

***Pteris vittata* Arsenic Accumulation Only Partially Explains Soil Arsenic Depletion during Field-Scale Phytoextraction**

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Supplemental Information

Micro-focused and bulk X-ray spectromicroscopy method details

X-ray microprobe analyses were performed at the Advanced Light Source XFM beamline 10.3.2 [1]. Thirty micron-thick thin sections were mounted with silicon vacuum grease onto a Peltier-cooling stage with a window film installed in front of the samples. N₂ gas flowed in front of the window to avoid frost buildup. All data were collected in fluorescence mode at -20°C and ambient pressure using a Canberra 7-element Ge solid state detector and processed using a suite of LabVIEW custom software available at the beamline.

μXRF Mapping: Arsenic and iron spatial distribution in the samples was determined using micro-focused X-ray fluorescence (μXRF) elemental mapping with a 12 keV incident beam, using a 50 ms/pixel dwell time, a 2 × 2.5 μm beam spot size, and a pixel size of 3 × 3 μm (Figure 3A), 7 × 7 μm (Figure 3B), or 10 × 10 μm (Figure 3C). Maps were deadtime-corrected. Decontamination was not required.

μXANES: In sample regions of interest, arsenic and iron speciation were determined using arsenic K-edge and iron K-edge micro-focused X-ray absorption near edge structure (μXANES) spectroscopy. Spectra were calibrated using sodium arsenate (E₀=11875 eV) and elemental iron (E₀=7110.75 eV), respectively. All spectra recorded in the range 11770-12177 eV (As) and 7011-7416 eV (Fe) were deadtime-corrected, deglitched, calibrated, pre-edge background subtracted, and post-edge normalized using

a suite of custom LabVIEW software available at the beamline. If counts were low, spectra were collected at 2 adjacent spots and merged.

Bulk XAS: Bulk XANES spectra were collected at SSRL beamline 7.3. Filter membranes were sealed and mounted on sample holders with Kapton tape. Data were collected in fluorescence mode using a cryostat sample holder at 10 K and a Canberra 30 element Ge solid state detector. Spectra collected in the range 11635-12877 eV (As) and 6880-8126 eV (Fe) were calibrated to a reference gold ($E_0=11919$ eV) foil (As) or iron ($E_0=7112$ eV) foil (Fe) in SixPak (Webb 2005), and normalized and plotted in Athena.

Least-square linear combination fitting: LSQ LC fitting of experimental XANES spectra (micro and bulk) was performed using custom LabVIEW software for arsenic in the range of 11770-12177 eV using a library of 68 standard arsenic compounds, and for iron in the range of 7011-7416 eV using a library of 79 standard iron compounds. The best LCF was obtained by minimizing the normalized sum of squares residuals (NSS=0=perfect fit), according to the formula $NSS = 100 \times \{\sum(\mu_{\text{exp}} - \mu_{\text{fit}})^2 / \sum (\mu_{\text{exp}})^2\}$ where μ represents the normalized absorbance. Some spectra were corrected for over-absorption induced distortion using $\mu_{\text{corrected}} = \mu_{\text{exp}} / (1 + a(1 - \mu_{\text{exp}}))$ where the parameter a was adjusted. The error on the percentages of species found via LC fitting is estimated to $\pm 10\%$. 2D scatter valence plots were generated from Fe and As XANES data using a Matlab code available at beamline 10.3.2. For iron valence plots, kappa and mu represent normalized absorption values at 7113 and 7117.5eV respectively. For arsenic

valence plots, kappa and mu represent normalized absorption values at 11870.8 and 11889.9 eV respectively. Standards are plotted as black empty squares and sample data points are in color.

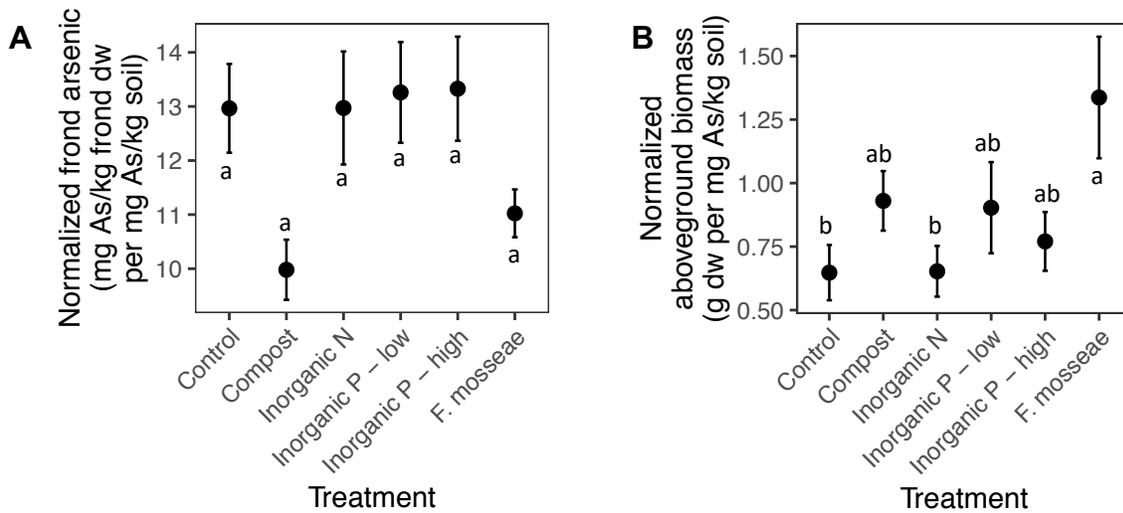


Figure S1. Mean fern arsenic concentrations (A) and fern aboveground biomass (B) by treatment, normalized to soil arsenic concentrations, at final harvest, 58 weeks after fern transplanting into arsenic-contaminated soils. n = 24. Symbols represent mean values *per* treatment, and error bars represent standard error of the mean. Means with the same letter are not significantly different (P<0.05).

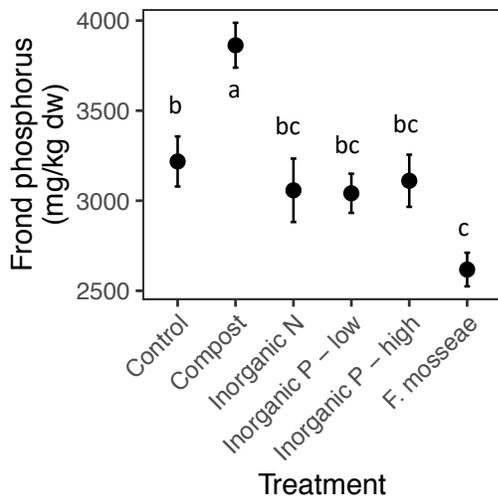


Figure S2. Mean phosphorus concentrations in dry fern aboveground biomass by treatment at final harvest, 58 weeks after fern transplanting into arsenic-contaminated soils. n = 24. Symbols represent mean values *per* treatment, and error bars represent standard error of the mean. Means with the same letter are not significantly different (P<0.05).

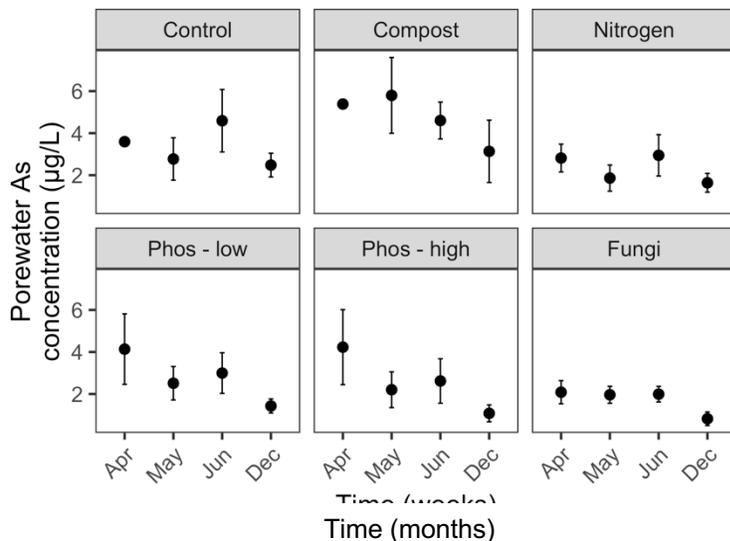


Figure S3. Temporal changes in mean arsenic concentrations in porewater collected from soil 0-10 cm deep 15 cm from fern crowns starting 5 months after fern transplant into arsenic-contaminated soils. Panels represent soil treatments. Error bars represent standard error of the mean. n = 6 plots/treatment.

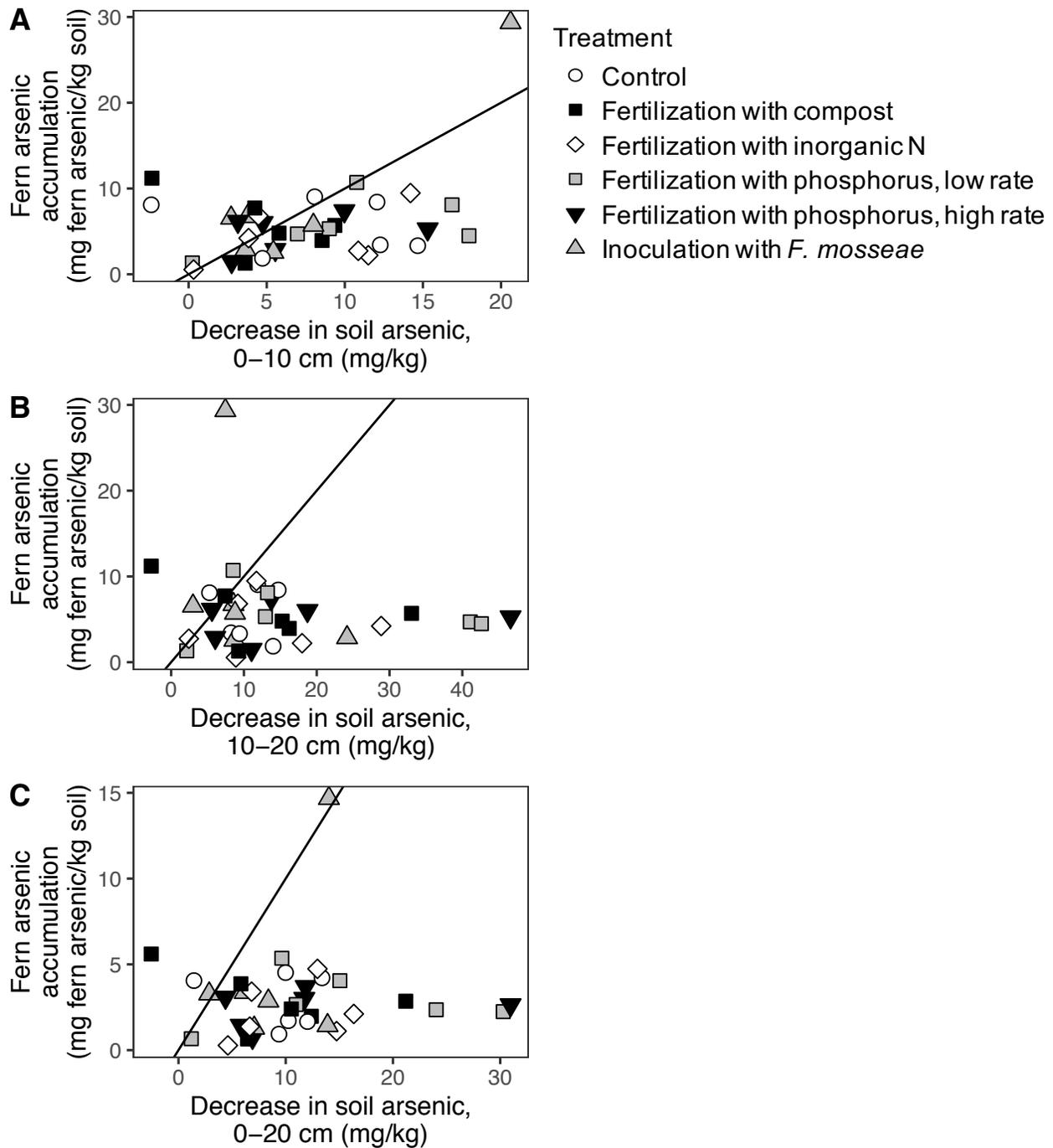


Figure S4. Mean arsenic contained *per* fern, normalized *per* kilogram soil, plotted by decrease in soil arsenic concentrations in (A) 0-10 and (B) 10-20 cm depth, assuming all arsenic came from 1 depth, and (C) across 0-20 cm depth. The line indicates a 1-1 relationship between fern arsenic accumulation (Y axis) and soil arsenic depletion (X axis). Fern arsenic uptake is mean of 4 replicates, soil arsenic mean final arsenic (3 replicates) subtracted from mean initial arsenic (3 replicates).

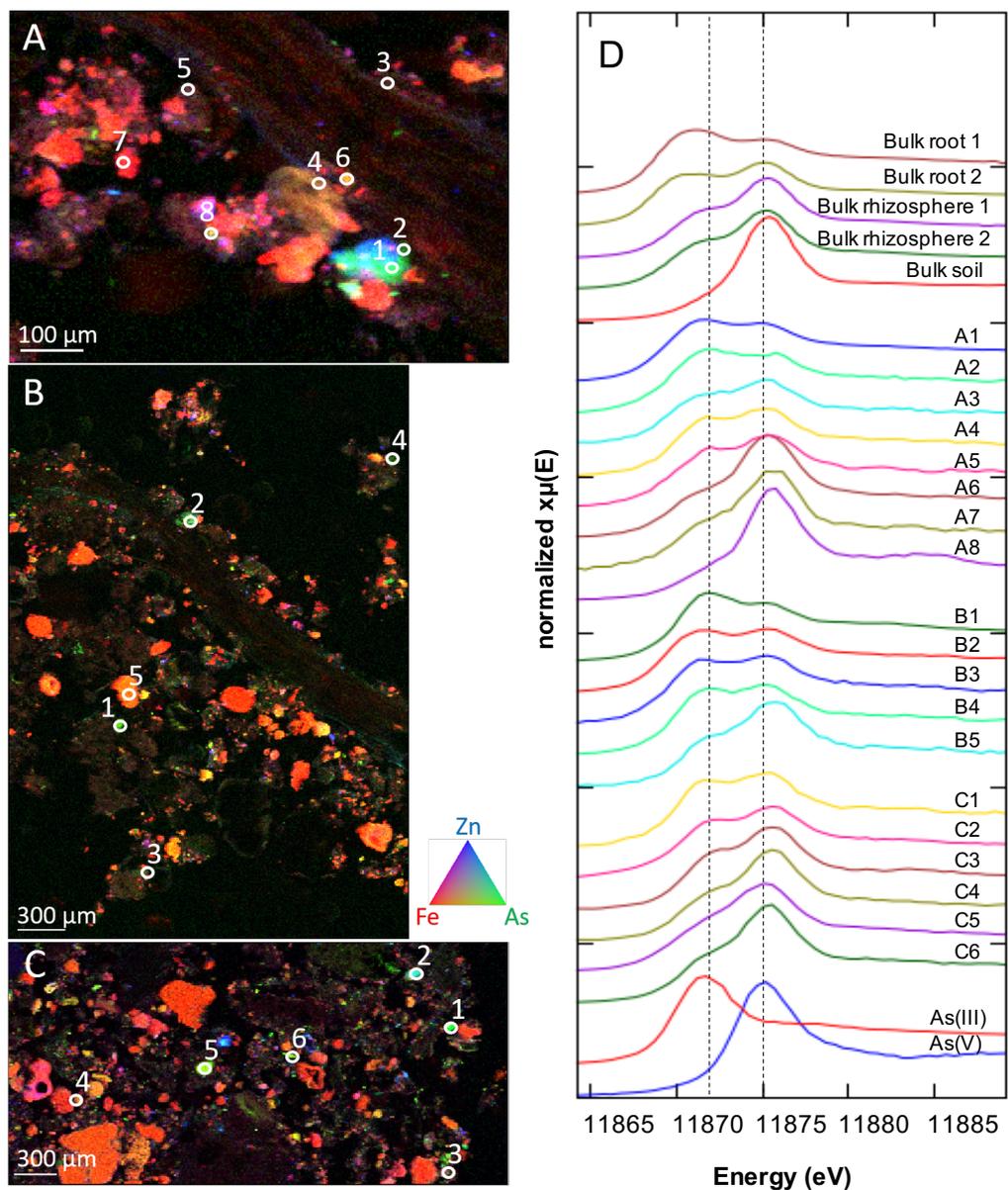


Figure S5. Tricolor-coded micro- X-ray fluorescence maps of As, Fe, and Zn in (a, b) untreated (control) roots with rhizosphere soil and (c) untreated (control) soil aggregate. The tri-color shield indicates color in panels A to C. Within an image, brighter colors indicate higher fluorescence signal. (d) Selected arsenic K-edge bulk and micro XANES data: bulk spectra from powdered untreated (control) roots, rhizosphere soil, and bulk soil; micro spectra for 8 spots across root cross-section A; 5 spots across root cross-section B; and 6 spots across aggregate cross-section C (spots 1-3 are on aggregate edge; spot 4 is approximately aggregate center). As(III) and As(V) standard spectra that were included in the best-fit results from LCF analysis are shown in panel D for reference. The complete library of XAS standards is available in the Supplemental Information. Vertical lines denote energies at 11872 and 11875 eV that were used to differentiate As(III) and As(V) species, respectively.

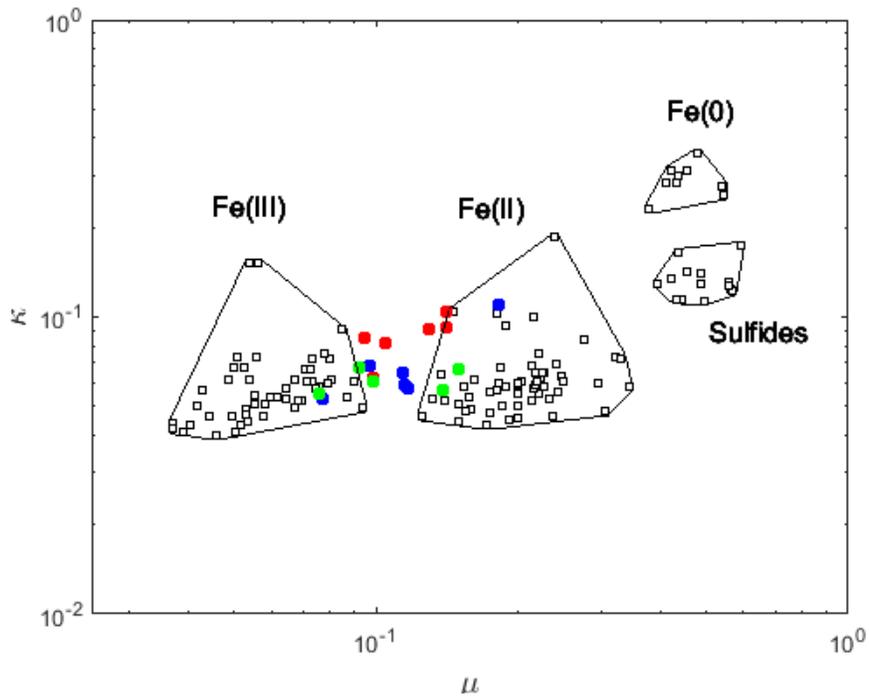
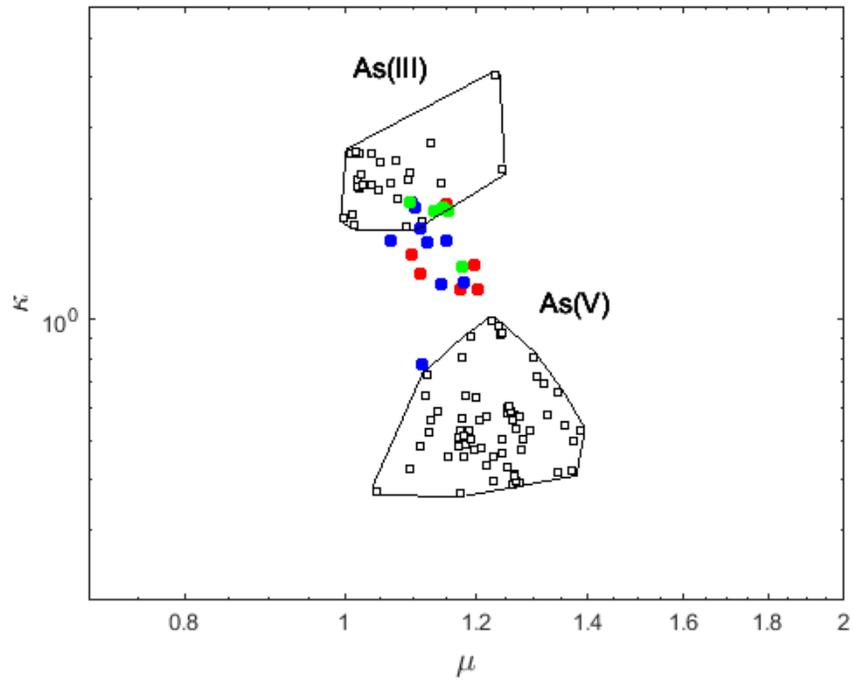


Figure S6. Valence scatter plots of arsenic (top) and iron (bottom) oxidation state in root and soil samples in Figure 3A (blue), Figure 3B (green), and Figure 3C (red). Standards are plotted as black empty squares. In the As plot, kappa and mu represent normalized absorption values at 11870.8 and 11889.9 eV respectively. In the Fe plot, Kappa and mu represent normalized absorption values at 7113 and 7117.5eV

Table S1. Least-square linear combination fits for arsenic K-edge bulk and micro (A1-C6) XAS spectra in Figure 3D.

Spectra	As(III) %	As(V) %	Normalized Sum of Squares
Bulk root 1	101.8	6.1	2.59E-04
Bulk root 2	75.5	26.3	2.90E-04
Bulk rhizosphere 1	48.6	52.5	4.04E-04
Bulk rhizosphere 2	48.3	50.4	4.25E-04
Bulk soil	9.9	102.9	4.20E-04
A1	71.5	29.1	1.81E-04
A2	71.2	30.8	6.41E-04
A3	57.7	42.6	8.26E-04
A4	55.0	46.1	5.76E-04
A5	54.1	46.9	1.05E-03
A6	30.4	70.5	5.06E-04
A7	31.7	71.0	1.56E-03
A8	12.8	89.8	1.51E-03
B1	70.3	31.8	4.93E-04
B2	67.6	34.3	3.40E-04
B3	64.0	38.3	7.75E-04
B4	61.5	40.3	5.42E-04
B5	42.9	60.1	1.47E-03
C1	64.8	37.7	8.15E-04
C2	53.4	46.9	4.60E-04
C3	49.1	52.0	7.11E-04
C4	40.0	61.0	6.08E-04
C5	38.4	63.8	4.75E-04
C6	27.9	73.9	3.44E-04

Table S2. Linear combination fits for iron K-edge bulk and micro (A1 -C6) XAS spectra collected from bulk samples or microXANES spots in Figure 3(A-C) and/or Figure SI-5(A-C). Continued on next page.

Spectra*	%	Mineral group	Component 1	%	Mineral group	Component 2	%	Mineral group	Component 3	Norm. Sum of Squares
Bulk root	25.7	Iron (III) oxyhydroxide	Goethite	53.0	Iron (III) oxide	Hematite	19.7	Iron (II-III) silicate	Illite-smectite mixed layer silicate	5.68E-05
Bulk rhizo-sphere 1	2.8	Iron (II-III) silicate	Chlorite (1)	91.0	Iron (III) oxide	Hematite	4.3	Iron (III) silicate	Smectite	3.57E-05
Bulk rhizo-sphere 2	3.5	Iron (II-III) silicate	Chlorite (1)	88.6	Iron (III) oxide	Hematite	19.4	Iron (II-III) silicate	Illite-smectite mixed layer silicate	5.73E-05
Bulk soil	1.2	Iron (II-III) silicate	Chlorite (1)	94.0	Iron (III) oxide	Hematite	2.8	Iron (III) silicate	Smectite	4.00E-05
A1	10.7	Iron (II-III) silicate	Chlorite (1)	10.6	Iron (II) sulfate	Iron sulfate	79.7	Iron (II-III) silicate	Illite-smectite mixed layer silicate	4.22E-04
A2	12.7	Iron (II-III) silicate	Chlorite (1)	49.8	Iron (II-III) silicate	Illite-smectite mixed layer silicate	37.7	Iron (II) sulfate	Melanterite	1.89E-04
A3	12.8	Iron (II-III) silicate	Chlorite (1)	62.2	Iron (II-III) silicate	Ferrosmeectite	24.5	Iron (II) sulfate	Iron sulfate	7.21E-05
A4	35.8	Iron (III) oxyhydroxide	Ferrihydrite	49.8	Iron (III) oxyhydroxide	Goethite	14.9	Iron (II) silicate	Hedenbergite	9.64E-05
B1	12.4	Iron (II-III) silicate	Chlorite (1)	61.4	Iron (II-III) silicate	Ferrosmeectite	25.8	Iron (II) sulfate	Melanterite	3.42E-05
B2	14.8	Iron (III) oxyhydroxide	Goethite	5.2	Iron (II) silicate	Hedenbergite	79.6	Iron (III) oxide	Hematite	4.11E-05
B3	38.5	Iron (II-III) silicate	Chlorite (2)	10.7	Iron (III) silicate	Gehlenite	52.7	Iron (III) oxyhydroxide	Goethite	1.35E-04

Table S2 con't. Linear combination fits for iron K-edge bulk and micro (A1-C6) XAS spectra collected from bulk samples or microXANES spots in Figure 3(A-C) and/or Figure SI-5(A-C).

Spectra*	%	Mineral group	Component 1	%	Mineral group	Component 2	%	Mineral group	Component 3	Norm. Sum of Squares
B4	10.4	Iron (III) silicate	Iron (III) silicate	51.1	Iron (II) sulfate	Iron sulfate	37.6	Iron (III) oxyhydroxide	Goethite	5.55E-05
C2	1.6	Iron (II) silicate	Ferrosilite	7.3	Iron (III) silicate	Kaolinite	93.0	Iron (III) oxide	Maghemite	5.08E-05
C3	28.0	Iron (III) carbonate	Coalingite	36.8	Iron (III) polysaccharide	Iron (III) dextrans	34.4	Iron (III) oxide	Hematite	3.35E-05
C4	1.7	Elemental iron	Iron foil	4.3	Iron (II) silicate	Hedenbergite	93.6	Iron (III) oxide	Hematite	1.01E-04
C5	11.9	Iron (II-III) silicate	Chlorite (1)	68.7	Iron (II-III) silicate	Ferrosmectite	18.7	Iron (II) sulfate	Melanterite	3.87E-05

Chlorite (1) = chlorite_r2137 standard

Chlorite (2) = chlorite_r2139 standard

*Satisfactory fits could not be obtained for all spots due to reference database limitations.

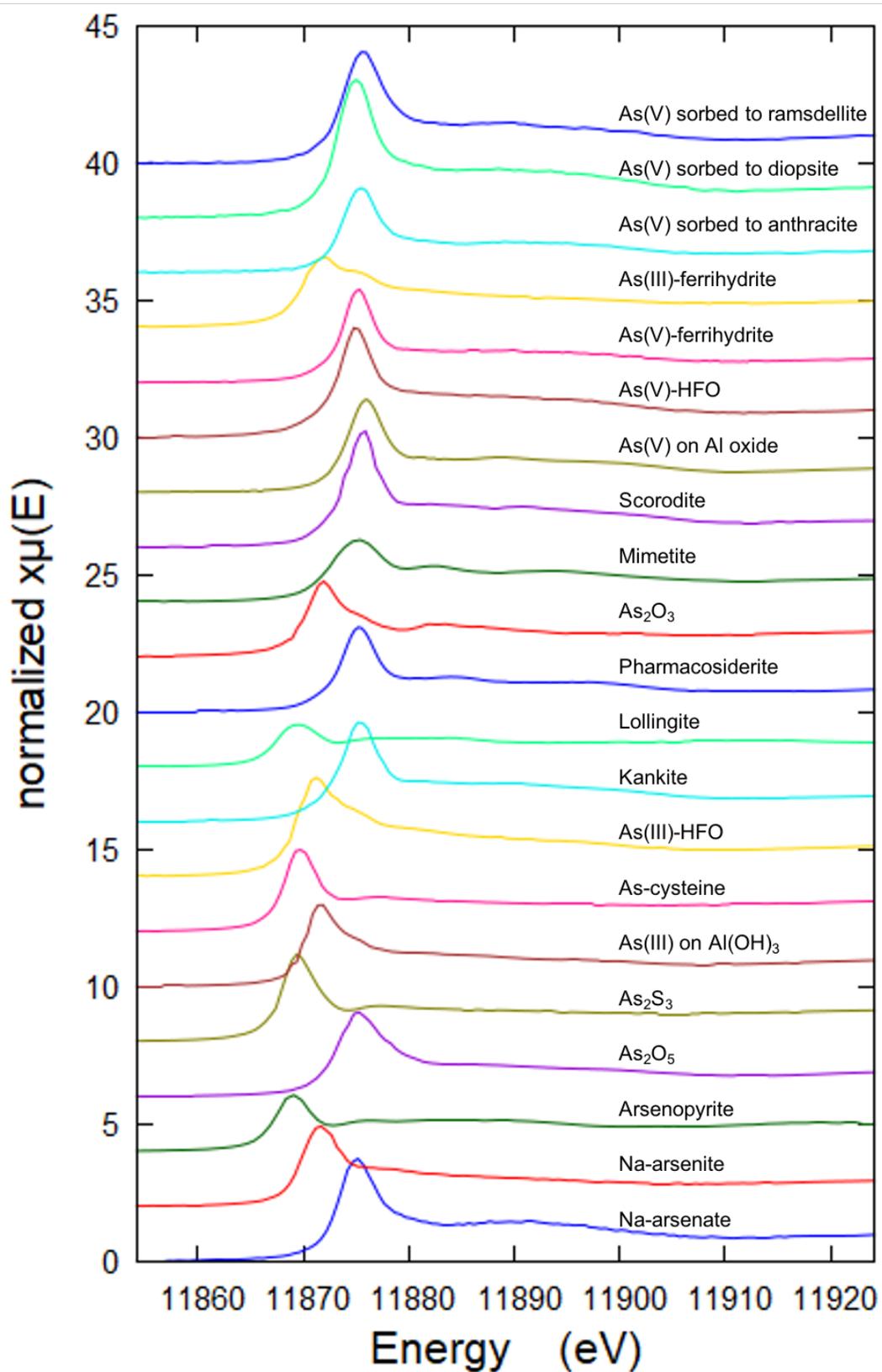


Figure S7. Standard spectra used in LC fitting of arsenic K-edge spectra shown in Figure 3D and Figure S5.

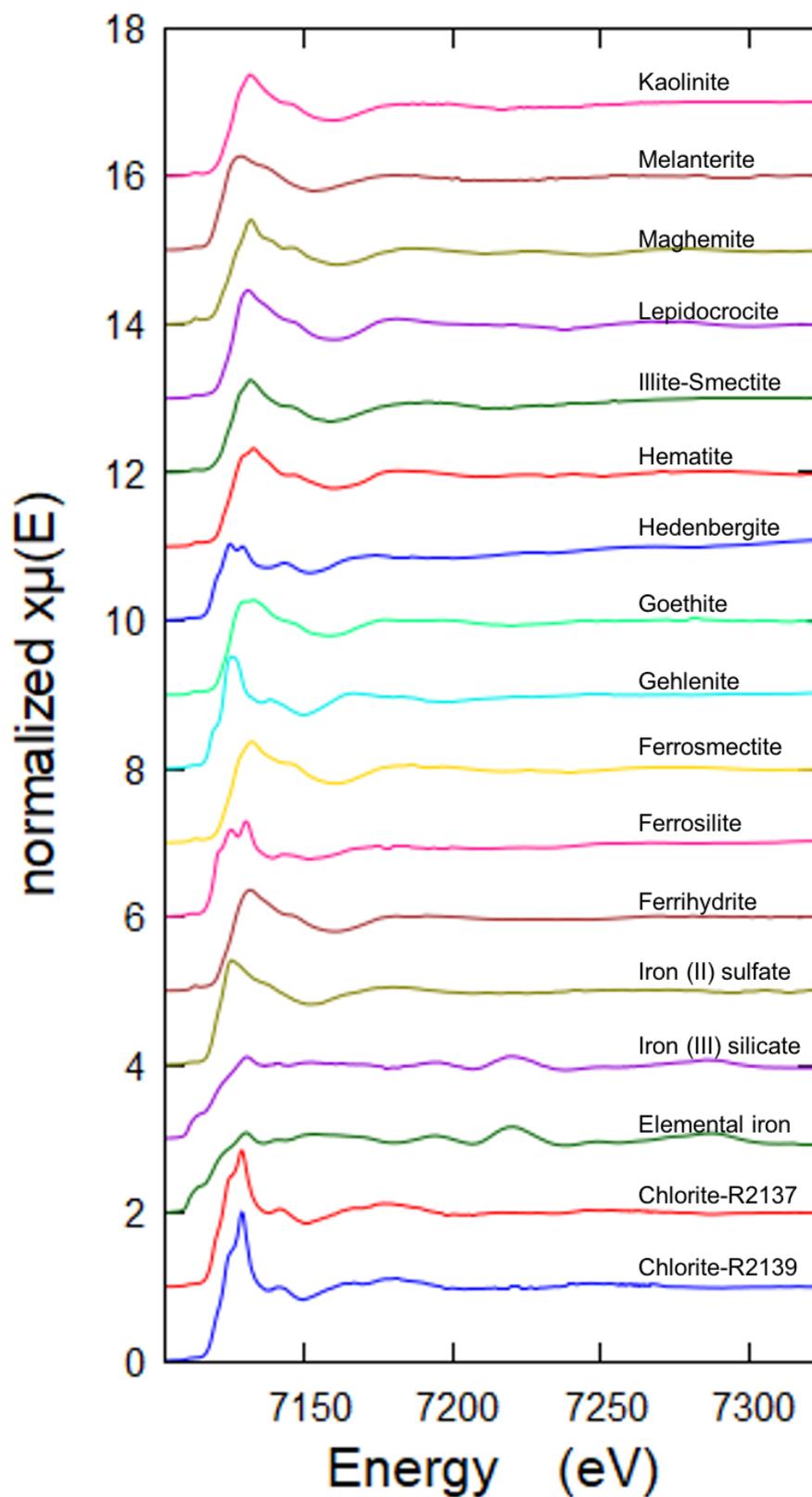


Figure S8. Standard spectra used in LC fitting of iron K-edge spectra, see results in Table S2.