NET4mPLASTIC PROJECT

WP4 - Activity 4.1 Monitoring plastic and microplastic wastes on coastal and marine environments

D 4.1.1

Sediments samplings, river and sea water samplings and biota sampling from the 4 macro-areas

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1 Introduction

Main objectives of the Work Package n.4 of the NET4mPLASTIC Project are to perform demonstration activities related to testing and improving the methodologies of characterization of micro plastics in coastal areas of the INTERREG Italy Croatia program. The activities carried out include the critical analysis of the available methodologies, identification of strengths and weaknesses and integration of the procedures with the introduction for the activities in the field of new instrumental facilities (drones, sensors) and digital systems for computerization and data management (adapting already existing methods or developing new innovative technologies). Activity 4.1 deals with the monitoring of plastic and microplastic waste in coastal (beach, lagoon, river mouth) and marine environments, in order to obtain physical, environmental data relating to their composition and distribution for the four pilot macro areas of the project (Po Delta, Pescara, Rijeka and Split).

To verify the distribution of macro and micro plastics in the different environments and evaluate the possible impacts of extreme meteorological events (storm surges, travelling downbursts, meteo-tsunami and / or floods), for the Activity 4.1 in situ investigations have been carried out twice a year and before and after extreme events. Several PPs (UNIFE, IZSAM, TIPH, RERA, UNIST-FGAG) contributed to these activities, carried out with a multidisciplinary approach. These activities produced a detailed physical characterization of the pilot sites and a collection of a large pool of samples, useful to map the distribution of the plastic and assess the extent of contamination.

In each macro-area, several sites were selected for carrying out surveys campaign in order to verify the following impacts within the distribution of marine litter and microplastics:

1. Po delta: in barrier islands, coastal lagoons (in dunes that surround them, on the shores of the lagoon affected by changes in tide level and in the aquatic folds), in the bars sediment, in the mouth banks and on the seabed. Four sites: Rosolina, Boccasette (Porto Tolle), Goro and Volano, both on the land, on the sea surface and close to mussel farms. The selected sites are representative of the various geomorphological environments of the Po Delta, an area of community interest for the protection of biodiversity. The project activities in this area, therefore, allow evaluating the risk factors connected to the relationship between sedimentation and erosion in a vulnerable environment, that receives a huge volumes of waste from the most important Italian river, whose banks are highly populated;
2. Pescara river coastal area: three test sites from Pineto Municipality (Teramo) to Pescara Municipality: “Torre del Cerrano” (Pineto) Site of Community Interest SCI IT7120215, Montesilvano, and Pescara-South beach (up to 5 km south from the mouth of the Pescara river), both on the land, on the seawater surface and close to mussel farms. For this project three sites have been chosen in a morphologically uniform environment, to verify the anthropic impact and to assess how much the establishment of a marine protected area can reduce the impact of exposure to microplastics;

3. Rijeka area in three sites: Meline Beach, Klimno, north-eastern coast of Krk Island (toward Vinodolski Channel, Rajska Plaža Beach (Island of Lopar), Island of Susak beach, both on the land, on the sea surface and close to mussel farms. These areas represent an interesting environment in limestone rocky coast whose chemical, physical micro- and macro-topographic features of rocky bottom habitat may strongly affect the distribution and the abundance of microplastic. Also the species are diversified in relation to seabed morphology on coast areas, so the related biodiversity level can change the probability of plastic ingest by marine biota;
4. Split area: located in the center of the central Dalmatian county, and home to more than 8/10 of its population, is located on the Marjana peninsula, between the Kaštela bay and the Split canals to the west at the mouth of Žrnovnica to the east. The central microregion of Split consists of the island of Šolta, whose coast is bordered by the limestone ridges of Opura, Kozjak, Mosor, Perun and Zagora. Along the coast there are narrow alluvial plains, sejute to leave marly in the slopes topped by hills have numerous water springs.

This document, therefore, will present all the sampling campaigns realized during the time frame of the project (2019-2022), describing also the different sampling and analysis methodologies used for the sediment, sea water and biota samples (except for the physical and geomorphological data and drone images that will be explained in the D.4.1.2 - Physical data and drone images).

Although it will be explained in more detail in the following paragraphs, it should be noted that not all types of data acquisition (and related methodologies described here) have been applied in all areas of interest, and sampling was not carried out simultaneously.

All the collected data has been imported in Excel dataset, that was then shared with partners (Hydra Solution and Prosoft) for the uploading on NET4mPLASTIC's database and related web platform (www.net4mplastic.net).

2 Study areas

The NET4mPLASTIC Project, as a cross-border international collaboration project between two of the countries facing the Adriatic Sea (Italy and Croatia), considering also the Partners involved in the Project and the differences in investigating area, choose as pilot sites 2 Italian sites (Delta Po and Pescara area) and 2 Croatian sites (Rijeka and Split areas).

2.1 Test site 1: Po Delta (IT)

The interested zone covers the coastal and maritime area between the Adige River mouth (belonging to the Venetian region) and Lido di Volano (beach and sea area in the Municipality of Comacchio, Ferrara Emilia Romagna Region).

The Po Delta is located on the northwest coast of the Adriatic Sea, and covers a surface of 610 Km^2, with a 64 km of coast. The current conformation of the Po delta is the result of the transformations suffered by this area over the millennia and depends on the different forces that insist on this territory. The succession and the overlapping of complex environmental events to which, especially in recent centuries, added the human activity (Simeoni and Bondesan, 1997; Simeoni and Corbau, 2009) characterize the evolution of Po Delta. The human requirements have resulted in the stabilization of the course of the Po, through the creation of artificial banks designed to keep the rivers within their beds and to simplify the hydrographic network, making the course of the river as straight as possible. The asymmetry of the lobes of the modern Delta depends on the loss of hydraulic efficiency of the northern river branches in comparison to the three southern branches. This condition started with the "cut of Porto Viro" or "Taglio novo", realized by the Republic of Venice at the beginning of the 17th century (Bondesan and Simeoni, 1983). This hydrographic change led to a very rapid development of the southern lobe of the Delta at the expense of the detriment of the northern one, less and less fed by the sediment contributions of the northern Po branches.

Over the past 100 years, a dynamic balance has been maintained between natural processes (full of rivers, tides, subsidence) and anthropic interventions, particularly accelerated with the large land reclamation work started in 1870 (embankments, drainage, infrastructure, water collection methane supply).

Nowadays the area of the Po Delta, which includes saltmarsh lands, lagoon areas and fishing farms in addition to the strictly marine coastal area, is in fact affected by several phenomena, including subsidence, water advancing with consequent variation of the coastline, flooding, high and low tide phases differentiated according to latitude and current action.

The Po Delta, is divided into 5 main branches (Po di Maistra, Po della Pila, Po delle Tolle, Po di Gnocca or Donzella and Po di Goro) corresponds to one of the areas with greater risk of flooding, especially in adverse weather conditions, such as the presence of strong depressions from the Atlantic (Bondesan et al., 1995) or prolonged strong Sirocco winds (from SE) that prevent rivers from pouring their waters

into the Adriatic Sea. Such occasional flood events can affect the naturalistic and dynamic aspect (geomorphological, biological and ecological).

The coasts bordering the Po Delta area are characterized by sandy beaches with low slopes, delimited towards land by dune systems. The Po Delta lagoons are separated from the sea by narrow tongues of sand (*sandspit-frecce litorali*) and by long and narrow barrier islands. In this specific area the coastal arrows are called "Scanni" while the barrier islands can be identified as "Lidi". In addition, some lagoons in the Delta are named "Sacche". The “Scanni” are very unstable and constantly evolving environments, since they are affected both by constructive phenomena linked to the contribution of sediments brought by rivers and coastal drift, and by destructive phenomena such as marine and wind erosion. This instability leads to the development of a vegetation that often does not take on a well-defined appearance and comparable with the classic dune series of psammofila vegetation (plants adapted to live in sandy environments). Being semi-enclosed coastal environments, the lagoons have a reduced hydrodynamism, which brings to a consequent high rate of sedimentation, accumulation of materials of various nature and increase in suspended particulate material.

The whole area is characterized by important altimetric variations, with the highest altitudes near the sand dunes (15 m s.l.m.) and the lower altitude close to the lagoon areas, especially in the western part, once submerged and now recovered from the reclamation, with values up to 2 m s.l.m. Currently the whole basin of the Po Delta is located at a lower altitude than the sea level and is kept dry thanks to the constant activity of water pumps (Province of Rovigo, 2008).

The main lagoons of the Po Delta are 8: four on the northern side (Caleri, Vallona, Barbamarco and Burcio lagoons) and four in the southern side (Basson, Canarin, Scardovari and Goro lagoons). The average depth of the lagoons is 50-60 cm (with a maximum of 150-200 cm).

The areas of the Po Delta, which fall within two Regional Parks (Parco regionale del Delta del Po dell'Emilia-Romagna and Parco regionale Veneto del Delta del Po), in addition to being areas of environmental and tourist interest, have a significant economic interest: productive activities linked to shellfish and fish farming, but also to agriculture and hunting, are very developed. The agricultural production is mainly focused on cereals such as corn and rice. The Po Delta area includes several Natura 2000 sites, among which we can mention “Sacca di Goro”, “Bosco di Volano”, “Delta del Po”, “Po di Goro”, including both Bird Directive Sites (SPA) and Habitat Directive Sites (pSCI, SCI, SAC).

The Po Delta test sites includes for this Project several locations, that we briefly describe in the next paragraphs with their main characteristics from North to South (*Figure 1*).

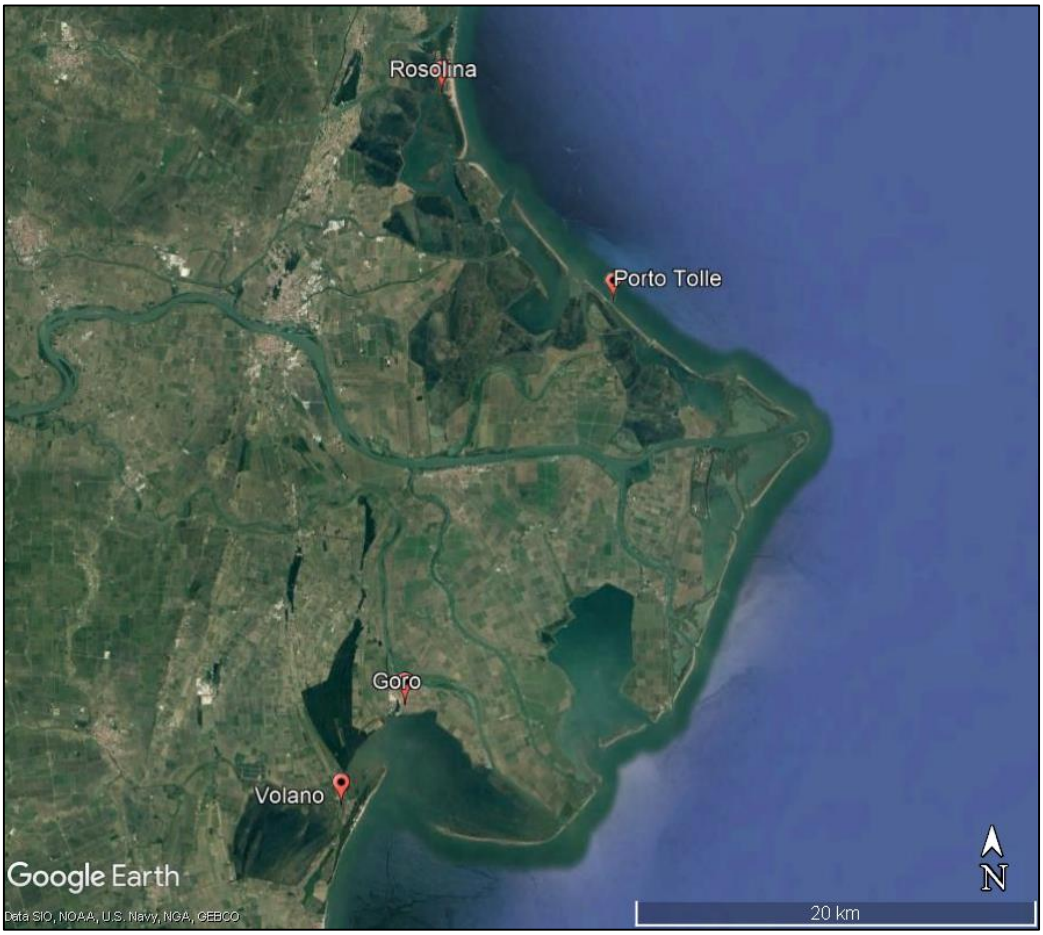


Figure 1:Test site 1 - Po Delta

2.1.1 Rosolina Mare (Rosolina - Rovigo, Italy)

The Adige River mouth borders the northernmost part of Rosolina Mare. River activity and solid transport in the river itself have contributed in the past and still contribute to the dynamics of the coastline itself. The mouth changed its appearance over the years. In the past the delta system of this river crossed its path with Po ones, and only after the Cucca break (589 AD) the mouth stabilized in its current position. The dismantling of the body also characterizes the current state, and this is certainly caused by the drastic decrease, compared to the past, of the sedimentary contributions. The current reduction of solid transport depends on the construction of anthropogenic devices, such as dams, embankments for the protection of adjacent settlements and excavations in the riverbed for construction and industrial purposes (ARPAV, 2012).

Rosolina Mare is a coastal strip of low and sandy beach, consisting of an articulated system of beach-dune. The whole area can be outlined by dividing the coast into three coastal areas, proceeding from north to south: the first section extends for about 2 km, the second section for another 3 km and

finally the southernmost section of 3 km. To the north the beach is practically absent, except for some parts where at most it reaches a width of 5-10 meters. The dunes, which persisted until last few years, are currently completely absent. This situation occurred as a result of a drastic decrease in the solid supply by the Adige river that in the past fed them, and the reinforcement of the mouth that has conveyed the sediments offshore, thus depriving the coastal area of its natural sedimentary supply. To counteract this phenomenon, permanent structures to protect the shore have been put in place, such as mass groynes with head, wooden groynes, structures adhering to other seasonal interventions, such as sandbags.

Currently, there is a constant retreat of the shoreline of the norther section (from a beach width of 60 m in 1955 to 10 m, or even absent), which highlights an erosion still ongoing; otherwise, the inner limit of the beach remained roughly stable, since it is bound by the presence of a campsite (Tiengo, 2012).

The central section, 3 km long, presents a distinctly different environment; here the beach is much wider with an extension that reaches in some points the 200 meters. The area, however, is completely man-made for tourist purposes, in fact in the summer the beach is flattened losing. In winter, thanks to the end of bathing activities, the beach returns to a more natural appearance, where, however, the obstacles to wind transport are made up does not give vegetation, but from windbreak nets and other constructions that during the summer are used for bathing services. The area is characterized by the presence of dunes, even of considerable height (7-10 m), but discontinuous as interrupted by manmade passages that hinder its natural defensive function. Also in this stretch, as well as the northern one, there are erosive phenomena, and in fact some defensive interventions have been proposed, such as filtering/degrading groynes in the area in front of the town of Rosolina Mare (*Figure 2*).

In the southernmost section, there is an area of considerable natural and environmental value. The coastal area stretches for about 3 km to the extreme southern tip of the peninsula, that is, it is a coastal spit. In this area the dominant morphological processes are marine-lagoon and river linked to the rivers Po and Adige. There is also a clear trend towards growth, which is confirmed by an accumulation offshore of about 10,000 m3/year of sediment with active dynamics. It is therefore a coastal stretch that, unlike those previously described, has preserved almost entirely the natural characteristics typical of these environments.

Figure 2: Rosolina (Po Delta site).

2.1.2 Boccasette (Porto Tolle – Rovigo, Italy)

Porto Tolle is included between the branches of Po di Maistra and Po di Gnocca and is crossed by the Po of Venice. These branches create in fact three islands: the island of Cà Venier, the island of Donzella and the island of Polesine Camerini. The territory of Porto Tolle is unique and is characterized by saltmarshes, "barene" (sandbanks), creeks, "scanni", islands with sandy beaches not yet inhabited, abandoned branches of the river, floodplains and lagoons.

The spit that divides the lagoon of Barbamarco from the northern Adriatic Sea is composed of a discontinuous and dynamic line of three bays, called Boccasette (or Palo), Gallo Nord and Gallo Sud (or Galletto), separated by two sea-lagoon connecting mouths. The current configuration of the lagoon of Barbamarco is the result of the recent evolution of the coast of the northern lobe of the Po Delta, which occurred from the second half of the last century.

Currently, the coastal dynamics is characterized by a longitudinal transport of sediment from South-East to North-West, parallel to the coast, fed by the shoreline and the consistent contribution of the Busa di Tramontana (Ruol and Pinato, 2016). The Po di Maistra carries a smaller amount of sediment

than the Busa di Tramontana. The 3 estuary bars that close the lagoon reflect these characteristics, and are thicker, taller and more massive to the South, wider and more elongated to the North (*Figure 3*). However, the coastal dynamics collide with the river one, with evident alternations in the morphological forms created by the reciprocal interaction: the growth of the spit from the South tends to diverts the outflow from the river to the North, which thus tends to channel below the coast and determine erosive events even consistent along the floor near the mouth.

The texture distribution of samples of the coastal area consists of medium or fine sand (0.5 mm to 0.125 mm). According to the classification of Shepard (1954), based on the relationships between the fractions of sand, silt and clay, instead, the samples of the seabed fall in the area of the sands. These data are consistent with what one would expect to find from the sediments of dune, emerged beach, submerged beach and submerged bar.

Figure 3: Boccasette (Po Delta site).

2.1.3 Goro (Ferrara, Italy)

The Sacca di Goro is one of the lagoons of the Po Delta. The Sacca was created by the progressive accretion of the Scanno, the sandspit that originates at the Po di Goro mouth and extends for more than 8 km westwards. The exchanges with the sea are guaranteed by two tidal vents separated by a barrier island, now submerged. The main vent is located to the west, while the secondary vent to the east of the barrier island (*Figure 4*).

The natural evolution of the Sacca tends to its progressive closure at sea due to the extension of the Scanno that causes, over time, an increasing reduction in the water exchange between internal and external waters, with the consequent reduction in the capacity of water recirculation, nutrients, oxygen, etc. In warm periods this causes a huge environmental damage to the Sacca for the establishment of eutrophic water conditions, with serious repercussions on the economy of the area linked to the decrease in shellfish production, the primary economic source of the area.

The Sacca di Goro, formed in the nineteenth century, is the southernmost lagoon connected to the apparatus of the Po delta. It extends for about 2600 ha, on an average depth of 1.5 m, and contains a volume of water of about 39,465,000 m^3.

In the Sacca di Goro the fresh waters coming from the Po di Volano flow to the west, from the Canal Bianco, and Po di Goro to the east, while to the south, through the year, converge the salt waters for the hydrodynamic lagoon-sea exchanges. Based on the chemical-physical, biological and hydrodynamic characteristics, the lagoon is conventionally divided into three zones: western (influenced by the Po di Volano with rapid fluctuations of salinity both in space and time); central (influenced by continuous and wide exchanges with the open sea that cause a marine salinity, morphologically flat and with mainly sandy sediment that helps the clams farming); eastern (area is bordered and influenced by the Po di Goro, which can be identified with the Gorino valley, characterized by fresh water and poor hydrodynamism).

The Sacca di Goro is an environment of transition from inland waters (fresh) to external waters (salt) and is subjected to the phenomenon of subsidence. The shallow seabed (on average 1.5 m) and the limited exchange from the vents cause a strong environmental risk mainly linked to the macroalgae bloom and the corresponding risk of water anoxia. The quality of the water in the lagoon is guaranteed by a network of sublagunar canals that helps the interchange with the sea and the increase of the internal circulation, and by various hydraulic constructions that control the intake of fresh water.

In the Goro area from the 50s there is a reduction in the amount of materials brought by the Po di Goro due to the intense collection of aggregates in the riverbed; this phenomenon, together with an increase in subsidence for the extraction of gas and water, determined the cessation of the process of growth of the mouth apparatus, which thus entered into crisis. Although in small quantities, the materials brought by the river and those coming from the erosion of the beaches in the left of the mouth, continued to be transferred westward by the wave motion. Currently, while the coast close to the Po delta are subjected to erosion, the rest of the Scanno, even if thinning, increases in length and wideness developing

a platform of swash, with a narrower shape close to the mouth and wider at the opposite end, near the tidal mouth (Meistrell, 1966).

The coastal drift of the Goro area, determined by the exposure to winds, occurs mainly along the east-west direction, taking charge of the sediments coming from the southern branches of the Po and mainly from the Po di Goro.

Therefore, in recent years there has been a continuous growth of the Scanno towards west, at the expense of a secondary vent opening that gradually reduced and moved westward, driven by the growth process of the spit. At the same time the barrier island has gone into erosion for several reasons: because it is no longer fed, due to subsidence; due to the erosive action of the sea and the interception of sediments in transit east-west by the head of the Scanno.

Figure 4:Goro (Po Delta site).

2.1.4 Volano (Comacchio – Ferrara, Italy)

From the Goro lagoon the Delta Po area leads toward south to the last zone covered by this study, that includes a coastal area which extends from the mouth of the Po di Volano to the so-called Lidi Ferraresi (till Porto Garibaldi). This coastal area, about 15 km long, is characterized by a direct coastal transport from the south to the north, whose greatest intake is given by the sands coming from the Reno river. In the section of the Lido of Volano the contribution is also given by the aforementioned Scanno of Goro (fed by the Po river). All the above mentioned contributions are however modest because of the presence of two main artificial structures: the jetty of Porto Garibaldi and the submarine channel of access to the Port of Goro. In general, it can be noted that the area of the beach of Volano results in erosion (retreat of the coastline). Along with the beach, the Lido di Volano presents a vast pine forest with a surface area of 169 ha, which is part of the state nature reserve named Po di Volano (*Figure 5*).

Figure 5: Volano (Po Delta site).

2.2 Test site 2: Pescara area (IT)

The area of interest was characterized from a geological point of view previously through the Geological map of Italy at the scale 1: 50.000, created within the CARG project (F. 351. Pescara – ISBN: 9788893110464) (Ori et al., 2016); subsequently, in 2019, the area of interest was characterized by the Coastal Defense Plan, for what concern erosion, effects of climate change and pollution in a map drafted by the Abruzzo Region, Department of Infrastructure, Transport, Mobility, Networks and Logistics of Maritime Works and Marine Water Service. This planning of coastal strip management interventions characterized coastal environments by analysing their quality status, including the impacts of climate change and anthropogenic pressure, but did not address the impact of marine litter, so the assessment activities of the diffusion of microplastics realized for the Net4mPLASTIC project can be included in the update of integrated management plan of the Abruzzo coast. The Abruzzo coast develops for a length of about 130 km. It is rather heterogeneous, with stretches characterized by a low and sandy coast and stretches characterized by a high and rocky coast, which is substantially inherent. along the Abruzzo coast, the Abruzzo Region in the plan has identified the following physiographic sub-units, whose boundaries are shown in the figure (*Figure 6*).

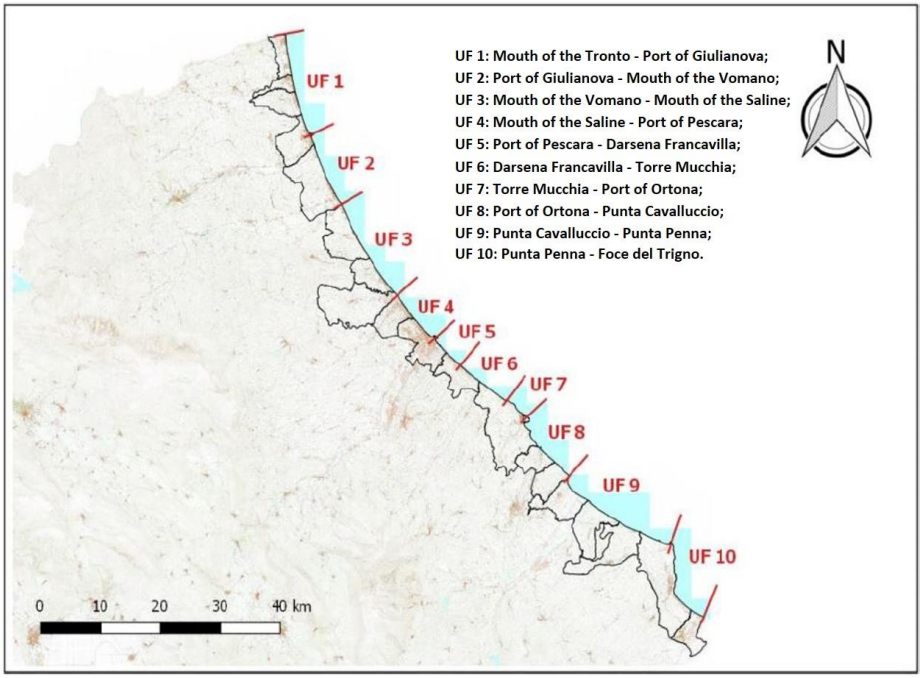


Figure 6: Longitudinal limits of anthropic physiographic units (UF). From PORTODIMARE geoPORtal of TOols & Data for sustaInable Management of coAstal and maRine Environment (ADRION205) Report, 2021.

Given that the analysis of microplastics in high-coast, mainly rocky environments, is undertaken in the Croatian area, three areas with low sandy coast have been selected for the Net4mPLASTIC project in the Pescara area. The Region has carried out the analysis of the waves, which clearly indicates an

Abruzzo coast subject to a bimodal climate with two main sectors of origin of the waves. The first one is characterized by waves generated by winds coming from the north, the bora coming from the Balkan region gives the maximum values of the geographical "fetch". The second one, characterized by the waves generated by the east winds, also includes the waves generated by the winds that blow from the SE. The SE winds in Abruzzo, due to the geographical screen operated by the Gargano, give rise to marine states coming mainly from the east on the coast. The study of wind trends is fundamental in the design of coastal erosion defences works and to controls the distribution of sediments but also of plastics.

The coastal strip in three sites: a) Site of community Interest IT7120215 “Torre del Cerrano” (district of Teramo) selected for its low anthropic impact and at about 9 km from the mouth of the Vomano river. The Torre del Cerrano Marine Protected Area, which identifies the protected coastal strip, as well as the external protection area, was established in Torre Cerrano on the border between Pineto and Silvi; b) the beach in Montesilvano Municipality (district of Pescara), south of the mouth to river Saline (about 2 km), is located in an urbanized area with several summer residences. c) an area of intense urbanization of Francavilla al Mare beach (district of Pescara), about 5 km from the mouth of the Pescara river. The two sites in the district of Pescara are characterized by the presence of marine erosion defences works that influence the distribution of sediments and plastics along the coast.

The three Net4mPLASTIC survey test sites are described below and shown in *Figure 7*.

Figure 7: Test site 2 (Pescara area).

2.2.1 Torre del Cerrano (Pineto – Teramo, Italy)

The Marine Protected Area (MPA) Torre del Cerrano was candidate as Site of Community Interest (SCI) by Regional Council with the Resolution n. 738 of 27.09.2010. The Ministry of the Environment validated the proposal in September 2011. The European Union approval and recognition as SIC for the Marine Protected Area (MPA) Torre del Cerrano came with the C (2012) 8135 (2013/23/EU) and with European Communities Union L24/58 on 26 January 2013.

The area is located between Silvi and Pineto municipalities (province of Teramo). It includes the coastal strip of 7 km extending from the mouth of the Calvano stream in Pineto and Padre Pio Square in Silvi, while the sea area extends up to 3 miles from the coast with a total area of 37 km^2. The test site owes the name "Torre del Cerrano" to the toponym - it was built at the mouth of the Cerrano stream fed by sediments from the hills of Atri and flows into the marina of Silvi - and from the defensive tower built in 1568 by the Spaniards to defend the incursions of the Saracen pirates. The tower was built in the hilly stretch close to the coastal dunes, due to its location in an area exposed to erosion it was probably beset by collapses and landslides witnessed by the numerous blocks of artificial conglomerates (***Errore. L'origine riferimento non è stata trovata.***).

Vallarola et al. 2015 have collected various historical and cartographic evidence on the basis of which they have reconstructed the trend of the variation of the coastline and of the sedimentary contributions in historical times, demonstrating that the coastal strip has undergone a significant contraction of its extension in recent decades due to the reduction of solid river flows, following the construction or expansion of maritime works, destruction of river basins, urbanization and incorrect coastal management. In addition to natural erosion there is the effect of cleaning activities carried out also for tourist purposes, which produces the removal of considerable quantities of material, including lamellibranch shells and marine litter. This activity is carried out with mechanized vehicles operated on the beaches near the protected marine area and used for bathing activities. The protected area does not have the rigid structures inserted north and south, as coastal protection works, that have modified the natural nourishment processes of the beaches. Above all, by interrupting the transport of light solid material from North to South, this configuration produce, under the effect of the prevailing (from SE and NE), a progressive erosion.

The MPA also includes the related coastal territories of the maritime state property and it is delimited by the following points of Latitude and Longitude:

A1	42° 36’ 45” N; 14° 03’ 55” E (in coast)
B	42° 37’ 10” N; 14° 05’ 20” E
C	42° 37’ 30” N; 14° 18’ 15” E
D	42° 35’ 40” N 14° 09’ 50” E
E	42° 34’ 00” N 14° 07’ 55” E
F1	42° 33’ 35” N 14° 06’ 55” E (in coast)

Art. 4 of the Regulation governing the activities permitted in the MPA Torre del Cerrano (D.M. 28 July 2009, n° 218) shows the Delimitation of the Marine Protected Area and the planned zoning divided into three areas subject to different protection regimes: ZONE B, ZONE C, ZONE D (*Figure 8*).

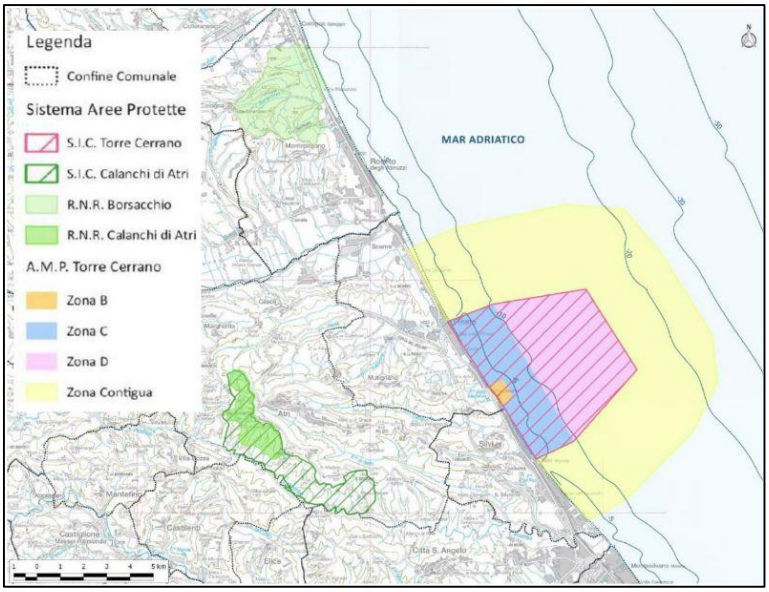


Figure 8: Extract from the thematic chart n.2 – Charts of protected areas system (Lombardi A., 2015) from Piano di Gestione SIC IT7120215 "Torre del Cerrano", Relazione non Tecnica, 2015.

In the MPA of Torre del Cerrano the Zone A of integral protection, which is usually present for environmental values and socio-economic needs of use, it was preferred not to include it.

The "Unauthorized activities", consistently with the provisions of art. 19, paragraph 3, of Law 394 of 6 December 1991 - Framework Law on Protected Areas, concern:

- any activity that could constitute danger or disturbance of plant and animal species, including bathing, scuba diving, navigation, anchoring, mooring, the use of jet skis or jet skis and similar means, practice of water skiing and similar water sports, underwater fishing, introduction of native species and active restocking;
- any activity involving the capture, collection and damage of specimens of animal and plant species, including hunting and fishing;
- any removal, even partial, and damage to archaeological finds and geological formations;
- any alteration, direct or indirect, caused by any means, of the geophysical environment and of the biochemical characteristics of the water, including the introduction of any toxic or polluting substance, the landfill of solid or liquid waste, aquaculture, '' introduction of discharges not in compliance with the most restrictive prescriptions provided for by the law in force
- introduction of weapons, explosives and any destructive or capture means, as well as toxic or polluting substances;
- the use of open fires.

Article 6 of the D.M. 21 October 2009 - "Regulations governing the activities permitted in the various areas" - allows for a protection strategy mediated from the most rigid, in Zone B, to the most tenuous, in Zone D.

2.2.2 Montesilvano beach (Montesilvano – Pescara, Italy)

The coastal area chosen as the test site is a beach bordered by the built-up area, which is part of the municipality of Montesilvano (*Figure 7*). From a geomorphological and hydrogeological point of view, this area can be divided into the following macro-areas:

1. Rocky substrate, consisting of sediments connected to the deep marine environment, modelled by exogenous agents, following the emergence of regressive series. They consist mainly of sandy deposits with medium-fine grain size, with, in places, fine silty inclusions and scattered gravels, consisting of heterometric calcareous pebbles from rounded to sub-rounded, generally flattened.
2. Sediments linked to fluvial and alluvial fan deposits, deposited in depressions which develop perpendicularly to the coast. The valley floor area of the Saline River is characterized by alluvial deposits of the Saline River, mainly gravelly-sandy, which have thicknesses that can reach a depth of about 25-35 m in the most distal portions with respect to the Saline River (alluvial terrace). The ends of the gravelly deposit are covered predominantly by silty-sandy soils with an average thickness of about 6 m.
3. Area in which dunes and coastal paleo-dunes sediments predominate, linked to the bathing environment and its evolution that develops with a dirt strip parallel to the coast above the rocky sedimentary substrate and not interrupted by the incisions of the river mouths.
4. Beach deposits continually emerge along the entire coastline and from the coastline including beach, dunes and paleo-dunes, with a transversal width ranging from a few tens of meters to over 1 km. They are affected by intense anthropization, which has spared the current beach with a variable band between a few tens of meters to 100 m. The deposits consist of medium-fine, loose or thickened sands, and locally of gravels with heterometric rounded to sub-rounded pebbles, generally flattened. Gravel deposits prevail near the mouth of the Saline River. The thickness of the beach deposits reaches a maximum of 10 m, passing in depth to the silty clayey geological substratum. The stretch of coast is defended by three series of detached barriers, emerging from natural boulders, built starting from the 1960s. The coastal defences barriers were built in the years 1982-1983, 1988 and 1992-1993 to prevent erosion.

The Montesilvano mussel farm is identified with the code 015CH230 G.R. Abruzzo Region n. 807 of 05.12.2014 and it consist of rows, with an area of approx. 2,000,000 (two million) delimited by the following coordinates: Vertex A -42 ° 31'60''N - 14 ° 11'75'E; Summit B - 42 ° 31'90''N - 14 ° 12'35'E; Summit C - 42 ° 31'00''N - 14 ° 13'25'E; Summit D - 42 ° 30'65''N - 14 ° 12'65'E. Center point Z 42 ° 31'25''N - 14 ° 12'50'E. In this mussel farm - "ATLANTIDE DI ENNIO DI GIOVANNI S.A.S." - samples of "*Mytilus galloprovincialis*" were collected.

2.2.3 Beach of Pescara (Southern side of Pescara, Italy)

The stretch of coast south of the tourist port of Pescara, which includes also the coastal strip of the municipality of Francavilla al Mare, is characterized by the presence of barriers emerging from natural boulders, built in the years 1960-1965, 1982-1983, 1988 and 1992-1993. Most detached barriers have an oblique orientation (north-north-west), with respect to the average course of the coastline, and almost parallel to the more intense and more frequent wave motion direction. Defences works are also present I order to stabilize the coast (*Figure 7*). The deposition of sediments reduced the depth of the seabed in the areas located between the third defence structure and the beach.

2.3 Test site 3: Rijeka area (HR)

The third NET4mPLASTIC pilot site corresponds to the coastal and marine area surrounding the Municipality of Rijeka, in Primorje-Goranska Kotar County (*Figure 9*).

The total length of the coast of Primorje-Goranska Kotar County in Croatia is 1065 km, 133 km of land-sea coast and 932 km of islands-sea coast. The sea area of the County includes the Kvarner Bay, with the Gulf of Rijeka, Kvarner and Kvarnerić, and the islands of Krk, Cres, Rab, Lošinj, Susak, Unije and part of Pag, with the Vela vrata, Srednja vrata, Vinodol Channel and Rab Channel.

The sea area in the County corresponds to 434414 km^2, which makes 55% of the County area. The island series Cres - Lošinj and Krk - Rab - Pag divides the sea area into Kvarner, Kvarnerić, Rijeka Bay, Vinodol Channel and Velebit Channel and the open continental part of the Adriatic Sea. The sea area in this part of Adriatic is strongly influenced by meteorological, oceanographic and hydrological conditions, prevailing in the wider area. In addition, the directions and magnitude of waves and sea currents, but also seasonal and annual variations in temperature and salinity are significantly affected by the described separation of the waters by island series and relatively narrow passages between them.

In spring, a vortex circulation is established in the northern part of the Adriatic Sea, as a result of a stronger inflow of fresh water, which can result in a surface current passing in the direction of Kvarner - Rijeka Bay through the Vela Vrata pass. In such circulation regime, diluted waters are distributed throughout the sea area belonging to the Primorje-Gorski Kotar County.

In winter, in the Gulf of Rijeka, currents are directed from Kvarnerić and the Vinodol Channel towards the Gulf of Rijeka, and from there towards Kvarner and the open part of the Adriatic Sea. At the end of spring, the exchange of water masses between Kvarner, Kvarnerić and the Gulf of Rijeka is at a minimum, and in summer there is a flow in two layers, where the direction of bottom currents is cyclonic and surface currents are anticyclonic.

The impact of freshwater inflow in the Kvarner waters is very significant. This is especially true for the Gulf of Rijeka and the Vinodol-Velebit Channel. There are numerous springs in the coastal area. The two main rivers in this area are Rječina and Dubračina.

The coastal area is densely populated and therefore could be considered endangered in many ways. On the coastal part of this area, near the city of Rijeka, the main source of pollution is transport and industry, while on the islands the main source is tourism.

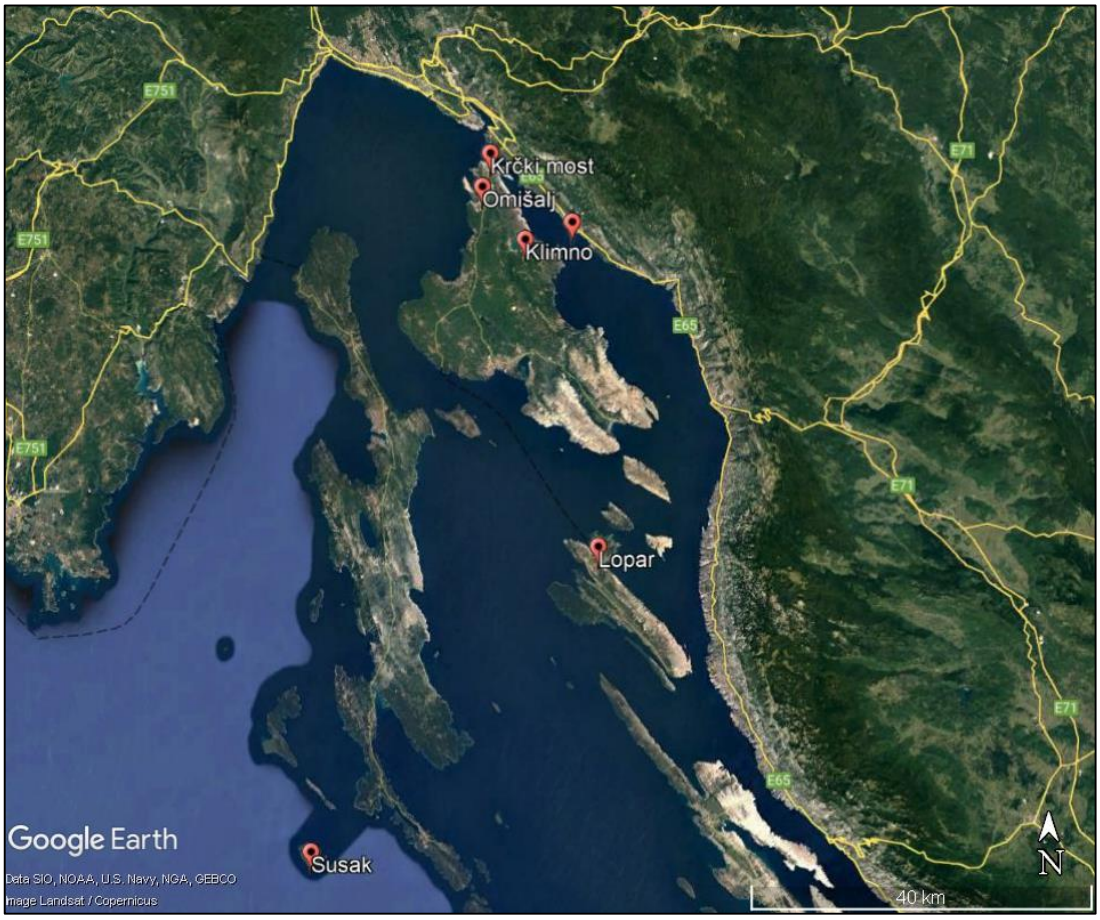


Figure 9: Test site 3 (Rijeka area)

The main climatological data of Rijeka area are described in the following Table (*Table 1*).

It is visible from the table that this area has moderate climate with lots of rain through the year. In the last decade the number of days with rain is decreasing, while the number of hot days is increasing.

Table 1: Climatological data of Rijeka. Values registered in 1948-2020 (https://www.meteo.hr).

1948-2020		Jan	Feb	Mar	Apr	May	Jun	Jul	Aug	Sep	Oct	Nov	Dec
AIR TEMPERATURE	Mean [°C]	5,8	6,3	9,0	12,8	17,2	21	23,6	23,4	19,2	14,6	10,3	7
	Abs. maximum [°C]	20	21,4	25	28,9	33,7	36,7	40	39,5	34,8	28,8	25,5	20,4
	Date (day/year)	20/1974	22/1990	29/2017	28/2012	25/2009	12/2003	19/2007	5/2017	4/1949	1/1956	2/2004	4/1979
	Abs. minimum [°C]	-11,4	-12,8	-7,7	-0,2	2,1	7,4	10,4	9,1	4,8	-1,2	-4,5	-8,9
	Date (day/year)	9/1985	10/1956	5/1971	14/1986	12/1978	8/1962	16/1970	28/1995	29/1977	30/2012	15/1983	28/1996
INSOLATION	Duration [h]	108.8	123.0	153.1	180.3	234.0	256.6	301.6	280.4	203.6	162.8	103.3	98.8
PRECIPITATION	Total Prec. [mm]	132.0	121.6	108.8	108.8	105.9	102.1	78.1	97.2	172.6	182.4	190.3	157.7
	Max. Snow Cower [cm]	28	17	52	-	-	-	-	-	-	-	8	14
	Date (day/year)	15/1985	23/2013	10/1976	- / -	- / -	- / -	- / -	**/ -	**/ -	- / -	4/1980	18/2010
NUMBER OF DAYS WITH	Clear Sky	6	6	5	4	4	5	9	10	8	7	4	6
	Fog	1	1	1	0	0	0	0	0	0	0	0	1
	Rain	10	9	10	12	12	11	9	9	10	11	13	11
	Frost	9	7	4	0	0	0	0	0	0	0	4	9
	Snow	1	1	1	0	0	0	0	0	0	0	0	1
	Min. Temp ≤ -10°C	0	0	0	0	0	0	0	0	0	0	0	0
	Max. Temp < 0°C	0	1	0	0	0	0	0	0	0	0	0	0
	Min. Temp < 0°C	6	5	2	0	0	0	0	0	0	0	1	4
	Max. Temp ≥ 25°C	0	0	0	1	5	17	26	25	11	1	0	0
	Max. Temp ≥ 30°C	0	0	0	0	0	4	11	11	1	0	0	0

2.4 Test site 4: Split area (HR)

The last test site of the NET4mPLASTIC Project corresponds to the area surrounding the City of Split, on the eastern coast of Croatia.

In contrast to the western Adriatic coast, which was formed under the influence of fluvial deposits accumulated in the Quaternary (Županović and Jardas, 1989), the eastern coast is morphologically much more indented with 1.246 islands, islets, and rocks whose southern shores rise steeply from the depths of the sea. The rows of island chains are parallel to the coastline, and this is known worldwide as a Dalmatian type of coast (Fairbridge, 1968). The geomorphology of the western part is characterized by low, mostly sandy, sedimentary shores while the eastern coast is karstic, generally high, rugged, and rocky with only 5% covered with coastal silt, sand and gravel.

Due to the density gradient in the water column, the current regime in the Adriatic Sea is usually observed in three layers: surface, intermediate and demersal. The prevailing surface circulation consists of a northerly flow along the eastern coast (EAC-East Adriatic Current) and a southerly return flow along the western coast (WAC-West Adriatic Current) (Artegiani et al., 1997) with the occurrence of several cyclonic gyres, the most prominent of which are in the southern and central Adriatic (Orlić et al., 1992). In addition to the density gradient, water masses circulation is also triggered and regulated by seasonal winds, mistral (NW) in summer and bora (NE) and sirocco (SE) in winter.

As the Po, Adige and Isonzo rivers have a crucial impact on the northern Adriatic and the western coast, the south-eastern part of the Adriatic is highly influenced by the Buna-Bojana shed and the Neretva river, both in terms of freshwater inflow and in terms of land-based litter washed from landfills along the riverbanks. Knowing the circulation of the Adriatic, it is expected that after a long period of strong southerly winds, the accumulation of litter along the southern shores of the exposed islands (Vis, Mljet, Korčula and Lastovo) and the peninsula of Pelješac will increase, and a large part of the floating litter will be washed ashore.

The locations for sampling microplastics at the sea surface and in the beach sediment were selected considering a potential increased accumulation of microplastic particles due to prevailing winds and currents and the proximity of the Cetina and Neretva river mouths. Microplastics in beach sediment were sampled at two stations in the central (Duće beach and Zaglav beach, island of Vis) and two stations in the southern Adriatic (Prapratno beach and Komin estuary beach). Sampling of microplastics at the sea surface was also carried out at two selected stations in the central (Brač and Hvar Channel) and two stations in the southern Adriatic (Neretva Channel, southwest side of the island of Mljet). These sites are close to sampling sites for beach sediment (*Figure 10*).

Biota samples were collected from a mussel farm and a natural habitat in the central (Site A) and also in the southern Adriatic (Site B). Site A included the area of Brač Island where live mussels (*Mytilus galloprovincialis*) were collected from the mussel farm in Maslinova Bay and the nearby Lučica Bay as their natural habitat. Site B included two sites in Mali Ston Bay, a mussel farm in Bistrina and a natural bank in Luka Bay on the Pelješac Peninsula (*Figure 10*).

Mali Ston Bay is an elongated, sparsely populated and industrialized bay located between the mainland and the Pelješac peninsula. Its geomorphological characteristics, as well as its hydrographic and production features, make it unique along the eastern Adriatic coastline. The freshwater inflow from the Neretva River affects mainly the outer part of the bay, and the circulation pattern mostly depends on the direction of local winds (Matić, 2005). The strong seawater currents, considerable freshwater inflow from the Neretva River and several submarine springs situated inside the Mali Ston Bay provide optimal conditions for high productivity (Kršinić et al., 2016).

Figure 10: Map of sampled stations in the central and southern Adriatic Sea (yellow lines – microplastics at the sea surface; red dots – microplastics in beach sediments; white dots – microplastics in shellfish).

In addition, the transparency of the sea, the absence of large phytoplankton blooms, and the aeration of the water column characterize this area as a stable environment suitable for shellfish farming (Viličić, 2017). As an extremely important area for shellfish farming, not only in the Adriatic but also in the Mediterranean, Mali Ston Bay was protected as a special marine reserve in 1983. Aquaculture in the bay mainly includes mussel and oyster farming and accounts for over 85% of the total Croatian farming. In 2019, the total production of mussels in Croatia was 947 tons and oysters (*Ostrea edulis*) 61 tons.

The other mussel farm studied is located on the southern side of the island of Brač in the central Adriatic Sea. In contrast to the large aquaculture area in Mali Ston Bay, there are relatively few shellfish pergolas in Maslinova Bay, as it is mainly sea bass (*Dicentrarchus labrax*) and sea bream (*Sparus aurata*) that are farmed there. The cultivation of mussels for commercial purposes stopped at the beginning of 2020, but there are still pergolas in the sea where small quantities of mussels (*Mytilus galloprovincialis*) are grown.

The sea currents in this area are typical of channel waters and flow along the coast from east to west. The direction is changed by the shape of the seabed, ensuring a relatively good exchange of water masses. In winter the most frequent wind is the bora from the direction NE, while in summer the winds of the coastal circulation are the most frequent (ESE-W-NW).

3 Methodology

Several methodologies have been developed in the last years with the main aim of a definition of a joint methodology for macro and microplastic sampling, monitoring and analyses. For this Project, starting from the literature, it has been produced in numerous deliverables a State of the Art on these issues (see Deliverables of WP3), comparing different methods, and linking them with the main characteristics of the four Project Pilot sites.

This chapter will describe briefly the methodologies used, in relation to the Pilot site and the type of samples to be collected. Sediment samples were collected according to a standard MSFD methodology, developed, tested and used in the Adriatic within the DeFishGear project (Palatinus et al., 2015). In general, we can say that NET4mPLASTIC sampling adapted the already tested DeFishGear protocols, to better fit the specific sites and the Project aims and outputs. Furthermore, the standard sampling protocols have been sided by innovative methods (numerical model, marine drone, holo-sensor – see WP3 and WP5 results and deliverables).

3.1 Sediment

Beach sampling have been performed in all four Pilot sites, in different years, seasons, locations, meteo-marine conditions. In line with DeFishGear protocols, with some adaptations, macrolitter and Large and Small Microplastic (LMP and SMP) have been sampled and analysed with the following procedures.

3.1.1 Macrolitter (1000m stretch, Items size: > 50 cm)

A 1000m length zone was selected in different locations in order to identify marine litter (bigger than 50 cm). In this case, considering that the size of the identified objects did not allow the collection, all the items have been photographed with a dGPS (Leica GS16) (georeferenced pictures) in order to have an overall picture on the most common items, their materials/composition, and the possible sources that can produce and transport them in place.

Subsequently, all pictures are imported in a GIS environment in order to map and statistically analyse the distribution of the ML items.

3.1.2 Macrolitter (100m stretch, Items size: < 50cm)

The locations of the sampling areas have been selected according to the following criteria: minimum length of 100 m longshore for a fixed 100-metre stretch; low/moderate slope (~ 1.5 – 4.5°); breakwaters or jetties absence; easy beach access guaranteed all year round; no/few additional human cleaning activities. On-field sampling areas covered a 100 m long shore-parallel line, divided in 10 transects (10 m wide each) with a variable length depending on hydrodynamic conditions (*Figure 11*). All

the macrolitter identified within a transect (items smaller than 50 cm) have been collected in separate plastic bags, in addition dividing the items found in the different seashore zones.

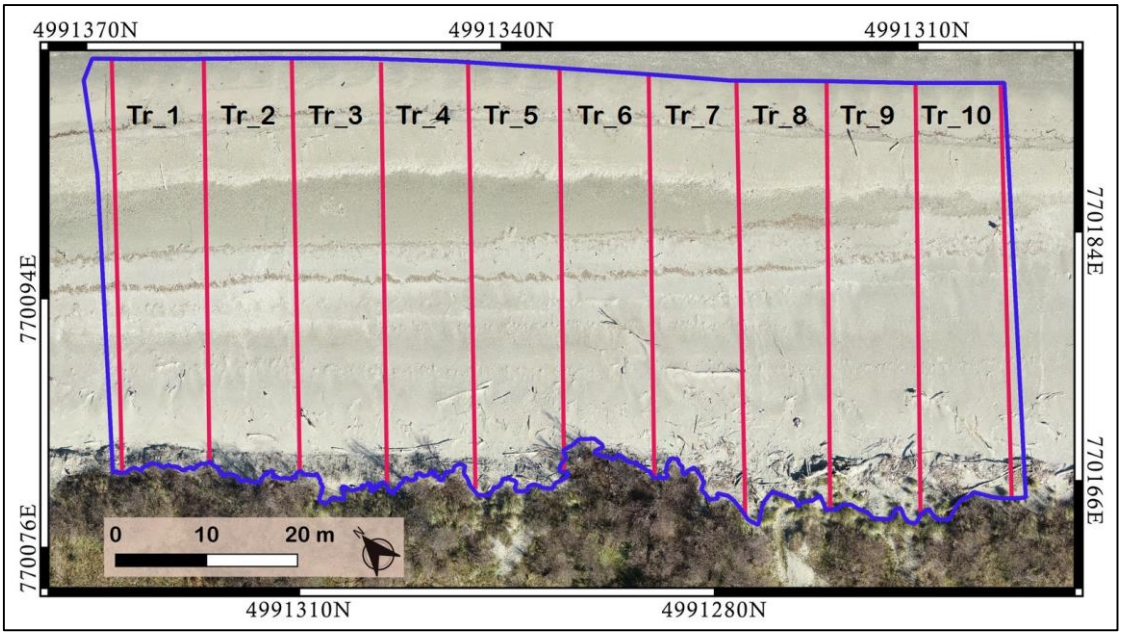


Figure 11: Example of the sampling area divided in 10 transects.

The macro litter items (size < 50 cm) were sorted and counted according to "*Master List of Categories of Litter Items*" (Palatinus et al., 2015). For each sample, a photo of collected marine litter items was taken (*Figure 12*). All this information was recorded in an Excel dataset (for statistical analysis), that was then shared with partners for the uploading on NET4mPLASTIC's web platform.

Figure 12: Example of image of marine litter lab analysis.

3.1.3 LMP – Large Micro Plastic (Items size: 1 – 5 mm)

The LMP samples (1 - 5 mm) have been collected within the 100m-length zone selected for the macrolitter collection. Following the standard procedure, 5 squares (1x1 m) have been randomly selected on the back shore of the beach, at least 2.5 meters one from each other.

Minimum 3 L of sand has been collected within the first 3 cm depth from each square, using a small metal shovel. In case of dry sand, the collected volume has been sieved in situ through 5 mm and 1 mm mesh size sieves (*Figure 13*). In case of wet sand, the collected sample has been properly labelled in situ, and then air-dried (for 2 or 3 days) and sieved in laboratory (*Figure 14*). The mixed residual particles (LMPs with shells, sand, and twigs) found between the two sieves have been stored in paper bags properly labelled. In laboratory, the mixed particles have been manually sorted to separate the LMP items from the others (*Figure 15*). The 3-dimensions of LMP items (length, width and height) have been measured with an electronic digital calibre or with an imaging software for stereomicroscope (measuring usually the longest diagonal). Furthermore, all LMP items identified have been numerated, counted and defined by colours and aspect. For each sample analysed a photo of identified LMP items was taken (*Figure 16*). All this information was recorded in an Excel dataset, that was then shared with partners for the uploading on NET4mPLASTIC's web platform. Finally, a part of LMP samples was sent to UNITS for spectroscopic analysis.

Figure 13: Example of LMP's dry sample sieved in situ (through 5 mm and 1 mm mesh size sieves) and stored in paper bags.

Figure 14: Example of LMP's wet sample air-dried and sieved in laboratory.

Figure 15: Manual sorting of LMP items from other particles and measure of LMP items with electronic digital calibre.

Figure 16: Example of LMP items identified from a NET4mPLASTIC sample.

3.1.4 SMP – Small Micro Plastic (Items size: 20 µm – 1 mm)

Five samples of SMP (20 µm – 1 mm) have been collected on 1 corner of each of the 5 squares (1x1m) used to sampling the LMP. For each sample, 250 ml of sand has been collected from the beach surface layer with a metal spoon and stored in glass jars, properly labelled (*Figure 17*). Then, each 250 ml sediment sample has been separated in laboratory in 5 sub-samples of 50 ml. Each sub-sample has been mixed with 200 ml of saturated NaCl solution (previously prepared) in a glass beaker (with a glass or aluminium lid), with the magnetic stirrer for 2 minutes. Then, the mixed solution has been left to settle for the sedimentation needed time as long as the greatest part of sand particles precipitate (same time for each sample).

The supernatant, which contains the SMP-items, has been filtered through glass fibre filters placed on the filtration ramp with electric vacuum pump (or on the Büchner filtrating funnel). The obtained filters have been stored in sealed petri dish until to dry them. The stereomicroscope analysis (magnification 80x) have been later performed by examining each sub-sample's filter. All SMP-items identified have been numerated, counted, defined by colours, aspect, and dimensions (measuring usually the longest diagonal. The majority of the SMP samples has been analysed by the imaging software for stereomicroscope and several of the identified items have been photographed (*Figure 18*). All this information was recorded in an Excel dataset, that was then shared with partners for the uploading on NET4mPLASTIC's web platform. Finally, a part of SMP samples was using for testing further methods of identification (e.g. RAMAN and spectroscopic analysis).

Figure 17: Example of SMP sample collected with metal spoon on the corner of LMP-square, and stored in a glass jar.

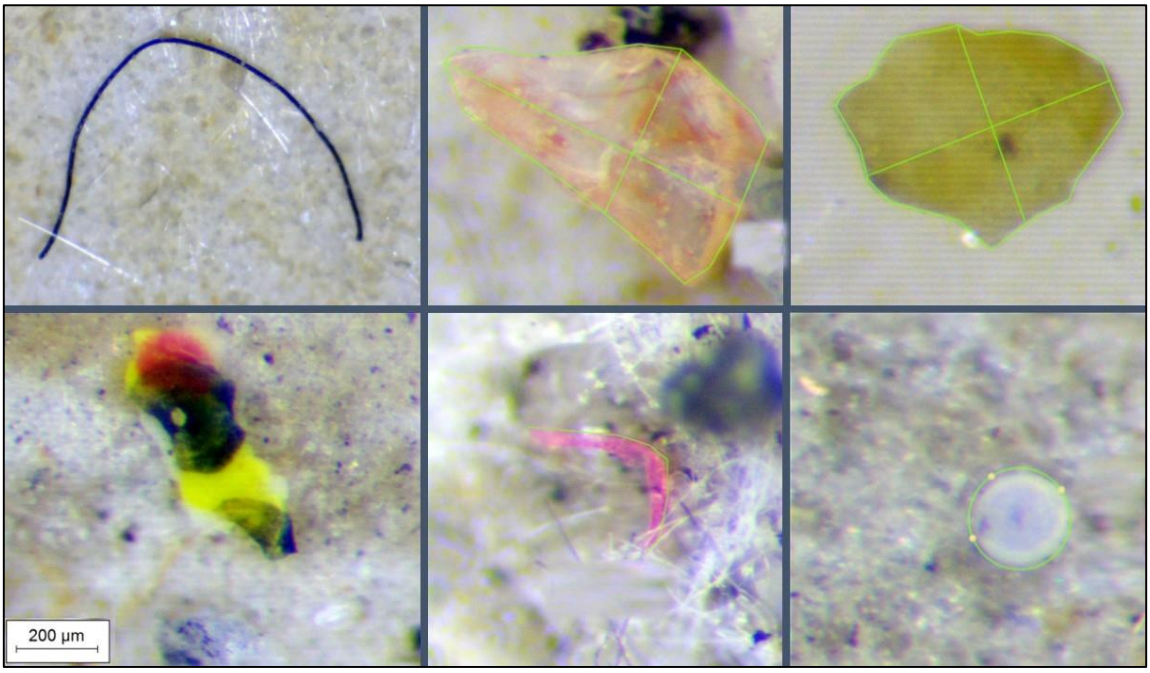


Figure 18: Example of SMP-items identified with stereomicroscope imaging software.

3.2 River and Sea water

3.2.1 MP-SSL: micro Plastic from Sea Surface Layer sampling (Items size: > 300 µm)

Five transects perpendicular to the coastline have been selected for each sampling area (close to the river mouths when possible). For each transect realised *in situ* (at least 4 on 5 previously mentioned), the manta net (mesh size: 300 µm) it has been deployed at the side of the vessel, out of the wake zone (around 4 m distance from the boat, in order to prevent the collection of water affected by turbulence) (*Figure 19*). A field datasheet with useful information has been filled up for each transect (*Figure 20*), for example: starting and ending point (GPS coordinates and time), sea-weather condition, travel speed, flowmeter code, etc. The sampling duration was around 30 min, but in case of huge amount of natural material (as twigs or plankton bloom), it has been reduced. At the end of the sampling time, the manta-net has been hoisted on the vessel and carefully rinsed from the outside of the net with sea water (or boat water reservoir water) through a high-pressure pump (*Figure 21*). The rinse has been performed from the manta mouth to the collector, in order to prevent external contamination and to concentrate all particles adhered to the net into the ending-collector (*Figure 22*). The collector has been later safely removed over a metal bucket (precaution to catch any spillage) and rinsed several times to prevent that some particles could adhered and remain inside. The collected sample has been put in some glass jars (500 ml), properly labelled, by metallic funnel, and then added with an alcoholic solution (ethanol 70%) (*Figure 23*). The sample has been stored into the portable cool box and then put into the fridge.

Subsequently, each sample has been filtered in laboratory with a metallic sieve (≤ 300 µm mesh size), in order to separate the solid fraction from the liquid one. Using visual identification and tweezers, all items of > 5 mm size (natural or artificial) have been rinsed with distilled water, removed from the sample, and stored in separate glass jars (or glass Petri dishes) in order to dry them (*Figure 24*). All the remaining pieces on the sieve have been put into another glass container (with ethanol 70%) and observed under the stereomicroscope (magnification 20x - 80x), in order to separate the micro plastic items (mP-SSL) from the other ones.

Each identified mP-item has been categorized according to the protocol, numerated, counted, defined by colours, aspect, and dimensions (measuring usually the longest diagonal). All the mP samples has been analysed by the imaging software for stereomicroscope and the identified items have been photographed (*Figure 25*). All this information was recorded in an Excel dataset, that was then shared with partners for the uploading on NET4mPLASTIC's web platform.

Figure 19: The manta net deployed at the side of the vessel, out of the wake zone.

DATA SHEET – SEA SURFACE SAMPLING

Reaserchers present	Partner / Organisation	Sampling date	Weather condition						Country	Description of the location
			Sunny		Windy		Rainy			
			Cloudy		Partly cloudy		Foggy			
Other circumstances that influenced the sampling:										
Sample ID (yyyymmdd_SITE_A1/A2)										
Start Point – Time										
Start Point – GPS Lat (N)										
Start Point – GPS Lon (E)										
Start Point – Flowmeter n.										
Stop Point – Time										
Stop Point – GPS Lat (N)										
Stop Point – GPS Lon (E)										
Stop Point – Flowmeter n.										
Average speed (kn)										
Transport length (nm)										
Sea state (0-4 Dougl.)										
Wind direction										
Depth start point										

Figure 20: NET4mPLASTIC field datasheet.

Figure 21: Manta-net has hoisted on the vessel and carefully rinsed with sea water, using a high-pressure pump.

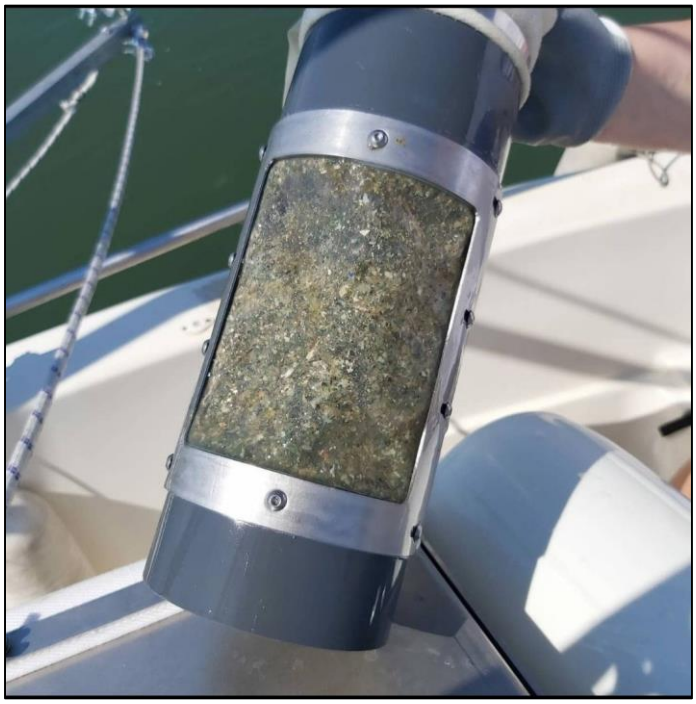


Figure 22: All sample concentrated into the ending-collector.

Figure 23: Sample collected and stored in 3x500 ml glass jars properly labelled (already added with ethanol 70%).

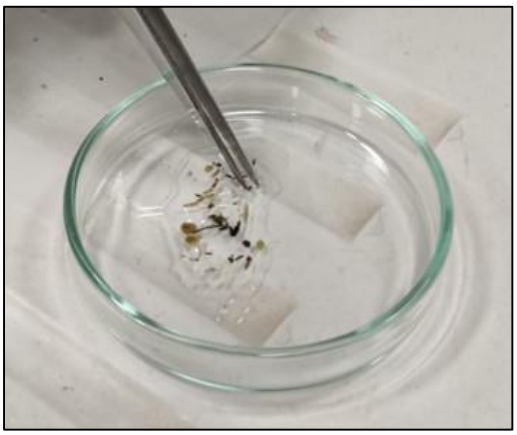


Figure 24: Visual identification to separate natural and artificial items, stored in glass Petri dishes before the dry.

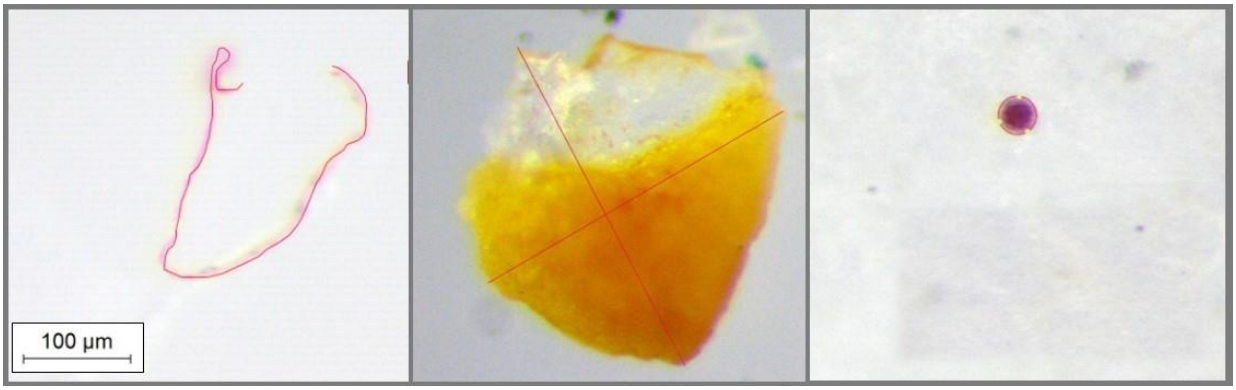


Figure 25: Example of mP-SSL items identified with stereomicroscope imaging software.

3.2.1.1 Marine drone field campaign

In three of the Project Pilot sites (Po Delta, Rijeka and Split area) Hydra Solution, with the collaboration of UNIFE, Prosoft, RERA, TIPH performed some sampling campaigns, in order to test the integrated system (*Figure 26*) developed in WP3 and WP5 consisting in an autonomous vehicle (marine drone) equipped with an holo sensor (SEQUOIA LISST-HOLO2), a mini manta, and an OBU – On Board Unit (probes).

Figure 26: Integrated system (marine drone + holo sensor + OBU + mini-manta).

The Holo sensor was fixed below the drone hull (*Figure 27*), and its main characteristics, as well as the description of its functioning and the results obtained are described in WP5 deliverables. The OBU acquired physical properties measurements (Turbidity and Dissolved Oxygen, Temperature, Salinity, Conductivity), and its results, togheter with the main holo sensor results, are described in the D.4.1.2.

Figure 27: LISST-HOLO Sequoia sensor, mounted below the marine drone.

The mini manta (*Figure 28*) has been dragged by the marine drone, about 1.5m behind the AUV. Differently from the previously described procedures of acquisition of sea surface samples, this “mini-manta” has a mouth of 30x15 cm (same mesh size: 300 µm), and the sampling duration was between 15 and 30 min for each transect, depending on the holo-sensor acquisition/marine condition. After the acquisition of each transect (1 or 2 in total depending on the site and the marine condition) the same procedure described in chapter 2.2.1 has been followed on field.

Figure 28: "Mini-manta" dragged by the marine drone.

3.3 Biota

Regarding the biota sampling, the Veterinary Public Health Institute of Abruzzo and Molise Regions (IZSAM) performed the sampling campaigns (and related analyses, see D.4.4.3) for the Italian sites (Po Delta and Pescara area), Teaching Institute for Public Health, Primorje-Gorski Kotar County (TIPH) performed sampling campaigns in Rijeka area, and the Institute of Oceanography and Fisheries (IOR, collaborating with RERA) performed data collection and analyses in Split area.

In this paragraph are briefly described the methodologies used for collecting the mussel samples in mussel's farms. A similar procedure has been used for the mussel's collection on natural banks, but considering that this specific location interested only the 3rd and 4th pilot sites (Rijeka and Split), that operation is better described in the last site paragraphs.

For each sampling point, about 2-3 kg of mussels (commercial size: 4-7 cm) were collected for the three depth levels of the water column (greater depth, intermediate, 50 cm from the surface).

The 3 elementary samples, after appropriate separation of the mussels from the long line, being careful not to damage the byssus to avoid stressing them, were carefully mixed and used to compose a single sample to be used both for qualitative and quantitative research analyses of microplastics in molluscs bivalves and for the detection of chemical contaminants such as polycyclic aromatic hydrocarbons (PAHs), polychlorinated biphenyls (PCBs), dioxins and heavy metals (lead, cadmium and mercury) (*Figure 29*).

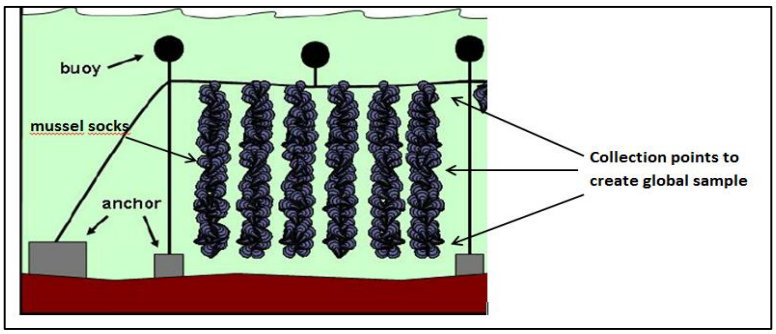
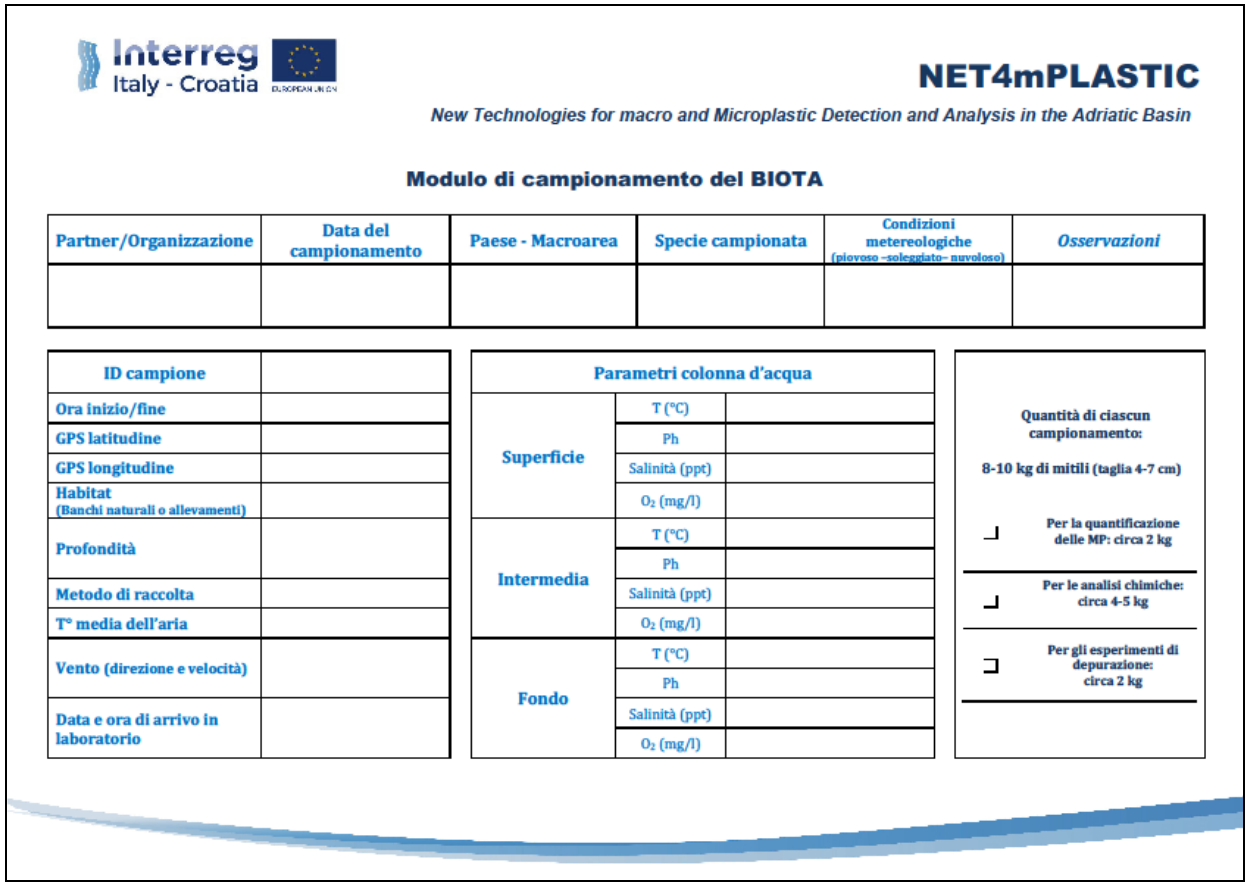


Figure 29: Collection points of samples for mussels.

The equipment needed for sampling was the following:

- Aluminum foil
- Balance
- Blotting paper
- Caliper
- Latex gloves without powder
- Multi-parameter probe
- Portable refrigerator
- Scissors
- Scalpels
- Water resistant pens

In order uniform the information collected during the sampling, it was used the following form for the accompanying data collection (*Figure 30*).



Interreg Italy - Croatia

NET4mPLASTIC

New Technologies for macro and Microplastic Detection and Analysis in the Adriatic Basin

Modulo di campionamento del BIOTA

Partner/Organizzazione	Data del campionamento	Paese - Macroarea	Specie campionata	Condizioni metereologiche (piovoso –soleggiato– nuvoloso)	*Osservazioni*

ID campione	
Ora inizio/fine	
GPS latitudine	
GPS longitudine	
Habitat (Banchi naturali o allevamenti)	
Profondità	
Metodo di raccolta	
T° media dell'aria	
Vento (direzione e velocità)	
Data e ora di arrivo in laboratorio	

Parametri colonna d'acqua		
Superficie	T (°C)	
	Ph	
	Salinità (ppt)	
	O_2 (mg/l)	
Intermedia	T (°C)	
	Ph	
	Salinità (ppt)	
	O_2 (mg/l)	
Fondo	T (°C)	
	Ph	
	Salinità (ppt)	
	O_2 (mg/l)	

Quantità di ciascun campionamento:

8-10 kg di mitili (taglia 4-7 cm)

☐ Per la quantificazione delle MP: circa 2 kg

☐ Per le analisi chimiche: circa 4-5 kg

☐ Per gli esperimenti di depurazione: circa 2 kg

Figure 30: Sampling template - biota.

The taken samples were stored in refrigerated thermal containers (4 ± 2 °C) and transferred within 24 hours to the laboratories of the IZSAM.

Upon arrival at the laboratory, the samples were analysed within 24 hours following collection or stored in the freezer at -20 ± 5 °C until the test is performed.

4 Sampling campaigns

Regarding the fieldwork realized for NET4mPLASTIC Project, as previously mentioned, it should be noted that not all types of data acquisition (and related methodologies) have been applied in all areas of interest, and sampling was not carried out simultaneously. In some cases, in the same test site campaigns have been performed in all the years of the project, and in different seasons. In other cases, especially during and after the pandemic Covid-19 situation, it was not possible to carry out or to repeat some measurements in all the locations.

4.1 Test site 1: Delta Po (IT)

Several sampling campaigns have been carried out during the entire duration of the project (2019-2022) in the Po Delta area: sediment, sea water and biota (*Table 2*). The activities have been performed by the Lead Partner, University of Ferrara (UNIFE-GEO Team, for sediment and sea water sampling) and by the Veterinary Public Health Institute of Abruzzo and Molise Regions (IZSAM – for the biota sampling). In the following paragraphs the data collection activities on the beach and marine areas are described in detail. The main results of the data elaboration are included in D.4.2.2 (for sediment and sea water sampling) and D.4.4.3 (for biota sampling).

In the municipality of Comacchio some samples were performed (not for all methodologies) on the beaches of Lidi Ferraresi: Volano, Nazioni, Pomposa, Porto Garibaldi, Estensi and Logonovo. More details will be explained in the following paragraphs.

Table 2: Recap table of sampling activities carried out with different methods in Po Delta pilot site. The coordinates identify the site on the beach, and generally in front this point (on the sea side) sea surface sampling has been performed. ML= marine litter; SMP= Small Micro Plastic; LMP: Large Micro Plastic; mP-SSL= micro Plastic from Sea Surface Layer.

Site	Lat (N)	Lon (E)	ML > 50 cm	ML < 50 cm	LMP	SMP	mP-SSL	BIOTA
Rosolina	45,188311	12,3311981	X	X	X	X	X	-
Porto Tolle	45,022542	12,430712	X	X	X	X	X	-
Goro	44,786866	12,334729	X	X	X	X	X	X
Comacchio	44,801329	12,272639	-	X	X	X	X	-

4.1.1 Sediment

4.1.1.1 Macrolitter (1000m stretch, items size: > 50 cm)

The campaigns performed in Po Delta area for the *in situ* monitoring of marine litter (ML, items size: > 50 cm) are listed in the table below (*Table 3*).

Table 3: Performed campaigns for ML (items size: > 50 cm) in Po Delta area.

Date	Site	Note
06/10/2020	Porto Tolle	Data elaborated and paper submitted (2022).
08/10/2020	Goro	Data elaboration in progress.
05/11/2020	Volano beach (Comacchio)	Results published in https://doi.org/10.3390/drones5040140
11/11/2020	Rosolina	Data elaboration in progress.
25/06/2020	Porto Tolle	Data elaboration in progress.

4.1.1.2 Macrolitter (100m stretch, items size: < 50 cm)

After the first sampling activities, the operators noticed that the marine litter (ML) accumulated differently, depending on the geomorphological features of the different beach zones (*Figure 31*).

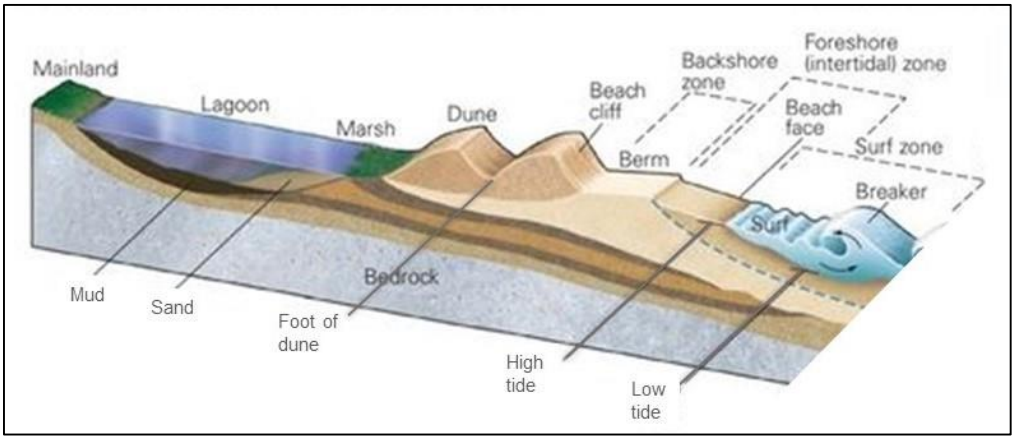


Figure 31: Costal geomorphological sections (edited from Marshak, 2015).

In particular, along the perpendicular axis to the shoreline, field observations suggest that:

1. The **intertidal zone**, as it is more affected by the action of waves and by tidal variations, is the area with less abundance of ML (both bigger and smaller than 50 cm);
2. The **backshore zone**, as mostly affected by winds activity, if there are no obstacles able to catch ML of medium size, is generally empty of "volatile" or "light" items. However, this area is scarcely covered by large objects (e.g., buoys and houseware, besides branches and trunks).
3. The **emerged beach** (between backshore and foot of dune), especially during winter season, is covered by branches and trunks (also bigger than 2x1 m), which favours the trapping of large and medium ML. They turned to be more abundant in this area, than in the previously described ones.
4. **Foot of dune "zone"** (within 1m from the foot of dune "line" toward the sea), despite is not a wide zone, turned out to be the one with the greater abundance of ML, of all dimensional categories. In particular, mixed piles of branches and trunks (often smaller than 1 m long) have been often observed in association with ML.
5. The **dune**, as it is a fragile ecosystem often under protection and covered with vegetation, were less investigated. However, in some places around the dune vegetation, accumulations of ML have been observed.

In order to verify the field observations, the 100m-stretch was divided in 10 transects perpendicular to the shoreline (in accordance with the standard sampling protocol) and in further 4/5 sub-transects parallel to the shoreline, depending on the geomorphological sectors identified in the investigated site. Based on the field campaigns, further analysis and correlations between ML and different costal zones will be implemented in the future.

The performed campaigns for ML items (< 50 cm) are listed in *Table 4.*

Table 4: Performed campaigns for ML (items size: < 50 cm) in Po Delta area. In detail for each sampling campaign: date and site, N° of standard transects, geomorphological sectors sampled (IZ = intertidal zone, BZ = backshore zone, EB = emerged beach, FDZ = foot of dune zone, D = dune) and note.

Date	Site	N° of Standard Transects	Geomorphological sectors	Note
30/07/2018	Goro	10	BZ	Samples collected before the beginning of the project, used as protocol test in the first stage of the project.
10/06/2019	Goro	10	BZ	
25/10/2019	Volano beach (Comacchio)	10	BZ - D	
14/11/2019	Porto Tolle	10	BZ	At the beginning 1st sea-storm, high-water and flooding of Po.
06/12/2019	Porto Tolle	10	BZ	After 1st sea-storm; declining phase of high-water and flooding of Po.
20/02/2020	Porto Tolle	10	IZ – BZ – EB - FDZ	After 2nd sea-storm.
25/06/2020	Porto Tolle	10	IZ – BZ – EB – FDZ - D	After Covid-19 lockdown and before tourist season.
06/10/2020	Porto Tolle	10	IZ – BZ – EB – FDZ - D	After tourist season.
08/10/2020	Goro	10	IZ – BZ – EB – FDZ - D	A whole stranded tree (not fall in place), present on one of the transect, covered sectors IZ – BZ – EB – FDZ. ML sorting was performed *in situ*, without the possibility of collecting the ML items trapped within branches and roots or under the trunk. Moreover, in the IZ, ML items were present only in 3 transects over 10.
05/11/2020	Volano beach (Comacchio)	10	IZ –EB – FDZ	Results published in https://doi.org/10.3390/drones5040140
11/11/2020	Rosolina	10	IZ – BZ – EB – FDZ - D	

4.1.1.3 LMP – Large Micro Plastic (Items size: 1 – 5 mm)

As reported in the previous paragraph, after the initial survey campaigns also the standard protocol for LMP sampling (items size: 1 – 5 mm) has been modified. The performed campaigns for LMP items in Delta Po area are listed in the following table (*Table 5*).

Table 5: Performed campaigns for LMP (items size: 1 – 5 mm) in Po Delta area. In detail for each sampling campaign: date and site, N° of collected samples, geomorphological sectors sampled (IZ = intertidal zone, BZ = backshore zone, EB = emerged beach, FDZ = foot of dune zone, D = dune) and note.

Date	Site	N° of collected samples	Geomorphological sectors	Note
30/07/2018	Goro	5	BZ	Samples collected before the beginning of the project, used as protocol test in the first stage of the project.
15/11/2018	Goro	10	BZ - EB	Samples collected before the beginning of the project, used as protocol test in the first stage of the project.
10/06/2019	Goro	7	BZ - EB	
25/10/2019	Volano beach (Comacchio)	5	BZ	
14/11/2019	Porto Tolle	5	BZ	
06/12/2019	Porto Tolle	5	BZ	
20/02/2020	Porto Tolle	5	BZ	
25/06/2020	Porto Tolle	14	BZ – EB – D	Samples in dune sector were collected both from a zone with vegetation and from another zone without.
21/07/2020	Goro	6	BZ – EB – FDZ	2 samples collected for each sector; far at least 2,5 m from each other.
06/10/2020	Porto Tolle	8	BZ – EB – D	Samples in dune sector were collected both from a zone with vegetation and from another zone without.
08/10/2020	Goro	8	BZ – EB – D	Samples in dune sector were collected both from a zone with vegetation and from another zone without.

11/11/2020	Rosolina	8	BZ – EB – D	Samples in dune sector were collected both from a zone with vegetation and from another zone without.
20/05/2021	Goro	6	BZ – FDZ – D	Collected samples were sieved *in*. No LMP items were found from samples collected at the FDZ.
20/10/2021	Goro	5	IZ (sandbar)	Samples collected on a sandbar, in high tide conditions, about 2 m east of the groyne (*Figure 32*)

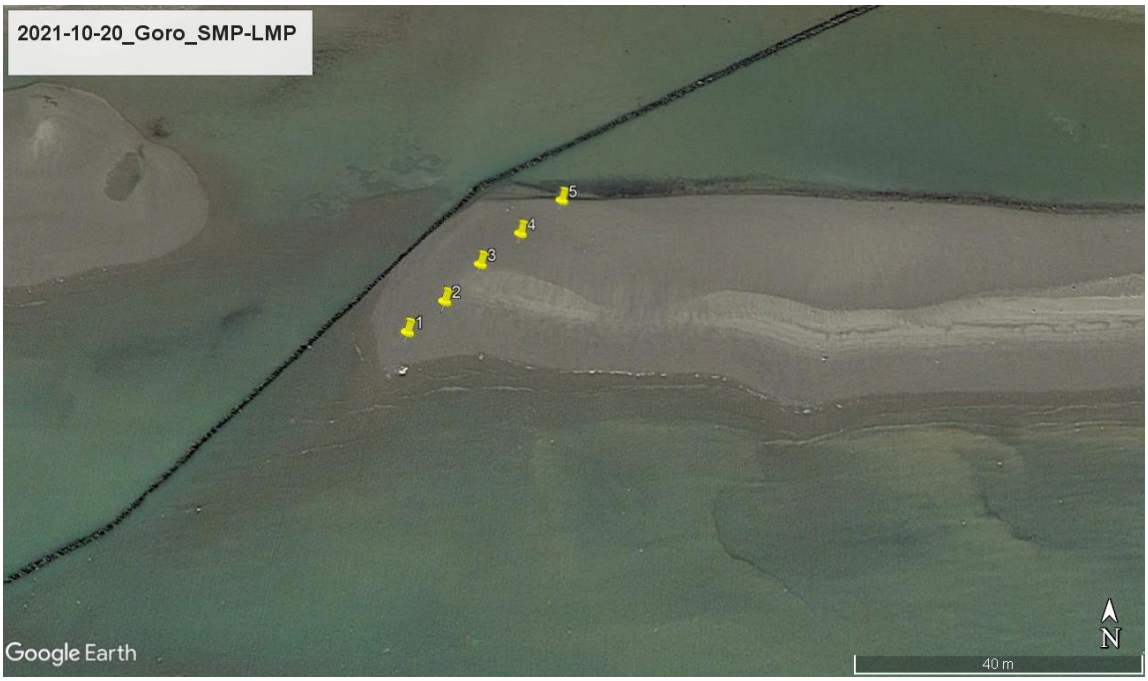


Figure 32: Goro (Ferrara, Italy): LMP and SMP sampling location on sandbar, in high tide conditions.

4.1.1.4 SMP – Small Micro Plastic (Items size: 20 µm – 1 mm)

As reported in the previous paragraphs, after the initial survey campaigns, also the standard protocol for SMP sampling (items size: 20 µm – 1 mm) has been modified. The performed campaigns for SMP items in Delta Po area are listed in the table below (*Table 6*).

Due to the Covid-19 lab access restrictions, some samples collected in the same pilot area before the beginning of the project have been used for the SMP analyses (considering that they were already prepared), to achieve the NET4mPLASTIC project output (10.000 MP items). Some samples collected within the NET4mPLASTIC project, could not be analysed due to the Covid-19 restrictions. Data elaboration is still in progress. All these samples are highlighted and listed in *Table 6*.

Table 6: Performed campaigns for SMP (items size: 20 µm – 1 mm) in Po Delta area. In detail for each sampling campaign: date and site, N° of collected samples, geomorphological sectors sampled (IZ = intertidal zone, BZ = backshore zone, EB = emerged beach, FDZ= foot of dune zone, D = dune) and note. Samples marked with (*) were collected but the data elaboration is still in progress.

Date	Site	N° of collected samples	Geomorphological sectors	Note
06/12/2017	Pomposa beach (Comacchio)	5	BZ	Samples collected before the beginning of the project, used as protocol test in the first stage of the project.
05/02/2018	Rosolina	5	BZ	Samples collected before the beginning of the project.
28/06/2018	Rosolina	5	BZ	Samples collected before the beginning of the project.
01/07/2018	Goro	5	BZ	Samples collected before the beginning of the project.
15/11/2018	Goro	10	BZ - EB	Samples collected before the beginning of the project, used as protocol test in the first stage of the project.
26/06/2019	Volano beach (Comacchio)	5	BZ	Samples used as protocol test in the first stage of the project.
15/07/2019	Nazioni Beach (Comacchio)	5	BZ	
16/07/2019	Pomposa beach (Comacchio)	5	BZ	
17/07/2019	Porto Garibaldi beach (Comacchio)	5	BZ	

18/07/2019	Estensi beach (Comacchio)	5	BZ	
18/07/2019	Logonovo beach (Comacchio)	5	BZ	
19/07/2019	Volano beach (Comacchio)	5	BZ	
14/11/2019	Porto Tolle	5 (*)	BZ	Data elaboration in progress.
05/12/2019	Volano beach (Comacchio)	5	BZ	
06/12/2019	Porto Tolle	5 (*)	BZ	Data elaboration in progress.
20/02/2020	Porto Tolle	5 (*)	BZ	Data elaboration in progress.
25/06/2020	Porto Tolle	14 (*)	BZ – EB – D	Samples in D were collected both from a zone with vegetation and from another zone without. Data elaboration in progress.
21/07/2020	Goro	6 (*)	BZ – EB – FDZ	2 samples collected for each sector; far at least 2,5 m from each other. Data elaboration in progress.
21/07/2020	Volano beach (Comacchio)	6	BZ – EB – FDZ	2 samples collected for each sector; far at least 2,5 m from each other.
06/10/2020	Porto Tolle	8	BZ – EB – D	Samples in dune sector were collected both from a zone with vegetation and from another zone without.
08/10/2020	Goro	8 (*)	BZ – EB – D	Samples in dune sector were collected both from a zone with vegetation and from another zone without. Data elaboration in progress.
05/11/2020	Volano beach (Comacchio)	5	BZ	
11/11/2020	Rosolina	8	BZ – EB – D	Samples in dune sector were collected both from a zone with vegetation and from another zone without.
20/05/2021	Goro	5 (*)	BZ	Data elaboration in progress.

20/10/2021	Goro	5	IZ (sandbar)	Samples were collected on a sandbar, in high tide conditions, about 2 m east of the groyne (*Figure 32*).
20/10/2021	Volano beach (Comacchio)	5 (*)	BZ – EB – FDZ	Samples collected at 2,5 m from each other, of which 2 in BZ, 2 in EB and 1 on FDZ. Data elaboration in progress.

4.1.2 River and Sea water

4.1.2.1 MP-SSL: micro Plastic from Sea Surface Layer sampling (Items size: > 300 µm)

The campaigns performed in Po Delta area for the micro plastics from sea surface layer (mP-SSL, items size: > 300 µm) are listed in the table below (*Table 7*).

Table 7: Performed campaigns for mP-SSL (items size: > 300 µm) in Po Delta area. In detail for each sampling campaign: date and site, N° of transects performed and note.

Date	Site	N° of transect	Note
21/07/2020	Goro	1	Protocols and instrumentation testing.
06/10/2020	Porto Tolle	4	Data elaboration in progress.
09/10/2020	Goro	4	Data elaboration in progress.
20/05/2021	Goro	4	19/05/2021 farm mussel sampling by IZSAM in Goro Site. Data elaboration in progress.
18/10/2021	Rosolina	4	Physical data collected by multi-parameter probe.
19/10/2021	Porto Tolle	4	Physical data collected by multi-parameter probe.
20/10/2021	Goro	3	Physical data collected by multi-parameter probe.
20/10/2021	Volano Beach (Comacchio)	2	Physical data collected by multi-parameter probe.

4.1.2.2 Marine drone field campaign

The main information related to the sampling carried out in the area of the Po Delta with the integrated system of the marine drone are described in the following Table.

Table 8: Main chacacteristics of the marine drone campaigns in Po Delta area.

Date	Site	Holo-sensor	OBU	Mini-manta	Note
23/10/2021	Volano	X		X	Integrated system testing phase. Manta: 1 transect/15min
12/05/2022	Goro		X	X	Manta: 2 transect/30min OBU: mounted on marine drone

4.1.3 Biota

For the biota sampling in the Delta Po area (Sacca di Goro - Northern Adriatic Sea) (*Table 9*), two sampling points were chosen within the same mussel farm (*Mytilus galloprovincialis*), part of the Goro Fishermen's Cooperative (CO.PE.GO.) (*Figure 33*).

Table 9: Biota sampling information in the Delta Po area.

Site (macro-area)	Sampling point	Coordinate	Habitat
Sacca di Goro	Sampling point 1 – Long-line closer to the coast - 2.5 nautical miles from the coast	N 44°44.920' E012°17.936'	CO.PE.GO farm
	Sampling point 2 – Long-line further from the coast - 3.5 nautical miles from the coast	N 44°45.031' E012°17.699'	CO.PE.GO farm

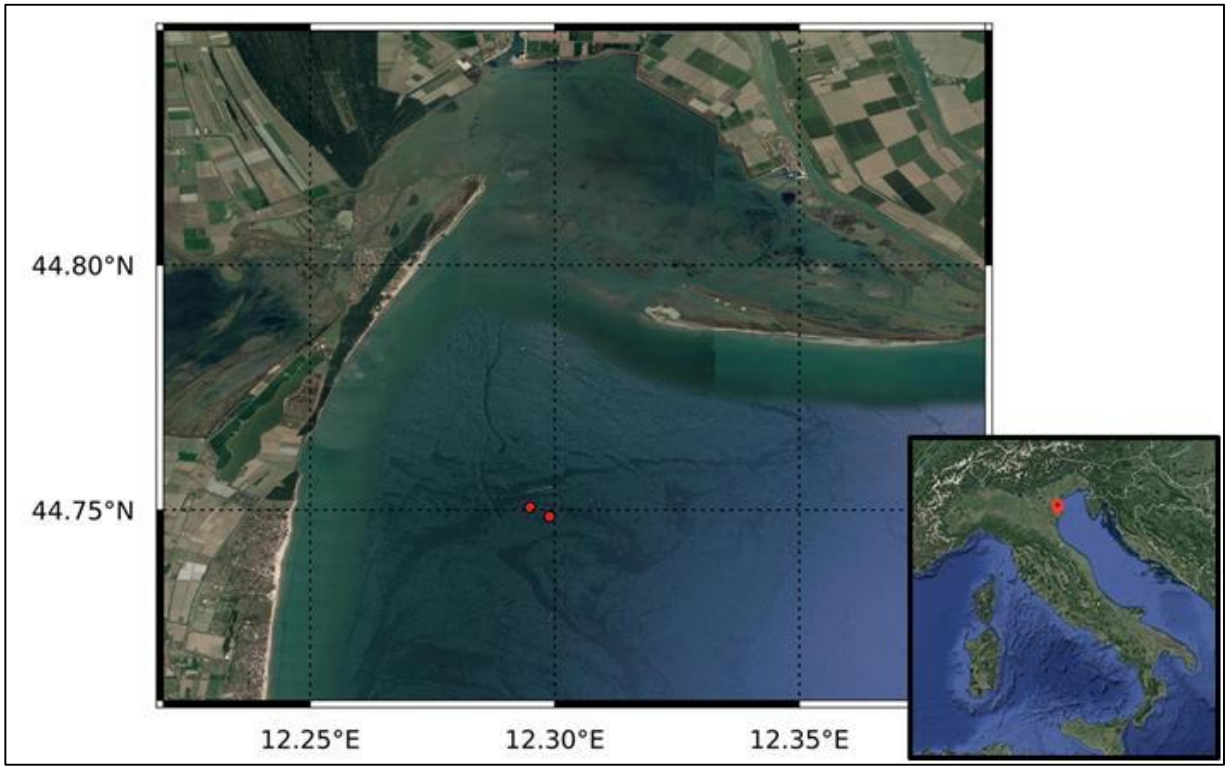


Figure 33: Map of sampling sites in the Sacca di Goro (northern Adriatic coast)

The samplings of the biota in the Delta Po area (Goro Sacca) started in December 2019 and ended in May 2022. The samplings were scheduled for two annually, one in the autumn period (October-November) and a second in the spring period (April-May) (*Table 10*).

Table 10: Biota sampling campaignes performed in the Delta Po area.

Sampling period	Sampling date	Sampling date	Habitat	Sample ID
Autumn/Winter 2019	05/12/2019	Sacca di Goro	CO.PE.GO Farm 2,5 nm	1640/1641
	05/12/2019	Sacca di Goro	CO.PE.GO Farm 3,5 nm	1642/1643
Spring/Summer 2020	04/06/2020	Sacca di Goro	CO.PE.GO Farm 2,5 nm	611/612
	04/06/2020	Sacca di Goro	CO.PE.GO Farm 3,5 nm	615/617
Autumn/Winter 2020	06/11/2020	Sacca di Goro	CO.PE.GO Farm 2,5 nm	1348/1350
	06/11/2020	Sacca di Goro	CO.PE.GO Farm 3,5 nm	1349/1351
Spring/Summer 2021	19/05/2021	Sacca di Goro	CO.PE.GO Farm 2,5 nm	621/622
	19/05/2021	Sacca di Goro	CO.PE.GO Farm 3,5 nm	623/624
Autumn/Winter 2021	07/12/2021	Sacca di Goro	CO.PE.GO Farm 2,5 nm	1929/1930
	07/12/2021	Sacca di Goro	CO.PE.GO Farm 3,5 nm	1931/1934
Spring/Summer 2022	May 2022	Sacca di Goro	CO.PE.GO Farm 2,5 nm	379/380

4.2 Test site 2: Pescara area (IT)

Several sampling campaigns have been carried out during the entire duration of the project (2019-2022) in the Pescara area: sediment, sea water and biota. The activities have been performed by the Lead Partner, University of Ferrara (UNIFE-GEO Team, for sediment and sea water sampling) and by the Veterinary Public Health Institute of Abruzzo and Molise Regions (IZSAM – for the biota sampling). In the following paragraphs the data collection activities on the beach and marine areas are described in detail. The main results of the data elaboration are included in D.4.2.2 (for sediment and sea water sampling) and D.4.4.3 (for biota sampling).

Table 11: Recap table of sampling activities carried out with different methods in Pescara area pilot site. The coordinates identify the site on the beach, and generally in front this point (on the sea side) the sea surface sampling has been performed. ML= marine litter; SMP= Small Micro Plastic; LMP: Large Micro Plastic; mP-SSL= micro Plastic from Sea Surface Layer.

Site	Lat (N)	Lon (E)	ML < 50 cm	LMP	SMP	mP-SSL	BIOTA
Pineto	42,576261	14,097713	X	X	X	-	-
Montesilvano	42,514096	14,165181	-	X	X	X	X
Pescara (S)	42,560317	14.246374	X	X	X	-	-

4.2.1 Sediment

4.2.1.1 Macrolitter (100m stretch, Items size: < 50 cm)

In Pescara area the marine litter campaigns (items size: < 50 cm) have been performed according to the geomorphological sectors described in paragraph *4.1.1.2*. The sampling campaigns for ML items are listed in *Table 12*.

In particular, as Pescara beach (southern side of Pescara River area) is highly anthropized, the 100m-stretch was positioned in a free-access beach (not privately managed) between two beach facilities (privately managed). Moreover, it was not possible to identify a proper dune system, as the dune in that site should be considered artificial and confined by a fence built up to separate the beach area from the street behind.

Table 12: Performed campaigns for ML (items size: < 50 cm) in Pescara area. In detail for each sampling campaign: date and site, N° of standard transects, geomorphological sectors sampled (IZ = intertidal zone, BZ = backshore zone, EB = emerged beach, FDZ = foot of dune zone, D = dune) and note.

Date	Site	N° of Standard Transects	Geomorphological sectors	Note
25/05/2021	Torre del Cerrano (Pineto)	10	IZ – BZ – EB – FDZ - D	
26/05/2021	Pescara (South)	10	IZ – BZ – EB	Predominance of blue and white nylon strapping bands, probably due to the presence of the sunshades in one of the beach facilities. ML items present only in 8/10 transects of IZ, and 9/10 transects of BZ.

4.2.1.2 LMP – Large Micro Plastic (Items size: 1 – 5 mm)

The performed campaigns for LMP items in Pescara area are listed in *Table 13.*

Also in this case, the standard protocol for the sampling of LMP (items size: 1 – 5 mm) have been integrated and adapted on the base of the different geomorphological sectors identified.

In particular, as Pescara beach (southern side of Pescara River area) is highly anthropized, the LMP samples have been collected between 2 beach facilities. Moreover, it was not possible to identify a proper dune system, as the dune in this site should be considered artificial and confined by a fence built up to separate the beach area from the street behind.

Therefore, what have been identified and considered during the sampling campaigns as "Foot of dune zone" should be considered artificial (sediment transported and accumulated by meteo-marine agents at the base of the fence).

Montesilvano presents an anthropized beach, as the one just described (Pescara beach) and the sampling campaigns were performed around the beach facilities and a slipway for small fishing vessels. Moreover, the beach appears very close to the street and to the pedestrian walkway and no dunes are present. For these reasons, the area that was designated as "Foot of dune zone" should be considered artificial.

Table 13: Performed campaigns for LMP (items size: 1 – 5 mm) in Pescara area. In detail for each sampling campaign: date and site, N° of collected samples, geomorphological sectors sampled (IZ = intertidal zone, BZ = backshore zone, EB = emerged beach, FDZ = foot of dune zone, D = dune) and note.

Date	Site	N° of Standard square	Geomorphological sectors	Note
25/05/2021	Pineto	8	BZ – EB – FDZ - D	
25/05/2021	Montesilvano	7	IZ – BZ – EB – FDZ	Anthropized beach near beach facilities and a slipway for small fishing vessels; close to the street and the pedestrian walkway; artificial FDZ.
26/05/2021	Pescara (South)	7	IZ – BZ – EB – FDZ	Free access anthropized beach between two beach facilities; artificial dune confined by a fence to separate the beach from the street.
10/02/2022	Pineto	5	IZ – BZ – EB – FDZ - D	Data elaboration in progress.
10/02/2022	Montesilvano	5	IZ – BZ – EB – FDZ	Anthropized beach near beach facilities and a slipway for small fishing vessels; close to the street and the pedestrian walkway;

				artificial FDZ. Data elaboration in progress.
10/02/2022	Pescara (South)	5	IZ – BZ – EB – FDZ	Free access anthropized beach between two beach facilities; artificial dune confined by a fence to separate the beach from the street. Data elaboration in progress.

4.2.1.3 SMP – Small Micro Plastic (Items size: 20 µm – 1 mm)

The performed campaigns for SMP items in Pescara area are listed in *Table 14*.

Also in this case, the standard protocol for the sampling of *SMP (items size:* 20 µm – 1 mm*)* has been integrated and adapted on the base of the different geomorphological sectors identified.

In particular, as Pescara beach (southern side of Pescara River area) is highly anthropized, the LMP samples have been collected between 2 beach facilities. Moreover, it was not possible to identify a proper dune system, as the dune in this site should be considered artificial and confined by a fence built up to separate the beach area from the street behind.

Therefore, what have been identified and considered during the sampling campaigns as "Foot of dune zone" should be considered artificial (sediment transported and accumulated by meteo-marine agents at the base of the fence).

Montesilvano presents an anthropized beach, as the one just described (Pescara beach) and the sampling campaigns were performed around the beach facilities and a slipway for small fishing vessels. Moreover, the beach appears very close to the street and to the pedestrian walkway and no dunes are present. For these reasons, the area that was designated as "Foot of dune zone" should be considered artificial.

Table 14: Performed campaigns for SMP (items size: 20 µm – 1 mm) in Pescara area. In detail for each sampling campaign: date and site, N° of collected samples, geomorphological sectors sampled (IZ = intertidal zone, BZ = backshore zone, EB = emerged beach, FDZ= foot of dune zone, D = dune) and note.

Date	Site	N° of Standard square	Geomorphological sectors	Note
25/05/2021	Pineto	8	BZ – EB – FDZ - D	Data elaboration in progress.
25/05/2021	Montesilvano	7	IZ – BZ – EB – FDZ	Anthropized beach near beach facilities and a slipway for small fishing vessels; close to the street and the pedestrian walkway; artificial FDZ. Data elaboration in progress.
26/05/2021	Pescara (South)	7	IZ – BZ – EB – FDZ	Free access anthropized beach between two beach facilities; artificial dune confined by a fence to separate the beach from the street. Data elaboration in progress.
10/02/2022	Pineto	5	IZ – BZ – EB – FDZ - D	Data elaboration in progress.
10/02/2022	Montesilvano	5	IZ – BZ – EB – FDZ	Anthropized beach near beach facilities and a slipway for small

				fishing vessels; close to the street and the pedestrian walkway; artificial FDZ. Data elaboration in progress.
10/02/2022	Pescara (South)	5	IZ – BZ – EB – FDZ	Free access anthropized beach between two beach facilities; artificial dune confined by a fence to separate the beach from the street. Data elaboration in progress.

4.2.2 River and Sea water

4.2.2.1 MP-SSL: micro Plastic from Sea Surface Layer sampling (Items size: > 300 µm)

The campaigns performed in Pescara area for the micro plastics from sea surface layer (mP-SSL, items size: > 300 µm) are listed in the table below (*Table 15*).

Table 15: Performed campaigns for mP-SSL (items size: > 300 µm) in Pescara area. In detail for each sampling campaign: date and site, N° of transects performed and note.

Date	Site	N° of transect	Note
26/05/2021	Montesilvano	4	26/05/2021 farm mussel sampling by IZSAM in Pescara site. Physical data collected by multi-parameter probe. Data elaboration in progress.
09/02/2022	Montesilvano	4	Physical data collected by multi-parameter probe. In 2/4 transects a current of fresh water have been crossed. Along slide current an elongated sea surface layer of ML accumulation has been produced. Data elaboration in progress.

4.2.3 Biota

For the biota sampling in the Pescara area (near the Pescara river) a single sampling point was chosen within the mussel farm (*Mytilus galloprovincialis*) "ATLANTIDE DI ENNIO DI GIOVANNI S.A.S." (*Figure 34*) (*Table 16*).

Table 16: Biota sampling information in the Pescara area.

Site (macro-area)	Sampling point	Coordinate	Habitat
Pescara	Sampling point 1 – Long-line at 1 nautical miles	N 42°31.344' E014°12.235'	Atlantide farm

Figure 34: Map of sampling site in Pescara (central Adriatic coast)

The samplings of the biota in the Pescara area started in June 2020 and ended in May 2022. The samplings were scheduled for two annually, one in the autumn period (October-November) and a second in the spring period (April-May).

Table 17: Biota sampling campaignes performed in the Pescara area.

Sampling period	Sampling date	Sampling date	Habitat	Sample ID
Spring/Summer 2020	08/06/2020	Pescara	Atlantide Farm 1,0 nm	555/556
Autumn/Winter 2020	16/12/2020	Pescara	Atlantide Farm 1,0 nm	1501/1502
Spring/Summer 2021	26/05/2021	Pescara	Atlantide Farm 1,0 nm	698/699
Autumn/Winter 2021	26/10/2021	Pescara	Atlantide Farm 1,0 nm	1742/1743
Spring/Summer 2022	May 2022	Pescara	Atlantide Farm 1,0 nm	411/412

4.3 Test site 3: Rijeka area (HR)

Several sampling campaigns have been carried out in the last 2 years of the Project (2021 and 2022) in Rijeka area: sediment, sea water and biota. The activities have been performed by the Teaching Institute for Public Health, Primorje-Gorski Kotar County (TIPH). In the following paragraphs the data collection activities on the beach and marine areas are described in detail. The main results of the data elaboration are included in D.4.2.2.

4.3.1 Sediment

In Rijeka area the samples of beach sediment were collected on three beaches: Beach Meline on the Island of Krk (Soline Bay, Klimno), Beach Rajska Plaža on the island of Rab and on the beach of the Island of Susak. In this part of Croatia there are not many sandy beaches so the choice of locations was limited by the geographical charasteristics of the area (*Figure 35*).

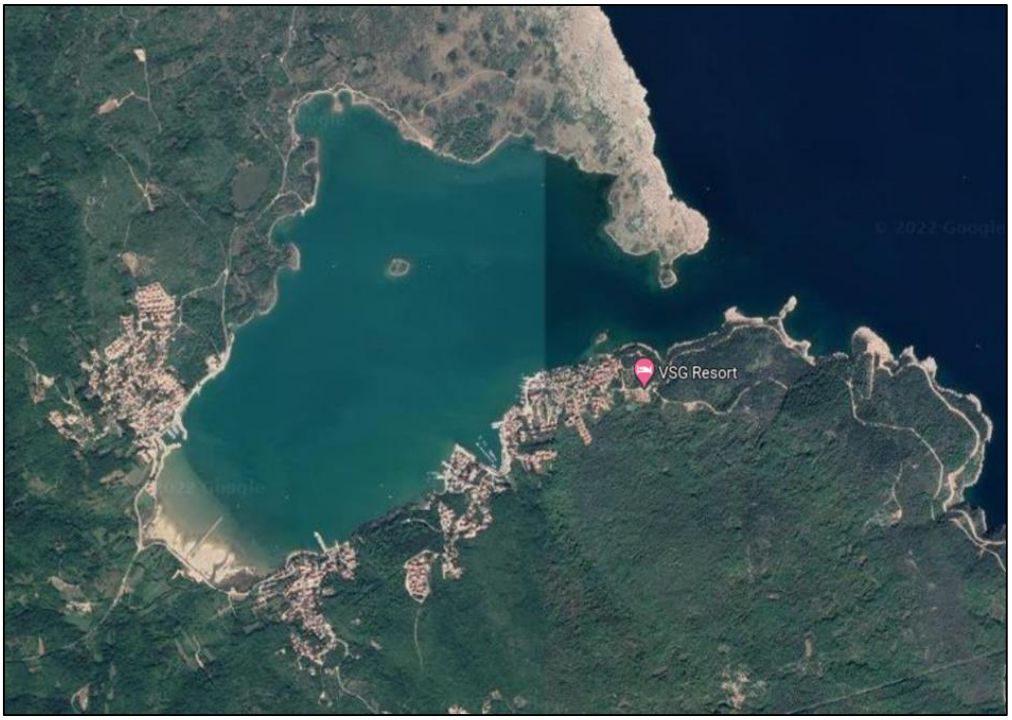


Figure 35: Klimno, Soline Bay (Isle of Krk).

Soline Bay is about 1.5 km long and up to 1 km wide (*Figure 36* and *Figure 37*). It is very shallow bay. The depth of the seabed is only around 10 m deep at the entrance of the bay. The Beach Meline is about 600 m long. During the winter it is often completely flooded by the sea, and it becomes the sea bottom. During the summer it is very popular touristic destination. Around the bay there are many houses

but most of them are not inhabited during the winter. This represents a great danger of local pollution and probably the most important source of microplastics.

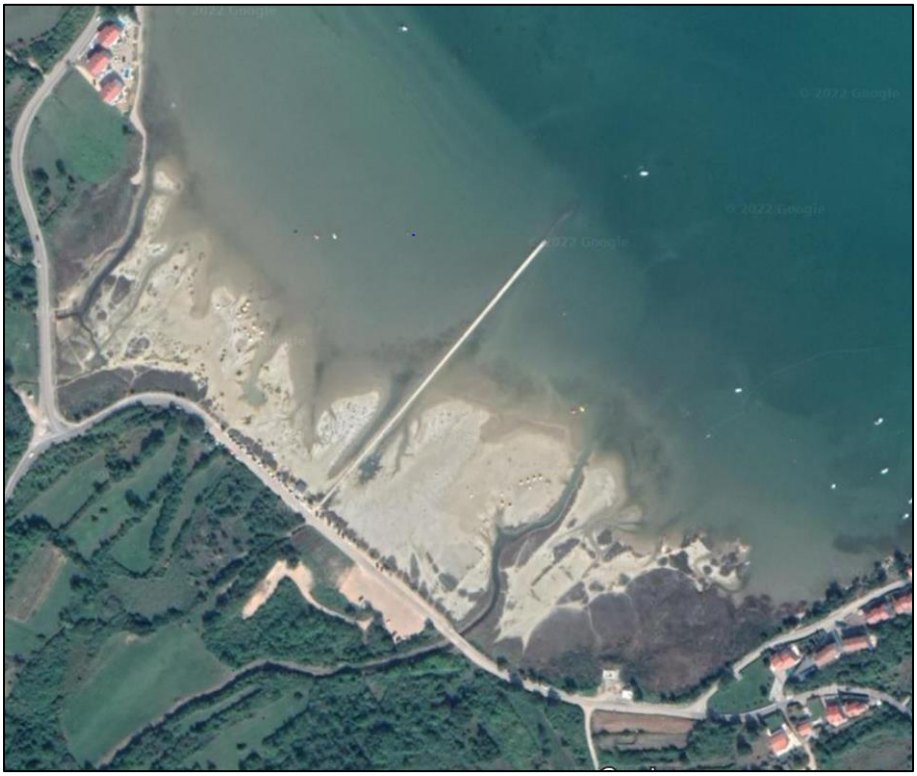


Figure 36: Beach Meline, Soline Bay.

Figure 37: Sampling location on the beach Meline.

The northwest coast of the island of Rab has an indented coast (*Figure 38*). There are three large bays and several smaller coves (Lopar, Supetarska and Kamporska Draga Bays). The capes of the coves are rocky and steep. Lopar Bay, in the northwestern part of the isthmus, is very shallow and completely altered by embankment. It is greatly influenced by man. On the southwest coast of Lopar there are numerous bays with sandy beaches. In the bay of Crnika on the northeastern part of the peninsula there is the longest sandy beach in the Kvarner area. The seabed to a depth of 10 m is sandy. Influences of inland water are also observed in the coastal part. Rajska plaža (Paradise Beach) is 1,5 km long and 14 km from the town of Rab. The sea in front of the beach is very shallow.

Figure 38: Island of Rab and Rajska plaza in Lopar.

Figure 39: Lopar – Rajska plaza.

Figure 40: Sampling location on Rjska plaza, Lopar

The island of Susak has mostly rocky, but gently sloping shores, in the background of which are very steep slopes formed in loess. In the area of the port of Susak, the coast is sandy and extremely shallow (*Figure 41*) (*Figure 42*) (*Figure 43*).

Figure 41: Island of Susak.

Figure 42: Susak beach.

Figure 43: Sampling location on the Island of Susak.

The samples were taken before the beginning of the bathing season and at the end of the bathing season, May/September 2021, except on the beach on the Island of Susak, where one sample was taken in the middle of the bathing season (*Table 18*). The samples were taken and analysed according to the standard methodology described in the section 2.1 of this document. Sampling was conducted on sandy beaches above the wreck or intertidal zone/line were possible. Certain volume of surface top 3 cm of sediment was collected with a metal spoon from the area of 100 cm x 100 cm. Samples were sieved through 5 and 1 cm sieves. Larger particles remained on the 5 cm sieve, while microplastic particles remained on the 1 cm sieve. Samples were stored in glass jars and properly labelled. At each point 250 g of send was collected for the analysis of particles smaller than 1 cm. Samples were stored in glass jars. Analysis of sediment were performed later in the laboratory by examining the samples under a stereomicroscope.

Table 18: Beach sediments sampling in Rijeka area.

Sampling location	Geographical coordinates	Date of sampling
Beach Meline, Klimno, Island of Krk	45.150351 N 14.600663 E	21.05.2021 27.09.2021
Beach Rajska plaža, Island of Lopar	44.824372 N 14.742410 E	26.05.2021 24.09.2021
Beach on the Island of Susak	44.510492 N 14.308676 E	28.07.2021 28.09.2021

4.3.2 River and Sea water

The samples of microplastics from sea water were taken on six locations, three of which are situated in Vinodolski kanal from Krčki most to Crikvenica (sampling sites near Crikvenica, Šilo and Krčki most) and in the bay Soline, Island of Krkr, in front of the beach Rajska plaža and in front of the beach on the Island of Susak (*Figure 44*).

From Krčki most to bay Stinica, the coast of Vinodolski kanal and Velebitski kanal is rocky and quite steep. Along the coast, gravel beaches are common: Jadranovo, Kačjak, Selce, Klenovica, Novi Vinodolski, Povile, Žrnovnica, Sibinj, Jablanac, Stinica. Near Crikvenica Dubračina River flows in the Adriatic Sea. The sea bottom in this area is covered with coarse sand and small pebbles. In deeper parts and up to 200 m from the coast with reduced flow the bottom is covered with terrigenous mud.

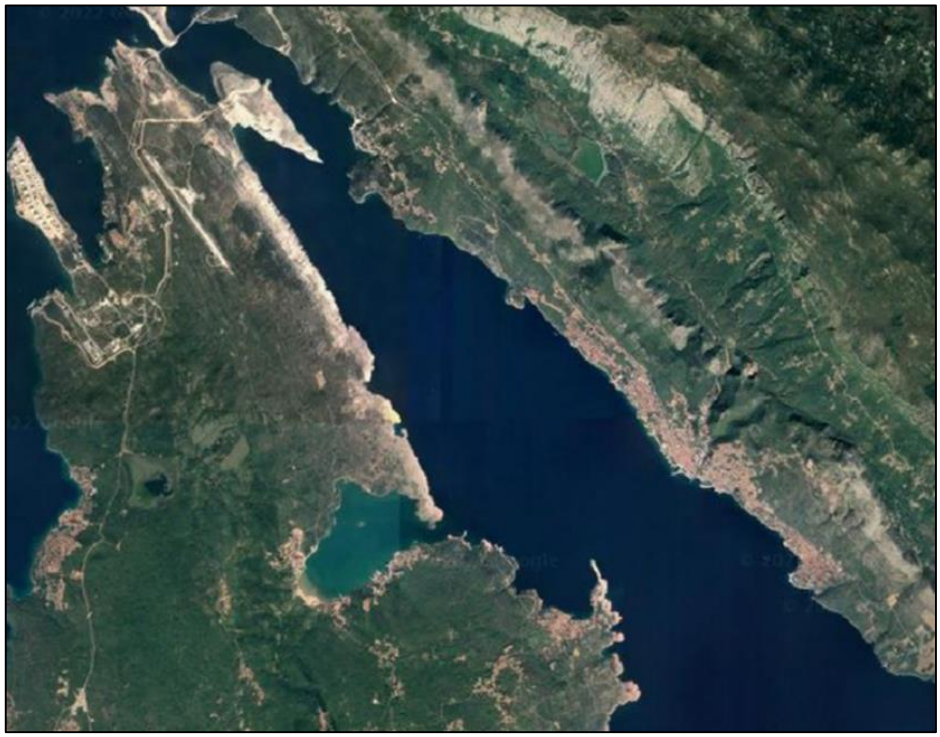


Figure 44: Vinodolski channel.

The samples of microplastics from sea surface were collected from May to October 2021 and in May 2022. The samples were collected according to the methodology already described in the Methods section of this document. Manta net was used for the collection of microplastics from the sea surface. The net opening is 15 x 30 cm, and the mesh size is 300 µm. It was pulled from the side of the vessel for half an hour in one straight direction with speed of app. 2 - 3 knots, collecting samples of the filtered surface water in a beaker at the bottom of the net. During each sampling campaign two samples were taken and stored in ethyl alcohol. Stereozoom microscope with different magnifications was used for the identification of microplastics.

Table 19: Sampling locations of surface sea water sampling in Rijeka aerea.

Sampling location	Geographical coordinates	Dates of sampling
Uvala Soline	45.164073836 N 14.61571733 E	10/06/2021 27/10/2021
Lopar	44.818142 N 14.548351 E	26/05/2021 24/09/2021
Susak	44.818142 N 14.743231 E	28/07/2021 28/09/2021
Vinodolski channel – Crikvenica - Novi Vinodolski	45.126460 N 14.733594 E	10/06/2021
Vinodolski channel - Šilo	45.161218 N 14.662726 E	10/06/2021
Krčki most	45.2484727 N 14.5697527 E	10/06/2021 27/10/2021
Omišalj	45.208086 N 14.549098 E	04/05/2022

4.3.2.1 Marine drone field campaign

In the Rijeka area two drone campaigns were conducted: the first one on October 27th, 2021, and the second on May 4th, 2022. In the first drone campaign microplastics samples were collected from the sea surface at two stations: in Soline Bay and near Krčki most in Vinodolski kanal. Second time the samples were collected near Omišalj on the Island of Krk. The samples were collected using Manta net. The Manta net (same size as for the sea surface sampling) was attached to the drone and collected samples following the drone's trajectory. Samples were stored in glass jars and preserved with 96% ethyl alcohol. Analysis was performed in the laboratory by examining the samples under a stereomicroscope.

Table 20: Drone campaign.

Sampling location	Geographical coordinates	Dates of sampling
Uvala Soline	45.164073836 N; 14.61571733 E	27/10/2021
Vinodolski kanal - Krčki most	45.2484727 N; 14.5697527 E	27/10/2021
Omišalj	45.208086 N; 14.549098 E	04/05/2022

The main information related to the sampling carried out in the area of Rijeka with the integrated system of the marine drone are described in the following Table.

Table 21: Main chacacteristics of the marine drone campaigns in Rijeka area.

Date	Site	Holo-sensor	OBU	Mini-manta	Note
27/10/2021	Rijeka	X	X	X	Manta: 2 transect/15 and 30 min OBU: on board of the ship
04/05/2022	Rijeka		X	X	Manta: 2 transect/30 min OBU: mounted on marine drone

4.3.3 Biota

The samples of mussels were taken on the natural banks on four locations at the depth of 0.5 m – in Lopar on the Island of Rab, in Bakar, Smokvica and Stinica. In this area of Adriatic Sea there are no mussels farm, so the samples were taken from the natural banks by diving and picking them by hand. Bigger individuals were collected were possible. There were very small number of mussels in Stinica in spring 2022, so have been collected mussels in Smokvica instead. The samples were taken in winter and spring in 2022, except for Lopar, where the samples were taken during autumn 2021 and spring 2022. At each sampling site three to five kilos of mussels were taken. Several analyses have been carried out on these samples. Concentration of PAHs, PCBs and heavy metals was determined in the soft tissue of the mussels. The number of microplastics was determined in the samples from Stinica, Smokvica and Lopar.

Table 22: Mussels sampling locations.

Sampling location	Geographical coordinates	Date of sampling
Lopar	44.823901 N 14.752949 E	24/09/2021 02/02/2022
Bakar	45.306629 N 14.535454 E	08/02/2022 04/05/2022
Smokvica	45.086883 N 14.847759 E	03/05/2022
Stinica	44.726567 N 14.896468 E	03/02/2022

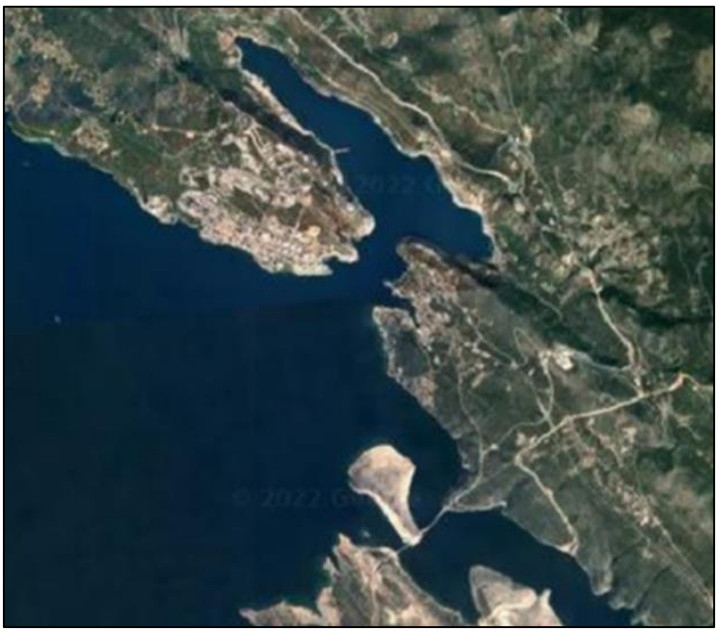


Figure 45: Bakarski zaljev – Bakar.

Figure 48: Sampling location of mussels in town Bakar.

Figure 49: Stinica

Figure 47: Sampling location of mussels in Stinica.

Figure 46: Sampling location of mussels in Smokvica.

Figure 50: Sampling location of mussels in Lopar, Island of Rab.

4.4 Test site 4: Split area (HR)

Several sampling campaigns have been carried out in 2020 and 2021 the Split area: sediment, sea water and biota. The activities have been performed by the Institute of Oceanography and Fisheries (IOR), collaborating with the Department for EU Projects Planning and Implementation, Public Institution RERA S.D. for Coordination and Development of Split Dalmatia County (RERA). In the following paragraphs the data collection activities on the beach and marine areas are described in detail. The main results of the data elaboration are included in D.4.2.2.

4.4.1 Sediment

Sampling of LMP microplastics from sandy beach sediments was conducted in June 2020, February/March, and September/October 2021 at two locations in the central Adriatic and two in the southern Adriatic (*Figure 10*). Station name, location and coordinates are given in *Table 23*. Sediment samples were collected according to a standard MSFD methodology, developed, tested and used in the Adriatic within the DeFishGear project, chapter "3.2.2 Sediment sampling of large microplastics (LMP) (1 mm - 5 mm)" from the protocol "Recommendation on regional approach to monitoring and assessment of microplastic in the marine environment" (Kovač Viršek et al., 2015). Sampling was conducted on sandy beaches above the wreck or intertidal zone/line by collecting certain volume of surface 5 cm of sediment and passing it through 5 and 1 cm sieves. Larger particles remained on the 5 cm sieve, while microplastic particles remained on the 1 cm sieve. Samples were stored in paper bags and properly labelled. Analysis is later performed in the laboratory by examining the sample under a stereomicroscope.

Table 23: Overview of research locations with corresponding station names and coordinates where microplastics from the beach sediment were sampled.

Sampling compartment	Area	Location	Station name	Station coordinates
Microplastics from the beach sediment	Central Adriatic	Duće beach	LMP-Duće	43.439131 N 16.675890 E
Microplastics from the beach sediment	Central Adriatic	Zaglav beach, Island of Vis	LMP-Zaglav	43.032804 N 16.228386 E
Microplastics from the beach sediment	Southern Adriatic	Komin estuary beach	LMP-Neretva	43.020281 N 17.448558 E
Microplastics from the beach sediment	Southern Adriatic	Prapratno beach	LMP-Prapratno	42.816926 N 17.676361 E

4.4.2 River and Sea water

Sampling of microplastics from the sea surface was conducted in July 2020, March/May, and September/October 2021 at four pre-determined locations near those where sediment microplastics were sampled. Station name, location, and coordinates are given in *Table 24*. Samples were collected following the methodology developed as a part of the DeFishGear project "Recommendation on a regional approach to monitoring and assessment of microplastics in the marine environment" (Kovač Viršek et al., 2015) by pulling the Manta net (mouth width 60 cm; mesh size ~300 µm) from the side of the vessel (m/b Navicula) for half an hour and collecting samples of the filtered surface water in a beaker at the bottom of the net. The collected material was stored in a glass container and preserved with 96% ethyl alcohol. The analysis was performed in the laboratory, by examining the sample under a stereomicroscope.

Table 24: Overview of research locations where microplastics were sampled from the sea surface, with the name of the corresponding station and coordinates of the start- and endpoint of the transect.

Sampling compartment	Area	Location	Station name	Startpoint transect coordinates	Endpoint transect coordinates
Sea surface microplastics	Central Adriatic	Hvar Channel	MikroP-Hvarski kanal	43.264389 N 16.359781 E	43.279281 N 16.389831 E
Sea surface microplastics	Central Adriatic	Brač Channel	MikroP-Brački kanal	43.415583 N 16.63115 E	43.421558 N 16.595147 E
Sea surface microplastics	Southern Adriatic	Neretva Channel	MikroP-Neretvanski kanal	43.02625 N 17.3714 E	43.040744 N 17.340592 E
Sea surface microplastics	Southern Adriatic	Mljet, SW side of the island	MikroP-Mljet	42.77355 N 17.316533 E	42.762144 N 17.3499 E

The main information related to the sampling carried out in the area of Split with the integrated system of the marine drone are described in the following Table.

Table 25: Main chacacteristics of the marine drone campaigns in Split area.

Date	Site	Holo-sensor	OBU	Mini-manta	Note
29/10/2921	Split	X	X	X	Manta: 2 transect/30 min OBU: on board of the ship

4.4.2.1 Marine drone field campaign

In the Split region the drone campaign was conducted on October 29, 2021. Microplastic samples were collected from the sea surface at two stations near the Katalinića brig (ST1) and Stobreč (ST2) sewage outflows. The samples were collected using two different Manta nets (*Figure 51*). The smaller Manta net was attached to the drone and collected samples following the drone's trajectory, while the standard Manta net (mouth width 60 cm; mesh size ~300 µm) collected samples in the usual way from the side of

the vessel following the drone. During sampling, a flowmeter was attached to a large Manta net so that we could measure the total volume of water that flowed through the net. Samples were stored on board in glass jars and preserved with 96% ethyl alcohol, while analysis was performed later in the laboratory by examining the samples under a stereomicroscope. Sewage outflow coordinates, as well as surface area and volume of filtered seawater of the investigated transects are given in *Table 26*.

Figure 51: Split area: map of sampled transects near Katalinića brig (ST1) and Stobreč (ST2) sewage outflows (yellow lines – microplastics transects; red dots – sewage outflows

Table 26: Overview of research locations during the drone campaign with the name of the corresponding station, sewage outflow coordinates, surface area (km2) and volume (L) of filtered seawater.

Sampling compartment	Area	Location	Station name	Sewage outflow coordinates	Surface (km^2)	Volume (L)
Drone campaign - sea surface microplastics	Central Adriatic	Katalinića brig	ST1	43.304722 N 16.463611 E	0,00076	123561,6
Drone campaign - sea surface microplastics	Central Adriatic	Stobreč	ST2	43.311389 N 16.445833 E	0,000797	129651,84

4.4.3 Biota

Biota samples were collected from a mussel farm and a natural habitat in the central (Site A) and also in the southern Adriatic (Site B). Site A included the area of Brač Island where live mussels (*Mytilus galloprovincialis*) were collected from the mussel farm in Maslinova Bay and the nearby Lučica Bay as their natural habitat. Site B included two sites in Mali Ston Bay, a mussel farm in Bistrina and a natural bank in Luka Bay on the Pelješac Peninsula (*Figure 52*). Biota sampling was conducted at two sites: Maslinova mussel farm and natural bank in Lučica Bay (Site A) and mussel farm in Bistrina Bay and natural habitat in Luka Bay (Site B). Mussels (*Mytilus galloprovincialis*) were sampled relatively close to shore, 0.02-0.03 nautical miles from the coast at Site A and 0.05-0.1 nautical miles from the coast at Site B (*Figure 52*). Approximately 5 kg of mussels were collected at each location using the methodology provided by the project partners. At the mussel farms, specimens were collected from pergolas at three different depths: surface, 1-2 m and 3-5 m depth. Because it was difficult to find mussels in their natural habitat, most specimens were collected from the underside of mooring buoys at 1 to 2 m depth. Specimens from mussel farms were generally larger (> 5 cm) than those from natural habitats. All specimens were collected by snorkelling and hand picking. Details of the locations, dates and quantities of mussels collected are provided in

Table 27.

The collected mussels were properly labelled and frozen as soon as possible, and as such delivered to the project partners in Rijeka for quantification of microplastics and in Teramo for chemical analysis of PAHs, PCBs, dioxins and heavy metals.

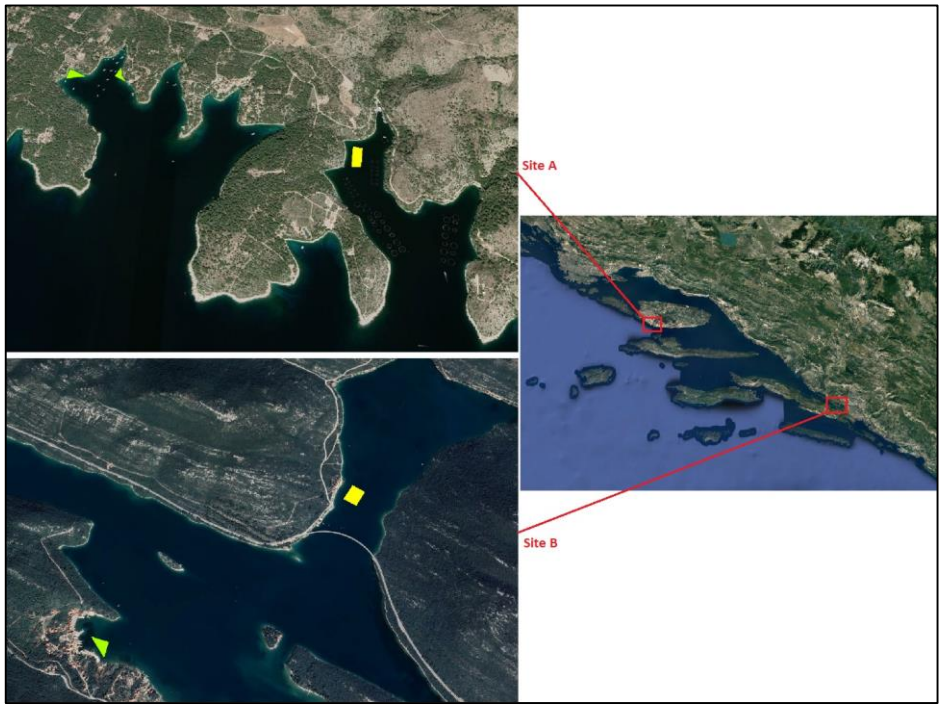


Figure 52: Map of Site A and Site B where mussels were sampled from mussel farms (yellow area) and natural habitats (green area).

Table 27: Overview of research locations during the drone campaign with the name and coordinates of the corresponding station, sampling dates, surface area (km2) and volume (L) of filtered seawater.

Area	Location	Station name	Station coordinates	Dates	Quantity for MP analyses	Quantity for chemical analyses	Quantity for electron microscopy
CA	Island of Brač, Maslinova bay	MF site A	43.304722 N 16.463611 E	7/7/21 19/1/22	2 x 3 kg	2 x 2 kg	6 individuals/ 36 tissue samples
CA	Island of Brač, Lučica bay	NB site A	43.311389 N 16.445833 E	7/7/21 19/1/22	2 x 3 kg	2 x 2 kg	3 individuals/ 18 tissue samples
SA	Mali Ston Bay, Bistrina bay	MF site B	42.870456 N 17.701556 E	27/5/21 20/1/22	2 x 3 kg	2 x 2 kg	6 individuals/ 36 tissue samples
SA	Mali Ston Bay, Luka bay	NB site B	42.862581 N 17.681864 E	27/5/21 20/1/22	2 x 3 kg	2 x 2 kg	3 individuals/ 18 tissue samples

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