

Due to intensive industrial activities, significant acreage of land, scattered all over the world has been heavily polluted with metals. As a result, the biodiversity of organisms inhabiting such environments exhibited significant losses. These areas have become a major threat to human health and well-being. Limiting the pollution by restricting metal availability and their dispersion has become an important challenge.

During evolution, a small number of organisms have evolved the ability to inhabit even extremely degraded environments. A good example of such adaptation are plants inhabiting post-mining wastes. The deposition of metals: Pb, Cd, Zn and Fe in such environments often exceeds tolerable concentrations by a few orders of magnitude. A plant species commonly present on post-mining waste dumps in the South of Poland is *Arabidopsis arenosa*. According to our research, a specific group of symbiotic microorganisms-endophytic fungi play an important role in the adaptation of this species and plants in general to vegetation in the extremely hostile environment of a mine dump. These microorganisms have evolved tolerance to high concentrations of metals deposited in the substrate and the ability to facilitate plant growth. Recently, the importance of symbiotic microorganisms in the regulation of numerous aspects of not only plant, but all multicellular organisms aspects of physiology and behavior is gaining the recognition of the scientific community. Nevertheless, our knowledge of the role of these symbiotic microbes is scarce. It is assumed that the loss of this biodiversity may be a serious indication of pathology or degradation.

The aim of the planned investigations is to assess the biodiversity of microorganisms inhabiting *Arabidopsis arenosa* from the mine dump "Boleslaw". This will allow the selection of microorganisms with highest metal tolerance potential and highest ability to confer metal toxicity resistance in its host plant. In result, the selected endophytic fungi may be utilized in optimization of degraded environment restoration strategies, as supplementary means for phytoremediation. According to our previous studies we know that: 1) these symbiotic microorganisms are not species specific; they possess the ability to interact in a mutual relationship with various, often phylogenetically distant plants and their role in plant adaptation is not restricted to conferring toxic metal tolerance; 2) endophyte induced stress tolerance is accomplished by facilitating plant nutrient (mainly phosphorus) and water acquisition by increasing the root surface area of its host; 3) decreasing metal uptake, as well as optimization of metal distribution within the plant and cellular compartment. The latter is a result of endophyte dependent regulation of the expression of metal transporters involved in metal efflux, root to shoot translocation and sequestration in the plant vacuole-a mechanism that allows the plant to protect vulnerable cell compartments against metal toxicity by spatial isolation.

One of the most important aims of the planned study is to describe the mechanisms underlying endophyte regulation of the expression of the mentioned above proteins. According to our previous and preliminary studies endophyte interference with plant hormone ethylene synthesis and/or signaling activates the expression of the metal transporter proteins. Additionally, endophyte induced metal tolerance regulation is affected by specific populations of regulatory, non-protein coding RNA (npcRNA) molecules that are involved in a mutual regulation mechanism (probably feedback loop) with ethylene signaling/synthesis. The planned investigations are designed to evaluate the role of the ethylene-npcRNA interplay in endophyte induced plant adaptation to the hostile conditions of a post-mining waste dump. This will allow to improve our general understanding of metal tolerance in plants and the role of symbiosis in this process. Additionally, the plant-endophytic fungi interaction is exclusively studied in single inoculation experiments. A situation that is hard to imagine in natural environments. Here, it is planned to perform experiments in double and triple inoculation setups. This approach will allow to study endophytic fungi biology in a more realistic model.

A wide array of molecular and analytical methods are planned to be utilized in environmental research. NGS (New Generation Sequencing), qPCR (quantitative Polymerase Chain Reaction), confocal microscopy, molecular cloning, *in vitro* culture, high performance liquid chromatography (HPLC) and analytical methods (inorganic chemistry) such as the Kjeldahl method, absorption atomic spectroscopy will be used. The role of genes targeted in genome wide expression analysis (RNAseq) (and/or previously described) will be verified with *A. thaliana* mutants and transgenic plants, generated in our laboratories.

The obtained results will broaden our understanding of the processes leading to plant adaptation to metals toxicity. They will also be a good beginning point in studying the possibility of utilizing microorganisms in re-cultivation of polluted environments. The effects of water and land pollution make this objective of research of particular significance.