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# Arsenic removal from As-hyperaccumulator *Pteris vittata* biomass: Coupling extraction with precipitation

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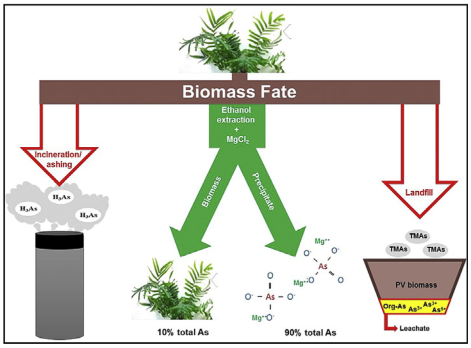
## HIGHLIGHTS

- Extraction was coupled with precipitation to remove As from *P. vittata* biomass.
- Optimized extraction was based on ethanol/water with particle size <1 mm for 2 h.
- The method extracted ~90% As from *P. vittata* biomass.
- Magnesium was added to precipitate soluble As from *P. vittata* to form $Mg_3(AsO_4)_2$.
- The optimized precipitation removed ~98% of soluble As.

## GRAPHICAL ABSTRACT



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## ABSTRACT

Proper disposal of As-hyperaccumulator *Pteris vittata* biomass (Chinese brake fern) enhances its application in phytoremediation. The goal of this study was to optimize As removal from *P. vittata* (PV) biomass by testing different particle sizes, extractants, extraction times and solid-to-liquid ratios. PV biomass was extracted using different extractants followed by different Mg-salts to recover soluble As via precipitation. Water-soluble As in PV biomass varied from 6.8% to 61% of total As depending on extraction time, with 99% of As being arsenate (AsV). Extraction with 2.1% HCl, 2.1% $H_3PO_4$, 1 M NaOH and 50% ethanol recovered 81, 78, 47 and 14% of As from PV biomass. A follow-up extraction using HCl recovered 27–32% with ethanol recovering only 5%. Though ethanol showed the lowest extractable As, residual As in the biomass was also the lowest. Among the extractants, 35% ethanol was the best to remove As from PV biomass. Approximately 90% As was removed from PV biomass using particle size <1 mm at solid:liquid ratio 1:50 and pH 6 for 2 h. Adding $MgCl_2$ at As:Mg ratio of 1:400 with pH 9.5 was effective to precipitate soluble As, resulting in 98% removal. Effective removal of As from PV biomass prior to disposal helps make phytoremediation more feasible.

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## 1. Introduction

Arsenic (As) is naturally present in low concentrations in soils, ranging from 0.1 to 67 mg $kg^{-1}$ (Mandal and Suzuki, 2002).

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Anthropogenic activities such as the use of pesticides and fertilizers, mining activity, coal combustion, and wood treatment have all contributed to As elevation in soils, increasing its risk to humans (Gress et al., 2016; Roychowdhury et al., 2002). It is estimated that ~36 million people worldwide live in As-contaminated areas, making it the #1 hazardous substance on the USEPA priority list (ATSDR, 2007). Arsenic toxicity in soils depends on its oxidation state, redox potential and pH, with Fe/Al (hydr)oxides controlling As availability in soils (Walthan and Eick, 2004).

Human exposure to arsenic occurs via many pathways, with consumption of contaminated food and water being the most important (Gress et al., 2016). Arsenic exposure may cause various cancers. Generally, As toxicity decreases following the order of arsenite (AsIII) > arsenate (AsV) > monomethylarsonic acid (MMA) > dimethylarsinic acid (DMA) (ATSDR, 2007). In addition, plant exposure to arsenic can reduce its biomass production. Plants have developed strategies to tolerate As by accumulating it in the roots (Chen et al., 2017; Lessl et al., 2014), or changing phosphate transporters to reduce its uptake (El-Zohri et al., 2015; Wang et al., 2015). However, some plants can accumulate high As concentrations, making them hyperaccumulators (Ma et al., 2001).

Phytoremediation is a low-cost technology that utilizes hyperaccumulator plants to extract metals from soil. *Pteris vittata* (PV; Chinese brake fern) is the first known As-hyperaccumulator and it can accumulate up to 23,000 mg $kg^{-1}$ As in the fronds (Tu and Ma, 2002). The advantages of phytoremediation include minimum disturbance of the area, low environmental impact and favorable aesthetics (Nedelkoska and Doran, 2000). The efficiency of phytoremediation is affected by soil properties, metal bioavailability, and the plants' ability to accumulate metals (Krämer, 2005).

Proper husbandry practices enhance PV's effectiveness to remediate As-contaminated sites (Kertulis-Tartar et al., 2006; Lessl and Ma, 2013). *P. vittata* takes up As and rapidly translocates it into the fronds, the main site of As accumulation (Danh et al., 2014; Yang et al., 2007). Though most of the As in the fronds is present as AsIII, it is oxidized to AsV in dry biomass (Tu et al., 2003). The disposal of PV biomass can be a drawback for its application in phytoremediation. Usually, the biomass is either disposed at regulated landfills or incinerated (Chaney et al., 2007). Other methods include compaction, pyrolysis, ashing, and liquid extraction; however, they require specialized equipment and transport of large amounts of biomass. Therefore, an effective As recovery method is needed.

Various chemicals including acid, base and chelate have been used to recover As, with HCl and NaOH being efficient in solubilizing As from plants at 90–92% (Alam et al., 2001; Jang et al., 2007; Sullivan et al., 2003). In addition, methanol and ethanol have been used to extract As from plant biomass for speciation (Amaral et al., 2013; Zhang et al., 2002). Zhao et al. (2015) showed that 1:3 ethanol:water was effective to recover ~80% of As from PV fronds. Besides extraction, As can be precipitated as $Mg_3(AsO_4)_2$ (Park et al., 2010), which can be used to separate As from the solution.

The goal of this study was to optimize As removal from PV biomass. The specific objectives were: 1) to optimize As removal from PV biomass by testing different extractants, extraction times, particle sizes and pH; and 2) to recover soluble As by precipitation with different Mg salts. Removing most of the As from PV biomass can help to improve its application in phytoremediation.

## 2. Materials and methods

### 2.1. Chemical reagents and PV biomass

All chemicals were of analytical grade or better. Nitric acid (trace metal grade), $H_2O_2$, NaOH, $H_3PO_4$, and ethanol were obtained from Fisher Scientific (Waltham, MA). The Sep-Pak AccellPlus QMA Plus Short cartridges were obtained from Waters Corporation (WAT020545, Milford, MA). Before use, all labware was washed and soaked in 1 M nitric acid for 24 h and rinsed several times with DI water.

*P. vittata* biomass was obtained from a long-term phytoremediation experiment with 126 mg $kg^{-1}$ As in As–contaminated soil (Lessl et al., 2014). PV fronds were harvested every six months, oven dried at 65 °C and shredded to <2 mm size. Concentrations of As and other elements in PV fronds are shown in Table 1 (dry weight).

### 2.2. Water-soluble As in PV biomass

To determine arsenic concentration, PV biomass was digested using $HNO_3/H_2O_2$ via USEPA Method 3050B on a hot block (Environmental Express, Ventura, CA). Briefly, 0.5 g of dry plant biomass was suspended in 15 mL 1:1 nitric acid and heated at 105 °C for 6 h. After cooling, 1 mL 30% $H_2O_2$ was added and digested for an additional 30 min before bringing samples to a 50 mL volume with DI water. Arsenic concentration was analyzed using inductively coupled plasma mass spectrometry (ICP-MS, Perkin-Elmer Corp., Norwalk, CT).

Water-soluble As in dry PV biomass was extracted using double-distilled water (pH ~6.5) at a solid:liquid ratio of 1:25 in 50 mL plastic bottles for 2, 4, 8, 12, 16 or 20 h. Half of the samples were shaken at 60 rpm in a rotary shaker and the other half were kept static. Speciation of water-soluble As was determined after centrifugation at 4200 rpm for 15 min and filtration with Whatman N. 42 filter paper using an As speciation cartridge (WAT020545, Waters Corporation, Milford, MA), which retains arsenate (Mathews et al., 2011). Total As and AsIII was determined using ICP-MS, with AsV being the difference between total As and AsIII.

### 2.3. Optimization of As extraction from PV biomass

Preliminary studies showed that 80 °C was most efficient to extract As from PV biomass (data not shown), so all experiments were conducted at 80 °C. Based on the literature (Alam et al., 2001; Jang et al., 2007; Sullivan et al., 2003), the following extractants were chosen: 2.1% HCl, 2.1% $H_3PO_4$, 1 M NaOH and 50% ethanol, which was followed by a second extraction using 2.1% HCl. To extract As, 1 g of PV dry biomass was placed in a 50 mL vial. After adding 25 mL of extractant, the samples were placed in a water bath at 80 °C for 15 h and supernatant was collected after centrifugation at 4200 rpm for 15 min and filtration with Whatman N. 42 filters. For the second extraction, after adding 25 mL of HCl, the samples were placed in a water bath at 80 °C for 2 h. Arsenic concentration was determined using ICP-MS after centrifugation at

**Table 1**
Elemental concentrations in six month old PV frond biomass obtained from a long-term phytoremediation experiment of As–contaminated soil (n = 3).

| Element | Concentration |
|---|---|
| pH (0.01 M $CaCl_2$) | 5.14 |
|  | mg $kg^{-1}$ |
| As | 2665 ± 31 |
| Cu | 4.5 ± 0.2 |
| Mn | 67 ± 2.0 |
| Zn | 53 ± 2.0 |
| Fe | 69 ± 1.2 |
| P | 1193 ± 57 |
| Ca | 2235 ± 113 |
| Mg | 2630 ± 100 |
| K | 12,016 ± 827 |

4200 rpm for 15 min and filtration with Whatman N. 42 filters.

After finding that ethanol was the best extractant, efforts were made to optimize its efficiency. Different conditions were tested, including extraction time (0.5, 2, 8, 15 or 24 h), particle size (<1, <2 or >2 mm), solid:liquid ratio (S:L, 1:25, 1:50, 1:100, 1:200 or 1:300) and pH (6, 7 or 8). Extraction time was optimized using particle size <1 mm PV dry biomass and 35% ethanol at S:L 1:25 and pH 6. Similarly, particle size was determined based on optimal extraction time and 35% ethanol at S:L 1:25 and pH 6. Optimal S:L ratio was assessed using the optimized time and particle size, and 35% ethanol at pH 6. Finally, optimal pH was determined using the optimal time, particle size and S:L ratio, and 35% ethanol.

### 2.4. *Precipitation of water-soluble As from PV biomass*

During As extraction using ethanol solution, colloidal precipitate was formed, lowering As concentration in solution (data not shown). Ethanol has been used in chlorophyll extraction, which is denatured above 60 °C (Ritchie, 2006). Based on stoichiomotry, the precipitate was inferred to be magnesium arsenate [$Mg_3(AsO_4)_2$] (data not shown). To optimize $Mg_3(AsO_4)_2$ precipitation, we used different As:Mg ratios (1:3, 1:10, 1:50, 1:200 or 1:400), pH (7, 8, 8.5, 9 or 9.5) and different salts ($Mg(OH)_2$, $MgCl_2$, MgO, $MgCO_3$ and $MgSO_4$). The solution was centrifuged at 4200 rpm for 15 min and filtered with Whatman N. 42 filters. The As in solution was determined using ICP-MS. In addition, standard reference materials from the National Institute of Science and Technology (NIST 1547 — peach leaves, Gaithersburg, MD) and appropriate reagent blanks, internal standards and spikes were used as quality checks to ensure method accuracy and precision.

### 2.5. *Statistical analyses*

All data are presented as the mean of three replicates with standard deviation. Significant differences were determined by using one-way analysis of variance (ANOVA) and treatment means were compared by Tukey's multiple range tests at $p < 0.05$ using software (R 3.2.2) (Team, 2005).

## 3. Results and discussion

### 3.1. *Water-soluble As in PV biomass*

Arsenic and Mg concentrations in PV biomass were 2665 and 2630 mg $kg^{-1}$ (Table 1). Water-soluble As varied with extraction time (6.8–61%) with no difference between shaking versus no shaking treatments (Table 2 and Fig. 1), showing a potential for secondary contamination if the biomass is not properly handled. Arsenic in dry biomass consisted of ~99% AsV although AsIII is the primary form in fresh PV biomass (Duan et al., 2005; Zhang et al., 2002). As plants senesce, AsIII is oxidized to AsV (Chrobok et al., 2016; Tu et al., 2003). During extraction, solution pH increased with time (Table 2). As pH increases, organic compounds become more negatively charged, facilitating anion desorption (Carbonell-Barrachina et al., 1999). Besides, after 20-h extraction, solution pH at 6.8 was close to the arsenic acid $pK_{a2}$ of 6.96, favoring formation of $H_2AsO_4^-$ and $HAsO_4^{-2}$, thereby increasing As extraction.

**Table 2**
Water-soluble arsenic (%) and solution pH as a function of time and extraction method from PV frond biomass (shaking and no shaking) (n = 3).

| Time (h) | No shaking | pH | Shaking | pH |
|---|---|---|---|---|
| 2 | 6.8 ± 1.6 B | 4.9 ± 0.5 | 7.6 ± 0.5 b | 5.7 ± 0.2 |
| 4 | 6.1 ± 0.7 B | 5.9 ± 0.1 | 6.1 ± 0.2 b | 6.3 ± 0.0 |
| 8 | 5.3 ± 0.5 B | 6.4 ± 0.0 | 7.6 ± 0.9 b | 6.7 ± 0.2 |
| 12 | 7.4 ± 1.1 B | 6.3 ± 0.3 | 7.2 ± 0.4 b | 6.0 ± 0.1 |
| 16 | 7.4 ± 3.8 B | 6.1 ± 0.6 | 6.6 ± 0.3 b | 6.3 ± 0.3 |
| 20 | 60.8 ± 1.2 A | 6.8 ± 0.6 | 57.4 ± 1.8 a | 6.8 ± 0.1 |

Treatments followed by the same letters are not significantly different at $p < 0.05$.

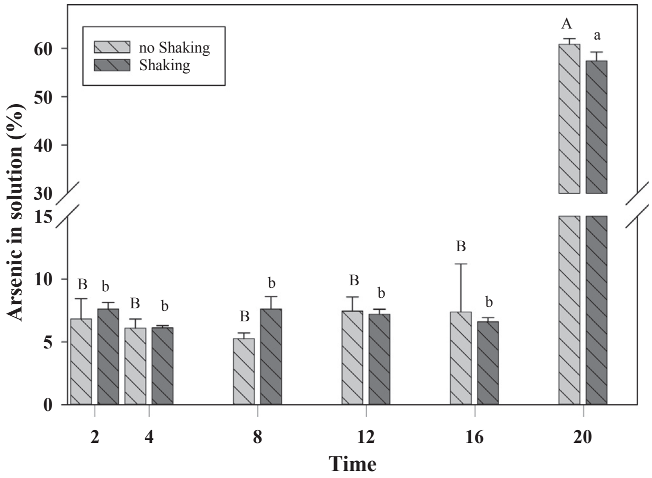

**Fig. 1.** Water-soluble arsenic from PV biomass as a function of time and extraction method (static or shaking at 60 rpm in a rotary shaker) at 1:25 solid:liquid ratio (n = 3). Treatments followed by the same letters are not significantly different at $p < 0.05$.

These results indicate a potential for secondary contamination if disposed in a landfill. Microbial activities in mildly alkaline and anaerobic conditions can reduce AsV to AsIII, further increasing its mobility (Kjeldsen et al., 2002). Addition of organic amendment rich in sulfur compounds and amorphous Fe oxide can be used to reduce As mobility (Carbonell-Barrachina et al., 1999; Dixit and Hering, 2003).

### 3.2. *Arsenic extraction from PV biomass*

Common extractants for As in plants include 2.1% HCl, 2.1% $H_3PO_4$, 1 M NaOH and 50% ethanol (Alam et al., 2001; Zhao et al., 2015). To determine the most efficient method, their extraction efficiency was tested (Fig. 2). During the first extraction, HCl and $H_3PO_4$ were most effective, with ethanol being least effective. The recoveries by HCl, $H_3PO_4$, NaOH and ethanol were 81, 78, 47 and 14%, respectively, with 5–32% being recovered in the second extraction by HCl. After two extractions, ethanol had the lowest recovery at 20%; however, it also showed the least As remaining in PV biomass. Low As recovery was probably attributed to As precipitation with Mg as $Mg_3(AsO_4)_2$ or absorption onto $Mg(OH)_2$ (Park et al., 2010).

Acid extraction is efficient and widely used for As speciation in plants at low As concentration (Narukawa and Chiba, 2010; Williams et al., 2005). However, difference in tissue matrices and As concentration impact its efficiency (Heitkemper et al., 2001). Extraction with $HNO_3$ at 90 °C recovered >90% of As from PV biomass (Zhao et al., 2015). However, some acids including HCl may interfere in As determination using ICP-MS (Cai et al., 2000).

Dilute $H_3PO_4$ is efficient in extracting As from organic compounds, sediments and soils (Bohari et al., 2002; Giacomino et al., 2010; Tokunaga and Hakuta, 2002). However, As extraction from plant material is variable with recovery ranging from 0 to 94% (Bohari et al., 2002; Foster et al., 2007; Kuehnelt et al., 2001). At 2.1%, $H_3PO_4$ was efficient in extracting 78% As from PV biomass (Fig. 2). However, it presents a drawback when recovering As from the solution by competing for the sorbent with much greater

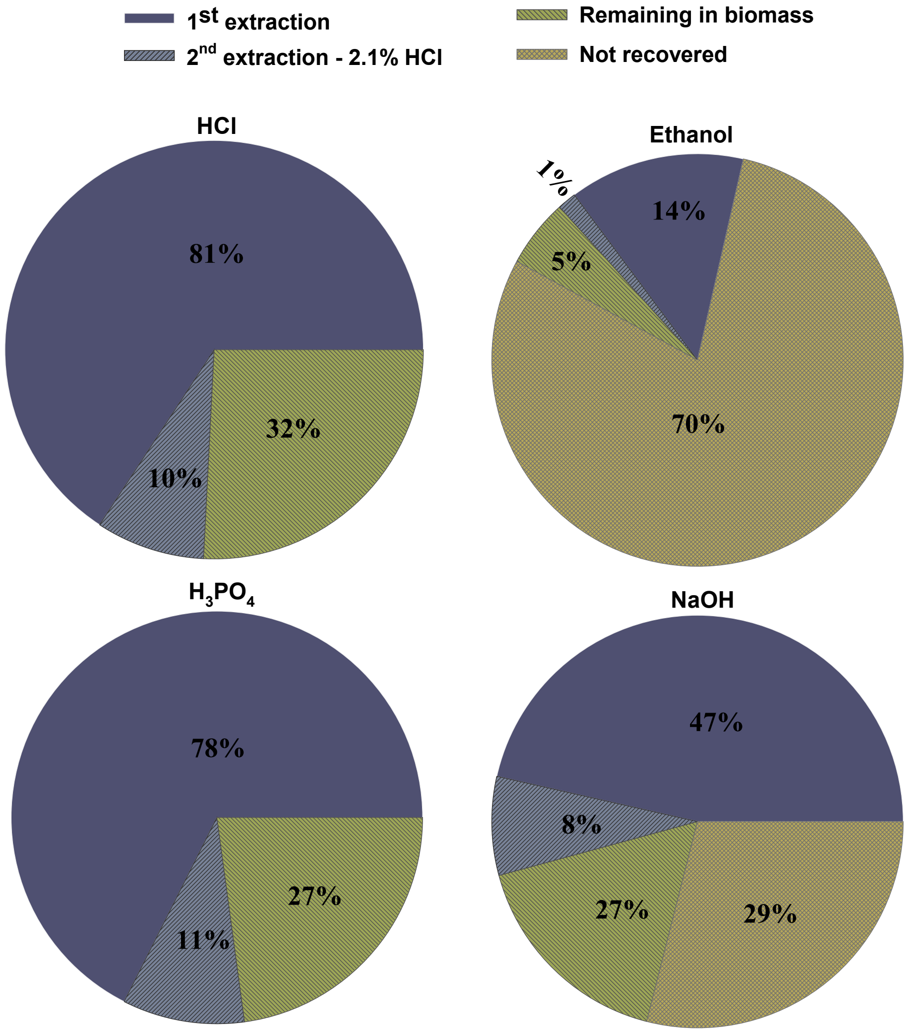

**Fig. 2.** Arsenic extraction from PV frond biomass using different extractants followed by HCl extraction (recovery of 123, 116, 82 and 20% for 2.1% HCl, 2.1% $H_3PO_4$, 1 M NaOH and 50% ethanol, respectively) (n = 3).

concentration than As. Dilute NaOH was also efficient in plant As extraction (He et al., 2002). NaOH removes As by breaking S—As bonds and by hydroxyl ion ligand replacement, with the high pH avoiding its readsorption (Bohari et al., 2002; Jang et al., 2005). However, NaOH was ineffective on PV biomass, extracting only 47%. This was probably due to As precipitation with Mg as $Mg_3(AsO_4)_2$ or absorption onto $Mg(OH)_2$ at pH > 12 (Fig. 2) (Park et al., 2010). Also, NaOH partially solubilized the PV biomass, making it difficult to separate biomass from solution and, thus, it is impractical.

Ethanol is a powerful and non-toxic extractant for As speciation (Amaral et al., 2013). Zhao et al. (2015) obtained satisfactory As recovery in PV biomass using ethanol coupled with sonication when compared to other methods including methanol. In this study, ethanol was least effective, with 20% recovery (Fig. 2). Interestingly, it also had the least residual As in the biomass, leaving 70% unknown. Upon reviewing the procedure, a colloidal precipitate was noticed in the vial bottom. Thus, low recovery was probably related to chlorophyllic Mg release into solution, resulting in formation of $Mg_3(AsO_4)_2$ at pH 7–10. Based on As removal at > 90% via spontaneous precipitation, ethanol was the best extractant.

### 3.3. Optimization of ethanol extraction from PV biomass

Based on a preliminary test, 35% ethanol was optimal after one extraction (data not shown), which was different from Zhao et al. (2015) who reported 25% ethanol. The use of sonication in their study compared to higher temperature in our study may explain the difference. Other factors include time, particle size and pH. Zhao et al. (2015) reported >90% As recovery after 0.5 h compared to 72% recovery in this study (Fig. 3a). The difference might be attributed to differences in As speciation in PV biomass. In Zhao et al. (2015), As was 93% AsIII in fresh PV biomass compared to 99% as AsV in dry PV biomass in this study. Compared to AsIII, AsV is more accumulated in cell walls, thus requiring more time to extract (Yuan et al., 2005; Zhao et al., 2015). In fact, after increasing extraction time to 2 h, no difference in As recovery compared to Zhao et al. (2015) was noticed (Fig. 3a). Therefore, 2 h was chosen as extract time. In terms of particle size, there was a difference with smaller particles resulting in greater recovery (Fig. 3b). This result was expected based on the larger surface area of smaller particles, which enhanced As extraction.

Similar to particle size, solid-to-liquid ratio affected As extraction with 1:50 ratio being most effective at 90–100% recovery (Fig. 3c). Zhao et al. (2015) used 1:300 S:L ratio, which recovered <90% As in this study. Given the large amount of biomass, lower S:L is better. Though it was expected that higher pH would result in higher As recovery, this was not the case (Fig. 3d). Hydroxyl ions can replace AsV at high pH, thereby increasing its extraction (Jang

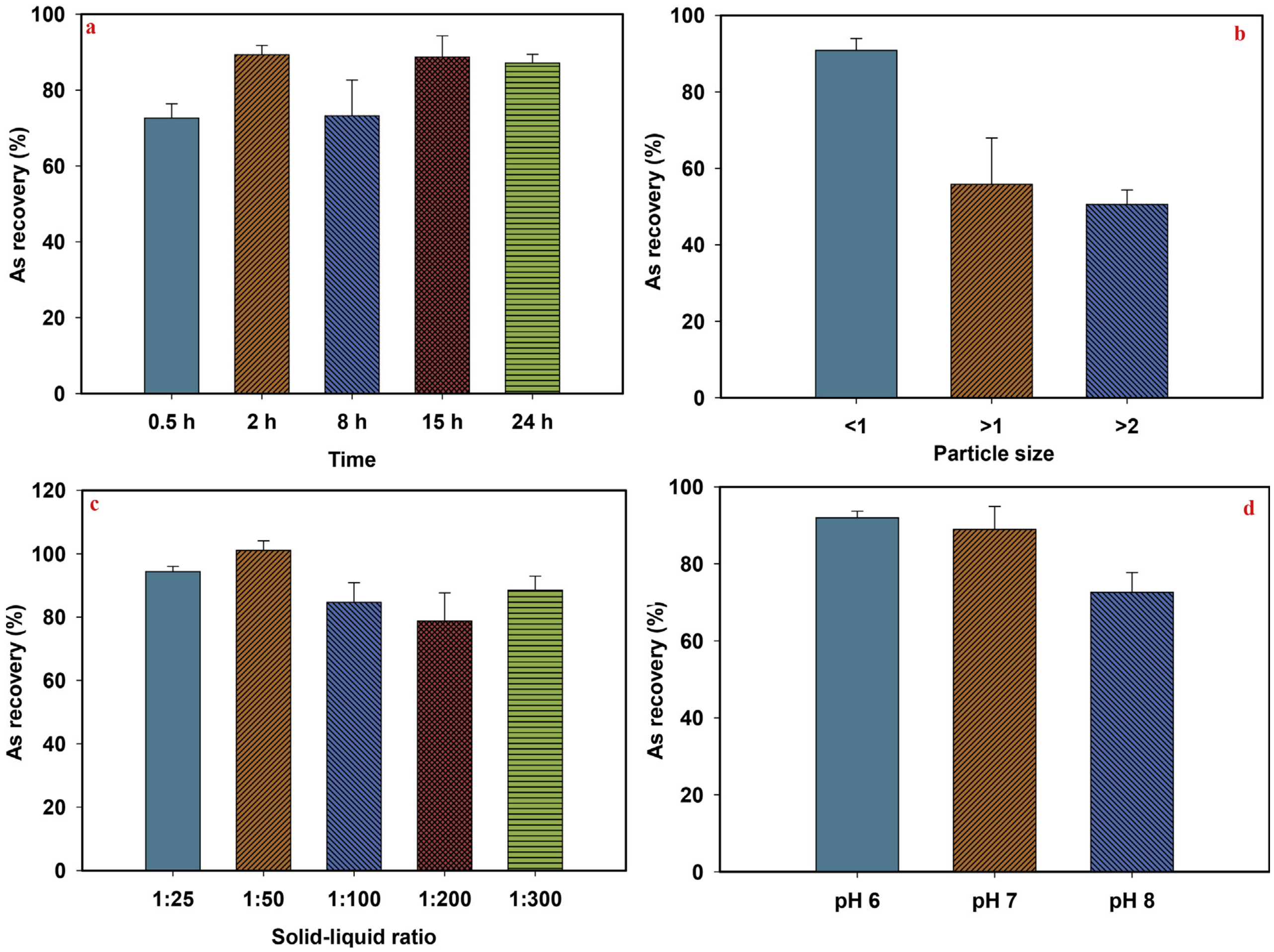

**Fig. 3.** Effect of time (a), particle size (b), solid-to-liquid ratio (c) and pH (d) on As extraction from *P. vittata* biomass using 35% ethanol. Time was tested using particle size <1 mm, S:L ratio 1:25 and pH 6. Particle size was tested using 2 h extraction, S:L ratio 1:25 and pH 6. Solid-to-liquid ratio was tested using 2 h extraction, particle size <1 mm and pH 6. pH was tested using 2 h extraction, particle size <1 mm and S:L ratio 1:50. (n = 3).

et al., 2005). It was possible that formation of $Mg_3(AsO_4)_2$ or absorption onto $Mg(OH)_2$ might explain the difference. Based on our data, the optimal As removal procedure (2 h, particle size < 1 mm, S:L of 1:50 and pH 6) produced satisfactory results, removing ~90% As from PV biomass.

### 3.4. *Precipitation of water-soluble As from PV biomass*

After extraction, recovered soluble As needs to be treated. Arsenic concentration in the ethanol extraction solution was 28.7 mg $L^{-1}$ (Table 3). Technologies used to remove As from effluents include adsorption, precipitation with Fe oxides, and electrocoagulation (Bissen and Frimmel, 2003; Sullivan et al., 2003). In addition, AsV can be precipitated with Mg, which presents low solubility (Magalhaes, 2002).

Hoernesite [$Mg_3(AsO_4)_2$], which has $K_{sp}$ of $10^{-30.32}$ and forms in pH range of 7–10, has been widely studied (Raposo et al., 2004; Zhu et al., 2005). However, its application in As removal is limited. Under alkaline conditions at stoichiometric As:Mg ratio > 0.5, precipitation of $Mg_3(AsO_4)_2$ may occur (Park et al., 2010). In fact, spontaneous precipitation was observed at effluent pH > 7 and As:Mg ratio of 1:1.9 in our study, decreasing As concentration. Thus, this process was optimized using different Mg salts.

There was no difference among Mg salts even though they have different solubility constants ($5.61 \times 10^{-12}$, $2.37 \times 10^{-8}$ and $6.82 \times 10^{-6}$ for $Mg(OH)_2$, MgO and $MgCO_3$, respectively; Haynes, 2014) (Fig. 4a). One advantage of using $Mg(OH)_2$ or $MgCO_3$ was the potential to increase pH by 0.7 and 1.9 units, respectively. However, their low solubility requires addition of large amounts to be effective. Therefore, $MgCl_2$ was chosen due to its high solubility ($k_{sp}$ 738) and low cost.

Arsenic concentration was reduced from 28.7 mg $L^{-1}$ to <2 mg $L^{-1}$ at pH 9 with As:Mg ratio of 1:400 (Fig. 4b). Speciation modeling predicted As concentration of 0.8 mg $L^{-1}$ at pH 9.5 and As:Mg ratio of 1:3 (Park et al., 2010). In this study, As:Mg ratio at 1:3 decreased As concentration by ~35%. This difference might be

**Table 3**
Characterization of ethanol extraction effluent (n = 3).

| pH | As | Mg | P | Ca | Fe | As:Mg molar ratio |
|---|---|---|---|---|---|---|
| mg $L^{-1}$ | | | | | | |
| 5.51 ± 0.02 | 28.7 ± 3.7 | 18.0 ± 0.27 | 9.46 ± 1.4 | 8.24 ± 0.14 | 0.04 ± 0.01 | 1:1.9 |

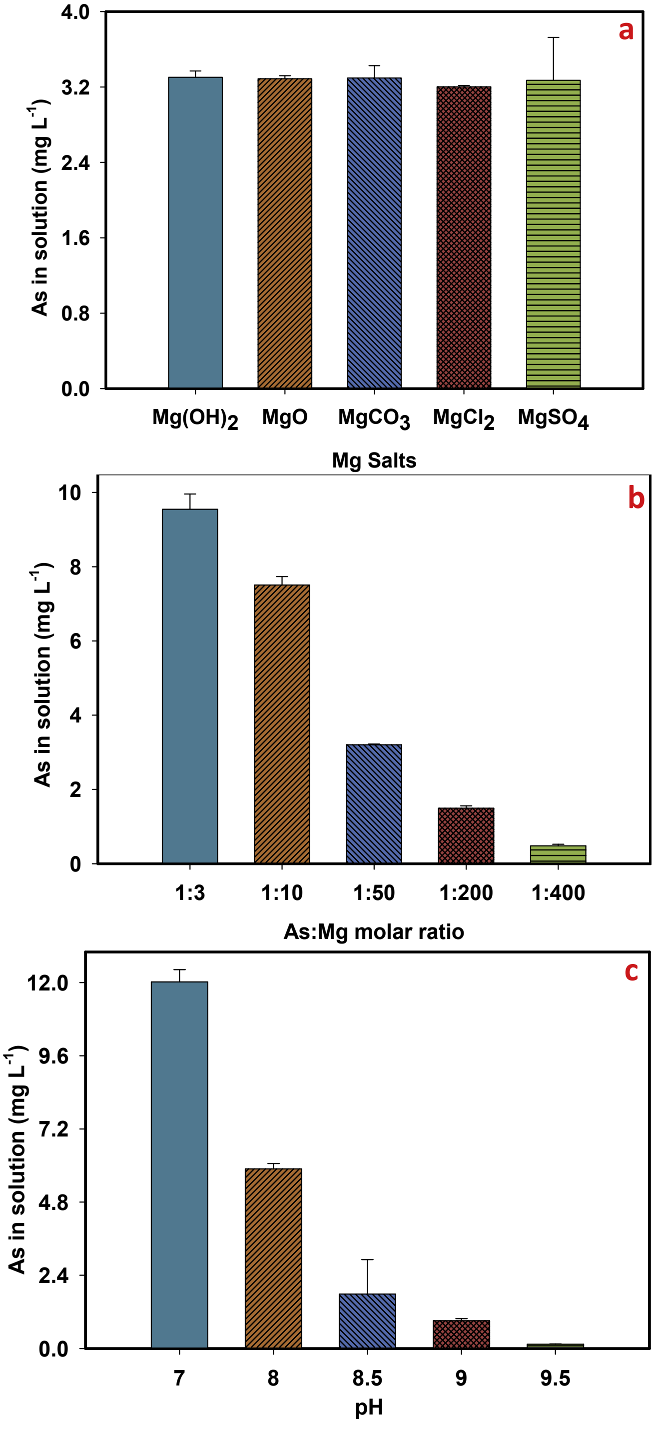

**Fig. 4.** Effect of Mg salts (a), As:Mg ratio (b) and pH (c) in Mg–As precipitation (n = 3). Effect of Mg salts was tested using As:Mg ratio of 1:50 and pH 9. As:Mg ratio was tested using $MgCl_2$ and pH 9. Optimal pH was tested using $MgCl_2$ and As:Mg ratio of 1:400.

explained by As competition with phosphate ions ($HPO_4^{-2}$) and the presence of organic ligands. Phosphate and arsenate are analogues so phosphate presence decreases the effectiveness of AsV removal. Besides, precipitation of $Mg_3(PO_4)_2$ may occur at pH 6–10 and P:Mg ratio of 3:2 (Tamimi et al., 2011).

Among all factors, pH was the master variable in As removal, with the optimal pH being 9.5 (Fig. 4c), which is similar to Park et al. (2010). As pH increased, soluble As concentration decreased from 12 mg $L^{-1}$ to 0.4 mg $L^{-1}$. Besides, at pH > 9.5, $Mg(OH)_2$ can also precipitate (Tabelin et al., 2013), which has positive charge to sorb oxyanions such as AsV. However, at pH > 11, As was again soluble due to higher stability of $Mg(OH)_2$ (Park et al., 2010).

Addition of $MgCl_2$ was efficient to remove As from ethanol extraction solution from PV biomass. The precipitated $Mg_3(AsO_4)_2$ can be reused or sent for waste disposal, but in much smaller quantity. Further, after removing As precipitate, pH can be increased to >11 to precipitate Mg as $Mg(OH)_2$, allowing it to be reused in As removal after acid dissolution.

## 4. Conclusion

An alternative method to remove As from As-laden PV biomass was assessed using 35% ethanol extraction followed by As precipitation as $Mg_3(AsO_4)_2$. Optimal ethanol extraction was achieved using 2 h, particle size <1 mm, S:L ratio 1:50 at pH 6. After extraction, As removal from the solution was achieved using $MgCl_2$ at As:Mg ratio of 1:400 and pH 9.5. Approximately 60% As from dry frond biomass of *P. vittata* was water soluble, with 99% As being AsV. Ethanol was effective in As removal (>90%) followed by spontaneous precipitation. $MgCl_2$ addition in the effluent decreased As concentration from 28.7 mg $L^{-1}$ to ~0.4 mg $L^{-1}$.

Ethanol extraction coupled with $Mg_3(AsO_4)_2$ precipitation was fast and efficient to recover As from As-laden PV biomass. However, for large-scale application, the remaining 10% As in biomass still can pose risk. Thus, further research is being conducted including anaerobic decomposition of the low-As biomass with potential methane production and recovery of the remaining As.

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## References

Alam, M.G.M., Tokunaga, S., Maekawa, T., 2001. Extraction of arsenic in a synthetic arsenic-contaminated soil using phosphate. Chemosphere 43, 1035–1041.

Amaral, C.D.B., Nóbrega, J.A., Nogueira, A.R.A., 2013. Sample preparation for arsenic speciation in terrestrial plants—a review. Talanta 115, 291–299.

ATSDR, 2007. Toxicological Profile for Arsenic. Agency Toxic Subst. Dis. Regist. Div. Toxicol., Atlanta, GA.

Bissen, M., Frimmel, F.H., 2003. Arsenic—a review. Part II: oxidation of arsenic and its removal in water treatment. CLEAN – Soil Air Water 31, 97–107.

Bohari, Y., Lobos, G., Pinochet, H., Pannier, F., Astruc, A., Potin-Gautier, M., 2002. Speciation of arsenic in plants by HPLC-HG-AFS: extraction optimisation on CRM materials and application to cultivated samples. J. Environ. Monit. 4, 596–602.

Cai, Y., Georgiadis, M., Fourqurean, J.W., 2000. Determination of arsenic in seagrass using inductively coupled plasma mass spectrometry. Spectrochim. Acta Part B At. Spectrosc. 55, 1411–1422.

Carbonell-Barrachina, Á.A., Burló, F., Valero, D., López, E., Martinez-Romero, D., Martínez-Sánchez, F., 1999. Arsenic toxicity and accumulation in turnip as affected by arsenic chemical speciation. J. Agric. Food Chem. 47, 2288–2294.

Chaney, R.L., Angle, J.S., Broadhurst, C.L., Peters, C.A., Tappero, R.V., Sparks, D.L., 2007. Improved understanding of hyperaccumulation yields commercial phytoextraction and phytomining technologies. J. Environ. Qual. 36, 1429–1443. https://doi.org/10.2134/jeq2006.0514.

Chen, Y., Han, Y.-H., Cao, Y., Zhu, Y.-G., Rathinasabapathi, B., Ma, L., 2017. Arsenic transport in rice and biological solutions to reduce arsenic risk from rice. Front. Plant Sci. https://doi.org/10.3389/FPLS.2017.00268. Feb.

Chrobok, D., Law, S.R., Brouwer, B., Lindén, P., Ziolkowska, A., Liebsch, D., Narsai, R., Szal, B., Moritz, T., Rouhier, N., others, 2016. Dissecting the metabolic role of mitochondria during developmental leaf senescence. Plant Physiol. 01463.

Danh, L.T., Truong, P., Mammucari, R., Foster, N., 2014. A critical review of the arsenic uptake mechanisms and phytoremediation potential of Pteris vittata. Int. J. Phytoremediation 16, 429–453.

Dixit, S., Hering, J.G., 2003. Comparison of arsenic (V) and arsenic (III) sorption onto iron oxide minerals: implications for arsenic mobility. Environ. Sci. Technol. 37, 4182–4189.

Duan, G.-L., Zhu, Y.-G., Tong, Y.-P., Cai, C., Kneer, R., 2005. Characterization of arsenate reductase in the extract of roots and fronds of Chinese brake fern, an arsenic hyperaccumulator. Plant Physiol. 138, 461–469.

El-Zohri, M., Odjegba, V., Ma, L., Rathinasabapathi, B., 2015. Sulfate influx transporters in Arabidopsis thaliana are not involved in arsenate uptake but critical for tissue nutrient status and arsenate tolerance. Planta 241, 1109–1118. https://doi.org/10.1007/s00425-015-2241-4.

Foster, S., Maher, W., Krikowa, F., Apte, S., 2007. A microwave-assisted sequential extraction of water and dilute acid soluble arsenic species from marine plant and animal tissues. Talanta 71, 537–549.

Giacomino, A., Malandrino, M., Abollino, O., Velayutham, M., Chinnathangavel, T., Mentasti, E., 2010. An approach for arsenic in a contaminated soil: speciation, fractionation, extraction and effluent decontamination. Environ. Pollut. 158, 416–423.

Gress, J., da Silva, E.B., de Oliveira, L.M., Zhao, D., Anderson, G., Heard, D., Stuchal, L.D., Ma, L.Q., 2016. Potential arsenic exposures in 25 species of zoo animals living in CCA-wood enclosures. Sci. Total Environ. 551–552, 614–621. https://doi.org/10.1016/j.scitotenv.2016.02.065.

Haynes, W.M., 2014. CRC Handbook of Chemistry and Physics. CRC Press.

He, B., Fang, Y., Jiang, G., Ni, Z., 2002. Optimization of the extraction for the determination of arsenic species in plant materials by high-performance liquid chromatography coupled with hydride generation atomic fluorescence spectrometry. Spectrochim. Acta Part B At. Spectrosc. 57, 1705–1711. https://doi.org/10.1016/S0584-8547(02)00167-2.

Heitkemper, D.T., Vela, N.P., Stewart, K.R., Westphal, C.S., 2001. Determination of total and speciated arsenic in rice by ion chromatography and inductively coupled plasma mass spectrometry. J. Anal. At. Spectrom. 16, 299–306.

Jang, M., Hwang, J.S., Choi, S.I., 2007. Sequential soil washing techniques using hydrochloric acid and sodium hydroxide for remediating arsenic-contaminated soils in abandoned iron-ore mines. Chemosphere 66, 8–17.

Jang, M., Hwang, J.S., Choi, S.I., Park, J.K., 2005. Remediation of arsenic-contaminated soils and washing effluents. Chemosphere 60, 344–354.

Kertulis-Tartar, G.M., Ma, L.Q., Tu, C., Chirenje, T., 2006. Phytoremediation of an arsenic-contaminated site using Pteris vittata L.: a two-year study. Int. J. Phytoremediation 8, 311–322.

Kjeldsen, P., Barlaz, M.A., Rooker, A.P., Baun, A., Ledin, A., Christensen, T.H., 2002. Present and long-term composition of MSW landfill leachate: a review. Crit. Rev. Environ. Sci. Technol. 32, 297–336.

Krämer, U., 2005. Phytoremediation: novel approaches to cleaning up polluted soils. Curr. Opin. Biotechnol. 16, 133–141.

Kuehnelt, D., Irgolic, K.J., Goessler, W., 2001. Comparison of three methods for the extraction of arsenic compounds from the NRCC standard reference material DORM-2 and the brown alga Hijiki fuziforme. Appl. Organomet. Chem. 15, 445–456.

Lessl, J.T., Luo, J., Ma, L.Q., 2014. Pteris vittata continuously removed arsenic from non-labile fraction in three contaminated-soils during 3.5 years of phytoextraction. J. Hazard. Mater. 279, 485–492. https://doi.org/10.1016/j.jhazmat.2014.06.056.

Lessl, J.T., Ma, L.Q., 2013. Sparingly-soluble phosphate rock induced significant plant growth and arsenic uptake by Pteris vittata from three contaminated soils. Environ. Sci. Technol. 47, 5311–5318.

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

Magalhaes, M., 2002. Arsenic. An environmental problem limited by solubility. Pure Appl. Chem. 74, 1843–1850.

Mandal, B.K., Suzuki, K.T., 2002. Arsenic round the world: a review. Talanta 58, 201–235.

Mathews, S., Rathinasabapathi, B., Ma, L.Q., 2011. Uptake and translocation of arsenite by Pteris vittata L.: effects of glycerol, antimonite and silver. Environ. Pollut. 159, 3490–3495. https://doi.org/10.1016/j.envpol.2011.08.027.

Narukawa, T., Chiba, K., 2010. Heat-assisted aqueous extraction of rice flour for arsenic speciation analysis. J. Agric. Food Chem. 58, 8183–8188.

Nedelkoska, T.V., Doran, P.M., 2000. Characteristics of heavy metal uptake by plant species with potential for phytoremediation and phytomining. Miner. Eng. 13, 549–561.

Park, Y.Y., Tran, T., Lee, Y.H., Nam, Y.I., Senanayake, G., Kim, M.J., 2010. Selective removal of arsenic (V) from a molybdate plant liquor by precipitation of magnesium arsenate. Hydrometallurgy 104, 290–297.

Raposo, J.C., Zuloaga, O., Olazabal, M.A., Madariaga, J.M., 2004. Study of the precipitation equilibria of arsenate anion with calcium and magnesium in sodium perchlorate at 25 C. Appl. Geochem. 19, 855–862.

Ritchie, R.J., 2006. Consistent sets of spectrophotometric chlorophyll equations for acetone, methanol and ethanol solvents. Photosynth. Res. 89, 27–41.

Roychowdhury, T., Uchino, T., Tokunaga, H., Ando, M., 2002. Arsenic and other heavy metals in soils from an arsenic-affected area of West Bengal, India. Chemosphere 49, 605–618.

Sullivan, E.J., Bowman, R.S., Legiec, I.A., 2003. Sorption of arsenic from soil-washing leachate by surfactant-modified zeolite. J. Environ. Qual. 32, 2387–2391.

Tabelin, C.B., Igarashi, T., Yoneda, T., Tamamura, S., 2013. Utilization of natural and artificial adsorbents in the mitigation of arsenic leached from hydrothermally altered rock. Eng. Geol. 156, 58–67.

Tamimi, F., Le Nihouannen, D., Bassett, D.C., Ibasco, S., Gbureck, U., Knowles, J., Wright, A., Flynn, A., Komarova, S.V., Barralet, J.E., 2011. Biocompatibility of magnesium phosphate minerals and their stability under physiological conditions. Acta Biomater. 7, 2678–2685.

Team, R.D.C, 2005. Development Core Team. 2005. R: a Language and Environment for Statistical Computing. The R Foundation for Statistical Computing, Vienna, Austria.

Tokunaga, S., Hakuta, T., 2002. Acid washing and stabilization of an artificial arsenic-contaminated soil. Chemosphere 46, 31–38.

Tu, C., Ma, L.Q., 2002. Effects of arsenic concentrations and forms on arsenic uptake by the hyperaccumulator ladder brake. J. Environ. Qual. 31, 641–647.

Tu, C., Ma, L.Q., Zhang, W., Cai, Y., Harris, W.G., 2003. Arsenic species and leachability in the fronds of the hyperaccumulator Chinese brake (Pteris vittata L.). Environ. Pollut. 124, 223–230. https://doi.org/10.1016/S0269-7491(02)00470-0.

Walthan, A.C., Eick, M.J., 2004. Kinetics of chromate adsorption on goethite in the presence of sorbed silicic acid. J. Environ. Qual. 33, 1703–1708. https://doi.org/10.2136/sssaj2002.0818.

Wang, X., Peng, B., Tan, C., Ma, L., Rathinasabapathi, B., 2015. Recent advances in arsenic bioavailability, transport, and speciation in rice. Environ. Sci. Pollut. Res. 22, 5742–5750. https://doi.org/10.1007/s11356-014-4065-3.

Williams, P.N., Price, A.H., Raab, A., Hossain, S.A., Feldmann, J., Meharg, A.A., 2005. Variation in arsenic speciation and concentration in paddy rice related to dietary exposure. Environ. Sci. Technol. 39, 5531–5540.

Yang, X., Chen, H., Xu, W., He, Z., Ma, M., 2007. Hyperaccumulation of arsenic by callus, sporophytes and gametophytes of Pteris vittata cultured in vitro. Plant Cell Rep. 26, 1889–1897.

Yuan, C., Jiang, G., He, B., 2005. Evaluation of the extraction methods for arsenic speciation in rice straw, Oryza sativa L., and analysis by HPLC-HG-AFS. J. Anal. At. Spectrom. 20, 103–110.

Zhang, W., Cai, Y., Tu, C., Ma, L.Q., 2002. Arsenic speciation and distribution in an arsenic hyperaccumulating plant. Sci. Total Environ. 300, 167–177.

Zhao, D.I., Li, H.-B., Xu, J.-Y., Luo, J., Ma, L.Q., 2015. Arsenic extraction and speciation in plants: method comparison and development. Sci. Total Environ. 523, 138–145.

Zhu, Y., Zhang, X., Xie, Q., Chen, Y., Wang, D., Liang, Y., Lu, J., 2005. Solubility and stability of barium arsenate and barium hydrogen arsenate at 25 C. J. Hazard. Mater. 120, 37–44.