Combined effects of heavy metals and drought in the early stages of sunflower ontogenesis

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Abstract. The paper presents the results of a study on the effect of various soil moisture supply conditions and increasing concentrations of the most common agricultural soil pollutants - cadmium (Cd) and lead (Pb) on the growth of *Helianthus annuus* L. 'Poseidon 625' seedlings. It was found that Cd absorption by seedlings under soil moisture deficiency conditions increases compared to sufficient watering conditions, while Pb absorption decreases. It was determined that simulating drought conditions with an osmotic solution (7.4% sucrose) prevents the growth of the aboveground part of seedlings and activates the growth of the root system. It was shown that under the influence of various cadmium concentrations in the soil, the development of the root system of 8-day-old seedlings is blocked, but active development of adventitious roots of the "goose foot" type is observed, which contributes to the adsorption and accumulation of Cd^{2+} in the phytomass. It was found that maximum concentrations of lead suppress the growth of both the main and adventitious roots of sunflower seedlings and simultaneously stimulate the growth of their above-ground part, which probably indicates the activation of the storage function of the stem, as the next line of defense of seedlings from the damaging effects of combined stress.

1 Introduction

Environmental pollution with heavy metals (HM) due to anthropogenic activities has attracted increasing attention from the scientific community. Heavy metals, especially carcinogenic ones, including chromium (Cr), cadmium (Cd), mercury (Hg), aluminum (Al), lead (Pb) and arsenic (As), have been found to be the most significant air, water and soil pollutants that adversely affect the quantity, quality and safety of crop production worldwide (Rahman et al, 2022, Zhang et al, 2024, Sharma et al, 2024).

Sunflower (*Helianthus annuus* L.) is the main oilseed crop grown worldwide. However, its yield is negatively affected by abiotic and biotic stresses such as drought, heavy metals (especially cadmium). In recent decades, this toxic heavy metal has become a serious threat to agricultural production and human health due to industrial and anthropogenic activities (Li et al, 2021). Studying the mechanism of drought tolerance in sunflower and understanding its physiological responses to drought stress is of great practical importance

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for accelerating the breeding of new drought-resistant (tolerant) varieties and ensuring high and stable sunflower yields (Shi et al, 2024).

Oil crops, including sunflower, have a good accumulation capacity for a number of metals, high biomass, which allows them to be used for phytoremediation purposes. Therefore, the issue of accumulation of heavy metals in sunflower plants is currently the focus of many studies (Yang et al, 2020).

There are many studies in the literature devoted to the accumulation of heavy metals by sunflower. Cadmium and lead are toxic pollutants that seriously inhibit its growth and productivity (Chen et al, 2021). It is noted that elevated concentrations of Me in the soil contribute to their accumulation not only in the phytomass, but also in the grain of this crop, which has a direct impact on the quality of the oil produced. (Dhiman et al, 2017). Agricultural land contaminated with Cd threatens food security and safety by inhibiting crop growth and accumulating Cd in edible parts. The results of the study showed that the order of Cd accumulation ability of the three oilseed crops was sunflower > rapeseed > soybean. The yield and quality were reduced by treatment with different Cd concentrations (Wang et al, 2022).

Despite the abundance of information on the isolated effect of drought or heavy metals on the growth and development of sunflower, there is very little data in the literature on how drought affects the accumulation of heavy metals in the plant. The Orenburg region belongs to the risky farming zone due to the lack of soil moisture and frequent droughts. Therefore, our study aimed at studying the effect of combined stress, namely, various moisture conditions and increasing concentrations of the most common pollutants of agricultural soils - Cd and Pb on the accumulation of pollutants in phytomass and the formation of sunflower seedlings, is of great importance. The results of this study can be used by farmers to assess the risks of agricultural production in regions with both limited and sufficient moisture.

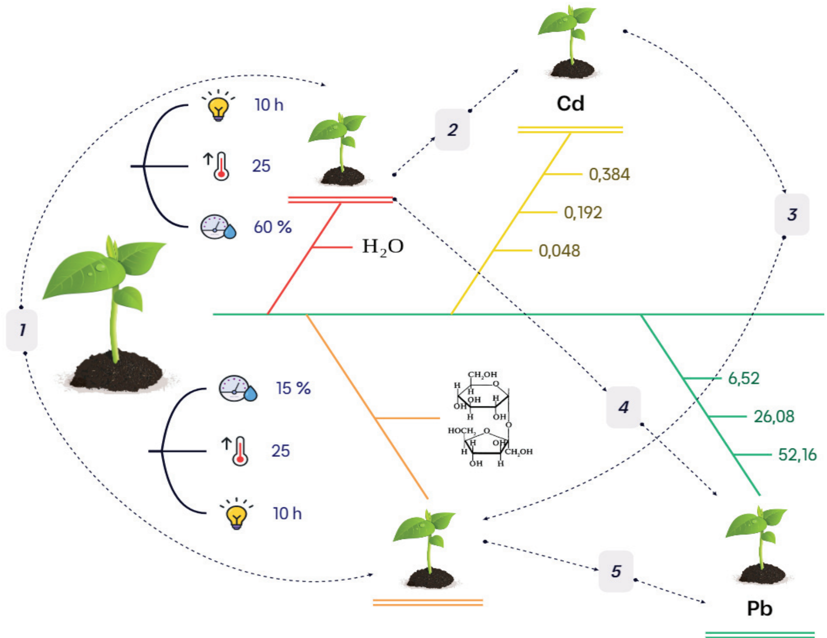
2 Materials and methods

The object of the study is *Helianthus annuus* L. 'Poseidon 625'.

A series of laboratory experiments were conducted in a JIUPO BPC500 climate chamber. Moistened soil was placed in containers with subsequent sowing of sunflower seeds. Control samples were grown at a chamber humidity of 60% with daily watering with distilled water. The experiments were conducted under constant conditions: 10-hour photoperiod, temperature +25 ºC. A total of 5 series of experiments were conducted in triplicate, the scheme of which is presented in Figure 1

Drought conditions were simulated by reducing the humidity in the chamber to 15% with watering with distilled water during the first 2 days after sowing, and then with a solution of an osmotic substance (sucrose 7.4%) - osmopriming, until the seedlings reached the age of 8 days.

To study the characteristics of heavy metal accumulation in sunflower phytomass and to assess the degree of their influence on seedling growth, solutions of cadmium (Cd) and lead (Pb) acetate salts were added to the soil in quantities containing 0.048; 0.0192; 0.384 mg Cd and 6.52; 26.08; 52.16 Pb per 1 kg of air-dry soil. The soil was thoroughly mixed and infused for two weeks for better distribution of metals.



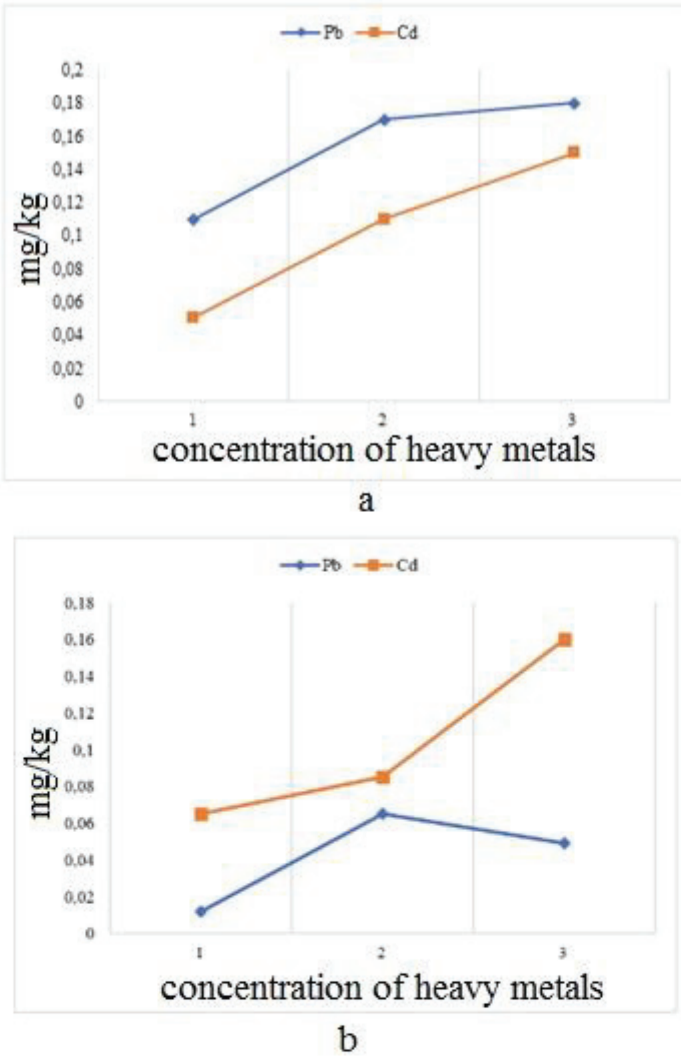
1-Normal soil moisture conditions and osmopriming; 2-Application of cadmium acetate solution in increasing concentrations to the soil while ensuring normal moisture conditions; 3-Application of cadmium acetate solution in increasing concentrations to the soil under osmopriming conditions; 4-Application of lead acetate solution in increasing concentrations to the soil while ensuring normal moisture conditions; 5-Application of lead acetate solution in increasing concentrations to the soil under osmopriming conditions

Fig. 1. Experimental research scheme.

The concentration of heavy metals in the phytomass of seedlings was determined by the atomic absorption method on a KVANT-2AT spectrometer in accordance with GOST 30692-2000 (2001). Morphometric parameters were determined using an ADA Mechanic 150 Pro electronic caliper with an accuracy of 0.1 mm. Statistical data processing includes the calculation of standard mathematical indicators - the mean value with an error and the variation coefficient. Tukey's HSD test was used to establish the degree of influence of drought stress and various Me concentrations on the growth indicators of seedlings. Spearman's rank correlation coefficient was used to establish the relationship between various morphometric parameters of seedlings under different soil moisture supply conditions.

3 Results and Discussion

When incubating sunflower seedlings in soil containing increasing concentrations of cadmium (Cd) and lead (Pb), it was found that under conditions of normal moisture supply, the concentration of heavy metal ions in sunflower phytomass increases proportionally to the increase in their concentration in the soil solution - from 0.051 mg / kg to 0.15 mg / kg when Cd is added to the soil and from 0.1 to 0.18 mg / kg when increasing concentrations of Pb are added. However, under the influence of combined stress (heavy metals + osmotic stress simulating conditions of soil moisture deficiency), it was noted that Cd absorption by seedlings increases from 0.07 mg / kg to 0.2 mg / kg compared to conditions of normal soil moisture supply. Under the same conditions, Pb absorption is significantly reduced - to 0.05 mg / kg at the maximum concentration of Me in the soil. In this case, as in the case of normal soil moisture, the tendency for Cd to increase its content in the phytomass of seedlings is maintained, and with an increase in the concentration of Pb in the soil, the opposite tendency is observed - the adsorption of lead ions from the soil solution decreases. Probably, increased doses of Pb in combination with drought cause changes in the anatomical and morphological structure of the root system of seedlings, significantly inhibiting its work (Figure 2 a, b).



1 – Pb concentration 6.52 mg/kg, 2 – Pb 26.08 mg/kg, 3 – Pb 52.16 mg/kg; 1 – concentration of Cd 0.048 mg/kg, Cd 0.192 mg/kg, Cd 0.384 mg/kg

Fig. 2. Content of cadmium (Cd) and lead (Pb) in the phytomass of 8-day-old sunflower seedlings under conditions of: a – sufficient moisture, b – physiological drought.

The study of growth indices of sunflower seedlings under the conditions of Experiment 1 allowed us to establish that water deficiency leads to changes in their descriptive indices. Under conditions of sufficient soil moisture, the size of the aboveground part of sunflower seedlings is almost twice the size of the root system. The growth of the aboveground part is ensured by the rate of hypocotyl growth regardless of moisture conditions ($r = 0.9$, at $p < 0.05$ according to Spearman), and the formation of the epicotyl and, accordingly, the formation of the first true leaves depends on the degree of growth of the photosynthetic surface of the cotyledons (r = 0.8, at $p < 0.05$ according to Spearman), and not on the growth rate of the hypocotyl knee.

Simulation of drought conditions with a sucrose solution prevents the growth of the aboveground part of seedlings and promotes the activation of the root system growth. Under the influence of drought stress, the height of seedlings, the size of the root system, epicotyl, hypocotyl, length and width of cotyledons decreases by 25% (r from 0.0002 to 0.3 at $p < 0.05$ according to Tukey's HSD test). Powerful development of the root system is aimed at ensuring the growth and development of the hypocotyl (r = 0.9, at $p < 0.05$), and not at increasing the parameters of the cotyledons and / or the formation of the first true leaves, which significantly slows down the growth and development of seedlings. Due to the inhibition of epicotyl development, an increase in the size of the hypocotyl knee and an increase in the area of the photosynthetic surface of the cotyledons occurs (r = - 1.0) to ensure photosynthetic activity and normalize metabolic processes under the critically increasing effect of the drought factor.

Under conditions of osmotic stress, the root system actively grows, but an acute moisture deficit in the soil inhibits the growth of the hypocotyl with the simultaneous growth of the cotyledon mesophyll (r = 0.8), which promotes the accumulation of water and nutrients in the cotyledons to ensure further growth of seedlings and the formation of the first and second pairs of true leaves (Table 1).

Table 1. Morphometric indices of seedlings under conditions of different levels of soil moisture supply.

Samples	Morphometric parameters, cm													
	sprouts								cotyledons					
	aboveground		underground		epicotyl		hypocotyl		length		width		thickness, mm	
	average	Cv, %	average	Cv, %	average	Cv, %	average	Cv, %	average	Cv, %	average	Cv, %	average	Cv, %
normal hydration														
Control 1*	9.6 ±1.7	19	6.9 ±1.7	24	1.6 ±1.5	40	9.0 ±1.7	19	3.2 ±0.7	25	1.8 ±0.3	18	1.12 ±0.2	16
osmopriming														
Control 2**	8.1 ±1.5	18	15.0 ±4.8	33	0.5 ±0.2	38	7.4 ±1.1	15	2.3 ±0.3	11	1.6 ±0.2	12	1.13 ±0.1	7

* - daily watering with distilled water; ** - watering with 7.4% sucrose solution from the third day of the experiment

In experiment 2, under normal moisture conditions, the introduction of increasing tested concentrations of cadmium into the soil promotes powerful development of the root system with simultaneous suppression of the aboveground part. A reliable decrease in the hypocotyl length was noted with an increase in the Cd concentration in the soil (r from 0.02 to 0.3 at $p < 0.05$ according to the Tukey HSD test). The morphometry of the cotyledons changes insignificantly. The obtained data indicate the fact of manifestation of the barrier function by the root in relation to the entry of cadmium into the plant. While such a defense mechanism, provided by the growth of the root system, is active, the root is able to deposit cadmium, as a result of which the seedling grows and develops normally. However, with an increase in concentration to values of 0.192 and 0.384 mg / kg, despite the continuing growth of the root system, cadmium overcomes the protective barrier of the root and the growth of seedlings is suppressed more significantly.

In experiment 3, with the simultaneous effect of two stress factors - drought and testing concentrations of cadmium, inhibition of the growth of the main root by more than two times, compared with the conditions of normal moisture supply, and an increase in the length of the above-ground part of the seedlings (due to an increase in the hypocotyl) are determined - 0.002 and 0.004 at $p < 0.05$ according to the Tukey HSD test. However, a significant increase in the number of lateral roots and an increase in the root system in volume are observed, which confirms the fact that the main mechanism of protection from Cd ions is the work of the root system. Also, as in the case of sufficient moisture, high concentrations of cadmium (0.384 mg / kg) critically weaken the protective systems of the root and the growth processes of seedlings are blocked. The morphometry of cotyledons under combined cadmium and drought stress changes insignificantly, with the exception of their length, which significantly increases with an increase in the cadmium concentration in the soil (r from 0.01 to 0.009 at $p < 0.05$ according to Tukey's HSD test) (Table 2).

Table 2. Morphometric indices of seedlings under conditions of mutual influence of different moisture conditions and cadmium stress.

Samples	Morphometric parameters, cm													
	sprouts								cotyledons					
	aboveground		underground		epicotyl		hypocotyl		length		width		thickness, mm	
	average	Cv, %	average	Cv, %	average	Cv, %	average	Cv, %	average	Cv, %	average	Cv, %	average	Cv, %
normal hydration														
Cd 0.048	8.0 ±1.1	13	10.7 ±2.5	23	0.6 ±0.2	35	7.4 ±1.2	16	2.8 ±0.4	13	1.8 ±0.2	13	1.03 ±0.1	14
Cd 0.192	7.2 ±1.5	20	10.8 ±2.4	22	0.8 ±0.4	56	6.4 ±1.4	22	2.9 ±0.4	13	2.0 ±0.2	11	1.12 ±0.1	10
Cd 0.384	7.4 ±1.6	18	13.0 ±6.0	46	0.9 ±0.4	43	8.4 ±1.4	16	3.0 ±0.4	15	1.8 ±0.2	12	1.14 ±0.1	12
osmopriming														
Cd 0.048	9.0 ±2.0	22	5.0 ±0.8	14	0.7 ±0.2	31	8.3 ±2.0	24	2.8 ±0.1	5	1.7 ±0.1	9	1.07 ±0.2	17
Cd 0.192	10.2 ±2.1	21	4.0 ±0.8	20	0.6 ±0.1	20	9.6 ±2.1	22	2.6 ±0.2	6	1.7 ±0.1	8	1.07 ±0.2	17
Cd 0.384	7.5 ±2.7	36	6.0 ±1.2	20	0.8 ±0.2	28	6.7 ±2.5	37	3.2 ±0.7	21	2.0 ±0.1	6	1.13 ±0.2	14

In experiment 4, when studying test concentrations of lead on sunflower seedlings under conditions of sufficient moisture supply, the same tendency in growth processes as with cadmium is observed - the root is the main barrier to lead entering the above-ground part. Even a low concentration of Pb activates significant growth of the root system compared to the control, which ensures normal development of seedlings (r from 0.003 to 0.008 at $p < 0.05$ according to Tukey's HSD test). However, under the condition of sufficient moisture supply, at the maximum studied concentration (52.16 mg / kg), the power of root system development is reduced by half. This indicates the suppression of the reserve function of the root in relation to lead.

In experiment 5, under the influence of different lead concentrations under conditions of insufficient soil moisture, a significant inhibition of the root system function is observed (r from 0.002 at $p < 0.05$ according to Tukey's HSD test) along with an increase in the above-ground part of the seedlings (r 0.0007 at $p < 0.05$ according to Tukey's HSD test). This indicates that lead begins to actively enter the above-ground part of the plant due to the weakening of the barrier function of the root. At the same time, a tendency towards a decrease in the morphometric parameters of the cotyledons is observed, especially their thickness (r from 0.0002 to 0.003 at $p < 0.05$ according to Tukey's HSD test), which indicates the activation of the depositing function of the stem with respect to lead. At the maximum studied concentration of lead (52.16 mg/kg), blocking of seedling growth is observed, which indicates a critical accumulation of Pb both in the root system and in the phytomass (Table 3).

Table 3. Morphometric indices of seedlings under conditions of mutual influence of different moisture conditions and lead stress.

Samples	Morphometric parameters, cm													
	sprouts								cotyledons					
	aboveground		underground		epicotyl		hypocotyl		length		width		thickness, mm	
	average	Cv, %	average	Cv, %	average	Cv, %	average	Cv, %	average	Cv, %	average	Cv, %	average	Cv, %
normal hydration														
Pb 6.52	9.5 ±1.5	16	14.2 ±3.7	26	1.0 ±0.4	42	8.5 ±1.5	17	2.9 ±0.3	12	2.1 ±0.3	14	1.10 ±0.1	10
Pb 26.08	9.4 ±2.0	21	16.0 ±5.4	34	0.6 ±0.2	34	8.7 ±1.8	20	2.9 ±0.4	15	1.8 ±0.2	14	1.09 ±0.1	13
Pb 52.16	9.0 ±1.5	15	9.5 ±1.7	18	1.1 ±0.6	57	8.0 ±1.4	15	3.1 ±0.7	21	1.9 ±0.4	20	1.06 ±0.1	12
osmopriming														
Pb 6.52	8.4 ±1.8	21	7.5 ±0.7	10	2.1 ±1.1	52	6.3 ±0.7	12	2.5 ±0.2	7	1.7 ±0.1	5	0.74 ±0.12	11
Pb 26.08	11.9 ±1.8	15	4.7 ±0.8	18	2.7 ±0.8	30	9.2 ±1.4	16	2.7 ±0.4	16	1.7 ±0.2	12	0.98 ±0.1	10
Pb 52.16	10.0 ±0.7	7	4.5 ±1.0	23	1.6 ±0.4	8	8.4 ±0.7	8	2.2 ±0.3	15	1.5 ±0.2	11	0.69 ±0.1	18

4 Conclusion

Based on the results of the conducted study, a number of conclusions were formulated:

- It was established that the absorption of cadmium by seedlings under conditions of soil moisture deficiency increases almost 2 times compared to conditions of normal soil moisture supply with an increase in the concentration of Cd^{2+} in the soil solution. At the same time, under these same conditions, the absorption of Pb^{2+} decreases significantly - on average more than 5 times.
- It was revealed that simulating drought conditions with a 7.4% sucrose solution prevents the growth of the above-ground part of seedlings compared to normal moisture and promotes the activation of root system growth by 2.2 times.
- It was proven that the introduction of increasing doses of cadmium into the soil under normal moisture supply has a significant effect on seedling growth. The size of the aboveground part decreases by 1.1, 1.2 and 1.3 times, and the size of the root system increases by 1.5, 1.6 and 2 times with an increase in the concentration of Me in the soil compared to the control. Under the influence of combined stress (Cd + drought), the growth of roots in length is blocked. The size of the main root decreases by 2.5 times under the influence of maximum doses of cadmium in the soil. However, active development of adventitious roots of the "goose foot" type is observed, which contributes to the active absorption and accumulation of Cd^{2+} in the phytomass of seedlings even under the condition of a deficit of soil moisture.
- It has been shown that the introduction of increasing doses of lead into the soil under normal moisture supply has an effect on the growth of seedlings similar to Cd. Under conditions of moisture deficit, maximum lead concentrations suppress the growth of sunflower seedling roots by more than 3 times and simultaneously stimulate the growth of the above-ground part - by 1.2 times compared to the control. Probably, high

concentrations of Pb contribute to the suppression of the barrier function of the root in relation to heavy metals and the activation of the storage function of the stem, as the next line of protection of seedlings from the damaging effect of combined stress.

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References

1. S.U. Rahman, M.F. Nawaz, S. Gul, G. Yasin, B. Hussain, Y. Li, H. Cheng, State-of-the-art OMICS strategies against toxic effects of heavy metals in plants: A review. Ecotoxicol Environ Saf., **242**, 113952 (2022) doi: 10.1016/j.ecoenv.2022.113952
2. Y. Zhang, C. Yang, S. Liu, Z. Xie, H. Chang, T. Wu, Phytohormones-mediated strategies for mitigation of heavy metals toxicity in plants focused on sustainable production. Plant Cell Rep, **43**(**4**), 99 (2024) doi: 10.1007/s00299-024-03189-9
3. J. Sharma, S. Kumar, P. Singh, V. Kumar, S. Verma, P. Khyalia, A. Sharma, Emerging role of osmoprotectant glycine betaine to mitigate heavy metals toxicity in plants: a systematic review. Biol Futur., **75**(**2**), 159-176 (2024) doi: 10.1007/s42977-023-00198-9
4. J. Li, X. Li, P. Han, H. Liu, J. Gong, W. Zhou, B. Shi, A. Liu, L. Xu, Genome-wide investigation of bHLH genes and expression analysis under different biotic and abiotic stresses in Helianthus annuus L. Int J Biol Macromol, **189**, 72-83 (2021) doi: 10.1016/j.ijbiomac.2021.08.072
5. H. Shi, J. Hou, D. Li, H. Hu, Y. Wang, Y. Wu, L. Yi, Comparative transcriptome and coexpression network analysis reveals key pathways and hub candidate genes associated with sunflower (Helianthus annuus L.) drought tolerance. BMC Plant Biol, **24**(**1**), 224 (2024) doi: 10.1186/s12870-024-04932-w
6. Y. Yang, X. Zhou, B. Tie, L. Peng, H. Li, K. Wang, Q. Zeng, Comparison of three types of oil crop rotation systems for effective use and remediation of heavy metal contaminated agricultural soil. Chemosphere, **188**, 148-156 (2017)
7. J. Zhou, L.H. Chen, L. Peng, S. Luo, Q.R. Zeng, Phytoremediation of heavy metals under an oil crop rotation and treatment of biochar from contaminated biomass for safe use. Chemosphere (2020) doi: 10.1016/j.chemosphere.2020.125856
8. Chen, I. Muhammad, Y. Zhang, W. Hu, Q. Lu, W. Wang, B. Huang, M. Hao, Transfer of heavy metals in fruits and vegetables grown in greenhouse cultivation systems and their health risks in Northwest China Sci. Total Environ., **766**, 142663 (2021)
9. S.S. Dhiman, X. Zhao, J. Li, D. Kim, V.C. Kalia, I.W. Kim, J.Y. Kim, J.K. Lee, Metal accumulation by sunflower (Helianthus annuus L.) and the efficacy of its biomass in enzymatic saccharification. PLoS One (2017) doi: 10.1371/journal.pone.0175845
10. K. Wang, Y. Li, C. Liang, Closed-loop evaluation on potential of three oil crops in remediation of Cd-contaminated soil, J Environ Manage (2022) doi: 10.1016/j.jenvman.2022.115123
11. GOST 30692-2000 Feed, compound feed, compound feed raw materials. Atomic absorption method for determining the content of copper, lead, zinc and cadmium, Publishing house of standards, Moscow, 8 (2001)