

Final Report

The Use of Neutrons for the Characterization of Heavy Metal Binding by Functional Groups Used in Phytoremediation

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1. Introduction

One of the major challenges of the 21st century is to provide sufficient potable water to sustain life on earth. On the basis of current population models that include increases in life expectancy, fertility, and immigration. It is expected that the population in the United States will grow an average of 38% by the year 2040 [1]. The same study shows that the water demand will increase an average of 38% for domestic and public use [1]. This potentially large demand in water resources over the next half century necessitates the development of new strategies to unlock new freshwater resources, as well as efficient filtering methods for cleaning and restoration of freshwater from existing watersheds [1],[2][GEO-3 synthesis report, 2002; Brown, 2000].

Major efforts will have to be undertaken to protect the freshwater resources in the United States. Of particular interest is the remediation of heavy metals from existing contaminated sites since many of these metals and semimetals are highly toxic even in small concentrations. For example, Nickel and Zinc which have been identified as carcinogens and mutagens [3] are introduced into the environment by a variety of widespread industrial processes, such as metal plating, mining operations, steel works, and corrosion of galvanized piping [4]. The wastes from these processes have initiated the difficult and costly problem of heavy metal remediation. The continued contamination of the environment poses a pressing problem to society and policy makers considering the adverse physiological effects of heavy metals even when present in low concentrations. Techniques such as chemical precipitation, solvent extraction, adsorption, and reverse osmosis have been used in an attempt to address this problem. These techniques, however, have intrinsic complications such as unpredictable metal removal, high material cost, and the generation of toxic sludge

The use of plants and plant material in the cleanup of sites contaminated with heavy metals (phytoremediation) such as copper (Cu), chromium (Cr), and nickel (Ni) has gained the interest of environmental scientists over the past decade [1]. However, much of the work is still empirical in nature and a detailed understanding of the transport mechanism of metals in the soil and how they are finally sequestered within the plant system are lacking. These processes operate

on the atomic and nano scale, and once they are understood, they will open new insights into phytoremediation. This observation nurtures the hope that active research of the underlying physical and chemical principles as they are utilized in naturally occurring biological systems will aid in optimizing these processes to design more efficient extraction methods that may be used in water management and water restoration practices and thereby help to meet the increasing freshwater demands in the United States in the 21st century.

Several functional groups are believed to be involved in metal binding by biomaterials, including the carboxyl, hydroxyl, sulfhydryl, amino, and phosphate groups (Figure 1). One particular aspect of this research effort is the characterization of the effects of the sequestered metal on the bond topology and its physical and chemical properties. If known, this would not only allow us to establish selection criteria for systems that possess a

higher affinity for sequestering specific metals but also provide important prerequisites for synthesis experiments of these biomaterials and suggest directions for further characterization in laboratory experiments. The necessary experiments are non-trivial and pose a great challenge for experimentalists. Traditional X-ray and neutron diffraction techniques are more than capable of discerning the bonding environment of the metal within chemical complexes, however, a low signal superimposed on a large background make the interpretation of the results difficult. However, some researchers have had success by probing the extended X-ray Absorption Fine Structure (EXAFS) [5]. While this technique is capable of revealing the metals local bond environment (usually the first coordination shell), it contains only limited information on the intermediate and long-range effects of metal/functional group interactions.

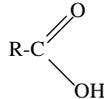
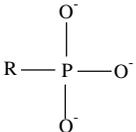
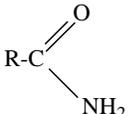
Carboxyl 	Phosphate 
Hydroxyl R-OH	Amine R-NH ₂ , R-NH ⁻ , R-N ₂ ⁻
Sulfhydryl R-SH	Amide 

Figure 1 - shows the functional groups believed important for metal sequestration in phytoremediation.

Previous studies at the Stanford Synchrotron Radiation Laboratory (SSRL) on *Larrea Tridentata* (creosote bush) has demonstrated that it is capable of copper and lead uptake from contaminated soils. In these studies *L. Tridentata* was collected from six copper and lead contaminated sites as well as from two control sites. Fresh *L. Tridentata* and a copper phytochelatin sample, which is thought to be involved in metal detoxification in higher plants [6] were compared. The copper phytochelatin data are typical of the metallothionein class of cuprous-thiolate binding proteins, with predominantly Cu-S coordination and short Cu-Cu interactions at 2.7 Å [6]. The copper EXAFS data indicates the presence of Cu-S and, unlike the phytochelatin, no short Cu-Cu interaction.. The shoulder peak reported at 3.2 Å for site 2 is clearly different from the corresponding Cu-Cu

interaction peaks centered at about 3.7 Å for site 4. This suggests that the biochemical mechanisms responsible for uptake and binding are different depending on the plant growth conditions. Based on these data, binding of the heavy metal copper in *L. Tridentata* is different than what the available literature suggests for other biological systems. EXAFS, however, has shown difficulty in separating out oxygen interactions as well as the inability to exceed the first coordination shell of the metal. The use of neutrons as a probe allows for structural determination past the first coordination shell and by use of isotopic substitution allows for contrast that is unavailable to x-rays, thereby allowing for the direct probing of the bound metal.

2. The Difference Between X-ray and Neutron Interactions with Matter

Neutron and X-ray diffraction are complementary probes of condensed matter. This is a result of the differences in the scattering mechanism between the two radiation types. While neutrons scatter from the nucleus,[7,8] X-rays scatter from the electron cloud. The scattering of X-rays reflects the number of electrons surrounding the nucleus and so the scattering intensity increases with atomic number. The strength of the scattering interaction for both X-rays and neutrons are shown in figure 2. Neutrons scatter poorly but relatively evenly for light and heavy elements. Collection of both X-ray and neutron data sets provides information that is weighted to the number of electrons, and hence the Z number, for x-rays and information that is weighted to the scattering length in the case of neutrons.

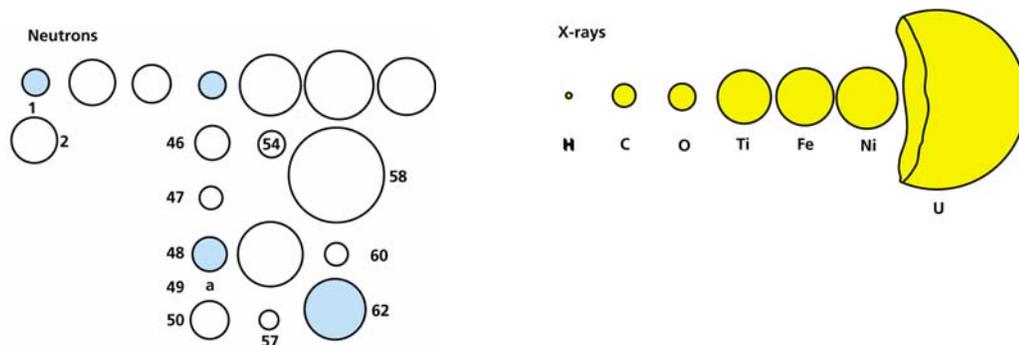


Figure 2: Comparison between the intrinsic scattering interaction strength between neutrons^{7,8} and X-rays

A striking consequence of these differences may be seen in the diffraction data for an oxide system (ref. figure 3). For an oxide glass, such as Yttrium-Aluminum Oxide (YAG), the major contribution to the overall diffraction signal is from oxygen. Implementing the PDF analysis technique the real space transforms of the diffraction data are compared. It is demonstrated that the neutrons probe the metal-oxygen and oxygen-oxygen correlations quite nicely while the X-ray data set elucidates the metal-metal correlations (those of higher Z). Also, Neutrons are sensitive to hydrogen and its isotopes. This is important, as x-rays are not

able to do so. Therefore complementary neutron and X-ray diffraction can prove to be a useful tool in examining hydrogenous samples.

3. The Experiment

The experiment involved a physical characterization of certain functional groups embedded in a commercially available ion-exchange resin, which had been exposed to a heavy metal. The functional groups used were those believed to be most responsible for heavy metal uptake in certain plants [9,10,11], including *L. Tridentata*. The three groups characterized with isotopic substitution were carboxyl, amino, and sulfhydryl (see figure 1). Each group consisted of two samples, one binding ^{58}Ni and the other binding ^{62}Ni at atomic concentrations greater than 1%. Nickel was chosen as the metal to bind because the difference between the scattering length densities of the isotopes is the largest between isotopes in the periodic table. This would enhance the signal relative to background when the first order difference to isolate the metals environment is taken. This characterization will serve as a baseline for the future study of the same functional groups within actual plant matter which has been allowed the uptake of heavy metals.

The experiment made use of isotopic substitution to determine the environment surrounding the metal atom. The large presence of hydrogen within the resin required the use of two identical samples containing different metal isotopes (^{58}Ni and ^{62}Ni in this case). This would permit the extraction of the metal atom's partial structure factors, allowing for a characterization of its environment (i.e. coordination number, neighbors, distances, and bond angles). Low order Chebyshev polynomials would then be used to remove the self scattering of the hydrogen containing samples.

Preparation of the two identical nickel containing polymer resins (^{58}Ni and ^{62}Ni) involved reacting the ion exchange resin at pH 5.0 (the maximum binding pH for nickel) with an aqueous solution containing the metal for one hour. Analysis of the supernatant by atomic absorption allowed a determination of the metal concentration on the resin. Once prepared the sample was freeze dried to remove any trace of water.

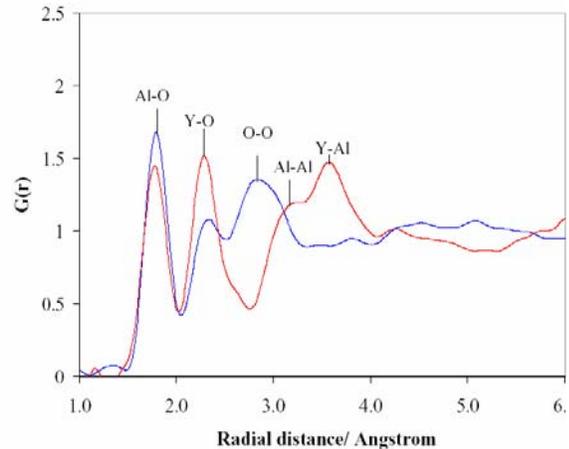


Figure 3 – Yttrium-Aluminum Oxide glass real space distribution functions as probed by x-rays (red) and neutrons (blue)

4. Results and Discussion

The samples were run on the Glass Liquid and Amorphous Diffractometer (GLAD) at the Intense Pulsed Neutron Source at Argonne National Laboratory (Figure 4). GLAD is a time of flight instrument that looks at a 28K solid methane moderator. This provides an incident neutron pulse comprised of a wavelength range of 0.1 – 7.0 Å, ideal for looking at intermediate range ordering. The instrument has a total of 235 ³He detectors contained within 7 detector banks isolated from each other by radial collimators which serve to substantially reduce instrument background.

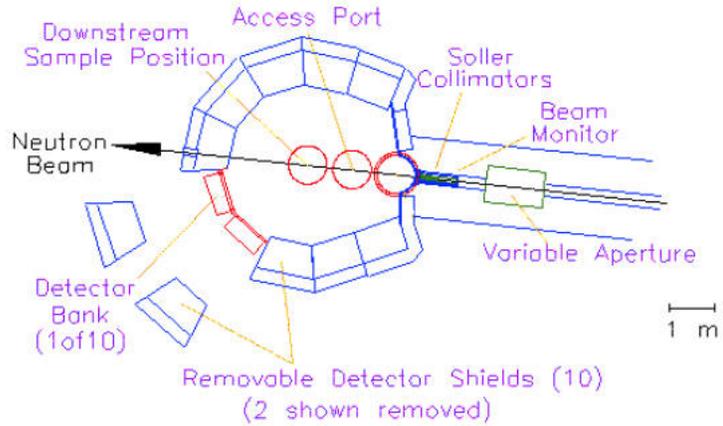


Figure 4 – The Glass Liquid and Amorphous Diffractometer

Two experiments were run for each functional group; one bound with ⁵⁸Ni and the other bound with ⁶²Ni. In addition an empty container, an instrument background, and a vanadium standard to normalize the incident neutron spectrum were taken. The scattering function, prior to corrections for multiple scattering, container geometry, attenuation, inelastic contributions, and deadtime, is given by:

$$S(Q) \cong \frac{\text{Sample} - \text{MT}_{\text{Cell}}}{\text{Background} - \text{Vanadium}_{\text{Std}}} \quad \text{Eq. 1}$$

The first order difference is then given by:

$$\Delta S(Q) = S_{\text{Ni-62}}(Q) - S_{\text{Ni-58}}(Q) \quad \text{Eq. 2}$$

This function may then be Fourier transformed to arrive at the real space distribution of Ni in the sample. Figure 5 shows the sulfhydryl spectra as represented by equation 1. Note the rapid rise at low values of momentum transfer indicative of a high inelastic contribution to the scattering. Figure 6 shows similar spectra but for the carboxyl group bound to ⁵⁸Ni and ⁶²Ni. The striking similarity of the two spectra is expected as both samples are binding nickel, just different isotopes. However, the rapid rise at low Q dominates the scattering spectra and was unsuccessfully corrected for. At present there is no analytical solution for the inelastic correction and typically a low order polynomial is fit to the data and subtracted away. Unfortunately, when this was done for the spectra taken in this experimental run the result was a straight line, within experimental error. This is not typical as the difference in scattering length

densities between ^{58}Ni ($b_c=14.4$) and ^{62}Ni ($b_c=-8.7$) is the largest amongst the elements. The failure of the present experiment was two-fold. First, it is quite apparent from the data that not all of the hydrogen in the polymer backbone was displaced during despite the vendors claim of 99.9% deuteration. If this had been the case then there would not have been the rapid rise at low values of Q and the inelastic contribution would have been minimal. Second, the high similarity in relative intensity is suggestive of not having completely displaced the sodium present in the ion exchange resin. The very large difference in scattering length densities should have resulted in spectra with much larger differences in relative intensities than is depicted in figure 6. To correct for these problems we have prepared our own deuterated ion-exchange resin and are confident that the problems associated with the inelastic contribution will be resolved. Also, the new samples don't suffer from underloading of the binding metal. The new samples should provide the structural information of the bound metal out to the third or fourth coordination shell as all of the sodium has been displaced. Understanding the bonding environment could potentially provide new avenues for understanding the process of phytoremediation. This may have important implications for optimizing current technologies and developing new methods for watershed management and the remediation of contaminated sites.

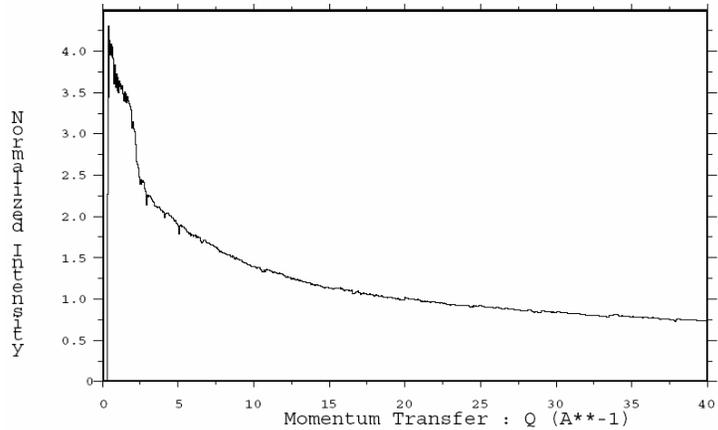


Figure 5 – Sulfhydryl functional group attached to the commercial ion exchange resin bound to ^{62}Ni . The rapid rise at low values of Q is indicative of a large inelastic contribution.

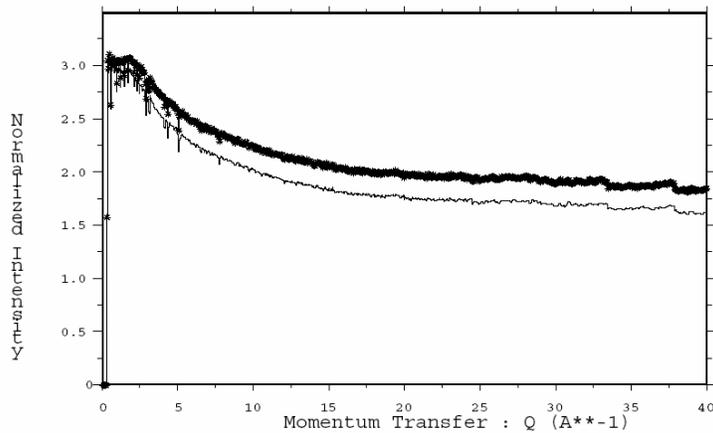


Figure 6 – Carboxyl functional group attached to the commercial ion exchange resin bound to natural nickel (bottom) and ^{62}Ni (top).

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