Riccione	2108	27 % Mussels nets, Oyster nets
	Fasano	1360	22 % Cigarette butts and filters
	Canovella degli Zoppoli	1279	19 % Mussels nets, Oyster nets
	Grado Pineta	1095	30 % Polystyrene pieces 2.5cm><50cm
	Punta	629	21 % Polystyrene pieces 2.5cm><50cm
	Ugento	346	32 % Cigarette butts and filters

The average amount of litter concentration was 12 item/m. The highest was at Zambratija beach with an average of 44 item/m and the lower of 1.2 item/m was at Ugento beach (Figure 2).

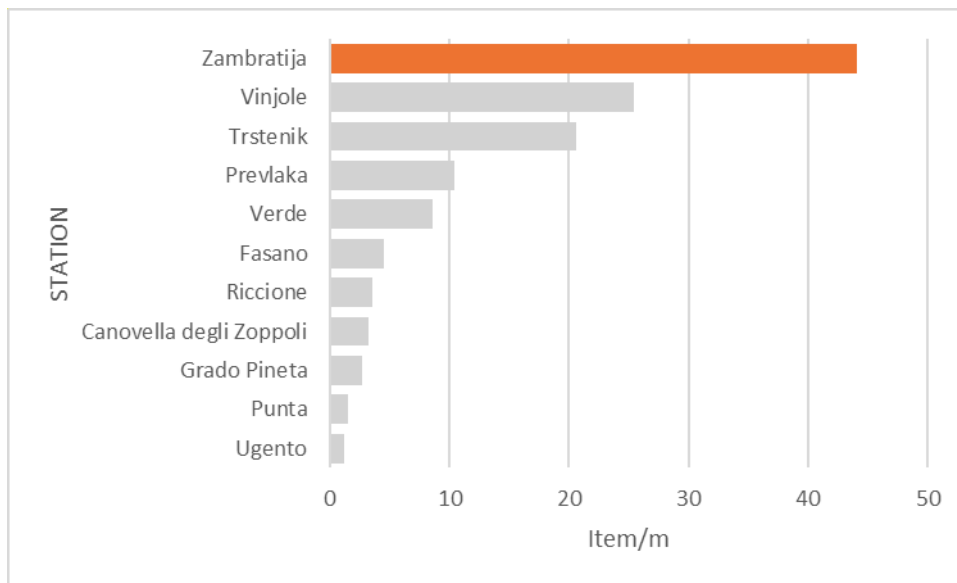


Figure 2 - Average Item/m per station

d. The MARLESS app to dig in the marine litter dataset

In the frame of the MARLESS project, the volume of data collected and the detail of the information harvested cannot be fully explored according to a static approach, that is with a report, even if in digital format. So, an interactive tool has been developed and let available to the whole community by way of a World Wide Web service. To access the service, follow this link:

https://public.tableau.com/app/profile/interregithr.arpafvg/viz/InterregIT-HRMARLESSCampaigns/MARLESS_campaigns

The data are accessible according four main access points that aim to ease the exploration of the whole dataset. Entering each of those points, menus are available to select data collected in each sampling transect, according to campaign dates and type of marine Litter. (Figure 3)

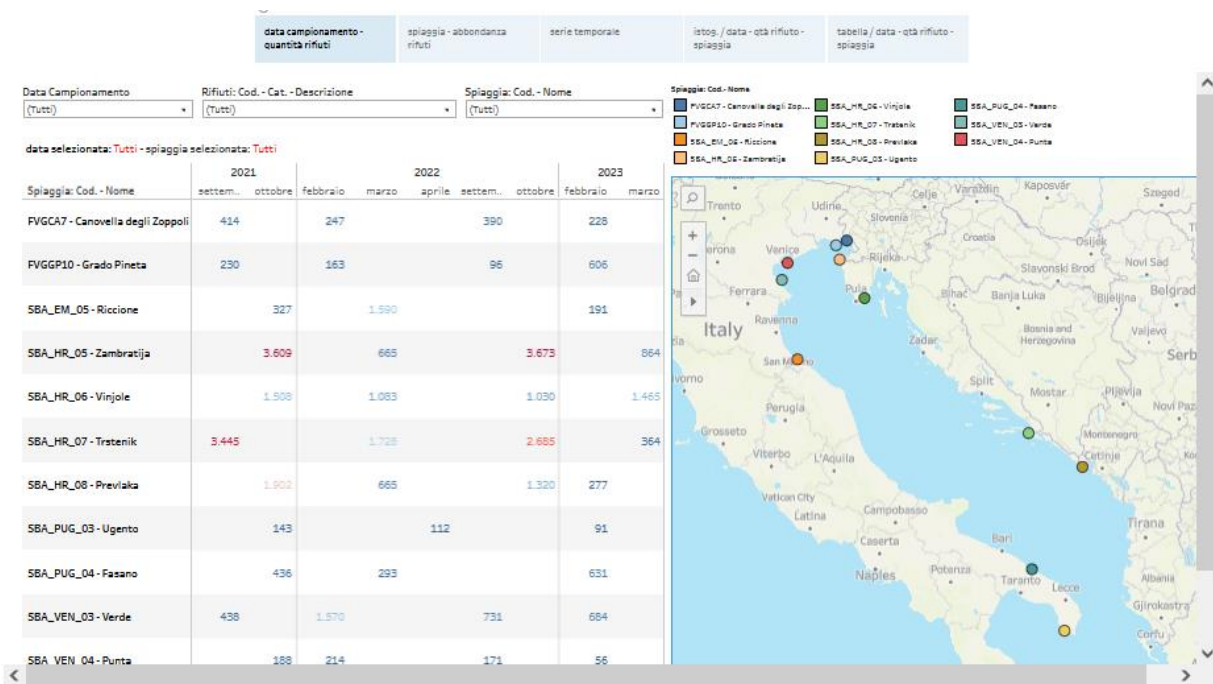


Figure 3 – Web interface of MARLESS database

All the results (Figure 4) are presented automatically as sum of the items found in the campaigns, since the total number of waste material is considered the piece of information permeating the analyses. The count of the objects is presented when data are available.

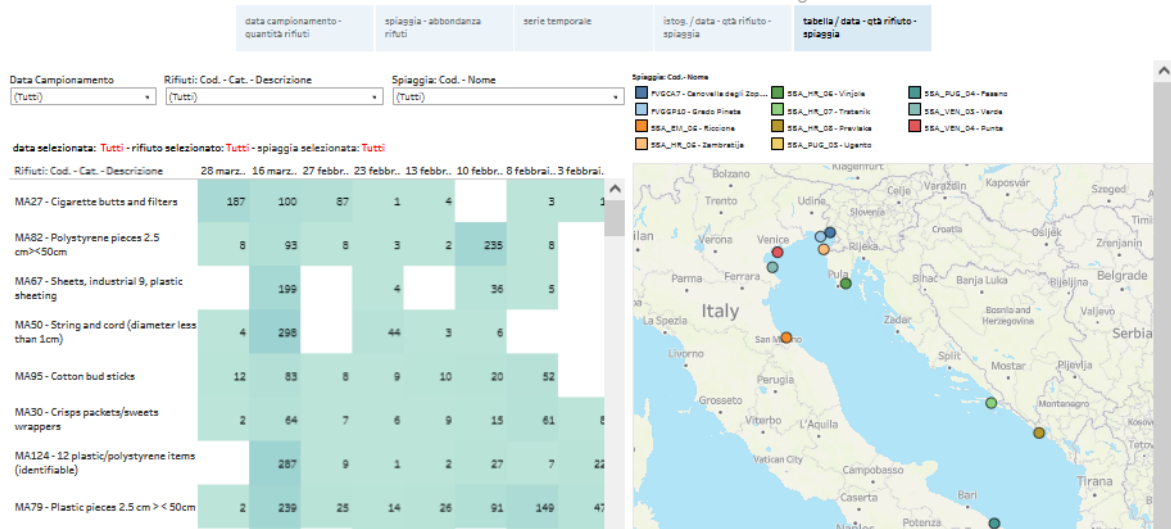


Figure 4 – MARLESS campaign dataset

3. Floating Litter

a. Study area

The survey area is defined by transect width and length. Width of the monitoring transect was 10 m. The transect length was determined from GPS coordinates of its starting and ending point. The same areas were monitored during all surveys. The studies areas are shown in Figure 5.

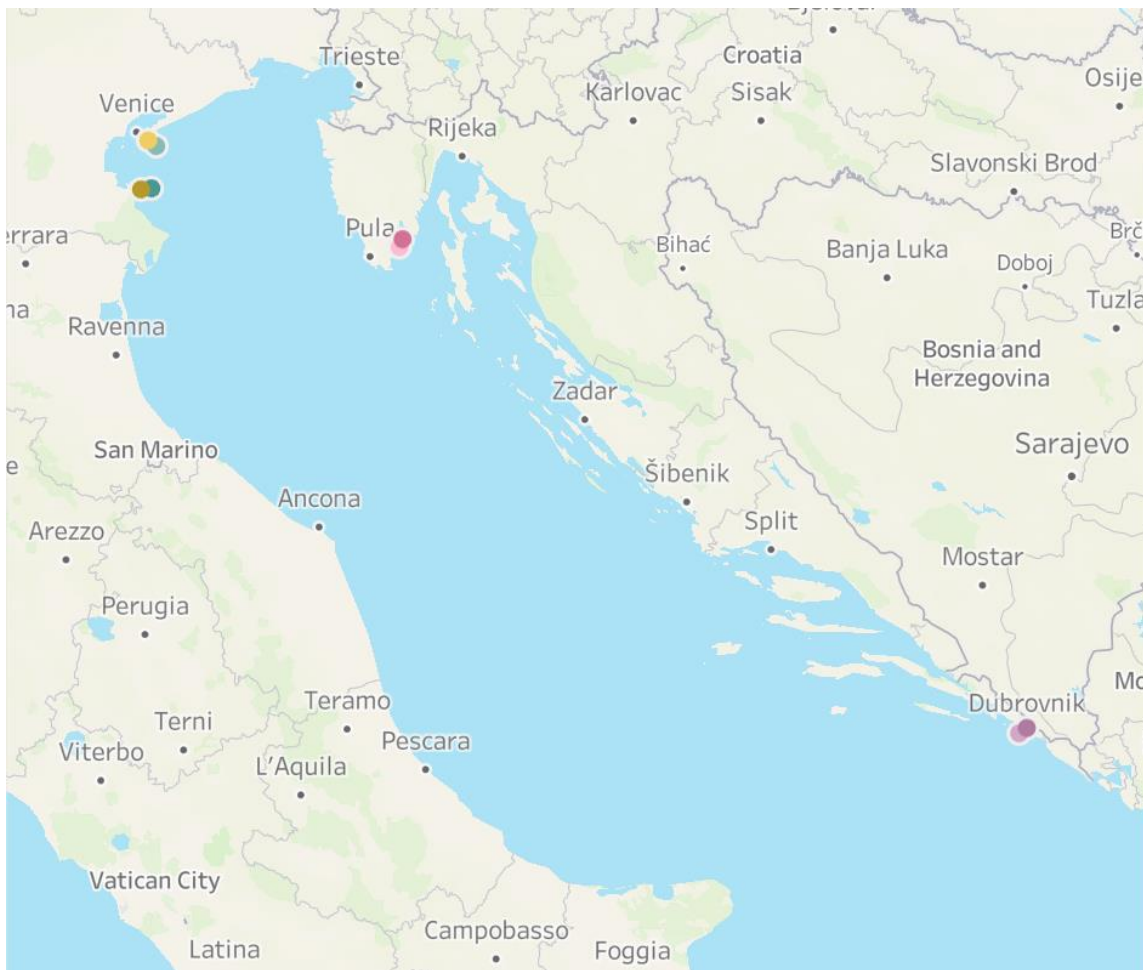


Figure 5 - Monitoring floating litter sites.

b. Methodology

All surveys performed followed the “Methodology for Monitoring Floating Litter (Macro-Debris >2.5 cm)” that was agreed within the framework of the MARLESS project. The methodology was prepared based on the IPA-Adriatic DeFishGear project (2014), the EU MSFD TG10 “Guidance on Monitoring of Marine Litter in European Seas” (Galvani et al., 2013), the OSPAR “Guideline for Monitoring Marine Litter on the Beaches in the OSPAR Maritime Area” (OSPAR, 2010) and the NOAA “Marine Debris Monitoring and Assessment: Recommendations for Monitoring Debris Trends in the Marine Environment (Lippiatt et al., 2013), taking into consideration the draft UNEP/MAP MEDPOL “Monitoring Guidance Document on Ecological Objective 10: Marine Litter (UNEP/MAP MEDPOL, 2014)”. A total of two surveys were carried out in a year: before and after the touristic season. Observed litter size was between 2.5 and 50 cm. As a result of that and the 10 m width of the observation transect, the speed of the observation boat didn’t exceed 3 knots. Litter observation was carried out in the front area of the boat with the direction of observation perpendicular to the pathway of the boat (Figure 6).

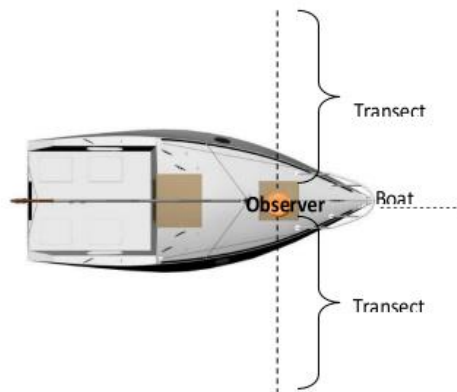


Figure 6- Direction of litter observation as opposed to boat pathway (source: IPA-Adriatic DeFishGear, 2014. “Methodology for Monitoring Floating Litter”)

All litter items observed inside the survey area were entered in the ‘MARLESS Floating Litter Monitoring Sheet’. Each type of item in the sheet has been assigned a unique identification number. Litter items that couldn’t be identified or are not listed in the survey sheet were noted in the appropriate “other” item box. The unit used for assessing the amount of litter on the sea surface is number of litter items per square kilometer (litter items/km²).

c. Abundance and composition

Total number of 57 plastic items were spotted in 6 surveyed sites in 4 sampling periods, with the average density of 190 item/km² (Figure 7). The minimum abundance detected on Veneto and Dubrovnik Region sites were 0 items/km² in two seasons where the maximum was detected in Istra Region with 967 items/km² density. The most abundant material found was plastic with 100 % contribution, where the most common items were plastic pieces and fragments.

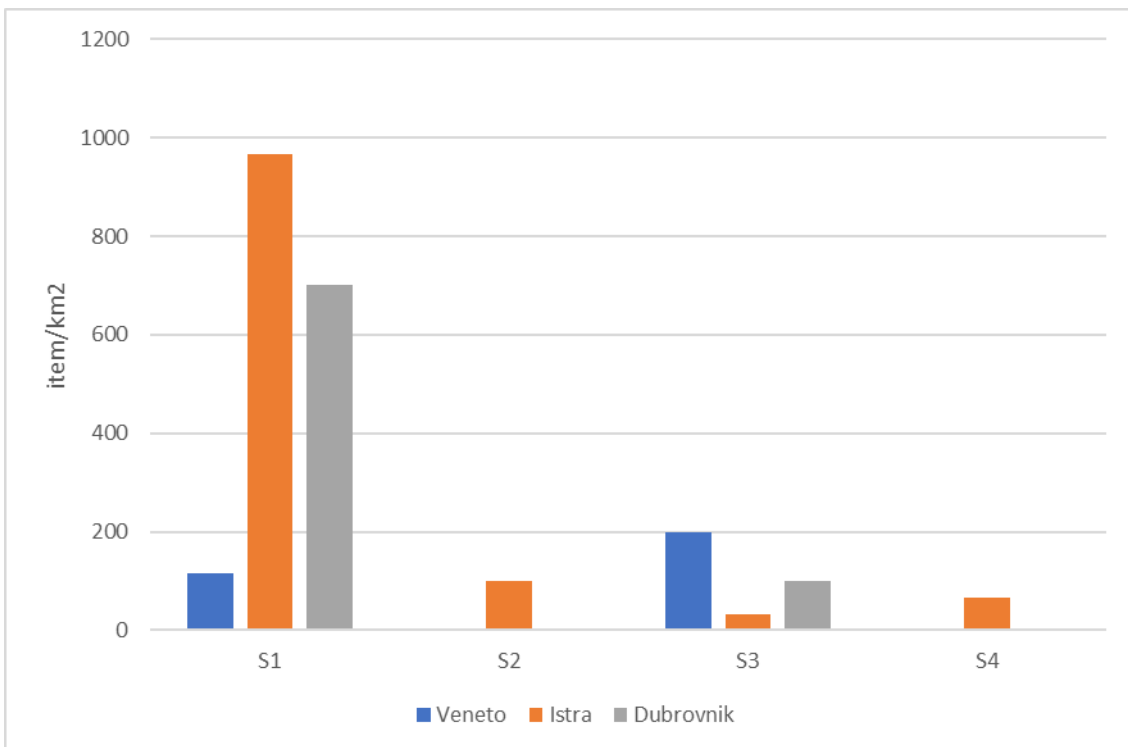


Figure 7- Floating litter abundance in monitoring sites (S – season)

4. Surface Microplastics

a. Study area

Microplastic is a term used to describe solid material that is less than 5 mm in length, differently dispersed in the environment. The sampling activities and laboratory analyses listed below are aimed at assessing the abundance and, if possible, the composition of the microplastic present in seawater, especially that of microplastics.

The monitoring surveys included at least 2 sampling stations in every involved region taking into account accumulation areas and source points (Figure 8).

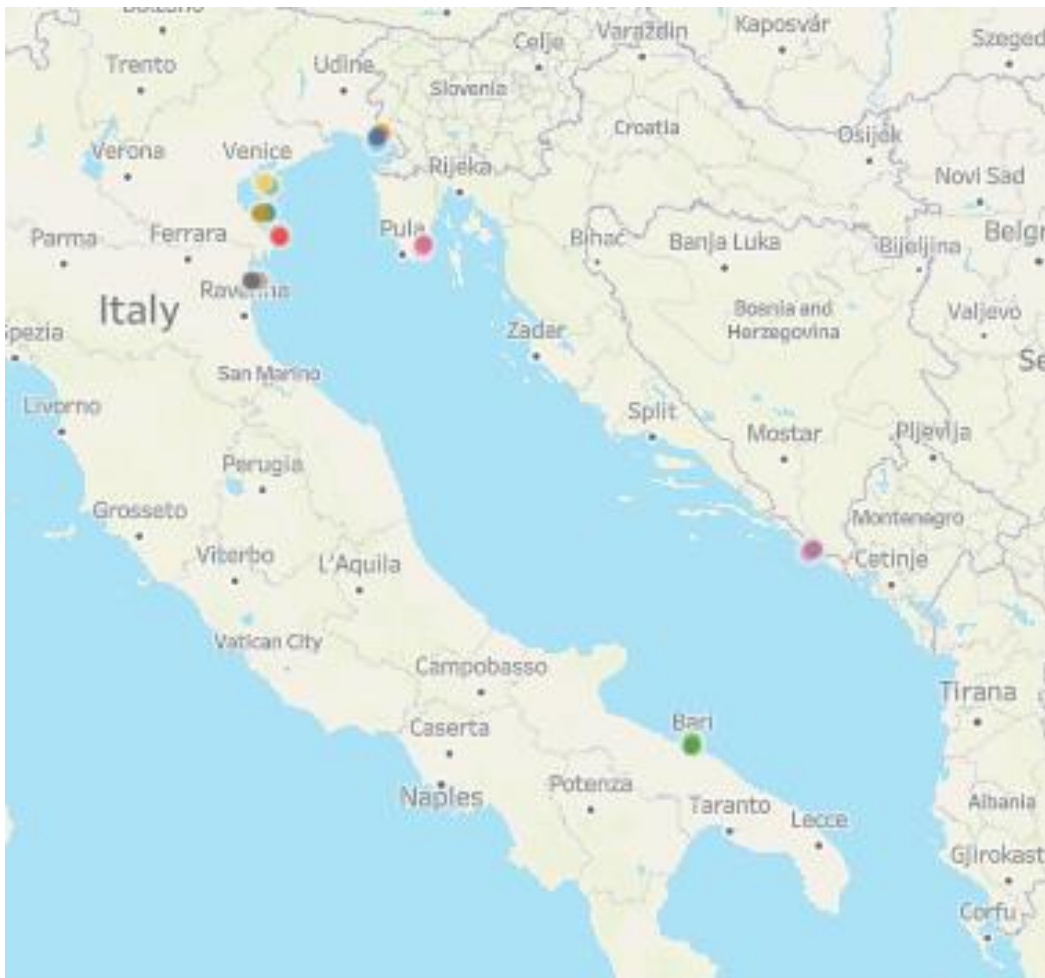


Figure 8 - Floating microplastic sampling sites

b. Methodology

All surveys performed followed the “Methodology for Monitoring Micro Litter” that was agreed within the framework of the MARLESS project. The methodology was prepared based on the IPA-Adriatic DeFishGear project (2014), the EU MSFD TG10 “Guidance on Monitoring of Marine Litter in European Seas” (Galvani et al., 2013), the OSPAR “Guideline for Monitoring Marine Litter on the Beaches in the OSPAR Maritime Area” (OSPAR, 2010) and the NOAA “Marine Debris Monitoring and Assessment: Recommendations for Monitoring Debris Trends in the Marine Environment (Lippiatt et al., 2013), taking into consideration the draft UNEP/MAP MEDPOL “Monitoring Guidance Document on Ecological Objective 10: Marine Litter (UNEP/MAP MEDPOL, 2014)”. Due to their exceedingly small size, lightweight properties and relative density, microplastics tend to accumulate mostly on the sea surface and then in the basal zone of the thermocline. For this reason, a "manta trawl net" is deployed (Figure 9 - Manta net. This type of net is specifically designed to be towed on the surface layer of the water column and hence to collect samples within the layer affected by the wave motion. The manta trawl net consists of a rectangular metal aperture, or mouth, with a cone-shaped net attached to it and a collecting cup or container at the cod end. The most used mouth size is 25 cm high by 50 cm wide with a net length of about 2.5 m. The dimensions refer to the internal dimensions of the mouth; that is, the part to which the net is connected. The advised mesh size was 330 μm (Figure 9).



Figure 9 - Manta net

The net was lowered and hauled for 20 - 30 minutes along a linear path at a speed between 1 and 2 knots, but never more than 3 knots, so as to allow the net to filter the water without regurgitation. After the samples were collected, they were sieved $\leq 300\mu\text{m}$ mesh size and stored in EtOH 70%. All natural or artificial litter objects larger than 5 mm (macro and mezzo litter) were removed from the sample. Visual identification was performed on stereomicroscope with the help of tweezers. Items were categorized by size, shape and color (Table 3). Their abundance was expressed as number of Item/m².

Table 3 - Microlitter categories

Micro litter categories	Colour of plastic items
Fragments (G103, G104, G105, G106)	White
Pellets (G107, G108, G109, G110, G111)	Clear-white-cream
Granules (G116)	Red
Filaments (G113)	Orange
Films (G114)	Blue
Foam (G115, G117)	Black
Other (nonplastic materials) (G217)	Grey
Uncategorized plastic pieces*	Brown
	Green
	Pink
	Tan
	Yellow

c. Abundance and composition

In 18 surveyed sites, during the 4 surveys, the average density of 0.057 items/m² (Figure 10). The maximum abundance were detected on SBA_PUG_07-Bari 4M with 0.346 items/m², where the minimum at SBA_FVG_09 - MARI1 with 0.01 item/m² density. The most common found items were fragments and filaments (> 0.55 item/m² in total) also fibers were very common (> 0.30 item/m² in total). The most common color found was black. Transparent item were more common than opaque items.

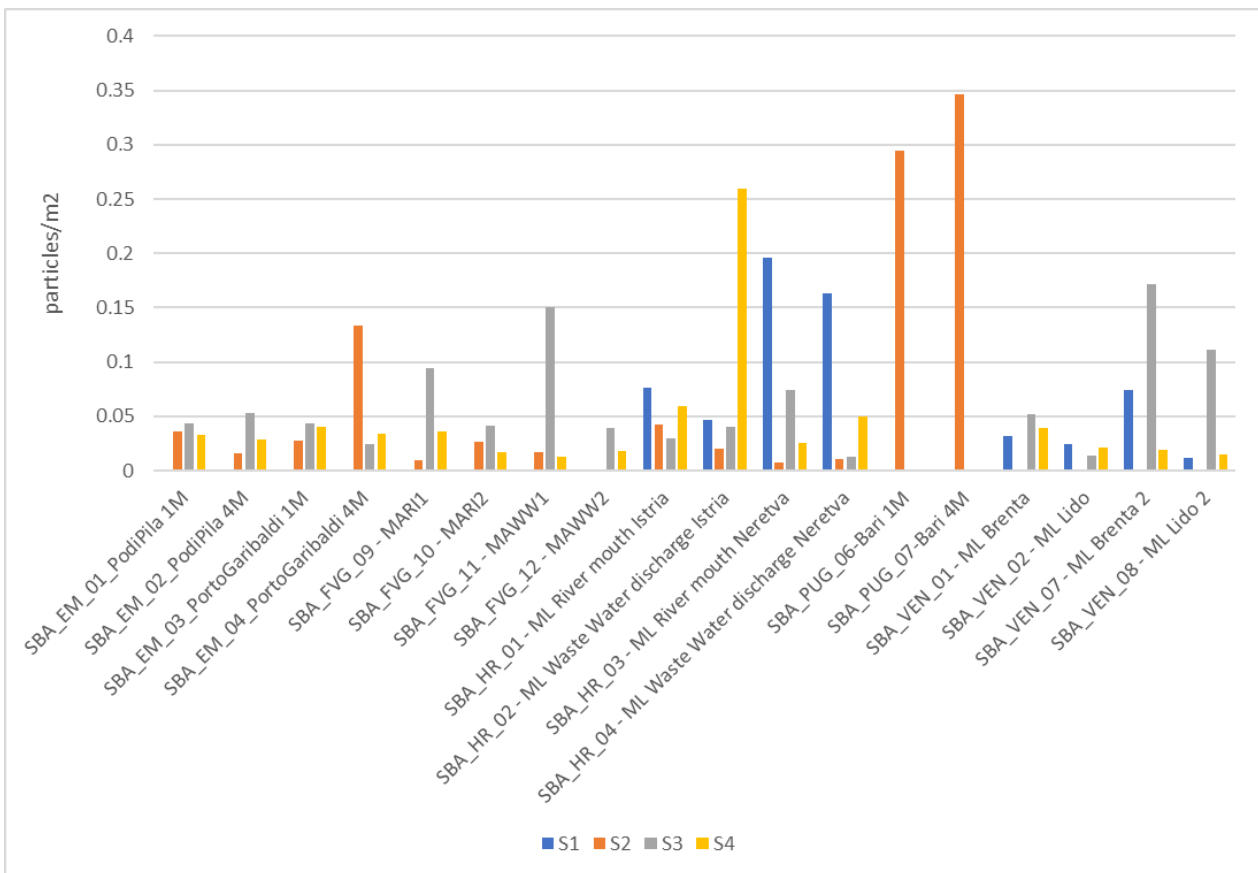


Figure 10 - Microlitter density on investigated sites during MARLESS project (S - season)

d. The MARLESS app to dig in the micro plastic dataset.

In the frame of the MARLESS project, the volume of data collected, and the detail of the information harvested cannot be fully explored according to a static approach, that is with a report, even if in digital format. So, an interactive tool has been developed and let available to the whole community by way of a World Wide Web service. To access the service, follow this link:

https://public.tableau.com/app/profile/interregithr.arpa/viz/InterregIT-HRMARLESSMicroplastiche/marless_microplastiche

The data are accessible according four main access points that aim to ease the exploration of the whole dataset. Entering each of those points, menus are available to select data collected in each sampling transect, according to campaign dates and type of micro plastic marine Litter (Figure 11).

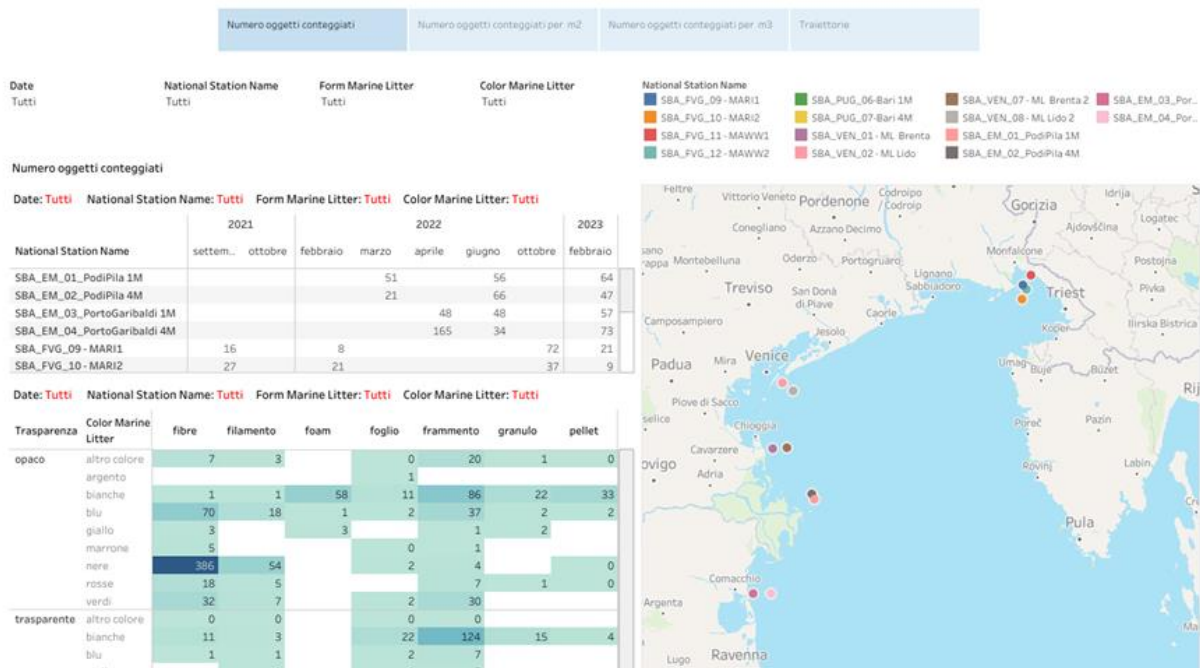


Figure 11 - Web interface of MARLESS database

A map is always available to geographically locate the focus of the analysis (Figure 12), the trajectories reporting the sampling transects are plotted and clicking on their position, on the map, tables and plots are presented for the specific sampling. Features of each trajectory are available too.

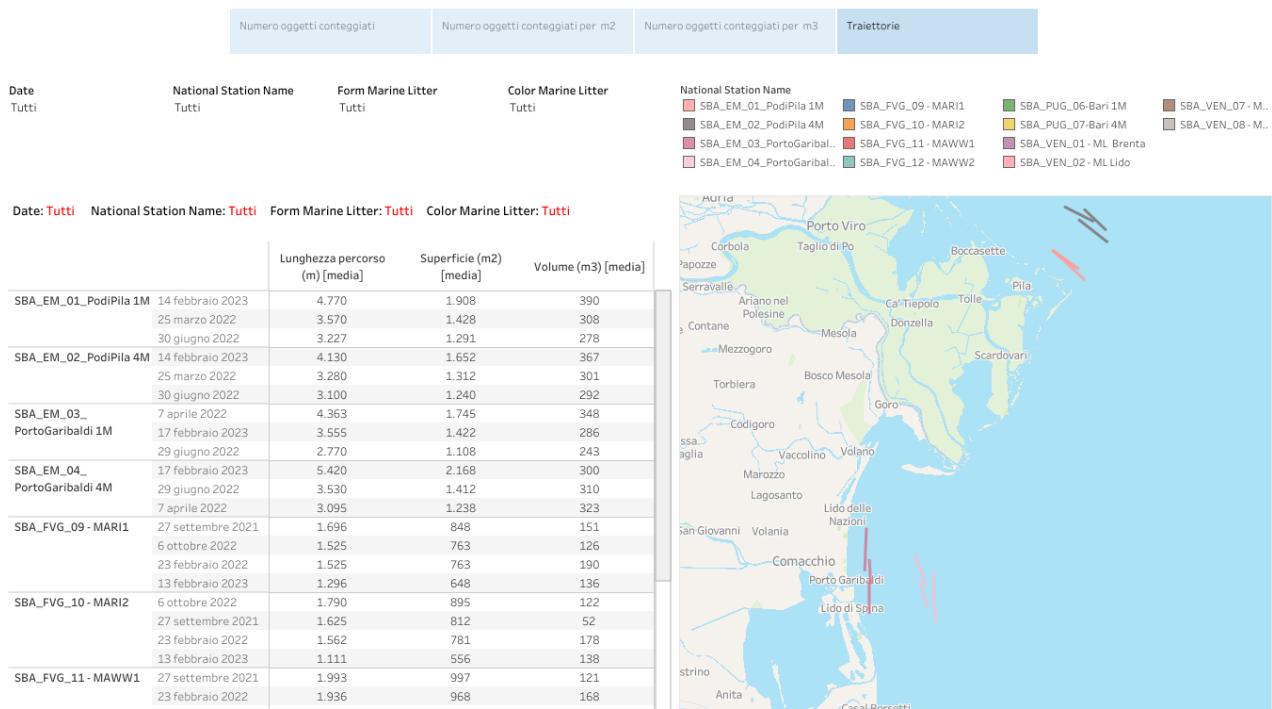


Figure 12 - Example of site map

All the results are presented automatically as sum of the items found in the campaigns, since the total number of waste material is considered the piece of information permeating the analyses. The count of the objects is presented when data are available. For all the campaigns the normalized information per unit of surface or volume is presented and the statistics of each of the micro plastic classes of items is explorable filtering the dataset (Figure 13).

Numero oggetti conteggiati	Numero oggetti conteggiati per m2	Numero oggetti conteggiati per m3	Traiettorie
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Date: Tutti National Station Name: Tutti Form Marine Litter: Tutti Color Marine Litter: Tutti

National Station Name
 SBA_FVG_09 - MARI1 SBA_PUG_06-Bari 1M SBA_VEN_07 - ML Brenta 2 SBA_EM_03_Por...
 SBA_FVG_10 - MARI2 SBA_PUG_07-Bari 4M SBA_VEN_08 - ML Lido 2 SBA_EM_04_Por...
 SBA_FVG_11 - MAWW1 SBA_VEN_01 - ML Brenta SBA_EM_01_PodiPila 1M
 SBA_FVG_12 - MAWW2 SBA_VEN_02 - ML Lido SBA_EM_02_PodiPila 4M

Numero oggetti conteggiati

Date: Tutti National Station Name: Tutti Form Marine Litter: Tutti Color Marine Litter: Tutti

National Station Name	2021		2022				2023	
	settem..	ottobre	febbraio	marzo	aprile	giugno	ottobre	febbraio
SBA_EM_01_PodiPila 1M				51		56		64
SBA_EM_02_PodiPila 4M				21		66		47
SBA_EM_03_PortoGaribaldi 1M					48	48		57
SBA_EM_04_PortoGaribaldi 4M					165	34		73
SBA_FVG_09 - MARI1	16		8				72	21
SBA_FVG_10 - MARI2	27		21				37	9

Date: Tutti National Station Name: Tutti Form Marine Litter: Tutti Color Marine Litter: Tutti

Trasparenza	Color Marine Litter	fibre							
		filamento	foam	foglio	frammento	granulo	pellet		
opaco	altro colore	7	3		0	20	1	0	
	argento				1				
	bianche	1	1	58	11	86	22	33	
	blu	70	18	1	2	37	2	2	
	giallo	3		3		1	2		
	marrone	5			0	1			
opaco	nere	386	54		2	4		0	
	rosse	18	5		7		1	0	
	verdi	32	7		2	30			
trasparente	altro colore	0	0		0	0			
	bianche	11	3		22	124	15	4	
	blu	1	1		2	7			

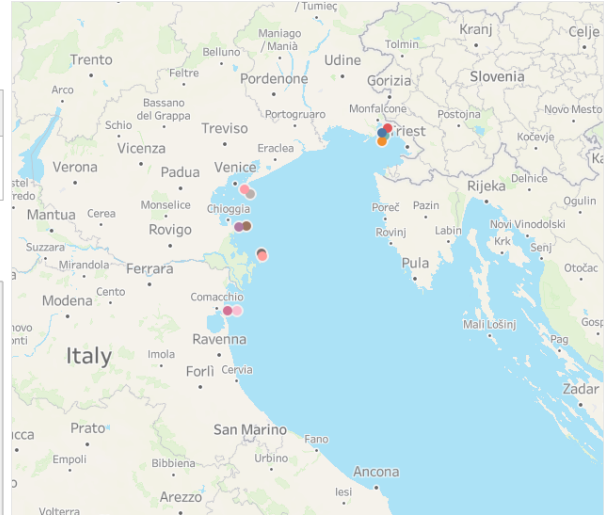


Figure 13 - Exemple of MARLESS dataset

5. Bottom litter

a. Study area

Selected sites had to meet the following criteria: uniform substrate, areas that accumulate litter, without the risk of exerting damage on endangered and protected species. The sampling is conducted in shell zones up to 20 m depth. The size of the sampling area was defined by each partner. Suggested sampling periods were autumn and spring. In shallow waters (< 20 m depth), underwater collection surveys with scuba diving/snorkeling were performed. The selected sites with different antropogenic pressure are shown in Figure 14

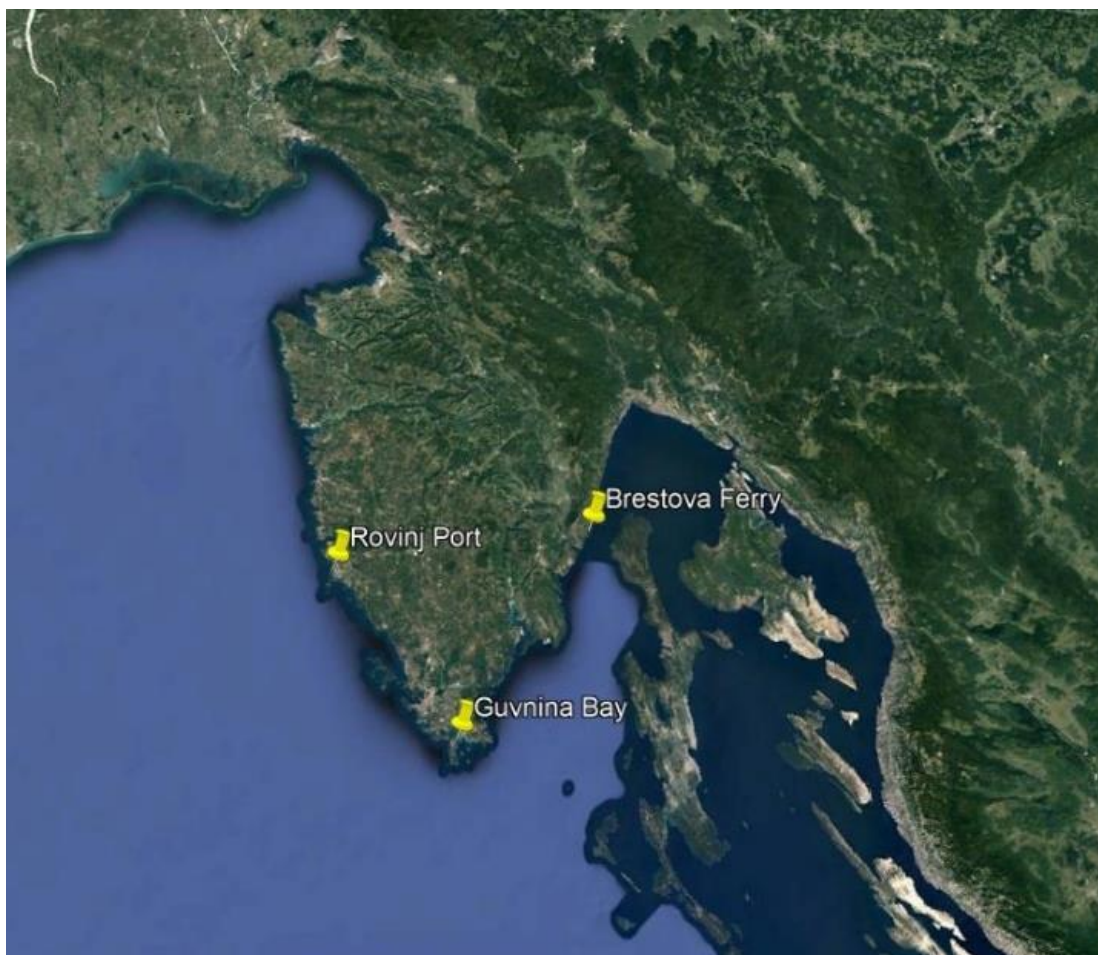


Figure 14- Geographical location of sampling sites in Northern Croatia

b. Methodology

All surveys performed followed the “Methodology for Monitoring Bottom Marine Litter (Macro-Debris >2.5 cm)” that was agreed within the framework of the MARLESS project. While conducting underwater surveys with SCUBA divers, lighter litter items were collected (while larger items were just marked), brought ashore and entered the ‘Bottom Litter Monitoring Sheet’.

c. Abundance and composition

In Northern Croatia, bottom litter monitoring via SCUBA diving was conducted in collaboration with local diving centers in Rovinj, Medulin and Rabac.

There were 3 sampling sites: Rovinj port (10000m²) (Figure 15), Guvnina bay (9100m²) (Figure 7) and Brestova ferry (4500m²) (Figure 16). All sites were sampled two times in total (mid-June-start of July and September during the year of 2022) (Figure 17).



Figure 15 - Sampling site-Rovinj port



Figure 16 - Sampling site Guvnina bay

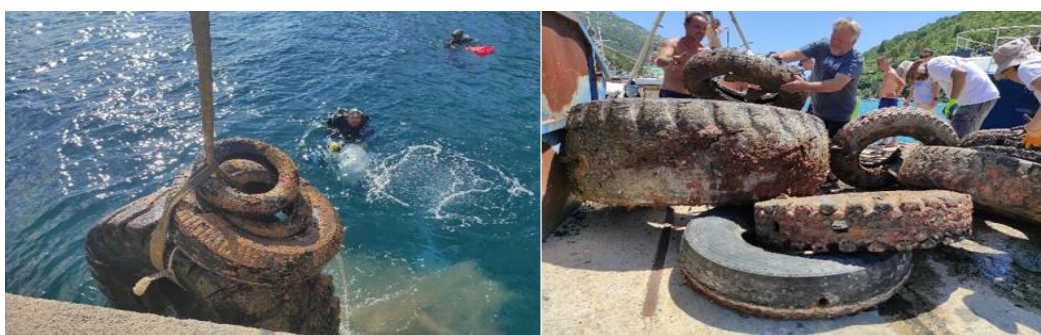


Figure 17 - Sampling site Brestova ferry port was full of truck tires

Each selected site represents an area that's under specific anthropogenic influence. Guvnina bay is the representation of the impact of tourism, Rovinj port represents the impact of naval influence coupled with tourism and Brestova ferry port, an area under the influence of boat traffic. As it is shown in Table 4, Brestova ferry port was the most abundant site while Rovinj port was the least abundant.

Table 4- Sampling sites in Northern Croatia with sampling dates, total number of items, total weight, sampling area and items per Km²

SAMPLING SITE	SEASON	TOTAL ITEM	TOTAL WEIGHT	SAMPLING AREA (m2)	ITEMS PER Km2
Guvnina bay	22.5.2022	977	> 530 kg	9100	9.3
	11.9.2022	713	> 285 kg		12.8
Rovinj port	4.6.2022	5534	> 1100 kg	10000	1.8
	3.9.2022	4038	> 500 kg		2.5
Brestova ferry port	26.6.2022	25	> 3000 kg	4500	180.0
	18.9.2022	35	> 5100 kg		128.6

Considering the composition of the samples, Brestova ferry port had the most unified samples that consisted mostly out of truck tires (Figure 17), plastic (23-29%) dominated the samples in Rovinj port (Table 5) while glass bottles dominated in Guvnina bay (30-48%) (Table 6). Rovinj port had the highest number of collected samples (5534, 4038), while Brestova ferry port had the lowest number of samples (25, 35).

Table 5- Rovinj port with the top five item categories sampled along with their percentage

ROVINJ PORT	No	Total item	CODE	General name	%		No	Total item	CODE	General name	%
04.06.2022.	1	1602	G79	Plastic pieces 2.5cm > < 50cm	29	03.09.2022.	1	935	G79	Plastic pieces 2.5cm > < 50cm	23
	2	733	G33	Cups and cup lids	14		2	770	G73	Cups and cup lids	19
	3	667	G200	Bottles incl. Pieces	12		3	304	G10	Food containers incl. fast food containers	8
	4	263	G175	Cans (beverage)	5		4	221	G175	Cans (beverage)	5
	5	260	G198	Other metal pieces < 50cm	5		5	216	G200	Bottles incl. Pieces	5

Table 6 - Guvnina bay with the top five item categories sampled along with their percentage.

GUVNINA BAY	No	Total item	CODE	General name	%		No	Total item	CODE	General name	%
22.05.2022.	1	470	G200	Bottles incl. Pieces	48	11.09.2022.	1	217	G200	Bottles incl. Pieces	
	2	58	G79	Plastic pieces 2.5cm > < 50cm	6		2	62	G79	Plastic pieces 2.5cm > < 50cm	
	3	45	G124	Other plastic/polystyrene items (identifiable)	5		3	54	G71	Shoes/sandals	
	4	43	G71	Shoes/sandals	4		4	42	G124	Other plastic/polystyrene items (identifiable)	
	5	39	G201	Jars, including pieces	4		5	33	G201	Jars, including pieces	

6. Microplastics in biota

a. Study area

Microplastic particles (MPs) smaller than 5 mm in size are one of the most widespread pollutant and an emerging threat to marine ecosystem ([Mercogliano et al., 2021](#)). Mussels have been widely used for biomonitoring studies in the marine environment due to their broad geographical distribution and easy accessibility ([Avio et al., 2017](#); [Pizzurro et al., 2022](#)). For this pilot action, mussels *Mytilus galloprovincialis* were sampled in 6 different stations located in the Adriatic Sea with a total of 176 analyzed mussels. MP were found in mussels from all investigated regions Figure 18.



Figure 18 - Investigated regions

b. Methodology

Collection of 30 *M. galloprovincialis* individuals 4-6 cm in length per site in 6 different regions and analyzed in the laboratories of the Marine Research Center, IRB in Rovinj, Croatia. In order to avoid contamination, the experiment was performed in an environment where the MP contamination was minimized. Instead of plastic dishes and tips, glassware was used and two blanks (dry and wet) were added to each batch of samples in order to get more accurate results. Mussels were freshly dissected and then stored at - 20°C or frozen and dissected after thawing right before the experiment. The dissected tissue was digested in 35% H₂O₂, and then heated at 60°C till the tissue digestion had completed. Saturated solution of NaCl was added to each sample and stirred for 2 minutes prior filtration. Surface layer of sample was collected with a pipette and filtered over a 0,2 µm pore size cellulose filter. Filters were then transferred into a glass petri dish and left in the desiccator to dry. Dry samples were examined under a stereomicroscope. MP fragments were categorized by color, shape and size.

c. Abundance and composition

MPs are found in all the sampled regions. Results showed that the average frequency of MP occurrence (%F) in mussels is 80% (Figure 19) with an average numerical abundance (%N) of 3 MP per mussel (Figure 20). Filaments are found to be the most prevalent group followed by plastic fragments while pellets were found only in one sample.

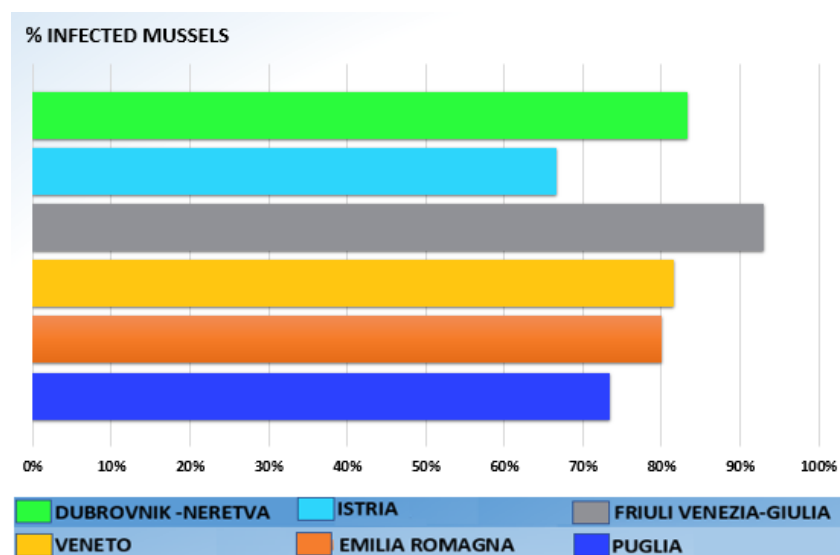


Figure 19 - Frequency of infected mussels per region

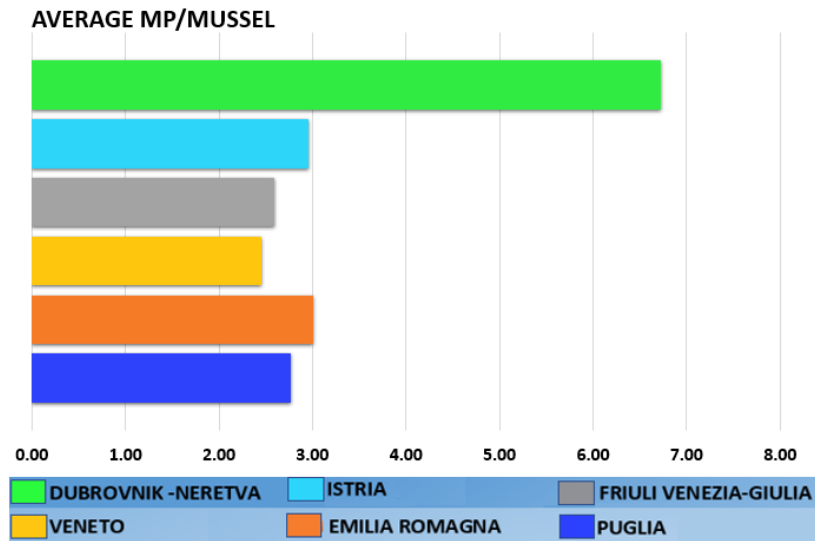


Figure 20 - Average of microplastic particles found per mussel.

MP were found in mussels from all investigated regions. Friuli Venezia Giulia region has the highest infected rate but second lowest average MP/mussel number which is negatively correlated to the average surface MP load of the region. On the other side, Dubrovnik Neretva region, with the second highest rate of infected mussels has the highest average of MP/mussel but is negatively correlated with the average surface MP load (Figure 21).

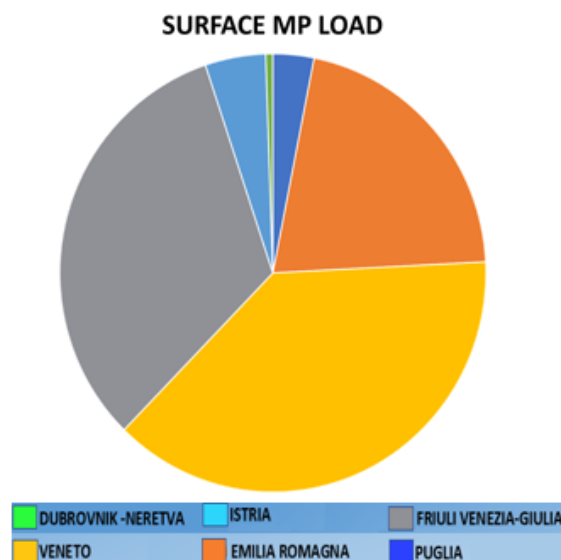


Figure 21 - Average surface microplastic particles load per region (particles per km²) source: LITTERBASE/AWI

LITERATURE

- Avio, C.G., Gorbi, S., Berlino, M., Regoli, F., 2017. Presence, Distribution, and Characterization of Microplastics in Commercial Organisms From Adriatic Sea, in: Fate and Impact of Microplastics in Marine Ecosystems. pp. 102–103.
- Commission, O., 2010. Guideline for monitoring marine litter on the beaches in the OSPAR maritime area.
- Galgani, F., Hanke, G., Werner, S., Oosterbaan, L., Nilsson, P., Fleet, D., Kinsey, S., Thompson, R., Van-Franeker, J., Vlachogianni, T., Scoullou, M., Veiga, J., Palatinus, A., Mattidi, M., Maes, T., Korpinen, S., Budziak, A., Leslie, H., Gago, J., Liebezeit, G., 2013. Monitoring guidance for marine litter in European seas. MSFD GES Technical Subgroup on Marine Litter (TSG-ML). DRAFT REPORT, 120p.
- Lippiatt, S., Opfer, S., Arthur, C., 2013. Marine debris monitoring and assessment: recommendations for monitoring debris trends in the marine environment. repository.library.noaa.gov.
- Litter, M.T.S., 2013. Guidance on monitoring of marine litter in European seas. European Commission . <https://doi.org/10.2788/99475>
- Mercogliano, R., Santonicola, S., Raimo, G., Gasperi, M., Colavita, G., 2021. Extraction and identification of microplastics from mussels: Method development and preliminary results. Ital J Food Saf 10, 9264.
- Pizzurro, F., Recchi, S., Nerone, E., Salini, R., Barile, N.B., 2022. Accumulation Evaluation of Potential Microplastic Particles in *Mytilus galloprovincialis* from the Goro Sacca (Adriatic Sea, Italy). Microplastics. <https://doi.org/10.3390/microplastics1020022>
- Vlachogianni, T., Zeri, C., Ronchi, F., Fortibuoni, T., Anastasopoulou, A., 2016. Marine Litter Assessment in the Adriatic and Ionian Seas. The IPA-Adriatic DeFishGear Project.