0163-4984/(Online) 1559-0720/07/11503–0277 $30.00

# Multivariate Analysis of Elements in Chinese Brake Fern as Determined Using Neutron Activation Analysis

CHAO-YANG WEI*,[1] AND ZHI-YONG ZHANG[2]

*[1]Institute of Geographical Sciences and Natural Resources Research, and [2]Institute of High Energy Physics, Chinese Academy of Sciences, Beijing 100101 China*

Received May 25, 2006; Accepted June 16, 2006

## ABSTRACT

Pytoremediaton of arsenic (As) contamination using Chinese brake fern (*Pteris vittata* L.), an As hyperaccumulator has proven potential because of its cost-effectiveness and environmental harmonies. Aiming to investigate the elemental correlation in Chinese brake fern, 20 elements (As, Br, Ca, Ce, Co, Cr, Eu, Fe, Hf, La, Na, Nd, K, Rb, Se, Sm, Sr, Th, Yb and Zn) were measured in the fronds and roots of the fern by neutron activation analysis. The ferns were sampled from two sites with high geogenic As levels: Zimudang (ZMD) and Lanmuchang (LMC) in Guizhou Province, China. Multivariate statistic analysis was performed to explore the interrelationship between these elements, especially between As and other elements. As was found to be positively related to K, Na, La, and Sm in both the roots and the fronds, suggesting that these four elements might operate as synergies to As during uptake and transportation processes. Se was positively related to most of the other cations measured, except in the fronds of the fern at ZMD, where Br replaced Se as positively related to the other cations. The difference of As and Se in correlation with other cationic elements suggested that the two anionic elements play different roles in elemental uptake processes. Our findings of elemental correlation highlight the importance of the anion–cation balance in Chinese brake fern.

**Index Entries:** Arsenic; anion–cation balance; antagonism; selenium; synergy; cluster analysis; principle component analysis.

## INTRODUCTION

Arsenic (As) is ubiquitous but toxic in the environment, and As contamination has caused severe global epidemic As poisonings and arsencosis,

*Author to whom all correspondence and reprint requests should be addressed.

especially in Bangladesh, India, Vietnam, and China *(1)*. Up to the present, there appears to be no cost-effective method for the *in situ* cleanup of As-contaminated soils and groundwater. The Chinese brake fern (*Pteris vittata* L.) is a newly identified As hyperaccumulator and has proven potential in remediation of As contamination in soil and water *(2–4)*. The fern has great ability for tolerance and accumulation of As; it can accumulate more than 1000 mg/kg As in its fronds when growing on the tailings with 23,400 mg/kg As in the field and soils spiked with 1500 mg/kg As in greenhouse conditions *(5,6)*. A growing interest has been focused on the mechanisms of As uptake and accumulation by this fern; however, much has been left unresolved *(7–11)*.

Plants uptake metal or metalloids through channels in their roots; hydrponic experiments demonstrated that some As-tolerant plants, including *Holcus lanatus*, *Deschampsia cespitosa*, and *Agrostis capillaris*, as well as the As-hyperaccumulating plant the Chinese brake fern uptake As and P via the same channel, as the two elements are analogues in chemistry *(9,12–17)*. High-performance liquid chromatography–mass spectrometry (HPLC-MS) and synchrotron radiation extended X-ray absorption fine structure EXAFS studies have reveled that As exists mainly as inorganic arsenite and arsenate forms in the Chinese brake fern, with arsenite as the major form *(18–20)*. These highlight the importance of the correlation between As and other elements, as plants usually take up elements in a synergic or antagonistic way in their transportation and, through these, to get an ion balance *(21)*. As is toxic to most of the plants; the concentration in ordinary plants is low *(22)*. The abnormally high As concentration in the Chinese brake fern might rely on the anion–cation balance effect between As and other elements. Little is known about the Chinese brake fern in this respect, the present study aimed to explore the correlation between As and other elements in the Chinese brake fern from two sites with high As levels. Instrumental neutron activation analysis (NAA) was employed for elements analysis, as this method has been proven to be useful in the multielemental analysis in plants *(23)*.

## MATERIALS AND METHODS

### *Sites and Sampling Methods*

Detailed site description and sampling methods have been illustrated in our previous report *(24)*. In brief, samples of the Chinese brake fern were collected from two sites—Zimudang (ZMD) and Lanmuchang (LMC)—with high geogenic As levels in Guizhou Province, China.

### *Determination of Elements in the Chinese Brake Fern*

Fern samples were separated into fronds (aboveground) and roots (below ground), washed, dried, and powdered before analysis *(24)*. Each

Table 1
Comparison of Analytic Results of As by NAA and ICP-AES

| | NAA | | | | ICP-AES | | | | Pearson coefficient (NAA vs ICP-AES) |
|---|---|---|---|---|---|---|---|---|---|
| | Mean | Min. | Max. | SD | Mean | Min. | Max. | SD | |
| ZMD fronds | 15.74 | 4.97 | 51.9 | 13.37 | 1104 | 362 | 3599 | 837 | 0.949** |
| roots | 18.5 | 3.4 | 38.7 | 14.11 | 690 | 265 | 1691 | 428 | 0.587* |
| LMC fronds | 8.42 | 1.62 | 33.13 | 9.97 | 810 | 266 | 1832 | 451 | 0.914** |
| roots | 0.46 | 0.2 | 1.2 | 0.27 | 536 | 197 | 1291 | 307 | NS |

* Significant level at $p<0.05$.
** Significant level at $p<0.01$.

powdered sample was packed with two layers of aluminum foil. Samples were irradiated for 8 h in a heavy-water nuclear reactor at the Chinese Institute of Atomic Energy (CIAE) at a thermal neutron flux of $3.35\times10^{13}$ $n/cm^2/s$. After decaying for 5 and 20 d, the samples were counted twice for medium and long-lived nuclides by a high-purity germanium (HPGe) detector. Analytical quality control was assured by using two certified reference materials (CRMs), including GBW08501 Chinese Peach Leaf and GBW08505 Chinese Tea (Center for Standard Reference of China). A total of 20 elements (As, Br, Ca, Ce, Co, Cr, Eu, Fe, Hf, La, Na, Nd, K, Rb, Se, Sm, Sr, Th, Yb, and Zn) were recorded for their concentrations.

### *Statistical Analysis*

Statistical analysis was carried out using SPSS 11.0. Correlations were evaluated using the bivariation method, with two-tailed significance and Pearson correlation coefficients. Cluster analysis (CA) with furthest neighbor and Pearson correlation and principle components analysis (PCA) with rotation of maximum variance were performed to test the correlation between As and other elements in the Chinese brake fern.

## RESULTS

### *Comparison of As Measurement Between NAA and ICP-AES*

The analytical results of As concentration determined by NAA were remarkably lower than that by ICP-AES (Table 1). However, generally there existed a clear correlation between the results of NAA and inductively coupled plasma–atomic emission spectrometer (ICP-AES), especially in the fronds. Because As can be volatile in high temperatures during the radiation process in the heavy-water nuclear reactor, NAA methods give a much lower concentration for the Chinese brake fern.

### Correlation Analysis

Correlation analysis gave common but slightly different results on the relationship between As and other elements in the Chinese brake fern, between fronds and roots, and between the ZMD and LMC sites. In the fronds of the Chinese brake fern, correlation analysis showed that As was positively correlated to K, Na, and Sm but negatively correlated to Ca at ZMD, and positively correlated to La, Na, and Sm at LMC; In the roots of the Chinese brake fern, La and Sm were found to be positively correlated to As, whereas Br was found to be negatively correlated to As at ZMD, and only La was positively correlated to As at LMC (Table 2).

Among the 20 elements tested, only As, Br, and Se are anions. Numerous significant correlations between Se and Br with other cations, were found especially Se, correlation was found between Se with K, Na, La, and Sm, which were generally positively correlated to As in the Chinese brake fern, both in the fronds and roots at the two sites. In the fronds of the Chinese brake fern, Br was positively correlated to Ce, Co, Cr, Eu, Fe, Hf, Nd, Sb, Sc, Th, Yb, and Zn at ZMD, whereas Se was positively correlated to Ce, Co, Cr, Eu, Fe, Hf, Nd, Sc, Tb, and Th at LMC. In the roots of the Chinese brake fern, Se was positively correlated to most of the cations, including Cr, Eu, Fe, Hf, Nd, Sb, Sc, Th, and Yb at both sites, plus Ca, Ce, and Co at ZMD.

### Cluster Analysis

In CA, La, Sm, As, K, and Na were classified into a group in the fronds of the Chinese brake fern at the two sites, however, slightly differences appeared in the roots, with Rb added to the group at ZMD, and Sm and Na removed but Ca added at LMC (Fig. 1).

### Principle Component Analysis

A selection of 10 elements, including As, Co, Cr, Fe, Hf, K, La, Na, Se, and Sm, were evaluated using PCA. The Varimax rotated component matrix clearly indicated that As appeared with K, La, Sm, and Na in the same component, with the only exception being in the roots of the Chinese brake fern at LMC, where Na was found to appear in another component. Se appeared with Co, Cr, Fe, and Hf in a same component in different parts of the fern at two sites, except in the fronds of the Chinese brake fern at ZMD (Table 3).

## DISCUSSION

Although the As concentrations measured by NAA were much lower than that by ICP-AES, the data obtained by NAA could be regarded as reasonable for statistic analysis; this is because As was proportionally lost during the volatile process at high temperatures (Table 1).

Table 2
Elemental Correlation in the Fronds and Roots at ZMD and LMC (Pearson Coefficients, Two-Tailed)
ZMD Fronds ($n$=14)

| | As | Ba | Br | Ca | Ce | Co | Cr | Cs | Eu | Fe | Hf | K | La | Na | Nd | Ni | Rb | Sb | Sc | Se | Sm | Sr | Tb | Th | Yb | Zn |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **As** | 1.000 | | | | | | | | | | | | | | | | | | | | | | | | | |
| **Ba** | -0.237 | 1.000 | | | | | | | | | | | | | | | | | | | | | | | | |
| **Br** | -0.218 | 0.288 | 1.000 | | | | | | | | | | | | | | | | | | | | | | | |
| **Ca** | -0.538* | 0.336 | 0.103 | 1.000 | | | | | | | | | | | | | | | | | | | | | | |
| **Ce** | -0.236 | 0.294 | 0.655* | 0.312 | 1.000 | | | | | | | | | | | | | | | | | | | | | |
| **Co** | -0.148 | 0.297 | 0.588* | 0.198 | 0.900** | 1.000 | | | | | | | | | | | | | | | | | | | | |
| **Cr** | -0.159 | 0.287 | 0.765** | 0.241 | 0.777** | 0.787** | 1.000 | | | | | | | | | | | | | | | | | | | |
| **Cs** | -0.485 | 0.639* | 0.526 | 0.392 | 0.598* | 0.600* | 0.430 | 1.000 | | | | | | | | | | | | | | | | | | |
| **Eu** | -0.098 | 0.267 | 0.590* | 0.174 | 0.911** | 0.979** | 0.803** | 0.623* | 1.000 | | | | | | | | | | | | | | | | | |
| **Fe** | -0.100 | 0.305 | 0.710** | 0.240 | 0.939** | 0.945** | 0.905** | 0.557* | 0.962** | 1.000 | | | | | | | | | | | | | | | | |
| **Hf** | -0.081 | 0.224 | 0.715** | 0.207 | 0.945** | 0.893** | 0.870** | 0.520 | 0.937** | 0.979** | 1.000 | | | | | | | | | | | | | | | |
| **K** | 0.707** | 0.060 | 0.005 | -0.111 | -0.119 | -0.110 | 0.084 | -0.282 | -0.090 | 0.016 | -0.014 | 1.000 | | | | | | | | | | | | | | |
| **La** | 0.526 | 0.105 | -0.278 | 0.125 | 0.155 | 0.032 | 0.016 | -0.154 | 0.108 | 0.118 | 0.173 | 0.564* | 1.000 | | | | | | | | | | | | | |
| **Na** | 0.639* | -0.649* | -0.294 | -0.636* | -0.403 | -0.393 | -0.357 | -0.777** | -0.359 | -0.347 | -0.250 | 0.114 | 0.082 | 1.000 | | | | | | | | | | | | |
| **Nd** | -0.266 | 0.605 | 0.551* | 0.291 | 0.802** | 0.790** | 0.679* | 0.681* | 0.781** | 0.767** | 0.732** | -0.200 | 0.166 | -0.546* | 1.000 | | | | | | | | | | | |
| **Ni** | -0.007 | 0.056 | 0.334 | -0.034 | 0.630* | 0.810** | 0.525 | 0.521 | 0.805** | 0.691** | 0.617 | -0.172 | -0.135 | -0.247 | 0.515 | 1.000 | | | | | | | | | | |
| **Rb** | -0.115 | 0.207 | 0.225 | 0.385 | 0.483 | 0.427 | 0.243 | 0.483 | 0.482 | 0.436 | 0.515 | -0.042 | 0.389 | -0.217 | 0.350 | 0.184 | 1.000 | | | | | | | | | |
| **Sb** | -0.317 | 0.646* | 0.604* | 0.492 | 0.588* | 0.548* | 0.546* | 0.694** | 0.566* | 0.609* | 0.624* | -0.218 | 0.033 | -0.408 | 0.699 | 0.219 | 0.643* | 1.000 | | | | | | | | |
| **Sc** | -0.101 | 0.271 | 0.670** | 0.193 | 0.946** | 0.966** | 0.851** | 0.580* | 0.982** | 0.991** | 0.972** | -0.042 | 0.092 | -0.327 | 0.756 | 0.748** | 0.454 | 0.582* | 1.000 | | | | | | | |
| **Se** | 0.066 | 0.352 | 0.047 | -0.179 | 0.218 | 0.417 | 0.168 | 0.148 | 0.327 | 0.243 | 0.148 | 0.102 | 0.152 | -0.283 | 0.519 | 0.214 | -0.072 | 0.019 | 0.256 | 1.000 | | | | | | |
| **Sm** | 0.646* | -0.127 | -0.252 | -0.078 | 0.238 | 0.191 | 0.076 | -0.145 | 0.298 | 0.257 | 0.306 | 0.520 | 0.847** | 0.211 | 0.082 | 0.169 | 0.275 | -0.126 | 0.268 | 0.113 | 1.000 | | | | | |
| **Sr** | -0.583 | 0.665* | 0.530 | 0.603* | 0.616* | 0.586* | 0.592* | 0.756** | 0.591* | 0.611* | 0.589* | -0.388 | -0.054 | -0.662** | 0.792 | 0.263 | 0.474 | 0.875** | 0.579* | 0.176 | -0.183 | 1.000 | | | | |
| **Tb** | -0.078 | 0.218 | 0.412 | 0.095 | 0.844** | 0.940** | 0.681** | 0.498 | 0.941** | 0.884** | 0.842** | -0.110 | 0.082 | -0.296 | 0.679 | 0.775** | 0.335 | 0.392 | 0.923** | 0.424 | 0.344 | 0.459 | 1.000 | | | |
| **Th** | -0.119 | 0.291 | 0.685** | 0.257 | 0.930** | 0.952** | 0.884** | 0.548* | 0.964** | 0.992** | 0.971** | -0.061 | 0.089 | -0.317 | 0.776 | 0.703** | 0.441 | 0.638* | 0.987** | 0.239 | 0.235 | 0.645* | 0.889* | 1.000 | | |
| **Yb** | -0.064 | 0.060 | 0.632* | 0.259 | 0.899** | 0.782** | 0.786** | 0.471 | 0.850** | 0.899** | 0.934** | 0.003 | 0.168 | -0.205 | 0.563 | 0.644** | 0.468 | 0.488 | 0.899** | -0.131 | 0.351 | 0.440 | 0.746** | 0.882** | 1.000 | |
| **Zn** | -0.212 | 0.545* | 0.754** | 0.095 | 0.705** | 0.628* | 0.562* | 0.636* | 0.630* | 0.658* | 0.691** | -0.194 | -0.006 | -0.301 | 0.815 | 0.280 | 0.445 | 0.800** | 0.654* | 0.326 | -0.115 | 0.684** | 0.498 | 0.659* | 0.503 | 1.000 |

*(Table continues)*

Table 2
*(continued)*
LMC Fronds ($n$=14)

| | As | Ba | Br | Ca | Ce | Co | Cr | Cs | Eu | Fe | Hf | K | La | Na | Nd | Rb | Sb | Sc | Se | Sm | Sr | Tb | Th | Yb | Zn |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **As** | 1.000 | | | | | | | | | | | | | | | | | | | | | | | | |
| **Ba** | 0.308 | 1.000 | | | | | | | | | | | | | | | | | | | | | | | |
| **Br** | -0.511 | -0.343 | 1.000 | | | | | | | | | | | | | | | | | | | | | | |
| **Ca** | 0.286 | 0.496 | -0.198 | 1.000 | | | | | | | | | | | | | | | | | | | | | |
| **Ce** | -0.077 | 0.294 | 0.161 | 0.093 | 1.000 | | | | | | | | | | | | | | | | | | | | |
| **Co** | -0.143 | 0.014 | 0.113 | 0.069 | 0.909 | 1.000 | | | | | | | | | | | | | | | | | | | |
| **Cr** | 0.031 | 0.535* | 0.031 | 0.139 | 0.893** | 0.674** | 1.000 | | | | | | | | | | | | | | | | | | |
| **Cs** | -0.237 | 0.104 | 0.046 | 0.290 | -0.064 | -0.082 | 0.019 | 1.000 | | | | | | | | | | | | | | | | | |
| **Eu** | -0.111 | 0.292 | 0.101 | 0.097 | 0.977** | 0.910** | 0.870** | 0.088 | 1.000 | | | | | | | | | | | | | | | | |
| **Fe** | -0.078 | 0.188 | 0.104 | 0.032 | 0.963* | 0.956** | 0.777** | -0.143 | 0.946 | 1.000 | | | | | | | | | | | | | | | |
| **Hf** | -0.068 | 0.404 | 0.116 | 0.179 | 0.979** | 0.859** | 0.912** | 0.090 | 0.984** | 0.917** | 1.000 | | | | | | | | | | | | | | |
| **K** | 0.470 | 0.215 | -0.717** | 0.206 | -0.167 | -0.199 | -0.064 | 0.126 | -0.129 | -0.188 | -0.140 | 1.000 | | | | | | | | | | | | | |
| **La** | 0.986** | 0.329 | -0.482 | 0.327 | 0.029 | -0.039 | 0.107 | -0.330 | -0.028 | 0.027 | 0.023 | 0.441 | 1.000 | | | | | | | | | | | | |
| **Na** | 0.540* | 0.040 | -0.378 | 0.251 | 0.538* | 0.607* | 0.421 | -0.209 | 0.528 | 0.579* | 0.478 | 0.469 | 0.604* | 1.000 | | | | | | | | | | | |
| **Nd** | 0.066 | 0.170 | 0.131 | -0.079 | 0.949** | 0.862** | 0.837** | -0.183 | 0.900** | 0.923** | 0.898** | -0.088 | 0.166 | 0.609* | 1.000 | | | | | | | | | | |
| **Rb** | -0.230 | -0.077 | 0.088 | -0.430 | 0.340 | 0.319 | 0.240 | 0.345 | 0.454 | 0.336 | 0.363 | 0.216 | -0.270 | 0.186 | 0.357 | 1.000 | | | | | | | | | |
| **Sb** | 0.090 | -0.014 | -0.118 | 0.096 | 0.527 | 0.580* | 0.442 | 0.377 | 0.595* | 0.578* | 0.534* | 0.156 | 0.086 | 0.536* | 0.527 | 0.334 | 1.000 | | | | | | | | |
| **Sc** | -0.147 | 0.222 | 0.141 | 0.038 | 0.981** | 0.935** | 0.820** | -0.062 | 0.978** | 0.980** | 0.953 | -0.153 | -0.046 | 0.547* | 0.919** | 0.433 | 0.537* | 1.000 | | | | | | | |
| **Se** | -0.171 | 0.507 | 0.024 | 0.226 | 0.736** | 0.586* | 0.772** | 0.352 | 0.767** | 0.671** | 0.792** | 0.036 | -0.127 | 0.280 | 0.638* | 0.433 | 0.380 | 0.733** | 1.000 | | | | | | |
| **Sm** | 0.982** | 0.378 | -0.465 | 0.343 | 0.061 | -0.014 | 0.140 | -0.295 | 0.011 | 0.058 | 0.064 | 0.419 | 0.995** | 0.597* | 0.189 | -0.231 | 0.082 | -0.011 | -0.061 | 1.000 | | | | | |
| **Sr** | -0.069 | 0.793** | -0.241 | 0.301 | 0.203 | -0.056 | 0.457 | 0.355 | 0.268 | 0.091 | 0.313 | 0.296 | -0.091 | -0.075 | 0.019 | 0.280 | 0.057 | 0.196 | 0.559 | -0.048 | 1.000 | | | | |
| **Tb** | -0.136 | 0.287 | 0.116 | 0.077 | 0.958** | 0.873** | 0.849** | -0.032 | 0.946** | 0.958** | 0.935** | -0.159 | -0.040 | 0.503 | 0.890** | 0.337 | 0.600* | 0.967** | 0.762** | -0.011 | 0.264 | 1.000 | | | |
| **Th** | -0.055 | 0.433 | 0.073 | 0.155 | 0.975** | 0.847** | 0.903** | 0.062 | 0.977** | 0.930** | 0.991** | -0.096 | 0.035 | 0.487 | 0.901** | 0.386 | 0.541* | 0.961** | 0.818** | 0.078 | 0.348 | 0.958** | 1.000 | | |
| **Yb** | -0.155 | -0.304 | 0.173 | -0.370 | 0.043 | 0.144 | -0.138 | -0.147 | 0.095 | 0.044 | 0.055 | -0.282 | -0.149 | -0.087 | 0.066 | 0.325 | -0.371 | 0.097 | -0.160 | -0.129 | -0.338 | -0.102 | 0.009 | 1.000 | |
| **Zn** | 0.229 | 0.034 | -0.398 | 0.131 | 0.381 | 0.450 | 0.317 | -0.085 | 0.385 | 0.412 | 0.354 | 0.337 | 0.274 | 0.572 | 0.405 | -0.004 | 0.652 | 0.358 | -0.007 | 0.231 | -0.072 | 0.339 | 0.337 | -0.183 | 1.000 |

Table 2
*(continued)*
ZMD Roots ($n$=13)

| As | Ba | Br | Ca | Ce | Co | Cr | Cs | Eu | Fe | Hf | K | La | Na | Nd | Rb | Sb | Sc | Se | Sm | Sr | Tb | Th | Yb | Zn |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 1.000 | | | | | | | | | | | | | | | | | | | | | | | | |
| -0.270 | 1.000 | | | | | | | | | | | | | | | | | | | | | | | |
| -0.840** | 0.291 | 1.000 | | | | | | | | | | | | | | | | | | | | | | |
| -0.447 | 0.498 | 0.719** | 1.000 | | | | | | | | | | | | | | | | | | | | | |
| -0.442 | 0.627* | 0.460 | 0.737** | 1.000 | | | | | | | | | | | | | | | | | | | | |
| -0.416 | 0.698 | 0.483 | 0.795** | 0.938** | 1.000 | | | | | | | | | | | | | | | | | | | |
| -0.424 | 0.752** | 0.416 | 0.697** | 0.947** | 0.956** | 1.000 | | | | | | | | | | | | | | | | | | |
| -0.404 | 0.787** | 0.314 | 0.393 | 0.447 | 0.597 | 0.650* | 1.000 | | | | | | | | | | | | | | | | | |
| -0.398 | 0.753 | 0.310 | 0.543 | 0.777** | 0.790** | 0.850** | 0.572* | 1.000 | | | | | | | | | | | | | | | | |
| -0.397 | 0.769** | 0.427 | 0.736** | 0.926** | 0.964** | 0.985** | 0.672* | 0.806** | 1.000 | | | | | | | | | | | | | | | |
| -0.353 | 0.805** | 0.331 | 0.661* | 0.883** | 0.912** | 0.945** | 0.643* | 0.960** | 0.926** | 1.000 | | | | | | | | | | | | | | |
| 0.651 | -0.244 | -0.787** | -0.560* | -0.373 | -0.342 | -0.258 | -0.091 | -0.406 | -0.248 | -0.336 | 1.000 | | | | | | | | | | | | | |
| 0.804** | 0.035 | -0.836 | -0.362 | -0.163 | -0.140 | -0.127 | -0.188 | -0.119 | -0.093 | -0.059 | 0.742* | 1.000 | | | | | | | | | | | | |
| 0.715* | 0.055 | -0.690** | -0.203 | 0.077 | 0.039 | 0.035 | -0.288 | 0.078 | -0.004 | 0.113 | 0.482 | 0.830** | 1.000 | | | | | | | | | | | |
| -0.446 | 0.744** | 0.412 | 0.595* | 0.756** | 0.801** | 0.826** | 0.663* | 0.930** | 0.797** | 0.917** | -0.496 | -0.264 | -0.086 | 1.000 | | | | | | | | | | |
| 0.236 | -0.363 | -0.446 | -0.654* | -0.687** | -0.602* | -0.559* | 0.099 | -0.625* | -0.524 | -0.615* | 0.580* | 0.134 | -0.218 | -0.539 | 1.000 | | | | | | | | | |
| -0.187 | 0.814 | 0.230 | 0.602* | 0.688** | 0.817** | 0.832** | 0.770** | 0.843** | 0.862** | 0.907** | -0.227 | 0.004 | 0.021 | 0.872** | -0.365 | 1.000 | | | | | | | | |
| -0.349 | 0.731** | 0.473 | 0.807** | 0.876** | 0.936** | 0.932** | 0.651* | 0.667* | 0.965** | 0.831** | -0.186 | -0.082 | -0.021 | 0.680 | -0.493 | 0.789** | 1.000 | | | | | | | |
| -0.247 | 0.464 | 0.367 | 0.734** | 0.756** | 0.883** | 0.795** | 0.411 | 0.587* | 0.851** | 0.727** | -0.154 | 0.004 | 0.031 | 0.602* | -0.497 | 0.720** | 0.861** | 1.000 | | | | | | |
| 0.818** | 0.015 | -0.844** | -0.364 | -0.116 | -0.103 | -0.091 | -0.224 | -0.095 | -0.065 | -0.034 | 0.720** | 0.987** | 0.862* | -0.226 | 0.090 | 0.021 | -0.068 | 0.049 | 1.000 | | | | | |
| -0.354 | 0.569* | 0.652* | 0.944** | 0.781** | 0.837** | 0.743** | 0.397 | 0.540 | 0.772** | 0.680* | -0.479 | -0.261 | -0.064 | 0.634 | -0.687** | 0.612* | 0.852** | 0.767** | -0.235 | 1.000 | | | | |
| -0.330 | 0.753** | 0.250 | 0.522 | 0.790** | 0.803** | 0.856** | 0.564* | 0.993** | 0.815** | 0.967** | -0.376 | -0.064 | 0.143 | 0.931** | -0.615* | 0.862** | 0.670* | 0.599* | -0.030 | 0.535 | 1.000 | | | |
| -0.411 | 0.645 | 0.529 | 0.814** | 0.894** | 0.948** | 0.936** | 0.593* | 0.668* | 0.963** | 0.822** | -0.241 | -0.172 | -0.082 | 0.681* | -0.524 | 0.762** | 0.985** | 0.894** | -0.144 | 0.846** | 0.669* | 1.000 | | |
| -0.334 | 0.740** | 0.229 | 0.535 | 0.823** | 0.816** | 0.876** | 0.628* | 0.961* | 0.836** | 0.967** | -0.331 | -0.073 | 0.117 | 0.924** | -0.529 | 0.870** | 0.699** | 0.579* | -0.047 | 0.533 | 0.974** | 0.691** | 1.000 | |
| -0.156 | 0.472 | 0.008 | 0.065 | 0.296 | 0.303 | 0.320 | 0.284 | 0.565* | 0.247 | 0.479 | -0.116 | 0.135 | 0.290 | 0.549 | -0.375 | 0.315 | 0.169 | 0.088 | 0.110 | 0.181 | 0.548 | 0.115 | 0.513 | 1.000 |

*(Table continues)*

Table 2
*(continued)*
LMC Roots ($n$=13)

| | As | Br | Ca | Ce | Co | Cr | Eu | Fe | Hf | K | La | Na | Nd | Rb | Se | Sm | Sr | Th | Yb | Zn |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **As** | 1.000 | | | | | | | | | | | | | | | | | | | |
| **Br** | -0.128 | 1.000 | | | | | | | | | | | | | | | | | | |
| **Ca** | 0.222 | 0.310 | 1.000 | | | | | | | | | | | | | | | | | |
| **Ce** | -0.311 | -0.096 | -0.145 | 1.000 | | | | | | | | | | | | | | | | |
| **Co** | -0.507 | 0.448 | 0.231 | -0.008 | 1.000 | | | | | | | | | | | | | | | |
| **Cr** | 0.225 | -0.191 | -0.268 | -0.287 | -0.313 | 1.000 | | | | | | | | | | | | | | |
| **Eu** | -0.143 | 0.426 | -0.085 | -0.330 | 0.283 | 0.652* | 1.000 | | | | | | | | | | | | | |
| **Fe** | -0.277 | 0.368 | -0.250 | 0.158 | 0.205 | 0.206 | 0.429 | 1.000 | | | | | | | | | | | | |
| **Hf** | -0.027 | -0.036 | -0.340 | -0.316 | -0.118 | 0.534 | 0.630* | 0.612* | 1.000 | | | | | | | | | | | |
| **K** | 0.317 | 0.366 | 0.348 | 0.339 | 0.007 | -0.276 | -0.086 | 0.402 | 0.105 | 1.000 | | | | | | | | | | |
| **La** | 0.991** | -0.108 | 0.214 | -0.348 | -0.460 | 0.156 | -0.165 | -0.234 | 0.008 | 0.347 | 1.000 | | | | | | | | | |
| **Na** | -0.098 | -0.027 | 0.231 | -0.338 | 0.180 | 0.220 | 0.414 | 0.425 | 0.710** | 0.197 | -0.042 | 1.000 | | | | | | | | |
| **Nd** | -0.155 | 0.412 | -0.054 | -0.330 | 0.352 | 0.578* | 0.983** | 0.483 | 0.694** | -0.013 | -0.158 | 0.535 | 1.000 | | | | | | | |
| **Rb** | 0.208 | -0.339 | -0.460 | 0.403 | -0.726** | 0.038 | -0.358 | -0.326 | -0.185 | -0.033 | 0.160 | -0.487 | -0.436 | 1.000 | | | | | | |
| Se | 0.200 | 0.064 | -0.152 | -0.294 | -0.281 | 0.581* | 0.554* | 0.656* | 0.864** | 0.194 | 0.216 | 0.588* | 0.589* | -0.194 | 1.000 | | | | | |
| **Sm** | -0.342 | 0.487 | 0.325 | -0.095 | 0.794** | -0.347 | 0.226 | 0.092 | -0.132 | -0.067 | -0.291 | 0.156 | 0.297 | -0.611* | -0.142 | 1.000 | | | | |
| **Sr** | -0.008 | 0.292 | 0.021 | -0.327 | -0.054 | 0.354 | 0.641* | 0.580* | 0.800** | 0.181 | 0.021 | 0.687** | 0.694** | -0.294 | 0.882** | 0.103 | 1.000 | | | |
| **Th** | -0.042 | -0.083 | -0.326 | -0.144 | -0.221 | 0.590* | 0.576* | 0.694** | 0.946** | 0.114 | -0.024 | 0.673* | 0.626* | -0.122 | 0.914** | -0.197 | 0.803** | 1.000 | | |
| **Yb** | -0.116 | 0.018 | -0.253 | -0.140 | 0.065 | 0.721** | 0.866** | 0.463 | 0.794** | -0.089 | -0.137 | 0.549 | 0.885** | -0.197 | 0.697* | 0.032 | 0.698 | 0.806** | 1.000 | |
| **Zn** | -0.465 | 0.051 | -0.070 | 0.012 | -0.174 | -0.311 | -0.199 | 0.318 | 0.228 | 0.051 | -0.427 | 0.232 | -0.177 | -0.025 | 0.208 | -0.218 | 0.319 | 0.256 | -0.155 | 1.000 |

* Significant level at $p<0.05$.
** Significant level at $p<0.01$.

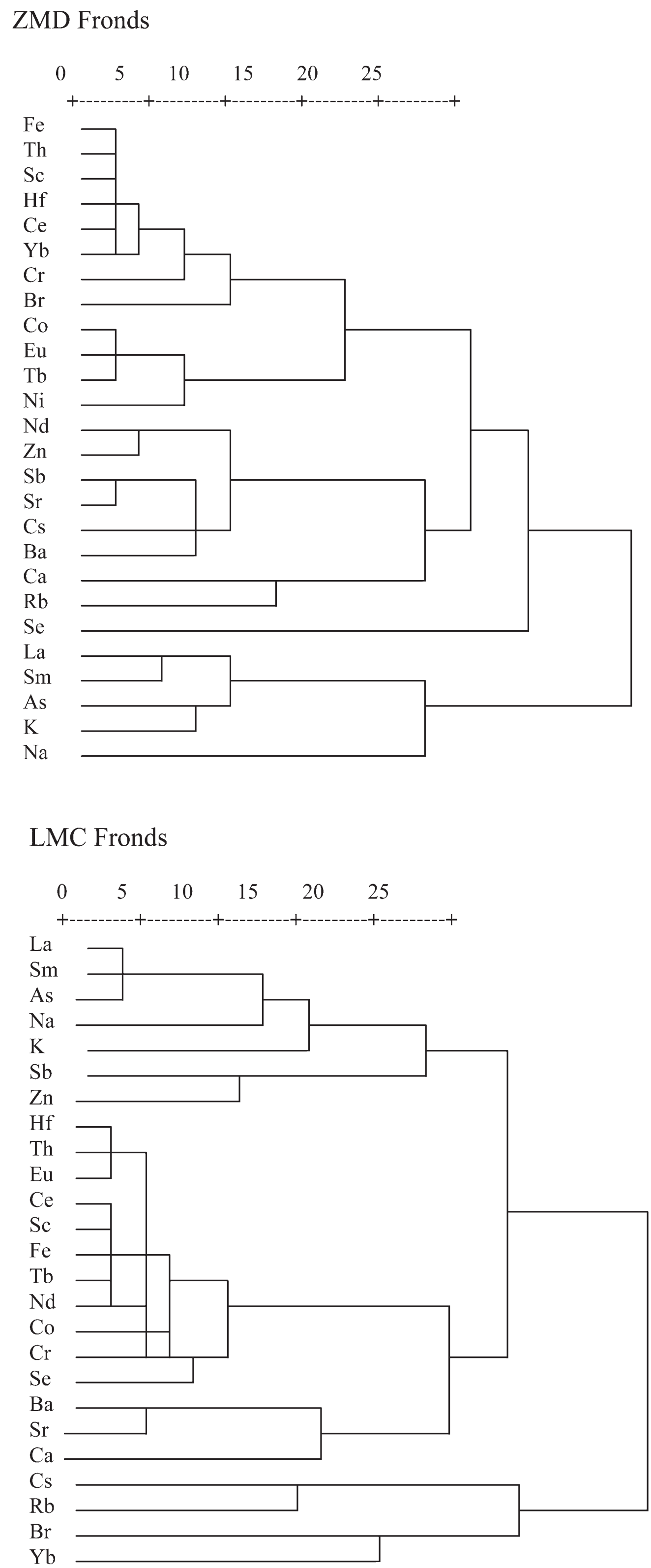
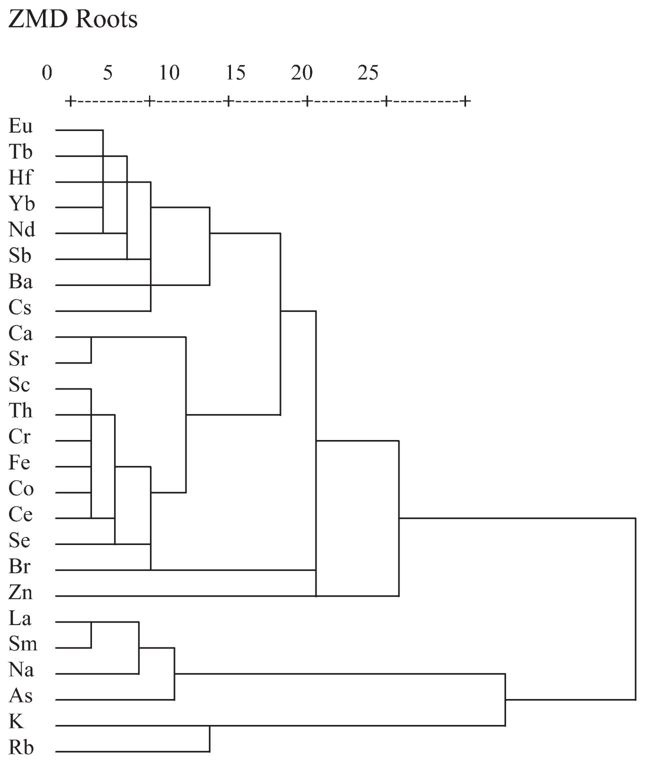



Fig. 1. Cluster tree of elements using CA based on Pearson's correlation coefficients. *(Figure continues)*



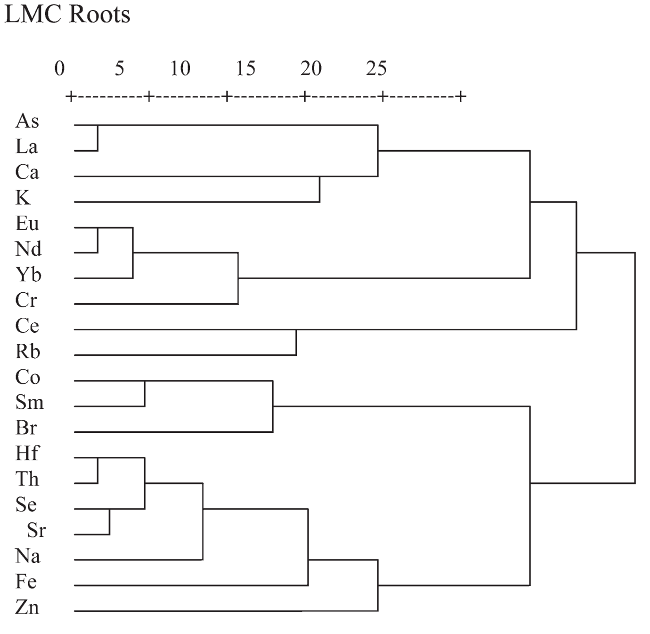




Fig. 1. (continued)

Table 3
Principal Component Loadings from PCA After Rotation for the Maximum Variance

| | ZMD Fronds | | | LMC Fronds | | ZMD Roots | | LMC Roots | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | PC1 | PC2 | PC3 | PC1 | PC2 | PC1 | PC2 | PC1 | PC2 | PC3 |
| As | -0.128 | 0.863 | -0.290 | -0.085 | 0.972 | -0.327 | 0.861 | -0.103 | -0.454 | 0.829 |
| Co | 0.907 | -0.012 | 0.294 | 0.918 | -0.044 | 0.980 | -0.123 | 0.066 | 0.836 | -0.281 |
| Cr | 0.915 | -0.030 | 0.107 | 0.889 | 0.076 | 0.973 | -0.106 | 0.468 | -0.689 | -0.173 |
| Fe | 0.985 | 0.060 | 0.142 | 0.961 | 0.004 | 0.981 | -0.091 | 0.814 | 0.243 | -0.019 |
| Hf | 0.984 | 0.085 | 0.025 | 0.976 | -0.004 | 0.947 | -0.060 | 0.917 | -0.233 | -0.047 |
| K | -0.051 | 0.795 | 0.067 | -0.119 | 0.610 | -0.239 | 0.769 | 0.275 | 0.354 | 0.758 |
| La | 0.067 | 0.852 | 0.137 | 0.010 | 0.976 | -0.010 | 0.977 | -0.065 | -0.384 | 0.858 |
| Na | -0.273 | 0.345 | -0.738 | 0.560 | 0.680 | 0.129 | 0.884 | 0.784 | 0.146 | 0.023 |
| Se | 0.109 | 0.208 | 0.806 | 0.808 | -0.107 | 0.883 | 0.015 | 0.886 | -0.317 | 0.155 |
| Sm | 0.221 | 0.876 | -0.032 | 0.050 | 0.966 | 0.027 | 0.986 | 0.037 | 0.774 | -0.174 |

However, routine analysis of As with NAA for the determination of As contamination or screening for As hyperaccumulators is invalid.

Numerous studies have confirmed the As-hyperaccumulation property of the Chinese brake fern; however, little is known about the correlation between As and other elements in this fern *(2–11).* Two experiments reported that K increased and Ca decreased in the fronds of the Chinese brake fern when As was added to the pots *(25,26).* Chen et al. *(27)* found a positive correlation between As and K in the pinnae of *Pteris nervosa* (Cretan brake fern) using the synchrotron radiation X-ray fluorescence spectroscopy (SRXRF) method. However, because this method only measures 10 elements as a percentage of the total, the possible correlation between As with other elements had not been explored in depth, In the present study, through analysis of the expanding lists of elements, we found that As was closely correlated to K, Na, La, and Sm. The results of NAA confirmed our previous results using ICP-AES: that K and Na were probably taken up with As, as the two elements are relatively mobile and easy for uptake and transport in plants *(24,28).* Palmer and Bacon *(21)* had suggested that there existed an anion–cation balance effect in mustard leaves *(Sinapis alba).* As has been demonstrated to be taken up by and stored in an inorganic form in the Chinese brake fern, suggesting the anion–cation balance effect during elemental uptake. In this study, the correlation between As and K, Na, La, and Sm revealed by multivariate analysis might suggest that the four cations act as antagonists to As during uptake and transportation. An EXAFS study found that As tends to accumulate in the cell walls and vacuoles in the Chinese brake fern with K *(19,20),* whereas Na is not an essential element for the plant, but it also tends to be stored in the cell walls and vacuoles *(29).* La and Sm belong to rare earth elements (REEs) and plants tend to uptake and accumulate them *(30).* A study on wheat (*Triticum aestivum* L.) accumulation of REEs found that the content of the total REEs in the leaves of wheat dramatically decreased with increasing levels of phosphate applied *(31)*, suggesting the interaction between REE and P (a chemical analogue of As). An EXAFS study found that La accumulated in the cell walls of wheat *(32)*, which is similar to the results of Cd and Zn localization in Cd and Zn accumulation in plants *(33–35)*. All of the above results might imply that As can be taken up with K, Na, La, and Sm in the same way and stored in the same place; this might be involved in the mechanism of detoxification of As by the Chinese brake fern.

Selenium was found to be positive to most of the cations in the Chinese brake fern, which suggests that Se might operate as an antagonist to most of the cations. In PCA, Se was also found to appear with these cations, except in the fronds at ZMD, where Br was found to be positively correlated to most of the cations in the elemental correlation analysis, which might suggest that Br occasionally substitutes Se as an antagonist. However, the reason for substitution of Se to Br in the role of the ion counterbalance is not clear from this study.

Our previous studies have found that the Chinese brake fern could accumulate a high concentration of Fe, with increasing As levels in its growing soils *(24,28)*. However, both our previous and present results have not found significant correlation between As and Fe. To the contrary, we found in the present study that Fe was positively correlated to Se in the Chinese brake fern. This might suggest that although high As levels in the growing medium could induced an increasing accumulation of Fe in the Chinese brake fern, the two elements did not actually rely on each other on a physiological base.

In CA, Rb appeared in the group with As, K, Na, La, and Sm in the roots of the Chinese brake fern at ZMD. As the greatest As concentrations were recorded in the roots of the Chinese brake fern at ZMD (data not shown), the coappearance of Rb with As might suggest that Rb operates as an antagonist to As, similar to that of K. Because K and Rb are chemical analogues, plants tend to take up Rb when the K pool is depleted *(22,36,37)*. The coexistence of As with Rb during uptake and accumulation in the roots of the Chinese brake fern at ZMD might imply that an enhanced uptake of mono-cation ions of K, or Rb when K is depleted, was necessary to enable the greater As accumulation by this fern, as is necessary for anion–cation counterbalance.

In summary, NAA can be used as an effective tool to explore the elemental relationship in the As-hyperaccumulator Chinese brake fern. Through the multivariate analysis approach, we conclude that the anion–cation counterbalance effect operates on As hyperaccumulation by the Chinese brake fern, the mono-ion cations of K, Rb, and Na, together with the two REEs La and Sm, acted as antagonists to As during their uptake and accumulation in this fern.

## ACKNOWLEDGMENTS

This work was financially supported by the National Natural Science Foundation of China (grant no. 40271099), the project of the Laboratory of

Nuclear Analysis Techniques, Chinese Academy of Sciences (grant no. K-111), and the Renovation Project of the Institute of Geographical Sciences and Natural Resources Research, Chinese Academy of Sciences (grant no. CXIOG-C04-02). The authors express thanks to Mr. C. D. Sun and Mr. S. Fang for their kind help with the field sampling and NAA analysis.

## REFERENCES

1. B. K. Mandal and K. T. Suzuki, Arsenic round the world: a review, *Talanta* **58,** 201–235 (2002).
2. C. Tu, L. Q. Ma, and B. Bondada, Arsenic accumulation in the hyperaccumulator Chinese brake and its utilization potential for phytoremediation, *J. Environ. Qual.* **31,** 1671–1675 (2002)
3. S. Tu, L. Q. Ma, A. O. Fayiga, and E. J. Zillioux, Phytoremediation of arsenic-contaminated groundwater by the arsenic hyperaccumulating fern *Pteris vittata* L., *Int. J. Phytoremediat.* **6,** 35–47 (2004).
4. C. Y. Wei and T. B. Chen, Arsenic accumulation by two brake ferns growing on an arsenic mine and their potential in phytoremediation, *Chemosphere* **63,** 1048–1053 (2006).
5. L. Q. Ma, M. K. Kenneth, C. Tu, W. H. Zhang, Y. Cai, and E. D. Kennelley, A fern that hyperaccumulates arsenic, *Nature* **409,** 579 (2001).
6. T. B. Chen, C. Y. Wei, Z. C. Huang, Q. F. Huang, and Q. G. Lu, Arsenic hyperaccumulator *Pteris vittata* L. and its arsenic accumulation, *Chin. Sci. Bull.* **47,** 902–905 (2002).
7. A. A. Meharg, Variation in As accumulation–hyperaccumulation in ferns and their allies, *New Phytol.* **157,** 25–31 (2002).
8. F. J. Zhao, S. J. Duham, and S. P. McGrath, As hyperaccumulation by different fern species, *New Phytol.* **156,** 27–31 (2002).
9. J. Wang, F. J. Zhao, A. A. Meharg, A. Raab, J. Feldmann, and S. P. McGrath, Mechanisms of arsenic hyperaccumulation in *Pteris vittata.* Uptake kinetics, interactions with phosphate, and arsenic speciation, *Plant Physiol.* **130,** 1552–1561 (2002).
10. W. J. Fitz, W. W. Wenzel, H. Zhang, et al., Rhizosphere characteristics of the arsenic hyperaccumulator *Pteris vittata* L. and monitoring of phytoremoval efficiency, *Environ. Sci. Technol.* **37,** 5008–5014 (2003).
11. G. L. Duan, Y. G. Zhu, Y. P. Tong, C. Cai, and R. Kneer, Characterization of arsenate reductase in the extract of roots and fronds of Chinese brake fern, an arsenic hyperaccumulator, *Plant Physiol.* **138,** 461–469 (2005).
12. C. J. Asher and P. F. Reay, Arsenic uptake by barley seedlings, *Aust. J. Plant Physiol.* **6,** 459–466 (1979)
13. A. A. Meharg and M. R. Macnair, The mechanisms of arsenate tolerance in *Deschampsia cespitosa* (L.) Beauv and *Agrostis capillaris* L., *New Phytol.* **119,** 291–297 (1991)
14. A. A. Meharg and M. R. Macnair, Suppression of the high-affinity phosphate uptake system: a mechanism of arsenate tolerance in *Holcus lanatus* L., *J. Exp. Bot.* **43,** 519–524 (1992)
15. A. A. Meharg, J. Naylor, and M. R. Macnair, Phosphorus nutrition of arsenate tolerant and nontolerant phenotypes of velvetgrass, *J. Environ. Qual.* **23,** 234–238 (1994).
16. M. J. Abedin, J. Feldmann, and A. A. Meharg, Uptake kinetics of arsenic species in rice plants, *Plant Physiol.* **128,** 1120–1128 (2002).
17. A. A. Meharg and J. Hartley-Whitaker, Arsenic uptake and metabolism in arsenic resistant and nonresistant plant species, *New Phytol.* **154,** 29–43 (2002)
18. W. H Zhang, Y. Cai, C. Tu, and L. Q. Ma, Arsenic speciation and distribution in an arsenic hyperaccumulating plant, *Sci. Total Environ.* **300,** 167–177 (2002).

19. E. Lombi, F. J. Zhao, M. Fuhrmann, LQ. Ma, and S. P. McGrath, Arsenic distribution and speciation in the fronds of the hyperaccumulator *Pteris vittata, New Phytol.* **156,** 195–203 (2002)
20. T. B. Chen, X. L. Yan, X. Y. Liao, et al., Subcellular distribution and compartmentalization of arsenic in *Pteris vittata* L., *Chin. Sci. Bull.* **50,** 2843–2849 (2005)
21. M. J. Palmer and J. S. D. Bacon, The effect of illumination on the malic acid content and anion/cation balance of mustard leaves *(Sinapis alba), Biochem. J.* **102,** 304–312 (1967).
22. Kabata-Pendias and H. Pendias, *Trace Elements in Soils and Plants,* 3rd ed., CRC, Boca Raton, FL (2001).
23. S. B. Sarmani, I. Abugassa, A. Hamzah, and M. D.Yahya, Elemental analysis of herbal preparations for traditional medicines by neutron activation analysis with the k0 standardization method, *Biol. Trace Element Res.* **71–72,** 365–376 (1999).
24. C. Y. Wei, X. Sun, C. Wang, and W. Y. Wang, Factors influencing arsenic accumulation by *Pteris vittata:* a comparative field study at two sites, *Environ. Pollut,* **141,** 488–493 (2006).
25. X. Y. Liao, X. Y. Xiao, and T. B. Chan Effects of Ca and As addition on As, P and Ca uptake by hyperaccumulator *Pteris vittata* L. under sand culture (in Chinese), *Acta Ecol. Sin.* **3,** 2057–2065 (2003).
26. X. D. Cao, L. Q Ma, and C. Tu, Antioxidative responses to arsenic in the arsenic-hyperaccumulator Chinese brake fern (*Pteris vittata* L.), *Environ. Pollut.* **128,** 317–325 (2004).
27. T. B. Chen, Z. C. Huang, Y. Y. Huang, H. Xie, and X. Y. Liao, Cellular distribution of arsenic and other elements in hyperaccumulator *Pteris nervosa* and their relations to arsenic accumulation, *Chin. Sci. Bull.* **48,** 1586–1591 (2003).
28. C. Y. Wei, C. Wang, X. Sun, and W. Y. Wang, Arsenic accumulation by ferns: a field survey in southern China, *Environ. Geochem. Health.* (2006, in press).
29. M. A. Kader and S. Lindberg, Uptake of sodium in protoplasts of salt-sensitive and salt-tolerant cultivars of rice, *Oryza sativa* L. determined by the fluorescent dye SBFI, *J. Exp. Bot.* **56,** 3149–3158 (2005).
30. G. Tyler, Rare earth elements in soil and plant systems—a review, *Plant Soil* **267,** 191–206 (2004).
31. S. M. Ding, T. Liang, C. S. Zhang, J. Yan, Z. Zhang, and Q. Sun, Role of ligands in accumulation and fractionation of rare earth elements in plants: examples of phosphate and citrate, *Biol. Trace Element Res.* **107,** 73–86 (2005).
32. S. M. Ding, T. Liang, C. S. Zhang, Z. C. Huang, Y. N. Xie, and T. B. Chen, Fractionation mechanisms of rare earth elements (REEs) in hydroponic wheat: an application for metal accumulation by plants, *Environ. Sci. Technol.* **40,** 2686–2691 (2006).
33. C. Cosio, L. DeSantis, B. Frey, S. Diallo, and C. Keller. Distribution of cadmium in leaves of *Thlaspi caerulescens, J. Exp. Bot.* **56,** 765–775 (2005).
34. U. Galli, H. Schüepp, and C. Brunold, Heavy metal binding by mycorrhizal fungi, *Physiol. Plant.* **92,** 364–368 (1994).
35. B. Frey, C. Keller, K. Zierold, and R. Schulin, Distribution of Zn in functionally different leaf epidermal cells of the hyperaccumulator *Thlaspi caerulescens, Plant Cell Environ.* **23,** 675–687 (2000).
36. A. A. Very and H. Sentenac, Molecular mechanisms and regulation of $K^+$ transport in higher plants, *Annu. Rev. Plant Biol.* **54,** 575–603 (2003).
37. A. M. Sheikh, T. C. Broyer, and A. Ulrich, Interaction of rubidium or sodium with potassium in absorption by intact sugar beet plants, *Plant Physiol.* **47,** 709–712 (1971).