# Advancing Hybrid Nature-Based Solutions for Biodiversity Enhancement and Emerging Contaminant Mitigation


## Summary:

Aquatic ecosystems are increasingly threatened by anthropogenic pressures, including persistent emerging contaminants (ECs) like pesticides, pharmaceuticals, and PFAS. These micropollutants pose significant risks to non-target species, disrupting aquatic ecosystem structure and function. Conventional wastewater treatment plants are ineffective in removing certain ECs, highlighting the urgency for innovative, cost-effective, and environmentally sustainable solutions. NATUREBIOPROMO project was approved under Biodiversa+ call to address these challenges. This project aligns with key international policies, including the EU Water Framework Directive and the Sustainable Development Goals (SDG 6, 7 and 15). The proposed PhD project will address this challenge through 1) the integration of hybrid Nature-Based Solutions (NBS)—such as constructed wetlands (CWs), artificial floating islands (AFIs), and microalgae ponds—under real conditions to enhance pollutant and nutrient removal efficiency. It also emphasizes 2) the dual role of NBS in wastewater treatment and biodiversity conservation, supporting biodiversity-oriented management aligned with the EU's Green Deal and Biodiversity Strategy for 2030. The main objectives include:

O1: Assessment of real-scale spatial and temporal performance of our case study NBS to evaluate its ecological significance;

O2: Seasonal and temporal characterisations of biodiversity levels and their functional attributes;

O3: Evaluation of present and future contributions of the NBS, regarding biodiversity enrichment, reduction of ECs and nutrients.


## Main Metodologies:

WP1 - Assessment of real-scale spatial and temporal performance of our case study NBS to evaluate its ecological significance 1.1 - Environmental surveys of the case study Field surveys to a CW integrated into a municipal wastewater treatment plant located in the North Portugal will be planned in a seasonal approach during the first year for measuring physicochemical parameters (e.g. temperature, pH, oxygen, water flow), nutrient concentrations (N, P), and ECs concentrations from the influent and the effluent of our case study. 1.2 - Biodiversity analyses Biodiversity analysis will be divided into the microbiological (bacteria and fungi) and macrofauna diversity associated with the NBS. Microbiological diversity: Microbial communities will be assessed through sequencing of 16S rRNA gene amplicons and ITS following the procedures as previously described1,2,3,4. Macrofauna diversity: Macrofauna samples will be collected using standardized protocols, sorted, identified as far as possible to species level and counted. These data will be analysed using different taxonomic and functional diversity metrics5,6 that reflect their richness, dispersion, and evenness7. For functional diversity, species will be classified according to functional traits5. 1.3 - Environmental characterization (water, substrate and plants) Water quality: Samples will be transported to the laboratory in a cool box, and preserved at 4 ºC until analysis. Target compounds will be extracted by solid phase extraction (SPE) and later, analysed. Substrate quality: Collected samples will be used for quantification of organic matter (450 ºC, 8h) and the remaining for later quantification of target ECs. Plants quality: For quantification of ECs in plant tissues, they will be first freeze-dried and then homogenized for later quantification. Plants Dynamics: Characterizing plant traits (e.g. density, plant growth parameters, root traits) & photosynthesis efficiency (via PAM fluorometry)8, pigments (chlorophyll a, b), and EC bioaccumulation will be done whenever possible.

WP2 - Evaluation of present and future contributions of the NBS, regarding biodiversity enrichment, reduction of ECs and nutrients 2.1 Selection and implementation of an optimized solution Based on the results of WP1, different enhancement solutions can be considered (e.g. application of artificial floating islands (AFI), 2) use of polycultures of wetland plants; 3) Integration of CW units with a microalgae tank. The analysis will use water quality data - such as EC levels, nutrients, water volume and basic information about the systems and their surroundings. It will also consider the main properties of the detected ECs. Based on this information and local conditions, the most suitable treatment technologies will be identified. 2.2 Evaluation of efficiency of enhanced solutions in NBS During one-year, seasonal field surveys will be planned, to study the effect of the optimization strategies based on the biodiversity change and the improvement of ECs removal. A similar sampling strategy will be adopted as in 1.1.

WP3 – Toxicity effects of contaminants exposure 3.1 - Toxicity effects of ECs exposure on non-target species For this study aquatic model species will be selected. Based on the results of ECs concentrations on the effluents of the case study, before and after the optimized solution, the model species will be exposed to different combinations of ECs concentrations for 21 days. DNA damages through citogenotoxicity analysis9 and effects on gonads maturation dynamics through histological analyses10 will be evaluated.

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## References:

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## Place of Work

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## Will the Proposal research idea be funded by a specific project?

Yes, by Biodiversa2023-935

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