Bioaccumulation Potential And Speciation Of Arsenic Ions In Vigna Radiate Root Biomass Using Microwave Assisted Extraction By HPLC-ICP-MS

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Abstract-Arsenic ions accumulation and speciation of Vigna radiate root biomass using microwave assisted extraction analysis has been studied. Plants were cultured in a synthetic water with modified 0.25 M concentration of Hoagland’s nutrient solution at pH 6.8 supplemented with 0, 3, 5, and 10 mg As/L solution of As₂O₃. The maximum extraction efficiency of arsenic ions was found to be 85.14% from root biomass using 10% (v/v) tetramethylammonium hydroxide solution at 30 °C for 30 minutes after 21 days. After treatment of plants then the root biomass was characterized using high performance liquid chromatography, inductively coupled plasma mass spectroscopy, scanning electron microscope and fourier-transform infrared spectroscopy technique.

Keywords: Microwave assisted extraction; Arsenic ions speciation; Vigna radiate; Inductively coupled plasma mass spectroscopy; Root biomass

1. INTRODUCTION

Heavy metal pollution by natural as well as anthropogenic activities is significant threats to the living and nonliving environment. Arsenic is one of the most hazardous metalloid pollutants which are emitted by natural and anthropogenic sources like biological activity, geochemical reactions, smelting of non-ferrous metals and burning of fossil fuels [1-2]. Arsenic exists in environment in both inorganic as well as organic forms. Inorganic arsenic can occur in the environment in several forms, e.g. metalloid arsenic As (⁰), arsenite (AsO₃³⁻) and arsenate (AsO₄³⁻). As (III), As (V), methylarsonic acid (MAA) and dimethylarsinic acid (DMA) are the predominant arsenic species found in green plants [3,4]. Chronic exposure to arsenic, particularly inorganic arsenite (As (III)) and arsenate (As (V)) has been implicated in many physiological disorders and various types of cancers. Therefore, World Health Organization (WHO) has recommended the maximum permissible limit of arsenic in drinking water as 10 μgL⁻¹ [5-6]. The major advantages of bioremediation technique are the environmentally friendly, cost-effective, aesthetically pleasing, technologically feasible, long-term applicability, and ecological aspect [7-8]. The extraction of arsenic ions from solid matrices is a critical process since high recoveries as well as species preservation are required. Speciation is generally accomplished in three steps: sample preparation, species separation and detection. Most of the speciation work done of different arsenic species in marine organism. Enzymatic digestion or methanol extractants with agitation and microwave heating extraction have been successfully used to extract the organic arsenic species [9-12]. The use of microwave radiation and high pressure allows to accelerate sample digestion and to minimize contamination and losses of volatile elements. Conventional extraction procedures as well as ultrasound assisted extraction with acids were used for the direct determination of trace elements in various plant samples by atomic absorption spectrometry. The extraction in an open system was performed with conc. HCl or diluted HNO₃ for dissolution of Al, Ca, Cd, Cu, Fe, Mg, Pb and Zn from plant materials [13-16]. Microwave assisted extraction was applied mainly for elemental analysis of soils, sludge and sediments of metals in these materials. In plants, arsenic is strongly bound to -SH groups of cytosolic proteins and macromolecular constituents. Several comprehensive evaluations of techniques for extracting arsenic species from terrestrial plant samples have been reported [17-19]. In order to be applicable to risk assessment approaches to arsenic impacted sites, these methods need to be highly reproducible, rugged, and use instrumentation that is readily available, and cost effective, in commercial laboratories. The aim of the present study is (i) to evaluate the uptake of different arsenic ions (in the range of mg kg⁻¹) with different concentration by Vigna radiate root from aqueous solution, (ii) to evaluate the efficiency of extraction solution extracted arsenic ions using a complete microwave wet digestion process by ICP-MS. (iii) to evaluate extracted materials were characterized by HPLC, SEM-EDX and FTIR techniques to know the arsenic ions.
2. MATERIALS AND METHODS

2.1. Experimental Procedure

*Vigna radiate* dried matured seeds were collected from the Department of Plant Breeding and Genetics, Odisha University of Agriculture and Technology, Odisha, India. Seeds were treated with detergent solution and washed with running tap water for 15 min. Seeds were sterilized with 0.1% aqueous mercuric chloride solution for 20 min and were sown over plastic nets on glass trays (12 x 10 x 8 cm) (Borosil, India) containing the Hoagland nutrient solution. The ratio of the seeds and the solution used was 1:30. The trays were kept in a growth room temperature at 25 ± 2°C under cool, white fluorescent lamp (55 µmol m² s⁻¹) with 16 hours’ photoperiod. pH (Orion two star, USA) of the nutrient solution was adjusted to 6.8 using 0.1 N HCl or 0.1N KOH; the solution was changed regularly at 3 days’ intervals to maintain the pH. The concentrations were selected on the basis of preliminary experiment within a range that produced growth inhibition. Arsenic trioxide (As₂O₃) was used as [(0 (control), 3, 5, and 10 mg L⁻¹]. The experiment was laid in a completely randomized block design with three replications. The length of the primary root and the shoot numbers were measured at 7th day intervals from the date of root emergence up to the 28th day. The rate of root length in each experiment was determined by subtracting the length of the root recorded on the day of germination from that noted on the 28th day. The treated plants were harvested after 7, 14, 21 and 28 days. Deionised water was added daily to compensate the water loss through plant transpiration, sampling and evaporation. After completed test duration each plant with approximately fresh weight of 200g having root length of 8 to 10 cm and shoot length of 10 to 12 cm were selected and analyzed for arsenic ions accumulation and toxicity. In addition, the residual arsenic ions in the solution were measured to assess the removal potential of *Vigna radiate* plant.

2.2. Preparation Of Hoagland Nutrient Solution

The modified 0.25 molar concentration of Hoagland nutrient solution consists of 4.0mM Ca(NO₃)₂, 2.0mM MgSO₄, 4.0mM KNO₃, 0.4mM (NH₄)₂SO₄, 2µM MnSO₄, 0.3µM CuSO₄, 0.8µM ZnSO₄, 30µM NaCl, 0.1µM Na₂MoO₄, 1.43µM KH₂PO₄, 10 µM H₂BO₃ and 20 µM Fe- Na-EDTA (All obtained from Merck Specialties) in 1 L of double distilled water. The pH of the nutrient solution was adjusted to 6.8 using 0.1 N HCl or 0.1 N KOH. The solution was changed regularly at three days’ intervals to maintain the desired pH [20].

2.3. Microwave Extraction Analysis Followed ICP-MS

All reagents were of analytical-reagent grade.1M HNO₃ (Merck, Germany),1M HCl (Merck, Germany) and 30 % H₂O₂ were applied for the preparation of the leaching solutions. In all experiments, double distilled water (Milli-Q Millipore 18.2 MΩ cm⁻¹ conductivity) was used for preparation, dilution and analytical purposes of solutions. A solution of 1000 mgL⁻¹ of arsenite stock solution was prepared by dissolving 1.320 g of arsenic trioxide (As₂O₃; Merck Chemicals, Germany) in deionized water containing 4 g NaOH in 1 L of double distilled water. The stock solutions were preserved with 2% (v/v) trace metal grade nitric acid. Subsequently, arsenite different working solutions of required concentrations were prepared by proper dilution. The pH was adjusted to the desired level with 0.1 N HCl or 0.1N KOH solutions. Before use all lab ware was subjected to a cleaning procedure. Microwave assisted wet digestion extraction processes were conducted with Shanghai Sineo MAS-II oven delivering a maximum power of 1000 W. The microwave system was programmed to heat the sample to a specified temperature and maintained for a specified period. To optimize the microwave-assisted extraction of arsenic ions from root parts of plant material was conducted consisting of 3 heating temperatures (40 °C, 60 °C and 80 °C) and heating times of 5, 15 and 25 minutes for each of the 2 different extraction solutions. The two extracting solutions were used i.e. 10% (v/v) tetramethylammonium hydroxide (TMAH) and a modified protein extracting solution. Arsenic ions containing dried *Vigna radiate* root powder (0.5 g) was weighed into a 50 mL reaction vessels to which 10 mL of concentrated nitric acid was added. The digested mixture was heated at 60 °C for 30 min and filtered into a volumetric flask with the help of Whatman 42 filter paper [10]. After complete digestion each sample was added 10 mL extractant solution. The microwave system was programmed to heat the sample to a specified temperature, and the temperature was maintained for a specified period. After microwave heating, the samples were allowed to cool to room temperature. The flasks were allowed to cool to room temperature and made up to a final volume (10 mL) with deionized water. To measure arsenic ions concentration in digested samples, 1 mL of digest was mixed with 9 mL of reducing solution consisting of 1.5% (w/v) potassium iodide, 1.5% (w/v) ascorbic acid and 10% (v/v) hydrochloric acid.
This mixture was then heated at 40 °C for 1 h. Arsenic ions were determined by ICP-MS (model PE ELAN 6000, Perkin-Elmer, USA). The carrier solution was 10% (v/v) hydrochloric acid, and the reductant solution consisted of 0.2% (w/v) sodium borohydride and 0.05% (w/v) sodium hydroxide. The carrier solution was 1% (v/v) nitric acid; samples were made up in solution of 10 µg rhodium L-1 which served as an internal standard [21-22]. All data were expressed as mean ± SEM of three separate experiments. The mean of metal ions concentration was calculated using MINITAB.

2.4. Arsenic Ions Speciation Using HPLC
Arsenic ions speciation of Vigna radiate root biomass during absorption process was observed with the help of high performance liquid chromatography (Hamilton PRP-X100 anion-exchange column chromatogram). Dry 0.5 g arsenic containing samples were extracted using microwave assisted digestion with 5 mL of 1:1 methanol/water for 2 hours. The samples were centrifuged then the supernatant was decanted into a 50 mL volumetric flask. The procedure was repeated with the residual pellet and the two extracts were combined. The residue was rinsed three times with 5 mL of water, and all supernatants were combined. Mobile phase was 20 mM ammonium phosphate (NH₄H₂PO₄) buffer at pH 6.0 and flow rate 1.5mLmin⁻¹.

3. RESULTS AND DISCUSSION
3.1. Extraction Efficiency Of Arsenic Ions
Extraction is an important step for separation of constituents from the plant material. Microwave digestion technique is a simple, inexpensive and valuable tool used in applied chemistry which requires lesser amount of solvent, simplified manipulation and higher purity of final product with lower cost. Microwave extraction is becoming the choice for the extraction of solid matrices for organic analyte analysis by ICP-MS techniques. In the present study, extraction of arsenic ions from Vigna radiate root biomass using 10% (v/v) tetramethylammonium hydroxide (TMAH), and a modified protein extracting solution has been conducted at three different temperatures for three different times after 21 days of experiment and the results are presented in Figure 1a and Figure 1b.

![Figure 1. Arsenic ions extraction efficiency for Vigna radiate root biomass using (a) modified protein extracting solution, (b) 10% TMAH after 21 days’ experiment. Data represents the mean ± S.D (n=3, “n” stands for the number of experiment replicates.)](image)

The efficiency of the extraction of arsenic ions increased with increasing temperature in modified protein extracting solution at all concentration of arsenite. In tetramethylammonium hydroxide extracting solution efficiency increased with increase in temperature with the highest value at a temperature of 60 °C after 21th days experimentation in treated with 5 mg/L arsenite solution. It is evident that at high temperature tetramethylammonium hydroxide (TMAH) is expected to break down the strong As-SH (thiol) bonds present in plant cells. For arsenic ions accumulation once inside the plant must be translocated through the cytoplasm /vacuoles of adjacent cells at high concentrations and that does not disrupt cytoplasmic function. The electrochemical species of arsenic ions changed in different forms in
the plant shoot tissue. Sulphydryl enzymes enhanced electrochemically oxidize in plant tissue of arsenite to arsenate. The bacterial arsenate reductase (ArsC) catalyzes the electrochemical reduction of arsenate to arsenite. The bacterial \(\gamma\)-glutamylcysteinesynthetase (\(\gamma\)-ECS) catalyzes the formation of \(\gamma\)-glutamylcysteine (\(\gamma\)-EC) from the amino acids glutamate and cysteine and is the committed step in the synthesis of glutathione (GSH) and phytochelatine (PCs) (indicated by three arrows) [18, 24, 19]. Reduced arsenite can bind organic thiols (RS) such as those in \(\gamma\)-EC, GSH, and PCs through the replacement of oxygen by organic sulfur species. The scheme of reaction mechanism is presented in Figure 2. Metal ions penetrated plants by passive process, mostly by exchange of cations which occurred in the cell wall. All heavy metals were taken up by plants through absorption, translocation and released by excretion. It can be proposed that the roots reached saturation during the period and there exists some mechanism in roots that could detoxify heavy metals or transfer them to aerial parts [25-29].

![Figure 2. Hypothetical model and reaction mechanism of arsenic ions changed in different forms in Vigna radiate root tissue.](image)

3.2. Stability And Speciation Of Arsenic Ions

Arsenic species were separated on the Hamilton PRP x 100 anion-exchange column. In present study, the plant material was used to evaluate the stability of arsenic species during the extraction procedure. Therefore, the method adopted in this study was tested on root biomass of Vigna radiate extracted by 10% (v/v) tetramethylammonium hydroxide (TMAH) with yield of 85.14%, and extracted by a modified protein extracting solution with yield of 88.92%. Chromatograms are obtained for arsenic species in plant root biomass with modified protein extraction and TMAH extract solution by using HPLC after 21 days’ experiment and results are shown in Figure 3a and Figure 3b. Vigna radiate root biomass reveals maximum amount of inorganic arsenic species and very less amount of organic arsenic species consists as it is indicated from the above figures. Arsenic (III) are present in maximum quantity, arsenic (V) in minimum quantity and the organic arsenic like monomethylarsonic (MMA) and dimethylarsinic acid (DMA) are very less at 5 mg/L in different experimentation days and results are presented in Table 1. Recent studies have described the formation of As-phytochelatin complexes in several terrestrial plants upon exposure to arsenate. Phytochelatins (PCs) are thiol (SH) - rich peptides derived from glutathione (GSH) and are considered to be involved in the mechanism of detoxifying heavy metals in
higher plants. Arenic (III) readily forms complexes with thiol groups which is supported by the results [27, 25, 30-31].

![Graph](image)

<table>
<thead>
<tr>
<th>Days</th>
<th>Mean±S.D. (%)</th>
<th>Arsenite As(III)</th>
<th>Arsenate As(V)</th>
<th>Methylarsionic acid (MAA)</th>
<th>Dimethylarsionic acid (DMA)</th>
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</thead>
<tbody>
<tr>
<td>7 days</td>
<td>35.21±0.42</td>
<td>1.23±0.11</td>
<td>0.0001±0.01</td>
<td>0.0001±0.02</td>
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<tr>
<td>14 day</td>
<td>54.22± 0.11</td>
<td>4.21±0.32</td>
<td>0.0012±0.02</td>
<td>0.0001±0.01</td>
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<tr>
<td>21 days</td>
<td>80.10± 0.76</td>
<td>5.03±0.31</td>
<td>0.0032±0.03</td>
<td>0.00011±0.02</td>
<td></td>
</tr>
<tr>
<td>28 days</td>
<td>72.11± 0.45</td>
<td>10.23±0.09</td>
<td>0.0010±0.01</td>
<td>0.00010±0.01</td>
<td></td>
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S.D. = Standard deviation
The standard deviation has been obtained for n=3

3.3. Characterization Of Extract Material
3.3.1. SEM-EDX Analysis
The surface morphology of Vigna radiate root biomass without and with extract of arsenic ions during absorption process was observed with the help of SEM-EDX (JOEL model JSM-6480LV (Japan).) and presented in Figure 4a. It is show that the surface texture and pores in the species without absorption of arsenic ions. Figure 4b shows the morphological changes with respect to shape and size of the materials after absorption of arsenic ions respectively. It can be clearly observed that the surface of materials shape has been changed into a new shiny bulky particle and whitish patches structure. The EDX spectra of arsenic ions are shown in Figure.4a and Figure 4b. So, it was concluded that, arsenic ions were adsorbed on the surface of the extract materials. These results are further confirmed with the results of FTIR spectra analysis.
3.3.2. **FTIR Analysis**

FTIR (Perkin Elmer FT-IR, Spectrometer Spectrum RX-I.) spectra of extract material of arsenic determine which functional groups may have contributed to the arsenic ions as shown in Figure 5. The spectra of extract material exhibit a broad absorption band at 3672.17 cm\(^{-1}\) due to bonded –OH stretching vibration which complexation of –OH groups with arsenic ions. The peak at 1,596.07 cm\(^{-1}\) may be due to the complexation of carboxylic group with arsenic ions. The absorbance peaks at 1498.80 cm\(^{-1}\) and 1473.68 cm\(^{-1}\) attributed due to N-H stretching vibration, -CH\(_2\) scissoring or –CH\(_3\) anti symmetrical bending vibration and O-H deformation is shifted to lower frequency may be due to the complexation of arsenic ions. Another peak at 1,231.24 cm\(^{-1}\), 1167.68 cm\(^{-1}\), 1069.70 cm\(^{-1}\) and 1,022.57 cm\(^{-1}\) may be due the interaction of nitrogen from amino group with arsenic ions. The other weak absorption peak 809.43 cm\(^{-1}\) to 751.50 cm\(^{-1}\) and 689.90 cm\(^{-1}\) corresponding to the thiol or sulphydryl group with arsenic ions [34-35]. This clearly manifests the binding of arsenic ions to the extract materials.

![Figure 5. FTIR spectra of arsenic ions absorption of extracted materials.](image)
4. CONCLUSION
Nutrient culture is an efficient method for screening toxic element tolerant capacity of Vigna radiate plant in aqueous solution treatment. The arsenic ions uptake is ascertained by using inductively coupled plasma mass spectroscopy. Maximum removal percentage of arsenite is 85.14% after 21 days’ treatments using 10% (v/v) tetramethylammonium hydroxide (TMAH) solution at 30 °C for 30 minutes. Arsenic ions species speciation using high performance liquid chromatography and arsenite ions were reveals maximum percentage among other arsenic species. Before and after accumulation of arsenic ions were characterized by SEM-EDX technique. The FTIR spectroscopic analysis confirmed that the –OH stretching vibration, complexation of carboxylic group, interaction of nitrogen from amino group and corresponding to the thiol or sulfhydryl group with arsenic ions. Great removal efficiency and high arsenic ions accumulation capacity make Vigna radiate is an excellent choice for phytoremediation processes.

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REFERENCES


