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Determination of elemental content of cyanobacteria *Spirulina platensis* and *Nostoc linckia* using neutron activation analysis

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Abstract. The cyanobacteria *Spirulina platensis* and *Nostoc linckia* are high-nutrition products with broad potential for use in the food and pharmaceutical industries, as well as in bioremediation. However, their high capacity for metal, including potentially toxic, bioaccumulation necessitates monitoring of their elemental composition. Multi-element neutron activation analysis, a powerful, non-destructive analytical chemistry technique, at the IBR-2 reactor of Frank Laboratory of Neutron Physics of the Joint Institute for Nuclear Research in Dubna, Russia, was used to study the elemental composition of two strains of *Spirulina platensis* and cyanobacterium *Nostoc linckia*. The content of 23 elements, including Mg, Al, Cl, Ca, Na, K, Sc, Cr, Mn, Fe, Ni, Co, Zn, Br, As, Se, Rb, Sb, Ba, Cs, and U, was determined in each cyanobacterial strain. The level of toxic metals in the microbial biomass did not exceed the limits for daily intake set by the World Health Organization. Possible mechanisms of metal ions uptake by cyanobacterial biomass were discussed.

Keywords: neutron activation analysis; *Spirulina platensis*; *Nostoc linckia*; elemental content

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1. Introduction

Promising new fields in medicine, pharmacology, and biotechnology are being intensively developed for disease treatment and prevention, along with environmental bioremediation (Assidi et al., 2022; Santos-Beneit, 2024). A significant biotechnological approach involves the application of cyanobacteria, a rich source of bioactive compounds for the production of vitamins, enzymes, and pharmaceuticals (Bouyahya et al., 2024; Vijayakumar & Menakha, 2015; Żymańczyk-Duda et al., 2022). The filamentous cyanobacterium *Spirulina platensis* is particularly notable, having gained international importance for its high-value phytonutrients and pigments (Bourais et al., 2022; Mary Leema et al., 2010). Spirulina is extensively used to produce biomass enriched with trace elements (Se, I, Cr, V, Fe, etc.), which is used to treat deficiencies of these elements and their related ailments (Cepoi et al., 2017; Mondal et al., 2024; Podgórska-Kryszczuk, 2024; Yang et al., 2024).

Members of cyanobacteria belonging to the family Nostocaceae are considered the most impressive “biochemical factories” of the biological world. The genus Nostoc is a valuable source of a wide spectrum of

secondary metabolites, such as fatty acids, that have many different potential uses as anticancer, anti-HIV, antimalarial, antifungal and/or antimicrobial drugs (Galhano et al., 2011; Shalaby et al., 2019; Thuan et al., 2019).

The rate of cyanobacteria biomass growth and its composition depend on many factors, the most important of which are the composition of nutrient medium, temperature and light. Recently, different types of cultivation medium have been developed to produce cyanobacteria biomass (Raoof et al., 2006). The application of cyanobacteria biomass in medicine and drug production requires careful control of its biochemical and chemical composition. The main part of the research in the field is devoted to the biochemical characterization of biomass (Cepoi et al., 2022; Raoof et al., 2006; Spínola et al., 2024). At the same time, several studies reported high content of elements with no biological functions in cyanobacteria biomass, which can have a negative impact on human health (Sochacka et al., 2025) .

Therefore, in the present study, the elemental composition of two types of *Spirulina platensis* biomass, produced in Moldova and Russia, and *Nostoc linkia* biomass was determined using neutron activation analysis, a powerful analytical technique for multi-element surveys. It should be mentioned that the elemental composition of cyanobacteria *Nostoc linckia* was determined for the first time.

2. Materials and methods

Materials

In the present study, algological pure cultures of cyanobacteria *Spirulina platensis* CNM-CB-02 (Spirulina I) (Cepoi, Zinicovscaia, Rudi, et al., 2022) and *Nostoc linckia* CNM-CB-03 strain (Cepoi et al., 2022) from the National Collection of Nonpathogenic Microorganisms (Institute of Microbiology and Biotechnology, Technical University of Moldova) and Spirulina platensis (Spirulina II) from the Moscow State University Collection (Moscow, Russia) were used.

Biomass cultivation

The cultivation of Spirulina (I) was carried out in an open-type tank with a volume of 60 L in the SP-1 nutritive medium (Cepoi et al., 2020) at a temperature of 32-35 ºC, illumination 37-55 µmoles of photons·m^{-2}·s^{-1}, pH 8-9 and at constant mixing. The cultivation of Spirulina (II) was carried out in an open-type tank with a volume of 1500 L (surface area 15 m^2, the depth of the nutrient medium 0.1 m) in the following nutritive medium (in g·L^{-1}): KNO_3-3.0; $NaHCO_3$-15.0; NaCl-1.0; K_2SO_4-0.5; K_2HPO_4-0.6; $MgSO_4·7H_2O$-0.5; H_3BO_3-2.86; $MnCl_2·4H_2O$-1.81, $CuSO_4·5H_2O$ -0.08 L; $ZnSO_4·7H_2O$-0.22; MoO_3–0.015 mg·L^{-1}; and FeEDTA – 1 g·L^{-1} at a temperature of 25-35 ºC, pH 9-11 and at constant mixing. Illumination during a 24-hour period changed from 0 to 100,000 lx. Selected illumination mode simulated a 24-hour light cycle, changing from 0 to 100,000 lux, represents the natural progression from a night (0 lux) through twilight (around 1-100 lux) to bright daylight (100 00-100,000 lux).

The culture of *Nostoc linckia (Roth) Born et Flah CNM-CB-03* was cultivated in laboratory conditions on mineral medium Gromov 6 (Cepoi, Zinicovscaia, Valuta, et al., 2022), stirring daily, at a temperature of 25-27 ºC, pH 6.8-7.2 and continuous illumination (a light intensity of 37-55 µmoles of photons·m^{-2}·s^{-1}). In the stationary growth phase (6^{th} day for *S. platensis* and 14^{th} day for *N. linckia*) the cyanobacteria biomass was separated from the culture medium by centrifugation, washed several times with bi-distilated water to remove media salts/impurities and dried. Next, the obtained biomass was used for elemental analysis.

Neutron activation analysis (NAA)

Neutron activation analysis was carried out at the pulsed fast reactor IBR-2 of the Frank Laboratory of Neutron Physics, JINR, Dubna, Russia. The temperature in the irradiation channels of the reactor IBR-2 does not exceed 60–70 ^{0}C, which allows irradiation of biological samples. A total of 23 elements (Mg, Al, Cl, Ca, Na, K, Sc, Cr, Mn, Fe, Ni, Co, Zn, Br, As, Se, Rb, Sb, Ba, Cs, and U) were determined using both short and long-time activation. To determine elements with short-lived isotopes: Mg, Al, Cl, Ca, and I, samples were irradiated for 3 min under a thermal neutron fluency rate of approximately $1.2·10^{12}$ n cm^{-2} s^{-1} and measured for 15 min. In the case of long-lived

isotopes: Na, K, Sc, Cr, Mn, Fe, Ni, Co, Zn, Br, As, Se, Rb, Sb, Ba, Cs, La, Ce, Hg, and U samples were irradiated for 4 days under a thermal neutron fluence rate of approximately $1.1 \cdot 10^{11}$ cm^2 s^{-1} and their activity was then measured in 4 and 20 days, respectively. γ-ray spectra were measured using a large-volume high-purity germanium detector with a resolution of 1.96 keV for the 1332.4-keV line of ^{60}Co.

The quality control of analytical measurements was provided by the National Institute of Standards and Technology (NIST, Gaithersburg, MD, USA) certified reference materials – SRM 1633b (constituent elements in coal fly ash), SRM 1572 (citrus leaves), SRM 1566b (Oyster tissue) (NIST 1566b). The difference between the certified and measured content of elements of the certified material varied between 1 and 10%. The NAA data processing and determination of element concentrations were performed using the software developed in FLNP JINR. More details about the irradiation of biological samples can be found in (Zinicovscaia et al., 2016, 2018).

Determination of the biochemical composition of Nostoc linkia

Protein content in the *Nosctoc linckia* biomass was determined spectrophotometrically by the Lowry method. Carbohydrate amount was determined by a spectrophotometric method using an anthrone reagent. Quantitative determination of lipids was carried out spectrophotometrically using the phospho–vanillin reagent. Phycobiliprotein level was calculated on the basis of the formula of Siegelman and Kycia. Pigments in biomass were quantified spectrophotometrically. More details about the determination of the biochemical composition of biomass can be found in (Cepoi et al., 2021).

Statistical analysis

All experiments were replicated 3 times. Statistical analysis was performed with a one-way analysis of variance (ANOVA) by using Statistica 10. Data are shown as Mean value± SD.

3. Results

In Table 1, the content of major and trace elements in *S. platensis* biomass are presented. Data obtained in the present work were compared with data present in Mosulishvili et al. (2004), Campanella et al. (1998), Ortega-Calvo et al. (1993) , and Al-Dhabi and Arasu (2013) studies. In total, it was possible to determine 22 elements, including major (Na, K, and Mg), minor (Fe, Zn, Se, Cr, Ni, and I), and elements that have no biological function.

The strain *Nostoc linckia (Roth.) BORN. et FLAH.* CNM-CB-03 cultivated on the Gromov -6 medium is characterized by the following biochemical composition: proteins – 15-25 %, carbohydrate – 35-50 %, phycoerythrin – 2.0-4.0 %, phycocyanin – 0.5-1.0 %, alophycocyanin – 0.5 -1.0 %, lipids – 2 %, carotene – 0.2-0.4 %, and xanthophylls - 0.4-0.7 %.

Table 1. Content of elements in the *Spirulina platensis* biomass and literature data

	Concentration, mg·kg^{-1} dry weight							
Element	*S. platensis* (I)	*S. platensis* (II)	*S. platensis* Mosulishvili et al. (2004)	*S. platensis* Campanella et al. (1998)	Spirulina A Ortega-Calvo et al. (1993)	Spirulina B Ortega-Calvo et al. (1993)	Spirulina C Ortega-Calvo et al. (1993)	Spirulina (25 samples) Al-Dhabi and Arasu (2013)
Method*	NAA	NAA	NAA	INAA and ICP-AES	AAS	AAS	AAS	ICP–MS
Na	10600±420	4133±270	15485±2300	12300±400	13630	17680	1230	–
Mg	5380±270	3013±160	1640±320	120±50	3420	5030	4610	–
Al	130±6	47±5	94±11	–	–	–	–	–
Cl	6430±385	667±37	5690±570	630±30	–	–	–	–
K	18600±1670	7037±350	18025±1800	890±40	18970	16490	11340	–
Ca	21100±2300	4637±244	937±230	720±10	9600	15410	6870	–
Sc	0.01±0.002	n.d.	0.01±0.002	0.25±0.02	–	–	–	–
Cr	8.9±0.9	<1.9	6.2±0.9	9.0±0.3	3.3	7.1	5	–
Mn	117±5	34±2	48±5	54.5±0.4	117	64	36	0.08-2.2
Fe	4610±230	517±30	1360±120	1116±25	751	2016	945	–
Ni	4.4±0.4	1.1±0.2	4.7±1.3	–	5.4	5.8	6.1	0.2-4.7
Co	0.12±0.01	0.22±0.01	0.98±0.01	0.68±0.05	1.7	0.5	0.9	–
Zn	34±3	17±1.1	115±18	240±11	50	29	24	0.5-6.2
As	0.44±0.02	0.100±0.001	0.57±0.17	–	–	–	–	–
Se	0.12±0.03	<0.3	n.d	0.120±0.006	–	–	–	–
Br	1.9±0.2	1.2±0.1	0.7±0.1	19.2±0.4	–	–	–	–
Rb	0.32±0.07	0.8±0.1	0.41±0.06	–	–	–	–	–
Sb	0.060±0.003	0.010±0.001	0.10±0.01	0.14±0.02	–	–	–	–
I	4.1±0.7	0.21±0.01	0.4±0.1	–	–	–	–	–
Ba	25±1.3	n.d	n.d	–	–	–	–	–
Cs	0.009±0.002	0.023± 0.001	n.d	0.060±0.002	–	–	–	–
V	n.d	n.d	0.42±0.08	3.2±0.4	–	–	–	–
U	0.041±0.003	0.024±0.001	n.d	n.d	–	–	–	–

Table 2 shows the macromineral and trace elements content in the *Nostoc linkia* biomass. A total of 23 elements were determined in biomass by NAA. The obtained data were compared with data obtained for *Nostoc flagelliforme* (Gao, 1998) and allowed daily intake levels of metals recommended by the National Institutes of Health (Office of Dietary Supplements (ODS), 2025) and the World Health Organization (Trace Elements in Human Nutrition and Health, 1996).

Table 2. Content of elements in the *Nostoc linkia* biomass, mg·kg^{-1}

Element	*Nostoc linkia*	*Nostoc flagelliforme* (Gao, 1998)	Allowed daily intake
Na	5500±220	–	–
Mg	5360±260	2700	–
Al	140±7	–	2-14 mg·day^{-1}
Cl	9310±745	–	–
K	63600±5090	–	3500 mg·day^{-1}
Ca	9300±1210	1830	200-1300 mg·day^{-1}
Cr	6.0±0.7	–	0.2-120 µg·day^{-1}
Mn	131±7	23±6.5	2-5 mg·day^{-1}
Fe	480±62	300	0.2-27 mg·day^{-1}
Ni	15.2±1.5	10.6±2.3	<1 mg·day^{-1}
Co	1.81±0.01	2.8±0.09	5-8 µg·day^{-1}
Zn	30±2	12.8±4.2	2-12 mg·day^{-1}
As	0.70±0.02	–	15-70 µg·day^{-1}
Se	0.30±0.05	–	15-70 µg·day^{-1}
Br	4.8±0.6	–	–
Rb	4.82±0.08	–	–
Sb	0.100±0.005	–	–
Ba	17.3±1.4	–	–
La	0.20±0.03	–	–
Ce	2.7±1.7	–	–
Sm	0.030±0.004	–	–
Cs	0.30±0.01	–	–
U	0.040±0.003	–	–

Discussion

All *S. platensis* samples showed a relatively high Na and K content; however, the Na/K ratio in all cases was below 1.5, approximately 0.6 for both types of spirulina samples. Potassium (K) is a cofactor for many enzymes and is involved in protein synthesis and osmotic regulation (Harris, 2012). The Na content in biomass depends mainly on the Na amount in salts added in the formulation of the cultivation medium. Magnesium (Mg) level in the studied samples was in the range of data reported in Ortega-Calvo et al. (1993), but higher than the data presented by Mosulishvili et al. (2004) and Campanella et al. (1998). Magnesium (Mg) occupies a strategic position in the photosynthetic apparatus as the centre of the chlorophyll molecule; therefore, all cyanobacterial species have an absolute requirement of this element. Apart from this, it has a role in the aggregation of ribosome into functional units, and the formation of catalase (Ahmed et al., 2023; Salman et al., 2023).

Trace elements: Fe, Zn, Se, Cr, Ni, and I, which play an important role in the metabolism and vital functions of living organisms. Iron is essential for the functioning of many biochemical processes, including electron transfer reactions, gene regulation, binding and transport of oxygen, and regulation of cell growth and differentiation (Beard, 2001; Lieu et al., 2001). The difference in Fe content was very pronounced in the studied spirulina samples: 4610 mg·kg^{-1} for Spirulina I and 517

mg·kg^{-1} for Spirulina II. Zinc (Zn), the second after iron most abundant microelement in all living organisms, is the co-factor of more than 300 enzymes, providing structural stability of a large number of proteins (Chasapis et al., 2020). Zinc content in Spirulina I sample (34 mg·kg^{-1}) was two times higher than in Spirulina II sample (17 mg·kg^{-1}). Obtained Zn levels were lower than data reported by Mosulishvili et al. (2004) and Campanella et al. (1998) and similar to data obtained by Ortega-Calvo et al. (1993). In the case of Cr, its amount in the Spirulina II sample was below the method's detection limit. In Spirulina I sample Cr content was similar to the data reported in Campanella et al. (1998) and higher than the values reported I other studies. Chromium is an essential nutrient that potentiates insulin action and thus influences carbohydrate, lipid and protein metabolism (Chromium - Health Professional Fact Sheet, 2025). Manganese (Mn) is both an activator and a constituent of several enzymes. Manganese level in Spirulina I and Spirulina II samples, 117 and 43 mg·kg^{-1}, respectively, was in the range of data presented in Ortega-Calvo et al. (1993) study. Iodine (I) level in spirulina samples was highly variable: 4 mg·kg^{-1} for Spirulina I and 0.2 mg·kg^{-1} for Spirulina II. It should be mentioned that I was determined only in spirulina samples, which were analyzed using NAA.

The ultra-trace element Ni is both essential and toxic for animals and humans. A Ni-poor nutrition of < 0.1 mg·kg^{-1} dry matter led to Ni deficiency symptoms (Chasapis et al., 2012). The lowest Ni content (1.1 mg·kg^{-1}) was determined in the Spirulina II sample, while its amount in the Spirulina I sample (4.7 mg·kg^{-1}) was in the range of data presented in other works. While some of trace elements are important from the nutritional point of view, other ones (As, Rb, Sb, U) are considered toxins for cells. Trace amount of these elements appear in chemical reagents used to prepare nutrient medium. Rubidium (Rb) and U were determined only in spirulina samples analyzed by NAA. WHO data show that the concentration of toxic elements in the analyzed samples did not exceed allowed daily intake (Table. 2) (Trace Elements in Human Nutrition and Health, 1996)

Comparing two types of spirulina samples, it was observed that the content of all elements in Spirulina (II), except Rb and Co, were lower than in Spirulina (I). These differences can be explained by the particularity of biomass cultivation, the differences in the composition of the cultivation medium used for these two strains and light intensity. It is known that constant light intensity promotes biomass growth parameters, which results in higher pigments and proteins as well as minerals accumulation (Soni et al., 2017). Production of Spirulina biomass depends on many factors, the most important of which are nutrient availability, temperature and light. Kumar et al. (2011) reported that the highest biomass in Spirulina was obtained at 35°C and 2,000 lux light intensity. Spirulina I was cultivated under constant light intensity and temperature, while in the case of Spirulina II light intensity and temperature varied in a wide range. Low temperature and illumination lead to low cell productivities as a result the accumulation of metals from nutrient medium can be also reduced.

In case of *Nostoc linckia* the level of elements in the analyzed strain was higher than in *Nostoc flagelliforme* (Gao, 1998). The presence of toxic elements as As, Sb, La, Ce, Sm, etc in the biomass can be explained by their introduction in the cultivation medium with chemical reagents used to prepare it. Therefore, only high-purity reagents must be used to produce biomass for pharmaceutical and medical purposes. The content of toxic metals in the microbial biomass did not exceed the limits for daily intake set by the World Health Organization.

Essential metals are actively taken up by cyanobacteria through specialized uptake systems, but non-essential metals may also be taken up because they are mistaken for an essential metal (Ledin, 2000). Cyanobacteria can accumulate metals through biosorption or bioaccumulation (fig. 1). Metal ions biosorption mechanisms includes physical adsorption (weak forces), electrostatic attraction, ion exchange, complexation, chelation, and surface precipitation. The functional groups of carbohydrates, proteins and lipids include amino, carboxyl, thio ether, sulfhydryl, imidazole group of histidine, the oxygen, phosphate, phenolic, nitrogen of the peptide bond and amide moieties are responsible for the coordinating bond with the metallic ions (Rangabhashiyam & Balasubramanian, 2019).

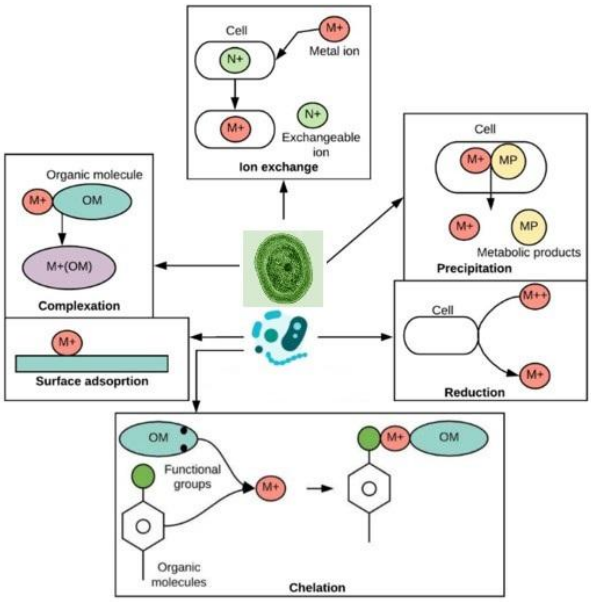

Figure 1. Main mechanism of metal ions uptake by cyanobacteria (Ramírez Calderón et al., 2020)

5. Conclusion

Neutron activation analysis is very efficient analytical technique for performing quantitative multi-elemental analysis of major, minor and traces components in biological samples. The concentrations of 23-24 elements were determined simultaneously in each cyanobacteria strain. The elemental composition of cyanobacteria *Nostoc linckia* was determined for the first time. The elemental content of the studied spirulina samples (Spirulina I and Spirulina II) was within the variability range of similar products reported in the literature. Calcium (Ca), Mg and Fe concentration in Spirulina I were higher in comparison with other samples. For Spirulina II concentrations of Al, Cl, Cr, Ni, and Zn were lower in comparison with other samples. Concentration of all determined elements in Spirulina II, except Rb and Co, was lower than in Spirulina I. The uptake of elements by biomass depends on experimental parameters and is controlled by the line of chemical mechanisms, including ion exchange, complexation, chelation, and surface precipitation. *Spirulina platensis* and *Nostoc linckia* can be successfully used for nutrition and pharmaceuticals production as safety source of trace elements. Both cyanobacteria strains present great interest for medicine and food technology. In future studies, it is necessary to assess the impact of varying experimental conditions on biomass growth as well as to assess the transfer of elements in the living organisms (animals, humans).

6. Supplementary Materials: No supplementary material.

7. Author Contributions

Conceptualization - I.Z.; methodology - I.Z.; data processing - I.Z.; validation - I.Z.; formal analysis - I.Z.; investigation - I.Z..; resources - I.Z.; data curation - I.Z.; writing - original draft preparation - I.Z..; writing - review and editing - I.Z.; visualization - I.Z.

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Нейтрондық активация талдауын қолдана отырып, *Spirulina platensis* және *Nostoc linckia* цианобактерияларының элементтік құрамын анықтау

Инга Зиньковская

Аңдатпа. *Spirulina platensis* және *Nostoc linckia* цианобактериялары тамақ және фармацевтика өнеркәсібінде, сондай-ақ биоремедиацияда қолдануға кең әлеуеті бар жоғары қоректік заттар болып табылады. Дегенмен, олардың металға, соның ішінде улы биоаккумуляцияға жоғары қабілеті олардың элементтік құрамын бақылауды қажет етеді. Ресейдің Дубна қаласындағы Біріккен Ядролық зерттеулер институты, Франк нейтрондық физика зертханасының IBR-2 реакторындағы көп элементті нейтрондық активация талдауы Spirulina platensis және цианобактерия Nostoc linckia екі штамының элементтік құрамын зерттеу үшін пайдаланылды. Әрбір цианобактерия штаммында Mg, Al, Cl, Ca, Na, K, Sc, Cr, Mn, Fe, Ni, Co, Zn, Br, As, Se, Rb, Sb, Ba, Cs және U сияқты 23 элементтің құрамы анықталды. Микробтық биомассадағы улы металдардың мөлшері Дүниежүзілік денсаулық сақтау ұйымы белгілеген күнделікті тұтыну шегінен аспады.

Түйін сөздер: нейтронды активациялау талдауы; *Spirulina platensis*; *Nostoc linckia*; элементтік құрамы

Определение элементного состава цианобактерий *Spirulina platensis* и *Nostoc linckia* методом нейтронно-активационного анализа

Инга Зиньковская

Аннотация. Цианобактерии *Spirulina platensis* и *Nostoc linckia* являются высокопитательными продуктами с широким потенциалом использования в пищевой и фармацевтической промышленности, а также для задач биоремедиации. Однако их высокая способность к биоаккумуляции металлов обусловливает необходимость контроля элементного состава. Многоэлементный нейтронно-активационный анализ на реакторе ИБР-2 Лаборатории нейтронной физики им. И.М. Франка Объединенного института ядерных исследований в Дубне (Россия) был использован для изучения элементного состава двух штаммов *Spirulina platensis* и цианобактерии *Nostoc linckia*. В каждом штамме цианобактерий было определено содержание 22-23 элементов, включая Mg, Al, Cl, Ca, Na, K, Sc, Cr, Mn, Fe, Ni, Co, Zn, Br, As, Se, Rb, Sb, Ba, Cs и U. Содержание токсичных металлов в микробной биомассе не превышало предельно допустимых норм суточного потребления, установленных Всемирной организацией здравоохранения.

Ключевые слова: нейтронно-активационный анализ; *Spirulina platensis*; *Nostoc linckia*; элементный состав