# ENVIRONMENTAL BEHAVIORS OF NANOPARTICLES: DISTRIBUTION, BIOTRANSFORMATION AND ECOTOXICITY

by

## LIWEN ZHANG

(Under the Direction of Qingguo Huang)

### ABSTRACT

The increased production, use and disposal of nanoparticles (NPs) will ultimately lead to the environmental release of these materials, and thus understanding their environmental behaviors is necessary for an accurate environmental risk assessment. Phase distribution of NPs between aqueous and solid phases determines in which environmental compartment the NPs are most likely to reside; however, the system containing solid phase was merely been studied previously. Our results showed that the interaction between inorganic clay minerals and MWCNTs can be explained by van der Waal's, electrostatic, and acid-base interactions and modeled by extended-DLVO theory, regardless of the mineral type (kaolinite or smectite). Diagenetically young organic matter, peat, sorbed MWCNTs only when cation was added, while released dissolved organic matter (DOM) that helped stabilize MWCNTs. However, diagenetically old organic matter in shale strongly sorbed MWCNTs. Meanwhile, smaller MWCNTs showed higher tendency to aggregate, but DOM impaired this tendency. Our results suggest that MWCNTs are not likely to be stably dispersed in hard water or seawater; instead, they preferentially accumulate in soil or sediment, unless there is DOM present. In addition, we at the first time reported that a bacteria community mineralized MWCNTs, i.e., degrade into CO<sub>2</sub>, which was confirmed by C14-labeling. This mineralization is likely performed by several bacteria species, including *Burkholderia kururiensis*, *Delftia acidovorans*, and *Stenotrophomonas maltophilia*, through co-metabolism that requires carbon source other than MWCNTs. The intermediates of low-molecular aromatic organic compounds were also detected. This indicates that microbes may modify the long-term fate of MWCNTs.

The effect of nano-TiO<sub>2</sub> to the growth of one major crop, wheat, was shown to be dependent on the type and dosage of nano-TiO<sub>2</sub>, the spiking method, the incubation time and the wheat genotype, which varied from negative, neutral to positive. The photosynthesis efficiency maintained a high level across the tested conditions, indicating the effect of nano-TiO<sub>2</sub> may not last for long term. The uptake and translocation of nano-TiO<sub>2</sub> to the root and shoot of wheat were also observed. Elevated level of peroxidase activity in root at 5 and 50 mg kg<sup>-1</sup> soil dosage indicated the root is under oxidative stress.

INDEX WORDS: Nanoparticles, Multiwall carbon nanotubes, Titanium dioxide nanoparticles, Fate, Sorption, Biotransformation, Toxicity

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# DEDICATION

I would like to dedicate this dissertation to my parents, Mr. Baoming Zhang and Mrs. Huijiao Li, for their everlasting love, support, encouragement, and wise advice through my life.

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## CHAPTER I

## INTRODUCTION

Nanoparticles (NPs), such as metal/metal oxide nanoparticles, carbon nanoparticles, quantum dots, are featured by their small size in nanoscale (1-100 nm). When reaching this specific size range several special phenomena arise, e.g., statistical mechanical effects, quantum mechanical effects, and the mechanical, thermal and catalytic properties altered by the increase in surface area to volume ratio.<sup>1</sup> Due to these unique physicochemical, optical, and mechanical properties, NPs have been applied in various areas such as electronics, catalyst, composite materials, leading to technology advancement as well as rapid growth in their production.<sup>1, 2</sup> This fact suggests the high likelihood of the manufactured NPs to enter and interact with the environment.<sup>3-5</sup> As a result, the concerns on the potential consequences of NPs released to the environment have also been raised by many researchers.<sup>1, 6, 7</sup> To help address these concerns, we studied the environmental distribution, transformation and toxicity of two representative NPs, i.e., multiwall carbon nanotubes (MWCNTs), a typical carbon nanomaterial, and TiO<sub>2</sub> nanoparticles (nano-TiO<sub>2</sub>), one representative metal oxide nanoparticles. These two types of NPs were chosen because of their popularity in research and application. The goal of this study is to provide scientific information for the precise and comprehensive life-cycle analysis and environmental assessment of NPs, as well as to probe the influential factors and mechanisms governing the interactions between NPs and the environment.

#### BACKGROUND

Carbon nanotubes (CNTs) are one type of carbon-based nanoparticles with cylindrical shape formed by graphene layer(s), in which multiwall carbon nanotubes (MWCNTs) are composed by a series of coaxially-arranged graphene layers. The potential applications of MWCNTs include reinforced composites, conductive materials, sensors, drug delivery vessels, and sorbents.<sup>8</sup> The global market for MWCNTs is estimated to rise from \$219 million in 2012 to \$292 million in 2016.<sup>9</sup> Upon this fast growth rate, it is very likely that this material will be ultimately released to the environment during manufacturing, transportation of raw CNTs, or via product use and disposal.

Similarly, nano-sized titanium dioxide (nano-TiO<sub>2</sub>) is among the most widely used NPs. The consumer products based on nano-TiO<sub>2</sub> mainly include two categories: 1) UV light attenuator such as sunscreens and other cosmetics; 2) photocatalysis or semiconductor such as self-cleaning coatings, air sanitizers or filtration devices, environmental remediation of pollutants.<sup>10</sup> The increasing manufacture amount has already leaded to the release of this material to various environmental compartments.<sup>11</sup> Nano-TiO<sub>2</sub> is predicted to have environmental concentrations of 21 ng L<sup>-1</sup> for surface waters, 4  $\mu$ g L<sup>-1</sup> for sewage treatment effluents, and 89  $\mu$ g kg<sup>-1</sup> for soil.<sup>12</sup> One study also reported the detection of nano-TiO<sub>2</sub> in water leaching from exterior facades.<sup>13</sup> These releases will consequently cause nano-TiO<sub>2</sub> exposure to aquatic, marine and terrestrial organisms.<sup>14</sup>

### **ENVIRONMENTAL BEHAVIORS: PHASE DISTRIBUTION**

Due to their high potential to enter the environment, research on the ecotoxicity, aggregation and transport of MWCNTs and nano-TiO<sub>2</sub> has been conducted in recent years to provide information on their impact to and fate in the environment.<sup>4, 15-17</sup> The summary of the

related literature results will be listed in the next chapter. One of the most concerned topics is the toxicity of NPs. The general understanding is that nanoparticles exhibit special properties that are different with their counterparts of the same composition at either molecular or bulk scales. As such their interactions with various receptors may not be readily postulated from our current knowledge platform, and need to be carefully evaluated.<sup>4, 7, 18</sup> However, one important prerequisite on these toxicity studies is the fate and distribution of NPs in the environment, i.e., where the particles are most likely to reside. This determines what ecological receptors are susceptive to NPs exposure, and different receptors may result in very different responses. For example, bioaccumulation of CNTs by terrestrial and benthic organisms was minimal in spiked soils or sediments,<sup>19-23</sup> while *Daphnia magna*, an aquatic invertebrate, had nanotube body burdens of up to 6.3% on a dry mass basis after being exposed to MWCNTs suspended in water.<sup>24, 25</sup> As such, information on the phase distribution of NPs between water and sediment is essential to assess their environmental risks in natural systems.

Because of their size range, NPs most likely adopt Brownian motion and can be dispersed stably in water to form colloidal dispersion. However, aggregation will increase their size and lead to settling, especially for CNTs that have not only large specific surface area for van der Waals attraction but also strong hydrophobic interaction as well. On the contrary, electrostatic repulsion can act against the attraction forces and prevent aggregation. This electrostatic repulsion mainly comes from surface functional groups.<sup>26-28</sup> Previous research has elucidated the colloidal behaviors of NPs in laboratory prepared aqueous samples,<sup>7, 26</sup> but investigation on systems having a solid phase like soil or sediment was very rare. This knowledge gap is partially due to that characterization and quantification of NPs in environmental samples are very difficult, especially for CNTs because of the ubiquitous existence of background carbon. Therefore in this

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study we investigated the interactions between MWCNTs and soil/sediment as a major thrust. To solve the difficulty on quantification, we used C14-labeling in the backbone structure of MWCNTs for to unambiguously quantification without modifying their physical/chemical properties. Such labeling is particularly powerful to study the interaction of CNTs with natural organic matters that are commonly found in soil/sediment as well as natural water.

The intrinsic complexity of soil and sediment has also hindered the study on the interaction between CNTs and soil/sediments. Soil/sediment is heterogeneous and usually composed of different components with diverse properties. In order to better understand the mechanisms governing the interactions, we investigated the typical components of soil/sediment, i.e., soil organic matter (young and aged), and clay minerals (kaolinite and smectite), separately. At the same time, the effects of varying aqueous conditions, i.e., pH, cation concentrations and dissolved organic matter, were also studied to model natural conditions. Further, the influence of the properties of MWCNTs such as particle size on their phase distribution was examined as well. These investigations are reported respectively in Chapter III to V, and the results provide important information for toxicity and environmental risk assessment of MWCNTs.

### ENVIRONMENTAL BEHAVIORS: TRANSFORMATION

Whether and how NPs may transform in the environment is another critical question to answer, because transformation of NPs may alter the chemical and/or physical properties of NPs and consequently influence their fate.<sup>26, 29</sup> For example, the species and amount of surface oxide resulted from different chemical/biochemical modification processes<sup>30</sup> can strongly influence the disperse of MWCNTs in water.<sup>27</sup>

The transformation of CNTs is a particularly interesting yet challenging topic, because carbon has four electrons available for covalent bonding providing abundant variation in reaction pathways. As a carbon-based nanoparticle, it is also important to know whether CNTs can be transformed to  $CO_2$  in the environment. If such transformation is possible, they can potentially fit in the carbon cycle and would not accumulate in the environment in the long run to cause adverse effects.

So far the environmental transformation of CNTs has not yet been fully investigated due to their high chemical stability as well as challenges with CNT quantification. The basic structure of CNTs is aromatic rings fused by sp<sup>2</sup>-hybridized carbon which are analogous to polycyclic aromatic hydrocarbons (PAHs) and considered to be stable, whereas the associated defects such as pentagon-heptagon pairs (Stone-Wales defects), sp<sup>3</sup>-hybridized carbon atoms, vacancies in the nanotube lattice, and open ends can provide additional reactivity to CNTs.<sup>31-35</sup> Thus these defects may serve as reaction sites that lead to CNTs deformation. Previous research has been summarized in the next chapter.

Microorganisms are well-known decomposers and some of them can degrade persistent organic chemicals such as PAHs and lignin. In this study we attempted to explore their potential to degrade CNTs. The degrader candidates we tested included three species from white-rot fungi, two types of activated sludge, bacteria community, and two types of enzymes. Moreover, the <sup>14</sup>C-labeling technique that we have used in this study is a powerful tool to unambiguously trace the end product of degradation by measuring the released <sup>14</sup>CO<sub>2</sub>. The result will be discussed in Chapter VI.

### ENVIRONMENTAL BEHAVIORS: TOXICITY

As will be illustrated in Chapter III and IV, soil/sediment is likely in most cases the ultimate sink of NPs and thus organisms reside in these environmental compartments have high likelihood of being exposed to NPs. Among these organisms, the toxicity of NPs to plants, especially crops and vegetables, are of particular concern because of their close relationship to human life. First, NPs may directly exert toxic effects on these plants, thus reducing their production. Second, crops and vegetables may uptake and accumulate NPs, consequently making consumption of NP-contaminated edible plants a potential exposure pathway to animals and humans. Thus the toxicity and bioaccumulation of NPs to these plants have attracted research interests.<sup>10, 18, 36</sup> However, most of the studies were conducted in hydroponic conditions, which may not be readily applied to soil systems. Therefore we tested the toxicity and bioaccumulation of MWCNTs and nano-TiO<sub>2</sub> by wheat as a model major crop in soil system. The preliminary experiment showed that the effect of MWCNTs on wheat growth was not significant. The result is presented in Chapter V.

### **SUMMARY**

The three interrelated aspects of the environmental behaviors of NPs can together help understanding the fate of NPs (MWCNTs and nano-TiO<sub>2</sub>) in the environment, consequently provide important information to their life cycle analysis and environmental risk assessment. Through analyzing the differences caused by various environmental conditions, NPs properties, etc., the mechanisms governing their behaviors and impacts were discussed. The results in Chapter III to V respectively address: 1) How aqueous conditions, i.e., cation concentration, pH, and dissolved organic matter, may influence MWCNTs dispersing in aqueous phase; 2) How different soil components interact with MWCNTs; 3) How the size of MWCNTs influence the stability of their water dispersion. Chapter VI mainly discusses the possibility of MWCNTs biotransformation into  $CO_2$  and the possible pathways. Chapter VII investigates the toxicity and bioaccumulation of nano-TiO<sub>2</sub> to wheat and probes the influential factors using statistical analysis. Chapter VIII summarizes the major conclusions and discusses the environmental risks of NPs.

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## CHAPTER II

### LITERATURE REVIEW

## PHASE DISTRIBUTION OF NANOPARTICLES: AGGREGATION AND SETTLING

As stated in Chapter I, one important aspect of the environmental study on nanoparticles is their phase distribution, e.g., in which environmental compartment the nanoparticles are more likely to reside. This section will use carbon nanotubes (CNTs) as an example to introduce the processes that control the distribution of nanoparticles between solid and aqueous phases in two steps: 1) the colloidal stability of CNTs in aqueous phase; 2) the effect of solid phase, i.e., sorption.

### Colloidal stability

Because nanoparticles are essentially colloids although towards the lower end of the size range (Figure 2.1), the colloid science can provide a basis for studying nanoparticles. CNTs dispersed in water form a colloidal dispersion that differs from a true solution in nature. A colloidal dispersion refers to one phase (e.g., solid) homogeneously distributed in another phase, e.g., water.<sup>1</sup> In a stable colloidal dispersion, CNTs can undergo Brownian motion and remain suspended in water over certain time scales as long as the settling velocity is equal to, or less than, the Brownian displacement. Generally the Brownian motion applies to a particle range of 1 nm to 1 µm. When the particle size is beyond this range, the particles (or aggregates) begin settling. The settling rate for a spherical or near-spherical particle is proportional to the square of the particle diameter. Thus the size is one of the governing factors to determine the colloidal stability of CNTs in aqueous phase. CNTs have large specific surface areas that are hydrophobic

in nature, and are thus easy to aggregate leading to size increasing and consequent particle settling. Therefore aggregation is a crucial process to study the colloidal stability of CNTs.

Colloidal dispersions are dynamic non-equilibrium systems, and are often sensitive to physical or chemical disturbances that result in aggregation of particles.<sup>2</sup> There are two major steps involved in aggregation: particle transport (collision) and attachment.<sup>1, 3</sup> The first step can be originated from three fundamental processes: Brownian diffusion of particles leads to perikinetic aggregation; shear flow transport particles at different velocities causes orthokinetic (shear) aggregation; and particles of different size or density undergo differential settling (Figure 2.2). After initial aggregation, particle-cluster and cluster–cluster aggregation processes also take place.<sup>1</sup>

Pristine CNTs have a strong tendency to attach and aggregate when they collide with each other as a result of attractive van der Waals force. Experiments indicated that raw multiwall carbon nanotubes (MWCNTs) settled more rapidly than carbon black and activated carbon particles, which may be attributable to that the much greater aspect ratio of MWCNTs (1:1,000) allows multiple contact points between particles, greater entanglement, and increased van der Waals forces, leading to aggregates of increased mass.<sup>4</sup> However, negative surface functional groups on CNT surface tend to mitigate aggregation as mentioned in Chapter I. Different types of surface functional groups can be added to CNTs during various purification and dispersion processes.<sup>5-9</sup> Such functional groups are in general hydrophilic and acidic (e.g., carboxyl, hydroxyl and carbonyl groups).<sup>10-20</sup> Carboxyl groups, in particular, have low pKa values (~3.5), and are thus dissociated around common aqueous conditions rendering negative charges on CNT surfaces, although the charge densities may vary depending on the CNT synthesis and purification procedures. Smith et al. found that the zeta-potential is proportional to the amount of carboxyl groups on CNT surfaces ( $\mathbb{R}^2 = 0.89$ ).<sup>21</sup> The speciation of surface functional groups is another influencing factor, but inconsistent observations have been made. For MWCNTs, Kennedy et al. showed that the stabilizing ability of hydroxyl groups is greater than carboxyl groups,<sup>4</sup> whereas Smith et al. found that carboxyl groups are more influential than hydroxyl groups or carbonyl groups.<sup>21</sup> Nevertheless, these functional groups increase the hydrophilicity of CNTs, and the amount of surface oxygen concentration correlates with surface charge density,<sup>21</sup> and thus increase the stability of CNTs in aqueous phase.

It has been found in several studies that the attachment efficiencies of CNTs due in large measure to their surface charges, can be fairly well modeled using the Derjaguin—Landau— Verwey—Overbeek (DLVO) theory that describes the interactions of charged spherical colloidal particles. Although DLVO theory was derived for ideal spherical particles with evenly distributed surface charge and other surface properties, the aggregation behavior of CNTs can still be reasonably well modeled by DLVO theory in many aspects despite their cylindrical shapes.<sup>22, 23</sup>

## Influential factors: Counter-ion and pH

The charges on CNT surfaces lead to repulsion forces that prevent CNT aggregation, and the surface potential of CNTs is correlated to their stability in aqueous systems.<sup>21-23</sup> It thus follows that any factor that can influence CNT surface potential may result in changes in the stability of CNTs in water. Consistent results have been reached in different studies that cations present in solution facilitated CNT aggregation.<sup>22-26</sup> As the concentration of electrolytes increases, the surface potential indicated by electophoretic mobility<sup>23, 27, 28</sup> or zeta-potential<sup>23</sup> of CNTs becomes less negative. This suggests that the repulsive force between CNTs is reduced, and the attachment efficiency between CNTs would increase due to the decreasing energy barrier

to aggregation. Once the concentration of electrolytes reaches or exceeds a certain value, i.e., the critical coagulation concentration (CCC), the particle surface charge becomes completely screened, thus eliminating the energy barrier to aggregation so that attractive forces between particles, e.g., van der Waals force, become dominant. Aggregation kinetics of the CNTs exhibited slow (reaction-limited or unfavorable) and fast (diffusion-limited or favorable) regimes in the presence of these cations, the intersection of which is the calculated CCC (Figure 2.3).<sup>22, 29</sup> Comparing the CCC values of SWCNT and MWCNT can provide information on the relative aqueous stability of these CNTs. The CCC values were 20 mM NaCl<sup>30</sup> or 37 mM NaCl<sup>25</sup> for SWCNTs, while 93 mM NaCl and 98 mM Na2SO4 for MWCNT,<sup>23</sup> indicating an greater stability of MWCNT than SWCNT. The CCC values of divalent cation Ca<sup>2+</sup> for SWCNT ( $\sim$ 2 mM)<sup>32</sup> and MWCNT (1.2 mM)<sup>23</sup> were similar with each other, while that for fullerene was higher (6.1 mM).<sup>31, 32</sup>

The ability of divalent cations, e.g., Mg<sup>2+</sup> or Ca<sup>2+</sup>, to induce aggregation is dramatically stronger than that of monovalent cations, e.g., Na<sup>+</sup> or K<sup>+</sup>.<sup>23, 25</sup> The CCC of Na<sup>+</sup> for fullerene aggregation was 120 mM, while that of Ca<sup>2+</sup> was 4.8 mM.<sup>27</sup> According to the Schulze-Hardy rule, CCC is proportional to Z<sup>-6</sup> for surfaces with high charge densities, or Z<sup>-2</sup> for those with low charge densities (where Z is the counterion valence).<sup>25, 33, 34</sup> Observations with colloidal particles showed the CCC value dependence on Z ranged between Z<sup>-6</sup> and Z<sup>-2</sup> (Figure 2.4).<sup>3</sup> In a study with acid-treated MWCNTs, Smith et al. found that the ratios of CCCs of MgCl<sub>2</sub> and CaCl<sub>2</sub> over that of NaCl were 2<sup>-5.7</sup> and 2<sup>-6.3</sup>, respectively,<sup>23</sup> very close to the theoretical value of 2<sup>-6</sup>. However, this theoretical ratio of 1/64 is not only determined by the valence of cations, but also the symmetry of electrolytes and the shape of CNTs. Researchers showed that for symmetric 2:2

 $CaCl_2$ , the ratio should be 1/42.<sup>34, 35</sup> Chen et al. found the ratio of CCC values for colloidal particles with  $CaCl_2$  over NaCl is 1/40,<sup>34</sup> consistent with the 1/42 prediction. However, there are some experimental results not in good agreement with the 1/60 or 1/42 value, for example, 1/17 for MgCl<sub>2</sub>.<sup>22</sup> These discrepancies may be caused by different surface charge densities.

While mono- and di-valent cations have large difference in CCCs, the difference between cations with the same valence is minor. The CCC values for an acid-treated MWCNT (with carboxyl groups on surface) are 1.8 mM for MgCl<sub>2</sub> and 1.2 mM for CaCl<sub>2</sub>.<sup>23</sup> Moreover, the type and valence of anions have little influence. The CCC values for the acid-treated MWCNTs are 93 mM Na<sup>+</sup> in the form of NaCl and 98 mM Na<sup>+</sup> for Na<sub>2</sub>SO<sub>4</sub>.<sup>23</sup>

Although solution ionic conditions strongly influence the electric interactions between CNTs, the van der Waals forces are quite independent of solution conditions and CNT surface chemistry. Hamaker constant, a parameter delineating van der Waals potential between two particles, does not change with solution conditions. The fitted Hamaker constants from two experiments investigating the aggregation of fullerene nanoparticles in aqueous medium were 6.7  $\times 10^{-21}$  J at pH 5.2  $\pm 0.1$  (NaCl concentration range of about 50 mM to 500 mM)<sup>27</sup> and 8.5  $\times 10^{-21}$  J at pH 5.5 (KCl concentration range of about 20 mM to 1000 mM).<sup>36</sup>

CNT aggregation is also strongly influenced by pH, mainly because pH influences the protonation/deprotonation of surface functional groups that contributes to surface charges. For example, the zeta-potential of CNTs became more negative when pH increased from 3 to 11, indicating more charges added, which led to smaller aggregate sizes and more stable suspension.<sup>22, 37</sup> Acid-treated MWCNTs were unstable at pH 0 but the stability increased when pH increased from 4 to 10.<sup>23, 38</sup> However, the electrophoretic mobility did not change much when

pH was above 6,<sup>23</sup> indicating that electrophoretic mobility is not necessarily consistent with colloidal stability.<sup>21, 23</sup>

## Influential factors: Natural organic matter (NOM)

Natural organic matter (NOM) ubiquitously exists in natural or engineered aquatic systems. It has been found in many studies that the presence of NOM significantly enhances the stability of CNTs in water. With the presence of Suwannee River NOM (humic acid and fulvic acid) at the concentrations from 1 mg/L to 100 mg/L, CNTs (both SWCNTs and MWCNTs, pristine and functionalized) are much more stable,<sup>4, 22, 26, 32, 39</sup> indicated by reduced aggregation rates and increased aqueous concentration. Similar results were obtained in aggregation experiments using natural river water containing NOM<sup>39, 40</sup> and dissolved soluble soil humic substances (Aldrich humic acid dissolved at 150 mg/L and water-extractable Catlin soil humic substances at 300 mg/L).<sup>41</sup> To quantitatively investigate the effect of NOM, experiments were carried out with various NOM concentrations and a fixed initial MWCNT concentration. It was found that, after 24 hours of settling, the concentration of MWCNTs remaining in water was linearly correlated with the NOM concentration varying from 0 to 100 g/L.<sup>4, 39</sup> In general, the concentration of MWCNT stably suspended in water is dependent on the amount of NOM adsorbed per unit mass of MWCNT.<sup>42</sup> The reason for this phenomenon is that NOM can cause disaggregation of CNTs demonstrated by microscopic and dynamic light scattering, which makes CNTs individually dispersed,<sup>39</sup> and such effect increases with increasing NOM concentration.<sup>43</sup>

Two components are thus involved in the mechanism of NOM enhancing CNT stability in aqueous phase: 1) sorption of NOM on CNTs, and 2) steric or electrosteric stabilization imposed by NOM.<sup>39, 42, 44, 45</sup> NOM is surface active, and, because of this nature, their interactions with CNTs tend to take the form where the hydrophobic moieties of NOM associate with the CNT surface, likely through pi-pi or CH-pi stacking, while their hydrophilic moieties are exposed to the water.<sup>14, 40-42, 44-47</sup> Studies showed that NOM association on CNTs was an exothermic equilibrium process, similar to spontaneous adsorbate-adsorbent interaction,<sup>39, 40, 42</sup> and appeared to follow pseudo-first-order rate kinetics.<sup>40</sup> Hyung et al. demonstrated that the adsorption of NOM on CNT was proportional to the aromatic carbon content and molecular weight of the NOM.<sup>42</sup> Pi-pi stacking is likely more powerful than CH-pi stacking, e.g., the stabilization effect of sodium dodecyl sulfate (SDS) through CH-pi stacking is substantially weaker than that of NOM that elicit pi-pi stacking.<sup>48</sup>

Organic matter imposes steric and/or electrosteric stabilization to CNTs, and thus prevent CNT attachment and consequent aggregation.<sup>22, 32, 44</sup> The steric stabilization results from displacement of water molecules into the bulk phase caused by the interpenetration of the hydrophilic portion of the NOM that extends into the solution phase when two CNP particles approaching.<sup>44</sup> This stabilization is relatively inert to the change of ionic strength in solution phase. For example, the stability of fullerenes in aqueous phase didn't change significantly across a range of NaCl concentrations in the presence of NOM.<sup>29</sup> If the organic matter is ionic, both steric and electrostatic repulsion will likely take place and the latter is influenced by the ionic strength of solution.<sup>44</sup> On the contrary, when having divalent cation such as CaCl<sub>2</sub> present in the solution, bridging effect (complex formation) may occur and destabilize CNTs in the presence of NOM.<sup>32, 44</sup> Such enhanced aggregation was attributed to organic molecule bridging by Ca<sup>2+, 32</sup> Such bridging effect by Ca<sup>2+</sup> was also observed in humic acid stabilized C<sub>60</sub> nanoparticles.<sup>29</sup> However, this bridging effect was not observed with other di-valent cations, e.g., Mg<sup>2+, 49</sup>
### PHASE DISTRIBUTION OF NANOPARTICLES: SORPTION

Solid phases (sediment or soil) ubiquitously exist in natural aquatic systems. The association of CNTs with these solid phases, or sorption, is another important process governing the partition of CNTs between water and solid phases, which in turn will contribute to its bioavailability and toxicity. Yet sorption of NPs, especially CNTs, has not been fully investigated. Fullerene can serve as an analogous to CNTs, and its sorption by soils has been found to follow a linear isotherm with solid phase concentration proportional to aqueous phase concentration.<sup>50, 51</sup> Soil organic matter (SOM) plays an important role in the sorption of nC<sub>60</sub>, and the sorption capacity strongly depends on the organic content of the soil.<sup>50, 51</sup> The sorption of  $C_{60}$ to SOM was also found to depend on the SOM type and properties. If the organic matter is hydrophilic or surface active in nature, steric hindrance may take place and thus exert a stabilizing effect. For example, in a deposition study with silica as a solid phase in solution containing NaCl, the attachment efficiencies between  $C_{60}$  and silica surface was mitigated when the silica surface was pre-coated with dissolvable humic acid or alginate.<sup>29</sup> This result implies that the negatively charged organic moieties or functional groups coated on soil minerals may reduce the sorption of fullerenes by soil.

Studies related to CNT sorption on soil are limited and little is known about the interactions involved in the sorption. An inorganic clay particle, kaolin, was found to improve MWCNT removal from aqueous phase when its concentration was high in the influent water.<sup>52</sup> Soil can also affect CNT stability in water through indirectly. In a study investigating the interaction between clay minerals (kaolinite and montmorillonite) and MWCNT suspensions stabilized by surfactant (SDBS, CTAB, and TX100), clay minerals reduced the stability of MWCNTs in two ways: 1) competitive adsorption of surfactants thus reducing their stabilizing

effect and 2) bridging between clay mineral and MWCNTs by surfactants.<sup>53</sup> These effects depend on the properties of surfactants and the sorption capability of clay minerals. Additional research is needed to investigate the sorption effect of different soils or soil components under different aqueous conditions.

# TRANSFORMATION

Possible CNTs transformation in natural or engineered systems can change the properties of CNTs and consequently affect their mobility and bioavailability. Transformation of CNTs under natural conditions has not been fully investigated. There are, however, investigations regarding CNT reactions in chemistry and chemical engineering studies, and this information may suggest likely routes of CNT transformation in natural environments.

The graphene structures of CNPs, although inert in general, are still open to covalent reactions to a certain extent, which is primarily driven by the strain engendered by the curvature of CNTs and spherical geometry of fullerenes caps at the end.<sup>20, 54</sup> For an sp2-hybridized (trigonal) carbon atom, planarity is strongly preferred, described by a so-called pyramidalization angle of  $\theta_p = 0^{\circ}$  (Figure 2.5); whereas an sp3-hybridized (tetrahedral) carbon atom requires  $\theta_p = 19.5^{\circ}.^{54}$  According to the geometry of C<sub>60</sub>, all of the sp2-hybridized carbon atoms have  $\theta_p = 11.6^{\circ}$ , which is closer to the tetrahedral structure. Thus, the conversion of sp2- to sp3-hybridization can release the strain and mitigate the strain of the rest of the 59 atoms,<sup>54</sup> which is consequently favorable to covalent addition.<sup>54, 55</sup> The end caps of nanotubes, if not closed by the catalyst particle, tend to be composed of highly curved (and hence unstable) fullerene-like hemispheres that are much more reactive than the sidewalls.<sup>20, 56</sup> The reactivity of these end caps is similar to fullerene, depending on the degree of pyramidalization in that the strain energy of

pyramidalization is roughly proportional to  $\theta_p^{2}$ .<sup>20</sup> Thus, the smaller the diameter of fullerenes or fullerene end caps, the larger the curvature is and consequently the more reactive it is.

The reactivity of CNT sidewalls comes from the curvature-induced pyramidalization, analogous to but much weaker than fullerene. In addition, misalignment of the pi-orbitals between adjacent pairs of conjugated carbon atoms would also contribute to CNT side-wall reactivity,<sup>57, 58</sup> for which calculations of torsional strain energies in conjugated organic molecules has provided some theoretical support.<sup>20, 59</sup>

# Oxidization

Oxidization is a common form of covalent reactions occurring to CNTs and has been studied the most. Certain treatments under harsh conditions can even destroy CNPs.<sup>60</sup> However, these oxidation conditions are not likely to appear in surface earth processes. Some oxidization processes are commonly employed in CNT purification to remove impurities, and these processes generally lead to reduction of CNT sizes and addition of oxygen functional groups to CNT surfaces, changes that tend to enhance mobility and perhaps bioavailability and toxicity. The unit backbone structure of CNT sidewalls, a six-numbered conjugated sp2 carbon ring, is relatively inert to oxidation. However, the sidewalls contain defect sites such as pentagonheptagon pairs called Stone-Wales defects, sp3-hybridized defects, and vacancies in the nanotube lattice.<sup>56, 61</sup> The end caps and the defects on sidewalls are expected to be sites more susceptible to oxidization.<sup>13, 15, 20</sup>

Major types of oxidants include strong acids, e.g., concentrated  $HNO_3$ ,<sup>12-14, 62</sup> mixtures of concentrated  $HNO_3/H_2SO_4$ ,<sup>7, 8, 13, 16</sup> KMnO<sub>4</sub>/  $H_2SO_4$ ,<sup>16, 63</sup> K<sub>2</sub>Cr<sub>2</sub>O<sub>7</sub>/  $H_2SO_4$ ,<sup>63</sup> OsO<sub>4</sub>,<sup>20, 63</sup> and  $H_2O_2/H_2SO_4$ ,<sup>10</sup> as well as strong energy inputs such as ultrasonication.<sup>8, 59</sup> However, these strong oxidative forces are unlikely to frequently occur naturally in the environment. Oxidative

substances used in waste water treatments, e.g., ozone,<sup>64, 65</sup> and radical-generating reagents such as Fenton's reagent<sup>11, 15, 66</sup> and photophenton reagent,<sup>67</sup> are also effective in causing CNT oxidation. Photooxidation is one possible process occurring to CNTs in the environment. For example, when exposed to the sunlight or to lamps that emit light only within the solar spectrum, carboxylated SWCNT solution produced reactive oxygen species (ROS) such as <sup>1</sup>O<sub>2</sub>, O<sub>2</sub><sup>•</sup>, and <sup>•</sup>OH,<sup>68</sup> and these radicals can oxidize CNTs and modify their surfaces.

The common results of oxidation are to open the end cap and introduce oxygen-containing surface functional groups like carboxyl, hydroxyl, carbonyl, and ester to attach on either the ends or the sidewalls of CNTs,<sup>10-19, 67</sup> although minor differences exist with various treatments. For example, Fenton's reagent ( $Fe^{2+}$ -  $H_2O_2$ ) is effective in introducing both carboxyl and hydroxyl groups, while Photophenton and UV/ $H_2O_2$  processes mostly produce hydroxyl groups.<sup>19</sup> The strong oxidation processes were also found to disrupt the aromatic ring system of CNTs,<sup>13</sup> for example, sonicated MWCNTs were shorter and exhibited a narrower length distribution<sup>22, 23</sup>. The consequence of these modifications is to increase the surface charge and thus stability in water.

In addition to adding surface functional groups, the oxidative treatment of fullerene-like caps and graphene layers generated oxidized polycyclic aromatic substances, which are like fulvic acids and remained sorbed on MWCNT surfaces in acidic and neutral solutions.<sup>14</sup> As mentioned before, these sorbed organic matters can also help to stabilize MWCNTs in water.

# **Biodegradation**

Recent studies indicate that carboxylated CNTs can be transformed via mediation by common enzymes such as horseradish peroxidase, but such transformation did not seem to occur in pristine CNTs.<sup>69-73</sup> During such transformations, SWCNT lengths were shortened, carboxyl groups were added to SWCNT surfaces, and  $CO_2$  was produced.<sup>70</sup> The products of the enzymatic

degradation were identified as shown in Figure 2.6.<sup>70</sup> The same group has also found that the neutrophil myeloperoxidase, a peroxidase generated inside human cells, can degrade SWCNTs and the resulting nanotubes do not generate an inflammatory response when aspirated into the lungs of mice.<sup>74</sup>

Degradation of MWCNTs by HRP was also observed.<sup>71, 72</sup> Zhao et al. (2011) proposed a degradation procedure of MWCNTs in which the MWCNTs were attacked at multiple sites on surface and the side walls was removed layer by layer, demonstrated by the phenomenon that the diameter shifted to lower range after degradation.<sup>71</sup>

### <u>TOXICITY</u>

A number of research efforts have been directed to investigate the interactions between NPs and various ecological receptors,<sup>75-78</sup> but studies relating to higher plants have been rather limited, particularly those on food crops. Most studies in this area are still limited to report observations and sometimes different or conflict conclusions have been drawn from different studies. In this section the phyto- and cyto- toxicity and bioaccumulation of nano-TiO<sub>2</sub> to vascular plants are summarized, and possible influencing factors are discussed.

# Phyto- and Cyto-toxicity

The phyto- and cyto-toxicity parameters that have been most commonly used in the study so far include germination rate and root elongation, activity of antitoxin or antioxidant enzymes, and photosynthesis parameters such as chlorophyll content and photochemical reactions. The studies on the effect of nano-TiO<sub>2</sub> on plant have grown rapidly in recent years, but yielded inconsistent results varying from negative, neutral to beneficial effects. Toxic findings include that *Zea mays L*. and *Vicia narbonensis L* after short-term exposure of 0.2‰ to 4.0‰ nano-TiO<sub>2</sub> solution showed delayed germination progression for the first 24 h, while root elongation was affected only after treatment with the higher nano-TiO<sub>2</sub> concentration.<sup>79</sup> Other exposure pathway such as foliar application also lead to toxic effect. For example, leaf spray of 0.1%, 0.2% and 0.4% nano- TiO<sub>2</sub> (anatase) solutions lowered the net photosynthetic rate of *Ulmus elongata*, comparing with the control without spraying.<sup>80</sup> Jacob et al. (2013) observed that exposure to nano-TiO<sub>2</sub> of 0, 6, 18 mmol Ti L<sup>-1</sup> did not affect biomass production of *Triticum aestivum* (wheat) and *Phaseolus vulgaris* (bean), but significantly increased root Ti sorption and uptake.<sup>81</sup> Neutral or beneficial effects appeared to have been reported more frequently. Nano-TiO<sub>2</sub> (rutile, 0.25%) was reported to promote photosynthesis of spinach, possibly by activation of photochemical reaction of chloroplasts, demonstrated by that the reduction rate of ferricyanide (FeCy) and the rate of oxygen evolution of chloroplasts was accelerated, the chloroplast coupling was improved, etc..<sup>82</sup> Similar promotion effect to naturally aged spinach seeds was also observed using lower dosage of rutile nano- TiO<sub>2</sub> at 0.25-4‰: increased germination rate and vigor indexes as well as elevated plant dry weight, the chlorophyll formation, the ribulosebisphosphate carboxylase/oxygenase activity, and the photosynthetic rate during the growth stage.<sup>83</sup> The optimal concentration in the tested range was 2.5‰.<sup>83</sup> Anatase-treated spinach also showed greatly increased level of the electron transfer, oxygen evolution, and photophosphorylation of chloroplast (Chl) under visible light and ultraviolet light illumination.<sup>84</sup> It seems that the effect of nano-TiO<sub>2</sub> regardless of being negative, neutral or positive, tend to be at least partially attributed to their nanoscale size. The results indicating the effect of nano-TiO<sub>2</sub> to spinach growth mentioned above was not significant with bulk TiO<sub>2</sub>.<sup>79, 82, 83</sup> Another study comparing the cytotoxic and genotoxic potential of nano-TiO<sub>2</sub> and bulk TiO<sub>2</sub> in human lymphocytes revealed that nano-TiO<sub>2</sub> were genotoxic at a low dose of 0.25 mmol L<sup>-1</sup> followed by a decrease in extent of DNA damage at higher concentrations; while bulk TiO<sub>2</sub> reveal a genotoxic effect only at dose 1.25 mmol  $L^{-1}$  and above.<sup>85</sup>

The variable results that have been observed suggested that the impact of nano-TiO<sub>2</sub> on plants might be dependent on multiple factors including plant species, nano-TiO<sub>2</sub> concentration and properties, as well as environmental conditions. These factors or the mechanisms governing the effect of nano-TiO<sub>2</sub> require further study.

One possible factor is the concentration of nano-TiO<sub>2</sub>. Song et al. reported that nano-TiO<sub>2</sub> stimulated the growth of the duckweed, *Lemna minor* at low concentrations (10-500 mg/L) under hydroponic condition, but inhibited plant growth at high concentrations (2000 mg/L).<sup>86</sup> The antioxidant enzymes (peroxidase [POD], catalase [CAT], and superoxide dismutase [SOD] activities) are possibly the defense mechanism of the nano-TiO<sub>2</sub> toxicity. Their activity increased when nano-TiO<sub>2</sub> concentration was lower than 200 mg/L to eliminate accumulated reactive oxygen species in plant cells.<sup>86</sup> However this defense mechanism has a limit. When the nano-TiO<sub>2</sub> concentration was higher than 200 mg/L, the SOD activity decreased and the plant cell membrane encountered serious damage from 500 mg/L nano-TiO<sub>2</sub> in the culture medium.<sup>86</sup> Another study reported that the phytotoxicity of 15-nm anatase nano-TiO<sub>2</sub> assayed using seeds of *Linum usitatissimum* seeds was also different according to the concentration. After 72 h of exposure, the reduction of biomass production at 100 mg L<sup>-1</sup> is much less than that of 0.01 mg L<sup>-1</sup>.<sup>80</sup>

Other important factors that can influence NP toxicity to plants may include the properties of NPs, e.g., size and crystal structure. The EC<sub>50</sub> of nano-TiO<sub>2</sub> to *Daphnia magna* increased from  $1.3 \pm 0.21$  to  $3.44 \pm 0.67$  mg L<sup>-1</sup> when the size increased from 15 nm to 32 nm.<sup>80</sup> Yin et al. have also observed growth inhibition and cell damage of a common grass, *Lolium multiflorum*, by exposure to AgNPs in a hydroponic culture, and the toxicity effects were negatively correlated to sizes.<sup>87</sup> Another possible factor is crystal phase. As mentioned earlier, both rutile and anatase

nano-TiO<sub>2</sub> can promote photosynthesis and improve growth of spinach.<sup>82-84</sup> However these two crystalline form showed different toxicity effects on *Linum usitatissimum*: because of lipophilicity, the rutile crystalline structure of nano-TiO<sub>2</sub> form larger aggregates in aqueous medium; thus they are less toxic to biological organisms than the anatase  $TiO_2$ .<sup>88</sup>

Based on the results mentioned above, it also can be concluded that different plant species react differently to nano- $TiO_2$  exposure. This suggested that some species are either more resistant or susceptive to NPs exposure.

### **Genotoxicity**

Potential genetic toxicity of NPs on plants represents a great risk and has recently attracted research interests. The genotoxicity of nano-TiO<sub>2</sub> to various plants has been observed, including *Allium cepa, Zea mays*, and *Vicia narbonensis*.<sup>79, 85</sup> Genotoxicity indicators used in these studeis include mitotic index, comet assay, DNA laddering, chromosomal abberation, micronucleus, and random amplified polymorphic DNA analysis.<sup>89</sup> Nano-TiO<sub>2</sub> is a photocatalyst and can thus cause oxidative stress to plants; therefore they may induce damage to DNA bases by both attacking on isolated purines and pyrimidines in the nucleotide pool and on intact duplex DNA.<sup>90</sup> Ghosh et al. (2010) detected increased level of malondialdehyde (MDA) concentration in *Allium cepa* at 4 mmol L<sup>-1</sup> treatment dose of nano-TiO<sub>2</sub>, indicating that lipid peroxidation usually caused by elevated oxidative stress could be involved as one of the mechanism leading to DNA damage.<sup>85</sup> Uptake and translocation

Possible uptake and translocation of NPs in plants, especially to the edible tissues, are of great concern due to the consequences of trophic transfer and exposure to human. There have been a few studies examining the uptake of NPs in plants using species including *Triticum aestivum* (wheat), *Phaseolus vulgaris* (bean), *Rumex crispus* (a wetland species), *Elodea* 

canadensis (Canadian waterweed, a floating aquatic plant), Cucurbita pepo (pumpkin),

*Cucurbita maxima* (squash), and *Oryza sativa* (rice).<sup>75, 81, 89</sup> Here the results of nano-TiO<sub>2</sub> are summarized. One study showed that the plant *Arabidopsis* can uptake anatase nano-TiO<sub>2</sub> with surface modification using Alizarin red S and sucrose.<sup>91</sup> Larue et al. (2012) also showed that wheat and rapeseed plantlets accumulated Ti when exposed to 14 nm or 25 nm anatase nano-TiO<sub>2</sub> in hydroponic conditions, either through root or leaf exposure.<sup>92</sup> In field condition, the nano-TiO<sub>2</sub> nanoparticles were retained in the soil for long periods and primarily adhered to cell walls of wheat and reduced the wheat biomass.<sup>93</sup>

Nano-TiO<sub>2</sub> accumulation and translocation by plant depend on their agglomeration state,<sup>92</sup> diameter and crystal phase.<sup>94</sup> Larue et al. (2012) reported that nano-TiO<sub>2</sub> accumulate in roots and distribute through whole plant tissues without dissolution or crystal phase modification. The threshold diameters for root uptake and shoot translocation above which NPs are no longer accumulated in these plant tissues were suggested to be 140 nm and 36 nm for root and shoot, respectively.<sup>94</sup> However, these studies were almost exclusively performed under hydroponic conditions. The bioaccumulation of nano-TiO<sub>2</sub> in soil media, especially the influencing factors and mechanisms require further study.

### ENVIRONMENTAL IMPLICATIONS AND FURTHER STUDIES

Despite increasing research effort on investigating the environmental behavior and impact of NPs, knowledge gaps still widely exist. For example, most studies on NP aggregation focused on homoaggregation, i.e., aggregation among NPs, whereas in natural aquatic systems heteroaggregation between CNPs and natural occurring colloids is more likely to dominate since natural colloids would largely exceed the amount of NPs.<sup>44</sup> The collision rate for perikinetic aggregation and differential settling is lower when particles are of the same size; hence a

monodispersed dispersion tends to be more stable than polydispersed dispersions.<sup>1</sup> This implies that in natural water where solid phases such as colloids or microbes are present, NP aggregation tendency may be stronger, thus increasing the possibility of NPs residing in the solid phase (soil/sediment). The interaction between these solid phase and NPs is of great importance but yet largely unknown.

Aggregation and sorption of NPs is governed by the interaction of a number of factors, including NP surface chemistry, aquatic conditions such as ionic strength, pH, NOM concentration, and solid phase properties. Similarly, NPs toxicity also depends on a series of factors including receptor species, NPs properties and concentrations. These factors not only affect NPs, but also enhance or compensate the effects of other factors, making the evaluation of NPs environmental behaviors and toxicity complicated. For instance, ionic strengths typical to many natural waters tend to favor deposition and thus reduce the potential exposure of NPs. Without NOM being present in water, the stable suspension of NPs can be easily eliminated by divalent cations at low concentrations. However, NOM was found to counteract the effect of cations and dramatically stabilize NPs at environmentally relevant concentrations (e.g., 5 mg/L). The stabilization effect of NOM is of paramount significance with regard to the potential mobility and exposure of NPs in natural aquatic systems, for example, enabling NPs to be transported through a longer distance and spread in a wider range. There are, however, beneficial outcomes of NOM stabilized NPs. Studies showed that nC<sub>60</sub> stabilized by dissolved humic substances lost the toxicity typically associated with  $nC_{60}$ .<sup>50</sup> The presence of NOM thus could have strong impacts on both the mobility and toxicity of NPs, the two factors determining the potential environmental risks.

28

The transformation occurring to CNTs in natural environment is special due to the nature of carbon. In general, the oxidation processes tend to reduce CNT sizes and add hydrophilic groups. Such changes can lead to greater CNT mobility and perhaps greater bioavailability and toxicity as well. Templeton et al. found smaller, more mobile fractions of SWCNTs were more toxic towards an estuarine copepod than the larger fractions.<sup>95</sup> Lovern and Klaper found a similar inverse relationship between the aggregate particle size and the oxicity of fullerenes to *Daphnia magna*.<sup>96</sup> It is thus important when assessing the long-term environmental risks of NPs to take into account potential transformation of NPs in the environmental systems.

Based on a thorough review of previous studies, we designed our experiment to provide information filling the knowledge gap in the interactions between NPs and the environment. Our study provides understanding at a mechanistic level, thus some of the conclusions drawn from MWCNTs and/or nano-TiO<sub>2</sub> in this study may also help interpreting the environmental behaviors of other NPs.

### FIGURES AND TABLES



FIGURE 2.1 Size domains and typical representatives of natural colloids and nanoparticles. The vertical line represents the operationally defined cut-off given by filtration at 0.45  $\mu$ m.<sup>97</sup>



FIGURE 2.2 The three collision mechanisms and associated rate coefficients for the aggregation of 1  $\mu$ m particles with particles of diameter d<sub>p</sub>; temperature is 12 °C, with particle density of 2.6 g/ml and shear rate of 35/s. The cartoons represent the processes of perikinetic, orthokinetic, or differential settling, respectively. Dotted arrow indicates the graph relating to each cartoon (process).<sup>1</sup>



FIGURE 2.3 (a) Attachment efficiencies of fullerene nanoparticles as a function of NaCl concentration at pH 5.2. The critical coagulation concentration (CCC) based on these data is 120 mM NaCl. (b) Attachment efficiencies of fullerene nanoparticles as a function of CaCl<sub>2</sub> concentration at pH 5.2. The CCC based on these data is 4.8 mM CaCl<sub>2</sub>. The lines (used as eye guides) are extrapolated from the reaction-limited and diffusion limited regimes, and their intersections yield the respective CCC.<sup>27</sup>



FIGURE 2.4 Double logarithmic plot of the critical coagulation concentrations (CCC) against the cation valance. The solid line has a slope of -6.<sup>25</sup>



FIGURE 2.5 Pyramidalization angle.<sup>20</sup>



FIGURE 2.6 Products identified by LC-MS in the HRP degradation, including oxidized PAHs such as benzaldehyde (1), 1,2-benzenediol (2), cinnamaldehyde (3), and diphenylacetic acid (4).<sup>70</sup>

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# CHAPTER III

# PHASE DISTRIBUTION OF 14C-LABELED MULTI-WALLED CARBON NANOTUBES IN AQUEOUS SYSTEMS CONTAINING MODEL SOLIDS: PEAT<sup>1</sup>

<sup>&</sup>lt;sup>1</sup> Zhang, L.; Petersen, E.J. and Q. Huang. 2011. *Environmental Science and Technology*. 45 (4): 1356-1362. Reprinted here with permission of the publisher.

### **ABSTRACT**

Understanding the phase distribution of nanoparticles between soils or sediments and water is a critical factor in determining their fate in environmental systems. As such, we examined the interactions of <sup>14</sup>C-labeled multi-walled carbon nanotubes (MWCNTs) in aqueous systems with peat as a model solid organic material. The MWCNTs that had been treated with strong oxidative acids possessed negative charges on their surfaces and were relatively stable in de-ionized water. In the absence of peat, MWCNTs aggregated when the concentration of sodium cations was above 4.0 mM or at a solution pH of 4.0. Dissolved organic matter (DOM) from peat effectively stabilized MWCNTs in solution by making the suspended nanotubes less sensitive to changes in sodium concentrations or solution pH. Direct sorption interactions between MWCNTs and solid peat were not observed in the absence of sodium cations, whereas became apparent when the ionic strength was sufficiently high to reduce charge repulsion between peat and MWCNTs.

### **INTRODUCTION**

Carbon nanotubes (CNTs) are long (often µm scale), thin (nm scale), hollow cylinders comprising single or multiple coaxial tube(s) of hexagonal graphitic sheet(s). Those composed of a single cylinder are known as single-walled carbon nanotubes (SWNT), while multiple-layered nanotubes are called multi-walled carbon nanotubes (MWCNTs). CNTs possess exceptional physicochemical, optical, and mechanical properties,<sup>1, 2</sup> which enable numerous applications such as reinforced composites, conductive materials, sensors, drug delivery vessels, hydrogen storage media, and sorbents.<sup>1, 3</sup> The total mass of CNTs synthesized worldwide during the 2007/2008 year was estimated to be 350 tons,<sup>4</sup> and the global market for CNTs is expected to grow exponentially at an annual rate of 73.8%,<sup>5</sup> indicating substantial CNT production and rapid

predicted growth. This gives rise to concerns about the potential environmental impacts of CNTs after incidental release during manufacturing, transportation of raw CNTs, or via product use and disposal.

One important component of understanding the risks CNTs may pose in ecosystems is the extent to which they are accumulated by organisms. Earlier studies have shown that organisms in the water column accumulate substantially higher masses of CNTs compared to those in soils or sediments.<sup>6-12</sup> For example, bioaccumulation of CNTs by terrestrial and benthic organisms was minimal in spiked soils or sediments,<sup>6-10</sup> while *Daphnia magna* had nanotube body burdens of up to 6.3% on a dry mass basis after being exposed to MWCNTs suspended in water.<sup>11, 12</sup> As such, information on the phase distribution of CNTs between water and sediment is essential to assessing their environmental risks in natural aquatic systems, but the interactions between nanotubes and soils or sediments are currently not well understood.

Aggregation appears to be an important process governing the phase distribution of CNTs in aqueous systems. It is generally accepted that pristine CNTs have a strong tendency to aggregate as a result of attractive van der Waals forces acting across their large surface areas. However, in practice, defects typically occur during CNT synthesis and subsequent purification processes, which usually impart the CNTs with hydroxyl or carboxyl surface functional groups.<sup>13, 14</sup> In addition, it is often necessary to modify CNT surfaces to increase aqueous solubility and biocompatibility for various applications.<sup>15, 16</sup> While the introduction of hydrophilic surface functional groups are highly sensitive to solution ionic strength. Cations present in the solution facilitate CNT aggregation, seemingly via compression of the electric double layers surrounding CNTs.<sup>17, 18</sup> In contrast, dissolved organic matter (DOM) can effectively stabilize CNTs in solution, likely

through surface adsorption of amphiphilic DOM molecules.<sup>19, 20</sup> When DOM and cations are both present, such as in natural aquatic systems, the impact of different concentrations of DOM and cations on CNT aggregation is unclear.

Potential sorption of CNTs on sediments is another process expected to govern the phase distribution of CNTs in natural aquatic systems. One indirect mechanism through which sediments may influence surfactant-stabilized MWCNTs is through competitively absorbing those surfactants as was previously found with clay particles.<sup>21</sup> However, there are no studies yet to our knowledge on direct interactions between CNTs and soil or sediment particles. Interactions between CNTs and sediments may be highly complex, because, in addition to direct sorption to the solid matter itself, sediments may also release cations or DOM, which may themselves affect CNT phase distributions.

The lack of previous studies on CNT sorption to soils and sediments is likely due, in large part, to the challenges related to quantification of CNTs in samples containing solid materials. In the present study, we used carbon-14 labeled CNTs to overcome this difficulty, and investigated the phase distribution of MWCNTs in aquatic systems containing peat as a model soil component. Our objectives were to quantify and compare the influences of the solid and dissolved components of peat on the phase distribution of MWCNTs under various conditions (i.e., ionic strength and pH) and to probe the interactions between MWCNTs and solid peat. This paper marks the first study to our knowledge of the phase distribution of CNTs between water and soil or sediment materials.

### MATERIALS AND METHODS

# MWCNT synthesis and suspension preparation

Carbon-14 labeled carbon nanotubes were synthesized using a modified chemical vapor deposition technique,<sup>7</sup> purified, and treated with a 3:1 mixture of sulfuric to nitric acid<sup>6, 22</sup> as described in Supporting Information. These surface-modified MWCNTs have a specific radioactivity of 0.1 mCi/g as determined by liquid scintillation counting after biological oxidation (OX 500; R. J. Harvey Instrument Co. Tappan, NY). The surface area of the acid-treated MWCNTs is 111 m<sup>2</sup>/g as measured by the standard Brunauer–Emmett–Teller (BET) method using nitrogen adsorption at 77 K (Micromeritics Gemini 2375, Norcross, GA), which accords with a previous result of 118 m<sup>2</sup>/g for purified but not acid-treated MWCNTs

To prepare a stable stock solution, 100 mg of MWCNTs was dispersed in 1 L of deionized water by ultrasonication (200 Watt, Cole-Parmer CV33) for 6 hr, and then left at room temperature for 6 hr. The stable supernatant with a concentration of 74 mg/L was collected and kept at room temperature as a stock solution for all subsequent experiments. Before experiments, a sample of the stock solution was centrifuged at 3500 g for 5 min, and the supernatant with a concentration of 67 mg/L was collected and diluted to predetermined concentrations. To assess the extent to which the ultrasonication process damaged the MWCNTs, the initial MWCNT powder and the stock solution of suspended MWCNTs were examined using thermal gravimetric analysis (TGA) (Pyris 1 TGA, Perkin Elmer) and x-ray photoelectron spectroscopy (XPS) (Kratos Analytical Axis Ultra X-ray photoelectron spectrometer).

# Model solid phase: Canadian peat

Peat, an organic component frequently present in soil and sediments, was used as a model organic material in this study. Peat is usually composed of recently deposited plant organic materials, and is thus a diagenetically young organic material.<sup>23</sup> Canadian peat (Quebec, Canada) that had been sieved to obtain a particle size fraction of less than 2 mm was used in our experiments. The total organic carbon fraction of this material was previously determined to be 47.5%.<sup>23</sup> Its specific surface area is 1.8 m<sup>2</sup>/g as determined by the BET method described above. MWCNT and peat characterization

Microscopic investigations of MWCNTs and peat were performed with a FEI Inspect F50 FEG scanning electron microscope (SEM) operating at an accelerating voltage of 10.00 kV. Zeta ( $\zeta$ ) potentials of MWCNTs were measured on a Malvern Zetasizer Nano ZS instrument. Point of zero charge (PZC) of the MWCNT stock solution was determined using ten points from pH 1.00 to pH 5.83. Concentrations of metal ions released by peat were determined by an inductively coupled plasma-mass spectrometry (ICP-MS) (Elan 9000, Perkin-Elmer, MA). MWCNT phase distribution

Experiments were conducted to examine the distribution of MWCNTs between solid and aqueous phases in 150-mL flasks as batch reactors. MWCNT concentrations were quantified by radioactivity using a Beckman LS 5801 liquid scintillation counter (Brea, CA) after mixing a 3-ml sample with 3 ml of scintillation cocktail (Insta-Gel Plus, PerkinElmer, MA). The detection limit for the radioactivity quantification method was 0.04 mg/L; this value was determined by mixing 3 mL of de-ionized water with 3 mL of scintillation cocktail (n=6), measuring their radioactivity, and defining the detection limit as three times the standard deviation of these measurements.

Peat can be divided into two components: one that readily dissolves in water and one that remains as a solid phase, both of which may have different effects on the phase distribution of MWCNTs. To separate the effects of these two components, three types of treatments were examined. In the blank (control) treatment, the solution was prepared using de-ionized water without peat; in the peat treatment, the solution was prepared using de-ionized water with 50 mg peat added to the reactor; and in the DOM treatment, the solution contained DOM extracted from 50 mg of peat but not the solid peat itself (see Supporting Information for additional details about DOM solution preparation). This DOM treatment was designed to imitate the effect of DOM released by peat in the reactor. Thus, differences between DOM and blank treatments can be attributed to effects from DOM, while differences between peat and DOM treatments stem from the presence of solid peat. For each system, two sets of varying solution conditions were examined. One set involved three different Na<sup>+</sup> concentrations (0, 4 and 40 mM) that were prepared by the addition of NaCl. The other set was prepared at three different pHs (4.0, 6.0 and 8.0). The pH 4.0 solution was prepared with a 1 mM sodium acetate buffer, and the pH 6.0 and 8.0 solutions with a 1 mM sodium phosphate buffer. The actual pH values for various conditions are listed in Table S3.1a. To maintain a constant ionic strength at different pH values, an appropriate mass of NaCl was added to each buffer solution to adjust the final Na<sup>+</sup> concentration to 4 mM. For each condition tested, reactors containing 10 mL of solution were prepared with ten different initial concentrations of MWCNTs from 0.15 to 67 mg/L (See Table S3.1b for exact concentrations).

Experiments were performed at least twice to ensure that the patterns observed were reproducible. The reactors were hand shaken for mixing when all components were added, and then left without shaking in the dark at room temperature for 7 days. We selected this period

based on our preliminary time-course tests which showed that most of the change for the solid phase distribution pattern occurred within the first 7 days thus indicating that a pseudo-steady state had been reached (Figure S3.1). After 7 days, 5 mL of the supernatant in each reactor was transferred to a centrifuge tube and centrifuged at 3500 g for 3 min to ensure the separation of solid and liquid phases. Preliminary tests showed that sedimentation for 6 hr was not sufficient to achieve good separation. A 3-mL liquid phase sample was taken for radioactivity measurements as described above. The total mass of MWCNTs that had distributed into solid phase in each treatment,  $M_s$  (mg), was then obtained by

$$M_{\rm s} = (C_0 - C_{aq}) \times V \tag{1}$$

where  $C_0$  (mg/L) and  $C_{aq}$  (mg/L) are the initial and final concentrations of MWCNTs, respectively, and V is the sample volume (10 mL). While  $M_s$  resulted primarily from aggregative settling in the blank and DOM treatments, both sorption and aggregation may contribute to the  $M_s$  in a system containing solid peat.

We also calculated an apparent solid phase MWCNT concentration  $q_a$  (mg/g peat) for systems containing solid peat (peat treatment),

$$q_a = \frac{M_s^P - M_s^D}{D} \tag{2}$$

where  $M_s^{p}$  and  $M_s^{D}$  are the total mass of MWCNTs in solid phase in peat treatment and DOM treatment, respectively, and D is the peat dosage of 0.050 g. The apparent solid phase concentration thus obtained approximates the solid phase partitioning of MWCNTs directly attributable to the presence of solid peat by excluding the mass of settled MWCNTs. For certain discussions, we also estimated the total solid phase MWCNT concentration,  $q_t$  (mg/g peat), by equation 3, in which the contribution of MWCNT settling was not excluded.

$$q_t = \frac{M_s^P}{D} \tag{3}$$

# **RESULTS AND DISCUSSION**

We experimentally examined the phase distribution of MWCNTs in three types of systems, under a series of same pH and ionic conditions, including 1) systems without organic matter present; 2) systems containing DOM extracted from peat but not the peat particles themselves, and 3) systems with peat particles present. Our goal was to, through the comparison of systems 1 and 2, identify the effect of DOM on MWCNT aggregation, and to probe the direct interaction between solid peat and MWCNTs by comparing systems 2 and 3. We present below the results of MWCNT characterization followed by data collected respectively for each of the three systems, in sequence, along with quantitative comparison and analysis.

### **MWCNT** Characterization

MWCNTs were thoroughly characterized and the effects of sonication were investigated. Given that amorphous carbon impurities generally burn at lower temperatures than carbon nanotubes, peaks of the derivative of the mass change with respect to temperature at lower temperatures than the principal MWCNT peak represent the oxidation of carbon impurities in TGA analyses. As shown in the TGA graphs in Figure S3.2, the amount of metal catalyst remaining in the nanotube samples was nearly identical before and after sonication, and an amorphous carbon peak was not observed. This indicates that sonication did not produce a detectable increase in the fraction of amorphous carbon, a result which corresponds to the high purity of the MWCNT samples as determined by scanning electron microscopy (SEM) (Figure S3.1 part a, Figure S3.3). XPS high resolution C (1s) spectra for the powder and the sonicated MWCNTs suggests that the total fraction of carbon atoms associated with oxygenated functional
groups increased slightly from roughly 7.4 to 8.6% after sonication, and that the total oxygen percentage increased from 7.5 to 8.6%. The MWCNT length distribution is provided in Figure 3.1 part b, and the pH resulting in the point-of-zero charge of MWCNTs in Figure 3.1 part c. Effect of cation concentrations on MWCNT settling

MWCNT settling in solutions prepared by de-ionized water in the absence of peat is shown in Figure 3.2 part a. In the absence of sodium cations,  $22.1\pm8.6\%$  (n=20; uncertainties always represent standard deviations) of MWCNTs settled after the 7-day experiments and centrifugation, indicating that the acid-treated MWCNTs were relatively stable in aqueous phase. If the sodium concentration is increased to 4 mM, an environmentally relevant cation concentration in fresh water, the fractions of deposited MWCNTs ranged from 60.4 to 90.6% (71.2±9.9%, n=20) for the tests with different initial MWCNT dosages, whereas with 40 mM sodium, MWCNTs were almost entirely eliminated from the aqueous phase for all cases. This trend is consistent with earlier observations that cations such as sodium, potassium, calcium, and magnesium can induce MWCNT aggregation by reducing the MWCNT surface electrostatic potential, indicated by zeta potential.<sup>2, 17, 18</sup> Aggregated MWCNTs are larger and thus are more easily removed by settling or centrifugation. Our measurements indicated the zeta potentials of MWCNTs were -29.7  $\pm 0.9$  (mV), -20.3  $\pm 3.1$  (mV), and -12.0  $\pm 0.8$  (mV) under sodium concentrations of 0, 4, and 40 mM, respectively. Figure 3.1c shows the point of zero charge value for the MWCNTs is near pH 1.57 given that the zeta-potential at this pH value was -0.068 ±0.83 mV.

## Effects of peat DOM on MWCNT settling

The presence of DOM dramatically decreased MWCNT settling at sodium concentrations of 4 and 40 mM as shown in Figure 3.2 part a, a result consistent with past research on DOM interactions with CNTs.<sup>24</sup> The TOC value of DOM solution released for the 0, 4 and 40 mM sodium experiments were 73, 70, and 66 µg/mL, respectively. The enhanced MWCNT stabilization in DOM solutions occurred despite the fact that the DOM solutions also contained additional cations that peat released (see Table S3.2). These findings support previous research indicating that DOM can stabilize MWCNTs in a manner similar to surfactants.<sup>24</sup> Molecular DOM are adsorbed by their aromatic rings or aliphatic chains binding to MWCNTs' surface carbon rings via pi-pi or CH-pi interactions, while their hydrophilic moieties are exposed to water.<sup>25, 26</sup> TEM images have indicated adsorption of Suwanee River NOM by CNTs.<sup>19, 20</sup> We also conduct an experiment to verify the sorption of DOM on MWCNTs by TOC analysis (Supporting information). DOM sorption by MWCNTs was 0.26  $\pm$  0.13, 0.41  $\pm$  0.12, and 0.49  $\pm$ 0.13 mg C DOM/ mg MWCNT (n = 4) when there was 0, 4 or 40 mM sodium added, respectively. DOM adsorption changed MWCNT surfaces from being dominated by a hydrophobic aromatic-like structure to possessing an extended surface of hydrophilic organic functional groups in DOM. The sorbed DOM thus helped inhibit aggregation between the CNTs through steric hindrance as well as electrostatic repulsion. Increasing cation concentrations are expected to significantly reduce electrostatic repulsion by double layer compression. However, more NOM was adsorbed by MWCNTs with increasing ionic strength, a result that accords with previous studies.<sup>19, 20</sup> As such, the net result of higher ionic strengths in the presence of DOM may be that the steric hindrance from NOM still maintains a significant effect although electrostatic repulsion is impaired by cations. Therefore, these DOM-MWCNT composites are much less susceptible to the effects of higher cation concentrations as shown in our data (Figure 3.2 part a).

## Effects of solid peat

Sorption of MWCNTs to solid peat was also investigated (Figure 3.2 part b). The apparent solid phase MWCNT concentration (defined in equation 2) was calculated with MWCNT settling subtracted, thus approximating the direct effect from the solid peat on the MWCNTs. Solid peat did not appear to adsorb MWCNTs in the absence of sodium ions, indicating that the DOM-MWCNT composites exhibit a greater affinity towards water than the solid phase. The pH for this un-buffered peat-water system was 5.17 indicating the acidic nature of peat. The solid peat and DOM were thus likely charged negatively as a result of deprotonation. Electrostatic repulsion between the DOM-MWCNT composites and peat surfaces, in combination with the hydrophilic nature of the functional groups extending from the DOM wrapped on the nanotubes, may have led to the greater affinity of DOM-MWCNT composites with water than with peat.

However, solid peat sorbed MWCNTs when sodium cations were added as shown in Figure 3.2 part b, and this effect became more pronounced with increasing sodium concentrations. This result indicates that the presence of sodium cations increased the relative affinity between solid peat and DOM-MWCNT composites. Cations such as sodium can decrease the charge potential of solid peat surfaces as well as of DOM-MWCNT composites, a change expected to facilitate interactions between the solid peat and DOM-MWCNTs through van de Waals attractions and/or hydrophobic interactions.

## Phase distribution modeling

While the above discussion focuses on the direct interaction between MWCNTs and solid peat by examining the apparent solid phase concentration  $q_a$ , we have also calculated the total solid phase MWCNT concentrations,  $q_t$ , using equation 3 to investigate the overall solid phase distribution of MWCNTs effected by both settling and peat sorption (Figure 3.2 part c). In an attempt to quantify the relationship between  $q_t$  and the MWCNT concentration remaining in aqueous phase ( $C_{aq}$ ), we fit the data to the equation  $q_t = KC_{aq}^n$ . Simulation parameters thus obtained (K, n) were used to compare the phase distribution behaviors among systems having different sodium concentrations. The simulation parameter n reflects to what extent the solid phase MWCNT concentration is linearly correlated to its aqueous phase concentration, while the parameter K denotes the tendency of MWCNTs to enter the solid phase. This modeling is not intended to indicate that these are equilibrium constants, because equilibrium was not reached under our experimental condition. Instead, these parameters were used to allow for comparison of sorption behaviors under various conditions after seven days of incubation, a period in which the sorption approached to pseudo-steady state (Figure S3.1).

Table 3.1 shows the simulation parameters, *K* and *n*, obtained by data fitting, along with their 95% confidence intervals and coefficients of determination,  $R^2$ . The data were fitted quite well to the equation as indicated by high  $R^2$  values. The higher sodium concentrations increased not only the amount of solid phase MWCNTs as evidenced by increasing *K*, but also the linearity of the phase distribution equation as shown by *n* becoming closer to 1. A linear relationship indicates the overall forces that drive the solid phase distribution of MWCNTs, i.e., aggregation and peat sorption, are uniform with varying MWCNT concentrations.<sup>27</sup> This can result from the electric screening effect at higher ionic strengths that effectively reduces the heterogeneity in charge potential distribution on the surfaces of both MWCNTs and peat particles. When the repulsive electrostatic forces are reduced, van der Waals attraction and/or hydrophobic interactions become dominant and thus cause greater MWCNT solid phase distribution. We also similarly analyzed the apparent solid phase concentration, *q<sub>a</sub>* (Figure 3.2 part b), by fitting the

data to  $q_a = KC_{aq}^n$ , and the same trend was obtained that higher sodium concentration leads to greater linearity of the equation (Table S3.3).

## Effects of pH

MWCNTs are negatively charged across the solution pH ranging from 4.0 to 8.0. At a sodium concentration of 4 mM, the zeta-potential did not change substantially with -11.9  $\pm$  1.3, - 18.0  $\pm$  1.4, and -19.3  $\pm$  0.2 mV at pH values of 4.0, 6.0, and 8.0, respectively. These values accord with those previously measured for acid-treated MWCNTs by this same synthesis method in de-ionized water at pH values of 3, 7, and 11.<sup>6</sup>

The effect of pH on CNTs aggregation has been previously investigated, and it is clear that decreasing pH from 10 to 3 will enhance aggregation.<sup>17, 18</sup> Our data for the systems without DOM present showed that a change in the pH from 8.0 to 6.0 did not substantially impact the final aqueous concentration of MWCNTs, but more settling occurred at a pH of 4.0 (see Figure 3.3 part a).

In the presence of DOM, increasing the pH from 4 to 8 did not significantly influence the phase distribution of MWCNTs (Figure 3.3 part a). This occurred despite an increase in TOC from the released peat DOM with increasing pH: 80.1, 86.5, and 95.4 µg/mL at pHs of 4, 6, and 8, respectively. We also conducted additional experiments in which the pH was adjusted after extracting the DOM by water to yield a consistent DOM concentration in solutions across the pH range, and there was no significant difference in the phase distribution observed, as shown in Figure S3.4. When experiments were conducted in the presence of solid peat, a difference was still not evident across this pH range (Figure 3.3 part b). These results suggest that the surface charge of solid peat and DOM-MWCNT composites does not change significantly within the pH range investigated here.

## ENVIRONMENTAL IMPLICATIONS

The phase distribution behaviors observed in this study reveal the complex interactions between MWCNTs and peat across a range of environmentally relevant aqueous phase conditions. The dissolved fraction of peat can assist MWCNT dispersion by preventing their aggregation even with high sodium concentration (40 mM), a concentration beyond those relevant to fresh water systems. Therefore, the presence of DOM in natural water is expected to increase the duration that MWCNTs remain in suspension, consequently increasing their exposure risk for human and ecological receptors in the water column. Solid peat can have the opposite effect by sorbing MWCNTs under environmentally relevant ionic strength conditions. Although the impact of ionic strength on MWCNT aggregation is significantly reduced by DOM, sorption is enhanced by increasing ionic strength. These results suggest that solid soil organic matter has the potential to sorb MWCNTs during their contact with soil and sediment particles.

The overall effect of increasing ionic strength is that more MWCNTs will transfer out of the aqueous phase. The environmental consequence of this result is that MWCNTs are unlikely to transport long distances in seawater or hard freshwater. Changes in pH are unlikely to have a major effect on MWCNT suspensions, especially considering the relatively steady pH in most natural water systems.

Understanding the phase distribution of MWCNTs in a peat-water system provides useful information towards elucidating the environment fate of MWCNTs in real soil- or sediment-water ecosystems. This work also helps identify important factors in conducting such sorption experiments and provides a method for future investigations. Additional work is needed to investigate sorption of CNTs to other components of soils and sediments and thus eventually

yield a comprehensive understanding of the behaviors and fate of CNTs in the natural environment.

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# SUPPORTING INFORMATION AVAILABLE

Chemicals used in the experiments (I), detailed experimental methods for MWCNT synthesis, purification, and acid treatment procedures (II), DOM only solution preparation (III), and sorption of DOM on MWCNTs (IV), and additional figures and tables about MWCNT phase distribution at certain conditions and periods, TGA analysis, SEM images of the peat particles, solution conditions of certain experimental systems, and certain parameters obtained by data fitting (V) are available in the Supporting Information. This information is available free of charge via the Internet at http://pubs.acs.org.

## FIGURES AND TABLES



FIGURE 3.1 a) Scanning electron microscopy (SEM) images showing the morphology of suspended MWCNTs at 16kx magnification, b) Initial length distribution of MWCNT suspension measured by SEM (n = 797), and c) Zeta potentials of suspended MWCNTs after ultrasonication at pHs varying from 1.00 to 5.83. Error bars represent the standard deviation from two measurements made of the same sample at each pH value; each run consisted of 50 to 100 sub-runs. The pH was adjusted by HCl, and the cation concentration was kept constant at 0.1 mol/L by adding NaCl.



FIGURE 3.2 MWCNT phase distributions in different solutions with varying sodium concentrations (0, 4, 40 mM): a) MWCNT settling in DOM solutions (DOM, filled symbols) or water (Blank, open symbols), and b) the apparent solid phase concentration ( $q_a$ ) of MWCNTs in peat-containing solutions (Peat). The apparent solid phase concentration was calculated by equation 2 whereby the contribution of MWCNT settling was subtracted, and c) overall phase distribution of MWCNTs between peat and water modeled using the parameters from Table 3.1.



FIGURE 3.3 Phase distribution of MWCNTs across a range of pH values: a) Control treatments are denoted as "blank", and those with DOM extracted under various pHs denoted as "DOM"; b) Phase distribution of MWCNTs with peat under different pHs.

<b>TABLE 3.1 Parameters obtained by data fitting to the equation</b> $q_t = KC_{aq}^n$				
Sodium Concentration (mM)	K (mg/g peat) <sup>(1-n)</sup>	<i>n</i> dimensionless	$\mathbf{R}^2$	
0	0.207	0.395	0.729	
$N^b = 20$	$(0.196, 0.219)^a$	(0.369, 0.421)		
4	0.209	0.654	0.929	
N = 20	(0.198, 0.221)	(0.630, 0.678)		
40	0.393	0.981	0.929	
N = 10	(0.348, 0.982)	(0.914, 1.0493)		

<sup>*a*</sup> Values in parentheses are 95% confidence intervals

<sup>b</sup> Number of data points used in data fitting

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# CHAPTER IV

# INTERACTIONS OF 14C-LABELED MULTI-WALLED CARBON NANOTUBES WITH SOIL MINERALS IN WATER<sup>2</sup>

<sup>&</sup>lt;sup>2</sup> Zhang, L.; Petersen, E.J.; Zhang, W.; Chen, Y.; Cabrera, M. and Q. Huang. 2012. *Environmental Pollution*. 166: 75-81. Reprinted here with permission of the publisher.

## **ABSTRACT**

Carbon nanotubes are often modified to be stable in the aqueous phase by adding extensive hydrophilic surface functional groups. The stability of such CNTs in water with soil or sediment is one critical factor controlling their environmental fate. We conducted a series of experiments to quantitatively assess the association between water dispersed multi-walled carbon nanotubes (MWCNTs) and three soil minerals (kaolinite, smectite, or shale) in aqueous solution under different sodium concentrations. <sup>14</sup>C-labeling was used in these experiments to unambiguously quantify MWCNTs. The results showed that increasing ionic strength strongly promoted the removal of MWCNTs from aqueous phase. The removal tendency is inversely correlated with the soil minerals' surface potential and directly correlated with their hydrophobicity. This removal can be interpreted by the extended Derjaguin–Landau–Verwey– Overbeek (EDLVO) theory especially for kaolinite and smectite. Shale, which contains large and insoluble organic materials, sorbed MWCNTs the most strongly.

#### INTRODUCTION

Carbon nanotubes (CNTs) are one of the most popular classes of nanoparticles as a result of their exceptional properties. A CNT is a micrometer-scale graphene sheet rolled into a cylinder with a nanoscale diameter.<sup>1</sup> There are two main types of carbon nanotubes: singlewalled carbon nanotubes (SWCNTs) and multi-walled carbon nanotubes (MWCNTs), depending upon the number of graphene layers. CNTs show extraordinary mechanical, electrical, and thermal properties because of their C-C covalent bonding and seamless hexagonal network architecture, which make them widely researched for commercial applications.<sup>2</sup> Some potential CNT applications like drug delivery vessels, reinforced composites,<sup>3, 4</sup> biomedical applications,<sup>5, 6</sup> and environmental applications<sup>1</sup> require a stable and homogeneous dispersion of CNTs in polar mediums such as water or polymeric resins.<sup>7</sup> For these applications, CNTs are often shortened and functionalized with carboxyl, hydroxyl and/or carbonyl surface functional groups to make them more hydrophilic and stable in water,<sup>8</sup> which is supposed to increase their mobility in the environment and influences their potential environmental impacts.<sup>4</sup> Thus, the need to better understand the environmental fate of such modified CNTs is evident.

Stability of CNTs in natural water systems containing soil or sediment is one important aspect of their environmental fate. The extent to which CNTs are removed from water is influenced by the colloidal characteristics of CNTs such as the surface charge.<sup>9-12</sup> Oxygen containing groups, especially carboxyl groups, make the CNT surface more hydrophilic and also change the surface charge.<sup>11</sup> Due to electrostatic repulsion, hydrophilic CNTs can be stabilized as described by the Derjaguin-Landau-Verwey-Overbeek (DLVO) theory.<sup>10, 12-16</sup> Thus, the presence of common cations in the environment such as Na<sup>+</sup>, K<sup>+</sup>, Ca<sup>2+</sup>, and Mg<sup>2+</sup> can decrease their stability (e.g., induce aggregation) by suppressing electrostatic repulsion between the CNTs.

Most previous studies have investigated the stability of CNTs in water without a solid phase,<sup>17-20</sup> whereas in natural ecosystems the interaction between CNTs and soil or sediment particles contributes to their fate. Soil or sediment particles such as peat, one common type of soil organic matter, can sorb MWCNTs with the presence of sodium cations.<sup>21</sup> Higher concentrations of kaolin were found to improve MWCNT removal from water.<sup>20</sup> However, these phenomena have thus far rarely been studied in large part as a result of analytical method limitations for CNT quantification.<sup>21-23</sup> An earlier study on the sorption of functionalized MWCNTs by soils revealed a linear sorption pattern, but the contribution of different soil

components are still unclear.<sup>24</sup> Clay minerals are a major building block of natural soils, and play an important role in the removal of various organic or inorganic pollutants via adsorption onto their large surface areas.<sup>25</sup> The capacity for clays to remove CNTs in the natural environment has not yet been fully investigated. A previous study indicated that clay minerals can destabilize surfactants-dispersed MWCNT by two mechanisms: (1) removal of surfactants by clay minerals from solution and MWCNT surfaces and (2) bridging between clay mineral and MWCNT by surfactants.<sup>26</sup> Nonetheless, a quantitative study of the colloidal interactions between soil minerals and CNTs is needed to further elucidate the fundamental mechanisms of the environmental behaviors of CNTs.

We in this study examined the stability of MWCNTs in aqueous solutions containing three minerals (kaolinite, smectite, and shale) under varying sodium concentrations. In this system composed by both an aqueous and solid phase, the mechanisms of MWCNTs removal were 1) settling due to aggregation and 2) favorable interactions between solid phases and MWCNTs or MWCNTs aggregates, which is termed as "sorption" hereafter. These two processes occur concurrently and cannot be separated experimentally. Our hypothesis is that these processes are additive, so that quantity of MWCNTS sorbed to the solid phase can be determined by comparing results from systems with and without solids present. According to previous studies, van der Waals and electrostatic forces are likely to play roles in determining the stability of MWCNTs.<sup>16</sup> Thus, the extended DLVO theory (EDLVO) was applied to interpret the interactions. The EDLVO theory incorporates the acid-base force in addition to van der Waals and electrostatic forces to describe the effects of hydrophobic and hydrophilic interactions, and has been proven to yield more precise predictions of many colloidal systems than the traditional DLVO simulation.<sup>27-29</sup> The overall goal of this study was to provide insights into the

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fundamental mechanisms governing the aqueous stability of MWCNTs with the presence of solid phase, and to quantitatively estimate their interactions, thereby contributing towards a scientific understanding of the environmental fate of MWCNTs.

# MATERIALS ANS METHODS

# <sup>14</sup>C labeled MWCNT

Radioactive MWCNTs were synthesized using modified chemical vapor deposition (CVD) with mixtures of <sup>14</sup>C-labeled and regular methane gas as the carbon source. This synthesis method has been thoroughly described previously.<sup>30, 31</sup> To remove metal catalyst impurities, MWCNTs were bath sonicated with concentrated hydrochloric acid (11.1 mol/L) for 1 h. These nanotubes were then bath sonicated in an acid mixture of concentrated sulfuric (14.8 mol/L) and nitric (15.6 mol/L) acids by a 3:1 (V:V) ratio for 2 h to make them more stable in water by imparting additional oxygen-containing functional groups.<sup>32, 33</sup> These surface-modified MWCNTs have a specific radioactivity of 0.1 mCi/g determined by liquid scintillation counting after biological oxidation (OX 500; R. J. Harvey Instrument Co. Tappan, NY). The BET MWCNT surface area is  $111 \text{ m}^2/\text{g}$ ,<sup>21</sup> which is comparable to previous results with purified but not acid-treated MWCNTs synthesized by the same technique.<sup>34</sup> Thermal gravimetric analysis (TGA) revealed that these acid-treated carbon nanotubes were (99.7  $\pm$  0.2) % pure with respect to metal catalyst impurities and indicated an absence of amorphous carbon;<sup>33</sup> it was previously shown using TGA that ultrasonication for 6 h only slightly increased the percentage of oxygen and did not produce detectable quantities of amorphous carbon.<sup>21</sup> Diameters of these MWCNTs were previously measured with SEM to range predominately from 30 to 70 nm.<sup>30, 31</sup>

To prepare a stable stock suspension, MWCNTs (100 mg) were dispersed in 1 L of deionized water (> 18 M $\Omega$ ) by ultrasonication (200 W; Cole-Parmer CV33, IL) for 2 h, and then left at room temperature for 6 h. The stable portion was collected as the stock solution. The average length of MWCNTs was  $(353 \pm 452)$  nm (n=836; uncertainties always represent standard deviations) measured using an FEI Inspect F50 FEG scanning electron microscope (SEM) operating at an accelerating voltage of 15.00 kV. According to x-ray photoelectron spectroscopy (XPS), the percentage of carbon atoms associated with oxygenated functional groups and total oxygen percentage were both approximately 7.4% for the acid-treated MWCNTs.<sup>21</sup>

## Soil minerals: Kaolinite, smectite and shale

Clay minerals are generally composed of two building blocks, silicon tetrahedron  $(Si_2O_5^{2-})$ and aluminum octahedron  $(Al(OH)_6^{3-})$ , and are particles with sizes of less than 1 or 2  $\mu$ m.<sup>35</sup> Kaolinite, a common clay material in most soils,<sup>36</sup> was obtained from Washington County, Georgia and was sieved and purified. Kaolinite belongs to the 1:1 clay minerals which contain one tetrahedral and one octahedral sheet per clay layer.

Smectite was obtained from Sigma-Aldrich (CAS number of 1318-93-0). This product is a calcium montmorillonite that has been treated by hydrochloric acid. Smectite is a typical 2:1 clay mineral which contains two silicon sheets with one aluminum layer in between,<sup>36</sup> and has a permanent layer charge resulting from isomorphous substitution. The surface area was (240  $\pm$  15) m<sup>2</sup>/g and thus larger than that of kaolinite ((16.2  $\pm$  0.7) m<sup>2</sup>/g) (n=3 for both clays).

Lachine shale was collected from the Paxton Quarry west of Alpena, MI. The shale was crushed and sieved, and has been thoroughly characterized in previous studies.<sup>37, 38</sup> Shale contains diagenetically old organic material such as kerogen, one type of organic matter with a large molecular weight (> 1,000 D), relatively low O/H ratio and high H/C ratio.<sup>37, 38</sup> The organic carbon content of this shale is 8.27 %.<sup>37</sup> The surface area of this material ((6.60  $\pm$  0.15) m<sup>2</sup>/g

(n=3)) is similar to that of kaolinite. The weight loss of the dry shale after heating at 600  $^{\circ}$ C overnight was (13.8 ±4.9) % (n = 3).

To remove the extra salts associated with clay particles as well as simplify the types of cations bonded with the clay particles, potassium saturated clay was prepared by soaking all three clay minerals in 0.1 mol/L KCl four times for 12 h each time, then extensively washing by de-ionized water until no Cl<sup>-</sup> was eluted by AgNO<sub>3</sub> test in the filtered solution.<sup>39</sup> The organic carbon present in the smectite particles was minimum, indicated by a weight loss of  $(4.9 \pm 0.6)$  % (n=3) after heating at 600 °C by muffle furnace. The weight loss of kaolinite under the same conditions was larger  $(12.9 \pm 5.7)$  % (n = 3), probably due to the loss of CO<sub>2</sub> from carbonates. The clay and shale samples were also analyzed by scanning electron microscopy-energy dispersive spectroscopy (SEM-EDS) (FEI Inspect F FEG-SEM equipped with EDAX). Images and surface element compositions are shown in Figure S4.1 and S4.2, and Table S4.6 summarizes the EDS results.

## Quantification and characterization

The concentrations of MWCNTs were determined based on their radioactivity. A 3-mL aliquot of each sample was mixed with 3 mL of scintillation cocktail (Insta-Gel Plus, PerkinElmer, MA), and the radioactivity was measured with a Beckman LS 5801 liquid scintillation counter (Brea, CA). The detection limit for this method was 0.04 mg/L, determined by mixing 3 mL of de-ionized water with 3 mL of scintillation cocktail (n=6), measuring their radioactivity, and defining the detection limit as three times the standard deviation; lower detection limits can be achieved using larger volumes of solution. The electrophoretic mobility was determined from duplicate samples per condition using a Malvern Zetasizer Nano ZS instrument operating at 25 °C.

The contact angles of the three types of mineral particles were measured by flattening them as thin layers on 5 cm × 5 cm clean glass cover slips.<sup>40, 41</sup> The glass cover slips were immersed in 2 % (v/v) nitric acid solution for 30 min and then rinsed vigorously with 90 % (v/v) ethanol (VWR Histology reagent alcohol 95%). A 2-mL aliquot of each clay suspension (approximately 1 g/L concentration) was dropped on the cover slip, which was then spun at 100 g for 5 min on a spin coater device (WS-400E, Laurell Technologies Corporation, US). A thin layer of the mineral particles was achieved after air drying for 5 to 10 min. Equilibrium contact angles ( $\theta_L$ ) of the clay layers were measured on a Model 250 Ram é-hart goniometer at ambient conditions. Three types of suspensions were prepared for each mineral for this test using three different probe solvents (de-ionized water, glycerol, and formamide) which surface tension properties were summarized in Table S3.1.

## Association of MWCNTs with solid phase in different aqueous systems

The stability of MWCNTs in aqueous phase with or without solid phase was investigated using a bottle-point technique as described previously.<sup>21</sup> Ten initial concentrations of MWCNTs were selected from 0.1 mg/L to 16 mg/L. In each treatment, 10-mL of MWCNT suspension was added to a 150-mL flask with 50 mg of kaolinite, smectite, or shale; experiments were also conducted with 10 mg of shale. The contents were mixed and set still for 7 d when the aqueous concentrations of MWCNTs reached a quasi-equilibrium as indicated in a previous study.<sup>21</sup> Each treatment was sampled and centrifuged at 3,500 g for 1 min to remove suspended soil minerals, conditions shown in a preliminary test for effective removal of the mineral particles. Without the centrifugation step, some fraction of the soil minerals remained in suspension, thus the centrifugation step was necessary to quantify the nanotubes remaining in aqueous phase. The radioactivity in 3 mL of solution was then measured to quantify the MWCNT concentration.

Each experiment was repeated three times with each condition to confirm the reproducibility of the results. Control treatments without solid phase were also tested using the same method with sodium concentrations of 0, 0.4, 4, and 40 mmol/L to characterize MWCNT removal in the absence of the solid phase. The removal of MWCNT from water during the 7d period and the centrifugation step in systems without a solid phase will be referred to hereafter as "settling."

The total mass of MWCNTs that had distributed into solid phase in each treatment,  $M_s$  (mg), was obtained by mass balance using equation 1

$$M_{\rm s} = (C_0 - C_{\rm e}) \times V \tag{1}$$

where  $C_0$  and  $C_e$  are the initial and final aqueous concentrations of MWCNTs (mg/L), respectively, and V is the sample volume (0.010 L). For the control treatments,  $M_s$  represents the mass of nanotubes that have settled out of solution, while in clay treatments,  $M_s$  also includes the association between soil minerals and MWCNTs.

The solid phase MWCNT concentration  $(q_s)$  is defined as

$$q_s = \frac{M_s}{D} \tag{2}$$

where D is the dosage of soil minerals (0.050 g).

A linear model was used to quantify the relationship between  $q_s$  and  $C_e$ . The linear parameter, *a*, was calculated as

$$q_s = aC_e \tag{3}$$

Parameter *a* demonstrates the tendency of MWCNTs to be removed from the aqueous phase. Control treatments gave  $a_1$  which shows the settling of aggregated MWCNTs caused by sodium, while the treatments with solid phase gave  $a_2$  which includes both settling and sorption of MWCNTs under different sodium concentrations. A new parameter,  $\Delta a$ , was calculated as

$$\Delta a = a_2 - a_1 \tag{4}$$

and indicates the contribution of sorption to the solid phase.

The influence of dissolved material from all three soil minerals on MWCNT stability was also investigated as described in Supplementary Material, because dissolved organic matter from peat was shown in our previous study to substantially reduce MWCNT settling.<sup>21</sup>

Homogeneity of slope tests were performed using SAS 9.2 to test the differences of the values of *a* between: 1) different sodium concentrations; 2) different soil minerals; 3) with or without soil minerals, i.e.,  $a_2$  and  $a_1$ ; and 4) with or without extractable materials from soil minerals. The null hypothesis for each test was that each pair of slopes were the same, and the p-values of this assumption were calculated accordingly. Statistically significant values were determined using  $\alpha$ =0.05.

## Interaction energy calculation by the EDLVO theory

The EDLVO theory was used to estimate the MWCNT-clay particle interaction energy by considering the non-retarded van der Waals (vdW) attraction, electrical double layer (EDL) repulsion, and Lewis acid-base (AB) interaction.<sup>28, 42</sup> The mathematical expressions for the van der Waals and electrostatic forces vary with the geometry of the interacting entities. Because the mineral particles are at least 1.5 to 2  $\mu$ m in one dimension while the length of MWCNTs is in the range of a few hundred nanometers and diameters were measured to typically range between 30 to 70 nm, the clay-MWCNT interaction was approximated as a particle–plate geometry. Definitions and determinations of all other parameters are provided in supplementary material sections S4.2 and S4.3 and Tables S4.2 through S4.4.

#### RESULTS AND DISCUSSION

## Settling and Sorption of MWCNT with different soil minerals: Model analysis

Figure 4.1 illustrates the overall removal of MWCNTs in the presence of kaolinite (part a), smectite (part b), or shale (part c) or without a solid phase (part d). A linear regression model was proposed to describe the pattern of MWCNTs distributed between the aqueous and solid phases (see Table 4.1). The final concentrations of MWCNTs ranged from 0.05 mg/L to 10 mg/L. Within this range, the linear model fits sorption data equally well as the Freundlich model or Langmuir model, models that typically yield more robust fits of sorption behaviors. Moreover, previous studies showed nearly linear sorption pattern of MWCNTs by peat or three different types of soils.<sup>21, 24</sup>

A larger value of *a* indicates a higher tendency of MWCNTs to be removed from the aqueous phase by settling or sorption. The value of *a* increased significantly (as shown in Table S4.9) with increasing sodium concentration for all three minerals, demonstrating the effect of sodium cations to promote MWCNT removal from the aqueous phase, while MWCNTs tended to remain suspended without the addition of sodium ions.

When the sodium concentration reached a certain level, MWCNTs were almost completely eliminated from solution (less than 10% remaining in the aqueous phase). Above this threshold, there were not significantly different changes in *a* by increasing the sodium concentration (see Table S4.9). The threshold sodium concentrations were 4 mmol/L and 40 mmol/L for kaolinite and smectite, respectively. When the dose of shale was 50 mg, MWCNTs were almost entirely eliminated from the aqueous phase regardless of the sodium concentration, indicating the strong association tendency between MWCNTs and shale. To more carefully investigate the effects of varying sodium concentrations, the shale dose was reduced from 50 mg to 10 mg. For a shale dose of 10 mg, the threshold value of sodium concentration for nearly complete removal was 4 mmol/L.

The effects of the three types of soil minerals at the same sodium concentration (4 mmol/L) and mineral dosage (50 mg) were also directly compared in Figure 4.1 part e. Similar to the results shown in Figure 4.1 parts a, b, and c, smectite demonstrated the lowest tendency to associate with MWCNTs, while the MWCNTs were almost eliminated from the aqueous phase with the presence of kaolinite or shale. However, there was no statistical difference in the MWCNT stability among the different soil minerals without added sodium or at a sodium concentration of 40 mmol/L, because the MWCNTs were either highly stable or almost entirely removed from the aqueous phase, respectively.

The important effect of cation concentration on MWCNT removal from the aqueous phase differs to some extent from our previous study with peat.<sup>21</sup> Peat released substantial concentrations of dissolved organic matter to water, which helped the MWCNTs remain in solution and mitigated the effects of the sodium cations to a certain extent.<sup>21</sup> There was no statistically significant difference with or without dissolved components of the three minerals except for kaolinite at 0.4 mol/L sodium ions (see Supplementary Material for more information). EDLVO modeling and mechanisms influencing MWCNTs stability

One of the important processes influencing the stability of MWCNTs is electrostatic interactions. Given that both minerals and MWCNTs have negative charges under almost all of the conditions tested (see Table 4.3), the electrostatic interaction between them was repulsive. Van der Waal's forces, which universally exists between two interacting materials, also play an important role in the interaction between MWCNTs and soil minerals or between individual MWCNTs. Furthermore, to account for the effects of hydrophobic and hydrophilic interactions,

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Lewis acid-base (AB) interactions were also included in the EDLVO theory in addition to van der Waal's and electrostatic forces. This model was applied to calculate the interaction energies under different sodium concentrations in our study. The energy potentials with respect to interaction distances are shown in Figure 4.2.

As shown in Figure 4.2 part a to c, there were energy barriers separating two minima of potential energy between MWCNTs and clay particles under each condition except for the treatment with kaolinite and 40-mmol/L sodium concentration. The positive energy barrier indicates that the association of MWCNTs with clay particles is thermodynamically unfavorable. The height of the energy barrier indicates the potential energy between clay particles and MWCNTs, which must be overcome for the association process to occur and thus the height of the energy barrier may strongly influence the fate of MWCNTs. The values of the energy barrier decreased with increasing sodium concentrations due to decreased electric double layer repulsion. In other words, increasing ionic strength raised the likelihood of MWCNT associations with the mineral particles and decreased the stability of MWCNTs in the aqueous phase. According to the calculation, the interaction energies between these acid-treated MWCNTs were always negative when the sodium concentrations were 0, 0.4, 4, and 40 mmol/L (see Figure 4.2d). This result indicates a favorable association between MWCNTs, and explains the strong settling tendency of the MWCNTs due to aggregation.

To correlate the tendency of MWCNTs to enter the solid phase with the thermodynamics from the EDLVO theory, the common log of the linear model parameter, a, was plotted with the values of the energy barrier, as shown in Figure 4.3. Clearly, log a was inversely correlated with the energy barrier value for each mineral. According to Figure 3, there was a linear trend between the log a and the energy barrier. If we fit Log a to the energy barrier of both clay

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minerals together (excluding shale), the correlation factor, *R*, which indicates the strength of that relationship was 0.98. This result shows the ability of the EDLVO theory to predict the trend of the association of MWCNTs with solid phase under different conditions for kaolinite and smectite. The major interaction forces between the clay particles and MWCNTs can be well summarized by the acid-base interaction, the electrostatic force, and van der Waals force. However, the pattern of shale which contains organic matters, differed from those of the other two inorganic minerals. This suggests that when organic matter is present as for shale, additional non-DLVO forces such as steric interactions may exist, which are needed to accurately reflect the complex interactions occurring.

## Contribution of mineral particles

Values for the parameter indicating only sorption,  $\Delta a$ , are listed in Table 4.2. When the sodium concentration was 0 mmol/L, the effect of kaolinite or smectite on the stability of MWCNTs was not pronounced, which differs from the effects of shale. The net effect of smectite across the range of sodium concentrations was to stabilize MWCNTs in aqueous phase, while those of kaolinite and shale were to promote the removal of MWCNTs.

The increase of affinity towards MWCNTs in the order of smectite, kaolinite, and shale under the same sodium concentration can be explained by the surface charge and hydrophobicity of different soil minerals. This trend corresponds to the increase of hydrophobicity indicated by the contact angles (see Table S4.2). Compared to smectite, kaolinite is less charged demonstrated by the absolute value of the electrophoretic mobility of kaolinite being less than that of smectite for each sodium concentration (see Table 4.3). The negative charge on the clay surface can repulse the negatively charged MWCNTs, and consequently prevent them from coming into contact. Therefore, the more negatively charged smectite renders the MWCNTs more stable than the less negatively charged kaolinite. Interestingly, shale has a higher electrostatic potential than kaolinite and smectite as evidenced by its electrophoretic mobility, but more association affinity with MWCNTs. This is probably due to the organic content of shale that can strongly sorb MWCNTs, which dominates MWCNT-shale interactions. Our previous study also demonstrated that the diagenetically young soil organic matter, peat, sorbed MWCNTs when sodium ions were present.<sup>21</sup> The high affinity between MWCNTs and shale is thus probably ascribed to the sorption ability of soil organic matter.

## ENVIRONMENTAL IMPLICATIONS

Minerals like clay are extensively distributed on the earth surface as important components of soils and sediments. Therefore, understanding the interactions between MWCNTs and such minerals is of significance in predicting the fate of MWCNTs in environment matrices. For clay particles without organic matters, surface charge is critical in these interactions. Therefore environmental conditions that can change the surface charges such as ionic strength can also influence the effect of clay particles on MWCNTs. In sea water and hard water, MWCNTs will likely be removed from the aqueous phase by settling or association with soil or sediment particles, whereas in fresh water with low cation concentrations, MWCNTs are more likely to remain in the aqueous phase. A much more favorable association between the shale and MWCNTs was observed in comparison to inorganic minerals. The relative effects of these minerals also depend on their concentrations in soils and sediments, which is an interesting topic for further researches.

We also demonstrated the capability of the EDLVO theory to describe the interaction between MWCNTs and soil minerals. The calculated energy barrier based on the EDLVO theory was well correlated with the tendency of MWCNT association with solid phase (the parameter *a*)

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and accurately described the behavior of inorganic clay particles under different sodium concentrations, regardless of the clay type. Thus, the EDLVO theory may serve as a powerful tool to quantitatively study the stability of MWCNTs in the environment. However, approximations were also adopted in the EDLVO calculation (summarized in Supplementary Material S4.2). The effect of these approximations is left for future investigations.

## **ACKNOWLEDGEMENT**

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# FIGURES AND TABLES



FIGURE 4.1 Sorption of MWCNTs by clay minerals under sodium concentrations of 0, 0.4, 4, and 40 mmol/L: a) kaolinite; b) smectite; and c) shale. In a) and b), the dose of clay particles is 50 mg/10 ml, while in c) the dose is either 50 mg/10 ml or 10 mg/10 ml. d) A control treatment without clay minerals. e) Sorption of MWCNTs under sodium concentrations of 4 mmol/L by three type of clay particles. Uncertainty analysis of the plots is provided by the standard errors of the fitted linear model in Table 4.1.



FIGURE 4.2 Interaction energies between MWCNT and a) kaolinite; b) smectite; c) shale; and d) another MWCNT under different ionic strengths using the EDLVO model.



FIGURE 4.3 The relationship between the Langmuir model parameter, *a*, and the energy barrier calculated by the EDLVO theory. The linear fit line is for kaolinite and smectite only.

	Kaolinite		Smectite		Shale	
Sodium Conc. (mmol/L)	a (mg/g clay)	$R^2$	a (mg/g clay)	$R^2$	a (mg/g clay)	$R^2$
0	0.035	0.17	0.049	0.665	0.651	0.816
	$(0.008)^{a}$		(0.004)		(0.044)	
0.4	0.225	0.75	0.120	0.809	0.741	0.556
	(0.022)		(0.007)		(0.007)	
4	2.514	0.39	0.546	0.729	11.146	0.063
	(0.370)		(0.037)		(1.875)	
40	16.705	0.01	7.3858	0.490	12.424	0.232
	(3.474)		(0.816)		(2.756)	

TABLE 4.1 Parameters obtained by data fitting to the equation  $q_t = aC_{aq}$ 

<sup>*a*</sup> Values in parentheses are standard error. The number of samples tested ranged from 20 to 30. The dose of clay particles for kaolinite and smectite was 50 mg, while for shale is 10 mg.

	Kaolinite	Smectite	Shale (10 mg)
Sodium Conc. (mmol/L)	∆a	∆a	∆a
	(mg/g clay)	(mg/g clay)	(mg/g clay)
0	-0.041*	-0.027*	$0.575^*$
	$(0.001)^{a}$	(0.001)	(0.005)
0.4	$0.0705^*$	-0.065*	$0.556^{*}$
	(0.038)	(0.023)	(0.086)
4	$1.771^*$	-0.197	$10.404^{*}$
	(0.432)	(0.098)	(1.936)
40	9.251	-0.068	$4.970^{*}$
	(4.134)	(1.476)	(3.416)

TABLE 4.2 The contribution of clay particles indicated by the difference of model parameter,  $\Delta a$ .

<sup>*a*</sup> Values in parentheses are standard errors calculated using Gauss error propagation. The number of samples tested ranged from 20 to 30. The dose of clay particles for kaolinite and smectite was 50 mg, while for shale it was 10 mg.

<sup>\*</sup>Values are significantly different from zero ( $\alpha$ =0.05), tested by comparing the *a* values with and without solid phase.

TABLE 4.3 Electrophoretic mobility values of clay particles and MWCNTs (  $\mu m \ cm/Vs$ )

Sodium Conc. (mM)	Kaolinite	Smectite	Shale	MWCNTs
0	-2.17 (0.03) <sup>a</sup>	-2.78 (0.29)	-3.18 (0.57)	-1.51 (0.02)
0.4	-1.32 (0.02)	-2.14 (0.06)	-3.60 (0.73)	-1.10 (0.08)
4	-0.52 (0.02)	-1.18 (0.01)	-3.69 (0.61)	-0.95 (0.14)
40	0.01 (0.37)	-0.65 (0.08)	-3.11 (1.29)	-0.94 (0.06)

<sup>*a*</sup> Values in parentheses are standard errors (n = 2).

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# CHAPTER V

# PRIMARY PARTICLE SIZE ON COLLOIDAL STABILITY OF 14C-LABELED MULTIWALL CARBON NANOTUBES<sup>3</sup>

<sup>&</sup>lt;sup>3</sup> Zhang, L.; Pan, Z. and Q. Huang. Submitted to *Water Science and Technology*, Jun 2013.

#### **ABSTRACT**

Multiwall carbon nanotubes (MWCNTs) are one type of nanoparticles that have various special properties and potential applications. Due to their increasing production and potential toxicological effects, the environmental behavior and transport of MWCNTs have become important research topics. Particle size is one of the important properties of nanoparticles, yet its effects on MWCNTs' environmental behaviors have not been fully investigated. In this study, we tested how the length of MWCNTs influenced their settling in the presence of Na<sup>+</sup> and/or natural organic matter (NOM), and postulated the governing mechanisms. The results showed that when adding Na<sup>+</sup> the shorter MWCNTs exhibited preferential aggregation and settling. One possible reason could be that shorter MWCNTs possess larger specific surface area and consequently stronger attraction forces. However, NOM strongly mitigated such aggregation, and helps dispersing MWCNTs regardless of their length and aqueous conditions.

#### **INTRODUCTION**

The development of nanotechnology has resulted in successful applications of various nanomaterials and will encourage further expansion.<sup>1</sup> Carbon nanotubes (CNTs) are among the nanomaterials that have been studied extensively due to their prominent electronic, physicochemical, optical, and mechanical properties.<sup>2</sup> CNTs are allotropes of carbon that can be illustrated as rolling graphene sheet(s) into a cylindrical shape, while multiwall carbon nanotubes (MWCNTs) are composed by a series of coaxially-arranged graphene sheets. In some of the applications, such as drug delivery vessel and sorbents,<sup>2, 3</sup> CNTs are required to be dispersed

stably in aqueous phase. Upon release to environmental aquatic bodies, whether CNTs can stably suspend in water will influence their mobility, environmental distribution and bioavailability.<sup>1, 4, 5</sup>

The size of MWCNTs falls into the colloidal range. Studies showed that MWCNTs with hydrophilic surface functional groups, i.e., carboxyl, hydroxyl, carbonyl, etc., can stably suspend in water to form a colloidal dispersion. However, common cations such as Na<sup>+</sup>, K<sup>+</sup>, Ca<sup>2+</sup>, and Mg<sup>2+</sup> can induce their aggregation by suppressing electrostatic repulsion between the CNTs, and this process can be reasonably modeled using the Derjaguin-Landau-Verwey-Overbeek (DLVO) theory.<sup>6-8</sup> MWCNTs aggregation, when reach certain sizes, can readily settle from water, and consequently their mobility is reduced. Therefore aggregation is an important process governing the environmental fate of MWCNTs in aqueous systems.

In addition to aqueous conditions, e.g., cation valence, cation concentration, and pH, MWCNT properties, e.g., surface charge and functional groups, also affect their colloidal stability.<sup>9, 10</sup> However, the impact of other properties such as size and morphology has not been fully investigated, yet may be important factors controlling CNT aggregation and stability according to the traditional colloid science.<sup>11-14</sup> The research on the effect of particle size to colloidal stability usually studied particles of a spherical shape or that can be approximated or assumed as a spherical shape. CNTs were however rod-shaped.<sup>15</sup> The effect of size to the colloidal stability of tubular particles is largely unknown. Moreover, in literature, the size of CNTs was often described by equivalent spherical size, e.g., hydrodynamic diameter, a way that does not differentiate individual particles and their aggregates. To precisely describe a CNT size requires two parameters, i.e., diameter and length, both of which may pose impact on the CNT behavior in aqueous phase. For example, O'Driscoll *et al.*, (2010) has found that decreasing

diameter enhanced the colloidal stability of MWCNTs dispersed by natural organic matter (NOM), whereas the effect of MWCNTs length is still unknown.<sup>16</sup>

In order to investigate the size effect to the colloidal stability of a polydispersed MWCNTs suspension, we used scanning electron microscope (SEM) to characterize the length distribution of primary MWCNTs, as well as dynamic light scattering (DLS) to measure the hydrodynamic diameter. The size effect was examined under two aqueous conditions: varying sodium ion concentrations (0, 4 and 40 mmol  $L^{-1}$ ) and the absence or presence of NOM. Possible mechanisms of the phenomenon were discussed.

### MATERIALS AND METHODS

# <sup>14</sup>C labeled MWCNT

Carbon-14 labeling was used to unambiguously quantify MWCNTs in aqueous phase. <sup>14</sup>C-labeled MWCNTs were synthesized using a modified chemical vapor deposition technique as described in our previous study.<sup>17, 18</sup> The MWCNTs were treated with hydrochloric acid followed by a mixture of concentrated sulfuric and nitric acid (3:1 v/v) to remove impurities and add hydrophilic functional groups to the nanotube surfaces. The MWCNTs were then ultrasonicated (34.7  $\pm$  0.8 W; uncertainty indicates the standard error) in de-ionized (DI) water (> 18 MΩ) for 6 h, centrifuged at 3500 g for 5 min to produce a stable dispersion of 67 mg L<sup>-1</sup> determined by the radioactivity. This MWCNTs dispersion was fully characterized in our previous study.<sup>17</sup> Briefly, the surface oxygen contents were determined to be ~8.6% by X-ray photoelectron spectroscopy (XPS) (Kratos Analytical Axis Ultra X-ray photoelectron spectrometer). The point of zero charge (PZC) is around pH 1.5 in a monocation solution at 0.1 mol L<sup>-1</sup>.

The MWCNTs concentration was diluted to ~10 mg  $L^{-1}$  before initiating the experiments. MWCNT concentrations were quantified by radioactivity using a Beckman LS 5801 liquid scintillation counter (Brea, CA) after mixing a 3-ml sample with 3 ml of scintillation cocktail (Insta-Gel Plus, PerkinElmer, MA).<sup>17</sup> These initial dispersions were sampled and subjected to the scanning electron microscope (SEM) measurement as described in Particle size characterization: Length.

## Settling Experiment

The effect of particle length on the colloidal stability of MWCNTs was investigated by comparing the length distribution of MWCNTs before and after settling. If MWCNTs in certain size range are more stable than those in other ranges, the relative abundance of them will increase after the settling and be reflected in the length distribution histogram. Aside from length, we also examined the effect of two aqueous conditions using a factorial design, i.e., Na<sup>+</sup> concentrations and with or without NOM (in Figure 1 and 2 noted as "Blank" or "NOM", respectively). The experiment was conducted in 150-mL flasks; each contained 10 mL of a MWCNT suspension. The tested Na<sup>+</sup> concentrations were 0, 4 and 40 mmol L<sup>-1</sup> adjusted by adding NaCl. The NOM is the water-soluble portion of a natural soil organic matter, Canadian peat (Quebec, Canada). Peat is one typical soil organic matter usually composed of recently deposited plant organic materials, and is thus a diagenetically young organic matter.<sup>19</sup> Thus it contains relatively higher oxygen to carbon content and less compact structure compared to the aged organic matter. The total organic carbon content of the final solution containing this NOM was 7.67 mg C L<sup>-1</sup>. Each condition was prepared in triplicate. The samples were hand shaken initially and then set still in room temperature for 7 d to allow settling. At the end of the 7 d period, a 5-mL portion was taken from each sample and centrifuged at 3500 g for 5 min in a

glass centrifuge tube of 1.2 cm diameter to obtain a stable suspension. The supernatant of the top 3-cm layer was sampled from the centrifuge tube for further measurements: 1) 0.2 mL for length distribution of the MWCNTs by Scanning electron microscope (SEM); 2) 3 mL for quantification of MWCNTs by C14 radioactivity.

#### Particle size characterization: Length

Scanning electron microscope (SEM) was employed to measure sizes of individual MWCNTs. The MWCNTs examined by SEM were from the initial dispersion without adding NaCl or NOM or from the stable dispersions after settling experiments. 0.2 mL of each sample was mixed with 1.8 mL methanol; 10 µL of the mixture was applied on a silicon substrate and vacuum dried. The substrate was then examined using a FEI Inspect F50 FEG scanning electron microscope (SEM) at an accelerating voltage of 10.00 kV or 15.00 kV according to image clarity. 20kx magnification images were taken for each sample at random positions on the silicon substrate. The lengths of individual MWCNTs on the microscopic images were quantified using SigmaScan Pro. 5.0. The curved MWCNTs were measured using a customized ruler. The length distribution histogram was number-averaged by at least 150 MWCNTs for each sample. The differences among the settling percentage or the relative abundances of MWCNTs were evaluated by pair-wised comparisons using Tuckey's test at the confidence level of 0.01 or 0.05, respectively. The statistical analysis was performed using SAS ver. 9.2.

#### Particle size characterization: Hydrodynamic diameter

The average sizes of MWCNTs and their aggregates were described using the hydrodynamic diameter as well as polydispersity index (PDI) measured by dynamic light scattering (DLS) on a Malvern Zetasizer Nano ZS instrument (Malvern Instruments, UK) operating at 25 °C. MWCNTs dispersions were measured immediately after the addition of NaCl

or after the settling experiment for each condition. Samples were transferred to a macro-cuvette (pass length: 10 mm) and the scattering angle was 173°. The instrument automatically chose the number of runs performed on each sample depending on the quality of the autocorrelation function; typically 10 to 30 runs were performed.

# **RESULTS AND DISCUSSION**

#### Effect of individual length

The mass of <sup>14</sup>C-MWCNT that remained in the dispersion after settling was measured using liquid scintillation counter (LSC) and the percentage was calculated according to the radioactivity (Figure 5.1). In the absence of Na<sup>+</sup> and NOM, 13.3  $\pm$  8.2% (n = 3, uncertainties represent standard deviations) of MWCNTs were removed during the settling. The addition of Na<sup>+</sup> significantly increased the removal of MWCNTs to 61.2  $\pm$  18.9% and 89.3  $\pm$  2.5% for 4 and 40 mmol L<sup>-1</sup> Na<sup>+</sup>, respectively.

Scanning electron micrographs of the initial dispersed MWCNTs are shown in Figure S5.1 a. According to the graph, most of MWCNTs were individually dispersed. By visually comparing the SEM images before and after settling (Figure S5.1 a and c), the amount of individually dispersed MWCNTs after the addition of NaCl is less than the initial sample; instead, most of them exist in bundles. This showed that the cation induced the MWCNT aggregation, and the aggregation is likely to cause the removal of MWCNTs from aqueous phase.

The initial length distribution (Figure 5.1 and Figure S5.2) indicates a broad range of MWCNT length ranging from less than 100 nm to more than 2000 nm, a result similar to that previously determined for a different set of MWCNTs prepared using the same synthesis method.<sup>20</sup> 73.2% of the MWCNTs were shorter than 400 nm, and the average length (by number) is approximately 353 nm. Diameters ranged mainly from 16 to 92 nm with a mean at  $34.8 \pm 13.3$ 

nm (n = 50; uncertainties indicate standard deviations). The polydispersed nature of the MWCNTs suspension makes it difficult to separately track the behaviors of MWCNTs within different length range. Instead, the relative abundance of MWCNTs in each length range is quantifiable. Its change can reflect the preferential removal of MWCNTs with regard to length. Therefore the length distributions after settling under different condition were analyzed and are shown in Figure 5.2. Given the resolution of the SEM images and challenges associated with distinguishing between MWCNTs and dust or debris on the wafer, MWCNTs less than 100-nm long were not considered.

Figure 5.2 shows the change of the number-averaged percentage of MWCNTs in different length ranges before and after settling. As shown in Figure 5.2a, after settling, the relative abundance of shorter fraction of MWCNTs significantly decreased, and the decrement was negatively correlated with Na<sup>+</sup> concentration. This suggests the preferential removal of the primary MWCNTs with shorter length. Generally speaking, the MWCNTs falling in the 100 to 400 nm length range were relatively less stable than those in other length ranges in this experiment, showing higher tendency to be removed from aqueous phase and consequently lower mobility in the environment. Aggregation is the prerequisite of the MWCNT settling, and the factors influencing the aggregation can also influence the settling. Therefore the preferred settling of shorter MWCNTs to the longer ones is likely resulted from the preferential aggregation of shorter MWCNTs in the lower length range. This result is consistent with the colloidal stability studies of small particles in nanoscale.<sup>11, 12, 14</sup> A study using standard material, polystyrene microspheres (mean diameter ranged from 76 nm to 1010 nm) stabilized by negative sulfate charges revealed that at a given salt concentration, stability of this colloid increases, reaches a maximum, and then decreases as a function of size increase.<sup>14</sup> Kallay et al., (2002)

concluded that for the very small colloid particles in nanometer range, smaller particles are less stable by theoretical calculation using the Br ønsted concept based on the Transition State Theory.<sup>12</sup> This theorem has been experimentally verified by hematite particles (radius of 12, 32 and 65 nm).<sup>11</sup> Instead, within a larger diameter range such as 890  $\pm$  0.2 nm, this trend was reversed in that the secondary-minimum aggregation of hematite particles was enhanced by increasing particle size.<sup>13</sup> Thus in the lower size range, the spherical particles with smaller diameter are easier to aggregate. Our result showed that this phenomenon may also be applied to non-spherical particles such as MWCNTs.

In addition, MWCNTs of different length may form aggregates with different density, which partially explains the preferential removal of the shorter MWCNTs. A previous study reported that the acid-treated single-walled carbon nanotubes (SWCNTs) yielded looser aggregate structures at large length scale and more compact structures at smaller length scale.<sup>21</sup> This conclusion could also be applied to MWCNTs. In this case, with increased density the aggregates of shorter MWCNTs tend to have greater settling velocity according to the Stokes' Law as described in Supplementary Material, and consequently become less stable than the longer MWCNTs.

As shown in Figure 5.1, MWCNTs dispersed in the NOM solution showed 28.8  $\pm$ 8.8% settling, larger than the amount of the control without NOM addition (13.3  $\pm$ 8.2%). This may resulted from the mineral salts associated with the NOM. The enhanced settling compared to control has also caused the percentage of the shorter MWCNTs to slightly decrease, however not significantly (Figure 5.2b). In contrast to the treatments without NOM present, increasing the cation concentration did not significantly increase the amount of MWCNTs settling, 33.0  $\pm$ 7.0% and 38.1  $\pm$ 6.5% for 4 and 40 mmol L<sup>-1</sup> Na<sup>+</sup>, respectively.

With the presence of NOM, even if the concentration of Na<sup>+</sup> reached 40 mmol L<sup>-1</sup>, the length distribution did not change significantly after 7 d with one exception, i.e., length range between 400 to 700 nm when NaCl concentration was 40 mmol L<sup>-1</sup> (Figure 5.1b). This phenomenon suggested the prominent ability of NOM to disperse MWCNTs in water regardless of the length. Previous studies have shown that NOM can be adsorbed on MWCNT surfaces and stabilize MWCNTs by steric hindrance and/or electrostatic repulsion.<sup>17, 22, 23</sup> Through such NOM adsorption, the MWCNTs were mostly individually dispersed and the aggregation was significantly reduced. However, the adsorption of NOM also increased the size of the MWCNTs NOM composite, which may be one of the reasons that contributed to the MWCNTs settling in the presence of NOM.

The preferential aggregation of the shorter MWCNTs could be explained by the fact that longer length may result in lower specific surface area. Lower specific surface area will in turn lead to weaker van der Waals attractions and hydrophobic interactions between MWCNTs, which result in less aggregation and settling. However, higher specific surface area will also lead to stronger interactions between NOM and MWCNTs, and consequently the smaller MWCNTs will adsorb more NOM per unit mass. This may be the reason why MWCNTs with smaller diameter are preferentially suspended in NOM solutions according to O'Driscoll et al., (2010). In our study, the adsorption of NOM compensated the extra instability of shorter MWCNTs and made them equally stable even under the "stress" of relatively high Na<sup>+</sup> concentration. <u>Hydrodynamic diameter and polydispersity of MWCNT dispersions</u>

The average hydrodynamic diameters and polydispersity factors of MWCNT dispersions at the initial state of settling and stable state after settling were shown in Table 5.1. The hydrodynamic diameter measures an effective size of a particle in liquid environment that

includes the thickness of the thin, electric dipole layer adhering to the particle surface. Thus it is usually larger than the actual size of particle measured by microscopic techniques. In addition, as mentioned before, it does not differentiate primary particles and their aggregates. However, it is an important parameter when considering particle's movement in aqueous phase. We can see in Table 5.1 that the average hydrodynamic diameter at the initial state of settling showed a significant increase corresponding to the increasing Na<sup>+</sup> concentration, which means the addition of Na<sup>+</sup> immediately initiated the aggregation process and increased the apparent size of MWCNTs, especially when the Na<sup>+</sup> concentration was high, e.g. 40 mmol L<sup>-1</sup>. However the MWCNTs aggregates with larger size were unstable and settled out during the 7-d period, leaving the smaller ones suspended in the aqueous phase. Thus the average hydrodynamic diameters decreased, and the difference between various Na<sup>+</sup> concentrations was far less pronounced as when the Na<sup>+</sup> was first added.

Polydispersity index (PDI) value indicates the extent that the MWCNTs suspension diverged from monodispersed solution that has very narrow size distribution. Given the wide length range of the MWCNTs revealed by SEM, the polydispersity in the suspensions of MWCNT is likely greater than the monodispersed spherical nanoparticles. In addition, the hydrodynamic diameter determined by the DLS method for high aspect ratio nanoparticles, such as MWCNT, will be neither the length nor the width of that particle, and is orientation-dependent, because it involves a calculation based on the assumption that the particles are spherical. The tube-like shapes orientate themselves in different ways in aqueous phase and thus appear to have wider size distribution. After 7-day settling, the polydispersity indices decreased, suggesting that the hydrodynamic sizes of the dispersed particles became more consistent. It is likely because

that the smaller MWCNTs tended to aggregate, consequently approaching the stable size range while the aggregates larger than the stable size range tended to settle.

#### **CONCLUSIONS**

Size is one important physical property of CNTs influencing many important phenomena, e.g., cellular interactions and uptake, percolation thresholds, strength of interactions with external fields, and self-assembly, and optical.<sup>24, 25</sup> The effect of MWCNTs sizes on their environmental behaviors and impact is of great significance. For example, only SWCNTs shorter than 200 nm can negatively affect the metabolism of several mammalian cell lines.<sup>24</sup> Smaller fraction of SWCNTs can reduce the life cycle of the estuarine copepod *Amiphiascus tenuiremis* and increase the mortality.<sup>26</sup> Our research showed that the smaller fraction of MWCNTs below 400-nm long is less stable than the longer fraction, and consequently easier to be removed from the aqueous phase in wastewater treatment.

However, with the presence of NOM, this preferential removal is mitigated. Besides, CNTs with smaller diameter were shown to be preferentially suspended in NOM solutions,<sup>16</sup> likely due to the larger specific area associated with smaller diameter. In addition, the settling of MWCNTs was significantly reduced by NOM with the presence of Na<sup>+</sup>. Therefore the smaller CNTs are likely to be more available for transport and dispersion in aquatic ecosystems with NOM.

Our work studied relatively small MWCNTs with the majority lengths less than 1000 nm, the lower end of colloidal particle sizes, and our results showed that the smaller size MWCNTs tend to preferentially aggregate. According to the traditional colloidal study with regard to spherical particles, the behavior of preferential aggregation differs for particles with sizes smaller and larger than 890  $\pm$  0.2 nm. For tubular shape nanoparticles like MWCNTs, whether there is

such a transition in preferential aggregation and what is the threshold size is an interesting topic for further investigation.

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FIGURE 5.1 Mass percentage of MWCNTs settled during experiment process with different aqueous conditions, i.e., NaCl concentration and NOM addition. The error bars represent the standard deviations of the triplicates. The different letters (i.e., a and b) above the bars indicate statistically significant difference with each other, at the confidence level of 0.1 according to the pair-wised comparisons among conditions across the same length range using Tuckey's test.



FIGURE 5.2 Length distributions of MWCNTs with different aqueous conditions after settling for 7 d, as measured by SEM. "NOM" in (b) denotes treatments with peat NOM in solution and "Blank" in (a) and (b) means without NOM in the solution. The concentrations indicated i.e., 0, 4 or 40 mM (mmol  $L^{-1}$ ), are for the NaCl added. The error bars represent the standard deviations of the triplicates. The different letters (e.g., a and b) above the bars indicate statistically significant difference with each other, at the confidence level of 0.05 according to the pair-wised comparisons among conditions across the same length range using Tuckey's test.

	Initial state		Stable state	
Na+ conc.	Z-average*	Z-average*		
(mmol L		Polydispersity		Polydispersity
1)	<b>d</b> (nm)	index	<b>d</b> ( <b>nm</b> )	index
0	$162.3(1.8)^{a}$	0.302	190.9 (1.9) <sup>A</sup>	0.272
4	202.8 (9.5) <sup>b</sup>	0.305	200.4 (1.3) <sup>B</sup>	0.278
40	818.9 (20.7) <sup>c</sup>	0.375	250.5 (2.9) <sup>C</sup>	0.288

TABLE 5.1 Hydrodynamic diameters and polydispersity indices of the initial state and stable state of MWCNTs under different NaCl concentrations.

<sup>\*</sup> Values in parentheses are standard error. The Z-average diameter defines as the 'harmonic intensity averaged particle diameter'. It is an intensity-based calculated value also known as the cumulants mean. The calculation is defined in ISO 13321 and ISO 22412. The different letters in superscript (e.g., a, b or A, B) indicate statistically significant difference with each other, at the confidence level of 0.05 according to the pair-wised comparisons among various Na<sup>+</sup> concentrations using Tuckey's test.

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# CHAPTER VI

# DEGRADATION OF MULTIWALL CARBON NANOTUBES BY BACTERIA $^{\rm 4}$

<sup>&</sup>lt;sup>4</sup> Zhang, L.; Petersen, E.J.; Habteselassie, M.Y.; Mao, L. and Q. Huang. Accepted by *Environmental Pollution*, June 2013. Reprinted here with permission of the publisher.

#### ABSTRACT

Understanding the environmental transformation of multiwall carbon nanotubes (MWCNTs) is important to their life cycle assessment and potential environmental impacts. We report that a bacterial community is capable of degrading <sup>14</sup>C-labeled MWCNTs into <sup>14</sup>CO<sub>2</sub> in the presence of an external carbon source via co-metabolism. Multiple intermediate products were detected, and genotypic characterization revealed three possible microbial degraders: *Burkholderia kururiensis, Delftia acidovorans,* and *Stenotrophomonas maltophilia*. This result suggests that microbe/MWCNTs interaction may impact the long-term fate of MWCNTs.

#### **INTRODUCTION**

Carbon nanotubes (CNTs) are one type of carbon-based nanomaterial formed by rolling graphene sheet(s) into a cylindrical shape. Multiwall carbon nanotubes (MWCNTs) are a series of coaxially-arranged graphene sheets. Due to their unique physicochemical, optical, and mechanical properties, CNTs can be applied to many fields such as reinforced composites, conductive materials, sensors, drug delivery vessels, and sorbents.<sup>1</sup> Research on their ecotoxicity, aggregation and transport has been conducted in recent years to provide information on their impact to and fate in the environment.<sup>2-4</sup> One critical question is the extent and rate of CNT biodegradation to CO<sub>2</sub>, which has not yet been fully investigated due to their high chemical stability and challenges with CNT quantification.

The basic structure of MWCNTs is aromatic rings fused by sp<sup>2</sup>-hybridized carbon which is analogous to polycyclic aromatic hydrocarbons (PAHs) and considered to be stable. However, defects such as pentagon-heptagon pairs (Stone-Wales defects), sp<sup>3</sup>-hybridized carbon atoms, vacancies in the nanotube lattice, and open ends are always associated with CNTs.<sup>5-8</sup> These defects are expected to make CNTs more reactive,<sup>8,9</sup> and thus may serve as sites that enzymes can attack. Researchers have shown that fullerols can be degraded by two types of white rot fungi,<sup>10</sup> and carbon nanotubes can be degraded by an enzyme, horseradish peroxidase (HRP)<sup>11-14</sup> and by neutrophil myeloperoxidase<sup>15</sup> in the presence of H<sub>2</sub>O<sub>2</sub>. However, the extent to which MWCNTs can be degraded by microbes under natural conditions is unknown. In this study, we used <sup>14</sup>C-labeling to trace the end product of MWCNTs microbial degradation by measuring the released <sup>14</sup>CO<sub>2</sub>, and report a bacterial community that is capable of degrading MWCNTs into CO<sub>2</sub>.

#### MATERIALS AND METHODS

# <sup>14</sup>C labeled Multiwall Carbon Nanotubes (MWCNTs) Synthesis and Characterization

The <sup>14</sup>C-labeled MWCNTs were synthesized using a modified chemical vapor deposition technique,<sup>16</sup> purified, and treated with a 3:1 (volume fraction) mixture of sulfuric to nitric acid as described in our previous study (see Supplementary Material I for details).<sup>17</sup> The <sup>14</sup>C accounts for ca. 0.002% of the total MWCNT carbon. These MWCNTs were dispersed stably in water with a concentration of 9.5 mg L<sup>-1</sup> by ultrasonication for 2 h and were previously thoroughly characterized.<sup>18</sup> The obtained MWCNTs have a surface oxygen content of 8.6% determined by X-ray photoelectron spectroscopy (XPS) (Kratos Analytical Axis Ultra X-ray photoelectron spectrometer). See Supplementary Material I for additional characterization information.

#### MWCNTs Degradation Experiments

The experiment set-up is briefly summarized as follow (see Supplementary Material II for details). In the treatment flasks, a 5-mL dispersion of unsterilized <sup>14</sup>C-labeled MWCNTs were added to 45 mL of a defined culture medium (composition listed in Table S6.1) containing

all common nutrients required for microbial growth and incubated at 39 °C for 7 d. No additional microbe source was added to the medium. The different concentrations of the 5-mL MWCNTs were achieved by diluting the stock dispersion using sterilized de-ionized water. The flask was connected to a test tube containing NaOH solution (10 mL, 0.5 mol  $L^{-1}$ ) to capture CO<sub>2</sub> if any evolved from the incubation. The system was aerated daily with O<sub>2</sub> (34 KPa) via the gas inlet for 30 min. Control flasks contained the same substances except 1) with sterilized MWCNTs, or 2) without MWCNTs but with the MWCNTs degrading bacteria from treatment flasks. After 7 d, the NaOH solution was mixed with 10 mL of scintillation cocktail (Insta-Gel Plus, PerkinElmer, MA), and its radioactivity was measured by a Beckman LS 5801 liquid scintillation counter (CA, U.S.). The radioactivity readings of the two controls were not significantly different from solutions with deionized water or the NaOH solution mixed with Insta-Gel cocktail. The degradation mass or percentage was obtained by subtracting the radioactivity of the treatment samples by the background radioactivity from the control samples. The bacteria concentration after 7-d incubation was determined using light absorbance at 650 nm to be approximately 9.8  $\pm$  $4.7 \times 10^8$  cell mL<sup>-1</sup> (n = 3, uncertainty value represents the standard deviation) for treatment flasks; no significant microbial population was observed in control 1. Microorganisms were identified as described in the Supplementary Material V.

#### Intermediate Product Identification

The detection of possible intermediate products was performed by liquid chromatography-tandem mass spectrometry (LC-MS/MS, Waters Micromass Quattro) and gas chromatography-mass spectrometry (GC-MS, Hewlett Packard 5971). Detailed sample preparation methods and instrument set-up were included in Supplementary Material III. The structures of the detected intermediate product were deduced according to their MS/MS spectra (example shown in Supplementary Material III).

### **RESULTS AND DISCUSSION**

# <sup>14</sup>C-labeled MWCNTs Microbial Degradation.

The <sup>14</sup>C-labeled MWCNTs were incubated in various cultures under different conditions in an attempt to screen MWCNT-degrading microorganisms (see Supplementary Material II). The incubation was conducted in a setup that allowed the capture of the released end product, <sup>14</sup>CO<sub>2</sub>. Among the different systems tested, we observed microbial activity and significant MWCNTs degradation in the system that had unsterilized MWCNTs in a sterilized culture medium. Figure 6.1 shows the quantity and percentage of MWCNTs that were released as <sup>14</sup>CO<sub>2</sub> (radioactivity values are provided in Table S6.2) after 7 d of incubation with different initial MWCNTs concentrations (0.06 to 1.0 mg L<sup>-1</sup>). In each case, a significant fraction of MWCNTs, ranging from 2.0% to 6.8%, was transformed into <sup>14</sup>CO<sub>2</sub>. Scanning electron microscope (SEM, FEI Inspect F50 FEG) and transmission electron microscope (TEM, FEI Technai 20) were used to characterize any morphological changes of the MWCNTs after incubation (see Supplementary Material VI Figure S6.6 and S6.7). The remaining MWCNTs showed different degrees of oxidation, but most retained a tubular shape.

#### Degradation Pathway

Although the ultimate end product of the microbial degradation of MWCNTs is  $CO_2$ , degradation products other than or as precursors of  $CO_2$  are likely to exist. We extracted the culture media after 7-d incubation using ethyl acetate or dichloromethane and analyzed the extracts by HPLC-MS or GC/MS (Supplementary Material III). Figure 6.2 shows selected ion chromatograms of LC-MS for two intermediate products and those of additional four products

are shown in Figure S6.3, as well as the original LC chromatographs. All figures were obtained by subtracting the chromatograms of the treatment by the chromatograms of two controls: one was incubation without bacteria and the other was without MWCNTs. Table 6.1 summarizes the products identified by LC-MS/MS as well as their deduced molecular formulas and structures, including 2-naphthol, 2-methoxy naphthalene, isophthalic acid, and cinnamaldehyde. These intermediate products were confirmed by comparing their LC-MS/MS spectra to those of high purity (> 99 %, Sigma Aldrich) chemical standards. The LC-MS/MS spectra comparison for isophthalic acid is shown in Figure 6.2 and those for other products are given in Figure S6.5. Some of the same molecular ions, such as m/z = 165, were also detected by GC-MS. These various oxidized organic compounds residing in the degrading mixture are similar to those found during microbial degradation of PAHs under aerobic conditions,<sup>19</sup> and HRP degradation of single-walled carbon nanotubes.<sup>14</sup> The multiple intermediate products are likely due to the complexity of the degradation process, in that they came either from different steps in pathways or from parallel steps mediated by different enzymes/microbes. The presence of intermediate products indicates that small molecules were first flaked off from MWCNTs and then subjected to further degradation. This is similar to the depolymerization step in the microbial degradation of biopolymers which is also the rate-limiting step.<sup>20</sup>

Given the large size of MWCNTs, the first degradation step is most likely to occur extracellularly through enzymatic reaction. Three types of extracellular enzymes were tested: horseradish peroxidase (HRP type I, Sigma-Aldrich), which has been reported to degrade MWCNTs,<sup>11, 12, 14</sup> laccase (Sigma-Aldrich) and tyrosinase (Sigma-Aldrich), which are from representative PAH-degrading enzyme categories;<sup>19</sup> a detailed experimental method is provided in Supplementary Material IV. However, none of the enzymes resulted in significant production

of <sup>14</sup>CO<sub>2</sub>, which shows that these enzymes alone cannot degrade the MWCNTs into CO<sub>2</sub>. Interestingly, a previous study showed significant removal of MWCNTs by HRP.<sup>11, 12</sup> These two different results may be due to the different properties of MWCNTs used, such as the species and contents of the surface functional groups and/or defects. For example, the catalytic pathway of HRP and laccase often involves radicals formed by phenol groups,<sup>21, 22</sup> and thus the hydroxyl groups on MWCNTs surface are very likely to be sites attacked by these enzymes. On the contrary, the MWCNTs with large portion of carboxyl groups may not be vulnerable to these enzymes. This is an interesting area for further investigation and would have strong potential implications in environmental risk assessment.<sup>12, 23, 24</sup>

#### Microorganism Identification

Since the medium that resulted in MWCNTs biodegradation described above had other carbon sources in it (*e.g.*, glucose, veratryl alcohol), we repeated the biodegradation study in which MWCNTs were the sole carbon source to selectively enrich the microorganisms that specifically degrade MWCNTs. However, after incubation for two months, no <sup>14</sup>CO<sub>2</sub> release was detected, indicating that the need for additional carbon source for the MWCNTs biodegradation to proceed via co-metabolism.

Because selective enrichment of MWCNTs degrader(s) in the sole carbon source medium was not possible, we used polymerase chain reaction (PCR)-based technique to survey the microbial pool in the culture. The genomic DNA of the microorganisms was extracted, purified, and then subjected to PCR by targeting the 16S-rDNA with a universal bacterial primer pair.<sup>25</sup> The fungi universal primer pair nu-SSU-0817F and nu-SSU-1196R<sup>19</sup> was also used but no product was detected, which indicated that the potential degraders were all bacteria. The PCR amplicons were then separated using cloning, sequenced, and compared with sequences in the

GenBank database of the National Center for Biotechnology Information (NCBI) by BLAST search. Three bacteria species were identified: *Burkholderia kururiensis, Delftia acidovorans*, and *Stenotrophomonas maltophilia*. These bacteria are commonly found in the natural environment (*e.g.*, ground water, surface water, soil rhizosphere, *etc.*), and previous studies have shown their capability to degrade various persistent organic contaminants. *D. acidovorans* has been shown to degrade sulfophenylcarboxylates and herbicides such as linuron, phenoxypropionate, and phenoxyacetate,<sup>26-28</sup> either individually or in community. *S. maltophilia* is known to metabolize phenols, benzoic acids and complex compounds like PAHs and oil refinery residuals.<sup>29-31</sup> In addition, the combinations of strains from these species were able to degrade more compounds than when they are present individually. For example, *S. maltophilia* in combination with other species in *Burkholderia* genus were found to degrade chemicals such as PAHs, 2,4-DNT, and dodecyldimethylamine.<sup>32, 33</sup>

In order to confirm their ability to degrade MWCNTs, we made an attempt to isolate the individual bacteria and incubated them with MWCNTs. To this end, the mixed culture was diluted ( $10^{-3}$ ,  $10^{-4}$ ,  $10^{-5}$ , and  $10^{-6}$ ) and spread-plated on a TGY (tryptone/glucose/yeast) medium (1% tryptone, 0.5% yeast extract, 0.1% glucose, and 2.0% agar). Fifteen well isolated colonies from the plates were selected and identified using the PCR-based technique described above. The results showed that they belonged to two of the three previously identified isolates, *Burkholderia kururiensis* and *Stenotrophomonas maltophilia*. The pure cultures of *B. kururiensis* and *S. maltophilia* (100 µl, cell concentration approximately  $10^8$  cell mL<sup>-1</sup>) were then inoculated either individually or together into the medium containing MWCNTs (51.5 µg MWCNTs in 50 mL medium) and glucose (the same as MWCNTs degradation study, Table S6.1) to test their ability to degrade MWCNTs. No production of <sup>14</sup>CO<sub>2</sub> was detected with *B. kururiensis*. Inoculation of *S.* 

*maltophilia*, however, resulted in some production of <sup>14</sup>CO<sub>2</sub>, *i.e.*, 0.57 %  $\pm$  0.20 % (n=3; uncertainty indicates standard deviation) at an initial MWCNT concentration of 0.6 mg L<sup>-1</sup>. This was ten times lower than that of the original bacteria community in the mixed culture, and could not be significantly enhanced by varying incubating conditions, including addition of the isolated *B. kururiensis*. This suggests that a community of microorganisms containing more than the two isolated strains is required to effectively degrade MWCNTs; and the missing species could not probably be isolated under the culture conditions we have used.

#### **CONCLUSIONS**

In this study, we demonstrated the ability of some bacteria to degrade acid-treated MWCNTs under environmentally relevant conditions, which would decrease their environmental persistence. This degradation appears to require external carbon source involving co-metabolism and the cooperation of several microorganisms. Additional research is needed to further explore the degradation potential of the MWCNTs in the natural environment, and to elucidate possible dependence of MWCNTs biodegradation on their surface chemistry.

#### ACKNOWLEDGEMENT

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(NIST) nor does it imply that any of the materials, instruments or equipment identified are necessarily the best available for the purpose.

#### Degraded MWCNT Mass (µg) Mass transformed to CO<sub>2</sub> Percentage transformed to CO<sub>2</sub> Percentage (%) Initial MWCNT Mass (µg)

FIGURES AND TABLES

FIGURE 6.1 Biodegradation of <sup>14</sup>C-MWCNTs after 7-d incubation at different initial dosages. Left y-axis shows the mass of MWCNTs that has been fully degraded (calculated based on the amount of <sup>14</sup>CO<sub>2</sub>, values shown in Table S2). Right y-axis shows the percentage (by mass) of biodegraded MWCNTs. Error bars of the two highest initial concentration show the propagated standard deviation of uncertainty from the scintillation counter and two replicates. Single measurements were performed at the lower concentrations and thus the error bars only include instrument variability.



FIGURE 6.2 Selected ion chromatograms of LC-MS and spectra of LC-MS/MS for two intermediate products. a) Molecular ion mass of 165, extracted by ethyl acetate; b) molecular ion mass of 117, extracted by dichloromethane. The red lines relate to the samples degraded by microbes, while the blue lines and black lines are the controls incubated with culture medium but without MWCNTs or without microbes, respectively. The secondary MS spectra of: c) the intermediate products with m/z value in the primary MS as 165; d) comparison between intermediate product (up) and high purity chemicals purchased from Sigma-Aldrich (down) with m/z = 165; The structures of the intermediate product are shown in the upper-left corner. Structures of the other products are listed in Table 6.1.

# TABLE 6.1

Extraction Solvent	m/z	Formula	Structures
	143	C <sub>10</sub> H <sub>8</sub> O	ОН
	157	$C_{11}H_{10}O$	
Ethyl acetate	165	$C_8H_6O_4$	ощон он
	171	$C_{11}H_8O_2$	о
Dichloromethane	117	$C_{5}H_{10}O_{3}$	ОН
	131	C <sub>9</sub> H <sub>8</sub> O	

Summary of intermediate products identified by LC-MS/MS

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## CHAPTER VII

# EFFECT OF TITANIUM DIOXIDE NANOPARTICLES ON THE WHEAT SPECIES,

# TRITICUM AESTIVUM L.<sup>5</sup>

<sup>&</sup>lt;sup>5</sup> Zhang, L.; Petersen, E.J.; Hu, C.; Johnson, J. and Q. Huang. To be submitted to *Environmental Health*.

#### **ABSTRACT**

As one of the first engineered nanoparticles detected in the environment, nano-sized titanium dioxide (nano-TiO<sub>2</sub>) raised numerous concerns, such as their bioavailability and toxicity to various ecological receptors. Crops are of particular importance among the receptors of concern because they are source of staple food directly consumed by human and other species. To investigate the possible effect of nano-TiO<sub>2</sub> to wheat *Triticum aestivum* L., seeds of two cultivars were planted in a soil spiked with different types and concentrations of nano-TiO<sub>2</sub> and incubated for 28 days. Results and statistical analysis show that the photosynthesis efficiency of wheat was not affected by nano-TiO<sub>2</sub> across the conditions tested, whereas the root and shoot length showed different responses which varied from elongation (500 mg kg<sup>-1</sup> soil, root only) to neutral (150 mg kg<sup>-1</sup> soil), and to reduction (5 and/or 50 mg kg<sup>-1</sup> soil) depending on the treatment conditions. The response of dry biomass follows the same trend with root or shoot length but at less magnitude. Degussa P25 nano-TiO<sub>2</sub> affected the plant growth more significantly than anatase nano-TiO<sub>2</sub>. The spiking method, i.e., mixing soil with nano-TiO<sub>2</sub> powder or dispersion, also showed different influences likely due to different actual particle sizes resulting from different preparation procedures. Between the two wheat cultivars, Pioneer® variety 26R61 was less sensitive than the AGS 2000 under the same conditions, indicating different resistant mechanisms. One possible cause of the toxic effect is the oxidative stress induced by nano-TiO<sub>2</sub>, and the peroxidase activity could serve as a sensitive indicator. The uptake and translocation of nano-TiO<sub>2</sub> to root and shoot were also detected. This study provides information for environmental risk assessment and potential applications of nano-TiO<sub>2</sub>.

#### **INTRODUCTION**

Titanium dioxide (TiO<sub>2</sub>) has a long history of application and its basic chemical/physical properties have been thoroughly studied. TiO<sub>2</sub> naturally occurs as crystalline phases: anatase, rutile, and brookite, in which anatase and rutile are the most commonly encountered.<sup>1</sup> Rutile is the thermodynamically stable form of TiO<sub>2</sub> under normal pressures. Its melting point ranges from 1830  $\$  to 1850  $\$ .<sup>1</sup> Phase change from anatase to rutile occurs upon heating before melting, across a broad temperature range of about 500  $\$  to 900  $\$ .<sup>1</sup> The refractive indexes of rutile and anatase are approximately 2.75 and 2.54, respectively.<sup>1</sup> Because of their thermal stability and refractive indexes, macroscale TiO<sub>2</sub> has been used as white pigment for centuries.

When the size reduces to nanoscale, the physical and chemical properties of  $TiO_2$  change dramatically, such as largely increased reactivity due to the increased specific surface area and free surface electrons.<sup>2</sup> Contrary to pigmentary  $TiO_2$  which is a visible light attenuator, nanosized titanium dioxide (nano- $TiO_2$ ) is a UV light attenuator.<sup>1</sup> Nano- $TiO_2$  has been utilized in many fields. Consumer products that use nano- $TiO_2$  to attenuate UV light include sunscreens or similar cosmetics, various plastic-based products and containers, and clothing. Applications that treat nano- $TiO_2$  as a catalyst include cleaning products, self-cleaning coatings, air filtration devices, electronics (e.g., computer keyboard and mouse), and hair styling devices. Emerging applications of nano- $TiO_2$  also include environmental remediation of pollutants and solar cells that use nano- $TiO_2$  for its electron transfer properties.

With the development of nanotechnology the production of nano-TiO<sub>2</sub> has been and will be growing fast in these decades.<sup>3, 4</sup> As mentioned in Chapter I, nano-TiO<sub>2</sub> has already been detected in the environment.<sup>5, 6</sup> Therefore information on the ecotixicity of nano-TiO<sub>2</sub> is necessary to assess its environmental risk and to better exploit its promised benefits.<sup>7</sup> Compare to

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aquatic and marine species, the effect of TiO<sub>2</sub> to terrestrial organisms is less known.<sup>8,9</sup> Vascular plants are of particular importance because they constitute one of the main exposure routes for higher species like human, i.e., directly consumption by human or accumulation through the food chain. Moreover, plants closely contact with soil, water and the atmosphere, which makes them highly possible to receive the exposure of nano- $TiO_2$  released in various environmental compartments. Thus the concerns on the interactions between nano-TiO<sub>2</sub> and vascular plant have been heightened in recent year; but contradictory evidences have been obtained in different studies that added to its complexity. On one hand, toxic effects of nano-TiO<sub>2</sub> (100 nm) have been observed in two plant species, Allium cepa (onion) and Nicotiana tabacum (tobacco), including induced DNA damage, inhibited growth, and increased lipid peroxidation.<sup>10</sup> On the other hand, Klančnik reported that nano-TiO<sub>2</sub> (size of 15 nm, anatase) had no effect on macroscopic parameters (number of roots per bulb, the average length of roots, etc.) and microscopic parameters (shares/portions of mitotic phases, chromosome aberrations, etc.) of Allium cepa up to 1 mg  $L^{-1}$ , but affected the mitotic index in root tips.<sup>11</sup> Beneficial effects of nano-TiO<sub>2</sub> on plant growth have also been reported. For example, TiO<sub>2</sub> nanoparticles were shown to improve the growth of spinach by enhancing photosynthesis in leaves,<sup>12</sup> nitrogen-absorbing capability in roots,<sup>13</sup> increasing the electron transfer, oxygen evolution, and photophosphorylation of chloroplast (Chl) under visible light and ultraviolet light illumination.<sup>14</sup> These variable results suggest that the impact of nano-TiO<sub>2</sub> on plants may be dependent on multiple factors that have not been fully understood, and some species are either more resistant or susceptive to nano- $TiO_2$ exposure.

Wheat (*Triticum spp.*) is a cereal grain cultivated worldwide. In 2012, world production of wheat was 655.43 million tons, making it the third most-produced cereal after maize and

rice.<sup>15</sup> Thus the possible effect of nano-TiO<sub>2</sub> to wheat is an important topic to investigate. Larue et al. reported that 14 nm or 25 nm anatase nano-TiO<sub>2</sub> exposure (10, 50, and 100 mg  $L^{-1}$ ) with hydroponic condition induced increased root elongation but did not affect germination, evapotranspiration, and plant biomass.<sup>16, 17</sup> Jacob et al. also found exposure to TiO<sub>2</sub> nanoparticles  $(0, 6, and 18 \text{ mmol Ti } L^{-1})$  did not affect biomass of wheat.<sup>18</sup> Other study showed that nano-TiO<sub>2</sub> at certain concentration (2 and 10 ppm) could promote the seed germination of wheat in comparison to bulk TiO<sub>2</sub>, but at high concentrations (100 and 500 ppm) it had no or inhibitory effect on germination.<sup>19</sup> However, these studies have been conducted under hydroponic conditions, whereas soil is a very different medium and more complicated than the defined hydroponic culture; thus the conclusions of hydroponic studies cannot be simply extrapolated to soil systems. For example, the soil exposure of silver nanoparticles (AgNPs) caused less germination inhibition than hydroponic exposure.<sup>20</sup> In a study comparing the effect of AgNPs to Phaseolus radiatus in agar or soil as the growth medium, the growth rate was not affected in the soil whereas concentration-dependent growth inhibition was observed in agar.<sup>21</sup> These differences was attributed to that the properties of nanoparticles have been changed in soil, which in turn changed the toxicity to plants. As soil is actually used in crop growing, it is of great importance to elucidate the effect of nano-TiO<sub>2</sub> in experiments using soil as the growth medium.

In this study, we tested the effect of nano-TiO<sub>2</sub> of different dosages to the growth of wheat at early stage (28 days) using indicators including photosynthesis efficiency, root and shoot length, and dry biomass. The contributions of wheat genotype, nano-TiO<sub>2</sub> type, spiking method, and cultivation time were investigated. Because nano-TiO<sub>2</sub> is photocatalyst, we hypothesize that it can cause oxidative stress to wheat in soil. To test this hypothesis, two indices

of plant responses to oxidative stress, i.e., lipid peroxidation and peroxidase activity were measured. Plant uptake and translocation were also quantified. ANOVA analysis and pair-wise comparison (Tuckey's test) were performed by SAS 9.2 to elucidate the significance of the results at confidence level of 0.05.

#### MATERIALS AND METHODS

#### Nano-TiO<sub>2</sub> Spiked Soil

The top-layer (20 cm) soil was collected from Griffin, Georgia, dried under room temperature for 10 days, and sieved using 2-mm (9-mesh) sieve. The soil was classified as loamy sand with the sand, silt and clay contents of 85.8%, 10.2% and 4.1%, respectively. The pH value was 5.14. The organic carbon concentration was 0.5%, and the contents of native Ti element were 608.9 mg kg<sup>-1</sup>.

Two types of nano-TiO<sub>2</sub> were applied in this study. The SRM-TiO<sub>2</sub> (Standard Reference Material® 1898, Degussa P25, <100 nm) was purchased from The National Institute of Standards & Technology (NIST) which has been fully characterized and applied in eco-toxicology studies of nano-TiO<sub>2</sub> (<u>https://www-s.nist.gov/srmors/view\_detail.cfm?srm=1898</u>).<sup>22</sup> NIST standard reference materials also help with inter-laboratory comparisons. This nano-TiO<sub>2</sub> contains 76 ± 3% anatase and 24 ± 3% rutile with crystallite size of 19 ± 2 nm and 36 ± 6 nm, respectively. The point of zero charge is between 7 and 7.1, thus can be stabilized electrostatically in acidic media at low ionic strength. The anatase nano-TiO<sub>2</sub> (<25 nm, purity 99.7%) was purchased from Aldrich.

The nano-TiO<sub>2</sub> spiked soil samples were prepared using two methods. In the "powder spiked" method, the nano-TiO<sub>2</sub> powder was grinded and directly mixed with soil using mixer; while in the "dispersion spiked" method the nano-TiO<sub>2</sub> was first dispersed in deionized water

and then mixed with soil. The dispersions were prepared according to Taurozzi, et al.<sup>23</sup> In brief, TiO<sub>2</sub> powder of 10 g was suspended in 1000-mL deionized water (> 18 M $\Omega$ ·cm) by ultrasonication (60%, equivalent power approximately is ~50 W) for 15 min using an 80 % on / 20% off pulsed operation mode. The aqueous dispersions were transferred to 1000-mL amber glass bottles and stored free from UV light at ambient temperature. The hydrodynamic diameter of the dispersions was 113.9 ± 5.1 and 278.3 ± 19.0 nm (n = 3, uncertainties indicate standard deviations) for SRM- and Aldrich-TiO<sub>2</sub>, respectively, determined by dynamic light scattering (Malvern Zetasizer Nano ZS, UK). The zeta-potential that is related to the surface charge was -15.33 ± 1.75 and -11.37 ± 1.01 (n = 3) for SRM- and Aldrich-TiO<sub>2</sub>, respectively. The TiO<sub>2</sub> dispersion was serial diluted and mixed with the sieved soil in mixer (concentration from low to high) with water to soil ratio as 100 mL to 1 kg. The resulted concentrations were 5, 50, 150, and 500 mg kg<sup>-1</sup> soil. Control soil samples were mixed with equal amount of deionized water. Approximately 300-g (dry weight) soil was transferred to each pot for exposure experiment. The pot size was 8 cm × 8 cm.

#### Wheat Genotypes and Exposure

Two genotypes of wheat, AGS 2000 (AGS) and Pioneer® variety 26R61 (PR), were studied. They both belong to the soft red winter wheat and are considered two standard wheat varieties. Six seeds were planted in each pot in approximately 1-cm depth containing TiO<sub>2</sub> concentration as designed. The experiment was conducted using full factorial design for most of the factors except for dosage. Each treatment was triplicated. The experiment was carried out in a growth chamber under controlled conditions: day/night photoperiod (16/8 h), light intensity of ~140  $\mu$ mol m<sup>-2</sup>s<sup>-1</sup>, with the temperature of 23 °C for day period and 16 °C for night period. The pots were watered thoroughly every day.

#### Effect of Nano-TiO<sub>2</sub> on Plant Growth

After 14 d and 28 d, the photosynthesis efficiency was evaluated by fluorescence induction monitor (FIM 1500 Analytical Development Company Limited, UK) according to the procedures. Each leaf was clipped in to a leafclip (32 mm wide and 80 mm long) with the shutter plate closed to induce dark-adaption for 30 min. An array of six high intensity light emitting diodes (LEDs) provided red light with a peak wavelength of 650 nm to illuminate the exposed leaf area (4 mm diameter) with a maximum intensity of approximately 3000 m<sup>-2</sup>s<sup>-1</sup>.

After recording the measured fluorescence parameters, the soil was carefully removed from the root surface followed by extensive washing of the root by water. The root and shoot lengths of each wheat plant were measured using a ruler. The total length of primary roots was recorded. Four out of the six plants in each pot were oven-dried at 105 °C overnight and their dry masses were measured. Some of the oven-dried samples were microwave digested according to EPA method 3052: microwave assisted acid digestion of siliceous and organically based matrices, and measured using inductively coupled argon plasma axially viewed atomic emission spectrometer (ICP-AVAES, Spectro Analytical Instruments, Inc., Mahwah N.J.).

Two fresh plants out of each pot were weighed, cut into small pieces and the possible effect of oxidative stress was evaluated by measuring lipid peroxidation and peroxidase activity. Lipid peroxidation (LPO) was determined by measuring the amount of malondialdehyde (MDA).<sup>10</sup> An amount of 0.1 g root or shoot tissues from the control and treated plants were mixed with 1 mL of 5% trichloro-acetic acid (TCA) solution, transferred into fresh tubes and centrifuged at 6000 g for 15 min at ambient temperature. Then 0.75 mL of the supernatants were mixed with equal volumes of 0.5% thiobarbituric acid (TBAR) in 20% TCA solution (freshly prepared) in a new tube and incubated at 96 °C for 25 min. The tubes were instantly transferred

into ice bath and incubated for 10 min and then centrifuged at 8000 g for 5 min.

Malondialdehyde (MDA) content was determined using the absorbance of the supernatant at 532 nm (Beckman UV-vis spectrometer) and corrected for non-specific turbidity by subtracting the absorbance at 600 nm.<sup>10</sup> 0.25% TBAR in 10% TCA solution was used as the blank. The extinction coefficient of MDA is 155 mM<sup>-1</sup> cm<sup>-1</sup>. The peroxidase activity was measured using a colorimetric assay in which 2,2'-Azino-bis(3-Ethylbenzthiazoline-6-Sulfonic Acid) (ABTS) was transformed to oxidized form mediated by peroxidases. Fresh root or shoot samples of 0.2 g were homogenized in 5 ml of cold 50 mM Na-phosphate buffer (pH 7.0).<sup>24</sup> The homogenates were centrifuged at 6000 g for 15 min and the supernatant was used for the assay. The assay mixture in a total volume of 3.4 mL containing 3.0 mL 2 mM ABTS in 10 mM pH 6.0 Na-phosphate buffer, 100  $\mu$ l sample, and the assay was started by adding 0.3 mL 10 mM H<sub>2</sub>O<sub>2</sub> to the cuvette. The absorbance was recorded every 10 s for 60 s at 405 nm. The extinction coefficient is 18.6 mM<sup>-1</sup> cm<sup>-1</sup>.

#### Statistical Analysis

Data obtained from experiments was summarized into descriptive statistics (mean, range, standard deviation), and reported in appropriately formatted graphs and tables. Analysis of variance (ANOVA) on the data to differentiate effects observed in various treatments within the same experiment was performed using SAS ver. 9.2 at the 95% significance level. Correlation analysis was employed to investigate possible correlations between root or shoot lengths with influential factors.

#### **RESULTS AND DISCUSSIONS**

#### Effect of Nano-TiO<sub>2</sub> Exposure on Plant Growth

The effect of nano-TiO<sub>2</sub> exposure with different concentrations (5 to 500 mg kg<sup>-1</sup> soil) on wheat growth was investigated by evaluation of the photosynthesis efficiency, root and shoot length, and dry biomass (Table 7.1 and Table 7.2). The two wheat cultivars maintained active photosynthetic capacity under the various conditions tested in this study, as indicated by the constant levels of initial chlorophyll fluorescence  $(F_0)$ , maximum fluorescence  $(F_m)$  and the variable to maximum fluorescence  $(F_v/F_m)$  (Figure 7.1). The differences between two cultivars (AGS and PR), two growing time periods (14 and 28 days), and two spiking methods (dispersion or powder spiking) were not significant and thus the data were not all presented. Photosynthesis involves a process of light harvesting to provide energy for synthesizing organic compounds. Since the energy in a photon is high for cellular molecules, the absorbed energy beyond the photosynthesis requirement must be dissipated; otherwise the photosynthetic apparatus can be damaged. Especially when under stress, plants utilize less light energy for photosynthesis.<sup>25</sup> Thus, the plants under stress need to have an active system for efficient allocation of excess energy to protect photosynthetic systems, which can be achieved by excitation transfer to neighboring pigments, thermal de-excitation, and quenched into chlorophyll fluorescence emission (as indicated by the F values in this study), particularly in photosystem II (PSII) and subsequent electron carriers.<sup>26, 27</sup>  $F_0$  is the initial fluorescence measured at the onset of illumination and its sources include *Chl a* molecules in PSII and attached light harvesting complex.<sup>25</sup> In contrast to  $F_0$ ,  $F_m$  is measured after excess light illumination and achieved when the electrons were transferred from the PSII reaction center to other acceptors (i.e., pheophytin a,  $Q_A$ ,  $Q_B$ , and PQ pool) and the acceptors were fully reduced.<sup>28</sup> The decrease in  $F_{\rm m}$  usually suggests irreversible or

slowly reversible damage to the photosynthesis system, which is termed "photoinhibition".<sup>27</sup> The  $F_v/F_m$  ratio is related to the quantum efficiency (enhanced carbon assimilation and oxygen evolution per quantum use) and consequently higher biomass production. The range of  $F_v/F_m$  for various monocot and dicot families with different life styles has been reported to be from 0.80 (early in the growing season) to 0.60 or less (latter part of growing season).<sup>25, 29</sup> The high levels of  $F_v/F_m$  (0.79–0.84 across all tested conditions, Table 7.3) indicate that nano-TiO<sub>2</sub> did not impair the photochemical efficiency at the tested levels in this study.

The effect of nano-TiO<sub>2</sub> to the root and shoot length depends not only on the dosage but also on the other factors including wheat genotype, type of nano-TiO<sub>2</sub>, spiking method and growth time (Table 7.1 and 7.2). To better illustrate the effects of these factors and comparison between different treatments, all figures were normalized by the corresponding controls to show relative changes. Figure 7.2 is the dose-response relationship between nano-TiO<sub>2</sub> and the root or shoot length of the wheat cultivar AGS2000 in soil spiked with SRM-TiO<sub>2</sub> dispersion. According to Figure 7.2 and Table 7.2, the root length of AGS2000 was reduced after 28 d by ~40% or ~20% of corresponding control at low dosages (5 or 50 mg kg<sup>-1</sup>), not affected at 150 mg kg<sup>-1</sup> and enhanced by ~90% at 500 mg kg<sup>-1</sup>. This trend was similar between the results of 14 and 28 days. Similarly the shoot length was also reduced at two lower dosages across the incubation period, ~60% and ~30% respectively. However the enhancing effect at 500 mg kg<sup>-1</sup> dosage was absent for shoot; instead at 14 days decreased shoot lengths (~40%) were observed. This may suggest that the high dosage of SRM-TiO<sub>2</sub> (500 mg kg<sup>-1</sup>) can cause preferential root growth in AGS wheat cultivar during the early stage (<14 d) and thus retarded shoot growth, but such effect disappeared after further growth to 28 days. Our result is different from those drawn from studies using hydroponic media. Several investigators reported adverse effects of nano-TiO<sub>2</sub> on plant

development at higher concentrations to maize (0.3-1 g L<sup>-1</sup>) and *Vicia narbonensis L*. (0.2-4 g L<sup>-1</sup>),<sup>30, 31</sup> while others found inductive effects to wheat and spinach<sup>12, 16, 17</sup> or no effects to willow trees at lower concentrations (1 to 100 mg L<sup>-1</sup>)<sup>32</sup>. Such difference suggests that whether the growth medium is soil or nutrient solution will lead to different response of plant to nano-TiO<sub>2</sub> exposure, either due to the contribution of soil minerals and/or organic matters, or the possible changes in nano-TiO<sub>2</sub> properties in soil compared to in water. Thus the interaction between soil and nano-TiO<sub>2</sub> is an interesting topic to explore in order to understand the mechanisms of the effect of nano-TiO<sub>2</sub> on plant growth.

The variation of root or shoot dry biomass followed the same trend as the length, but at relatively less magnitudes (Figure 7.3 and Table 7.1-7.2). Larue et al. (2012) also reported that the impact of nano-TiO<sub>2</sub> on the dry biomass of wheat was not observed at the dosages of 50 and 100 mg L<sup>-1</sup> in a hydroponic medium despite induced root elongation.<sup>16</sup> These results show that nano-TiO<sub>2</sub> may cause stimulation to wheat growth, but will not significantly influence the biomass production. It has been suggested that the root elongation might be caused by that nano-TiO<sub>2</sub>, having high surface reactivity, enlarges root pores or create additional ones, leading to higher hydromineral flows in roots and elevated nutrient uptake.<sup>16</sup> However, the plant biomass production mainly depends on the accumulation of carbon products through photosynthesis. As stated above, with (or without) the nano-TiO<sub>2</sub> exposure the photochemical efficiency of wheat maintained at a high level throughout the growth period indicated by the  $F_v/F_m$  values of 0.79-0.84. This may be the reason why the biomass was affected by nano-TiO<sub>2</sub> less significantly than the length. The high photochemical efficiency may also explain why the reduction effect of SRM-TiO<sub>2</sub> to AGS shoots in 14 days diminished in 28 days, which suggests that the impact of nano-Ti $O_2$  to the plant growth may not endure for long term.

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### Effect of Wheat Genotype, Type and Spiking Method of Nano-TiO<sub>2</sub>

Under the same treatment conditions, the wheat cultivar PR was less sensitive to nano-TiO<sub>2</sub> than AGS. Figure 7.4 compares the root and shoots length of these two genotypes growing in soil spiked with SRM-TiO<sub>2</sub> dispersion. Although the reduced root length of PR at 5 mg kg<sup>-1</sup> soil was comparable to that of AGS, the enhanced root length at 500 mg kg<sup>-1</sup> and the reduced shoot length at 5 mg kg<sup>-1</sup> soil were absent for PR. The root or shoot dry biomass of PR also showed similar changes with that of lengths. Thus the wheat cultivar PR may have stronger stress response mechanism to resist the impact caused by nano-TiO<sub>2</sub>.

The effect of nano-TiO<sub>2</sub> to wheat also depends on the type of nanoparticle and the spiking method, i.e., soil mixed with SRM-TiO<sub>2</sub> dispersion or powder. If compare the two spiking methods using SRM-TiO<sub>2</sub> as shown in Figure 7.5 and Table 7.2, the adverse effect to root or shoot length was evident with "dispersion spiking" at 5 and 50 mg kg<sup>-1</sup> but not with "powder spiking". Across the exposure dosage range, soil spiked with SRM-TiO<sub>2</sub> powder did not exert significant impact to root or shoot growth during the 28-day growth; the only exception was the shoot length was increased at dosage of 500 mg kg<sup>-1</sup>. In contrast to SRM-TiO<sub>2</sub>, the 500 mg kg<sup>-1</sup> of Aldrich-TiO<sub>2</sub> powder reduced the root length of wheat while the other dosages resulted in no significant effect, and by "dispersion spiking" the Aldrich-TiO<sub>2</sub> affected wheat growth significantly only at 50 mg kg<sup>-1</sup>, i.e., decreased the root length. In summary, mixing soil with nano-TiO<sub>2</sub> dispersion resulted in more significant effect to wheat growth than mixing with powder, and the wheat growth appeared to be more sensitive to SRM-TiO<sub>2</sub> than to Aldrich-TiO<sub>2</sub>. One possible reason that may have caused these differences is the actual size of TiO<sub>2</sub> particles resulted from aggregation. In general, the dispersivity of TiO<sub>2</sub> powder would be much weaker than that of TiO<sub>2</sub> dispersion as shown by the representative scanning electron microscopic

images in Figure 7.6. Thus the TiO<sub>2</sub> in soil prepared using "powder mixing" would more likely be the sizes of their agglomerates. In regard of the two types of nano-TiO<sub>2</sub>, in dispersions the average value of the actual size of SRM-TiO<sub>2</sub> dispersion is smaller than that of Aldrich-TiO<sub>2</sub> dispersion as measured by dynamic light scattering (DLS). The hydrodynamic diameter of SRM-TiO<sub>2</sub> dispersion was close to the primary particle size (113.9  $\pm$  5.1 nm, n = 3, compared to <100 nm), indicating that the SRM-TiO<sub>2</sub> was well dispersed; whereas agglomerates persisted in the Aldrich-TiO<sub>2</sub> suspension demonstrated by the much larger hydrodynamic diameter than the particle size (278.3  $\pm$  19.0 nm, n = 3, compared to <25 nm). The polydispersity indices were  $0.253 \pm 0.007$  and  $0.466 \pm 0.100$  (n = 3) for SRM- and Aldrich-TiO<sub>2</sub> dispersions respectively. As discussed in Chapter V, in the lower end of colloidal range, smaller particles have larger specific surface area and consequently easier to aggregate. Thus the smaller Aldrich-TiO<sub>2</sub> was more easily to aggregate than SRM-TiO<sub>2</sub> and had larger actual sizes in dispersions. According to these results, wheat is likely more sensitive to smaller  $TiO_2$  particles, possibly because the surface area is negatively correlated with surface reactivity. However, why the soil spiked with Aldrich-TiO<sub>2</sub> powder at 500 mg kg<sup>-1</sup> dosage showed an adverse effect to wheat root development is still unknown.

#### Possible Mechanisms: Bioaccumulation and Oxidative Stress

The phytotoxicity of nanoparticles was proposed to resulted either from the uptake of the nanoparticles themselves or the ability of the nanoparticles to deliver dissolved metal ions to critical biotic receptors.<sup>33</sup> However,  $TiO_2$  does not readily dissolve in water, and consequently does not release significant amount of metal ion to the soil solution. Therefore if the effect of the nano-TiO<sub>2</sub> comes from uptake it is likely due to the direct uptake of nanoparticles themselves. According to Figure 7.7 (a), the uptake of nano-TiO<sub>2</sub> (powder spiking) by wheat increased with

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dosage, but was significant only when the dosage was beyond 150 mg kg<sup>-1</sup>. The bioaccumulation amount by root is much higher than by shoot, suggesting barriers between root and shoot to prevent the translocation of nano-TiO<sub>2</sub>. The uptake of nano-TiO<sub>2</sub> differs between two spiking methods as shown in Figure 7.7 (b). The bioaccumulation amount of Ti using dispersion spiking increased with dosage, reached the apex at 50 mg kg<sup>-1</sup>, and dropped at 500 mg kg<sup>-1</sup>. It is worth noting that the level of Ti uptake was the same with the dosages of 5 and 500 mg kg<sup>-1</sup>. Comparing Figure 7.5 and 7.7, the increase of bioaccumulation amount is generally correlated to the reduction of root or shoot length, suggesting the possible connection between uptake and toxic effect of nano-TiO<sub>2</sub>.

Nano-TiO<sub>2</sub> is well known as a photocatalyst and can thus cause oxidative stress to plants. To test this hypothesis, two parameters were measured. The lipid peroxidation is oxidative degradation of lipid and reflects the damage potential to cell membrane. Figure 7.8 shows the level of lipid peroxidation measured in terms of MDA contents of wheat tissues exposed to different dosages of SRM-TiO<sub>2</sub>. Although the value changed slightly when dosage increased and reached the apex at 5 mg kg<sup>-1</sup>, the difference was not statistically significant partially due to the large variation. In addition, there was no clear correlation between the lipid peroxidation and the root or shoot length as shown in Figure 7.8 (b). The Pearson correlation coefficients between length (cm) and lipid peroxidation (mmol g<sup>-1</sup>) were -0.281 (*p* value = 0.245) and -0.177 (*p* value = 0.483) for root and shoot, respectively. On the contrary, the change of another parameter, the peroxidase activity, is more significant especially for root (Figure 7.9). The Pearson correlation coefficients between length (cm) and peroxidase activity (unit mL<sup>-1</sup>) when treated with SRM-TiO<sub>2</sub> dispersion were -0.817 (*p* value = 0.013) and -0.017 (*p* value = 0.969) for root and shoot, respectively. The 5 and 50 mg kg<sup>-1</sup> dosages of nano-TiO<sub>2</sub> caused significant elevation of

peroxidase activity in wheat root tissue, indicating that the plant is under oxidative stress and thus secrete peroxidases to mediate redox reactions in order to mitigate the stress. This is likely to be one of the defense mechanisms adopted by plant in the changing environment. With nano-TiO<sub>2</sub> dosage of 500 mg kg<sup>-1</sup> the peroxidase activity was not different with the control, suggesting the irrelevance between the root elongation and the peroxidase activity. When treated with SRM-TiO<sub>2</sub> powder the peroxidase activity remained stable at 2.24  $\pm$ 0.26 unit mL<sup>-1</sup> for root and 0.84  $\pm$ 0.22 unit mL<sup>-1</sup> for shoot when the dosage increased, which is consistent with the lack of reduction in length. Our results show that the peroxidase activity can serve as a sensitive indicator to reflect the oxidative stress of wheat and is more sensitive than lipid peroxidation. Moreover, the response of root is stronger than shoot, which may be because the bioaccumulation of nano-TiO<sub>2</sub> in root is greater than in shoot, and the nano-TiO<sub>2</sub> in soil may also cause extra oxidative stress to roots.

#### FIGURES AND TABLES



FIGURE 7.1 Initial and maximum chlorophyll fluorescence index ( $F_0$  and  $F_m$ ), and ratio between variable and maximum chlorophyll fluorescence ( $F_v/F_m$ ) of wheat (AGS2000) leaves at different dosages of nano-TiO<sub>2</sub> after 14 days.



FIGURE 7.2 Dose-response relationships between nano-TiO<sub>2</sub> and the root or shoot length of AGS2000 wheat planted in soil spiked with SRM-TiO<sub>2</sub> dispersion. The root or shoot length ratio was calculated using the average root or shoot length resulted from each dosage level divided by the average length of corresponding controls.



FIGURE 7.3 Dose-response relationships between nano-TiO<sub>2</sub> and the root or shoot dry weight of AGS2000 wheat planted in soil spiked with SRM-TiO<sub>2</sub> dispersion.



FIGURE 7.4 Comparison between two wheat genotypes, PR and AGS, of their response to nano-TiO<sub>2</sub> exposure in soil spiked with SRM-TiO<sub>2</sub> dispersion. The root (left) and shoot (right) length ratio to corresponding controls are illustrated.



FIGURE 7.5 Comparison between the effects of two types of nano-TiO<sub>2</sub>, SRM1898 Degussa P25 and Aldrich anatase, and spiking methods, soil mixed with dispersion or powder of nano-TiO<sub>2</sub> to the relative (a) root or (b) shoot length of wheat cultivar AGS.



FIGURE 7.6 Scanning electron micrographs of Aldrich-TiO<sub>2</sub> as powder (a, b) and as dispersion (c, d).



FIGURE 7.7 Bioaccumulation of  $TiO_2$  by wheat: (a) the amount of Ti accumulated in root and shoot tissue exposed to Aldrich-TiO<sub>2</sub> powder; (b) the amount of Ti accumulated in shoot tissue when the wheat was exposed to the soil spiked with Aldrich-TiO<sub>2</sub> dispersion or powder.



FIGURE 7.8 The effect of nano-TiO<sub>2</sub> on the lipid peroxidation of wheat: (a) changing with time and dosage (wheat cultivar AGS, SRM-TiO<sub>2</sub> dispersion); (b) overall trend of the lipid peroxidation of two wheat cultivars exposed to SRM-TiO<sub>2</sub> dispersion.



FIGURE 7.9 The effect of nano-TiO<sub>2</sub> (SRM-TiO<sub>2</sub> dispersion) on the peroxidase activity of wheat cultivar AGS.

	Root			Shoot		
		Dry	Lipid		Dry	Lipid
	Length	Weight	Peroxidation	Length	Weight	Peroxidation
Genotype	**	*	**	**	NS	NS
Dosage	****	NS	*	*	NS	NS
Time	*	NS	**	****	NS	NS
Spiking	NS			NS		
			Interaction			
Genotype*Dosage	***	NS	NS	***	NS	NS
Genotype*Time	NS	NS	NS	NS	NS	NS
Dosage*Time	NS	NS	NS	NS	NS	NS
Dosage*Spiking	***			NS		
NS = not significant						
* = 0.05						
** = 0.01						

TABLE 7.1 The ANOVA test of the contribution of various factors using general linear model procedure

\*\* = 0.01

\*\*\* = 0.001

\*\*\*\* = < 0.0001

TABLE 7.2 The average values of the ratios normalized by corresponding controls and the pairwise comparison using Tukey's test among dosages under various conditions; the same letter indicates no significant difference at  $\alpha = 0.05$ 

Dosage <sup>*</sup>	Root				
$(mg kg^{-1})$	Longth	Dry	Lipid	Peroxidase	
(ing kg )	Length	Weight	Peroxidation	Activity	
0	1.000 <sup>BC</sup>	1.000 <sup>AB</sup>	$1.000^{AB}$	1.000 <sup>B</sup>	
5	0.525 <sup>D</sup>	0.856 <sup>AB</sup>	$1.208^{A}$	1.831 <sup>A</sup>	
50	$0.748^{\text{CD}}$	0.769 <sup>B</sup>	0.653 <sup>B</sup>	1.047 <sup>B</sup>	
150	1.106 <sup>B</sup>	1.104 <sup>A</sup>	$0.822^{AB}$	1.176 <sup>B</sup>	
500	1.416 <sup>A</sup>	$1.071^{AB}$	0.612 <sup>B</sup>	0.900 <sup>B</sup>	
Dosage			Shoot		
$(ma ka^{-1})$	Lanath	Dry	Lipid	Peroxidase	
(iiig kg )	Length	Weight	Peroxidation	Activity	
0	1.000 <sup>A</sup>	1.000 <sup>A</sup>	$1.000^{A}$	1.000 <sup>A</sup>	
5	0.530 <sup>B</sup>	0.750 <sup>A</sup>	0.716 <sup>A</sup>	1.199 <sup>A</sup>	
50	$0.774^{\operatorname{AB}}$	$0.778^{A}$	$0.558^{\mathrm{A}}$	1.179 <sup>A</sup>	
150	0.953 <sup>AB</sup>	1.045 <sup>A</sup>	0.415 <sup>A</sup>	1.041 <sup>A</sup>	
500	$0.769^{AB}$	0.959 <sup>A</sup>	0.596 <sup>A</sup>	1.100 <sup>A</sup>	

\*Overall comparison between dosages using means calculated for all factors including genotype, time, spiking methods and type of nano-TiO<sub>2</sub>.

Dosage <sup>*</sup>	Root				
$(ma ka^{-1})$	Longth	Dry	Lipid	Peroxidase	
(ing kg )	Length	Weight	Peroxidation	Activity	
0	1.000 <sup>B</sup>	1.000 <sup>A</sup>	$1.000^{AB}$	1.000 <sup>C</sup>	
5	0.584 <sup>C</sup>	0.698 <sup>B</sup>	1.267 <sup>A</sup>	2.498 <sup>A</sup>	
50	0.796 <sup>C</sup>	0.769 <sup>B</sup>	0.414 <sup>C</sup>	2.264 <sup>B</sup>	
150	1.118 <sup>B</sup>	1.104 <sup>A</sup>	$0.412^{\rm C}$	1.176 <sup>C</sup>	
500	1.901 <sup>A</sup>	1.228 <sup>A</sup>	0.732 <sup>BC</sup>	0.972 <sup>C</sup>	
Anesod		Shoot			
$(ma ka^{-1})$	Longth	Dry	Lipid	Peroxidase	
(ing kg )	Length	Weight	Peroxidation	Activity	
0	1.000 <sup>A</sup>	А	$1.000^{A}$	$1.000^{B}$	
5	0.403 <sup>B</sup>	В	$0.897^{\mathrm{A}}$	1.187 <sup>A</sup>	
50	0.613 <sup>B</sup>	AB	$0.596^{\mathrm{A}}$	0.793 <sup>B</sup>	
150	0.953 <sup>AB</sup>	А	$0.730^{A}$	1.041 <sup>B</sup>	
500	0.932 <sup>A</sup>	А	0.415 <sup>A</sup>	0.652 <sup>B</sup>	

\*Comparison between dosages using means calculated for wheat cultivar AGS exposed to soil spiked with SRM-TiO<sub>2</sub> dispersion.

	SRM D	ispersion	SRM Powder		
Dosage (mg/kg	Root Length	Shoot Length	Root Length	Shoot Length	
soil)	Ratio	Ratio	Ratio	Ratio	
0	$1.000^{B}$	0.999 <sup>AB</sup>	$1.000^{\rm A}$	$1.000^{B}$	
5	0.479 <sup>C</sup>	0.393 <sup>B</sup>	1.366 <sup>A</sup>	1.366 <sup>B</sup>	
50	$0.676^{\rm C}$	0.534 <sup>B</sup>	0.914 <sup>A</sup>	0.933 <sup>B</sup>	
500	1.874 <sup>A</sup>	1.318 <sup>A</sup>	1.682 <sup>A</sup>	1.802 <sup>A</sup>	
	Aldrich I	Disporsion	Aldrich Powder		
	Alunchi	Dispersion	Additon	rowdei	
Dosage (mg/kg	Root Length	Shoot Length	Root Length	Shoot Length	
Dosage (mg/kg soil)	Root Length Ratio	Shoot Length Ratio	Root Length Ratio	Shoot Length Ratio	
Dosage (mg/kg soil) 0	Root Length Ratio	Shoot Length Ratio 1.000 <sup>A</sup>	Root Length Ratio	Shoot Length Ratio 1.000 <sup>A</sup>	
Dosage (mg/kg soil) 0 5	Root Length Ratio 1.000 <sup>A</sup> 0.698 <sup>AB</sup>	Shoot Length Ratio 1.000 <sup>A</sup> 0.983 <sup>A</sup>	Root Length Ratio 1.000 <sup>A</sup> 0.699 <sup>AB</sup>	Shoot Length Ratio 1.000 <sup>A</sup> 1.119 <sup>A</sup>	
Dosage (mg/kg soil) 0 5 50	Root Length Ratio 1.000 <sup>A</sup> 0.698 <sup>AB</sup> 0.397 <sup>B</sup>	Shoot Length Ratio 1.000 <sup>A</sup> 0.983 <sup>A</sup> 0.811 <sup>A</sup>	Root Length Ratio 1.000 <sup>A</sup> 0.699 <sup>AB</sup> 0.945 <sup>A</sup>	Shoot Length Ratio 1.000 <sup>A</sup> 1.119 <sup>A</sup> 0.987 <sup>A</sup>	

\*Comparison between dosages using means calculated for wheat cultivar AGS exposed to soil spiked with two types of  $TiO_2$  either in dispersion or as powder. The growth time was 28 days.

Dosage	14 days			28 days		
(mg/kg soil)	$F_0$	$F_m$	Fv/Fm	F0	Fm	Fv/Fm
0		3626	0.832	622	3333	0.812
	606 (32)*	(129)	(0.004)	(67)	(331)	(0.025)
5		3089	0.832	659	3524	0.813
	516 (151)	(167)	(0.003)	(47)	(175)	(0.006)
500		3809	0.836	619	3279	0.811
	624 (20)	(115)	(0.001)	(29)	(132)	(0.002)

TABLE 7.3 The photosynthetic efficiency of wheat cultivar Pioneer® variety 26R61

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#### CHAPTER VIII

### CONCLUSIONS

This study investigated the environmental behaviors of typical nanoparticles from three aspects to provide information on their life-cycle analysis and risk assessment: the phase distribution between solid and aqueous phase, the biodegradation and the toxicity.

Hydrophilic surface functional groups render multiwall carbon nanotubes (MWCNTs) dispersible in water and the dispersion behavior can be reasonably described by the basic rules of colloidal science. This colloidal stability is due to the electrostatic repulsion by these functional groups which compensates the attraction interactions between MWCNTs, i.e., van der Waal's and hydrophobic interactions. Cations such as Na<sup>+</sup> can shelter the electrostatic repulsion, and, when reach certain level (4 and 40 mmol  $L^{-1}$  in our experiment), can induce aggregation and consequent settling of MWCNTs. However, the cation effect can be largely impaired by dissolved organic matter (DOM) sorbed on CNT surface that exert steric hindrance as well as electrostatic repulsion between MWCNTs. Especially the steric hindrance would not be sensitively affected by ion concentration. This was demonstrated by the fact that when the Na<sup>+</sup> concentration increased to 4 and 40 mmol  $L^{-1}$  the stability of MWCNTs was not significantly changed in the presence of DOM. When contacting with solid phase, if the solid phase is inorganic such as kaolinite or smectite tested in this study, the interaction between MWCNTs and the clay minerals is likely to follow the extended DLVO model and their interaction can be described by combination of electrostatic, van der Waal's, and acid-base interactions. However, if the solid phase contains organic matter the scenario would be more complex. For the diagenetically young organic matter as peat, because it readily release dissolvable portion to water which strongly increases the colloidal stability of MWCNTs, the sorption by the undissolvable peat is largely compensated. The sorption only became observable when adding Na<sup>+</sup>, possibly because the electrostatic repulsion from the surface charges on both MWCNTs and peat was reduced. On the contrary, shale contains the diagenetically old organic matter such as kerogen whose solubility in water is very low. Therefore shale can sorb MWCNTs much stronger than the other soil components tested in this study. The sorption isotherms of MWCNTs by the soil components showed a linear trend within the tested range. Not only the aqueous and solid phase conditions, but also the MWCNTs properties influenced their colloidal stability. In our experiment, when the length of MWCNTs was less than 2000 nm, the shorter the MWCNTs, the less their colloidal stability would be, maybe due to the increased specific surface area which caused stronger aggregation. However, this instability can be overcome by DOM. These results showed that MWCNTs, especially smaller ones, can be easily removed by the cations in the natural water and/or water treatment conditions, or sorbed to soil/sediment especially those which contain insoluble organic matter. Under these circumstances the soil/sediment is very likely to be the sink of MWCNTs in the environment. However, if DOM is present at a significant level the MWCNTs could be stable and transportable via water ways.

When released to the hydrosphere, the MWCNTs can possibly be mineralized, i.e., degraded to  $CO_2$ , by bacteria community under environmentally relevant conditions despite their high chemical stability. This result suggests the possibility that MWCNTs can be fit in the carbon cycle and not accumulated in the environment in the long run. The degradation pathway was also investigated. Before transformed into  $CO_2$ , small flakes were first trimmed off from MWCNTs. Aromatic organic compounds with low molecular weight were also detected in the

degradation process. Furthermore, this degradation requires additional carbon source, and likely by cooperation of different bacteria species but not fungus. The possible degraders include *Burkholderia kururiensis*, *Delftia acidovorans*, and *Stenotrophomonas maltophilia*. However, there is still undiscovered species that contributed to the degradation. Other questions like to what extent the MWCNTs can be degraded, and what is the turnover time still remains to be studied.

MWCNTs showed insignificant effects to the growth of wheat, Triticum aestivum L., while the nano-TiO<sub>2</sub> had various influences on the wheat growth according to the treatment conditions. The effect of nano-TiO<sub>2</sub> to root and shoot length of wheat depends on the type and dosage of nano-TiO<sub>2</sub>, the spiking method, and the genotype of wheat; the effects on root or shoot biomass followed the same trend but less significant. After exposed to dosages ranged from 5 to 500 mg kg<sup>-1</sup> soil for 14 or 28 days, the wheat leaves maintained high photochemical efficiency as indicated by the chlorophyll fluorescence, which indicates the effect of nano-TiO $_2$  to wheat biomass production may not last for long term. One type of nano-TiO<sub>2</sub>, Degussa P25, promoted root elongation of wheat cultivar AGS2000 at 500 mg kg<sup>-1</sup> dosage, whereas had no effect at 150 mg kg<sup>-1</sup> and a negative effect at 5 and/or 50 mg kg<sup>-1</sup>, when spiked with soil as dispersion. The effect on shoot length was similar but with less magnitude, except for the 500 mg kg<sup>-1</sup> dosage at which the shoot length was first reduced in 14 days and turned to no effect in 28 days. The two types of nano-TiO<sub>2</sub> showed different influence on the wheat growth: the Degussa P25 nano-TiO<sub>2</sub> induced stronger responses than the anatase nano-TiO<sub>2</sub>. When comparing with the same type of nano-TiO<sub>2</sub>, spiked with soil as dispersion tended to have stronger effect than spiked as powder. These differences were likely due to the different size distribution of nano-TiO<sub>2</sub> resulting from the two preparation methods in that the smaller ones had more surface reactivity and thus were

more influential to wheat growth. In addition, another wheat cultivar, Pioneer® variety 26R61, was more inert to nano-TiO<sub>2</sub> than AGS and mostly showed no changes in the tested parameters across the dosages. The possible causes of the toxic effect include the oxidative stress induced by nano-TiO<sub>2</sub> which was suggested by the elevated peroxidase activity in plant tissue, and the uptake and translocation of nano-TiO<sub>2</sub> to root and shoot. Further exploration of the interactions between soil and nano-TiO<sub>2</sub> would help to better understand the mechanisms of nano-TiO<sub>2</sub> to plants.

This study provides information that is critical to science-informed environmental risk assessments and responsible applications of two important nanoparticles, MWCNTs and nano-TiO2. The environmental behaviors and impacts of nanoparticles are very complicated and depend on many factors. Further study into understanding the mechanisms underlying those phenomena will help achieve the goal of accurate modeling and predicting the fate and ecotoxicological effects of nanoparticles in the environment.
## APPENDIX A

# SUPPORTING INFORMATION:

# PHASE DISTRIBUTION OF 14C-LABELED MULTI-WALLED CARBON NANOTUBES IN AQUEOUS SYSTEMS CONTAINING MODEL SOLIDS: PEAT<sup>6</sup>

<sup>&</sup>lt;sup>6</sup> Zhang, L.; Petersen, E.J. and Q. Huang. 2011. *Environmental Science and Technology*. 45 (4): 1356-1362. Reprinted here with permission of the publisher.

#### <u>CHEMICALS</u>

Hydrogen gas (99.95% purity), argon gas (99.998% purity), and the <sup>14</sup>C-labeled and nonlabeled methane gas (99.97% purity) were purchased from Cryogenic Gases. Nickel nitrate hexahydrate (99%), magnesium nitrate hexahydrate (reagent grade), hydrochloric acid (11.1 M), concentrated sulfuric (14.8 M), nitric (15.6 M) acids, and sodium chloride (reagent grade) were purchased from Fisher. Sodium phosphate dibasic (hexahydrate) (99.0 – 101.0%), sodium phosphate monobasic (monohydrate) (98.0 – 102.0%), and sodium acetate trihydrate (99.0%) were from Sigma Aldrich.

#### MWCNT SYNTHESIS, PURIFICATION AND ACID TREATMENT

To make the catalyst, nickel nitrate, magnesium nitrate, citric acid, and 20 mL of Milli-Q water were mixed, dried at 100 °C, and calcined. To synthesize the MWCNTs, 100 mg of catalyst was added to a quartz boat and heated in a tube furnace to 600 °C under a flow of hydrogen gas. The hydrogen gas was then switched to a mixture of <sup>14</sup>C-labeled and non-labeled methane and the gas flow (300 mL/min) was maintained for 30 min. The reactor was cooled to room temperature under a stream of argon gas. To remove the catalyst impurities, the MWCNTs were bath sonicated in a hydrochloric acid bath (11.1 M) for 1 hr and washed with boiling water. The MWCNTs were sonicated again in an acid mixture composed of concentrated sulfuric (14.8 M) and nitric (15.6 M) acids in a 3:1 volume ratio, a process known to introduce functional groups (e.g., carboxyl groups) to the nanotube surface.

#### PREPARATION OF PEAT DOM SOLUTIONS

The Canadian peat we used in this study has the following elemental composition: 47.5% C, 44.11% O, 5.37% H, 0.71 N, 0.11 S and 4.08% ash. To prepare a solution with only the DOM fraction of peat, 500 mg of peat was added to 50 mL water for 3 days, a duration shown in

preliminary tests for equilibrium of the DOM dissolution to be reached, and then the solid phase was removed by filtration (0.45  $\mu$ m, Millipore, Millipore Corp., Bedford, MA). The resulting solution containing both dissolved organic matter and solid peat particles less than 0.45 $\mu$ m that can stably suspend in water is called "DOM solution" in this paper. According to TOC measurements, the portion of organic particles between 0.45 and 0.22  $\mu$ m is 3.6 $\pm$ 1.6% (n = 6), thus indicating a relatively small fraction of peat particles.

This peat-to-water ratio is twice that of the experimental condition for the peat treatment. In DOM treatment, 5 mL of this DOM solution was mixed with 5 mL of a MWCNT solution to reach a final MWCNT concentration equivalent for blank and peat treatments. In a preliminary experiment, the DOM concentration determined by UV spectra is linearly proportional to the peat-to-water ratio from 2 mg/mL to 20 mg/mL, thus confirming that diluting the DOM concentration by a factor of two when mixing the DOM and MWCNT solutions would yield DOM concentrations that were equivalent to those in the peat treatments. This process was repeated for each water condition tested in the experiment: Na<sup>+</sup> concentrations of 0, 4, and 40 mM, and pH values of 4.0, 6.0, and 8.0.

#### DOM SORPTIONS ON MWCNTS

An experiment was performed to assess DOM sorption by MWCNTs using 0.22  $\mu$ m membrane filtration in conjunction with total organic carbon (TOC) and carbon-14 MWCNT radioactivity measurements. The MWCNT suspension and DOM solution were mixed together to reach a 10 ml volume with nanotube and NOM concentrations of 2.57 mg MWCNT/L and 7.67 mg C/L, respectively. After 3 days this mixture was filtered using a 0.22  $\mu$ m filter (Millipore 47mm), and the TOC of the filtrate was measured to yield TOC<sub>mix</sub>. The carbon-14 MWCNT concentration in the filtrate was measured by radioactivity measurements; this

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radioactivity was then converted using a calibration factor to the corresponding MWCNT TOC value, and the resulting value was termed  $TOC_{MWCNT}$ . The amount of DOM that passed through the filter is thus  $TOC_{mix} - TOC_{MWCNT}$ . We also tested a control treatment in which the DOM solution without MWCNTs was filtered, and the TOC of the filtrate was measured, yielding a value called  $TOC_{control}$ . DOM sorption by MWCNTs was then calculated using the following formula.

$$TOC_{sorbed} = TOC_{control} - (TOC_{mix} - TOC_{MWCNT})$$
(1)

This experiment was performed with three sodium concentrations (0, 4, and 40 mM) with four replications for each treatment. The TOC<sub>sorbed</sub> were 0.61  $\pm$ 0.40, 1.34  $\pm$ 0.32, and 1.54  $\pm$ 0.33 mg C/L (n = 4; uncertainties represent standard deviations) when 0, 4, or 40 mM NaCl was added, respectively. To determine the mass of sorbed DOM per nanotube mass, TOC<sub>sorbed</sub> was divided by the concentration of residual MWCNTs to yield 0.26  $\pm$ 0.13, 0.41  $\pm$ 0.12, and 0.49  $\pm$ 0.13 mg C/ mg MWCNT (n = 4) when 0, 4 , or 40 mM NaCl was added, respectively.

#### FIGURES AND TABLES



FIGURE S3.1 Phase distribution of MWCNTs in 10 ml of solution containing 20 mM Na<sup>+</sup> and 50 mg peat after incubation for 1, 3, 7 and 12 days.



FIGURE S3.2 Thermal gravimetric analysis (TGA) graph of the MWCNT powder (blue) and the dispersed MWCNTs after ultrasonication (orange).



(a)

(b)



(c)

FIGURE S3.3 Scanning electron microscopy (SEM) images showing the morphology of peat particles (1k magnification) (a and b) and un-sonicated MWCNT powder (100k magnification) (c).



FIGURE S3.4 Phase distribution of MWCNT at different pHs with the same DOM concentration (140  $\mu$ g/L).

TABLE S3.1a Initial concentration	on (mg/L)
Varying sodium concentrations	Varying pHs
0.329	0.265
0.516	0.442
1.831	0.884
3.703	2.267
7.495	4.378
11.222	8.309
17.202	12.046
28.424	20.087
41.658	27.396
66.914	43.780

TABLE S3.1 a) Initial MWCNT concentrations of various treatments; b) pH values in various treatments.

TABLE S3.1b Actual pHs in different treatments						
рН	DOM	blank	$Na^{+}(mM)$	DOM	Blank	
4	3.76	3.62	0	5.17	6.28	
6	5.04	5.45	4	4.66	6.07	
8	7.71	8.13	40	4.58	5.67	

	Peat solution (ppb)
Na	811
Si	368
Κ	304
Mg	110
Zn	82.2
Al	58.9
В	36.0
Ti	12.7
Bi	11.0
Sr	10.6
Pb	3.99
Cu	3.42
Rb	1.88
Мо	0.07

TABLE S3.2 Concentrations of metal ions in a peat solution prepared by de-ionized water (5g peat/L)

TABLE S3.3 Parameters obtained by data fitting to the equation  $q_a = KC_{aq}^n$ 

Sodium Conc. (mM)	K (mg/g peat) <sup>(1-n)</sup>	N (dimensionless)	R <sup>2</sup>
4	0.082	0.701	0.877
$N^{a} = 18$	$(0.0762, 0.0881)^{\rm b}$	(0.668, 0.733)	
40	0.232	0.952	0.938
N=10	(0.198, 0.272)	(0.901, 1.000)	

\*The data set collected at 0 sodium concentration was not used for data fitting because certain  $q_a$  values were negative

<sup>*a*</sup> Number of data points used in the fitting.

<sup>b</sup> Values in parentheses are 95% confidence intervals.

### APPENDIX B

## SUPPORTING INFORMATION:

# INTERACTIONS OF 14C-LABELED MULTI-WALLED CARBON NANOTUBES WITH

# SOIL MINERALS IN WATER<sup>7</sup>

<sup>&</sup>lt;sup>7</sup> Zhang, L.; Petersen, E.J.; Zhang, W.; Chen, Y.; Cabrera, M. and Q. Huang. 2012. *Environmental Pollution*. 166: 75-81. Reprinted here with permission of the publisher.

# S1. Testing the effects of dissolved substances from the shale, kaolinite, and smectite on MWCNT stability in solution

Soil mineral solution was used to test the effect of dissolvable substances on the aqueous stability of MWCNTs. In these experiments, 100 ml of de-ionized water was mixed with 500 mg of kaolinite, smectite or shale (the same water-to-clay ratio as in the treatments testing sorption with the solid phases) and with NaCl concentrations of 0, 0.4, 4 and 40 mmol/L, respectively. The solution was filtered through a 0.22  $\mu$ m membrane after 7 days of equilibration to remove the solid soil minerals.

The effect of these solutions was compared with the control treatments with NaCl concentrations of 0, 0.4, 4 and 40 mmol/L, accordingly. The values of linear regression parameter, *a*, were not significantly different with and without soil solutions ( $\alpha$ =0.05) except for kaolinite with 0.4 mmol/L NaCl (see Table S4.8). The result for 0.4 mmol/L NaCl with kaolinite was unexpected and the significance is unclear.

These results were generally as expected for smectite and kaolinite, but was surprising for shale given its high percentage of carbon (8.27 %).<sup>1</sup> However, no dissolved organic matter was detected by UV absorbance at 400 nm, unlike what was previously observed for peat. Shale's organic components are mainly large molecules and insoluble.<sup>1-3</sup> Thus, they do not dissolve in water and did not help stabilize the MWCNTs.

#### S2. Definitions and Determination of Parameter in Equation 3a to 3e

Under the Derjaguin integration approximation, the total EDLVO interaction energy  $(U_{CwM}^{TOT})$  between the clay surface (denoted *C*) and a MWCNT (denoted *M*) in water (denoted *w*) were written as follows:<sup>4-7</sup>

$$U_{CwM}^{TOT} = U_{CwM}^{vdW} + U_{CwM}^{EL} + U_{CwM}^{AB}$$
(5a)

$$U_{CwM}^{\nu dW}(D) = -\frac{A_{CwM}}{6} \left[ \frac{R}{D} + \frac{R}{D+2R} + \ln \frac{D}{(D+2R)} \right]$$
(5b)

$$U_{CwM}^{EL}(D) = 64\pi\varepsilon\varepsilon_0 R \left[\frac{k_B T}{ze}\right]^2 \tanh\left[\frac{ze\varphi_C}{4k_B T}\right] \tanh\left[\frac{ze\varphi_M}{4k_B T}\right] \exp\left(-\kappa D\right)$$
(5c)

$$\kappa^{-1} = \sqrt{\frac{\varepsilon \varepsilon_0 k_B T}{2N_A I e^2}}$$
(5d)

$$U_{CwM}^{AB}(D) = \pi R \lambda \Delta G_{CwM,D_0}^{AB} \exp\left(\frac{D_0 - D}{\lambda}\right)$$
(5e)

where  $U_{CwM}^{vdW}$  is the van der Waals interaction energy  $(k_BT)$ ,  $U_{CwM}^{EL}$  is the electrostatic interaction energy  $(k_BT)$ , and  $U_{CwM}^{AB}$  is the acid-base interaction energy  $(k_BT)$ . *D* is the interacting distance (nm);  $A_{CwM}$  is the Clay-MWCNT Hamaker constant in water (w)(detailed determination of the Hamaker constant in water is provided in the section S4.2 of SI); *R* is the hydrodynamic radius of the MWCNT particles, and other parameters  $k_B$  is the Boltzmann constant,  $1.38 \times 10^{-23}$  J/K; *T* is the absolute temperature (298 K); *z* is the electrolyte valence; *e* is the unit charge  $(1.602 \times 10^{-19} \text{ C})$ ;  $\varphi_C$  and  $\varphi_M$  are the surface potentials (mV) for clay particles and MWCNT determined from measured electrophoretic mobilities and the Debye-H ückel approximation,<sup>8,9</sup>

 $\varphi = \zeta(1 + d / R) \exp(\kappa d)$ , where *d* is the distance from the particle's surface to the slipping layer, a distance that is generally of the order of  $\approx 3$  Å to 5 Å,  $\kappa$  is the reciprocal of the Debye length (nm<sup>-1</sup>);  $\varepsilon_0$  is the dielectric permittivity of a vacuum,  $8.854 \times 10^{-12} \text{ CV}^{-1} \text{m}^{-1}$ ,  $\varepsilon$  is the dielectric constant of water, 78.5 (dimensionless),  $N_A$  is Avogadro's number,  $6.02 \times 10^{23} \text{ mol}^{-1}$ , *I* is ionic strength (M),  $I=0.5 \cdot \Sigma c_i Z_i^2$ , where  $c_i$  is the molar concentration of one species ions (*i*),  $\lambda$  is the correlation length, or decay length, of the molecules of the liquid medium. For pure water, it is approximately 0.6 nm.<sup>9</sup>  $\Delta G_{H_{WE},D_0}^{AB}$  is the standard acid-base free energy of interaction between clay particles and MWCNT in water (*w*) at the distance  $(D_0)$ ,<sup>10</sup> and  $D_0$  is the minimum equilibrium distance due to Born repulsion, 0.157 nm.<sup>11</sup>

# <u>S3. Determination of $A_{CwM}$ and $\Delta G_{CwM,D_0}^{AB}$ </u>

The Hamaker constant for interaction between three types of clay (*C*) and MWCNT (*M*) in water (*w*) is can be computed by the method of van Oss:<sup>9</sup>

$$A_{CwM} = \left(\sqrt{A_{CC}} - \sqrt{A_{ww}}\right) \left(\sqrt{A_{MM}} - \sqrt{A_{ww}}\right)$$
(6a)

$$A_{ii} = 24\pi D_0^2 \cdot \gamma_i^{LW} \tag{6b}$$

$$(1 + \cos \theta_L) \cdot \gamma_L = 2(\sqrt{\gamma_i^{LW} \gamma_L^{LW}} + \sqrt{\gamma_i^+ \gamma_L^-} + \sqrt{\gamma_i^- \gamma_L^+})$$
(6c)

$$\gamma_i^{AB} = 2\sqrt{\gamma^+ \gamma^-} \tag{6d}$$

$$\gamma_i = \gamma_i^{LW} + \gamma_i^{AB} \tag{6e}$$

where  $\gamma_i^{LW}$  is the Lifshitz-van der Waals apolar component of the surface energy and  $D_0$  is the minimum equilibrium distance (0.157 nm). According to Eq. (6d),  $\gamma_i^{LW}$  as well as the polar surface tension components: electron-acceptor ( $\gamma^+$ ) and electron-donor ( $\gamma^-$ ) can be treated as three unknown and be calculated from the contact angles (see Table S4.2) using the three probe liquid. The results of  $\gamma_i^{LW}$ ,  $\gamma^+$  and  $\gamma^-$  are summarized in Table S4.3 and the acid-base interaction surface tension ( $\gamma_i^{AB}$ ) and the surface tension ( $\gamma_i$ ) of each clay particles can be further determined by Eqs (6d) and (6e).

The Hamaker constants ( $A_{ii}$ ) of the three kinds of clay particles can be derived from Eq. (6c). Although there is no reported value of Hamaker constant of MWCNTs in vacuum, Chen and Elimelech showed that fullerene ( $C_{60}$ ) NPs in aqueous media have a Hamaker constant around 7.5 × 10<sup>-20</sup> J.<sup>12, 13</sup> Because of the strong similarity between the MWCNT and fullerene

NPs, we used  $7.5 \times 10^{-20}$  J to represent the Hamaker constant of our MWCNT, which has a comparable magnitude with the Hamaker constants commonly assumed for carbon nanotubes in vacuum (2.3-6.0 × 10<sup>-19</sup> J).<sup>14</sup> Using Eq. (6b), we calculated the Hamaker constants for the interactions between clay particles and MWCNT and those between clay and MWCNT themselves in water and show the results in Table S4.4. Interestingly, a negative Hamaker constant was obtained for Kadinite-MWCNT interactions. In fact, negative non-retarded Hamaker constants, indicating a repulsive van der Waals interaction, were recently demonstrated by direct measurements of repulsive van der Waals forces.<sup>15, 16</sup>

The standard polar acid-base free energies ( $\Delta G_{CwM,D_0}^{AB}$ ) for different interacting entities were calculated according to the Dupr éequation (S4.7) and are shown in Table S4.3:

$$\Delta G_{CwM,D_0}^{AB} = 2\left[\left(\sqrt{\gamma_C^+} - \sqrt{\gamma_M^+}\right)\left(\sqrt{\gamma_C^-} - \sqrt{\gamma_M^-}\right) - \left(\sqrt{\gamma_C^+} - \sqrt{\gamma_w^+}\right)\left(\sqrt{\gamma_C^-} - \sqrt{\gamma_w^-}\right) - \left(\sqrt{\gamma_M^+} - \sqrt{\gamma_w^+}\right)\left(\sqrt{\gamma_M^-} - \sqrt{\gamma_w^-}\right)\right]$$

$$(7)$$

#### S4. Limitations of EDLVO calculation

Extended DLVO or EDLVO theory considers Lewis acid-base interactions, which is an important addition to DLVO theory and the predictions were found to be consistent with many coagulation observations.<sup>9, 17</sup> The limitations of EDLVO theory generally includes first, that the XDLVO interaction energy between spherical particles (i.e., CNT) and plain surface (i.e., clay) is obtained from the surface element integration (SEI) technique,<sup>11</sup> which assumes CNTs and clay surfaces are perfectly spherical and plain respectively. This assumption is undoubtedly not realistic but such simplifications facilitate the estimate of interaction energies. Second, viewing the interactions between CNTs and clay surfaces as particle-plain geometry may also be questionable in light of the rod-shaped geometry of CNTs. Third, the EDLVO as well as DLVO theories have preferably been used for monovalent salts at relatively low concentrations.

#### S5. The relative importance of Brownian motion and sedimentation in aggregation

The relative importance of Brownian motion and sedimentation at the initial state of aggregation in static aqueous environment can be evaluated by Peclet number, which is expressed as <sup>18</sup>:

$$P_e = \frac{\pi \Delta \rho g d^4}{24kT}$$

where  $\Delta \rho$  is the density difference between MWCNTs and water, *g* is the acceleration due to gravity. When  $P_e \ll 1$ , Brownian motion dominates and aggregation is pure perikinetic, whereas at  $P_e \gg 1$  aggregation is mainly orthokinetic, due to differential sedimentation. By taking  $P_e = 1$  the critical diameter of particles can be estimated as 1306.4 nm. The Peclect numbers according to different sizes of MWCNTs with four sodium concentrations are all less than 1 as shown in Table S4.7, indicating that after 7-day period, sedimentation of aggregated MWCNTs remained in aqueous phase should be negligible in the aqueous system.<sup>18</sup>

# S6. Figures and Tables



(c)

FIGURE S4.1. Images measured by FEI Inspect F50 FEG scanning electron microscope (SEM) operating at an accelerating voltage of 15.00 kV: a) kaolinite; b) smectite; c) shale.



FIGURE S4.2 The typical spectra of three types of clay particles measured by scanning electron microscopy-energy dispersive spectroscopy (SEM-EDS) (FEI Inspect F FEG-SEM equipped with EDAX EDS) : a) kaolinite; b) smectite; c) shale.

	$\gamma_L$	$\gamma_i^{LW}$	$\gamma^+_L$	$\gamma_L^-$	$\gamma^{AB}$
Ultrapure water	72.8 <sup>a</sup>	21.8	25.5	25.5	51.0
Glycerol	64.0	34.0	3.9	57.4	30.0
Formamide	58.0	39.0	2.3	39.6	19.0

TABLE S4.1 Surface tension properties  $(mJ/m^2)$  of probe liquids at 20°C <sup>9</sup>

<sup>a</sup> Uncertainties for these parameters were not provided in this reference.

TABLE S4.2 Contact angles of three probe liquid on different types of clay layers

	Formamide	Glycerol	Water
Kaolinite	0±0	14.9±0.5	0±0
Smectite	0±0	0±0	0±0
Shale	0±0	11.9±0.7	22.3±0.8
MWCNT*	18.5±7.6	49.7±4.7	67.4±1.1

\* Cited from ref.<sup>19</sup> The results are represented by mean values and standard deviations that were calculated based on at least three independent contact angle measurements.

TABLE S4.3 Surface energy components and Hamaker constants of three types of clay and MWCNT.

Interacting surfaces	$\gamma_i^{LW}$ (mI/m <sup>2</sup> )	$A_{ii}$ (mJ/m <sup>2</sup> )	$\gamma_i$ (mI/m <sup>2</sup> )	Polar energy (mJ/m	surface y compo ( <sup>2</sup> )	onents	$\Delta G^{AB}_{iwi,D_0}$ (mI/m <sup>2</sup> )	$\Delta G^{AB}_{CwM,D_0}$ (mI/m <sup>2</sup> )
	(1113/111 )		(1113/111 )	$\gamma^+$	$\gamma^-$	$\gamma_i^{AB}$	(1115/111 )	(1113/111)
Water <sup>20</sup>	21.8	4.0×10 <sup>-20</sup>	72.8	25.5	25.5	51.0	-	
MWCNT <sup>12, 14</sup>	16.2	$7.5 \times 10^{-20}$	16.2	17.6	0	0	-35.5	
Kaolinite	26.6	$4.9 \times 10^{-20}$	26.6	61.6	76.8	0	-41.6	34.6
Smectite	16.7	$3.1 \times 10^{-20}$	66.1	11.7	52.1	49.4	14.1	-12.7
Shale	9.1	$1.7 \times 10^{-20}$	9.1	10.9	0	0	-35.3	-26.3

Cited from ref.<sup>20</sup><sup>12, 14</sup>

TABLE S4.4 Hamaker constants ( $A_{CwM}$ ) between different interacting entities.

(×10 <sup>-20</sup> J)	MWCNT	Kaolinite	Smectite	Shale
MWCNT	5.5	1.6	-1.8	-5.1
Clay 1 (Kaodinite)	1.6	0.5	-	-
Clay 2 (Smectite)	-1.8	-	0.6	-
Clay 3 (Shale)	-5.1	-	-	4.8

$A_{CwM}$	Clay-MWCNT Hamaker constant in water ( <i>w</i> ). Detailed determination is provided in
	the section S2 of SI.
R	The hydrodynamic radius of the MWCNT particles.
$k_B$	Boltzmann constant, $1.38 \times 10^{-23}$ J/K.
Т	The absolute temperature, 298 K.
Zi	The valence of the i <sup>th</sup> ion.
e	Unit charge, $1.602 \times 10^{-19}$ C.
<i>φ</i> <sub>C</sub> , <i>φ</i> <sub>M</sub>	Surface potentials (mV) for clay particles and MWCNT. From measured zeta
	potential ( $\zeta$ ), the surface potentials can be determined by the Debye-Hückel
	approximation, <sup>21, 22</sup> $\varphi = \zeta (1 + d/R) \exp(-\kappa d)$ , where d is the distance from the
	particle's surface to the slipping layer, a distance that is generally of the order of $\approx 3$
	to 5 Å.
κ	The reciprocal of the Debye length (nm <sup>-1</sup> ).
<u>80</u>	The dielectric permittivity of a vacuum, $8.854 \times 10^{-12} \text{ CV}^{-1} \text{m}^{-1}$ .
З	The dielectric constant of water, 78.5 (dimensionless).
$N_A$	<u>Avogadro's number</u> , $6.02 \times 10^{23}$ mol <sup>-1</sup> .
Ι	Ionic strength (M), $I=0.5 \cdot \Sigma c_i Z_i^2$ , where $c_i$ is the molar concentration of one species
	ions (i).
λ	The correlation length, or decay length, of the molecules of the liquid medium. For
	pure water, it is approximately 0.6 nm. <sup>9</sup>
$\Delta G^{AB}_{H_{WF}D_{e}}$	The polar or acid-base free energy of interaction between clay particles and MWCNT
11,20	in water (w) at the distance $(D_0)^{10}$ ). Detailed determination of $\Delta G_{H_{WE},D_0}^{AB}$ is provided
	in the section S2 of SI.
$D_0$	The minimum equilibrium distance due to Born repulsion, 0.157 nm. <sup>11</sup>

TALBE S4.5 Parameters of relevance in EDLVO theory calculations

TABLE S4.6 Elemental composition of the minerals. Values indicate the mean atomic percentage and uncertainties represent standard deviations (n=3) determined using SEM-EDS. ND indicates not detected.

	kaolinite	smectite	shale
Element	At%	At%	At%
С	64 ±12	$24\ \pm 19$	69 ±6
0	24 ±11	$54 \pm 13$	$0.86\ \pm 1.05$
Na	$0.44 \pm 0.4$	$0.56\ \pm 0.03$	$23 \pm 4$
Mg	ND	$0.53 \pm 0.22$	$0.1\ \pm 0.02$
Al	$5.3 \pm 3.2$	$3.3 \pm 1.5$	$0.31~\pm0.05$
Si	$3.7 \pm 1.6$	$16 \pm 4.3$	$1.5 \pm 0.5$
Cl	$0.3 \pm 0.2$	$0.42\ \pm 0.21$	ND
Fe	ND	$0.48\ \pm 0.22$	$0.39 \pm 0.25$
Р	ND	ND	$4.4 \pm 1.6$

		1	1	
-	Na+ conc.	Z-average hydrodynamic	Polydispersity	Peclet
-	(mmol/L)	diameter (nm)	index	number
	0	81.15 (1.8)	0.302	1.49E-05
	0.4	82.47 (3.7)	0.293	1.59E-05
	4	101.42 (9.5)	0.305	3.63E-05
_	40	409.45 (20.7)	0.375	9.65E-03

TABLE S4.7 Hydrodynamic diameter of MWCNTs aggregates in aqueous phase determined by zetasizer. Standard deviations of duplicates were included in parenthesis.

TABLE S4.8 Statistical analysis of the effect of dissolved materials from soil mineral on MWCNT stability. Values provided are the probability (p values) that the slope, a, with soil mineral solution is the same as control treatment without soil mineral solution, under different Na<sup>+</sup> concentrations. Small values indicate significant differences (i.e., soil mineral solution affected the stability of MWCNTs).

	Kaolinite	Smectite	Shale
$Na^+$			
Conc.	Kaolinite	Smectite	Shale
(mmol/L)			
0	0.5762	0.9397	0.3635
0.4	0.0352	0.5047	
4	0.6263	0.1693	0.7441
40	0.6856	0.3037	0.5222

Na <sup>+</sup> Conc. (mmol/L)	0	0.4	4	40
0		< 0.001	< 0.001	< 0.001
0.4			< 0.001	0.0149
4				0.0964
40				
	Smectite			
Na <sup>+</sup> Conc.				
(mmol/L)	0	0.4	4	40
0		< 0.001	< 0.001	< 0.001
0.4			< 0.001	< 0.001
4				< 0.001
40				
	Shale			
Na <sup>+</sup> Conc.				
(mmol/L)	0	0.4	4	40
0		0.9015	0.0006	0.0295
0.4			0.0818	0.0253
4				0.9838
40				

TABLE S4.9 Comparison of the slope a with different Na<sup>+</sup> concentrations to assess if the a values are significantly different. Small p values indicate significantly different slopes. Kaolinite

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## APPENDIX C

# SUPPORTING INFORMATION:

# PRIMARY PARTICLE SIZE ON COLLOIDAL STABILITY OF 14C-LABELED

MULTIWALL CARBON NANOTUBES  $^{8}$ 

<sup>&</sup>lt;sup>8</sup> Liwen Zhang, Zhengwei Pan, Qingguo Huang. Submitted to *Water Science and Technology*, Jun 2013.

#### Effect of density to the settling of the MWCNTs aggregates

Stokes' Law is widely applied in simulating the particle movement, i.e., the settling, in aqueous system. We can apply the derivative equation of Stokes' Law to our system:

$$v_s = \frac{1}{18} \frac{(\rho_p - \rho_w)}{\mu} g D^2$$

where  $v_s$  is the particles' settling velocity;  $\rho_p$  and  $\rho_w$  are the mass densities of the particle and water/solution, respectively; *D* is the equivalent hydrodynamic diameter of the particle or aggregates measured by DLS.  $\mu$  is the viscosity of water which is  $1.0020 \times 10^{-3}$  N s/m<sup>2</sup> at 20 °C; *g* is the gravitational acceleration; these parameters can be viewed as constant in the conditions applied in our experiment. The MWCNTs suspension was centrifuged at 3500g for 5 min, which means the parameter *g* is  $3500 \times g \approx 3.325 \times 10^4$  m/s<sup>2</sup>. In our experiment, the supernatant after centrifugation at the top 3 cm portion was sampled. Thus the cut-off value of the settling velocity  $v_s$  is approximately 3 cm  $\div$  5 min =  $1 \times 10^{-4}$  m/s, which means that the particles with velocity higher than this value are considered to be unstable in this system, while those with lower velocity can remain in the supernatant. The settling velocity  $v_s$  is critical in determine whether or not the particle can be stably suspended in the aqueous system. According to this equation, the particles with larger density will have higher settling velocity if other parameters are the same.

As a result of the aggregation induced by the addition of NaCl, shorter MWCNTs not only have higher extend of aggregation, but also more compact aggregates, consequently higher particle density. When considering the dispersion of colloidal particles, the density of the particle is always thought to be a constant. Thus according to the equation, the settling velocity is proportional to the hydrodynamic diameter, i.e., the size of the particle. In this context, since the cut-off velocity in our experiment with different Na<sup>+</sup> concentrations is the same, the cut-off hydrodynamic diameter should also be the same, which contradict to our result in Table 1. However, with the presence of cation, MWCNTs will aggregate and the mass density is no longer the density of the primary particles which is relative universal; instead, it is the density of the aggregates and becomes polydispersed. In this sense, both the diameter and the density are polydispersed, which adds to the complexity of the MWCNT suspension system.

Generally speaking, when particles aggregate the overall density will decrease due to the formation of pores in the aggregates. The larger the aggregates, the lower the density will be. The addition of Na<sup>+</sup> caused the decrease of the number of primary MWCNTs while the increase of the amount of MWCNTs aggregates, and consequently a lower overall density of the MWCNTs, which can partially cancel the effect caused by the increment of the diameter. The extent of this effect is more pronounced with 40 mmol L<sup>-1</sup> than 4 mmol L<sup>-1</sup> Na<sup>+</sup>. Therefore the overall density of MWCNTs decreased, and consequently the value of the stable hydrodynamic diameter increased with the Na<sup>+</sup> concentration as shown in Table 1. According to the cut-off velocity and hydrodynamic diameter, the simulated overall mass density of the MWCNTs were  $2.45 \times 10^3$  kg m<sup>-3</sup>,  $2.31 \times 10^3$  kg m<sup>-3</sup>, and  $1.84 \times 10^3$  kg m<sup>-3</sup> under Na<sup>+</sup> concentration of 0, 4 or 40 mmol L<sup>-1</sup>, respectively. This result shows that when modeling the MWCNTs removal from aqueous phase by diffusion, the change of density due to aggregation need to be considered, and the size range of stable MWCNTs may be larger than the calculated value using the mass density of primary MWCNTs.



FIGURE S5.1 Scanning electron micrographs of: a) MWCNT suspension; b) MWCNT suspension with peat NOM; c) MWCNT suspension with NaCl. The magnifications are 10 k, 20 k and 40 k for a), b) and c), respectively. The black material in graph b) is likely to be NOM. The crystals formed in graph c) are NaCl. Most of the MWCNTs in graph a) and b) are individually dispersed, while in graph c) MWCNTs are agglomerated.



FIGURE S5.2 Length distribution of MWCNT suspension measured by SEM.

## APPENDIX D

# SUPPORTING INFORMATION:

# DEGRADATION OF MULTIWALL CARBON NANOTUBES BY BACTERIA9

<sup>&</sup>lt;sup>9</sup> Zhang, L.; Petersen, E.J.; Habteselassie, M.Y.; Mao, L. and Q. Huang. Accepted by *Environmental Pollution*, June 2013. Reprinted here with permission of the publisher.

#### I. MWCNT Purification and Characterization.

Purification and characterization of the MWCNTs were previously described,<sup>23</sup> and this information is repeated here. Briefly, MWCNTs synthesized from the CVD method were bath sonicated in hydrochloric acid (11.1 M) for 1 h followed by washing with boiling water to remove catalyst impurities associated with the CVD synthesis. The MWCNTs were subsequently sonicated for 2 h in an acid mixture composed of concentrated sulfuric (14.8 M) and nitric (15.6 M) acids in a 3:1 volume ratio and the samples were then washed again with boiling water.<sup>23, 24</sup> This purification process is known to introduce functional groups (e.g., carboxyl groups) to the nanotube surface. To make a stable stock dispersion, the MWCNTs were then ultrasonicated for 2 h and centrifuged at 3500 g for 5 min; the ultrasonication power applied to the dispersion was measured using the method described by Taurozzi and coworkers and was found to be 34.7 W  $\pm$  0.8 W (uncertainty indicates the standard error from the linear regression analysis). The supernatant was collected as the stock dispersion.<sup>25</sup>

The MWCNTs were characterized using several techniques as previously described.<sup>23</sup> The surface area of the acid-treated MWCNTs is 111 m<sup>2</sup>/g as measured by the standard Brunauer–Emmett–Teller (BET) method using nitrogen adsorption at 77 K (Micromeritics Gemini 2375, Norcross, GA).<sup>23</sup> The carbon purity was evaluated using thermal gravimetric analysis (TGA) (Pyris 1 TGA, Perkin-Elmer). Amorphous carbon impurities generally burn at lower temperatures than carbon nanotubes, and thus the lack of a peak of the derivative of the mass change with respect to temperature at lower temperatures than the principal MWCNT peak indicates the lack of amorphous carbon (see Fig. S6.1 part a). As shown in the TGA graphs in Fig. S6.1 part a, the percentage of metal catalysts was  $1 \pm 1\%$ .<sup>23, 26</sup> TGA analysis of MWCNTs after 6 h sonication, which is longer than the 2 h sonication period used in this study, did not

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indicate an increase in the presence of amorphous carbon. According to images obtained from scanning electron microscopy (SEM) analysis (Fig. S6.1 part b), the majority of MWCNTs were individually dispersed and maintained the MWCNT morphology.<sup>23</sup> The MWCNT length distribution was measured using SEM and provided in Fig. S6.1 part c. The average diameter was  $36.5 \pm 12.7$  nm (n = 80, the uncertainty indicates the standard deviation value) ranging from 20 nm to 90 nm. Electrophoretic mobilities of MWCNTs were measured on a Malvern Zetasizer Nano ZS instrument. Point of zero charge (PZC) of the MWCNT stock dispersion was determined using ten points from pH 1.00 to pH 5.83 and was approximately pH 1.5. The result is shown in Fig. S6.1 part d.



Fig. S6.1 part a



Fig. S6.1 part b



Fig. S6.1 part c



Fig. S6.1 part d

**FIG. S6.1** MWCNT characterization. a) Thermal gravimetric analysis (TGA) graph of the MWCNT powder (blue) and the dispersed MWCNTs after ultrasonication for 6 h (orange). b) Scanning electron microscopy (SEM) images at 16 kx magnification showing the morphology of suspended MWCNTs. c) Initial length distribution of MWCNT suspension measured by SEM (n = 797). d) Electrophoretic mobilities of suspended MWCNTs at pH values varying from 1.00 to 5.83. Error bars represent the standard deviation from two measurements made of the same sample at each pH value; each run consisted of 50 to 100 sub-runs. The pH was adjusted by HCl, and the cation concentration was kept constant at 0.1mol/L by adding NaCl. This figure is reprinted and modified with permission from [1] copyright (2011) American Chemical Society.

#### II. MWCNT Degradation

The experiment set-up is illustrated in Fig. S2. All devices and chemicals were sterilized except when stated otherwise. The flask was air tight with a sealed gas inlet and a gas outlet connected to a test tube containing 10 mL of 0.5 mol  $L^{-1}$  NaOH solution to capture CO<sub>2</sub> if any evolved from the incubation. The gas produced in the flask passed through an additional bottle before the test tube with NaOH solution to prevent the possible contamination between the NaOH solution and culture medium. After 7 d, the radioactivity of the NaOH solution was mixed with scintillation cocktail (Insta-Gel Plus) and measured by a Beckman LS 5801 liquid scintillation counter (LSC). The counting time for LSC was 10 min for each run. The chemiluminescence was monitored by the Beckman LS5801 counter. The data was tolerated if less than 10% of the reading came from chemiluminescence; otherwise, the mixture of NaOH and cocktail was diluted using more cocktail. The <sup>14</sup>C background level calculated from the control samples was  $43.4 \pm 1.9$  counts per minute (n = 5; uncertainty represents standard deviation). The degradation mass or percentage was obtained by subtracting the radioactivity of the treatment samples by the background radioactivity. The actual radioactivity values in counts per minute (CPM) are listed in Table S2.

To screen possible degraders of MWCNTs, 5 mL of the sterilized MWCNTs dispersion was added to 45 mL culture medium (composition listed in Table S1). Then this mixture was inoculated with 100 µl solution containing various organisms including fungus spore solution of *Phanerochaete chrysosporium*, *Trametes versicolor* or *Pycnoporus* sp. SYBC-L3, belonging to *Basidiomycetes* (spore solution absorbance at 650nm > 0.1, approximately  $5 \times 10^5$  spores ml<sup>-1</sup>)<sup>27</sup>, soil extraction by pH 7.0 PBS buffer (loamy sand or sandy loam from Griffin, GA, using  $10^{-2}$ ,  $10^{-3}$ ,  $10^{-4}$ , and  $10^{-5}$  dilutions) prepared according to Zuberer (1994), <sup>28</sup> and anaerobic or aerobic sludge from potato creek wastewater treatment plant (Griffin, GA, U.S.). In another system, instead of 5 mL of sterilized MWCNTs, 5 mL of unsterilized MWCNTs without any other microbes was added to the culture medium. In this last system, microbial activity and degradation of MWCNTs were found. There were two control systems: 1) 5 mL of sterilized MWCNTs dispersion added to 45 mL of sterile culture medium, and 2) 5 mL de-ionized water added to 45 mL of culture media, and then 10- $\mu$ l solution containing the MWCNTs degrading bacteria community. No significant amount of <sup>14</sup>CO<sub>2</sub> was produced from these flasks after a 7-d period if compared to the controls.

	concentration in 50 mL	sterilization method	
Chemicals	(g/L)		
KH <sub>2</sub> PO <sub>4</sub>	2		
MgSO <sub>4</sub>	0.5		
CaCl <sub>2</sub>	0.1		
$MgSO_4$	0.21		
MnSO <sub>4</sub>	0.035	Autoclave	
NaCl	0.07		
FeSO <sub>4</sub> .7H <sub>2</sub> O	0.007		
CuSO <sub>4</sub>	0.007		
AlK(SO <sub>4</sub> ) <sub>2</sub> .12H <sub>2</sub> O	0.00007		
H <sub>3</sub> BO <sub>3</sub>	0.00007		
Na <sub>2</sub> MoO <sub>4</sub> .2H <sub>2</sub> O	0.00007	0.22 µm filter-sterilization	
Nitrilotriacetate	0.105		
Glucose	10	Autoclave	
Thiamin	0.001	0.22 µm filter-sterilization	
Ammonium tartrate	0.2	Autoclave	
Veratryl alcohol	0.04 M	0.22 µm filter-sterilization	
2,2- dimethylsuccinate,	0.01 M, pH4.2	$0.22 \ \mu m$ filter-sterilization	

TABLE S6.1 Contents of culture medium



FIG. S6.2 Experiment set up for MWCNT degradation.

**TABLE S6.2** The radioactivity readings in counts per minutes (CPM) of the initial  $^{14}$ C-MWCNT dispersions and the product  $^{14}$ CO<sub>2</sub>.

Total initial C <sup>14</sup> -MWCNT radioactivity <sup>a</sup>	Initial mass (µg)	Total $CO_2$ radioactivity <sup>b</sup>	Degraded mass (µg)	Percentage (%)
$34305\ \pm 119$	51.5	$2211 \pm 13.9$	3.32	6.44
$34305\ \pm 119$	51.5	$3376 \pm 9.3$	5.07	9.84
$18956 \pm 66$	28.5	$845\ \pm 2.2$	1.27	4.46
$18956 \pm 66$	28.5	$1168\ \pm 1.6$	1.75	6.16
$6660 \pm 23$	10.0	$132 \pm 9.4$	0.20	1.98
$3791\ \pm 13$	5.7	$96 \pm 2.3$	0.14	2.53
$2637\ \pm9$	4.0	$79\pm 6.0$	0.12	3.00
$2109\ \pm 7$	3.2	$83\ \pm 5.0$	0.12	3.93

<sup>a</sup> The initial concentrations were calculated from dilutions of the stock dispersion.

<sup>b</sup> Uncertainties represent standard deviation values calculated from three measurements by LSC.

## III. LC-MS, LC-MS/MS and GC-MS Measurement

The detection of possible intermediate products was performed by liquid

chromatography-tandem mass spectrometry (LC-MS/MS, Waters Micromass QuattroMass) and

gas chromatography-mass spectrometry (GC-MS, Hewlett Packard 5971). One mL of the solution phase from the microbial degradation mixture after 7-d incubation was sampled after centrifugation at 5000 g for 5 min (Eppendorf Centrifuge 5804, Germany). The samples were prepared by liquid-liquid extraction using either ethyl acetate or dichloromethane. The solution was sequentially mixed with 3 mL, 3 mL, and 5 mL of the extracting solvent and was shaken thoroughly. The resulting extractants were combined and concentrated using gentle nitrogen gas blow, added to 10 mL methanol, and reduced to 1 mL. Approximately 10 µL of each sample was injected into an LC unit (Waters 2690) equipped with an Ascentic C18 reverse phase column  $(250 \times 4 \text{ mm}, 5 \mu\text{m}; \text{Supelco}, \text{St. Louis, MO})$ . The mobile phase was a mixture of water and acetonitrile in a water-to-acetonitrile ratio changing from 5:5 to 1:9 at a rate of 1 mL/min. The LC unit was interfaced with a quadruple mass analyzer (Waters Quattro Micromass) through electrospray ionization in a negative mode, which usually creates molecular ions with one negative charge by losing a proton. Thus, the resulting m/z ratio is the molecular mass with one mass unit less. By comparing the treatment samples with control samples without bacteria or without MWCNTs, the m/z ratio of the intermediate products were identified and then subjected to LC-MS/MS measurement with the same LC condition, and collision energy ranged from 11 to 15 eV according to different molecules. The LC chromatograms of two products are shown in Fig. 2 and those of additional four products given in Fig. S3.

GC-MS (Hewlett Packard 5971) measurements were made using an Alltech Econocap EC-5 column, (30 m, 0.25 mm ID, 0.25 um film) with a helium flow rate of 1 mL/min. GC started at 70  $^{\circ}$ C for 3 min, then ramped at 10 degrees/min to 280  $^{\circ}$ C and held for 1 min. The MS scanned from 30 to 500 m/z using electron ionization.










Part c



Part d



Part e





**FIG. S6.3** Selected ion chromatograms of LC-MS for four intermediate products: a-c) extracted by ethyl acetate and d) extracted by dichloromethane. The m/z values are on the upper left of each chromatogram. All four chromatograms were subtracted by the chromatograms of two controls: one control was incubation without bacteria and the other was without MWCNTs. Overall LC chromatographs extracted by e) ethyl acetate and f) dichloromethane.

The secondary MS spectrum of the parent ion m/z = 165 is shown in Fig. S4 as an example. The most abundant peak in the spectrum, m/z = 148, is likely the fragment resulting from the loss of  $\cdot$ OH (M = 17). The next two abundant peak clusters, m/z = 120 and m/z = 102, were likely formed by further losing the CO $\cdot$ , and the H<sub>2</sub>O from the m/z 148 ion. The reason why it could not be 1, 2-benzenedicarboxylic acid is that the loss of neutral H<sub>2</sub>O of this acid is too favorable so that the molecular ion peak, m/z = 165, would be eliminated. Interpretation of the other spectra was similarly conducted. In order to further confirm the product structures, we have tested high purity standards (> 99 %, Sigma-Aldrich) on the LC-MS/MS for verification (Fig. S5). The results provided a definitive confirmation.



**FIG. S6.4** The secondary MS spectrum of a possible intermediate product with m/z = 165 in the primary MS. The postulated structures of the intermediate product are shown in the up-left corner.





Part b



Part d

**FIG. S6.5** The secondary MS spectrum of the intermediate products and high purity chemicals purchased from Sigma-Aldrich with m/z value in the primary MS as: a) m/z = 143; b) m/z = 158; c) m/z = 165; d) m/z = 131. The structures of the intermediate product are shown in the upper-left corner.

## IV. Method for Enzymatic Degradation Experiments

The reactions were carried out in a similar setup to that of the microbial degradation experiments with a reduced solution volume of 20 mL in 150-mL flasks. The MWCNT concentration used was 1 mg/L. Three enzyme concentrations were tested: 0.01, 0.1, and 1 unit/mL. Ten mmol/L concentrations of buffer were used to maintain the pH. Phosphorous buffer was used to maintain pH values of 7.0 or 6.5 for HRP and tyrosinase, respectively, while citric acid buffer was used to maintain a pH of 4.5 for laccase. For HRP experiment, initial concentration of H<sub>2</sub>O<sub>2</sub> (Sigma-Aldrich, 30 wt. %) was 0.09 mmol/L, and 200  $\mu$ l of 0.9-mmol/L H<sub>2</sub>O<sub>2</sub> solution was added every day, while for laccase and tyrosinase experiments aeration was done for 20 min every day for 7 d (34 kPa). The enzyme activity in solution was tested each time before each treatment using ABTS as the substrate for HRP, 2,6-dimethoxyphenol for laccase, and L-3,4-dihydroxyphenylalanine for tyrosinase.

## V. Identification of Potential MWCNT Degraders

The genomic DNA of the microorganisms was extracted and purified using DNAzol genomic DNA isolation reagent kit (Molecular Research Center, OH). The DNA extract was then subjected to polymerase chain reaction (PCR) by targeting the 16S-rDNA gene with the bacterial universal primer pair 27f (5'-GAGAGTTTGATCCTGGCTCAG-3') and 1495r (5'-CTACGGCTACCTTGTTACGA-3')<sup>29</sup>, which were synthesized by Integrated DNA

Technologies (IDT, U.S.). The PCR had a total reaction volume of 25  $\mu$ L, containing 12.5  $\mu$ L GoTaq® Colorless Universal PCR Master Mix (Promega, Germany), approximately 75 ng of DNA and 1 mmol/L of each primer. The PCR conditions were as follows: an initial denaturation step at 95 °C for 5 min followed by 34 cycles of denaturation at 95 °C for 1 min, primer annealing at 59 °C for 1 min, and extension at 72 °C for 1.5 min, and a final extension at 72 °C for 10 min <sup>29</sup>. The PCR amplicons were cloned into the pGEM-T Easy vector (Promega, Germany) following the manufacturer's instructions. Fifteen colonies that contained the amplicons were selected, transferred to a standard LB medium, and incubated at 37 °C for 2 d. The recombinant plasmid was extracted, digested with restriction enzyme EcoRI, and the products of expected length (1495 bp) were detected by gel electrophoresis. The 15 plasmid with inserts, ranging in concentrations between 390 and 450 ng/ $\mu$ L were sent to Georgia Genomics Facility, University of Georgia, for sequencing using M13 forward and reverse primers. Sequencing results were analyzed through BLAST and species were assigned using criteria of 99% sequence identity.

## VI. SEM and TEM Characterization before and after Degradation

Scanning electron microscope (SEM, FEI Inspect F50 FEG) and transmission electron microscope (TEM, FEI Technai 20) were used to characterize the morphological change of MWCNTs before and after incubation. The SEM measurement was operated at an accelerating voltage of 10.00 kV or 15.00 kV (see Fig. S1 part b for before degradation and Fig. S6 for after degradation).



Fig. S6.6 part a



Fig. S6.6 part b



Fig. S6.6 part c

**FIG. S6.6** SEM images of MWCNTs after degradation: a) MWCNTs maintained the tubular morphology and attached to microbe surface; b) Free MWCNTs not attached to microbes; c) MWCNTs marked using red boxes and microbes. It is clear that more than one bacteria species were presented in the solution.

Transmission electron microscopy was also used to examine the morphology and structure of the MWCNTs before and after degradation. As shown in Fig. S7 a-b, before degradation the MWCNTs showed a tubular morphology with thin inner diameter and thick carbon-lattice layers. The TEM images of the MWCNTs after degradation are shown in Fig. S7 c-f. Graph d) was taken from the open end of the MWCNT in c) and shows the transition from the regular carbon lattice crystal structure to the amorphous carbon without any pattern. This amorphous carbon is likely to be one of the intermediate steps during the degradation process. Aside from the amorphous carbon, pieces with the carbon-lattice structure as of MWCNTs but having lost the tubular shape were also observed (Fig. S7 e). This could be a by-product of

MWCNTs degradation. Amorphous carbon was also found to be associated with the outside layer of MWCNTs as shown in graph f). Moreover, we can see the differential etching depth of degradation activity indicated by staggering from appearance to disappearance of the carbon lattice. These results suggest that the degradation may start from both the outer layer and the open end.





**FIG. S6.7** TEM images of MWCNTs **a-b**) before and **c-f**) after degradation for 7 day. d) and e) are two parts taken from c) as shown by red boxes and arrows. The red box in f) shows the disruption of carbon crystal lattice on MWCNT surface.