IN VITRO ASSESSMENT OF THE BIOSORPTION POTENTIAL OF SOME REPRESENTATIVES OF THE GENUS *BACILLUS* DURING INTERACTION WITH LEAD CATIONS

SIZENTSOV, A. N.[1] – GALAKTIONOVA, L. V.[2] – DAVYDOVA, O. K.[1] – NIKIYAN, H. N.[3*]

[1]*Department of Biochemistry and Microbiology, Orenburg State University, 460018, 13 Pobedy Ave., Orenburg, Russia*

[2]*Department of Biology and Soil Science, Orenburg State University, 460018, 13 Pobedy Ave., Orenburg, Russia*

[3]*Department of Biophysics and Condensed Matter Physics, Orenburg State University, 460018, 13 Pobedy Ave., Orenburg, Russia*

**Corresponding author*
e-mail: nikiyan@yahoo.com, phone: +7-905-819-2681

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Abstract. The article presents studies on the detoxification of lead by bacteria of the genus *Bacillus*. In the experimental part. a combination of methods was used to fully assess the toxicity of lead cations and their effects on the growth of *Bacillus* bacteria in a model experiment. The data obtained indicate an inhibitory effect of $Pb(NO_3)_2$ and $Pb(CH_3COO)_2$ at concentrations from 1 mM to 0.063 mM on the test organisms. The presence of these cations in the nutrient substrate indicates the effect of lead on the growth of microorganisms. as the increase in optical density is due to their high sorption properties. Atomic absorption spectrophotometry and atomic force microscopy were used to study the biosorption of lead from the substrate. Assessment of detoxification. expressed in active accumulation of lead on microbial cell surfaces up to 65% during the stationary growth phase suggests the potential use of these microorganisms as microbial bio-remediators and bio-correctors to reduce excess lead content in bodies or ecosystems.
Keywords: *detoxification, accumulation, lead salts, heavy metal removal, bioremediation*

Introduction

Studies on the biological role of metals in ecosystems indicate that they have a complex effect on biological organisms. Metals are essential for normal functioning, but in high levels, they can become toxic and affect vital processes of organisms negatively (Sarubbo et al., 2015; Rai et al., 2018; Giangrande et al., 2017; Küpper et al., 2016; Chertko et al., 2012; Sheibak, 2016). The boundary between safe and toxic concentrations is not clearly defined. making it difficult to study their impact on ecosystems. The toxicity of some metals depends not only on their concentration but also on their chemical properties and their role in biochemical cycles (Mustafa and Komatsu, 2016; George, 2018; Polyak and Shigaeva, 2017; Honcharuk and Zagoskina, 2017).

Natural sources of heavy metals, such as volcanic emissions and continental dust transport, as well as rock weathering due to prolonged exposure to air, significantly increase the amount of these metals in soils (Chen et al., 2015; Luo et al., 2015; Marrugo-Negrete et al., 2017). In addition, human activities such as metallurgical mining, smelting, industry, atmospheric deposition. agriculture. and waste management can also lead to the introduction and accumulation of heavy metals in

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soil. These activities include the release of metals such as arsenic (As), cadmium (Cd), lead (Pb), mercury (Hg), chromium (Cr), cobalt (Co), copper (Cu), nickel (Ni) and zinc (Zn) (Dunea et al., 2016; Quinteros et al., 2017; Shao et al., 2017; Golik et al., 2018).

The solubility of heavy metals can lead to differences in their biological significance and toxicity, depending on specific conditions and interactions with macronutrients within living organisms. Because of their low solubility. trivalent and tetravalent metal cations such as Sn, Ce, Ga, Zr and Th do not have any special biological significance. Of the remaining metals, Fe, Mo and Mn are trace elements that have low toxicity and are important for certain biological functions. Zn, Ni, Cu, V, Co, W and Cr are also trace elements. but they have higher toxicity and significance in biological processes. As, Ag, Sb, Cd, Hg, U and Pb do not serve any significant biological function but can be harmful to cells (Kovda, 1976; Bashirova and Chernova, 2016).

Thus, lead is found in all components of the natural environment. with 0.0016% of it being in the earth's crust. It participates in atmospheric and hydrospheric transport, most of it deposited with dust and less with atmospheric precipitation (less than 40%). Lead enters plants from the soil and water and enters the animal body when plants and water are consumed, as well as through food, water and dust in the human body. At the same time, leaded gasoline engines are the main source of lead pollution, with exhaust gases containing tetraethyl lead. Other sources include thermal power plants operating on coal, mining, metallurgical and chemical industries, such as the use of lead to extinguish the Chernobyl nuclear power plant reactor, which entered the air and dispersed. leading to lead concentrations in industrial areas 10.000 times higher than natural levels. Surface waters receive up to 300.000 tons of lead per year. Residents of industrialized countries have significantly higher levels of lead in their bodies compared to residents of agricultural countries. This accumulation of lead in bones. hair and liver can increase over time. Lead is eliminated from the body through excretion through the intestines and kidneys with up to 0.05 mg/L being excreted in urine. While this level may seem small, it is generally considered safe for health (Kovda, 1976; Bashirova and Chernova, 2016).

The mechanisms of metal toxicity are generally well understood, but they can be difficult to classify for individual metals. One such mechanism is a competitive relationship between essential and potentially toxic metals for binding to proteins. Metal ions can stabilize and activate proteins, being part of enzyme systems (Bashirova and Chernova, 2016). Some proteins also have unoccupied sulfhydryl (SH) groups that can interact with toxic metal ions like cadmium, lead and mercury. This interaction can lead to toxic effects. However, the manifestation of metal ion toxicity in different organs and tissues does not always correlate with their level of accumulation. For instance, lead ions are mostly immobilized in bone tissue, accounting for more than 90% of total body content. Nevertheless, toxicity is mainly manifested from 10% of lead content in other body tissues. Therefore, the immobilization of lead ions in bone tissue can be seen as a result of the detoxification process (Gilmiyarova et al., 2017; Bokova and Gracheva, 2000).

For many microorganisms the effectiveness of biological detoxification of metals through targeted microbial activity depends on various factors, such as culture age, cell shape, pH, contact time and initial metal concentrations in the solution (Matta and Gjyli, 2016). Several studies have shown that actively growing cells can effectively remove heavy metal ions from nutrient media, especially when metal concentrations are

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relatively low (Wang and Shen, 2016; Mishra and Malik, 2012). Mishra and Malik in 2012 reported that the *Aspergillus lentulus* strain removed 34% of Ni^{2+} ions and 71-78% of Cu^{2+}, Cr^{3+} and Pb^{2+} ions. Similar results were obtained with *Zygosaccharomyces rouxii* with a removal efficiency of up to 94% for Cd^{2+} at an initial concentration of less than 0.04 mM (Li et al., 2013). *Beauveria bassiana* showed a removal efficiency between 61% and 75% for Cu^{2+}, $Cd^{2+\cdot}$ and Zn^{2+} (Gola et al., 2016). The above studies demonstrate that the bioaccumulation of heavy metals by growing cells of microscopic fungi could be a promising technology for wastewater treatment. In addition, using actively growing cells directly simplifies system management and reduces operational costs, eliminating the need for a separate process of producing biomass (Chojnacka, 2010).

Several strategies have been described by microorganisms to overcome the harmful effects of toxic heavy metals. These include: (1) elimination of metals through permeable barriers, (2) active transport of metals out of the cell, (3) intracellular sequestration of metals by binding, (4) extracellular sequestration, (5) enzymatic detoxification of metals to less toxic forms, (6) reduced sensitivity of cellular targets to metal ions (Ipatova et al., 2015; Pishchik et al., 2016; Savannah et al., 2018). One or more of these detoxification mechanisms may be used simultaneously for a specific metal or group of metals. The characteristics of microorganisms strongly influence the choice of detoxification mechanism. For instance. some microbes have specific genes that regulate resistance to toxic metals and are located on plasmids or chromosomes. The regulatory system for metal resistance is generally based on chromosomal genes. making it more complex than that found on plasmids. On the other hand, plasmid-encoded systems often interact with the process of toxic ion efflux (Silver, 1996).

It has been reported that the factors responsible for plasmid-mediated resistance to toxic metal ions. such as copper, are induced (Xu et al., 2009). For instance, lead-resistant *Enterococcus faecalis* retains its resistance to lead even after the elimination of all plasmids, indicating that the genes responsible for heavy metal resistance are located in the bacterial chromosome (Macur et al., 2004).

Haloalkalophilic *Bacillus* species found in saline soils produce compatible solvents and exopolymers to help them survive in changing haloalkane conditions (Pöther et al., 2013). These extracellular molecules may give haloalkalophilic Bacilli a competitive advantage in removing heavy metals through biosorption.

Based on the analysis of literature data, our goal is to study the mechanisms of lead ion biosorption by *Bacillus* representatives from a nutrient medium in the presence of a high concentration of cations in the environment. and to evaluate their potential as microbial bioremediation agents in an in vitro model.

Materials and methods

Research objects

Six strains of microorganisms from the genus *Bacillus*. which are included in probiotic preparations, were used as research objects: Sporobacterin (*B. subtilis* 534; Bakoren LLC, Russia), Bactisubtil (*B. cereus* IP 5832; Sanofi-Aventis, France), Vetom 1.1 (*B. subtilis* 10641), Vetom 2 (*B. licheniformis* 7048), Vetom 3 (*B. amyloliquefaciens* 10642), Vetom 4 (*B. amyloliquefaciens* 10643; NFP “Research Center,. Russia).

Pure cultures isolated from these preparations were used in experimental studies.

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Research methods

To achieve this goal. we used the following methods: agar well method combined with serial dilution, nephelometric method, atomic force microscopy and atomic absorption spectrometry.

Agar diffusion method

The agar diffusion method is the most revealing and easy-to-perform method that allows not only to assess the degree of dissociation of chemical compounds, but also to determine the level of biological effects of metal cations on the studied microorganisms. In our study, we used the agar well method combined with the method of sequential dilutions of the studied element. The advantage of this method is to assess the effect of different lead concentrations on the organisms tested under similar conditions (substrate composition, pH of the medium, temperature, etc.), which makes it possible to assess the inhibitory effect of lead with a high level of reliability (due to performing the experiment more than 10 times repeated for each of the concentrations).

To implement the method GRM agar was used as a nutrient medium, since all the studied microorganisms are chemo organoheterotrophs and this substrate provides their physiological needs. Sterile aqueous solutions of $Pb(NO_3)$ and $Pb(CH_3COO)_2$ were used in the experiment, the selection criterion for these chemical compounds is their high level/dissociation constants in aqueous solutions, which makes it possible to create a high concentration of lead cations in the substrate (mobile forms) in a short period of time.

Daily cultures of microorganisms were sown on sterile culture media. Previously, a series of dilutions of the studied chemical compounds was prepared in concentrations from 1 M to 0.063 M. Wells with a diameter of 5 mm at a distance of 1.5 cm from the edge of the Petri dish and 3 cm between the wells in the amount of 7 per cup were made using a sterile microbiological template punch in the thickness of the agarized medium. 30 μL of solutions containing various metal concentrations were introduced into the wells using a sterile automatic pipette (clockwise with the minimum concentration in the center of the cup). Bacterial cell cultures in the presence of metal were incubated for 24 h at a temperature of 37°C. The results were evaluated by measuring the diameter of the growth inhibition zones of test organisms. The use of this technique made it possible to determine the working concentrations of lead chemical compounds (which do not have a pronounced inhibitory effect) for each of the studied microorganisms.

Determination of the growth kinetics of bacterial cultures

Determining the growth rate of a microbial population in a batch culture is a complex and time-consuming process. Counting individual cells is difficult and does not eliminate errors in their determination. Considering that the experiment requires determining not the number of cells, but the time at which the population reaches the plateau at maximum concentration, we used a nephelometric method for determination. The principle of this method is that, with increasing microbial biomass, less light passes through the cell suspension. The timing of the onset of the stationary growth phase was determined by measuring optical density every 3 h in daily cultures with added working concentrations of lead salts relative to controls (intact samples) in order to conduct a comparative analysis of the degree of influence of xenobiotic elements and their anionic components on population size and the rate of onset of the maximum growth phase. For

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each series of experiments, sterile nutrient medium GRM broth was prepared and 15 ml was poured into sterile vials. A suspension of microorganisms was prepared according to a turbidity standard of 0.5 McFarland in saline solution. and 150 μl of this suspension was added to each vial. Background measurements were performed immediately after the suspensions were applied, followed by incubation at 37°C for 30 min before measurement to obtain homogeneous suspensions. Measurements were taken in plastic transparent wells on a Stat Fax 303 + photometer (Awareness, USA).

Determination of Pb content in solutions

The ability of microorganisms to bioregulate lead was investigated by measuring the lead content of their biomass. The percentage difference in the element content of a nutrient substrate with and without microorganisms was determined using atomic absorption spectrometry (AAS). The studies were conducted using an atomic absorption spectrometer (AAS-1) with flame atomization from Analytik Jena, Germany. Test samples were prepared by periodically cultivating experimental test organisms in the presence of lead salts until they reached the stationary growth phase. For this purpose, 300 mL of sterile liquid nutrient medium (GRM broth). working concentrations of metal in terms of volume. and 3 mL of a suspension of microorganisms corresponding to the McFarland turbidity standard (0.5) were added to 400-mL vials. The vials were then incubated until the stationary phase at 37°C. Afterwards, the contents were centrifuged for 30 min at 3.000 revolutions per minute (rpm) to precipitate the microbial biomass. The supernatant was separated from the biomass. The cell mass was lysed by adding 5% potassium hydroxide (KOH) and kept for 20 min in a boiling water bath. Then, both the biomass and the supernatants were analyzed to determine the lead concentration of the samples.

Atomic force microscopy

Atomic force microscopy was used to obtain experimental data on the spatial localization of metals and to evaluate the morphometric characteristics of the genus *Bacillus* bacteria as part of the final stage of our research. To accomplish this task, we used an original approach to sample preparation. combining it with the method of agar wells and the diffusion of an active substance into the depth of the agarized substrate.

The preparation of samples involved fixing a freshly cut mica plate measuring 5 × 5 mm, ensuring a clean and atomically smooth surface. The plate was then placed in a holder and brought into contact with the surface of a nutrient medium in a Petri dish. This was done in two locations: (1) in the zone of growth inhibition near the well and (2) in the border area between the zone of suppression and the area where the microbial population grows on a dense nutrient medium in the environment. The samples were scanned using an SMM-2000 atomic force microscope (PROTON-MIET, Russia) in contact mode. using general-purpose MSCT cantilevers (Bruker, USA), with a stiffness of 0.01 N/m and a nominal radius of curvature of the probe of 10 nm.

Quantitative morphometric analysis of the obtained images was performed using standard software provided by the microscope. The data obtained during the experiments were statistically processed using AtteStat program (Center for Statistical Technologies LLC). A probability criterion of p less than 0.05 was considered sufficient for a significant difference between the results of the experimental and control studies.

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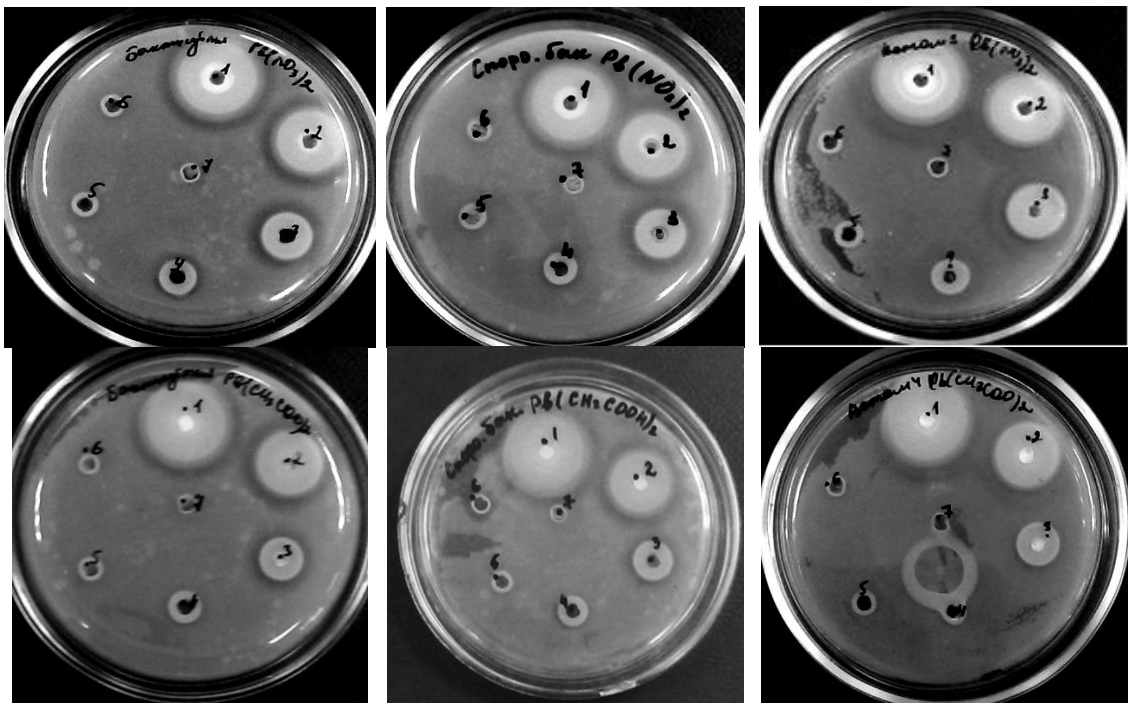
Results and discussion

Assessment of the toxicity of $Pb(NO_3)_2$ and $Pb(CH_3COO)_2$ on the test organisms

The first stage of the research was to evaluate the inhibitory effect of lead chemical compounds with a high level of dissociation in aqueous solutions. The choice of metal salts is associated with the need to create a maximum cationic load in the nutrient substrate. indirectly affecting the test organisms under study. Data obtained indicate the presence of a pronounced inhibitory effect from high concentrations (1 M to 0.063 M) of $Pb(NO_3)_2$ and $Pb(CH_3COO)_2$ on all studied bacteria (*Fig. 1*). At the same time. *B. subtilis* 10641 is the most resistant strain to these chemicals at the maximum concentration. with a growth suppression zone that is 57.38% less than that of the most sensitive *B. amyloliquefaciens* strain 10463 (*Table 1*), while concentrations of 0.31 M and 0.16 M of the compounds did not show a pronounced effect.



1 2 3

Figure 1. *Effect of $Pb(NO_3)_2$ (top row) and $Pb(CH_3COO)_2$ (bottom row) on the growth of the studied microorganisms (in columns): 1 - B. cereus IP 5832; 2 - B. subtilis 534; 3 - B. amyloliquefaciens 10643 at concentrations from 1M to 0.016M (clockwise)*

The growth suppression zones of the studied strains did not significantly differ in the presence of $Pb(NO_3)_2$ and $Pb(CH_3COO)_2$, with the most pronounced toxic effect being observed at the maximum concentration of $Pb(CH_3COO)_2$. High sensitivity to its high concentrations of *B. amyloliquefaciens* has been experimentally established, as well as a relatively high level of resistance in *B. subtilis* 10641, in relation to solutions ranging from 1 M to 0.063 M, which probably indicates the existence of individual biochemical characteristics of this microorganism that allow it to show maximum resistance to high levels of lead ions.

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Table 1. *Resistance (diameter of the suppression zone, mm) of representatives of the genus Bacillus to lead cations*

Studied strains	Concentration of the analyte, M					
	1	**0.5**	**0.25**	**0.125**	**0.063**	**0.031**
$Pb(NO_3)_2$						
B. licheniformis 7048	31.0±1.0	27.3±0.3*	20.3±0.7***	15.7±0.3**	7.0±1.5**	R
B. cereus 5832	30.3±0.3	25.3±0.7**	19.3±0.3***	10.7±1.2***	7.3±0.3*	R
B. subtilis 534	30.0±0.01	26.0±0.6**	17.0±0.6***	9.7±0.3***	7.0±0.6*	R
B. subtilis 10641	23.7±0.3	18.7±0.3***	14.7±0.3***	9.7±0.3***	6.7±0.3***	R
B. amyloliquefaciens 10642	36.0±0.6	27.7±1.2**	24.7±2.2	10.7±0.3**	7.3±0.3***	R
B. amyloliquefaciens 10643	37.3±1.5	30.3±0.9**	20.0±2.9*	10.7±0.3*	6.7±0.3***	R
$Pb(CH_3COO)_2$						
B. licheniformis 7048	32.0±0.01	27.0±1.0**	20.7±1.7	12.3±0.3**	9.1±1.9	R
B. cereus 5832	32.3±0.3	22.3±1.5**	14.0±1.4**	9.7±0.3*	7.7±0.3**	R
B. subtilis 534	32.0±0.6	25.3±0.3***	13.3±0.9***	10.3±0.3*	6.7±0.3***	R
B. subtilis 10641	23.3±0.3	19.3±0.3***	13.0±0.6***	9.7±0.3**	6.7±0.3***	R
B. amyloliquefaciens 10642	38.7±0.3	30.3±0.9***	23.3±0.9**	10.3±0.3***	7.0±0.6**	R
B. amyloliquefaciens 10643	31.7±1.7	22.3±0.3**	13.3±0.3***	8.7±0.9**	5.7±0.7*	R

*p < 0.05; **p < 0.01; ***p < 0.001 relative to the previous indicator. R - absence of a growth inhibition zone (resistant)

Evaluation of the effect of lead exposure on the growth characteristics of the studied strains in a batch culture

The next stage of our research was to evaluate the effect of the lead compounds on the growth of microorganisms under conditions of periodic cultivation in a liquid medium. Our previous studies allowed us to determine a concentration of the chemicals that did not have a significant inhibitory effect on the bacterial strains - 0.031 M. We studied the degree of influence of lead on bacterial population growth using a nephelometric method and plotted graphs to visualize the effect of xenobiotic elements on bacterial test organisms based on average values (*Table 2, fig. A1*).

The need for this study arises from the determination of the onset of the maximum concentration phase, which is characterized by a high level of detoxification exhibited by representatives of this type of microorganism in connection with the accumulation of characteristics. Experimental data indicate that populations of *B. subtilis* 534, *B. cereus* IP 5832 and *B. licheniformis* 7048 have achieved maximum density under conditions of an increased cationic load. Lead does not participate in the metabolic processes of these microorganisms; therefore. it is not possible to testify to a stimulating effect of this element on the increase in biomass in the presented microorganisms. This phenomenon is hypothetically due to the high sorption properties on the surface structures of cells in these strains, leading to the formation of aggregates with a higher light scattering level. The remaining strains did not show significant differences in cell density compared to control parameters. A stepwise change in population density of all strains studied in the exponential phase is due to restructuring of enzyme systems as the substrate depletes. When describing the rate of entry into the stationary phase, it should be noted that there is a general tendency towards stabilization of indicators of bacterial biomass by the 24th hour of the experiment.

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Table 2. *Effect of $Pb(NO_3)_2$ on the growth dynamics of the studied microorganisms*

Time, h	***B. subtilis* 534**		***B. subtilis* 10641**	
	Growth control	**$Pb(NO_3)_2$**	**Growth control**	**$Pb(NO_3)_2$**
0	0.001 ± 0.0001	0.005 ± 0.0003***	0.003 ± 0.0003	0.005 ± 0.0003**
3	0.028 ± 0.0003	0.068 ± 0.0003***	0.004 ± 00006	0.045 ± 0.0001***
6	0.041 ± 0.0009	0.117 ± 0.0033***	0.043 ± 0.0035	0.055 ± 0.0006*
9	0.061 ± 0.0006	0.152 ± 0.0005***	0.055 ± 0.0003	0.056 ± 0.0003
12	0.087 ± 0.0003	0.155 ± 0.0007***	0.072 ± 0.0003	0.091 ± 0.0003***
15	0.087 ± 0.0065	0.175 ± 0.0003***	0.084 ± 0.0005	0.097 ± 0.031***
18	0.113 ± 0.0003	0.184 ± 0.0009***	0.110 ± 0.0003	0.123 ± 0.0006***
21	0.139 ± 0.0002	0.196 ± 0.0003***	0.128 ± 0.0006	0.160 ± 0.0006***
24	0.164 ± 0.0003	0.196 ± 0.0005***	0.140 ± 0.0006	0.160 ± 0.0006***
27	0.164 ± 0.0001	0.196 ± 0.0004***	0.140 ± 0.0003	0.160 ± 0.0003***
30	0.164 ± 0.0001	0.196 ± 0.0002***	0.140 ± 0.0004	0.160 ± 0.0001***
33	0.164 ± 0.0002	0.196 ± 0.0002***	0.140 ± 0.0006	0.160 ± 0.0005***
36	0.164 ± 0.0002	0.196 ± 0.0003***	0.140 ± 0.0003	0.160 ± 0.0006***
39	0.164 ± 0.0001	0.196 ± 0.0006***	0.140 ± 0.0005	0.160 ± 0.0006***
	***B. cereus* IP 5832**		***B. amyloliquefaciens* 10643**	
	Growth control	**$Pb(NO_3)_2$**	**Growth control**	**$Pb(NO_3)_2$**
0	0.003 ± 0.0006	0.015 ± 0.0003***	0.001 ± 0.0003	0.001 ± 0.0003
3	0.024 ± 0.0007	0.038 ± 0.0003***	0.014 ± 0.0003	0.068 ± 0.0003***
6	0.033 ± 0.0006	0.049 ± 0.0003***	0.033 ± 0.0003	0.075 ± 0.0003***
9	0.047 ± 0.0009	0.065 ± 0.0007***	0.046 ± 0.0003	0.085 ± 0.0036***
12	0.053 ± 0.0007	0.078 ± 0.0003***	0.054 ± 0.0003	0.154 ± 0.0003***
15	0.082 ± 0.0003	0.091 ± 0.0003***	0.057 ± 0.0006	0.157 ± 0.0003***
18	0.097 ± 0.0003	0.133 ± 0.0015***	0.075 ± 0.0003	0.175 ± 0.0003***
21	0.114 ± 0.0003	0.150 ± 0.0003***	0.088 ± 0.0003	0.183 ± 0.0003***
24	0.144 ± 0.006	0.150 ± 0.0003***	0.089 ± 0.0006	0.185 ± 0.0003***
27	0.145 ± 0.0007	0.150 ± 0.0006**	0.124 ± 0.0007	0.185 ± 0.0003***
30	0.145 ± 0.0003	0.150 ± 0.0006***	0.155 ± 0.0003	0.185 ± 0.0001***
33	0.145 ± 0.0003	0.150 ± 0.0006***	0.155 ± 0.0001	0.185 ± 0.0003***
36	0.145 ± 0.0003	0.150 ± 0.0003***	0.155 ± 0.0003	0.185 ± 0.0006***
39	0.145 ± 0.0001	0.150 ± 0.0001***	0.155 ± 0.0003	0.185 ± 0.0003***
	***B. licheniformis* 7038**		***B. amyloliquefaciens* 10642**	
	Growth control	**$Pb(NO_3)_2$**	**Growth control**	**$Pb(NO_3)_2$**
0	0.001 ± 0.0002	0.001 ± 0.0003	0.003 ± 0.0003	0.004 ± 0.0003
3	0.014 ± 0.0006	0.063 ± 0.0003***	0.014 ± 0.0006	0.048 ± 0.0001***
6	0.032 ± 0.0003	0.079 ± 0.0003***	0.032 ± 0.0003	0.065 ± 0.0004***
9	0.056 ± 0.0006	0.087 ± 0.0032***	0.051 ± 0.0007	0.076 ± 0.0003***
12	0.066 ± 0.0006	0.155 ± 0.0003***	0.054 ± 0.0006	0.094 ± 0.0003***
15	0.074 ± 0.0003	0.159 ± 0.0004***	0.066 ± 0.0003	0.097 ± 0.0007***
18	0.076 ± 0.0003	0.178 ± 0.0003***	0.072 ± 0.0007	0.138 ± 0.0006***
21	0.076 ± 0.0007	0.183 ± 0.0002***	0.088 ± 0.0006	0.156 ± 0.0003***
24	0.093 ± 0.0003	0.187 ± 0.0003***	0.110 ± 0.0003	0.161 ± 0.0006***
27	0.140 ± 0.0003	0.187 ± 0.0002***	0.131 ± 0.0003	0.162 ± 0.0005***
30	0.140 ± 0.0003	0.187 ± 0.0001***	0.137 ± 0.0007	0.161 ± 0.0002***
33	0.140 ± 0.0001	0.187 ± 0.0004***	0.141 ± 0.0003	0.162 ± 0.0004***
36	0.140 ± 0.0003	0.187 ± 0.0004***	0.141 ± 0.0003	0.162 ± 0.0003***
39	0.140 ± 0.0002	0.187 ± 0.0003***	0.141 ± 0.0003	0.162 ± 0.0005***

*p < 0.05; **p < 0.01; ***p < 0.001

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Biosorption characteristics of microorganisms, spatial localization of lead on the surface of bacterial cells and its effect on morphological and physiological characteristics

The final stage of the research. which included the use of atomic force microscopy combined with the agar diffusion method. allowed us to identify the species and strains that accumulate lead, as well as to conduct a visual assessment of the cytotoxicity of $Pb(NO_3)_2$ and $Pb(CH_3COO)_2$ and the effects of lead cations on bacterial cell morphology (*Fig. 2*). The experimental data obtained indicate a high level of lead diffusion into the agarized nutrient medium. While it should be noted that xenobiotic elements interact with substrate components, their maximum deposition occurs on the surface of the medium (*Fig. 2A*).

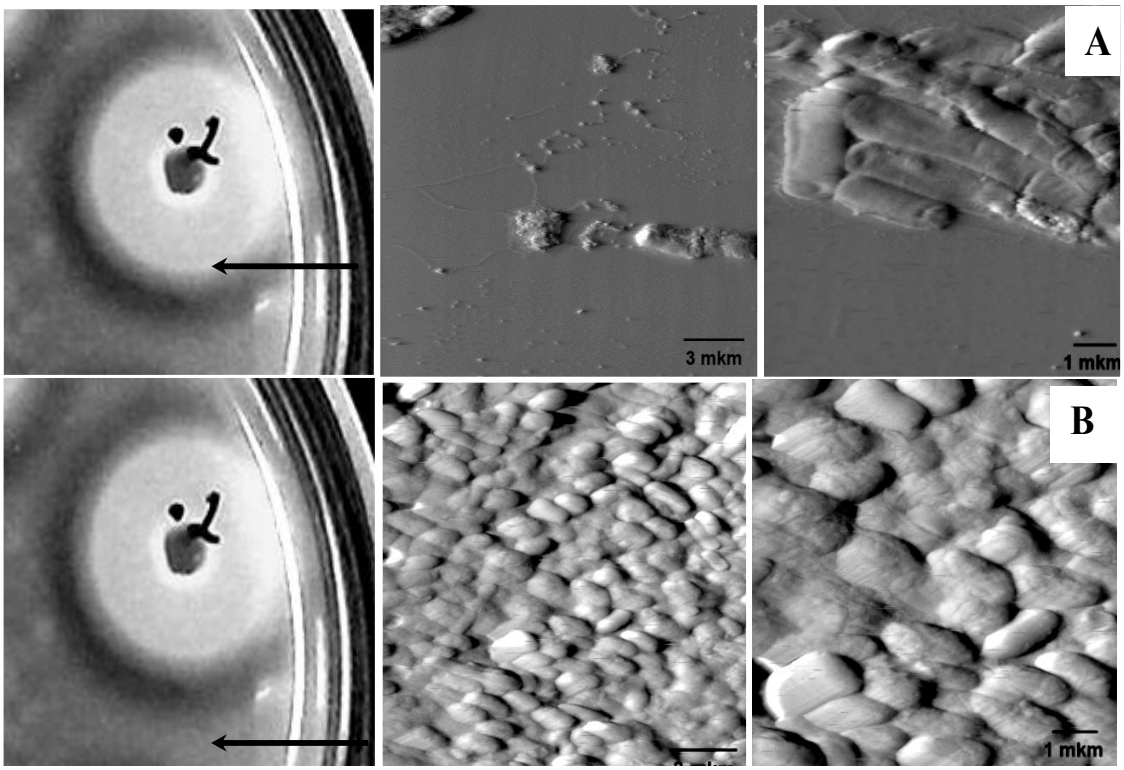

Figure 2. *Influence of lead cations on example B. subtilis 534 using atomic force microscopy: A - sampling zone for AFM in the growth inhibition zone, B - sampling for AFM at the growth boundary of the studied microorganisms*

It is difficult to assess the viability of strains in the zones of localization of elements, as cells with maximum interaction with lead cations can be visually identified without changes in their morphological characteristics. However, there are no spores or vegetative forms of microorganisms in these areas. Hypothetically, this phenomenon could be explained as follows: a high level of xenobiotic cationic load on the substrate provokes adaptive and physiological mechanisms in individual representatives of population groups, but at the same time blocks their metabolic processes and spore formation mechanisms, as a result of which bacterial cells lose their ability to divide and form dormant forms.

As we move further away from the central well, a region forms that is characterized by both a lack of visible effect of lead ions on the substrate and a lack of population

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growth of the microorganisms. This allowed us to define the next boundary region for the selection of experimental samples (*Fig. 2B*). A visual assessment of the impact of sub-inhibitory lead concentrations on the organisms under study shows a high degree of damage to the sorption of the element onto the cell surface. In contrast, in the growth inhibition zone sample, active growth of vegetative forms and spore formation of bacterial strains are observed, indicating a negative impact of the xenobiotic on physiological and adaptive resistance mechanisms to environmental stressors. The concentration of lead ions formed in this area has a significant impact on the morphological features of the studied microorganisms, which is evident in the form of shorter bacterial cells and the lack of a typical arrangement of cells in relation to each other (streptobacilli). The elimination of toxic lead effects is caused by the formation of inactive metal forms on the surface of bacteria, which in turn ensures their survival. To confirm the hypothesis about the pathway of detoxification of lead-related elements on the cell surface, we conducted a study to evaluate the bioaccumulation characteristics of the microorganisms under study with respect to lead ions (*Fig. 3*). For this purpose, the method of atomic absorption spectroscopy was used.

Under experimental conditions. $Pb(NO_3)_2$ and $Pb(CH_3COO)_2$ were added to the liquid substrate before the introduction of tested microorganisms and at a dose of 0.031 M. This was followed by the incubation of bacterial strains in the presence of xenobiotic elements for 30 h at 37°C. The results were obtained based on the differences between the doses of introduced elements and their determination in biomass and supernatant samples.

Analysis of the experimental data obtained indicates a pronounced biosorptive activity of all the studied strains towards lead cations in the substrate. At the same time, there were no significant differences between the samples when $Pb(NO_3)_2$ and $Pb(CH_3COO)_2$ were present. The heterogeneity of the results prevents us from determining a general pattern of the influence of anionic components on sorption levels. However, we identified species and strains that accumulate individual representatives of *Bacillus* during the study.

Thus, maximum sorption values were recorded for representatives of the *B. subtilis* species. The percentage of biosorption during the stationary growth phase was on average 65.3% for *B. subtilis* 534 and 66.2% in the presence of both $Pb(NO_3)_2$ and $Pb(CH_3COO)_2$, respectively, for the *B. subtilis* 10641 strain. These values were 60.9% and 62.6%, respectively. Representatives of *B. amyloliquefaciens* have the lowest accumulating characteristics among all studied microorganisms. However, it is promising to use all bacterial strains for implementing projects for using bacterial strains of the genus *Bacillus* as bio-remediators for biologically active forms of lead in ecological systems at various levels of organization.

The study of detoxification mechanisms associated with the sorption of elements on the surface of bacterial slime in an inactive form has been confirmed by the results obtained using atomic force microscopy (AFM) which coincides with previously published literature data (Ramya et al., 2018; Raize et al., 2004). Gayatri et al., for example. experimentally established that isolates of *B. licheniformis*, *B. cereus* and *B. subtilis* have a maximum lead adsorption on their surfaces, as determined by atomic absorption spectroscopy and scanning electron microscopy, respectively (Gayatri et al., 2017). The binding mechanisms involve a combination of ion exchange, chelation and occasionally reduction reactions, accompanied by the deposition of metallic lead on the cell walls. It is believed that during ion exchange processes Ca, Mg, H, Na and K

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cations are replaced by heavy metals in the cell wall material. It was found that lead biosorption alters the carboxyl, hydroxyl, amino, sulfhydryl and sulfate groups of peptidoglycan. where other metal ions cannot compete. giving it a greater affinity (Chang and Huang, 1998; Gadd, 1993).

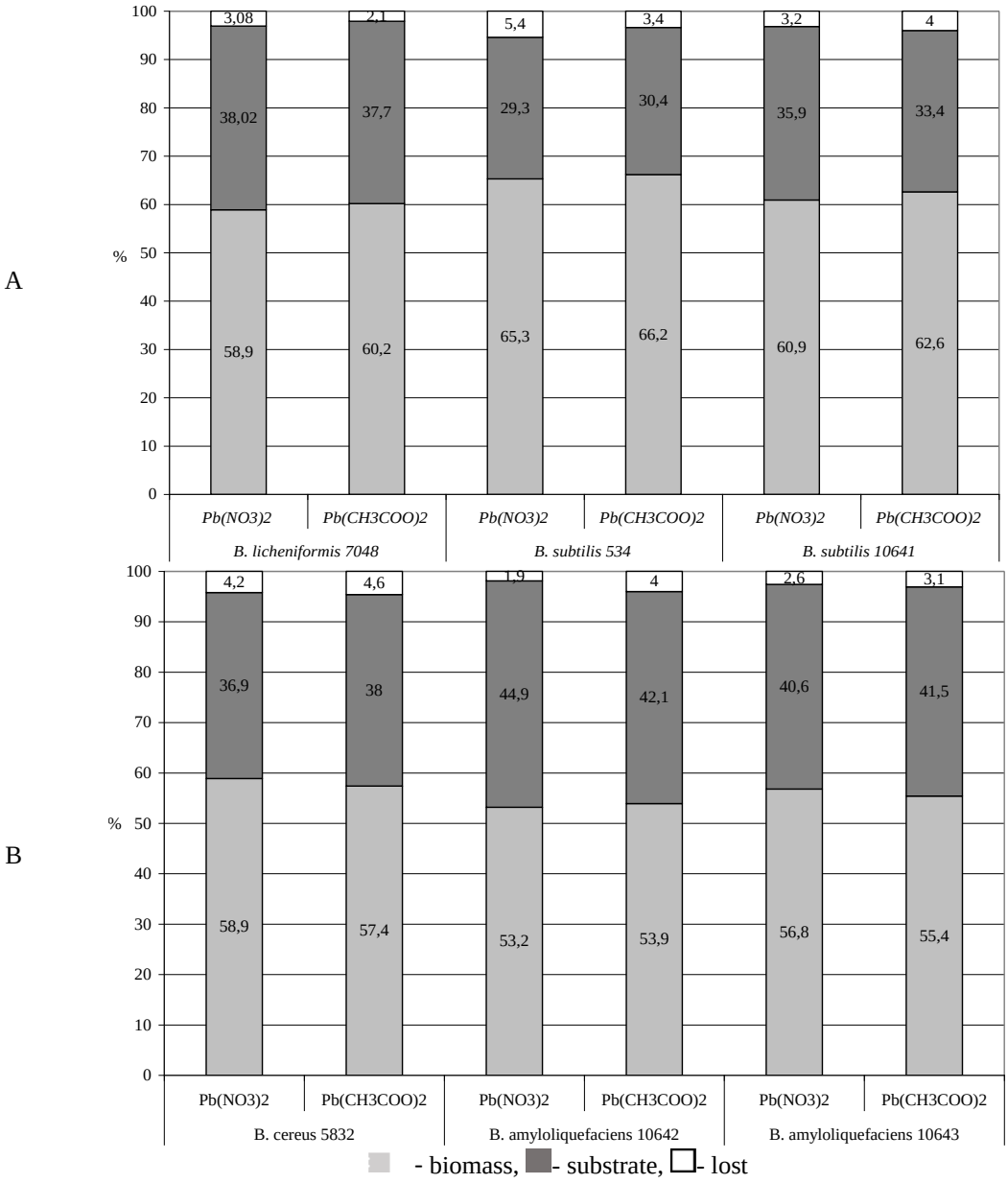

Figure 3. *Assessment of sorption properties of bacteria of the genus Bacillus to lead cations from a substrate containing $Pb(NO_3)_2$ and $Pb(CH_3COO)_2$. A - accumulation of lead cations by strains of B. licheniformis 7048, B. subtilis 534 and B. subtilus 1064. B - accumulation of lead cations by strains B. cereus IP 5832, B. amyloliquefaciens 10642, B. amyloliquefaciens 10643*

The combination of methods used allowed us to fully assess the toxicity and effects of lead cations on microbial growth in a model experiment. Atomic adsorption spectroscopy and atomic force microscopy were used to estimate biosorption of xenobiotic elements from a substrate.

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Conclusions

The experimental data obtained indicate a direct correlation between the toxicity, effect on growth and sorption characteristics of the studied strains. Resistance of *B. amyloliquefaciens* to $Pb(NO_3)_2$ and $Pb(CH_3COO)_2$ has been demonstrated. with *B. subtilis* 10641 being the most sensitive at the maximum concentration of metal ions. The effect of lead on the growth of these strains in a periodic culture is expressed by a dynamic increase in biomass over 24 h at a minimum salt concentration of 0.031 M. which has no pronounced inhibitory effect on the experimental strains. These data were used to study the biosorption characteristics of the tested microorganisms with respect to lead cations and determine their localization and impact on morphological and physiological characteristics. As a result, it was established that all tested microorganisms have a distribution of biosorption indicators in the presence of $Pb(NO_3)_2$ and $Pb(CH_3COO)_2$ substrates for *B. subtilis* 534 and *B. subtilis* 1064, *B. licheniformis* 7048 and *B. cereus* IP5832, *B. amyloliquefaciens* 1063 and *B. amylolliquefaciaens* 1062, respectively. The absence of a significant effect on the toxicity and sorption levels of anionic components in the chemical compounds has been experimentally confirmed. Summarizing the above, there is a high interest in studying the biosorption characteristics of microorganisms for heavy metal removal, with the prospect of using them as bioremediators in ecological systems at various levels of organization. that is, to solve the problems of cleaning up the natural environment.

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APPENDIX

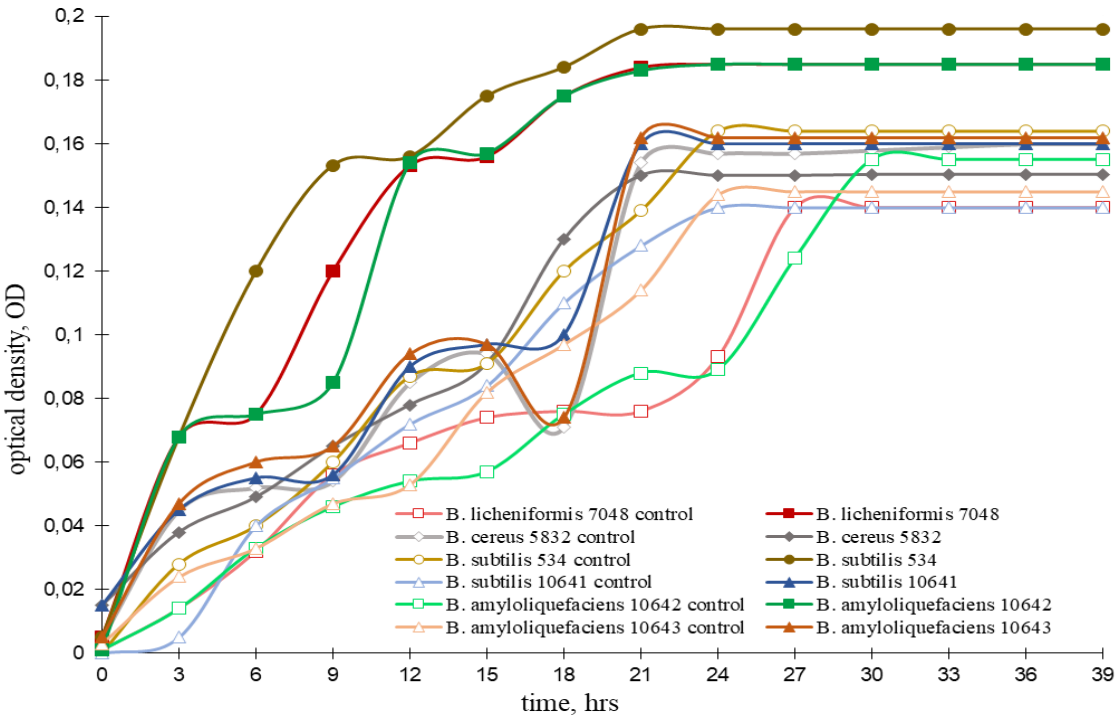

Figure A1. *The effect of $Pb(NO_3)_2$ at a concentration of 0.31 M on the growth dynamics of the studied microorganisms*

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