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## Co-cropping and inoculating hyperaccumulator plants with PGPR: how to improve metal phytoremediation?

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**ABSTRACT**: Phytomining technologies could be developed to ensure production of strategic metals, while preserving soil functions, by decreasing their metal toxicity. These processes would provide a range of economic, social and ecosystem services from materials and lands of initial low value. Agromining is the conception of agro-metallurgical production chains based on cropping hyperaccumulator plants on contaminated matrices (soils) or naturally rich in metals (ultramafic soils) to produce high value metal compounds and decreasing metal toxicity in soils.

Recently, the role of rhizosphere microorganisms in metal phytoextraction processes was stressed. Hyperaccumulator crops are included in theses approaches. Among microorganisms of potential interest (PGPR), some promote plant germination and facilitate the development of root biomass *via* the production of hormone-like molecules. Some also promote the resistance of plants to the stress exerted by the pollutant *via* the production of ACC deaminase. Such expected effects should ensure a better plant development and improve plant growth, biomass production and/or metal uptake and accumulation. But, in many cases the effects of these plant-microbial associations have been shown to be plant-species specific, thus underlining the importance of the origin of strains and the choice of inoculated plants.

Therefore, a series of experiments was designed to assess the effect of co-cropping of hyperaccumulators on the phytoextraction process (biomass production, nickel uptake). Results showed that there is positive effect in several cases of associations. The co-crop of hyperaccumulators *Bornmuellera tymphaea - Noccaea tymphaea* was the most successful plant association. The next step was to look for potential PGPR in the same associations that occur in situ (Balkans).

Plant growth promoting rhizobacteria were isolated from the in situ rhizosphere of two different natural plant associations (*B. tymphaea – N. tymphaea* and *B. tymphaea – Alyssum murale*) collected in Greece in 2013. The effect of field-isolated PGPR on phytoextraction was studied (i.e. plant biomass production and nickel phytoextraction) on the same plant associations as in original field conditions. The screening of isolates from both plant associations targeted the following PGPR traits: indole-3-acetic acid (IAA) production, siderophores production, and 1-aminocylopropane-1-carboxylate deaminase (ACCd) activity. The screening revealed two powerful PGPR strains (AB30 and NB24). Moreover, the genetic characterization showed that both strains were affiliated to *Variovorax paradoxus*. After 5 months of culture, inoculation of the associations *B. tymphaea – N. tymphaea* and *B. tymphaea – A. murale* with the selected PGPR was performed and the plants were allowed to grow for one more month before harvest.

Results show a significant improvement of root growth and an increase in Ni uptake after inoculation with selected PGPR strains. The combination between the hyperaccumulator plants *Noccaea-Bornmuellera* inoculated by the PGPR strain NB24, seemed to be the best choice for an efficient phytoextraction *in situ*. Further experiments will be conducted in field conditions to implement both co-cropping and PGPR assisted phytoextraction in agromining cropping systems.