Original Article

ACTIVITY OF NITROGEN-FIXING CYANOBACTERIA UNDER SALINITY AND HEAVY METALS STRESS

Aziza Usmonkulova[1], Tulkin Shonakhunov[2], Gulchekhra Kadirova[3]
[1,2,3]Department of Phytovirology, Institute of Microbiology of the Academy of Sciences of the Republic of Uzbekistan, Tashkent 100128, Uzbekistan
Email: usmonkulova.aziza@mail.ru

Abstract

In this article, the effects of different concentrations of heavy metals Cu (II), Co(II) and Cd(II) cations and NaCl salt stress on the morphological characteristics, biomass, chlorophyll a, exopolysaccharides and phytohormones production capacity of native salt-tolerant nitrogen-fixing cyanobacteria Nostoc calcicola 25 and Anabaena variabilis 21 strains observed. It was found that the strains were resistant to NaCl concentration from 100 to 800 mmol/L and maintained a sufficient amount of biomass at NaCl concentration from 100 to 500 mmol/L. Nostoc calcicola 25 and Anabaena variabilis 21 strains can synthesize exopolysaccharide, gibberellin and IAA under the influence of salinity with NaCl and heavy metals. Specifically, Nostoc calcicola 25 strain indole-3-acetic acid production was 117 mg/l at Cu (II) 20 mmol/L and tryptophan 2.5 mg/ml concentration, 98 mg/l Cu (II) at 20 mmol/L and 0.5 mg/ml tryptophan concentration, gibberellin content at Cu (II) 20 mmol/L was 39 mg/L. Anabaena variabilis 21 produced exopolysaccharides 5 times higher than the control on the 5th day of cultivation at 200 mmol/L salinity and 50 mmol/L Cd (II). The obtained results make it possible to create biological fertilizers for remediation of saline soils contaminated with heavy metals based on local strains of cyanobacteria and to recommend them for practice.

Keywords: Cyanobacteria, salinity, resistance, indole-3-acetic acid, heavy metals, exopolysaccharides, chlorophylla, gibberellin.

1. INTRODUCTION

The role of cyanobacteria (blue-green algae) in biocenoses developing under extreme conditions is especially important as an accumulator of organic matter, a molecular nitrogen fixer and a stimulator of soil microbiological activity (Genuario et al., 2018; Singh et al., 2016). Considering the problems related to agroecosystems and the environment, recent advances in biotechnology offer more reliable approaches to solve the problem of food security for future generations, as well as to solve complex environmental problems (Morrissey et al., 2004; Hosseini et al., 2015; Stal 2007). Cyanobacteria is important as a valuable biological resource for the sustainable development of agrobioecosystems due to increase soil fertility, reducing greenhouse gas emissions, and migitating stress conditions with their unique properties, such as oxygenic photosynthesis, high productivity of biomass, growth in plowed and irrigated soils, survival in various adverse environmental stress conditions, synthesing of useful biologically active substances (Singh et al., 2017; Tyagi et al., 2014; Zulpa et al., 2008).

Due to their strong adaptability and effective defense mechanisms against various abiotic stresses, cyanobacteria colonize and survive in various terrestrial and aquatic habitats, including extreme and polluted areas (Abdullah Al-Amin et al., 2021; Cain et al., 2008; Raungsomboon et al., 2006). Different representatives of cyanobacteria resistant to heavy metal ions were isolated from soil contaminated with metal. Isolated cyanobacteria have several heavy metal tolerance mechanisms such as extracellular binding or precipitation as well as internal detoxification (Priyanka et al., 2020).

Address for correspondence: Aziza Usmonkulova,
Institute of Microbiology of the Academy of Sciences of the Republic of
Uzbekistan, Tashkent 100128, Uzbekistan
Email: usmonkulova.aziza@mail.ru

For reprints contact: pnrjournal@gmail.com

How to cite this article: Aziza Usmonkulova, Tulkin Shonakhunov, Gulchekhra Kadirova, ACTIVITY OF NITROGEN-FIXING CYANOBACTERIA UNDER SALINITY AND HEAVY METALS STRESS, J PHARM NEGATIVE RESULTS 2022;13:355-363.

Access this article online
Quick Response Code:
Website:
www.pnrjournal.com
DOI:
10.47750/pnr.2022.13.03.055

In addition, El-Enany and Issa (2000), Vanhoudt et al. (2018) and De Philippis et al. (2011) revealed that cyanobacteria effectively remove heavy metal ions from the water environment due to the release of some extracellular polymeric substances that chelate free metal ions or hold them on the extracellular surface. Many cyanobacteria produce extracellular polymeric substances (exopolysaccharides) and metal-binding proteins (metallothioneins), which are mainly polysaccharide in nature. These exopolysaccharides (EPS) can be attached to the cell surface in the form of membranes, capsules/mucilages or released into the environment as polysaccharides (Pereira et al., 2011). Polysaccharides usually have a high binding property with metal ions and they can be used as promising chelating agents to remove heavy metals from water (Zhang et al., 2016; Cepoi et al., 2019; De Philippis et al., 2000; Suresh et al., 2007; Nowruzi et al., 2021; Ni et al., 2019). Shuhong Y (2014) and Delattre (2016) clarified the effect of O-H, C = O, C-O-C and C = OC groups of EPS in binding Cu(II) , Pb(II) Cr(VI) ions.

In addition, cyanobacteria are resistant to strong salinity and adapt well to adverse environmental conditions from halophilic to cryophilic (Rampelotto 2013).

The purpose of the study was to investigate the effects of different concentrations of NaCl and heavy metal ions (Co(II), Cu(II), Cd(II)) on the growth of Nostoc calcicola 25 and Anabaena variabilis 21 strains, synthesis of chlorophyll a, exopolysaccharides and production activity of gibberellin and indole-3-acetic acid (IAA).

2. MATERIALS AND METHODS

Objects of study

The objects of the study were local strains of nitrogen-fixing cyanobacteria of genera Nostoc and Anabaena, isolated from saline sirozem soils contaminated with pesticides and heavy metals in the Kashkadarya and Syrdarya regions of Uzbekistan (Kadirova et al., 2012).

Growing conditions

Nostoc calcicola 25 and Anabaena variabilis 21 strains were grown in liquid medium BG11 (without nitrogen) (Rippka et al., 1979) supplemented with different concentrations of heavy metals and NaCl and the pH was 7.1. Heavy metal salts (CuCl2 x 5H2O, CdSO4 x 8H2O and CoCl2 x 6H2O) were added to the nutrient medium at concentrations of 10, 20, 30, 40, 50, 70 and 100 mmol/L and NaCl salt from 100 to 800 mmol/L. A medium without metal ions and NaCl salt was used as a control medium. Cultures were grown in a luminostat for 14 days at 28 °C under 15 μmol m -2 s -1 light (Liu et al., 2014).

Determination of cell morphology and biomass

Morphological characteristics of cyanobacteria grown for 7 days under the influence of heavy metals were studied using a LEICA DM 1000 (Germany) microscope. For biomass determination, cells were collected and dried in an oven at 100°C. Cell dry weight was measured periodically during 5, 7, 14 days of cultivation (Nowruzi et al., 2013; Okmen et al., 2011).

Isolation of exopolysaccharides (EPS)

Nostoc calcicola 25 and Anabaena variabilis 21 strains were harvested from the nutrient medium after 5, 7 and 9 days using a centrifuge at 6000 rpm for 30 minutes. The culture fluid separated from the biomass was precipitated by adding ethanol and stored at 4 °C for 24 h. The precipitates were collected and placed in a drying cabinet to evaporate the remaining ethanol. Finally, the dry mass of the sediment was determined relative to the control (Sardari et al., 2017; Okmen et al., 2011).

Determination of chlorophyll a content

Spectrophotometric method recommended by Okmen et al. (2011) was used to determine of chlorophyll a content under conditions of heavy metal and salt stress. Cultures were grown for 9 days in a nutrient medium containing 200 mmol/L of NaCl salt and 20 mmol/L and 50 mmol/L of cadmium, copper and cobalt ions, and of chlorophyll a content was periodically checked for 5, 7 and 14 days compared to the control.

Investigation of IAA and gibberellin synthesis at different concentrations of heavy metal ions and NaCl

IAA production by cyanobacterial strains in the presence of 0.5 and 2.5 mg/ml L-tryptophan under saline conditions (200 mMol/L NaCl) and pollution with copper, cobalt and cadmium ions (20 and 50 mmol/L) were examined according to the guidelines (Ahmad F et al. 2005). IAA content was checked periodically on the 5th, 7th and 14th days of cultivation.

The gibberellin synthesis of cyanobacteria was determined periodically during 5, 7 and 14 days of cultivation according to the spectrophotometric method recommended by Berrios et al. (2014).

Statistical Analysis

The statistical significance of data was tested by the analysis of variance of the Microsoft Excel 2010 package. Mean comparisons were conducted using the least significant difference test ($p= 0.05$). The average values of average values of microorganisms' growth and development parameters, EPS, IAA and gibberelin production and the standard deviation were counted based on several replications.

3. RESULTS AND DISCUSSION

Previously, nitrogen-fixing cyanobacteria of the genera Nostoc and Anabaena were isolated from samples of saline and pesticide-contaminated soils on the basis of morphological, cultural and molecular genetic features (Fig. 1).

The results of the study showed that the strains Anabaena variabilis 21 and Nostoc calcicola 25 were resistant to NaCl up to 800 mmol/L (Fig. 2). However the best growth was observed in a medium with NaCl 100, 200 and 300 mmol/L, while the first colonies were found on the 7-8th day of cultivation on the agar medium.

Figure 1. Microscopic photographs of cyanobacteria trichomes: A) Anabaena variabilis 21; B) Nostoc calcicola 25. (Magnification: 100 x 13.5)

It should be noted that when NaCl concentrations were 100 and 200 mmol/L, the biomass of cyanobacteria Nostoc calcicola 25 and Anabaena variabilis 21 did not decrease compared to the control, which shows that this concentration of NaCl did not significantly affect their growth. It was found that Nostoc calcicola 25 and Anabaena variabilis 21strains maintained a sufficient amount of biomass when NaCl content was from 100 to 500 mmol/L. The biomass dry weight of the initial culture of Nostoc calcicola 25 was 152 mg per 100 ml, and at 500 mmol/L salinity, the biomass was reduced by only 14% compared to the control.

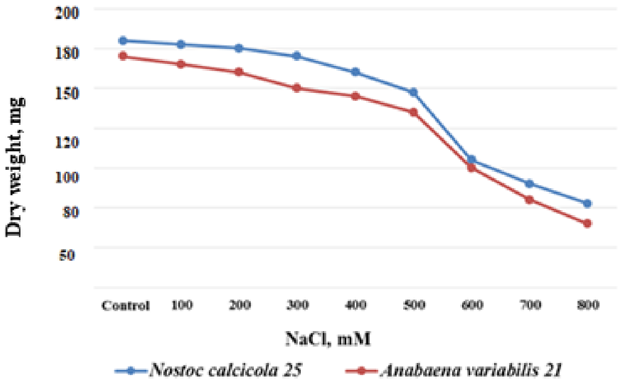
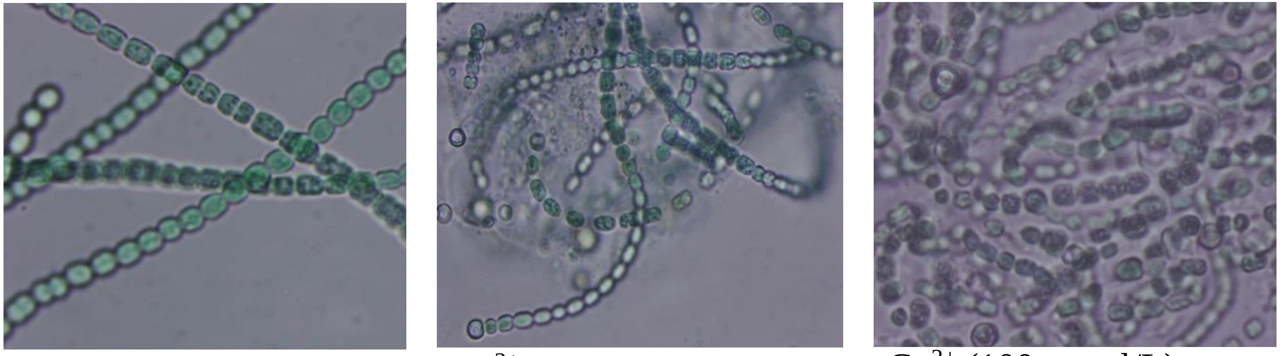


Figure 2. Growth of cyanobacteria Noctoc calcicola 25 and Anabaena variabilis 21 under salt stress

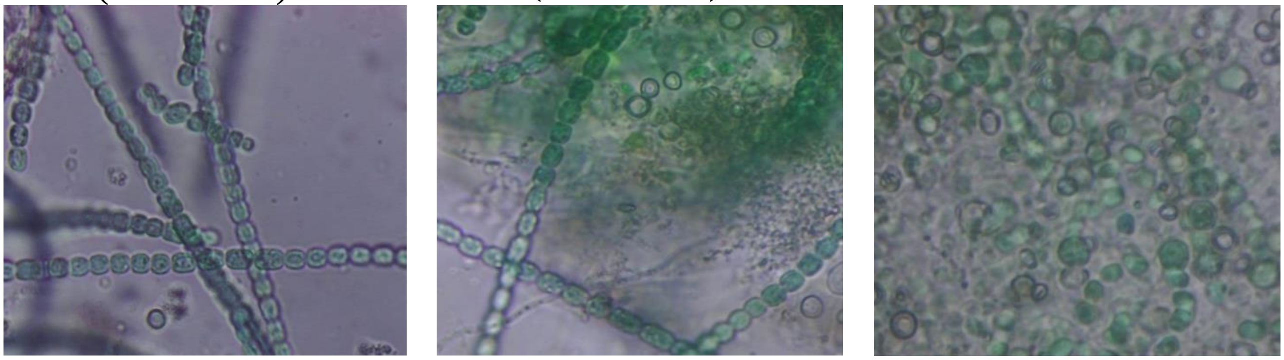
The most halophilic cyanobacteria were found among representatives of the genus Cyanothece, for which the optimal NaCl concentration is 10%. Many cyanobacteria are halotolerant. The maximum concentration of NaCl at which the growth of cyanobacteria was observed under natural conditions is 10-25% (Nagle et al., 2010).

Next, we studied the growth of Nostoc calcicola 25 and Anabaena variabilis 21 in nutrient media with Cu(II), Cd(II) and Co(II) - 10, 20, 30, 40, 50, 70 and 100 mmol/L heavy metal ions. It should be noted that the studied cultures grow well in the presence of heavy metal ions from 10 to 50 mM, but at 70 and 100 mmol/L, the biomass of cells decreased by 15 and 10%, respectively, compared to the control. It seems that heavy metals primarily caused disturbances in photosynthesis and nitrogen fixation in cyanobacteria. After 7 days of growth in Cu(II) and Co(II) concentrations of 70 and 100 mM, Nostoc calcicola 25 and Anabaena variabilis 21 strains changed to yellow-green color. The data obtained are consistent with the work of Gornostaeva (2015), where it was shown that under the action of high concentrations of copper and nickel ions in the cells of soil cyanobacteria, the intensity of biochemiluminescence, dehydrogenase and catalase activities, and the concentration of chlorophyll a decrease; the concentrations of pheophytin and malondialdehyde, a product of lipid peroxidation, increase. Figure 3 shows microscopic photographs of Nostoc calcicola 25 cells in the presence of 20, 50, and 100 mmol/L copper, cobalt, and cadmium ions. At a concentration of 20 mmol/L of copper, cobalt and cadmium, the morphology of Nostoc calcicola 25 cells practically did not differ from the original cells (Fig. 1), at 50 mmol/L, individual cells located in trichomes became thicker, and the cell diameter increased, and at the same time, long trichomes turned into short chains. It should be noted that at a high copper concentration of 100 mmol/L, cells located in trichomes thickened and lost their blue-green color. At a concentration of 100 mmol/L of cobalt and cadmium, the cyanobacterial cells almost completely disintegrated into single cells and slightly lost their blue-green color (Fig. 3). Microbiological analysis of cyanobacteria showed that the morphological characteristics of cyanobacteria cells change under the influence of high concentration of HM.

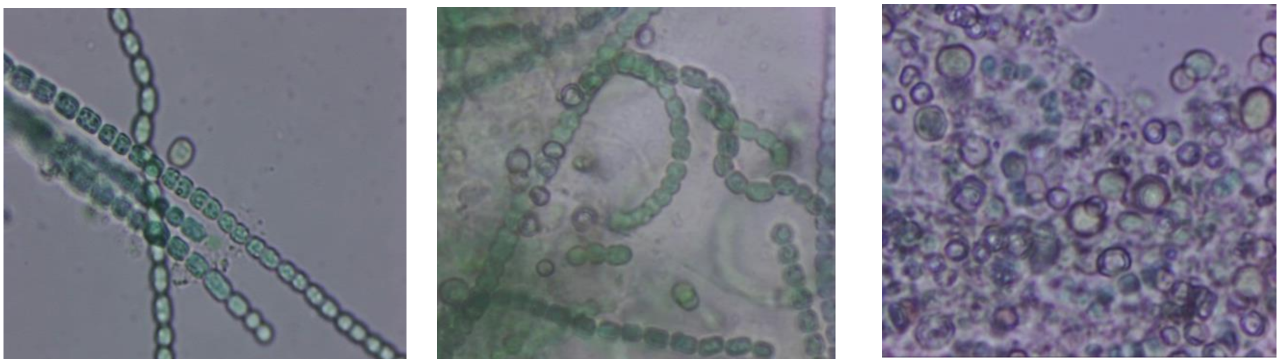
The results of the experiment aimed at determining the amount of chlorophyll a under the conditions of heavy metal stress also showed that the amount of chlorophyll a decreases with the increase in the concentration of heavy metals in the environment (table). Many heavy metals play important roles in living photosynthetic organisms and are required in very low concentrations for optimal growth. Small amounts of Fe, Cu, Mn, Co, Zn and Ni heavy metal cations often play an important role in organisms, as they participate in complex biochemical reactions. At high concentrations, they are toxic because they form non-specific complexes in cells (Bagaeva et al., 2013). Thus, excessive heavy metals cause serious toxic effects depending on the type of organism, the nature and concentration of the metal.



Cu^{2+} (20 mmol/L) Cu^{2+} (50 mmol/L) Cu^{2+} (100 mmol/L)



Co(II) (20 mmol/L) Co(II) (50 mmol/L) Co(II) (100 mmol/L)



Cd(II) (20 mmol/L) Cd(II) (50 mmol/L) Cd(II) (100 mmol/L)

Figure 3. Microscopic photographs of Nostoc calcicola 25 cyanobacteria cells exposed to various concentrations of copper, cobalt and cadmium ions. (Magnification: 100 x 13.5)

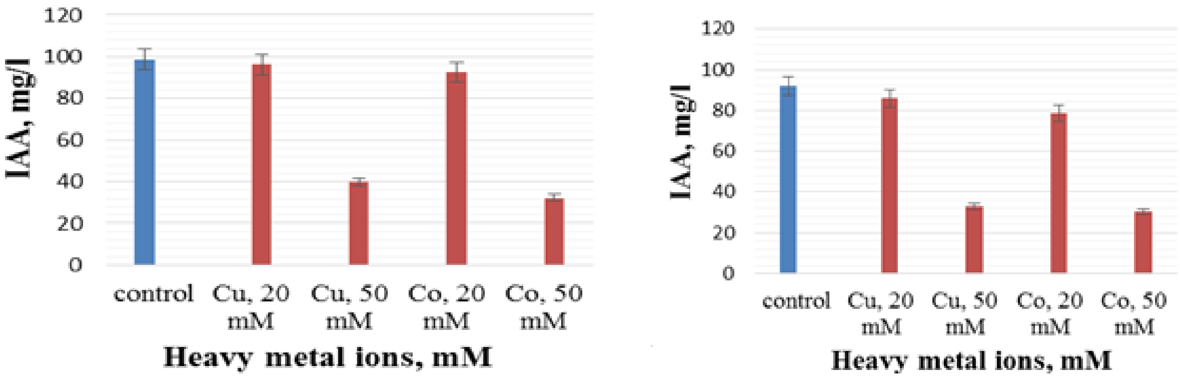
Determination of the physiological status of cyanobacteria under salt stress and in the presence of heavy metal ions was based on the ability of chlorophyll content, exopolysaccharides and phytohormones production of salt-resistant nitrogen-fixing strains of cyanobacteria. The production of EPS under the conditions of heavy metal and NaCl stress increased in accordance with the increasing concentration of heavy metals. Specialty, EPS synthesis in Anabaena variabilis 21 strain grown at 200 mmol/L of NaCl and 20 and 50 mmol/L of Cd cations was observed to increase by 3 and 5 times, respectively, compared to the control on the 7th day of cultivation. Nostoc calcicola 25 strain produced 6 times more EPS than the control at the concentration of Co(II) and Cu(II) cations of 50 mmol/L on the 5th day of cultivation (table). The obtained results show that exopolysaccharides ensure adaptation and resistance of cyanobacteria to various stress conditions (Micheletti et al., 2008). Due to the large amount of negative charges in the outer cell layers, EPS-producing cyanobacteria are very promising as chelating agents to remove positively charged heavy metal ions from aqueous solutions (De Philippis et al., 2011).

Table. Some properties of cyanobacteria under conditions of heavy metal and NaCl (200 mmol/L) salinity (5th day)

Cyanobacterial strains	**Heavy metals, mMol/L**	**Quruq biomassa, mg/ml**	**Chlorophyll a content, mg/ml**	**Exopolysaccharide content, mg/ml**	**Gibberellin content, mg/L**
Nostoc calcicola 25	Control	2.4 ±0.8	4.7 ± 0.89	7±0.1	88±0.3
	Cd(II) (20)	2.0 ±0.4	1.3 ±0.07	18±0.02	35±0.03
	Cd (II) (50)	0.005 ±0.003	0.73 ±0.03	27±0.01	17±0.012
	Cu(II) (20)	2.1 ±0.5	3.3 ± 0.04	23±0.08	39±0.05
	Cu(II) (50)	0.018 ±0.003	1.51 ±0.025	42±0.03	21±0.08
	Co(II) (20)	1.3 ±0.001	0.03 ± 0.006	31±0.09	32±0.07
	Co(II) (50)	0,3 ± 0.01	0.013 ±0.002	45±0.4	19±0.04
Anabaena variabilis 21	Control	2.7 ±0.1	9.2 ± 0.3	13±0.05	75±0.02
	Cd(II) (20)	0.08 ±0.001	3.1 ± 0.01	39±0.03	29±0.001
	Cd (II) (50)	0.01 ± 0009	0.18 ±0.01	62±0.07	13±0.03
	Cu(II) (20)	1.2 ±0.1	3.7 ± 0.12	22±0.3	34±0.001
	Cu(II) (50)	0.8 ±0.01	1.1 ± 0.12	35±0.001	16±0.05
	Co(II) (20)	0.2 ±0.0002	4.1 ± 0.01	32±0.005	19±0.09
	Co(II) (50)	0.3 ±0.001	1.58 ±0.01	46±0.028	11±0.001

Gibberellin synthesis of Anabaena variabilis 21 and Nostoc calcicola 25 strains grown at a concentration of heavy metals (Cu(II), Cd(II) and Co(II)) and NaCl at 200 mmol/L decreased as the concentration of heavy metals increased (table). It was observed that Anabaena variabilis 21 and Nostoc calcicola 25 strains synthesized 1.5-2 times higher gibberellin on the 5th day of cultivation at Cu(II) concentration of 20 mmol/L compared to other heavy metal conditions. Gibberellins can also reduce the harmful effects of heavy metals in soil by controlling oxidative stress and stimulating plant antioxidant systems (Zhu et al., 2012). Addition of gibberellin to T. aestivum seedlings had toxic effects on growth, chlorophyll level, and carbonic anhydrase activity and alleviated Ni(II) cation oxidative stress (Siddiqui et al., 2011).

Some characteristics of local strains of cyanobacteria determined for 14 days under salinity and heavy metal stress conditions, dry biomass, chlorophyll a, exopolysaccharide, auxin and gibberellin content showed the highest values on the 5th day of cultivation. Based on the obtained results, local salt-resistant Nostoc calcicola 25 and Anabaena variabilis 21 strains of cyanobacteria are producers of auxin and gibberellin, exopolysaccharides under the influence of double stress - heavy metals (copper, cadmium, cobalt) and salinity with NaCl was determined. The presence of genes for resistance to heavy metals in bacteria ensures their survival in adverse environmental conditions. In addition, phytohormones produced by microorganisms may be an important role in enhancing plant growth under heavy metal-polluted conditions (Ayangbenro et al., 2020; Rodriguez et al., 2006; Singh 2014). In our further studies, the synthesis of IAA by N. calcicola 25 and A. variabilis 21 under saline conditions was 98.6 mg/L and 91.8 mg/L (with tryptophan 0.5 mg/ml), 128.8 mg/L, and 122.6 mg/l (with tryptophan 2.5 mg/ml), respectively. It should be noted that the production of IAA by N. calcicola strain 25 in the presence of Cu2+ (20 mmol/L) at a concentration of 2.5 mg/ml tryptophan increased by 17% compared to the data obtained with 0.5 mg/ml tryptophan. IAA production by N. calcicola 25 strain in the presence of Co2+ 20 mmol/L and 50 mmol/L was 92.4 and 32.2 mg/L (L-tryptophan 0.5 mg/ml) and 98.2 and 54,6 mg/L (L-tryptophan 2.5 mg/ml) respectively (Fig. 4, 5).



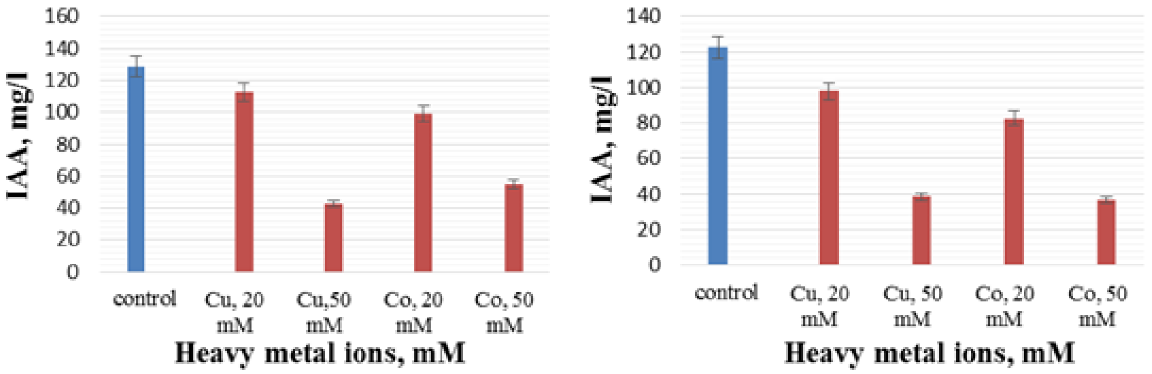
A

B

Figure 4. The production of IAA by strains N. calcicola 25 (A) and A. variabilis 21 (B) under conditions of salinization with NaCl 200 mM in the presence of heavy metal ions and L-tryptophan 0.5 mg/ml

Thus, it was noted that when L-tryptophan was added to the culture medium in the amount of 2.5 mg/ml at Co+2 concentration of 50 mmol/L, the production of IAA by the strain N. calcicola 25 increased by 50% as compared to the data obtained with L-tryptophan 0.5 mg/ml in the culture medium. It should be noted that the production of IAA by strains N. calcicola 25 at a concentration of Cd(II) 20 mmol/L and tryptophan 0.5 mg/ml was 84.6 mg/L and at a concentration of Cd2+ 50 mmol/L and tryptophan 2.5 mg/ml - 38 mg/L.

It is well known that tryptophan is a precursor of IAA synthesis, and the addition of this amino acid to bacterial cultures results in higher IAA production. Therefore, the addition of tryptophan to bacterial cultures significantly enhanced the tryptophan-dependent IAA production (Ahmad et al., 2005; Kamilova et al., 2006; Prasanna et al., 2010). The production of IAA by the strain A. variabilis 21 under saline conditions in the presence of L-tryptophan 2.5 mg/ml, as well as Cu(II) 50 mMol/l and Co (II) 50 mMol/l in the culture medium increased by 17.7 and 21% as compared to the data obtained for L-tryptophan 0.5 mg/ml in medium.



A

B

Figure 5. The production of IAA by the strains N. calcicola 25 (A) and A. variabilis 21 (B) under saline conditions (NaCl 200 mM) in the presence of L-tryptophan 2.5 mg/ml

This is consistent with the conclusions of other researchers that tryptophan is a precursor of IAA synthesis and enhances the production and accumulation of IAA in cyanobacterial cells (Ahmad et al., 2005; Kamilova et al., 2006; Prasanna et al., 2010; Sergeeva et al., 2002). It should be noted that, regardless of the growth conditions, both in the absence and in the presence of salinity, tryptophan, and HM ions, IAA production was found in N. calcicola 25 and A. variabilis 21 cultures.

The growth improvement of Helianthus annuus L. and

Brassica juncea L. when exogenous indolyl-3-acetic acid was added to Pb and As heavy metals showed that auxins are effective in mitigating the toxicity of heavy metals (Krishnamurthy et al., 2015). Similarly, tryptophan supplementation of O. sativa seedlings grown in Cd-contaminated soil improved plant growth and yield (Farooq et al., 2015). Beneficial effects of exogenously applied auxin were observed in Acutodesmus obliquus exposed to Pb stress (Bajguz et al., 2020). Together, these studies suggest that heavy metal-induced cytokinins and auxin synthesis may help plants cope with stress and improve growth and development.

Nadeem et al. (2014) and Forni et al. (2017) reported about mechanisms of microorganisms' positive effect on plants growth and improvement of their resistance to stress under extreme environmental conditions. It is known that there are several mechanisms of plant growth stimulation, plant protection and salt stress mitigation by rhizobacteria, such as nitrogen fixation, synthesis of osmoprotectors, exopolysaccharides, 1-aminocyclopropane-1-carboxylate (ACC) deaminase, phytohormones; solubilization of minerals, such as phosphorus and potassium [Berg et al., 2013; Mishra et al., 2017; Wang et al., 2016). It was shown that metabolites synthesized by bacteria, including phytohormones (auxins, cytokinins, gibberellins, and abscisic acid), play a vital role in plant growth, nutrition, and also provide resistance to various abiotic and biotic stress factors (Ruiz-Lozano et al., 2012; Sorty et al., 2016).

Plant-associated bacteria play a key role in host adaptation to adverse environmental conditions, thereby altering plant cell metabolism or promoting plant growth. The effective rhizobacteria producing IAA, which stimulate plant growth, are widely used to accelerate phytoremediation of soil contaminated with heavy metals (Khan et al., 2009; Kuffner et al., 2010; Ma et al., 2011; Sayyed et al., 2010; Spaepen et al., 2007).

4. CONCLUSION

Various representatives of bacteria participate in the biotic activity of the soil ecosystem, making it more dynamic for high plant productivity. The various negative environmental consequences of using chemical fertilizers in the fields lead to the search for environmentally friendly bacterial fertilizers that have a positive effect on plant growth and productivity. Cyanobacteria are excellent accumulators or destructors of various environmental pollutants, such as heavy metals, pesticides, and oil-containing compounds. Bioremediation of heavy metals using cyanobacteria is recognized as a cheaper, more efficient and environmentally friendly alternative to traditional physicochemical remediation methods. It should be noted that the biological properties of local strains of nitrogen-fixing cyanobacteria, such as the synthesis of physiologically active substances IAA and gibberellin, growth under salinity conditions at concentrations of NaCl from 100 to 800 mmol / L and at different concentrations of heavy metal ions, the production of chelating EPS for the removal of heavy metal ions indicates that cyanobacteria are promising candidates for use in agrobiotechnology.

AUTHOR`S CONTRIBUTION

GK and AU carried out the experiments. AU analyzed data. GK statistically analyzed results. TSh and AU wrote the draft of the manuscript. TSh conducted the critical revision of the manuscript. GK worked out the concept and design and supervised and funded the experiments. All the authors contributed equally to this manuscript and agreed to submit it for publication.

FUNDING

There is no funding to report.

CONFLICTS OF INTEREST

The authors report no financial or any other conflicts of interest in this work.

ETHICAL APPROVALS

This study does not involve experiments on animals or human subjects.

REFERENCES

1. Abdullah Al-Amin, Fahmida Parvin, Joydeep Chakraborty & Yong-Ick Kim (2021). Cyanobacteria mediated heavy metal removal: a review on mechanism, biosynthesis and removal capability. Environmental Technology Reviews. 10(1): 44-57.
2. Ahmad F, Ahmad I, Khan M.S (2005). Indole Acetic Acid Production by the Indigenous Izolates of Azotobacter and Fluorescent Pseudomonas in the Presence and Absense of Tryptophan. Turk. J. Biol. 29: 29-34.
3. Ayangbenro A.S, Babalola O.O (2020). Genomic analysis of Bacillus cereus NWUAB01 and its heavy metal removal from polluted soil. Scientific reports.10(1); 19660- 19671
4. Bajguz A, Urszula Kotowska, Elżbieta Zambrzycka-Szelewa (2020). Auxins and Cytokinins Regulate Phytohormone Homeostasis and Thiol-Mediated Detoxification in the Green Alga Acutodesmus obliquus Exposed to Lead Stress. Scientific Reports. 10(1).
5. Berrios. J, Illanes.A, German E Aroca (2004). Spectrophotometric method for determining gibberellic acid in fermentation broths. Biotechnology Letters. 26(1):67-70
6. Berg G, Alavi P, Schmidt C. S, Zachow C, Egamberdieva D, Kamilova F, Lugtenberg BJJ (2013). Biocontrol and osmoprotection for plants under salinated conditions. In Molecular Microbial Ecology of the Rhizosphere. 587-592.
7. Cain A, Vannela R, Woo L.K (2008). Cyanobacteria as a biosorbent for mercuric ion. Bioresource Technology. 99 (14): 6578-6586.
8. Cepoi L. V, Zinicovscaia I, Chiriac T, Rudi L, Yushin N, Miscu V (2019). Silver and gold ions recovery from batch systems using Spirulina platensis biomass. Ecol Chem Eng S. 26(2):229–240
9. De Philippis R, Faraloni C, Margheri MC, Sili C, Herdman M, Vincenzini M (2000). Morphological and biochemical characterization of the exocellular investments of polysaccharide-producing Nostoc strains from the Pasteur Culture Collection. World J Microbiol Biotechnol. 16(7):655–661.

10. De Philippis R, Colica G. and Micheletti E (2011). Exopolysaccharide-producing cyanobacteria in heavy metal removal from water: molecular basis and practical applicability of the biosorption process. Applied Microbiology and Biotechnology. 92(4): 697-708.
11. Delattre C, Per G, Laroche C, Michaud P (2016). Production, extraction and characterization of microalgal and cyanobacterial exopolysaccharides. Biotechnol Adv. 34(7):1159–79.
12. El-Enany, A.E. and Issa, A.A (2000). Cyanobacteria as a biosorbent of heavy metals in sewage water. Environmental toxicology and pharmacology. 8 (2): 95-101.
13. Farooq M, Hussain M, Wakeel A, Siddique K. H. M. (2015): Salt stress in maize: effects, resistance mechanisms, and management. A review. – Agronomy for Sustainable Development. 35: 461-481.
14. Forni C, Duca D and Glick B. R (2017). Mechanisms of plant response to salt and drought stress and their alteration by rhizobacteria. Plant Soil. 410: 335–356.
15. Genuario Diego B, Marcelo G.M.V. Vaz, Suikinai N. Santos, Vanessa N. Kavamura, Itamar S. Melo (2018). Cyanobacteria From Brazilian Extreme Environments: Toward Functional Exploitation. Microbial Diversity in the Genomic Era, Academic Press, E-book; Chapter 16: 265-284.
16. Hosseini Nezhad M, Shafiabadi J, Hussain M.A (2015). Microbial resources to
safeguard future food security. Advances in food technology and nutritional sciences. 1: 8-13
17. Kadirova G.Kh, Kim A.A, Lorenz A, Rasulov B (2012). Functioning of Salt Tolerant Anabaena variabilis and Nostoc calcicola Strains in Salt Stress, Destructors of Hexachlorocyclohexane (HCH) in Saline Conditions. Environment and Natural Resources Research. 1: 63-72.
18. Kamilova F, Kravchenko L.V, Shaposhnikov A.I, Azarova T, Makarova N, Lugtenberg B (2006). Organic acids, sugars, and L-tryptophane in exudates of vegetables growing on stonewool and their effects on activities of rhizosphere bacteria. Mol. Plant-Microbe Interact. 19: 250-256.
19. Khan M, Zaidi A, Wani P, Oves M (2009). Role of plant growth promoting rhizobacteria in the remediation of metal contaminated soils. Environ Chem Lett. 7: 1–19.
20. Kuffner M, De Maria S, Puschenreiter M, Fallmann K, Wieshammer G, et al (2010). Culturable bacteria from Zn-and Cd-accumulating Salix caprea with differential effects on plant growth and heavy metal availability. Journal App. Microbiol. 108: 1471–1484.
21. Krishnamurthy A, Rathinasabapathi B (2013). Auxin and its transport play a role in plant tolerance to arsenite induced oxidative stress in Arabidopsis thaliana. Plant Cell and Environment. 36(10).
22. Liu L, Jokela J, Wahlsten M, Nowruzi B, Permi P, Zhang YZ, et al (2014). Nostosins, trypsin inhibitors isolated from the terrestrial cyanobacterium Nostoc sp. strain FSN. J Nat Prod. 77(8):1784–90
23. Ma Y, Prasad M, Rajkumar M, Freitas H (2011). Plant growth promoting rhizobacteria and endophytes accelerate phytoremediation of metalliferous soils. Biotechnol Adv. 29: 248–258.
24. Morrissey J. P, Dow J. M, Mark G. L & O'Gara F (2004). Are microbes at the root of a solution to world food production? Rational exploitation of interactions between microbes and plants can help to transform agriculture. EMBO reports. 5(10): 922–926.
25. Mishra S. K, Khan M. H, Misra S, Dixit V. K, Khare P, Srivastava S, & Chauhan P.S (2017). Characterisation of Pseudomonas spp. and Ochrobactrum sp. isolated from volcanic soil. Antonie Van Leeuwenhoek. 11: 253–270.
26. Micheletti E, Colica G, Viti C, Tamagnini P, De Philippis R (2008). Selectivity in the heavy metal removal by exopolysaccharide-producing cyanobacteria. J Appl Microbiol. 105(1):88-94.
27. Nagle V.L, Mhalsecar N.M, Jagtap T.J (2010). Izolation, optimization of selected Cyanophycean. Indian journal of Marine Sciences. 39: 212-218.
28. Nadeem S. M, Ahmad M, Zahir Z. A, Javaid A, Ashraf M (2014). The role of mycorrhizae and plant growth promoting rhizobacteria (PGPR) in improving crop productivity under stressful environments. Biotechnol. Adv. 32: 429–448.
29. Nowruzi B, Bouaïcha N, Metcalf JS, Porzani SJ, Konur O (2021). Plant-cyanobacteria interactions: Beneficial and harmful effects of cyanobacterial bioactive compounds on soil-plant systems and subsequent risk to animal and human health. Phytochemistry. 192:112959.
30. Nowruzi B, Khavari-Nejad R, Sivonen K, Kazemi B, Najafi F, Nejadsattari T (2013). Optimization of cultivation conditions to maximize extracellular investments of two Nostoc strains. Arch Hydrobiol Suppl Algol Stud. 142(1):63–76.
31. Ni L, Gu G, Rong S, Hu L, Wang P, Li S, et al (2019). Effects of cyanobacteria decomposition on the remobilization and ecological risk of heavy metals in Taihu Lake. Environ Sci Pollut Res. 26(35):35860–35870.
32. Okmen G, Bozanta.E, Ugur A , Ceyhan N (2011). Zinc effect on chlorophyll a, total carbohydrate, total protein contents and biomass of cyanobacterial species. Journal of applied biological sciences. 5 (2): 67-73.
33. Pereira S, Micheletti E, Zille A, Santos A, Moradas-Ferreira P, Tamagnini, P. De Philippis, R (2011). Using extracellular polymeric substances (EPS)-producing cyanobacteria for the bioremediation of heavy metals: do cations compete for the EPS functional groups and also accumulate inside the cell? Microbiology. 157(2): 451-458.
34. Priyanka Cash Kumar, Antra Chatterjee, Wang Wenjing, Deepanker Yadav, Prashant Kumar Sing (2020). Cyanobacteria: potential and role for environmental remediation. Abatement of Environmental Pollutants. 10: 193-202.
35. Prasanna R, Joshi M, Rana A, Nain L (2010). Modulation of IAA Production in Cyanobacteria by Tryptophan and Light. Polish Journal of Microbiology. 59: 99-105.
36. Prasanna R, Sood A, Jaiswal P, Nayak S, Gupta V, Chaudhary V, Joshi M, Natarajan C (2010). Rediscovering cyanobacteria as valuable sources of bioactive compounds. Appl. Biochem. Microbiol. 46: 119-134.
37. Raungsomboon S, Chidthaisong A, Bunnag B, Inthorn D, Harvey N.W (2006). Production, composition and Pb2+ adsorption characteristics of capsular polysaccharides extracted from a cyanobacterium Gloeocapsa gelatinosa. Water Research. 40 (20): 3759-3766.
38. Rampelotto P.H (2013). Extremophiles and extreme environments. Life (Basel, Switzerland). 3(3): 482–485.
39. Rippka R, Deruelles J, Waterbury JB, Herdman M, Stanier RY (1979). Generic assignments, strain histories and properties of pure cultures of cyanobacteria. Microbiology. 111(1):1–61.
40. Ruiz-Lozano J.M, Porcel R, Azcon R, Aroca R (2012). Regulation by arbuscular mycorrhizae of the integrated physiological response to salinity in plants. New challenges in physiological and molecular studies. J. Exp. Bot. 63: 4033–4044.
41. Rodriguez A.A, Stella A.M, Storni M. M, Zulpa G, & Zaccaro M.C (2006). Effects of cyanobacterial extracellular products and gibberellic acid on salinity tolerance in Oryza sativa L. Saline systems. 2: 7-10.
42. Sayyed R, Chincholkar S (2010). Growth and siderophores production in Alcaligenes faecalis is regulated by metal ions. Indian H Microbiol. 50: 179–182.
43. Sardari R, Kulcinskaja E, Ron EY, Björnsdóttir S, Friðjónsson ÓH, Hreggviðsson GÓ, et al (2017). Evaluation of the production of exopolysaccharides by two strains of the thermophilic bacterium Rhodothermus marinus. Carbohyd Polym. 156:1–8.
44. Singh J.Sh, Kumar A, Rai A.N, Singh D. P (2016). Cyanobacteria: A Precious Bio-resource in Agriculture, Ecosystem and Environmental Sustainability. Frontiers in Microbiology. 7: 529.
45. Singh R, Parihar P, Singh M, Bajguz A, Kumar J, Singh S, Singh V.P, Prasad S.M (2017). Uncovering Potential Applications of Cyanobacteria and Algal Metabolites in Biology, Agriculture and Medicine: Current Status and Future Prospects. Front Microbiol. 8(515): 1-37.
46. Singh S (2014). Review on possible elicitor molecules of cyanobacteria: their role in improving plant growth and providing tolerance against biotic or abiotic stress. Journal of Applied Microbiology. 117: 1221-1244.
47. Siddiqui M , H Al-Whaibi M, O Basalah M (2011). Interactive effect of calcium and gibberellin on nickel tolerance in relation to antioxidant systems in Triticum aestivum L. National library of medicine. 248(3):503-11.
48. Stal L.J (2007). Cyanobacteria: Diversity and versatility, clues to life in extreme environment. Algae and Cyanobacteria in Extreme

Environment. 659–680.

49. Sorty A.M, Meena K.K, Choudhary K, Bitla U.M, Minhas P.S, Krishnani K.K (2016). Effect of plant growth promoting bacteria associated with halophytic weed (Psoralea corylifolia L.) on germination and seedling growth of wheat under saline conditions. Appl. Biochem. Biotechnol. 180: 872–882.
50. Suresh Kumar A, Mody K, Jha B (2007). Bacterial exopolysaccharides–a perception. J Basic Microbiol. 47(2):103–117.
51. Shuhong Y, Meiping Z, Xong Y, Xan V, Shan X, Yan L and other (2014). Biosorption of Cu2+, Pb2+ and Cr6+ by a novel exopolysaccharide from Arthrobacter ps-5. Carbohydrate polymn. 101:50–6
52. Tyagi R, Kaushik D.B, Kumar J (2014). Antimicrobial activity of some cyanobacteria. Microbial Diversity and Biotechnology in Food Security. 463-470.
53. Vanhoudt N, Vandenhove H, Leys N. and Janssen P (2018). Potential of higher plants, algae, and cyanobacteria for remediation of radioactively contaminated waters. Chemosphere. 207: 239-254.
54. Wang Q, Dodd I. C, Belimov A. A. & Jiang F (2016). Rhizosphere bacteria containing 1-aminocyclopropane-1-carboxylate deaminase increase growth and photosynthesis of pea plants under salt stress by limiting Na+ accumulation. Functional Plant Biology. 43.2: 161-172.
55. Zhang X, Liu L, Zhang S, Pan Y, Li J, Pan H, Xu S, Luo F (2016). Biodegradation of dimethyl phthalate by freshwater unicellular cyanobacteria. BioMed Research International. 1-8.
56. Zulpa G, Siciliano M.F, Zaccaro M.C, Storni M, Palma M (2008). Effect of cyanobacteria on the soil microflora activity and maize remains degradation in a culture chamber experiment. International Journal Agriculture and Biology. 10: 388–392.
57. Zhu X , Jiang T, Wang Z, Lei G, Shi Y, Li G, Zheng Sh(2012). Gibberellic acid alleviates cadmium toxicity by reducing nitric oxide accumulation and expression of IRT1 in Arabidopsis thaliana. J Hazard Mater. 239-240:302-7.
58. Bagaeva T.V., Ionova N.E., Nadeeva G.V. (2013). Microbiological remediation of natural systems from heavy metals: educational method. allowance /. - Kazan: Kazan University. - 56 p.
59. Gornostaeva E.A. (2015). Effect of copper and nickel ions on soil cyanobacteria and cyanobacterial communities. Dissertation for the degree of Candidate of Biological Sciences. 189