multi-Risk sciEnce for resilienT commUnities undeR a changiNgclimate

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1 Technical references

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1.1 Document history

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2 ABSTRACT

The main objective of Environmental degradation SPOKE-VS4 within the RETURN Project – Multi-Risk Science for Resilient Communities under a Changing Climate is the development of an integrated, multidisciplinary approach to quantify, mitigate, and prevent environmental degradation across terrestrial, aquatic, and transitional ecosystems. The Proofs of Concept (PoCs) include the evaluation of cumulative effects through laboratory experiments and modelling approaches, the development of a structured framework for practical implementation, and the translation of scientific outcomes for comparison with field observations. Three main operational phases are identified:

(1) Observation, monitoring, and assessment through field and laboratory activities.

(2) Understanding, simulation, and prediction through in silico analyses.

(3) Prevention, mitigation, and remediation actions.

The primary focus of the Proofs of Concept (PoCs) is the assessment and management of the cumulative impacts of chemical stressors and climate change on ecosystems. To this end, innovative methodologies are applied to track contaminant pathways from sources to final receptors and to analyse and characterise the interaction processes between multiple stressors and potentially exposed ecological endpoints, taking into account future scenarios representative of climate change.

Observation, monitoring, and assessment activities are conducted in several national geographical areas representative of the different model systems:

(1) River basin.

(2) Transitional and coastal environment.

(3) Coastal city and port infrastructure.

The study areas include the Sarno River Basin (Campania) for the river basin system; the Grado-Marano lagoonal system and the Orbetello Lagoon for the transitional and coastal environment system; and the ports of Ischia, Genoa, and Trieste for the coastal cities and port infrastructure system. Moreover, the Aeolian hydrothermal vents (Vulcano, Panarea, and Lipari) were used as natural laboratories to investigate the impacts of chemical stressors under variable pH and temperature regimes. The study focuses on multiple chemical stressors, selected according to site-specific characteristics. Particular attention is devoted to the quantification of: Potentially Toxic Elements (PTEs); organic contaminants (e.g. PCBs); microplastics; bioplastics; and emerging contaminants (e.g. additives and PFAS). Across the different sites, the following aspects were investigated: the potential effects of chemical contamination under hypoxic conditions, ocean acidification and elevated temperatures on aquatic and benthic organisms, as well as on *Posidonia* seagrass meadows; and the effects of reduced pH and increased temperature on the alteration and degradation of plastics and bioplastics in environmental matrices. In addition, during site characterization for contaminant monitoring, discrete measurements were integrated with data obtained using passive sampling techniques.

3 Table of contents

4 Introduction and scope of work

Environmental degradation represents a major and growing challenge for territorial systems and communities, driven by the combined effects of anthropogenic pressures and climate change. Chemical pollution, habitat alteration, urbanization, and extreme climatic events interact across spatial and temporal scales, leading to the progressive loss of ecosystem integrity, functionality, and services. These processes directly affect environmental quality, human health, economic activities, and the resilience of natural and social systems, particularly in highly vulnerable and human-dominated areas. In this context, environmental degradation is increasingly characterized by the coexistence and interaction of multiple hazards and stressors. Chemical, physical, and climatic drivers act simultaneously, generating cumulative and potentially non-linear impacts that cannot be adequately addressed through traditional single-risk or sectoral assessment approaches. This complexity highlights the need for integrated frameworks capable of capturing interdependencies, cascading effects, and long-term consequences under current and future climate conditions. The multi-risk assessment paradigm provides a structured and comprehensive approach to address these challenges. By integrating multiple hazards, exposure pathways, vulnerabilities, and impacts within a unified framework, multi-risk assessment supports a more realistic and robust evaluation of environmental risks. This approach is fully aligned with the objectives of the RETURN Project – *Multi-Risk Science for Resilient Communities under a Changing Climate* – which aims to strengthen scientific knowledge, technological innovation, and decision-support capacity for risk management and resilience building at the territorial scale. Within the RETURN Project, the Proofs of Concept (PoCs) represents a key operational tool for implementing and validating the multi-risk assessment framework. PoCs are designed to translate scientific advances into applied solutions through the integration of field observations, laboratory analyses, and modelling activities. They enable the assessment of cumulative impacts arising from chemical stressors and climate-related drivers, supporting the identification of sources, pathways, receptors, and potential risk hotspots in representative environmental and socio-technical systems. The PoCs also play a strategic role in testing the interoperability and scalability of methods, data, and models developed within the project. By applying harmonized methodologies across different model systems, PoCs contribute to the reduction of uncertainties and to the consolidation of shared assessment protocols. This approach enhances the transferability of results and supports the development of standardized tools and indicators for environmental monitoring and risk assessment. From a PNRR perspective, the implementation of PoCs generates tangible benefits in terms of innovation, capacity building, and policy support. PoCs foster collaboration among research institutions, disciplines, and stakeholders, reinforcing the integration of scientific excellence with operational needs. Moreover, they provide evidence-based knowledge and decision-support tools that can inform environmental management strategies, mitigation and remediation actions, and public policies aimed at promoting resilience and sustainable development. By bridging research and application, the Proofs of Concept contribute to the overarching goals of RETURN and PNRR, supporting the transition towards more resilient territories and communities capable of addressing environmental degradation and climate-related risks in an integrated and forward-looking manner.

5 Innovative Approach

The innovative approach tested within the Proof of Concept (PoC) is focused on the analysis of interactions between chemical stressors and ecological endpoints under variable climatic forcings in three different model ecosystems: River basin, Transitional and coastal environment and Coastal city and port infrastructure. The PoC aims to evaluate the cumulative and interacting effects of multiple stressors by integrating field observations, experimental activities, and targeted monitoring tools across different environmental systems. The approach has been implemented through the analysis of specific interaction mechanisms in the representative model systems, as detailed below.

5.1 Application of passive sampling techniques for contaminant monitoring

Passive samples were applied for the monitoring of organic and inorganic contaminants in transitional and coastal environments, as well as in coastal cities and port infrastructures. Monitoring activities were conducted in the Grado and Orbetello lagoons, in the ports of Ischia, Genoa, and Trieste, and in the Aeolian hydrothermal vent systems, integrating passive sampling with conventional sampling. The integration of passive samples into the exposure characterization phase ensures a more accurate assessment of environmental contamination levels, as it allows the identification of the bioavailable fraction of contaminants and the detection of substances present at very low environmental concentrations. Although the effects of these contaminants may be negligible when considered individually, they can contribute substantially to the toxicity of realistic environmental mixtures, through synergistic interactions with co-occurring contaminants.

5.2 Assessment of Potentially Toxic Elements (PTEs) effects under hypoxic conditions in water and sediments.

The effects of oxygen depletion and chemical contamination were investigated in lagoon environments. The study was conducted in the Grado and Orbetello lagoons through the characterization of chemical exposure concentrations in the water column and the evaluation of associated biological effects. In the Grado Lagoon, hypoxic conditions were experimentally induced using mesocosm systems to assess the impact of mercury on the structure and functioning of benthic communities. In the Orbetello Lagoon, the multidisciplinary approach integrating abiotic (water and sediment) and biotic matrices (fish and mussels) was applied to assess the distribution and impacts of both legacy contaminants (heavy metals and Organochlorines) and emerging contaminants (phthalate esters, bisphenol A and PFAS), including microplastics in lagoon ecosystems. *Mussel Watch* experiments were carried out to evaluate bioaccumulation patterns and biological responses.

5.3 Assessment of PTE effects on Posidonia seagrass meadows under elevated temperature and salinity conditions.

The combined effects of mercury contamination and climate-related stressors were evaluated on Posidonia seagrass meadows in environments characterized by high variability in salinity and temperature.

5.4 Assessment of contaminant mixtures in riverine environments and evaluation of contaminant transport and redistribution in flooding-prone areas.

The PoC included the analysis of mixtures of organic and inorganic contaminants in riverine systems, with a specific focus on contaminant source localization, pathways analysis, and their spatial redistribution during flooding events. Activities were conducted in the Sarno River Basin, an area characterized by intense anthropogenic pressure related to urbanization and industrial activities. The study aimed at addressing the influence of climate change and extreme meteorological events on contaminant mobilization and redistribution from source areas to mid-basin flooding zones and alluvial plains. The approach combines extensive historical datasets with newly acquired data on surface waters, stream sediments, and riparian soils, enabling a comprehensive evaluation of both short-term and mid-term environmental dynamics.

5.5 Assessment of PTE effects on macrophytes under elevated temperature and low pH conditions.

An integrated approach including passive and active monitoring techniques was used to assess the combined effects of chemical contamination (trace elements and sulfur) and global change drivers (warming and ocean acidification) on habitat-forming macrophytes in coastal volcanic areas. These natural acidified environments, widely used to assess the impacts of future ocean acidification on the marine ecosystems, actually share several features with projected global climate change scenarios in marine systems (e.g., elevated temperature and CO_2 levels, resulting in reduced pH and high concentrations of toxic compounds such as sulfides and trace metals). These environments therefore represent valuable natural laboratories for investigating the effects of multiple stressors on short- and long-term biological and ecological responses at different hierarchical levels of organization (species, communities, and ecosystems). Experimental activities were conducted in the shallow hydrothermal systems of Panarea Island, characterized by different pH, temperature, and toxic chemical composition of the fluids, and in the coastal waters of Salina Island (Aeolian Archipelago), used as control areas without CO_2 emissions.

5.6 Experimental evaluation of the alteration and degradation of plastics and bioplastics under increased pH and temperature conditions.

Laboratory experiments were conducted using stainless steel cylinders containing different environmental matrices exposed at selected sites within the Aeolian hydrothermal vent systems (Vulcano and Lipari). The interactions among plastic pollution, ocean acidification, global warming, and anthropogenic pressures are often studied separately and rarely in truly synergistic contexts. By using conventional and biodegradable polymers at sites with natural gradients in pH, temperature, and anthropogenic contamination, this approach simulates future climate scenarios while capturing site-specific variability. This enables the evaluation of non-linear responses, such as accelerated degradation or increased toxicity, with ecological realism that is more difficult to achieve in laboratory experiments under controlled conditions. Unlike traditional studies that focus on single stressors (e.g., UV exposure or mechanical fragmentation), this proof of concept examines the combined effects of natural (CO_2 emissions causing acidification) and anthropogenic (e.g., PAHs and port-related metals) factors, reflects climate model predictions (e.g., IPCC scenarios) in which warming and acidification amplify pollutant dynamics.

6 Model System

6.1 River Basin (Sarno River Basin, Campania)

The objective was to test an appropriate methodology among the available risk assessment approaches for the co-presence of chemical contaminants and environmental stressors. After acquiring historical data on the presence of potentially toxic elements in the sediments and soil of the river basin, an additional monitoring campaign of river stream water, sediments and riparian soils was carried out to fill in the missing information in areas not previously studied and to compare the evolution of contamination patterns during the last two decades as potential function of the climate change. The samples collected underwent both analytical assessments of the main potentially toxic metals and ecotoxicological effects assessments. The concentration of potentially toxic metals was assessed in all environmental sample types. In addition, stream sediments were also analyzed to determine the concentration of Organochlorine Pesticides (OCPs), Polycyclic Aromatic Hydrocarbons (PAHs), Polychlorobyphenils (PCBs), and Organotin compounds (OTINs). The environmental samples collected were tested for their effects on a variety of freshwater species belonging to different levels of the food chain, from primary producers (microalgae and plants) to consumers (crustaceans) and degraders (bacteria), through acute and chronic exposures combined with innovative diagnostic tools based on sub-lethal effects. The degradation potential of bioplastics in river sediments was studied to verify their transformation, the release of products and to assess their toxic effects. The data collected and measured were processed to define the background levels of potentially toxic elements and raise awareness of the actual contamination of the basin. Priority contaminant mixtures to be considered in the risk assessment of the sampled areas were also defined by comparing hypothetical and actual mixtures. The assessment of contamination footprints in different environmental matrices was carried out using advanced statistical techniques. This PoC adopted a multidisciplinary approach, including field surveys, laboratory analyses and data modelling techniques.

6.2 Transitional and coastal environment (Grado Lagoon, Marano Lagoon and Orbetello Lagoon)

Lagoon systems or similar ecosystems are fragile ecosystems that are particularly susceptible to the combined effects of human activities and climate change. Through a multidisciplinary approach, this POC aimed to test and validate an integrated investigation methodology for assessing the environmental risks arising from human activities and their synergistic effect with the impact of climate change on the quality and functionality of ecosystems, as well as to assess the possible impacts related to the alteration of ecosystem services. This knowledge is crucial for the development of mitigation and adaptation strategies that can be transferred and capitalized on in other particularly sensitive areas of the territory. The approach involved the collection of historical and field data on traditional contaminants (such as heavy metals, in particular Hg, and organochlorines such as PCBs, HCBs and DDT) and/or emerging contaminants (such as microplastics, additives and PFAS), analyzing the different environmental matrices. Integrated monitoring systems, such as passive samplers, were also used over time for both organic and inorganic contaminants. Experiments inducing hypoxia conditions, using mesocosms set up in areas of interest subject to historical mercury contamination, allowed for a particularly effective study of the combined action of stressors on the structure and function of benthic communities at different trophic levels. Lagoon areas characterized by high temperature and salinity were used to study the influence of these parameters, in combination with the presence of trace contaminants (metals), on the structure and function of Posidonia meadows. Particular attention was paid to the potential (eco)toxicological effects of individual substances of interest and real environmental matrices, as well as to the integration of the biological responses obtained for the purpose of an integrated risk assessment. A risk analysis model based on the Weight Of Evidence (WOE) approach was applied, implemented within a specific informatic tool capable of processing, using standardized procedures, the results of numerous types of surveys highlighted in the environmental context under consideration. The assessment of the decrease in ecosystem services provided as a function of the calculated ecological risk provided a measure of the potential damage resulting from the combined action of multiple stressors.

6.3 Coastal city and port infrastructure (Genova, Trieste and Ischia)

Port areas generally combine commercial, industrial and service functions, which can generate various environmental problems. In addition to the possible spillage of pollutants into the water column (e.g. petroleum refining products, chemicals present in ships, etc.) and the suspension of sediments with consequent contamination of water, sediment and marine organisms, rainwater must also be considered. Rainwater causes runoff from roads and, within the port, from vehicle transit and parking areas, as well as from material handling and storage areas. These impacts are generally subject to seasonal and annual monitoring of water and sediment. This PoC aimed to verify the use of passive sampling techniques compared to active monitoring in order to fully characterize the presence and type of contaminants, even in trace amounts. By integrating the contaminants present over time, this technique makes it possible to identify the presence of compounds that would otherwise be unmeasurable because they are below the quantification limit. The application protocol is completed by the ecotoxicological characterization of the sampled matrices and eluates, to also compare the results derived from mixed contaminants. The data were collected from three seasonal sampling points in the Port of Genoa and two collection points in the Port of Trieste. The sediments collected, with a view to possible remediation, particularly in Genoa, were also subjected to a micoremediation approach to assess their potential recovery. All the data collected were subjected to an assessment of the ecological risk and possible alteration of ecosystem services in the surrounding coastal areas.

7 Activities in field & lab

The observation, monitoring, and assessment activities were carried out at the selected sites of the Sarno River Basin, the Grado–Marano and Orbetello Lagoons, and the ports of Ischia, Genoa, and Trieste. In addition, the hydrothermal systems of the Aeolian Islands were used as natural laboratories to analyze the effects of chemical stressors under variable pH and temperature conditions. The research focused on multiple chemical contaminants, including potentially toxic elements, organic contaminants, microplastics, bioplastics, and emerging contaminants (such as PFAS and additives). The effects of contamination under hypoxic conditions and elevated temperatures were evaluated on aquatic and benthic organisms, on *Posidonia* seagrass meadows, as well as on the alteration and degradation processes of plastics and bioplastics. Monitoring activities integrated discrete measurements with passive sampling techniques across all observation sites.

7.1 Passive Sampling and exposure characterization

Passive samplers represent an innovative monitoring tool for the time-integrated measurement of bioavailable contaminants (i.e., inorganic and organic compounds) in water and sediment (Vrana et al., 2005). These devices are proving to be reliable, robust and cost-effective and can be very useful for a proper evaluation of the ecotoxicological impact.
Organic compounds were sampled using Semi-Permeable Membrane Device (SPMD) and Polar Organic Chemical Integrative Sampler (POCIS) for lipophilic and hydrophilic compounds, respectively, and were operated for 21 days (Huckins et al., 1990; Alvarez et al., 2004). Additionally, Diffusive Gradients in Thin films (DGT) were deployed for 7 days to sample metals and mercury (Schintu et al., 2008; Vrana et al., 2005; Zhang and Davison, 2000).

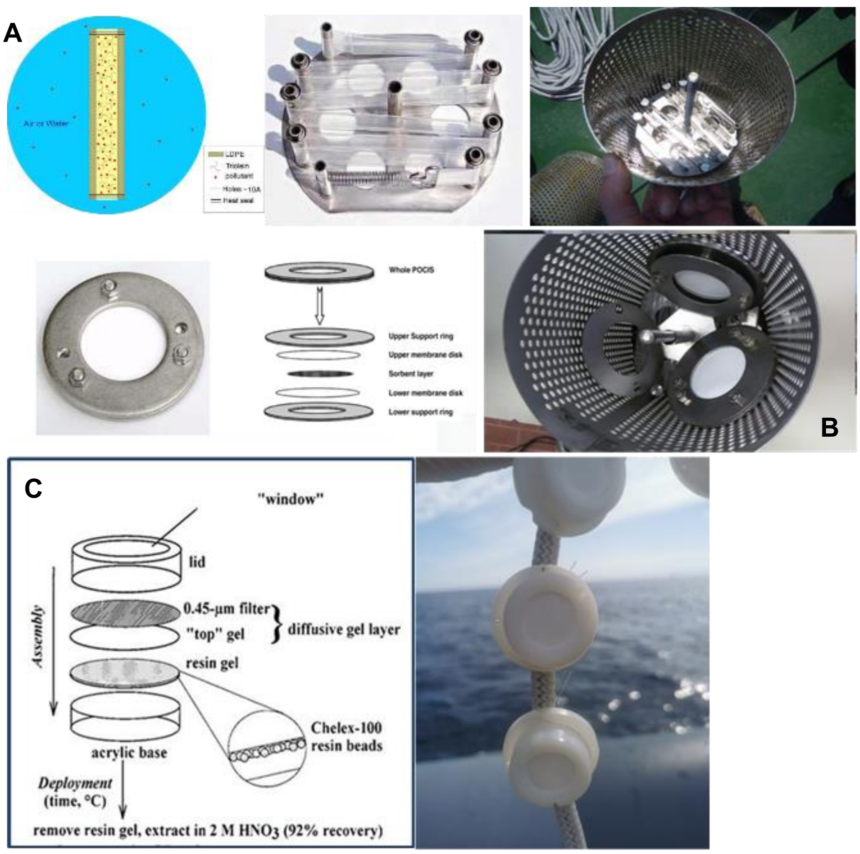

Figure 1. Images that show how SPMD (A), POCIS (B) and DGT (C) are composed, respectively

Through diffusion (DGT) or adsorption (SPMD and POCIS), they capture the labile fraction of the elements, thus simulating their accumulation in marine biota, allowing to estimate their impact on the environment and organisms.
For metal analysis, the extremely complex marine matrix often prevents the detection of many trace elements, such as Cr, Cd, and Pb, during seawater analysis. The same is true for Hg: the low concentrations of this analyte in seawater samples and the high salt content of these samples do not allow for accurate analyses of mercury to be carried out with the analytical instruments generally used. Therefore, DGT system offers an effective method for their detection, and for estimating their availability. For PAHs, at almost all sites, the various components could be detected more effectively than in seawater samples.
Although these devices also have a few disadvantages, such as the inability to identify point events, hydrodynamic conditions that can be highly variable (currents, waves, tides), and, under certain conditions, greater difficulty in implementation, recovery, and logistics, they nevertheless prove to be an useful alternative to the generally used dynamic methods, thanks to the significant simplification of field sampling. Furthermore, time-integrated data obtained by these devices, and in-depth research on biological factors and substance availability, along with ecotoxicology experiments to examine the impacts of chemical mixtures in the marine environment, will be used to improve chemical monitoring and ecological risk assessment of chemical pollution.

7.1.1 Port of Trieste

The Port of Trieste has been subject to a long-term environmental monitoring program since 2021, targeting approximately 200 contaminants in seawater and sediments. Monitoring activities include solvents, hydrocarbons, pesticides, dioxins, heavy metals, asbestos, and emerging contaminants. Although most analytes are generally reported below detection limits in conventional water samples, the port is characterized by significant anthropogenic pressures, including shipping activities, industrial operations, and urban inputs. For this reason, passive sampling techniques were introduced to improve the sensitivity of contaminant detection and to provide time-integrated measurements that better reflect environmental exposure.

Among the four monitoring sites, station BM2 (45°37’38.5’’N, 013°45’43.9’’E) was selected as representative of the most anthropogenically impacted area within the port. Station BM4 (45°38’33’’N, 013°44’13’’E), located outside the port basin, was used as a reference site.

7.1.1.1 Methods

7.1.1.1.1 Passive Samplers

Three types of passive samplers were employed:

- **SPMDs (Semi-Permeable Membrane Devices)** for hydrophobic organic contaminants such as PAHs and PCBs
- **POCIS (Polar Organic Chemical Integrative Samplers)** for polar organic contaminants
- **DGT (Diffusive Gradients in Thin Films)** for dissolved and bioavailable metals

All samplers were deployed at a depth of 1 meter below the sea surface. At each deployment and retrieval, water samples were collected at the same depth:

- 2 L for organic contaminant analysis
- 50 mL (acidified with HNO_3) for metal analysis
- 500 mL for ecotoxicological testing

The first deployment occurred on 4 July 2024. DGT samplers were retrieved after 7 days, while SPMDs and POCIS were retrieved on 31 July 2024. However, at BM2, SPMDs and POCIS were lost together with part of the DGT samplers. A second deployment was carried out on 7 October 2024; during this campaign, DGT samplers were lost at both stations due to strong currents, although SPMDs and POCIS were successfully retrieved on 7 November 2024.

A third campaign was conducted between March and May 2025, during which all devices were successfully deployed and recovered. DGT samplers were retrieved after 6 days, while SPMDs and POCIS remained in the water column for approximately 40 days.

Sediment samples were collected once during routine annual monitoring near the port breakwaters. Among twelve sediment stations, S09 (nearest to BM2) and S01 (nearest to BM4) were selected for detailed contaminants and ecotoxicological analyses.

Figure 2. Positions of investigated stations inside the Port of Trieste

7.1.1.1.2 Ecotoxicological Assessment

To complement chemical analyses, ecotoxicological tests were performed using a battery of organisms representing different trophic levels and biological endpoints:

- *Vibrio fischeri* (bioluminescence inhibition – acute toxicity)
- *Dunaliella tertiolecta* (algal growth inhibition – primary producers)
- *Artemia salina* (mortality test – secondary consumers)

To integrate the results obtained from different bioassays and provide a synthetic evaluation of ecological risk, the Toxicity Battery Index (TBI) was applied. The TBI combines normalized responses from each biological test into a single composite index, allowing comparison among stations and sampling periods while reducing endpoint-specific bias.

7.1.1.2 Results

7.1.1.2.1 Organic Contaminants

Passive samplers proved capable of detecting organic contaminants that were often below detection limits in grab samples.
Polycyclic Aromatic Hydrocarbons (PAHs) were detected at significant concentrations. The total PAH concentration (ΣPAH) reached 1311.59 ng/L at BM4. The most abundant compounds included naphthalene (434.55 ng/L), fluoranthene (303.86 ng/L), phenanthrene (78.48 ng/L), and perylene (71.95 ng/L). These compounds are commonly associated with petroleum-derived inputs and combustion processes, suggesting contributions from shipping activities and urban emissions.
Polychlorinated Biphenyls (PCBs) were also detected. The total PCB concentration (ΣPCB) reached 459.24 ng/L, including 11.08 ng/L of dioxin-like congeners, which are of particular toxicological concern. The persistence of these compounds reflects historical industrial contamination.
Organochlorine pesticides (OCPs), including α-endosulfan (586.24 ng/L), β-endosulfan (64.68 ng/L), 4,4'-DDD (69.03 ng/L), and 2,4'-DDT (45.63 ng/L), were also detected. Although banned or restricted for decades, these substances remain present due to their high environmental persistence.
Overall, the passive sampling approach revealed background contamination that would not have been fully captured by conventional monitoring.

7.1.1.2.2 Metals in Seawater and Bioavailability

Dissolved metals were measured at both BM2 and BM4. Nickel concentrations were approximately 0.19 mg/L at both stations, copper ranged between 0.84 and 0.92 mg/L, manganese was around 1.7 mg/L, arsenic approximately 1.4 mg/L, and uranium around 3.4 mg/L.
DGT samplers provided information on the labile and potentially bioavailable fraction of metals. In some cases, DGT-derived concentrations were comparable to or higher than directly measured dissolved concentrations, indicating dynamic metal exchange processes in the water column.
Mercury was detected at low but measurable levels, with concentrations of 1.3 ng/L at BM2 and 1.0 ng/L at BM4. Although relatively low, mercury remains of concern due to its bioaccumulative nature.

7.1.1.2.3 Sediment contamination

Sediment analysis revealed spatial differences between the impacted and reference areas. Station S09, located near BM2, exhibited higher concentrations of several metals compared to S01. Iron reached approximately 36,814 mg/kg, while zinc (285 mg/kg), lead (155 mg/kg), chromium (131 mg/kg), and mercury (1.39 mg/kg) indicated moderate enrichment.

Organotin compounds were also higher at S09, suggesting historical antifouling paint contamination associated with maritime activities.

These findings indicate that sediments may act as both sinks and potential secondary sources of contaminants.

7.1.1.2.4 Ecotoxicity

The ecotoxicological results indicate spatial differences in biological effects, with generally low acute toxicity in the water column and more pronounced effects in sediments. At BM2, algal inhibition was limited (10%), with no negative effect on *Artemia salina* and a mild hormetic response (10%) in *Vibrio fischeri*, suggesting low but detectable contaminant stress. BM4, although considered a reference site, showed higher algal inhibition (25%) and a stronger hormetic response in bacteria (20%), indicating background contamination likely influenced by diffuse maritime activities. In contrast, sediment samples showed the highest effects: S1 and S9 induced strong inhibition in *Dunaliella tertiolecta* (52% and 55%, respectively), while *Artemia* and *V. fischeri* mainly exhibited hormetic or slight effects. These biological responses are consistent with the chemical profile of the sites, where PAHs, PCBs (including dioxin-like congeners), organochlorine pesticides (e.g., endosulfan and DDT derivatives), metals (Zn, Pb, Cr, Hg), and organotin compounds were detected. Such contaminants are known to interfere particularly with primary producers, explaining the greater sensitivity of algae. The stronger sediment toxicity confirms that sediments act as reservoirs of persistent pollutants and may represent a long-term source of chronic exposure, highlighting mixture effects rather than acute single-compound toxicity.

The Toxicity Battery Index (TBI) provides an integrated evaluation of the ecotoxicological results by combining the responses of all tested organisms into a single value, allowing a clearer classification of environmental risk. According to the TBI values, BM2 (2.1) falls within the “absent risk” category, indicating negligible overall ecotoxicological impact in the water column at this station. BM4 shows a TBI of 9.1, corresponding to a low ecotoxicological risk, suggesting the presence of background contamination capable of inducing measurable but limited biological effects. In contrast, sediment stations exhibit substantially higher TBI values: S1 reaches 18.6, classified as moderate risk, while S9 shows the highest value (20.7), indicating elevated ecotoxicological risk. These results confirm that sediments represent the most critical environmental compartment in the study area, particularly near the more anthropogenically influenced station (S9). The TBI pattern is consistent with the chemical findings, which highlighted higher concentrations of persistent organic pollutants (PAHs, PCBs, organochlorine pesticides), metals, and organotin compounds in sediments. Overall, the integrated TBI assessment strengthens the conclusion that, although water column toxicity is limited, sediment-associated contamination poses a more significant and ecologically relevant risk.

Table 1. Synthesis of the measured risk in the study sites

TBI	PT%	RISK
BM2	2,1	absent
BM4	9,1	low ecotoxicological risk
S1	18,6	moderate risk
S9	20,7	elevate risk

7.1.1.3 Conclusions

The application of passive samplers in the Port of Trieste significantly enhanced contaminant detection compared to conventional water sampling. Persistent organic pollutants, including PAHs, PCBs, and organochlorine pesticides, were detected despite generally low concentrations in routine monitoring data.

DGT samplers confirmed the presence of bioavailable metal fractions, while sediment analyses revealed localized contamination near the more anthropogenically influenced site.

Most importantly, ecotoxicological assays demonstrated substantial biological effects, particularly on primary producers, underscoring the ecological relevance of the detected contamination.

Overall, the results indicate that the Port of Trieste presents a complex contamination profile characterized by persistent organic pollutants, dissolved metals, and biologically significant effects. Continued integrated monitoring using both passive sampling and ecotoxicological tools is strongly recommended to assess long-term trends and support environmental management strategies.

7.2 Lagoons

7.2.1 Grado lagoon

Stressors: Oxygen depletion and Hg contamination

7.2.1.1 Ecosystem-based approach

The Grado lagoon is historically contaminated by mercury and periodically exposed to hypoxia or anoxia events, particularly in its northernmost, confined areas. In this context, this lagoon represents an ideal case study for investigating the synergistic effects of combined stressors, i.e. oxygen depletion and mercury (Hg) contamination, on the benthic-pelagic coupling and the lagoon overall ecosystem functioning. The experiment was performed in June 2024 at a severely Hg-contaminated shallow site. Specific lagoon sections were secluded using mesocosms, which limit the horizontal exchanges with oxygen-enriched seawater, induce water stagnation and dissolved oxygen depletion, to mimic the increase in the resident time of lagoon water that occurs in these confined areas in summer, simulating potential future climate change scenarios. Eighteen cylindrical mesocosms (Ø100 cm, vol. ~0.8 m^3 each), divided into three clusters (R1, R2, R3) were used for the experiment. Sampling was performed at the start (T_0), after 4 days (T_1), and after 10 days (T_2). A holistic approach was applied by simultaneously investigating several contaminants (also using passive samplers), the physical-chemical features of water (temperature, pH, O_2, salinity, inorganic nutrient and Chl a) and sediments (grain-size, total organic C and total N), pelagic and benthic communities' abundance and composition (using classical taxonomy and molecular tools), and the main biological processes (primary production, prokaryotic heterotrophic C production and community respiration).

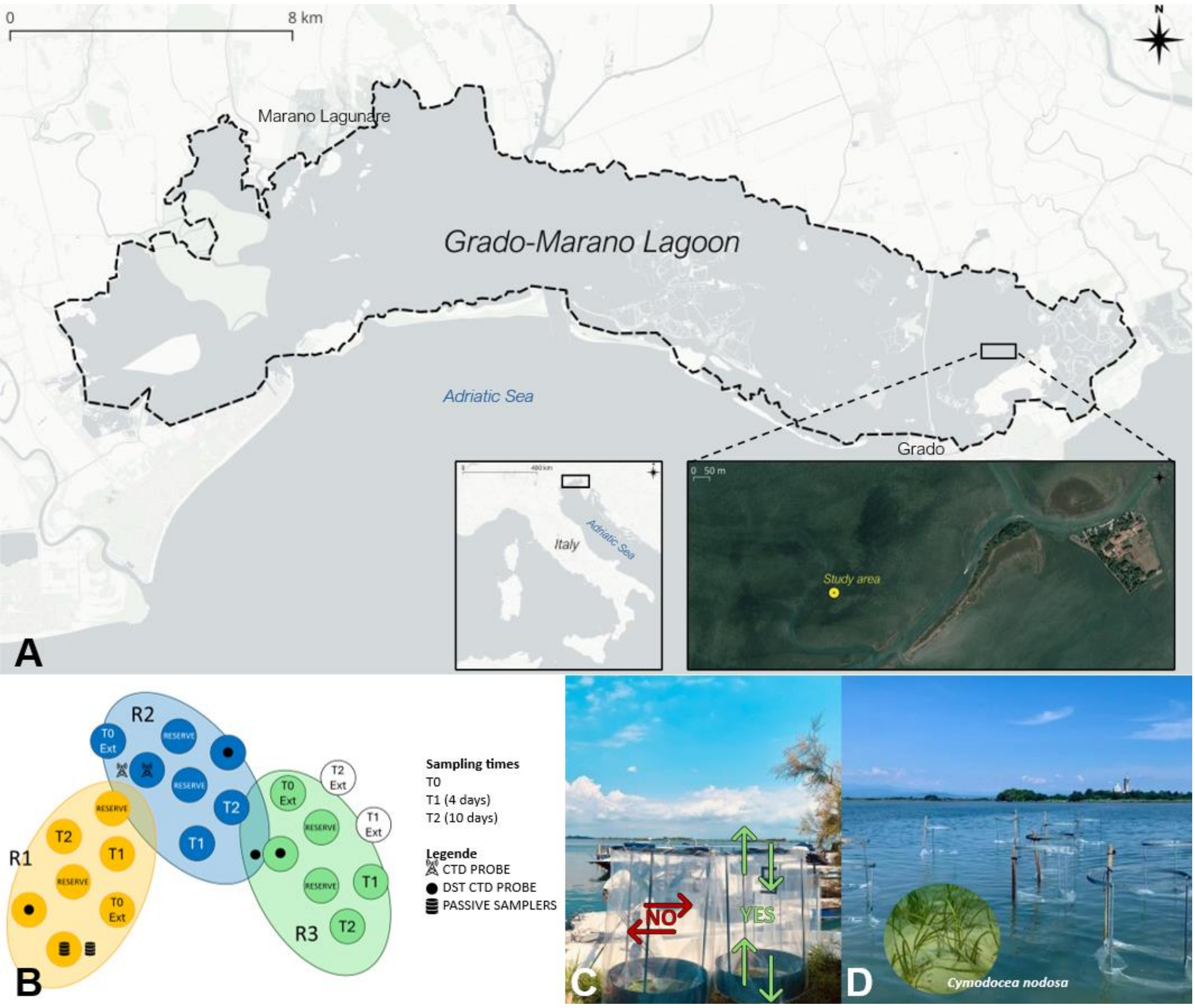

Figure 3. Experimental design: Study area (A), mesocosms positions (B), mesocosms structure, the vertical exchanges are allowed while the horizontal ones are blocked (C), mesocosms during the experiment (D)

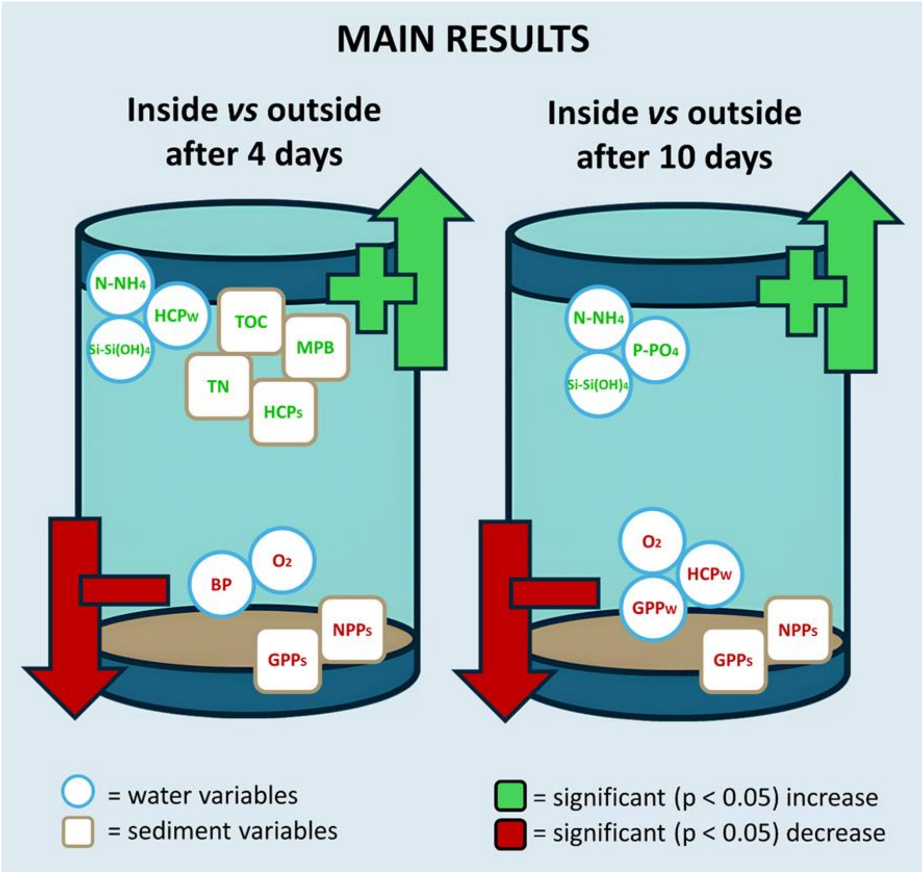

Figure 4. Graphical abstract: Significant changes in the investigated variables between inside and outside the mesocosms after 4 (T1) and 10 (T2) days. Abbreviations: MPB, microphytobenthos; TOC, Total Organic C; TN, Total N; HCPw and HCPs, Heterotrophic C Production in water and sediments, respectively; BP, Phytoplankton Biomass; GPPs and NPPs, Gross and Net Primary Production in sediments, respectively

7.2.1.2 Results

The induced confinement progressively triggered water stagnation and dissolved oxygen depletion, mimicking the increase in the residence time of lagoonal water that occurs in the most confined areas in summer. The passive samplers evidenced an enrichment of some metals, especially of Hg, inside the mesocosms. This suggests that the stagnation and water stratification, induced by the enclosure, already cause the transfer of Hg from sediments to the water column, even without having reached proper hypoxic conditions. This finding may have important implications for the lagoon ecosystem functioning, especially in its most confined areas where water stagnation is common in summer and autumn.

We observed increased benthic respiration and strongly reduced primary production over time, shifting system trophy towards heterotrophy. In contrast, external conditions, with natural seawater exchange, remained autotrophic for the duration of the experiment. Our results indicate that already a moderate reduction in oxygen concentration, compared to natural conditions, is enough to trigger significant cascading effects on biological and biogeochemical processes, leading to an imbalance in the benthic-pelagic coupling of the ecosystem. The presence of an abundant phototrophic microbial community, and particularly its swift biomass increase at the early signs of adverse conditions likely favoured lagoon ecosystem resilience. This finding highlights the extreme sensitivity of Hg contaminated, confined lagoons to water stagnation and moderate variations in oxygen concentration, suggesting that they might be precursors to disrupting effects on the ecosystem, leading to a dystrophic event at the beginning of summer, already. These findings provide evidence on the vulnerability, yet short-term adaptive capacity of coastal lagoons exposed to multiple stressors and highlight the enclosure set-up as a powerful tool to understand complex ecosystem responses to contamination and global changes.

7.2.2 Orbetello lagoon

The E-TRACE project applied a multidisciplinary approach articulated into five work packages (WPs) tested and applied in a relevant ecological area, the Orbetello Lagoon (Fig. 5), characterized by a fragile ecosystem particularly sensitive to the combined effects of anthropogenic activities and climate change.

In detail, the results obtained within the different WPs, concerning contamination by microplastics (MPs) (WP1), emerging and legacy contaminants (WP2), provided data on the distribution, abundance, and characterization of these contaminants in the different environmental matrices considered (sediment, water column) as well as in organisms. A set of ecotoxicological endpoints on bioindicator organisms (WP3) have also applied to assess the ecotoxicological status of the lagoon by considering alterations in cellular detoxification and biotransformation processes, oxidative stress, neurotoxicity, and endocrine-metabolic toxicity associated with exposure to single compounds or mixtures of substances monitored in the previous WPs. By integrating the different biological responses obtained through toxicity assays and *in vitro* exposures of human cells (via laboratory tests), data provided insights into the actual impacts across various levels of structural complexity.

Through metagenomics and DNA metabarcoding, it has been possible to assess impacts on biodiversity in relation to the results obtained on the contamination status of the different sites, to investigate the presence of associations and drivers of environmental degradation, such as anthropogenic impacts and climate change.

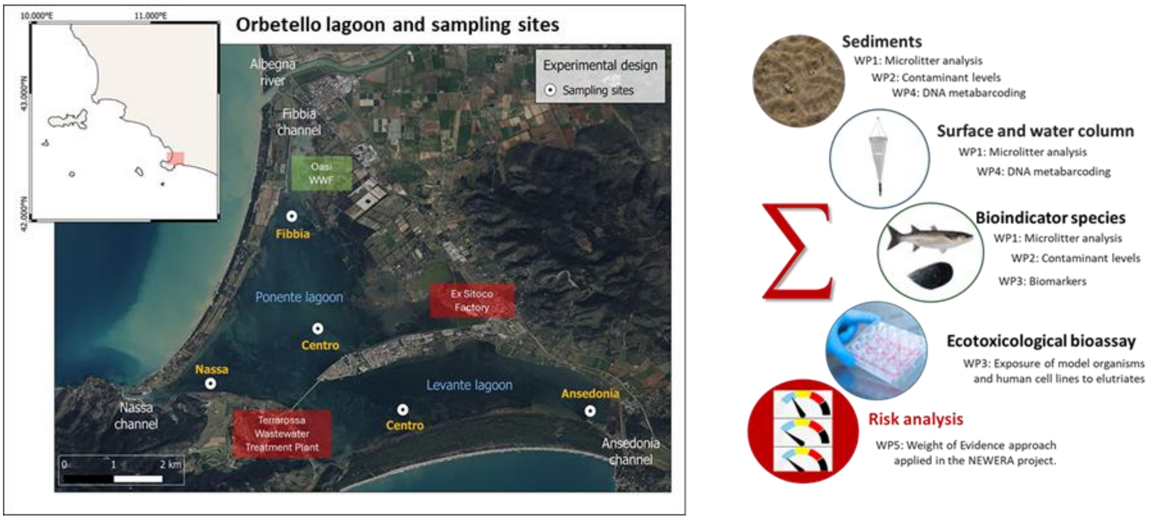

Figure 5. Map of the Orbetello Lagoon showing potential sources of contamination (red), sampling sites included in this study (yellow), the WWF natural Reserve (green) and the lagoon's connections with the open sea. On the right the outline of theWPs and the analysis performed

In detail, two sampling campaigns were carried out in different seasons (autumn 2024/winter 2025 and spring/summer 2025) by collecting biotic matrices (surface water, water column, and sediment) during both seasons and subsequently analyzed to determine microplastic abundance and characteristics. The concentrations of microplastics in both sediment and water varied across the two seasons (in both the two halves of the lagoon) and are in line with data found in the Mediterranean area (Fig. 6).

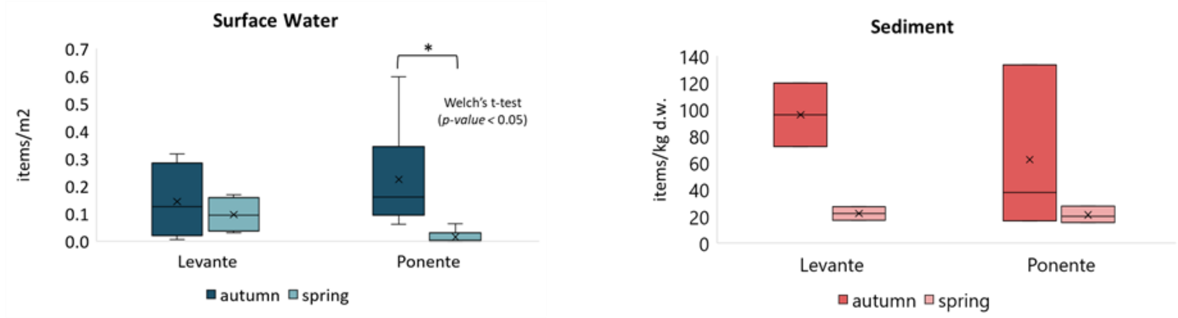

Figure 6. Microplastics abundance in water and sediment

Regarding the organisms, 86 specimens of *Chelon labrosus* were collected in autumn 2024 and spring 2025 from two sampling stations (one for half-lagoon: Nassa channel and Ansedonia channel). Additional control organisms were obtained from a control site (the Diaccia Botrona Nature Reserve). Specimens of *Mytilus galloprovincialis* were transplanted, caged and exposed for approximately one month at four sites within the Orbetello Lagoon. The exposure experiments were carried out in winter and spring 2025.

The analyses of organochlorine contaminants, heavy metals (Hg, Cd, and Pb), and emerging contaminants (PAEs, BPA, and PFAS) were conducted on sediment samples (N = 10), as well as on specimens of *C. labrosus* (N = 86) and *M. galloprovincialis* (N ≈ 180 per contaminant class), collected from the Orbetello Lagoon in two different seasons, as described above. The levels of OCs, Hg and Phthalates were higher in the sediment of the Levante Lagoon, in particular in autumn. Regarding the levels of accumulation in the tissue of *C. labrosus* and *M. galloprovincialis*, any statistical difference was found and the levels vary according to the season (Fig. 7).

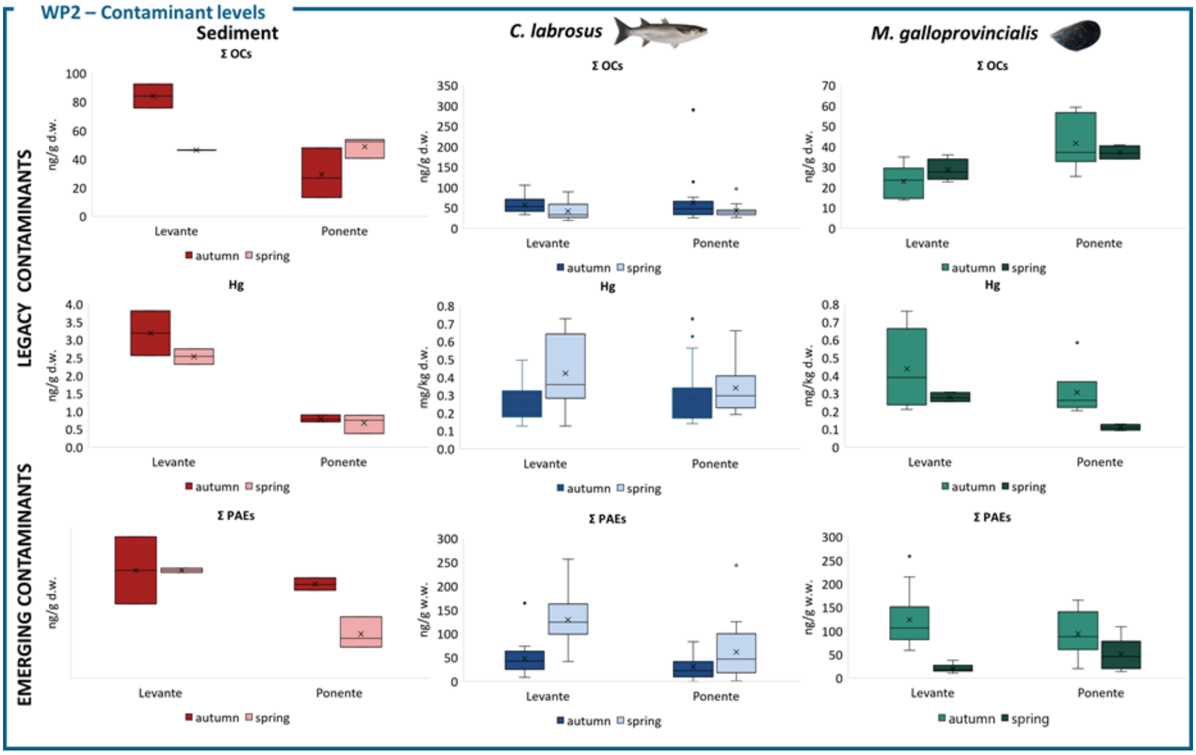

Figure 7. Contaminant levels in sediment and bioindicator species in the two halves of the lagoon and in the two sampling seasons

C. labrosus (N = 86) and *M. galloprovincialis* (N ≈ 180), collected during both seasons, were analyzed to evaluate the biological effects by using biomarkers of oxidative stress (e.g. lipid peroxidation), immunotoxicity (gene expression of immune related genes), neurotoxicity (e.g. AChE), and lipid and energy metabolism in various tissues (Fig. 8).

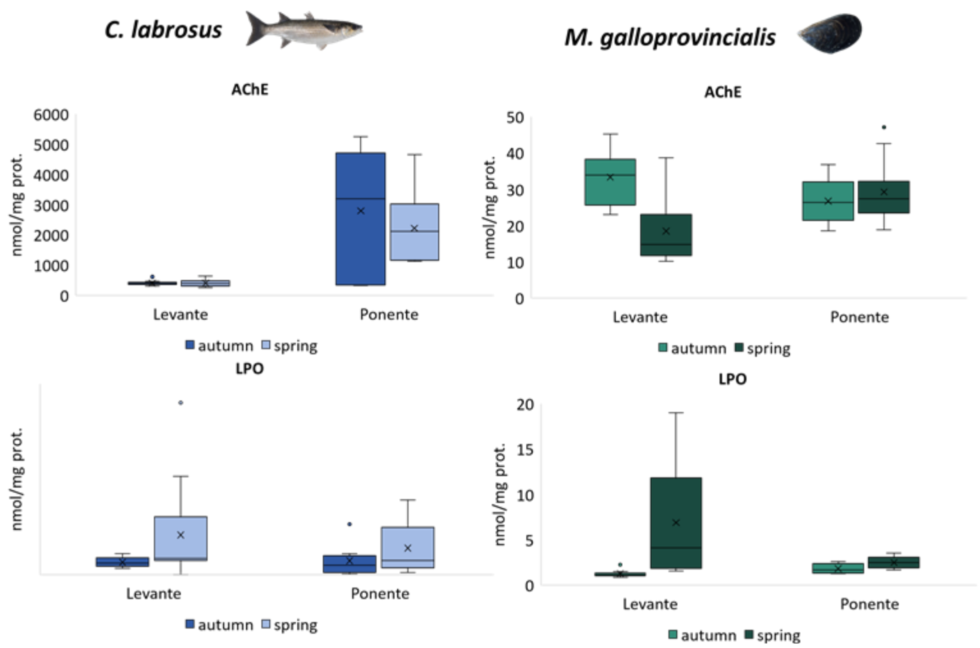

Figure 8. Some biomarker responses in bioindicator species (C. labrosus and M. galloprovincialis) in the two halves of the lagoon and in the two sampling seasons

Sediment samples collected during the two seasons were also used to prepare eluates, which were subsequently employed in toxicity assays at different trophic levels by exposing *Aliivibrio fischeri* and performing a bioluminescence inhibition assay, *Ficopomatus enigmaticus* by performing an embriotoxicy assay, *Phaeodactylum tricornutum* by measuring the algal growth inhibition and *Acartia tonsa* by assessing the inhibition nauplii mobility.

Moreover, Water (N = 30) and sediment (N = 30) samples were collected at the five sampling sites (in triplicates) and subjected to DNA sequencing to assess biodiversity in the Orbetello Lagoon in relation to contamination levels. Metagenomic analyses conducted on sediment revealed microbial diversity detecting 83 species (4 Archaea, 79 Bacteria). By analyzing alpha (Shannon Index) and beta diversity (Bray-Curtis Index) a different pattern of biodiversity was observed across the 5 sampling sites and according to the sampling season (Fig. 9).

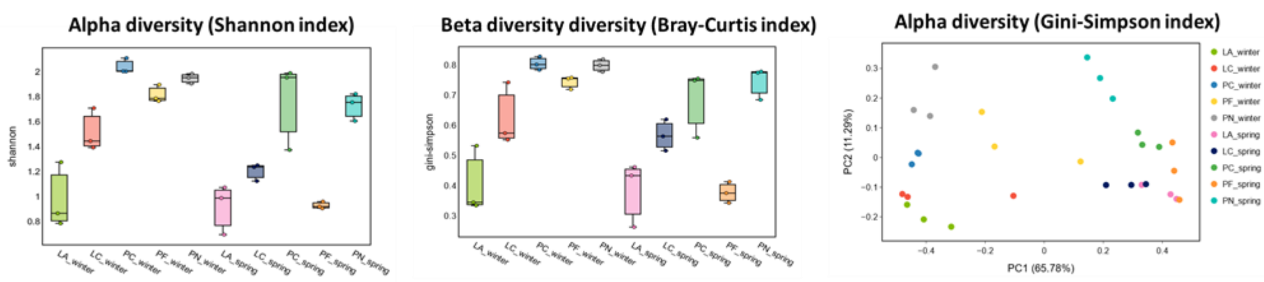

Figure 9. Biodiversity indexes for microbial biodiversity in sediment by metagenomic analysis

DNA metabarcoding (4 targets: 12S, 16S, 18S and COI) was performed on the water samples to assess metazoan biodiversity. The most efficient target resulted the 16S for vertebrates and 18S for invertebrates. Together they allowed us to detect more than 70 metazoan species, amongst which: 4 cnidarians, 22 annelids, 5 arthropods, 5 bryozoans, 24 molluscs, 9 teleosts and 3 birds.
The integration of the whole results enabled the development of schemes and models for assessing the health status of the ecosystems under consideration, based on the parameters evaluated in the different environmental matrices and in synergies with the Weight of Evidence approach applied in the NEWERA project. The Weight of Evidence approach allowed to integrate the collected data, classified into six different Lines of Evidence (LOEs) and determine a "moderate" status of the ecotoxicological impact of the Orbetello lagoon (Fig. 10).

		LOE 1		LOE 2		LOE 3		LOE 4		LOE 5				LOE 6			
Site	Sample	Chemical Sediments		Bioavailability		Biomarkers		Toxicological Bioassays		MPs Seawater		MPs Sediments		MPS Biota		Weight Of Evidence integration	Level
		Level	*Contr %*	*Level*	*Contr %*	*Level*	*Contr %*	*Level*	*Contr %*	*Level*	*Contr %*	*Level*	*Contr %*	*Level*	*Contr %*		
Levante Ansedonia	ET LA	SEVERE	28,1	SLIGHT	10,9	SLIGHT	9,2	MODERATE	14,1	SLIGHT	7,3	SLIGHT	9,1	MAJOR	21	MODERATE	46,78
Levante Centro	ET LC	SEVERE	28,6	MODERATE	14,3	SLIGHT	10,3	ABSENT	0,7	MAJOR	17,5	SLIGHT	7,8	MODERATE	20,5	MODERATE	45,89
Ponente Centro	ET PC	SEVERE	23,9	SLIGHT	10,6	MODERATE	12,2	ABSENT	0,9	MAJOR	21,2	SLIGHT	9,5	MAJOR	21,4	MODERATE	46,69
Ponente Fibbia	ET PF	SEVERE	23,1	MODERATE	12,5	MODERATE	13,1	SLIGHT	7,8	SLIGHT	9,3	SLIGHT	5,6	SEVERE	28,2	MODERATE	50,46
Ponente Nassa	ET PN	MAJOR	25,5	SLIGHT	12	MODERATE	14	ABSENT	1,3	MODERATE	14,4	SLIGHT	7,7	MAJOR	24,7	MODERATE	40,61

Figure 10. The Weight of Evidence approach in synergies with the Newera project based on the collected data, classified into six different Lines of Evidence (LOEs)

7.3 Multistressors effects in shallow hydrothermal vents

Shallow hydrothermal vents are among the most powerful in situ analogues of global change scenarios, as they simultaneously reproduce several key drivers of projected ocean conditions, including elevated temperature and CO_2 concentrations, reduced pH and the presence of toxic compounds like sulfides, and high trace metal concentrations. Nevertheless, research conducted in these systems has predominantly focused on the effects of ocean acidification (OA) on marine ecosystems. This PoC aimed to validate the use of these areas as natural laboratories to assess the combined impact of climate-related drivers (OA and warming) and toxic chemicals on plastic debris, on the bivalve *Mitylus galloprovincialis* and on the response, resilience and persistence of habitat-forming species (*P. oceanica* and *E. brachycarpa*). The methodological framework integrates field activities, in situ experiments, and laboratory-based analyses. The significance of this PoC lies in the strategic use of these uniquely stressed areas as experimental systems, allowing field-based testing of ecological responses to combined global change drivers. By applying established methodologies within these naturally acidified environments, the PoC generates mechanistic insights with strong global relevance and provides a scientific basis for developing conservation and management strategies to enhance the resilience of marine ecosystems facing the compounded pressures of climate change and pollution.

7.3.1 Panarea and Salina islands: effects of multistressors on macrophytes

Stressors: ocean acidification, high temperatures and chemical contamination (trace elements and sulfur)

Panarea island and its shallow hydrothermal system, composed by numerous active vent sites characterized by CO_2-dominated fluid emissions, provide well-characterized natural laboratories where multiple stressors (acidification, warming and toxic chemical) co-occur under real environmental conditions. In this Poc, these vent systems are used as experimental sites to assess the impacts of the combined effects of these multiple stressors on the marine ecosystem and in particular on macrophytes (*P. oceanica* and *E. brachycarpa*) and the associated living communities. Two sampling campaigns (September 2024 and May 2025) were conducted in three representative shallow hydrothermal sites of Panarea island, selected based on their different physico-chemical characteristics, presence of one of the two target macrophytes and depth, and four control sites unaffected by hydrothermal emissions, one in Panarea and three along the southern coast of Salina Island (Fig. 11).

Island	Site	Depth (m)	Target species	Stressor
Panarea	Hot/cold	8-10	*P. oceanica*	acidification, temperature of the seafloor, sulfur, metals
	Campo 21	16 and 21	*P. oceanica*	acidification, sulfur, metals
	Bottaro crater	10	*E. brachycarpa*	acidification, sulfur, metals
	Lisca nera	7	*E. brachycarpa*	control
Salina	Tre pietre	8-10	*P. oceanica*	control
	Lingua	21	*P. oceanica*	control
	Punta Megna	8-10	*P. oceanica*	control

Figure 11. Investigated sites and species

7.3.1.1 Methods

The investigation was conducted using a comprehensive and integrated analytical framework, combining passive and active environmental monitoring with chemical and ecological assessments across water, sediments, and biotic compartments. The approach included characterization of physical-chemical parameters of the water column (temperature, pH, dissolved O_2, salinity, inorganic nutrients, and carbonate system), interstitial water (temperature, pH, redox potential, and main cations and anions), and sediments (grain-size, total organic C and total N), as well as trace metals dynamics in abiotic and biotic matrices and ecotoxicology. This was complemented by isotopic signature patterns of $\delta^{34}S$, $\delta^{13}C$, and $\delta^{15}N$ in seagrass tissue, plant performance descriptors (phenology, lepidocrinology) and structural analyses of associated macrofaunal communities.

Figure 12. Different phases of the experiment

7.3.1.2 Results

The integrated assessment of seawater, sediment, interstitial chemistry, and macrophyte responses demonstrates that the Panarea hydrothermal system functions as a highly dynamic, multi-stressor environment shaped by persistent volcanic CO_2 emissions. Water column monitoring revealed strong spatial heterogeneity and temporal variability in pH, with extreme short-term acidification events (down to pH ~6.8) at vent sites, while control sites at Salina showed stable, non-hydrothermal Mediterranean conditions. Acidification at Panarea was consistently associated with reduced carbonate saturation states and altered nutrient regimes, confirming its role as a natural analogue for future ocean acidification scenarios. Hydrothermal influence extended to sediments, which were characterized by elevated temperatures (up to 54°C), strongly reducing conditions, increased ammonium, suppressed nitrate, and higher bicarbonate concentrations. Trace element enrichment, particularly for Hg and As, further distinguished vent-influenced sediments, with multivariate analyses highlighting strong small-scale spatial structure rather than uniform hydrothermal forcing. Biological responses of *Posidonia oceanica* closely tracked these environmental gradients, demonstrating the ecological relevance of this natural experimental setting. Meadows at vent sites showed reduced shoot density, simplified population structure, altered growth dynamics, increased trace element accumulation, particularly in the rhizomes, and isotopic signatures indicative of modified carbon and sulfur cycling. Integrated modeling approaches further demonstrated that plant performance was shaped by the combined effects of trace metal bioavailability, altered sulfur dynamics, and low pH, confirming the importance of considering interacting stressors. Overall, the results indicate that while *P. oceanica* can persist under sustained multi-stressor exposure, hydrothermal conditions reduce meadow structural complexity and may compromise long-term ecosystem stability, enhancing our understanding of the capacity of *P. oceanica* meadows to act as biological buffers under multi-stressor conditions. Moreover, the tight coupling observed among water column chemistry, sediment processes, and seagrass responses illustrates how natural CO_2-enriched environments can provide mechanistic insights into ecosystem resilience under projected climate change and contamination scenarios. This approach reinforces the importance of whole-system assessments in predicting the future functioning of Mediterranean coastal habitats.

7.3.2 Combined effects of contaminants, warming and acidification on degradation of plastics and bioplastics (Lipari and Vulcano Islands)

Coastal ecosystems are increasingly exposed to multiple natural and anthropogenic stressors, such as acidification, warming, and varying anthropogenic pressure. In this context, Vulcano Island serves as an ideal natural laboratory for investigating the synergistic effects of these stressors on polymer degradation dynamics and ecosystem responses, selected for their contrasting environmental conditions (pH, temperature) and different levels of anthropogenic pressure.

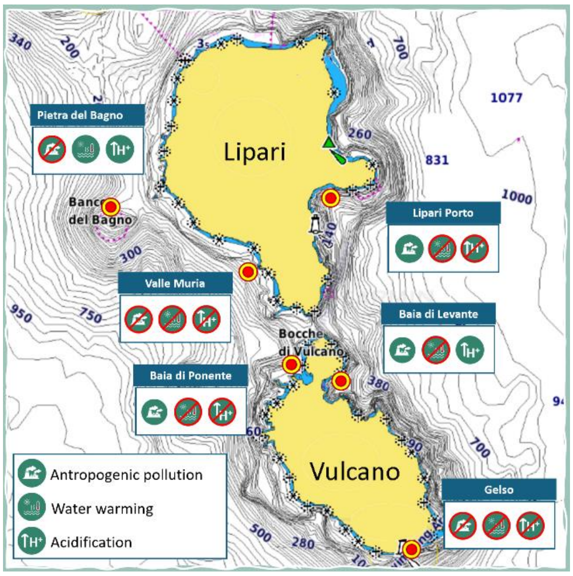

Figure 13 Study area

Table 2. Study sites and investigated stressors

ISLAND	SITE	STRESSOR	DEPTH (m)
Vulcano	Baia di Levante	Antropogenic pollution, acidification	3-4
Vulcano	Baia di Ponente	Antropogenic pollution	3-4
Vulcano – Gelso	Spiaggia Punta dell'Asino	Control	3-4
Lipari	Porto	Antropogenic pollution, acidification	3-4
Lipari	Spiaggia Valle Muria	Control	3-4
Lipari	Pietra del Bagno	Acidification, water warming	12

7.3.2.1 Methods

At each site, stainless steel cylinders containing four types of polymers were deployed. Microplastic fragments were prepared from virgin, sterilized macrocontainers of the same polymers and inserted into the exposure units to prevent external contamination. Samples were collected every four months. A multidisciplinary approach was applied to investigate (i) macrofouling and microfouling communities' development on polymer surfaces (plastisphere), (ii) physical degradation processes of the materials, and (iii) ecotoxicological responses to the exposed microplastics. In parallel, biochemical and nutritional analyses were conducted on Oblada melanurus to assess metabolic, protein, and lipid responses to environmental stressors. At the time of cylinder deployment, sediment

Figure 14. Stainless cylinders used in the study

and seawater samples were collected at each site for chemical characterization of the study areas.

7.3.2.2 Results

The results of this experiment highlight the importance of using hydrothermal areas as natural laboratories to assess the effects of acidification as well as surface water warming and chemical contaminants, not only on marine ecosystem but also on microplastic polymers. As demonstrated by the conducted experiment in short- and long-term, different from laboratory experiments conducted on controlled conditions, the synergy of natural stressors (acidification, hydrothermal inputs) and anthropogenic pressures (port activity, local pollution) accelerated the degradation process of bioplastic with first signs of additive loss, surface oxidation, and structural modification, accompanied by a marked increase in harmful compounds released into marine environment. Chemical and ecotoxicological analyses also showed that biological communities responded consistently to local environmental gradients, reinforcing the close link between polymer behavior, contaminant availability, and ecosystem functioning.

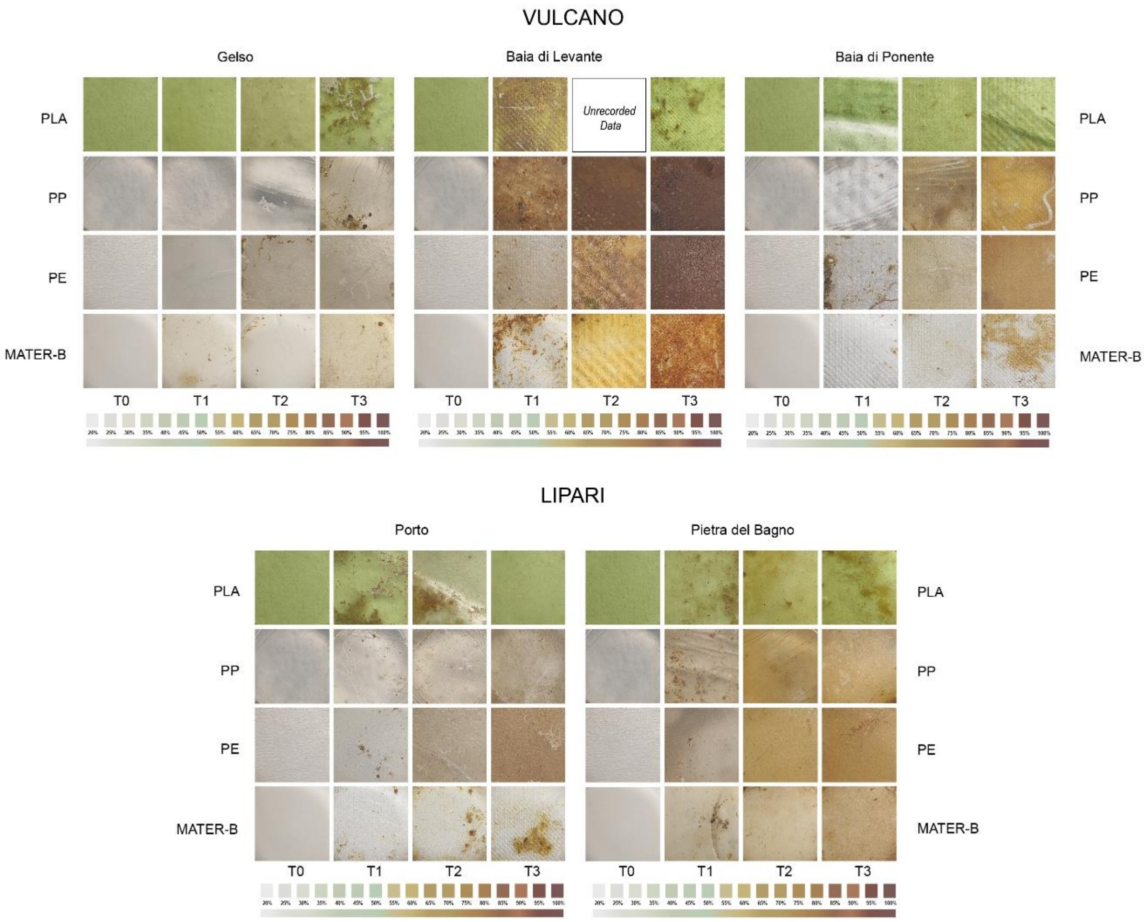

Figure 15. Synthesis of the behavior of the different polymers

7.3.3 Multi-stressors effects of ocean acidification and chemical contamination in mussels: a One Health perspective (Ischia CO_2 vents)

Stressors: ocean acidification, trace metals and organic contaminants

Model organism: *Mytilus galloprovincialis*

Approach: integrated environmental–ecotoxicological–human health assessment

The shallow CO_2 vent system of Ischia provides a natural gradient of reduced pH conditions co-occurring with site-specific chemical contamination linked to port activities, maritime traffic, and coastal urbanization. Within this PoC, the vent system was used as a field-based platform to evaluate how ocean acidification interacts with environmental contaminants, influencing bioaccumulation processes in mussels and their potential implications for human exposure.

7.3.3.1 Methods

Mussels were deployed at sites characterized by:

- Reference pH conditions (mean pH ~8.1),
- Acidified conditions representative of near-future scenarios (mean pH ~7.7),

during two seasonal exposure periods, allowing the assessment of temporal variability. Passive sampling techniques were applied in parallel to characterize the bioavailable fraction of inorganic and organic contaminants in the water column.

After exposure, mussel tissues were analyzed for:

- Potentially Toxic Elements (PTEs),
- Selected organic contaminants,
- Biomarkers of physiological stress.

To extend the assessment beyond the environmental compartment, mussel tissue extracts were processed and tested on human intestinal epithelial cell lines, within a One Health framework, in order to evaluate potential cytotoxic and inflammatory responses.

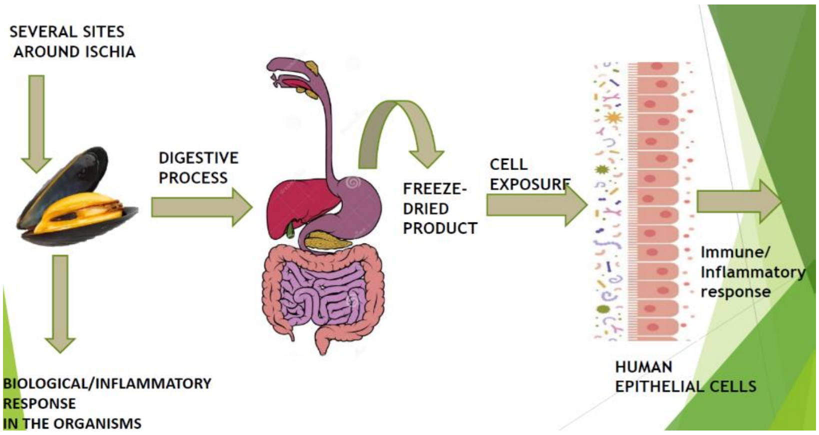

Figure 16. Workflow

7.3.3.2 Results

The results indicate that reduced pH conditions influence contaminant accumulation patterns in mussels.

Specifically:

- Differential accumulation of trace metals was observed under acidified conditions, consistent with changes in metal speciation and bioavailability.
- Site-dependent variability reflected the interaction between local contamination sources and seawater chemistry.
- Seasonal differences highlighted the dynamic nature of exposure and uptake processes.

Biological responses in mussels exposed to acidified conditions suggested altered physiological status, indicating that ocean acidification can modulate contaminant uptake and internal regulation mechanisms.

Importantly, in vitro exposure of human intestinal cell lines to mussel-derived extracts revealed measurable cellular responses, including modulation of inflammatory pathways. In particular, climate change can amplify the epigenetic effects of contaminants, increasing susceptibility to chronic diseases and enhancing their transgenerational transmissibility

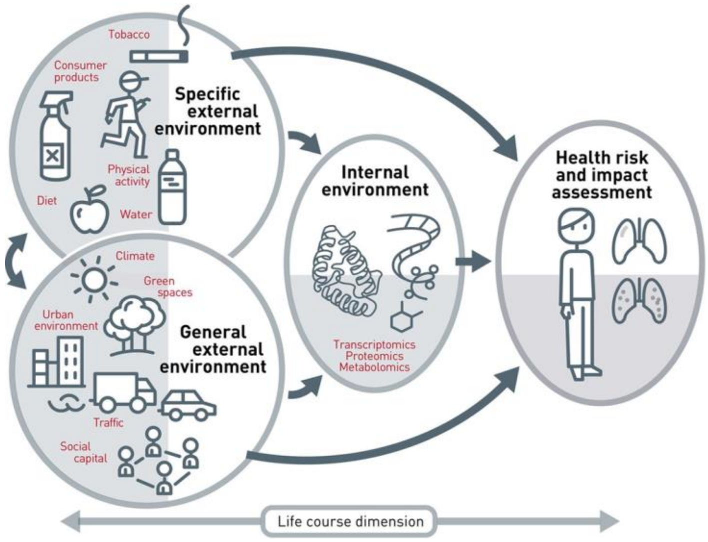

Figure 17. "The exposome is the environmental complement of the genome."

These findings demonstrate the relevance of integrating ecological and human health endpoints in contaminated coastal systems.

7.3.3.3 Integrated interpretation within the multi-risk framework

This PoC confirms that climate-related drivers, such as ocean acidification, can modify contaminant dynamics along the source–pathway–receptor continuum. Reduced pH conditions may enhance contaminant bioavailability and influence biological processing in sentinel organisms, potentially altering exposure levels for higher trophic levels, including humans.

The results support the application of the exposome concept, recognizing the cumulative contribution of environmental exposures to health outcomes. Within this perspective, climate change may act as an amplifier of contaminant effects, reinforcing the interconnection between environmental quality, ecosystem health, and human well-being.

Implications for risk assessment and management

The integration of:

- Field-based natural gradients,
- Passive sampling for exposure characterization,
- Bioaccumulation assessment in sentinel species,
- In vitro human cell line testing,

demonstrates the feasibility of an operational One Health approach within a multi-risk assessment framework.

This PoC validates the use of volcanic CO_2 vent systems as natural laboratories to assess combined effects of acidification and contamination and provides a transferable methodological model for coastal monitoring and seafood safety evaluation under future climate scenarios.

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Ministero dell'Università e della Ricerca
Italiadomani PIANO NAZIONALE DI RIPRESA E RESILIENZA

7.4 Multiple stressors effects in sediments of Sarno River

Stressors: Chemical contamination (Potentially Toxic Elements and Organic Compounds)

Approach: integrated environmental–ecotoxicological assessment

Within the framework of the POC project, the Sarno River basin was selected as a case study to test the proposed environmental risk prioritization techniques. This basin is characterized by intense anthropogenic pressure associated with tannery and food-processing industries, intensive agriculture, and a dense road network heavily affected by residential and commercial traffic.

In this context, the study area represents a complex and highly vulnerable territorial system, where multiple sources of potentially toxic elements (PTEs) and other contaminants interact with surface and subsurface environmental compartments. The Sarno basin was therefore used to verify whether the proposed risk prioritization methodologies can support more effective territorial management and decision-making processes, particularly in areas where environmental quality is already compromised.

Furthermore, the surface dynamics of the basin are frequently influenced by climate change–related phenomena, including extreme rainfall events, flooding, and increased runoff. These processes may enhance the mobilization, redistribution, and bioavailability of contaminants, thereby amplifying environmental and ecological risks. In such a scenario, the application of structured and quantitative risk prioritization approaches becomes essential to identify critical hotspots, optimize monitoring strategies, and support sustainable land-use planning.

Figure 18. Study area

7.4.1 Methods

In the first stage of the research activity, chemical concentration data for PTEs and other major, minor, and trace elements were compiled from previous geochemical prospecting campaigns conducted across the river basin. Subsequently, new investigations were planned and carried out on stream water and sediments, which were collected at 24 locations. In addition, riparian soils were also collected and analyzed at 16 locations within the framework of the MONSECO project by personnel from the TECNOBIOS laboratory.

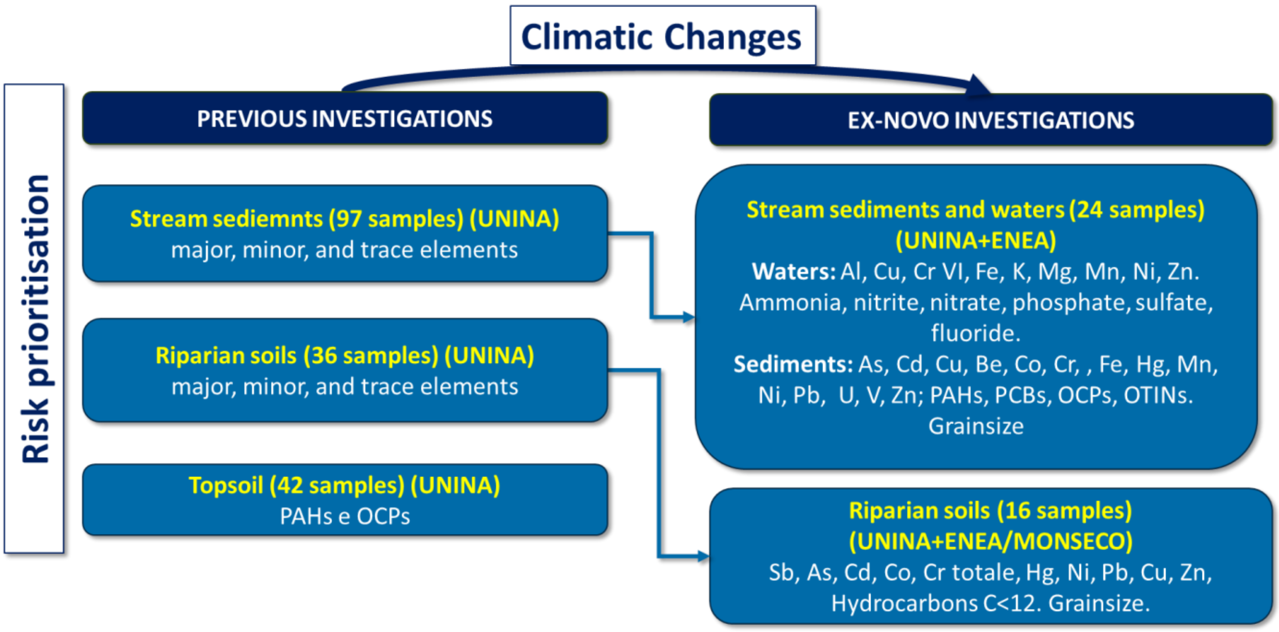


Figure 19. Workflow

Historically available data were used for two main purposes:

- Establishing the environmental status of the basin twenty years prior to the present
- Developing a spatial analysis method to reduce the dilution effect of surface transport, accounting for local geochemical baseline (Sample Catchment Basin approach - SCB) (Iannone et al., 2025).

Ex-novo data were used for:

- Assessing the actual environmental status of the basin (ENEA & Dipartimento di Scienze della Terra, Ambiente e Risorse, Università degli Studi di Napoli Federico II, 2026)
- Establishing a methodological approach to assess the impact of climate change on the distribution patterns of contaminants in soils and sediments over the past 20 years, based on the comparison with previous data.

7.4.2 Results

The integration of the Sample Catchment Basin (SCB) approach with multivariate statistical analysis and paired comparison tests proved to be an effective framework for interpreting spatial and temporal variability of potentially toxic elements (PTEs) in fluvial sediments. By explicitly incorporating the hydrological and geomorphological structure of the contributing catchments, the SCB method enhances the understanding of contaminant dilution processes driven by surface transport dynamics. In morphodynamically active systems, precipitation-induced runoff promotes erosion, sediment redistribution, and selective removal of fine-grained fractions, which are typically more enriched in metal-bearing phases. Consequently, downstream decreases in concentration may reflect dilution and sediment mixing processes rather than genuine reductions in anthropogenic emissions.

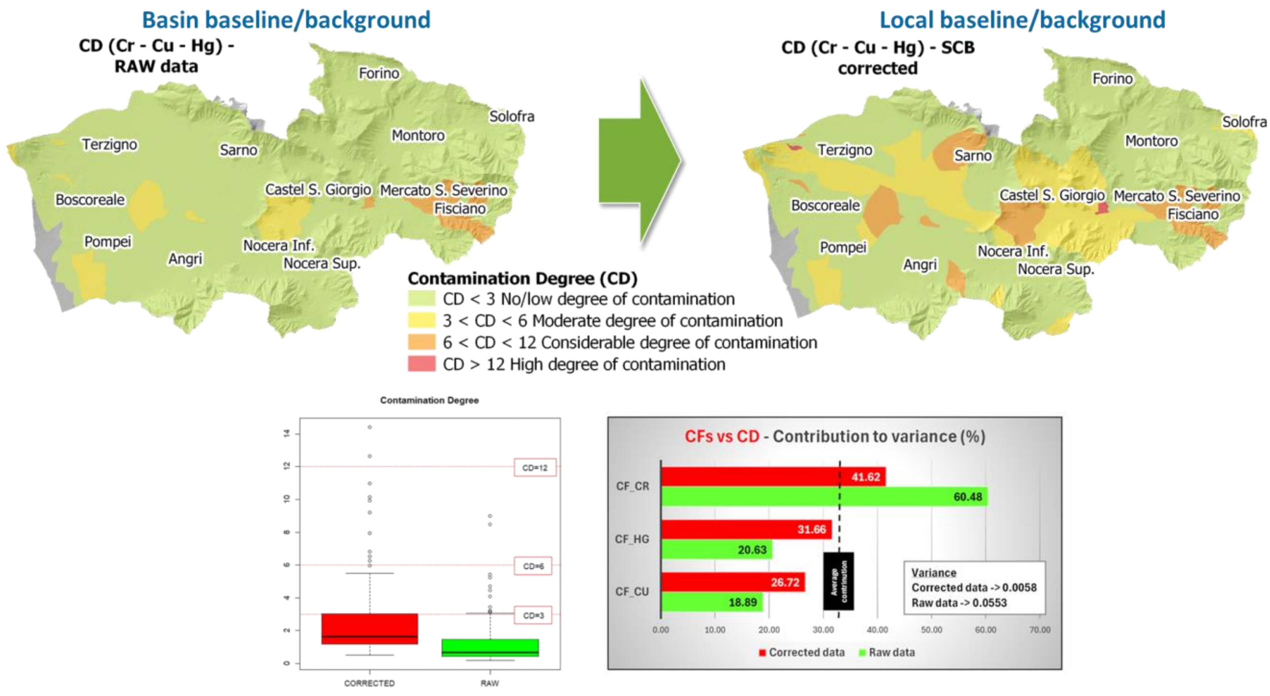
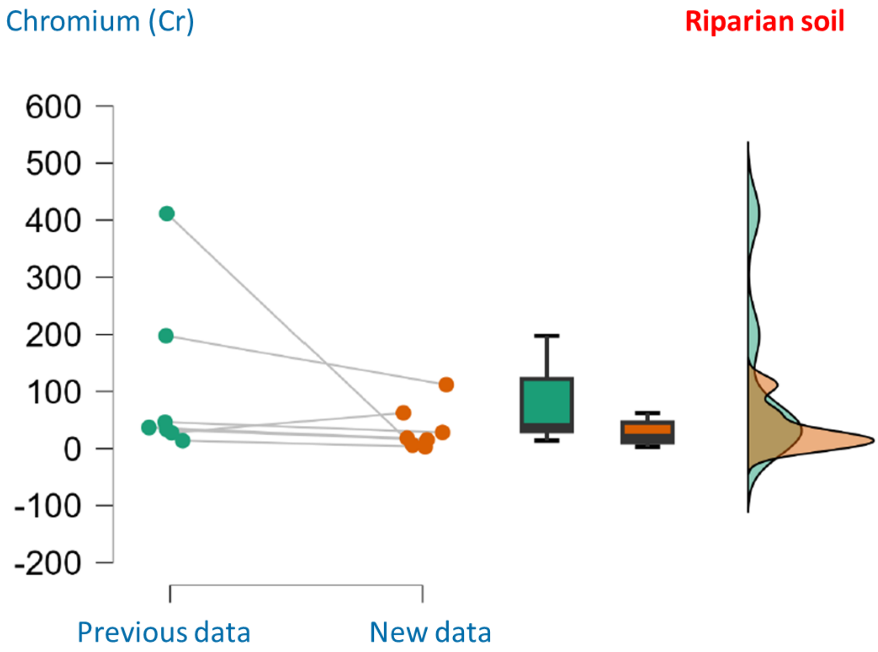


Figure 20. Contamination degree

The application of multivariate statistics and parametric and non-parametric comparison tests to temporally coupled datasets allowed the detection of subtle changes in geochemical patterns over time. In the Sarno River case study, only selected elements exhibited significant modifications in their statistical distribution over the last two decades. When interpreted in relation to extreme climatic events, these variations suggest that high-energy transport episodes may reduce the apparent dispersion of anthropogenic elements such as Cu and Cr by removing fine sediment fractions preferentially capable of adsorbing dissolved metallic ions.



Measure 1		Measure 2	Test	Statistic	z	df	p
Cr_OLD-BS	-	Cr_NEW-BS	Student	1.313		6	0.237
			Wilcoxon	23.000	1.521		0.156

Figure 21. Statistical results

Beyond the local case study, these findings demonstrate the importance of embedding geochemical assessments within a physically based, basin-scale framework. The proposed methodological integration improves the identification of true contamination hotspots, reduces the risk of misinterpreting dilution effects as emission trends, and provides a robust basis for environmental risk prioritization. Such an approach is transferable to other fluvial systems and is particularly relevant in the context of increasing climate variability, where sediment dynamics and contaminant redistribution are expected to become progressively more complex.

7.5 Combined effects of contaminants, warming and acidification on degradation of plastics and bioplastics

Bioplastic products are increasingly marketed as a more sustainable alternative to conventional plastics. However, like any lightweight single-use item, they can still be mismanaged, dispersed by wind and runoff, and ultimately contaminate soils, freshwater systems, and marine ecosystems.
Plastic pollution remains a global environmental issue. The United Nations Environment Program estimates that 19–23 million tons of plastic waste leak into aquatic ecosystems every year, polluting rivers, lakes, and seas. Even materials labelled as "biodegradable" do not necessarily degrade rapidly in natural environments. Their environmental fate strongly depends on polymer composition and site-specific conditions, and biodegradation in marine environments can be particularly slow. For example, Dilkes-Hoffman et al. (2019) estimated that a polyhydroxyalkanoate (PHA) bottle may require 1.5–3.5 years to fully biodegrade under marine conditions.
At the same time, the bioplastics sector is expanding rapidly, with global production capacity estimated at approximately 2.47 million tons in 2024 and projected to increase further in the coming years.

Given these uncertainties and the limited evidence regarding ecosystem- and organism-level impacts, two laboratory-scale experimental campaigns were conducted to assess the degradation behavior of commercial bioplastic products under controlled conditions simulating marine exposure.

7.5.1 Methods

Two experimental campaigns were conducted at laboratory scale to assess the degradation of commercial bioplastic products under controlled conditions simulating exposure in seawater. Four different bioplastic products were selected: a plate (PL), a coffee capsule (CA), a shopper bag (SH), a sticker (ET) and packing chips (CH). The products were characterized using a combination of FT-IR and TGA, and the results showed the following compositions: PL was primarily PLA (polylactic acid) with carbonate fillers; CA and ET were mainly composed of PLA and PBS (polybutylene succinate) with talc fillers; CH was composed of thermoplastic starch (TPS); SH consisted mainly of PLA and PBAT (polybutylene adipate terephthalate).
The seawater experimental campaign was conducted in accordance with ISO 19679. Natural seawater and sediments were collected along the Lazio coast, and artificial seawater was also prepared for comparison using deionized water and ultrapure NaCl. SH samples were tested at three different concentrations (0.3, 1 and 6 $gTOC_{SH}/L$) both in natural and artificial seawater using 250 mL airtight glass reactors, which were incubated at 18 °C for 12 months. Samples were collected after 3 (t1), 6 (t2) and 12 (t3) months. Degradation was evaluated based on CO_2 production, mass loss, and chemical changes assessed by FTIR analysis. In addition, potential ecotoxicological effects on selected target organisms were investigated, seawater samples were analyzed by ICP-MS to measure metals concentration and microplastic leachates were extracted by SPE and analyzed by UPLC.

7.5.2 Results

The degree of aerobic biodegradation and mass loss of commercial bioplastic products in seawater was negligible throughout the experimental campaigns. Negligible variations in enzyme activities, basal respiration and DNA yield was observed after 3 and 6 months compared with the blank tests. An acidification effect was observed for tests with natural seawater in time (**Errore. L'origine riferimento non è stata trovata.**).

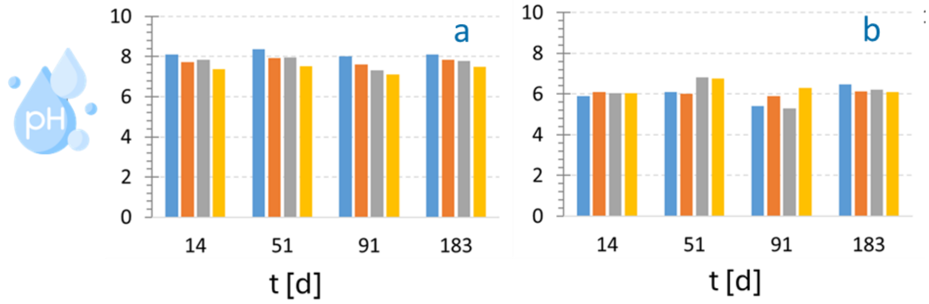


Figure 22. pH values for tests with a) natural seawater and b) artificial seawater

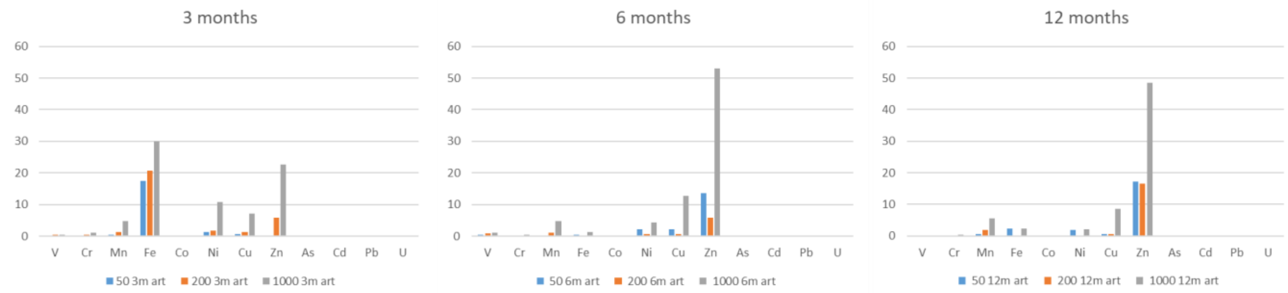


Figure 24. ICP-MS results measuring metals concentration in artificial seawater samples after 3, 6 and 12 months of test for the different SH concentrations

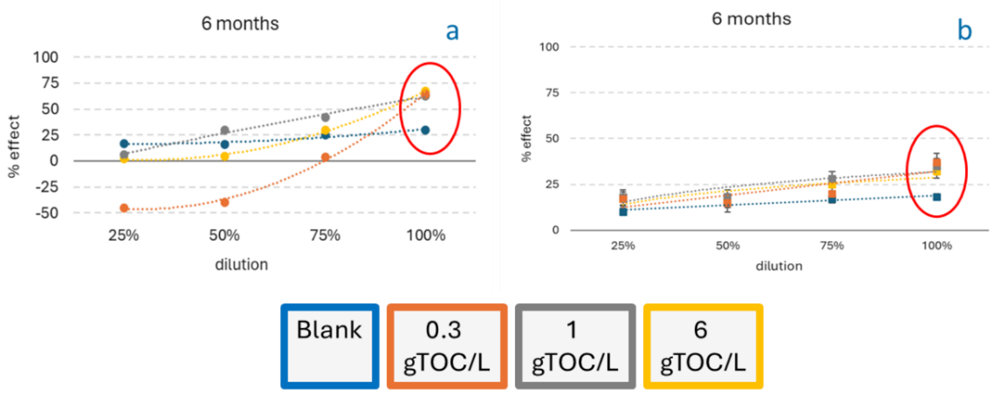


Figure 23. Ecotoxicity effects of natural seawater samples on a) D. tertiolecta and b) A. Salina biomass growth after 6 months of exposure. Sample dilutions range from 25% to 100% (v/v), where 100% corresponded to the undiluted sample

A progressive increase in metal (in particular Zn) concentrations over time and with higher SH concentrations was also observed (**Errore. L'origine riferimento non è stata trovata.**). This release was unsurprising as zinc is extensively used in bioplastic films.

Ecotoxicity effects were investigated by monitoring *D. tertiolecta* and *A. Salina* biomass growth. In **Errore. L'origine riferimento non è stata trovata.** the effect on the two target organisms of natural seawater sampled after 6 months of exposure to SH is reported. Ecotoxicity was assessed using sample dilutions ranging from 25% to 100% (v/v),

where 100% corresponded to the undiluted sample. It can be observed that, as dilution increased, a slight beneficial effect emerged for the water sample containing the lowest SH concentration in *D. tertiolecta*. In contrast, the undiluted natural seawater samples showed a clear negative effect, irrespective of the SH concentration tested. A similar pattern was observed for *A. salina*.

8 Conclusions

The Proof of Concept has highlighted several key outcomes that underscore the complexity of environmental systems exposed to multiple, interacting stressors. The results clearly demonstrate that the adoption of a multidisciplinary approach is essential for the robust assessment and interpretation of cumulative and combined impacts. The integration of diverse methodologies and expertise has significantly enhanced scientific understanding, enabling the generation of novel insights into environmental effects, associated risks, and the development of advanced methodological frameworks.

The findings confirm that multiple stressors can produce synergistic effects that exceed those expected from the simple additive contribution of individual factors. In particular, mercury was shown to markedly amplify ecological and ecotoxicological impacts when combined with low oxygen concentrations or acidification conditions. Emerging contaminants, including microplastics and (bio)plastics, exhibited degradation pathways and toxicological responses that are strongly modulated by environmental stressors such as temperature, pH, and oxygen availability.

Bioindicator organisms, such as mussels, proved to be highly sensitive sentinels of contaminant bioavailability, effectively reflecting environmental exposure and providing valuable insight into potential risks for human health. Overall, these results support the exposome concept, highlighting the tight interconnection between environmental, animal, and human health within a One Health perspective.

Furthermore, the outcomes indicate that climate change may act as an additional amplifier of contaminant effects, including epigenetic alterations, potentially increasing susceptibility to chronic diseases and their intergenerational transmission. These findings emphasize the need for integrated, cross-disciplinary cooperation to effectively address current and emerging environmental challenges and to inform future risk assessment and management strategies.

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