

# Impact of titanium dioxide nanoparticles (TiO<sub>2</sub>-NPs) on growth and mineral nutrient uptake of wheat (*Triticum vulgare* L.)

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## Abstract

Titanium dioxide nanoparticles (TiO<sub>2</sub>-NPs) among metallic NPs are one of the most produced and consumed NPs in the world. Thus, TiO<sub>2</sub>-NPs release into the environment is inevitable. Their impact on food source cereals is still unclear. The purpose of this study was to assess the phytotoxic impacts of TiO<sub>2</sub>-NPs on growth and some mineral nutrient (zinc (Zn), iron (Fe), manganese (Mn), copper (Cu), nitrogen (N), phosphorous (P), potassium (K)) uptake of the wheat (*Triticum vulgare* L.) plant. The TiO<sub>2</sub>-NP suspensions at increasing concentrations (0, 5, 10, 20 and 40 mg L<sup>-1</sup>) were applied to wheat plant Yunus cultivar grown in hydroponic culture under controlled conditions for 21 days. The results indicate that the TiO<sub>2</sub>-NPs have not statistically significant effects on plant growth. The chlorophyll content was decreased with increasing TiO<sub>2</sub>-NP treatment, except for 5 mg L<sup>-1</sup> TiO<sub>2</sub>-NPs treatment. The higher N (4.58%), P (0.78%), Zn (87.50 mg kg<sup>-1</sup>), and Cu (12.90 mg kg<sup>-1</sup>) concentration were recorded at 40 mg L<sup>-1</sup> TiO<sub>2</sub>-NPs treatment. On the other hand, K concentration was decreased at 20 and 40 mg L<sup>-1</sup> TiO<sub>2</sub>-NPs treatments. This study has shown that exposure to TiO<sub>2</sub>-NPs causes no significant phytotoxic effect on wheat.

## Introduction

In the last decade, the fast development in nanotechnology and its impacts have raised concerns about the application of nanoparticles in agriculture and environment (Rafique et al., 2014). The most commonly used metallic NPs in industries are TiO<sub>2</sub>, ZnO, CuO, Au, Fe<sub>3</sub>O<sub>4</sub> and CeO<sub>2</sub>. Among these NPs, TiO<sub>2</sub>-NPs are one of the most produced and consumed materials in the world. Up to 2 million tons per year of TiO<sub>2</sub>-NPs are produced in the worldwide (Larue et al., 2011). According to the group of Swiss Federal Laboratories for Materials Testing and Research (Empa)-ETH and the University of Zurich, the estimated annual production of TiO<sub>2</sub>-NPs in Europe is 39.000 metric tons (SNSF, 2016). Today, TiO<sub>2</sub>-NPs are widely used as the pigment in paint, paper, ink and plastics industries (Larue et al., 2011), and also in sunscreen due to their UV protective properties in cosmetic products (Mahmoodzadeh et al., 2013, Fan

et al., 2016). This extensive production is postulated to result in the release into the environment with subsequent contamination of water, air, soils and plants (Aliabadi et al., 2016; Gottschalk et al., 2009; Shafea et al., 2017). It has been expected that these amounts will increase every year and they will accumulate in the ecosystems.

Plants may provide a potential pathway for NPs to enter the food chain. The accumulation of NPs in plants can cause a potential risk for human health and ecosystems due to their gross contamination into plant-based materials (Larue et al., 2018).

The metallic NPs play an essential role in plant growth and development at very low concentrations within the tolerance limits of plants. The response of plants to metal NPs varies according to the characteristics of NPs (size, shape, concentrations, exposure time and so on), the plant species and the growth stage of plants (Aliabadi et al., 2016; Aslani et al.,

2014; Hasanpour et al., 2015). Some researchers reported that the excessive of TiO<sub>2</sub>-NPs in the long term caused a phytotoxic effect on seed germination, chlorophyll content, photosynthesis activity, plant growth and so on (Dogaroglu & Koleli, 2017a; Samadi et al., 2014; Yaqoob et al., 2018). In literature, there are contradictory reports about the potential toxicity of TiO<sub>2</sub>-NPs in plants. Some reports proved that it has positive effect (Feizi et al., 2013; Landa et al., 2012; Servin et al., 2013; Singh et al., 2012; Song et al., 2013), some of them mentioned its adverse effects (Asli & Neumann, 2009; Cai et al., 2017; Castiglione et al., 2011; Du et al., 2011) and the others reported even no effect (García et al., 2011; Jacob et al., 2013; Landa et al., 2012; Larue et al., 2011, 2012; Seeger et al., 2009; Song et al., 2013; Zheng et al., 2005). Most studies on metallic NPs effects have been done in petri dishes through one week (Dogaroglu & Koleli, 2017a, 2017b). On the other hand, studies related to TiO<sub>2</sub>-NPs phytotoxicity and accumulation in crop plants in hydroponic culture are still lacking (Lin & Xing, 2008; Song et al., 2013; Yang et al., 2006; Zhu et al., 2008). However, to simulate results closer to actual field conditions, the hydroponic or pot experiments should be verified in the controlled conditions. Hydroponic culture makes it possible to study with more plants in a short time, even if not as much as toxicity test in the petri dishes.

Wheat among cereals is the most essential food source for more than half of the world's population. Despite the fact being an important food source for human consumption all over the world, the impact of TiO<sub>2</sub>-NPs on growth and some mineral nutrient uptake of cereals, including wheat is still unclear. Therefore, there is a need for a better understanding of the current knowledge about the effects of TiO<sub>2</sub>-NPs on not only growth but also some mineral nutrient uptake in wheat under hydroponic conditions. In the present study, we examined the effects of TiO<sub>2</sub>-NPs on growth and some mineral nutrient uptake in wheat (*Triticum vulgare* L.) in hydroponic culture.

## Materials and Methods

### Nanomaterial

The TiO<sub>2</sub>-NPs (~30-50 nm) were prepared with the aqueous sol-gel method (Gokhale et al., 2009), which was modified by Dr. Karakaya. The identification of TiO<sub>2</sub>-NPs as one of the primary nanomaterials by the Organization for Economic Cooperation and Development (OECD) is the most essential criterion in selecting the NPs to be used in this study (OECD, 2013).

The size, morphology and elemental analysis of TiO<sub>2</sub>-NPs were determined by using Scanning electron microscopy (SEM) and energy - dispersive X-ray spectroscopy (EDX) (Carl Zeiss, Supra 55). The particle size and size distribution of the TiO<sub>2</sub>-NPs in the solution was analyzed by using Zetasizer (Nano ZS, Malvern Instruments, UK).

### Plant material

The seeds of Yunus cultivar (bread wheat) were used as plant material in this study. The seeds were purchased from the Transitional Zone Agricultural Research Institute, Eskisehir Province, Turkey.

### Hydroponic experiment

The wheat seeds were germinated in a mixture of peat and perlite (1:1 w/w) until the seedlings were getting hairy rooted and 2-3 leaves. Afterwards, twenty-five of the wheat seedlings (5 plants each branch × 5 branches, each pot) were transferred into the 4.5 L polyethylene pots supplied with the Hoagland nutrient solution medium. The nutrient solution was contained as essential macro (1 mM KH<sub>2</sub>PO<sub>4</sub>, 3 mM KNO<sub>3</sub>, 0.25 mM MgSO<sub>4</sub>·7H<sub>2</sub>O, 2 mM Ca(NO<sub>3</sub>)<sub>2</sub>·4H<sub>2</sub>O and 2.5×10<sup>-2</sup> mM KCl) and micronutrients (1 μM MnSO<sub>4</sub>·H<sub>2</sub>O, 1 μM ZnSO<sub>4</sub>·H<sub>2</sub>O, 0.25 μM CuSO<sub>4</sub>·H<sub>2</sub>O, 0.25 μM (NH<sub>4</sub>)<sub>6</sub>Mo<sub>7</sub>O<sub>24</sub>, and 0.125 μM H<sub>3</sub>BO<sub>3</sub>). The pH of the nutrient solution was adjusted to 5.2. Increasing doses (0, 5, 10, 20 and 40 mg L<sup>-1</sup>) of TiO<sub>2</sub>-NPs were added to the nutrient solution 5 days after transferring of seedlings into the pots. The suspensions of each pot were changed every 2-3 days. The experiment was designed as a randomized block method with three replications. The hydroponic experiment was conducted under controlled environmental conditions (16/8h light/dark period, 25/20°C, 60% humidity and 10 Klux light intensity) for 21 days.

### Morphological observations and chlorophyll contents

The morphological changes of wheat plants under TiO<sub>2</sub>-NPs exposure were observed throughout the experiment. Additionally, the effects of TiO<sub>2</sub>-NP treatment on the leaf chlorophyll contents were measured by the Konica Minolta SPAD-502 chlorophyll meter as soil plant analysis development value (SPAD value) (Dağhan, 2018).

### Elemental analysis in plants

The plants were harvested as shoots and roots separately 21 days after planting. Then, all plant samples were washed with distilled water and dried at 65 °C in an oven. When the samples reached a constant weight, their dry weights (DW) were taken and then, they were ground in the agate mill (Retsch MM301 Mixer Mill, Retsch, Nordrhein-Westfalen, Germany). Grounded plant samples were digested using a wet digestion method with HNO<sub>3</sub> and H<sub>2</sub>O<sub>2</sub> in the microwave oven (MarsXpres CEM, Matthews, USA) for elemental analysis. Total essential element (potassium (K), phosphorus (P), zinc (Zn), iron (Fe), copper (Cu), and manganese (Mn)), and Ti concentrations of the extracted samples were determined using the Inductively Coupled Plasma Mass Spectrometry (ICP-MS) (Agilent 7500ce, Agilent Technologies, Santa Clara, USA).

The Ti contents of roots were calculated by multiplying root dry weights and Ti concentrations as below;

Ti content ( $\mu\text{g root}^{-1}$ ) = Ti concentration in the root ( $\text{mg kg}^{-1}$  DW)  $\times$  root DW (g)

The nitrogen (N) concentration of the shoots was determined, according to Nelson & Sommers (1980). Certified reference materials (SRM 1573A, SRM 1547) were analyzed to check the accuracy of the extraction technique.

### Statistical analysis

The variance analysis of the data was statistically analyzed by using the SPSS-20 statistical analysis package program to determine the significance of levels and grouped by Duncan test at the 0.05 probability level.

## Results and Discussion

### Characterization of TiO<sub>2</sub>-NP

The SEM image of the synthesized TiO<sub>2</sub>-NPs is shown in Figure 1. The average size of TiO<sub>2</sub>-NPs was measured at about 30-50 nm. Besides titanium (Ti), carbon (C) and oxygen (O) elements were detected in the EDX spectrum (Figure 1). The SEM images spectrums showed that the TiO<sub>2</sub>-NPs were round in shape and tend to agglomerate; this tendency might be based on its hydrophobic surface properties (Dogaroglu & Koleli, 2017a; Nia et al. 2015).

The particle size and size distribution of TiO<sub>2</sub>-NPs were measured by using a Zetasizer Nano ZS (Malvern Instruments Ltd., UK). The Zetasizer results showed that the mean particle size of TiO<sub>2</sub>-NPs was around 50nm (Figure 2).

### Hydroponic experiment

Among the plants, wheat, lettuce, cucumber, red clover and radish are the most recommended plants to determine the effects of NPs toxicity, by the US Environmental Protection Agency (US EPA), the US Food and Drug Administration (US FDA), and OECD (Faraji &

Sepehri, 2018). Therefore, the wheat plant (*Triticum vulgare* L. var. Yunus) was chosen as a model and widely used to evaluate the effect of the TiO<sub>2</sub>-NPs.

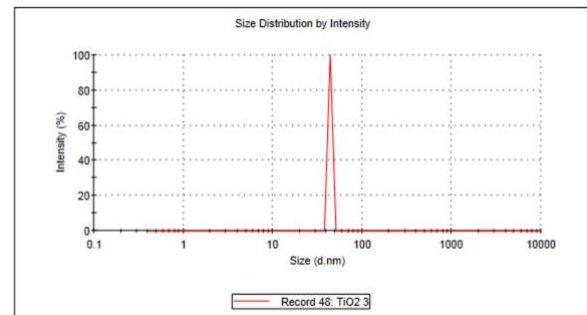


Figure 2. Particle size distribution by intensity of TiO<sub>2</sub>-NPs.

### Morphological observations and chlorophyll contents

The effects TiO<sub>2</sub>-NPs on morphological changes of wheat plants were observed throughout the experiment. The wheat plants did not show any visual symptoms such as chlorosis or necrosis under the increasing doses of TiO<sub>2</sub>-NPs treatments (Figure 3).

Chlorophyll, which gives green color to plants, is a vital and the most abundant pigment for plants. The primary function of chlorophyll is absorbing light to provide energy for photosynthesis (Tan et al., 2018). Determination of chlorophyll content, which is a vital pigment molecule for photosynthesis, is an important indicator for observation of plant growth, especially under stress conditions. In this study, the chlorophyll content of leaf was measured for determination of the effect of TiO<sub>2</sub>-NPs treatment on plant growth.

The 5 mg L<sup>-1</sup> TiO<sub>2</sub>-NP treatment increased chlorophyll content compared to control. On the other hand, the chlorophyll contents of plant decreased post 5 mg L<sup>-1</sup> TiO<sub>2</sub>-NP treatments ( $p < 0.01$ ). The highest chlorophyll content (43.1 SPAD Unit) was obtained at 5 mg L<sup>-1</sup> TiO<sub>2</sub>-NP treatment and the lowest chlorophyll content (40.9 SPAD Unit) was obtained at 40 mg L<sup>-1</sup> TiO<sub>2</sub>-NP treatment (Figure 4).

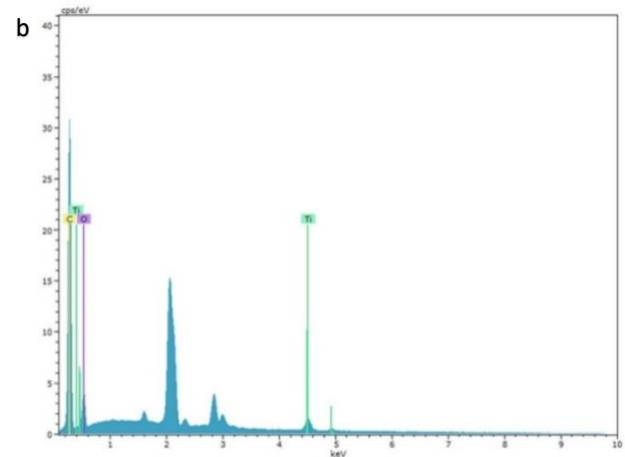
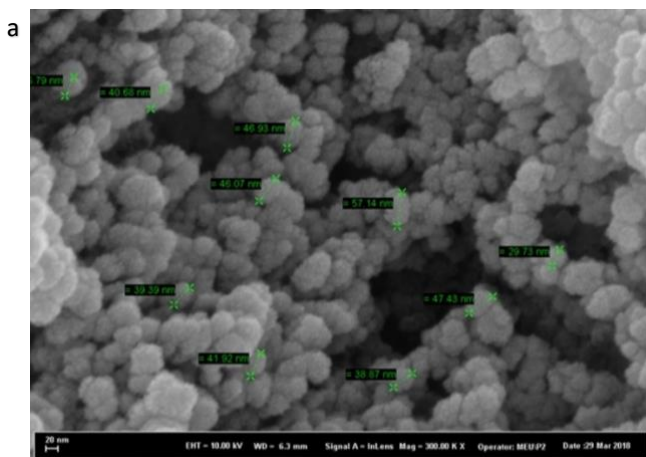
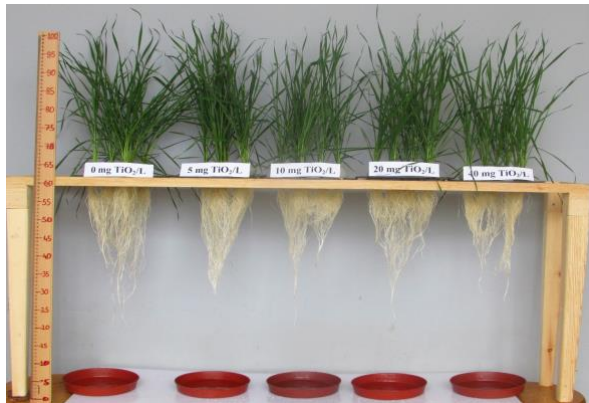
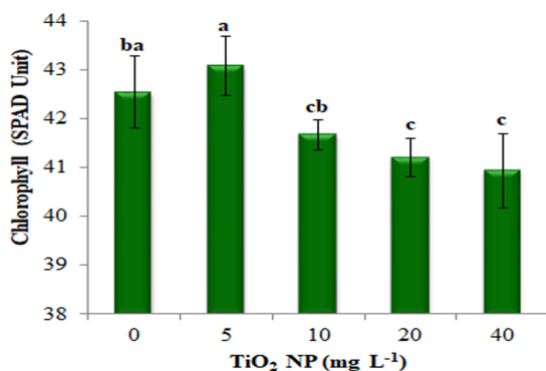


Figure 1. SEM image and EDX spectrum of synthesized the TiO<sub>2</sub>-NPs. Scanning Electron Microscopic image of TiO<sub>2</sub>-NPs (a) and elemental analysis of the TiO<sub>2</sub>-NPs (b).

Many conflicting results in the literature reported decreasing or increasing chlorophyll content in wheat under TiO<sub>2</sub>-NPs treatments. Dogaroglu & Koleli (2017a) notified that the chlorophyll contents of barley decreased at 20 mg kg<sup>-1</sup> TiO<sub>2</sub>-NPs treatments. They also found that the chlorophyll content of wheat was negatively affected by increasing doses of TiO<sub>2</sub>-NPs treatments (Dogaroglu & Koleli, 2017b). We had a similar result with Dogaroglu & Koleli (2017a, b). Aliabadi et al. (2016) investigated the effects of nano TiO<sub>2</sub> (0, 100, 1000, 2000 mg L<sup>-1</sup>) and nano Al<sub>2</sub>O<sub>3</sub> (0, 100, 500, 1000 mg L<sup>-1</sup>) on plant growth as a spray to the wheat leaves two times in a week. Compared to the control treatment, nano TiO<sub>2</sub> and Al<sub>2</sub>O<sub>3</sub> treatments significantly reduced chlorophyll a and b at high concentrations. The chlorophyll content (chlorophyll a and b) of wheat increased only at 100 mg L<sup>-1</sup> nano TiO<sub>2</sub> treatment. The photosynthetic abilities may be reduced decrease because of increased reactive oxygen species (ROS) and lipid peroxidation, thus the chlorophyll content decreases (Hou et al., 2018).



**Figure 3.** The effects of increased TiO<sub>2</sub>-NP suspensions on the shoot and root biomass of wheat plant grown in hydroponic culture after 21 days.



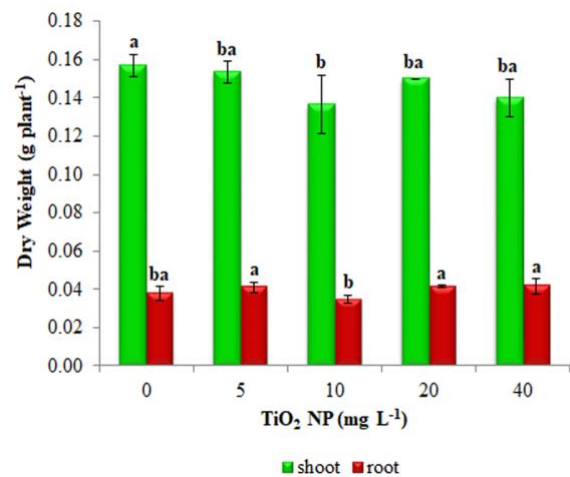
**Figure 4.** The effects of increased TiO<sub>2</sub>-NP suspensions on the chlorophyll content of wheat plant leaves (n=3). Letter(s) on each bar show significant level (p<0.01).

On the contrary to our result, many studies reported that TiO<sub>2</sub>-NP treatments increased photosynthetic activity and chlorophyll content of plants such as spinach (Hong et al., 2005; Yang et al., 2006), cucumber (Servin et al., 2012, 2013), Arabidopsis

(Lenaghan et al., 2013), rapeseed (Li et al., 2015). Yang et al. (2006) and Siddiqui et al. (2015) reported the TiO<sub>2</sub>-NPs enhanced the chlorophyll formation and light absorption, so the photosynthetic time of chloroplast and plant growth/development increased. Besides that, Hong et al. (2005) and Jaberzadeh et al. (2013) reported that TiO<sub>2</sub>-NPs positively affected photosynthetic activity and so the chlorophyll content of spinach and corn, respectively.

#### Plant dry weight (DW)

TiO<sub>2</sub>-NP treatments did not significantly affect shoots and roots dry weights (DW) of wheat plants (Figure 5). The recorded maximum and minimum shoot dry weights were obtained as 0.157 g at the control group and 0.137 g at 10 mg L<sup>-1</sup> TiO<sub>2</sub>-NPs treatment respectively. The effects of increased dose of TiO<sub>2</sub>-NPs on dry weights of shoots were not statistically significant. However, the shoot produced higher biomass than the roots (Figure 4). The lowest root dry weight (0.0383 g) was obtained in control and the highest root dry weight (0.0417 g) was recorded at 40 mg L<sup>-1</sup> TiO<sub>2</sub>-NPs treatment.



**Figure 5.** The TiO<sub>2</sub>-NP treatments effects on shoot and root DWs of the wheat plant. Letter(s) on each bar show significant level (n=3).

Studies in the literature showed that TiO<sub>2</sub>-NPs had positive effects sufficient or low concentrations and had adverse effects in high concentrations (Mahmoodzadeh et al., 2013; Rafique et al., 2014). Du et al. (2011) reported that the application of 10 mg kg<sup>-1</sup> TiO<sub>2</sub>-NPs into the soil reduced wheat growth and soil enzyme activities under field conditions. Feizi et al. (2012) showed a promoting effect of low concentrations of TiO<sub>2</sub>-NPs for seed germination of wheat. Jacob et al. (2013) did not observe differences in the wheat dry matter upon exposure to TiO<sub>2</sub>-NPs in a hydroponic system.

On the other hand, Du et al. (2011) have reported reduced wheat dry matters at a concentration of approximately 90 mg kg<sup>-1</sup> TiO<sub>2</sub>-NPs.

### Ti concentration

There is limited information about uptake, transport, accumulation and tolerable level of Ti and TiO<sub>2</sub>NPs by plants in literature. Lyu et al. (2017) reported that the concentration of Ti in above-ground parts of some plant species, which are grown in soils can vary between 1 to 578 mg kg<sup>-1</sup>. Kelemen et al. (1993) were found that Ti accumulates in roots and only a small amount of it transported to the shoots.

The Ti concentrations were not detected in the parts of the shoot in all treatments because it was under the detection limits (data not shown). However, the Ti concentration of roots was increased with increasing concentration of TiO<sub>2</sub>-NPs (Table 1).

**Table 1.** The TiO<sub>2</sub>-NP treatment's effects on Ti concentration (mg kg<sup>-1</sup>DW) and content (μg plant<sup>-1</sup>) of wheat roots (n=3)

Plant	TiO <sub>2</sub> NP (mg L <sup>-1</sup> )	Dry matter (g plant <sup>-1</sup> )	Ti concentration (mg kg <sup>-1</sup> DW)	Ti content (μg plant <sup>-1</sup> )
Wheat	0	0.0383 <sup>ba</sup>	n.d.	n.d.
	5	0.0409 <sup>a</sup>	1980 <sup>d</sup>	80.98 <sup>b</sup>
	10	0.0347 <sup>b</sup>	2285 <sup>c</sup>	79,29 <sup>b</sup>
	20	0.0414 <sup>a</sup>	2431 <sup>b</sup>	100.64 <sup>a</sup>
	40	0.0417 <sup>a</sup>	2603 <sup>a</sup>	108,55 <sup>a</sup>
Dose	F	3.272 <sup>n.s.</sup>	578 <sup>**</sup>	285 <sup>**</sup>

n.d.: not determined, n.s.: not significant, \*\*: p≤0.01

The highest Ti concentration (2603 mg kg<sup>-1</sup>) was obtained in 40 mg L<sup>-1</sup> TiO<sub>2</sub>-NP treatment, whereas the lowest (1980 mg kg<sup>-1</sup> DM) was obtained in 5 mg L<sup>-1</sup> TiO<sub>2</sub>-NP treatment (Table 1). This result may be indicated that TiO<sub>2</sub>-NPs could not be transferred from roots to the shoots of wheat due to its size or the lower exposure concentration relative to other studies (Larue et al., 2012). Larue et al. (2012) indicated that the upper threshold diameter of TiO<sub>2</sub>-NPs for root uptake was 140 nm and translocation in the shoot of wheat was 36 nm. Tan et al. (2017) reported that the TiO<sub>2</sub>-NPs, which have smaller particle sizes than 36 nm, could translocate from root to shoots.

### Ti content

The Ti content refers to total Ti concentration of root or shoot and it is directly related to the total dry mass of plant parts and Ti concentration.

The root Ti content was increased with the increasing TiO<sub>2</sub>-NP treatments (p≤0.01). The highest Ti content (108.55 μg plant<sup>-1</sup>) was shown in 40 mg L<sup>-1</sup> TiO<sub>2</sub>-NP treatment, whereas the lowest (79.29 μg plant<sup>-1</sup>) was

shown in 5 mg L<sup>-1</sup> TiO<sub>2</sub>-NP treatment (Table 1). The results were the same with results reported by Feizi et al. (2012). Consequently, due to TiO<sub>2</sub>-NPs accumulation in roots and less transfer to the shoots of wheat may not have caused any toxic effects on plants. Cox et al. (2017) reported similar results. Plants shown response to hydroponics exposure to NPs may differ from response to NP-contaminated soil exposure (Larue et al., 2012).

### Mineral nutrition uptake

In the literature, the metallic nanoparticle effects on plant mineral nutrition uptake were studied very few (Larue et al., 2018). However, any phytotoxicity may affect the plant mineral nutrient uptake as a synergistic, antagonistic or neutral. For this reason, the effects of increasing TiO<sub>2</sub>-NPs doses on macro (N, P, and K) and micro (Cu, Fe, Mn, and Zn) nutrient element concentrations of the shoot (Table 2) and root (Table 3) were investigated. This study ensured our understanding of the effects of the TiO<sub>2</sub>-NPs on some essential mineral nutrition uptake, such as N, P, K, Fe, Zn, Cu, and Mn of wheat.

The highest N (4.58%), P (0.78%), Zn (87.5 mg kg<sup>-1</sup>) and Cu (12.90 mg kg<sup>-1</sup>) concentrations were recorded at 40 mg L<sup>-1</sup> TiO<sub>2</sub>-NP treatment in shoots of wheat. However, K concentration was decreased at 20 and 40 mg L<sup>-1</sup> TiO<sub>2</sub>-NPs treatments and Mn concentration was not affected by TiO<sub>2</sub>-NP treatments. Except Mn concentration of shoot, mineral nutrient concentrations of shoot results were statistically important (p≤0.01). According to Jones et al. (1991), the shoots of wheat plant nutrients concentrations were determined to be sufficient Zn (21-70 mg kg<sup>-1</sup>) except for 40 mg of TiO<sub>2</sub> L<sup>-1</sup>, Fe (10-300 mg kg<sup>-1</sup>), Mn (16-200 mg kg<sup>-1</sup>), and Cu (5-50 mg kg<sup>-1</sup>). On the other hand, macronutrients (N (>3%), P (0.5-0.8%) and K (>5%)) concentrations were determined higher than the critical concentration of these nutrients in wheat. These results indicated that TiO<sub>2</sub>-NPs application was promoted N, P and K uptake of the plant shoots. Hong et al. (2005) and Yang et al. (2006) reported similar positive effects. Potassium, Zn, Mn, and Fe concentration of the roots were significantly decreased with TiO<sub>2</sub>-NP treatments compared to the control plant (p≤0.01). The higher K (4.94%), Zn (195 mg kg<sup>-1</sup>), Fe (3491 mg kg<sup>-1</sup>), and Mn (134.4 mg kg<sup>-1</sup>) were obtained in control treatments (Table 3). The highest P (2.21%) and Cu (32.1 mg kg<sup>-1</sup>) concentrations were obtained at 40 mg L<sup>-1</sup> TiO<sub>2</sub>-NP treatment. Other researchers reported

**Table 2.** The effects of increased TiO<sub>2</sub>-NP suspensions on some mineral nutrient concentration of wheat shoot (n=3)

Plant	TiO <sub>2</sub> NP (mg L <sup>-1</sup> )	N	P	K	Zn	Fe	Mn	Cu
		(% )			(mg kg <sup>-1</sup> )			
Wheat	0	4.31 <sup>b</sup>	0.65 <sup>d</sup>	6.03 <sup>a</sup>	46.4 <sup>b</sup>	165 <sup>c</sup>	147.3 <sup>a</sup>	9.70 <sup>c</sup>
	5	4.53 <sup>a</sup>	0.70 <sup>b</sup>	6.01 <sup>a</sup>	41.2 <sup>c</sup>	204 <sup>a</sup>	140.3 <sup>b</sup>	10.57 <sup>b</sup>
	10	4.35 <sup>b</sup>	0.69 <sup>cb</sup>	6.05 <sup>a</sup>	35.9 <sup>d</sup>	190 <sup>b</sup>	141.3 <sup>ba</sup>	10.00 <sup>c</sup>
	20	4.27 <sup>b</sup>	0.67 <sup>dc</sup>	5.82 <sup>b</sup>	41.6 <sup>cb</sup>	170 <sup>c</sup>	139.7 <sup>b</sup>	10.60 <sup>b</sup>
	40	4.58 <sup>a</sup>	0.78 <sup>a</sup>	5.72 <sup>b</sup>	87.5 <sup>a</sup>	158 <sup>c</sup>	141.3 <sup>ba</sup>	12.90 <sup>a</sup>
Dose	F	20.2 <sup>**</sup>	39.5 <sup>**</sup>	17.1 <sup>**</sup>	222 <sup>**</sup>	27.9 <sup>**</sup>	2.67 <sup>n.s.</sup>	67.3 <sup>**</sup>

\*\* : p≤0.01, n.s.: not significant

**Table 3.** The effects of increased TiO<sub>2</sub>-NP suspensions on some mineral nutrient concentration of root (n=3)

Plant	TiO <sub>2</sub> NP (mg kg <sup>-1</sup> )	P	K (%)	Zn	Fe	Mn (mg kg <sup>-1</sup> )	Cu
Wheat	0	1.87 <sup>d</sup>	4.94 <sup>a</sup>	195 <sup>a</sup>	3491 <sup>a</sup>	134.4 <sup>a</sup>	28.3 <sup>cb</sup>
	5	2.05 <sup>c</sup>	4.72 <sup>cb</sup>	105 <sup>b</sup>	1855 <sup>b</sup>	129.7 <sup>b</sup>	26.6 <sup>c</sup>
	10	1.82 <sup>e</sup>	4.80 <sup>b</sup>	94.0 <sup>c</sup>	1808 <sup>b</sup>	129.4 <sup>b</sup>	28.6 <sup>cb</sup>
	20	2.11 <sup>b</sup>	4.79 <sup>b</sup>	100 <sup>cb</sup>	710 <sup>c</sup>	129.3 <sup>b</sup>	29.3 <sup>b</sup>
	40	2.21 <sup>a</sup>	4.66 <sup>c</sup>	96.1 <sup>c</sup>	355 <sup>d</sup>	130.4 <sup>b</sup>	32.1 <sup>a</sup>
Dose	F	231 <sup>**</sup>	10.5 <sup>**</sup>	365 <sup>**</sup>	7251 <sup>**</sup>	7.12 <sup>**</sup>	11.0 <sup>**</sup>

n.s.: not significant \*\*: p<0.01

similar results. The application of increasing doses of TiO<sub>2</sub>-NP (250, 500, and 750 mg kg<sup>-1</sup>) to the soil was rising the accumulation of K and P concentration in cucumber fruit (Servin et al., 2013).

Mattiello & Marchiol (2017) reported that the TiO<sub>2</sub>-NP treatments (0, 500, 1000, and 2000 mg kg<sup>-1</sup>) to the soil affect mineral nutrition uptake of the barley (*Hordeum vulgare* L. var. Tunika) plant compared to the control treatment. The N, S, Ca, Fe, Mn, and Zn concentrations of barley seeds increased compared to the control treatment while K concentration decreased with TiO<sub>2</sub>-NP treatments. On the other hand, P, and Mg, B, and Cu concentrations did not affect the incensement of TiO<sub>2</sub>-NP treatments (Mattiello & Marchiol, 2017). Tan et al. (2017) reported that the unmodified TiO<sub>2</sub>-NPs enhanced Cu (104%) and Fe (90%); hydrophilic TiO<sub>2</sub>-NPs enhanced Fe (90%). However, Mn (339%) concentration raised while, Ca (71%), Cu (58%), and P (40%) concentration of basil plants decreased with 500 mg kg<sup>-1</sup> hydrophobic TiO<sub>2</sub> particles treatment to the soil. Pošćić et al. (2016), investigated that the application of increasing doses (0, 500, and 1000 mg kg<sup>-1</sup>) of CeO<sub>2</sub> and TiO<sub>2</sub>-NPs effects on the nutrient concentration of barley (*Hordeum vulgare* L). They significantly were obtained with increased Ca and significantly decreased sulfur (S) in both TiO<sub>2</sub>-NP treatments. Potassium concentration was decreased at 1000 mg kg<sup>-1</sup> TiO<sub>2</sub>-NP. Mn and Zn concentrations were increased at 500 mg kg<sup>-1</sup> TiO<sub>2</sub>-NPs treatment. All these results showed that increasing dose TiO<sub>2</sub>-NPs application positively affected nutrient uptake of wheat plant.

## Conclusion

Among metallic NPs, TiO<sub>2</sub>-NPs is the most produced and consumed materials by far. This extensive production and consumption are postulated to result in the release into the environment with subsequent contamination of soils and plants (agroecosystems). Thus, plants may be a potential entry point for TiO<sub>2</sub>-NPs in the food chain. Today TiO<sub>2</sub>-NPs contamination and accumulation in plants may be a severe problem around the world in the future due to the potential threat to food safety and its detrimental effects on the agroecosystems and human health.

This study provides new information about the effects of TiO<sub>2</sub>-NPs on growth and mineral nutrient uptake of shoots and roots of wheat (*Triticum vulgare* L.) in a hydroponic culture trial. The possible phytotoxic effect of TiO<sub>2</sub>-NPs on chlorophyll content, plant growth and mineral nutrient uptake of the wheat plant was investigated in this study. The TiO<sub>2</sub>-NPs reduced chlorophyll content over 5 mg L<sup>-1</sup> TiO<sub>2</sub>-NP treatment. Plant growth was not statistically affected by increasing doses of TiO<sub>2</sub>-NPs treatments. The dose of increased TiO<sub>2</sub>-NPs resulted in increased N, P, Zn, and Cu concentrations. Nevertheless, it caused K and Mn concentrations of the plant to reduce. Further study of the effects of lower and higher dosage TiO<sub>2</sub>-NPs on cereals and food crops should be needed to be undertaken.

Furthermore, for clarification of possible phytotoxic effects of TiO<sub>2</sub>-NPs on the wheat plant, more studies need to investigate genotypic differences of wheat plants. Besides these, the mobility, transportation, accumulation and risk assessments of NPs on the environment and living organisms should be urgently studied both in the field and in hydroponic culture experiments.

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