

Rhizobia and arbuscular mycorrhizal fungi (AMF) as the microbiota potentially improving the growth of legumes in heavy metal polluted areas

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The metalliferous tailings in southern Poland are spontaneously colonized by metal-tolerant *Anthyllis vulneraria* L. (*Fabaceae*), which can form simultaneously symbiotic association with nitrogen-fixing rhizobia and phosphorus-acquiring arbuscular mycorrhizal fungi (AMF). So far, the diversity of symbiotic microbiota of legumes colonizing the tailings have been poorly studied.

The aim of this study was to characterize the rhizobia, their plant growth promoting (PGP) traits, and AMF associated with *A. vulneraria* calamine ecotype. The results indicated that plants are nodulated by metal-resistant *Bradyrhizobium liaoningense* and *Rhizobium metallidurans*, which showed PGP traits thus may induce *A. vulneraria* growth on metal contaminated sites directly by nitrogen fixation, IAA and ammonium production, phosphate solubilization, siderophore formation or lowering ethylene levels. Moreover, the nodulated roots were intensively colonized by AMF (mycorrhizal frequency 86.3% and relative mycorrhizal intensity 20.53%) with the *Arum*-type of mycorrhiza. Molecular identification of AMF using PCR-DGGE analysis based on the 18S rDNA ribosomal gene by nested-PCR revealed *Rhizophagus* sp., *R. fasciculatus*, and *R. iranicus* in *Anthyllis* roots. Heavy metal excess had no negative effect on the number of AMF spores, the amounts of glomalin-related soil proteins and AMF species composition. AMF may influence the plant growth directly providing nutrients and/or decreasing metal uptake and translocation by glomalin production. Metal ions were accumulated mainly in the nodules and intraradical fungal structures rather than thick plant cell walls.

The results of this work indicate the presence of unique symbionts, which may enhance *A. vulneraria* tolerance to heavy metal stress and plant adaptation to extreme conditions on calamine tailings.