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THE BIOREMEDIATION POTENTIAL OF IRON-BASED INORGANIC COAGULANTS FOR THE CONTROL OF CYANOBACTERIAL GROWTH

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Cyanobacteria are among the oldest photosynthetic organisms on Earth and play a key role in aquatic ecosystems as primary producers of organic matter. However, under favorable environmental conditions, they can proliferate excessively, leading to harmful algal blooms (HABs), which deteriorate water quality, reduce dissolved oxygen levels, and pose threats to aquatic organisms and human health. In addition, cyanobacteria produce a wide range of secondary metabolites, including cyanotoxins that belong to various groups, such as neurotoxins, hepatotoxins, dermatotoxins, and cytotoxins. The most widespread cyanotoxins include microcystins, anatoxins, and cylindrospermopsins, which can damage the liver and nervous system and may even contribute to the development of oncological diseases. Consequently, cyanobacterial blooms represent a particularly serious problem for drinking water sources.

To mitigate this phenomenon, physical, biological, and chemical control methods are employed. Among chemical approaches, coagulation is an effective method for the removal of cyanobacterial cells and the reduction of intracellular toxin concentrations. Iron-based coagulants, such as iron sulfate ($FeSO_4$) and the chelated form Fe-EDTA, interact with cyanobacterial cells, promoting their aggregation and floc formation. The efficiency of the coagulation process depends on several factors, including pH, coagulant concentration, cyanobacterial species, and the chemical composition of the water.

The aim of this study was to evaluate the effects of $FeSO_4$ and Fe-EDTA on cultures of Nostoc commune. The culture was grown in Fitzgerald medium to a concentration of 5.4×10^6 cells/mL, after which the addition of the coagulants was followed by an assessment of changes in culture density, pH, cell morphology, and the ratio of live to dead cells. The results demonstrated that Fe-EDTA promoted the effective formation of stable flocs and a substantial reduction in cell abundance without significant cell mortality, whereas $FeSO_4$ exhibited pronounced toxicity (51.9% dead cells) and resulted in unstable floc formation. Even at the highest concentrations tested, the application of Fe-EDTA caused the death of less than 19% of cells, indicating its greater ecological safety.

Thus, the chelated iron form Fe-EDTA is a promising coagulant for controlling cyanobacterial abundance, as it combines high cell removal efficiency with a minimal risk of cyanotoxin release into the water. This approach supports the maintenance of ecological balance in aquatic ecosystems and ensures safer application in water treatment processes.

Keywords: cyanobacteria, Nostoc commune, coagulants, $FeSO_4$, Fe-EDTA, flocculation, toxicity, environmental safety

Introduction. Cyanobacteria are among the oldest photosynthetic organisms on the planet. They play an important role in the functioning of aquatic ecosystems as primary producers of organic matter. However, under certain environmental conditions, their rapid and massive proliferation can lead to a phenomenon known as *water bloom*. This process is accompanied by changes in water color and quality, as well as the formation of biofilms on the water surface. One of the main consequences of water blooms is a reduction in dissolved oxygen levels in water bodies, which negatively affects aquatic organisms, deteriorates drinking water quality, and may cause mass poisoning among consumers (Bláha et al., 2009; Boopathi and Ki, 2014).

Cyanobacteria are well known for their ability to produce compounds such as 2-methylisoborneol and geosmin, which can impart unpleasant odors and tastes to drinking water. However, over the past two decades, scientific research has increasingly focused on harmful metabolites that these microorganisms are also capable of synthesizing. Because cyanotoxins have been associated with numerous cases of poisoning in humans and animals, they have become a serious environmental problem and a significant public health concern (Rodgers et al., 2018).

Cyanobacteria are capable of producing a wide variety of secondary metabolites that exhibit diverse biological and biochemical activities, some of which are potent toxins known as cyanotoxins. These toxins constitute a group of compounds that differ in their chemical structures and toxicological properties. Depending on their toxicological targets, cyanobacterial toxins are classified into neurotoxins, hepatotoxins, dermatotoxins, cytotoxins, as well as

irritant toxins (Huertas and Mallén-Ponce, 2022; Boopathi and Ki, 2014).

The most common cyanotoxins include microcystins, anatoxins, cylindrospermopsins, and lipopolysaccharides, among others (Erratt et al., 2022). Cyanotoxins are characterized by a broad spectrum of effects, ranging from damage to the liver and nervous system to the development of oncological diseases. This issue is particularly critical in water bodies used as sources of drinking water, as cyanotoxins may persist in the water even after conventional treatment processes, thereby posing a threat to public health.

Various approaches are employed to control the phenomenon of water blooms, which can be broadly classified into physical, chemical, and biological methods. Physical methods include aeration, filtration, ultraviolet irradiation, and the application of ultrasonic waves. Biological methods involve the use of natural antagonists of cyanobacteria, such as other microorganisms or specific aquatic plants. Chemical methods involve the application of various chemical substances, among which coagulants play a particularly important role (El Bouaidi et al., 2022; Ho et al., 2012).

Coagulants are substances capable of reducing the concentration of suspended particles, including cyanobacteria, by promoting floc formation and subsequent sedimentation. Coagulation and flocculation are among the most widely used and effective water treatment methods (Yang et al., 2024; El Bouaidi et al., 2022; Gu et al., 2019; Ahmad et al., 2011). Since cyanobacterial cells typically carry a negative surface charge, coagulants are added to water to neutralize these charges, causing cell destabilization and subsequent sedimentation. This process allows for the simultaneous removal of both cyanobacterial cells and their metabolites.

However, at high cyanobacterial concentrations, sticky extracellular secretions can extend beyond the cell boundaries due to their small size, forming complex colloids with coagulants. This can hinder cell destabilization and reduce coagulation efficiency. The process of coagulation and flocculation using chemical coagulants, such as aluminum salts or ferric chloride, promotes particle agglomeration, forming flocs. This method can effectively remove up to 90% of intracellular cyanotoxins, representing a significant achievement (Yang et al., 2024; Zhang et al., 2017). Coagulants form bridges between particles, creating flakes that capture cyanobacterial cells, which then settle through the water column, increasing the number of entrapped cells.

The efficiency of coagulation depends on various factors, including cyanobacterial species, temperature, mixing intensity, and the chemical composition of the water, as not all coagulants are equally effective for different water types. Studies have shown that water pH also significantly affects the coagulation efficiency for most coagulants (Yang et al., 2024). Despite their effectiveness in reducing turbidity and cyanobacterial cells, chemical coagulants and flocculants have certain drawbacks, including environmental pollution from improper sludge disposal, human health risks due to residual alum in treated water, high costs of sludge management, and broader ecological impacts.

The main components contributing to cyanobacterial coagulation include polysaccharides, glycoproteins, functional proteins, polyphenols, and/or proteolytic enzymes (Ho et al., 2012). Natural coagulants and flocculants offer advantages due to their biodegradability, cost-effectiveness, safety, and reduced sludge production compared to conventional chemical methods. Particular attention has been given to iron-based coagulants, such as iron sulfate ($FeSO_4$), ferric chloride ($FeCl_3$), and the chelated form of iron (Fe-EDTA) (Addison et al., 2021). Their mechanism of action is based on interactions with cyanobacterial cells and their metabolic products, enabling effective cell sedimentation without causing significant harm to the ecosystem. The use of iron-containing coagulants is a promising approach for controlling water blooms, as they not only promote the removal of cyanobacterial cells but also help reduce cyanotoxin levels in water (Shortle et al., 2020; Ahmad et al., 2011).

Therefore, the aim of this study was to evaluate the effects of iron-based inorganic coagulants on the cyanobacterium *Nostoc commune* for potential application in controlling water blooms.

Materials and methods. The study utilized a culture of the cyanobacterium *Nostoc commune*, maintained in the collection of the Department of Biochemistry and Biotechnology at Chernivtsi National University. This is a macroscopic, non-toxic cyanobacterium belonging to the genus *Nostoc*. It is commonly found on various soil types, rocks, wetlands, and in freshwater environments. In a hydrated state, this species exhibits shades of blue-green, olive-green, or brown, whereas in a desiccated state, it becomes inconspicuous, forming a brown film [https://www.algaebase.org/]. Colonies of *N. commune* used in bioassays possess the unique ability to retain viability for over 100 years after drying. This species occurs in tropical, temperate, and polar regions of both hemispheres.

For cyanobacterial cultivation, Fitzgerald medium modified by Zender and Gorham was used. The medium contained the following components (g/L): $NaNO_3$ – 0.496 (alternatively, 1.054 g urea per 6 L of medium); K_2HPO_4 – 0.039; $MgSO_4$ –

0.075; $CaCl_2$ – 0.036; Na_2SiO_3 – 0.058; Na_2CO_3 – 0.020; $C_6H_5O_7Fe \cdot nH_2O$ – 0.006; $C_6H_8O_7$ – 0.006; Trilon B – 0.001; microelement stock solution – 0.08 mL (containing Fe, Zn, Mn, B, Si, Cu, Mo, Cl, Co, and V).

The accumulation cultivation was conducted over 21 days at 20 ± 2 °C under a 16-hour photoperiod. At the end of the cultivation period, the cell density in the culture reached 5.4×10^6 cells/mL. Cell counting was performed using a Fuchs-Rosenthal counting chamber and an XS-2610 LED MICROmed microscope.

The study employed iron-based coagulants: $FeSO_4$ and the chelated form Fe-EDTA. $FeSO_4$ (iron sulfate) is one of the most commonly used inorganic coagulants in water treatment. When dissolved in water, it releases Fe^{2+} ions, which interact with microorganisms, forming precipitates and promoting bacterial cell aggregation. A key property of $FeSO_4$ is its ability to oxidize rapidly in the presence of oxygen, leading to the formation of a more stable precipitate. However, at high pH values, its effectiveness may decrease, resulting in less efficient coagulation (Addison et al., 2021; Andrews et al., 2003).

Fe-EDTA (iron chelated with ethylenediaminetetraacetic acid) is a coagulant in which iron is stabilized in a chelated form. In this configuration, iron is bound to the ligand EDTA, which maintains it in a soluble state and increases resistance to oxidation. Although Fe-EDTA does not precipitate as quickly as other iron forms, it provides long-term stability and allows for controlled iron release within the system. This is beneficial for maintaining optimal coagulation conditions and promoting effective algal removal. One of the advantages of Fe-EDTA is the ability to regulate iron solubility and bioavailability during water treatment (Zhang et al., 2017).

Solutions of coagulants were added to cyanobacterial cultures in test tubes to achieve Fe concentrations ranging from 0.1 to 1.6 mg Fe/L. Cultures of Nostoc commune with different forms of iron were incubated under controlled conditions for 7 days. Temperature, illumination, and photoperiod duration were maintained at optimal conditions for the growth of N. commune. On the seventh day of incubation, the cultures were assessed for the effects of the coagulants, including observations of culture appearance (color, flocculation), pH measurements, optical density, microscopic examination, and counts of live and dead cells.

All manipulations with cyanobacterial cultures were conducted in a laminar flow cabinet under strict microbiological safety conditions. Prior to the experiments, the laminar box was exposed to ultraviolet (UV) light for 30 minutes. Subsequent operations were performed no earlier than 20–30 minutes afterward to allow for the inactivation of free radicals generated by UV exposure. The surface of the cabinet was wiped with 70% ethanol.

Culture density of *N. commune* was measured using a KFK-2 photoelectric colorimeter at a wavelength of 750 nm. This method allows estimation of the number of cells suspended in the culture medium. The pH of the culture medium was determined using a portable ADWA AD11 pH meter.

Microscopic analysis of *N. commune* samples was performed after the incubation period with coagulants using an XS-2610 LED MICROmed microscope. A clean microscope slide was prepared by placing a single drop of culture medium from the test tube containing N. commune cells treated with coagulants. The drop was covered with a coverslip, avoiding air bubble formation that could interfere with observation. Excess liquid was carefully removed using filter paper to ensure uniform contact between the coverslip and the slide. The prepared slide was placed on the microscope stage for examination. Observations were conducted at 200× magnification (20× eyepiece and 10× objective).

To assess the ratio of live to dead *Nostoc commune* cells, a differential staining method using methylene blue and neutral red dyes was employed. This approach allows determination of cell viability based on the ability of cells to absorb or repel the dyes. After incubation, a 1 mL sample of the culture medium was taken for analysis. To this, 1 mL of each dye solution, prepared at a 1:5000 dilution, was added. The mixture was incubated for 1 hour at room temperature to ensure complete interaction of the cells with the dyes. Cell counting was performed using a Fuchs-Rosenthal counting chamber, which allows accurate determination of cell numbers in a defined sample volume. Live cells acquired a red hue, while dead cells stained bright blue due to the dye penetrating the damaged cell membrane.

The toxicity coefficient was assessed by evaluating the ratio of live to dead cells after their incubation with coagulants. This was based on the cell counts obtained using the Fuchs-Rosenthal chamber.

Statistical analysis of the results was performed using standard methods with Microsoft Excel software. All experiments were conducted in quadruplicate. The significance of the results was evaluated using Student's t-test. In graphs and tables, data are presented as mean values for all replicates, with standard deviations indicated.

Results. This study investigated the effects of iron-based inorganic coagulants ($FeSO_4$ and Fe-

EDTA) on *Nostoc commune* cells. A laboratory model of cyanobacterial bloom conditions was established. To this end, *Nostoc commune* was cultured in a modified Fitzgerald medium until reaching a cell density of 5.4 × 10^6 cells/mL, corresponding to bloom conditions. The use of coagulants was aimed not only at cell sedimentation but also at reducing cell viability, thereby contributing to an effective mitigation of the problem. Initial assessments focused on visual changes in test tubes containing *N. commune* cultures incubated with iron-based coagulants (Fig. 1).

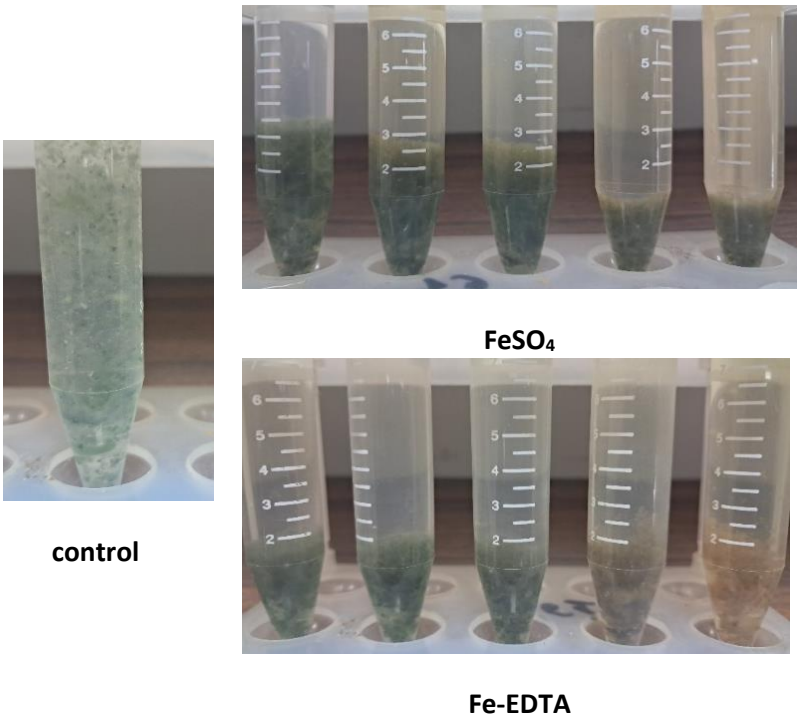

Fig. 1. Coagulation process of Nostoc commune cells under the influence of iron-based inorganic coagulants

As early as the third day of incubation, loose aggregates of cyanobacterial cells were observed in the test tubes. A color change of the cells from blue-green to dirty green and brown was also noted. With increasing incubation time and coagulant concentration, these changes became more pronounced. After a seven-day incubation period, the impact of the coagulants on the density of the N. commune culture was evident (Fig. 2).

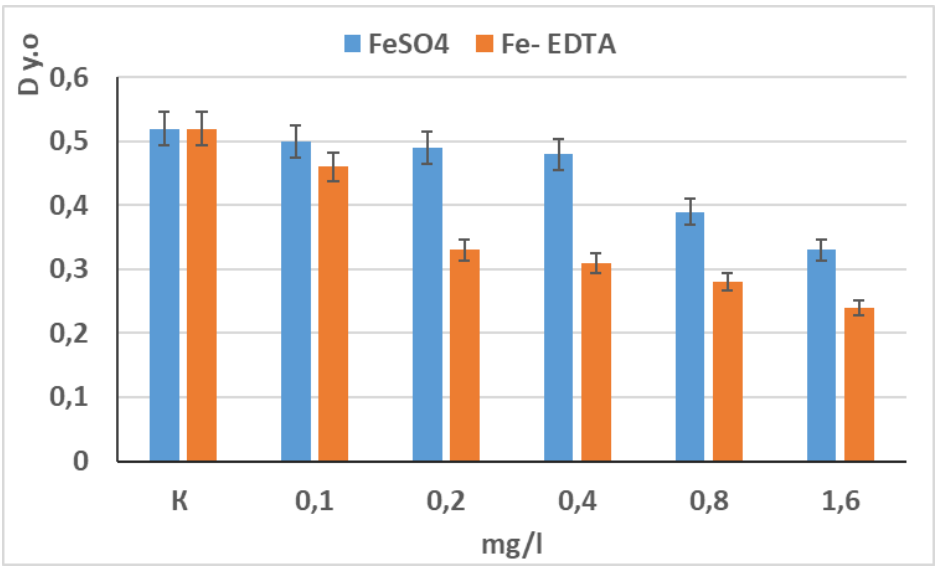

Fig. 2. Density of N. commune culture under the influence of iron-based inorganic coagulants

The density of the *N. commune* culture decreased depending on the concentration of the added coagulant. A difference was observed depending on the nature of the coagulant. In the case of Fe-EDTA, this process was more pronounced, with the density decreasing twofold relative to the control values.

The next step involved measuring the pH levels of the samples, the results of which are presented in Table 1. pH determination is a crucial stage of the experiment, as the acid–base balance significantly affects the viability of cyanobacteria and the efficiency of coagulants. Sharp pH fluctuations can induce stress or lead to the death of *N. commune* cells. Furthermore, pH measurement allows for the assessment of the potential impact of coagulants on aquatic ecosystems, since excessive acidification of water may have adverse effects on other aquatic organisms and the overall ecological status.

Table 1.

pH changes in N. commune culture under the influence of Iron-based inorganic coagulants

Experimental conditions	**$FeSO_4$**		**Fe-EDTA**	
	1 day	**7 day**	**1 day**	**7 day**
control	7,8±0,01	7,0±0,01	7,5±0,02	7,1±0,03
0,1 mg/l	7,5±0,06	7,1±0,07	7,7±0,01	6,9±0,21
0,2 mg/l		6,7±0,22		6,0±0,18
0,4 mg/l		5,9±0,27		5,5±0,09
0,8 mg/l		4,7±0,19		5,0±0,12
1,6 mg/l		3,8±0,13		5,0±0,11

The research results demonstrated a significant effect of the selected coagulants on the pH of the *N. commune* culture. It was observed that an increase in coagulant concentration led to a decrease in pH, which can be attributed to their chemical properties and reactions in an aqueous environment [2,10]. When $FeSO_4$ was used, the pH decreased twofold compared to the control sample, in which no coagulants were added. This indicates a strong acidic effect of this reagent on the surrounding medium.

In contrast, the use of Fe-EDTA yielded more stable results. The reduction in pH in samples treated with this coagulant was moderate-approximately 1.5-fold compared to the controls. Such a change in acidity is considered acceptable both for the effective elimination of cyanobacteria and for maintaining the ecological balance of the water body. The obtained data suggest that Fe-EDTA exerts a lower impact on the acid-base balance, making it safer for use in natural ecosystems (Zhang et al., 2017).

Microscopy was one of the primary methods in this study, as it allowed the evaluation of morphological changes in the *N. commune* culture under the influence of coagulants and the investigation of the structure and shape of the formed flocs. Following incubation with different coagulants, microscopic analysis provided valuable information on cellular changes and aggregation. In samples treated with ferric sulfate, a color change of the culture to orange-yellow was observed, with color intensity increasing with higher coagulant concentrations. This likely indicates a disruption of cyanobacterial viability and the accumulation of secondary metabolites in response to stress conditions. Furthermore, these samples exhibited the formation of loose structures that did not form well-defined flocs, suggesting lower efficiency in aggregation and sedimentation.

In the case of Fe-EDTA treatment, the culture retained its initial appearance almost entirely, indicating minimal impact on the cyanobacterial pigments. Upon inverting the test tube, the formed flocs remained distinct and preserved their shape even after agitation. Under the microscope, these flocs exhibited a well-defined structure and a high level of cellular aggregation, demonstrating the effectiveness of this coagulant in sedimenting *N. commune* cells.

Thus, at this stage of the study, the advantage of the Fe-EDTA chelate complex in forming stable flocs compared to ferric sulfate was confirmed.

Investigating the ratio of live to dead cells is an important aspect of the study, as it allows assessment of the coagulants' impact on cyanobacterial viability. Staining results with methylene blue and neutral red showed that the use of ferric sulfate ($FeSO_4$) caused a significant increase in dead cells, particularly at high coagulant concentrations (Fig. 3).

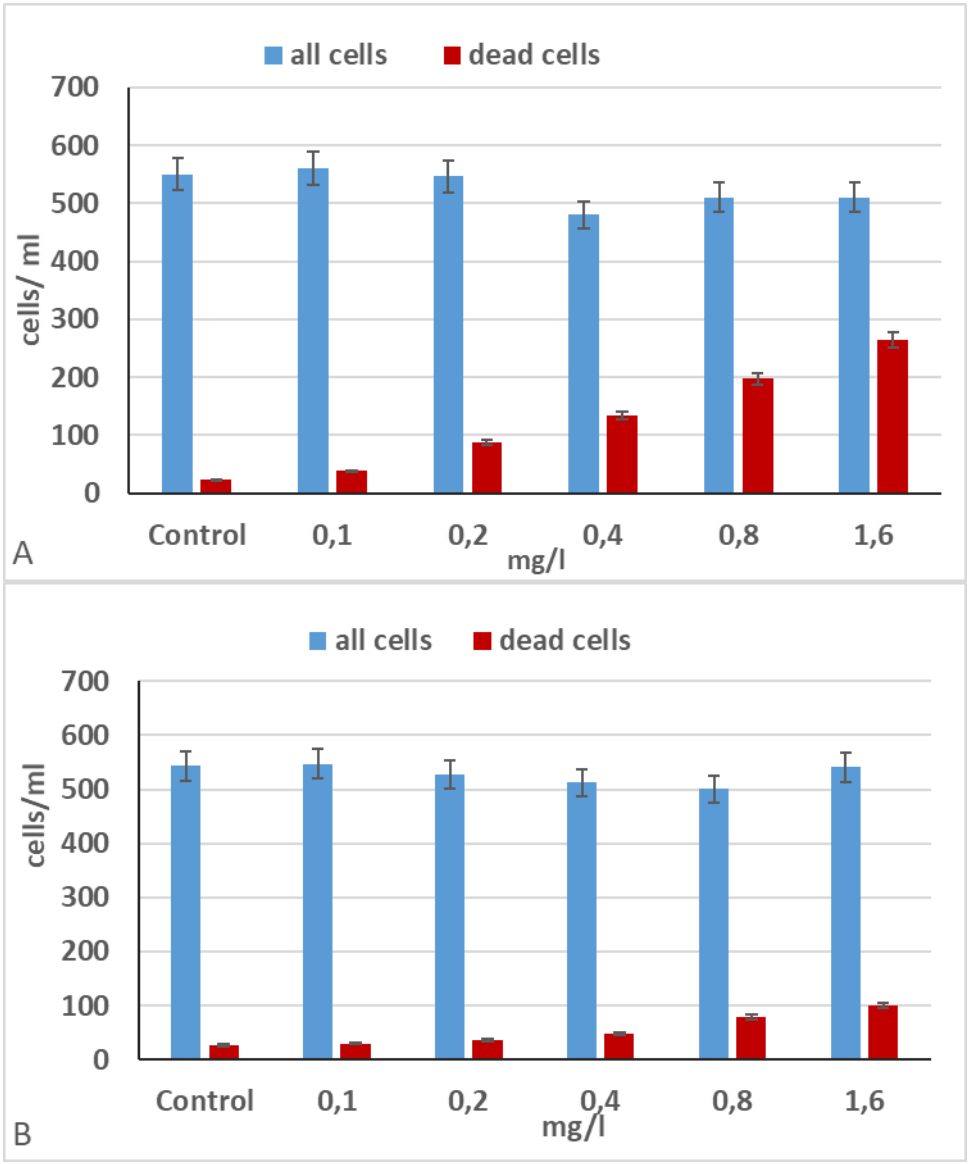

Fig. 3. Ratio of live to dead cells in N. commune culture under the influence of iron-based inorganic coagulants – $FeSO_4$ (A) and Fe-EDTA (B)

The results indicate the detrimental effects of $FeSO_4$ on the cellular structure and metabolism of cyanobacteria. The preservation of the majority of cells in a viable state after treatment is critically important, as cyanobacterial cell death may lead to the release of cyanotoxins into the environment, posing a significant threat to the ecosystem. Other results were obtained in samples treated with Fe-EDTA. Following staining, the cells exhibited pronounced contrast, facilitating the assessment of their condition. Even at high concentrations of this coagulant, the proportion of dead cells remained low. This suggests that the use of Fe-EDTA for floc formation promoted the aggregation of cells in a viable state, minimizing structural damage.

Thus, the study results confirm that the chelated Fe-EDTA complex is the most effective coagulant for preserving the viability of *N. commune* cells, reducing the risk of cyanotoxin release into the surrounding environment. A key aspect of the study is the analysis of coagulant toxicity, as an effective agent for cyanobacterial removal should not only promote floc formation but also cause minimal harm to microorganisms and the ecosystem. We calculated the toxicity of the applied compounds (Table 2).

The calculation was performed taking into account the total cell count in the culture and the number of dead cells following coagulant treatment. Excessive toxicity may lead to cell death, which in turn increases the risk of cyanotoxin release into the surrounding environment. Indeed, $FeSO_4$ proved to be more toxic than Fe-EDTA. At the maximum concentration, the toxicity coefficient of $FeSO_4$ for *N. commune* cells reached 51.9%, whereas under the same conditions, Fe-EDTA caused the death of only 18.7% of *N. commune* cells.

Table 2.

Concentration-dependent toxicity coefficients of various coagulants, %

Coagulant concentration, mg/l	$FeSO_4$	Fe-EDTA
0,1	6,8	5,5
0,2	15,9	7,0
0,4	27,9	9,4
0,8	38,8	15,8
1,6	51,9	18,7

The use of inorganic coagulants is among the most effective methods for cyanobacterial removal from water. However, it is crucial to recognize that the choice of coagulant should be based not only on its removal efficiency but also on its safety for the ecosystem. Severe cellular damage may lead to toxin release, necessitating a detailed analysis of coagulant effects on both the cells and the aquatic environment as a whole. Therefore, the effective implementation of coagulants in water treatment requires a comprehensive approach that considers their chemical properties, mode of action, and potential ecological consequences.

Conclusions. The introduction of coagulants into *N. commune* cultures induces morphological transformations, including changes in cell color, medium viscosity, sediment formation, and the development of well-defined flocs, which are clearly visible under microscopy. The most pronounced changes were observed during incubation with Fe-EDTA, resulting in distinctly formed, mechanically stable flocs.

In samples treated with FeSO, significant alterations in the composition of the growth medium were recorded, leading to the formation of unstable flocs and gradual death of the *N. commune* culture. The toxicity of $FeSO_4$ for *N. commune* cells was 51.9%.

Incubation of *N. commune* with the chelated form of iron (Fe-EDTA), even at high coagulant concentrations, significantly reduced the number of cells without causing substantial cell death. The proportion of dead cells in the culture at the maximum Fe-EDTA concentration did not exceed 19%.

For the regulation of cyanobacterial abundance, Fe-EDTA can be applied at a concentration of 1.6 mg/L, followed by the separation of the settled biomass.

***Conflict of Interest**: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.*

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БІОРЕМЕДІАЦІЙНИЙ ПОТЕНЦІАЛ НЕОРГАНІЧНИХ ЗАЛІЗОВМІСНИХ КОАГУЛЯНТІВ ЗАДЛЯ КОНТРОЛЮ РОСТУ ЦІАНОБАКТЕРІЙ

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Ціанобактерії є одними з найдавніших фотосинтезуючих організмів на Землі та відіграють ключову роль у водних екосистемах як продуценти органічної сировини. Проте за сприятливих умов вони можуть масово розмножуватися, спричиняючи явище «цвітіння води», яке погіршує якість води, знижує рівень кисню та становить загрозу для водних організмів і людини. Крім того, ціанобактерії виробляють вторинні метаболіти, включно з ціанотоксинами, які належать до різних груп – нейротоксинів, гепатотоксинів, дерматотоксинів та цитотоксинів. Найбільш поширеними є мікроцистини, анатоксини та циліндроспермопсини, які здатні завдавати шкоди печінці, нервовій системі та навіть сприяти розвитку онкологічних захворювань. Це робить проблему цвітіння води особливо актуальною для питних джерел.

Для боротьби з цим явищем застосовують фізичні, біологічні та хімічні методи. До хімічних підходів належить коагуляція – ефективний спосіб осадження ціанобактерій та зниження концентрації внутрішньоклітинних токсинів. Коагулянти на основі заліза, такі як сульфат заліза ($FeSO_4$) та хелатна форма Fe-EDTA, взаємодіють із клітинами цианобактерій, сприяючи їх агрегації та утворенню флокул. При цьому ефективність процесу залежить від pH, концентрації коагулянту, виду ціанобактерій та хімічного складу води.

Метою дослідження було оцінити вплив $FeSO_4$ та Fe-EDTA на культуру Nostoc commune. Культуру вирощували на середовищі Фітцджеральда до концентрації $5{,}4\times10^6$ кл/мл, і після додавання коагулянтів оцінювали зміни щільності культури, pH, морфології клітин, а також співвідношення живих і мертвих клітин. Результати показали, що Fe-EDTA забезпечував ефективне формування стабільних флокул і суттєве зменшення кількості клітин без значної загибелі, тоді як $FeSO_4$ спричиняв значну токсичність (51,9% мертвих клітин) та нестійкі флокули. Застосування Fe-EDTA навіть у максимальних концентраціях призводило до загибелі менше ніж 19% клітин, що робить його більш безпечним для екосистеми.

Отже, хелатна форма заліза Fe-EDTA є перспективним коагулянтом для контролю чисельності ціанобактерій, оскільки поєднує високу ефективність осадження клітин із мінімальним ризиком вивільнення ціанотоксинів у воду. Це дозволяє підтримувати екологічний баланс водойм і забезпечує безпечне застосування у водоочищенні.

Ключові слова: ціанобактерії, Nostoc commune, коагулянти, $FeSO_4$, Fe-EDTA, флокуляція, токсичність, екологічна безпека

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