

Interactive Effects of Lead and Potassium on Seed Germination and Early Seedling Development of *Malva parviflora* L. Under Salinity Stress

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SUMMARY

Salinity and heavy metal toxicity are among the most critical abiotic stressors affecting plant growth, yet their interactive effects remain poorly understood. This study provides novel insights into the combined impact of salinity, potassium (K), and lead (Pb) toxicity on seed germination and early seedling development of *Malva parviflora*, a species with potential applications in stress tolerance and phytoremediation. A controlled pot experiment was conducted, subjecting plants to varying salinity and heavy metal concentrations. Our results reveal a significant reduction in seed germination under stress, with the highest germination rate observed in control plants (4.67%) and a drastic decline in the 100 mM + HHM treatment (0.34%). Biomass accumulation was notably affected, with the highest root fresh weight (RFW) recorded in the 100 mM + HHM treatment (3.33%) compared to the control (0.12%). The 50 mM + LHM treatment exhibited the highest root dry weight (RDW) and the longest root length (RL) (2%), while the control recorded the longest root length (RL) (6.5%). Leaf biomass showed a complex response, with the maximum leaf fresh weight (LFW) and leaf dry weight (LDW) in the 100 mM treatment. The findings suggest that salinity exacerbates Pb-induced toxicity, leading to severe physiological impairments in *M. parviflora*. These novel insights enhance our understanding of stress tolerance mechanisms in halophytes and highlight the potential of *M. parviflora* as a candidate for phytoremediation in metal-contaminated saline soils.

Keywords: *Malva parviflora*, salinity, heavy metal toxicity, potassium, lead, seed

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INTRODUCTION

Metals and metalloids with atomic densities greater than 6 g/cm³, collectively termed heavy metals (HMs), pose a dual challenge in plant ecology. While certain heavy metals, such as copper (Cu), chromium (Cr), and zinc (Zn), serve as essential

micronutrients in trace amounts, others, including lead (Pb), cadmium (Cd), and mercury (Hg), exhibit severe toxicity even at low concentrations (Adrees et al., 2015).

Recent studies underscore the dynamic role of heavy metal interactions in plant stress responses. They show that metal-induced oxidative stress can activate complex signaling cascades that influence growth, metabolism, and even epigenetic regulation (Kumar et al., 2019a). However, the boundary between the essentiality of micronutrients and their phytotoxic effects is still not well-defined, especially under varying environmental conditions like salinity. Gaining a deeper understanding of these intricate mechanisms is essential for developing stress-resilient crops and leveraging plant-microbe interactions for bioremediation applications (Misu et al., 2025).

Industrial and agricultural activities release heavy metals that accumulate in the environment, disrupting microbial communities and essential biogeochemical cycles. Non-point sources like fertilizers, pesticides, and sewage sludge degrade soil health, reduce microbial diversity, and impair nutrient availability. This contamination lowers crop productivity, threatens food security, and poses risks to human and animal health through bioaccumulation. Sustainable management is crucial to mitigating these impacts (Crişan et al., 2024). Trace/heavy metals in soils are not degradable; they accumulate in soils and consequently pass to plants through the food chain (Galal et al., 2019).

Soil degradation by heavy metals causes significant issues for the biotic and abiotic components of the ecosystem (Tianjiao et al., 2023). Plants growing in HM-contaminated soils show signs of stunted growth, chlorosis, root browning, and sometimes even death (Apergis and Ozturk, 2015). The accumulation of HMs consequently impacts soil health and texture due to the variation in soil pH and the ratio of essential to non-essential components, which is a global concern for agricultural output (Hafeez et al., 2023).

Lead, a toxic metal, can harm plants without causing noticeable changes in appearance or yield. In some cases, lead levels can surpass safe limits for human consumption, raising concerns about its introduction into the food chain. This has led to an increased interest in researching lead accumulation in vegetables (Raheem et al., 2014). Heavy metal toxicity impacts all plant tissues and stages of growth, particularly during seed germination and root development. The physicochemical conditions of the rhizosphere can reduce seed germination and vigor, ultimately hindering seedling growth in many cases. This underscores the significant effects of heavy metal toxicity (Adrees et al., 2015).

Lead toxicity disrupts seed morphology, physiology, and germination by inhibiting key hydrolytic enzymes like amylases and proteases, which are essential for starch hydrolysis and protein mobilization. Similar effects have been observed with other heavy metals (HMs), such as cadmium and chromium, which impair metabolic pathways and suppress early seedling growth. Recent studies highlight HM-induced oxidative stress and hormonal imbalances, particularly in gibberellin and abscisic acid signaling, further hindering germination. These findings provide new insights into the biochemical disruptions caused by HMs, emphasizing the need for targeted seed treatments and bioremediation strategies to enhance crop resilience in contaminated soils. In addition to the seeds, changes in root architecture have been

observed in plants growing in soils contaminated with heavy metals. For instance, the presence of heavy metals such as Cu, Pb, Cr, and Zn has been linked to reduced root elongation and increased development of lateral roots. Heavy metal toxicity in plants also affects leaf characteristics, including area, number, color, and thickness, by disrupting water relations. This disruption subsequently impacts various physiological processes, such as transpiration and photosynthesis (Li et al., 2023).

Potassium (K^+), a highly active ion in the soil system, is crucial for plant development. Phosphorus is considered essential to plant physiology, as is nitrogen, with nitrogen levels ranging from 1 to 3 percent by weight in plant tissue. Potassium is relatively mobile in the soil but highly mobile in the plant system, and like lithium, sodium, and chlorine, it does not combine to generate compounds (Wakeel et al., 2022). K^+ plays a crucial role in plant systems through three processes: osmoregulation, charge balancing, and enzymatic reaction activation. Incorporating ^{15}N -labelled inorganic nitrogen into protein fractions demonstrates K^+ 's involvement in protein synthesis. Within 5 hours, K^+ -sufficient tobacco plants showed 21% more ^{15}N incorporation into protein than K^+ -deficient plants (Sahi and Mostajeran, 2021).

Additionally, potassium is essential for protein synthesis and facilitates the intake of amino acids (Khare et al., 2025). According to Jose (2023) Starch synthase, the enzyme responsible for starch synthesis, requires potassium ions for activation. Low potassium ion levels lead to decreased starch concentration and the accumulation of soluble carbohydrates and nitrogen molecules in potassium ion-deficient plants, likely due to the enzyme's potassium ion requirement.

The electrochemical distinction between the cytosol and the external medium is crucial for ion transport. Plant cell membranes are relatively permeable to potassium ions because they include a variety of selective potassium ion (K^+) channels (Khan et al., 2023). K^+ is necessary for charge balancing in the cell's numerous functions. As long as the cytosol's electrochemical potential is lower than that of the outside medium, potassium ions are imported into the cell. The electrochemical potential rises with the import of potassium ions and eventually reaches equilibrium (Barlas, 2023).

The plasma lemma H^+ pump continuously releases H^+ ions from the cytosol into the apoplast, keeping the cytosol's negative charge constant and the negative membrane potential between 120 and 200 mV. According to Cochavi et al (2020), Potassium is also essential for the movement of water, minerals, metabolites, and phytohormones via the xylem and phloem. When the K^+ supply is decreased, translocation processes become less active. Potassium helps stabilize the pH between 7 and 7.5, which is ideal for most enzymatic activity. It also neutralizes numerous organic anions and other chemicals within the plant. Similarly, K^+ is necessary for the movement of organic acids through the phloem (Barlas, 2023).

Dryland salinity, also known as secondary salinity, occurs when salt accumulates in soil due to natural processes. In contrast, tertiary salinity, also known as irrigation salinity, occurs when excessive irrigation leads to salt buildup (Clarke et al., 2002). Salinity in rivers, lakes, and oceans is the amount of salt dissolved in water, including sodium chloride, magnesium sulfate, potassium nitrate, and sodium bicarbonate. Chlorinity refers to the quantity of chloride ions in a solution. Soil salinity impacts agricultural production, affecting 95 million hectares globally, but

accurately describing and quantifying it presents technical challenges (Kumar et al., 2019b; Szabolcs, 1994).

This study sheds important light on the interactive effects of salinity, lead (Pb), and potassium (K) toxicity on the early developmental stages of *Malva parviflora*, a species with potential applications in stress tolerance and phytoremediation. While the individual impacts of salinity and heavy metals on plant growth are well-documented, their combined effects remain poorly understood. This research aims to bridge this gap by examining how varying saline concentrations influence Pb and K toxicity, affecting key physiological parameters such as seed germination, root development, and biomass accumulation. To achieve this, we assessed root fresh weight (RFW), root dry weight (RDW), leaf fresh weight (LFW), leaf dry weight (LDW), and plant biomass (PBM) under different stress conditions. By elucidating whether salinity exacerbates or mitigates Pb and K toxicity, this study provides new insights into plant stress physiology and adaptive mechanisms, contributing to the development of salt and metal-tolerant plant systems for sustainable agriculture and environmental remediation.

MATERIALS AND METHODS

EXPERIMENTAL DESIGN

The pot experiment was conducted in the Botany Lab, Plant Tissue Culture, and Physiology Lab at the University of Malakand to examine the impact of K and Pb and salt stress on *Malva parviflora*. The soil was created using the following proportions: 50% clay, 30% humus, and 20% sand. The ingredients were combined and vigorously agitated until they reached a dark hue. Following this, soil was added to each container.

SELECTION OF VIABLE SEED

To select viable seeds were immersed in water, the seeds floating on the water's surface were discarded, and those that sank to the bottom were sown.

SOWING OF SEED

The soil in the pot was wet with saline water, and then, in each pot, five seeds were sown. After sowing the seeds, the control group was treated with regular tap water (0% salt), while the other groups were treated with different concentrations: 50 mM, 100 mM, 50 mM + LHM, and 100 mM + HHM.

PARAMETERS STUDIED

Shoots, Leaves and Roots Length

The length of the leaves was measured using a centimeter scale when the plants were 28 days old, while the shoot length was measured from the base of the stem to the higher tips of the plant using a scale meter at each pot. The length of the roots was measured by uprooting the plants after 28 days of germination using a digital balance.

PHYSIOLOGICAL STUDY PARAMETERS

The chlorophyll content was determined using the method of (Palta, 1990).

CHEMICALS USED

The substances used were prepared as follows: ammonium hydroxide (NH₄OH) in aqueous form, commonly referred to as ammonia water; distilled water; and an 80% aqueous solution of acetone.

PREPARATION OF AMMONIUM HYDROXIDE ACETONE SOLUTION

A solution of 0.1 N NH₄OH was combined with acetone in a volume-to-volume ratio of 1:9 (NH₄OH: acetone). Preparation of 80% acetone solution in water: Combine distilled water with reagent-grade acetone in a volumetric ratio of 2 parts water to 8 parts acetone.

PROCEDURES

The photosynthetic tissue of the plants was immersed in a solution consisting of 1 part 0.1 Normal (N) ammonium hydroxide solution and 3 parts acetone (volume to volume ratio). The mixture was then placed in a centrifuge to separate the supernatant from the mixture. The volume of the supernatant was increased to 10 ml by adding 80% acetone solution. The solution was placed in a test tube and kept at a temperature of 4°C for 24 hours. Subsequently, the absorbance was measured using the Lambda Bio method at 663 nanometers (nm and 645 nm wavelengths). The absorbance readings should fall within the range of 0.2 to 0.8. At these specific wavelengths, the absorbance of each solution was determined, and the concentrations of chlorophyll A and B were estimated using the following formulas. Absorbance data at particular wavelength values were used to estimate the chlorophyll content. The formula for chlorophyll A concentration (in mg/ml) is given as $12.7A_{663} = 7A_{663} - 2.69A_{645}$. Similarly, the formula for chlorophyll B concentration (in mg/ml) is $22.9 * A_{645} - 4.68 * A_{663}$. In the present work, the absorbance at the wavelength of 645 nm is represented by A₆₄₅, while that at 663 nm is represented by A₆₆₃. These formulas are used to measure the amount of chlorophyll in a particular sample.

RESULTS AND DISCUSSION

Malva parviflora L. is a widely distributed herbaceous plant with a remarkable ability to thrive in diverse climatic conditions, ranging from temperate to arid regions. Native to Northern Africa, Europe, and Asia, it has successfully adapted to various ecological niches, including saline and metal-contaminated soils. Beyond its ecological significance, *M. parviflora* holds substantial medicinal and nutritional value. Traditionally, its leaves and roots have been utilized in ethnomedicine for the treatment of respiratory ailments such as asthma, dermatological conditions like eczema, and inflammatory disorders. Recent pharmacological studies suggest that *M. parviflora* possesses potent antioxidant, antimicrobial, and anti-inflammatory properties, making it a promising candidate for novel therapeutic applications. However, despite its widespread use, limited research has explored its physiological adaptations to environmental stressors, particularly in saline and heavy metal-polluted habitats. This knowledge gap underscores the need for further investigation into its

biochemical resilience, stress tolerance mechanisms, and potential applications in phytoremediation and sustainable agriculture (Raheem et al., 2014).

Potassium (K^+) is a crucial element that can function as a catalyst. It arranges extra molecules in a manner that allows chemical reactions to occur. Potassium activates a minimum of 60 enzymes that are involved in plant development. Potassium, which is electrostatically attached to the enzyme surface, enhances enzymatic activity by modifying the shape of the enzyme molecule and exposing the appropriate active regions for reaction (Ahmed and Hasnain, 2014). Tobacco plants with sufficient potassium (K^+) levels show better growth compared to tobacco plants with deficient potassium (K^+) level. Potassium is essential for the absorption of amino acids and plays a crucial role in the synthesis of proteins. Leigh and Storey (1991) proposed that starch synthase is the enzyme responsible for starch synthesis and emphasized the essential role of K^+ in activating this enzyme. Therefore, when there is insufficient potassium (K^+), the amount of starch decreases while the concentration of soluble carbohydrates increases. The presence of low levels of potassium (K^+) in plants leads to an increase in the concentration of soluble carbohydrates and nitrogen compounds, while the concentration of starch decreases. This is believed to be due to the need for K^+ in enzymes like pyruvate kinase and phosphofructokinase. In the enzyme dialkyl-glycine decarboxylase, the K^+ ion is positioned in the center of an octahedron, with oxygen atoms at each of the six corners. The oxygen atoms are contributed by three amino acids, one water molecule, and the oxygen atoms from the serine and aspartate hydroxyl groups (Eisenmann and Miller, 2023).

Lead, a hazardous heavy metal, can be damaging to plants. However, plants can often accumulate significant quantities of lead without noticeable alterations in their physical appearance or productivity. Many plants can accumulate Pb at several hundred times higher than the maximum threshold allowed for human consumption. Incorporating Pb into the food chain can potentially impact human health. Therefore, investigations are necessary. The significance of Pb accumulation in vegetables is growing by Raheem et al. (2014).

The current study documented the effects of potassium (K), lead (Pb), and salt stress on *Malva parviflora*. A pot experiment was conducted in the plant tissue culture and physiology lab at the University of Malakand to investigate the impact of high and low potassium (K) and lead (Pb) levels on plants under salt stress. Salt stress affects seed germination and early seedling growth. The seed germination of *Malva parviflora* L. was different according to the environmental conditions, whether they were controlled or under stress. The current study examined the variability in seed germination and physiological characteristics of *Malva parviflora* L. seeds, which were cultivated under various stress levels: 0mM, 50mM, and 100mM of NaCl, as well as 50mM + LHM and 100mM + HHM. Data analysis indicated that increased levels of salinity, potassium (K), and lead (Pb) led to a gradual decline in seed germination. Additionally, found that the highest fresh and dry weights of roots occurred under elevated salinity, potassium, and lead levels. However, the length of the roots consistently decreased as salinity and the concentrations of potassium and lead increased. In the PBM study, the highest mean values for potassium and lead were also noted at higher salinity levels. The observed increase in plant biomass, both

fresh and dry weight, in response to salt stress and heavy metal exposure is attributed to the accumulation of metal ions in the leaf and shoot tissues of the plant.

ANOVA STUDY TRAITS

The mean square of the ANOVA indicates that the treatment significantly impacted seed germination and the fresh and dry weight of the leaves. Table 1 shows the measurements for root length, root dry weight, plant biomass, and plant biomass dry weight. These measurements are categorized as least significant, significant, and non-significant.

Table 1: Mean square of ANOVA for studied traits.

Sources	DF	GE	RFW	RDW	RL	LFW	LDW	PBM	PBDW
Replicate	2	2.07	0.3	0.002	0.3	0.02	1.92	0.096	0.0006
Treatment	4	9.77**	0.9	0.00*	23.2	0.247**	6.04**	0.649*	0.001*
Error	8	0.32	0.4	0.003	0.4	0.01	2.92	0.04	0.0002
Total	14	4	14	4	14	4	4	4	4

Keywords: Degree of freedom (DF), Germination (GE), Root fresh weight (RFW), Root dry weight (RDW), Root length (RL), Leaf fresh weight (LFW), Leaf dry weight (LDW), Plant biomass (PBM), Plant biomass dryweight (PBDW).

DESCRIPTIVE STATISTICS OF MORPHOLOGICAL CHARACTERS

The present study reveals a significant disparity in seed germination and early seedling growth between control plants and those subjected to salinity and heavy metal stress. Seed germination rates exhibited a progressive decline under increasing stress conditions, with the control group showing the highest germination rate (4.67%), followed by 50 mM NaCl (3.67%), 100 mM NaCl (2.67%), and 50 mM + LHM (1%). The most pronounced reduction was observed in the 100 mM + HHM treatment (0.34%), suggesting a synergistic toxic effect of lead and high salinity on seed viability. Biomass accumulation followed a distinct trend, with the highest root fresh weight (RFW) recorded in the 100 mM + HHM treatment (3.33%), indicating a potential compensatory response to stress. However, this was accompanied by significant reductions in root dry weight (RDW) (Figure 1) and root length (RL) (Figure 2), as, with the control group maintaining the longest roots (6.5%).

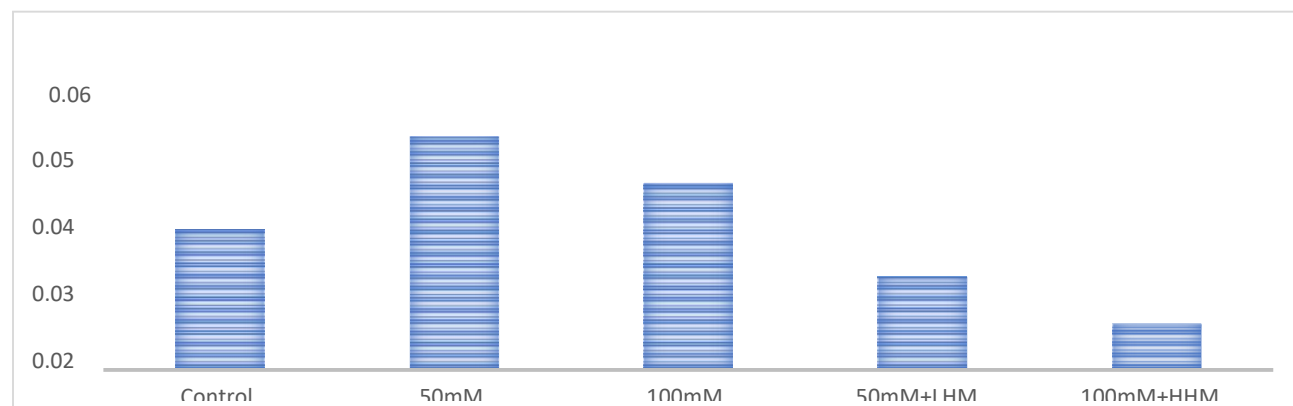


Figure 1: Graphical representation of Root Dry Weight.

Notably, plants exposed to moderate salinity (50 mM + LHM) exhibited the highest RDW (2%), highlighting a potential threshold where K⁺ supplementation mitigates lead toxicity effects. These findings suggest that while *M. parviflora* exhibits a degree of tolerance to salinity and heavy metal stress, extreme conditions severely compromise seed germination and biomass development. The differential response to varying stress intensities underscores the importance of investigating molecular and physiological mechanisms underlying stress adaptation. Future studies integrating transcriptomic, proteomic, and metabolomic analyses could provide deeper insights into the regulatory networks governing stress resilience in *M. parviflora*, with implications for phytoremediation and the development of stress-tolerant crop species.

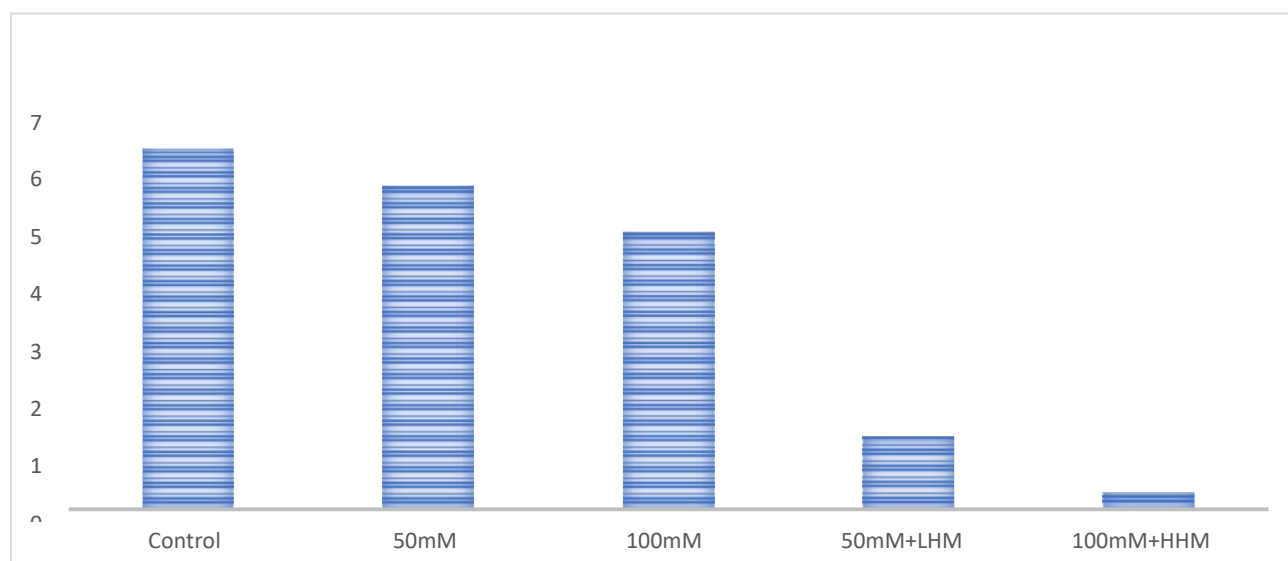


Figure 2: Graphical representation of Root length.

The control group had the highest recorded value (6.5%), followed by the 50 mM group (5.83%), the 100 mM group (5.0%), the 50 mM+LHM group (1.3%), and the lowest value was seen in the 100 mM+HHM group (0.3%). The highest mean value of leaf fresh weight (LFW) (Figure 3) was recorded in the 100 mM treatment (0.6%), followed by the 50 mM treatment (0.59%), the control group (0.36%), the 50 mM treatment with LHM (0.36%), and the lowest value was seen in the 100 mM treatment with HHM (0%). There was a variance in the leaf dry weight (LDW) among different treatments. The treatment with the most significant mean LDW was 50 mM+LHM (0.07%), followed by 50 mM (0.03%), control, and 100 mM (0.02%), while the lowest LDW was seen in 100 mM+HHM (0%). The most outstanding average value of plant biomass (PBM) was observed in the 100 mM+HHM treatments, with a mean value of 4.33%. This was followed by the 50 mM treatment, which had a mean value of 1.03%, and the 50 mM+LHM treatment, which had a mean value of 0.11%.

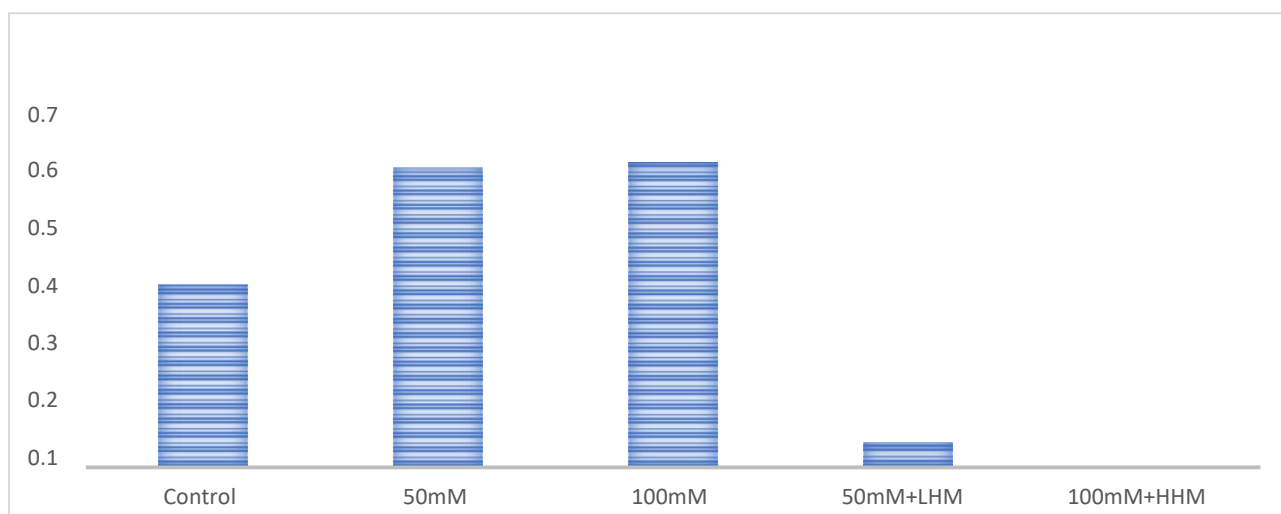


Figure 3: Graphical representation of leaf fresh weight.

The prevalence rates of the condition were 0.9% and 0.72% in the experimental and control groups, respectively. The 100 mM+ HHM treatments had the highest mean value of plant biomass dry weight (PBMDW) (1.67%), followed by the 50mM treatment (0.06%) and the 100mM treatment (0.04%). The lowest PBMCW values were recorded in the 50mM+LHM and control treatments (0.03%), as shown in Table 2.

SEED GERMINATION

The seed germination of *Malva parviflora* exhibited significant heterogeneity in the current experiment. Under salt stress, the germination rate of *Malva parviflora* seeds was markedly lower than in the control environment. Furthermore, the combination of salinity stress and heavy metal treatment had an even more detrimental effect on seed germination, as shown in Figure 4, compared to both the control and salt stress conditions.

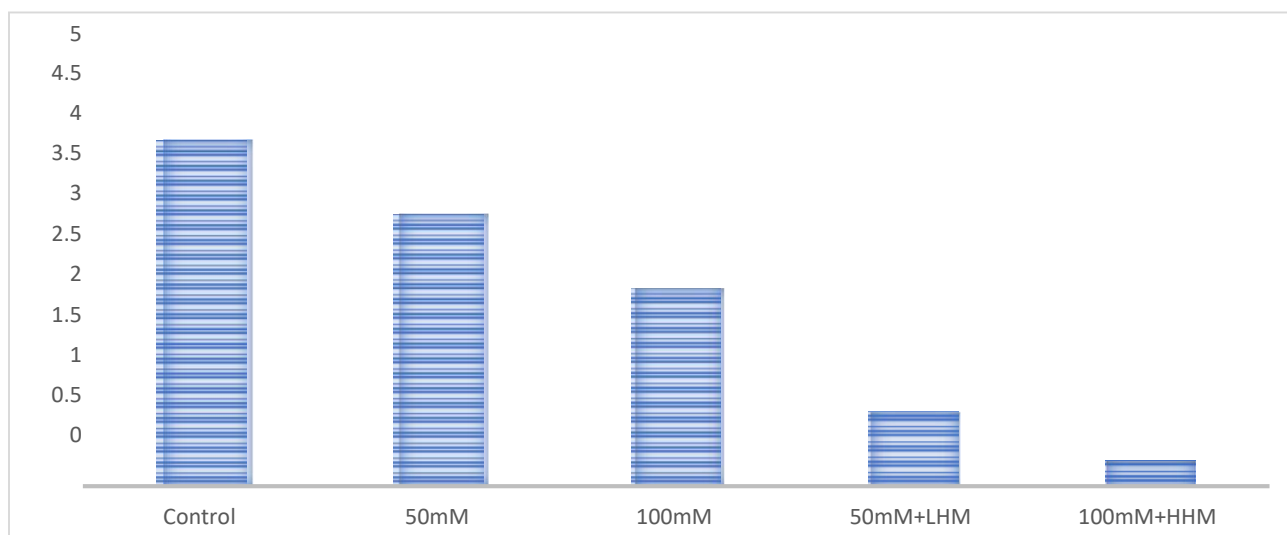


Figure 4: Graphical representation of salt stress, control, and heavy metal seed germination.

Table 2: Descriptive statistics of morphological attributes studied of *Malva parviflora*.

Traits	Treatment	Mean	SD	CV	Minimum	Maximum
GP	Control	4.67	0.58	12.37	4	5
	T1	3.67	0.58	15.7	3	0.4
	T2	2.67	1.15	43.3	2	4
	T3	1	1	100	0	2
	T4	0.34	0.58	173.2	0	1
RFW	Control	0.52	0.07	52.87	0.05	0.17
	T1	0.43	0.45	104.8	0.13	0.94
	T2	0.15	0.09	62.84	0.04	0.21
	T3	0.02	0.01	79.51	3	0.03
	T4	0.03	5.77	173.2	0	0.11
RDW	Control	0.03	0.02	65.4	0.01	0.06
	T1	0.05	0.02	48.71	0.03	0.08
	T2	0.04	0.03	59.75	0.01	0.06
	T3	0.02	1	50	1	0.03
	T4	0.01	1.73	173.2	0	0.03
RL	Control	6.5	0.5	7.69	6	7
	T1	5.83	1.04	17.84	5	7
	T2	5	0.5	10	4.5	5.5
	T3	1.3	0.2	15.38	1.1	1.5
	T4	0.3	0.51	173.2	0.02	0.9
LFW	Control	0.36	0.09	27.3	0.3	0.47
	T1	0.59	0.19	32.1	0.41	0.79
	T2	0.6	0.11	18.32	0.51	0.72
	T3	0.05	0.05	102.4	0.02	0.11
	T4	0	0	0	0	0
LDW	Control	0.02	5.5	28.5	0.04	0.02
	T1	0.03	0.01	35.83	0.02	0.05
	T2	0.02	0.01	41.83	0.01	0.03
	T3	0.07	2.517	37.75	0.04	0.09
	T4	0	0	0	0	0
PBM	Control	0.72	0.23	32.14	0.46	0.86
	T1	1.03	0.24	23.24	0.89	1.3
	T2	0.9	0.41	46.1	0.43	1.22
	T3	0.11	0.05	46.08	0.06	0.17
	T4	4.33	7.5	173.2	0.5	6.2

PBMW	Control	0.03	0.02	54.9	0.01	0.05
	T1	0.06	0.02	32.7	0.05	0.083
	T2	0.04	0.02	60.3	0.01	0.06
	T3	0.03	0.01	53.44	0.01	0.04
	T4	1.67	2.89	173.2	0.04	5

Key: Plant biomass dry weight (PBMDW), Plant biomass (PBM), Leaf dry weight (LDW), Leaf fresh weight (LFW), Root length (RL), Root dry weight (RDW), Fresh leaf weight (RFW), Germination percentage (GP).

CONCLUSION

This study investigated the salinity tolerance of *Malva parviflora* and barley in both indoor and outdoor greenhouse conditions, focusing on how environmental variables influence plant response to salinity. Barley plants were subjected to varying salinity levels (0 mM, 50 mM, 100 mM, 50 mM + LHM, and 100 mM + HHM) during the early seedling and germination stages. The results demonstrated a significant reduction in both structural and functional characteristics of barley under salinity stress, with fresh weight declining as salinity increased. Elevated soil salinity decreased the osmotic potential of the soil solution, exacerbating osmotic stress and hindering plant growth. Barley exhibited moderate sensitivity to salinity, underscoring the importance of precise control over irrigation practices and salinity levels to mitigate the negative impacts of salt stress on barley growth. These findings highlight the critical need to manage salinity in agricultural systems to ensure optimal crop productivity in saline environments.

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