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## 1. Executive Summary

The objective of this project was to develop and apply a direct speciation method based on X-Ray Absorption Spectroscopy (XAS) for determining the speciation of Ni in laboratory spiked sediments and support laboratory findings of Ni ecotoxicity. One of the primary emphases of this project was to determine whether Ni was effectively bound to sulfides in amended sediments. For this purpose, we used XAS to probe the chemical speciation of Ni from sediment samples that were used in ecotoxicological tests and field studies. The ecotoxicity experiments were performed by the USGS laboratory located in Columbia (MI) whereas the field studies were carried out by the Universities of Michigan and Wright State. In addition, we tested the significance of the AVS/SEM protocol for assessing the bioavailability of Ni in sediments. For this purpose, we probed the local environment of Ni in samples before and after the leaching step corresponding to release of sulfides from sediments.

The collection of Ni XAS spectra on sediment samples is challenging because of the fluorescence coming from elements of lower atomic number than Ni - in particular Fe that is present in large quantities - tends to overwhelm the response of the detector. We selected the optimal data collection method based on various trial experiments that allowed us to obtain qualitative as well as quantitative speciation results on Ni on sediments that were used in the evaluation of Ni toxicity. The chemical speciation of Ni in sediments was obtained by performing a spectral decomposition of the signal using the spectra of reference compounds, *i.e.*, the minerals and amorphous compounds that are likely to bind Ni in sediments. Our first experiments showed that NiS was undergoing rapid oxidation if the samples were exposed to oxygenated waters or air. As a consequence, we carried all our experiments in the laboratory under controlled N<sub>2</sub> atmosphere. Using a library of reference compounds and a multivariate analysis method based on the Principal Component Analysis (PCA) method, we were able to assess the speciation of Ni in samples. In addition, we use x-ray near edge absorption fine structure (XANES) to identify the major phases under which Ni is present in the samples that we studied.

In all the samples, from the USGS ecotoxicity studies and the Universities of Michigan and Wright State field studies, we found that some NiS was present. However, Ni does not appear to be bound exclusively to sulfide but it is also coordinated with O. Our data therefore, do not support the conventional “wisdom” that Ni will be uniquely bound to sulfide in anaerobic sediments when sulfides are present in excess.

The test of the SEM-AVS method that we performed showed that a significant fraction of NiS is not soluble 6N HCl but that some of the other fractions – Ni bound to carbonate or to oxides – are. In many instances the spectroscopic data showed that Ni was predominantly present under the form of a sulfide in the residual sediment left after reaction with HCl. Upon oxidation, no detectable Ni remained in the residual sediment fraction indicating that NiS was dissolved. These results question the applicability of the SEM-AVS method in the case of Ni.

## 2. Introduction

The goal of this proposal is to develop and apply a direct speciation method based on X-Ray Absorption Spectroscopy (XAS) for determining the speciation of Ni in contaminated sediments. In addition, we propose to compare and contrast these direct speciation results with more traditional approaches based on sediment leaching experiments or sequential extraction methods. One of the primary emphases of this project is to determine if Ni is effectively bound to sulfides minerals in amended sediments used in toxicity tests.

The processes that control the mobility and bioavailability of Ni, as many other metals, in freshwater sediments remain poorly understood. One of the primary reasons is that it is often difficult to assess the chemical speciation of metals in sediments because they encompass a complex chemical mixture of various well crystallized as well as many ill-defined, poorly crystalline/amorphous solid phases. The solubility of any metal within the sediment pore waters is controlled by the presence of these precipitates, dissolved ligands that can complex the metal, and also the formation of metal-surface complexes. The determination of the chemical speciation of the metal aims at establishing how the metal partitions between these different chemical forms. In most environmental situations, the fraction of a metal that is attached to or occluded in sediment particles is large compare to the part that is present in solution pore waters. The solubility and the lability of the solid phases influence therefore greatly the fate of the metal within sediments. Ni can be present under a wide variety of chemical forms as a result of diagenetic reactions that are driven by microbial processes fueled by the decomposition of organic matter. Consequently, nickel can be associated to various solid phases such as: carbonates oxides or hydroxides, and sulfides or sorbed to other metal precipitates, for example iron or manganese oxides and aluminosilicates.

The few studies that have investigated the behavior of Ni in sedimentary systems suggest that Ni tends to be more mobile than most other metals. However, little remains known about the nature of the chemical phases that bind Ni in sediments. Most of the available information has been inferred from operationally defined wet chemical extraction procedures such as Sequential Extraction – the Tessier (Tessier, Campbell et al. 1979) or BCR schemes - or leaching methods related to the characterization of a specific phase such as the SEM/AVS (Simultaneously Extracted Metals/Acid Volatile Sulfides) protocol (Allen, Fu et al. 1993). As we have documented, these methods are prone to artifacts and can lead to erroneous conclusions for the speciation of metals in sediments (Peltier, Dahl et al. 2005). Another contentious issue in this field is the relation between AVS – Acid Volatile Sulfides – and metals present in sediments. Given that metal sulfides are characterized by low solubility products, the microbial evolution of sedimentary sulfides is viewed as an effective way to immobilize metals. The concentrations of the metals that are simultaneously liberated during the acid leaching of sediments, for liberating the sulfide gas, are conventionally used to assess if these metals are bound to sulfides. Various arguments based on the solubility of the various metal sulfide phases have been used to that effect (Cooper and Morse 1999). However, no one has reported direct evidence of the presence of these pure solid phases within sediments, even the ones that constitute the most significant pool: the Fe-sulfide minerals. Given

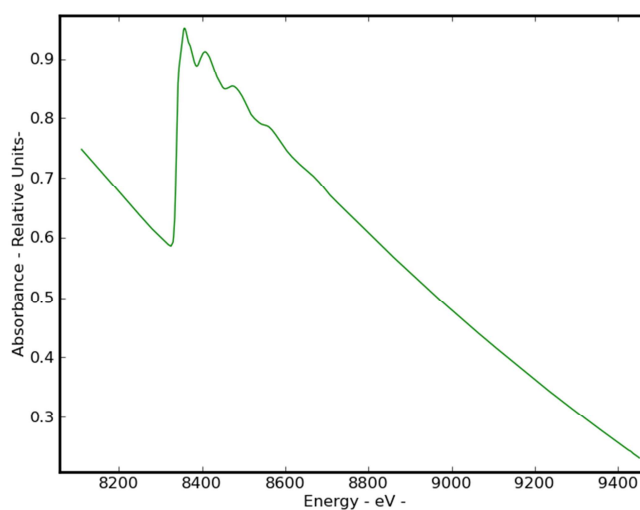
the limitations of these approaches, the chemical speciation of Ni in sediments remains a challenging issue. In particular, it is not clear if the proposed SEM/AVS model is appropriate for Ni.

To address these issues, we have determined the chemical speciation of Ni in sediments using a direct method based on a spectroscopic technique: X-ray Absorption Spectroscopy (XAS). This is a technique that we have successfully used in the past to study the chemical speciation of Zn in freshwater sediments contaminated by the operation of a smelter (Gaillard, Webb et al. 2001; Webb, Gaillard et al. 2001; Peltier, Webb et al. 2003; Webb, Gaillard et al. 2003; Peltier, Dahl et al. 2005; Gaillard 2007; Gough, Dahl et al. 2008; Gough, Dahl et al. 2008).

### **2.1.X-Ray Absorption Spectroscopy (XAS)**

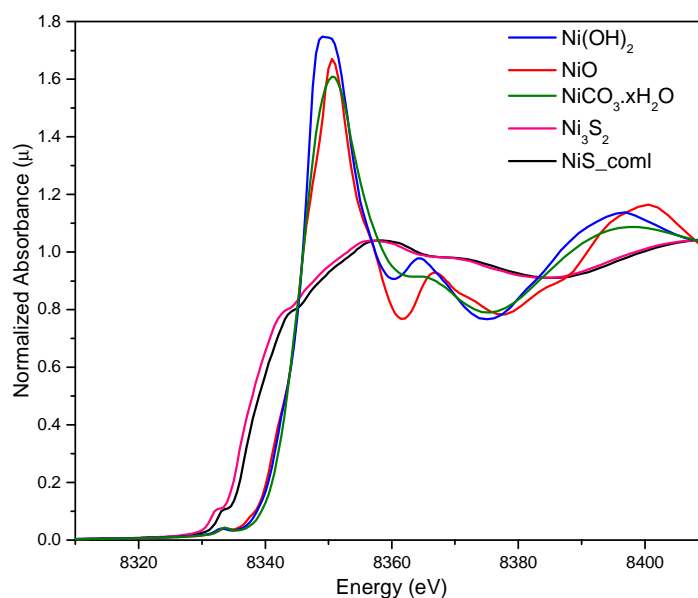
XAS is an element specific technique that can be used with virtually every element in the periodic table; it is independent of the physical state of samples, and it can be used as a direct probe, since it requires little sample preparation (Peterson, Brown et al. 1997; Gaillard 2007; Kelly, Hesterberg et al. 2008). It has been widely used in the general speciation of metals in the environment, since it can provide direct evidence of the presence of specific phases in a variety of natural samples (Peterson, Brown et al. 1997; O'Day, Carroll et al. 2000; Wilke, Farges et al. 2001; Manceau, Marcus et al. 2002a; Peltier, Dahl et al. 2005; Gaillard 2007; Gough, Dahl et al. 2008).

During an X-ray absorption experiments, x-ray photons of increasing energy produce an abrupt jump in the absorption coefficient since they interact with core electrons of the element of interest. An absorption edge is observed at a characteristic energy for each chemical element (Figure 1). This jump occurs when photons of enough energy are absorbed by the element and eject core shell electrons to the continuum. The resulting photoelectron then interacts with neighbor atoms, producing characteristic features that reflect the coordination environment of the central atom. Consequently, XAS is composed of two spectral regions, both of which arise from the interaction of the x-ray photons with the absorbing atom: the X-ray absorption near edge structure (XANES) and the extended x-ray absorption fine structure (EXAFS) (Peterson, Brown et al. 1997; Gaillard 2007; Kelly, Hesterberg et al. 2008).



**Figure 1:** A XAS spectra collected at the Ni K-edge. It shows the abrupt increase in the absorption coefficient at a specific energy, as well as the modulation of this coefficient as a result of the backscattering of the photoelectron by nearest neighbors. These oscillations are characteristic of the coordination environment of the element and can be used to determine provide structural information in the case of pure compounds.

The XANES region provides information about the oxidation state and the geometry of an element (Peterson, Brown et al. 1997; Gaillard 2007; Kelly, Hesterberg et al. 2008), and the shape of the absorption edge can be affected by the metal coordination environment. This is the case with nickel: when bound to oxygen (O) or nitrogen (N), nickel's edge jump is larger and sharper than when the metal is bound to sulfur (S) (Figure 2) (Eidsness, Sullivan et al. 1988). This is so because there are more bound states available in the oxygen or nitrogen hybridized orbitals than in the sulfur orbitals, making it possible for the nickel core electron to move to one of these bound states before being ejected to the continuum.



**Figure 2:** XANES spectra from various Ni compounds. Note the significant difference between the spectra of Ni-sulfides and the other Ni compounds such as Ni oxide or hydroxide as well as the Ni-carbonate where Ni is coordinated to an O in the first shell. These spectral differences provide an easy way to fingerprint species in samples.

The EXAFS region is used to determine the element's local coordination environment. The EXAFS spectral features depend on constructive and destructive interferences resulting from the interactions between the ejected photoelectron and elements present around nickel (Peterson, Brown et al. 1997; Gaillard 2007; Kelly, Hesterberg et al. 2008). Ni-EXAFS has been used to successfully study, for example, the inclusion of Ni into layered double hydroxides, including the determination of coordination number and bond distances between Ni-O, Ni-Ni, and Ni-Al (Peltier, Allada et al. 2006) and the effect of humic acid in the formation of such Ni-layered structures (Nachtegaal and Sparks 2003). Manceau and colleagues have also studied the behavior of nickel (and other metals) in complex layered minerals using XANES and EXAFS (Manceau, Gorshkov et al. 1992a; Manceau, Gorshkov et al. 1992b; Manceau, Tamura et al. 2002b).

### 3. Method

#### 3.1. Principle of Nickel Speciation by XAS

The EXAFS features can be considered a fingerprint of individual nickel compounds. Since the XAS signal represents an average of the metal coordination environment in the sample being probed, in the case of mixtures of nickel compounds the spectra can be decomposed into individual components. This is very useful in the study of metal speciation in natural samples, where the metal is rarely present as a single compound, but in more than one phase.

The EXAFS signal of a mixture of nickel compounds can thus be expressed as (Gaillard, Webb et al. 2001; Manceau, Marcus et al. 2002a)

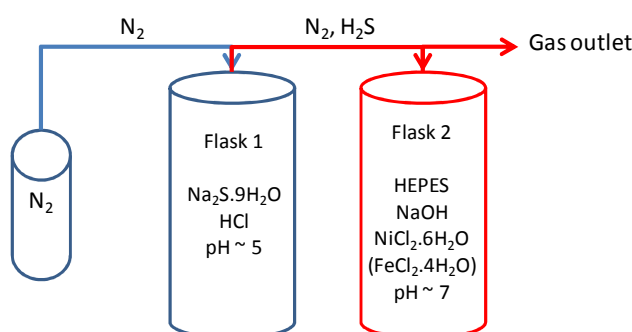
$$\chi_{mixture} = \sum_{i=1}^n f_i \chi_i$$

Where  $f_i$  represents the fraction of each individual compound  $i$  present in the mixture and  $\chi_i$  ( $\chi_i$ ) represents the EXAFS function of each compound. Therefore, in order to perform the spectral decomposition of a mixture, one needs to have the spectra of all the individual compounds that are present in the sample. If one were to miss a significant reference compound, then the spectral decomposition will be incomplete. For this work, nickel standards were obtained both through laboratory synthesis and commercially available sources.

## 3.2. Synthesis of Standard Nickel Compounds

### 3.2.1. Nickel Sulfides

Nickel sulfide and its co-precipitate phase with iron sulfide were synthesized under a nitrogen atmosphere through a procedure similar to the synthesis of mackinawite (FeS) (Cooper and Morse 1999), illustrated in Figure 3.



**Figure 3.** Simplified scheme used to synthesize Ni sulfide compounds.

After purging the system with nitrogen for at least 30 min, a solution of about 0.01 M sulfide was obtained by washing and weighing a sodium sulfide pellet ( $\text{Na}_2\text{S}\cdot 9\text{H}_2\text{O}$ ) to the approximate desired mass and dissolving it into deoxygenated Milli-Q water containing 0.02 M HCl. The evolved sulfide was trapped into a second solution, which contained HEPES (4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid), NaOH, and the metal(s) of interest ( $\text{NiCl}_2\cdot 6\text{H}_2\text{O}$  for  $\text{Ni}^{2+}$  and  $\text{FeCl}_2\cdot 4\text{H}_2\text{O}$  for  $\text{Fe}^{2+}$  when preparing a 1:1 mixture of the two metal sulfides). The total concentration of metals Ni and Fe in the second solution was 0.01 M. The nickel and iron salts were the last reagents added to the system and the mixture was stirred for about 30 min. HEPES buffer and NaOH were used to prevent acidification of the solution (due to the presence of the metals) and consequent loss of sulfide gas. The precipitates were washed through three steps of centrifugation and addition of deoxygenated Milli-Q water, and then



recovered by lyophilization. All the materials used were previously washed in acid to prevent contamination. Contact with oxygen was prevented by flushing the system with N<sub>2</sub> gas, as well as manipulation of the synthesized material in a N<sub>2</sub>/H<sub>2</sub> atmosphere anaerobic chamber.

The nickel sulfide phase expected to form through this procedure is NiS. However, other phases such as Ni<sub>3</sub>S<sub>2</sub> may also form. The products were stored under nitrogen until characterization by XAS and X-ray diffraction (XRD). XAS measurements are described below, and XRD measurements were performed at Northwestern's J.B. Cohen X-ray Diffraction facility.

### **3.2.2. Standards of Nickel bound to Oxygen**

Additional synthesis of nickel carbonate and nickel phosphate compounds was achieved following a similar procedure, changing the anion solution according to the desired solid phase, without the need to use two separate bottles or avoiding oxygen atmosphere. NaHCO<sub>3</sub> was used for carbonates and NaH<sub>2</sub>PO<sub>4</sub> for phosphates, adding CaCl<sub>2</sub> for additional co-precipitation in the case of nickel carbonate synthesis.

Nickel adsorbed onto Fe(OH)<sub>3</sub> has been achieved by reacting nickel chloride with a slurry of the iron hydroxide. Fe(OH)<sub>3</sub> was obtained by adding ~ 1 g FeCl<sub>3</sub>·6H<sub>2</sub>O and pellets of NaOH into 100 mL of Milli-Q water until a brown-red precipitate formed. The final pH of the mixture was between 5 and 7. The precipitate was separated from excess water by settling and decanting, and it was washed with Milli-Q water. A subset of the product was mixed with Ni<sup>2+</sup> solution magnetically stirred for about 2 hours, after which the water was removed by settling and decantation, followed by filtration through a 0.2 μm membrane. Since this is a hydroxide sample, it was not necessary to be manipulated under N<sub>2</sub> atmosphere.

A solution of 10<sup>-2</sup> M of nickel chloride and 10<sup>-2</sup> M of sodium citrate was used to represent the complexation of nickel by organic carboxyl groups.

Nickel chloride in solution (10<sup>-2</sup> M) and commercially available compounds of nickel (NiCO<sub>3</sub>·xH<sub>2</sub>O, Ni(OH)<sub>2</sub>, NiS<sub>2</sub>) were also used as standards.

### **3.3.XAS Data Collection**

Commercial and synthesized Ni standards were grinded and smeared on Kapton® tape. Liquid standards were placed inside liquid cells with a Kapton® tape window. Sediment samples were passed through filter paper to retain the solids and placed in between Kapton® tape. Standards and samples containing sulfides were manipulated and analyzed under nitrogen atmosphere. A list of nickel standards analyzed is shown in Table 1.

Table 1. List of nickel standards.

<b>Standard</b>	<b>Source</b>
NiS_coml	Alfa Aesar
NiS_lab	Synthesized in the laboratory
Ni <sub>x</sub> Fe <sub>(1-x)</sub> S	Synthesized in the laboratory
NiS <sub>2</sub>	Alfa Aesar
Ni <sub>3</sub> S <sub>2</sub>	Sigma Aldrich
Ni_Birnessite (manganese oxide mineral)	Synthesized in the laboratory
Ni_Solution	Synthesized in the laboratory
Ni_Citrate	Synthesized in the laboratory
NiO	Aldrich
Ni(OH) <sub>2</sub>	Aldrich
NiOOH	Synthesized in Prof. Poeppelmeier's laboratory
NiCO <sub>3</sub> .xH <sub>2</sub> O_coml	Johnson Matthey
NiCO <sub>3</sub> .xH <sub>2</sub> O_lab	Synthesized in the laboratory
Ni_CaCO <sub>3</sub>	Synthesized in the laboratory
Ni <sub>3</sub> (PO <sub>4</sub> ) <sub>2</sub>	Synthesized in the laboratory
Ni_Fe(OH) <sub>3</sub>	Synthesized in the laboratory

XAS data were collected at the Ni K-edge (~ 8.3 keV) using the bending magnet beamline of the DuPont-Northwestern-Dow Collaborative Access Team at the Advanced Photon Source of Argonne National Laboratory. X-ray energy scans were performed using a Si(1,1,1) double-crystal monochromator detuned to 70% intensity to reduce harmonics interference to a negligible level. Depending on signal strength or sample concentration, the signal was collected in transmission or fluorescence mode. Two types of detectors were used to collect fluorescence signals, in order to study which one was the best option for this project: a solid state Canberra 13 element Germanium detector that collected the entire fluorescence emanating from the samples and a bent-Laue spiral filter with an attached Canberra photomultiplier tube to collect the fluorescent energy specific of Ni, removing effectively the fluorescence coming from elements with atomic numbers below Ni.

One of the greatest concerns in measuring Ni XAS data in fluorescence mode is the presence of large concentration of iron compared to Ni in the samples. The K-beta1 fluorescence of Fe is at 7059 eV and a fraction of that signal interferes with the K-alpha1 fluorescence of Ni, which is at 7480 eV. The use of the 13 element detector allows the definition of an energy window through which one can collect the relevant signal, i.e., the signal originating from the fluorescence of Ni. However, this window is about 300 eV wide in energy and some overlap is present between the Fe fluorescence signal and the Ni signal. Since Fe is usually present in abundance in natural samples, and Ni is expected in concentrations much lower than Fe, this increases significantly the counting times for the acquisition of Ni XAS spectra because of the large fluorescence signal coming from Fe that contributes to the dead-time of the detector.

The bent-Laue spiral filter is a Si (1,1,1) crystal about 500 $\mu\text{m}$  thick. A spiral filter made specifically to collect Ni fluorescence signal may have a signal collection window of about 20eV, which removes effectively the Fe interference when compared to the 13 element detector. The disadvantage of this filter is that, due to its thickness, it greatly absorbs part of the Ni fluorescence signal, leaving only a fraction of it to be detected by the photomultiplier. The X-ray beam hitting the sample needs to be only a few microns in diameter – point source - for geometric reasons related to the operation of a bent-Laue spiral filter. In order to use such a focused beam one needs to either use an injection device or undulator beamline that is usually in higher demand or a bending magnet with sagittal focusing using a bender. Our trial experiments with the bent-Laue spiral filter resulted in Ni XAS spectra that were noisier than with the 13 element detector – primarily because the number of x-ray photon collected was much lower than expected. Therefore, we decided to collect all our spectra from various samples using the 13 element detector. Scans were collected using a narrower detection window to reduce interference of the Fe signal and we increase the collection times in order to obtain the best signal/noise ratio possible within the allocated beamtime.

### 3.4.XAS Spectral Decomposition

#### 3.4.1. Normalization and background removal

Normalization of XAS data was performed using the software Athena, which is built on IFEFFIT(Newville 2001; Ravel and Newville 2005; Ravel and Newville 2005). Scans of each sample were aligned, averaged, energy calibrated and normalized. Energy calibration was performed with respect to the first inflection point of the Ni metal foil absorption edge, which was determined by its first derivative and set to 8333 eV. Normalization was performed by fitting pre- and post-edge functions to each scan, then setting the difference between these functions at the edge energy to one absorption coefficient unit. To ensure consistency in the normalization, the same energy range and polynomial type of the pre- and post-edge lines were used for all samples.

Traditional EXAFS data treatment consists of the extraction of the EXAFS data through the removal of a background function after normalization of the spectra. Commonly used data treatment programs have built in features for background removal, usually based on the algorithm Autobk (Newville 2001). The algorithm calculates a background function for each data set, *i.e.*, for each normalized average. Each background function has its own sinusoidal features along the EXAFS region, so that the extracted EXAFS - graphed as  $\chi(k)$  as a function of modulus of the wavevector  $k(\text{\AA}^{-1})$  - for each averaged spectra waves symmetrically around zero. This facilitates further modeling of the data and is very useful for the study and modeling of pure compounds. However, it is not good practice for the speciation of metals in natural samples, as in the present work.

The removal of different background functions from different averaged spectra can cause artifacts in the extracted EXAFS data that may be critical for the speciation analysis. We have found that speciation results are more reliable when a single background function is used to extract the EXAFS data from the spectra of all standards and all samples. Therefore, normalized data were exported from Athena into the software Origin (OriginLab 2007) for the removal of a fixed background function. The extracted EXAFS were then weighted by  $k^2$  to account for the decrease of the signal when moving away from the edge.

### 3.4.2. Principal Component Analysis and Least Squares Fitting

Principal Component Analysis (PCA) was carried out through the software SixPack (Webb 2005), which is also built on IFEFFIT. The objective of this multivariate analysis is to determine the number of components, or principal axes, that can explain the set of spectroscopic signals obtained from the samples. In essence, it provides a number of components that are originally equal to the number of samples being analyzed, but allows one to reduce this set to fewer components that explain most of the variance present in the data set. Each component is mathematically defined by a linear combination of the samples and has its own “significance” in representing the group of samples. The number of *principal* components chosen for a given group of samples is selected by looking at the structure of the signal that it represents. The principal components that do not show any specific spectroscopic features, i.e., for which the Fourier Transform resembles “white noise”, are not retained in the analysis. In addition, the selection of the principal components to retain is achieved through the calculation of various parameters such as the spoil factor (Malinowski 2002; Manceau, Marcus et al. 2002a).

After defining the principal components from the set of samples’ spectra, target transformation was used to select from the pool of standards the ones that were relevant to the group of samples being studied. Target transformation consists in verifying whether the function describing a standard reference spectrum belongs to the same space of functions describing the group of samples being studied and, consequently, whether it can be reconstructed by the same components found by PCA. If the resultant reconstructed function matches well with that of the standard, the standard can be a possible species in the unknown samples.

Three parameters are used to characterize a standard during a target transformation: chi-square, R value, and spoil value. The chi-square is a traditional sum of the squared differentials and represents the difference between the target transform spectrum and the original standard spectrum (it should not be confused with the  $\chi(k)$  or  $\chi(k)$  that defines the EXAFS function). The R value is a measure of the percent misfit between the two spectra, and the spoil value is parameter that allows one to decide acceptable thresholds for considering target, i.e., reference spectra to include in the spectral decomposition (Malinowski 2002). The lower these values, the closer the standard is to the group of samples, which means the compound can be part of the samples composition. Therefore, target transformation is used to select the standards that will be used in the next step of spectral decomposition: linear combination fitting.

In a group of samples with a fairly well defined composition, finding the compounds that are likely part of their composition can be easy because their chi-square, R and spoil values will be several orders of magnitude lower than the standards that are not present in the samples. A sharp cut value, e.g. < 1% misfit, can be used to select the standards to be part of a linear combination fitting. However, in natural samples the metal of interest may be partitioned into several different phases and a sharp cut value for the selection of standards is not as obvious.

Once the main components likely to be in the group of samples were selected, they were used in a linear combination fitting for each sample through the Least Square Fitting feature in the software SixPack (Webb 2005). Fits were forced to be summed to 1 and to be non-negative.

### 3.4.3. Comparison between different methods of background removal

In order to evaluate the reliability of the XAS data treatment in terms of nickel speciation results, a set of 30 hypothetical samples (HS) of known composition were created and submitted through the data treatment process. These samples were mathematical mixtures of four nickel standards: NiCO<sub>3</sub>.xH<sub>2</sub>O (commercial), Ni(OH)<sub>2</sub>, Ni<sup>2+</sup> in solution, and nickel sulfide synthesized in the laboratory (NiS\_lab). These HS samples were created through simple linear combination of varying fractions of the standards spectra, with the concentration of NiS\_lab varying from 5% to 95% (Table 2). Therefore, the EXAFS function of these samples can be expressed as described by a  $\chi_{\text{mixture}}$  as explained above.

**Table 2.** Mathematical composition of hypothetical samples.

Hypothetical Sample	Fraction of standard				Total
	NiS_lab	NiCO <sub>3</sub> _coml	Ni(OH) <sub>2</sub>	Ni_Solution	
HS1	0.05	0.1	0.15	0.7	1
HS2	0.05	0.25	0.45	0.25	1
HS3	0.1	0.35	0.35	0.2	1
HS4	0.1	0.7	0.15	0.05	1
HS5	0.15	0.05	0.5	0.3	1
HS6	0.15	0.4	0.4	0.05	1
HS7	0.2	0.65	0.05	0.1	1
HS8	0.2	0.05	0.7	0.05	1
HS9	0.25	0.5	0.15	0.1	1
HS10	0.3	0.3	0.2	0.2	1
HS11	0.3	0.6	0.05	0.05	1
HS12	0.35	0.15	0.25	0.25	1
HS13	0.4	0.05	0.5	0.05	1
HS14	0.4	0.4	0.1	0.1	1
HS15	0.45	0.05	0.1	0.4	1
HS16	0.5	0.2	0.15	0.15	1
HS17	0.5	0.15	0.3	0.05	1
HS18	0.55	0.35	0.05	0.05	1
HS19	0.6	0.05	0.25	0.1	1
HS20	0.65	0.05	0.15	0.15	1
HS21	0.65	0.2	0.1	0.05	1
HS22	0.7	0.1	0.05	0.15	1
HS23	0.75	0.05	0.05	0.15	1
HS24	0.75	0.1	0.1	0.05	1
HS25	0.8	0.1	0.05	0.05	1
HS26	0.85	0.05	0.05	0.05	1
HS27	0.85	0	0.05	0.1	1
HS28	0.9	0.05	0.05	0	1
HS29	0.95	0	0	0.05	1
HS30	0.95	0	0.05	0	1

Four background removal processes were independently applied to the hypothetical samples, followed by PCA, target transformation, and least squares fitting. The main components identified by target transformation and their concentrations in each hypothetical sample given by least squares fitting were then compared to the mathematical composition of the samples.

The four background removal processes tested were:

- a) Autobk background removal done in the software Athena: as explained earlier, this background removal utilizes a specific background function for each spectrum, each one with their own features around the EXAFS region. In order to ensure the maximum consistency possible, the same spline range and clamps were set to all samples (spline k range: 0.0 to 9.949; no clamps for both low and high energy). Spline clamps define how tight to the data the spline (*i.e.*, the background function) should be. In datasets with EXAFS structure up to the end of the spectrum, a loose spline (with weak or no clamps) is usually advisable, because it will in principle not match the wiggles in the data, therefore preserving the structure.
- b) Removal of the background function given by Autobk for the Ni(OH)<sub>2</sub> spectrum: without spline clamps in both low and high energies.
- c) Removal of the background function given by Autobk to the NiS lab spectrum: without spline clamps in both low and high energies.
- d) Removal of a smoother background function given Autobk to the NiS lab spectrum: with a rigid spline clamp in low energy and no clamps in high energy.

### 3.5. Selective chemical extraction of nickel from sediments

A metals' contaminated lake sediment - Lake DePue, Illinois (Webb, Leppard et al. 2000; Gough, Dahl et al. 2008; Gough, Dahl et al. 2008) - and a clay (Kaolinite from Georgia) were amended with known concentrations of nickel standards, and subjected to a two-step chemical extraction process. The speciation of Ni in the amended and residual sediment fractions were determined by XAS. In this case, we focused on the SEM-AVS method to assess whether or not it was providing reliable information on the chemical make-up of the sediment.

#### 3.5.1. Nickel-amended sediments

Amendments were performed under anaerobic conditions, *i.e.*, in the anaerobic chamber to avoid any oxidation of NiS. Samples were amended with 1000 ppm (0.1%) of nickel sulfide, 500 ppm (0.05%) of nickel carbonate and 500 ppm (0.05%) of nickel hydroxide. Three "types" of nickel sulfide were used separately for each sediment matrix: NiS\_lab, NiS\_coml, and Ni<sub>3</sub>S<sub>2</sub>. Nickel carbonate and nickel hydroxide used in this experiment were both from commercial sources. About 10g of each sediment sample were measured and the appropriate mass of each nickel compound was added to the sample. The mixture was homogenized using mortar and pestle and let stand overnight in a closed flask. A small sample of each sample was collected for XAS analysis.

#### 3.5.2. Chemical extraction of Ni-amended sediments

After each of the two extraction steps described below, a sample of the residual sediment was collected and submitted to XAS analysis.

##### 3.5.2.1. Step 1 – $\Sigma$ SEM/AVS

The  $\Sigma$ SEM/AVS protocol recommended by EPA has been described elsewhere (Allen, Fu et al. 1993; U.S.EPA 2005). A simplified scheme of the system is shown in Figure 4. The extraction system was set up

in a fume hood. All glassware and plastic ware were rinsed with 5% HNO<sub>3</sub> prior to use to avoid contamination.

Briefly, 100mL of Milli-Q water was added to the reaction flask and 80mL of 0.05M NaOH was added to each of the H<sub>2</sub>S trap flasks. The system was purged with N<sub>2</sub> for about 1h. In the anaerobic chamber, about 5g of sediment sample was weighed on a 5 x 5 cm parafilm, which was kept under anaerobic conditions until it was added to the reaction flask. The system was purged again with N<sub>2</sub> for about 10min, and 20mL of 6M HCl was added to the reaction flask through one of the septa. The reaction mixture was then magnetically mixed for about 1h under the N<sub>2</sub> flow.

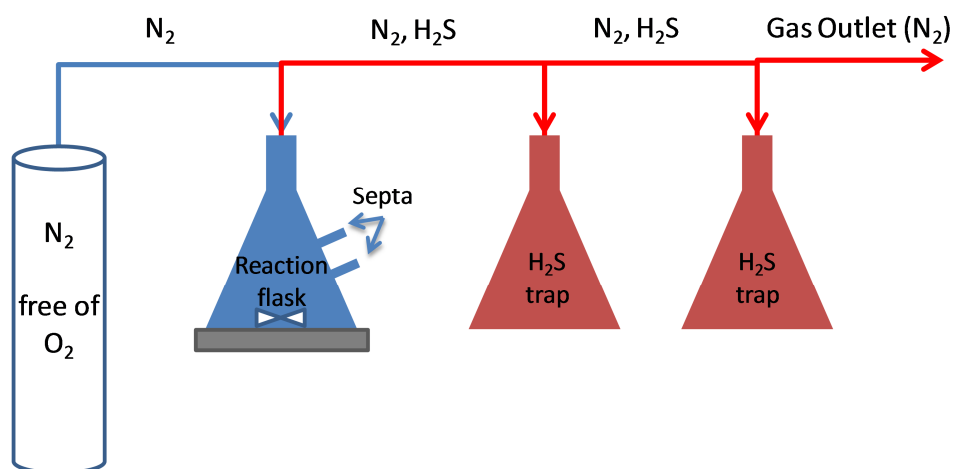


Figure 4. Simplified scheme of the system used for ΣSEM/AVS extraction.

After 1h, the system was closed to prevent oxidation and the flasks were transferred to the anaerobic chamber. Supernatant of the reaction flask was collected using a syringe and filtered through 0.2 μm pore size filters into plastic flasks for SEM analysis. The residual solids were transferred to plastic flasks. Solutions from the H<sub>2</sub>S traps were transferred to 100mL volumetric flasks for determination of AVS.

#### 3.5.2.2. Step 2 – Oxidation

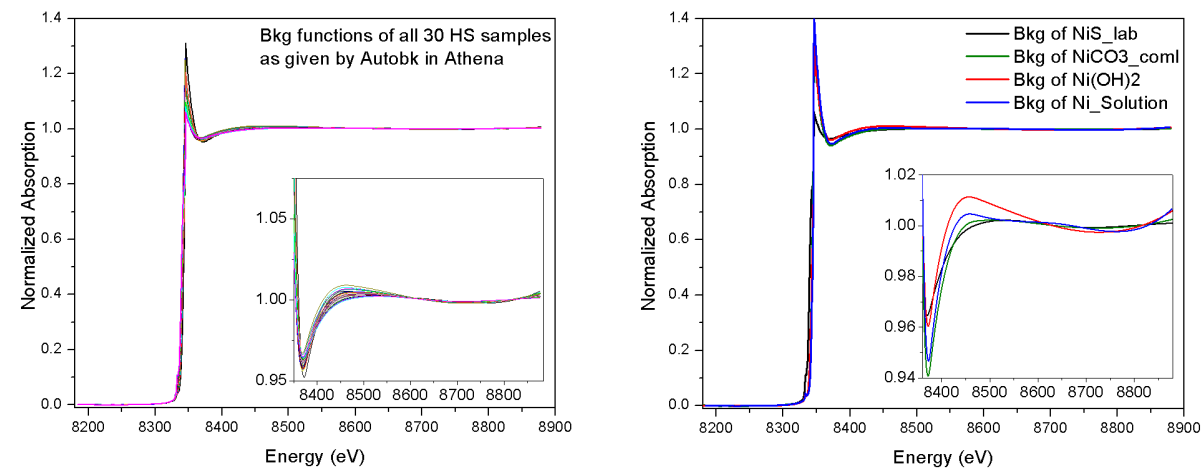
The residual sediments from ΣSEM/AVS were subjected to an oxidation step similar to the one used by the European Community Bureau of Reference (BCR) method for extraction of metals from sediment samples (Ure, Quevauviller et al. 1993; Boughriet, Proix et al. 2007).

Of the residual from ΣSEM/AVS, 0.5g was weighed and transferred into 55 mL PFA microwave tubes, to which 10 mL of 8.8M (30%) H<sub>2</sub>O<sub>2</sub> were added. The digestion was carried out for 1h at room temperature and then in a MARS system microwave (CEM Corp) at 85°C for 1h. The contents were then transferred to a 125 mL Erlenmeyer, and 50mL of 2M ammonium acetate adjusted to pH 2 with 70% HNO<sub>3</sub> were added. After 16h of digestion at room temperature with occasional, gentle agitation, the mixture was centrifuged at 3000 rpm for 10 min. The supernatant was collected using a syringe and filtered through 0.2μm filters into plastic flasks for analysis of nickel.

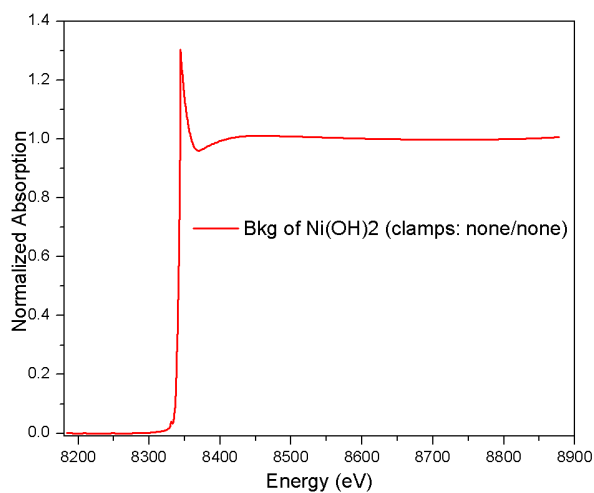
## 4. Results and Discussion

### 4.1. The importance of a consistent background removal method

The different shapes of background functions used for the four types of background removal processes described in section 2.5.3 (Comparison between different methods of background removal) are shown in Figure 5.

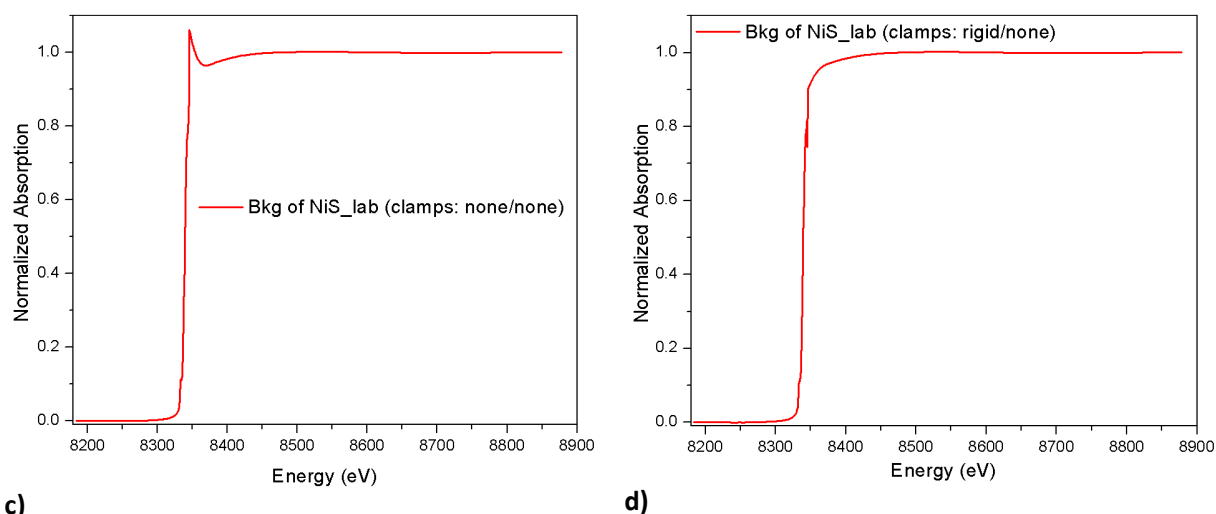


a)



b)





**c)** **d)**  
**Figure 5.** Shape of the background functions used to test the reliability of the background removal process when studying Ni speciation in complex samples. (a) Autobk background functions given by the software Athena; (b) background function given by Autobk for the Ni(OH)<sub>2</sub> spectrum; (c) background function given by Autobk to the NiS\_lab spectrum; (d) smoother background function given by Autobk to the NiS\_lab spectrum.

Following PCA, target transformation was performed in all standards available, including those that were not part of the HS samples composition. For background removals performed with a single background function removed from all spectra (background removal processes b, c, and d described above), the standards that were in fact used to construct the samples were clearly identified by the Target Transformation as part of the same mathematical space as the HS samples, whereas the standards that were not part of the HS samples composition were not recognized as belonging to the same space of functions (Table 3). Furthermore, through least squares fitting the correct concentration of the standards used to make up the samples were identified. These results were independent of the shape of the background function used, *i.e.*, as long as one single background function was used to extract the EXAFS data of HS samples and standards, the spectral decomposition results were reliable.

However, for the EXAFS functions extracted by the automatic Autobk process (process (a) described above), the results were unreliable. Through target transformation, it was difficult to find good criteria to define which standards belonged to the mathematical space of the HS samples. Consequently, wrong standards were identified and some of the standards that should have been identified as important were not (Table 3). Carrying these standards over to the Least Squares Fitting, the expected composition of the samples was also not correctly identified. This is due to the fact that, as it can be seen in Figure 5 and mentioned previously, each background function has its own shape and can remove important detailed features from the EXAFS spectra, making further analysis difficult. On the one hand, the automatic Autobk background removal is very well suited for EXAFS analysis of pure compounds or less complex samples, for which the resulting  $\chi$  spectra are brought to modeling software that minimize the  $\chi$  function and allow study of structural properties. On another hand, the present results show that this background removal process is not well suited for *speciation analysis of metals* in more complex matrices.

For this reason, background removal involving natural samples was performed using a single background function for treatment of all spectra.

**Table 3:** Comparison between target transformation based on fixed background function removal and on Autobk automatic background removal. Correct standards were identified by the former process, but not by the latter.

Standard	Removal of a fixed background function*			Background removal done automatically by Autobk in the software Athena		
	Chi Sq.	R. Value	Spoil	Chi Sq.	R. Value	Spoil
NiO	0.24426	0.34119	2.41e14	208.13301	0.54341	28.4333
Ni(OH) <sub>2</sub>	3.04e-30	6.87e-30	1.5393	4.59104	0.03628	22.5011
NiOOH	0.00170	0.00461	8.16e13	2.49835	0.02858	17.2240
NiCO <sub>3</sub> _coml	2.18e-30	7.08e-30	1.6325	2.08063	0.03243	30.0081
NiCO <sub>3</sub> _lab	0.00670	0.01879	8.31e13	1.23033	0.01759	19.8557
Ni <sub>3</sub> (PO <sub>4</sub> ) <sub>2</sub>	0.00493	0.01236	1.1e14	2.72336	0.03309	15.1326
NiS <sub>2</sub>	0.02423	0.15277	1.46e14	31.49104	0.51616	91.1224
Ni <sub>3</sub> S <sub>2</sub>	0.01376	0.11523	1.69e14	14.69488	0.22334	42.7659
NiS_coml	0.01534	0.12677	1.79e14	16.36512	0.23602	41.9709
NiS_lab	4.13e-31	3.90e-30	1.2855	2.83e-10	7.48e-12	19.9265
Ni <sub>x</sub> Fe <sub>(1-x)</sub> S	0.00143	0.00981	8.59e13	1.61379	0.03061	36.1500
Ni_Fe(OH) <sub>3</sub>	0.00360	0.00937	6.91e13	5.59716	0.06078	34.5028
Ni_Birnessite	0.03505	0.08727	8.19e13	35.42177	0.35335	54.7331
Ni_CaCO <sub>3</sub>	0.00529	0.01592	7.31e13	3.18742	0.04422	31.1860
Ni_Solution	3.55e-30	9.75e-30	1.1954	0.11962	0.00140	86.3368
Ni_Citrate	0.00696	0.01724	6.24e13	4.88567	0.05407	62.1385

\* All three methods of background removal using a fixed background function provided the same end results; the numbers shown here are the ones using the background function given by Autobk for the Ni(OH)<sub>2</sub> spectrum.

## 4.2.USGS samples – XAS Studies

Samples provided by the USGS and that served as a basis for toxicity studies as well as samples received from the University of Michigan were analyzed. In addition, sediment samples from a Ni contaminated lake in the Sudbury region in Ontario (Canada) were also analyzed during beam times that extended until February of 2010. The samples from the University of Michigan were re-labeled at Northwestern to facilitate sample ID - Table 4.

**Table 4:** Simplified sample labels for the University of Michigan Study

Michigan Label	NU Label
Ni9-SJ-High-56d-TOP	SM1
Ni9-SJ-High-56d-BOT	SM2
Ni9-LM-SPR-High-56d-TOP	SM3
Ni9-LM-SPR-High-56d-BOT	SM4
Ni9- SJ -High-28d-TOP	SM5
Ni9- SJ -High-28d-BOT	SM6
Ni9-LM-DowHigh-56d-TOP	SM7
Ni9-LM-DowHigh-56d-BOT	SM8
Ni9-RR-High-56d-TOP	SM9
Ni9-RR-High-56d-BOT	SM10
Ni9-LM-STM-High-56d-TOP	SM11
Ni9-LM-STM-High-56d-BOT	SM12

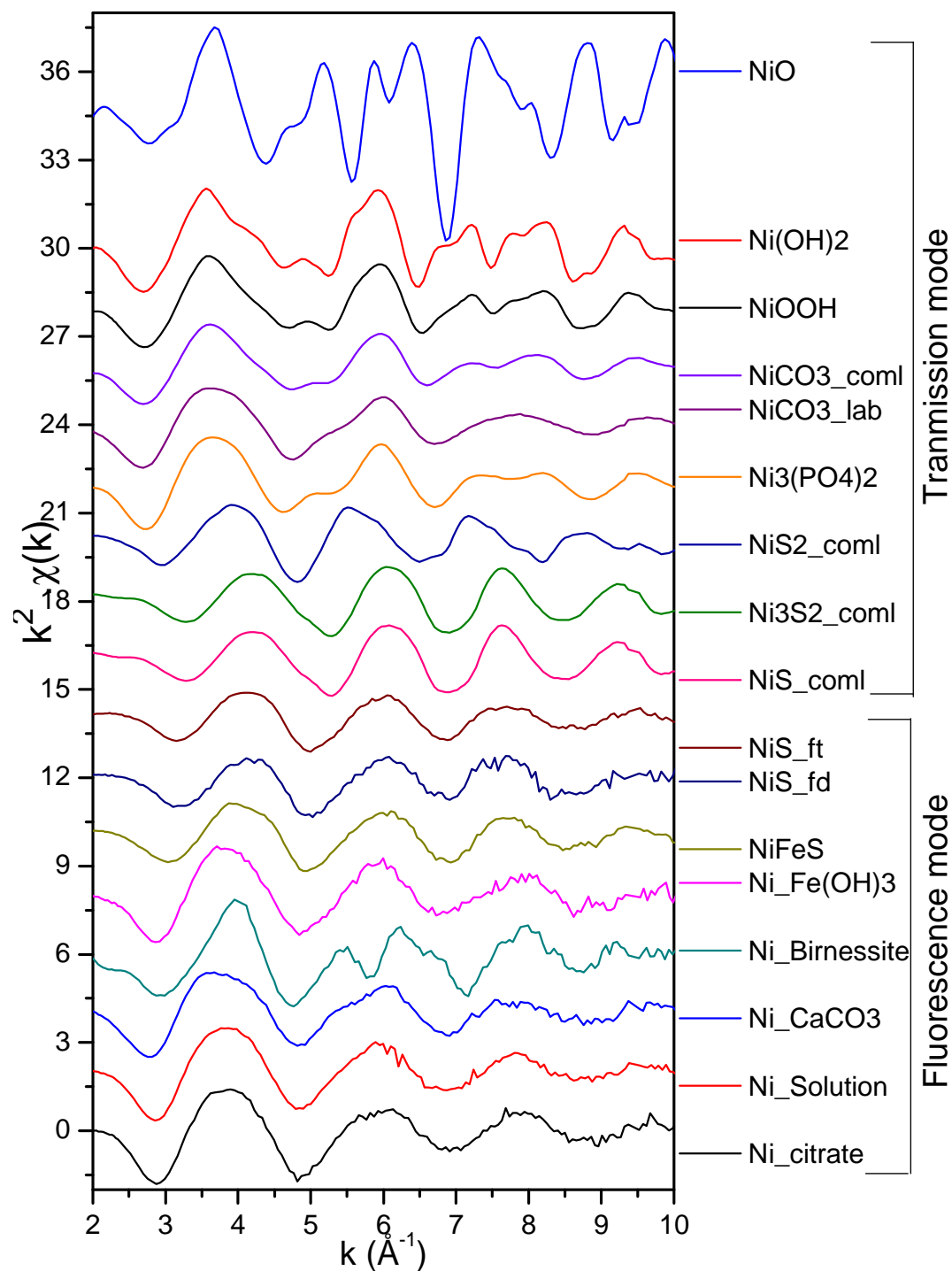
The EXAFS spectra that were obtained from this set of samples are presented, in part, in Figure 6b whereas the list of spectra for the reference spectra that we have collected is given in Figure 6a. The results of the multivariate analysis to select the suite of standard reference material that can explain the spectral signatures observed are presented in Table 5 and the results of the spectral decomposition based on this set of standard material is presented in Table 6.

These direct measurements of the chemical speciation of Ni in sediments show that when we are able to get an XAS spectrum that one can interpret, Ni is most often if not always present in multiple coordination shells. These results show therefore that Ni mineral phases co-exist in the sediment samples that we were able to analyze. Our spectroscopic data do not support the hypothesis that only one form of Ni is present – an hypothesis that is often made when metals are in presence of excess of AVS. The highest fraction of NiS measured on the samples prepared for ecotoxicity tests by the USGS were found in the samples containing the highest values of AVS, samples S8, S10, and S12; the sample S12 containing about 92% (molar fraction) of Ni bound to sulfide. In the other set of samples – S2, S4, and S6 – our results suggest a predominance of phosphate and oxide phases and some sulfide coordination, although the uncertainties attached to these values are high. Actually in the samples the signal obtained can be quite low that makes the spectral decomposition difficult since there is a large amount of noise in the data. As for the measurements carried out on the sediment samples taken from the field experiments performed by the Universities of Michigan and Wright State, the surface sediments did not contain enough Ni to measure any spectroscopic data. Therefore, all the

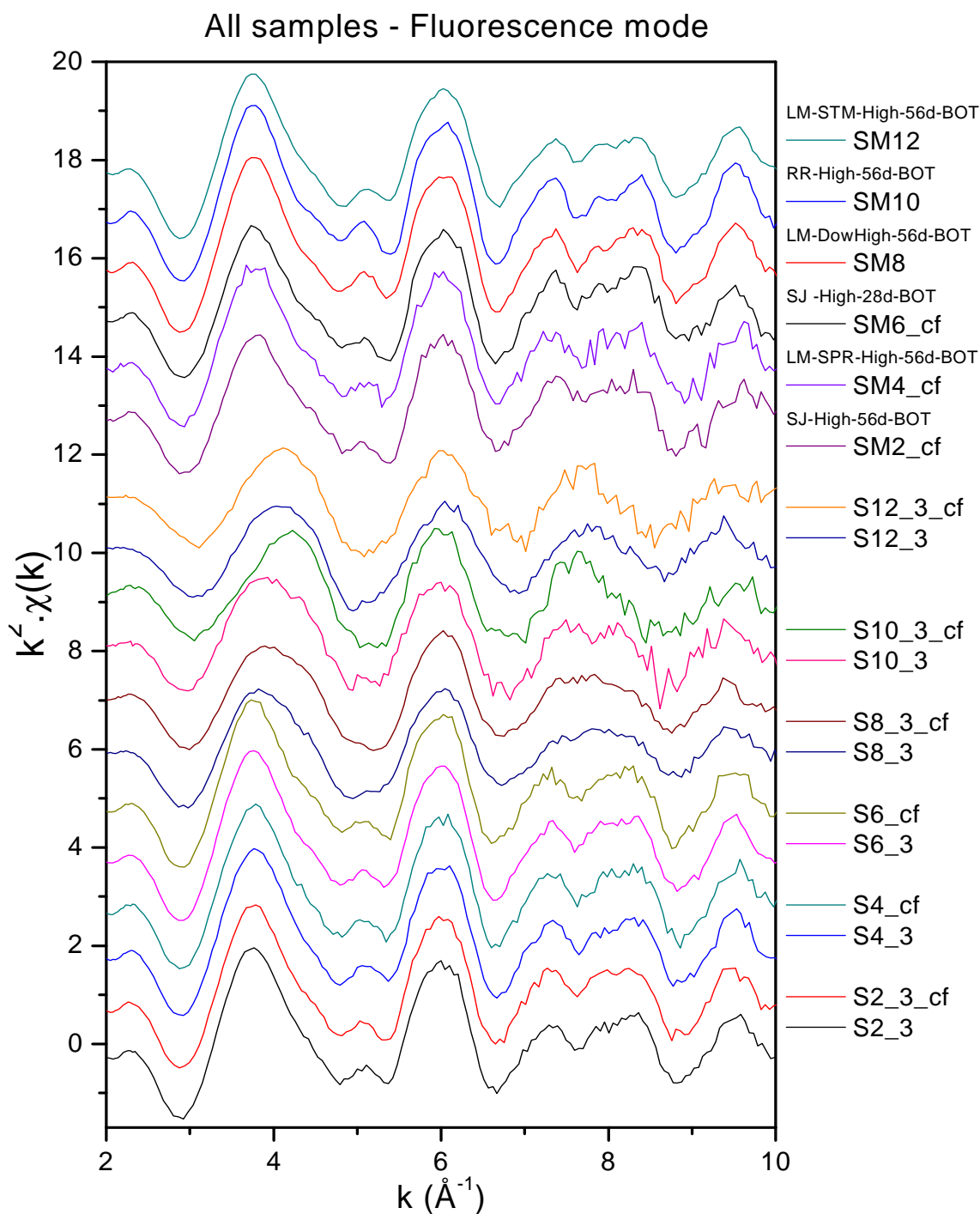
measurements that we did were completed on the bottom sediment samples labeled –BOT in Table 4, *i.e.*, labeled SM2, SM4, SM6, SM8, SM 10, and SM12. In the case of these sediments, most of the Ni was present as Ni sulfides (either as NiS or  $\text{Ni}_x\text{Fe}_{(1-x)}\text{S}$  compounds) with additional coordination environments with phosphate and carbonate groups. The spectral decompositions for the SM samples are however not very reliable, as is indicated by the large uncertainties reported in Table 6. These results reflect the difficulty in obtaining good spectral information at the Ni K-edge on sediment samples.

Some of the results obtained are quite questionable in the sense that the error on the fit obtained is larger than the value of the fraction of the component reported. This usually happens when the set of spectra representing the reference compounds selected by PCA and target transformation is incomplete, *i.e.*, missing one or more components. This is exemplified in Figure 7, where we show the outcome of a “good” and a “bad” fit through the linear combination of reference spectra. In part a, the results of the fit are visually “pleasing” and the analysis of the structure of the residuals through their Fourier transform, shows that most of the spectral signatures are explained. On the other hand, in Figure 7b, the misfit is obvious and the residuals show that some spectral signatures are not present in the vector basis constituted by the components chosen. In this case, the spectra of additional reference materials need to be considered and collected. Since Ni is also thought to be complexed by natural organic matter, we have to perform additional XAS data collection on Ni coordinated to various functional groups which was the beyond the scope of the work proposed within this project.

Concurrently, we are also revisiting our background removal process to see if we can improve it.



**Figure 6a:** EXAFS spectra for the reference materials used in this study. These spectra were acquired either in transmission or in fluorescence mode, the fluorescence mode was necessary for samples with more overall “dilute” Ni concentration.



**Figure 6b:** EXAFS spectra for the samples sent by the USGS and the University of Michigan. These spectra were all acquired in fluorescence mode as required by the concentration level of Ni in the samples.

**Table 5:** List of standard reference material after target transformation. This table is organized by increasing R. value. Only the standards with the lower Chi Square were retained for spectral decomposition.

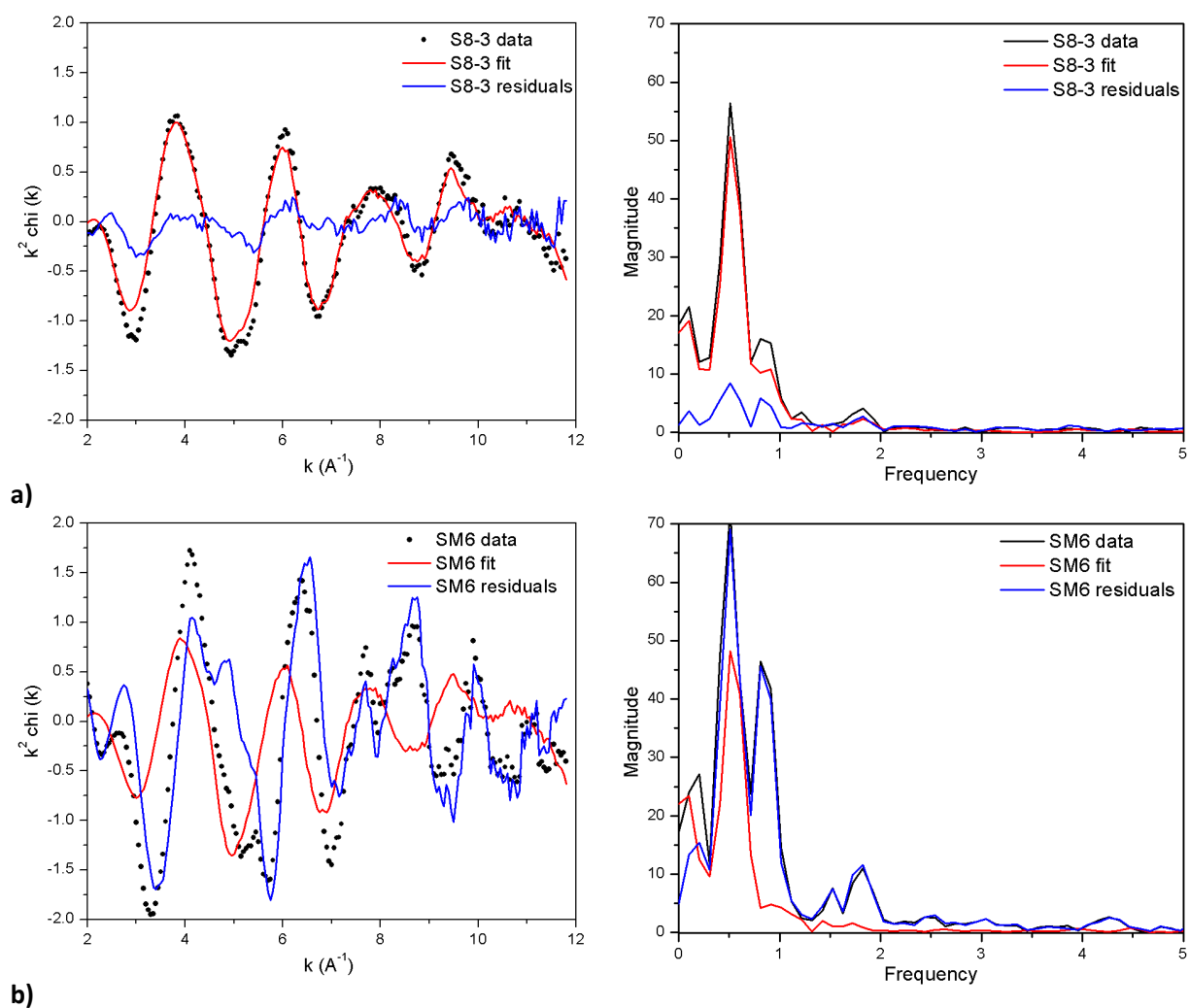
Standard	Chi Sq.	R. Value = % mismatch	Spoil
NiOOH	6.54822	0.07713	2.5454
Ni <sub>x</sub> Fe <sub>(1-x)</sub> S	6.06780	0.10159	2.1029
Ni <sub>3</sub> (PO <sub>4</sub> ) <sub>2</sub>	9.33807	0.11010	3.1835
NiS_lab	5.96546	0.12977	2.2476
NiCO <sub>3</sub> _coml	8.64460	0.13176	3.4218
Ni(OH) <sub>2</sub>	21.66821	0.17971	4.3388
Ni_Fe(OH) <sub>3</sub>	17.37105	0.18096	5.5071
Ni_citrate	19.23239	0.18341	5.6414
Ni_Solution	18.22288	0.20474	5.7183
Ni_CaCO <sub>3</sub>	16.47045	0.21370	5.8084
Ni <sub>3</sub> S <sub>2</sub>	17.65284	0.23804	2.5220
NiCO <sub>3</sub> _lab	18.17874	0.24457	5.8511
NiS_coml	18.98002	0.24728	2.5775
Ni_Birnessite	49.85758	0.39569	9.7491
NiS <sub>2</sub>	34.78350	0.55862	6.5469
NiO	248.59441	0.62104	6.8704

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**Table 6:** EXAFS Linear Combination results of natural sediment samples: “S” samples are from the first USGS batch; “SM” samples are from the second USGS (received from Michigan); “B2” samples are from a lake in Sudbury, Canada.

Sample	Ni compound concentration (fraction of total Ni)					Chi Sq.	Red. Chi Sq.
	NiS_lab	Ni <sub>x</sub> Fe <sub>(1-x)</sub> S	Ni <sub>3</sub> (PO <sub>4</sub> ) <sub>2</sub>	NiCO <sub>3</sub> _coml	NiOOH		
S2-3	0.018768 +/- 0.116810	0.115460 +/- 0.125202	0.383773 +/- 0.120420	-	0.482007 +/- 0.098067	10.9646	0.0571
S4-3	0.040126 +/- 0.124001	0.138104 +/- 0.132909	0.429987 +/- 0.127833	-	0.391792 +/- 0.104104	12.35601	0.0644
S6-3	0.005114 +/- 0.120812	0.132747 +/- 0.129491	0.378369 +/- 0.124545	-	0.483780 +/- 0.101426	11.72866	0.0611
S8-3	0.184702 +/- 0.065651	0.395228 +/- 0.070368	0.153663 +/- 0.067680	-	0.266411 +/- 0.055117	3.4635	0.0180
S10-3	0.238007 +/- 0.138507	0.352676 +/- 0.148458	0.073638 +/- 0.142788	-	0.335681 +/- 0.116283	15.4161	0.0803
S12-3	0.466054 +/- 0.078930	0.451184 +/- 0.084600	-	0.072143 +/- 0.102925	0.010622 +/- 0.066264	5.0062	0.0261
SM2	0.425109 +/- 0.355066	0.375480 +/- 0.380574	0.081761 +/- 0.366038	0.117630 +/- 0.463008	-	101.3081	0.5276
SM4	0.419038 +/- 0.362455	0.387396 +/- 0.388494	0.093627 +/- 0.373655	0.099930 +/- 0.472643	-	105.5685	0.5498
SM6	0.418402 +/- 0.367942	0.383009 +/- 0.394376	0.090988 +/- 0.379313	0.107587 +/- 0.479799	-	108.7893	0.5666
SM8	0.416303 +/- 0.381736	0.391519 +/- 0.409161	0.096796 +/- 0.393533	0.095375 +/- 0.497787	-	117.0991	0.6099
SM10	0.411166 +/- 0.378997	0.397957 +/- 0.406225	0.104198 +/- 0.390709	0.086683 +/- 0.494214	-	115.4244	0.6012
SM12	0.419358 +/- 0.347296	0.384449 +/- 0.372246	0.089608 +/- 0.358028	0.106582 +/- 0.452876	-	96.9226	0.5048
B2 0-5cm	0.481878 +/- 0.295921	0.393391 +/- 0.317181	-	0.117823 +/- 0.385883	0.006889 +/- 0.248438	70.3685	0.3665024 81785
B2 5-10cm	0.474044 +/- 0.299937	0.398940 +/- 0.321485	-	0.121787 +/- 0.391119	0.005214 +/- 0.251809	72.2913	0.3765
B2 10-15cm	0.467153 +/- 0.455507	0.392750 +/- 0.488231	-	0.119435 +/- 0.593983	0.020643 +/- 0.382416	166.7307	0.8684





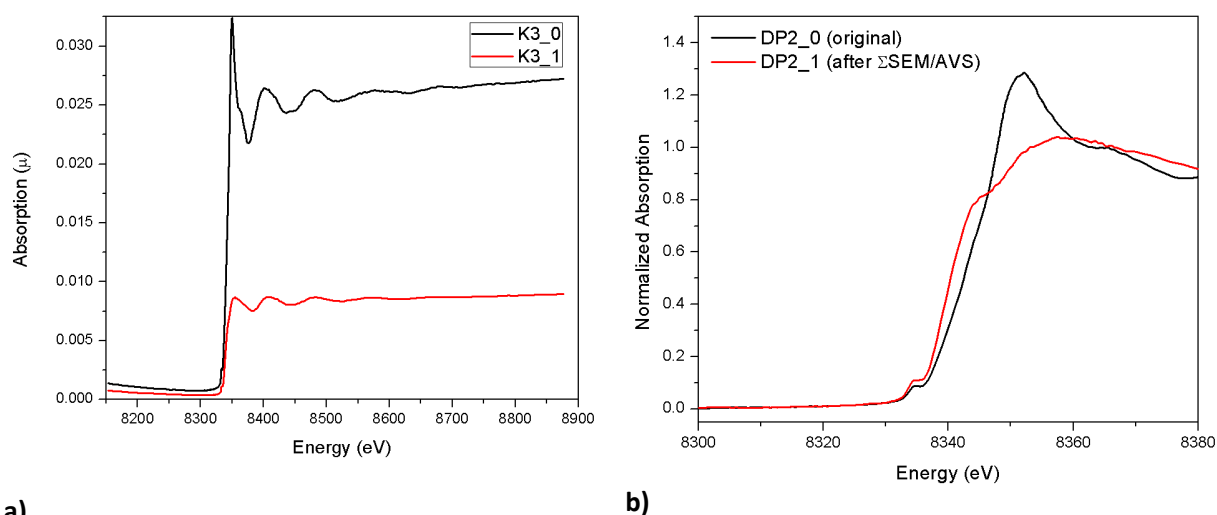
**Figure 7:** Examples of LC-EXAFS fits and residuals of USGS sediment samples. Samples from the first batch (“S” samples) were more easily fitted than samples from the second batch (“SM” samples). The Fourier Transform of residuals show that the residuals of “SM” samples have significant structure, indicating that the fit is missing important components of nickel speciation in those samples.

### 4.3. Testing the effectiveness of $\Sigma$ SEM/AVS through XAS

Given the questions raised in the literature regarding low solubility of nickel sulfides in the extraction medium of  $\Sigma$ SEM/AVS, we tested the protocol using XAS as a direct method to confirm the effectiveness of the extraction. To do this, anoxic sediments from Lake DePue and Kaolinite from Georgia were amended with nickel compounds, including three types of sulfide, and submitted to the process. In addition, the residuals of the  $\Sigma$ SEM/AVS were submitted to an oxidative digestion. Samples of the sediments and clays were collected before and after every step for XAS analysis, as a way to follow the speciation of nickel during the process.

Samples without the addition of nickel compounds showed no measurable Ni signal in the XAS analysis. Samples amended with nickel submitted to  $\Sigma$ SEM/AVS were not expected to produce any Ni signal. However, good quality spectra were obtained, showing that the extraction did not remove nickel from the sediment samples effectively. The residue of  $\Sigma$ SEM/AVS submitted to oxidation did not produce measurable Ni signal, indicating that most of the nickel remaining in the sediments was removed by this second extraction step.

XANES spectra showed that (1) the total concentration of nickel in the sediments decreased by about 70% after  $\Sigma$ SEM/AVS (Figure 8 a), which was estimated by the difference in Delta  $\mu$  of the non-normalized spectra before and after the extraction, and (2) amended sediments initially showed XANES characteristics of mixtures of nickel bound to oxygen and to sulfur, but after  $\Sigma$ SEM/AVS the remaining nickel is primarily bound to sulfur (Figure 8 b).

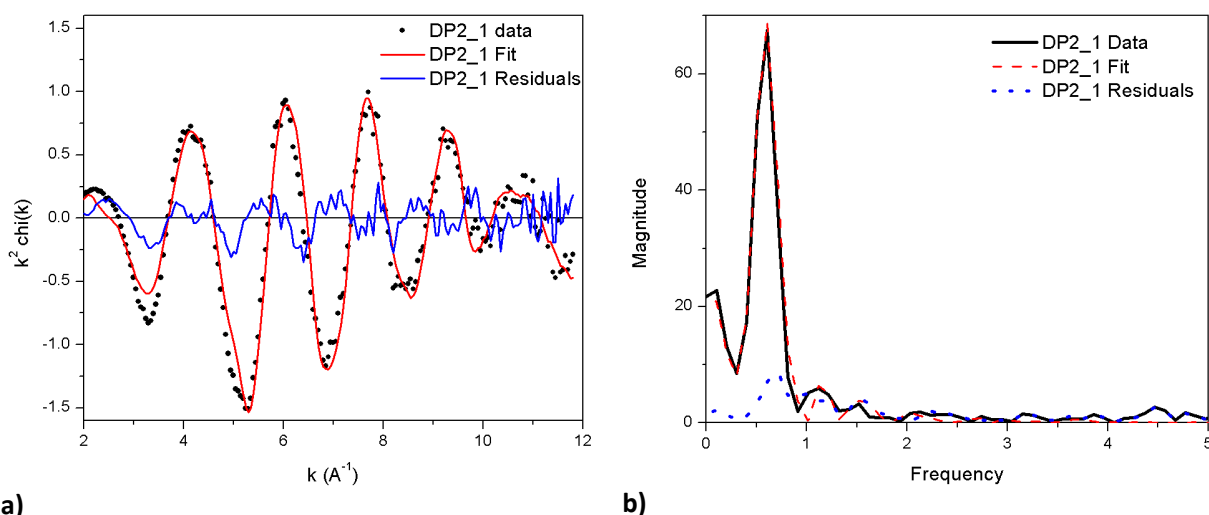


**Figure 8.** General trend of sediments XAS spectra before and after  $\Sigma$ SEM/AVS. **a)** Non-normalized spectra of Kaolinite amended with  $\text{Ni}_3\text{S}_2$ ,  $\text{NiCO}_3 \cdot x\text{H}_2\text{O}$  and  $\text{Ni}(\text{OH})_2$  before (black line, K3\_0) and after (red line, K3\_1)  $\Sigma$ SEM/AVS: the total concentration of nickel in the sediments decreases, but the metal is not completely removed by this extraction method. **b)** Normalized XANES spectra of Lake DePue sediments amended with  $\text{NiS}_{\text{coml}}$ ,  $\text{NiCO}_3 \cdot x\text{H}_2\text{O}$  and  $\text{Ni}(\text{OH})_2$  before (black line, DP2\_0) and after (red line, DP2\_1)  $\Sigma$ SEM/AVS: the remaining nickel in the sediments is primarily in the form of sulfides.

Spectral decomposition of EXAFS spectra of the original samples (Table 7) is in good agreement with the expected results, based on the real mass of various nickel compounds added to each sample. The one exception was sample DP1 (Lake DePue sediments amended with NiS\_lab, NiCO<sub>3</sub>.xH<sub>2</sub>O\_coml, and Ni(OH)<sub>2</sub>), where it seems that nickel sulfide suffers a re-speciation when it is added to the sample.

Since the NiS made in the laboratory is a very fine powder, consisting of colloidal-type particles and amorphous structure, its characteristics are closer to the NiS that possibly forms in anoxic sediments than any of the commercial sulfides. The fact that it re-speciates when in presence of a complex natural sediment matrix, in addition to the difficulties in finding direct evidence of natural NiS formation in sediments, is evidence that NiS is in fact not very stable and can be broken down to liberate Ni<sup>2+</sup> to form other species in the environment. In addition, in lake DePue sediments, the presence of other metals (Webb, Leppard et al. 2000; Gough, Dahl et al. 2008; Gough, Dahl et al. 2008) in relatively large quantity is likely to induce a re-speciation, as observed on a small time scale in this case.

An example of the EXAFS spectral decomposition fit is shown in Figure 9. To confirm that the fit residuals were negligible, a Fast Fourier Transform (FFT) was performed using the software Origin (OriginLab 2007). If the fit were missing any specific components (i.e., any nickel species not included in the fit), fit residuals peaks in the Magnitude versus Frequency graph would be observed. However, no peaks were observed in the FFT of the fit residuals, i.e., the residuals do not seem to have any specific structure. This indicates that the fits did not miss any significant nickel components (Figure 9 b).



**Figure 9. a)** Example of Least Squares Fit obtained using the software SixPack for sample DP2\_1 (Lake DePue sediment amended with NiS\_coml, NiCO<sub>3</sub>.xH<sub>2</sub>O and Ni(OH)<sub>2</sub> after ΣSEM/AVS. **b)** Fast Fourier Transform of the data in (a): the data and the fit show a first shell peak, while no peak is obtained for the residuals, indicating the fit did not miss any important nickel components.

EXAFS spectral decomposition corroborates and quantifies the observations from the XANES region of the spectra: the fraction of nickel carbonate and hydroxide generally decreased after ΣSEM/AVS, while

the fraction of nickel sulfide present in the samples increased (Table 7). In other words,  $\Sigma\text{SEM}/\text{AVS}$  was able to remove most of the nickel present in the sediments as carbonate or hydroxide, but not all of the nickel present as sulfides.

**Table 7.** Nickel amended sediments and Least Squares Fitting (LC-EXAFS) of XAS data before and after  $\Sigma$ SEM/AVS. Sediments without addition of nickel and the residuals of the oxidative digestion did not produce measurable signals in XAS, indicating no presence of nickel.

Conditions	Sample ID		DP1	DP2	K1	K2	K3
	Sediment matrix		Lake DePue	Lake DePue	Kaolinite	Kaolinite	Kaolinite
	Type of sulfide		NiS_lab	NiS_coml	NiS_lab	NiS_coml	Ni3S2
Based on mass added to samples	Fraction	sulfide	0.61	0.68	0.53	0.52	0.59
	of	carbonate	0.15	0.15	0.32	0.17	0.17
	nickel	hydroxide	0.24	0.17	0.15	0.30	0.24
LC-EXAFS of original samples	Fraction	sulfide	0.25 ± 0.05	0.59 ± 0.02	0.47 ± 0.04	0.55 ± 0.02	0.54 ± 0.02
	of	carbonate	0.37 ± 0.12	0.18 ± 0.05	0.46 ± 0.08	0.18 ± 0.04	0.19 ± 0.04
	nickel	hydroxide	0.38 ± 0.08	0.23 ± 0.04	0.07 ± 0.05	0.27 ± 0.03	0.28 ± 0.03
LC-EXAFS after $\Sigma$ SEM/AVS	Fraction	sulfide	0.75 ± 0.04	0.89 ± 0.01	0.55 ± 0.03	0.67 ± 0.04	0.83 ± 0.01
	of	carbonate	0.17 ± 0.10	0.05 ± 0.03	0.15 ± 0.07	0.13 ± 0.09	0.13 ± 0.03
	nickel	hydroxide	0.08 ± 0.07	0.07 ± 0.02	0.29 ± 0.05	0.19 ± 0.06	0.03 ± 0.02

## 5. Conclusions

The primary objectives of this research project were achieved. We were able to obtain direct evidence of the presence of NiS in the sediments that were used for toxicity tests by the USGS.

However Ni was not exclusively bound to sulfur, to form sulfides, since large fractions of Ni were found to be coordinated with O, as one can expect when Ni is present under the form of oxides – or bound to oxide surfaces – or carbonates. The spectroscopic information provided through the analysis of the XANES corroborated the information obtained from the EXAFS region of the XAS spectra, in most of the samples that were amended with Ni, one can expect to find this element in multiple chemical forms. As a consequence, we do not think that is always correct to assume that in presence of excess sulfide Ni would only be present in sediments as nickel sulfide.

The quantification of the different compounds under which Ni is present in sediments is difficult because of the presence of other metals – particularly Fe – that have lower atomic number than Ni and that contribute significantly to the spectroscopic signal. In some instances, our XAS speciation results are not reliable because of the poor quality of the signal or because we are still missing a binding phase for Ni in sediments. However, for many samples the chemical speciation that we have determined is relatively well defined and leads to results that are sound. This is particularly the case when we assessed the efficacy of the AVS/ESM protocol for Ni.

Based on our spectroscopic assessment of the speciation of Ni in sediments subjected to AVS/SEM we can conclude that a relatively large fraction of NiS is not extracted by 6N HCl, the reagent that is used to volatilize sulfide as H<sub>2</sub>S. It turns out that the spectral features obtained on the residual sediment, *i.e.*, the sediment fractions that remains after reaction with HCl, show that it is mostly nickel sulfide that stays behind. Upon oxidation, this NiS is readily leached. Therefore one potential method for assessing the most stable NiS fraction present in sediments would consist in submitting the residual sediment after AVS/SEM to an oxidative step to release NiS.

Overall, our results are challenging some of the principles on which the assessment of Ni toxicity in sediments is based using the AVS/SEM protocol. In presence of excess AVS, it is likely that Ni is not solely bound to sulfides since other coordinative environments for Ni were observed. Therefore, the premise of thermodynamic equilibrium does not seem to hold, which is often the case in aquatic systems. In addition, the AVS/SEM method does not completely extract nickel sulfides from sediments but seems to remove the other Ni species more effectively.

## 6. References

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