DOI 10.31489/2022BMG1/96-101

UDC 620.3:634:615.9

A.T. Serikbai[1]*, A.M. Aitkulov[1], A.K. Zeynidenov[1], Z.T. Kystaubaeva[2]

[1]*Karagandy University of the name of academician E.A. Buketov, Karaganda, Kazakhstan;*
[2]*Kazakh University of Technology and Business, Nur-Sultan, Kazakhstan*
**Corresponding author: arailym_serikbai@mail.ru*

Evaluation of macroparticles and nanoparticles of zinc and zinc oxide's toxicity based on the *Artemia salina* model

This article is devoted to the study of the effect of macroparticles and nanoparticles of zinc and zinc oxide on animals. The toxicity of pollutants to the fauna of aquatic ecosystems was assessed in course of a laboratory experiment. The test objects, the larvae of crustaceans of *Artemia salina,* were exposed to the particles of zinc (Zn) and zinc oxide (ZnO). The particle content in the water was 5 mg, 10 mg, 20 mg per 100 ml. The obtained results of the research indicate that the zinc and zinc oxide nanoparticles in the studied concentrations show a slight degree of toxicity during the 48-hour experiment period. The macroparticles of these substances have higher toxic properties for eggs and larvae of *Artemia salina*.

Keywords: macroparticles, nanoparticles, zinc, zinc oxide, *Artemia salina*, aquatic ecosystems.

Introduction

In recent years, intensive work has been done worldwide to obtain and apply nanoscale materials in various branches of science, industry, and agriculture. Numerous works indicate that nanoparticles also exhibit a biological effect in addition to the required physical and chemical properties. This effect can be both positive and negative for the biological system. In this regard, the problem of assessing the impact of nanoparticles on the environment is of vital importance.

The results of the studies related to the possible effects of nanoparticles on the body of animals and the mechanism of their biological action do not have an unambiguous interpretation and often demonstrate contradictory results. The importance of specialized studies of the biological properties of nanoparticles and materials is explained by the fact that the physical, chemical, and biological properties of most substances change during the transition from macro- to the nanoscale, and the nanomaterials can cause a completely different, sometimes opposite effect when contacted and penetrated biological structures. In addition, nanomaterials are characterized by high bioavailability: they easily penetrate through any biological barriers and are impermeable to particles of microscopic size. The studies conducted using various types of biological systems (cells, bacteria, and living organisms) show that nanoparticles can have an eco-toxicological effect. These features are important for assessing and predicting the biological and environmental safety of nanoscale materials [1].

The nanoparticles of biophilic elements, namely zinc, are of particular interest. L.V. Zhong in his study [2] noted that nanoparticles of ZnO have a large number of nanostructured variants, which might explain the fact that the influence of these nanoparticles on animal organisms causes both positive and negative effects.

The positive effect of zinc nanoparticles and their compounds on various organisms was especially widely published in biomedical studies. In many works, it has been proved that the use of pure suspensions of zinc nanoparticles, as well as in combination with chitosan, activates the processes of regeneration of mammalian skin wounds [3, 4]. Another research of the above-mentioned authors demonstrated the antibacterial effect of zinc nanoparticles on the strains of staphylococcus. The positive effect of ZnO nanoparticles is found not only in mammals but also in birds, which is associated with an improvement in the physiological parameters of the intestinal barrier and biochemical characteristics of antioxidant protection [3–5].

However, these substances can have a toxic effect in higher doses. There are numerous studies describing the negative effect of zinc nanoparticles on various organisms. For example, in the works of I.I. Tomilin, nano- and micro-particles and zinc ions have a toxic effect on freshwater hydrobionts of different trophic levels: ceriodaphnia branched crustaceans (*Ceriodaphnia affinis*), larvae of dipteran chironomids (*Chironomus riparius*) and aquarium fish (*Brachydanio rerio*). Zhao et al. (2016) and Chun et al. (2017) observed some negative effects of nanoparticles and demonstrate that as the concentration of nanoparticles in water bodies increases, the rate of development of embryos and the hatching rates of Danio fish decreases [6, 7]. Choi et al. (2016) showed that the cause of this may be a violation of the gene expression of the Danio fish embryos. It is also shown that, unlike $ZnSO_4$, ZnO nanoparticles caused inflammatory processes of the pericardium, tail and yolk sac and disruption of the immune system. Other species of fish, in particular carps, also respond by reducing innate and adaptive immunity to feeding ZnO nanoparticles [8–10].

Zinc nanoparticles have the properties of high bioavailability for animal organisms, which is demonstrated in the work of R.V. Raspopov [11], where the distribution of zinc nanoparticles by organs and tissues was revealed by the method of radioactive tracers.

In addition, nanoparticles, in particular, zinc oxide ones, are steadily accumulated in the body of animals. It is shown that the presence of ZnO nanoparticles in water resulted in accumulation in the gills and intestines of fish (a rain trout) even after cleaning the medium, which caused oxidative stress in the gills and disruption of liver antioxidant protection mechanisms even in case of quick removal of the pollutant from that medium. Apart from the liver and gills, when nanoparticles are found in water, their accumulation is established in other organs of animals. Zn was observed to be accumulated in the brain, liver and muscles, which led to disruption of normal metabolic functioning of the liver. Some authors mentioned that the ZnO nanoparticles remain stable at the high pH values, but when the pH drops, Zn ions began to be generated. In the study of Du et al. (2016) dedicated to the study of the response of Danio embryos to the exposure of nanoparticles, more damage was recorded during the growth and development of larvae, in which both chemicals were present compared to the system exposed to two separate agents [12, 13].

Similar results were obtained in the experiments conducted with other species of fish. The effects of different concentrations, small (10–30 nm) and large (100 nm) ZnO nanoparticles, on tilapia as (*Oreochromis niloticus*) caused a significant accumulation of both small and large ZnO nanoparticles in the tissues of the liver, gills, intestines, kidneys, brain and muscles, which led to a violation of oxidative metabolism. The fact that, under the same exposure conditions, the accumulation of small ZnO nanoparticles was significantly higher compared to the larger nanoparticles of this substance is of particular importance [14].

The problem of the multidirectional manifestation of zinc oxide nanoparticles' impact was well-described in the works of D.R. Vishnu et al. [15]. In the comparative experiments on rodents and fish, the importance of studying the toxicity of nanoparticles of this compound is proved with similar conclusions reached by Chen et al. [16]. These studies emphasized the need to study the toxic effects of ZnO nanoparticles on terrestrial and aquatic animals. This results in the need to reduce the number of nanoparticles in animal diets and to revise the concentrations of these pollutants in natural environments, especially in the presence of over one pollutant, which can lead to the manifestation of synergetic toxicity. The possibility of synergy is associated with the high reactivity of nanoparticles and their ability to easily form complexes with other substances.

It was noted that in Kazakhstan there were several publications devoted to the disclosure of the physical and chemical properties of nanoparticles, but the study of their biological effect and toxicity for living organisms has not previously been done.

Thus, the study of the effects of metal nanoparticles on biological objects, including hydrobionts, remained an interesting and practically significant task. The effect of concentrations of macro- and nanoparticles of zinc and zinc oxide on the *Artemia salina* nauplii were studied in this research in an aqueous saline solution.

Experimental

The suspensions of zinc and zinc oxide particles of different concentrations (5, 10, 20 mg per 100 ml of water) were used in the experiment. The particle size was 80–100 nm (nanoparticles) and 500–1000 nm (macroparticles). As the test objects, the *Artemia salina* crustacean, lived in salty reservoirs, were used in the experiment. The dry eggs of *Artemia salina* (12 g of eggs per 500 cm^3 of water) were poured with settled tap water at the required concentration of NaCl. Under favorable conditions, *Artemia salina* nauplii hatched from eggs through 1–2 days. For the experiment, the hatched nauplii, 4 individuals each, were placed in 30 ml glass cups in a saline solution with different concentrations of zinc or zinc oxide macroparticles and nanoparticles. The experiment was conducted with 5-fold repetition with 20 individuals in each experimental and control group [17]. The duration of exposure to the pollutants was 48 hours long. After 48 hours, the alive and dead individuals were counted and compared against the indicators of the control group.

Results and Discussion

The study of the effect of macroparticles on the hatching of crustaceans indicates that the presence of zinc and zinc oxide macroparticles in the aquatic medium leads to a decrease in the percentage of hatched *Artemia salina* eggs.

The content of Zn macroparticles at the concentration of 20 mg /100 ml led to a maximum reduction in hatches by 55 % compared to the control group (Tab. 1).

Table 1

Percentage of *Artemia salina* nauplii hatched after exposure to zinc macroparticles

Sample	Control	5 mg/100 ml	10 mg/100 ml	20 mg/100 ml
1	4	2	2	
2	4	3	3	1
3	4	1	1	3
4	4	3	3	3
5	4	2	2	2
Total	20	11	11	9
A, %	100	55	55	45

The presence of ZnO macroparticles in the medium also led to a decrease in the hatched crustaceans, but to a lesser extent compared to the zinc macroparticles (Tab. 2). The maximum decrease in hatching percentage was observed at the concentrations of 5 and 20 mg per 100 ml. In other experimental groups exposed to the smaller and higher concentrations of the substance, a significant decrease of larval density by 45 % was also observed.

Table 2

Percentage of *Artemia salina* larvae hatched after exposure to zinc oxide macroparticles

Sample	Control	5 mg /100 ml	10 mg /100 ml	20 mg /100 ml
1	4	3	2	2
2	4	3	2	3
3	4	2	2	2
4	4	1	5	2
5	4	2	3	2
Total	20	11	14	11
A, %	100	55	70	55

Nanoparticles of zinc in most of the studied concentrations illustrated the same survival rate of crustacean larvae for both control and experimental groups with no toxic properties (Tab. 3). The exception was the maximum concentration of zinc nanoparticles in the medium of 20 mg per 100 ml, which caused a decrease in egg pecking by 10 %.

Table 3

Percentage of *Artemia salina* larvae hatched when exposed to zinc nanoparticles

Sample	Control	5 mg /100 ml	10 mg /100 ml	20 mg /100 ml
1	4	4	4	4
2	4	4	4	4
3	4	4	4	2
4	4	4	4	4
5	4	4	4	4
Total	20	20	20	18
A, %	100	100	100	90

After studying the effects of zinc oxide nanoparticles, it was noted that only the lowest concentration of these particles (5 mg/100 ml) demonstrated some toxic effect. The higher concentrations of the substance caused the same hatching of larvae showing absent of toxicity (Tab. 4).

Table 4

Percentage of *Artemia salina* larvae hatched when exposed to zinc oxide nanoparticles

Sample	Control	5 mg /100 ml	10 mg /100 ml	20 mg /100 ml
1	4	2	4	4
2	4	4	4	4
3	4	4	4	4
4	4	4	4	4
5	4	4	4	4
Total	20	18	20	20
A, %	100	90	100	100

Based on the study, it can be stated that zinc and zinc oxide nanoparticles in the studied concentrations demonstrated a slight degree of toxicity during the experiment period of 48 hours. The macroparticles of these substances have higher toxic properties for eggs and larvae of *Artemia salina*.

Conclusions

The effect of metal particles on the representatives of the aquatic fauna of the invertebrate *Artemia salina* depends not only on the chemical composition of the agents, their concentration in the medium but also on the size of the active particles. Macro- and nanoparticles of zinc and zinc oxide show a different level of toxicity. A large toxic effect on *Artemia salina* eggs is posed by the zinc macroparticles. A smaller but significant level of toxic reaction on the crustacean eggs was caused by zinc oxide macroparticles. Nanoparticles of zinc and zinc oxide exhibit negligible toxicity.

Based on the study, it is assumed that the effect of these toxicants may vary depending on the duration of the experiment and the stage of development of the crustacean. In this regard, it is planned to continue research in order to obtain the data of how both macro-and nanoparticles of zinc and its oxide may affect the later stages of development of *Artemia salina.*

References

1 Томилина И.И. Морфологические деформации сильнохитинизированных структур ротового аппарата личинок рода *Chironomusriparius* как показатель органического загрязнения пресных водоемов / И.И. Томилина, Л.П. Гребенюк // Биология внутренних вод. — 2014. — № 3. — С. 79–91.

2 Zhong L.W. Zinc oxide nanostructures: growth, properties and applications / L.W. Zhong // Journal of physics: condensed matter. — 2004. — Vol. 16, No. 25. — P. 829–858.

3 Бабушкина И.В. Регенерация экспериментальной раны под влиянием наночастиц цинка / И.В. Бабушкина, Е.В. Гладкова, И.А. Мамонова, С.В. Белова, Е.В. Карякина // Вестн. нов. мед. технол. — 2012. — Т. XIX, № 4. — С. 16.

4 Бабушкина И.В. Влияние наночастиц цинка на бактериальные клетки / И.В. Бабушкина, Е.Г. Чеботарева, М. Эльбубу, С.Б. Орлов, Е.В. Бородулина, В.Б. Бородулин // Вестн. РУДН. Сер. Медицина. — 2012. — № 3. — С. 22–25.

5 Zhaogang Y. The cytotoxicity of zinc oxide nanoparticles to 3D brain organoids results from excessive intracellular zinc ions and defective autophagy / Y. Zhaogang, L. Liangliang, W. Junkang, Zh. Jiaqi, H. Chaobo // Cell Biology and Toxicology. — 2021. https://doi.org/10.1007/s10565-021-09678-x

6 Zhao X. Zinc oxide nanoparticles induce oxidative DNA damage and ros-triggered mitochondria-mediated apoptosis in zebra fish embryos / X. Zhao, X. Ren, R. Zhu, Z. Luo, B. Ren // Aquat. Toxicol. — 2016. — Vol. 180. — P. 56–70.

7 Chun H.S. Two zinc-aminoclays' In-vitro cytotoxicity assessment in hela cells and In-vivo embryotoxicity assay in zebra fish / H.S. Chun, D. Park, S. Eun Lim, K.H. Jeong, J.S. Park, H.J. Park, S. Kang // Ecotoxicol. Environ. Saf. — 2017. — Vol. 137. — P. 103–112.

8 Choi J.S. Developmental toxicity of zinc oxide nanoparticles to zebrafish (*Danio rerio*): a transcriptomic analysis / J.S. Choi, R.O. Kim, S. Yoon, W.K. Kim // PLoS One. — 2016. — Vol. 11, № 8. — e0160763.

9 Ruttkay-Nedecky B. Nanoparticles based on essential metals and their phytotoxicity / B. Ruttkay-Nedecky, O. Krystofova, L. Nejdl, V. Adam // Journal of Nanobiotechnology. — 2017. — Vol. 15. — P. 33–35.

10 Chupani L. Effects of chronic dietary exposure of zinc oxide nanoparticles on the serum protein profile of juvenile common carp (*Cyprinus carpio* L.) / L. Chupani, E. Zusková, H. Niksirat, A. Panáček, V. Lünsmann, S.-B. Haange, M. von Bergen, N. Jehmlich // Sci. Total. Environ. — 2017. — Vol. 579. — P. 1504–1511.

11 Распопов Р.В. Характеристика эффективности использования наночастиц оксида цинка в питании / Р.В. Распопов, Э.Н. Трушина, О.К. Мустафина // Эксперименты на лабораторных животных. — 2011. — № 5. — С. 46–51.

12 Du J. Effects of ZnO nanoparticles on perfluorooctanesulfonate induced thyroid-disrupting on zebra fish larvae / J. Du, S. Wang, H. You, Z. Liu // J. Environ. Sci. — 2016. — Vol. 47. — P. 153–164.

13 Yin Y. Elevated CO_2 levels increase the toxicity of ZnO nanoparticles to goldfish (*Carassius auratus*) in a water-sediment ecosystem / Y. Yin, Z. Hu, W. Du, F. Ai, R. Ji, J.L. Gardea-Torresdey, H. Guo // J. Hazard. Mater. — 2017. — Vol. 327. — P. 64–70.

14 Hasan K. Effects of zinc oxide nanoparticles on bioaccumulation and oxidative stress in different organs of tilapia (*Oreo chromisniloticus*) / K. Hasan, A. Fatix, G. Mert, Y. Sevdan, A. Mexmet, T. Veysel // Environmental Toxicology and Pharmacology. — 2015. — Vol. 40. — P. 936–947.

15 Vishnu D. Rajputa Effects of zinc-oxide nanoparticles on soil, plants, animals and soil organisms: A review / Vishnu D. Rajputa // Environmental Nanotechnology, Monitoring & Management. — 2018. — Vol. 9. — P. 76–78.

16 Chen A. Evaluation of the effect of time on the distribution of zinc oxide nanoparticles in tissues of rats and mice: a systematic review / A. Chen, L. Shao, X. Feng, S. An, Y. Zhang, T. Sun // IET Nanobiotechnol. — 2016. — Vol. 10, No. 3. — P. 97–106.

17 Терехова В.А. Методика определения токсичности по выживаемости солоноватоводных рачков *Artemia salina* / В.А. Терехова, Е.Ф. Исакова, Т.А. Самойлова. — М.: МГУ, 2009. — 28 с.

А.Т. Серікбай, А.М. Айткулов, А.К. Зейниденов, З. Т.Кыстаубаева

Мырыш пен мырыш оксидінің макробөлшектері мен нанобөлшектерінің *Artemia salina* үлгісінде уыттылығын бағалау

Мақала мырыш пен мырыш оксидінің макробөлшектерінің (МБ) және нанобөлшектерінің (НБ) жануарларға әсерін зерттеуге арналған. Су экожүйелерінің фаунасы үшін ластаушы заттардың уыттылық деңгейін бағалау зертханалық тәжірибеде жүргізілді, онда зерттелетін тест-объектісі — шаян тәрізділерінің дернәсілдері *Artemia salina* мырыш (Zn) және мырыш оксидінің (ZnO) бөлшектерінің әсеріне ұшырады. Су бөлшектерінің мөлшері 100 мл-де 5, 10, 20 мг болды. Зерттеу нәтижелері зерттелген концентрациялардағы мырыш пен мырыш оксидінің нанобөлшектері 48 сағаттық тәжірибе кезеңінде елеусіз уыттылық дәрежесін көрсетті. Мырыштың макробөлшектері *Artemia salina* жұмыртқалары мен дернәсілдері үшін салыстырмалы түрде жоғарырақ улы қасиетке ие.

Кілт сөздер: макробөлшектер, нанобөлшектер, мырыш, мырыш оксиді, *Artemia salina*, су экожүйелері.

А.Т. Серикбай, А.М. Айткулов, А.К. Зейниденов, З.Т. Кыстаубаева

Оценка токсичности макрочастиц и наночастиц цинка и оксида цинка на модели *Artemia salina*

Статья посвящена исследованию влияния макрочастиц и наночастиц цинка и оксида цинка на животных. Оценку уровня токсичности поллютантов для фауны водных экосистем проводили в условиях лабораторного эксперимента, где тест-объекты — личинки рачков *Artemia salina* подвергались воздействию частиц цинка и оксида цинка. Содержание частиц в воде составляло 5, 10, 20 мг на 100 мл. Результаты исследования свидетельствуют, что наночастицы цинка и оксида цинка в изученных концентрациях проявляют незначительную степень токсичности в течение 48 ч эксперимента. Авторами сделан вывод, что макрочастицы этих веществ обладают более высокими токсичными свойствами для яиц и личинок *Artemia salina*.

Ключевые слова: макрочастицы, наночастицы, цинк, оксид цинка, *Artemia salina*, водные экосистемы.

References

1 Tomilina, I.I., & Grebeniuk, L.P. (2014). Morfologicheskie deformatsii silnokhitinizirovannykh struktur rotovogo apparata lichinok roda *Chironomusriparius* kak pokazatel organicheskogo zagriazneniia presnykh vodoemov [Morphological deformations of highly chitinized structures of the oral apparatus of larvae of the genus *Chironomusriparius* as an indicator of organic pollution of fresh water bodies]. *Biologiia vnutrennikh vod — Biology of Inland Waters, 3*, 79–91 [in Russian].

2 Zhong, L.W. (2004). Zinc oxide nanostructures: growth, properties and applications. *Journal of physics: condensed matter, 16 (25)*; 829–858.

3 Babushkina, I.V., Gladkova, E.V., Mamonova, I.A., Belova, S.V., & Kariakina, E.V. (2012). Regeneratsiia eksperimentalnoi rany pod vliianiem nanochastits tsinka [Regeneration of experimental wound under the influence of zinc nanoparticles]. *Vestnik novykh meditsinskikh tekhnologii — Bulletin of medical technologies, 19 (4),* 16 [in Russian].

4 Babushkina, I.V., Chebotareva, E.G., Elbubu, M., Orlov, S.B, Borodulina, E.V., & Borodulin, V.B. (2012). Vliianie nanochastits tsinka na bakterialnye kletki [Effects of zinc nanoparticles on bacterial cells]. *Vestnik RUDN. Seriia Meditsina — RUDN Journal of Medicine, 3,* 22–25 [in Russian].

5 Zhaogang, Y., Liangliang, L., Junkang, W., Jiaqi, Zh., & Chaobo, H. (2021). The cytotoxicity of zinc oxide nanoparticles to 3D brain organoids results from excessive intracellular zinc ions and defective autophagy. *Cell Biology and Toxicology, 2021*. https://doi.org/10.1007/s10565-021-09678-x

6 Zhao, X., Ren, X., Zhu, R., Luo, Z., & Ren, B. (2016). Zinc oxide nanoparticles induce oxidative DNA damage and ROS-triggered mitochondria-mediated apoptosis in zebrafish embryos. *Aquatic toxicology (Amsterdam, Netherlands)*, *180*, 56–70. https://doi.org/10.1016/j.aquatox.2016.09.013

7 Chun, H.S., Park, D., Eun Lim, S., Jeong, K.H., Park, J.S., & Park, H.J. et al. (2017). Two zinc-aminoclays' in-vitro cytotoxicity assessment in HeLa cells and in-vivo embryotoxicity assay in zebrafish. *Ecotoxicology and environmental safety*, *137*, 103–112. https://doi.org/10.1016/j.ecoenv.2016.11.022

8 Choi, J.S., Kim, R.O., Yoon, S., & Kim, W.K. (2016). Developmental Toxicity of Zinc Oxide Nanoparticles to Zebrafish (Danio rerio): A Transcriptomic Analysis. *PloS one*, *11*(8), e0160763. https://doi.org/10.1371/journal.pone.0160763

9 Ruttkay-Nedecky, B., Krystofova, O., Nejdl, L. & Adam, V. (2017). Nanoparticles based on essential metals and their phytotoxicity. *Journal of Nanobiotechnology, 15*; 33–35.

10 Chupani, L., Zusková, E., Niksirat, H., Panáček, A., Lünsmann, V., Haange, S. B., von Bergen, M., & Jehmlich, N. (2017). Effects of chronic dietary exposure of zinc oxide nanoparticles on the serum protein profile of juvenile common carp (Cyprinus carpio L.). *The Science of the total environment*, *579*, 1504–1511. https://doi.org/10.1016/j.scitotenv.2016.11.154

11 Raspopov, R.V., Trushina, E.N., & Mustafina, O.K. (2011). Kharakteristika effektivnosti ispolzovaniia nanochastits oksida tsinka v pitanii [Characterization of the nutritional efficiency of zinc oxide nanoparticles]. *Eksperimenty na laboratornykh zhivotnykh — Experiments on laboratorial animals, 5,* 46–51 [in Russian].

12 Du, J., Wang, S., You, H., & Liu, Z. (2016). Effects of ZnO nanoparticles on perfluorooctane sulfonate induced thyroid-disrupting on zebrafish larvae. *Journal of environmental sciences (China)*, *47*, 153–164. https://doi.org/10.1016/j.jes.2016.01.018

13 Yin, Y., Hu, Z., Du, W., Ai, F., Ji, R., Gardea-Torresdey, J.L. & Guo, H. (2017). Elevated CO_2 levels increase the toxicity of ZnO nanoparticles to goldfish (*Carassius auratus*) in a water-sediment ecosystem. *J. Hazard. Mater., 327*; 64–70.

14 Hasan, K., Fatix, A., Mert, G., Sevdan, Y., Mexmet, A. & Veysel, T. (2015). Effects of zinc oxide nanoparticles on bioaccumulation and oxidative stress in different organs of tilapia (*Oreo chromisniloticus*). *Environmental Toxicology and Pharmacology, 40*; 936–947.

15 Vishnu, D. Rajputa (2018). Effects of zinc-oxide nanoparticles on soil, plants, animals and soil organisms: A review. *Environmental Nanotechnology, Monitoring & Management, 9*; 76–78.

16 Chen, A., Feng, X., Sun, T., Zhang, Y., An, S., & Shao, L. (2016). Evaluation of the effect of time on the distribution of zinc oxide nanoparticles in tissues of rats and mice: a systematic review. *IET nanobiotechnology*, *10*(3), 97–106. https://doi.org/10.1049/iet-nbt.2015.0006

17 Terekhova, V.A., Isakova, E.F., & Samoilova, T.A. (2009). *Metodika opredeleniia toksichnosti po vyzhivaemosti solonovatovodnykh rachkov Artemia salina [Methodology for determining the survival toxicity of brackish crustaceans Artemia salina]*. Moscow: Moscow State University [in Russian].