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Experimental tests of application of zinc-containing preparations based on probiotic strains of microorganisms

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Development of mineral deficient conditions in the body of farm animals and, as a consequence, in humans leads to a decrease in the biological capacities and development of pathological conditions, some of which are fatal. Development of deficient conditions may occur as a result of geochemical characteristics (deficient states and excessive content of elements-antagonists) as well an effect of unbalanced diet. Analysis of the obtained experimental data gives an indication of the effectiveness of the application of our prototype used as a micronutrient preparation. So, in the experimental groups, we recorded increases in the concentrations of zinc in the studied tissue samples throughout the period of application of the preparation. It should be noted that the 10 day's experiment values of the experimental groups exceed the integral content of the elements in the tissues of groups K_0 and K_1. Discontinuation of the preparation application on the experimental groups affected the results of zinc content in the observed tissues at the final stage of the study, since in both studied groups, the element content decreased, which, in our view, was due to treating animals on a mineral diet and, as a consequence, reduction in the general level of elements in the tissues. The 15 day's study values of the experimental groups exceeded the value of group K_0. We were the first to conduct an experimental research based on the use of microorganisms' features to deposit excessive contents of essential elements in the bioavailable form, which in turn allowed us to experimentally prove the effectiveness of their application as micronutrients with an increase in the content of this element in the tissues up to 42.8 %, that can serve as a basis for correction of zinc deficient states in farm animals.

Keywords: Correction of Mineral-Deficient States; Micronutrients; Probiotics; Zinc; Zinc-deficient State; Zinc Bioaccumulation.

INTRODUCTION

Zinc belongs to essential elements actively involved in various biological processes in different life forms, ranging from prokaryotes to the human body. In humans and animals, this trace element performs a regulatory function in the metabolism of proteins, carbohydrates, and fats. This element is required for the functioning of many metalloenzymes (Salnikova, 2016, Skalny A.V. et al., 2016, Skalny A.A. et al., 2017a).

An annual increase in the number of population sickness rate resulting from mineral deficient conditions including zinc deficiency is not only the result of unbalanced nutrition but also of the features of geochemical distribution of this element, as well as the competition between elements, so for example, in relation to zinc, elements antagonists are copper, manganese, iron, and cadmium, excessive content of which significantly affects the level of zinc biological intake in the body (Skalny A.A. et al., 2017b). Athletes, diabetics, vegetarians, as well as alcohol abusers face an increased risk of zinc deficiency (Skalny A.V. et al., 2017).

The anthropogenic factor, providing a significant zinc intake in the biosphere, does not cover the reference values for mobile forms of zinc in most regions of the Russian Federation, which ultimately leads to a deficiency of this element in residents' diet (Skalny A.V., 2003, Skalny A.V. and Rudakov, 2004). So, for example, zinc deficient states in the body of the representatives of the male population living in the Orenburg region significantly increase the risk of emergence and development of pathological changes in the reproductive system, diabetes, and neoplasms (Paules IT, Saier M, 2004).

One of the possible options for elemental status regulation of an animal and human organism is the application of biotic preparations based on waste products of transient micro flora with high sorption characteristics (Skalny A.V., 2004, Sizentsov et al., 2013, 2014, Klimova et al., 2017).

Bioaccumulation of essential micronutrients including metals by bacterial cells has two-phase nature. The first phase is characterized by biosorption of elements by the components of the cell wall and is not related to the energy status of the cell. The second phase is energy-dependent intracellular accumulation occurring with the participation of enzymes which are ion carriers (Paules and Saier, 2004, Klimova et al., 2017, 2018).

Literature review testifies exploring the different characteristics of heavy metal sorption by microorganisms, including zinc (Peshkov et al., 2015). For example, the Department of Microbiology of the University of Port Harcourt in Nigeria conducted a study on the accumulating characteristics of bacteria *B. subtilis, S. albus,* and *P. aeruginosa* used as zinc biosorbents in river water with the aim to clean. In the course of their studies, they discovered that the shares of zinc accumulation after 24-hour impact were: to 91.6 %, 68.1 %, and 52.9 %, respectively (Peshkov et al., 2015).

Active sorption characteristics of zinc cations were observed in *B. sphaericus* and *B. cereus*, where the concentration of accumulation amounted to 11.8 mol/g of dry biomass and to 4.6 mol/g, respectively (Green-Ruiz, 2006, Montes, 2006). In studies on accumulation and removal from aqueous solutions of zinc cations by bacterium *B. circulans*, it was found that the maximum sorption of this element (in periodic cultivation) occurs at the end of the exponential phase and initial stage of phase M of concentration of growth and was 70% for 96th h of incubation (Ince Yilmaz, 2003, Waihung et al., 2007).

On the basis of the presented literature, one may conclude that our research does not contradict the overall picture of ongoing research.

Summing up the above, the goal of our research was to explore the application of probiotic strains to create preparations with high zinc bioavailability.

MATERIALS AND METHODS

Welfare of Animals

Ethical approval: All applicable international, national, and institutional guidelines for the care and use of animals were followed.

Diet and feeding

For the experimental part of our study, we chose the following probiotic strains: *B. subtilis 10641* ("Vetom 1.1") and *B. amyloliquefaciens 10642* ("Vetom 3"), both manufactured by LLC SPC "Issledovatelsky Tcentr" (Russia).

As the source of the essential element in the first phase of the study we used zinc nitrate, chloride, sulfate, and acetate, a common criterion for selection of these salts was their high level of salt dissociation in aqueous solutions and, as a consequence, the creation of peak concentrations of zinc cations in substrates. As objects of study of in vivo model, we used rats Wistar in conditions of the experimental biological clinic (vivarium) of FSBEI "Orenburg State University". Treatment to animals and running procedures when carrying out the studies was in accordance with the requirements and recommendations of Russian rules (order of the Ministry of the health of the USSR № 755 of 12.08.1977) and "The Guide for the Care and Use of Laboratory Animals (National Academy Press Washington, D.C. 1996)". To perform the experimental studies, we selected 96 rats, which formed four groups of identical counterparts in sex, age, and weight. To assess the effectiveness of application of preparations with high zinc bioavailability derived from the experimental cultivation of bacterial strains in presence of excessive amount of zinc cations in the substrate ($ZnSO_4$), we caused mineral deficiency states in animals of three groups, for 20 days before the start of the experiment and throughout the study period we used a diet consisting of distilled water and boiled-off within 15 minutes polished rice, followed by washing with distilled water (Khanafari et al., 2008). In order to avoid the development of avitaminosis, the diet of animals included complete multivitamin drugs. The group of intact animals was on the diet in accordance with the requirements and recommendations of Russian rules (order of the Ministry of the health of the USSR № 163 of 10.03.1966).

The first block of the experimental research aimed to evaluate the biosorption characteristics of the studied bacterial strains and to define the working concentrations of zinc sulfate to create the experimental preparation. For the implementation of this phase of the research, we used the diffusion method of agar basins. This method combines two methods: the method of basins in the agar layer and the method of serial dilutions. As a source of excessive cation concentrations, there were used zinc salts (sulfates, chlorides, nitrates, acetates) with a high level of dissociation in aqueous solutions. An advantage of this method is a visual estimate of the chemical toxicity in different concentrations under identical conditions, in addition, this technique is not only about quality, but also about quantification of biotoxicity of the studied chemical compounds (Fomina et al., 2011).

To determine the optimal time of metal sorption from the nutrient substrate, we studied the influence of zinc salts with a various anionic component on the growth of experimental bacterial strains, necessity for this research was the fact that in the process of growth in periodic culture due to depletion of the nutrient substrate, bacteria are not only forced to search for alternative sources of energy and substrate but also to activate mechanisms of detoxification of secondary metabolites and other toxigenic factors. Based on this, one of the stages of our research was to determine the start of the phase M concentration of growth in the presence of zinc salts with a various anionic component. We used the colorimetric method for this task (Sizentsov et al., 2018). Evaluation of the effect of zinc on the growth of microorganisms and the time of the start of the phase M concentration of growth were carried out by measuring the optical density (OD) of bacterial suspensions with an interval of 3 h, starting with the background research and continued to receive three identical values that stated the fact of starting the phase M concentration of growth. To assess the purity of the experiment, at all check-points of the study we carried out Gram stain. We drew the growth charts for the studied strains with a view to visualize the obtained results of assessing the impact of zinc on the growth of the studied bacterial strains and the time of the start of phase M concentration of growth. Each point of the chart represented an average of three measurements of parallel conducted investigations.

The final phase of the first block of the experiments was to evaluate zinc ion sorption characteristics of probiotic strains. For this task, we used the atomic absorption method based on a property to absorb light of a specific wavelength of atoms, emerging when ash solutions get sprayed in acetylene-air flame. The studies were performed using an atomic-absorption spectrophotometer - the device of type AAS-1 (GDR) with a set of spectral lamps (Fomina et al., 2011). Preparing the samples, we conducted a preliminary cultivation of the studied bacterial strains in liquid nutrient substrate adding the working concentration of zinc (no bactericidal and bacteriostatic effect) the periodic cultivation was carried out before the M phase concentration of growth, followed by separation of the contents of the vials on biomass and supernatant by means of centrifugation within 10 minutes at 3000 rpm. The resulting supernatant was separated from biomass with an automatic pipette. The bacterium biomass was lysed using a 5% KOH solution, followed by boiling in a water bath within 20 minutes. To obtain reliable data, there was made an analysis of determining the concentration of metal both in the biomass and the supernatants. The average value of the series of measurements was supposed to be the final result of the analysis.

The experiments of the first block of the research allowed us to embark on the second (main) block of the research, which was aimed at studying the prospective use of probiotic preparations with the accumulating ability to create preparations with a high level of zinc bioavailability.

Preparation of test samples was conducted by the cultivation of the studied bacterial strains in the liquid substrate in the presence of the working concentrations of the metal before the phase M concentration of growth. However, at this stage, we met the need to inactivate the microorganisms for maximal release of the essential element and in order to avoid competition with macroorganism. The technique of preparation was in addition of the working zinc sulfate concentration of 0.03 mol/l to liquid substrate concentration and the suspension of the daily culture of the studied bacterial strains with further incubation within 24 h at a temperature of 37°C. The biomass of bacteria was attained to by a similar technique of sample preparation for atomic adsorption studies with following inactivation of microorganisms by autoclaving for 40 min. The resulting biomass was used for an individual oral introduction to laboratory animals.

For assessment of the prospective application of transient bacterial strains as a regulatory factor of the elemental status in the development of zinc deficient states, we formed four groups of animals counterparts – two control and two experimental ones with 24 animals in each. The first control group was on a mineral-deficiency diet (K_1). The second control group of intact animals was on a normal diet and acted as criterial assessment of the physiological norm (K_2). The experimental groups like group K_1 received the mineral diet leading to the development of zinc deficient states for 20 days. The diet of the first experimental group (O_1) included the biomass of inactivated *B. subtilis 10641* (Vetom 1.1) after cultivation in the liquid substrate in the presence of excessive content of $ZnSO_4$ in a dosage of 1 l-3 per capita within 10 days from the start of the experiment. In the second experimental group (O_2), *B. amyloliquefaciens 10642* (Vetom 3) served as the source of zinc ions. Introduction and dosage, in this case, were the same. The study was conducted by the comparative method of biological research, i.e., animals were in identical conditions at the same time.

The impact of the application of the experimental preparations was measured, using atomic absorption spectrophotometry. For this purpose, we picked over biological materials throughout the experiment with a periodicity of five days (a background study, the fifth, the tenth and the fifteenth day). We chose the cutaneous covering, the muscle and the bone tissue as the biological materials in quantities of 5 g. each of the studied samples.

Statistical processing of the obtained data was conducted by the adopted techniques using software “Statistica 10.0”, including assessment of the arithmetic mean value (M), standard error (m)

RESULTS AND DISCUSSION

Studying biotoxicity of zinc salts with a various anionic component, we discovered that excessive concentrations of this essential element do not have a distinct bactericidal effect in relation to the studied microorganisms, which, in our view, is associated with the detoxication mechanism of bacterial strains.

It should be noted that as the concentration of the analyzed element decreased, the pronounced bactericidal effect got registered regardless of the anionic component in the structure of chemical compounds (Table 1), which, in our opinion, is related to biological activity of this element in the vital activity of microorganisms and, as a consequence, lack of protection mechanisms.

The data presented in table 1 shows the pronounced bactericidal effect of zinc cations against the studied bacterial strains. Significant differences between the effects of cations in the structure of salts with a various anionic component were not revealed. It should be noted that the maximal resistance of the microorganisms was observed in zinc sulfate with no toxic effect at concentrations of 0.03 mol/l, which was determined as the working concentration for further research.

One of the determinant parameters of heavy metal sorption of from substrates in periodic cultivation is finding the time of the start of phase M concentration of growth (Figure 1).

An analysis of the experimental data obtained from the studies of the effects of zinc cations in structure of $ZnSO_4$ when introduced into the nutrient substrate in the concentration of 0.03 mol/l leads to a suggestion that this element in an excessive concentration inhibits the growth of microorganisms and in both cases shortens the beginning of the phase M concentration of growth.

To assess the reliability of the results of the experimental research, we calculated Student criterion, which allowed to note statistically valid changes of optical density values for the studied bacterial strains in the presence of zinc ions, approaching to and throughout the phase M concentration of growth ($p \leq 0.5$, $r \leq 0.01$). This data allowed to find out the time (30 h) for selection of the experimental samples for further research.

The next stage of our research was to study the bioaccumulative capacity of the bacterial strains in the presence of excessive content of zinc cations in the liquid substrate. To exclude a possible impact on any of the results, we carried out a study of zinc content in the composition of the liquid substrate (Table 2).

Table 1. Effect of zinc salts on growth of bacteria of the genus *Bacillus*

Salts	1 Mole	0.5 Mole	0.25 Mole	0.125 Mole	0.063 Mole
	B. amyloliquefaciens 10642				
$ZnSO_4$	31.3±1.68	27.7±2.94	25.7±1.88	17.0±1.51	15.7±0.90
$Zn(CH_3COO)_2$	37.0±1.53	36.0±1.00	30.0±2.08	24.7±2.67	21.3±1.33
$ZnCl_2$	32.7±2.67	29.3±0.67	28.0±0.00	25.3±1.36	21.3±1.67
$Zn(NO_3)_2$	33.0±1.68	31.0±1.61	26.3±2.21	23.3±0.84	20.7±1.36
	B. subtilis 10641				
$ZnSO_4$	32.7±1.85	30.7±2.60	26.2±0.66	11.7±1.33	5.5±1.33
$Zn(CH_3COO)_2$	34.8±0.69	31.3±1.33	26.0±1.00	24.3±1.96	19.7±1.33
$ZnCl_2$	34.0±1.69	33.3±0.56	20.3±1.98	12.3±1.18	7.4±2.88
$Zn(NO_3)_2$	35.0±1.59	32.9±0.20	30.6±2.06	22.3±1.17	19.0±2.66

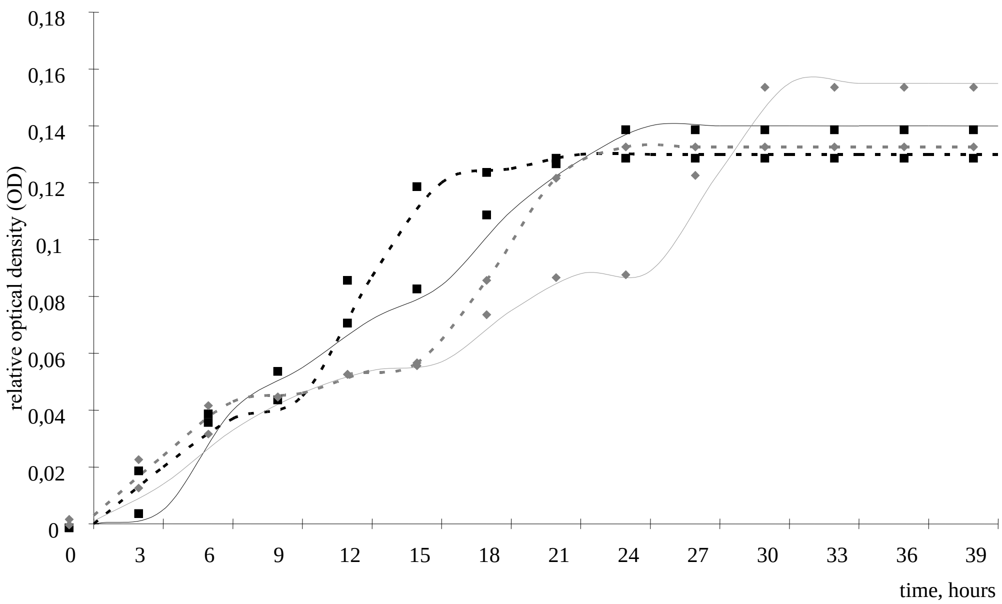

Figure 1. Effects of zinc sulfate on growth dynamics of the microorganisms

control of growth of B. subtilis 10641

growth of B. subtilis 10641 in the presence of $ZnSO_4$

control of growth B. amyloliquefaciens 10642

growth of B. amyloliquefaciens 10642 in the presence of $ZnSO_4$

Table 2. The quantitative content of zinc in the substrate before the introduction of the working salt concentration

Metal	C(Me), introduced into the nutrient medium, mol/l	C_{min}(Me), able to be determined with AAS, mol/l	C_{min}(Me) in the nutrient medium, mol/l
Zn	0.03	0.0000030	-

Table 3. The concentration of zinc ions in biological samples on different dates of the study, g-3/g+3

Groups	Background research	In 5 days	In 10 days	In 15 days
Zinc content in the cutaneous covering				
K_0	0.23 ± 0.0028	0.24 ± 0.0047	0.23 ± 0.0028	0.22 ± 0.0047
K_1	0.43 ± 0.0027	0.41 ± 0.0048	0.44 ± 0.0078	0.41 ± 0.0028
O_1	0.21 ± 0.0028	0.32 ± 0.0049*	0.40 ± 0.0058**	0.36 ± 0.0075*
O_2	0.20 ± 0.0069	0.34 ± 0.0078*	0.43 ± 0.0077**	0.39 ± 0.0028*
Zinc content in the muscular tissue				
K_0	0.25 ± 0.0026	0.26 ± 0.0046	0.25 ± 0.0029	0.24 ± 0.0047
K_1	0.44 ± 0.0027	0.44 ± 0.0047	0.43 ± 0.0047	0.44 ± 0.0078
O_1	0.22 ± 0.0028	0.39 ± 0.0078*	0.40 ± 0.0078***	0.37 ± 0.0048*
O_2	0.26 ± 0.0029	0.38 ± 0.0048*	0.45 ± 0.0058**	0.43 ± 0.0028**
Zinc content in the bone tissue				
K_0	0.19 ± 0.0049	0.18 ± 0.0077	0.19 ± 0.0028	0.19 ± 0.0028
K_1	0.35 ± 0.0077	0.33 ± 0.0058	0.31 ± 0.0029*	0.31 ± 0.0047*
O_1	0.20 ± 0.0047	0.32 ± 0.0048**	0.35 ± 0.0078**	0.32 ± 0.0028**
O_2	0.19 ± 0.0028	0.33 ± 0.0031***	0.36 ± 0.0028***	0.31 ± 0.0048***

*p < 0.5; **p < 0.01
Comparison of group K_1 to the experimental groups

The study revealed that both of the studied strains actively sorb zinc cations from the nutrient substrate, the sorption level for *B. subtilis* 10641 was 44.3 % of the total mass of the introduced composition, and for *B. amyloliquefaciens* 10642 - 40.6 %, respectively.

At the final stage of our studies, we assessed the effectiveness of the application of transient probiotic bacterial strains to create preparations with a high level of bioavailable zinc (Table 3).

The analysis of the obtained experimental data gives an indication of the effectiveness of the resulting prototype used as preparation-micronutrient.

So, we recorded increases of the zinc concentrations in the studied tissue samples in the experimental groups throughout the period of application of preparations. It should be noted that the 10-day values of the experimental groups exceeded the sum of the elements in the tissues of group K_0 to 41.74 % and 45.97 % in groups O_1 and O_2, and K_1 group to 3.78 % and 10.48 %, respectively. Discontinuation of the application on the experimental groups affected the results of zinc content in the researched tissues at the final stage of the study, since in both groups zinc decreased to 8.70 % and 8.87 %, which, in our view, was due to the treatment on the mineral diet and, as a consequence, reduction in the general level of elements in the tissues. The 15-day values of the experimental groups exceeded the value of group K_0 to 38.10 % and 42.78 % for O_1 and O_2, respectively.

CONCLUSION

Summarizing the results of the research, it should be noted that application of probiotic strains with high accumulative characteristics as a source of creating micronutrient preparations for correction of zinc deficient states is very promising. The obtained experimental data indicates that the application of such compounds can significantly increase the percentage of zinc content in the tissues and consequently prevent the development of pathological states associated with deficiency of this element. We were the first to conduct an experimental research based on the use of microorganisms' features to deposit excessive contents of essential elements in the bioavailable form, which in turn allowed us to experimentally prove the effectiveness of their application as micronutrients with an increase in the content of this element in the tissues up to 42.8 %, that can serve as a basis for correction of zinc deficient states in farm animals.

CONFLICT OF INTEREST

The authors declared 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

SAN wrote a manuscript. SyaA and KTA performed an experiment. BES, SEV and KOV data analysis. TAA and DGK designed experiments and reviewed the manuscript. All authors read and approved the final version.

REFERENCES

Fomina MV, Kvan OV, Akimov SS, Sukhanova ON, 2011. Study on the effect of nutritional dietary factors on blood indices of laboratory animals using nonparametric statistics. Vestnik of Orenburg State University 15(134): 150–152 (in Russian).

Green-Ruiz C, 2006. Mercury (II) removal from aqueous solutions by nonviable Bacillus sp. from a tropical estuary. Bioresour Technol 97(10): 1907–1911.

Ince Yilmaz E, 2003. Metal tolerance and biosorption capacity of Bacillus circulans strain EB1. Res Microbiol 154(6): 409–415.

Khanafari A, Eshghdoost S, Mashinchian A, 2008. Removal of Lead and Chromium from Aqueous Solution by Bacillus circulans. Iranian J Environ Health Sci Engineering 5(3): 195–200.

Klimova TA, Barysheva ES, Morozova NV, Korobova IV, 2017. The application of probiotic preparations based on bacteria of the genus Bacillus in the delivery system of iron and zinc in the body of laboratory animals. Vestnik of Orenburg State University 9(209): 65–69 (in Russian).

Klimova TA, Barysheva ES, Sizentsov AN, Bykov AV, Kvan OV. March 7, 2018. A way to increase iron content in the body of animals. Application for a patent of Russian Federation. Registration number 2018108489 of 07.03.2018 (in Russian).

Montes D, 2006. Removal of mercury (II) from aqueous solutions of non-viable cells of Bacillus sp. Biotechnol 97(7): 1907-1911.

Paules IT, Saier M, 2004. A novel family of ubiquiotous heavy metal ion transport protein. J Membr Biol 156(5): 99–103.

Peshkov SA, Sizentsov AN, Nikiyan HN, Kobzev GI, 2015. Study on the bioaccumulation of heavy metals by bacteria of the genus Bacillus. using XRF analysis and atomic force microscopy. Modern problems of science and education 4: 1–7 (in Russian).

Salnikova EV, 2016. Human's need for zinc and its sources (review). Microelements in medicine 17: 11–15 (in Russian).

Sizentsov AN, Galchenko TA, Martynovich YI, 2013. Accumulation of heavy metals by probiotic preparations based on bacteria of the genus Bacillus in vitro. Scientific notes of Bauman Kazan State Academy of Veterinary Medicine 216: 303–307 (in Russian).

Sizentsov AN, Kvan OV, Miroshnikova EP, Gavrish IA, Serdaeva VA, Bykov AV, 2018. Assessment of biotoxicity of Cu nanoparticles with respect to probiotic strains of microorganisms and representatives of the normal flora of the intestine of broiler chickens. Environ Sci Pollut Res 25(16): 15765-15773.

Sizentsov AN, Kvan OV, Notova SV, Galchenko TA, 2014. The efficiency of application of probiotic preparations based on bacteria of the genus Bacillus in the iron delivery system. Herald of restorative medicine 2: 66–75 (in Russian).

Skalny AA, Karganov My, Skalny AV, Fomina MA, Nikonorov AA, 2017a. The effect of zinc supplementation on the zinc and selenium status in exercise. J Trace Elem Med Biol 41(Suppl 1): 39.

Skalny AA, Radysh IV, Skalny AV et al., 2017b. Zinc supplementation modifies trace element status in exercised rats. J Appl Biomed 15(1): 39–47.

Skalny AV, 2003. Zinc and human health. Editorialpublisher department of OSU, Orenburg, Russia (in Russian).

Skalny AV, 2004. Chemical elements in physiology and ecology of a human. Publishing house “ONYX 21 century”: Mir, Moscow, Russia (in Russian).

Skalny AV, Grabeklis AR, Tinkov AA, Skalnaya MG, Skalnaya AA, 2017. Zinc deficiency as a mediator of toxic effects of alcohol abuse. Eur J Nutr 57(7): 1–10.

Skalny AV, Rudakov IA, 2004. Bioelements in medicine. Publishing house “ONYX 21 century”: Mir, Moscow, Russia (in Russian).

Skalny AV, Salnikova EV, Kvan OV, Sizentsov AN, Salnikov IA, 2016. Studying the relationship of zinc bioaccumulation in food and human body on the territory of the Orenburg region. Vestnik of Orenburg State University 10: 79–81 (in Russian).

Waihung Lo N, Mei Lau H, Peter HF, 2007. Biosorption and desorption of copper (II) ions by Bacillus sp. Appl Biochem Biotechnol 107(3): 581–591.