1.4 = Rhizosphere response to Ni stress in the facultative hyperaccumulator *Alyssoides utriculata* (L.) Medik.

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The contamination of metals like Nickel (Ni) represents a serious threat worldwide in the soil. To counteract this phenomenon, hyperaccumulator plant species, able to remove metal from soil and store it at high concentration in shoots, are employed for metal phytoremediation purposes. Native microbial communities occurring in the rhizosphere of hyperaccumulators often promote plant growth and metal uptake.

So far, each abiotic and biotic rhizospheric components (soil, root system and microbiota) have been used without considering the reciprocal interactions within the responses to Ni stress. The present study aims to develop for the first time an innovative and multidisciplinary approach to examine the rhizosphere of Ni-hyperaccumulators as a holistic model, promoting the plant development and the Ni uptake.

Among metalliferous soils, specific attention was given to serpentine which display extremely hostile conditions (nutrient shortage and highly toxic concentration of metals - e.g., Ni, Cr, Co, Mg) for most plants except for some hyperaccumulator species.

Early response to Ni in plant development was assessed with micro- and mesocosm germination tests under Ni stress in the Ni-hyperaccumulator species *Alyssoides utriculata* (L.) Medik., *Noccaea caerulescens* (J. Presl & C. Presl) F. K. Mey., *Odontarrhena bertolonii* (Desv.) L. Cecchi & Selvi, and nonaccumulator species *Alyssum montanum* L., and *Thlaspi arvense* L. used for comparison. Afterwards, the response to increasing Ni concentrations in terms of root surface area, root and shoot biomass and photosynthetic efficiency was evaluated.

Subsequently, *A. utriculata* was selected as a good candidate to study rhizospheric components because of its Ni-facultative hyperaccumulation traits and its ability to thrive in harsh metalliferous soils. Related rhizosphere and bare soil samples were collected from serpentine and non-serpentine sites.

Plant and soil samples were processed and analysed with specific attention to isolation and identification of cultivable microbiota, then selected for their Ni-tolerance and Plant Growth Promoting (PGP) traits.

Results demonstrate that increasing Ni concentrations can induce marked inhibition of germination in hyperaccumulator species, despite their accumulation ability. However, hyperaccumulator species exhibit a positive response in terms of root surface area, biomass and photosynthetic efficiency, compared to non-hyperaccumulator species in which there is a dose-response effect by Ni, except for *T. arvense* in pot test.

Specifically, *A. utriculata* reveals an increased aboveground biomass and sample vitality in pot test, suggesting an adaptation to harsh environmental conditions.

Microbiota isolates (bacteria and fungi) are more abundant in non-serpentinitic and rhizospheric soil, without selectivity between microorganisms and Ni.

Some bacterial and fungal strains (*Pseudomonas* sp. SERP1, *Streptomyces* sp. SERP4 and *Penicillium ochrochloron* Biourage Serp03S, *Trichoderma harzianum* Rifai Serp05S respectively) reveal high Ni tolerance (up to 20 nM) and PGP traits. The selected strains could be promising candidates as natural chelators in the rhizosphere of *A. utriculata*, to enhance plant development and Ni uptake.

This research represents the first step of integrated plant-bacteria-fungi tool, in the perspective to improve Ni uptake from polluted soil, using native Ni-hyperaccumulator species and associated rhizobiota, although further investigations are required to ascertain the efficiency of the field application.