

The effects of cadmium, cobalt and copper on the germination of *Helianthus annuus* L. seeds

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Manuscript received: 29 November 2024; revised: 3 December 2024; accepted: 4 December 2024

Abstract

Heavy metals adversely affect the sunflower crop due to rising pollution, wherein most of the pollution is caused by industrialization and the use of herbicides, pesticides, and chemical fertilizers, which are harmful to the environment and, in particular, to the crop plants. In this respect, we have focused our attention in this work on the study of Cd²⁺, Co²⁺, and Cu²⁺ ions' effects on the germination of sunflower seeds with particular emphasis on the threats to plant development. The effect of heavy metals was determined on sterilized sunflower seeds at three different concentrations: 1 mM, 2 mM, and 4 mM of cadmium chloride (CdCl₂), cobalt chloride (CoCl₂), and copper sulfate (CuSO₄). All the experiments were conducted in the laboratory, having five repetitions with 20 sunflower seeds in each repetition. In this regard, results showed that increased heavy metal concentration significantly inhibited sunflower seed germination capacity ($p < 0.05$). Cadmium chloride, on one hand, had the highest inhibiting effect of the heavy metals used during this experiment ($p < 0.05$), having the lowest germination rates of 33% at the 4 millimolar concentration, whereas concomitant applications of both cobalt chloride and copper sulfate had inhibitive effects, depressing germination rates to as low as 51% and 63%, respectively, at the 4 millimolar concentration.

These results suggest that preventing soil contamination from heavy metals is essential for crop safety. This study recommends further studies on the development of sustainable agricultural technologies and bioremediation approaches to mitigate heavy metals in soil.

Keywords: sunflower, Cadmium, Cobalt, Copper, heavy metal salts, germination impact.

Introduction

Sunflower (*Helianthus annuus*, 2n=34) is of major importance for both Romanian and global agriculture, being used for the production of oils for food and industrial consumption [16], as well as for fatty acids such as oleic, linoleic, palmitic and stearic [12]. Globally, sunflower production of over 54 million tons was recorded on a total cultivated area of over 29 million hectares with an average yield of 1855 kg/ha. In 2023, over 1 million hectares were cultivated with a total production of just over 2 million tons with a yield of 1870 kg/ha, lower than in 2021, when the average yield per hectare was 2530 kg/ha [11].

The World Health Organization (WHO) reports the devastating effects of pollution on human health, highlighting serious problems for both adults and children: premature deaths caused by air pollution, with approximately 7 million deaths attributed to air pollution, originating from both external sources (vehicles, factories) and internal sources such as the burning of wood and traditional fuels. This type of pollution significantly increases the risk of chronic respiratory diseases, chronic bronchitis, asthma and lung cancer. In the case of children, an extremely vulnerable category, according to WHO data, 93% of them breathe air with levels of pollutants that exceed recommended limits, which affects their lung development, causes cognitive and physical delays and increases the risk of respiratory infections and allergies [9]. Exposure to pollution also increases the risk of cardiovascular diseases, heart attacks and strokes [7]. According to the WHO, it is estimated that due to air pollution, the rate of deaths caused by vascular accidents is 24% and that of deaths associated with ischemic disease is 25% [14]. The consumption of contaminated water increases the risk of spreading numerous infectious diseases. The WHO estimates that 1.8 billion people are exposed to the consumption of contaminated water, causing approximately 485,000 deaths per year [18].

The increase in pollution due to industrialization affects the soil, water and air with various contaminants. Heavy metal pollution is a problem of great interest for the environment at a global level that affects the health of living organisms, agricultural productivity and the quality of abiotic factors [8]. Heavy metals such as

cadmium (Cd^{2+}), cobalt (Co^{2+}) and copper (Cu^{2+}) are found in various substances from various sources such as industrial waste, agricultural chemicals such as pesticides and herbicides and mining [19]. Their release into the environment contaminates water, soil and air, and through water they are absorbed by plants. Once accumulated in the organs of the plant such as fruits, leaves, seeds, stems, roots of plants intended for human or animal consumption, they pose a risk to public health, food safety and affect consumer confidence in agricultural products [19]. Other factors affected by heavy metal contamination are soil fertility and drinking water [4], their bio-remediation being a slow and expensive process that is still continuing through remediation studies and research to develop sustainable solutions to protect ecosystems and maintain agricultural productivity [5].

European environmental protection directives impose legal limits for heavy metals in soil, which are 1-3 mg/kg for cadmium, 10-20 mg/kg for cobalt and 20-100 mg/kg for copper. Legal limits for heavy metals in water are 0.005 mg/l for cadmium, 0.01 mg/l for cobalt and 2 mg/l for copper. Limits for heavy metals in air are 5 ng/m³ for cadmium and 0.0001 mg/m³ for cobalt. There are no directly regulated values for copper, but these may vary depending on industrial sources [1]. In soils contaminated with heavy metals, they are absorbed by plants through their roots, being stored in cells and organs, affecting their growth and development and causing significant damage. Water contaminated with heavy metals also poses a danger to humans, causing cardiovascular, neuronal, and renal diseases, as well as risks of cancer and diabetes [2]. Exposure to heavy metals affects organisms at the cellular and genomic levels. The effect of heavy metals on sunflower plants leads to the blockage of the cell division spindle and therefore affects plant growth if they are present above the permissible doses, causing oxidative stress at the cellular level. Heavy metals can reach the fruits and, therefore, the human body. Therefore, thorough monitoring of soil contamination levels is necessary [20].

The heavy metal compounds for this experiment are from the category of salts through which the effect of three different concentrations on seed germination was studied, the salts used were: Cadmium Chloride (CdCl_2), Cobalt Chloride (CoCl_2) and Copper Sulfate (CuSO_4).

This study proposed to follow up by testing the effect of heavy metals on sunflower seeds, at different concentrations to establish which metals and at what concentration have the greatest impact on germination.

Material and Method

Germplasm

For the experiment seeds from commercial sunflower hybrid FAVORIT developed in INCDA Fundulea [24] were used, from which 100 seeds were selected for 5 repetitions of 20 seeds for testing germination capacity and were exposed to treatments with heavy metal salts.

Favorit hybrid is a conventional hybrid with semi-early maturing group, medium height, abundant dark green foliage, medium to large head, and medium-sized seeds with oval-elongated shape, dry and heat resistant, TKW of 65-70 g.

Experimental Design

The experimental setup utilised heavy metal salts, specifically copper sulfate (CuSO_4), cadmium chloride (CdCl_2), and cobalt chloride (CoCl_2), all procured from SilverChemicals, Bucharest.

For the experiment, seeds were sown in 10 repetitions in Petri dishes with filter paper substrate until germination. The seedlings were then transplanted into trays with soil substrate, where 5 ml of water was added with a laboratory pipette every 3 days for 21 calendar days. The experiment was carried out in 10 repetitions of 10 seeds for each heavy metal salt solution concentration used, with solution concentrations of 1 mM, 2 mM, and 4 mM, by diluting the stock solutions of 150 mM with distilled water.

The experiments were conducted in a controlled environment, at a constant temperature of 23°C and a relative humidity of 60%, to ensure optimal germination conditions. Light was provided for 12 hours a day, simulating natural sunlight conditions.

Data Collection and Results Analysis

A statistical analysis was conducted using a two-way ANOVA test (multivariate analysis of variance) to examine how the plants responded to various levels of toxicity and concentrations of CdCl_2 , CoCl_2 , and CuSO_4 in the early stages of growth and development. The significance was determined at a confidence level of $p < 0.05$. The results were subjected to a comparative analysis using the Tukey test. Statistical interpretation was performed using the RStudio 4.3.2 software [13].

Results and Discussion

During the experiment, following the application of treatments with varying concentrations of heavy metal salts, a reduction in germination was observed 10 days from the start of the experiment. Germination decreased depending on the type of salt used ($p < 0.05$), and directly proportional with the concentration ($p <$

0.05). The analysis of both factors, namely the type of salt and concentration taken together, revealed a significant influence on the germination capacity (Table 1).

Table 1. ANOVA test regarding the effect of the type of salt and concentration on the germination of metal salt concentrations.

Source of variance	Df	Sum Sq	Mean Sq	F value	Pr(>F)
Salt Type	3	381.6	127.21	124.782	< 2e-16 ***
Concentration	2	83.5	41.73	40.937	5.86e-14 ***
Salt Type:Concentration	6	32.8	5.47	5.362	7.03e-05 ***
Residuals	108	110.1	1.02		

“Ns”—not significant, “*”— $p < 0.05$, “***”— $p < 0.01$, “****”— $p < 0.001$.

Comparative analyses on seedlings germination rates revealed significant differences on salt type and salt concentration.

The control samples (H₂O dist) revealed an average result of 9.40 seedlings grown having a very significant difference between all the variants using heavy metals salts.

On the other hand the lowest percentage of seedlings grown were present using the treatment based on solution with CdCl₂ having lower averages of grown seedlings at any type of concentration: 65% at 1 mM, 37% at 2mM and 33% at 4mM.

For the CoCl₂ treatments the germination was significant difference between concentration of 75% at 1 mM and 55% at 2mM and 51% at 4mM.

On the other hand the lowest impact, although still significant compared to the control, was observed as the concentration of CuSO₄ increased. The germination rate decreased to 83% at 1 mM, 67% at 2 mM, and 63% at 4 mM (Table 2).

Table 2. Results regarding the effect of the type of salt and concentration on the germination of the seedlings

Salt Type	Concentration	Average	Standard deviation
CdCl₂	1 mM	65% cde	0,97
	2 mM	37% fg	0,95
	4 mM	33% g	1,16
CoCl₂	1 mM	75% bc	0,85
	2 mM	55% de	1,43
	4 mM	51% ef	1,10
CuSO₄	1 mM	83% ab	1,25
	2 mM	67% cd	1,34
	4 mM	63% cde	0,95
H₂O	Control	94% a	0,52

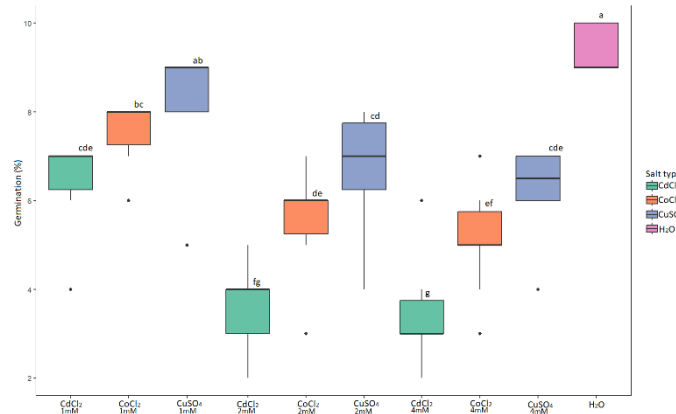
Note: Superscript letters, assigned based on Tukey's pairwise comparison test, indicate significance levels for each column value, reflecting the salt type's effect on germination. The highest values start with 'a,' and shared letters denote non-significant differences ($p > 0.05$).

The results are revealing that as high the concentration heavy metal increases the rate of viable seedlings becomes lower showing that the concentration of heavy metal salts affects direct proportionally the normal germination and development of the seedlings (Figure 1).

The results showed a significant decrease in germination capacity for sunflower seeds, depending on the concentration and type of salt used, revealing that pollution with CuSO₄, CoCl₂, and CdCl₂ salts can negatively impact plant growth.

These results are consistent with previous studies that have shown that cadmium has an inhibitory effect on plant germination. According to the study conducted by Rehman et al. [15], cadmium disrupts the absorption of water and nutrients, which explains the observed reduction in the germination rate in this experiment. Therefore, it is essential for farmers to conduct soil analyses before planting crops sensitive to heavy metals, as the results revealed a decrease in germination rates after treatment [22].

It is known that the concentrations of heavy metal might be indirectly ingested by people through plants, as the studies of R. Singh et al. [18] and Rehman et al. [15] confirm. High concentrations of heavy metals from the soil remaining in plants crops and increasing the risks of low development, low production, premature death of the crops shown by Edo et al [3].



Note: Superscript letters, assigned based on Tukey's pairwise comparison test, indicate significance levels for each column value, reflecting the salt type's effect on germination. The highest values start with 'a,' and shared letters denote non-significant differences ($p > 0.05$).

Figure 1. Boxplot representation regarding the effects of heavy metal salts on seedling germination.

Furthermore, a limitation of the experiment is that it was conducted under controlled laboratory conditions as temperature, light, and watering differing from field conditions. In the field, plants may also be affected by additional stress factors such as variable temperature, weather conditions, interactions with other soil substances, the influence of previous crops, and any treatments previously applied.

In addition to the studies of Nordberg et al. showing that the influence of preceding crops and previously applied treatments can affect the results, a factor not evaluated in this study [10]. Uncontrolled application of treatments and pesticides in previous years on earlier crops may negatively impact the biochemical state of the soil. The discharge of industrial waste without compliance with environmental regulations authorities affects the quality of groundwater and surface water, which, through irrigation, ends up being administered to the sunflower crop [21].

Also, the chemical and biochemical status of the soil and use the herbicides and pesticides influence the impact of the pollutions leading to a cumulative effect on the plants. Therefore is recommended that farmers to contribute with the methods of soil pollution decreasing, such as integrated pest management (IPM), organic amendments, crop rotation and cover crops, precision agriculture, soil testing and monitoring [17].

It is confirmed that the exposure of heavy metals in long term affects seriously the health and developing of living organisms, the effects shown on *Drosophila melanogaster* exposed to different concentrations of heavy metals [6].

Future studies in the sunflower breeding programs should be considered on creating hybrids and lines that do not require treatments with heavily dangerous substances that have a long lasting remanence in soil

Conclusions

The current study expressed the relationship between heavy metal salt concentration and sunflower seeds germination. The results indicated a highly significant impact on seedling development using CdCl₂, CoCl₂, and CuSO₄ and the levels of concentration 1mM, 2mM, and 4mM. The higher the concentration of heavy metal salt, the higher the effect would be on seed germination. In all treatments with salts, a remarkable effect could be observed as compared to the control variant: starting from the lowest concentration used-1 mM and increasingly affective germination with the increase of concentration up to 2 mM and then up to 4 mM. The strongest impact was recorded in the variants treated with cadmium chloride, followed by cobalt chloride, while for cuprum sulfate, it was the weakest; however, it remarkably reduced the rate of germination.

These results create awareness among farmers and agricultural producers of the need to implement sustainable agronomic practices in order not to compromise crop plants, soil, and water. Long-term studies regarding these metals should be undertaken in relation to crop productivity, possibly on their bioremediation.

Acknowledgements

The authors would like to thank the publishing of this journal supported by the Institute for Plant Biotechnology of the University of Life Sciences "King Mihai I" from Timisoara.

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