

For purposeful control of the adsorption process, a comprehensive study of the properties of the original cells and the effect of metal ions on them is necessary. In this regard, the features of the adsorption of Cr(III) ions on the cell surface of Spirulina platensis algae were studied. FTIR spectroscopy revealed that the main functional groups responsible for the binding of Cr(III) ions are carboxyl, hydroxyl, amino, and phosphate groups on the surface of algae. The adsorption data were processed using the Langmuir and Freundlich models. It is shown that the maximum adsorption of Cr(III) ions on the surface of algae cells is 31.25 mg/g. The Freundlich constant $1/n$ is 0.65.

The study of the effect of the concentration of Cr(III) ions on the Zeta-potential of algae cells revealed an abnormal increase in the negative value of the ζ – potential at 10^{-5} mol/L, caused by the release of an additional amount of anionic functional groups to the surface. A further increase in the concentration of Cr(III) ions in the algae suspension leads to a decrease in the ζ – potential and recharge of the surface at $C > 10^{-2}$ mol/L. It was found that the adsorption of Cr(III) ions also affects the morphology of the cell surface. If before contact with Cr(III) ions, the surface of algae cells is represented as a uniform green grid, after adsorption of Cr(III) ions, the surface becomes green-brown, with swollen spirals. The study of the effect of pH on the adsorption and desorption processes shows an increase in the desorption of Cr(III) ions from the surface of algae during acidification of the medium. The adsorption reaches a maximum value in the pH range of 6–7. In the region of optimal Cr(III)/biosorbent ion ratios, the recovery rate of Cr(III) reaches 98.5–99.3 %

Keywords: algae, Spirulina platensis, Cr(III) ions, removal degree, adsorption, Zeta-potential

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ANALYSIS OF CR(III) IONS ADSORPTION ON THE SURFACE OF ALGAE: IMPLICATIONS FOR THE REMOVAL OF HEAVY METAL IONS FROM WATER

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1. Introduction

The presence of heavy metal ions in wastewaters has become one of the urgent environmental issues. Despite their well-known toxic impacts, heavy metal applications are still growing in industrial sectors. Once heavy metals containing contaminants are released in the environment, they persist indefinitely in the environment, causing pollution of air, water, and soil. Heavy metal contamination has exceeded the acceptable limit, posing serious hazards to human health and the environment. In recent years, the strong environmental law compels industry to invent sustainable technologies to bring down the concentration of hazardous metal ions below the discharge limits. This is due to the widespread use of heavy metal ions in various industries, agriculture, and medicine.

One of the most toxic and widespread elements in the environment is Cr(III) which enters the sewage from the waste of the leather industry, dyeing and metal processing. Among them, chromium is one of the familiar contaminants which gains importance owing to its highly toxic character even at a very low concentration. Chromium exists in oxidation states of Cr(II), Cr(III) and Cr(VI), where the most stable and commonly found forms of chromium in nature are trivalent (Cr(III)) and hexavalent (Cr(VI)), which have differing physical and chemical characteristics. Commonly used methods for the removal of chromium (VI) from wastewaters include precipitation, evaporation, ion-exchange, electro-dialysis and membrane processes. But disadvantages like incomplete metal removal, high reagent and energy requirements, generation of toxic sludge or other waste products that require careful disposal have made it imperative for

a cost-effective treatment method that is capable of removing chromium from aqueous effluents.

In this regard, the most convenient adsorbents for wastewater treatment from chromium compounds are microbial cells. However, a significant factor constraining the use of biotechnological techniques in water purification is the lack of comprehensive studies that include the consideration of cells as biosorbents in close connection with their chemical composition and surface properties.

2. Literature review and problem statement

Among the methods of wastewater treatment from heavy metal ions, the most effective is adsorption [1]. Synthetic and natural ion exchangers, residues from the processing of agricultural products, plant biomass and microbial cells are widely used as sorbents [2]. The use of microbial cells for the extraction of metal ions from solutions and wastewater is of particular interest because of their huge surface area and high sorption capacity. In addition, the adsorption capacity can vary widely, for example, the adsorption capacity of microalgae varies from 0.42 mg/g to 275.5 mg/g for different cells [3]. It is reported [4] that in the case of *Chlamydomonas reinhardtii* algae cells immobilized on alginate, the adsorption value reaches 380.7 mg/g. *Sargassum sp.* cells immobilized on Ca-alginate beads showed a higher sorption capacity than free cells.

An interesting idea of cell surface engineering is discussed in [5]. The authors suggested that traditional methods of physical and chemical treatment are ineffective at low concentrations of heavy metal ions. In order to create effective biosorbents for the removal and reduction of heavy metal ions, various metal-binding proteins/peptides were successfully fixed on the surface of microbial cells, and these composite sorbents demonstrated increased biosorption of heavy metal ions.

Experimental results of the adsorption of metal ions on the surface of *Saccharomyces cerevisiae* yeast cells show that the capture of metal ions is a fast process at pH values of 5.0–6.0, and the order of accumulation of metal ions is as follows: Pb(II) > Zn(II) > Cr(III) > Co(II) > Cd(II) > Cu(II) [6]. It was noticed that many microorganisms have a negative surface charge [7]. Negatively charged groups that participate in the adsorption of metal ions are alcohol, amine, carboxyl, ester, hydroxyl, sulfhydryl, phosphoryl, sulfonate, thioester and thiol. The metal ion binding process involves various mechanisms, including electrostatic interaction, ion exchange, precipitation, redox processes and surface complexation.

The key role of various microorganisms: bacteria, fungi, algae in bioremediation is reported [8]. The ability of algae to sorb Cr(III) ions has been shown [9]. The cells of the algae *Spirulina* and *Spirogyra* absorb Cr(III) ions by 93.4 % and 87.8 %, respectively. In the case of green microalgae *Scenedesmus quadricauda* cells, the recovery rate for Cr(III) and Cr(VI) ions was 98.3 % and 47.6 % [10]. The maximum degree of extraction of Cr(III) ions by *Ulva lactuca* algae cells is 84 % at pH=5 [11]. It is obvious that algae cells with a wide variety of functional groups have great prospects for using Cr(III) ions as sorbents.

In the considered works, the main attention is paid to determining the degree of extraction of Cr(III) ions from solutions and wastewater using microbial cells. At the same time, the use of microbial cells in water purification from Cr(III) ions requires data on their sorption capacity, optimization of the conditions for desorption of Cr(III) ions from their surface by changing the pH of the medium, as well as knowledge of the adsorption me-

chanism. A way to overcome these difficulties can be purposeful regulation of the degree of extraction of Cr(III) ions, studying the composition of the cell surface, as well as the mechanism of adsorption, determining the sensitivity of cells to metal ions by changing the zeta potential. There is also no data on the study of the toxicity of metal ions for cells as living organisms. Such information is important for the reuse of microbial cells. The toxicity of metal ions to cells can be judged by the change in the morphology of their surface. All this makes it necessary to comprehensively study the process of removing Cr(III) ions with the help of microbial cells, taking into account their influence on the physicochemical, surface and regenerative properties.

3. The aim and objectives of the study

The aim of the study is to characterize the process of adsorption of Cr(III) ions on the surface of *Spirulina platensis* algae cells in order to achieve a degree of water purification corresponding to acceptable standards and to determine the relative toxicity of Cr(III) ions for algae cells.

To achieve this aim, the following objectives are accomplished:

- to estimate the adsorption capacity of *Spirulina platensis* algae cells in close relationship with their chemical composition, and zeta potential;
- to determine cell's morphology and survival rates, tolerance to Cr(III) ions;
- to study the sensitivity of the adsorption process to changes in the pH of the medium;
- to optimize the conditions for water purification from Cr(III) ions using *Spirulina platensis* algae cells.

4. Materials and methods

4.1. Object of research

Chromium nitrate Cr(NO₃)₃·9H₂O purchased from Sigma Aldrich (USA) was used to prepare solutions containing Cr(III) ions.

Spirulina platensis algae cells were cultured in a lighted incubator at 308 K in a nutrient medium containing: NaNO₃ 2.50 g/L; K₂HPO₄ 0.50 g/L; NaHCO₃ 10.00 g/L; NaCl 1.00 g/L; MgSO₄·7H₂O 0.2 g/L; CaCl₂·2H₂O 0.02 g/L; FeSO₄·7H₂O 0.01 g/L. The cells were separated from the culture medium by centrifugation (6,000 rpm, 5 min) and subsequently washed three times with excess of distilled water to remove residual salts. The cells were then dried at room temperature for 48 hours.

4.2. Research methods

4.2.1. Carrying out the process of adsorption and desorption

To carry out adsorption experiments, 0.125 g of dried cells of *Spirulina platensis* algae were added to 20 mL of Cr(NO₃)₃ solution of various concentrations. The resulting suspension was shaken for 24 hours at 298 K. After that, the adsorbent was separated from the solutions in which the concentration of Cr(III) ions was afterward determined. The analysis of the content of Cr(III) ions was carried out on an Agilent 240FS atomic adsorption spectrophotometer (USA) with a measurement accuracy of 1–3 %. The adsorption A of Cr(III) ions on the surface of *Spirulina platensis* algae cells was calculated by using the equation:

$$A = \frac{(C_i - C_{eq}) \cdot V}{m},$$

where C_i and C_{eq} – the initial and equilibrium concentration of Cr(III) ions, mg/L; V – the volume of the solution, L; m – the mass of the adsorbent, g. All experiments were carried out at room temperature of 298 ± 2 K.

To carry out desorption experiments of Cr(III) ions, the adsorbent biomass was separated from the equilibrium solution and placed in the medium at different pH values. The mixture was shaken for 60 minutes on a rotary shaker and left for 24 hours. After that, the biomass of the adsorbent was separated from the eluent by filtration, and the eluent was analyzed for the content of Cr(III) ions. For desorption calculations, the results of 3 experiments were averaged. The number of desorbed (D) Cr(III) ions on the surface of *Spirulina platensis* algae cells was calculated by using the following formula:

$$D = \frac{(C_i - C_{eq})}{A} \cdot 100,$$

where D – the value of desorption, %; A – the value of adsorption, mg/g.

4. 2. 2. FTIR spectroscopy

For FTIR spectroscopic studies, the algae cells were dried at 353 K for 24 h. Subsequently, samples were prepared by diluting dried powders in KBr disks, containing 1 mg of algae cells and 100 mg of KBr (≈ 1 %, w/w). The analysis was carried out using a PerkinElmer Spectrum 65 FTIR instrument (USA). The investigations were carried out in the range of vibration frequencies from 450 to $4,000 \text{ cm}^{-1}$.

4. 2. 3. Determination of the composition of cells

The cell composition was determined using a Fe-ray microanalyzer on an X-ray fluorescence spectrometer Focus 2M (Russia) with a measurement accuracy of 1–3 %. All experiments were carried out at a temperature of 298 ± 2 K.

4. 2. 4. Measurement of electrokinetic potential

The ζ – potential of the algal cultures before and after the adsorption of Cr(III) ions was determined by electrophoretic mobility measurements combined with light scattering using Malvern ZetaSizer NanoZS (Malvern, UK) as described elsewhere. To carry out experiments, 10 mL of $\text{Cr}(\text{NO}_3)_3$ solutions with a concentration of 10^{-5} – 10^{-1} mol/L were mixed with algae cells weighing 0.125 g. The resulting mixture was shaken for 24 hours at 298 K. The electrophoretic mobility values of particles were transformed into electrokinetic potential by the classical Smoluchowski formula using the software supplied with the instrument. Such a procedure is widely accepted in the literature, and it can serve for comparison purposes, i. e. to follow the effect of different variables on the electrophoretic mobility and ζ – potential values.

4. 2. 5. Obtaining optical microscopic images of cells

The microscopic analysis of the surface of *Spirulina platensis* algae cells before and after the adsorption of Cr(III) ions was carried out on an Axio Vert A.1 instrument (Carl Zeiss, Germany) at different magnifications. Prior to the analysis, samples of algae cells were dried at room temperature for 48 hours. Subsequently, the dry samples were placed on a glass slide and few drops of water were added to moisten

the dry sample. All photographs were taken with a magnification of $\times 50$ and $\times 500$.

5. Results

5. 1. Adsorption of Cr(III) ions on the surface of algae cells

Spirulina platensis is a filamentous cyanobacterium that has been successfully commercialized thanks to its high productivity, high protein content and strong bioactivity [12]. The study of the adsorption of Cr(III) ions from solutions on the surface of *Spirulina platensis* algae cells showed that the isotherm possesses a form typical for porous adsorbents (Fig. 1). It is an upward curve with no plateau which would reveal the surface saturation. Similar adsorption isotherms were reported for the microbial cells [13]. Algae have some advantages over bacteria and fungi as a biosorbent, as they usually do not produce toxic substances and have low nutrient requirements. Being autotrophic, they produce a large amount of biomass. Sorption of metals by algae generally depends on ionic charge of the metal ion, algal species and chemical composition of the metal ions in solution. They have different bioactive substances such as polyphenols, polysaccharides, lipids, proteins, vitamins, and carotenoids which have amine and several negative charge active groups, for example, carboxyl, phosphate and hydroxyl that create strong links between them and hazardous metals [14, 15].

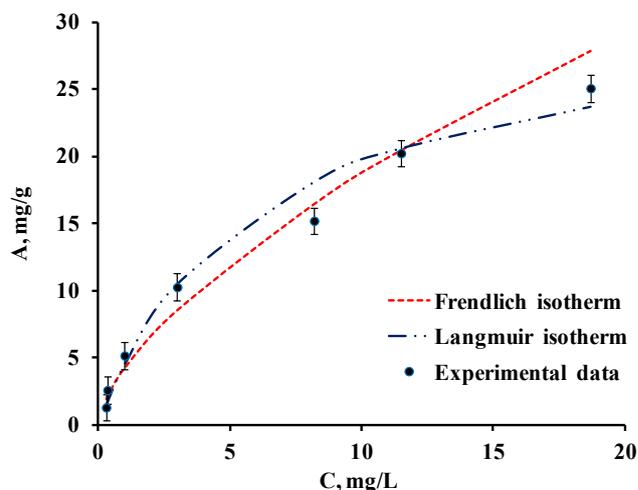


Fig. 1. Adsorption isotherm of Cr(III) ions on the surface of *Spirulina platensis* algae cells (pH: 6.5; temperature: 298 K; contact time: 24 h; adsorbent dose: 0.125 g/L)

The experimental results were described using Freundlich and Langmuir adsorption models [16]. The Langmuir type equation was transformed into the linear equation:

$$\frac{C}{A} = \frac{1}{A_{\max} \cdot K} + \frac{C}{A_{\max}}$$

Freundlich isotherm model is an empirical equation which is used to describe adsorption on heterogenous surface. To calculate the Freundlich constants, the equation was applied,

$$A = K_F \cdot C^{1/n},$$

where K_F – the equilibrium constant characterizing the adsorption capacity of the adsorbent; $1/n$ – a parameter

indicating the intensity of the adsorbate – adsorbent interactions.

In addition, $1/n$ value ranges between 0 and 1 and describes the surface heterogeneity. If its value gets nearer to zero, the adsorbent surface is described as more heterogeneous. The values of $1/n$ are less than one for Cr(III) ions, which indicates the degree of nonlinearity. A comparison of the (R^2) values shows that the experimental data on the adsorption of Cr(III) ions are very well described by the Freundlich isotherm model. It follows that the adsorbent is heterogeneous due to the heterogeneity of the biomass surface [16].

Also in that case, the equation was linearized. The values of calculated parameters from both equations are presented in Table 1.

Table 1

Adsorption parameters of Freundlich and Langmuir isotherms for the adsorption of Cr(III) ions on the surface of *Spirulina platensis* algae cells

Adsorbent	T, K	Langmuir model			Freundlich model		
		K_L , L·mg ⁻¹	A_{max} , mg·g ⁻¹	R^2	$1/n$	K_F , mg·g ⁻¹	R^2
<i>Spirulina platensis</i> cells	298	0.17	31.25	0.95	0.65	4.19	0.96

The Langmuir correlation coefficient values (R^2) are lower than the Freundlich correlation coefficient values obtained, but, at a low concentration, the experimental data coincide with the Langmuir approach line. The dimensionless factor $K_L < 1$, which indicates favorable adsorption and corresponds to a single-layer process [17]. In accordance with [18, 19], an adsorbent should be considered acceptable for the given adsorption processes if the value of $1/n$ is in the range of 0.1–1.0. In the case of adsorption of Cr(III) ions on the surface of *Spirulina platensis* cells, the $1/n$ value is equal to 0.65, confirming the usefulness of the adsorbent for the removal of Cr(III) ions. Comparing the experimental data with model isotherms (Fig. 1), it can be stated that both Langmuir and Freundlich approaches describe the adsorption process of Cr(III) ions on algae in a satisfactory way. It was found that the value of the maximum adsorption (A_{max}) is 31.25 mg/g. This is a rather high value, so it can be assumed that Cr(III) ions are prone to bind to algal cells.

To establish the mechanism of interaction of *Spirulina platensis* cells with Cr(III) ions, FTIR-spectroscopic studies were carried out for the cells prior and after their contact with metal ions. The infrared spectra of *Spirulina platensis* cells are shown in Fig. 2, a. The most noticeable bands for the original algae cells were recorded at the oscillation frequencies of 3,472 cm⁻¹, 1,679 cm⁻¹, 1,432 cm⁻¹, 1,384 cm⁻¹, 1,290 cm⁻¹, 1,153 cm⁻¹, 849 cm⁻¹, 599 cm⁻¹. In the high-frequency zone, the broad band at 3,472 cm⁻¹ is assigned to the OH⁻ groups of adsorbed water, carboxyl and hydroxyl groups [20, 21]. Vibrations in the range of 2,900–2,400 cm⁻¹ are related to the presence of C-H and NH₂ groups in the composition of algae, and the bands with vibration frequencies of 1,679 and 1,384 cm⁻¹ are attributed to primary amino and sulfonate groups of proteins and CN⁻ groups of polypeptides. In addition, the band at 1,432 cm⁻¹ corresponds to C=O and CH₂ groups. At the same time, at a vibration frequency of 1,290 cm⁻¹, C-H bonds appear, and at 1,153 cm⁻¹ and C-O-C bonds of ether groups [21].

The small band at 1048 cm⁻¹ belongs to phosphate groups in the algal proteins. A sharp band at 849 cm⁻¹ corresponds to phosphate groups, while a broad and intense band at 599 cm⁻¹ describes the deformation vibrations of C-H bonds of hydrocarbons and N-H bonds of primary amino groups. The bulk of the cell wall is a protein bound to polysaccharides. Proteins contain functional groups such as amine, carboxyl, sulfate and hydroxyl, which are significantly involved in the biosorption process. Peptidoglycan, phospholipids, and lipopolysaccharides are among the anionic functional compounds and are actively involved in metal binding [22].

Noticeable changes are observed in the FTIR spectrum of algae, after adsorption of Cr(III) ions (Fig. 2, b). The band at a vibration frequency of 3 472 cm⁻¹, corresponding to O-H bonds, shifts to 3 432 cm⁻¹. This is evidence of the interaction of hydroxyl and carboxyl groups of algae surfaces with Cr(III) ions. The band at a vibration frequency of 2 892 cm⁻¹, belonging to C-H bonds, shifts to 2 852 cm⁻¹. In addition, the intensity and location of the band at an oscillation frequency of 1 679 cm⁻¹ are shifted to the lower frequency of 1 655 cm⁻¹. The most probable cause of this shift is the complexation of amino groups with Cr(III) ions. For the same reason, the width and intensity of the band at 1 384 cm⁻¹ change to 1 380 cm⁻¹. Here, the interaction of the sulfonate groups of proteins with metal ions can also be manifested [23, 24]. In addition, the disappearance of the band at 1 432 cm⁻¹ is evidence of the interaction of C=O and CH₂ groups with Cr(III) ions. In [25], when studying the infrared spectra of *Spirulina sp.* before and after the biosorption of Cr(VI) ions, the participation of carboxyl, carbonyl, hydroxyl, and amine groups in the sorption was revealed.

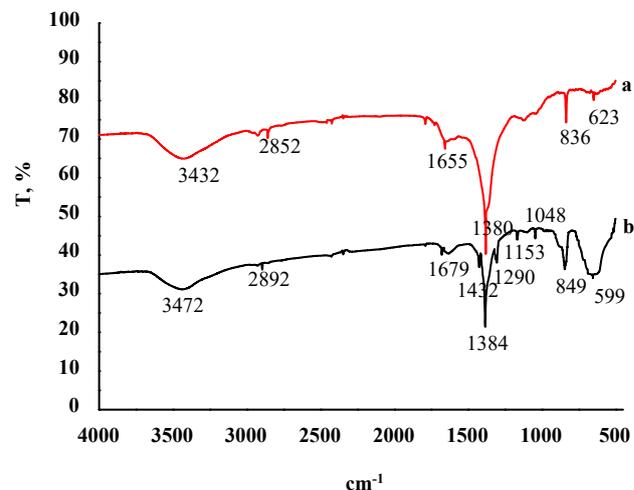


Fig. 2. FTIR spectra: a – *Spirulina platensis* algae cells before adsorption of Cr(III) ions; b – *Spirulina platensis* algae cells after adsorption of Cr(III) ions (pH: 8; temperature: 298 K; contact time: 24 h; Cr(NO₃)₃ concentration: 10⁻² mol/L; adsorbent dose: 0.125 g/L)

Further, the disappearance of the band at 1,048 cm⁻¹, as well as a decrease in the intensity of the band at 849 cm⁻¹ and a shift of its position to 836 cm⁻¹ is evidence of the interaction of phosphate and sulfonate groups with Cr(III) ions. A noticeable change after adsorption of Cr(III) ions occurs in the intensity and position of the band at 599 cm⁻¹, which corresponds to the bending vibrations of C-H and N-H groups. It shifts to 623 cm⁻¹, while its intensity decreases. These changes are caused by the complexation of Cr(III) ions with

amino groups, as well as by binding with sulfide groups. It should be noted that compounds of Cr(III) ions with phosphate and sulfide groups have the lowest solubility products [26]. Even chromium (III) phosphate is an insoluble compound, which is the basis for its use in heterogeneous catalysis. It is also known to be used for the catalysis of cation exchange in sorption processes to reduce the toxicity of the metal. Chromium (III) sulfide is a trigonal structure and brown-black solid compound. It has magnetic, magnetoresistance, and thermoelectric properties and is also an insoluble substance [27].

An elemental analysis of the samples of *Spirulina platensis* algae prior and after adsorption of Cr(III) ions on their surface was carried out. It should be noted that the EDX system will only detect the elements with an atomic mass above 30. As it can be seen from the data presented in Table 2, the composition of cells is different for the samples measured prior and after the adsorption. After the adsorption of Cr(III) ions, the cell composition undergoes significant changes. Moreover, the content of some elements increases, and the number of others decreases. The most noticeable changes are observed in the K content, which can be explained by the replacement of single-charged K⁺ ions with multi-charged Cr(III) ions.

Table 2

Elemental composition of *Spirulina platensis* algae cells before and after of Cr(III) ions adsorption

Base elements	K	Ca	Fe	Cl	P	Mn	S	Cr
Pristine cells, %	69.77	0.01	3.77	21.93	1.86	0.241	2.33	0.09
Cells after adsorption of Cr(III) ions, %	12.14	1.37	1.44	4.80	2.37	–	1.68	76.2

The electrical surface properties of the cells of microorganisms can serve as an indicator of the adverse effect of environmental components on it, and in particular, of heavy metals. Therefore, the influence of the concentration of Cr(III) ions on the electrokinetic potential of *Spirulina platensis* algae cells was studied. Fig. 3 shows the dependence of the Zeta-potential of cells on the concentration of Cr(III) ions. The surface of the algae cells has a negative charge with a zeta potential of –13.2 mV at pH 8. This value of the electrokinetic potential is close to the literature data, for example, for the cells of the algae *Coelastrrella sp.* and *Chlorella vulgaris* ζ – potential values range from –15 mV to –20 mV at pH=6–9 [28]. It is known that both gram-positive and gram-negative bacteria carry a negative charge on their surface. The total negative charge of the cells is the result of the balance of 10⁷–10⁸ negative and 10⁶–10⁷ positive charges per one cell [29]. The negative charge of the cell surface is due to the presence of carboxyl, hydroxyl, phosphate, sulfonate and other anionic groups.

At the same time, the general form of the Zeta-potential dependence on the concentration of Cr(III) ions differs from that for typical dispersed systems. The difference lies in the presence of a concentration region with an abnormal increase in the negative value of the electrokinetic potential in response to the addition of chromium cations into the suspension. This increase is observed in the Cr(III) concentrations range of 10⁻⁵–10⁻³ mol/L, and only above the concentration of 10⁻³ mol/L the system shows a behavior characteristic of dispersed systems, i.e. the electrokinetic potential decreases monotonically. A further increase in the concentration of Cr(III) ions leads to an overcharge of the cell surface.

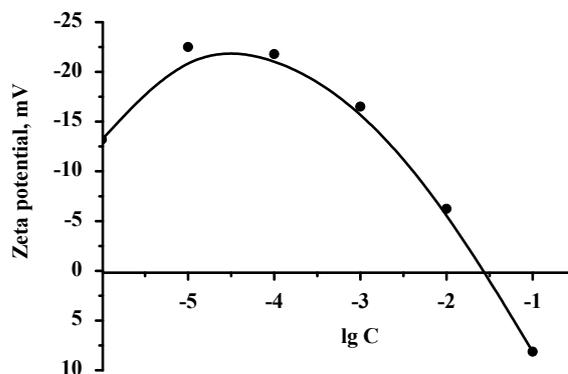


Fig. 3. Dependence of Zeta-potential of *Spirulina platensis* algae cells on the concentration (C) of Cr(III) ions (pH: 8; temperature: 298 K; contact time: 24 h)

5. 2. Effect of Cr(III) ions on the surface morphology of algae cells

The adsorption of Cr(III) ions on the surface of living cells of microorganisms affects their appearance and basic functions. The cell morphology of *Spirulina platensis* algae was studied by phase contrast microscopy using a light microscope (Fig. 4, 5).

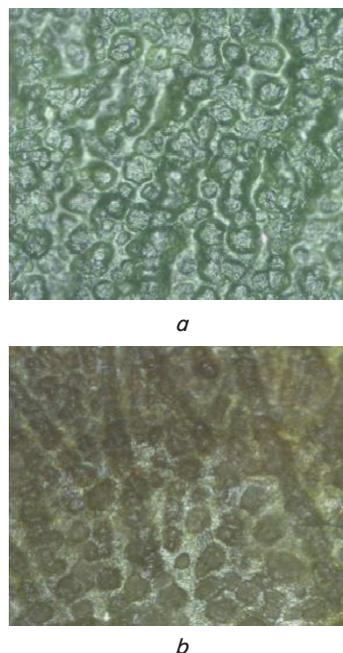


Fig. 4. Optical microscopic images: a – *Spirulina platensis* algae cells before adsorption of Cr(III) ions from the solution of concentration 10⁻³ mol/L; b – after adsorption of Cr(III) ions. Magnification ×50

In Fig. 4, images of *Spirulina platensis* algae cells are shown before and after the adsorption of Cr(III) ions on their surface at a salt concentration of 10⁻³ mol/L. The surface of algae before the contact with chromium ions is presented in the form of a green homogeneous network, after adsorption of Cr(III) ions the cell wall becomes green-brown, the filaments of the spirals are swelled and clearly visible. Apparently, Cr(III) ions penetrate into the cells, and their hydration leads to the swelling of the helices.

In Fig. 5, at a 500-fold magnification, it can be seen that new algae cells appear in the cell wall 7 days after the adsorption of Cr(III) ions. It is known that high concentrations of toxic substances kill living organisms, and low ones stimulate them to survive [30].

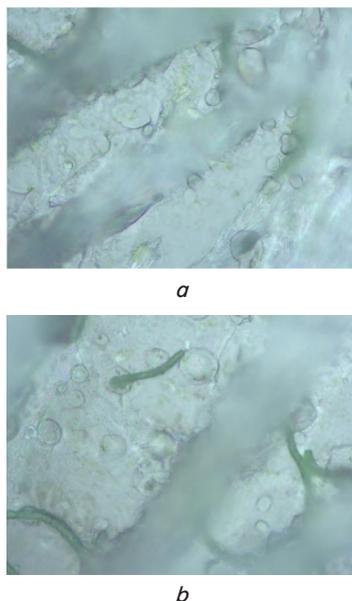


Fig. 5. Optical microscopic images of *Spirulina platensis* algae cells after adsorption of Cr^{3+} ions at a concentration of 10^{-3} mol/L: *a* – after 1 day, *b* – after 7 days. Magnification $\times 500$

Thus, the adsorption of Cr(III) ions affects the morphology of the cell surface. Before adsorption of Cr(III) ions, the surface of algae cells is represented as a uniform green grid, after adsorption of Cr(III) ions, the surface becomes green-brown, with swollen spirals. After keeping the system of algae cells *Sp. platensis* – Cr(III) ions for 7 days, new cells appear on the surface of the algae. Such a change in the morphology of the cell surface is justified by the struggle of microorganisms for survival and the development of a strategy for existence in a toxic environment.

5. 3. Effect of medium pH on the adsorption/desorption of Cr(III) ions

Studies on the adsorption of metal ions on various microorganisms have shown that the pH of the medium is the most important factor influencing the adsorption effects. Therefore, the effect of the pH of the medium on the adsorption of Cr(III) ions on the surface of *Spirulina platensis* algae was studied. As can be seen from Fig. 6, acidification of the medium leads to a decrease in the amount of adsorption. In the pH range of 6, 7, the adsorption reaches its maximum value, while in an alkaline medium it decreases slightly. In an acidic medium, the dissociation of acidic groups is suppressed by H^+ ions, therefore, the course of ion exchange reactions or electrostatic attraction of metal cations to acidic groups is hampered. Moreover, at low pH, amine groups are protonated and this prevents the complexation reaction between them and the Cr(III) ions. Nevertheless, some of the Cr(III) ions are still adsorbed on the surface of algae and the adsorption value in the pH range of 2–5 is in the range of 12–18 mg/g.

The surface of the cells of microorganisms possesses a fairly high negative charge, which remains practically unchanged

both in the strongly acidic pH range and in the strongly alkaline medium [31]. As shown by the data of FTIR spectroscopy (Fig. 2), the formation of a charge on the cell surface is owing to the presence of dissociated amine, hydroxyl and phosphoric acid groups. Phosphate groups are found mainly in glycoproteins and play an important role in adsorption, because they can exhibit a negative charge in an environment with $\text{pH} > 3$ [32].

Studies on the desorption of Cr(III) ions from the surface of *Spirulina platensis* algae were carried out and the results are presented in Fig. 7. Samples of algae with an adsorption value of 25.0 mg/g were used in these experiments. The desorption rate increases with decreasing pH of the medium, reaching a maximum value at pH 1.

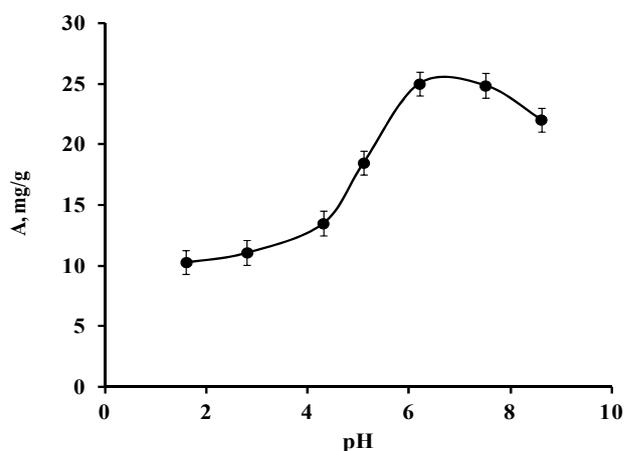


Fig. 6. Dependence of the adsorption of Cr(III) ions on the surface of *Spirulina platensis* algae on the pH of the medium (temperature: 298 K; contact time: 24 h; metal concentration: 10^{-2} mol/L; adsorbent dose: 0.125 g/L)

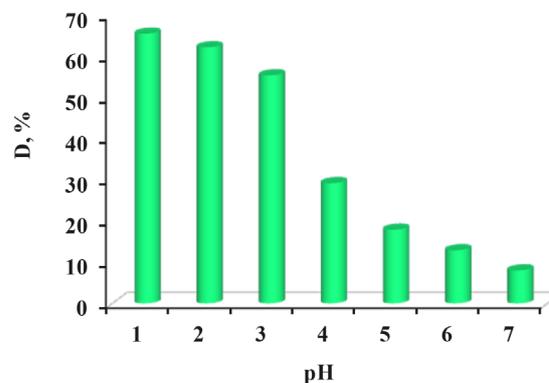


Fig. 7. Dependence of the desorption of Cr(III) ions from the surface of *Spirulina platensis* algae on the pH of the medium (temperature: 298 K; contact time: 24 h; metal concentration: 10^{-2} mol/L; adsorbent dose: 0.125 g/L)

Obviously, in an acidic environment, the H^+ ions of the introduced acid compete with the Cr(III) ions for binding to the phosphate and carboxyl groups in algae, and this leads to the desorption of metal ions.

5. 4. Effect of initial Cr(III) ions concentration on the removal efficiency

For the maximum removal of Cr(III) ions from aqueous solutions, it is necessary to introduce a sufficient number of

cells to bind the metal ions in solution. Indeed, as it can be seen from Fig. 8, with an initial concentration of Cr(III) ions in a solution of 0.1–0.5 mg/L, the degree of removal efficiency of metal ions is 98.5–99.3 %. However, with a further increase in the concentration of Cr(III) ions and a constant mass of algae (0.125 g), the degree of removal monotonically decreases and at a concentration of 20 mg/L it drops to 96.4 %.

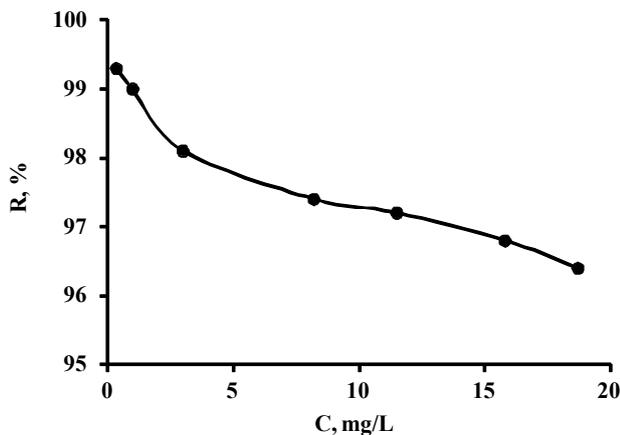


Fig. 8. Removal of Cr(III) ions from solution with *Spirulina platensis* algae cells (temperature: 298 K; contact time: 24 h; adsorbent dose: 0.125 g/L)

It results from the fact that at an initial concentration of Cr(III) ions in a solution of 0.1–0.5 mg/L, the ratio between the number of metal ions and the cell surface area is optimal. With an increase in the concentration of Cr(III) ions, their excess appears in relation to functional groups capable of interacting with them. Therefore, some of them remain free in solution, which leads to a decrease in the degree of removal of Cr(III) ions from the solution.

6. Discussion of experimental results of adsorption of Cr(III) ions on the surface of *Spirulina platensis* algae cells

The results of studying the adsorption of Cr(III) ions on the surface of *Spirulina platensis* algae cells are shown in Fig. 1. The Langmuir and Freundlich models have shown that the maximum adsorption of Cr(III) ions on the surface of algae cells is 31.25 mg/g (Table 1). The Freundlich constant $1/n$ is 0.65. According to [23], the adsorption of Cr(III) ions on the cell surface of *Spirulina platensis* and *Chlorella vulgaris* algae is 10–40 mg/g. For example, maximum adsorption of Cr(III) ions on algal cells *Spirogyra spp.* is 30.21 mg/g [33]. The maximum adsorption of Cr(III) ions on the surface of marine macro-algae *H. clathratus* and *C. barbata* are 7.19 mg/g and 7.30 mg/g, respectively [16].

The processing of the adsorption data in the framework of the Langmuir and Freundlich models shows that *Spirulina platensis* algae are efficient as sorbent of Cr(III) ions. The maximum adsorption value is within the limits typical for the adsorption of Cr(III) ions on the surface of microbial cells.

It can be stated that the main changes in the FTIR spectrum of *Spirulina platensis* algae cells after the adsorption of Cr(III) ions are due to changes in the position and intensity of the bands corresponding to OH^- , -NH , COO^- and PO_4^{3-} groups (Fig. 2). The interaction of these groups with Cr(III)

ions is caused by electrostatic attraction, ion exchange and complexation.

XRF is used as an indirect method for assessing the adsorption of cations on the microorganisms' cells [34]. The decrease in the amount of Cl in the composition of algae cells can be justified by the participation of amino groups of their surface in the donor-acceptor interaction with Cr(III) ions, as a result of which their counterions will be released (Table 2). The decrease in the sulfur content indicates the formation of a poorly soluble compound-chromium sulfide, which is removed from the surface when washing the adsorbent. The increase in the amount of Ca and P in cells can be explained by the fact that the appearance of multicharged Cr(III) ions in the system stimulates not only the processes of ion exchange, but also some biochemical processes, in particular, the appearance and growth of new algae cells.

An abnormal increase in the ζ -potential of cells (Fig. 3) can be explained by biochemical processes, the release of negatively charged functional groups to the surface to capture metal ions. The decrease in the Zeta-potential is due to physicochemical processes, specifically – the compression of the diffuse part of the electric double layer due to the specific adsorption of metal ions. Upon completion of the biochemical processes, the cell loses its resistance to the action of metal ions and behaves as a non-living system. To explain changes in the Zeta-potential of cells under the influence of metal ions note that such phenomenon was found in [35] for yeast cells.

Moreover, the cells of microorganisms are able to accumulate metal ions for use as a nutrient [36]. Probably, the appearance of new algae cells at a concentration of Cr(III) ions 10^{-3} mol/L is due to the struggle of cells for survival. For example, the survival rate of the simplest *Euplotes mutabilis* (ciliate protozoa) in industrial wastewater containing high concentrations of heavy metals (cadmium, lead, copper, and chromium) was estimated in [37]. The ciliate showed tolerance to cadmium (22 mg/mL), chromium (60 mg/mL), lead (75 mg/mL), and copper (22 mg/mL). From the results revealed, it follows that algae cells are able to develop their own mechanisms of coexistence with toxic Cr(III) ions, while maintaining their functions of vital activity and reproduction.

Of course, the data on the effect of chromium ions on the morphology of the surface of algae cells, the appearance and growth of their new chains are not enough to judge their survival in an environment containing Cr(III) ions (Fig. 4, 5). Therefore, further development of research in this direction will consist in the quantitative assessment of the ability of cells to reproduce. In addition, such studies will allow determining toxicity thresholds of Cr(III) ions for *Spirulina platensis* cells.

As can be seen from Fig. 6, acidification and alkalization of the medium lead to a decrease in the adsorption value. In the pH range of the medium 6–7, the adsorption reaches its maximum value, and when moving away from the maximum, it decreases slightly. This may be due to the fact that in an acidic environment, the dissociation of acidic groups of the algae surface is suppressed by H^+ ions. On the other hand, H^+ ions protonate amino groups, competing with Cr(III) ions for binding to them. In an alkaline medium, phosphate and carboxyl groups are in a dissociated state, which is convenient for binding to Cr(III) ions. However, here OH^- ions can compete with them. Therefore, the highest values of adsorption are achieved at pH 6–7.

The isoelectric point (pI) for the microalgae *Spirulina platensis* is 2.8–3.5 [38]. However, the adsorption isotherm

did not reveal changes due to the difference in the mechanism of adsorption of Cr(III) ions below and above the pH. Obviously, the role of phosphate groups in the binding of Cr(III) ions by *Spirulina platensis* algae dominates. The decrease in the adsorption values in the range of pH>6 can be explained by the deposition of Cr(III) ions in the form of Cr(OH)₃ that prevents the adsorption of metal ions on the surface of algae. Furthermore, in this pH region, Cr(III) ions can exist in the form of negatively charged complex ions [Cr(OH)₄(H₂O)₃]⁻, [Cr(OH)₅(H₂O)]²⁻, and [Cr(OH)₆]³⁻ which will be repelled from the anionic phosphate groups [27, 39].

Thus, at low pH values, a protonation of amine groups is possible, which causes the destruction of coordination complexes and the release of Cr(III) ions into the aqueous medium. An increase in the pH of the medium promotes the dissociation of phosphate and carboxyl groups, which can bind to Cr(III) ions, therefore the desorption decreases. In this connection, changing the pH of the medium is an effective way to regulate the processes of adsorption and desorption of Cr(III) ions on and from the surface of *Spirulina platensis* algae cells (Fig. 7). As can be seen from Fig. 7, the acidification of the medium leads to an increase in the degree of desorption, which is the result of the competition of H⁺ and Cr³⁺ ions for binding to amino groups. However, in order to obtain more reliable data on the mechanism of binding of Cr(III) ions to the surface of algae cells, additional studies are needed to determine the state of chromium ions in solution. Therefore, further research in this direction will be devoted to determining the state of chromium ions at different pH values of the medium.

Using dry biomass of *Spirulina platensis* for removing Cr(III) ions showed [40] that a high degree of removal efficiency of 93 and 86 % was achieved at a concentration of Cr(III) ions of 25 and 35 mg/L, respectively, while at a higher concentration, the removal was significantly lower (61 and 43 % at C₀=50 and 75 mg/L, respectively). Nevertheless, it is interesting that in such a wide range of Cr(III) ion concentration – from 0.1 mg/L to 20.0 mg/L – the recovery degree decreases insignificantly – from 99.3 to 96.4 % (Fig. 8). Probably, the biological specificity of the sorbent manifests itself here, which consists in the fact that cells output their functional groups to the surface to capture metal ions as a source of nutrients.

According to [41], the maximum permissible concentration of Cr(III) ions in wastewater is 5 mg/L. After removal of Cr(III) ions from solutions with an initial concentration of 20.0 mg/L (Fig. 8) using *Spirulina platensis* algae cells, the residual concentration is 0.72 mg/L. Therefore, the use of *Spirulina platensis* algae cells with an optimal ratio of metal ions to the mass of the biosorbent can provide water purification from Cr(III) ions to a level corresponding to acceptable standards.

Thus, although a fairly wide range of chromium salt concentration was chosen to optimize the conditions for the extraction of Cr(III) ions from solutions – from 0.1 to 20 mg/L, the maximum degree of Cr(III) ions removing (98.5–99.3 %) is achieved in the range of their concentrations of 0.1–0.5 mg/L.

7. Conclusions

1. The features of adsorption of Cr(III) ions on the surface of *Spirulina platensis* algae have been studied. The adsorption results are analyzed in the framework of the Langmuir and Freundlich adsorption models. It was found that the value of the maximum adsorption is 31.25 mg/g, and the Freundlich constant 1/n is 0.65, which indicates a favorable utilization of algae for the sorption of Cr(III) ions. It has been shown by FTIR spectroscopy and X-ray fluorescence analysis that the carboxyl, hydroxyl, amino and phosphate groups of the algae surface are mostly responsible for binding to Cr(III) ions. For the cells of *Spirulina platensis* algae, the phenomenon of an abnormal increase in the negative value of the Zeta-potential in the region of the low concentration (10⁻⁵ mol/L) of Cr(III) ions was found, due to the release of an additional amount of anionic functional groups to the surface. A further increase in the concentration of Cr(III) ions in the algal suspension leads to a decrease in the ζ – potential up to a surface recharge at C>10⁻² mol/L, which is associated with the compression of the diffuse part of the electrical double layer as a result of specific adsorption of Cr(III) ions.

2. The effect of Cr(III) ions on cell morphology was studied. It is shown that after contact with Cr(III) ions for 7 days, new cell sprouts appear on the surface of algae cells, which is justified by the stimulating effect of metal ions on the cells. The survival ability of *Spirulina platensis* algae cells in a medium containing Cr(III) ions was evaluated. It has been shown that these microorganisms develop strategies that allow them to transfer, resist, or detoxify heavy metals, which is very important when used as biological sorbents.

3. The medium pH affects substantially the adsorption/desorption processes. It was revealed that the adsorption decreases with acidification of the medium and has a maximum value in the range of pH 6–7. The desorption of Cr(III) ions increases in an acidic environment and decreases in an alkaline one. A decrease in the adsorption values and an increase in the desorption of Cr(III) ions in an acidic medium are explained by the suppression of the dissociation of carboxyl and phosphate groups, as well as by the protonation of amino groups on the surface of algae cells. The increase in adsorption values with increasing pH is caused by the ionization of acidic groups by OH⁻ groups in the medium.

4. At the optimal ratios of chromium ions/biosorbent *Spirulina platensis*, the highest degree of removal of Cr(III) ions of 98.5–99.3 % is achieved at their initial concentration of 0.1–0.5 mg/L and a constant mass of algae (0.125 g). A further increase in the concentration of Cr(III) ions leads to a decrease in the degree of removal, which is associated with the appearance of an excess of metal ions in relation to the adsorption sites on the surface of algae cells.

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References

1. Mustapha, M. U., Halimoon, N. (2015). Microorganisms and Biosorption of Heavy Metals in the Environment: A Review Paper. *Journal of Microbial & Biochemical Technology*, 7 (5). doi: <http://doi.org/10.4172/1948-5948.1000219>

2. Barakat, M. A. (2011). New trends in removing heavy metals from industrial wastewater. *Arabian Journal of Chemistry*, 4 (4), 361–377. doi: <http://doi.org/10.1016/j.arabjc.2010.07.019>
3. Ayangbenro, A., Babalola, O. (2017). A New Strategy for Heavy Metal Polluted Environments: A Review of Microbial Biosorbents. *International Journal of Environmental Research and Public Health*, 14 (1), 94. doi: <http://doi.org/10.3390/ijerph14010094>
4. Suresh Kumar, K., Dahms, H.-U., Won, E.-J., Lee, J.-S., Shin, K.-H. (2015). Microalgae – A promising tool for heavy metal remediation. *Ecotoxicology and Environmental Safety*, 113, 329–352. doi: <http://doi.org/10.1016/j.ecoenv.2014.12.019>
5. Li, P.-S., Tao, H.-C. (2013). Cell surface engineering of microorganisms towards adsorption of heavy metals. *Critical Reviews in Microbiology*, 41 (2), 140–149. doi: <http://doi.org/10.3109/1040841x.2013.813898>
6. Farhan, S. N., Khadom, A. A. (2015). Biosorption of heavy metals from aqueous solutions by *Saccharomyces Cerevisiae*. *International Journal of Industrial Chemistry*, 6 (2), 119–130. doi: <http://doi.org/10.1007/s40090-015-0038-8>
7. Yin, K., Wang, Q., Lv, M., Chen, L. (2019). Microorganism remediation strategies towards heavy metals. *Chemical Engineering Journal*, 360, 1553–1563. doi: <http://doi.org/10.1016/j.cej.2018.10.226>
8. Ramírez Calderón, O. A., Abdeldayem, O. M., Pugazhendhi, A., Rene, E. R. (2020). Current Updates and Perspectives of Biosorption Technology: an Alternative for the Removal of Heavy Metals from Wastewater. *Current Pollution Reports*, 6 (1), 8–27. doi: <http://doi.org/10.1007/s40726-020-00135-7>
9. Samuel, R. T., Menon, L. P., Bhavana, V., Sathya, P. (2019). Studies on Phycoremediation of *Chlorpyrifos* and Heavy Metal Chromium Using Algae. *International Journal of Pharmacy and Biological Sciences*, 9, 2230–7605. Available at: <https://www.researchgate.net/publication/330619850>
10. Shokri Khoubestani, R., Mirghaffari, N., Farhadian, O. (2014). Removal of three and hexavalent chromium from aqueous solutions using a microalgae biomass-derived biosorbent. *Environmental Progress & Sustainable Energy*, 34 (4), 949–956. doi: <http://doi.org/10.1002/ep.12071>
11. Asnaoui, H., Khalis, M., Laaziri, A., Elbougarrani, O. (2014). Decontamination of a solution of chromium IV by marine algae (*Ulva lactuca*). *International Journal of Innovative Research in Advanced Engineering*, 1, 62.
12. Prasad, A., Singh, A. K., Chand, S., Chanotiya, C. S., Patra, D. D. (2010). Effect of Chromium and Lead on Yield, Chemical Composition of Essential Oil, and Accumulation of Heavy Metals of Mint Species. *Communications in Soil Science and Plant Analysis*, 41 (18), 2170–2186. doi: <http://doi.org/10.1080/00103624.2010.504798>
13. Bulgariu, D., Bulgariu, L. (2012). Equilibrium and kinetics studies of heavy metal ions biosorption on green algae waste biomass. *Bioresource Technology*, 103 (1), 489–493. doi: <http://doi.org/10.1016/j.biortech.2011.10.016>
14. Hassan, S. W., Kassas, H. Y. (2012). Biosorption of cadmium from aqueous solutions using a local fungus *Aspergillus cristatus* (Glaucus Group). *African Journal of Biotechnology*, 11 (9), 2276–2286. doi: <http://doi.org/10.5897/ajb11.3140>
15. Jencarova, J., Luptakova, A. (2012). The elimination of heavy metal ions from waters by biogenic iron sulphide. *Chemical Engineering Transaction*, 28, 205–210. doi: <https://doi.org/10.3303/CET1228035>
16. Ali, H. S., Kandil, N. F. E. S., Ibraheem, I. B. M. (2020). Biosorption of Pb^{2+} and Cr^{3+} ions from aqueous solution by two brown marine macroalgae: an equilibrium and kinetic study. *Desalination and water treatment*, 206, 250–262. doi: <http://doi.org/10.5004/dwt.2020.26314>
17. Hu, Q., Liu, Y., Gu, X., Zhao, Y. (2017). Adsorption behavior and mechanism of different arsenic species on mesoporous $MnFe_2O_4$ magnetic nanoparticles. *Chemosphere*, 181, 328–336. doi: <http://doi.org/10.1016/j.chemosphere.2017.04.049>
18. Tavengwa, N., Cukrowska, E., Chimuka, L. (2014). Synthesis of bulk ion-imprinted polymers (IIPs) embedded with oleic acid coated Fe_3O_4 for selective extraction of hexavalent uranium. *Water SA*, 40 (4), 623–630. doi: <http://doi.org/10.4314/wsa.v40i4.7>
19. Tounsadi, H., Khalidi, A., Abdennouri, M., Barka, N. (2015). Biosorption potential of *Diplotaxis harra* and *Glebionis coronaria* L. biomasses for the removal of $Cd(II)$ and $Co(II)$ from aqueous solutions. *Journal of Environmental Chemical Engineering*, 3 (2), 822–830. doi: <http://doi.org/10.1016/j.jece.2015.03.022>
20. Rahman, M. S., Sathasivam, K. V. (2015). Heavy Metal Adsorption onto *Kappaphycussp.* from Aqueous Solutions: The Use of Error Functions for Validation of Isotherm and Kinetics Models. *BioMed Research International*, 2015, 1–13. doi: <http://doi.org/10.1155/2015/126298>
21. Dmytryk, A., Saeid, A., Chojnacka, K. (2014). Biosorption of Microelements by *Spirulina*: Towards Technology of Mineral Feed Supplements. *The Scientific World Journal*, 2014, 1–15. doi: <http://doi.org/10.1155/2014/356328>
22. Marzbali, M. H., Mir, A. A., Pazoki, M., Pourjamshidian, R., & Tabeshnia, M. (2017). Removal of direct yellow 12 from aqueous solution by adsorption onto spirulina algae as a high-efficiency adsorbent. *Journal of Environmental Chemical Engineering*, 5 (2), 1946–1956. doi: <http://doi.org/10.1016/j.jece.2017.03.018>
23. Chojnacka, K., Chojnacki, A., Górecka, H. (2005). Biosorption of Cr^{3+} , Cd^{2+} and Cu^{2+} ions by blue-green algae *Spirulina sp.*: kinetics, equilibrium and the mechanism of the process. *Chemosphere*, 59 (1), 75–84. doi: <http://doi.org/10.1016/j.chemosphere.2004.10.005>
24. Lakhbayeva, Zh., Kurmangazhy, G., Tazhibayeva, S., Artykova, D., Musabekov, K. (2019). Possibility of water purification from Cu^{2+} , Pb^{2+} and Cr^{3+} by using vermiculite. *Journal of Chemical Technology and Metallurgy*, 54 (3), 603–609.
25. Rezaei, H. (2016). Biosorption of chromium by using *Spirulina sp.* *Arabian Journal of Chemistry*, 9 (6), 846–853. doi: <http://doi.org/10.1016/j.arabjc.2013.11.008>
26. Leusbrock, I., Metz, S. J., Rexwinkel, G., Versteeg, G. F. (2010). The solubilities of phosphate and sulfate salts in supercritical water. *The Journal of Supercritical Fluids*, 54 (1), 1–8. doi: <http://doi.org/10.1016/j.supflu.2010.03.003>

27. Maignan, A., Bréard, Y., Guilmeau, E., Gascoin, F. (2012). Transport, thermoelectric, and magnetic properties of a dense Cr₂S₃ ceramic. *Journal of Applied Physics*, 112 (1), 013716. doi: <http://doi.org/10.1063/1.4736417>
28. Gojkovic, Z., Shchukarev, A., Ramstedt, M., Funk, C. (2020). Cryogenic X-ray photoelectron spectroscopy determines surface composition of algal cells and gives insights into their spontaneous sedimentation. *Algal Research*, 47, 101836. doi: <http://doi.org/10.1016/j.algal.2020.101836>
29. Hadjoudja, S., Deluchat, V., Baudu, M. (2010). Cell surface characterisation of *Microcystis aeruginosa* and *Chlorella vulgaris*. *Journal of Colloid and Interface Science*, 342 (2), 293–299. doi: <http://doi.org/10.1016/j.jcis.2009.10.078>
30. Sigel, A., Sigel, H., Sigel, R. K. O. (2011). *Metal Ions in Life Sciences*. Royal Society of Chemistry, 8.
31. Gupta, V. K., Nayak, A., Agarwal, S. (2015). Bioadsorbents for remediation of heavy metals: Current status and their future prospects. *Environmental Engineering Research*, 20 (1), 1–18. doi: <http://doi.org/10.4491/eer.2015.018>
32. Monteiro, C. M., Castro, P. M. L., Malcata, F. X. (2012). Metal uptake by microalgae: Underlying mechanisms and practical applications. *Biotechnology Progress*, 28 (2), 299–311. doi: <http://doi.org/10.1002/btpr.1504>
33. Bishnoi, N. R., Kumar, R., Kumar, S., Rani, S. (2007). Biosorption of Cr(III) from aqueous solution using algal biomass *spirogyra* spp. *Journal of Hazardous Materials*, 145 (1-2), 142–147. doi: <http://doi.org/10.1016/j.jhazmat.2006.10.093>
34. Bertagnolli, C., da Silva, M. G. C., Guibal, E. (2014). Chromium biosorption using the residue of alginate extraction from *Sargassum filipendula*. *Chemical Engineering Journal*, 237, 362–371. doi: <http://doi.org/10.1016/j.cej.2013.10.024>
35. Podolskaya, V. I., Voytenko, E. Yu., Yakubenko, L. N., Ulberg, Z. R., TSYganovich, E. A., Ermakov, V. N., Grischenko, N. I. (2010). Vliyaniye slabogo impulsnogo elektricheskogo polya na vzaimodeystvie nekotorykh mikroorganizmov s ionami serebra i medi. *Nanostrukturalnoe materialovedeniye*, 2, 64–72. Available at: <http://dspace.nbuv.gov.ua/handle/123456789/62715>
36. König-Péter, A., Kilar, F., Felinger, A., Pernyeszi, T. (2015). Biosorption characteristics of *Spirulina* and *Chlorella* cells to accumulate heavy metals. *Journal of the Serbian Chemical Society*, 80 (3), 407–419. doi: <http://doi.org/10.2298/jsc140321060p>
37. Rehman, A., Shakoori, F. R., Shakoori, A. R. (2008). Heavy metal resistant freshwater ciliate, *Euplotes mutabilis*, isolated from industrial effluents has potential to decontaminate wastewater of toxic metals. *Bioresource Technology*, 99 (9), 3890–3895. doi: <http://doi.org/10.1016/j.biortech.2007.08.007>
38. Gagrai, M. K., Das, C., Golder, A. K. (2013). Reduction of Cr(VI) into Cr(III) by *Spirulina* dead biomass in aqueous solution: Kinetic studies. *Chemosphere*, 93 (7), 1366–1371. doi: <http://doi.org/10.1016/j.chemosphere.2013.08.021>
39. Zhang, R., Tian, Y. (2020). Characteristics of natural biopolymers and their derivative as sorbents for chromium adsorption: a review. *Journal of Leather Science and Engineering*, 2 (1). doi: <http://doi.org/10.1186/s42825-020-00038-9>
40. Lodi, A., Soletto, D., Solisio, C., Converti, A. (2008). Chromium(III) removal by *Spirulina platensis* biomass. *Chemical Engineering Journal*, 136 (2-3), 151–155. doi: <http://doi.org/10.1016/j.cej.2007.03.032>
41. Jobby, R., Jha, P., Yadav, A. K., Desai, N. (2018). Biosorption and biotransformation of hexavalent chromium [Cr(VI)]: A comprehensive review. *Chemosphere*, 207, 255–266. doi: <http://doi.org/10.1016/j.chemosphere.2018.05.050>