BIORESOURCES FOR SOIL QUALITY: MICROBIAL SPECIES APPLICATION FOR RESTORING SOIL QUALITY IN CO-CONTAMINATED SITES

Ph.D. Student: Roberto Giovannini
Ph.D. Tutor: Pr. Anna Maria Persiani

OBJECTIVES OF THE PROJECT

Soil contamination is a research of the upmost interest, as rising numbers of contaminated sites are identified and the estimate is nearing 3 millions in the EU("EEA Signals 2020 — Towards Zero Pollution in Europe — European Environment Agency"). Among these, impacts from present and past warfare represent more than 3% of identified sites and often involve multiple contaminations from both organic pollutants and toxic elements (EEA 2020). The efficiency of known bioremediation techniques in presence of multiple contaminants cannot be reliably predicted as the effects of interactions are still largely unexplored (Ceci et al. 2019; Ye et al. 2017). This project aims at tackling this issue by testing the potentiality of microbial bioresources in restoring co-contaminated soil, so that contaminated sites can be return to the community.

The main objective of this project is the selection of an effective treatment that remediates polycyclic aromatic hydrocarbons (PAHs) polluted soils in conditions of co-contamination with potentially toxic metals and metalloids typical of military sites. Fungi has been reported in literature to be effective in treating a wide variety of organic pollutants, due to their array of enzymatic capabilities, including PAH (Winquist et al. 2014), pesticides (Spina et al. 2018) and chlorinated hydrocarbons (Marco-Urrea, García-Romera, and Aranda 2015), with concrete potentialities of real-world application in restoring soil quality. Yet, the effect of co-contaminations on degradation efficiency is largely undocumented. In order to fill-in the lack of knowledge, a primary aim is gathering new information on effective fungal bioresources isolated from co-contaminated soils.

A second goal is comparing the effectiveness of microbial consortia against single strains. Naturally occurring microbial communities are pivotal geoactive agents and studies have highlighted how consortia can be more effective on organic pollutants than single-strain treatments (Zafra et al. 2017; Hassan et al. 2020). In fact, bacteria can take advantage of fungal mycelium capacity of bridging air gaps (fungal highways)(Simon et al. 2015), enhancing bioavailability and the overall degradation efficiency. The potentialities of combining the unique traits of fungi and bacteria is yet to be extensively tested on soil contaminated by both organic and inorganic pollutants, so providing new insights can open new biotechnological uses.

Thirdly, in order to explore the potentialities of the microbiome, evaluating the effectiveness of multi-organism approaches that include both plants and soil microbiota is yet another aim of the project. In recent years, combined techniques such as Microbial-assisted Phytoremediation have risen in interests (Dotaniya et al. 2018; Yang et al. 2020; García-Sánchez et al. 2018), as they allow to take advantages of both the largely diverse metabolic assets and the interactions between plants and microbes to tackle both inorganic and organic pollutants simultaneously. Verifying the applicability of such techniques in co-contaminated sites can bring more interest towards bioremediation of complex contaminations using biotechnological means. Furthermore, as military areas are increasingly described as hosting habitats largely unimpacted by human activities (Ellwanger et al. 2016; European Commission 2005; Zentelis and Lindenmayer 2015) in their exclusion zones – buffer areas, unaccessible by civilian but outside of the area of warfare activities -, local flora will be evaluated to find potential candidates for biotechnological applications.

Lastly, a more long-term objective is the assessment of realistic possibilities of future industrial scale-up, as new technologies are needed to recover polluted sites impacted by a wide array of contaminants.

MAIN PHASES OF THE PROJECT

The project is made up of four main phases: sampling and chemical characterisation of the site, isolation of microbial community, microcosm experiments and mesocosms.

The sampling of soil from a military site impacted by a co-contamination event will be carried out in the initial months of the first year of the project. The site will be chosen among those without active sources of contamination, as to make sure that the autochthonous microbial community has adapted to the presence of contaminants, thus providing valuable candidates for bioremediation. Sampling plan will be devised based on various factors, including the extent of the contaminated area and whether the contamination is punctual or diffuse, and the most appropriate sampling grid will be applied. Sampling will be focused on the topsoil, as most of the microbial biomass occur in the topmost horizon of soil(Tedersoo et al. 2014). A representative pool of soil cores (5cm diameter and 5cm deep) will be collected on the site and sieved to remove coarse roots and stones. Chemical analysis will be performed on quotas to assess current contamination levels. A survey of the plant community of the site will be planned and performed to evaluate the presence of potential candidates for mesocosms within the impacted plant community.

Subsequently, the isolation of the microbiota will be carried out. The chosen methods are the dilution plate and enrichment culture selective methods. Water/soil dilution plate is an extensively described method for isolating soil microbial community that yields highly repeatable and comparable data(Ceci et al. 2012), while enrichment culture is a selective method of rising interest that allows to isolate strains specialized in using pollutants as carbon source(Spini et al. 2018) A suspension of contaminated soil will be cultured in mineral medium, providing representatives of the organic contaminants found in site's soil as the sole carbon source for microbial growth. Selected strains are then moved to pure culture. Morphologically different fungal strains are moved to Malt Extract Agar (MEA) plates and identified through a polyphasic approach that includes the identification through morphological characteristics and a subsequential DNA extraction and sequencing of ITS regions 1-4. Bacterial strains are moved to pure culture in Trypticase Soy Agart (TSA) medium to be then collected through an incubation loop for DNA extraction and sequencing of the 16S region of the ribosomial RNA. Selected strains are screened for the constitution of microbial consortia to be used in microcosm experiments.

In the second year, selected strains and consortia are evaluated for their degradation effectiveness. This phase involves degradation experiments in microcosms. Selected strains and consortia will be tested for their degradation efficiency in pot experiments under controlled growth conditions. Initially, sterilized soil is amended with representatives of site contaminants to simulate concentrations found in site and inoculated with microbial treatments. Ex-ante and ex-post chemical analyses and ecotoxicological assayes will be used to evaluate degradation and detoxification efficiency. The most effective treatment will be chosen by analysing data for significant differences through suitable statistical analyses.

The last phase of the project involves testing the best treatment selected in the microcosms in a co-contaminated environment under less controlled conditions at mesocosm level, applying the microbial-assisted (MAP) technique which is gaining interest in soil decontamination literature (Yang et al. 2020; Dotaniya et al. 2018; García-Sánchez et al. 2018). Based on the results of the survey of site's plant community, potential candidates among locally occurring plant species will be evaluated. The selected microbial treatment will be inoculated in sterile soil that replicates site's contamination conditions and then cultivated together with the chosen plant. Chemical and ecotoxicological assessment will be carried out to determine the efficiency of the treatment. Statistical analysis will be carried out on results of chemical and ecotoxicological analyses to highlight significant differences between microbial and MAP treatment.