Proceedings of the Annual International Conference on Soils, Sediments, Water and Energy

Volume 12 Article 26

January 2010

Phytoextraction And Phytovolatilization Of Arsenic From As-Contaminated Soils By Pteris vittata

Masayuki Sakakibara
Ehime University

Aya Watanabe
Ehime University

Masahiro Inoue
Ehime University

Sakae Sano
Ehime University

Toshikazu Kaise
Tokyo University of Pharmacy and Life Science

Chapter 25

PHYTOEXTRACTION AND PHYTOVOLATILI-ZATION OF ARSENIC FROM AS-CONTAMI-NATED SOILS BY *PTERIS VITTATA*

Masayuki Sakakibara[1], Aya Watanabe[1], Sakae Sano[2], Masahiro Inoue[1], and Toshikazu Kaise[3]
[1]*Graduate School of Sciences and Engineering, Ehime University, 2-5 Bunkyo-cho, Matsuyama, 790-8577 Japan;* [2]*Earth Science Laboratory, Faculty of Education, Ehime University,* [3]*Tokyo University of Pharmacy and Life Science, 1432-1 Horinouchi, Hachioji 192-0392, Japan*

Abstract: Phytoremediation of metal contaminated soil and water is a low cost method of remediation, which allows some extracted metals to be recycled for economic use. The use of phytoextraction and phytovolatilization of metals by plants offers a viable remediation on commercial projects. Arsenic is of great environmental concern due to its extensive contamination and toxicity. Ma *et al.* (2001) recently discovered the first known and extremely efficient arsenic hyperaccumulating plant, *Pteris vittata*. If we can use phytovolatilization for As-contaminated soils, it could minimize the production of As-laden plant material and the duration of remediation. Several greenhouse experiments conducted by us have shown that the material balance of As between uptake by *P. vittata* and reduction from As-polluted soils has not been maintained. Vapor samples were collected to determine volatilization of arsenic compounds from fronds of *P. vittata* grown in As-polluted soil. A low-density polyethylene bottle was placed over a stem and sealed at the open bottleneck with sealant and tape to produce a tight seal around the stem and chambers for a period of 2–7 days. Concentration of arsenic in trap samples was measured by ICP-MS and speciation of arsenic was analyzed using HPLC/ICP-MS system. Concentrations in collected water samples (ca. 0.6–5.9ml) reached 10.7–30.8 μg-As/L (2–7 days). Percentages of arsenic components in one sample were 37% for arsenite and 63% for arsenate. Our results suggest that *P. vittata* is a plant species that is effective at volatilizing As; it removed about 90% of the total uptake of As from As-contaminated soils in the greenhouse, where the environment was similar to the subtropics. However, if a large amount of arsenic had been released from the contaminated site into the atmosphere by the fern, the process may have caused a secondary As-contamination to the surrounding environments.

Key words: Arsenic, *Pteris vittata*, phytoextraction, volatilization, arsenite, arsenate, greenhouse experiment

1. INTRODUCTION

Some metal contaminants such as As, Hg, and Se also exist as gaseous species in the environment. In the recent years, researchers have searched for naturally occurring or genetically modified plants that are capable of absorbing elemental forms of these metals from the soil, biologically converting them into gaseous species within the plant, and releasing them into the atmosphere. This process is called phytovolatilization, the most controversial phytoremediation technology. Since Hg and Se are toxic (Wilber, 1980; Suszcynsky and Shann, 1995) there is an uncertainty whether the volatilization of these elements into the atmosphere is safe (Watanabe, 1997). Se phytovolatilization is the process by which gaseous Se is produced from inorganic or organic Se compounds (Lewis *et al.* 1966; Terry *et al.* 1992; Bañuelos *et al.*, 1993a, b; McGrath, 1998). Futhermore, the volatilization is particularly attractive for the phytoremediation of Se-contaminated environment because this element is a serious problem in many parts of the world with Se-rich soil (Brooks, 1998). Moreover, in recent years, there has been a considerable effort to insert bacterial Hg ion reductase genes into plants for Hg phytovolatilization (Rugh *et al.*, 1996; Heaton *et al.*, 1998; Rugh *et al.*, 1998; Bizily *et al.*, 1999). On the other hand, there have been no efforts to genetically engineer plants that volatilize As. The most efficacious remediation of arsenic has been suggested where plants extract arsenic from soil and hyperaccumulate it aboveground in fronds and stems.

Pollution is a worldwide problem and there is tremendous demand for the cleanup of As-contaminated soil and water. Some plant species growing on As-contaminated soils are As tolerant and accumulate very high concentrations of As (As accumulators). An arsenic hyperaccumulator, *Pteris vittata* L. (Chinese brake), was first discovered in China by means of a field survey and greenhouse cultivation. The plant's abilities to produce large quantities of root exudates (to solubilize soil arsenic), effectively translocate arsenic to the fronds (up to 95%), reduce arsenic from arsenate-As(V) to arsenite-As(III) (up to 100% arsenite) in the fronds, and keep high concentration of P in the roots have contributed to its capability to hyperaccumulate arsenic, making it a prime candidate for use in phytoremediation of As-contaminated sites (Ma *et al.*, 2001).

The objective of this study was to determine *P. vittata*'s ability to metabolize As into volatile As forms (phytovolatilization) along with the application of phytoremediation for the cleanup of As-contaminated environments.

2. MATERIAL AND METHODS

2.1 Greenhouse experiment

The As-contaminated soil used in this experiment was collected from a deposit site of neutralized acid mine drainage in Japan. It accumulated up to 6,540±380 mg As/kg-dry weight (DW).

One healthy fern with 6–7 fronds, about six months old, was planted in each pot containing about 12 kg of soil. The plants were grown for 18 months in a greenhouse where the average temperature varied from 25 (night) to 45ºC (day) in summer and 10 (night) and 25ºC (day) in winter. The concentration of As in the fronds ranged from 3,830 to 11,020 mg/kg-DW.

Water vapor samples were collected to determine volatilization of arsenic compounds from leaves of the fern to the atmosphere. A low-density polyethylene bottle (20L) was placed over a stem and sealed at the open bottleneck with sealant and tape to produce a tight seal around the stem and chambers during a 2–7 day period (Fig. 1). The bottles were fixed in the same location during sample collection.

Figure 1. Pteris vittata for the experiment of volatilization of arsenic. a. the transplanted fern in the As-contaminated soil, b. the fern growing up, c. the frond for the volatilizing experiment, d. experiment for collecting vapor from the fern.

2.2 Analytical methods

The speciation and quantification of As were accomplished by using HPLC to separate the species and ICP-MS to detect them. Detection of arsenic species was accomplished with an ELAN® DRC-e ICP-MS (PerkinElmer SCIEX) at Tokyo University of Pharmacy and Life Science (Kamidate *et al.*, 2000). Total arsenic concentrations were also measured without speciation, using conventional nebulization into the ICP-MS at Ehime University (Sano *et al.*, 2005).

3. RESULTS

Three volatile water samples from the fern were collected for chemical analysis by ICP-MS. As-concentrations in the collected water samples were 0.74 μg-As/L in 0.6 cc (2 days), 16.1 μg-As/L in 5.9 cc (7 days), and 30.8 μg-As/L in 4.1 cc (7 days). As-concentrations of blank were 0.15 μg-As/L in 30 cc (7 days). The result of the experiment revealed that *P. vittata* is a plant species that releases As from the fronds.

Chromatographical analysis using HPLC-ICP-MS for arsenic of volatilized water detected only arsenite and arsenate. Chromatograms are presented in Fig. 2, showing that good separations were achieved for the As species. Percentages of arsenic components in one sample were 37% for arsenite and 63% for arsenate.

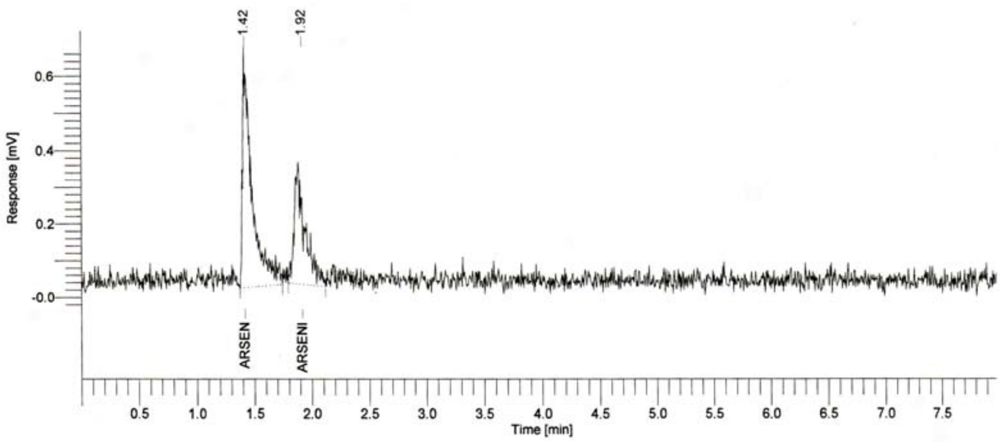

Figure 2. Chromatograms (HPLC-ICP-MS) of vapor collected from *Pteris vittata* for As speciation

4. DISCUSSION

For phytoextraction, the total amount of arsenic accumulation by the fern should be considered because it takes into account both biomass production and As concentration, measuring the potential effectiveness of a plant for phytoextraction. The amount of arsenic found in the plant tissue was well explained by the decrease in total soil arsenic after growing *P. vittata* (Tu *et al.*, 2002). Moreover, the most striking feature associated with the As hyperaccumulation by *P. vittata* lies in the exceedingly efficient transport from roots to shoots. In the depletion experiments (8 h) by Wang *et al.* (2002), between 50% and 78% of As taken up had already been transported from the culture to the fronds.

On the other hand, only 15–20% of the total As removed from the soil can be accounted for that accumulated by the fern in our recent experiments (Sakakibara *et al.*, 2004a; Watanabe *et al.*, 2005; and unpublished data).

Speciation in plant tissues by HPLC-ICP-MS is conducted on methanol/water extracts, including previous studies of As-hyperaccumulating ferns, where only arsenate and arsenite have been detected (Ma *et al.*, 2001; Francesconi *et al.*, 2002). A few studies have reported that a reduction of arsenic, arsenate ion (As^{5+}) to arsenite ion (As^{3+}), occurred in the plant body (Ma *et al.*, 2001; Lombi *et al.*, 2002; Wang *et al.*, 2002; Huang *et al.*, 2004). Wang *et al.* (2002) concluded that arsenate was taken up by *P. vittata* via the phosphate transporters, reduced to arsenite, and then sequestered in the fronds primarily as As(III). Huang *et al.* (2004) reported that the As in the fern were 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, by using synchrotron radiation extended X-ray absorption fine structure (SR EXAFS). Based on our experiments, *P. vittata* accumulated large amounts of inorganic As compounds, mainly arsenite and arsenate, in their tissues and released large amounts of As from the fronds. *P. vittata* is promising for the phytoremediation of As-contaminated soil because of its remarkable ability to phytoextract As and release the accumulated As in the fronds. However, the process of volatilization of arsenic from *Pteris vittata* remains unknown.

P. vittata may release their secretions including arsenic compounds, through secretory glands at the edge of the fronds. Hokura *et al.* (2006) revealed that the arsenic distributions of the fern were successfully measured by the µ-SR-XRF analysis, and As(V) in the culture medium was reduced to As(III) in the tissue after absorption. The As contents are characteristically high at the edge of the frond concentrating secretory glands, based on the As imaging of XRF. However, if large amount of arsenic would have been removed from the contaminated site into the atmosphere by the fern, then the process would have caused a secondary As-contamination to the surrounding environment.

5. CONCLUSION

It was determined that vapor released from the frond of *P. vittata* included arsenic compounds, arsenite and arsenate. The results suggest that *P. vittata* effectively volatilizes As; it removed a maximum ratio of 90% of the total uptake of As from As-contaminated soils in greenhouse, where the environment was similar to the subtropics. However, if a large amount of arsenic had been released from the contaminated site into the atmosphere by the fern, the process may have caused a secondary As-contamination to the surrounding environment.

ACKNOWLEDGEMENTS

We would like to thank the Grant for Research and Development Assistance of Ehime University for their support. We also thank Dr. Kuramoto for his assistance in ICP-MS analyses and Mr. Toshihito Kondo in FUJITA Co. Ltd. for providing the seedlings of *P. vittata*.

REFERENCES

Bañuelos, G.S., Cardon, G., Mackey, B., Ben-Asher, J., Wu, L.P., Beuselinck, P., Akohoue, S., and Zambrzuski, S. 1993a. Boron and selenium removal in B-laden soils by four sprinkler irrigated plant species. J. Environ. Qual. 22, 786-797.

Bañuelos, G.S., Mead, R.R., and Hoffman, G.J. 1993b. Accumulation of selenium in wild mustard irrigated with agricultural effluent. Agri. Ecosys. Environ. 43, 119-126.

Bizily, S.P., Rugh, C.L., Summers, A.O., and Meagher, R.B. 1999. Phytoremediation of methylmercury pollution: *Mer B* expression in *Arabidopsis thaliana* confers resistance to organomerculials. Proc. Natl. Acad. Sci. USA. 96, 6808-6813.

Brooks, R.R. 1998. Plants that hyperaccumulate heavy metals. Wallingford, CAB International., 384.

Francesconi, K., Visoottiviseth, P., Sridokchan, W., and Goessler, W. 2002. Arsenic species in an arsenic hyperaccumulating fern, Pityrogramma calomelanos: a potential phytoremediator of arsenic-contaminated soils. Sci. Total Environ. 284,
27-35.

Heaton, A.C.P., Rugh, C.L., Wang, N., and Meagher, R.B. 1998. Phytoremediation of mercury - and methylmercury - polluted soils using genetically engineered plants. J. Soil Contam. 7, 497-510.

Hokura, A., Onuma, R., Terada, Y., Kitajima, N., Abe, T., Saito, H., Yoshida, S., and Nakai, I. 2006. Arsenic distribution and speciation in an arsenic hyperaccumulator fern by X-ray spectrometry utilizing a synchrotron radiation source. J. Anal. Atom. Spectr. 21, 321-328.

Huang, Z-C., Chen, T-B., Lei, M., and Hum T-D. 2004. Direct determination of arsenic species in arsenic hyperaccumulator *Pteris vittata* by EXAFS. Acta Botan. Sin. 46, 46-50.

Kaimidate, Y., Yamada, M., Furusho, Y., Kuroiwa,T., Kaise, T., and Fujiwara, K. 2000. A rapid method for the detection of arsenic compounds in biological samples with semimicro column and an inductively coupled plasma mass spectrometer. Biomed. Res. Trace Elem. 11, 445-446 (Japanese with English abstract).

Lewis, B.G., Johnson, C.M., and Delwiche, C.C. 1966. Release of volatile selenium compounds by plants: collection procedures and preliminary observations. J. Agri. Food Chem. 14, 638- 640.

Lombi, E., Zhao, F.J., Fuhrmann, M., Ma, L.Q., and McGrath S.P. 2002. Arsenic distribution and speciation in the fronds of the hyperaccumulator Pteris vittata. New Phytrogist, 156, 195-203.

Ma, L.Q., Komar, K.M., Tu, U.C., Zhang, W., Cai, Y., and Kennelley, E.D. 2001. A fern that hyperaccumulates arsenic. Nature 409, 579.

Mcgrath, S.P. 1998. Phytoextraction for soil remediation. In: Plants that hyperaccumulate heavy metals: their role in phytoremediation, microbiology, archaeology, mineral exploration and phytomining, pp. 261-288 (Brooks, R.R., Ed.), New York, CAB International.

McGrath, S.P., Zhao, F.J., and Lombi, E. 2002. Phytoremediation of metals, metalloids, and radionuclides. Adv. Agron. 75, 1-56.

Rugh, C.L., Wilde, H.D., Stacks, N.M., Thompson, D.M., Summers, A.O., and Meagher, R.B. 1996. Mercuric ion reduction and resistance in transgenic Arabidopsis thaliana plants expressing a modified bacterial merA gene. Proc. Natl. Acad. Sci. USA. 93, 3182-3187.

Rugh, C.L., Gragson, G.M., Meagher, R.B., and Merkle, S.A. 1998. Toxic mercury reduction and remediation using transgenic plants with a modified bacterial gene. Hortsci. 33, 618-621.

Sakakibara, M., Takagi, K., Inoue, M., Kubota, R., Hori, R.S., and Sano, S. 2004. Preliminary experiments on phytoremediation by Pteris vittata L. of altered including arsenopyrite. Proc. 14th Symp. Geo-Environ. Geo-Tech. 11-20
(Japanese with English abstract).

Sano, S., Sakakibara. S., and Watanabe, A. 2005. Quantitative analyses of arsenic in plants by instrumental neutron activation analyses and inductively coupled plasma-mass spectrometry. Mem. Fac. Sci. Ehime Univ., 11, 1-6 (Japanese with English abstract).

Suszcynsky, E.M. and Shann, J.R. 1995. Phytotoxicity and accumulation of mercury subjected to different exposure routes. Environ. Toxic. Chem. 14, 61-67.

Terry, N., Carlson, C., Raab, T.K., and Zayed, A. 1992. Rates of selenium volatilization among crop species. J. Environ. Qual. 21, 341-344.

Tu, C., Ma, L.Q. and Bondada, B. 2002. Arsenic accumulation in the hyper-accumulator Chinese Brake and its utilization potential for phytoremediation. J. Environ. Qual., 31, 1671-1675.

Wang, J., Zhao, F.J., Meharg, A.A., Raab, A., Feldmann, J., and McGrath, S.P. 2002. Mechanisms of Arsenic Hyperaccumulation in *Pteris vittata*. Uptake Kinetics, Interactions with Phosphate, and Arsenic Speciation. Plant. Physiol. 130, 1552-1561.

Watanabe, A., Sakakibara, M., Sano, S., Kondo T., Inoue, M., Hori, R. S., Suzuki, T., and Takehana, D. 2005. Greenhouse pot experiment of phytoremediation of mudstone leaching arsenic by *Pteris vittata* L. Mem. Fac. Sci. Ehime Univ. 11, 13-18 (Japanese with English abstract).

Watanabe, M.E. 1997. Phytoremediation on the brink of commercialization. Environ. Sci. Techn. 31, 182-186.

Wilber, C.G., 1980, Toxicology of selenium: a review. Clin. Toxicol. 17, 171-230.