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Arsenic extraction and speciation in plants: Method comparison and development

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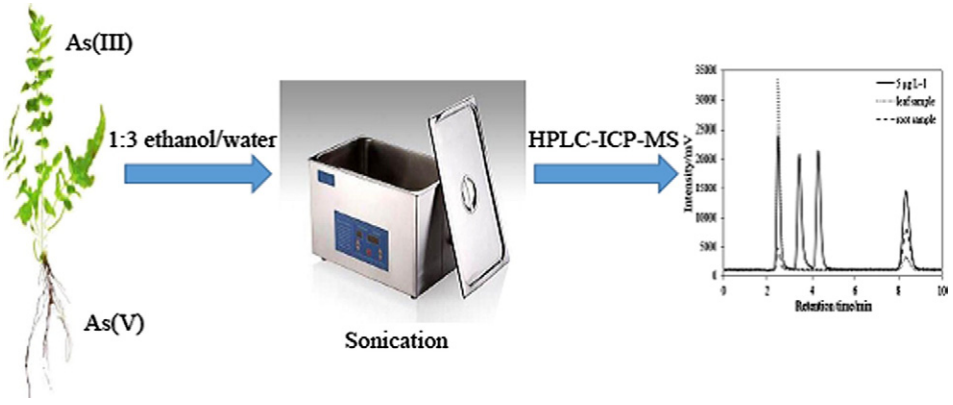
HIGHLIGHTS

- An optimized extraction method for As speciation in plants based on three different plants and four different methods was developed.
- The optimized method was based on ethanol/water extraction and used 50% less ethanol and 38% less time.
- The optimized method produced satisfactory extraction efficiency (~80% for the roots and >85% for the fronds).
- The optimized method has the potential to be used on other plant samples for As speciation.

GRAPHICAL ABSTRACT



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ABSTRACT

We compared four methods to extract arsenic (As) from three different plants containing different As levels for As speciation with the goal of developing a more efficient method, i.e., As-hyperaccumulator *Pteris vittata* at 459–7714 mg kg^{-1}, rice seedling at 53.4–574 mg kg^{-1}, and tobacco leaf at 0.32–0.35 mg kg^{-1}. The four methods included heating with dilute HNO_3, and sonication with phosphate buffered solution, methanol/water, and ethanol/water, with As being analyzed using high-performance liquid chromatography coupled with inductively-coupled plasma mass spectrometry (HPLC–ICP-MS). Among the four methods, the ethanol/water method produced the most satisfactory extraction efficiency (~80% for the roots and >85% for the fronds) without changing As species based on *P. vittata*. The lower extraction efficiency from *P. vittata* roots was attributed to its dominance by arsenate (82%) while arsenite dominated in the fronds (89%). The ethanol/water method used sample:solution ratio of 1:200 (0.05 g:10 mL) with 50% ethanol and 2 h sonication. Based on different extraction times (0.5–2 h), ethanol concentrations (25–100%) and sample:solution ratios (1:50–1:300), the optimized ethanol/water method used less ethanol (25%) and time (0.5 h for the fronds and 2 h for the roots). Satisfactory extraction was also obtained for tobacco leaf (78–92%) and rice seedlings (~70%) using the optimized method, which was better than the other three methods. Based on satisfactory extraction efficiency with little change in As species during extraction from three plants containing different As levels, the optimized method has the potential to be used for As speciation in other plants.

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1. Introduction

Arsenic (As) is a toxic pollutant in the environment, resulting from both natural and anthropogenic sources. Plants growing in As-contaminated soils accumulate As in their biomass. Arsenic in plants causes toxicity such as leaf chlorosis and necrosis, and reduces growth (Abedin et al., 2002; Caille et al., 2005). Although As in plants is mainly present as inorganic forms including arsenite (AsIII) and arsenate (AsV), small amounts of organic species including dimethylarsinic acid (DMA) and monomethylarsonic acid (MMA) have also been found (Jedynak et al., 2009; Bergqvist and Greger, 2012). Different As species in plants show different toxicity (B'Hymer and Caruso, 2004), so it is important to identify As species in plants to better understand their metabolism.

Pteris vittata (PV; Chinese brake fern) is the first-known As hyperaccumulator (Ma et al., 2001). It can accumulate up to 23,000 mg kg^{-1} As in its fronds when growing in an As-contaminated soil containing 1500 mg kg^{-1} As (Ma et al., 2001). Since the plant can effectively accumulate large amounts of As quickly, it has potential for phytoremediation of As-contaminated soils and waters (Cao et al., 2003; Tu et al., 2004). In PV, As is mainly present in inorganic forms, with AsIII dominating in the fronds and AsV in the roots (Ma et al., 2001). Understanding As speciation helps to better examine its uptake, transformation, and detoxification mechanisms in PV. However, the extraction efficiency for As speciation in PV is unsatisfactory, especially for the roots at ~60% (Zhang et al., 2002).

Analysis of As speciation in plants is challenging, as the procedure needs to extract all As from the matrix without altering their species (Burguera and Burguera, 1997). Various extraction methods have been developed for As speciation in plants (Amaral et al., 2013). Mild extractants including methanol, water, and methanol/water mixture have been used to extract As by shaking, sonication, or microwave (He et al., 2002; Ruiz-Chancho et al., 2008). For some plants, trifluoroacetic acid (TFA) is more acceptable than methanol. However, the method reduces 20% of AsV to AsIII in the plant (Abedin et al., 2002). Though HCl helps to solubilize As in plants by breaking up the bonds between AsIII and thiol groups (Muñoz et al., 1999), the method suffers from chlorine interference during As analysis using inductively coupled plasma-mass spectrometry (ICP–MS) (Heitkemper et al., 1989).

Many studies investigated As extraction methods in plants, but few focused on As hyperaccumulators. Zhang et al. (2002) developed a methanol/water method to extract As in PV, with recovery of 85–100% in the fronds and ~60% in the roots. The low As recovery in the roots is unsatisfactory so it is important to develop a more efficient method for As extraction in PV. Several extraction methods have been developed using certified reference materials, which are then applied to plant samples without further tests (Bohari et al., 2002; Raber et al., 2012). For example, Heitkemper et al. (2001) obtained 95–105% recovery for NIST standard reference material (SRM) 1568a rice flour based on methanol/water method, but only 24–36% was obtained for enriched long-grain white rice. This might be attributed to the different sample matrices between SRM and plant samples such as different plant species and the amounts of As present in the samples. Therefore, to develop a robust As extraction method for plant samples, the method should be tested using real plant samples in addition to SRM.

To develop a satisfactory As extraction method from plants, we compared four extraction procedures including sonication with phosphate buffered solution, heating with HNO_3, and sonication with methanol/water and ethanol/water mixture (Zhang et al., 2002; Su et al., 2008; Sun et al., 2008). The specific objectives of this research were to: (1) compare extraction efficiency of the four methods in extracting As from *P. vittata*; (2) develop an effective method to extract As from *P. vittata* roots by optimizing analysis parameters; and (3) test the developed method in other plant samples including tobacco leaf and rice seedling.

2. Materials and methods

2.1. Plant materials

Three plant materials were used in this experiment including the roots and fronds of As-hyperaccumulator *P. vittata* (PV), tobacco leaf, and rice seedlings. One batch of PV plants was obtained from Xu et al. (2014). The plants grew for 60 d in a soil spiked with 200 mg kg^{-1} AsIII ($NaAsO_2$) or AsV (Na_2HAsO_4 $7H_2O$), and a soil with no As. Another batch of PV plants was obtained after growing in 0.2-strength Hoagland nutrient solution containing 0, 1, or 10 mg L^{-1} of AsV for 8 d. Rice seedlings were obtained by growing in a nutrient solution containing 2.5 mg L^{-1} AsV (Na_2HAsO_4 $7H_2O$) for two weeks (Ren et al., 2014).

After harvest, all PV and rice seeding plants were washed thoroughly with Milli-Q water, separated into roots and fronds. Both PV and rice plants were freeze-dried for 48 h and ground into fine powder using liquid nitrogen. To compare the difference in sample preparation, part of a fresh PV plant was ground into fine powder using liquid nitrogen. In addition to fresh plants, dried tobacco leaves were also used, which were obtained from cigarettes purchased from a supermarket in Nanjing. The tobacco leaves and freeze-dried PV and rice seedling samples were passed through a 150 μm nylon sieve to obtain uniform size before extraction. For all speciation analysis, freeze-dried PV and rice samples were used unless specified otherwise.

2.2. Comparison of four extraction methods

Four common methods (Zhang et al., 2002; Su et al., 2008; Sun et al., 2008) were compared in extracting As from freeze-dried roots and fronds of PV after growing in a soil spiked with 200 mg kg^{-1} AsV for 60 d.

Sonication with phosphate buffered solution (PBS): 50 mg of ground PV roots or fronds were weighed into a centrifuge tube and 10 mL of PBS (2 mM NaH_2PO_4, 0.2 mM Na_2EDTA, pH 6.0) were added to extract the samples for 1 h under sonication followed by 15 min centrifugation at 4000 rpm to retrieve supernatant.

Heating with HNO_3 (HNO_3): 5 mL of 1% HNO_3 were added into 50 mg of ground PV and the mixture was allowed to stand overnight in the dark to minimize As transformation. Then the samples were heated following the procedure using a hot block digestion system: 10 min at 55 °C, 10 min at 75 °C, and 30 min at 9 °C. The samples were centrifuged at 4000 rpm for 15 min.

Sonication with methanol/water (methanol): 50 mg of ground PV were ultrasonically extracted with 10 mL 1:1 methanol/water for 2 h. The samples were then centrifuged at 4000 rpm for 15 min, and the supernatant was decanted into a 50 mL centrifuge tube. The procedure was repeated twice and the three extracts were combined. The temperature was set at 25–30 °C during sonication extraction.

Sonication with ethanol/water (ethanol): the same as methanol method except that 1:1 ethanol/water was used.

For all four methods, the extracts were diluted to 50 mL with Milli-Q water and filtered through 0.22 μm filters for As speciation analysis. In addition, As stability during extraction using the four methods was investigated using a PV sample cultivated in a clean soil with low As concentration. AsIII and AsV at 10 mg L^{-1} were spiked into the mixture of the PV plants and extraction solution, which was subjected to the same extraction procedure as the four methods.

2.3. Optimization of ethanol/water extraction method

After finding that the ethanol/water method provided more satisfactory extraction efficiency for PV roots (~80%) and fronds (>90%) than the other three methods as well as the preservation of As species, we