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Biological effects of CeO_2 nanoparticles on components of water microbiocenosis

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A growing number of research in the scientific world community is devoted to nanoparticles (NPs) of metals and their influence on living systems, including plant organisms. Inclusion of NPs in plant cells requires they pass through a cell wall that has a pore size of at most 5 nm in most species, while their transfer from roots to shoot is blocked by Casparian strips in roots. Nano-CeO_2 is widely used in many industries. Interest in this metal antioxidant is due to its ability to be directly involved in the oxidation-reduction cycle and to rapidly change its oxidation state ($Ce3^+$ and $Ce4^+$). In general, numerous studies have shown that cerium oxide nanoparticles have low cytotoxicity and high antioxidant properties, although high concentrations may have the opposite effect. The main objective of the current study is to assess the toxicity of cerium oxide nanoparticles for the main representatives of water biocenosis.

Keywords: Cerium oxide nanoparticles, active oxygen species, DNA, cell viability, duckweed, *Stylonychia mytilus.*

INTRODUCTION

A growing number of research in the scientific world community is devoted to nanoparticles (NPs) of metals and their influence on living systems, including plant organisms (Papagiannis, 2004; Kuznetsov, 2006; Venzhik, 2017). Works that reveal mechanisms of nanometal influence on aquatic plants are particularly relevant, since many NPs tend to show higher toxicity precisely in the water phase (Limbach, 2008; El-Temsah, 2010). Thus, when water composition changes, the degree of NP filtration through soil may change, and water vapor can significantly change the number of facets in them (Zhu, 2016). Inclusion of NPs in plant cells requires they pass through a cell wall that has a pore size of at most 5 nm in most species (Miralles, 2012), while their transfer from roots to shoot is blocked by Casparian strips in roots. Despite these barriers, it was reported that NPs larger than 5 nm could also be transferred from roots to shoots, avoiding Casparian strips (Rico, 2011; Majumdar, 2014).

In this light, it is very little known about the behavior of cerium dioxide nanoparticles (CeO_2) in an aqueous medium. Nano-CeO_2 is widely used in many industries and as an antioxidant in biomedicine and a photosynthetic stimulant in agriculture (Croy, 2007; Das, 2007; Limbach, 2008; Giraldo, 2014). Interest in this metal antioxidant is due to its ability to be directly involved in the oxidation-reduction cycle and to rapidly change its oxidation state (Ce3 + and Ce4 +) (Cassee, 2011).

In general, numerous studies have shown that cerium oxide nanoparticles have low cytotoxicity and high antioxidant properties, although high concentrations may have the opposite effect. In particular, the toxic properties of nanocerium are shown in *E. coli*, which is associated with the formation of reactive oxygen species (ROS) on the metal surface after the $Ce^{3+} \rightarrow Ce^{4+}$ conversion (Singh, 2011). Using plants, it has been shown

that treating tomato with relatively low concentrations of CeO_2 NPs (10 mg / L) affects life cycle of plants, reduces biomass and water transpiration, slightly increases ROS content and leads to an increase in the number of root hairs (Wang, 2013). Previously, several toxicity mechanisms of nanocerium have been proposed: interference with the nutrient transport functions of the membrane, mechanical damage membrane, ROS generation and induction of oxidative stress (Rogers, 2010; Rodea-Palomares, 2011; Rodea-Palomares, 2012). According to the published data, CeO_2 NPs reduces the amount of Fe and antioxidant substances in *Oryza sativa L.* (except flavonoids) (Rico, 2013). Wang et al. conducted an interesting study: it was established that plants of the second generation after treatment with CeO_2 NPs had a lower biomass, lower water transpiration, and a higher ROS content than the first generation plants (Wang, 2013). Some science works reported that Ce3 + ions lead to oxidation-reduction transformations up to the formation of highly toxic ROS- H_2O_2 and hydroxyl radicals (Heckert, 2008). A recent comprehensive study showed that CeO_2 NPs do not cause acute toxicity during germination and early ontogenesis (Andersen, 2016). The existing contradictory information about whether nanocerium acts as an oxidizer (and causes toxicity) or as an antioxidant (and protects cells from oxidative damage) makes it more relevant to study the mechanisms of their effect on cellular and molecular levels in details.

Although acute toxicity has been studied, and in general, the biological properties of CeO_2 NPs for plants have been described in detail, their effects in water environment are still unknown and little discussed (Schwabe, 2013; Collin, 2014; Du, 2015; Zhao, 2015). Very few works devoted to the problems of the development of biological effects in water organisms, incl. plants, after incubation with cerium oxide nanoparticles (Blinova, 2017). In fact, most works on the biological effects of nanocerium on plants was carried out in soil and produced conflicting results. However, in aqueous media, it is much more difficult to understand the behavior of nanoparticles, since they can significantly change their structure, shape and size because of aggregation, solubilization or adsorption phenomena (Handy, 2008). The chemical composition of water determines the particle agglomeration rate and, as a result, the bioavailability (and toxicity) of metal oxide nanoparticles in aquatic ecosystems (Nowack, 2012).

Thus, data on the levels of nanoparticles exposure in aqueous media are still lacking, and the lack of tools for identifying and quantifying NPs in aquatic environments is the main restraint on describing the degree of exposure and risks associated with the spread of NPs in the environment. Research work in this area is a priority and extremely relevant.

The main objective of the current study is to assess the toxicity of cerium oxide nanoparticles for the main representatives of water biocenosis. In particular, *Stylonychia mytilus* infusoria as a key species in indicating the state of aquatic environment and an important body model in ecotoxicology and *Lemna minor* duckweed, as a representative of another trophic level in aquatic ecosystem (primary producers). Experimental studies using *Stylonychia mytilus* were performed both in conditions of full water biocenosis and isolated in vitro. Advantages of choosing duckweed as a test aquatic organism are the following: (1) macrophytes are one of the main autotrophs in the ecosystems of small lakes and rivers, (2) high reproductive rate, small size and ease of growth, (3) growth inhibition test with *Lemna sp.* is widely used in ecotoxicological studies of nanomaterials (Li, 2013; Üçüncü, 2013). In addition, the biotoxic state of cerium oxide nanoparticles was estimated using reporter strains with a constitutive and inducible character of bacterial luminescence. Such complex experiments are extremely necessary to assess the risks associated with the use of nano-sized cerium oxide for cleaning domestic waste in situ and / or caused by accidental release into the environment.

MATERIALS AND METHODS

Material science certification of cerium oxide nanoparticles used in the study.

Sample preparation

In this work three types of nanoparticles were used (Table 1). The nanoparticles were manufactured by “Advanced powder technologies LLC”, Russian Federation.

NPs were stored in loose bulk condition in a glass container. Immediately before the experiments, according to TU 931800-4270760-96, many NP suspensions were prepared in distilled water and intensively pipetted. To maintain the required dispersity of suspensions, the samples were processed in an ultrasonic bath (Sapphire TTTC, Russia) at a frequency of 35 kHz for 20 minutes. Ultrasonic processing eliminates the addition of impurity stabilizers, which distort the result of

bioactivity of NP. However, in the presence of any electron donor in the solution, $O_2^{\bullet -}$ generation can occur, which can affect the characteristics of the samples under study (Godymchuk, 2012).

Atomic-force microscopy study

For morphological study of nanoparticle and its contact with bacterial cells, an aliquots (20 μL) of nanoparticle` aqueous suspensions, were applied to freshly prepared mica. The samples were incubated at 93% relative humidity and 20−22°C and scanned with atomic force microscope Certus V Light (Nanoscan technology, Russian Federation) equipped by cantilevers NSG 10 (nominal spring constant 37,6 N/m; curvature radius < 10 nm) (Tips Nano, Estonia). The sample was scanned in air using the tapping mode.

Zeta-potential measurements

The size and zeta-potential of nanoparticles dispersed in aqueous suspensions were assessed with a laser autocorrelation analyzer Photocor (Photocor, Russian Federation). The samples were placed in 10 × 10 × 45 mm polystyrene cuvettes and were illuminated by a 633 nm helium-neon laser. The light scattering was measured at angle of 90°, and the particles size distribution was calculated from the diffusion coefficient according to Smoluchowski equation. The average diameter ± width (nm) of nanoparticle aggregates in aqueous suspensions was calculated according to volume size distribution data using the software of the instrument.

Toxicological study CeO_2 in vitro. Bioluminescence method

Biological activity of nanomaterials was tested involving lux-biosensors with constitutive and inducible characteristics of bacterial luminescence. *Escherichia coli* strain K12 TG1 pF1 (“Ecolum”, Russia) was used in constitutive method of fluorescence. To assess activity of nanoparticles against *E. coli* K12 TG1 pF1 cells we used a previously described version of bioluminescent analysis for carbon-based nanomaterials. Doing similar biotesting we rehydrated commercially available freeze-dried products lux-biosensors *E. coli* K12 TG1 pF1 (“Ecolum”, Russia) by adding 1.5% solution of NaC1 or distilled water chilled up to 8C, after that we kept them at the temperature of 2-4C during 30 minutes. Briefly, aqueous suspensions of nanoparticles (μg/ μL) were added to the wells of a “Microlite 2+” microplate with non-transparent side walls (Thermo, USA), wherein they were further doubly diluted in sterile deionized water, from 1:1 to 1:1024, up to a final volume of 50 μL. To the filled wells were then added 50 μL of a previously prepared suspension of constitutively luminescent *E. coli* K12 TG1 lac::luxCDABE cells. Wells filled with sterile deionized water and containing an appropriate amount of bacterial biosensor were used as negative controls. *E. coli* K12 MG1655 pkatG'::lux (for a detection of peroxide of hydrogen) and *E. coli* K12 MG1655 psoxS'::lux (for a detection of superoxide anion) strains were used in inducible method of bacterial luminescence.

The work used strains grown from LB-broth with 20 μg/ μL of ampicillin at 37C during 16-18 hours. Directly before the experiment the culture was additionally grown in the same fresh substratum at 1:20 and was incubated for 3-5 hours, then it was suspended in 0.5% solution of NaC1 to reach OD 450=0.05. The received suspensions at the amount of 50 μL were put into the tablet pits which contained nanomaterials prepared in advance at the amount of 50 μL; the suspensions were kept for 15 min, after that 100 μL of LB-broth were additionally put in each pit.

Bioluminescence measurements were carried out using the Infinite PROF200 (TECAN, Austria) microplate reader, which dynamically registered the luminescence intensity of the samples for 180 min, estimated in relative light units (RLU). The data were analyzed using the software provided with the instrument. To quantify the bioluminescence inhibition index (I) due to nanoparticle toxicity were calculated according to the algorithm (1).

$$I = RLUc0 \times RLUtn/RLUcn \times RLUt0, \quad [1]$$

where *c* and *t* are the RLU values of the control and test samples at the 0-th and n-th minute of measurement.

5 Toxicological study CeO_2 in vitro. Study on test cultures

As a test object, a culture of freshwater infusorian *Stylonychia mytilus* (wild strain) was used in the phase of exponential growth. The number of test functions included: survival, number (biomass). The initial culture of *Stylonychia mytilus* was cultivated on Lozin-Lozinsky medium, (1 g per 1 liter of distilled water) with the addition of yeast (*Saccharomyces cerevisiae*) nutrient medium.

The 950 ml of distilled water was added to 50 ml of concentrated medium to prepare the medium with a working concentration. The

washed culture in a narrow part of the neck of the flask, was used for analysis.

Sensitivity of *Stylonychia mytilus* to the action of the toxicant was determined by the time of their death. The latter was diagnosed by the cessation of protozoa movement, which was accompanied by violation of the integrity and lysosomes of the cell. The number of cells in 5 mL of medium containing an intact culture of infusorians (without the addition of nanoparticles) served as a control in all the experiments. Total number of cells in 5 mL of medium containing infusoria was counted by using a light microscope (MT 5300L). Cells taken in a stationary growth phase were incubated at the temperature of 20±2°C in a medium supplemented with substances for 24 hours. Interim counting was carried out in 1,6,12 and 24 hours.

Studies in the conditions of aquabiocenosis. Studies of microbial trophic network and Stylonychia mytilus

The performed research of composition of microbial trophic network communities, which transform the main amount of primary aquatic production, were focused at the study of the quantitative distribution of sanitary-indicatory bacteria and protozoa in micro water reservoir.

Toxicant (CeO_2) at a concentration of 1, 10 and 100 mg/l was added to a separate aquarium and exposed for 4 weeks. The control toxicant was not added to the reservoir, so the dynamics of the change in the number of microorganisms in it showed a natural process of their growth and dying out. After the exposure, plating of water samples for selective nutrient media from each reservoir was performed. Sampling was carried out on 7, 14 and 30 day. The determination of the total microbial count (TMC) was carried out according to MUK 2.1.4.1184-03 (2003). The number of test functions for protozoa included: survival, number (biomass).

Study on Lemna minor L. Peculiarities of growing plants

The study used an aquatic plant *Lemna minor L.*, which was treated with NPs of cerium oxide for 7, 14 and 30 days. The plants were grown for 3 days in an aqueous medium without metal, and then NPs were added at a concentration of 1, 10 and 100 mg / l. Preliminary tests revealed that the concentration of 10 mg / l is threshold, or sub-toxic, which corresponds to the safety certificate for cerium oxide (GOST 30333, 2007).

To assess the biological effect of nano-cerium, an average sample was taken, which consisted of leaves of 3 plants in three biological replications.

At the end of the experiment, the number of leaves was counted and dry biomass of plants was determined. Then it growth inhibition was estimated compared to control for each test concentration of CeO_2 NPs, expressed as % of growth inhibition (OECD Guideline fo Testing of Chemicals, #221, 2006).

Assessment of antioxidant status of plants

The amount of products of lipid peroxidation (LPO) in the soluble fraction of homogenate and directly in tissues was determined according to the content of Malondialdehyde (MDA) according to Heath and Packer (1968). The method is based on the reaction of MDA with 2-thiobarbituric acid, TBA ("Lenreactiv", Russia), which at high temperature and acid pH - in the presence of trichloroacetic acid, TCA (Reachim, Russia) forms a colored trimethine complex. This complex has a characteristic absorption spectrum with a maximum at λ = 532 nm (Sibgatullina, 2011) (Appendix 2, Figure 7). It should be taken into account that some of TBA products are formed in the analyzed sample during the analytical procedure (Esterbauer, 1991).

For the analysis, 100 mg of duckweed leaves were taken and triturated with 200 µl of 20% TCA. The resulting homogenate was centrifuged for 5 minutes at 12,000 g. The resulting supernatant was introduced into two tightly closed tubes in an amount of 100 µl: 100 µl of 20% TCA was added in the first tube, using it as a control, in the other 100 µl of 0.5% TBA was added. The samples were incubated in a boiling water bath (100 ° C) for 30 minutes and cooled at room temperature. The measurements were carried out on a spectrophotometer at 532 nm and additionally at 600 nm (to correct the non-specific absorption of the carbonyl compounds). The calculation was made using the formula:

$C = ((OD/155)\cdot X\cdot V)/(m\cdot l)$ [2]

where C is content of MDA, mmol / g wet weight, OD is the optical density of the sample at 532 nm, 155 is MDA extinction coefficient at 532 nm, мМ-1cm-1, X is the dilution (the ratio of the total volume of the reaction mixture to the amount of sample), V is the volume of the extract, ml, m is the mass of the wet sample, g, l is the optical path length, cm (Sibgatullina, 2011).

The level of lipid peroxidation was expressed as a percentage, the amount of TBA-reacted

products contained in the cells of the original roots was taken for 100%.

Currently, one of sensitive methods of ROS detection is a method using 2,7- Dichloro-dihydro-fluorescein diacetate (DCFH-DA), which in the presence of oxidizers (H_2O_2, $HO^•$, $ROO^•$, $ONOO^-$, but not $^1O_2$ и $O_2^{··}$) transforms in fluorescent 2',7'-dichlorofluorescein (DCF) (Bonini, 2006; Faisal, 2013). The dye is more likely an indicator of overall oxidative stress, rather than specific types of ROS (Gomes, 2005). Reagent DCFN-DA ("Acros organics", USA) was prepared by dilution in ethanol (96%) to a concentration of 10 microM (Bonini, 2006), providing that the concentration of ethanol does not exceed 0.1% in a sample. Then 3 roots (40 mg) were taken and triturated with Teflon pestle for 10 minutes in tubes of "Eppendorf" type in 500 mcl of 0.1M cooled FSB (pH 7,4) ("Ambresco" tablets, USA). The mixture was then centrifuged for 5 min at 3000g. The supernatant was decanted, and the remaining precipitate was washed twice with the same amount of the FSB. The precipitate for precipitation of protein fractions was cooled for 10 min on ice. Then, the sample was centrifuged again, resuspended in 100µl of the FSC, and taken up in 96-well plate.

The resulting samples were incubated with 10µM dye DCFN-DA in ratio 10:1 at 25 °C in a thermal shaker for 20 min and then washed twice by 100 µl excess fluorochrome FSB (Bonini, 2006). Prepared suspensions were stored in the dark at room temperature and used within a maximum of 4 h.

Fluorescence detection was carried out at a temperature of 25 °C in shaking mode at λexc = 488 nm and the λem = 524 nm on a multitray plate reader (Infinite 200 PROro) (Tecan, Austria). The values obtained were expressed in relative fluorescence intensity units of the samples.

Hydroxyl radical (·OH) has been considered as one of the main active radicals in the oxidizing mechanism, particularly DNA molecules. To detect ·OH, we measured the surface fluorescence of 7-hydroxycoumarin-3-carboxylic acid (7-OH-CCA, umbelliferone) which is formed after reaction with coumarin-3-carboxylic acid (CCA) with HO (Manevich, 2002). For the analysis, cell suspension was prepared preliminarily. 40 mg of raw material was triturated with 250 µl of FSB for 5 min, centrifuged at 10,000 rpm/min for 5 min. 200 µl of supernatant was added to a microplate well and mixed with 20 µl of CCA to a final concentration of 10^{-4} M. Samples were incubated at room temperature in the dark for 1 h. The reaction was terminated by addition of 10 mM Tris base (pH = 9.0) (Yoon, 2010; Miller, 2014).

Determination of viability of Lemna minor L cells

Cell viability was analyzed according to the change of reductase enzyme activity was performed according to the manufacturer's protocol (WST-8 patent no. 2.251.850, Canada) using Cell Counting Kit-8 (CCK-8) highly sensitive test (Sigma-Aldrich, USA), based on using water-soluble tetrazolium salt (WST-8)[42]. For carrying CCK-8-test analysis, cell suspensions were prepared, leaves of 3 plants weighting 40 mg were triturated for 10 min in 120 µl FSB, centrifuged for 5 min at 10000 rev/min. Then, 100 µl of supernatant was collected and mixed with 10 µl of CCK-8 dye in a 96-well plate. The obtained samples were incubated for 1 h at 37 °C. Thereafter, absorbance was measured at λ=450 nm (WST-8 patent no. 2.251.850, Canada) every 15 min using a microplate reader (Tecan, Austria) in shaking and heating mode up to 37°C. Number of viable cells (in %) was calculated according to the formula from the ratio of the experimental samples (ES) to control values (ESc) minus background absorption CCK-8 reagent kit:

Cell viability, % = $((ES_o - ES_c)/(ES_c - CCK8))\cdot 100\%$ [3]

Study of electrophoretic mobility of DNA molecules

DNA extraction from leaves of *L. minor* was carried out with the help of a set of reagents "DNA-Extran-3" according to the isolation protocol developed by the enterprise "Synthol" (Russia). 500 mg of raw material were added to the tubes with one steel ball 7 mm in diameter and frozen in a refrigerator at -70°C for 30 minutes. The samples were then ground in a homogenizer "TissueLyser LT" ("Qiagen Hilden", Germany) at a frequency of 50Hz for 15 seconds. The concentration and quality of DNA was determined spectrophotometrically with the help of NanoDrop-1000 ("Fisher", USA) for the ratio of $ES_{260/280}$ and $ES_{260/240}$. If $ES_{260/280} \geq 1.8$-2, then the preparation of DNA is sufficiently pure and does not contain protein contaminant.

Separation and analysis of DNA fragments was carried out by horizontal electrophoresis in 1% universal agarose I (Helicon, Russia). To identify the length of fragment and concentration of the sample and to determine the molecular weight of DNA sample, 1 µl of a 1 Kb DNA marker

(M12) from “Sibenzym” (Russia) was added into outer wells of the gel plate, consisting of a mixture of 13 fragments of special plasmids cleaved with specific enzymes HindIII and EcoRI with a length of 250 to 10,000 bp. In the remaining wells, 10 µl of test samples were added. Bromophenol blue or xylene cyanol was not added to the wells to avoid changing oxidation-reduction reactions and additional binding of DNA to its components.

Electrophoresis was performed in a horizontal chamber SE-1 (Helicon, Russia) with 4-10 V/cm voltage of gel, 80 W and a current strength of 200 mA, preset by a power source “Elf-8” (Helicon, Russia) within 40 minutes. Tris-borate buffer (Wilson, Walker, 2015) was used as the electrolyte, which is suitable for electrophoresis of small DNA fragments (<1 Kb) and consists of: 0.04 M Tris-HCl (Applichem, Germany), 1 mM EDTA (Reachim, Russia) and 0.089 M boric acid ("Tula Pharmaceutical Factory", Russia) (pH 7.2). After the run was completed, the gel was soaked for 20 minutes in a 1% solution of ethidium bromide on a tris-borate buffer. The gel was then carefully washed in distilled water and visualized using the video documentation of Gel-Doc gels ("Bio-Rad", USA). The gels were then photographed and processed using universal computer program ImageJ (National Institutes of Health, USA), where the intensity of linear profiles and the total fluorescence area of tracks were analyzed.

All tests were carried out in 2-3 independent experiments with 3-4 repetitions per sample and subjected to statistical processing. The data obtained was processed using a variational statistics methods with the Statistica V10 (StatSoft Inc., USA).

RESULTS

Physical and chemical characteristics

The manufacturer of nanoparticle indicates the size of the cerium dioxide in the range 15.8 nm (Table 1). However, according to dynamic light scattering data, the CeO2 particles in aqueous suspensions form three fractions with different dimensions parameter (Table 2). In particular, only 45 % of the particles dispersed in the aqueous suspension have diameter 192 ± 62 nm, while most have a diameter between 100-200 nm. In turn, the zeta potential of the CeO2 particles characterized by a negative value.

Visualization using the atomic-force microscopy has made it possible to characterize the cerium particles as large agreggates with average diameter about 1.2± 0.5 µm (Fig.1).

Bioluminescence results

Metal particles of CeO_2 in the studied concentrations of 3-0.000195 M did not cause changes in the dynamics of bacterial bioluminescence (Fig 2).

An increase of concentrations up to 4 M did not cause a toxic effect, which characterized cerium dioxide particles as a conventionally safe substance for a living organisms.

The use of lux-biosensors with inducible characteristic of bacterial luminescence proved the data received earlier. Nanoparticles of ceric dioxide didn’t have the oxidative activity and were neutral to the bacterial cell (Fig. 3 a, b).

The toxic properties of cerium compounds depends on particle size and related to the surface charge density. Direct contact of cerium oxide particles with the bacteria mediates by electrostatic attraction between the cell wall and positively charged with cerium particles, which will lead to external destabilization of the bacterial membrane and development of cytotoxic effect (Zholobak, 2014). As revealed by DLS the zeta potential of used sample of CeO_2 was negative low value what are determines their aggregation and absence of toxicity for bacteria.

Results of assessment of biological action of CeO_2 NPs on Stylonychia mytilus

The present study was carried out on a model of hydrobiont (representative of ciliated protozoans *Stylonychia mytilus*), which was exposed to CeO2 NPs at concentrations of 1, 10 and 100 mg/l within 7, 14 and 30 days.

The introduction of CeO2 NPs in aqueous medium at a concentration of 1 mg/l for 7 to 30 days did not significantly affect the culture of *Stylonychia mytilus* cells relative to the corresponding control group ($P \leq 0.05$), the values did not exceed an average of 15-25% irrespective of exposure time (Table 3).

Analysis of activity of CeO2 NPs demonstrated that concentration of 10 and 100 mg/l at the 30th day of exposure were most toxic, causing a decrease in activity of *S. mytilus*. The effect of 10 mg/l of CeO2 NPs on the 14th day of exposure resulted in a smoother decrease in activity.

The 1 mg/l of CeO2 NPs during the entire period had little effect on the population of *Stylonychia mytilus* cells.

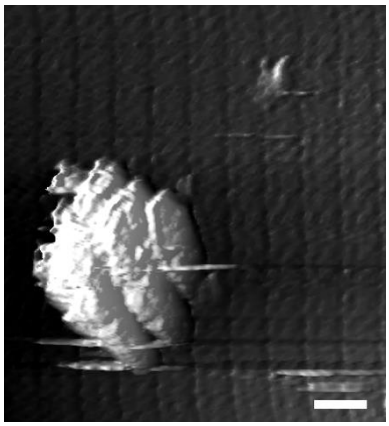

Figure 1. Microscopy visualization of metal particles dispersed in water and dried on mica. AFM-images of cerium oxide. Bar scale – 500 nm.

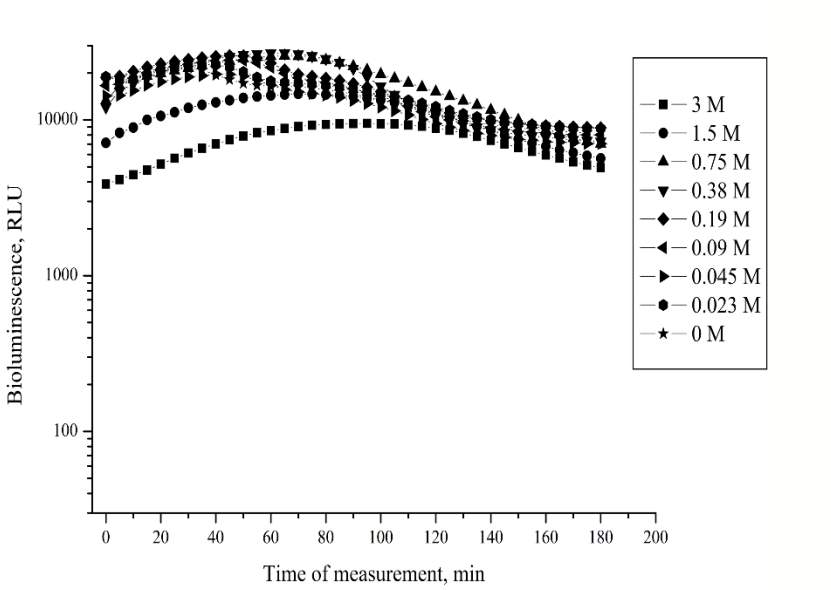

Figure 2. Dynamics of luminescence of *E. coli K12 TG1 pF1* ("Ecolum") treated with CeO_2 nanoparticles taken at various concentrations

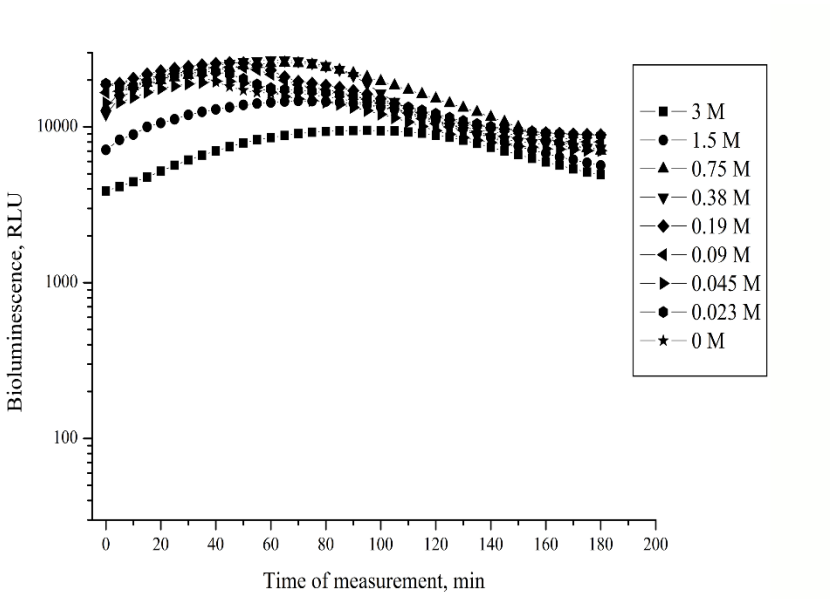

Figure 3. Dynamics of bioluminescence of lux-biosensors (a – *E. coli K12 MG1655 psoxS'::lux*; b – *E. coli K12 MG1655 pkatG'::lux*) treated with CeO_2 nanoparticles taken at various concentrations

Table 1. Characteristics of the CeO_2 according to the manufacturer

Nanoparticles	Diameter, nm	Chemical and phase composition	The method of synthesis	Specific surface area (S_{SP}, M^2/G)
CeO_2	15,8	CeO_2: 99,8%	Gas-phase synthesis	49,6

Table 2. The main physical characteristics of nanoparticle preparations

Samples	Particle distribution, %	Diameter, nm	Zeta-potential, mV
CeO_2	45	192 ± 62	- 15,98 ± 0,19

Table 3: Effects of nanoparticles CeO_2 for a population of cells *S. mytillus*

Time of exposition, day	Concentration: 0 mg/l	Concentration: 1 mg/l
	CeO_2	CeO_2
7	NOEC	NOEC
14	NOEC	NOEC
30	NOEC	NOEC
	Concentration: 10 mg/l	Concentration: 100 mg/l
7	NOEC	NOEC
14	NOEC	LOEC
30	LOEC	LOEC

Note. Tox – the concentration causing 0-39 % survival object; LC50 – concentration causing 50% survival object; LOEC – the concentrate, causing 40-69% survival rate of the object; NOEC – the concentration causing 70-100% survival object[44].

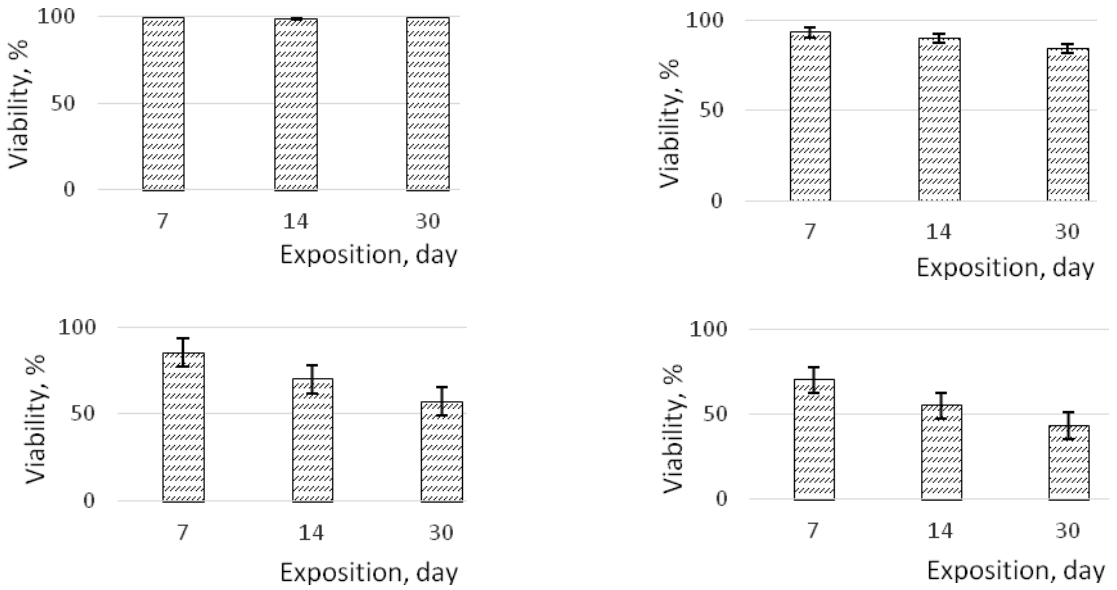

Figure 4. Toxicity as percent viability of L. *minor* after 7, 14 and 30 days of exposure of different concentrations of NPs CeO2: control= 0 mg/L, 1 mg/L, 10 mg/L and 100 mg/L; Vertical bar around the mean is the standard deviation; * significantly different from control (P≤0,05).

Analysis of concentration effects showed that CeO2 NPs cause maximum inhibition of protozoa cell growth, which was observed at a concentration of 100 m/l on the 30th day of incubation.

Evaluation of time periods when any effect was observed showed that effect of the analyzed CeO2 NPs appears on the 30th day of incubation at all concentrations, excluding 1 mg/l CeO2 NPs.

A high survival rate of *S. mytilus* cells was registered during a period from 7 to 30 days at a concentration of 1 mg/l CeO2 NPs, relative to the control group. The maximum survival rate of *S. mytilus* was fixed at a concentration of 10 mg/l CeO2 NPs throughout the period from 7 to 14 days, which in quantitative terms was no more than 15 and 30%, respectively, relative to control. The toxic effect of CeO2 NPs at a concentration of 10 mg/l was maintained until the 15th day of incubation, which was expressed by a value no more than 30% respectively compared to the control. With each subsequent day of incubation, the number of living cells decreased. Thus, the number of surviving specimens on day 30 of the experiment was about 57% (Fig. 4).

The number of surviving *S. mytilus* species decreased by 30 day of exposure at 100 mg/l CeO2 NPs, the values did not exceed 43% when compared to the control. For a period from 7 to 14 day at the same concentration of CeO2 NPs, the survival rate remained the same, relative to control (P≤0.05), but the values did not exceed 30% on day 7 of incubation and 45% on day 14 of incubation.

Definitely, the mortality of cells was associated with destructive membrane pathology, which occurred on the 30th day of incubation after the exposure at a concentration of 100 mg/l of CeO2 NPs.

Thus, there was no pronounced dose-dependent effect of CeO2 NPs on growth and survival of *Stylonychia mytilus* population in the case of the entire time range. At a concentration of 1 mg/l of CeO2 NPs for shorter timeframes (7 and 14 days of incubation) no changes in the biomass amount of the *S. mytillus* cell population were observed, the protozoan cells retained activity. However, on the 30th day of incubation, the concentration of CeO2 NPs in the amount of 1 mg / L led to changes to a slight decrease in biomass.

The effect of 10 mg/l CeO2 NPs on the 7th and 14th day of incubation slightly reduced the population biomass, which in numerical terms was 17% and 28%, respectively, with respect to the control. The CeO2 nanoparticles at a concentration of 10 mg/l on the 30th day of incubation reduced the biomass of *S. mytillus* cell population by 36%, relative to the control sample.

A decrease in biomass of *S. mytillus* population was promoted by a concentration of 100 mg/l of CeO2 on the 14th and 30th days by 28 and 36%, respectively, when compared to the

control sample. While on the 7th day of incubation, the given concentration of CeO2 NPs had no visible effect (Fig. 5).

Results for protozoa and microorganisms in aquarium conditions

Analysis of silicon and cerium oxides has not shown significant bactericidal and bacteriostatic effects. The overall microbial number was slightly lower than in the control. The literature data also confirm non-toxicity of cerium dioxide for microorganisms (Lushchaeva, 2009).

Nanoparticles of cerium oxide did not manifest negative effect on protozoan cells, throughout the entire experiment (7-84 days), there was no change in their number, the overall dynamics of infusoria number was maintained throughout the experiment (Table 4).

Study of biological activity of CeO2 nanoparticles on Lemna minor L. Analysis of morphometric characteristics of L. minor L

Adding CeO2 NPs to the aqueous medium (1, 10 and 100 mg / l) within 7 up to 30 days contributed slight inhibition of growth of *Lemna minor L.* in relation to the corresponding control (P≤0.05), but the values did not exceed 20% irrespective of the exposure time and the morphological parts of plant (Fig. 6). In addition, there was no dose-dependent effect of CeO2 NPs on the growth of duckweed within the entire time range.

Analysis of the pro- and / or antioxidant properties of CeO2 nanoparticles

The ability of CeO2 NPs to increase the amount of ROS even at low concentrations (10 mg/l) has been shown (15-18). Therefore, in the second series of experiments, the effect of nanocerium on the development of pro-antioxidant effects in leaves of *L. minor L.* was studied. Thus, an analysis of the degree of lipid peroxidation showed the maximum accumulation of malonic dialdehyde (MDA) in a vegetative body against the background of all dilutions from 1 up to 100 mg/l) after 30 days of exposure - from 35% to 44% above the control, respectively (Table 5). At the same time, there was a tendency to decrease MDA in the leaves after 7 days of metal treatment in minimal concentrations of 1 and 10 mg / l - by 18% and 12% compared to the control samples, respectively (P <0.05).

In addition, the fluorimetric measurement of DCFH-DA showed a slight increase in the total amount of active oxygen species (AOS) in duckweed leaves in the case of a maximum concentration of 100 mg/l of cerium oxide after 14 and 30 days of exposure, by 19.2% and 12.8 % in comparison with the control (P <0.05), respectively (Table 5). In this case, a small accumulation of $HO^{\bullet}$ radicals have also been fixed - up to 5.5% and 7.3%.

It is generally accepted that some metal nanoparticles lead to the development of AFK-secondary signal messengers, which lead to transcriptional regulation of secondary metabolism, incl. phenolic compounds (Marslin, 2017). Analysis of the content of phenolic compounds showed an ambiguous effect of nano-sized cerium oxide on low-molecular antioxidants. In particular, when metal was exposed for 7 and 14 days, there was no significant change in the control (Table 5). On the contrary, after 30 days of exposure, there was a multidirectional change in the content of phenolic metabolites, depending on the dose of the added metal: a decrease in metabolites to 18.5 and 38% relative to control was registered after exposure with 1 and 10 mg / l of CeO2 NPs, and after using 100 mg/l there was a slight increase to 17.5%.

Thus, CeO2 NPs have an ambiguous effect on antioxidant system of duckweed, the effect primarily depends on the concentration and time of metal exposure and is characterized by a shift in the oxidation-reduction balance towards increasing prooxidants - AOS and MDA (Figure 7).

Study of viability of plant cells

Analysis of reductase activity using a water-soluble tetrazolium salt demonstrated that 10 and 100 mg/l CeO2 NPs proved to be the most toxic, causing a decrease in the yield of formazan after 14 days of exposure by 27% and after 30 days by 31% compared to the control, respectively. On the contrary, the effect of 1 mg/l CeO2 NPs resulted in a smoother decrease of living systems (no more than 10% of the control) (Fig. 8).

Some studies have shown that nanoparticles of metal oxides readily interact with DNA molecules, contributing to their oxidative damage (Bhattacharya, 2009; Tulpan, 2010). Therefore, in continuation of the study on the mechanism of biological activity of CeO2 NPs, the degradation degree of DNA molecules extracted from leaves of *Lemna minor L.* was analyzed. The set of *in vivo* data did not show significant changes in the electrophoretic mobility of DNA extracted from leaves of *Lemna minor L.* after 7 (the electrophoregram is not represented). However,

after exposure with CeO2 NPs for 14 and 30 days, a dose-dependent degradation of DNA was registered, manifested as a "track" (or "smear") from immobile nucleotides of variable molecular weight less than 1000 n.p. up to 38.5%, and a simultaneous decrease in high-molecular fragments with a mass of 10,000-3,000 n.p. up to 64%. Moreover, the most intensive destruction of DNA relative to the intact samples occurred at the maximum analyzed dilutions of CeO2 NPs (10 and 100 mg / l): on day 14 from 10 to 26% and on day 30 - from 34 to 38.5%, respectively (Fig. 9, 10). Loft monotonous luminosity peaks approaching the control variant are seen on the linear profiles of sample with 1 mg/l CeO2 NPs, but the intensity of tracks in the area less than 250 n.p increases to a dilution of 10 and 100 mg/l, and the curves become split into two parts of peaks (Fig. 11).

Table 4. Effects of nanoparticles CeO_2 for a population of cells *S. mytillus* in aquarium conditions

Substance	n, mg/dm³	Dosage, mg/dm³		
		n	10n	100n
Contact time: 7 day				
CeO_2	10	NOEC	NOEC	-
Contact time: 28 days				
CeO_2	10	NOEC	NOEC	-
Contact time: 56 days				
CeO_2	10	NOEC	NOEC	-
Contact time: 84 days				
CeO_2	10	NOEC	NOEC	-

Table 5. Effect of CeO2 NPs on the realization of pro- /antioxidant effects in leaves of *Lemna minor L*.

Concentration of NPs CeO2, mg/l		MDA	ROS	PC
7 days	1	↓	↑	↓
	10	↓	↑	↓
	100	↑	↑ (8,6%)	↓
14 days	1	↑	↑	↓
	10	↑	↑ (8,7%)	↓
	100	↑	↑ (19,2%)	↓
30 days	1	↑↑ (37%)	↑ (8,2%)	↓ (18,5%)
	10	↑↑ (38%)	↑ (9,5%)	↓↓ (38%)
	100	↑↑ (44%)	↑ (12,8%)	↑ (17,5%)

Note: "↓/↑"a slight decrease or increase (less than 20%); "↓↓ / ↑↑" moderate decrease or increase (more than 30%); PC - phenolic compounds; MDA - malondialdehyde; ROS - reactive oxygen species; significance from control P <0.05

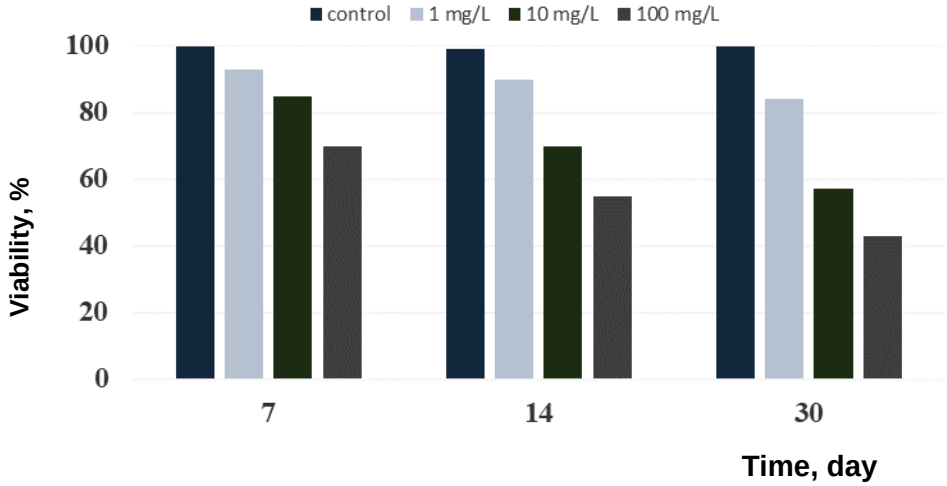

Figure 5 Toxicity as biomass percentage of S. *mytillus* after 7, 14 and 30 days of exposure with different concentrations of CeO_2 NPs: control= 0 mg/L, 1 mg/L, 10 mg/L and 100 mg/L; Vertical bar around the mean is the standard deviation; * significantly different from control (P≤0,05).

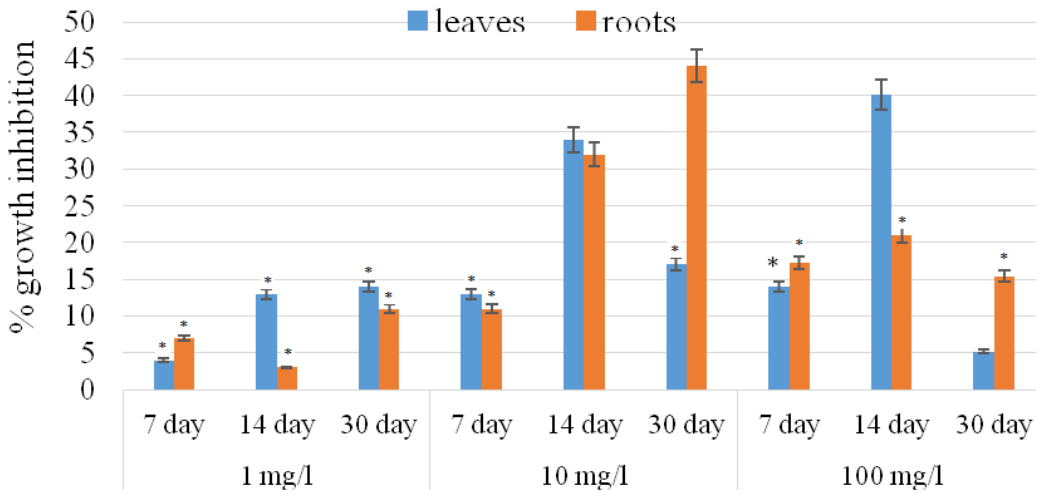

Figure 6. The percentage of growth inhibition (%) of L. minor after exposure with CeO2 NPs (1, 10 and 100 mg / L), * a variant significantly different from the control (value P≤0.05)

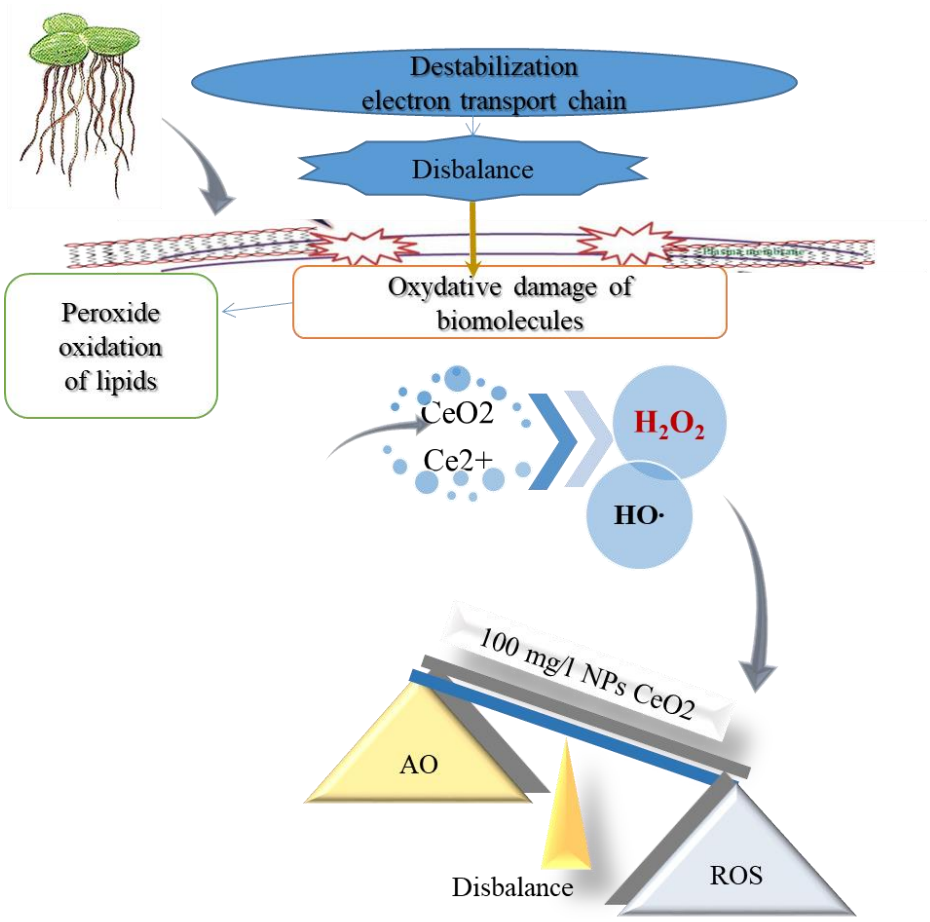

Figure 7. Proposed mechanisms for prooxidant action of 100 mg / l of CeO2 NPs on *Lemna minor L.* after 30 days of exposure

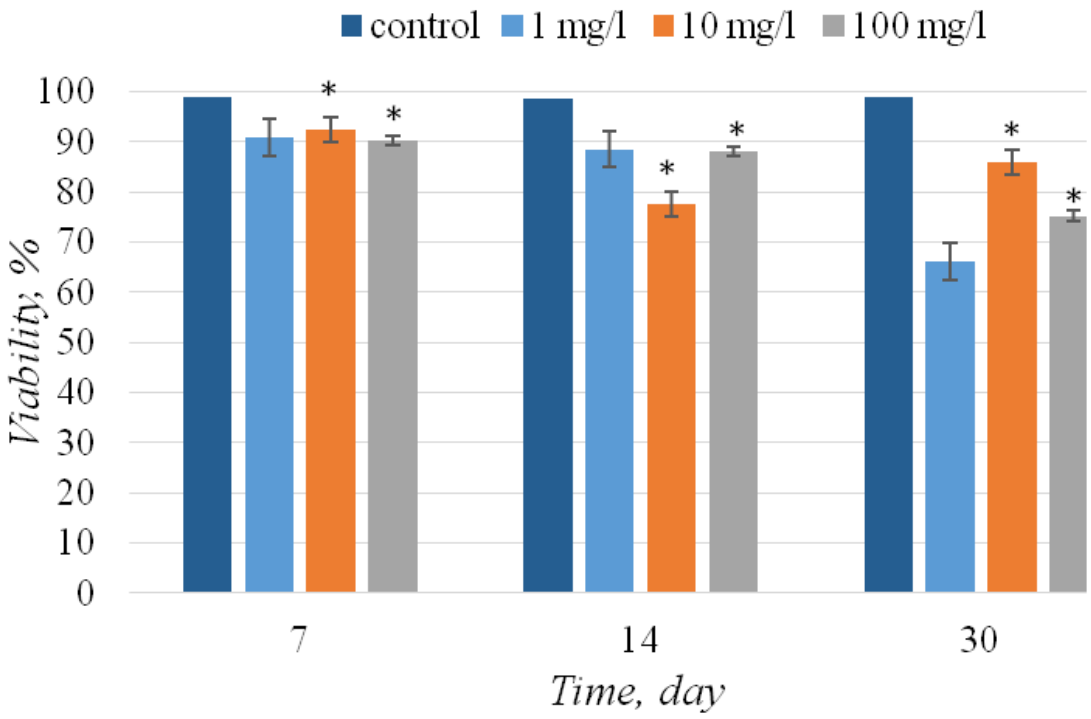

Figure 8 Toxicity as percent viability of L. *minor* after 7, 14 and 30 days of exposure of different concentrations of NPs CeO2: control= 0 mg/L, 1 mg/L, 10 mg/L and 100 mg/L; Vertical bar around the mean is the standard deviation; * variant significantly different from the control (value P≤0.05)

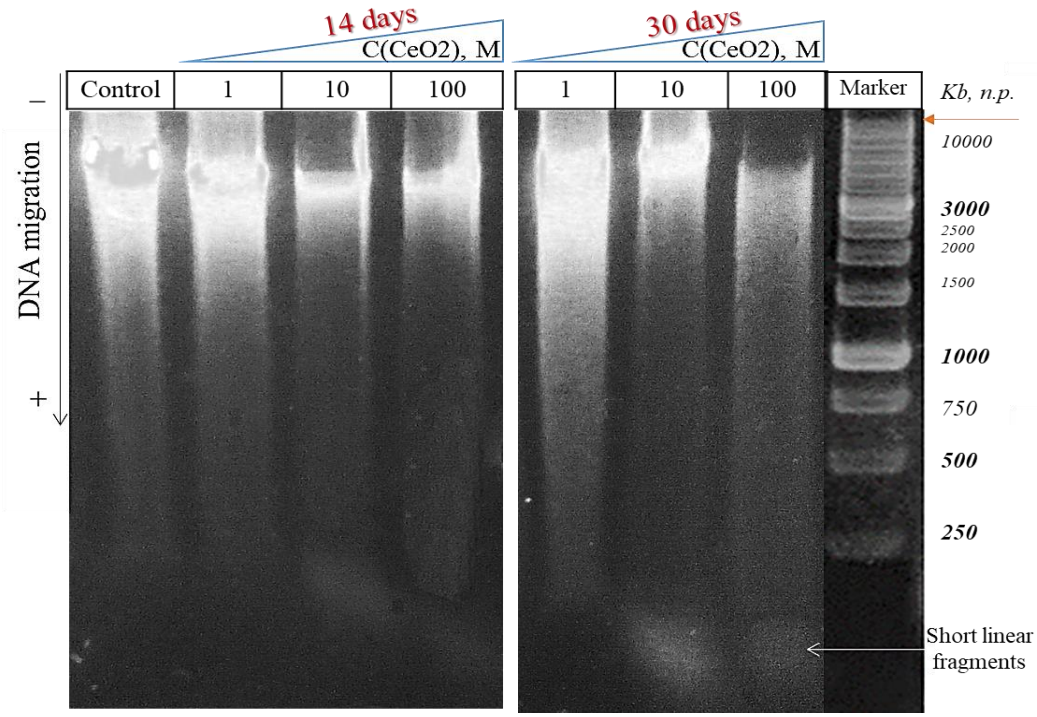

Figure 9. Electrophoretogram of DNA extracted from leaves of *Lemna minor L.* after 14 and 30 days of growing with CeO2 NPs at concentrations of 1, 10 and 100 mg/l *in vivo*

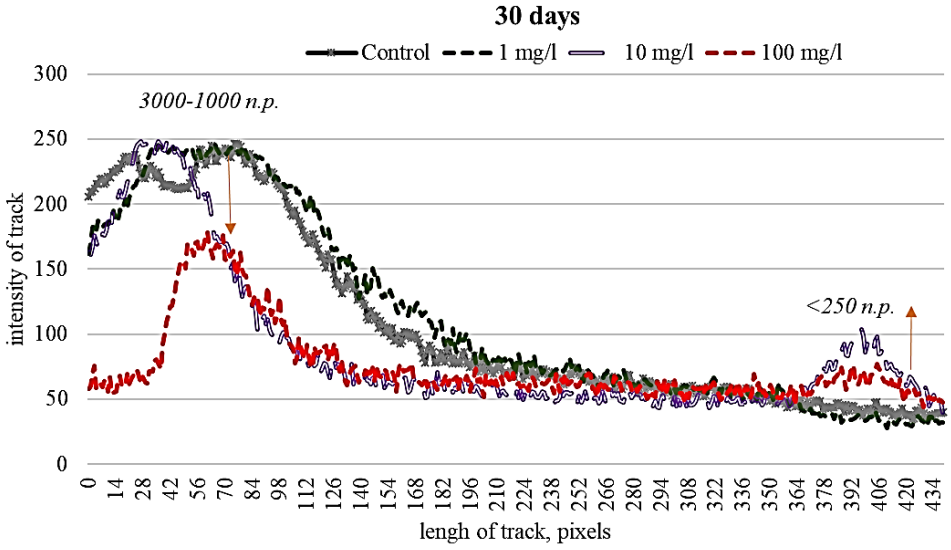

Figure 10. Linear profiles of bands of DNA isolated from the leaves of *Lemna minor L.* after 30 days of exposure with CeO_2 NPs in concentrations of 1, 10 and 100 mg/l *in vivo* (profiles obtained in ImageJ program): the graph shows the intensity of luminescence of DNA bands (*axis* of abscissas) on each pixel of the track of the electrophoregram (axis of ordinates)

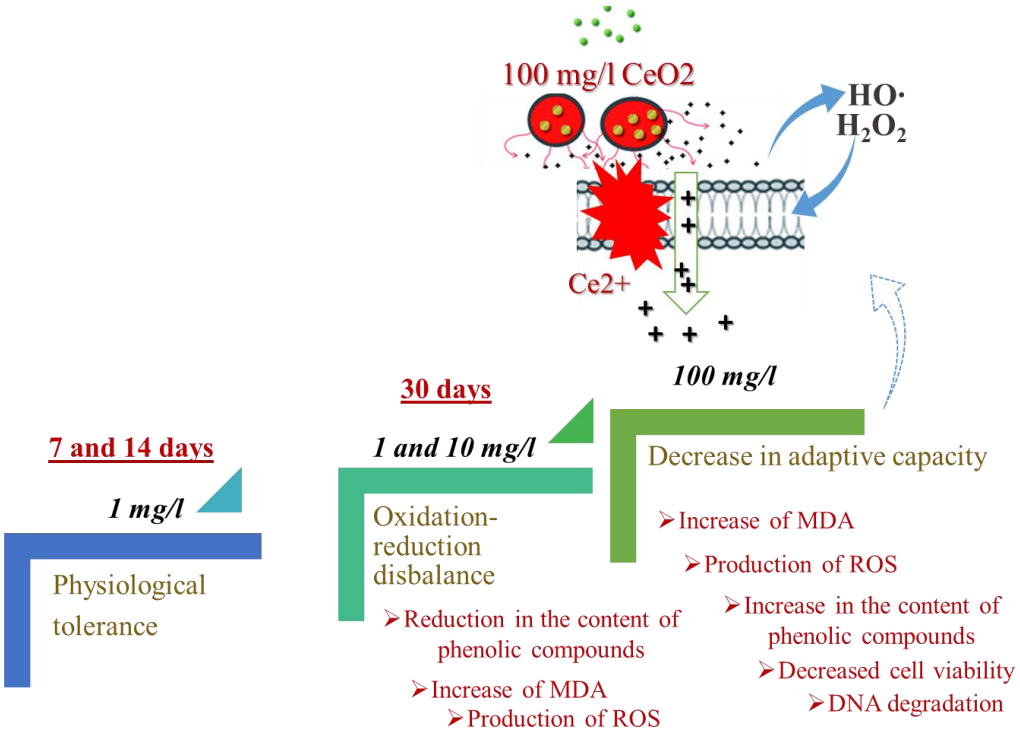

Figure 11. The resulting scheme of biological effects of CeO_2 NPs in relation to *Lemna minor L.*

DISCUSSION

According to the biotic concept, environmental monitoring according to biota reaction is the most reliable characteristic of ecosystem ecological quality, while laboratory testing and responses of standardized test organisms in many cases can serve as an indicator anticipating the manifestation of negative effects in natural conditions. The method of water biotesting according to the degree of paramecia survival is quite widely used. Paramecium caudatum refers to the most complex protozoa, very sensitive to changes in the chemical composition of medium. The short life cycle of infusorians makes it possible to reveal the character of toxicant action quickly. Suppression of growth rate of infusoria cells and their death is the indicator of toxicity of the used sample.

We assume that in the case of pollution of water bodies, most of the suspended metal nanoparticles will be concentrated in the bottom area and may change the quality of bottom habitats, in particular, affect aquatic organisms.

In the current study, a parallel test of nanocerium acute toxicity on the test culture showed a very low toxicity of the nano-sized cerium oxide.

Previous work also demonstrates the intensity of oxidative stress reactions dependent on CeO_2 concentration, and what is more, at high concentrations, CeO_2 NPs induce composite modifications in the root xylem of cereals (Rico, 2013; Rico, 2015).

The absence of toxicity of nanoparticles in dose of 1 mg/l and a slight redox imbalance after 7 days of metal exposure at a dilution of 10 mg/l may be caused by the sedimentation of ashes in medium (Schwabe, 2013).

At the same time, pores of the primary roots have an average diameter of 6.6 nm, so CeO_2 NPs with a smaller diameter than the latter can not penetrate into the root (Zhao, 2012). Probably, nanoparticles sank in aquarium and were accumulated by the plant to a small extent. In addition, in this case, adhesion of nanoparticles on the surface of plant roots or with root exudates is unlikely, as it was done with other substrates (Judy, 2012).

The data obtained conform to the results of a three-week study of corn grown in the presence of CeO_2 nanoparticles, which also shows the accumulation of H_2O_2 in shoots (in phloem, xylem and epidermal cells) (Zhao, 2012).

Previously, it was shown that the bioavailability of CeO_2 NPs in deionized water is higher than in agar media, but the sensitivity of plants to metal was lower (Ma, 2010; Zhang, 2013; Cui, 2014).

It is known that the pore size of cell wall varies from 2 to 30 nm, therefore, metal NPs studied by us probably remain on the root surface and act indirectly.

Toxicity was probably associated with biotransformation of CeO_2 NPs and high sensitivity of duckweed plants to released Ce3 + ions. Other authors registered similar phenomena (Gui, 2015).

However, such results do not agree with the results obtained by Lopez-Moreno et al., (López-Moreno, 2010). It is well known that the behavior of metallic nanoparticles strongly depends on their physicochemical properties (Nowack, 2007). Thus, in this case it is likely that contradictory results are simply related to the use of particles of different sizes. The size of nanoparticles affects their zeta potential. In this case, this facilitates the agglomeration of $nCeO_2$, which leads to low bioavailability and no phytotoxic effect on the treated seeds.

Surprisingly, Ce 100 on plants were lower than those of Ce 10. The same evidence was documented for this concentration Such an apparent paradox is very likely due to aggregation processes (denoted by the dynamic light scattering analysis) that occurred between Ce nanoparticles in water treated with a higher dose of $nCeO_2$. The presence of more aggregated Ce nanostructures leads to a smaller bioavailable $nCeO_2$. In our case, the final result is a non-linear dose-response relationship in which low and high doses of a given agent or stressor can act in opposite directions.

It is assumed that in the long-term effect of CeO_2 NPs at the maximum concentration, the earlier plant response in the form of large-scale necrotic DNA degradation took, which is known to manifest much earlier than internucleosomal (Koukalova, 1997; Tulpan, 2010). This is probably caused by the above-mentioned excessive production of AOS, incl. HO· and the accumulation of oxo-derived DNA molecules. At the same time, it is possible that redox imbalance is associated with the ability of highly dispersed metals to release a small fraction of Ce4 + ions and exert an additive effect on the destabilization of DNA. Thus, Lopez-Moreno shows the deposition of cerium ions from CeO_2 NPs in the cell walls and intercellular spaces of the root (López-Moreno, 2010).

High resistance of plants is shown by the

example of many metal oxides of CeO_2 (Giraldo, 2014).

Thus, the results of the biological effect of CeO_2 NPs demonstrate the absence of an acute toxic effect, since a lower effect was exhibited in the concentration of 1 mg/l of CeO_2 during the entire exposure time. The toxic effect of 10 mg/l of CeO_2 NPs increased up to 14 days of exposure and lasted up to 30 days. The maximum effect of CeO_2 NPs was established after 30 days of exposure at a concentration of 100 mg/l. At a concentration of 1 mg/l CeO_2 NPs, biomass of protozoan cells was comparable to the control during the entire period. At 10 mg/l CeO_2 NPs no changes in the biomass of *S. mytillus* cells at earlier time intervals (7 days of exposure) were registered. The concentration of 100 mg/l contributed to a gradual decrease in the biomass of protozoan cells (by 58%) on the 30th day of exposure.

Nanoparticles of CeO_2 at a concentration of 1 mg/l manifested no negative effect on protozoan cells within the experiment (7-30 days of exposure). While, the concentration of 10 mg/l influenced decrease in the number of test object on the 14th and 30th days of exposure by 30 and 43%, relative to the control sample. At the 30th day of exposure, the maximum cell death (43%) was registered, it was caused by a concentration of 100 mg/l CeO_2 NPs.

Thus, cyto- and DNA-damaging properties of CeO_2 NPs were assessed. The investigated substances in small concentrations (1 and 10 mg/l) showed no significant cytotoxic and DNA damaging effect on model organisms (duckweed) up to 30 days of exposure. However, metal had the ability to stimulate redox processes towards increase of pro-oxidants. Moreover, CeO_2 NPs at a maximum concentration of 100 mg/l after 30 days of exposure can cause oxidative stress up to a percent damage to DNA and a percent decrease in cell viability.

Thus, the accumulation of CeO_2 NPs in aquatic environment can have a negative effect on not only aquatic organisms but probably also other species. However, even if the influence of CeO_2 only on duckweed is registered, it may already affect other aquatic inhabitants through competitive interactions. The current study confirmed that a comprehensive study of the effect of CeO_2 NPs on early stages of aquatic organisms development is of practical value for risk assessment.

CONCLUSION

Thus, the use of nanometals is accompanied by universal stress reactions of *T. vulgare* sprouts, such as redox status shifts in the form of quantitative and qualitative changes in the content of enzymatic and non-enzymatic antioxidants as well as peroxidation products of membranes. It is possible to single out the specific action of nanoparticles of metal and their oxides in mechanisms of changing antioxidant content and physiological consequences of this change. Depending on the organ of plant and, accordingly, the composition and concentration of metal, action of different components of AOS is not the same. In roots, where the main amount of metal NPs absorbed by the sprouts is accumulated, detoxification of AOS goes mainly due to an increase of activity of enzyme antioxidants. In contrast to roots, the main role in preventing oxidative stress belongs to non-enzymatic low-molecular compounds, such as carotenoids and FS in the leaves of plants. It can be assumed that changes in the redox profile from the position of selective isolation of AOS in the cells of wheat roots after the addition of Cu°, CuO and Ni° *apriori* lead to changes in the number of normally functioning cells. It formed the basis for further revealing the mechanisms of development of toxic effects of these nanometals in the root system as the main "target" of their action.

CONFLICT OF INTEREST

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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AUTHOR CONTRIBUTIONS

KDB performed an experiment to assess the biological activity of the *E. coli* K12 TG1 pF1 cells and also wrote a manuscript. KAM developed and conducted an experiment to assess the biological activity of CeO_2 on *L. minor* and performed a data analysis. REA has identified survival and number (biomass) a culture of freshwater infusorian *S. mytilus*. All authors read and approved the final version.

REFERENCES

Andersen, C.P., King, G., Plocher, M., Storm, M., Pokhrel, L.R., Johnson, M.G., and Rygiewicz, P.T 2016. Germination and early plant development of ten plant species exposed to titanium dioxide and cerium oxide nanoparticles. Environ Toxico Chem. 35:222–2229.

Bhattacharya, K., Davoren, M., Boertz, J., and Dopp E. 2009. Titanium dioxide nanoparticles induce oxidative stress and DNA-adduct formation but not DNA-breakage in human lung cells. Part. Fibre. Toxicol. 6:10-17.

Blinova, I., Kanarbik, L., Irha, N., and Kahru, A. 2017. Ecotoxicity of nanosized magnetite to crustacean *Daphnia magna* and duckweed *Lemna minor*. Hydrobiologia. 798:141–149.

Bonini, M.G., Rota, C., Tomasi, A., and Mason, R.P. 2006. The oxidation of 2',7'-dichlorofluorescin to reactive oxygen species: a self-fulfilling prophesy? Free Radic Biol Med. 40(6):968-975.

Cassee, F. R., van Balen, E. C., Singh, C., Green, D., Muijser, H., Weinstein, J. and Dreher, K. 2011. Exposure, health and ecological effects review of engineered nanoscale cerium and cerium oxide associated with its use as a fuel additive. Crit. Rev. Toxicol. 41(3):213–229.

Castro-Concha, L.A., Tuyub-Che, J., Moo-Mukul, A., Vazquez-Flota, F.A., Miranda-Ham, M.L. 2014. Antioxidant capacity and total phenolic content in fruit tissues from accessions of Capsicum chinense Jacq. (Habanero pepper) at different stages of ripening. Sci. World J. 14:1-5.

Collin, B, Auffan, M., Johnson, A.C., Kaur, I., Keller, A.A., Lazareva, A., Lead, J.R., Ruth, C., Merrifield, R.C., Svendsen, C., White, J.C., Unrine, J.M. 2014. Environmental release, fate and ecotoxicological effects of manufactured ceria nanomaterials. Environ. Sci. Nano. 1: 533-540

Croy, J. R. 2007. Support dependence of MeOH decomposition over size-selected Pt nanoparticles. Catalysis Letters. 119:209–216.

Cui, D., Zhang, P., Ma, Y., He, X., and Li, Y. 2014. Effect of cerium oxide nanoparticles on asparagus lettuce cultured in an agar medium. Environmental Science: Nano. 1:459–465.

Das, M. 2007. Auto-catalytic ceria nanoparticles offer neuroprotection to adult rat spinal cord neurons. Biomaterials. 28:1918–1925.

Du, W.C., Gardea-Torresdey, J.L., Ji, R., Yin, Y., Zhu, J.G., Peralta-Videa, J.R., and Guo, H.Y. 2015. Physiological and biochemical changes imposed by CeO_2 nanoparticles on wheat: a life cycle field study. Environ Sci Technol. 49:11884–11893.

El-Temsah, Y. S. 2010. Impact of Fe and Ag nanoparticles on seed germination and differences in bioavailability during exposure in aqueous suspention and soil. Environmental Toxicity. 10:42-49.

Esterbauer, H., Schaur, R.J., and Zollner, H. 1991. Chemistry and biochemistry of 4-hydroxynonenal, malonaldehyde and related aldehydes. Free Radic Biol Med. 11(1):81-128.

Faisal, M., Saquib, Q., Alatar, A.A., Al-Khedhairy, A.A., Heqazy, A.K., and Musarrat, J. 2013. Phytotoxic hazards of NiO-nanoparticles in tomato: a study on mechanism of cell death. J Hazard Mater. 250: 318-332.

Giraldo, J. P. 2014. Plant nanobionics approach to augment photosynthesis and biochemical sensing. Nat. Mater. 89:10-17.

Godymchuk, A.Yu. 2012. Ecology of Nanomaterials: A Training Manual. Moscow, BINOM. Laboratory of Knowledge. pp 326-340.

Gomes, A., Fernandes, E., and Lima, J.L. 2005. Fluorescence probes used for detection of reactive oxygen species. J Biochem Biophys Methods. 65:45-80.

Gui, X., Zhang, Z., Liu, S., Ma, Y., Zhang, P., He, X., Li, Y., Zhang, J., Li, H., Rui, Y., Liu, L., and Cao, W. 2015. Fate and Phytotoxicity of CeO_2 Nanoparticles on Lettuce Cultured in the Potting Soil Environment. Plos One. 10(8):134-158.

Handy, R.D., Owen, R., Valsami-Jones, E. 2008. The ecotoxicology of nanoparticles and nanomaterials: current status, knowledge gaps, challenges, and future needs. Ecotoxicology. 17(5):315-25.

Heckert, E. G., Karakoti, A. S., Seal, S., and Self, W. T. 2008. The role of cerium redox state in the SOD mimetic activity of nanoceria. Biomaterials. 29:2705–2709.

Jackson, P., Jacobsen, R. N., Baun A., Deev, R., Kühnel, D., and Jensen, K.A., Vogel, U., and Wallin H. 2013. Bioaccumulation ecotoxicity and nanotubes. Chemistry Central AUD carbon. 7(1):150-154.

Judy, J.D., Unrine, J.M., Rao, W., Wirick, S., and Bertsch, P.M. 2012. Bioavailability of gold nanomaterials to plants: importance of particle size and surface coating. Environ Sci Technol. 46:8467–8474.

Koukalova, B. 1997. Chromatin fragmentation associated with apoptotic changes in tobacco cells exposed to cold stress. FEBS Lett. 414:289-292.

Kuznetsov, V. and Dmitrieva, G. (2006) Plant physiology. Vysshaya shkola, Moscow., pp 145-157.

Li, L., Sillanpaa, M., Tuominen, M., Lounatmaa, K., and Schultz, E. 2013. Behavior of titanium dioxide nanoparticles in *Lemna minor* growth test conditions. Ecotoxicol. Environ. Saf. 88: 89–94.

Limbach, L.K., Bereiter, R., Müller, E., Krebs, R., Gälli, R., and Stark, W.J. 2008. Removal of oxide nanoparticles in a model wastewater treatment plant: influence of agglomeration and surfactants on clearing efficiency. Environ. Sci. Technol. 42:5828–5833.

Limbach, L.K., Bereiter, R., Müller, E., Krebs, R., Gälli, R., and Stark, W.J. 2008. Removal of Oxide Nanoparticles in a Model Wastewater Treatment Plant: Influence of Agglomeration and Surfactants on Clearing Efficiency. Environmental Science & Technology. 42 (15):5828-5833.

López-Moreno, M.L., de La Rosar, G., Hernández-Viezcas, J.R., Peralta-Videa, J.R., and Gardea-Torresdey, J.L. 2010. X-ray absorption spectroscopy (XAS) corroboration of the uptake and storage of CeO_2 nanoparticles and assessment of their differential toxicity in four edible plant species. J. Agric. Food Chem. 58:3689-93.

Lushchaeva, I.V., Morgalev, S.N. 2009. Study of the effects of nanoparticles TiO_2 and Al_2O_3 on bacteria Pseudomonas fluorescens and Bacillus mucilaginosus. Bulletin of Tomsk state University. Biology. 4(8):97-105.

Ma, Y., Kuang, L., He, X., Bai, W., and Ding, Y. 2010. Effects of rare earth oxide nanoparticles on root elongation of plants. Chemosphere. 78:273–279.

Majumdar, S., Peralta-Videa, J. R., Bandyopadhyay, S., CastilloMichel, H., Hernandez-Viezcas, J.-A., Sahi, S. and GardeaTorresdey, J. L. 2014. Exposure of cerium oxide nanoparticles to kidney bean shows disturbance in the plant defense mechanisms. J. Hazard. Mater. 278: 279–287.

Manevich, Y., Sweitzer, T., Pak, J.H., Feinstein, S.I., Muzykantov, V., and Fisher, A.B. 2002. 1-Cys peroxiredoxin overexpression protects cells against phospholipid peroxidation-mediated membrane damage. Proc Natl Acad Sci USA. 99:11599–11604

Marslin, G., Sheeba, C.J., and Franklin, G. 2017. Nanoparticles Alter Secondary Metabolism in Plants via ROS. Burst Front Plant Sci. 8:820-832.

Miller, T.R., Wiek, A., Sarewitz, D., Robinson, J., Olsson, L., Krieble, D., and Loorbach, K. 2014. The future of sustainability science: a solution-oriented research agenda Sustain. Sci. 9:239-246.

Miralles, P., Church, T. L. and Harris, A. T. 2012. Toxicity, Uptake, and Translocation of Engineered Nanomaterials in Vascular plants. Environ. Sci. Technol. 46(17): 9224–9239.

Nowack, B., and Bucheli, T.D. 2007. Occurrence, behavior and effects of nanoparticles in the environment. Environ. Pollut. 150:5-22.

Nowack, B., Ranville, J. F., Diamond, S., Gallego-Urrea, J. A., Metcalfe, C., Rose, J., Horne, N., Koelmans, A. A., and Klaine, S. J. 2012. Potential scenarios for nanomaterial release and subsequent alteration in the environment. Environmental Toxicology and Chemistry. 31:50–59.

Papagiannis, L., Kagalou, L., Leonardos, J.,

Petridis, D., and Kalfakakou, V. 2004. Copper and zinc in four freshwater fish species from lake Pamvotis (Greece). Environ. Int. 30:357–362.

Rico, C. M., Majumdar, S., Duarte-Gardea, M., Peralta-Videa, J. R. and Gardea-Torresdey J. L. 2011. Interaction of Nanoparticles with Edible Plants and Their Possible Implications in the Food Chain. J. Agric. Food Chem. 59(8): 3485–3498.

Rico, C.M., Hong, J., Morales, M. I., Zhao, L., Barrios, A.C., Zhang, J-Y., Peralta-Videa, J.R., and Gardea-Torresdey, J.L. 2013. Effect of Cerium Oxide Nanoparticles on Rice: A Study Involving the Antioxidant Defense System and In Vivo Fluorescence Imaging. Environ. Sci. Technol. 47 (11):5635–5642.

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

Rico, C.M., Peralta-Videa, J.R., and Gardea-Torresdey, J.L. 2015. Differential Effects of Cerium Oxide Nanoparticles on Rice, Wheat, and Barley Roots: A Fourier Transform Infrared (FT-IR) Microspectroscopy Study. Applied Spectroscopy. 69:143-150.

Rodea-Palomares, I. 2011. Physicochemical characterization and ecotoxicological assessment of CeO2 nanoparticles using two aquatic microorganisms. Toxicological sciences: an official journal of the Society of Toxicology. 119:135–145.

Rodea-Palomares, I. 2012. An insight into the mechanisms of nanoceria toxicity in aquatic photosynthetic organisms. Aquatic toxicology. 122:133–143.

Rogers, N. J. 2010. Physico-chemical behaviour and algal toxicity of nanoparticulate CeO2 in freshwater. Environmental Chemistry. 7:50–60.

Safety Data Sheet of Cerium (IV) oxide ROTI®REMETIC 99.99% in accordance with Regulation (EC) No 1907/2006 (REACH), as amended by 453/2010 / EC. GOST 30333 2007.

Schwabe, F., Schulin, R., Limbach, L.K., Stark, W., and Bürge, D. 2013. Influence of two types of organic matter on interaction of CeO_2 nanoparticles with plants in hydroponic culture. Chemosphere. 91:512–520.

Sibgatullina, G. V., Khaertdinova, L. R., Gumerova, E. A., Akulov, A. N., Kostyukova, Y. A., Nikonorova, N. A., and Rumyantsev, N. 2011. Methods for determining the redox status of cultivated plant cells: training. Kazan, Kazan (Volga) Federal University. pp 15-32.

Singh, S., Dosani, T., Karakoti, A. S., Kumar, A., Seal, S. and Self, W. T. 2011. A phosphate-dependent shift in redox state of cerium oxide nanoparticles and its effects on catalytic properties. Biomaterials. 32(28): 6745–6753.

Tulpan, D. 2010. Free energy estimation of short DNA duplex hybridizations. BMC Bioinformatics. 11:99-105.

Üçüncü, E., Tunca, E., Fikirdesici, S., and Altındag˘, A. 2013. Decrease and increase profile of Cu, Cr and Pb during stable phase of removal by duckweed (*Lemna minor L.*). Int. J. Phytoremediat. 15:376–384.

Venzhik, Yu., Titov, A., and Talanova, V. 2017. Wheat plants react to the combined action of low temperature and cadmium. Proceedings of Karelian research centre of RAS. 12: 54-60.

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

Yoon, J., Chang, S.T., Park, J.S., Kim, Y.H., and Min, J. 2010. Functional characterization of starvation-induced lysosomal activity in Saccharomyces cerevisiae. Appl Microbiol Biotechnol. 88(1):283-289.

Zhang, P., Ma, Y., Zhang, Z., He, X., and Li, Y. 2013. Species-specific toxicity of ceria nanoparticles to Lactuca plants. Nanotoxicology. 12:1–8.

Zhao, L., Peng, B., Hernandez-Viezcas, J.A., Rico, C., Sun, Y., Peralta-Videa, J.R., Tang, X., Niu, G., Jin, L., Varela-Ramirez, A., Zhang, J.Y., and Gardea-Torresdey, J.L. 2012. Stress response and tolerance of *Zea mays* to CeO_2 nanoparticles: cross talk among H_2O_2, heat shock protein, and lipid peroxidation. ACS Nano. 6:9615–9622.

Zhao, L., Peralta-Videa, J.R., Ren, M., Varela-Ramirez, A., Li, C., Hernandez-Viezcas, J.A., Aguilera, R.J., Gardea-Torresdey, J.L. 2012. Transport of Zn in a sandy loam soil treated with ZnO NPs and uptake by corn plants: electron microprobe and confocal microscopy studies. Chem Eng J. 184:1–8.

Zhao, L.J., Sun, Y., Hernandez-Viezcas, J.A.,

Hong, J., Majumdar, S., Niu, G.H., Duarte-Gardea, M., Peralta-Videa, J.R., and Gardea-Torresdey. J.L. 2015. Monitoring the environmental effects of CeO_2 and ZnO nanoparticles through the life cycle of corn (Zea mays) plants and in situ μ-XRF mapping of nutrients in kernels. Environ Sci Technol. 49:2921–2928.

Zholobak, N.M., Shcherbakov, A.B., Bogorad-Kobelska, A.S., Ivanova, O.S., Baranchikov, A.Y., Spivak, N.Y., and Ivanov, V.K. 2014. Panthenolstabilized cerium dioxide nanoparticles for cosmeceutic formulations against ROS-induced and UV-induced damage. J Photochem Photobiol B. 130: 102-108.

Zhu, B., Xu, Z., Wang, C., and Gao, Y. 2016. Shape evolution of metal nanoparticles in water vapor environment. Nano letters. 16:2628-2632.