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Mechanisms of Arsenic Sequestration by *Prosopis juliflora* during the Phytostabilization of Metalliferous Mine Tailings

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Abstract

Phytostabilization is a cost-effective long-term bioremediation technique for the immobilization of metalliferous mine tailings. However, the biogeochemical processes affecting metal(loid) molecular stabilization and mobility in the root zone remain poorly resolved. The roots of *Prosopis juliflora* grown for up to 36 months in compost-amended pyritic mine tailings from a federal Superfund site were investigated by microscale and bulk synchrotron X-ray absorption spectroscopy (XAS) and multiple energy micro-X-ray fluorescence imaging to determine iron, arsenic, and sulfur speciation, abundance, and spatial distribution. Whereas ferrihydrite-bound As(V) species predominated in the initial bulk mine tailings, the rhizosphere speciation of arsenic was distinctly different. Root-associated As(V) was immobilized on the root epidermis bound to ferric sulfate precipitates and within root vacuoles as trivalent As(III)–(SR)₃ tris-thiolate complexes. Molar Fe-to-As ratios of root epidermis tissue were two times higher than the 15% compost-amended bulk tailings growth medium. Rhizoplane-associated ferric sulfate phases that showed a high capacity to scavenge As(V) were dissimilar from the bulk-tailings mineralogy as shown by XAS and X-ray diffraction, indicating a root-surface mechanism for their formation or accumulation.

Graphical abstract

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Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at DOI:10.1021/acs.est.7b04363. Experimental details. Figures showing a definition of root components, sulfur XANES results, correlation plots, semi-quantitative analysis of elemental content, XRD results, shell-by-shell fit, and wavelet analysis. Tables showing Gaussian fits, XANES matrix, linear combination fit parameters, and shell-by-shell fits. (PDF)

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Notes

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1. INTRODUCTION

Arsenic is a metalloid of significant concern in the Earth's critical zone because of its proven toxic effects on humans and animals and disruption to plant metabolism.^{1–3} In base-metal mining regions in the arid and semiarid southwestern United States, arsenic is naturally abundant as arsenopyrite (FeAsS). When exposed to oxygen and water by natural weathering, arsenopyrite dissolves oxidatively, releasing arsenate (AsO_4^{3-}) and protons to solution. This geochemical transformation is observed at the legacy mine tailings located in the Iron King Mine and Humboldt Smelter Superfund site (IKMHSS, EPA no. AZ0000309013) in central AZ.⁴ The surficial mineralogy of the IKMHSS tailings is dominated by high iron and sulfur content with the major contaminant of concern being arsenic (ca. 2100 mmol kg^{-1} Fe, 3100 mmol kg^{-1} S, and 40 mmol kg^{-1} As).^{4,5} Under the oxidizing conditions of the surficial IKMHSS tailings, ferrous sulfides naturally weather to form ferric (oxyhydr)oxides and (hydroxy)sulfates.^{4,5} These arsenic enriched secondary minerals have the potential for off-site transport as geo-dust in wind-driven erosion.^{6–9} One potential low-cost, long-term remediation method proposed for such abandoned mine tailings involves phytostabilization, i.e., the establishment of a sustainable vegetation “cap” with which to effectively contain legacy tailings particles and the associated metal(loid)s including arsenic, thereby diminishing contaminant exposure to adjacent communities.^{10–15} Compost-assisted direct planting during tailings phytostabilization has the goals of immobilizing contaminants against leaching or off-site particulate transport, establishing a positive feedback to improved soil health and fertility while decreasing contaminant leaching to groundwater.^{16,17} However, the changes in metal(loid) speciation that occur as a result of root proliferation in the porous tailings media, and that control stabilization at the molecular scale, remain poorly resolved.

Prior studies have shown that both arsenate ($\text{H}_x\text{AsO}_4^{x-3}$) and arsenite ($\text{H}_x\text{AsO}_3^{x-3}$) may be assimilated by plant roots, with arsenate subsequently being reduced to arsenite.^{3;18–20} Arsenic observed within plant tissue has been characterized as As(III) bound by three thiolate groups – defined hereafter as “As(III)-(SR)₃” – often attributed to phytochelatins, glutathione, or cysteine-like compounds.^{18,19,21–33} Prior work proposes that arsenic-bound thiol(ate) complexes may be immobilized and sequestered in root cell vacuoles as a detoxifying mechanism leading to potentially highly localized accumulation in arsenic tolerant species, but direct evidence is scant.^{2,21,33,34} Among such plants studied previously in laboratory systems is *Prosopis juliflora* (mesquite). This halophytic tree, tolerant to

growth in compost-amended IKMHSS tailings, is part of a field-scale experiment assessing long-term feasibility of a direct planting phytostabilization.³⁵ *P. juliflora* plants grown under stress in arsenic-spiked media¹⁰ have been shown to exhibit both As(V) and As(III)–S complexes either associated with or in root tissue,¹⁹ but there are no prior studies of rhizosphere arsenic speciation deriving from arsenic-bearing mine tailings systems.

Additional mechanisms of As detoxification have been reported. For example, the wetland plant species rice and cattail, which translocate oxygen to the root zone in water-logged anoxic paddy soils, have been shown to immobilize arsenic via root-associated ferric deposits or iron plaques with high arsenate-sorption affinity.³² This root-associated ferric iron³⁷ has been characterized as ferric (hydr)oxides similar to ferrihydrite and goethite.^{29,32,37–41} However, root zone biogeochemical conditions in semiarid and arid mine tailings are distinctly different from paddies and wetlands, and there are no prior detailed investigations of the partitioning and speciation of arsenic in root tissue of *P. juliflora* as it occurs in situ during mine-tailings phytostabilization.

Herein, we report the observation of Fe(III) sulfate plaques on root surfaces in a sulfate-rich environment and the direct observation of As(III)–(SR)₃ in root vacuoles using micro-imaging (1 μm^2 pixel) technology. The aim of the present study was to determine the contribution of *P. juliflora* root chemical activity to the long-term phytostabilization of arsenic in pyritic mine tailings in a semiarid climate. By combining X-ray absorption spectroscopy (XAS), multiple energy micro X-ray fluorescence ((ME)- μXRF) imaging, and bulk elemental analysis, this study reveals long-term (up to 3 years) stabilization of arsenic by *P. juliflora*. Arsenic sequestration is detected in two distinct speciation pools spatially partitioned as As(V) associated with Fe(III) sulfate plaques on the root surface and thiolate-bound As(III) in the vacuoles of the root cortex.

2. MATERIALS AND METHODS

2.1. Sample Collection

Compost-amended and unamended tailings were collected at the IKMHSS phytostabilization field site, which is described in the Supporting Information and detailed elsewhere.^{4,5,10} Briefly, the federal Superfund site is a pyritic tailings pile containing bulk arsenic concentrations of ca. 4 g kg^{−1} from arsenopyrite that has undergone oxidative weathering in the top 2 m over the past 50 years since deposition. The site is now the focus of a large-scale phytostabilization trial. The root systems of two field site mesquite (*P. juliflora*) plants were harvested at 1 and 3 years of growth, transported on ice to the laboratory, and stored at −15 °C until analysis. Additionally, to mimic early plant growth under field conditions, greenhouse plants were grown in surficial tailings (0–20 cm depth) from IKMHSS with the same field site mixture of compost amendment (Arizona Dairy Compost LLC, Anthem, AZ), at a mass concentration of 150 g kg^{−1}. Following previous work, *P. juliflora* seeds (Desert Nursery, Phoenix, AZ) were sown in 4 replicate pots (30 each) at a depth of 0.5 cm.³⁵ Quadruplicate pots produced 6 ± 3 plants, and 1 to 3 plants were harvested from each pot on days 41, 76, and 102 for the analysis of metal(loid) phytoaccumulation. Greenhouse plant materials were segregated into roots, shoots, and leaves. All plant samples were freeze-dried and sectioned using a stainless steel blade. For

bulk analysis, tissue was hand-ground by mortar and pestle (2013 field samples) or mechanically ground using a dedicated KitchenAid blade coffee grinder (model no. BCG211OB) with a spice-grinder attachment (2015 field samples and greenhouse samples). Samples were stored at -15°C and transported on ice leading up to analysis. A total of three grab samples of the tailings-compost mixture were freeze-dried, subjected to microwave-assisted digestion (CEM Corporation, MARS 6, Matthews, NC) following EPA method no. 3051A and analyzed for total metal(loid) content by inductively coupled plasma mass spectrometry (ICP-MS, PerkinElmer, ELAN, Waltham, MA) and total sulfur content by CHNSO analyzer (Costech Analytical Tech, Inc., ECS 4010, Valencia, CA). Additional detail regarding plant sample collection and tissue preparation is described in the Supporting Information.

2.2. X-ray Spectroscopy

Root samples were analyzed with K-edge XAS for speciation of arsenic, iron and sulfur. Spectra were collected at Stanford Synchrotron Radiation Lightsource (SSRL) on beamlines (BL) 11–2 for As and Fe and BL 4–3 for S. Beam energy was calibrated on an As foil with the main edge inflection assigned 11867 eV, Fe foil with the first edge inflection assigned 7112 eV, and sodium thiosulfate with the first peak (S–S) assigned 2472.02 eV. At BL 11–2, fluorescence was quantified with a 100-element solid-state Ge detector with a LN_2 cryostat sample holder ($\sim 77\text{ K}$; see the Supporting Information for XAS setup and analysis details). Sulfur XAS on BL 4–3 was monitored under $\text{He}_{(g)}$ with a passivated implanted planar silicon (PIPS) detector at room temperature. Spectral processing was performed with SixPack⁴² (average and deadtime correction) and Athena⁴³ (normalization and background subtractions), and spectral fitting was conducted with Athena and Artemis.⁴³ Additional details of bulk XAS collection and data reduction are provided in the Supporting Information.

2.3. μXRF Imaging Collection and Analysis

A pair of field-collected root segments were washed and dried as described (see the Supporting Information) prior to embedding in Paraplast Plus wax with no fixing agent, microtomed to $30\text{ }\mu\text{m}$ sections, and placed on quartz slides (part no. CGQ-0640-01 Chemglass, Inc.). Thin sections were imaged and analyzed with μXRF , μXAS , and XANES at the SSRL BL 2–3 (As, Fe, and K) with a step size of $2.5\text{ }\mu\text{m}^2$, a dwell time of 50 ms, and on BL 14–3 (S) with a step size of $5.0\text{ }\mu\text{m}^2$ and a dwell time of 75 ms. Speciation maps for As were collected at multiple energies across the absorption edge, and μXANES scans were collected at points of interest (see the Supporting Information for imaging details). Higher-resolution As speciation maps were collected at Brookhaven National Laboratory National Synchrotron Light Source II (NSLS-II) SRX beamline with a step size of $1.0\text{ }\mu\text{m}^2$ and a dwell time of 300 ms. Data were collected at multiple energies across the As edge and μXANES spectra were collected at select spots (see the Supporting Information for details). Maps were processed (deadtime corrected, normalized, PCA, and XANES imaging) using the software package SMAK.⁴⁴ Speciation maps were fit by applying a matrix of normalized spectral intensity of reference end-member spectra defined by the linear combination fit (LCF) of μXANES spot analysis from the root sample to the multiple energy stacked map. Data reduction of all μXANES was performed as above for bulk XAS.

3. RESULTS AND DISCUSSION

3.1. Elemental Distribution

Root tissue components (epidermis, cortex, and stele) of *P. juliflora* plants grown in IKMHSS tailings media exhibited dissimilar concentrations of As, S, and Fe (Table 1), where components are morphologically defined in Figure S1. To gauge bulk-scale phytoaccumulation by *P. juliflora*, As, S, and Fe molar concentrations as well as molar ratios (Fe-to-As and S-to-Fe) within plant tissue were compared with reference standard plant average values,⁴⁵ and relevant growth media for this study (i.e., compost, unamended tailings, and 15% compost-amended tailings; Table 1). Notably, the total sulfur content of all root components were within the range reported for plant averages with leaves, whole roots, epidermis, and cortex exhibiting concentrations an order of magnitude higher than the shoot or root stele. The root components of epidermis, cortex, and stele for larger roots (2–4 and 5–15 mm diameters) of plants grown in field conditions in IKMHSS tailings exhibited preferential sequestration of As and Fe in the following order: epidermis > cortex > stele (Table 1). Both arsenic and iron exceeded the standard plant average values for whole roots and epidermis (Table 1). Arsenic was preferentially phytoaccumulated in root tissue compared to shoots or leaves, signifying subsurface arsenic sequestration. However, Fe-to-As molar ratios increased with growth time in the latter (Table 1). The Fe-to-As molar ratio of whole-root tissue and epidermis were about the same or slightly lower than those of bulk tailings and half the value of 15% compost-amended tailings mixture that the plants were grown in indicating enrichment of As relative to Fe in the rhizoplane (Table 1). While arsenic is enhanced with respect to iron in the epidermis compared to the 15% compost amended tailings growth medium, the S-to-Fe molar ratio of the epidermis tissue is similar to that of the growth medium. Sulfur and iron concentrations are both higher in the root epidermis compared with the internal root and above-ground biomass samples due to development of iron and sulfur containing minerals strongly associated with the root surface (Table 1).

3.2. X-ray Fluorescence

Roots of *P. juliflora* were further investigated for arsenic microscale phytoaccumulation mechanisms. A light micrograph of *P. juliflora* in thin sections grown at the IKMHSS tailings field site for 12 months displays epidermis, cortex, and stele external and internal structure (Figure 1a). The water and nutrient transport channel of the stele is resolved by a strong potassium fluorescence intensity (Figure 1b). The total sulfur K-edge μ XRF imaging reveals that sulfur is ubiquitous throughout the root cross-section (Figure 1c). Gaussian peak fitting of bulk S XANES collected on mesquite root samples 2–4 and 5–15 mm in diameter exhibit a strong sulfate signal and co-localization of sulfate and organic thiol in all three root components (epidermis, cortex, and stele; see Figure S2 and Table S1).

3.2.1. Fe Speciation—Iron μ XRF imaging shows most Fe associated with the root epidermis (Figure 1d). Regions of interest selected for Fe μ XANES are identified by circles (Figure 1d). The averaged Fe K-edge μ XANES and the bulk Fe K-edge EXAFS reveal that the iron plaque deposit at the epidermis from Figure 1d is well-described by a LCF by the ferric sulfate minerals jarosite, $\text{XFe}_3^{\text{III}}(\text{SO}_4)_2(\text{OH})_6$, and schwertmannite, $(\text{Fe}_8^{\text{III}}\text{O}_8(\text{SO}_4))$

(OH)₆ (Figure 2b, fit parameters reported in Table S3). The root-bark Fe K-edge EXAFS is well fit to jarosite (50.4%), schwertmannite (38.9%), and chlorite (5.9%), as are the Fe K-edge normalized XANES and first-derivative XANES data (Figure 2b,c). X-ray diffraction (XRD) of the bulk root bark reveals amorphous or poorly crystalline morphology of the ferric sulfate root plaque with a small crystalline contribution corresponding to the highest intensity peaks for jarosite (Figure S5; see the Supporting Information for XRD collection and data reduction details). While a jarosite signal in the XRD of the bark is evident, signal contribution from poorly crystalline schwertmannite and high arsenic content likely account for the amorphous morphology detected for the root-associated iron plaque.^{46,47}

3.2.2. As Speciation—Principal components analysis (PCA) conducted on the As μ XRF multiple energy maps^{48,49} revealed the existence of two unique arsenic component pools in the root thin section sample. Arsenic K-edge μ XANES performed with a 2.5 μm^2 beam size were collected from both pools according to regions of interest (ROIs) 1–10 shown (Figure 1e). Arsenic speciation of two components As(V)–O and As(III)–S was determined from LCFs using endmember As K-edge μ XANES spectra (Figure 2a) collected from the thin section sample. Arsenic XANES maps for As(V) and As(III)–S (Figure 1e,f) were produced from As μ XRF images collected at 11869, 11872, 11875, and 11880 eV by applying a XANES signal intensity matrix of normalized references (Table S2). The dominant arsenic species associated with the root epidermis was As(V) (Figure 1e), whereas As(III)–S was concentrated in the cortex (Figure 1f). A tricolor plot of Fe, As(V), and As(III)–S with color intensity scales ranging from 0 to 500 counts for Fe (red) and 3–50 counts for As(V) (blue) and As(III)–S (green) was constructed from the μ XRF maps and shows strong colocalization of Fe and As(V) at the root epidermis, displayed as purple (Figure 1g; see Figure S3 for quantification of the co-localization). Thiolate-bound arsenic, As(III)–S, appears to be compartmentalized in pockets located in the cortex at the 2.5 μm^2 pixel size (Figure 1g). The breakout images (Figure 1h–k) show that further probing of the root thin section by As μ XANES and μ XRF imaging using the same method but with a 1 μm^2 pixel size, confirmed the presence of concentrated pockets of thiolate-bound arsenic (green) in the cortex and strong co-localization between As(V) (blue) and Fe(III) (red) in a tricolor plot (Figure 1k) compiled from ROIs i–viii (Figure 1i). Imaging with a 1 μm^2 beam spot size provides ca. 6-fold enhancement of spatial resolution compared with the 2.5 μm^2 beam spot maps. At this higher spatial resolution, the As(III)–S is shown to be isolated in pockets of approximately 9 μm cross-sectional diameter and isolated from the Fe(III) and As(V) concentrated at the root epidermis (indicated by dotted white line) (Figure 1i–k). At the lower resolution, As(III)–S could only be observed as diffused throughout the cortex. Resolving these small structural features of isolated As(III)–S storage provides evidence for immobilization and detoxification. Plant vacuole size is highly variable, but it is commonly reported that they can take up to 80% of a plant cell's volume in a plant cell that has been shown to measure approximately 10 μm in diameter in mesquite roots.⁵⁰ Arsenic μ XANES collected at the ROIs identified in Figure 1e,i were fit by linear combinations of end-member spectra collected from the thin section (Figure 2a and Table 2). End-members were identified for As(III)–S (1, i) and As(V) (10, vii) (Figure 2a and Table 2). Semiquantitative analysis of As, Fe, and K concentrations of a mesquite root grown for one year at the tailings field-site shows As 0–1, Fe 0–100, and K 0–100 $\mu\text{g cm}^{-2}$ (Figure S4).

Arsenic K-edge bulk EXAFS data were collected for *P. juliflora* cortex tissue of roots 2–4 mm in diameter from a plant grown at the IKMHSS field site to measure the oxidation state and speciation of arsenic to confirm the presence and proportional abundance of As(III)–(SR)₃ complexes (Figure 3). Shell-by-shell fit results indicate that cortex tissue is composed of a mixture of 63.5% As(III)–(SR)₃, in which arsenic is trigonally coordinated to sulfur and 36.5% As(V) with As tetrahedrally coordinated with oxygen (Figure 3, with fit shown in Table S4). Wavelet analysis of the shell-by-shell fit of bulk *P. juliflora* root cortex tissue As k³-weighted EXAFS identifies two distinct arsenic species contributions (As(V)–O and As(III)–S) and is provided in Figure S7. FEFF path contributions are attributed to single scattering paths As(V)–O (CN = 4, tetrahedral, 1.69 Å interatomic distance) and a multiple scattering contribution corresponding to the As(V)–O–O path (3.09 Å interatomic distance) within the arsenate tetrahedron and As^{III}–S (CN = 3, trigonal, 2.28 Å interatomic distance) (Figure 3, with fits shown in Table S3).

The observed interatomic distance of 2.28 Å between As(III) and S in the plant cortex is in agreement with previous observations of As(III)–(SR)₃ complexes found in plant tissue and organic peat samples that have been reported to range from 2.24 to 2.34 Å.^{19,21,24,25,36} For validation, As–Tris–DMSA, As–Tris–cysteine, and As–Tris–glutathione were synthesized,²¹ analyzed by As EXAFS, and fit by the same method to confirm the As–S single scattering shell of the mesquite cortex sample. The chemical structures of DMSA, L-cysteine, and glutathione are provided in the Supporting Information along with the shell-by-shell fit of As–Tris–glutathione and the corresponding fit statistics (Figure S6 and Table S5). Arsenic has been shown to coordinate with three sulfhydryl groups of humic acid (As–S interatomic distances of 2.24–2.34 Å),²⁴ and with three organic sulfur groups in peat (As–S interatomic distances of 2.24–2.25 Å).²⁵ Similar arsenic–thiol interactions have been shown to occur within the tissue of plants grown in arsenic-contaminated growth media. Previous studies of mesquite plants grown under stress of high-arsenic-spiked agar medium^{21,36} and soil^{19,26} exhibit As(III)–(SR)₃ complexation in the root interior with an As–S interatomic distance of 2.24–2.26 Å.¹⁹ However, we show in situ distribution of As(III)–(SR)₃ in discrete ~9 μm pockets, supporting the common claim that root vacuoles serve as sinks for As(III)–(SR)₃ compartmentalization and storage as a potential detoxifying mechanism for high-arsenic-containing environments.

Ecosystem-scale impacts of these sequestration mechanisms can be evaluated based on previous research on root biomass in a mature (mean parameters: 3.4 ± 0.1 m in height, 5.1 ± 0.2 m in canopy diameter, and 5.8 ± 0.4 basal stems) mesquite savanna of north Texas (mean annual precipitation of 665 mm).⁵¹ Assuming a total root biomass of 11 ± 3.6 kg per tree contained to a volume of 25.44 m³ of soil, as reported in that study, indicates an approximate subsurface arsenic phytostabilization capacity by *P. juliflora* roots of 11 mmol As per tree. The concentration of arsenic in the IKMHSS tailings in the top half-meter is 53 mmol kg⁻¹, while the bulk density of the IKMHSS tailings is about 1.5 kg dm⁻³.^{4,10} Therefore, the total arsenic that would be contained in the live mesquite root growth volume determined by Ansley et al.⁵¹ represents ca. 0.001% of the total arsenic in the bulk tailings. This value does not account for the high-fractional fine root turnover that occurs on an annual basis, and that could lead to an accumulation of the observed arsenic species in senescent root tissue. This work reveals key features of arsenic phytostabilization in mine

tailings that have not been previously reported, including the coexistence in close proximity of plant-root stabilized arsenic partitioned into distinct thiolate- and ferric-sulfate bound species whose formation was evidently promoted by root biogeochemistry (Figure 1). Previous research on the IKMHSS tailings revealed that the limited water through-flux at the semiarid site leads to persistence of sulfate that, in turn, enhances the thermodynamic stability of Fe(III)-sulfate minerals, dominantly jarosite, as products of pyrite (and arsenopyrite) weathering (Figure 4).⁵

Arsenic and iron K-edge XANES, EXAFS, and XRD data (Figure 2) suggest a disordered, arsenic-enriched, schwertmannite-like ferric hydroxysulfate structure for root epidermis-associated iron plaque that is distinct and chemically different from the surrounding growth medium of 15% compost-amended IKMHSS mine tailings. Although previous research has shown Fe strongly associated with root surfaces as Fe-(oxyhydr)oxide minerals such as ferrihydrite or goethite, a study of the binding affinity of As to schwertmannite found that under acidic conditions (pH 3–4), such as those found in the IKMHSS tailings surface (pH 2–3) As(V) introduction to the schwertmannite mineral structure, can inhibit the weathering of schwertmannite to goethite and may explain the persistence of Fe(III)-sulfate minerals in the rhizosphere.⁵⁴ While iron uptake and internal biomineralization of jarosite by the plant *Imperata cylindrical* has been reported,⁵⁵ this study reports observation of a poorly crystalline Fe(III)-sulfate root plaque that sequesters arsenic during phytostabilization by *P. juliflora* (Figure 4).

This investigation applied multi-element μ XANES imaging to resolve spatial partitioning of arsenic, iron, and sulfur spatial distribution and speciation in plant tissue to reveal two distinct mechanisms of apparent arsenic detoxification in the *P. juliflora* rhizosphere. Ferric sulfate plaques formed on root surfaces comprise elevated (relative to growth medium) concentrations of co-precipitated arsenate substituting for sulfate in the mineral structure, effectively limiting plant uptake of this toxic element in the above-ground biomass. Arsenate that nonetheless penetrates the root is reduced to As(III) and bound into As(III)-(SR)₃ complexes in root vacuoles, preventing interaction with root cellular metabolism. The latter mechanism had been postulated but not confirmed by direct analysis prior to this study. These findings present new applications for (ME)- μ XANES imaging analysis for probing metal(loid) biogeochemistry relating to plant-soil interactions.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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ABBREVIATIONS

XRF	X-ray fluorescence
XAS	X-ray absorption spectroscopy
ROI	region of interest

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Figure 1.

Microscale XRF imaging analysis of *P. juliflora* root. Images depict μ XRF maps and (ME)- μ XRF imaging for a 30 μ m thick *P. juliflora* root thin section from a plant grown at the IKMHSS tailings amended with 15% compost and lime for 1 year. Panels show (a) a light-microscope image with dotted lines delineating the root epidermis, cortex, and stele; (b) total potassium; (c) total sulfur; (d) total iron; (e) As(V); (f) As(III)-S; and (g) a tricolor plot overlaying Fe, As(V), and As(III)-S in a 10:1:1 ratio of intensity scales. The inset shows (h) total iron; (i) As(V); (j) As(III)-S; and (k) a tricolor plot overlaying Fe, As(V), and As(III)-S collected with a 6.25 $\times$ increase in spatial resolution. Circles identify regions of interest (ROIs) that were probed by μ XANES analysis (see Figure 2). White dotted lines in h-k delineate the root interior from the epidermis. Color intensity corresponds to the fluorescence signal of each chemical component per volume in each pixel, mapped at 2.5 μ m² (panels b–g) and 1 μ m² (panels h–k). Micro-XRF maps were collected at 11 880 eV (K, Fe) and 2487.5 eV (S). Arsenic speciation maps were generated from μ XANES matrix analysis from μ XRF maps collected at 11 869, 11 872, 11 8750, and 11 880 eV (Table S2).

Figure 2.

Speciation analysis of As and Fe in *P. juliflora* roots and IKMHSS tailings by XAS. (a) Normalized arsenic μXANES are shown for regions of interest (ROIs) identified in Figure 1 and were collected with a (1–10) 2.5 μm^2 and (i–vii) 1 μm^2 beam. Linear combination fits (LCF) depicted by red dotted lines were performed using ROI end-member spectra identified at the top and bottom by solid black lines with no fit represented, and values are tabulated in Table 2. The “Bulk” As XANES of the surficial tailings amended with 15% compost is shown for reference to the growth medium in panel a. The significant contribution of both As(III)–S (dark gray) and As(V) (light gray) are highlighted for the As μXANES LCF of ROIs 8 and v. Iron XAS LCF are shown for the root “Plaque” composite signal from the four thin section ROIs, the root “Bark” collected using bulk XAS, and the “Bulk” amended IKMHSS tailings for (b) Fe K-edge normalized and 1st derivative XANES and (c) Fe K-edge k^3 -weighted EXAFS. Iron XAS LCF values are reported in Table S3. Solid black lines are data; stippled red lines are least-squares best fits. References provided for comparison include ferrihydrite (Fh.), hydronium jarosite, (Jar.), schwertmannite (Sch.), chlorite (Chl.), and pyrite (Pyt.). Shaded gray panels are intended to identify peak energies for As species (As(III)–S and As(V)) and Fe minerals.

Figure 3.

Molecular-scale characterization As stored in *P. juliflora* root cortex. Arsenic k^3 -weighted EXAFS (inset), Fourier transform (FT) EXAFS uncorrected for phase shift, and the real FT components were fit using the shell by shell method. Solid black lines are data; stippled lines are least-squares best fits (fit details and wavelet transform in Table S4 and Figure S7).

Figure 4.

Modes of As immobilization by *P. juliflora* during growth in the oxidizing pyritic IKMHSS tailings. Oxidation of the IKMHSS tailings weathers the mineral-bound Fe(II), As, and S deposited as pyritic mineral to As(V)-associated poorly crystalline ferric sulfate minerals composed of Fe(III) and SO_4 . (a) Establishment of a vegetation cap on mine tailings provides a physical barrier to erosion that aids in As (blue) and Fe (red) subsurface containment in which the paired accumulation is displayed in purple. (b) Externally, arsenic bearing ferric sulfate mineral products of arsenopyrite oxidative weathering develop on the root epidermis, presumably as a result of As(V) (blue) scavenging and immobilization from pore waters during ferric plaque formation (red), where purple indicates a prominence of As(V) and Fe(III) spatial co-localization. (c) Internally, reduced As(III), which was never detected in the bulk tailings or on the root exterior, is immobilized by complexation with thiolates such as phytochelates that were modeled by As(III)-(GLU)₃ (green) and stored in vacuoles in the root cortex. Mineral structures were taken from published models.^{52,53}

Table 1

Total Elemental Concentrations for *P. juliflora* Samples

	growth time (months)	total concentration ($\mu\text{mol g}^{-1}$) ^a			Fe	molar ratio of Fe/As	molar ratio of S/Fe
		S	As				
whole root ^b	1	167 (53)	0.29 (0.14)		11 (4)	37	15
whole root ^b	2	114 (38)	0.50 (0.31)		20 (13)	40	6
whole root ^b	3	105 (32)	0.20 (0.11)		9 (5)	44	12
whole root ^c	12	202 (1)	2.15 (0.33)		41 (6)	19	5
whole root ^c	36	166 (6)	1.03 (0.47)		35 (14)	34	5
shoot ^b	1	47 (1)	0.01 (0.00)		0.58 (0.14)	54	81
shoot ^b	2	35 (7)	0.01 (0.00)		0.79 (0.44)	73	45
shoot ^b	3	23 (5)	0.00 (0.00)		0.52 (0.18)	70	44
leaves ^b	1	70 (4)	0.11 (0.04)		3.11 (0.87)	29	23
leaves ^b	2	132 (26)	0.08 (0.03)		4.23 (0.44)	56	31
leaves ^b	3	140 (27)	0.09 (0.03)		6.53 (1.68)	71	21
epidermis, 2–4 mm diameter ^c	12	153 (22)	1.61 (0.21)		51.7 (6.7)	32	3
epidermis, 2–4 mm diameter ^c	36	202 (6)	3.13 (0.34)		99.9 (9.8)	32	2
epidermis, 5–15 mm diameter ^c	12	204 (12)	3.64 (0.76)		117.8 (18)	32	1.7
epidermis, 5–15 mm diameter ^c	36	165 ^d	2.75 (0.13)		105 (8)	38	1.6
cortex, 2–4 mm diameter ^c	12	101 (0)	0.21 (0.04)		4.89 (1.44)	23	21
cortex, 2–4 mm diameter ^c	36	125 (32)	0.06 (0.02)		2.04 (0.11)	33	61
cortex, 5–15 mm diameter ^c	12	55 (23)	0.15 (0.03)		4.97 (1.34)	33	11
cortex, 5–15 mm diameter ^c	36	47 (2)	0.08 (0.01)		2.5 (0.2)	30	19
stele 2–4 mm diameter ^c	12	86 (17)	0.12 (0.04)		2.67 (0.5)	23	32
stele 2–4 mm diameter ^c	36	36 (2)	0.02 (0.01)		0.55 (0.09)	25	65
stele 5–15 mm diameter ^c	12	39 (1)	0.07 (0.03)		2.17 (0.35)	31	18
stele 5–15 mm diameter ^c	36	20 (4)	0.02 (0.01)		0.57 (0.08)	30	36

	total concentration ($\mu\text{mol g}^{-1}\text{dw}$)			molar ratio of Fe/As	molar ratio of S/Fe
	S	As	Fe		
standard plant average ^e	18.7–311.9	0.00–0.02	0.09–3.58	–	–
bulk tailings 0–20 cm ^f	2500 (300)	54 (6)	1950 (200)	36	1.3
15 wt % compost mixture ^f	2050 (80)	27.4 (0.4)	1589 (25)	58	1.3
compost ^f	274 (26)	0.013 (0.004)	65 (19)	5000	4.2

^aTotal elemental analysis by ICP-MS after microwave digestion of subsectioned components.

^bGreenhouse-grown *P. juliflora*, average and standard deviation (in parentheses) of quadruplicate samples.

^c*P. juliflora* from IKMHSS field site, with average and standard deviation reported from duplicate composite sample.

^d $n = 1$ due to limited samples.

^eRange reported is the average content of elements expected to occur in tissue of a typical plant.⁴⁵

^fResults from triplicate analysis.

Table 2Arsenic K-edge μ XANES Linear Combination Fit Statistics^a

2.5 μm^2 beam As K-edge normalized μXANES				
As-XANES point	fit (%)		ΣAs_i	
	As(III)–S	As(V)	total	red χ^2
1 As(III)–S end-member	100	0	100	na
2	96	3	99	0.002
3	95	3	98	0.002
4	94	3	97	0.003
5	94	4	98	0.002
6	87	11	98	0.002
7	81	17	98	0.002
8	41	55	96	0.001
9	9	85	94	0.003
10 As(V) end-member	0	100	100	na

1 μm^2 beam As K-edge normalized μXANES				
As-XANES point	fit (%)		ΣAs_i	
	As(m)–S	As(V)	total	red χ^2
i As(III)–S end-member	100	0	100	na
ii	100	2	102	0.001
iii	99	2	101	0.001
iv	93	7	100	0.001
v	72	28	100	0.001
vi	7	91	98	0.001
vii	1	97	98	0.001
viii As(V) end-member	0	100	100	na

^aSamples correspond to ROIs defined in Figure 1e,i. na: not applicable.^bPercent fit (%) for contributing component species, total fit (ΣAs_i), and reduced chi squared (χ^2) using end-member XANES collected from the thin-section root sample are reported for spectra collected using 2.5 and 1.0 μm^2 beams.