EFFECTS OF TiO_2 AND ZnO NANOPARTICLES ON GERMINATION AND ANTIOXIDANT SYSTEM OF WHEAT (*TRITICUM AESTIVUM* L.)

DOĞAROĞLU, Z. G.* – KÖLELI, N.

Department of Environmental Engineering, Faculty of Engineering, Mersin University, Ciftlikkoy Campus, TR-33343, Mersin, Turkey
(phone: +90-324-361-0001; fax: +90-324-361-0032)

**Corresponding author*
e-mail: gorkemgulmez@gmail.com

(Received 23rd Feb 2017; accepted 19th May 2017)

Abstract. In this study, effects of two types of nanoparticles (titanium dioxide and zinc oxide) suspensions on root-shoot elongation, chlorophyll content, metal uptake, and antioxidant enzymes activities of bread wheat (*Triticum aestivum* L. Michelangelo) were investigated. Wheat seeds were germinated in petri dishes with or without different concentrations of nanoparticles (0, 5, 10, 20, 40, and 80 mg/kg). After 7 days the germinated seedlings were transferred to pots containing turf and grown for 15 days.The results showed that TiO_2 and ZnO nanoparticles had no effect on seed germination. Both TiO_2 and ZnO nanoparticles effected root-shoot elongation, antioxidant enzymes, and the whole TiO_2 nanoparticles concentrations caused oxidative stress. Our data showed that both type of nanoparticles were toxic for wheat.
Keywords: *antioxidant enzymes; chlorophyll; germination; SOD; plant*

Introduction

Titanium dioxide (TiO_2) and zinc oxide (ZnO) nanoparticles (NPs) are the most commonly used nanoparticles in many industries because of their unique physical and chemical properties. They have expanded the application into the drug industry for white pigment, into cosmetic industry for sunscreen, paint industry and electronic industry (Kim et al., 2010; Lin and Xing, 2007). Nanoparticles occur in the environment because of their increasing use. The production, use, and disposal of nanomaterials will fatefully cause their release into atmospheric, aquatic and terrestrial environment (Lin and Xing, 2007). Plants are the most common-used materials to determine the environmental effect of nanoparticles. Some bacteria such as *E. coli* (Kumar et al., 2011), and *Scenedesmus obliquus* (Dalai et al., 2013; Wei et al., 2010), some aquatic invertebrates such as *Daphnia magna* (Zhu et al., 2010; Kim et al., 2010) and some aquatic vertebrates such as zebra-fish (*Danio rerio*) (Ma et al., 2013) are also used. In the environment, plants and microorganisms can internalize the natural NPs but the engineered nanoparticles may cause toxic effect on the organisms. Plants use various amount of antioxidant enzymes in an attempt to protect itself from toxicity (Asada and Takahashi, 1987; Ye et al., 2000).

There is an increasing amount of investigation on effect of nanoparticles on different organisms. There is scarce knowledge on the effect of metallic NPs especially TiO_2 NPs on plants (Larue et al., 2012; Du et al., 2011; Song et al., 2013; Gubbins et al., 2011) compared to animals (Feizi et al., 2013). The toxic effect of ZnO NPs was more than the other nanoparticles (e.g. TiO_2, CuO) on organisms. The investigation effect of TiO_2 and ZnO NPs determined at three different concentrations on root meristem cells of *Allium*

http://www.aloki.hu ● ISSN 1589 1623 (Print) ● ISSN 1785 0037 (Online)
DOI: http://dx.doi.org/10.15666/aeer/1503_14991510

cepa. The researcher showed that the nanoparticles caused DNA damage at100 and 1000 mg/L ZnO and 1000 mg/L TiO_2 nanoparticles concentrations (Demir et al., 2014).

There are currently studies investigating the effects of different sizes of TiO_2 nanoparticles on wheat and different plants. However there is no comparative study has been found in the literature on wheat. TiO_2 and ZnO NPs' potential phytotoxicity has been evaluated in this study. Wheat (*Triticum aestivum* L. Michelangelo) plants were chosen because of wheat is one of the most common-used consumption plants in the world. The size of nanoparticles used in industries plays an important role to determine their toxicity. In this study, the phytotoxicity of TiO_2 and ZnO NPs in 30 nm mean size is investigated because the most common-used NPs size in many industries are 30 nm. TiO_2 and ZnO NPs uptake and antioxidant enzyme levels were examined in this study in addition to the effects on root-shoot elongation and seed germination. This approach enhances our understanding of the toxicity of the TiO_2 and ZnO NPs on wheat.

Material and Methods

Chemicals

Titanium dioxide (~30 nm) nanoparticles were synthesized using a combination of sol-gel and hydrothermal synthesized methods at a research center (BME-Kocaeli/Turkey) (the method details are not given here due to patent application). Zinc oxide nanoparticles (~30 nm) were synthesized at Mersin University Advanced Technology of Education, Research and Application Center (MEITAM). ZnO synthesized according to the method of Ito et al., (2008) with small changes. In the method, 5 g (22 mmol) Zinc acetate dihydrate ($(CH_3COO)_2Zn.2H_2O$) was dissolved in 3 mL glycerol and then the mixture was heated at 150 °C during an hour. After the cooling process 8 mL 1-butanol, 0.2 mL glycerol, 5 mL triethylamine and 0.1 mL deionized water was added. The mixture was stirred at 35 °C for 3 days and then this mixture was sintered at 500 °C for 2 hours. The morphology of ZnO and TiO_2 nanoparticles was observed by Field Emission Scanning Electron Microscopy (FE-SEM) (ZEISS, Sigma 500).

Seed germination and root elongation assay

In this experiment, the seeds were sterilized in 70 % ethanol solution for 30 s and then exposed to 3 % sodium hypochlorite solution for 10 min, after the sterilization shaken in ultrapure water 5 times for 5 min. The seeds selected were uniform size to minimize error in seed germination. Filter paper cut to fit regular petri dishes was used as inert material. A double-layer of filter paper was placed in the petri dish, ten wheat seeds were placed in every petri dish and were separately treated with different concentrations (0, 5, 10, 20, 40 and 80 mg/kg) of 5 mL TiO_2 or ZnO nanoparticles suspensions. The petri dishes were incubated through 7 days in the dark at 25 ^{o}C. After the seed germination, average five wheat plants were chosen in every petri dish and root-shoot length were measured by graph paper.

Chlorophyll content

Germinated plants in petri dishes were transferred to the pots which contained 50 g turf. Every pot included five plants. Chlorophyll contents of leaves were determined

http://www.aloki.hu ● ISSN 1589 1623 (Print) ● ISSN 1785 0037 (Online)
DOI: http://dx.doi.org/10.15666/aeer/1503_14991510

using Konica-Minolta SPAD-502 chlorophyll meter after 21 days planting. Results are given as SPAD units.

After 21 days of planting, plant shoots were harvested 1 cm above the ground for determining the Ti and Zn uptake by plants. Possible contaminants were removed by brief rinsing in deionized water. The shoots were oven-dried at 65 ^oC to a constant weight. Dry plant samples were digested with 5 mL 12 M HNO_3 in hot plate at 220 ^oC. The all sample solutions were analyzed by Inductively Coupled Plasma Mass Spectrometry (ICP-MS) (Agilent 7500ce Model ICP-MS) and triplicated samples were measured for each condition.

Antioxidant enzymes analysis

Antioxidant (superoxide dismutase-SOD, ascorbate peroxidase-APX, catalase-CAT, proline and glutathione-GSH) levels analyses were done using fresh biomass of plants. All spectrophotometric analyses were conducted by UV-Visible Spectrophotometer (PG Instruments, T90+ model). Harvested wheat plants, as noted earlier that how harvested, were homogenized in 50 mM potassium phosphate buffer solution (pH 7.6) which contained 0.1 mM EDTA. The homogenate was centrifuged at 15000 x g at 4 ^oC for 10 min. The supernatant was used for SOD, CAT and APX analyses.

Superoxide dismutase (SOD)

SOD activity was determined by *p*-nitro blue tetrazolium chloride (NBT) photochemical assay as described by Cakmak and Marschner (1992) and Cakmak (1994). In this method 5 mL reaction mixture containing 50 mM potassium phosphate buffer (pH 7.6), 5 mM NBT, 0.1 mM riboflavin 12 mM L-methionine, 0.5 mM Na_2CO_3 solutions and 200 µL samples were added into glass tubes and the reaction was started with a light for 8-10 min. After the reaction time, absorbance was measured at 560 nm.

Ascorbate peroxidase (APX)

Ascorbate peroxidase activity was determined as described by Cakmak and Marschner (1992). The reaction mixture containing 50 mM potassium phosphate buffer (pH 7.6), 0.5 mM ascorbic acid, 12 mM H_2O_2 with EDTA and 0.1 ml of enzyme extract. The activity of ascorbate peroxidase was measuring the change in absorbance at 290 nm for 2 min interval and calculated using the extinction coefficient (2.8 mM/cm).

Catalase (CAT)

Catalase activity was determined as described by Cakmak and Marschner (1992). The decomposition of H_2O_2 was followed by decline in absorbance at 240 nm. One mL of reaction mixture containing 50 mM potassium phosphate buffer (pH 7.6), 10 mM H_2O_2 and 0.1 mL of enzyme extract. The activity of catalase was calculated using the extinction coefficient (39.4 mM/cm).

Glutathione (GSH)

The concentration of GSH in wheat plants homogenates was determined via DTNB method which described by Sedlak and Lindsay (1968) and the results have been expressed as µM/g of fresh plants. In this method, 0.5 g harvested plants were homogenized in 5 mL 5% meta-P acid. The samples were centrifuged at 400 rpm for 30

http://www.aloki.hu ● ISSN 1589 1623 (Print) ● ISSN 1785 0037 (Online)
DOI: http://dx.doi.org/10.15666/aeer/1503_14991510

minutes. 2.5 mL phosphate buffer (pH 7.5) with EDTA and 0.5 mL DTNB was added in 0.5 mL supernatant and after 15-20 minute the absorbance was measured at 412 nm. The glutathione concentration was determined by a standard curve prepared with reduced glutathione standard.

Proline analysis

Proline analysis was performed as described by Bates et al. (1973). Harvested wheat plants were homogenized in 3 % sulfosalicylic acid and the homogenate was centrifuged at 12 000 x g at 4 ^{o}C for 10 min. The supernatant was used for proline analysis. The reaction medium was containing 2 mL acid-ninhydrin, 2 mL glacial acetic acid and 2 mL of enzyme extract. Test tube was incubated for 1 hour at 100 °C, and then the reaction was stopped in an ice bath. The reaction mixture was extracted with 4 mL toluene, and mixed with a stirrer for 15-20 sec. The absorbance read at 520 nm using toluene for a blank. The proline concentration was determined by a standard curve.

Statistical analysis

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

Results and Discussion

Seed germination and root elongation

Seed germination and root elongation tests are the standard indictor tests to determine the phytotoxicity (Ma et al., 2010). Titanium dioxide and ZnO nanoparticles application did not have remarkable effects on wheat seed germinations. All seeds were germinated after the nanoparticles exposure and seedling yield was 100 %. Experimental data was similarly in literature which investigated effect of TiO_2 and ZnO nanoparticles on seed germination of different plant species (Feizi et al., 2012; Li et al., 2012; Boonyanitipong et al., 2011; Lopez-Moreno et al., 2010).

Metallic oxide nanoparticles (e.g. ZnO, TiO_2) were shown to be inhibitive effect at different growth stages (e.g seed germination and root elongation) of plants (Ma et al., 2010). Because of these reasons, it was determined the root and shoot elongation of wheat plants. As compare with the control wheat plants root and shoot elongation decreased except 40 mg/L TiO_2 nanoparticles exposure (*Fig. 1*).

http://www.aloki.hu ● ISSN 1589 1623 (Print) ● ISSN 1785 0037 (Online)
DOI: http://dx.doi.org/10.15666/aeer/1503_14991510

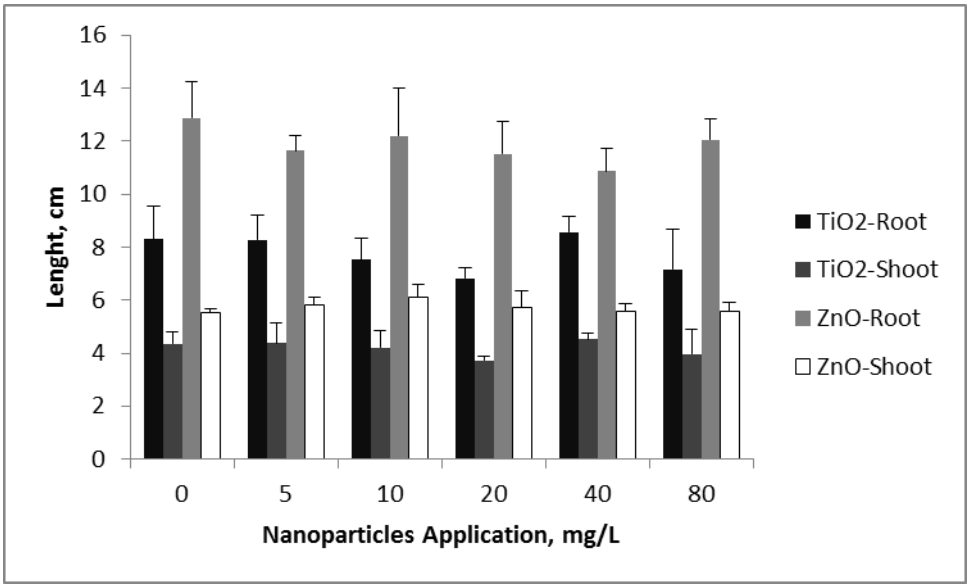


Figure 1. *Effect of different TiO_2 and ZnO nanoparticles concentrations on root and shoot elongation of wheat (The significance between the groups indicate with different letters)*

Application of 10 mg/L ZnO NPs increased shoot elongation but decreased root elongation as compared with the control (*Fig.1*). We determined that there were some putrefaction on roots and weaknesses on shoots at 5 mg/L ZnO NPs application. Li et al. (2012) determined that there were not any adverse effect on shoot length as compare with control at 0.5 and 1 mM (65.4 mg/L) Zn ions concentrations, but they indicated that there was significant inhibition on shoot length at 3 mM Zn ions application on wheat. For the root elongation they indicated that there was more inhibition with increasing Zn concentration. Boonyanitipong et al. (2011) indicated that the increasing ZnO nanoparticles concentrations caused decreasing root elongation and the number of hairy roots.

Chlorophyll content, growth, and uptake of metal by wheat

All concentrations of nanoparticles significantly affected the shoot dry weight. The highest shoot dry weight was found at concentration of 10 mg/L and lowest shoot dry weight was found at concentration of 40 mg/L TiO_2 nanoparticles. In contrast, the highest shoot dry weight was found at concentration of 40 mg/L and lowest shoot dry weight was found at concentration of 10 mg/L ZnO nanoparticles.

Chlorophyll content of wheat plants at tillering stage (according to zadox scale) growing at different concentrations of TiO_2 and ZnO nanoparticles were determined. The minimum chlorophyll content of leaves was determined at concentration of 5 mg/kg for both ZnO and TiO_2 nanoparticles (31.45 and 28.55 SPAD Unit, respectively) ($p \leq 0.05$) and the maximum content was measured in control plants of both ZnO and TiO_2 nanoparticles (32.8 and 34.55 SPAD Unit, respectively) (*Fig.2*).

Some researchers reported that Zn uptake decrease in chlorophyll content of cluster bean (*Cyamopsis tetragonoloba*) (Manivasagaperumal et al., 2011), pea (*Pisum sativum*) (Doncheva et al., 2001) and rye grass (*Lolium perenne*) (Bonnet et al., 2000). The same trend was observed at our data.

http://www.aloki.hu ● ISSN 1589 1623 (Print) ● ISSN 1785 0037 (Online)
DOI: http://dx.doi.org/10.15666/aeer/1503_14991510

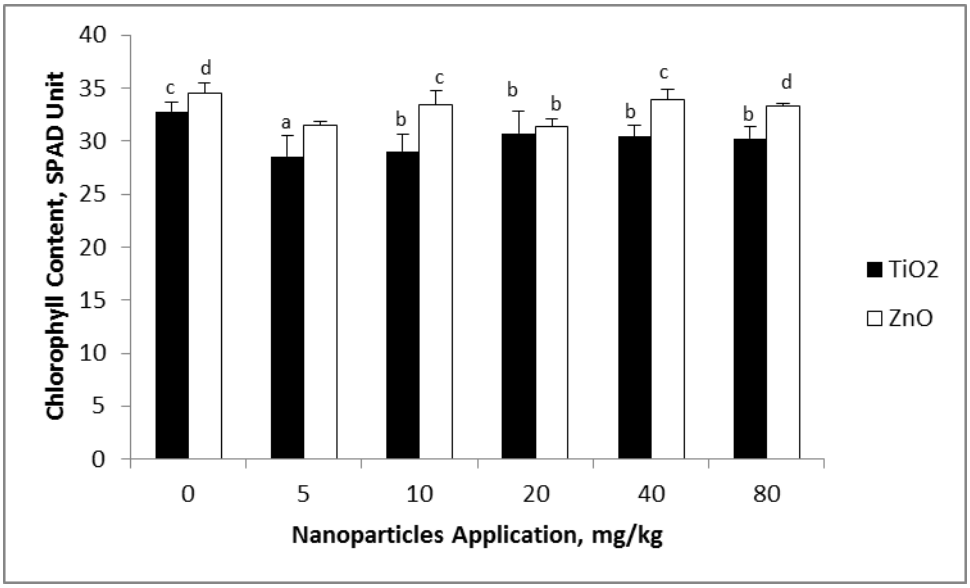


Figure 2. *Effect of different TiO_2 and ZnO nanoparticles concentrations on chlorophyll content (in SPAD units) of wheat (The significance between the groups indicate with different letters)*

The effect of TiO_2 and ZnO nanoparticles on Ti and Zn uptake by wheat plants were analyzed. The highest Ti values were determined at concentration of 40 mg/kg (1.377 mg/kg Ti) TiO_2 nanoparticles ($p \leq 0.05$) when the highest Zn values were determined at concentration of 10 mg/kg (30.68 mg/kg Zn) and 20 mg/kg (42.41 mg/kg Zn) ZnO nanoparticles ($p \leq 0.05$). Zinc is an essential nutrient for plants so it might cause the average Zn content was upper than Ti. These results were fitted to the results which obtained by some researchers (Brennan, 2005; Alloway, 2008; Manivasagaperumal et al., 2011). Zhu et al. (2008), which showed for the first time that pumpkin roots were in interaction with Fe_3O_4, thus the nanoparticles translocated in the plant tissues.

Antioxidant enzymes activities

The antioxidant enzymes activities (SOD, CAT and APX) were determined at tillering stage. APX, CAT and SOD can be used as biomarkers of phytotoxicity. The CAT, APX and SOD enzymatic responses are shown in *Fig. 3*. The CAT activity was significantly increased at concentration of 10 mg/kg (1.525 mmole/min) TiO_2 and also it was significantly decreased at concentration of 10 mg/kg (0.578 mmole/min) ZnO nanoparticles as compared with control ($p \leq 0.05$).

Some researchers indicated that the CAT activity was reduced by ZnO nanoparticles application with compared to control in sand-grown wheat plants (Dimkpa et al., 2012). Increasing and decreasing CAT activity shows that there were stress indicators at both nanoparticles types at different concentrations.

The APX activity was decreased at the both nanoparticles exposure for all concentrations. There was a significant decrease especially at concentration of 10 mg/kg (0.019 mmole/min) TiO_2 and 40 mg/kg (0.018 mmole/min) ZnO nanoparticles ($p \leq 0.05$) (*Fig. 3*). Nair and Chung (2014) showed that Ag nanoparticles at concentration of 0.2 mg/L were not effective in rice root and shoots CAT and APX activity.

http://www.aloki.hu ● ISSN 1589 1623 (Print) ● ISSN 1785 0037 (Online)
DOI: http://dx.doi.org/10.15666/aeer/1503_14991510

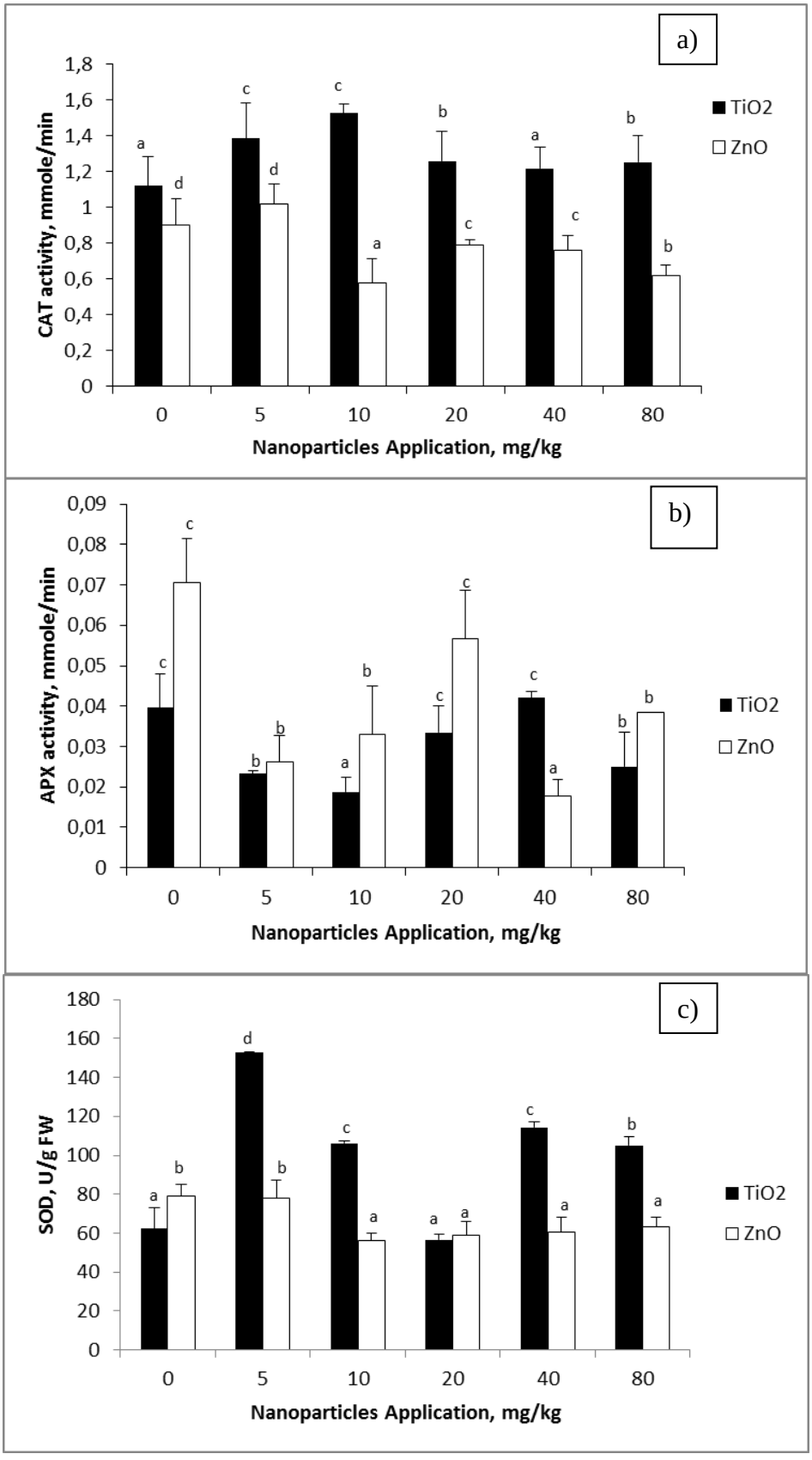


Figure 3. *a) Catalase (CAT), b) Ascorbate peroxidase (APX) and c) Superoxide dismutase (SOD) activities in wheat (The significance between the groups indicate with different letters)*

http://www.aloki.hu ● ISSN 1589 1623 (Print) ● ISSN 1785 0037 (Online)
DOI: http://dx.doi.org/10.15666/aeer/1503_14991510

Some researchers showed that the CAT activities in cucumber leaves were significantly induced at different TiO_2 nanoparticles concentration compared to control and also showed no differences on APX activities except the 500 mg/kg. There was a significant induction on the CAT and APX activities in velvet mesquite leaves at 4000 mg/L, and no increase of the CAT activity at 500 mg/L ZnO nanoparticles application (Ma et al., 2015).

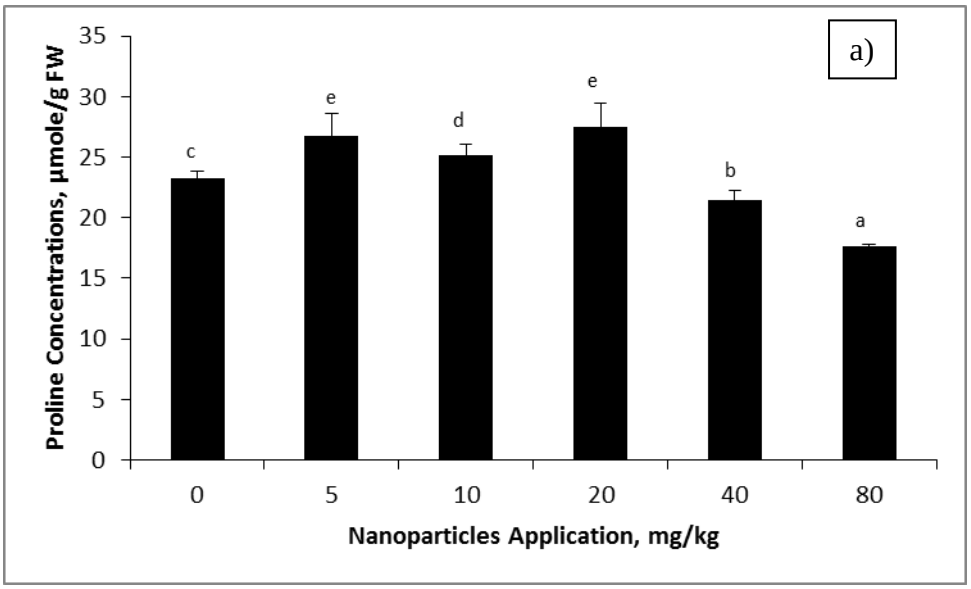
The SOD is one of the powerful stress enzymes which catalyze the dismutation of O_2^- to H_2O_2. The SOD activity increased except at concentration of 20 mg/kg (56.66 U/g FW) TiO_2 nanoparticles exposure and it was decreased at whole ZnO nanoparticles concentrations compared to control ($p \leq 0.05$). The SOD inhibition was shown in *Fig. 3*.

Lei et al. (2008) showed that the SOD, CAT, APX and GPX activities in spinach increased and Song et al. (2012) showed GPX, SOD, CAT activities increased in *L. minor*, but decreased the GR and APX activities in *V. Faba* with TiO_2 nanoparticles exposure, stated by Foltete et al. (2011). The same trend was observed in our data.

The level of proline and glutathione (GSH) are shown in *Fig.4* and *Fig. 5*, respectively. Proline level were increased till 20 mg/kg (27.49 μmole/g FW) and decreased at concentration of 40 and 80 mg/kg TiO_2 nanoparticles, minimum proline level was determined at concentration of 80 mg/kg (17.62 μmole/g FW) ($p \leq 0.05$). The level of proline were on the increase of 20 mg/kg (0.150 μmole/g FW), than it was decreasing slightly at concentration of 40 and 80 mg/kg ZnO nanoparticles ($p \leq 0.05$) (*Fig. 4a* and *4b*).

Various investigations in the literature proved that there are direct proportion between proline synthesis and developing resistance to stress in plants, such as in tobacco, in wheat, in citrus fruits, in sugar cane and in other various plants (Krasavina et al., 2014).

The glutathione concentrations were significantly decreased after the exposure of TiO_2 and ZnO nanoparticles compared to control also except at concentration of 5 mg/kg (218.8 μM/g FW) ZnO nanoparticles (*Fig. 5*).



http://www.aloki.hu ● ISSN 1589 1623 (Print) ● ISSN 1785 0037 (Online)
DOI: http://dx.doi.org/10.15666/aeer/1503_14991510

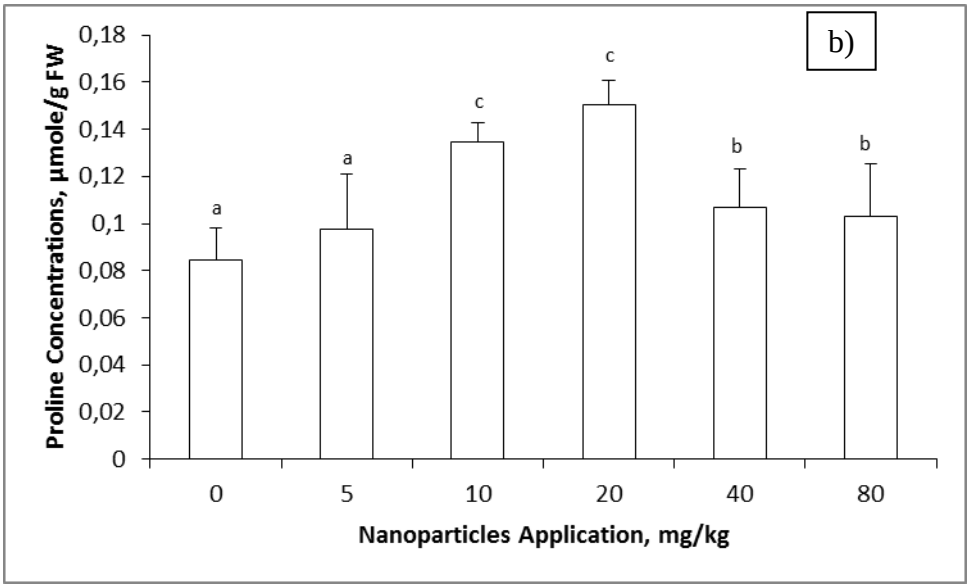


Figure 4. *The proline concentrations of wheat plants, a) TiO_2 and b) ZnO nanoparticles (The significance between the groups indicate with different letters)*

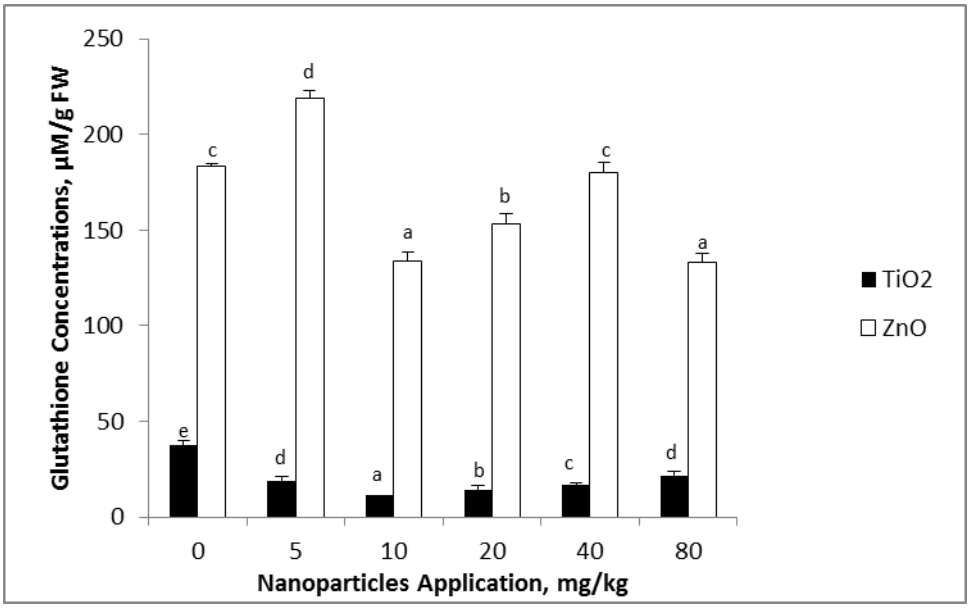


Figure 5. *Glutathione concentrations of wheat plants (The significance between the groups indicate with different letters)*

The application of TiO_2 and ZnO nanoparticles exposure caused the increase or decrease of the oxidative enzymes. Different nanoparticles affect different enzymes systems and this makes it difficult to conclude which nanoparticles affect which enzymes (Rico et al., 2015). Glutathione have an important role in detoxification of plant cell under heavy metals stress. Heavy metals cause inhibitions or activations some enzymes in the plant cells and these inhibitions may occur either by the formation of bonds between the metals and the sulfhydryl groups or by the exchange of toxic metals with the metals in the enzymes (Servin et al., 2013).

http://www.aloki.hu ● ISSN 1589 1623 (Print) ● ISSN 1785 0037 (Online)
DOI: http://dx.doi.org/10.15666/aeer/1503_14991510

Conclusions

This study proved that the potential adverse effects of TiO_2 and ZnO nanoparticles on wheat. In this paper, we showed that TiO_2 and ZnO nanoparticles application did not have remarkable effects on wheat seed germinations but after the seed germination stage, both TiO_2 and ZnO nanoparticles negatively affected chlorophyll content and antioxidant enzymes. The whole TiO_2 and ZnO nanoparticles application caused oxidative stress to the tested wheat plants. Our data thus showed that these nanoparticles were toxic for wheat plants so for ecosystem and need to more investigation on this area, especially at low concentration.

Acknowledgements. The authors wish to thank Mersin University Scientific Research Fund (BAP, Project No: BAP-FBE ÇMB (ZGD) 2013-1 DR)) for financial support. The authors also would like to thank Dr. Birol Karakaya for preparing the TiO_2 nanoparticles and Dr. K. Ocakoglu for preparing the ZnO nanoparticles.

REFERENCES

[1] Alloway, B.J. (2008): Zinc in Soils and Crop Nutrition. International Zinc Association Communications. - IZA Publications, Brussels.

[2] Asada, K., Takahashi, M. (1987): Production and scavenging of active oxygen in chloroplasts. - In: Kyle, D.J., Osmond, C.B., Arntzen, C.J. (ed.) Photoinhibition. Elsevier, Amsterdam.

[3] Bates, L.S., Waldren, R. P., Teare, I.D. (1973): Rapid determination of free proline for water-stress studies. - Plant and Soil 39: 205-207.

[4] Bonnet, M., Camares, O., Veisseire, P. (2000): Effects of zinc and influence of *Acremonium lolii* on growth parameters, chlorophyll a fluorescence and antioxidant enzyme activities of ryegrass (*Lolium perenne* L. cvApollo). - Journal of Experimental Botany 51(346): 945–953.

[5] Boonyanitipong, P., Kositsup, B., Kumar, P., Baruah, S., Dutta, J. (2011): Toxicity of ZnO and TiO_2Nanoparticles on Germinating Rice Seed *Oryzasativa* L. - International Journal of Bioscience, Biochemistry and Bioinformatics 1(4): 282-285.

[6] Brennan, R.F. (2005): Zinc Application and Its Availability to Plants. - Degree of Doctor of Philosophy Thesis of Murdoch University, Australia.

[7] Cakmak, I. (1994): Activity of ascorbate-dependent H_2O_2-scavenging enzymes and leaf chlorosis are enhanced in magnesium and potassium deficient leaves, but not in phosphorus deficient leaves. - Journal of Experimental Botany 45:1259-1266.

[8] Cakmak, I., Marschner, H. (1992): Magnesium deficiency and highlight intensity enhance activities of superoxide dismutase, ascorbate peroxidase and glutathione reductase in bean leaves. - Plant Physiology 98:1222-1226.

[9] Dalai, S., Pakrashi, S., Nirmala, M.J., Chaudhri, A., Chandrasekaran, N., Mandal, A.B., Mukherjee, A. (2013): Cytotoxicity of TiO_2 nanoparticles and their detoxification in a freshwater system. - Aquatic Toxicology 138-139:1-11.

[10] Demir, E., Kaya, N., Kaya, B. (2014): Genotoxic effects of zinc oxide and titanium dioxide nanoparticles on root meristem cells of *Allium cepa* by comet assay. - Turkish Journal of Biology 38: 31-39.

[11] Dimkpa, C.O., McLean, J.E., Latta, D.E., Manangon, E., Britt, D.W., Johnson, W.P., Boyanov, M.I., Anderson, A.J. (2012): CuO and ZnO nanoparticles: phytotoxicity, metal speciation, and induction of oxidative stress in sand-grown wheat. - Journal of Nanoparticles Research 14:1125.

[12] Doncheva, S., Stoynova, Z., Velikova, V. (2001): Influence of succinate on zinc toxicity of pea plants. - Journal of Plant Nutrition 24(6):789–804.

http://www.aloki.hu ● ISSN 1589 1623 (Print) ● ISSN 1785 0037 (Online)
DOI: http://dx.doi.org/10.15666/aeer/1503_14991510

[13] 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 13:822-828.
[14] Feizi, H., Amirmoradi, S., Abdollahi, F., Pour, S.J. (2013): Comparative effects of nano sized and bulk Titanium dioxide concentrations on medicinal plant *Salvia officinalis* L. - Annual Review and Research in Biology 3(4):814-824.
[15] Feizi, H., Rezvani, M.P., Shahtahmassebi, N., Fotovat, A. (2012): Impact of bulk and nano sized titanium dioxide (TiO_2) on wheat seed germination and seedling growth. - Biological Trace Element Research 146:101-106.
[16] Foltete, A.S., Masfaraud, J.F., Bigorgne, E., Nahmani, J., Chaurand, P., Botta, C., Labille, J., Rose, J., Ferard, J.F., Cotelle, S. (2011): Environmental impact of sunscreen nanomaterials: Ecotoxicity and genotoxicity of altered TiO_2 nanocomposites on *Vicia faba*. - Environmental Pollution 159:2515-2522.
[17] Gubbins, E.J., Batty, L.J., Lead, J.R. (2011): Phytotoxicity of silver nanoparticles to *Lemna minor* L. - Environmental Pollution 159:1551-1559.
[18] Ito, D., Jespersen, M.L., Hutchison, J.E. (2008): Selective growth of vertical ZnO nanowire arrays using chemically anchored gold nanoparticles. - ACS Nano, 2(10):2001–2006.
[19] Kim, K.T., Klaine, S.J., Cho, J., Kim, S.H., Kim, S.D. (2010): Oxidative stress responses of *Daphnia magna* exposed to TiO_2 nanoparticles according to size fraction. - Science of the Total Environment 408:2268-2272.
[20] Krasavina, M.S., Burmistrova, N.A., Raldugina, G.N. (2014): The Role of Carbohydrates in Plant Resistance to Abiotic Stresses. In: Ahmad, P., Rasool, S. (ed.) Emerging Technologies and Management of Crop Stress Tolerance: Volume 1: Biological Techniques, Elsevier.
[21] Kumar, A., Pandey, A.K., Singh, S.S., Shanker, R., Dhawan, A. (2011): Engineered ZnO and TiO_2 nanoparticles induced oxidative stress and DNA damage leading to reduced viability of *Escherichia coli.* - Free Radical Biology and Medicine 51:1872-1881.
[22] Larue, C., Laurette, J., Herlin-Boime, N., Khodja, H., Fayard, B., Flank, A.M., et al. (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 43:197–208.
[23] Lei, Z., Mingyu, S., Xiao, W., Chao, L., Chunxiang, Q., Liang, C., Hao, H., Xiaoqing, L. and Fashui, H. (2008): Antioxidant Stress is Promoted by Nano-anatase in Spinach Chloroplasts Under UV-B Radiation. - Biol Trace Elem Res 121, 69–79.
[24] Li, X.N., Ma, H.Z., Jia, P.X., Wang, J., Jia, L.Y., Zhang, T.G., Yang, Y.L., Chen, H.J. and Wei, X. (2012): Responses of seedling growth and antioxidant activity to excess iron and copper in *Triticum aestivum* L. - Ecotoxicol.Environ.Saf. 86:47–53.
[25] Lin, D., Xing, B. (2007): Phytotoxicity of Nanoparticles: Inhibition of Seed Germination and Root Growth. - Environmental Pollution 150:243-250.
[26] Lopez-Moreno, M.L., De La Rosa, G., Hernandez-Viezcas, J.A., Castillo-Michel, H., Botez, C.E., Peralta-Videa, J.R., Gardea-Torresdey, J.L. (2010): Evidence of the differential biotransformation and genotoxicity of ZnO and CeO_2 nanoparticles on soybean (*Glycine max*) plants. - Environmental Science and Technology 44:7315–7320.
[27] Ma, C., White, J.C., Dhankher, O.P., Xing, B. (2015): Metal-based nanotoxicity and detoxification pathways in higher plants. - Environmental Science and Technology 49:7109-7122.
[28] Ma, H., Williams, P.L., Diamond, S.A. (2013): Ecotoxicity of manufactured ZnO nanoparticles-A Review. - Environmental Pollution 172:76-85.
[29] Ma, X., Geisler-Lee, J., Deng, Y., Kolmakov, A. (2010): Interactions between engineered nanoparticles (ENPs) and plants: Phytotoxicity, uptake and accumulation-Review. - Science of the Total Environment 408(16):3053–3061.

http://www.aloki.hu ● ISSN 1589 1623 (Print) ● ISSN 1785 0037 (Online)
DOI: http://dx.doi.org/10.15666/aeer/1503_14991510

[30] Manivasagaperumal, R., Balamurugan, S., Thiyagarajan, G., Sekar, J. (2011): Effect of zinc on germination, seedling growth and biochemical content of cluster bean (*Cyamopsis tetragonoloba*(L.) Taub). - Current Botany 2(5):11–15.
[31] Nair, P.N.G., Chung, M. (2014): Physiological and molecular level effects of silver nanoparticles exposure in rice (*Oryza sativa L.*) seedlings. - Chemosphere 112:105-113.
[32] Rico, C.M., Peralta-Videa, J.R., Gardea-Torresdey, J.L. (2015): Chemistry, biochemistry of nanoparticles, and their role in antioxidative defense system in plants. - In: Siddiqui, M.H., Al-Whaibi, M.H., Mohammad, F. (ed.) Nanotechnology and Plant Sciences, Springer.
[33] Sedlak, J., Lindsay, R.H. (1968): Estimation of total, protein-bound, and nonprotein sulfhydryl groups in tissue with Ellman's reagent. - Analytical Biochemistry 25(1):192-205.
[34] 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 and Technology 47(20):11592-11598.
[35] 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.
[36] Song, U., Jun, H., Waldman, B., Roh, J., Kim, Y., Yi, J., et al. (2013): Functional analyses of nanoparticle toxicity: A comparative study of the effects of TiO_2 and Ag on tomatoes (*Lycopersiconesculentum*). - Ecotoxicology and Environmental Safety 93:60-67.
[37] Wei, C., Zhang, Y., Guo, J., Han, B., Yang, X., Yuan, J. (2010): Effects of silica nanoparticles on growth and photosynthetic pigment contents of *Scenedesmus obliquus*. - Journal of Environmental Sciences 22(1):155–160.
[38] Ye, Z., Rodriguez, R., Tran, A., Hoang, H., Los Santos, D.D., Brown, S., Vellanoweth, R.L. (2000): The developmental transition to flowering repsesses ascorbate peroxidase activity and induced enzymatic lipid peroxidation in leaf tissue in Arobidopsis thaliana. - Plant Science 158:115-127.
[39] Zhu, H., Han, J., Xiao, J.Q., Jin, Y. (2008): Uptake, Translocation and Accumulation of Manufactured Iron Nanoparticles by Pumpkin Plants. - Journal of Environmental Monitoring 10:713-717.
[40] Zhu, X., Chang, Y., Chen, Y. (2010): Toxicity and bioaccumulation of TiO_2 nanoparticle aggregates in *Daphnia magna*. - Chemosphere 78:209–215.

http://www.aloki.hu ● ISSN 1589 1623 (Print) ● ISSN 1785 0037 (Online)
DOI: http://dx.doi.org/10.15666/aeer/1503_14991510