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Summary of the doctoral dissertation

Assessment of the potential of using microorganisms to improve the effectiveness of selected phytotechnologies in the remediation of soils and waste contaminated with metals

Keywords: bacteria, biodiversity, phytoremediation, inoculation, trace elements, smelter waste piles

Smelter waste piles and soils contaminated with elements such as zinc, lead, cadmium and arsenic create a serious environmental problem in areas with a high density of smelting industry. One of the strategies of limiting the negative impact of such areas is phytoremediation. It is aimed at creating a permanent plant cover that limits the dispersion of metals, which can be based on the use of both remediation plants and spontaneous vegetation. The role of microorganisms in the colonization of smelter waste piles by plants may also be significant, which has not been fully recognized. Moreover, the biodiversity of native microorganisms is extremely important for explaining their role in the colonization of such soils and landfills, and is a source of strains adapted to stressful conditions and potentially supporting plants.

The main aim of the research was to identify the role of microorganisms in phytoremediation of soils and smelter waste deposits contaminated with metals and assess potential of bacteria in optimizing phytoremediation treatments. To achieve the main goal, it was necessary to meet the following specific objectives: (1) assessment of the long-term phytostabilization of smelter waste pile in Piekary Śląskie on the enzymatic and metabolic activity of bacteria; (2) identification and acquisition of microorganisms potentially useful in phytoremediation; (3) assessment of the interaction between the diversity and activity of microorganisms and the development of vegetation spontaneously inhabiting non-remediated smelter waste heaps; (4) assessment of the potential for further increase in effectiveness of phytoremediation by using bacteria that are isolated from various contaminated sites.

The scope of the work was divided into three research tasks. Each task corresponded to specific objectives and research hypotheses set out in this dissertation. The **first research task** included testing soil samples from the slag waste pile in Piekary Śląskie, reclaimed in 1996 with the use of sewage sludge and waste lime and selected varieties of grasses. The monitoring

of this site has shown that the reclamation methods used ensure the permanent plant cover of the pile and the reduction of secondary emission of pollutants. However, there were no data available on the biological activity of the soil and the presence of bacterial groups with key functions for the resistance of such reclaimed wasteland, such as nitrogen-fixing bacteria or bacteria involved in phosphorus transformation. The research proved that the applied reclamation method enables long-term functioning of the ecosystem created on the waste heap, characterized by diversity of microorganisms, which additionally supports the resistance of the plant cover to chemical and weather stresses. The activity and abundance of the various groups of microorganisms were highest in the plots where the combination of sludge and lime was applied. Since the reclaimed heaps are not fertilized regularly, the processes of microbiological nitrogen release are key to maintaining vegetation. Bacteria of the genus *Azotobacter* were found only in plots where sewage sludge was combined with lime. Moreover, the combined use of sewage sludge and lime ensured the highest number of ammonification bacteria. A clear increase in the metabolic and genetic diversity of the soil microbiome was also demonstrated as a result of the application of sludge and lime. Bacteria involved in the nitrogen cycling and phosphorus solubilization were isolated from the plots and identified. On the basis of the obtained data, it can be concluded that the stimulation of the microbial diversity and activity on the reclaimed site is necessary to achieve the effectiveness of the treatments.

In the **second research task**, it was assumed that the natural waste pile colonisation by plants are of great importance for the possibility of explaining the role of microorganisms in the these processes and their interaction with plants in phytostabilisation. Therefore, plant material and soil samples were collected from two non-reclaimed waste dumps in the region of Piekary Śląskie from root zone of plant species dominant in natural revegetation processes. The chemical properties and the total number of bacteria and fungi as well as the number of individual groups of bacteria were determined in soil samples. Next generation sequencing (NGS) analysis was used for molecular identification of bacterial populations and metabolic diversity was determined using the Biolog®EcoPlates method. Additionally, a biotest was carried out to assess the level of biosorption of trace elements by microorganisms in the plant root zone. The task showed that the proportion of unclassified bacteria in the soil was high, especially in the control slag samples from the area without plants. This proves the specific structure of the bacterial population in the slag waste. The processes of waste colonization by plants transform the soil microbiome into more similar to that characteristic of soils. It can also be assumed that the interaction of plants and microorganisms stimulates the processes supporting plant growth, similar to those occurring in soils. Actinobacteriota were the most

abundant type of bacteria, accounting for at least 25% of the total population, and among the types of bacteria, *Blastococcus*, *Nocardioides* and *Pseudonocardia* were the most abundant. Research has shown that interactions between plants and microorganisms lead to acceleration of the activity of microorganisms and changes in the utilisation of carbon substrates. These interactions also lead to mobilisation of nutrients including phosphorus, potassium and nitrogen. It can be assumed that these processes enable the survival of vegetation in this contaminated and poor in nutrients environment. The research also showed a statistically significant contribution of microorganisms to the biological immobilization of trace elements.

The **third research task** included testing the effectiveness of selected bacterial strains in the optimization of phytostabilization treatments of smelter waste. The bacterial strains used were previously isolated from the reclaimed landfill in Piekary Śląskie and from contaminated soils in Spain. In a pot experiment, the influence of bacterial strains on the development of grasses and the solubility of trace elements were tested. The strains were applied in combination with soil amendments commonly used in phytostabilization (compost, calcium phosphate, iron oxide). As the individual treatment, all tested strains improved the growth and yield of Italian ryegrass. They were also effective in supporting the growth of plants on slag with the addition of compost and calcium phosphate. The study has shown that there is a potential for further improving the efficiency of phytostabilization by using selected strains of bacteria. Better development of plant biomass in the waste pile after phytostabilisation treatments means in practice greater durability and effectiveness of remediation and limitation of the dispersion of potentially toxic elements.

In summary, the research results prompted the following final conclusions:

(1) The use of sewage sludge and waste lime in the stabilisation of smelter waste piles permanently restores the biological activity and soil functions related to the activity of microorganisms, ensuring an ecosystem resistant to environmental pressures.

(2) Interactions between the soil microbiome and plants inhabiting the waste piles are crucial for the restoration of biological life and for ensuring conditions for plant growth in landfills, both in the phytostabilization approach and in the natural attenuation processes.

(3) The use of selected strains of bacteria may increase the effectiveness of assisted phytostabilization, by supporting plant development and influencing the bioavailability of nutrients and trace elements. The applied waste inoculation with bacteria had a positive effect on the plant yield under controlled conditions, signaling their potential in optimizing phytostabilization processes.