ДОПОВІДІ
НАЦІОНАЛЬНОЇ
АКАДЕМІЇ НАУК
УКРАЇНИ

https://doi.org/10.15407/dopovidi2025.03.083
UDC 57.04:581.5

N.P. Vedenicheva, https://orcid.org/0000-0002-0579-0342

M.G. Kholodny Institute of Botany of the NAS of Ukraine, Kyiv, Ukraine
E-mail: vedenicheva@ukr.net

Impact of heavy metals on germination and seedling growth of Triticale plants after seed priming with zeatin

Presented by a Corresponding Member of the NAS of Ukraine O.E. Khodosovtsev

The effect of heavy metal compounds (cadmium, cobalt, manganese) on germination and seedlings growth of Triticale (×Triticosecale Wittmack, cv. ADM9 Synthetic) after pre-sowing seed priming with cytokinin was studied.

Contamination of agricultural land with pollutants of anthropogenic origin has become a particular threat in Ukraine, where the content of heavy metal compounds in soil has increased significantly as a result of military actions. Plants, especially cereals, absorb heavy metals and accumulate them in themselves, which prevents their growth and poses a health hazard to consumers. One way to mitigate the negative effects of heavy metals on plants is an application of exogenous phytohormones. In the present research, the effect of $CdCl_2$, $Co(NO_3)_2$ and $MnSO_4$ solutions at concentrations of 50 μM, 100 μM, 250 μM on germination and growth of Triticale seedlings after priming seeds with a zeatin solution (10^{-6} M) was studied.

The experiments showed that heavy metals negatively affect the germination of Triticale seeds and the further seedling growth. They had a particularly detrimental effect on the development of the root system. Cadmium, cobalt and manganese at different concentrations altered the final germination rate and the seedling linear parameters differently. Cadmium demonstrated the most toxic effect on seedling growth whereas manganese was not toxic at low concentrations. All the elements studied had a harmful impact at a concentration of 250 μM. The results of seed priming with cytokinins to mitigate the inhibitory effect of heavy metals on Triticale plant growth depended on the nature of the metal and its concentration. The obatined data can be taken into account in the future in developing experimental designs for continuing research aimed at developing biotechnologies to overcome the consequences of soil contamination with cadmium, cobalt and manganese compounds.

Keywords: *heavy metal, Triticale, germination, growth, priming, zeatin.*

Introduction. One of the most pressing problems of our time is environmental contamination by pollutants of anthropogenic origin. Dangerous toxicants get into the soil, air or water as a result of emissions from industrial enterprises, mining, traffic, etc. Among them, the most hazardous are heavy metal compounds. In Ukraine, the problem of such pollution has become extremely acute as a result of military actions [1]. For instance, in the Kharkiv region, soil contamination with

Citation: Vedenicheva N.P. Impact of heavy metals on germination and seedling growth of Triticale plants after seed priming with zeatin. *Dopov. Nac. akad. nauk Ukr.* 2025. No. 3. P. 83—92. https://doi.org/10.15407/dopovidi2025.03.083

cadmium exceeded 200 % as a result of the russian invasion [2]. The presence of heavy metals in agricultural soils threatens crop yields, food quality and the sustainability of agriculture in general. Cereals, as a vital source of nutrition, are of special interest since they can absorb heavy metals from the soil and accumulate them in edible parts, which is extremely harmful for the health of consumers of agricultural products. Plants themselves are also affected by heavy metals and react to their exposure with growth retardation, yellowing and other negative symptoms [3]. Hazardous pollutants include elements such as cadmium (Cd), cobalt (Co), and manganese (Mn). Cd ions are easily absorbed by plant roots, inducing membrane damage, generation of reactive oxygen species, disorder in respiration and photosynthesis [4]. Cd is detrimental for plants and significantly reduces their productivity, although its effect depends on concentration, soil type, and plant genotype [5]. Co is essential microelement for many lower plants, as well as for leguminous plants, in the latter it is involved in nitrogen fixation. The role of Co in plant development depends on its content in the soil. At high concentrations, this element is toxic, causing iron deficiency and leaf discoloration [6]. Mn is an essential element that acts as a cofactor for many enzyme systems and is necessary for vital processes in plants, including photosynthesis. However, in excessive amounts, Mn is toxic, especially in poor and acidic soils [7]. Given the ability of plants to accumulate heavy metals, their impact certainly provokes severe stress, to overcome which plants have developed defense strategies, in particular, detoxification and adaptation mechanisms [8]. However, with high levels of contaminants in the soil, additional measures should be taken to help plants mitigate the negative consequences from the heavy metal impact. One of the effective ways to mitigate such harmful effect on the growth and productivity of crops is treatment with exogenous phytohormones [9]. Phytohormones are known to be signaling biomolecules of a different chemical structure and physicochemical properties that regulate all physiological and metabolic processes of plants. All of them to some extent affect plant stress tolerance after exogenous treatments. Seed priming or foliar application of phytohormones to cereals contributed to the mitigation of both abiotic and biotic stresses [10]. One of the most important classes of phytohormones are cytokinins, which regulate a wide range of physiological activities in plants at all stages of ontogenesis, from seed germination to plant senescence. Cytokinins provide physiological protection against heat and cold stresses, water deficit, drought, salinity in cereals [11]. Cytokinin treatment also increases tolerance to heavy metals in different plant species. For example, 6-benzylaminopurine (BAP) ameliorated the growth of young bamboo plants exposed to Cd by increasing antioxidant activity, improving photosynthesis properties, and reducing metal accumulation and translocation from root to shoot [12]. Exogenous *trans*-zeatin riboside increased plant growth, stomatal conductance, net photosynthesis, total ascorbate content and decreased malondialdehyde and ethylene levels in Zn- and Cd-treated wetland halophyte *Kosteletzkya pentacarpos* plants [13]. Addition of *trans*-zeatin to the culture of freshwater single-celled alga *Euglena gracilis* exposed to nickel, lead, or cadmium alleviated metal toxicity due to regulation of endogenous cytokinins [14]. Introduction of BAP into hydroponic nutrient solution decreased Co toxic effect on tomato seedlings by regulating metal absorption and translocation [15]. Unfortunately, information about involvement of cytokinins in mitigation heavy metal stress in cereals is obscure.

The aim of this work was to study the effect of heavy metal compounds with varying degrees of toxicity (cadmium, cobalt and manganese) on seed germination and development of Triticale seedlings, as well as to study the effect of seed priming in cytokinin solution on these processes under the influence of heavy metals.

Materials and method. Triticale plants (×*Triticosecale* Wittmack, cv. ADM 9 Synthetic), created by crossing rye with durum wheat in V.M. Remeslo Myronivka Institute of Wheat of the NAAS of Ukraine, were studied. Triticale is a wheat-rye amphidiploid and one of the first artificially created crops, which differs from other cereals by large grain size, unique combination of the best economic and biological traits of wheat and rye [16]. Triticale plants are characterized by high yield potential of grain and green mass, as well as increased adaptive properties to adverse conditions (winter and drought resistance, undemanding to soils, resistance to fungal diseases) [17]. In our experiments, we used Triticale plants as a model object, primarily investigating the typical and general response of this grain crop to heavy metal exposure and zeatin treatment and not focusing on the biological properties of a particular hybrid or variety. Grains were sterilized with 80 % ethanol, washed with purified water, and then soaked in zeatin solution (10^{-6} M) or water for 3 h. Seeds were washed again with purified water and placed on wet filter paper in Petri dishes in water (control) or $CdCl_2 \cdot 6H_2O$, $Co(NO_3)_2 \cdot 4H_2O$, $MnSO_4 \cdot 5H_2O$ solutions of various concentrations (50 μM, 100 μM, 250 μM, 500 μM). Total amount of seeds per one dish was 100 units. In choosing the concentrations of metal compounds, we were guided by the results of other researchers studying their effect on seed germination [18, 19].

For further germination, they were transferred to a climate chamber Vötsch (Germany) at a temperature of +16 °C, a photoperiod of 16/8 h, day/night (light intensity 190 μmol/($m^2 \cdot s$)) and humidity of 60 %. After 7 days, the linear parameters of the seedlings were measured by the standard linear measurement method. The final germination rate (FG) was calculated using the formula: FG rate = Total germinating seed number/Total tested seed number × 100 % [4].

All experiments were carried out in five biological replicates. The data were processed by standard methods of variation statistics using Microsoft Excel 2007 program. In the Figures, the average values and standard errors are presented. Values of $P < 0.05$ were considered significant.

Results and discussion. Triticale seeds were characterized by a high germination capacity, which reached almost 80 %. It is evident that values of FG rate and seedling linear parameters varied depending on the metal nature and its concentration. All the metals tested at a concentration of 500 μM almost completely inhibited root emergence; the germination of the grains was no more than 5 % with the subsequent formation of deformed dwarf seedlings. At cadmium chloride concentrations of 50 and 100 μM, the germination rate of Triticale seeds decreased by 21 %, and at a concentration of 250 μM — by 34 % (fig. 1). Seed priming in zeatin solution had a negative effect on seed germination both under control conditions and under cadmium chloride exposure.

When seeds were incubated in cobalt nitrate solution, a slight negative effect on germination was observed at a concentration of 250 μM (11 %) (fig. 2). At low concentrations of 50 and 100 μM, cobalt nitrate eliminated the inhibition of seed germination after priming with zeatin. At the same time, at a concentration of cobalt nitrate solution of 250 μM, the inhibitory effect of zeatin on seed germination was manifested in the same way as in the control.

Among the metal compounds studied, manganese sulfate solution had the most negative effect on seed germination (fig. 3). As the solution concentration enhanced, a decrease in the germination rate to 54 % was observed. At the same time, manganese sulfate significantly activated the germination of seeds primed with zeatin solution. At a manganese sulfate concentration of 50 μM, the germination rate of primed seeds increased to almost 100 %, and at the concentration of 250 μM — by 29 % compared to unprimed seeds.

The influence of heavy metals significantly affected the habitus of Triticale seedlings. Thus, exposure to cadmium chloride provoked a decrease in shoot elongation by 15—58 % at the

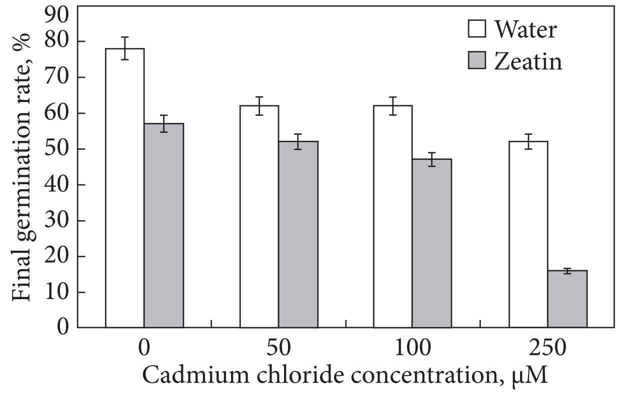

Fig. 1. Effect of $CdCl_2$ and seed priming in zeatin solution (10^{-6} M) on Triticale seed germination

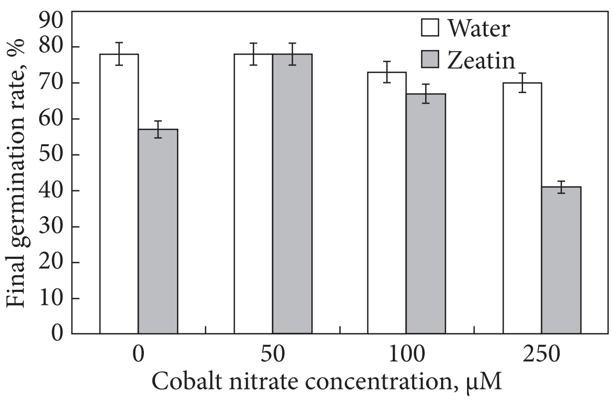

Fig. 2. Effect of $Co(NO_3)_2$ and seed priming in zeatin solution (10^{-6} M) on Triticale seed germination

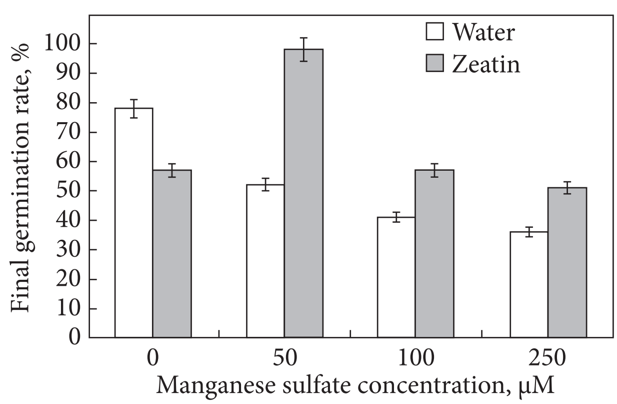

Fig. 3. Effect of $MnSO_4$ and seed priming in zeatin solution (10^{-6} M) on Triticale seed germination

concentration range from 50 to 250 μM (fig. 4). The root system was more vulnerable, and its length was reduced by 22—85 % under the same conditions. Priming the seeds with zeatin solution led to a decrease in the linear parameters of both control and cadmium chloride-treated seedlings. The greatest size inhibition was recorded for roots — from 30 % in the control to 89 % under the influence of 250 μM $CdCl_2$.

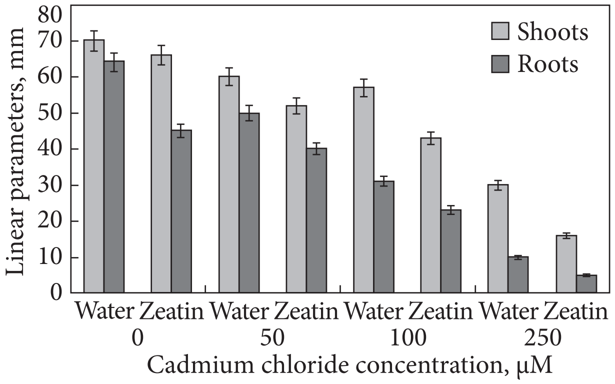

Fig. 4. Effect of $CdCl_2$ and seed priming in zeatin solution (10^{-6} M) on linear parameters of 7-day-old Triticale seedlings

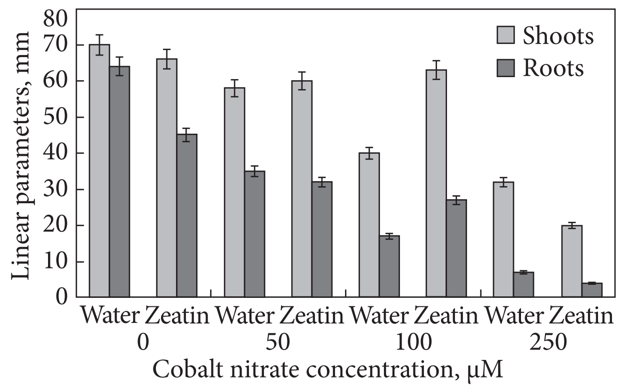

Fig. 5. Effect of $Co(NO_3)_2$ and seed priming in zeatin solution (10^{-6} M) on linear parameters of 7-day-old Triticale seedlings

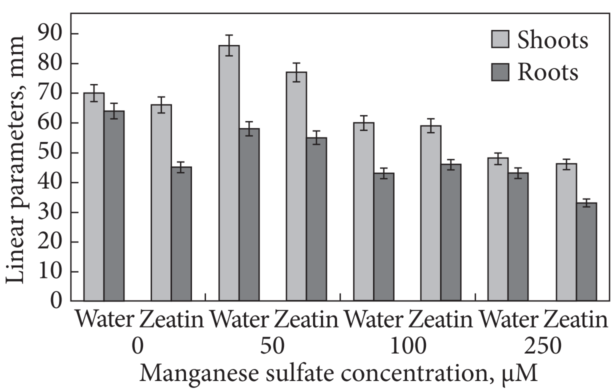

Fig. 6. Effect of $MnSO_4$ and seed priming in zeatin solution (10^{-6} M) on linear parameters of 7-day-old Triticale seedlings

Incubation in cobalt nitrate solution affected the linear parameters of the above-ground part of Triticale seedlings (fig. 5). The greatest decrease in shoot size was recorded at concentrations of 100 μM (by 43 %) and 250 μM (by 55 %). The effect of cobalt nitrate on root growth was even more detrimental. At the mentioned concentrations, the inhibition of root linear dimensions was 74 and 89 %, respectively. Priming seeds with zeatin caused a positive effect on the growth of juvenile Triticale plants at a $Co(NO_3)_2$ concentration of 100 μM: the above-ground part of seedlings grown from primed seeds was 43 % taller and the roots were 42 % longer than those grown from seeds soaked in water.

Incubation in 50 μM manganese sulfate solution stimulated the growth of the above-ground part of seedlings grown from both unprimed seeds (by 19 %) and zeatin-treated seeds (by 15 %) (fig. 6). An increase in the concentration of $MnSO_4$ caused a slowdown in shoot growth by up to 32 % under the influence of 250 μM salt. Inhibition of root growth was detected at all concentrations of $MnSO_4$, the greatest was in a solution of 250 μM (33 %). Priming the seeds with a zeatin solution did not allow overcoming the negative effect of manganese sulfate on seedling growth.

Recently, plant tolerance to abiotic stresses has come to the forefront of research, especially with regard to anthropogenic pollution factors. Triticale plants exceed other cereals due to their increased resistance to adverse environment [20, 21]. Therefore, the study of the heavy metal negative impact on the growth and juvenile development of Triticale plants and the search for agents to mitigate it is an urgent task. Unfortunately, information on this issue is presented only in a few reports. Absorption and accumulation of heavy metals (zinc, cadmium, lead) by Triticale plants from contaminated soil was revealed [22]. The differentiated effect of Cu^{2+}, Zn^{2+} and Cd^{+} ions on the growth of Triticale in the first 9 days after germination was established and the cadmium compound was found to have the most toxic effect [18]. In this research, the changes in germination rate and morphological alterations of Triticale seedlings exposed to heavy metal were demonstrated. The negative effect on seed germination increased in the following sequence: $Co^{2+} < Cd^{2+} < Mn^{2+}$. Simultaneously, the impact on the further plant development varied significantly: from strictly suppressive for Cd to stimulating for Mn at low concentrations. High amounts of all studied metals definitely inhibited Triticale seed sprouting. Numerous previous investigations have shown an adverse impact of different heavy metal on seed germination and seedling development for many plant species including cereals [23]. While the effects of Cd have been studied thoroughly, Co and Mn have received less attention. The mechanisms of toxic effects of heavy metals on plants are associated with changes in the antioxidant system, protein catabolism, damage to photosynthesis, and decreased α-amylase activity [5, 23]. The toxicity of heavy metal compounds depends largely on the concentration of a solution and possibly on the plant species. For instance, in rice, full suppression of root emergence was recorded at a $CdCl_2$ concentration of 200 μM [24]. In wheat, inhibition of seed germination was found after exposure to a solution 10 μM of $CdCl_2$, but even 1000 μM of $CdCl_2$ did not stop seedling growth [4]. In our experiments, the inhibitory effect on germination and growth of Triticale seeds was established at a minimum concentration of $CdCl_2$ 50 μM. Although Co is an essential element in some living organisms as an enzyme cofactor, its effect on cereal growth has not been well described. Recently, a decrease in the length and weight of maize roots and shoots was reported at Co concentration 200—400 μM [25]. In present research, the growth-limiting concentration of $Co(NO_3)_2$ for Triticale seedlings was 250 μM. Mn is an essential element for plants, its deficit causes a disorder in photosynthetic machinery and reduces biomass [7]. In wheat, seed priming with Mn at low concentration (0,1 mg/L) significantly increased the germination percentage, growth traits and grain yield per plant under lead stress [26]. Mn toxicity induces negative symptoms with chlorotic leaves and necrotic spots, but toxic Mn concentrations are highly dependent on plant species and genotypes [7]. In Triticale, a 50 μM $MnSO_4$ soolution inhibited seed germination, but noticeable slowdown of seedling development was observed at concentration 250 μM.

The role of exogenous cytokinin treatment in the amelioration of abiotic stresses has been well documented in the literature [27]. In particular, exogenous cytokinins mitigate salt-induced senescence [28], improve nutrient uptake, leaf chlorophyll content and photosynthesis in droughtstressed wheat plants [29], simulate the effect of acclimation in response to overheating, enhancing the thermotolerance of rice plants [30]. Cytokinin protection against different stresses in cereals was provided mainly by foliar application and spraying [11]. Simultaneously, seed priming with phytohormones has recently been considered as a promising method for increasing tolerance and yield of crop plants as well [31]. For instance, wheat grain soaked in BAP solution demonstrated better growth and increased content of soluble sugars, chlorophyll, and amylase activity under salinity [32]. Sweet sorghum (*Sorghum bicolor*) seed priming with kinetin improved germination and growth parameters, chlorophyll content, and the antioxidant system under salt stress [33]. In present research, priming of Triticale seeds with zeatin slowed down the final germination rate and unsignificantly decreased seedlings height. Root length was affected to a greater extent (more than 20 %). Cytokinins are known to be negative regulators of root development, also cytokinin treatment suppresses root elongation [34]. Reduction of the root system results reduces nutrients and water availability and may be one of the reasons for the decrease in germination energy and further growth. Nevertheless, cytokinins are considered as a potential modulator of heavy metal stress tolerance in plants [35]. They play a vital role in the formation of adaptive response to heavy metal stress by activating antioxidant system, maintaining ion balance and regulating the expression of stress-related genes [36]. In our investigation, the differentiated influence of zeatin priming on seedling growth was found. At all studied concentrations of the most toxic metal Cd, zeatin treatment was not effective in improving Triticale young plant growth. In the case of Co treatment, zeatin exerted a protective effect on shoot and root growth at metal concentrations of 50 μM and 100 μM, but it was not effective in improving growth at metal concentration 250 μM. Synergistic action of zeatin and Mn in stimulation of Triticale seedling growth was revealed at low metal concentrations, whereas no amelioration in growth parameters was observed at 250 μM $MnSO_4$. The data obtained indicate that at early stage of Triticale plant development, the action of cytokinins as a protective agent is strongly dependent on the heavy metal nature and its toxicity. Perhaps, the efficiency of cytokinins in Triticale growth maintenance would be more significant with foliar treatment of older plants with a more developed and formed root system. In addition, it should be taken into account that during exogenous treatment, excess cytokinins are stored in plant tissues in the form of a mobile *O*-glucoside, which is easily converted into active forms of the hormone if necessary [37]. Although the data on the effects of heavy metals on the balance of endogenous cytokinins are contradictory [35], it is most likely that stress affect it negatively. It can be assumed that stored during seed priming zeatin-*O*-glucoside could be used in Triticale plants for regulation of growth and development at tillering stage to compensate cytokinin deficit through the heavy metal influence. Thus, the positive role of zeatin priming could be manifested at later stages of plant development. Unfortunately, we are not aware of any literature data on the protective role of cytokinins in heavy metal stress in cereals. Therefore, any results regarding the selection of the plant development stage and the method of treatment with phytohormones for the protection against heavy metals are of great importance and will serve as a basis for further experiments.

Conclusion. Thus, the experiments performed showed that heavy metals negatively affect the germination of Triticale seeds and further seedling growth. They had a particularly detrimental effect on the development of the root system. Cadmium, cobalt and manganese at different concentrations altered the final germination rate and the seedling linear parameters differently. Cadmium demonstrated the most toxic influence on seedling growth whereas manganese was not toxic at low concentrations. All the elements studied had a harmful impact at concentration 250 μM.

The results of seed priming with cytokinins to mitigate the inhibitory effect of heavy metals on Triticale plant growth depended on the nature of the metal and its concentration. The data obtained can be taken into account in the future development of experimental designs for the continuation of research aimed at the development biotechnologies to overcome the consequences of soil contamination by cadmium, cobalt, and manganese compounds.

The study was carried out within the framework of the planned scientific research "Natural growth regulators in the induction of resistance of cereal plants to heavy metals" funded by the National Academy of Sciences of Ukraine No III-90-19.489 (2024—2028).

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Received 24.04.2025

Н.П. Веденичова, https://orcid.org/0000-0002-0579-0342

Інститут ботаніки ім. М.Г. Холодного НАН України, Київ, Україна
E-mail: vedenicheva@ukr.net

ВПЛИВ ВАЖКИХ МЕТАЛІВ НА ПРОРОСТАННЯ І РІСТ ПРОРОСТКІВ ТРИТИКАЛЕ ЗА ПРАЙМУВАННЯ НАСІННЯ ЗЕАТИНОМ

Досліджено вплив сполук важких металів (кадмію, кобальту, мангану) на проростання та ріст проростків тритикале *(×Triticosecale Wittmack*, сорт ADM9 Синтетик) за передпосівного праймування насіння цитокініном.

Забруднення сільськогосподарських угідь полютантами антропогенного походження набуло загрозливих масштабів в Україні, де вміст сполук важких металів у ґрунті внаслідок воєнних дій значно зріс. Рослини, зокрема злаки, поглинають важкі метали й накопичують їх у своїх частинах, що сповільнює їхній ріст і становить небезпеку для здоров'я споживачів. Одним із шляхів подолання негативного впливу важких металів на рослини є застосування екзогенних фітогормонів. У статті наведено результати вивчення впливу розчинів $CdCl_2$, $Co(NO_3)_2$ і $MnSO_4$ у концентраціях 50, 100, 250 мкМ на проростання та ріст проростків тритикале за праймування насіння розчином зеатину (10^{-6} М). Показано, що важкі метали негативно впливали на проростання насіння тритикале й подальший ріст проростків. Особливо згубно вони діяли на розвиток кореневої системи. Кадмій, кобальт та манган у різних концентраціях змінювали швидкість проростання та лінійні параметри проростків диференційовано. Кадмій виявляв найбільш токсичний вплив на ріст проростків, тоді як манган у низьких концентраціях не був токсичним. Усі досліджені елементи спричиняли шкідливу дію за концентрації 250 мкМ. Результати праймування насіння цитокінінами для подолання інгібувального впливу важких металів на ріст рослин тритикале залежали від природи металу і його концентрації. Отримані дані можуть бути враховані в майбутньому під час складання дизайну експериментів для продовження досліджень, спрямованих на розроблення біотехнологій подолання наслідків забруднення ґрунтів сполуками кадмію, кобальту та мангану.

Ключові слова: *важкі метали, Triticale, проростання, ріст, праймування, зеатин.*