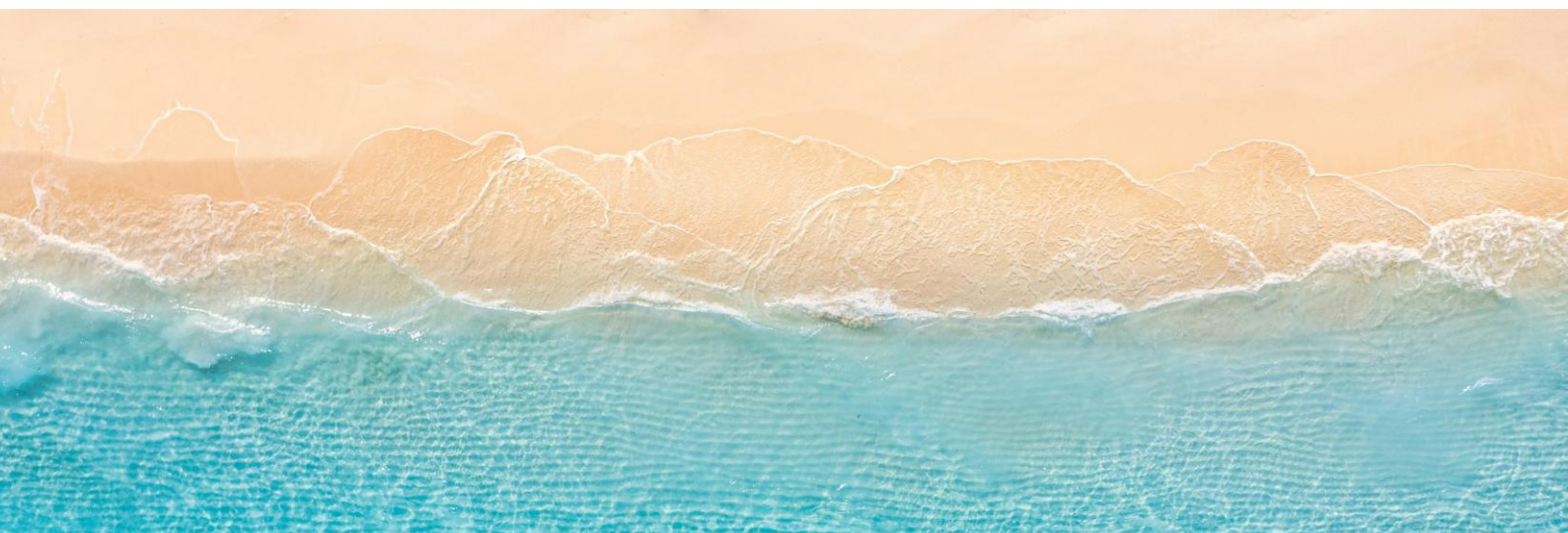




***Open Call for “Associated Regions”
ANNEX 1.1 - Technical Specification***

Submission deadline: May 31st, 2025, 17.00 CET



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TABLE OF CONTENTS

Acronyms	5
1.0 Introduction	6
2.0 The RHE-MEDIation Methodology	6
3.0 RHE-MEDIation innovative solutions	8
3.1 Innovative solutions: micro-algae-based water remediation technology	9
3.2 Innovative solutions: water quality monitoring technologies	12
3.3 Innovative solutions: design for integration of RHE-MEDIation measured data with the Digital Twin Ocean and Water Knowledge management platforms	17
4.0 Design and implementation of a wide stakeholder engagement	19
5.0 Positioning of the project in terms of R&I maturity	20
6.0 Demonstration cases overview	20
6.1 Demonstration Site 1 - Sea WTP pilot, Taranto (IT)	22
6.2 Demonstration Site 2 - Thriasio Wastewater Treatment Plant, Municipalities of Aspropyrgos, Elefsina and Mandra-Idyllia, Attica (GR)	23
6.3 Demonstration Site 3 - Dilovasi Municipal Wastewater Treatment Plant, Dilovasi (TK)	24
7.0 Checklist	26
8.0 Conclusion	27

LIST OF FIGURES

Figure 1. RHE-MEDIation process and methodological approach.....	7
Figure 2. RHE-MEDIation step development plan	7
Figure 3. RHE-MEDIation step development plan	8
Figure 4. The RHE-MEDIation mission strategy	9
Figure 5. First preview of the final scaled-up GREEN DUNE® Photobioreactor.	10
Figure 6. Final design of the GREEN DUNE® Photobioreactor.....	12
Figure 7. GREEN DUNE® development and deployment in Greece.	12
Figure 8. Front and back side view of the SCW water sensor. Measurement of the six different metals with their catalytic properties processed by ML algorithms contributes to the discrimination of chemical compounds.....	13
Figure 9. MDM CIRCE Unit.	14
Figure 10. MDM CARIDDI Unit.....	15
Figure 11. MDM CIRCE external probes connections	16
Figure 12. MDM CIRCE Units assembled to be used as RHE-MEDIation fixed nodes.....	17
Figure 13. RHE-MEDIation Ingestion Service.	19
Figure 14. RHE-MEDIation Technology Basket TRL expected up-scale.	20
Figure 15. RHE-MEDIation demonstration sites characteristics.	21
Figure 16. RHE-MEDIation demonstration sites characteristics.	22
Figure 17. Aerial view of the Taranto Bay (Italy).....	23
Figure 18. Thriasion Plain and Elefsis Gulf areas.....	24

Figure 19. View of İzmit Bay, Marmara Sea26

ACRONYMS

API	Application Programming Interface
BOD	Biochemical Oxygen Demand
COD	Chemical Oxygen Demand
CPU	Computational Capability
DWWTP	Dilovası Municipal Wastewater Treatment Plant
EIS	Electrical Impedance Spectrometer
EMODnet	European Marine Observation and Data Network
ENI	Ente Nazionale Idrocarburi
KPI	Key Performance Indicator
MBR	Membrane BioReactor
NGEU	Next Generation EU
OC4ARs	Open Call for Associated Regions
ORP	Oxidation Reduction Potential
PAHs	Polycyclic aromatic hydrocarbons
PCBs	Polychlorinated Biphenyls
PFAS	PerFluorinated Alkylated Substances
SCW	Smart Cable Water
SWE	Sensor Web Enablement
TDS	Total Dissolved Solids
TRL	Technology Readiness Levels
TSS	Turkish Straits System
TÜBİTAK MAM	Scientific and Technological Research Council of Türkiye - Marmara Research Center
TWWTP	Thrasio Wastewater Treatment Plant
WPs	Work Packages
WTP	Wastewater Treatment Plan
WWTPs	Waste Water Treatment Plants

1.0 Introduction

The RHE-MEDIation Open Call for “Associated Regions” (OC4ARs) has a total budget of €500,000 to fund up to five (5) projects and aims to:

- Securing the interest of local authorities managing semi-confined water bodies affected by persistent chemical pollution (defined as HOT SPOTs) due to the low recirculation and the persistent polluted discharge from human activities on land and who are willing to acquire knowledge about remediation technologies, such as those proposed by the RHE-MEDIation project, thereby setting the basis for a roadmap to plan future scenarios of de-stress solutions implementation.

This document, referred to as ANNEX 1.1 Technical Specification, offers a comprehensive outline of:

- RHE-MEDIation methodology,
- RHE-MEDIation innovative solutions, and
- RHE-MEDIation demonstration cases overview.

The information provided in this document is intended to assist Third Parties in developing their replication site technologies as described in their proposals (Annex 2). This document should be considered as an extension of Annex 1 – RHE-MEDIation Guidelines for Applicants.

1.1 The RHE-MEDIation Methodology

The RHE-MEDIation Hub aims to enhance the capability to mitigate chemical pollution in the Mediterranean Sea across three Member States, as part of the interdisciplinary approach mandated by the Lighthouse governance to foster cooperation within the EC Missions through the Implementation Charter. The goal is to extend this initiative to five additional Mediterranean countries, with the intention of replicating activities and facilitating further expansion throughout the EU.

The main innovations introduced by RHE-MEDIation are:

- a. The creation of a universal, one-stop-shop technology utilizing a customized microalgae solution designed for integration within existing infrastructures. This technology offers a simple, cost-effective, and circular economy-compliant method for addressing both historical and contemporary chemical threats, including heavy metals, pesticides, pharmaceuticals, PFAS, and other persistent organic pollutants.
- b. The integration and deployment of advanced microsensor technology with exceptional discrimination capabilities across a wide range of chemical substances. Powered by machine learning for component signature characterization, this technology will be incorporated into on both fixed and mobile measurement stations, enabling real-time, on-site data acquisition and processing. This will facilitate mapping of contamination presence, quantity, and spatial distribution, with full exportability and usability by current monitoring facilities at both local and EU levels.
- c. Demonstration of these technologies and key performance indicators (KPIs) at three pilot sites in Italy, Greece, and Turkey, with plans for replication in five additional Mediterranean

countries.

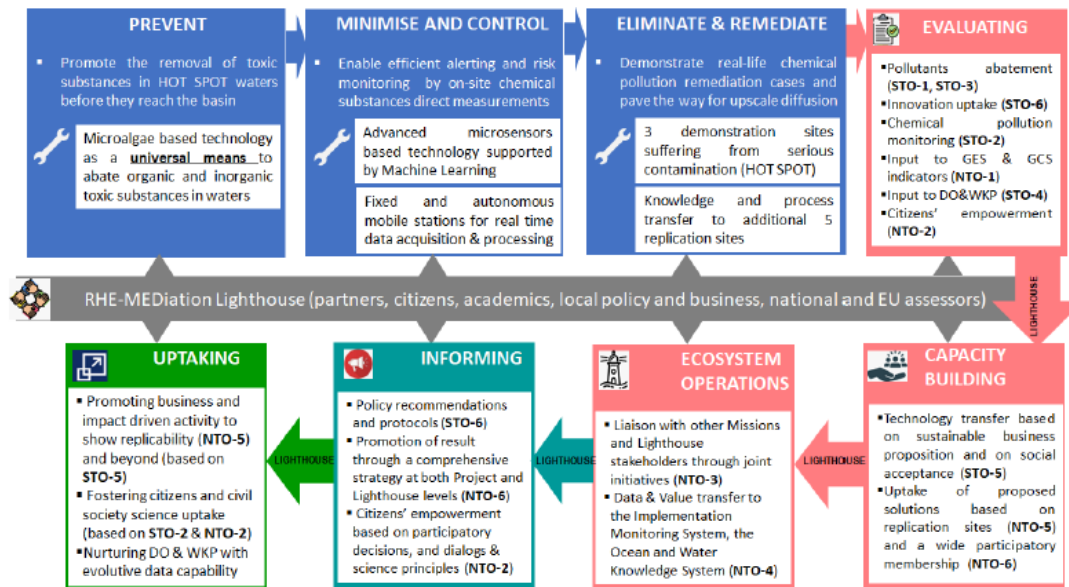


Figure 1. RHE-MEDIation process and methodological approach

The project will span 36 months and consist of 8 work packages (WPs). A five-step methodology has been developed to ensure successful project delivery and achievement of Technology Readiness Levels (TRLs). These steps are illustrated in Figure 2. The sixth step, which extends beyond the project timeline, emphasizes potential funding opportunities to support the scaling up of demo sites in alignment with 2030 benchmarks.

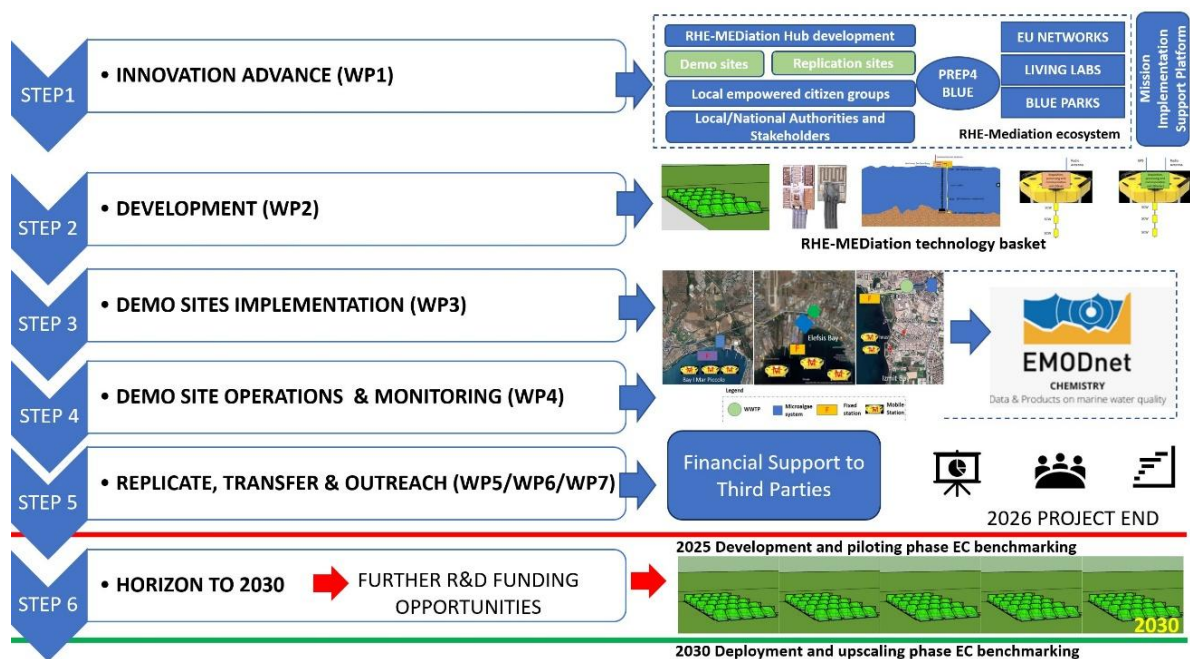


Figure 2. RHE-MEDIation step development plan

2.0 RHE-MEDIation innovative solutions

RHE-MEDIation will assist policymakers by developing advanced remediation solutions for Mediterranean hot spots, along with tools and methods for monitoring and control, while also fostering different stakeholders engagement in this initiative. RHE-MEDIation will serve as a key resource for policymakers, citizens, and various stakeholders, facilitating a paradigm shift in the Mediterranean. In this role, it will be the primary source of knowledge generated and shared about our seas, in line with the principles outlined in Figure 3.

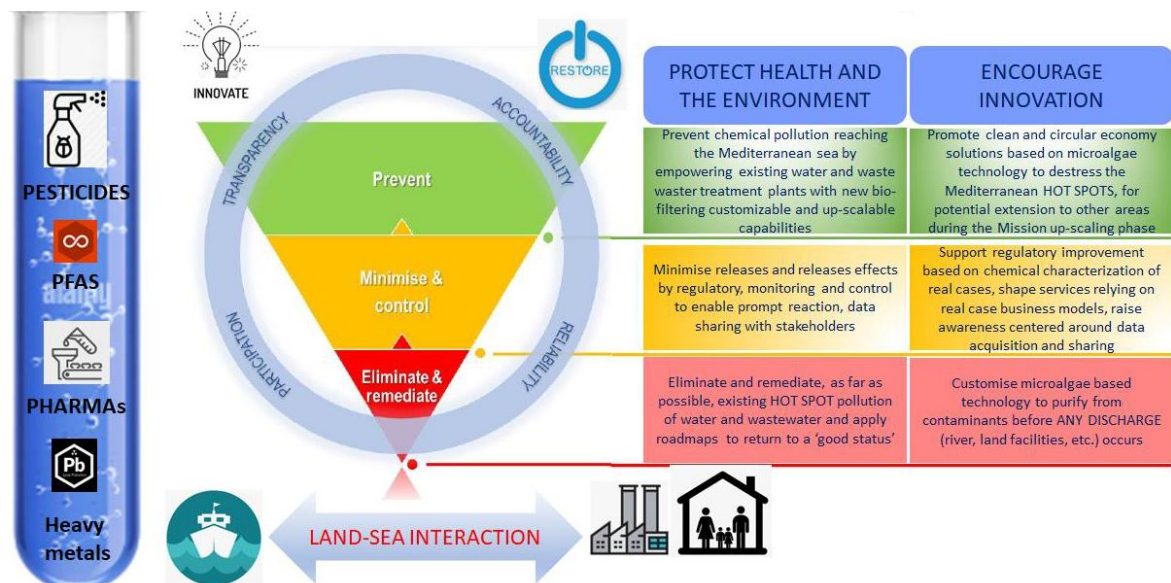


Figure 3. RHE-MEDIation step development plan

PREVENT chemical pollution reaching the Mediterranean Sea and tackle environmental challenges with innovative technologies along the whole discharge networks and spanning across different sectors and industries, in urban, rural, water flows and sea contexts. The RHE-MEDIation hub will address solutions and practices to empower existing water & wastewater treatment sites with new customizable and up-scalable bio-filtering capabilities. It will enhance the establishment of clean and circular economy solutions based on microalgae technology to prevent chemical inflow in the Mediterranean HOT SPOTS, for potential extension to other similar areas during the Mission up-scaling phase. Solutions and data, after measurement, will be validated through three demo-sites (Mar Piccolo-Taranto-IT, Elefsis Bay-GR, Izmit Bay, TK).

MINIMIZE & CONTROL emissions and their impacts through regulation, monitoring, and control to facilitate rapid responses in Mediterranean hot spots, while also encouraging data sharing with stakeholders at demonstration sites. The RHE-MEDIation initiative will enhance regulatory frameworks based on the chemical characterization of real cases, helping to develop services grounded in practical business models and increasing awareness of data acquisition and sharing.

ELIMINATE & REMEDIATE existing polluted hot spots by addressing the sources of major pollution, aiming for a long-term return to a “good status” through improved dilution conditions. The RHE-MEDIation initiative will tailor microalgae-based technologies to purify water from contaminants before any discharges (such as into rivers or land facilities)

occur, with a particular focus on high-priority substances like heavy metals, PAHs, PCBs, PFAS, and pharmaceuticals. Guided by the RHE-MEDIation hub, efforts in upstream prevention, elimination, remediation, and monitoring of chemical pollution will align with key policies, including the Water Framework Directive, Marine Strategy Framework Directive, Circular Economy Action Plan, EU Strategy for Plastics in the Circular Economy, EU Chemicals Strategy for Sustainability, the European Bio-economy Strategy and its 2022 Progress Report, as well as the Urban Wastewater Treatment Directive. Enhanced infrastructure for water and wastewater collection, treatment, and reuse will support the European Commission’s objectives for 2030 in both rural and urban areas, addressing household and industrial needs while leveraging Next Generation EU (NGEU) funding to prevent untreated discharges into rivers and the sea.

The RHE-MEDIation Hub aims to achieve the ambitious goal of reducing chemical stress in the Mediterranean Sea by promoting: 1) a culture of water clearance before any discharge occurs by implementing sustainable and circular economy technologies, 2) continuous monitoring and control from treatment plant nodes to the final water destination, and 3) engagement of European stakeholders and citizens to collaboratively ensure the mission's success (see Figure 4).

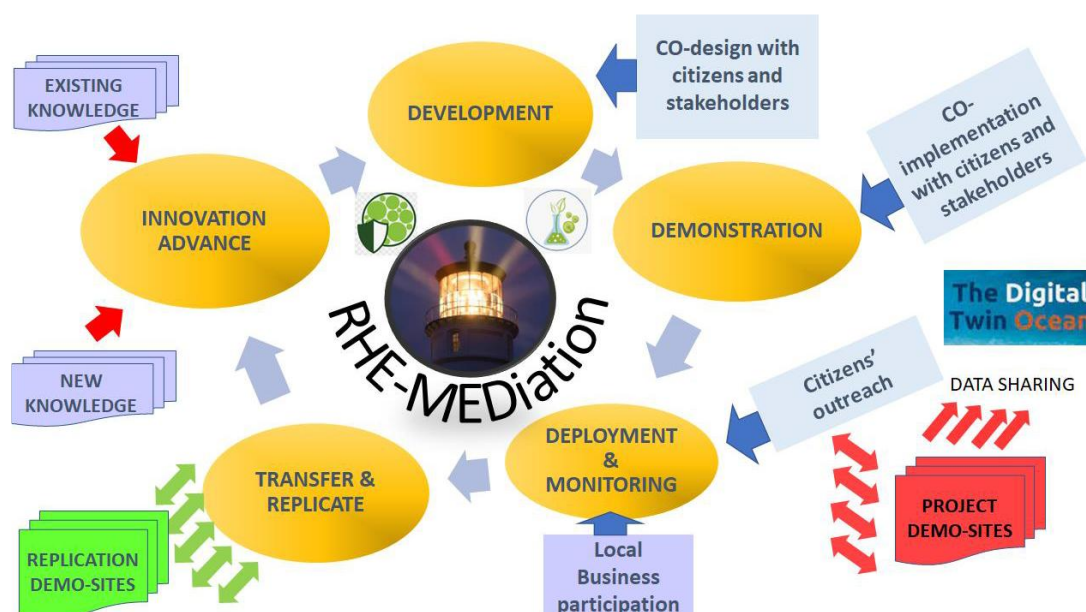


Figure 4. The RHE-MEDIation mission strategy

Leveraging on the design and implementation phases of the relevant basket of technologies analyzed within WP2, the following section will present an insight into such technologies and on the results assessed within the project’s framework. Below, an insight into such technologies, being:

- GREEN DUNE® Photobioreactor technology
- MDM Circe and MDM Cariddi Solution
- RHE-MEDIation Ingestion Service

2.1 Innovative solutions: micro-algae-based water remediation technology

This technology is capable of bioremediating a wide range of chemically contaminated waters, thus guaranteeing cost-efficient and circular abatement of pollution before it is discharged. This represents a sustainable solution to de-stress hot spot areas from chemical pollution.

Various pilot tests have been successfully conducted in different wastewater treatment plants throughout Portugal using photobioreactors (PBR). The previous versions of the photobioreactors have treated leachate, industrial wastewater, urban wastewater (sewage), and even a hospital effluent. The pilot tests revealed outstanding results in decreasing Biochemical Oxygen Demand (BOD), Chemical Oxygen Demand (COD), Nitrogen nutrients (N) and Total Phosphorus (TP), the most common undesired components of leachates. The previous wastewater treatment plant (WWTP) pilot tests also eliminated more than 99% of medicine residue from the hospital's wastewater. The elimination of contaminants in all the pilot tests complied with all Emission Limit Values (ELV) established by the EU for the compounds present in the leachates and wastewaters to be discharged. In these pilot tests, the previous layouts demonstrated decreased energy costs in wastewater treatment by more than 60% and lowered operational costs by more than 40% compared to traditional WWTPs.

In 2017 a redesign of the GREEN DUNE® Photobioreactor was developed. It turned from the previous tubular shape to a more suitable prismatic shape to be incorporated into the ALGAMATER® WWTP. The photobioreactor's front surface angle calculation was made to maximize the productivity and the sun exposure to the microalgae. The scale-up of the photobioreactor was made to bring its volume from 200 l to 450 l per unit, increasing its capacity and production of microalgae

The first preview of the final design of the GREEN DUNE® Photobioreactor – which definitively overcomes some limitations of the previous version – is provided in Figure 5.



Figure 5. First preview of the final scaled-up GREEN DUNE® Photobioreactor.

This design includes a UV protected acrylic, offering more sunlight resistance to the reactors; it will be possible to completely empty the reactor through a better positioned bottom discharge; the previous air and CO₂ diffusion tube had a tendency to clog, while the new tube will have more diffusion efficiency and less clogging potential; it will have a simple removable mechanism to open/close the PBR system – in some cases, such as wastewater treatment, a thorough control is not needed and the system can be open, while for cultivation of certain microalgae strains, the system must be closed.

The photobioreactor, produced in two pieces, is joined and sealed using a sealant which is resistant to mechanical and/or microbiological loads and is permanently elastic. The prismatic upper part is transparent, and the lower part is an opaque or transparent basin that serves as the base. The two pieces

are produced in two different molds. Bluemater studied the best production solution for some time to achieve the final configuration.

In addition, the previous manufacturing process was more costly, since it was based on handcrafted production of small quantities. Nevertheless, the handcrafted photobioreactor already had much cheaper production costs when compared to the other available tubular photobioreactors. With a new high-scale manufacturing process, the production cost of the photobioreactor decreases in approximately 50% after mold amortization and instead of 4-5 photobioreactors / day it is possible to produce 80 units / day with a single mold. This allows us to produce in high quantities at a lower price compared to its previous handcrafted version, turning this technology into a financially feasible investment for large-scale applications. The daily production can be easily doubled with the production of an extra mold.

The technology was redesigned after the limitations shown in the previous version of tubular photobioreactors. In fact, as evidenced by the two pilot tests performed on leachate wastewater and on hospital effluent, the need for a higher volume with a simultaneous wider superficial area was made clear, to effectively expose the liquid to sunlight. Secondly, we noticed that some microalgae adhered to the photobioreactors' walls, making sunlight breaking through the photobioreactor more difficult, thus lowering the productivity of microalgae growth and the overall efficiency of the treatment. A further challenge faced referred to the shade projection that each tubular photobioreactor caused on the photobioreactors nearby, due to their height and slenderness, reducing the efficiency and feasibility of the technology for the leachate and wastewater treatment.

The design improvements were achieved within the GREEN DUNE® Photobioreactor, developed by Bluemater (International Application No. PCT/IB2022/059191, Publication Number WO / 2023 / 073454). The final shape was eventually upgraded, lifting the PBR base, due to new lower entry and exit points, designed for easy maintenance, cleaning, and biomass removal.

The Photobioreactor presents an optimized prismatic shape, to facilitate transport and shipping. Furthermore, each photobioreactor allows for a total water volume of 500 liters. The implemented material is Clear High-Impact Acrylic, a type of extruded acrylic six-times more resistant than standard acrylic sheets. The material is also known for its excellent mechanical properties, high gloss, and resistance to low temperatures. Moreover, it can be thermoformed quickly, and is recyclable. The upper and lower parts of the PBR are glued together (see Figure 6).

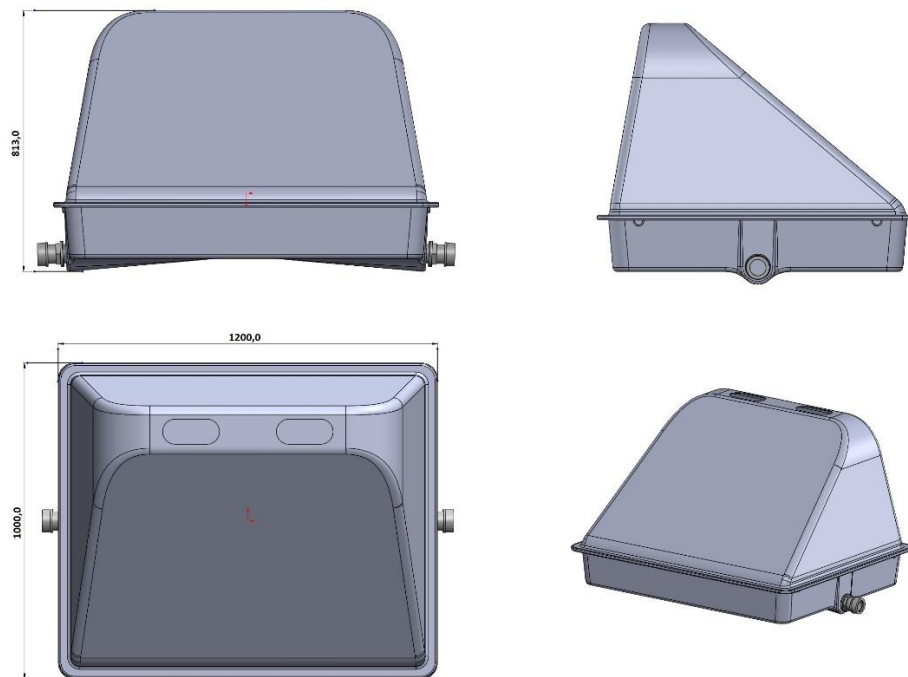


Figure 6. Final design of the GREEN DUNE® Photobioreactor.

Innovation is evident not only in technological upgrades but also in the commitment to sustainability and cost-effectiveness. The GREEN DUNE® Photobioreactor, now positioned as a financially viable solution for large-scale applications, aligns seamlessly with the water/wastewater pollutants cleaning framework. The RHE-MEDIation project, through these advancements, emerges as a promising and sustainable solution in the ongoing mission to alleviate chemical pollution in the Mediterranean Sea.

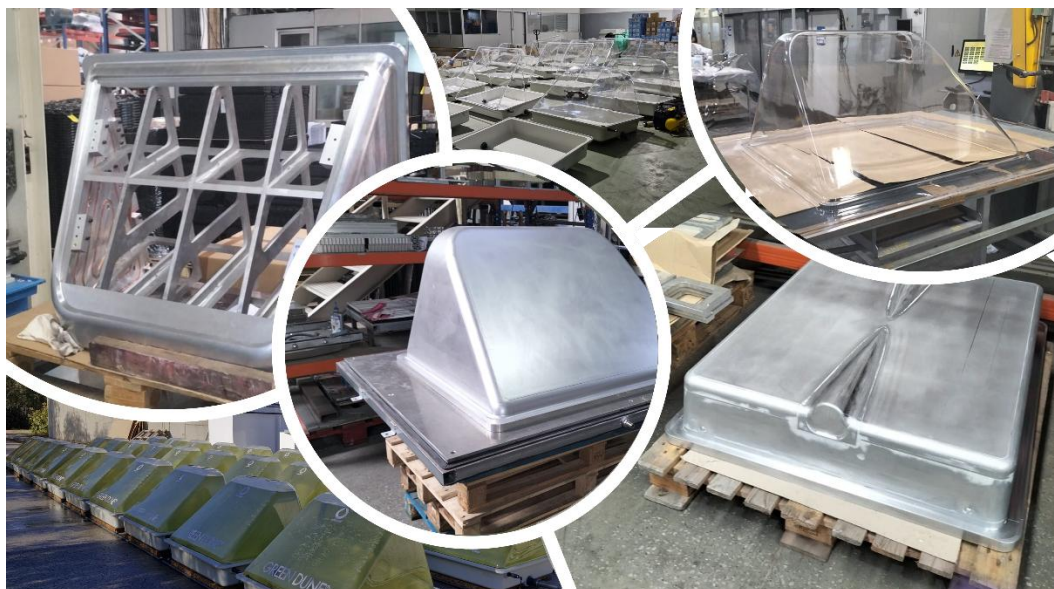


Figure 7. GREEN DUNE® development and deployment in Greece.

2.2 Innovative solutions: water quality monitoring technologies

The MDM monitoring nodes are based on available systems by MDM, previously developed within a national R&D projects aimed at creating Smart Drifters to be used for oceanographic purposes.

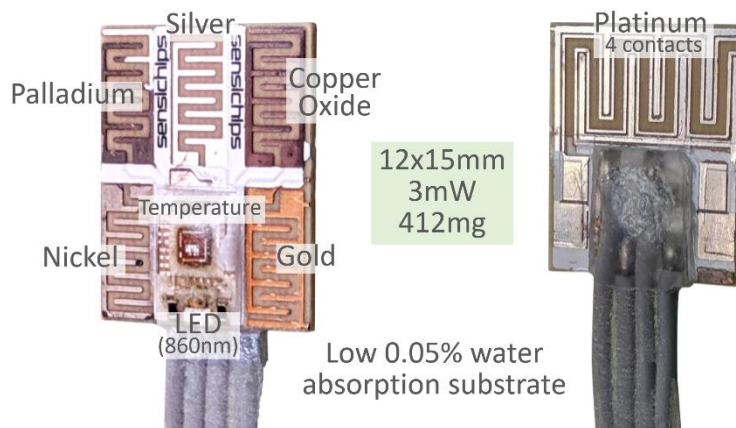


Figure 8. Front and back side view of the SCW water sensor. Measurement of the six different metals with their catalytic properties processed by ML algorithms contributes to the discrimination of chemical compounds.

For the RHE-MEDIation project, Sensichips manufactured 20+10 SCW water sensors and 10 SCA air sensors. Initially, measurement and API software for the measurement of generic water quality parameters were improved, such as: Temperature, Electrical Conductivity, Electrical Permittivity, Total Dissolved Solids (TDS), Salinity, Oxidation/Reduction potential (ORP), Turbidity. Subsequently Sensichips performed training campaigns (still ongoing) for the detection and quantification of pharmaceuticals such as Aspirine, Niflam, Clavulanic Acid antibiotics, and heavy metals with main focus on Copper ions. Sensichips also performed training campaigns for the SCA Air Sensor to detect hydrocarbons contaminants like petrol derivatives which cannot be detected with water sensors. More specifically, those trained sensors do not have the sensitivity required to detect trace level concentrations required for water quality monitoring. Sensichips is developing new techniques based on electrochemical Squarewave Voltammetry and machine learning algorithms to improve such performance.

The technology consists of an IoT system with extensive and variable sampling capability, large variety of payload interconnection and full support of the integrated Sensichips smart probes.

In particular, remote sensing units were modified and updated, so to perform continuous sampling process of water data, including Sensichips probes to perform lately classification of pollution compounds.

In fact, different probes, encompassing those for temperature, pH, and electrical conductivity, along with the Sensichips probe, were incorporated into a stationary buoy system named CIRCE, developed by MDM.

Specifically, the CIRCE unit sends data over the cloud utilizing an LTE/4G connection, with a sample period that can be adjusted through a web cloud interface (MDM data portal). Additionally, a mobile unit (unmanned surface vehicle) known as MDM Cariddi is deployed to collect data along a user-defined path, enabling the collection of information from areas of interest. The overall system allows to perform a classification of pollution, detected by the sensing units, so to perform continuous long-term sampling, sending the gathered water data over cloud to enable partner post processing and digital twin synchronization. In particular, the site presents the following configuration:

RHE-MEDIation project will make use of different monitoring nodes to capture data from water.

These nodes can be divided into two classes:

- 2 fixed buoys inside the container (one at the inlet and one at the outlet of the photobioreactors)
- 2 buoys at sea (one fixed and one mobile)

Fixed nodes

The RHE-Mediation fixed nodes are based on the MDM CIRCE unit. Such a system is composed of:

- 1x GPS Receiver (to acquire geodetic position)
- 1x LTE/4G Modem (to transmit data to cloud)
- 1x Microcontroller ESP32 (to perform calculation and arrangements of data)
- 1x SD Card (to store unit configuration and data that cannot be sent for lack of communication)
- 1-3x Probes (additional water quality sensors, i.e. pH, Electrical Conductivity etc.),
- 1x Battery Pack (to store energy from solar panels)
- 1x Solar panel system (to gather energy from solar radiation)
- 1x Sensichips Microcontroller + Probes (to collect raw, water quality and classified data).

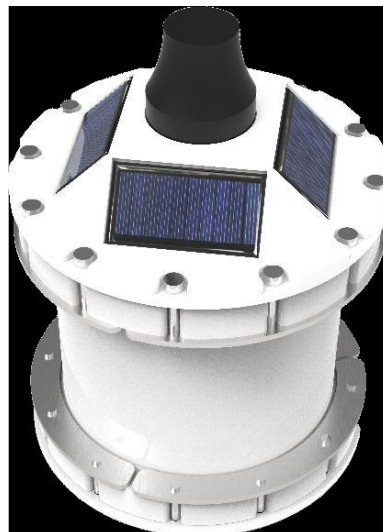


Figure 9. MDM CIRCE Unit.

The previous Figure 8 is representing a MDM Circe Unit.

The MDM Circe system, originally designed as an oceanographic drifting unit, has been intensively modified both on mechanical, electrical and software aspects. The result is an IoT system with extensive and variable sampling capability, large variety of payload interconnection and full support of Sensichips smart probes.

Such a system is able to provide real-time measurements of water treatment plants and nearby coastal areas and it represents a relevant feedback technology within the RHE-Mediation project.

The system has been tested for more than a month (cumulatively) both on sea and lake. During

the lake tests, Sensichips probes have been successfully integrated, delivering data over the cloud with a sampling period of 10 minutes. There are occasional anomalies in the measurement system that will be investigated.

The system has been properly tuned and adjusted to preserve battery charge over deep-sleep phases and the cloud system has been modified to allow third party users to collect data using HTTP post APIs.

Mobile nodes

The RHE-Mediation mobile nodes are based on MDM SCYLLA (Passive) and CARIDDI (Active) drifter units.

The CARIDDI unit is equipped with:

- ARM64 CPU (to perform all necessary calculations including guidance navigation and control)
- 2 or 3 thrusters (to enable unit to move on the sea surface)
- GPS receiver (to gather information on geodetic position)
- Inertial Measurement Unit (to obtain a heading reference system for control purposes)
- WiFi 2.4GHz (to perform communication with the control station and the user)

and will carry, additionally, the same electronics of the CIRCE unit to sample water data.

The MDM CARIDDI Unit is an Unmanned Surface Vehicle (USV) (Figure 9) designed to carry different sort of payloads and to move along designed paths. The unit will establish a link with WiFi 2.4Ghz to the control station where user will operate the vehicle either manually or autonomously.



Figure 10. MDM CARIDDI Unit.

The two fixed nodes will be placed at the inlet and outlet of the Photobioreactors; at the inlet a quantitative measurement of the state of the water before being processed will be provided, while at the outlet variations of water data affected by the water plant will be monitored. Moreover, the mobile unit will be used at sea as a fixed monitoring unit, providing an insight into the water released into the sea. The data will be marked with related geospatial information, (i.e. latitude, longitude, speed and course over ground).

The buoy systems incorporate Smart Cable Water (SCW) sensors, a multi-sensor microsystem created by SENSICHIPS. These sensors enable the real-time detection of toxic chemicals (TICs), pollutants and complex compounds in water.

Once recovered from the sensors, the gathered data is transmitted and dispatched within the cloud system by using HTTP post through a dedicated API. The cloud system decrypts, verifies, and saves the data into various time-series database entries. Once the data is retrieved and processed, the cloud offers access to primary data analytics, categorized into trackers and datasets. Specifically, the tracker view provides access to the most recent unit collected data and geotime signature, whilst the dataset view allows to combine sets of MDM Circe data within a selected timeframe, representing the main tool through which it is possible to inspect trackers of interest.

Setup for Probe Replacement and Ongoing Upgrades

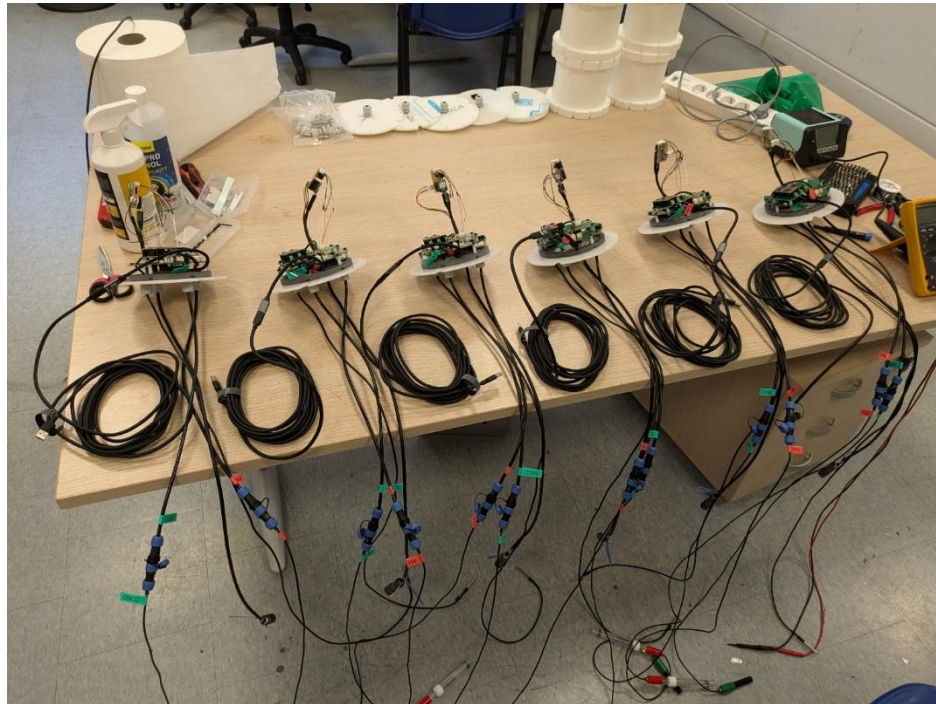


Figure 11. MDM CIRCE external probes connections

All the fixed units have been configured with external connectors for the installed probes. This will simplify maintenance over time, given the possibility to replace the probes during the future. In addition, a custom water-tight connector has been installed to accommodate firmware upgrade for the Sensichips microcontroller installed onboard. Since for each probe there exists a dedicated electronic component to acquire, process and transmit data to MDM Team Circe unit, a proper cable labelling has been adopted to reduce the chance of mistakes when probes are replaced.



Figure 12. MDM CIRCE Units assembled to be used as RHE-MEDIation fixed nodes.

All the probes installed into the fixed nodes have been calibrated using three different liquids provided by the sensor manufacturing industry. Calibration procedure follows an internal, proprietary algorithm provided by the sensor manufacturing industry. MDM Team has incorporated the proprietary protocol to engage calibration procedure in order to facilitate probe replacement and re-calibration.

2.3 Innovative solutions: design for integration of RHE-MEDIation measured data with the Digital Twin Ocean and Water Knowledge management platforms

The RHE-MEDIation project, through contaminant data collected from the three key pilot sites, will contribute to the EMODnet objectives of producing new contaminant maps, in alignment with relevant EU Environmental Directives. This will be possible thanks to the RHE-MEDIation Ingestion Service, as part of the RHE-MEDIation Monitoring System.

The RHE-MEDIation Ingestion Service, developed in T2.3, consists of a data acquisition process, a data transformation component, and a data loading mechanism. It utilizes an ERDDAP server to deliver datasets to the EMODnet Map Viewer via REST GET requests. ERDDAP is a scientific data server that allows users to upload and download datasets in widelyused file formats. The datasets shared by RHE-MEDIation with EMODnet may include pollutants like pharmaceuticals, pesticides, heavy metals, PAHs, PCBs, and PFAS, along with other parameters such as temperature, salinity, oxidation-reduction potential, total dissolved solids, and hydrogen potential. These datasets will be made available to EMODnet under the Creative Commons Attribution 4.0 International license.

Following an assessment between two different versions of the RHE-MEDIation Ingestion Service, the chosen solution involves utilizing a MySQL database for security purposes, hosted on a separate machine from the ERDDAP Server and managed by Microsoft Azure for MySQL Database application. This document includes a detailed low-level design in terms of Java classes, elaborating on the high-level modules previously discussed, and identifies the following components:

- ✓ DataCollector: Responsible for gathering data from the MDM Server.
- ✓ DataLoader: Facilitates GET requests that allow the EMODnet application to access RHE-MEDIation data.
- ✓ Credentials: Manages authentication and authorization mechanisms.
- ✓ Main: Implements the overall flow of the RHE-MEDIation Ingestion server, including data collection, transformation, and uploading.
- ✓ Data Transformer: Handles the conversion of data formats.
- ✓ HttpClient: Manages HTTPS communication.
- ✓ HttpsParam: Manages all parameters required by the HttpClient class.
- ✓ Configuration: Loads and stores all configuration parameters from a file.

The confidentiality, integrity and availability of the overall application is guaranteed by three components: Microsoft Azure for MySQL Database, Active Directory and Microsoft Azure Front End.

The technology consists in the RHE-MEDIation Ingestion Service, providing physical and chemical measured data to EMODnet, the European Commission in situ marine data service (EC) in situ marine data service of the EC Directorate-General Maritime Affairs and Fisheries (EC DG MARE). In particular, RHE-MEDIation project, thanks to the three parameters collected in the three relevant pilots from the mobile and fixed installation sensors will be delivered to the MDM Web application server and then to the RHE-MEDIation Ingestion Service.

In particular, the technology in scope consists of the following modules:

- The data acquisition module that requests to the MDM Server the JSON file with water quality parameters measured via fixed and mobile sensors,
- the data transformation module that converts the JSON file in a format compatible with EMODnet,
- the data loading module that integrates the received datasets into the RHE-MEDIation ERDDAP Server.

The overall system architecture is made up of the following elements, having the first server hosting:

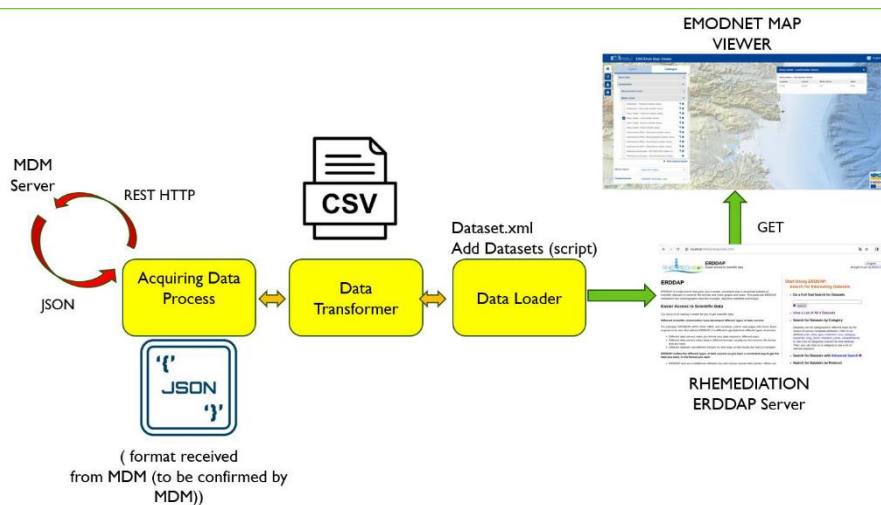


Figure 13. RHE-MEDIation Ingestion Service.

- Red Hat Linux server operating system,
- Docker engine that contains Tomcat and ERDDAP server application,
- Data collector Daemon that is a RINA-C developed SW that retrieves data from MDM Server using HTTPS Rest requests,
- RINA-C Active Directory,
- Azure Front-door.

Furthermore, the content of the second server has been selected considering a MYSQL database storing the data to be published on EMODnet. In this case, the Data Collector daemon saves in the database measured data received from the MDM Server. Then this information is retrieved from the ERDDAP Server and sent to the EMODnet Application.

2.4 Design and implementation of a wide stakeholder engagement

The involvement of citizens and various stakeholders (such as chemical and environmental protection scientists, wastewater treatment operators, authorities, industry representatives, etc.) will be crucial. Awareness of remediation solutions should be maintained and developed through regular participation in dedicated workshops and webinars, which should be supported by local authorities and the National Authority.

Data will also play a central role: transparency regarding past investigations in the basin, the remediation plan implemented within the project, and the associated measured data is essential. Monitoring and up-scaling efforts to completely avoid dangerous chemical discharge into water bodies should be shared.

The model under discussion and subsequent agreement will focus on the following core areas:

- Finding ways to make remediation actions sustainable and cost-effective at the WTP/WWTP level, including providing participatory budget opportunities for citizens;
- Benchmarking the evolution of environmental status over time against set targets;
- Defining roles and responsibilities for oversight by citizens and other stakeholder representatives, and establishing KPIs to measure expected synergies.

2.5 Positioning of the project in terms of R&I maturity

The RHE-MEDIation Technology Basket proposed hinges upon a set of 7 assets, whose initial TRL is illustrated in Figure 11. The average Technology Readiness Level (TRL) of the individual technologies is currently at 5. Through the design, integration, implementation, validation, and demonstration activities planned in the project, the final average TRL of the integrated package, validated in a pilot configuration within a real environment, is expected to reach an intermediate level between 6 and 7: as a result, applicants for the replication sites will have the availability of a TRL 7 technology.

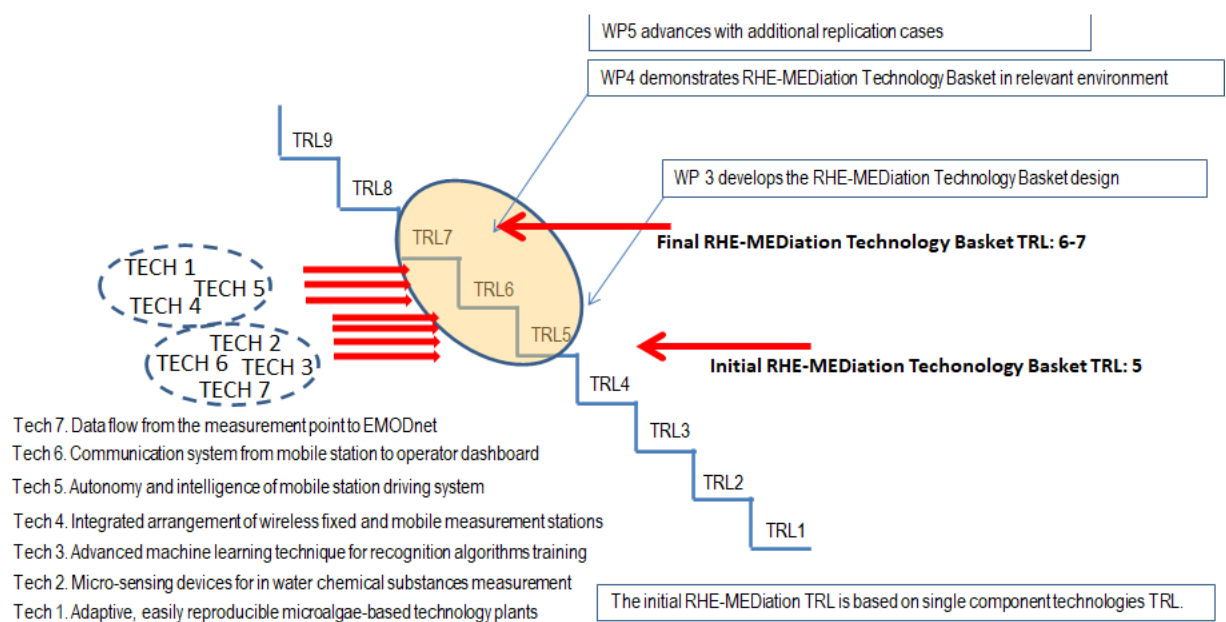


Figure 14. RHE-MEDIation Technology Basket TRL expected up-scale.

2.6 Demonstration cases overview

RHE-MEDIation features three complex demonstration cases, selected in different Mediterranean countries: Italy, Greece, and Turkey, represented by Mar Piccolo Bay, Elefsis Bay, and Izmit Bay, respectively. These locations are well-known hotspots of chemical pollution in the Ionian, Aegean, and Marmara seas.

Following the RHE-MEDIation strategy, which aims to demonstrate the ability to reduce pollutants in both seawater and wastewater before it reaches the sea, these three distinct demonstration cases have been incorporated into the project (see Figure 12).

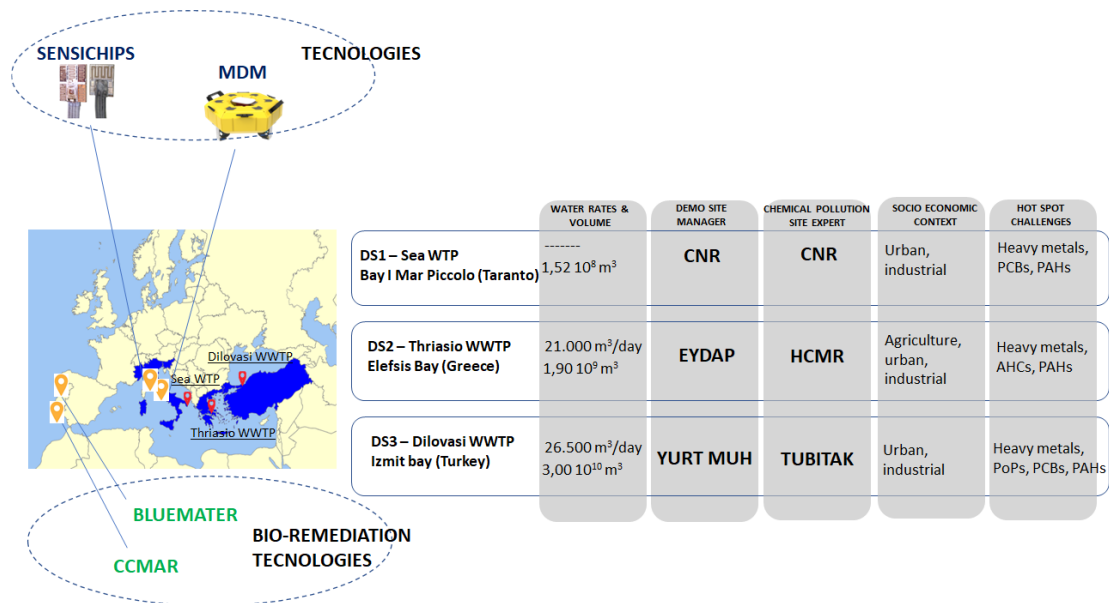


Figure 15. RHE-MEDIation demonstration sites characteristics.

The micro-algae-based technology will specifically be used to:

- remove pollutants from freshwater entering the **Mar Piccolo Bay**,
- remove pollutants at the **Thriasio Wastewater Treatment Plant**, which serves the municipalities of Aspropyrgos, Elefsina, and Mandra-Idyllia in Attica, and discharges treated water into Elefsis Bay,
- remove pollutants at the **Dilovasi Municipal Wastewater Treatment Plant**, which discharges treated water into the Dilovasi rivers that flow into Elefsis Bay.

The representativeness of these case studies ensures wide applicability of the project’s proposed solutions, as the microalgae-based technology will be tested and validated under different conditions. These selected sites have been thoroughly studied and investigated in recent years, with available data providing the necessary background for initiating the project. Figure 13 outlines the design for the deployment of the RHE-MEDIation systems.

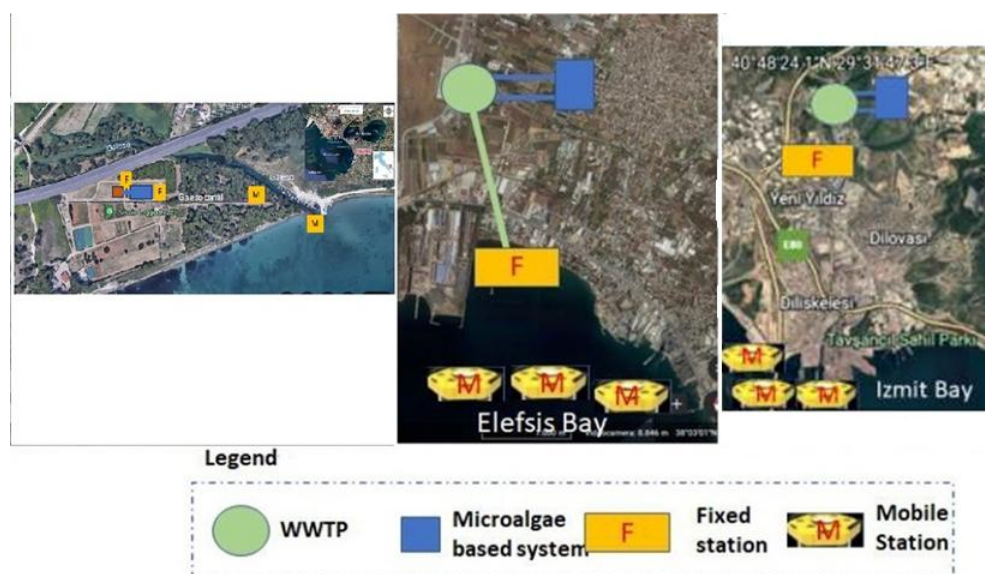


Figure 16. RHE-Mediation demonstration sites characteristics.

2.7 Demonstration Site 1 - Sea WTP pilot, Taranto (IT)

Mar Piccolo is a coastal basin located north of the city of Taranto, covering an area of approximately 20.7 km² with a total volume of 0.152 km³ (Figure 14). The basin is divided into two sections: the first bay (Bay I) and the second bay (Bay II), separated by the promontories of Punta Penna and Punta Pizzone. Mar Piccolo represents a coastal marine ecosystem example, whose biological balances have been modified step by step, in relation to anthropic development and, in particular, to the big industry settlement. Moreover, the basin reflects the negative effects of pollution because of its semienclosed shape with remarkable problems of water exchange, which are mainly due to moderate sea tides.

The high urbanization and the massive industrialization of the Taranto area have caused, in the years, sediment contamination by different organic compounds and heavy metals. The industrial and harbour activities are two of the main employers in the area. These activities have heavily affected the coastal marine environment, especially the sediment quality: The benthic sediments contain pollutants at concentrations that often exceed those of the overlying water column by several orders of magnitude. In such a situation, contaminated sediments can represent a significant, long-term source of contaminants to the overlying water column and the aquatic biota.

Various chemical characterizations and research confirmed high levels in sediments of priority contaminants such trace metals (lead, cadmium, mercury, copper, zinc, etc.) and organic pollutants (polycyclic aromatic hydrocarbons– IPA, polychlorinated dibenzo-p-dioxins and dibenzofurans–PCDD/Fs, polychlorinated biphenyls–PCBs). Moreover, high levels of these chemicals have also been found in the aquatic biota with a significant risk especially for human health.

However, pollutants such as pesticides, pharmaceuticals, and PFAS have not yet been thoroughly investigated, though their presence is likely.

In this scenario, special programs have just been started to plan actions for sediment remediation and pollution reduction. National programs also include actions for the characterization and recovery of surrounding sites that may indirectly influence the quality of the basin. Moreover, other activities include protection of biodiversity, the study of the presence and the accidental introduction of alien species, and transfer of pollutants through the food chain. All these factors made the Mar Piccolo basin a model ecosystem identified by many projects to assess the environmental impacts and identify remediation actions.

As regards the remediation of contaminated marine sediments, the remediation is almost exclusively addressed by transferring the dredged material to controlled landfills in mostly port areas. Recently, an innovative technology has been tested in Mar Piccolo, as part of the Life program, on a pilot scale, an innovative technology alternative to the most invasive technologies such as dredging and capping, based on tangential microfiltration, aimed at the in situ removal of only the fine fraction of sediments (i.e., the most contaminated) without making changes or damage to the marine ecosystem. Tangential microfiltration is characterized by a high separation capacity, being able to discriminate the different species in solution based on their molecular weight or their surface charge. In addition, cross-flow membrane filtration technologies allow the recovery of both permeate effluent and concentrate without the use of heat, solvents and chemical reagents, and therefore clean and defined technologies are the best available technologies.

As regards programs that include actions for the characterization and recovery of surrounding sites that may indirectly influence the quality of the basin, the pilot project to be implemented in Taranto,

Italy, will be different from the other two sites, because it will not treat wastewater but water from the Galeso river that flows into the Inlet of Mar Piccolo.

Main challenge: The primary goal is to reduce harmful substances such as heavy metals, PCBs, PAHs, pesticides, PFAS, and other persistent chemicals (which are likely present, even though they haven't been measured yet) from fresh water entering into Mar Piccolo.

RHE-MEDIation solution: The new functionality will:

- a) Demonstrate the reduction of chemical pollutants in the fresh water entering into Mar Piccolo;
- b) Reduce stress on Mar Piccolo from heavy metals, PCBs, PAHs, PFAS, and other persistent chemicals

RHE-MEDIation data measurement scenario: This involves two fixed installations and two mobile autonomous installations in the Bay I basin for intermittent measurements at key locations where past data is available.



Figure 17. Aerial view of the Taranto Bay (Italy).

2.8 Demonstration Site 2 - Thriasio Wastewater Treatment Plant, Municipalities of Aspropyrgos, Elefsina and Mandra-Idyllia, Attica (GR)

The Thriasion Plain is located 25 km west of Athens on the Attica Peninsula, covering an area of 120 km² and including the towns of Elefsis, Aspropyrgos, Mandra, and Magoula (Figure 18).

The plain represents 27.3% of the area and 52% of the population of Western Attica, and accounts for 2.1% of the total population of the Attica Region, which includes Athens. With a population of 78,302, the region has seen significant growth of 31.9% between the 2001 and 2011 censuses. This growth rate far exceeds that of Western Attica (20.7%) and the Attica Region as a whole (6.6%). Urban expansion has been driven by industrial development, peaking in the early 2000s, with heavy industries such as oil refineries, plastic and rubber production, chemicals and petrochemicals, iron and steel manufacturing, machinery, transport, logistics, scrap metal recycling, and two of Greece's largest shipyards operating in

the area. Despite the heavy contamination from these industrial activities, no remediation has been implemented for the seawater and marine sediments in Elefsis Gulf.

The Thriasio Wastewater Treatment Plant (TWWTP), operational since 2012 (Phase A), serves the municipalities of Aspropyrgos, Elefsina, and Mandra-Idyllia. It also handles pre-treated liquid waste from local industries and businesses. Treated sewage is discharged into Elefsis Gulf through a 1,560-meter pipeline. The TWWTP has eliminated the issues of cesspools and uncontrolled sewage discharges, leading to gradual improvement in Elefsis Bay's condition and enhanced quality of life for residents. Phase A of the TWWTP has a capacity of 117,000 equivalent inhabitants, treating up to 21,000 m³ of wastewater per day. Expansion plans (Phase B) will increase this capacity to 42,000 m³/day, with pre-treatment facilities, buildings, and infrastructure already underway.

Main challenge: The RHE-MEDIation initiative aims to optimize the TWWTP by adding features to systematically treat wastewater to:

- a) Prevent harmful substances from entering Elefsis Bay through the discharge pipeline;
- b) Reduce stress on the Bay from heavy metals, pharmaceuticals, PCBs, PAHs, PFAS, and other persistent chemicals, which may be present even if not yet measured, due to improved dilution conditions.

RHE-MEDIation solution: The initiative will enhance the TWWTP with additional remediation capabilities.

RHE-MEDIation data measurement scenario: The project will include one fixed installation at the wastewater outlet and three mobile autonomous installations in Elefsis Bay for intermittent measurements at key locations where previous data is available.

Expected benefits: With a microalgae-based remediation capacity of 10 m³/day, the expected benefits during the project demonstration period (1 year) include:

- Over 90% reduction in chemical contaminants;
- Cleaning of 3,650 m³ (4.8% of the wastewater treatment capacity required).

Upscale: To meet 50% of the wastewater treatment capacity needs for Phase A, the RHE-MEDIation system must be scaled up by a factor of 1,050. For Phase B, the system would need to be scaled up by a factor of 2,000. This latter configuration would impact Elefsis Bay's water volume by approximately 3.8% over a 10-year period.



Figure 18. Thriasio Plain and Elefsis Gulf areas.

2.9 Demonstration Site 3 - Dilovası Municipal Wastewater Treatment Plant,

Dilovasi (TK)

The Bosphorus, the Sea of Marmara, and the Dardanelles are collectively known as the "Turkish Straits System" (TSS). This complex and dynamic system facilitates the transfer of water, mass, heat, and materials between the Aegean and Black Seas via the Marmara Sea and the Istanbul and Dardanelles Straits (see Figure 16). Izmit Bay (IB) is a semi-enclosed bay located in the northeastern part of the Marmara Sea. It is divided into three sections (western, central, and eastern) connected by narrow openings, with a total volume of $3 \times 10^{10} \text{ m}^3$.

Since the 1980s, residential and industrial development in the region has tripled. Future anthropogenic pressures are expected to rise due to planned expansions in transportation and industry. Izmit Bay has been under monitoring by TUBITAK MAM since 2007 through both regional projects (Monitoring of Izmit Bay Water Quality and Terrestrial Inputs and Developing Recommendations for Pollution Prevention) and national initiatives (Integrated Monitoring and Assessment Programme). Seasonal monitoring of physical, chemical, and biological parameters has revealed contamination of sediments with heavy metals (Pb, Ni, Zn, Cu), polycyclic aromatic hydrocarbons (PAHs), and polychlorinated biphenyls (PCBs).

The Dilovası Municipal Wastewater Treatment Plant (WWTP) is one of the major source of pollution in Izmit Bay, with its discharge flowing into the bay via Dilderesi River. The plant processes 26,500 m^3/day of wastewater, with a planned capacity increase to 40,000 m^3/day over the next decade. Approximately 60-70% of the plant's wastewater inflow comes from industrial facilities (chemicals, plastics, metals, etc.), resulting in wastewater that is more heavily polluted than typical municipal waste.

The WWTP employs an extended aeration activated sludge process with phosphorus removal capabilities and also includes rapid sand filtration and UV disinfection for additional treatment.

Main challenge: The RHE-MEDIation initiative aims to optimize the Dilovası Municipal Wastewater Treatment Plant (DWWTP) through systematic wastewater treatment to:

- a) Prevent harmful substances from entering Izmit Bay via the Dilderesi River;
- b) Alleviate the contamination of Izmit Bay from heavy metals, PCBs, and PAHs already present and transported by Dilderesi River, as well as PFAS and other persistent chemicals, which are likely present even if not yet physically measured.

RHE-MEDIation data measurement scenario: This involves one fixed installation at the wastewater outlet and three mobile autonomous installations in Izmit Bay for intermittent measurements at key locations where previous data is available.

RHE-MEDIation solution: The initiative will equip the DWWTP with remediation capabilities.

Expected benefits: With an anticipated microalgae-based remediation capacity of 10 m^3/day , the projected benefits during the 1-year project demonstration period are:

- Over 90% reduction in chemical contaminants
- Cleaning of 3,650 m^3 (0.038% of the wastewater treatment capacity).

Upscale: To meet 50% of the current wastewater treatment capacity needs, the RHE-MEDIation system must be scaled up by a factor of 1,325. For the future scenario, the system would need to be scaled up by a factor of 2,000. This latter configuration would result in an approximate 0.24% impact on Izmit Bay's water volume over a 10-year period.



Figure 19. View of İzmit Bay, Marmara Sea.

2.10 Checklist

The following checklist provides an example of the steps required to frame RHE-MEDIation replication sites checklist. This checklist can support Third Parties in developing their own replication sites by helping them better understand the type of information needed, pre-setup, and processes required to effectively plan and manage a replication site development. Candidates should develop their own replication site based on the 9-month RHE-MEDIation Technical Support Program period.

RHE-MEDIation replication site checklist		
#	Title	Description
1	Replication site objectives	Define the objectives of this replication site. <i>Example: The main goal of this replication site is to frame a replication plant based on Project RHE-MEDIation-inspired innovative technological solutions for this hot spot.</i>
2	Place where the replication site will be developed	Specify the geographical location where the replication site will be established, including any unique characteristics pertinent to the site, the facilities where the replication activities will take place, etc.
3	Existing facility (if any) within the replication site	Which kind of contaminants does the existing facility measure/discriminate? Please, provide specific features and indicators.
4	Identify compounds and parameters that can be monitored or addressed	Provide description of the chemical pollutants, e.g. heavy metals, pesticides and PFAS, etc.
5	Impact of the compounds	What are the potential consequences if these pollutants are not monitored and treated?
6	Technologies to be implemented and replicated in the replication site	Connect the RHE-MEDIation solution that could be tested, on the bases of the replication site requirements. The preliminary efforts made during the beneficiary's participation in the RHE-MEDIation program should demonstrate the feasibility, replicability, and scalability of the solution(s).

7	Essential requirements that must be addressed during the implementation of the replication site	Requirements for the user (<i>Documented requirements and expectations that a system must satisfy for its users</i>). Functional requirements (<i>Explain WHAT the system is expected to accomplish according to the user requirements</i>).
8	Replication site Key Performance Indicators	Describe which KPI's have been chosen and how as it is expected that they will be achieved.
9	Expected constraints	Specify any limitations or constraints, such as technological, budgetary, temporal, etc. For example: Budget cap of €100,000, project must be completed within 9 months.
10	Line-up to regulatory and technical existing frameworks	1) Regulations and directives that the use case must adhere to 2) Data management requirements 3) Risk assessment procedures 4) Any regulatory approvals required, including the timeline for obtaining them.
11	Stakeholder identification and engagement	Concisely describe what each stakeholder category (chemical and environmental protection scientists, wastewater treatment operators, authorities and citizens, etc.) expects from this replication site. E.g.: science communities can access pollution remediation data and analysis, local authorities can rely on a stronger basis for decision-making process, etc.
12	Expected final outcome	What should be the final outcome of this replication site, both shortly after the project concludes and in the medium term (within 3 years)?
13	Timeline	Provide a roadmap with the activities to be carried out and their expected timeline, along the duration of the 9-months financial support within the RHE-MEDIation programme.

2.11 Conclusion

In summary, this document offers a comprehensive overview of the RHE-MEDIation methodology, innovative solutions, and the strategy for deploying replication cases. It complements Annex 1, the RHE-MEDIation Guidelines for applicants, by providing an in-depth understanding of the innovative solutions and replication sites specifications from the RHE-MEDIation consortium.

This information is intended to assist Third Parties in developing their own replication sites.

Funded by the European Union's Horizon Europe program, RHE-MEDIation is a 5.8 million euros, three-year project that will be a responsive hub for deploying long-term governance centered on the mission to de-stress the Mediterranean Sea from chemical pollution, including peak concentrations in known HOT SPOTS.

In particular, the project will test and validate a chemical pollution remediation technology based on micro-algae solutions that will be integrated within existing water/wastewater treatment systems that will enhance the removal of heavy metals, pesticides, and PFAS and forever chemicals. Moreover, it will be complemented with mobile and fixed sensing systems to identify and measure the presence of chemical substances in both land and marine waters, being measured data delivered to the EC EMODnet platform to contribute to the Digital Twin of the Ocean.

Three sites (Italy, Greece and Turkey) have been chosen for demonstration and once realized, five replication sites will be added to the list for further investigation. To this end, local stakeholders will be empowered to manage this transformation.



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