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THE EFFECT OF HEAVY METALS ON THE NITROGEN FIXATION ACTIVITY OF CYANOBACTERIA

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Abstract

In this study, the effect of heavy metals Cu^{2+} and Co^{2+} on the nitrogen fixation activity of salt-tolerant cyanobacteria Nostoc calcicola and Anabaena variabilis at concentrations of 10–50 mM was studied. According to the results determined based on the acetylene reductase method, nitrogen fixation activity was maintained at 85.30–97.26% at concentrations in the range of 10–30 mM, while at a concentration of 50 mM, nitrogen fixation activity in different strains decreased to an average of 71.54%. The differential resistance of cyanobacteria to Cu^{2+} and Co^{2+} ions allows them to be considered as promising microorganisms for bioremediation processes in areas contaminated with metals.

Keywords: Cyanobacteria, heavy metals, nitrogen fixation, acetylene reductase, Cu^{2+}, Co^{2+}, bioremediation, Nostoc calcicola, Anabaena variabilis.

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Introduction

In recent decades, as a result of the rapid pace of industrialization, the expansion of mining and metallurgy, chemical industry, agriculture and municipal activities, the level of heavy metals entering the biosphere components has increased sharply [1,2]. Such pollutants, in particular, elements such as copper (Cu), cobalt (Co), lead (Pb), chromium (Cr), cadmium (Cd) and zinc (Zn), are characterized by their long-term persistence in ecological systems, bioaccumulation and high levels along the trophic chain [3]. Their non-biodegradability makes heavy metal ions a global environmental threat. These metals lead to a violation of the redox balance in living organisms, disruption of protein biosynthesis and enzymatic processes, inhibition of cell division and growth processes [4].

Cyanobacteria are photosynthetic prokaryotes of great ecological importance. As the main microorganisms carrying out the nitrogen fixation process, they are an important component of primary production in agricultural ecosystems, water bodies and desert landscapes [5]. With the participation of heterocyst-forming species, molecular nitrogen from the atmosphere is converted into biologically active forms, which is an integral part of the natural nitrogen cycle. At the same time, cyanobacteria produce polysaccharide extracellular polymers (EPS), which enhance the mechanism of resistance to heavy metal stress by binding metal ions and providing membrane stability [6,7].

From an ecological point of view, the study of heavy metal-resistant cyanobacteria is of particular importance in the development of technologies for the bioremediation of inorganic toxicants from contaminated soil and water systems using microorganisms. Against the background of increased heavy metal pollution in a number of regions of Uzbekistan due to industrial effluents, mine wastes, and irrigation systems, it is scientifically and practically relevant to determine the degree of adaptation of local microbial collections to these conditions [8].

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In this regard, the present study investigated the effects of heavy metal ions Cu^{2+} and Co^{2+} on the nitrogen fixation activity of local salt-tolerant cyanobacterial strains in a wide concentration range (10–50 mM). The main objectives of the study were to determine the tolerance threshold of these strains to toxic metal ions, assess changes in their nitrogen fixation metabolism, and determine their potential for bioremediation.

Materials and Methods

The cyanobacteria Nostoc calcicola and Anabaena variabilis, obtained from local collections, were used as the object of the study. Cyanobacteria were grown in standard mineral nutrient medium under sterile conditions. In order to assess the effect of heavy metal ions, solutions of Cu2+ and Co2+ salts with concentrations of 10, 30 and 50 mM were prepared and used in accordance with the experimental variants. The cultivation process lasted 7-20 days, during which the visual growth rate, pigmentation level and general physiological condition of cyanobacteria were monitored.

Nitrogen fixation activity was determined by the acetylene reductase reaction (ARA), acetylene gas was injected into each strain, and the amount of ethylene produced was recorded using gas chromatography. The results were compared with standard samples and expressed as a percentage of the initial control activity. To ensure the reliability of the experimental data, each measurement was repeated at least three times, the average values were calculated, and the statistical errors (±SE) were determined. Differences between parameters were evaluated using analysis of variance (ANOVA).

Results and Discussion

During the study, the nitrogen fixation activity (acetylene reductase reaction - ARA) of cyanobacterial strains was determined at different concentrations of heavy metal ions such as Cu^{2+} and Co^{2+}, and their physiological tolerance limits

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were assessed. The results were systematized in the form of Figures 1–2 and Table 1–2.

Effect of Cu^{2+} ions

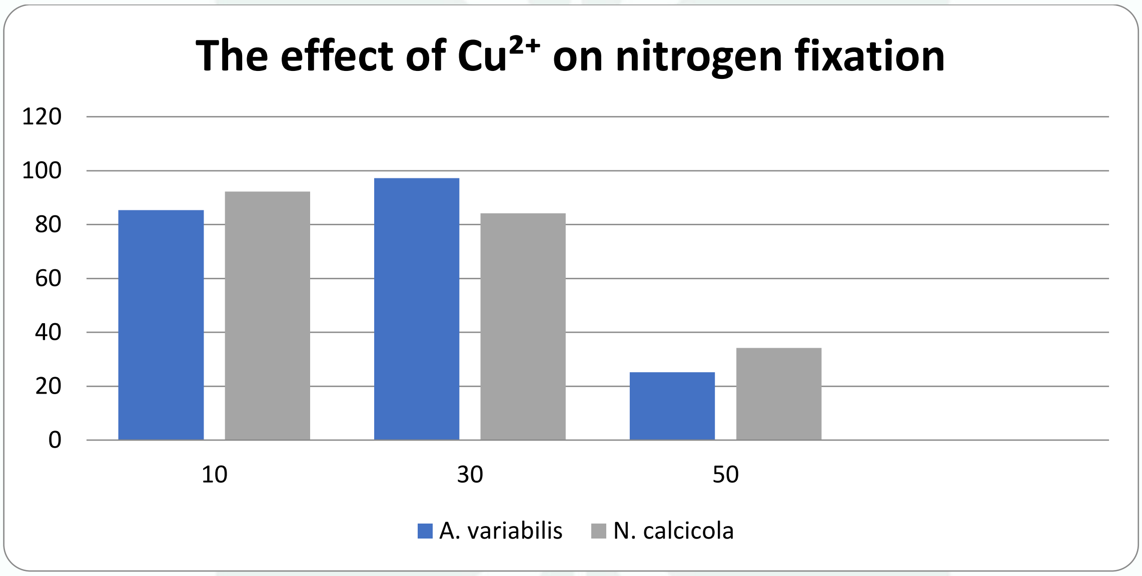

Figure 1. Effect of Cu^{2+} ion on nitrogen fixation activity of cyanobacteria at concentrations of 10–50 mM

A. variabilis — 10 mM (85.30 %), 30 mM (97.26 %), 50 mM (25.20 %)

N. calcicola — 10 mM (92.18 %), 30 mM (84.21 %), 50 mM (34.17 %)

According to the data presented in the figure, although ARA activity was significantly preserved in the strains studied at 10–30 mM concentrations of Cu^{2+}, a 50 mM concentration of the ion had a toxic effect for all strains.

Table 1. ARA activity at different concentrations of Cu^{2+} ion (% relative to the initial value)

Strain	10 mM	30 mM	50 mM
A. variabilis	85.30 %	97.26 %	25.20 %
N. calcicola 25	92.18 %	84.21 %	34.17 %

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The relatively stimulating effect of Cu^{2+} at low concentrations is due to the role of the metal as a cofactor of plastocyanin, nitrate reductase and some oxidoreductase complexes. However, at a level of 50 mM, i.e. in thylakoid membranes, lipid peroxidation, disruption of photosynthetic electron transport, degradation of Fe–S clusters are observed, which undermines the stability of the nitrogenase enzyme complex. As a result, ARA activity decreases sharply [5].
The highest resistance among the strains was noted in A. variabilis and N. pruniforme. This is explained by the fact that these strains synthesize more EPS (extracellular polysaccharides).

Effect of Co^{2+} ions

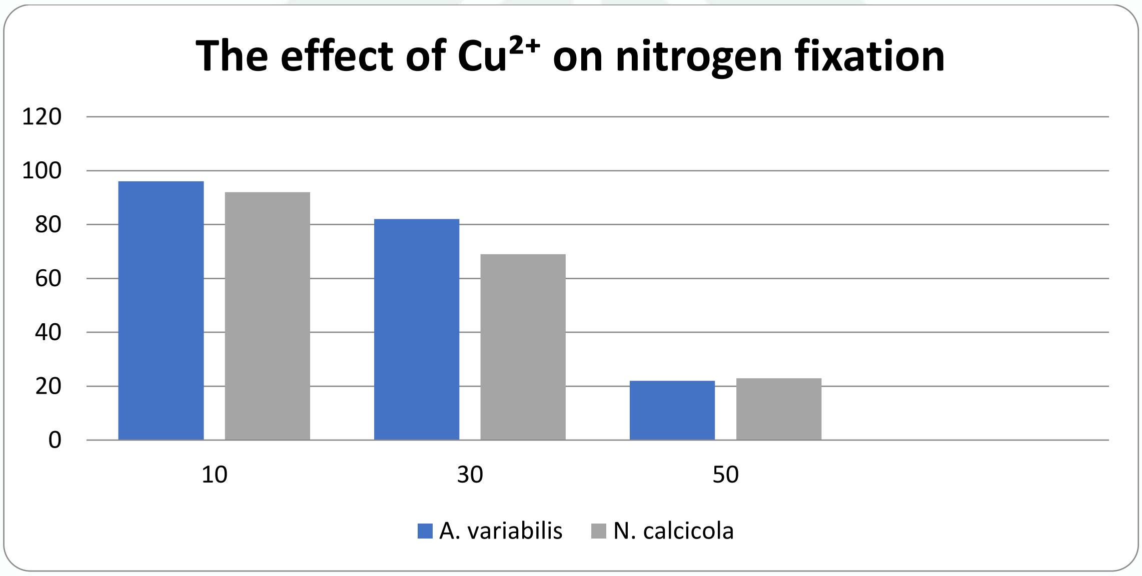

Figure 2. Effect of Co^{2+} ion on cyanobacterial ARA activity at concentrations of 10–50 mM

10 mM: 92–97%, 30 mM: 66–82.5%, 50 mM: average 21–30% reduction (67% reduction)
The biological role of Co^{2+} is different from Cu^{2+}: it is an important element in the biosynthesis of cobalamin (Vitamin B_{12}) and is directly involved in the process

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of nitrogen fixation. Therefore, at low concentrations, a stimulating effect of Co^{2+} has been observed.

Table -2ARA activity of Co^{2+} ion at different concentrations (in %)

Shtamm	10 mM	30 mM	50 mM
N. calcicola	97.32 %	83.15 %	23.12 %
A. variabilis	93.22 %	70.18 %	24.16 %

At 10 mM Co^{2+}, the nitrogen fixation process is almost completely preserved. This is due to the support of cobalamin synthesis, the stability of nitrogenase cofactors, and the processes of DNA replication and cell division, while at 30 mM, due to the excessive accumulation of the ion, ribonucleoprotein complexes, ferredoxin exchange, and energy metabolism are slowed down.

At a concentration of 50 mM, Co^{2+} disrupts cell stability and a violation of the membrane potential is observed as a result of ion transport.

Growth dynamics and physiological state During the experiment, good growth was observed in all strains for 7–8 days, and degradation and autolysis of cells were observed for 15–20 days.

This indicates an increase in oxidative stress associated with heavy metal ions. Especially in the 50 mM variants, a decrease in chlorophyll a, degradation of carotenoids, and thinning of the cell wall were observed.

The data obtained in this study are significant in that they more accurately indicate the tolerance limits of cyanobacteria to heavy metal ions. The results obtained from the perspective of bioremediation show that A. variabilis, N. pruniforme, N. calcicola strains are the most promising microorganisms for bioremediation in heavy metal-contaminated soils and water bodies due to their ability to accumulate heavy metal ions, produce EPS, and maintain metabolic activity to a certain limit.

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Conclusion

Cu^{2+} and Co^{2+} ions significantly affect the nitrogen fixation activity of cyanobacteria. 10–30 mM concentrations are the tolerant range, and the strains maintain 80.30–97.26% of nitrogen fixation. 50 mM concentration is the toxic range, and nitrogen fixation activity decreases sharply. The studied strains have a moderately high resistance to heavy metals and can be used as bioremediation agents in areas contaminated with metals.

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