

DOCTORAL THESIS

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**Arsenic Uptake, Accumulation and Tolerance in Chinese
Brake Fern (*Pteris vittata* L., an Arsenic
Hyperaccumulator) under the Influence of Phosphate**

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**A thesis submitted in partial fulfillment of the requirements
for the degree of
Doctor of Philosophy**

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ABSTRACT

The major aims of the present study are to investigate arsenic (As) uptake, accumulation and mechanisms of As tolerance, and also effects of phosphate (P) on As uptake, accumulation and root exudates of Chinese brake fern (*P. vittata* L.).

Ethylene diamine triacetic acid (EDTA), hydroxyl ethylene diamine triacetic acid (HEDTA) enhanced Cu, Zn, Pb, but lowered As accumulation in Chinese brake fern and rostrate sesbania (*Sesbania rostrata* L.), while oxalic acid (OA) enhanced As accumulation in vetiver (*Vetiveria zizanioides* L.) in their aboveground parts significantly. In addition to As, Chinese brake fern also accumulated the highest concentrations of Cu, Pb and Zn in its aboveground parts among the three plant species grown in metal-contaminated soil. This species, therefore, can be used to clean up As, Cu, Pb and Zn simultaneously from co-contaminated soil.

High concentrations of As inhibited root and frond growth of semi-pinnated brake fern (*P. semipinnata* L.). Uptake of As by Chinese brake fern was significantly higher ($p < 0.05$) than that of semi-pinnated brake fern. Arsenic accumulation in the roots increased with increasing As concentrations for both species. Most of the As (82-90%) accumulated was distributed in the fronds of Chinese brake fern, while only 29-41% in the fronds of semi-pinnated brake fern.

There was a significant correlation ($p < 0.05$) between the increase in hydrogen peroxide (H₂O₂) contents with the increase of As content in plants and also As exposure time in both plant species, especially the midrib of semi-pinnated brake fern.

Semi-pinnated brake fern showed higher concentrations of H₂O₂ in the pinnae, midribs and roots than those of Chinese brake fern. The relative electrical conductivity (REC, %) values in the root and pinnae followed a similar trend as plant H₂O₂ contents. The results indicated that high doses of As resulted in oxidative damages in both plant species.

Arsenic uptake by Chinese brake fern was significantly influenced by both the chemical form and concentration of As in the nutrient solution. Higher concentrations of As in plants treated with arsenate were observed, with a positive correlation between As concentrations in plant tissues and in nutrient solution. The short-term (< 24 h) and low concentrations (< 25 μmol l⁻¹) uptake kinetics in Chinese brake fern were nonsaturated which could be divided into linear and saturable components. The saturable part of As uptake could be described by Michaelis-Menten kinetics/model.

Arsenic concentrations in fronds and roots of Chinese brake fern were significantly ($p < 0.05$) decreased ($p < 0.05$) with P in nutrient solution under both arsenate and arsenite treatments, especially the former. Phosphate uptake was significantly ($p < 0.05$) inhibited by 1000 μmol l⁻¹As. Arsenic treatments enhanced As and P transport to fronds, while increasing P inhibited their transportation. Arsenic concentrations were negatively correlated with P in roots and fronds of Chinese brake fern treated with arsenate, as well as in roots of plants treated with arsenite.

pH values in the nutrient solution increased with increasing exposure time, and decreased with increasing P levels. Dissolved organic carbon (DOC) contents (dry weight basis) in nutrient solution decreased with increasing P levels. The addition of As

and deficiency of P in nutrient solution resulted in the increase in DOC contents in root exudates of Chinese brake fern. Six organic acids were detected in the root exudates of Chinese brake fern, with oxalic and malic acids being the most dominant.

Arsenate and arsenite caused significant ($p < 0.05$) reductions in total soluble-protein (TSP) contents in pinnae of Chinese brake fern. Exposure to arsenate increased TSP contents in midribs and roots of plants. AR activities were increased when exposed to arsenite and arsenate for 10 days, with 79 and 68 times higher in the pinnae treated with arsenate than that of the control. Although Chinese brake fern accumulate extraordinary amounts of As in its fronds, there is no symptom of toxicity. Internal detoxification must exist. It was suggested that AR plays an important role in As distribution and detoxification in plant.

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