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# $TiO_2$ and ZnO Nanoparticles Toxicity in Barley (*Hordeum vulgare* L.)

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**In this study, the effects of $TiO_2$ and ZnO nanoparticles (0, 5, 10, 20, 40, and 80 mg $kg^{-1}$) on seed germination, root elongation, chlorophyll content, and the activities of antioxidant enzymes of barley (*Hordeum vulgare* L.) are studied. The number of seeds germinated and roots elongated in germinated plants (tillering stage) are determined on day 7. Seedlings are transferred to pots containing 50 g turf and grown for 21 days. Antioxidant levels (superoxide dismutase, catalase, ascorbate peroxidase, glutathione, and proline) are determined on day 21 after planting. The results shows that seed germination and root elongation are not significantly affected by types and concentrations of ZnO and $TiO_2$ nanoparticles. However, the antioxidant enzyme activities are diversely affected. Furthermore, the results also show that the ZnO nanoparticles are more toxic to barley plants than the $TiO_2$ nanoparticles.**

## 1. Introduction

Nowadays, researchers' studies on the possible effect of engineered nanoparticles on organisms have increased intensively since these particles can occur in the aquatic, terrestrial, and atmospheric environment. In this stage, the impact mechanism is important to determine the complex effects of nanoparticles such as composition, surface charge, physicochemical properties in shape and size. Some of these properties can be toxic to living organisms.[1,2] Release of nanoparticles into the environment can occur naturally, accidentally (such as, from industrial process) or deliberately (such as, from pesticides or fertilizers). The increasing usage of various nanoparticles in different industries needs more investigation to obtain more knowledge about their adverse effects on different plant species.

It is well known that nanoparticles can be transported by or can travel in aquatic environment and accumulate in soil[3–5] and pass through to the food chain via plants.[6,7] The soil-plant systems profile may change due to the interferences of nanoparticles in soil.[1] Plants can internalize the nanoparticles, so they can be translocated to other parts of the plants via the interior distribution system—phloem and xylem.[8,9] This internalization and translocation are related with the nanoparticles size and type. The concentrations, size, and characteristic features of nanoparticles as well as chemistry of the soil affect the seed germination and growth/development of plants.[10]

Accumulation of metal nanoparticles in soil may induce adverse effects on plant development. Some metals (such as Mn, Fe, Zn, and Cu) are essential micronutrients for plants and have an important role in life activities.[11] Like bulk metals, nanoparticles may also cause toxic effects on plant development at threshold concentrations. Furthermore shape, agglomeration and/or aggregation properties, surface characteristics, chemical composition, and especially the size of nanoparticles can change their toxic effects. Nanoparticles generally impact the antioxidant defense system in plants.[12–15] The change of reactive oxygen species (ROS) level in cells is an indicator of phytotoxic effects. It is believed that oxygen derivatives are one of the oldest stress sources for the living organisms.[16] Plants have different defense systems to protect itself from the oxidative stress.[17–20] These defense systems include enzymatic and nonenzymatic ROS scavenging mechanisms. The major antioxidative enzymes are superoxide dismutase (SOD), catalase (CAT), and ascorbate peroxidase (APX) and the major low molecular weight antioxidative metabolites are proline, glutathione, and ascorbic acid.[17]

Metallic nanoparticles like $TiO_2$ and ZnO are incorporated into agricultural products day by day, including nanofertilizers,[21] nanopesticides,[22] nanosensors,[23] soil remediation additives,[24] and exposure to wastewater.[25] Titanium dioxide and zinc oxide phytotoxicity were reported in different plant species in the literature. Some antioxidant enzyme activities and lipid peroxidation were induced because of ROS production increasing in *Vicia faba* and *Nicotiana tabacum* cells which were exposed to 0.2, 0.4, and 0.8 g $L^{-1}$ ZnO nanoparticles.[2] Similar studies demonstrated significant toxic effects of different metal and metal oxide nanoparticles because of their solubility and aggregation behavior in suspensions. For example the soybean seedlings gave different reaction to $Al_2O_3$ (500 mg $L^{-1}$), ZnO (500 mg $L^{-1}$), and Ag (50 mg $L^{-1}$) nanoparticles.[10]

Plants can uptake and translocate the metal oxide nanoparticles via root (through the apoplastic and symplastic pathway) or leaf (through the stomata or cuticle and then through the phloem) pathways.[1,26] There are several studies showing that metal oxide nanoparticles had adverse effects

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(physiological, antioxidant activities, and photosynthetic) in plants.[1,12,14] These studies showed that different nanoparticles have different impact in plants. For example magnetite nanoparticles did not affect the seed germination of *Triticum aestivum* L.[3] and also Ag nanoparticles have adverse effect on the seed germination of *Lolium perenne* L., *Hordeum vulgare* L., and *Linum usitatissimum* L.[27] The authors reported shoot elongation after the exposure to Ag nanoparticles was a better parameter to determine toxicity than the seed germination.[28]

ZnO and $TiO_2$ nanoparticles are the most common used nanoparticles in the consumer products and their potential phytotoxicity has been evaluated in this study. Barley is one of the major crops which has widespread cultivation around the world; so this plant was chosen as a model plant to evaluate the effect of these nanoparticles. The aim of this study was to evaluate the phytotoxic effects of ZnO and $TiO_2$ nanoparticles ($30\,nm^{-1}$) on seed germination and early stage of barley plant growth. Seed germination, root elongation, chlorophyll content, and antioxidants (SOD, CAT, APX, glutathione, and proline) in barley shoots were determined in this study.

## 2. Experimental Section

### 2.1. Chemicals

Titanium dioxide ($\sim 30\,nm^{-1}$) nanoparticles were synthesized by Dr. Birol Karakaya through using a combination of sol-gel and hydrothermal synthesis with small changes in traditional methods at a research center (BME-Kocaeli/Turkey) (method is not given here due to patent application). Zinc oxide nanoparticles ($\sim 30\,nm^{-1}$) were synthesized at Mersin University Advanced Technology of Education, Research, and Application Center (MEITAM). ZnO was synthesized according to the method of Ito et al.[29] with small changes. In the method, 5 g ($22\,mmol^{-1}$) zinc acetate dihydrate ($[CH_3COO_]2Zn \cdot 2\,H_2O$) was dissolved in $3\,mL^{-1}$ glycerol and then the mixture was heated at 150°C for 1 h. After the cooling process $8\,mL^{-1}$ 1-butanol (99.8%), $0.2\,mL^{-1}$ glycerol (≥99.5%), $5\,mL^{-1}$ trimethylamine (≥99.5%), and $0.1\,mL^{-1}$ deionized water were added. The mixture was stirred at 35°C for 3 days and then this mixture was sintered at 500°C for 2 h. The morphology of ZnO and $TiO_2$ nanoparticles was observed by field emission scanning electron microscopy (FE-SEM) (ZEISS, Sigma 500).

### 2.2. Seed Germination and Root Elongation Assay

According to US Environmental Protection Agency, there are two valid methods for determining phytotoxicity; seed germination and root elongation.[22] In the seed germination experiments, selected seeds were of uniform size to minimize error in germination. The seeds were sterilized in 70% ethanol for 30 s and then exposed to 3% sodium hypochlorite for $10\,min^{-1}$. After the sterilization, they were shaken in ultrapure water five times for $5\,min^{-1}$. Filter paper was cut to fit regular Petri dishes and it was used as inert material. A double layer of filter paper was placed in every Petri dish and then 10 barley seeds were placed in the Petri dishes. Then, the seeds were treated with $5\,mL^{-1}$ $TiO_2$ or ZnO nanoparticles suspension with different concentrations (0, 1, 5, 10, 20, 40, and $80\,mg\,L^{-1}$). The Petri dishes were incubated for 7 days in the dark at 25°C. Thereafter, on average five barley plants were chosen in every Petri dish and root length was measured by placing roots on graph paper with $1\,mm^{-1}$ squares.

### 2.3. Zinc and Titanium Analysis in Plants

Plants, germinated in Petri dishes for 7 days, were transferred to the pots which contained 50 g turf, obtained commercially (pH 6–8, total N: 0.2–0.45%, water holding capacity: 300–450%). Every pot included five barley plants and all the plants growing processes were conducted at room temperature. The plants were watered with tap water (pH 7.32 and conductivity $109.1\,\mu S\,cm^{-1}$) every second day. For determining Zn and Ti amount in barley shoots on day 21 after planting, they were harvested $1\,cm^{-1}$ above the ground and the possible contaminants were removed using deionized water. The plant shoots were digested with $5\,mL^{-1}$ of 12 M $HNO_3$ on hot plate at 220°C after oven-dried at 65°C for ca. 2 h. All the sample solutions were diluted to $10\,mL^{-1}$ with deionized water. Zn and Ti uptake by plants was determined by inductively coupled plasma MS (ICP-MS) (Agilent 7500ce Model ICP-MS) in three replications.

### 2.4. Chlorophyll Content

Chlorophyll content of leaves was measured on day 21 after planting and determined using a SPAD-502 chlorophyll meter (Konica–Minolta, Japan, 0.06 $cm^{-2}$ measurement area and its accuracy is ±1.0 SPAD units). The SPAD-502 calculates the chlorophyll content as soil plant analysis development value (SPAD value).

### 2.5. Antioxidant Analysis

The antioxidants, including SOD, CAT, APX, proline, and glutathione (GSH) in plant fresh biomass were measured on day 21 after planting. All spectrophotometric analyses were conducted by UV-vis spectrophotometer (PG Instruments, T90+ model). Barley plant shoots were harvested from $1\,cm^{-1}$ above the ground to prevent the possible contamination and then harvested plants were homogenized in $50\,mM^{-1}$ potassium phosphate buffer solution (pH 7.6) which contained $0.1\,mM^{-1}$ EDTA. The homogenate was centrifuged at $15000 \times g$ at 4°C for $10\,min^{-1}$. The supernatant was used for SOD, CAT, and APX analyses.

#### *2.5.1. Superoxide Dismutase (SOD) Activity*

SOD activity was determined by the *p*-nitro blue tetrazolium chloride (NBT) photochemical assay as described by Cakmak and Marschner[30] and Cakmak.[31] In this method, $5\,mL^{-1}$ reaction mixture included $50\,mM^{-1}$ potassium phosphate buffer (pH 7.6), $5\,mM^{-1}$ NBT, $0.1\,mM^{-1}$ riboflavin, $12\,mM^{-1}$ L-methionine,

0.5 $mM^{-1}$ $Na_2CO_3$, and 200 $\mu L^{-1}$ samples were added into glass tubes and the reaction started with light for 8–10 $min^{-1}$. After the reaction time, absorbance was measured at 560 $nm^{-1}$.

#### 2.5.2. Ascorbate Peroxidase (APX) Activity

Ascorbate peroxidase activity was determined as described by Cakmak and Marschner.[30] The reaction mixture included 50 $mM^{-1}$ potassium phosphate buffer (pH 7.6), 0.5 $mM^{-1}$ ascorbic acid, 12 $mM^{-1}$ $H_2O_2$ with EDTA and 0.1 $mL^{-1}$ of enzyme extract. The activity of ascorbate peroxidase was measured at 290 $nm^{-1}$ and calculated using the extinction coefficient (2.8 mM $cm^{-1}$).

#### 2.5.3. Catalase (CAT) Activity

Catalase activity was determined as described by Cakmak and Marschner.[30] The decomposition of $H_2O_2$ was followed by decline in absorbance at 240 $nm^{-1}$. A 1 $mL^{-1}$ of reaction mixture included 50 $mM^{-1}$ potassium phosphate buffer (pH 7.6), 10 $mM^{-1}$ $H_2O_2$ and 0.1 $mL^{-1}$ of enzyme extract. The activity of catalase was calculated using the extinction coefficient (39.4 mM $cm^{-1}$).

#### 2.5.4. Proline Analysis

Proline analysis was performed as described by Bates et al.[32] Harvested barley plants were homogenized in 3% sulfosalicylic acid and the homogenate was centrifuged at 12 000×*g* at 4°C for 10 $min^{-1}$. The supernatant was used for proline analysis. The reaction medium contained 2 $mL^{-1}$ acid-ninhydrin (1.25 g ninhydrin in 30 $mL^{-1}$ glacial acetic acid and 20 $mL^{-1}$ 6 M phosphoric acid), 2 $mL^{-1}$ glacial acetic acid (≥99.85%) and 2 $mL^{-1}$ of enzyme extract. Test tube was incubated for 1 h at 100°C, and then the reaction was stopped in an ice bath. The reaction mixture was extracted with 4 $mL^{-1}$ toluene (≥99%) and mixed with a stirrer for 15–20 s. The absorbance was read at 520 $nm^{-1}$ using toluene as a blank. The proline concentration was determined by a standard curve.

#### 2.5.5. Glutathione (GSH) Analysis

The concentration of GSH in barley plants homogenates was predicted by evaluating GSH, via the 5,5′-dithiobis (2-nitrobenzoic acid) (DTNB) method which is described by Sedlak and Lindsay[33] and the results were expressed as $\mu M\ g^{-1}$ GSH $g^{-1}$ fresh plant. In this method, 0.5 g harvested plants were homogenized in 5 $mL^{-1}$ of 5% *meta*-P acid. The samples were centrifuged at 400 $rpm^{-1}$ for 30 $min^{-1}$. A 2.5 $mL^{-1}$ phosphate buffer (pH 7.5) with EDTA and 0.5 $mL^{-1}$ of 6 $mM^{-1}$ DTNB was added to 0.5 $mL^{-1}$ supernatant and after 15–20 $min^{-1}$ the absorbance was measured at 412 $nm^{-1}$. The glutathione concentration was determined by a standard curve prepared with reduced glutathione standard.

### 2.6. Statistical Analysis

Statistical analyses were performed using SPSS Version 21 software (SPSS, USA). For the samples applied, one-way analysis of variance (ANOVA) was performed in a completely randomized design in three replications. The data were subjected to the analysis of variance at the 5% level of significance shown as $p < 0.05$. The significant levels of difference for all measured traits were calculated and the mean was compared by the least significant difference, LSD, test at 5% level.

## 3. Results and Discussions

### 3.1. Nanoparticles Characterization

The FE-SEM image of synthesized ZnO and $TiO_2$ nanoparticles is shown in **Figure 1** and the average size of nanoparticles was about 30 $nm^{-1}$. The FE-SEM image showed that the ZnO nanoparticles have hexagonal or octagonal structure and the $TiO_2$ nanoparticles tend to agglomerate and this might be based on its hydrophobic surface properties.[34]

### 3.2. Seed Germination and Root Elongation

Under the experimental conditions, the seed germination was not affected by application of ZnO and $TiO_2$ nanoparticles with respect to control ($p > 0.05$). The average seed germination rate was 96% for all nanoparticles applications. These results fit with reports in the literature.[14,22,35,36]

The difference between control and all the concentrations of ZnO and $TiO_2$ nanoparticles in root elongation was not significant ($p > 0.05$) (**Figure 2**). The maximum root elongation was observed at 5 mg $L^{-1}$ ZnO nanoparticles concentration as 17.8 $cm^{-1}$ and the control plants root length was 15.9 $cm^{-1}$. The minimum root length was observed at 80 mg $L^{-1}$ $TiO_2$ nanoparticles concentration as 10.9 $cm^{-1}$, whereas the control plants' length was 13.1 $cm^{-1}$.

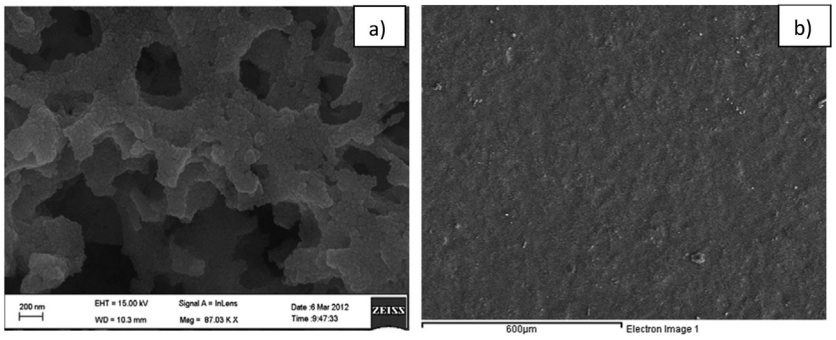


**Figure 1.** SEM image of synthesized nanoparticles; a) ZnO and b) $TiO_2$.

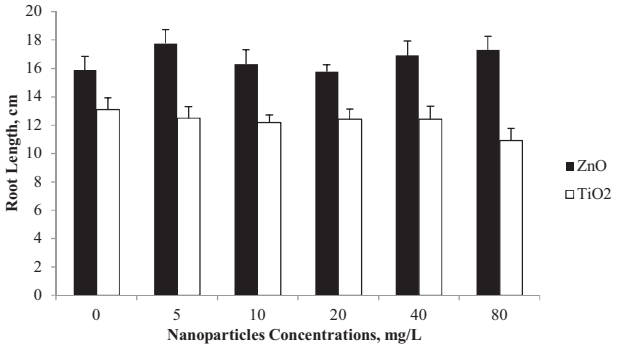


**Figure 2.** Effect of ZnO and $TiO_2$ nanoparticles on root length of barley.

Various studies on physiological and biological effects of different nanoparticles have shown positive or negative effects on root and shoot elongation in different plant species like adverse effects of 0.5 $mM^{-1}$ CuO nanoparticles on barley root and shoot elongation,[12] adverse effect of ZnO nanoparticles on root elongation of rice (100, 500, and 1000 mg $L^{-1}$), blue pardo verde (4000 mg $L^{-1}$), velvet mesquite (500, 1000, 2000, and 4000 mg $L^{-1}$) and tumbleweed (500 and 2000 mg $L^{-1}$)[13,37] and positive effects of ZnO nanoparticles (1000 mg $L^{-1}$) on root elongation of peanut.[38] On the other hand some reports proved that nanoparticles had no effect on seed germination and root elongation as in the report of Shaw et al.[12] and Boonyanitipong et al.[39]

### 3.3. Uptake of Metals by Barley

To determine the effects of $TiO_2$ and ZnO nanoparticles on barley plants, the uptake of Ti and Zn ions in barley shoots were also analyzed. Quantity of titanium in barley shoots was 100 times higher than the control groups at a concentration of 40 mg $kg^{-1}$ $TiO_2$ nanoparticles as 1 $mg^{-1}$ Ti $kg^{-1}$. The minimum titanium uptake was observed at concentration of 20 mg $kg^{-1}$ $TiO_2$ nanoparticles as 0.35 $mg^{-1}$ Ti $kg^{-1}$ ($p < 0.05$) and Zn uptake as 7.78 $mg^{-1}$ Zn $kg^{-1}$ at a concentration of 20 mg $kg^{-1}$ ZnO nanoparticles ($p < 0.05$). The maximum zinc uptake by barley was observed at a concentration of 80 mg $kg^{-1}$ ZnO nanoparticles as 16.51 mg $kg^{-1}$ and this value is 2.68 times higher than the control ($p < 0.05$). The uptake of both nanoparticles by barley increased with increasing concentrations, showing a drop at 20 mg $kg^{-1}$ and then increased again (**Figure 3**).

### 3.4. Chlorophyll Content

The general phytotoxicity symptoms on early growth stage of plants are decrease of chlorophyll content and growth retardations.[3] These symptoms were related to the size of $TiO_2$ nanoparticles, chemical composition, surface properties, and ionization of the surface[3,40] and effects of ZnO nanoparticles on photosynthesis also depending on different conditions, especially plant species, and concentration of nanoparticles.[1,41–43] Rao and Shekhawat reported that the bulk

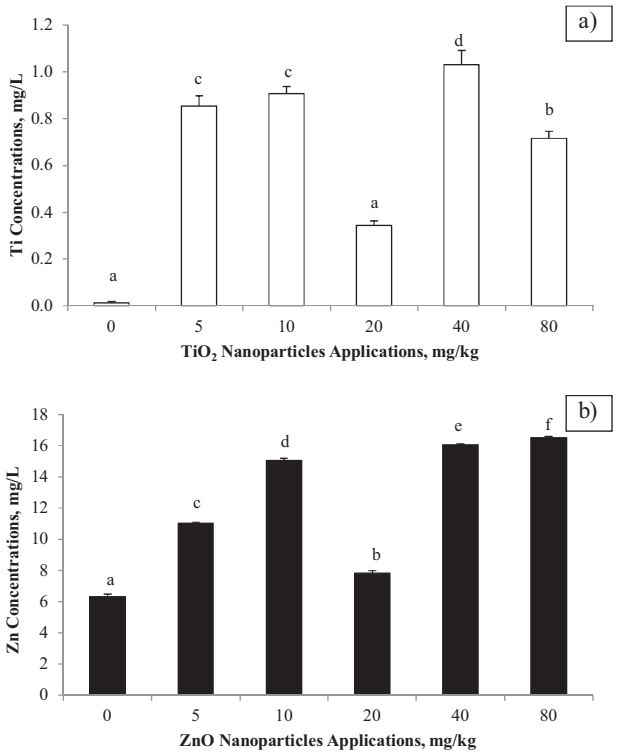


**Figure 3.** Uptake of metals by barley; a) Ti and b) Zn (the significance between the groups is indicated by different letters).

$Zn^{+}$ ions and ZnO nanoparticles caused phytotoxicity as retardation of growth.[15] The chlorophyll contents of barley exposed to $TiO_2$ and ZnO nanoparticles suspensions tend to decrease as compared to control, especially at a concentration of 20 mg $kg^{-1}$ nanoparticles ($p < 0.05$) (**Figure 4**). The minimum chlorophyll content of leaves was observed at this concentration for ZnO nanoparticles 22.3 SPAD units and for $TiO_2$ nanoparticles 16.75 SPAD units ($p < 0.05$). Shaw et al. reported that the chlorophyll content in barley leaves which were exposed to CuO nanoparticles was not affected for the first ten days, but it decreased after 20 days.[12]

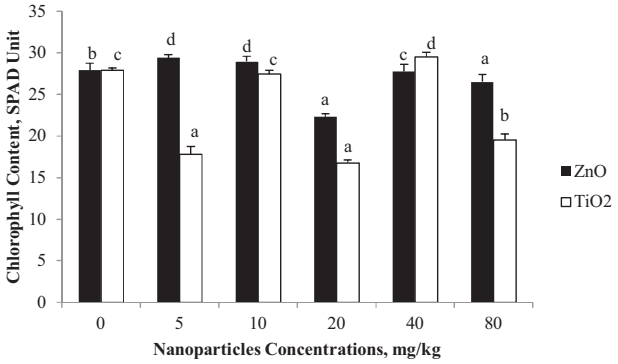


**Figure 4.** Chlorophyll content of barley leaves after application of ZnO and $TiO_2$ nanoparticles (the significance between the groups is indicated by different letters).

### 3.5. Antioxidant Enzyme Activities

The major antioxidant enzymes—SOD, CAT, and APX are responsible for scavenging of ROS—were measured to determine the oxidative stress in barley plants after nanoparticles' application. In the literature, several reports show that the antioxidant enzymes activities increase because of nanoparticles application on plants. However, studies proved that the antioxidant enzymes gave different reactions to different nanoparticles. The changes in enzymes activities may occur at germination stage or growth, at development stage exposure pathway, and based on concentration of nanoparticles. SOD activity in barley, after exposure to $TiO_2$ nanoparticles, decreased at low concentrations in contrast to ZnO nanoparticles application ($p < 0.05$). The maximum and minimum SOD activity were observed at 40 mg kg$^{-1}$ (68.97 U g$^{-1}$ fresh weight [FW]) and at 10 mg kg$^{-1}$ (22.63 U g$^{-1}$ FW) for $TiO_2$ nanoparticles, respectively, and at 10 mg kg$^{-1}$ (92.23 U g$^{-1}$ FW) and 40 mg kg$^{-1}$ (63.09 U g$^{-1}$ FW) for ZnO nanoparticles, respectively (**Figure 5**).

The sensitivity of enzymatic reactions depends on the functions of each enzyme; for example, catalase is responsible for the transformation of $H_2O_2$ to $H_2O$ and $O_2$.[44] The presence of hydroxyl radicals causes an increase in CAT activity.[45] In this study, CAT activity significantly increased after both $TiO_2$ and ZnO nanoparticles exposure at all concentrations ($p < 0.05$). The minimum activity was observed for 20 mg kg$^{-1}$ ZnO nanoparticles as 0.63 mmol min$^{-1}$ and for 40 mg kg$^{-1}$ $TiO_2$ nanoparticles as 1.760 mmol min$^{-1}$. It is predicted that the hydroxyl radicals caused this increase in CAT activity (**Figure 6**). Servin et al. reported that the $TiO_2$ nanoparticles exposure caused an increase in CAT activity in cucumber leaves.[35]

The maximum APX activity in barley plants was determined for 10 mg kg$^{-1}$ (0.05 mmol min$^{-1}$) $TiO_2$ nanoparticles ($p < 0.05$) and 5 mg kg$^{-1}$ (0.079 mmol min$^{-1}$) ZnO nanoparticles ($p > 0.05$). The general situation of APX activity showed a falling tendency after the application of both $TiO_2$ and ZnO nanoparticles (**Figure 7**).

The changes in the antioxidant enzymes response depend on the type and concentration of plant species and exposure pathways of metallic nanoparticles. Shaw and Hossain,[14] and

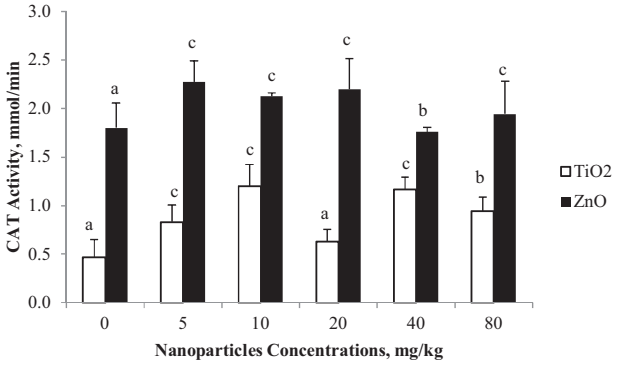


**Figure 6.** CAT activity in barley plants after application of ZnO and $TiO_2$ nanoparticles (the significance between the groups is indicated by different letters).

Shaw et al.[12] reported application of 1 and 1.5 mM$^{-1}$ CuO nanoparticles caused significantly increases in APX activity but it was not significantly affected for 0.5 mM$^{-1}$ CuO nanoparticles application in rice and barley plants.

### 3.6. Proline and Glutathione Levels

Proline is a component of the defense system in living organisms against oxidative stress and accumulates in organisms under heavy metals exposure. It is known that accumulation of proline under stress conditions has a role as membrane fixing agent.[15,42,44,46] In the present study, proline content was significantly affected by application of $TiO_2$ nanoparticles at which the maximum proline content was determined for 5 mg kg$^{-1}$ (17.0 μmol g$^{-1}$ FW) ($p < 0.05$). On the other hand, the proline content tended to decrease in ZnO nanoparticles treated plants in comparison to control plants ($p > 0.05$) (**Figure 8**a and b). Ozden et al. demonstrated that proline had a positive effect on antioxidant enzymes and played a protective role in grapevine leaves against oxidative stress effects.[44]

Glutathione (GSH) content was significantly affected at 40 mg kg$^{-1}$ $TiO_2$ nanoparticles ($p < 0.05$), and it significantly decreased at 10 mg kg$^{-1}$ ZnO nanoparticles ($p < 0.05$) (**Figure 9**).

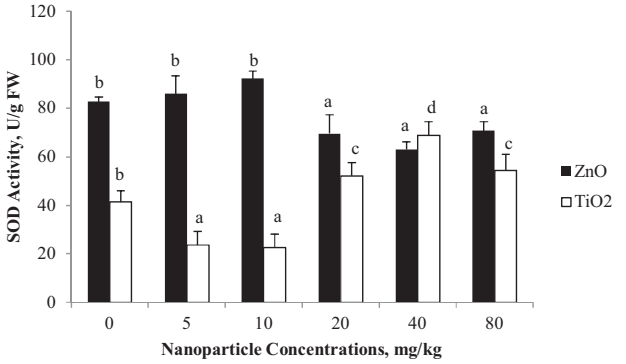


**Figure 5.** SOD activity in barley plants after application of ZnO and $TiO_2$ nanoparticles (the significance between the groups is indicated by different letters).

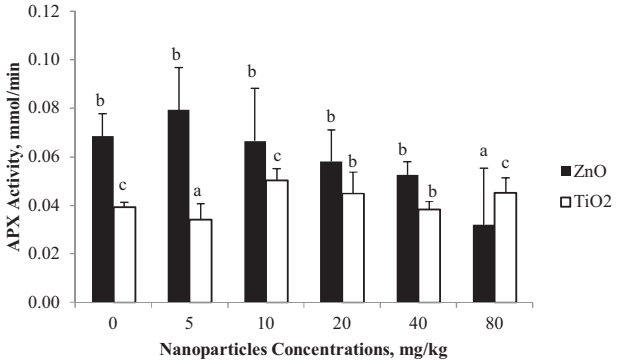


**Figure 7.** APX activity in barley plants after application of ZnO and $TiO_2$ nanoparticles (the significance between the groups is indicated by different letters).

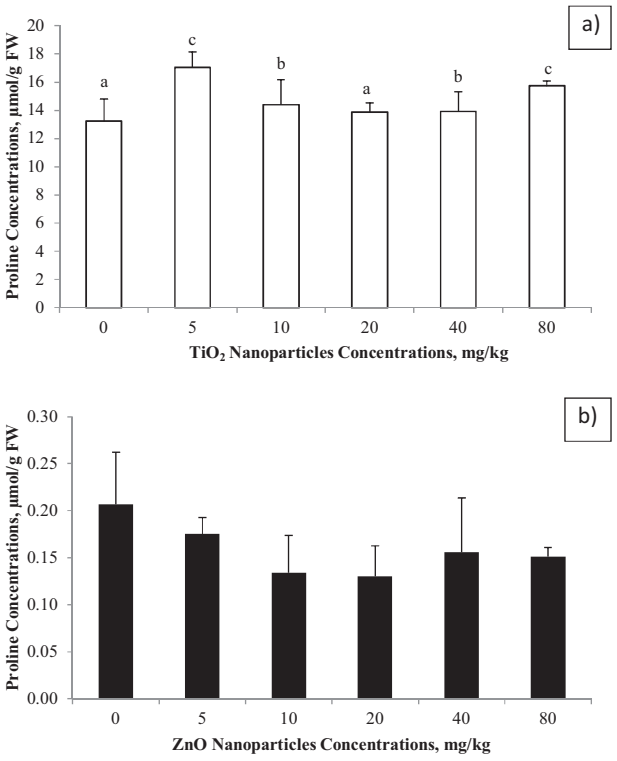


**Figure 8.** Proline concentrations in barley plants after application of nanoparticles; a) $TiO_2$ and b) ZnO (the significance between the groups is indicated by different letters).

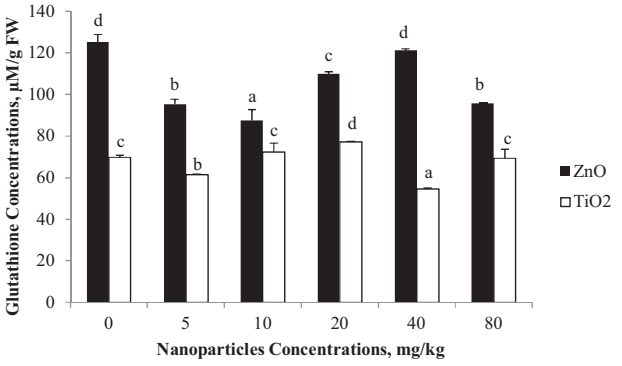


**Figure 9.** Glutathione concentrations of barley after application of ZnO and $TiO_2$ nanoparticles (the significance between the groups is indicated by different letters).

A similar result was presented by Shaw et al. in barley plants after exposure to CuO nanoparticles.[12]

## 4. Concluding Remarks

In this study, the phytotoxic effects of $TiO_2$ and ZnO nanoparticles on barley were evaluated. It is clear that the effect of nanoparticles depend on plant species, nanoparticles concentrations and also exposure pathways. Zinc oxide and $TiO_2$ nanoparticles had no effect on seed germination of barley. Both of these nanoparticles affected the chlorophyll content and antioxidant levels at seedling stage (21 days after planting). Superoxide dismutase enzymes activities showed a tendency to decrease with increasing ZnO nanoparticles concentrations in contrast to $TiO_2$. The results of chlorophyll content, antioxidant levels, and concentrations of Ti and Zn in the plants demonstrated that the plant was stressed at a concentration of 20 mg $kg^{-1}$ $TiO_2$ and ZnO nanoparticles. The current knowledge on effects and stress mechanisms of nanoparticles is not well known. For this reason more studies should be done on plants. The results demonstrated that the ZnO nanoparticles are more toxic to barley than the $TiO_2$ nanoparticles. More studies should be done in different crop plants to verify the toxicological effects of $TiO_2$ and ZnO nanoparticles especially between concentrations of 10 and 40 mg $kg^{-1}$.

## Abbreviations

APX, ascorbate peroxidase; CAT, catalase; DTNB, 5,5′-dithiobis (2-nitrobenzoic acid); FE-SEM, field emission scanning electron microscopy; FW, fresh weight; GSH, glutathione; ICP-MS, inductively coupled plasma mass spectrometry; NBT, *p*-nitro blue tetrazolium chloride; ROS, reactive oxygen species; SOD, superoxide dismutase; SPAD, soil-plant analyses development.

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## Ethical Conflict

The authors have declared no conflict of interest.

## Keywords

Chlorophyll content, Nanoparticles, Oxidative stress, Phytotoxicity, Seed germination

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[1] W. Du, W. Tan, J. R. Peralta-Videa, J. L. Gardea-Torresdey, R. Ji, Y. Yin, H. Guo, *Plant Physiol. Biochem*. **2017**, *110*, 210.
[2] M. Ghosh, A. Jana, S. Sinha, M. Jothiramajayam, A. Nag, A. Chakraborty, A. Mukherjee, A. Mukherjee, *Mutat. Res*. **2016**, *807*, 25.
[3] M. F. Iannone, M. D. Groppa, M. E. de Sousa, M. B. F. van Raap, M. P. Benavides, *Environ. Exp. Bot*. **2016**, *131*, 77.
[4] Z. Hossain, G. Mustafa, S. Komatsu, *Int. J. Mol. Sci*. **2015**, *16*, 26644.
[5] C. Ma, J. C. White, O. P. Dhankher, B. Xing, *Environ. Sci. Technol*. **2015**, *49*, 7109.
[6] C. Remedios, F. Rosario, V. Bastos, *J. Bot*. **2012**, *1–8*, 751686.

 

[7] M. Hatami, K. Kariman, M. Ghorbanpour, *Sci. Total Environ*. **2016**, *571*, 275.
[8] Z. Cifuentes, L. Custardoy, J. M. de la Fuente, C. Marquina, M. R. Ibarra, D. Rubiales, A. Pérez-de-Luque, *J. Nanobiotechnol*. **2010**, *26*, 1.
[9] X. Ma, J. Geisler-Lee, J. Deng, A. Kolmakov, *Sci. Total Environ*. **2010**, *408*, 3053.
[10] Z. Hossain, G. Mustafa, K. Sakata, S. Komatsu, *J. Hazard. Mater.* **2016**, *304*, 291.
[11] M. Gil-Díaz, A. Gonzalez, J. Alonso, M. C. Lobo, *J. Environ. Manage*. **2016**, *165*, 150.
[12] A. K. Shaw, S. Ghosh, H. M. Kalaji, K. Bosa, M. Brestic, M. Zivcak, Z. Hossain, *Environ. Exp. Bot*. **2014**, *102*, 37.
[13] C. M. Rico, J. Hong, M. I. Morales, L. Zhao, A. C. Barrios, J. Y. Zhang, J. R. Peralta-Videa, J. L. Gardea-Torresdey, *Enviro. Sci. Technol* **2013**, *47*, 5635.
[14] A. K. Shaw, Z. Hossain, *Chemosphere* **2013**, *93*, 906.
[15] S. Rao, G. S. Shekhawat, *J. Environ. Chem. Eng*. **2014**, *2*, 105.
[16] D. K. Dowling, L. W. Simmons, *Proc. R. Soc. London, Ser. B* **2009**, *276*, 1737.
[17] V. Demidchik, *Environ. Exp. Bot*. **2015**, *109*, 212.
[18] K. Asada, M. Takahashi, *Photoinhibition, in Production and Scavenging of Active Oxygen in Chloroplasts*. (Eds: D. J. Kyle, C. B. Osmond, C. J. Arntzen). Elsevier, Amsterdam **1987**, p. 227.
[19] Z. Ye, R. Rodriguez, A. Tran, H. Hoang, D. D. Los Santos, S. Brown, R. L. Vellanoweth, *Plant Sci*. **2000**, *158*, 115.
[20] S. Teotia, D. Singh, *Approaches to Plant Stress and Their Management*. (Eds: R. K. Gaur, P. Sharma). Springer, New Delhi, India **2014**, p. 243.
[21] M. R. Naderi, A. Danesh- Shahraki, *Int. J. Agric. Crop Sci* **2013**, *5*, 2229.
[22] L. R. Khot, S. Sankaran, J. M. Maja, R. Ehsani, E. W. Schuster, *Crop Prot*. **2012**, *35*, 64.
[23] V. Rai, S. Acharya, N. Dey, *J. Biomater. Nanobiotechnol*. **2012**, *3*, 315.
[24] B. Pan, B. Xing, *Eur. J. Soil Sci*. **2012**, *63*, 437.
[25] K. S. Brar, M. Verma, R. D. Tyagi, R. Y. Surampalli, *Waste Manage*. **2010**, *30*, 504.
[26] C. Larue, H. Castillo-Michel, S. Sobanska, N. Trcera, S. Sorieul, L. Cecillon, L. Ouerdanef, S. Legrosg, G. Sarreta, *J. Hazard. Mater.* **2014**, *273*, 17.
[27] Y. S. El-Temsah, E. J. Joner, *Environ. Toxicol*. **2012**, *27*, 42.
[28] A. Cox, P. Venkatachalam, S. Sahi, N. Sharma, *Plant Physiol. Biochem*. **2016**, *107*, 147.
[29] D. Ito, M. L. Jespersen, J. E. Hutchison, *ACS Nano* **2008**, *2*, 2001.
[30] I. Cakmak, H. Marschner, *Plant Physiol*. **1992**, *98*, 1222.
[31] I. Cakmak, *J. Exp. Bot*. **1994**, *45*, 1259.
[32] L. S. Bates, R. P. Waldren, I. D. Teare, *Plant Soil* **1973**, *39*, 205.
[33] J. Sedlak, R. H. Lindsay, *Anal. Biochem*. **1968**, *25*, 192.
[34] M. H. Nia, M. Rezaei-Tavirani, A. R. Nikoofar, H. Masoumi, R. Nasr, H. Hasanzadeh, M. Jadidi, M. Shadnush, *J. Paramed. Sci*. **2015**, *6*, 96.
[35] A. D. Servin, M. I. Morales, H. Castillo-Michel, J. A. Hernandez-Viezcas, B. Munoz, L. Zhao, J. E. Nunez, J. R. Peralta-Videa, J. L. Gardea-Torresdey, *Environ. Sci. Technol* **2013**, *47*, 11592.
[36] D. Lin, B. Xing, *Environ. Pollut* **2007**, *150*, 243.
[37] G. de la Rosa, M. L. Lopez–Moreno, J. Hernandez-Viezcas, M. O. Montes, J. R. Peralta Videa, *Int. J. Nanotechnol*. **2011**, *8*, 492.
[38] T. N. V. K. V. Prasad, P. Sudhakar, Y. Sreenivasulu, P. Latha, V. Munaswamy, K. Raja Reddy, T. S. Sreeprasad, P. R. Sajanlal, T. Pradeep, *J. Plant Nutr* **2012**, *35*, 905.
[39] P. Boonyanitipong, B. Kositsup, P. Kumar, S. Baruah, J. Dutta, *Int. J. Biosci. Biochem. Bioinf*. **2011**, *1*, 282.
[40] M. H. Ghafariyan, M. J. Malakouti, M. R. Dadpour, P. Stroeve, M. Mahmoudi, *Environ. Sci. Technol*. **2013**, *47*, 10645.
[41] L. J. Zhao, Y. P. Sun, J. A. Hernandez-Viezcas, A. D. Servin, J. Hong, G. H. Niu, J. R. Peralta-Videa, M. Duarte-Gardea, J. L. Gardea-Torresdey, *J. Agric. Food Chem* **2013**, *61*, 11945.
[42] C. Poschenrieder, J. Barcelo, *Heavy metal stress in plants*. (Ed: M. N. V. Prasad). Narosa Publishing House, New Delhi **2004**, p. 249.
[43] C. O. Dimkpa, J. E. McLean, D. E. Latta, E. Manangon, D. W. Britt, W. P. Johnson, M. I. Boyanov, A. J. Anderson, *J. Nanopart. Res*. **2012**, *14*, 1.
[44] M. Ozden, U. Demirel, A. Kahraman, *Sci. Hort*. **2009**, *119*, 163.
[45] K. T. Kim, S. J. Klaine, J. Cho, S.-H. Kim, S. D. Kim, *Sci. Total Environ*. **2010**, *408*, 2268.
[46] G. Kaur, B. Asthir, *Biol. Plant* **2015**, *59*, 609.

 