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DOSE-DEPENDENT EFFECT OF IMIDAZOLINONE HERBICIDES ON THE MONOCULTURE OF DESMODESMUS ARMATUS

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This study investigates the dose-dependent effects of imidazolinone herbicides, particularly imazamox, on a monoculture of the green microalga Desmodesmus armatus, which is a sensitive test organism for ecotoxicological assessments. The relevance of the research is обусловлена the increasing risk of herbicide entry into freshwater bodies as a result of agricultural runoff and anthropogenic disturbances, which may adversely affect primary producers in aquatic ecosystems.

The toxic effects of imazamox were evaluated based on culture growth parameters (cell density and optical density) and the state of the photosynthetic apparatus by determining the contents of chlorophylls a and b and carotenoids over 14–28 days of exposure. It was established that the response of Desmodesmus armatus is clearly concentration-dependent. Low concentrations (0.01–0.06 mg/L) induced a short-term hormetic effect, characterized by stimulation of growth and the pigment system. Medium concentrations (0.1–1 mg/L) caused latent toxicity, manifested by gradual growth inhibition and a decrease in chlorophyll content. High concentrations (2.5–10 mg/L) resulted in persistent suppression of photosynthetic activity, degradation of the pigment system, and the absence of adaptive responses.

The obtained results confirm the suitability of Desmodesmus armatus as a bioindicator for assessing the toxicity of imidazolinone herbicides and emphasize the importance of considering the prolonged effects of pesticides when evaluating their ecological safety.

Keywords: imidazolinone herbicides, imazamox, Desmodesmus armatus, microalgae, dose-dependent effect, photosynthetic pigments, freshwater ecosystems

Introduction. The extensive use of herbicides in modern agriculture is accompanied by the continuous input of their residues into freshwater ecosystems. Water resources, which are critically important as sources of drinking water, are increasingly exposed to organic pollutants of anthropogenic origin, particularly pesticides (Husk et al., 2019; Hasenbein et al., 2017). Pesticides may contaminate aquatic environments through surface runoff, wastewater discharge, and return flows from agricultural and irrigated lands. They can enter water bodies either directly, when applied for the control of aquatic weeds, or indirectly via transport from treated agricultural areas. Surface runoff from agricultural lands represents one of the main pathways of pesticide entry into freshwater systems (Gonçalves-Filho et al., 2020).

The environmental fate of herbicides is determined by a complex set of processes, including retention (adsorption, absorption, sedimentation), transformation (decomposition and degradation), and transport (leaching, volatilization, surface runoff, and dispersion), as well as their interactions within environmental compartments (Herrero-Hernández et al., 2017). Due to their high solubility in water, herbicides can readily migrate into surface and groundwater systems, including rivers, lakes, and soil horizons, posing a potential threat to aquatic organisms (Vonk and Kraak, 2020). These compounds may exert toxic effects on autotrophic aquatic organisms by disrupting photosynthetic processes, reducing oxygen production, and triggering cascading alterations in the trophic structure of aquatic ecosystems (Onyango et al., 2024).

In the context of military activities, this problem becomes even more severe, as the uncontrolled release of herbicides resulting from the destruction of storage facilities, infrastructure damage, or fires may cause their rapid and large-scale introduction into the environment. Under such conditions, herbicides effectively act as military-related pollutants, capable of rapid dispersion within aquatic systems and of generating additional risks to the ecological stability and functioning of freshwater ecosystems. Primary producers, particularly microalgae, exhibit the highest sensitivity to herbicide exposure, as they are responsible for the majority of biomass production and oxygen generation in freshwater biocenoses. As the foundation of aquatic trophic pyramids, microalgae play a crucial role in maintaining ecosystem stability; therefore, inhibition of their growth by xenobiotics can lead to systemic disruptions of ecological balance (Grasso et al., 2022). The accumulation of pesticides in the cells of aquatic

organisms, including algae, may result in deficiencies of key metabolites, reduced amino acid metabolism, and inhibition of protein synthesis, thereby indirectly affecting higher trophic levels through the impairment of food web structure (Mugudamani et al., 2023).

Among herbicides, compounds of the imidazolinone group are of particular importance, with imazamox being a key active ingredient that inhibits the enzyme acetolactate synthase (ALS, EC 4.1.3.18). This enzyme catalyzes the first step in the biosynthesis of branched-chain amino acids - valine, leucine, and isoleucine - in plants. Inhibition of ALS results in a deficiency of these essential amino acids, leading to suppressed protein synthesis and growth arrest in susceptible species (Tan et al., 2005). Imazamox is absorbed through both leaves and roots, translocated to meristematic tissues, and disrupts the functioning of growth zones. Despite its targeted application for weed control, imidazolinone herbicides may enter aquatic environments via surface runoff, thereby causing unintended adverse effects on aquatic biota (Rojano-Delgado et al., 2015).

Recent studies have demonstrated that imazamox-based herbicides, in addition to their effects on higher plants, can inhibit algal growth and alter metabolic activity, inducing toxic effects at the cellular level. These effects are primarily associated with disruptions of photosynthetic processes and metabolic pathways in algal cells upon exposure to the herbicide. In light of these findings, further research in this area is essential for a comprehensive assessment of the ecological safety of imidazolinone herbicides (Huang et al., 2024).

Therefore, the aim of this study was to determine the dose-dependent effects of imidazolinone derivatives on a monoculture of Desmodesmus armatus.

Materials and methods. The material for this study was an algologically pure culture of the green microalga *D. armatus*, maintained in the collection of the Department of Biochemistry and Biotechnology at CNU.

Fig. 1. Microphotograph of Desmodesmus armatus (Chodat) E.H. Hegewald (Culture Collection of Algae)

This typical representative of freshwater phytoplankton is highly sensitive to pollutants and is widely used as a model test organism in toxicological studies of aquatic ecosystems.

In this study, the systemic post-emergence herbicide "Eurolighting" was used, containing 33 g/L of imazamox and 15 g/L of imazapyr. The concentration of imazamox was considered the key indicator of biological activity. Imazamox was applied at the concentrations indicated in the slide, allowing coverage of a range of low to moderate contamination levels characteristic of water bodies exposed to agricultural runoff.

The growth dynamics of *Desmodesmus armatus* cultures were monitored by counting the number of cells per 1 mL of culture suspension using a Fuchs-Rosenthal counting chamber and a Micromed XS-3300 trinocular microscope. The initial inoculum cell density was 1.5×10^6 cells/mL.

Exposure was carried out for 14 days, with cell counts recorded every 3 days. To assess long-term effects, cultivation was extended up to 28 days, with measurements taken on days 14, 21, and 28.

On days 14, 21, and 28, the contents of chlorophyll a, chlorophyll b, and carotenoids were determined. To evaluate pigment content, the biomass pellet was extracted with absolute acetone at a 2:1 ratio. The extracts were aliquoted into tubes and incubated in the dark for 72 hours.

Pigment concentrations were determined spectrophotometrically: carotenoids at 450 nm, chlorophyll a and b at 650 and 665 nm, respectively, and calculated using standard formulas.

All experimental studies were conducted in quadruplicate (n=4). Data are presented as the mean (M) ± standard error of the mean (m) (M ± m).

Statistical analysis was performed using standard methods in Microsoft Excel. Significance of differences was assessed using Student's t-test at a significance level of $p \leq 0.05$.

Results. The study of herbicide effects on green microalgae is of significant importance, as these organisms represent a key component of aquatic ecosystems, contributing to photosynthesis, primary production of organic matter, and the maintenance of oxygen balance in water bodies. Green algae (Chlorophyta), particularly representatives of the genera *Scenedesmus*, *Chlorella*, and *Pseudokirchneriella*, are widely used as test organisms for assessing the ecotoxicity of pesticides (Ceschin et al., 2021). Herbicides can affect algae through various mechanisms, including disruption of photosynthesis, enzyme inhibition, or induction of oxidative stress. Most commonly, they inhibit photosystem II, resulting in reduced photosynthetic efficiency, slower cell growth, and increased

production of reactive oxygen species (Fassiano et al., 2022).

Herbicide toxicity is typically assessed based on the growth activity of microalgae, since even slight inhibition can disrupt trophic interactions in freshwater ecosystems. In our study, control samples, which were not exposed to the herbicide, demonstrated stable increases in cell density of *Desmodesmus armatus*: from 1.57 × 10⁶ cells/mL on day 0 to 3.85 × 10⁶ cells/mL on day 14 of the experiment. This indicates optimal cultivation conditions and the absence of inhibitory factors, ensuring high culture viability.

In the experimental samples treated with the herbicide, a clear concentration-dependent response of the algae was observed. At the lowest concentration (0.06 mg/L), accelerated growth was already noted on day 3, with cell density reaching 2.85 × 10⁶ cells/mL, and peaking on day 6 at 3.28 × 10⁶ cells/mL (Fig. 2). This stimulatory effect may be explained by the low herbicide concentration activating internal adaptive or protective mechanisms in the cells, thereby promoting enhanced growth during the initial stage.

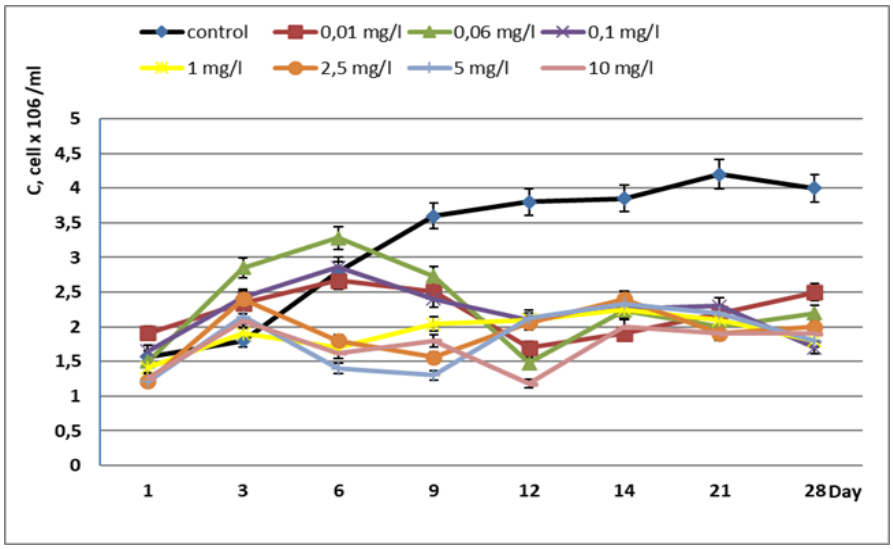

Fig. 2. Dynamics of Desmodesmus armatus monoculture cell numbers at different imazamox concentrations

Concentrations in the range of 0.1–1 mg/L did not induce noticeable growth stimulation; the population remained relatively stable for 6–9 days, after which a gradual decline in cell density or maintenance at a level below the control was observed. This pattern indicates a latent toxic effect, likely associated with the inhibition of the acetolactate synthase (ALS) enzyme, which impedes the synthesis of essential amino acids (valine, leucine, isoleucine) required for normal cell growth.

At concentrations of 2.5–10 mg/L, the maximal toxic effect was observed, manifested as an absence of significant population increase or only a slight rise in cell numbers by day 3. From day 6, a sharp decrease in cell density was noted, indicating disruption of photosynthetic and metabolic processes. In samples with low concentrations (0.01–0.1 mg/L), partial growth recovery was observed on days 12–14, likely due to adaptive mechanisms or herbicide degradation in the medium. In contrast, high doses (5–10 mg/L) maintained an inhibitory effect until the end of the experiment, suggesting the absence of an adaptive cellular response.

The results of this study confirm that *Desmodesmus armatus* is a suitable model organism for ecotoxicological assessments due to its high growth rate and ability to adapt to different cultivation conditions. The impact of imazamox on algae was clearly concentration-dependent: low doses (0.01–0.1 mg/L) promoted partial growth recovery, likely through the activation of internal adaptive or protective mechanisms; intermediate concentrations (0.1–1 mg/L) caused latent toxicity, manifested as a gradual decline in cell density relative to the control; and the highest concentrations (2.5–10 mg/L) induced maximal toxic effects, characterized by pronounced inhibition of photosynthetic and metabolic processes and the absence of adaptive responses throughout the experiment. Nevertheless, even the highest herbicide concentrations did not result in culture death, indicating the need for further studies on the prolonged effects of the pollutant on the model organism.

These observations confirm that *Desmodesmus armatus* is a sensitive bioindicator for evaluating the toxicity of imidazolinone-based herbicides and highlight its importance for assessing pesticide safety in freshwater ecosystems. Prolonged exposure to imidazolinone-derived herbicides elicited a complex, multiphase response in *D. armatus*,

dependent on both toxicant concentration and exposure duration. In control cultures, where the herbicide was absent, cell numbers and optical density exhibited a stable positive trend: the initial density of 1.57 × 10⁶ cells/mL gradually increased to 3.8–4.0 × 10⁶ cells/mL by days 21–28, while optical density slightly decreased from 0.32 a.u. on day 14 to 0.25 a.u. on day 28, indicating the attainment of the stationary growth phase and optimal cultivation conditions (Fig. 3). These values are consistent with literature data for species of the genus *Scenedesmus*, where maximal cell density and stable optical density indicate the absence of stress (Fassiano et al., 2022).

Variants with low herbicide concentrations (0.01–0.06 mg/L) demonstrated slightly stimulated cell growth and corresponding increases in optical density during the early exposure stages, consistent with the hormesis phenomenon described for *Chlorella vulgaris* and *Scenedesmus obliquus* under toxic stress (Li et al., 2020). The hormetic effect appears only during the early stages of exposure and disappears with prolonged toxicant action—a pattern also observed in our experiment.

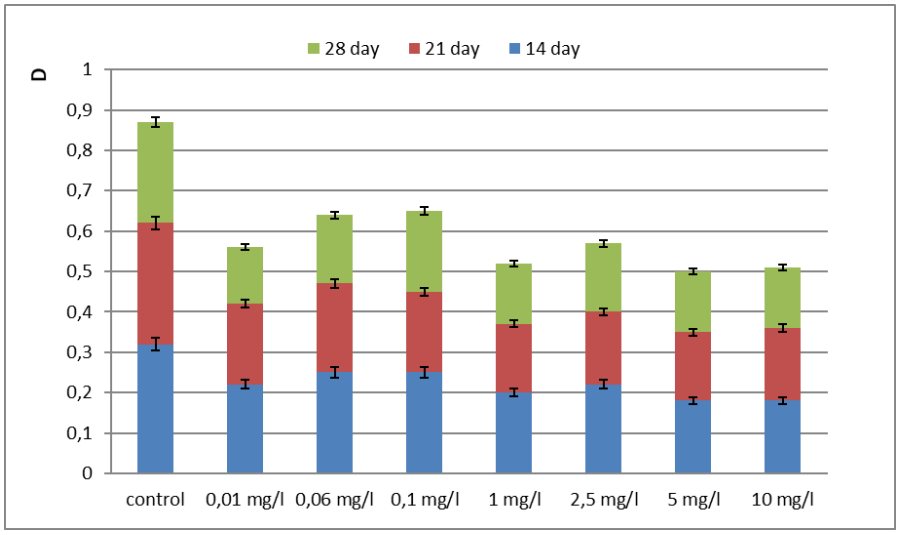

Fig. 3. Optical density of Desmodesmus armatus monocultures at different imazamox concentrations

After day 14, the culture exhibited a temporary decline in growth rate and optical density; however, by days 21–28, cell density partially recovered to 2.1–2.5 × 10⁶ cells/mL, with optical density maintained at 0.17–0.20 a.u., indicating successful adaptation to sub-inhibitory concentrations. This recovery likely reflects partial degradation of imazamox, enhanced antioxidant defenses, and reorganization of the pigment apparatus, allowing cells to sustain photosynthetic activity under mild toxic stress.

Intermediate concentrations (0.1–1 mg/L) induced latent toxicity: cell density and optical density remained relatively stable during the first 6–9 days, but by days 12–14, a sustained growth inhibition was observed, with cell density decreasing to 1.6–1.9 × 10⁶ cells/mL and optical density to 0.16–0.20 a.u. By days 21–28, the culture remained at a consistently low level without full recovery. This effect corresponds to imazamox's mechanism of blocking the synthesis of essential amino acids (valine, leucine, isoleucine), gradually limiting resources for growth, while cells maintain viability despite reduced photosynthetic activity, consistent with findings in *Raphidocelis subcapitata* (Gómez-Martínez et al., 2023).

High concentrations (2.5–10 mg/L) caused the strongest toxic effects. Cell density remained nearly unchanged during the first 3–6 days and declined to 1.3–1.5 × 10⁶ cells/mL by days 12–14, while optical density dropped to 0.15–0.16 a.u., indicating severe impairment of the photosynthetic apparatus and growth inhibition. By days 21–28, no recovery was observed, with cell density stable at 1.4–1.7 × 10⁶ cells/mL and optical density unchanged, confirming the absence of adaptive compensation. These results align with previous observations in *Scenedesmus quadricauda*, where high ALS-inhibitor concentrations caused prolonged inhibition of growth and photosynthetic activity (Dosnon-Olette et al., 2010).

Overall, analysis of cell density and optical density demonstrates that prolonged exposure to imidazolinone herbicides exerts concentration-dependent effects on *Desmodesmus armatus*: low concentrations induce transient hormetic stimulation followed by growth stabilization, intermediate concentrations cause latent toxicity with gradual growth suppression, and high concentrations result in sustained inhibition. These findings underscore the necessity of long-term studies for accurate ecological risk assessment, as short-term

experiments fail to capture the full spectrum of adaptive and toxic responses.

The strong correlation between growth and optical density highlights the link between growth activity and photosynthetic function, emphasizing the importance of further investigating pigment composition and photosynthetic performance. Prolonged exposure to imidazolinone herbicides not only affects growth, but also induces significant restructuring of the photosynthetic apparatus - a sensitive indicator of toxic stress. Since photosynthesis provides essential energy and structural components for cells, analysis of chlorophyll a, chlorophyll b, and carotenoid content offers valuable insights into the mechanisms underlying adaptive and stress responses to long-term imazamox exposure, complementing the growth observations described above.

The obtained results demonstrated a clear concentration-dependent response of *Desmodesmus armatus*, which closely correlated with changes in cell density and optical density of the culture. Analysis of the pigment profile over 14, 21, and 28 days of exposure revealed three main response scenarios: hormesis at low concentrations, latent or moderate toxicity at intermediate concentrations, and sustained suppression of the photosynthetic apparatus at high herbicide concentrations.

Chlorophyll a serves as a sensitive indicator of the functional state of the photosynthetic apparatus. As the primary pigment of the reaction centers of photosystems, chlorophyll a provides the most informative measure of overall photosynthetic performance.

In our study, low concentrations (0.01–0.06 mg/L) resulted in a moderate increase in chlorophyll a content compared to the control by day 14 (Fig. 4). This transient stimulatory effect, or hormesis, is typical for low-dose toxicant exposure and is consistent with trends observed in growth parameters. By day 21, stimulation was most pronounced, while by day 28, a partial decline in pigment concentration was observed, although levels remained higher than those in the intermediate and high imazamox treatments. These results indicate the activation of adaptive mechanisms in the cells, including enhanced antioxidant defenses, reorganization of photosynthetic complexes, and increased light absorption efficiency.

At intermediate concentrations (0.1–1 mg/L), the dynamics of chlorophyll a reflected latent toxicity. During the early stages of the experiment (day 14), pigment content decreased slightly, whereas by days 21 and 28, a stable reduction was observed. Pigment suppression was not critical, indicating partial retention of photosystem function, although their activity was limited.

At high concentrations (2.5–10 mg/L), a pronounced toxic effect was observed at all stages of exposure: chlorophyll a content decreased by almost 50% relative to the control already by day 14, and the further decline observed on days 21–28 indicated degradation of the photosynthetic membranes and inhibition of pigment biosynthesis. The absence of recovery throughout the experimental period confirms the irreversible nature of the damage.

Comparison of chlorophyll a and b revealed that the latter was even more sensitive to imazamox. Changes in chlorophyll b generally followed the dynamics of chlorophyll a but exhibited greater amplitude, reflecting deeper structural rearrangements in the pigment apparatus. At 0.01–0.06 mg/L, chlorophyll b showed a noticeable decline on day 14, but levels nearly recovered or even exceeded the day-21 control values by days 21 and 28 (particularly at 0.06 mg/L). This likely reflects expansion of light-harvesting antennae and enhanced light absorption efficiency—a typical adaptive response during early stages of toxicant exposure. Thus, low doses may stimulate the pigment apparatus during prolonged adaptation.

At 0.1 mg/L, a decline on day 14 followed by partial recovery at later time points was also observed, although adaptation was less complete than at lower concentrations. Intermediate concentrations caused a more pronounced suppression of chlorophyll b compared to chlorophyll a, likely due to the high sensitivity of light-harvesting complexes to protein imbalances.

At 1–10 mg/L, chlorophyll b content was reduced at all exposure times. A particularly strong decrease occurred at 5 mg/L, while 10 mg/L also caused marked suppression, though partial recovery was observed by day 28. High herbicide concentrations led to a rapid decline in chlorophyll b already during early exposure, indicating destabilization of light-harvesting complexes and impairment of energy supply to the photosystems.

Overall, the dynamics of chlorophyll b confirm that light-harvesting complexes are sensitive indicators of prolonged stress, with the degree of change directly depending on imazamox concentration. Low concentrations induce adaptive reorganization, intermediate concentrations disrupt the stability of the light-harvesting complexes, and high concentrations lead to structural degradation of the antenna apparatus.

Thus, comparison of chlorophylls a and b demonstrates that chlorophyll b responds more rapidly and strongly, making it a reliable early marker of photosynthetic system disruption under imidazolinone herbicide exposure.

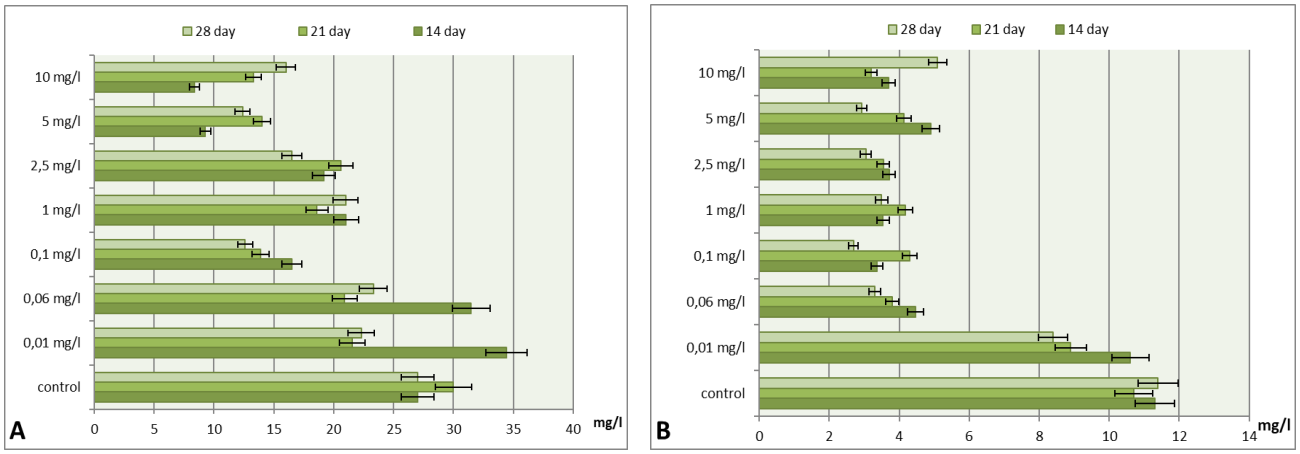

Fig. 4. Content of chlorophyll a (A) and chlorophyll b (B) in Desmodesmus armatus cells exposed to imazamox

Carotenoids reflect the antioxidant status and photoprotective function of the cells. They perform two key roles: contributing to the formation of photosynthetic complexes and protecting chloroplasts from excess light and reactive oxygen species. Therefore, their dynamics are an important indicator for assessing toxic effects.

In the control, carotenoid levels remained stable throughout the exposure period, which is typical for *Scenedesmus* species under non-stress conditions (Fig. 5). This stability confirms consistent cultivation conditions and the absence of endogenous oxidative stress.

Under low concentrations (0.01–0.06 mg/L), a moderate increase in carotenoid content was observed, coinciding with the initial hormetic effect seen for chlorophylls. This transient stimulation of the pigment apparatus likely reflects activation of the carotenoid components of the light-harvesting complexes to compensate for stress signals.

More pronounced changes occurred at intermediate concentrations (0.1–1 mg/L). During the first few days, carotenoid levels remained close to the control, but by days 12–14, a substantial increase was observed. Such a response is typical for photosynthetic organisms under prolonged stress: carotenoids scavenge reactive oxygen species, stabilize thylakoid membranes, and prevent photooxidative damage to chlorophylls. Literature reports indicate that during extended exposure to ALS inhibitors, carotenoid content may increase as part of a compensatory response, even when chlorophyll levels are already depressed (Lopes et al., 2010).

The most pronounced changes were observed at high concentrations (2.5–10 mg/L). During the early stages of exposure, the sharp decline in chlorophyll content was accompanied by a compensatory increase in carotenoids, indicating activation of the maximal photoprotective response.

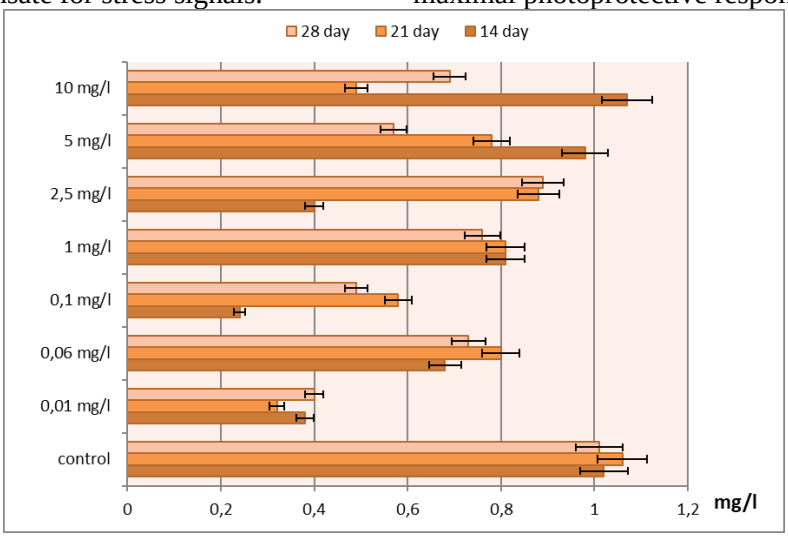

Fig. 5. Carotenoid content in Desmodesmus armatus cells under imazamox exposure

However, at later stages (days 21–28), carotenoid levels stabilized or even slightly declined, likely reflecting depletion of cellular resources. Since imazamox inhibits the synthesis of essential amino acids, cells are limited in their capacity to sustain high rates of carotenoid biosynthesis over prolonged periods.

When considered alongside chlorophyll dynamics, this carotenoid response suggests that the photosynthetic apparatus of *Desmodesmus armatus* can partially adapt to sub-inhibitory doses of imazamox, whereas high concentrations cause systemic suppression of cellular function.

Thus, analysis of the main photosynthetic pigments indicates that the response of Desmodesmus armatus to prolonged imazamox exposure is clearly concentration-dependent. Low concentrations induced a transient stimulation of the pigment apparatus, intermediate concentrations caused suppression with elements of adaptation, and high concentrations resulted in sustained impairment of photosynthetic function.

Importantly, changes in pigment composition closely correlate with previously described alterations in cell density and optical density. This demonstrates a strong functional link between culture growth and the state of the photosynthetic apparatus, which determines the energetic capacity and adaptive potential of the cells.

Despite the reduction in cell numbers, indicating decreased growth activity, optical density and chlorophyll content suggest a gradual adaptation of the culture to the prolonged presence of the herbicide in the medium..

Conclusions. Imazamox significantly inhibits the growth of *Desmodesmus armatus*, as evidenced by reductions in cell density and slowed biomass accumulation. The strongest inhibitory effect was observed at an imazamox concentration of 2.0 mg/L.

As a result of imazamox exposure, the pigment system of *Desmodesmus armatus* underwent both degradation and subsequent compensatory changes: chlorophylls a and b decreased, with chlorophyll b showing greater sensitivity, indicating disruption of light-harvesting complexes, while carotenoids initially declined but later increased above control levels, reflecting activation of photoprotective and antioxidant mechanisms.

***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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ДОЗОЗАЛЕЖНИЙ ВПЛИВ ІМІДАЗОЛІНОНОВИХ ГЕРБІЦИДІВ НА МОНОКУЛЬТУРУ DESMODESMUS ARMATUS

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У роботі досліджено дозозалежний вплив гербіцидів групи імідазолінонів, зокрема імазамоксу, на монокультуру зеленої мікроводорості Desmodesmus armatus, яка є чутливим тест-організмом для екотоксикологічних оцінок. Актуальність дослідження зумовлена зростанням ризику потрапляння гербіцидів у прісні водойми внаслідок сільськогосподарського стоку та антропогенних порушень, що може негативно впливати на первинних продуцентів водних екосистем.

Оцінку токсичної дії імазамоксу проводили за показниками росту культури (чисельність клітин, оптична густина) та станом фотосинтетичного апарату, визначаючи вміст хлорофілів a, b і каротиноїдів упродовж 14–28 діб експозиції. Встановлено, що реакція Desmodesmus armatus має чіткий концентраційно залежний характер. Низькі концентрації (0,01–0,06 мг/л) спричиняли короткочасний горметичний ефект зі стимуляцією росту та пігментного апарату. Середні концентрації (0,1–1 мг/л) викликали приховану токсичність, що проявлялася поступовим пригніченням росту та зниженням вмісту хлорофілів. Високі концентрації (2,5–10 мг/л) зумовлювали стійке пригнічення фотосинтетичної активності, деградацію пігментної системи та відсутність адаптаційних реакцій.

Отримані результати підтверджують придатність Desmodesmus armatus як біоіндикатора токсичності імідазолінонових гербіцидів та підкреслюють необхідність урахування пролонгованої дії пестицидів при оцінці їх екологічної безпеки.

Ключові слова: імідазолінонові гербіциди, імазамокс, Desmodesmus armatus, мікроводорості, дозозалежний ефект, фотосинтетичні пігменти, прісноводні екосистеми

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