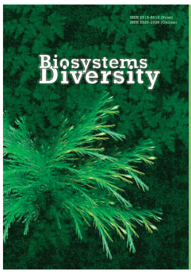

Biosystems Diversity

ISSN 2519-8513 (Print)
ISSN 2520-2529 (Online)
Biosyst. Divers., 26(3), 227–232
doi: 10.15421/011834

Influence of lead salts on some morphological and physiological parameters of filamentous cyanobacteria

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Article info

Received 14.07.2018
Received in revised form 20.08.2018
Accepted 23.08.2018

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Galperina, A. R., & Soprunova, O. B. (2018). Influence of lead salts on some morphological and physiological parameters of filamentous cyanobacteria. Biosystems Diversity, 26(3), 227–232. doi: 10.15421/011834

In the conditions of a model laboratory experiment the influence of lead salts (an acetate and nitrate) on morphological and physiological parameters of filamentous cyanobacteria was studied. During the experiment we estimated features of formation of biomass, structure of trichomes, form and the size of cells, content of chlorophyll *a*, carotenoids and phycobiliproteins. It is noted that in the presence of lead acetate of up to 5 maximum allowable concentrations there is a formation of a biomass in the form of attached and free films, and presence of a nitrate form of lead at the same concentration promotes formation of filaments, fixed from one side. At the same time, the increase of concentration of both acetate, and nitrate forms of lead promotes formation of rarefied films of one layer multidirectional trichomes; to disintegration of trichomes on the fragments and separate cells united by an external mucilaginous envelope. Content of lead acetate in concentration of 15 times the maximum allowable concentration, and lead nitrate at 10 times the maximum allowable concentration leads to formation of abnormally long cells up to 10.0–10.5 µm long. It is established that lead acetate has a stimulating effect on formation of a biomass and synthesis of photosynthetic pigments. The biomass growth of up to 223.7% of the control was observed at concentration up to 15 times the maximum allowable concentration inclusive. The content of chlorophyll *a* grew by 30.6%, carotenoids – by 24.0% at one maximum allowable concentration. Lead nitrate stimulates a biomass gain much more weakly – up to 70.0% at 5 times the maximum allowable concentration and also has the expressed inhibiting effect on synthesis of photosynthetic pigments. Depression of concentration of chlorophyll *a* and carotenoids by 38.8% and 79.4% respectively was observed already at one maximum allowable concentration. The stimulating effect of lead acetate is noted on synthesis of phycocyanin (by 94.0%) and allophycocyanin (by 120.0%) in concentration up to 5 times the maximum allowable concentration; the stimulating effect of lead nitrate was observed on synthesis of phycocyanin (by 64.7%) in concentration up to 5 times the maximum allowable concentration and on synthesis of allophycocyanin (up to 140.0%) and on phycoerythrin (up to 228.0%) at concentration up to 10 times the maximum allowable concentration. Comparison of influence of various lead salts on filamentous cyanobacteria revealed a more expressed inhibiting effect of the nitrate form of lead in comparison with acetate.

Keywords: heavy metals; cyanobacteria; lead; photosynthetic pigments.

Introduction

One of the most significant sources of the environmental pollution is salts of lead. Annually, around 3.5 Mt of lead is released into the environment, and if we take into account the re-extraction of lead from wastes, the production of lead is 4.1 Mt. Pollution of natural objects occurs as a result of combustion and melting of lead ore, during combustion of coal, wood and other organic materials, including municipal wastes and fuel (Da Costa & Duta, 2001; Kapkov et al., 2017; Rozanov, 2017). Lead performs no biological functions. It is toxic, belongs to the Ist class of hazard and is able to accumulate in food chains of living organisms. At the same time, in contrast to organic compounds, lead does not decompose, and is only able to redistribute between components of the natural environment (Kul'bachko et al., 2015; Pischik et al., 2016; Shulman et al., 2017). Cyanobacteria are promising objects to apply for cleaning discharge water from compounds of heavy metals in general, and lead in particular, one of the most significant functions of which is the capacity of bioaccumulation and detoxication of heavy metals (Abdullah & Loo, 2006). Also, their response reactions (sizes, morphology, ultrastructure of the cells and physiologic-biochemical features) to the impact of anthropogenic factors are the fastest and can be representative indicators of the condition of aquatic and soil ecosystems (Bekasova et al., 2002; Tay et al., 2003; Dubey et al., 2011; Shilpi et al., 2014; Fokina et al., 2015; Fokina et al., 2017). Therefore, research focused on the morphological and physiological-biochemical parameters of cyanobacteria under the impact of heavy metals is of great relevance. Earlier, research on sensitivity of filamentous cyanobacteria to heavy metals recorded increased resistivity of the bacteriologic culture to lead acetate (Galperina, 2017). At the same time, different anions are able to reduce or strengthen toxic effects of the heavy metal (Temraleeva & Pinskii, 2010; Vijayakumar, 2012; Bilal et al., 2018).

The objective of this paper was evaluation of impact of different concentrations of lead salts (acetate and nitrate) on some morphological and physiological parameters of filamentous cyanobacteria.

Materials and methods

The object of the research was a phycologically pure culture of filamentous cyanobacteria selected from a laboratory cyanobacterial community. The cyanobacteria were cultivated on a BG-11 media in Erlenmeyer flasks at the temperature of 20 ± 2 ˚C and in constant artificial illumination of 1500 lux. Lead was introduced to the environment in acetate ($Pb(CH_3COO)_2$) and nitrate form ($Pb(NO_3)_2$). In the experiment, we used 1, 5, 10, 15, 20 MPC concentrations of lead (Maximum Permissible Concentration, MPC, of lead for surface water of water bodies is 0.1 mg/l). Growth of cyanobacteria was checked by increase in raw biomass and visually. The colouring of biomass and character of growth in the growth media were recorded. Morphological parameters of cyanobacteria were studied using an optical microscope (at 700 times magnification). We recorded the form and size of the cells and characteristics of the trichomes. The pigment complex of the cyanobacteria was assessed according to the content of chlorophyll *a*, carotenoids and phycobiliproteins. The content of photosynthetic pigments in the cells of the studied cyanobacteria was determined using a colorimetric method. Chlorophyll *a* and carotenoids were extracted using 70.0% ethyl spirit (Sirenko, 1975). For determining concentrations of pigments in extract, we used formulas of Vernon (1960) and Wettstein (1957).

Phycobiliproteins – phycocyanin, allophycocyanin and phycoerythrin were extracted using phosphate buffer with 6-time freezing. The concentration of phycobiliproteins was calculated using the Siegman and Kycia formulae (Siegelman & Kycia, 1978).

Statistical analysis was made in Statistica 6.1 pack (StatSoft Inc., USA). The data were presented in the form of x ± SD. For comparing two independent selections, we used Student's t-criterion (at $P < 0.05$ differences were considered reliable, the data was previously checked on the normal distribution). For morphological parameters, the volume of selection equaled 35 replications for each taxon.

Results

During the study, we observed that presence of lead of up to 5 MPC did not inhibit the development of cyanobacteria. Membranes and teniae were formed by long trichomes of saturated blue-green colour. The cells in the trichomes equalled 2.6–3.8 × 1.8–2.5 µm, but there were thinner cells, which can indicate intense process of growth and cell division (Table 1).

Table 1
Change in morphology of cells of cyanobacteria exposed to lead salts (volume of selection equaled 35 replications for each taxon)

Lead concentration	Cell sizes, µm			
	lead acetate		lead nitrate	
	length of cells	width of cells	length of cells	width of cells
Control	3.8 ± 0.45	2.4 ± 0.20	3.8 ± 0.45	2.4 ± 0.20
1 MPC	2.8 ± 0.20*	1.8 ± 0.08**	3.2 ± 0.20**	1.8 ± 0.08**
5 MPC	2.8 ± 0.50	1.6 ± 0.04**	2.8 ± 0.50	1.6 ± 0.04**
10 MPC	2.3 ± 0.20*	1.6 ± 0.15**	10.0 ± 3.00*	2.2 ± 0.20
15 MPC	10.5 ± 3.10*	2.4 ± 0.20	–	–

Note: * – $P < 0,05$, ** – $P < 0.01$ compared to the control, "–" – absence of living cells of cyanobacteria in microecosystem.

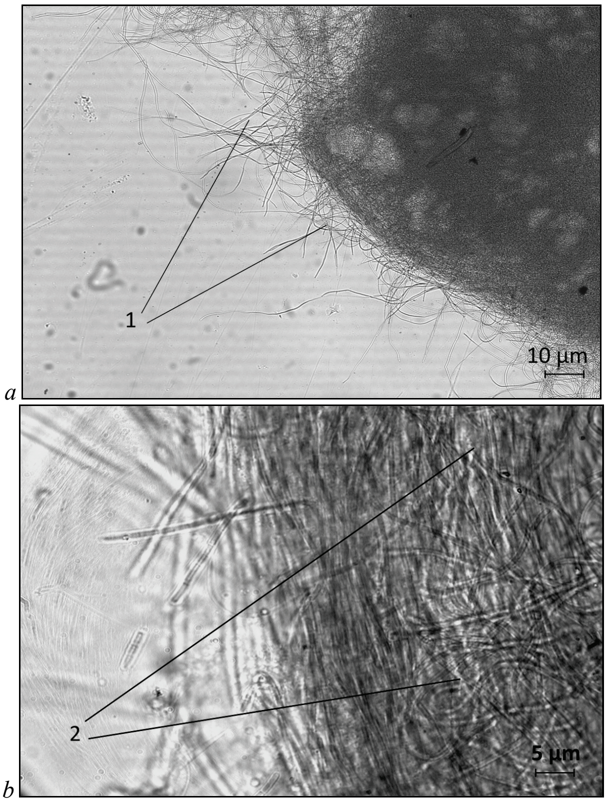
Development of cyanobacteria biomass in the presence of lead acetate and nitrate was different. Introduction of lead acetate in concentrations of up to 5 TLV contributes to formation of membranes and teniae both on the surface of the media and attached to the bed and walls. By contrast, at the same concentrations of lead nitrate in the environment, intense formation of 2–4 mm long clusters occurred, which were tighly attached on one end of a tenia. Examination under the microscope revealled that clusters were formed by a dense interlaced bundle of trichomes (Fig. 1).

At increase in lead concentration, both in nitrate and acetate forms, signs of growth inhibition were observed in the medium: discolouration of trichomes, their thinned out density, and there was also observed a deformation of cells and increase in their sizes – to 10.0–10.5 µm (Table 1, Fig. 2). There was also observed a reliable significant decrease in the length of trichomes down to its decomposition into fragments composed of 4–8 cells ($P < 0.05$, $t = 2.14$). In the media with lead acetate, cyanobacteria developed at concentration up to 15 MPC inclusive, and in the presence of nitrate – up to 10 MPC inclusively.

The pigment complex was assessed in relation to change in the content of chlorophyll *a*, carotenoids and phycobiliproteins. In the studied culture, sensitivity of the pigment apparatus to the impact of different salts of lead was found.

The greatest decrease in the concentration of chlorophyll *a* was observed after introducing lead acetate and nitrate at 20 and 25 MPC, respectively. At the same time, introduction of lead acetate in the amount of 1 MPC stimulated synthesis of chlorophyll a by 30.6%. Further increase in the concentration of the metal to 25 MPC almost totally inhibited its synthesis.

The number of carotenoids compared to the control decreased in the all variants. At the same time, it was observed that the most significant inhibiting effect on the formation of carotenoids was caused by lead nitrate. At 1 MPC concentration, decrease by 79.4% in synthesis of carotenoids occurred. The following increase in lead concentration almost completely inhibited synthesis of carotenoids. Thus, during the experiments, we observed that compared to the synthesis of carotenoids, the process of biosynthesis of chlorophyll a is less sensitive to the impact of lead salts. At the same time, increased synthesis of the pigment can be related to the protective reaction of cells against the toxicant and indicates presence of a mechanism of adaptation to lead in the studied cyanobacteria (Table 2, Fig. 3, 4).



a
b
Fig. 1. Cluster formed by trichomes of cyanobacteria developed by cultivation with 5 MPC lead nitrate: *1* – external trichomes of cyanobacteria, *2* – internal trichomes of cyanobacteria, which form clusters

Fig. 2. Trichomes of cyanobacteria cultivated in the presence of 10 MPC lead acetate: *1* – anomalous increased cell of cyanobacteria

The ratio of sum of carotenoids and chlorophyll *a* (pigment index) is one of the main characteristics of the physiological condition of the cells. Under the influence of lead acetate, this parameter increased up to 7 times compared to the control (Table 3).

Increase in pigment index can indicate an inhibited condition and reduced activity of cyanobacteria.

In the study of the influence of lead on the complex of phycobilin pigments, a stimulating effect of both salts at concentrations of up to 5 MPC was observed (Table 4, Fig. 5). An exception was the concentration of phycoerythrin in the presence of lead acetate (Fig. 5*c*). Increase in lead concentration above 10 MPC totally inhibited the synthesis of phycobiliproteins.

Table 2
Content of pigments in biomass of filamentous cyanobacteria during growth in media with lead acetate and nitrate

Variant	Pigments, mg/g of raw mass			
	chlorophyll *a*		carotenoids	
	lead acetate	nitrate acetate	lead acetate	lead nitrate
Control	0.124 ± 0.006	0.124 ± 0.006	0.125 ± 0.006	0.125 ± 0.006
1 MPC	0.162 ± 0.008*	0.076 ± 0.004**	0.155 ± 0.008*	0.027 ± 0.001**
5 MPC	0.106 ± 0.005	0.080 ± 0.004**	0.109 ± 0.005	0.027 ± 0.001*
10 MPC	0.046 ± 0.002**	0.012 ± 0.001**	0.068 ± 0.003**	0.006 ± 0.001**
15 MPC	0.057 ± 0.003**	0.018 ± 0.001**	0.061 ± 0.003**	0.006 ± 0.003**
20 MPC	0.007 ± 0.001**	0.012 ± 0.001**	0.054 ± 0.001*	0.005 ± 0.001*
25 MPC	0.026 ± 0.001*	0.008 ± 0.001	0.058 ± 0.001*	0.006 ± 0.001

Note: * – P < 0.05, ** – P < 0.01 compared to the control.

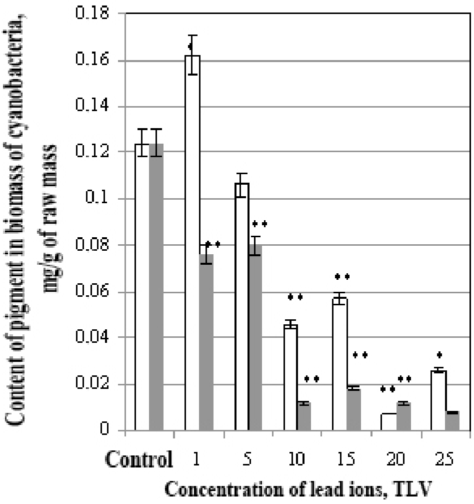


Fig. 3. Content of chlorophyll *a* in the biomass of the cyanobacteria: x ± SD, n = 5, * – values reliably different from the control at P < 0.05; ** – values reliably different from the control at P < 0.01; □ – media with lead acetate, ■ – media with lead nitrate

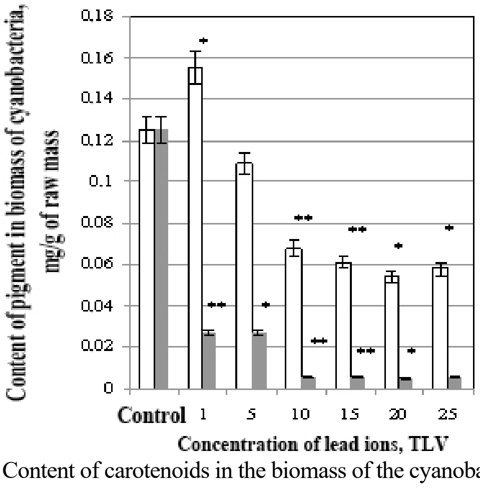


Fig. 4. Content of carotenoids in the biomass of the cyanobacteria: x ± SD, n = 5, * – values reliably different compared to the control at P < 0.05; ** – values reliably different compared to the control at P < 0.01; □ – media with lead acetate, ■ – media with lead nitrate

Investigation of the increase in biomass of the cyanobacteria revealed that lead nitrate demonstrates the higher toxic effect. Lead acetate stimulated a threefold increase in biomass at concentration of up to 5 MPC; inhibition of cyanobacteria growth was observed only at 20 MPC, whereas lead nitrate was significantly weaker in stimulating growth in low concentrations of 1 to 5 MPC (up to 1.5 in total) and inhibited growth of cyanobacteria at 10 MPC (Table 5, Fig. 6).

Table 3
Pigment index of cyanobacteria grown in media with lead acetate and nitrate

Variant	Pigment index	
	lead acetate	lead nitrate
Control	1.01 ± 0.06	1.01 ± 0.06
1 MPC	0.96 ± 0.05	0.35 ± 0.01**
5 MPC	1.03 ± 0.06	0.33 ± 0.01**
10 MPC	1.48 ± 0.08**	0.50 ± 0.03**
15 MPC	1.07 ± 0.07	0.33 ± 0.02**
20 MPC	7.70 ± 0.50**	0.42 ± 0.03**
25 MPC	2.30 ± 0.12**	0.75 ± 0.04**

Note: * – P < 0.05, ** – P < 0.01 compared to the control.

Table 4
Content of phycobilin pigments in biomass of filamentous cyanobacteria grown in media with lead acetate and nitrate

Variant	Pigments, mg/g of raw mass					
	phycocyanin		allophycocyanin		phycoerythrin	
	lead acetate	lead nitrate	lead acetate	lead nitrate	lead acetate	lead nitrate
Control	0.017 ± 0.0009	0.017 ± 0.001	0.005 ± 0.0001	0.005 ± 0.0001	0.014 ± 0.0007	0.014 ± 0.0007
1 MPC	0.033 ± 0.0016*	0.016 ± 0.0008	0.011 ± 0.0005*	0.012 ± 0.0006*	0.004 ± 0.0001*	0.046 ± 0.0023*
5 MPC	0.017 ± 0.0009	0.028 ± 0.0014*	0.006 ± 0.0001	0.011 ± 0.0005*	0.002 ± 0.0001	0.026 ± 0.0013*
10 MPC	0.013 ± 0.0007	0.005 ± 0.0001*	0.006 ± 0.0001	0.005 ± 0.0001	0.002 ± 0.0001	0.021 ± 0.0010*
15 MPC	–	–	0.010 ± 0.0001	–	0.005 ± 0.0001	–
20 MPC	–	–	–	–	–	–
25 MPC	–	–	–	–	–	–

Note: * – P < 0.05, "–" – absence of living cells of cyanobacteria in microecosystem.

Table 5
Increase in biomass of filamentous cyanobacteria (g of raw mass) grown in media with lead acetate and lead nitrate

Variant	Lead acetate	Lead nitrate
Control	2.18 ± 0.01	2.18 ± 0.01
1 MPC	4.72 ± 0.05**	3.43 ± 0.03**
5 MPC	7.07 ± 0.04**	3.72 ± 0.04**
10 MPC	6.67 ± 0.05**	1.99 ± 0.03**
15 MPC	5.31 ± 0.03**	0.34 ± 0.07**
20 MPC	0.66 ± 0.07*	0.65 ± 0.06*
25 MPC	1.08 ± 0.04	0.25 ± 0.07

Note: * – P < 0.05, ** – P < 0.01 compared to the control.

Discussion

During microscopic study of cyanobacteria which developed in the presence of different salts of lead, it was observed that teniae and clusters formed in the process of development were formed by trichomes of cyanobacteria. In the media with lead acetate (1–5 MPC), thick membranes were formed. Every layer of the membrane was formed by the trichomes orientated in the same direction. In the following layer, regulated trichomes were positioned at an angle in relation to the previous layer. At the same time, separate trichomes were observed, which performed a function of transversal conjunctions between the layers. They were arranged in a wavy form and penetrated several surfaces together at the same time or one after another (Fig. 7). In the medium with lead nitrate (1–5 MPC), the cyanobacteria, apart from membranes, formed clusters of the trichomes' flagella.

Development of cyanobacteria in the form of clusters can indicate the formation of a complex spatial structure, where microorganisms interact between one another, performing a cooperative consumption of the substrate and other processes. Similar "collective" behaviour of bacteria has been termed "quorum sensing". Quorum sensing allows bacteria to regulate behaviour and respond to changes in the environment at the level of population (Waters & Bassier, 2005). In clusters formed by dense interlacings of trichomes, a structure is formed which has external and internal layers. At the same time, toxic impact affects only external cells and the total harm to the population is lower. Therefore, cyanobacteria demonstrate the presence of adaptive mechanisms to the toxic influence of lead.

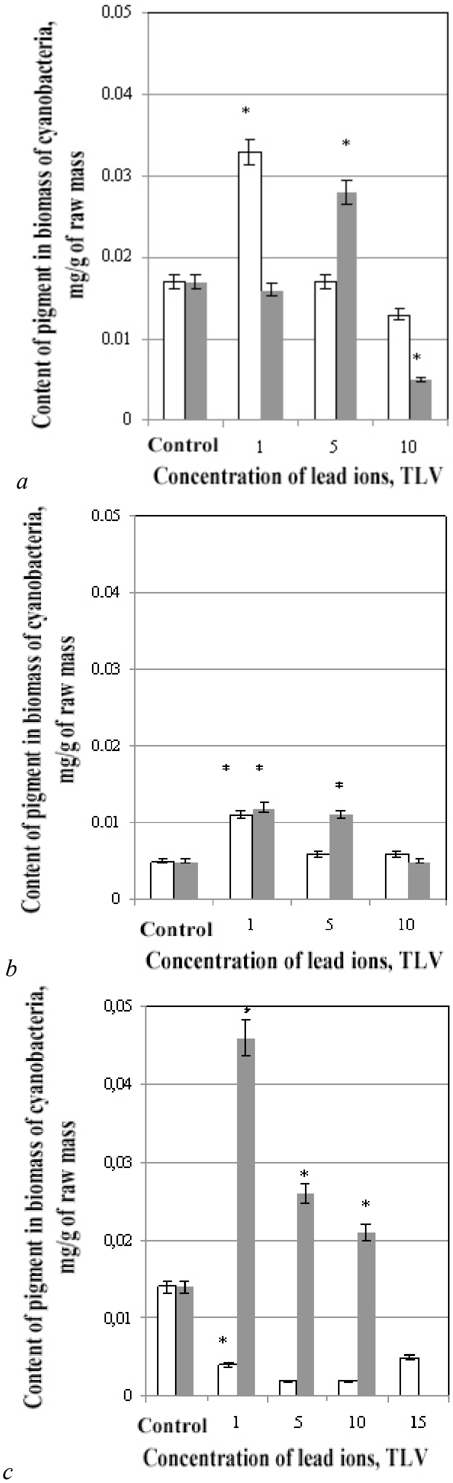


Fig. 5. Content of phycobilin pigments in biomass of cyanobacteria: *a* – phycocyanin, *b* – allophycocyanin, *c* – phycoerythrin; x ± SD, n = 5, * – values reliably different from the control at P < 0,05; □ – media with lead acetate, ■ – media with lead nitrate

Increase in the content of lead above 5 MPC both in nitrate and in acetate forms contributes to formation of teniae from one layer of differently-orientated trichomes. Also, there was observed a decomposition of trichomes into fragments and separate cells united by a common cover. Lead acetate concentration of 15 MPC and lead nitrate at 10 MPC cause formation of anomalously long cells of up to 10.0–10.5 µm length. Deformation of cells and trichomes under the impact of ions of lead is proved by the data on changes in cells of cyanobacteria affected by heavy metals such as nickel, cadmium, cuprum and mercury (Bekasova et al., 2002; Lefebvre et al., 2007; Arunakumara & Xuecheng, 2008). Significant increase in the cell sizes could be caused by disorder in the process of cell division, i.e. separation of processes of growth and division (Pereira et al., 2011; Shanab et al., 2012; Mota et al., 2015). The morphological changes in cyanobacteria which we observed indicate the toxic effect of lead at a concentration of over 5 MPC, both with acetate and nitrate forms.

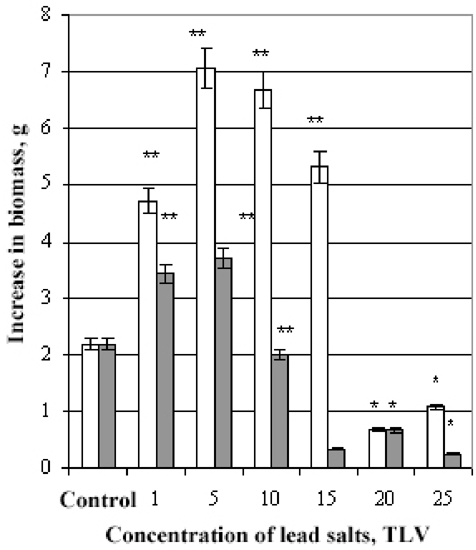


Fig. 6. Increase in biomass of cyanobacteria in presence of different lead salts: x ± SD, n = 5; * – values reliably different from the control at P < 0.05; ** – values reliably different from the control at P < 0.01; □ – media with lead acetate, ■ – media with lead nitrate

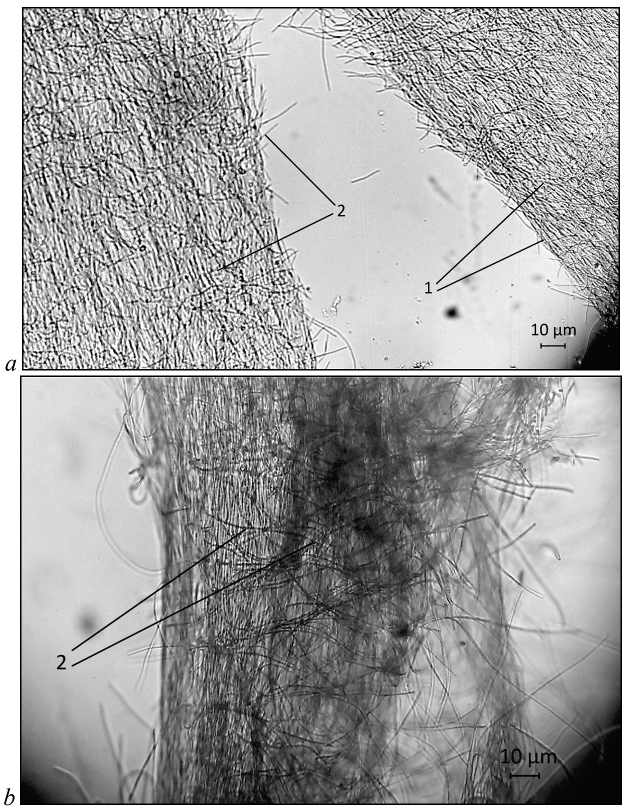


Fig. 7. Tenia of cyanobacteria cultivated in presence of 5 MPC lead acetate: *1* – layer of single direction orientated trichomes, *2* – wavy arrangement of trichomes which penetrate the layer of trichomes orientated in one direction

During the experiment, it was observed that lead had an influence on the pigment complex of cyanobacteria. Lead acetate in 1 MPC concentration stimulated synthesis of chlorophyll a and carotenoids by 30.6% and 24.0% respectively. Further increase in the concentration of lead acetate caused inhibition of synthesis of chlorophyll a by 94.4%, and carotenoids – by 56.8%. Lead nitrate showed a clear inhibiting effect on the synthesis of photosynthetic pigments. Concentration of 1 MPC decreased synthesis of chlorophyll *a* by 38.8%, and carotenoids – by 79.4%. Further increase in the concentration causes practically total inhibition of synthesis of these pigments. Decrease in the intensity of photosynthetic processes due to inhibition of synthesizing chlorophyll *a* is one of the first signs of intoxication with heavy metals. It could be caused by change in the functional structure of the thylakoids: increase in the space between the thylakoids and accumulation of particles of the metals there, formation of membrane vesicles. Intense decrease in concentration of both chlorophyll *a* and carotenoids can indicate the irreversible degradation effect of the toxicant (Heng et al., 2004; Ajayan et al., 2011; Wong et al., 2014; Deep et al., 2016).

The impact of lead on phycobiliproteins is variable. We observed a stimulating effect of lead acetate on synthesis of phycocyanin and allophycocyanin in concentrations of up to 10 MPC; lead nitrate on synthesis of phycocyanin in concentrations of up to 5 MPC and on synthesis of allophycocyanin and especially phycoerythrin – in concentrations of up to 10 MPC. Increased synthesis of some pigments can be related to manifestation of mechanisms of adaptation to lead among cyanobacteria (El-Sheekh et al., 2005; Kiran et al., 2008). Some of them can be manifested in transformation of heavy metal ions into less toxic particles due to phycobilin pigments (Bekasova et al., 2002; Andrade et al., 2004; Clares et al., 2015).

Investigation of the impact of salts of lead on the increase in the biomass of cyanobacteria revealled a stimulating effect of lead acetate of up to 15 MPC concentration inclusive. Maximum increase in biomass by 223.8% was observed after adding lead acetate in the amount of 5 MPC. At the same time, addition of lead nitrate stimulated development of biomass to a far lower extent, having maximum increase in biomass by 70.3% only at 5 MPC. Increase in the concentration contributed to inhibition of cyanobacteria' growth.

Thus, comparing the impact of different salts of lead on morphological and physiological parameters of cyanobacteria, a stronger inhibitting effect was demonstrated by lead nitrate than by lead acetate. This could be related to the fact that when lead nitrate is added to the solution, a large part of the metals is in Pb^{2+} forms (up to 40%) and $Pb(OH)^{+}$ (up to 20%) (Minkina et al., 2014). When lead acetate is added, a strong complex cation Pb(Ac) is formed, the toxicity of which is perhaps lower than that of ion Pb^{2+} (Temraleeva & Pinskii, 2010). The literature also mentions that acetate ion can intensify absorption of positive lead particles on the surface of biomass of cyanobacteria and therefore prevent penetration of metal ions into the cells (Zinicovscaia et al., 2017). Also, acetate ion can stimulate the development of heterotrophic associations of cyanobacteria which participate in inactivation of heavy metal ions (Bekasova et al., 2002; Temraleeva & Pinskii, 2010).

Through our experimental studies, we determined the threshold values of lead salts (1–5 MPC), when the stimulating effect on growth and development of filamentous bacteria was observed, which manifests in maximum increase in biomass and synthesis of chlorophyll *a* and carotenoids. Above those concentrations, we observed a stable inhibition of increase in biomass, content of photosynthetic pigments, and also morphological changes in the cells of the cyanobacteria, which indicate the unfavourable effect of the toxicant. Concentration of lead salts of 20–25 MPC totally inhibit the vitality of cyanobacteria.

Conclusions

Low concentrations (up to 5 MPC) of lead salts (acetate and nitrate) can stimulate growth and development of filamentous cyanobacteria, which manifests in increase in biomass, synthesis of chlorophyll *a*, carotenoids and phycobilin pigments. Lead acetate can form biomass in the form of membranes and teniae, and lead nitrate clusters. Increase in lead concentration of up to 10–25 MPC inhibits growth and development of cyanobacteria down to total discolouration of teniae and absence of vital cells, which was proven by luminescence microscopy. At the same time, the accumulation of biomass slows the concentration of photosynthetic pigments in the cells decreases and morphological changes are observed: increase in the length of the cells and decomposition of trichomes into separate fragments. Maximum inhibiting effect was demonstrated for lead nitrate.

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