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Direct Determination of Arsenic Species in Arsenic Hyperaccumulator *Pteris vittata* by EXAFS

HUANG Ze-Chun[1], CHEN Tong-Bin[1*], LEI Mei[1], HU Tian-Dou[2]

(1. *Laboratory of Environmental Remediation, Institute of Geographical Sciences and Natural Resources Research, The Chinese Academy of Sciences*, Beijing 100101, China;
2. *Laboratory of Synchrotron Radiation, Institute of High Energy Physics, The Chinese Academy of Sciences*, Beijing 100039, China)

Abstract: Synchrotron radiation extended X-ray absorption fine structure (SR EXAFS) was used to study the transformation of coordination and redox state of arsenic (As) in a newly discovered As hyperaccumulator, *Pteris vittata* L., which is considered to have great potential for phytoremediating the As contaminated soil. It is shown that the As in this plant was mainly coordinated with oxygen in the reduced state, As (III), and the reduction of As (V) occurred in the root after it was taken up. No oxidation of As (III) was found during the translocation of As, from root to shoot. Only a small amount of As was coordinated with sulfur in root and petiole, but not distinct in pinna.

Key words: arsenic species; extended X-ray absorption fine structure (EXAFS); hyperaccumulator; *Pteris vittata* (Chinese brake); reduction; translocation

Arsenic (As) pollution has received increasing attention recently (Nordstrom, 2002). Remediation of arsenic contaminated soils has become a major environmental issue. As a economic, efficient and environmental friendly method for the remediation of contaminated soils and waters, more and more attention has been paid to the phytoremediation of arsenic contaminated soils (Chen *et al*., 2002). Three arsenic hyperaccumulators, i.e. *Pteris vittata* (Ma *et al*., 2001; Chen *et al*., 2002), *P. cretica* (Wei *et al*., 2002) and *Pityrogramma calomelanos* (Visoottiviseth *et al*., 2002), have been discovered recently, and provide hopeful candidates for the phytoremediation of arsenic contaminated soils.

It has been reported that *P. vittata* has extremely high trend to take up arsenic and transports it from root to shoot. However, why this plant has such special character still remains unknown. Study on species transformation of arsenic during root uptake and root to shoot translocation within this plant might play an important role in understanding the hyperaccumulating nature of *P. vittata*, and help to understand the arsenic uptake and transport mechanisms in the plant and arsenic detoxification inside the plant cells. The extended X-ray absorption fine structure (EXAFS) provides a promising tool to determinate elemental oxidation state and local coordination environments in samples directly, whereas most other commonly used methods such as chromatographic are indirect, requiring time consuming and cumbersome preparation, which may alter elemental species originally presented in samples and lead to false results. EXAFS has been newly used in biological and environmental sciences for studying the transformation of metal and metalloid ions in plants, and has been considered as a powerful and unique technique to study the physiological actions of elements in living plant (Pickering *et al.*, 2000; Parson *et al*., 2002).

The arsenic uptake and its species in different tissues of *P. vittata* treated with two inorganic arsenic species, arsenate and arsenite, usually found in soils, were studied in the present study by using EXAFS method.

1 Materials and Methods

1.1 Plant culture and arsenic treatment

Spores of *Pteris vittata* L. obtained from Hunan Province of China were sprinkled onto a moist soil in a seedbed covered with a plastic cling film to maintain moisture. After the spores germinated, and grew into sporelings with true leaves about 2 cm in height. The plant was pre-cultured for one week in a modified Hoagland nutrient solution containing 2.0 mmol/L of $Ca(NO_3)_2$, 0.75 mmol/L of K_2SO_4, 0.1 mmol/L of KCl, 0.25 mmol/L of KH_2PO_4, 0.65 mmol/L of $MgSO_4$, 0.1 mmol/L of EDTA-Fe(II), 0.01 mmol/L of H_3BO_3, 0.001 mmol/L of $MnSO_4$, 0.001 mmol/L of $ZnSO_4$, 0.000 1 mmol/L of $CuSO_4$ and 5.0×10^{-6} mmol/L of $(NH_4)Mo_7O_{24}$. Two plants were transplanted into a plastic pot containing 0.7 kg quartz sand, which was pretreated with dilute HCl and washed with deionized water. The plant in individual

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* Author for correspondence. E-mail: <chentb@igsnrr.ac.cn>.

treatment was cultured in the sandy media containing the nutrient solution with 10 mg/L of As, either arsenite or arsenate. The solution was renewed every 3 d to maintain the arsenic concentration and species during the culture period. The plant culture was conducted in a growth chamber with the following conditions: 16 h of light period with a light intensity of 300 $\mu E \cdot m^{-2} \cdot s^{-1}$, temperature of 26 ℃/15 ℃ (day/night), and average relative humidity of 60%. Each treatment was repeated triplicately.

After 4-week' s cultivating, the plant was harvested and washed with tap-water followed by three rinses with deionized water. The plant sample was freeze-dried and ground, and divided into two parts: one part for the analysis of arsenic concentration and another for EXAFS measurement. The grounded sample (about 0.1 g) was weighted and digested with a mixture of HNO_3-$HClO_4$-H_2SO_4 following the method 3050B of USEPA (1996). The determination of arsenic concentration was performed on an atomic fluorescence spectrometer (AFS-2202). The sample for EXAFS was stored in a sealed bag and kept in a refrigerator before the EXAFS experiment.

1.2 EXAFS measurement

The plant sample was packed into a 3.0 cm × 0.7 cm sample holder, the solutions of model arsenic compounds (sodium arsenite, sodium arsenate dibasic and As (Ⅲ)- glutathione (GSH)) were pipetted into a Lucite liquid holder of the same size. The complex of As (Ⅲ)-GSH was composed by adding a 10-fold molar excess of glutathione to a solution of sodium arsenite, following the method mentioned by Pickering *et al.* (2000).

Arsenic K-edge (11 867 eV) X-ray absorption spectra collection were performed using a double crystal monochromator (Si 111) in fluorescence mode at room temperature at EXAFS station on Beamline 4W1B of Beijing Synchrotron Radiation Facility (BSRF). The electron storage ring was operated at 2.2 GeV.

Arsenic K-edge (11 867 eV) X-ray absorption spectra reduction and analysis were performed using following procedures. Pre-edge background was removed from the spectra and then normalized. The resulting data were converted from *E* space to *k* space and weighted by k^3. EXAFS spectra obtained by the steps mentioned above were calculated with Cerious2-XAFS software (Fig.1A). Fourier transformation was then performed to obtain the radial structural function (RSF)(Fig.1B). Final fitting of the spectra was done on Fourier transformed k^3 weighted spectra in *R* space to get the value of interatomic distance (R) and coordination number (N)(Arai *et al.*, 2001).

2 Results

Symptom of arsenic toxicity was found to appear in fronds of *P. vittata* treated with arsenic, both arsenite and arsenate, after three weeks of the culture. The symptom, dark brown coloration at the tips and the margins of pinnae, was observed mainly in the bottom pinnae of senescent fronds while young pinnae did not show any symptom of arsenic toxicity. This toxicity was not too serious that the plants could survive throughout the whole culture period. No significant difference between arsenite and arsenate treatments was found in both aboveground and root biomass (data not shown).

Hyperaccumulation of arsenic in *P. vittata* was demonstrated clearly in this experiment, with As concentrations in the pinnae reaching up to more than 3 000 mg/kg DW, the concentration of As in the shoots was always greater than that in the roots, and the translocation factors (i.e., shoot/root ratio of As concentration) were about 10, in both treatments (Table 1). As concentration in the plant treated with arsenate was significantly higher ($P<0.05$) than that in the plant treated with arsenite, for all the tissues except normal pinna (Table 1).

Figure 1A shows the k^3 weighted χ functions of several model compounds and samples from *P. vittata*. Fine signal-to-noise arsenic K-edge EXAFS spectra were obtained in most of the plant samples, except in the root (Fig.1d) and mature petiole (Fig.1e) in arsenite treatment, with As concentration lower than 400 mg/kg (Table 1). It can be concluded that interpretable EXAFS spectrum with *K*-space ranging between 3-13 Åcould be obtained from samples containing As concentrations higher than 400 mg/kg.

Figure 1B shows the corresponding Fourier transforms (radial distribution functions, RDFs) and best fitting of EXAFS spectra. Final calculated coordination numbers, interatomic distances, and fitting parameters are presented in Table 2. Only one coordination shell was discovered in each of those three arsenic model compounds, As-S shell

Table 1 Arsenic (As) in tissue of *Pteris vittata* treated with either arsenite or arsenate*

	As concentration (mg/kg DW)	
	Arsenite added	Arsenate added
Root	349.5±119.2a	416.6±165.4b
Mature petiole	216.2±31.8a	390.1±39.5b
Young petiole	884.2±130.1a	1 075±109b
Normal pinna	3 306±932a	3 466±1 178a
Pinna with As-toxic symptom	3 573±1 007a	5 019±1 693b

*, values are Means ± *SE* (n = 3). Values followed by different letters are significantly different between treatments at $P<0.005$.

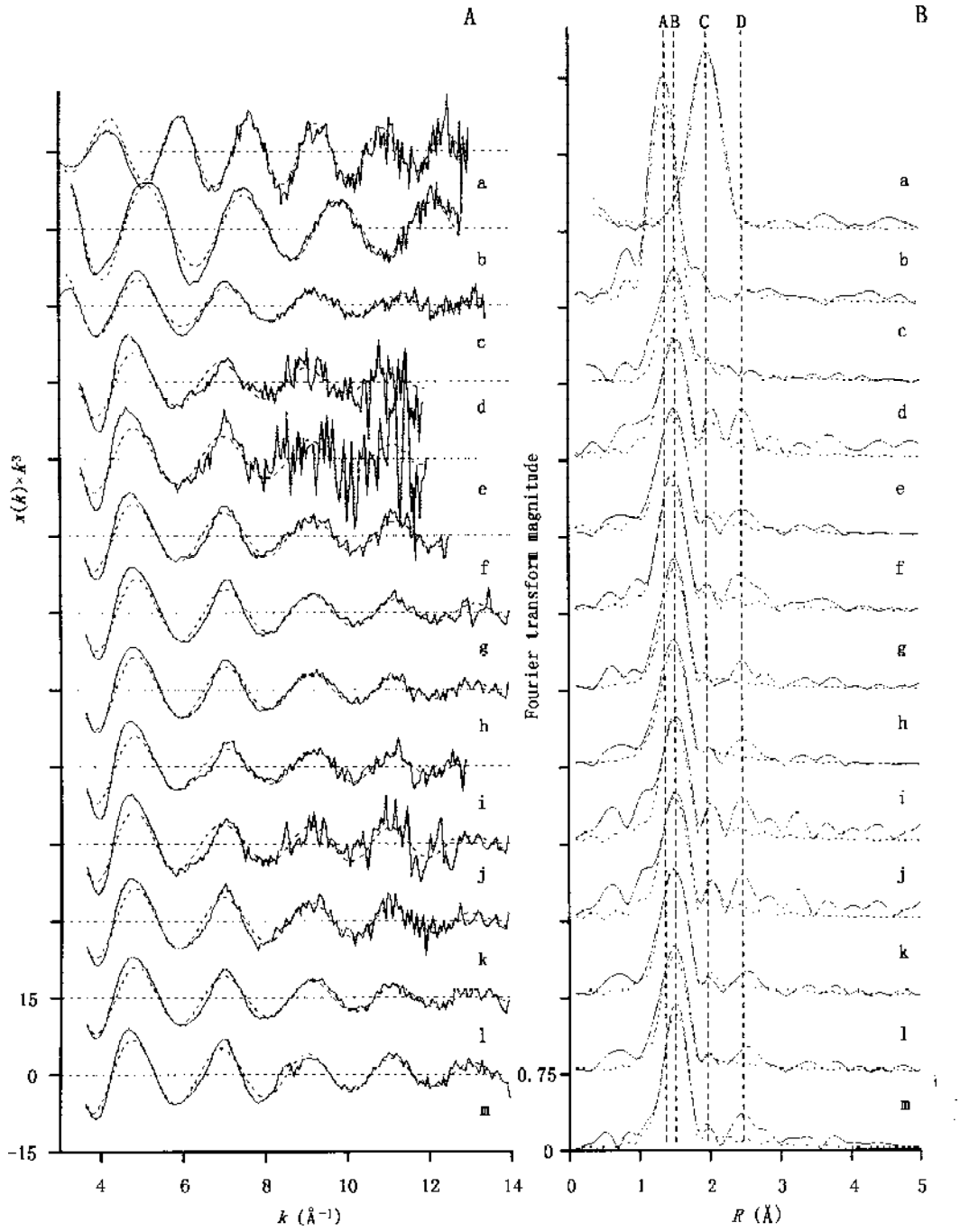

Fig.1. Arsenic K-edge EXAFS (**A**) and corresponding Fourier transforms (phase shift uncorrected)(**B**) of samples from *Pteris vittata* and arsenic model compound. a, As (**III**)-GSH; b, sodium arsenite; c, sodium arsenate dibasic; d-h, plant treated with arsenite (d, root; e, mature petiole; f, young petiole; g, normal pinna; h, pinna with As-toxic symptom); i-m, plant treated with arsenate (i, root; j, mature petiole; k, young petiole; l, normal pinna; m, pinna with As-toxic symptom). Solid lines show the data and the dashed lines the best fit (Table 2). The spectra were offset vertically but were plotted with the same relative scale.

for As(**III**)-GSH with N=3 and R=2.26, As-O shell for arsenate with N=4 and R=1.70, and As-O shell for arsenite with N=3 and R=1.78, respectively. The arsenic in all samples was coordinated with oxygen in the first coordination shell at a distance of 1.79 ± 0.01Å, which was similar to that of arsenite, but significant longer than that of arsenate, means that As (V) was reduced to As (**III**) immediately after it was taken up by the root of *P. vittata* and no oxidation from As (**III**) to As (V) happened during the process of translocation from root to shoot. A second As-S coordination shell with interatomic distance of 2.26 ± 0.01Åmatching that of As (**III**)-GSH is found in most of the plant samples. In comparison with other samples, the complex of As (**III**)-GSH was more distinct in root treated with both arsenic species

Table 2 Results of fitting arsenic K-edge EXAFS of *Pteris vittata* and model compounds*

Treatment	Sample or model compound	Type	N	*R* (Å)	σ^2
Arsenite [a]	Root	As-O	3.0	1.79	0.010
		As-S	0.3	2.27	0.004
	Mature petiole	As-O	2.9	1.79	0.006
	Young petiole	As-O	3.0	1.79	0.006
	Normal pinna	As-O	3.2	1.79	0.008
	Pinna with As-toxic symptom	As-O	3.3	1.77	0.009
Arsenate [b]	Root	As-O	2.9	1.79	0.008
		As-S	0.3	2.25	0.006
	Mature petiole	As-O	3.0	1.79	0.007
		As-S	0.3	2.25	0.005
	Young petiole	As-O	3.1	1.79	0.006
	Normal pinna	As-O	3.0	1.79	0.007
	Pinna with As-toxic symptom	As-O	3.3	1.80	0.006
Model compound	As (III)-GSH	As-S	3.0	2.26	0.007
	Arsenate	As-O	4.0	1.70	0.002
	Arsenite	As-O	3.0	1.78	0.007

a, the plant was treated with arsenite; b, the plant was treated with arsenate; σ^2, the measure of the static disorder of the shell; N, the coordination number; *R*, interatomic distance; Type, the coordination type.

and mature petiole treated with arsenate (Fig.1B, d, i, j). The coordination number of As-S is significant smaller than that of As-O in all plant samples (Table 2), indicating that only a small amount of As (**III**) combined with sulfur in plant. Additionally, a third undefined coordination shell dashed with line D in Fig.1B was also found almost in all the plant samples, which does not appear in the RDFs of all the arsenic model compounds including crystalloid sodium arsenite (data not shown) in the present study.

3 Discussion

Electron microscopes such as SEM and TEM coupled with elemental probes are usually employed to observe elemental distribution in plant tissues at the scale larger than several microns (Chen *et al*., 2003). However, the EXAFS technology is direct technology with less pretreatment to investigate the arsenic species at the scale of atomic level.

It is shown by the results of EXAFS that As (**III**) coordinated with oxygen was the predominant species in *P. vittata*, the reduction of As (V) to As (**III**) in arsenate treatment mainly occurred in root after it was taken up, and no oxidation As (**III**) was discovered during its translocation from root to shoot in both arsenic species treatments. As (**III**)-S coordination was also noted in the root and a part of petiole of *P. vittata*, but it was not distinct in pinnae where arsenic was mainly stored.

Although the arsenic species in *P. vittata* was studied with X-ray absorption spectrum by Lombi *et al*. (2002) and Webb *et al*. (2003), none of them obtained any interpretable spectrum from plant tissues other than pinna. Therefore, it is not possible to trace the transformation of arsenic species in the process of root uptake and translocation, and to identify where As (V) was reduced to As (**III**) in plant.

The reduction of As (V) in *P. vittata* may be an important mechanism remained to be elucidated. Arsenic reduction in higher plant was also observed in *Brassica juncea* by other researchers (Pickering *et al*., 2000). It was also hypothesized that thiol groups might be the possible reductant for As (V) (Pickering *et al*., 2000; Schmöger *et al*., 2000). However, all those studies based on arsenic non-hyperaccumulators and the arsenic appeared to be entirely combined with thiols in the form of As (**III**)-tris-glutathione or As (**III**)-PCs (phytochelatins) in those plants, which was different to arsenic in *P. vittata*. Whether the thiol compound in arsenic hyperaccumulator playing an important role in arsenic reduction or not is still a question needed to be studied in the future.

4 Conclusion

It is found from EXAFS study of *P. vittata* that As (V) was reduced to As (**III**) after it was taken up and the arsenic was mainly presented as As (**III**)-O in the plant, no matter arsenate or arsenite was added in sandy culture. Arsenic was kept as As (**III**) during the translocation from root to shoot. Different from that arsenic mainly combined with thiols in some arsenic non-hyperaccumulator, only a small amount of arsenic was coordinated with sulfur, in root and petiole of *P. vittata*, and no distinct As-S was found in pinna where arsenic was mainly stored.

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超富集植物蜈蚣草中砷化学形态的 EXAFS 研究

黄泽春[1] 陈同斌[1*] 雷 梅[1] 胡天斗[2]

(1. 中国科学院地理科学与资源研究所环境修复室, 北京 100101;
2. 中国科学院高能物理研究所同步辐射实验室, 北京 100039)

摘要: 采用同步辐射扩展X射线吸收精细结构(SR EXAFS)技术研究了超富集植物蜈蚣草(*Pteris vittata* L.)中As的化学形态及其在转运过程中的变化。结果表明, 蜈蚣草中的As主要以As (Ⅲ)与O配位的形态存在。As(Ⅴ)被植物吸收后, 很快转化为As (Ⅲ), 其转化过程主要发生在根部。As (Ⅲ)向地上部转运的过程中价态基本不变。在植物的根部和部分叶柄中存在少量与As-GSH相似的As-S结合方式, 但是在As含量最高的羽叶中基本上未发现这种结合方式。与需要提取和分离过程的化学方法相比, 采用EXAFS方法研究植物中的砷形态不需经过预分离或化学预处理就可以直接测定植物样品中元素的化学形态, 因此可以避免样品预处理过程对As形态的干扰, 并获得可靠的砷化学形态方面的信息。

关键词: 砷形态; EXAFS; 超富集植物; 蜈蚣草(*Pteris vittata*); 还原; 转运

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*通讯作者。E-mail: <chentb@igsnrr.ac.cn>。

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