Interactions between rhizosphere microorganisms and spontaneous plant species inhabiting smelter wastelands

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Abstract
The role of microorganisms in colonizing toxic smelter waste deposits by plants has not been sufficiently understood. The aim of the work was to assess interactions between microorganisms and most frequent spontaneous plants inhabiting two waste deposits in Piekary, Poland. The samples were collected in the summer of 2018 from rhizosphere of 8 plant species inhabiting the waste pile: 1. *Thymus serpyllum*; 2. *Silene vulgaris*; 3. *Solidago virgaurea*; 4. *Echium vulgare*; 5. *Rumex acetosa*; 6. *Verbascum thapsus*; 7. *Solidago gigantea*; 8. *Eupatorium cannabinum*. The samples were subjected to analyzes of enzymatic activity, abundance of microorganisms, functional (System Biolog) and genetic diversity (NGS), physicochemical properties (pH, metal content and solubility, nutrient content) and subsequently compared with the unplanted reference samples. Plant samples were analyzed for metals and nutrient contents.
Keywords: microorganisms, rhizosphere, smelter wasteland, trace metals

1. Introduction

Processes of spontaneous vegetation of toxic zinc (Zn) and lead (Pb) smelter waste deposits are slow. However, permanent plant cover, effectively limiting dispersion of metals, should be based both on introduced grasses and spontaneous species to ensure greater plant diversity and resistance of such ecosystem (Siebielec et al., 2018). The role of microorganisms in colonizing smelter wastelands by plants has not been sufficiently explored. Most of Zn and Pb waste deposits are almost barren but sparse spontaneous plants provide valuable information on relationships between plants and rhizosphere microorganisms helping plants to establish their growth. The growing conditions for both plants and microorganisms in such waste are extremely harsh due to the high content of heavy metals and deficit of nutrients and water. The aim of the work was to assess interactions between microorganisms and most frequent spontaneous plants inhabiting a waste deposit in Piekary, Poland.

2. Materials and Methods

2.1. Top layer sampling

The soil samples were collected in the summer of 2018 from two waste deposits (slag and flotation waste) to represent rhizosphere of 8 plant species inhabiting the smelter wasteland: 1. *Thymus serpyllum*; 2. *Silene vulgaris*; 3. *Solidago virgaurea*; 4. *Echium vulgare*; 5. *Rumex acetosa*; 6. *Verbascum thapsus*; 7. *Solidago gigantea*; 8. *Eupatorium cannabinum*. The samples were collected from a 0-15 cm depth, from the rhizosphere soil the plants and two locations representing barren area, serving in the study as reference samples. The material was thoroughly mixed, homogenized and transported to the laboratory, where the material was sieved through a 2mm mesh and subdivided into two portions: one to be dried for chemical analysis and the second stored fresh in the refrigerator at ±4°C for microbiological and biochemical analysis.

2.2. Soil biochemical and microbiological analyses

In order to characterize biochemical activity of the amended wasteland, activities of three enzymes (dehydrogenases, acidic and alkaline phosphatases) were measured using standard protocols (Tabatabai, 1994).

The total count of bacteria and actinobacteria, ammonification bacteria, bacteria of *Azotobacter* and total count of fungi were determined by the plate dilution using standard protocols.

The assessment of metabolic profile (phenotypic) of soil samples was carried out using BIOLOG EcoPlate® System (Biolog TM, USA). The method is based on direct inoculation of BIOLOG plates, containing range of carbon sources with microbial suspension from soil. The change of purple tetrazolium color is an indicator of the degradation of the given carbon source. Absorbance at 590 nm was measured using BIOLOG Microstation after 144 h incubation.

The genetic characteristics of bacterial population was done after isolation of genomic DNA directly from soil sample with the use of ready-to-use kits, such as FastDNA® SPIN Kit for Soil (MP Biomedicals). The

isolated and purified genomic DNA was subjected to an amplification reaction of the fragment of gene 16S rDNA.

2.2. Soil physicochemical properties

Air dried samples were subjected to pH using a combined glass electrode in a slurry with a 1:2 v/v soil/water ratio. Organic matter (OM) content was measured by a loss on ignition in a muffle oven at 480^{o}C within 16 h. The texture of the samples was done by the laser diffraction method. Total trace element contents were measured after digestion of a sample in a 3:1 mixture of concentrated HNO_3:HCl in teflon PFA vessels in a microwave accelerated reaction system (MarsXpress; CEM Corp., USA) followed measurements of elements in extracts by ICP-MS (Agilent 7500ce). Analysis of metals solubility was done by extraction in deionized water (1:2 soil/water ratio, shaken for 2 h at room temperature) and subsequent measurement of metal concentrations by ICP-MS.

3. Results

Total contents of potentially toxic metals in the waste were extremely high and represented the following range for Zn, Pb and Cd: 13,200 – 116,130; 7,500 – 33,200 and 127 – 1,060 mg kg^{-1}, respectively. Soil pH was alkaline: 7.9 – 8.6. Statistically significant differences in the activity of the enzymes in soils representing different plant species were observed. Highest activity of dehydrogenases, and both acid and alkaline phosphatase were found for the *T. serpyllum*, *S. vulgaris* and *S. virgaurea*. Rhizosphere samples of all tested plants were more active than control soils. especially as compared to control areas. Differences in the intensity of carbon source decay indicate that the highest biochemical activity was characteristic to samples taken from rhizosphere of *V. thapsus* and *E. cannabinum*, especially when compared to the control areas (Fig. 1.). They were collected from the slag waste.

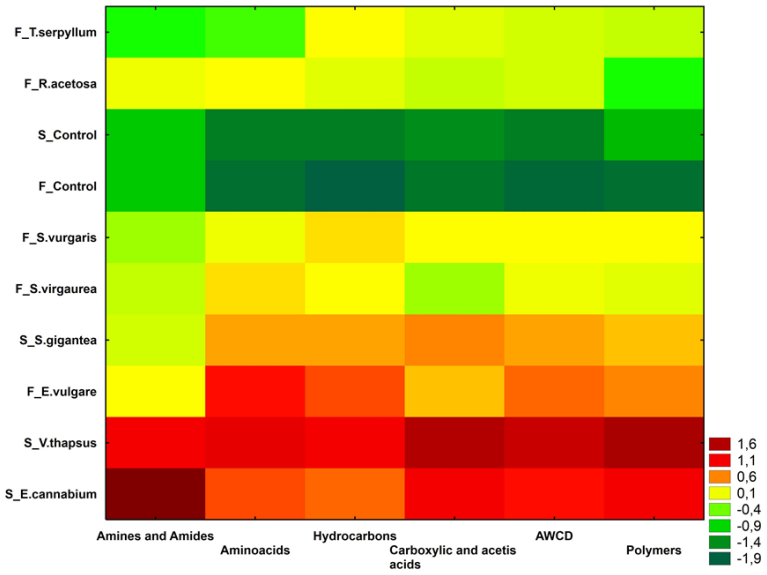

Figure 1. Metabolic profile of soil collected from rhizosphere of spontaneous plants

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