Annals of Biology **40** (2) : 291-299, 2024

Impact Score : 0.32 (Scopus)

Assessing Heavy Metal Impacts on Seed Germination in Brinjal and Cowpea Plants

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(Received: March 15, 2024; Accepted: July 15, 2024)

ABSTRACT

The present investigation was carried to assess heavy metals (chromium and lead) pollutants effects on the seed germination in plants. The plants selected for the study were cowpea (*Vigna unguiculata*) and brinjal (*Solanum melongena*). The heavy metals were applied in four concentrations viz., 20, 40, 100 and 200 ppm solutions. Maximum seed germination frequency (60%) was observed with 40 ppm concentration of lead (Pb) and 20 ppm concentration of chromium (Cr) with stimulatory effect on seed germination in brinjal seeds. One hundred and 200 ppm concentrations were stimulatory in the beginning but later on became inhibitory. Around 100% germination of cowpea seeds was observed in control. The maximum seed germination frequency was observed with Pb (40 ppm) and Cr (40 ppm). All concentrations of Pb and Cr, in general, had inhibitory effects on cowpea seed germination except 20 ppm concentration of Cr after 48 and 72 h and 40 ppm concentration of Cr at 48 h. The study outlined how heavy metal contamination affected seed germination in brinjal and cowpea plants, emphasizing species-specific responses and temporal dynamics. Through experimentation and analysis, significant influences of heavy metal concentration on seed germination outcomes were observed. The analysis underscored the importance of considering plant diversity and conducting longitudinal assessments to accurately evaluate environmental impacts. By integrating findings with existing literature, insights are gained into plant responses to environmental stressors, informing strategies for sustainable land management and conservation practices. This study provides a succinct overview of the research, contributing to the broader understanding of heavy metal pollution's ecological consequences.

Key words: Heavy metals, chromium, lead, germination, brinjal, cowpea, regression

INTRODUCTION

The heavy metals pollution became a big threat to the environment sustainability, which affects flora and fauna at various levels of development (Kumar *et al.*, 2021; Arora *et al.*, 2023). The natural water as well as ground water are now contaminated by heavy metal ions due to anthropogenic and natural activities (Hansa *et al.*, 2024). The bio-magnification process of these metals in the food chain reached to an extent, where they behave as toxic materials (Raj *et al.*, 2024). Generally, metals that can form sulphides and have atomic densities more than 6 g/cm^3 are often referred to as heavy metals. All metals having an atomic number greater than 23 are considered heavy metals, with the exception of Rb, Sr, Y, Cs, Ba and Fr. This category includes roughly 40 elements (Briffa *et al.*, 2020). Even while many heavy metals are necessary micronutrients for both plants and animals, when present in high concentrations, they can be harmful. It has been declared that

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Cr, Co, Ni, Cd, Hg and Pb are the metals that humanity is most concerned about, mostly because of the pollution they produce and the fact that they are discharged into aquatic environments. Heavy metals enter the food web after being discharged into the soil matrix (Luo *et al.*, 2023). In a natural undisturbed ecosystem, the primary sources of most heavy metals are the (i) underlying bedrock, or (ii) surface materials transported in the environment.

Zhi *et al.* (2015) studied the impact of the heavy metals: Ni, Hg, Cr, Pb and Cd on seed germination and early seedling growth in the oil crop *Eruca sativa*. They found that only Ni, at higher concentrations (1 mM and above), significantly and dose-dependently reduced *Eruca* seed germination. According to Baruh *et al.* (2019), tomato seeds exhibited highest sensitivity to Cd and Cu during the germination stage. Wheat displayed maximum tolerance to Cu and Cd, while pea seedlings demonstrated a greater tolerance to Pb. Plants are sensitive to chromium, as evidenced by early seedling development or decreased seed germination. Therefore, the early phases of a seedling's growth are crucial markers of the toxicity effects of heavy metals on plants. Lead is also a highly toxic pollutant. Besides the uptake from soil and water, lead may enter the plant through aerial parts including leaf surface (Zulfiqar *et al.,* 2019). Deposition of lead on plants causes a significant reduction in seed and fruit production as well as other parameters including yield (Sultana *et al.*, 2020). Hence, this investigation was designed to evaluate the effects of Pb as well as Cr on the seed germination of brinjal and cowpea crops.

MATERIALS AND METHODS

The seeds of cowpea [(*Vigna unguiculata* (L.) Walpers ssp. *Cylindrical*] and the brinjal [(*Solanum melongena* Linn. (L.) van Eseltine], were procured from National Seeds Corporation (NSC), Meerut. Pusa-1 and Gomati varieties of brinjal and cowpea, respectively, were used. Ten plastic trays with dimensions of 25 by 30 cm were used for the experiments. For a week, the trays were kept adequately moist by filling partially with garden soil and watering them daily. Set I of the studies utilized five trays for brinjal seeds, whereas Set II of the experiment employed five trays for cowpea seeds. In each tray, 20 seeds were sown in four rows of five seeds each, with the spacing between the seeds being almost equal. Before sowing the seeds, care was taken to ensure they were apparently healthy and had been sterilized with 0.1% $HgCl_2$ for 2 min followed by sufficient washing with distilled water. Four of the five trays received treatments, while the fifth tray served as the control and received only distilled water treatment (Arora *et al.,* 2023).

The aqueous solutions of required concentrations of lead, as lead nitrate (one each for 20, 40, 100 and 200 ppm solutions) were prepared. Similarly, the aquatic solutions of chromium, as chromium sulfate (one each for 20, 40, 100 and 200 ppm solutions), were also prepared. The aqueous solutions of required pH values (5.6, 4.5, 3.5 and 2.5) were made through a dilution technique by adding the required quantities of concentrated stock solution to distilled water following the standard protocol. The pH of the prepared working acid solution for SAR experiments was further enumerated and calibrated *in vitro* using a digital pH meter (EI-111).

One tray of each set was used as a control and treated with distilled water, while the remaining four trays of each set were provided treatments. The trays with brinjal seeds (Set I) were treated with aquatic solutions of different concentrations (20, 40, 100 and 200 ppm) of both the metals. Similarly, the trays of Set II (planted with cowpea seeds) were also treated in the same manner.

Generally, after one day, the seeds began to germinate. The emergence of a radicle (1-2 mm) was considered as successful germination. The number of germinated seeds was daily counted until the count stabilized, indicating no further germination. Various treatments were administered to the seedlings on alternate days; alternating between distilled water and the required heavy metal solution. This treatment regimen continued for six days. From the data collected, the germination percentage, mean germination frequency and seed vigour were calculated.

Germination percentage = 100 x [(Number of seeds germinated)/(total number of seeds)]

Rate of germination or mean germination frequency = (Maximum number of seeds germinated)/(minimum period in which maximum seeds germinated)

Seed vigour an index of seed germination = (Σ Quitient of daily counts)/(number of days of germination).

The seed germination data obtained from the experiments were analyzed using regression statistics. To capture the behaviour of germination, multi-regression analysis was employed to determine the effects of pH and time on seed germination percentages of brinjal and cowpea plants. Main-effect plots were created to illustrate the variation in germination percentage by considering one factor at a time. Regression statistics was generated to ensure the efficacy of the model fitment, along with the modelling of statistical equations to generalize the overall behaviour of germination for both the plants. Necessary trends and effects have been illustrated using a colourful heat map.

RESULTS AND DISCUSSION

Initially, at 24 h, Pb and Cr significantly inhibited germination (Table 1). However, by 48 h, germination rates increased, though outcomes varied with metal concentration and exposure duration. Pb at 200 ppm resulted in seed rotting at 120 and 144 h, indicating severe toxicity. Mean germination frequency decreased with higher metal concentrations and exposure duration, indicating dose-dependent inhibition. Similarly, seed vigour declined notably with increased metal concentrations and exposure. Pb exhibited a stronger inhibitory effect than Cr, particularly at higher concentrations and longer exposures. Brinjal seed germination peaked at 45.0% under control conditions. Lead (Pb) and chromium (Cr) at concentrations of 20 and 40 ppm generally stimulated germination. However, at 100 ppm concentrations, they initially stimulated but later inhibited germination, sometimes causing seedling rot. At 200 ppm, Pb initially had no effect but later strongly inhibited germination. Optimal germination frequency occurred at 40 ppm Pb and 20 ppm Cr, with maximum seed vigour observed at 40 ppm for both the metals. These findings underscore the necessity of addressing heavy metal pollution for optimal crop growth and highlight the need for further research into mitigation strategies for sustainable agriculture.

Initially, at 24 h, most Pb and Cr concentrations showed lower germination rates than the control, indicating inhibition (Table 2). However, as exposure increased, germination generally improved, though reductions occurred at higher concentrations; notably 200 ppm, leading to decreased germination and seed rotting. Mean germination frequency declined consistently with heavy metal concentration, indicating a dose-dependent impact. Seed vigour decreased with higher metal concentrations, notably at 200 ppm. Cr treatments showed slightly higher germination rates than Pb, especially at lower concentrations and longer exposures. The control group exhibited maximum cowpea seed germination (100%). Most Pb and Cr concentrations inhibited germination, except for 20 ppm Cr at 48 and 72 h, and 40 ppm Cr at 48 h. The maximum germination frequency was observed with Pb (200 ppm) and Cr (40 ppm). The seed vigour of treated seeds was lower than that of the control.

Some seeds rotted or died with 100 ppm Pb and 100-200 ppm Cr. These findings underscore

Table 1. Effect of treatment with heavy metals (Pb and Cr) on germination percentage, mean germination frequency and vigour of brinjal seeds

Hours	Control (% germination)	Concentration of Pb (ppm) (% germination)				Concentration of Cr (ppm) (% germination)			
		20	40	100	200	20	40	100	200
24	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil
48	10.00	25.00	60.00	25.00	10.00	10.00	40.00	15.00	10.00
72	10.00	25.00	60.00	25.00	10.00	15.00	40.00	15.00	10.00
96	40.00	25.00	60.00	25.00	10.00	20.00	40.00	15.00	15.00
120	45.00	30.00	60.00	20.00(R)	10.00	60.00	45.00	20.00	15.00
144	45.00	30.00	60.00	20.00(R)	Nil	60.00	60.00	15.00	15.00
Mean germination frequency	9.00	6.00	30.00	12.50	5.00	12.00	10.00	4.00	3.75
Seed vigour	25	24.16	50	19.16	6.66	27.50	37.50	15.83	10.83

R–Rotting.

Table 2. Effect of treatment with heavy metals (Pb and Cr) on germination percentage, mean germination frequency and seed vigour of cowpea seeds

Hours	Control (% germination)	Concentration of Pb (ppm) (% germination)				Concentration of Cr (ppm) (% germination)			
		20	40	100	200	20	40	100	200
24	45.00	30.00	5.00	5.00	Nil	Nil	Nil	45.00	30.00
48	65.00	45.00	40.00	40.00	Nil	70.00	95.00	30.00	25.00
72	85.00	70.00	60.00	55.00	20.00	95.00	60.00	45.00	20.00
96	95.00	90.00	80.00	60.00	35.00	95.00	60.00	45.00	20.00
120	100.00	90.00	85.00	60.00	25.00	95.00	65.00	45.00	20.00
144	100.00	90.00	95.00	60.00	15.00	95.00	65.00	40.00 (R)	10.00 (R)
Mean germination frequency	20.00	22.50	15.83	15.00	8.75	31.66	47.50	45.00	12.50
Seed vigour	81.66	69.16	60.83	46.66	15.83	75	57.50	43.33	20.83

R–Rotting.

the adverse effects of Pb and Cr contamination on cowpea germination and vigour, highlighting the need to mitigate heavy metal pollution for optimal crop productivity.

The regression analysis on seed germination data reveals the impact of factors such as heavy metal concentration, seed type (brinjal or cowpea) and heavy metal type (Cr or Pb) on germination percentage over time. Time (hours) positively influenced germination, while heavy metal concentration had an insignificant effect on it, except for a marginally significant impact of Pb. Cowpea seeds exhibited higher germination rates than brinjal seeds. The presence of Pb significantly enhanced germination. Interaction effects indicated that higher heavy metal concentrations impeded the positive influence of time on germination. The interaction between cowpea seeds and Pb significantly enhanced germination, while with Cr, it was insignificant. Overall, the model explained 80.47% of the variability in germination, supported by significant ANOVA results.

The regression analysis adjusted sums of squares (Adj SS) and means squares (Adj MS), F-values and associated p-values for various predictors and their interactions in explaining seed germination behaviour. Overall, the regression model was significant ($P < 0.001$), with significant effects observed for time (hours), plant seed type and interactions between time and concentration (ppm) and between time, plant seed type and heavy metal type ($P < 00.05$). However, the concentration of heavy metals and certain interactions did not significantly impact seed germination ($P > 0.05$).

The regression equations focus on seed germination dynamics concerning time, heavy metal concentration, and plant seed, as well as heavy metal types (Table 3). Positive coefficients for time suggested a consistent increase in germination percentage over time. However, heavy metal concentration impacted germination differently, with negative coefficients indicating potential decrease in germination with higher concentrations, although significance varied. Variations between plant seed and heavy metal types

Table 3. Regression model equations for seed germination (%)

Plant seed (Type)	Heavy metal (Type)	Modelled equation for seed germination (%)
Brinjal	Control	- 34.4 + 1.161 time (h) - 0.055 conc. (ppm) - 0.004358 time (h) × time (h) + 0.000311 conc. (ppm) × conc. (ppm) - 0.001881 time (h) × conc. (ppm)
Brinjal	Cr	-24.1 + 1.186 time (h) - 0.055 conc. (ppm) - 0.004358 time (h) × time (h) + 0.000311 conc. (ppm) × conc. (ppm) - 0.001881 time (h) × conc. (ppm)
Brinjal	Pb	-11.7 + 1.065 time (h) - 0.055 conc. (ppm) - 0.004358 time (h) × time (h) + 0.000311 conc. (ppm) × conc. (ppm) - 0.001881 time (h) × conc. (ppm)
Cowpea	Control	19.2 + 1.196 time (h) - 0.089 conc. (ppm) - 0.004358 time (h) × time (h) + 0.000311 conc. (ppm) × conc. (ppm) - 0.003136 time (h) × Conc. (ppm)
Cowpea	Cr	12.1 + 1.220 time (h) - 0.089 conc. (ppm) - 0.004358 time (h) × time (h) + 0.000311 conc. (ppm) × conc. (ppm)- 0.003136 time (h) × conc. (ppm)
Cowpea	Pb	-10.4 + 1.480 time (h)- 0.089 conc. (ppm) - 0.004358 time (h) × time (h) + 0.000311 conc. (ppm) × conc. (ppm) - 0.003136 time (h) × conc. (ppm)

suggested unique germination patterns and responses to contamination. These models were valuable for understanding and potentially mitigating heavy metal effects on germination, informing tailored agricultural practices and environmental management strategies (Singh *et al.*, 2022).

The Pareto chart highlights influential predictors and interactions affecting seed germination percentage (Fig. 1). "Time (Hrs)" exhibited the highest standardized effect (5.84), followed by the interaction "Time (Hrs) x Time (Hrs)" (4.92), emphasizing time duration's compounded impact. "Plant Seed (Type)" ranked next (3.08), accentuating plant species' importance. Interactions like "Plant Seed (Type) x Heavy Metal (Type)" (CD) contributed significantly to germination variability, with a standardized effect of 2.85. The Pareto chart ranked "Heavy Metal (Type)" (D) with a standardized effect of 1.31 and "Conc. (ppm)" (B) with a standardized effect of 0.49, indicating their lesser impact. These insights prioritized factors for optimizing seed germination strategies.

Plant seed type significantly impacted germination outcomes. Brinjal seeds showed minimal germination across all conditions, while cowpea seeds displayed higher rates, especially without heavy metals. Time duration enhanced germination for both the seeds. Heavy metal type affected germination differently; brinjal germination remained inhibited across all concentrations of lead and chromium (Fig. 2).

Heavy metal presence affected cowpea seeds differently, with chromium impacting germination more than lead. Higher metal concentrations correlated with lower germination rates, particularly for cowpea seeds. The main effect plots revealed the complex interaction of plant seed type, time, heavy metal type and concentration on germination, underscoring the necessity for comprehensive management strategies to mitigate adverse effects and ensure optimal crop yield.

Interaction plots visually represented the interplay between predictors on the response variable in regression analysis. They showed how relationships changed across different levels of predictors. The significant interaction between time (Hrs) and conc. (ppm) ($P < 0.05$) indicated their combined effect on seed germination. Similarly, the plant seed (type) and heavy metal (type) interaction ($P = 0.006$) suggested combined effects on germination (Fig. 3). These findings emphasized

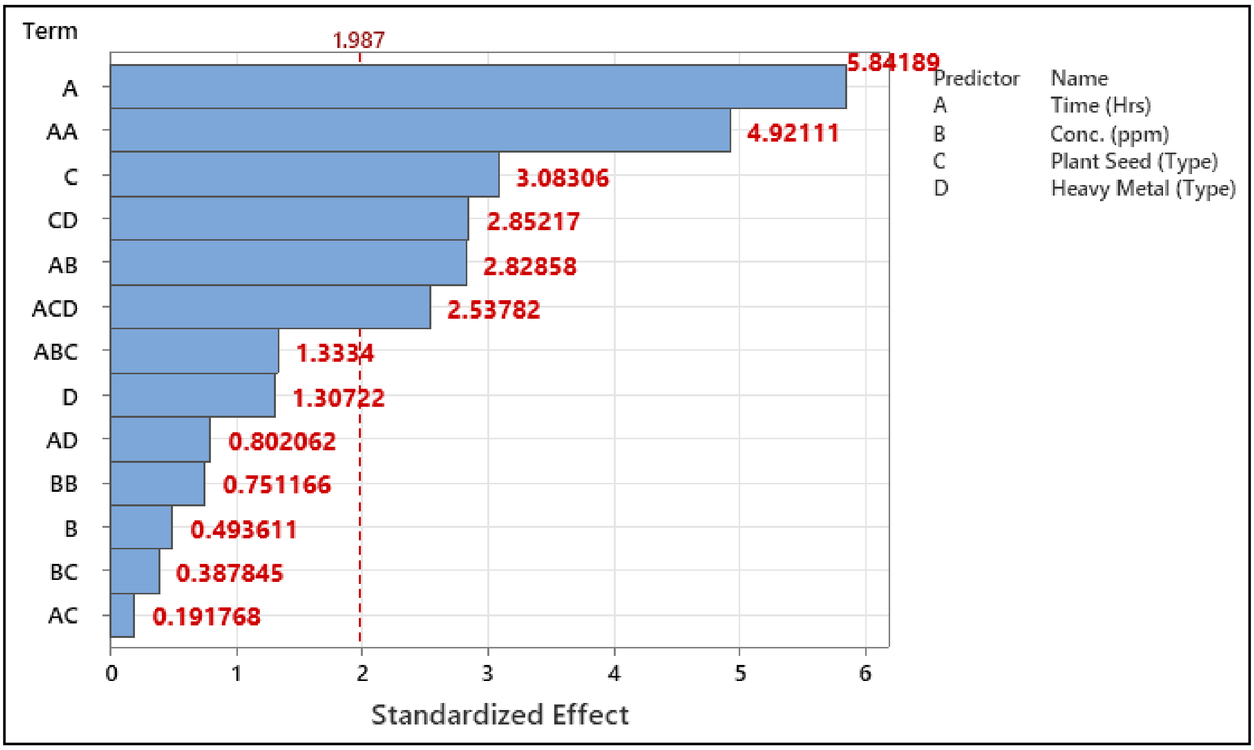

Fig. 1. Pareto chart for seed germination.

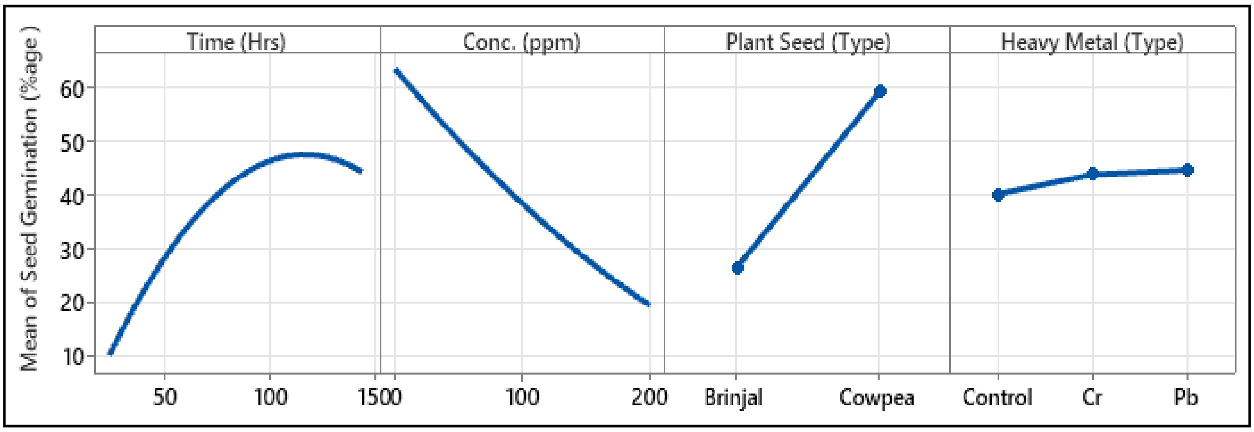

Fig. 2. Main effect plots for seed germination.

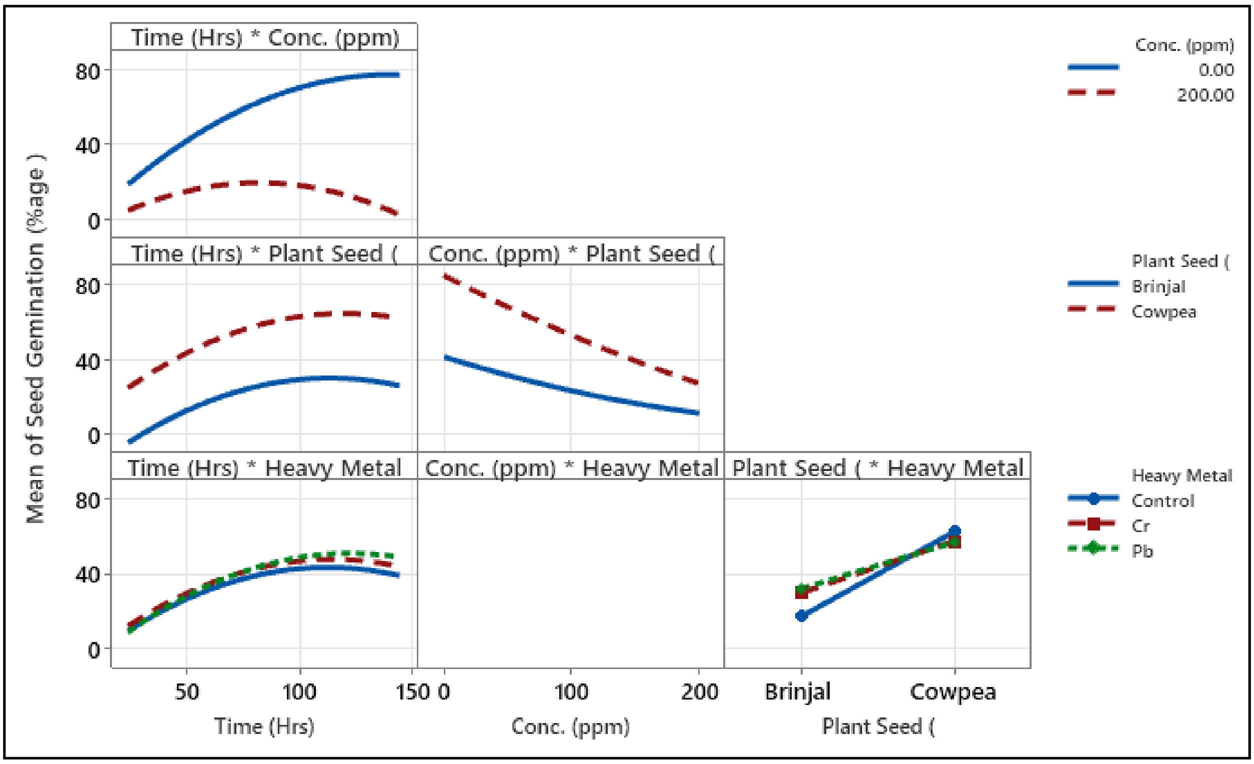

Fig. 3. Interaction plots for per cent seed germination.

considering multiple predictors for understanding their impact on seed germination. Further research is needed to fully grasp these interactions and their implications across varied conditions.

The interaction analysis suggested a significant combined effect of time, plant seed type and heavy metal type on seed germination (P=0.013), underlining their complex interplay. The heat map revealed higher germination percentages for control conditions, contrasting with lower percentages in the presence of Pb or Cr (Fig. 4). Brinjal seeds consistently exhibited low germination percentages across all conditions, while cowpea seeds showed more variation, with higher percentages over time, especially without heavy metals. Increasing Pb or Cr concentration correlated with reduced germination in cowpea seeds, emphasizing heavy metals' detrimental impact. This visualization underscored the importance of considering various factors for optimizing seed germination and crop yield.

In the control condition, both brinjal and cowpea seeds showed higher germination rates compared to heavy metal exposure. Brinjal seeds maintained consistently low germination percentages across time

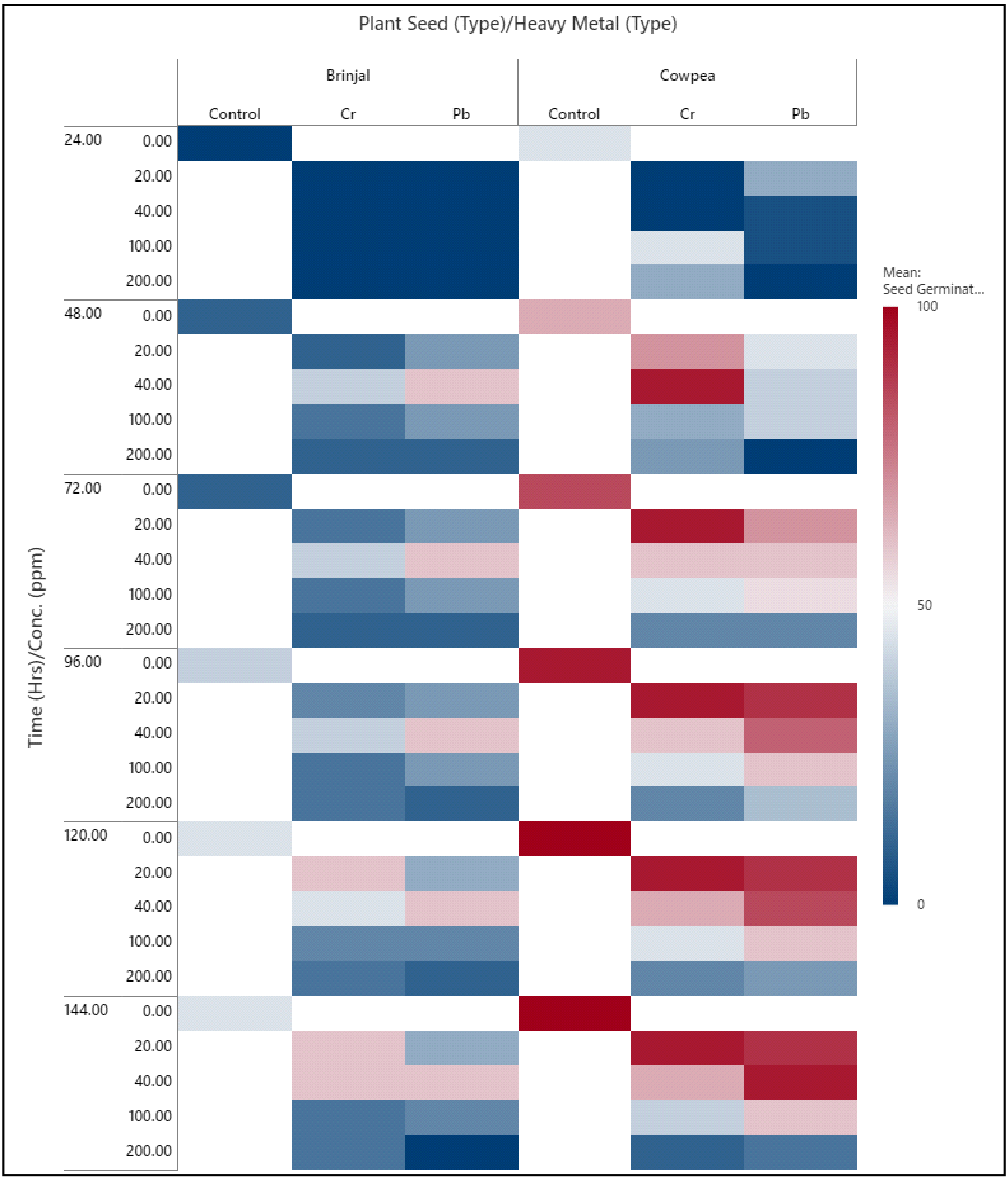

Fig. 4. Heat map.

durations, warranting further investigation. Conversely, cowpea seeds exhibited variable but generally increasing germination rates over time, suggesting their resilience in uncontaminated environments. This underscored the detrimental impact of heavy metal exposure on seed germination and emphasized the need for uncontaminated soil for optimal crop yield.

In the presence of chromium (Cr), both brinjal and cowpea seeds exhibited decreased germination compared to the control. Brinjal seeds showed a consistent decline in germination with increasing chromium concentration, indicating sensitivity to contamination. Cowpea seeds also demonstrated reduced germination, especially at higher concentrations. However, cowpea seeds exhibited some resilience to lower chromium levels compared to brinjal seeds. Overall, chromium contamination negatively affected seed germination, emphasizing the need to mitigate pollution for optimal crop growth.

In the presence of lead (Pb), both brinjal and cowpea seeds showed varied germination responses compared to the control. Brinjal seeds exhibited fluctuating germination percentages with increasing lead concentration, while cowpea seeds consistently decreased in germination percentage. At lower lead concentrations (20 ppm), brinjal seeds showed increased germination, but this effect diminished as lead concentration rose. Cowpea seeds displayed a consistent decrease in germination with higher lead concentrations across all exposure durations. Monitoring lead levels is crucial for mitigating its adverse effects on crop productivity and ecosystem health.

In contrast to the expected outright inhibition of seed germination by heavy metals, a number of reports exists indicating that many heavy metals (at least at very low concentrations) rather stimulate the germination of seeds (Bae *et al.*, 2016). It depends on the plant species as well as the specific metal and its concentration whether inhibition or stimulation of seed germination would occur. For seed germination, Sultana *et al.* (2020) selected the kenaf and mesta types. Pb and Cr chemicals were combined at concentrations of (0,0), (60,60), (80,80), (100,100), and (120,120) mg/l. When compared to the control, the germination percentage and primary growth parameters steadily decreased with increased levels of lead and chromium. According to Zhi *et al.* (2015), heavy metals other than zinc and nickel mostly affected root length, followed by shoot length or fresh seedling weight, while seed germination in *Eruca sativa* was always the last to be affected. It was found that while low concentrations of Pb and Cr are stimulatory for seed germination of brinjal, these are inhibitory for cowpea. Thus, at lower concentrations, heavy metals have micronutrient-like effects on the plants. Salt stress can seriously weaken plant cell walls and interfere with normal plant growth and development, drastically lowering yield and productivity. The primary components of the cell wall, cellulose, pectins, hemicelluloses, lignin, and suberin, are affected in terms of biosynthesis and deposition (Dabravolski and Isayenkov, 2023; Arora *et al.* 2024). Seed germination in many food crops, including *Cajanus cajan* (pigeon pea), *Zea mays* (maize), *Oryza sativa* (rice), *Triticum aestivum* (wheat), *Brassica napus* (rapeseed), *Vigna radiata* (mung bean), *Lens culinaris*/*Lens esculenta* (lentil) and *Nigella sativa* (black cumin), has been found to be negatively impacted by heavy metal-contaminated soil (Rai *et al.*, 2023).

CONCLUSION

Heavy metals pose significant threats to ecosystem health and crop growth due to their toxicity. The present study on the germination behaviour of brinjal and cowpea crops revealed that lead (Pb) and chromium (Cr) exhibited both stimulatory and inhibitory effects on seed germination, depending on their concentrations. Higher levels of Pb and Cr impaired seed germination and seedling vigour in both the crops. The importance of preventing heavy metal contamination in water and soil has been emphasized to maintain a healthy ecosystem conducive to crop growth. The research underscores the need for plant-specific assessments in environmental studies and highlights the temporal dynamics of heavy metal effects on seed germination. Soil monitoring and remediation efforts are crucial for mitigating environmental contamination and ensuring sustainable crop production. The integration of present findings with existing literature, contributed to understand plant responses to environmental stressors, guiding land management and conservation practices toward sustainable environmental stewardship.

ACKNOWLEDGEMENTS

The authors are thankful to the authorities of Maharishi Markandeshwar (Deemed to be University), Mullana, Ambala (Haryana), India, for providing necessary facilities for completing this research.

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