Biological risk assessment of the use of ultrafine particles of metals and their oxides to create new microelements preparations

E S Aleshina², E A Drozdova², A S Pavlinova², E A Sizova¹², K.S. Nechitaylo¹
¹ Federal Research Centre of Biological Systems and Agrotechnologies of the Russian Academy of Sciences, 29, Yanvarya 9 str., Orenburg, 460000, Russia,
² Orenburg State University, 13, Pobedy ave., Orenburg, 460018, Russia

E-mail: esaleshina@mail.ru

Annotation. The use of nanomaterials is now included in many areas of human activity. A promising area of research for ecology, biology, agriculture and medicine is currently the development in the field of nanotechnology related to the production and use of ultrafine particles of metals and their oxides. In particular, a very broad trend in their use is associated with the creation of new microelements based on them. In this regard, of interest are only those forms of ultrafine particles, which do not have their own toxic effect, but have a more pronounced positive effect compared with substances in macrodispers form. That is why it becomes relevant to study the safety issues of ultrafine particles of metals-trace elements and assess the possibility of biological risks associated with these applications, for example, as food additives. In this work, the hazard / safety of ultrafine particles of metals and their oxides on the recombinant Escherichia coli K12 TG1 strain with cloned luxCDABE Vibrio fischeri genes was investigated using bioluminescent analysis and the forms of ultrafine particles and their concentrations suitable for further use in agriculture and medicine were determined.

1. Introduction
For the normal development and functioning of any biological systems, including the human or agricultural animals, the presence of mineral substances is important [1-6]. Currently, in addition to the organic forms of sources of microelements, ultradispersed metal particles are characterized by a certain interest, which can be considered as an alternative to substances in the macrodispers phase [7].

The ultrafine particles are characterized by a high level of biological activity, other physicochemical parameters and toxic properties when compared with particulates of the same elements [8, 9]. That is why it is very important to establish the possible biological risks of using ultrafine particles and to determine the concentrations at which they can be used in the agro-industrial complex, medicine, and other branches of science and technology.

Even though the use of ultrafine particles is rapidly expanding, there is a need for more complete coverage of the positive and negative aspects of this issue [10]. Today, there is evidence that nanomaterials can be toxic to animals and humans, especially for ultrafine particles, which are trace elements of living organisms [11-14].
There is also evidence that ultrafine particles can have a protective effect on living organisms, increasing the body's resistance to various toxicants [15]. Data on the use of ultrafine particles of trace metals in agriculture, particularly in crop production, suggests that they have bactericidal and fungicidal effects, and can also be used as elements of the mineral nutrition of long-lasting plants, which as a result increases their adaptive potential. [sixteen]. However, at certain concentrations in the environment, they are capable of provoking oxidative reactions in plant cells that can cause severe damage [17]. For the purpose of environmental protection, nanochips and biosensors based on ultrafine metal particles [18] have been developed. Also, ultrafine particles are used in the food industry with the purpose of increasing the shelf life and production of food with a given chemical composition and other indicators [19]. Wide prospects for the use of ultrafine particles cops there are in medicine. T...

| ultrafine particles | Diameter, nm | S.gr., m²/g | Z-potential, mV | Particle shape | Sample purity, % | Colour        |
|---------------------|-------------|-------------|-----------------|----------------|------------------|--------------|
| Zn                  | 98.0 ± 2.1  | 5.3         | 25±0.5          | spherical      | 99.9             | dark grey    |
| ZnO                 | 95.0 ± 2.0  | 9.0         | 25.4 ± 3.66     | spherical      | 96.0             | white        |
| Cu                  | 99.0 ± 2.0  | 24.0        | 31±0.1          | spherical      | 96.0             | dark brown   |
| CuO                 | 50.0 ± 2.0  | 14.0        | 47±0.1          | spherical      | 99.9             | dark brown   |
| Ti                  | 80.0 ± 1.8  | 13.8        | -33.2 ± 0.96    | spherical      | 99.8             | Gray         |
| TiO₂                | 90.0± 0.9   | 90.0        | -18.50 ± 0.50   | spherical      | 99.8             | white        |
| Al                  | 90.0 ± 2.0  | 35.0        | 34.00 ± 0.65    | spherical      | 94.0             | Gray         |
| Al₂O₃               | 54.0± 0.2   | 40.0        | 30.00 ± 0.10    | spherical      | 97.0             | white        |
| Mo                  | 50.0± 1.5   | 14.0        | -43.00 ± 0.52   | cubic          | 99.7             | dark grey    |
| MoO₃                | 92.0 ± 2.0  | 12.0        | -42.00 ± 0.52   | spherical      | 99.7             | light gray   |
| Ni                  | 70.0 ± 0.3  | 6.0         | 25.00 ± 0.50    | spherical      | 99.8             | dark grey    |
| NiO                 | 94.0 ± 0.7  | 12          | 29.00 ± 0.50    | spherical      | 99.6             | light green  |
The impact of ultrafine particles on Escherichia coli K12 TG1 with cloned luxCDABE Vibrio fischeri genes was assessed using bioluminescence analysis [26].

The luminescing strain Escherichia coli K12 TG1, constitutively expressing the luxCDABE genes of the natural marine microorganism Vibrio fischeri, was used as an object of study. The bacterial strain used is produced by NVO Immunotech (Russia) in a lyophilized state under the commercial name Ecolum and is a lyophilized culture of luminescent bacteria or preparations of bacterial luciferase contained in inert gases in glass bottles. The lyophilized form of microorganisms was diluted with distilled water to obtain a bacterial suspension.

An 8 × 12 microplate for Infinite PROF200 microplate analyzer (Tecan Group Ltd., Switzerland) was used for bioluminescence. 200 μl of a suspension of ultrafine particles were introduced into the cells of the plate and a series of twofold dilutions were performed in subsequent cells of the row, adding 100 μl of distilled water each. After introducing the ultrafine particles into the microplate, 100 μl of the bacterial biosensor was added to each cell. The control sample contained 100 μl of distilled water and 100 μl of the bacterial suspension. The plates were placed in a measuring unit of a microplate analyzer, recording the luminescence intensity for 180 minutes with an interval of 5 minutes. Based on the data obtained, we calculated the toxicological parameters of the EU20 and EC50, corresponding to the concentrations of ultrafine particles, causing 20% or 50% inhibition of the biosensor luminescence compared to the control. The results of the influence of nanomaterials on the intensity of bacterial bioluminescence were evaluated by the change in the luminosity of the control and experimental samples at the zero and n-th second of the experiment.

The main data obtained in the studies were performed in triplicate and processed using Excel and Statistica V8 (StatSoft Inc., USA).

3. Results and Discussion

In the study of biotoxicity, the maximum possible duration of the study was 180 minutes, after which the luminescence of the used bacterial biosensor in control samples was observed to extinguish its own, due to the lack of energy substrates for the bioluminescence reaction. The biological effect of ultrafine particles in relation to the used sensory microorganisms had a gradual and time-evolving dynamics, which determined the kinetic measurement of bioluminescence during the entire specified period.

The study of the toxic effect of ultrafine particles of titanium and its oxide (Figure 1a) showed the absence of their toxic effect in the whole range of concentrations studied. The change in quenching of the glow occurred within 20% of the control values, which allows us to characterize these samples as non-toxic with respect to living target cells. However, ultrafine particles of titanium oxide still caused a slightly greater decrease in the intensity of the luminescence of the sensory strain as compared with its metallic form. The obtained data confirm the possibility of its use as inert additives in the pharmaceutical and food industry [27]. However, there is evidence of the negative effect of high concentrations of ultrafine particles of titanium dioxide on some indicators of plants [28]. All this makes it possible to further use of these ultrafine particles in agriculture and other areas of science with a refinement of the concentrations used.
Figure 1. The emission dynamics of E. coli K12 TG1 when exposed to ultrafine particles of Ti and TiO\textsubscript{2} (a), Al and Al\textsubscript{2}O\textsubscript{3} (b), Cu and CuO (c), Ni and NiO (d), Mo and MoO\textsubscript{3} (e), Zn and ZnO (f) at the 180th minute of the experiment: 1 - ultrafine particles of metals, 2 - their oxides

Among the ultrafine particles of aluminum and its oxide (Figure 1b), ultrafine particles of aluminum were also less toxic. In this case, both forms of ultrafine particles led to the quenching of luminescence by more than 20% of the control values, which made it possible to calculate EC\textsubscript{20} values, which amounted to 0.25 and 0.016 M, respectively, for ultrafine particles of aluminum and its oxide. Our data on the weak toxicity of ultrafine particles of aluminum and its oxide are confirmed by some authors [29], who point to the fact that nanoscale powders of iron and aluminum oxides are extensively and
intensively investigated from the point of view of application in medicine and biotechnology. In particular, the alumina nano-powder can be used as a contrast agent in ultrasound diagnosis [30].

The use of ultrafine copper oxide particles (Figure 1c) had a less pronounced toxic effect on the test object compared to the sample of ultrafine copper particles. Thus, the bioluminescence decreases by 20% and then by 50% from the control values was observed already at concentrations of ultrafine copper oxide particles of 0.0625 and 0.25 M, respectively. However, when exposed to ultrafine copper particles, intensive extinguishing of bioluminescence could be observed already at the 120th minute of the experiment, and at the 180th minute the EC50 values were already 0.0625 M. According to the results, these samples became possible to be toxic to living cells, which allows them to be used to create antibacterial and wound healing agents [31]. It should be especially careful when they are introduced into the body to regulate the microelement composition [32, 33].

In the study of the biotoxicity of ultrafine nickel particles and its oxide (Figure 1d) using the bacterial strain Escherichia coli K12 TG1, it was found that both materials have a toxic effect, manifested in an intense decrease in the luminescence of the studied samples, which allowed us to determine the EC20 and EC50 parameters equal to 0.031 and 0.13 M for ultrafine nickel particles and 0.016 and 0.13 M for its oxide, respectively. As the mechanisms of the antibacterial effect of nickel particles, it should be noted that it can penetrate inside the cell and cause damage to the genetic material by direct interaction with DNA, block the functions of aquaporins and thereby cause cell death. In contrast, the genotoxic effect of ultrafine nickel oxide particles is probably realized due to the electrostatic interaction of positively charged nickel ions Ni2+ released from their particle matrix and the phosphate groups of the polyanion DNA [34]. Everything is of interest for use in medicine as antibacterial drugs and substances [35], restoring sensitivity to antibiotics of certain clinical strains of microorganisms [36].

Ultradisperse particles of molybdenum and its oxide had an even more pronounced toxic effect (Figure 1e). The toxicological parameters EC20 and EC50 calculated for them were 0.008 and 0.016 M for molybdenum and 0.0037 and 0.0039 for its oxide, respectively. The results obtained were, overall, quite expected due to the fact that an excess of this element in a macro dispersive form leads to the development of asthma [37], alveolar and bronchial adenomas and carcinomas [38]. At the same time, there are studies on the positive effect of ultrafine molybdenum particles on the growth and development of chickpea plants [39]. However, information on the effects of the interaction of molybdenum-containing nanomaterials has not yet been fully studied, including in more complex models [40, 41].

Ultrafine particles of zinc and its oxide had a practically identical toxic effect (Figure 1f), causing a complete suppression of the constitutive bioluminescence of the test object, starting with minimal concentrations. On this basis, both samples of ultrafine particles can be characterized as highly toxic, which will allow them to be used as antibacterial agents [42, 43]. However, because zinc belongs to essential trace elements, it is necessary to investigate in more detail the possibility of its use as food additives.

In the course of the research it was found that among this series of ultrafine particles (Ti and TiO2, Mo and MoO3, Al and Al2O3, Ni and NiO), predominantly metal oxides have the greatest inhibitory effect on the luminescence of microorganisms than their metals. Perhaps this is because the proposed mechanism of ultrafine particles of redox stress develops more rapidly when exposed to oxygen. The oxygen radical can have a detrimental effect on the life processes occurring in the cell, which, presumably, occurred with a decrease in the bioluminescent feature of the recombinant strain [44]. In general, the toxicity of ultrafine particles depends on several factors, among which the most important are particle size, crystal structure, surface properties, and chemical structure. In this case, the toxicity of water-soluble ultrafine metal particles is associated primarily with high redox potential of ions and oxidation of biological molecules. The most important factor is the ability of ultrafine particles to induce oxidative stress, that is, generate ROS [45, 46]. The established dynamics of the manifestation of the effects of toxicological effects on luminous bacteria with a corresponding change in the activity of their bioluminescent system is individual for each concentration of ultrafine particles of metal and oxide.
4. Conclusion

Among all the ultradispersed particles of metals and their oxides studied, ultradisperse particles of zinc and its oxide turned out to be the most toxic. The severity of the biological effect of these ultrafine particles increased in the following toxicity series: Ti < Al < Cu < Ni < Mo < Zn and TiO2 < Al2O3 < CuO < NiO < MoO3 < ZnO. Thus, in terms of the severity of the biological effect, regardless of the form of ultrafine particles, the cation toxicity series turned out to be the same.

The study showed that the effect of ultrafine particles and their oxides on Escherichia coli K12 TG1 in various concentrations is accompanied by ambiguous biological effects. Based on the data obtained, it was established that ultrafine particles of Ti and TiO2, Al and Al2O3 did not have a pronounced negative effect on the bioluminescence of the studied strain in all the range of concentrations studied. However, due to the weak manifestation of toxicity of ultrafine particles of Al and Al2O3, the most optimal for these purposes are concentrations in the range from 4 × 10−5 to 0.25 M for aluminum, and from 4 × 10−5 to 7 × 10−3 M for its oxide. Ultrafine particles of Cu and CuO, Ni and NiO, Mo and MoO3, Zn and ZnO, on the contrary, demonstrate a pronounced toxic effect. In this regard, their use will find wide application as a basis for products with bacteriostatic or bactericidal effects. Thus, ultrafine particles of zinc and its oxide are toxic in the whole range of concentrations (from 4 × 10−5 to 0.5 M), and ultrafine particles of copper and its oxide from 0.125 to 0.5 M; Ultrafine particles of nickel and its oxide - in concentrations from 0.0625 to 0.5 M. Minimum concentrations of ultrafine particles of molybdenum and its oxide (7 × 10−3 and 3 × 10−3 M) can be used in the agricultural sector and other fields.

Given the promising use of microelements, such as ultrafine particles, in agriculture and other areas of science, our data can be further used to develop appropriate solutions to minimize their negative impact on living organisms and the environment.

Acknowledgements

The studies were performed in accordance with the plan of research works Federal Research Centre of Biological Systems and Agrotechnologies of the Russian Academy of Sciences No. 0761-2019-0005.

References

[1] Fisinin V I, Miroshnikov S A, Sizova E A, Ushakov A S and Miroshnikova E P 2018 Poultry Sci. J. 74(3) 523–40
[2] Sizova E, Miroshnikov S, Heusa Ya E and Polyakova V 2015, Health Responsibility and Health Care Center for BioMed Research International 243 173
[3] Sizova E, Yausheva E, Miroshnikov S, Lebedev S and Duskaev G 2015 Element Status in Rats at Intramuscular Injection of Iron. Nanoparticles Biosciences Biotechnology Res. Asia 12 119–27
[4] Sizova E, Miroshnikov S, Lebedev S, Kudasheva A and Ryabov 2016 For example Agricultural Biology 51(4) 553–62
[5] Levy L, Sahoo Y, Kim K, Bergey E J and Prasad P N 2012 Nanochemistry: Synthesis and Characterization of Multifunctional Nanoclincs for Biological Applications 14(9) 3715–21
[6] Yausheva E, Sizova E, Gavrish I, Lebedev S, Kayumov F 2017 Effect of Al2O3 nanoparticles on soil
[7] Miroshnikov S, Yausheva E, Sizova E and Miroshnikova E 2015 Comparative assessment of the effect of copper nano and microparticles in chicken. Oriental J. of Chemistry 31(4) 2327–36
[8] Sizova E A, Korolev V V, Makaev Sh A, Miroshnikova E P and Shakhov V A 2016 Agricultural Biology 51(6) 903–11
[9] Rakhmanin Yu A, Khrypach L V, Mikhailova R I and Koganova Z I 2014 Comparative analysis of the influence of nano- and ionic forms of silver on the biochemical indicators of laboratory animals Hygiene and Sanitation 145–50
[10] Rusakova E, Kosyan D, Sizova E and Miroshnikov S 2015 Comparative Evaluation of Zinc, copper and their nanosystems using Stylonychia mytilus Oriental J. of Chem. 31 105–12
[11] Handy R D, Owen R and Valsami-Jones E 2008 The ecotoxicology of nanoparticles and nanomaterials: current status, knowledge gaps, challenges, and future needs *Ecotoxicology* **17**(5) 315–25
[12] Lystsov V N and Murzin N V 2007 *Problems of nanotechnology security* (Moscow: MEPI)
[13] Buzea C, Blandino I P and Robbie K 2007 Nanomaterials and nanoparticles: Sources and toxicity *Biointerphases* **2**(4) 17–31
[14] Schrand A M 2010 Metal-based nanoparticles and their toxicity assessment *Wiley Interdisciplinary Reviews: Nanomedicine and Nanobiotechnology* **2**(5) 544–68.
[15] Korotkova A, Sizova E, Lebedev S, Kosyan D and Rusakova E 2015 Influence of Triticum vulgarius on ecology *Environment and Conservation* **21** 101–11
[16] Lebedev S V Gavrish I A and Gubaydullina I Z 2019 Different chrome sources influence on morphobiochemical indicators and activity of digestive enzymes in wistar rats *Agricultural Biology* **54**(2) 304–15 doi: 10.15389/agrobiology.2019.2.304Eng.
[17] Dykman L A and Shchegolev S Yu 2017 Interaction of plants with nanoparticles of noble metals *Agricultural biology* **1** 13–24
[18] Lebedev S V, Gavrish I A, Galaktionova L V, Korotkova A M and Sizova E A 2018 *Geochemistry and Health* doi: 10.1007 / s10653-018-0171-3
[19] Nanotechnology in agri-food production: an overview of Nanotechnology 2014 *Science Applications* 7 31–53
[20] Pankhurst Q A, Connolly J, Jones S K ans Dobson J 2013 Applications of magnetic nanoparticles in biomedicine *J. of Physics D: Applied Physics* **13** 87
[21] Chatterjee D K, Diagaradjane P and Krishnan S 2011 Nanoparticle-mediated hyperthermia in cancer therapy *TherDeliv* **2**(8) 1001–14
[22] Yaushcheva E V, Miroshnikov SA, Kosyan DB and Sizova E A 2016 *Nanoparticles in combination with chicken and spirits extracts* (Iler) **51**(6) 912–20
[23] Rein M J, Renouf M, Cruz-Hernandez and Cacits-Goreta L 2013 Bioavailability of bioactive food compounds: a challenging journey to bioefficacy *Br. J. ClinPharmacol.* **75**(3) 588–602
[24] Mishra B, Patel B B and Tiwari S 2010 Colloidal Nanocarriers *Nanomed-Nanotechnol*. 69–24
[25] Raspopov R V, Trushina E N, Gmoshinskiil V and Khotimchenko S A 2011 Bioavailability of nanoparticles of ferric oxide when used in nutrition. Experimental results in rats *Vopr. Pitan.* **80**(3) 25–30
[26] Duskaev G.K., Deryabin D.G., Karimov I., Kosyan D.B., Notova S.V. 2018 Assessment of (in vitro) Toxicity of Quorum Sensing Inhibitor Molecules of Quercus cortex *Journal of Pharmaceutical Sciences and Research* 10(1) 91-95.
[27] Kuburovic N D, Golubovic A V and Babincev L M 2018, Development of new smart metal nanomaterials based on titanium dioxide for volatile and antimicrobial activities *Vojnoteh. glas.* 4 771–835
[28] Varduni T V, Sereda M M and Kapralova O A 2017 The influence of titanium dioxide nanoparticles on the growth and development of tomato (lycopersic conesculentum) in in vitro culture *Modern problems of science and education* 6 26–33
[29] Mikhailova E A, Mironov A Yu, Fomina M V and Kirgizova S V 2017 Escherichia coli’s ability to form biofilms in the presence of aluminum oxide nanoparticles *Clinical laboratory diagnostics* 6 381–4
[30] Sheyda E., Sipaylova O., Kvan O., Notova S., Nesterov D., Rusakova E., Kosyan D., Duskaev G. 2014 Functional properties of antimicrobial peptides extracted from hens’ platelets. *Life Science Journal* **11**(9),25 180-184
[31] Rakhmetova A A, Bogoslovskaya O A and Olkhovskaya I P 2012 Wound-healing agents of a new generation based on copper nanoparticles *Herald Health and Education in the 21st Century* **11** 327–8
[32] Bogoslovskaya O A 2006 The influence of copper and iron nanoparticles on the growth of microbial cells Scientific-practical conference “New technological platform for biomedical research” (Rostov-on-Don) pp 72–3

[33] Arsentieva P 2007 Certification and use of metal nanoparticles as biologically active drugs Nanotechnology Specialist. the issue of “Nanotechnology-medicine” 2(10) 72–7

[34] Korotkova A, Lebedev S and Gavrish I 2017 Nanoparticle CuO nanoparticle CuO in order to test the seed roots of the Triticum vulgare Environ Sci Pollut Res 24(11) 10220–33

[35] Mamonova I A and Babushkina I B 2012 Antibacterial activity of nickel nanoparticles Infection and immunity 1–2 225

[36] Mamonova I A, Babushkina I V, Gladkova E V 2014 The effect of metal nanoparticles on the antibiotic sensitivity of clinical strains of Escherichia coli PSU Bulletin Biology 4 104–8

[37] Huang X, Xie J, Cui X, Zhou Y, Wu X, Lu W, Shen Y, Yuan J and Chen W 2016 A case-control study in Wuhan, China. PLoS ONE, 11 5

[38] Chan P C, Herbert RA, Roycroft J H and Haseman J K 1998 Lung tumor induction by inhalation exposure to molybdenum trioxide in rats and mice Toxicological Sciences 1 58–65

[39] Gonchar E N, Shcherbakov A V, Lopatko K G, Gonchar L N 2013 Increasing the efficiency of microbial plant symbiosis by creating composite biopreparations using nanoparticles of biogenic metals Achievements of Sci. and Technol. APC 12 30–4

[40] Lebedev S, Yausheva E, Galaktionova L, Sizova E 2016 Impact of molybdenum nanoparticles on survival, activity of enzymes, and chemical elements in Eisenia fetida using test on artificial substrata. Environmental Science and Pollution Research. 23(18) 18099-18110

[41] Kosyan D, Rusakova E, Miroshnikov S, Sizova E 2016 The toxic effect of the International Journal of Geometry 23(11) 2170–6.

[42] Babushkina I V, Gladkova Ye V, Mamonova I A, Belova S V and Karyakina E V 2012 Regulation of an experimental wound under the influence of zinc nanoparticles Herald of new medical technol. 416–18

[43] Babushkina I V, Gladkova Ye V, Mamonova I A and Belova S V 2017 Comparative study of complex powder preparations for the regeneration of soft tissues Saratov J. of Med. Sci. Res. 3 705–10

[44] Suvorov O A, Volozhaninova S Y, Balandin G V and Frolova Y V 2017 Antibacterial effect of colloidal solutions on microorganisms of cereal crops Foods and Raw materials 1 100–7

[45] Sitnikova O G, Nazarov S B and Dyzhev Zh A 2013 The study of the influence of various types of nano-sized silicon dioxide on the development of oxidative stress and antioxidant activity in vitro Bull. of new medical technol. 3 17–20

[46] Sizova E, Miroshnikov S, Yausheva E and Kosyan D 2015 Comparative using the bacterial bioluminescence Biosci. Biotechnol. Res. Asia 12 361–8