Aquatic Pollutants

FUNDED PROJECTS BOOKLET
JOINT TRANSNATIONAL CALL 2020

Risks posed to human health and the environment by pollutants and pathogens present in water resources

Water JPI

JPI OCEANS

jpiamr

CONTENTS

PRESENTATION OF THE AQUATICPOLLUTANTS
JOINT TRANSNATIONAL CALL 2020

Within the framework of ERA-NET Co-fund AquaticPollutants, the three Joint Programming Initiatives (JPIs) on Water, Oceans and Antimicrobial Resistance (AMR) collaborated to implement a joint transnational call for research and innovation projects on risks posed to human health and the environment by pollutants and pathogens present in the water resources.

There are still major risks associated with the occurrence of emerging contaminants, pathogens and antimicrobial resistant bacteria in our water bodies and oceans. To face these challenges in a comprehensive way and to develop multidisciplinary and practical solutions for the provision of safe drinking water and healthy aquatic environments, the 2020 joint transnational call aims to support the research communities of freshwater sector, the marine sector and the health sector to work together and create synergies for joint approaches.

Eighteen projects with 20M€ in requested budget were selected for funding in response to the Aquatic Pollutants joint call. The funded projects, involving 22 countries, are presented in the booklet.

OBJECTIVES OF THE JOINT TRANSNATIONAL CALL

The call will support research and innovation projects that establish integrated and cross-sectoral approaches for risk-management combining the research areas of contaminants of emerging concerns (CECs), pathogens and antimicrobial resistance. The whole water cycle, from the source through the river basins and eventually to the estuaries and oceans, is considered.

The main research and innovation objectives of the AquaticPollutants 2020 Joint Transnational Call are:

▶ to **establish** integrated and cross-sectoral **approaches for risk management** combining the research areas of emerging pollutants, pathogens and antimicrobial resistance under the overall topic "from the source to the mouth"

▶ to **analyse the spread** of CECs and pathogens related to antimicrobial resistance from the different sources (e.g. urban areas) that leads to impacts and risks on the aquatic ecosystem, environment and human health

▶ to **describe the transformation** of such CECs and pathogens and their effects when entering the different aquatic systems and accumulating in the food chain

▶ to **improve strategies and develop/ evaluate technologies** (incl. digital technologies) for reducing CECs and pathogens at the sources, on their pathways and end-of-pipe; and

▶ to **develop/ integrate innovative methodologies and tools** to allow policy-makers to develop more effective policies and efficient regulations.
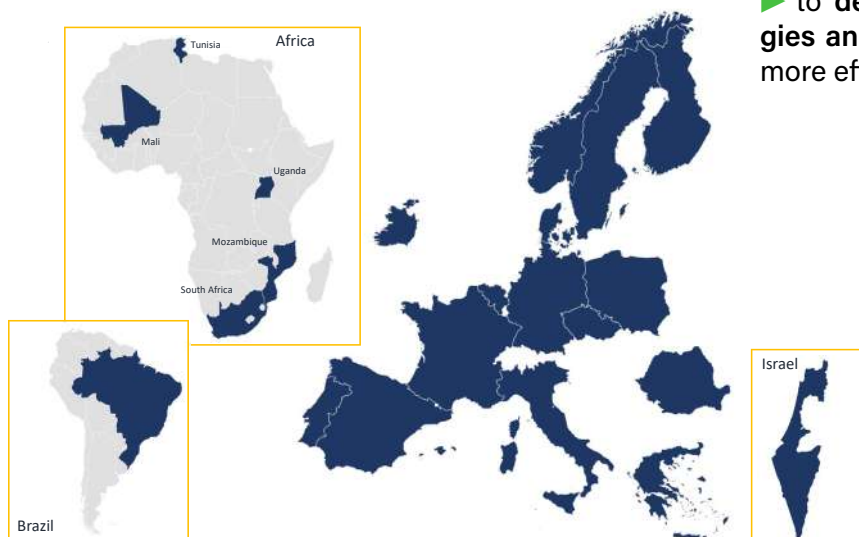
THEMES OF THE JOINT TRANSNATIONAL CALL

Funded Research & innovation projects address at least one of the following themes:

Theme 1 – **Measuring** - Environmental behaviour of contaminants of emerging concern (CECs), pathogens and antimicrobial resistant bacteria in aquatic ecosystems

▶ **Subtheme 1.1 -** Assessment of the significance of different potential sources, reservoirs and pathways of CECs and pathogens including antimicrobial resistant bacteria

▶ **Subtheme 1.2 -** Understanding and predicting the environmental and cumulative behaviours of contaminants of emerging concern (CECs) and pathogens including antimicrobial resistant bacteria, including the development of tools and digital solutions

Theme 2 – **Evaluating** - Risk Assessment and Management of contaminants of emerging concern (CECs), pathogens and antimicrobial resistant bacteria from aquatic ecosystems (inland, coastal and marine) to human health and environment

▶ **Subtheme 2.1 -** Characterising the exposure routes and effects of CECs and pathogens including antimicrobial resistant bacteria, on aquatic ecosystems and on human health

▶ **Subtheme 2.2 -** Development of integrated risk assessment and risk management procedures

▶ **Subtheme 2.3 -** Parameters and strategies for monitoring potential antimicrobial resistant bacteria

Theme 3 – **Taking Actions** - Strategies to reduce contaminants of emerging concern (CECs), pathogens and antimicrobial resistant bacteria in aquatic ecosystems (inland, coastal and marine)

▶ **Subtheme 3.1 –** Implementation of strategies to reduce CECs and pathogens, including antimicrobial resistant bacteria at the source

▶ **Subtheme 3.2 –** Development of methods for preventing the spread of CECs and pathogens, including antimicrobial resistant bacteria

DISCLAIMER

THEME 1

MEASURING

Environmental behaviour of contaminants of emerging concern (CECs), pathogens and antimicrobial resistant bacteria in aquatic ecosystems

ARENA

Antibiotic Resistance and Pathogenic Signature in Marine and Freshwater Aquaculture Systems

PROJECT COORDINATOR

Dr Gian Marco LUNA
National Research Counci (CNR) - Italy

PROJECT PARTNERS

Catalan Institute for Water Research (ICRA) - Spain
—
University of Applied Sciences Aachen - Germany
—
University of Liège - Belgium
—
KU Leuven - Belgium

DURATION
3 years

STARTING
September 2021

CONTACT
Dr Elisa BALDRIGHI
elisa.baldrighi@irbim.cnr.it

ABSTRACT

The project aims to improve the **quality assessment of aquaculture practices and products by exploring the fate of antibiotics (AB) and microbial contaminant**s across the water cycle.

Contamination levels will be assessed from sources (effluents, river outflows) to end points (aquaculture plants) and final food products (fish fillets), along with the antibiotic resistome and pathogenic signature in farm surrounding aquatic environments in open (mariculture) and recirculating aquaculture systems (RAS), which mainly consist of indoor tanks in which fish is grown using low or no ABs or chemicals. For mariculture only, the assessment of the same contaminants will be also performed in benthic biota beneath fish cages; moreover, in order to take into account seasonal variations, we will perform analyses at contrasting seasons (e.g., summer, generally characterized by a higher anthropogenic pressure, and winter, with a lower anthropogenic pressure).

Novel early-warning tools for the rapid detection of antibiotic residues (flow cytometry-based), **antibiotic-resistance genes** (ARGs) (high-throughput sequencing-based), and **microbial pathogens** (sensor-based) in environmental and biological samples will be optimized.

ARENA will contribute to **elucidate the significance of antibiotic-related issues**, finally providing a **cross-disciplinary approach for risk assessment and future operative efforts to mitigate the magnification of antibiotic resistance** and pathogenicity in aquaculture settings.

OUTCOMES AND EXPECTED IMPACT

Aquaculture represents a key source of fish food for human consumption, and is among the fastest growing food sectors globally. Although EU aquaculture is renowned for its high quality and sustainability, **increasing concerns arise from the release of ABs in the environment via aquatic routes** and determining possible contaminations of fish farms and products. **Assessing the sources, reservoirs and pathways of ABs, AMR and pathogens** is of primary importance, and the **development of fast and reliable tools for their detection is urgently needed**.

ARENA will provide knowledge and tools for future operative efforts to decrease the spread and magnification of ARB and pathogens in aquaculture.

Within the ARENA project, we will produce a complete overview of the AB-resistome (ARGs content) and pathogenic signature (pathogenic bacteria content) of open and closed fish farms and their potential contamination sources and pathways. Different innovative approaches will be optimized and tested for their capability of rapidly detecting AB residues, ARGs, ARB, and other pathogens in the environment as well as in the fish products (i.e., sensors, nanopore sequencing).
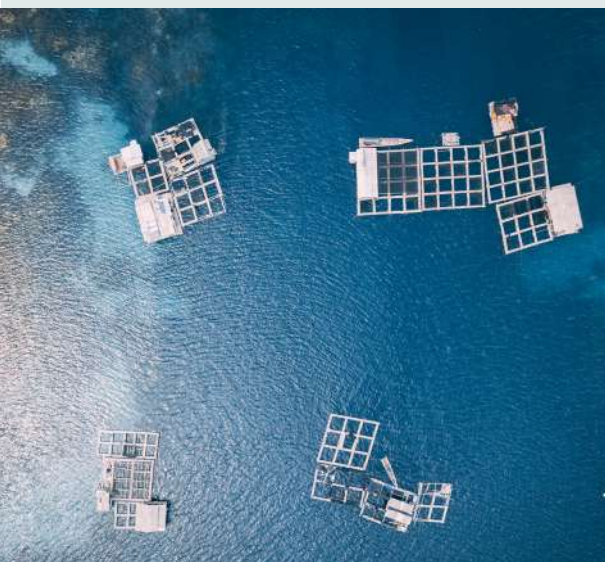
PROJECT STRUCTURE

The ARENA project is divided into five work packages:

WP1 – Project Management
WP1 will assure and facilitate the realization of the project's activities and goals.

WP2- Source and fate of antibiotic resistance
In WP2 we will explore and define the contamination sources and pathways in RAS and mariculture systems, characterized by different features and potential risks of contamination. The variety of samples that will be analyzed will allow to better define the sources and routes of ARGs, antibiotic resistant bacteria (ARB) and pathogenic bacteria (PB) in different types of aquaculture.
WP2 will also deal with the on-site evaluation of novel bacteria-sensing and sequencing-based tools developed in WP4.

WP3 – Antibiotic contamination in aquaculture
The main activities in this WP concern the assessment of the presence and concentration of ABs and their metabolites in marine and RAS aquaculture by means of powerful and sensitive multi-residue analytical methodologies. A novel methodology based on flow cytometry will be tested for the rapid detection of AB residues in edible products from fish farming.

WP4 – Sensors for on-site bacterial detection
WP4 is dedicated to the development of sensor tools for the selective on-site quantification of bacterial pathogens and contamination monitoring in aquaculture. The activities will be built around three central tasks:
- The synthesis of surface-imprinted polymers as bacteria receptors, using a novel surface-imprinting route that combines the (existing) master-stamping approach.
- The development of an on-site bacterial sensing instrument, based on impedimetric detection and aiming at measuring bacterial concentrations
- down to trace levels.
- The development of the early warning tool for bacterial contamination in aquaculture. The major challenge is in the autonomous functioning of this tool during extended times ranging from a few days to ideally a month.

WP5 – Exploitation and Dissemination
The activities foreseen in WP5 relate mainly to the ARENA website and the final project workshop.

EXPERIMENTS / CASE STUDIES

Research activities will be focused on **marine aquaculture systems as well as on closed Recirculating Aquaculture Systems** (RAS), located in Italy, Belgium and/or Norway.

Several Associated Partners will support the ARENA project by providing seawater samples, by doing sampling campaigns together, and by helping to validate the developed sensors. NORCE, as subcontractor, will cover the set-up, optimization and validation of a nanopore-based method for the assessment of ARGs and ARBs in aquaculture settings.
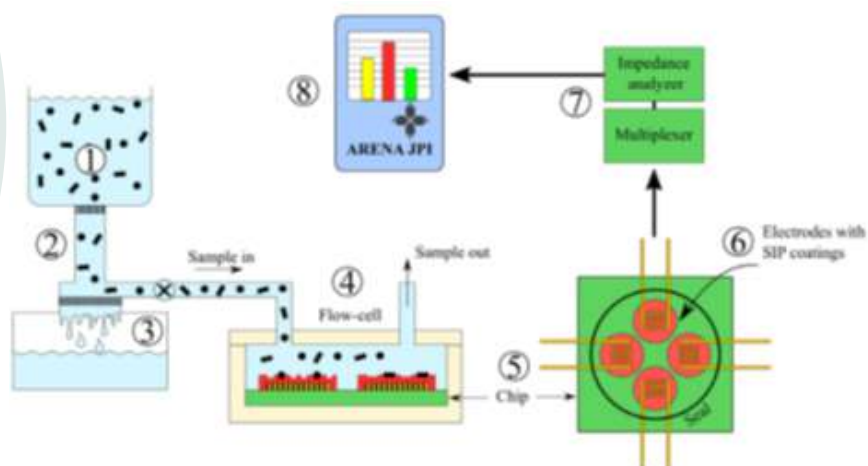
PROJECT CONTRIBUTION TO POLICIES

ARENA is fully in line with the vision and guidelines defined by the EU and WHO to tackle the **global fight against AMR** and in recognizing the threats by AMR on human and environmental health (EU **One Health Action Plan against AMR**, 2017; **WHO Global action plan on antimicrobial resistance**, 2015).

ARENA will contribute to **UN SDG 14 (Life below water)**, which deals with the conservation and sustainable use the oceans, seas and marine resources for sustainable development.

FUNDING INSTITUTIONS
AEI (Spain), BMBF (Germany), FNRS (Belgium), FWO (Belgium), MUR (Italy)

FOREWARN

Development of a smart forewarning system to assess the occurrence, fate and behaviour of contaminants of emerging concern and pathogens, in waters

PROJECT COORDINATOR

Esteban ABAD & Marinella FARRÉ
Institute of Environmental Assessment and
Water Research (IDAEA-CSIC) - Spain

PROJECT PARTNERS

University of Helsinki - Finland

—

French Agency for Food, Environmental and
Occupational Health & Safety (ANSES) - France

—

Dublin City University - Ireland

—

Attikon University Hospital - Greece

DURATION
3 years

STARTING
September 2021

CONTACT
Esteban ABAD
eahqam@cid.csic.es
Marinella FARRÉ
mfuqam@cid.csic.es

ABSTRACT

FOREWARN will assess the occurrence, fate and behaviour of contaminants of emerging concern (CECs) and pathogens, and **develop machine-learning methods to model their transfer and behaviour and build a decision support system** (DSS) for predicting risks and propose mitigation strategies. FOREWARN will be focussed on CECs such as antibiotics and pathogens such as antibiotic-resistant bacteria (ARB), antibiotic resistance genes (ARG) and emerging viruses, such as SARS-CoV-2.

Using large datasets from previous research, FOREWARN will establish the basis of the relationships between the conditions of a particular aquatic environment such as the hydrological and geological conditions, clime, drought and floods, the anthropogenic stressors such as water supply, wastewater discharges, type of wastewater discharges, among many others, and the emerging pollution that is CECs and emerging pathogens. The project will consider 2 types of case studies:

1. **In-silico case studies** will be selected from previous results and dataset obtained in past or ongoing EU projects. Data will be used to develop the models and algorithms to feed and **develop the DSS system to better understanding the sources, transport, degradation of CECs and pathogens and modelling their behaviour**.

2. The adaptive DSS system will be refined and tested under real environmental conditions to achieve TRL5 in **real environment case studies**.

OUTCOMES AND EXPECTED IMPACT

The final DSS, developed in FOREWARN, will facilitate decision-making processes, technologies to apply and the selection of wastewater treatments needed in each case. The system will be adaptable to different environments using deep-learning algorithms. Outputs from models and supporting data will be presented to stakeholders via a web-based decision support dashboard.

Therefore, FOREWARN is expected to:

- **Improve the understanding of the occurrence, fate and behaviour of CECs and their influence on ARBs, ARG,** and the transferability of the emerging virus through the water by developing innovative modelling approaches.
- **Provide an early warning system for rapid detection of hot points** needing remediation or preventive actions. The application of machine learning as a base for a decision support system is a new quality step in the science of CECs, ARBs, ARGs, and emerging virus in the aquatic environment.
- **Provide a foundation for prevention and mitigation solutions** based on better understanding the sources and distribution of CECs and emerging pathogens, access to models of their environmental fate and degradation pathways and improved understanding of relevant human and environmental health of the problem of emerging pollution.
- **Contribute to support the decision-making processes** in the short term thanks to the FOREWARN-DSS (punctual emissions, potential situations potential risk) and provide support on technical aspects such as wastewater treatments required in particular areas.
- **Contribute to protecting human and environmental health** by quantifying human health and environmental exposure, impact and behaviour.
- Support the implementation of European policies.
- **Contribute to capacity building** and to improve the capabilities of recently qualified students, to enlarge their working options, technicians at related industries, and in general personnel of the main stakeholders' groups.

PROJECT STRUCTURE

The FOREWARN project is divided into six work packages:

WP1 – Data collection on CECs and Pathogens and modelling
In this WP, data from previous monitoring studies performed in Europe will be compiled and modelled. This WP aims to carry out the "in-silico case studies" for CECs and pathogens to establish models of occurrence, transport, and resilience to relate their presence with environmental and anthropogenic parameters.

WP2- ARB and ARG
The presence and diversity of pathogens, as well as the presence, diversity and spread of AR genes, will be evaluated, in order to assess the contribution of Wastewater Treatment Plants to the environmental and human resistome. Samples of wastewater and waters from receiving environments will be collected in Spain, Greece, Ireland, France and Finland for antibiotics analysis, chemical characterization and the creation of an internal biobank.

WP3- DSS development
WP3 will use data collected and produced in WP1, 2, and 4 to develop a set of software tools to support decision making regarding CECs and pathogens. This will include a set of predictive models that will use historical data to attempt to predict new emergence and impact of CECs and pathogens. An easy-to-use graphical user interface will be developed to allow policy advisors and decision makers to interact with the software and visualize the predictions of the model.

WP4- Experimental case studies and DSS validation
In WP4, two real-environment case studies will be carried out in order to refine, test and validate the operability of the DSS platform: I) The Ebro Delta and II) Vantaa River.

WP5- Dissemination
WP5 is focused on the dissemination activities of the project to a wide international audience thanks to the multiple contacts of project partners including stakeholders such as policymakers, scientific community, industry, local authorities, and NGOs working in the fields of waste management, environmental and human health protection, and public policy.

WP6- Coordination
WP6 is dedicated to the management and coordination of the project.

EXPERIMENTS / CASE STUDIES

The project will consider 2 types of case studies:
1. **In-silico case studies**: from previous results and dataset obtained in past or ongoing EU projects.
2. **Real environmental case studies** (6 months) to achieve TRL5

- **Ebro Delta** (North East of Spain): It is the third-largest delta in the Mediterranean Sea and comprises a wetland area of 320 km. It is mainly devoted to aquaculture, tourism and agriculture, but it is also stressed by different Wastewater Treatment Plants with a fluctuant population..
- **Vantaa River** (South of Finland): The river passes through the most densely populated area in the metropolitan district of Southern Finland and ends in the Baltic Sea in Helsinki. Several smaller towns are situated along the river, and the wastewater effluents of some municipal wastewater treatment plants are discharged into the river. The catchment area is 1680 kms.

PROJECT CONTRIBUTION TO POLICIES

FOREWARN will support current EU policies such as the **Water Framework Directive** and the new initiatives such as the **Zero Pollution Action Plan**, which aims to secure clean air, water, and soil, healthy ecosystems and a healthy living environment for Europeans through the prevention and by suggesting efficient mitigation measures of emerging pollution (chemical contaminants and pathogens) within the major circular economy routes to support the EU transition towards a **pollution-free circular economy**.

The results of FOREWARN will support the implementation of the **UN SDG 13** (Climate Action), **14** (Life below water) and **15** (Life on Land).
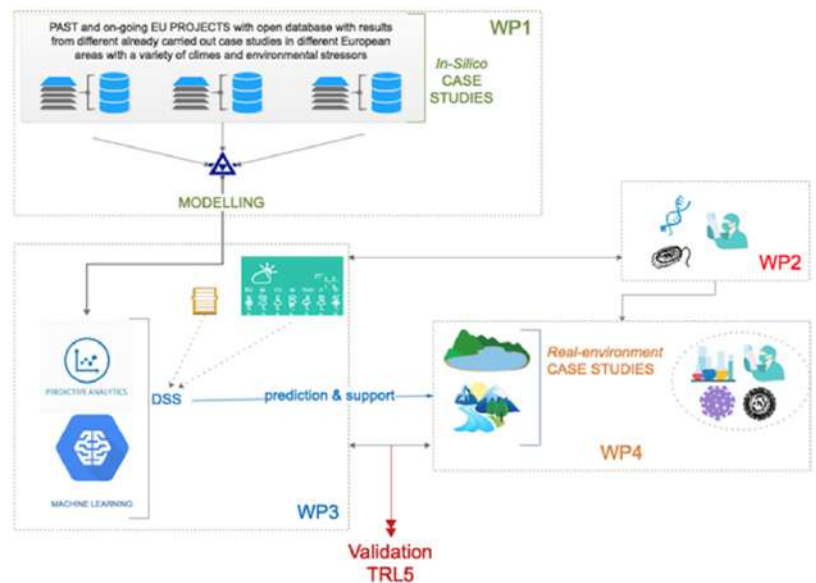
FUNDING INSTITUTIONS

AEI (Spain), AKA (Finland), ANR (France), EPA (Ireland), GSRT (Greece)

MAPMAR

Marine Plasmids Driving the Spread of Antibiotic Resistances

PROJECT COORDINATOR

Fernando DE LA CRUZ
University of Cantabria, Institute for Biomedicine and Biotechnology (IBBTEC) - Spain

PROJECT PARTNERS

Israel Oceanographic and Limnological Research (IOLR), National Center for Mariculture (NCM) - Israel

—

Karlsruhe Institute of Technology (KIT). Institute for Biological Interfaces (IBG5) - Germany

DURATION
3 years

STARTING
September 2021

CONTACT
Raul FERNANDEZ LOPEZ
fernandr@unican.es

ABSTRACT

Antibiotic resistance genes (ARGs) are one of the **most challenging contaminants of emerging concern** (CECs). Instead of being directly produced by human activity, ARGs emerge as consequence of antibiotic use in clinical settings, and residual antibiotic contamination. **ARGs spread through horizontal gene transfer and conjugative plasmids**, because their ability to cross inter-species barriers are key in this process.

Recent findings revealed the existence of marine plasmids (MAPS) of global distribution and broad host range. These MAPS can transmit ARGs across oceanic distances, and may reintroduce them to human food chains via marine products. They are, however, different to classical plasmids from clinical settings.

MAPMAR uses metagenomics, data science and single-cell sequencing to obtain a catalog of most prevalent and transmissible MAPs. **By testing methods to block their transmission, MAPMAR explores strategies to curtail the risk of oceans** acting as highways for ARG propagation.

OUTCOMES AND EXPECTED IMPACT

The overall aim of the present project is to **identify and evaluate the transmissibility of marine plasmids** (MAPs), **their association to antibiotic resistance genes** (ARGs), and their dispersal through oceanic waters, as well as **the risk of their reintroduction to the human population** through mariculture.

For this aim, MAPMAR project intents to find answers to the following questions:

1. What are the agents responsible for ARG transmission in oceanic environments? What is their prevalence among bacterial pathogens and other common marine species?
2. What is the transmission potential of marine mobile genetic elements (MGEs)? What is the transmissibility of MAPs, both in terms of in terms of the host range of bacteria they are able to invade, as well as the dynamics of their propagation?
3. What is the relationship between continental and oceanic ARG propagation? Are MAPs a subset of continental plasmids resulting from contaminated waters from sewage and agricultural wastewater? Do they form a separate subset of mobile genetic elements?
4. Are we creating a vicious cycle of ARG accumulation and transmission through mariculture?
5. Can we block re-entry of ARGs from aquatic environments into the food chain to prevent the antibiotic resistant infections in humans? Can we find a suitable strategy to attain a safer employment of continental and sea waters for human nutrition?

The expected impact of MAPMAR is to provide an appropriate **description of the pathways of ARG spread in freshwater and marine ecosystems**. These transmission pathways will shed light on the major routes and accumulation sinks of ARGs, since their origin in hospitals, farms and urban settlements until their eventual return to the food chain via marine products. The major impact outcomes expected are:

- A catalog of the marine plasmidome, and its relationship to the plasmidome present in continental and estuarine ecosystems. Such catalog will be key in tracing the major traffickers shuttling ARGs across ecosystems.
- A list of the most transmissible MAPs identified in aquatic ecosystems, together with an assessment of their host range. This in turn will serve to elaborate a risk index, indicating which plasmids and host species are more prone to act as super-spreaders of antibiotic resistances.
- An assessment on the ability of MAPs and antibiotic resistant bacteria present in marine waters to re-enter into the ARG cycle by their incorporation to marine products. The ability of conjugation inhibitors to break this vicious cycle will be investigated.
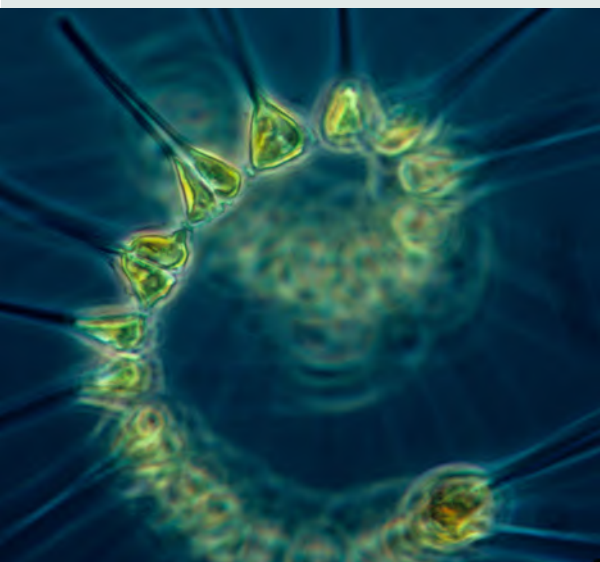
PROJECT STRUCTURE

The MAPMAR work plan is comprised of five work packages:

WP1 - Data Mining and In silico analysis of the marine plasmidome
This WP aims to elaborate an in silico database of genomic and metagenomic data of the oceanic, estuarine and coastal plasmidome. More than 5 TB of genomic and metagenomic data from marine samples will be scanned to identify i) antibiotic resistance genes (ARGs) and ii) plasmid-specific genes. Metagenome-assembled genomes for the most abundant plasmids will be retrieved, and a series of unique plasmid identifiyiers (UPIs) will be generated. These molecular barcodes will be then used for the targeted retrieval of specific plasmids from marine samples. This WP will allow us to identify the most abundant plasmids present in the sea environment, along with a series of molecular barcodes that will be used for their capture in WP2.

WP2 - Sampling, metagenomic analysis and targeted single cell-sequencing.
Water samples with detailed metadata information will be retrieved from a series of oceanic and continental sites. Metagenomes of these samples will be obtained, and in those cases where ARGs / Plasmids of interest are detected, targeted single-cell sequencing will be employed to retrieve the plasmid and host genomes by single cell sequencing. Briefly, the UPIs developed in WP-1 will be used to generate fluorescent tags that will allow the separation of the desired targets through FACs. Single-cell sequencing will be then applied to obtain the complete genome of the most prevalent plasmids in the oceans, alongside their host genomes.

WP3 - Reconstruction of marine plasmids using synthetic biology.
When possible, marine plasmids will be isolated from samples by conjugation to a series of model hosts. If isolation is not feasible, the genomes of the targeted plasmids will be reconstructed using genome synthesis techniques and propagated in a series of model bacteria. The most important phenotypic traits of the plasmids isolated/reconstructed, such as their host range and conjugation frequency, will be experimentally determined.

WP4 - Studying marine plasmid dynamics in mariculture ponds.
Those plasmids with the broadest host range /highest conjugation rate, will be selected for further analysis. Their ability to propagate antibiotic resistances, and eventually transmit them to the food chain, will be analyed in mariculture ponds. These ponds will be inoculated with a series of model bacteria containing the plasmids, and the spread potential of the plasmid and the antibiotic resistance will be monitored in time. In this WP we will also study the feasibility of minimizing the spread of antibiotic resistance genes using different interventions, including chemical compounds known to block plasmid conjugation.

WP5 – Project coordination
WP5 includes all project coordination activities, and all centralized administrative duties implicated in the management of the project.

PROJECT CONTRIBUTION TO POLICIES

MAPMAR results will contribute to **UN SDG 6** (Clean Water and Sanitation) and **14** (Life below Water) by safeguarding the provision of water, and ensuring healthy and clean marine and freshwater ecosystems.

The global spread of antimicrobial resistant organisms and genes pose one of the most serious threats for human health and well-being. Tackling the problem of antibiotic resistances can be only afforded from a **One-Health approach**.

EXPERIMENTS / CASE STUDIES
Experiments on the **ability of marine plasmids to shuttle antibiotic resistances to the food chain** via mariculture products will be performed by our partners at the Israeli National Center for Mariculture, in Eilat.
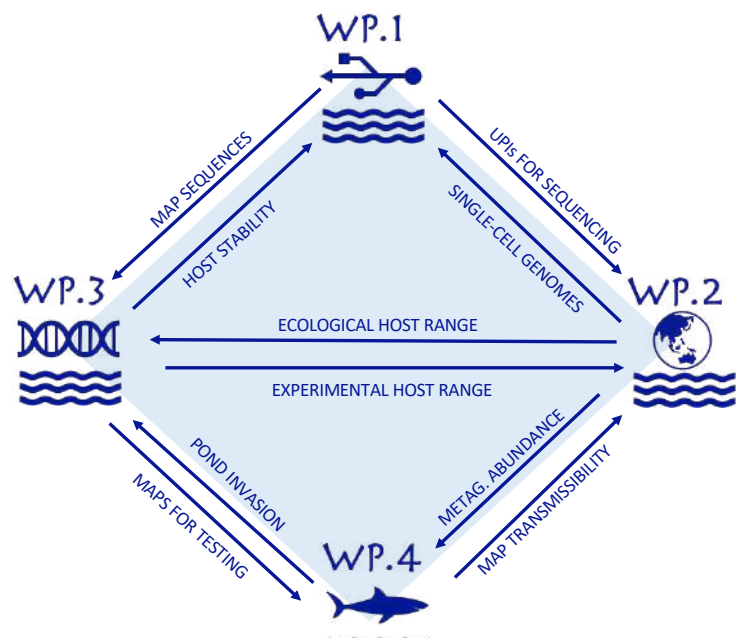

FUNDING INSTITUTIONS
AEI (Spain), BMBF (Germany), CSO-MOH (Israel)

PAIRWISE

Dispersal of antIbiotic resistance and antibiotics in water ecosystems and influence on livestock and aquatic wildlife

PROJECT COORDINATOR

Karl PEDERSEN
National Veterinary Insitute (SVA) - Sweden

PROJECT PARTNERS

Norwegian Institute for Water Research (NIVA) - Norway
—
Linköping University (LiU) - Sweden
—
Estación Biológica de Doñana, Consejo Superior de Investigaciones Cientificas (EBD-CSIC) - Spain
—
National Research Institute for Rural Engineering, Water, and Forestry (INRGREF) - Tunisia
—
Faculty of medicine Ibn Al-Jazzar Sousse (FMS) - Tunisia
—
Makerere University (MAK) - Uganda

DURATION
3 years

STARTING
September 2021

CONTACT
Karl PEDERSEN
karl.pedersen@sva.se

ABSTRACT

PAIRWISE aims to advance knowledge of antimicrobial resistance as a pollution in aquatic environments, wildlife, and livestock. PAIRWISE focuses on **dispersal and dynamics of antibiotic resistant bacteria, antibiotic resistance genes and antibiotics in aquatic environments affected by wastewater treatment plants** (WWTPs).

Overall goals are to understand:
- i) **dispersal of antibiotic** resistant bacteria, antibiotic resistance genes and antibiotics in surface waters downstream of WWTPs,
- ii) **carriage of antibiotic** resistant bacteria and antibiotic resistance genes **in livestock** linked to surface waters influenced by WWTPs,
- iii) **role of aquatic birds in dispersal** of antibiotic resistant bacteria and antibiotic resistance genes.

Issues from the JPI call tackled by PAIRWISE include, but are not limited to entry points and fate of antibiotic resistant bacteria (ARB), antibiotic resistance genes (ARGs) and antibiotics (ATB); identification of ARGs and ARB useful as indicators; providing insight to fate and transport of ARB, ARGs and ATB to, within, and from aquatic ecosystems, and the role of aquatic birds in such events.

PAIRWISE will assess the **influence of antimicrobial resistance in agricultural settings and improve understanding of the dissemination and sustainment of antimicrobial resistance** in the interface between humans, wildlife, and livestock **with a One Health perspective**. It will provide vital knowledge for policy makers and end-users, facilitating informed decisions on mitigation strategies.

OUTCOMES AND EXPECTED IMPACT

The overall aim of PAIRWISE is to provide **insight into the dispersal of antibiotics, antibiotic resistant bacteria and antimicrobial resistance genes downstream of WWTPs** by comparing different geographical/climatic regions, wastewater management practices and types of water bodies. PAIRWISE will also investigate the potential **influence that WWTPs have on occurrence of antibiotic resistant bacteria and antimicrobial resistance genes in livestock** downstream of the WWTPs.

Furthermore, by combining state-of-the-art phenotypic and molecular methodologies together with high-resolution telemetry data on animal movements, PAIRWISE will obtain ground-breaking knowledge about how **different point-sources affect occurrence in aquatic birds, and the role birds play in transfer of pollutants between aquatic habitats**, regionally and between countries and continents.

With the involvement of several key stakeholders and associated partners together with a plan of effective dissemination of our results, PAIRWISE provides novel and necessary tools needed to **facilitate the formation of potential mitigation strategies for environmental dissemination of antibiotic resistant bacteria, antimicrobial resistance genes and antibiotics**.

In this respect, PAIRWISE will contribute to the global combat against antimicrobial resistance in a truly **one-health dimension**.

PROJECT STRUCTURE

PAIRWISE has been organised in four integrated work-packages:

WP1 - Coordination, communication, data sharing
WP1 will focus on coordination, communication, data sharing and harmonization of protocols. Harmonized method protocols for PAIRWISE will be develop to ensure that analysis in involved laboratories are done in an equivalent way and that all data produced are comparable.

WP2 - Dispersal of antibiotics, antibiotic resistant bacteria and antibiotic resistance genes in the waterbodies downstream of wastewater treatment plants
WP2 will focus on dispersal of ARB, ARG and ATB in the water bodies downstream of WWTPs and investigate how this dispersal is affected by environmental, geographical, climate and temporal variation. This WP would also study the differences in occurrence and levels between countries that differ greatly in prevalence and types of ARBs and ARGs, and representing countries with low, high, and very high antibiotic usages.

WP3 - Antibiotic resistant bacteria and antibiotic resistance genes in grazing cattle and water
WP3 will focus on the occurrence of ARB and ARG in livestock in contact with surface waters influenced by effluents from WWTPs, to establish if these animals can acquire ARB and ARG from surface waters. The WP3 will be conducted in Southern, Central or Eastern Sweden, in the Doñana Biosphere reserve in South West Spain, and in Uganda.

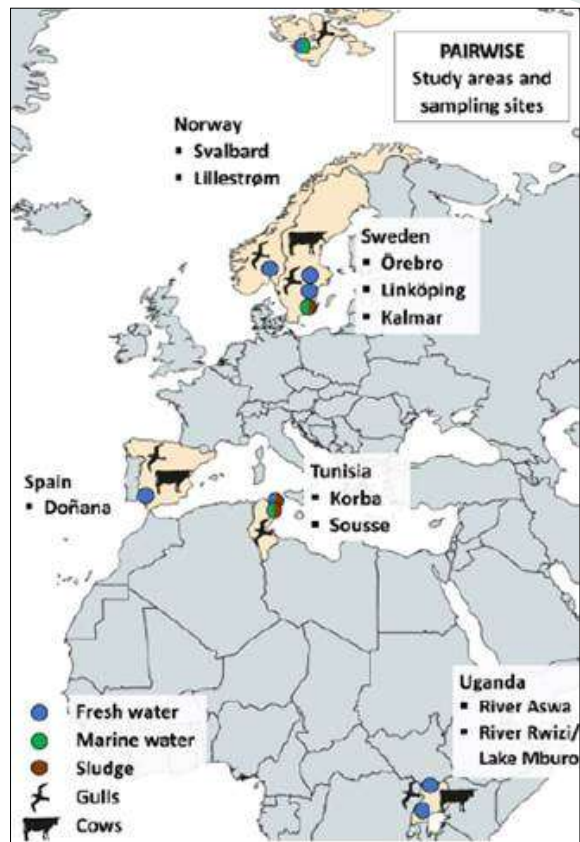### WP4 - Dispersal of antimicrobial resistance through aquatic birds
WP4 will focus on aquatic birds and their role in short- and long-distance dispersal between aquatic environments. The specific objectives are to characterize and quantify AMR (ARB and ARGs) among aquatic birds, identify important environmental pathways of AMR dissemination by birds and model the relative risk of dispersal of AMR through aquatic birds. Data collected will be used for spatial modelling of local and long-distance AMR dispersal pathways via aquatic birds.

PROJECT CONTRIBUTION TO POLICIES

The project will provide science-based proofs on the impact of effluents discharge on the dispersal of ATB, ARB and ARG in the environment which will be very useful for **setting future regulations on pollutants release in water and the development of tools and indicators for assessing environmental quality and impact of wastewater**.

The project contributes to the achievements of **UN SDG 6** (Clean Water and Sanitation).

EXPERIMENTS / CASE STUDIES

PAIRWISE includes an extremely intensive sampling of water, upstream and downstream of wastewater treatment plants, wild birds, and livestock grazing on grasslands.

These samples will be surface water samples, and faecal samples from birds and livestock. This sampling will take place at 10 different study areas located in **Uganda** (2 sites), **Tunisia** (2 sites), **Spain** (1 site), **Sweden** (3 sites), and **Norway** (2 sites), including both **freshwater** (lakes, lagoons, rivers, streams, and fjords) and **marine water** (Arctic Ocean/Barents Sea, Inner Oslofjord, Baltic Sea, Mediterranean Sea) located upstream and/or downstream of WWTPs, groundwater, hospital effluents will be collected for analysis.

The samples will be investigated for antibiotic resistant bacteria and antibiotic resistance genes – qualitatively and quantitatively, and the water samples will be examined for antibiotics and biocides.

PAIRWISE Study areas and sampling sites

FUNDING INSTITUTIONS
AEI (Spain), FORMAS (Sweden), MHESR (Tunisia), RCN (Norway), SIDA (Sweden), SRC (Sweden)

PARRTAE

Probing Antibiotic Residues and Resistance transfer in Aquatic Environments

PROJECT COORDINATOR

Åsa SJÖLING
Karolinska Institutet - Sweden

PROJECT PARTNERS

Universidad de Las Palmas de Gran
Canaria - Spain
—
ILVO Technology & Food - Belgium
—
Norwegian University of Science and
Technology (NTNU) - Norway
—
Swedish Universtiy of Agricultural
Sciences - Sweden

DURATION
3 years

STARTING
September 2021

CONTACT
Åsa SJÖLING
asa.sjoling@ki.se
Jonatan MARTIN RODRIGUEZ
jonatan.martin.rodriguez@ki.se

ABSTRACT

The project aims to **study bacteria, antibiotic resistance genes (ARGs) and antibiotic residues in groundwater, surface water, marine water environments** in the North Sea and Atlantic including ports and aquaculture facilities.

Samples will be collected from sites with high and low suspected loads of antibiotic residues. Culture-based and sequencing-based methods will be used to identify ARGs.
Transfer of ARG-containing plasmids will be analysed using indicator bacteria *Escherichia coli, Vibrio spp*, and *Shewanella algae* as recipients. LC-MS/MS will be used to quantify the levels of different antibiotics in the collected samples. We hypothezise that local water microbiota, antibiotic residues and recipient species will affect the type of plasmids transferred.

The effects of the acquired plasmids on the physiology of our bacterial models will be analysed in experiments lab systems, and their influence on fitness and virulence in a live host will be studied by host interactions in a shrimp (*Artemisia*) model.

The project will **determine common ARG plasmids circulating in European waters** and their inherent properties as a fundament to understand and prevent their dissemination.

OUTCOMES AND EXPECTED IMPACT

The impact of circulating ARG plasmids and antibiotic residues and their contribution to the emerging resistance problem has only begun to be understood.

This project aims to **detect emerging plasmids and other mobile elements and their corresponding bacterial hosts** by joining microbial expertise from the health, aquaculture, freshwater, and marine sectors.

We aim to **determine and measure the behavior of important ARG plasmids, corresponding genes and bacterial hosts in water and over time**, and experimentally analyze the driving sources, including antibiotic residues, behind the preponderance of such plasmids.

The PARRTAE project will:
- Provide a **broad overview of relative frequencies of ARGs** in different bacterial species in water environments.
- **Correlate the presence of antibiotic residues** to the presence and frequency of ARG indicator bacteria.
- **Determine occurrence and frequency of different ARG plasmids and their transferability** to recipient strains.
- **Determine the impact of plasmid gene content** on transfer frequencies, fitness and impact on water residing eukaryotic hosts using an *Artemisia* model.

PROJECT STRUCTURE

The PARRTAE work plan is comprised of five work packages:

WP1 – Monitorization of antibiotic resistance

The objective is to measure levels of ARG bacteria and antibiotic residues in a diverse set of water samples collected from sites with low and high levels of antibiotic residues and/or other types of contamination, to compare pristine areas with contaminated locations. Collection of bacterial isolates from water microbiota in different environments, isolation of strains followed by whole genome sequencing and metagenome analyses will be performed. Isolates will be analysed for ARGs and antibiotic residues will be measured from the water environments. The influence of antibiotic residues on microbiota will be analysed in controlled water microcosms.

WP2 – Sequencing and analysis of plasmid

Antibiotic resistance can emerge by mutations in target genes or by acquisition of ARGs. The most important concern in antibiotic resistance is the spread of ARGs by plasmids. In WP2, we will determine the type of plasmids transferred from different environments for transmission frequency and ARG profiles. Analysis of ARG isolates and their ability to transfer ARG plasmids to recipients (marker species *E. coli, S. algae and Vibrio spp*) will be performed. The plasmid content, and its methylome with impact on transfer frequencies will be analysed.

WP3 – Experimental analyses on fitness by transferred plasmids

In WP3, the interactions between the *E. coli, Vibrio* and *Shewanella* strains with the plasmids/resistant genes and the animal hosts will be investigated. Plasmid fitness and virulence will be analysed using a host model of *Artemisia*. Gene deletions of target genes involved in plasmid transfer and host adaptation and host immune responses will be analysed.
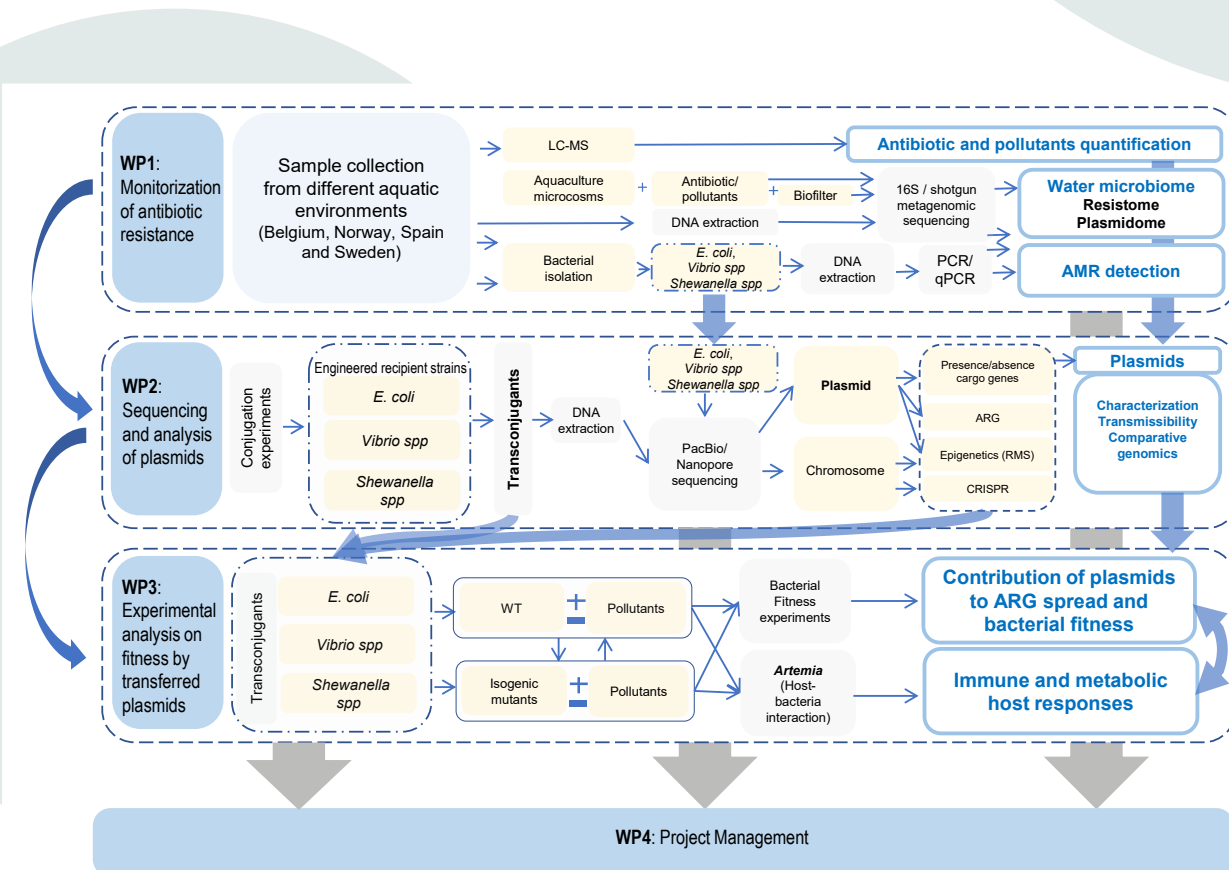
WP4 – Project Management

This WP is dedicated to project management.

PROJECT CONTRIBUTION TO POLICIES

The outputs of PARTAAE project on **prevalent and emerging plasmids and bacteria /pathogens** is of interest for policymakers from EU and outside EU.

The project and results can have implications for water management, agriculture and human health. They will contribute to **UN SDG 3** (Good Health and wellbeing), **6** (Clean water and sanitation) and **14** (Life below water).

FUNDING INSTITUTIONS

AEI (Spain), BELSPO (Belgium), RCN (Norway), SRC (Sweden)

SARA

Surveillance of Emerging Pathogens and Antibiotic Resistances in Aquatic Ecosystems

PROJECT COORDINATOR

Prof. Dr. Andreas TIEHM
DVGW-Technologiezentrum Wasser
(TZW) - Germany

PROJECT PARTNERS

Universitat de Barcelona (UB) - Spain

—

Ministry of Health (PHLTA) - Israel

—

École Centrale de Lyon, Laboratoire Ampère - France

—

European Union Reference Laboratory for Foodborne
Viruses, Swedish Food Agency (SFA) - Sweden

—

Universidade Lisboa,
Instituto Superior Tecnico (IST) - Portugal

—

Mbarara University of Science and Technology
(MUST) - Uganda

—

Eduardo Mondlane University (EMU) - Mozambique

DURATION
3 years

STARTING
September 2021

CONTACT
Prof. Dr. Andreas TIEHM
Andreas.Tiehm@tzw.de

ABSTRACT

Appropriate methods for **wastewater-based epidemiology** (WBE) and a better understanding of the fate of pathogenic viruses and antibiotic-resistant bacteria from the sources to river basins and estuaries are urgently required. Our project will determine the **prevalence of pathogenic viruses** (including SARS-CoV-2), **microbial indicators, antibiotic resistance, and microbial source tracking** (MST) markers in wastewater, surface water, coastal sea waters, sediment and bivalve molluscan shellfish (BMS) in catchments located in different climate areas (Sweden, Germany, France, Spain, Portugal, Israel, Mozambique, and Uganda).

The project aims are:
- (i) **method harmonization and training** of European and African partners,
- (ii) **SARS-CoV-2 detection in raw wastewater** as a biomarker of COVID-19 cases,
- (iii) enteric viruses, antibiotic resistances and MST **markers monitoring in aquatic environments**,
- (iv) **evaluation of sediments and BMS** as integral reservoirs,
- (v) determination of the **impact of climate and extreme weather events**, and
- (vi) **microbial risk assessment** for water resources.

Results and recommendations will be transferred to the scientific community by peer-reviewed papers and conference presentations. International health and environment organisations as well as authorities and waterworks that represent end-users on a global, European and African level will participate in the Stakeholder Forum.

OUTCOMES AND EXPECTED IMPACT

In the framework of the project, the **spread of viruses, antibiotic resistance bacteria (ARB) and antibiotic resistance genes (ARGs) and MST markers** from wastewater, WWTP effluent, streams and rivers to the sea will be evaluated. Culture, molecular biology and metagenomics methods will be applied.

Since the environmental and geographical conditions will influence the spread of the emerging pathogens in the environment, **ecosystems from different and well-defined geographical areas** will be sampled and investigated. **Northern region** with wet and temperate-cold climate and abundant rainfall (Sweden) Regions with continental climate characterised by a significant annual variation in temperature (hot summers and cold winters) and moderate amount of rainfall (Germany and France).
Southern European region with seasonal changes from humid to semi-arid conditions and alternating periods of rain and water scarcity (Spain, Portugal). Regions characterized by **tropical climate** with a dry, cooler season and a wet, hot season (Uganda, Mozambique), and semi-arid regions (Israel).

Innovative methodologies and tools will be used to help policymakers **develop more effective policies and efficient regulations**.

The results will help to **unravel the pathways involved in the spread of viruses and antibiotic resistance in the environment and facilitate risk assessment and mitigation measures** to evaluate and reduce human exposure.
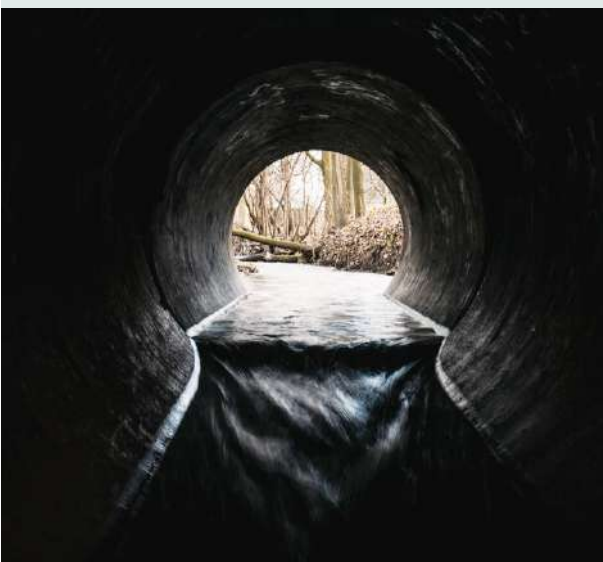
PROJECT STRUCTURE

The SARA project is structured into 9 different work packages:

WP1: Project coordination
The Management of the consortium, the organization of project meetings, and creation and organization of an external Advisory Board and Stakeholder Forum will be performed in WP1.

WP2: Harmonization of sampling and analysis
WP2 aims to define the best practices for sampling, the standard operation protocols for the analysis of cultural parameters, and to determine the sample preparation for the molecular biological methods.

WP3: SARS-CoV-2
WP3 is focussed on the monitoring of SARS-CoV-2 in wastewater and in surface water, the investigation of its infectivity, and the refinement and implementation of a wastewater-based epidemiology model to estimate total infections (symptomatic, pre-symptomatic, asymptomatic and undiagnosed shedders) in the population.

WP4: Enteric viruses
In WP4, we will evaluate and compare the presence of the selected enteric viruses with bacterial indicators in the different sampling locations and water matrices. Furthermore, the effect of extreme events (prolonged periods of drought or heavy rain) on the deterioration of water quality will be investigated.

WP5: Antibiotic resistance
A major aim of WP5 is to study the dynamics of ARGs and ARB in aquatic environment in Europe and Africa and evaluate effects of climatic conditions. The prevalence of key ARGs will be also evaluated in non-bacterial fractions from the water environment (extracellular DNA and bacteriophages).

WP6: Microbial source tracking
In WP6, the study of the occurrence and fate of MST markers in different aquatic environments will allow to assess the relationship with SARS-CoV-2, enteric viruses and antibiotic resistances. Potential new MST markers based on digital metagenomic analysis will also be identified.

WP7: Sediment and BMS for integrated monitoring
In WP7, Bivalve molluscan shellfish (BMS), as well as sediments, will be investigated and monitored for viruses, *E. coli* bacteria, MST markers and antibiotic resistance in order to use their integrative characteristics.

WP8: Risk assessment and recommendations
WP8 is dedicated to the implementation of a risk assessment and provision of recommendations for monitoring parameters.
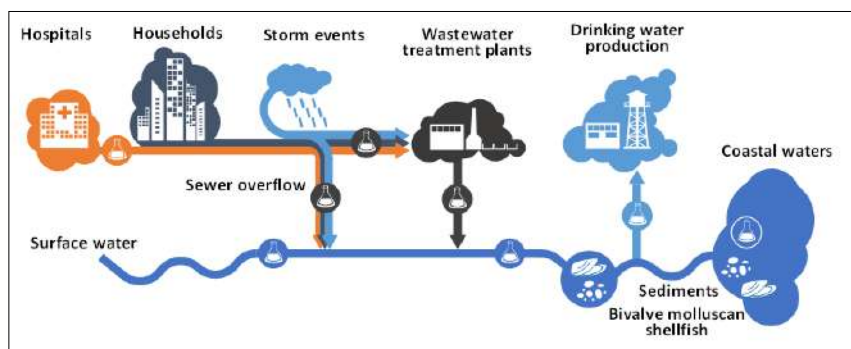
WP9: Training and dissemination
In WP9, various measures will be taken to implement the capacity building activities.

PROJECT CONTRIBUTION TO POLICIES

The project contributes to **a better understanding of microbiological water quality**.

Recommendations for monitoring key parameters, sampling sites and conditions will be developed. Results will be available for implementation of **national and European regulations**, such as the **Water Reuse** and **the Drinking Water Directives**. Trainings and dissemination on international level support the UN SDGs, in particular **UN SDG 3** (Good Health and well-being), **6** (Clean water and sanitation).

EXPERIMENTS / CASE STUDIES

Sampling campaigns will be performed in:
Göta Älv River, **Sweden** - Infulene River, **Mozambique** - Llobregat River, **Spain** - Rhine River, **Germany** - Rhône River, **France** - Rwizi River, **Uganda** - Tagus River, **Portugal** - Yarkon Stream, **Israel**

Raw wastewater, Wastewater Treatment Plants effluent, receiving surface waters including water for drinking water production and coastal seawaters as well as sediment and bivalve molluscan shellfish (BMS) will be sampled.

FUNDING INSTITUTIONS

AEI (Spain), ANR (France), BMBF (Germany), CSO-MOH (Israel), FCT (Portugal), FORMAS (Sweden), SIDA (Sweden)

SPARE-SEA

Environmental Spread and Persistence of Antibiotic Resistances in aquatic Systems Exposed to oyster Aquaculture

PROJECT COORDINATOR

Dr. K. Mathias WEGNER
Alfred Wegener Institute – Helmholtz centre for Polar and Marine Research - Germany

PROJECT PARTNERS

CNRS, Ifremer, Université de Montpellier, Université de Perpignan - France

—

National Research Council (CNR) - Italy

—

DISTAV, University of Genova - Italy

—

Insitut De Reverca I Technologia Agroalimentaria (IRTA) - Spain

DURATION
3 years

STARTING
September 2021

CONTACT
Dr. Karl ANDREE
karl.Andree@irta.es

ABSTRACT

Aquaculture has been identified as a gateway for antibiotic resistance (AR) spread, but little is known of AR in the oyster aquaculture environment. The biggest oyster aquaculture industry cultivates the Pacific oyster *Crassostrea gigas*, which is cultured in marine coastal areas that are often contaminated by AR determinants (antibiotics, resistance genes, and resistant bacteria) and other pollutants.

Moreover, antibiotics are used in hatcheries, and since oysters accumulate bacteria, c**onsumption of raw oysters can be a vector for AR into human microbiomes**. Also AR transmission to other species threatens the safety of coastal marine systems, the sustainability of shellfish farming and human health.

By combining human, animal and environmental health, SPARE-SEA implements a **One Health approach** to **identify environmental drivers and pathways of AR spread within and between environmental compartments** including known and emerging pathogens.

By **investigating the cumulative effects of human use of coastal ecosystems** along multiple gradients (e.g oyster farming intensity and agrochemical pollutant run-off) on the enrichment of AR in the oyster bio-reactor and its subsequent transfer routes within anthroposized coastal environments, we will link objectives of all three JPIs involved.

OUTCOMES AND EXPECTED IMPACT

The scientific output of SPARE-SEA will determine the future research lines in the field of AR in bivalve aquaculture by defining the impact of oysters' production on the surrounding environment, and by assessing **the effect of specific anthropogenic pressures on oyster aquaculture** on the EU scale.

SPARE-SEA perfectly covers important open questions and scientific needs in all the three JPIs involved in the call, enhancing innovation, integration and transfer of new and EU standardized knowledge.

As a more far-reaching impact, SPARE-SEA is the **first intra-EU action for an integrated analysis of the impact of oyster farming on the spread and selection of AR** in EU coastal waters and in oyster aquaculture.

We will not only target the microbiomes and their related resistomes and pathobiomes, but specifically target established and emerging animal and human pathogens commonly found in oysters as potential carriers of ARGs.
This will provide a comprehensive signal of the **risk associated to human and ecosystem health** by comparing the "environmental resistome" in several environmental compartments (e.g. water, sediment, particles) to the presence of ARB in the food product, thereby applying a "**one-health approach**" to the problem.

SPARE-SEA is definitely providing the necessary **knowledge to develop oyster farming while reducing AR in coastal waters, and improving public health and animal farming.**
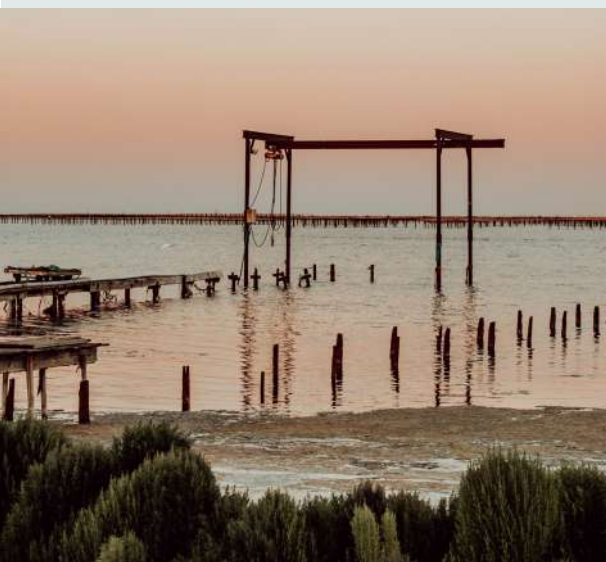
PROJECT STRUCTURE

The SPARE-SEA work plan is comprised of four work packages:

WP1 - Characterization of the oyster resistome
WP1 will decipher the baseline resistome diversity in microbial communities associated with oyster aquaculture, by analysing and comparing the presence of antibiotic resistance genes (ARGs) and antibiotic resistant bacteria (ARB) in whole tissue homogenates of oysters collected from four main geographic areas important for oyster culture that are impacted differently by anthropogenic influences.

WP2 – Cumulative effects of human use
Coastal areas highly exposed to contaminants (due to discharges of chemicals) are considered hotspots of ARB emergence as they increase selective pressure for maintenance of ARGs and/or increase the rate of ARG transfer through mobile genetic elements. These ARGs could concentrate in oysters (filter-feeders), which are cultured in highly anthroposized environments and accumulate both chemicals and microorganisms. WP2 will monitor ARBs/ARGs in regions differentially exposed to contaminants due to human exploitation of ecosystems.

WP3 – Experimental ARG enrichment
WP3 will employ an experimental approach to assess whether horizontal transfer of ARG is enhanced in the oyster bioreactor and how the cumulative effects of antibiotics and agrochemicals (heavy metals from vineyards, pesticides from rice culture) can select for the spread of ARGs.
We will conduct experiments exposing different life stages (larvae, adults) to antibiotics and contaminants for which ecological relevant concentrations will be determined in pilot experiments. Enrichment experiments will be conducted on different sources of oysters.

WP4 - Oyster ARGs in a One Health approach
Within a One Health paradigm it is necessary to determine where, when and why ARBs circulate in ecosystems exploited for oyster culture. For that, we need to evaluate ecosystems and their composite parts.
Samples from terrestrial and aquatic origin will be analyzed to identify common sources suggestive of direct ARG transfer. Gene flow through the habitat can be characterized in this way and the areas where anthropogenic forcing factors can be minimized or possibly eliminated will be identified so as to provide guidance for potential future legislation.

PROJECT CONTRIBUTION TO POLICIES

SPARE-SEA project will assess how clean and healthy coastal aquatic ecosystems are influenced by oyster aquaculture activities in terms of ARG transfer that can cross the human-animal barrier easily by oyster consumption.
The output of SPARE-SEA can turn **farming strategies into more sustainable procedures strengthening the competitiveness and growth of oyster producers** and their future access to European and global markets (see **EU Farm to Fork Strategy**).

It also offers knowledge and management tools to public administrators and civil society organisations, fitting into the guidelines of the new **European Green Deal** for a sustainable society as well as in the **UN SDG 3** (Good Health and well-being), **6** (Clean water and sanitation) and **14** (Life below water).
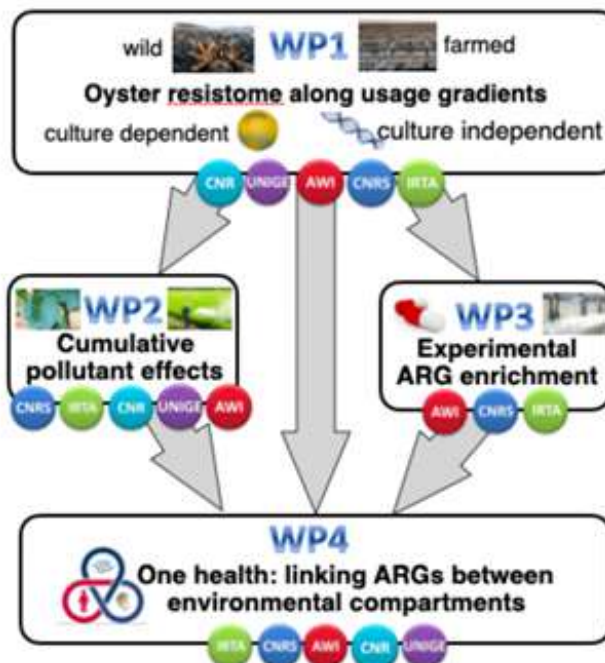
FUNDING INSTITUTIONS
AEI (Spain), ANR (France), BMBF (Germany), MUR (Italy)

EVALUATING

Risk assessment and management of CECs, pathogens and antimicrobial resistant bacteria from aquatic ecosystems (inland, coastal and marine) to human health and environment

AIHABs

Artificial Intelligence-powered Forecast for Harmful Algal Blooms

PROJECT COORDINATOR

Dr Ahmed NASR
Technological University Dublin
(TU Dublin) - Ireland

PROJECT PARTNERS

Norwegian University of Science and Technology
(NTNU) - Norway

—

Helmholtz Centre Potsdam - German Research
Centre for Geosciences (GFZ) - Germany

—

University of South Bohemia in České Budějovice
(USB) - Czech Republic

—

International Iberian Nanotechnology Laboratory
(INL) - Portugal

—

Universidad Autónoma de Madrid (UAM) - Spain

—

University of Santiago de Compostela - Spain

DURATION
3 years

STARTING
September 2021

CONTACT
Marcos Xosé ÁLVAREZ CID
marcos.alvarez@ntnu.no

ABSTRACT

Eutrophication of water bodies in Europe is contributing to the increase of Harmful Algal Blooms (HABs) which poses serious risk to human health. To address this problem, the AIHABs project will develop an **early warning system to forecast the occurrence, spread and fate of cyanotoxins caused by HABs in inland and coastal waters**, using Artificial Intelligence (AI) and the latest innovations in mathematical modelling, nanosensors, and remote sensing.

The novelty of this project lies in merging these tools with the joint purpose of providing an early warning system to decision-making authorities in terms of risk to the public. The model predictions will allow timely action to minimise the risks of consuming surface waters or using them as recreational resources when the waterbodies are prone to produce toxic cyanobacterial blooms.

A number of candidate sites with a history of HABs in the countries of the project partners will be evaluated using multi-criteria analysis in order to identify the most suitable inland and coastal water sites for use in the study. The main criteria for selecting the sites will be the availability of the required data for modelling and the strong evidence of historical HABs.

OUTCOMES AND EXPECTED IMPACT

HAB episodes generally pose great risk to freshwater and marine water and their manifestations are correlated to a significant impact on human health and socioeconomic uses of water bodies. Mitigating the HABs risk falls under the emerging area of environmental impact of chemicals. Addressing this area will both **protect human health and deliver on the zeropollution ambition for a toxin-free environment** proposed in the 2019 European Green Deal. Therefore the need for further research to **better understand the lifecycle of chemicals in the environment and to establish the links between chemicals in the environment and human health and wellbeing** has been prioritised.

The main outcome of the AIHABs project is to produce **a new Artificial Intelligence (AI)-based software for forecasting occurrence and spread of Harmful Algal Blooms** (HABs) in inland and coastal water bodies. Developing a tool to predict HABs will contribute to the HABs risk assessment. The development of methods for early detection and predictive models would allow resource managers time to respond more effectively to potentially harmful conditions.

Furthermore, the **modelling results and all data** required to calibrate and validate the tool will provide **good understanding of the governing processes and the pathways of pollution** by measuring and evaluating the resultant HABs.

In addition any mitigation scenarios to reduce the point source and the diffuse source pollution loads from the discharging catchment could be assessed using the **HABs developed models for inland and coastal waters**.
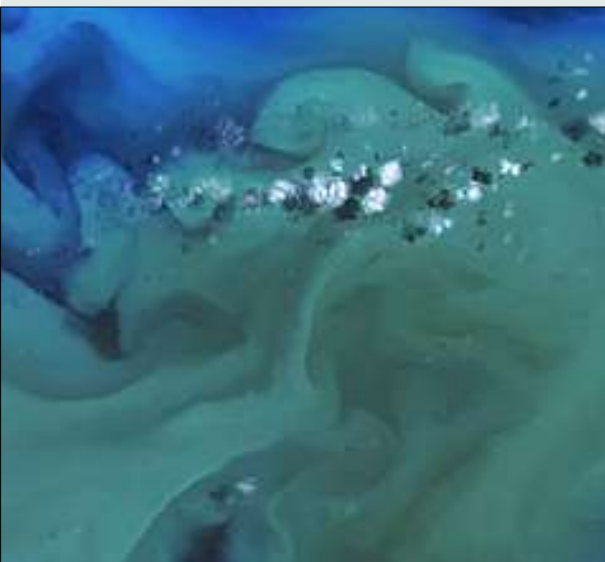
PROJECT STRUCTURE

The AIHABs work plan is comprised of seven work packages:

WP1: Project management and coordination
WP1 deals with all management activities of the project.

WP2: Characterisation of cyanobacteria
In this WP the cyanobacterial materials, both in the field and in laboratory cultures, will be characterised and provided to other WPs. Risk assessment of the proliferation of cyanobacteria will also be identified in the selected study sites which include inland and coastal water bodies.

WP3: Inland water and coastal modelling
Modelling systems will be applied to the inland water body (the MIKE11 model) and to coastal water body (the MIKE3FM model) of the selected study sites. For the inland water body, a hydrological modelling of water catchment will be followed by water quality modelling of parameters contributing to the growth of cyanobacteria in the aquatic environment. For the costal water, a hydrodynamic model will simulate the tidal and flow patterns and water quality simulations will simulate the transport and fate of toxins in these coastal waters.

WP4: Sensors
WP4 will develop and test of new efficient and fit-for-purpose tools for the early monitoring of toxic cyanobacterial blooms in coastal and freshwaters. A combined approach of remote and in-situ sensing will be performed to 1) adapt a portable immunosensing system for the monitoring of total microcystin in coastal and freshwater bodies, 2) develop a portable lab-on-a-chip electrochemical system for the identification and quantification of saxitoxin and 3) set up a hyperspectral imaging framework and pipeline to monitor the bioprocesses driven by cultured cyanobacteria.

WP5: AI-based water quality forecast
WP5 will develop all the activity necessary to set up an early warning system based on Artificial Intelligence (AI) techniques, such as Machine Learning (ML) and Deep Learning (DL). A repository management tool and user interfaces will be installed to collect and store all information, and data from other work packages. An advanced software allowing integration of the different subsystems involved in AIHABs to deliver the forecast result will be used.

WP6: Integration and validation
WP6 will integrate the results, procedures and approaches generated by the different WPs. Every result or designed procedure will be validated separately before validation of the complete integrated platform.

WP7: Communication, Dissemination & IPR
WP7 will deal with the strategic communication and dissemination of the project.

EXPERIMENTS / CASE STUDIES

An inland and a coastal water bodies showing frequently cyanobacterial and cyanotoxins will be selected from the following potential water bodies in order to validate and calibrate models for the AI- powered forecast system:

- **Minho River Estuary** (International waters) and upstream drinking water reservoirs (Belesar / Vila-souto) (PT, ES)
- **Guadiana River Estuary** (International waters) and upstream drinking water reservoirs (Alqueva/ Alange) (PT, ES)
- **Orlík and Švihov water reservoirs** (CZ)
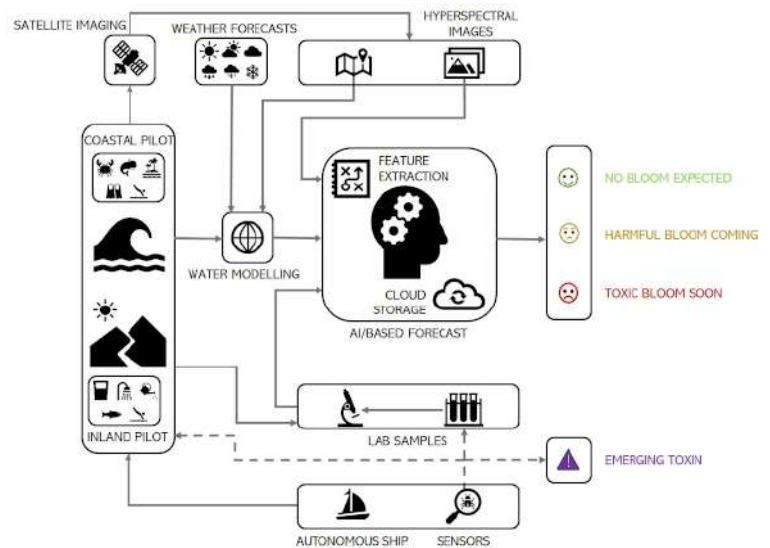- **Blackwater River Estuary** (IE)

PROJECT CONTRIBUTION TO POLICIES

The AI- tool will be utilised for monitoring the HABs in the freshwater and the marine water and this will enhance the capability of the **EU Water Framework Directive**, **EU Marine Strategy Framework Directive** and the **EU Bathing Water Directive**.

HABs data delivered by this project will play significant role for the **River Basin Management Plan**, the **OSPAR Convention** and the **EU Habitat Directive**.

Furthermore, the results of AIHABs project will directly contribute to the **UN SDG 6** (Clean Water and Sanitation) by addressing the negative impact on the water quality, **UN SDG 14** (Life Below Water) by quantifying the impact of anthropogenic factors on the HABs occurrence and spread in the marine water and **UN SDG 13** (Climate Action) through a powerful modelling framework to assess climate change impact on HABs.

FUNDING INSTITUTIONS

AEI (Spain), BMBF (Germany), EPA (Ireland), FCT(Portugal), RCN (Norway), TACR (Czech Republic)

BIOCIDE

Antibacterial biocides in the water cycle - an integrated approach to assess and manage risks for antibiotic resistance development

Aquatic Pollutants

PROJECT COORDINATOR
Joakim LARSSON
University of Gothenburg - Sweden

PROJECT PARTNERS
Institute of Marine Research, contaminants and biohazards - Norway

—

Federal Institute for Materials Research and Testing (BAM), Materials and Environment - Germany

—

Umeå University, Department of Chemistry - Sweden

—

Technical University of Denmark - Denmark

—

University of Bucharest - Romania

—

University of South Bohemia, Faculty of Fisheries and Protection of Waters - Czech republic

DURATION
3 years

STARTING
September 2021

CONTACT
Kristian KVINT
kristian.kvint@gu.se

ABSTRACT

The overall aim of BIOCIDE is to **determine how antibacterial biocides** (i.e., chemicals with antibacterial properties that are not used for treating infections) **contribute to the development and spread of antibiotic resistant bacteria in different aquatic/marine ecosystems**, and to **inform and enable measures that ultimately protects human health and safe water resources** for both humans and wildlife.

Generated data will include:
- exposure levels in different matrices,
- concentrations that are likely to co-select for antibiotic resistance and promote horizontal gene transfer,
- identification of predominant and novel genetic mechanisms for co-selection, as well as
- a risk assessment.

The knowledge created and its impact will reach well beyond the European setting. We will provide **means to guide action** both at the source (approval), and in other parts of the water cycle. Predicted No Effect Concentrations and new methodology will facilitate possible future inclusion in regulatory systems, in Europe and elsewhere.

The maritime sector will receive guidance to improve sustainable transports by a **better understanding of potential human health risks associated with the use of antifouling agents**. The research has high relevance for all three JPIs and for several themes within the call, particularly those related to risk assessment and management.

OUTCOMES AND EXPECTED IMPACT

Key outcomes of BIOCIDE will be :
- the **identification of biocides associated with high risk for promoting the development of antibiotic resistance**,
- a **risk evaluation scheme** for biocides based on concentrations that may drive resistance,
- **suggested incorporation** of this scheme **into the Biocidal Products Regulation, and**
- a **web-based resource for biocide resistance and antibiotic co-selection potential** building on the existing BacMet database infrastructure.

The results generated by BIOCIDE project will provide means to guide action both at the source (approval), and in other parts of the water cycle. Standardization of methodology will facilitate possible future inclusion in regulatory systems in Europe and elsewhere. Here, the involvement of stakeholders, particularly regulatory bodies, in the process is important.

The maritime sector will receive guidance to improve sustainable transports by a better understanding of potential human health risks associated with antifouling agents (also used in e.g. fish farming). While our ultimate goal is to protect the ability to prevent and treat bacterial infections using antibiotics, the generation of analytical tools, environmental exposure data and effect data for a range of microorganisms will also inform risks for ecological effects.

PROJECT STRUCTURE

The BIOCIDE project is divided into five work packages:

WP1 - Exposure
The main objective of WP1 is reflected by its three tasks:
- Prioritize antibacterial biocides to be investigated based on existing databases on sales, uses and detection in aquatic environments
- Develop state-of-the art chemical analysis protocols for a range of antibacterial biocides, applicable to different sample types, and
- Generate screening data for the presence and levels of antibacterial biocides in different environmental matrices from a set of different aquatic ecosystems in Europe and Africa.

WP2 - Effects
The main objectives will be:
- Generating a dose-response matrix for a large number of biocides and bacterial species in order to obtain Predicted No Effect Concentrations (PNECs) for growth that will also be protective against antibiotic resistance co-selection,
- Directly assesssing co-selection by competition experiments in complex aquatic communities using a subset of biocides, and
- Quantifying the potency of selected biocides to induce horizontal transfer of antibiotic resistance genes via conjugation and transformation.

WP3 - Mechanisms
This WP will allow to:
- Uncover the incidence and mechanisms of antibiotic resistance driven by metal-based antifouling agents used in international maritime traffic and marine aquaculture,
- Provide a first description of the predominant co- and cross-resistance mechanisms in bacterial isolates from aquatic ecosystems in Europe and Africa,
- Identify the genetic context and basis for resistance to several biocides using a functional (meta) genomics approach combined with exploration of public genomic data.

WP4 – Synthesis
The main objectives of WP4 are reflected in its three tasks
Incorporating extensive data on selective concentrations and co-selection opportunities into the BacMet database to make it useful for practical risk assessment and management
- Performing a preliminary assessment of risks for biocides to promote antibiotic resistance in aquatic environments based on generated exposure and effect data,
- Producing an evaluation scheme in collaboration with relevant authorities on how resistance risks formally could be incorporated in existing regulatory frameworks.

WP5 - Coordination
WP5 is dedicated to research coordination and communication activities.

PROJECT CONTRIBUTION TO POLICIES

We foresee that generated data on risks and approaches to assess risks will be of interest to relevant authorities engaged in the **EU Biocidal Products Regulation**, the **Water Framework Directive**, and the **Marine Strategy Framework Directive**.

An evaluation of risks that antifouling agents promote antibiotic resistance, and with that associated risks for human health, will be useful for the maritime sector globally.

The research performed with BIOCIDE will have direct implications for **UN SDG 3** (Good Health and Well-being), **6** (Clean Water and Sanitation), **14** (Life Below Water) and **15** (Life On Land), but indirectly also on a number of other goals.
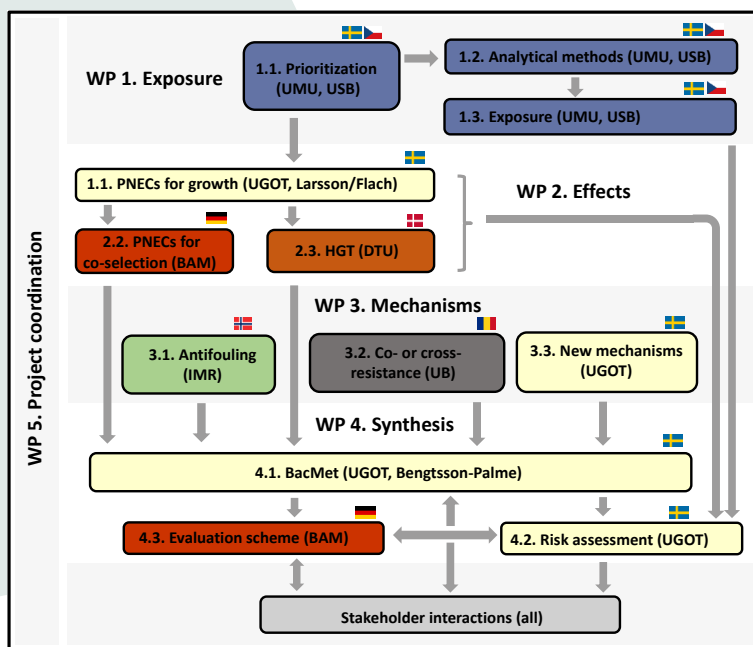
FUNDING INSTITUTIONS
BMBF (Germany), FORMAS (Sweden), IFD(Denmark), RCN (Norway), SRC (Sweden), UEFISCDI (Romania)

CONTACT

Consequences of antimicrobials and antiparasitics administration in fish farming for aquatic ecosystems

PROJECT COORDINATOR

Michael SCHLOTER
German Research Center for Environmental Health (GmbH)

Helmholtz Zentrum Muenchen - Germany

PROJECT PARTNERS

Technical University of Denmark, Institute for Health Technology - Denmark

—

University of Campinas; Institute for Analytical Chemistry - Brazil

—

École Centrale de Lyon / Université de Lyon; Laboratoire Ampère - France

—

The National Center for Mariculture, Microbiology and Water Quality – Israel

DURATION
3 years

STARTING
September 2021

CONTACT
Baerbel FOESEL
baerbel.foesel@helmholtz-muenchen.de

ABSTRACT

Aquaculture is an important source for food, nutrition, income and livelihoods for millions of people around the globe. **Intensive fish farming is often associated with pathogen outbreaks** and therefore high amounts of veterinary drugs are used worldwide.

As in many other environments, mostly application of antimicrobials triggers the development of (multi)resistant microbiota. This process might be fostered by co-selection as a consequence of the additional use of antiparasitics. **Usage of antimicrobials in aquaculture** does not only affect the cultured fish species, but – to a so far unknown extent - also **aquatic ecosystems connected to fish farms** including microbiota from water and sediment as well as its eukaryotes. Effects include increases in the number of (multi)resistant microbes, as well as complete shifts in microbial community structure and function. This dysbiosis might have pronounced consequences for the functioning of aquatic ecosystems.

Thus in the frame of this project we want to study **consequences of antimicrobial/-parastic application in aquaculture** for the cultured fish species as well as for the aquatic environments. To consider the variability of aquaculture practices worldwide **four showcases** representing typical systems from the tropics, the Mediterranean and the temperate zone will be studied including freshwater and marine environments. For one showcase a targeted mitigation approach to reduce the impact on aquatic ecosystems will be tested.

OUTCOMES AND EXPECTED IMPACT

The aim of CONTACT project is to **improve our understanding on the consequences of antimicrobials on Antibiotic Resistance Gene (ARG) emergence and dissemination from aquaculture activity into the aquatic ecosystems** with special respect to major routes of entry, hotspots of emergence and spread and potential co-selection e.g. due to additional application of antiparasitics.

The identification of metabolites and depletion of the antimicrobials in fish tissues will also be assessed using radiolabeled drugs in model studies. Thus the project will address questions related to the **spread of AMR as well as shifts in the microbiomes and food web structures**, both issues which are in line with the **One Health concept**.

Our working hypotheses include :
1. As a consequence of antimicrobials administration in aquaculture, we expect spread of ARGs and Mobile Genetic Elements (MGEs) into the aquatic environment and throughout all trophic layers.
2. The spread of AMR is accompanied by shifts in the structure and function of free-living microbiomes as well as specific host – microbe interaction patterns.
3. Co-selection of ARGs and MGEs due to administration of antiparasitics and other confounding factors such as disinfectants or heavy metals is likely to happen.
4. Community shifts and reduction of diversity throughout the different trophic levels (e.g. productivity loss of endemic water plants or animals, invasion and prevalence of neophytes and non-native animal species) could occur.
5. A faster spread in warmer climatic regions due to higher turnover and productivity and
6. differences between freshwater and marine systems can be expected.

In depth **understanding of the underlying mechanisms of ARG dissemination from aquaculture** due to veterinary drugs application will **help to pinpoint major threads for the aquatic environment** and **define necessary countermeasures** to be taken for more sustainable and environmentally friendly aquaculture practices.

PROJECT STRUCTURE

The overall concept is based on the idea to **study the effects of common antimicrobials and antiparasitics used in fish farming worldwide in four showcases across different climatic zones**. Considered routes of entry of veterinary drugs into the aquatic ecosystem are directly (from drug losses) or via the farmed animals and their excretions into the waterbody and the sediment, as well as throughout the food chain. The CONTACT work plan is comprised of six work packages:

WP1 - Project coordination
WP1 is dedicated to project management. An efficient coordination of the project is the basis for the success of CONTACT.

WP2 - Residue kinetics of administered drugs
WP2 will allow to assess the residue kinetics of the administered drugs in fish tissues and environmental samples from the field sites. The selected compounds are florfenicol as a model for antimicrobials and emamectin benzoate as an antiparasitic. Investigations will include the parent compounds and/or their metabolites and their behavior in the environment (spread, deposit, degradation).

WP3 - Host microbiomes
This WP will study the effects of the administered antimicrobial and antiparasitic compounds on the cultivated fish species and their associated microbiomes. The health status of cultivated fish will also be assessed.

WP4 - Free-living microbiomes
The consequences of the administered drugs for non target biota in aquatic ecosystems will be assessed. Further confounding factors which could drive the development of multi-resistance patterns of the aquatic microbiome as e.g. disinfectants or heavy metals will be considered where relevant in the monitoring studies.

WP5 - Mitigation strategies
The use of a plant-based biofilters as a possible mitigation strategy to reduce the need for therapeutics administration will be studied.

WP6 - Dissemination
Creating awareness for the consequences of antibiotic use in fish farming for human- and environmental health is the major aim of this work package.

PROJECT CONTRIBUTION TO POLICIES

The results of CONTACT project might **increase the awareness** about the role of antibiotic use in fish farming and could contribute to an **improved understanding of the One Health global collaborative platform**, endorsing human and ecosystem health.

The project will also contribute to **UN SDG 3** (Good Health and well-being), **6** (Clean water and sanitation) and **14** (Life below water).

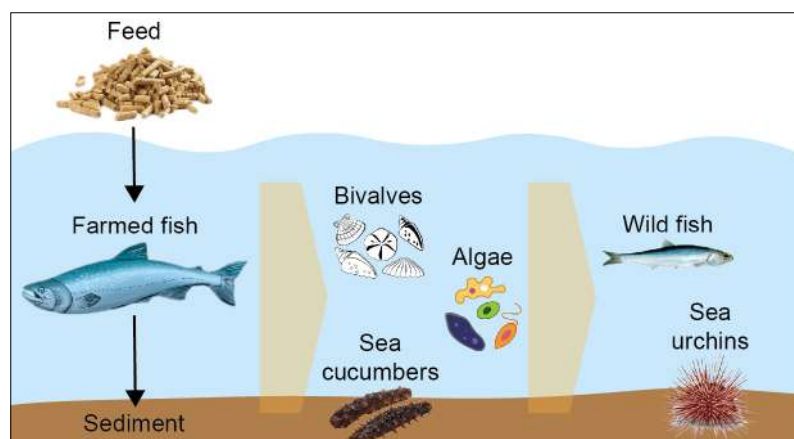# EXPERIMENTS / CASE STUDIES
The four case studies include:
1. **The tropical to subtropical climatic zone (Brazil)** with a focus on freshwater systems and nile tilapia (*Orechromis niloticus*).
2. **The temperate zone (Germany)**, with a focus on trouts -rainbow trout (*Oncorhynchus mykiss*), brown trout (*Salmo trutta fario*)- in freshwater systems.
3. **The Mediterranean region (Israel)**, with gilthead seabream (*Sparus aurata*) and purple sea urchin (*Paracentrotus lividus*) as a model in marine systems.
4. **The temperate-subpolar area (Norway)**, with salmonids (*Salmo salar* and *Salmo trutta trutta*) in marine settings.

Experiments will be performed under controlled conditions. In addition a monitoring program in the mentioned countries for antibiotic residues as well as potential consequences for non target biota will be included.

FUNDING INSTITUTIONS
ANR (France), BMBF (Germany), CSO-MOH (Israel), IFD (Denmark)

PHARMASEA

Presence, behavior and risk assessment of pharmaceuticals in marine ecosystems

Aquatic Pollutants

PHARMASEA

PROJECT COORDINATOR
Prof. Francesco REGOLI
Department of Life and Environmental Sciences -
Polytechnic University of Marche - Italy

PROJECT PARTNERS
University of Heidelberg - Germany

—

University of Stavanger - Norway

—

Instituto Universitario de Medio Ambiente (IUMA)
Universidade da Coruña - Spain

—

Spanish Institute of Oceanography of Murcia
(CSIC) - Spain

DURATION
3 years

STARTING
September 2021

CONTACT
Marica MEZZELANI
m.mezzelani@staff.univpm.it

ABSTRACT

PHARMASEA integrates international expertise to answer key research questions on **fate and biological effects of active pharmaceutical ingredients** (APIs), well recognized contaminants of emerging concern (CECs) for marine ecosystems.

The project will allow **in-depth studies on APIs distribution, effects and risks in four European coastal areas**, targeting:
- occurrence, uptake and trophic transfer along regional marine food webs;
- bioaccumulation/excretion kinetics, potential ecotoxicological effects from molecular to individual levels, and characterization of modes-of-action in model and selected marine species;
- development of specific risk assessment procedures for APIs.

The communication and connection with industries, private and public stakeholders as well as citizen engagement are important aims to promote public awareness, pre-normative research and implementation into European Directives.

The project is expected to have a major impact enhancing the scientific knowledge and awareness on interactions between human and environmental health. The results could influence prescribing and disposal practices of domestic medicines and increase market opportunities and competitiveness of European pharma industries investing on environmental sustainability of their products.

OUTCOMES AND EXPECTED IMPACT

The main tangible outcomes of the project include:
1. an **enhanced scientific knowledge on occurrence, fate and effects of APIs in European coastal ecosystems**, mapping priorities on a large geographical scale;
2. a reliable and flexible **strategy for their detection and monitoring in water systems**;
3. a **list of priority API** and their mixtures in regional coastal environments;
4. a scientifically sound **software-assisted tool for Environmental Risk Assessment.**

Using a collaborative approach and knowledge exchange between project partners and key stakeholders, the project will contribute to counteract the current environmental challenges caused from APIs and to create new sustainable behaviors including the correct disposal of unwanted medicines behavioural and market opportunities. PHARMASEA will **increase the general awareness on interactions between human and environmental health.**

The activities of the project include pre-normative research which might be of utility to develop reliable normative guidelines and appropriate strategies for environmental risk assessment for implementation into European Directives.

Various exploitation pathways can be highlighted, and multiple governmental, economical, scientific and societal end-users are expected to benefit from the project results, such as public authorities and regulatory bodies relevant to European Directives, European Agencies, private sector organizations and industries including pharmaceutical societies, WWTPs operators, water supply companies and aquaculture facilities investing in new technologies to obtain clean and safe water, further promoting its reuse, scientific community and academia in related fields of expertise, civil society and citizens to improve public awareness on the ecological risk of APIs and associated concern.

Water JPI | **JPI OCEANS** | **jpiamr**

PROJECT STRUCTURE

The PHARMASEA work plan is comprised of five work packages:

WP1 – Pharmaceuticals in European coasts
WP1 is based on field sampling activities on a wide geographical range across European Seas to provide qualitative and quantitative characterization of the presence, distribution, fate and behavior of APIs in seawater, sediments and biota. The main objectives are to identify potential sources and transfer of APIs contamination from land to the marine environment, unravelling spatial and temporal variations. Field data, combined with those from previous studies, will allow to define a priority list of APIs in marine ecosystems, and an adequate risk assessment procedure.

WP2 - Effects of APIs on aquatic species
WP2 will mostly be devoted to investigate, under controlled and ecologically relevant experimental conditions, the uptake, bioaccumulation, biotransformation and excretion kinetics of APIs as well as ecotoxicological adverse effects in aquatic, non-target model species. Understanding modes-of-actions of APIs through molecular analyses will help to define the most suitable biomarkers for early detection of exposure and onset of sub-lethal adverse effects.

WP3 - Weighted elaboration and weight-of-evidence (WOE) model
Multidisciplinary investigations based on integration of chemical and biological measurements would represent an added value to monitoring and management of APIs in aquatic environments, and this WP aims to standardize procedures for the elaboration and integration of complex datasets of heterogeneous results as an important basis toward the harmonization of a risk assessment procedure for APIs in marine environments. In this respect, the structure of a quantitative weight-of- evidence (WOE) model developed for traditional chemical pollutants, will provide an already validated theoretical and mathematical framework to be specifically adapted to APIs.

WP4 - Dissemination and stakeholder engagment
WP4 will disseminate project activities and results to different typologies of audiences, ranging from expert scientists, political decision-makers, pharmaceutical industries, citizenships and students. Stakeholder engagement will target relevant European and national bodies, intergovernmental organizations and industries through diverse communication pathways, including specifically-developed strategies and dedicated workshops.

WP5 - Project and data management
WP5 aims to ensure both the achievements of PHARMASEA objectives.

EXPERIMENTS / CASE STUDIES
Main experiments include:
1. **Seasonal field samplings** to characterize APIs distribution in water column, sediments and marine organisms from **Adriatic Sea, Western Mediterranean, Atlantic Ocean, North Sea**;
2. Laboratory studies to evaluate uptake, accumulation and excretion rates of APIs in aquatic invertebrate and fish model species;
3. Laboratory experiments to characterize the onset of effects induced by APIs under ecologically relevant exposure scenarios;
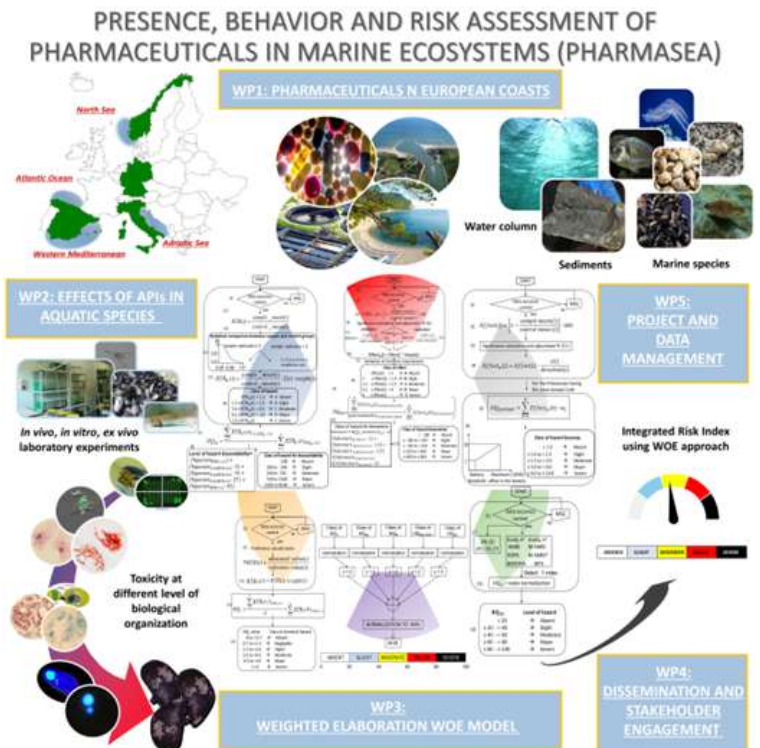4. Laboratory investigation on APIs toxicity in Early Life Stages (embryo and larval) of aquatic species.

PROJECT CONTRIBUTION TO POLICIES

PPHARMASEA outcomes will be useful to public authorities and regulatory bodies relevant to:

1. **European Directives**, including the newly adopted European Green Deal, the European Water Framework Directive, the Marine Strategy Framework Directive, the European Strategic Approach to Pharmaceuticals in the Environment, the Circular Economy Action Plan, the Chemicals Strategy for Sustainability, and the European Bioeconomy Strategy;
2. **Regional Sea Conventions** (Ospar, Barcelona, Helcom & Black Sea Commission, ICES);
3. **UN SDGs**

Sustainable Development

PRESENCE, BEHAVIOR AND RISK ASSESSMENT OF PHARMACEUTICALS IN MARINE ECOSYSTEMS (PHARMASEA)

FUNDING INSTITUTIONS
AEI (Spain), BMBF (Germany), MUR (Italy), RCN (Norway).

THEME 3

TAKING ACTIONS

Strategies to reduce CECs, pathogens and antimicrobial resistant bacteria in aquatic ecosystems (inland, coastal and marine)

AMROCE

Nanoenabled strategies to reduce the presence of contaminants of emergent concern in aquatic environments

PROJECT COORDINATOR

Prof. Tzanko TZANOV
Universitat Politècnica de Catalunya - Spain

PROJECT PARTNERS

Bar Ilan University - Israel
—
SINTEF - Norway
—
University of Milano-Bicocca - Italy
—
Project SAS - Italy
—
Technical University Crete - Greece
—
VTT Technical Research Centre of Finland Ltd - Finland
—
Polymemtech - Poland

DURATION
3 years

STARTING
September 2021

CONTACT
Prof. Tzanko TZANOV
tzanko.tzanov@upc.edu
Dr. Julio BASTOS
julio.bastos@upc.edu

ABSTRACT

Contaminant of emerging concern (CECs) such as antibiotics, pathogens and antimicrobial resistant (AMR) bacteria in water bodies associated to intensive fish and inland animal farming, represent a great threat to the environment and human health.

A common issue with the fish cage nets is the formation of biofoulings and biofilms, acting as a bacteria reservoir and reducing the water flow inside the cages. This implies frequent net cleaning and replacement, or the use of expensive antifouling agents that increase the operating costs of fish farming. **Nano-materials emerged as novel antimicrobial agents with demonstrated efficacy against AMR bacteria**.

AMROCE aims at **reducing antibiotic pollution and spread of AMR bacteria in the entire water cycle** through a platform of novel antibiotic-free antimicrobial products.

AMROCE will **develop antimicrobial/antibiofilm fish cage nets and wastewater filtration membranes** through polymer and surface nano-engineering. Marine-derived antimicrobial agents and antibiofilm enzymes will be nano-formulated as alternative to antibiotics for fish and animal feed supplement.

Human and environmental nanosafety during the manufacturing and use of the novel **nanotechnology-embedded products** will be continuously evaluated to anticipate nanosafety issues.

OUTCOMES AND EXPECTED IMPACT

AMROCE will target the following key performance indicators:
- **20% competitiveness increase in fish and animal farming** at EU level thanks to the manufacturing of high quality and cost-efficient functional products
- **80% reduction of CECs** (antibiotics and AMR bacteria) and 80% reduction of **AMR risk** by tackling the polluting source (livestock and aquaculture industries)
- > 8 log **reduction of the presence of bacteria** in effluents from wastewater treatment plants thanks to the novel water filtration membranes
- 15% reduction of the operation costs of water filtration membranes (WFM)
- **50% lower consumption of antibiotics** in aquaculture and livestock to decrease the antibiotic water contamination
- ≥90% antifouling efficiency of fish cage nets and membranes and reduced cleaning costs.

Novel antibiotic-free antimicrobial strategies will provide competitive advantages over the current remedial approaches for infection control in aquatic ecosystems.

Current membrane processes in water treatment plants reduce bacterial contamination by up to 7 log while the will achieve > 8 log bacterial elimination. Membrane cleaning due to biofouling represents 20% of the wastewater treatment cost and shortens by 50% the membrane lifetime. AMROCE WFM will reduce the biofouling by 80%, resulting in 15% decrease of the operation costs and 40% increased membrane durability.

AMROCE will alleviate by 90% the costs of biofouling in aquaculture. Additional cost burden related to reduce fish welfare by biofilm associated pathogens, low oxygen concentrations due to poor water exchange and increased stress during net changing, will be mitigated. Potential net failure and escapes, caused by drag and net deformation during cleaning, will be minimised.
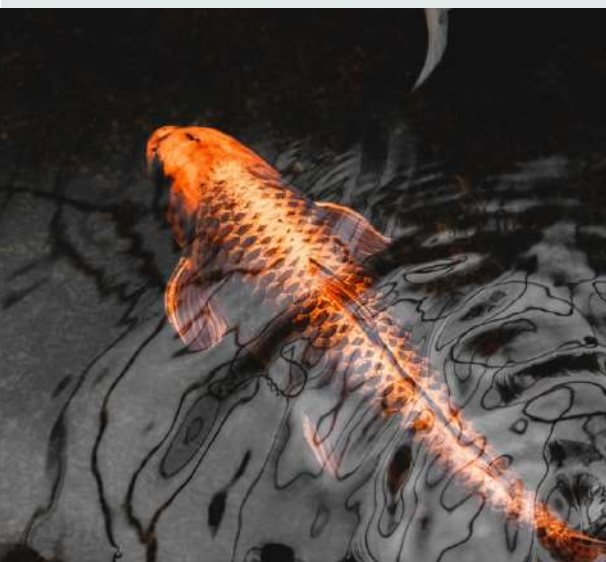
PROJECT STRUCTURE

The AMROCE work plan is comprised of five work packages:

WP1 – Nanoformulations for fish feed
WP1 is dedicated to the development of nano-formulations assembling marine/algae-derived antimicrobial lipids and Antimicrobial lipids and peptides (AMP) with Quorum Quenching Enzymes (QQE), as stable additives for fish and animal feed with antibiofilm and bactericidal efficacies that prevent from bacterial infections.

WP2 - Antimicrobial fish cage nets (FCN) and water filtration membranes (WFM) engineering
In WP2, bulk engineered or coated fish cage nets (FCN) and water filtration membranes (WFM) containing stable metal oxide nanoparticles (MeO NPs) with antimicrobial and antibiofilm activities will be generated.

WP3 - Nanosafety/risk assessment
WP3 will identify the parameters related to the safety of the nano-formulation process for both workers and users, considering not only the existing regulation, but also the gaps existing in the legislation and norms.
An improved approach to risk assessment based on hazard analysis in cooperation with the major stakeholder groups of the aquaculture industry and animal farming will be developed. Each operation will be discussed in detail, hazards will be assessed according to the existing environmental policies, risks associated with each task will be identified and evaluated and risk levels will be assigned. This approach will increase the likelihood for identifying possible new hazards and better assessing existing ones.

WP4 - Sustainability and economic impact
In WP4 the cost effectiveness and sustainability will be studied applying Life cycle assessment and costing (LCA/LCC) approaches to optimise the costs as well as the environmental and social impact of the novel technologies and products.
WP5 is dedicated to project management, communication and dissemination activities

WP5 - Dissemination and coordination
WP5 is dedicated to project management, communication and dissemination activities

PROJECT CONTRIBUTION TO POLICIES

Striving to reach good environmental status of the aquatic ecosystems in EU, the novel technological solutions of AMROCE for reduction of the antibiotic and AMR bacterial contamination will **foster the implementation of the national and EU environmental policies in the aquaculture and livestock industries**.

Life cycle assessment and environmental risk assessment will systematically monitor the progress within the ecological boundaries of the bio-economy and will **provide reliable information to environment-oriented policy makers and tools**, e.g. the EU Ecolabel, Product Environmental Footprint and Green Public Procurement

Applying an improved approach to risk assessment will result in a comprehensive list of hazards and **propose best practices and concrete mitigating actions**.

AMROCE
AGAINST CECs (AMR BACTERIA and ANTIBIOTICS) IN WATER BODIES
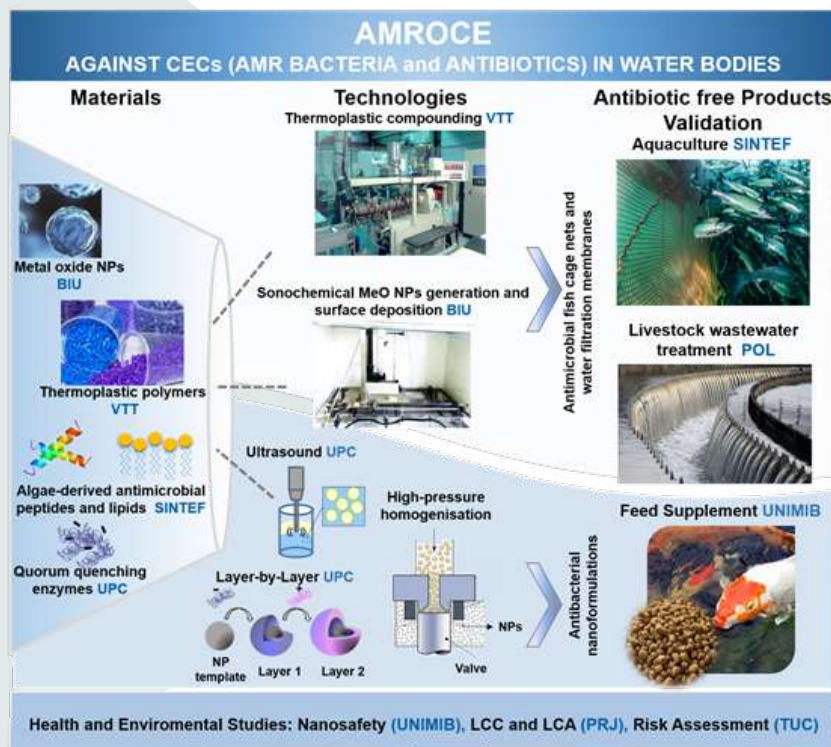
FUNDING INSTITUTIONS
AEI (Spain), AKA (Finland) CSO-MOH (Israel), GSRT (Greece), MUR (Italy), NCBR (Poland), RCN (Norway)

GREENWATERTECH

Green Ultrafiltration Water Cleaning Technologies

PROJECT COORDINATOR

Lars ÖSTERLUND
Uppsala University - Sweden

PROJECT PARTNERS

Swedish University of Agricultural Sciences
Molecular Sciences - Sweden
—
Institute of Inorganic Chemistry of the Czech
Academy of Sciences - Czech Republic
—
CNRS/IRCELYON - France
—
Ecole Normale Supérieure de Lyon - France
—
University of J.E. Purkyne - Czech Republic

DURATION
3 years

STARTING
June 2021

CONTACT
Marcus GRÜNERWALD
marcus.grunerwald@nanoformscience.se
Lars ÖSTERLUN
lars.osterlund@angstrom.uu.se

ABSTRACT

Clean water is a key challenge in the 21th century, articulated in the UN sustainable development goal (UN SDG 6). In close collaboration with industry and stakeholders, this project aims at **developing new types of sustainable water treatment techniques** that are, cheap, easy to maintain and can be applied in settings/countries where clean water is a challenge.

By **using non-critical and non-toxic materials to eliminate contaminants of emerging concern (CECs) and pathogens**, including antibiotic resistant bacteria (ARB), the project aims to avoid their further spread in a safe and sustainable way. We will **develop a water cleaning concept**, amenable for **decentralized use**, that will be validated on a small pilot scale comprising a series of modules:

1. An enzymatic step including nanoporous materials bearing enzymes and chelating functions to retain hazardous metals,
2. enzyme-mimetic nanomaterials,
3. nanoporous adsorbents, and finally
4. a photocatalytic step that ensures zero-discharge of contaminants.

Laboratory scale research will be implemented in reactor modules in collaboration with industrial partners. The **evaluation of different combinations of modules** will be an integral part of the project. Industrial partners will provide engineering solutions that allow testing of new materials/technologies. Associated partners will evaluate the technology and disseminate results, to various industries as well as stakeholders in the developing world.

OUTCOMES AND EXPECTED IMPACT

The goal of this project is to investigate and develop materials and techniques to make a **modular reactor** comprising several steps for **cleaning of water**, possessing a **broad-spectrum CEC and ARB action, with low energy consumption employing cost-effective and green materials**.

The aims are both to develop new ultrafiltration technologies to existing Wastewater Treatment Plants that enables zero-discharge and reuse of water, as well showing the limitation of the technologies and determine conditions for their employment as decentralized stand-alone water cleaning units.

The modular technology comprises:
- (i) hazardous metal cation binding functions with immobilized enzymatic functions on 3D structured substrate,
- (ii) enzyme-mimetic step for bacterial and virus removal,
- (iii) nanoporous adsorbents to capture CECs, and
- (iv) complete elimination of remaining CEC and ARB with photocatalytic 3D textiles and glass velvets. The modular reactor should lead to **complete decomposition of CEC and ARBs with release of only CO2 and H2O.**

The targeted research pilot reactor is planned to reach 1 m3/day capacity. System evaluation will be carried out in collaboration with industrial partners, associated partners and stakeholders in the consortium network.

It is expected that the project will contribute with v**alidation of new water cleaning methodologies relevant for delocalized used**, including industries and hospitals discharging CECs and ARBs, well as suggesting new ways for treating discharge water to utilize fresh water in a sustainable and efficient way. The industrial participation is expected to leverage validation, as well as possible upscaling and deployment possibilities.

PROJECT STRUCTURE

The GREENWATERTECH project is structured into five different work packages:

WP1: Enzyme immobilization
WP1 aims to produce hybrid materials with high water permeability, bearing immobilized oxidative enzymes effective for removing persistent pharmaceutical pollutants and pathogens.

WP2: Nanoporous adsorbents
WP2 deals with the synthesis and surface modification of mesoporous silica and porous oxide materials for heavy metals and CECs removal in waste water. The synthesized materials include nanostructured metal (hydr)oxides, phosphate-modified silica, TiO_2, CeO_2, MnO_x, various iron (hydr)oxides, their mixtures and composites with high surface area, well-developed porosity and suitable surface properties for the efficient adsorption of hazardous metal pollutants (i.e. ions of Cu, Co, Hg, As, Cd, Cr, Zn, Mn, Ni) and CEC (including various endocrine disrupting chemicals, pesticides, pharmaceuticals, and macrolide antibiotics) from waste water.
The adsorption ability of synthesized materials as well as the mechanism of adsorption or degradation will be evaluated. Further objective is scaling-up synthesis of promising materials and testing of their performance under varying conditions using simulated and real waste water.

WP3: Photocatalytic coatings
WP3 is dedicated to the synthesis and deposition of photocatalytic TiO_2, ZnO and Cu_2O based nanocomposite coatings on woven optical fibres, polymers and glass velvets and for effective degradation of CECs. Further development will be to up-scale the synthesis of photocatalytic materials to small pilot reactor.

WP4: Modular Hybrid Ultra-Filtration Pilot
WP4 aims to develop a modular small-scale ultra-filtration water cleaning pilot (about 1 m3/day) consisting of a modular hybrid unit comprising of a series of steps for complete removal of targeted CECs and ARB. The materials and coated supports from lab-scale research will be implemented in the reactor modules and each module will be evaluated in collaboration with industrial partners PHAREM, DEKONTA; TREFFLER, BROCHIER, and NANOFORM SCIENCE. Chembio analysis will be performed to evaluate CECs and ARB in effluents

WP5: Coordination and management
WP5 deals with project management and market study, exploitation and commercialization plans. All partners and associated partners (Blue Centre Gotland, Clean Projects International, Nanoform Science, Treffler Production) will be involved. Important inputs from the subcontractors PHAREM, DEKONTA and BROCHIER concerning up-scalable results and exploitation will be used in several tasks.

EXPERIMENTS / CASE STUDIES
The GreenWaterTech experiments include:
1. Testing of **enzymatic reactor** (Sweden -a)
2. Testing of **nanoporous reactor** (Czech Republic -b)
3. Testing of **photocatalytic reactor** (France -c)
4. Adaptation and development of **module hybrid reactor** (enzymatic, nanoporous, photocatalytic reactor) and testing the modularity of those (Sweden, Czech Republic and France - d)
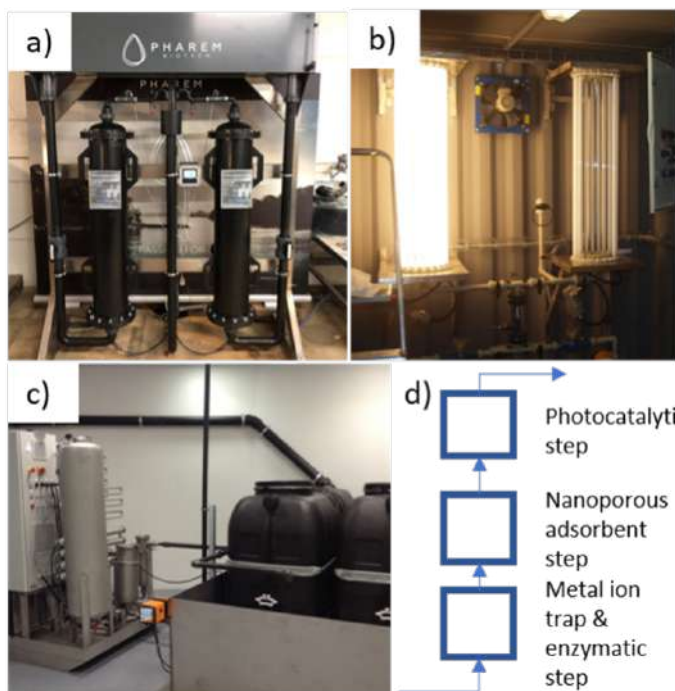
PROJECT CONTRIBUTION TO POLICIES

Lack of access to clean water is one of the World's major health issues. The need to develop new water cleaning technologies is clearly identified. The project contributes to **UN SDG 6** (Clean water and sanitation).

It addresses goals in the water treatment field to **reduce overall effluent emissions by reusing treated wastewater towards zero-discharge strategies,** minimising waste generation and disposal costs, towards sustainable water and wastewater management.
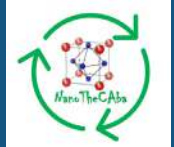
The proposed modular enzymatic, nanoporous and advanced oxidation processes (AOP) technique for complete removal of CECs and pathogens from wastewater, aligns with those goals and pushes the field beyond current state-of-the-art.

FUNDING INSTITUTIONS
ANR (France), FORMAS (Sweden), TACR (Czech Republic)

NANOTHEC-ABA

CECs and AMR bacteria pre-concentration by ultra-nano filtration and Abatement by ThermoCatalytic Nano-powders implementing circular economy solution

PROJECT COORDINATOR

Giuliana MAGNACCA
Torino University - Italy

PROJECT PARTNERS

National Research Council - Institute for the Study of Nanostructured Materials (CNR-ISMN) - Italy

—

Aalborg University - Denmark

—

Liqtech International A/S - Denmark

—

Centre for Nanotechnology and Smart Materials (CeNTI) - Portugal

DURATION
3 years

STARTING
September 2021

CONTACT
Maria Cristina PAGANINI
mariacristina@unito.it

ABSTRACT

The project will deliver an **energy efficient new integrated prototype system** for water purification, composed of:

- the first-to-be realised **ultra-stable silicon carbide** (SiC) UltraFiltration/ NanoFiltration (UF/NF) **membrane** for **pre-concentration of the CECs contaminated water**,
- an innovative nano-enabled **thermocatalytic energy efficient packed-bed reactor** (TPBR) for the generation of OH radicals **able to abate CECs and residues of AMR bacteria** via an Advanced Oxidation Process (AOP), and
- a nano-enabled **antimicrobial MicroFiltration (MF) membrane**.

The TPBR beads are obtained by recycling SiC membranes scraps deriving from the ordinary production process and will be coated with thermocatalytic perovskite-nano-powders **allowing full abatement of Contaminants of Emerging Concern (CECs) and of Antimicrobial Resistant (AMR) pathogens at mild temperatures** without need of chemicals and light sources. The MF membrane is coated with antibacterial titania-silica-core shell nanoparticles for inactivating AMR bacteria, while removing suspended solids. The UF/NF membrane separates the clean permeate stream, ready to be recycled or reused, from the toxic concentrate, which is purified by the TPBR, thus preventing discharge of CECs and pathogens in rivers and oceans.

The new system is **compact, amenable to scale-up, and ease to integrate** in mariculture, aquaculture, tannery, hospital, and other industrial wastewaters treatment facilities, providing safe and efficient operation. The integration of the three components allows the optimization of each system unit both alone and in combination, boosting the efficiency of the process and ensuring high water quality and safety, by enabling a water and SiC recycling multi-circular model.

OUTCOMES AND EXPECTED IMPACT

NanoTheC-Aba proposes a solution that **combines pre-concentration, antifouling, high filtration efficiency, CECs abatement, operation safety, simplicity, robustness** with respect to the Reverse Osmosis system complexity, **cost effectiveness** and **without consumption** or **waste of natural resources**.

The integrated one step water treatment prototype system aims at providing **abatement (> 99%) of the largest spectrum of CECs and AMR-pathogens**, regardless their chemical or biological nature, and the **complete reuse of process effluents**, thus minimizing disposal of wastewater in the environment, reducing the water treatment cost of at least 30% in the four case studies while keeping low the running costs of the process. The project involves the investigation of the following relevant aspects:

- A) **Generation of antibacterial SiC membranes for AMR pathogens abatement**, by using nanoparticules (NPs) with enhanced antibacterial activity immobilized on SiC flat membrane by spray coating
- B) **Functionalized beads for packed bed reactor**: The perovskite-based Nanopowder (NPW) will be deposited on beads produced by SiC-based membranes scraps through innovative sonochemical techniques following circular economy model.

NanoTheC-Aba project offers a viable implementation to solve the issue of the CECs abatement for human and environmental safety, providing a better water quality. This will benefit people at large providing a measurable **positive impact on society and health**. The integrated apparatus can be applied without any change in the existing waste water treatment system, covering different sectors such as hospitals, tanneries, aqua-mariculture, industrial wastewater, agriculture, utilities.

The involvement of one of the leading global company in SiC membranes production will offer the unique opportunity for global industrial exploitation of the developed results and their application in different industrial and public sectors. The technological impact is also relevant for the **development of the first SiC NF membrane** opening up revolutionary applications.

Providing differential scale filtering stages combined with the simultaneous abatement of CECs and AMR pathogens makes NanoTheC-Aba project a unique, **disruptive solution for future environmental and safety global challenges**.

PROJECT STRUCTURE

The NanoTheCAba is divided into five work packages:

WP1 - UF/NF membrane and prototype Development

WP1 will be focused on the design and development of the two innovative treatment stages of the pilot unit for water treatment: the unique SiC-based UF/NF membranes and the TPBR for CECs abatement.

A circular economy principle will be applied for the development of the TPBR for CECS and AMR pathogens oxidation and consequent abatement at mild temperatures, by recycling the ceramic scraps deriving by filters production. This provides and answers to the important economic and environmentally sustainable needs deriving form SiC membranes scrap production.

WP2 - Thermocatalytic NPW and AM NPs synthesis

In WP2, the synthesis of thermocatalytic Nanopowders to be coated onto the scraps-based SiC beads will be performed. The active thermocatalytic layer is a perovskite-type compound. For the antimicrobial MicroFiltration (MF) membrane, antimicrobial NPs will be purchased and/or synthesized and characterized for further antimicrobial performance enhancement.

This WP will be developed in conjunction with WP1, WP3 and WP5, by taking into consideration: the nanopowder catalytic activity, the amounts of NPWs to be produced per batch, and the nano-safety issues.

WP3 - Coating solutions for SiC membranes

The objectives of WP3 are the development of optimal sonochemical coating for functionalising the SiC beads with thermocatalytic NPW and the development of ultrasonic spray coating formulation to deposit antimicrobial NPs on MF membranes. Subcontracted Sonochemistry experts will help devising the optimal sonochemical coating parameters to functionalise the TPBR beads in order to achieve maximum deposition rate and maximum thermocatalytic functionality referring to processing temperature.

The membranes coatings by spray coating technology with antimicrobial metal oxides nanoparticles will also be developed. The NPs processability by spray coating will be investigated in detail.

WP4: System integration testing & validation

In WP4, A bench-scale apparatus will be designed, assembled and tested first with relevant wastewater samples. The bench scale apparatus will generate two streams of pure water, the membrane permeate and the TPBR effluent. Both streams are expected to not contain pathogens and have negligible concentrations of CECs. Then, the pilot will be installed in Italy, where NanoTheC-Aba Associated partners will provide real wastewater samples relevant for 4 industrial case studies (a fish farm, a textile industry, a tannery and a hospital).
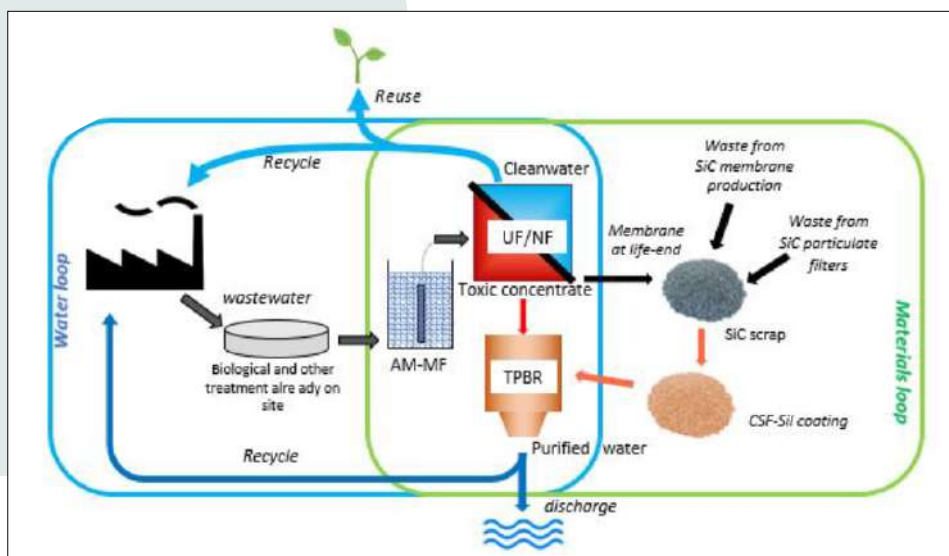
The activities will also address industrial upscaling.

WP5: Management, dissemination, exploitation

WP5 is dedicated to the management and the implementation of the communication and exploitation plans of the project.

The project would like to face policies involving **responsible water consume, sustainable water treatment and human health**. In this sense it pursues the **UN SDG 6** (Clean water and sanitation) and **12** (Responsible consumption and production).

FUNDING INSTITUTIONS

FCT (Portugal), IFD (Denmark), MUR (Italy)

NATURE

Nature-based solutions to reduce antibiotics, pathogens and antimicrobial resistance in aquatic ecosystems

PROJECT COORDINATOR

Victor MATAMOROS
Institute of Environmental Assessment and Water Research (IDAEA-CSIC) - Spain

PROJECT PARTNERS

AARHUS University - Denmark

—

CIIMAR - Interdisciplinary Centre of Marine and Environmental Research - Portugal

—

École Nationale d'Ingénieurs – Abderhamane BabaTouré (ENI-ABT) - Mali

—

Helmholtz Centre for Environmental Research – UFZ - Germany

—

Kilian Water Ltd. - Denmark

DURATION
3 years

STARTING
September 2021

CONTACT
Ana SOTRES
comunicacion.idaea@cid.csic.es

ABSTRACT

The NATURE project will **assess nature-based solutions (NBS) as management option for water treatment on the catchment scale.**
An array of NBS including conventional and high-end constructed wetlands, river re-naturalization, and restoration of wetlands will cover the continuum from urban sources to coastal biota in estuaries. We propose a **comprehensive quantification of the fate of ABs, pathogens, and AMR** in these systems together with ecotoxicological and human health assessments.

NBS performance will be analysed using multivariate modelling techniques to identify parameters with the greatest empirical influence on the attenuation of targeted pollutants.
The NATURE project will encompass three interconnected phases:
- An **experimental phase** in which the reduction of aquatic pollutants will be evaluated in NBS and compared with reference sites.
- In a **data modelling phase**, diagnostic indicators (indicative parameters from the first phase) will be identified for cost-effective future monitoring.
- In a **risk assessment phase**, the effect of aquatic pollutants on environment and human health will be evaluated, estimating its reduction due to NBS implementation.

The unique combination of advanced approaches from analytical chemistry, molecular microbiology, modelling and ecotoxicology will be of paramount importance for an accurate evaluation of NBS treatment performance. NATURE's key objective is to **promote the sustainable and green attenuation of aquatic pollutants.**

OUTCOMES AND EXPECTED IMPACT

The NATURE project will have a major impact on **enhancing the use of sustainable NBS for wastewater treatment and river management**. The application of NBS will become increasingly imperative in the coming years due to the economic costs of conventional water treatment and the need to re-naturalize ecosystems.

Specifically, NATURE will:
- **(i) increase the knowledge-base of NBS's effectiveness** in reducing risks associated with ABs, pathogens and AMR,
- **(ii) enhance the capacity of local stakeholders in the implementation of NBS**,
- **(iii) establish a sustainable network of scientists** among the EU countries working at the frontier of NBS research and development.

A detailed set of measures will be developed to maximize the dissemination, impacts, and exploitation of the NATURE project.

Specific impacts of the project include:
- **Novel, energy-efficient wastewater treatment NBS** (Microbial Electrochemical-based Constructed wetlands - METland CWs) to reduce the impact of aquatic pollutants on water bodies;
- **Implementation of NBS** as wastewater treatment and management measures in river basins;
- **Environmental safety and economic benefits for aquaculture** through the introduction of NBS for water treatment and management (including the safety of food products in estuarine areas), considering environmental, socio-economic, and legal constraints governing the adoption of these technologies;
- **Protection of ecosystem and public health** by transferring of NBS practices to key stakeholders such as government and public authorities,water utilities, health authorities.

PROJECT STRUCTURE

The NATURE work plan is comprised of six work packages:

WP1 – NBS optimization and implementation
WP1 will encompass the evaluation and optimization of seven NBS practices at five river catchment levels for the individual, secondary treatment, tertiary treatments, and river basin management measures at Spain, Portugal and Denmark sites and their conceptual translation to Mali (WP4).

WP2 – Analysis toolbox
WP2 incorporates all of the environmental quality and public health analyses at the implemented sites in Spain, Denmark and Portugal**.**

WP3 - Data modeling
In WP3, selected diagnostic indicators will be correlated with all of the data collected in WP2 to evaluate NBS (WP1), in addition to GIS-based watershed models to support WP4.

WP4 - Assessment of ecosystems and human health risk
WP4 will be devoted to the assessment of environmental and human health risks associated with the presence of pollutants in river and estuarine waters (aquaculture), estimating its reduction due to NBS implementation.

WP5 - Communication/Dissemination and Training
WP5 will include information transfer to local and international stakeholders (i.e. public health, environmental, aquaculture farmers, and Waste Water Treatment Plant managers), educational workshops and training events, scientific publications, and an international symposium on NBS.

WP6 - Project management
WP6 will encompass all of the scientific, administrative, and legal administration in the project.

PROJECT CONTRIBUTION TO POLICIES

The Nature project will tackle relevant remaining gaps in EU and national policies in the water, marine, health and agricultural and environmental sectors by the **implementation of nature based solution strategies to reduce aquatic pollutants** in aquatic ecosystems.

It involves specific **water policies** (**EU Water Framework and Urban Waste Water Treatment Directives**) and the **EU aquaculture policy** (building resilience to aquatic pollutants in estuarine areas),

NATURE project aims also to contribute to **UN SDG 6** (Clean water and sanitation) by using nature based solutions to ensure availability and sustainable management of water and sanitation for all.

EXPERIMENTS / CASE STUDIES

Several full-scale case studies will be assessed:

1. **Secondary wastewater treatment NBS**. A novel Constructed Wetland (CW) engineered development - METland - imetland.eu - and its capacity to attenuate pollutants will be compared to current best available CW technology for wastewater treatment, i.e. a vertical flow CW (VFCW). Both systems are located in Denmark (Orby and Styrnoby).
2. **Tertiary wastewater treatment NBS**. Two state-of-the-art wastewater tertiary treatment CW configurations (Horizontal Flow and Surface Flow) will be assessed located in Barcelona and Granollers (Spain).
3. **Restored wetland** Aarslev (100 ha) located in Aarhus (Denmark).
4. **Wastewater-dominated river re-naturalization**. A wastewater-dominated river 2.6 km from the WasteWater Treatment Plant discharging point to the river mouth will be studied (Senia river, Spain).
5. **Saltmarsh estuarine NBS** located in Lima River estuary and Ria de Aveiro (Portugal).
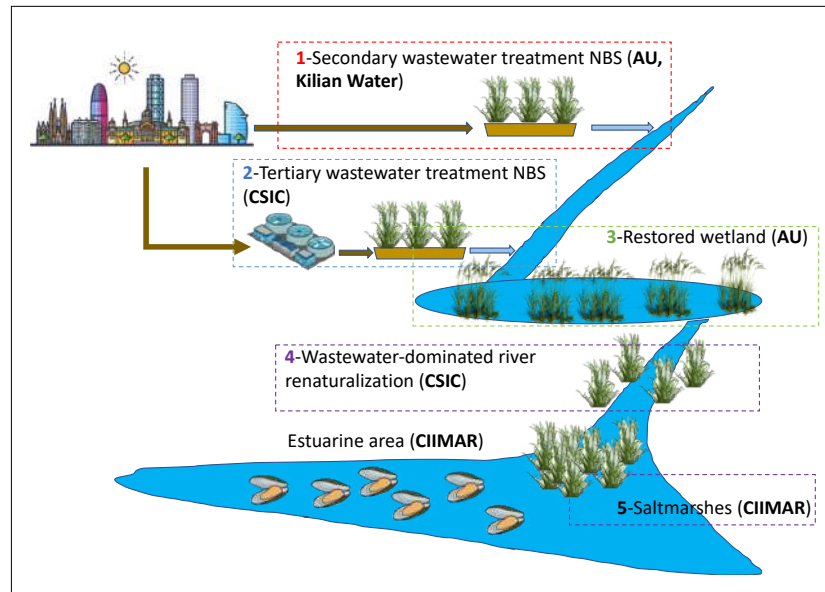
FUNDING INSTITUTIONS

AEI (Spain), BMBF (Germany), FCT (Portugal), IFD (Denmark), Sida (Sweden)

PRESAGE

Potential of decentralized wastewater treatment for preventing the spread of antibiotic resistance, organic micropollutants, pathogens and viruses

PROJECT COORDINATOR

Francisco Omil
University of Santiago de Compostela - Spain

PROJECT PARTNERS

University of Sao Paulo - Brazil

—

Technical University of Denmark - Denmark

—

University of Porto - Portugal

—

CNRS/Institut National polytechnique de Toulouse/École Nationale Supérieure Agronomique de Toulouse - France

—

Technische Universität Dresden (TU Dresden) - Germany

DURATION
3 years

STARTING
September 2021

CONTACT
Sonia SUAREZ
sonia.suarez@usc.es

ABSTRACT

New approaches are needed to **reduce the emission of contaminants of emerging concern** (CECs). Some sources contribute strongly to such emissions, which has driven the focus of PRESAGE on **innovative decentralized wastewater treatment** (WWT), based on anaerobic and aerobic compact systems.

An **integrated analysis of the behaviour of organic micropollutants** (OMPs), **antibiotic resistant microorganisms and genes** (ARMs/ARGs) **and pathogens** (viruses and bacteria) will be carried out. This will allow better understanding the relation between the operational parameters of reactors, the microbiological evolution in the system, the removal of OMPs and pathogens, and the development of ARMs and ARGs. The contribution of such a complex mixture on the final effluent ecotoxicity will be assessed.

The **technologies will be validated at 4 demosites treating black and grey water, and effluents from hospitals and an antibiotic industry**, in close collaboration with the industrial sector. This high readiness level anticipates a good impact of project results on wastewater innovation.

Experts in the field of advanced WWT, microbiology and ecotoxicology will strongly cooperate and participate in a mobility plan focusing on complementary skills. PRESAGE impacts society and economy, **boosting the water industry and protecting the environment from effluent discharges containing CECs.** In the proposed treatment strategy a minimum global impact is targeted, preferentially promoting the onsite water reuse.

OUTCOMES AND EXPECTED IMPACT

PRESAGE offers an original research perspective in which environmental and microbiological know-how will be integrated to d**evelop sustainable decentralized treatment processes**.

PRESAGE aims at **reducing the emission of ARMs, pathogens and pharmaceuticals** that can cause important adverse effects on endocrine (e.g. hormones) or nervous (e.g. tranquilizers) systems, **through new technological solutions** applied to sources containing high concentrations of CECs.

PRESAGE will generate different types of results for valorisation:

- **A new disinfection alternative** will be analysed as effective barrier for pathogens and ARMs. The developed innovative and sustainable technologies for decentralized WWT and the study of ecotoxicity of the generated effluents on aquatic ecosystems will be of interest for WWT companies and consulting services.
- The **cause – effect relation between the fate of OMPs** and the generation and transfer of ARMs during biological WWT will be investigated. Due to the novelty of such approach, the resulting **integrated analysis** will be of great interest for the scientific sector.
- The project will propose optimal and competitive management strategies for hospital, pharmaceutical industry and domestic WW that reduce the risk of CECs spread in the urban aquatic environment, evidencing its important economic and societal challenges. **Data on reachable effluent qualities through competitive developments** will be very relevant for water authorities to develop future policies.

The structure of the project highly facilitates transfer of results to full scale as research will be carried out at 4 demosites in collaboration with Aqualia, Veolia subsidiary, Krueger and Adict Solutions as associated partner.

PROJECT STRUCTURE

The project is structured into seven different work packages:

WP1 – Separated Black Water and Grey Water (Domestic WasteWater)
WP1 aims to maximize CECs removal during decentralized treatment of separated black water (BW) and grey water (GW) for a safer disposal and/or reuse. For this, the CECs removal during BW treatment will be evaluated in an anaerobic membrane bioreactor (AnMBR) and the effluents will be further treated with GW in a Hybrid anoxic/aerobic membrane bioreactor (Hybrid MBR) with and without the addition of Powdered Activated Carbon (PAC) to promote removal through sorption (Demosite 1).

WP2 - Industrial and hospital wastewaters
WP2 will compare aerobic suspended biomass and biofilms in terms of CECs removal using two technologies treating industrial and hospital wastewaters (Demosites 2 and 3) and evaluate different flow patterns on the removal of CECs in anaerobic biofilm reactors fed with hospital wastewater (synthetic and from hospital wastewater-demo-site 4).

WP3 - Postreatment
WP3 will validate for the minimization of the risk of Pathogens, ARM and OMPs the Particle Bed Biocidal Reactor (PBBR), which consists of 3-4 mm particles functionalized with an immobilized biocide, packed together inside a column reactor, and compare with conventional postreatment strategies using dissolved biocides (such as chlorine-based ones) (Demosite 1).

WP4 - OMPs selection and analytical methods
WP4 aims to select the targeted OMPs and validate the analytical procedure and methodologies in order to ensure comparable results on OMPs fate and behaviour between partners.

WP5 - ARMs/ARGs fate & transmission
WP5 will standardize the analysis and quantification of ARM and ARGs in order to determine removal efficiency for each demosite treatment procedure and to elucidate the effects of BW biomass and filtrate on ARM/ARG abundance in activated sludge.

WP6 - Emission of pathogens (bacteria/viruses)
WP6 will assess the fate of selected bacteria in order to determine their effectiveness for the removal of pathogens in the different treatment combinations and characterise the virome at the influents and effluents of the optimized treatments for domestic and hospital wastewater to evaluate possible removal differences among viruses with different characteristics.

WP7 - Ecotoxicity
WP7 is dedicated to ecotoxicological evaluation of the effluents produced at the different demosites.

EXPERIMENTS / CASE STUDIES
The decentralized treatment technologies will be validated at pilot scale at 4 demosites:
- **Demo 1**: It is located in Vigo (North West Spain) in an **office building** where **BW and GW are collected separately.**
- **Demo 2**: The effluent from a **fermentation based antibiotics productions** in Copenhagen.
- **Demo 3**: The WW from a **hospital site** in Zealand.
- **Demo 4**: The WW from the General **Hospital** of the Ribeirão Preto School of Medicine in Brazil.
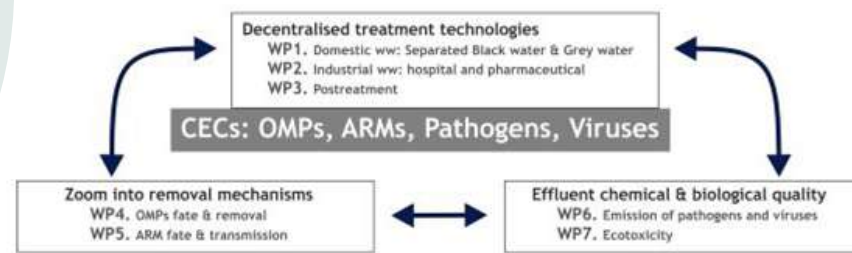
PROJECT CONTRIBUTION TO POLICIES

The presence in urban and hospital wastewaters of OMPs has been recognised as an emerging issue and several OMPs are included in the "Watch List" of the **Water Framework Directive.**
Antibiotic-resistant infections are one of the greatest threats to human health and global food security. In December 2019, the **European Green Deal** reinforced the **need to protect the health and well-being of citizens from environment-related risks and impacts**.
The proposed alternatives should promote **onsite water reuse**, without compromising human health.
The projects supposes an advance in the implementation of **UN SDG 6** (Clean Water and Sanitation) and **9** (Make industries sustainable, with increased resource-use efficiency).

Decentralised treatment technologies
WP1. Domestic ww: Separated Black water & Grey water
WP2. Industrial ww: hospital and pharmaceutical
WP3. Postreatment

CECs: OMPs, ARMs, Pathogens, Viruses

Zoom into removal mechanisms
WP4. OMPs fate & removal
WP5. ARM fate & transmission

Effluent chemical & biological quality
WP6. Emission of pathogens and viruses
WP7. Ecotoxicity

FUNDING INSTITUTIONS
AEI (Spain), ANR (France), BMBF (Germany), CONFAP / FAPESP (Brazil), FCT (Portugal), IFD (Denmark)

REWA

Reduction and assessment of antimicrobial resistance and emerging pollutants in natural-based water treatment systems

PROJECT COORDINATOR

Tiina LEIVISKÄ
University of Oulu - Finland

PROJECT PARTNERS

Galilee Research Institute (MIGAL) - Israel
—
University of Copenhagen - Denmark
—
University of KwaZulu-Natal - South Africa

DURATION
3 years

STARTING
September 2021

CONTACT
Bice MARTINCIGH
MARTINCI@ukzn.ac.za

ABSTRACT

The main goal of the REWA project is the strategic **development and implementation of sustainable and cost-effective technologies** for the removal of contaminants of emerging concern (CECs), metals, pathogens including antimicrobial resistant bacteria and antibiotic resistance genes (ARGs) from water.

The scientific and technological aims are to demonstrate:
1. **new concept in surface water treatment** based on state-of-the-art methods (nano-composites coagoflocculation and pathogens removal, photocatalysis oriented to visible light catalysts, and tailor-adapted sorbents),
2. **sewage effluent** polishing with **biosorbents and carbon-based nanomaterials**,
3. the use of **biocoagulants for metal-rich effluents**.

The efficiencies of the treatment steps for removal of CECs and antibiotic resistance (ARGs, mobile genetic elements and bacteria) will be assessed. Mitigation of selection pressures for antibiotic resistance/co-selection potentials will be investigated.

Special attention will be given to the raising of awareness via various channels and to the education by developing training material including an e-learning course.

OUTCOMES AND EXPECTED IMPACT

REWA project is expected to **reduce the environmental dissemination of Antimicrobial Resistance to nature** (rivers, lakes, oceans) by pushing forward **innovative and simple treatment technologies**.

The developed and validated water treatment technologies will be relevant in any country, where access to high quality water is limited or of concern.

The solutions are largely based on use of specifically prepared **nature-based materials** (clays, carbon-based materials and wood residues), which are well available worldwide. In underdeveloped countries, the proposed systems might considerably improve water quality, and reduce exposure to diseases, pollutants and other CECs. REWA project will offer local decision makers valuable indicators for water and wastewater management.

The project will create **new knowledge about the fate and removal of CECs and AMR from real waters** in new sustainable solutions. Specific impacts of the project include:

- **Novel concept for surface water treatment** using clay-polymer nanocomposites, photocatalysis and tailored-made specific sorbents;
- **Demonstration of the use of wood-based biosorbents and carbon-based materials** for sewage effluent polishing;
- **Demonstration of the use of biocoagulants** for metal-rich effluents;
- **Understanding of the mechanisms of the water treatment processes and their effects** on the microbiome and resistome;
- **Creating awareness of new treatment solutions** and developing training material.

Project will transfer **good practices** and project results will allow further innovation and development both among partners and beyond.

PROJECT STRUCTURE

The REWA project is divided into five work packages:

WP1 – Project management
WP1 comprises the administrative, legal, technical and financial project management.

WP2 – Material preparation and pilot design
In WP2, materials (coagulants, flocculants, sorbents and catalysts) will be prepared and characterised using previously developed synthesis routes in the consortium, and pilots used in case studies will be designed in detail. A very important part of WP2 is to create opportunities for students and water facilities operators to learn new characterization techniques and modification methods and to disseminate working procedures to actual water treatment stakeholders.

WP3 – Demonstration
WP3 will demonstrate selected water treatment technologies for polluted surface water, sewage effluents and metal-rich effluent. The objectives of the WP3 are to select representative recalcitrant CECs in case studies, to optimize treatment processes at lab scale in order to study the fate and removal of CECs and metals and understand the degradation pathways. In all case studies, the materials and concepts will be tested on lab scale. Then the pilot units will be installed and operated in the water source to treat polluted surface water and sewage effluent.

WP4 – Microbiome analyses
WP4 ultimately aims to assess water treatment technologies for their abilities to mitigate environmental dissemination of antibiotic resistance and to reduce whole-effluent toxicity and bioavailability of selected CECs and metals. The objectives of the WP4 are to optimize protocols for microbial indicators in order to study the fate and removal of antibiotic resistance genes (ARGs), mobile genetic elements (MGEs) and antibiotic resistant bacteria from real waters undergoing different water treatments. The co-selection potentials to antibiotic resistance in polluted waters before and after water treatment and in aquatic reference habitats will also be studied.

WP5 – Dissemination and communication
WP5 is dedicated to dissemination and communication of the project results to the targeted audiences.

PROJECT CONTRIBUTION TO POLICIES

REWA will support the regulation through the **development of new standards for quantification and mitigation of the antibiotic resistance**. REWA will mostly contribute to **UN SDG 6** (Clean Water and sanitation) through the development of new concept for water treatment and improvement of the existing processes in urban Wastewater Treatment Plants and metallurgical industry, which facilitates water reuse.

REWA will also contribute to **UN SDG 13** (Climate action) with our actions to improve awareness and promote the introduction of new solutions, including in underdeveloped countries
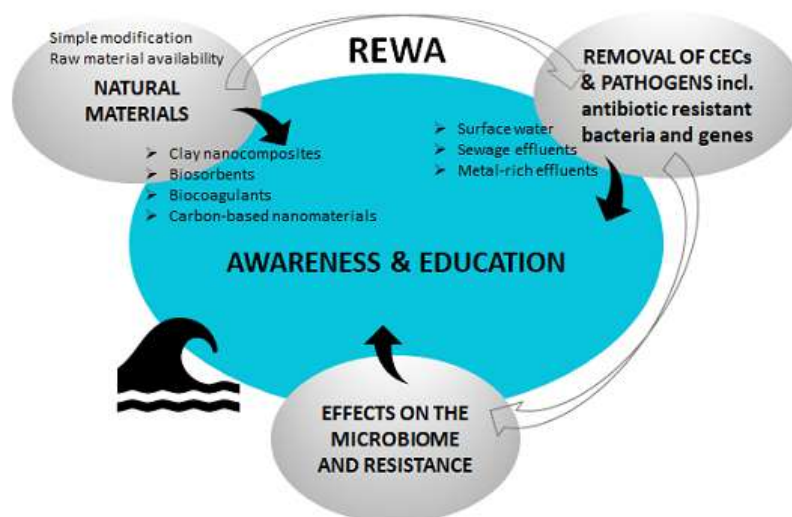
EXPERIMENTS / CASE STUDIES

This project proposes innovative demonstrations:
- Case study #1: **New concept for surface water treatment using clay-based products** (lab and pilot scale) – The Jordan river will be used as a source of polluted surface water (Israel).
- Case study #2: **Sewage effluent polishing by biosorbents** (lab and pilot scale) - Collection of Real wastewaters and pilot test will be performed in an urban Oulu's Wastewater Treatment Plant (Finland).
- Case study #3: **Sewage effluent polishing by carbon-based nanomaterials** (lab scale) - Real sewage effluents will be collected from Durban-based Wastewater Treatment Plants (South Africa).
- Case study #4: **Metal-rich effluent treatment by biocoagulants** (lab scale) - Samples will be collected from a metallurgical wastewater industry (Outokumpu Chrome Oy, Finland).

FUNDING INSTITUTIONS

SERPIC

Sustainable Electrochemical Reduction of contaminants of emerging concern and Pathogens in wastewater treatment plants effluent for Irrigation of Crops

PROJECT COORDINATOR

Lothar SCHÄFER
Fraunhofer Institute for Surface Engineering
and Thin Films - Germany

PROJECT PARTNERS

University of Ferrara - Italy
—
SolarSpring GmbH - Germany
—
University of Castilla La Mancha - Spain
—
Faculty of Engineering University of Porto - Portugal
—
Águas de Portugal (AdP) - Portugal
—
Norwegian Institute for Water Research (NIVA) - Norway
—
Stellenbosch University - South Africa

DURATION
3 years

STARTING
September 2021

CONTACT
Jan GÄBLER
jan.gaebler@ist.fraunhofer.de

ABSTRACT

SERPIC will develop an **integral technology, based on a multi-barrier approach, to treat the effluents of wastewater treatment plants** (WWTPs) to maximise the reduction of contaminants of emerging concern (CECs).

A **membrane nanofiltration (NF) technology** will be applied to **reduce CECs** in its permeate stream **by at least 90 %** while retaining the nutrients. A **residual disinfection** using chlorine dioxide produced electrochemically will be added to the stream used **for crops irrigation** (Route A). **The CECs in the polluted concentrate** (retentate) stream will be **reduced by at least 80 % by light driven electro-chemical oxidation**. When discharged into the aquatic system (route B), it will contribute to the quality improvement of the surface water body.

A **prototype treatment plant** will be set-up and evaluated for irrigation in long-term tests with the help of agricultural test pots. A **review investigation of CECs spread** will be performed **at four regional showcases** in Europe and Africa. It will include a detailed assessment of the individual situation and surrounding condition. Transfer concepts will be developed to transfer the results of the treatment technology to other regions, especially in low- and middle-income countries.

OUTCOMES AND EXPECTED IMPACT

The overall aim of the SERPIC project is to **investigate and minimise the spread of CECs and antibiotic resistant bacteria (ARB)/ antibiotic resistant genes (ARGs)** with a focus on additional water sources for food production.

SERPIC will **develop the concepts and technology** for the valorisation of a constantly available alternative water sources by **reuse of wastewater effluent for safe use in agricultural irrigation**.

Main outcomes include:
- **Review investigation of CECs spread and transformation** at four regional showcases in Europe (Italy, Spain and Portugal) and in Africa (South Africa)
- Selection of the six most representative pollutants relevant in the four showcase regions
- **Development of technology to reduce CECs from WWTP effluent** by membrane filtration and light driven electro-chemical processes
- A **prototype treatment plant validated in relevant environment (TRL5) and powered by photovoltaics**
- Results about breakdown and **transformation of CECs in the product water**
- Results about **irrigation with the treated water** via long-term agricultural growth tests
- **Transfer concepts for technology transfer to other regions and countries**, especially low- and middle-income countries
- Increase of knowledge and awareness in the European Research Area and beyond about CECs and technologies to reduce them
- Strengthening the competitiveness and growth of companies by developing an innovative product and process
- Contribution to economic growth as well as safeguarding and creating jobs, especially in low- and middle-income countries and regions like South Africa
- Contribution to fulfil the EU goal of efficient water resources management and maximisation of water reuse for irrigation

PROJECT STRUCTURE

The SERPIC project is divided into five work packages:

WP1 - Sources, spread and transformation
A review investigation of CECs spread and transformation will be performed in different matrices (water from raw municipal wastewater and treated effluent, soil and crops) at four regional showcases in Italy, Spain, Portugal and South Africa. Subsequently, the boundary parameters for the prototype test will be defined concerning WWTP effluent composition, irrigation method, crop selection and solar irradiance. A set of the most representative pollutants relevant in the four showcase regions will be selected. Six target CECs will be chosen: one from the group of ARBs, one from the ARGs and four other chemical compounds.
All analytical procedures will be developed in this WP. Toxicity assays will be also performed on the product water as the electro-chemical oxidation may lead to toxic CEC products.

WP2 - Treatment technology and prototype
This WP aims to develop and validate SERPIC technology. The treatment solution of SERPIC is based on membrane nanofiltration, splitting the water flow into a permeate with negligible amounts of CECs while preserving the nutrients for route A, and a concentrate that contains the rejected pollutants for route B. The oxidant chlorine-dioxide will be added to the stream of route A to receive a residual disinfection to avoid microbials until the water reaches the field. Powerful oxidants (peroxosulfate and chlorine dioxide) will be produced electrochemically, activated by deep UV, to minimise the CEC content in the stream of route B. Initially persulfate will be the target species for the membrane photoreactor and ClO2 will be used only in case persulfate does not operate properly.
Already available equipment will be used to develop optimised parameters for the individual technology modules. Then, the prototype will be built-up and validated.

WP3 - Transfer strategies
WP3 aims to develop transfer strategies for other regions of the world by assessing the applicability of the SERPIC system in the four showcase regions.

WP4 - Education, communication, exploitation
WP4 will ensure that the results will reach the relevant academic, economical and societal communities.

WP5 - Management
WP5 is dedicated to the coordination activities and project management.

PROJECT CONTRIBUTION TO POLICIES

SERPIC will address the mentioned SDGs and EU regulations:
- **EU Urban Wastewater Treatment Directive**,
- **Water Framework Directive**,
- European Parliament legislative resolution and **regulation on minimum requirements for water reuse** for agricultural irrigation,
- **Marine Strategy Framework Directive**,
- European Commission's Watch List of CECs
- European Green Deal,
- **Common Agricultural Policy**,
- **UN SDG 1** (No poverty), **2** (Zero hunger), **3** (Good health and well-being), **6** (Clean water and sanitation), and **14** (Life below water).

The project is guided by the One Water - One Health paradigm.

EXPERIMENTS / CASE STUDIES
To validate the effectiveness of the developed treatment technology, a **prototype treatment plant will be set-up on-site in Ciudad Real, Spain** and evaluated for irrigation in long-term field tests with the help of agricultural test pots. This disposition will allow to sample not only the vegetables but also the vertical pollutant profiles in the soil when the water will be applied in agricultural irrigation. Sampling of vegetables will differentiate roots, tails, and leaves or the edible products. Also, the quality of the water will be compared for the target CECs.
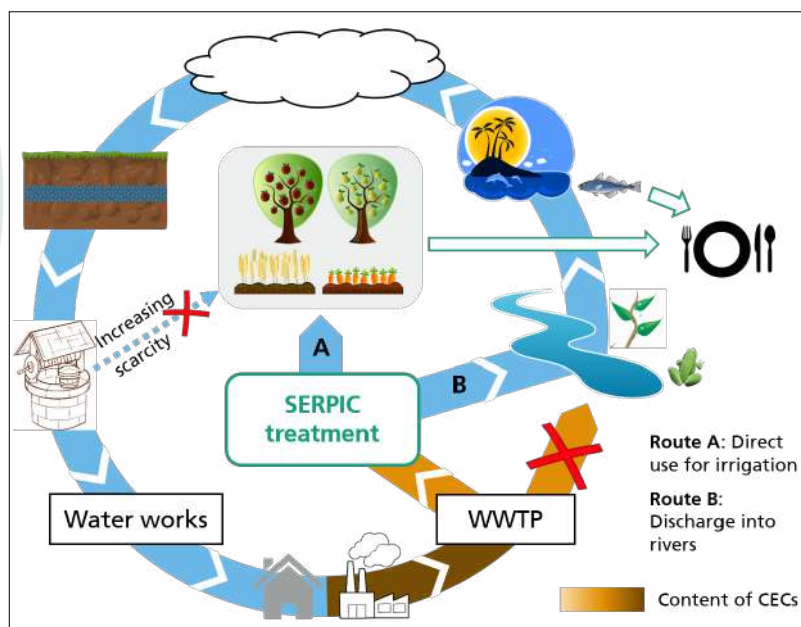
The prototype will use regenerative, sustainable energy thanks to photovoltaic modules.

FUNDING INSTITUTIONS
AEI (Spain), BMBF (Germany), FCT (Portugal), RCN (Norway), MUR (Italy), WRC (South Africa)

DISCLAIMER

ACKNOWLEDGMENTS

WEBSITES
www.waterjpi.eu / www.jpi-oceans.eu / www.jpiamr.eu

CONTRIBUTORS AND LAYOUT (ANR - WATER JPI)
Édith Santa-Cruz, Armelle Montrose

Photo Credits - Free Copyright: freepik.com - unsplash.com - rawpixel.com
- Landsat 8 / NASA (Public domain. License: CC-PD Mark 1.0)