BIO Web of Conferences **238**, 02009 (2026) https://doi.org/10.1051/bioconf/202623802009
ESDCA 2026

Analysis of the impact of soil pollution with heavy metals on the ontogenesis of oil crops in arid conditions (using *Helianthus annuus* L. as an example)

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Abstract. This paper presents the results of a study examining the combined effects of drought and increasing concentrations of the most common agricultural soil pollutants - cadmium (Cd) and lead (Pb) - on the vegetation of *Helianthus annuus* L. Phenological observations were conducted regularly in a field experiment throughout the entire vegetative development period and focused on recording the main phenophases, from seedling emergence to head maturation. A delay in seedling emergence was observed in soils contaminated with heavy metals and with a soil moisture deficit, as well as an increase in the sunflower vegetation period under the influence of Cd and a reduction under the influence of Pb, primarily due to an extension/shortening of the flowering and seed filling periods, respectively. It was established that the biological minimum temperatures required to initiate a certain phenophase under Pb soil contamination do not differ from the control, while under the influence of Cd, the opposite trend was revealed: the heat requirement of plants increases dose-dependently. Increased dose-dependent accumulation of Cd in sunflower seeds was established.

1 Introduction

Soil pollution with toxic heavy metals (HMs) is currently a serious environmental problem requiring increased attention. Due to the rapid pace of industrialization, it is only worsening year after year. Even in trace amounts, heavy metals and metalloids such as chromium, lead, mercury, cadmium, and arsenic are toxic and carcinogenic and pose a serious threat to agricultural production and human health [1].

There are currently numerous studies describing the negative impact of pollutants on metabolic and physiological processes in plants, as well as confirming the presence of HMs in final crop products. The yields of global food crops (barley, wheat, and sunflower) are declining due to agro-technogenic soil degradation, and the cumulative effects of heavy

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metals in food chains can negatively impact the health of humans and other living organisms [2-3].

The distribution of heavy metals in soil at various stages of crop growth and development is currently being actively studied in the global literature. For example, in wheat, the mechanism of soil response to the transformation of heavy metal forms depending on the vegetation phase (tillering, filling, and ripening) was studied, and the main periods and factors influencing the accumulation of cadmium and lead in wheat grain were identified [4-6]. The negative impact of lead ions during the reproductive development phase of sunflower has been described [7].

Along with heavy metal pollution, global climate change, particularly drought, poses another serious threat to agricultural productivity and food security. Abiotic stresses caused by climatic factors and/or pollutants cause significant damage to agriculture. Furthermore, climate change, which leads to aridity, will exacerbate the effects of heavy metals and increase their negative impact on crops. Drought, soil salinization, and exposure to heavy metals and pesticides are serious environmental problems that require close attention and comprehensive study [8-9].

Despite extensive literature on the impact of drought and heavy metals on crop growth and development, there is very little data on the combined effects of drought and heavy metals during different periods of crop development, including sunflower. The Orenburg region is considered a high-risk agricultural zone due to insufficient soil moisture and frequent droughts. Therefore, our study, which explores the impact of combined stress - specifically, drought and increasing concentrations of the most common agricultural soil pollutants, Cd and Pb - on the development of one of the world's food crops, is of significant importance. The results of this study can be used by farmers to predict risks in agricultural production.

2 Materials and methods

The study subject was *Helianthus annuus* L. 'Poseidon 625'.

The Orenburg region has a sharply continental climate. Situated in the dry steppe zone, the region is considered a risky agricultural zone due to its insufficient moisture.

The field experiment was conducted on the experimental plot of the Orenburg State University (OSU) Botanical Garden. The study subject was *Helianthus annuus* L. (annual sunflower).

The experimental groups were sown three times manually in pots with a previously restored soil horizon. The seeding depth was no more than 5 cm. The seeding rate was 80,000 seeds per hectare, with the value converted to an experimental pot with an area of 1 m2. Experimental variants:

1. Control (C)
2. Experimental groups of plants with Cd (addition of $Cd(CH_3COO)_2$ to the soil at concentrations of 0.384 mg/kg, 0.768 mg/kg, and 1.536 mg/kg).
3. Experimental groups of plants with Pb (addition of $Pb(CH_3COO)_2$ to the soil at concentrations of 13.04 mg/kg, 26.08 mg/kg, and 52.16 mg/kg) (Fig. 1).

Growing containers (V=150 L) were sunk deep into the soil to the horizon. Cd and Pb salts were added in the fall of the year preceding planting to ensure their uniform distribution within the soil structure. The soil in the containers was medium-humus, medium-deep, heavy loamy chernozem (the main soil type characteristic of the Orenburg region). Conditions of soil moisture deficiency were simulated by covering the root zone of plants at the edge of the growing container with polyethylene film.

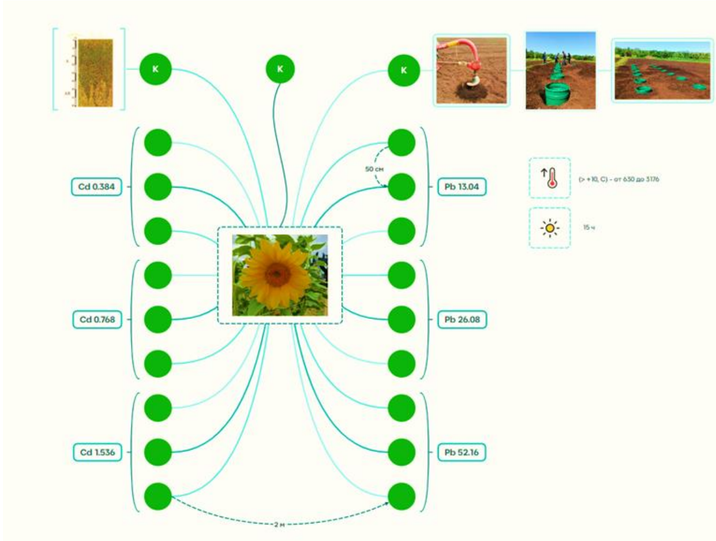

Figure 1. Field experiment layout diagram.

Phenological observations were conducted throughout the entire crop development period, recording the onset dates of eight developmental stages: 1 – sowing – seedlings, 2 – seedlings – first pair of true leaves, first pair of true leaves – second pair of true leaves, second pair of true leaves – inflorescence formation, 3 – inflorescence formation – flowering, 4 – flowering – achene formation, 5 – achene formation – achene filling, 6 – achene filling – achene maturation.

HM concentrations in seedling seeds were determined using the atomic absorption method on a KVANT-2AT spectrometer in accordance with GOST 30692-2000.

Tukey's HSD test was used to determine the impact of drought stress and various Me concentrations on the course of sunflower vegetative development. Spearman's rank correlation coefficient was used to establish relationships between different phases.

3 Research results

During observations of the course of sunflower vegetation in field experiments, it was established that soil contamination with initial concentrations of heavy metals affects the passage of interphase periods during the ontogenesis of the crop (Fig. 2).

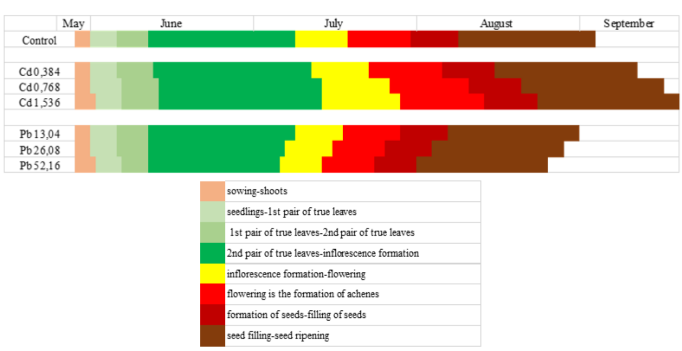

Figure 2. Phenospectrum of sunflower under conditions of soil contamination with heavy metals.

The maximum concentrations of cadmium and lead tested slightly inhibited cotyledon emergence, delaying seedling emergence by 1-2 days compared to the control. Intermediate and maximum cadmium concentrations increased the period from seedling emergence to the formation of the first pair of true leaves. The maximum lead concentration also had this effect. However, the effect of heavy metals was not statistically significant ($r = 0.32$). The presence of lead in the substrate had no effect on the duration of the interphase period from the formation of the first pair of true leaves to the formation of the second pair of true leaves, corresponding to the value in the control experiment and lasting approximately 6 days. Intermediate and maximum cadmium concentrations slightly increased this period, lasting approximately 7 days.

The period from the formation of the second pair of true leaves to inflorescence initiation was long, lasting approximately 28 days under the control experiment. However, when heavy metals were added to the substrate, significant differences were observed between the cadmium and lead experimental groups. The application of various cadmium concentrations significantly (at $p \leq 0.05$) increased the duration of the aforementioned period of sunflower vegetation, with a more significant increase at higher metal concentrations – by two days with 0.384 mg/kg Cd and by three days with 0.768 mg/kg Cd and 1.536 mg/kg Cd, compared to the control. The presence of lead in the soil has the opposite effect – the higher the metal concentration in the soil, the more the fourth period of vegetation is shortened. A Pb concentration of 52.16 mg/kg leads to a decrease in the number of days required for sunflower plants to initiate inflorescence, compared to the control, and amounts to approximately 25 days.

The duration of the flowering phase in the experimental groups of plants with the minimum lead concentration is the same as the control value and is approximately 10 days. The maximum studied lead concentration (52.16 mg/kg) contributes to the early termination of this phase by two days. Due to the increased period before inflorescence formation, plants in the Cd experimental group enter the flowering phase later than in the control and lead groups. The difference is significant (at $p \leq 0.05$) and, compared to the control, is approximately 3 days, and with the lead group – up to 6 days. The flowering duration of plants growing on a substrate with the addition of cadmium also differs: the higher the metal concentration in the soil, the longer the flowering period. At the maximum concentration, the flowering duration is significantly longer ($r=0.78$) and is 15 days, i.e., 5 days longer than in the control variant of the experiment (Fig. 3).

Figure 3. Flowering phase of experimental groups of sunflower plants.

The period from flowering to achenes formation in the control is 12 days. In the experimental groups of sunflower plants incubated in pots with lead added, this phase is shortened by up to 3 days compared to the control. The onset of this interphase period in this group also occurs significantly earlier than in the control (at $p \leq 0.05$) due to a shift in the preceding phases of the vegetation cycle. Due to the extension of the 4th and 5th vegetation phases, plants in the cadmium experimental group enter the achenes formation phase significantly later than others – the difference with the control ranges from 2 to 4 days, and with plants in the lead experimental group from 3 to 6 days, depending on the increase in the metal concentration in the substrate; i.e., the higher the Cd 2+ concentration in the soil, the longer the achenes formation period.

The period from formation to achenes filling also differs among plants in different experimental groups, but these differences are insignificant. If in the group of plants incubated on soil with the addition of Pb 2+ the period of seed filling practically corresponds to the control values and is about 8–9 days, then in the plants of the experimental group with cadmium this period increases somewhat and at the maximum concentration is about 10 days.

The period from seed filling to maturation of achenes in the control group and the group of plants incubated in soil with varying doses of cadmium was virtually identical, amounting to approximately 27 days. In plants in the lead-treated group, achenes matured significantly (at $p \le 0.05$) earlier, taking approximately 23 days. Moreover, the higher the lead concentration in the soil, the faster the achenes matured.

Thus, analyzing the course of sunflower ontogenesis under conditions of adequate moisture and combined stress (HM + drought), we observed that the vegetation period in soil contaminated with cadmium significantly (at $p \le 0.05$) increased dose-dependently (from 107 days at the minimum concentration to 115 days at the maximum) compared to the control (99 days). When lead is introduced into the soil, the opposite trend is observed: the vegetation period is significantly reduced in a dose-dependent manner (at $p \le 0.05$) compared to the control (from 96 days at the minimum studied concentration to 90 days at the maximum) (Fig. 4).

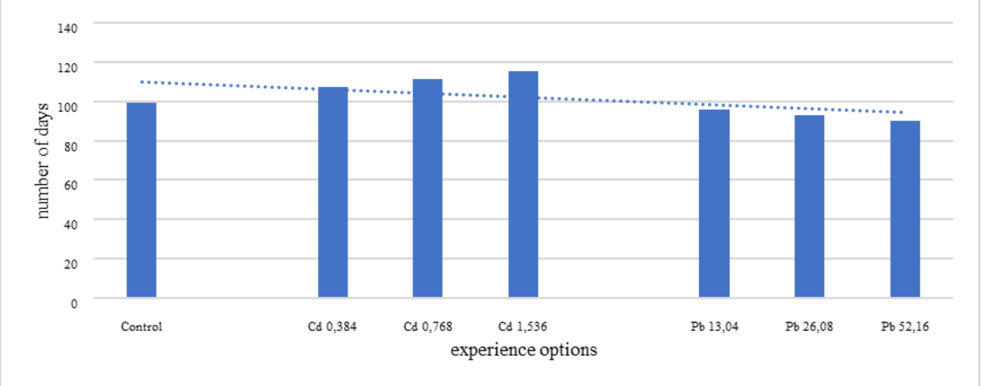

Figure 4. Duration of sunflower vegetation under conditions of sufficient moisture (control) and combined stress (TM + drought).

The amount of heat (sum of active temperatures, SAT) required for sunflower growth and development at a given stage of vegetation in soil contaminated with lead differs only slightly from control values. Pb has no significant effect on the biological minimum temperature required to initiate a given stage of plant development, and the shortening of the vegetation period is due to the plant's exhaustion in coping with lead stress. When incubating sunflower plants in cadmium-enriched soils, a significant (at $p \le 0.05$) dose-dependent increase in SAT is observed during the inflorescence formation, flowering, and seed formation phases. This suggests that cadmium exposure increases the plant's need to accumulate the heat necessary to initiate the aforementioned stages of ontogenesis, resulting in a prolonged vegetation period. Probably, due to the biovisualization ability of

Cd, sunflower plants do not experience the pronounced stress effects as in the case of Pb, therefore cadmium actively accumulates in sunflower seeds even when exposed to elevated temperatures (drought) (Fig. 5).

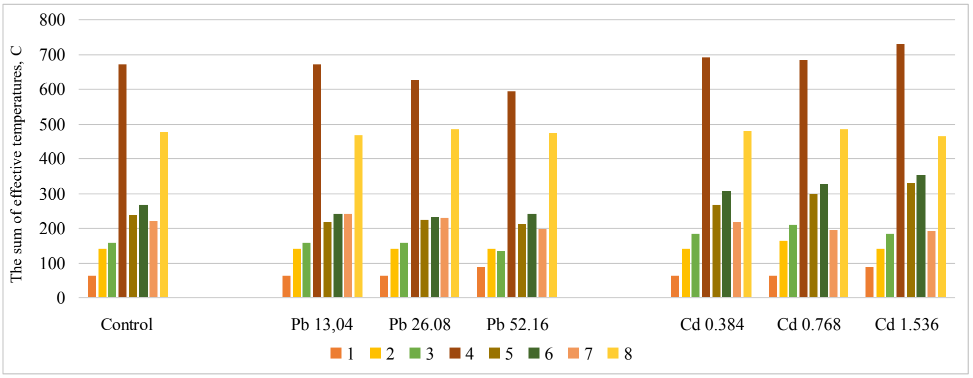

Figure 5. Fluctuations in the sum of accumulated active temperatures during the vegetation phases of sunflower under the influence of lead (top) and cadmium (bottom).

At the end of the growing season, after harvest, the concentration of Cd and Pb in sunflower seed samples was determined. Trace amounts of the studied Me in the background soil structure (control) were insignificant – within 0.003 mg/kg. In the experimental variant with plant incubation in soils containing $Cd(CH_3COO)_2$, a significant (at $p \leq 0.05$) dose-dependent increase in the Cd content in seeds was noted compared to the control – by 11-fold, 15-fold, and 16-fold using concentrations of 0.384 mg/kg, 0.768 mg/kg, and 1.536 mg/kg, respectively. This fact indicates that sunflower plants do not perceive Me as highly toxic, so cadmium freely penetrates the plant organism, bypassing its defense systems. Moreover, the sunflower vegetation period is extended. In the experimental variant incubating plants in soils supplemented with increasing doses of $Pb(CH_3COO)_2$, significantly lower lead accumulation in seeds was observed compared to Cd, indicating that sunflower plants block the element's uptake by activating various defense mechanisms. Due to the depletion of plant defenses in the fight against metal stress, sunflower vegetation is shortened. During the experiment, lead concentrations increased by an average of 5 times compared to the control, regardless of the Me concentration in the substrate. A trend toward decreased Pb accumulation in seeds was observed as its soil content increased (Fig. 6).

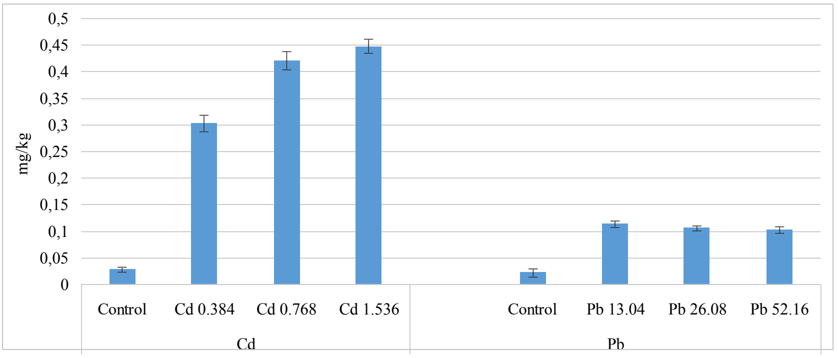

Figure 6. Cadmium (Cd) and lead (Pb) content in sunflower seed samples.

4 Conclusion

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

1. A delay in sunflower seed germination was observed in soil contaminated with Cd and Pb under conditions of soil moisture deficit. The progression of several phases of phenological development (true leaf initiation, flowering, and head formation and maturation) was increased under the influence of Cd, while it was reduced under the influence of Pb.
2. A significant (at $p \leq 0.05$) dose-dependent increase in sunflower growing season was observed in soil contaminated with Cd – up to 115 days at the maximum concentration compared to the control (99 days). A cadmium-exposed plant demonstrated an increased need to accumulate the sum of active temperatures necessary to initiate phenological phases. This may be limiting when cultivating the crop in regions with a short growing season. In soil contaminated with Pb, the sunflower growing season was significantly reduced – up to 90 days at the maximum concentration. The reduction in vegetation is caused by the general weakening of the plant organism under the influence of combined stress, which leads to plant desiccation and, consequently, premature termination of ontogenesis.
3. Increased accumulation of Cd in sunflower seeds compared to Pb has been demonstrated. The plant perceives Pb as a true toxicant and, by activating its defense systems, blocks its entry into the seeds. Cd has biovisualization properties, which facilitates its active accumulation in seeds.

Acknowledgments

This study was supported by grant No. 23-76-10060 from the Russian Science Foundation, https://rscf.ru/project/23-76-10060/

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