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Regular paper

Effect of ZnO, TiO_2, Al_2O_3 and ZrO_2 nanoparticles on wheat callus cells

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Effect of metal oxide nanoparticles on calli of two wheat varieties: Parabola (stress tolerant) and Raweta (sensitive) was studied. ZnO induced 10% larger membrane damage in Raweta calli. TiO_2, Al_2O_3, and ZrO_2 caused nearly 30% greater lactate dehydrogenase leakage for Raweta compared to Parabola. UV-irradiation of samples containing ZnO particles intensified this effect. Membrane lipid peroxidation in ZnO treated Raweta calli was twice as high as in Parabola and further increased after UV-irradiation. TiO_2, Al_2O_3, and ZrO_2 nanoparticles caused a 4-fold increase in malondialdehyde concentration in Raweta calli in comparison to Parabola calli. The nanoparticles studied damaged the cellular defense system by inactivating the antioxidative enzymes.

Key words: wheat, callus, metal oxide nanoparticles, cytotoxicity, UV irradiation

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Abbreviations: NPs, nanoparticles; LDH, lactate dehydrogenase; MDA, malondialdehyde; SOD, superoxide dismutase; POX, peroxidase; ROS, reactive oxygen species; MS, Murashige and Skoog medium

INTRODUCTION

Metal nanoparticles have always been naturally emitted into the atmosphere as a result of volcanic eruptions, fires and the burning of everyday use materials like fuels. However, with industrial development, the concentration of metal nanoparticles in the environment is still increasing, due to faster than ever development of nanotechnology and numerous applications of nanoparticles (Moore, 2006). Despite the safeguards, products and industrial waste often go to the aquatic environment (Daughton, 2004). Constant increase of nanoparticles` amount is observed not only in water reservoirs and soil but also in the air (Nations *et al.*, 2011). Undoubtedly, this is a reason for the growing exposure of plants to nanomaterials (Ge *et al.*, 2011).

Silver and gold nanoparticles are best studied because they show, among others, bactericidal properties. Metal oxides are a group of compounds with diversified chemical and physical properties. The metal oxides (e.g. ZnO, TiO_2, CuO) in form of nanoparticles are widely used in cosmetics, sunscreens, self-cleaning coverings and textiles. They are also applied as agents for water treatment, components of solar batteries and car catalytic converters. The biological activity of nanoparticles depends on their size, chemical composition, surface structure, solubility, shape and propensity to aggregate, as well as the environmental conditions (including pH or salinity), and plant species they interact with (Nel *et al.*, 2006; Handy *et al.*, 2008; Milewska-Hendel *et al.*, 2016).

In previous works, it has been demonstrated that significant impact of silver nanoparticles on generating an oxidation stress in wheat callus cells depends on the surface charge of silver nanoparticles (Barbasz *et al.*, 2016; Barbasz *et al.*, 2018). This work investigates the influence of metal oxide nanoparticles: zinc oxide (ZnO) of two particle sizes: >100 nm and >130 nm, aluminum oxide (Al_2O_3) (30–60 nm), titanium oxide (TiO_2) (<150 nm) and zirconium oxide (ZrO_2) (<100 nm) on callus cells of two varieties of wheat (*Triticum aestivum* L.) Parabola and Raweta. Wheat is one of the most commonly cultivated cereals, thus its exposure to adverse environmental factors (including nanomaterials) is inevitable (Curtis & Halford, 2014). The selected cell type allows linking the influence of the tested substances to cell defense mechanisms. The influence of UV radiation on the cytotoxicity of ZnO nanoparticles in the tested wheat cells has also been checked.

MATERIALS AND METHODS

Five commercial metal oxide nanoparticles: zinc oxide (ZnO-NPs) of two particle sizes (>100 nm and >130 nm), aluminum oxide (Al_2O_3-NPs) (30–60 nm), titanium oxide (TiO_2-NPs) (<150 nm) and zirconium oxide (ZrO_2-NPs) (<100 nm) were purchased from Sigma-Aldrich (USA).

Cell culture. Immature embryos were isolated from the seeds of two spring wheat (*Triticum aestivum* L.) varieties (drought stress tolerant Parabola and drought stress sensitive Raweta). Embryos were sterilized in 70% ethanol for 1 minute, then for 10 minutes in 10% solution of bleaching agent "Domestos". Next, they were rinsed in sterile water and placed in Petri dishes on Murashige and Skoog (MS) medium containing vitamins from Duchefa Biochemie (Murasnige & Skoog, 1962) supplemented with 2,4-Dichlorophenoxyacetic acid (2 mg/dm^3, Sigma-Aldrich) to promote growth of undifferentiated calli (Filek *et al.*, 2009). After three months of culture, 1 g of callus cells was transferred into 20 cm^3 MS media containing the investigated nanoparticles at concentrations 3, 6 and 12 mg/dm^3 and into the medium without additional supplementation (adopted as a control). A selection of samples (containing ZnO-NPs) was additionally exposed to 254 nm wavelength light emitted by a 30W mercury lamp (Sankyo Denki G30T8) for 30 minutes. Culture flasks were placed in a circular shaker inside a growth chamber and cultured at 25°C in the dark for 24 hours. Next, cy-

tological and biochemical analyses of the cultures were conducted.

LDH assay. Lactate dehydrogenase leakage (LDH assay) was used to determine cell viability after nanoparticles treatment. After 24 h incubation, the cells were centrifuged (5 min, 1000 × *g*), and the pellet was suspended in PBS (0.01 M Na_2HPO_4, 1.8 mM KH_2PO_4, 2.7 mM KCl and 13.7 mM NaCl). Next, the cells were vortexed for 1 minute and then lysed using ultrasonic bath (15 kHz) for 5 minutes and centrifuged (10 min, 10000 × *g*). 0.2 cm^3 of each of the supernatants (obtained after the centrifugation) were added to the mixture of 0.5 cm^3 0.75 M sodium pyruvate and 0.01 cm^3 of 0.14 mM NADH. The mixtures were incubated at 37°C for 30 minutes. Afterwards, 0.5 cm^3 of 1 mM solution of 2,4-dinitrophenylhydrazine in 1 M HCl was added to each sample. After 1 hour, the absorbance of formed hydrazone was measured at 450 nm using the microplate reader Epoch (BioTek Instruments).

Determination of lipid peroxidation. The lipid membrane peroxidation was determined according to method of Dhindsa and others (Dhindsa *et al*., 1981) which was then used in the study of cytotoxicity of silver nanoparticles (Barbasz *et al*., 2016). Approximately 1 g of callus cells after treatment was homogenized in 0.5% trichloroacetic acid (TCA). Next, after centrifugation at 19000 × *g*, 0.5 cm^3 of the supernatant was mixed with 2.5 cm^3 of 0.5% thiobarbituric acid (TBA) in 20% TCA and the samples were boiled for 30 min. Malondialdehyde (MDA) concentration (as the indicator of lipid peroxidation) was determined spectrophotometrically at λ=532 nm at room temperature, corrected for non-specific background by subtracting the absorbance at λ=600 nm. The concentration of MDA was calculated using the molar extinction coefficient of 155 mM^{-1} cm^{-1}.

Enzyme assays. To determine total superoxide dismutase activity [SOD; EC 1.15.11], about 1 g of callus cells was homogenized in a 0.05 M phosphate buffer (pH 7.2) containing 0.1% bovine serum albumin and 0.1 M EDTA. The homogenate was centrifuged for 10 minutes at 10000 × *g*. Then, supernatant and xanthine oxidase were added to a reaction mixture consisting of phosphate buffer (pH 7.2), 0.1 mM cytochrome *c*, 0.1 mM EDTA, and 0.1 mM xanthine. The absorbance measurements were conducted for two minutes at λ=550 nm. It was assumed that the unit of activity (1 unit, 1 unit of cytochrome) corresponded to the amount of enzyme which causes 50% inhibition of the reduction of cytochrome C at 25°C.

Measurements of total peroxidase activity [POX; EC 1.11.19] were performed spectrophotometrically according to the modified method of Lück (Lück, 1962). The assay mixture contained 2 cm^3 of 0.05 M phosphate buffer, 0.05 cm^3 of 1% *p*-phenylenediamine (pPD), and 0.05 cm^3 of enzyme extract. In the next step, 0.05 cm^3 of 0.03 M H_2O_2 was added to initiate the reaction, and absorbance was measured at λ=460 nm for 2 minutes. All the measurements were carried out using a UV-Vis spectrophotometer from Thermo Fisher Scientific. The activity of the studied enzymes was expressed relatively to the protein content in the supernatant. Protein concentration was determined by the method of Bradford (Bradford, 1976).

Statistical analysis. The results of biochemical analyses were presented as the mean values with standard deviation (±S.E.) calculated from three experiments. The statistical analysis was performed using Duncan's Multiple Range test at $p<0.05$ and PC SAS 8.0 software.

RESULTS AND DISCUSSION

To investigate the effect of nanoparticles` impact on callus cell viability, the LDH content in cells was measured. Lactate dehydrogenase, as a cytoplasmic enzyme, should be released from the cell as a result of its damage. The graphs show percentage changes in the content of LDH in cells (Fig. 1; the higher percentage means the greater number of damaged cells). The level of damage of ZnO-NPs-treated calli cells of stress sensitive and stress tolerant wheat varieties (in comparison to control) differed significantly. On average, it was 10% greater for the Raweta variety. The varieties tested reacted similarly to silver nanoparticles treatment (Barbasz *et al*., 2016). The variety sensitive to drought stress was definitely more sensitive also to the stress generated by the presence of metal nanoparticles. Padmavathy and Vijayaraghavan (Padmavathy & Vijayaraghavan, 2008) have shown that the toxicity of ZnO to bacteria depends on the nanoparticles' size (the smaller nanoparticles were more bactericidal). Interestingly, for callus cells, the larger nanoparticles (130 nm) were more toxic for tolerant variety, oppositely to the smaller ones (100 nm) which were more toxic for the sensitive variety. These differences between wheat varieties can be directly related to the structure of their cell membranes. As was shown by Filek and others (Filek *et al.,* 2012), the cell membranes of Parabola and Raweta varieties have different composition, with different content of saturated fatty acids. Other studies indicate that ZnO-NPs exhibited toxic effect also on other terrestrial plants. Exposure of *Brassica nigra* to high concentrations of ZnO-NPs (above 500 mg dm^{-3}) had a negative impact on seed germination, seedling growth and resulted in increased concentration of enzymatic and non-enzymatic antioxidants in cells (Zafar *et al*., 2016). ZnO-NPs were also toxic to *Arabidopsis thaliana*, causing a strong inhibition of seed germination, as well as root growth and a decrease in the number of leaves (Lee *et al*., 2010).

Literature reports point to UV radiation as an important environmental factor that may contribute to the increase in the cytotoxicity of metal oxide nanoparticles. As reported by Ma and others (Ma *et al.,* 2014), a photon with a wavelength of less than 368 nm has the potential to photoactivate zinc oxide, leading to the production of reactive oxygen species (ROS). It has also been proven that UV radiation accelerates the dissolution of ZnO-NPs (Han *et al*., 2010). Waves shorter than 368 nm constitute about 6% of solar energy reaching the Earth's surface, therefore the photochemical degradation of ZnO-NPs together with photoinduced generation of ROS significantly increases the potential risk connected with these nanomaterials entering the environment (Ma *et al*., 2014). Under the influence of UV light, ZnO-NPs show photocatalytic activity and act as antibacterial agents (Han *et al*., 2010; Ann *et al*., 2014). However, Lee & An (2013) in their studies on green algae (*Pseudokirchneriella subcapitata*) indicated that the toxicity of ZnO-NPs did not depend on ultraviolet radiation. In the present study, ZnO NPs of 130 nm diameter and at the highest tested concentration (12 mg dm^{-3}), exhibited greater cytotoxicity to both wheat varieties after exposure of the samples to UV radiation, the effect being particularly pronounced for Raweta.

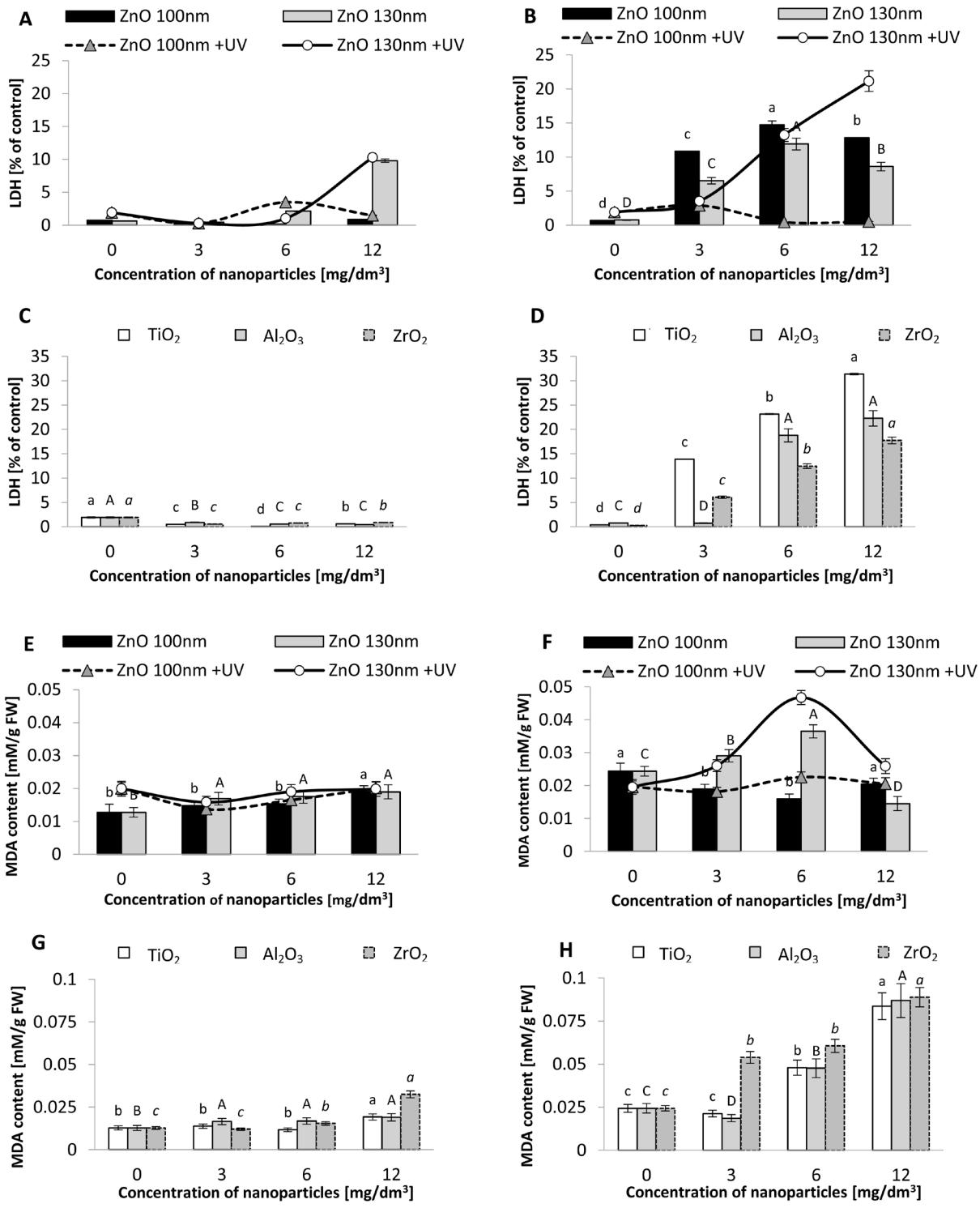

Figure 1. Lactate dehydrogenase leakage (LDH) from callus cells of Parabola (A, C) and Raweta (B, D) after 24 h exposure to metal oxide nanoparticles, as compared to control media (0).
Comparison of malondialdehyde (MDA) level in callus cells of Parabola (**E, G**) and Raweta (**F, H**) cultured on control media (0) and after 24 h exposure to metal oxide nanoparticles. Values are mean ±S.E. Different letters indicate significant (p<0.05) differences between treatments.

Table 1. Antioxidant activities (superoxide dismutase. SOD and peroxidase POX) in callus cells of two wheat varieties cultured in control medium (0) and after 24h exposure to metal oxide nanoparticles.
The values for callus cells after 1h UV-treatment and then 24h exposure to metal oxide nanoparticles are marked in gray. The presented values are mean ±S.E. Different letters indicate significant ($p<0.05$) differences between treatments.

Concentration [mg/dm^{-3}]	Parabola					Raweta				
	SOD [U/mg of proteins]									
	ZnO 100 nm	ZnO 130 nm	TiO_2	Al_2O_3	ZrO_2	ZnO 100 nm	ZnO 130 nm	TiO_2	Al_2O_3	ZrO_2
0	0.1176± 0.0065^{a}	0.1176± 0.0117^{a}	0.1176± 0.0694^{a}	0.1176± 0.0954^{a}	0.1176± 0.0099^{a}	0.1335± 0.0498^{a}	0.1335± 0.0257^{a}	0.1335± 0.0276^{a}	0.1335± 0.0034^{c}	0.1335± 0.0432^{c}
	0.0581± 0.0038^{D}	0.0581± 0.0054^{A}				0.0935± 0.0046^{A}	0.0935± 0.0087^{A}			
3	0.1171± 0.0153^{a}	0.0316± 0.0028^{b}	0.1020± 0.0429^{a}	0.1302± 0.0541^{a}	0.1069± 0.0386^{a}	0.1319± 0.0138^{a}	0.0850± 0.0105^{b}	0.1549± 0.0543^{a}	0.1755± 0.0654^{b}	0.2193± 0.0168^{a}
	0.1029± 0.0074^{A}	0.0374± 0.0047^{B}				0.0505± 0.0036^{B}	0.0239± 0.0025^{B}			
6	0.09746± 0.0038^{b}	0.0140± 0.0089^{c}	0.1102± 0.0046^{a}	0.1060± 0.0083^{a}	0.0825± 0.0086^{b}	0.0811± 0.0074^{b}	0.0769± 0.0104^{b}	0.1693± 0.0655^{a}	0.1846± 0.0065^{a}	0.1681± 0.0627^{b}
	0.0755± 0.0032^{B}	0.0032± 0.0002^{D}				0.0393± 0.0025^{C}	0.0155± 0.0047^{C}			
12	0.0809± 0.0096^{c}	0.0007± 0.0002^{d}	0.1244± 0.0943^{a}	0.1284± 0.0897^{a}	0.0823± 0.0064^{b}	0.0500± 0.0054^{c}	0.0700± 0.0085^{b}	0.1396± 0.0235^{a}	0.1834± 0.0146^{a}	0.1368± 0.0367^{c}
	0.0646± 0.0083^{C}	0.0069± 0.0004^{C}				0.0200± 0.0058^{D}	0.0100± 0.0010^{C}			

Concentration [mg/dm^{-3}]	Parabola					Raweta				
	POX [U/mg of proteins]									
	ZnO 100 nm	ZnO 130 nm	TiO_2	Al_2O_3	ZrO_2	ZnO 100 nm	ZnO 130 nm	TiO_2	Al_2O_3	ZrO_2
0	0.9347± 0.1005^{a}	0.9347± 0.1038^{a}	0.9347± 0.1017^{a}	0.9347± 0.0543^{a}	0.9347± 0.0763^{a}	0.6658± 0.0117^{a}	0.6657± 0.0654^{b}	0.6657± 0.0017^{a}	0.6657± 0.0329^{b}	0.6657± 0.0394^{c}
	0.7157± 0.0998^{C}	0.7157± 0.0573^{C}				0.4032± 0.0659^{B}	0.4032± 0.0579^{B}			
3	0.9893± 0.1390^{a}	0.5561± 0.0682^{b}	0.8866± 0.0478^{b}	1.0393± 0.0765^{a}	0.9279± 0.0754^{a}	0.4486± 0.0531^{b}	0.6003± 0.0076^{c}	0.8890± 0.0201^{a}	1.0522± 0.0992^{a}	1.6405± 0.0432^{a}
	1.7063± 0.2860^{A}	1.3234± 0.0765^{B}				0.8295± 0.0768^{A}	0.7013± 0.0105^{A}			
6	1.1065± 0.0965^{a}	0.4906± 0.0532^{c}	0.9479± 0.0394^{a}	0.8603± 0.0658^{a}	0.8357± 0.0578^{a}	0.4517± 0.0068^{b}	1.0679± 0.0049^{a}	1.2950± 0.7120^{a}	1.3238± 0.0392^{a}	1.4496± 0.8470^{a}
	1.3557± 0.1170^{B}	1.6567± 0.1648^{A}				0.4181± 0.0132^{B}	0.5307± 0.0580^{B}			
12	0.5357± 0.0362^{b}	0.2695± 0.0430^{d}	1.0016± 0.0997^{a}	1.1332± 0.3971^{a}	0.6717± 0.0276^{b}	0.2874± 0.0467^{c}	0.5525± 0.0038^{d}	0.9544± 0.1004^{a}	1.1139± 0.1149^{a}	1.0158± 0.1843^{b}
	0.6821± 0.0660^{C}	1.4526± 0.0763^{A}				0.7721± 0.0114^{A}	0.7222± 0.0077^{A}			

Other tested nanoparticles (TiO_2, Al_2O_3, ZrO_2) did not act cytotoxically on Parabola callus cells. For Raweta, the cytotoxic activity of these NPs correlated with concentration of the studied nanoparticles. The highest cytotoxicity, expressed as the degree of membrane damage, was found for TiO_2-NPs. Many studies have found that nanoparticles of metal oxides have a negative effect on mammalian cells and some aquatic organisms (Nations *et al.*, 2011). Al_2O_3-NPs showed no phytotoxicity in *Arabidopsis thaliana* (Lee *et al.*, 2010), but they were shown to inhibit cell growth and lower chlorophyll content in algae (*Scenedesmus* sp. and *Chlorella* sp.) (Sadiq *et al.*, 2011). Al_2O_3-NPs also had a negative effect on tobacco (*Nicotiana tabacum*), inhibiting seedling and root growth, decreasing plant biomass and significantly increasing the expression of genes involved in plant response to environmental stress (Burklew *et al.*, 2012). The increasing concentration of TiO_2-NPs led to a significant reduction of all growth parameters of *Lemna paucicostata* and *Spirodela polyrhiza*. In the latter, the changes in antioxidant enzymes activity were shown (Kim *et al.*, 2014; Movafeghi *et al.*, 2018). The effect of TiO_2-NPs strongly depends on the concentration and species of the plant. It has been shown that the nanoparticles of this metal oxide can both favorably and negatively affect germination of seeds and the growth of the root, shoot and cuttings, as well as the photosynthesis process (Kataria *et al., 2019).* The investigations of nano- and micro-particles of ZrO_2 showed that they were not cytotoxic (Karunakaran *et al.*, 2016). The effect of both TiO_2-NPs and ZrO_2-NPs on terrestrial plants is ambiguous.

The results of lipid peroxidation tests show that under the influence of the studied nanoparticles, callus cells undergo oxidative stress. After treatment of cells with 130 nm ZnO-NPs, a higher MDA content was found in the samples of the sensitive variety. UV radiation enhanced the toxic effect of ZnO-NPs. Interestingly, the highest concentration of MDA (more than twice in comparison to that caused by ZnO-NPs) was found after treatment of Raweta calli by TiO_2, Al_2O_3, ZrO_2 nanoparticles at a concentration of 12 mg dm^{-3}. This may be related to a significantly different content of unsaturated fatty acids in biological membranes of the tested varieties (Filek *et al.*, 2012).

The activity of SOD (expressed in relation to the protein content in the samples) decreased with increasing ZnO-NPs concentration (Table 1). This decrease was smaller for the Parabola variety. SOD activity increased after treating cells with other nanoparticles. TiO_2-, Al_2O_3-, ZrO_2-NPs at the lowest tested concentration (3 mg dm^{-3}) affected the increase of SOD activity in the analyzed cells of the Raweta variety. Superoxide dismutase is the first line of defense against reactive oxygen species and is considered to be the basic element of cell fight with oxidative stress (Alscher *et al.*, 2002). The decrease in its activity may be associated with the induction of too intense oxidative stress.

POX activity increases in the Parabola variety cells after UV irradiation of samples containing ZnO-NPs (Table 1). For the sensitive Raweta, TiO_2, Al_2O_3, ZrO_2 nanoparticles all enhance the POX activity. Peroxidases are involved in many processes in plant cells during their growth and development, and play an important role in plant's defense against pathogenic germs and insects; they also take part in wound healing (Chittoor *et al.*, 1997; Garcia-Laraa *et al.*, 2007; Mohan *et al.*, 1993). Literature reports confirm that the induction of ROS by nanoparticles is the main mechanism of their cytotoxicity. The disruption of cellular oxidation and reduction processes lead to damage to its structures and, as a consequence, to cell death (Rastogi *et al.*, 2017).

Based on the obtained results, it can be concluded that cell resistance to the cytotoxic effect of metal oxide nanoparticles depends on the sensitivity of the plant to environmental stress. The plant variety sensitive to drought stress is definitely more sensitive to stress generated by the presence of nanoparticles as well. ZnO-NPs act more cytotoxic as their diameter increases and in cells exposed to UV radiation. The differences in biological membranes structure can be the key to explaining the different reactions of callus cells to the tested nanoparticles. Generally considered to be non-toxic, metal oxide nanoparticles (TiO_2-NPs, Al_2O_3-NPs, ZrO_2-NPs) can generate cellular stress, damage cell membranes and significantly deactivate cellular defense systems. Currently, the growing pollution of the natural environment with nanoparticles can remarkably affect the productivity of agronomically important crops.

REFERENCES

Alscher RG, Erturk N, Heath LS (2002) Role of superoxide dismutases (SODs) in controlling oxidative stress in plants. *J Exp Bot* **53**: 1331–1341. https://doi.org/10.1093/jexbot/53.372.1331

Ann LC, Mahmud S, Bakhori SKM, Sirelkhatima A, Mohamadb D, Hasanc H, Seenid A, Rahmanc RA (2014) Effect of surface modification and UVA photoactivation on antibacterial bioactivity of zinc oxide powder. *Appl Surf Sci* **292**: 405–412. https://doi.org/10.1016/j.apsusc.2013.11.152

Barbasz A, Kreczmer B, Oćwieja M (2016) Effects of exposure of callus cells of two wheat varieties to silver nanoparticles and silver salt ($AgNO_3$). *Acta Physiol Plant* **38**: 76. https://doi.org/10.1007/s11738-016-2092-z

Barbasz A, Kreczmer B, Oćwieja M (2018) How the surface properties affect the nanocytotoxicity of silver? Study of the influence of three types of nanosilver on two wheat varieties. *Acta Physiol Plant* **40**: 31. https://doi.org/ doi.org/10.1007/s11738-018-2613-z

Burklew CE, Ashlock J, Winfrey WB, Zhang B (2012) Effects of aluminum oxide nanoparticles on the growth, development, and microrna expression of tobacco (nicotiana tabacum). *PLoS One* **7**: 1–9. https://doi.org/10.1371/journal.pone.0034783

Bradford MM (1976) A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. *Anal Biochem* **72**: 248–254. https://doi.org/10.1016/0003-2697(76)90527-3

Chittoor JM, Leach JE, White FF (1997) Differential induction of a peroxidase gene family during infection of rice by *Xanthomonas oryzae* pv. *oryzae*. *MPMI* **10**: 861–871. https://doi.org/10.1094/MPMI.1997.10.7.861

Curtis, T, Halford NG (2014) Food security: the challenge of increasing wheat yield and the importance of not compromising food safety. *Ann Appl Biol* **164**: 354–372. https://doi.org/10.1111/aab.12108

Daughton CG (2004) Non-regulated water contaminants: emerging research. *EIA Review* **24**: 711–732. https://doi.org/10.1016/j.eiar.2004.06.003

Filek M, Walas S, Mrowiec H, Rudolphy-Skórska E, Sieprawska A, Biesaga-Kościelniak J (2012) Membrane permeability and micro- and macroelement accumulation in spring wheat cultivars during the short-term effect of salinity-and PEG-induced water stress. *Acta Physiol Plant* **34**: 985–995. https://doi.org/10.1007/s11738-011-0895-5

Filek M, Zembala M, Hartikainen H, Miszalski Z, Kornaś A, Wietecka-Posluszny R, Walas P (2009) Changes in wheat plastid membrane properties induced by cadmium and selenium in presence/absence of 2,4-dichlorophenoxyacetic acid. *Plant Cell Tiss Org* **96**: 19–28. https://doi.org/10.1007/s11240-008-9455-0

García-Lara S, Arnason JT, Díaz-Pontones D, Gonzalez E, Bergvinson DJ (2007) Soluble peroxidase activity in maize endosperm associated with maize weevil resistance. *Crop sci* **47**: 1125–1130. https://doi.org/10.2135/cropsci2006.10.0687

Ge Y, Schimel JP, Holden PA (2011) Evidence for Negative Effects of TiO 2 and ZnO Nanoparticles on Soil Bacterial Communities. *Environ Sci Technol* **45**: 1659–1664. https://doi.org/10.1021/es103040t

Han J, Qiu W, Gao W (2010) Potential dissolution and photo-dissolution of ZnO thin films. *J Hazard Mater* **178**: 115–122. https://doi.org/10.1016/j.jhazmat.2010.01.050

Handy RD, Von der Kammer F, Lead JR, Hassellöv M, Owen R, Crane M (2008). The ecotoxicology and chemistry of manufactured nanoparticles. *Ecotoxicology* **17**: 287–314. https://doi.org/10.1007/s10646-008-0199-8

Kataria S, Jain M, Rastogi A, Živčák M, Brestic M, Liu S, Tripathi DK (2019) Role of Nanoparticles on Photosynthesis: Avenues and Applications. *Nanomaterials in Plants, Algae and Microorganisms* **2**: 103–127. https://doi.org/10.1016/B978-0-12-811488-9.00006-8

Karunakaran G, Suriyaprabha R, Rajendran V, Kannan N (2016) Influence of ZrO_2, SiO_2, Al_2O_3 and TiO_2 nanoparticles on maize seed germination under different growth conditions. *IET nanobiotechnol* **10**: https://doi.org/171-177. 10.1049/iet-nbt.2015.0007

Kim E, Kim SH, Kim HC, Lee SG, Lee SJ, Jeong SW (2014) Growth inhibition of aquatic plant caused by silver and titanium oxide nanoparticles. *Toxicol Environ Health Sci* **3**: 1–6. https://doi.org/10.1007/s13530-011-0071-8

Lee CW, Mahendra S, Zodrow K, Li D, Tsai YC, Braam J, Alvarez PJJ (2010) Developmental phytotoxicity of metal oxide nanoparticles to *Arabidopsis thaliana*. *Environ Toxicol Chem* **29**: 669–675. https://doi.org/10.1002/etc.58

Ma H, Wallis LK, Diamond S, Li S, Canas-Carrell J, Parra A (2014) Impact of solar UV radiation on toxicity of ZnO nanoparticles through photocatalytic reactive oxygen species (ROS) generation and photo-induced dissolution. *Environ Pollut* **193**: 165–172. https://doi.org/10.1016/j.envpol.2014.06.027

Milewska-Hendel A, Gawecki R, Zubko M, Stróż D, Kurczyńska E. (2016) Diverse influence of nanoparticles on plant growth with a particular emphasis on crop plants. *Acta Agrobot* **69**: 1694. http://dx.doi. org/10.5586/aa.1694

Mohan R, Bajar AM, Kolattukudy PE (1993) Induction of a tomato anionic peroxidase gene (tap1) by wounding in transgenic tobacco and activation of tap1/GUS and tap2/GUS chimeric gene fusions in transgenic tobacco by wounding and pathogen attack. *Plant Mol Biol* **21**: 341–354

Moore MN (2006) Do nanoparticles present ecotoxicological risks for the health of the aquatic environment? *Environ Int* **32**: 967–976. https://doi.org/10.1016/j.envint.2006.06.014

Movafeghi A, Khataee A, Abedi M, Tarrahil R, Dadpour M, Vafaeil F (2018) Effects of TiO_2 nanoparticles on the aquatic plant *Spirodela polyrrhiza*: Evaluation of growth parameters, pigment contents and

antioxidant enzyme activities. *J Environ Sci* **64**: 130–138. https://doi.org/10.1016/j.jes.2016.12.020

Murasnige T, Skoog F (1962) A revised medium for rapid growth and bio assays with tobacco tissue cultures. *Physio Planta* **15**: 473–497

Nations S, Wages M, Cañas JE, Maul J, Theodorakis C, Cobb GP (2011) Chemosphere acute effects of Fe_2O_3, TiO_2, ZnO and CuO nanomaterials on *Xenopus laevis*. *Chemosphere* **83**: 1053–1061. https://doi.org/10.1016/j.chemosphere.2011.01.061

Nel A, Xia T, Mädler L, Li N (2006). Toxic potential of materials at the nanolevel. *Science* **311**: 622–627. https://doi.org/10.1126/science.1114397

Padmavathy N, Vijayaraghavan R (2008) Enhanced bioactivity of ZnO nanoparticles- an antimicrobial study. *Sci Technol Adv Mater* **9**: 1–7. https://doi.org/10.1088/1468-6996/9/3/035004

Rastogi A, Zivcak M, Sytar O, Kalaji HM, He X, Mbarki S, Brestic M (2017) Impact of metal and metal oxide nanoparticles on plant: A critical review. *Front. Chem.* **5**: 78. https://doi.org/10.3389/fchem.2017.00078

Sadiq IM, Pakrashi S, Chandrasekaran N, Mukherjee A (2011) Studies on toxicity of aluminum oxide (Al_2O_3) nanoparticles to microalgae species: Scenedesmus sp. and Chlorella sp. *J Nanoparticle Res* **13**: 3287–3299. https://doi.org/10.1007/s11051-011-0243-0

Zafar H, Ali A, Ali JS, Haq IU, Zia M (2016) Effect of ZnO nanoparticles on *Brassica nigra* seedlings and stem explants: growth dynamics and antioxidative response. *Front Plant Sci* **7**: 1–8. https://doi.org/10.3389/fpls.2016.00535