

Influence of leaf senescence on arsenic species in *Pteris vittata* roots

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Introduction

Recently, phytoremediation by arsenic-hyperaccumulator, *Pteris vittata*, has gained interest for a clean-up technology of arsenic contaminated water [1]. However, the relationship between senescence and As accumulation has not yet been elucidated such that it is not clear whether the fern with progress of senescence can accumulate arsenic compare to that of normally growing fern.

In present study, Influence of senescence on accumulated arsenic species in the root was investigated [2]. Chlorophyll contents in the frond were measured as indicators of natural senescence or metalloids (As) stress-induced leaf senescence of the *P. vittata*.

Experimental procedure

P. vittata L. with 5 to 6 fronds was used for the following experiments. For XAS analysis, the fern was hydroponically cultivated in Hoagland medium supplemented with arsenate at a final concentration of 30 μM . Then, root was rinsed with 1L of milliQ-water and blotted dry three times. The amount of chlorophyll a and b were extracted from the fern pinnae with various developmental stages and concentrations of chlorophylls were determined.

The chemical state of arsenic in the living plant samples were analyzed using the resultant data of X-ray absorption near edge spectra (XANES) and extended X-ray absorption fine structure (EXAFS) at As K absorption edge. As reference material of arsenate and arsenite, aqueous solution of sodium salts, Na_2HAsO_4 and NaAsO_2 , was prepared. From the measured data of incident beam energy dependence of fluorescence intensity, the XANES spectra were obtained by normalizing using absorbance calculated under assumption as isolated atom of arsenic after subtracted backgrounds mainly due to the elastic scattering. The chemical state of arsenic in the fern samples was analyzed by comparing the energy position of absorption edge in the profile of XANES spectra for the samples with that for the arsenate and arsenite references.

Results and discussion

Chlorophyll a/b ratio in chloroplast is normally 3 in plants. It is also known that the ratio also decreased with advancing senescence [3]. In this experiment, the decrease in chlorophyll a/b ratio was observed as cultivation period was increased while there is no apparent change in total amount of chlorophyll in the frond (Table1). In present study, arsenite species of the root tips and root top were investigated with above mentioned ferns. In 2week incubated fern, arsenate

predominantly existed both in root tips and root top (Fig. 1a). On the other hand, there is no significant change of arsenate abundance in the root top (Fig. 1b).

Table 1: Chlorophyll and arsenic contents in the frond of the fern used in XANES analysis

Sample name	Chlorophyll content (μM)			Aboveground biomass (g)	Arsenic content in the whole root (ppm dry-weight)
	Chla	Chlb	a/b		
12week	1.76	0.91	1.93	10.6	10.4
8week	1.88	0.78	2.41	5.61	142
6week	2.25	0.92	2.45	10.1	17.9
4week	1.84	0.62	2.97	4.86	103
2week	n.d.	n.d.	n.d.	4.95	n.d.

*Chlorophyll data represent mean of ten independent measurements; n.d., not determined

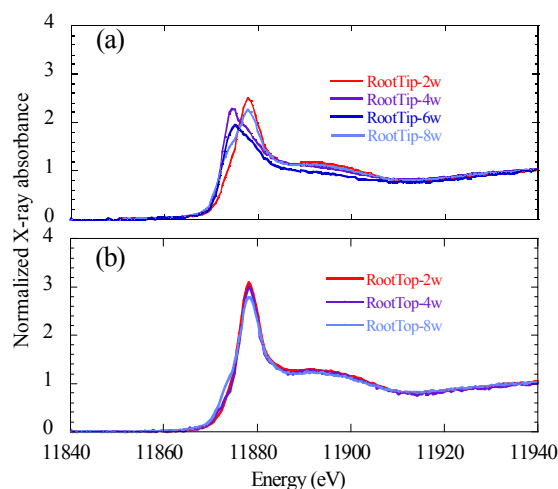


Figure 1 As K-edge XANES spectrum of As-accumulated *P.vittata* (a), root tip and; (b), root top. *P.vittata* were preincubated in the incubation solution for different time. Then, it was incubated with 20 μM arsenate (final conc.) for a week.

References

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