The Enhanced Mobility of Nanotech Waste in the Presences of Humic Acids and Natural Organic Matter

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Abstract

Carbon nano-materials from Nanotech waste may have the capacity to mobilize through the aquifer. The purpose of this experiment was to determine if natural organic matter in the form of humic acid can affect the mobility of nanomaterials. Using Right-Angle Light Scattering, Dynamic Light Scattering, and Zeta potential, measurements were made that indicated CNT mobility through the aquifer was possible in the presence of humic acid.

Section 1: Introduction and Purpose

1.1 Environmental Concern of Nanotech waste and other Nano-materials

Numerous industries are incorporating nanotechnology, from nanomedicine to nanorobotics. Nanomaterials (NMs) are being incorporated into many products of daily use - e.g. fillers, opacifiers, pharmaceuticals, lubricants, cosmetics, electronic devices, and appliances - and have the potential to spread quickly in the environment because of their growing use and disposal. The increasing production of and application potentials for carbon nanotubes (CNTs) suggests these materials will enter soil and sediment ecosystems in upcoming years. Estimates have it that by 2014, nanotechnologies will be widely used in our society and ten million new jobs will be created. Therefore, it becomes essential that society devises an integrated approach to research, cooperation, and communication strategies in order for it to pursue responsible and sustainable growth of nanotechnologies. Few studies have considered the environmental impacts of impurities and byproducts associated with low-efficiency nano-manufacturing processes. Also, the lack of standardized characterization and preparation methodologies for researchers to gain the needed information to assist risk assessors further complicates this problem. An approach to the treatment of nanotechnology waste requires an understanding of all its properties, not only chemical, but also physical and biological. There is a review that discusses specific issues related to handling of waste containing...
CNTs, large, long, tubular molecules, are well known to be insoluble. Consequently, it was thought that they will not impact the environment since they would not be mobile in aquifers or in surface waters. Recent results, however, have called this insolubility into question and there is indication that complexing organic molecules, natural organic matter (NOM), particularly humic acids (HAs), and could mobilize the nanoparticles (NPs), thus making them potentially hazardous in and around landfills and streams. The purpose of this work is to try to quantify some of the interactions of NPs and the environment by determining certain useful thermodynamic quantities that can help in environmental and biological modeling studies. Also, the study of HAs may lead to the development of new coatings and purification processes for industry and research. In a related note, such insolubility has limited useful applications of nanotubes in composite materials and surfactants and other materials including salmon sperm DNA have been studied to help separate, solubilize and incorporate nanotubes into materials. Increased mobility of CNTs may lead to an increased risk of CNTs impacting humans, plants, and animals. Preliminary studies are contradictory, but some indicate that CNTs are toxic to humans and organisms. NPs could impact the environment by changing the availability of toxins and nutrients to organisms, directly effecting biota, changing environmental microstructures, and indirect effects from interacting with NOM. The presence of CNTs in the environment affects the behavior of pollutants such as heavy metals, which is of environmental concern. The similarity in structure with asbestos suggests that CNTs may behave like these fibers and have a similar toxicity; in a specific study, MWNTs caused respiratory cell death. This effect is likely to be a problem not only in humans but in environmental organisms as well, although little is known about possible methods of exposure. In a specific study, higher concentrations of MWNTs showed toxicity to cells leading to irritations, phenotypic defects, and increased apoptosis in zebrafish embryos.

1.2 Background studies

For environmental modeling and monitoring, accurate and quantitative understanding of the NMs interaction is required. There is also some indication that NMs may accumulate up the food chain – a process called biomagnification by the United States Geological Survey - and that different types of particles do not behave the same - so detailed knowledge is necessary to model this. There is a review of CNT toxicity and assessment of potential occupational and environmental health risks. As a recent counterpoint to concerns of cytotoxicity, CNT ingestion into drosophila did not reduce viability and growth. The stability of multi-walled nanotubes (MWNTs) in an aqueous environment was largely dependent on the presence of sodium dodecyl sulfate (SDS) or NOM. Most NOM have a biological origin including materials released by plants, algae, and fungi. NOM may influence the surface speciation and charge of NPs, which would alter their aggregation and deposition properties. The adsorption capacity and strength is strongly dependent on both the type of NOM and activated carbon. Due to the polydispersity of NOM, different portions of NOM tend to adsorb in various degrees in the adsorbent. NOM adsorption is also affected by ionic strength and pH, which influence both the charge and configuration of NOM. The activated carbon consists of micro pores of various sizes that provide sites for NOM adsorption. The hydrophobic nature of CNTs and their large surface areas are believed to increase their affinity and aggregation properties. In a study, NOM was used as a more relevant and
biologically mild stabilizing agent relative to solvents and surfactants. In the results, it was suggested that MWNT toxicity could increase with functionalized MWNT.23

Generally speaking, the mobility and transport of contaminants adsorbed by CNTs may shed light on both potential environmental applications and understanding of the environmental impact of these materials.24 Further study with actual surface and ground waters that have relatively low NOM concentrations might be necessary for a more complete understanding of NOM and MWNTs.22 The understanding of how CNT interact with organic chemicals will provide important information on assessing CNT environmental risks and in exploring their applications.15

1.3 Theory

Quantitative examination of NP mobility for nanotubes and nanographenes (NG) can be achieved by quantifying the equilibrium complexation constant for the NP-humic acid complex. This is achieved by measuring the second virial coefficient using a ultrasensitive static light scattering spectrometer of our own construction.25 The second virial coefficient can be used to calculate the thermodynamic enthalpy of solution (Figure 2), which can determine the formation constants for complexation. These formation constants can then be put into environmental models and simulations to identify the effects and pathways of CNTs.

After plotting the data according to the Debye theory (see Figure 2), the solutions’ second virial coefficients (B2’s) and subsequently χ, the Flory-Huggins interaction parameter, can be calculated for each of the NT-solvent systems, which was performed on polystyrene (figure 3). A solution’s enthalpy of mixing is directly proportional to χ through the equation: \[ \Delta H_{\text{mix}} = k T N \phi \chi \]. Therefore, χ serves as a quantitative measure of the thermodynamic solubility of SWNTs in a given solvent, with a smaller χ indicating greater solubility.

The calculated χ values can be plotted against each of the solvents’ surface tensions: in the case of polystyrene the resulting plot had a strong negative peak in χ around a surface tension of 40 mJ/m² (Figure 4). This peak closely coincides with the solvent N-methyl pyrrolidone (NMP), which forms a SWNT solution with an actually negative value for χ. We plan to use this model to accurately calculate the surface energy of NTs and other fullerenes based
on solvent-NT surface tension matching and to further investigate the variability of \( \chi \) with solvent surface tension. In this study, we will investigate the surface energy of NOM solutions, such as aqueous humic acids, and compare them to those of high molecular weight NTs.

This experiment aimed to quantify \( B_2 \) and \( \chi \) for various different NOMs, specifically HAs. While \( \chi \) presents a quantitative measurement of the total enthalpic association between solvent and solute molecules, \( B_2 \) is related to only the intermolecular forces between solvent and solute. Therefore, an understanding of solution thermodynamics and solvent-solute interaction can be achieved by quantifying both \( B_2 \) and \( \chi \), and further parameters would give additional insight into solvent-solute interactions. These would include zeta potential and bundle size distribution data obtained through dynamic light scattering. Furthermore, \( \chi \) can be used to study the dissolution efficiency and thermodynamic stability of MWNTs around NOMs. An actual experiment would involve, for example, varying the concentration of HA in water, and making a series of samples with varying amounts of SWNTs or MWNTs. From this, \( \chi \) as a function of HA concentration could be calculated, as well as a bundling point of the CNT, if present.

**Section 2: Light scattering**

**2.1 Instrumentation**

The instrument was arranged so that a green laser would pass through a sealed cuvette containing the solvent. Uses lenses and a photomultiplier tube, the lighted scattered by the solution would be collected 90\(^\circ\) from the path of the laser. The light form the laser was dissipated into a black wall across the room. The instrument was kept in a dark room with a red lamp for operation. The following list contains the technical information of the instrument.
2.2 Reagents

- Leonardite: IHSS Leonardite Humic Acid Standard 1S104H
- Elliot: IHSS Elliot Soil Humic Acid Standard 1S102H
- Polystyrene standard: Scientific Polymer Products™, CAT# 772 - Mw = 1,300 - Mn = 1,180
- Methanol, Absolute: JT Baker® 9069-03 PHOTREX® reagent
- 1-Methyl-2-Pyrrolidinone (NMP): Sigma-Aldrich® 270458 CHROMASOLV® Plus for HPLC
- Toluene: Sigma Aldrich® 650579 CHROMASOLV® Plus for HPLC, >99.9%

2.3 Experiment

A series of humic acid (HA) solutions were made with NMP and methanol (Tables A1 and A2). About 0.3 mL of a sample was injected by syringe into the cuvette for each test. The samples were tested in series using the same solvent and same solute going from lowest to highest concentration. The cuvette was flushed with at least 3 mL of clean solvent after testing the highest concentration of a series to prepare for the next series. Whenever a new solvent was used, the cuvette was flushed with that solvent and was allowed to rest for one hour.

A computer collected voltage output over time on LoggerPRO® based on the intensity of the green light being scattered by the solution in the cuvette towards the PMT. The samples were collected for 10 minutes each in a dark room. Each sample was tested at least 2-3 times to verify the data. The data was plotted C/I versus C as in Figures 6 and 7.

Equation 1

\[
\frac{C}{I} = \frac{B_2}{K'} C + \frac{1}{M_w K''}
\]

\(C = \text{Concentration}\)
\(I = \text{Intensity of scattered light}\)
\(M_w = \text{Molecular weight}\)
\(K' \text{ and } K'' = \text{Instrument constants}\)

2.2 Results and Discussion

The instrument was unable to be calibrated using polystyrene in toluene due to the inadequate sensitivity of the PMT. Further work will be conducted to resolve this in order to provide useful quantitative results for the interactions of humic acid in various solvents, such as B_2 and \(\chi\). However, useful qualitative conclusions can be made from the data thus far.

The Debye plots for the humic acid can be seen in Figures 6 and 7. Even though the plots of intensity \(I\) versus concentration \(C\) had positive slopes with R-squareds of 0.9973 and 0.9984 respectively, Figures 6 and 7 depict how sensitive the Debye plots are to variations in intensity since the error is compounded in
Due to the negative slopes – thus negative $B_2$ - the $\chi$ values increase with molecular weight and the solutions are driven by entropy by equations 2 and 3, since $\chi$ is positively proportional to $\Delta H$.

Equation 2

$$\chi = \frac{1}{2} - B_2 \rho_2 M_w$$

Equation 3 (Gibbs’ Free Energy)

$$\Delta G = \Delta H - T \Delta S$$

Different humic acids have different solubilities and interactions with given solvents. Except for Leonardite in NMP, only a fraction of the humic acid solids would dissolve into the different solvents. This may be due to a saturation concentration in the solvent or due to some fraction of the humic acid being incapable of dissolving. As Section 1.2 mentioned, NOMs like HAs may influence the surface speciation and charge of NPs, which would alter their aggregation and deposition properties, thus changing their solubilities. NOMs have a range of properties and due to the range of polydispersity of NOMs, different portions of NOM tend to adsorb in varying degrees to the adsorbent.

2.5 Conclusion

Leonardite humic acid was completely soluble in NMP and partially soluble in methanol. The Leonardite was found to be driven into solution by entropy since the second virial coefficient was negative. The light scattering apparatus showed to be effective in measuring light scattering with respect to concentration. However, more work needs to be done to fully quantify the interactions between humic acids and nanotubes.

2.6 Future work

- Produce a reliable calibration plot with polystyrene in toluene
- Perform light scattering on Elliot in methanol
- Perform light scattering on humic acid in NMP with CNTs
- Perform light scattering on manure humic acid in water
Figure 6: Debye Plot of Leonardite in NMP. C is concentration in ppm at 25 C and I is right-angle light scattering in volts.

Leonardite in NMP unfiltered
Baseline of NMP = 1.363 V is subtracted
2.5 mg/mL PS = 0.590 V with NMP subtracted

\[ y = -0.1454x + 98.324 \]
\[ R^2 = 0.9327 \]

70 75 80 85 90 95 100

20 40 60 80 100 120 140

C/I

Concentration (ppm)

Leonardite in Methanol
Dilutions made from 219 ppm initial solution filtered with 0.45 um filter
(Baseline of methanol = 0.570 V is subtracted)

\[ y = -0.0504x + 78.845 \]
\[ R^2 = 0.5721 \]

0 50 100 150 200 250

0 10 20 30 40 50 60 70 80 90

C/I

Concentration (ppm)

Figure 7: Debye Plot of Leonardite in methanol. C is concentration in ppm at 25 C.
Section 3: Zeta Potential

3.1 Instrumentation

A Malvern© NanoZS ZEN3600 was used to determine Zeta potential and particle size. The instrument will be called a Zetasizer for this report. The glass cuvettes were 12 mm x 12 mm with inner dimensions of 1 cm X 1 cm and had a label 8G which always faced the user when inserted. For the Zeta potential measurements, a dip cell (model no. MAL1041992) was inserted into the glass cuvette, which applied a potential to the sample solution, automatically determined by the instrument.

3.2 Reagents

- Carbon nanotubes (CNTs): Cheaptubes, Inc. Multiwalled carbon nanotubes. Outer diameter 20-40 nm, length 10-30 µm, purity ≥ 95%wt, ash ≤ 1.5%wt
- Ultra pure water: produced using a Barnstead Easypure LF system that dispensed water at 18.3 MΩ-cm
- UWP manure humic acid: produced according Appendix B
- Leonardite: IHSS Leonardite Humic Acid Standard 1S104H
- Elliot: IHSS Elliot Soil Humic Acid Standard 1S102H

3.3 Experiment

A series of UWP manure humic acid (HA) and CNTs in ultra pure water were made (Table A3) and were given four months to sit. The supernatant of the solutions were tested individually in the Zetasizer in the cuvette. The instrument automatically measured and recorded the Zeta potential or the solute particle size distribution, depending on the command of the user. The size scans were 10 seconds each, with a minimum of 10 tests per run. Each sample was run three times. The Zeta potential scans were performed at least ten times per run, with three runs per sample.

Particle size measurement results produced the following: Z-average size (d-nm), polydispersity, the intercept, and the mean size for each peak. The Z-average size is the cumulants mean in terms of particle diameter in nanometers from dynamic light scattering (DLS). The polydispersity index (PdI which can be defined in the ISO standard document 13321:1996 E and 22412) on a range of zero to one indicates there are more peaks or wider peaks with a higher index value. The intercept is usually about 0.85 to 0.95 and can be used to indicate data quality.

Zeta potential measurement results produced the following y versus x parameters: Zeta potential (mV), Zeta standard deviation (mV), conductivity, and the mean potential of each peak. Zeta potential is a measure of solution stability. Solutions with Zeta potentials more than +30 mV and less than -30 mV are considered to be stable in solution. A Zeta potential of zero means that the sample is extremely unstable in solution. For this experiment, a high Zeta potential would indicate that the water is fully capable of mobilizing the solute(s) through aquifers and over long distances by excess precipitation runoff and in streams.
3.4 Results and Discussion

Figures 8 and 9 show Zeta potential and size distributions for 256 ppm UWP manure humic acid. The Zeta potential is measured by counting the number of particles that respond to each of the numerous potentials applied to the solution by the dip cell which is simplified in a distribution. Particle size is measured by dynamic light scattering of the solution which produced a distribution of the light intensity versus particle size.

Figure 8: Zeta potential distributions for six tests of 256 ppm manure humic acid in water. Mean Zeta potential is -24.9 mV.

Figure 9: Size distributions for six tests of 256 ppm manure humic acid in water. Mean diameter is 204.2 nm.

The aqueous HA and CNT solutions used in the experiment can be seen in the photographs shown in Figures 10, 11, and 12. Figure 10 shows a series of solutions with a constant CNT concentration of about...
10 ppm with an increasing concentration of HA going towards the right starting with zero and ending with about 200 ppm. The two solutions on the far right are HA solutions of 186 and 256 ppm with no CNTs. Without HAs, the CNTs have no color in solution. By themselves, HAs are yellow-orange in solution. When HA is added to the CNT solution, the solution becomes dark, and becomes more and more yellow-orange as the HA concentration is increased. Figure 11 shows a series of increasing CNT concentration with a constant HA concentration of about 200 ppm. For reference the left solution is only CNTs and the next two are 186 and 256 ppm HA. The solutions from the fourth placed from the left going right became more and more dark as the initial CNTs concentration was increased while the HA concentration remained constant, which indicates that the CNTs were indeed being taken into solution by the HA. Figure 12 shows that even at low concentrations of HA, a visible uptake of CNTs can be observed. Bottle 16 in Figures 10 and 11 containing the CNTs without HA did show to have X nm particles in the Zetasizer instrument. For visual inspection, this was used as the baseline.

The results of the Zetasizer size tests are in Figures 13 and 14. Figure 13 shows that as the concentration of humic acid was increased with a fixed concentration of CNT, the average size decreased. The lower two data sets showed that the instrument and results were consistent between March and April 2010. The upper data was from the dominating peak of the April 2010 data only, and gave indication to the distribution of the data between the peaks in each run. Figure 14 shows that as the concentration of CNT increased with a fixed concentration of humic acid, the average size increased. The layout is similar to that of Figure 13. In both cases, as the ratio of CNTs to HAs increased, the average size increased. On its own, UWP manure humic acid has an average size of 175 nm at a concentration of 181 ppm. The fact that the particle size increases with CNT concentration indicates that the humic acids could be adsorbing to the surface of the CNTs differently as more CNTs are available for adsorption, changing interactions and colloid size.

The results of the Zeta potential tests are in Figure 15. The Zeta potential of CNTs alone in solution was -11.5 mV. Like Figure 10, the lower two data sets showed that the instrument and results were consistent between March and April 2010. The upper data was from the dominating peak of the April 2010 data only, and gave indication to the distribution of the data between the peaks in each run. On its own, UWP manure humic acid has a Zeta potential of -31.5 mV at a concentration of 181 ppm. Referring to the red squares on the figure, starting with a low concentration of CNTs and a fixed HA concentration of 200
ppm, the initial Zeta potential was similar to that of HAs on their own. As the concentration of CNTs was increased, the Zeta potential was driven down to about -43 mV. Referring to the blue diamonds, starting with a low concentration of HA and a fixed concentration of 10 ppm CNTs, the Zeta potential started near -43 mV and increased to -32 mV as the concentration was increased to 186 ppm. It is interesting that the upper left red square data and the upper right blue diamond data both have a CNT to HA mass ratio of 0.052.

Figure 16 shows the results from Figure 15 in terms of mass ratios of CNTs to HAs. As stated just prior, the solutions with the lowest Zeta potential from each series had an identical CNT/HA ratio of 0.052. On the figure it can be seen that at this ratio, the Zeta potential is about -32 mV. The 0.35 CNT/HA samples had Zeta potentials of about -43 mV. It was noticed however, that between the 0.052 and 0.35, the Zeta potentials were highly variable on a range from -36 to -46 mV, which is lower than -32 mV and shows that there was a Zeta potential shift, but incapable of producing a clear proportional relationship. The obvious shift in Zeta potential may indicate that the HAs are encapsulating the formerly insoluble CNTs and producing colloids with more charge than before. More charge means better repulsion between colloids thus more stability in the aqueous solutions. Overall the CNTs are more stable in water when combined with HAs.

Figure 13: Effect of HA concentration on DLS size (CNT = 10 ppm). The mean of the dominating peak in April 2010 is indicated by the red squares. The data from March (blue diamonds) and April (green triangles) are collinear, which verified that the data is reproducible after one month.
Figure 14: Effect of CNT concentration on DLS size (HA = 200 ppm). The mean of the dominating peak in April 2010 is indicated by the red squares. The data from March (blue diamonds) and April (green triangles) are collinear, which verified that the data is reproducible after one month.

Figure 15: Results of Zeta potential tests. Red squares on left show varying concentrations of CNTs with a fixed concentration of 200 ppm HAs. Blue diamonds show varying concentrations of HAs with a fixed concentration 10 ppm CNTs.
3.5 Conclusion

The Zeta size and potential tests verified that the UWP manure HA was interacting with the CNTs. The interacting HAs and CNTs measured to be more stable in mixed solution than the manure humic acid alone. As stated earlier, the CNTs could be visibly observed in solution within four months of contacting the humic acid. The Zeta tests alongside the qualitative measurements produced consistent results that logically fit the original hypothesis that HAs would interact with CNTs and enable mobility in water.

Section 4: Final Remarks

Because the Leonardite was able to fully dissolve in the NMP, qualitatively the Leonardite can be concluded to have a surface tension similar to that of NMP and nanotubes (around 40 mJ/m²). More work must be conducted to determine the actual surface tension in order to model its interaction with CNTs.

The Zeta potential and size tests indicated that the manure humic acid was interacting with the CNTs. The interaction may have been the HAs and CNTs forming colloids that were more stable in solution that the HAs alone.

Manure is frequently used as fertilizer on agricultural lands, and although manure drastically varies on composition, the various humic acids can be carried off the land by excess precipitation runoff or seep into the soil by infiltrating precipitation. The presence of the humic acid is not threatening to the environment, as it is a naturally occurring organic matter in the top soil from animal droppings and degradation plant and organism remains. It is the possibility of Nanotech waste, in the form of CNTs or other nano-materials being carelessly, ignorantly, or accidentally dumped onto soil, that poses a potentially serious health risk.
Reflection of Student

The Wisconsin Department of Natural Resources (DNR) has numerous codes and regulations to ensure the water quality standard (WQS) of aquifers, streams, lakes, and rivers. The DNR also has numerous codes on solid and hazardous waste management and disposal. However, lack of enforcement and understanding has caused serious contaminations to occur.

The well-known large-scale tire fire in Watertown that lasted from July 19 to July 25, 2005 caused immense adverse environmental impact (Watertown Daily Times 07/20/2007). In this situation, innumerable wasted tires were allowed to build up on a pile with the goal of recycling someday. Local handlers did not view the tires as a serious threat. When the tires caught fire, as they typically do in piles, the toxic fumes and smoke billowed across the state. Ironically, as the firefighters struggled helplessly to quench the fire with water, they mistakenly perpetuated the problem by flushing the produced hazardous chemicals into the aquifer and nearby streams and ruined the quality of the water. The state had to intercede and spend millions of dollars to clean the contaminated water and soil. This is an extreme case of a substance that became mobile upon addition of heat and water although the substance was originally immobile. Nano-materials on a smaller scale can likewise be mobilized through the environment in the right conditions and potentially become severe health risks.

Since the DNR enforces codes on all solid wastes to some degree, the possibility of contamination is most likely to occur accidentally. In the future, as nano-materials are increasingly used and more truckloads are carried across the nation, more effort should be given to the cleanup of nano-materials than people would otherwise give, and it is visually similar to fine charcoal and activated carbon. Hazardous materials like asbestos were only given more stringent standards after the health of enough people declined due to the material. It is recommended that more investigation be given to the mobility and toxicity of emerging nano-materials to make wise decisions for their handling before the decisions are based on the ruined health of individuals.

Acknowledgments

I would like to thank Dr. Jasmine Erbs for her invaluable assistance with the Zetasizer experiment and input with the light scattering, Mr. Lester Lampert for his assistance with the technical setup and adjustments of the light scattering instrument, Dr. Timothy Zauche for his provision of the manure humic acid extraction procedure, and Dr. J Elmo Rawling for his input with the preliminary measurements with the thermo-gravimetric analyzer (supplemental report). I would especially like to thank Dr. James Hamilton for helping me get this project funded and for serving as my advisor.

Works Cited


Appendix A: Tables of solutions used in experiment

Table A:1 – Humic acid solutions in NMP for right-angle light scattering. Solutions were each sonicated for 10 minutes

<table>
<thead>
<tr>
<th>Bottle</th>
<th>Humic Acid Solute</th>
<th>Initial Concentration in NMP (ppm)</th>
<th>Visibly Soluble?</th>
</tr>
</thead>
<tbody>
<tr>
<td>50</td>
<td>Leonardite</td>
<td>2.93</td>
<td>Yes</td>
</tr>
<tr>
<td>49</td>
<td>Leonardite</td>
<td>10.9</td>
<td>Yes</td>
</tr>
<tr>
<td>48</td>
<td>Leonardite</td>
<td>15.5</td>
<td>Yes</td>
</tr>
<tr>
<td>47</td>
<td>Leonardite</td>
<td>37.0</td>
<td>Yes</td>
</tr>
<tr>
<td>44</td>
<td>Leonardite</td>
<td>64.8</td>
<td>Yes</td>
</tr>
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<td>45</td>
<td>Leonardite</td>
<td>81.5</td>
<td>Yes</td>
</tr>
<tr>
<td>46</td>
<td>Leonardite</td>
<td>111</td>
<td>Yes</td>
</tr>
<tr>
<td>42</td>
<td>Leonardite</td>
<td>132</td>
<td>Yes</td>
</tr>
<tr>
<td>43</td>
<td>Elliot</td>
<td>87.3</td>
<td>No</td>
</tr>
<tr>
<td>59</td>
<td>UWP Manure</td>
<td>150.0</td>
<td>No</td>
</tr>
</tbody>
</table>

Table A:2 – Humic acid solutions in methanol for right angle light scattering. Solutions were each sonicated for 10 minutes.

<table>
<thead>
<tr>
<th>Bottle</th>
<th>Humic Acid Solute</th>
<th>Initial Concentration in Methanol (ppm)</th>
<th>Visibly Soluble?</th>
</tr>
</thead>
<tbody>
<tr>
<td>39</td>
<td>Leonardite</td>
<td>40.1</td>
<td>Yes</td>
</tr>
<tr>
<td>54</td>
<td>Leonardite</td>
<td>40.1</td>
<td>Yes</td>
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<tr>
<td>55</td>
<td>Leonardite</td>
<td>40.1</td>
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<tr>
<td>57</td>
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</tr>
<tr>
<td>40</td>
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<td>79.1</td>
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</tr>
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<td>58</td>
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<td>102</td>
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</tr>
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<td>37</td>
<td>Leonardite</td>
<td>219</td>
<td>Yes</td>
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<td>41</td>
<td>Elliot</td>
<td>55.6</td>
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</tr>
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<td>38</td>
<td>Elliot</td>
<td>222</td>
<td>Yes</td>
</tr>
<tr>
<td>53</td>
<td>UWP Manure</td>
<td>177</td>
<td>No</td>
</tr>
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</table>
Table A:3 – Solutions of UWP manure humic acid and CNTs in water for Zeta tests. Solutions were each sonicated for 10 minutes and rested for at least four months.

<table>
<thead>
<tr>
<th>Bottle No.</th>
<th>Humic Acid</th>
<th>CNT</th>
<th>Zeta Potential (mV)</th>
<th>Peak 1 dia (nm)</th>
<th>Z-average dia (nm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Manure</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>17</td>
<td>28.2</td>
<td>10</td>
<td>-43.6</td>
<td>346</td>
<td>267</td>
</tr>
<tr>
<td>19</td>
<td>49.19</td>
<td>9.8</td>
<td>-37.2</td>
<td>320</td>
<td>255</td>
</tr>
<tr>
<td>20</td>
<td>65.07</td>
<td>9.8</td>
<td>-39.3</td>
<td>314</td>
<td>257</td>
</tr>
<tr>
<td>22</td>
<td>97.89</td>
<td>9.8</td>
<td>-41.4</td>
<td>291</td>
<td>235</td>
</tr>
<tr>
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<td>9.6</td>
<td>-33.8</td>
<td>293</td>
<td>218</td>
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<td>24</td>
<td>213.1</td>
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<td>298</td>
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<td>199.0</td>
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Appendix B: Procedure for extracting humic acid from manure

Provided by Dr. Timothy Zauche

Materials

Polyethersulfone filters: PALL® Life Sciences Supor® -200 0.2 µm 47 mm Membrane filters (P/N 60301)

Dialysis tubing: Sigma Aldrich® D9777 Dialysis tubing cellulose membrane (25 mm flat width)

Procedure

1. Obtain the sample and weigh
2. Drop the pH to 1 with 1.0 M HCl
   a. Add 0.1 M HCl until the volume is 10ml per gram of sample
   b. After one hour recheck the pH
3. Centrifuge at 1800 RPM for 10 minutes
4. Pipette out the supernatant and discard it
5. Scrape the solids back into their round bottom flask
6. Rinse the centrifuge tubes with 0.1 M NaOH
   a. Pour this rinsate into the round bottom flask
7. Add 1.0 M NaOH until the pH raises to 7 or 8
   a. Add 0.1 M NaOH until the volume is 10 mL per gram of initial sample
8. Purge with N₂ gas for 10 minutes and leave stirring with a magnetic stir bar for at least four hours
9. Discontinue stirring and allow to rest overnight
10. Centrifuge at 1800 RPM
11. Pipette out the supernatant and place it back into the round bottom flask
12. Recentrifuge the remaining solids at 2300 RPM for 10 minutes
13. Again remove the supernatant and place it with the rest
14. Discard the remaining solids
15. Drop the pH of the liquid to 1 with 6.0 M HCl
   a. Recheck the pH after at least one hour
16. Let rest overnight
17. Centrifuge at 1800 RPM for 10 minutes
18. Collect the supernatant and discard it
19. Recentrifuge the solids at 2000 RPM for 5 minutes
20. Again collect the supernatant and discard it
21. Scrape solids back into round bottom flask
22. Rinse the centrifuge tubes with 0.1 KOH
23. Add 0.1 M KOH until the humic acid becomes redissolved
   a. Usually at pH 7
24. Add solid KCl until solution is 0.3 M K
25. Place under N₂ gas for 15 minutes
26. Centrifuge at 1800 RPM for 10 minutes
27. Collect the supernatant and discard the solids
28. Drop the pH of the supernatant to 1 with 6.0 M HCl
29. Allow to rest overnight
30. Recheck the pH and begin filtration
31. Utilizing a vacuum filtration apparatus, pull the suspension through a 0.2 µm polyethersulfone membrane filter
   a. Rinsing was performed with 0.1 M HCl
32. Refiltered the liquid portion through the same membrane
33. Scrape HA residue and dissolve into a 50:50 mixture of 0.1 M NaOH and de-ionized water
   a. Some of the mixture can be poured out over the filter to dissolve any of the remaining humic acid
34. Pipette the liquid portion into dialysis tubing and seal with plastic clamps
35. Place tubing in de-ionized water until all tubing is submerged
36. Change water daily until no precipitation forms when a few drops of AgNO₃ is added
   a. Allow to occur overnight to be certain of reaction
37. Dispense contents of dialysis tubing into a flask and rinse with de-ionized water
38. Place Parafilm over the flask
39. Freeze dry the liquid with Parafilm so that the humic acid does not blow out of the flask
40. Gather humic acid into a closed container and store in a dry place