Supplementary Information For:

Cu, Pb, and Zn Sorption to Bacteriogenic Iron Oxyhydr(oxides) Formed in Circumneutral Environments

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This supporting information includes 12 pages, 1 methods section, 4 figures, 5 tables, and references.

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SI Methods

BIOS Composites

Once in the laboratory, the BIOS samples were centrifuged for 10 min at 10,000 × *g*. The supernatant was decanted and the BIOS were pooled into 50 mL centrifuge tubes by sampling date. The BIOS composites were then suspended in DI, followed by vortexing to ensure adequate homogenization. The BIOS were centrifuged one last time at 10,000 × *g* for 10 min. The supernatant was disposed of, and the BIOS composites were stored in the freezer at -20°C until further use.

2LFh Synthesis and Processing

A 0.331 M solution of $Fe(NO)_3 \bullet 9H_2O$ in DI was neutralized with 1 M KOH addition while under vigorous mixing on a stir plate. The pH of the suspension was held constant at 7.5 ± 0.5 for 30 minutes before transferring into multiple 50 mL PP centrifuge tubes. The ferrihydrite suspensions were then centrifuged at 10,000 × *g* for 10 min, the supernatant was discarded and the resulting 2LFh was rinsed with DI. This centrifuge-rinse cycle was repeated four more times. The 2LFh suspensions were combined into one 50 mL PP centrifuge tube, raised to volume with DI, and centrifuged a final time at 10,000 × *g* for 10 min. The supernatant was discarded, and the 2LFh composite was placed into the freezer at -20°C.

Characterization of BIOS and 2LFh

Both the BIOS and 2LFh were analyzed for elemental composition. The C and N content were determined via total combustion using a Perkin Elmer Series II-2400 CHNS/O analyzer. For digestions, 50 mg of BIOS and 2LFh were dissolved in 10 mL of concentrated HCl and 10 mL of concentrated HNO_3, and then diluted to 45 mL with DI water. After digestion, a 20 mL aliquot of the samples were filtered with a 0.22 μm nylon filter and analyzed with a Perkin Elmer Optima 8000 inductively coupled plasma-optical emission spectrophotometer (ICP-OES) for Fe, Al, Ca, Cu, K, Mg, Mn, Na, Pb, Zn, Si, P, and S. For synthetic 2LFh, only Fe was quantified.

BIOS and 2LFh mineral phase was examined via powder X-ray diffraction (XRD). Briefly, samples of 2LFh and BIOS were dried for 24 hr at 100°C, which has been determined not to cause a phase change in 2LFH [1]. After drying, the powders were ground with an agate mortar and pestle. Around 200 mg of sample was then loaded onto a glass sample holder and analyzed with a Rigaku SmartLab X-ray diffractometer with graphite monochromated Cu $K\alpha_1$ radiation. The powder samples were scanned from 20 to 90° 2θ with increments of 0.05° 2θ.

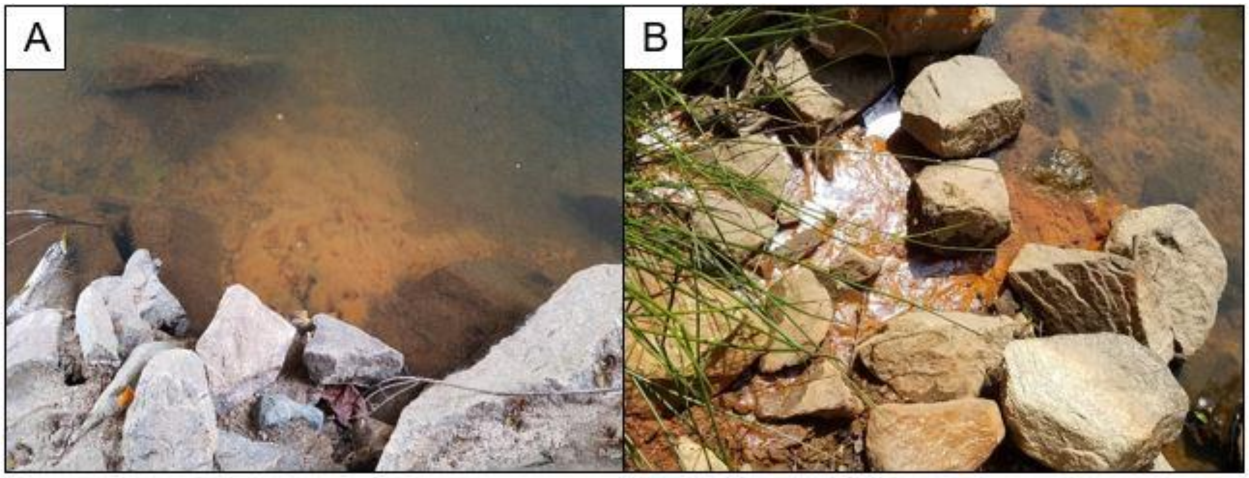

Figure S1. Visible differences in (A) fluffy BIOS and (B) clay-like BIOS sampled at Rocky Branch Creek (35°46′49″N 78°40′01″W; Raleigh, North Carolina) [2-4].

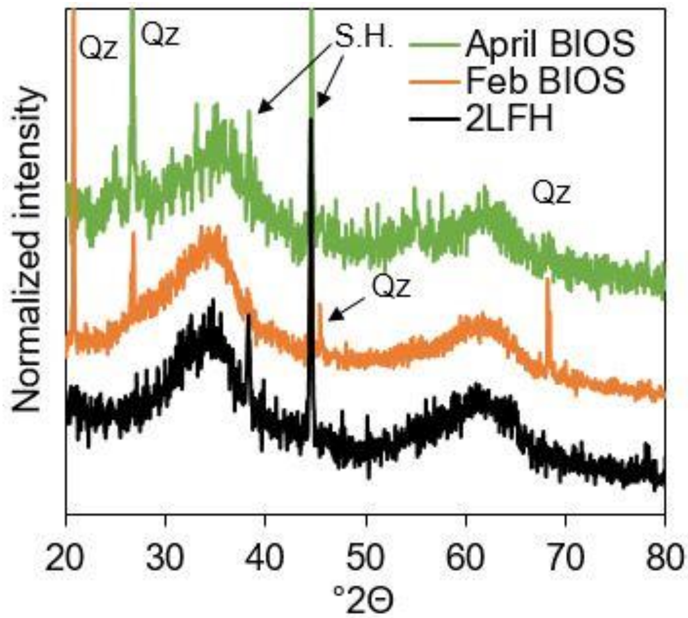

Figure S2. X-ray diffraction patterns for synthetic 2LFh and BIOS collected with a Rigaku SmartLab X-ray diffractometer with graphite monochromated Cu K-α radiation. For all samples, two broad diffraction inflections are seen at ~35 °2Θ and ~62 °2Θ which corresponds to d-spacings of 2.55 Å and 1.5 Å, respectively. The sharp peaks in the BIOS samples labeled Qz are due to the crystalline impurity quartz, whereas the sharp peaks labeled S.H. in all samples are due to the sample holder.

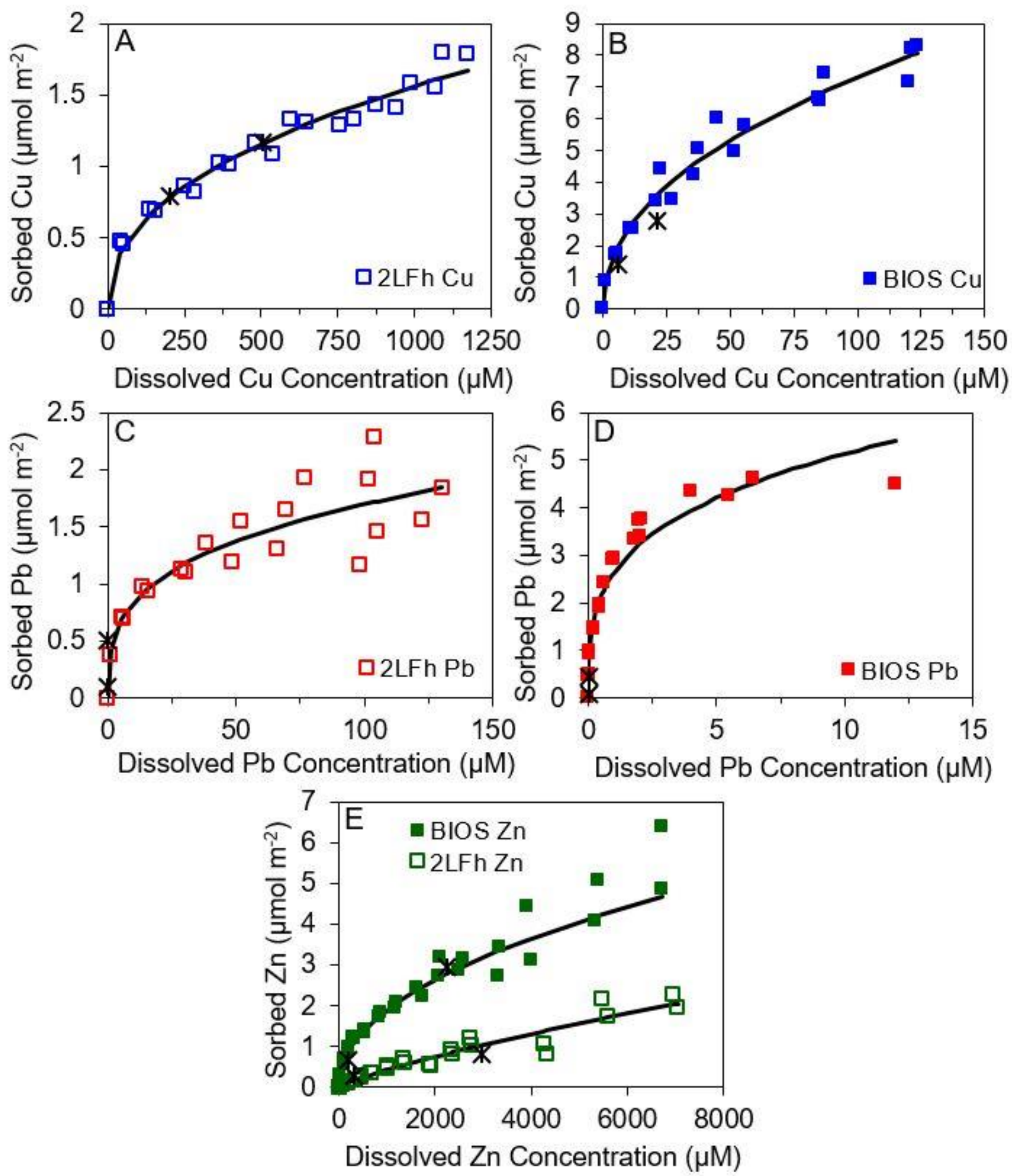

Figure S3. BET SSA normalized sorption of (A) Cu onto synthetic 2LFh (open triangle), (B) Cu onto BIOS (solid triangle), (C) Pb onto synthetic 2LFh (open square), (D) Pb onto BIOS (solid square), (E) Zn onto synthetic 2LFh (open circle) and BIOS (solid circle). Due to large differences in Cu and Pb equilibrium concentrations between BIOS and 2LFh, isotherms were plotted separately. The black ×'s represent the Feb BIOS and 2LFh surface loadings that were used for XAS studies. Data sets were modeled with a Freundlich sorption isotherm model (solid black line), with fit parameters shown in **Table S4**. Initial experimental conditions: 1 g L^{-1} (0.25 g L^{-1} for Pb) sorbent (dry weight basis), Cu(II) = 0-01.57 mM, Pb(II) = 0-0.24 mM, Zn(II) = 0-15.30 mM, I = 0.01 M $NaNO_3$, pH = 6.0 ± 0.1, Temp = 23 ± 2 ° C.

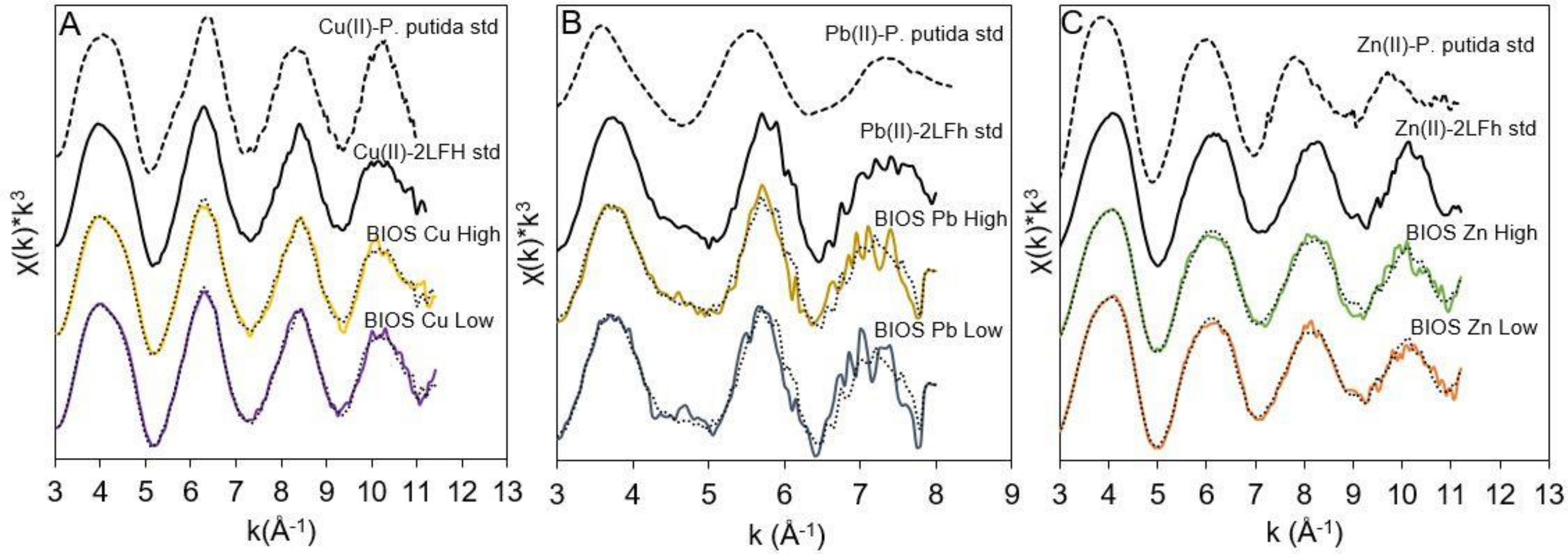

Figure S4. Linear combination fits of Cu (A), Pb (B), and Zn (C) BIOS EXAFS spectra of the low and high surface loadings plotted along with their respected Cu/Pb/Zn-ferrihydrite (2LFh) standard and Cu/Pb/Zn-biomass (*P. putida*) standard. Linear combination fitting was performed in SIXPACK and is represented by an overlain dotted line, with fit parameters shown in **Table S3.**

Table S1. Cu(II), Pb(II), and Zn(II) adsorption conditions utilized for 2LFh and Feb BIOS Low and High surface loadings examined with X-ray absorption spectroscopy. Conditions: pH = 6.0 ± 0.1, Sorbent solids loading = 1 g L^{-1}, I = 0.01 M (as $NaNO_3$)

Sorbent	Sorbate	$[Me^{2+}]_{initial}$ (μM)	Surface loading (μmol g^{-1})	Surface loading (μmol m^{-2})
2LFh Low	Cu(II)	393	192	0.79
Feb BIOS Low	Cu(II)	393	387	1.40
2LFh High	Cu(II)	787	283	1.17
Feb BIOS High	Cu(II)	787	767	2.77
2LFh Low	Pb(II)	24	24	0.10
Feb BIOS Low	Pb(II)	24	24	0.09
2LFh High	Pb(II)	121	121	0.50
Feb BIOS High	Pb(II)	121	121	0.44
2LFh Low	Zn(II)	382	67	0.28
Feb BIOS Low	Zn(II)	382	188	0.68
2LFh High	Zn(II)	3059	200	0.83
Feb BIOS High	Zn(II)	3059	813	2.94

Table S2. List of iron mineral standards [5-7] that were considered during the LCF fitting of the BIOS Fe K-edge EXAFS spectra. The bolded standards were used in the final fits.

Iron XAS Standards
Ferrihydrite
Hydrous ferric oxide
Hydrous ferric oxide with Si
Nano-goethite
Lepidocrocite
Hematite
Goethite
Magnetite
Fe(III) phosphate
Fe(III) chloride
Fe(III) peat
Pyrite
Siderite
Fe(III)-rhizoferrin (carboxylate complex)
Fe(III)-protochelin (catecholate complex)

Table S3. Elemental composition of the February (Feb) and April BIOS samples in g Kg^{-1} dry solid. Synthetic 2LFh Fe concentration was 611.0 g kg^{-1} solid. Reported values have an estimated error of approximately 10 %. Water chemistry for this site is reported elsewhere [2].

Sample	Fe	Al	Mn	Si	Ca	K	Mg	Na	Pb	Zn	Cu	P	S	C	N
Feb BIOS	464.2	0.7	0.5	2.0	4.9	0.2	0.3	0.1	<0.1	<0.1	<0.1	3.7	0.4	35.3	2.0
April BIOS	261.9	6.6	0.5	14.5	4.8	0.3	0.6	0.2	0.2	0.5	<0.1	1.8	1.1	88.2	3.2

Table S4. Freundlich parameters used to model Cu(II), Pb(II), and Zn(II) sorption to synthetic 2LFh and April BIOS samples normalized to BET SSA. K_f = sorption constant; n = exponential constant; E = model efficiency.

Adsorbate	Adsorbent	K_f (μmol Cu/Pb/Zn m^{-2})	n	E
Cu(II)	2LFh	0.08 ± 0.01	0.44 ± 0.02	0.979
Cu(II)	BIOS	0.9 ± 0.1	0.46 ± 0.03	0.986
Pb(II)	2LFh	0.42 ± 0.08	0.31 ± 0.05	0.871
Pb(II)	BIOS	2.64 ± 0.09	0.29 ± 0.02	0.943
Zn(II)	2LFh	0.002 ± 0.001	0.80 ± 0.08	0.888
Zn(II)	BIOS	0.07 ± 0.02	0.47 ± 0.03	0.949

Table S5. Copper and Zn K-edge and Pb L_3-edge EXAFS spectra linear combination fits for low and high Cu, Pb, and Zn surface loadings onto BIOS. LCFs were performed in SIXPACK and normalized to 100%, with raw fits summing to 90-110 ± 2-8%.

Sample	R factor	Fit components (%)	
		Cu/Pb/Zn-ferrihydrite	Cu/Pb/Zn-biomass†
BIOS Cu Low	0.0119	74 ± 3	26 ± 3
BIOS Cu High	0.0169	87 ± 3	13 ± 3
BIOS Pb Low	0.1239	79 ± 5	21 ± 7
BIOS Pb High	0.0957	86 ± 5	14 ± 6
BIOS Zn Low	0.0148	76 ± 2	24 ± 2
BIOS Zn High	0.0255	80 ± 2	20 ± 2

†. Cu- and Zn-biomass standards were provided by Peña et al. [8] and Toner et al. [9], respectively.

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