

# Using Basaltic Tuff for Decreasing the Growth Activity of Cyanobacteria

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**Abstract.** The article is devoted to the study of the cultivation of cyanobacteria *Microcystis aeruginosa* (Kützinger) Kützinger, *Microcystis pulvere*a (H.C.Wood) Forti. in the presence of basalt tuff. The possibility of using basalt tuff as an adsorbing material for regulating the number of cyanobacteria and preventing toxic “water bloom” in fluid circuits.

The study analyzes the change in the biomass amount and the dynamics of the proportion of dead cyanobacteria in response to the presence of basalt tuff in the culture medium. It is noted that the use of basalt tuff leads to a decrease in the growth activity of cultures of *Microcystis aeruginosa*, *Microcystis pulvere*a, manifested in an increase in the number of dead cells and slowdown the accumulation of cyanobacterial biomass. These effects are the result of a decrease in the amount of available nitrogen in the nutrient medium.

## Introduction

The problems caused by the “water bloom” and the massive development of cyanobacteria and microalgae are again attracting the attention of researchers all over the world. The progressive spread of common species of cyanobacteria and microalgae is a consequence of the uncontrolled use of natural water resources, which is now observed in most regions of both Ukraine and the whole world. The negative consequences of this process are aggravated by the fact that in some cases the “bloom” is accompanied by the release of biologically active and toxic substances into the aquatic environment. These compounds are pyrogenic and toxic, they cause skin irritation and allergic reactions in both people and animals [1,2], and can also be fatal in the population. Toxins that are in the cells of cyanobacteria, after the destruction of these cells enter the aquatic environment. They are very stable, not destroyed by chlorination of water, and toxins are stored in dry cells [3-5]. The use of algacidal preparations does not always make it possible to avoid the mass death of cyanobacteria and does not prevent the toxins from entering the aquatic environment.

Therefore, it is necessary to search for methods that allow regulating the number of cyanobacteria in the aquatic environment, while not allowing their mass reproduction and release of toxins into the environment. This can be avoided by filtration, water exchange, aeration, and adsorption methods [6-8]. The main thing when choosing a method is the ability to control the number of both vegetative cells and the spores of cyanobacteria. Unfortunately, most of these methods are cumbersome and expensive.

One of the alternative ways to prevent the massive development of cyanobacteria can be the use of adsorption methods. The need for materials with adsorbing properties puts forward a number of requirements relating to their technological efficiency, economic feasibility and biological activity. Adsorbing materials must have a high specific surface area, be non-toxic to invertebrates and fish and do not need complex sample preparation [9]. Promising adsorbing material with polyfunctional properties are basalt tuffs, which have found their application in various fields. Basalt tuffs are volcanogenic minerals that are structurally similar to zeolites in chemical composition. These are natural aluminosilicates, which in explored deposits are represented by igneous rocks and minerals. High chemical and thermal stability, significant deposits in the depths of Ukraine give reason to consider basalt tuff (BT) as a promising mineral raw material, and the near-surface tuff deposits make it possible to mine them in an open way [10,11].

It is known that tuff is a polyfunctional adsorbent and can be used to purify water from various types of contaminants. Basalt tuff, as a powerful natural sorbent, has a high selectivity of

absorption and the ability to separate the size of ions and molecules of various substances, a sufficiently high mechanical and chemical resistance. In the course of its operation, it changes little of its physicochemical properties, retains a high ion-exchange selectivity to a variety of chemical elements [12].

The aim of the work was to assess the effect of basaltic tuff on monocultures of cyanobacteria *Microcystis aeruginosa* (Kützinger) Kützinger, *Microcystis pulvereae* (H.C.Wood) Forti.

## Materials and Methods

The studies were conducted using cultures of cyanobacteria *Microcystis aeruginosa* (Kützinger) Kützinger. and *Microcystis pulvereae* (H.C.Wood) Forti. The original accumulative cultures were cultivated in a climatic room with a 16-hour photoperiod and a temperature of  $24 \pm 2^\circ\text{C}$  on a modified Fitzgerald medium until the exponential growth phase. These cultures served as a source of material for studying the effect of basaltic tuff on the growth activity of cyanobacteria. Layer waste water from RAS [13] was used as a nutrient medium. All manipulations with cyanobacterial cultures were carried out in laminar boxing conditions.

We used samples of minerals obtained from the Polytske-2 field. Samples are characterized by the following composition: zeolites 35-40%, montmorillonites 30-40%, feldspar 10-15%, silica 4-5%, hematite 3-5% [14]. Basalt tuff was introduced into the nutrient medium in the range from 5 to 50 g/l of medium. Concentrations were selected based on recommendations in the literature and previous developments of our laboratory.

The exposure of basalt tuff monocultures of *M. aeruginosa* and *M. pulvereae* was 5 days. In the course of cultivation, the pH of the nutrient medium, the density of algoculture, biomass accumulation, the change in the number of dead cells, the change in the number of different forms of nitrogen in the nutrient medium were monitored and the toxicity coefficient of basalt tuff on cyanobacteria cells was calculated.

Determination of the pH of the nutrient medium was carried out by potentiometric pH measurement using a glass electrode (U-160 MU ion meter).

The accumulation of biomass was analyzed by changing the optical density of the culture at a wavelength of 750 nm on CaryWin UV 60 (Agilent, USA). In the future, we carried out calculations of dry biomass through the experimentally established coefficient  $k$ :  $\text{ADB} = k \times D_{750}$  ( $k = \text{g} / \text{l} / \text{u.opt. density}$ ) [15].

Suspension cultures were colored with vital dyes (methylene blue and neutral red - 1: 5000). Cell counting was performed using a Fuchs-Rosenthal camera and a Micromed XS-3300 trinocular microscope. The percentage of dead cells was calculated as a percentage of the total number of cells [16].

The amount of nitrate, nitrite and ammonium nitrogen was determined by standard methods. The basis of these methods is the ability of nitrogen-containing compounds with appropriate reagents to form colored products, which are subsequently detected on a spectrophotometer. The quality reagent for the determination of nitrate nitrogen is Rochelle salt, nitrite is Griess reagent, and ammonium is potassium tetraiodomercurate [17].

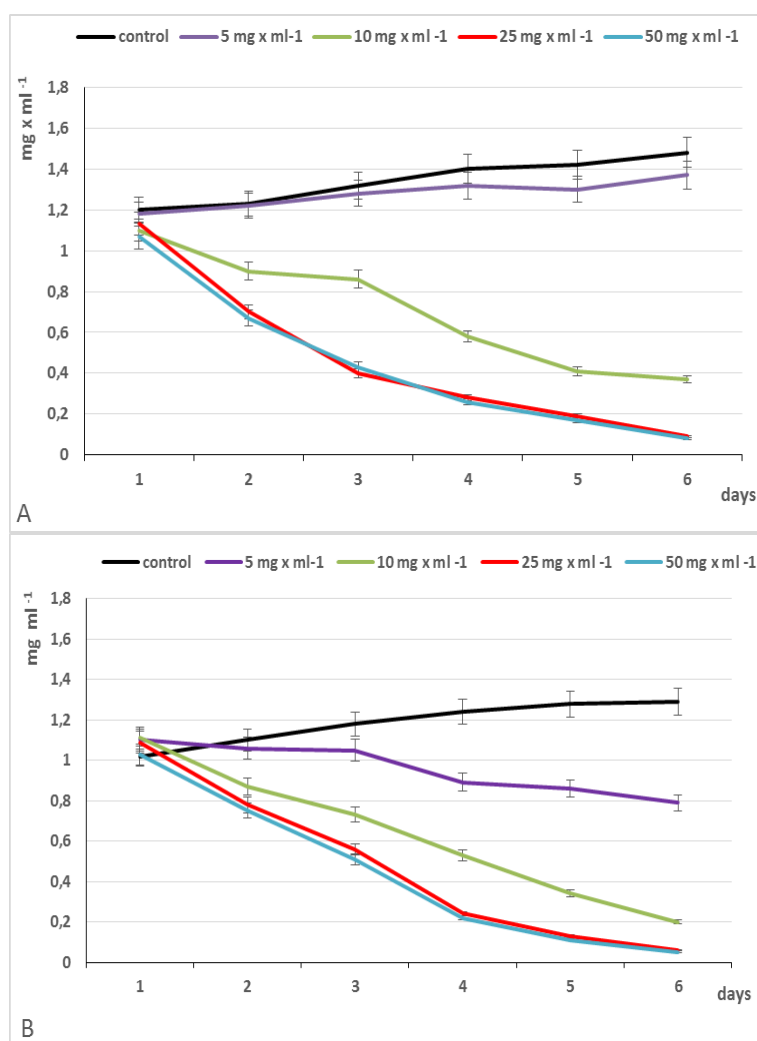
By the number of cells in the cyanobacteria culture, before and after exposure to basalt tuff, the coefficient of death of culture was calculated by the ratio of the number of dead cells to the total number of cells.

Statistical processing of the obtained results was performed using Microsoft Excel software. Differences in the results discussed in the work are likely at a significance level of  $p \leq 0.05$  by the Student's criterion. Quantitative determinations were carried out in 3-fold repeatability.

## Results and Discussion

Under laboratory conditions, a situation of mass development of cyanobacteria was modeled. To this end, the cyanobacteria *M. aeruginosa* and *M. pulvereana* were cultivated on Fitzgerald modified medium until the exponential growth phase was over. At this stage, monocultures were characterized by the number of cells in suspension at the level of  $3 \times 10^6$ – $5 \times 10^6$  cells / ml. Such a number of cells corresponds to the situation of uncontrolled mass development of cyanobacteria in natural waters with a high level of eutrophication [18,19].

Initially, the growth activity of the cultures was analyzed in terms of biomass accumulation. To determine the biomass its indirect study through an indicator of optical density of the culture was used. It has been established that the presence of basalt tuff in a nutrient medium leads to inhibition of the growth activity of both cultures of cyanobacteria (Fig. 1).

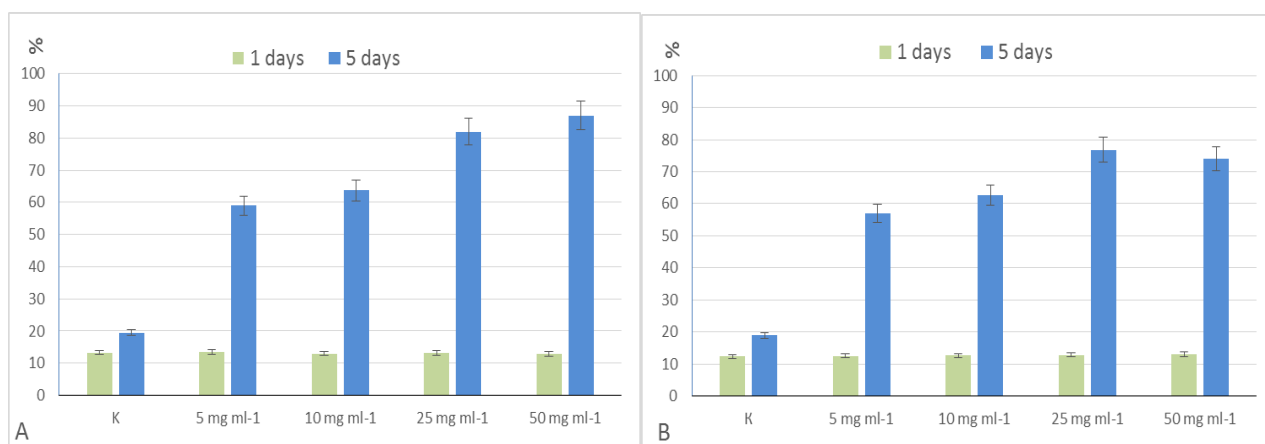


**Fig. 1.** Dynamics of biomass of cyanobacteria in the presence of basaltic tuff, where: A - *M. aeruginosa*, B - *M. pulvereana*

At the same time, there was a difference in the reaction to the presence of tuff depending on the species of the culture and the concentration of tuff in the culture fluid. Thus, the initial culture of *M. aeruginosa* was characterized by a slightly higher growth activity than the culture of *M. pulvereana*. This dependence persisted when the basalt tuff was introduced into the medium. The introduction of tuff at a concentration of 5 mg / ml had practically no effect on the accumulation of biomass, neither in the *M. aeruginosa* culture, nor in *M. pulvereana*. Significant differences in the suppression of growth activity at this concentration were observed only starting from the third day of cultivation. An increase in the amount of basalt tuff in the nutrient medium when growing cyanobacteria affected the intensity of biomass accumulation in both of the studied cultures.

A crucial decrease in the number of cells in the culture of *M. aeruginosa* and *M. pulvereana* was achieved when basalt tuff was used in the amount of 25 and 50 mg / ml of the nutrient medium. Under these conditions, a sharp decrease in the amount of biomass starting from 24 hours of exposure was observed in both studied cultures of cyanobacteria. So, for the culture of *M. aeruginosa*, if 25 mg / ml of basaltic tuff is added to the medium, a 15-fold decrease in biomass was established, and for *M. pulvereana* - 12-fold. The use of the adsorbent in the amount of 50 mg / ml did not lead to a significant suppression of growth activity.

Later, using differential staining of culture cells, we conducted a study of the viability of cyanobacterial cultures exposed to basalt tuff. When the membranes of unicellular organisms are damaged, the dye easily penetrates the cells, their contents turn blue. Such cells are considered dead. In living cells, the dye is localized on the surface apparatus of the cell, and they turn red (29,38). Against the background of the suppression of the biomass buildup, a gradual cell death was also observed in the culture of both cyanobacteria studied (Fig. 2). So, on the 5th day of exposure, the number of dead cells in the culture of *M. aeruginosa* increased 4.5 times with 10 mg / ml basalt tuff and 8.9 times with the maximum concentration of tuff in the nutrient medium. On the fifth day of incubation with 25 mg / ml basalt tuff, only about 10% of the cells in the *M. aeruginosa* culture remained viable.



**Fig. 2.** The proportion of dead cells in cyanobacterial cultures in the presence of basalt tuff, where: A - *M. aeruginosa*, B - *M. pulvereana*

Similar results, however somewhat less pronounced, were obtained for the *M. pulvereana* culture: here, viability after incubation in the presence of 25 mg / ml basalt tuff was maintained in about 20% of the culture. The use of twice the amount of the studied adsorbent (50 mg / ml) did not lead to a significant increase in the proportion of dead cells in both the *M. aeruginosa* culture and the *M. pulvereana* culture.

Reducing the proportion of living cells in culture allows us to predict that for a long time such a culture will adapt to the changed conditions of existence, its growth activity will remain low, which will avoid the mass development of cyanobacteria and, as a consequence, the release of toxins into the aquatic environment.

Based on the number of live and dead cells in the cyanobacteria culture, the coefficient of death of culture of basalt tuff was calculated (Table 1). As noted earlier, basalt tuff at a concentration of 5 mg / ml showed no toxic effect on cultures of the cyanobacteria studied. A slightly more pronounced toxic effect was when applying tuff at a concentration of 10 mg / ml. And only an increase in concentration to 25 and 50 mg / ml led to a significant suppression of growth activity and toxicity. At the same time, no significant difference was found when using these two concentrations of the adsorbent.

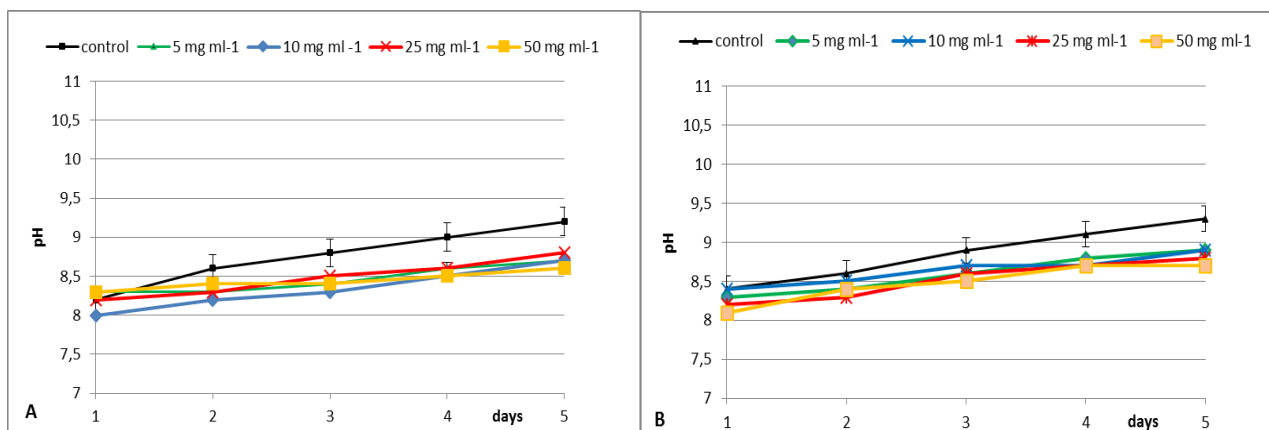
**Table 1.** Coefficient of death of culture of basalt tuff for cyanobacteria monocultures

Concentration of basalt tufts	Kd, %	
	<i>M. aeruginosa</i>	<i>M. pulvereae</i>
5 mg ml <sup>-1</sup>	10 ± 0,9	8 ± 0,5
10 mg ml <sup>-1</sup>	29 ± 1,6	24 ± 1,3
25 mg ml <sup>-1</sup>	88 ± 7,8	79 ± 6,9
50 mg ml <sup>-1</sup>	91 ± 8,2	80 ± 8,5

According to some authors [8,20], low concentrations of adsorbents do not lead to inhibition of growth and development of cyanobacteria, but, on the contrary, can stimulate biomass growth due to the positive effect on the dynamics of nutrient medium indicators. Similar results were obtained when studying the effect on ammonium sulfate mixtures on cyanobacteria together with copper sulfate. Under such conditions, even in fairly high concentrations of chemical reagents, the number of cyanobacteria cells in the studied natural water decreased by only 50% with an exposure of 5 days.

Another factor of positive impact can be the ion-exchange properties of basalt tufts [14]. Thus, in the study of the ion-exchange properties of tuff in water, the enrichment of the latter with bioactive elements was established. As a result of ion exchange, the content of iron, zinc, manganese, and potassium increased in water. As it is known, the main nutrient media for algae contain aqueous solutions of mineral salts, which provide algocultures with all the necessary elements. Their additional intake in small quantities may be the cause of a slight increase in biomass on the first days of cultivation of *M. aeruginosa* and *M. pulvereae* when using basalt tuff in the amount of 5 and 10 mg / ml.

The main limiting cultivation factor in the periodic system of microalgae is the depletion of the nutrient medium, the accumulation of target metabolites and metabolic products, which inevitably leads to changes in the pH of the cultivation medium. The temperature and pH of the medium is one of the most important abiotic factors regulating the rate of accumulation of the biomass of cyanobacteria. For each organism, there are optimal indicators of temperature and pH. The active response of the environment depends on the concentration of hydrogen, one of the most fundamental factors of life in its various manifestations. Resistance to changes in pH in different organisms is different; most cyanobacteria are characterized by a neutral or slightly alkaline reaction of the medium, the optimum pH is 7.2 - 7.5 [16]. The acidity of the environment affects the stability of the components of the nutrient medium, their availability to cyanobacteria, especially the absorption of growth factors. For most algocultures, it is precisely pH changes that are the driving factor for the attenuation of growth activity during long-term cultivation. The low growth activity of the *M. aeruginosa* and *M. pulvereae* cultures, which we noted when basalt tufts were used, could be related precisely to abrupt pH changes. However, we did not observe a similar phenomenon during the incubation of both the studied cultures in the presence of tuff (Fig. 3). On the contrary, the use of tuff allowed to maintain the pH value of the medium within the recommended limits. At the same time, a positive result did not depend on the concentration of the material used and was not significantly different for both types of cyanobacteria. Most likely, the inhibition of the growth activity of these algocultures is not associated with changes in pH.

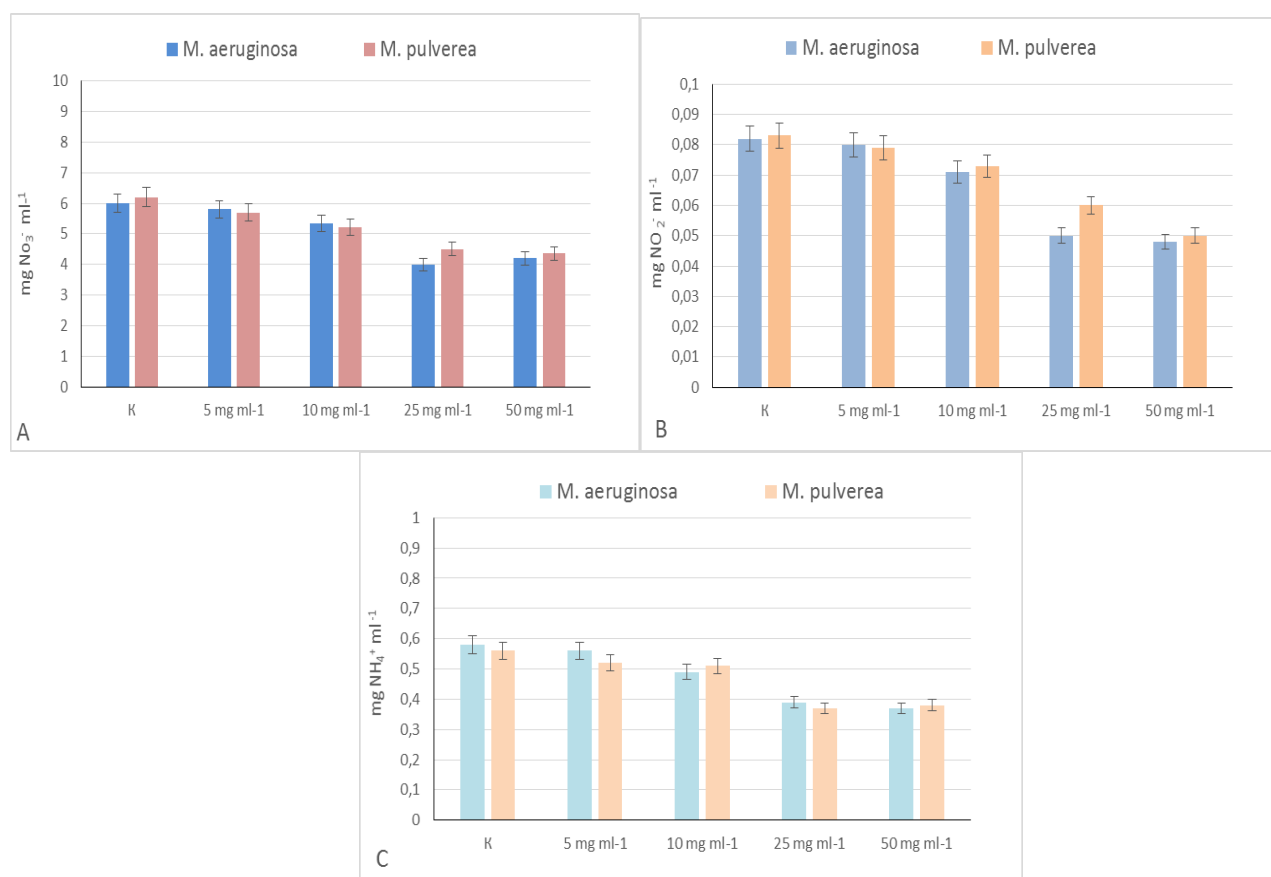


**Fig. 3.** Dynamics of pH in cyanobacterium cultures in the presence of basaltic tuff, where:  
A - *M. aeruginosa*, B - *M. pulvereae*

Considering the results obtained, our next step was to study the effect of the presence of basalt tuff on indicators of the amount of various forms of nitrogen in the nutrient medium for cyanobacteria. Previously, we showed the possibility of cultivating cyanobacteria on wastewater from a hatchery of a closed water supply as a nutrient medium with a high content of nutrients [21]. In most cases, nutrient media form the basis of aqueous solutions of inorganic salts containing phosphorus, nitrogen, potassium, and other macro- and microelements. As is known, it is the amount and ratio of various forms of nitrogen and phosphorus that are decisive in the composition of the nutrient medium for algocultures [22]. The high content of nutrients due to excess nitrogen and phosphorus contained in fertilizer-rich wastewater from agricultural land, wastewater from livestock farms, fish ponds, allows using these wastewaters as alternative nutrient media for the cultivation of microalgae. Both sea and freshwater algae can absorb inorganic nitrogen compounds ( $\text{NO}_3^-$  nitrates), nitrites ( $\text{NO}_2^-$ ) and ammonium ( $\text{NH}_4^+$ ). Regardless of the content in the water of certain forms of nitrogen, their assimilation and inclusion in the organic compounds of algal cells occurs only through the conversion of  $\text{NH}_4^+$ . This process has a two-stage character and is associated with an intracellular nitrate reductase complex. At the same time, free ammonia is poisonous, therefore, getting inside the cells, it does not accumulate, but is immediately used in biosynthetic processes [23]. The inclusion of ammonium ion in the process of biosynthesis of organic compounds occurs with the participation of the glutamine synthetase reaction. Under conditions of crucially reduced nitrogen content aquatic environment, the cells are nitrogen-deficient, the process of utilization of  $\text{NO}_3^-$  and  $\text{NH}_4^+$  can occur at night, but in daylight its intensity is particularly high.

As one of the most important nutrients, nitrogen (mainly in the form of nitrates) significantly affects the biological productivity of aquatic ecosystems. In optimal concentrations, it causes increased production of phytoplankton, phytobenthos, and higher aquatic plants. The lack of mineral nitrogen leads to a decrease in the intensity of photosynthesis in plants.

We analyzed the amount of various forms of nitrogen in the nutrient medium when *M. aeruginosa* and *M. pulvereae* were cultivated in the presence of basalt tuff (Fig. 4). It is noted that the introduction of basalt tuff into the nutrient medium reduces the amount of available nitrogen in various forms by 10–20%, depending on the amount of material used. A decrease in the amount of nitrate nitrogen in the medium was observed when tuff was used in the amount of 25 and 50 mg / ml.



**Fig. 4.** Changing the amount of nitrate (A), nitrite (B) and ammonium (C) nitrogen in the nutrient medium in the presence of basaltic tuff

When using tuff in lower concentrations (5 and 10 mg / ml), no significant changes in the amount of nitrate nitrogen were found. Similar trends were noted in the study of the amount of nitrite nitrogen in the nutrient medium during the cultivation of *M. aeruginosa* and *M. pulvereia*. However, significant changes are noted already with the use of tuff at a concentration of 10 mg / ml, while the use of the material under study in an amount of 25 and 50 mg / ml led to similar results. During 5 days of incubation, the amount of nitrite nitrogen in such conditions decreased by 1.7 times.

Similar patterns can be traced in the study of changes in the amount of ammonium nitrogen. During the incubation period of cyanobacteria with basalt tuff, about 35% of the total amount of NH<sub>4</sub><sup>+</sup> was derived from the nutrient medium. It should be noted that ammonium and nitrate nitrogen under certain conditions are equivalent sources of nitrogen for plants. The predominant absorption of ammonium nitrogen occurs when NH<sub>4</sub><sup>+</sup> is the only source of nitrogen [16,17]. The use of ammonium or nitrate nitrogen by plants depends on a number of factors, the most important of which are: the biological characteristics of the plant species, the availability of carbohydrates, the reaction of the environment, the presence of calcium, potassium and other nutrients, including trace elements.

In our opinion, it is the withdrawal of a sufficiently large amount of nitrogen-containing compounds from circulation that directly affects the growth activity of both cyanobacteria cultures studied. Since basalt tuff as a polyfunctional material actively adsorbs nitrogen-containing compounds, their amount is insufficient to meet the needs of algocultures.

So, using the example of two monocultures of cyanobacteria, we have shown the possibility of using basalt tuff as an adsorbing material for regulating the number of cyanobacteria and preventing toxic “water bloom” in aquatic systems. Such an approach will allow controlling mass outbreaks of cyanobacterial biomass accumulation both in controlled systems of the RAS type and in open water bodies.

## Conclusions

The inhibition of the growth activity of monocultures of cyanobacteria *M. aeruginosa* and *M. pulvereae* in the presence of basalt tuff in the nutrient medium has been established. During the 5 days of incubation with 25 mg / ml basalt tuff, the amount of biomass in the *M. aeruginosa* culture decreased 15 times, and in the *M. pulvereae* culture 12 times.

In the presence of basalt tuff in a nutrient medium against the background of a decrease in biomass, an increase in the number of dead cells in monocultures of cyanobacteria occurs. After incubation in the presence of 25 mg / ml basalt tuff, about 10% of the *M. aeruginosa* culture and 20% of the *M. pulvereae* culture remained viable.

The introduction of basalt tuff into the nutrient medium leads to a decrease in the number of available nitrogen forms for cyanobacteria by 10–20%, which results in the suppression of the growth activity of *M. aeruginosa* and *M. pulvereae*.

Basalt tuff at a concentration of 25 mg / ml of the nutrient medium is recommended to be used as an adsorption material for regulating the number of cyanobacteria.

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## Conflict of interest

There is no conflict of interest between authors in the publication of this paper.

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