

Review

Linking microbiome and hyperaccumulation in plants

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Abstract

Hyperaccumulating plants can take up extraordinarily large concentrations of one or more metal(loid)s from the soil and accumulate it/them in the aboveground tissues without exhibiting any visible toxicity symptoms. Among more than 700 plant taxa reported to have evolved this unique phenotype, the most common is the hyperaccumulation of nickel (Ni), and less common is the hyperaccumulation of arsenic (As), cadmium (Cd), cobalt (Co), chromium (Cr), copper (Cu), manganese (Mn), lead (Pb), antimony (Sb), selenium (Se), thallium (Tl) or zinc (Zn). Metal(loid) hyperaccumulation is a result of several independent evolutionary events and despite considerable efforts, none of the proposed hypotheses on the environmental constraints driving these events has been supported fully to date. Among several tolerance strategies enabling hyperaccumulation is the allocation of metal(loid)s to competent cell types, typically away from photosynthetic apparatus, to limit damage to plant metabolism. Recently, the involvement of microorganisms colonizing roots in hyperaccumulation phenomenon has achieved increased attention due to the role of microorganisms in the mobilization of metal(loid)s in the soil. The complex interactions between hyperaccumulation and belowground microbiome are of primary interest for phytoremediation, a promising green technology for removing or immobilisation of metal(loid)s in the soil with the help of plants. In this review, we discuss and complement current reports on the contribution of microorganisms to metal(loid) hyperaccumulation.

Keywords

hyperaccumulating plants; arbuscular mycorrhizal fungi; dark septate endophytes

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Povezava med rastlinskim mikrobiomom in hiperakumulacijo

Izvleček

Hiperakumulacijske vrste lahko iz tal v svoja nadzemna tkiva privzamejo zelo velike koncentracije ene ali več (pol)kovin brez opaznih škodljivih učinkov pri rastlini. Pri več kot 700 taksonih, za katere poročajo, da so razvili ta edinstven fenotip, je najpogostejša hiperakumulacija niklja (Ni), manj pogosta pa je hiperakumulacija arzena (As), kadmija (Cd), kobalta (Co), kroma (Cr), bakra (Cu), mangana (Mn), svinca (Pb), antimona (Sb), selena (Se), talija (Tl) in cinka (Zn). Hiperakumulacija (pol)kovin je posledica več neodvisnih evlucijskih dogodkov. Kljub precejšnjim prizadevanjem pa nobena od predlaganih hipotez, ki bi razložila evlucijsko prednost hiperakumulacije, do danes ni bila v celoti podprta. Med številnimi tolerančnimi mehanizmi, ki omogočajo hiperakumulacijo, je tudi kopičenje presežnih koncentracij (pol)kovin v določenih tipih rastlinskih celic, običajno stran od fotosinteznega aparata. V zadnjem desetletju v ospredje raziskav hiperakumulacije stopa vloga mikroorganizmov, ki kolonizirajo korenine, pri mobilizaciji (pol)kovin v tleh. Zapletene interakcije med hiperakumulacijo in podzemnim rastlinskim mikrobiomom so zanimive predvsem z vidika fitoremediacije, t.j. zelene tehnologije, ki s pomočjo rastlin na naraven način odstrani (pol)kovine iz tal ali pa jih stabilizira. V tem preglednem članku razpravljamo in dopolnjujemo dosedanje študije o prispevku talnih in/ ali simbiotičnih mikroorganizmov k hiperakumulaciji.

Ključne besede

hiperakumulacijske vrste; arbuskularne mikorizne glive; temni septirani endofiti

Introduction

The term hyperaccumulator was introduced by Brooks et al. (1977) to describe plants with exceptional concentrations of one or more metal(loid)s in the aboveground biomass without showing any visible toxicity symptoms (Brooks et al., 1977; Rascio, 1977). Initially, these unique plants were studied in relation to geobotany, while today, they have been at the centre of discussions concerning environmental pollution and phytoremediation. Hyperaccumulation is more than tolerance because a typical metal(loid)-tolerant plant aims to limit translocation of metal(loid) from roots to shoots, whereas in aboveground tissue of a hyperaccumulating plant, up to 1000-fold larger concentrations of metal(loid)s can be found compared to non-hyperaccumulating plants (Rascio, 1977; Reeves, 2006). Among hyperaccumulators, the largest number of species hyperaccumulate Ni, followed by other metal(loid)s like As, Cd, Co, Cr, Cu, Mn, Pb, Sb, Se, Tl and Zn (Table 1) (Baker et al., 2000; Baker & Brooks, 1989; Reeves et al., 2018; White & Pongrac, 2017). To warrant the designation of a plant as a hyperaccumulating species, the following conditions must be met: (i) the plant has to accumulate metal(loid) when grown in native soil, (ii) the shoot-to-root concentration ratio for a metal(loid) must be higher

than unity, and (iii) foliar concentration (in mg g⁻¹ dry weight) of a metal(loid) must exceed the thresholds 0.1 for Cd, 1 for As, Co, Cr, Cu, Ni, Pb, Sb, Se, Tl, and 10 for Mn and Zn (Baker et al., 2000; Baker & Brooks, 1989; Krämer, 2010; McGrath & Zhao, 2003; Rascio & Navari-Izzo, 2011). To date, more than 700 plant taxa are listed as hyperaccumulating, with several belonging to the Brassicaceae family (Baker et al., 2000; Krämer, 2010; Reeves et al., 2018; White & Pongrac, 2017).

Many authors argue that hyperaccumulation has independently evolved multiple times (Krämer, 2010; Reeves, 2006) as a response against herbivory and pathogens to which plants are constantly exposed (Boyd, 2007; Plaza et al., 2015). It is an extreme evolutionary trait, and although several hypotheses have been proposed, none of them is fully scientifically supported. However, the most accepted hypothesis among researchers remains the “defence against natural enemies” hypothesis, which predicts that plants (hyper)accumulate metal(loid)s in their aerial tissues to protect themselves from pathogens and herbivores. Furthermore, it also predicts the variety of defence organic compounds (Pollard, 2022; Rascio & Navari-Izzo, 2011) to complement large metal(loid) concentrations.

Although there are many studies on hyperaccumulating plants, many knowledge gaps still need to be filled to fully

Table 1. List of metal(loid)s, number of species that hyperaccumulate corresponding metal(loid) and selected hyperaccumulating species with corresponding references. Note that one species can hyperaccumulate more than one metal(loid) and that not all combinations are presented in the current table. The number of species was summarized by Reeves et al. (2018).

Tabela 1. Seznam kovin(loidov), število vrst, ki hiperakumulirajo ustrezne kovine(loide), in izbrane hiperakumulirajoče vrste z ustreznimi referencami. Upoštevajte, da lahko ena vrsta hiperakumulira več kot eno kovino(loid) in da v tej tabeli niso predstavljene vse kombinacije. Število vrst je bilo povzeto po Reeves et al. (2018).

Metal(loid)	Number of species	Selected plant species (family)	Reference(s)
As	5	<i>Pteris vittata</i> (Pteridaceae)	Xiao et al., 2021
Cd	7	<i>Arabidopsis halleri</i> , <i>Noccaea caerulescens</i> , <i>N. praecox</i> (Brassicaceae)	Bert et al., 2003; Vogel-Mikuš et al. 2005; Wójcik et al., 2005
Co	42	<i>Haumaniastrum robertii</i> (Lamiaceae)	Van Der Ent et al., 2019
Cr	1	<i>Spartina argentinensis</i> (Poaceae)	Redondo-Gómez et al., 2011
Cu	53	<i>Aeolanthus biformifolius</i> (Lamiaceae)	Van Der Ent et al., 2019
Mn	42	<i>Grevillea meisneri</i> (Proteaceae)	Bihanic et al., 2021
Ni	532	<i>Alyssum heldreichii</i> , <i>N. pindicum</i> (Brassicaceae)	Psaras et al., 2000
Sb	At least 1	<i>P. vittata</i> (Pteridaceae)	Wan et al., 2016
Se	41	<i>Stanleya pinnata</i> (Brassicaceae), <i>Symphytotrichum ericoides</i> (Asteraceae)	Cochran et al., 2018
Tl	2	<i>Biscutella laevigata</i> (Brassicaceae)	Pošćić et al., 2013
Zn	20	<i>A. halleri</i> , <i>N. caerulescens</i> , <i>N. praecox</i> (Brassicaceae)	Kozhevnikova et al., 2017; Mijovilovich et al., 2020; Vogel-Mikuš et al., 2008

understand the physiological mechanisms behind hyperaccumulation and furthermore, its complex interactions with the surrounding environment. One of the most prominent knowledge gaps is the understanding of the role microorganisms have in hyperaccumulation, tolerance, and/or the overall fitness of hyperaccumulators. Therefore, the main aim of this review is to appraise existing knowledge and complement current reports on the contribution of microorganisms to metal(loid) hyperaccumulation.

Plants are not alone

Seemingly, plants function as individuals. However, recent investigations revealed that they host numerous microorganisms (MO), referred to as phytomicrobiome. Phytomicrobiome consists of archaea, bacteria, fungi, nematodes, protists, and viruses (Bordenstein and Theis, 2015; Trivedi et al., 2020). Together with their plant host, they form the-so-called holobiont (Fig. 1). The interactions of MO with plant hosts can be (anthropocentrically) labelled either beneficial, harmful or neutral (Arnold et al., 2000;

Brundrett, 2006; Inácio et al., 2002; Lindow & Brandl, 2003; Rosenblueth & Martínez-Romero, 2006), although gradients can be observed, namely a neutral interaction can become either beneficial or harmful, depending on different intrinsic and/or external factors.

Phytomicrobiome colonizes different parts of the plant, both vertically (Fig. 1) and horizontally. The MO colonizing the aboveground parts of the plant (e.g., leaves) inhabit the phyllosphere (Schlaeppli & Bulgarelli, 2015; Whipps et al., 2008), and those colonizing the underground (e.g., plant roots) inhabit the rhizosphere (Schlaeppli & Bulgarelli, 2015). Microorganisms can be found either inside their host's tissues or on their surface and are therefore referred to as endophytic or epiphytic, respectively. Endophytic MO is associated with the host for the whole or only a part of their life cycle. The beneficial effects of phytomicrobiome on their host have been well documented and include (i) increased tolerance to abiotic stresses (Rolli et al., 2014), (ii) enhanced nutrient uptake (Van Der Heijden et al., 2015), (iii) priming plant immune system (van der Ent et al., 2009) and (iv) protection against pathogens (Ritpitakphong et al., 2016). Some authors hypothesise that the phytomicro-

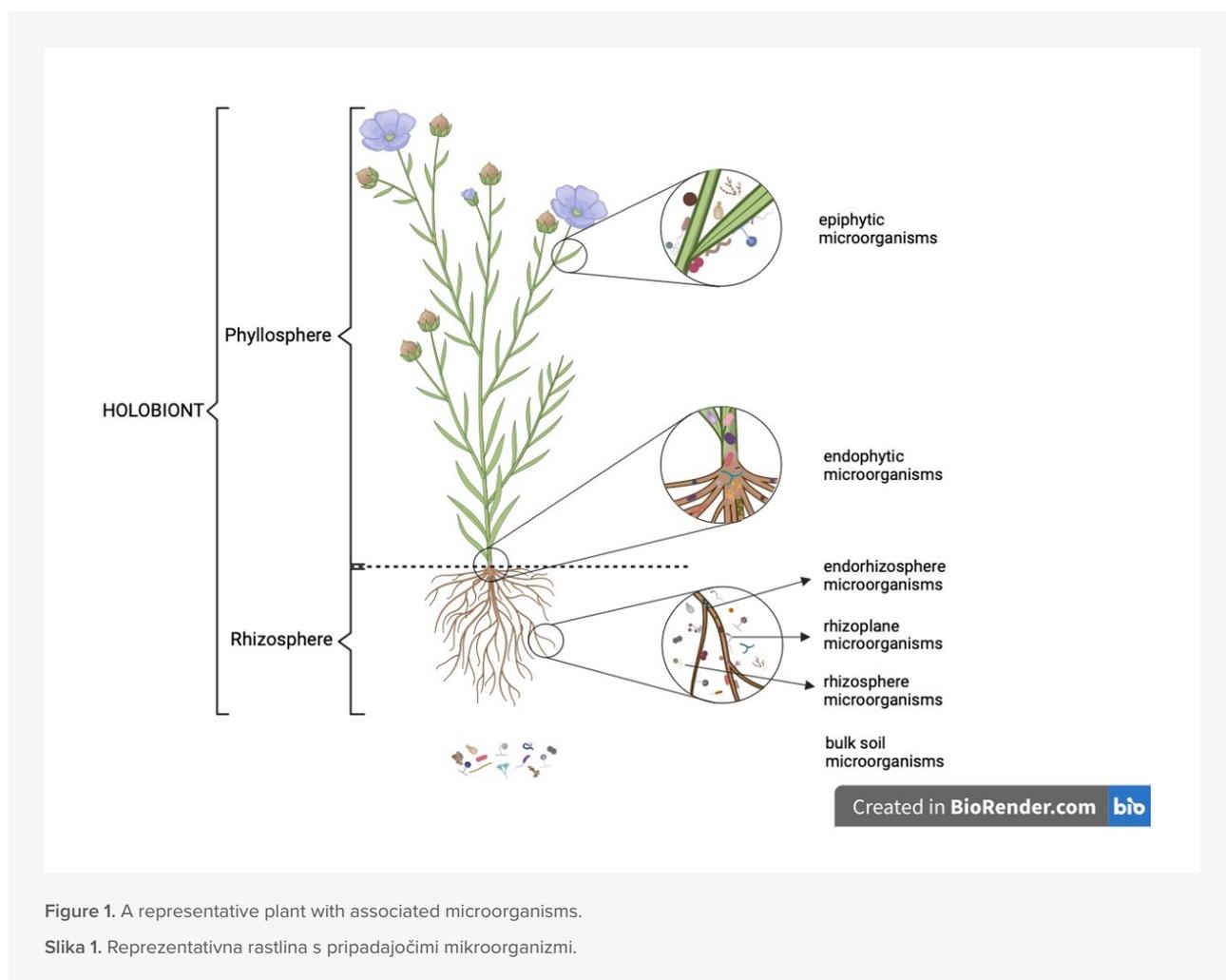
biome is an extension of the plant genome (Rosenberg & Zilber-Rosenberg, 2016; Schlaeppi & Bulgarelli, 2015).

The root system is a habitat for several MO, which inhabit both the surface of the roots and internal parts of this branched underground plant organ. Henceforth, we focus on three groups of rhizosphere-associated MO associated with hyperaccumulating plants: (i) mycorrhizal fungi, (ii) dark septate endophytes, and (iii) rhizoplane MO.

Do hyperaccumulators form mycorrhizal associations?

Mycorrhiza is one of the oldest and best-studied, presumably beneficial endophytic interactions between plants, and there is robust evidence that this interaction has played an important role in the evolution of land plants (Buscot, 2015; Martin & van der Heijden, 2024; Strullu-Derrien et al., 2018). Among different types of mycorrhiza, arbuscular mycor-

rhiza (AM) and arbuscular mycorrhizal fungi (AMF) are also associated with hyperaccumulating plants. Nevertheless, although they colonize most land plants, some plant families are believed not to form AM associations (Sharma et al., 2023). These include Brassicaceae, which hosts many known hyperaccumulators (Johnson et al., 1997; Harley & Harley, 1987; Veiga et al., 2013). However, some recent studies show that the non-mycorrhizal status of Brassicaceae might not be universal, at least in the sense of the plant family as a whole (Trautwig et al., 2023). For example, the presence of AMF was observed in *Biscutella laevigata* (Brassicaceae), a TI hyperaccumulating plant (Pošćić et al., 2013), both at metalliferous and non-metalliferous sites with all typical AMF structures, i.e., vesicles, coils, and arbuscules (Orłowska et al., 2002). A year later, Regvar et al. (2003) discovered that pennycresses (*Noccaea* spp.; Brassicaceae) form AMF associations too. The AMF colonization of a Cd and Zn hyperaccumulating *N. praecox*



was found at metal-polluted and non-polluted sites but was significantly lower or absent on the most polluted sites with the highest concentrations of Cd, Pb, and Zn (Vogel-Mikuš et al., 2005). This observation could mean there is a threshold of metal(loid) concentration that AMF can tolerate and above which mycorrhizal interactions cannot be formed. A greenhouse inoculation experiment on *N. praecox* with an indigenous AMF mixture (Vogel-Mikuš et al., 2006) indicated that AMF association is favoured during the reproductive period of the plant when the requirements for nutrients are elevated. Moreover, inoculated plants showed significant improvement in nutrient uptake and decreased uptake of Cd and Zn compared to non-inoculated plants (Vogel-Mikuš et al., 2006). Pongrac et al. (2007) later confirmed similar results under field conditions. The higher AMF colonization levels during the reproductive phase could protect the plant from potentially toxic metal(loid)s when plants invest the majority of photosynthates to seed production. The protective role of AMF was revealed by their direct effect on Cd accumulation in *N. praecox*, where the accumulation of Cd in the shoots was decreased, preventing potential over-accumulation in the seeds (Pongrac et al., 2007; Vogel-Mikuš et al., 2006). In addition, *N. praecox* (population from Žerjav, Slovenia) and a Cd, Zn, and Ni hyperaccumulating *N. caerulea* (Ganges ecotype) were successfully inoculated with AMF monospore inocula in a controlled pot experiment. The plants were grown for six months in the commercial substrate and in heavy-metal contaminated, field-collected soil from Žerjav as described previously but without metal amendments (Pongrac et al., 2009). In addition, inoculated treatments in which 100 g of monospore AMF inoculum of *Funneliformis mosseae* or *F. caledonium* (both obtained as *Glomus mosseae* and *G. caledonium* from International Collection of Vesicular Arbuscular Mycorrhizal Fungi; reference numbers UK115 and UK301, respectively) was mixed into the commercial substrate and 100 g of *N. praecox* indigenous fungal mixture to the heavy-metal contaminated, field-collected soil from Žerjav. After the first three months of growth, plants were exposed to lower temperatures to induce flowering, as this developmental stage has been shown to enhance the formation of the AM symbiosis in *N. praecox* (Pongrac et al., 2007; Vogel-Mikuš et al., 2006) and plants were allowed to grow for three more months. At harvest, roots and shoots were separated, and carefully washed with deionized water, and a subsample of roots from all treatments was retained for AMF staining (Phillips & Hayman, 1970) and

AMF colonization parameters (Vogel-Mikuš et al., 2006), whereas the rest of the shoots and roots were dried and weighed (dry weight). The concentrations of phosphorus (P), Zn, and Cd were determined after wet digestion using atomic absorption spectrometry (for Zn and Cd; (Pongrac, Zhao, et al., 2009)) and following the vanado-molybdate method according to Olsen & Sommers (1982) for P). In roots of inoculated *Noccaea* species AMF structures (hyphae, arbuscules, vesicles and coils) were observed (Fig. 2A). There was a stronger statistical difference between the species than between treatments observed in the dry biomass (Fig. 2B.). Only for *N. caerulea* there was a significant decrease in root dry weight for inoculated treatments of commercial soil (Fig. 2B).

Interestingly, inoculation with *F. caledonium* resulted in the highest shoot P, Zn and Cd concentrations for both species, compared to the control commercial substrate and the *F. mosseae*-inoculated commercial substrate (Fig. 3). In field-collected soil, the inoculation with *N. praecox* indigenous inoculum increased Cd concentration in shoots only. In root Cd concentration, there was a decrease in the commercial soil-inoculated treatments, indicating an increase in the Cd translocation factors (Fig. 3). Again, in roots from the polluted site in Žerjav, AMF structures with distinct arbuscules have been observed (Fig. 4A).

Other hyperaccumulating species have also been reported to form AM. For example, in As hyperaccumulating fern *Pteris vitatta* (Pteridaceae), an increase in the translocation of As has been observed in AMF-inoculated plants compared to non-AMF-inoculated plants (Trotta et al., 2006). In an effort to bridge the gap in our understanding of the distribution and the ecology of AMF, the GlobalAMFungi database (<https://globalamfungi.com>) has been recently developed (Větrovský et al., 2023). It will enable us to understand better the fungal taxa that form this remarkable interaction with plants.

Dark septate endophytes associated with hyperaccumulators

Another group of endophytic fungi has been discovered and studied extensively, especially with plants growing in extreme environmental conditions, namely dark septate endophytes (DSEs). DSEs comprise a miscellaneous group of ascomycetous anamorphic groups colonizing the plants inter- and/or intracellularly (Jumpponen, 2001). They have been found to colonize about 600 plant species, classified

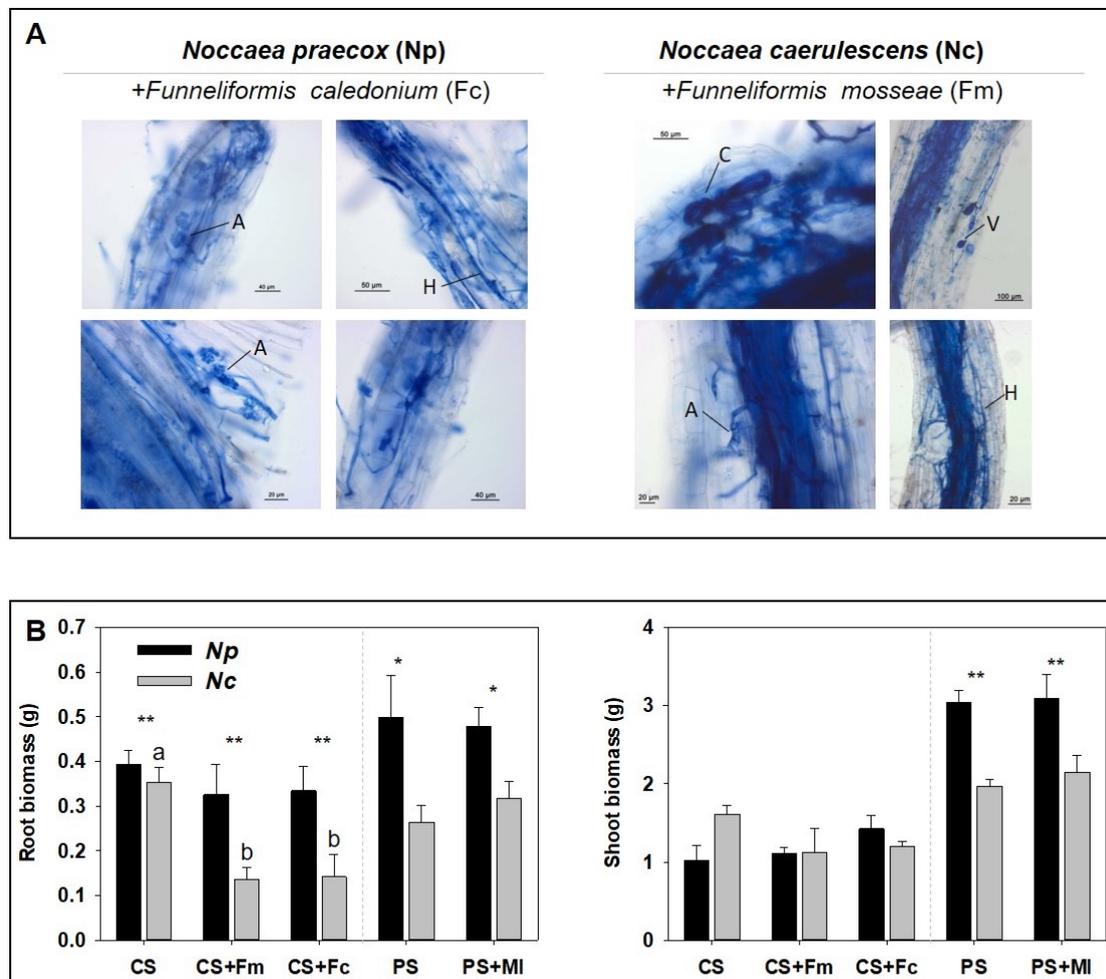


Figure 1. Arbuscular mycorrhizal (AM) structures (hyphae, H; arbuscules, A; coils, C and vesicles, V) in roots of *Nocceae praecox* (Np) and *N. caerulescens* (Nc) inoculated by AM fungi *Funneliformis mosseae* (Fm) or *F. caledonium* (Fc) in a pot experiment (A) and root and shoot biomass of six months old plants (B) grown in commercial soil (CS) and inoculated with *F. mosseae* (CS+Fm) or *F. caledonium* (CS+Fc) or grown in heavy-metal polluted, field-collected soil (PS) and inoculated with Np-indigenous AM inoculum (PS+MI). Different letters above columns present statistically significant differences between treatments (at $p < 0.05$) for CS and PS separately, and asterisks indicate statistically significant differences between species (*, $p < 0.05$; **, $p < 0.01$;) as determined by two-way ANOVA and Holm-Sidak post-hoc test. Shown are means ($n=3$) + standard errors.

Slika 1. Arbuskularne mikorizne (AM) strukture (hife, H; arbuskule, A; tuljave, C in vezikule, V) v koreninah *Nocceae praecox* (Np) in *N. caerulescens* (Nc), inokuliranih z AM glivami *Funneliformis mosseae* (Fm) ali *F. caledonium* (Fc) v lončnem poskusu (A) ter biomasa korenin in pogankov šest mesecev starih rastlin (B), gojenih v komercialni zemlji (CS) in cepljenih z glivami *F. mosseae* (CS+Fm) ali *F. caledonium* (CS+Fc) ali gojenih v s težkimi kovinami onesnaženi, na polju zbrani zemlji (PS) in cepljenih z inokulumom Np-indigenous AM (PS+MI). Različne črke nad stolpci predstavljajo statistično pomembne razlike med obravnavami (pri $p < 0,05$) ločeno za CS in PS, zvezdice pa označujejo statistično pomembne razlike med vrstami (*, $p < 0,05$; **, $p < 0,01$), kot je bilo določeno z dvosmerno ANOVA in Holm-Sidakovim post-hoc testom. Prikazana so povprečja ($n=3$) + standardne napake.

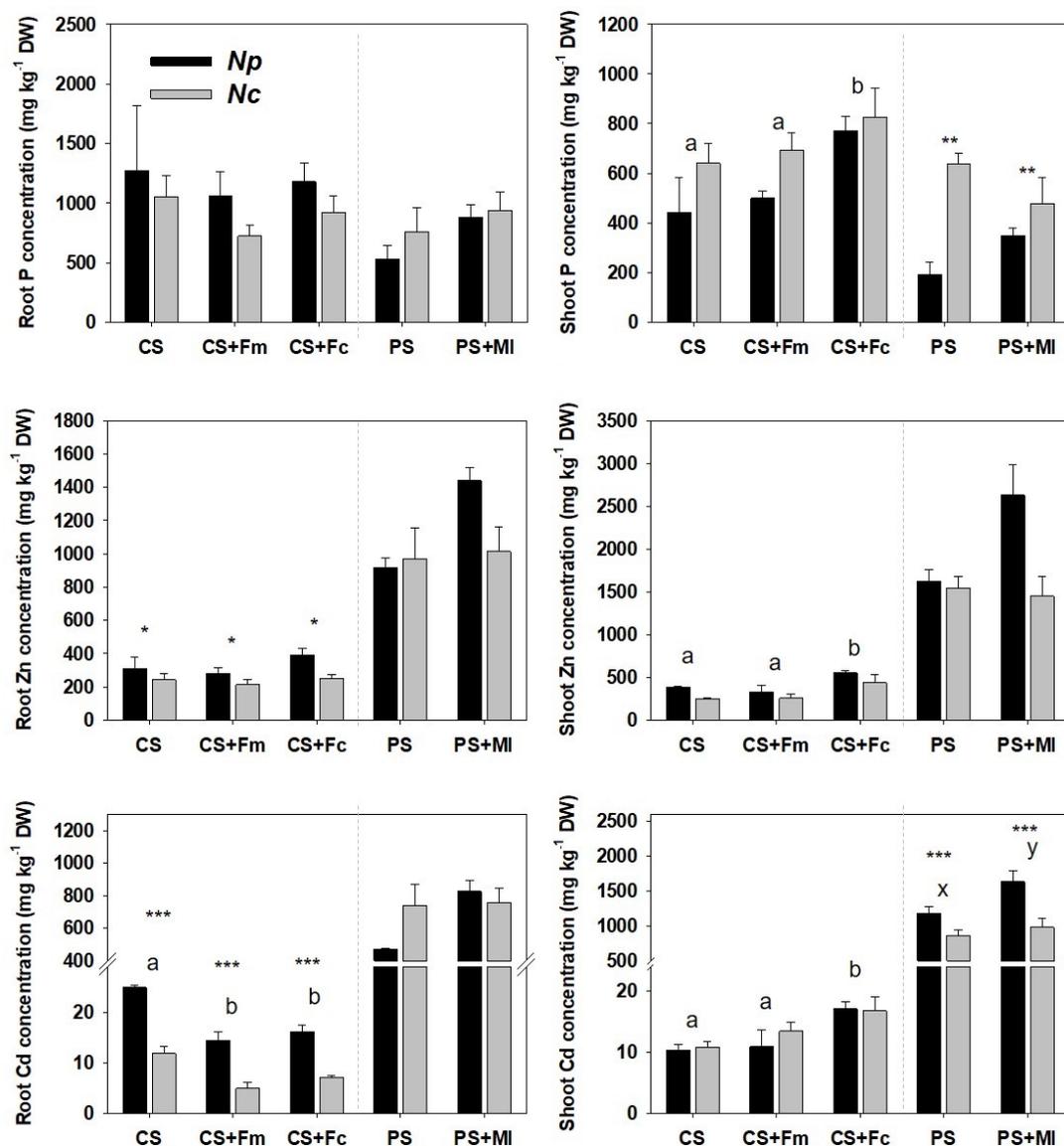


Figure 3. Concentration of phosphorus (P), zinc (Zn) and cadmium (Cd) in roots and shoots of *Noccaea praecox* (Np) and *N. caerulea* (Nc) grown in commercial soil (CS) and inoculated with monospore culture of arbuscular mycorrhizal fungi *Funneliformis mosseae* (CS+Fm) and *F. caledonium* (CS+Fm) or grown in heavy-metal polluted, field-collected soil (PS) and inoculated with Np-ingenous mycorrhizal inoculum (PS+MI). Different letters above columns present statistically significant differences between treatments (at $p < 0.05$) for CS and PS separately, and asterisks indicate statistically significant differences between species (*, $p < 0.05$; **, $p < 0.01$; ***, $p < 0.001$) as determined by two-way ANOVA and Holm-Sidak posthoc test. Shown are means ($n=3$) + standard errors. DW, dry weight.

Slika 3. Koncentracija fosforja (P), cinka (Zn) in kadmija (Cd) v koreninah in poganjkih *Noccaea praecox* (Np) in *N. caerulea* (Nc), gojenih v komercialni zemlji (CS) in inokuliranih z monosporno kulturo arbuskularnih mioriznih gliv *Funneliformis mosseae* (CS+Fm) in *F. caledonium* (CS+Fm) ali gojene v s težkimi kovinami onesnaženi, na polju zbrani zemlji (PS) in inokulirane z mikoriznim inokulumom Np-avtohtonih mikoriz (PS+MI). Različne črke nad stolpci predstavljajo statistično značilne razlike med tretiranj (pri $p < 0,05$) ločeno za CS in PS, zvezdice pa označujejo statistično značilne razlike med vrstami (*, $p < 0,05$; **, $p < 0,01$; ***, $p < 0,001$), določene z dvosmerno ANOVA in Holm-Sidakovim posthoc testom. Prikazana so povprečja ($n=3$) + standardne napake. DW, suha masa.

into 320 genera and 114 families (Jumpponen & Trappe, 1998). DSEs benefit their host by facilitating carbon, nitrogen, and phosphorus uptake (Vergara et al., 2018; Yakti et al., 2018). Furthermore, they can induce the release of phytohormones like auxins (Berthelot et al., 2016) and protect plants against abiotic stress (Wang et al., 2016). It is known that DSEs colonize extreme habitats, like high salinity sites (Sonjak et al., 2009), dry habitats (Barrow, 2003), and metal-enriched soils (Ban et al., 2012; Likar & Regvar, 2009, 2013). Although most studies have debated the beneficial role of DSEs (Ban et al., 2012; Likar & Regvar, 2009, 2013; Newsham, 2011), some studies proposed that DSE taxa can form parasitic associations with their host (Wilcox & Wang, 1987).

In metal-enriched soils, DSEs have been shown to positively impact plant fitness. A particular plant species where DSEs frequently colonize the roots, especially in highly metal-contaminated soil, is goat willow (*Salix caprea* L.; Salicaceae). The study by Likar & Regvar (2013) revealed smaller Cd and Zn leaf concentrations, larger chlorophyll concentrations and transpiration in DSE-inoculated *S. caprea* grown in metal-enriched soils compared to non-DSE-inoculated plants. It has been proposed that the binding of Cd and Zn to melanin in DSE may, through limiting the translocation of heavy metals, contribute to these observations (Potisek et al., 2021) in accordance with reports by Ban et al. (2012). These observations suggested that DSE associations are favorable for *S. caprea* under metal-enriched conditions. Furthermore, a larger diversity of DSE was found at sites with higher metal concentrations compared to low soil-metal concentrations (Likar & Regvar, 2009).

Although several studies of DSEs were performed on non-hyperaccumulators (Ban et al., 2012; Barrow, 2003; He et al., 2019; Likar & Regvar, 2013; Stoyke & Currah, 1991), little is known about the associations between DSEs and hyperaccumulators. In a recent study DSE strains isolated from the roots of poplar growing at different trace element contaminated sites were used to successfully inoculate *N. caerulescens* (Yung et al. 2021). Moreover, in the highly contaminated soil, a specific strain of DSEs significantly increased the root biomass of *N. caerulescens* compared to the non-inoculated plant without affecting the nutrient status of the plant (Yung et al., 2021). The positive effect of DSE strains was also observed in the accumulation of Zn and Cd, which was larger in the roots in highly contaminated soil with inoculation of DSE (30% and 90% more, respectively) compared to non-DSE-inoculated plants

(Yung et al., 2021). The DSEs were also observed in roots of *N. praecox*, a Cd and Zn hyperaccumulating plant, when grown on a highly contaminated site in Žerjav (Slovenia) (Fig. 4B) – an area still affected by the past mining and smelting activities (Bočaj et al., unpublished; Pongrac et al., 2009). DSEs may be important partners for the host, but more focused research is required, ideally by using DSE inoculum and performing controlled experiments to evaluate effects in different plants in different soils.

Are, therefore, DSEs mycorrhizal or not? Jumpponen (2001) claimed that at least under some conditions, DSE should be treated as a mutualistic partner, in agreement with Treu et al. (1996). By contrast, Brundrett (2006) disputes this by pointing out that in contrast to mycorrhizal symbiosis, the relationship between host plant and endophyte (e.g., DSE) lacks three features typical for arbuscular mycorrhiza: a cellular interface where specialized structures (e.g., arbuscules in AM) occur, but the same is true for ectomycorrhizal fungi. The synchronization of the development between the plant host and fungi and the benefits for both partners in the interaction are those that count. This demonstrates that there is a thin and often unclear line between beneficial or harmful, mycorrhizal or not.

Rhizosphere epiphytic microorganisms may also play an important role in hyperaccumulation

Sometimes overlooked in comparison to AMF and DSEs is the rhizosphere MO, one of the most complex microbial communities on the earth with a large number and rich diversity (Mendes et al., 2013) because they play a crucial role in plant growth and development, nutrient acquisition, pest prevention, and yield improvement (Mendes et al., 2011). The diversity and complexity of the soil as a substrate and a habitat are reflected in different microbial communities between bulk soil and rhizosphere MO, as well as between rhizosphere and rhizoplane (i.e., the external surface of the root, including closely adhering soil particles). The involvement of rhizosphere and rhizoplane MO in the ability of plants to hyperaccumulate metal(loid)s have been only poorly investigated so far. In a Cd and Zn hyperaccumulating *Sedum alfredii* (Crassulaceae), the diversity of four spatial compartments: bulk soil, rhizosphere, rhizoplane, and endosphere revealed that regardless of the soil type or genotype of *S. alfredii*, diversity diminished from rhizosphere to rhizoplane and

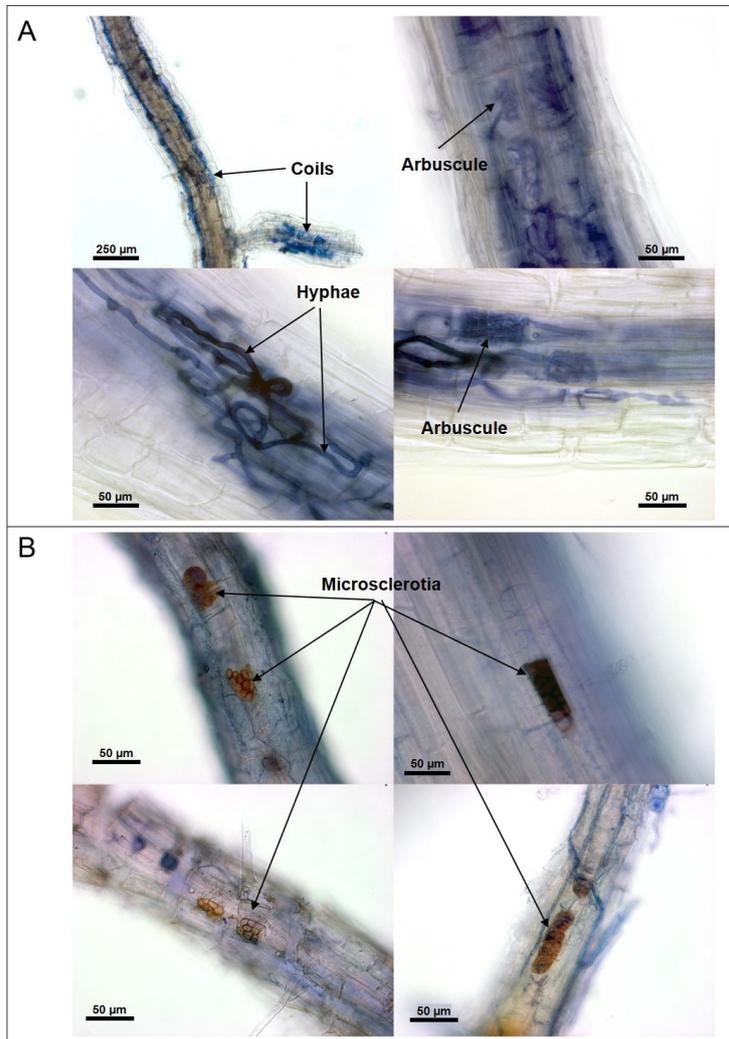


Figure 4. Representative structures of arbuscular mycorrhizal fungi (A) and dark septate endophytes (B) in roots of *Noccaea praecox* collected in Žerjav (Slovenia) in 2023. Roots were stained following the standard Tripian blue staining protocol (Philips & Haymann, 1970).

Slika 4. Reprezentativne strukture arbuskularnih mikoriznih gliv (A) in temnosemenskih endofitov (B) v koreninah *Noccaea praecox*, nabranih v Žerjavu (Slovenija) leta 2023. Korenine so bile obarvane po standardnem protokolu barvanja s Tripian blue (Philips & Haymann, 1970).

endosphere (Luo et al., 2017). By contrast, the diversity increased from the bulk soil to the rhizosphere, which was confirmed by Xiao et al. (2021) and later by Kushwaha et al. (2022), claiming that the diversity is larger close to the roots than in nearby soil. This may be attributed to plant exudates, microbial chemotaxis towards exudates and nutrients (García-Salamanca et al., 2012), and changes in pH caused by plant exudates (Fan et al., 2017).

Interestingly, the taxa richness of MO was reported to differ between hyperaccumulators and non-hyperaccumulators. For example, comparing several Se-hyperaccumulators and non-hyperaccumulators from the same family sharing the same growing site revealed substantial

differences in rhizosphere communities, with hyperaccumulators harboring a larger number of rhizobacterial species (Cochran et al., 2018). Similarly, a study by Martos et al. (2021) showed that in hyperaccumulators from the Brassicaceae an enrichment in previously described metal-tolerant bacteria and bacteria involved in nitrogen cycling was found compared to non-hyperaccumulating species (Martos et al., 2021). Unfortunately, these studies have so far been conducted on confamilial species and therefore, further in-depth species-specific investigations are required before meaningful conclusions can be made.

The importance of soil MO communities for hyperaccumulation was studied by Muehe et al. (2015), who

compared the microbial community of *A. halleri* growing on metal-contaminated and gamma-irradiated soil, the latter having decreased diversity and species richness. Although no differences in aboveground biomass were observed during the experiment between treatments, *A. halleri* grown in untreated soil accumulated significantly more Cd and Zn (100% and 15%, respectively) than when grown in gamma-irradiated soil (Muehe et al., 2015). Mechanisms by which MO improve accumulation may lie in their ability to improve the bioavailability of metal(loid)s (Audet & Charest, 2007). Whiting et al. (2001) used sterile soil where Zn was initially unavailable in water-soluble forms. Inoculation of *N. caerulea* seeds with bacteria increased the solubility of Zn in the rhizosphere, resulting in a higher concentration of water-extractable Zn compared to axenic conditions (Whiting et al., 2001). In highly contaminated sites where hyperaccumulation could cause toxicity to plants, MO can also play an important role in reducing the bioavailability of metal(loid)s. Such an effect was shown for the Ni accumulation in *N. caerulea* whose inoculation with Ni-resistant bacteria did not result in the increase in Ni uptake, but it reduced the bioavailability of Ni in the serpentine soil and promoted plant growth compared to the axenic control (Aboudrar et al., 2013).

All the above findings indicate the significant importance of soil MO on hyperaccumulation by altering metal(loid) bioavailability either positively or negatively.

Biological cleaners of metal(loid)s pollution and predictions

The use of hyperaccumulators to sustainably and in an environmentally friendly way remove toxic metal(loid) concentrations from the soil with commercial viability has been demonstrated for Ni only because plants with sufficient biomass and Ni hyperaccumulation capacity have been identified. For other metal(loid)s, the main constraint remains the small aboveground biomass of hyperaccumulators and/or limited accumulation capacity. Therefore, efforts into the discovery of new hyperaccumulators or enhancing biomass and hyperaccumulation capacity of known hyperaccumulators are essential if we are to remove other metal(loid)s using plants. According to Ernst (2005), an ideal plant for phytoextraction would have deep and

well-branched root systems with AMF associations to take up as much metal(loid)s as possible, have efficient root-to-shoot translocation with binding capacity in the roots smaller than in the shoots, without any biomass penalties. In addition, herbivores should not be attracted to these plants to prevent transmission through food chains, and harvesting should be easy and possible with conventional agricultural methods (Ernst, 2005). There is preliminary evidence showing that MO can affect several of these traits, therefore it may be viable to tailor MO associated with hyperaccumulators. However, our understanding of MO and their role in hyperaccumulation remains scarce. In this review, we underlined the indispensable role of MO for their hyperaccumulating hosts. Current knowledge of the link between functional profiles and ecological functions in the MO communities is still scarce, and several questions, including the AM status of hyperaccumulators from the Brassicaceae, remain open. With the advances in analytical methods, such as cheaper and high-throughput next-generation sequencing and metabolomics, we can expect ground-breaking advancements in this novel field of research. Furthermore, with a better understanding of the hyperaccumulating plants and their interactions with MO, phytoremediation efforts may be improved and developed to the point that it becomes applicable to a variety of environmental remediation strategies.

Author Contributions

Conceptualization, M.R. and P.P.; investigation, V.B.; writing—original draft preparation, V.B.; writing—review and editing, M.R. and P.P.; supervision, P.P.

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Conflicts of Interest

The authors declare no conflict of interest.

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