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RESEARCH ARTICLE

Impact of exogenous zeatin on the growth, pigment complex and capacity of sporophytes of *Salvinia natans* (*Salviniaceae*) for biological extraction of zinc from the water

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Abstract. The response of plants to heavy metals involves phytohormones, particularly cytokinins, with zeatin being one of the active forms. Exogenous phytohormones are believed to induce plant resistance to heavy metals and enhance phytoextraction. We investigated the impact of exogenous zeatin on the morpho-physiological characteristics of young and mature sporophytes of *Salvinia natans* and their ability to extract zinc ions from the aquatic environment. It has been shown that zeatin mitigated the adverse effect of zinc sulfate on dry weight accumulation, but did not alleviate its negative impact on fresh weight accumulation in both young and mature sporophytes. During intensive growth and sori formation and spore maturation stages under zinc loading, exogenous zeatin (at 10^{-6} M concentration) led to 40% and 50% increase in the dry weight of young and mature sporophytes, respectively. In the presence of zinc sulfate, the total chlorophyll content decreased by 23% in the fronds of young sporophytes and by 44% in the fronds of mature sporophytes, while total carotenoids decreased by 21% in both cases. Zeatin addition alleviated the negative impact of the metal on the pigment complex in young sporophyte fronds but exacerbated it in mature sporophyte fronds. The pigment complex of the sporophyte was more susceptible to metal action during sori formation and spore maturation, resulting in frond browning and pronounced chlorosis. However, chlorosis was less intense and localized upon zeatin addition. The capacity of *S. natans* sporophytes to extract zinc ions from the aqueous medium was demonstrated, with zinc concentration decreasing by over tenfold from 10 mg·L^{-1} to 0.6 mg·L^{-1}after 14 days of cultivation. The application of zeatin did not affect the efficiency of zinc ions extraction from water.

Keywords: biological extraction, growth indices, pigments, *Salvinia natans*, zeatin, zinc

Introduction

Water fern *Salvinia natans* (L.) All. belongs to the native species of Central and South-Eastern Europe. It exhibits rapid growth rates, adapts to various environmental conditions, actively spreads, and possesses the ability to remove both organic and inorganic pollutants from the aquatic environment

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(Jiang et al., 2009; Kosakivska et al., 2022; Polechońska et al., 2019; Török et al., 2023). *Salvinia natans* is recognized as a heavy metal (HM) hyperaccumulator, as confirmed by energy dispersive X-ray fluorescence analysis (EDXRF). When fresh fern biomass was introduced into wastewater samples over time, a gradual decrease in zinc (Zn), copper (Cu), nickel (Ni) and chromium (Cr) content by 84.8%, 73.8%, 56.8% and 41.4% respectively, was observed (Dhir, Srivastava, 2011). *Salvinia natans* demonstrates high tolerance to HM exposure. In the presence of cadmium (Cd), cobalt (Co), Cr, iron (Fe), lead (Pb) and Ni at a concentration of 30 mg L^{-1}, various plant indices, such as the biomass, photosynthesis efficiency, quantum yield, photochemical quenching, electron transport rate, and elemental composition (% C, H and N) remained unaffected. However, exposure to higher concentrations (50 mg L^{-1}) of Cu and Zn resulted in significant reductions of these indices (Dhir, Srivastava, 2013). Polish scientists Hołtra and Zamorska-Wojdyła (2014) demonstrated capacity of *S. natans* to extract copper (II) from a culture medium containing Cd. After phytoremediation of wastewater from electroplating processes *S. natans* biomass accumulated HM. When metal-loaded fern biomass was applied to soil with 10-day-old *Triticum aestivum* plants, significant reduction in chlorophyll content, glucose, and plant yield was observed. Nonetheless, the percentage content of carbon (C) in wheat plants remained unchanged, nitrogen (N) slightly increased, and hydrogen (H) decreased (Dhir, Srivastava, 2012). The physiological impact of zinc oxide nanoparticles on the *S. natans* sporophyte was demonstrated. After 7 days the activity of superoxide dismutase and catalase increased, while the content of photosynthetic pigments decreased, and the aggregation of nanoparticles was observed in submerged fern fronds (Hu et al., 2014). *Salvinia natans* exhibits significant phytoremediation capabilities in cleaning water bodies from nitrogen compounds. The content of total nitrogen, ammonium and nitrite decreased; moreover, a reduction in biochemical oxygen consumption was observed (Laabassi, Boudehane, 2019). Due to its biocompatibility with the herbicide 2,4-dichlorophenoxyacetic acid (2,4-D), *S. natans* enhances auxin metabolism, facilitating the bioaccumulation of sodium (Na) and arsenic (As) (Dolui et al., 2021, 2022). Additionally, *S. natans* actively binds complex aluminum compounds (Mandal et al., 2013) and nitrogen-containing dyes dissolved in water (Ohtani et al., 2020). The fern is characterized by a high efficiency antioxidant system and osmotic resistance of cells, minimizing intracellular damage from herbicide exposure (Pandey et al., 2016). The capacity of *Salvinia* sporophytes to extract up to 90% of zinc from polluted water has been reported in studies (Dhir et al., 2008; Dhir, Srivastava, 2011).

Understanding the mechanisms underlying plant growth and development depends on studying the hormonal regulation system. Presently, the hormonal system of hydrophyte ferns in the *Salviniaceae* family remains relatively understudied (Kosakivska et al., 2016, 2023). An effective approach to regulating growth and development involves treating plants with exogenous phytohormones (Kosakivska et al., 2022). Exogenous cytokinins, such as *trans*-zeatin and kinetin, have been shown to mitigate lead toxicity in the green alga *Acutodesmus obliquus* by modulating endogenous hormone levels and promoting the accumulation of organic acids involved in metal detoxification (Piotrowska-Niczyporuk et al., 2020). In hydroponic systems, the addition of kinetin during the cultivation of the fern *Pteris cretica* var. *nervosa* has been found to enhance arsenic (As) uptake and increase the activity of antioxidant enzymes, aiding the plant in overcoming pollutant-induced stress (Wang et al., 2021). The hyperaccumulation of As in this fern has been correlated with the content of *trans*-zeatin (Zhang et al., 2017). Various forms of cytokinins, including *trans*- and *cis*-zeatin, zeatin riboside, zeatin-*O*-glucoside, isopentenyladenosine and isopentenyladenine, have been identified in the vegetative and generative organs of *Salvinia natans* at different developmental stages. The highest levels of endogenous cytokinins were observed in the floating fronds during the phase of intensive sporophyte growth. Transitioning to reproductive development coincided with a decrease in *cis*-zeatin content in both floating and submerged fronds and the accumulation of zeatin-*O*-glucoside, a reserve form of cytokinins (Phytohormonal system…, 2019).

Indicators of the photosynthetic pigments content (chlorophyll *a* and *b*, carotenoids) and their ratios in plants serve as reliable markers of HM toxicity (Gomes et al., 2014). HMs suppress the activity of photosynthetic enzymes involved in chlorophyll biosynthesis, displace magnesium ions within the pigment molecule, disrupt electron transport in the light phase, and negatively impact enzyme activity during the dark phase of photosynthesis (Rai et al., 2016). Elevated HM concentrations hinder nutrient absorption, consequently affecting photosynthetic activity adversely (Siyar et al., 2020). In *S. natans* sporophytes, high levels of Cd, Cr, Cu, Ni, Zn, and the metalloid As have been observed to diminish the efficiency of the photosynthetic apparatus (Cui et al., 2022), Furthermore, the content of photosynthetic pigments decreases with increasing concentrations of Cd, Pb, Ni (Leblebici et al., 2018) and mercury (Hg) (Sitarska et al., 2023) in the nutrient medium, depending on the duration of exposure to the toxin.

In our previous research, we examined the influence of exogenous phytohormones and zinc sulfate on the morphophysiological characteristics of *S. natans*. Specifically, we demonstrated that simultaneous addition of phytohormones with zinc sulfate at the highest concentration partially adverse effects, reduced biomass degradation, and sustained sporophyte viability. Additionally, we identified qualitative changes in the fern phenotype indicative of the biotoxicity of excessive zinc concentrations, thus suggesting the potential use of *Salvinia natans* as a bioindicator of water pollution (Kosakivska et al., 2024). Given the prevalence of zinc pollution, particularly in water bodies adjacent to industrial sits, our study aimed to investigate the impact of exogenous zeatin on the growth, pigment complex, and the *S. natans* sporophyte ability to biologically extract this heavy metal.

Materials and Methods

Sporophytes of the heterosporous water fern *Salvinia natans* (L.) All. were studied. The plants were collected in June and August 2023 on the Prorva River, near the village of Hnidyn, Boryspil District, Kyiv Region. The average monthly air temperature in June and August during the sampling period was 26 and 28 °C, water — 23 and 25 °C, respectively. The analysis of water samples for the concentration of zinc ions was carried out using a Macherey-Nagel PF-12 Plus portable photometer with a software-integrated method for determining the concentration of this metal. The number of the working method in the device interface is 5981. Before measuring the indices, reagents from the Macherey-Nagel test set No. 5-98 (REF 931098) were added to the water samples in a volume of 5 ml to determine the concentration of zinc in accordance with the sequence indicated in the instructions for quantitative analysis Zn^{2+}. It was established that the content of zinc ions in water samples taken from the reservoir was below the limit of sensitivity of the method, which is 0.1 mg·L^{-1}.

Experiment design

Study of the zeatin and zinc sulfate effect on the growth of sporophytes of *Salvinia natans*

Sporophytes in the phase of intensive growth, which had 3–5 pairs of floating and submerged fronds attached to a horizontal stem, and mature sporophytes in the phase of sori formation and spore maturation, which had 14–15 pairs of fronds on the central part and two modules with 3–5 pairs of fronds, were studied. Young sporophytes, four to five individuals each, and mature sporophytes, one per plant, were placed in glass containers with a volume of 250 ml, filled with purified tap water with the addition of: (1) zeatin at a concentration of 10^{-6} M; (2) zinc sulfate (10 mg L^{-1} zinc); (3) zeatin and zinc sulfate. In our dose-response experiment, we tested various concentrations of zeatin and zinc sulfate. When comparing the biomass of plants grown in zinc solutions with and without addition of the phytohormone it has been found that the application of zeatin at 10^{-6} M reduced growth inhibition by 32.9% (Kosakivska et al., 2024). The effective concentration of zinc in water (10 mg·L^{-1}) was determined experimentally. At lower zinc concentrations, the adverse effects on the morphological state of *S. natans* sporophytes were minimal. However, at higher zinc concentrations, the damage progressed too rapidly. This zinc concentration enables *S. natans* sporophytes to survive for extended periods, albeit with noticeable morphological deterioration. Ferns were grown for seven days in a vegetation chamber (VOTSCH GmbH, Germany) at a temperature of +22 °C, illumination of 190 μmol·m^{-2} s^{-1}, a photoperiod of 16/8 h (day/night) and a relative humidity of 65 ± 5%. Biometric indicators were recorded at the beginning of the experiment and on the 7th day of cultivation. Plants grown in water without additives served as controls.

Study of the content of photosynthetic pigments

Plant material (200 mg) was collected on the seventh day of cultivation. Photosynthetic pigments were extracted using 100% acetone and quantified following the methods of Holm (1954) and von

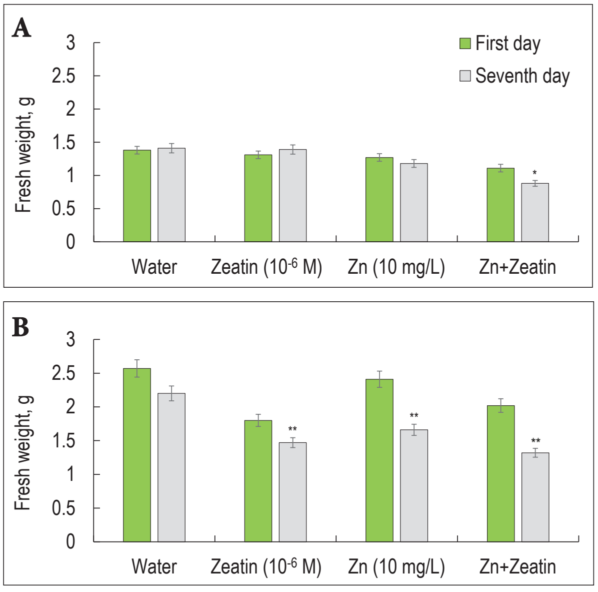

Fig. 1. Effect of zeatin and zinc sulfate on fresh weight accumulation of sporophytes of *Salvinia natans*. A: at the stage of intensive growth; B: at the stage of sori formation and spore maturation. n = 15; x ± standard error (SE). An asterisk (*) indicates significant differences between the indicators of the control and experimental groups (P ≤ 0.05)

Wettstein (1957). Measurements were taken with a Jenway UV-6850 spectrophotometer (Great Britain) at wavelengths of 662, 664, and 440.5 nm, with acetone as the control.

Determination of the ability of sporophytes of *S. natans* to extract zinc from the aqueous medium

Young sporophytes of *S. natans* (4 g) in the phase of intensive growth were placed in a container with a volume of 1 liter. Water samples were taken on the fourth, sixth, eighth, tenth, twelfth and fourteenth days of the experiment. Water samples with a volume of 5 mL were taken from the vegetation containers with a medical syringe, avoiding the ingress of solid residues. The selected samples were filtered through a membrane syringe filter made of regenerated cellulose with a pore size of 0.2 μm. Analysis of water samples for the concentration of zinc ions was carried out using a Macherey-Nagel PF-12 Plus portable photometer.

Statistical analysis

Experiments were performed in three biological and three analytical replicates. Results are presented as mean ± standard error. Differences in mean values were analyzed using two-way analysis of variance ANOVA. Tests with $P < 0.05$ were considered statistically significant. The analysis was carried out using the program Statistix v. 10.0 (Analytical Software, Tallahassee, FL, USA). Experimental data were displayed graphically using Microsoft Excel (Redmond, USA).

Results

Effect of exogenous zeatin and zinc sulfate on sporophyte morphometric parameters

Under control conditions, the fresh weight (FW) of young sporophytes remained relatively stable. Cultivation with the addition of zeatin induced a slight increase in FW (by 6.1%). Conversely, after growing ferns in a medium containing zinc sulfate, the FW decreased by 7.1%, and under the combined action of zeatin and zinc sulfate, the FW of the sporophyte diminished by 20.8% (Fig. 1A). After 7 days of vegetation under control conditions, the FW of mature sporophytes reduced by 14.4%. With the addition of zeatin it decreased by 18.3%, with zinc by 31.1%, and in the presence of zinc and zeatin by 34.7% (Fig. 1B).

At the stage of intensive growth and the stage of sori formation and spore maturation, the addition of zeatin to the culture medium induced a 11.8% and 9% increase in the dry weight (DW) of the sporophyte, whereas zinc sulfate caused a 41.2% and 45.5% decrease, respectively. With the addition of zeatin to the culture medium in the presence of zinc sulfate, the DW of the sporophyte raised by 40% and 50%, respectively, but did not reach the control indices and the variant with the addition of the hormone in the absence of zinc sulfate (Fig. 2).

In general, changes in morphometric indices recorded when zeatin and zinc sulfate were added to the culture medium were more pronounced in mature sporophytes. Changes in the phenotype of mature sporophytes were also more striking. On the seventh day of cultivation, signs of chlorosis and necrosis were detected in the floating fronds of mature sporophytes in a medium containing zinc sulfate (10 $mg \cdot L^{-1}$ zinc). The adaxial surface of floating fronds of mature sporophytes acquired a brownish-red color. Adding 10^{-6} M zeatin to the culture medium did not affect the growth of the sporophyte and did not remove the external displays of the negative effect of zinc (Fig. 3). The greatest manifestation of chlorosis was observed in mature sporophytes with the addition of zinc and zinc with zeatin (Fig. 3B). Conversely, in young and mature sporophytes, when zeatin was added, chlorosis became point-like and less intense (Fig. 3A). Overall, sporophytes of *S. natans* were more sensitive to the action of zinc sulfate at the stage of sori formation and spore maturation.

Effect of exogenous zeatin and zinc sulfate on the content of photosynthetic pigments

During the stages of intensive growth and sori formation and spore maturation with the aqueous medium exposed to zinc sulfate, the content of photosynthetic pigments in the floating fronds of *S. natans* sporophytes decreased. In fronds of young sporophytes, the total chlorophyll content decreased by 23%, and total carotenoids by 21%. In fronds of mature sporophytes, the total chlorophyll content reduced by 44%, and total carotenoids by 21%. The level of chlorophylls *a* and *b* in the fronds of young sporophytes was half as low as that of mature sporophytes, while the level of carotenoids remained unchanged.

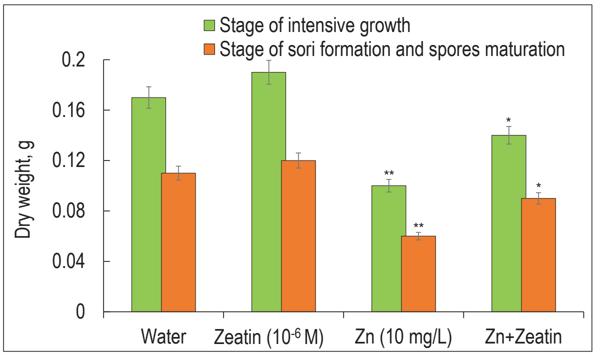

Fig. 2. Effect of zeatin and zinc sulfate on dry weight accumulation of sporophytes of *Salvinia natans*. n = 15; x ± standard error (SE). An asterisk (*) indicates significant differences between the indicators of the control and experimental groups ($P \leq 0.05$)

In the presence of a mixture of zeatin and zinc sulfate, the content of photosynthetic pigments in the floating fronds of the *S. natans* sporophyte decreased. The amount of chlorophylls *a+b* diminished by 1.3 times in the floating fronds of young sporophytes

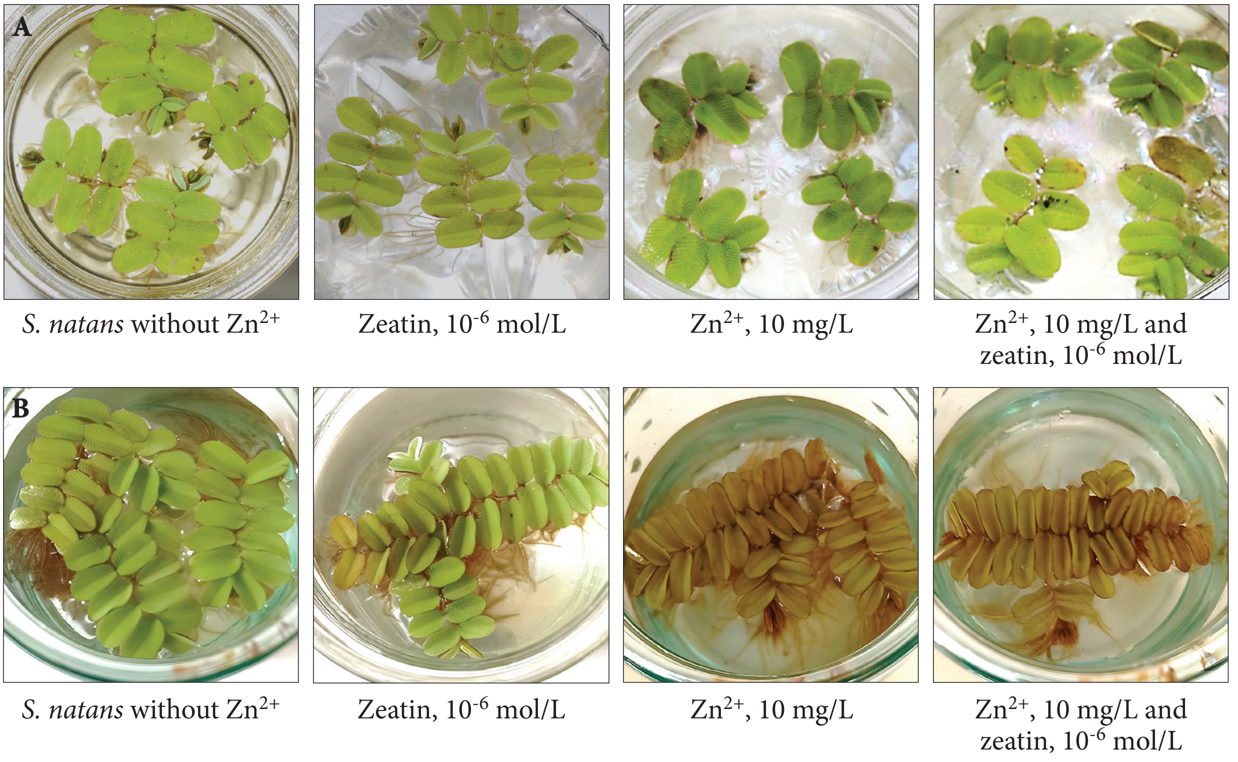

Fig. 3. Sporophytes of *Salvinia natans* during cultivation on media containing zeatin (10^{-6} M), zinc sulfate (the content of pure zinc per liter of water is indicated) and a mixture of zinc and zeatin on the seventh day of the experiment. A: at the stage of intensive growth; B: at the stage of formation of sori and maturation of spores

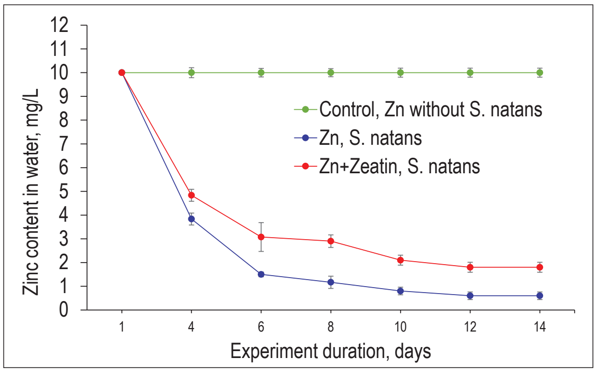

Fig. 4. Dynamics of biological extraction of zinc ions from water by sporophytes of *Salvinia natans*

when $ZnSO_4$ was added, while no significant differences were observed when a mixture of $ZnSO_4$ and zeatin was added compared to the control. In mature fronds of sporophytes, the amount of chlorophylls *a+b* decreased by 1.8 fold when $ZnSO_4$ was added and by 2.1 fold when the mixture of metal and hormone was applied. Under the influence of exogenous zeatin, the *a+b* indices dropped 1.2 times.

The ratio of the amount of chlorophylls *a+b* to carotenoids at the stage of intensive growth in the presence of zinc sulfate was within the control range, while in the variant $ZnSO_4$ + zeatin this indicator increased by 12%. During the stage of sori formation and spore maturation, a decrease in the ratio chlorophylls (*a+b*) to carotenoids was observed in all variants of the experiment: with the addition of $ZnSO_4$ — by 28%, with the combination of $ZnSO_4$ + zeatin — by 39%, in the presence of zeatin — by 19%.

Therefore, in the presence of zinc sulfate in the culture medium, the content of chlorophylls in the floating fronds of the *S. natans* sporophyte decreased, while the content of carotenoids was within the control range. The pigment complex of the mature sporophyte fronds turned out to be more sensitive to the action of the metal. The addition of zeatin mitigated the negative effect of the metal on the pigment complex in fronds of young sporophytes, but increased it in mature sporophytes fronds. A positive effect of zeatin on the pigment complex of mature fronds was revealed.

The effect of exogenous zeatin on the extraction of zinc ions from the aqueous medium by sporophytes of *S. natans*

Young sporophytes of *S. natans* at the stage of intensive growth effectively removed zinc ions from water (Fig. 4). For 12 days of cultivation, the concentration of zinc in water reduced by more than 90%, while with the addition of zeatin it decreased by 82%. At the end of the experiment, the content of zinc ions in the water was less than one milligram per liter, which is below the maximum permissible level of zinc in drinking water and water used for domestic purposes (1 mg·L^{-1}), accepted in Ukraine. The highest rates of extraction of zinc ions by *S. natans* sporophytes were observed between the first and sixth days, after which the rate of absorption slowed down. Starting from the 12th day, the zinc content in the water practically did not change. The addition of zeatin caused inhibition of the process of extracting zinc ions, which was especially noticeable in the time period between the fourth and tenth days of the experiment. The concentration of zinc in water in the presence of the hormone at the end of the experiment was 1.8 mg·L^{-1}. At the same time, in the variant without the addition of zeatin, three times less zinc remained in the water. In the control, the concentration of zinc ions in water remained unchanged throughout the experiment (Fig. 4). In general, the present study demonstrated the phytoremediation capacity of *S. natans* sporophytes for the biological extraction of Zn^{2+} ions from a polluted water environment.

Discussion

Zinc is an essential microelement vital for the normal growth and development of plants. However, elevated concentrations of zinc in soil or water bodies, particularly near lead-zinc production sites, ferrous metallurgy enterprises, and urban areas, can exert toxic effects on plants. Symptoms such as stunted root growth and chlorosis on young leaves can be manifested rapidly under such conditions (Khan, Khan, 2014; Balafrej et al., 2020). At extremely high zinc concentrations, leaves may undergo complete discoloration and eventual death (Fig. 5). Excessive zinc levels within plant cells hinder biomass accumulation by affecting the integrity of photosynthetic membranes and proteins (Zhao et al., 2012; Beyer et al., 2013; Ebbs et al., 2015) and impacting mineral nutrition, particularly the bioavailability of potassium ions (Hafeez et al., 2013). Despite being a divalent metal without redox properties, zinc indirectly induces oxidative stress through various mechanisms. Notably, it induces glutathione deficiency, binds to protein sulfhydryl groups, inhibits antioxidant enzymes, and

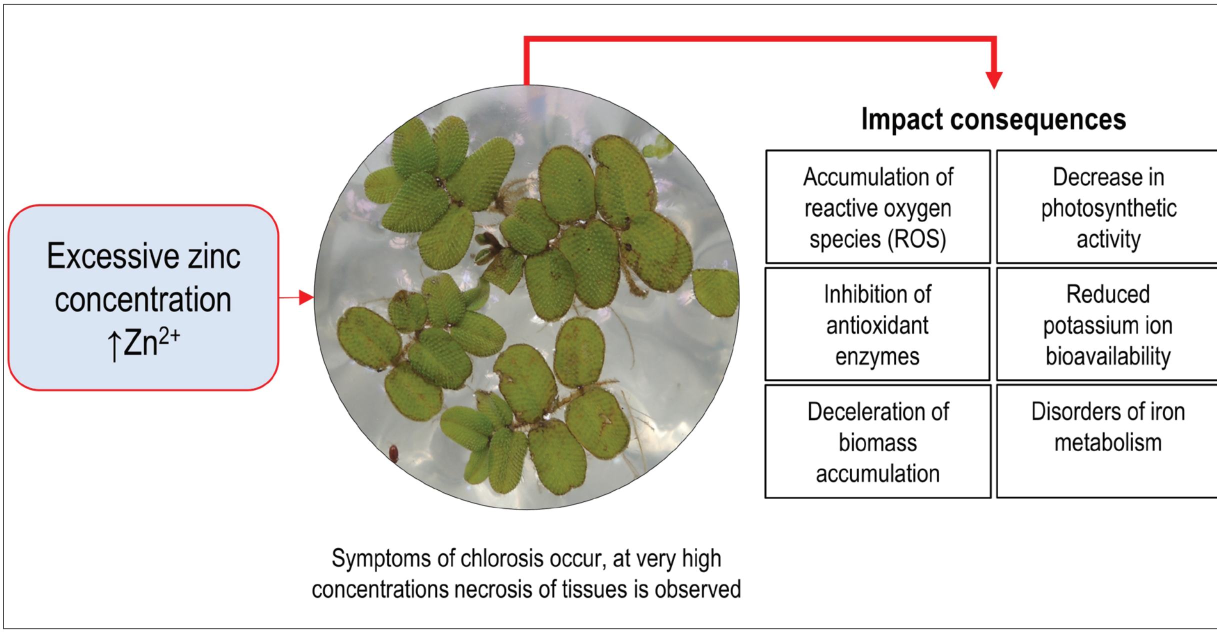

Fig. 5. Effects of excessive zinc concentrations in water and soil on plants

triggers enzymes involved in active oxygen forms production (Bielen et al., 2013). Plants response to zinc excess depends on species sensitivity to HM, water availability, soil pH, and the presence of other chemical elements in the soil or water solution (Noulas et al., 2018). The precise mechanism by which exogenous phytohormones impact phytoextraction remains unclear. However, it is hypothesized that they enhance the process by promoting plant growth and biomass accumulation while inhibiting the reproductive process (Tassi et al., 2008). For instance, treatment with cytokinin significantly enhanced the ability of *Alyssum murale* plants to extract nickel by increasing plant biomass (Cassina et al., 2011).

Exogenous zeatin affected the morphometric indices of *S. natans* sporophytes minimally. Nevertheless, it mitigated the negative effects of zinc, as evidenced by the increase in sporophyte DW when zeatin was added to the culture medium in the presence of zinc sulfate. On the 7th day of cultivation, zeatin (10^{-6} M) induced a 11.8% and 9% increase in the DW of young and mature *S. natans* sporophytes, while the addition of zinc sulfate (10 mg l^{-1} zinc) resulted in 41.2% and 45.5% reduction in a DW. When zeatin was added to the culture medium in the presence of zinc sulfate, the DW of the sporophyte increased by 40% and 50%. However, it did not reach the control levels observed in the variant without zinc sulfate (Fig. 2). These results highlight the negative impact of zinc on ferns and the mitigating effect of exogenous zeatin. In a study by Hołtra and Zamorska-Wojdyła (2014) a 42% decrease in the DW of the *Salvinia natans* sporophytes was reported on the 14th day of cultivation in a medium containing 20 mg Cu/dm^3. The adverse effects of zinc on the ferns were further evidenced by chlorosis symptoms and browning of the floating fronds, which became more pronounced over seven days of cultivation, particularly in mature sporophytes. Addition of zeatin mitigated chlorosis, resulting in less intense symptoms (Fig. 3).

Successful adaptation to environmental conditions largely depends on the optimal functioning of the photosynthetic apparatus, with key indicators being the content of photosynthetic pigments (Syvash, Zolotareva, 2017; Kosakivska et al., 2018). In metal-contaminated environments, chlorosis and stunted plant growth indicate disruptions in the biosynthesis pathways of photosynthetic pigments, impacting plastid development, photosynthetic efficiency, and general metabolism (Rai et al., 2016). Several studies have documented reductions in photosynthetic pigment content and negative impact on photosynthesis attributed to Cd, Pb, Ni and Hg exposure in *S. natans* sporophytes (Leblebici et al., 2018; Sitarska et al., 2023), iron in *Salvinia minima* Baker (Leal-Alvarado et al., 2016), lead in *S. biloba* Raddi (Loría et al., 2019), Cd and Pb in *S. cucullata* Bory (Phetsombat et al., 2006), chromium (Cr) in *S. auriculata* Aubl. (cited as *S. rotundifolia* Willd.) and *S. minima* (Prado et al., 2016). Zinc's impact on green pigment content may be attributed to the formation of metal-substituted chlorophyll, blockage of the electron transport chain, and reduced enzyme activity in chlorophyll biosynthesis, as observed by Rai et al. (2016). Changes in photosynthetic activity in Salvinia species depended on HM concentration and exposure duration. We found that zinc sulfate in the water led to decreased chlorophylls *a+b* content in *S. natans* sporophytes.

Cytokinins influence the activity of photosynthetic enzymes, chlorophyll biosynthesis, and the rate of photosynthesis (Hönig et al., 2018). Our study demonstrated that zeatin alleviated the negative impact of $ZnSO_4$ on the content of photosynthetic pigments in young sporophytes, but did not fully counteract the effect of HM, and in fact, slightly exacerbated it in mature sporophytes of *S. natans*. We did not observe a significant difference in the chlorophylls *a+b* during the stage of intensive growth. However, at the stage of sori formation and spore maturation, these indices substantially decreased. Moreover, the ratio of *a+b* / carotenoids in both developmental stages of *S. natans*, under the influence of zinc sulfate and zeatin addition, significantly decreased, particularly in mature sporophytes, indicating reduced photochemical activity. Dhir et al. (2011) demonstrated that Zn exposure leads to a significant reduction in photosynthetic pigments, a finding that aligns with our results.

Our study demonstrated that young *S. natans* sporophytes effectively reduce zinc ion levels in water. In the experimental group without zeatin, zinc concentration decreased by over 90% within 12 days, reaching less than one milligram per liter by the experiment's end, below the permissible level for drinking and domestic water in Ukraine (1 $mg \cdot L^{-1}$). Previous works (Dhir et al., 2008; Dhir, Srivastava, 2011) reported *Salvinia* sporophytes capable to extracting up to 90% of zinc from polluted water. However, adding zeatin noticeably inhibited zinc ion adsorption, particularly between the 4^{th} and 10^{th} day of the experiment (Fig. 5). At the experiment's conclusion, zinc concentration in water with zeatin was 1.8 $mg \cdot L^{-1}$, three times higher than without zeatin. The reason for such a slowdown may be that zeatin stimulates the process of cell proliferation in vegetative organs, particularly in floating and submerged fronds, leading to an increase in the mass of undifferentiated cells, which are most strongly affected by zinc toxicity. Simultaneously, there is a decrease in the mass of differentiated extracellular structures where chelation of adsorbed heavy metal ions occurs (Zhao et al., 2012; Mohebi, Nazari, 2021). This, in turn, slows down the removal of zinc from the water. In this experimental variant, the addition of zeatin acts as a stress factor. Specifically, the hormone interferes with the process of chelation of zinc ions by *S. natans* sporophytes and significantly hampers adaptive changes. Exogenously used phytohormones are known to be metabolized much faster than endogenous ones, and an excess of the hormone disrupts the hormonal balance, often perceived by plants as stress (Vedenicheva, Kosakivska, 2017). The results of the experiment suggest that the vegetative sporophytes of *S. natans* can effectively and economically purify water from zinc contamination. However, the addition of zeatin not necessarily improves the efficiency of using *S. natans* in extracting zinc ions from water. The introduction of exogenous zeatin likely increases the stress caused by the supply of excessive amounts of zinc.

Conclusions

The study investigated the impact of exogenous zeatin on morpho-physiological parameters and the ability of *Salvinia natans* sporophytes to biologically extract zinc ions from the aquatic environment.

During the stages of intensive growth and sori formation and spore maturation, exogenous zeatin induced an increase in the dry weight of *S. natans* sporophytes and alleviated the impact of zinc sulfate on this parameter. However, the hormone did not mitigate the adverse effect of zinc on fresh weight accumulation.

The addition of zeatin ameliorated the detrimental effects of zinc on the pigment complex of sporophytes during the intensive growth stage. The pigment complex exhibited greater sensitivity to HM exposure during sori formation and spore maturation, manifesting in leaf browning and more pronounced chlorosis. The addition of zeatin resulted in chlorosis adopting a punctate pattern and being less intense.

The capacity of *S. natans* sporophytes to biologically extract zinc ions from the aqueous medium was demonstrated. Over a 14-day cultivation period, the zinc concentration decreased by more than tenfold from 10 mg·L^{-1} to 0.6 mg·L^{-1}. The addition of zeatin did not impact the efficiency of zinc ions extraction from water.

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ETHICS DECLARATION

The authors declare no conflict of interest.

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Вплив екзогенного зеатину на ріст, пігментний комплекс і здатність спорофітів *Salvinia natans* (*Salviniaceae*) до біологічного вилучення іонів цинку із водного середовища

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Реферат. Реакція рослин на дію важких металів пов'язана з фітогормонами, зокрема цитокінінами, до активних форм яких належить зеатин. Припускають, що екзогенні фітогормони здатні індукувати стійкість рослин до дії важких металів та посилювати фітоекстракцію. В статті проаналізовано вплив екзогенного зеатину на морфо-фізіологічні характеристики молодих і зрілих спорофітів *Salvinia natans* та їхню здатність до біологічного вилучення іонів цинку з водного середовища. У фази інтенсивного росту та формування сорусів і дозрівання спор за умови цинкового навантаження екзогенний зеатин (10^{-6} М) індукував зростання маси сухої речовини спорофіту на 40% та 50%, відповідно. Водночас гормон не знімав негативної дії важкого металу на накопичення маси сирої речовини. За присутності сульфату цинку загальний вміст хлорофілів у ваях молодих і зрілих спорофітів зменшився відповідно на 21% і на 44%, тоді як вміст загальних каротиноїдів зменшився на 21% в обох випадках. Додавання зеатину пом'якшувало негативну дію металу на пігментний комплекс у ваях молодих спорофітів, проте посилювало у ваях зрілих спорофітів. Більш чутливим до дії металу виявився пігментний комплекс спорофіту у фазу формування сорусів і дозрівання спор, що проявилось у побурінні вай і більш виразному прояві хлорозу. При додаванні зеатину хлороз набував точкового характеру і був менш інтенсивним. Продемонстровано здатність спорофіта *S. natans* до біологічної вилучення іонів цинку з водного середовища. Через 14 діб культивування концентрація цинку зменшилась більш ніж у десять разів з 10 мг·л$^{-1}$ до 0,6 мг·л$^{-1}$. Додавання зеатину не впливало на ефективність вилучення іонів цинку з води.

Ключові слова: *Salvinia natans*, біологічне вилучення, зеатин, пігменти, ростові показники, цинк