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Exposure Of Commercial Titanium Dioxide And Copper Hydroxide Nanomaterials On Basil (ocimum Basilicum): A Life Cycle And Transgenerational Study

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EXPOSURE OF COMMERCIAL TITANIUM DIOXIDE AND COPPER HYDROXIDE NANOMATERIALS ON BASIL (*OCIMUM BASILICUM*): A LIFE CYCLE AND TRANSGENERATIONAL STUDY

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Dedication

To my parents, you are always the persons I would like to share my achievements with you.
Both of you are my models of love-learning.
I am proud of being your daughter.

To my husband, you are the best thing happened in my life.
Let’s accomplish more together.
I love you.

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Abstract

Although thousands of reports have shown that engineered nanomaterials (ENMs) can alter several agronomical, physiological, and biochemical parameters in plant and mammal systems, the effects of long-term exposure and varietal responses are scarce. Titanium dioxide nanoparticles (nano-TiO_2) and copper hydroxide nanoparticles [nano-$Cu(OH)_2$], which are widely used in agriculture, medicine, food industry, textile, among other areas, are among the ENMs with high production and consumption globally. Though reports indicate that these ENMs affected plant development and metabolism, their interactions with basil (*Ocimum basilicum*) are unknown. Basil is a popular culinary herb worldwide, featured in Asian and European cuisine and has more than 30 varieties. This research project was performed to investigate the changes in plant growth, nutritional compounds, biochemical responses, and the accumulation of Ti and Cu in long-term (life cycle and transgenerational) exposure. This research project was undertaken into three parts: Part I was aimed at examining the effects of root exposure of nano-TiO_2 with different surface properties (pristine, hydrophobic, and hydrophilic) at the concentration of nanoparticles ranging from 0~750 $mg \cdot kg^{-1}$. Part II was conducted to determine the transgenerational effects of the corresponding nano-TiO_2 at 750 $mg \cdot kg^{-1}$. Plants in Part I and II were harvested at the flowering stage and analyzed for element content, enzymatic activity, photosynthesis, and macromolecular content. In Part III, two varieties (Dark Opal and Dulce) of basil were analyzed for the responses to the foliar exposure of 4.8 mg Cu/pot of $Cu(OH)_2$ nanowires, CuPro [$Cu(OH)_2$ bulk], and $CuSO_4$ suspensions/solutions. In this part, plants were harvested at the pruning stage, and GC-MS analyses were conducted on leaves to investigate variety-dependent metabolic responses. Results from Part I suggest that these three nano-TiO_2 resulted in concentration-dependent Ti

accumulation in roots, with preferential uptake of hydrophobic nano-TiO_2. At 750 $mg \cdot kg^{-1}$, lower total sugar content was determined in the plants treated with pristine (32%), hydrophobic (38%), and hydrophilic (66%) nano-TiO_2, compared with control. Besides, lower reducing sugar (34%) and starch content (25%) was yielded by pristine and hydrophobic nano-TiO_2, respectively. In Part II, higher stomatal conductance was determined (214%) in the plants exposed to pristine nano-TiO_2 in two cycles, while hydrophobic and hydrophilic nano-TiO_2 resulted in lower total chlorophyll content in plants exposed to the corresponding nano-TiO_2 in the first cycle (24% and 30%, respectively). In addition, higher total sugar was determined in plants exposed to hydrophilic nano-TiO_2 in both cycles (80%). Compared with plants that were never exposed to nanoparticles, significantly higher Ti was determined in the roots of plants exposed to the three nanoparticles in the first cycle. Nevertheless, higher Ti was only determined in the roots of plants treated with pristine nano-TiO_2 in two consecutive cycles. None of the treatments in Part I and II resulted in higher Ti translocation to shoots, which indicates lower risks for humans to uptake these nanoparticles. Results of Part III showed that in low anthocyanin plants, copper remained in the leaves and none of the compounds affected the anthocyanin and essential oil content. In addition, $CuSO_4$ and $Cu(OH)_2$ nanowires increased six types of fatty acids, while CuPro decreased two types of fatty acids. In the high anthocyanin variety, copper translocated from leaves to stems and roots, and the three compounds reduced anthocyanin and essential oil contents. Moreover, six types of fatty acids were reduced by the three copper compounds at different degrees. The alterations in nutritional molecules by these ENMs suggest that people who rely on nutritional supplement, through dietary intake, might be affected. It is expected that these findings shed light on the long-term and transgenerational effects of ENMs in crop plants.

TABLE OF CONTENTS

List of Tables

List of Figures

Chapter 1: Introduction

Engineered nanomaterials (ENMs) are materials with a size ranging from 1 to 100 nm at least in one dimension and are produced for specific agricultural or industrial purposes (Ellenbecker and Tscai, 2011). Their unique properties, such as light weight, high surface-to-volume ratio, surface chemistry, as well as optical, electrical and antimicrobial properties make them different from their bulk and ionic counterparts (Murphy et al., 2005). These special properties allowed the use of ENMs in food industry, agriculture, cosmetics, wastewater treatment, pharmaceutics, textiles, and engineering (electronic, material, and mechanical), among others (Biswas et al., 2005; Chen et al., 2006; Roco, 2011).

The large number of applications lead to an overwhelming production and development of different types of ENMs. Keller and Lazareva (2013) estimated that approximately 374,000 tons of ENMs were produced globally in 2010, and this number is forecasted to increase. Roco (2011) estimated that by 2020, the market would reach about $3 trillion. Titanium dioxide nanoparticles (nano-TiO_2) are among the most essential metal-based ENMs (Welch and Compton, 2006; Piccinno et al., 2012). Nano-TiO_2 have three common crystal phases (anatase, rutile, and brookite). They are naturally white, very stable (low dissolution and high thermal stability), and have excellent optical and electrical properties (Chen and Mao, 2007). These unique properties of nano-TiO_2 lead to a wide range of applications as food additive, catalysis, water treatment, paints, solar cell, and personal care products, among others (Klaine et al., 2008; Johnson et al., 2011). Keller and Lazareva (2013) reported that the global production of nano-TiO_2 was approximately 88,000 tons in 2010.

Copper-based ENMs are widely used in the industry, agriculture, dyes, fungicide/bactericide, and in biocompatible devices (Cioffi et al., 2005; Mary et al., 2009; Cometa et al., 2013). In particular, copper hydroxide [$Cu(OH)_2$] nanowires are commercially available as a pesticide in agricultural field, as an alternative to Bordeaux mixture, Kocide, and CuPro (US Research Nanomaterials). Kocide and CuPro [$Cu(OH)_2$] are the most widely used bulk (with a size greater than 100 nm) copper-based pesticide. Based on the manufacturer's instructions, and assuming that 1% of the United States agricultural land is applied with these $Cu(OH)_2$, the annual consumption rate will reach 6,075 to 21,265 tons (Specimen).

The increasingly growing production and application of ENMs would result in their high release to the environment. As the major sink of ENMs, soil properties could affect the interactions between ENMs and plants. For example, low soil pH is considered to accelerate the dissolution of copper-based ENMs, while organic matter can slow their dissolution (Sekine et al., 2017). Plants are considered as one of the largest and important living organisms. Crop plants are essential dietary food and nutritional sources for human beings. Current literature has shown that plants would absorb ENMs or the respective ions through roots or leaves and transport them via xylem/phloem (Navarro et al., 2008).

Several studies have contributed to the understanding of the fate and transport of ENMs in plants (Hong et al., 2016; Lin et al., 2009; Wang et al., 2012; Hernandez-Viezcas et al., 2013). It is reported that alterations in the physiological/biochemical responses to ENMs are attributed to their dissolution and speciation. For example, a small percentage of nano-CeO_2 were found to biotransform from Ce(IV) into Ce(III) in kidney bean (Zhang et al., 2015) and soybean (Hernandez-Viezcas et al., 2013), while nano-ZnO was found as Zn-citrate, Zn-phosphate, histidine and phytate, in soybean (Hernandez-Viezcas et al., 2013), wheat (Dimpka et al., 2013),

and cowpea (Wang et al., 2012). Nano-CuO was taken by wheat shoot as Cu^{I}-sulfur (Dimpka et al., 2013). However, no papers reported the biotransformation of nano-TiO_2 in plants.

The literature has demonstrated that nano-TiO_2 and copper-based ENMs can affect plant growth in different forms (Du et al., 2017). Likewise, several studies have indicated that the interaction between plants and ENMs relies on various factors, including plant species/variety (Rico et al., 2015), type of ENMs (Dimpka et al., 2013), growth media (Majumdar et al., 2016), exposure mode (Raliya et al., 2015), particle size and crystal phase (Larue et al., 2012), and surface chemistry (Trujillo-Reyes et al., 2014; Tan et al., 2017). For example, Larue et al. (2012) reported that nano-TiO_2 with a smaller size (12 nm, 22 nm, or 25 nm) translocated from wheat roots to shoots, and particles with a size of 36 nm were present in the root parenchyma but did not enter the stele. Later on, Servin et al. (2012) found that rutile nano-TiO_2 resulted in a preferential absorption than the anatase phase in cucumber leaves. Moreover, Trujillo-Reyes et al. (2014) found that the core-shell nano-Cu/CuO resulted in higher copper accumulation in lettuce roots, compared with $CuSO_4 \cdot 5H_2O$. Cai et al. (2017) found that uncoated anatase nano-TiO_2 entered rice roots, whereas, uncoated rutile and hydrophilic particles did not and alleviated the lead accumulation in plants. Many reports have discussed the impacts of copper-based ENMs, such as nano-Cu (Stampoulis et al., 2009) and nano-CuO (Hong et al., 2016) in mung bean, wheat (Lee et al., 2008), zucchini (Stampoulis et al., 2009), radish, perennial and annual ryegrass (Atha et al., 2012), and rice (Wang et al., 2015). Researchers have found baseline information of the detoxification mechanisms of nano-TiO_2 or copper-based ENMs by studying the metabolic, metabolomics, or transcriptomic in tobacco (Frazier et al., 2014), lettuce (Zhao et al., 2016), cucumber (Zhao et al., 2017a), corn (Zhao et al., 2017b), spinach (Zhao et al., 2017c), *Arabidopsis thaliana* (Tumburu et al., 2017), and rice (Wu et al., 2017).

Basil (*Ocimum basilicum*) is considered as one of the most popular culinary herbs in Asian and European cuisine (Madaleno, 2015). Basil is primarily used for flavor, which is given by essential oils; however, it also contributes essential elements, antioxidants, nutritional compounds, and carbohydrates (Almeida et al., 2007). Diseases caused by fungi or bacteria, such as fusarium wilt, are common and lethal diseases for basil plants (Daferera et al., 2003). It is reported that nano-based products can play a positive role in controlling these diseases (Elmer et al., 2016). There are more than 30 basil varieties with different compositions and contents of aromatic compounds (Simon et al., 1999). This suggests that different effects could be observed if they are exposed to ENMs. In addition, to the best of the author's knowledge, no previous investigations have described the interaction of nano-TiO_2 or $Cu(OH)_2$ with basil plants. Thus, the long-term effects of nano-TiO_2 and $Cu(OH)_2$ and the role of plant variety are not well understood.

Due to their outstanding UVA/UVB absorbing properties (Sachtleben Chemie GmbH), rutile nano-TiO_2, UV-TITAN M212 (with a hydrophilic coating) and M262 (with a hydrophobic coating) are developed for personal care and cosmetics (PC&C) uses. Despite their widespread uses in the PC&C industry, very few studies have demonstrated the potential impacts of surface coated nano-TiO_2 in plants (Tan et al., 2017). It is assumed that nano-TiO_2 with surface coatings would generate different physiological and biochemical effects in plants, compared to the pristine nano-TiO_2. In addition, these effects on parent plants can yield sequential results in plants of the second generation. Part I and II were conducted to investigate the above hypotheses (Figure 1.1). On the other hand, $Cu(OH)_2$ based nanoproducts are developed as disease suppressors in agriculture. Nevertheless, some reports have attributed plant toxicity to the absorption of $Cu(OH)_2$ based compounds (Zhao et al., 2016a; Zhao et al., 2016b). To date, there are very limited reports about the interactions of $Cu(OH)_2$ nanopesticide with plants, especially targeting the foliar

exposure study and the role of plant varieties. Our hypothesis is that basil plants have a variety-dependent response towards $Cu(OH)_2$ nanopesticide, and Part III was performed to confirm this assumption (Figure 1.2). Plasma based techniques (ICP-OES/MS) were performed to determine the accumulation in Ti, Cu, and other micro-/macro-elements. Biochemical and colorimetric assays were conducted to probe the alterations in pigment contents (chlorophyll and anthocyanin), enzyme activities (catalase and ascorbate peroxidase), and carbohydrates (sucrose and starch). In addition, a chromatographic method (GC-MS) was employed to determine changes in plant metabolites (essential oils and fatty acids).

Specific objectives

The objectives of this research are:

(1) To understand the physiological/biochemical responses of basil plants to the exposure of pristine, hydrophobic, or hydrophilic titanium dioxide nanoparticles.

(2) To investigate the extent to which uncoated and coated titanium dioxide nanoparticles would affect parental and second generation plants.

(3) To examine whether the translocation and biochemical effects of copper hydroxide nano/bulk pesticides are variety- or size-dependent.

Working hypotheses

The working hypotheses of this research were:

(1) Titanium dioxide nanoparticles with surface coatings show more toxicity in basil plants, compared to pristine nanoparticles.

(2) The uptake and physiological/biochemical effects of titanium dioxide nanoparticles in parent plants generate sequential alterations in plants of the second generation.

(3) The effect of copper hydroxide nano/bulk is variety dependent in basil plants.

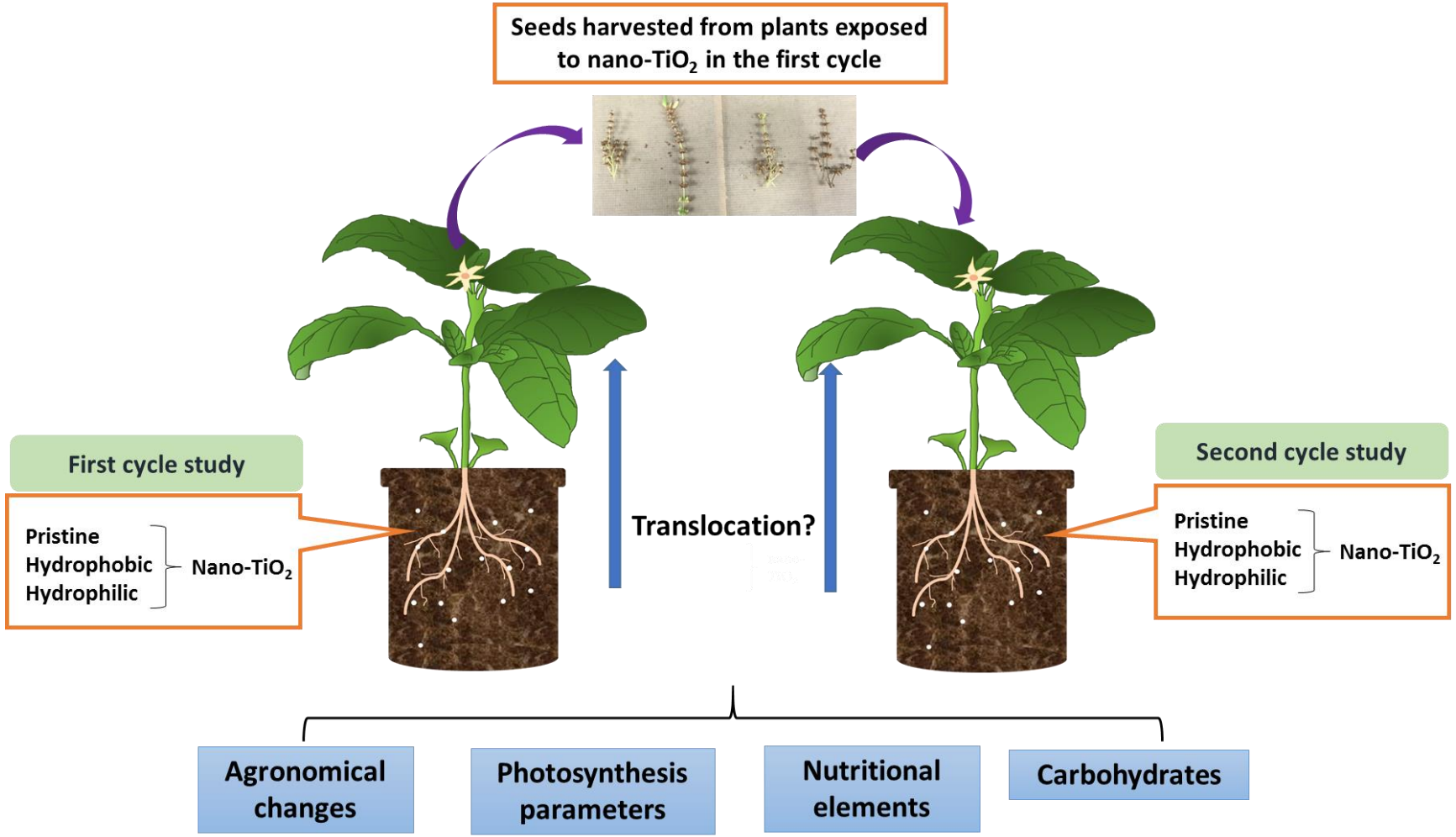


Figure 1.1 Schematic diagram for the life cycle and transgenerational studies of interactions between titanium oxide nanoparticles with different surface coatings and basils

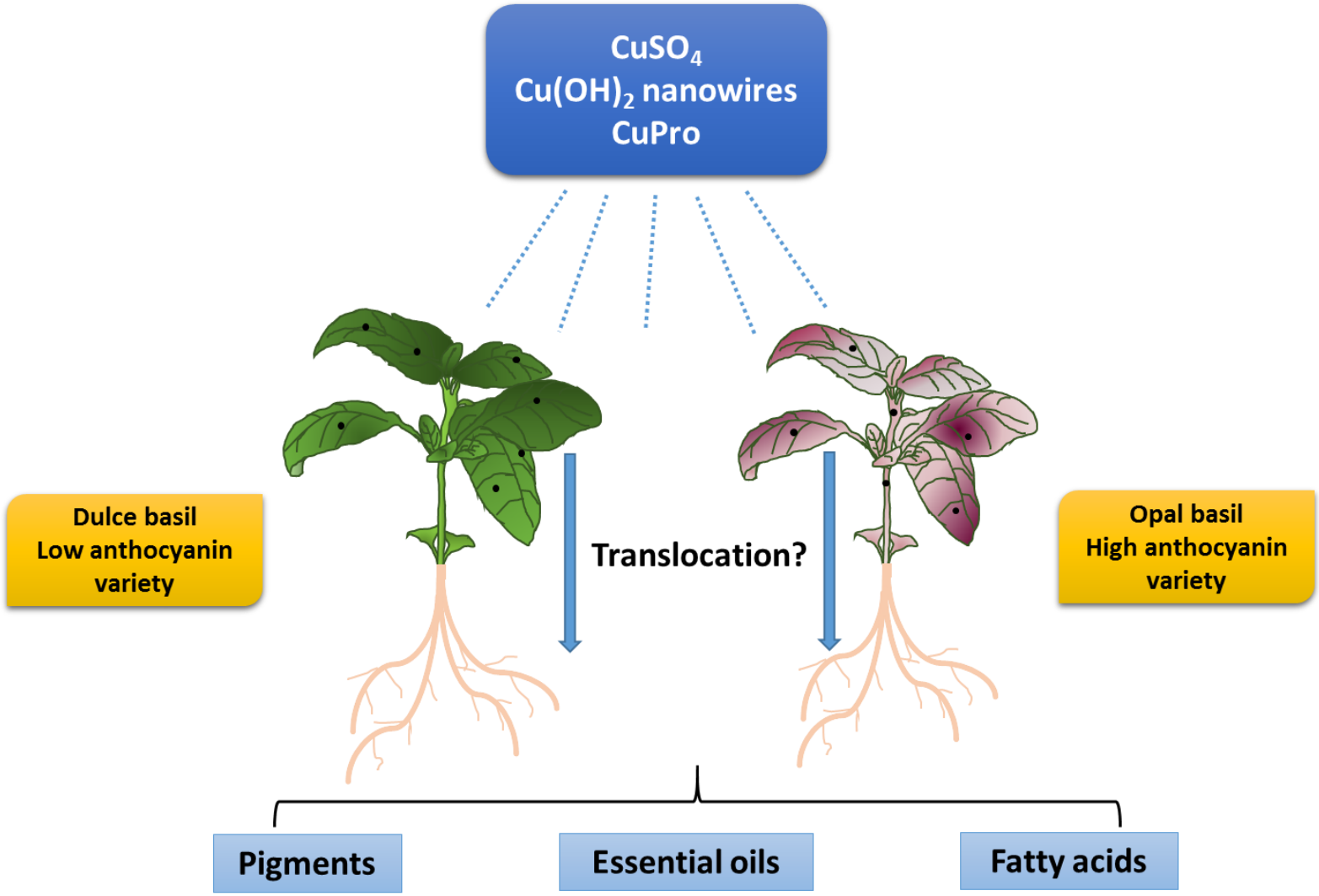


Figure 1.2 Schematic diagram for the study of interactions between copper hydroxide nanopesticides and basils with two different basil varieties (low and high anthocyanin contents)

Chapter 2: Surface Coating Changes the Physiological and Biochemical Impacts of nano-TiO_2 in Basil (*Ocimum basilicum*) Plants

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, and hydrophilic titanium dioxide nanoparticles (nano-TiO_2) at concentrations ranging from 0~750 $mg \cdot kg^{-1}$. 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-TiO_2 reduced seed germination by 41% and 59%, respectively, while unmodified and hydrophobic nano-TiO_2 decreased shoot biomass by 31% and 37%, respectively ($p \leq 0.05$). Roots exposed to hydrophobic particles at 750 $mg \cdot kg^{-1}$ had significantly more Ti, compared with the other particles; however, no differences were found in shoots. Although the three types of particles affected the homeostasis of essential elements, only hydrophobic nano-TiO_2 reduced root elongation by 53%. Unmodified, hydrophobic, and hydrophilic particles reduced total sugar by 39%, 38%, and 66%, respectively, compared with control. Moreover, unmodified particles decreased reducing sugar (34%), while hydrophobic particles reduced starch (35%). Overall, independently of surface properties, nano-TiO_2 impacted the physiology, biochemistry, and nutritional quality of basil plants.

Keyword: TiO_2 nanoparticles, Surface coating, Basil, Uptake, Nutritional value

Capsule: In general, TiO_2 nanoparticles significantly reduced total sugar; unmodified particles decreased reducing sugar and hydrophobic particles reduced starch in basil plants.

2.1 Introduction

There is an ever increasing production of engineer nanoparticles (ENPs) to fulfill the growing demand of nano-based consumer products. Titanium dioxide nanoparticles (nano-TiO_2) are one of the most consumed metal oxide ENPs (Chen and Mao, 2007 and Piccinno et al., 2012). Nano-TiO_2 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-TiO_2 into the environment. Keller and Lazareva (2014) estimated that the discharge of nano-TiO_2 into bodies of water around the San Francisco Bay area, California, USA exceeds 10,000 $kg \cdot year^{-1}$. Nano-TiO_2 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 $mg \cdot kg^{-1}$ nano-TiO_2 (Gottschalk et al., 2009), which suggests their potential buildup in agricultural soils.

Several researchers have reported that nano-TiO_2 affect organisms in different ways (Gardea-Torresdey et al., 2014, Ma et al., 2015 and Servin and White, 2016). Effects of nano-TiO_2 in plants depend on the concentration, size, and crystal phase (Larue et al., 2011, Larue et al., 2012a and Larue et al., 2012b). In addition, a few reports have shown that plants absorb and accumulate nano-TiO_2 without transformation (Larue et al., 2012a). Servin et al. (2012 and 2013) demonstrated that nano-TiO_2 was absorbed through the roots and translocated to the leaf trichomes and fruits of cucumber (*Cucumis sativus*) plants. Similar results have been reported for tomato (*Solanun lycopersicum*) plants (Raliya et al., 2015 and Song et al., 2013).

One of the major issues limiting the applications of nano-TiO_2 and other ENPs is aggregation. To solve this issue, they have been 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-TiO_2 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-TiO_2 in *Arabidopsis thaliana*. Larue et al. (2014) found that foliar applied paint-containing aged nano-TiO_2 (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 (Zhao et al., 2013, Trujillo-Reyes et al., 2013, Barrios et al., 2015 and Perreault et al., 2014).

UV-TITAN M212 and M262 nano-TiO_2 were developed for cosmetics to protect from UVA/UVB, and are among the most widely used nano-TiO_2 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-TiO_2 in food crops.

Basil (*Ocimum basilicum*) is a worldwide popular culinary herb featured in Asian and European cuisine. It is believed that the essential oil of basil leaves has great potential medical value due to its antioxidant and antimicrobial properties (Almeida et al., 2007 and Chiang et al., 2005). In traditional Asian culture, basil is commonly used as supplementary treatment for insomnia, asthma, and diabetes, among other diseases (Madaleno, 2015). As a culinary herb, basil is mainly used for flavor; however, it also contributes essential elements and carbohydrates. Although the heat of cooking could decompose the essential oil of leaves, dissipating the flavor, nutrient components such as carbohydrates and micro/macro-elements prevail.

In this study, the physiological and biochemical effects of pristine, hydrophobic (UV-TITAN M262), and hydrophilic (UV-TITAN M212) nano-TiO_2 were evaluated in basil plants cultivated in soil to full maturity. 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.

2.2 Materials and methods

2.2.1 Nano-TiO_2 suspensions' preparation

Pristine and surface modified nano-TiO_2 (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-TiO_2 (UV-TITAN M262) were firstly capped with Al_2O_3 (6 wt%) and encapsulated with dimethicone (2 wt%), while the hydrophilic nano-TiO_2 (UV-TITAN M212) were coated with Al_2O_3 (6 wt%) and encapsulated with glycerol (1 wt%).

Each nanomaterial was tested at 125, 250, 500, and 750 mg nano-TiO_2 kg^{-1} soil (Menard et al., 2011 and Servin et al., 2013). Unmodified and hydrophilic particles were suspended in Millipore water (MPW, 18 μΩ) and sonicated for 30 min at 20 ℃ in a water bath (120 V, 3 Amps, 50/60 Hz) before mixing with soil. The hydrophobic particles were mixed directly with soil.

2.2.2 Experimental soil

Portions of 2000 g of top soil (Garden Pro®, SI Table 1.1) were mixed with suspensions or powder and placed into general purpose plastic pots (20 cm × 17.5 cm × 15 cm). 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.

2.2.3 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 μmole m^{-2} 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).

2.2.4 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 HNO_3 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.

2.2.5 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 desscribed by Beyer and Fridovich (1987) and Gallego et al. (1996).

2.2.6 Uptake of Ti and nutritional elements

Quadruplicate samples of roots and shoots were dried at 60 ºC 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-TiO_2 were digested as mentioned above. The digested were analyzed by inductively coupled plasma-optical emission sprectrometry (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.

2.2.7 Statistical analyses.

All the pots were allocated in the growth chamber in a completely random design. The reported data are averages of four replicates ± 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.

2.3 Results and discussions

2.3.1 Nanoparticle characterization

Comprehensive physicochemical characterization data of all three TiO_2 particles is shown in Table 2.1 and Figures 2.1-2.2. TEM analysis demonstrated that all nano-TiO_2 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-TiO_2 particles had a surface area of ~50 m^2/g, considerable higher than that of the pristine sample (20-40 m^2/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-TiO_2 carried a negative zeta potential (-14.5 ± 0.5 mV) in the deionized water, both the hydrophobic and hydrophilic TiO_2 nanoparticles had a positive zeta potential of ~27.0 mV, likely due to the organic matter surface coating. The TGA analysis suggests that all TiO_2 nanoparticles had purity higher than 95%. The contribution of Al_2O_3 coating to ionic Al in the medium is shown in Supplemental Material (Figure 2.2). The Al^{3+} leakage from the surface coating after seven days in MPW was lower than 0.4 $mg \cdot kg^{-1}$, which is negligible, compared with the average Al^{3+} concentration in soil.

2.3.2 Titanium absorption

Ti concentrations in basil plants exposed to uncoated and coated nano-TiO_2 are shown in Figure 2.3A-B. As shown in Figure 2.3A, Ti accumulated in roots in a concentration-dependent manner, and at the highest concentration (750 $mg \cdot kg^{-1}$), 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, Ti is very persistent, and very likely, part of the Ti detected in roots was from the NPs stuck to the root epidermis (Larue et al., 2016).

Roots exposed to hydrophobic nano-TiO_2 at 250 and 500 mg kg^{-1} had ~287% and ~652% more Ti, compared with control ($p \leq 0.05$). Furthermore, at 750 $mg \cdot kg^{-1}$ treatments of pristine, hydrophilic, and hydrophobic particles, root Ti was ~461%, ~945%, and 642% higher, respectively, compared with control. Notably, hydrophobic nano-TiO_2 resulted in 87% and 40% more Ti than the pristine and hydrophilic nano-TiO_2, respectively. This could be due to differences

Table 2.1 Physical characterizations of nano-TiO_2 exposed to basil (*Ocimum basilicum*).

Properties	Characterization technique	Nanoparticles		
		Pristine TiO_2	Hydrophobic TiO_2 M262	Hydrophilic TiO_2 M212
Size (nm)	TEM	50 ± 25	50 ± 25	50 ± 25
Crystal phase	XRD	Tetragonal, rutile	Tetragonal, rutile	Tetragonal, rutile
Morphology	TEM	Elongated	Elongated	Elongated
Surface area (m^2/g)	BET	30 ± 10	47.6 ± 1.0	55.7 ± 2.0
Hydrodynamic size (nm)	DLS	341 ± 10	261 ± 5	282 ± 7
Zeta potential in DI water (mV)	DLS	-14.5 ± 0.5	27.0 ± 0 .9	26.9 ± 0.5
Purity (wt. %)	TGA	99.9	97.1	95.9

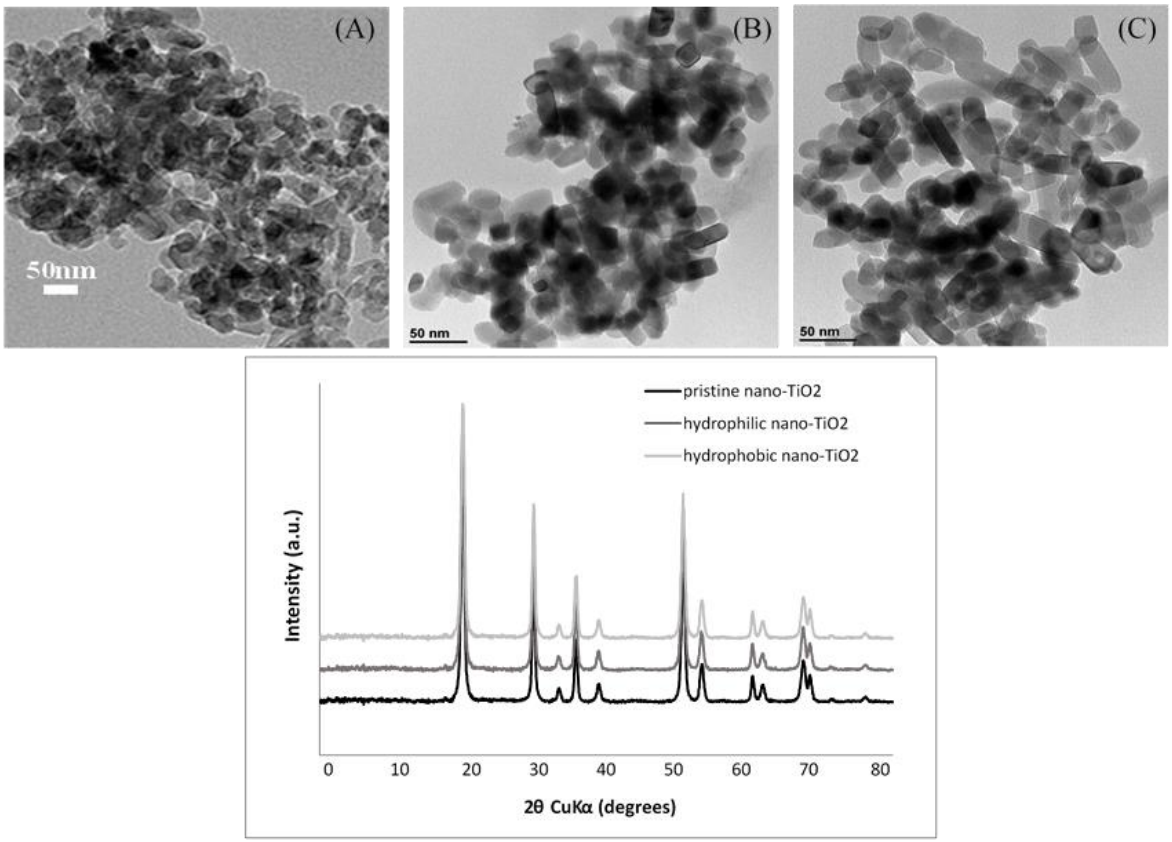


Figure 2.1 TEM images of unmodified (A), hydrophobic (B), and hydrophilic (C) nano-TiO_2, and XRD characterization (D) of unmodified (black), hydrophobic (grey), and hydrophilic (light grey) nano-TiO_2.

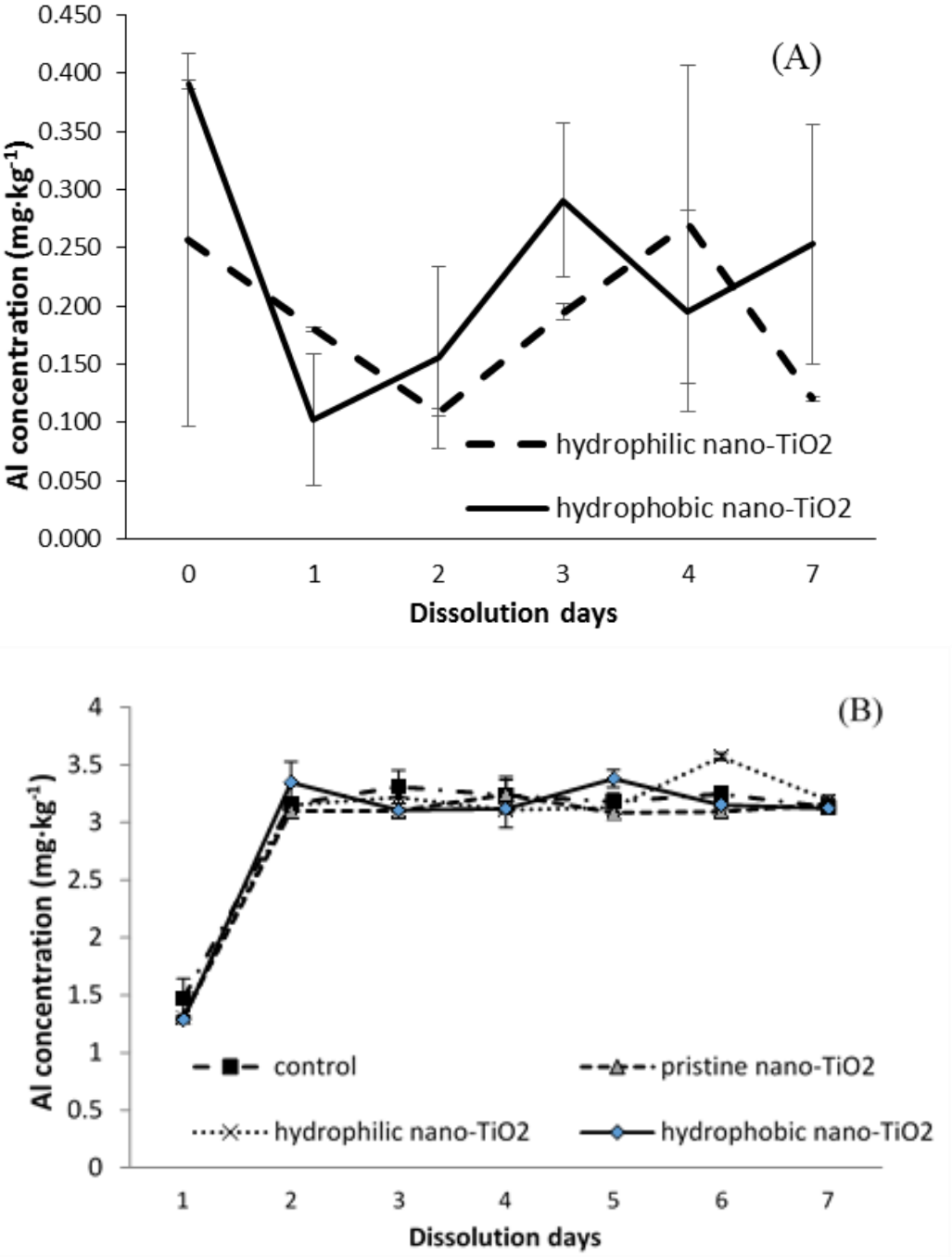


Figure 2.2 Release of Al^{3+} ions from nano-TiO_2 in the MPW (A) and soil (B) media up to seven days.

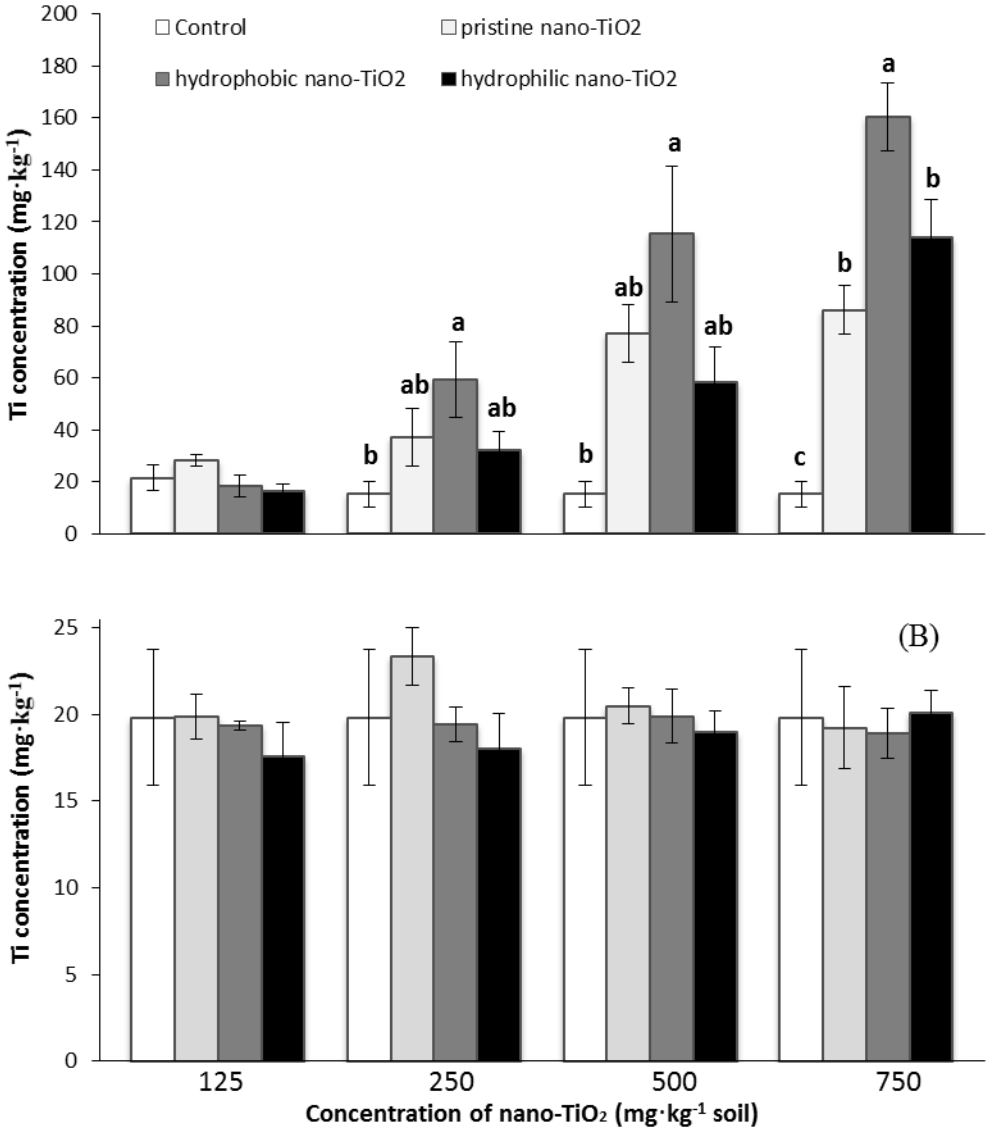


Figure 2.3 Ti concentration in roots (A) and shoots (B) of basil plants grown for 65 days in soil amended with unmodified and surface modified nano-TiO_2 at 0, 125, 250, 500, and 750 mg·kg^{-1} soil. Each value is mean ± SE of four replicates. Different letters represent statistically significant differences between control and treatments at the same concentration ($p \leq 0.05$).

in the interaction between the root surface and the surface properties of nano-TiO_2. The negative charge in plasma membranes of root surface cells (Wang et al., 2014) might exclude the negatively charged pristine nano-TiO_2 (-14.5 ± 0.5 mV, Table 2.1), and attract the positively charged hydrophobic (26.9 ± 0.5 mV) and hydrophilic nano-TiO_2 (27.0 ± 0.9 mV). Also, positively charged soil particles (Sparks, 2003) would electrostatically adsorb anions, in this case, pristine nano-TiO_2, 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-TiO_2 (Chen et al., 2010). In addition, there are large regions of uncharged and non-polar organic matters (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-TiO_2.

Ti concentrations in shoots was determined by ICP-MS and the results are displayed in Figure 2.3B. As see in this figure, none of the treatments produced significant differences in Ti shoot contents. 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-TiO_2 containing samples is hard to achieve. Moreover, only few reports have shown the translocation of nano-TiO_2 from root to shoot. Servin et al. (2012 and 2013) used synchrotron µ-X-ray fluorescence (µ-XRF) and µ-X-ray absorption near edge structure (µ-XANES) to show that unmodified nano-TiO_2 (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-TiO_2 and reported small amounts of Ti in stems. The literature indicates that Ti translocation from roots to shoots is lower, compared with other nanomaterials (nano-ZnO (Zhao et al., 2013), nano-CeO_2 (Barrios et al., 2016), and nano-CuO (Perreault et al., 2014). However, even with low translocation, nano-TiO_2 (especially with different surface properties) produced physiological and biochemical perturbations in basil plants.

2.3.3 Accumulation of nutritional elements

Concentrations of micro- and macro-elements in roots of basil plants exposed to uncoated and coated nano-TiO_2 are shown in Table 2.2. This table only lists elements that accumulated significantly more or less, compared to controls. As shown in Table 2.2, hydrophilic and hydrophobic particles interfered with the accumulation of macronutrients Ca and P. Hydrophilic particles significantly reduced Ca accumulation (71%) at 500 $mg \cdot kg^{-1}$, and P accumulation (25%) at 750 $mg \cdot kg^{-1}$. However, at 250 and 500 $mg \cdot kg^{-1}$, hydrophobic particles significantly increased P accumulation (40%). 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-TiO_2. Unmodified nano-TiO_2 significantly ($p \leq 0.05$) increased Cu (~80% at 250 $mg \cdot kg^{-1}$ and 104% at 500 $mg \cdot kg^{-1}$) and Fe (90% at 500 $mg \cdot kg^{-1}$ and 59% 750 $mg \cdot kg^{-1}$), compared to controls (Table 2.2). At all concentrations (except 750 $mg \cdot kg^{-1}$), hydrophobic particles significantly increased Mn (408%, 262%, and 339%, respectively), while at 500 $mg \cdot kg^{-1}$ reduced Cu by 58%. Hydrophilic particles significantly increased Fe (90%) at 500 and 750 $mg \cdot kg^{-1}$, Mn (60% at 750 $mg \cdot kg^{-1}$), and Zn (80% at 250 $mg \cdot kg^{-1}$).

Concentrations of macronutrients Ca, Mg, and P in basil shoots are shown in Table 2.3. As seen in this table, unmodified nano-TiO_2 significantly ($p \leq 0.05$) affected Mg in shoots at 125 and 250 $mg \cdot kg^{-1}$. On the other hand, at 125 $mg \cdot kg^{-1}$, hydrophobic nano-TiO_2 reduced the translocation

Table 2.2 Accumulations of micro- and macro-elements in basil roots grown in soil amended with unmodified and surface modified nano-TiO_2. Data are means of four replicates ± 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	Difference (%)
Ca	Control	918.9 ± 269.3	100 %
	Hydrophilic-500	187.4 ± 55.7	-71.0 %
P	Control	3567.9 ± 290.8	100 %
	Hydrophobic-250	5145.0 ± 126.0	+44.2 %
	Hydrophobic-500	4600.7 ± 259.4	+42.0 %
	Hydrophilic-750	2665.1 ± 72.8	-25.3 %
Cu	Control	25.2 ± 3.6	100 %
	Pristine-250	45.3 ± 3.3	+79.8 %
	Pristine-500	51.4 ± 3.1	+104.0 %
	Hydrophobic-500	10.6 ± 2.0	-57.9 %
Fe	Control	177.3 ± 9.7	100 %
	Pristine -500	337.0 ± 6.5	+90.1%
	Pristine -750	282.1 ± 8.0	+59.1%
	Hydrophilic-500	340.1 ± 39.9	+91.8%
	Hydrophilic-750	336.1 ± 47.3	+89.6%
Mn	Control	26.3 ± 0.7	100 %
	Hydrophobic-125	133.7 ± 18.2	+408.4 %
	Hydrophobic-250	95.2 ± 23.8	+262.0%
	Hydrophobic-500	115.4 ± 8.7	+338.8 %
	Hydrophilic-750	42.1 ± 3.7	+60.1 %
Zn	Control	45.4 ± 5.7	100 %
	Hydrophilic-250	81.6 ± 8.8	+79.7 %

of Ca (56%) and Mg (29% at 125 $mg \cdot kg^{-1}$ and 18% at 250 $mg \cdot kg^{-1}$). At all concentrations, hydrophilic nano-TiO_2 significantly reduced Mg translocation (19%, 27%, 28%, and 22%, respectively), Ca (62% at 125 $mg \cdot kg^{-1}$, 50% at 500 $mg \cdot kg^{-1}$, 46% and 750 $mg \cdot kg^{-1}$), and P translocation (~30% at 500 and 750 $mg \cdot kg^{-1}$, respectively).

Table 2.3 also shows the concentration of micronutrients Cu, Fe, Mn, and Se in basil shoots. As seen in this table, compared with controls, unmodified nano-TiO_2 reduced Se (74% at 125$mg \cdot kg^{-1}$ and 41% at 500 $mg \cdot kg^{-1}$) and Fe (28% at 125 $mg \cdot kg^{-1}$); while increased Cu (69% at 250 $mg \cdot kg^{-1}$) and Mn (67% at 500 $mg \cdot kg^{-1}$). Hydrophobic nano-TiO_2 reduced Fe (39% at 250 $mg \cdot kg^{-1}$ and 30% at 500 $mg \cdot kg^{-1}$) and Se (88% at 125 $mg \cdot kg^{-1}$ and 82% at 500 $mg \cdot kg^{-1}$), but increased Mn at all concentrations. Only hydrophilic nano-TiO_2 at 500 $mg \cdot kg^{-1}$ reduced Se by 85%.

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 $mg \cdot kg^{-1}$ of unmodified anatase-rutile nano-TiO_2. 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 $mg \cdot kg^{-1}$ of anatase nano-TiO_2.

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 $H_2PO_4^-$ or HPO_4^{2-}, glycosylphosphatidylinositol, nucleotide, and coenzyme (Armstrong, 1999). Phosphorus is absorbed from soil as $H_2PO_4^-$ or HPO_4^{2-} via Pht1 transporters (Nussaume et al., 2011). The data suggests that hydrophobic particles upregulate, while hydrophilic particles downregulate the synthesis of the Pht1 transporters. In shoots, P content was significantly increased by hydrophobic treatments but reduced at hydrophilic exposure. This

Table 2.3 Accumulations of micro- and macro-elements in basil shoots grown in soil amended with unmodified and surface modified nano-TiO_2. Data are means of four replicates ± 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	Influence
Ca	Control	3303.2 ± 369.2	100 %
	Hydrophobic-125	1441.4 ± 51.4	-56.4 %
	Hydrophilic-125	1260.5 ± 78.7	-61.8 %
	Hydrophilic-500	1666.2 ± 252.8	-49.6 %
	Hydrophilic-750	1779.4 ± 107.6	-46.1 %
Mg	Control	3464.7 ± 70.1	100 %
	Pristine -125	1739.7 ±93.6	-49.8 %
	Pristine -250	4402.4 ± 147.1	+27.1 %
	Hydrophobic-125	2545.9 ± 124.4	-29.3 %
	Hydrophobic-250	2834.3 ± 108.2	-18.2 %
	Hydrophilic-125	2810.7 ± 192.0	-18.9 %
	Hydrophilic-250	2545.9 ± 108.2	-26.5 %
	Hydrophilic-500	2507.9 ± 274.2	-27.6 %
	Hydrophilic-750	2720.4 ± 150.0	-21.5 %
P	Control	8915.2 ± 669.0	100 %
	Hydrophilic-500	6212.6 ± 453.7	-30.3 %
	Hydrophilic-750	6191.5 ± 319.3	-30.6 %
Cu	Control	9.3 ± 0.4	100 %
	Pristine-250	15.7 ± 1.6	+68.8 %
	Hydrophobic-125	6.2 ± 0.2	-33.3 %
Fe	Control	48.5 ± 1.5	100 %
	Pristine-125	34.8 ± 3.6	-28.2 %
	Hydrophobic-250	29.8 ± 2.6	-38.6 %
	Hydrophobic-500	33.8 ± 3.7	-30.3 %
Mn	Control	43.3 ±2.1	100 %
	Pristine-500	72.2 ± 3.8	+66.7 %
	Hydrophobic-125	133.5 ± 18.6	+208.3 %

	Hydrophobic-250	175.8 ±51.7	+306.0 %
	Hydrophobic-500	128.2 ± 2.5	+196.1 %
	Hydrophobic-750	62.2 ± 0.4	+43.6 %
Se	Control	11.5 ± 1.3	100 %
	Pristine-125	3.0 ± 1.1	-73.9 %
	Pristine-500	6.8 ± 1.2	-40.9 %
	Hydrophobic-125	1.3 ± 0.6	-88.7 %
	Hydrophobic-500	2.1 ± 1.4	-81.7 %
	Hydrophilic-500	1.7 ± 0.4	-85.2 %

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-TiO_2 affected the uptake and translocation of Mn in a similar pattern as they affected P uptake and translocation. It is assumed 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-TiO_2 has smaller zeta potential (Table 2.1), 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-TiO_2. This may be a result of the zeta potential of the nano-TiO_2. 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-TiO_2. Selenium is taken up by plants as SeO_4^{2-} via S transporters (Pilon-Smits and Quinn, 2010). It is possible that the hydrophilic particles interact with S transporters, reducing the movement of Se within the plant. The collected data did not show a trend in the effects of unmodified and modified nano-TiO_2 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.

2.3.4 Effects of nano-TiO_2 on basil plant growth

The physiological effects of coated ENPs on plants are still largely unknown. Table 2.4 displays the effects of the three nano-TiO_2 on germination, biomass/water content and elongation of basil plants. Compared with the other treatments, at 250 and 750 $mg \cdot kg^{-1}$, hydrophilic nano-TiO_2 reduced seed germination by 51% and 59%, respectively. Also, hydrophobic particles at 125 $mg \cdot kg^{-1}$ showed a significant reduction (46%). Reports have shown no effects, promotions, and reductions of seed germination by unmodified nano-TiO_2. 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-TiO_2 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-TiO_2 on seed germination; thus, the results cannot be compared to previous results. The reduction of seed germination by hydrophilic nano-TiO_2 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-TiO_2 particles. The hydrophobic particles reduced root length by approximately 32%, 53%, and 31% at 250, 500, and 750 $mg \cdot kg^{-1}$, respectively, compared to controls. However, at 250 $mg \cdot kg^{-1}$, these particles increased shoot length by 20%, and uncoated and hydrophilic particles at 750 $mg \cdot kg^{-1}$ also increased shoot length by 37% and 32%, respectively. However, statistically significant differences were only shown in roots. Previous reports have shown different effects of

Table 2.4 Seed germination, plant elongation, and biomass/water content of basil plants grown in soil amended with unmodified and surface modified nano-TiO_2. Data are means of four replicates ± SE. Different letters represent statistically significant differences between control and treatments at the same concentration ($p \leq 0.05$). Biomass + water content = 100%

	Treatment	125 mg·kg^{-1}	250 mg·kg^{-1}	500 mg·kg^{-1}	750 mg·kg^{-1}
Germinated seeds	Control	8.3 ± 0.9 a	8.3 ± 0.9 a	8.3 ± 0.9	8.3 ± 0.9 a
	Pristine	8.5 ± 0.3 a	7.5 ± 0.7 ab	6.8 ± 0.9	6.5 ± 0.9 ab
	Hydrophobic	4.5 ± 1.3 b	5.8 ± 0.5 ab	6.3 ± 1.4	6.0 ± 0.7 ab
	Hydrophilic	5.6 ± 0.7 ab	4.1 ± 1.3 b	3.4 ± 1.4	3.4 ± 0.9 b
Root length (cm)	Control	22.7 ± 1.3	22.7 ± 1.3 a	22.7 ± 1.3 a	22.7 ± 1.3 a
	Pristine	18.8 ± 2.2	18.5 ± 0.8 ab	19.5 ± 0.7 a	21.4 ± 1.5 a
	Hydrophobic	18.0 ± 2.1	15.2 ± 1.3 b	10.6 ± 0.4 b	15.6 ± 0.7 b
	Hydrophilic	16.9 ± 2.3	17.7 ± 2.8 ab	20.0 ± 1.7 a	20.8 ± 1.5 a
Shoot length (cm)	Control	17.5 ± 1.1	17.5 ± 1.1	17.5 ± 1.1	17.5 ± 1.1 ab
	Pristine	15.9 ± 2.2	19.5 ± 2.0	21.0 ± 1.4	23.9 ± 1.0 a
	Hydrophobic	16.1 ± 2.5	20.9 ± 3.3	18.1 ± 3.5	15.7 ± 1.2 b
	Hydrophilic	15.5 ± 1.8	17.5 ± 3.0	16.6 ± 1.9	23.1 ± 2.6 a
Biomass (%)	Control	15.6 ± 0.6 a	15.6 ± 0.6 a	15.6 ± 0.6 a	15.6 ± 0.6 a
	Pristine	13.1 ± 0.1 bc	10.8 ± 0.1 b	12.5 ± 0.6 b	11.9 ± 0.5 b
	Hydrophobic	11.6 ± 0.2 c	11.1 ± 1.1 b	11.8 ± 0.2 b	9.9 ± 0.6 b
	Hydrophilic	14.5 ± 0.9 ab	15.4 ± 0.2 a	13.8 ± 0.8 ab	15.9 ± 0.6 a
Water (%)	Control	84.4 ± 0.6 b	84.4 ± 0.6 b	84.4 ± 0.6 b	84.4 ± 0.6 c
	Pristine	87.7 ± 0.8 a	89.6 ± 0.3 a	88.4 ± 0.7 a	88.7 ± 0.1 b
	Hydrophobic	88.7 ± 0.2 a	88.9 ± 1.1 a	89.0 ± 0.6 a	91.1 ± 0.6 a
	Hydrophilic	85.2 ± 0.7 b	84.5 ± 0.1 b	85.7 ± 0.6 b	84.2 ± 0.4 c

nano-TiO_2 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 2.4, uncoated and hydrophobic nano-TiO_2 significantly ($p \leq 0.05$) decreased the biomass content (~16%) at all concentrations, compared with control. Rafique et al. (2015) reported a significant reduction of biomass in wheat exposed to pristine nano-TiO_2 (> 60 $mg \cdot kg^{-1}$). 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).

2.3.5 Effects of nano-TiO_2 on relative chlorophyll and carbohydrate contents

Figure 2.4 displays the relative chlorophyll and carbohydrate contents in leaves of basil plants cultivated in soil amended with uncoated, hydrophobic, and hydrophilic nano-TiO_2. The comparison of compounds at the same concentration did not show significant difference in relative chlorophyll content (Figure 2.4A). The three nano-TiO_2 particles reduced carbohydrate concentrations at varied levels (Figure 2.4B-D). Total sugar contents were reduced by pristine nano-TiO_2 at 500 (39%) and 750 $mg \cdot kg^{-1}$ (41%), by hydrophobic at 500 (31%) and 750 $mg \cdot kg^{-1}$ (38%), and by hydrophilic particles at 500 (41%) and 750 $mg \cdot kg^{-1}$ (66%). Reducing sugars were only significantly decreased by pristine nano-TiO_2 at 750 $mg \cdot kg^{-1}$ (34%). Only hydrophobic particles yielded significant reductions of starch at 250 (34%) and 750 $mg \cdot kg^{-1}$ (25%). 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-CeO_2. A reduction in starch content in rice (*Oryza sativa* L.) grains and cucumber fruits by nano-CeO_2 were

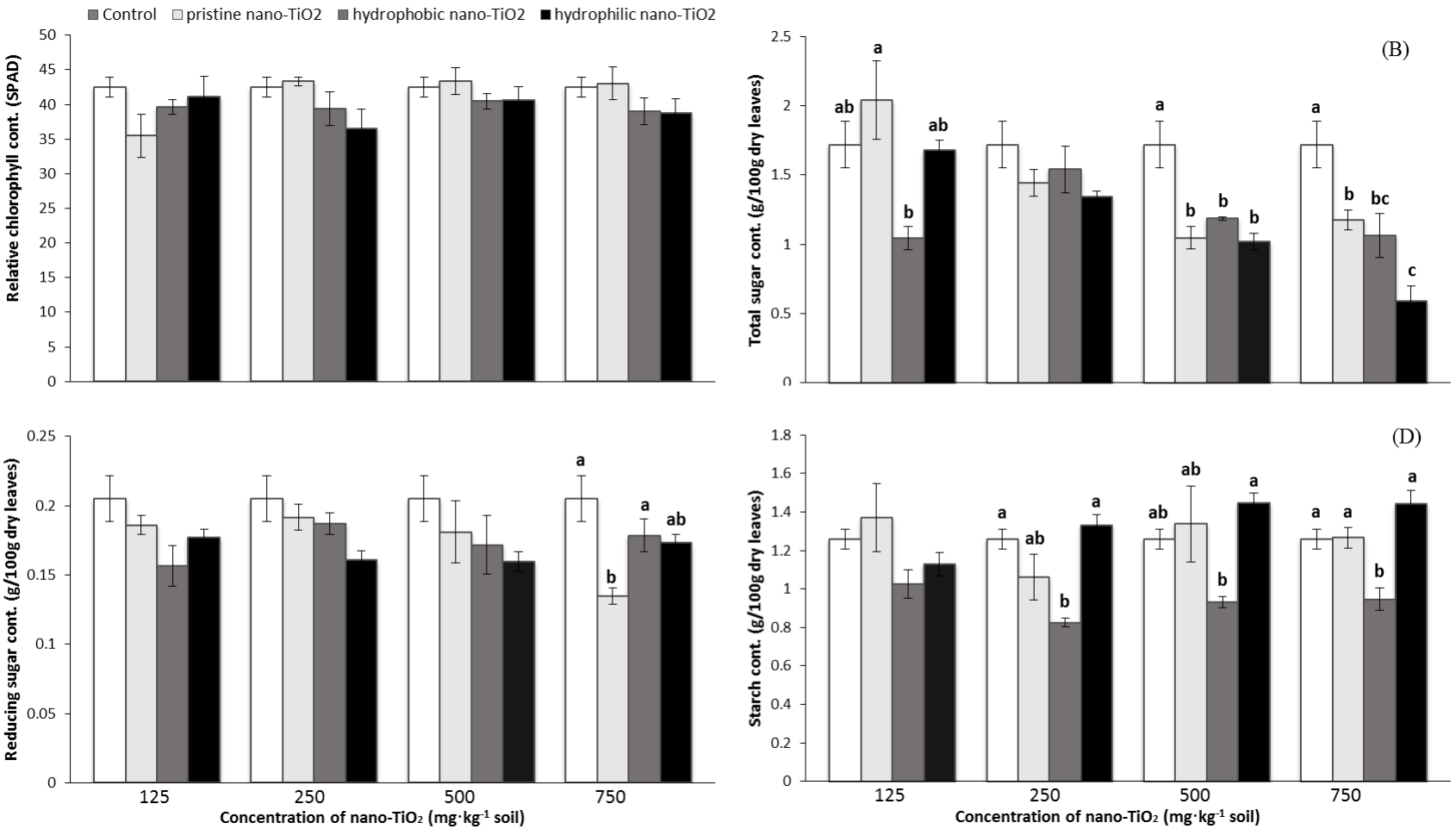


Figure 2.4 Relative chlorophyll (A), total sugar (B), reducing sugar (C) and starch (D) contents in basil plants grown for 65 days in soil amended with unmodified and surface modified nano-TiO_2 at 0, 125, 250, 500, and 750 mg·kg^{-1} soil. Each value is mean ± SE (n= 4). Different letters represent statistically significant differences between control and treatments at the same concentration ($p \leq 0.05$).

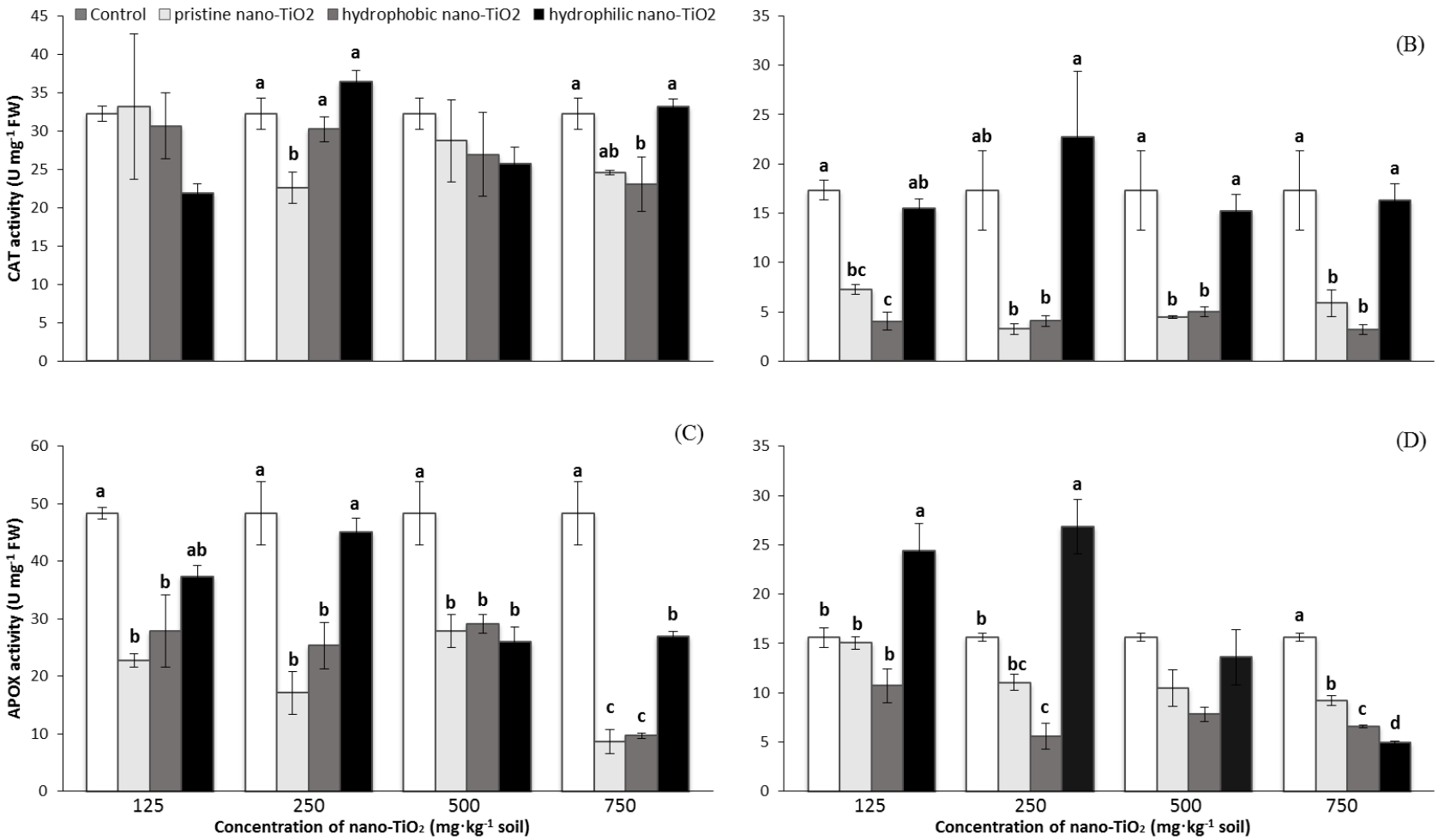


Figure 2.5 Catalase activity in leaves (A) and roots (B), and ascorbate peroxidase activity in shoots (C) and roots (D) of basil plants grown for 65 days in soil amended with unmodified and surface modified nano-TiO_2 at 125, 250, 500, and 750 mg·kg^{-1} soil. Each value is mean ± SE (n = 4). Different letters represent statistically significant differences between control and treatments at the same concentration ($p \leq 0.05$).

reported by Rico et al. (2013) and Zhao et al. (2014). The mechanism for carbohydrate disruption by ENPs is still unknown.

2.3.6 Effects of nano-TiO_2 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 2.5 displays the CAT and APOX activities in roots and leaves of basil plants cultivated in soil amended with unmodified, hydrophobic, and hydrophilic nano-TiO_2. In leaves, unmodified nano-TiO_2 significantly reduced CAT activity at 250 $mg \cdot kg^{-1}$ (30%) and APOX activity at all concentrations ($p \leq 0.05$), compared with controls. Hydrophobic particles significantly reduced CAT activity at 750 $mg \cdot kg^{-1}$ (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 $mg \cdot kg^{-1}$ only. In the roots, unmodified and hydrophobic nano-TiO_2 significantly decreased CAT and APOX activities at all concentrations, compared with controls. Hydrophilic nano-TiO_2 did not affect CAT but significantly increased APOX activity at 125 and 250 $mg \cdot kg^{-1}$ (72%) and decreased it at 750 $mg \cdot kg^{-1}$ (68%). Servin et al. (2013) reported that in cucumber, CAT activity was enhanced, while APOX activity was suppressed at 500 $mg \cdot kg^{-1}$ 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-TiO_2 might interfere the chemical equilibrium, causing less stress than the other nano-TiO_2. 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). Further studies should be performed to determine the role of surface properties in affecting the antioxidant response.

2.4 Conclusion

The ICP determinations showed Ti accumulation in both roots and shoots. However, only root Ti concentrations showed statistical differences under exposure to surface modified nano-TiO_2 at 500 and 750 mg kg^{-1}. This suggests that, independently of the surface modification, little amount of nano-TiO_2 reached the transport system of basil plants. Hydrophilic nano-TiO_2 showed a reduction in seed germination and hydrophobic nano-TiO_2 significantly reduced root elongation. This suggests that both hydrophilic and hydrophobic particles impact agronomic parameters in basil. The three types of nano-TiO_2 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-TiO_2 in roots and leaves, while hydrophilic nano-TiO_2 in roots. Hydrophilic nano-TiO_2 at 750 mg·kg^{-1} reduced total sugar content, while hydrophobic particles reduced starch, and unmodified particles decreased reducing sugars. Overall, independently of the surface properties, nano-TiO_2 has negative effects in the physiology and biochemistry of basil plants.

Chapter 3: Physiological and Biochemical Response of Basil (*Ocimum basilicum*) Exposed for Two Consecutive Generations to TiO_2 Nanoparticles of Different Surface Chemistry

Abstract

There is a lack of information about the transgenerational effects of titanium dioxide nanoparticles (nano-TiO_2) in plants. This study aimed to evaluate the impacts of successive exposure of nano-TiO_2 with different surface properties to basil (*Ocimum basilicum*). Seeds from plants exposed to pristine, hydrophobic, or hydrophilic nano-TiO_2 were cultivated for 65 days in soil unamended or amended with 750 $mg \cdot kg^{-1}$ of the respective particles. Plant growth, concentration of titanium and essential elements, as well as content of carbohydrates and chlorophyll were evaluated. There were no differences on Ti concentration in roots of plants sequentially exposed to pristine or hydrophobic nano-TiO_2, or in roots of plants exposed to the corresponding particle, only in the second cycle. However, sequential exposure to hydrophilic particles resulted in 65.2% less Ti in roots, compared to roots of plants exposed the same particles, only in the second cycle. The Ti concentrations in shoots were similar in all treatments. On the other hand, pristine and hydrophilic particles increased Mg in root by 115% and 81%, respectively, while pristine and hydrophobic particles reduced Ni in shoot by 84% and 75%, respectively, compared to unexposed plants in both cycles. Sequential exposure to pristine nano-TiO_2 increased stomatal conductance (214%, $p \leq 0.10$), compared to plants that were never exposed. Hydrophobic and hydrophilic nano-TiO_2 reduced chlorophyll *b* (52%) and total chlorophyll (30%) but increased total sugar (186%) and reducing sugar (145%), compared to unexposed plants in both cycles. Sequential exposure to hydrophobic or hydrophilic nano-TiO_2 resulted in more adverse effects on photosynthesis but in positive effects on plant growth, compared to pristine nano-TiO_2.

Keyword: TiO_2 nanoparticles, Surface chemistry, Basil, Transgenerational effects, Growth

3.1 Introduction

Titanium dioxide nanoparticles (nano-TiO_2) with different shape, size, crystalline phase, and surface properties, are widely used in skin care, painting, food additives, solar cells, gas sensors, and photocatalysis, among other fields (Makarova et al., 2000; Chae et al., 2003; Seo et al., 2007; Chen and Mao, 2007). The demand of nano-TiO_2 for personal care and cosmetic industry (PC&C) increased from 1300 metric tons in 2005 to 5000 metric tons in 2010 (Weir et al., 2012), which suggests higher environmental release of nano-TiO_2. Recent estimates indicated that the release of nano-TiO_2 equals one quarter of the total engineered nanomaterials (ENMs) emitted to the environment (Keller and Lazareva, 2013). Furthermore, nano-TiO_2 particles used in PC&C have been found in sludge in the range of 69 to 1500 mg kg^{-1} (Gottschalk et al., 2015). This suggests that plants can be unintentionally exposed to high concentrations of nano-TiO_2.

Reports from controlled condition experiments have shown that nano-TiO_2 can alter germination, seedling elongation, yield characteristic, photosynthesis process, and the antioxidant defense system (Du et al., 2017) in *Arabidopsis thaliana* (Ze et al., 2011 and Liu et al., 2017), wheat (Larue et al., 2012), spinach (Hong et al., 2005 and Zheng et al., 2005), lettuce (Larue et al., 2014), cucumber (Servin et al., 2013), tomato (Song et al., 2013), and soybean (Rezaei et al., 2015). For example, Wu et al. (2017) investigated metabolism alterations in rice treated with nano-TiO_2 (anatase) for 14 days. These researchers found that metabolites related to basic energy-generating pathways were enhanced, whereas the carbohydrate synthesis metabolism was suppressed. In studies with spinach, Hong et al. (2005) and Zheng et al. (2005) reported that rutile phase nano-TiO_2 promoted the photosynthesis rate and increased chlorophyll formation. However, in tomato treated with anatase/rutile mixture, Song et al. (2013) found no effects on root length, biomass, and chlorophyll content. Siddiqi and Husen (2017) pointed out that the response to nano-

TiO_2 depends on the dose and exposure time, while the accumulation and translocation indicates these particles are non-biodegradable. In flax (Clément et al., 2013), exposure to 100 mg/L of nano-TiO_2 for 72 h resulted in an enhancement of seed germination and root length; however, the biomass was reduced. Other reports have shown that the interaction between nano-TiO_2 and plants varies with particle size, crystal phase (Larue et al., 2012) and surface coatings (Tan et al., 2017). Larue et al. (2012) reported that particles larger than 30 nm penetrated wheat root cells, suggesting that nano-TiO_2 enlarged cell pores. Data from exposure to the rutile phase (uncoated and coated with dimethcone) showed preferential uptake in cucumber (Servin et al., 2012) and basil (Tan et al., 2017) respectively. However, no data from multi-generational exposure was found (Du et al., 2017; Siddiqi and Husen, 2017; Zuverza-Mena et al., 2017)

Due to the UVA/UVB absorbing properties, UV-TITAN M212 (with hydrophilic surface) and M262 (with hydrophobic surface) titanium dioxide nanoparticles (nano-TiO_2) are developed by PC&C (Sachtleben Chemie GmbH, Duisburg, Germany). Although the consumption of these nano-products is continually increasing, only very few papers have evaluated their impacts on biosystems. Few reports refer to the effects of these two nano-TiO_2 on animal or human cells (Hammond et al., 2103; Chen et al., 2016; Rahman et al., 2017), but only our previous publications dealt with plants (Tan et al., 2017).

Studies on the effects of continuous exposure to ENMs to plants are scarce. In tomato, second generation seedlings sequentially treated with 10 $mg \cdot kg^{-1}$ nano-CeO_2 had lower biomass and water transpiration (Wang et al., 2013). Third generation plants of *Brassica rapa,* continually exposed to nano-CeO_2, had lower seed production and higher oxidative stress (Ma et al., 2016).[24] Exposure to nano-CuO for two generations produced alterations in gene expression and higher Cu accumulation (Wang et al., 2016). In a two cultivation cycles study with wheat, Rico et al. (2017)

reported lower root cerium accumulation in plants only exposed to nano-CeO_2 in the first cycle. To the best of the authors' knowledge, such studies with nano-TiO_2 are yet to be published.

This is a follow-up of a previous study about the effects of pristine, hydrophilic (M212), and hydrophobic (M262) nano-TiO_2 on basil (Tan et al., 2017). Seeds collected from untreated and nano-TiO_2 treated plants in the first cycle were exposed to the same type of particles in a second growth cycle. After 65 days, Ti accumulation, agronomical makers, nutritional elements, gas exchange, chlorophyll, and carbohydrate contents were evaluated following previous protocols (Tan et al., 2017). To the authors' knowledge, this is the first report showing the effects of sequential exposure of uncoated and coated nano-TiO_2 to a culinary plant. The aim of this study was to determine the effects of the exposure of nano-TiO_2 upon successive generations of basil plants. Based on results from a previous study, the working hypothesis was that pristine nano-TiO_2 would result in less negative effects than hydrophobic and hydrophilic nano-TiO_2.

3.2 Materials and methods

3.2.1 Preparation of experimental soil

Pristine, hydrophobic (M262), and hydrophilic (M212) nano-TiO_2 were provided by US research Nanomaterials and Sachtleben Chemie, Germany, respectively. The detailed characterization of the nano-TiO_2 particles is shown in Tan et al. (2017). Briefly, three nano-TiO_2 used were in rutile phase, with a size of 50 ± 25 nm. The purchased hydrophobic and hydrophilic nano-TiO_2 came coated with Al_2O_3 (6 wt%) and encapsulated with dimethicone (2 wt%) (hydrophobic) or glycerol (1 wt%). The suspension was added to the soil to have a final concentration of 750 $mg \cdot kg^{-1}$. Suspensions were sonicated in a water bath (120 V, 3 Amps, 50/60 Hz) for 30 min at 20 ℃. The hydrophobic particles were directly amended with soil. Portions of 1200 g of potting soil (Miracle Gro®) (Barrios et al., 2016) were amended with the NP stock

suspensions or hydrophobic powder homogenously, and then placed into each plastic pot with a size of 20 cm × 17.5 cm × 15 cm. The potting mix soil was previously analyzed by Barrios et al. (2016). Detailed information is provided in the electronic supplementary information (S2 Table 2.1). Each treatment was replicated three times. Ten basil seeds were sown on the next day after soil preparation.

3.2.2 Seed germination and cultivation conditions

Basil seeds (*Ocimum basilicum*, summer basil, Willhite Seed) were harvested from control (not exposed to NPs in the first cycle) and plants treated with 750 $mg \cdot kg^{-1}$ of pristine, hydrophobic or hydrophilic nano-TiO_2 (Tan et al., 2017). The seeds were sown in soil unamended/amended with 750 $mg \cdot kg^{-1}$ of the same type of nano-TiO_2, as per Wang et al. (2013) (Table 3.1). The experimental design included the following treatments: (a) control-0 (no TiO_2 exposure in the first and second cultivation cycles), (b) control-750 (seeds exposed to 750 $mg \cdot kg^{-1}$ of pristine, hydrophobic or hydrophilic NPs only in the second crop cycle), (c) treated-0 (seeds harvested from NP exposed plants in the first cycle and grown without NPs in the second crop cycle), and (d) treated-750 (seeds collected from NP treated plants in the first cycle and exposed to the same type of nano-TiO_2 in the second crop cycle). Plants were cultivated in a growth chamber (Environmental Growth Chamber, Chagrin Falls, OH) set at 65% relative humidity, 25/20 °C day/night, 340 μmole m^{-2} s^{-1} light intensity, and 14 h photoperiod. No additional fertilizer or nutrient solution was applied for this study. All the plants were watered with 100 mL of MPW every other day and harvested when 75% of the control-0 plants had flowers (65 days).

3.2.3 Plant growth and biomass production

At harvest, plant samples were treated as previously described (Morales et al., 2013). To

Table 3.1 Experimental design of nano-TiO_2 exposed to basil (*Ocimum basilicum*)

Type of NPs	Name	First cycle	Second cycle
		Seeds from NP-treated plants (without, -; with, +)	NP (without, -; with, +)
	Control-0	-	-
Pristine nano-TiO_2	Prist-control-750	-	+
	Prist-treated-0	+	-
	Prist-treated-750	+	+
Hydrophobic nano-TiO_2	HB-control-750	-	+
	HB-treated-0	+	-
	HB-treated-750	+	+
Hydrophilic nano-TiO_2	HP-control-750	-	+
	HP-treated-0	+	-
	HP-treated-750	+	+

remove NPs adhere to the root surface, plant tissues were thoroughly rinsed with 0.01 M HNO_3 and MPW for six times. Fresh and oven-dried (60 °C, 72 h) shoot samples were measured to determine biomass and water content. The water content was determined as described elsewhere (Tan et al., 2017).

3.2.4 Chlorophyll content and gas exchange measurement

For chlorophyll determination, 200 mg of fresh chopped leaves per replicate were exposed to chilled acetone, until all the pigment was extracted. Absorbance of the supernatant was recorded at 645, 655 and 664 nm, respectively. Chlorophyll *a*, chlorophyll *b* and total chlorophyll contents were calculated according to Porra (1991). Gas exchange, net photosynthetic rate (*A*) and stomatal conductance (*gs*) were monitored with a portable gas exchange system (LI-6400, LiCor Biosciences, Lincoln, NE, USA) in 60-day old plants. The measurements were made in a 2 × 3 cm chamber with an LED light source at 25 °C, with a photosynthetic photon flux of 850 $\mu mol \cdot m^{-2} \cdot s^{-}$

^{1}, and concentration of CO_2 entering the measuring chamber of 475 μmol·mol^{-1}. These conditions were set to match the growth chamber environment in which the plants were growing. Before measurement, plants were watered to avoid the effects of water limitation. The data were collected until environmental conditions and gas exchange parameters in the cuvette were stable for five seconds. All gas exchange measurements were made between 9 am and 11 am. Three leaves from each pot located at approximately the same height on each plant were measured and the average value of these three measurements was used for statistical analysis.

3.2.5 Carbohydrate measurement

Carbohydrates (total sugar, reducing sugar, and starch) of fresh leaves were extracted as per Verma and Dubey (2001). Briefly, total sugar and reducing sugar were extracted by boiling for 30 min, in water bath (80°C), samples of 100 mg containing 10 mL 80% ethanol. After cooling, the samples were centrifuged at 22,000 × g for 20 min. The extraction was repeated three times and the supernatants combined. The combined supernatants were evaporated to 3 mL; then, the extract was diluted to 25 mL with MPW water. Residues from the previous extractions were added two mL of H_2SO_4 to extract starch. Concentrations of total sugar and starch were measured after Dubois et al. (1956) while reducing sugars were measured according to Nelson-Somogyi (1952).

3.2.6 Absorption of titanium and nutritional elements

Root and shoot tissue samples were dried at 60 ℃ for 72 h and digested following a previous described protocol (Larue et al., 2014). The recovery rate for Ti was between 67% (Larue et al., 2014) and 82% (Tan et al., 2017). Concentrations of Ti, Al, micro and macroelements (B, Cu, Fe, Mo, Mn, Ni, Se, Zn, Ca, K, Mg, P, and S) were determined by inductively coupled plasma-optical emission sprectrometry (ICP-OES, Perkin-Elmer Optima 4300 DV). Blank, spiked

samples, and standard reference materials (NIST-SRF 1570a and 1547, Metuchen, NJ) were used as calibration and QC/QA controls.

3.2.7 Statistical analysis

The reported data are means of three replicates ± standard errors. The experiment variance was determined by one-way ANOVA using the statistical package SPSS (Social Sciences 22.0, Chicago, IL). Statistical differences between treatments were analyzed with the Tukey Honestly Significant Difference ($p \leq 0.05$) or Duncan's Multiple Range tests ($p \leq 0.10$).

3.3 Results and Discussion

3.3.1 Impact of nano-TiO_2 on basil seed production

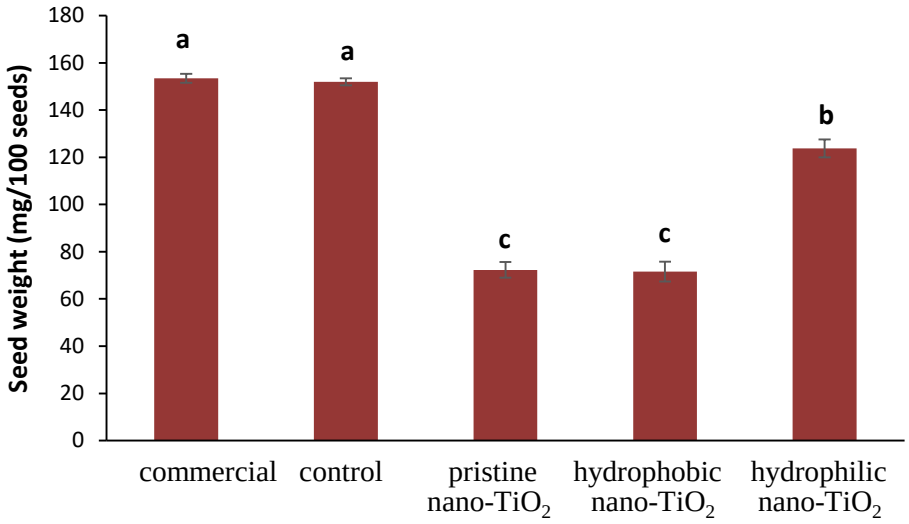
Figure 3.1A-B shows the biomass and an image of basil seeds harvested from control plants and plants exposed to 750 $mg \cdot kg^{-1}$ of nano-TiO_2 in the first crop cycle. Figure 3.1A shows that, in the first crop cycle, seeds from pristine, hydrophobic, and hydrophilic exposed plants resulted in significantly lower seed biomass (52.9%, 53.3% and 19.3%, respectively), compared with controls. Fig. 3.2B shows that plants exposed to pristine and hydrophobic nano-TiO_2 had smaller seeds, with drier hulls, and a slightly brown color. The yellow color of seeds coat is given, mainly, by two flavonol glycosides, kaempferol (3,4,5,4'-tetrahydroxyflavone)-3-O-β-D-glucopyranoside, and kaempferol 3-O-β-D-glucopyranoside-(2-1)-O-β-D-xylopyranoside (Beninger et al., 1998). This suggests that the pristine and hydrophobic nano-TiO_2 changed gene expression of these compounds. It could be also possible that imbalances in the hemostasis of essential elements (Ca, Cu, Mg, Mn and P) (Tan et al., 2017), contributed to the changes in the glycosides. Deficiencies in Ca, an essential element to maintain membrane integrity, may result in blossom end rot of developing fruits or seeds (Jones, 2016). Deficiencies in Mg and Mn can interfere in

photosynthesis, causing chlorosis, and inhibiting plant growth and development (Tewari et al., 2006 and Löhnis, 1951). A deficiency in P, essential element in energy transfer system, can considerably reduce plant growth (Jones, 2016) Additionally, a shortage in Cu, which is a constituent of proteins and enzymes, may result in small and poorly formed fruits or seeds (Jones, 2016 and Anderssen, 1932).

3.3.2 Titanium uptake

Titanium concentration in basil plant tissues of the second crop cycle is displayed in Fig. 3.2A-B. Comparisons were made between control and treatments within the same type of nanoparticles. As shown in Fig. 3.2A, roots of plants exposed to pristine particles only in the second crop cycle (control-750), or in both cycles (treated-750), had similar amounts of Ti (292 and 306 mg kg^{-1}, respectively). These Ti concentrations were 350% and 362% higher than the root Ti in plants exposed to pristine particles in the first crop cycle (treated-0) ($p \leq 0.05$). Similar results were found in *Arabidopsis thaliana* exposed to nano-CuO (Wang et al., 2016). Authors hypothesized that higher accumulation of copper in the second generation plants could be attributed to mutations of genes related to root growth and reactive oxygen species (Wang et al., 2016). On the other hand, hydrophobic nano-TiO_2 treatments showed no statistical differences in root Ti of plants consecutively exposed (treated-750) or exposed in the second crop cycle only (control-750). In addition, plants exposed to these particles in the second cycle (control-750), had 2435% more Ti, compared to treated-0 plants (exposed in the first cycle, not exposed in the second cycle). In case of hydrophilic nano-TiO_2, plants exposed only in the second cycle (control-750), had 187% more root Ti than plants consecutive exposed (treated-750). Additionally, there were no differences in root Ti between plants exposed only in the first cycle (treated-0) and plants consecutively exposed (treate-750). A comparison between particles showed that all of treated-0

(A)



(B)

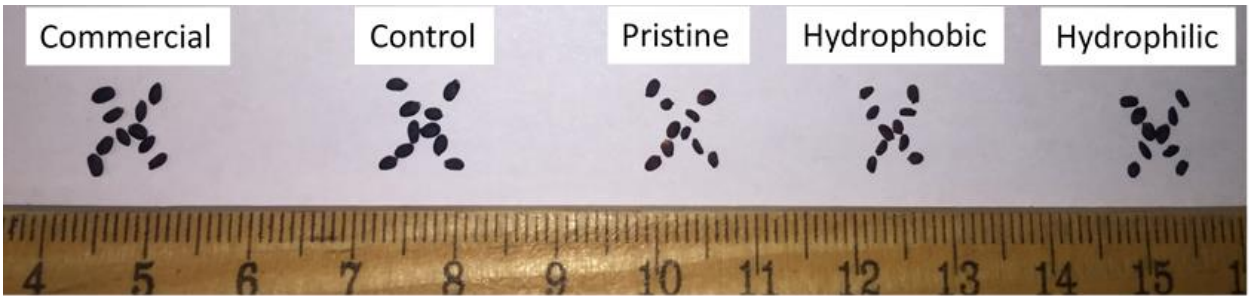


Figure 3.1 (A) Biomass of 100 basil seeds harvested from plants with no NP exposure or plants exposed to 750 mg·kg^{-1} of nano-TiO_2 (pristine, hydrophobic, and hydrophilic, respectively) in the first cycle. Each value is mean ± SE (n = 4). Different letters represent statistically significant differences compared with respect to commercials seeds at Tukey's test ($p \leq 0.05$). (B) Images for commercial seeds (A), and seeds harvested from plants with no NP exposure (B), or plants exposed to 750 mg·kg^{-1} pristine (C), hydrophobic (D), and hydrophilic (E) of nano-TiO_2, respectively, in the first cycle.

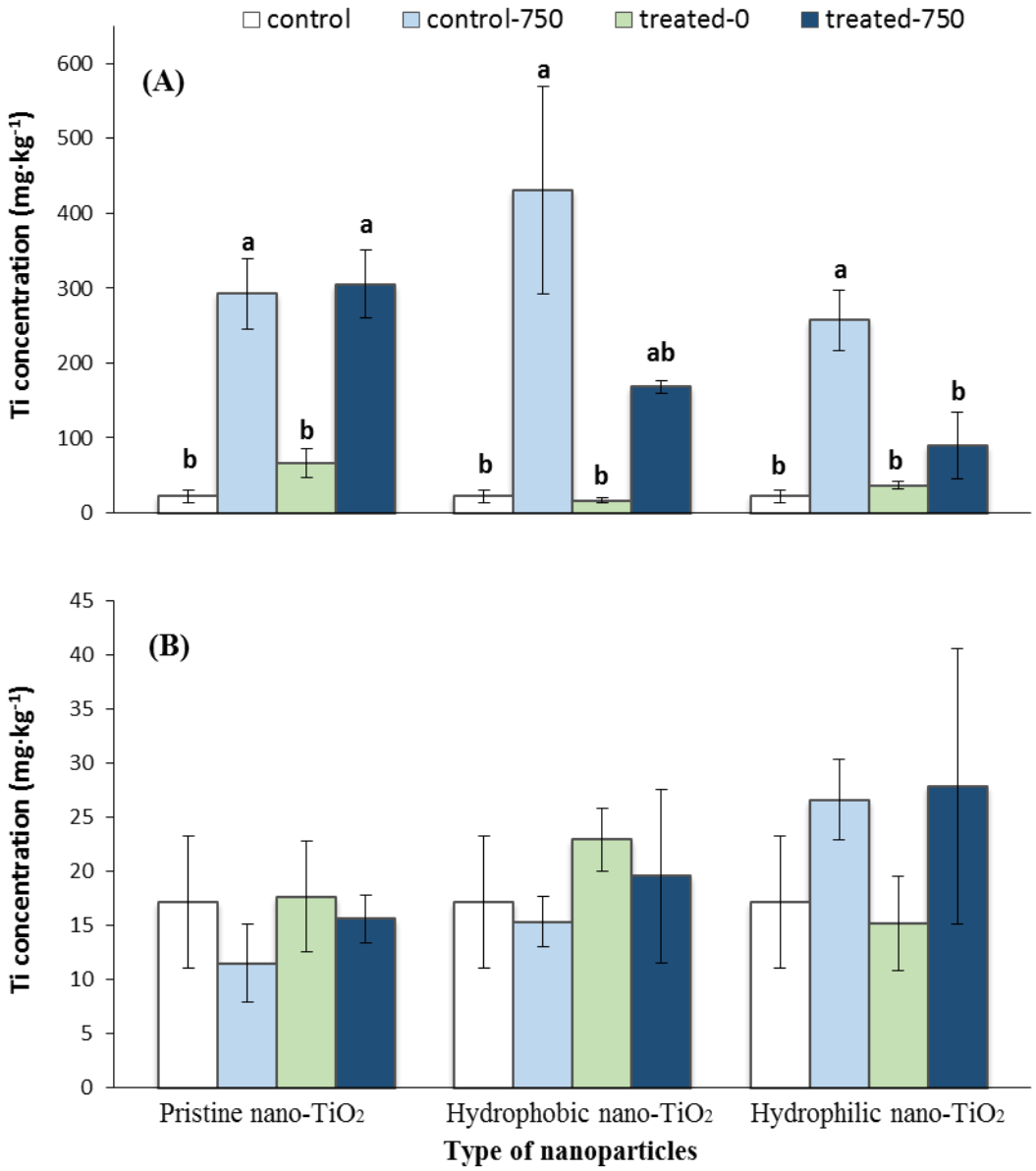


Figure 3.2 Ti content in roots (A) and shoots (B) of 65 day-old basil plants generated from untreated and treated seeds with/without sequential exposure of 750 $mg \cdot kg^{-1}$ of pristine, hydrophobic, and hydrophilic nano-TiO_2, respectively. Each value is mean ± SE (n = 3). Different letters represent statistically significant differences between control and treatments within the same nanoparticles at Tukey's test ($p \leq 0.05$).

plants (exposed in the first cycle, not exposed in the second cycle) had similar root Ti concentrations. There were also no differences between the root Ti of never exposed plants and plants exposed only in the first crop cycle to the three nano-TiO_2. This suggests that the three nano-TiO_2 particles have similar probabilities for transgenerational distribution.

In the previous study, it was found that roots from plants exposed to hydrophobic nano-TiO_2 had more Ti compared to pristine and hydrophilic nano-TiO_2. Similarly, in the present study, the highest Ti concentration was found in roots of plants exposed, in the second cycle only, to hydrophobic nano-TiO_2. This can be explained by a preferential uptake of hydrophobic nano-TiO_2, driven by the negatively charged plasma membranes of root surface cells (hydrophobic particles have a positive charged, 26.9 ± 0.5 mV) (Tan et al., 2017). However, in plants exposed to nano-TiO_2 in both cycles, only the pristine treatment resulted in significantly higher Ti concentration in roots. The positively charged hydrophobic and hydrophilic nano-TiO_2 (Tan et al., 2017) would be attracted by the negatively charged soil particles, while negatively charged pristine rutile nano-TiO_2 (Tan et al., 2017), repelled such particles. Moreover, hydrophilic nano-TiO_2 would show higher affinity with natural organic matter coated soil particles (hydrophilic) (Peralta-Videa et al., 2011). On the other hand, previous reports have shown that nano-TiO_2 can cause problems such as changes in mitotic index (Pakrashi et al., 2014) and chromosomal aberrations in onion (Ghosh et al., 2010), induce tubulin aggregation in *Arabidopsis thaliana* (Wang et al., 2011), change DNA sequences in zucchini (Moreno-Olivas et al., 2014), and mRNA expression in tobacco (Frazier et al., 2014), among others. It is possible that some of these alterations operate in the development of subsequent generations of TiO_2 exposed plants. In tomato, the second generation of plants exposed to the nano-CeO_2 had extensive root hairs, but no significantly high cerium concentration

(Wang et al., 2013). However, no previous studies on transgenerational effects of nano-TiO_2 were found in the literature.

The Ti content in shoots is shown in Figure 3.2B. As shown in this figure, none of the treatments showed differences in shoot Ti. Similar results were found in our previous study (Tan et al., 2017). Even though there were no differences in shoot Ti, previous studies have shown the translocation of nano-TiO_2 from root to the aboveground plant parts (Du et al., 2011; Larue et al., 2012; Servin et al., 2012; Song et al., 2013; Larue et al., 2016), although this seems to be a species-dependent process (Servin et al., 2012; Song et al., 2013; Larue et al., 2016). To this end, it is clear that more studies are needed to understand the mechanisms of uptake and transgenerational movement of TiO_2 particles with different surface coatings.

3.3.3 Accumulation of nutritional elements

Changes in element composition are important indicators of the biochemical impacts of ENMs in plants (Peralta-Videa et al., 2014). Concentrations of aluminum, micro- and macro-elements in basil plants are displayed in Fig. 3.3A-E (roots) and Fig. 3.3F (shoots). Only elements that accumulated significantly more or less in roots or shoots, compared with plants with no NP exposure in both cycles, are presented ($p \leq 0.5$). As shown in Fig. 3.3A, only plants derived from seed of unexposed plants, exposed to hydrophobic nano-TiO_2 in the second cycle (control-750), resulted in significant increase of root Al (212%). Aluminum is not an essential element associated with the growth improvement of some plants.

Compared with no NP exposure in both cycles, significant increase of root Cu (Fig. 3.3B) was observed in plants exposed to pristine nano-TiO_2 in both cycles (184%), and plants exposed to hydrophilic nano-TiO_2 (207%) in the first cycle only. The accumulation of root Mg was significantly increased in plants exposed to pristine nano-TiO_2 in the second cycle (control-750,

83%) and pristine treated plants in the first cycle, without/with NP exposure in the second cycle (treated-0, 80%; treated-750, 115%) (Fig. 3.3C). In addition, statistically higher root Mg was determined in plants exposed only in second cycle to hydrophilic nano-TiO_2 (74%), as well as hydrophilic treated plants in the first cycle, without/with NP exposure in the second cycle (70% and 81%, respectively; Fig. 3.3C). For Ca and Zn, plants obtained from pristine treatment in the first cycle, exposed again to pristine particles, resulted in significantly higher accumulation in roots (118% and 266%, respectively, Fig. 3.3D-E), as compared to control. Additionally, statistically higher Zn concentration was determined in plants exposed to hydrophobic nano-TiO_2 in both cycles (106%). In shoot samples, only significant reductions in Ni were shown. Significantly lower Ni concentrations were found in untreated plants exposed, only in the second cycle to pristine nano-TiO_2 (45%), as well as in pristine treated plants in the first cycle, without/with NP exposure in the second cycle (84% and 83%, respectively; Fig. 3.3F). Also, significantly lower Ni concentrations were found in untreated plants exposed, only in second cycle, to hydrophobic nano-TiO_2 (68%), and hydrophobic treated plants in the first cycle, without or with NP exposure in the second cycle (75% and 61%, respectively; Fig. 3.3F).

In our previous study, plants treated with pristine nano-TiO_2 resulted in significantly higher Cu concentration in roots, which is in agreement with our present study.[24] However, plants exposed to these particles in both cycles had higher concentrations of Al, Mg, Ca, and Zn in roots, and lower Ni in shoots, which for some elements, was contrary to the results found in the first crop cycle. In the first crop cycle, roots had significantly lower Ca, P, Mg, Fe, and Se (Tan et al., 2017). Nano-TiO_2 has been associated with the generation of reactive oxygen species (Ze et al., 2011; Pakrashi et al., 2014) and genotoxicity (Ghosh et al., 2010; Wang et al., 2011), including DNA aberrations and chromosomal breaks (Pakrashi et al., 2014). Thus, it is very likely that the

transporters or bonding ligands of minerals were affected by the presence of nano-TiO_2 (Drummond et al., 2006). In basil, the higher uptake of Al might be caused by organic ligands such as citrate and malate, which are also related to the uptake of Zn and Ni (Haydon and Cobbett, 2007; Sasaki et al., 2010). It is possible that changes in the expression of mugineic acid ligand transporters, yello-stripel1 (YS1) (Schaaf et al., 2004), modified the uptake of Cu and Zn and the transport of Ni. It is also possible that nano-TiO_2 increased the capacity of Ca binding proteins, CAS (Nomura and Shiina, 2014), AtMRS2-11 (Drummond et al., 2006); thus, increasing the concentration of Ca and Mg in basil tissues. Previous reports have shown increased concentrations of Ca, Fe, K, P, or S in plants exposed to a mixture of anatase and rutile (Servin et al., 2013) or anatase nano-TiO_2 (Sasaki et al., 2010). Cai et al. (2017) found that rutile nano-TiO_2, with hydrophilic or hydrophobic coating, did not enter rice seedlings but reduced lead uptake. This suggests that growth conditions, plants species, and the ENMs per se, affect the uptake of nutritional elements.

3.3.4 Impacts of nano-TiO_2 on basil plant growth

The agronomical parameters of basil plants are listed in Table 3.2. As can be seen in this table, none of the nanoparticles showed significant difference in seed germination and root length. However, some effects were noted in shoot growth. Compared to control plants (not NP treated plants), plants exposed to hydrophobic nano-TiO_2 in the first cycle, without/with sequential exposure to 750 $mg \cdot kg^{-1}$ nano-TiO_2 in the second cycle, resulted in longer shoot length (21% and 19%, respectively, $p \leq 0.10$, Table 3.2). These plants also had higher biomass production (4.5%) and, conversely, lower water content (4.7%, $p \leq 0.1$). Significant increase in stem length was shown in cucumber treated with uncoated nano-TiO_2 mixture (P25, mixture of 82% anatase and 18% rutile) (Servin et al., 2012). However, no significant difference in shoot lengths were observed

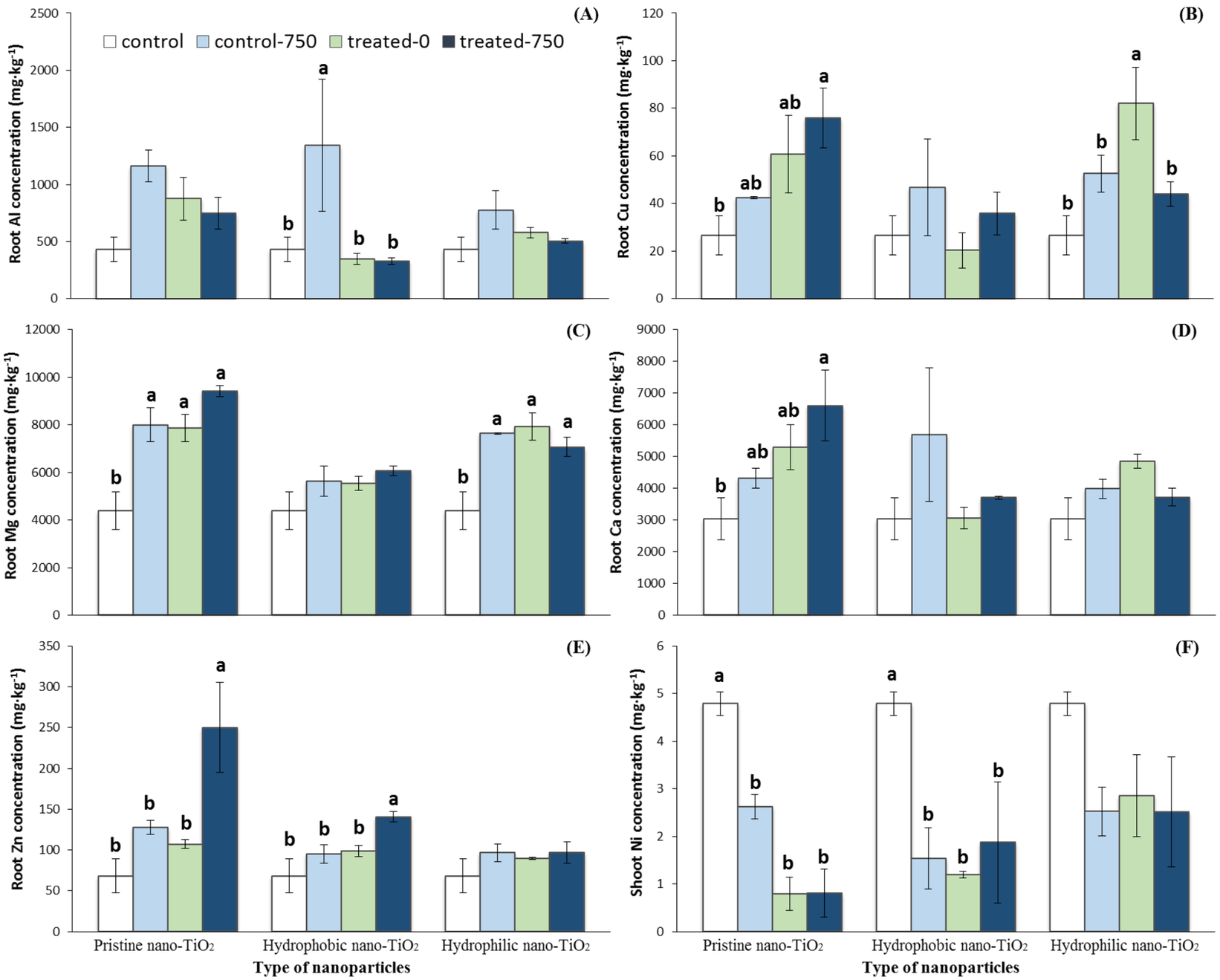


Figure 3.3 Nutrient elements modification of Al (A), Ca (B), Cu (C), Mg (D), Zn (E) in roots and Ni (F) in shoots of 65 day-old basil plants generated from untreated and treated seeds with/without sequential exposure of 750 mg·kg^{-1} of pristine, hydrophobic, and hydrophilic nano-TiO_2, respectively. Each value is mean ± SE (n = 3). Different letters represent statistically significant differences between control and treatments within the same nanoparticles at Duncan's test ($p \leq 0.10$).

in wheat (Feizi et al., 2012) and fennel (Feizi et al., 2013) grown in soil exposed uncoated nano-TiO_2 mixture.

The water content was also impacted by the nano-TiO_2 particles. Plants exposed to hydrophobic particles in the two consecutive cycles, and hydrophobic treated plants in the first cycle only, had significantly lower water content (5.8% and 5.6%), compared to plants with no NP exposure in both cycles ($p \leq 0.10$). This could be attributed to a decrease in root hydraulic conductivity (Asli and Neumann, 2009). Hydrophobic nano-TiO_2 (Fig. 3.2A) would repel water absorption in the roots, due to a poor affinity between root membrane and the nano-TiO_2. Hydrophobic nano-TiO_2 is positively charged, and positively charged hydrophobic molecules can create holes on the cell membrane (Hong et al., 2004). This could be a reason for the water loss. However, in willow trees treated with P25 nano-TiO_2, no significant differences in transpiration and water use efficiency were observed (Seeger et al., 2009). In wheat, none of the evapotranspiration parameters were changed by different sizes and crystal phases of nano-TiO_2 (Larue et al., 2012). The different responses would be attributed to differences in cultivation conditions and plant species.

3.3.5 Impacts of nano-TiO_2 on gas exchange

Nano-TiO_2 have high electronic band gaps (≥ 3.0 eV) that increase in energy as the size of particles decreases. Thus, nano-TiO_2 particles have high absorption in the UV region (Elghniji et al., 2012). However, particles varying in surface properties would affect photosynthesis parameters in different ways. Fig. 3.4A-B displays photosynthetic rate (*A*) and stomatal conductance (*gs*) in leaves of basil plants exposed to the three nano-TiO_2 particles. In this study, only plants treated with pristine nano-TiO_2, in both cycles, showed a significant difference, compared with control.

Table 3.2 Germination rate (%), elongation, and shoot biomass/water (%) content in soil-grown basil plants generated from untreated and treated seeds with/without sequential exposure to 750 $mg \cdot kg^{-1}$ of pristine, hydrophobic, and hydrophilic nano-TiO_2. Data are means ± SE (n=4). Different letters represent statistically significant differences between control and treatments at the same concentration at Duncan's test ($p \leq 0.1$). The absence of letters indicates no significant differences, compared with control.

	Treatment	Pristine	Hydrophobic	Hydrophilic
Germination rate (%)	Control-0	83.3 ± 6.7	83.3 ± 6.7 ab	83.3 ± 6.7
	Control-750	80.0 ± 5.8	90.0 ± 5.8 ab	83.3 ± 8.8
	Treated-0	73.3 ± 6.7	96.7 ± 3.3 a	83.3 ± 3.3
	Treated-750	66.7 ± 6.7	70.0 ± 10 b	73.3 ± 8.8
Root length (cm)	Control-0	24.6 ± 1.9	24.6 ± 1.9	24.6 ± 1.9
	Control-750	30.2 ± 3.2	25.5 ± 0.2	26.7 ± 0.9
	Treated-0	22.9 ± 1.4	23.3 ± 1.4	23.7 ± 0.3
	Treated-750	25.2 ± 1.5	25.9 ± 1.3	24.3 ± 0.7
Shoot length (cm)	Control-0	35.5 ± 2.2	35.5 ± 2.2 b	35.5 ± 2.2
	Control-750	38.0 ± 1.9	35.5 ± 1.5 b	37.1 ± 4.0
	Treated-0	39.4 ± 2.3	43.0 ± 1.6 a	35.6 ± 1.7
	Treated-750	39.3 ± 0.4	42.2 ± 0.6 a	35.5 ± 1.3
Shoot biomass (%)	Control-0	9.5 ± 0.8	9.5 ± 0.8 b	9.5 ± 0.8
	Control-750	11.0 ± 1.5	13.4 ± 1.6 ab	12.0 ± 1.5
	Treated-0	10.7 ± 1.0	13.2 ± 1.3 ab	12.3 ± 0.6
	Treated-750	9.9 ± 1.0	13.8 ± 0.5 a	12.1 ± 1.5
Water (%)	Control-0	90.5 ± 0.8	90.5 ± 0.8 a	90.5 ± 0.8
	Control-750	89.0 ± 1.6	86.6 ± 1.6 ab	88.0 ± 1.5
	Treated-0	89.3 ± 1.0	86.8 ± 1.3 ab	87.7 ± 0.6
	Treated-750	90.1 ± 1.0	86.2 ± 0.5 b	87.9 ± 1.5

Leaves of these plants showed a significant increase in stomatal conductance (214 %, $p \leq 0.10$, Fig. 3.4B). Stomatal conductance is proportional to water potential in guard cells, but inversely proportional to water content in subsidiary cells (Cowan, 1972). Variations in water potential of guard cell would result in stomatal closure, changing stomatal conductance, consequently, affecting the transpiration rate (Farquhar and Sharkey, 1982). Our results indicate that significantly higher stomatal conductance, and hence transpiration rate was only shown in plants treated with pristine nano-TiO_2, in both cycles (data not shown). A possible mechanism for this effect is that water uptake ability in basil plants was disrupted, which could be attributed to the nano-TiO_2 adhering onto plant roots, partially enlarged root cells and facilitated water uptake (Larue et al., 2011). Larue et al. (2011) reported that increased water flow would be induced by the enlargement of the root cell wall pores. However, other studies reported controversial results about the effects of nano-TiO_2 on water content. Asli et al. (2009) found the mixture of anatase and rutile nano-TiO_2 reduced root pore size, water flow, and root hydraulic potential in corn. Later on, Larue et al. (2012) reported that the evapotranspiration was not affected in wheat and rapeseed.

Several short-term and one generational studies revealed contradictory results. In the spinach study with rutile nano-TiO_2, lower the photosynthetic rate was observed when NPs concentration increased (Zheng et al., 2005). It might be attributed to reactive oxygen species produced by nano-TiO_2, which damage membrane sebaceous structure (Zheng et al., 2005). Photosynthetic rate and intercellular carbon dioxide concentration decreased at varied degrees in anatase nano-TiO_2 treated long raceme elm (Gao et al., 2013). Similar results were reported in a mixture of anatase and rutile nano-TiO_2 study in *Elegant clarkia*; authors hypothesized that energy transportation from photosystem II to Calvin cycle was interrupted (Conway et al., 2015). More studies focus on determining the role of surface property in affecting the photosynthesis functions

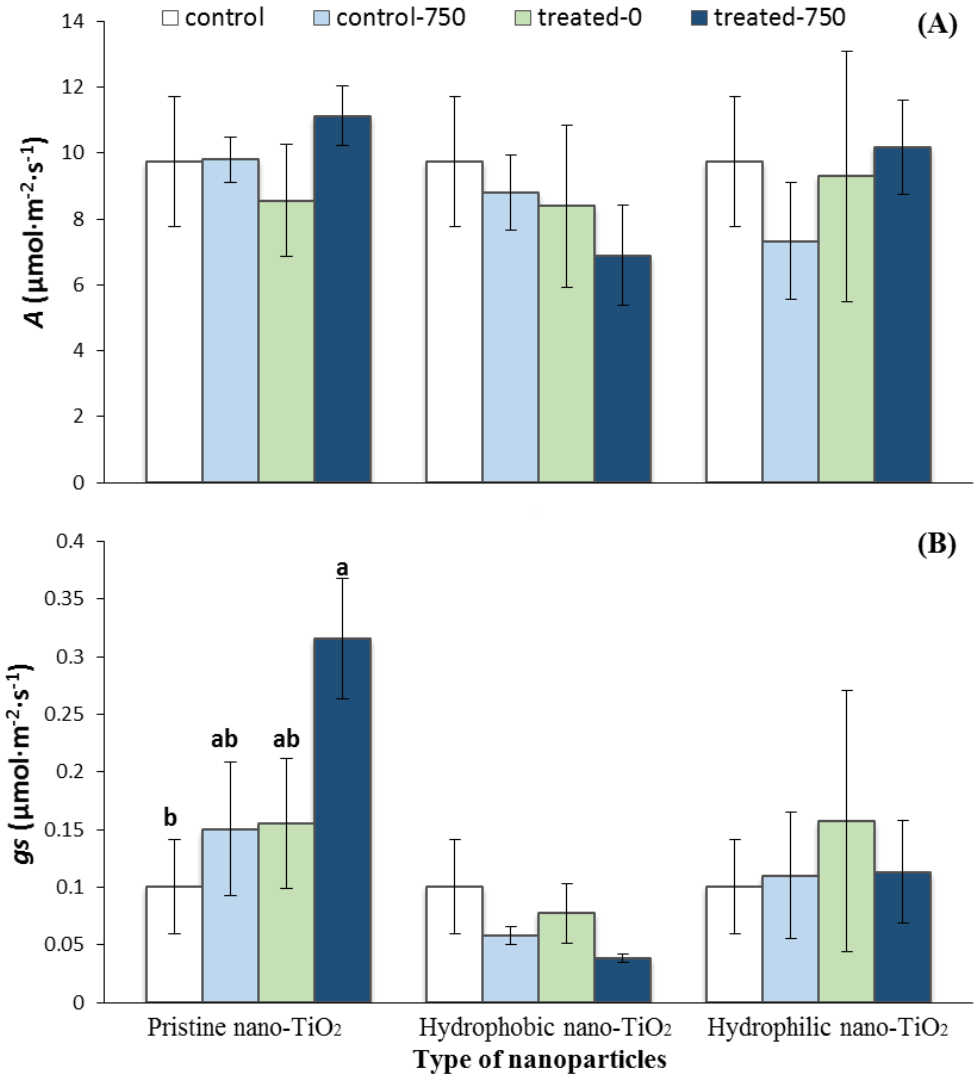


Figure 3.4 Photosynthetic rate (A) and stomatal conductance (B) in 60-day old basil leaves generated from untreated and treated seeds with/without sequential exposure of 750 mg·kg^{-1} of pristine, hydrophobic, and hydrophilic nano-TiO_2, respectively. Each value is mean ± SE (n = 3). Different letters represent statistically significant differences between control and treatments within the same nanoparticles at Duncan's test ($p \leq 0.10$).

of nano-TiO_2 in plant system are urgently needed.

3.3.6 Impacts of nano-TiO_2 on chlorophyll contents

Chlorophyll *a*, chlorophyll *b*, and total chlorophyll contents in leaves of basil plants exposed to the nano-TiO_2 particles are displayed in Fig. 3.5A-C. With respect to control, none of the treatments showed significant differences in chlorophyll *a*, which is the core reactive molecule in both the photosystem I and II (Blankenship, 2013). This suggests these two pathways were not significantly affected. However, hydrophobic nano-TiO_2 significantly reduced chlorophyll *b* in plants exposed in the first cycle, with/without sequential exposure in the second cycle (46% and 40%, respectively), or in plants exposed to the NPs only in the second cycle (42%, Fig. 3.5B). In addition, lower chlorophyll *b* was determined in hydrophilic treated plants in the first cycle only (52%, Fig. 3.5B). A similar trend was observed on total chlorophyll contents. Significantly lower total chlorophyll content was determined in untreated plants exposed to hydrophobic nano-TiO_2 in the second cycle (21%), and hydrophobic nano-TiO_2 treated in the first cycle, with/without sequential exposure in the second cycle (22%, and 24%, respectively). Also, a significant reduction in total chlorophyll was found in hydrophilic treated seeds in the first cycle only (30%, Fig. 5C). Chlorophyll *b* is more soluble in water than chlorophyll *a* (Raven et al., 2005). Then, we assumed the significantly lower water content in hydrophobic nano-TiO_2 treatments (Table 3.2) could cause the reductions in chlorophyll *b*. Chlorophyll *b* helps to extend the range of light wavelength and increase light amount to plants in photosynthesis (Blankenship, 2013). We assumed that the deficiency in chlorophyll *b* could result in negative effects in photosynthesis.

In other studies, significant reductions of chlorophyll content and lower membrane permeability of chloroplasts were induced in the rutile nano-TiO_2 treated spinach studies (Hong et al., 2005; Zheng et al., 2005). On the contrary, in the nano and bulk TiO_2 treated spinach study,

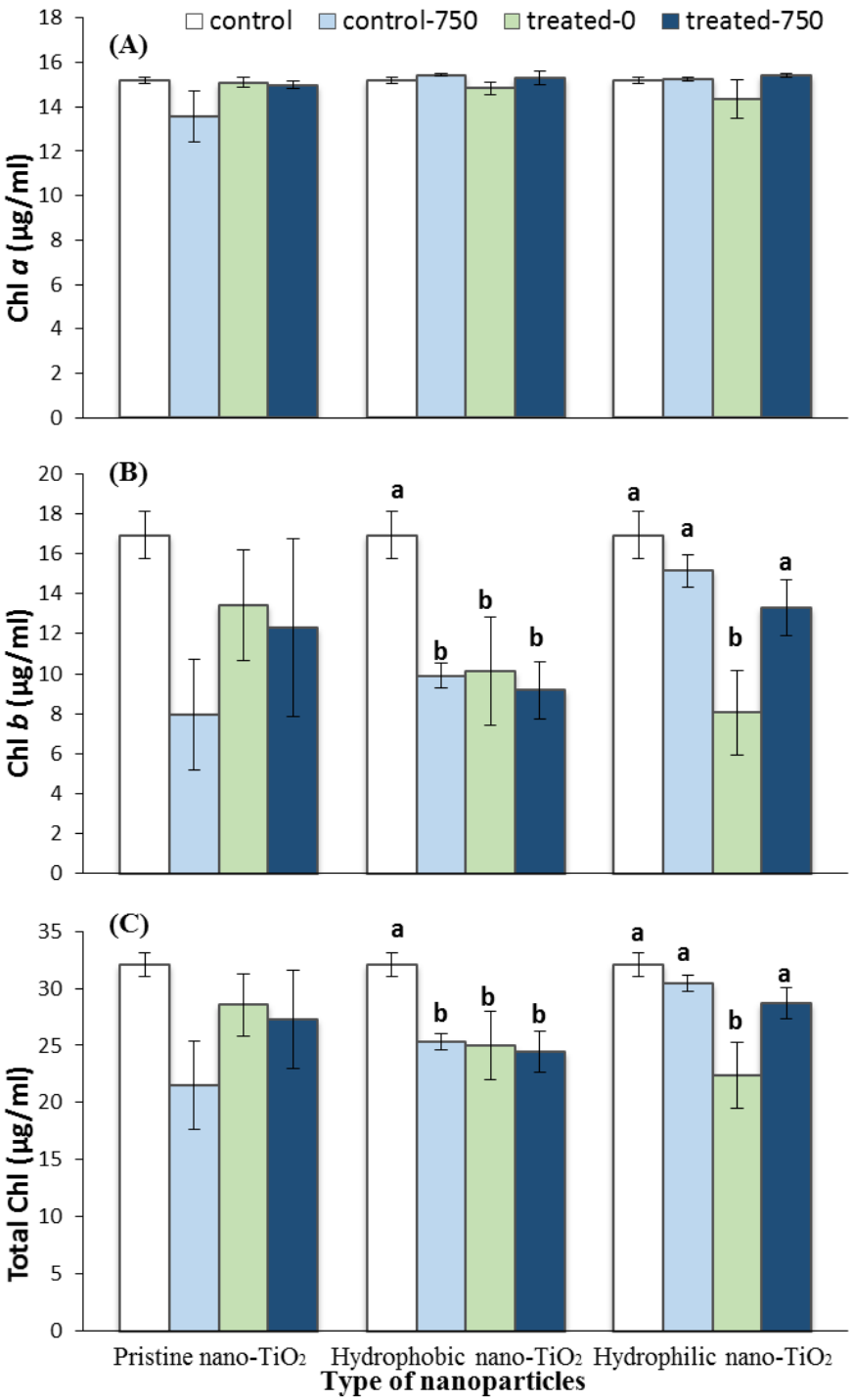


Figure 3.5 Chlorophyll *a* (A), chlorophyll *b* (B), and total chlorophyll (C) contents in 60-day old basil leaves generated from untreated and treated seeds with/without sequential exposure of 750 $mg \cdot kg^{-1}$ of pristine, hydrophobic, and hydrophilic nano-TiO_2, respectively. Each value is mean ± SE (n = 3). Different letters represent statistically significant differences between control and treatments within the same nanoparticles at Duncan's test ($p \leq 0.10$).

chlorophyll content was 37.48% and 13.34% higher than control (Yang et al., 2007). Similar results were shown in the study of cucumber (Servin et al., 2013) and soybean (Rezaei et al., 2015). More studies are needed to explore the mechanism of how nano-TiO_2 affect photosynthesis procedure in plant system, not only in successive generations but also focus on surface chemistry.

3.3.7 Impacts of nano-TiO_2 on carbohydrates

Carbohydrates (sugar, starch, and fiber) are the most abundant structural components for plant systems (Solomons and Fryhle, 2011). Total sugar, reducing sugar, and starch contents in leaves of basil plants are displayed in Fig. 3.6A-C. The comparison was made between control and treatments within the same nanoparticles. As can be seen in Fig. 3.6A, only hydrophilic nano-TiO_2 significantly affected total sugar production. With respect to control, hydrophilic treated plants in the first cycle, sequentially exposed to hydrophilic nano-TiO_2 in the second cycle, resulted in a significant increase on total sugar (80%, $p \leq 0.10$). For reducing sugar, compared to plants with no NP exposure in both cycles, exposed to hydrophobic nano-TiO_2 in the second cycle only, and plants sequentially exposed to hydrophobic nano-TiO_2 showed significantly increased reducing sugar (186% and 139%, respectively, $p \leq 0.10$, Fig. 3.6B). Furthermore, hydrophilic nano-TiO_2 treated plants only in the first cycle resulted in 145% significantly higher reducing sugar. In addition, no significant difference was shown in starch (Fig. 3.6C).

We found that reductions in chlorophyll *b* and total chlorophyll contents observed from hydrophobic and hydrophilic treatments, surprisingly caused higher total sugar and reducing sugar content in those NPs treatments. It has been reported that reduced chlorophyll *b* content corresponded to increased soluble sugars in corn (Khodary, 2004). Sugar and starch are the most important end products of photosynthesis in plant, and eventually sugar would convert into starch

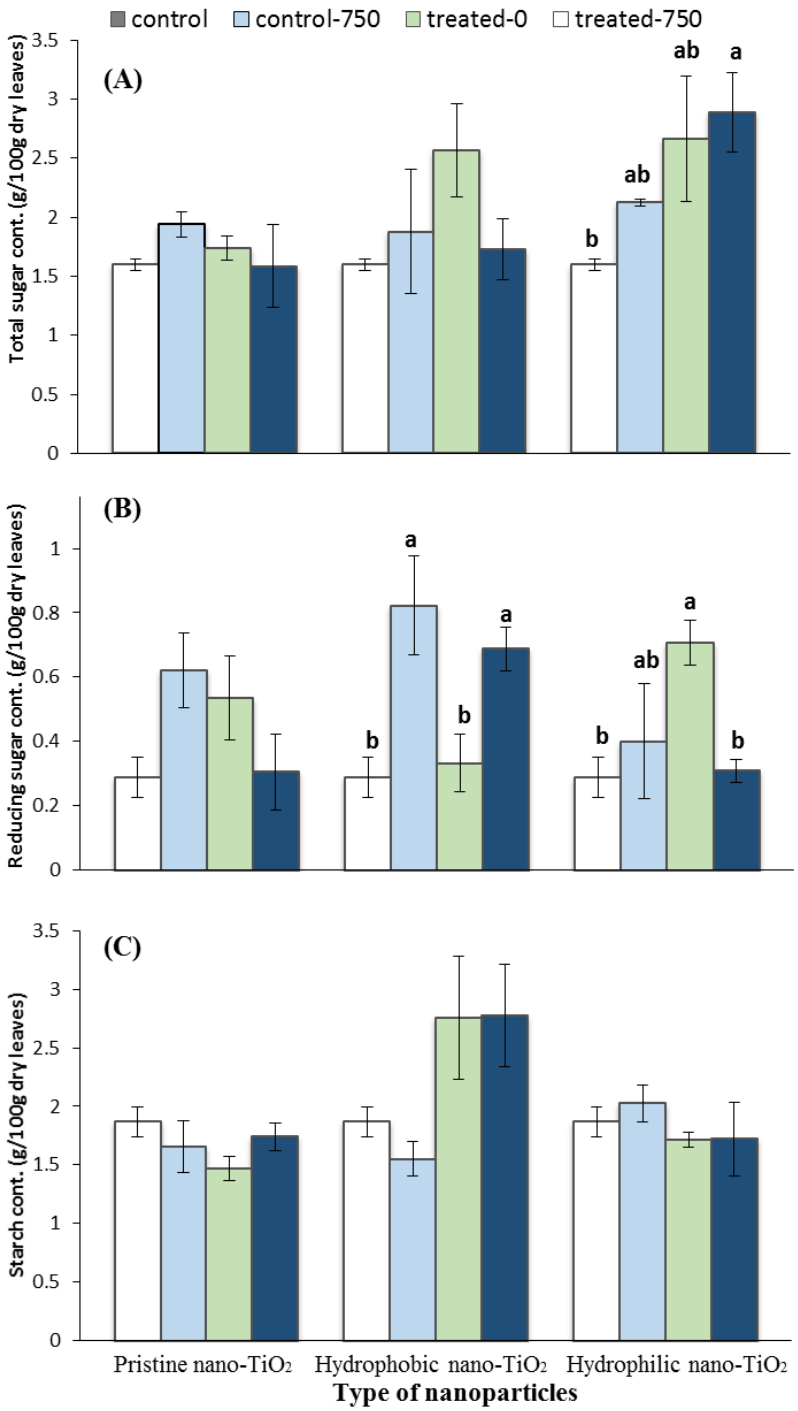


Figure 3.6 Total sugar (A), reducing sugar (B), and starch (C) contents in 65-day old basil leaves generated from untreated and treated seeds with/without sequential exposure of 750 mg·kg^{-1} of pristine, hydrophobic, and hydrophilic nano-TiO_2, respectively. Each value is mean ± SE (n = 3). Different letters represent statistically significant differences between control and treatments within the same nanoparticles at Duncan's test ($p \leq 0.10$).

in plant cells as energy storage (Blankenship, 2013). We assumed that hydrophobic and hydrophilic nano-TiO_2 disturbed the metabolic consumption of sugars to the starch transformations (Khodary, 2004). Active electrons transfer caused by nano-TiO_2 have capabilities to disrupt enzyme activities related to glycometabolism, such as GWD, BAM3, and AMY3.[78] It has been reported upregulation of BAM3, AMY3, or GWD mutants with higher sugar contents but lower starch (Yano et al., 2005; Kaplan and Guy, 2005; Thalmann et al., 2016). Whereas, the mechanism for carbohydrates disruption by NPs is still largely unknown. The glycometabolism is related to various vital processes, such as photosynthetic reaction, transpiration, and metabolism. Multiple biomolecules, such as enzymes, proteins, fatty acids, ribose, deoxy-ribose, and nucleic acid are involved in such processes (Dey and Harborne, 1997).

3.4 Conclusion

This two generations study showed that the long-term exposure to nano-TiO_2 with different surface coatings caused several alterations in Ti absorption, plant growth, photosynthesis, and nutrient accumulation in basil plants. After exposure for two consecutive cultivation cycles, none of the three nano-TiO_2 particles affected the Ti translocation to shoots, germination rate, root length, chlorophyll *a*, and starch content. Pristine and hydrophobic nano-TiO_2 resulted in a similar amount of Ti in roots of plants sequentially exposed to nano-TiO_2 and plants exposed to the corresponding particle only in the second cycle. Differently, sequential exposure to hydrophilic particles showed lower Ti in roots, compared to exposure only in the second cycle. Exposure to pristine particles in two cycles resulted in enhancement of gas exchange. On the other hand, plants exposed to hydrophobic particles in two cycles, significantly increased the length and biomass of shoots, but had less chlorophyll *b* and total chlorophyll contents. Furthermore, plants exposed to hydrophilic particles, only in the first cycle, showed lower chlorophyll *b* and total chlorophyll

contents, while exposure for two cycles resulted in higher total sugar content. These findings suggest important transgenerational impacts and alterations in basil plants under exposure to nano-TiO_2 with different surface properties. We believe these results provide insights into the understanding of long-term effects of nanomaterials in living organisms.

Chapter 4: Foliar Exposure of $Cu(OH)_2$ Nanopesticide to Basil (*Ocimum basilicum*): A Variety-dependent Copper Translocation and Biochemical Responses

Abstract

In this study, low and high anthocyanin basil (*Ocimum basilicum*) varieties (LAV and HAV) were sprayed with 4.8 mg Cu/per pot from $Cu(OH)_2$ nanowires, $Cu(OH)_2$ bulk (CuPro), or $CuSO_4$ and cultivated for 45 days. In both varieties, significantly higher Cu was determined in leaves of $CuSO_4$ exposed plants (691 and 672.6 mg/kg for LAV and HAV, respectively); however, only in roots of HAV, Cu was higher, compared to control ($p \leq 0.05$). Nanowires increased n-decanoic, dodecanoic, octanoic, and nonanoic acids in LAV, but reduced n-decanoic, dodecanoic, octanoic, and tetradecanoic acids in HAV, compared with control. In HAV, all compounds reduced eugenol (87%), 2-methylundecanal (71%), and anthocyanin (3%) ($p \leq 0.05$). In addition, in all plant tissues, of both varieties, nanowires and $CuSO_4$ reduced Mn, while CuPro increased chlorophyll contents, compared with controls ($p \leq 0.05$). Results suggest that the effects of $Cu(OH)_2$ pesticides are variety-and-compound-dependent.

Keyword: Basil, Varieties, $Cu(OH)_2$, Nanopesticide, Fatty acids, Essential oils, Anthocyanin

4.1 Introduction

Farmers have always fought against different pests to obtain high quality comestible products. Foliar spray of inorganic pesticides has been one of the most effective approaches to suppress crop damages (Servin et al., 2015; Zhao et al., 2017).[1,2] For example, European farmers have controlled fungi diseases for more than 100 years by spraying Bordeaux mixture [$Ca(OH)_2$ + $CuSO_4$].[3] However, the systematic application of Bordeaux has been linked to heavy metal pollution in European vineyard soils (297 mg/kg Cu in Italy wet plains).[4] Thus, alternative $Cu(OH)_2$ formulations have been developed to supplant Bordeaux mixture. For example, CuPro@2005, a $Cu(OH)_2$ formulation that contains 53.8% of Cu plus O, Na, Al, and Si as inert ingredients, is one the most commonly used Cu compounds to suppress gray leaf mold, leaf spots, and bacterial diseases.[5,6] In addition, $Cu(OH)_2$ nanowires are advertised as an alternative to Bordeaux mixture.[7] However, it is of great urgency to investigate the fate and effects of these products in plants and other organism.[6]

Different metabolic responses towards $Cu(OH)_2$ have been shown in several plants including lettuce,[8,9] cucumber,[10] corn,[2] and spinach.[12] In addition, excess copper has shown to affect the growth of radish[13] and Indian mustard,[14] protein content in eggplant,[15] secondary metabolites in oil palm,[16] chlorophyll content in kidney bean,[17] and gene expression in cucumber,[10] among others. Reports also mention that $Cu(OH)_2$ increases K concentration and polyamine in lettuce shoots[8,9] and upregulate antioxidant and detoxification-related genes.[10] The data suggest that the responses to Cu-based products are species-dependent.[5]

Different cultivars of plants have shown varied responses when exposed to nanomaterials or other abiotic stressors, such as salinity,[18] organic acids,[19] heavy metals,[20] or nanomaterials.[21] For example, Barbieri et al.[22] reported that salt stress affected in different way the relative

abundance of essential oil in basil cultivars. At 200 mM NaCl, more eugenol was determined in Genovese Gigante variety but not in Napolitano variety. In a full-life cycle study with CeO_2 nanoparticles, Rico et al.[21] found that rice with high amylose resulted in higher concentration of tetradecanoic acid, while rice with low amylose yielded higher pentanoic acid, compared with untreated grains. In addition, rice with medium amylose had lower contents of dodecanoic, hexadecanoic, oleic, and pentanoic acids. However, knowledge regarding the effects of $Cu(OH)_2$ nanopesticide in basil is still unknown.

There are more than 30 varieties of basil with different descriptors, physiological, and biochemical markers.[23] Basil contains essential oils that are popular in cosmetics, food industry, and medical treatments.[24] Eugenol and 2-methylundecanal are the most important essential oils, with ample application in anesthesia, perfumes, and hygiene products.[25, 26, 27] In addition, among all the culinary herbs, basil provides the highest content of omega-3 fatty acids,[28] which are polyunsaturated fatty acids (such as linolenic acid) essential in human metabolism. Other fatty acids are essential for plant cellular membrane fluidity and integrity.[29, 30] In the present study, we exposed $Cu(OH)_2$ nanowires, bulk CuPro@2005, and $CuSO_4$ (as ionic counterpart) to dark opal (Purpurascens, higher anthocyanin content, HAV) and dulce (Albahaca Dulce, lower anthocyanin content, LAV) basil verities and determined copper accumulation in tissues and the effects on pigments (chlorophyll and anthocyanin) and metabolite (fatty acid and essential oils) contents. The objectives of this study were to investigate whether the response of basil to these copper-based agricultural products are size- or variety-dependent. Based on the previously reported information, it is hypothesized that the copper products used will affect in different ways the essential characteristics of basil varieties. Spectroscopic (ICP-OES and GC-MS) and colorimetric (UV-vis) techniques were used to determine the effects of the treatments.

4.2 Materials and methods

4.2.1 Copper-based nanopesticide

The characterizations of CuPro@2005 and $Cu(OH)_2$ nanowires were provided by Hong et al.[5] and US Research Nanomaterials, respectively (Table 4.1 and SI Figure 3.1). Due to the morphology and size, CuPro@2005 is considered bulk material.[5] Besides the functional compound (53.8% of Cu), CuPro@2005 contains O, Na, Al, and Si as inert ingredients (46.2 %). To evaluate the effects of copper dissolution, $CuSO_4$ was selected as ionic counterpart. Suspensions/solutions containing 480 mg/L of Cu (1 pounds per acre) were prepared using $CuSO_4$, $Cu(OH)_2$ nanowires, and CuPro, following the manufacturer's instruction.[31] The dissolution analyses of $Cu(OH)_2$ nanowires and CuPro were conducted and the data are provided in the supporting information (SI Figure 3.2).

Table 4.1 Physical characterizations of copper compounds exposed to basil (*Ocimum basilicum*).

Properties	Characterization technique	CuPro@2005 (Hong et al., 2015)	$Cu(OH)_2$ nanowires
Size (nm)	TEM	10^4 -10^6	Diameter: 50 nm Length: 3-5 μm
Morphology	TEM	Spherical	Elongated
Hydrodynamic size (nm)	DLS	4779 ± 4767	2057 ± 154
Zeta potential in DI water (mV)	DLS	-47.8 ± 1.1	24.1 ± 0.32
Cu content (wt. %)		34.0	65.1

4.2.2 Seed germination and cultivation conditions

Two basil (*Ocimum basilicum*) verities dark opal 'Purpurascens' (high anthocyanin, HAV) and dulce 'Albahaca Dulce' (low anthocyanin, LAV) were used in this study. The seeds were

germinated in a starter germination kit (9GreenBox) and 20-day old seedlings were transplanted into plastic pots (20 cm × 17.5 cm × 15 cm) containing 1 kg of potting mix soil, two plants per pot. The soil analysis was previously reported by Barrios et al.[32] Plants were placed in a growth chamber (Environmental Growth Chamber, Chagrin Falls, OH) with 65% relative humidity, 25/20 °C day/night, 340 μmole m^{-2} s^{-1} light intensity, and 14 h photoperiod. Plants were provided with 100 mL Millipore water (MPW) every day and no additional nutrient solution or fertilizer was used. Two days after transplant, two mL of $Cu(OH)_2$ nanowires, CuPro@2005, or $CuSO_4$ stock solutions/suspensions were sprayed five times, every three days (a total of 10 mL), following the manufacturer's instructions, to give a total amount of 4.8 mg copper per pot.[31] Not covered soil was used to mimic field conditions. To evaluate the possible effects of Cu deposition in soil through the applications, three replicates of each treatment were covered or uncovered and the soil content determined. Three replicates with no copper exposure were set as controls. Plants were harvested at pruning stage (45 days) and the roots carefully removed with soil to avoid damaging plant tissues. Samples of roots, stems and leaves were sectioned and thoroughly washed with 0.01 M HNO_3 and MPW for six times, alternatively, to remove the nanoparticles adhered on the surface for further analysis.

4.2.3 Chlorophyll and total anthocyanin content analysis

For the chlorophyll determination, 200 mg of fresh chopped leaves per replicate were exposed to chilled acetone untill all the pigment was extracted. Absorbance of the supertants were recorded at 645, 655 and 664 nm, respectively. Chlorophyll *a*, chlorophyll *b*, and total chlorophyll contents were calculated according to Porra.[33] Total anthocyanin was extracted as per by Deubert et al.[34] Briefly, 100 mg of chopped basil leaves were blended with 2 mL of ethanol 95%-1.5 M HCl (85:15, *v/v*) and the supernatants read at 525 nm using UV/vis, as desscribed by Baublis et

al.[35] The total anthocyanin concentration was calculated based on pelargonidin-3-gucoside, following the equation c (mg/L) = (E × molecular weight of pelargonidin-3-gucoside × dilution factor × 1000)/ε and a molar absorptivity (ε) of 26,900 L mol^{-1} cm^{-1}.[36]

4.2.4 Determination of copper translocation

For absorption and translocation of Cu, samples of roots, stems, and leaves were dried at 60 ℃ for 72 h. The dried tissues were digested following the U.S. Environmental Protection Agency (EPA) protocol 3051 on the hot block by adding 4 mL plasma pure HNO_3 (65%) in plastic vessels. Copper concentrations, and nutritional elements (B, Fe, Mo, Mn, Ni, Se, Zn, Ca, K, Mg, P, and S) were determined by inductively coupled plasma-optical emission spectroscopy (ICP-OES). Blank, reference material (NIST-SRF 1570a and 1547, Metuchen, NJ), and spiked samples were used as calibration and QC/QA controls. The recovery rate of Cu is 93.9 ± 6.0%.

4.2.5 Essential oils and fatty acids determination

The extraction and methyl esterification of fatty acids in fresh basil leaves was performed according to Browse et al.[37] Essential oils and fatty acids in leaf extracts were determined using a gas chromatography- mass spectrometer (GC-MS) as described by Rico et al.[38] Briefly, a 6890N GC containing a HP-5MS column (30 m × 0.25 m × 0.25 μm) and 5973 mass selective detector (Agilent Technology, Santa Clara, CA, USA) were used for separation and detection, respectively. The empty thermal desorption tubes were spiked with samples of 5 μL and subjected to thermal desorption via a GERSTEL Twister TM Desorption Unit (TDU) with a CIS 4 cryo-injector (Gerstel, INC., Baltimore, MD, USA) in splitless mode. Quantification of essential oil and fatty acid compounds was made from peak area using the unique ion as default.

4.2.6 Statistical analyses

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

4.3 Results and discussions

4.3.1 Copper accumulation in basil tissues

Concentrations of copper in roots, stems, and leaves of LAV and HAV are shown in Figure 4.1(A-B). In LAV (Figure 4.1A), significantly higher copper accumulation was observed in leaves exposed to $CuSO_4$ (691.1 mg/kg), followed by $Cu(OH)_2$ nanowires (265.4 mg/kg), and CuPro (145.2 mg/kg, $p \leq 0.05$). However, only stems from plants exposed to $CuSO_4$ resulted in significantly higher Cu concentration, compared to controls (185.5 mg/kg, $p \leq 0.05$). Neither $Cu(OH)_2$ nanowires nor CuPro resulted in statistically different copper translocations to stems and roots, compared to controls. On the other hand, in HAV (Figure 4.1B), significantly higher copper accumulation was determined in leaves treated with $CuSO_4$ (672.6 mg/kg), $Cu(OH)_2$ nanowires (387.7 mg/kg), and CuPro (53.62 mg/kg). $CuSO_4$ and $Cu(OH)_2$ nanowires resulted in significantly higher copper concentrations to stems (776% and 221%, respectively), compared with controls. Different from LAV, all copper compounds showed significantly higher Cu translocation from shoots to roots ($CuSO_4$, 80%; $Cu(OH)_2$ nanowires, 133%; CuPro, 128%), compared to control. Although there was no difference in soil copper concentration between uncovered and covered

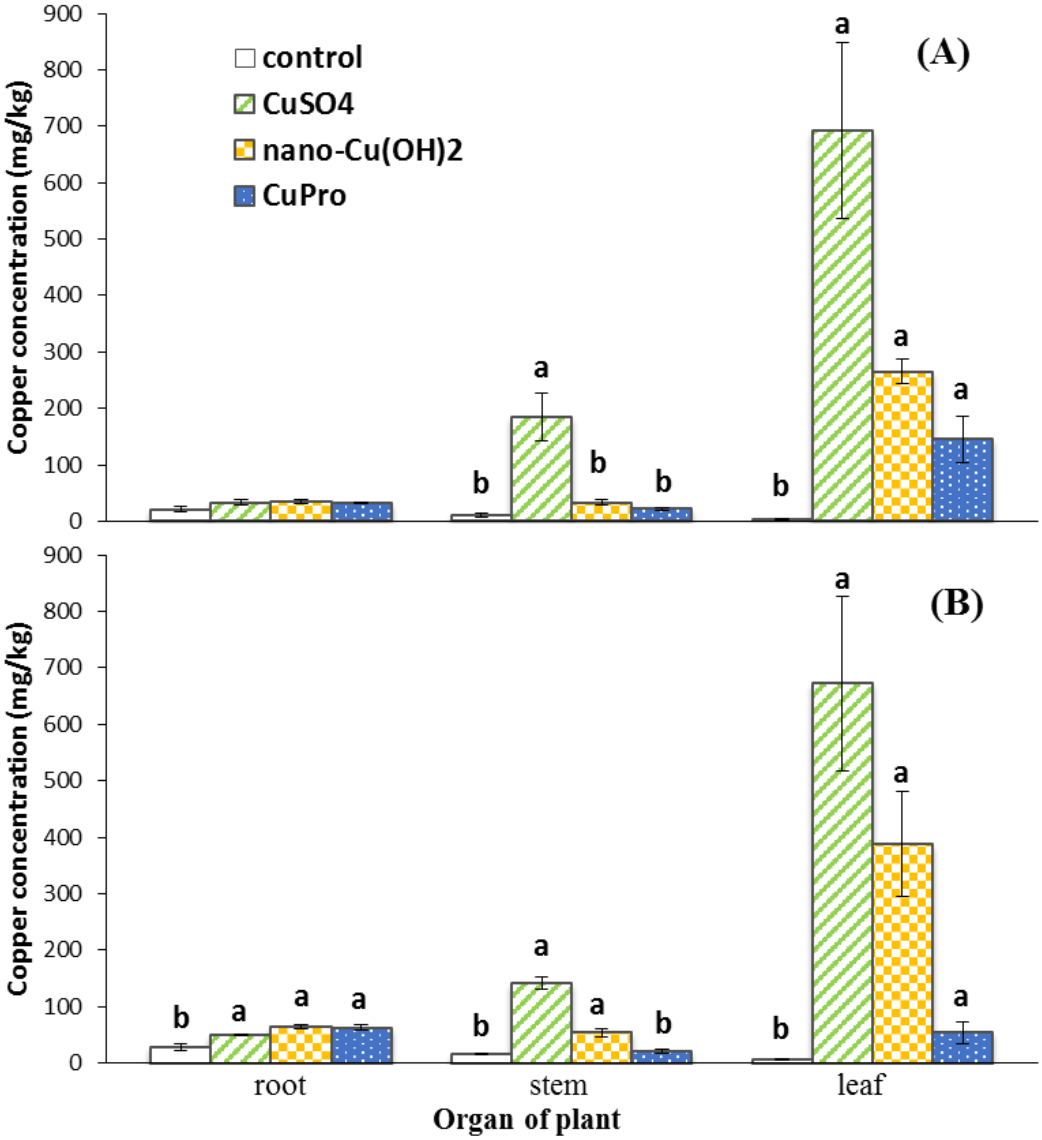


Figure 4.1 Copper concentrations in roots (green column), stems (yellow column), and leaves (blue column) of LAV (A) and HAV (B) basils with foliar exposure of 4.8 mg Cu/per pot from $CuSO_4$, $Cu(OH)_2$ nanowires, and CuPro for 2 weeks. Each value is mean ± SE of three replicates. Means with different letters stand for significant difference compared with controls ($p \leq 0.05$).

pots (SI Figure 3.3-3.4), it is possible that part of the Cu found in roots correspond to the copper fallen from the application. However, the low Cu amount applied to the shoots (total of 4.8 mg) does not seem high enough to cause the difference.

Due to the Cu^{2+} ionization, it is not surprising that the ionic counterpart resulted in highest copper concentration in leaves or stems of both varieties. The fact that plants treated with $Cu(OH)_2$ nanowires had higher copper concentration than CuPro, regardless the variety or organ (Figure 4.1), could be attributed to the smaller hydrodynamic size (2057 ± 154 nm *vs* 4779 ± 4767 nm, Table 4.1) and smaller size (diameter, 50 nm and length, 3-5 μm *vs* diameter, 10^4 -10^6 nm) of the nanowires. For all the copper compounds, the high copper accumulation in leaves in both species could be explained by three major reasons. First, ions or particles smaller than stomatal diameter (8-12 μm)[39] are very likely to enter the plant guard cell. Thus, in this case, $Cu(OH)_2$ nanowires had greater possibilities to enter leaf stomata than CuPro. Second, the released Cu^{2+} ions diffuse through cuticle and stomatal subsequently, they were transported through phloem from leaves to other tissues.[40] In an 18 day dissolution analysis at pH 7, with a suspension/solution of 480 mg/L, CuPro showed higher Cu release than $Cu(OH)_2$ nanowires (SI Figure 3.2). The copper concentrations (mg/L) in the supernatants at day 7, 15, and 18 were: 383.8 ± 6.04 *vs* 13.9 ± 1.53; 383.1 ± 33.6 *vs* 11.6 ± 1.88, and 429.0 ± 40.7 *vs* 11.0 ± 1.75 mg/L). Vencalek et al.[41] reported that Kocide 3000, a $Cu(OH)_2$ compound, released over 90% in DI water within 8 h. Since the nanowires have lower dissolution, due to their small size, it is likely that copper translocated into stem and roots as Cu ions and in the nano-form. Third, even though the plant tissues were thoroughly washed six times, alternatively with MPW and acid, Cu^{2+} ions and particles could still be persistent and attached onto plant leaves. To explain the variety-dependent copper translocation, we speculate it might be due to the different amount and types of leaf exudates in both species. In plants, the

anthocyanin pigments are restricted in an acidic environment.[42] The leaf cells of HAV basil are more acidic than LAV basil;[42] this suggests the acidic leaf environment could facilitate the dissolution of the copper compounds, resulting in higher copper translocation to stems and roots of Purpurascens, the high anthocyanin variety.

Previous studies demonstrated that the translocation of copper from $Cu(OH)_2$ in plants is species-dependent.[5] In soil exposure studies, significantly higher copper concentration was determined in both lettuce roots and shoots,[5] as well as in alfalfa shoots[5] and cilantro shoots,[11] indicating root-to-shoot translocation. In foliar exposure studies, copper was translocated from lettuce shoots to roots,[8] from spinach shoots to roots,[12] but not into cucumber roots.[10] These diverse results revealed that the shoot-to-root translocation of $Cu(OH)_2$ agricultural products depends on plant species and variety.

4.3.2 Effects of treatments on nutritional element content

As many other nutritional herbs, basil provides nutritional elements for human beings. The literature shows that ENMs have capabilities to alter the uptake of micro- and macro-elements by disrupting the metabolism[8] or gene expression[10] in plants. The literate has shown that these effects are associated with the growing media, the ENM, and plant species. In organic matter-enriched soil, bean exposed to hydrophobically-coated nano-ZnO and $ZnCl_2$ resulted in higher Zn, K, S, P, Mg, Ca, Fe, and Mn accumulation, compared to the seeds collected from natural soil.[43] Majumdar et al.[44] reported that after exposure of nano-CeO_2 to kidney bean, lower Mg, Fe, Zn and higher S contents were determined in both low organic matter soil (LOMS) and organic matter enriched soil (OMES). Whereas, less Ca and Cu concentrations were measured in LOMS, and reduced K and Al concentrations were found in OMES.

In basil, from all the nutrient elements analyzed, only Mn showed significant differences, compared with control. Lower Mn concentrations were found in plants exposed to $CuSO_4$ and $Cu(OH)_2$ nanowires in both basil varieties (Table 4.2). In LAV, Mn concentrations in plants exposed to $CuSO_4$ and $Cu(OH)_2$ nanowires were reduced by 65% and 68% in leaves, 74% and 75% in stems, and 85% and 84% in roots, respectively, compared with control ($p \leq 0.05$). In HAV, Mn concentrations in plants exposed to $CuSO_4$ and $Cu(OH)_2$ nanowires were significantly decreased by 82% and 79% in leaves and 78% and 75% in stems ($p \leq 0.05$).

The data regarding the reduction of Mn in plants exposed to Cu-based products is in some way consistent. Hong et al. reported a reduction of Mn in leaves of cucumber foliarly exposed to nano- and bulk CuO.[45] In a root exposure study with Cu/CuO NPs, with a core-shell structure, significantly lower Mn accumulation was detected in lettuce leaves.[46] In a study with beans cultivated in soil treated with CuO:ZnO mixture and nano-CuO: *Pc*O6 (a root bacterium, *Pseudomonas chlororaphis* O6) mixture, lower Mn was found in shoots (Dimkpa et al.)[47] Le Van et al.[48] found less Mn in shoots but more Mn in roots of conventional and transgenic cotton exposed to nano-CuO. Perhaps the difference was due to genetic material of transgenic plants, or to a specific condition of cotton. It is possible that the gene expressions of the Mn transporters, such as Nramp5[49] and IRT1[50], were interrupted by $CuSO_4$ and $Cu(OH)_2$ nanowires.

4.3.3 Effects of copper compounds on essential oils production

Concentrations of essential oils (eugenol and 2-methylundecanal) in leaves of LAV and HAV are shown in Figure 4.2(A-D). In LAV, no significant differences in the relative abundance of eugenol and 2-methylundecanal were observed. However, in HAV, significant decrease of these two essential oils were obtained under exposure to $CuSO_4$ (2-methylundecanal, 61%), $Cu(OH)_2$ nanowires (eugenol, 57%; 2-methylundecanal, 71%), and CuPro (eugenol, 87%; 2-

Table 4.2 Manganese concentrations in roots, stems, and leaves of LAV and HAV basils with foliar exposure of 4.8 mg Cu/per pot from CuSO4, CuPro, and $Cu(OH)_2$ nanowires for 2 weeks. Each value is mean ± SE of three replicates. Means with different letters stand for significant difference compared with controls ($p \leq 0.05$). The units of concentrations shown in are mg/L.

	LAV basil			HAV basil		
Compound	Root	Stem	Leaf	Root	Stem	Leaf
Control	114.02 ± 2.94 a	45.65 ± 3.36 a	70.37 ± 3.41 a	119.2 ± 13.3	67.92 ± 5.87 a	108.6 ± 26.0 a
$CuSO_4$	17.00 ± 5.64 b	11.63 ± 2.23 b	24.28 ± 2.01 b	86.09 ± 7.34	15.13 ± 1.22 b	19.88 ± 0.416 b
Nano-$Cu(OH)_2$	18.76 ± 5.11 b	11.33 ± 2.70 b	22.45 ± 1.13 b	94.66 ± 6.07	17.3 ± 1.64 b	23.03 ± 1.76 b
CuPro	137.11 ± 12.08 a	38.20 ± 7.24 a	82.2 ± 19.2 a	119.4 ± 2.05	92.92 ± 15.7 a	110.1 ± 1.92 a

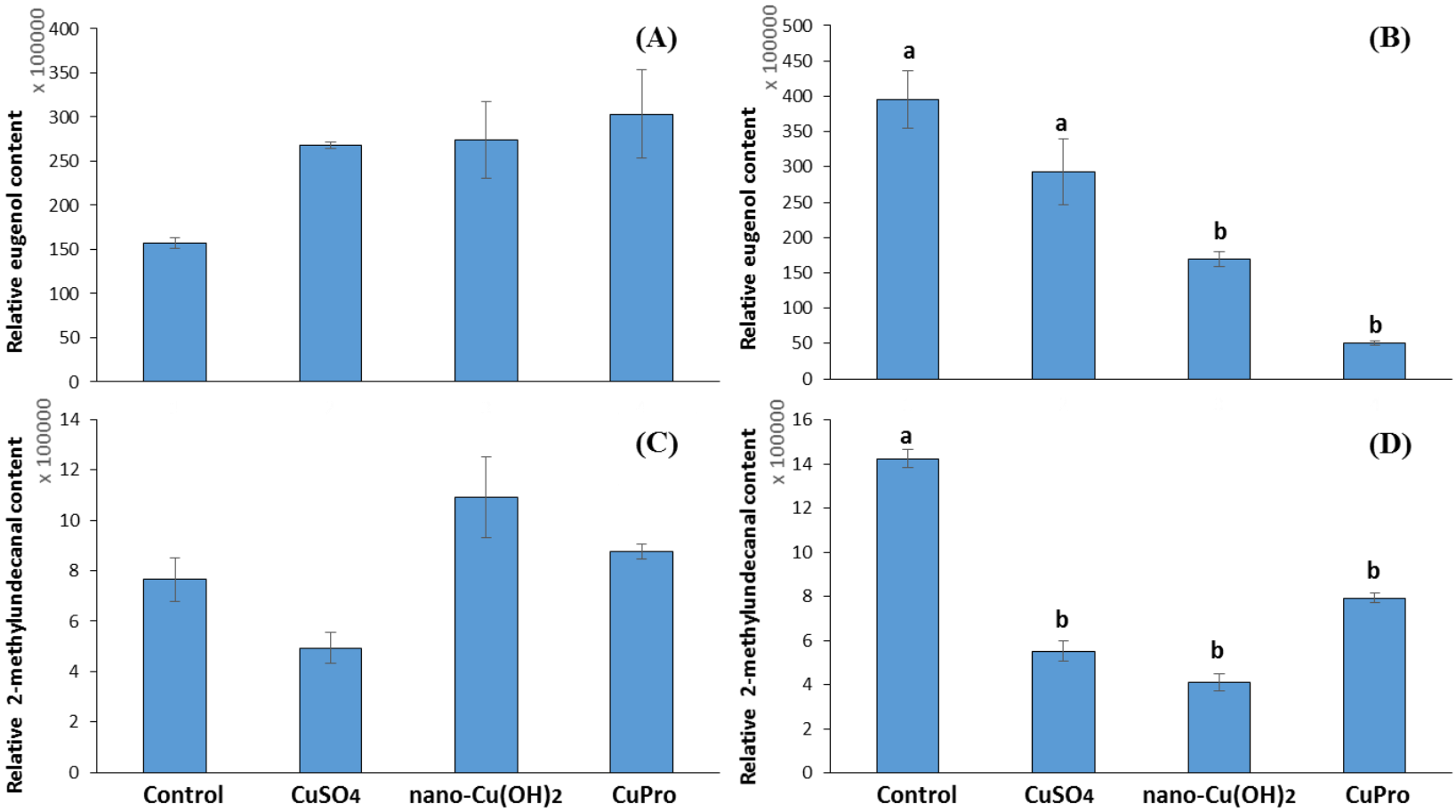


Figure 4.2 Relative abundance of essential oils in leaves of LAV (A, eugenol; C, 2-methylundecanal) and HAV (B, eugenol; D, 2-methylundecanal) basils with foliar exposure of 4.8 mg Cu/per pot from $CuSO_4$, $Cu(OH)_2$ nanowires, and CuPro for 2 weeks. Each value is mean ± SE of three replicates. Means with different letters stand for significant difference compared with controls ($p \leq 0.05$).

methylundecanal, 43%). Hojati et al.[51] reported that excess copper in roots of feverfew flower (*Tanacetum parthenium*) resulted in significantly lower essential oil contents. There was higher linalool and α-terpineol content, but lower β-caryophyllene content in broad leaf Italian basil treated with excess copper.[52] In another root exposure study, Broad leaf Italian basil treated with the combination of copper, lead, and cadmium resulted in lower essential oil content (14% in dry leaves).[53] In a foliar study with shell ginger, $CuSO_4$ increased volatile components including 1,8-cineol, camphor, borneol, and terpinene-4-ol.[54] These studies indicate that the response to copper exposure is species- or variety-dependent.[22] Our results suggest that anthocyanin has a role in the tolerance to copper toxicity.

4.3.4 Effects of copper compounds on fatty acids

The relative abundances of 11 fatty acids in leaves of LAV and HAV basils are shown in Figure 4.3-4.4. The relative abundances of eight saturated fatty acids (both medium-chain and long-chain fatty acids) were significantly increased or decreased by $CuSO_4$, $Cu(OH)_2$ nanowires, and CuPro in LAV (Figure 4.3), compared with control. In this variety, $CuSO_4$ resulted in significantly higher n-hexadecanoic (173%), dodecanoic (48%), and eicosanoid acids (31%, $p \leq 0.05$). $Cu(OH)_2$ nanowires resulted in significantly higher n-decanoic (69%), octanoic (61%), dodecanoic (198%), and nonanoic acids (90%, $p \leq 0.05$). CuPro significantly increased n-decanoic (113%), n-hexadecanoic (137%), dodecanoicd (107%), and tetradecanoic acids (112%), but decreased eicosanoic (56%) and octadecanoic acids (47%, $p \leq 0.05$, Figure 4.5). This indicates that CuPro had negative effects in the biosynthesis of saturated fatty acids with longer-chains (C $\geq$ 18) in LAV. On the other hand, in HAV, the relative abundances of six saturated fatty acids (both medium-chain and long-chain) were significantly decreased by $CuSO_4$, $Cu(OH)_2$ nanowires, and CuPro (Figure 4.4 and 4.6). $CuSO_4$ and $Cu(OH)_2$ nanowires showed significantly lower n-

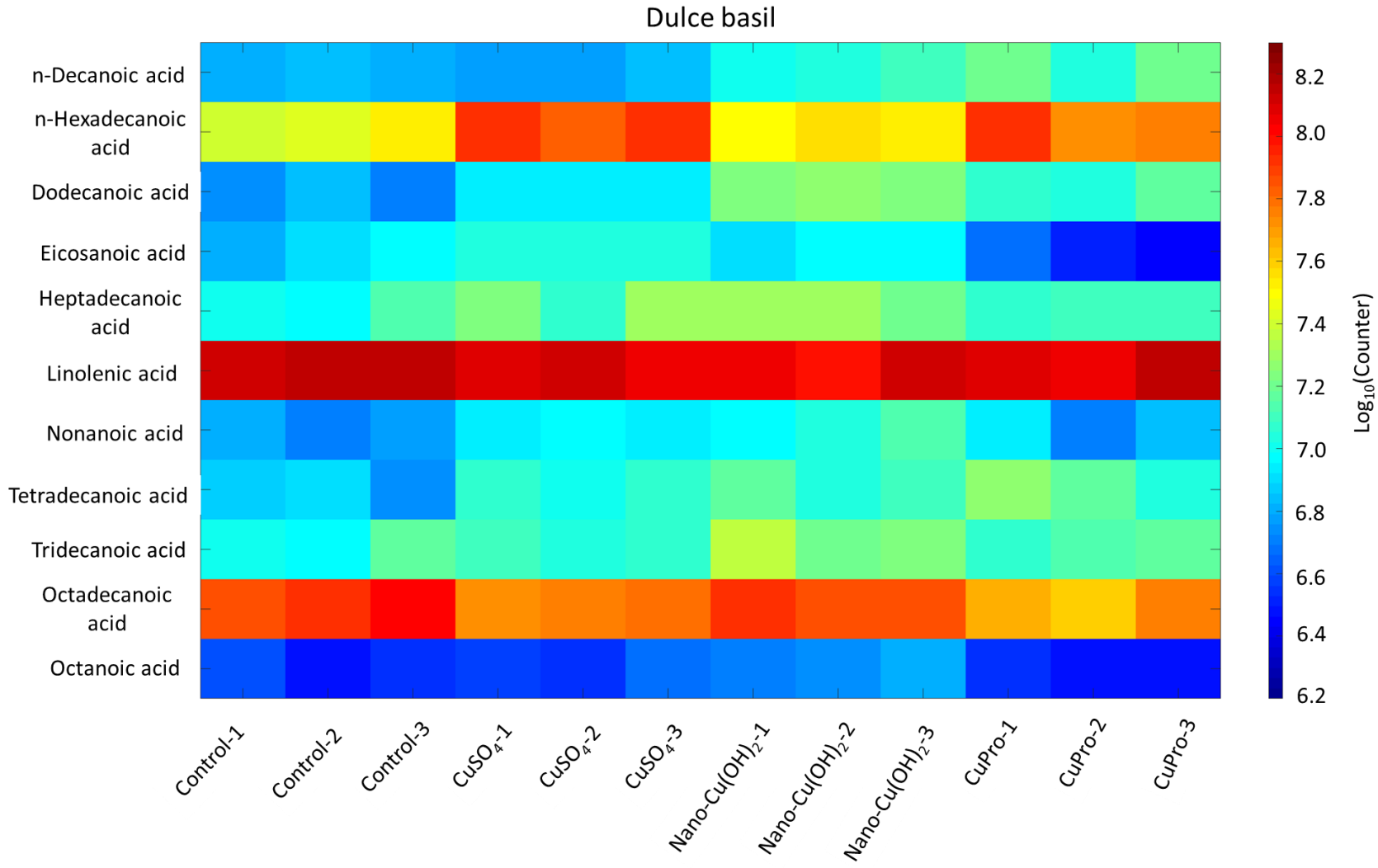


Figure 4.3 Heatmap presenting the relative abundance of 11 fatty acids in LAV basils with foliar exposure of 4.8 mg Cu/per pot from $CuSO_4$, $Cu(OH)_2$ nanowires, and CuPro for 2 weeks. The data are shown in log10 (count) per value. The relative abundance increases when the color changes from blue to red.

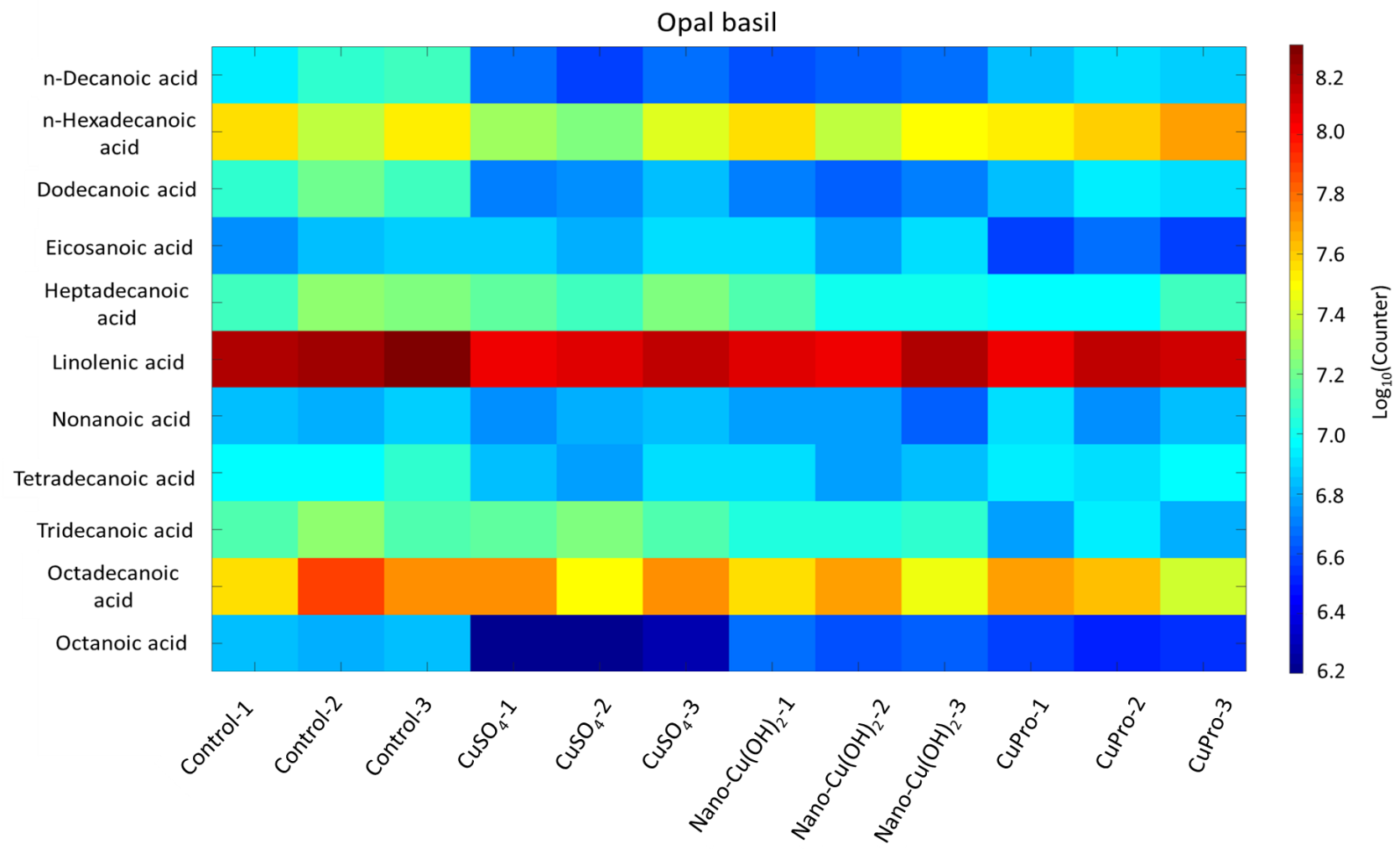


Figure 4.4 Heatmap presenting the relative abundance of 11 fatty acids in HAV basils with foliar exposure of 4.8 mg Cu/per pot from $CuSO_4$, $Cu(OH)_2$ nanowires, and CuPro for 2 weeks. The data are shown in log10 (count) per value. The relative abundance increases when the color changes from blue to red.

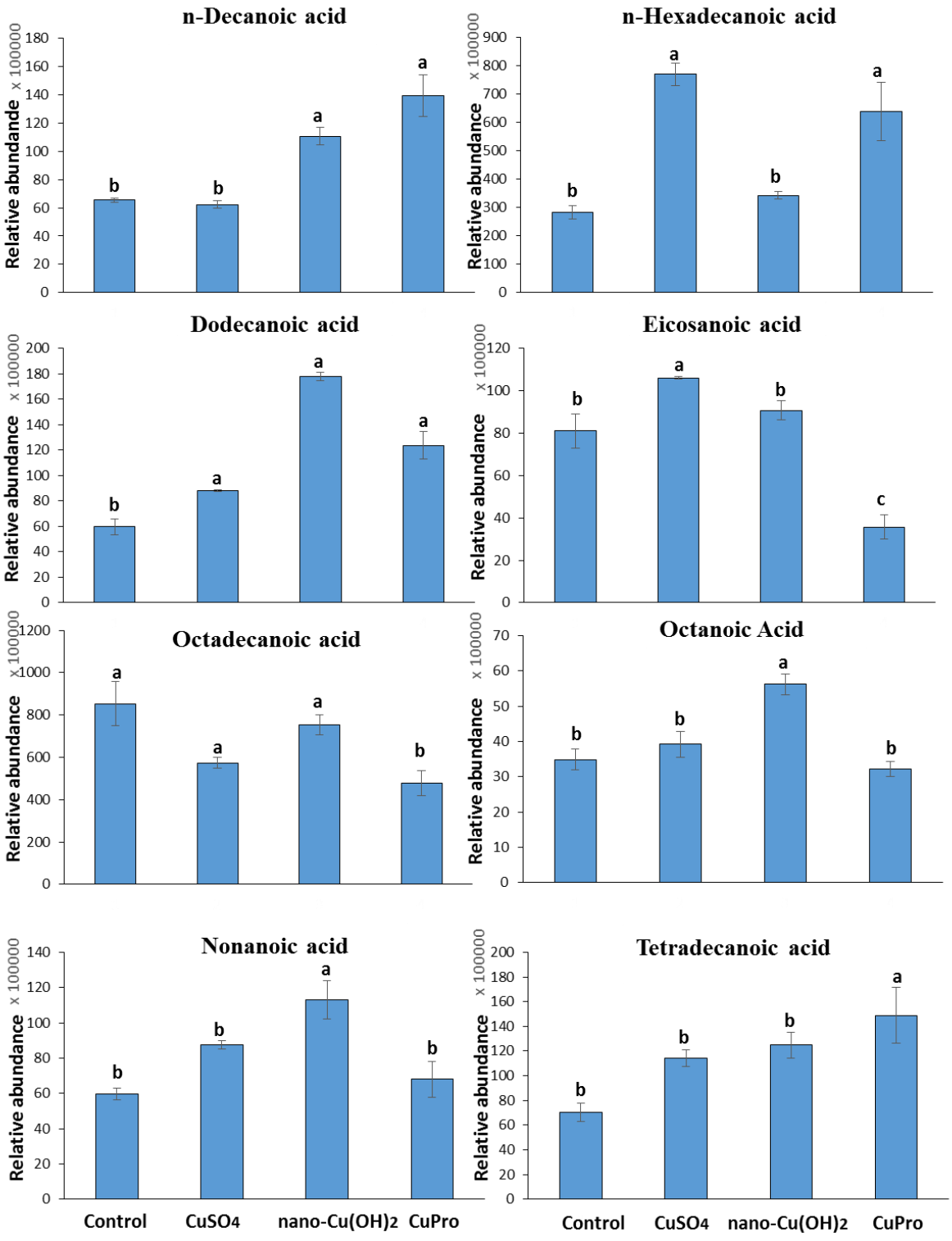


Figure 4.5 Relative abundance of fatty acids in leaves of LAV anthocyanin basils with foliar exposure of 4.8 mg Cu/per pot from $CuSO_4$, $Cu(OH)_2$ nanowires, and CuPro for 2 weeks. Each value is mean ± SE of three replicates. Means with different letters stand for significant difference compared with controls ($p \leq 0.05$).

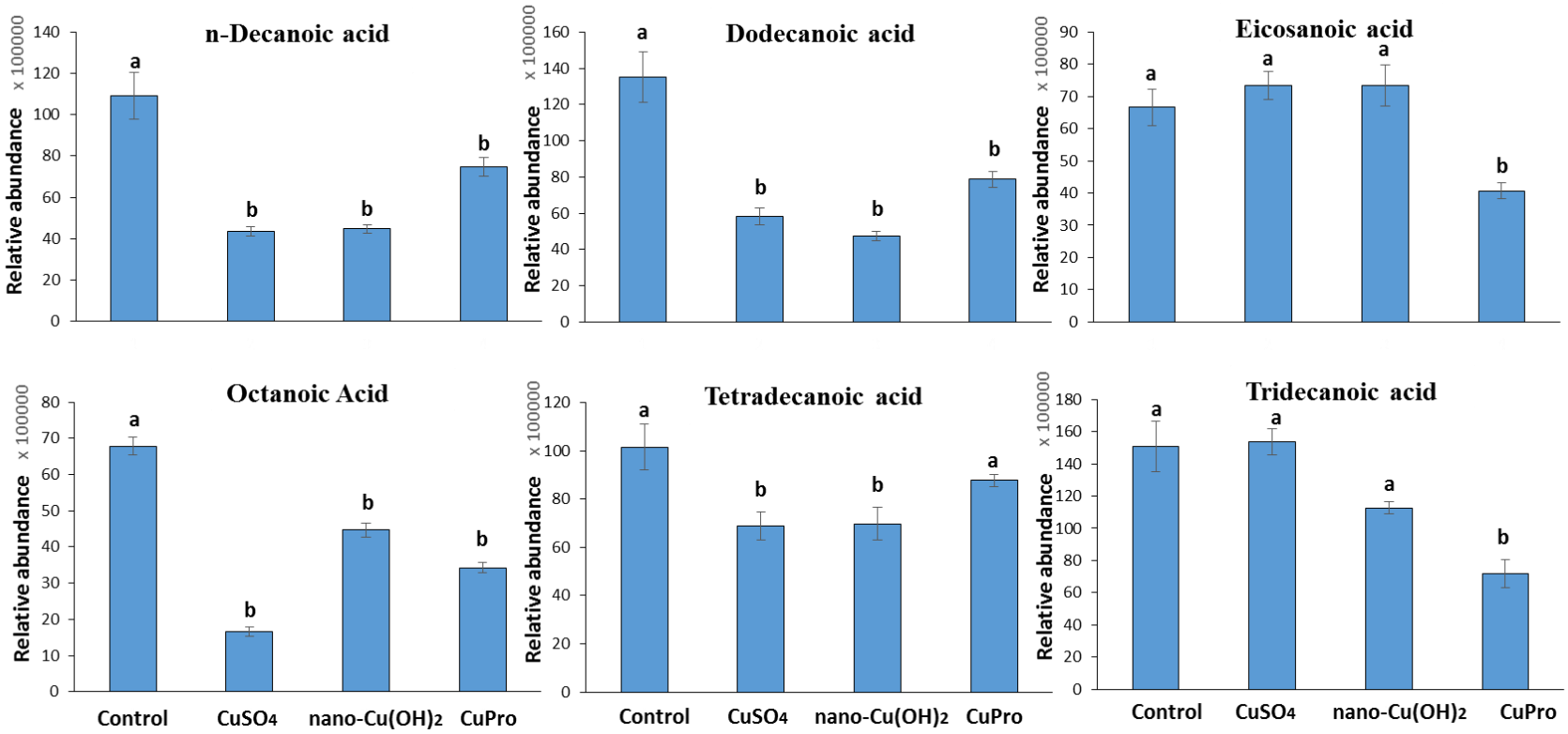


Figure 4.6 Relative abundance of fatty acids in leaves of HAV basils with foliar exposure of 4.8 mg Cu/per pot from $CuSO_4$, $Cu(OH)_2$ nanowires, and CuPro for 2 weeks. Each value is mean ± SE of three replicates. Means with different letters stand for significant difference compared with controls ($p \leq 0.05$).

decanoic (60% and 59%), dodecanoic (57% and 65%), octanoic aicds (76% and 34%) and tetradecanoic acid (32% and 31%). CuPro, not only significantly reduced n-decanoic (29%), dodecanoic (42%) and octanoic acids (50%), but also eicosanoic (39%), and tridecanoic acids (52%, $p \leq 0.05$). None of the copper compounds, in both varieties, affected linolenic acid, an unsaturated fatty acid. We assumed that differences in pH values in the cellular environment on both basil varieties are associated with the metabolic responses. In LAV, the three copper compounds significantly increased n-decanoic acid and dodecanoic acid; in contrast, significantly lower contents of these two fatty acids were determined in HAV.

Juntachote and Berghofer[55] found that the activity and stability of antioxidants are pH-dependent. Higher antioxidant activity was obtained in Holy basil and Galangal (*Alpinia galangal*) at neutral pH, compared to acidic pH. This may explain the higher relative abundance of fatty acids determined in LAV basil, which has a neutral cellular pH.[42] Besides, in a tomato study, Ouariti et al.[56] found that plants treated with excess copper resulted in higher saturated but lower unsaturated fatty acids. They found the alteration in fatty acids was associated with a decrease in lipid contents (glycolipids, phospholipids, and neutral lipids). In a study of the transcriptome responses to copper in rice, Lin et al.[57] reported that genes encoding lipoxygenase, NADH oxidase, and oxophytodienoic acid reductase were upregulated. In *A. thaliana*, changes in fatty acids were attributed to the reduced proton gradient caused by dissolution of nano-CuO.[58] Zhao et al.[2] found the precursor of n-hexadecanoic acid and octadecanoic acid, 1-monopalmitin and 1-monostearin, were significantly increased in maize treated with 100 mg $Cu(OH)_2$. Thus, the alterations in fatty acids could be attributed to the disruptions of their precursors stimulated by excess copper. Zhao et al.[8, 9, 12] found that foliar application of Kocide 3000 did not affect saturated and unsaturated fatty acids in lettuce and spinach, but affected the contents of carboxylic acid, carbohydrate,

polyphenols, polyamines, amino acid, and sucrose, which indicates the tricarboxylic acid cycle, sugar metabolism, and shikimate-phenylpropanoid pathway were interrupted. Later on, they found saturated fatty acids were elevated in maize, and unsaturated fatty acids were reduced in cucumber, which indicates the metallic response is species-dependent.[2]

4.3.5 Effects of copper compounds on pigments

Concentrations of pigments (anthocyanin and chlorophyll) in leaves are shown in Table 4.3-4.4, respectively. In LAV, none of the copper compounds affected anthocyanin and chlorophyll *a*, while CuPro increased chlorophyll *b* (63%), and total chlorophyll (29%), compared to controls ($p \leq 0.05$, Table 4.3). However, in HAV, $CuSO_4$ and $Cu(OH)_2$ nanowires reduced anthocyanin by 2% and 3%, respectively, compared with control ($p \leq 0.05$, Table 4.4). In addition, all treatments increased chlorophyll *a* ($CuSO_4$, 13%; nano-$Cu(OH)_2$, 12%), chlorophyll *b* ($CuSO_4$, 49%; nano-$Cu(OH)_2$, 70%; CuPro, 78%), and total chlorophyll contents ($CuSO_4$, 28%; nano-$Cu(OH)_2$, 36%; CuPro; 35%, $p \leq 0.05$), compared with control. The colour and chemical forms of anthocyanin degrade as the pH value increases.[42] After exposure to nano and bulk $Cu(OH)_2$ solutions, these compounds might interact with leaf exudates (such as oxalic acid and citric acid) forming water and other copper organic salts.[12] Due to the consumption of H^+ on leaf cuticle and stomata, a light decrease in pH value of leaves might occur; thus, the bioavailability and dissolution of anthocyanin may be reduced in HAV. In addition, copper is a vital element for proteins involved in electron transfer reactions and photosynthetic activity including plastocyanin and Ctyochrome-c-oxidase.[59] It is also important in the enzyme activity connected with photosynthesis process.[60] The increase on chlorophyll contents in HAV could be associated with the copper translocation (Figure 4.1). Copper is required to maintain the activity of ascorbate

Table 4.2 Anthocyanin concentrations in leaves of LAV basils with foliar exposure of 4.8 mg Cu/per pot from $CuSO_4$, CuPro, and $Cu(OH)_2$ nanowires for 2 weeks. Each value is mean ± SE of three replicates. Means with different letters stand for significant difference compared with controls ($p \leq 0.05$). The units of concentrations of anthocyanin shown in are mg/100g. The units of concentrations of chlorophyll shown in are mg/L.

Compound	Anthocyanin	Chlorophyll *a*	Chlorophyll *b*	Total chlorophyll
Control	8.87 ± 0.32	8.41 ± 0.41	6.12 ± 0.39 b	14.53 ± 0.75 b
$CuSO_4$	8.94 ± 0.35	9.18 ± 0.29	7.68 ± 1.16 ab	16.86 ± 1.44 ab
Nano-$Cu(OH)_2$	10.93 ± 0.77	8.13 ± 0.16	5.58 ± 0.79 b	13.71 ± 0.90 b
CuPro	8.98 ± 0.62	8.78 ± 0.016	9.95 ± 0.37 a	18.73 ± 0.35 a

Table 4.3 Anthocyanin concentrations in leaves of HAV basils with foliar exposure of 4.8 mg Cu/per pot from $CuSO_4$, CuPro, and $Cu(OH)_2$ nanowires for 2 weeks. Each value is mean ± SE of four replicates. Means with different letters stand for significant difference compared with controls ($p \leq 0.05$). The units of concentrations of anthocyanin shown in are mg/100g. The units of concentrations of chlorophyll shown in are mg/L.

Compound	Anthocyanin	Chlorophyll *a*	Chlorophyll *b*	Total chlorophyll
Control	35.46 ± 0.063 a	8.41 ± 0.41 b	6.12 ± 0.39 b	14.53 ± 0.75 b
$CuSO_4$	34.83 ± 0.082 b	9.51 ± 0.031 a	9.12 ± 0.55 a	18.63 ± 0.53 a
Nano-$Cu(OH)_2$	34.49 ± 0.075 c	9.38 ± 0.047 a	10.41 ± 0.51 a	19.80 ± 0.46 a
CuPro	35.38 ± 0.020 a	8.67 ± 0.028 ab	10.90 ± 0.27 a	19.57 ± 0.24 a

oxidase, which converts oxygen to water and produce dehydroascorbate (DHA) in an acidic environment. This suggests that the acidic environment and the higher copper accumulation had positive effects on the development of chloroplast in HAV.

4.4 Conclusion

This research has shown that the anthocyanin content affect the translocation of Cu from leaves to roots. In both varieties, significantly higher Cu was determined in leaves of $CuSO_4$ exposed plants (691 and 672.6 mg/kg for LA and HA, respectively). However, only in roots of HAV, higher Cu concentration was determined, compared to control ($p \leq 0.05$). Furthermore, $Cu(OH)_2$ nanowires significantly increased n-decanoic, dodecanoic, octanoic, and nonanoic acids in LAV, but reduced n-decanoic, dodecanoic, octanoic, and tetradecanoic acids in HAV, compared with control. In HAV, all compounds reduced eugenol (87%), 2-methylundecanal (71%), or anthocyanin (3%, $p \leq 0.05$). In addition, in all plant tissues, of both varieties, nanowires and $CuSO_4$ reduced Mn accumulation, while CuPro increased chlorophyll contents in leaves, compared with controls ($p \leq 0.05$). In summary, our findings indicate that CuPro (bulk) resulted in more biochemical alterations than $CuSO_4$ and $Cu(OH)_2$ nanowire, regardless of the varieties. This suggests that nanowires have a potential for agricultural use; however, more studies with other plants and growth media have to be performed in order to have a definitive result.

Chapter 5: Summary and Conclusions

Part I of this study showed that the accumulation of Ti and physiological/biochemical responses towards pristine, hydrophobic, and hydrophilic nano-TiO_2 were different in basil plants. Although the nano-TiO_2 resulted in concentration-dependent Ti accumulation in roots, with a preferential uptake from hydrophobic nano-TiO_2, none of nano-TiO_2 resulted in higher Ti concentration in shoots. Table 5.1 summarized the effects of the particles in several plant characteristics. As seen in this table, all the particles reduced the measured variables, but the hydrophobic particles had higher toxicity, since they reduced more parameters. Furthermore, the three types of nano-TiO_2 affected the homeostasis of essential elements (including Ca, P, Mg Cu, Fe, Mn, Se, and Zn), compared to controls.

Table 5.1 Effects of pristine, hydrophobic, and hydrophilic nano-TiO_2 in 65-day soil-grown basil

Type of NPs	Germination	Root length	Biomass	Total sugar	Reducing sugar	Starch	APOX		CAT	
							Root	Shoot	Root	Shoot
Pristine nano-TiO_2			↓	↓	↓		↓	↓	↓	↓
Hydrophobic nano-TiO_2	↓	↓	↓	↓		↓	↓		↓	↓
Hydrophilic nano-TiO_2	↓			↓				↓	↑↓	↓

Table 5.2 Effects of 750 $mg \cdot kg^{-1}$ of pristine, hydrophobic, and hydrophilic nano-TiO_2 on plants generated from untreated and treated seeds with/without sequential root exposure

Type of NPs	Treatment	Shoot length	Biomass	Chlorophyll	Total sugar	Reducing sugar	Gas exchange
Pristine nano-TiO_2	Prist-control-750						
	Prist-treated-0						
	Prist-treated-750						↑
Hydrophobic nano-TiO_2	HB-control-750			↓		↑	
	HB-treated-0	↑		↓			
	HB-treated-750	↑	↑	↓		↑	
Hydrophilic nano-TiO_2	HP-control-750						
	HP-treated-0			↓		↑	
	HP-treated-750				↑		

Part II showed that the sequential exposure to nano-TiO_2 with different surface coatings resulted in varied responses for plant growth, Ti absorption, and photosynthesis parameters. Table 5.2 summarizes the sequential effects of corresponding nano-TiO_2. This table shows that hydrophobic particles significantly reduced chlorophyll production in single or sequential applications, except increased sugar, biomass and shoot length. In general, this study indicates transgenerational effects of nano-TiO_2 on basil plants are specific to the surface coatings of nanoparticles.

Table 5.3 Effects of $CuSO_4$, $Cu(OH)_2$ nanowires, and CuPro in leaves of low and high anthocyanin basil varieties

Type of compounds	Low anthocyanin	High anthocyanin
$CuSO_4$	n-Hexadecanoic acid ↑ Dodecanoic acid ↑ Eicosanoic acid ↑	Anthocyanin ↓ Chlorophyll *a* ↑ Chlorophyll *b* ↑ Total chlorophyll ↑ Undecanal, 2-methyl- ↓ n-Decanoic acid ↓ Dodecanoic acid ↓ Octanoic acid ↓ Tetradecanoic acid ↓
$Cu(OH)_2$ nanowires	n-Decanoic acid ↑ Dodecanoic acid ↑ Octanoic acid ↑ Nonanoic acid ↑	Anthocyanin ↓ Chlorophyll *a* ↑ Chlorophyll *b* ↑ Total chlorophyll ↑ Eugenol ↓ Undecanal, 2-methyl- ↓ n-Decanoic acid ↓ Dodecanoic acid ↓ Octanoic acid ↓ Tetradecanoic acid ↓
CuPro	Chlorophyll *b* ↑ Total chlorophyll ↑ n-Decanoic acid ↑ n-Hexadecanoic acid ↑ Dodecanoic acid ↑ Tetradecanoic acid ↑ Eicosanoic acid ↓ Ocatadecanoic ↓	Chlorophyll *b* ↑ Total chlorophyll ↑ Eugenol ↓ Undecanal, 2-methyl- ↓ n-Decanoic acid ↓ Dodecanoic acid ↓ Eicosanoic acid ↓ Octanoic acid ↓ Tridecanoic acid ↓

Part III of this study showed that the response of basil plants to Cu-based ENMs or compounds is variety-dependent. Bulk $Cu(OH)_2$ resulted in more biochemical effects regardless the basil variety. In low anthocyanin basil, copper remained on the leaves, instead of translocating to stems or roots. Whereas, differently, significantly higher copper concentration was determined in leaves, stems, and roots in the HAV basil treated with the three copper compounds. The biochemical effects of $CuSO_4$, $Cu(OH)_2$ nanowires, and CuPro in both varieties of basils is shown in Table 5.3. On one hand, in LAV, anthocyanin, chlorophyll *a*, and essential oil compounds were not affected by $CuSO_4$, $Cu(OH)_2$ nanowires, and CuPro. With respect to relative abundance of fatty acids, only CuPro significantly reduced the relative abundance of eicosanoic acid and

octadecanoic acid, and six other types of fatty acids were increased by $CuSO_4$, $Cu(OH)_2$ nanowires, and CuPro to different degrees. On the other hand, in HAV, essential oils and anthocyanin contents were significantly reduced, but chlorophyll was significantly increased by $CuSO_4$, $Cu(OH)_2$ nanowires, or CuPro at varying degrees. Moreover, six fatty acid were reduced by these copper compounds at different degrees. In addition, in all plant tissues, of both varieties, nanowires and $CuSO_4$ reduced Mn accumulation.

In summary, the exposure of nano-TiO_2 of different surface chemistry caused varied effects on basil plants in both life-cycle and transgenerational studies. Hydrophobic nano-TiO_2 resulted in more physiological and biochemical alterations than pristine and hydrophilic particles in both cultivation cycles. In addition, the foliar application of $Cu(OH)_2$ nanowires resulted in less toxicity than the bulk $Cu(OH)_2$. Nano-TiO_2, with different surface properties, altered the nutritional elements/compounds, antioxidant activities, and photosynthesis in basil. In addition, copper-based pesticides affected pigments, essential oils, and fatty acids, which suggests that people who consume basil as nutritional supplement or medicinal treatment might be affected. Further studies have to be done to determine the toxicity to humans.

References

Chapter 1

Almeida, I., Alviano, D.S., Vieira, D.P., Alves, P.B., Blank, A.F., Lope,s A.H.C.S., Alviano, C.S., Rosa M.S.S., 2007. Antigiardial activity of *Ocimum basilicum* essential oil. Parasitology Research 101, 443-452.

Atha, D. H., Wang, H., Petersen, E. J., Cleveland, D., Holbrook, R. D., Jaruga, P., Dizdaroglu, M., Xing, B., Nelson, B., 2012. Copper oxide nanoparticle mediated DNA damage in terrestrial plant models. Environmental Science & Technology 46(3), 1819-1827.

Biswas, P., Wu, C.-Y., 2005. Nanoparticles and the environment. Journal of the Air & Waste Management Association 55(6), 708-746.

Cai, F., Wu, X., Zhang, H., Shen, X., Zhang, M., Chen, W., Gao, Q., White, J.C., Tao, S., Wang, X., 2017. Impact of TiO_2 nanoparticles on lead uptake and bioaccumulation in rice (*Oryza sativa* L.). NanoImpact 5, 101-108.

Chen, H., Weiss, J., Shahidi, F., 2006. Nanotechnology in nutraceuticals and functional foods. Food Technology.

Chen, X., Mao, S.S., 2007. Titanium dioxide nanomaterials: synthesis, properties, modifications, and applications. Chemical Reviews 107, 2891-2959.

Cioffi, N., Ditaranto, N., Torsi, L., Picca R.A., de Giglio, E., Sabbatini, L., Novello, L., Tantillo, G., Bleve-Zacheo, T., Zambonin, P.G., 2005. Synthesis, analytical characterization and bioactivity of Ag and Cu nanoparticles embedded in poly-vinyl-methyl-ketone films. Analytical and Bioanalytical Chemistry 382(8), 1912-1918.

Cometa, S., Iatta, R., Ricci, M.A., Ferretti, C., de Giglio, E., 2013. Analytical characterization and antimicrobial properties of novel copper nanoparticle-loaded electrosynthesized hydrogel coatings. Journal of Bioactive and Compatible Polymers 28(5), 508-522.

Dimkpa, C.O., Latta, D.E., McLean, J.E., Britt, D.W., Boyanov, M.I., Anderson, A.J., 2013. Fate of CuO and ZnO nano-and microparticles in the plant environment. Environmental Science & Technology 47(9), 4734-4742.

Daferera, D.J., Ziogas, B.N., Polissiou, M.G., 2003. The effectiveness of plant essential oils on the growth of Botrytis cinerea, *Fusarium* sp. and Clavibacter michiganensis subsp. michiganensis. Crop Protection 22(1), 39-44.

Ellenbecker, M.; and Tsai, S. 2011. Engineered nanoparticles: safer substitutes for toxic materials, or a new hazard? Journal of Cleaner Production 19(5), 483-487.

Elmer, W.H., White, J.C., 2016. The use of metallic oxide nanoparticles to enhance growth of tomatoes and eggplants in disease infested soil or soilless medium. Environmental Science: Nano 3(5), 1072-1079.

Frazier, T.P., Burklew, C.E., Zhang, B., 2014. Titanium dioxide nanoparticles affect the growth and microRNA expression of tobacco (*Nicotiana tabacum*). Functional & Integrative Genomics 14(1), 75-83.

Hernandez-Viezcas, J.A., Castillo-Michel, H., Andrews, J.C., Cotte, M., Rico, C., Peralta-Videa, J.R., Ge, Y., Priester, J.H., Holden, P.A., Gardea-Torresdey, J.L., 2013. In situ synchrotron X-ray fluorescence mapping and speciation of CeO_2 and ZnO nanoparticles in soil cultivated soybean (*Glycine max*). ACS nano 7(2), 1415-1423.

Hong, J., Wang, L., Sun, Y., Zhao, L., Niu, G., Tan, W., Rico, C.M., Peralta-Videa, J.R., Gardea-Torresdey, J.L. 2016. Foliar applied nanoscale and microscale CeO_2 and CuO alter cucumber (*Cucumis sativus*) fruit quality. Science of the Total Environment 563, 904-911.

Johnson, A.C., Bowes, M.J., Crossley, A., Jarvie, H.P., Jurkschat, K., Jürgens, M.D., Lawlor, A.J., Park, B., Rowland, P., Spurgeon, D., Svendsen, C., Thompson, I.P., Barnes, R.J., Williams, R.J., Xu, N., 2011. An assessment of the fate, behaviour and environmental risk associated with sunscreen TiO_2 nanoparticles in UK field scenarios. Science of the Total Environment 409, 2503-2510.

Keller, A.A., Lazareva, A., 2013. Predicted releases of engineered nanomaterials: from global to regional to local. Environmental Science & Technology Letters 1(1), 65-70.

Klaine, S.J., Alvarez, P.J., Batley, G.E., Fernandes, T.F., Handy, R.D., Lyon, D.Y., Mahendra, S., Mclaughlin, M.J., Lead, J.R., 2008. Nanomaterials in the environment: behavior, fate, bioavailability, and effects. Environmental Toxicology and Chemistry 27, 1825-1851.

Larue, C., Laurette, J., Herlin-Boime, N., Khodja, H., Fayard, B., Flank, A.M., Brisset, F., Carriere, M., 2012. Accumulation, translocation and impact of TiO_2 nanoparticles in wheat (*Triticum aestivum* spp.): influence of diameter and crystal phase. Science of the Total Environment 431, 197-208.

Lee, W. M., An, Y. J., Yoon, H., Kweon, H. S., 2008. Toxicity and bioavailability of copper nanoparticles to the terrestrial plants mung bean (*Phaseolus radiatus*) and wheat (*Triticum aestivum*): plant agar test for water-insoluble nanoparticles. Environmental Toxicology and Chemistry 27(9), 1915-1921.

Li, W., Ni, C., Lin, H., Huang, C., Shah, S.I., 2004. Size dependence of thermal stability of TiO_2 nanoparticles. Journal of Applied Physics 96, 6663-6668.

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

Majumdar, S., Peralta-Videa, J.R., Trujillo-Reyes, J., Sun, Y., Barrios, A.C., Niu, G., Flores-Margez, J.P., Gardea-Torresdey, J.L., 2016. Soil organic matter influences cerium translocation and physiological processes in kidney bean plants exposed to cerium oxide nanoparticles. Science of the Total Environment 569, 201-211.

Mary, G., Bajpai, S.K., Chand, N., 2009. Copper (II) ions and copper nanoparticles-loaded chemically modified cotton cellulose fibers with fair antibacterial properties. Journal of Applied Polymer Science 113(2), 757-766.

Murphy, C.J., Sau, T.K., Gole, A.M., Orendorff, C.J., Gao, J., Gou, L., Hunyadi, S.E., Li, T., 2005. Anisotropic metal nanoparticles: synthesis, assembly, and optical applications. Journal of Physics Chemistry B 109, 13857-13870

Navarro, E., Baun, A., Behra, R., Hartmann, N.B., Filser, J., Miao, A.J., Quigg, A., Santschi, P.H., Sigg, L., 2008. Environmental behavior and ecotoxicity of engineered nanoparticles to algae, plants, and fungi. Ecotoxicology 17(5), 372-386.

Piccinno, F., Gottschalk, F., Seeger, S., Nowack, B., 2012. Industrial production quantities and uses of ten engineered nanomaterials in Europe and the world. Journal of Nanoparticle Research 14(9), 1109.

Raliya, R., Nair, R., Chavalmane, S., Wang, W.N., Biswas, P., 2015. Mechanistic evaluation of translocation and physiological impact of titanium dioxide and zinc oxide nanoparticles on the tomato (*Solanum lycopersicum* L.) plant. Metallomics 7(12), 1584-1594.

Rico, C.M., Morales, M.I., Barrios, A.C., McCreary, R., Hong, J., Lee, W.Y., Nunez, J., Peralta-Videa, J.R., Gardea-Torresdey, J.L., 2013. Effect of cerium oxide nanoparticles on the quality of rice (*Oryza sativa* L.) grains. Journal of Agricultural and Food Chemistry 61(47), 11278-11285.

Roco, M.C., 2011. The long view of nanotechnology development: the National Nanotechnology Initiative at 10 years. Journal of Nanoparticle Research 13, 427-445.

Sachtleben Chemie GmbH, Duisburg, Germany, Product information UV-TITAN M212.

Sachtleben Chemie GmbH, Duisburg, Germany, Product information UV-TITAN M262.

Sekine, R., Marzouk, E. R., Khaksar, M., Scheckel, K. G., Stegemeier, J. P., Lowry, G.V., Donner, E., Lombi, E., 2017. Aging of dssolved copper and copper-based nanoparticles in five different Soils: Short-term kinetics vs. long-term fate. Journal of Environmental Quality doi:10.2134/jeq2016.12.0485.

Specimen Label CuPRO*2005 T/N/O

Stampoulis, D., Sinha, S. K., White, J. C., 2009. Assay-dependent phytotoxicity of nanoparticles to plants. Environmental Science & Technology 43(24), 9473-9479.

Trujillo-Reyes, J., Majumdar, S., Botez, C. E., Peralta-Videa, J. R., Gardea-Torresdey, J. L., 2014. Exposure studies of core–shell Fe/Fe_3O_4 and Cu/CuO NPs to lettuce (*Lactuca sativa*) plants: Are they a potential physiological and nutritional hazard? Journal of Hazardous Materials 267, 255-263.

Tumburu, L., Andersen, C.P., Rygiewicz, P.T., Reichman, J.R., 2017. Molecular and physiological responses to titanium dioxide and cerium oxide nanoparticles in *Arabidopsis*. Environmental Toxicology and Chemistry 36(1), 71-82.

Wang, Z., Xie, X., Zhao, J., Liu, X., Feng, W., White, J.C., Xing, B., 2012. Xylem-and phloem-based transport of CuO nanoparticles in maize (*Zea mays* L.). Environmental Science & Technology 46(8), 4434-4441.

Wang, P., Menzies, N.W., Lombi, E., McKenna, B.A., Johannessen, B., Glover, C.J., Kappen, P., Kopittke, P.M., 2013. Fate of ZnO nanoparticles in soils and cowpea (*Vigna unguiculata*). Environmental Science and Technology 47(23), 13822-13830.

Wang, S., Liu, H., Zhang, Y., Xin, H., 2015. The effect of CuO NPs on reactive oxygen species and cell cycle gene expression in roots of rice. Environmental Toxicology and Chemistry 34(3), 554-561.

Welch, C.M., Compton, R.G., 2006. The use of nanoparticles in electroanalysis: a review. Analytical and Bioanalytical Chemistry 384(3), 601-619.

Wu, B., Zhu, L., Le, X.C., 2017. Metabolomics analysis of TiO_2 nanoparticles induced toxicological effects on rice (*Oryza sativa* L.). Environmental Pollution 230, 302-310.

Zhao, L., Huang, Y., Hannah-Bick, C., Fulton, A.N., Keller, A.A., 2016a. Application of metabolomics to assess the impact of Cu $(OH)_2$ nanopesticide on the nutritional value of lettuce (*Lactuca sativa*): Enhanced Cu intake and reduced antioxidants. NanoImpact 3, 58-66.

Zhao, L., Ortiz, C., Adeleye, A.S., Hu, Q., Zhou, H., Huang, Y., Keller, A.A., 2016b. Metabolomics to detect response of lettuce (*Lactuca sativa*) to Cu $(OH)_2$ nanopesticides: Oxidative stress response and detoxification mechanisms. Environmental Science & Technology 50(17), 9697-9707.

Zhao, L., Hu, Q., Huang, Y., Fulton, A.N., Hannah-Bick, C., Adeleye, A.S., Keller, A.A., 2017a. Activation of antioxidant and detoxification gene expression in cucumber plants exposed to a Cu $(OH)_2$ nanopesticide. Environmental Science: Nano 4(8), 1750-1760.

Zhao, L., Huang, Y., Keller, A.A., 2017b. Comparative metabolic response between cucumber (*Cucumis sativus*) and corn (*Zea Mays*) to a Cu $(OH)_2$ nanopesticide. Journal of Agricultural and Food Chemistry DOI: 10.1021/acs.jafc.7b01306

Zhao, L., Huang, Y., Adeleye, A.S., Keller, A.A., 2017c. Metabolomics reveals $Cu(OH)_2$ nanopesticide-activated anti-oxidative pathways and decreased beneficial antioxidants in spinach leaves. Environmental Science & Technology 51(17)

Zhang, P., Ma, Y., Zhang, Z., He, X., Zhang, J., Guo, Z., Tai, R., Zhao, Y., Chai, Z., 2012. Bio-transformation of ceria nanoparticles in cucumber plants. ACS Nano 6, 9943-9950

Chapter 2

Almeida, I., Alviano, D.S., Vieira, D.P., Alves, P.B., Blank, A.F., Lopes, A.H.C.S., Alviano, C.S., Rosa, M.S.S., 2007. Antigiardial activity of *Ocimum basilicum* essential oil. Parasitology Research 101, 443-452.

Andersen, C.P., King, G., Plocher, M., Storm, M., Pokhrel, L.R., Johnson, M.G., Rygiewicz, P.T., 2016. Germination and early plant development of ten plant species exposed to TiO_2 and CeO_2 nanoparticles. Environmental Toxicology and Chemistry, 1-7.

Antisari, L.V., Carbone, S., Gatti, A., Vianello, G., Nannipieri, P., 2015. Uptake and translocation of metals and nutrients in tomato grown in soil polluted with metal oxide (CeO_2, Fe_3O_4, SnO_2, TiO_2) or metallic (Ag, Co, Ni) engineered nanoparticles. Environmental Science and Pollution Research 22(3), 1841-1853.

Armstrong, D.L., Eds., 1999. Functions of phosphorus in plant. Better crops 83, 6-7.

Barrios, A.C., Medina-Velo, I.A., Zuverza-Mena, N., Dominguez, O.E., Peralta-Videa, J.R., Gardea-Torresdey, J.L., 2016. Nutritional quality assessment of tomato fruits after exposure to uncoated and citric acid coated cerium oxide nanoparticles, bulk cerium oxide, cerium acetate and citric acid. Plant Physiology and Biochemistry, doi:10.1016/j.plaphy.2016.04.017.

Beyer, W.F., Fridovich, I., 1987. Assaying for superoxide dismutase activity: some large consequences of minor changes in conditions. Analytical Biochemistry 161, 559-566.

Bohn, H.L., Strawn, D.G., O'Connor, G.A., 2015. Soil chemistry, fourth ed. John Wiley & Sons, New York, United States, pp. 248.

Brady, N.C., Weil, R.R., 1996. The nature and properties of soils, eleventh ed. Prentice-Hall Inc.

Castiglione, M.R., Giorgetti, L., Geri, C., Cremonini, R., 2011. The effects of nano-TiO_2 on seed germination, development and mitosis of root tip cells of *Vicia narbonensis L*. and *Zea mays L*. Journal of Nanoparticle Research 13, 2443-2449.

Chae, S.Y., Park, M.K., Lee, S.K., Kim, T.Y., Kim, S.K., Lee, W.I., 2003. Preparation of size-controlled TiO_2 nanoparticles and derivation of optically transparent photocatalytic films. Chemistry of Materials 15(17), 3326-3331.

Chen, R., Ratnikova, T.A., Stone, M.B., Lin, S., Lard, M., Huang, G., Hudson, J.S., Ke, P.C., 2010. Differential uptake of carbon nanoparticles by plant and mammalian cells. Small 6, 612-617.

Chen, X., Mao, S.S., 2007. Titanium dioxide nanomaterials: synthesis, properties, modifications, and applications. Chemical Reviews 107, 2891-2959.

Chiang, L.C., Ng, L.T, Cheng, P.W., Chiang, W., Lin, C.C., 2005. Antiviral activities of extracts and selected pure constituents of *Ocimum basilicum*. Clinical and Experimental Pharmacology and Physiology 32, 811-816.

Daram, P., Brunner, S., Rausch, C., Steiner, C., Amrhein, N., Bucher, M., 1999. Pht2; 1 encodes a low-affinity phosphate transporter from Arabidopsis. Plant Cell 11(11), 2153-2166.

Du, W., Sun, Y., Ji, R., Zhu, J., Wu, J., Guo, H., 2011. TiO_2 and ZnO nanoparticles negatively affect wheat growth and soil enzyme activities in agricultural soil. Journal of Environmental Monitoring 13(4), 822-828.

Dubois, M., Gilles, K.A., Hamilton, J.K., Rebers, P., Smith, F., 1956. Colorimetric method for determination of sugars and related substances. Analytical Chemistry 28, 350-356.

Elghniji, K., Hentati, O., Mlaik, N., Mahfoudh, A., Ksibi, M., 2012. Photocatalytic degradation of 4-chlorophenol under P-modified TiO_2/UV system: Kinetics, intermediates, phytotoxicity and acute toxicity. Journal of Environmental Sciences 24(3), 479-487.

Fan, R., Huang, Y.C., Grusak, M.A., Huang, C.P., Sherrier, D.J., 2014. Effects of nano-TiO_2 on the agronomically-relevant Rhizobium–legume symbiosis. Science of the Total Environment 466, 503-512.

Gallego, S.M., Benavídes, M.P., Tomaro, M.L., 1996. Effect of heavy metal ion excess on sunflower leaves: evidence for involvement of oxidative stress. Plant Science 121, 151-159.

Gardea-Torresdey, J.L., Rico, C.M., White, J.C., 2014. Trophic transfer, transformation, and impact of engineered nanomaterials in terrestrial environments. Environmental Science & Technology 48, 2526-2540.

Gottschalk, F., Sonderer, T., Scholz, R.W., Nowack, B., 2009. Modeled environmental concentrations of engineered nanomaterials (TiO_2, ZnO, Ag, CNT, fullerenes) for different regions. Environmental Science & Technology 43, 9216-9222.

Guo, H., Zhou, H., Zhang, Y., Du, W., Sun, Y., Yin, Y., Pei, D.P., Ji, R., Wu, J.C., Wang, X.R., Zhu, J.G., 2015. Combination of elevated CO_2 levels and soil contaminants' stress in wheat and rice, in: R. Mahalingam (Eds.), Combined stresses in plants. Springer, New York, United States, pp. 71-92.

Hong, F., Yang, F., Liu, C., Gao, Q., Wan, Z., Gu, F., Wu, C., Ma, Z., Zhou, J., Yang, P., 2005. Influences of nano-TiO_2 on the chloroplast aging of spinach under light. Biologucal Trace Element Research 104, 249-260.

Johnson, A.C., Bowes, M.J., Crossley, A., Jarvie, H.P., Jurkschat, K., Jürgens, M.D., Lawlor, A.J., Park, B., Rowland, P., Spurgeon, D., Svendsen, C., Thompson, I.P., Barnes, R.J., Williams, R.J., Xu, N., 2011. An assessment of the fate, behaviour and environmental risk associated

with sunscreen TiO_2 nanoparticles in UK field scenarios. Science of the Total Environment 409, 2503-2510.

Kabata-Pendias, A., Mukherjee, A.B., 2007. Trace elements from soil to human. Springer Science & Business Media, Heidelberg, Germany, pp. 201-202.

Keller, A.A., Lazareva, A., 2013. Predicted releases of engineered nanomaterials: from global to regional to local. Environmental Science & Technology Letters 1, 65-70.

Klaine, S.J., Alvarez, P.J., Batley, G.E., Fernandes, T.F., Handy, R.D., Lyon, D.Y., Mahendra, S., Mclaughlin, M.J., Lead, J.R., 2008. Nanomaterials in the environment: behavior, fate, bioavailability, and effects. Environmental Toxicology and Chemistry 27, 1825-1851.

Kraynov, A., Müller, T.E., 2011. Concepts for the stabilization of metal nanoparticles in ionic liquids, applications of ionic liquids in science and technology, in: Handy S. (Ed.), Applications of Ionic Liquids in Science and Technology. InTech, 2011, pp. 235-254.

Kurepa, J., Paunesku, T., Vogt, S., Arora, H., Rabatic, B.M., Lu, J., Wanzer, M.B., Woloschak, G.E., Smalle, J.A., 2010. Uptake and distribution of ultrasmall anatase TiO_2 alizarin red S nanoconjugates in *Arabidopsis thaliana*. Nano Letter 10, 2296-2302.

Larue, C., Khodja, H., Herlin-Boime, N., Brisset, F., Flank, A., Fayard, B., Chaillou, S., Carriere, M., 2011. Investigation of titanium dioxide nanoparticles toxicity and uptake by plants. Journal of Physics: Conference Series IOP Publishing, 304(1), 012057.

Larue, C., Laurette, J., Herlin-Boime, N., Khodja, H., Fayard, B., Flank, A.M., Brisset, F., Carriere, M., 2012a. Accumulation, translocation and impact of TiO_2 nanoparticles in wheat (*Triticum aestivum spp*.): influence of diameter and crystal phase. Science of the Total Environment 431,197-208.

Larue, C., Veronesi, G., Flank, A.M., Surble, S., Herlin-Boime, N., Carrière, M., 2012b. Comparative uptake and impact of TiO_2 nanoparticles in wheat and rapeseed. Journal of Toxicology Environment Health Part A 75, 722-734.

Larue, C., Castillo-Michel, H., Sobanska, S., Trcera, N., Sorieul, S., Cécillon, L., Ouerdane, L., Legros, S., Sarrret, G., 2014. Fate of pristine TiO_2 nanoparticles and aged paint-containing TiO_2 nanoparticles in lettuce crop after foliar exposure. Journal of Hazardous Materials 273, 17-26.

Larue, C., Castillo-Michel, H., Stein, R.J., Fayard, B., Pouyet, E., Villanova, J., Magnin, V., Real, A.P., Trcera, N., Legros, S., Sorieul, S., Sarrest, G., 2016. Innovative combination of spectroscopic techniques to reveal nanoparticle fate in a crop plant. Spectrochimica Acta Part B: Atomic Spectroscopy 119, 17-24.

Lavado, R.S., Porcelli, C.A., Alvarez, R., 2011. Nutrient and heavy metal concentration and distribution in corn, soybean and wheat as affected by different tillage systems in the Argentine Pampas. Soil and Tillage Research 62, 55-60.

Li, W., Ni, C., Lin, H., Huang, C., Shah, S.I., 2004. Size dependence of thermal stability of TiO_2 nanoparticles. Journal of Applied Physics 96, 6663-6668.

Lopez-Moreno, J.L., Giménez, J.L., Moreno, A., Fuentes, J.L., Alcaraz, C.F., 1995. Plant biomass and fruit yield induction by Ti (IV) in P-stressed pepper crops. Fertilizer Research 43 (1-3), 131-136.

Ma, C., White, J.C., Dhankher, O.P., Xing, B., 2015. Metal-based nanotoxicity and detoxification pathways in higher plants. Environmental Science & Technology 49 (12), 7109–7122.

Maathuis, F.J.M., 2009. Physiological functions of mineral macronutrients. Current Opinion in Plant Biology 12 (3), 250-258.

Madaleno, I.M., 2015. Traditional Medicinal Knowledge in India and Malaysia. Pharmacognosy Communications 5, 116.

Makarova, O.V., Rajh, T., Thurnauer, M.C., Martin, A., Kemme, P.A., Cropek, D., 2000. Surface modification of TiO_2 nanoparticles for photochemical reduction of nitrobenzene. Environmental Science & Technology 34(22), 4797-4803.

Menard, A., Drobne, D., Jemec, A., 2011. Ecotoxicity of nanosized TiO_2. Review of in vivo data. Environmental Pollution 159, 677-684.

Millaleo, R., Reyes-Díaz, M., Ivanov, A.G., Mora, M.L., Alberdi, M., 2010. Manganese as essential and toxic element for plants: transport, accumulation and resistance mechanisms. Journal of Soil Science and Plant Nutrition 10(4), 470-481.

Morales, M.I., Rico, C.M., Hernandez-Viezcas, J.A., Nunez, J.E., Barrios, A.C., Tafoya, A., Flores-Marges, J.P., Peralta-Videa, J.R., Gardea-Torresdey, J.L., 2013. Toxicity assessment of cerium oxide nanoparticles in cilantro (*Coriandrum sativum L.*) plants grown in organic soil. Journal of Agricultural and Food Chemistry 61, 6224-6230.

Nussaume, L., Kanno, S., Javot, H., Marin, E., Pochon, N., Ayadi, A., Nakanishi, T.M., Thibaud, M.C., 2011. Phosphate import in plants: focus on the PHT1 Transporters. Frontiers in Plant Science 2(83), 235-244.

Panda, S., Choudhury, S., 2005. Chromium stress in plants. Brazilian Journal of Plant Physiology 17, 95-102.

Perreault, F., Popovic, R., Dewez, D., 2014. Different toxicity mechanisms between bare and polymer-coated copper oxide nanoparticles in Lemna gibba. Environmental Pollution 185, 219-227.

Piccinno, F., Gottschalk, F., Seeger, S., Nowack, B., 2012. Industrial production quantities and uses of ten engineered nanomaterials in Europe and the world. Journal of Nanoparticle Research 14(9), 1-11.

Pilon-Smits, E.A., Quinn, C.F., 2010. Selenium metabolism in plants, in: R. Hell, R.R. Mendel (Eds.), Cell biology of metals and nutrients. Springer, New York, United States, pp. 225-241.

Rafique, R., Arshad, M., Khokhar, M., Qazi, I., Hamza, A., Virk, N., 2015. Growth response of wheat to titania nanoparticles application. NUST Journal of Engineering Sciences 7, 42-46.

Raliya, R., Nair, R., Chavalmane, S., Wang, W.N., Biswas, P., 2015. Mechanistic evaluation of translocation and physiological impact of titanium dioxide and zinc oxide nanoparticles on the tomato (*Solanum lycopersicum L.*) plant. Metallomics 7, 1584-1594.

Rico, C.M., Morales, M.I., Barrios, A.C., McCreary, R., Hong, J., Lee, W.Y., Nunez, J., Peralta-Videa, J.R., Gardea-Torresdey, J.L., 2013. Effect of cerium oxide nanoparticles on the quality of rice (*Oryza sativa* L.) grains. Journal of Agricultural and Food Chemistry 61, 11278-11285.

Rittschof, D., Orihuela, B., Harder, T., Stafslien, S., Chisholm, B., Dickinson, G.H., 2011. Compounds from silicones alter enzyme activity in curing barnacle glue and model enzymes. PLoS One 6(2), e16487.

Sachtleben Chemie GmbH, Duisburg, Germany, Product information UV-TITAN M212.

Sachtleben Chemie GmbH, Duisburg, Germany, Product information UV-TITAN M262.

Seo, J., Chung, H., Kim, M., Lee, J., Choi, I.H., Cheon, J., 2007. Development of water-soluble single-crystalline TiO2 nanoparticles for photocatalytic cancer-cell treatment. Small 3(5), 850-853.

Servin, A.D., White, J.C., 2016. Nanotechnology in agriculture: next steps for understanding engineered nanoparticle exposure and risk. NanoImpact 1, 9-12.

Servin, A.D., Castillo-Michel, H., Hernandez-Viezcas, J.A., Diaz, B.C., Peralta-Videa, J.R., Gardea-Torresdey, J.L., 2012. Synchrotron micro-XRF and micro-XANES confirmation of the uptake and translocation of TiO_2 nanoparticles in cucumber (*Cucumis sativus*) plants. Environmental Science & Technology 46, 7637-7643.

Servin, A.D., Morales, M.I., Castillo-Michel, H., Hernandez-Viezcas, J.A., Munoz, B., Zhao, L., Nunez, J.E., Peralta-Videa, J.R., Gardea-Torresdey, J.L., 2013. Synchrotron verification of TiO_2 accumulation in cucumber fruit: a possible pathway of TiO_2 nanoparticle transfer from soil into the food chain. Environmental Science & Technology 47, 11592-11598.

Sharma, P., Jha, A.B., Dubey, R.S., Pessarakli, M., 2012. Reactive oxygen species, oxidative damage, and antioxidative defense mechanism in plants under stressful conditions. Journal of Botany 2012, 1-23.

Somogyi, M., 1952. Notes on sugar determination. Journal of Biological Chemistry 195, 19-23.

Song, U., Jun, H., Waldman, B., Roh, J., Kim, Y., Yi, J., Lee, E.J., 2013. Functional analyses of nanoparticle toxicity: a comparative study of the effects of TiO_2 and Ag on tomatoes (*Lycopersicon esculentum*). Ecotoxicology and Environmental Safety 93, 60-67.

Sparks, D.L., 2003. Environmental soil chemistry, second ed. Academic press, Massachustts, United States, pp. 184.

Trujillo-Reyes, J., Vilchis-Nestor, A.R., Majumdar, S., Peralta-Videa, J.R., Gardea-Torresdey, J.L., 2013. Citric acid modifies surface properties of commercial CeO_2 nanoparticles reducing their toxicity and cerium uptake in radish (*Raphanus sativus*) seedlings. Journal of Hazardous Materials 263, 677-684.

US research Nanomaterials, Inc., http://www.us-nano.com/inc/sdetail/7710.

Verma, S., Dubey, R., 2001. Effect of cadmium on soluble sugars and enzymes of their metabolism in rice. Biologua Plantarum 44, 117-123.

Wang, Y.M., Kinraide, T.B., Wang, P., Hao, X.Z., Zhou, D.M., 2014. Surface electrical potentials of root cell plasma membranes: implications for ion interactions, rhizotoxicity, and uptake. International Journal of Molecular Sciece 15, 22661-22677.

Warman, P., 1999. Evaluation of seed germination and growth tests for assessing compost maturity. Compost Science & Utilization 7, 33-37.

Weng, C.C., Wei, K.H., 2003. Selective distribution of surface-modified TiO_2 nanoparticles in polystyrene-b-poly(methyl methacrylate) diblock copolymer. Chemistry of Materials 15(15), 2936-2941.

Zangi, R., Berne, B., 2006. Aggregation and dispersion of small hydrophobic particles in aqueous electrolyte solutions. Journal of Physical Chemistry B 110, 22736-22741.

Zhao, L., Hernandez-Viezcas, J.A., Peralta-Videa, J.R., Bandyopadhyay, S., Peng, B., Munoz, B., Keller, A.A., Gardea-Torresdey, J.L., 2013. ZnO nanoparticle fate in soil and zinc bioaccumulation in corn plants (*Zea mays*) influenced by alginate. Environmental Science: Processes Impacts 15, 260-266.

Zhao, L., Peralta-Videa, J.R., Rico, C.M., Hernandez-Viezcas, J.A., Sun, Y., Niu, G., Servin, A.D., Nunes, J.E., Duarte-Gardea, M., Gardea-Torresdey, J.L., 2014. CeO_2 and ZnO nanoparticles change the nutritional qualities of cucumber (*Cucumis sativus*). Journal of Agricultural and Food Chemistry 62, 2752-2759.

Zheng, L., Hong, F., Lu, S., Liu, C., 2005. Effect of nano-TiO_2 on strength of naturally aged seeds and growth of spinach. Biological Trace Element Research 104(1), 83-91.

Zhou, X.T., Ji, H.B., Huang, X.J., 2012. Photocatalytic degradation of methyl orange over metalloporphyrins supported on TiO_2 degussa P25. Molecules 17, 1149-1158.

Chapter 3

Asli, S., Neumann, P.M., 2009. Colloidal suspensions of clay or titanium dioxide nanoparticles can inhibit leaf growth and transpiration via physical effects on root water transport. Plant, Cell & Environment 32(5), 577-584.

Barrios, A.C., Rico, C.M., Trujillo-Reyes, J., Medina-Velo, I.A., Peralta-Videa, J.R., Gardea-Torresdey, J. L., 2016. Effects of uncoated and citric acid coated cerium oxide nanoparticles, bulk cerium oxide, cerium acetate, and citric acid on tomato plants. Science of The Total Environment 563, 956-964.

Beninger, C.W., Hosfield, G.L., Nair, M.G., 1998. Flavonol Glycosides from the Seed Coat of a New Manteca-Type Dry Bean (*Phaseolus v ulgaris* L.). Journal of Agricultural and Food Chemistry 46(8), 2906-2910.

R. E. Blankenship, Molecular mechanisms of photosynthesis. John Wiley & Sons, New York, NY, 2013.

Cai, F., Wu, X., Zhang, H., Shen, X., Zhang, M., Chen, W., Gao, Q., White, J.C., Wang, X., 2017. Impact of TiO_2 nanoparticles on lead uptake and bioaccumulation in rice (*Oryza sativa* L.). NanoImpact 5, 101-108.

Castiglione, M.R., Giorgetti, L., Cremonini, R., Bottega, S., Spanò, C., 2014. Impact of TiO_2 nanoparticles on *Vicia narbonensis* L.: potential toxicity effects. Protoplasma 251(6), 1471-1479.

Chae, S.Y., Park, M.K., Lee, S.K., Kim, T.Y., Kim, S.K., Lee, W.I., 2003. Preparation of size-controlled TiO_2 nanoparticles and derivation of optically transparent photocatalytic films. Chemistry of Materials 15(17), 3326-3331.

Chen, C.W., Huang, J.H., Lai, T.C., Jan, Y.H., Hsiao,M., Chen, C.H., Hwu, Y., Liu, R.S., 2016. Evaluation of the intracellular uptake and cytotoxicity effect of TiO_2 nanostructures for various human oral and lung cells under dark conditions. Toxicology Research 5(1), 303-311.

Chen, X., Mao, S.S., 2007. Titanium dioxide nanomaterials: synthesis, properties, modifications, and applications. Chemical Reviews 107, 2891-2959.

Conway, J.R., Beaulieu, A.L., Beaulieu, N.L., Mazer, S.J., Keller, A.A., 2015. Environmental stresses increase photosynthetic disruption by metal oxide nanomaterials in a soil-grown plant. Acs Nano 9(12), 11737-11749.

Cowan, I.R., 1972. Oscillations in stomatal conductance and plant functioning associated with stomatal conductance: observations and a model. Planta 106(3), 185-219.

Dey, P.M., Harborne, J.B., ed. Plant biochemistry. Academic press, Waltham, MA, 1997, pp, 143-191.

Drummond, R.S.M., Tutone, A., Li, Y.C., Gardner, R.C., 2006. A putative magnesium transporter AtMRS2-11 is localized to the plant chloroplast envelope membrane system. Plant Science 170(1), 78-89.

Du, W., Sun, Y., Ji, R., Zhu, J., Wu, J., Guo, H., 2011. TiO_2 and ZnO nanoparticles negatively affect wheat growth and soil enzyme activities in agricultural soil. Journal of Environmental Monitoring 13(4), 822-828.

Du, W., Tan, W., Peralta-Videa, J.R., Gardea-Torresdey, J. L., Ji, R., Yin, Y., Guo, H., Interaction of metal oxide nanoparticles with higher terrestrial plants: physiological and biochemical aspects. Plant Physiology and Biochemistry 2017, 110: 210-225.

Dubois, M., Gilles, K.A., Hamilton, J.K., Rebers, P., Smith, F., 1956. Colorimetric method for determination of sugars and related substances. Analytical Chemistry 28, 350-356.

Elghniji, K., Hentati, O., Mlaik, N., Mahfoudh, A., Ksibi, M., 2012. Photocatalytic degradation of 4-chlorophenol under P-modified TiO_2/UV system: Kinetics, intermediates, phytotoxicity and acute toxicity. Journal of Environmental Sciences 24(3), 479-487.

Farquhar, G.D., Sharkey, T.D., 1982. Stomatal conductance and photosynthesis. Annual Review of Plant Physiology 33(1), 317-345.

Frazier, T.P., Burklew, C.E., Zhang, B., 2014. Titanium dioxide nanoparticles affect the growth and microRNA expression of tobacco (*Nicotiana tabacum*). Functional & Integrative Genomics 14(1), 75-83.

Feizi, H., Moghaddam, P. R., Shahtahmassebi, N., Fotovat, A., 2012. Impact of bulk and nanosized titanium dioxide (TiO_2) on wheat seed germination and seedling growth. Biological Trace Element Research 146(1), 101-106.

Feizi, H., Kamali, M., Jafari, L., Moghaddam, P.R., 2013. Phytotoxicity and stimulatory impacts of nanosized and bulk titanium dioxide on fennel (*Foeniculum vulgare Mill*). Chemosphere 91(4), 506-511.

Gao, J., Xu, G., Qian, H., Liu, P., Zhao, P., Hu, Y., 2013. Effects of nano-TiO_2 on photosynthetic characteristics of *Ulmus elongata* seedlings. Environmental Pollution 176, 63-70.

Ghosh, M., Bandyopadhyay, M., Mukherjee, A., 2010. Genotoxicity of titanium dioxide (TiO_2) nanoparticles at two trophic levels: plant and human lymphocytes. Chemosphere 81(10), 1253-1262.

Gottschalk, F., Lassen, C., Kjoelholt, J., Christensen, F., Nowack, B., Modeling flows and concentrations of nine engineered nanomaterials in the Danish environment. International Journal of Environmental Research and Public Health 2015, 12(5): 5581-5602.

Hammond, S.A., Veldhoen, N., Helbing, C.C., 2015. Influence of temperature on thyroid hormone signaling and endocrine disruptor action in Rana (*Lithobates*) catesbeiana tadpoles. General and Comparative Endocrinology 219, 6-15.

Haydon, M.J., Cobbett, C.S., 2007. Transporters of ligands for essential metal ions in plants. New Phytologist 174(3), 499-506.

Hong, F., Yang, F., Liu, C., Gao, Q., Wan, Z., Gu, F., Wu, C., Ma, Z., Zhou, J., Yang, P., 2005. Influences of nano-TiO_2 on the chloroplast aging of spinach under light. Biologucal Trace Element Research 104, 249-260.

Hong, S., Bielinska, A. U., Mecke, A., Keszler, B., Beals, J. L., Shi, X., Balogh, L., Orr, B.G., Baker, J.R., Banaszak Holl, M. M., 2004. Interaction of poly(amidoamine) dendrimers with supported lipid bilayers and cells: hole formation and the relation to transport. Bioconjugate Chemistry 15(4), 774-782.

Jones, J. B. Jr, ed. Hydroponics: a practical guide for the soilless grower. CRC press, Boca Raton, Florida, 2016.

Kaplan, F., Guy, C.L., 2005. RNA interference of Arabidopsis beta - amylase8 prevents maltose accumulation upon cold shock and increases sensitivity of PSII photochemical efficiency to freezing stress. The Plant Journal 44(5), 730-743.

Keller, A.A., Lazareva, A., 2013. Predicted releases of engineered nanomaterials: from global to regional to local. Environmental Science & Technology Letters 1, 65-70.

Khodary, S.E.A., 2004. Effect of salicylic acid on the growth, photosynthesis and carbohydrate metabolism in salt-stressed maize plants. International Journal of Agriculture and Biology 6(1), 5-8.

Larue, C., Laurette, J., Herlin-Boime, N., Khodja, H., Fayard, B., Flank, A.M., Brisset, F., Carriere, M., 2012. Accumulation, translocation and impact of TiO_2 nanoparticles in wheat (*Triticum aestivum spp*.): influence of diameter and crystal phase. Science of the Total Environment 431,197-208.

Larue, C., Castillo-Michel, H., Sobanska, S., Trcera, N., Sorieul, S., Cécillon, L., Ouerdane, L., Legros, S., Sarret, G., 2014. Fate of pristine TiO_2 nanoparticles and aged paint-containing TiO_2 nanoparticles in lettuce crop after foliar exposure. Journal of Hazardous Materials 273, 17-26.

Larue, C., Castillo-Michel, H., Stein, R.J., Fayard, B., Pouyet, E., Villanova, J., Magnin, V., Real, A.P., Trcera, N., Legros, S., Sorieul, S., Sarrest, G., 2016. Innovative combination of

spectroscopic techniques to reveal nanoparticle fate in a crop plant. Spectrochimica Acta Part B: Atomic Spectroscopy 119, 17-24.

Liu, H., Ma, C., Chen, G., White, J.C., Wang, Z., Xing, B., Dhankher, O.P., 2017. Titanium Dioxide Nanoparticles Alleviate Tetracycline Toxicity to *Arabidopsis thaliana* (L.). ACS Sustainable Chemistry & Engineering, 5(4), 3204-3213.

Löhnis, M.P., 1951. Manganese toxicity in field and market garden grops. Plant and Soil 3(3), 193-222.

Ma, X., Wang, Q., Rossi, L., Ebbs, S.D., White, J.C., 2016. Multigenerational exposure to cerium oxide nanoparticles: physiological and biochemical analysis reveals transmissible changes in rapid cycling *Brassica rapa*. NanoImpact 1, 46-54.

Makarova, O.V., Rajh, T., Thurnauer, M.C., Martin, A., Kemme, P.A., Cropek, D., 2000. Surface modification of TiO_2 nanoparticles for photochemical reduction of nitrobenzene. Environmental Science & Technology 34(22), 4797-4803.

Morales, M.I., Rico, C.M., Hernandez-Viezcas, J.A., Nunez, J.E., Barrios, A.C., Tafoya, A., Flores-Marges, J.P., Peralta-Videa, J.R., Gardea-Torresdey, J.L., 2013. Toxicity assessment of cerium oxide nanoparticles in cilantro (*Coriandrum sativum L.*) plants grown in organic soil. Journal of Agricultural and Food Chemistry 61, 6224-6230.

Moreno-Olivas, F., Gant, V.U., Johnson, K.L., Peralta-Videa, J. R., Gardea-Torresdey, J.L., 2014. Random amplified polymorphic DNA reveals that TiO_2 nanoparticles are genotoxic to *Cucurbita pepo*. Journal of Zhejiang University SCIENCE A 15(8), 618-623.

Nomura, H., Shiina, T., 2014. Calcium signaling in plant endosymbiotic organelles: mechanism and role in physiology. Molecular Plant 7(7), 1094-1104.

Pakrashi, S., Jain, N., Dalai, S., Jayakumar, J., Chandrasekaran, P.T., Raichur, A.M., Chandrasekaran, N., Mukherjee, A., 2014. *In vivo* genotoxicity assessment of titanium dioxide nanoparticles by *Allium cepa* root tip assay at high exposure concentrations. PLoS One 9(2), e87789.

Peralta-Videa, J.R., Hernandez-Viezcas, J.A., Zhao, L., Diaz, B.C., Ge, Y., Priester, J.H., Holden, P.A., Gardea-Torresdey, J.L., 2014. Cerium dioxide and zinc oxide nanoparticles alter the nutritional value of soil cultivated soybean plants. Plant Physiology and Biochemistry 80, 128-135.

Porra, R.J., 1991. Recent advances and re-assessments in chlorophyll extraction and assay procedures for terrestrial, aquatic, and marine organisms, including recalcitrant algae. Chlorophylls 31.

Rahman, L., Wu, D., Johnston, M., Williams, A., Halappanavar, S., 2016. Toxicogenomics analysis of mouse lung responses following exposure to titanium dioxide nanomaterials reveal their disease potential at high doses. Mutagenesis 32(1), 59-76.

Raven, P.H., Evert, R.F., Eichhorn, S.E., in Biology of Plants, W.H. Freeman. Gordonsville, VA, 7th ed., 2005, Photosynthesis, Light, and Life, pp. 119–127.

Rezaei, F., Moaveni, P., Mozafari, H., 2015. Effect of different concentrations and time of nano TiO2 spraying on quantitative and qualitative yield of soybean (*Glycine max* L.) at Shahr-e-Qods, Iran. Biological Forum 7(1), 957.

Rico, C.M., Johnson, M.G., Marcus, M.A., Andersen, C.P., 2017. Intergenerational responses of wheat (*Triticum aestivum* L.) to cerium oxide nanoparticles exposure. Environmental Science: Nano, 4(3), 700-711.

Sachtleben Chemie GmbH, Duisburg, Germany, Product information UV-TITAN M212.

Sachtleben Chemie GmbH, Duisburg, Germany, Product information UV-TITAN M262.

Sasaki, T., Mori, I. C., Furuichi, T., Munemasa, S., Toyooka, K., Matsuoka, K., Murata, Y., Yamamoto, Y., 2010. Closing plant stomata requires a homolog of an aluminum-activated malate transporter. Plant and Cell Physiology 51(3), 354-365.

Schaaf, G., Ludewig, U., Erenoglu, B. E., Mori, S., Kitahara, T., von Wirén, N., 2004. ZmYS1 functions as a proton-coupled symporter for phytosiderophore-and nicotianamine-chelated metals. Journal of Biological Chemistry 279(10), 9091-9096.

Seo, J., Chung, H., Kim, M., Lee, J., Choi, I.H., Cheon, J., 2007. Development of water-soluble single-crystalline TiO_2 nanoparticles for photocatalytic cancer-cell treatment. Small 3(5), 850-853.

Seeger, E. M., Baun, A., Kästner, M., Trapp, S., 2009. Insignificant acute toxicity of TiO_2 nanoparticles to willow trees. Journal of Soils and Sediments 9(1), 46-53.

Servin, A.D., Castillo-Michel, H., Hernandez-Viezcas, J.A., Diaz, B.C., Peralta-Videa, J.R., Gardea-Torresdey, J.L., 2012. Synchrotron micro-XRF and micro-XANES confirmation of the uptake and translocation of TiO_2 nanoparticles in cucumber (*Cucumis sativus*) plants. Environmental Science & Technology 46, 7637-7643.

Servin, A.D., Morales, M.I., Castillo-Michel, H., Hernandez-Viezcas, J.A., Munoz, B., Zhao, L., Nunez, J.E., Peralta-Videa, J.R., Gardea-Torresdey, J.L., 2013. Synchrotron verification of TiO_2 accumulation in cucumber fruit: a possible pathway of TiO_2 nanoparticle transfer from soil into the food chain. Environmental Science & Technology 47, 11592-11598.

Siddiqi, K.S., Husen, A., 2017. Plant Response to Engineered Metal Oxide Nanoparticles. Nanoscale Research Letters 12(1), 92.

Solomons, T. W. G., Fryhle, C. B., Carbohydrates. Organic Chemistry, 10 ed. John Wiley & Sons, Inc., 2011, pp. 1001-1002.

Somogyi, M., 1952. Notes on sugar determination. Journal of Biological Chemistry 195, 19-23.

Song, G., Gao, Y., Wu, H., Hou, W., Zhang, C., Ma, H., 2012. Physiological effect of anatase TiO_2 nanoparticles on *Lemna minor*. Environmental Toxicology and Chemistry 31(9), 2147-2152.

Song, U., Jun, H., Waldman, B., Roh, J., Kim, Y., Yi, J., Lee, E.J., 2013. Functional analyses of nanoparticle toxicity: a comparative study of the effects of TiO_2 and Ag on tomatoes (*Lycopersicon esculentum*). Ecotoxicology and Environmental Safety 93, 60-67.

Tan, W., Du, W., Barrios, A.C., Armendariz, R., Zuverza-Mena, N., Ji, Z., Hernandez-Viezcas, J.A., Peralta-Videa, J.R., Gardea-Torresdey, Gardea-Torresdey, J.L., 2017. Surface coating changes the physiological and biochemical impacts of nano-TiO_2 in basil (*Ocimum basilicum*) plants. Environmental Pollution 222, 64-72.

Tewari, R.K., Kumar, P., Sharma, P.N., 2006. Magnesium deficiency induced oxidative stress and antioxidant responses in mulberry plants. Scientia Horticulturae 108(1), 7-14.

Thalmann, M.R., Pazmino, D., Seung, D., Horrer, D., Nigro, A., Meier, T., Kölling, K., Pfeifhofer, H.W., Zeeman S.C., Santelia, D., 2016. Regulation of leaf starch degradation by abscisic acid is important for osmotic stress tolerance in plants. The Plant Cell, 29(9).

Verma, S., Dubey, R., 2001. Effect of cadmium on soluble sugars and enzymes of their metabolism in rice. Biologua Plantarum 44, 117-123.

Wang, S., Kurepa, J., Smalle, J.A., 2011. Ultra-small TiO_2 nanoparticles disrupt microtubular networks in *Arabidopsis thaliana*. Plant, Cell & Environment 34(5), 811-820.

Wang, Q., Ebbs, S. D., Chen, Y., Ma, X., 2013. Trans-generational impact of cerium oxide nanoparticles on tomato plants. Metallomics 5(6), 753-759.

Wang, Z., Xu, L., Zhao, J., Wang, X., White, J. C., Xing, B., 2016. CuO Nanoparticle interaction with Arabidopsis thaliana: Toxicity, parent-progeny transfer, and gene expression. Environmental Science & Technology 50(11), 6008-6016.

Weir, A., Westerhoff, P., Fabricius, L., Hristovski, K., von Goetz, N., 2012. Titanium dioxide nanoparticles in food and personal care products. Environmental Science & Technology 2012, 46(4), 2242-2250.

Zuverza-Mena, N., Martínez-Fernández, D., Du, W., Hernandez-Viezcas, J. A., Bonilla-Bird, N., López-Moreno, M. L., Komárek, M., Gardea-Torresdey, J.L., 2017. Exposure of engineered nanomaterials to plants: Insights into the physiological and biochemical responses-A review. Plant Physiology and Biochemistry 110, 236-264.

Yano, R., Nakamura, M., Yoneyama, T., Nishida, I., 2005. Starch-related α-glucan/water dikinase is involved in the cold-induced development of freezing tolerance in Arabidopsis. Plant Physiology 138(2), 837-846.

Yang, F., Liu, C., Gao, F., Su, M., Wu, X., Zheng, L., Hong F.,Yang, P., 2007. The improvement of spinach growth by nano-anatase TiO_2 treatment is related to nitrogen photoreduction. Biological Trace Element Research 119(1), 77-88.

Ze, Y., Liu, C., Wang, L., Hong, M., Hong, F., 2011. The regulation of TiO_2 nanoparticles on the expression of light-harvesting complex II and photosynthesis of chloroplasts of *Arabidopsis thaliana*. Biological Trace Element Research 143(2), 1131-1141.

Zheng, L., Hong, F., Lu, S., Liu, C., 2005. Effect of nano-TiO_2 on strength of naturally aged seeds and growth of spinach. Biological Trace Element Research 104(1), 83-91.

(1) Servin, A.; Elmer, W.; Mukherjee, A.; De la Torre-Roche, R.; Hamdi, H.; White, J. C.; Bindraban, P.; Dimkpa, C. A review of the use of engineered nanomaterials to suppress plant disease and enhance crop yield. *J. Nanopart. Res*. **2015**, 17(2), 92.

(2) Zhao, L.; Huang, Y.; Keller, A. A. Comparative metabolic response between cucumber (*Cucumis sativus*) and corn (*Zea Mays*) to a $Cu(OH)_2$ nanopesticide. *J. Agric. Food Chem*. **2017**. DOI: 10.1021/acs.jafc.7b01306.

(3) Clark, J. F. On the toxic properties of some copper compounds with special reference to Bordeaux mixture. *Botanical Gazette*, 1902, *33*(1), 26-48.

(4) Brun, L. A.; Maillet, J.; Richarte, J.; Herrmann, P.; Remy, J. C. Relationships between extractable copper, soil properties and copper uptake by wild plants in vineyard soils. *Environ. Pollut*. 1998, *102*(2), 151-161.

(5) Hong, J.; Rico, C. M.; Zhao, L.; Adeleye, A. S.; Keller, A. A.; Peralta-Videa, J. R.; Gardea-Torresdey, J. L. Toxic effects of copper-based nanoparticles or compounds to lettuce (*Lactuca sativa*) and alfalfa (*Medicago sativa*). *Environ. Sci. Processes Impacts* **2015**, 17(1), 177-185.

(6) Keller, A. A.; Adeleye, A. S.; Conway, J. R.; Garner, K. L.; Zhao, L.; Cherr, G.; Hong, J.; Gardea-Torresdey, J. L.; Godwina, H. A.; Hanna, S.; Ji, Z.; Kaweeteerawat, C.; Lin, S.; Lenihan, H. S.; Miller, R. J.; Nel, A. E.; Peralta-Videa, J. R.; Walker, S. L.; Taylor, A. A.; Torres-Duarte, C.; Zink, J. I.; Zuverza-Mena, N. Comparative environmental fate and toxicity of copper nanomaterials. *NanoImpact*, **2017**, 7, 28-40.

(7) US Research Nanomaterials Inc. http://www.us-nano.com/inc/sdetail/29132

(8) Zhao, L.; Huang, Y.; Hannah-Bick, C.; Fulton, A. N.; Keller, A. A. Application of metabolomics to assess the impact of $Cu(OH)_2$ nanopesticide on the nutritional value of lettuce (*Lactuca sativa*): Enhanced Cu intake and reduced antioxidants. *NanoImpact*, **2016**, 3, 58-66.

(9) Zhao, L.; Ortiz, C.; Adeleye, A. S.; Hu, Q.; Zhou, H.; Huang, Y.; Keller, A. A. Metabolomics to detect response of lettuce (*Lactuca sativa*) to $Cu(OH)_2$ nanopesticides: oxidative stress response and detoxification mechanisms. *Environ. Sci. Technol*. **2016**, 50(17), 9697-9707.

(10) Zhao, L.; Hu, Q.; Huang, Y.; Fulton, A. N.; Hannah-Bick, C.; Adeleye, A. S.; Keller, A. A. Activation of antioxidant and detoxification gene expression in cucumber plants exposed to a $Cu(OH)_2$ nanopesticide. *Environ. Sci. Nano* **2017**, 4(8), 1750-1760.

(11) Zuverza-Mena, N.; Medina-Velo, I. A.; Barrios, A. C.; Tan, W.; Peralta-Videa, J. R.; Gardea-Torresdey, J. L. Copper nanoparticles/compounds impact agronomic and physiological parameters in cilantro (*Coriandrum sativum*). *Environ. Sci. Processes Impacts* **2015**, 17(10), 1783-1793.

(12) Zhao, L.; Huang, Y.; Adeleye, A. S.; Keller, A. A. Metabolomics Reveals $Cu(OH)_2$ nanopesticide-activated anti-oxidative pathways and decreased beneficial antioxidants in spinach leaves. *Environ. Sci. Technol*. **2017**, 51(17).

(13) Wu, S. G.; Huang, L.; Head, J.; Chen, D. R.; Kong, I. C.; Tang, Y. J. Phytotoxicity of metal oxide nanoparticles is related to both dissolved metals ions and adsorption of particles on seed surfaces. *J. Pet. Environ. Biotechnol*. **2012**, 3, 126.

(14) Nair, P. M. G.; Chung, I. M. Study on the correlation between copper oxide nanoparticles induced growth suppression and enhanced lignification in Indian mustard (*Brassica juncea* L.). *Ecotoxicol. Environ. Saf.* **2015**, 113, 302-313.

(15) Körpe, D. A.; Aras, S. Evaluation of copper-induced stress on eggplant (*Solanum melongena* L.) seedlings at the molecular and population levels by use of various biomarkers. *Mutat. Res. Genet. Toxicol. Environ. Mutagen*. **2011**, 719(1), 29-34.

(16) Bivi, M. R.; Siti Noor Farhana, M. D.; Khairulmazmi, A.; Idris, A. S.; Susilawati, K.; Sariah, M. Assessment of plant secondary metabolites in oil palm seedlings after being treated with calcium, copper ions and salicylic acid. *Arch. Phytopathology Plant Prot*. **2014**, 47(9), 1120-1135.

(17) Apodaca, S. A.; Tan, W.; Dominguez, O. E.; Hernandez-Viezcas, J. A.; Peralta-Videa, J. R.; Gardea-Torresdey, J. L. Physiological and biochemical effects of nanoparticulate copper, bulk copper, copper chloride, and kinetin in kidney bean (*Phaseolus vulgaris*) plants. *Sci. Total Environ*. **2017**, 599, 2085-2094.

(18) Amirjani, M. R. Effect of salinity stress on growth, sugar content, pigments and enzyme activity of rice. *Int. J. Bot*. **2011**, 7, 73-81.

(19) Idrees, M.; Khan, M. M. A.; Aftab, T.; Naeem, M.; Hashmi, N. Salicylic acid-induced physiological and biochemical changes in lemongrass varieties under water stress. *J. Plant Interac*. **2010**, 5(4), 293-303.

(20) Norton, G. J.; Dasgupta, T.; Islam, M. R.; Islam, S.; Deacon, C. M.; Zhao, F. J.; Stroud, J. L.; Mcgrath, S. P.; Feldmann, J.; Price, A. H.; Meharg, A. A. Arsenic influence on genetic variation

in grain trace-element nutrient content in Bengal Delta grown rice. *Environ. Sci. Technol*. **2010**, 44, 8284-8288.

(21) Rico, C. M.; Morales, M. I.; Barrios, A. C.; McCreary, R.; Hong, J.; Lee, W. Y.; Nunez, J.; Peralta-Videa, J. R.; Gardea-Torresdey, J. L. Effect of cerium oxide nanoparticles on the quality of rice (*Oryza sativa* L.) grains. *J. Agric. Food Chem*. **2013**, 61(47), 11278-11285.

(22) Barbieri, G.; Vallone, S.; Orsini, F.; Paradiso, R.; De Pascale, S.; Negre-Zakharov, F.; Maggio, A. Stomatal density and metabolic determinants mediate salt stress adaptation and water use efficiency in basil (*Ocimum basilicum* L.). *J. Plant Physiol*. **2012**, 169(17), 1737-1746.

(23) Simon, J. E.; Morales, M. R.; Phippen, W. B.;Vieira, R. F.; Hao, Z. Basil: a source of aroma compounds and a popular culinary and ornamental herb. *Perspect. New Crops New Uses* **1999**, 499-505.

(24) Prasad, G.; Kumar, A.; Singh, A. K.; Bhattacharya, A. K.; Singh, K.; Sharma, V. D. Antimicrobial activity of essential oils of some *Ocimum* species and clove oil. *Fitoterapia*, **1986**, 57, 429-432.

(25) Rastogi, S. C.; Lepoittevin, J. P.; Johansen, J. D.; Frosch, P. J.; Menné, T.; Bruze, M.; Dreier, B.; Andersen, K. E.; White, I. R. Fragrances and other materials in deodorants: search for potentially sensitizing molecules using combined GC-MS and structure activity relationship (SAR) analysis. *Contact Dermatitis* **1998**, 39(6), 293-303.

(26) Bertram, H. J.; Obrocki, T.; Luders, S. U.S. Patent Application, 2007, No. 11/854,854.

(27) Tago, A.; Yokoyama, S.; Ishikawa, M.; Koshio, S. Pharmacokinetics of eugenol in Japanese flounder, *Paralichthys olivaceus*. *J. World Aquacult. Soc*. **2017**. DOI: 10.1111/jwas.12438.

(28) SelfNutritionData.http://nutritiondata.self.com/foods00214000000000000000.html?max Count=25.

(29) Upchurch, R. G. Fatty acid unsaturation, mobilization, and regulation in the response of plants to stress. *Biotechnol. Lett*. **2008**, 30 (6) 967-977.

(30) Peetla, C.; Vijayaraghavalu, S.; Labhasetwar, V. Biophysics of cell membrane lipids in cancer drug resistance: implications for drug transport and drug delivery with nanoparticles. *Adv. Drug Delivery Rev*. **2013**, 65(13-14), 1686-1698.

(31) [CuPro* 2005T/N/O]

(32) Barrios, A. C.; Rico, C. M.; Trujillo-Reyes, J.; Medina-Velo, I. A.; Peralta-Videa, J. R.; Gardea-Torresdey, J. L. Effects of uncoated and citric acid coated cerium oxide nanoparticles, bulk cerium oxide, cerium acetate, and citric acid on tomato plants. *Sci. Total Environ.* **2016**, 563, 956-964.

(33) Porra, R.J. Recent advances and re-assessments in chlorophyll extraction and assay procedures for terrestrial, aquatic, and marine organisms, including recalcitrant algae. *Chlorophylls*, **1991**, 31.

(34) Deubert, K.H. A rapid method for the extraction and quantitation of total anthocyanin of cranberry fruit. *J. Agric. Food Chem.* **1978**, 26(6), 1452-1453.

(35) Baublis, A.; Spomer, A. R. T.; Berber-Jimenez, M. D. Anthocyanin pigments: comparison of extract stability. *J. Food Sci*. **1994**, 59(6), 1219-1221.

(36) Klopotek, Y.; Otto, K.; Böhm, V. Processing strawberries to different products alters contents of vitamin C, total phenolics, total anthocyanins, and antioxidant capacity. *J. Agric. Food Chem*. **2005**, 53(14), 5640-5646.

(37) Browse, J.; McCourt, P. J.; Somerville, C. R. Fatty acid composition of leaf lipids determined after combined digestion and fatty acid methyl ester formation from fresh tissue. *Anal. Biochem*. **1986**, 152, 141-145

(38) Rico, C. M.; Morales, M. I.; McCreary, R.; Castillo-Michel, H.; Barrios, A. C.; Hong, J.; Tafoya, A.; Lee, W-Y.; Varela-Ramirez, A.; Peralta-Videa, J. R.; Gardea-Torresdey, J. L. Cerium oxide nanoparticles modify the antioxidative stress enzyme activities and macromolecule composition in rice seedlings. *Environ. Sci. Technol*. **2013**, 47(24), 14110-14118.

(39) Krajíčková, A.; Mejstřik, V. The effect of fly ash particles on the plugging of stomata. *Environ. Pollut. Series A, Ecol. Biol*. **1984**, 36(1), 83-93.

(40) Wang, Z.; Xie, X.; Zhao, J.; Liu, X.; Feng, W.; White, J. C.; Xing, B. Xylem-and phloem-based transport of CuO nanoparticles in maize (*Zea mays* L.). *Environ. Sci. Technol.* **2012**, *46*(8), 4434-4441.

(41) Vencalek, B. E.; Laughton, S. N.; Spielman-Sun, E.; Rodrigues, S. M.; Unrine, J. M.; Lowry, G. V.; Gregory, K. B., In situ measurement of CuO and $Cu(OH)_2$ nanoparticle dissolution rates in quiescent freshwater mesocosms. *Environ. Sci. Technol. Lett*. **2016**, 3(10), 375-380.

(42) Castañeda-Ovando, A.; de Lourdes Pacheco-Hernández, M.; Páez-Hernández, M. E.; Rodríguez, J. A.; Galán-Vidal, C. A. Chemical studies of anthocyanins: A review. *Food Chem.* **2009**, 113(4), 859-871.

(43) Medina-Velo, I. A.; Dominguez, O. E.; Ochoa, L.; Barrios, A. C.; Hernández-Viezcas, J. A.; White, J. C.; **Peralta-Videa**, **J. R.;** Gardea-Torresdey, J. L. Nutritional quality of bean seeds harvested from plants grown in different soils amended with coated and uncoated zinc oxide nanomaterials. *Environ. Sci.: Nano*, **2017**. DOI: 10.1039/C7EN00495H

(44) Majumdar, S.; Peralta-Videa, J. R.; Trujillo-Reyes, J.; Sun, Y.; Barrios, A. C.; Niu, G., Flores-Margez, J. P.; Gardea-Torresdey, J. L. Soil organic matter influences cerium translocation and physiological processes in kidney bean plants exposed to cerium oxide nanoparticles. *Sci. Total Environ.* **2016**, 569, 201-211.

(45) Hong, J.; Wang, L.; Sun, Y.; Zhao, L.; Niu, G.; Tan, W.; Rico, C. M.; Peralta-Videa, J. R.; Gardea-Torresdey, J. L. Foliar applied nanoscale and microscale CeO_2 and CuO alter cucumber (*Cucumis sativus*) fruit quality. *Sci. Total Environ.* **2016**, 563, 904-911.

(46) Trujillo-Reyes, J.; Majumdar, S.; Botez, C. E.; Peralta-Videa, J. R.; Gardea-Torresdey, J. L. Exposure studies of core–shell Fe/Fe_3O_4 and Cu/CuO NPs to lettuce (*Lactuca sativa*) plants: Are they a potential physiological and nutritional hazard? *J. Hazard. Mater.* **2014**, 267, 255-263.

(47) Dimkpa, C. O.; McLean, J. E.; Britt, D. W.; Anderson, A. J. Nano-CuO and interaction with nano-ZnO or soil bacterium provide evidence for the interference of nanoparticles in metal nutrition of plants. *Ecotoxicology*, **2015**, 24(1), 119-129.

(48) Le Van, N.; Ma, C.; Shang, J.; Rui, Y.; Liu, S.; Xing, B. Effects of CuO nanoparticles on insecticidal activity and phytotoxicity in conventional and transgenic cotton. *Chemosphere*, **2016**, 144, 661-670.

(49) Sasaki, A.; Yamaji, N.; Yokosho, K.; Ma, J. F. Nramp5 is a major transporter responsible for manganese and cadmium uptake in rice. *Plant Cell*, **2012**, *24*(5), 2155-2167.

(50) Vert, G.; Grotz, N.; Dédaldéchamp, F.; Gaymard, F.; Guerinot, M. L.; Briat, J. F.; Curie, C. IRT1, an *Arabidopsis* transporter essential for iron uptake from the soil and for plant growth. *Plant Cell*, **2002**, 14(6), 1223-1233.

(51) Hojati, M.; Modarres-Sanavy, S. A. M.; Enferadi, S. T.; Majdi, M.; Ghanati, F.; Farzadfar, S.; Pazoki, A., Cadmium and copper induced changes in growth, oxidative metabolism and terpenoids of *Tanacetum parthenium*. *Environ. Sci. Pollut. Res*. **2017**, 24(13), 12261-12272.

(52) Zheljazkov, V. D.; Warman, P. R. Application of high Cu compost to Swiss chard and basil. *Sci. Total Environ*. **2003**, 302(1), 13-26.

(53) Zheljazkov, V. D.; Craker, L. E.; Xing, B. Effects of Cd, Pb, and Cu on growth and essential oil contents in dill, peppermint, and basil. *Environ. Exp. Bot*. **2006**, 58(1), 9-16.

(54) Elzaawely, A. A.; Xuan, T. D.; Tawata, S. Changes in essential oil, kava pyrones and total phenolics of *Alpinia zerumbet* (Pers.) BL Burtt. & RM Sm. leaves exposed to copper sulphate. *Environ. Exp. Bot.* **2007**, 59(3), 347-353.

(55) Juntachote, T.; Berghofer, E. Antioxidative properties and stability of ethanolic extracts of Holy basil and Galangal. *Food Chem*. **2005**, 92(2), 193-202.

(56) Ouariti, O.; Boussama, N.; Zarrouk, M.; Cherif, A.; Ghorbal, M. H., Cadmium-and copper-induced changes in tomato membrane lipids. *Phytochemistry*, **1997**, 45(7), 1343-1350.

(57) Lin, C. Y.; Trinh, N. N.; Fu, S. F.; Hsiung, Y. C.; Chia, L. C.; Lin, C. W.; Huang, H. J., Comparison of early transcriptome responses to copper and cadmium in rice roots. *Plant Mol. Boil.* **2013**, 81(4-5), 507-522.

(58) Yuan, J.; He, A.; Huang, S.; Hua, J.; Sheng, G. D. Internalization and phytotoxic effects of CuO nanoparticles in *Arabidopsis thaliana* as revealed by fatty acid profiles. *Environ. Sci. Technol*. **2016**, 50(19), 10437-10447.

(59) Clemens, S. Molecular mechanism of plant metal tolerance and homeostasis. *Planta* **2001**, 212, 475-486

(60) Savini, I.; D'Alessio, S.; Giartosio, A.; Morpurgo, L.; Avigliano, L. The role of copper in the stability of ascorbate oxidase towards denaturing agents. *The FEBS J.* **1990**, 190(3), 491-495.

Appendix

1. SUPPORTING INFORMATION FOR CHAPTER 2: Surface Coating Changes the Physiological and Biochemical Impacts of nano-TiO_2 in Basil (*Ocimum basilicum*) Plants

1.1 Materials and Methods

1.1.1 Characterization of TiO_2 nanoparticles. Transmission electron microscopy (TEM, FEI Techni T12, accelerating voltage 80 kV) was used to determine the size and shape of the three nano-TiO_2 particles. For the TEM analysis, a drop of nano-TiO_2 (50 μg/mL suspended in deionized water) was placed on the grids and air-dried at room temperature. BET surface area was measured using a Quantachrome Instruments (QUADRASORB SI) with nitrogen as the adsorption gas. For identification of the crystal structure of each material, powder X-ray diffraction (XRD) spectra were obtained on a Philips X'Pert Pro diffractometer, equipped with Cu Kα radiation. Hydrodynamic size and zeta-potential measurement of the TiO_2 nanoparticle suspensions in water were performed using a ZetaPALS instrument (Zeta Potential Analyzer, Brookhaven Instruments, Holtsville, NY). Material impurity was quantified using thermal gravimetric analysis (TGA) on a Pyris Diamond TG/ DTA, Perkin Elmer.

1.1.2 Al^{3+} leakage determinations. Suspensions of hydrophobic and hydrophilic nano-TiO_2 were prepared at 750 $mg \cdot kg^{-1}$ in MPW. The release of Al^{3+} was determined by suspending the nano-TiO_2 in MPW or soil. Each measurement was performed three times. Two mL of suspensions, stored at room temperature, were centrifuged at 15 000 × g for 60 min at 0, 1, 2, 3, 4, and 7 days. The supernatant (1 mL) of each sample was transferred to clean tubes (SC475, Environmental Express) for acid digestion. Digestion was carried out with 10 mL of concentrated HNO_3 (65-70%, Trace Metal Grade, Fisher Scientific) at 95 °C overnight in a HotBlock (SC100, Environmental Express). Once all digested solutions dried out, the samples were cooled at room

temperature and subsequently diluted with 2 % (v/v) HNO_3 at 95 °C for 3 h to extract the analytes. For soil medium, 5 g of soil were mixed with nano-TiO_2 suspension in 20 mL MPW and shaked for 24 h. After 4 h, the supernatants were collected and centrifuged at 5 000 rpm for 30 min (Eppendorf AG 5417R, Hamburg, Germany) to avoid the interference of particulate matters. Then, two mL of supernatants were centrifuged at 14 000 rpm for 30 min. The supernatants were transferred and centrifuged at 14 000 rpm for 30 min. The centrifugation process was repeated three times. The supernatants from both media were diluted into 15 mL with the 2% (v/v) HNO_3, and the release of Al^{3+} was measured by ICP-OES as described below.

1.1.3 Carbohydrate and enzyme activity measurement. Fresh roots and leaves (~200 mg) were ground in liquid nitrogen and homogenized with 2 mL 50 mM potassium phosphate buffer (pH=7.4), which contained 1 mM ethylenediaminetetraacetic acid (EDTA), 1% polyvynilpyrrolidone (PVP), and 0.5% (v/v) Triton X-100. The crude extracts were centrifuged at 10 000 rpm (Eppendorf AG bench centrifuge 5417R, Hamburg, Germany) for 10 min at 4℃, collected, and stored at -80℃.

1.2 Results and Discussion

1.2.1 Leakage of Al^{3+} from the surface coating

It is believed that the coating of Al_2O_3 on the NP surface could enhance the optical properties of nano-TiO_2 (Fabregat-Santiago et al., 2004). However, the chemistry property of Al_2O_3 on the surface of NPs and the possible release of Al^{3+} are suspected to play certain roles in the interaction between nano-TiO_2 and plants. The Al^{3+} leakage from the surface coating is displayed in Figure 2.2. On one hand, after seven days, the dissolution Al^{3+} from M212 and M262 particles in MPW was lower than 0.4 $mg \cdot kg^{-1}$ (Figure 2.2). The concentration of exchangeable Al^{3+} in soil in United States is 10.8 $mg \cdot kg^{-1}$ and in United Kingdom is 270 $mg \cdot kg^{-1}$ (Sparks 2003).

This indicates the contributions of NPs to Al^{3+} concentration in soil could be neglected. On the other hand, in soil medium, Al concentrations of nano-TiO_2 up to seven days showed no significant differences compared with blank soil (Figure 2.2). These results revealed the Al_2O_3 coatings were well encapsulated by the organic matters. Meanwhile, results of Al accumulation in plants revealed no significant difference between NPs treatments and controls (SI Table 1.3 and 1.4). Also, the determinations of Ti absorption (Figure 2.3 and SI Table 1.2), growth parameters (Table 2.4 and SI Table 1.5), carbohydrates contents (Figure 2.4 and SI Table 1.6) and enzyme activities (Figure 2.5 and SI Table 1.7) showed contrasting results between hydrophilic and hydrophobic nanoparticles. Such results suggest that the coating of Al_2O_3 did not play a major role in affecting the Ti uptake, physiological and biochemical parameters of basil plants. This also supports the lack of treatments with ionic Al^{3+} in the experimental setup.

SI Table 1.1 Characterizations of top soil Garden Pro® (total concentration of micro-/macro-elements, pH, total dissolve solid and electro-conductivity). Data are means of four replicates ± SE.

Element	Concentration ($mg \cdot kg^{1}$)	Parameters	Value
Al	4872.4 ± 452.3	pH	6.75 ± 0.15
Cu	42.8 ± 9.1	Total dissolve solid	2855 ± 225 $mg \cdot kg^{-1}$
Fe	6794.9 ± 235.5	Electro-conductivity	5715 ± 445 $\mu S \cdot cm^{-1}$
Mn	90.0 ± 14.3		
Se	76.0 ± 3.7		
Zn	50.2 ± 14.6		
Ca	29086.9 ± 2292.2		
Mg	3869.6 ± 714.6		
P	5181.5 ± 734.9		
S	9525.5 ± 1445.2		
Ti	137.8 ± 29.2		

SI Table 1.2 Ti concentration in roots and shoots of basil plants grown for 65 days in soil amended with 0 - 750 $mg \cdot kg^{-1}$ of unmodified, hydrophobic, and hydrophilic nano-TiO_2. Data are means of four replicates ± SE. Different letters indicate statistically significant differences between control and treatment concentrations ($p \leq 0.05$).

Particle type	Nano-TiO_2 in soil ($mg \cdot kg^{-1}$)	Ti concentration ($mg \cdot kg^{-1}$)	
		root	shoot
Pristine nano-TiO_2	0	15.3 ± 5.0 b	19.8 ± 3.9
	125	28.2 ± 2.3 b	19.9 ± 1.3
	250	37.0 ± 11.2 b	23.4 ± 1.7
	500	77.1 ± 11.0 a	20.5 ± 1.0
	750	86.1 ± 9.4 a	19.2 ± 2.3
Hydrophobic nano-TiO_2	0	15.3 ± 5.0 b	19.8 ± 3.9
	125	18.4 ± 4.1 b	19.3 ± 0.3
	250	59.4 ± 14.6 b	19.4 ± 1.0
	500	115.5 ± 26.1 a	19.9 ± 1.6
	750	160.4 ± 13.2 a	18.9 ± 1.4
Hydrophilic nano-TiO_2	0	15.3 ± 5.0 c	19.8 ± 3.9
	125	16.6 ± 2.3 c	17.6 ± 2.0
	250	32.1 ± 7.2 bc	18.0 ± 2.0
	500	58.5 ± 13.4 b	19.0 ± 1.2
	750	113.9 ± 14.9 a	20.1 ± 1.3

SI Table 1.3 Concentration ($mg \cdot kg^{-1}$) of micro- and macro-elements in roots of basil plants exposed for 65 days to unmodified, hydrophobic, and hydrophilic nano-TiO_2. Data are means of four replicates ± SE, and different letters represent statistically significant differences between control and treatment concentrations ($p \leq 0.05$). Only Aluminum and essetial element concentrations showing statistical differences are listed.

Particle type	Treatment concent. ($mg \cdot kg^{-1}$)	Al	Ca	Cu	P	Mn	Zn
Pristine nano-TiO_2	0	252.6 ± 33.0	918.9 ± 269.2	25.2 ± 3.6 c	3567.9 ± 290.8ab	26.3 ± 0.7 b	45.4 ± 5.7
	125	333.9 ± 114.4	1150.0 ± 277.9	31.9 ± 6.6 bc	2958.5 ± 59.1 b	25.4 ± 2.1 b	62.2 ± 12.6
	250	290.5 ± 89.1	1232.8 ± 352.0	45.3 ± 3.3 ab	3159.4 ± 209.3b	25.5 ± 1.2 b	58.2 ± 3.8
	500	272.3 ± 29.8	1251.9 ± 82.5	51.4 ± 3.1 a	4150.5 ± 218.3a	37.1 ± 2.1 a	51.0 ± 5.4
	750	356.6 ± 23.7	1549.0 ± 283.9	30.5 ± 3.4 bc	3928.7 ± 251.0ab	32.4 ± 2.8 ab	74.6 ± 17.8
Hydrophobic nano-TiO_2	0	252.6 ± 33.0	918.9 ± 269.2 a	25.2 ± 3.6 a	3567.9 ± 290.8 b	26.3 ± 0.7 b	45.4 ± 5.7
	125	267.4 ± 73.6	266.8 ± 83.9 b	13.5 ± 3.3 ab	4600.7 ± 259.4ab	133.7 ± 18.2 a	56.1 ± 6.6
	250	156.9 ± 16.3	604.7 ± 108.7 ab	15.2 ± 1.3 ab	5145.0 ± 126.0 a	95.2 ± 23.8 ab	71.9 ± 7.9
	500	367.3 ± 69.1	473.1 ± 60.3 ab	10.6 ± 2.0 b	5064.9 ± 601.6 a	115.4 ± 24.8 a	48.7 ± 6.6
	750	153.3 ± 11.5	254.9 ± 23.5 b	24.0 ± 3.1 a	4155.6 ± 152.0ab	32.4 ± 2.4 b	54.8 ± 4.7
Hydrophilic nano-TiO_2	0	252.6 ± 33.0	918.9 ± 269.2 ab	25.2 ± 3.6	3567.9 ± 290.8ab	26.3 ± 0.7 b	45.4 ± 5.7 b
	125	137.1 ± 45.1	341.8 ± 153.5 b	23.0 ± 5.4	2968.2 ± 54.1 bc	11.5 ± 0.5 c	73.9 ± 10.0 ab
	250	371.0 ± 75.8	1409.1 ± 369.8 a	27.7 ± 3.9	3373.1 ± 134.3ab	18.4 ± 1.5 c	81.6 ± 8.8 a
	500	340.4 ± 52.0	187.5 ± 55.7 b	20.5 ± 1.2	3961.1 ± 95.3 a	17.5 ± 1.8 c	66.6 ± 8.3 ab
	750	402.7 ± 104.5	679.9 ± 4.2 ab	13.3 ± 1.0	2665.1 ± 72.8 c	42.2 ± 3.7 a	56.7 ± 3.0 ab

SI Table 1.4 Concentration ($mg \cdot kg^{-1}$) of micro- and macro-elements in shoots of basil exposed for 65 days to unmodified, hydrophobic, and hydrophilic nano-TiO_2. Data are means of four replicates ± SE, and different letters represent statistically significant differences between control and treatment concentrations ($p \leq 0.05$). Only Aluminum and essential element concentrations showing statistical differences are listed.

Particle type	Treatment concent. ($mg \cdot kg^{-1}$)	Al	Ca	Cu	Fe	Mg	Mn	P	Se
Pristine nano-TiO_2	0	50.4 ± 1.3	3303.2 ± 369.2	9.3 ± 0.4 bc	48.5 ± 1.5 ab	3464.7 ± 70.1 b	43.3 ± 2.1 b	8915.2 ± 669.0 ab	11.53 ± 1.3 a
	125	51.0 ± 2.8	1627.9 ± 684.4	6.2 ± 1.1 c	34.8 ± 3.6 b	1739.7 ± 93.6 c	14.3 ± 2.8 c	6438.9 ± 183.7 b	3.0 ± 1.1 b
	250	44.8 ± 4.9	2971.9 ± 103.3	15.7 ± 1.6 a	53.9 ± 6.6 a	4402.4 ± 147.1 a	77.1 ± 4.8 a	10340.3±1243.9 a	5.9 ± 1.7 ab
	500	56.1 ± 17.7	2580.2 ± 193.3	10.5 ± 0.4 b	48.2 ± 1.6 ab	3772.1± 161.5 b	72.2 ± 3.8 a	8712.9 ± 417.7 ab	6.8 ± 1.0 ab
	750	54.4 ± 6.5	2389.9 ± 125.1	8.5 ± 0.5 bc	37.1 ± 2.7 b	3173.8± 202.2 b	41.7 ± 5.5 b	8980.7 ± 400.1 ab	11.5 ± 1.0 a
Hydrophobic nano-TiO_2	0	50.4 ± 1.3	3303.2 ± 369.2 a	9.3 ± 0.4 a	48.5 ± 1.5 a	3464.7 ± 70.1 ab	43.3 ± 2.1 b	8915.2 ± 669.0	11.53 ± 1.3
	125	55.6 ± 15.3	1441.4 ± 51.4 b	6.2 ± 0.2	38.1 ± 1.5 abc	2450.1 ± 124.4 c	133.5 ± 18.6 ab	8256.0 ± 1025.7	1.3 ± 0.6
	250	65.9 ± 23.9	2493.8 ± 328.9 a	8.7 ± 1.3	28.8 ± 2.6c	2834.3± 192.0 bc	175.8 ± 51.7 a	8842.9 ± 489.3	6.8 ± 1.9
	500	47.2 ± 1.4	2746.4 ± 213.8 a	7.6 ± 0.6	33.7 ± 3.7 bc	3342.8± 277.9 ab	128.2 ± 2.5 ab	9501.8 ± 644.1	2.1 ± 1.4
	750	59.1 ± 9.0	2742.7 ± 59.1 a	9.4 ± 0.7	41.7 ± 2.7 ab	4088.7 ± 186.5 a	62.2 ± 0.4 b	8371.3 ± 546.6	13.2 ± 6.2
Hydrophilic nano-TiO_2	0	50.4 ± 1.3	3303.2 ± 369.2 a	9.3 ± 0.4	48.5 ± 1.5	3464.7 ± 70.1a	43.3 ± 2.1	8915.2 ± 669.0 a	11.53 ± 1.3 a
	125	42.9 ± 1.6	1260.5 ± 136.3 c	11.1 ± 0.2	44.6 ± 4.0	2810.7 ± 128.0b	22.0 ± 5.9	8106.7 ± 419.5 a	6.2 ± 2.5 b
	250	40.4 ± 4.9	2311.1± 184.9 b	9.6 ± 1.8	40.3 ± 2.3	2545.9 ± 108.2b	35.2 ± 6.3	6125.8 ± 135.7 b	12.2 ± 0.3 a
	500	55.5 ± 11.6	1666.2± 252.8 bc	8.4 ± 1.3	50.4 ± 5.0	2507.9 ± 274.3b	21.6 ± 2.2	6212.6 ± 453.7 b	1.7 ± 0.4 b
	750	53.2 ± 13.4	1779.4± 107.6 bc	7.7 ± 1.1	40.0 ± 5.8	2720.4 ± 150.0b	29.9 ± 6.5	6191.5 ± 319.3 b	1.2 ± 0.6 b

SI Table 1.5 Seed germination, plant elongation, and biomass/water content of basil plants grown in soil amended with unmodified and surface modified nano-TiO_2. Data are means of four replicates ± SE. Different letters represent statistically significant differences between control and treatments at the same concentration ($p \leq 0.05$). Biomass + water content = 100%

	Treatment	125 mg·kg^{-1}	250 mg·kg^{-1}	500 mg·kg^{-1}	750 mg·kg^{-1}
Germinated seeds	Control	8.3 ± 0.9 a	8.3 ± 0.9 a	8.3 ± 0.9	8.3 ± 0.9 a
	Pristine	8.5 ± 0.3 a	7.5 ± 0.7 ab	6.8 ± 0.9	6.5 ± 0.9 ab
	Hydrophobic	4.5 ± 1.3 b	5.8 ± 0.5 ab	6.3 ± 1.4	6.0 ± 0.7 ab
	Hydrophilic	5.6 ± 0.7 ab	4.1 ± 1.3 b	3.4 ± 1.4	3.4 ± 0.9 b
Root length (cm)	Control	22.7 ± 1.3	22.7 ± 1.3 a	22.7 ± 1.3 a	22.7 ± 1.3 a
	Pristine	18.8 ± 2.2	18.5 ± 0.8 ab	19.5 ± 0.7 a	21.4 ± 1.5 a
	Hydrophobic	18.0 ± 2.1	15.2 ± 1.3 b	10.6 ± 0.4 b	15.6 ± 0.7 b
	Hydrophilic	16.9 ± 2.3	17.7 ± 2.8 ab	20.0 ± 1.7 a	20.8 ± 1.5 a
Shoot length (cm)	Control	17.5 ± 1.1	17.5 ± 1.1	17.5 ± 1.1	17.5 ± 1.1 ab
	Pristine	15.9 ± 2.2	19.5 ± 2.0	21.0 ± 1.4	23.9 ± 1.0 a
	Hydrophobic	16.1 ± 2.5	20.9 ± 3.3	18.1 ± 3.5	15.7 ± 1.2 b
	Hydrophilic	15.5 ± 1.8	17.5 ± 3.0	16.6 ± 1.9	23.1 ± 2.6 a
Biomass (%)	Control	15.6 ± 0.6 a	15.6 ± 0.6 a	15.6 ± 0.6 a	15.6 ± 0.6 a
	Pristine	13.1 ± 0.1 bc	10.8 ± 0.1 b	12.5 ± 0.6 b	11.9 ± 0.5 b
	Hydrophobic	11.6 ± 0.2 c	11.1 ± 1.1 b	11.8 ± 0.2 b	9.9 ± 0.6 b
	Hydrophilic	14.5 ± 0.9 ab	15.4 ± 0.2 a	13.8 ± 0.8 ab	15.9 ± 0.6 a
Water (%)	Control	84.4 ± 0.6 b	84.4 ± 0.6 b	84.4 ± 0.6 b	84.4 ± 0.6 c
	Pristine	87.7 ± 0.8 a	89.6 ± 0.3 a	88.4 ± 0.7 a	88.7 ± 0.1 b
	Hydrophobic	88.7 ± 0.2 a	88.9 ± 1.1 a	89.0 ± 0.6 a	91.1 ± 0.6 a
	Hydrophilic	85.2 ± 0.7 b	84.5 ± 0.1 b	85.7 ± 0.6 b	84.2 ± 0.4 c

SI Table 1.6 Relative chlorophyll, total sugar, reducing sugar and starch contents in leaves of 65 day-old basil plants grown in soil amended with unmodified, hydrophobic, and hydrophilic nano-TiO_2 at 0-750 mg kg^{-1}. Data are means of four replicates ± SE. Different letters indicate statistically significant differences between each particle concentration and control ($p \leq 0.05$).

Particle type	Nano-TiO_2 concent. ($mg \cdot kg^{-1}$)	Chlorophyll (SPAD)	Total sugar (g/100g dry leaves)	Reducing sugar (g/100g dry leaves)	Starch (g/100g dry leaves)
Pristine nano-TiO_2	0	42.52 ± 1.45	1.72 ± 0.17 ab	0.21 ± 0.02 a	1.26 ± 0.05
	125	37.86 ± 2.86	2.04 ± 0.28 a	0.19 ± 0.01 ab	1.37 ± 0.18
	250	42.70 ± 0.02	1.45 ± 0.09 ab	0.19 ± 0.01 ab	1.06 ± 0.12
	500	45.23 ± 0.92	1.05 ± 0.08 ab	0.18 ± 0.02 ab	1.34 ± 0.20
	750	41.32 ± 2.30	1.17 ± 0.07 b	0.13 ± 0.01 b	1.27 ± 0.05
Hydrophobic nano-TiO_2	0	42.52 ± 1.45	1.72 ± 0.17 a	0.21 ± 0.02	1.26 ± 0.05 a
	125	40.63 ± 0.57	1.05 ± 0.08 b	0.16 ± 0.01	1.03 ± 0.08 b
	250	41.10 ± 2.38	1.54 ± 0.17 ab	0.19 ± 0.01	0.83 ± 0.02 b
	500	41.51 ± 0.59	1.19 ± 0.01 ab	0.17 ± 0.02	0.93 ± 0.03 b
	750	40.63 ± 1.40	1.06 ± 0.16 b	0.18 ± 0.01	1.00 ± 0.04 b
Hydrophilic nano-TiO_2	0	42.52 ± 1.45 a	1.72 ± 0.17 a	0.21 ± 0.02 a	1.26 ± 0.05 ab
	125	38.57 ± 1.51 ab	1.68 ± 0.07 a	0.18 ± 0.01 ab	1.13 ±0.06 b
	250	33.91 ± 1.89 b	1.35 ± 0.04 ab	0.16 ± 0.01 b	1.33 ± 0.06 ab
	500	38.73 ± 0.35 ab	1.02 ± 0.06 b	0.16 ± 0.01 b	1.45 ± 0.05 a
	750	36.71 ± 1.49 b	0.59 ± 0.11 c	0.17 ± 0.01 ab	1.44 ± 0.07 a

SI Table 1.7 Antioxidant activity of catalase (CAT) and ascorbate peroxidase (APOX) in fresh roots and leaves of 65 day-old basil plants grown in soil amended with unmodified, hydrophobic, and hydrophilic nano-TiO_2 at 0-750 mg kg^{-1}. Data are means of four replicates ± SE. Different letters indicate statistically significant differences between each particle concentration and ($p \leq 0.05$.

Particle type	Nano-TiO_2 concent. ($mg \cdot kg^{-1}$)	CAT ($U \cdot mg^{-1}$ FW)		APOX ($U \cdot mg^{-1}$ FW)	
		root	shoot	root	shoot
Pristine nano-TiO_2	0	17.32 ± 4.01 a	32.25 ± 2.01 a	15.61 ± 0.42 a	48.30 ± 5.50 a
	125	7.23 ± 0.50 b	33.16 ± 9.51 a	15.06 ± 0.63 a	22.77 ± 5.50 bc
	250	3.26 ± 0.56 b	22.56 ± 2.04 b	11.05 ± 0.81 b	17.14 ± 3.72 bc
	500	4.45 ± 0.17 b	28.72 ± 5.37 a	10.50 ± 1.86 b	27.82 ± 2.87 b
	750	5.88 ± 1.33 b	24.57 ± 0.27 b	9.20 ± 0.53 b	8.62 ± 2.14 c
Hydrophobic nano-TiO_2	0	17.32 ± 4.01 a	32.25 ± 2.01	15.61 ± 0.42 a	48.30 ± 5.50 a
	125	4.04 ± 0.89 b	30.67 ± 4.28	10.73 ± 1.73 b	27.85 ± 6.27 b
	250	4.07 ± 0.52 b	30.23 ± 1.62	5.57 ± 1.30 c	25.36 ± 4.04 bc
	500	5.04 ± 0.49 b	26.95 ± 5.50	7.81 ± 0.75 bc	29.06 ± 1.64 b
	750	3.20 ± 0.47 b	23.01 ± 3.55	6.58 ± 0.14 bc	9.68 ± 0.46 c
Hydrophilic nano-TiO_2	0	17.32 ± 4.01 a	32.25 ± 2.01 a	15.61 ± 0.42 b	48.30 ± 5.50 a
	125	15.45 ± 0.99 b	21.87 ± 1.12 b	24.42 ± 2.71 a	37.29 ± 2.04 ab
	250	22.71 ± 6.71 b	36.49 ± 1.39 a	26.86 ± 2.74 a	45.11 ± 2.36 a
	500	15.21 ± 1.69 b	25.78 ± 2.10 b	13.62 ± 2.81 b	26.07 ± 2.50 b
	750	16.28 ± 1.66 b	33.21 ± 1.03 a	4.95 ± 0.17 c	27.03 ± 0.74 b

Reference

Fabregat-Santiago, F., Jorge, G. C., Palomares, E., Clifford, J. N., Haque, S.A., Durrant, J. R., Garcia-Belmonte, G., Bisquert, J., 2004. The origin of slow electron recombination processes in dye-sensitized solar cells with alumina barrier coatings. Journal of Applied Physics 96(11), 6903-6907.

Sparks, D.L., 2003. Environmental soil chemistry, second ed. Academic press, Massachusetts, United States, pp. 184.

2. SUPPORTING INFORMATION FOR CHAPTER 3: Physiological and Biochemical Response of Basil (*Ocimum basilicum*) Exposed for Two Consecutive Generations to TiO_2 Nanoparticles of Different Surface Chemistry

SI Table 2.1 Nutrition composition and pH value of potting soil[1]

Nutritional element	Concentration (mg/kg)
	Average ± SE
Al	7551.28 ± 447.58
Ca	29570.39 ± 3406.41
Cu	30.52 ± 4.97
Fe	4653.38 ± 404.12
K	1868.65 ± 92.83
Mg	3110.12 ±789.19
Mn	197.67 ± 12.08
P	1818.36 ± 261.48
Zn	44.22 ± 5.22

This product is formulated from processed forest products, sphagnum peat moss, peat, perlite, fertilizer, and a wetting agent. It contains 0.21% total nitrogen (0.113% ammoniacal nitrogen and 0.097% nitrate nitrogen), 0.11% available phosphate (P_2O_5), and 0.16% soluble potash (K_2O). Soil pH= 6.8-7.2. Information is provided by Miracle Gro®.

SI Table 2.2 Ti concentration in roots and shoots of 65 day-old basil plants from untreated and treated seeds with/without sequential exposure of pristine, hydrophobic, and hydrophilic nano-TiO_2, respectively. Data are means of four replicates ± SE. Different letters indicate statistically significant differences between control and treatment concentrations at Tukey's test ($p \leq 0.05$).

Treatment	Nano-TiO_2 type	Ti concentration ($mg \cdot kg^{-1}$)	
		Root	Shoot
Control-750	Control	22.09 ± 8.030 b	17.20 ± 6.102
	Pristine	292.5 ± 47.44 ab	11.52 ± 3.570
	Hydrophobic	431.1 ± 138.0 a	15.34 ± 1.083
	Hydrophilic	257.7 ± 40.14 ab	26.62 ± 3.737
Treated-0	Control	22.09 ± 8.030	17.20 ± 6.102
	Pristine	66.37 ± 19.75	17.67 ± 5.130
	Hydrophobic	16.51 ± 2.901	22.97 ± 2.899
	Hydrophilic	36.82 ± 4.752	15.14 ± 4.362
Treated-750	Control	22.09 ± 8.030 c	17.20 ± 6.102
	Pristine	305.7 ± 44.71 a	15.65 ± 2.222
	Hydrophobic	168.2 ± 7.912 b	19.56 ± 8.060
	Hydrophilic	89.72 ± 44.59 bc	27.83 ± 12.74

SI Table 2.3 Accumulations of micro- and macro-elements in basil roots and shoots of 65 day-old basil plants from untreated and treated seeds with/without sequential exposure of pristine, hydrophobic, and hydrophilic nano-TiO_2, respectively. Data are means of four replicates ± SE, and different letters indicate statistically significant differences compared with control within the same treatment at Tukey's test ($p \leq 0.05$). Only the element concentrations showing statistical differences and Al concentrations are listed. All units of concentrations shown in are $mg \cdot kg^{-1}$.

Treatment	Nano-TiO_2 type	Root					Shoot
		Al	Ca	Cu	Mg	Zn	Ni
Control-750	Control	431.3 ±107.6	3030 ± 658.6	26.75 ± 8.215	4384 ± 794.0 b	68.38 ± 20.78	4.790 ± 0.2538 a
	Pristine	1161 ± 140.7	4316 ± 313.0	42.54 ± 0.4471	8003 ± 699.0 a	128.0 ± 8.716	2.626 ± 0.2550 b
	Hydrophobic	1344 ± 579.1	5692 ± 2106	46.71 ± 20.35	5632 ± 643.2 ab	95.41 ± 11.44	1.539 ± 0.6445 b
	Hydrophilic	776.4 ± 168.9	3985 ± 297.2	52.52 ± 7.759	7637 ± 38.68 a	96.72 ± 11.11	2.525 ± 0.5111 b
Treated-0	Control	431.3 ±107.6 ab	3030 ± 658.6	26.75 ± 8.215 b	4384 ± 794.0 b	68.38 ± 20.78	4.790 ± 0.2538 a
	Pristine	875.2 ± 188.8 a	5290 ± 716.0	60.88 ± 16.27 ab	7872 ± 570.3 a	107.2 ± 5.556	0.7892 ± 0.3498 c
	Hydrophobic	348.3 ± 47.09 b	3066 ± 332.7	20.33 ± 7.505 b	5530 ± 294.9 ab	98.72 ± 6.888	1.194 ± 0.0677 bc
	Hydrophilic	577.3 ± 47.41 ab	4851 ± 218.6	82.00 ± 15.10 a	7918 ± 578.1 a	89.53 ± 1.228	2.857 ± 0.8578 b
Treated-750	Control	431.3 ±107.6 ab	3030 ± 658.6 b	26.75 ± 8.215 b	4384 ± 794.0 c	68.38 ± 20.78 b	4.790 ± 0.2538
	Pristine	750.5 ± 139.2 a	6599 ± 1116 a	75.91 ± 12.45 a	9407 ± 230.4 a	250.3 ± 55.34 a	0.8144 ± 0.5070
	Hydrophobic	329.0 ± 30.02 b	3702 ± 49.29 b	35.81 ± 8.919 b	6074 ± 205.9 bc	140.8 ± 5.973 ab	1.873 ± 1.266
	Hydrophilic	505.3 ± 17.05 ab	4722 ± 280.6 b	43.94 ± 5.100 ab	7060 ± 403.1 b	96.90 ± 13.39 b	2.517 ± 1.156

SI Table 2.4 Germination number, elongation and shoot biomass/water content in leaves of 65 day-old basil plants from untreated and treated seeds with/without sequential exposure of pristine, hydrophobic, and hydrophilic nano-TiO_2, respectively. Different letters indicate statistically significant differences compared with control within the same treatment at Duncan's test ($p \leq 0.10$). The units of NPs concentrations shown in are $mg \cdot kg^{-1}$.

Treatment	Nano-TiO_2 type	Germination number	Shoot biomass content (%)	Water content (%)	Elongation (cm)	
					Root	Shoot
Control-750	Control	8.3 ± 0.7	9.5 ± 0.8	90.5 ± 0.8	24.6 ± 1.9	35.5 ± 2.2
	Pristine	8.0 ± 0.6	11.0 ± 1.5	89.0 ± 1.6	30.2 ± 3.2	38.0 ± 1.9
	Hydrophobic	9.0 ± 0.6	13.4 ± 1.6	86.6 ± 1.6	25.5 ± 0.2	35.5 ± 1.5
	Hydrophilic	8.3 ± 0.9	12.0 ± 1.5	88.0 ± 1.5	26.7 ± 0.9	37.1 ± 4.0
Treated-0	Control	8.3 ± 0.7 ab	9.5 ± 0.8 b	90.5 ± 0.8 a	24.6 ± 1.9	35.5 ± 2.2
	Pristine	7.3 ± 0.7 b	10.7 ± 1.0 ab	89.3 ± 1.0 ab	22.9 ± 1.4	39.4 ± 2.3
	Hydrophobic	9.7 ± 0.3 a	13.2 ± 1.3 a	86.8 ± 1.3 b	23.3 ± 1.4	43.0 ± 1.6
	Hydrophilic	8.3 ± 0.3 ab	12.3 ± 0.6 ab	87.7 ± 0.6 ab	23.7 ± 0.3	35.6 ± 1.7
Treated-750	Control	8.3 ± 0.7	9.5 ± 0.8 b	90.5 ± 0.8 a	24.6 ± 1.9	35.5 ± 2.2 b
	Pristine	6.7 ± 0.7	9.9 ± 1.0 b	90.1 ± 1.0 a	25.2 ± 1.5	39.3 ± 0.4 ab
	Hydrophobic	7 ± 1.0	13.8 ± 0.5 a	86.2 ± 0.5 b	25.9 ± 1.3	42.2 ± 0.6 a
	Hydrophilic	7.3 ± 0.9	12.1 ± 1.5 ab	87.9 ± 1.5 ab	24.3 ± 0.7	35.5 ± 1.3 b

SI Table 2.5 Photosynthetic rate (*A*) and stomatal conductance (*gs*) in 60-day old basil leaves from untreated and treated seeds with/without sequential exposure of pristine, hydrophobic, and hydrophilic nano-TiO_2, respectively. Each value is mean ± SE (n = 3). Different letters indicate statistically significant differences compared with control within the same treatment at Duncan's test ($p \leq 0.10$). The units of NPs concentrations shown in are $mg \cdot kg^{-1}$.

Treatment	Nano-TiO_2 type	Photosynthetic rate	Stomatal conductance
Control-750	Control	9.7 ± 2.0	0.101 ± 0.04
	Pristine	9.8 ± 0.7	0.151 ± 0.06
	Hydrophobic	8.8 ± 1.1	0.058 ± 0.01
	Hydrophilic	7.3 ± 1.8	0.110 ± 0.05
Treated-0	Control	9.7 ± 2.0	0.101 ± 0.04
	Pristine	8.6 ± 1.7	0.155 ± 0.06
	Hydrophobic	8.4 ± 2.5	0.077 ± 0.03
	Hydrophilic	9.3 ± 3.8	0.157 ± 0.11
Treated-750	Control	9.7 ± 2.0	0.101 ± 0.04 b
	Pristine	11.1 ± 0.9	0.316 ± 0.05 a
	Hydrophobic	6.9 ± 1.5	0.038 ± 0.00 b
	Hydrophilic	10.2 ± 1.4	0.110 ± 0.04 b

SI Table 2.6 Chlorophyll *a*, chlorophyll *b*, and total chlorophyll contents in leaves of 65 day-old basil plants from untreated and treated seeds with/without sequential exposure of pristine, hydrophobic, and hydrophilic nano-TiO_2, respectively. Each value is mean ± SE (n=3). Different letters indicate statistically significant differences compared with control within the same treatment at Tukey's test ($p \leq 0.05$). The units of NPs concentrations shown in are $mg \cdot kg^{-1}$.

Treatment	Nano-TiO_2 type	Chlorophyll *a*	Chlorophyll *b*	Total chlorophyll
Control-750	Control	15.2 ± 0.13	16.9 ± 1.17 a	32.1 ± 1.04 a
	Pristine	15.3 ± 0.10	15.15 ± 0.81 a	30.4 ± 0.72 a
	Hydrophobic	15.4 ± 0.07	9.89 ± 0.62 b	25.3 ± 0.69 ab
	Hydrophilic	13.6 ± 1.14	7.94 ± 2.75 b	21.5 ± 3.81 b
Treated-0	Control	15.2 ± 0.13	16.9 ± 1.17 a	32.1 ± 1.04
	Pristine	14.3 ± 0.85	8.1 ± 2.13 b	22.4 ± 2.89
	Hydrophobic	14.8 ± 0.27	10.1 ± 2.71 ab	25.0 ± 2.97
	Hydrophilic	15.1 ± 0.22	13.4 ± 2.78 ab	28.5 ± 2.72
Treated-750	Control	15.2 ± 0.13	16.9 ± 1.17	32.1 ± 1.04
	Pristine	15.4 ± 0.09	13.3 ± 1.41	28.7 ± 1.32
	Hydrophobic	15.3 ± 0.30	9.2 ± 1.45	24.5 ± 1.75
	Hydrophilic	15.0 ± 0.17	12.3 ± 4.47	27.3 ± 4.31

SI Table 2.7 Carbohydrates (Total sugar, reducing sugar and starch) content in fresh leaves in 65-day old basil leaves from untreated and treated seeds with/without sequential exposure of pristine, hydrophobic, and hydrophilic nano-TiO_2, respectively. Each value is mean ± SE (n = 3). Different letters indicate statistically significant differences compared with control within the same treatment at Duncan's test ($p \leq 0.10$). The units of NPs concentrations shown in are $mg \cdot kg^{-1}$.

Treatment	Nano-TiO_2 type	Total sugar	Reducing sugar	Starch
Control-750	Control	1.60 ± 0.05	0.29 ± 0.06 b	1.87 ± 0.13
	Pristine	1.94 ± 0.11	0.62 ± 0.12 ab	1.66 ± 0.22
	Hydrophobic	1.88 ± 0.53	0.82 ± 0.15 a	1.55 ± 0.15
	Hydrophilic	2.13 ± 0.03	0.40 ± 0.18 ab	2.02 ± 0.16
Treated-0	Control	1.60 ± 0.05	0.29 ± 0.06 b	1.87 ± 0.13 ab
	Pristine	1.74 ± 0.10	0.54 ± 0.13 ab	1.47 ± 0.10 b
	Hydrophobic	2.57 ± 0.39	0.33 ± 0.09 b	2.75 ± 0.53 a
	Hydrophilic	2.66 ± 0.53	0.71 ± 0.07 a	1.72 ± 0.06 b
Treated-750	Control	1.60 ± 0.05 b	0.29 ± 0.06 b	1.87 ± 0.13
	Pristine	1.59 ± 0.35 b	0.31 ± 0.12 b	1.74 ± 0.11
	Hydrophobic	1.73 ± 0.26 b	0.69 ± 0.07 a	2.78 ± 0.44
	Hydrophilic	2.89 ± 0.33 a	0.31 ± 0.04 b	1.72 ± 0.31

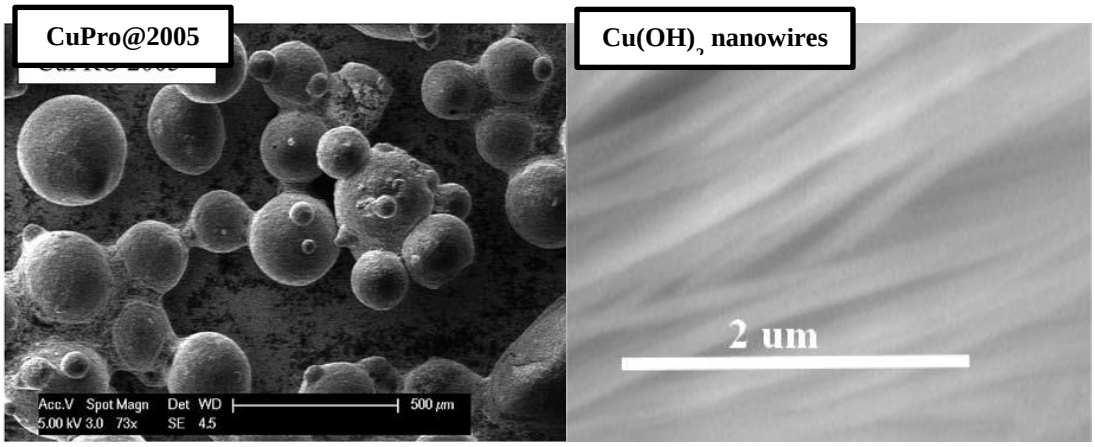
Reference

A. C. Barrios, C. M. Rico, J. Trujillo-Reyes, I. A. Medina-Velo, J. R. Peralta-Videa and J. L. Gardea-Torresdey, *Sci. Total Environ.*, 2016, **563**, 956-964.

3. SUPPORTING INFORMATION FOR CHAPTER 4: Foliar Exposure of $Cu(OH)_2$ Nanopesticide to Basil (*Ocimum basilicum*): A Variety-Dependent Copper Translocation and Biochemical Responses

3.1. Materials and Methods

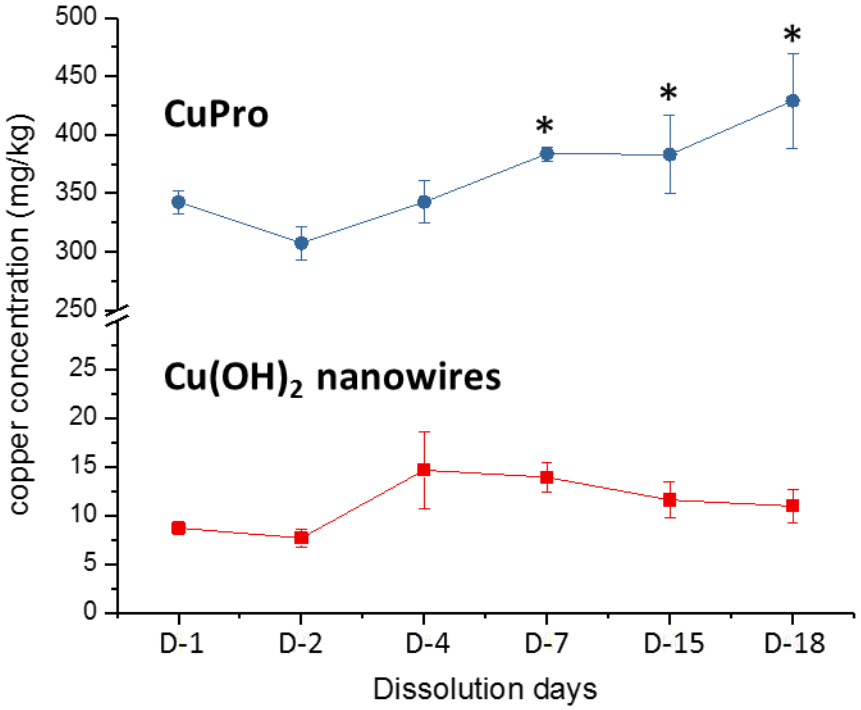
3.1.1 Characterizations of $Cu(OH)_2$ suspension.



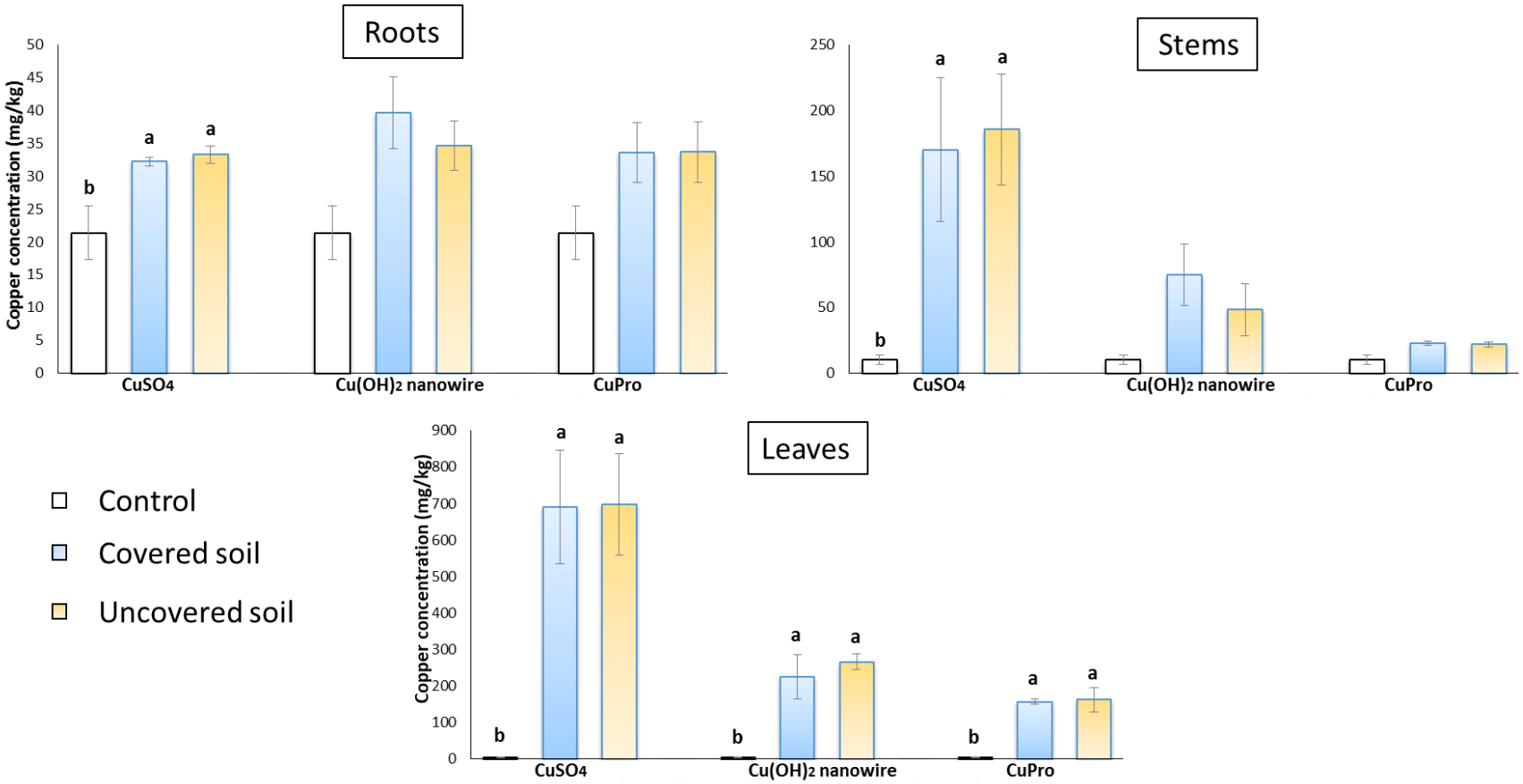
SI Figure 3.1 Scanning electron microcopy images of CuPro@2005 (Hong et al., 2005) and $Cu(OH)_2$ nanowires (US Research Nanomaterials).

3.1.2 Cu^{2+} leakage determination.

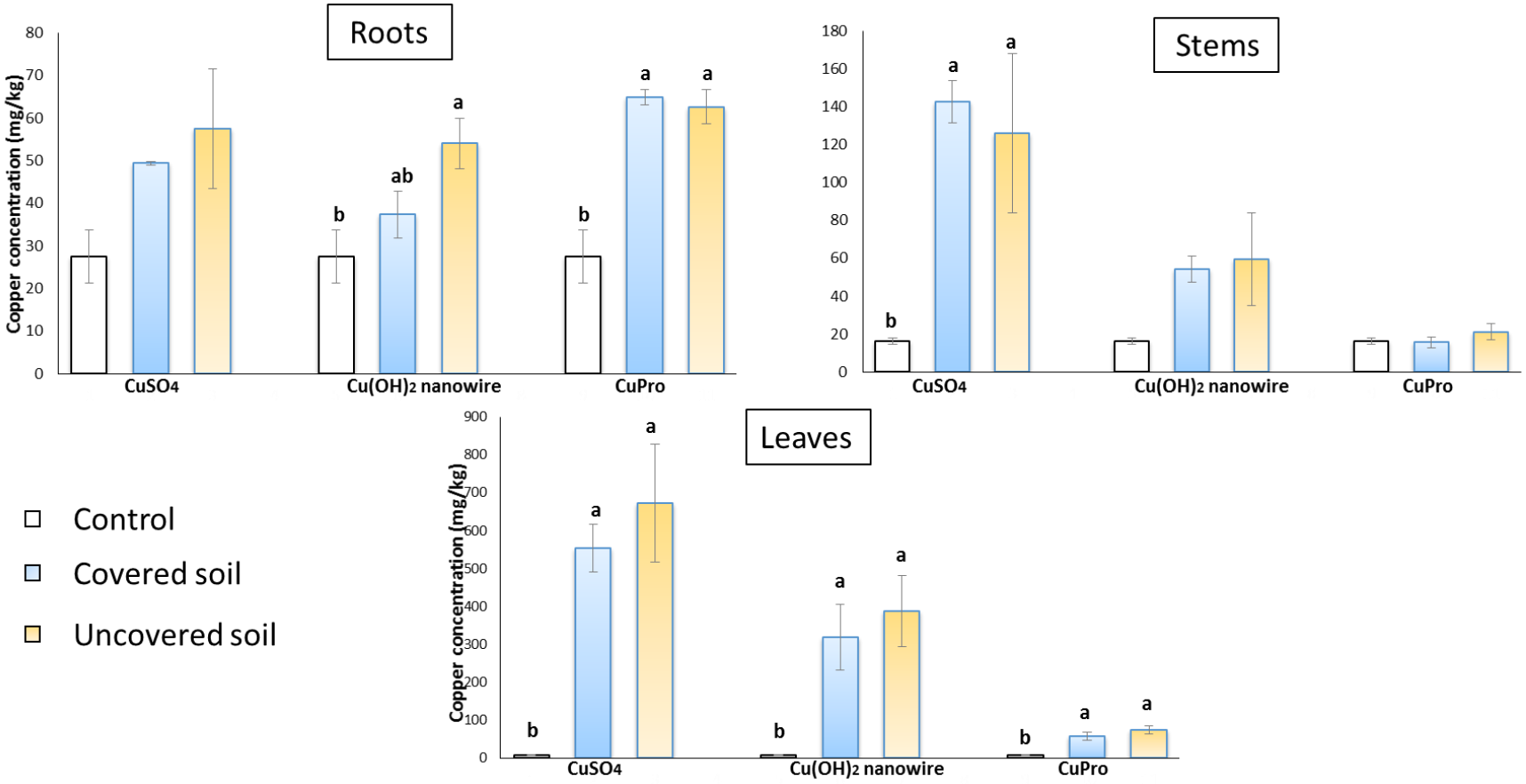
The dissolution of CuPro@2005 and $Cu(OH)_2$ nanowires were determined by dispersing the CuPro@2005 in MPW and kept shaking for 15 days. It was performed in three replicates. In order to avoid the interference of particulate matters (Bandyopadhyay et al., 2015), after 4 h, the supernatants were collected and centrifuged at 5 000 rpm for 30 min (Eppendorf AG bench centrifuge 5417R. Hamburg, Germany). Then, 2 mL of supernatants were taken up and centrifuged at 14 000 rpm for 30 min. The supernatants were transferred and centrifuged at 14 000 rpm for 30 min. The centrifuge process was repeated for three times. The supernatants from both media were diluted into 20 mL with the 2% (v/v) HNO_3, and the amount of Cu^{2+} was measured by inductively coupled plasma-optical emission sprectrometry (ICP-OES, Perkin-Elmer Optima 4300 DV).



SI Figure 3.2 Release of Cu^{2+} ion of CuPro (Blue curve) and $Cu(OH)_2$ nanowires (Red curve) in the MPW media up to fifteen days.



SI Figure 3.3 Copper concentrations in basil roots, stems, and leaves of low anthocyanin basils with foliar exposure of 4.8 mg Cu/per pot from $CuSO_4$, $Cu(OH)_2$ nanowires, and CuPro for 2 weeks. White column stands for control, blue column stands for treatments covered soil, and yellow column stands for treatments uncovered soil. Each value is mean ± SE of four replicates. Means with different letters stand for significant difference compared with controls ($p \leq 0.05$).



SI Figure 3.4 Copper concentrations in basil roots, stems, and leaves of high anthocyanin basils with foliar exposure of 4.8 mg Cu/per pot from $CuSO_4$, $Cu(OH)_2$ nanowires, and CuPro for 2 weeks. White column stands for control, blue column stands for treatments covered soil, and yellow column stands for treatments uncovered soil. Each value is mean ± SE of four replicates. Means with different letters stand for significant difference compared with controls ($p \leq 0.05$).

References

Bandyopadhyay, S.; Plascencia-Villa, G.; Mukherjee, A.; Rico, C. M.; José-Yacamán, M.; Peralta-Videa, J. R.; Gardea-Torresdey, J. L. Comparative phytotoxicity of ZnO NPs, bulk ZnO, and ionic zinc onto the alfalfa plants symbiotically associated with *Sinorhizobium meliloti* in soil. *Sci. Total Environ*. 2015, 515 60-69.

Hong, J.; Rico, C. M.; Zhao, L.; Adeleye, A. S.; Keller, A. A.; Peralta-Videa, J. R.; Gardea-Torresdey, J. L. Toxic effects of copper-based nanoparticles or compounds to lettuce (*Lactuca sativa*) and alfalfa (*Medicago sativa*). *Environ. Sci. Processes & Impacts* 2015, 17(1), 177-185.

US Research Nanomaterials, Inc. http://www.us-nano.com/inc/sdetail/29132.

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Vita

Wenjuan Tan was born in Wujiaqu, Xinjiang, China. She received her Bachelor of Science in Applied Chemistry in H Southwest Forestry University in China, 2010. Then she earned her Master degree from Ocean University of China in Inorganic Chemistry in 2013. Afterwards, she joined the Doctoral Program in Chemistry Department and switched to Environmental Science and Engineering at The University of Texas at El Paso. Her research project has been under the supervision of Dr. Jorge Gardea Torresdey.

During these 4 years, her collaborative work with other scholars resulted in 7 peer-review journal papers (*Environmental Pollution, Science of the Total Environment, Plant Physiology and Biochemistry,* and *Environmental Science & Process Impact*) and 2 book chapters (Springer-Verlag GmbH and Springer International Publishing) as first author and co-author. Also, she has submitted two manuscripts to *Environmental Science: Nano* and one other manuscript which is almost ready to submit.

Wenjuan is a member of the University of California's Center for Environmental Implications of Nanotechnology (UC CEIN) and was invited to attend site visit and workshops twice. She has presented her research at local or national conference meetings, including the American Chemical Society's National Meeting, Annual Conference of Sustainable Nanotechnology Organization and International Nanotoxicology Congress, College of Science Conference at University of Texas at San Antonio.

While pursuing her PhD, Mrs. Wenjuan Tan worked as a Teaching Assistant in the chemistry department. Also, she was the vice president (2014-2015) of UTEP Chinese Students and Scholars Association (CSSA). She also served as a volunteer in several campus research events, such as research expo and COURI.

Publications

1. **Tan, W.**, Feng, L., Wang, X., Li, D., Huang, F., Li. X., Research progress of one-dimensional nanomaterials and whiskers of magnesium borate. Chemical industry and engineering progress, 2012, 31, 182-186. (In Chinese)
2. Huang, F., Feng, L., Yang, W., **Tan, W.**, Xu, K., Li, C., Research progress of preparation of liquid fuel by direct liquefaction from the algae. Chemical industry and engineering progress, 2012, 31(10), 2197-2212. (In Chinese)
3. Huang, F., Feng, L., **Tan, W.**, Xu, K., Li, C., Progress of hydrothermal liquefaction decomposition by using *Enteromorpha*, Journal of Ocean University of China. 2013, 43(4), 067-072. (In Chinese)
4. Rico, C.M., Barrios, A.C., **Tan, W.**, Rubenecia, R., Lee, S.C., Varela- Ramirez, A., Peralta-Videa, J.R., Gardea-Torresdey, J.L., Physiological and biochemical response of soil-grown barley (*Hordeum vulgare L.*) to cerium oxide nanoparticles. *Environmental Science and Pollution Research*, 2015, 22 (14), 10551-10558.
5. Zuverza-Mena, N., Medina-Velo, I.A., Barrios, A.C., **Tan, W.**, Peralta-Videa, J.R., Gardea-Torresdey, J.L., Copper nanoparticles/compounds impact agronomic and physiological parameters in cilantro (*Coriandrum sativum*). *Environmental Science & Process Impact*, 2015, 17, 1783-1793.
6. Hong, J., Wang, L., Sun, Y., Zhao, L., Niu, G., **Tan, W.**, Rico, C.M., Peralta-Videa, J.R., Gardea-Torresdey, J.L., Foliar applied nanoscale and microscale CeO_2 and CuO alter cucumber (*Cucumis sativus*) fruit quality. *Science of the Total Environment*, 2016, 563, 904-911.
7. Du, W., **Tan, W.**, Peralta-Videa, J.R., Gardea-Torresdey, J.L., Ji, R., Yin, Y., Guo, H., Interaction of Metal Oxide Nanoparticles with Higher Terrestrial Plants: Physiological and Biochemical Aspects. *Plant Physiology and Biochemistry*, 2017, 110, 210-225.
8. Medina-Velo, I.A., Zuverza-Mena, N., **Tan**, **W.,** Hernandez-Viezcas, J.A., Peralta-Videa, J.R., Gardea-Torresdey, J.L., Biophysical methods of detection and quantification of uptake, translocation and accumulation. *Plant Nanotechnology-Principles and Practices*, 29-63.
9. **Tan, W.**, Du, W., Barrios, A.C., Armendariz, Jr. R., Zuverza-Mena, N., Ji, Z., Chang, C.H., Zink, J.I., Hernandez-Viezcas, J.A., Peralta-Videa, J.R., Gardea-Torresdey, J.L., Surface coating changes the physiological and biochemical impacts of nano-TiO_2 in basil (*Ocimum basilicum*) plants. *Environmental Pollution*, 2017, 222, 64-72.
10. S Apodaca, S., **Tan**, **W.,** Dominguez, O.E., Hernandez-Viezcas, J. A., Peralta-Videa, J.R., Gardea-Torresdey, J.L., Physiological and biochemical effects of nanoparticulate copper, bulk copper, copper chloride, and kinetin in kidney bean (*Phaseolus vulgaris*) plants. *The Science of the Total Environment* 2017, 599, 2085.
11. Rawat, S., Apodaca, S., **Tan**, **W.,** Peralta-Videa, J.R., Gardea-Torresdey, J.L., Terrestrial nanotoxicology: Evaluating the nano-biointeractions in vascular plants. In: Yan, B., Zhou, H., Gardea-Torresdey, J.L. (Eds.) *Bioactivity of Engineered Nanoparticles*. Springer-Verlag GmbH, Berlin, Germany (In press), 2017.

12. Du, W., **Tan, W.,** Peralta-Videa, J.R., Gardea-Torresdey, J.L., Ji, R., Guo, H., Nanoparticles/microparticles on plant growth and nutritional quality of Oregano (*Origanum vulgare*). (Science of the Total Environment, 2017, in press)
13. **Tan, W.**, Du, W., Darrouzet-Nardi, A.J., Hernandez-Viezcas, J.A., Peralta-Videa, J.R., Gardea-Torresdey, J.L., Physiological and biochemical response of basil (*Ocimum basilicum*) exposed for two consecutive generations to TiO_2 nanoparticles of different surface chemistry. (Submitted to Environmental Science: Nano)
14. **Tan, W.,** Peralta-Videa, J.R., Gardea-Torresdey, J.L., Interaction of titanium dioxide nanoparticles with soil components and plants: Current knowledge and future research needs-A critical review (Submitted to Environmental Science: Nano)
15. **Tan, W.,** Gao, Q., Deng, C., Wang, Y., Lee, W.Y., Hernandez-Viezcas, J.A., Peralta-Videa, J.R., Gardea-Torresdey, J.L., Foliar exposure of $Cu(OH)_2$ nanopesticide to basil (*Ocimum basilicum*): A variety-dependent copper translocation and biochemical responses. (To be submitted)

Presentations

1. Huang, F., Feng, L., **Tan, W.**, Xu, K., Li, C., Preparation of liquid fuel by direct liquefaction by using *Enteromorpha*. Analytical Testing Annual Meeting, Qingdao, Shangdong, China, December, 2011. (Excellent Academic Paper)

2. Rico, C. M., Barrios, A. C., **Tan, W.,** J.; Peralta-Videa, J. R., Gardea-Torresdey, J. L. Cerium oxide nanoparticles modify crop physiology and grain quality in cereals. UTEP Graduate Research Expo, November 14, 2014, El Paso, Texas. (Oral presentation)

3. **Tan, W.**, Du, W., Barrios, A.C., Armendariz, Jr. R., Zuverza-Mena, N., Ji, Z., Chang, C.H., Zink, J.I., Hernandez-Viezcas, J. A., Peralta-Videa, J.R., Gardea-Torresdey, J.L., Biochemical effects of nano-TiO_2 with different surface properties on basil. 8th International Nanotoxicology Congress, Boston, Massachusetts, June 1-4, 2016. (Poster presentation)

4. **Tan, W.**, Du, W., Barrios, A.C., Armendariz, Jr. R., Zuverza-Mena, N., Ji, Z., Chang, C.H., Zink, J.I., Hernandez-Viezcas, J.A., Peralta-Videa, J.R., Gardea-Torresdey, J.L., Effects of surface chemistry on the physiological and biochemical interactions between nano-TiO_2 and basil. 252nd American Chemical Society National Meeting & Exposition, Philadelphia, PA, August 21-25, 2016. (Poster presentation in Sci-mix and Environmental Chemistry Division sessions)

5. **Tan, W.**, Du, W., Dominguez, O.E., Molina, M.H., Darrouzet-Nardi, A.J., Hernandez-Viezcas, J.A., Peralta-Videa, J.R., Gardea-Torresdey, J.L., Trans-generational effects of nano-TiO_2 with different surface properties on basil. 5th Annual Sustainable

Nanotechnology Organization Conference, Orlando, Florida, November 10-12, 2016. (Oral presentation)

6. **Tan, W.**, Gao, Q., Deng, C., Wang, Y., Lee, W.Y., Hernandez-Viezcas, J.A., Peralta-Videa, J.R., Gardea-Torresdey, J.L., Foliar exposure of $Cu(OH)_2$ nanopesticide to basil (*Ocimum basilicum*): Variety-dependent metabolic responses. University of Texas at San Antonio College of Science Research Conference, San Antonio, TX, October 6th, 2017. (Oral and poster presentation)

7. **Tan, W.**, Deng, C., Wang, Y., Hernandez-Viezcas, J.A., Peralta-Videa, J.R., Gardea-Torresdey, J.L., Biochemical responses of basil (*Ocimum basilicum*) to $Cu(OH)_2$ nanopesticide: A foliar exposure study. 6th Annual Sustainable Nanotechnology Organization Conference, Los Angeles, California, November 5-7, 2017. (Oral presentation)

8. Deng, C., Wang, Y., Sun, Y., **Tan, W**., Rawat, S., Hernandez-Viezcas, J.A., Niu, G., Jose R. Peralta-Videa J.R., Gardea-Torresdey, J.L., Effect of copper nanoparticles on Rosie and Green bok choy (*Brassica rapa*) varieties: physiological and biochemical responses. 6th Annual Sustainable Nanotechnology Organization Conference, Los Angeles, California, November 5-7, 2017. (Poster presentation)

Awards and Scholarships

1. ***Student Travel Award***, 5th Sustainable Nanotechnology Organization Conference, Nov. 2016, Orlando, Florida.
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