DOCTORAL THESIS

Colonization and species diversity of arbuscular mycorrhizal fungi and their efffects on metal tolerance and metal accumulation in two metal hyperaccumulators, Pteris vittata L. and Sedum alfredii Hance

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Colonization and Species Diversity of Arbuscular Mycorrhizal Fungi and Their Effects on Metal Tolerance and Metal Accumulation in Two Metal Hyperaccumulators, *Pteris vittata* L. and *Sedum alfredii* Hance

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Abstract

The major objectives of the present study were to: (1) investigate AM fungal species diversity and community structure of two metal hyperaccumulators, *Pteris vittata* L. and *Sedum alfredii* Hance, growing on metal-contaminated and uncontaminated soils in southeast China; (2) determine the ability of *S. alfredii* to accumulate arsenic (As), lead (Pb), zinc (Zn), cadmium (Cd) and copper (Cu) in field and compare intraspecific variation in As, Pb and Zn accumulation and tolerance in *P. vittata*; and (3) investigate the effects of AM fungi on As accumulation and tolerance in *P. vittata*.

Metallicolous populations of *P. vittata* and *S. alfredii* were collected from As and Pb/Zn mining and/or smelting sites located in Quzhou (QZ), Zhejiang Province, Chongyang (CY) and Lechang (LC), Guangdong Province and Dashunlong (DSL), Jinchuantang (JCT) and Yejiwei (YJW), Hunan Province. Nonmetallicolous populations of *P. vittata* and *S. alfredii* were also sampled from two uncontaminated sites, Hong Kong (HK), and Hangzhou (HZ), Zhejiang Province, respectively. Up to 1,373 As, 680 Pb, 376 Zn, 4.8 Cd, 169 Cu mg kg^{-1} in fronds of *P. vittata* and 358 As, 2,290 Pb, 23,403 Zn, 708 Cd, 342 Cu mg kg^{-1} in shoots of *S. alfredii* were detected. Constitutive properties of As and Zn hyperaccumulation in metallicolous populations of *P. vittata* and *S. alfredii*, respectively, were confirmed. However, Cd hyperaccumulation in *S. alfredii* varied among populations. The two hyperaccumulators varied in their efficiency in taking up Pb, Cd and Cu. In addition, low to moderate levels of AM colonization were observed in roots of *P. vittata* (4.2-12.8 %) and *S. alfredii* (8.5-45.8 %). The abundance of AM fungal spores ranged

from 16 to 190 spores per 25 g soil. *Glomus microaggregatum*, *G. mosseae*, *G. brohultii* and *G. geosporum* were the most common species associated with both *P. vittata* and *S. alfredii*. To our knowledge, this is the first report on AM fungal status in rhizosphere of *P. vittata* and *S. alfredii*.

Both metallicolous (CY, DSL, JCT, LC and YJW) and nonmetallicolous (HK) populations of *P. vittata* exhibited high-level As tolerance when grown in 20 % Hoagland's nutrient solution containing different As concentrations. The metallicolous populations did not exhibit higher Pb and Zn tolerance than the nonmetallicolous population. Moreover, the six populations possessed metal-specific variation in tolerance and accumulation of As, Pb and Zn. The nonmetallicolous population was found to possess significantly higher rates As accumulation than the metallicolous populations. After growing on As-contaminated soils for 12 weeks, the fronds of HK population had As concentrations ranging from 1,060 to 1,639 mg kg^{-1}, about 2.6-3.6 fold for DSL and 3.0-5.4 fold for JCT populations. It was concluded that As, Pb and Zn tolerances in *P. vittata* were largely metal-specific and could be regard as constitutive properties, although there was variation among populations, and that the nonmetallicolous population exhibits more effective As accumulation than the metallicolous populations.

Two predominant fungal species, *G. mosseae* and *G. geosporum*, and a rapidly sporulating fungal species, *G. etunicatum*, associated with *P. vittata* were isolated from metal-contaminated soils. Two isolates of *G. mosseae* with contrasting tolerance to As were used to investigate As accumulation and tolerance in two populations (HK and JCT) of *P. vittata* under hydroponic culture and pot trials. At lower levels of As exposure (50 - 200 μM), both uncontaminated and metal-contaminated isolates significantly increased short-term As influx into roots. However, at higher levels of As exposure (400 – 1,000 μM), only uncontaminated isolates significantly increased

short-term As influx into roots. Uncontaminated isolates also significantly increased As accumulation in roots of HK population and in fronds of JCT population growing on 100 mg As kg^{-1} soils. Furthermore, both metal-contaminated and uncontaminated isolates increased phosphorus (P) uptake. It was concluded that metal-contaminated isolates of AM fungi conferred As tolerance on *P. vittata* through P acquisition for association at the cost of no increase in As accumulation.

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