University of Nevada Cooperative Extension

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# Detecting Multiwall Carbon Nanotube Uptake and Translocation in Lettuce to Enhance Food Safety Assessment


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***Reclaiming Water for Urban Foodsheds*** *integrates basic scientific research with extension outreach to examine the feasibility of using reclaimed water resources for irrigated agriculture in urban environments. Funded by a grant [2017-69007-26309] from the USDA National Institute of Food and Agriculture, research is conducted in University of Nevada, Reno campus laboratories and the Nevada Agricultural Experiment Station Greenhouse Complex. The research reported in this fact sheet also received funding from USDA (Grant No. 2015-67018- 23120) and DOE (Grant No.DE-SC0014275).*


## Introduction

Carbon nanotubes (CNTs) are engineered nanomaterials (a nanometer is one-billionth of a meter) shaped as cylinders made up of carbon atoms. CNTs are stronger than steel but are only about one-sixth the weight. The unique thermal, magnetic and electrical properties of CNTs make them useful in a multitude of products we use daily, including electrical (batteries), renewable energy (solar cell technologies), construction (rebar for concrete reinforcement), transportation (planes, boats, car parts), biomedical (imaging technology, pharmaceutical carriers, implants), cosmetic (anti-aging products, sunscreen), and recreational applications (golf clubs, bicycles, skis, toys).

CNTs can be made of a single sheet of graphite material (single-walled carbon nanotubes; SWCNTs), or can be made of multiple layers to form multi-walled carbon nanotubes (MWCNTs). MWCNTs are exceptionally strong and can vary in length, shape and metal composition, making the assessment of environmental risk particularly difficult. Yet MWCNTs are the most commonly produced carbonaceous nanomaterials, with an estimated global production of 9,400 tons in 2015 alone (De Volder et al., 2013).

An increase in the number of new applications for CNTs has led to a rapid increase in their production (De Volder et al., 2013). With increasing production and use of CNTs, and their subsequent release during the life cycle of CNT-based products, these engineered nanomaterials are likely to accumulate in surface waters, wastewater treatment plant effluents, biosolids, sediments and soils. Subsequent and unintended uptake of CNTs by agricultural crops could increase the risk of human exposure through the food chain (Das et al., 2018; Miralles et al., 2012). It is estimated that more than 50 percent of released CNTs will enter soils, resulting in an annual release of 20−40 tons to soils by 2030 (Gottschalk et al., 2013). Still, relatively little is known about the effects of CNT on soils and water (Ge et al., 2016). While toxicology studies are underway to identify specific CNT risks to human health, the focus is primarily on occupational exposure and biomedical applications (Donaldson et al., 2006). Therefore, accurate detection of CNTs in agricultural plants is necessary to understand potential pathways for human exposure through the food chain and to assess impacts on the environment.

A recent study shows that more than 1.25 milligram per kilogram (mg/kg) MWCNTs can accumulate in leaves of rice (*Oryza sativa)*, maize (*Zea mays*) and soybean (*Glycine max*) plants when these plants are grown in hydroponic systems with 2.25 milligram per liter (mg/L) of MWCNTs (Lin et al., 2009; Zhao et al., 2017). The chemical properties of CNTs, among many other factors, can influence their uptake and translocation in plants. For instance, dispersion and accumulation patterns in maize tissue and cells were different for pristine MWCNT (p-MWCNT), without any reactive groups on tube surface and MWCNTs containing carboxylic functional groups (c-MWCNT) on the surface (Zhai et al., 2015). Interactions between CNTs and soil components also greatly influence their uptake in plants.

Nanotechnology has received recent attention for helping to control agricultural production input costs while encouraging sustainable agriculture through improved targeted fertilizer delivery and release (Raliya et al., 2017). In addition, its use in accelerating and regulating plant growth may improve the production of biofuel and food crops, thereby improving both energy and food security at a global scale (Mukesh and Jha, 2017). However, responses of plants to CNTs depend on species, growth medium and environmental conditions, as well as CNT concentration and type (Mukherjee et al., 2016). Despite nanotechnology being used more frequently in food production, its potential effects on the environment and human health are not clear. Additional scientific research is needed to better understand these risks (Raliya et al., 2017).

**Methods**

It is unclear how CNTs enter the plant roots and how CNTs are subsequently translocated to aboveground organs (stem, leaves and fruits). Our understanding of CNT uptake by agricultural plants is largely hindered by technical difficulties associated with detecting and quantifying CNTs in biological tissues. To address this challenge, we developed an analytical method that included enhanced plant material digestion in combination with Raman spectroscopic analysis. Digestion breaks down the plant tissue and releases CNTs that can then be studied with analytical techniques such as Raman spectroscopy. Raman spectroscopy is commonly used to identify specific molecules.

We applied our analytical method to study MWCNT uptake and translocation in lettuce grown under hydroponic conditions. Lettuce was grown in the Nevada Agricultural Experiment Station Greenhouse Complex, while chemical analyses were performed in the Harry Reid Engineering Laboratory on the University of Nevada, Reno campus. For this first study of its kind, we chose lettuce (*Lactuca sativa* L.) as a model agricultural plant because it is relatively easy to digest, and as a result, most likely produces the clearest Raman signal of any MWCNTs present in the plant tissues.

Plants were exposed to different concentrations of reactive and non-reactive MWCNT added to the hydroponic solution. At harvest, a number of plant responses were determined, including water use, dry biomass and root biomass. Harvested plant tissues were treated with several reagents, or chemical substances, to enhance our ability to detect MWCNTs. Acidic, alkaline and oxidative reagents, including sulfuric, hydrochloric and nitric acid; ammonium hydroxide; and hydrogen peroxide, can hydrolyze and dissolve plant cell wall biopolymers, such as cellulose, hemicellulose and lignin, thereby reducing interference with the Raman spectroscopy measurements.

After 12-hour digestion of leaf or root tissues that do not contain MWCNTs, clear suspensions were obtained using nitric acid treatments. Treatments with sulfuric and hydrochloric acid resulted in dark suspensions, and treatments with ammonium hydroxide and hydrogen peroxide resulted in white and brown residues, indicating incomplete removal of materials that could interfere with MWCNT analysis. Further Raman analysis indicated that nitric acid digestion was most effective in removing any material that interfered with the MWCNT analysis.

## Results

For lettuce tissues that were spiked[1] with 2500 mg/kg dry weight[2] of nonreactive MWCNT (p-MWCNT) or reactive MWCNT (c-MWCNT) by mixing lettuce tissues with MWCNTs suspensions and subsequently digested by various reagents [as described above], both p-MWCNT and c-MWCNT were detected in the root, stem and leaf tissues of exposed lettuce plants (see Figure 1). This indicated that our plant tissue digestion methods were able to effectively remove potentially interfering plant compounds, allowing for clear detection of MWCNTs with Raman spectroscopy.

[1] The suspension of CNT (prepared in double deionized water (DDW)) was added to dry powder of plant tissues. The plants were collected from a local nursery, cleaned with DDW, dried at 80 C for 12 hours, and ground and sieved with 0.25 mm mesh. The CNT suspension was prepared by adding CNT powder in DDW and sonicating for 12 hours.

[2] We added 25 µg CNT in 10 mg dry plant tissues. This is the highest applied concentration used for method development. We used three orders magnitude lower than this concentration to show the effectiveness of the method. The applied lowest concentration was 25 mg/kg. We have not determined the detection limit explicitly.

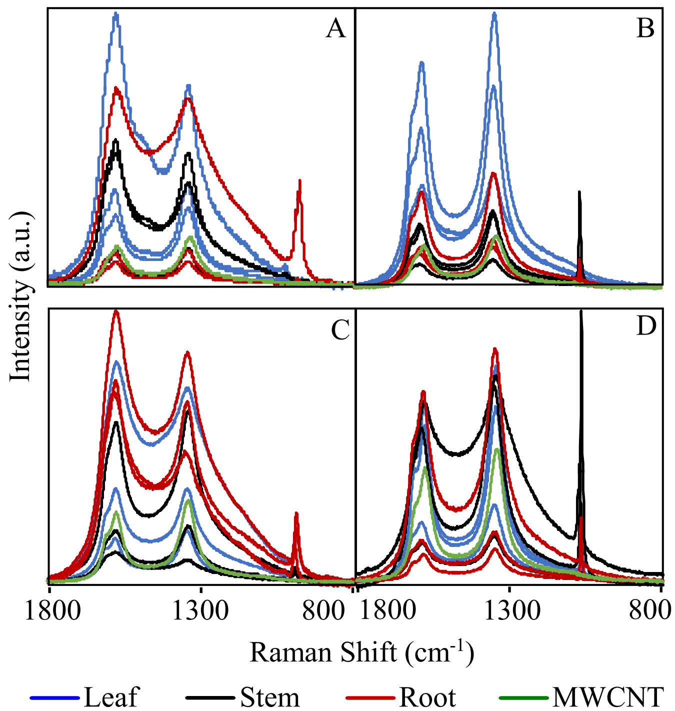


**Figure 1.** Raman spectra of lettuce tissues that were spiked with 2500 mg/kg dry weight of p-MWCNT (A, B) or c-MWCNT (C, D) and digested with $H_2SO_4$ (A, C) or $HNO_3$ (B, D). Triplicate spectra of leaf, stem and root samples are shown for each condition, along with pure MWCNT digested using the same reagent (see Das et al., 2018). The digestion with acidic reagents removed interferences of plant tissues. This process helped to facilitate detection of MWCNTs as the two fingerprint Raman peaks, as shown, appear at 1300-1350 and 1550-1600 $cm^{-1}$.

Our results demonstrate that nitric acid plant digestion in conjunction with Raman analysis is an effective approach for detecting MWCNTs in food. As a result, this method holds promise for developing new analytical techniques for studying the environmental fate of MWCNTs in the soil-plant system and human exposure through food-chain pathways.

### *Effects of MWCNTs on the growth and physiology of lettuce*

Most of the plants that were exposed to MWCNTs had less dry biomass than the control, nontreated plants. Only plants treated with 10 mg/L c-MWCNTs demonstrated physiological effects, such as higher root length. However, MWCNTs greatly affected root system architecture of lettuce plants by stimulating the development of lateral roots.

### *Detection of MWCNT uptake and translocation in lettuce*

To determine MWCNT uptake, we applied our analytical approach to lettuce plants grown hydroponically for 18 days. Trace levels of MWCNTs were detected in most root, stem, and leaf tissues of the plants exposed to either p-MWCNT or c-MWCNT, except for leaves of the plants exposed **to the highest applied concentrations of (20 mg/L) p-MWCNT** and stems of the **plants exposed to the highest c-MWCNT concentration (20 mg/L).** The fact that MWCNTs were identified in roots of all exposed plants and in stem and leaf tissue suggests that uptake is possible in lettuce for both MWCNTs and at exposure levels of 5, 10 and 20 mg/L MWCNTs in culture solution.

## Conclusions and Future Research

Agricultural plants can be exposed to CNTs through application of wastewater and biosolids. Increasing production and application of CNTs for industry and consumer products will likely lead to the accumulation of CNTs in soils and increase exposure to agricultural plants. This may have implications for human and animal health as plants are consumed. Our study showed that plants may take up CNTs, and that CNTs may accumulate in plant tissue (through processes indicated in Figure 2), which could potentially indicate a health concern. However, some recent studies also demonstrate positive effects of

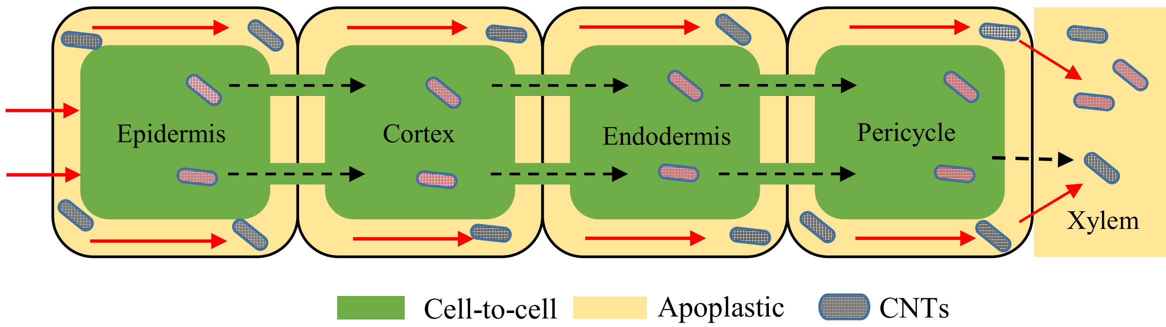


**Figure 2.** A proposed and simplified scheme for apoplastic and cell-to-cell uptake and translocation of CNTs. These pathways are similar to water and solute transport from the soil solution (left) to the xylem vessels (right), which can lead to transportation to the shoot and other organs. The grey hatch and red 'CNTs' are used to indicate the apoplastic and cell-to-cell pathways, respectively.

CNTs on plant growth, suggesting some potential for the application of CNTs in agriculture.

Therefore, unambiguous detection and quantification of CNTs in plant tissues are critical for evaluating CNT uptake by agricultural plants and associated environmental effects in order to optimize their agricultural application and evaluate potential health risks.

In this fact sheet, we report on the development of an effective method for detecting and analyzing MWCNTs in plant tissues by coupling optimized plant digestion using nitric acid with Raman spectroscopy, contributing to the potential development of new analytical platforms for studying the environmental fate of CNTs in the soil–plant system and ultimately human exposure to CNTs (Das et al., 2018). Applying this method, we demonstrated the uptake and translocation of p-MWCNT and c-MWCNT in all lettuce plants grown in hydroponic media. Uptake and translocation of CNTs in lettuce appears to be at least partly dependent on the surface chemistry of MWCNTs.

Our method could be used to quantitatively evaluate the uptake and translocation of CNTs by other agricultural plants. In order for CNTs to establish competitive advantage in agricultural industries, education outreach must be based on rigorous scientific research. CNT industries must work closely with public university research and outreach to explore the potential benefits and risks of CNT to humans, plants and the environment in order to enhance food security while ensuring food safety.

## References

Das, K., You, Y., Torres, M., Barrios-Masias, F., Wang, S., Tao, S., Xing, B. and Yang, Y. (2018). Development and application of a digestion-Raman analysis approach for studying multiwall carbon nanotube uptake in lettuce. *Environmental Science: Nano,* 5, 659-668.

De Volder, M.F.L., Tawfick, S.H., Baughman, R.H. and Hart, A.J. (2013). Carbon nanotubes: present and future commercial applications. *Science*, *339*, 535-539.

Donaldson, K., Aitken, R., Tran, L., Stone, V., Duffin, R., Forrest, G. and Alexander, A. (2006). Carbon nanotubes: A review of their properties in relation to pulmonary toxicology and workplace safety. *Toxicological Sciences*, *92* (1), 5-22.

Ge, Y., Priester, J.H., Mortimer, M., Chang, C.H., Zhaoxia, J., Schimel J.P. and Holden, P.A. (2016). Long-Term Effects of Multiwalled Carbon Nanotubes and Graphene on Microbial Communities in Dry Soil. *Environmental Science and Technology*, *50* (7), 3965–3974.

Gottschalk, F., Sun, T.Y. and Nowack, B. (2013). Environmental concentrations of engineered nanomaterials: review of modeling and analytical studies. *Environmental Pollutants*, *181*, 287-300.

Lin, S.J., Reppert, J., Hu, Q., Hudson, J.S., Reid, M.L., Ratnikova, T.A., Rao, A.M., Luo, H. and Ke, P.C. (2009) Uptake, translocation, and transmission of carbon nanomaterials in rice plants. *Small, 5* (10), 1128-1132.

Miralles, P., Church, T.L. and Harris, A.T. (2012). Toxicity, uptake, and translocation of engineered nanomaterials in vascular plants. *Environmental Science and Technology, 46* (17), 9224-9239.

Mukesh, T. and Jha, A.K. (2017). A Review on: Carbon Nanotubes Are Vital for Plant Growth. *American Journal of Agriculture and Forestry*. Special Issue: Pest Science, Vol. 5, No. 5-1, 1-9.

Mukherjee, A., Majumdar, S., Servin, A.D., Pagano, L., Dhankher, O.P. and White, J.C. (2016). Carbon nanotubes in agriculture: a critical review. *Frontiers in Plant Science*, *7*, 172.

Raliya, R., Saharan, V., Dimkpa, C. and Biswas, P. (2017). Nanofertilizer for Precision and Sustainable Agriculture: Current State and Future Perspectives. Article ASAP. Journal of Agricultural and Food Chemistry, DOI:10.1021/acs.jafc.7b02178.

Zhai, G.S., Gutowski, S.M., Walters, K.S., Yan, B. and Schnoor, J.L. (2015). Charge, size, and cellular selectivity for multiwall carbon nanotubes by maize and soybean. *Environmental Science and Technology, 49* (12), 7380-7390.

Zhao, Q., Ma, C., White, J.C., Dhankher, O.P., Zhang, X., Zhang, S. and Xing, B. (2017). Quantitative evaluation of multi-wall carbon nanotube uptake by terrestrial plants. *Carbon, 114*,