

IRSTI 34.31.27

¹G.I. Yernazarova, ^{1*}S.K. Turasheva, ²A.A. Sartayeva, ¹S.B. Orazova,
³A.A. Bazargaliyeva, ²E.M. Imanova, ²G.K. Omarova, ⁴S. Yancheva

¹Laboratory of Environmental Biotechnology, Almaty, Kazakhstan

²Department of Biology, Kazakh State Women's Teacher Training University, Almaty, Kazakhstan

³Department of Biology, Aktobe Regional University named after K. Zhubanov, Aktobe, Kazakhstan

⁴Agricultural University-Plovdiv, Plovdiv, Bulgaria

*e-mail: svetlana.turasheva@kaznu.kz

Absorption of chromium by mono- and mixed cultures of microalgae

Abstract: In the article the sorption ability of microalgae cultures to chromium are discussed. The objects of the study were microalgae from the collection of the Department of Biotechnology of Al-Farabi Kazakh National University, related to cyanoprokaryotes (*Cyanoprokaryota/ Cyanobacteria*): *Anabaena flos-aquae*, *Anabaena arnoldii*, *Nostoc linckia*, *Calothrix parietina* as well as their two-species mixtures *A. flos-aquae* x *C. parietina*, *N. linckia* x *C. parietina*, *A. flos-aquae* x *N. Linckia*, *A. flos-aquae* x *A. arnoldii* and *Scenedesmus quadricauda*. Also two green (*Scenedesmus quadricauda* and *Chlorhormidium sp.*) and one diatomaceous (*Nitzshia sp.*) algae were studied. The duration of cultivation was 20 days. Potassium bichromate was added to the nutrient medium at concentrations 0.01-0.2 mg/ml calculated per unit of chromium. The concentration of chromium in the filtrates of the studied cultures in some cases significantly decreased by the end of the cultivation period, which indicates the biosorption of this element by the microalgae cells. The investigated strains of microalgae absorb chromium from the medium in varying degrees. The most active biosorbents among the explored cultures were *A. flos-aquae*, *N. linckia* and *C. parietina*. A microalgae *A. arnoldii* extracts chromium from the medium in smaller quantities. The listed strains refer to cyanoprokaryotes (cyanobacteria), from other cultures, the *Scenedesmus quadricauda* absorbs chromium quite actively. The highest intensity of chromium biosorption is characteristic of *Nostoc (N. linckia)*, which extracts from the medium 60.8-74.6% chromium at initial concentrations 0.05-0.1 mg/ml respectively. The most active biosorbents of chromium were four species of Cyanobacteria. In this regard, these strains have been selected by us for future study of the processes of sorption and metabolic activity in mono- and mixed cultures of microalgae.

Key words: microalgae, chromium, sorption, stability, antagonism, algoflora.

Introduction

The growth of cities and industrial production leads to environmental pollution by chemical elements. The consequence of this is the increasing spread of ecosystems, disturbed as a result of human technical activities. Pollution of the environment by potentially dangerous chemicals, especially various metal compounds, creates extreme conditions for living organisms, including humans [1].

The main sources of air, soil and water contamination with metals are fossil fuel combustion products and industrial emissions, especially mining, metallurgical and chemical. In the zones of influence of metallurgical and some other enterprises in water, soils and plants, a large number of metals can accumulate (Ag, Al, Be, Cd, Co, Cr, Cu, Fe, Mn, Mo, Ni, Pb, Sb, Sn, Ti, V, Zn and etc.) [2; 3]. Some of them

cause death or inhibit the growth of plants, and when precipitated from the air, they are sucked from the surface of the soil by the roots of plants, impair the quality of vegetables and fruits, have harmful effects, plant animal feed resources and microorganisms involved in soil self-cleaning, with surface runoff in water and have a disastrous effect on fish and their food resources. They are filtered through the soil and at a low level of water in the reservoirs in winter with the groundwater enter rivers and lakes and have a harmful effect on aquatic organisms [4]. Heavy metals coming from anthropogenic sources of pollution have a great impact on aquatic ecosystems. In reservoirs that are testing the constantly increasing level of ecotoxicants in the water, they accumulate in the tissues and organs of plants and animals, a steady decrease in the number of individual components of the biocenosis, which can lead to a violation of the

balance of biological processes and the death of the ecosystem [5-9].

Recently, the ability of microorganisms, including microalgae, to biosorb heavy metals has been proven. The intensity of the process of metal sorption depends, first of all, on the physiological state of the culture. The actively metabolizing populations of microalgae store and macrophytes more intensively heavy metals than inactive cultures [1; 8].

Algae are an exceptionally convenient model object for studying the general patterns of the influence of toxicants on the cellular and population levels simultaneously [10-13].

Among the test objects of different trophic levels studied in the comparative toxicological experiment (seaweed *Phaeodactylum tricornutum* and *Coscinodiscus janishii* A.S., the fusiform *Infusoria Euplotes patella lemani* Dragesco, the early naupliar stages of the trophic lobster crustaceans *Artemia salina* L., the fertilized eggs of the bivalve mollusks *Mytilus galloprovincialis* L.) unicellular algae in general – the most sensitive to potassium dichromate group of organisms [14]. In this regard, algae, as one of the main test objects, are included in the methodological documents for the assessment of the toxicity of the polluted aquatic environment [11; 12]. In studies on the effects of harmful substances on aquatic organisms, potassium dichromate is used as a reference toxicant [13; 14].

Growth and development of microalgae in a certain range of concentrations of TM, due to genetic characteristics, can be characterized as tolerance. Resistance of microalgae populations to TM concentrations outside the tolerant zone is characterized as resistance. The concept of resistance is closely related to adaptation – the ability to experience unfavorable conditions that caused the death of a given organism.

It has been established that laboratory cultures of green microalgae (*Scenedesmus* sp., *Chlorella* sp.) are more resistant to chromium than diatomaceous algae cultures (*Fragillaria crotonensis*) [12; 15]. In natural populations of phytoplankton at elevated Cr²⁺ concentrations, dominance in some algal flora of some water bodies also changed from diatom and blue-green to green algae [16]. At the same time, the exceptional resistance of blue-green algae with gel-like mucous membranes on the surface of cells is also emphasized, and among others it performs the function of chelating many elements [17; 18].

Materials and methods

The microalgae species from the collection of the Department of Biotechnology of Al-Farabi Kazakh

National University, related to Cyanoprokaryotes *A. flos-aquae*, *A. arnoldii*, *N. linckia*, *C. parietina* as well as their two-species mixtures, two green (*Scenedesmus quadricauda* and *Chlorhormidium* sp.) and one diatomaceous (*Nitzshia* sp.) algae were used as the objects of investigation.

Microalgae in the form of monocultures and two-species mixtures were grown on Fitzgerald medium at 24-hour illumination. The duration of cultivation was 20 days. Potassium dichromate was added to the nutrient medium at concentrations of 0.01-0.2 mg/ml in terms of chromium.

The chromium content in the medium and the biomass of plant objects was determined on an AAS-IN atomic absorption spectrophotometer, Carl Zeiss, using a flame atomizer. The plant samples were kept in an oven for 1 hour at 130 °C. The samples were mineralized in a muffle furnace at 450 °C. The dish with the ash was cooled to room temperature and 1 ml of nitric acid solution was wetted with sulfur ashes. Then, the acid was evaporated to dryness on an electric cooker with a mild heating, and again a cup with a sample was placed in an electric oven at a temperature of 250 °C. The temperature was gradually brought to 450 °C and held for 1 hour. The mineralization was considered complete when the ash became white or slightly colored, without charred particles. In the presence of charred particles, the treatment of the ash was repeated with a solution of nitric acid or water. The metal concentration was determined by the following formula:

$$C\% = \frac{nVk100}{P},$$

where n is the determined concentration of metal in solution (µg/ml), is determined from the calibration curve; V is the volume of the solution (ml); P is the weight of the sample (g); k is the dilution factor [14].

The amount of metal in the medium was calculated by the formula:

$$C = \frac{nV_2}{V_1},$$

where n is the concentration of the element found in the solution (µg / ml), is determined from the calibration curve; V_1 – initial volume (ml); V_2 is the final volume (ml) [17]. To determine the dry mass of algae, several samples with 10 ml of medium were taken at the beginning and end of the experiment

and passed through a membrane filter. The weight of filters with algae and without algae was determined after repeated drying in bucks at 130 ° C. By the difference, a dry mass of algae was determined in each variant of the experiment at the beginning and end of the experiment.

The data were processed statistically and reliably at $P > 0.95$. The experimental data show the arithmetic mean values of the three experiments and their standard deviations.

Results and discussion

In this study were tested strains of microalgae from the collection of the Department of Biotechnology of Al-Farabi Kazakh National University. 9 strains of microalgae, belonging to the majority of Cyanoprokaryotes (6 strains), 2 green (*Scenedesmus quadricauda* and *Chlorhormidium sp.*) and 1 diatomaceous (*Nitzshia sp.*) were used in this investigation. The microalgae were cultured for 25 days on a Fitzgerald medium with a chromium content of 0,05 and 0.1 mg/ml. The concentration of chromium in the filtrates of the studied cultures in some cases significantly decreased by the end of the cultivation period, which indicates the biosorption of this element by the microalgae cells. In accordance with Figure 1, the investigated microalgae strains absorb chromium from the medium to some extent.

The most active biosorbents among the studied cultures were *A. flos-aquae*, *N. linckia* and *C. paryethina*. A little in smaller quantities extracts chromium from the environment of *A. arnoldii*. The listed strains refer to cyanoprokaryotes (cyanobacteria). Other cultures like *Scenedesmus quadricauda* is quite actively absorbed chromium and had the highest intensity of biosorption

Further follows *A. flos-aquae* (53.6-55.6%). It is interesting to note that the degree of extraction of chromium from the medium is not always directly proportional to its concentration in the medium. In *Calothrix parietina*, the amount of absorbed chromium decreases slightly (from 53 to 51%) with an increase in its initial concentration in the medium. A decrease in the biosorption of chromium is also observed in the case of *A. arnoldii*, *Scenedesmus quadricauda*, *Anabaena variabilis*, *Chlorhormidium sp.*, *Phormidium uncinatum*. The decrease in the intensity of metal absorption from the medium with increasing its concentration may be due to inhibition of growth processes and a decrease in the metabolic activity of the algae.

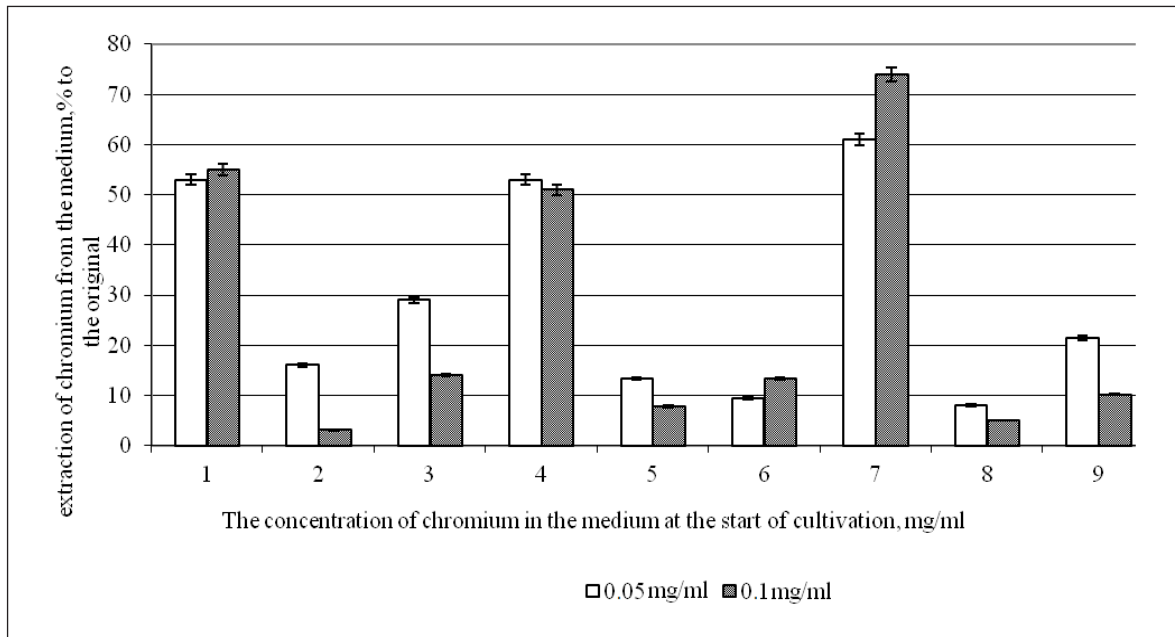
As noted above, the most active chromosome biosorbents have been shown to be 4 species of blue-green algae. In connection with this, these strains were chosen by us for the subsequent study of the processes of sorption and metabolic activity in mono- and mixed cultures of microalgae.

Some works indicate that mixed cultures of certain species of microalgae show higher growth activity than monocultures [3; 4]. This fact should be taken into account when selecting species that actively absorb heavy metals in mixed populations. To clarify the activity of chromium absorption, we studied not only the monocultures of the above four microalgae strains, but also their mixed two-species cultures. In cultures of 20 days of age, a growth curve appears on the plateau, which is the beginning of the stationary phase, when the metabolic processes are still quite active. It is known that there is some rhythm in the sorption-desorption-reabsorption processes. According to J. Fogg [4; 5], through the isolation of exometabolites, algae perform dissolution, complexation, reduction of various elements, as well as obtaining specific exogenous complexes ready for absorption.

In subsequent experiments, we studied the intensity of the processes of chromium sorption in correlation with the intensity of metabolic processes.

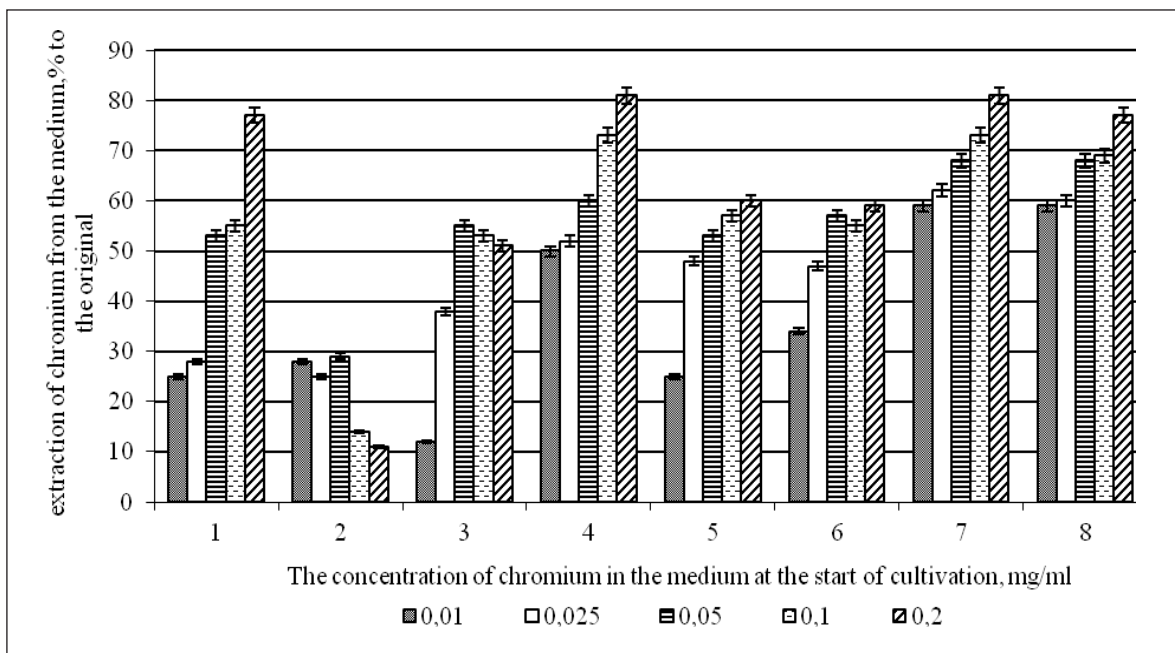
According to the Figure 2, where the results of the determination of chromium extraction from the medium at a greater concentration change (0.01-0.2 mg/ml) are presented, among the monocultures of the studied microalgae species, the most sorptive activity with respect to chromium was Nostoc, in the filtrate of which even at a very high initial concentration (0.2 mg / ml), only 19.5% of the initial amount of the element was found, which corresponds to an extraction of 80.6% (Figure 2). The value of the *A. flos-aquae* sorption activity at the maximum chromium concentration in the medium approaches the values determined for Nostoc, although at chromium concentrations from 0.01 to 0.05 mg/ml, the intensity of chromate absorption by anabenium was significantly lower than for Nostoc. Significantly less sorption activity was shown by *C. parietina*. With an increase in the concentration of chromium from 0.05 to 0.2 mg/ml, the rate of its extraction from the medium decreases.

According to the intensity of biosorption of chromium, *A. arnoldii* showed the lowest activity in these experiments in comparison with the above species.



1 – *A. flos-aquae*, 2 – *Anabaena variabilis*, 3 – *A. arnoldii*, 4 – *C. parietina*, 5 – *Chlorhormidium sp.*, 6 – *Nitzschia sp.*, 7 – *N. linckia*, 8 – *Phormidium uncinatum*, 9 – *Scenedesmus quadricauda*

Figure 1 – Screening of microalgae on the biosorption of chromium from the chromium environment is typical for nostoc, which extracts from the medium 60.8 – 74.6 % chromium at initial concentrations of 0.05 – 0.1 mg/ml, respectively



1 – *A. flos-aquae*, 2 – *A. arnoldii*, 3 – *C. parietina*, 4 – *N. Linckia*, 5 – *A. flos-aquae* x *C. parietina*, 6 – *N. linckia* x *C. parietina*, 7 – *A. flos-aquae* x *N. Linckia*, 8 – *A. flos-aquae* x *A. arnoldii*

Figure 2 – Absorption of chromium by microalgae

In mixed cultures with negative allelopathy, the intensity of chromium uptake from the medium is, for the most part, comparable to the intensity of biosorption of one of the partners of the mixture or represents a certain average value between the extraction values in monocultures of both partners. At the same time, in mixtures with positive allelopathy, there is an increase in sorption activity in comparison with monocultures. In a mixture of *A. flos-aquae* x *N. linckia* as well as in a mixture of *A. flos-aquae* x *A. arnoldii*, a linear dependence of the extraction of chromium on its concentration in the medium is observed. The greatest amount of chromium was extracted at a concentration of 0.2 mg/ml of microalgae in the first 5 days of cultivation. The change in the intensity of sorption processes in the cultures of microalgae studied confirms this regularity. As an example, we give the results of analyzes carried out on the culture of *C. parietina*. Table 1 shows the determination of chromium content in various fractions: 1) culture liquid, i.e. dissolved, residual chromium; 2) microalgae cells = absorbed chromium and 3) washings with a 5% solution of NH_4OH sediment of algal cells on the membrane filters (Table 1). The amount of chromium found in

the wash solution was evaluated as adsorbed on the cell surface.

Data given in Table 1 show that at the first day of cultivation (24 hours) only 22% of the initial chromium content remains in the medium. 64% is absorbed by the cells, while a certain amount (14%) is adsorbed on the cell surface.

With the increase in the period of cultivation, gradual desorption of chromium from the cells into the medium is observed, which leads to an increase in the amount of dissolved metal to 66% by the 5th day of cultivation (120 hours). The amount of chromium adsorbed on the cell surface to 26% also increases. By this time of cultivation the chromium content in cells is reduced to 8%.

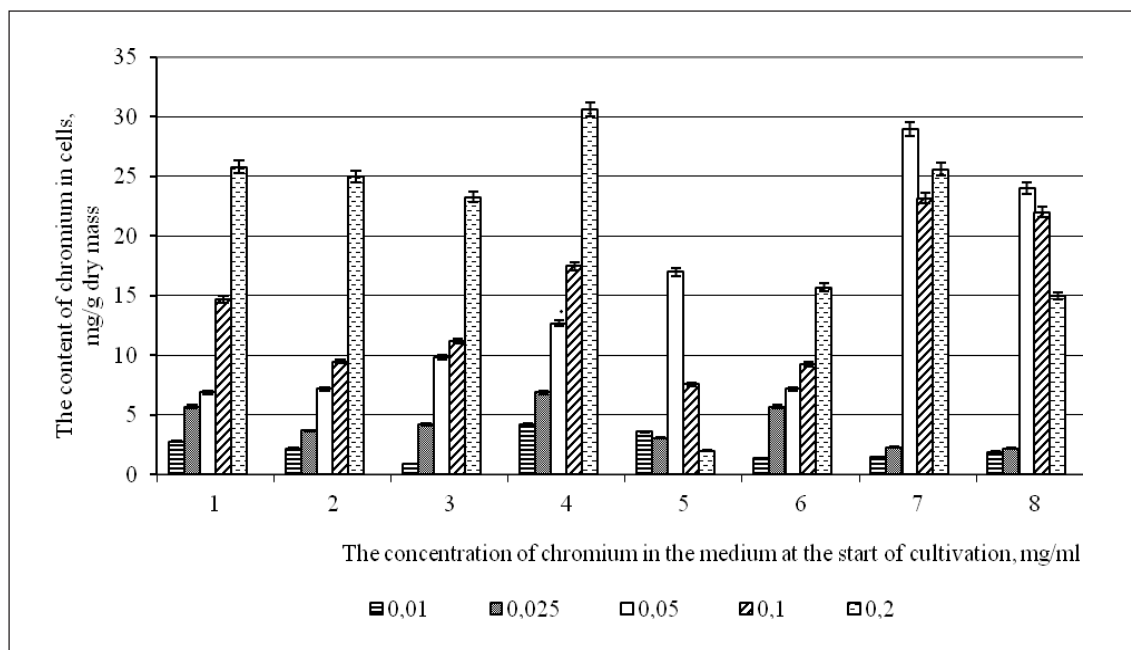
In accordance with Figure 3, where the accumulation of chromium is shown by the cells of the blue-green algae studied, a gradual increase in chromium content in the cells is observed in monocultures as the concentration of this element in the medium increases. The largest quantities were found in variants with a starting content of 0.2 mg/ml of chromium. Monocultures of blue-green algae in this variant of the experiment accumulate in the cells 15.0-25.0 mg of chromium per gram of dry mass.

Table 1 – Dynamics of chromium content in culture of *C. parietina* in the initial period of cultivation (at an initial concentration 0.05 mg/ml)

Day	Dissolved chromium in the medium (residual)		Adsorbed chromium		Absorbed cells chromium	
	mg/ml	% to the original	mg/ml	% to the original	mg/ml	% to the original
1	0.011±0.003	22	0.007±0.001	14	0.032±0.002	64
2	0.016±0.002	32	0.007±0.001	14	0.027±0.003	54
4	0.019±0.002	38	0.008±0.002	16	0.023±0.004	46
5	0.026±0.001	52	0.014±0.003	28	0.010±0.003	20
6	0.033±0.003	66	0.013±0.003	26	0.004±0.001	8

In mixed cultures with negative allelopathy, the tendency to increase the chromium content in cells with an increase in its concentration in the medium does not persist. Thus, in a mixture of *A. flos-aquae* x *C. parietina*, the maximum chromium content in cells was found in the variant 0.05 mg/ml, which is 17.2 mg/g. A further increase in the initial concentra-

tion of chromium in the medium leads to a decrease in the concentration of chromium in the cells, which is obviously explained by a decrease in the cell viability level in this mixture, as shown above. Cell death in another mixture with negative allelopathy (*N. linckia* x *C. parietina*) leads to rather low values of chromium absorbed by the cells.



1 – *A. flos-aquae*, 2 – *A. arnoldii*, 3 – *C. parietina*, 4 – *N. Linckia*, 5 – *A. flos-aquae* x *C. parietina*, 6 – *N. linckia* x *C. parietina*, 7 – *A. flos-aquae* x *N. Linckia*, 8 – *A. flos-aquae* x *A. arnoldii*

Figure 3 – Chromium content in cells of mono- and mixed cultures of microalgae

With an increase in the chromium concentration in the medium, the chromium content in the cells is further reduced. In the other two-species cultures, much higher amounts of chromium were detected in the cells. Allelopathy with positive interference of species, leading to active growth and division of cells of both partners, promotes intensive absorption of chromium from the environment. It can only be noted that at low chromium concentrations (0.01-0.025 mg/ml), the cells absorb rather small amounts of chromium. However, a further increase in chromium concentration in the medium leads to its accumulation in cells at sufficiently high concentrations. The highest chromium content in *A. flos-aquae* x *N. linckia* cells was found in variants with a starting content of 0.05 and 0.2 mg/ml. At a concentration of 0.1 mg/l decrease in the chromium content of the cells is observed, which is rather difficult to explain. According to some authors, the concentration absorption curves of metals can have several peaks, very often no linear dependence is observed. This character of metal absorption is explained by the biological specificity of the given object and the physicochemical features of the behavior of metal ions in solutions [1, p.84].

It is interesting to consider how the chromium extracted from the medium is distributed: whether it is absorbed by the cells. To do this, we used as an example to determine the chromium content in a 20-

day mixed culture of *N. linckia* x *C. parietina*. In Table 2 (similarly to Table 1), the determination of chromium content in different fractions is given: 1) culture liquid, i.e. dissolved, residual chromium; 2) microalgae cells = absorbed chromium and 3) washings with a 5% solution of NH_4OH sediment of algal cells on membrane filters (Table 2). The amount of chromium found in the wash solution was evaluated as adsorbed on the surface of the cells.

As can be seen from the data presented in Table 2, after a 20-day cultivation of chrome microalgae, the medium remains between 28 and 66%, depending on the initial concentration of chromium in the medium. The lowest degree of sorption is characteristic for both the minimum concentration (0.01 mg/ml) and its maximum concentration (0.02 mg/ml). Much more intensively, the cells absorb chromium in the 0.025 mg/l variant, reducing the amount of residual chromium in the medium to 28%. An increase in the chromium concentration to 0.05 and 0.1 mg/ml resulted in an increase in chromium content in the medium. The adsorption of chromium on the surface of cells increases in an abrupt way with increasing concentration. The greatest number was found in the variants with 0.025 mg/ml and 0.2 mg/ml of chromium. The chromium content in the cells decreased with increasing initial concentration in the medium, reaching a minimum of 9% at 0.2 mg/ml.

Table 2 – Distribution of chromium by fractions in a 20-day mixed culture *N. linckia x C. pariethina*

The initial concentration of chromium in the medium (mg/l)	Chromium (residual) dissolved in the medium		Adsorbed chromium		Cell absorbed chrome	
	mg/l	% to the original	mg/l	% to the original	mg/l	% to the original
0.010	0.0062±0.0009	66.0	0.0010±0.0007	14.0	0.0042±0.0008	30
0.025	0.0071±0.0011	28.0	0.0081±0.0005	32.0	0.0120±0.0006	40
0.050	0.0210±0.0006	42.0	0.0141±0.0008	28.0	0.0153±0.0004	30
0.100	0.0520±0.0004	52.0	0.0192±0.0006	19.0	0.0291±0.0003	29
0.200	0.1270±0.0010	63.5	0.0552±0.0007	27.5	0.0182±0.0010	9

If we compare these data with those shown in Figure 1, the change in the intensity of chromium sorption from the medium by the cells of the studied mixed culture can be explained by the intensification and retardation of cell division. With the activation of growth processes at a concentration of 0.025 mg/ml, the uptake of chromium was increased to 72% (in total), with almost 45% of this amount adsorbed on the cell surface.

Inside the cells only 55% of the sorbed chromium penetrates. With increasing chromium concentration in the medium, the intensity of chromium uptake from the medium drops to 36.5%.

Conclusion

Most of this quantity (75.3%) remains adsorbed on the surface, and only 9% of sorbed chromium permeates the cells, which confirms the data of the researchers [6], who found that active metabolizing cells actively activate the biosorption of metals.

The obtained results indicate that the intensity of sorption is directly proportional to its concentration in the medium. Thus, as the results of studying of phytotoxicity tests, it has been established that cultivation in media with high concentrations of chromium, microalgae extract chromate ions from the medium from 25 to 80% of its original quantity. Up to 50 percent or more of the extracted chromium is adsorbed on the surface of the cells, while in chromium biomass chromium is detected in an amount not exceeding 30 mg/g dry weight. In the processes of intracellular absorption of chromium, mixtures with positive allelopathy are again leading. Perhaps, chromium binds to intracellular complexes such as polyphosphate bodies, designed to store substances and, thus, is rendered harmless.

References

1. Brigida D'Abrosca, Marina Dellagrega, Antonio Fiorentino, Marina Isidori, Pietro Monaco, Severina Pacifico (2006) Chemical constituents of aquatic plant *Schoenoplectus lacustris*. *J Chem Ecol.*, vol. 32, no. 1, pp. 317-323.
2. Gadd G.M. (2010) Metals, minerals and microbes: geomicrobiology and bioremediation. *Microbiol.*, vol. 156, pp. 609-643.
3. Adam S., Kumar Ps., Santhanam P., Kumar S.D., Prabhavathi (2015) Bioremediation of Tannery wastewater using immobilized marine microalga *Tetraselmis sp.*: experimental studies and pseudo-second order kinetics. *Int conf Mar Biol Oceanogr.*, pp. 37-38.
4. Gadd G.M. (2009) Heavy metals pollutants: environmental and biotechnological aspects. In: *Encyclopedia of microbiology*. 3rd ed. Elsevier, Oxford, pp. 321-334.
5. Pratiush K.D. (2017) Microalgal technology for biosorption of hexavalent chromium in contaminated water bodies: opportunities and challenges. *National Seminar on Role of microalgae in Waste Water treatment (RMWT)-2017, India.*
6. Ghinwa Naja, Bohumil Volesky (2011) The Mechanism of metal cation and anion biosorption. In: *Microbial biosorption of metals*, Ch 3, pp. 19-38.
7. Muzichkina R.A., Korulkin D.J., Abilov Zh.A. (2012) Metodologia issledovania rastitelnih metabolitov. *Monographiya [Methodology of investigation of plant metabolites. Monograph]*. Almaty: MV-Print, p. 324.
8. Malathy R., Shaleesha A. Stanley (2015) Studies on the potential therapeutic effects on the aquatic macrophytes namely *Cabomba aquatica*, *Ceratophyllum demersum* and *Hygrophila corymbosa*. *J Chem Pharmaceut Res*, vol. 7, no. 4, pp. 479-483.

9. Gihan A. El Shoubaky, Essam A Salem (2014) Terpenes and sterols composition of marine brown algae *Padina pavonica* (dictyotales) and *Hormophysa triquetra* (fucales). *Int J pharmacognosy phytochem res.*, vol. 15, no. 6, pp. 894-900.
10. Braginskiy L.P., Velichko M.M., Sherban E.P. (1987) Freshwater plankton in toxic environment, Kiev: Naukova dumka, p. 179.
11. Adam S., Kumar Ps., Santhanam P., Kumar S., Prabhavathi D. (2015) Bioremediation of Tannery wastewater using immobilized marine microalga *Tetraselmis* sp.: experimental studies and pseudo-second order kinetics. *Int conf Mar Biol Oceanogr.*, p. 37.
12. Wallen D.G. (1996) Adaptation of the growth of the diatom *Fragillaria crotonensis* (Kitton) and the phytoplankton assemblage of Lake Eric to chromium. *J Great Lakes Res.*, vol. 22, pp. 55-62.
13. Morsi H., Ebd-El-Monem, Corradi M.G. (1998) Toxicity of copper and zinc to two strains of *Scenedesmus acutus* having different sensitivity to chromium. *Env and Exp Bot.*, vol. 40, pp. 59-66.
14. Ospanova A.K., Kaliyeva A.B., Anuarova L.E, Bazargaliyeva A.A., Yernazarova G.I., Ramazanova A.A., Sekenov I.E. (2017) Mildew of oleaster (*Elaeagnus oxycarpa* Schlecht.) registered in large industrial cities (Pavlodar, Aksu, Ekibastuz) of the Pavlodar region. *Saudi J Biol Sci*, in press, available at <http://www.sciencedirect.com/science/article/pii/S1319562X16301152>.
15. Eduardo Pérez-Rodríguez, José Aguilera, Félix L Figueroa (2003) Tissular localization of coumarins in the green alga *Dasycladus vermicularis* (Scopoli) Krasser: a photoprotective role. *J Exp Bot.*, vol. 54, no. 384, pp. 1093-1100.
16. Dinesh S., Kumara Santhanama, Nandakumara P., Anantha R., Nithyac S., Dhanalakshmic P. (2016) An integrated approach. *Desalination and Water Treatment*, vol. 1, pp. 1-10.
17. Yernazarova G.I. (2016) Razrabotka effektivnoy mineralnoy pitatelnoy smesi dlya intensivnitsii rosta diatomovih vodorosley presnovodnih vodoev / Creation of the effective mineral nutrition medium for increasing growth of diatom algae from freshwater reservoirs. *Science and Art*, vol. 39, no. 4, pp. 95-103.
18. Dzhokebaeva S.A., Orazova S.B., Karpeynyuk T.A., Goncharova A.V., Tsurkan Y.S., Kalbaeva A.M., Beisembaeva R.U. (2012) The use of microalgae and higher aquatic plants in the accumulation of chromium. *5th Int Symp Biosorp Bioremed*. Prague, Czech Republic, pp. 9-13.