The aim of this project is to investigate whether the bioaugmentation of agricultural areas contaminated with herbicides (or, in the future, with herbicidal ionic liquids – HILs) will lead to an unintended transfer of herbicide resistance to weed or crop plants by specialized bacteria. Furthermore, the novelty of our project is based on the comparison of herbicide biodegradation (classic and a new group of herbicidal ionic liquids) by microorganisms isolated from the phyllosphere, rhizosphere and endosphere of resistant and non-resistant plants. The studies to date focused solely on specific aspects, hence there is a lack of a multidimensional approach to this topic.

By employing next generation sequencing we will be able to monitor genes associated with the degradation of these xenobiotics in the environment. Moreover, we will establish the occurrence frequency of genes responsible for the biodegradation of selected herbicides among microorganisms present on the plant (phyllosphere), in the plant (endosphere), in the plant root system (rhizosphere) and finally in the plant independent soil matrix. We will compare microorganisms for herbicide resistant and non-resistant plants. Additionally, we will compare the biodegradation efficiency of the isolated bacteria under laboratory and field conditions. The ultimate verification will be the investigation of whether plants acquired herbicide resistance due to bioaugmentation with specialized bacteria (both indirectly, by the presence of efficiently biodegrading microorganisms, and directly *via* genetic mutations).

The foundation for conducting the studies is associated with the fact that modern agriculture depends on the application of herbicides, which are characterized by limited diversity. The common application of herbicides has a multidimensional negative effect on the environment. Under unfavourable conditions herbicides may accumulate in soil and migrate to surface waters. The public opinion pays special attention to the presence of herbicides in food and beverages. Furthermore, several disadvantages impede the necessity to modify herbicides by transforming them into ionic liquids. Their synthesis allows to design compounds with desired biological and physicochemical properties (e.g. practically completely reducing their volatility) and due to the use of appropriate cations and anions their efficiency may be easily increased with a simultaneous reduction of the effective dose.

Nevertheless, the permanent presence of classic herbicides in agricultural soils has contributed to the evolution of herbicide resistance in weeds, which is a major concern for agriculture – similar to the issue of antibiotic resistance in medicine. Such weeds cause a reduction of crop yield and quality, which results in a lower economic profit. Moreover, some herbicide resistant weed species are very expansive and rapidly colonize new niches. Currently, there are approx. 100 known resistant weed species, among which there are 34 species resistant to glyphosate only. The mechanism of spontaneous acquisition of herbicide resistance is not entirely known. Additionally, the resistance may be based on the efficient use of microorganisms for detoxification of the plant or to directly transfer the resistance genes into the plant. This aspect further complicates the introduction of intentionally genetically-modified crop plants e.g. Roundup Ready maize, which may potentially be a source of resistance genes into other species.

The fact that the presence of numerous bacteria capable of biodegrading herbicides was established in soil is not surprising. Attempts to use such microorganisms for environmental clean-up (especially in case of aqueous environments) via bioaugmentation were undertaken with diverse efficiency. Recent years introduced methods to monitor such processes using next generation sequencing with emphasis on tracing genetic expression as a tool for conducting successful bioaugmentation. Furthermore, there is more data regarding the mutual interaction between the microorganisms and plants. Initially, the role of bacteria in the root zone (rhizosphere) was highlighted as a buffer which protects the plant against xenobiotics present in soil, which also had a notable impact on the plant. Currently, there is a growing interest in microorganisms isolated from plant tissues (endophytes), which directly influence the plant metabolism. Moreover, in a properly functioning terrestrial environment the plant along with its endophytes, phyllosphere and rhizosphere exists in a soil matrix colonized by various microbial communities. The presence of xenobiotics in soil notably influences all populations by decreasing their biodiversity and favouring the growth of species capable of survival under stressful conditions. This phenomenon specifically applies to microorganisms which degrade xenobiotics, inducing herbicides. This leads to a situation when the plant is subjected to constant stress by the presence of herbicides and permanent contact with bacteria possessing genes responsible for herbicide resistance and their degradation. In consequence, it can be expected that the plant will also gain resistance by efficient utilization of microorganisms for the detoxification or resistance based on the modification of its own generic material. This sort of unintended acquisition of resistance by plants is the most crucial issue for agriculture, which became the foundation to propose this project.