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Spectroscopic Study of the Impact of Arsenic Speciation on Arsenic/Phosphorus Uptake and Plant Growth in Tumbleweed (*Salsola kali*)

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This manuscript reports the toxic effects of As_2O_3 (arsenic trioxide) and As_2O_5 (arsenic pentoxide) on *S. kali* as well as the arsenic and phosphate uptake and arsenic coordination within plant tissues. Plants were germinated and grown for 15 days on a Hoagland-modified medium containing either As(III) (arsenic trioxide) or As(V) (arsenic pentoxide). Subsequently, the seedlings were measured and analyzed using inductively coupled plasma optical emission spectroscopy and X-ray absorption spectroscopy techniques. Plants stressed with 2 mg L^{-1} of whichever As-(III) or As(V) concentrated 245 ± 19, 30 ± 1, and 60 ± 3 mg As kg^{-1} dry weight or 70 ± 6, 10 ± 0.3, and 27 ± 3 mg As kg^{-1} dry weight in roots, stems, and leaves, respectively. Arsenate was less toxic, and more As translocation occurred from the roots to the leaves. All treatments reduced P concentration at root level; however, only As(V) at 2 and 4 mg L^{-1} reduced P concentration at leaf level. Regardless the arsenic species supplied to the plants, arsenic was found in plant tissues as As(III) coordinated to three sulfur ligands with an interatomic distance of approximately 2.25 Å.

Introduction

The presence of arsenic in soils and waters provides a route for human and animal exposure to this element. Thus, the restoration of arsenic polluted environments becomes an important task to reduce health risks. Traditional methods to remediate arsenic-laden soils are expensive and the soils still have to be disposed of after treatment (*1*, *2*). An emerging technology for the remediation of soils and waters is phytoremediation, defined as the use of plants for cleaning or reducing the toxicity of contaminated environments (*3*). Phytoremediation technologies include phytorestoration, which is the revegetation of barren soils to avoid the dispersion of contaminated soil particles (*3*). Phytorestoration of arsenic contaminated lands is sometimes complicated since many polluted areas lack mycorrhizal species, thus reducing the establishment of mycorrhizal-dependent plants (*4*). In addition, few plant species are tolerant to elevated As concentrations and low nutrient availability (*5*).

Several plants with the ability to uptake heavy metals or toxic chemicals have been described in the literature (*6*−*12*). However, in the case of arsenic, only one hyperaccumulator plant has been reported to date (*8*). Even when several species including *Holcus lanatus* and Basin wildrye have demonstrated tolerance to this element (*5, 13*), they form mycorrhizal association. In this context, the identification of plant species suitable for phytorestoration and/or phytoextraction of As from polluted soils with or without mycorrhizal association is especially important. In addition, the understanding of the mechanisms of tolerance and hyperaccumulation is of paramount importance since this will lead to the improvement of phytoremediation techniques.

Although a number of metal-contaminated sites are located in desert areas, to our knowledge, little is known about the toxicity and uptake of As by desert plant species. Consequently, the information about the mechanisms of As accumulation in these plants is limited. Several techniques (*14*−*19*) including X-ray absorption spectroscopy (XAS) have been used to determine the speciation and coordination of arsenic taken up by plants (*15*, *20*, *21*). In this study, *Salsola kali*, a drought-tolerant bush, which does not form mycorrhizal association and that has proven beneficial for the restoration of disturbed sites (*22*), was studied for its ability to uptake arsenic. Arsenic and phosphorus were determined in plants treated with arsenic trioxide and arsenic pentoxide. In addition, XAS studies were performed to determine the oxidation state and the coordination environment of the arsenic within the plants. Important data is been provided herein since tumbleweed may be used in the revegetation of disturbed sites contaminated with arsenic.

Materials and Methods

Plant Growth. A modified Hoagland nutrient medium was used to germinate and grow seedlings of tumbleweed in different concentrations of As_2O_3 (arsenic trioxide, As(III)) and As_2O_5 (arsenic pentoxide, As(V)). The nutrient medium prepared as described by Peralta et al. (*23*), was amended with either As(III) or As(V) to obtain concentrations of 0, 1, 2, and 4 mg L^{-1} As. After the pH was adjusted to 5.8, the agar was added (0.5% w/v) and the media was poured into 250-mL jars and sterilized. Approximately 40 seeds of tumbleweed previously disinfected with an antibiotic/antimycotic solution (A 9909, Sigma, St Louis, MO) were planted for every treatment. The jars were placed under a 12-h photoperiod with a photon irradiance of 39.5 μmol m^{-2} s^{-1} and a temperature of 25 °C. The seed germination was evaluated after 4 days, and the uptake study was performed after 15 days, at which time the plants were removed from the growth media and the root and shoot length was measured and kept for further evaluation. Every treatment was in triplicate for statistical purposes.

Determination of As(III) and As(V) Effect on Plant Growth. Complete plants were rinsed using 0.01 M HNO_3 and washed with deionized (DI) water. Afterward, the root and shoot length of 10 plants per replicate/treatment were

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measured and separated into roots, stems, and leaves. The samples were then oven dried for 72 h at 60 °C to avoid As volatilization (*24*), and the dry mass was recorded. Data were statistically analyzed to determine the effect of the treatments on plant growth and biomass accumulation.

Digestion. Oven-dried samples were weighed and acid digested to destroy organic matter and to solubilize the elements for chemical quantification. Digestion was performed using 5.0 mL trace pure HNO_3 via microwave assistance and following EPA method 3051 with minor modifications (*25*). A temperature of 120 °C was used in order to avoid As volatilization (*26*). The digested samples were then diluted to a final volume of 10 mL using deionized water.

Inductively Coupled Plasma Optical Emission Spectroscopy (ICP-OES) Analysis. Total As and P in the digested samples were determined using an ICP-OES Optima 4300 DV (Perkin-Elmer, Shelton, CT). Analyses were performed under optimized conditions according to EPA method 200.7 (*27*) with minor modifications. The wavelengths 197.197 and 178.221 nm used to determine the total As and P, respectively, were selected based on correlation coefficients and detection limits. Calibration curves (with correlation coefficients ≥0.9999) were obtained using the blank and four concentrations of each element. Spiked control samples were used every 10 samples for quality assurance/quality control.

Statistical Analyses. One-way analysis of variance (ANOVA), Tukey's honestly significant difference (HSD) test, *t*-test analyses, and Pearson correlation coefficients were used as statistical tools (SPSS 11.0, Chicago, IL).

XAS Sample Preparation. Plant tissues were immersed in liquid nitrogen for 45 min and lyophilized at −45 °C and 69×10^{-3} Mbar pressure (LabConco Freeze Zone 4.5, Kansas City, MO). After lyophilization, the samples were ground into a fine powder and packed into 1-mm aluminum sample plates with Kapton tape windows. Samples were analyzed at the Stanford Synchrotron Radiation Laboratory (SSRL, Menlo Park, CA).

XAS Data Acquisition. The samples were run on Beamline 7-3 at SSRL using a 13-element Ge detector (Canberra Industries, Meriden, CT) and an Oxford liquid helium cryostat (Oxford Instruments, Livingstone, UK) to reduce thermal disorder in the samples. The beamline conditions were as follows: energy of 60 mA, beam current of 2.3 GeV, and a Si(220, φ90) double-crystal monochromator. The samples (ran in fluorescence mode) and model compounds (ran in transmission mode) were scanned twice to reduce noise and verify the obtained spectra. Both the samples and model compounds were run using an internal As(0) foil for calibration purposes. The model compounds were ground using a mortar and pestle until a homogeneous mixture was obtained. The dilution of the model compounds was performed using a predetermined mass of boron nitride to obtain a 1 absorption unit change across the absorption edge.

XAS Data Analysis. The XAS data analyses were performed using the WinXAS software and standard methods (*28*). The samples were averaged and calibrated using a second-degree derivative of the internal arsenic foil (energy of 11.898 keV). The pre-edge region of sample spectra were background corrected using a one-degree polynomial fitting, and the post-edge regions were background corrected using a five-degree polynomial fitting. The X-ray absorption near-edge structure (XANES) spectra of both the model compounds and the samples were extracted from the background corrected spectra from 11.80 to 11.96 keV. The extended X-ray absorption fine structure (EXAFS) were extracted from the background corrected spectra by first converting the spectra to k space, based on the energy of the photoelectron ejected from the sample. This was calculated using a second-degree derivative of the individual sample and model compound absorption edges. The extracted EXAFS spectra were subsequently k weighted to three and Fourier transformed using a Bessel window. The back transforms of the Fourier-transformed EXAFS were based on the first coordination shell of the sample spectra and were back-transformed and fitted using FEFF V 8.10 (The FEFF Project, University of Washington, Seattle, WA). The input files for the FEFF calculations were based on crystallographic data of the model compounds input into the ATOMS software (Version 2.5, Naval Research Laboratory, Washington, DC). From the FEFF output files, the interatomic distances, coordination numbers, and Debye−Waller factors were calculated.

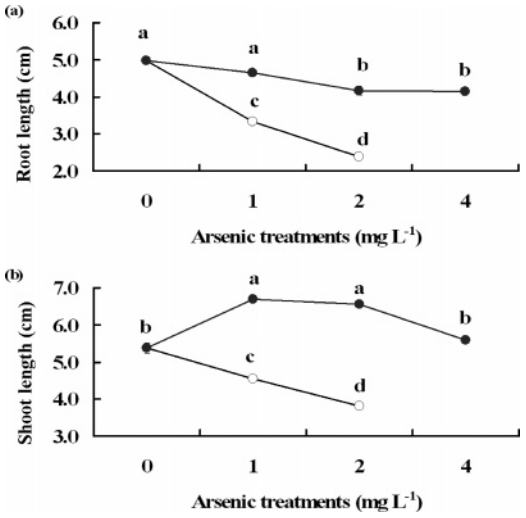

FIGURE 1. Root (a) and shoot (b) length of tumbleweed plants treated with different concentrations of As(III) (○) and As(V) (●). Data are mean of three replicates of 10 plants each ± standard error Different letters indicate statistically significant differences between treatments according to Tukey's HSD test at $P < 0.05$.

Results and Discussion

The maximum allowed concentration for arsenic in soils in the U.S.A. is of 0.4 mg kg^{-1} (*29*). We decided to test concentrations up to 10 times that amount in order to have a worst-case scenario of As toxicity and accumulation in *S. kali*. Thus, we tested As concentrations of 0, 1, 2, and 4 mg L^{-1}.

Effect of Arsenic on Tumbleweed Growth. In this study, none of the treatments significantly reduced seed germination and As(V) allowed seedlings development at concentrations up to 4 mg L^{-1}. However, it was found that, above 2 mg L^{-1}, As(III) was lethal for tumbleweed seedlings (data not shown). Neither As(III) nor As(V) treatments reduced root and leaf biomass accumulation. For all treatments, roots mass was between 3.8 ± 0.12 and 5.1 ± 0.5 mg, while the mass of the leaves ranged from 5.47 ± 0.43 to 6.77 ± 0.24 mg, with no significant differences ($P < 0.05$) between treatments. In terms of biomass accumulation, stem tissues were positively sensitive to As(V) but not to As(III). The mass of control stems and those treated with 1 and 2 mg As(III) L^{-1} were of 8.23 ± 0.43, 7.43 ± 0.54, and 7.30 ± 0.7 mg, respectively, with no significant differences ($P < 0.05$). However, an increase in biomass accumulation was detected in stems of tumbleweed plants grown in As(V), presenting masses of 9.90 ± 0.42, 10.43 ± 0.29, and 9.17 ± 0.32 mg for the 1, 2, and 4 mg As(V) L^{-1}, respectively.

According to the results, all of the As(III) treatments used in this study significantly decreased ($P < 0.05$) root and shoot elongation as compared to control and As(V) treated plants (parts a and b of Figure 1). Root elongation in plants exposed to 2 mg As(III) L^{-1} decreased about 50% as compared to control plants. It appears that shoots were less sensitive to

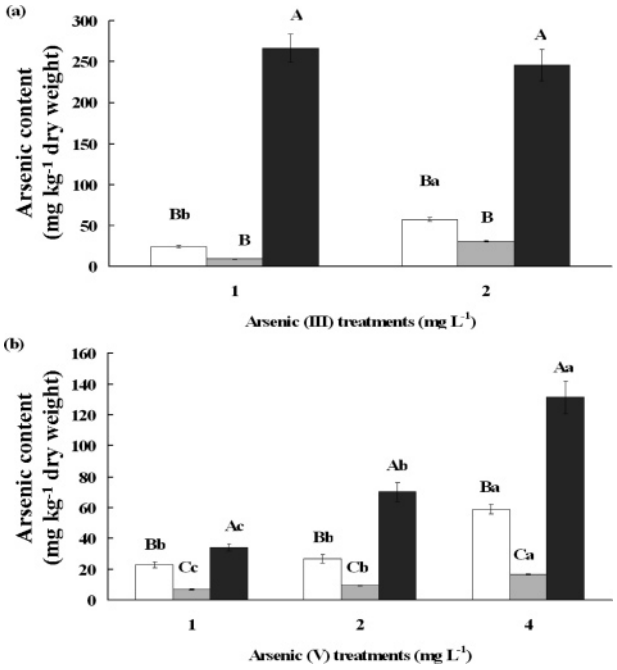

FIGURE 2. Arsenic content in leaves (□), stems (gray shaded box), and roots (■) of tumbleweed plants grown for 15 days in agar media with different concentrations of (a) As(III) and (b) As(V). Data are the mean of three replicates ± standard error. Lowercase letters indicate statistically significant differences in As content in the same tissue and different treatments; uppercase letters indicate statistically significant differences in As content between tissues of the same treatment at $P < 0.05$.

As(III) levels since the elongation of this part of the plants only decreased by about 30% as compared to control shoots. However, Figure 1b shows an increase of about 20% in shoot length in plants treated with 1 and 2 mg As(V) L^{-1} as compared to control plants; such effect is called hormesis and has been observed in a variety of living organisms (*30*). Pearson correlation coefficients calculated for root and shoot elongation against As concentration in the media were of −0.926 and −0.8, respectively (2-tailed, $P < 0.01$). Thus, the negative effect of As(III) on plant growth was noticeable. On the other hand, the correlation value for As(V) concentration in the media and root length was of −0.5 (2-tailed, $P < 0.01$). This means that As(V) did not affect root growth as much as As(III). No significant relationship was found for shoot elongation in plants treated with As(V). According to Tu et al. (*31*), the As hyperaccumulator *Pteris vittata* L. increased its biomass when transplanted to an As(V)-contaminated soil, and Carbonell-Barrachina et al. (*32*) found that As(V) increased the growth of *S. patents* and *S. alterniflora* plants. On the other hand, As(III) affected plant growth in *Oryza sativa* L. (*26*). None of the treatments produced chlorosis in tumbleweed seedlings.

Uptake of As Species by Tumbleweed. As previously explained, As(III) at 4 mg L^{-1} was lethal for tumbleweed plants. Figure 2a shows that plants exposed to 2 mg As(III) L^{-1} accumulated 245 ± 19, 30 ± 1, and 60 ± 3 mg As kg^{-1} dry weight in roots, stems, and leaves, respectively. The lack of significant differences between As concentrations in the roots of the two As(III) treatments might indicate that tumbleweed plants reached the maximum As storage concentration when exposed to As(III). However, doubling the arsenic concentration in the growth media resulted in 2- and 2.5-fold the As concentration in stems and leaves, respectively. No significant differences ($P < 0.05$) between the amounts of As accumulated in stems and leaves were found (one-way ANOVA, Tukey's HSD). Nevertheless, the trend indicates that the plant might possess the ability to move As from stems to leaves.

TABLE 1: Phosphorus Concentrations in Tumbleweed Plants Grown for 15 Days in a Modified Hoagland Nutrient Medium (*23*) Containing Either As(III) or As(V)[a]

treatments (mg L^{-1} As)	As species	mg P (kg dry weight)$^{-1}$ roots	stems	leaves
0		$11869 \pm 823_{aA}$	8272 ± 280	$8222 \pm 490_{A}$
1	As(III)	$8320 \pm 147_{b}$	7440 ± 440	7060 ± 100
	As(V)	$8453 \pm 220_{B}$	7380 ± 310	$7160 \pm 260_{AB}$
2	As(III)	$6900 \pm 310_{b}$	7130 ± 310	7200 ± 230
	As(V)	$7830 \pm 77_{B}$	7610 ± 196	$6770 \pm 55_{B}$
4	As(III)			
	As(V)	$6380 \pm 420_{B}$	7760 ± 162	$6520 \pm 67_{B}$

[a] One-way ANOVA and Tukey's test were used to separate averages. Lowercase letters indicate significant differences ($P < 0.05$) in P content between control plants and those treated with As(III). Uppercase letters indicate significant differences ($P < 0.05$) in P content between control plants and those treated with As(V). Data are mean ± standard error.

The arsenic concentration in plants stressed with As(V) is shown in Figure 2b. The data suggest that the As concentrations in leaves were statistically higher ($P < 0.05$) than the As concentrations in stems, which could indicate that As is more easily translocated from stems to leaves when supplied as As(V). According to these data, As speciation significantly affected the final concentration of this element in plant tissues. By comparison of the arsenic concentration in root tissue of plants grown with 1 mg L^{-1}As, an 8-fold amount was found in plants treated with As(III) as compared to those grown in As(V). In addition, when As was supplied at 2 mg L^{-1} level, a 3- and 2-fold increase in As concentration in stems and leaves, respectively, was found in plants treated with As(III) as compared to plants stressed with As(V) (Figure 2). The results presented herein differ from those reported by Aldrich et al. (*33*), who found a higher concentration of As in tissues of *Prosopis* spp. when the plants were grown in agar media containing As(V).

By assumption that all the arsenic in the agar is water soluble, the As bioconcentration factor (BF) in plants treated with 2 mg L^{-1} was about 170 and 54 for the As(III) and As(V) treatments, respectively. Even when arsenic concentrations found in *S. kali* are not as high as those reported for Chinese brake (*Pteris vittata*) and other fern species (*31, 34*), tumbleweed has demonstrated a better As uptake ability than other plants. For example, leaves of honey mesquite grown in an agar media containing 5 mg As(V) L^{-1} accumulated only a fifth the amount of As accumulated by tumbleweed exposed to the 4 mg As L^{-1} treatment (*32*). In addition, leaves of Indian mustard plants exposed to a 250 μM (18.75 mg As(V) L^{-1}) solution for 5 days, accumulated only a third the amount reported herein for tumbleweed leaves (*14*).

Effect of Arsenic Speciation on Phosphorus Uptake. The phosphorus uptake data (Table 1) show that the presence of both arsenic species in the growth media significantly ($P < 0.05$) reduced phosphorus content in roots as compared to controls plants. On the other hand, no significant differences ($P < 0.05$) were observed in the phosphorus concentration at the stem level. The presence of As(V) in the growth media significantly reduced phosphorus concentrations in leaf tissue. In plants treated with As(III), the Pearson correlation analysis (Table 2) revealed a negative relationship between As and P concentration in root tissue (−0.865, $P < 0.01$, 2-tailed). However, As(V) significantly reduced phosphorus content in roots and leaves (Pearson correlation coefficients of −0.871, and −0.739, respectively, at $P < 0.01$, 2-tailed). Thus, phosphorus absorption was also affected by arsenic speciation. The results indicate that more As was translocated from the As(V) medium than the As(III) growth medium

TABLE 2: Pearson Correlation Coefficients for Arsenic and Phosphorus Content in Tumbleweed Plants

plant tissue	As treatment	Pearson correlation coefficient	significance
roots	As(III)	−0.865[a]	0.003
	As(V)	−0.871[a]	0.000
stems	As(III)	−0.621	0.074
	As(V)	−0.329	0.296
leaves	As(III)	−0.564	0.114
	As(V)	−0.739[a]	0.006

[a] Correlation is significant at the 0.01 level (2-tailed).

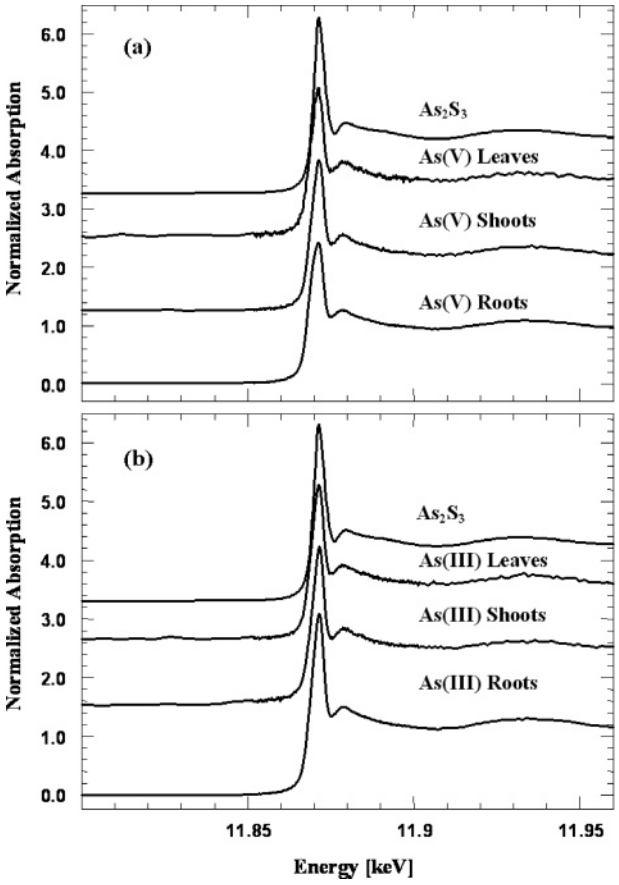

FIGURE 3. (a) XANES of the As_2S_3 model compound and of the As(V) accumulated by tumbleweed in roots, shoots, and leaves. (b) XANES of the As_2S_3 model compound and of As(III) accumulated by tumbleweed in roots, shoots, and leaves.

(Figure 2), which may be related to the decrease in phosphorus content in leaves of plants treated with As(V). Studies have reported on the reduction on phosphate uptake in plants exposed to arsenate, which indicates that arsenate uptake occurs via the phosphate system even replacing P in the phosphate groups of biomolecules (*35*−*37*). It has also been proposed that arsenate uptake via the phosphate system occurs in some arsenic nontolerant plants, while in tolerant plants such system might be altered (*35*). Therefore, in the case of As(V), it is very likely that the metalloid entered tumbleweed plants via phosphate uptake, thus reducing phosphorus concentration in roots and its subsequent transport to the leaves.

XAS Studies. The XANES spectra of As in tumbleweed plants treated with As(V) and As(III) are displayed in parts a and b of Figure 3, respectively. Previous data obtained using XAS analysis (*33*) have shown that 84% of the As(V) supplied

TABLE 3: EXAFS Fittings of the As Present in Roots, Shoots, and Leaves of Tumbleweed Plants, the As Found in the Agar, and the As Model Compounds[a]

sample	bond	N	*R* (Å)	σ^2 (Å^2)
As(V) roots	As−S	3.0	2.24	0.0030
As(V) shoots	As−S	2.0	2.26	0.0043
As(V) leaves	As−S	3.0	2.26	0.0030
As(III) roots	As−S	3.0	2.24	0.0055
As(III) shoots	As−S	2.91	2.26	0.0045
As(III) leaves	As−S	2.8	2.25	0.0044
As_2O_3	As−O	3.0	1.79	0.0026
As_2O_5	As−O	3.9	1.67	0.0015
As_2S_3	As−S	2.99	2.27	0.0015
$NaHAsO_4$	As−O	4.1	1.68	0.0034
As(V) agar	As−O	4.0	1.68	0.0040
As(III) agar	As−O	2.9	1.78	0.0015

[a] *N* is the coordination number, *R* is the interatomic distance give in angstroms, and σ^2 is the Debye−Waller factor.

to the agar media remains as As(V). Thus, important amounts of As(V) should be found in plant tissues if the plants were not able to reduced it. However, the XANES of the As in plant samples (Figure 3) indicated that As(V) was reduced to As(III) and complexed to sulfur within all sections of the plant. Nevertheless, the complex formed within the plants was not As_2S_3 as a difference in the geometry of the two different As−S complexes was observed (See Figure 1a, Supporting Information). The XANES studies of the As(III) uptake by tumbleweed shown in Figure 3b display similar results to the uptake of the As(V) from the growth media. The absorption edge shows up at the same energy as the As_2S_3 model compound, indicating that the coordination of arsenic in the plants is with sulfur and the arsenic is present as As(III). Similar to the XANES results of As in plant samples treated with As(V), different geometries for As(III)−S complex and the As_2S_3 compound was observed (See Figure 1b, Supporting Information).

The EXAFS fittings for As(V) taken up by tumbleweed in roots, shoots, and leaves are shown in Table 3. The results of EXAFS studies show that As(V) taken up by tumbleweed is coordinated solely to sulfur ligands. In addition, from the FEFF fittings of the absorbed arsenic (Table 3), the coordination of sulfur to the arsenic indicates that there are three sulfur backscatters at interatomic distances ranging from 2.24 to 2.26 Å, very similar to the As(III) sulfide. However, the As(III) taken up and bound to the sulfur within the plants is missing the As−As coordination observed at approximately 3.2 Å. The transformation of the arsenic from an oxygen ligand complex to a sulfur ligand complex might mainly occur within the roots. This arsenic−sulfur-containing complex is found throughout the entire plant (roots, stems, and leaves) as can be observed in Table 3. The EXAFS of roots, stems, and leaves of tumbleweed treated with As(III) showed that, similar to As(V), the absorbed As(III) is found within the entire plant in a sulfur-containing complex with 3 sulfur ligands at an approximate interatomic distance of 2.25 Å (Table 3, see also parts a and b of Figure 2 in Supporting Information).

The results presented herein showed that a complete biotransformation of both As(III) and As(V) to As−S complexes within all portions of the plants occurred, indicating that this is a specific response of tumbleweed to arsenic contamination. Pickering et al. (*14*) were able to identify the arsenate present in the xylem sap of *B. juncea* plants exposed for 5 days to a 250 μM arsenate treatment. Since the XAS data of this study were collected at the end of the experimental period and the results indicated the presence of solely As(III)−S chelated compounds in all plant parts, it is possible that, in the first days of treatment, part of the As(V) was transported in the arsenate form and was subsequently

reduced to the As(III)−S complexes. However, further studies are needed in order to corroborate this hypothesis.

It is known that inorganic As(III) is more toxic than inorganic As(V) mainly because As(III) readily binds to sulfur and nitrogen groups disrupting normal functions of proteins and enzymes (*38*, *39*). Thus, it is expected that As(III) rapidly reacts with proteins and enzymes present in the plasma membrane affecting structural integrity. Important data for the uptake and resistance to arsenic in *S. cerevisiae* has been obtained (*40*). However, the uptake mechanism, transport, and biotransformation of arsenic species in plants are still unclear. Furthermore, As(III) should be taken up and transformed in a different pathway than As(V). As mentioned before, part of the As(III) absorbed by the roots probably reacts with proteins and enzymes. In *S. cerevisiae*, the FPS1 gene encodes a membrane protein similar to bacterial and mammalian aquaglyceroporin involved in arsenite uptake (*41*). Thus, it is very likely that the transport of As(III) through plasmalemma in tumbleweed plants is facilitated by a type of aquaglyceroporin, similar to the one that has been found in loblolly pine (*Pinus taeda*, gi 15777892) and in common tobacco (*Nicotiana tabacum*, gi 4584428). In the biotransformation of As−O to As−S, it is very possible that low molecular weight thiols (including glutathione) react with As(III) to form complexes of the type $As(R-S)_3$. The resulting complex might be stored in vacuoles and/or transported to stems and leaves in a much less toxic form of As(III). Our hypothesis is based on Ghosh et al. (*40*), who reported that in *S. cerevisiae* the As(III) resistance includes extrusion and vacuolar sequestration (in the form of the As−$glutathione_3$ complex). These two mechanisms in this yeast are mediated by the ACR (arsenic compounds resistance) and the YCF1 gene products. Even though the YCF gene has been found in plants including *Arabidopsis thaliana, Pinus tunbergii*, *Zea mays*, and *Nicotiana tabacum* (*42*), the lack of similarity at the translated protein level suggests that the specificity of that protein is fairly different from that in ACR. In summary, more research is needed in order to identify the homologous genes in plants as well as their physiological function.

Extensive research has been performed in order to elucidate the pathway for arsenate reduction to arsenite (*14*, *43*−*45*). However, much of this work has been performed in bacteria and yeast where some families of arsenate reductases (ArsC) have been identified (*44*). To our knowledge, those enzymes have not been reported in plants (*42*). However they may be used to model the mechanism of arsenate to arsenite reduction in plants. In any case, the moieties present in the enzymes, which are responsible for the arsenate reduction, include functional groups containing sulfur. After reduction, the resulting arsenite species are over again complexed by sulfur entities, which in plants might include glutathione-related compounds such as phytochelatins, and protein residues. Some researchers have identified phytochelatins (which are glutathione-related compounds) as important molecules possibly related to As resistance in plants (*46*). Moreover, Raab et al. (*47*) reported the extraction of As−phytochelatin complexes obtained from the arsenic tolerant plant (*H. lanatus*) and the arsenic hyperaccumulator plant (*P. cretica*). The data obtained in this research suggested that tumbleweed may also produce thiol-related compounds to chelate and inactivate the absorbed arsenic.

Even when at this point tumbleweed cannot be considered an arsenic hyperaccumulator, its tolerance to the arsenate levels used in this study indicate that this plant may assist in the revegetation of disturbed sites contaminated with arsenic at low levels. In addition, different strategies including genetic engineering and the use of chelating agents can be explored in order to improve arsenic uptake by this plant species. Recently, Dhankher et al. (*48*) have reported the overexpression of two bacterial genes in *Arabidopsis thaliana*. By this manipulation, As translocation to the leaves has been improved. On the other hand, Pickering et al. (*14*) showed that mercaptosuccinate added to the growth media significantly improved As translocation in Indian mustard. This study showed that tumbleweed tolerate moderate amounts of As(V). In addition, the data provided by this research is important since few studies have been performed in desert plant species nonforming mycorrhizal association. *S. kali* is a Cd hyperaccumulator that can tolerate moderate amounts of As and thus is well suited for the remediation of mixed contaminant sites and it transforms As(V) to As(III).

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Supporting Information Available

Expanded view of the XANES region from 11.87 to 11.89 keV showing the slight deviation in geometry of the As_2S_3 and As(V) accumulated by tumbleweed plants; expanded view of the XANES region from 11.87 to 11.89 keV showing the slight deviation in geometry of the As_2S_3 and As(III) accumulated by tumbleweed plants; non-phase and amplitude-corrected Fourier transformed EXAFS of As(V) accumulated by tumbleweed roots, shoots, leaves, and As_2S_3; and non-phase and amplitude-corrected Fourier-transformed EXAFS of As(III) accumulated by tumbleweed roots, shoots, leaves, and As_2S_3. This material is available free of charge via the Internet at http://pubs.acs.org.

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