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

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CONTENTS

PRESENTATION OF THE AQUATICPOLLUTANTS JOINT TRANSNATIONAL CALL 2020

Within the framework of ERA-NET Cofund 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.

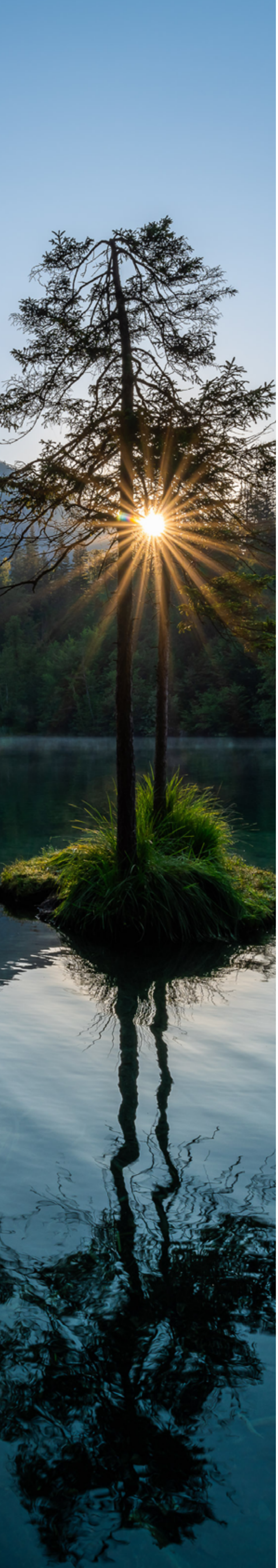
Eighteen projects with 20M€ in requested budget were selected for funding in response to the Aquatic Pollutants joint call. The funded projects, involving 22 countries, are presented in the booklet.

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

This project has received funding from the European Union’s Horizon 2020 research and innovation programme under grant agreement No 869178-AquaticPollutants.

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

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DURATION
3 years

STARTING
September 2021

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MOTIVATION BEHIND THE PROJECT

Over the past decade, aquaculture has expanded significantly, becoming a key source of fish for human consumption. However, concerns have grown over antibiotic residues, emerging pollutants, resistant bacteria, and pathogens in farmed fish, posing potential risks to human and environmental health. Understanding the sources and transmission of these contaminants in open and closed aquaculture systems is essential. ARENA aims to develop rapid detection tools to ensure safer food production, protect ecosystems, and support treatment strategies and epidemiological monitoring, contributing to sustainable aquaculture and mitigating antimicrobial resistance.

PROJECT OBJECTIVES

- Understanding the fate of antibiotics, antibiotic resistance genes, antibiotic resistant and other pathogenic bacteria in the aquaculture chain
- Assessing contamination sources and pathways in both mariculture systems and Recirculating Aquaculture Systems (RAS)
- Understanding the levels of contamination both in the environment and in the final food product to assess potential risks to human health
- Developing/optimizing early-warning tools, including (i) a novel flow cytometry assay for rapid detection of AB residues, (ii) an innovative sequencing-based approach to analyze ARGs contamination, (iii) a sensor-based platform for real-time monitoring in aquaculture environments

RESEARCH METHODOLOGY AND IMPLEMENTATION SUMMARY

- Shotgun metagenomic sequencing and qPCR for AB resistome;
- Cytofluorimetric-based immunoassay for screening antibiotic residues in fish tissues;
- High-Resolution Mass Spectrometry together with predictive tools to identify additional associated contaminants;
- Nanopore-based detection of AMR genes in environmental samples;
- Sensor platform for on-site detection of pathogenic bacteria

SUMMARY OF MAIN RESULTS

Common antibiotics such as sulfonamides and macrolides, especially azithromycin and clarithromycin, have been found in fish, sediments, and seawater in mariculture and Recirculating Aquaculture Systems (RAS), posing environmental hazards. Antibiotic resistance genes (AMR) and microbial activity were higher near aquaculture activities; mariculture displayed extensive AMR in the sediments beneath fish cages, while RAS displayed a variety of AMR in internal sites. AMR gene diversity was higher in RAS, according to a metagenomic study. Nanopore sequencing for AMR detection and a biosensor for E. coli demonstrated potential for monitoring environmental and microbial health in aquaculture settings.

KEY EXPLOITABLE OUTCOME / OUTPUT	DESCRIPTION
Result 1 Metagenomic data	A dataset consisting of 106 metagenomes from mariculture and RAS environment has been produced and deposited in the public Sequence Read Archive (SRA)
Result 2 Synthetic bioreceptors for bacteria	A novel master-stamping technique overcomes production limits and low receptor site density in surface-imprinted polymers, enabling high-density, “template bacteria-free” fabrication for improved detection efficiency.
Result 3 Sensor chip for bacteria detection	Two biosensor platforms were developed using novel imprinting on interdigitated and QCM electrodes, enabling bacterial detection via electrochemical impedance spectroscopy and quartz crystal microbalance with dissipation monitoring.
Result 4 Sensor chip for “environmental” parameters	A microfluidic chip-based sensor was developed to monitor temperature, pH, and conductivity in aquaculture water, demonstrating effective performance in field sample measurements.

OUTLOOK / PERSPECTIVES

The ARENA project's future perspectives include advancing the understanding and management of antibiotic resistance and microbial contamination in aquaculture environments. This project, which aimed to assess and mitigate the impacts of antibiotics and pathogens in aquaculture systems, has developed innovative tools such as biosensors and sequencing-based methods to monitor these contaminants that we expect will contribute significantly to environmental health and ensure the sustainability and safety of aquaculture practices.

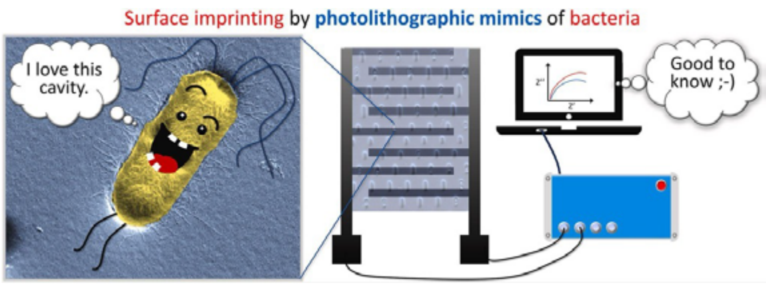

Figure 1. Graphical abstract regarding the work on developing a biomimetic SIP-based biosensor system to detect bacteria (e.g., E. coli). The sensor was fabricated by new method consisting of a positive master stamp including photolithographic mimics of the template bacteria, which enables reproducible fabrication of the SIP surface without the need for “real” bacteria (Source: Özsoylu D., et al., 2024, Biosensors & Bioelectronics, 261, 116491, DOI: 10.1016/j.bios.2024.116491).

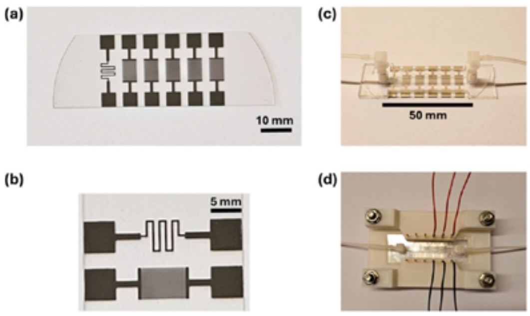

Figure 2. Microfluidic, chip-based sensor system for monitoring “environmental” parameters of water samples in aquacultures. (a) A glass chip features a meander-shaped temperature sensor composed of platinum (left) and five interdigitated electrode structures (IDEs). Panel (b) provides a close-up view of (a). One IDE structure is adequate for measuring the electrical conductivity of the medium, while the other four can be modified for other functionalities. (c) A microfluidic channel is installed, complete with an inlet and outlet for fluids. (d) The channel includes electrical connections with 3D-printed connectors for conducting temperature and impedance measurements (Source: Fereshteh A, Özsoylu D., et al, 2024, Micromachines, 15[6], 755, DOI: 10.3390/mi15060755).

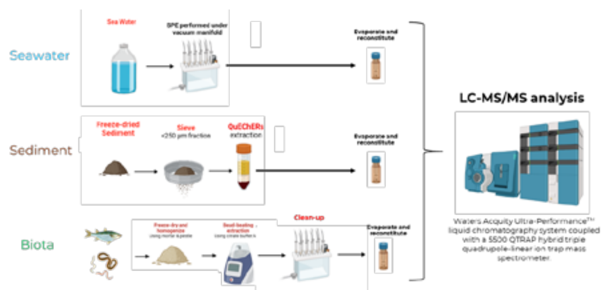

Figure 3. Experimental workflow for the extraction and analysis of antibiotics in three environmental matrices: seawater, sediment, and biota. Water samples were pre-concentrated using solid-phase extraction (SPE) under vacuum. Freeze-dried sediment samples, specifically the fraction smaller than 250 µm, were extracted using a QuEChERS methodology. Fish and benthic biota were processed using a zirconium bead-beating extraction method, followed by a cleanup step with SPE. Each sample was carefully conditioned according to its matrix-specific protocol and analyzed using liquid chromatography-tandem mass spectrometry (LC-MS/MS) to detect and quantify antibiotics. Detailed information on the methodology is provided in Deliverable 3.1.

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

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DURATION
3 years

STARTING
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MOTIVATION BEHIND THE PROJECT

Antimicrobial resistance (AMR) has emerged as one of the principal public health problems of the 21st century that threatens the effective prevention and treatment of an ever-increasing range of infections. The primary objective of FOREWARN was to assess the occurrence and behaviour of contaminants of emerging concern (CECs) and emerging pathogens such as antimicrobial resistant bacteria (ARB), to model their transfer and behaviour, develop new predictive models, and build a decision support system (DSS) for predicting risks and proposing mitigation strategies.

PROJECT OBJECTIVES

The main objective has been to study, assess, and model the occurrence, fate and behavior of pathogens including viruses, such as SARS-CoV-2, CECs, and the development of ARB, and ARGs under different environmental, climatic, and hydrological situations.

RESEARCH METHODOLOGY AND IMPLEMENTATION SUMMARY

To fulfil this objective, two types of case studies are considered:1-In-silico, and 2-Environmental case studies. In silico case studies compile previous results and datasets obtained in past or ongoing EU projects. These data were considered to develop the models and algorithms to feed and develop the DSS. However, the lack of complete datasets evaluating the same samples for pathogens, CECs, and environmental conditions led to complementing previous data with extra new data generated during the FOREWARN project. The adaptive DSS system was finally refined and tested under real environmental conditions.

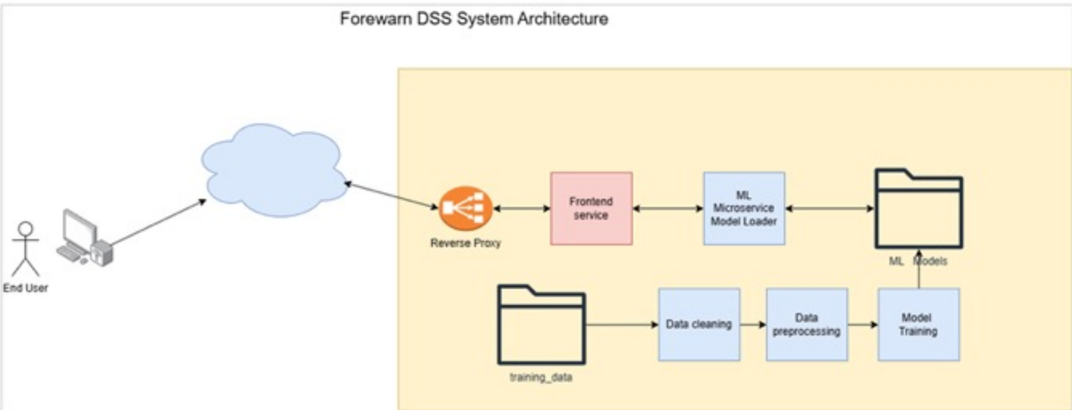
SUMMARY OF MAIN RESULTS

The project has achieved most of its main objectives. However, due to different drawbacks encountered during the project, inevitable delays occurred concerning the DSS evaluation at TRL5 (tested in one relevant environment), one of the project's more relevant outputs. However, a four-month extension was approved, and during this period, the consortium fulfils all the project's results and outputs, as well as the deliverables and milestones postponed for the end. The project required close collaboration between partners from different scientific fields, including environmental sciences, analytical chemistry, microbiology, virology, computational sciences, machine learning, and IT. Furthermore, individual efforts could not have yielded the combined results obtained, such as the FOREWARN-DB, the extended information regarding CECs levels, emerging viruses, ARBs, ARGs, and the DSS. The multidisciplinary approach was possible thanks to the close collaboration, continuous consortium and focus meetings, and interchange of personnel between institutions.

KEY EXPLOITABLE OUTCOME / OUTPUT	DESCRIPTION
Result 1	Improve the knowledge on CECs combined with emerging pathogens in aquatic environments
Result 2	FOREWARN-DB. The first database compiles data reporting at least one of the following information: the levels of ARBs, ARGs, Antibiotics and pathogens in the aquatic environment. The has been transferred to the NORMAN net to ensure the data availability and maintenance. This also contribute to the initiation of a new database in the NORMAN network dedicated to viruses. End Users: Education & Training, researchers, environmental managers, water utilities, policy makers / decision makers, and the civil society.
Result 3	Decision Support System (DSS) evaluated at TRL5 (tested in one relevant environment) The DSS will 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. The FOREWARN-DSS supports the decision-making processes in the short term (punctual emissions, potential situations potential risk) and provide support on technical aspects such as wastewater treatments required in particular areas. End Users: Research, environmental managers, water utilities, policy makers / decision makers, and the civil society.

OUTLOOK / PERSPECTIVES

FOREWARN have impacts on three essential points: 1) Scientific Impact: Creating high-quality new knowledge; Increasing information available about CECs and emerging pathogens, including ARGs and ARBs. Strengthening human capital in research and innovation through summer school and capacity building and diffusion activities. Fostering diffusion of knowledge and Open source: Data is available through the NORMAN network, ensuring their open availability and maintenance after the project ends.
2) Societal impact through environmental protection, supporting the EU policy priorities, and global challenges: By developing a DSS to predict the risk of emerging contamination and antimicrobial resistance.
3) Socio-economic impact and use: The FOREWARN project objective was to develop and validate a DSS at TRL5, which means tested in just one relevant environment. However, in the middle term, it is planned to train and test the system in different environments to reach a TRL7-8 near commercialization with their consequent socio-economic impact. On the other hand, throughout the project, various actions have been carried out in terms of capacity building.



FUNDING INSTITUTIONS

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

MAPMAR

Marine Plasmids Driving the Spread of Antibiotic Resistances

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DURATION
3 years

STARTING
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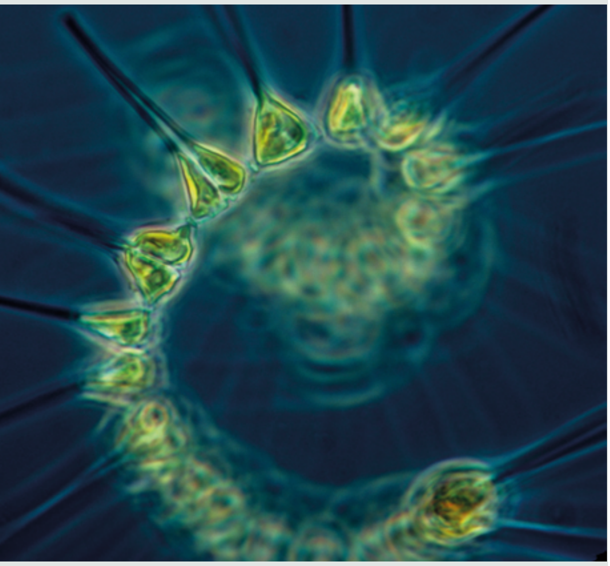


MOTIVATION BEHIND THE PROJECT

MAPMAR studies how conjugative and mobilizable plasmids contribute to the spread of antibiotic resistance (AR) in marine environments. While AR genes are common in terrestrial and contaminated aquatic ecosystems, their presence in marine bacteria remains unclear. MAPMAR analyzed over 1,100 metagenomes and 29K bacterial genomes, revealing that transmissible plasmids are scarcer in marine ecosystems than in terrestrial ones, leading to lower AR levels. However, sewage contamination is introducing plasmids and AR genes into the seas. Although marine AR contamination is still low, urgent action is needed to prevent its spread, as some cosmopolitan plasmids could facilitate AR propagation globally.

PROJECT OBJECTIVES

- Characterize marine plasmids to determine their nature and antibiotic resistance (AR) load.
- Compare marine and terrestrial plasmids to assess taxonomic overlap and transmission potential.
- Reconstruct plasmid backbones using genomic and metagenomic data.
- Analyze global metagenomic datasets (1,100+ metagenomes and 29K bacterial genomes) to identify transmissible plasmids in marine ecosystems.
- Determine AR gene prevalence in marine environments versus human-dominated ecosystems.
- Evaluate the impact of human sewage on AR gene introduction into the ocean.

RESEARCH METHODOLOGY AND IMPLEMENTATION SUMMARY

The research project investigated the prevalence and distribution of conjugative and mobilizable plasmids in marine ecosystems using metagenomic assembled genomes (MAGs) and metagenomic samples. Key methods included:

1. MAG Analysis: A dataset of 26,293 marine MAGs was curated, filtering out low-quality and archaeal genomes. Relaxase (RLX) genes, used as markers for plasmids, were identified using bioinformatic tools like Prodigal and MOBscan. Comparisons were made with MAGs from human, pig, and chicken gut microbiomes (GM) to assess RLX abundance and MOB class distribution.
2. Metagenomic Sampling: 370 marine metagenomic samples from the Tara Oceans Expedition were analyzed, alongside river, soil, sewage, and GM samples. RLX abundance was quantified using a probabilistic scoring method, and taxonomic composition was assessed using Kraken.
3. ARG Detection: Antibiotic resistance genes (ARGs) were identified using ABRicate and compared across environments. Plasmid content was classified using geNomad, revealing lower plasmid abundance in marine ecosystems.
4. Novel Plasmid Identification: A bioinformatic pipeline was developed to identify novel plasmid sequences from marine metagenomic data, revealing a largely unexplored marine plasmidome.
5. Aquaculture Experiments: Plasmid transmission in aquaculture environments was studied using Sparus aurata fish and seawater samples, with DNA extraction and sequencing to assess ARG spread.
6. Single-Cell Sequencing: Fluorescence-activated cell sorting (FACS) and single-cell genome sequencing were employed to validate plasmid detection and study horizontal gene transfer in marine environments.

Overall, the project combined bioinformatics, metagenomics, and experimental approaches to explore plasmid dynamics, ARG distribution, and horizontal gene transfer in marine ecosystems.

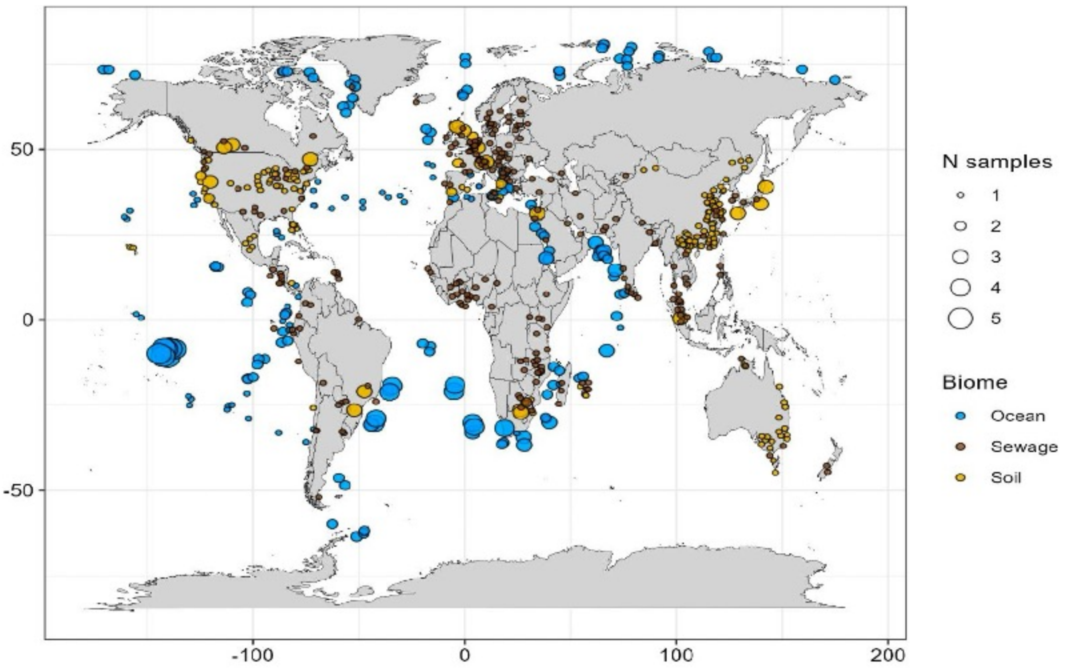
SUMMARY OF MAIN RESULTS

The study revealed that marine ecosystems have significantly fewer conjugative and mobilizable plasmids compared to human, pig, and chicken gut microbiomes. Relaxase (RLX) genes, used as plasmid markers, were 30-80 times scarcer in marine environments than in sewage and gut samples. Marine plasmids were enriched in specific MOB classes (-B, -F) but depleted in others (-V, -T). Antibiotic resistance genes (ARGs) were also less abundant in marine samples, with sulfonamide-resistant genes being the most common. Novel plasmid sequences dominated the marine plasmidome, indicating limited overlap with known plasmids. Aquaculture experiments and single-cell sequencing confirmed horizontal gene transfer via conjugation in marine environments

KEY EXPLOITABLE OUTCOME / OUTPUT	DESCRIPTION
Result 1 Database of Marine Plasmids	Database containing plasmids identified in marine metagenomes, including their conjugative potential and antibiotic resistance
Result 2 Report on the transmissibility of antibiotic resistance genes in aquaculture	Data on the transmissibility of several model plasmids in aquaculture systems for Spaurus aurata.

OUTLOOK / PERSPECTIVES

Future research should focus on expanding the exploration of marine plasmid diversity, particularly in understudied environments like deep-sea and oxygen minimum zones. Enhanced sequencing techniques, such as long-read and hybrid assembly, could improve plasmid recovery and characterization. Investigating the ecological roles of marine plasmids, including their contribution to horizontal gene transfer and adaptation, is crucial. Additionally, studying the impact of anthropogenic activities, such as aquaculture and pollution, on plasmid-mediated antibiotic resistance in marine ecosystems is essential for understanding and mitigating risks. Developing standardized protocols for plasmid detection and single-cell sequencing will further advance our understanding of plasmid dynamics and their role in marine microbial communities.



Metagenomic samples analyzed in the project

FUNDING INSTITUTIONS

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

PAIRWISE

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

PROJECT COORDINATOR

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Estación Biológica de Doñana, Consejo Superior de Investigaciones Cientificas (EBD-CSIC) - Spain

National Research Institute for Rural Engineering, Water, and Forestry (INRGREF) - Tunisia

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DURATION
3 years

STARTING
September 2021

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MOTIVATION BEHIND THE PROJECT

Antimicrobial resistance (AMR) is an important threat to human and animal health. Thus, it is alarming that the presence of antibiotic-resistant bacteria (ARB) increases all over the world and that they occur in all types of environments. An important route of introduction of ARB and antibiotics (ATB) to the environment is via wastewater treatment plants (WWTPs). The knowledge of AMR emanating from WWTPs as a pollutant of aquatic environments, wildlife and livestock is, however, limited. Thus, PAIRWISE focused on dispersal and dynamics of ARB, antibiotic resistance genes (ARG) and ATB in aquatic environments affected by WWTPs.

PROJECT OBJECTIVES

The objectives were to obtain knowledge on:

- Dispersal of ARB, ARG and ATB in surface waters downstream of WWTPs
- Carriage of ARB and ARG in grazing livestock linked to surface waters influenced by WWTP discharges
- Role of aquatic birds in the dispersal of ARB and ARG

RESEARCH METHODOLOGY AND IMPLEMENTATION SUMMARY

Water and faecal samples were taken from grazing cattle and aquatic birds at several places and occasions. The samples were cultivated to identify Escherichia coli and Klebsiella pneumoniae producing extended spectrum beta-lactamases (ESBL) or carbapenemases (CARBA), which indicate resistance against important ATB. Such bacteria were characterized by whole genome sequencing. The samples were also analyzed for a selection of ARG using high-throughput HT-qPCR smartchip analyses. Moreover, water samples were analyzed for ATB using HPLC-MS-MS. In Spain and Sweden, aquatic birds were fitted with GPS transmitters and their movements were followed in relation to presence of WWTPs and similar sites.

SUMMARY OF MAIN RESULTS

The data indicate that presence of ARB producing ESBL or CARBA in water closely associated with WWTPs was, in general, rather common but varied between regions studied. Moreover, the risk for grazing cattle to acquire ARB and ARG from surface waters was considered high in Uganda but low in Spain and Sweden. The findings also suggest that aquatic birds could act as vectors for dissemination of ARB and ARG (including CARBA-producing Enterobacterales) from WWTPs and landfills to environments with potential human contact. Thus, identification of ways to avoid such spread is important.

Sampling of faeces from gulls.
Photo: Hanna Wokseпp

KEY EXPLOITABLE OUTCOME / OUTPUT	DESCRIPTION
Result 1 Dispersal of antibiotic resistance in water ecosystems	Monitoring water environments in 5 countries generated data on AMR dispersal in water ecosystems. This highlights the role of human activity in AMR spread and provides information crucial for environmental and health policies.
Result 2 Spread of AMR from WWTPs to grazing cattle	The risk for grazing cattle to acquire AMR from surface waters downstream of WWTPs or waters contaminated with human waste was considered high in Uganda but low in Spain and Sweden.
Result 3 Aquatic and synanthropic birds as vectors for transmission of AMR	The findings suggest that black-headed gulls and synanthropic birds can act as vectors for dissemination of resistant bacteria from WWTPs, landfills and wastes to environments with potential human contact.
Result 4 Datasets on gull movements and migrations	GPS-tracking of black-headed gulls generated novel datasets on the movements of gulls in Spain and Sweden. Through Movebank, these are publicly visible which will be useful for other research projects.

OUTLOOK / PERSPECTIVES

It is our belief that the knowledge generated in this project will facilitate formation of potential mitigation strategies for environmental dissemination of antimicrobial resistance. Thus, PAIRWISE may contribute to the global combat against antimicrobial resistance in a One Health dimension.

1. Cattle grazing by a river downstream of a WWTP. Photo: Anna Olsson.
2. WWTPs can act as point sources for AMR. Photo: Andy J. Green.

FUNDING INSTITUTIONS

AEI (Spain), FORMAS (Sweden), MHESR (Tunisa), RCN (Norway), SIDA (Sweden), SRC (Sweden)

PARRTAE

Probing Antibiotic Residues and Resistance transfer in Aquatic Environments

PROJECT COORDINATOR

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DURATION
3 years

STARTING
September 2021

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MOTIVATION BEHIND THE PROJECT

The PARRTAE project studied water resident bacteria, antibiotic resistance genes (ARGs) and antibiotic residues in several water environments including fresh water and ground water, the Baltic Sea, the North Sea and the Atlantic, and wastewater treatment plants (WWTPs) in Sweden, Norway, Belgium and Spain (Gran Canaria). The studies focused on indicator bacterial species *Escherichia coli*, *Vibrio* spp., and *Shewanella* spp. with the aim to determine prevalence of ARGs, ability to transfer ARGs on mobile elements to new recipients. The effects of the acquired plasmids on the physiology of the bacterial model recipients were analyzed using the brine shrimp (*Artemia*) model.

PROJECT OBJECTIVES

- Determine presence and prevalence of resistance genes in indicator bacterial species Escherichia coli, Vibrio spp., and Shewanella spp. as well as presence of antibiotic residues in the collected water samples.
- Determine transfer of mobile genetic elements between isolates, with focus on plasmids.
- Determine the impact of transferred mobile elements with ARGs on the physiology of the receiving bacterial isolate, particularly the influence on virulence.

RESEARCH METHODOLOGY AND IMPLEMENTATION SUMMARY

The consortium collected and analyzed > 1000 E. coli and > 500 Shewanella and Vibrio spp. from all included water locations. Phenotypic and genotypic antibiotic resistance was determined, and a subset of the isolates were whole genome sequenced. Horizontal transfer abilities of ARGs were studied and found in ca 40% of E. coli. The effects of the acquired plasmids on receiving isolates on host immunity and virulence were analyzed using the Artemia model system.

SUMMARY OF MAIN RESULTS

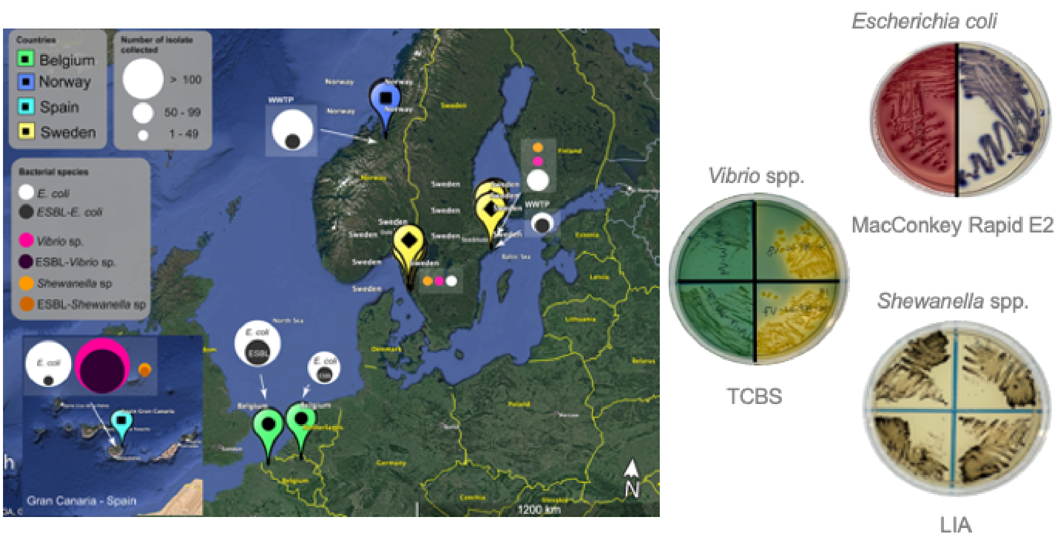
The results showed lower levels of ARGs in isolates from areas with low anthropogenic impact. Surface waters from human-impacted sampling sites had higher levels of bacteria and presence of ARGs. A majority of *E. coli* isolates from WWTPs were ESBL-positive and multiresistant and several were pathogenic. High levels of AGRs were found in *Vibrios* isolated from marine samples
Presence of antibiotic residues were found in fresh and brackish surface waters in all countries with the highest concentrations in WWTPs and brackish sediments.
ESBL positive *E. coli* could transfer ARG plasmids to recipient *E. coli* and *V. campbellii*. *Vibrio* transconjugants were more virulent in the Artemia model than its corresponding recipient isolate without plasmid.

KEY EXPLOITABLE OUTCOME / OUTPUT	DESCRIPTION
Result 1 Scientific publications	The impact of results generated are mainly expected to be scientific, findings on antibiotic resistance- and virulence genes in *E. coli, Shewanella* spp and *Vibrio* spp are expected to generate impact in the form of several publications.
Result 2 WWTP and authorities interactions	Project partners have initiated discussions with WWTPs and other authorities in Belgium regarding the findings of high levels of lincomycin-, fluoroquinolone- and sulfonamide-residues in water.
Result 3 Human and animal pathogen monitoring	The project unexpectedly isolated several novel species of Shewanella spp and emerging pathogenic species and clones of *Escherichia* spp. These findings might have impact on warning systems for emergence of novel pathogenic species, particularly in relation to global warming.
Result 4 Development of methods	Two novel methodologies to measure antibiotic residues in water and in sediment samples have been developed by the Belgian partners.

OUTLOOK / PERSPECTIVES

There are still relatively low levels of ARGs in natural water-residing *E. coli* and *Shewanella* in fresh and brackish waters but high levels of AGRs were found in *Vibrios* and *Shewanella* isolated from marine samples. Notably, ESBL *Vibrios* were identified from sea water, while Shewanella spp. might harbour novel variants of eg OXA resistance genes. The study confirmed the role of WWTPs as a main source of ESBL and multiresistant *E. coli*, and we found that ESBL positive E. coli could transfer ARG plasmids to recipient *E. coli* and *V. campbellii* but not to S. algae. The Vibrio transconjugants with acquired plasmid(s) were more virulent in the *Artemia* model. Hence, future studies on the impact of horizontal gene transfer needs to take a broader approach and study virulence aspects in relation to risks in eg. aquaculture animal health.

Probing Antibiotic Residues and Resistance in Aquatic Environments (PARRTAE)



FUNDING INSTITUTIONS

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

SARA

Surveillance of Emerging Pathogens and Antibiotic Resistances in Aquatic Ecosystems

PROJECT COORDINATOR

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PROJECT PARTNERS

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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
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MOTIVATION BEHIND THE PROJECT

Appropriate methods for wastewater-based epidemiology and a better understanding of the fate of pathogenic viruses and antibiotic resistant bacteria from source to river basin and estuary are urgently needed. The SARA project determined the prevalence of pathogenic viruses (including SARS-CoV-2), microbial indicators, antibiotic resistance and microbial source tracking (MST) markers in wastewater, surface water, coastal seawater, sediment and bivalve molluscan shellfish (BMS) in catchments located in different climatic zones (Sweden, Germany, France, Spain, Portugal, Israel, Mozambique and Uganda).

PROJECT OBJECTIVES

- Checking the suitability of SARS-CoV-2 detection in raw wastewater as an early warning tool and allowing an accurate estimation of COVID-19 infectivity and spread
- Method harmonisation for cultivation methods (indicators and antimicrobial resistances) and nucleic acid extraction
- Better understanding of the occurrence and fate of enteric viruses, antibiotic resistances and microbial source tracking markers in the aquatic environment
- Evaluation of the role of sediment and BMS as integral monitoring element and reservoir of viruses, antibiotic resistant bacteria, and antibiotic resistance genes
- Determination of the impact of climate and extreme weather events on the transmission of emerging pathogens and antibiotic resistances
- Microbial risk assessment for water resources
- Development of monitoring recommendations
- Dissemination of results to the scientific and end-user communities

RESEARCH METHODOLOGY AND IMPLEMENTATION SUMMARY

The project used culture and molecular methods to monitor the different model areas. The culture-based analyses were performed in a timely manner in all partner laboratories. For molecular analysis, concentration and extraction were performed promptly. The resulting nucleic acid extracts were stored at -80°C until sent to the specialised partner laboratory for final analysis. To ensure comparability of results, these procedures were harmonised during the first phase of the project and summarised in a booklet (available on the SARA website www.sara-project.info).

SUMMARY OF MAIN RESULTS

The project results show i) the benefits of wastewater-based surveillance for SARS-CoV-2, ii) differences in the prevalence and relative abundance of extended-spectrum beta-lactamase-producing E. coli and antimicrobial resistance genes between the model sites, with the highest prevalence in Uganda and the lowest prevalence in Sweden, iii) the limited reduction of antibiotic resistance in the Ugandan stabilisation ponds, iv) the increase in microbial contamination following heavy rainfall after dry periods, demonstrating increased risk due to climate change, and v) the high persistence of antibiotic resistant environmental bacteria in surface waters, vi) the suitability of crAssphage as a sensitive marker for the identification of human faecal contamination, and vii) the increasing evidence that bacteriophages play a relevant role in the spread of antimicrobial resistance in aquatic ecosystems.

KEY EXPLOITABLE OUTCOME / OUTPUT	DESCRIPTION
Result 1 Establishment of African partners laboratories	The European partners helped to develop the African partner laboratories and provided training. This included culture and molecular detection of antibiotic resistant bacteria and indicator microorganisms.
Result 2 Suitability of wastewater-based epidemiology	The project demonstrated the usefulness of wastewater-based surveillance for SARS-CoV-2 and antibiotic resistance genes. The knowledge gained was fed directly into the international GLOWACON network.
Result 3 Comparative analysis of the environmental antimicrobial resistance situation in the study areas	Levels of antibiotic resistance were highest in Uganda and lowest in Sweden (see Figure 1). In line with Sweden's strategic programme, SARA recommends strict regulation of antibiotic use in humans and animals.
Result 4 Antimicrobial resistance monitoring recommendations	The SARA results suggest the use of (i) extended-spectrum beta-lactamase (ESBL)-producing E. coli, (ii) environmental ESBL-producing bacteria (see Figure 2), (iii) specific ARGs detected by PCR, and metagenomic analyses for antibiotic resistance monitoring.
Result 5 Identification of human faecal contaminations	SARA results supports the use of crAssphage as a primary MST marker for identifying human faecal contamination, owing to its superior reliability and higher concentrations and prevalence across all samples (see Figure 3).

OUTLOOK / PERSPECTIVES

The project shows that wastewater monitoring can detect the spread of zoonoses and the early onset of pandemics, and that microbial source tracking can identify human contamination of water resources and help develop protective measures. Data on antibiotic resistance genes and bacteria show the impact on the aquatic ecosystem, also from a One Health perspective, and demonstrate the importance of environmental bacteria and viruses in the spread of antibiotic resistance. The SARA project has demonstrated how standardised monitoring can be established. The results and methods will help policy makers to develop standardised monitoring programmes and improve regulation.

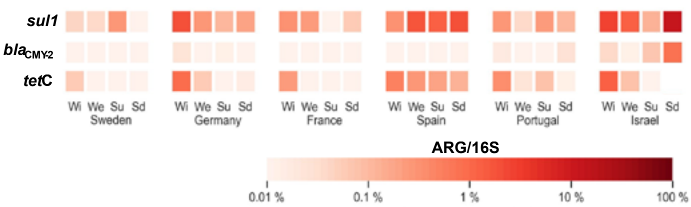

Figure 1. Heatmap of the relative abundance (normalised to 16S rRNA gene) of key antibiotic resistance genes in the different catchment areas (Wi – wastewater treatment plant influent (WWTP), We – WWTP effluent, Su – surface water upstream WWTP, Sd – surface water downstream WWTP). The results show that the relative abundance of antibiotic resistance genes was higher in Uganda. In contrast, significantly fewer antibiotic resistance genes were detected in samples from the Swedish model area.

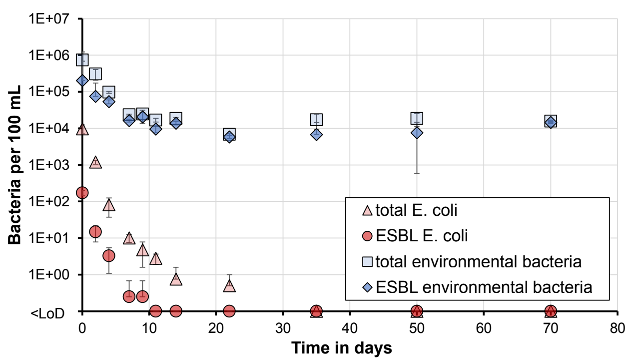

Figure 2. Results of a surface water microcosm experiment. Data show the concentration of total E. coli, extended beta-lactamase (ESBL)-producing E. coli, total oligotrophic bacteria and ESBL-producing oligotrophic bacteria over the incubation period of 70 days. The results show that human-associated antibiotic-resistant bacteria, such as ESBL-producing E. coli, die off faster and that environmental ESBL-producing bacteria persist in aquatic ecosystems.

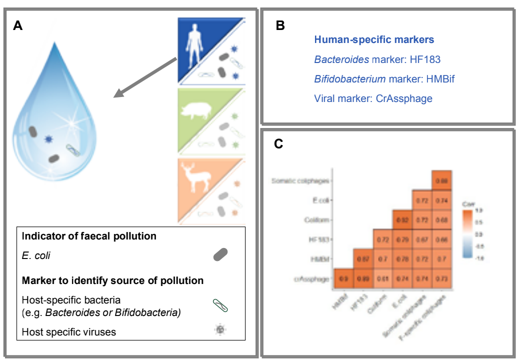

Figure 3. (A) Principle of microbial source tracking (MST) using characteristics of microorganisms strongly associated with specific host species to trace faecal pollution to specific animal species (including humans), (B) human-specific MST markers, and (C) Spearman correlation indices between culturable parameters and MST molecular markers showing significant correlations between faecal indicators and human-specific source tracking markers in the study areas.

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

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PROJECT PARTNERS

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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

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MOTIVATION BEHIND THE PROJECT

Aquaculture of Pacific oysters (*Magallana gigas*) coupled to their raw consumption could be a main gateway for spread of antibiotic resistance (AR), but only little is known about AR in oyster aquaculture. Oysters are cultured in highly anthropized and partly polluted coastal environments, and as filter-feeders they can act as bio-reactors that concentrate contaminants and microorganisms, thereby accelerating horizontal gene transfer and the spread of antimicrobial resistance genes (ARGs). SPARE-SEA investigates whether the explicit "One health'-connection of environmental, animal and human health of oyster aquaculture in intensively used coastal areas can accelerate antibiotic resistance transfer, especially towards potential pathogens.

PROJECT OBJECTIVES

- Catalogue ARGs associated to oyster aquaculture by combining classical microbiological assays with state-of-the-art "omics" techniques
- Identify factors that influence sources, sinks, and transfer routes of antibiotic resistance within and between environmental compartments exposed to oyster aquaculture along a gradient of usage intensity and agrochemical contaminant input
- Experimentally confirm the cumulative effects of pollutants and environmental factors as drivers of ARG spread
- Assess the effect of specific anthropogenic pressures on the potential for spread of ARGs in oyster aquaculture on the EU scale

RESEARCH METHODOLOGY AND IMPLEMENTATION SUMMARY

SPARE-SEA investigated oyster culture sites along gradients of anthropogenic pollution (BREST: pig farming, harbor: residual antibiotics / heavy metals; THAU: vineyards: copper; EBRO: rice paddies: agrochemicals; SYLT: pristine national park: none) and characterized oyster and environmental resistomes by resistance phenotyping and metagenomic sequencing. Samples were molecularly profiled using target enrichment protocols combined with culture based sequencing of pooled isolates and single genomes. The cumulative effects of target pollutants and environmental variation on ARG enrichment were tested in experimental setups replicated in all study sites. Connectivity of oyster resistomes was synthesized across different environmental compartments in mesocosms and the wild.

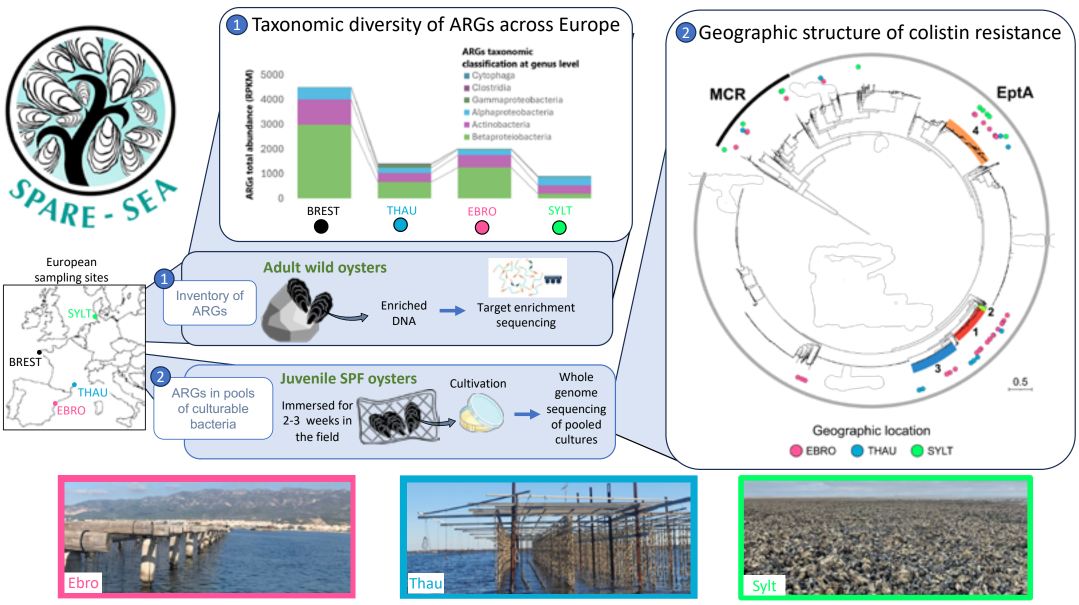
SUMMARY OF MAIN RESULTS

Metagenomic DNA enrichment and sequencing of pooled isolates showed site-specific resistomes with high complexity and diversity (11,225 ARGs in 276 gene families) that assembled within a few weeks when the same oyster cohorts were exposed to the different environmental conditions at each site. Detailed analyses of highly diversified eptA resistance genes revealed a novel resistance mechanism against the last resort antibiotic *colistin*. Together, these results suggest a highly dynamic nature of oyster resistome assembly by a combination of environmental drivers and anthropogenic pollutants like copper and pesticides that can co-select for AMR and further drive the spread of ARGs.

KEY EXPLOITABLE OUTCOME / OUTPUT	DESCRIPTION
Result 1 Novel resistance mechanisms against last-resort antibiotics	*Vibrio* can be a source of diversified antibiotic resistance genes employing novel resistance mechanisms against colistin. These genes confer intrinsic resistance, but represented a low risk of mobilization.
Result 2 Impact of oyster transfer practices on ARG spread across Europe	Oysters acquired environment colistin and ampicillin resistance genes specific to each European site after only two weeks in the field. The unregulated practice of oyster transport could thus contribute to ARG spread.
Result 3 Copper can co-select for ARGs in coastal areas used for oyster farming	Several ARGs co-occured with heavy metal resistance. High copper concentrations in oyster tissues from the Mediterranean suggests that environmental contamination by heavy metals can drive the co-selection of ARGs.
Result 4 Antibiotic exposure of oyster larvae has long-lasting effects on immunity and susceptibility to pathogens	Treatment of oyster larvae with Nuflor did not increase in the prevalence of resistant bacteria, but altered adult oysters' susceptibility to pathogens, indicating potential long-term effects on their immune system.

OUTLOOK / PERSPECTIVES

It is crucial to identify the selective pressures that generate diversity in novel antimicrobial resistance mechanisms, and characterize their mobility and transferability to raise awareness of potential risks. The dynamic acquisition of resistant strains from the environment along with the low genetic mobility suggests that oysters are only a minor source of AMR in the coastal environment. Nevertheless, the common, yet still unregulated practice of oyster transfer between culture sites represents a potential vehicle for ARG transport, and therefore have important implications for the spread of AMR in European coastal environments, calling for adjusted risk management strategies.



FUNDING INSTITUTIONS

AEI (Spain), ANR (France), BMBF (Germany), MUR (Italy)

THEME 2

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,
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PROJECT PARTNERS

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—

GFZ German Research Centre for Geosciences - Germany

—

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

—

International Iberian Nanotechnology Laboratory (INL) - Portugal

—

Universidad Autónoma de Madrid (UAM) - Spain

—

University of Santiago de Compostela (USC) - Spain

DURATION
40 months

STARTING
September 2021

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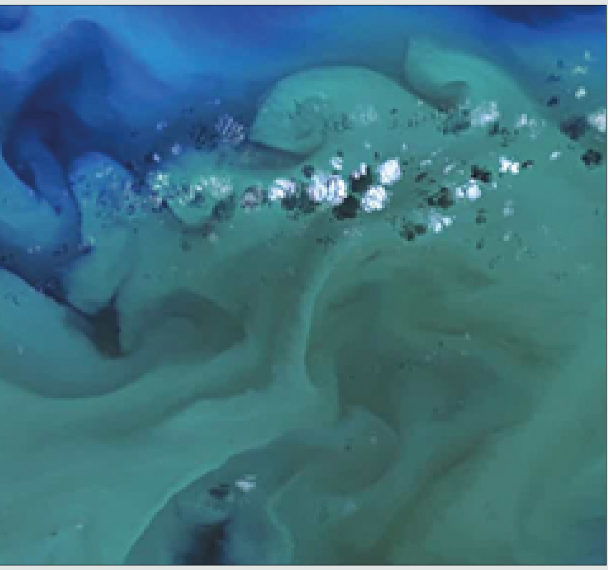


MOTIVATION BEHIND THE PROJECT

Eutrophication of water bodies significantly contributes to the proliferation of Harmful Algal Blooms (HABs) dominated by toxin-producing cyanobacteria, posing substantial risks to human health and the integrity of aquatic ecosystems. Mitigating the occurrence of harmful cyanobacterial blooms is crucial for maintaining and sustainably developing functional ecosystems. To this end, timely prediction of the occurrence and spread of cyanotoxins resulting from HABs is paramount, enabling the implementation of effective control measures. Accurate prediction necessitates the development of a robust modelling framework grounded in a thorough understanding of the key processes that drive HAB formation.

PROJECT OBJECTIVES

The AIHABs project aims to develop an integrated Artificial Intelligence (AI) model for predicting Harmful Algal Blooms (HABs) in freshwater and coastal ecosystems by pursuing the following objectives:

- Characterising HABs at selected study sites using physicochemical, biological, and genetic data.
- Developing mathematical models to simulate Chlorophyll-a as an indicator of HABs at these sites.
- Acquiring hyperspectral and multispectral imagery of the study sites and analysing it to characterise HABs.
- Applying machine learning algorithms to create a predictive model for Chlorophyll-a, a key indicator of HABs.

RESEARCH METHODOLOGY AND IMPLEMENTATION SUMMARY

The AIHABs technical work consists of five work packages designed to: (i) select study sites, conduct water quality sampling, and acquire multispectral and hyperspectral images using remote sensing technologies (drones and orbital satellites); (ii) perform mathematical modelling of the selected sites; (iii) develop portable sensors for the in situ detection of cyanotoxin species, specifically microcystin; and (iv) build an AI model for forecasting harmful algal blooms (HABs) by integrating results from field measurements, mathematical modelling, and remote sensing.

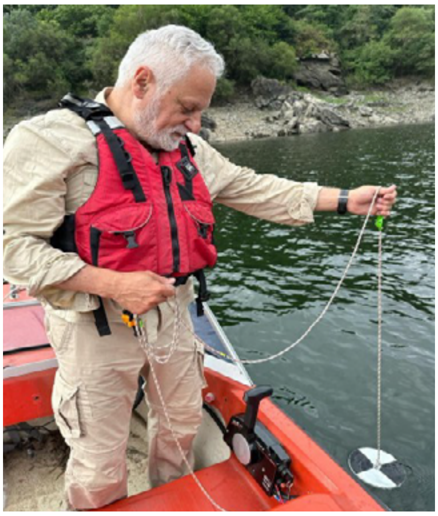
SUMMARY OF MAIN RESULTS

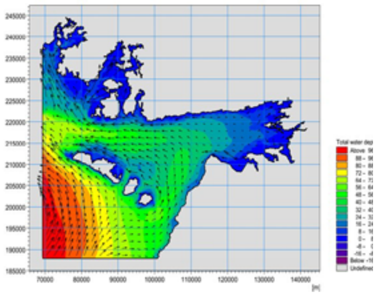
Three sites—Belesar Reservoir, Miño Estuary (Spain), and Orlík Reservoir (Czech Republic)—were studied to assess water quality and cyanotoxins. Cyanobacteria species and toxins were identified through genetic analyses, while metagenomics and fluorometry characterised blooms. MIKE-based water quality models simulated Chl-a dynamics using geomorphological, meteorological, and hydrological data. A portable microcystin sensor was developed and tested, with initial progress on a saxitoxin sensor. Hyperspectral imagery from satellites and drones, combined with machine learning models, predicted Chl-a concentrations. By integrating measured data and modeling results, the study advanced methods for assessing and predicting cyanobacterial bloom risks.

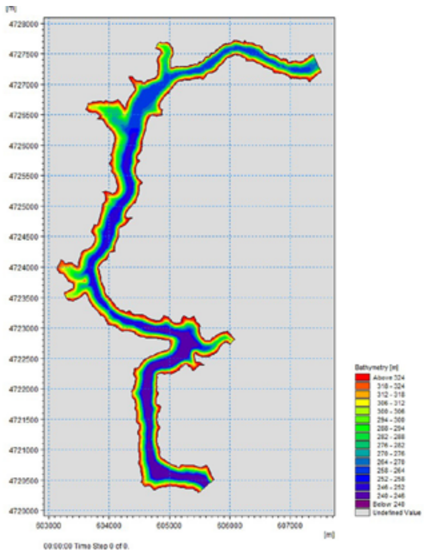
KEY EXPLOITABLE OUTCOME / OUTPUT	DESCRIPTION
Result 1 Water Quality Data for Cyanobacteria Characterisation	Field data on physicochemical parameters, nutrients (N, P), and Chl-a were collected, alongside chemical analysis to identify cyanotoxins.
Result 2 Simulated Nutrient and Chl-a Concentrations	Calibrated water quality models provide spatial and temporal distributions of nutrient concentrations (N, P) and Chl-a, offering key insights into ecosystem dynamics.
Result 3 Portable Microcystin Sensor	Automated portable measuring system for in situ microcystin monitoring allows 1-month operator-free analysis of microcystin total content in waters compliant with regulatory levels (less than 1 μg/L)
Result 4 Portable Saxitoxin Sensor	A portable detection system for saxitoxin was demonstrated to work for high concentrations. Ongoing work is being done to improve the detection and robustness limits.

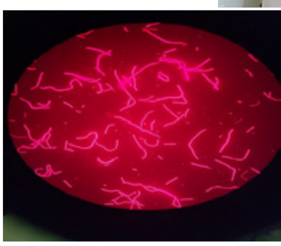
OUTLOOK / PERSPECTIVES

The AIHABs project delivers a cutting-edge model to characterise key features of Harmful Algal Blooms (HABs), enabling predictions of their occurrence. This facilitates timely actions to reduce risks associated with toxic cyanobacterial blooms in freshwater and marine environments, protecting public health and recreational water use. The model supports mitigation of HABs' socio-economic impacts on fisheries, tourism, and water management. It also enhances water quality in line with the EU Marine Strategy Framework Directive and the EU Bathing Water Directive, contributing to improved marine and bathing water standards and promoting sustainable use of aquatic resources.









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

PROJECT COORDINATOR

Joakim LARSSON
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PROJECT PARTNERS

Institute of Marine Research - Norway

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Umeå university, Sweden

—

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

—

Technical University of Denmark - Denmark

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University of Bucharest - Romania

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University of South Bohemia in České Budějovice - Czech Republic (self-funded partner)

DURATION
3 years

STARTING
September 2021

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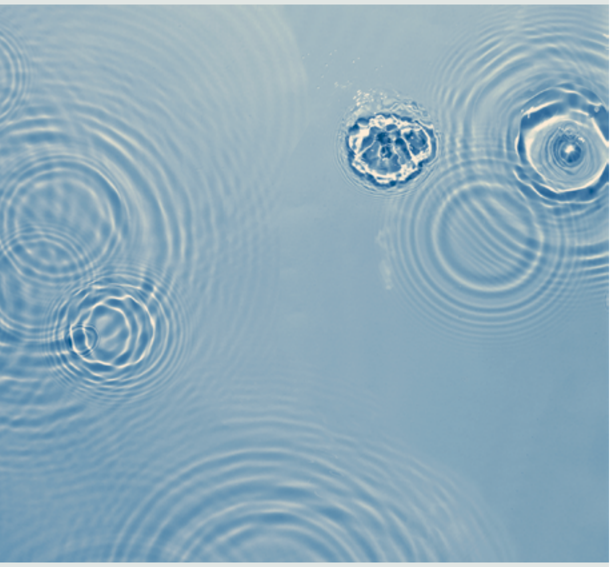


MOTIVATION BEHIND THE PROJECT

It is well known that antibiotics can select for and drive antibiotic resistance development. However, antibacterial biocides (i.e., chemicals with antibacterial properties that are not used for treating infections) can also do this through a process called co-selection. The overall aim of BIOCIDE is to determine how antibacterial biocides contribute to the development and spread of antibiotic-resistant bacteria in different aquatic/marine ecosystems, and to inform and enable measures that ultimately protect human health and safe water resources for both humans and wildlife.

PROJECT OBJECTIVES

1. Prioritize biocides for investigation based on existing databases.
2. Develop chemical analysis protocols for biocides in various samples.
3. Screen for biocide presence and levels in aquatic ecosystems in Europe and elsewhere.
4. Establish a dose-response matrix for biocides and bacteria to predict PNECs for growth that will also be protective against antibiotic resistance co-selection.
5. Validate the relationship between PNECs for growth and resistance selection in aquatic communities.
6. Assess biocides' ability to induce HGT of antibiotic resistance genes.
7. Study antibiotic resistance driven by metal-based antifouling agents.
8. Describe co- and cross-resistance mechanisms in bacteria from aquatic environments.
9. Identify genetic bases for biocide resistance using functional metagenomics.
10. Integrate biocide-specific data on e.g. potential for co-selection into the BacMet database, to expand its use for risk assessment.
11. Assess risks of biocides promoting antibiotic resistance in aquatic environments.
12. Develop an evaluation scheme with authorities for incorporating resistance risks into regulations.

RESEARCH METHODOLOGY AND IMPLEMENTATION SUMMARY

The project employed state-of-the-art analytical chemistry methods and adopted them for antibacterial biocides. A wide set of samples from different aquatic environments and wastewaters across the world were possible to analyze through collaborations and by taking advantage of sampling campaigns originally launched for other purposes. Conceptually novel approaches, including massively parallel growth analyses, intricate genetic labelling-strategies and novel gene-transfer analyses were adopted for generating effect levels for risk assessment. AI-based methods have greatly increased our knowledge about the vast diversity of biocide resistance mechanisms. Novel experimental approaches for functional screening of unknown resistance genes were developed.

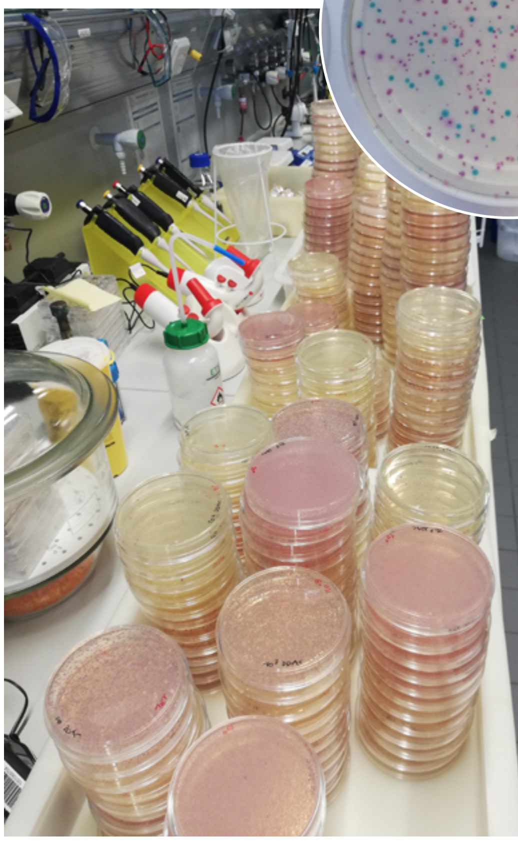
SUMMARY OF MAIN RESULTS

We have developed state-of-the art chemical analysis protocols for 32 organic antibacterial biocides and measured these in aquatic environments including wastewaters from ca 50 countries. To facilitate risk assessment, we have generated effect data for growth, selection and gene transfer, utilizing over 50 bacterial species individually as well as complex communities. We have studied co-selection on ship hulls painted with copper- and zinc-based antifouling paints. We are making a major update and revision of an existing database on biocide-resistance genes, called BacMet, also with the intention to be a resource for a wider set of users.

KEY EXPLOITABLE OUTCOME / OUTPUT	DESCRIPTION
Result 1 Analytical methods for antibacterial biocides	Quality-assured chemical analytical methods for a large set of prioritized antibacterial biocides, applicable to a wide set of matrixes, will be useful for both academics, regulators and industry.
Result 2 Effect data for antibacterial biocides	Effect data for growth, selection and horizontal gene transfer for a large set of prioritized antibacterial biocides will greatly improve possibilities to assess and manage risks for antibiotic resistance development
Result 3 Update of BACMET	The update of the BacMet database includes AI-based predictions of resistance genes, and adaptations to become a resource for a wider set of users, including academics, regulatory bodies and industry.
Result 4 Resistance task force	The establishment of a dedicated "resistance task force" within a standardization committee in the EU will increase possibilities to consider risks for co-selection of resistance during approval of biocides

OUTLOOK / PERSPECTIVES

The progress made in the BIOCIDE project has enabled better opportunities to assess risks for antibiotic resistance development driven by exposure to antibacterial biocides in different settings. A better understanding of risks can in turn inform and direct mitigations. Having said that, there are still major knowledge gaps in what role biocides play as potential indirect drivers of antibiotic resistance. The research performed has also led to new ideas and research, including a recently funded Swedish project on potential co-selection by rare-earth metals.



FUNDING INSTITUTIONS

BMBF (Germany), FORMAS (Sweden), IFD(Denmark), RCN (Norway), SRC (Sweden), UEFISCDI (Romania)

Bundesministerium für Bildung und Forschung FORMAS Innovation Fund Denmark The Research Council of Norway Swedish Research Council uefiscdi

CONTACT

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

PROJECT COORDINATOR

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Helmholtz Zentrum Muenchen - Germany

PROJECT PARTNERS

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Lior Guthmann, The Hebrew University of Jerusalem - Israel

Susanne Rath, University of Campinas - Brazil

Ole Lund, Technical University of Denmark - Denmark

DURATION
3 years

STARTING
September 2021

CONTACT
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MOTIVATION BEHIND THE PROJECT

The use 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 consequences for the functioning of aquatic ecosystems. The effects might be more pronounced if different compounds with antimicrobial properties are applied in combination like antibiotics against bacterial pathogens and eucaryotic pathogens.

PROJECT OBJECTIVES

- Assessment of nontarget effects of antimicrobial compounds used in aquaculture
- Analysis of combined effects of dual antibiotics use in aquaculture also for the treated fish species
- Compare responses across different climatic zones
- Define targeted mitigation strategies and sustainable forms for aquaculture.

RESEARCH METHODOLOGY AND IMPLEMENTATION SUMMARY

We study the consequences of antimicrobial/-parasitic (florfenicol and peracetic acid) application in aquaculture for cultured fish species and for the aquatic environments using controlled model systems. To consider the variability of aquaculture practices worldwide several showcases have been selected, including freshwater and marine aquaculture systems located in warmer and temperate regions respectively. For one showcase a targeted mitigation to reduce the pathogen pressure and subsequently the use of antimicrobials, applying an integrated multitrophic approach was tested. We complemented the obtained data with results from samplings which were done near real fish farms in the countries of the different showcases.

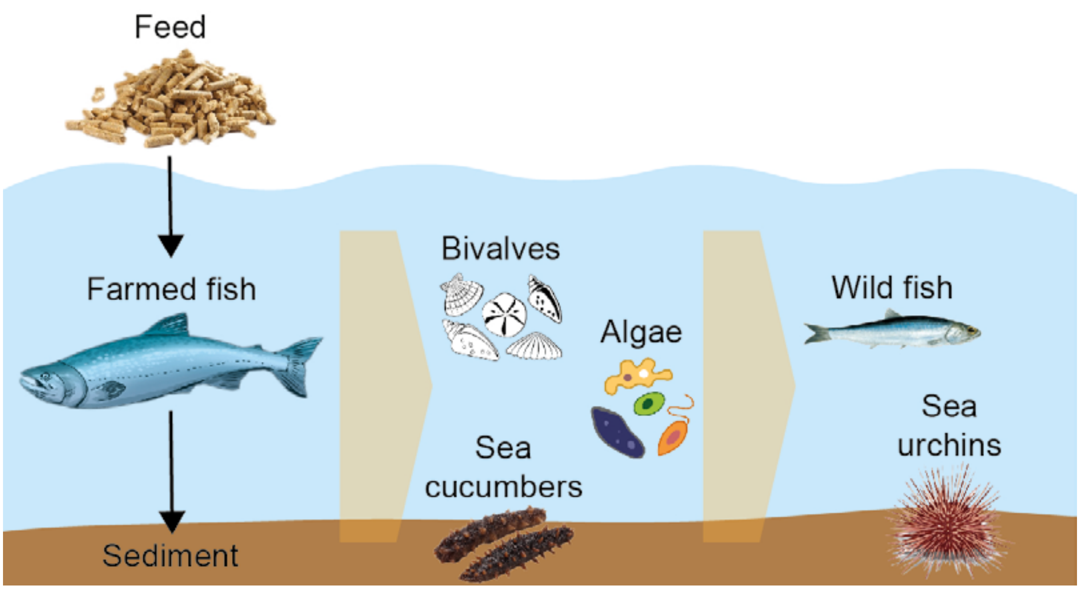
SUMMARY OF MAIN RESULTS

Despite the antimicrobial compounds were applied for each showcase in a comparable dose, our data indicates that the consequence of the antibiotic therapy strongly depends on the studied aquaculture system. The application of an integrated multitrophic approach to mitigate effects of aquaculture induced clear shifts in the microbiome of the fish used for aquaculture as well as the aquatic environment compared to control systems

KEY EXPLOITABLE OUTCOME / OUTPUT	DESCRIPTION
Result 1	In general effects on aquaculture systems in warmer regions microbiomes and resistomes differed compared to the responses temperate regions
Result 2	The age of the fish was a strong modulating factor. Changes in the microbiome structure and function for juvenile fish species were more pronounced and also visible in the post treatment phase compared to older
Result 3	Microbiomes of non-target organisms of the studied aquatic ecosystems including mussels and urchins were significantly affected by the applied antibiotic
Result 4	The application of an antiparasitic compound increased the effects of the antibiotics mainly in the water body

OUTLOOK / PERSPECTIVES

Our results have been discussed with local stakeholders in Germany, Brazil and Israel, resulting in first approaches to reduce fish densities in aquaculture tank systems to avoid the spread of parasites and fish pathogens, thus avoiding the use of compounds with antimicrobial properties – this approach for a more sustainable aquaculture needs to be further promoted in the future.



FUNDING INSTITUTIONS

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

PHARMASEA

Presence, behavior and risk assessment of pharmaceuticals in marine ecosystems

PROJECT COORDINATOR

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PROJECT PARTNERS

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University of Stavanger, Department of Chemistry, Bioscience and Environmental Engineering - Norway

University of A Coruña, Instituto Universitario de Medio Ambiente - Spain

Institute of Oceanography–Spanish National Research Council, Centro Oceanográfico de Murcia - Spain.

DURATION
3 years

STARTING
September 2021

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MOTIVATION BEHIND THE PROJECT

Active Pharmaceutical Ingredients (APIs) have been recognized as important contaminants of emerging concern in aquatic ecosystems. Their presence originates from large consumption in human and veterinary medicine, agriculture, aquaculture and limited removal by wastewater treatment plants. The risk of pharmaceuticals in coastal areas has been ignored for a long time on the presumption that oceans have an unlimited dilution capacity. However, the increasing number of studies pointing out the ubiquitous occurrence of APIs in worldwide water systems, highlighted the need to better investigate their occurrence and distribution especially in coastal areas, as well as their potential impact in marine species.

PROJECT OBJECTIVES

- To enhance scientific knowledge on occurrence, fate and effects of apis in european coastal ecosystems, mapping priorities on a large geographical scale,
- To provide a reliable and flexible strategy for apis detection and monitoring in water systems,
- To identify a list of priority apis and their mixtures in regional coastal environments,
- Develop a scientifically sound software-assisted tool for environmental risk assessment of pharmaceuticals,
- To increase the general awareness on interactions between human and environmental health.

RESEARCH METHODOLOGY AND IMPLEMENTATION SUMMARY

PHARMASEA activities are divided in 5 different but deeply interconnected Work Packages aimed to answer multidisciplinary and integrated questions on the environmental presence of these substances, their bioavailability to non-target species, and the onset of adverse effects at different levels of biological organization, (i.e. from molecular alterations to organism health impairment), thus representing a novel and fundamental approach for developing an effect-based environmental risk assessment procedure for pharmaceuticals.

SUMMARY OF MAIN RESULTS

- Selection of the 67 more relevant emerging pollutants and development of analytical procedures for their detection in environmental matrices;
- Mapping occurrence and distribution of selected apis in more than 450 environmental samples seasonally collected over three years in the adriatic, western mediterranean, north sea, and north atlantic ocean;
- Effects characterization of 25 apis and 10 mixtures on 12 aquatic species through the execution of more than 20 experimental designs;
- Development of an integrated risk assessment procedure for apis in marine ecosystems;
- Communication and dissemination to > 100K people (scientists, public/private bodies, pharma industries, NGOs, citizens, teachers students).

KEY EXPLOITABLE OUTCOME / OUTPUT	DESCRIPTION
Result 1 Development of analytical protocols for APIs detection	Analytical protocols for APIs extraction, purification, separation and detection allow to measure more than 60 pharmaceuticals in seawater, sediments and marine species.
Result 2 Characterization of APIs effects in different species	A large number of sublethal effects has been described thanks to laboratory studies carried out within PHARMASEA, allowing to clarify most biologically reactive compounds and APIs mixtures.
Result 3 Software development for APIs environmental risk assessment	This tool enables the elaboration of heterogeneous typologies of data providing a science-based environmental risk assessment procedure for pharmaceuticals. It will be helpful for regulatory bodies and public authorities.
Result 4 Dissemination and stakeholders' engagement tools	The developed dissemination material can be downloaded from the website and includes Project Brochures, Roll-Up, poster, 2 PHARMASEA Surveys, 2 board games with instructions, explanation and cards to play.

OUTLOOK / PERSPECTIVES

PHARMASEA results produced both scientific and societal impacts as demontrated by the 100,000 reached people through games, podcast, workshops, exhibitions and suveys. The increased awareness on this environmental issue, facilitate the adoption of correct behaviours and future mitigation strategies to reduce pharmaceutical pollution. The delivery of the list of priority API, along with its proper dissemination, could influence the revision of the list of priority substances present in the Watch list of the WFD. Similarly, the overall elaboration of results with the WOE model can promote at a European level the development of specific monitoring strategies for such compounds.

The PHARMASEA Consortium at the AquaticPollutants final Conference. Frankfurt, October 2024.

The PHARMASEA Team Returns to European Researchers Night. Ancona, September 2024

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

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PROJECT PARTNERS

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SINTEF - Norway

University of Milano-Bicocca - Italy

Project SAS - Italy

VTT Technical Research Centre of Finland Ltd - Finland

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DURATION
3 years

STARTING
September 2021

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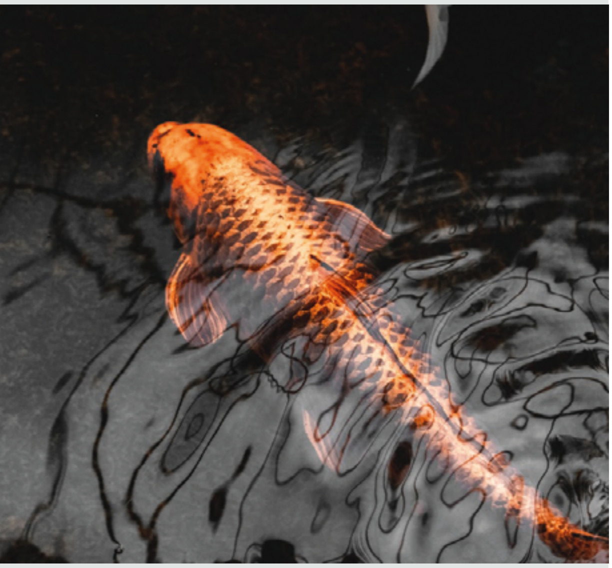


MOTIVATION BEHIND THE PROJECT

Contaminant of emerging concern (CECs) such as antibiotics, pathogens and antimicrobial resistant (AMR) bacteria in water bodies associated to intensive fish and animal farming, represent a great threat to the environment and human health. AMROCE project aims at developing **novel antibiotic-free antimicrobial strategies** to improve the animal and fish living conditions, reducing the use of antibiotics, the accumulation of CECs in the environment and AMR emergence. AMROCE innovations include **nano-formulated antibiofilm enzymes and marine-derived antimicrobials** for fish and animal feed, **antimicrobial and antibiofilm fish cage nets (FCN) and water filtration membranes (WFM)** embedded with metal oxide nanoparticles (MeO NPs).

PROJECT OBJECTIVES

- **Identification and extraction of antimicrobial marine-derived lipids and peptides**
- **Nano-formulation of antimicrobial enzymes, lipids and peptides for fish and animal feed** using simple and versatile nano-enabling technologies
- **Engineering of antimicrobial and antibiofilm FCN and WFM** from nano-enabled thermoplastic polymers and antimicrobial coatings
- **Validation of the novel nano-enabled materials** in exploitation environments
- **Nano-toxicity assessment** of the novel nano-enabled products.
- **Life cycle assessment and costing** (LCA/LCC) of the novel antimicrobial products

RESEARCH METHODOLOGY AND IMPLEMENTATION SUMMARY

AMROCE project comprises five work packages (WPs). In WP1, antimicrobial and antibiofilm nano-formulations of marine-derived lipids and peptides, and quorum quenching enzymes, as fish and animal feed additives are developed. Bulk or surface nano-engineered FCN and WFM incorporating MeO NPs with antimicrobial and antibiofilm activities are produced in WP2. WP3 evaluates the safety of the nano-enabling processes and products for workers and end users. LCA/LCC are employed in WP4 to optimise costs and minimise environmental impact of the novel manufacturing technologies and products. WP5 is dedicated to project management, communication and dissemination.

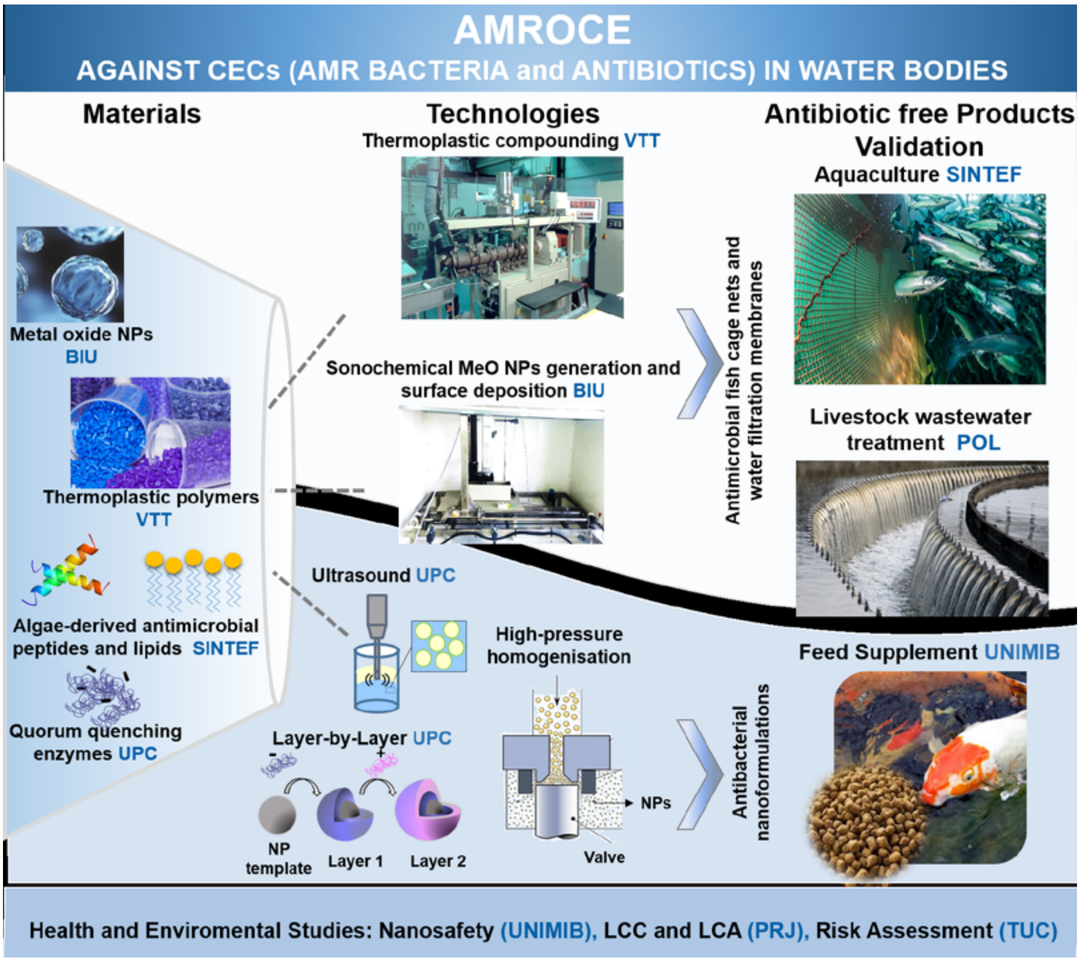
SUMMARY OF MAIN RESULTS

Antibiotic-free antimicrobial nanoformulations from marine-derived lipids and peptides, hybridised with antibiofilm enzymes demonstrated strong antibacterial and antibiofilm activities against human and fish pathogens, without inducing AMR. Nano-enabled FCN and WFM with durable antimicrobial and anti-biofilm properties were validated in exploitation environments. Zebrafish embryo toxicity tests demonstrated safety of the nanoformulated feed and nano-enabled FCN and WFM. Nanotoxicological studies next to life cycle assessments ensured the environmental and human safety of these products. Through technological innovation, AMROCE addressed major challenges related to AMR and environmental contamination, paving the way for sustainable practices in aquaculture and water treatment.

KEY EXPLOITABLE OUTCOME / OUTPUT	DESCRIPTION
Result 1 Novel marine-derived antimicrobials	Marine biomass-derived antimicrobial lipids and peptides are efficient against both Gram-positive and Gram-negative bacteria, showing lower potential for AMR development than conventional antibiotics.
Result 2 Bio-based antimicrobial nanoformulations	Novel antibiotic-free lipid and peptide nanoparticles, hybridised with anti-infective enzymes exhibit significantly improved antibacterial and antibiofilm properties, coupled to human/ environmental safety and lack of AMR.
Result 3 Antimicrobial nano-enabled FCN	Nano-enabled FCN embedded with antimicrobial CuO NPs efficiently and durably inhibit bacterial growth and biofilm formation, providing bacteria- and antibiotic-free aquaculture environment, and reducing the maintenance cost of the nets.
Result 4 Antimicrobial nano-enabled WFM	Nano-enabled polymer WFM embedded with antimicrobial CuO NPs reduce bacteria and biofilm attachment on their surface, ensuring durable filtration capacity, and bacteria-free reclaimed water from waste water treatment plants.

OUTLOOK / PERSPECTIVES

AMROCE project presents promising advancements in sustainable aquaculture and farming through the development of antibiotic-free antimicrobial technologies and products (TRL5-6). Future perspectives include scaling up the production of nano-enabled FCN, WFM and animal feed supplements while optimising their cost-effectiveness, nanosafety and environmental sustainability. Further research will focus on refining their antimicrobial efficacy, durability and regulatory alignment towards industry adoption and access to market, contributing to reduced antibiotic pollution and AMR globally.



FUNDING INSTITUTIONS

AEI (Spain), AKA (Finland) CSO-MOH (Israel), MUR (Italy), NCBR (Poland), RCN (Norway)

GreenWaterTech

Green Ultrafiltration Water Cleaning Technologies

PROJECT COORDINATOR

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PROJECT PARTNERS

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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

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MOTIVATION BEHIND THE PROJECT

Persistent chemicals of emerging concern and antibiotics are major threat to environment and humans. They include pharmaceuticals, PFAS, plasticizers, pesticides, etc. These chemicals are transported in water and accumulate in the entire ecosystem. There are no mechanisms for nature to decompose these chemicals and their concentration are now so high that it is necessary to find ways to separate them from water bodies, and ultimately completely and safely decompose them into harmless products that nature can handle.

PROJECT OBJECTIVES

- Produce hybrid materials with high water permeability, bearing immobilized oxidative enzymes effective for removing persistent pharmaceutical pollutants and pathogens.
- Synthesis and surface modification of mesoporous silica and porous oxide materials for heavy metal and CECs removal in wastewater including nanostructured metal (hydr)oxides, phosphate-modified silica and their mixtures and composites with high surface area, well-developed porosity and suitable surface properties for the efficient adsorption of hazardous metal pollutants and CEC from wastewater.
- Synthesis and deposition of photocatalytic TiO2, ZnO and Cu2O based nanocomposite coatings on woven optical fibres, polymers and glass velvets and for effective degradation of CECs. Up-scaling to small pilot scale synthesis of photocatalytic materials.
- Develop a modular small-scale ultra-filtration water cleaning pilot (about 1 m^3/day) consisting of a modular hybrid unit comprising of a series of steps for complete removal of targeted CEC and ARB. The materials will be implemented in the reactor modules in collaboration with industrial partners.
- Chembio analysis to evaluate CES and ARB in effluents in lab and real WWTP waters.
- Disseminate and exploit project results withing Aquatic Pollutant network and own networks, and beyond.

RESEARCH METHODOLOGY AND IMPLEMENTATION SUMMARY

Sustainable methods and material have been employed to develop a modular water cleaning technology consisting of three steps. In Step 1, nanoceria composite adsorbents were synthesized by low-temperature water-based precipitation methods. In Step 2 immobilization methods of enzymes on cost-effective substrates enzymes were developed. In Step 3, photocatalytic sulphated-titania and Cu-Zn nanocomposites were synthesized and deposited on high surface area glass fiber velvets and textiles. Semi-pilot tests of the 3-step modular reactor were performed on lab-scale and wastewater from a treatment plant (WWTP) in Stockholm. The work was designed to include industry and stakeholders for implementation and exploitation of results.

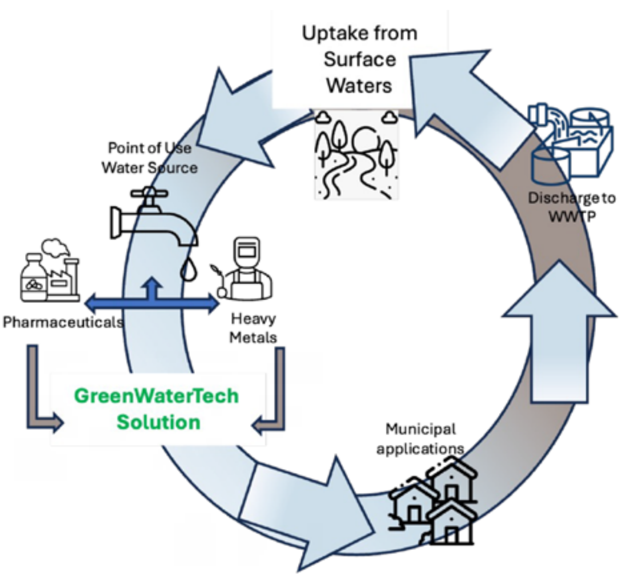
SUMMARY OF MAIN RESULTS

The GreenWaterTech project have performed research and developed remediation technologies to remove persistent chemicals in water. A new type of modular technology for ultra-filtration of water consisting of three serial treatment steps has been developed in the project. In the project we have used sustainable methods and materials, and devise “green” water remediation technologies that do not add extra environmental risks. Our results show that persistent chemicals and antibiotic in wasterwater from treatment plant effluents can be effectively removed. We have demonstrated a modular semi-pilot scale reactor that show that the technology can be up-scaled.

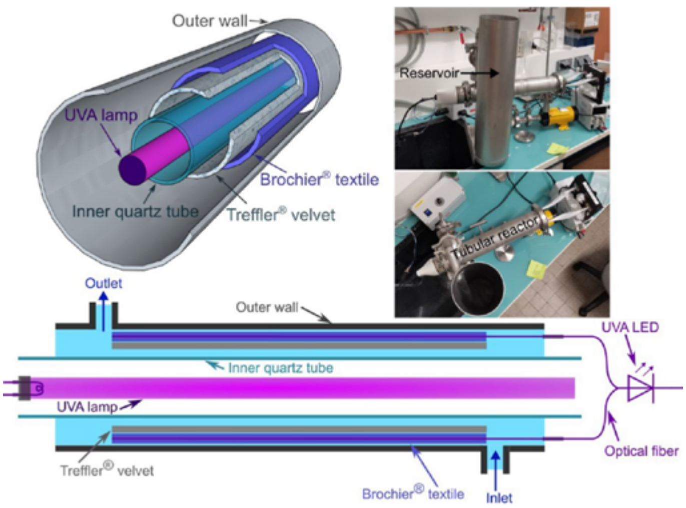
KEY EXPLOITABLE OUTCOME / OUTPUT	DESCRIPTION
Result 1	Development of materials and processes for synthesizing enzyme hybrid materials with improved CEC removal properties
Result 2	Development of materials and processes for synthesizing pseudo-enzymatic nanoceria adsorbent with CEC removal and antimicrobial properties
Result 3	Development of materials and processes for synthesizing sulfated titania photocatalysts with CEC degradation and antimicrobial properties
Result 4	Development of a 3-step modular hybrid reactor consiting of adsorbents, grafted enzymes and photocatalysts, on a semi-pilot scale reaching 1 m3/day cleaning of WWPT water.

OUTLOOK / PERSPECTIVES

Project results demonstrate that the modular configured adsorption and catalytic technologies can be used as final treatment of waterwater from WWTPs, or as a decentralised unit to clean point sources. The added advantage is that the technology is "green" and do not lead to secondary environmental problems such as waste deposit, new chemicals, and is safe to use. At present, the technology requires some basic engineering skills. Further development reducing the complexity and utilizing renewable energy source for powering and operation can however readily be done to make the GreenWatertech technology more user-friendly and even more energy effcient – beyond state-of-the-art.



GreenWaterTech's modular solution is suitable for achieving drinkable water directly at point of use.



Parallel designed configuration photocatalytsis reactor with self-luminous Brochier® textile and Treffler® glass fiber velvet. Yige Yan, et al. J. Catal. 438, 2024, 115704 (OA https://doi.org/10.1016/j.jcat.2024.115704).

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

B4Ceramics (B4C) - Denmark

Centre for Nanotechnology and Smart Materials (CeNTI) - Portugal

DURATION
3 years

STARTING
September 2021

CONTACT
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MOTIVATION BEHIND THE PROJECT

NanoTheC-Aba proposes a suitable solution to guarantee clean water from streams typically containing pathogens, toxic organic compounds deriving from natural processes or anthropic activities (wastewaters from hospital, fish farm, industries, white/black waters, contaminated groundwaters). The plant has been designed to mechanically remove particles, pathogens, large and small molecules, through a system of membranes with antimicrobial (AM) behaviors. The retentate of this process is not simply removed and accumulated as a solid waste, but is submitted to a chemical abatement in a thermocatalytic packed-bed reactor (TPBR), in which the catalytic process is activated by a mild heating.

PROJECT OBJECTIVES

The main objective of the project is to realise a pilot plant for wastewater treatment that is:

- Versatile (easy applicable to different wastewaters);
- Easy to use (suitable also for non-specialised users);
- Easy to maintain (using long-life membranes with a limited tendency to fouling);
- Efficient (possibility to perform several cycles of treatment to reach the maximum efficiency in both separation and abatement processes);
- As low energy-consuming as possible;
- In-flow to process large volumes of wastewater.

In addition the plant was designed to show two additional advantages;

- It has a modular structure and multipath connections that allows the use of just one unit or all units depending on the process to activate (the modular structure is also useful for checking the efficiency of each unit individually and to optimise each unit individually for new setups);
- The MF unit allows to house home-made or commercial flat membranes with the maximum size of 80x50 cm, the UF unit allows to house up to three home-made or commercial tubular membranes, the NF unit allows to house one home-made or commercial tubular membrane, the thermocatalyst can be packed in columns of variable size depending on the needs.

RESEARCH METHODOLOGY AND IMPLEMENTATION SUMMARY

Commercial TiO_2, chosen among other nanomaterials, covers the flat membrane, tested in antimicrobial process carried out in flow before the integration in the plant; NF/UF unit assembles one commercial NF membrane and one ZrO_2/SiC-UF membrane optimised at Liqtech in the first part of the project, CSF-Sil nanopowder was synthesized, tested and optimised in thermocatalytic experiments, then loaded on the SiC scraps tested in batch experiments before the use in the packed bed reactor. Finally, each unit was tested individually to optimize them one by one before the subsequent integration.

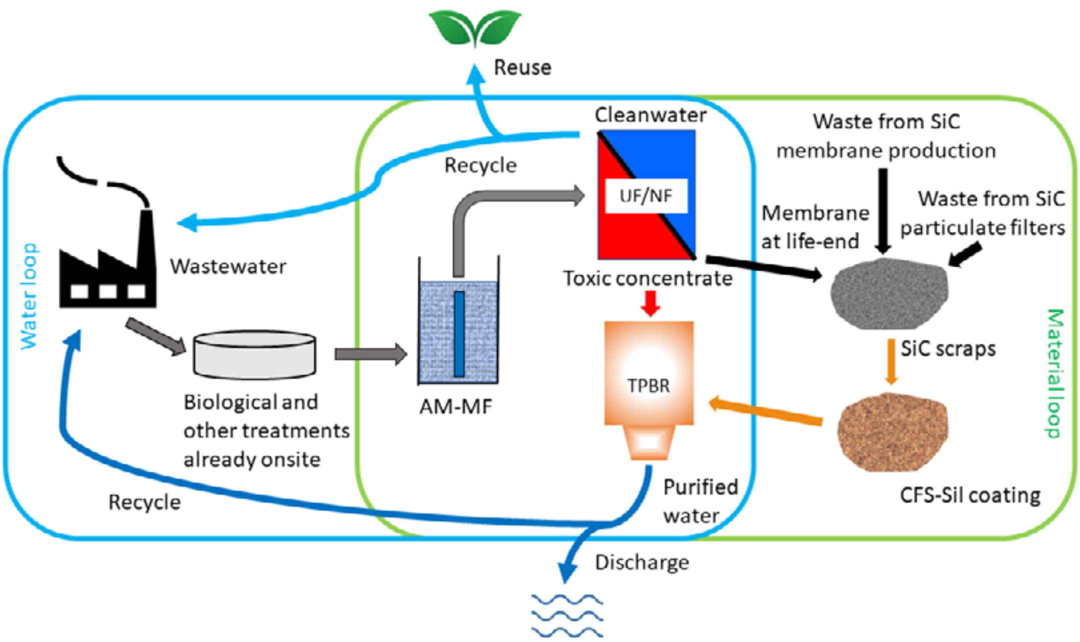
SUMMARY OF MAIN RESULTS

MF/UF processes reached good efficiency as SiC membranes chosen for filtering, compared to other ceramic and polymeric membranes present on the market, possess low filtration resistance (related to limited power consumptions) and high durability (related to lower overall costs for the lower replacement frequency).
All the functionalized membranes maintain the expected filtering ability after functionalization.
The membranes were efficiently functionalized modifying only limitedly the behaviors of the active nanomaterials (antimicrobial TiO_2 and thermocatalytic CSF-Sil).
SiC scraps, from membrane production byproducts, revealed a good support for CSF-Sil, increasing the sustainability of the entire process in a circular economy view.

KEY EXPLOITABLE OUTCOME / OUTPUT	DESCRIPTION
Result 1 Papers publication	The aspects related to synthesis, physico-chemical characterization and activity of the developed materials will be considered for the publication of scientific papers.
Result 2 NanoTheCAba pilot plant	A pilot plant composed of an Antimicrobial/Microfiltration unit, an Ultra and Nanofiltration unit and a Thermocatalytic packed-bed reactor was assembled and tested in wastewater treatment.

OUTLOOK / PERSPECTIVES

NanoTheC-Aba plant allows to remove pathogens and abate organic contaminants producing high quality water. The actual setup suffers from an issue at the level of the pump of the AM/MF unit that limits the possibility to work completely "in-flow": in the future we expect solving the problem to make the process more efficient.
The plant was widely tested with model contaminated solutions and/or with real water spiked with contaminants (pharmaceuticals and dyes), but a more extensive campaign to check real wastewaters is needed. We expect the plant can be transferred to a company for in situ wastewater treatment.



FUNDING INSTITUTIONS

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

FCT Fundação para a Ciência e a Tecnologia
/nnovation Fund Denmark
MUR

NATURE

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

PROJECT COORDINATOR

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PROJECT PARTNERS

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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
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MOTIVATION BEHIND THE PROJECT

Antibiotics are detected in two-thirds of the world's rivers, and waterborne drug concentrations are expected to rise by 65% by 2050. This contamination contributes to antimicrobial resistance (AMR), threatening ecosystems and human health. AMR is responsible for 33,000 deaths annually in the EU and costs €1.5 billion in healthcare and productivity losses. The NATURE project addresses this urgent issue by showcasing nature-based solutions (NBS) to reduce antibiotics, AMR, and pathogens from wastewater treatment facilities to estuaries. In comparison to current solutions, nature-inspired, costeffective
approaches provide significant environmental, social, and economic advantages, while enhancing resilience in our ecosystems.

PROJECT OBJECTIVES

The NATURE project provided scientific evidence about the use of NBS for water treatment at the catchment scale, from urban sources to coastal biota in estuaries, to reduce antibiotics, AMR and pathogens. Specific objectives include:

- Evaluation of secondary and tertiary wastewater treatment nature-based solutions to reduce selected aquatic pollutants.
- Assessment of different nature-based solutions in river basins to reduce aquatic Pollutants,
- Monitoring pollutant transformation by-products, scarcely studied so far in naturebased solutions,
- Identification of diagnostic indicators for future monitoring assessment of naturebased solutions, and
- Impact of nature-based solutions implementation on improving environment and human health

RESEARCH METHODOLOGY AND IMPLEMENTATION SUMMARY

The effectiveness of NBS for removing aquatic pollutants was assessed in three countries. In Denmark, decentralized NBS for wastewater treatment and a restored wetland were evaluated over two years. In Spain, two full-scale NBS used as tertiary treatments were compared seasonally with benchmark technologies, alongside the renaturalization of wastewater effluent-dominated streams. In Portugal, saltmarshes positive role on estuarine water and seafood quality was examined. Analytical methods for detecting antibiotics and ARGs were applied to water and biota samples. A risk assessment approach
incorporated toxicity data and bioassays to evaluate risks to seafood and human health.

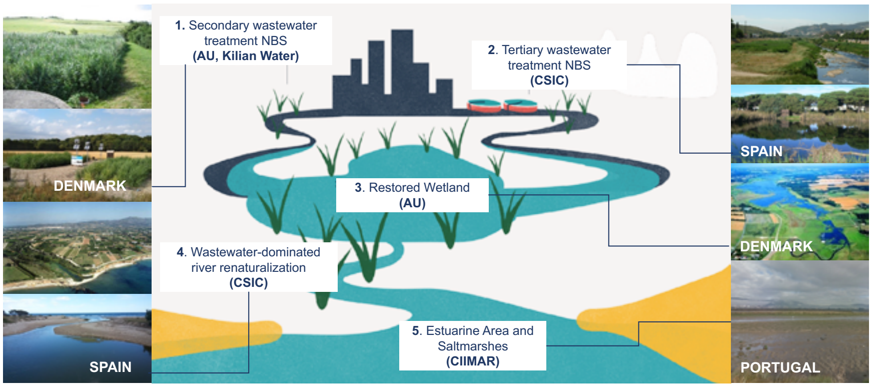
SUMMARY OF MAIN RESULTS

The study detected several antibiotics and ARGs in wastewater and surface water samples from Denmark, Spain, Portugal, and Mali, with azithromycin and trimethoprim being the most common. Decentralized NBS for wastewater treatment showed great effectiveness, and NBS were also more efficient than conventional methods for tertiary treatment, removing 70-80% of antibiotics and reducing AMR by 2-3 log units, compared to 40% and 1.5 log units in conventional treatments. Renaturalizing wastewater effluent-dominated streams improved antibiotic reduction by 30-40% compared to control streams. NBS also lowered water toxicity, reducing a possible impact on seafood.

KEY EXPLOITABLE OUTCOME / OUTPUT	DESCRIPTION
Result 1 NBS effectiveness	Studies from Spain, Denmark, and Portugal show that NBS, such as constructed wetlands and river restoration, significantly reduce antibiotics and AMR, providing an eco-friendly, cost-effective alternative to traditional wastewater treatments
Result 2 NBS microbiological impact	Microbiological assessments demonstrate that NBS improve water quality and significantly reduce the environmental impact of WWTPs on receiving water bodies. This highlights the potential of NBS as a sustainable alternative for wastewater management
Result 3 NBS toxicity	The implementation of NBS effectively mitigates the impact of pollutants on aquatic life and seafood, promoting healthier ecosystems and enhancing food safety.
Result 4 Toxicological assessment	The developed micro algae bioassay provide a reliable method for assessing the toxicity of water, enabling effective monitoring of pollutant impacts on aquatic ecosystems and facilitating environmental management.

OUTLOOK / PERSPECTIVES

The Knowledge Output is relevant to key European directives, including the WFD, UWWTD, Biodiversity Strategy, and Marine Strategy Framework Directive (MSFD). It highlights how NBS can reduce antibiotics and AMR in water bodies, contributing to the WFD's goal of good water quality. NBS also support UWWTD objectives by offering alternative wastewater management methods while ensuring compliance with water standards. Furthermore, NBS promote biodiversity, aligning with the Biodiversity Strategy, and reduce pollutants in river estuaries before they affect marine ecosystems, helping achieve the MSFD's aim of good environmental status for EU waters. We expect that the results from Nature project will be used to enhance the implementation of NBS.



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

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University of Santiago de Compostela - Spain

PROJECT PARTNERS

University of Santiago de Compostela, (coordinator) - Spain

University of São Paulo - Brazil

Technical University of Denmark - Denmark

University of Porto - Portugal

CNRS/Institut National Polytechnique de Toulouse (INP Toulouse), Ecole Nationale Supérieure Agronomique de Toulouse - France

TU Dresden - Germany

DURATION
3 years

STARTING
September 2021

CONTACT

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MOTIVATION BEHIND THE PROJECT

Some wastewater (WW) streams contribute to a higher extent to the spread of organic micropollutants (OMPs). In separated domestic WW collection systems, grey water (GW) accounts for up to 75% of the total flow and, compared to black water (BW), contains a lower concentration of OMPs (mainly personal care products). Residues of pharmaceuticals are mainly discharged through BW in more concentrated streams. Hospitals have also been identified as important sources of OMPs, generating between 4 and 12 times higher WW flows than households, significantly loaded with microorganisms, pharmaceuticals and other toxic chemicals. Some works suggest that they also represent a potential hotspot for antibiotic resistant genes (ARGs) that are often multidrug-resistant. In such a context, decentralized treatment strategies could provide optimized solutions to tackle the removal of emerging contaminants.

PROJECT OBJECTIVES

- To assess the fate of selected contaminants of emerging concern (CECs) comprising OMPs, ARGs and pathogens (including bacteria and viruses) in target wastewater matrixes
- To develop innovative technologies for decentralized wastewater treatment (from domestic, hospital and industrial origin)
- To optimize integrated treatment schemes by understanding the influence of operating parameters on the fate and behaviour of the target CECs.
- To provide final effluents suitable for reuse, complying with safe chemical and biological water quality (trough chemical analyses and ecotoxicological tests).

RESEARCH METHODOLOGY AND IMPLEMENTATION SUMMARY

A series of advanced secondary wastewater treatment technologies have been developed based on the use of bioreactors with high retention of biomass (biofilms and membranes). In addition, a water disinfection technology based on the use of functionalised microparticles that avoid the release of biocides was set-up. Monitoring tools for selected antibiotics, ARGs and pathogens (including bacteria and viruses) were adapted to assess the removal efficiencies reached in the different treatment systems. The effects of treated wastewater on aquatic ecosystems was evaluated for the different treatment schemes by dedicated ecotoxicity studies. The project was applied at pilot scale for the treatment of real wastewater from source-separated urban sewage, as well as effluents from hospitals and a vancomycin production industry.

SUMMARY OF MAIN RESULTS

The pilot plants have shown to be efficient in removal of conventional pollutants (>90% organic matter and ~70% nitrogen). Besides, the removal of antibiotics, including sulfamethoxazole (SMX), trimethoprim (TMP) and ciprofloxacin (CIP), was in the moderate to high range, which could be further improved using activated carbon in bioreactors. Almost complete removals were even achieved for wastewater containing high vancomycin concentration (0.1-0.2 g/L).
For ARGs, removals were >70% (relative abundances) in all treatment systems. ARGs abundance was especially relevant in sludges and biofilms, highlighting the important barrier that supposes the final membrane. Overall, the decentralized treatments efficiently eliminated bacteria and enteric viruses, although some species (ex. certain Enterococcus spp.) were detected in the final effluents.
For this reason, higher water quality was targeted by disinfection. This allowed further remove antibiotics, inactivate known resilient bacteria and high ARG removal.
The appropriateness of the proposed treatment strategies for safe water reuse was confirmed from the ecotoxicological point of view, with results highlighting their effectiveness in mitigating toxicity and genotoxicity.

KEY EXPLOITABLE OUTCOME / OUTPUT	DESCRIPTION
Result 1 WP1 - Treatment train AnMBR/H MBR	Two-step membrane biological processes with different redox environments and biomass conformations. It reaches high removal of COD (>90%) and TN (~70%). Moderate to high removal for a set of OMPs, that can be increased by addition of powdered activated carbon.
Result 2 WP2 - Membrane & biofilm based staged treatment of ARGs	Staged biofilm and membrane reactor to use as a barriers for ARGs spread in the downstream of WWTP and wastewater from antibiotic production industry. Finally, IFASMBR/MBBR treatment configuration was better on removing ARGs than MBR/MBBR.
Result 3 WP2 - Innovative anaerobic reactors aiming at micropollutant removal	The immobilized-cell reactors maintained a high cell concentration and a favorable ratio between cell retention time and hydraulic retention time, leading to a more stable system compared to conventional anaerobic reactors. As a result, the removal efficiencies of micropollutants were significantly enhanced.
Result 4 WP3-Functionalised microparticles for final water disinfection	Effective use of immobilized biocides with no release, while keeping a high disinfection capacity. Decrease in the horizontal antibiotic gene transfer when using functionalized microparticles
Result 5 WP5 - Standardized protocol for ARG quantification	An optimised panel of genes has been selected based on empirical and experimental evidence to allow the detection of highly relevant genes.
Result 6 WP6 - Emission of pathogens (bacteria and viruses)	The methodologies for detection of multiresistant Klebsiella and Enterococcus as well as for detection and quantification of enteric viruses have been optimised and could be used to assess the efficiency of the decentralized treatment.
Result 7 WP7 - Use of gut microbiota of Xenopus laevis as assessment tool of antibiotic resistance reduction	This methodology is new and could be used as a standardized test to assess variation of resistome when expose to substances and or effluent wastewater. Gut microbiota Xenopus laevis seems to be a good indicator but further research is needed

OUTLOOK / PERSPECTIVES

The presence in urban and hospital wastewaters of organic micropollutants (OMPs), many of them of xenobiotic nature, has been recognised as an emerging issue. This has been already evidenced by the EU when publishing the “Watch List” of the Water Framework Directive and will have future implications in the new Water Directive. Antibiotics are of special concern due to the potential development of antibiotic resistant microorganisms (ARMs) and the transfer of antibiotic resistant genes (ARGs) in wastewater treatment plants (WWTPs). In 2017, the World Health Organization published its first ever list of antibiotic-resistant “priority pathogens”.
Some wastewater streams contribute to a higher extent to the spread of such pollutants. So, in this context it is expected that decentralised treatment and postreatment is fundamental to correctly manage these effluents. In parallel, reliable and robust methodologies to asses the occurence of emerging contaminants as well as the final water ecotoxicity are essential to support such strategies.

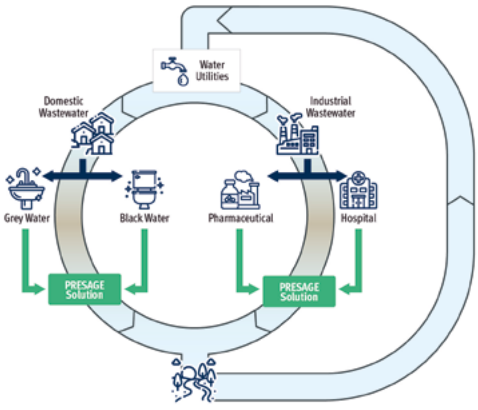

Figure 1. PRESAGE develops decentralized solutions to treat contaminatedwastewaters coming from hospitals, industry, and municipalities.

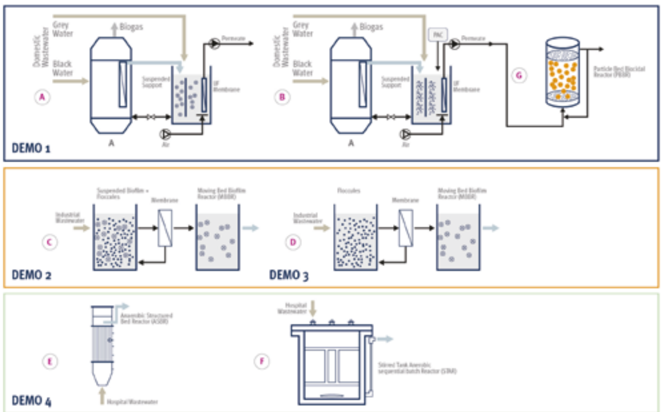

Figure 2: Treatment configurations applied in the different demosites (DEMO1: Spain; DEMO2 & DEMO3: Denmark; DEMO4: Brazil).

FUNDING INSTITUTIONS

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

Agencia Estatal de Investigación · anr agence nationale de la recherche · Bundesministerium für Bildung und Forschung · CONFAP · FAPESP · FCT Fundação para a Ciência e a Tecnologia · Innovation Fund Denmark

REWA

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

PROJECT COORDINATOR

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PROJECT PARTNERS

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DURATION
3 years

STARTING
September 2021

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MOTIVATION BEHIND THE PROJECT

Humans are at significant risk of contracting antibiotic resistance via exposure to contaminated waters, including drinking water, and there is thus a strong need for environmental solutions to mitigate the looming antibiotic resistance crisis. Moreover, there is a need for cost-effective and sustainable systems to improve the removal of contaminants of emerging concern (CEC) and pathogens, which do not require high investing costs. The REWA project has addressed these challenges by assessing a set of technologies and developing sustainable integrated treatment concepts capable of recovering high-quality water from different sources.

PROJECT OBJECTIVES

The main goal of the project was the strategic development and implementation of sustainable and cost-effective technologies for the removal of CECs, pathogens including antimicrobial resistant bacteria and antibiotic resistance genes from water and wastewater at a series of sources. Novel approaches were based on use of specifically prepared natural-based materials for coagulation and adsorption with advanced oxidation processes. The specific objectives were:

- Develop and demonstrate novel concept for surface water treatment using clay-polymer nanocomposites, photocatalysis and tailored-made specific sorbents.
- Demonstrate the use of wood-based biosorbents and carbon-based materials for sewage effluent polishing.
- Demonstrate the use of biocoagulants for metal-rich effluents.
- Assess effects of treatment processes on the microbiome and resistome.
- Understand the mechanisms of the water treatment processes.
- Create awareness of new treatment solutions and develop training material.

RESEARCH METHODOLOGY AND IMPLEMENTATION SUMMARY

The project focused on synthesizing and characterizing various materials, along with conducting four case studies to assess their effectiveness in water treatment:

- Case study #1. New concept for surface water treatment using clay-based products
- Case study #2 Sewage effluent polishing by biosorbents
- Case study #3 Sewage effluent polishing by carbon-based nanomaterials
- Case study #4 Metal-rich effluent treatment by biocoagulants

In the case studies, the efficiencies of the treatment steps for removal of CECs and antibiotic resistance were assessed. In addition, mitigation of selection pressures for antibiotic resistance/co-selection potentials was investigated.

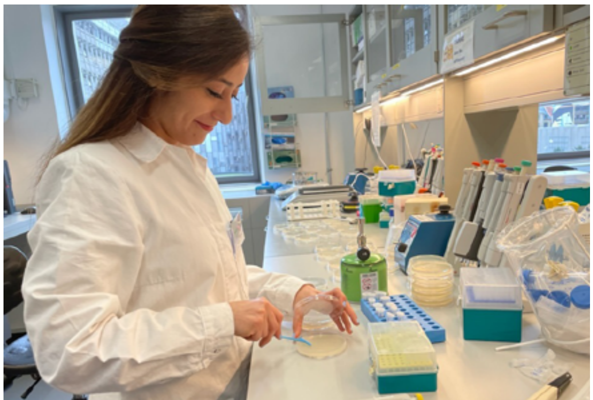
SUMMARY OF MAIN RESULTS

The case studies demonstrated the effectiveness of selected water treatment technologies for treating polluted surface waters, sewage effluents, and metal-rich effluent. Various materials including clay-based adsorbents, biosorbents, tannin-based coagulant and tin-vanadate-based and bismuth oxyhalide photocatalysts were tested for the removal or degradation of CECs from water. Pilot-scale studies with the magnetite pine bark showed effective removal of various antibiotics from real sewage effluent, while the use of Nitrosomonas europaea bioreporter strain revealed that the wastewater was generally non-toxic and regeneration caused short-time toxicity just after regeneration. Moreover, Aeromonas spp. turned out to be promising indicator organisms for water quality assessment and for evaluating water treatment impacts on antimicrobial resistance.

KEY EXPLOITABLE OUTCOME / OUTPUT	DESCRIPTION
Result 1 Quercetin-based adsorbent	Quercetin-based adsorbent was able to selectively adsorb zinc ions from industrial effluent.
Result 2 Biosorbents	Developed biosorbents, magnetite-pine bark and iron-modified peat, showed a high ability to adsorb antibiotics. Pilot-scale studies with the magnetite pine bark showed effective removal of various antibiotics from real sewage effluent.
Result 3 Tin-vanadate-based photocatalysts	Tin-vanadate-based photocatalysts and bismuth oxyhalide photocatalysts absorb visible light and can degrade CECs with relatively high efficiencies.
Result 4 *Aeromonas spp.* indicator organism	*Aeromonas spp.* constitutes promising indicator organisms for water quality assessment and for evaluating water treatment impacts on mitigating environmental dissemination of antibiotic resistance.

OUTLOOK / PERSPECTIVES

The technologies studied in the REWA project, are able to reduce the environmental dissemination of antimicrobial resistance to nature (i.e. aquatic recipients such rivers, lakes, and oceans). The project created new knowledge about the fate and removal of CECs from real waters in new sustainable solutions. REWA supports the regulation through the new knowledge and methodologies for evaluating impacts of water treatment processes on environmental dissemination of antimicrobial resistance.



FUNDING INSTITUTIONS

AKA (Finland), CSO-MOH (Israel), IFD (Denmark) WRC (South Africa)

SERPIC

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

PROJECT COORDINATOR

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Universidade do Porto, Portugal (UP)

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Norwegian Institute for Water Research, Norway (NIVA)

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DURATION
3 years

STARTING
September 2021

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MOTIVATION BEHIND THE PROJECT

Increasing **water scarcity** is one of the biggest challenges worldwide. The provision of safe water in sufficient amounts is essential for both human health (drinking water and sanitation) and – via irrigation of crops – for a sufficient human nutrition. SERPIC connects the urban water cycle with the water cycle in agriculture and the natural water cycle. The reuse of the effluents of wastewater treatment plants (WWTPs) constitutes a significant and constantly available water resource that can be safely used for irrigation. This is the motivation for the SERPIC project to develop technologies to reduce CECs from conventional WWTP effluent.

PROJECT OBJECTIVES

- **Objective 1:** minimize the spread and transformation of CECs including ARB and ARG, within the water cycle from households and industries to WWTPs effluents, and afterwards via irrigation into the food chain, into soil and groundwater and into river basins, estuaries, coastal areas, and oceans;
- **Objective 2:** reduce CECs from WWTP effluent by developing an innovative treatment technology, based on membrane filtration and light driven electro-chemical processes. Reduction targets: Route A > 90 %, Route B > 80 %
- **Objective 3:** elaborate methodologies and tools for monitoring, health and environmental risk assessment and for the implementation of new reuse concepts including new treatment technologies, as a basis for better policy and decision-making, for regulatory issues and new standards.

RESEARCH METHODOLOGY AND IMPLEMENTATION SUMMARY

With the SERPIC technological multibarrier approach, secondary effluent of a municipal wastewater plant underwent a sequence of reclaiming treatments: A nanofiltration separated the effluent into a high quality permeate stream (Route A), and a more polluted concentrate stream (Route B). Route A stream was disinfected by ozone gas, electrochemically generated using diamond electrodes, for safe irrigation of crops. The CECs in Route B stream were degraded by persulfate, electrochemically generated using diamond electrodes, for safe discharge into the aquatic environment. A prototype plant was set-up and tested in a long-term field test by irrigating crops with the treated water.

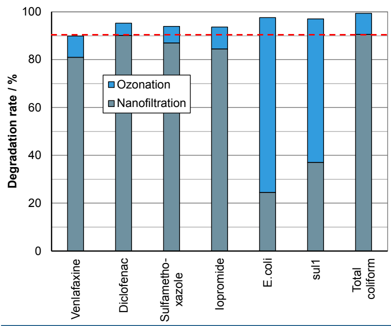
SUMMARY OF MAIN RESULTS

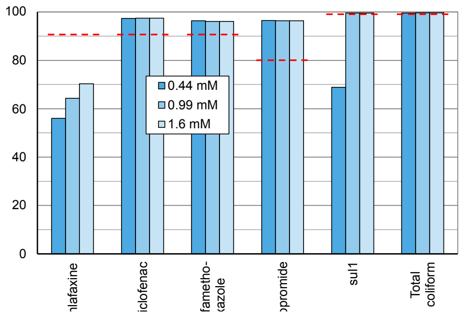
A methodology was developed to select relevant target CECs for the analysis to prove the performance of the technology. Process components were developed and built. A prototype plant was commissioned and put to operation. It could process 34 L/h of water with an energy consumption of 630 W. The CECs in Route A water for irrigation were reduced by 90-99 %. The CECs in Route B water for discharge were reduced by 70-99 %. LCA and LCC were done as well as a risk analysis of the plant operation. An exploitation of the results was planned and discussed with stakeholders.

KEY EXPLOITABLE OUTCOME / OUTPUT	DESCRIPTION
Result 1 Target CEC selection methodology	Designed to assess the usefulness of a technology for a specific location and the risk for environment and crops. It is based on occurrence, persistence, bioaccumulation and toxicity.
Result 2 SERPIC prototype and performance	This output includes a full set-up of a water treatment on prototype level to reduce CECs and AMR bacteria.
Result 3 Nanofiltration selection and performance	Knowledge has been elaborated on CEC removal performance of a specifically selected nanofiltration membrane.
Result 4 Persulfate electrolyser design and performance	- Computer-aided design (CAD), computational fluid dynamics simulation (CFD) and 3D printing to support the production process and achieve maximum electrogeneration efficiencies. - Design features that increase persulfate production efficiency: minimum distance between electrodes, uniform fluid distribution by distribution fins, conical inlets and outlets for the evacuation of gases generated in the process. Easily adapted to a single or double compartment. - Definition of optimal operating conditions, achieving production efficiencies higher than similar reactors without the need for any turbulence promoter. Capable of working in various discontinuous and continuous operation modes, regulating the inlet and outlet flow rate for future industrial application.
Result 5 Ozone gas electrolyser design and performance	- Custom design of an electrochemical cell for ozone production using Computer Aided Design and printed with 3D printing technology. - Proton Exchange Membrane type cell with a commercial Membrane Electrode Assembly specially designed to evacuate the gases formed during the electrolysis reaction, avoiding decomposition reactions and at the same time maintaining a good flow distribution within the electrochemical cell. - Optimization of the system variables to obtain the maximum ozone production conditions, but with the minimum energy consumption required.
Result 6 Persulfate oxidation photoreactor design and performance	- Technology optimized for the advanced treatment of nanofiltration retentate stream - Homogeneous oxidant distribution (radial and axial) via dosing through porous ceramic - Simulation tool for optimization of reflective surfaces in tubular UVC-photoreactors - Definition of operational conditions to ensure compliance with legal discharge limit values

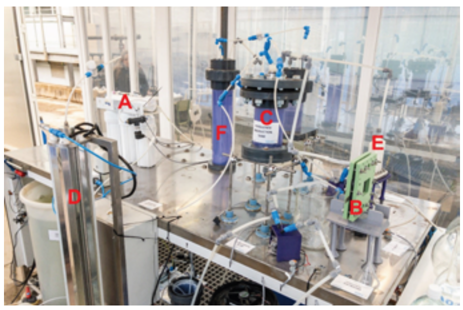
OUTLOOK / PERSPECTIVES

Disinfection by electrogenerated ozone gas is regarded as a promising alternative to gas ozonation with ozone produced from liquid oxygen.
The results offer new opportunities for nanofiltration as central CEC separation method. Nanofiltration also enables a very clean permeate that could be used for irrigation and also for industry.
Water treatment with electrochemically generated persulfate offers a low-energy alternative with much higher CEC degradation rates compared to activated carbon adsorption and ozonation.
A special advantage of SERPIC is offered for applications requiring the treatment of smaller flow rates, e.g. decentralised wastewater treatments, like remote places or areas without sewer system.





SERPIC prototype plant; A nanofiltration, B persulfate electrolyser, C persulfate intermediate tank, D UV photoreactor, E ozone gas electrolyser, F disinfection unit with ozone contactor.



FUNDING INSTITUTIONS

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

TRANSFER PROJECT CALL

Research and development for supporting knowledge transfer, scientific networking, and public engagement based on AquaticPollutants joint transnational projects

AquaticPollutantsTransNet

Successful knowledge transfer and networking strategies to minimise potential risks of aquatic pollutants

PROJECT COORDINATOR

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Institut für sozial-ökologische Forschung (ISOE) - Germany (Sub-contractor)

DURATION
4 years

STARTING
June 1st, 2021

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MOTIVATION BEHIND THE PROJECT

AquaticPollutantsTransNet is a transfer project and is fostering the knowledge transfer and exchange from local to European scale and beyond. It supports the AquaticPollutants projects to create synergies and to maximise impacts of their research results through collective strategic dissemination, exploitation and communication. Innovative science communication approaches elaborated will strengthen knowledge transfer and exchange with stakeholders. This will help to improve policy making and to reduce risks by aquatic pollutants.

PROJECT OBJECTIVES

AquaticPollutantsTransNet supports the funded AquaticPollutants research projects by:

- Identify key stakeholders and their knowledge gaps
- Develop innovative approaches for enhanced knowledge transfer and exchange
- Create synergies amongst the AquaticPollutants projects and strengthen stakeholder collaboration
- Improve knowledge transfer from the research projects to stakeholders
- Implement multiple dissemination and exploitation routes

RESEARCH METHODOLOGY AND IMPLEMENTATION SUMMARY

To improve the uptake of research results, APTransNet conducted over 50 stakeholder interviews, organized two national workshops, and analyzed relevant national and EU regulations. It identified specific knowledge demands from stakeholders (e.g., authorities, industry, NGOs) and matched them with project outputs, classified by country, sector, and pollutant type (e.g., CECs, AMR).
This process led to the creation of the “TransNet Viewer”, an online tool for researchers that connects research outputs with stakeholder needs and tailors communication strategies accordingly. The project also used a variety of communication channels (website, podcasts, newsletters, conferences, etc.) to raise awareness, support knowledge uptake, and highlight future research needs.
Through these activities, APTransNet strengthens the bridge between science and practice and contributes to broader societal awareness and solution-oriented understanding of aquatic pollutants.

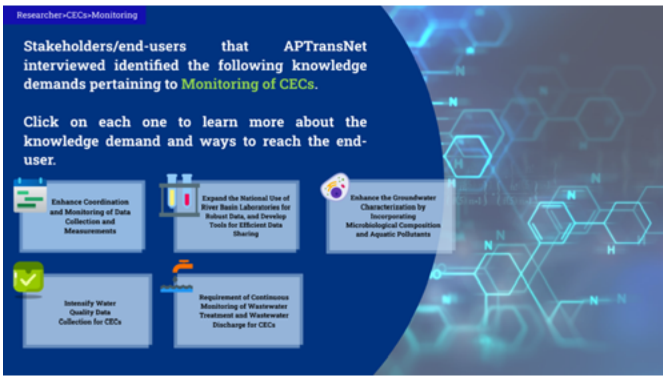
SUMMARY OF MAIN RESULTS

The APTransNet project achieved several key exploitable outcomes to enhance the impact of research on aquatic pollutants. First, it identified knowledge gaps and stakeholder needs by analyzing the mismatch between existing scientific knowledge and end-user demands in participating countries. This included mapping key water stakeholders at both EU and national levels, with the findings integrated into the interactive TransNet Viewer tool. Second, the project addressed the urgent issue of antimicrobial resistance (AMR) in aquatic environments through the development of a policy brief and the organization of a targeted policy event, supporting evidence-based decision-making. Third, APTransNet focused on the dissemination of research results by producing a range of accessible communication materials—such as podcasts, factsheets, and Layman's report—designed to effectively translate scientific insights for stakeholders and bridge the gap between research and practical application.

KEY EXPLOITABLE OUTCOME / OUTPUT	DESCRIPTION
Result 1 Identification of Knowledge Gaps and Stakeholder Needs on Aquatic Pollutants	Analyze gaps between scientific knowledge on aquatic pollutants and stakeholder needs including the mapping of key water stakeholders at EU level and national levels and the assessment end-user groups demands and knowledge gaps for participating countries. All these results are displayed in the TransNet Viewer.
Result 2 Strategies to Combat Antimicrobial Resistance (AMR) in aquatic ecosystems	Preparation of a policy brief and implementation of a policy event with the focus on addressing the pressing risks posed by AMR, a critical global health challenge.
Result 3 Dissemination of exploitable results	Creation of publications, public relations, communication, dissemination, and exploitation of knowledge related to the AquaticPollutants programme (e.g. Podcasts, Factsheets, Layman's report etc. as products that bridge the gap between science end users)

OUTLOOK / PERSPECTIVES

Building on its achievements, AquaticPollutantsTransNet offers significant potential for future development and long-term impact. The methodologies and tools developed—particularly the TransNet Viewer—provide a solid foundation for ongoing and future knowledge transfer activities across environmental and health-related domains. The insights gained through the identification of stakeholder knowledge gaps, combined with tailored communication strategies, can inform future research funding priorities, shape more responsive policy frameworks, and support a sustainable, science-policy interface in water management.
Looking ahead, the project's approach could be scaled to other environmental challenges such as soil, air, or climate pollutants. Moreover, the strong stakeholder engagement model can serve as a blueprint for other interdisciplinary research programmes seeking to improve uptake and societal relevance. Finally, by maintaining and expanding the established communication platforms and networks, APTransNet has the potential to become a central European hub for aquatic pollutant knowledge transfer, ensuring continued impact well beyond the project's lifetime.



FUNDING INSTITUTIONS

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DISCLAIMER

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WEBSITES

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

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