

Summary

Arbuscular mycorrhizal fungi (AMF) are the obligatory symbionts of most known plant species. In exchange for the photosynthetically assimilated carbon, they provide a host plant with mineral nutrients and stimulate its growth and viability, also under stress conditions. The positive influence of AMF on plants have gained much interest in the field of research on phytoremediation of soils contaminated with hydrocarbons, showing synergetic interactions between AMF, a plant host and hydrocarbon-degrading bacteria.

The aims of this study were to: characterize the biodiversity and development of AMF in roots and soil associated with *Ph. australis* and *P. trivialis* growing in the environment contaminated with phenol and polynuclear hydrocarbons (PAHs) and in the uncontaminated environment evaluate the biomass of other groups of soil microorganisms, assess the potential of AMF species isolated from the contaminated site in enhancing the growth of *Lolium perenne* in the presence of phenol and PAHs.

AMF development in the studied environments was evaluated on the basis of the mycorrhizal colonization of roots, the number of SSU rDNA gene copies in roots, the length of extramatrical mycelium, the number of AMF spores, the concentrations of glomalin-related soil proteins and marker neutral fatty acid 16:1 ω 5c in soil. Biomass of the main groups of soil microorganisms was evaluated by the analysis of the content of marker phospholipid fatty acids in soil. The biodiversity of AMF communities was analyzed based on the DGGE (denaturing gradient gel electrophoresis) profiles of SSU rDNA gene and the next generation sequencing of LSU rDNA metagenomic libraries. Isolation of AMF from environmental samples was performed using trap cultures. Isolated AMF strains were affiliated to species based on the sequence of SSU rDNA – ITS – LSU rDNA genes. AMF species isolated from the contaminated environment: *Funneliformis caledonium*, *Claroideoglossum walkeri* and *Diversispora varaderoana*, were used as single-species and multi-species inocula in *L. perenne* cultures contaminated with the mixture of phenol and PAHs. Final concentrations of the contaminants in culture medium were : 0/0, 5/20, 15/60, 30/120 mg phenol/PAHs kg⁻¹. Development of mycorrhiza in the cultures was evaluated by the assessment of mycorrhizal root colonization and the number of AMF spores. Isolated spores were used for the analysis of oxidative stress and the activity of antioxidative enzymes in AMF. The effect of AMF on plant growth was evaluated on the basis of shoot height, root Bioróżnorodność i rozwój grzybów and shoot biomass, the level of oxidative stress and the activity of antioxidative enzymes in plant tissues.

Soil contamination with phenol and PAHs had a negative effect on mycorrhizal root colonization, AMF biomass in soil and the biodiversity of AMF communities, especially those associated with *Ph. australis*. There were no significant differences between the biodiversity and structure of AMF communities in roots and soil. Soil contamination was identified as the main factor affecting the species structure of AMF communities. Host plant species had a significant effect on the structure of AMF communities only in the uncontaminated site. In both studied environments, a strong domination of AMF assigned to the family *Paraglomeraceae* was revealed. Moreover, AMF communities in the contaminated site were dominated by genera *Funneliformis*, *Rhizophagus* and *Claroideoglossum*, described previously as ruderal and stress tolerant AMF. Beside the family *Paraglomeraceae*, other AMF which dominated in the communities associated with the uncontaminated site represented the genus *Archaeosporaceae* and rarely identified AMF from the family *Glomeraceae*, eg. *Dominikia* sp., found previously in the environments undominated by ruderal AMF.

Soil contamination with phenol and PAHs had no significant influence on the total biomass of soil microorganisms. Biomass of the main groups of soil microorganisms depended on the interaction between the studied site and the host plant species. There were no significant differences, in the biomass of saprophytic fungi and the main groups of bacteria associated with *P. trivialis* between both studied sites. On the other hand, the contaminated soil associated with *Ph. australis* was characterized by a higher biomass of Gram-positive bacteria and actinobacteria, compared to the uncontaminated soil. Beside phenol and PAHs, actinobacteria might be another factor which

suppressed the development of AMF, because their biomass was negatively correlated with the biomass of AMF in soil.

Excluding *Dominikia* sp., AMF isolated in trap cultures did not represent dominant species which defined the studied AMF communities. Spores in the laboratory conditions were produced by AMF species which relative abundance in the studied communities was marginal. *Funneliformis caledonium*, *Diversispora varaderoana* and *Claroideoglossum walkeri*, isolated from the contaminated site, were used as inocula in the study on the effect of phenol and PAHs on the development of AMF and *L. perenne*. Plants inoculated with single AMF species reached higher growth and biomass, regardless of the concentration of contaminants, compared to plants inoculated with the mixture of AMF species, where a parasitic interaction between *L. perenne* and *C. walkeri* was observed. Although the mycorrhizal inoculation had a positive effect on plant growth, it did not contribute to a higher activity of antioxidative enzymes and a lower level of oxidative stress in plant tissues. AMF response to low and medium contamination with phenol and PAHs was the allocation of biomass to intraradical mycelium. High concentration of phenol and PAHs had a negative effect on mycorrhizal root colonization, production of spores and the level of oxidative stress in AMF spores.

The presented results constitute a firm background for further application research on the potential of native AMF strains and microorganisms cooperating with them in the enhancement of phytoremediation of soils contaminated with toxic organic substances.