Surface coating changes the physiological and biochemical impacts of nano-TiO₂ in basil (*Ocimum basilicum*) plants

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Abstract

Little is known about the effects of surface coating on the interaction of engineered nanoparticles (ENPs) with plants. In this study, basil (*Ocimum basilicum*) was cultivated for 65 days in soil amended with unmodified, hydrophobic (coated with aluminum oxide and dimethicone), and hydrophilic (coated with aluminum oxide and glycerol) titanium dioxide nanoparticles (nano-TiO₂) at 125, 250, 500, and 750 mg nano-TiO₂ kg⁻¹ soil. ICP-OES/MS, SPAD meter, and UV/Vis spectrometry were used to determine Ti and essential elements in tissues, relative chlorophyll content, carbohydrates, and antioxidant response, respectively. Compared with control, hydrophobic and hydrophilic nano-TiO₂ significantly reduced seed germination by 41% and 59%, respectively, while unmodified and hydrophobic nano-TiO₂ significantly decreased shoot biomass by 31% and 37%, respectively ($p \leq 0.05$). Roots exposed to hydrophobic particles at 750 mg·kg⁻¹ had 87% and 40% more Ti than the pristine and hydrophilic nano-TiO₂; however, no differences were found in shoots. The three types of particles affected the homeostasis of essential elements: at 500 mg/kg, unmodified particles increased Cu (104%) and Fe (90%); hydrophilic increased Fe (90%); while hydrophobic increased Mn (339%) but reduced Ca (71%), Cu (58%), and P 40%). However, only hydrophobic particles significantly reduced root elongation by 53%. Unmodified, hydrophobic, and hydrophilic particles significantly reduced total sugar by 39%, 38%, and 66%, respectively, compared with control. Moreover, unmodified particles significantly decreased reducing sugar (34%), while hydrophobic particles significantly reduced starch (35%). Although the three particles affected basil plants, coated particles impacted the most its nutritional quality, since they altered more essential elements, starch, and reducing sugars.

Keyword: TiO₂ nanoparticles, Surface coating, Basil, Uptake, Nutritional value

Capsule: In general, TiO₂ nanoparticles significantly reduced total sugar; unmodified particles decreased reducing sugar and hydrophobic particles reduced starch in basil plants.

1. Introduction

There is an ever increasing production of engineered nanoparticles (ENPs) to fulfill the growing demand of nano-based consumer products. Titanium dioxide nanoparticles (nano-TiO₂) are one of the most consumed metal oxide ENPs (Chen and Mao, 2007 and Piccinno et al., 2012). In 2010, the global production was estimated to be 88,000 tons/year (Keller et al., 2013). Nano-TiO₂ are used in catalysis, paints, plastics, water treatment, nutritional supplements, and personal care products, among others (Chen and Mao, 2007, Johnson et al., 2011 and Klaine et al., 2008). This variety of applications leads to a high production and, consequently, high release of nano-TiO₂ into the environment. Keller and Lazareva (2014) estimated that the discharge of nano-TiO₂ into bodies of water around the San Francisco Bay area, California, USA exceeds 10,000 kg·year⁻¹. Nano-TiO₂ are very stable (Li et al., 2004); consequently, they are found in wastewater and accumulate in sewage sludge that, as biosolids, end up in agricultural fields. Estimates indicate that sewage sludges contain between 107-802 mg·kg⁻¹ nano-TiO₂ (Gottschalk et al., 2009), which suggests their potential accumulation in agricultural soils.

Several researchers have reported that the unmodified nano-TiO₂ affect plant systems in different ways (Gardea-Torresdey et al., 2014; Ma et al., 2015; Servin and White, 2016). Effects have been associated with the concentration, size, and crystal phase (Larue et al., 2011; Larue et al., 2012a; Larue et al., 2012b). Gao et al. (2013) reported a concentration-dependent uptake in *Ulmus elongata* seedlings, and observed a dose-dependent reduction of nitrogen isotope values. Larue et al. (2012a) examined the uptake of difference sized nano-TiO₂ in wheat and found that particles of 14 nm accumulated and distributed in all plant tissues without transformation; particles of 36 nm did not reach the stele and only accumulated in root parenchyma, while those of 140 nm were absent in roots. Servin et al. (2012, 2013) exposed a mixture of anatase (82%)

and rutile (18%) nano-TiO₂ to cucumber (*Cucumis sativus*) roots and found Ti in the leaf trichomes and fruits. In such study, rutile had a preferential uptake compared with anatase. Similar results were reported for tomato (*Solanum lycopersicum*) plants (Raliya et al., 2015; Song et al., 2013).

One of the major issues limiting the applications of nano-TiO₂ and other ENPs is aggregation. Aggregation also complicates the toxicity assessment, as mentioned by Du et al. (2011) who reported that pristine nano-TiO₂ adhered to the periderm cell wall of wheat as aggregates reducing plant biomass. To address this issue, particles may be coated with several organic and inorganic materials (Chen and Mao, 2007). In addition, ENPs are covered with molecules of low interaction force to stabilize surface energy (Kraynov and Müller, 2011). Nano-TiO₂ with different surface modifications can be used in many fields including photocatalysts, photodegradation, and plastics (Seo et al., 2007, Chae et al., 2003, Weng et al., 2003, Makarova et al., 2000 and Elghniji et al., 2012). However, little is known about the effects of surface coating and stabilization in the interaction of ENPs with plants. Kurepa et al. (2010) reported cell wall penetration and subcellular accumulation of alizarin red S stained nano-TiO₂ in *Arabidopsis thaliana*. Larue et al. (2014) found that foliar applied paint-containing aged nano-TiO₂ (hydrophobic) penetrated through leaf stomata and translocated to all plant tissues in lettuce (*Lactuca sativa*). Studies with other surface coated ENPs have shown different physiological effects on plants. Zhao et al. (2013) reported that alginate coated nano-ZnO increased the chlorophyll content of corn (*Zea mays*) seedlings cultivated in soil. Trujillo-Reyes et al. (2013) and Barrios et al. (2015) found that citric acid coated nano-CeO₂ affected more the nutritional elements of radish (*Raphanus sativus*) and tomato (*Lycopersicon esculentum*), compared to the

uncoated NPs. Perreault et al. (2014) reported that nano-CuO with a poly(styrene-co-butyl acrylate) shell imposed four times more oxidative stress to *Lemna gibba* than pristine NPs.

Basil (*Ocimum basilicum*) is a worldwide popular culinary herb featured in Asian and European cuisine. In traditional Asian culture, basil is commonly used as supplementary treatment for insomnia, asthma, and diabetes, among other diseases (Madaleno, 2015). UV-TITAN M212 and M262 nano-TiO₂ were developed for cosmetics to protect from UVA/UVB, and are among the most widely used nano-TiO₂ products (Sachtleben Chemie GmbH). To the best of authors' knowledge, this is the first study aimed to determine the physiological and biochemical effects of the surface chemistry of nano-TiO₂ in food crops. In this study, the physiological and biochemical effects of pristine, hydrophobic (UV-TITAN M262), and hydrophilic (UV-TITAN M212) nano-TiO₂ were evaluated in basil plants cultivated in soil to the flowering stage. Total chlorophyll content, carbohydrate concentration, antioxidant enzyme activities and accumulation of Ti, micro- and macro-elements were determined by using different spectroscopic and biochemical techniques.

2. Materials and Methods

2.1 Nano-TiO₂ suspensions' preparation. Pristine and surface modified nano-TiO₂ (rutile, 50 ± 25 nm) were provided by US Research Nanomaterials (<http://www.us-nano.com/inc/sdetail/7710>) and Sachtleben Chemie GmbH, Germany, respectively. The hydrophobic nano-TiO₂ (UV-TITAN M262) were firstly capped with Al₂O₃ (6 wt%) and encapsulated with dimethicone (2 wt%), while the hydrophilic nano-TiO₂ (UV-TITAN M212) were coated with Al₂O₃ (6 wt%) and encapsulated with glycerol (1 wt%). The methodology and

results of nanoparticles characterization and release of Al^{3+} are shown in the Supplementa Material (Table S1 and Figure S1-2, SM)

Each nanomaterial was tested at 125, 250, 500, and 750 mg nano- TiO_2 kg^{-1} soil (Menard et al., 2011; Servin et al., 2013). In the coated exposures, the actual concentrations of TiO_2 were slightly lower than pristine treatment due to the 7-8% coating mass. To ensure the homogeneity of nano- TiO_2 in the soil, unmodified and hydrophilic particles were suspended in Millipore water (MPW, 18 μ) and sonicated for 30 min at 20 °C in a water bath (120 V, 3 Amps, 50/60 Hz) before mixing with soil. The hydrophobic particles were mixed directly with the soil.”

2.2 Experimental soil. Portions of 2000 g of top soil (Garden Pro®) were mixed with suspensions or powder and placed into general purpose plastic pots (20 cm × 17.5 cm × 15 cm). Characterizations of top soil are provided in Supplementary Material (Table S2, SM). The soil was continuously mixed by hand (20 min) with the nano- TiO_2 suspensions/powder. Each treatment was replicated four times. The soil mixed with nanoparticles was kept overnight for stabilization and basil seeds were planted the next day. Four replicates of untreated soil were set as controls.

2.3 Seed germination and cultivation conditions. Ten basil seeds (Summer basil, Willhite Seed) per pot were sown at 1 cm depth. All the pots were watered with MPW and placed in a growth chamber (Environmental Growth Chamber, Chagrin Falls, OH) with 65% relative humidity, 25/20 °C day/night, 340 $\mu\text{mole m}^{-2} \text{s}^{-1}$ light intensity, and 14 h photoperiod. No additional fertilizer or nutrient solution was used for this study. Seed germination was evaluated when 50% of control treatment had germinated (Warman, 1999). All the plants were harvested when 75% of the control treatment had flowers (65 days).

2.4 Plant growth and biomass production. At harvest, roots were carefully separated from soil to avoid damage. Four plants of similar size were selected in each treatment and severed at the crown for elongation measurement (Morales et al., 2013). Samples of roots and shoots were thoroughly washed six times with 0.01 M HNO₃ and MPW to remove the nanoparticles adhere onto the surface. The weight of fresh and oven-dried (60 °C, 72 h) tissues were measured to investigate the biomass and water content.

2.5 Relative chlorophyll, carbohydrate, and enzyme activity measurement. For the relative chlorophyll determination, five leaves per replicate were measured with a Chlorophyll Meter SPAD-502 (Minolta, Japan). Carbohydrates of fresh leaves were extracted as per Verma and Dubey (2001). Total sugar and starch concentrations were determined according to Dubois et al. (1956), while reducing sugars were determined after Nelson-Somogyi (1952). Enzyme extractions were performed as per Gallego et al. (1996). The CAT and APOX activities were determined by UV/vis, as described by Beyer and Fridovich (1987) and Gallego et al. (1996). All procedures mentioned are provided in Supplemental Material.

2.6 Uptake of Ti and nutritional elements. Quadruplicate samples of roots and shoots were dried at 60 °C for 72 h. Dry tissues were digested following a previous described protocol (Larue et al., 2014). To validate the digestion method, four replicates of 0.1 g pure nano-TiO₂ were digested as mentioned above. The digested were analyzed by inductively coupled plasma-optical emission spectrometry (ICP-OES, Perkin-Elmer Optima 4300 DV). The recovery rate was 82.3 ± 2.1%. Concentrations of Ti, Al, micro and macroelements (Ca, Mg, K, P, Fe, Mn, Cu, Zn, Se, Mo, B and S) were determined by ICP-OES. Blank, reference material (NIST-SRF 1570a and 1547, Metuchen, NJ), and spiked samples were used as calibration and QC/QA controls.

2.7 Statistical analyses. All the pots were arranged in the growth chamber in a completely random design. The reported data are averages of four replicates $\pm$ standard errors. The experiment variance was determined through one-way ANOVA, and differences between treatments were determined with the Tukey-HSD test using the statistical package SPSS (Social Sciences 22.0, Chicago, IL). Statistical significance were calculated with a probability of 5%, unless otherwise stated.

3. Results and Discussion

3.1 Nanoparticle characterization

Comprehensive physicochemical characterization data of all three TiO₂ particles is shown in Table S1 and Figures S1-2. TEM analysis demonstrated that all nano-TiO₂ samples (pristine, hydrophobic, and hydrophilic) show a similar size in the range of 25-70 nm with elongated morphologies. XRD analysis confirmed the high crystallinity with tetragonal rutile identified as the only phase for all samples (JCPDS 086-0147). Both hydrophobic and hydrophilic nano-TiO₂ particles had a surface area of $\sim 50 \text{ m}^2/\text{g}$, considerably higher than that of the pristine sample ($20\text{--}40 \text{ m}^2/\text{g}$). When suspended in the deionized water, all particles agglomerated to some degree with hydrodynamic diameters ranging from 261 to 341 nm. While the pristine nano-TiO₂ carried a negative zeta potential ($-14.5 \pm 0.5 \text{ mV}$) in the deionized water, both the hydrophobic and hydrophilic TiO₂ nanoparticles had a positive zeta potential of $\sim 27.0 \text{ mV}$, likely due to the organic surface coating. The TGA analysis suggests that all TiO₂ nanoparticles had purity higher than 95%. The contribution of Al₂O₃ coating to ionic Al in the medium is shown in Supplemental Material (Figure S2, SM). The Al³⁺ leakage from the surface coating after seven days in MPW was lower than $0.4 \text{ mg}\cdot\text{kg}^{-1}$, which is negligible, compared with the average Al³⁺ concentration in soil.

3.2 Titanium absorption

Concentrations of Ti in basil plants exposed to uncoated and coated nano-TiO₂ are shown in Figure 1A-B. Supplementary material (Table S3, SM) shows the statistical analysis of Ti concentration in tissues of plants exposed to the different treatments. As shown in Figure 1A, Ti accumulated in roots in a concentration-dependent manner, and at the highest concentration (750 mg·kg⁻¹), plants exposed to the three particles had more Ti, compared with control ($p \leq 0.05$). Although the roots were acid washed and rinsed six times, TiO₂ NPs are very persistent and some of them remain stuck to the root epidermis; thus, part of the Ti detected in roots could be adsorbed to the epidermal cells (Larue et al., 2016). No differences were observed at 0-250 mg·kg⁻¹, this could be due to the high concentration of Ti in the soil (SM, Table S2).

Roots exposed to hydrophobic nano-TiO₂ resulted in 87% and 40% more Ti than the pristine and hydrophilic nano-TiO₂, respectively. This could be due to differences in the interaction between the root surface and the surface properties of nano-TiO₂. The negative charge in plasma membranes of root surface cells (Wang et al., 2014) might exclude the negatively charged pristine nano-TiO₂ (-14.5 ± 0.5 mV, Table S1, SM), and attract the positively charged hydrophobic (26.9 ± 0.5 mV) and hydrophilic nano-TiO₂ (27.0 ± 0.9 mV). Also, positively charged soil particles (Sparks, 2003) would electrostatically adsorb anions, in this case, pristine nano-TiO₂, reducing their bioavailability for root uptake. Although the difference in hydrodynamic size between hydrophilic and hydrophobic particles was not so great (261 ± 5 vs 282 ± 7 nm), there was the possibility that it contributed to the difference in Ti accumulation. The hydrophobic cell wall might have preferential uptake of hydrophobic nano-TiO₂ (Chen et al., 2010). In addition, there are large regions of uncharged and non-polar organic matter (including hydrophobic and hydrophilic parts) in the soil that can play non-neglected roles (Bohn

et al., 2015). Further and detailed studies have to be performed in order to confirm this assumption, and to determine the role of soil components on the fate and bioavailability of nano-TiO₂.

Ti in shoots was determined by ICP-MS and the results are displayed in Figure 1B. As seen in this figure, none of the treatments produced significant differences in shoot Ti. Similar results were previously reported from lettuce (Larue et al., 2016) and wheat (Larue et al., 2012a and Du et al., 2011). Very few studies have reported Ti absorption by ICP-OES/MS since the digestion of nano-TiO₂ containing samples is hard to achieve. Moreover, only few reports have shown the translocation of nano-TiO₂ from root to shoot. Servin et al. (2012, 2013) used synchrotron μ -X-ray fluorescence (μ -XRF) and μ -X-ray absorption near edge structure (μ -XANES) to show that unmodified nano-TiO₂ (mixture of 82% anatase and 18% rutile) were taken up by cucumber root and translocated to fruits. Surprisingly, rutile was preferentially translocated to the leaves (Servin et al., 2012). Larue et al. (2016) also used μ -XRF to analyze samples of lettuce exposed to unmodified nano-TiO₂ and reported small amounts of Ti in stems. The literature indicates that Ti translocation from roots to shoots is lower, compared with other nanomaterials such as nano-CeO₂ (Barrios et al., 2016), and nano-CuO (Perreault et al., 2014). Although the surface coatings did not affect Ti translocation, nano-TiO₂ produced physiological and biochemical perturbations in basil plants.

3.3 Accumulation of nutritional elements

Concentrations of micro- and macro-elements in roots of basil plants exposed to uncoated and coated nano-TiO₂ are shown in Table 1. This table only lists elements that accumulated significantly more or less, compared to controls. Tables S4-S5 (SM) show statistical comparisons of elements' concentrations affected by each nano-TiO₂, compared with control. As shown in

Table 1, hydrophilic and hydrophobic particles interfered with the accumulation of macronutrients Ca and P. Hydrophilic particles significantly reduced Ca accumulation (71%) at 500 mg·kg⁻¹, and P accumulation (25%) at 750 mg·kg⁻¹. However, at 250 and 500 mg·kg⁻¹, hydrophobic particles significantly increased P accumulation (40%). Statistical comparison between control and each nano-TiO₂, showed that Ca concentration was significantly reduced in roots exposed to hydrophobic particles, and phosphorus uptake was greater in plants exposed to hydrophobic nano-TiO₂ (Table S4, SM). These and the other results will be further discussed.

Accumulation of micronutrients Cu, Fe, Mn, and Zn in roots was also differentially affected by unmodified and surface modified nano-TiO₂. Unmodified nano-TiO₂ significantly ($p \leq 0.05$) increased Cu (~80% at 250 mg·kg⁻¹ and 104% at 500 mg·kg⁻¹) and Fe (90% at 500 mg·kg⁻¹ and 59% 750 mg·kg⁻¹), compared to controls (Table 1). At all concentrations (except 750 mg·kg⁻¹), hydrophobic particles significantly increased Mn (408%, 262%, and 339%, respectively), while at 500 mg·kg⁻¹ reduced Cu by 58%. Hydrophilic particles significantly increased Fe (90%) at 500 and 750 mg·kg⁻¹, Mn (60% at 750 mg·kg⁻¹), and Zn (80% at 250 mg·kg⁻¹). Statistical comparisons between control and each nano-TiO₂, individually, showed that Cu was statistically lower, while Mn was higher in hydrophobic exposure ($p \leq 0.05$, Table S4, SM).

Concentrations of macronutrients Ca, Mg, and P in basil shoots are shown in Table 2. As seen in this table, unmodified nano-TiO₂ significantly ($p \leq 0.05$) affected Mg in shoots at 125 and 250 mg·kg⁻¹. On the other hand, at 125 mg·kg⁻¹, hydrophobic nano-TiO₂ reduced the translocation of Ca (56%) and Mg (29% at 125 mg·kg⁻¹ and 18% at 250 mg·kg⁻¹). At all concentrations, hydrophilic nano-TiO₂ significantly reduced Mg translocation (19%, 27%, 28%, and 22%, respectively), Ca (62% at 125 mg·kg⁻¹, 50% at 500 mg·kg⁻¹, 46% and 750 mg·kg⁻¹), and

P translocation (~30% at 500 and 750 mg·kg⁻¹, respectively). Statistical comparison between control and each nano-TiO₂ showed hydrophilic treatments significantly reduced Ca, Mg, and P in shoots ($p \leq 0.05$, Table, S5, SM).

Table 2 also shows the concentration of micronutrients Cu, Fe, Mn, and Se in basil shoots. As seen in this table, compared with controls, unmodified nano-TiO₂ reduced Se (74% at 125 mg·kg⁻¹ and 41% at 500 mg·kg⁻¹) and Fe (28% at 125 mg·kg⁻¹); while increasing Cu (69% at 250 mg·kg⁻¹) and Mn (67% at 500 mg·kg⁻¹). Hydrophobic nano-TiO₂ reduced Fe (39% at 250 mg·kg⁻¹ and 30% at 500 mg·kg⁻¹) and Se (88% at 125 mg·kg⁻¹ and 82% at 500 mg·kg⁻¹), but increased Mn at all concentrations. Only hydrophilic nano-TiO₂ at 500 mg·kg⁻¹ reduced Se by 85%. Statistical comparison between control and each nano-TiO₂ showed that Fe was lower in hydrophobic exposed plants, while Se translocation was lower in hydrophilic treated plants ($p \leq 0.05$, Table S5, SM).

An increase in P accumulation was reported in *Capsicum annuum* L. in the presence of Ti(IV) (Lopez-Moreno et al., 1995). Servin et al. (2013) reported a significant increase of K and P in cucumber fruit of plants exposed to 500 mg·kg⁻¹ of unmodified anatase-rutile nano-TiO₂. Larue et al. (2016) reported an increase in the accumulation of Ca, Fe, P, and S in the root epidermis of lettuce exposed to 1000 mg·kg⁻¹ of anatase nano-TiO₂.

The uptake of micro- and macro-nutrients could be affected by a combination of factors including plant species, soil condition, water deficit, and climate (Brady and Weil, 1996, Guo et al., 2015, Lavado et al., 2001 and Maathuis, 2009), among others. Phosphorus exists in plant systems as H₂PO₄⁻ or HPO₄²⁻, glycosylphosphatidylinositol, nucleotide, and coenzyme (Armstrong, 1999). Phosphorus is absorbed from soil as H₂PO₄⁻ or HPO₄²⁻ via Pht1 transporters (Nussaume et al., 2011). It is possible that root exudates increased the release of Al ions

complexing P. In shoots, P content was significantly increased by hydrophobic treatments but reduced at hydrophilic exposure. This suggests that hydrophobic and hydrophilic particles also affected the Pht2;1 transporters, which are in charge of P translocation. This also suggests that both particles affected Pht1 transporters in similar way (Daram et al., 1999). Manganese is absorbed by roots through an active transport system and translocated within the plant as Mn^{2+} or Mn^{4+} (Kabata-Pendias and Mukherjee, 2007 and Millaleo et al., 2010). Hydrophobic and hydrophilic nano-TiO₂ affected the uptake and translocation of Mn in a similar pattern as they affected P uptake and translocation. It is hypothesized that high charged ions, like HPO_4^{2-} and Mn^{4+} , increase the possibility of aggregation of hydrophobic particles (Zangi and Berne, 2006); thus, P and Mn ions have higher chances of absorption by roots. Since uncoated nano-TiO₂ has smaller zeta potential (Table S1, SM), they can also aggregate, having less interaction with charged ions, increasing the potential for them to be taken up by roots. On the other hand, the uptake and translocation of Ca^{2+} and Mg^{2+} was mainly reduced by hydrophobic and hydrophilic nano-TiO₂. This may be a result of the zeta potential of the nano-TiO₂. The positively charged hydrophilic and hydrophobic particles can be attracted onto the negative root surface, repelling positively charged ions. This might also happen with the uptake of Fe^{2+}/Fe^{3+} and Cu^{2+} . The concentration of Se in shoots was mainly reduced by hydrophilic nano-TiO₂. Selenium is taken up by plants as SeO_4^{2-} via S transporters (Pilon-Smits and Quinn, 2010). It is possible that the nanoparticles have blocked uptake channels inducing stress responses due to limitation of essential nutrient uptake.

The collected data did not show a trend in the effects of unmodified and modified nano-TiO₂ on the uptake and translocation of essential elements in basil. Further studies using other type of soils and other treatment concentrations are needed in order to clarify the responses.

3.4 Effects of nano-TiO₂ on basil plant growth

The physiological effects of coated ENPs on plants are still largely unknown. Table S6-7 (SM) displays the effects of the three nano-TiO₂ on germination, biomass/water content and elongation of basil plants. Compared with the other treatments (Table S6, SM), at 250 and 750 mg·kg⁻¹, hydrophilic nano-TiO₂ reduced seed germination by 51% and 59%, respectively. Also, hydrophobic particles at 125 mg·kg⁻¹ showed a significant reduction (46%). Statistical comparison between control and each nano-TiO₂ revealed that only hydrophilic resulted in a reduction at high concentration treatments (500 and 750 mg·kg⁻¹) ($p \leq 0.05$, Table S7, SM). Reports have shown no effects, promotions, and reductions of seed germination by unmodified nano-TiO₂. Germination was promoted in spinach (*Spinacia oleracea*) (Zheng et al., 2005), cabbage (*Brassica oleracea*), and oat (*Avena sativa*), while it was reduced in cucumber and soybean (*Glycine max*) (Andersen et al., 2016). Castiglione et al. (2011) found nano-TiO₂ delayed germination time of narbon bean (*Vicia narbonensis*) and corn (*Zea mays*). Fan et al. (2014) reported no effects on pea (*Pisum sativum*) seed germination. Similar results were reported in wheat (*Triticum aestivum*), rapeseed (*Brassica napus*), *A. thaliana* and tomatoes (Larue et al., 2011, Larue et al., 2012a, Larue et al., 2012b and Song et al., 2013). To the best of authors' knowledge, there are no previous reports on the effects of surface coated nano-TiO₂ on seed germination; thus, the results cannot be compared to previous results. The reduction of seed germination by hydrophilic nano-TiO₂ could be a result of physical blockage of water uptake by the seed coat. Further studies are needed in order to clarify this result.

Differential effects were observed on root and shoot growth of basil exposed to the three nano-TiO₂ particles. Statistically significant differences were only shown in roots. The hydrophobic particles significantly reduced root length by approximately 32%, 53%, and 31% at

250, 500, and 750 mg·kg⁻¹, respectively, compared to controls. Previous reports have shown different effects of nano-TiO₂ in root growth. Increased root lengths were reported in cucumber and wheat by Servin et al. (2012) and Larue et al. (2011, 2012a and 2012b), whereas no effects were reported in tomatoes (Song et al., 2013 and Antisari et al., 2015).

As seen in Table S6 (SM), uncoated and hydrophobic nano-TiO₂ significantly ($p \leq 0.05$) decreased the biomass content (~16%) at all concentrations, compared with control. A comparison between treatments at all concentrations (Table S7, SM) showed similar trends in biomass and water content. Rafique et al. (2015) reported a significant reduction of biomass in wheat exposed to pristine nano-TiO₂ (> 60 mg·kg⁻¹). Similar results were found in tomato (Song et al., 2013). However, no significant effects were reported in wheat, rapeseed (Larue et al., 2012a and Larue et al., 2012b), and lettuce (Larue et al., 2014).

3.5 Effects of nano-TiO₂ on relative chlorophyll and carbohydrate contents

Figure 2 displays the relative chlorophyll and carbohydrate contents in leaves of basil plants cultivated in soil amended with uncoated, hydrophobic, and hydrophilic nano-TiO₂. Chlorophyll is essential for photosynthesis in plants. The comparison of compounds at the same concentration did not show significant difference in relative chlorophyll content. However, comparison of control and each particle (Table S8, SM) showed that only the hydrophilic particles significantly ($p \leq 0.05$) decreased relative chlorophyll content at 250 and 750 mg·kg⁻¹ (24% and 18%, respectively). The data is concurrent with the results found in a spinach study (Zheng et al., 2005), but is inconsistent with results from cucumber exposed to a mixture of anatase-rutile (Servin et al., 2012 and 2013). The contrasting results might be due to changes in optical properties operated in the surface modified particles and/or differences in the anatase-

rutile mixture. This mixture has greater surface area (35-65 m²/g) (Zhou et al., 2012) than the rutile phase (20-40 m²/g) (Table S1, SM) and different UV absorbance ranges.

Carbohydrates play numerous roles in human bodies, such as energy storage, structural components, and co-enzyme, among others (Maton et al., 1993). The three nano-TiO₂ particles reduced carbohydrate concentrations at varied levels (Figure 2A-C). Total sugar contents were reduced by pristine nano-TiO₂ at 500 (39%) and 750 mg·kg⁻¹ (41%), by hydrophobic at 500 (31%) and 750 mg·kg⁻¹ (38%), and by hydrophilic particles at 500 (41%) and 750 mg·kg⁻¹ (66%). Reducing sugars were only significantly decreased by pristine nano-TiO₂ at 750 mg·kg⁻¹ (34%). Only hydrophobic particles yielded significant reductions of starch at 250 (34%) and 750 mg·kg⁻¹ (25%). Thus, nano-TiO₂ affected basil carbohydrates, independently of the surface coating. Studies with other ENPs have shown to reduce carbohydrates. Barrios et al. (2016) found significant decreases of total and reducing sugar in tomato treated by citric acid coated nano-CeO₂. A reduction in starch content in rice (*Oryza sativa* L.) grains and cucumber fruits by nano-CeO₂ were reported by Rico et al. (2013) and Zhao et al. (2014). The mechanism for carbohydrate disruption by ENPs is still unknown.

3.6 Effects of nano-TiO₂ on enzymatic activity

Catalase (CAT) and ascorbate peroxidase (APOX) are the most common enzymes that fight against reactive oxygen species (Panda and Choudhury, 2005). Figure 3 displays the CAT and APOX activities in roots and leaves of basil plants cultivated in soil amended with unmodified, hydrophobic, and hydrophilic nano-TiO₂. Table S9 (SM) displays the statistical differences in CAT and APOX activities in shoot and root tissues, as affected by each nano-TiO₂. In leaves, unmodified nano-TiO₂ significantly reduced CAT activity at 250 mg·kg⁻¹ (30%) and APOX activity at all concentrations ($p \leq 0.05$), compared with controls. Hydrophobic

particles significantly reduced CAT activity at $750 \text{ mg} \cdot \text{kg}^{-1}$ (29%) and APOX activity at all concentrations. Hydrophilic particles affected CAT and APOX activities the least in leaves, showing lower APOX activity (44%) at 500 and $750 \text{ mg} \cdot \text{kg}^{-1}$ only. In the roots, unmodified and hydrophobic nano-TiO₂ significantly decreased CAT and APOX activities at all concentrations, compared with controls. Hydrophilic nano-TiO₂ did not affect CAT but significantly increased APOX activity at 125 and $250 \text{ mg} \cdot \text{kg}^{-1}$ (72%) and decreased it at $750 \text{ mg} \cdot \text{kg}^{-1}$ (68%). Servin et al. (2013) reported that in cucumber, CAT activity was enhanced, while APOX activity was suppressed at $500 \text{ mg} \cdot \text{kg}^{-1}$ of anatase-rutile mixture treatment. Similarly, it showed a promotion of SOD, CAT, and POD activities in spinach (Hong et al., 2005). Hydrophobic/hydrophilic property would affect particle surfaces' affinity towards water molecules and, thus, the generation of ROS species. The -OH functional group provided by hydrophilic nano-TiO₂ might interfere the chemical equilibrium, causing less stress than the other nano-TiO₂. Also, another secondary metabolite, phenol which has -OH, can decrease the membrane fluidity and change lipid packing order so that restricts peroxidative reactions (Sharma et al., 2012). In addition, it is reported that silicone compounds (in this case, dimethicone present in hydrophobic particles) can alter enzyme properties (Rittschof et al., 2011). The data did not show a trend in the effects of unmodified and modified nano-TiO₂ on stress enzyme activities in basil. Further studies should be performed to determine the role of surface properties in affecting the antioxidant response.

4. Conclusions

The ICP data showed that Ti concentrations were significantly different only in roots of plants exposed to surface modified nano-TiO₂ (both hydrophilic and hydrophobic) at 500 and 750 mg kg^{-1} . This suggests that, independently of the surface modification, little amount of nano-TiO₂ reached the transport system of basil plants. Hydrophilic nano-TiO₂ showed a reduction in

seed germination and hydrophobic nano-TiO₂ significantly reduced root elongation. This suggests that both hydrophilic and hydrophobic particles impact agronomic parameters in basil. The three types of nano-TiO₂ affected the homeostasis of essential elements Ca, P, Mg Cu, Fe, Mn, Se, and Zn at different levels. In addition, all the particles caused oxidative stress: unmodified and hydrophobic nano-TiO₂ in roots and leaves, while hydrophilic nano-TiO₂ in roots. Hydrophilic nano-TiO₂ at 750 mg·kg⁻¹ reduced total sugar content, while hydrophobic particles reduced starch, and unmodified particles decreased reducing sugars. Although the three particles affected basil plants, coated particles impacted the most its nutritional quality, since they altered more essential elements, starch, and reducing sugars. It is premature to make a judgment about the effects of such changes on human nutrition.

Acknowledgements

This material is based upon work supported by the National Science Foundation and the Environmental Protection Agency under Cooperative Agreement Number DBI-1266377. Any opinions, findings, and conclusions or recommendations expressed in this material are those of the author(s) and do not necessarily reflect the views of the National Science Foundation or the Environmental Protection Agency. This work has not been subjected to EPA review and no official endorsement should be inferred. The authors also acknowledge the USDA grant 2016-67021-24985 and the NSF Grants EEC-1449500, CHE-0840525 and DBI-1429708. Partial funding was provided by the NSF ERC on Nanotechnology-Enabled Water Treatment (EEC-1449500). This work was also supported by Grant 2G12MD007592 from the National Institutes on Minority Health and Health Disparities (NIMHD), a component of the National Institutes of

Health (NIH). J. L. Gardea-Torresdey acknowledges the Dudley family for the Endowed Research Professorship and the Academy of Applied Science/US Army Research Office, Research and Engineering Apprenticeship program (REAP) at UTEP, grant #W11NF-10-2-0076, sub-grant 13-7. W. Du acknowledges the support of National Natural Science Foundation of China (21307056 and 21177058). A. C. Barrios acknowledges the Louis Stokes Alliance for Minority Participation (LSAMP) Bridge to the Doctorate Fellowship-NSF grant HRD-1202008.

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Figure legends

Figure 1. Ti concentration in roots of basil plants grown for 65 days in soil amended with unmodified and surface modified nano-TiO₂ at 0, 125, 250, 500, and 750 mg·kg⁻¹ soil. Each value is mean ± SE of four replicates. Different letters represent statistically significant differences between control and treatments at the same concentration ($p \leq 0.05$).

Figure 2. Total sugar (A), reducing sugar (B) and starch (C) contents in basil plants grown for 65 days in soil amended with unmodified and surface modified nano-TiO₂ at 0, 125, 250, 500, and 750 mg·kg⁻¹ soil. Each value is mean ± SE (n = 4). Different letters represent statistically significant differences between control and treatments at the same concentration ($p \leq 0.05$).

Figure 3. Catalase activity in leaves (A) and roots (B), and ascorbate peroxidase activity in shoots (C) and roots (D) of basil plants grown for 65 days in soil amended with unmodified and surface modified nano-TiO₂ at 0, 125, 250, 500, and 750 mg·kg⁻¹ soil. Each value is mean ± SE of four replicates. Different letters represent statistically significant differences between control and treatments at the same concentration ($p \leq 0.05$).

Table 1. Accumulations of micro- and macro-elements in basil roots grown in soil amended with unmodified and surface modified nano-TiO₂. Data are means of four replicates $\pm$ SE. Comparison were made with respect to control. Symbols “+” and “-” stand for the percent of increase and decrease in each element concentration with respect to the control.

Element	Treatment	Concentration ($\times 10^3 \text{ mg}\cdot\text{kg}^{-1}$)	Difference (%)
Ca	Control	0.919	100
	Hydrophilic-500	0.187	-71.0
P	Control	3.57	100
	Hydrophobic-250	5.15	+44.2
	Hydrophobic-500	4.60	+42.0
	Hydrophilic-750	2.67	-25.3
Cu	Control	0.0252	100
	Pristine-250	0.0453	+79.8
	Pristine-500	0.0514	+104.0
	Hydrophobic-500	0.0106	-57.9
Fe	Control	0.1773	100
	Pristine -500	0.337	+90.1
	Pristine -750	0.282	+59.1
	Hydrophilic-500	0.340	+91.8
	Hydrophilic-750	0.336	+89.6
Mn	Control	0.0263	100
	Hydrophobic-125	0.134	+408.4
	Hydrophobic-250	0.0952	+262.0
	Hydrophobic-500	0.115	+338.8
	Hydrophilic-750	0.0421	+60.1
Zn	Control	0.0454	100
	Hydrophilic-250	0.0816	+79.7

Table 2. Accumulations of micro- and macro-elements in basil shoots grown in soil amended with unmodified and surface modified nano-TiO₂. Data are means of four replicates $\pm$ SE. Comparison were made with respect to control. Symbols “+” and “-” stand for the percent of increase and decrease in each element concentration with respect to the control.

Element	Treatment	Concentration ($\times 10^3 \text{ mg}\cdot\text{kg}^{-1}$)	Influence
Ca	Control	3.30	100
	Hydrophobic-125	1.44	-56.4
	Hydrophilic-125	1.26	-61.8
	Hydrophilic-500	1.67	-49.6
	Hydrophilic-750	1.78	-46.1
Mg	Control	3.46	100
	Pristine -125	1.74	-49.8
	Pristine -250	4.40	+27.1
	Hydrophobic-125	2.54	-29.3
	Hydrophobic-250	2.83	-18.2
	Hydrophilic-125	2.81	-18.9
	Hydrophilic-250	2.54	-26.5
	Hydrophilic-500	2.51	-27.6
	Hydrophilic-750	2.72	-21.5
P	Control	8.92	100
	Hydrophilic-500	6.21	-30.3
	Hydrophilic-750	6.19	-30.6
Cu	Control	0.00930	100
	Pristine-250	0.0157	+68.8
	Hydrophobic-125	0.00622	-33.3
Fe	Control	0.0485	100
	Pristine-125	0.0348	-28.2
	Hydrophobic-250	0.0298	-38.6
	Hydrophobic-500	0.0338	-30.3
Mn	Control	0.0433	100
	Pristine-500	0.0722	+66.7
	Hydrophobic-125	0.134	+208.3
	Hydrophobic-250	0.176	+306.0
	Hydrophobic-500	0.128	+196.1

	Hydrophobic-750	0.0622	+43.6
	Control	0.0115	100
	Pristine-125	0.00302	-73.9
	Pristine-500	0.00681	-40.9
Se	Hydrophobic-125	0.00133	-88.7
	Hydrophobic-500	0.00209	-81.7
	Hydrophilic-500	0.00171	-85.2 %

Figure 1.

Figure 2.

Figure 3.

750 mg·kg ⁻¹		
Physiological and biochemical effects		
Pristine	↓	Biomass
		Reducing sugar
		CAT, APOX
Hydrophobic	↓	Germination
		Biomass
		Root length
		Starch
		CAT, APOX
Hydrophilic	↓	Germination
		Total sugar
		APOX

Ti accumulation in root (mg·kg ⁻¹)	
Control	15.3 ± 5.0 c
Pristine	86.1 ± 9.4 b
Hydrophobic	160.4 ± 13.2 a
Hydrophilic	113.9 ± 14.9 b