

MUHANDISLIK

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fan va ta'limga oid ilmiy-amaliy jurnal

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- 05.01.03 – Informatikaning nazariy asoslari
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Ma'lumot uchun, OAK

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MUNDARIJA

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NITROGEN FIXATION CHARACTERISTICS OF AZOLLA CAROLINIANA UNDER THE INFLUENCE OF HEAVY METAL (Cr^{6+})

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Abstract. This study investigated the nitrogen fixation activity of the aquatic plant *Azolla caroliniana* in different nutrient media (D and W) under the influence of various concentrations of hexavalent chromium (Cr^{6+}). During the experiments, biomass production, growth dynamics, and nitrogen fixation activity of *Azolla* were evaluated using the acetylene reduction method. The results showed that in chromium-free nutrient media, nitrogen fixation activity gradually increased over 10–30 days and exhibited a declining trend after day 35. Nitrogen fixation activity in the W-type nutrient medium was higher compared to the D medium. Although increasing chromium concentrations significantly reduced nitrogen fixation activity, the process did not completely cease even at high concentrations (3 mg/L). The results indicate that *Azolla caroliniana* is a relatively stable biological object in water environments contaminated with heavy metals.

Keywords: *Azolla caroliniana*, nitrogen fixation, chromium (Cr^{6+}), nutrient medium, biomass, acetylene reduction method, *Anabaena azollae*.

Annotatsiya. Ushbu tadqiqotda suv o'simligi *Azolla caroliniana* ning azot fiksatsiyasi faolligi turli oziqlantiruvchi muhitlarda (D va W) olti valentli xrom (Cr^{6+}) ning turli konsentratsiyalari ta'siri ostida o'rganildi. Tajribalar davomida *Azolla* ning biomassa hosil qilishi, o'sish dinamikasi va azot fiksatsiyasi faolligi atsetilenni qaytarish usuli yordamida baholandi. Natijalar shuni ko'rsatdiki, xrom mavjud bo'lmagan oziqlantiruvchi muhitlarda azot fiksatsiyasi faolligi 10–30 kun davomida bosqichma-bosqich oshgan va 35-kundan keyin pasayish tendensiyasini namoyon etgan. W turidagi oziqlantiruvchi muhitda azot fiksatsiyasi faolligi D muhitiga nisbatan yuqori bo'lgan. Xrom konsentratsiyasining ortishi azot fiksatsiyasi faolligini sezilarli darajada kamaytirgan bo'lsa-da, jarayon hatto yuqori konsentratsiyalarda (3 mg/l) ham to'liq to'xtamagan. Olingan natijalar *Azolla caroliniana* og'ir metallar bilan ifloslangan suv muhitlarida nisbatan barqaror biologik obyekt ekanligini ko'rsatadi.

Kalit so'zlar: *Azolla caroliniana*, azot fiksatsiyasi, xrom (Cr^{6+}), oziqlantiruvchi muhit, biomassa, atsetilenni qaytarish usuli, *Anabaena azollae*.

Аннотация. В данном исследовании изучалась активность фиксации азота водным растением *Azolla caroliniana* в различных питательных средах (D и W) под воздействием различных концентраций шестивалентного хрома (Cr^{6+}). В ходе экспериментов оценивались продукция биомассы, динамика роста и активность фиксации азота *Azolla* с использованием метода восстановления ацетилена. Результаты показали, что в питательных средах без хрома активность фиксации азота постепенно возрастала в течение 10–30 дней и демонстрировала тенденцию к снижению после 35-го дня. Активность фиксации азота в питательной среде типа W была выше по сравнению со средой D. Несмотря на то что увеличение концентрации хрома значительно снижало активность фиксации азота, процесс полностью не прекращался даже при высоких концентрациях (3 мг/л). Полученные результаты свидетельствуют о том, что *Azolla caroliniana* является относительно устойчивым биологическим объектом в водных средах, загрязнённых тяжёлыми металлами.

Ключевые слова: *Azolla caroliniana*, фиксация азота, хром (Cr^{6+}), питательная среда, биомасса, метод восстановления ацетилена, *Anabaena azollae*.



INTRODUCTION

Globally, large volumes of wastewater containing harmful chemical substances generated by various industrial sectors pose serious ecological problems. Biological treatment of wastewater plays a crucial role in maintaining the stability of anthropogenic ecosystems. In particular, the purification of wastewater contaminated with harmful chemicals using higher aquatic plants is of great importance for ecology and environmental protection. Wastewater discharged from leather-processing enterprises, paper mills, coal mines, and thermal power plants contains chromium (Cr), which poses a significant threat to aquatic ecosystems.

Worldwide, scientific studies have focused on the severe environmental problems arising from incomplete removal of chemical pollutants in wastewater from leather-processing industries. Special attention has been paid to determining the toxic properties of chromium in wastewater, its widespread use as a key component in light industry electroplating, leather processing, and metal preparation and polishing, as well as improving biological treatment methods for wastewater that is only partially treated by physico-chemical methods. The generation of large amounts of sulfites, ammonium nitrogen, and protein compounds in leather-processing enterprises further contributes to significant changes in ecosystems.

In Uzbekistan, especially after gaining independence, significant progress has been made in stabilizing the ecological situation, ensuring rational use of natural resources and water reserves, and implementing innovative water-saving technologies. These efforts aim to protect water resources from various types of pollution and prevent human exposure to harmful substances.

The Action Strategy for the further development of the Republic of Uzbekistan emphasizes: "...ensuring people live in an ecologically safe environment, constructing and modernizing household waste recycling complexes, strengthening their material and technical base, and providing the population with modern facilities for waste disposal..."

Based on these objectives, the integrated use of biological methods alongside widely applied physico-chemical methods for wastewater treatment in industrial enterprises has significant scientific and practical importance.

REVIEW OF LITERATURE ON THE SUBJECT

Biological nitrogen fixation has long been recognized as a critical process for sustaining soil fertility and agricultural productivity, particularly in low-input and sustainable farming systems. Early foundational studies by Bottomley (1920) demonstrated the essential role of organic matter in stimulating the growth of aquatic plants, thereby laying the groundwork for subsequent research on symbiotic nitrogen-fixing systems. Later, Hardy et al. (1968) and Hardy et al. (1971) significantly advanced methodological approaches to quantifying nitrogen fixation, emphasizing its global importance for protein production and ecosystem stability.

Within this context, *Azolla* species have attracted considerable scientific attention due to their unique symbiosis with nitrogen-fixing cyanobacteria. Hamdi (1982) highlighted the applicability of nitrogen-fixing systems, including *Azolla*, in soil improvement and sustainable land management. Kangmin (1988) further demonstrated the practical relevance of *Azolla* in integrated rice–fish farming systems, underscoring its role in enhancing nitrogen availability while supporting aquatic agroecosystems.

Beyond their agronomic benefits, *Azolla* species have been extensively studied for their capacity to tolerate and accumulate heavy metals. Benaroya et al. (2004) reported significant lead accumulation in *Azolla filiculoides*, indicating the plant's strong metal uptake potential. Similarly, Bennicelli et al. (2004) demonstrated that *Azolla caroliniana* effectively removes various heavy metals, including Cr(III) and Cr(VI), from municipal wastewater, suggesting its suitability for phytoremediation applications. These findings are further supported by Babu et al. (2014), who analyzed the kinetic, equilibrium, and thermodynamic aspects of chromium biosorption using *Azolla*, confirming its high affinity for Cr(VI) under aqueous conditions.

The interaction between nitrogen metabolism and heavy metal stress has also been explored in related biotechnological and environmental studies. Cohen (2006) emphasized the role of microbial systems in reducing the cost of metal removal from industrial waste streams, indirectly highlighting the importance of plant–microbe interactions in contaminated environments. In wastewater treatment contexts, Basset et al. (2016) illustrated how microbial biomass selection can be integrated with nitrogen removal processes, providing a relevant conceptual framework for understanding nitrogen transformation under stress conditions.

Physiological and biochemical responses of *Azolla* under environmental constraints have gained attention in recent years. Brouwer et al. (2015) investigated lipid yield and composition in *Azolla filiculoides*, revealing that environmental factors substantially influence metabolic pathways. Although focused on bioenergy potential, their findings imply that heavy metal stress may similarly alter nitrogen fixation efficiency and associated metabolic processes.

Collectively, the reviewed literature indicates that *Azolla caroliniana* possesses a dual functional capacity: effective biological nitrogen fixation and significant tolerance to heavy metal contamination, particularly chromium in its hexavalent form. However, while heavy metal accumulation by *Azolla* has been well documented, fewer studies explicitly address how Cr^{6+} stress affects nitrogen fixation characteristics at physiological and biochemical levels. This gap underscores the scientific relevance of investigating nitrogen fixation dynamics in *Azolla caroliniana* under chromium exposure, especially in the context of sustainable agriculture and phytoremediation strategies.

RESEARCH METHODOLOGY

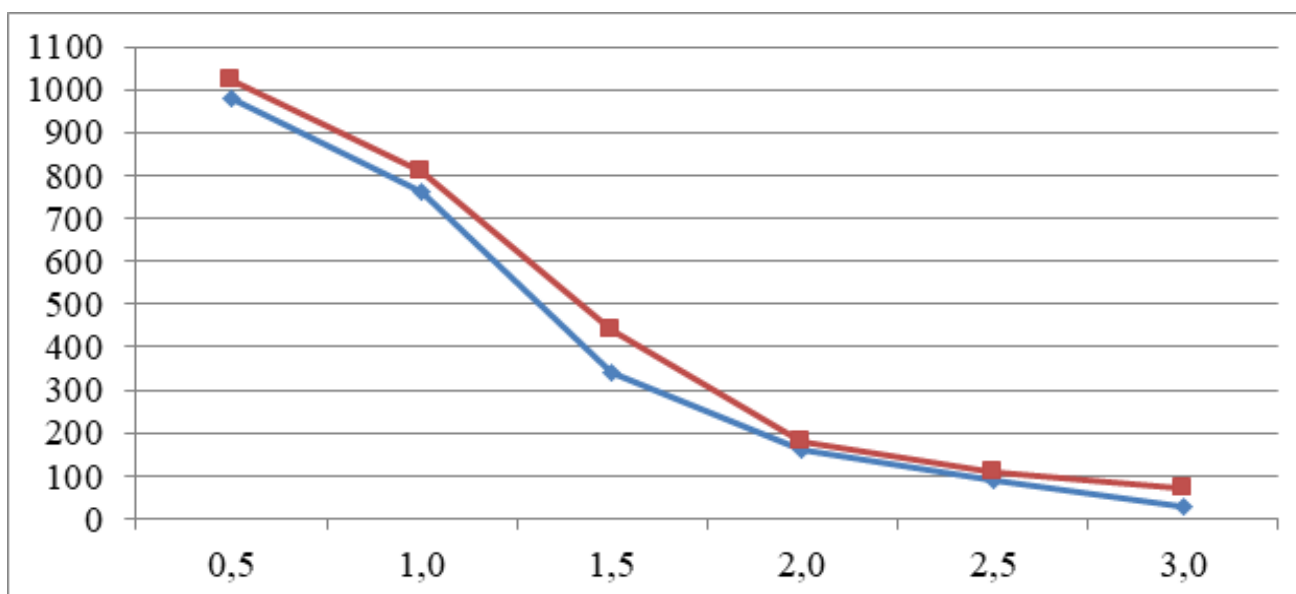
The aquatic plant *Azolla caroliniana* was selected as the study object. Experiments were conducted under laboratory conditions using D and W types of nutrient media, with both chromium-free and chromium-supplemented variants (0.5–3.0 mg/L Cr^{6+}). *Azolla* cultures were grown for 40 days, and nitrogen fixation activity was determined at different stages of the growth period (days 10, 15, 20, 25, 30, 35, and 40). Nitrogen fixation activity was assessed using the acetylene reduction method, with ethylene (C_2H_4) production expressed in nM C_2H_4 /flask/day. Additionally, biomass accumulation, plant morphological parameters (leaf and root system development), and the effect of chromium concentration on the physiological status of *Azolla* were comparatively analyzed. All data were statistically evaluated and presented in graphical and tabular formats.

ANALYSIS AND RESULTS

Analysis of the results showed that chromium addition to the nutrient media significantly affected biomass production, primarily by influencing the development of the leaf and root systems of the *Azolla* culture. Table X presents the leaf and root sizes, as well as their branching patterns, of cultures grown in D and W nutrient media compared to the control.

Since growth and total protein and nitrogen content of *Azolla* cultures in chromium-contaminated media showed minimal changes, further investigations focused on one of the most important traits of the cultures—nitrogen fixation activity. Nitrogen fixation characteristics were analyzed for *Azolla* grown in both chromium-free and chromium-supplemented nutrient media.

In the chromium-free D medium, nitrogen fixation activity of *Azolla* cultures was 2900 nM C_2H_4 /flask/day on day 10, increasing to 3200 nM C_2H_4 /flask/day on day 15 and 3280 nM C_2H_4 /flask/day on day 20. By day 30, nitrogen fixation activity slightly increased to 3460 nM C_2H_4 /flask/day. However, starting from day 35, nitrogen fixation activity sharply decreased to 3300 nM C_2H_4 /flask/day and continued to decline, reaching 2090 nM C_2H_4 /flask/day by day 40 (Figure 1).



W feed in red, D feed in blue

Figure 1. Nitrogen fixation activity of *Azolla caroliniana* in chromium-rich water

During the study, it was observed that nitrogen fixation activity of *Azolla* cultures grown in the W chromium-free nutrient medium was slightly higher compared to those grown in the D chromium-free medium. Specifically,



on the first 10 days of cultivation, nitrogen fixation activity in the D medium was 2900 nM C_2H_4 /flask/day, whereas in the W medium it reached 3600 nM C_2H_4 /flask/day, demonstrating a clear difference between the two media.

Furthermore, on day 15 (D – 3200 nM C_2H_4 /flask/day; W – 3570 nM C_2H_4 /flask/day), day 20 (D – 3280 nM C_2H_4 /flask/day; W – 3580 nM C_2H_4 /flask/day), day 25 (D – 3400 nM C_2H_4 /flask/day; W – 3660 nM C_2H_4 /flask/day), and day 30 (D – 3460 nM C_2H_4 /flask/day; W – 3720 nM C_2H_4 /flask/day), nitrogen fixation activity showed a gradual increasing trend.

From day 35 onwards, a decreasing trend in nitrogen fixation activity was observed in *Azolla* cultures in both nutrient media. On day 35, nitrogen fixation activity in the D medium was 3300 nM C_2H_4 /flask/day, while in the W medium it reached 3350 nM C_2H_4 /flask/day.

By day 40, nitrogen fixation activity decreased to 2090 nM C_2H_4 /flask/day in the D medium and to 1130 nM C_2H_4 /flask/day in the W medium.

These experimental results indicate that *Azolla* cultures exhibit varying nitrogen fixation activity depending on the composition of the nutrient medium, with the W medium supporting higher activity than the D medium under chromium-free conditions (Figure 2).

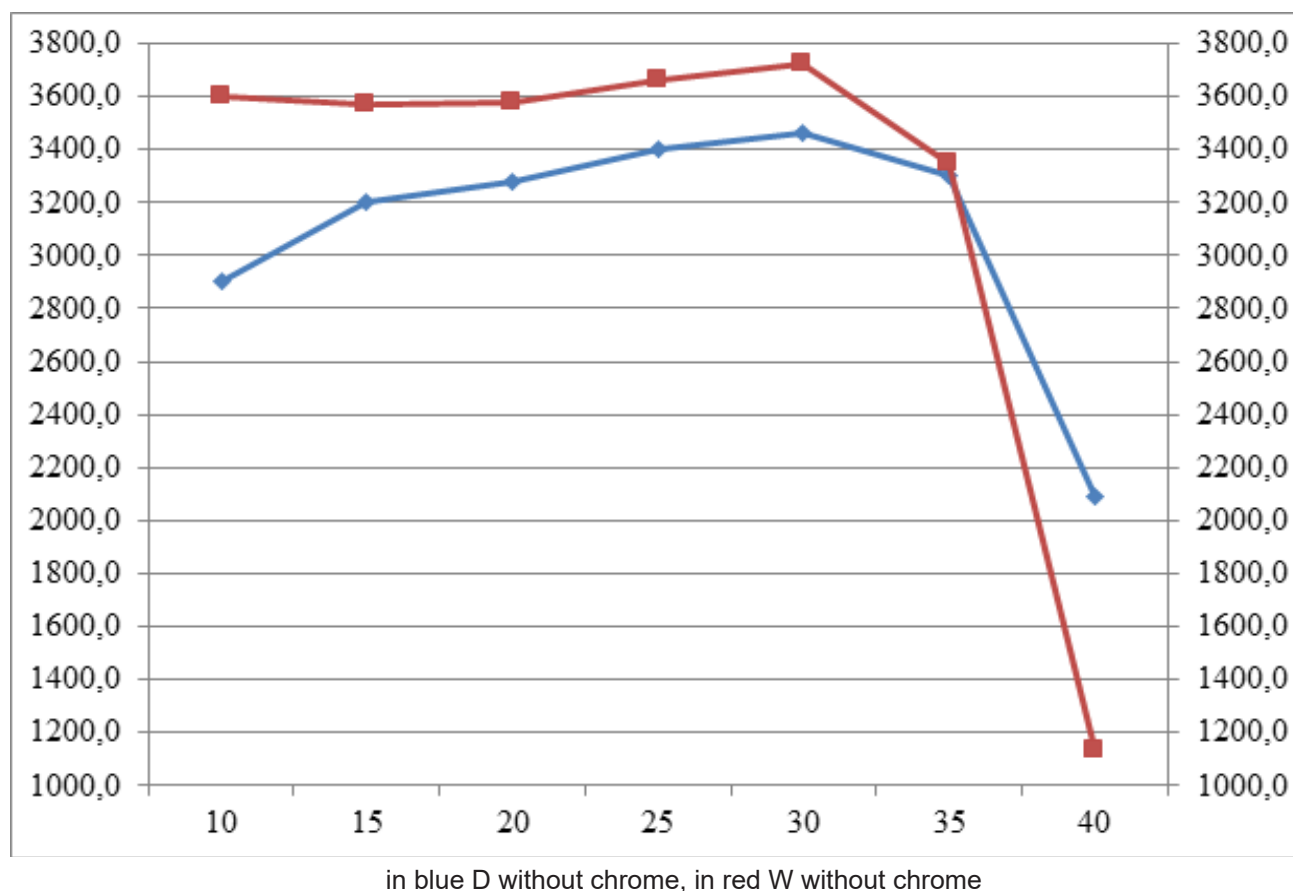


Figure 2. Nitrification activity of *A. caroliniana* in different nutrient media

In subsequent experiments, nitrogen fixation activity of *Azolla caroliniana* was investigated in D and W nutrient media supplemented with varying concentrations of hexavalent chromium (Cr^{6+}). The results are presented in Figure 2.

The findings indicated that *A. caroliniana* is highly sensitive to different chromium concentrations. In the D medium containing 0.5 mg/L Cr^{6+} , nitrogen fixation activity was 980 nM C_2H_4 /flask/day, while in 1.0 mg/L Cr it decreased to 760 nM C_2H_4 /flask/day. In media containing 1.5 and 2.0 mg/L Cr, nitrogen fixation activity further declined to 340 and 160 nM C_2H_4 /flask/day, respectively.

Similarly, in the W medium, nitrogen fixation activity was 1010 nM C_2H_4 /flask/day at 0.5 mg/L Cr, 810 nM C_2H_4 /flask/day at 1.0 mg/L Cr, and 440 nM C_2H_4 /flask/day at 1.5 mg/L Cr. At higher concentrations, 2.0 mg/L, 2.5 mg/L, and 3.0 mg/L Cr, nitrogen fixation activity in the D medium decreased to 160, 90, and 30 nM C_2H_4 /flask/day, respectively, while in the W medium it reached 180, 110, and 70 nM C_2H_4 /flask/day.

These results demonstrate that nitrogen fixation activity of *A. caroliniana* is dependent on cultivation duration. From day 10 to day 30, nitrogen fixation activity gradually increased, whereas from day 35 onward, a declining trend was observed. The gradual increase in activity can be explained by the progressive growth of the culture's biomass, including the number and size of leaves. Conversely, the decline after day 35 is associated with a reduction in nutrients in the liquid medium and an increase in necrotic or dead biomass.

A comparative analysis of nitrogen fixation activity revealed the pronounced inhibitory effect of chromium on *Azolla*. In the chromium-free D medium, activity increased from 2900 to 3460 nM C_2H_4 /flask/day, whereas in the W medium, it increased from 3600 to 3720 nM C_2H_4 /flask/day. In the D medium with 0.5 mg/L Cr, activity reached a maximum of 980 nM C_2H_4 /flask/day, but dropped to a minimum of 30 nM C_2H_4 /flask/day at 3 mg/L Cr. In the W medium, nitrogen fixation ranged from 1010 nM C_2H_4 /flask/day at 0.5 mg/L Cr to 70 nM C_2H_4 /flask/day at 3 mg/L Cr, yet activity was not completely abolished, likely due to the composition of the nutrient medium.

It is also well documented that cyanobacteria, such as *Anabaena azollae*, which live in symbiosis with *Azolla*, exhibit high nitrogen fixation activity. Therefore, in further experiments, *Azolla* cultures exposed to 3 mg/L Cr—where biomass (growth coefficient 1:0.39; maximum biomass 1.79 g/m²) and nitrogen fixation activity (70 nM C_2H_4 /flask/day) were significantly reduced, but 52% viability was retained—were selected as the experimental object.

CONCLUSIONS AND SUGGESTIONS

The study demonstrated that nitrogen fixation activity of *Azolla caroliniana* is directly influenced by nutrient medium composition, cultivation duration, and chromium concentration. In chromium-free conditions, particularly in the W medium, nitrogen fixation activity reached its highest value (3720 nM C_2H_4 /flask/day). Low chromium concentrations (0.5–1.0 mg/L) maintained nitrogen fixation, but higher concentrations caused a sharp decline. Notably, even at 3 mg/L Cr, nitrogen fixation was not completely abolished, highlighting *Azolla*'s ecological adaptability. These results scientifically confirm that *Azolla caroliniana* and its symbiotic cyanobacteria *Anabaena azollae* are promising agents for bioremediation and phytoremediation in heavy metal-contaminated aquatic ecosystems.

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