# A Novel Humic Acid-Functionalized Particle as a Tool for Research into Soil Contamination

Alexis Nawotka, Jr.

Introduction

In geologic terminology, a "soil" can be a simple or a complex feature, with anywhere from a single layer of approximately homogenous material, to many layers that are physically and chemically distinct from each other. Although the presence of an organic layer is not required to define a soil, such is frequently found as part of the assemblage, as a result of the decay of leaf fall, dying vegetation, and detrital animal matter. Ultimately, through chemical and biological decay processes, the nature of this organic layer tends toward a mixture of several distinct substances, including what are referred to as humic acids and fulvic acids. These are categories, broadly speaking, rather than specific compounds. Both possess long chains and rings of bonded carbon atoms, oxygen, and hydrogen, consisting of hundreds of compounds of various sizes and complexities. A representation of an example form can be constructed (figure 1).

Both of these acids can be characterized as having the same chain-ending functional groups such as phenols, alcohols, and carboxylic acid groups, although the proportions of these vary between the two acids; both are found to be soluble in basic solution (high pH) in spite of their general hydrophobicity (MacCarthy, 2001). Humic acid is distinguished from fulvic acid in general by its higher average molecular weight, due to its longer chains and complex structure, and averages at 2000 Daltons and above; fulvic acid has been reported to average 600 to 900 Da (MacCarthy, 2001). Humic acid also shows a tendency towards fewer functional groups with oxygen (Tang et al., 2014). However, it is not possible to identify a single compound which can be labeled
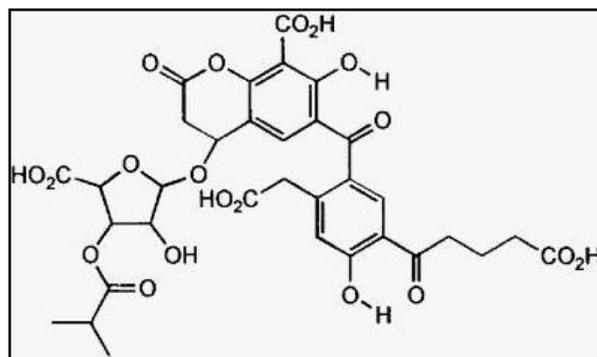
Figure 1. One of many possible structures of fulvic acid. After MacCarthy (2001), their figure 1.

definitively as either "humic acid" or "fulvic acid", and by necessity there are many that can be labeled as "humic acid" from their chemical properties (MacCarthy, 2001).

Due to the presence of many and varied functional groups, humic and fulvic acids can interact with metal ions and other organic matter, and are known to do so by a number of mechanisms: ion exchange, complexation, and redox reactions being amongst the more common (Perminova and Hatfield, 2005). Humic substances are capable of forming adducts with organic compounds and stable complexes with metals. Since humic and fulvic acids are major components of the organic portion of soils, these reactions with metals and organic materials become significant in the context of environmental geology, such as in the event of a spill of a hazardous chemical or contaminant. In such an occurrence, the contaminant will infiltrate the soil – potentially to a considerable depth – and may become bound to it at a molecular level, via one or more of these reactions with humic substances. The quantity of contaminant that is captured this way, and how tightly it is held, depend in large part on the interactions between it and humic substances; hence, study of these interactions can greatly contribute to the effective remediation of contaminated soil, and also to the general scientific understanding of soil chemistry.

In practice, these interactions are modeled by laboratory experimentation. However, when these experiments are carried out in solution, humic substances are noted to be difficult to isolate from the solution itself for analysis; this has subsequently led to research in the immobilization of humic material on larger organic or inorganic particles (Koopal et al., 1998). This immobilization allows for easy isolation of the humic substances by separating out the particles to which they are attached. In the study discussed herein, humic acid is immobilized on silica nanoparticles via an intermediary layer of poly-L-lysine (PLL), a novel construction which shows potential for future investigation of the interactions between contaminant chemicals and humic substances.

Background

A wide variety of chemical ions and compounds may be considered "contaminants" when their presence in soil above a certain concentration is deemed undesirable, including those with detrimental effects on human health. These include ions such as heavy metals, including lead, arsenic, chromium, and cadmium; they also include inorganic and organic

compounds, all of which may enter into soil from a wide variety of sources such as landfills, agricultural facilities, and industrial zones (Wuana and Okieimen, 2011). In the context of soil and general chemistry, an "organic" compound is defined as one whose structure is primarily composed of carbon, with the near-ubiquitous inclusion of hydrogen and oxygen and frequent inclusion of nitrogen; an example of such a compound would be glycine, an amino acid. Organic chemicals not only bind to soil particles, but can also display synergistic interactions with heavy metals, in which the presence of metals delays or hinders their breakdown by microbial activity (Wuana and Okieimen, 2011). Another group of compounds includes those with one or more organic components covalently bonded to a metal ion, an example of which would be tetraethyl lead, an obsolete gasoline additive. These different categories of contaminants may exhibit a variety of mechanisms by which they interact with soil and humic substances.

At sites known to be contaminated with heavy metals, the most common elements present are generally lead, chromium, and arsenic (Wuana and Okieimen, 2011). The interactions of these metals with soil material are strongly dependent on the metal's oxidation state and the identities of any chemical species bound to it. However, conditions within the soil such as oxygen availability and the presence of oxidizing or reducing environments will also influence the speciation of the metal (Wuana and Okieimen, 2011). Lead, for example, is most commonly found in the +2 oxidation state (Pb^{2+}), and is least soluble in water when in the form of lead carbonate, lead phosphate, or lead oxide; under reducing conditions with sufficient availability of sulfur, it will instead form lead sulfide, with a different affinity for humic substances than lead oxide or lead carbonate (Wuana and Okieimen, 2011). Chromium presents a challenge in that its most soluble ion, Cr^{6+}, is the most mobile in soil and groundwater and is also highly toxic. This form can be reduced to Cr^{3+}, which is relatively insoluble and non-toxic, but the reduced form can then be oxidized into a hazardous form again, and these interconversions are not dependent on humic substances, rather being influenced by iron and manganese oxides and hydroxides (James, 1996). Arsenic has been known to be toxic from antiquity and forms a great number of different complexes, including many with oxygen which then chelate to other metals; additionally, many arsenic species sorb strongly to soil particles and humic substances

(Wuana and Okieimen, 2011). The interactions of heavy metals such as lead and arsenic with soil can then be further diversified by the presence of organic contaminants.

The range of possible organic chemicals that can contaminate soil is sizable, and the compounds likely to be found in a given area are largely dictated by nearby sources: for example, pesticides and fertilizers from agricultural land, solvents from industrial plants, and an unpredictable variety of contaminants from landfills. Many other sources also exist. The intermolecular interactions between humic material and organic contaminants are dependent partly on the properties of the soil such as pH, clay content, and content of humic material, and partly on the properties of the contaminant such as hydrophobicity (preference for non-polar environments), acidity, and molecular size (Haigh, 1996). In general, compounds with a higher degree of hydrophobic character adsorb more strongly to soil particles, as humic material is also hydrophobic to a degree (figure 1), and the amount of contaminant that adsorbs increases with the overall organic content of the soil (Haigh, 1996). This adsorption can result in the partial or near-complete immobilization of the compound, creating a region of contaminated soil with a slow output of the contaminant to groundwater. While adsorbed, the compound is much less available to microbes which could potentially decompose it; simultaneously, a small but continuous amount can enter groundwater systems, as all organic contaminants retain some solubility in water, even if almost negligible (Haigh, 1996). One particular class of organic chemicals, the polycyclic aromatic hydrocarbons (PAHs), are considered to be of high concern as they adsorb strongly to soil particles in this manner, and are known to be potential carcinogens or teratogens at low concentrations (Garcia-Flores et al., 2016).
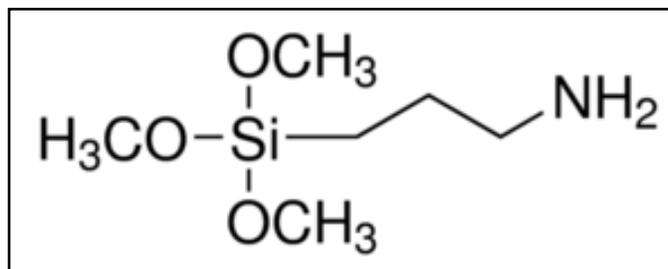
In order to elucidate the interactions between humic materials and the contaminants discussed herein, it is necessary to carry out experiments with humic material. Immobilizing the humic substances on a substrate provides a platform to carry out reactions and improves recovery of the material from experiments in solution, and enables application of humic substances to routes of investigation that require a stationary phase, such as high-performance liquid chromatography (Ayyildiz, 2015). Humic acids in particular possess many different functional groups including phenolic and hydroxyl, amine, and aliphatic and aromatic carboxyl groups, and several of these could potentially be utilized to anchor the humic acid molecules to the substrate (Ayyildiz, 2015). Several

different substrates have been previously investigated as well, including zeolite mineral structures, mediated by cationic surfactants (Lin et al., 2011). Humic acids have also been immobilized on cross-linked polystyrene resin and silica, of which there exist several functionalized derivatives (Koopal et al., 1998). In all of these instances, preparation was required for the substrate surface prior to functionalization with humic acids.

Zeolite structures have been investigated as substrates due to their relatively low cost and high capacity for cation exchange, although humic acids do not adsorb or bond to the structure without an intermediary (Lin et al., 2011). As naturally occurring minerals, zeolites possess a framework silicate (tectosilicate) structure with an overall negative charge which is normally balanced by cations present in the environment, such as sodium and calcium. A surfactant such as cetylpyridinium bromide can be loaded into the framework, displacing the natural cations, and the humic acids may then be immobilized by reaction with the prepared zeolite (Lin et al., 2011). This construction is likely to be very effective in uptake of heavy metal ions, as the ions not only bind to the humic acids but also enter cation sites within the mineral framework where the surfactant molecules cannot enter (Lin et al., 2011).

Based on a survey of current literature, cross-linked polystyrene resin has not been an active subject of research as a potential substrate for immobilization of humic acids. It is stable for use in experimentation, but not suitable for large-scale production (Koopal et al., 1998). The use of silica as a substrate, however, has been extensively investigated, and humic acids have been bonded and adsorbed to silica with a variety of intermediaries. This interest is due in part to the environmental applicability of silica, to its chemical stability, and to its well-understood mechanical properties; silica is a common material on the Earth's surface and is often found in the same natural environments as humic acids, and it is stable over a reasonable range of pH values (Koopal et al., 1998). Many of the properties of silica, including surface area, particle size, and pore size, can be effectively controlled within experimental parameters (Koopal et al., 1998). Silica is also already utilized for purposes such as high-performance liquid chromatography.

Functionalization of the silica surface before addition of humic acids has been accomplished with several intermediaries, including 3-aminopropyltrimethoxysilane, N,N-dimethylformamide, and glutaraldehyde. Humic material can then be bonded directly to these compounds or adsorbed, and the attachment can be made through different functional groups, such as through phenolic or carboxylic groups (Koopal et al., 1998). A widely-used preparation utilizes 3-aminopropyltrimethoxysilane as the

Figure 2. The structure of 3-aminopropyltrimethoxysilane.

intermediate between silica and humic acids, and these are frequently abbreviated to "aminopropyl" silica preparations, although this procedure is time-consuming and requires curing time (Koopal et al., 1998). Therefore, in this study is presented a new preparation for adsorption of humic acids mediated by poly-L-lysine polymer. This preparation was originally developed for functionalization of bare silica surfaces for use in a quartz crystal microbalance (Chen and Elimelech, 2008). The procedure is here adapted for preparation of the surfaces of silica nanoparticles, nominally 100 nm in diameter, which serve as the substrate for immobilization of humic acids.

Materials and Methods

Silica substrate.

The silica substrate consisted of 100-nanometer diameter silica particles, Nissan Chemical, lot 150117, product MP-1040H, with a SiO_2 content of 40.4 wt%. Solution was mixed well and diluted 1 to 8 with deionized water to produce a 5% stock solution. Further preparations were made as necessary from this stock. For characterization of the bare silica particles, the 5% stock was diluted 1 to 50 with deionized water. Particle size distributions and zeta potential values were determined in triplicate with a dynamic light scattering (DLS) instrument: a Malvern Zetasizer Nano ZSP, Temple University ID T345026.

Intermediate SiO₂ and poly-L-lysine solutions.

      In keeping with the original preparation of Chen and Elimelech (2008), intermediate solutions were prepared with HEPES buffer and sodium chloride. HEPES buffer was supplied by Fisher Scientific, lot 165542, received May 26, 2017, and sodium chloride supplied by EMD, lot UK04HZEMS, opened May 27, 2014. The SiO_2 intermediate solution was prepared in a 100 mL volumetric flask with 2 mL of 5% SiO_2 stock solution, sufficient HEPES to make a final solution containing 10 mM HEPES, and sufficient NaCl to make a final solution containing 100 mM of NaCl. Poly-L-lysine (PLL) was supplied by Sigma-Aldrich, lot SLBQ0620V, received June 6, 2017. The PLL intermediate solution was prepared in a 100 mL volumetric flask with 10 mg of PLL, sufficient HEPES to make a final concentration of 10 mM HEPES, and sufficient NaCl to make a final concentration of 100 mM NaCl. Both intermediate solutions were prepared with deionized water.

Preparation of humic acid solutions.

      Humic acid used was Aldrich Chemical, lot 01828JZ, technical grade, which required purification before use in order to isolate humic acids from fulvic acids and humin. This was accomplished with the procedure outlined by Koopal et al. (1998). Five grams of Aldrich humic acid was added to 500 mL of deionized water adjusted to pH 11 with sodium hydroxide (Fisher Scientific lot 134065, received Oct. 15, 2013). This was stirred overnight with no heat applied, then centrifuged at 4700 rpm for 30 minutes to separate insoluble material from soluble humic acids. The pH of the supernatant was adjusted to 2 using a solution of 1M HCl (self-prepared by dilution of HCl, Fisher Scientific lot 103794). Solution was stirred for 24 hours without heat, then centrifuged at 10,000 rpm for 30 minutes, and the supernatant discarded. The remaining material was re-suspended in 0.01M HCl (self-prepared, same stock solution as above) and stirred for two hours, then centrifuged again at 10,000 rpm for 30 minutes; the supernatant was again discarded. This 0.01M HCl wash was repeated two more times, and after the final supernatant was discarded, the solid material was allowed to dry completely overnight. It was then ground fine with a mortar and pestle.

      The intermediate humic acid solution was prepared in accord with Chen and Elimelech (2008). Dissolved 28.4 mg of purified humic acid powder in 52 mL of deionized

water by stirring for two hours, then filtered the solution through a 0.2 µm cellulose acetate filter, and adjusted the pH of the resulting solution to 5.5 with sodium hydroxide solution (self-prepared, same stock chemical as above).

Proportional SiO_2:PLL solutions.

A series of solutions were prepared from the above intermediate solutions in order to determine the proportions of SiO_2 and PLL that would result in an approximate monolayer of PLL on the silica nanoparticles. Intermediate solutions for SiO_2 and PLL were mixed to produce concentrations of SiO_2:PLL ranging from 1:5 up through 100:1. Particle size distributions and zeta potential values were again determined in triplicate with a Malvern Zetasizer Nano ZSP, ID number T345026.

Assembled nanoparticles with SiO_2, PLL, and purified humic acids.

After determination of an appropriate concentration for SiO_2:PLL, the reasoning for which is given below in Results and Discussion, purified humic acid was added to the PLL-coated particles. Silica and PLL intermediate solutions were first mixed to produce a 50:1 concentration of SiO_2:PLL; this is consistent for all solutions prepared with humic acid. Purified humic acid solution was then added to produce concentrations ranging from 5:1 SiO_2/PLL:HA up through 1:6 SiO_2/PLL:HA. Particle size distributions and zeta potential values were determined here with a Malvern Zetasizer Nano-ZS, S/N MAL1151891, functionally equivalent to the above Malvern Zetasizer Nano ZSP.

In addition, three solutions were chosen for thermogravimetric analysis (TGA): a portion of the purified humic acid solution, a solution of 50:1 SiO_2:PLL, and a solution of 1:2 SiO_2/PLL:HA. These were centrifuged at 4700 rpm for 30 minutes to settle out the particles, the supernatant was decanted, and the remaining solid material dried overnight. The residues underwent TGA on a TA Instruments TGA 2950 analyzer.
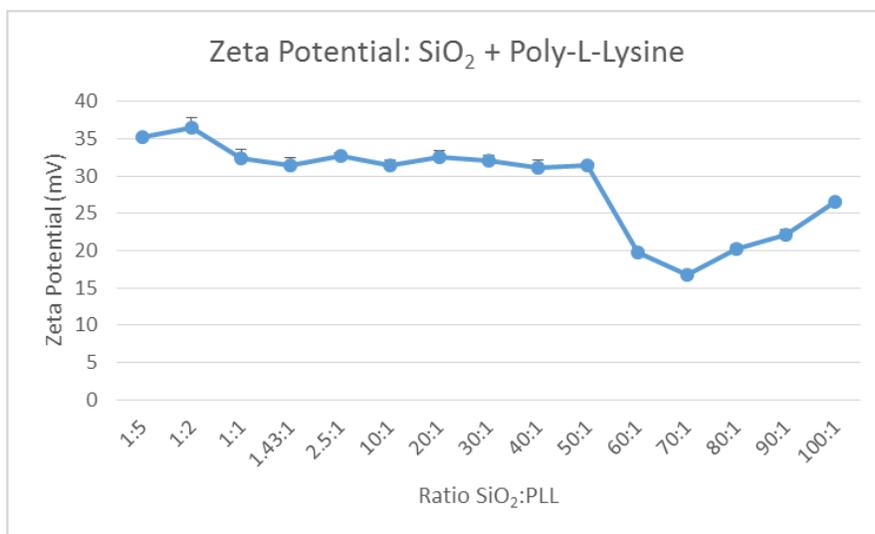
Results and Discussion

Characterization of the bare silica nanoparticles by DLS produced an average particle size of 120.6 nm and an average zeta potential of -63.3 mV. These are in agreement

with anticipated results since the starting material consisted of 100 nm diameter particles, and silica possesses a negative surface charge which is clearly reflected in the zeta potential. When solutions with only SiO_2 and PLL are analyzed by DLS, the average particle size remains approximately 120 – 140 nm at all concentration ratios from 1:5 to 50:1 SiO_2:PLL (figure 3). Beginning with 60:1 SiO_2:PLL, particle size becomes erratic with large margins of error, most likely due to the inability of PLL chains to completely cover the silica particles. This would result in chains "draping" across multiple particles.
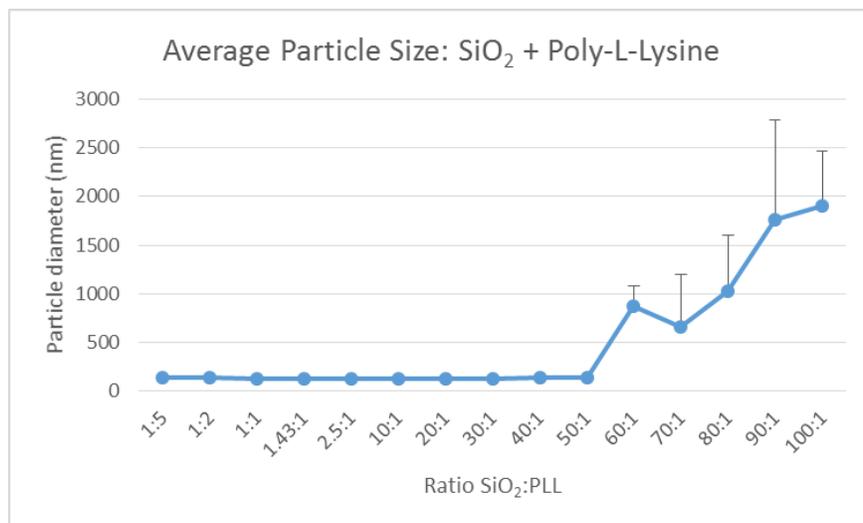
Figure 3. Average particle sizes determined by DLS for proportional SiO_2:PLL solutions. Error bars shown.

Zeta potential results for solutions containing only SiO_2 and PLL display unsteady values between approximately +38 mV and +32 mV for solutions between 1:5 and 50:1 SiO_2:PLL (figure 4). This indicates successful coating of the particles by PLL, as the polymer chains possess a positive charge which now is detectable by the instrument, much different from the original bare silica zeta potential of -63.3 mV. After a concentration of 50:1 SiO_2:PLL, the zeta potential begins to fall and reaches a low at 70:1 SiO_2:PLL before rising again.

The concentration ratio of 50:1 SiO_2:PLL was chosen as representing a monolayer of PLL on the silica nanoparticles. Figure 3 indicates that the particles are fully coated up until this point; the drop in zeta potential after this concentration may indicate an increased prevalence of negative charge as bare silica becomes exposed, although it is uncertain why the zeta potential begins to climb again. Using the 50:1 concentration ratio, humic acid was then adsorbed onto the SiO_2-PLL combination.

The average particle size for the humic acid-functionalized particles (figure 5) shows no clear pattern, falling to 209 nm at a concentration ratio of 1:3 SiO_2/PLL:HA before rising rapidly again at a 1:1 concentration ratio, then falling again until reaching 4:1 SiO_2/PLL:HA. The zeta potential (figure 6) displays a swing from negative to positive values as the amount of humic acid in solution decreases; since a solution of humic acid alone will have a negative zeta potential, this implies that the solutions with more humic acid have adequate material to be fully coated. The solution with 1:3 SiO_2/PLL:HA has the most negative zeta potential *and* the smallest particle size, again implying the most even layer of humic acid.
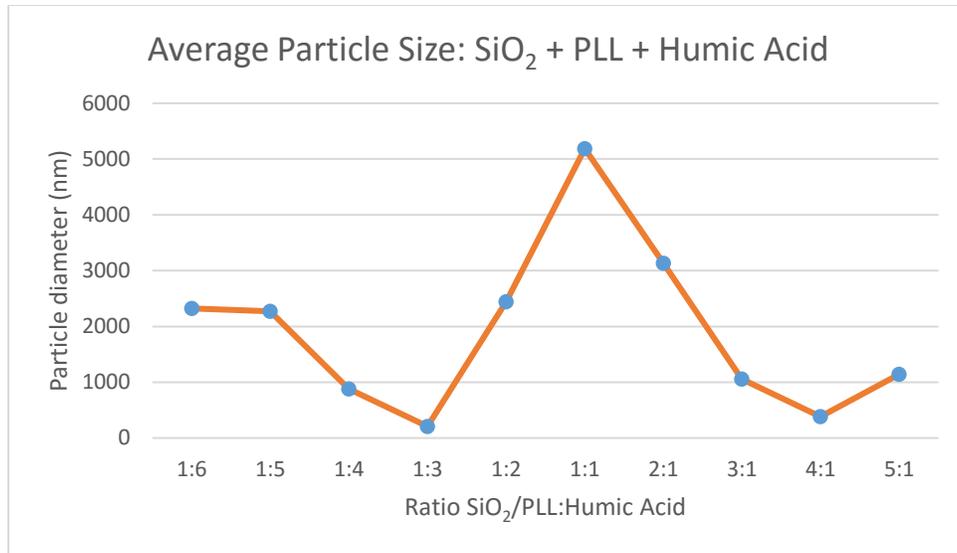
Figure 5. Average particle sizes for solutions of humic acid-functionalized particles.
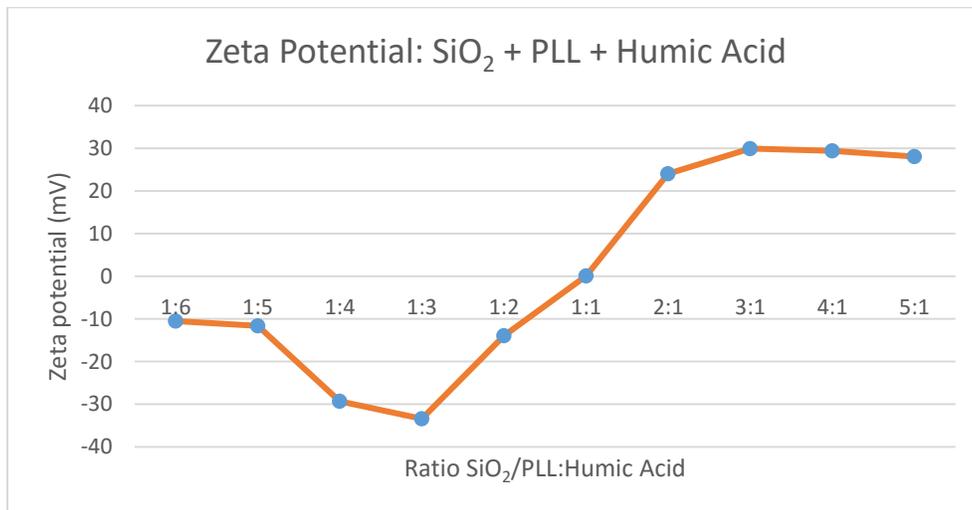
Figure 6. Zeta potential values for solutions of humic acid-functionalized particles.

Thermogravimetric analysis of the three particle residues are characteristic for their compositions (figure 7). Humic acid alone, composed solely of organic material, is entirely eliminated around 600°C, whereas the PLL-coated silica particles show a single step down in weight around 300°C and never drop below approximately 95% of their original mass, even at the end of the temperature run at 800°C. The particles with both PLL and humic acid show a possible shallow step down in mass at 100°C and another at 300°C, and never

drop below 93.5% of their original mass. These results are tentatively consistent with the proposed structure of their respective particles.
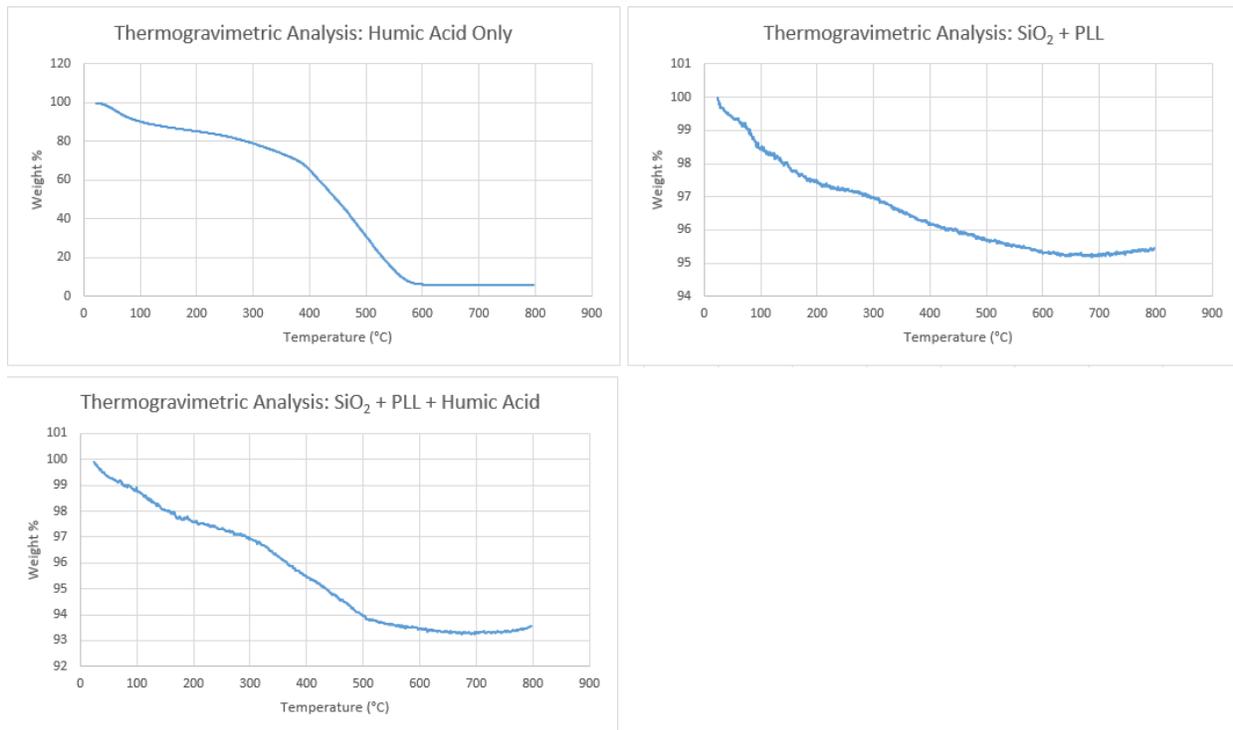
Figure 7. Thermogravimetric analysis for humic acid residue, SiO₂/PLL particle residue, and SiO₂/PLL + humic acid residue.

Future Research

Follow-up on this research project could follow numerous possible lines. Firstly, the DLS results for humic acid-functionalized particles require further investigation: zeta potential results and TGA results are suggestive of a successfully coated particle, but size results are ambiguous. Confirmation of particle size and morphology could be carried out by cryo-transmission electron microscopy (cryo-TEM). If the particles are confirmed to be successfully coated, they could play a role as a soil simulant material for research into interactions between contaminants and natural humic substances. Such research could include heavy metal uptake and adsorption of organic compounds, and could potentially measure the capacity of soil to immobilize these species.

In order to improve this material's accuracy as a soil simulant, it becomes necessary to include other materials found in natural soil such as clay minerals, and possibly even soil

fauna such as microbes and fungi, although including fauna may be beyond the practical scope of laboratory work. It would also be imperative to determine the long-term stability of the humic acid-functionalized particles. All of these proposed concepts are potential components of a number of research projects, the results of which could play a role in soil science and in understanding the ultimate fate of contaminants in nature.

References

Ayyildiz, H. F. (2015). Evaluation of new silica-based humic acid stationary phase for the separation of tocopherols in cold-pressed oils by normal-phase high-performance liquid chromatography. Journal of Separation Science, 38(5), 813-820.

Chen, K. L., & Elimelech, M. (2008). Interaction of fullerene (C60) nanoparticles with humic acid and alginate coated silica surfaces: Measurements, mechanisms, and environmental implications. Environmental Science & Technology, 42(20), 7607.

Garcia-Flores, E., Wakida, F. T., Rodríguez-Mendivil, D. D., & Espinoza-Gomez, H. (2016). Polycyclic aromatic hydrocarbons in road-deposited sediments and roadside soil in tijuana, mexico. Soil and Sediment Contamination: An International Journal, 25(2), 223-239.

Haigh, S. D. (1996). A review of the interaction of surfactants with organic contaminants in soil doi:https://doi.org/10.1016/0048-9697(95)05049-3.

James, B. (1996). The challenge of remediating chromium-contaminated soil. Environmental Science & Technology, 30(6), 248A-251A.

Koopal, L. K., Yang, Y., Minnaard, A. J., Theunissen, P. L. M., & Van Riemsdijk, W. H. (1998). Chemical immobilisation of humic acid on silica. Colloids and Surfaces A: Physicochemical and Engineering Aspects, 141(3), 385-395.

Lin, J., Zhan, Y., & Zhu, Z. (2011). Adsorption characteristics of copper (II) ions from aqueous solution onto humic acid-immobilized surfactant-modified zeolite. Colloids and Surfaces A: Physicochemical and Engineering Aspects, 384(1), 9-16.

Maccarthy, P. (2001). The principles of humic substances. Soil Science, 166(11), 738-751.

Perminova, I. V., & Hatfield, K. (2005). Remediation chemistry of humic substances: Theory and implications for technology. Use of Humic Substances to Remediate Polluted Environments: From Theory to Practice, pp. 3-36.

Tang, W., Zeng, G., Gong, J., Liang, J., Xu, P., Zhang, C., et al. (2014). Impact of humic/fulvic acid on the removal of heavy metals from aqueous solutions using nanomaterials: A review doi:https://doi.org/10.1016/j.scitotenv.2013.09.044.

Wuana, R. A., & Okieimen, F. E. (2011). Heavy metals in contaminated soils: A review of sources, chemistry, risks and best available strategies for remediation. Isrn Ecology, 2011.