

FINAL REPORT

Development of an Electrochemical Surrogate
For Copper, Lead, and Zinc Bioaccessibility
in Aquatic Sediments

SERDP Project ER-1748

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U.S. Geological Survey

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14. ABSTRACT

To help enable the use of metal bioavailability as a site cleanup criterion, the goal of this project was to measure the chemical stability and molecular diffusivity of sub-micromolar concentrations of dissolved Cu(II), Pb(II), and Zn(II) in model estuarine sediment pore waters using scanned stripping chronopotentiometry (SSCP). The criteria for success of the project are (1) automation of data acquisition and processing, (2) determination of chemical stability and diffusivity using a physicochemical model of experimental data, (3) qualitative agreement between expected and SSCP-derived chemical stabilities, and (4) capability of SSCP to predict relative differences in the outcome of a bioavailability process; specifically, Cu-, Pb-, and Zn sulfide precipitation. To evaluate the success of this project on the basis of these criteria, we needed to perform SSCP measurements in synthetic, chemically defined media in the laboratory to (a) perform enough SSCP measurements to determine the method's capabilities and improve its execution, (b) ensure that we knew beforehand the relative chemical stabilities of dissolved metal-organic complexes, which we achieved by using well-characterized organic matter isolated from natural waters as well as synthetic organic compounds, and (c) examine metal-precipitation across a range of metal-organic ligand ratios at a fixed sulfide concentration. In addition to being able to measure dissolved metal speciation at the 0.25 $\mu\text{mol/L}$ concentration level (16 $\mu\text{g Cu}$ and Zn/L and 52 $\mu\text{g Pb/L}$), the SSCP results confirmed what we expected would be the relative chemical stabilities of the metal-DOM complexes and discerned these stabilities across conditions that subtly varied with respect to carbon aromaticity and organic sulfur content. We also successfully designed a custom-made procedure within commercially available electrochemistry software to automate SSCP analysis and clarified which physicochemical model to use to derive chemical stability coefficients and diffusivities from SSCP data. Accordingly, we believe metal speciation parameters derived from SSCP should be compared to biological indicators of metal bioavailability under controlled laboratory conditions and that we should collect sediment pore water at DoD metals-contamination sites to test our method under natural conditions. Together, these follow-on tasks would provide the scientific basis to conclude that SSCP can predict metal bioavailability in sediments.

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List of Symbols and Acronyms

A	amperes
c_L	ligand concentration
D	diffusion coefficient
$\overline{D}$	weighted average diffusion coefficient (of all metal species)
DP	differential pulse (stripping voltammetry)
DOM	dissolved organic matter
δ	thickness of the diffusion layer at the electrode-water interface
E	electrical potential in volts relative to the Ag/AgCl/3M KCl reference potential
E_d	deposition (preconcentration) potential
E^0	standard redox potential
I_d	deposition (metal preconcentration) current
I_s	stripping (metal oxidation) current
L	ligand
M	free metal ion

M^0	reduced metal
$M^0(\text{Hg})$	metal amalgam
MDE	mercury drop electrode
ML	metal-ligand complex
n	number of electrons transferred per atom of metal reduced at the electrode
r_o	radius of the electrode
SSCP	<u>s</u> canned <u>s</u> tripping <u>c</u> hronopotentiometry
t_d	deposition (metal preconcentration) time
τ	transition time of chronopotentiometric scan
τ^*	maximum transition time
τ_d	characteristic time constant of the metal deposition process
θ	ratio of oxidized to reduced metal at the electrode surface

Keywords

Bioavailability, bioaccessibility, copper, electrochemistry, electrode, lead, metal, organic, speciation, sulfide, voltammetry, zinc

Abstract

To help enable the use of metal bioavailability as a site cleanup criterion, the goal of this project was to measure the chemical stability and molecular diffusivity of sub-micromolar concentrations of dissolved Cu(II), Pb(II), and Zn(II) in model estuarine sediment pore waters using scanned stripping chronopotentiometry (SSCP). The criteria for success of the project are (1) automation of data acquisition and processing, (2) determination of chemical stability and diffusivity using a physicochemical model of experimental data, (3) qualitative agreement between expected and SSCP-derived chemical stabilities, and (4) capability of SSCP to predict relative differences in the outcome of a bioavailability process; specifically, Cu-, Pb-, and Zn-sulfide precipitation. To evaluate the success of this project on the basis of these criteria, we needed to perform SSCP measurements in synthetic, chemically defined media in the laboratory to (a) perform enough SSCP measurements to determine the method's capabilities and improve its execution, (b) ensure that we knew beforehand the relative chemical stabilities of dissolved metal-organic complexes, which we achieved by using well-characterized organic matter isolated from natural waters as well as synthetic organic compounds, and (c) examine metal-precipitation across a range of metal-organic ligand ratios at a fixed sulfide concentration. In addition to being able to measure dissolved metal speciation at the 0.25 $\mu\text{mol/L}$ concentration level (16 μg Cu and Zn/L and 52 μg Pb/L), the SSCP results confirmed what we expected would be the relative chemical stabilities of the metal-DOM complexes and discerned these stabilities across conditions that subtly varied with respect to carbon aromaticity and organic sulfur content. We also successfully designed a custom-made procedure within commercially available electrochemistry software to automate SSCP analysis and clarified which physicochemical model to use to derive chemical stability coefficients and diffusivities from SSCP data. Accordingly, we believe metal speciation parameters derived from SSCP should be compared to biological indicators of metal bioavailability under controlled laboratory conditions and that we should collect sediment pore water at DoD metals-contamination sites to test our method under natural conditions. Together, these follow-on tasks would provide the scientific basis to conclude that SSCP can predict metal bioavailability in sediments.

1. Objective

To help enable the use of metal bioavailability as a site cleanup criterion, the goal of this limited scope project was to measure the chemical stability and molecular diffusivity of sub-micromolar concentrations of dissolved Cu(II), Pb(II), and Zn(II) in model estuarine sediment pore waters using scanned stripping chronopotentiometry (SSCP). The criteria for success of the project are

1. automation of data acquisition and processing,
2. determinations of chemical stability and diffusivity using a physicochemical model of experimental data,
3. qualitative agreement between expected and SSCP-derived chemical stabilities, and
4. capability of SSCP to predict relative differences in the outcome of a bioavailability process; specifically, Cu-, Pb-, and Zn-sulfide precipitation.

To evaluate the success of this project on the basis of these criteria, we needed to perform SSCP measurements in synthetic, chemically defined media in the laboratory to

- (a) perform enough SSCP measurements to determine the method's capabilities and improve its execution,
- (b) ensure that we knew qualitatively the relative chemical stabilities of dissolved metal-organic complexes, which we achieved by using well-characterized organic matter isolated from natural waters as well as synthetic organic compounds, and
- (c) examine metal-precipitation across a range of metal-organic ligand ratios at a fixed sulfide concentration.

2. Background

Since the late 1950s, the physicochemical speciation of a metal has been thought to affect its bioaccessibility, bioavailability, and toxicity in aquatic systems. Evidence to support this hypothesis began to mount in the mid-1970s from laboratory studies in which algae were grown in chemically defined media amended with copper and synthetic and naturally occurring ligands (1, 2). These studies found that copper toxicity was *not* directly related to total copper concentration, nor the concentration of complexed copper, but rather to the concentration of free cupric ion. In several laboratory toxicity studies, investigators added unnaturally high concentrations of metals to quantify free metal ions with ion-selective electrodes because ISEs are not quantitative below 1 $\mu\text{mol/L}$ unless extreme care is taken (2-5). Free metal ion concentrations were otherwise calculated with unverifiable thermodynamic models (1, 6). Under these exaggerated conditions, the concentration of metal resulted in a small fraction (<1%), yet still toxic concentration, of free metal ion. Many synthetic chelators bind metals more strongly than natural organic ligands, and the underlying assumption that organisms respond to metals complexed with synthetic ligands such as EDTA as they do to metals complexed by natural organic ligands is dubious (7-13). As a result, prevalent use of synthetic chelators in laboratory

studies has lead us to mistakenly believe that complexed metals in sediment and the water column are not bioavailable (14), and free metal ion determination has unfortunately become the primary objective of many metal bioavailability assessments. Although laboratory studies under unrealistic or exaggerated conditions show a clear relationship between free metal ion concentrations and toxicity, metal toxicity under realistic conditions continues to remain elusive.

Although the <1% metal fraction that might exist as a free ion would unquestionably increase health risk to benthic and pelagic organisms, what risk might the other 99+% pose? If we consider organisms of trophic status above algae, namely zooplankton, invertebrates, and vertebrates, uptake of nutrients and contaminants following ingestion often involve active chemical pathways (15, 16). We should expect some fraction of complexed metals to be bioavailable through ligand-exchange reactions (6, 17). In formulating the Free Ion Activity Model (FIAM) to predict metal bioavailability, Morel and Hering (1993) included ligand-exchange reactions at the cell-water interface. However, since no stability constants could be reliably measured under realistic conditions, this component of the model—arguably representing the dominant pathway of metal uptake—if often overlooked. To predict metal bioavailability in sediments, we need a tool that can detect and speciate dissolved metals at *in situ* concentrations so that we can stop ignoring that other 99+%.

A key question arises from the Department of Defense's (DoD) recognition of the physical, chemical, and biological complexity of sediments and the need for new technologies to better determine metal bioavailability (18): how many environmental factors do we need to understand at a site to remediate on the basis of bioavailability? While technological development is critical to moving forward, it is also advisable to not overly complicate site assessment. The electrochemical method developed during this limited scope project promises to measure enough metal speciation parameters to predict metal bioavailability—no more, no less. The two most important of these characteristics are the chemical stability and diffusivity of metal-organic complexes, which likely dominate the fraction of dissolved, and therefore potentially bioavailable, metals in sediment pore water. The method is designed to stand alone, and the results of this preliminary study suggest that it could begin to be used in site assessments today to provide unprecedented insight into metal speciation without caveats arising from signal distortions that have plagued other stripping voltammetry methods. To ultimately be accepted by stakeholders, its ability to predict bioavailability processes such as algal growth inhibition and (in)vertebrate metal assimilation needs to be studied, as discussed in section 5. In short, this physical-science-based method promises to meet the challenge of determining metal bioavailability at appropriate levels of scientific rigor and ease of use to be acceptable to site managers and stakeholders.

Voltammetry is currently the only non-perturbing way to measure physicochemical properties of metals at concentrations found in contaminated sediments. Voltammetry is typically known for operationally defining metals according to their 'electro-activity.' An electro-active metal species is small enough—less than 50 nanometers (8)—to diffuse to the electrode and chemically weak enough to break apart and oxidize or reduce (most metal-ligand complexes cannot be directly reduced or oxidized (8)). While these characteristics might significantly correlate to

bioavailability, distortion of voltammetric signals (19), combined with other unwanted complications such as sample handling, may obscure any causal relationship needed to make a site-management decision. In some cases, these limitations have not prevented regulatory buy-in; for instance, the voltammetric characterization of copper and nickel by Donat et al. (20) enabled the Environmental Protection Agency to raise the permissible level for these metals in South San Francisco Bay (21). In this case, however, the regulatory modification covered a relatively small geographic area because the Cu and Ni speciation results were site-specific. To prioritize site restoration, the current challenge is to provide non-site-specific bioavailability indices across numerous DoD sediment metals contamination sites. To do that, matrix-specific distortions that befuddle data interpretation need to be minimized by improving voltammetric techniques and developing better physicochemical models to describe voltammetric data.

A physicochemical (mathematical) model to speciate metals with voltammetry emerged in 1990 and was subsequently refined (7, 22); however, this model applies under restrictions that few, if any, sediment pore waters conform. A decade later, investigators determined that a new technique—stripping chronopotentiometry (SCP)—was not subject to signal distortion under chemically defined, simplified conditions (19, 23, 24). While electrochemical technology has advanced, physicochemical models to calculate metal speciation parameters from SCP data continue to improve (25-27). We seek to help realize metal bioavailability as a viable site remediation criterion by determining the capability of SCP to speciate metals under increasingly realistic conditions using currently available physicochemical models.

3. Materials and Methods

Although in other projects we use voltammetry to analyze minor redox species in sediments (28), we needed to focus on chemically defined, liquid-only aqueous systems in the current project to meet our study objectives, as explained in section 1. Divalent copper, lead, and/or zinc were added in known quantity to solutions containing NaClO₄ (an indifferent electrolyte) amended with either no pH buffer, Na₂HPO₄, or 4-(2-hydroxyethyl)-1-piperazine-ethanesulfonic acid (HEPES) at 0.005 M and 0.01 M, respectively. All solutions were adjusted to pH 7.5 with KOH and HClO₄ and had an ionic strength of 0.1 M, an intermediate ionic strength between fresh and seawater (e.g., as in an estuary). The organic compounds used in this study and selected characteristics are listed in Table 1.

Liquid samples were placed in a closed glass reaction vessel and de-aerated with ultra-high-purity N₂. To achieve sub-micro-molar sensitivity, Cu, Pb, and Zn were concentrated by reduction and amalgamation at a constant potential (E) in stirred solution for 4 to 10 min using a Metrohm 663 VA renewable electrode and Autolab potentiostat/galvanostat (Metrohm-Autolab B.V., Utrecht). To quantify the preconcentrated metal after the preconcentration step, E was scanned in a positive direction to oxidize the metal at a constant oxidizing current (1 to 2×10^{-9} A). The sequential preconcentration and oxidation steps are referred to as stripping chronopotentiometry (SCP). SCP was repeated at different ‘deposition’ potentials (E_d) and the overall procedure is called scanned stripping chronopotentiometry (SSCP). Data acquisition and processing was performed with Nova 1.6 software (Metrohm-Autolab). Additional calculations,

curve fitting, and modeling was performed using custom procedures in Igor Pro (Wavemetrics, Portland, OR).

Table 1. Organic compounds added to chemically defined aqueous media.

	Name	Composition	Ligand functional groups	Aromaticity (% total functional groups)	Reduced S (mol% total S)
1	Glycolic acid ^a	C ₂ H ₄ O ₃	1 carboxyl and 1 phenol	0	0
2	Thioglycolic acid	C ₂ H ₄ O ₂ S	1 thiol and 1 carboxyl	0	100
<i>Natural organic matter isolates</i>			<i>Site description</i>		
3	Suwannee River fulvic acid ^b	53% C, 0.72% N, 0.46 % S	Black water river draining Okefenokee Swamp, Fargo, Georgia	22.9	16.6
4	F1 Hydrophobic Acid ^c	52% C, 1.5% N, 1.7% S	Eutrophied marshland, northern Everglades	25.4	28.7
5	Pacific Ocean fulvic acid ^b	56% C, 1.1% N 0.4% S	Collected at 100 m depth, 170 km southwest of Pearl Harbor, O'ahu, Hawai'i	7.3	12.5

^a Glycolic acid is one of the main forms of organic carbon exuded by algae (29)

^b Obtained commercially

^c Isolated from natural water as described by Aiken et al. (30)

4. Results and Discussion

4.1 Elementary features of stripping chronopotentiometry

Chronopotentiometry is a voltammetric technique, but instead of controlling electrical potential (E) according to a time-dependent function, I controlled E to maintain a constant current (i) at the electrode. The potential is controlled by a potentiostat capable of maintaining a ± 10 -volt potential at the electrode versus a stable, industry-standard Ag/AgCl reference electrode protected by an inert electrolyte-filled salt bridge. The redox potential of typical porewaters are two orders of magnitude lower than this compliance voltage, and so in no way interferes with electrode control. E vs time relationships are our 'raw' data. During the deposition step, the mobile and labile fraction of metal is reduced and absorbed into the mercury electrode. Once this step is complete (typically 4 to 20 min), E is scanned from the deposition potential (E_d) toward more oxidizing potentials, as shown by the blue line in Fig. 1. Once the reduced metal starts to oxidize, the potential is swept more slowly to maintain a low preset current (flatter portion of the E vs. t curve; Fig. 1a). Although not the focus of the current study, we can separate the SCP signals of different metals in a mixture due to the metals' different characteristic redox potentials (Fig. 2). The current is set low enough to ensure that (1) most, if not all, of the metal is oxidized and (2) signal distortions arising from the association of newly oxidized (free) metal with organic ligands is minimized (31, 32). On the other hand, the current

is set high enough to ensure that the electrode is doing all of the oxidation and not trace amounts of oxygen. The optimal current lies within a range where the stripping charge does not vary with current, as is determined from auxiliary measurements. Once the electrode is depleted of metal, E is swept more rapidly to a preset cutoff. The metal oxidation step can take about 1 to 5 min. The current during this step arises from two processes: (1) changes in electrical charge at the electrode-water interface and (2) oxidation of metals. The oxidation, or ‘transition,’ time (τ) is determined by inverting the time derivative of E , plotting dt/dE against E , and calculating the baseline-corrected peak area (Fig. 1b). An appropriate stripping current ensures that the baseline of this plot represents the capacitive component of i , while any peak strongly correlates to metal oxidation. And since i is fixed, τ should be directly proportional to the quantity of metal that was oxidized and the amount of metal originally deposited onto the electrode during the preconcentration step. The key innovation of SCP is that it provides a more robust quantitative link than previous methods between (a) the electrochemical force inducing metal diffusion and dissociation and (b) the amount of metal that yielded to that force. Older stripping voltammetric methods rely on a tentative, often unverifiable, relationship between (a) and (b). To probe the entire range of metal-organic interactions, the preconcentration-SCP sequence is repeated about twenty times, starting with increasingly reductive deposition potentials. The oxidation times, corresponding to the quantity of metal deposited onto the electrode, are compiled and plotted as a function of E_d (Fig. 3a). The physicochemical model of this SSCP wave to derive chemical stability and diffusivity is introduced below and detailed in Appendix A.

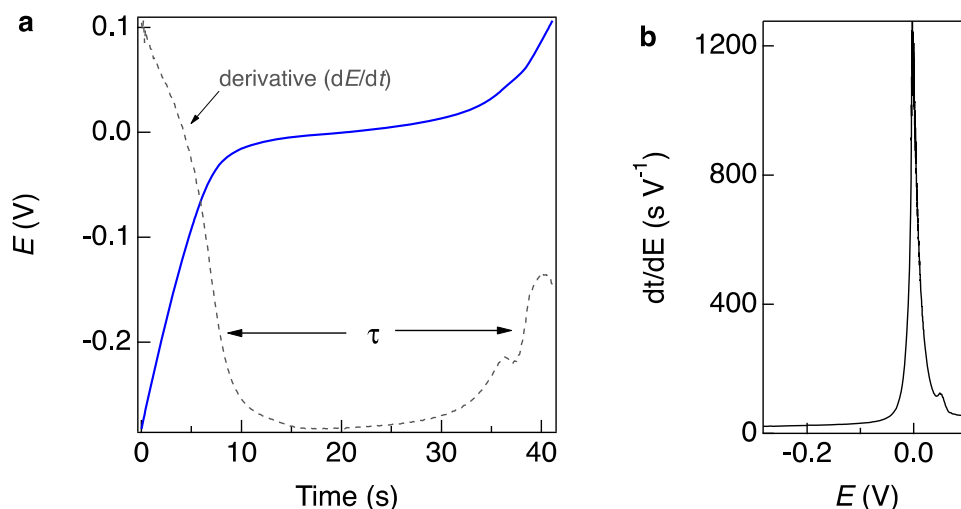


Figure 1. (a) Electrical potential (E) vs. time scan (blue line) for aqueous Cu(II)-glycolate solution to maintain a constant oxidizing current and its time derivative (dashed line); the flatter portion of the curve indicates oxidation of Cu^0 . **(b)** metal oxidation time (τ) determination from peak area of the inverted, baseline-corrected time-derivative (dt/dE). 0.25 $\mu\text{mol/L}$ Cu (15.9 $\mu\text{g/L}$), 0.56 mmol/L Na-glycolate, 0.005 mol/L phosphate, 0.095 mol/L NaClO_4 , pH 7.5.

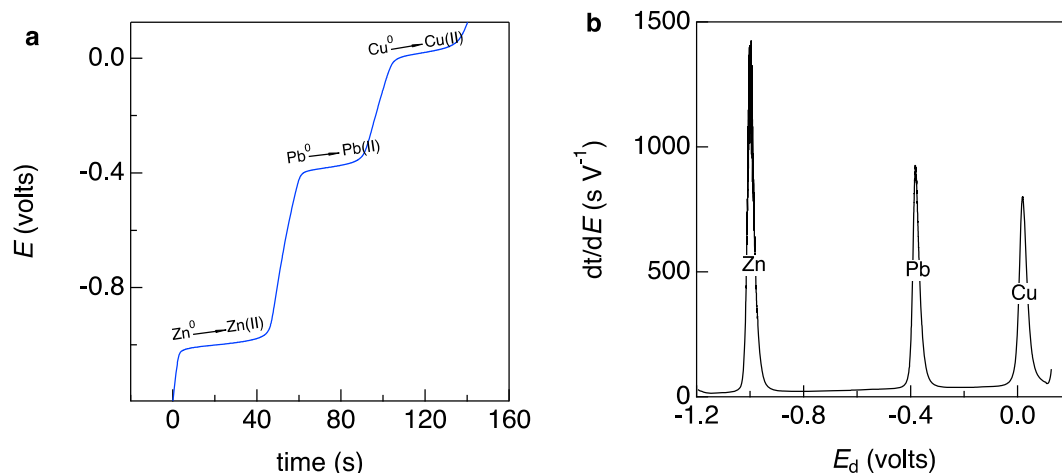


Figure 2. (a) Electrical potential (E) vs. time scan for a mixture of $0.25 \mu\text{mol/L}$ aqueous Cu(II) , Pb(II) , and Zn(II) ; the flatter portions of the curve indicate oxidation of (left to right) Zn^0 , Pb^0 , and Cu^0 . **(b)** inverted time derivatives of E to determine oxidation time (τ).

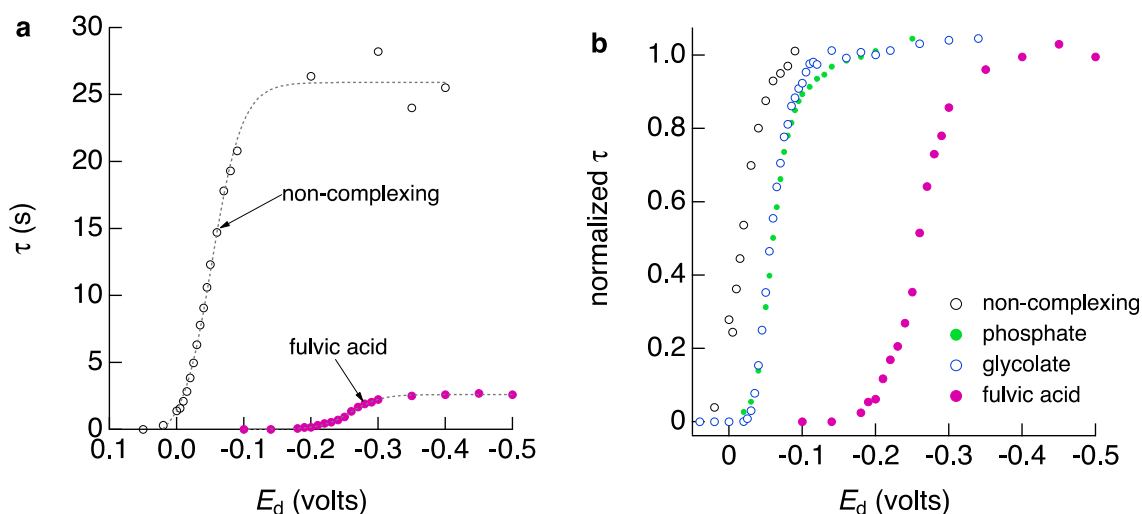


Figure 3. (a) Plot of Cu^0 oxidation time (τ) at constant current, corresponding to the quantity of Cu(II) accumulated in the electrode during the deposition step, as a function of deposition potential (E_d) for Cu^{2+} (non-complexing medium) and Cu(II) -fulvic acid complexes (Suwannee River isolate). **(b)** Cu^0 oxidation time normalized by the maximum respective τ values for Cu complexes with phosphate and dissolved organic matter. $0.25 \mu\text{mol/L}$ Cu ($15.9 \mu\text{g/L}$), 0.56 milli-carboxyl equivalents per L, 0.095 mol/L NaClO_4 , 0.005 mol/L phosphate (where indicated), pH 7.5.

4.2 Effect of dissolved organic matter on dissolved Cu, Pb, and Zn speciation

As shown in Fig. 3a, the SSCP ‘wave’ of a Cu -fulvic acid complex is greatly diminished compared to that of Cu^{2+} . The decrease is primarily due to the slower diffusion of larger Cu(II) -fulvic complexes compared to Cu^{2+} (8):

$$\bar{D}/D_M = (\tau_{M+L}^*/\tau_M^*)^2 \quad (\text{eqn 1})$$

where $\bar{D}$ is the weighted average diffusivity, D_M is the diffusivity of the free metal ion, τ_M^* is the maximum oxidation time of metal in a non-complexing medium and τ_{M+L}^* is the maximum oxidation time in the presence of a complexing ligand (L). The shift of the wave to more negative potentials reflects the greater electrochemical driving force needed to dissociate Cu(II)-fulvic complexes to enable Cu^{2+} reduction and subsequent detection. The shift in the position of E_d relative to Cu^{2+} (measured in a non-complexing medium), taken at half τ^* ($\Delta E_{d,1/2}$), is used to compute the weighed-average chemical stability coefficient of the Cu(II)-fulvic complexes (33):

$$\ln(1 + K') = -(nF / RT) \Delta E_{d,1/2} - \ln(\tau_{M+L}^* / \tau_M^*) \quad (\text{eqn 2})$$

where $K' = K^* c_L$ (eqn 3) and c_L is the ligand concentration.

To graphically clarify the relative chemical stabilities of metal complexes, SSCP waves are normalized by τ^* as shown in Fig. 3b to more easily compare $E_{d,1/2}$. In Fig. 3b, SSCP shows that glycolate, a simple organic anion, forms complexes with Cu^{2+} that are similar in strength to phosphate, both of which are much weaker than Cu(II)-fulvic acid complexes. The chemical stability coefficients and diffusivity ratios calculated from the SSCP data are summarized in Table 2. SSCP analysis also reveals that a lower concentration of Cu(II) relative to fulvic acid results in stronger overall chemical stability (Fig. 4). This phenomenon is due to preferential binding of Cu by high-affinity, but less abundant, ligands within the mixture of compounds that comprise natural dissolved organic matter (DOM).

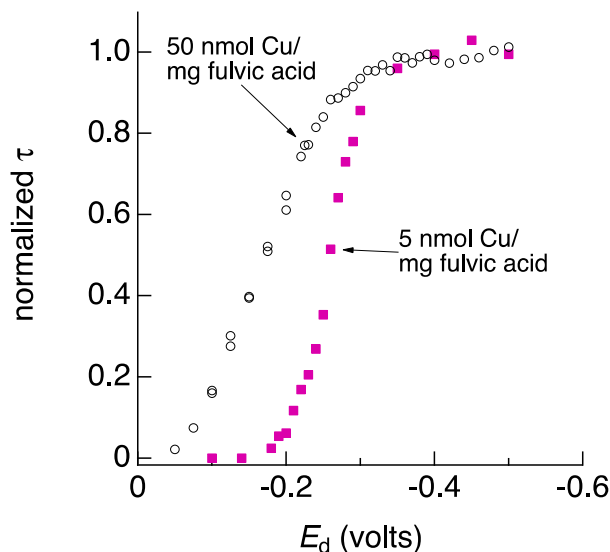


Figure 4. Plot of Cu^0 oxidation time normalized by the maximum respective τ values for Cu(II)-fulvic acid complexes (Suwannee River isolate) at two different metal-ligand ratios. $0.25 \mu\text{mol/L}$ Cu ($15.9 \mu\text{g/L}$), 0.56 milli-carboxyl equivalents per L, 0.095 mol/L NaClO_4 , 0.005 mol/L phosphate, pH 7.5.

Table 2. Weighted-average chemical stability coefficients (K^*) and diffusivity ($\bar{D}$) relative to that of the free metal ion calculated from SSCP data using equations 1-3.

Sample	Log K^* (L mol ⁻¹)	$\bar{D}/D_M$
Cu(II)-fulvic acid (Suwannee River)	10.4	-
Pb(II)-glycolate	3.51	-
Pb(II) + 99% glycolate, 1% thioglycolate	3.56	0.88
Pb(II) + 90% glycolate, 10% thioglycolate	4.67	0.90
Pb(II) + 100% thioglycolate	5.99	0.82
50 nmol Pb(II)/mg Suwannee River fulvic acid	6.7	-
5 nmol Pb(II)/mg Suwannee River fulvic acid ^a	6.81	-
5 nmol Pb(II)/mg Suwannee River fulvic acid ^b	6.21	-
5 nmol Pb(II)/mg Pacific Ocean fulvic acid ^b	5.36	-
5 nmol Pb(II)/mg Florida Everglades hydrophobic organic acid ^b	7.99	-
Zn(II)-glycolate	2.85	-
Zn(II)-thioglycolate	3.76	0.79
50 nmol Zn(II)/mg Suwannee River fulvic acid	2.8	0.41
5 nmol Zn(II)/mg Suwannee River fulvic acid ^a (phosphate)	3.79	0.12
5 nmol Zn(II)/mg Suwannee River fulvic acid ^b	4.31	0.25
5 nmol Zn(II)/mg Pacific Ocean fulvic acid ^b	3.98	-
5 nmol Zn(II)/mg Florida Everglades hydrophobic organic acid ^b	4.74	-

All solutions contain 0.25 μ mol/L Cu, Pb, or Zn, pH 7.5, 0.1 M ionic strength (NaClO₄)

^a system contained 0.005 mol/L phosphate

^b system contained 0.01 mol/L HEPES

Pb(II)- and Zn(II) complexes formed with a diverse set of DOM are more chemically stable than Pb²⁺ and Zn²⁺, on the basis of SSCP analysis (Figs. 5 and 6). Aside from being able to measure dissolved metal speciation at the 0.25 μ mol/L (16 μ g Cu and Zn/L and 52 μ g Pb/L) concentration level, it is remarkable how SSCP can correctly discern the relative chemical stabilities of Pb(II)- and Zn(II)-DOM complexes as we would expect given the properties of the different types of DOM used in this study. For instance, the increasing chemical stability of both Pb(II) and Zn(II)-glycolate, Pacific Ocean fulvic acid, Suwannee River fulvic acid, and Florida Everglades DOM follows the increasing carbon aromaticity and organic sulfur content of the DOM (Table 1). Aromatic organic moieties bind metals more strongly, as do sulfur-bearing moieties in the case of B-type metal cations like Pb(II) and Zn(II). Aromatic moieties tend to be substituents in larger organic molecules or clusters, which may diminish metal transport across cell membranes. The sensitivity of SSCP to the effect of reduced sulfur on chemical stability is

confirmed by comparison of SSCP waves of solutions containing Pb(II) and varying proportions of glycolate and thioglycolate (Fig. 7). The close systematic trend of $\log K^*$ with thiol concentration under simplified conditions, even down to 1% changes in reduced sulfur, indicates that SSCP will also be sensitive to metal-organic sulfur interactions in sediments. In summary, since the SSCP results confirmed what I expected would be the relative chemical stabilities of the metal-DOM complexes, and discerned these stabilities across subtly varying carbon aromaticity and organic sulfur content, I believe SSCP can be tested with sediment pore water and biological systems to predict metal bioavailability.

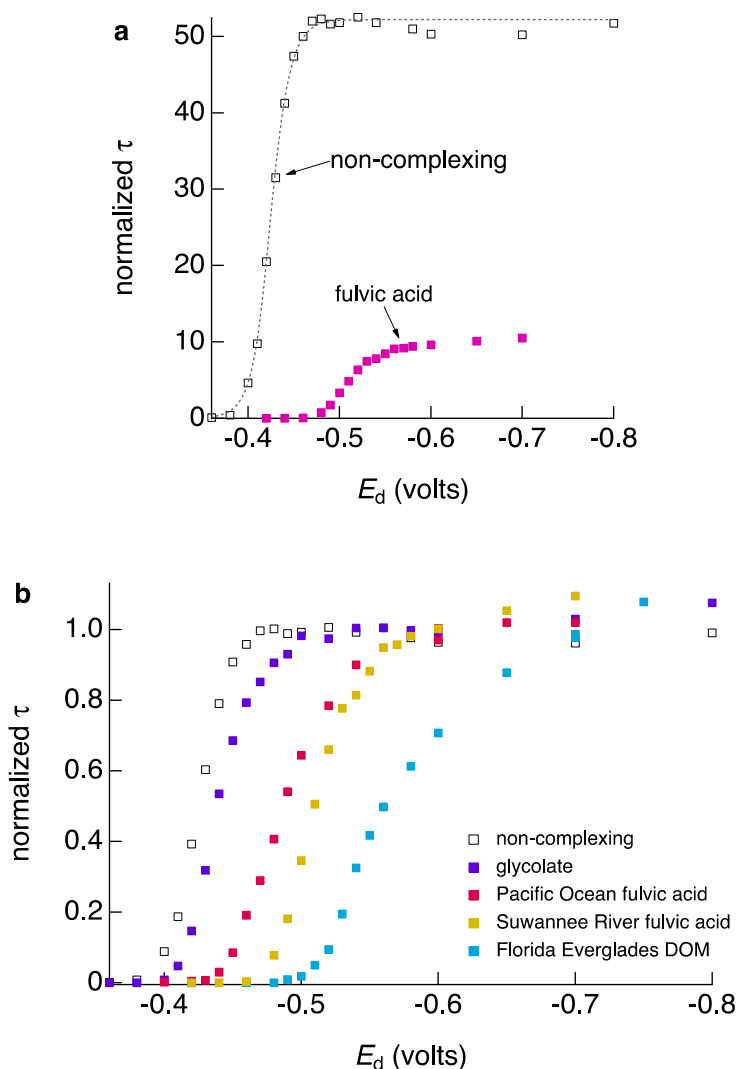


Figure 5. (a) Plot of Pb⁰ oxidation time (τ) at constant current, corresponding to the quantity of Pb(II) accumulated in the electrode during the deposition step, as a function of deposition potential (E_d) for Pb²⁺ (non-complexing medium) and Pb(II)-fulvic acid complexes (Suwannee River isolate). **(b)** Pb⁰ oxidation time normalized by the maximum τ for Pb(II) complexes with a variety of dissolved organic matter. 0.25 $\mu\text{mol/L}$ Pb (52 $\mu\text{g/L}$), 0.56 milli-carboxyl equivalents per L, 0.095 mol/L NaClO₄, 0.01 mol/L HEPES, pH 7.5.

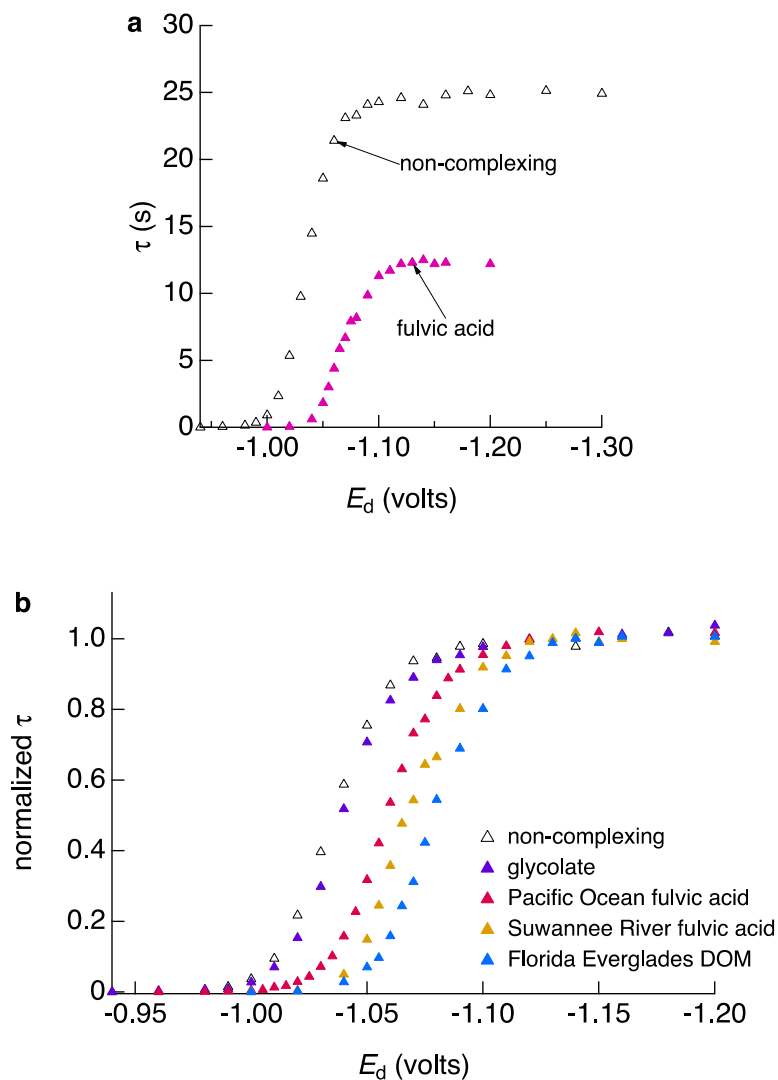


Figure 6. (a) Plot of Zn^0 oxidation time (τ) at constant current, corresponding to the quantity of Zn(II) accumulated in the electrode during the deposition step, as a function of deposition potential (E_d) for Zn^{2+} (non-complexing medium) and Zn(II) -fulvic acid complexes (Suwannee River isolate). **(b)** Zn^0 oxidation time normalized by the maximum τ for Zn(II) complexes with a variety of dissolved organic matter. 0.25 $\mu\text{mol/L}$ Zn (16.4 $\mu\text{g/L}$), 0.56 milli-carboxyl equivalents per L, 0.095 mol/L NaClO_4 , 0.01 mol/L HEPES, pH 7.5.

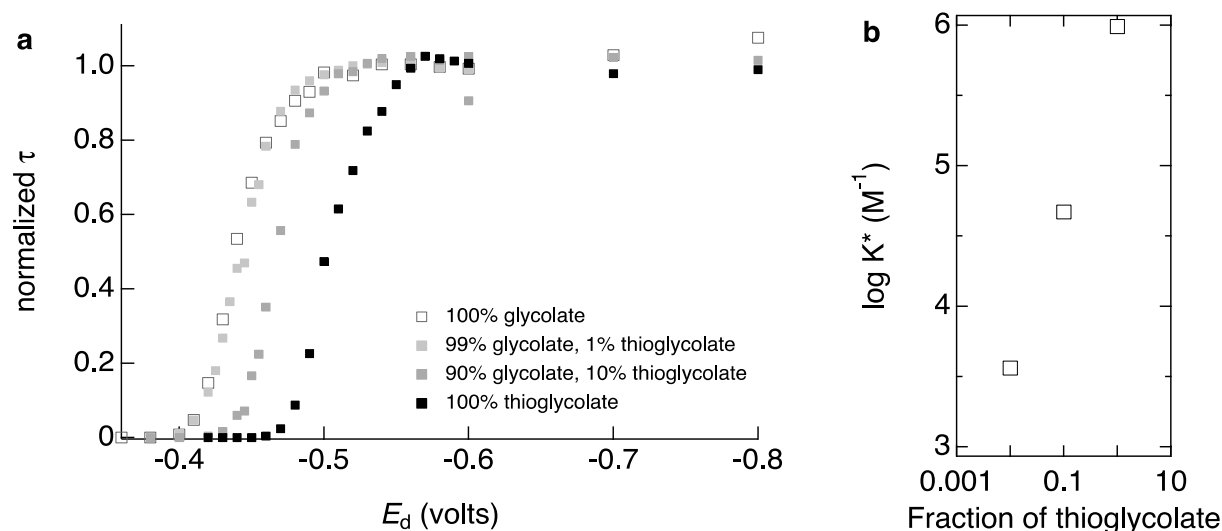


Figure 7. (a) Plot of normalized Pb^0 oxidation time at constant current, corresponding to the quantity of Pb(II) accumulated in the electrode during the deposition step, as a function of deposition potential (E_d) for Pb(II) -(thio)glycolate complexes containing varying amounts of thiols (reduced organic sulfur). **(b)** Plot of weighted average equilibrium constant of Pb(II) -(thio)glycolate acid complexes versus the amount of thioglycolate as a fraction of the total 0.56 mmol/L organic carbon. 0.25 $\mu\text{mol/L}$ Pb (52 $\mu\text{g/L}$), 0.095 mol/L NaClO_4 , 0.01 mol/L HEPES, pH 7.5.

5. Conclusions and Implications for Future Research

Here is how I met the criteria for success of this project:

1. Automation of measurements, data acquisition, and data processing

I successfully designed a custom-made procedure for the Nova electrochemistry software to automate numerous elementary steps involved with SSCP analysis. This procedure enables the user to put the sample in the voltammetric analyzer, initiate the analysis, and return later when the analysis is complete to load the next sample. It can take about 1.5 to 3 h to analyze one sample, which is quite reasonable to determine speciation of dissolved metals at sub-micromolar concentrations. While our Nova program automatically initiates peak fitting, baseline fits usually need to be corrected by the user. The algorithms of voltammetric data processing and analysis software continue to evolve, and I see a number of ways to increasingly automate these tasks and are poised to work with the instrument manufacturer's software developers to do so.

2. Determination of chemical stability and diffusivity using a physicochemical model of SSCP data

By clarifying which physicochemical model to use to derive chemical stability and diffusivity from SSCP data (Appendix A and equations 1-3), I successfully calculated these parameters within a simple, yet powerful, GUI-driven computing framework on an inexpensive, commercially available, cross-platform application (Igor Pro). Once I began analyzing the data, we learned how to refine our measurements, particularly to facilitate diffusivity calculations. I also gained an appreciation of physicochemical model variations that account for the complexity of natural organic matter interactions with metals at

electrodes. Thus, while speciation derived from more simply modeled SSCP data should be compared in follow-on work with bioavailability process outcomes, I see an opportunity to improve the science behind speciation parameter determination.

3. Qualitative agreement between expected and SSCP-derived chemical stabilities

As discussed in section 4, SSCP reliably predicted the relative chemical stabilities of Pb(II) and Zn(II) complexes on the basis of DOM aromaticity and reduced sulfur content. To our knowledge, this has never been shown before. I did not expect to discern stability differences in systems that varied so subtly as the Suwannee River fulvic acid and Florida Everglades DOM, and so consider our findings a big success according to this criterion.

4. Capability of SSCP to predict relative differences in the outcome of a bioavailability process; specifically, Cu-, Pb-, and Zn-sulfide precipitation.

At the time this report was first drafted, we had not had sufficient access to a synchrotron radiation facility to perform enough X-ray absorption spectroscopic characterizations of Cu-, Pb-, and Zn-sulfides to evaluate this criterion. Since then, we have collected a substantial amount of spectroscopic data, which are currently being analyzed (select data are appended). Preliminary EXAFS analysis reveals that CuS and ZnS formed from more chemically stable Cu- and Zn-organic solutions are qualitatively more disordered, as we would expect. A quantitative correlation of dissolved-phase metal-organic chemical stability and solid-phase metal-sulfide disorder is in progress.

The next logical step in this work is to compare SSCP-derived metal speciation with biological indicators of metal bioavailability under controlled laboratory conditions. For example, if dissolved metal chemical stability and diffusivity strongly correlate to diminished algal growth rate or invertebrate metal assimilation, then SSCP should be able to predict metal bioavailability. In addition, we should work directly with DoD to collect sediment pore water at metals-contamination sites to test our method under natural conditions. If chemical stabilities and diffusivities derived from SSCP data follow similar trends as in chemically defined media, then SSCP can measure physicochemical metal speciation under naturally complex conditions. Meeting both of these objectives would constitute the scientific basis to conclude that SSCP can discern physicochemical properties of metals that are directly relevant to, and therefore predictive of, bioavailability in sediments.

In addition to the science behind the method, there is a need for technological development. Although we have used a (commercially available) bench-top electrode system for the present work, a miniaturized version of this type of electrode exists but is only made by a small company in Italy (no domestic supplier exists). We are interested in developing an industrial partnership to fabricate microelectrodes to perform *in situ* metal speciation measurements in the field.

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Appendix A. Physicochemical model of the SSCP data

$$\tau = \frac{I_d^* \tau_d}{I_s} \left[1 - \exp\left(\frac{-t_d}{\tau_d}\right) \right] \quad (\text{eqn A1})$$

$$I_d^* = \frac{\tau^* I_s}{t_d} \quad (\text{eqn A2})$$

$$\tau_d = \frac{r_o^2 \delta_M}{3(r_o + \delta_M) D \theta} \quad (\text{eqn A3})$$

$$\theta = \exp\left[\frac{nF}{RT}(E_d - E^o)\right] \quad (\text{eqn A4})$$

Appendix B. Spectroscopic analysis of CuS precipitates

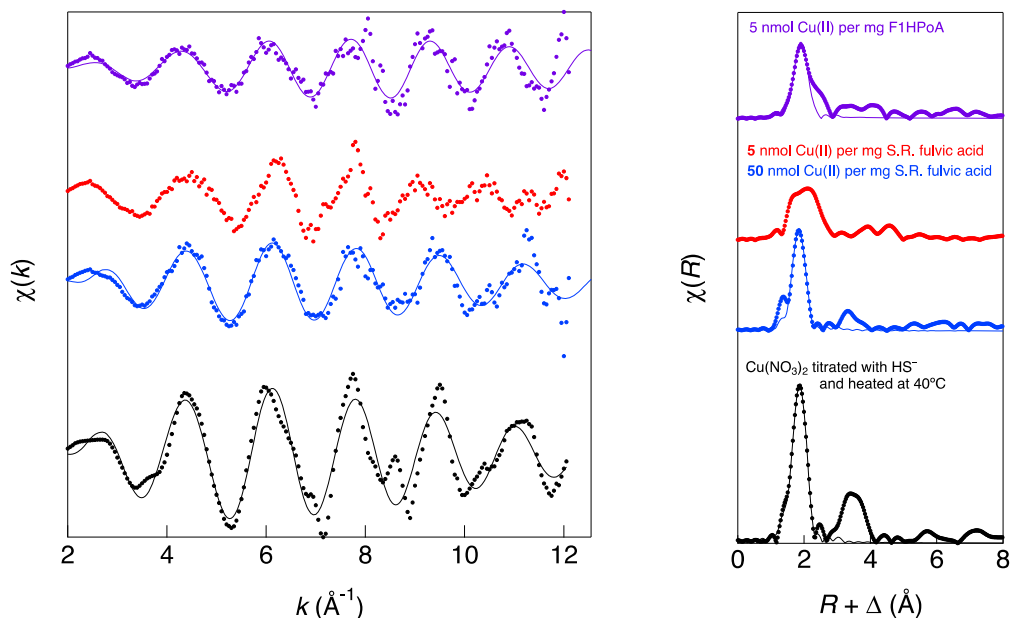


Figure B1. Structural analysis of CuS precipitates with extended X-ray absorption fine structure (EXAFS) spectroscopy. The EXAFS spectra indicate that CuS formed in the presence of dissolved organic matter is more disordered and therefore potentially more soluble than CuS formed in the absence of organic matter. The structural disorder of CuS increases as the Cu-to-DOM concentration ratio decreases. The type of organic matter affects the structure of CuS precipitates, as indicated by EXAFS measurements of CuS formed in the presence of dissolved organic matter isolated from the Florida Everglades versus the Suwannee River.

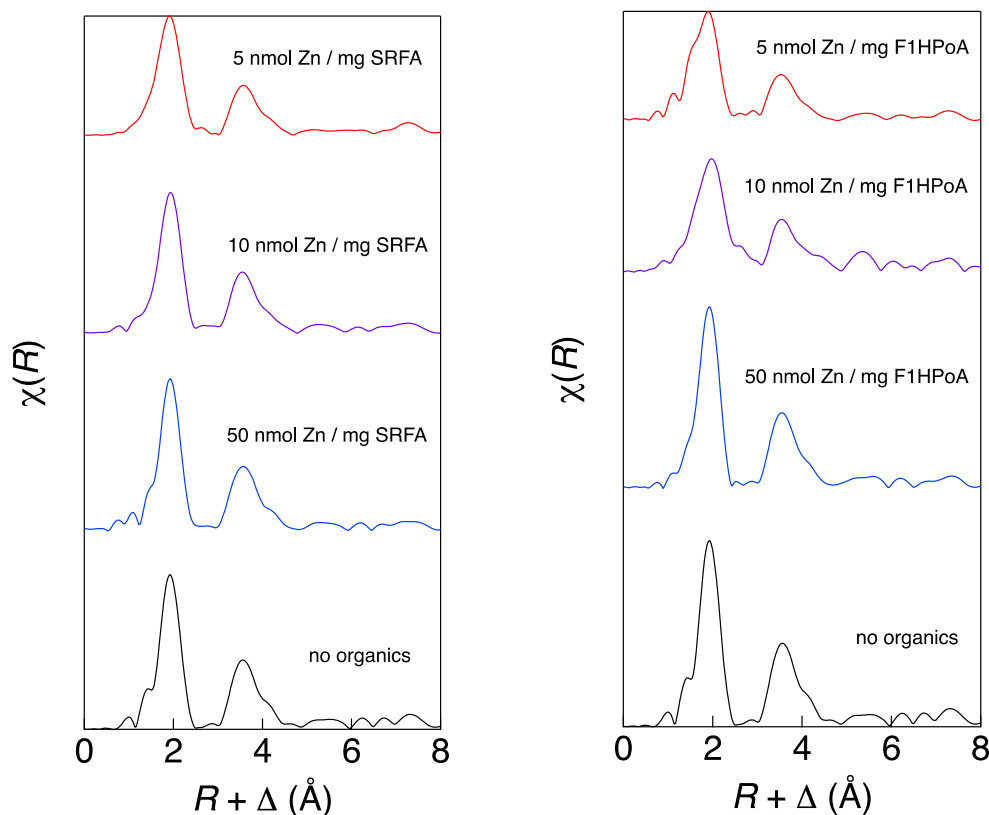


Figure B2. Structural analysis of ZnS precipitates with extended X-ray absorption fine structure (EXAFS) spectroscopy. The EXAFS spectra indicate that ZnS formed in the presence of dissolved organic matter is more disordered and therefore potentially more soluble than ZnS formed in the absence of organic matter. The structural disorder of ZnS increases as the Zn-to-DOM concentration ratio decreases. The type of organic matter affects the structure of ZnS precipitates, as indicated by EXAFS measurements of ZnS formed in the presence of dissolved organic matter isolated from the Florida Everglades versus the Suwannee River.