multi-Risk sciEnce for resilienT commUnities undeR a changiNgclimate

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1. Technical references

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2. ABSTRACT

Deliverable (DV) 4.3.3 "Improved methodologies for contaminant monitoring in marine coastal area" represents the first document to be delivered in the frame of Task 4.3.2 "Contaminants and microplastic fate and transport in coastal and marine areas and their bioaccumulation and magnification: novel observation methodologies; modelling space-time distribution of emerging contaminants; bottom sea distribution and vertical fluxes of plastic; plastic food web; reducing uncertainty" of Work Package (WP) 4.3 in the vertical Spoke VS4 "Environmental degradation". DV4.3.3 describes the research activities and preliminary results achieved during the first 15 months of the Return Project.

In line with the aims of WP 4.3, activities envisaged in Task 4.3.2 are focused on the analysis processes related to pollutants distribution, transport, and their bioaccumulation and magnification in marine and coastal areas and related matrices (i.e., sediments, water column, and biota).

Task research activities take into account diverse categories of contaminants, with special attention to microplastic (MPs) and mercury (Hg). The content and behavior of bioplastics in marine water solutions are also themes under investigation. Furthermore, the analysis and the processes affecting the distribution of beach litter, which includes any persistent, manufactured, or processed solid material discarded, disposed or abandoned in the marine and coastal environment, are among the scientific topics included in the Task.

The suitability of innovative non-invasive, non-destructive sensing technology based on hyperspectral imaging, coupled with chemometric and machine learning strategies, has been tested for the rapid detection and classification of MPs and large polymers. In situ monitoring activities have been carried out using HSI sensors properly designed for field-scale applications.

During the first period of the project, performed activities have been focused on the definition of sampling procedures, to be tailored according to the specific characteristics of the investigated sites. Furthermore, a suite of modeling tools to simulate the pollutants' fate, transport, and bioaccumulation have been also set up.

In synthesis, the Task activities have been aimed at the following objectives:

- definition of novel observation and monitoring methodologies for supporting the assessment of coastal and marine environmental quality,
- development of analytical protocols,
- setting up of innovative procedures for modelling the transport and distribution of pollutants,
- implementation of in vitro and in vivo tests for the determination of pollutant bioaccumulation and biomagnification.

The Task activities will increase the overall knowledge of the various processes affecting the fate of contaminants in coastal and marine environments, which are crucial for assessing their environmental impact, biodegradability, and ecological consequences, and to plan tailored policy actions. Therefore, the multi-disciplinary monitoring of pollutants in the diverse matrices is a fundamental step for evaluating potential sources and supporting the identification of effective risk mitigation strategies, thus significantly contributing to the development of sustainable solutions for the protection of marine ecosystems and related services. I

Task participants, which belong to six research groups (OGS, UNIBA, UNIFI, UNIGE, UNIPA, and UNIROMA1), have contributed to the deliverable preparation by summarizing the proposed methodological approaches and their scientific preliminary results in the form of a dedicated thematic chapter. The document is therefore composed of six thematic chapters, each of them referred to a single research group. Ongoing collaborations and joint activities among two or more research groups are also highlighted in the text.

3. Table of contents

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4. Improved methodologies for contaminant monitoring in marine coastal area

4.1 Fate, transport, and bioaccumulation of contaminants: modeling tools for coastal- marine environments (OGS)

4.1.1 Introduction

Investigating the dynamics of contaminants from point and diffuse sources to living marine organisms requires a multidisciplinary approach. Deterministic models, by synthesizing the knowledge of environmental dynamics acquired from different disciplines into a holistic scheme described by mathematical differential equations, provide a rigorous framework to support the interpretation of field/laboratory data, test hypotheses about processes, and identify gaps in knowledge. In addition, based on the relationships described in the equations, models can complement field data by providing information on areas/periods that have not been directly assessed, including the future. Therefore, improving the capabilities to simulate contaminants behavior across environmental compartments is crucial to tackle ecosystems degradation and human exposure, and to predict possible implications of climate change.

OGS is developing a suite of modeling tools to simulate the fate, transport, and bioaccumulation of mercury (Hg) and other emerging pollutants in the Mediterranean Sea basin, focusing on the Adriatic Sea and the Venice Lagoon. Mercury cycling in the marine environment is a good example of the complexity and non-linearity of contaminants trajectories in the environment, and it is of particular concern due to microbial conversion of Hg into the neurotoxic species methylmercury (MeHg). MeHg is taken up by phytoplankton and efficiently transferred with trophic interactions, causing bioaccumulation (i.e., the concentration of contaminant in specimens increases with exposure time) and biomagnification in marine food webs (i.e., the concentration of contaminant in organisms tends to increase along with the trophic level) that ultimately result in global human exposure to mercury (Li et al., 2022). Hg cycling involves several interactions between the biotic and abiotic compartments so it can be anticipated that climate change will likely affect it both directly and indirectly (e.g., Cossa et al., 2022). The ongoing increase in radiative forcing driven by carbon dioxide emissions is already causing alterations in marine ecosystems that are particularly perceivable in shallow water environments such as coasts and lagoons, which are also known to be sinks for land-based emissions of contaminants from industrial and urban facilities. Along Italian coasts, several sites have accumulated substantial amounts of contaminants in the sediment due to historical pollution. Although in most cases the primary sources of pollution have been phased out, sediments that are highly enriched in contaminants can act as a secondary source of contamination to the water, depending on local environmental conditions, and may bioaccumulate in food webs with deleterious effects on wildlife and humans and possible loss of ecosystem services.

4.1.2 Case study description

The Mediterranean Sea is a semi-enclosed basin characterized by thermohaline circulation and a decreasing west–east gradient of primary production (Crise et al., 1999). Due to its enclosed nature, and the peculiar physical and biogeochemical conditions, the basin is particularly sensitive to pollution. It has been reported to have the highest density of plastic waste in the world (Suaria et al., 2016) and elevated Hg bioaccumulation, higher than adjacent systems such as the Atlantic Ocean (Chouvelon et al., 2018; Cossa et al., 2012) or the Black Sea (Harmelin-Vivien et al., 2009). Also, observed rates of climate change for the basin exceed global trends for most variables (Cramer et al., 2018).

Within the Mediterranean Sea, the Northern Adriatic Sea is characterized by the presence of macrotidal coastal lagoons, such as the Venice Lagoon and the Marano-Grado Lagoon, which offer nursery and refuge area for several marine organisms and seabirds supporting lively ecosystems. These shallow water ecosystems are closely connected to watersheds and exchange waters with the sea through lagoon inlets, thus also transporting biological and inorganic materials. The Venice Lagoon receives freshwater inflows from 12 main tributaries and exchanges water with the Adriatic Sea through three inlets. Its average depth is 1 m and the surface area is approximately 550 km^2. The lagoon ecosystem supports artisanal and small-scale fishery and is subjected to multiple anthropogenic pressures (Canu et al., 2010; Libralato et al., 2004; Solidoro et al., 2010). High loadings of various pollutants from the industrial area of Porto Marghera during the industrialization period resulted in their accumulation in sediment, posing a need for monitoring the risk for the ecosystem (Apitz et al., 2007; Bellucci et al., 2002; Masiol et al., 2014; Pavoni et al., 1987), particularly concerning Hg (Critto et al., 2005), for which substantial sediment enrichment and bioaccumulation is observed because of historical emissions from a chlor-alkali plant (Rosati et al., 2020).

4.1.3 Methodologies

2.1.1. 4.1.3.1 Analytical methods

A literature review was carried out to select a suitable food web model to simulate bioaccumulation in the Adriatic Sea, and to improve the understanding of key environmental processes concerning emerging pollutants, while for Hg -a persistent priority pollutant of global concern- and oil a strong expertise has been already gained in recent years.

4.1.3.2 Experimental methods

n.a.

4.1.3.3 Modelling approaches

The Ecopath with Ecosim suite (EwE; www.ecopath.org) is a software suite for developing food web models. The EwE suite is composed of 3 core modules: the mass balance model (Ecopath), the temporal dynamic (Ecosim) and the spatial-temporal 2D dynamic model (Ecospace) of the food web (Pauly et al., 2000). EwE can be used for analyses of marine ecosystems and trophodynamic and spatial simulations, and has been used globally to quantitatively describe aquatic systems and the ecosystem impacts of fishing (Christensen and Walters, 2004; 2015; Link and Marshak, 2022). EwE includes also a module for simulating the bioaccumulation of pollutants in the food web, called Ecotracer (Walters and Christensen, 2018). Given that EwE is also representing the fishing activities, moreover, Ecotracer allows to account for dilution, removal and accumulation also through this pathway, highlighting commercial fished species with potential critical contamination for the consumers. The EwE model for the Adriatic Sea (Libralato et al., in prep; Project FAIRSEA https://programming14-20.italy-croatia.eu/web/fairsea) will be used. This model describes the food web of the Adriatic Sea with 73 functional groups including mammals (4), sea turtle (1), seabirds (1), finfish (33), jellyfish (1), crustacean invertebrates (15), mollusc invertebrate (7), zooplankton (3), primary producers plankton (2), primary producers seagrass and seaweed (2), bacteria (1), fishery discard (1) and detritus (3). The model characterizes in detail the fishing activities in the Adriatic Sea and is fitted to 20 years of fisheries and biological data.

A numerical study on the fate and transport of mercury species in the Venice Lagoon was carried out by developing and applying a finite element coupled hydrodynamic-sediment-mercury model (SHYFEM-Hg) for simulating environmental dynamics at a fine spatial resolution. The model will be further coupled to a zero-dimensional model for bioaccumulation in the clam *R. philippinarum*, which is an important socio-economic resource for the area.

The SHYFEM-Hg model is a dynamic model simulating Hg and sediment dynamics coupled to hydrodynamic (Figure 4.1.1). The model is particularly suitable for coastal sites as it allows to resolve processes at a high spatial resolution and resolves the benthic pelagic coupling. The model is spatially discretized over an unstructured mesh of the Venice Lagoon with variable element resolution, ranging from about 10 m in the more morphologically complex areas and the channel systems to 1 km over homogeneous tidal flats. The model simulates the dynamics of three Hg species (oxidized Hg - HgII, methylmercury - MeHg, and elemental Hg - Hg0) dissolved or associated to three different kinds of particles (fine inorganic particles - silt, refractory organic matter, and labile organic matter) resolving biogeochemical transformations and transport of Hg species linked to sediment erosion and deposition and to water properties and transport. Biogeochemical transformations of Hg species involve both photochemical and biological processes and are parameterized as first-order kinetics corrected either for light availability or temperature (Melaku Canu et al., 2015). Modeled transport processes include loadings from the watershed and the atmosphere, volatilization of gaseous Hg0, tidal exchanges and internal hydrodynamics transport of dissolved and particulate Hg species, and deposition/resuspension of particulate Hg species. Due to the limited availability of observations, a non-synoptic setup was built to perform model calibration against field observations collected in the early 2000s of water concentrations of mercury and methylmercury (2001-2003) (Bloom et al., 2004) and suspended particulate matter (2005) (MAV-CVN, 2005). The model was initialized with spatially variable sediment concentrations of mercury and methylmercury collected in 2008 (Zonta et al., 2018) and forced with a meteorological and hydrodynamic setup of 2005. After calibration, a second run is performed to simulate a more recent year (2019). The modeled concentrations of mercury species in organic matter at sites intended for clam farming and harvesting will be extracted from the SHYFEM-Hg model and used to force the bioaccumulation model for the clam *R. philippinarum*, which is being developed by integrating uptake and excretion dynamics for Hg and MeHg into an existing clam bioenergetic model (Canu et al., 2011; Solidoro et al., 2000).

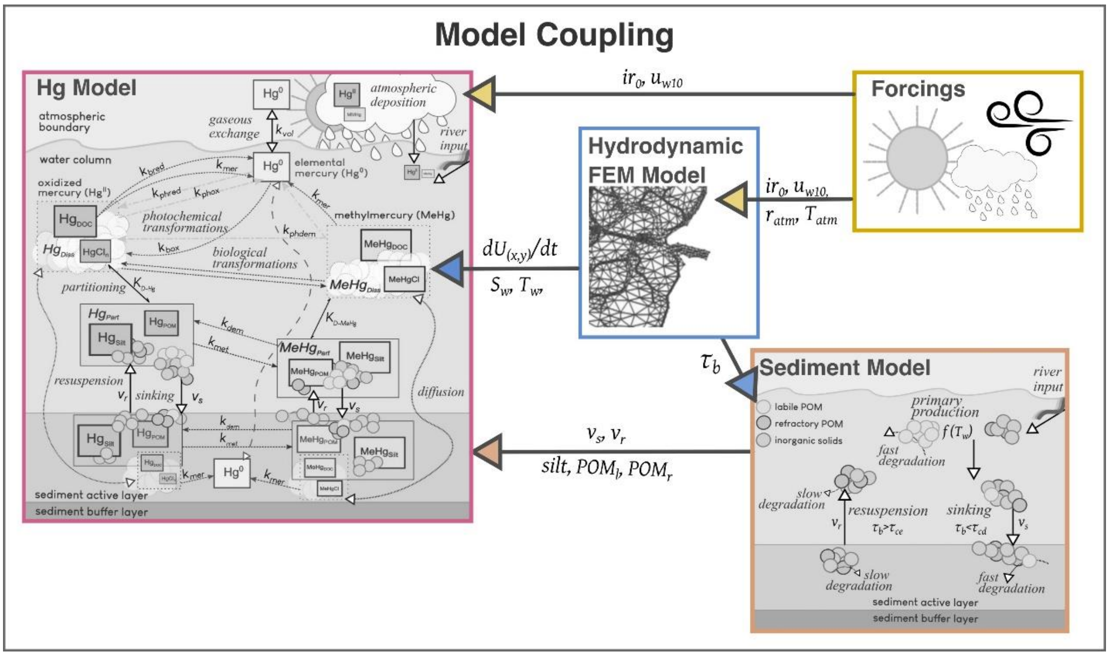

Figure 4.1.1: Scheme of the SHYFEM-Hg model showing the coupling among the hydrodynamic, sediment, and Hg modules, and the meteorological forcings applied.

A setup for the coupled OGSTM-BFM-Hg model (Rosati et al., 2022) was prepared to simulate long-term mercury dynamics in the Mediterranean Sea under climate change scenarios up to the end of the century. The climate forcings are based on Reale at al., 2022, Hg boundary conditions are from Rosati et al., (2022), and initial conditions are obtained from a 12-year model spin-up. The setup is stored in the HPC computing infrastructure Galileo100 maintained by CINECA.

The OGSTM-BFM-Hg model couples Hg biogeochemistry to the ecosystem-transport model OGSTM-BFM, a NPZD model simulating carbon and nutrient fluxes among environmental compartments up to the planktonic food web. The OGSTM-BFM has been used to investigate biogeochemical properties of the Mediterranean Sea (Cossarini et al., 2015, 2021; Lazzari et al., 2012, 2016, 2021) and potential impacts of climate change (Lazzari et al., 2014; Reale et al., 2022; Solidoro et al., 2022), and the OGSTM-BFM-Hg has been previously applied and validated for this study area. The key processes related to Hg species transformations and transport are resolved as for the SHYFEM Hg model, however, in this model more focus is given to the bioaccumulation in the plankton food web, while the benthic pelagic coupling is neglected, due to its limited impact in the open sea. The OGSTM-BFM-Hg model will be used to investigate the impact of climate change on Hg dynamics, under the two scenarios RCP4.5 and RCP8.5. Due to the complexity of the coupled transport-biogeochemical model, which numerically integrates a system of several partial differential equations, and the large number of uncertainty sources, including the prescribed initial and boundary conditions, it is likely that the model, just like any other model, would show some multi-year trend in some of its state variables when run over a period as long as 100 years (Solidoro et al., 2022) Therefore, a Control simulation is also implemented to quantify possible internal drift of the model due to the long term run and discern them from the climatic signal. In the Control simulation, the climatic forcings from the first 15 years are applied until the end of the century, and then the differences between the climate scenarios and the Control simulation are calculated to quantify the climate-related signal (Reale et al., 2022; Solidoro et al., 2022). The procedure to obtain the climatic forcings for the Control simulation has been improved by setting up an algorithm that randomly assigns each year of the 100-years series to a year from the 2005-2020 series. With respect to the method previously adopted, which recursively applied the first 15 years, the new procedure of random selection represents an improvement as it also removes the decadal variability.

4.1.4 Results

The Ecopath with Ecotracer model was identified as the most suitable food web model to simulate bioaccumulation at the state of the art. Ecotracer is fully coupled with the core modules of EwE and allows to simulate bioaccumulation of different pollutants (Booth et al., 2021; Li et al., 2022). For example, Ecotracer has been efficiently used for representing food web bioaccumulation of heavy metals such as mercury (Booth and Zeller, 2005), the dispersion in the food web of radioactive pollutants (Walters and Christensen, 2018) and the accumulation along the marine food chain of microplastics (Ma and You, 2021). The Ecotracer approach is based on the parametrization and dynamics of the food web from EwE but then it is highly flexible and adaptable to represent the direct uptake of the pollutant from the environment (water or sediment) from all the organisms of the food web, as well as the uptake through the food (via feeding interactions). Based on solid differential equation of direct uptake, food uptake, excretion, and reaction in the organisms, Ecotracer simulates toxicant-species specific accumulation processes (see Walters and Christensen, 2018).

The literature review highlighted that knowledge about environmental persistence, behavior and health effects is limited for most Emerging Contaminants (ECs), hampering environmental regulations. It also showed that modeling applications for ECs in marine ecosystems are scarce, except for plastics which has been investigated by various research groups (Everaert et al., 2020; Kaandorp et al., 2021; Tsiaras et al., 2021). For other compounds, only three relevant studies were identified based on research on Scopus. One study applied a hydrodynamic model to simulate the input of ECs from wastewater and their dispersal in Puget Sound (U.S.), relating distribution patterns to observed levels of ECs in wild mussels (James et al., 2020). The other studies developed coupled hydrodynamic-biogeochemical model to simulate the fate and transport of antibiotics and Bisphenol A in relation to detritus and phytoplankton dynamics, also considering degradation and conversion processes (Tong et al., 2021; Wang et al., 2024). The concept of "emerging contaminants" encompasses both contaminants that appeared only recently and contaminants that have been in the environment for a while but for which concerns have been raised much more recently (Sauvé & Desrosiers, 2014). ECs thus include various classes of compounds with different pyhsico-chemical properties, such as pharmaceuticals and drugs, plastic and plasticizers, pesticides, as well as new-generation flame retardants and short-chain PFAS synthetized to substitute more bioaccumulative and persistent pollutants such as PCBs and long chain PFAS (Christensen et al., 2022). One feature common to most EC is the inefficient removal from wastewater and water supply, due to the hydrophilicity of their polar molecules (Christensen et al., 2022; Patel et al., 2019).

We applied the SHYFEM-Hg model, recently developed at OGS, to simulate the spatio-temporal evolution of Hg species concentrations in the sediments, water, and porewater of the Venice Lagoon. The modeled seasonal distributions of Hg species in water and suspended particulate matter (SPM) for the year 2019 (Figure 4.1.2) highlight that MeHg dynamics differ from those of inorganic Hg. Inorganic Hg has a high affinity for particles (parameterized through a high partition coefficient K_D), which, in combination with the strong benthic-pelagic coupling that characterizes the lagoon, leads to a large spatial and temporal variability of concentrations. The spatial correlation between Hg^{II} and SPM is high and significant throughout the simulation, with a mean Pearson's $r = 0.67$ ($p<0.05$), indicating the importance of sediment deposition and resuspension dynamics in controlling Hg concentrations in water.

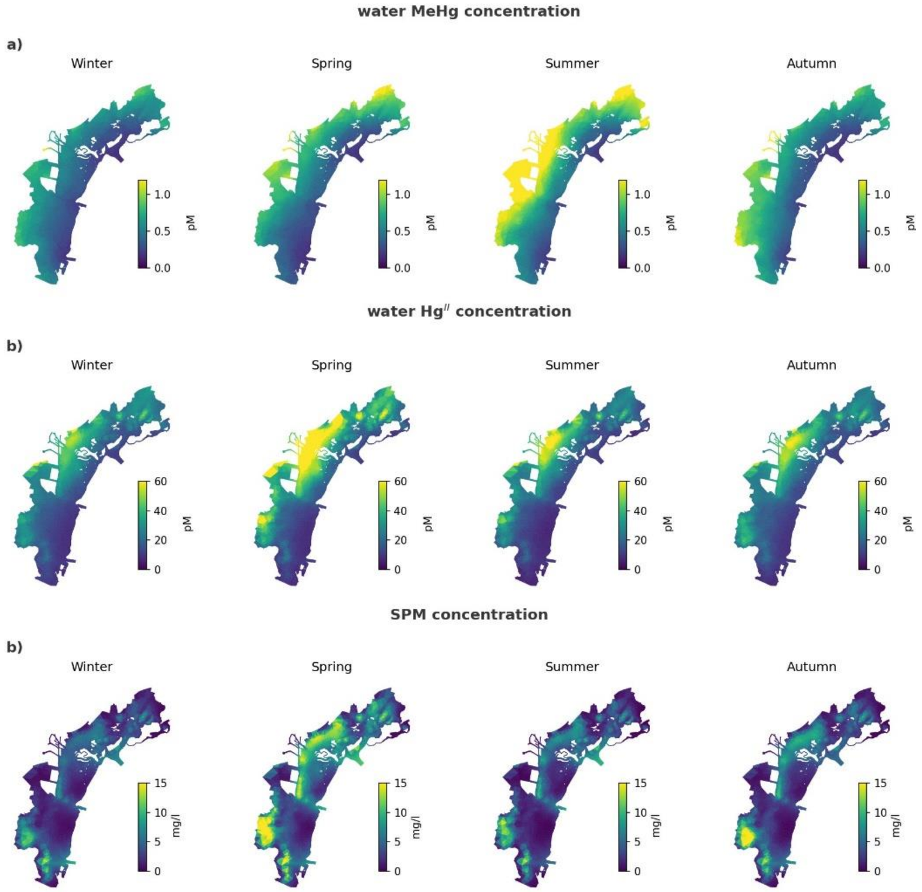

Figure 4.1.2: Modeled seasonal distributions of water concentrations of a) methylmercury (MeHg), b) inorganic mercury (Hg), and c) suspended particulate matter (SPM) in lagoon water simulated with the SHYFEM-Hg model for 2019.

In contrast, MeHg is less influenced by sediment resuspension/deposition dynamics than Hg^{II} (the correlation coefficient between MeHg and SPM is $r = 0.02$, $p>0.05$) and tends to have maximum concentrations in the inner parts of the lagoon due to the combination of river input and high-water residence time. The model was previously implemented for the simulation of the early 2000s to validate it with the available field data of Hg species (Bloom et al., 2004) and SPM (MAV-CVN, 2005). The modeled concentrations for the early 2000s agree satisfactorily with the observations of total Hg and methylmercury in water measured in different seasons from 2001 to 2003 (Figure 4.1.3).

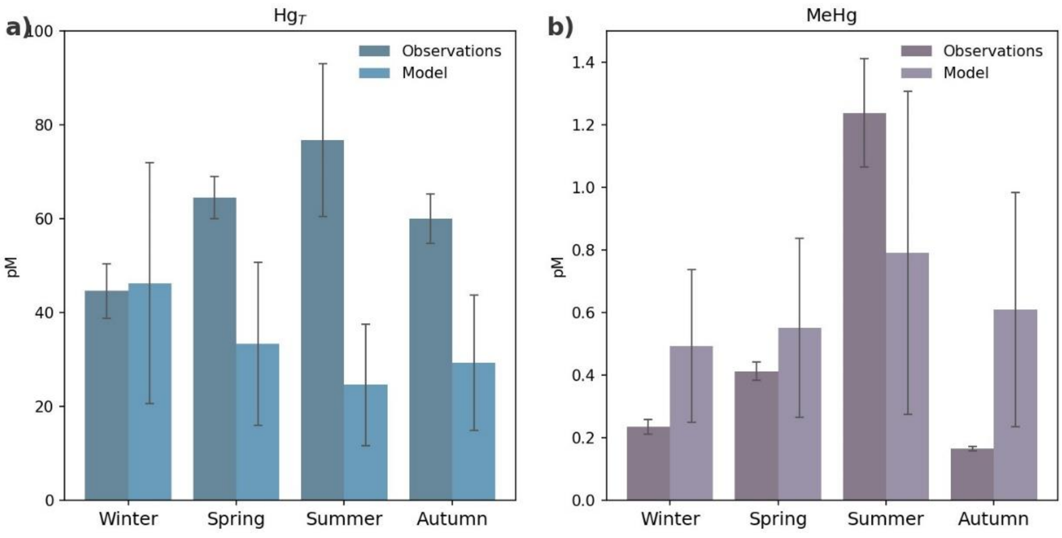

Figure 4.1.3: Comparison between modeled and observed (Bloom et al., 2004) water concentrations of HgT and MeHg in the northern stations of the Venice lagoon in different seasons (averages and standard deviation) for the early 2000s.

Overall, the total Hg concentration is slightly underestimated by the model, except in winter, where it is slightly overestimated (14%). The discrepancy is smaller in spring and autumn (-38% and -34% respectively) and largest in summer (-54%), due to the underestimation of concentrations of suspended particulate matter in this season (not shown). Indeed, sediment resuspension in the lagoon during summer is mainly caused by anthropogenic activities such as shipping (Bloom et al., 2004), which are not considered in the model. As for MeHg, the model underestimates water concentrations in summer by 35% (corresponding to an absolute error of 45 pM) and tends to overestimate concentrations in the other seasons (55% to 260%). The higher errors in predicting MeHg compared to inorganic Hg reflect the lack of mechanistic understanding of Hg methylation/demethylation processes, which are currently parameterized as first order reactions modulated by temperature (as a proxy for bacterial activity). The model also tracks changes in sediment Hg concentrations caused by the resuspension, transport and deposition of particles and associated Hg species. As a result of these dynamics, some areas, particularly those with the highest initial concentrations (Figure 4.1.4a,b), show a decrease in concentrations of total Hg and methylmercury of up to 20% (Figure 4.1.4d,e), while in areas where net deposition occurs over the course of a year, (Figure 4.1.4f) concentrations of Hg species tend to increase. The increase is strongest (40%) in areas of the southern lagoon where resuspension is limited by the presence of saltmarshes (Figure 4.1.4c). These model results are consistent over the simulations (early 2000s, and 2019), and agree with the observed long-term changes in Hg sediment concentrations (Bernardello et al., 2006; Molinaroli et al., 2013; Zonta et al., 2018).

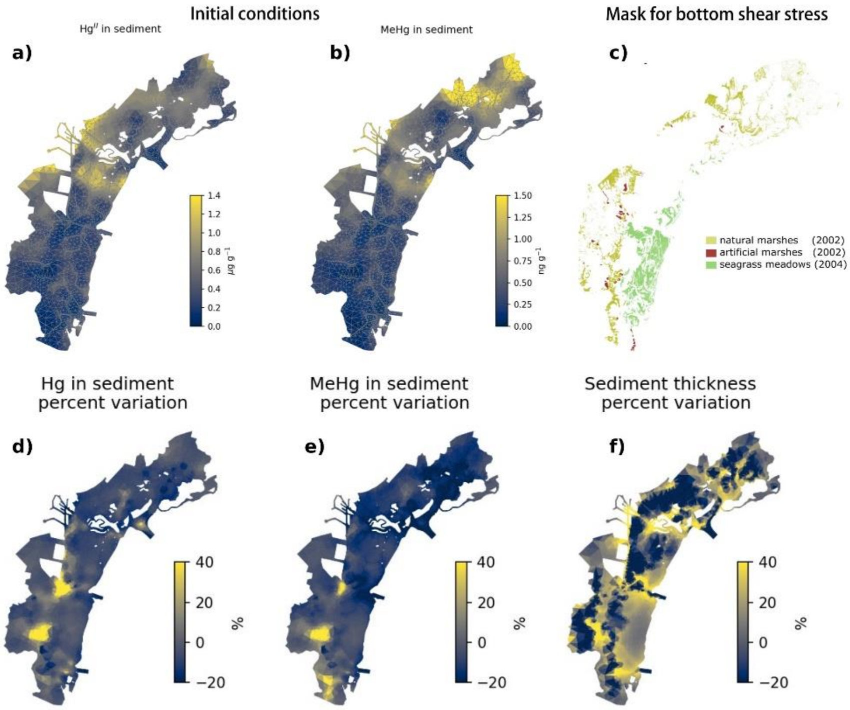

Figure 4.1.4: Maps of the spatial domain of the Venice lagoon in the SHYFEM-Hg model showing: the spatially variable initial condition for sediment concentrations of a) Hg^{II} ($\mu g\ g^{-1}$) and b) MeHg ($ng\ g^{-1}$), c) the mask of salt marshes and seagrasses applied to reduce sediment resuspension in vegetated areas, and the percentage variation in d) Hg^{II} and e) MeHg concentrations resulting from the f) morphological evolution of the seabed.

We prepared a setup to simulate the impact of climate change on Hg dynamics in the Mediterranean Sea with the OGSTM-BFM-Hg model (Rosati et al., 2022). The setup is aimed at running two simulations for the climate change scenarios RCP4.5 and RCP8.5 and one Control simulation. Preliminary tests have been run on the setup up to mid-century.

4.1.5 Scientific products and dissemination

Presentations at conferences and workshops:

Rosati G., Canu D., Lazzari P., Reale M., and Solidoro C. (2023). How will climate change affect mercury biogeochemistry in the ocean? Projected changes for the Mediterranean Sea under RCP4.5 and RCP8.5 emission scenarios. Goldschmidt Conference (9-14 July 2023, Lyon, France).

Rosati G., Canu D., Lazzari P., Reale M., Solidoro C. (2023). Mercury fate and transport in the Mediterranean Sea: current state and projected changes under RCP4.5 and RCP8.5 emission scenarios. ICRC-CORDEX 2023, International Conference on Regional Climate, (25-29 September 2023, Trieste, Italy).

Rosati G., Laurent C., Lazzari P., Aveytua Alcazar L., Solidoro C., Canu D. (2023). Developing new tools for contamination assessment and management in coastal-marine ecosystems. PredictOnTime workshop (11-13 May 2023, Lecce, Italy).

4.2 Analysis of plastics and microplastics distribution in coastal and marine environments (UNIBA)

4.2.1 Introduction

Marine and beach litter, which is defined as "any persistent, manufactured or processed solid material discarded, disposed of or abandoned in the marine and coastal environment" (UNEP, 2009), constitutes a constantly growing worldwide environmental issue for managers and researchers due to the million tons of litter entering every year oceans and seas from different sources. In this context, the high density of beach litter (BL) items and microplastics (MPs) pose a constant environmental risk to coastal areas and related ecosystems. Effective monitoring programs and management plans are therefore required to enhance coastal conservation and protection.

In this research, an integrated approach to identify and characterize BL (i.e., items >2.5 cm) along sandy beaches and MPs (i.e., particles < 5 mm) in coastal areas and marine sediments is proposed. Field surveys and laboratory analyses, coupled with the exploitation of UAV images, hyperspectral data, and machine learning tools, contribute to assessing both BL and MPs impacts on the coastal environments. Sampling procedures and analysis approaches applied to characterize the environmental status of the investigated coastal sites are schematically illustrated in Figure 4.2.1.

The assessment of BL distribution is carried out by applying both standard in-situ procedures and innovative methods based on the use of drones and imaging classification approaches (both manual and automatic) that solve the logistic limitations related to the in-situ surveys. Furthermore, the analyses are integrated with the assessment of the predominant morphodynamical processes (in terms of coastal erosion and/or accretion process), providing a useful informative layer for identifying coastal sectors prone to be affected by the accumulation and burial of BL items.

The assessment of the MPs content in coastal and marine sediments has required the definition of tailored sampling procedures. Furthermore, both standard (e.g., µRaman) and innovative techniques are applied for the MPs classification. As far as this last aspect is concerned, it is worth noting that during the first period of the project, UNIBA team started a research collaboration with UNIROMA1 team. Activities performed in the frame of such collaboration are focused on the testing of innovative procedures for automatic polymer classification based on the hyperspectral imaging (HSI) approach.

During the first period on the project, field and sampling activities were performed in different coastal sites in Apulia Region (Southern Italy).

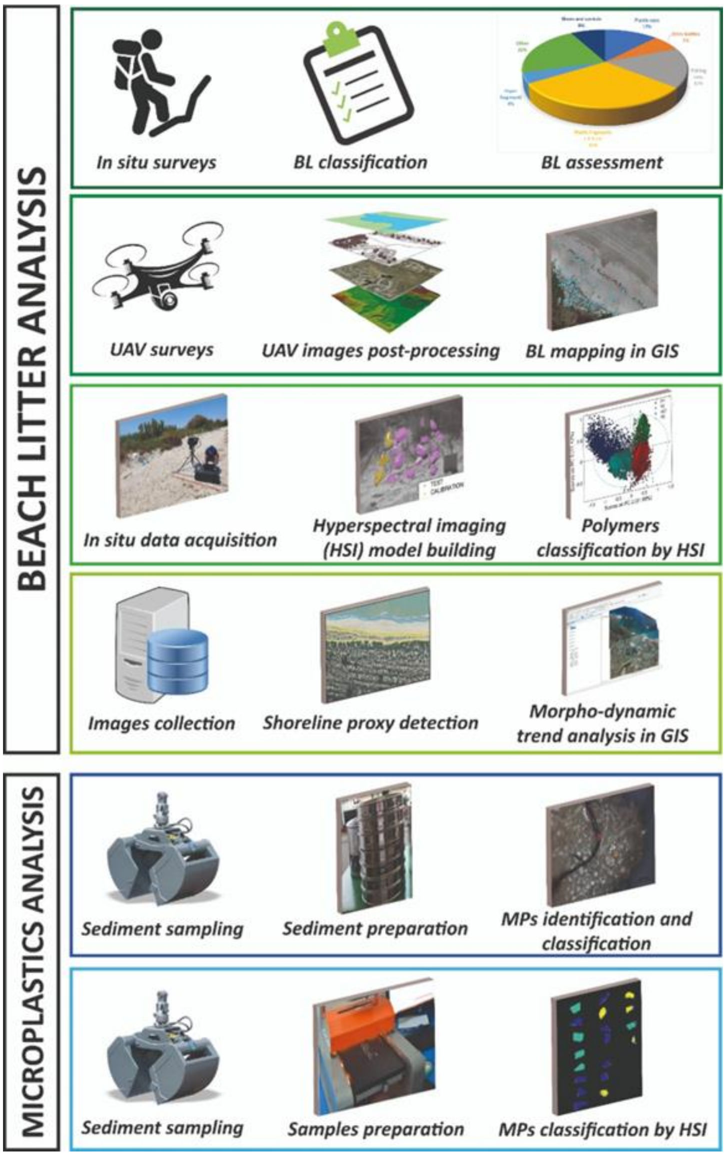

Figure 4.2.1: Schematic representation of the procedures proposed for the analysis and mapping of BL on sandy beaches and MPs in marine and coastal sediment samples. Polymer classification approaches are also indicated.

4.2.2 Case study description

Research activities were carried out in several coastal sites of the Apulia region (Figure 4.2.2). In detail, the BL analyses were performed on the Adriatic coast at Torre Guaceto beach (Brindisi province) while sediment samples were collected both in emerged and submerged environments, on the Adriatic side (Torre Guaceto beach and Adraitic offshore area) and the Ionian side (Gulf of Taranto) of the Apulian coast.

Torre Guaceto beach (Figure 4.2.2a, b) is included in a natural protected area established to preserve the local habitat biodiversity and protect a wide wetland separated from the sea by the dune system. The area was selected as test site since not subject to scheduled cleaning activities, due to the lack of touristic and recreational services. Furthermore, the investigated coastal sector is characterized by the presence of the mouth of "Canale Reale", which is a 48 km long creek that acts as a potential litter source, especially during the occurrence of heavy rain events (Galgani et al., 2015).

The Gulf of Taranto, and in particular the Mar Grande and the Mar Piccolo basins (Figure 4.2.2c), are semi-enclosed basins characterized by the presence of high-density anthropogenic activities, including industrial districts, shipyards and arsenals, and intensive mussel aquaculture plants, which have led to major environmental modifications. For this reason, the marine and coastal area of Taranto is included in the list of Sites of National Interest, for which urgent remediation activities are required.

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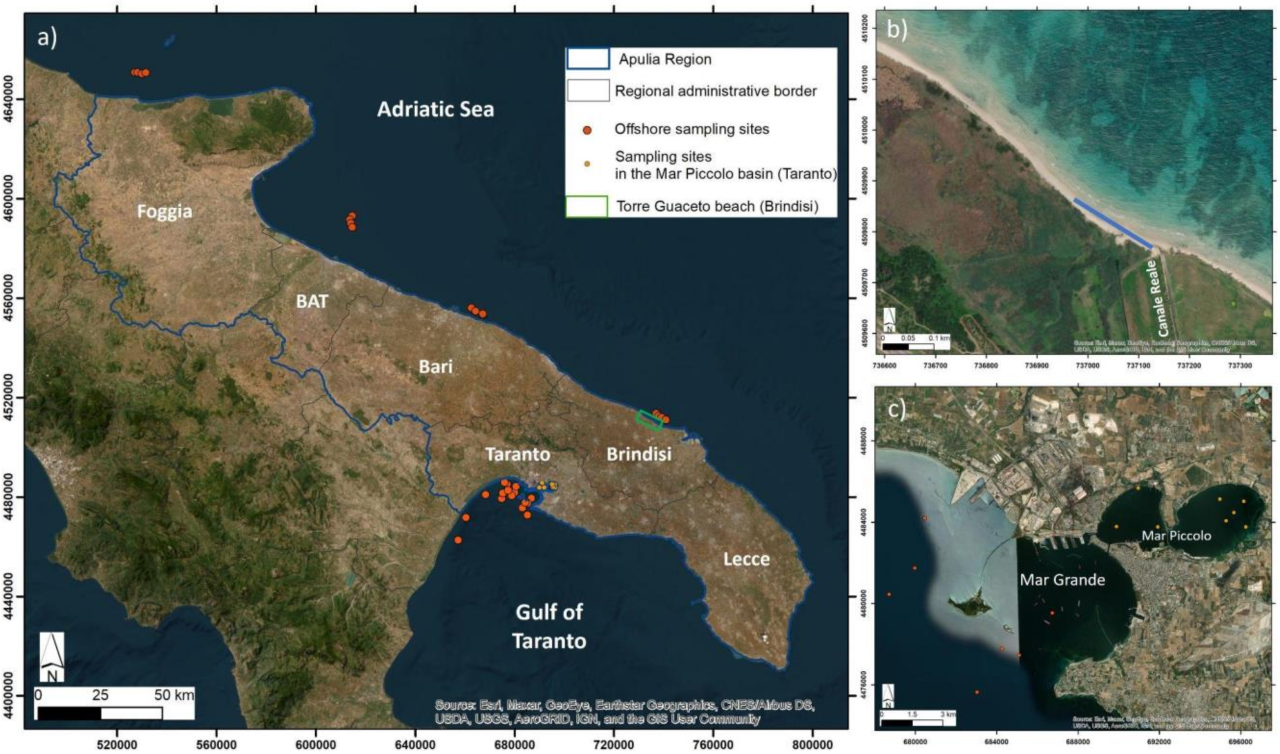

Figure 4.2.2: Study area location: a) investigated sites are located along both sides of the Apulian Region (on the Adriatic coast and in the Gulf of Taranto); b) the Torre Guaceto beach is located in the Brindisi province, in proximity of the Canale Reale mouth (the blue line identifies the investigated beach stretch); c) sediment sampling sites in the Mar Piccolo and Mar Grande basins (Taranto).

4.2.3 Methodologies

4.2.3.1 Analytical methods

The in-situ BL visual assessment was conducted following the international guidelines (Hanke et al., 2013; Vlachogianni, 2017), which suggest investigating a 100 m long coastal sector, from the shoreline to the inland natural or anthropic limit (cf. Figure 4.2.1). All BL items bigger than 2.5 cm were classified according to the Joint List of Litter Categories for marine macro-litter monitoring proposed by the European Commission (Fleet et al., 2021). In the case of the Torre Guaceto beach (cf. Figure 4.2.2b), litter abundance was assessed along the backshore area up to the foredune limit (Figure 4.3.3a). In-situ surveys were performed in spring, during favorable weather conditions.

To assess the MPs content in beach sediments, samples were collected in different points along the backshore. The sampling points were selected along transects perpendicular to the shoreline, at the morphological steps of the berms and at the base of the dune (Figure 4.2.3b). By using an ASTM Sieve (4000 microns), a first sieve was carried out directly on the beach to collect the sediment fraction representative of the MPs size.

The marine sediment samples were collected using a sediment grab installed on board of ships (Figure 4.2.3c). The offshore sampling activities have been supported by the Marina Militare Italiana (Italian Navy).

Offshore sampling sites have been selected based on the location of the main harbors and river mouths (Figure 4.2.2a). Sites in the Gulf of Taranto were selected based on a preliminary analysis of the marine substrate derived by the interpretation of morpho-acoustic data, while in the Mar Piccolo basin (Figure 4.2.2b,c) sites were identified mainly accounting for the distribution of the mussel farm plants, that limits the ship navigation.

Microplastics were analyzed on marine sediments collected in offshore sites. All samples were transported and stored at UNIBA laboratories. Analyzed samples were firstly weighted and then dried at temperature between 30 and 50° C for at least three consecutive days. The temperature range was set to avoid modifications in the polymers' physical and structural properties.

Sediment grain-size analyses were carried out by following international standard procedures. For the sieving, a set of ASTM sieves with meshes of ½ phi from 4 mm to the minimum granulometric fraction was used. Subsequently, each retained fraction was weighed and the results were processed with a specific application for Microsoft Excel (Gradistat© v8), which yield distribution cumulative curves, histograms, and statistical evaluation of the main textural parameters. Grain-size analyses of the fraction < 63 µm were conducted by the use of Coulter counter that works on dispersing samples.

Subsequently, the sediments were treated with 10% H_2O_2 solution in glass beakers for 24 h in order to remove the organic matter. Density separation of plastic particles was performed using a $ZnCl_2$ solution, which was added to the sediment up to 1 cm from the top of the beaker. After 24 hours, a progressive amount of $ZnCl_2$ solution was gently added to the solution to promote overflow of the suspended particles. The overflow solution was filtered through 0.8 µm paper filters and the collected particles were dried at 50°C, separated from the filter, and placed in a clean petri dish to be analyzed at optical microscope. MPs were isolated and divided according to shape (fibers, fragment, and film), size (LMPs and SMPs), and color (Vianello et al., 2013; Fastelli et al., 2016; Horton et al., 2017; Firdaus et al., 2020). Their concentration was calculated as the number of MPs (items) per kg considering the weight of the whole sample (hereafter MPs kg-1).

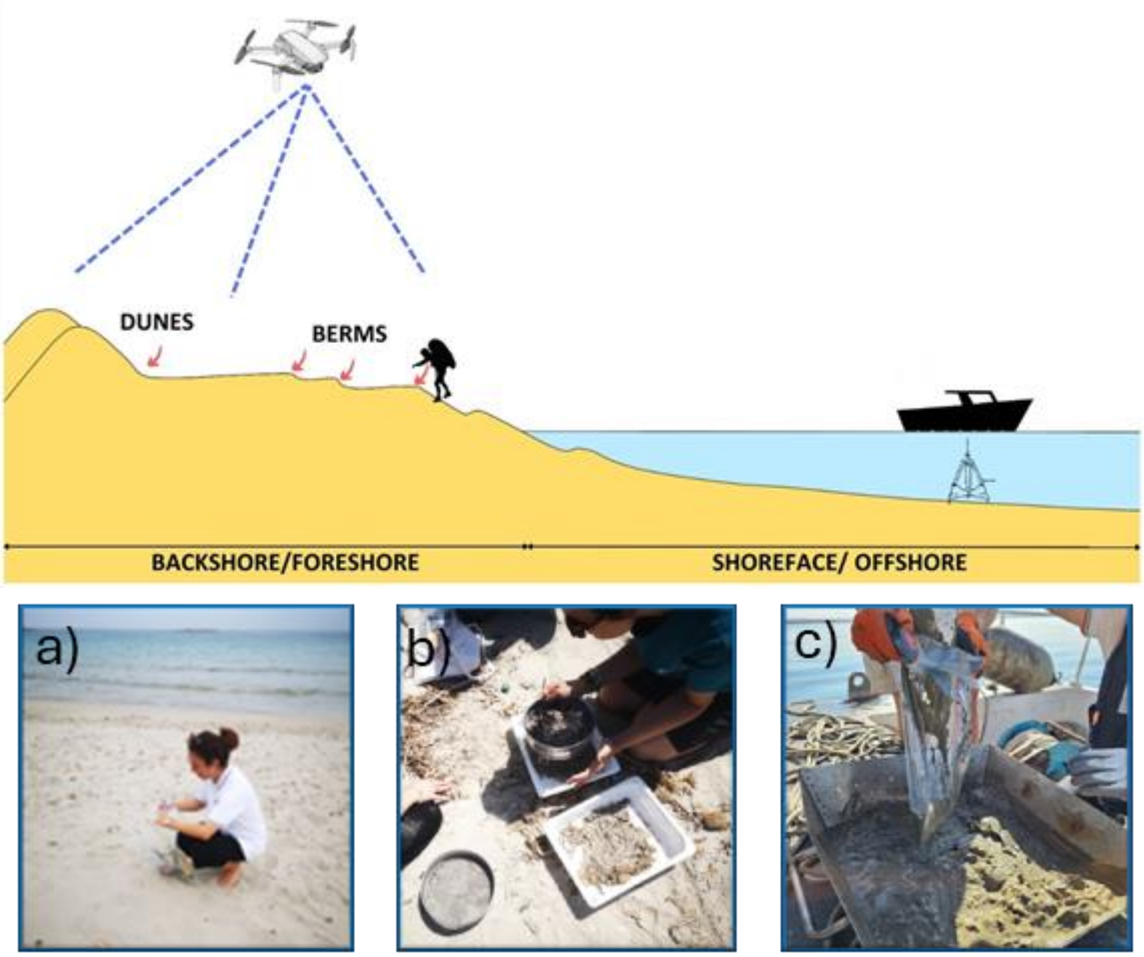

Figure 4.2.3: Sampling procedures: a) direct beach litter surveys were carried out along the backshore and foreshore sectors, whereas samples for MPs analysis were collected both in b) emerged (backshore) and c) submerged (shoreface/offshore) environments.

4.2.3.2 Experimental methods

A random subsample of MPs was selected to be analyzed by µ-FTIR and µ-Raman techniques. A Thermo Fisher Scientific µ-FTIR Nicolet iN10 MX, equipped with a mercury cadmium telluride detector was used. To automatically select, analyze, identify, and count the microplastics on the filter, the "Microparticle WIZARD function" of OMNIC Picta software was used. Firstly, a visual mosaic image was realized for the plastic debris on the petri dish. Then, all particles with size lower than 10 µm were analyzed. The software automatically collected one spectrum from each particle at a spectral resolution of 4 cm-1, averaging 16 scans for each spectrum, i.e. 5.58 s per spectrum. µ-Raman analyses of MPs were performed using a Thermo Fisher Scientific Nicolet DXR3TM Raman microscope, equipped with a 532 nm laser. To select, analyze, identify, and count the MPs present on the filter, the Particle Analysis function of the OMNIC TM Atlus software was used. First, the software acquires visual mosaic images, constructed from many individual fields of view, automatically recognizes particles, and acquires one spectrum from each. A library search algorithm based on the proprietary Thermo Fisher Scientific library is then used to identify all the acquired spectra. The power of the laser was set 10 mW on the sample and the exposure time was set at 3 s with three exposures. Innovative methods are also applied for the characterization of polymers identified in sediment samples and are based on the application of hyperspectral imaging (HSI) approaches, which are described in Thematic Chapter 4.6.

4.2.3.3 Modelling approaches

Innovative BL assessment procedures exploit the use of UAV photogrammetric images and their analysis. In detail, images were acquired by using a multi-rotor quadcopter "DJI Inspire 2" equipped with a "DJI Zenmuse X5S" optical camera (20.8 MP, DJI MFT 15 mm/1.7 ASPH supported lens, 4/3" CMOS sensor, FOV 72◦ and image resolution 5280×3956 pixel). To define the best setting, UAV surveys were carried out at different flight heights (ranging from 5 to 20 m). Furthermore, the high-precision georeferencing procedure was ensured by the use of the GCP coordinates collected on the field with vertical and horizontal accuracy of about 0.02 m and 0.01 m, respectively. To comply with monitoring guidelines, 100 m long sectors were surveyed. Then, image post-processing was executed using Agisoft Metashape Professional, which allowed for obtaining the digital elevation model and the orthomosaics of the investigated sites. BL identification and classification on orthomosaics were performed by applying both a manual visual screening and an automatic procedure based on the proposal of a tailored Machine Learning (ML) algorithm. The visual screening, carried out in GIS environment, allowed to obtain a database of detectable elements and to produce the distribution and density BL maps (cf. Figure 4.2.1)

For automatic identification and classification, an algorithm that exploits Mask Region-based Convolutional Neural Network (Mask-RCNN) was proposed (Scarrica et al., 2022; Sozio et al., 2023). Its architecture is based on Faster-RCNN and performs an instance segmentation based on a pre-training phase on the COCO dataset (Lin et al., 2014). The automatic identification was conducted by splitting the available dataset into training and test sets, which consisted of multiple tiles obtained from orthomosaics with associated polygon shapefiles, relative to the BL items. In detail, the training phase consisted of manually labeling litter objects on images and cataloging them in 5 classes. The manually labeled objects were used by the algorithm to produce a train model file, containing all information to differentiate objects of each class. In the testing phase, the algorithm exploited the train model file to automatically identify and classify objects on images not used before. To evaluate the performance of the proposed method, the mAP@IoU (Everingham et al., 2010) was used. This parameter expresses the overlapping area between the predicted masks produced by the algorithm and the manually digitized reference polygon (adopted as ground truth). Finally, the script of the proposed algorithm was also implemented with a further feature for georeferencing the segmentation outputs.

4.2.4 Results

During the most recent field activities conducted at Torre Guaceto beach in May 2023, before the high-season touristic period started to avoid the influence of beachgoers on litter abundance, both direct monitoring and drone surveys were performed. A total amount of 295 images were acquired and their post-processing allowed the definition of a RGB color scale orthomosaic with a resolution of 2.1 mm/pixel (GSD). Once the orthomosaic was imported into a GIS platform, the manual image screening was performed. This step led to identification of 382 BL items in a total investigated area of 1300 m^2. To this end, a dedicated database was created as a point shapefile. Each item was identified by a point manually inserted by the operator at the center of the item itself. Furthermore, items were briefly described and classified according to the most recent classification codes (Fleet et al., 2021) for the application of a common classification system. Considering all the items identified in the investigated area, a BL density of 0.29 items/m^2 was calculated (Rizzo et al., 2023). For what concerns the BL distribution along the beach profile, the results showed that the highest number of items (243 items, corresponding to 1.24 items/m^2) characterized the inner part of the beach, which identifies the area from the embryonic dune to the foredune limit while very few items (3) were detected on the upper part of the foreshore (beachface). This means that, excluding this section from the calculation, the BL density value estimated for the backshore area increased up to 0.48 items/m^2. The beach section between the first (i.e., the most recent one) to the last strandline/berm (i.e., the inner one), as well as the section from the last strandline up to the seaward limit of the embryo dune (here identified as the first line of incipient vegetation) were characterized by a similar BL distribution, with a density value of 0.20 items/m^2. Based on the acquired data, it emerged that the higher number of items is represented by plastic (336 items), followed by the glass and textiles categories (13 items in each category), metals (8 items), wood and paper (5 and 4 items, respectively) and, finally, rubber and not identified items (2 items in both cases). Plastic items represented 88% of all identified BL, being the most represented items included in the classes J79 (164 items), J45 (39 items), J8/J7 (37 items), J9 (30 items), J22/J23 (19 items). Regarding the polymer classification task, an innovative approach was tested during the field activities carried out at Torre Guaceto in May 2023. Details on this task are provided in Thematic Chapter 4.6, since these activities are coordinated by the UNIROMA1 team.

For what concerns the automatic BL item identification and classification, the comparative analysis between two machine-learning techniques (Mask-RCCN-based algorithm and Meanshift/SVM algorithms, available in QGIS) allowed the evaluation of performance, strengths, and limitations of both methodologies. The Mask-RCNN-based algorithm turned out to be the most suitable tool for the detection task, despite the need for future improvements. Ongoing activities are therefore focused on the increasing of automatic classification performance.

Grain size analysis performed on samples collected from the water/sediment interface did not show significant differences in sediment distribution between the semi-enclosed basin and the open sea. In detail, the samples from the First Bay of Mar Piccolo are characterized by very coarse silt and coarse sand, while samples collected in the Second Bay are characterized by slightly finer sediments, between medium silt and very fine sand. The Mar Grande/Gulf of Taranto samples' grain-size analyses reveal the presence of very fine sand. In general, the sediments appear poorly sorted, and the distribution curves are very coarse skewed, and very leptokurtic; it seems to be influenced by the presence of mussel thanatocenosis on the seafloor.

The analysis of MPs distribution in the Mediterranean basin was integrated with a literature review, which showed that the greatest concentration of MPs can be observed in the water column and marine sediments, especially in the offshore areas (Figure 4.2.4).

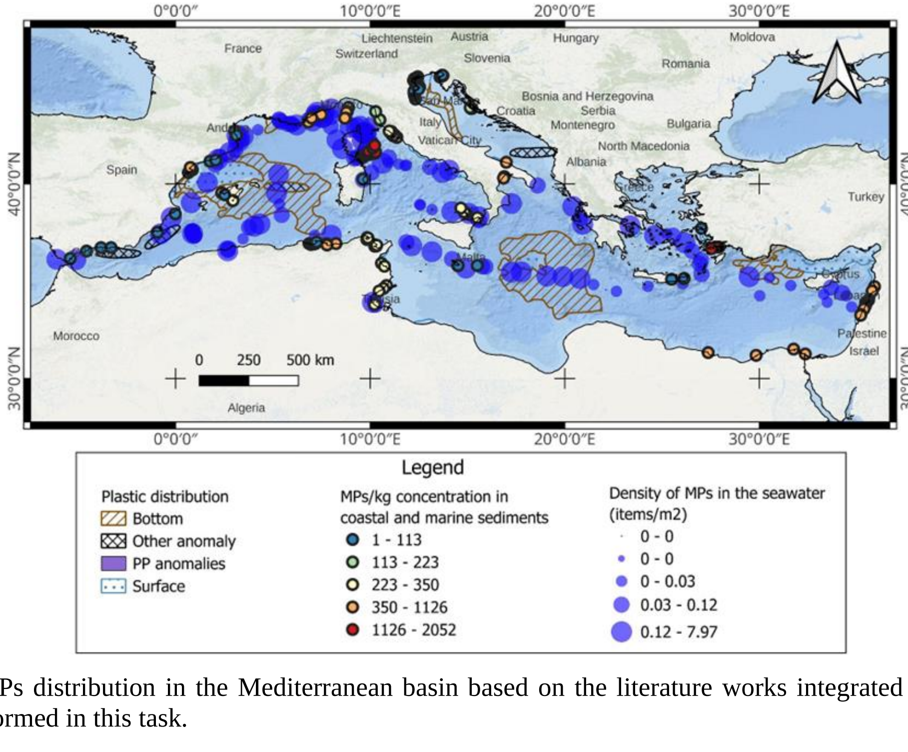

Figure 4.2.4: MPs distribution in the Mediterranean basin based on the literature works integrated with microplastic analysis performed in this task.

MPs analyses performed in this study revealed a high presence of fragments and films with size ascribed between 5 and 1 mm. In the offshore area of the Gulf of Taranto, the zone corresponding to the mouth of the Bradano river exhibits a concentration of 1246 MPs kg^{-1}, whereas sediments collected in proximity of the mouth of the Basento river exhibit a concentration of 581 MPs kg^{-1}; black fibers are most frequently observed, with a minor content of blue, red, and transparent fibers and an additional white fragment. Along the Adriatic Sea, sediment collected from the Bari offshore area exhibits concentrations of 209 and 257 MPs kg^{-1}, with a prevalence of black, red, and blue fibers, but also with films and fragments. The results of the μ-FTIR and μ-Raman analyses revealed that a large proportion of MPs in sediment probably originate from textile fibers. According to De Falco et a. (2019), many microfibers of cellulosic composition are released during the washing of clothes made with a blend of polyester and cellulose.

Polymers detected in the Apulian marine sediments seem to be related to clothing production, suggesting that textiles from wastewater discharge are a major cause of MPs pollution in inland and offshore areas. Finally, the polymer identification task on beach sediment collected at Torre Guaceto beach is under development by UNIROMA1 team.

4.2.5 Scientific products and dissemination

Published papers:

Anfuso, G., Álvarez, O., Dilauro, G., Sabato, G., Scardino, G., Sozio, A., & Rizzo, A. (2024). A First Attempt to Describe the Real-Time Behavior and Fate of Marine Litter Items in the Nearshore and Foreshore under Low Energetic Marine Conditions. Water, 16(3), 409.

Cofano, V., Mele, D., Lacalamita, M., Di Leo, P., Scardino, G., Bravo, B., ... & Capolongo, D. (2023). Microplastics in inland and offshore sediments in the Apulo-Lucanian region (Southern Italy). Marine Pollution Bulletin, 197, 115775.

Marsico, A., Rizzo, A., Capolongo, D., De Giosa, F., Di Leo, A., Lisco, S., ... & Scicchitano, G. (2023). Spatial Distribution of Trace Elements in Sub-Surficial Marine Sediments: New Insights from Bay I of the Mar Piccolo of Taranto (Southern Italy). Water, 15(20), 3642.

Rizzo, A., Sozio, A., Anfuso, G., La Salandra, M. (2023). The Use of UAV Images to Assess Preliminary Relationships between Spatial Litter Distribution and Beach Morphodynamic Trends: The Case Study of Torre Guaceto Beach (Apulia Region, Southern Italy). Geografia Fisica e Dinamica Quaternaria 2022, 45, 237–250

Sozio, A., Scarrica, V. M., Aucelli, P. P., Scicchitano, G., Staiano, A., & Rizzo, A. (2023). Comparing Meanshift/SVM and Mask-RCNN algorithms for beach litter detection on UAVs images. In 2023 IEEE International Workshop on Metrology for the Sea; Learning to Measure Sea Health Parameters (MetroSea) (pp. 483-487). IEEE.

Presentations at International Conferences:

Bonifazi, G., Capobianco, G., Cocozza, P., Mastronuzzi, G., Rizzo, A., Serranti, S. (2023). Identification of plastic debris on beaches by ground-based hyperspectral imaging. Presented at the International conference ECDS 2023: Emerging Concepts & Design for Sustainability (8-11 October 2023, Villers-sur-Mer, Calvados, France).

Cocozza, P., Serranti, S., Setini, A., Cucuzza, P., Bonifazi, G. (2023). Monitoring of contamination by microplastics on sandy beaches at Vulcano island (Italy) by different spectroscopic techniques. Presented at the 5th International Conference on Pollutant Toxic Ions and Molecules (6-9 November 2023, Caparica, Portugal). The poster is winner of the Best Poster Award.

Sozio A., Scarrica V.M., Aucelli P.P.C., Scicchitano G., Staiano A., Rizzo A., (2023). Comparing Meanshift/SVM and Mask-RCNN algorithms for beach litter detection on UAVs images. Conference paper at 2023 IEEE International Workshop on "Metrology for the Sea", (4-6 October 2023, La Valletta, Malta).

Abstracts accepted for presentations at International Conferences:

Bonifazi, G., Cucuzza, P., Lisco, S., Marsico, A., Mastronuzzi, G., Rizzo, A., Serranti, S. (2024). Application of hyperspectral imaging and machine learning for the automatic identification of microplastics on sandy beaches. SPIE Defence + Commercial Sensing conference (21-25 April 2024, Maryland, United States). Abstract accepted.

Cucuzza, P., Gorga, E., Serranti, S., Rizzo, A., Lapietra, I., Mastronuzzi, G., Mele, D., Bonifazi, G. (2024). Hyperspectral imaging applied to monitoring microplastics collected from sediments of Mar Piccolo basin (Taranto, Southern Italy). 11th International Conference on Sustainable Solid Waste Management (19-22 June 2024, Rhodes, Greece). Abstract accepted.

Liso, I.S., Balestra, V., Didonna, F., Fiorillo, F., Maurano, F., Rizzo, A., Vigna, B., Parise, M. (2024). Italian research initiatives on microplastics in caves and karst. 7th EuroSpeleo Symposium (9-13 September 2024, Kosice, Slovakia). Abstract accepted.

Rizzo, A., Sozio, A., Anfuso, G., La Salandra, M., Staiano, A., Sasso, C. (2024). Analysis of the interactions between coastal morphodynamical processes and Beach Litter distribution. EGU General Assembly 2024 (14-19 April 2024, Vienna, Austria). Abstract accepted.

Sozio, A., Rizzo, A., Scarrica, V.M., Aucelli, P.P.C., Anfuso, G., Barracane, G., Dimuccio, L., Ferreira, R., La Salandra, M., Staiano, A., Tarantino, M. P., Scicchitano, G. (2024). An innovative SAM-ViT based tool for the automatic detection of litter items on sandy beaches. EGU General Assembly 2024 (14-19 April 2024, Vienna, Austria). Abstract accepted.

Dissemination:

Project activities have been presented at:

- AIGEO (Associazione Italiana di Geografia fisica e Geomorfologia) workshop (Napoli, 24 June 2023)
- the European Researcher's Night (ERN) (Taranto, 29 September 2023) https://taranto.ern-bari.it/eventi/le-geoscienze-per-la-tutela-dellambiente-costiero/
- Return Project workshop (Torino, 1-2 February 2024).

4.3 Enhanced sample treatment for microplastic monitoring in the Mediterranean basin (UNIFI)

4.3.1 Introduction

The presence of microplastics (MPs) in marine ecosystems is a global environmental issue. The main negative effects associated with this type of pollution can affect fauna and vegetation that reside in this habitat and, through ingestion, pose a threat to human health. Especially in marine environment, MPs can easily be mistaken for natural preys by marine fauna and consequently be ingested, posing serious problems to their health (Clause et al., 2021). These effects can occur physically (choking, false sense of satiety) or chemically, through the release of toxic compounds. Concerning the chemical risk, MPs can adsorb other toxic chemicals, already present in the aquatic environment or contained in the plastics themselves (e.g., additives, dyes, flame retardants), which can be released into organisms once ingested (Hahladakis et al., 2018).

The Mediterranean Sea, due to its particular semi-enclosed conformation, acts as an excellent reservoir for MPs and other contaminants, highlighting why it is important to monitor their presence and the effect they may have (Santini et al., 2022). Most of the MP load in the Mediterranean basin is due to the population density of the coastal areas and the transport due to major rivers that discharge into the Mediterranean Sea (e.g., Po, Nile) (Liubartseva et al., 2018; Prevenios et al., 2018). In particular, the investigation of MP ingestion by marine species, especially edible ones, is essential to increase awareness of the impact of this contamination on the marine ecosystem and to achieve a more sustainable use of marine biological resources.

4.3.2 Case study description

In collaboration with the MICROPLASMED project, UNIFI investigated the distribution of MPs in water and commercial fish species, such as the red mullet species (*Mullus Barbatus*). Samples were collected at several sites in the Mediterranean basin, corresponding to the main commercial ports. Red mullet specimens were chosen because they are ubiquitous species in the Mediterranean basin, detritivores and considered as delicacies in the Mediterranean diet. The potential ingestion of MPs by these species was assessed by examining their digestive tract. The validation of an optimal digestion method for biotic matrices was also evaluated, to enable chemical characterization and avoid possible interference due to the biological matrix. The comparison of MP abundances in water and fish samples will be crucial in understanding the actual level of pollution in the Mediterranean basin, and to enable the implementation of a risk analysis concerning the ingestion of MPs by red mullet. Furthermore, the risk assessment associated with MPs ingestion by fish will be indicative of possible effects on human health through trophic chain.

4.3.3 Methodologies

4.3.3.1 Analytical methods

Biotic samples digestive tracts were treated with an oxidative approach, validating the method in terms of recovery and repeatability. For both samples, seawater and fish, the abundance and physical properties of the polymeric items found were assessed. Through chemical characterization, it was possible to identify the typology of polymers and suggest a possible source of their release. A protocol for contamination control was followed throughout the whole process of sample treatment and analysis.

4.3.3.2 Experimental methods

Development of a digestion method for biotic matrices with a double oxidative step and filtration. Direct filtration of an aliquot of seawater samples. All filters were subjected to stereomicroscope overview, where items were classified by shape, color and dimension. The chemical characterization was performed through micro-FTIR (2D imaging Fourier Transform Infrared spectroscopy coupled with microscopy).

4.3.3.2 Modelling approaches

n.a.

4.3.4 Results

Abundances of MPs vary significantly among sampling sites, both for water and fauna samples. The higher abundances can be correlated with commercial ports with a high population density and where maritime trade is particularly high. Chemical composition found in waters and digestive tracts of *Mullus Barbatus* will contribute to understanding the impact of MPs pollution in the Mediterranean basin and on the marine microbiota, contributing to assess the potential implication for fish species like the red mullet.

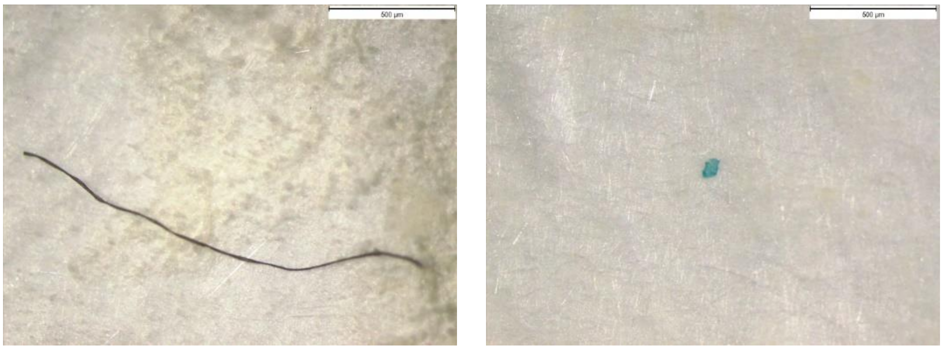

Figure 4.3.1: Stereomicroscope images of a fiber (left panel) and a fragment (right panel) found in the digestive tract of a red mullet specimens collected from two sampling sites.

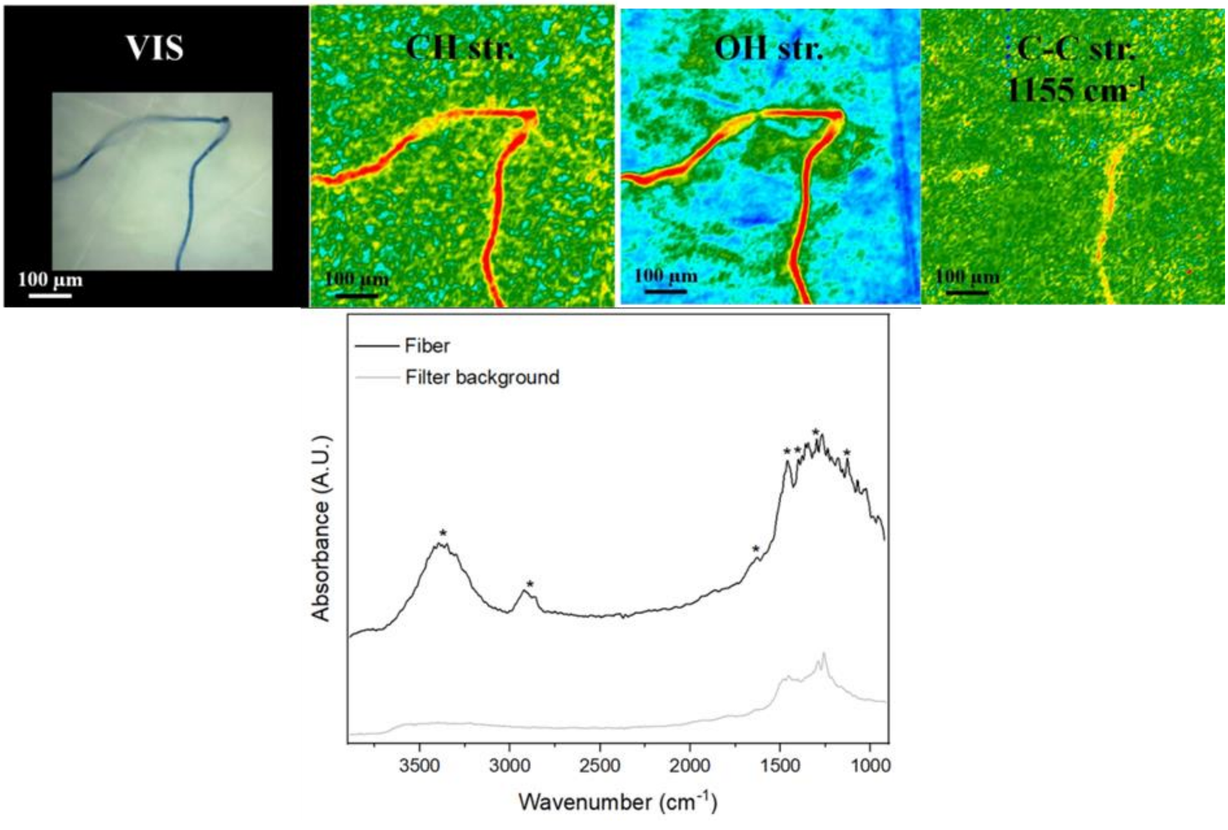

Figure 4.3.2: Example of data obtained with micro-FTIR analysis in a water sample. Top panels: Visible light map and 2D imaging maps of characteristic absorption frequencies of a fiber identified as cellulose. (Bottom panel: Spectrum of the fiber compared to that of the filter background. The stars in the spectrum indicate the characteristic absorption peaks that allowed for the chemical characterization.

4.3.5 Scientific products and dissemination

n.a.

4.4 Monitoring microplastics in bottom sediment and water column (UNIGE)

4.4.1 Introduction

Microplastics (MPs) are emerging pollutants that are widespread in all environments, land (Dioses-Salinas et al., 2020), air (Zhang et al., 2020), sea (Ruiz-Orejon et al., 2018), and biota (Lusher et al. 2017). Consequently, MPs are now monitored in all environments, including ports, and in all matrices to quantify their presence and determine their polymers to get a realistic picture of the contamination and thus attempt to find possible mitigation solutions, and also to study the problems related to their presence in the environment (damage to marine organisms, transport of other pollutants, etc.).

In the global scenario of MPs monitoring, a continuous effort is spent by the scientific community to find shared and universal sampling and analysis protocols that are applicable to all and that make the results obtained in different parts of the world reliable and comparable.

With regard to marine matrices such as water and sediments, various sampling techniques exploit oceanographic instruments commonly used to study other parameters (Manta net, Niskin bottle, etc.) and that are adapted to the MP sampling (Cutroneo et al., 2020). Instruments adapted for MP sampling are often not applicable in the context of closed basins, such as ports, where the presence of piers and ship traffic make their use impossible. For example, the Manta net, which is towed by a boat along transects for the sampling of plastics floating on the surface, requires large straight spaces that are often not present in port basins. For this and other reasons, such as the fact that many of the canonical instruments are made of plastic materials, new instruments dedicated exclusively to MP sampling have been studied and designed in recent years. An example is the prototype created within the recent European project titled SPlasH! "Stop to Plastics in H_2O!" (Interreg Italy-France Maritime 2014-2020 program) for the MP sampling at different depths in the water column inside a port.

The methodologies for the analysis of items sampled in seawater and sediments and the detection and recognition of plastic polymers, in most cases, involve a first step of classification of items under an optical microscope, and the use of two internationally established techniques: the Fourier-transform infrared (FTIR) spectroscopy and the Raman scattering spectroscopy (Chen et al., 2020; Liu et al., 2023). Both microscope analysis and the two techniques require the use of a long time. In fact, both FTIR and Raman spectroscopy involve the analysis of every single item found and isolated (or at least a representative percentage of them such as 20%; Galgany et al., 2013). This does not allow for a picture of the MP contamination in the short term. Therefore, the study of more rapid and automatic techniques is the subject of attention in the global panorama of research applied to MPs.

This chapter presents the MP sampling and analysis protocol chosen after an analysis of the main and most recent scientific bibliography by UNIGE and applied to the pilot site of the Port of Genoa.

4.4.2 Case study description

The Port of Genoa (Liguria - Italy) stretches continuously for 22 km along the coast, with a total area of 6 million m^2 and 14.5 million m^2 of water surface, and with 25 terminals equipped to accommodate all types of ships for all types of goods (containers, miscellaneous goods, perishable products, metals, forestry, solid and liquid bulk, and petroleum products) and passengers, with a full range of complementary services (ship repairs, ship fitting out, etc.). The Port of Genoa is a complex basin in which commercial, industrial, recreational and port service functions coexist; it is a leader in the traffic of conventional goods, transported by ships with differentiated characteristics (traditional, ro-ro, specialized). In recent years, it has undergone changes aimed at implementing the growing containerization market as well. Among these important changes is first and foremost the construction of the new breakwater of the port (infrastructure financed under the PNRR) which will base on a seabed of approximately 50 m of depth and which will make the Port of Genoa an open-air laboratory.

Because of these characteristics, there are many possible sources of pollution in the port waters and sediments, including plastic pollution. One contributor certainly comes from rain that causes runoff from the streets of the city of Genoa overlooking the port, and, within the port, from all the commercial and industrial activities. In addition, the Port of Genoa receives the water of two important streams (Polcevera and Bisagno Streams) and numerous minor streams and urban discharges.

Two sites within the Port of Genoa were chosen for water and sediment sampling for the quantification of MPs: FL and FP, at the eastern and western entrance to the port, respectively (Figure 4.4.1). These two sites are also involved in other activities, including the environmental monitoring of the construction of the new breakwater of the port, and the activities for the proposal of new monitoring protocols for multiple inorganic and organic contaminants in other RETURN tasks (VS4 WP 4.2). At the two chosen sites, there are two fixed monitoring stations equipped with current meters ADCP and multiparametric probes CTD that continuously record data on dynamics, turbidity, and temperature of the water masses. This information is useful to understand the influence of both the two nearby streams and the two entrances to the port in the MP distribution inside the port.

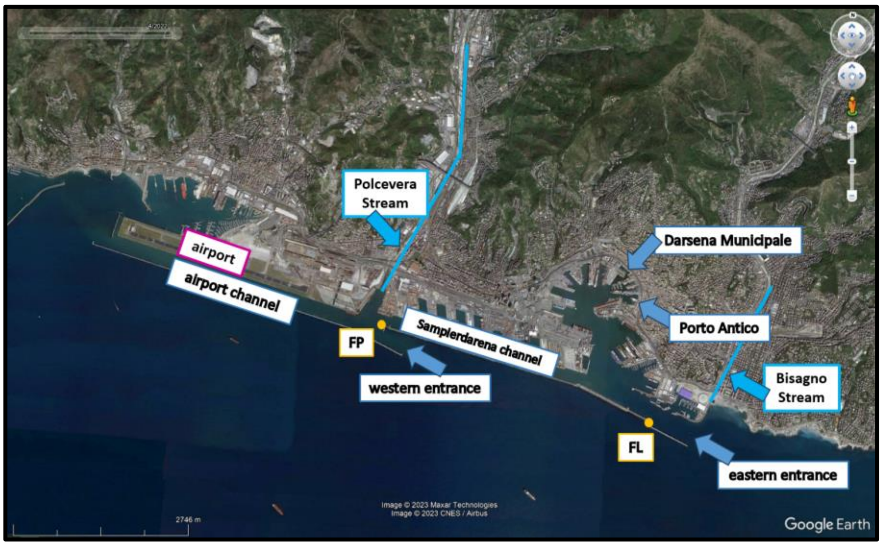

Figure 4.4.1: The Port of Genoa and localization of the two monitoring fixed stations FL and FP.

4.4.3 Methodologies

4.4.3.1 Analytical methods

For MP sampling in the water column, UNIGE chose to capitalize on the prototype created in the European Project SPlasH!, implement, and use it in the MPs monitoring in the Port of Genoa. The modified SPlasH! instrument (Figure 4.4.2) was assembled and consists of:

- an immersion pump for lifting seawater up the water column, with adjustable flow, maximum depth 20 m, and 2-mm impurity inlet grid;
- flexible hose;
- termination with metal funnel, inverted;
- stainless steel frame with support rings for sieve battery;
- a counter for determining the liters filtered;
- metal sieve battery, diameter 10 cm, with decreasing mesh size of 2000, 1000, 500, 250, 125, 63, 32 µm.

The material required for sampling is as follow:

- pre-filtered water to rinse the sieve and collect all the items retained by the sieves;
- glass jars rinsed with filtered water, to collect the items.

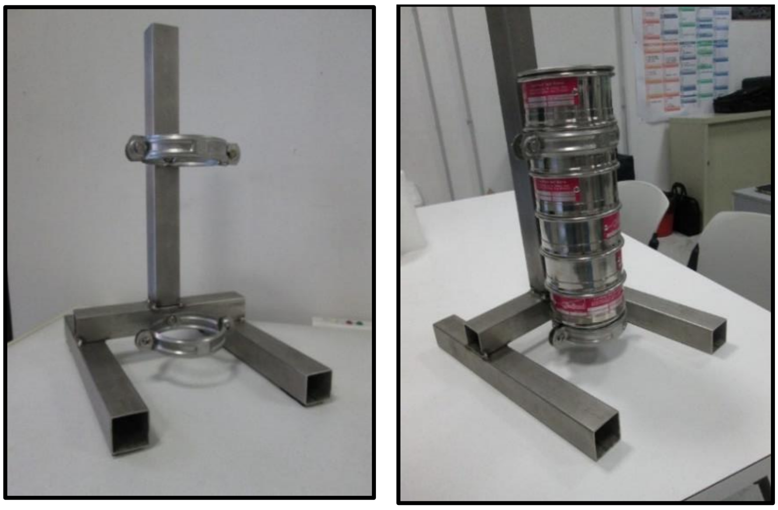

Figure 4.4.2: The sampling instrument. On the left, the stainless-steel structure; on the right, the battery of sieves on the structure.

Water sampling at different depths follows the following procedure:

- the extremity of the hose is positioned at the desired depth;
- a known quantity of seawater (calculated using a direct reading water flow meter) is pumped through the system and poured onto the sieve battery;
- each individual sieve is removed, and the items collected on the sieve are transferred to a glass jar with pre-filtered fresh water;
- the contents of each jar are filtered on a GF/F glass microfiber filter (porosity 0.7 µm; Whatman™);
- the filter was rinsed with 2 L of micro-filtered fresh water to eliminate salts residues;
- filters were finally dried and then placed in glass Petri dishes.

For bottom sediment sampling in the Port of Genoa, UNIGE selected a 5 L stainless steel Van Veen grab and the following procedure:

- the grab is lowered by an operator from a boat;
- the sediment collected from the grab is poured into a plastic tub and only the part that does not touch the tub is taken out with a metal spoon, so as to avoid any contamination of the sample;
- sediment is stored in glass jars rinsed beforehand with filtered water.
- The following treatment procedure, described by Cutroneo et al. (2022) and modified to make it suitable for port sediments, is applied to sediment samples:
- a portion of dry sediment is taken from each sample, placed inside a beaker and covered with watch glass dish;
- 200 mL of saturated magnesium chloride ($MgCl_2$) water solution (density 1.31 g cm^{-3}) is added to the sediment for density separation (Cutroneo et al., 2021);
- the mix is stirred with a glass stick for 5 min and then allows settling for 48 h;
- 10 mL of supernatant are taken and placed inside a glass jar.

The separation process is carried out 3 times for each sample, obtaining 30 mL of supernatant for each sediment sample.

- 30 mL of H_2O_2 are added to the supernatant to dissolve organic matter;
- supernatant is filtered on a GF/F glass microfiber filter (porosity 0.7 µm; Whatman™);
- the filter is rinsed with 2 L of micro-filtered fresh water to eliminate salts residues.
- filters are finally dried and placed in glass Petri dishes.

All the laboratory material used is previously rinsed with microfiltered water and the operators wear a cotton lab coat and remove everything plastic made to avoid the contamination of the sample. Furthermore, every time the sample is exposed to air, a control filter is placed alongside it, to be able to quantify any eventual contamination.

The bottom sediment was sampled at the two FL and FP points inside the Port of Genoa on 12 January 2024.

4.4.3.2 Experimental methods

The procedure chosen for the analysis of the findings collected on the filters obtained from sediment and water sampling involves first the investigation under an optical microscope and then the analysis with a µRaman.

As previously said, items over the filters are observed with a Leica Z16 microscope, photographed with a Leica Application Suite software, and then measured and classified according to shape (filaments, spheres, granules, fragments, and other type), color and size. Only items with dimensions between 40 µm and 5 mm are considered.

For the µRaman spectroscopy, UNIGE is equipped with an Xplora™ Plus Raman spectrometer with a 785 nm-laser, with Confocal Raman Microscope using a 100× lens, Horiba. The spectra obtained are classified according to the Bio-Rad KnowItAll® Raman Spectral Library. Only spectra with correspondence >70% to the reference spectra are accepted and considered in the results (Cutroneo et al., 2022).

During the next monitoring activities, the results obtained with the application of the method presented in this Chapter will be compared with those obtained with the application of a more expeditious method, the multispectral analysis developed by UNIROMA1 (see Thematic Chapter 4.6).

4.4.3.3 Modelling approaches

n.a.

4.4.4 Results

In this first phase of the RETURN project, UNIGE concentrated on selecting the best tools and methodologies applicable to the sampling of MPs in a port environment, such as the Port of Genoa.

As regards seawater sampling, UNIGE capitalized the SPlasH! prototype and improved in its functionality for the sampling of MP in the water column inside a port. Using a set of sieves with different mesh sizes, the items collected and filtered on the filters are already divided into specific size classes (range of dimensions). This makes it possible to avoid the measurement of each individual item under the optical microscope and considerably accelerates the classification of items. Having the items already divided by size is even more advantageous in terms of analysis time when dealing with fibers, and especially when the fibers are very long, have folds or are twisted. In addition, the method allows MPs to be sampled at the desired depth along the water column within the port basin.

For sediment sampling, UNIGE opted for the use of a 5-L stainless steel Van Veen grab, which can easily be deployed by hand by an operator on a boat and makes the operation quick even in confined areas such as ports.

Regarding the preparation of the supersaturated solution required for the density separation of MPs from the sediment, the use of $MgCl_2$ was chosen. $MgCl_2$ is a salt also used in the food industry and therefore non-hazardous for operators, which allows a solution with a density of 1.3 g cm^{-3} to be obtained and thus also extracts higher-density polymers that cannot be separated with the more usual supersaturated solution obtained with NaCl (1.2 g cm^{-3} density maximum).

The classic methodology of analyzing sampled items using firstly optical microscope and after the Raman spectroscopy technique in the laboratory was chosen by UNIGE to be applied to items extracted by sediment and water samples. Classification under an optical microscope provides the detail of shape, color and size of isolated items. Raman analysis makes it possible to recognize the composition of the items and to be certain that they really are made of plastic polymers. The combination of a polymer analysis method such as Raman spectroscopy with optical analysis is essential in the monitoring of MPs to obtain certain results. In fact, otherwise, even items that are not plastic, but which are mistaken as such by the human eye, could be considered MPs. Furthermore, the use of a hot needle which, in some studies, is used to verify the presence of MPs is not very applicable in the case of samples deriving from a matrix such as sediment or seawater in which small MPs are also present and therefore, they cannot be touched with the tip of a needle.

The coupling of microscope and µRaman also allows us to recognize the items present on the control filter (the filter used to verify the contamination of the sample due to the treatment of the sample in the laboratory) and therefore be able to eliminate them from the count of MPs found in the sample. This is not feasible with other methods, such as those that use computerized analysis of the microscope image for counting items.

MP monitoring results obtained with this methodology chosen by UNIGE will be compared to results obtained with the application of another more expeditious method (multispectral analysis) for the recognition of MPs and their polymers improved by UNIROMA1.

4.4.5 Scientific products and dissemination

Abstracts accepted for presentations at International Conferences:

Cutroneo L., Benedetti B., Caiazzo L., Cecchi G., Di Bella G., Di Carro M., Di Piazza S., Di Trapani D., Gaggero L., Geneselli I., Magi E., Manzo S., Montereali M., Parrella L., Schiavo S., Serranti S., Zotti M., Capello M. (2024). The Italian National Recovery and Resilience Plan (PNRR) RETURN Project: proposal of new monitoring and bioremediation protocols in the pilot site of the Port of Genoa. 11th International Conference on Sustainable Solid Waste Management (19-22 June 2024, Rhodes, Greece). Abstract accepted.

4.5 On the Detection of Bioplastic Content in Marine Water Using Microbiological, Analytical, Immunobiological and Spectroradiometric Techniques: state of the art and experimental design (UNIPA)

4.5.1 Introduction

Bioplastics, hailed as a contemporary alternative to traditional petrochemical-based plastics, have garnered significant attention. With projections indicating a notable surge in bioplastic production in the foreseeable future, it becomes imperative to scrutinize whether the environmental challenges associated with conventional plastics are merely being transferred to bioplastics. To unravel this conundrum, our focal point revolves around unravelling the myriad factors influencing the biodegradation mechanisms of bioplastics within marine ecosystems, with a specific emphasis on one of the most prevalent bio-based and biodegradable biopolymers of today: polylactic acid (PLA).

The term "bioplastics" encompasses materials derived from a spectrum of renewable biomass sources, including but not limited to vegetable fats and oils, corn starch, straw, woodchips, sawdust, and repurposed food waste. These bioplastics can be broadly classified into biodegradable, bio-based, or a hybrid of both. Biodegradable bioplastics undergo degradation in aqueous environments under the catalytic influence of bacterial activity, culminating in the formation of carbon dioxide and water as the ultimate byproducts. On the other hand, bio-based bioplastics, whether wholly or partially, trace their origins back to biomass-based syntheses. A prominent exemplar within this domain is PLA, a thermoplastic polymer nestled within the aliphatic polyesters' cohort, synthesized from α-hydroxy acids. The production of PLA unfolds through two principal routes: bacterial fermentation of renewable plant-based sources to yield lactic acid, or the polymerization of cyclic lactide dimer following a ring-opening reaction. PLA stands out in the commercial arena due to its commendable mechanical properties, processability, renewable nature, and benign toxicity profile. Its durability surpasses that of many biodegradable polymers, while its superior transparency and mechanical robustness contribute to its burgeoning global production footprint.

At the heart of bioplastic appeal lies its renewable sourcing, albeit not without attendant challenges. Biomass cultivation necessitates substantial land resources, substantial water consumption, and intensive agricultural practices geared towards optimizing production yields. Consequently, the production phase of bioplastics may inadvertently introduce environmental stressors such as pesticide application in agriculture and chemical usage during processing, underscoring the urgency for eco-friendly synthesis alternatives. Conversely, the innate biodegradability of bioplastics obviates the need for filler materials aimed at bolstering mechanical attributes, a stark departure from conventional plastic formulations. Despite the growing demand for bioplastics, hurdles impeding widespread adoption stem from their prohibitively expensive production and recycling methodologies.

Given the growing demand for bio-based polymers in the global market, it is imperative to investigate their real behavior and degradation under different environmental conditions. Moreover, to investigate if and how structural and physico-chemical properties can influence the bio-polymers behavior in marine ecosystems with eventual interaction with the living organism, different immunological markers good indicators of the impact on the biota, have been carried out on the bivalves mussel *Mytilus galloprovincialis*. We chose *M. galloprovincialis* as sentinel specimens since their extensive filter-feeding activity and sessile behavior. When *Mytilus galloprovincialis* is exposed directly to microparticles (MPs) can bioaccumulate them in soft tissues and organs (hemolymph, digestive gland, foot, and mantle), at different developmental stages. This can lead to their transfer, through the marine trophic web, to the human food chain. Preliminary results have shown that bioplastic particles behave in a very similar way to fossil plastic triggering the immuno-system and activating the pathways for elimination of non-self particles via cellular response.

4.5.2 Case study description

The case study is approached through an in-depth bibliographic review of the state of the art from the last five years and the preparation of the experimental plan for conducting characterization studies of bioplastics in laboratory systems, which simulate marine environmental conditions, using chemical, microbiological, immunobiological and spectral methodologies.

Degradation of bioplastics was studied as a valuable tool in assessing the environmental impact of bioplastic waste and devising appropriate measures for implementing waste legislation and policies. Although the main degradation mechanisms of bioplastics are known, the biodegradability of the material, and its decomposition may not occur uniformly under all circumstances or environmental conditions. Numerous factors influence the biodegradation process, including microbial density and environmental parameters such as temperature and humidity. The impact of these factors, which encompass polymer composition, molecular weight, crystallinity, pH, chemical structure, morphology, hydrophilicity, and breakdown products, on biodegradation remains incompletely understood. Degradation of these materials cause implies their entry into the environment in the form of small particles, potentially resulting in significant biological and toxicological ramifications. Consequently, quantifying and characterizing particles smaller than 10 µm pose a considerable challenge. The subsequent stage in the potential environmental pollution caused by bioplastics involves the production of solid microparticles and soluble compounds as a result of the degradation process.

Analytically, identifying and quantifying bioplastic degradation products in both solid and soluble forms presents complex challenges. Hence, we want to perform the experimental design reported with the aim of exploring the feasibility of developing an analytical methodology for the qualitative and quantitative analysis of bioplastic degradation products. The materials will be spectrally characterised through a laboratory experiment. The spectral signatures on the virgin bioplastics and thereafter their degradation (consequent to the microorganisms' action) will be compared with the spectra of plastic polymers (non-biodegradable) allowing us to evaluate the main differences between the different types of materials. The spectral signatures will be analysed individuating the wavelengths useful for the remote detection and, consequently, the promising sensors for their detection.

4.5.3 Methodologies

4.5.3.1 Analytical methods

The methodologies used are:

- **Plastic degradation ability of microbial communities.** To evaluate the biodegradation of plastics by marine microorganisms, a field experiment and laboratory tests were carried out. A field experiment will be performed to recruit the plastisphere communities on commercial products (PLA caps and PLA bags) after short and medium-term exposure in the coastal area of Palermo (Italy), at 2 m seawater depth. After exposure for 60 and 120 days, the plastic samples were collected. To avoid contamination between biofilm typologies, the scaffolds will be handled with sterile gloves. During the carriage to the laboratory, each biofilm treatment will be kept in a separate tank filled with seawater from the same area. 60 and 120-day-old microbial biofilms will be collected by scraping off the top face of each scaffold sample with a sterile scalpel. Microbial communities will be inoculated on 500ml sterilized glass flasks containing approximately 100ml of sterile seawater. The microcosms will be supplemented with fragments of PLA materials sterilized with 70% ethanol and UV for 30 min. A flask without any inoculum will be used as an abiotic control. All flasks were incubated at 30°C for 60 days. Plastic-associated biofilm-bacteria were identified by a culture-dependent approach by using ONR7a medium and plastics as unique carbon sources. PDLA/PLLA or PLLA fragments, and residual liquid medium (80 ml approximately) from each microcosm were stored at −20°C for molecular or chemical analyses.
- **Analytical detection.** Since preliminary investigations suggest that lactic acid is the final degradation product of PLA, we will optimise the method for extracting lactic acid from seawater. Specifically, we will test the efficiency of lactic acid extraction using Solid-Phase Extraction (SPE). We will work with seawater solutions containing known concentrations of lactic acid and conduct recovery tests using two different types of SPE cartridges to determine the best extraction method. The analytical technique involved in the analysis will be high-performance liquid chromatography (HPLC) coupled with high-resolution mass spectrometry (HRMS) through an Electrospray type source interfacing system (ESI). In particular, we will use a high-resolution mass spectrometer, such as the Orbitrap mass analyzer, which has a very high-resolution capacity which allows it to discriminate masses even to the fourth decimal digit, effectively working on exact monoisotopic masses, allowing for high sensitivity and accuracy analytical. The Orbitrap's ability to work at high resolution makes it the ideal instrument for our particularly complex matrices, as in the case of biological or environmental ones, in which it is necessary to discriminate molecules in trace and ultra-trace concentrations.

- **Spectroradiometric analyses** The commercial products, PLA caps and PLA bags, undergoing the degradation by microbial communities will be spectroradiometrically analysed by carrying out an indoor laboratory experiment. The spectral signature of the samples will be collected before exposing them to the microbiological action (T_0), after 60 (T_{60}) and 120 (T_{120}) days. The data will be analysed determining the main differences and the useful wavelengths for remote sensing detection. The spectra will be acquired by positioning the samples within a white box in which diffusive illumination conditions will be realized by rotating upward two ASD pro - Illuminator Reflectance Lamp Halogen, equipped with Single-Ended Quartz JC14.5V-50WC lamps, characterized by an irradiance curve simulating that of the sun. Figure 4.5. shows the box and the lamp's configuration.

Figure 4.5.1: Experimental setup.

The reflectance of the samples will be measured using the FieldSpec 4 Hi-Res spectroradiometer by ASD (Analytical Spectral Devices). It is composed by three spectroradiometers, one operating in the visible VNIR range (350-1000 nm) with a spectral resolution of 3 nm and the other two in different infrared ranges: shortwave 1 (1001-1800 nm) and shortwave 2 (1801-2500 nm) having a spectral resolution of 8 nm. Therefore, the spectral signatures of the samples are measured across the entire solar-reflected spectrum, from 300 to 2500 nm. The comparison between the reflectance acquired at T_0, T_{60} and T_{120} will allow us to evaluate the influence of the microorganisms on the spectral signatures. These data, jointly with the spectral signatures of plastic polymers (not biodegradable and collected on the beaches) will enable the identification of the most effective remote sensing techniques for *in situ* monitoring.

- **Immunobiological, enzymatic and tissue damage analyses.** Analysis on different immunological markers, good indicators of the impact on the biota, will be study on the bivalve mussel *Mytilus galloprovincialis*. Indeed, the impacts of biopolymer particles on the marine environment has been explored by evaluating in vitro the cellular response of *M. galloprovincialis* haemocytes in terms of their phagocytic and/or encapsulation activity. Considering that not all bio-based materials are biodegradable, or biodegradable in a very short timeframe, beyond the in vitro investigation of cellular recognition, we plan to investigate their interaction with filter-feeding mussels. In order to assess the eventual detrimental effects of these bioplastics on living feeder organisms, we organized in vivo experiments, placing the *M. galloprovincialis* specimens in water in presence of plastics, in order to evaluate eventual effect of an acute exposure stress. We plan to study the effects of microplastics on the bivalve specimens, through investigation of different biomarker: the number of free haemocytes circulating in the haemolymph, their phagocytic activity, potential histological variations in the morphology of the digestive glands, as well as the activity of several immune-related enzymes in the digestive gland and haemolymph (i.e., phenoloxidase, glutathione peroxidase, lysozyme, alkaline phosphatase and esterase). These markers were chosen, firstly, because bivalve haemocytes are easily influenced by exogenous factors as they regulate functional cell-mediated immune responses. Furthermore, enzymes produced in the digestive gland are known to assist, modulate and accelerate immunological processes in haemocytes. The digestive gland is a source of innate immune molecules and is involved in pathogen clearance, antigen processing and infection-induced metabolic changes. In detail, hydrolase enzymes, normally involved in detoxification, inflammatory and digestive processes, the phenoloxidase cascade and reactive oxygen species (ROS) scavenging, are recognized as immune parameters potentially influenced by environmental factors. Lysozyme activity is a phylogenetically conserved humoral response and has been studied in the mucus, tissues and haemolymph of many invertebrate species. It corresponds to the primary and rapid defence of organisms against pathogens and is a bactericidal hydrolytic enzyme which hydrolyses the β-1,4 glycosidic bonds of peptidoglycan (an important component of the cell wall of Gram-negative bacteria), resulting in the rupture of bacterial walls due to destabilization of the membrane.

4.5.3.2 Experimental methods

n.a.

4.5.3.3 Modelling approaches

n.a.

4.5.4 Results

An in-depth bibliographic study to thoroughly understand the nature of potential issues associated with the increasingly significant use of bioplastics and their potential environmental impact was realized. We have thus selected keywords for bibliographic research and have limited the study to the last five years, focusing on the isomeric forms of polylactic acid as the polymer of investigation. The result of the bibliographic inquiry has highlighted some interesting aspects that have informed our choices in preparing the experimental plan we intend to propose for studying the content and behavior of bioplastics in marine environments. In particular, we will refer to specific aspects.

In the sea, plastic degradation can be categorized into abiotic and biotic degradation processes. Generally, long polymer chains are initially broken down abiotically into shorter molecules (e.g., through ultraviolet (UV) radiation, wave action, and salt exposure) and subsequently undergo biodegradation (assimilation and mineralization) by microbial activity. However, comprehensive studies on the actual biodegradation rate in the marine environment are lacking. In addition, despite commercial bioplastic materials are commonly produced by different biopolymers and blends, research on biodegradable plastics is limited and primarily focused only on polylactic acid (PLA) and polyhydroxybutyrate (PHB) polymers. Therefore, as bioplastics are considered a replacement for recalcitrant fossil-based materials, assessing the natural biodegradability of commercial bio-based products is essential to prevent an unintentional shift from non-degradable conventional plastics to non-degradable bio-based plastics. Therefore, the project aims to study the biodegradation of commercial bio-based products through laboratory studies involving incubation with microorganisms collected from marine ecosystems. The main purpose is to determine the degradation kinetics by measuring the possible hydrolysis end-products. Additionally, the project intends to conduct experiments to assess weight variations at different scales, from the laboratory to the marine site.

Another concern is evaluating the actual presence of bioplastic materials in the environment, particularly within marine ecosystems. Currently, the detection of petroleum-based plastics in the sea remains a challenge and relies primarily on visual observation from boats or ships. Moreover, specific methods for bioplastic identification are lacking. Consequently, there is a pressing need to develop standardized methods for recognizing both conventional plastics and bioplastics on a larger scale than the microscale (i.e., locally, within a few dozen meters). Recently, *in situ* monitoring campaigns were conducted using Unmanned Aerial Vehicles (UAV) that allow estimating the quantities of beach litter through the application of unsupervised and supervised techniques. A lack of knowledge concerning the marine litter spectral signature was underlined by different studies, driving the scientific community to fill this scientific gap. Garaba and Dierssen (2018), realized a spectra library of marine-harvested macroplastics and microplastics, individuating the wavelengths useful for remote sensing detection. In Corbari et al. (2020), spectral signatures of virgin microplastics both in dry and wet conditions were acquired individuating the wavelengths and sensors more promising for marine litter detection. Since several studies reported that the end-of-life options for both fossil fuel-based plastics and bioplastics are similar, except for composting, the project aims to implement remote sensing techniques to detect bioplastics in sea conditions. The challenge is to assess changes in spectra between pristine bioplastic and the same materials exposed to the sea environment. These experimental investigations will aid in determining the most suitable spectral bands for distinguishing between these materials in natural conditions. Detecting plastics, including bioplastic and micro-(bio)-plastics, at sea surfaces is vital for enhancing early detection of plastic hotspots. This information aids in proactive mitigation strategies, safeguarding coastal social, economic, and environmental systems.

PLLA (poly-L-lactide) and PDLA (poly-D-lactide) forms are two isomers of the polylactic acid (PLA) polymer. These forms are optically active, which means they can interact with polarized light and can crystallize, forming an ordered and crystalline structure. However, unmodified PLLA and PDLA polymers have some limitations in practical applications. They are brittle and exhibit relatively poor resistance when exposed to oxygen. This makes them less suitable for applications that require durability and mechanical strength, especially in environments with oxygen presence. Furthermore, another characteristic that can affect the use of PLLA and PDLA is their slow degradation rate at room temperature. Due to their hydrophobic nature, and the presence of a methyl group that creates steric hindrance in the ester linkage, the process of hydrolysis of the ester linkage through water, occurs at a very slow rate. Since the properties of PLA are strongly influenced by the stereoisomeric L/D ratio of the lactate units, PDLLA (which is an amorphous form of the polymer) is commonly adopted in manufacturing, at which the L/D ratio is varied using appropriate additions of PLLA to more effectively control the crystallization, morphology, and nature of the hydrolysis of the polymer. This type of biopolymer is very widespread in the biomedical sector, the use of which began in the 1960s - both in homopolymeric form and as mixtures - for the slow release of drugs and proteins and applications in the surgical field. Today the fields of application of PLA are multiple as it is a material that can be used for packaging, producing disposable tableware, in clothing, the production of bottles, injection moulded products, extrusion coatings, and so on. In agriculture, PLA is used in the form of mulch films to allow the slow release of pesticides and fertilizers. Since PHAs and PLA biopolymers often find application in the form of mixtures, it seems appropriate to deepen the study of degradative processes also considering the different compositions that the finished products can assume.

4.5.5 Scientific products and dissemination

Dissemination:

Project activities have been presented at:

- Return Project workshop (Torino, 1-2 February 2024)

4.6 Identification and classification of macro - and microplastics dispersed in marine and coastal environments by fast and efficient innovative non-invasive, non-destructive sensing technologies (UNIROMA1)

4.6.1 Introduction

The aim of this research line is to develop innovative strategies for the identification of macro- and microplastics, dispersed in marine and coastal environments, based on the application of hyperspectral imaging (HSI) technology coupled with chemometrics. The possibility of having a fast and efficient analytical method allowing the classification of the polymer types constituting macro- and microplastics is an essential step of the environmental monitoring strategies. Several techniques are currently used for the characterization of microplastics (MPs) (Huang et al., 2023; Kavya et al., 2020), the most diffused being FTIR spectroscopy and Raman spectroscopy. Considering that the identification of polymers by these techniques is very tedious and time-consuming, in the last years the use of hyperspectral imaging (HSI) was proposed as a fast and effective solution (Serranti et al., 2018; Serranti et al., 2019; Fiore et al., 2022; Faltynkova et al., 2023). The main advantage of HSI is the possibility to rapidly acquire spectral images with different fields of view and pixel-resolution according to the size of the samples, with a minimal sample preparation. However, considering the large variability of samples that can be acquired in a short time by HSI, advanced processing strategies are needed to maximize the collected spectral information. It is thus essential to develop the proper analytical logics based on the specific problem.

A methodological study has been carried out to define the best analytical strategies for the characterization of MPs by HSI, testing different set up with reference to 1) spatial resolution, 2) wavelength range and 3) classification model.

Furthermore, in order to test the HSI-based analytical procedures, they have been applied to different case studies related to the classification of macro- and microplastic dispersed in marine and coastal environments:

- **Case Study 1: *In situ* macroplastic litter classification on coastal areas (Torre Guaceto, Brindisi, Italy).** Field testing at Torre Guaceto of a ground-based hyperspectral sensor for macroplastic litter monitoring on sandy beaches, in collaboration with UNIBA project partner (Bonifazi et al., 2023).
- **Case Study 2: Microplastic litter classification on sandy beaches at laboratory-scale (Vulcano Island, Italy).** Laboratory-scale monitoring of contamination by microplastics on sandy beaches at Vulcano Island by different spectroscopic techniques (Cocozza et al., 2023; the poster is winner of the Best Poster Award).
- **Case Study 3: Microplastic litter identification on sandy beaches at laboratory-scale (Torre Guaceto, Brindisi, Italy).** Investigation of sand samples collected at Torre Guaceto for laboratory-scale monitoring of the presence of microplastics by HSI, in collaboration with UNIBA project partner (Bonifazi et al., 2024, abstract accepted for presentation).
- **Case Study 4: Microplastic litter identification in marine sediments at laboratory-scale (Mar Piccolo, Taranto, Italy).** Laboratory-scale identification of microplastics by HSI in marine sediments from Mar Piccolo in collaboration with UNIBA project partner (Cucuzza et al., 2024, abstract accepted for presentation).
- **Case study 5: Microplastic litter identification from the water column at laboratory-scale (Port of Genoa, Liguria region, Italy).** Laboratory-scale identification by HSI of microplastics collected from the Port of Genoa, in collaboration with UNIGE project partner (Cutroneo et al., abstract accepted for presentation).

4.6.2 Case study description

Case studies 1 and 2 are closed, whereas case studies 3, 4 and 5 are still under development. A summary of the closed case studies is reported in the following.

Case Study 1: *In situ* macroplastic litter classification on coastal areas (Torre Guaceto, Brindisi, Italy)

A methodological approach and a valid solution to identify and monitor plastic litter based on HSI working in the near infrared (NIR) range was tested, along a foredune system at Torre Guaceto, a natural protected area, located along the Adriatic flank of the Apulia region (Brindisi, Italy) (Figure 4.6.1). Plastic items characterized by different color, size, shape and polymer (polyethylene - PE, polypropylene- PP, polyethylene terephthalate - PET and polystyrene - PS) were selected among those commonly found in coastal litter (i.e., bottles, containers, plates, glass, trays, etc.), and placed on a target area of about 2x3 m^2 along the foredune system. Hyperspectral images acquisition in the NIR range (1000-1700 nm) was carried out by a ground based HSI system (DV Optics, Italy) at about 3 meters from the objects. Data processing was performed using the PLS_toolbox (Eigenvector Research, Inc., Wenatchee, WA, USA) in MATLAB® environment (The Mathworks, Inc., Natick, MA, USA). Principal Component Analysis (PCA) was applied to evaluate the best spectral pre-processing strategy to identify polymers. A classification model based on Partial Least Square-Discriminant Analysis (PLS-DA) was built to identify the four different types of polymers constituting plastic litter. The good model's performance was assessed through the evaluation of *Sensitivity* and *Specificity* parameters calculated from confusion matrix. The results shown that employing ground based HSI in the NIR range can be a promising solution for a rapid identification of macroplastic debris along the coastlines.

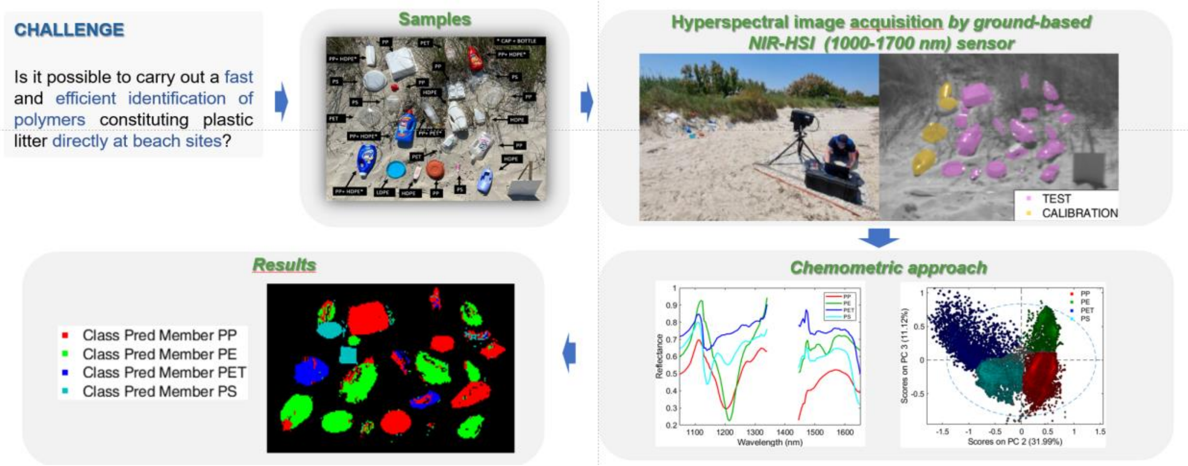

Figure 4.6.1: Case study 1 – In situ macroplastic litter classification on coastal areas (Torre Guaceto, Brindisi, Italy).

Case Study 2: Microplastic litter classification on sandy beaches at laboratory-scale (Vulcano Island, Italy)

The aim of this work was to carry out, for the first time, the characterization of microplastics in sandy beaches from a site designated for the establishment of a new marine protected area at Vulcano Island (Aeolian Islands, Sicily, Italy) (Figure 4.6.2). Microplastic samples were collected from two different beaches (Cannitello and Sabbie Nere) and characterized in terms of abundance, distribution, color, category (i.e.: pellet, fragment, filament, film, foam, etc.), polymer type, morphological and morphometrical characteristics. For the polymer identification, two different analytical techniques were applied: the innovative approach based on HSI (working in the short-wave infrared range - SWIR: 1000-2500 nm) compared to the conventional spectroscopic technique, i.e., FT-IR spectroscopy (4000-400 cm^{-1}). Concerning the morphological and morphometrical characterization, digital images of microplastic samples were acquired using a Nikon D5200 camera and processed using the software MATLAB® (The Mathworks, Inc., Natick, MA, USA). For each microplastic particle, measurements were taken for area, perimeter, minimum, maximum and mean Feret diameter, circularity, aspect ratio, and roundness. HSI data processing was carried out using PLS_toolbox (Eigenvector Research, Inc., Wenatchee, WA, USA) running in MATLAB® environment. To identify polymers, starting from the collected hyperspectral images, after image background removal using the information obtained by PCA, a hierarchical model based on PLS-DA was built. This classification model included six classes of polymers, among the most widespread polymers in the aquatic environment (Andrady, 2015; Serranti et al., 2018; Serranti et al., 2019): polyethylene (PE), polypropylene (PP), expanded polystyrene (EPS), polyamide (PA), polystyrene (PS) and polyethylene terephthalate (PET). The correctness of classification results obtained by HSI were confirmed by those of the FT-IR analysis. Finally, the results obtained in terms of category, morphology, morphometry, abundance and polymer were evaluated.

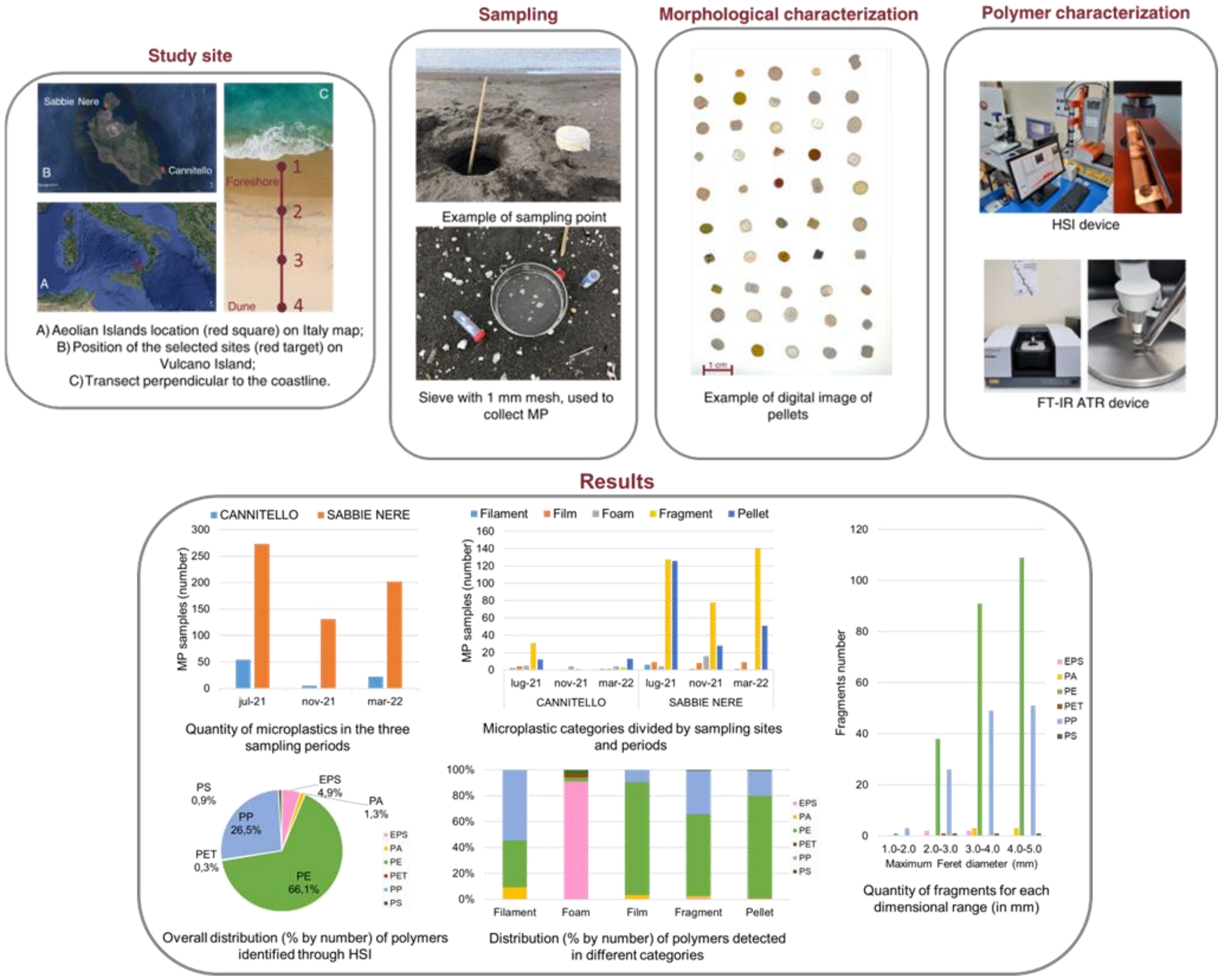

Figure 4.6.2: Case study 2 – Monitoring of contamination by microplastics on sandy beaches at Vulcano island (Italy) by different spectroscopic techniques.

4.6.3 Methodologies

4.6.3.1 Analytical methods

In the following it is described the methodological work carried out for the definition of the optimal strategies for the characterization of MPs by HSI, testing different set up with reference to: 1) spatial resolution, 2) wavelength range and 3) classification model.

It was preliminary created a MPs dataset by shredding post-consumer plastic packaging samples selected among the most widespread polymers in the environment, i.e. PS, PP and PE, using a cutting mill (SM 2000, Retsch GmbH, Haan, Germany). For each studied polymer, 3 size classes were prepared using stacked sieves, i.e., size 1: -2 mm +1 mm; size 2: -1 mm +0.5 mm; size 3: <0.5 mm. Hyperspectral images acquisition was carried out using the SisuCHEMA XL™ Chemical Imaging Workstation embedding an ImSpector™ N25E (Specim®, Finland) operating in the SWIR range (1000–2500 nm), coupled with a MCT camera (320x240 pixels). Hyperspectral images were acquired at two different magnifications, using two different objectives: a 31 mm lens, covering a 5 cm field of view (FOV) corresponding to a spatial resolution of 150 μm, and a macro lens, covering a 1 cm FOV, corresponding to a spatial resolution of 30 μm. Moreover, two spectral ranges were considered: 1000-1700 nm (NIR) and 1000-2500 nm (SWIR). Different classification models were tested on the same acquired images in order to evaluate their performances: partial least square-discriminant analysis (PLS-DA) (Ballabio et al., 2013), error-correcting output support vector machine (ECOC-SVM) (Zhou et al., 2018) and neural network pattern recognition (NNPR) (Gopi, 2010). The HSI data processing was carried out using different tools running inside MATLAB® environment (version R2022b, The Mathworks, Inc., Natick, MA, USA), namely: 1) PLS toolbox (ver. 9.2 Eigenvector Research, Inc., Wenatchee, WA, USA) for principal component analysis (PCA) (Bro et al., 2014) and PLS-DA and 2) Statistics and Machine Learning Toolbox™ for ECOC-SVM and NNPR (Gopi, 2010). The correctness of the developed classification models was evaluated by recall and specificity values.

4.6.3.2 Experimental methods

n.a.

4.6.3.3 Modelling approaches

n.a

4.6.4 Results

The raw average reflectance spectra of PS, PP and PE and background classes, acquired at two different spectral ranges (1000-2500 nm and 1000-1700 nm) and two different spatial resolutions (150 and 30 µm), are reported in Figure 4.6.3a-d. The spectral differences between the spectra acquired at a resolution of 150 µm and those acquired at a resolution of 30 µm are very low and mainly related to the reflectance levels, as a consequence of the different acquisition set-up. In Figure 4.6.3e-h, the PCA score plots of the calibration dataset obtained with a resolution of 150 and 30 µm from 1000 to 2500 and from 1000 to 1700 nm, are reported. PCA score plots show in all cases the presence of four clouds corresponding to the three different polymers (i.e., PS, PP and PE) and background classes.

Starting from the variability detected in PCA, three classification models based on PLS-DA, ECOSSVM and NNPR for each wavelength range and resolution (i.e. 150 and 30 µm) were developed. Finally, the models were applied to all considered MPs particle sizes. In Figure 4.6.4, it is shown a summary of the results obtained from the 3 classification models applied to the three particle size class ranges for the two wavelength ranges at 150 µm (Figure 4.6.4a-b) and at 30 µm (Figure 4.6.4c-d). The results show the high efficiency of the proposed models, with very good classification results for the 3 polymers. The comparison of prediction results obtained by the 3 models highlights some differences. More in detail, PLS-DA provided the best performances for the MPs acquired with a spatial resolution of 150 µm, whereas the ECOC-SVM model showed the best classification results on the samples acquired at 30 µm. Regarding the two different spectral ranges, the results showed few differences between the NIR (1000-1700 nm) and the SWIR (1000-2500 nm) ranges.

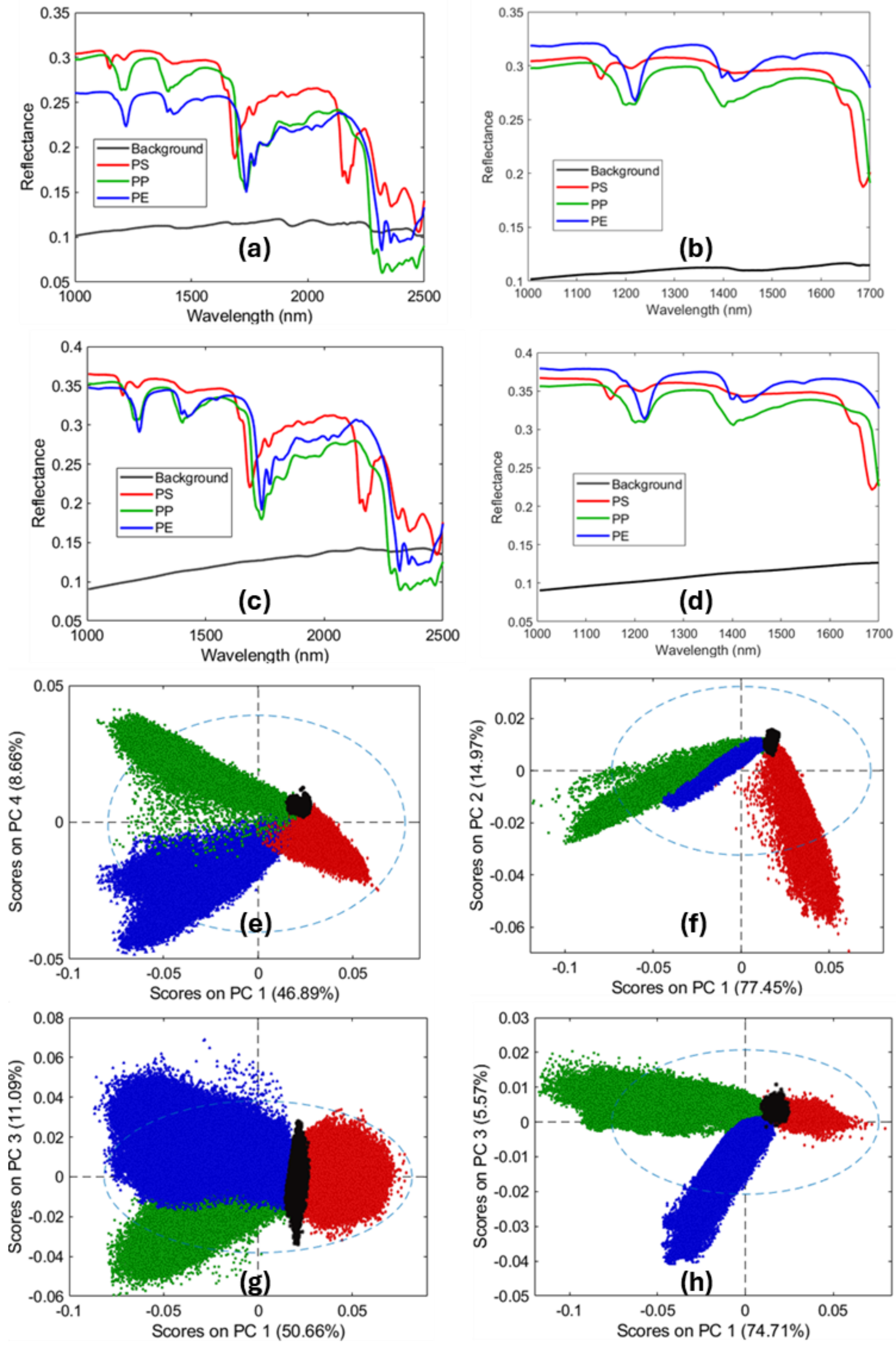

Figure 4.6.3: Raw average reflectance spectra from 1000 to 2500 nm (a) and from 1000 to 1700 nm (b) of samples acquired at 150 µm; raw average reflectance spectra from 1000 to 2500 nm (c) and from 1000 to 1700 nm (d) of samples acquired at 30 µm; PCA score plot of data acquired from 1000 to 2500 nm (e) and from 1000 to 1700 nm (f) at resolution of 150 µm and PCA score plot of data acquired from 1000 to 2500 nm (g) and from 1000 to 1700 nm (h) at resolution of 30 µm.

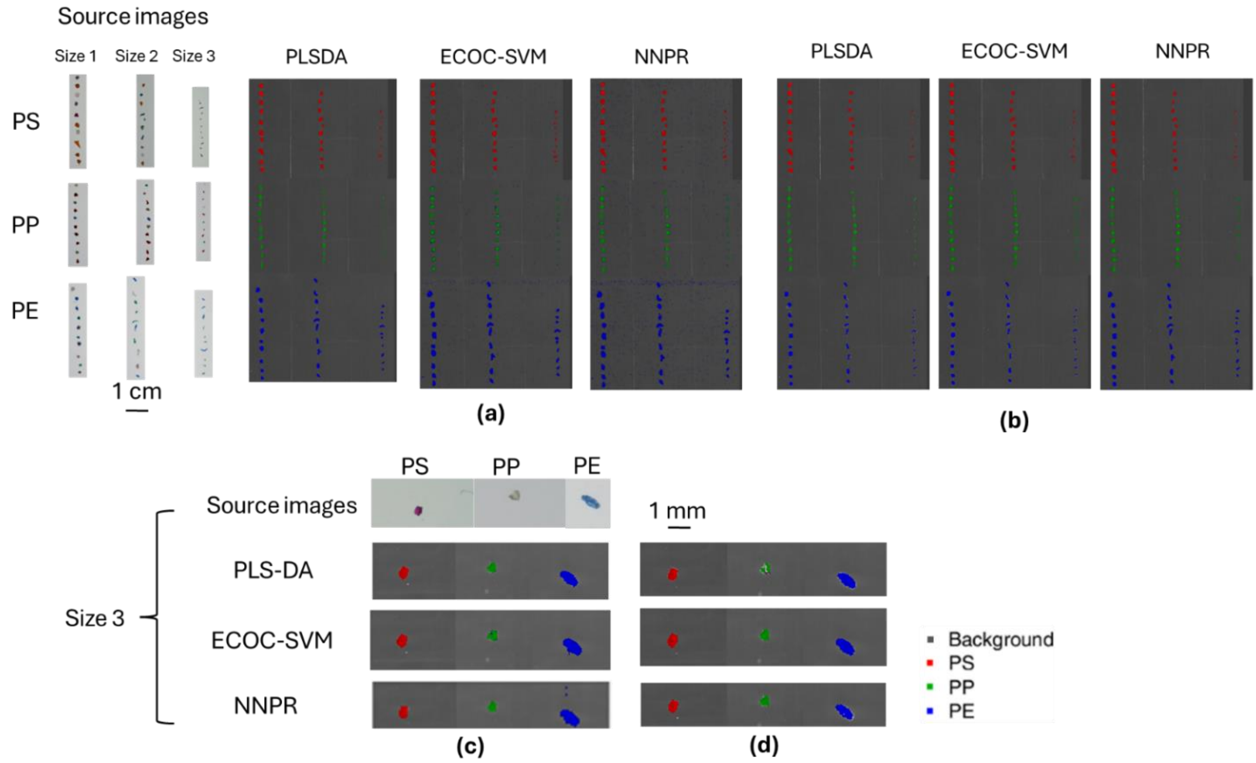

Figure 4.6.4: Summary of the results obtained by the applications of 3 different classification models (PLS-DA, ECOC-SVM and NNPR) to the MPs validation datasets acquired with a spatial resolution of 150 µm in the spectral range of 1000-2500 nm (a) and 1000-1700 nm (b), and with a spatial resolution of 30 µm in the spectral range of 1000-2500 nm (c) and 1000-1700 nm (d).

4.6.5 Scientific products and dissemination

Presentations at International Conferences:

Bonifazi, G., Capobianco, G., Cocozza, P., Mastronuzzi, G., Rizzo, A., Serranti, S. (2023). Identification of plastic debris on beaches by ground-based hyperspectral imaging. Presented at the International conference ECDS 2023: Emerging Concepts & Design for Sustainability (8-11 October 2023, Villers-sur-Mer, Calvados, France).

Cocozza, P., Serranti, S., Setini, A., Cucuzza, P., Bonifazi, G. (2023). Monitoring of contamination by microplastics on sandy beaches at Vulcano island (Italy) by different spectroscopic techniques. Presented at the 5th International Conference on Pollutant Toxic Ions and Molecules (6-9 November 2023, Caparica, Portugal). The poster is winner of the Best Poster Award.

Abstracts accepted for presentations at International Conferences:

Bonifazi, G., Cucuzza, P., Lisco, S., Marsico, A., Mastronuzzi, G., Rizzo, A., Serranti, S. (2024). Application of hyperspectral imaging and machine learning for the automatic identification of microplastics on sandy beaches. SPIE Defence + Commercial Sensing conference (21-25 April 2024, Maryland, United States). Abstract accepted.

Cucuzza, P., Gorga, E., Serranti, S., Rizzo, A., Lapietra, I., Mastronuzzi, G., Mele, D., Bonifazi, G. (2024). Hyperspectral imaging applied to monitoring microplastics collected from sediments of Mar Piccolo basin (Taranto, Southern Italy). 11th International Conference on Sustainable Solid Waste Management (19-22 June 2024, Rhodes, Greece). Abstract accepted.

Cutroneo L., Benedetti B., Caiazzo L., Cecchi G., Di Bella G., Di Carro M., Di Piazza S., Di Trapani D., Gaggero L., Geneselli I., Magi E., Manzo S., Montereali M., Parrella L., Schiavo S., Serranti S., Zotti M., Capello M. (2024). The Italian National Recovery and Resilience Plan (PNRR) RETURN Project: proposal of new monitoring and bioremediation protocols in the pilot site of the Port of Genoa. 11th International Conference on Sustainable Solid Waste Management (19-22 June 2024, Rhodes, Greece). Abstract accepted.

Dissemination:

Project activities have been presented at:

- Return Project workshop (Torino, 1-2 February 2024)

5. Conclusions

In line with the aims of the Marine Strategy Framework Directive (MSFD) "to maintain clean, healthy, productive, and resilient marine ecosystems while securing a more sustainable use of marine resources", the overall objective of Task 4.3.2 is to identify sustainable solutions to reduce coastal and marine pollution and to preserve marine biodiversity. In this context, activities scheduled by the research teams involved in the Task were focused on the definition of novel and innovative procedures to assess pollutants distribution, transport, degradation, and bioaccumulation in marine and coastal environments. Furthermore, special attention was also given to the polymers' characterization. Regarding the selection of the compounds to be analyzed, the researcher groups have been working on different classes of pollutants.

OGS team has focused on developing a suite of modeling tools to simulate the fate, transport, and bioaccumulation of mercury (Hg), a persistent priority pollutant of global concern, and other priority and emerging pollutants, also taking into account the potential impact of climate change. The selected study area is the Northern Adriatic Sea, characterized by the presence of macrotidal coastal lagoons, such as the Venice Lagoon and the Marano-Grado Lagoon, which offer nursery and refuge area for several marine organisms and seabirds. A numerical study on the fate and transport of Hg was carried out thanks to the development of a finite element coupled hydrodynamic-sediment-mercury model (SHYFEM-Hg) to simulate the spatio-temporal evolution of Hg species concentrations in sediments, water, and porewater at a fine spatial resolution. The model will be further coupled to a zero-dimensional model for bioaccumulation in the clam *R. philippinarum*, which is an important socio-economic resource for the investigated area. The team is also working on the setup for the coupled OGSTM-BFM-Hg model to simulate long-term Hg dynamics in the Mediterranean Sea under climate change scenarios up to the end of the century.

UNIBA, UNIFI, and UNIGE teams has focused on the MPs abundance and characterization in the diverse marine matrices. In details, UNIBA has investigated MPs distribution by collecting sediment samples both offshore, along the Adriatic and Ionian coast of the Apulia Region, and on the backshore of selected coastal sites. The offshore sampling sites are defined taking into account the proximity to the main harbors and river mouths. Furthermore, several samples come from the Taranto seas (Mar Grande and Mar Piccolo basins), which are characterized by strong anthropogenic impacts. After standard laboratory analyses, particles collected from the samples were analyzed at the optical microscope to assess the MPs concentration and then further analyzed by μ-FTIR and μ-Raman spectroscopy to determine their composition. UNIFI investigated the distribution of MPs in water samples and the ingestion in commercial fish species, such as the red mullet species (*Mullus Barbatus*). Samples were collected at several sites in the Mediterranean basin, corresponding to the main commercial ports. UNIGE team concentrated on selecting the best tools and methodologies applicable to the sampling of MPs. The team defined a sampling protocol for two different matrices (water and sediment) in the marine environment and for the treatment and analysis of the collected samples. Choice of the protocol was addressed in particular to the marine port environment, where the complex morphology of the basins and the presence of moving ships and nautical vehicles restrict the field of instruments that can be used for sampling. The application site of the chosen protocol is the Harbor of Genoa, where water will be sampled at different depths using the modified SplasH! prototype and bottom sediments will be collected with a small steel Van Veen grab. MPs items extracted from samples will be classified using an optical microscope, while the polymers recognition will be performed using μRaman spectroscopy.

UNIPA team has focused the activities on the analysis of biodegradation processes to understand the degradation of bio-polymers in marine environments, particularly those based on polylactic acid (PLA), which is one of the most prevalent bio-based and biodegradable biopolymers used nowadays due to its mechanical properties and renewability. The team activities are based on the development of an experimental plan to investigate the behavior of bioplastics in marine environments using analytical, microbiological, immunobiological and spectroscopic methodologies.

As far as coastal contamination is concerned, UNIBA team also has focused on the analysis of macro-litter (>5cm) distribution. To this end, standard beach litter (BL) monitoring guidelines were coupled with innovative approaches based on the exploitation of UAVs images and automatic systems for the identification and classification of BL items. The definition of the BL density along the investigated coastal sectors allow to identify hot spot areas of litter accumulation and therefore it supports the definition of tailored management actions.

UNIROMA1 team has carried out test analysis to evaluate the performances of hyperspectral imaging (HIS) coupled with chemometric logics, for the identification of three main polymers (PS, PP, and PE) usually found in MPs from coastal and marine environment, testing three different classification models, two spectral ranges, i.e., 1000-2500 and 1000-1700 nm, and two different spatial resolutions (150 and 30 µm) of the device. All the three classification models provided good results, at both spectral ranges and spatial resolutions, in the recognition of PE, PP and PS microplastic particles, as demonstrated by the prediction images and performance parameter values. Concerning the spectral range, the possibility to use the NIR range, providing the same classification performance of the SWIR one, can allow to reduce time and cost of the analysis. As regards the spatial resolution, PLS-DA provided the best results for the resolution of 150 µm, whereas ECOC-SVM showed the best performances for the resolution of 30 µm. Based on the achieved results, to optimize the analytical procedure, it is suggested to select the classification model depending on the spatial resolution of the acquired hyperspectral images and, therefore, on the size of the investigated MPs. For example, it is better to acquire particles with a diameter > 300 µm using a spatial resolution of 150 µm (at least two pixels for each particle), corresponding to a greater FOV (5 cm), reducing in this way acquisition and processing time and to use PLS-DA as classification model. On the contrary, for particles with a diameter < 300 µm it is suggested to use a spatial resolution of 30 µm, corresponding to a FOV of 1 cm and the application of the ECOC-SVM model.

The highly promising analysis approach proposed by the UNIROMA1 team has facilitated the establishment of fruitful collaborations with other research groups. It is worth noting that UNIBA and UNIROMA1 teams have performed a first in situ field campaign at the Torre Guaceto beach (Brindisi) in May 2023 during which a ground-based hyperspectral sensor was tested for macroplastic litter monitoring on sandy beaches. Furthermore, part of the sediment samples collected at the Apulian sites were also sent to UNIROMA1 laboratory to test the application of HIS for the identification of MPs. A similar collaboration was initiated between UNIROMA1 and UNIGE teams, with the program to carry out joint field activities to samples collections and MPs characterization.

In conclusion, in the first period of the RETURN project, Task 4.3.2 activities have been focused on the development of analytical protocols for the monitoring of contaminants in coastal and marine environments. The tuning of tailored procedures for the collection of water and sediment samples represents one of the main outcomes of the performed activities. The main results have been presented in the frame of six international conferences in Europe and have been accepted for future presentations at four international conferences in Europe and in USA. Currently, five contributions have been published as peer-reviewed paper on international journals.

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