



ALMA MATER STUDIORUM  
UNIVERSITÀ DI BOLOGNA

DIPARTIMENTO  
DI FARMACIA  
E BIOTECNOLOGIE

Piano Formativo Assegno di Ricerca:

### **Microbiomes characterization innovative system-driven bioremediation of Polluted Habitats and Environment**

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In this task the microbiomes collected from different biological resources within the project Nymphe will be characterized. These will include: i) the microbiomes sampled through a cross-sectional (case control) and a longitudinal approach from the different matrices, i.e., soils, groundwater and aquifer sediments, hyporheic zone and wastewater of the Nymphe sites; ii) the microbiomes of plant and animal holobionts selected in the project; iii) the microbiomes of the multi-component systems. DNA will be extracted from all samples using well established methods successfully implemented and used in previous funded FP and H2020 projects, and DNA's quality and concentration will be assessed using spectrophotometric measurements (e.g., nanodrop). All microbiomes will be first characterized through sequencing of the V3-V4 regions of the 16S rRNA gene on MiSeq Illumina platform to evaluate the level of internal bacterial diversity. This first screening will be instrumental for adjusting sequencing depth for the following shotgun metagenomics on NexSeq Illumina platform. Metagenomics will be performed using a  $2 \times 150$  bp paired-end protocol. Multiplexing will be adjusted to obtain a minimal sequencing output of 5 Gb per sample. High-quality paired-end sequences will be uploaded to the SRA repository. Metagenomics will be also coupled with marker gene NGS of selected functional molecular markers (e.g., phthalate CoA transferases and decarboxylases), using the Illumina MiSeq platform. The metagenomics data produced will be utilized for the de novo construction metagenome-assembled genomes (MAGs) and their taxonomic affiliation and function investigated with well established methods. The identification of metagenomics species with prominent degradation capacity will be done by performing Hidden Markov Model and DIAMOND-BLASTP search tools against a curated databases with taxonomically diverse protein sequences featuring key enzyme families, potentially attacking hundreds of different types of pollutants.