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MITIGATION OF ADVERSE EFFECTS OF CHROMIUM CHLORIDE TOXICITY IN FLAX SEEDS (*LINUM USITATISSIMUM* L.) THROUGH FOLIAR APPLICATION OF FOLIC ACID

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Abstract

This study aims to evaluate the anatomical and morphological strategies of Flaxseed (Linum usitatissimum L.) under the chromium (Cr) toxicity in the presence of folic acid (FA). The experiment was designed as a CRD (completely randomized design) to assess the morphological and anatomical traits under different concentrations of Cr toxicity (5 µM, 10 µM, 15 µM) along with two levels of FA (20 mg/L and 40 mg/L). It was hypothesized that Flaxseed (Linum usitatissimum L.) developed structural adaptations to overcome Cr stress with foliar application of FA. Morpho-anatomical attributes of Linum usitatissimum L. were negatively affected due to Cr toxicity by disrupting nutrient uptake, inducing oxidative damage, inhibiting photosynthesis and altering tissue structure. The results of this study showed that at 15 µM root length was reduced by 46% and a 24% decline was assessed in shoot length. A decrease was observed in plant height by 56%, leaf area by 45% and dry weight by 78% at 15 µM compared to the control group. Folic acid at 20mg/L improved growth attributes under Cr stress and 40mg/L FA offered the maximum resistance. Plant height, leaf area and biomass were improved and they followed a similar trend. Anatomical parameters were also adversely affected under Cr stress. At 15 µM maximum reduction was noted in the thickness of epidermis(37%), cortex(23%), xylem(14%), phloem(78%) and pith(12%) compared to the control group. Conclusively, Cr stress alone severely impaired Linum usitatissimum L. morphology and anatomical structures, whereas 40 mg/L folic acid mitigated these adverse effects.

Introduction

Flaxseed (*Linum usitatissimum* L.) is an annual herbaceous plant cultivated globally and has been traditionally valued for both its fiber and oil-rich seeds. It belongs to the family Linaceae (Nowak & Jeziork, 2023; Ayub et al., 2024). In nature, heavy metals are either found in extremely low concentrations or are trapped in rocks until industrialization makes them accessible, useful, and accumulable (Akram et al., 2022). Heavy metal concentrations in soil that are too high can be harmful to plants. Heavy metal pollution in

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soil has primarily come from mining, exhaust emissions, sewage irrigation, and the use of metal items (Abbas et al., 2022). Over the past few decades, heavy metal contamination in agricultural systems has increased and gotten worse worldwide, and its reach and extent are somewhat correlated with each nation's understanding of environmental protection (Javeed et al., 2023).Metal ions reduce the pH of the soil and cause acidification in intact soil. The seventeenth most hazardous element, chromium (Cr), is a contaminant. One of the dangerous substances (Ayoub et al., 2025). It is 7.15 g/cm3 in weight and naturally varied between 10 and 50 mg/kg of soil. Fertilizers and wastewater are included as the primary sources of Cr. Because of its many oxidation states, Cr is hazardous to living things (Feng et al., 2023; Naqvi et al., 2024). However, the toxicity of Cr to different plant species depends on its concentration, focal plant, and absorption methods (Atta et al., 2023). Lower concentrations of Cr had no effect on plant attributes, at the concentrations of Cr, the trichome, number of stomata, length of long and short cells, and trichome size all decreased and root epidermis, the cortical cells, the xylem artery also affected (Hassan et al., 2023; Iqbal et al., 2025). Naqvi et al., (2024) reported that both Cr and Pb have a major effect on the many development traits of exposed plants (wheat, maize, barley, sunflower, mustard, and soybean) as well as seed inhibition. Germination, plant height, root and shoot length, seedling fresh-dry weight, tolerance index, the mobility and chemical activity of heavy metals affect soil changes, especially when these elements exceed set limitations (Ayub et al., 2024; Hassan et al., 2024). Metal ions cause undamaged soil to become acidic by lowering its pH (Ishfaq et al., 2025).Because of their ability to penetrate the food chain, heavy metals harm human health by preventing plants from growing and causing a variety of harmful symptoms in plants (Abbas et al., 2022). However, for improved plant growth and output, the metal contaminants might be used in fertilizer, herbicides, fungicides, and nematodes (Hassan et al., 2023; Hassan et al., 2024). One of the main sources of the plants' growth is these treatments. Increased plant growth and biomass result in metal deposition in the soil (Aziz et al., 2025; Nowwar et al., 2025). The phytotoxicity of heavy metals in plants is influenced by their chemical makeup, the kind of plant and its species, and the pH of the metals (Akram et al., 2026). Through the direct and indirect toxicity of heavy metals, oxidative stress and a significant amount of reacting oxygen species are generated in the following ways: (a) electron reduction, (b) irregular metabolic pathway management, (c) decreased anti-oxidative enzyme activity, and (d) depletion of lower molecular weight compounds (Shahid et al., 2023; Fatima et al., 2026).Flax is also vulnerable to a range of eukaryotic plant diseases, which compromise both seed and fiber quality. Diseases such as pasmo (caused by *Septoria linicola*), rust (*Melampsora lini*), and Fusarium wilt are among the most damaging, often leading to significant economic losses (Moyse et al., 2023). Agricultural sustainability may be jeopardized by heavy metal phyto-toxicity such as that brought on by cadmium, which can hinder plant photosynthesis, nitrogen uptake, and enzyme activity (Ali et al., 2022; Akram et al., 2026). In reproductive organs, metal accumulation can lead to pollen sterility, reduced flower and fruit set, and impaired seed formation, thereby significantly lowering crop yields (Iqbal et al., 2024). One of the most significant oilseed crops for industrial, culinary, feed, and fiber uses is flaxseed. Commercial use is made of nearly every portion of the flaxseed plant, either raw or processed. The stem produces high-quality fiber that is extremely durable and strong (Lorenc et al., 2022).One of the first crops to be domesticated, flax provides animal feed, functional meals, and health-promoting seed oil and stem fiber. The textile industry uses its stem fiber. The value of natural products and growing knowledge of flaxseed's health advantages could spark a comeback even though flax production has decreased globally (Hellebois et al., 2021). Superior flax varieties with significant features are being developed through the application of cutting-edge procedures such as genome editing, genomic tools, and marker-assisted breeding. For

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the foreseeable future, flax is expected to keep up its standing as a "wonder crop (Panda et al., 2025).Since plants reproduce by seeds, the first stage that is affected by the heavy metal chromium is seed germination, which is also the main indicator of the plant's chromium tolerance. Hexavalent chromium in the fertilizer solution decreased seed germination by causing stress.

It is generally thought that the presence of chromium affects plant photosynthesis and disrupts plant growth (AO et al., 2022). Chromium enters plants through the root system and is partially transported to the above-ground parts of the plants along with nutrients, influencing the growth of organs like stems and leaves.At the same time, it was found that high chromium concentrations caused abnormal stomatal conductivity, reduced intercellular space, and reduced growth and yield of plants (Saud et al., 2022).

The water-soluble vitamin B9, folic acid and its derivatives (folates), is widely produced in the kingdom of plants. They have a role in connecting the nitrogen and carbon metabolisms in plants (Ali et al., 2024). Folates are also essential for one-carbon transfer processes and DNA synthesis. Because folate deficiency affects the pace of cell division and decreases genome stability, plants cannot grow normally (Akyo et al., 2020). The capacity of folates to promote a variety of precursors and intermediate processes, including the formation of porphyrins, S-adenosylmethionine (SAM), and isoprenoids, explains these effects (Alsamadany et al., 2022). However, under a variety of abiotic stressors, such as drought, salt, and heavy metals, exogenous folic acid has shown potent antioxidant qualities that are in charge of lowering oxidative damage in a number of plant species (Parveen at al., 2023; Akram et al., 2026). In general, additional harmful molecules like methylglyoxal (MG) can be reduced when plants under abiotic stress are treated with a potent antioxidant. This tactic is crucial for controlling plant signaling, growth and development, and a number of metabolic pathways when stress is present (Aljuaid et al., 2022; Nazar et al., 2026).

The main objective of the research is to investigate the effect of $CrCl_2$ stress, evaluate the potential of folic acid to reduce $CrCl_2$ stress, to determine the optimal concentration of folic acid for reducing $CrCl_2$ stress accumulation, examine the effects of folic acid on the different growth parameters and development and study the effects of the combine application of folic acid and $CrCl_2$ on the flax plant.

Materials and Methods

Plant material and experimental design

The experiment was conducted in the winter season of 2024 in the Botanical Garden at The Islamia University Bahawalpur. The edible seeds of the fiber and oilseed producing plant *Linum usitatissimum* L. belonging to the family Linaceae, were used as an experimental material. Flax is one of the oldest cultivated crops and is grown primarily for its seeds and strong fibers. For this research work, polythene bags were used to grow flax plants, ensuring suitable soil conditions and consistent care throughout the growth period. Flax seeds were sown in polythene bags filled with canal soil, and the plants were irrigated three-four times a week under typical growing conditions. The trial was designed using a completely randomized design (CRD). Each of the ten treatments (T0-T9) was replicated five times with replications (R1-R5).

Morphological analysis

Linum usitatissimum's various morphological characteristics were examined. The manual measuring scale was used to measure the root length (cm), shoot length (cm), plant height (cm), and leaf area (cm^2). A computerized weighing balance was used to calculate the fresh weight (g) and dry weight (g). Both the number of branches and the number of leaves per plant were recorded.

Anatomical analysis

Samples from anatomical studies were collected from the middle portion of the leaf lamina, the internodal base of the main stem and the primary root region near the root-shoot junction. Stem samples from the third internode of the main

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stem. Using scissors, the roots, stems, and leaves were cut into small sections without causing any damage to the surrounding tissue. The FAA solution (formalin-acetic acid-alcohol), consisting of ethanol, acetic acid and formaldehyde) for anatomical research. The samples are added after 48 hours, the samples were transferred to 3:1 alcohol-acetic acid solution for preservation. Digital photographs of prepared slides were captured using a smartphone-mounted microscope camera. The following anatomical traits were recorded:

Roots: xylem, cortex, pericycle, endodermis, pith diameter, phloem area, epiblema thickness.

Stems: cambium, stem epidermis, xylem, phloem, stem pith, cortex.

Leaf: spongy mesophyll, palisade mesophyll, upper epidermis thickness, lower epidermis,vascular bundle diameter.

Statistical analysis

The study employed a completely randomized design (CRD), with five replicates of each. $CrCl_2$, $CrCl_3$ and folic acid treatments were analyzed. After organizing the data, statistical analysis was performed using Microsoft Excel and Statistix 8.1. Mean values and Standard error (SE) were calculated, and results were presented in tables, graphs, and figure.

Results

Morphological attributes:

The morphological characteristics of *Linum usitatissimum* were significantly ($p < 0.05$) diminished by chromium chloride stress (Figure 1). Under $CrCl_2$ stress, root and shoot lengths reduced. Significant differences were observed at different levels of $CrCl_2$ as compared to the first block i.e T0. Plant height was reduced with the increasing concentration of $CrCl_2$ stress. The ANOVA results revealed that both heavy metal stress and folic acid application had statistically significant effects ($p < 0.05$) on plant height. Root Length was reduced with the increasing concentration of $CrCl_2$ stress. Fresh weight was reduced with the increasing concentration of $CrCl_2$ stress. Plants at second block under T1 and T2 show smallest reduction in fresh weight which is 1.626 and 1.517 respectively, while plants at T3 show maximum reduction that is 0.765 due to $CrCl_2$ stress. It indicates that more the concentration of salt, the greater the reduction Fresh weight as compared to the plants of T0 (Control Block) that is 1.663. Number of leaves was reduced with the increasing concentration of $CrCl_2$ stress. Plants at second block under T1 and T2 show smallest reduction in number of leaves which is 40 and 50 respectively, while plants at T3 show maximum reduction that is 30 due to $CrCl_2$ stress. In comparison to their corresponding control or $CrCl_2$-only groups (T1, T2, and T3), T4 (70 leaves), T5 (80 leaves), and T6 (80 leaves) exhibit the least amount of leaf count decline in the third block. These numbers, however, are still lower than those in the fourth block (T7: 115, T8: 120, T9: 125), where the highest concentration of folic Acid enhancer was used. Plant shoot length is greatly reduced by increased concentrations of $CrCl_2$ stress; however, the use of folic acid, particularly at maximal levels, helps counteract this impact and promotes growth. The use of folic acid effectively mitigates these losses, with the maximum dose offering the greatest recovery when compared to the minimum dose and non-enhanced controls, even though $CrCl_2$ stress dramatically reduces plant dry weight as salt concentration increases.

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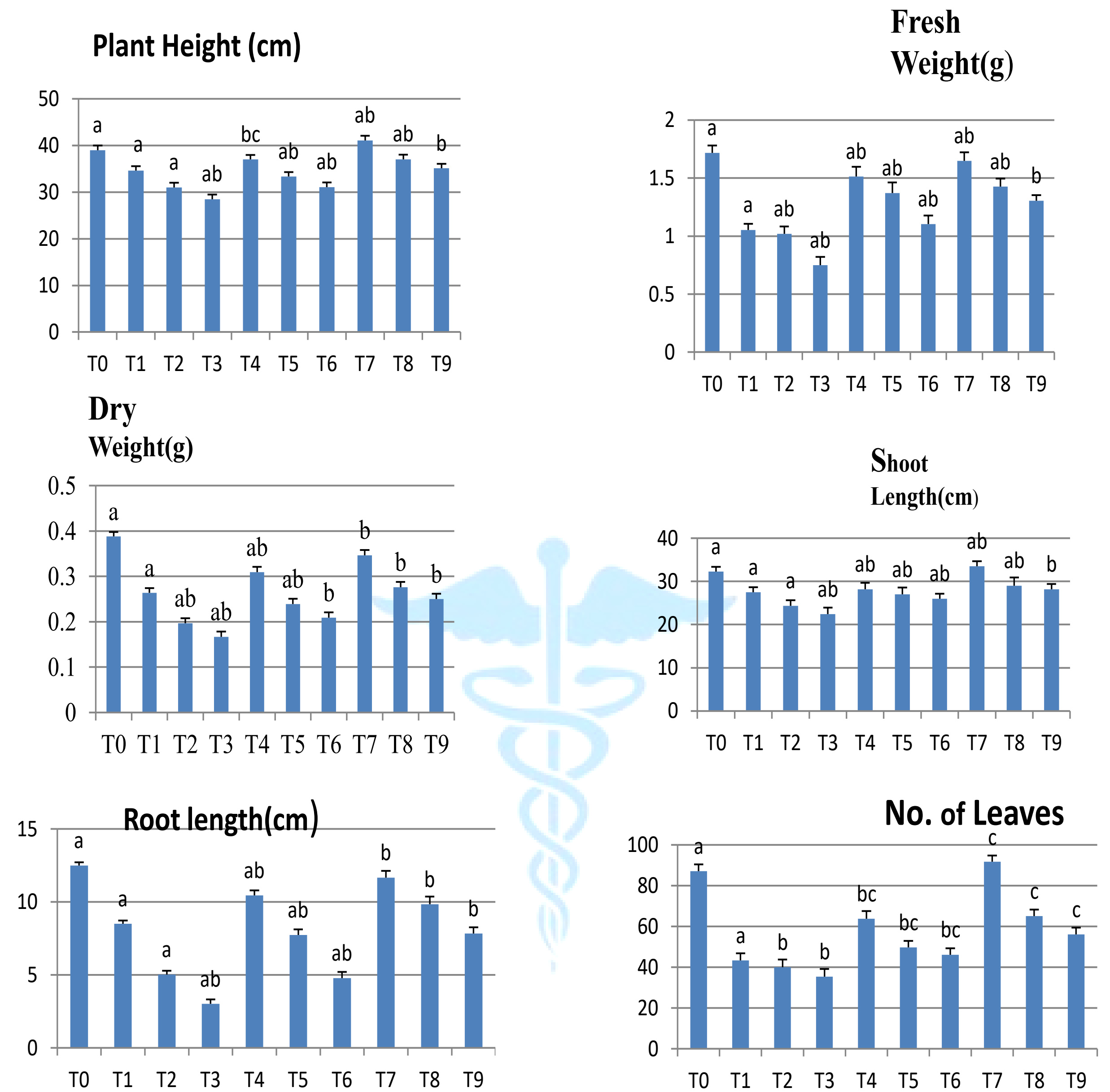

Figure:1 shows Morphological attributes of *Linum usitatissimum* L. subjected to different treatments of $CrCl_2$ and FA . The graph represents means ± SE, with treatments designated as ; T0(control),T1(5µM $CrCl_2$),T2(10µM$CrCl_2$), T3(15µM $CrCl_2$), T4(5µM$CrCl_2$+20mM Folic Acid),T5(10 µM $CrCl_2$+20 mM Folic Acid),T6(15 µM $CrCl_2$+20 mM Folic Acid),T7(5 µM $CrCl_2$+40 mM Folic Acid),T8(10µM $CrCl_2$+40mM Folic Acid),T9(15µM$CrCl_2$+40mM Folic Acid).

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Table 1: Morphological parameters of *Linum usitatissimum*, subjected to different levels of CrCl2 and folic acid.

Tr	PH (cm)	FW(g)	SL (cm)	DW(g)	RL (cm)	LN (µm)
T0t0	39a	1.32a	32.27a	0.39a	12.5a	87a
T1t0	35a	0.80a	27.5a	0.26a	8.5a	43.3a
T2t0	35a	0.62ab	24.3a	0.20ab	5.02a	40b
T3t0	35a	0.43ab	22.4ab	0.17ab	3.01ab	35.3b
T1t1	36a	1.1ab	28.1ab	0.31ab	10.4ab	63.6bc
T2t1	36ab	0.98ab	27.0ab	0.24ab	7.72ab	49.6bc
T3t1	37ab	0.8ab	26.0ab	0.21b	4.7ab	46bc
T1t2	38ab	1.2ab	33.5ab	0.35b	11.6b	91.6c
T2t2	39ab	1.04ab	29.0ab	0.28b	9.8b	65c
T3t2	38a	0.89b	28.0b	0.25b	7.8b	56c

T0(control),T1(5µM $CrCl_2$),T2(10µM$CrCl_2$), T3(15µM $CrCl_2$), T4(5µM$CrCl_2$+20mM Folic Acid),T5(10 µM $CrCl_2$+20 mM Folic Acid),T6(15 µM $CrCl_2$+20 mM Folic Acid),T7(5 µM $CrCl_2$+40 mM Folic Acid),T8(10µM $CrCl_2$+40mM Folic Acid),T9(15µM$CrCl_2$+40mM Folic Acid).Tr= Treatment, PH = Plant Height, FW =Fresh Weight, SL= Shoot Length, DW= Dry Weight, RL= Root Length, LN= Leaves Number Means following different alphabets differ significantly at $p < 0.05$.

Anatomical attributes:

Stem Anatomy:

Cambium thickness decreased concentration-dependently with exposure to $CrCl_2$ stress, falling from the 8.2µm control T° to a minimum of 6.9µm (T3).This structural inhibition was successfully reduced by folic acid intake, indicating a dose-dependent protective function in preserving vascular tissue integrity. The $CrCl_2$ + folic acid (max) groups (T7-T9) showed the greatest recovery, with T9 surpassing the control baseline and peaking at 9.2µm. Exposure to $CrCl_2$ stress caused a noticeable increase in epidermal thickness, reflecting the plant's defensive response to heavy metal toxicity through enhanced cuticular and epidermal cell layer formation. Among the $CrCl_2$-only treatments, T_3 (17.1 µm) exhibited the maximum mean epidermal thickness, followed by T_2 (16.5 µm) and T_1 (16.03 µm). This pattern suggests that elevated $CrCl_2$ concentrations stimulated stress-induced thickening of the epidermis as a protective mechanism against metal uptake and tissue damage. In contrast, the application of folic acid showed a restorative and regulatory effect on the epidermal structure. Exposure to $CrCl_2$ stress led to a decline in cortex thickness, suggesting cytotoxic damage and inhibited cell growth. Among treatments with $CrCl_2$, T_3 (115 µm) showed the greatest reduction in cortex thickness, indicating severe tissue shrinkage. Treatments T_1 and T_2 also exhibited significant reductions, highlighting the dose-dependent effects of $CrCl_2$. Conversely, the addition of folic acid improved cortex thickness in $CrCl_2$ + folic acid treatments (T_4–T_6), with values rising from 130 µm to 140 µm, indicating partial recovery. $CrCl_2$ + Folic Acid(max) treatments (T_7–T_9) showed even greater improvements, where T_9 reached a maximum mean thickness of 185 µm, demonstrating significant structural recovery and enhanced cell growth due to higher folic acid concentrations.

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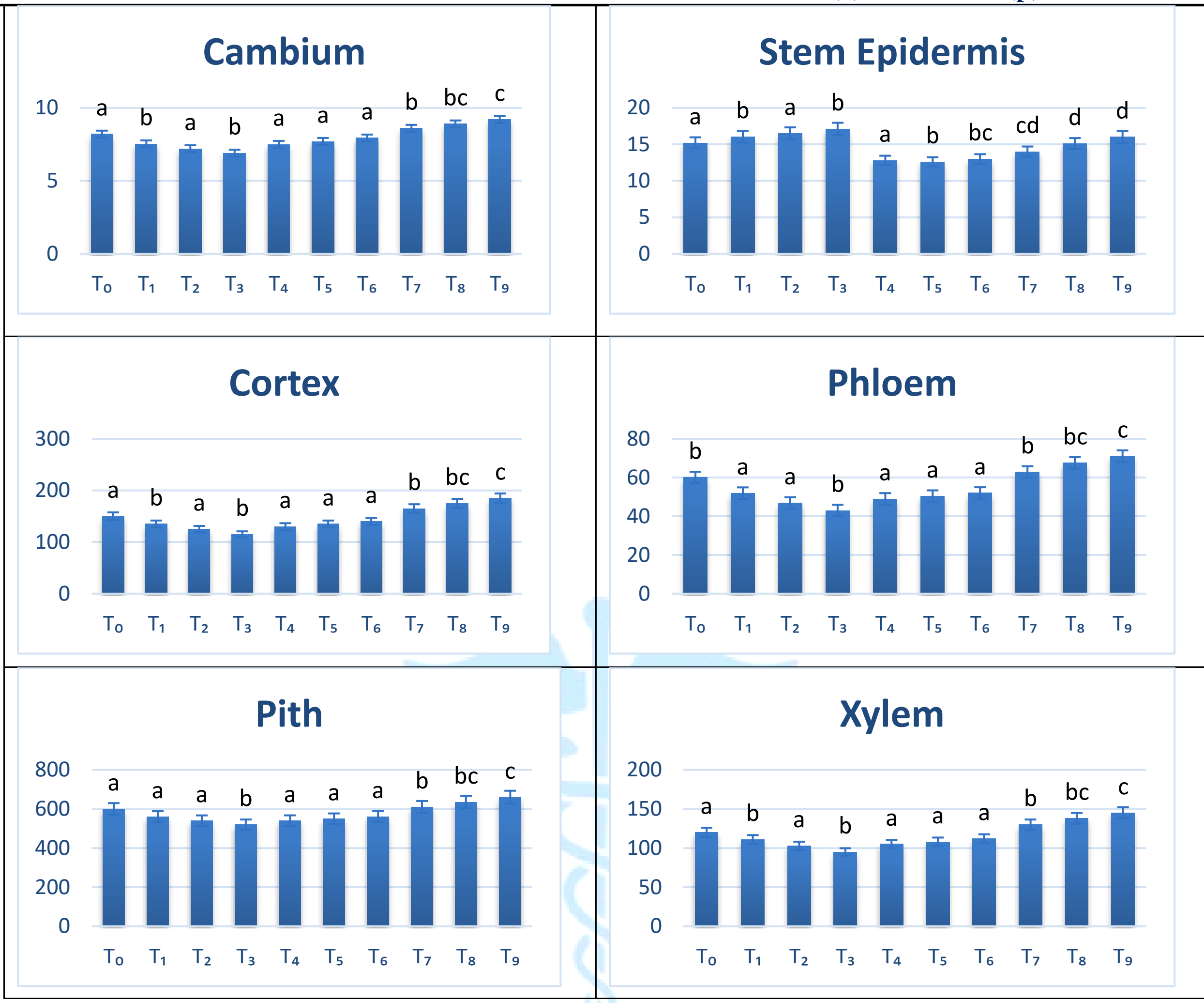

Figure 2: Different stem anatomical parameters of *Linum usitatissimum*, subjected to different levels of $CrCl_2$ and folic acid .

T0(control),T1(5µM $CrCl_2$),T2(10µM$CrCl_2$), T3(15µM $CrCl_2$), T4(5µM$CrCl_2$+20mM Folic Acid),T5(10 µM $CrCl_2$+20 mM Folic Acid),T6(15 µM $CrCl_2$+20 mM Folic Acid),T7(5 µM $CrCl_2$+40 mM Folic Acid),T8(10µM $CrCl_2$+40mM Folic Acid),T9(15µM$CrCl_2$+40mM Folic Acid).

Table 2: Stem anatomical parameters of *Linum usitatissimum*, subjected to different levels of $CrCl_2$ and folic acid.

Tr	CT(µm)	SET(µm)	CT(µm)	PT(µm)	PT (µm)	XT(µm)
T0t0	8.2a	15.2a	150a	60.06b	600a	120a
T1t0	7.5b	16.03b	135b	51.9a	560.3a	111b
T2t0	7.2a	16.5a	125a	46.9a	540a	103a

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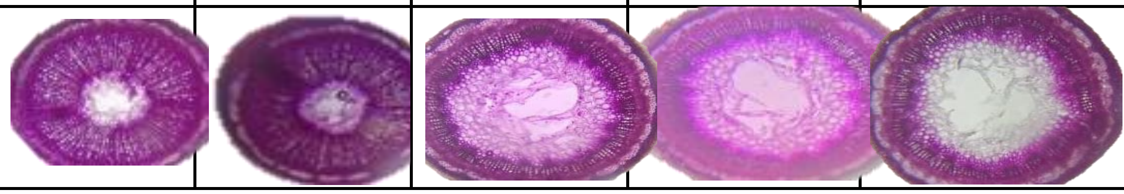
T3t0	6.9b	17.1b	115b	43b	520b	95b
T1t1	7.5a	15.8a	13a	49a	540.3a	105a
T2t1	7.7a	15.6b	135a	50.4a	549.6a	108a
T3t1	7.9a	15.5bc	140a	52a	561a	112a
T1t2	8.6b	15.3cd	165b	62.9b	610.3b	130b
T2t2	8.9bc	15.1d	175bc	67.5bc	635bc	138bc
T3t2	15c	18.5d	71.1c	9.2c	145c	660.3c

T0(control),T1(5µM $CrCl_2$),T2(10µM$CrCl_2$), T3(15µM $CrCl_2$), T4(5µM$CrCl_2$+20mM Folic Acid),T5(10 µM $CrCl_2$+20 mM Folic Acid),T6(15 µM $CrCl_2$+20 mM Folic Acid),T7(5 µM $CrCl_2$+40 mM Folic Acid),T8(10µM $CrCl_2$+40mM Folic Acid),T9(15µM$CrCl_2$+40mM Folic Acid). Parameters of the stem are represented as Tr= Treatment, CT= Cambium thickness,SET= stem epidermis thickness, CT= cortex thickness, PT= phloem thickness, PT= pith thickness, XT= xylem thickness. Means following different alphabets differ significantly at $p < 0.05$.

The control treatment (T_0) showed a mean phloem thickness of 60.07 µm, indicating healthy vascular tissue. Under $CrCl_2$ stress, phloem thickness declined, with T_3 (43 µm) experiencing the most severe suppression. T_1 (51.97 µm) and T_2 (46.9 µm) also exhibited reductions, confirming a dose-dependent response to $CrCl_2$. Folic acid inclusion improved phloem thickness in treatments T_4–T_6, ranging from 49.0 µm to 52.03 µm, suggesting structural restoration and reduced oxidative stress. Notably, T_9 in the $CrCl_2$ + Folic Acid(max) group reached a mean thickness of 71.1 µm, surpassing the control, demonstrating the effectiveness of higher folic acid concentrations in promoting vascular tissue health. Exposure to $CrCl_2$ stress resulted in a substantial reduction in pith diameter, indicating cellular damage, shrinkage, and reduced turgidity of parenchyma cells within the pith region. The treatments with $CrCl_2$ + folic acid (min) (T_4–T_6) exhibited moderate increases in pith diameter, ranging between 540.33 µm (T_4) and 561 µm (T_6). This improvement suggests that folic acid supplementation effectively alleviated the stress-induced tissue contraction by enhancing cell vitality and water retention capacity in the pith region.

	T0t0	T1t0	T2t0	T3t0	T4t1
4X					

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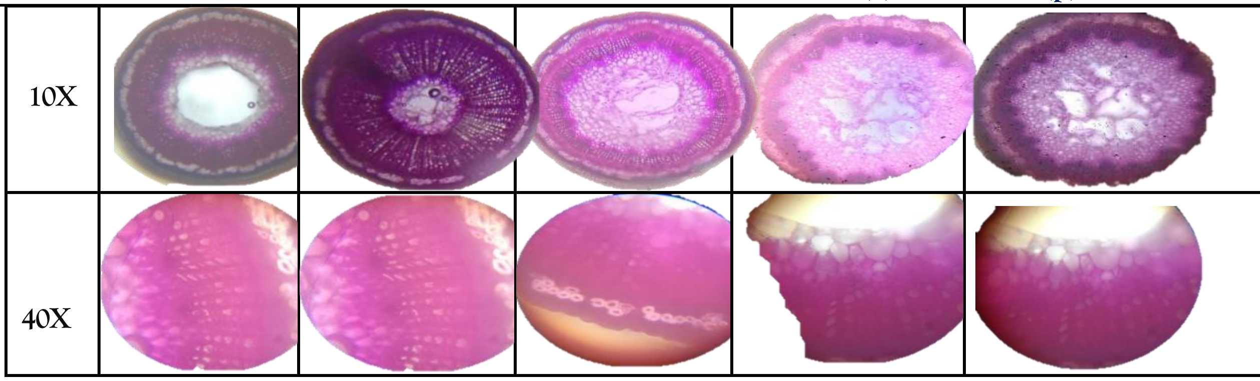

Figure 3:Stem Anatomy of *Linum usitatissimum* (Flax) under the treatment of the Different Levels of $CrCl_2$ Stress. . [T0t0 (Control), T1t0 (5 µM metal), T2t0 (10 µM metal), T3t0 (15 µM metal) [T4t1 (15 µM metal + 20mM) .

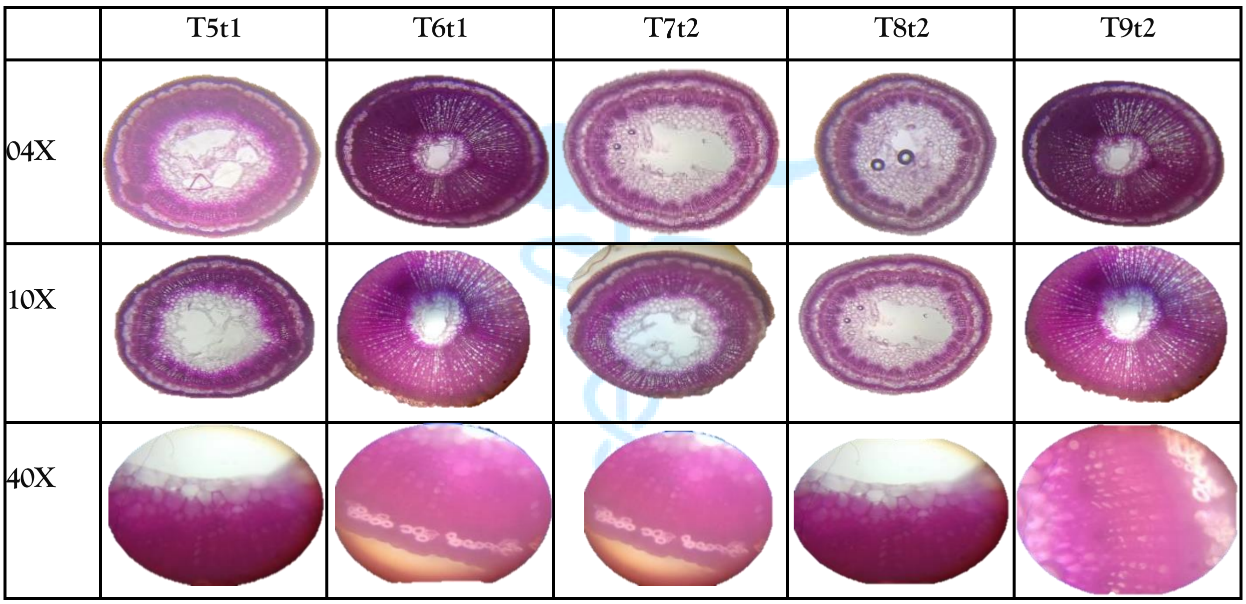

Figure 4:Stem Anatomy of *Linum usitatissimum* (Flax) under the treatment of the Different Levels of $CrCl_2$ Stress. T5t1 (10 µM metal + 20mM), T6t1 (15 µM metal + 20mM), T7t2 (5 µM metal+20mM) T8t2(10 µM metal + 40mM), T9t2 (15 µM metal + 40mM).

Leaf Anatomy:
Chromium chloride stress significantly reduced leaf anatomical traits. As a defensive response to heavy metal stress, exposure to $CrCl_2$ resulted in a concentration-dependent thickening of the upper epidermis. This structural damage was effectively mitigated and epidermal thickness was restored to levels close to control when folic acid was introduced, particularly at maximum concentrations. The control (T_0) exhibited a mean thickness of 42.567 µm, representing the normal, healthy structure of the palisade layer under non-stressed conditions. With increasing concentrations of $CrCl_2$ (T_1–T_3), there was a progressive decline in tissue thickness, reaching the lowest value of 36 µm in T_3, indicating a marked inhibitory effect of chromium toxicity on cellular elongation and chloroplast density. $CrCl_2$ exposure gradually reduced the thickness of the spongy mesophyll. On the other hand,

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folic acid supplementation was successful in reversing this damage, with concentrations that were higher than those in the control and improved tissue integrity through cellular stabilization. The vascular bundle diameter decreased gradually upon exposure to $CrCl_2$, eventually reaching a minimum of 21.1 m. This decrease is indicative of the toxicity of chromium, which prevents cell elongation and interferes with vital pathways for the transport of nutrients.

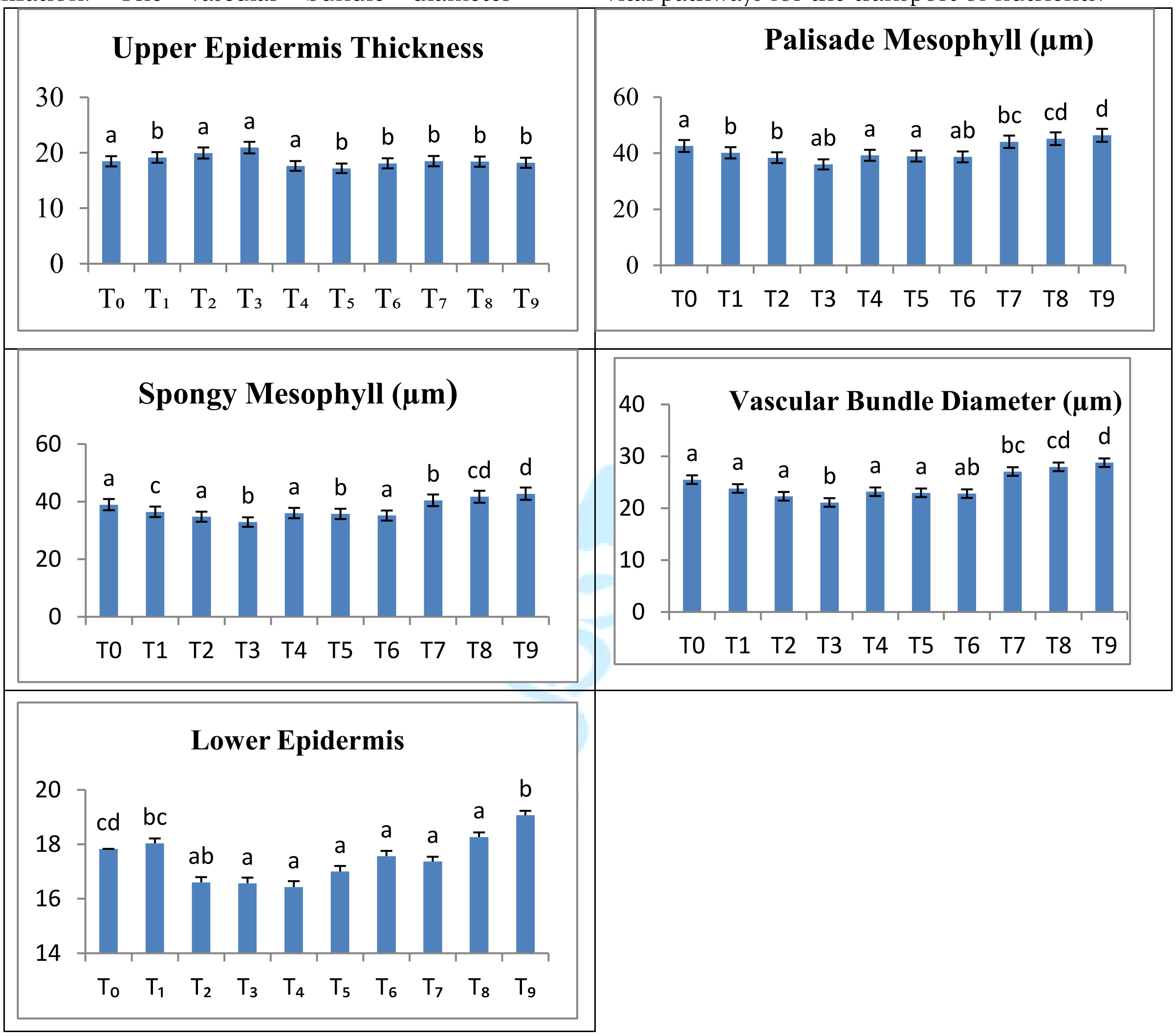

Figure 5: Different Leaf anatomical parameters of *Linum usitatissimum*, subjected to different levels of $CrCl_2$ and folic acid . T0(control),T1(5µM $CrCl_2$),T2(10µM$CrCl_2$), T3(15µM $CrCl_2$), T4(5µM$CrCl_2$+20mM Folic Acid),T5(10 µM $CrCl_2$+20 mM Folic Acid),T6(15 µM $CrCl_2$+20 mM Folic Acid),T7(5 µM $CrCl_2$+40 mM Folic Acid),T8(10µM $CrCl_2$+40mM Folic Acid),T9(15µM$CrCl_2$+40mM Folic Acid).

Table 3: Leaf anatomical parameters of *Linum usitatissimum*, subjected to different levels of $CrCl_2$ and folic acid

Tr	UET(µm)	PMT(µm)	SMT(µm)	VBD(µm)	LET(µm)
T0t0	18.5a	42.6a	38.9a	25.5a	17.8cd
T1t0	19.2b	40.1b	36.5c	23.8a	18.03bc

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T2t0	19.9a	38.4b	34.7a	22.3a	16.6ab
T3t0	20.9a	36ab	32.9b	21.1b	16.5a
T1t1	17.6a	39.2a	36.03a	23.2a	16.43a
T2t1	17.2b	38.9a	35.7b	22.9a	17a
T3t1	18.1b	38.6ab	35.2a	22.8ab	17.5a
T1t2	18.5b	44.06bc	40.5b	27.067bc	17.4a
T2t2	18.4b	45.06bc	41.7cd	27.967cd	18.3a
T3t2	18.2b	46.4d	442.7d	28.76d	19.1b

T0(control),T1(5µM $CrCl_2$),T2(10µM$CrCl_2$), T3(15µM $CrCl_2$), T4(5µM$CrCl_2$+20mM Folic Acid),T5(10 µM $CrCl_2$+20 mM Folic Acid),T6(15 µM $CrCl_2$+20 mM Folic Acid),T7(5 µM $CrCl_2$+40 mM Folic Acid),T8(10µM $CrCl_2$+40mM folic acid),T9(15µM$CrCl_2$+40mM folic acid). Parameters of the leaf are represented as Tr= Treatment, UET= upper epidermis thickness, PMT= palisade mesophyll thickness, SMT= spongy mesophyll thickness, VBD= vascular bundle diameter, LET= lower epidermis thickness. Means following different alphabets differ significantly at $p < 0.05$.

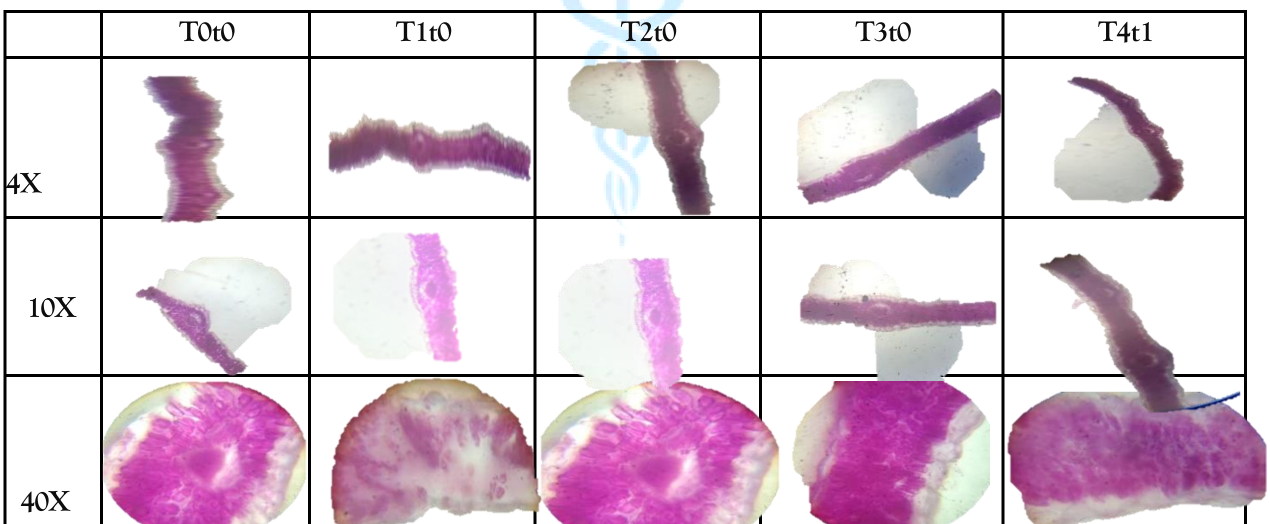

Figure 6:Leaf Anatomy of *Linum usitatissimum* (Flax) under the treatment of the Different Levels of CrCl2 Stress. . [T0t0 (Control), T1t0 (5 µM metal), T2t0 (10 µM metal), T3t0 (15 µM metal) [T4t1 (15 µM metal + 20mM) .

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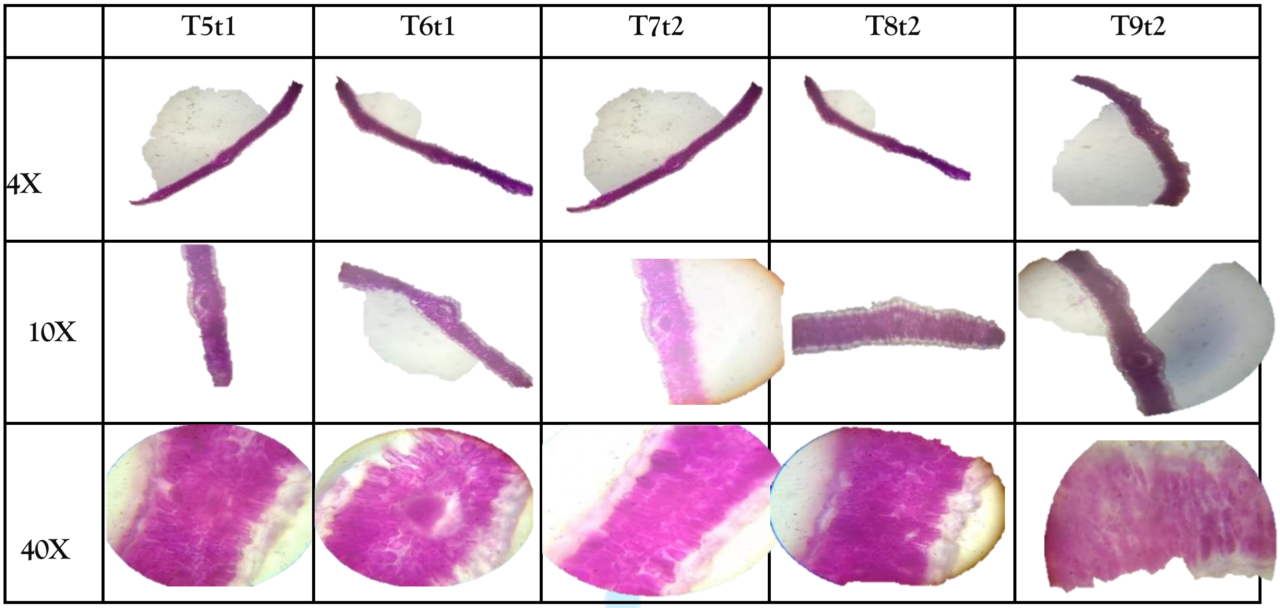

Figure 7: Leaf Anatomy of *Linum usitatissimum* (Flax) under the treatment of the Different Levels of $CrCl_2$ Stress. T5t1 (10 µM metal + 20mM), T6t1 (15 µM metal + 20mM), T7t2 (5 µM metal+20mM) T8t2(10 µM metal + 40mM), T9t2 (15 µM metal + 40mM).

Root Anatomy:

Root anatomy of *Linum usitatissimum* L was significantly affected by chromium chloride ($CrCl_2$) stress ($p \leq 0.05$), as evidenced by marked reductions in tissue thickness and cellular dimensions (Table 2; Figures 9–11). A progressive decline in anatomical parameters was observed with increasing $CrCl_2$ concentrations, with the most severe impairments recorded at the highest stress level (15 mg $CrCl_2$). Under this treatment, epiblema thickness (ET) declined by 66%, xylem thickness (ZT) by 15%, Phloem area (PA) by 96%, Cortical thickness (CT) by 43%, and phloem thickness (PT) by 85% compared with the control. In contrast, plants exposed to the lowest $CrCl_2$ concentration exhibited only slight anatomical alterations, indicating a clear dose-dependent response.

Exogenous application of folic acid (FA) significantly ameliorated Cr-induced anatomical damage in root tissues. Both minimum (20 mL) and maximum (40 mL) FA treatments enhanced epiblema thickness by 66% and 93%, respectively, while corresponding increases of 36% and 56% were observed in phloem thickness relative to untreated controls. Among combined treatments, plants receiving low $CrCl_2$ in conjunction with high FA concentrations displayed the greatest structural preservation, showing minimal reductions of 38% in ET, 1.3% in CT, 2.6% in EdT, 2% in XT, and 23% in PT. Even under severe Cr stress, application of maximum FA markedly reduced the extent of anatomical deterioration compared with $CrCl_2$ treatment alone, confirming the protective role of FA under heavy metal stress.

Chromium stress also led to a pronounced decline in root cellular dimensions, with the greatest reductions observed at the highest $CrCl_2$ level, whereas minimal declines occurred under mildly stress. FA supplementation effectively counteracted these effects, increasing Phloem area (PA) by 16% and 12% and endodermal cell area (EDCA) by 22% and 12% under minimum and maximum FA treatments, respectively. Nevertheless, under combined high $CrCl_2$ and FA treatments, moderate reductions persisted in epidermal (12%), collenchyma (1.4%), xylem (12%), and pericycle (10%) cell areas.

Chromium chloride stress severely disrupted root anatomical structures in *Linum usitatissimum* L; however, exogenous FA application substantially

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improved tissue thickness and cellular integrity across varying stress intensities, highlighting its potential role in enhancing root tolerance against heavy-metal-induced toxicity.

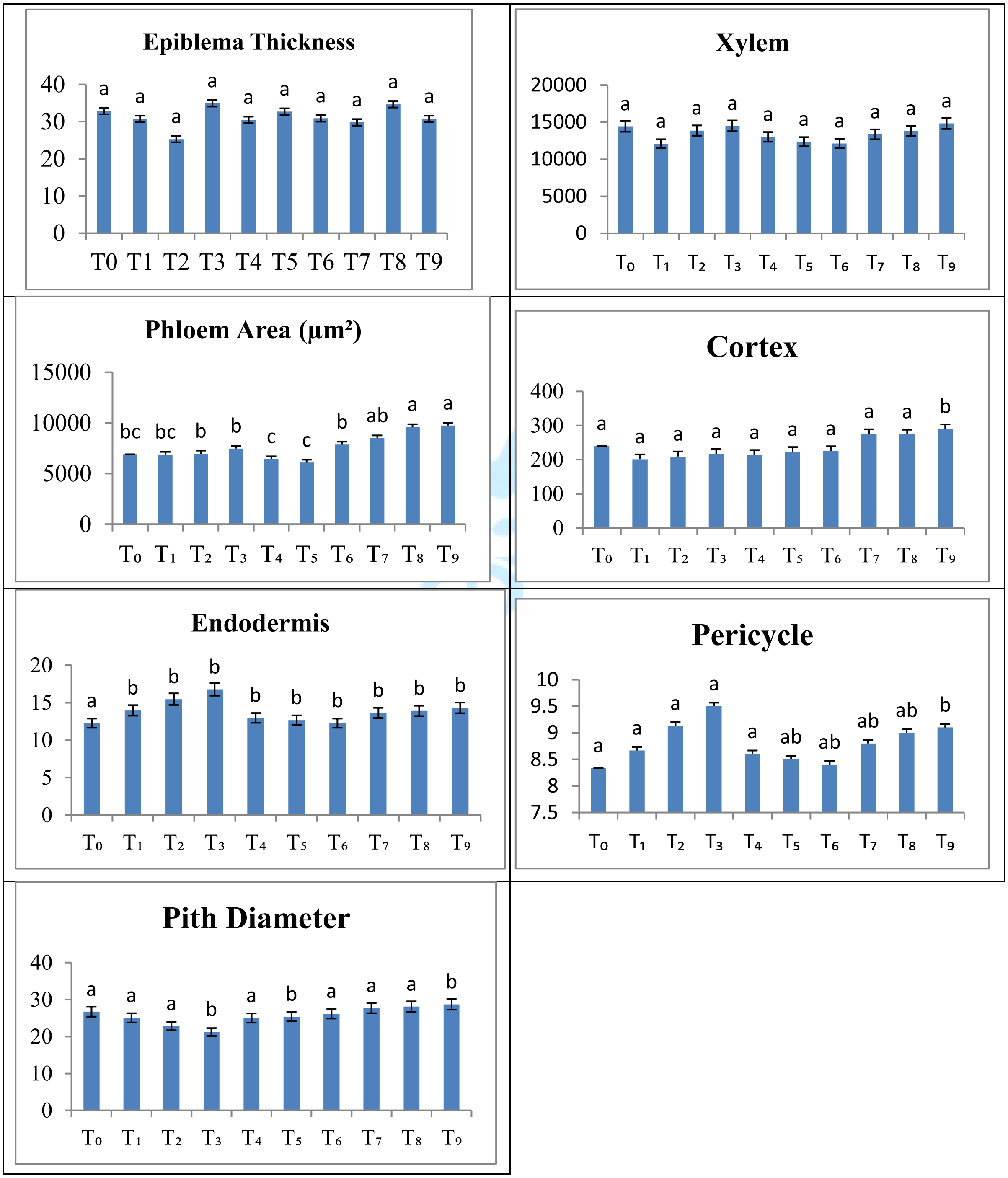

Figure 8: Different root anatomical parameters of *Linum usitatissimum*, subjected to different levels of CrCl2 and folic acid

T0(control),T1(5μM $CrCl_2$),T2(10μM$CrCl_2$), T3(15μM $CrCl_2$), T4(5μM$CrCl_2$+20mM Folic Acid),T5(10 μM $CrCl_2$+20 mM Folic Acid),T6(15 μM $CrCl_2$+20 mM Folic Acid),T7(5 μM $CrCl_2$+40 mM Folic Acid),T8(10μM $CrCl_2$+40mM Folic Acid),T9(15μM$CrCl_2$+40mM Folic Acid).

Table 4: Root anatomical parameters of *Linum usitatissimum*, subjected to different levels of CrCl2 and folic acid

Tr	ET(μm)	XT(μm)	PA(cm^2)	CT (μm)	ET (μm)	PT (μm)	PD (μm)
T0to	32.8a	14429a	6896.7bc	239.7a	12.3a	8.3a	26.7a
T1t0	30.7a	12086a	6877.1bc	201.5a	13.9b	8.7a	25.03a
T2t0	25.3a	13867a	6959.3b	209.3a	15.4b	9.1a	22.8a
T3t0	34.9a	14501a	7467b	216.7a	16.7b	9.5a	21.2b
T1t1	30.5a	13011a	6423.3c	213.9a	12.9b	8.6a	25a
T2t1	32.7a	12359a	6072c	222.8a	12.7b	8.5ab	25.4b
T3t1	30.8a	12129a	7850.7b	225.4a	12.3b	8.4ab	26.2a
T1t2	29.8a	13352a	8482.3ab	275.2a	13.63b	8.8ab	27.7a
T2t2	34.6a	13813a	9592a	274.4a	13.9b	9ab	28.1a
T3t2	30.7a	14826a	9739a	290.03b	14.3b	9.1b	28.7b

T0(control),T1(5μM $CrCl_2$),T2(10μM$CrCl_2$), T3(15μM $CrCl_2$), T4(5μM$CrCl_2$+20mM Folic Acid),T5(10 μM $CrCl_2$+20 mM Folic Acid),T6(15 μM $CrCl_2$+20 mM Folic Acid),T7(5 μM $CrCl_2$+40 mM Folic Acid),T8(10μM $CrCl_2$+40mM Folic Acid),T9(15μM$CrCl_2$+40mM Folic Acid). Parameters of the root are represented as Tr= Treatment, ET= epiblema thickness, XT= xylem thickness, PA= phloem area, CT= cortex thickness, ET= endodermis thickness, PD= pericycle diameter. Means following different alphabets differ significantly at $p < 0.05$.

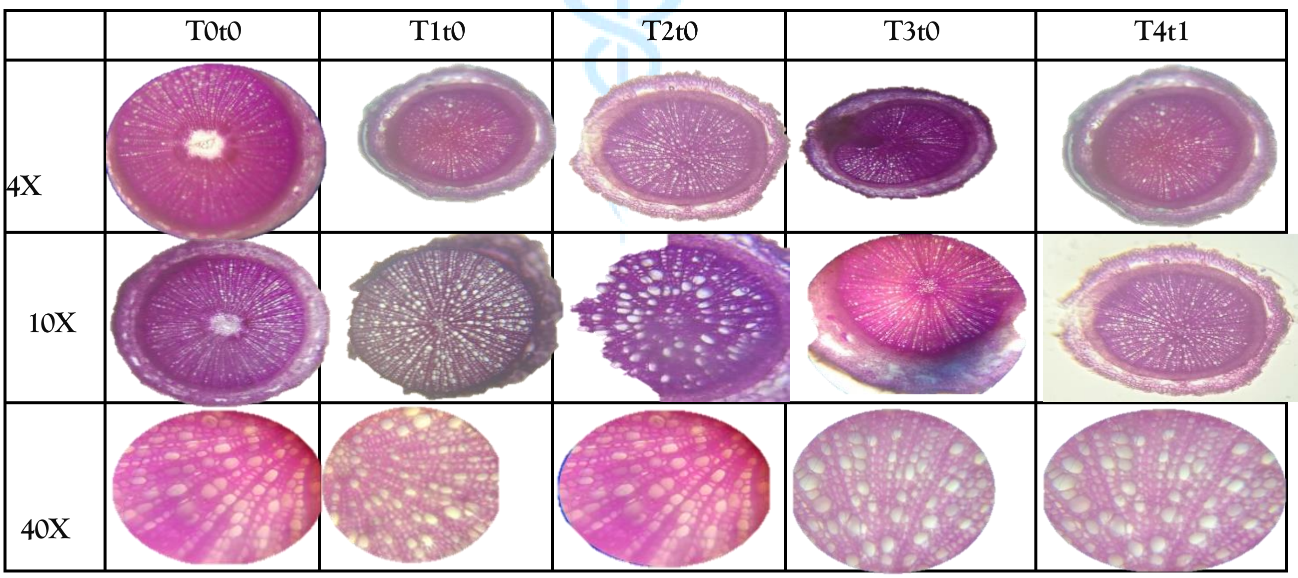

Figure 9: Root Anatomy of *Linum usitatissimum* (Flax) under the treatment of the Different Levels of $CrCl_2$ Stress. . [T0t0 (Control), T1t0 (5 μM metal), T2t0 (10 μM metal), T3t0 (15 μM metal) [T4t1 (15 μM metal + 20mM) .

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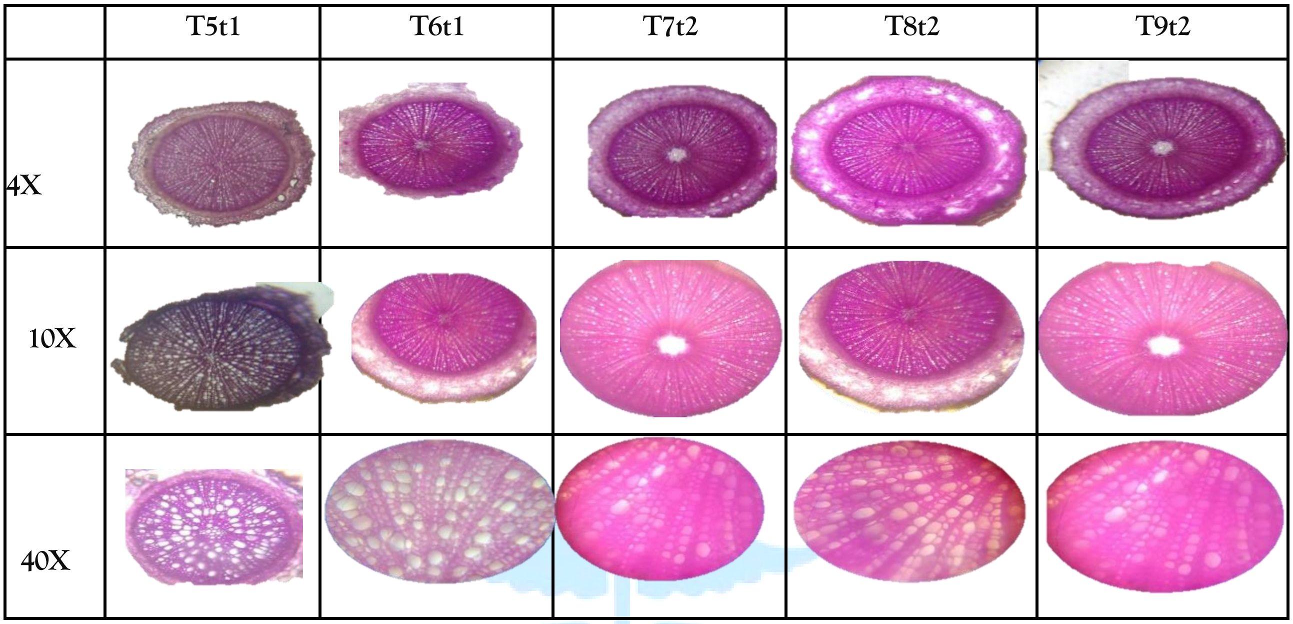

Figure 10: Root Anatomy of *Linum usitatissimum* (Flax) under the treatment of the Different Levels of $CrCl_2$ Stress. T5t1 (10 µM metal + 20mM), T6t1 (15 µM metal + 20mM), T7t2 (5 µM metal+20mM) T8t2(10 µM metal + 40mM), T9t2 (15 µM metal + 40mM).

Discussion

Heavy metal stress is a group of nonbiodegradable, persistent inorganic chemical constituents with an atomic mass greater than 20 and a density greater than 5 g/cm3 (Ahmad et al., 2022). Both terrestrial and aquatic life are severely impacted by heavy metal pollution (Pushkar et al., 2021). Plant height decreases in relation to the control when heavy metal stress increases. The plant's height is discovered to decrease under high levels of $CrCl_2$ stress (Iqbal et al., 2025). The results indicate that salinity had a significant impact on the height of plant seedlings lend credence to this theory (Zulfiqar et al., 2023; Shahid et al., 2023). In comparison to the control, it was observed that the leaf length and number of leaves decreased as the metals concentration increased. This study demonstrated that *Linum usitatissimum* has strong $CrCl_2$ tolerance and $CrCl_2$ accumulation capacity (Hassan et al., 2024). Cr indicated a delay in root maturation, elongation, and cell division. Root development is inhibited by high Cr concentrations, whereas lower Cr concentrations (20–40 µM) encourage it (Ahmad et al., 2022). *Linum usitatissimum* showed a phenomenon of “growth promotion in low concentrations and growth inhibition in high concentrations” under Cd treatment (Hu et al., 2023; Javeed et al., 2023). Cr may result in shorter coleoptiles, decreased radical length, and inhibited bud growth (Adhikari et al., 2020). The responses to $CrCl_2$ stress of the above-ground and below-ground parts of Linum usitatissimum were distinct. In roots, mainly $CrCl_2$ stress tolerance was exerted by reduced enzyme production(Hassan et al., 2023). Under $CrCl_2$ treatment, the total photosynthetic limitations were primarily caused by diffusion limitations—stomatal limitations and mesophyll conductance limitations (Ahmad et al., 2022; Maqsood et al., 2023). The inhibition of the photosynthetic efficiency of *Linum usitatissimum* under high $CrCl_2$ concentrations was indirectly caused by the

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impact of $CrCl_2$ on leaf morphology (Hu et al., 2023). Cr is responsible for decreasing the amounts of protein and photosynthetic pigments in plant leaves and can have an indirect impact on foliar plant parts (Hassan et al., 2026).

Under heavy metal stress of $CrCl_2$, it is observed that metal stress reduced the epidermal thickness with the increasing concentration (Nazar et al., 2026). However, $CrCl_2$ at higher concentration reduced much epidermal thickness because heavy metals inhibits cell division, cell elongation and cause oxidative damage (Dominic et al., 2022; Hassan et al., 2023). Cortical cell area, metaxylem area and phloem area of roots decreased significantly with increasing concentrations of $CrCl_2$ while epidermal thickness and cell area increased with increasing concentrations of $CrCl_2$ (GracePavithra et al., 2019; Akram et al., 2026). An increase in the thickness of the epidermis is associated with an increase in the root tissues' mechanical strength (Ghafoor et al., 2019).

Conclusion

Linum usitatissimum growth indices, such as fresh and dry weight and root-shoot length, were found to be significantly affected by the $CrCl_2$ stress in this study. *Linum usitatissimum's* anatomical characteristics were also altered by the $CrCl_2$ stress. The results of our study suggest that when *Linum usitatissimum* was subjected to $CrCl_2$ stress, growth characteristics were enhanced by applying foliar sprays of folic acid in sufficient quantities. Poisoning with chromium has a significant impact on both structural development and growth characteristics. Investigations on quick, economical, and effective ways to remove $CrCl_2$ from soil and other environmental areas are required.

DECLARATIONS: This study does not include human or animal subjects.

STATEMENT ON GUIDELINES: All experimental studies and experimental materials involved in this research are in full compliance with relevant institutional, national, and international guidelines and legislation.

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