Removing Heavy Metals in Water: The Interaction of Cactus Mucilage and Arsenate (As (V))

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Supporting Information

ABSTRACT: High concentrations of arsenic in groundwater continue to pose health threats to millions of consumers worldwide. Particularly, affected communities in the developing world need accessible technologies for arsenic removal from drinking water. We explore the application of cactus mucilage, pectic polysaccharide extracts from Opuntia ficus-indica for arsenic removal. Synthetic arsenate (As (V)) solutions were treated with two extracts, a gelling extract (GE) and a nongelling extract (NE) in batch trials. The arsenic concentration at the air−water interface was measured after equilibration. The GE and NE treated solutions showed on average 14% and 9% increases in arsenic concentration at the air−water interface respectively indicating that the mucilage bonded and transported the arsenic to the air−water interface. FTIR studies showed that the −CO groups (carboxyl and carbonyl groups) and −OH (hydroxyl) functional groups of the mucilage were involved in the interaction with the arsenate. Mucilage activity was greater in weakly basic (pH 9) and weakly acidic (pH 5.5) pH. This interaction can be optimized and harnessed for the removal of arsenic from drinking water. This work breaks the ground for the application of natural pectic materials to the removal of anionic metallic species from water.

INTRODUCTION

Contamination of groundwater with arsenic from natural leaching of arsenic-bearing minerals is an environmental phenomenon, which affects millions of people in different parts of the world. However, the health impact is most severe in East Asia where approximately 100 million people in the Bengal Basin of Bangladesh and West Bengal, India are exposed to the detrimental health effects of long-term consumption of water from contaminated wells. The majority of the affected communities have no access to conventional water treatment. The World Health Organization (WHO) recommended that the maximum concentration of arsenic in drinking water should not exceed 10 μg/L. This large scale arsenic poisoning has fueled research into accessible technologies for arsenic removal which need to be inexpensive, robust, reliable, and easy to maintain, while requiring little or no fossil fuel for operation. Plant-based materials are especially attractive since they are renewable, biodegradable, and can be sustainably managed, all of which contribute to making them suitable for accessible technologies. Pectins and pectinaceous plant materials for metal sorption, cactus mucilage and cactus pectin extracted from the pads of the Opuntia ficus-indica, were investigated in this work as a low cost material for arsenic removal. The two cactus extracts are distinguishable by the ability of the cactus pectin to form gels in the presence of Ca2+ ions, and is referred to as the gelling extract (GE). The cactus mucilage, a pectic polysaccharide is referred to as the nongelling extract (NE). Several researchers have shown that while both extracts contain arabinose, galactose, xylose, rhamnose, and galacturonic acid, the major difference between them is that GE is mainly comprised of galacturonic acid. McGarvie and Parolis proposed a polygalacturonic acid and rhamnose backbone for the NE structure with branched side chains of galactose and rhamnose; the branches being composed of arabinose, and xylose. Cardenas et al. reported that the gelling extract consisted mainly of poly-D-galacturonic acid (polyGA).
acid. For both of these pectic polysaccharides the main functional groups are carbonyl, carboxyl, and hydroxyl groups.

Despite the widespread application of plant pectins to the removal of cationic heavy metal contaminants, they have not been used with anionic contaminants. This is possibly due to concerns that the electrostatic repulsion would hinder interaction between the polyanionic pectins and anionic metal contaminants and lead to low loading capacity. However, electrostatic challenges are not the only determining factor in interaction, as evidenced by natural organic matter (NOM) binding with arsenic species.23–26 NOM, particularly humic acids, share carboxylic and alcohol functional groups with pectic polysaccharides and as such the latter are also expected to be able to bind arsenic species in solution.

While cactus mucilage has been explored for water purification,27,28 our research group was the first to demonstrate an interaction between cactus mucilage and arsenic.29,30 Solutions of arsenate (As(V)), which were treated with mucilage showed differences in arsenic concentration at the air–water interface over time. These concentration differences were attributed to the mucilage binding the arsenic and transporting it either toward or away from the air–water interface.

The current work looks at the interaction of the cactus mucilage with As(V), using a combination of batch tests and molecular spectroscopy. ATR-FTIR and UV-Vis spectroscopy showed that the −CO groups (carboxyl and carbonyl groups) and −OH (hydroxyl) functional groups of the mucilage were involved in reacting with arsenate. The gelling extract (GE) performed better on average than the nongelling extract (NE) and the pH was shown to affect the mucilage performance.

The vision for this technology is to develop low-cost arsenic removal filters for household point-of-use applications as well as community level treatment for low-income communities. The simplicity of the mucilage extraction makes its manufacture widely accessible. Potentially, this work will add to the overlap of water remediation materials and natural products since the material may become a new water treatment material and also expands the use for pectinaceous carbohydrate polymers.

## MATERIALS AND METHODS

Mucilage was extracted from the modified stems (pads) of the Opuntia ficus-indica cactus. Fresh pads were obtained from the private nursery of Dr. Norma Alcantar in Tampa, Florida, U.S. The plants were grown from pads originally purchased from Living Stones Nursery, Tucson AZ. Water intake was controlled to ensure they all grew at the same rate. No other controls were necessary since different growing conditions only affect the mucilage and pectin content of the pads.31 There was no significant variation from plant to plant.

All chemicals used were analytical grade or better and purchased from Fisher Scientific (Pittsburgh, PA).

### Mucilage Extraction. Cactus Pad Preparation.
Two mucilage fractions were extracted from the cactus pads; a pectic gelling extract (GE) and a nongelling extract (NE). The NE extraction was done following a modification of the method used by Goycoolea et al.18 The pads were first cleaned by removing thorns and brown spots, washed, dried, and weighed. The pads were diced and heated for 20 min at 80–85 °C in 1% NaCl solution (1:1 mass to volume ratio) in order to inactivate enzymes. After cooling, the mixture was liquidized for 45–50 s at the highest speed in a commercial blender (Osterizer), then neutralized with 1 M sodium hydroxide (NaOH). The mixture was then centrifuged (Fisher Scientific accuSpin 400) at 4000 rpm for 10 min to separate the liquid from the solids. The supernatant, containing the NE was decanted leaving the solid residue for GE extraction.

### Nongelling Extract (NE) Preparation. Sodium chloride (NaCl) was added to the liquid supernatant produced in the pad preparation step to obtain a final 1 M NaCl concentration. The liquid was then filtered using Whatman 41 filter paper. By visual observation, if the liquid were too viscous to flow easily through the Whatman 41 filter, then a knitted polyester cloth filter (Polx 1200, Berkshire Corp., Great Barrington, MA) was used. NE was precipitated from the filtrate using acetone or isopropanol in a 2:3 ratio of supernatant to solvent. The precipitate was washed with ethanol–water mixtures in a graded series (70%, 80%, 90%, 95% ethanol and absolute ethanol) to remove any remaining impurities. The precipitate was left to dry at room temperature overnight, followed by an overnight drying in an air oven (Yamato DX-41, Japan). The material was pulverized with a ceramic mortar and pestle and stored in a closed container at room temperature.

### Gelling Extract (GE) Preparation. The GE extraction procedure was an adaptation of a method developed by Turquis et al.32 The differences we used to improve our yield were separating by centrifugation and vacuum filtration, using a different dosage of chelating agent and a shorter sequestering time, and using acetone as the precipitating solvent.

The solid residue from the cactus pad preparation was mixed with 7.5 g/L sodium hexametaphosphate [(NaPO3)6] in 50 mM NaOH, in a 1:1 mass-to-volume ratio of residue to solution. The mixture was stirred for 1 h, then vacuum filtered with cloth to obtain the filtrate, which contains the dissolved GE. The filtrate pH was lowered to 2.0 by titration with hydrochloric acid (HCl) and refrigerated overnight (~5 °C) in order to precipitate the GE. The precipitate was separated by centrifugation and resuspended in sufficient DI water to cover it. The pH was adjusted to 8.0 with 1 M NaOH to redissolve the precipitate. The resulting solution was purified by filtering through a 1.2 μm and a 0.45 μm membrane. The GE was reprecipitated with acetone or isopropanol in a 2:3 liquid-to-solvent ratio, then washed and dried in the same manner as for NE.

### Batch Experiments. As(V) solutions, 10 mL of 60–80 μg/L As, were treated with GE and NE in 15 mL centrifuge tubes to attain final mucilage concentrations of 5–100 mg/L. An equilibration time of 20 h was established in previous unpublished work. After 36 h equilibration, 1 mL sample aliquots were removed from the air–water interface using an automatic pipet. The total As concentration was determined by Hydride Generation–Atomic Fluorescence Spectroscopy (HG-AFS). Two types of control experiments were run; As(V) solutions without mucilage, and mucilage solutions without As(V), with all other test conditions and concentrations kept constant. The As(V) stock solution used for experiments was prepared by dissolving solid sodium arsenate in sufficient deionized water to bring the final concentration to 1000 μg/L. The stock solution was continuously aerated using an aquarium aerator, which maintained the dissolved arsenic in the oxidized arsenate form. This was done to represent the natural aeration of groundwater when pumped above ground. When pH was a variable, pH was adjusted using sodium hydroxide and hydrochloric acid.

Results were reported in terms of increase in As concentration, calculated according to the following:
percent increase = \frac{\text{(test solution concentration} – \text{control solution concentration})}{\text{control solution concentration}} \times 100\%

Twenty experiments were run with samples in triplicate or better. Statistically significant difference between mucilage-treated solutions and untreated controls was determined using Student’s t test paired two sample for means, with \( \alpha = 0.1 \).

Total Organic Carbon (TOC). Ten mL samples of 100 \( \mu \text{g/L} \) As were treated with 50 mg/L GE in 15 mL centrifuge tubes. Sample aliquots were taken from the top and bottom of the tubes for TOC determination. The TOC of the bulk solution was calculated as the difference between TOC of the entire solution and that of the top and bottom combined. TOC was measured using a TOC analyzer TOC–V equipped with an automatic sample injector (Shimadzu, Japan). Potassium hydrogen phthalate standard solution was used for calibration of the system. The TOC detection limit was 50 \( \mu \text{g/L} \).

Attenuated Total Reflection Fourier Transform Infrared (ATR-FTIR) Studies. Mucilage films were prepared to characterize the functional groups by Attenuated Total Reflection (ATR). Mucilage films were prepared by pipetting 100 \( \mu \text{L} \) of a 5 g/L mucilage suspension directly onto a zinc selenide (ZnSe) crystal for use with a horizontal ATR accessory (Pike Technologies, Madison WI, USA). The suspension was allowed to air-dry before observing the spectra. Films were also prepared to observe the interaction of mucilage with arsenic, made from solutions containing 10 mg/L As(V) and 5 g/L mucilage. ATR-FTIR spectra for the samples were observed using a Nicolet 6700 spectrometer (Thermo Fisher, Madison WI, USA), using 100 scans at a resolution of 4 cm\(^{-1}\). The experiment was repeated three times.

RESULTS AND DISCUSSION

Effect of Mucilage Type and Concentration. Both GE- and NE-treated solutions showed higher concentrations of As at the air–water interface than the control solution, shown in Figure 1. The observed increase in As concentration was independent of the mucilage concentration. However, both extracts showed considerable variation in performance.

The GE showed an average 14% increase in As concentration at the air–water interface, while the NE showed an average 9% increase. Twenty experiments were run with two or more concentration levels for both GE and NE mucilage fractions. Each determination was done in triplicate or better. As such, more than 240 replicates are represented in Figure 1. 94% of the NE trials and 81% of the GE trials showed mucilage activity in transporting the As to the air–water interface. For both extracts, 56% of the trials showed mucilage activity greater than the average performance of each extract. We observed that sometimes the GE was able to separate up to 50% of the arsenic in the water, and other times, it only separated about 5%. We believe this variance is due to the difference in the performance expected in natural materials. GE showed a wider response range and higher maximum increase of 34% than NE, which had a maximum increase of 17%, indicating that the increase in concentration is extract dependent. GE was consequently chosen for further experiments as the more reactive/responsive extract.

One probable reason for the lack of response to increasing mucilage concentration could be that at higher concentrations, the mucilage tends to aggregate and form larger molecular

![Figure 1. As concentrations at air–water interface of mucilage-treated solutions relative to untreated control solutions as a function of mucilage extract and concentration.](image)

Table 1. Arsenic Sorption Capacity of Selected Sorbents

<table>
<thead>
<tr>
<th>sorbent</th>
<th>capacity (mg/g)</th>
<th>source</th>
</tr>
</thead>
<tbody>
<tr>
<td>Acacia nilotica powdered stem</td>
<td>50.8</td>
<td>34</td>
</tr>
<tr>
<td>Sorghum biomass</td>
<td>2.765</td>
<td>35</td>
</tr>
<tr>
<td>waste tea fungal biomass</td>
<td>0.55</td>
<td>36</td>
</tr>
<tr>
<td>human hairs</td>
<td>0.012</td>
<td>37</td>
</tr>
<tr>
<td>cactus mucilage</td>
<td>2.8 - 0.14</td>
<td>this study</td>
</tr>
</tbody>
</table>

These results present an unexplored interaction between pectic polysaccharides and metal oxyanions. Since the mucilage is not a source of As, the increase of As concentration at the air–water interface directly shows the mucilage interaction with As.

Pectins have been extensively shown to interact with metal cations; however, interaction of cactus mucilage with As(V) has only been demonstrated by our group.\(^{30}\) In the current study, we explore how the mucilage interacts with the As (V) in order to extend the technological application of pectins to treating new anionic metallic water pollutants, and give insight into how the interaction may be optimized. The transport of the arsenic to the air–water interface is thought to occur due to an increase in the hydrophobicity of the mucilage. Our proposed mechanism is that the hydrophilic mucilage binds to the
As(V) ions by using its ionizable hydroxyl, carboxyl, and carbonyl groups. Normally, these ionizable groups would have been used to stabilize the mucilage in the bulk of the aqueous solution by attracting counterions. On interaction with As the groups are no longer available and, thus destabilized and more hydrophobic, the mucilage-As complex is repelled to the air–water interface.

**TOC Analysis.** TOC analysis was done to determine the distribution of the mucilage in the solutions in order to definitively link the mucilage to the transport of As to the air–water interface. As shown in Figure 2, the TOC concentration in the entire solution, which correlates to the total mucilage concentration, does not change on addition of As. However, the distribution of mucilage in the solution does change in the presence of As. We determined the TOC in the bulk solution by subtracting the TOC of the combined top and bottom of the solution from that of the entire solution. Figure 2 shows a larger proportion of TOC in the bulk of the control solutions than in the mucilage-treated As solutions. This means that mucilage migrated from the bulk of the solution primarily to the air–water interface due to the presence of As.

**Effect of pH.** Three batch trials were run in triplicate or better at pH 5, 5.5, 6, 7, 8, and 9 to investigate the effect of pH on the interaction between mucilage and arsenic. We chose these pH values to reflect the pH of natural waters. Note that pH 5.5 is the initial solution pH.

As shown in Figure 3, only at pH 5.5 and 9, were small but statistically significant differences observed between mucilage-treated solutions and nonmucilage treated controls. Further, significant difference was observed between the mucilage-treated solutions at these two pH levels. These results indicate that solution pH does affect the interaction between the GE and arsenate and further, gives insight into the nature of the interaction. GE, an anionic polyelectrolyte, becomes increasingly negatively charged with increasing pH due to ionization of –OH groups from alcoholic and carboxylic groups.16,20,33

The As(V) oxyanion is polyprotic and changes protonation level with pH. In weakly acidic conditions, such as pH 5.5, it exists as dihydrogen arsenate H2AsO4−, while in weakly basic conditions (such as pH 9) it exists as hydrogen arsenate HAsO42−.38 Our proposed mechanism of interaction is via hydrogen bonded bridging between the protons associated with the As species and the ionized carbonyl and carboxyl groups on the mucilage. At lower pH, the mucilage is less ionized, but there is a higher number of protons on the H2AsO4−. At higher pH, the mucilage is more ionized, but the arsenate has less protons. The mucilage appears to work equally well in both scenarios.

The activity in these pH regions versus the lack of activity over the pH range from 6 to 8 may be due to poorer charge transfer while the pH is in the neutral region. The mucilage is therefore expected to perform better in solutions with higher ionic strength to facilitate charge transfer.

**FTIR Insight into Mucilage Interaction with Arsenic.** ATR-FTIR spectra provide a chemical fingerprint of materials by correlating their absorption frequencies with known absorption frequencies of bonds. The spectra of both GE and NE, shown in Figure 4, depict the characteristic group frequencies of pectic polysaccharides; that is, carboxylic acid, carboxylate, ether, and alcohol groups.

In the GE spectrum, there are four main features: the first is the broad band at 3350 cm−1 which corresponds to OH stretching of alcohol and carboxylic acid –OH groups involved in intermolecular hydrogen bonding. The second is two bands at 1609 and 1416 cm−1 corresponding to the antisymmetric and symmetric COO− stretch characteristic of carboxylic acid salts. Third, the bands at 1250 and 1140 cm−1 correspond to C–O–C ether stretch. Lastly, two strong bands at 1140 and 1100 cm−1 are due to C–O stretch of secondary alcohols and C–O–H stretch in cyclic alcohols, respectively.

Significant similarities exist between the spectra of GE and NE; however, four notable differences distinguish NE from GE. A more pronounced band is observed at 2929 cm−1 for the CH stretching. Further, NE shows the expected carbonyl C=O stretch at 1727 cm−1 that is absent in GE. The most intense
band for NE occurs at 1041 cm\(^{-1}\) due to HC=O–H stretch of cyclic alcohols. The most notable difference between the two extracts is seen in the region 1250 to 850 cm\(^{-1}\). Coimbra et al.\(^3\) showed that the intensity of the bands at 1100 and 1018 cm\(^{-1}\) correlated with the uronic acid content of pectic polysaccharides; on this basis, GE is deduced to have a higher uronic acid content than NE.

In FTIR absorbance spectroscopy, peak shifts and intensity changes are important for inferring chemical interaction. Peak shifts signify a change in the chemical environment of a functional group. The coupled appearance and disappearance of absorption bands indicate a reaction involving the corresponding functional group. On reaction with the As (V) oxyanion, significant changes occur in the spectra of GE and NE; shown in Figure 5. These changes indicate the participation of the carbonyl, carboxyl, and hydroxyl functional groups in the interaction with As (V).

The OH band of GE at 3350 cm\(^{-1}\) was shifted higher to 3364 cm\(^{-1}\) indicating a change in the hydrogen bonding network probably due to the direct involvement of the alcohol and carboxylic –OH groups. The 1609 cm\(^{-1}\) band shifted to 1640 cm\(^{-1}\) further corroborating hydrogen bonding as an interaction mechanism.\(^4\) This shift is shown more clearly in Figure 6. The appearance of band at 1733 cm\(^{-1}\) coupled with the decreased intensity of the 1640 cm\(^{-1}\) band indicate that the –C=O from carboxylic acid is the binding site for the As (V) oxyanion.

Similar changes were observed in the spectrum of NE after reaction. An analogous band at 1615 cm\(^{-1}\) shifted to 1645 cm\(^{-1}\) indicating hydrogen bonding. This band’s intensity decreased while the band at 1727 cm\(^{-1}\) assigned to C=O stretch shifted to 1735 cm\(^{-1}\) and increased in intensity (see Figure 6), providing further proof that the C=O group was a binding site for arsenate.

Having established that the major functionality in the pectic polysaccharides are carbonyl, carboxyl, and hydroxyl groups, we
deduced that mucilage binds As using these groups. At this point, we propose it occurs through a combination of mechanisms: hydrogen bonding between the protons of the OH groups on mucilage and O groups of arsenate, and electrostatic attraction or complexation between the positive metal center of As (V) and the electron-rich CO groups of the mucilage on account of the tetrahedral geometry of the arsenate oxyanion.\textsuperscript{41} FTIR data show that for both GE and NE, CO sites were important. Further, other researchers using entirely different biosorbents with similar functional groups have also reported the involvement of CO and OH groups in As binding.\textsuperscript{35,42,43}

Taken together, these findings suggest that the interaction of the mucilage with As increases the hydrophobicity of the mucilage by occupying its ionizable groups, which would have stabilized it in the bulk of the aqueous suspension. The mucilage-As complex is consequently repelled to the air–water interface on account of increased hydrophobicity, resulting in the observed increase in As at the air–water interface.

In order to realize the mucilage’s potential as a complexant for arsenic, further work is needed in designing an efficient heterogeneous contacting system which will allow easy separation of the mucilage-As complex.

\section*{ASSOCIATED CONTENT}

\subsection*{Supporting Information}

\textsuperscript{UV–vis} method, discussion and figures. This material is available free of charge via the Internet at http://pubs.acs.org.

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\textbf{Notes}

The authors declare no competing financial interest.

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\section*{REFERENCES}


