

## WP1. Selection, isolation, characterization of biologics to degrade the target pollutants

**The first work package in NYMPHE is a fundamental step in the project since it is dedicated to the characterization and selection of all biologics that are then considered for improvement and/or assembly, then integration in rationally developed bioremediation systems in further stages of the project.**

Twelve NYMPHE partners worked in this context to address biologics of a wide range, including degradative enzymes, microbial strains and consortia (bacteria, fungi, microalgae), plants and their associated microbiota, bivalves and earthworms. The capacity of these biologics to clean wastewater, groundwater, river sediments and soils from diverse organic contaminants was comprehensively assessed and characterized in work package 1.

### *Enzymes*

Bioinformatics and computational tools were used over own and publicly available information on genetic sequences to pre-select enzymes that potentially can assist the degradation of different contaminants of different NYMPHE matrices, including pharmaceuticals, several types of hydrocarbons, plastics and pesticides. Hundreds million sequences were screened for a selection of more than 500 enzymes of different classes, which were synthesized and tested in vitro for their capacity to degrade the target contaminants.

The large majority of these enzymes were able to degrade targeted contaminants by more than 80%. This is a high-level portfolio for improving microbial biologics by adding on-demand degradative capacity for application in bioremediation of the NYMPHE matrices in further stages of the project.

### *Microbial biologics*

Bacteria, fungi and green microalgae were characterized for selecting biologics with enhanced capacity to clean wastewater, river sediments, agricultural and industrial soils from pharmaceuticals and their degradation products, several types of hydrocarbons, microplastics and pesticides.

Bacteria and consortia were acquired in most cases using diverse enrichment strategies, based on the use of the NYMPHE contaminated matrices to extract the organisms thriving under the challenge of the targeted contaminants. These were isolated and tested for their degradative capacity over these contaminants. In other cases, microbial biologics for characterization were gathered from own collections of the partners based on background information available on their potential to degrade the target classes of contaminants.

Concerning effective degradation of pharmaceuticals, including antibiotics, in wastewater and riverine sediments, NYMPHE partners selected and successfully characterized 5 bacteria strains and 50 microbial consortia, while for degrading plastics and plasticizers in wastewater, more than 30 bacteria strains and more than 5 microbial consortia were obtained. An addition was made to the set of microorganisms capable of promoting the degradation of pharmaceuticals in wastewater, with the characterization of such capacity by 3 microalgae strains.

Aiming to achieve high degradation rates for several hydrocarbon contaminants in soils and groundwater of industrial areas, NYMPHE partners selected and characterized at least 13 microbial consortia and 15 bacteria strains. Concerning agricultural soils, relevant plastics degradation was achieved with 2 microbial consortia and 1 fungal strain, while pesticides degradation was promoted by at least 30 bacteria strains. These results open great perspectives for using selected microbial biologies to integrate new combined bioremediation tools that can more efficiently clean different contaminated matrices.

### ***Plants and their microbiota***

The known capacity of some plants to thrive in heavily contaminated soils in a joint venture with their rhizosphere microbiota was explored to develop biologies capable of attenuating the burden of industrial soils in several types of hydrocarbons. Several plant species were tested for their tolerance to the NYMPHE industrial soil, and sunflower was selected for the recruitment of bacteria with degradative capacity over the target soil contaminants and/or plant growth promoting activity. More than 150 of these bacteria strains were isolated from the NYMPHE soil or recovered from own collections, and a significant part of them were successfully characterised as hydrocarbon effective degraders/metabolizers and/or plant growth promoters.

In an additional effort towards developing effective bioremediation technologies based on plants, a genomically edited variant of the tobacco plant confirmedly degrading other organic contaminants was selected as per its potential to have the necessary features to degrade also target hydrocarbons contaminating the NYMPHE industrial soil.

### ***Animal biologies***

Animals were addressed as biologies in NYMPHE, namely bivalves targeting pharmaceuticals contamination of wastewater and earthworms targeting pesticide contamination in agricultural soils.

Concerning bivalves, both their biofiltration activity and the biosorption capacity of their shells were addressed. Although living bivalves showed a limited capacity to remove several pharmaceuticals from the water and limited survival to the NYMPHE raw wastewater, bivalve shells showed a promising capacity to remove several pharmaceuticals from water and wastewater, especially after treatment by

pyrolysis and in some cases depending on the water pH. This opens avenues to develop alternative biosorbents for refining the treatment of wastewater, using an abundant biological resource that can also be sourced as a waste from the food production and processing industries.

Three species of earthworms, widely known as soil engineers as per their burrowing activity, were assessed for their capacity to stimulate microbial degraders of pesticides. Burrows, cast and soil were confirmedly found to have different microbiomes, which changes differentially when pesticides are added to the soil.

The degradation of target pesticides was accelerated by two of the earthworm species tested, and the combined effect of the three earthworm species together was shown to be synergistic, improving markedly pesticide degradation, enlarging the scope for the use of earthworms as biologics enhancing bioremediation tools developed for agricultural soils.

## **About Nymphe project**

Nymphe is a four years European project dedicated to tackling environmental pollution by developing innovative bioremediation solutions funded by the **European Union**.

## **New system-driven bioremediation of polluted habitats and environment**

**Topic:** Environmental services – improved bioremediation and revitalization strategies for soil, sediments and water for a clean environment and zero pollution.

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