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Article

The effect of manganese and cadmium on morphological and biochemical parameters of barley seedlings

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Abstract. The influence of various concentrations of Mn^{2+} (100–800 mg/l) and Cd^{2+} (10–80 mg/l) on spring barley *Hordeum vulgare* L., variety 'Rodnik Prikamya' was studied in the roll culture under laboratory conditions. Seed germination, seedling biomass, and the content of free and bound polyphenols in plant biomass were estimated. The effect of low-dose manganese (100 mg/l) turned out to be stimulating: seed germination and biomass of barley seedlings increased by 35 and 51% compared to the control. Elevated concentrations of Mn^{2+} (200–800 mg/l) suppressed seed germination and biomass (by 55–75% and 60–79%). Cadmium (10–80 mg/l) made solely inhibitory impact: seed germination decreased by 20–70% and seedling biomass by 23–78%. Biochemical responses of plants to stress caused by these elements differed. For instance, application of Mn^{2+} increased the proportion of bound polyphenols in the total polyphenol of seedlings by 8, 12, 7, and 8% compared to the control. Cadmium stress was compensated by polyphenols only at its lowest tested concentration (20 mg/L); the proportion of bound polyphenols increased by 3.3%, as compared to the control. In case with the highest concentrations of the studied metal ions (500–800 mg/L for Mn^{2+} and 50–80 mg/L for Cd^{2+}), the content of polyphenols dropped to control values thereby indicating the depletion of adaptive reserves of plants.

Keywords: phytotesting, *Hordeum vulgare*, biomass, germination, polyphenols, toxicity

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Научная статья

Влияние марганца и кадмия на морфофизиологические и биохимические показатели проростков ячменя

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Аннотация. В лабораторном опыте исследовали действие различных концентраций Mn^{2+} (100–800 мг/л) и Cd^{2+} (10–80 мг/л) на растения ярового ячменя *Hordeum vulgare* L., сорт ‘Родник Прикамья’. Опыт проводили в рулонной культуре. Оценивали всхожесть семян, биомассу проростков, содержание свободных и связанных полифенолов в биомассе растений. Эффект марганца в низкой концентрации (100 мг/л) был стимулирующим: всхожесть семян и биомасса проростков ячменя увеличилась на 35 и 51% по сравнению с контролем. Повышенная концентрация Mn^{2+} (200–800 мг/л) ингибировала всхожесть семян и биомассу проростков ячменя (на 55–75% и 60–79%). Действие Cd^{2+} в пределах 10–80 мг/л было только угнетающим: всхожесть семян снизилась на 20–70%, биомасса проростков – на 23–78%. Биохимические реакции растений на стресс, вызванный марганцем и кадмием, различались. Марганец вызывал увеличение доли связанных полифенолов в общем полифенольном профиле проростков на 8, 12, 7 и 8% по сравнению с контролем. Кадмиевый стресс был компенсирован полифенолами только в наименьшей тестируемой концентрации (20 мг/л): доля связанных полифенолов возросла на 3.3% к контролю. При наиболее высоких концентрациях ионов исследуемых металлов (500–800 мг/л Mn^{2+} и 50–80 мг/л Cd^{2+}) происходило снижение содержания полифенолов до контрольных значений, что свидетельствует об истощении адаптационных резервов растений.

Ключевые слова: фитотестирование, *Hordeum vulgare*, биомасса, всхожесть, полифенолы, токсичность

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Introduction

In laboratory biotesting, plants are often used as test organisms due to their high sensitivity to a wide range of environmental conditions (Agrawal and Agrawal, 1999; Qaderi et al., 2023). Studies suggest that plants quickly respond to changes in lighting, temperature, humidity, carbon dioxide concentration and other parameters (Driesen et al., 2020). In plant cultivation, the assessment of characteristics of seed germination and further growth of seedlings is an integral part of diagnostics of plant resistance to various stress factors (Alekseychuk and Laman, 2005).

Both, in national and international practices, numerous certified phytotesting methods are based on accounting the alterations of major morphophysiological parameters of plants. Among them, seed germination and root length of plants are most often assessed (Terekhova et al., 2016). Suppression of seed germination and root length reduction were recorded in response to oil and salt pollution (Arzamazova et al., 2020), while inhibition of root and shoot elongation – to antibiotics present in soil (Kosmacheva, 2020). Toxicity and dose-dependent effect of fungicides (Saneeva et al., 2022), heavy metals (Alyabysheva, 2023), contaminated sludge sediments (Bostubaeva and Nauanova, 2022), and other toxicants (Koval and Ogorodnikova, 2023) were determined from a set of germination indicators, i.e. the seed germination energy, root and shoot length of plants. Therefore, the use of plants in the environmental monitoring of technogenically disturbed soils (Krasnoperova, 2015), urbanozems (Zykova et al., 2017), natural ecosystems and agrocenoses (Morozova et al., 2020) has been scientifically substantiated.

In biotesting, the employment of morphophysiological reactions of plants to stress factors as test functions is considered to be a simple and reliable approach. However, various stressors can be responsible for a reduced germination, slower growth of roots and seedlings, their external damage and death of plants at the beginning of ontogenesis. It is worth noting that biochemical responses are the first stage of plant adaptation to the changing environment, preceding the morphophysiological transformations. When stress is low, biochemical alterations may occur without external deviations in plant development. In some cases, biochemical indicators form specific combinations corresponding to a certain impact or its level. This is the scientific basis for biomarking. Radicals (reactive oxygen species), ions (Na^+, $Ca^{2+,}$ NO_3^-, NH_4^+, H^+), inorganic (H_2O, CO_2) and organic molecules (phytohormones, volatile compounds, pigments, RNA molecules) can serve as plant biomarkers (Tan et al., 2023).

Polyphenols are among the low-molecular secondary metabolites that exert a protective effect in response to biotic and abiotic stresses. The spectrum of polyphenolic compounds in plants is quite wide. In plant tissues, they exist both in the form of glycosides (bound fraction) and aglycones (free fraction).

The role of polyphenols in plant resistance has not been fully understood. On the one hand, it is associated with the species- and variety specificity of plants. At the same time, non-specific hormetic activation of various protective systems by low concentrations of toxicants is insufficiently studied (Erofeeva, 2022). Of practical interest is also a comparison of classical morphophysiological responses of plants to chemical stress and biochemical changes in plant test organisms.

Despite the accumulated data on characteristics of individual heavy metals and their impact on living organisms, identification of differences and similarities in the behavior of these elements still remains an urgent task in solving the problems of environmental pollution.

This study is aimed at the comparison of morphophysiological and biochemical reactions of spring barley seedlings *Hordeum vulgare* L. to various concentrations of manganese and cadmium in order to supplement the information about the mechanisms of plants' protection from chemical effects.

Materials and methods

Model experiment

In laboratory experiments, we studied the influence of various concentrations of manganese and cadmium ions on spring barley seedlings *Hordeum vulgare* L. For testing, barley seeds of the 'Rodnik Prikamya' variety (originator: FGBNU FANTS Severo-Vostok) were used. Laboratory seed germination made up 92%[1].

Phytotesting was implemented according to the method described in GOST 12038-84 with some modifications. Seeds were germinated in the roll culture. Before testing, barley seeds were soaked

[1] GOST 12038-84 Seeds of agricultural crops. Methods for determining germination.

in a distilled water for 16 hours. Then, swollen seeds (35 pcs.) were laid out on sheets of filter paper (15×50 cm), preheated in a dry-heat oven for 2 hours at a temperature of 105 °C. Seeds were oriented with the embryos down (2–3 cm from the upper edge) and covered with a similarly prepared filter paper of 5×50 cm size. Paper strips with seeds were loosely rolled and placed vertically in 500 ml glass beakers filled with salt solutions of manganese ($MnSO_4 \cdot 5\ H_2O$) and cadmium ($CdSO_4 \cdot 2.7\ H_2O$) of various concentrations (Table 1).

Cadmium was chosen for experiments due to its toxicity and hazard for human health (Wang et al., 2021), while manganese because of its prevalence and high content in soils (from 221 to 1428 mg/kg) (Simonova et al., 2019). Modeling of the increased manganese concentration was explained by its lower hazard class (class 3) compared to cadmium (class 1)[2].

A distilled water served as a laboratory control. Seed germination and subsequent plant cultivation were carried out at room temperature of 20 ± 2 °C with a photoperiod of 16 hours of light / 8 hours of darkness. The 10-day experiment was repeated three times.

Evaluated indicators

Following the exposure, we removed seedlings from rolls, counted normally germinated seeds and estimated raw biomass of seedlings.

After drying and grinding the sprouts, a combined sample of plant material was prepared, and polyphenols were extracted. The total content of polyphenols was determined in alkaline (2N NaOH solution, t = 80 °C, τ = 2 h) and free polyphenols in aqueous-alcoholic extracts (70% C_2H_5OH solution, t = 5 °C, τ = 16 h). The substrate/extractant ratio was 1 : 100. The polyphenol content was defined spectrophotometrically[3]. Gallic acid was employed as a standard. The mass fraction of bound (glycoside) polyphenol forms was found from the difference between the total content and free fraction of polyphenols.

Reliability and mathematical treatment of results

Each experimental variant was performed in triplicate with the result represented as the mean value and its standard deviation.

Marked on the graphs with Latin letters, significance of differences between the data sets was proved by the one-way analysis of variance (ANOVA) method. The significance level made up 0.05.

Pearson correlation coefficients (*r*) were calculated using Microsoft Excel 2007.

Results

Seed germination and biomass of barley seedlings under chemical stress

The negative effect of Mn^{2+} and Cd^{2+} on barley plants was determined from the reduced (compared to the control) seed germination and seedling biomass (Figs. 1**A, B**). Note that with the increase in ions concentration, the studied parameters in solutions decreased.

In experiments with the lowest concentration of Mn^{2+} (100 mg/l), we observed a stimulating effect expressed in the growing seed germination and seedling biomass (by 35 and 51%, respectively). At higher concentrations (200–800 mg/l), manganese, on the contrary, negatively affected the morphophysiological indices of barley growth, i.e. seed germination dropped by 25–75% and seedling biomass by 21.4–79%.

The influence of Cd^{2+} and Mn^{2+} on test organisms was similar. However, in the experiment with cadmium, all the studied concentrations made an inhibitory effect on seed germination (by 20–70%) and seedling biomass (by 23–78%). It should be noted that the obtained morphophysiological parameters significantly differed from the control values in all experiments, except for that with the lowest tested concentration of Cd^{2+} (10 mg/l).

The conducted series of experiments suggests that the selected dose ranges for Mn^{2+} and Cd^{2+} are efficient, i.e. they do make an effect on test organisms. The use of the same bioassays has made it possible to find out the way of changing the phenolic status of a phytoobject.

[2] GOST 17.4.1.02-83. Environmental protection, classification of chemicals for pollution control.

[3] GOST R 55488-2013. Propolis. Method for determining polyphenols.

Table 1. Experimental variants. Dash means the absence of a standard.

Ion	Estimated concentration, mg/l				MPC / APC in soil (total form), mg/kg[4]
Mn(II)	100	200	500	800	1500 / –
Cd(II)	10	20	50	80	– / 0.5–2.0

Phenolic status of barley under chemical stress

It is known that inhibition of barley seedling growth influenced by heavy metals largely occurs due to non-specific oxidative stress (Juknys et al., 2012). In this regard, among the studied parameters, polyphenols, as markers of chemical stress, are of particular interest.

Manganese and cadmium in the selected concentration ranges had a similar effect on the polyphenol profile of barley seedlings (Figs. 2**A, B**).

In experiments with the lowest concentrations (100 mg/l for Mn^{2+} and 10 mg/l for Cd^{2+}), no significant differences in the polyphenol content in barley biomass were recorded. With a 2-fold increase in the concentration (relative to the lowest one), the studied parameter significantly increased, as compared to the control (by 4 and 2.2 mg/g, respectively).

At higher concentrations of Mn^{2+} in the medium, the content of total polyphenolic compounds in biomass dropped to the control level. In the variant with maximum Cd^{2+} concentrations (80 mg/l), a tendency towards a decrease (compared to the control) in the study parameter was noted.

To clarify the mechanism of biochemical adaptation of plants to chemical stress, the proportional ratio of free and bound polyphenols in their total pool was estimated (Figs. 3**A, B**).

In the experiment with Mn^{2+}, as its concentration increased, the proportion of free polyphenols in the total content significantly decreased, whereas the share of bound ones in seedlings, on the contrary, increased (by 8, 12, 7, 8% compared to the control).

Bearing in mind that free polyphenols have a higher complex-forming potential than the bound ones (Eghbaliferiz and Iranshahi, 2016), excess manganese could bind to aglycones and increase the proportion of the bound fraction, including the total polyphenols in seedlings (Fig. 2).

Unlike manganese, cadmium did not have a significant effect on the ratio of polyphenol fractions. The exception was the experiment with the lowest concentration of Cd^{2+} in the solution (10 mg/l). Here, the proportion of free polyphenols in barley seedlings increased by 3.3% compared to the control.

Discussion

The roll culture-based phytotesting in the medium with manganese and cadmium sulfate solutions (100–800 and 10–80 mg/l by metal cations) demonstrates that seed germination and biomass of barley seedlings evidently and consistently change with increasing concentrations of the studied metal ions. In response to Cd^{2+} and Mn^{2+} effects, biomass ($r = -0.82$ and $r = -0.86$) and seedling germination ($r = -0.82$ and $r = -0.86$) significantly decrease.

In terms of total polyphenols and its components, the biochemical reaction of barley appears to be smoothed (as compared to the morphophysiological indexes) that is logical since the reaction norm of plant growth characteristics within one species is, of course, wider than that of biochemical indicators ensuring internal homeostasis. However, this fact makes the identified fluctuations in the phenolic status of phytoobjects extremely important. With a consistent elevation of concentrations of Mn^{2+} (to 200 mg/l) and Cd^{2+} (to 20 mg/l), the total content of polyphenols in seedlings grows. It is due to the property of plants to increase the content of polyphenolic compounds under stress conditions (Tuladhar et al., 2021). The researchers explain this phenomenon by the pronounced antioxidant features of polyphenols, protecting cells from oxidative damage by metals (Chen et al., 2020).

In the culture medium, a subsequent increase in concentrations of Mn^{2+} and Cd^{2+} (above 200 mg/l and 20 mg/l, respectively) results in the total polyphenols decline in seedlings to the control level.

[4] SanPiN 1.2.3685-21. On approval of sanitary rules and regulations "Hygienic standards and requirements for ensuring safety and (or) harmlessness of environmental factors for humans".

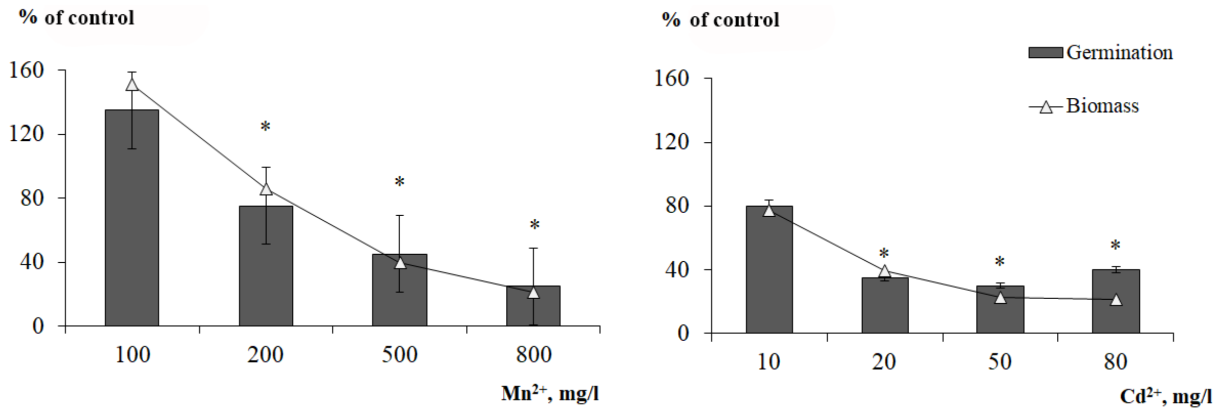

Fig. 1. Changes in morphophysiological parameters of barley sprout growth influenced by various concentrations of manganese and cadmium. * – a significant level of differences in parameters compared to the control (p < 0/05).

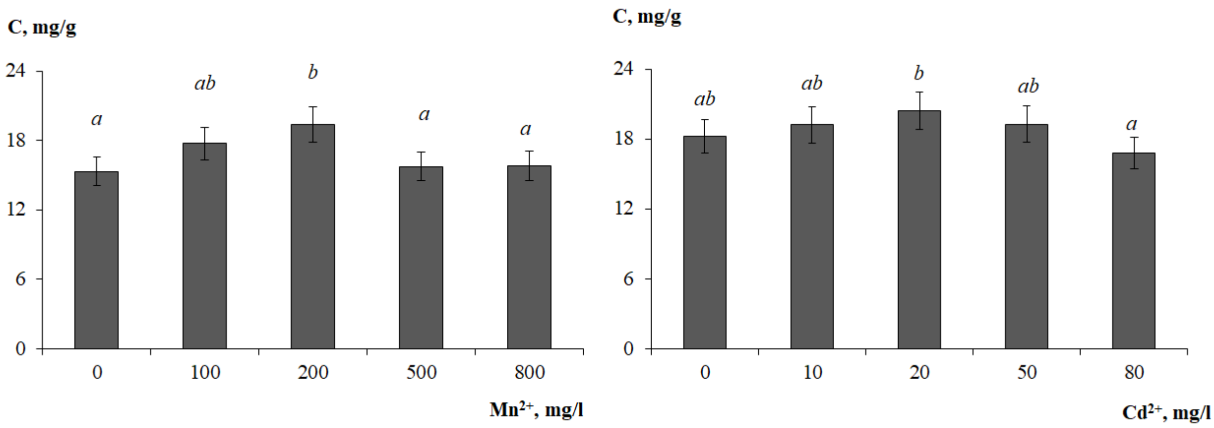

Fig. 2. Changes in the total content of polyphenols in barley sprouts influenced by various concentrations of manganese and cadmium. Different letters indicate significant differences ($p < 0.05$).

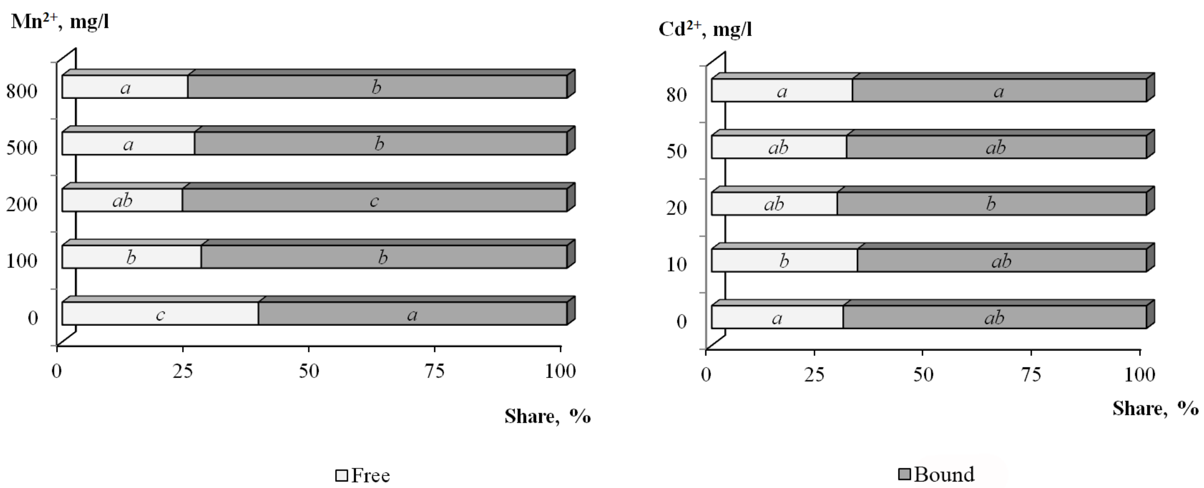

Fig. 3. The ratio of free and bound fractions of polyphenols in barley sprouts influenced by various concentrations of manganese and cadmium. Different letters indicate significant differences ($p < 0.05$).

Apparently, chemical loads (that excess the adaptive plant reserves) bring to a complex disruption of the antioxidant system and biosynthesis slowdown of polyphenols (Goncharuk and Zagoskina, 2023).

The mechanism of the biochemical response to a chemical stress shows low correlation coefficients with the studied metal concentrations: $r = -0.023$ ($p = 0.93$) for Mn^{2+} and $r = -0.07$ ($p = 0.82$) for Cd^{2+}.

The distribution of free and bound forms of polyphenols in their total pool differed, depending on active ions. As a biogenic element, manganese was neutralized by barley through the complexation with polyphenols resulting in the increase of bound polyphenols compared to free ones. Such a pattern was not established in experiments with cadmium. This fact and the tendency towards the falling content of total polyphenols in plants (Fig. 2) against the background of significant inhibition of morphophysiological indicators (Fig. 1) is evidence of a plant inability to neutralize the toxic effect of cadmium because of its binding with polyphenols. Thus, as a xenobiotic, cadmium disrupts the adaptive capabilities of the organism.

It is common knowledge that induction of biosynthesis of non-enzymatic antioxidants may depend on the type of metal and its concentration. Previously, in experiments with wheat seedlings, anthocyanin production rise was detected under moderate cadmium (25 μmol $CdCl_2$) – induced stress, whereas suppression of their antioxidant properties and drop in protective effect under severe one (50 μmol $CdCl_2$) (Shoeva and Khlestkina, 2018).

Conclusion

The conventional group of chemical elements classified as heavy metals includes both essential elements necessary for living organisms (Mn, Cu, Zn) and highly toxic ones (Cd, Pb, Hg). The significance of the latter for biotas is not fully understood. In this study, an attempt was made to compare the effect of needed for living organism manganese and cadmium, being at the same time potentially toxic even in low concentrations. When exposed to Mn^{2+} (100–800 mg/l) and Cd^{2+} (10–80 mg/l) solutions, biomass of barley seedlings decreased and polyphenol profile changed.

The sensitivity of barley seedlings to increasing concentrations of Mn^{2+} and Cd^{2+} was revealed from transformations of morphophysiological indexes. For instance, the correlation coefficients of "germination-concentration" and "biomass-concentration" parameters made up $r = -0.90$ and $r = -0.55$ for manganese, $r = -0.91$ and $r = -0.81$ for cadmium, respectively. Therefore, an excessive essential element (manganese) in plants causes the reactions similar to the effects of a highly toxic element (cadmium).

A difference in biochemical reactions of plants to stress induced by Mn^{2+} and Cd^{2+} is shown. In barley seedlings, manganese increases the proportion of bound polyphenols in the total polyphenol profile (by 8, 12, 7 and 8% for successively increasing concentrations). This suggests the antioxidant system response to manganese stress and worsening of its performance with growing chemical loads. Cadmium provides the increased proportion of bound polyphenols only at the lowest tested concentration (by 3.3% compared to the control). At maximum concentrations of Cd^{2+} (500–800 mg/l and 50–80 mg/l, respectively), the content of polyphenols drops to control values thereby indicating the depletion of adaptive reserves of plants.

Thus, the mechanisms of the toxic effect of elevated concentrations of Mn^{2+} and Cd^{2} were revealed due to morphophysiological and biochemical reactions of plants.

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