

The oil refinery industry is involved in the **global processes** of exploration, extraction, transporting and marketing petroleum products. Crude oil and petroleum are also the raw material for many chemical products, including pharmaceuticals, solvents, fertilizers, pesticides, and plastics. Oil and its derivatives (such as polycyclic aromatic hydrocarbons, PAHs) are among very significant and dangerous sources of ecosystem contaminants. Oil derivatives are a threat to human health as well as a hazard to all living beings. The main condition for effective microbiological degradation of PAHs in soil is the presence of strains capable not only of catabolic degradation of contaminants, but also of a number of other properties confirming their adaptation to the pollution and co-metabolic degradation. How nature can clean up its own resources is still unexplained and extremely interesting. In addition, no research has yet been carried out on an object with over 100 years of contamination with a natural remediation process. In the course of many years of contamination, the soil is richly populated by **ruderal (relict) vegetation** that spontaneously inhabits these areas. The rhizosphere and endorhizosphere of these plants are very unique and diverse habitats for bacteria and fungi capable of growing in contaminated conditions and having biotechnological potential to promote plant growth and development. **Both microorganisms and plants adapting to such conditions can create a number of defense and adaptive mechanisms.** Ruderal vegetation is characterized by a large variety of genetic, physiological and metabolomic profiles. Plants adapted to grow in the contaminated place may have (or develop) **unique features.** The assessment of ruderal vegetation inhabiting long-term contaminated areas, combined with knowledge of the microbiome, mycobiome and metavirome of soil and plants (including its rhizosphere and endorhizosphere) is an important and innovative research testing ground to expand knowledge in the field of soil bioremediation.

The main of the present project is to define the role of ruderal vegetation, their rhizosphere, endorhizosphere, indigenous microbes (bacteria and fungi) and bacteriophages in the processes of natural bioremediation of oil-contaminated soils. The specific goals are: explaining how ruderal plants develop mechanisms that adapt them to contamination over the years; to define the role and explain mechanisms of rhizosphere and endorhizosphere microorganisms and bacteriophages in **long-term natural bioremediation.** In the first stage of the project, soils and ruderal plants will be collected from the oil wells at the historical Crude Oil Mines in Węglówka. Therefore, the area after the mine was heavily contaminated and degraded (more than 100 years of pollution). Samples will be taken from the selected 9 oldest oil wells at the historical places in Crude Oil Mine. Oil production was not carried out anymore and the site itself has naturally remedied to the present day. Nevertheless, from the Oil Mine closure to the present time, crude oil flows spontaneously from the oil wells. Constant crude oil flow causes permanently contamination of the area with simultaneous natural remediation. This area is overgrown with meadow vegetation. Soil samples will be taken from selected 9 oldest oil wells. Five species of ruderal plants will be selected for research. DNA will be isolated directly from the soil, rhizosphere and endorhizosphere of plants. Isolation on selective media and characteristics of bacterial and fungal strains from rhizosphere and endorhizosphere of selected plants will be carried. Bacterial and fungal strains will be evaluated based of morphological, biochemical and genetic tests. The following analysis will be made: the genetic diversity using the Biolog system, Next Generation Sequencing (NGS) of variable regions (16S rRNA for bacteria and ITS for fungi) and NGS of soil metavirome (Shotgun Sequencing). In addition, the chemical parameters of plant and soil samples will be determined ( $C_{org}$ ,  $N_{min}$ ,  $\Sigma 16$  PAHs and trace elements). In plants will be assessed: the content of biological activity of selected secondary metabolites, accumulation of photosynthetic pigments, functioning of the photosynthetic apparatus, determination of the metabolomics profile and phenolic compounds. In addition to plants selected from contaminated areas, control plants (taken from unpolluted areas) will be used for testing.

This project will be one of the first attempts to broadly identify the effects of long-term soil contamination on physicochemical and biological properties as well as soil and plant biodiversity in conjunction with profiling of bacteria, fungi and bacteriophages. The new knowledge generated by the project will perfectly fill the biodiversity assessment gap regarding the still-undiscovered biological and molecular characteristics of oil-contaminated soils and plants. Furthermore, this project will be the first such detailed approach to evaluating the interactions between the microbiome, mycobiome and metavirome of the rhizosphere and endorhizosphere of ruderal plants and their contribution to bioremediation processes.