BIO-AGRONOMIC AND REMEDIAL IMPACT OF VERMICOMPOST ON GERMINATION AND PHYSIO-BIOCHEMICAL RESPONSES OF MAIZE SEEDLINGS UNDER Pb TOXICITY

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ABSTRACT

Lead (Pb) contamination in the environment poses significant risks to ecosystems and human health. Effective management strategies are crucial for mitigating its impact. Maize (*Zea mays* L.) is a major cereal crop cultivated globally and threatened by Pb toxicity. This study was conducted (a two factorial experiment under CRD) to examine the impact of vermicompost (VC) application (0, 4, 6 and 8 tons ha^{-1}) on growth and physio-biochemical indicators of maize seedlings under four Pb levels (control, 100, 200, and 300 mg kg^{-1} soil). A pot experiment was conducted to evaluate the impact of VC treatment on maize growth. The addition of VC mainly 8 tons ha^{-1} improved maize seedling emergence by increasing the germination rate. Electrolyte leakage (EL), malondialdehyde (MDA), and hydrogen peroxide (H_2O_2) were increased by 113.81%, 268.15%, and 1.97%, respectively, under Pb stress (300 mg kg^{-1} soil Pb). In comparison with the control, the application of VC (4<6<8 tons ha^{-1}) improved the photosynthetic attributes of Chl. a, b, and carotenoid content (13.52%, 19.08% and 15.55%) and relative water content (RWC) by 17.73%, respectively. Soil amended with VC reduced oxidative damage resulting from lead-induced accumulation of reactive oxygen species by decreasing H_2O_2 and MDA levels and increasing antioxidant enzyme activities. The application of VC also significantly improved maize yield indices. Overall, the addition of VC enhanced maize growth, alleviated Pb toxicity, and offered a practical strategy for managing Pb-contaminated soils. Collectively, the results demonstrate that VC is an effective organic amendment for mitigating Pb toxicity and promoting sustainable maize production in contaminated agroecosystems.

Keywords: Vermicompost, Bio-agronomic potential, Germination rate, Boosted antioxidants, Enhanced productivity

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INTRODUCTION

Global hunger has risen in the twenty-first century, with Pakistan experiencing significant increases due to economic downturns caused by natural disasters. Food scarcity and starvation associated with climate change could be addressed by expanding the availability of nutritious foods through organic farming, particularly in developing countries (Mrabet, 2023). Agriculture is affected by climate change in two primary ways. First, yields of major cereal crops are declining globally due to climate change. Second, essential natural resources such as fertile land and freshwater supplies are diminishing. In Central and South Asia, crop yields may decrease by up to 30% by 2050 (Shahbaz *et al*., 2021).

Maize *(Zea mays* L.), a significant crop that is produced annually. Maize is an important cereal crop belonging to the family Poaceae and is cultivated globally (Kaushal *et al*., 2023). It ranks among the most significant crops in terms of annual global yield (Shiferaw *et al*., 2011). As the world's most widely grown grain crop, maize provides substantial protein and calories for both animal feed and human consumption.

Human-induced disruptions of the biosphere have led to a wide range of global impacts, including rapid industrialization, intensive farming, widespread mining, and a growing population. In addition to severely affecting the supply of natural resources, urbanization has also led to extensive and serious pollution of the crucial components that support life on Earth. A significant issue arising from human disruption of natural biogeochemical cycles is the increased accumulation of heavy metals (HMs), which have ecological, nutritional, and environmental consequences (Singha and Deka, 2024). Pollution from heavy metals poses a severe risk to the environment and human health. Maintaining healthy soil is essential for growing food crops because excessive heavy metal deposition in soil reduces plant yields and endangers human health through biomagnification in food chains (AbdElgawad *et al*., 2020).

Pb is a persistent environmental contaminant and one of the most hazardous inorganic metal pollutants in the world (Wang *et al*., 2020). Numerous manufacturing processes generate Pb, which then combines with soil, and water (Mehran *et al*., 2025; Zhang *et al*., 2024). One of the non-essential ions in plants; Pb is poisonous and inhibits seed germination, limits plant development, and lowers crop output. Pb poisoning significantly impairs the absorption of essential nutrients and decreases chlorophyll concentrations in plants (Aslam *et al*., 2021a).

Numerous processes in plant life cycle, including membrane permeability and chlorophyll synthesis, are affected by Pb toxicity (Collin *et al*., 2022). Additionally, a greater Pb level decreases enzyme activity, disrupts the water balance, alters membrane permeability, lowers mineral nutrition (Fan *et al*., 2020). Various physio-chemical and biological practices are used worldwide to remove heavy metals from polluted areas (Zulfiqar *et al*., 2023; Shahbaz *et al*., 2025). An economical and sustainable method of improving soil quality and microbial biodiversity is VC application, which produces through the symbiotic relationships between microorganisms and earthworms (Vyas *et al*., 2022; Souffront *et al*., 2022).

Previous research has shown that Pb toxicity harms maize. However, there is limited information regarding the use of crop residue-based VC as an environmentally sustainable strategy to mitigate Pb stress. In particular, the impact of VC on seedling emergence, photosynthetic pigment content, antioxidant defense mechanisms, and osmolyte regulation under Pb contamination has not been thoroughly investigated. Furthermore, limited research has explored the relationship between physiological responses and yield improvement in Pb-contaminated soils. In order to address this gap, the current study investigates the impact of VC application on maize seedling emergence and physio-biochemical responses under Pb stress.

MATERIALS AND METHODS

Experimental Design: A two-factor factorial experiment was conducted (during the winter of 2024 at the Old Botanical Garden of the University of Agriculture, Faisalabad (31.4336 latitude and 73.0683 longitude) under controlled conditions to assess the toxic effects and organic amendment due to Pb on maize plant. A completely randomized design was followed for a total of 48 experimental units each with 3 replications. Four different concentrations (0, 100, 200, and 300 mg kg^{-1}) of factor A (Pb) contamination was applied in soil. The factor B involved the application of VC at four levels: 0, 4, 6, and 8 tons ha^{-1}. Tables 1 and 2 present the physicochemical indices of the soil and VC employed in this trial.

Seeds Sowing and Plant Growth Conditions: Maize seeds (Sahiwal Gold, obtained from the Ayub Agricultural Research Institute, Faisalabad) were sown in earthen pots (16 kg soil pot^{-1}) at a depth of 3.81-5.08 cm, with nine seeds per pot. All VC doses, and Pb stress levels (lead acetate was dissolved in deionized water (dH_2O), thoroughly mixed) were applied before sowing in all pots (dimension; 30 cm height × 25 cm upper inner diameter × 21 cm lower inner diameter) except those designated as controls. The soil was then allowed to spike for ten days before sowing to ensure metal stability. Irrigation was applied every 3 to 5 days to maintain soil moisture, with frequency adjusted according to environmental conditions. The experiment took place under natural light and at ambient temperature throughout. Weeds were removed manually each week. Plants were monitored regularly for pests. All agronomic practices were implemented uniformly across treatments to ensure consistent growth conditions. At 30^{th} days after sowing, during the vegetative stage, three plants from each treatment replicate were randomly selected for physiological and biochemical analyses while yield parameters were measured at maturity stage.

Emergence Parameters: The experiment was monitored daily after sowing. Each day, the number of germinated seeds was recorded until no further germination occurred and the count remained constant.

Time to start germination (days): The day on which the first seedling emerged was recorded as the start of emergence.

Time to 50% germination (days): Time for 50% emergence was calculated by following the equation formulated by Coolbear *et al*. (1984) and later modified by Farooq *et al*. (2005).

$$E50 = ti + [\frac{\frac{N}{2} - ni}{nj - ni}] * (tj - ti)$$

Where,
N = Final/total number of germinated seeds
ni and nj = The number of emerged seeds between two specific time points, ti and tj were determined by counting them.

Mean germination time (days): MGT was calculated using the equation formulated by Ellis and Roberts (1981).
Mean emergence time = ($\sum$ Dn / $\sum$n)
Where
n = number of germinated seeds
D = number of days counted for emergence of first seedling

Emergence index (days): The Association of Official Seed Analyst, 1990 developed a formula to calculate the emergence index.
EI = (total emerged seeds/days for first seedling count) + (emerged seeds/days for last count)

Final emergence percentage (%): The final emergence percentage was determined according to the following formula:

$$\text{Final emergence percentage } = \frac{\text{Total emerged seeds}}{\text{total number of seeds sown}} \times 100$$

Chlorophyll Contents: Arnon's method (1949) was employed to quantify carotenoids, chl. a, b, a/b ratio, and total chl. content. Fresh leaf samples (0.1 g) were homogenized in 80% acetone, and the absorbance of the extract was measured at 663 nm for chl. a, 645 nm for chl. b, and 480 nm for carotenoids.

Relative Water Content: To ascertain RWC, fresh leaves were promptly excised from each treatment, duplicate, and genotype, and their fresh weight was recorded simultaneously, then immersed half of their piece in distilled water for 12 hours. After blotting away extra water, the leaves were weighted in order to ascertain their totally turgid weight, then put through a 24-hour oven drying process at 70°C to get the dry weight. The RWC was estimated using the formula Turner (1986) proposed, which is:

$$\text{RWC (\%)} = \frac{\text{fresh weight} - \text{oven dry weight}}{\text{turgid weight} - \text{oven dry weight}} \times 100$$

Oxidative Stress Determinants: H_2O_2 content was determined according to the procedure of Velikova *et al*. (2000). Lipid peroxidation was evaluated by measuring MDA levels following the procedure of Camak and Host (1991).

Membrane Stability Index and Electrolyte Leakage: MSI was determined following the formula described in the study by Sairam (1994).

$$Membrane\ stability\ index = [1 - (C1/C2)] \times 100$$

EL was measured using the method described by Lutts *et al*. (2004). EL (%) was calculated using the following formula:

$$EL = (EC1/EC2) \times 100$$

Organic Osmolytes: The Bradford (1976) method was used to determine total soluble protein (TSP) content in maize plants. Green leaves (250 g) were ground in 5 mL of K_3PO_4 using a chilled mortar and pestle. The homogenized extract was transferred to an Eppendorf tube and centrifuged at 12,000 rpm for 15 min. The resulting supernatant was collected in a separate Eppendorf tube. Bradford reagent (5 mL) was combined with 0.1 mL of the sample extract in a test tube. Following mixing, absorbance at 595 nm was determined using a WELab-721 spectrophotometer. While Total soluble sugars (TSS) were quantified using the method described by Yoshida *et al*. (1976).

Enzymatic Antioxidants: In order to prepare the samples, 250 mg of fresh maize leaves were ground in a mortar and pestle with 5 mL of potassium phosphate buffer at 15°C. Following homogenization, the material was transferred to Eppendorf tubes and centrifuged at 12,000 rpm for 12 min. The resulting supernatant was collected in a separate Eppendorf tube and stored at 15°C. The activities of catalase (CAT), superoxide dismutase (SOD), and peroxidase (POD) were quantified from this extract following the established protocols by Chance and Maehly (1955); described a method to measure CAT and POD activity. SOD activity was quantified using the method of Spitz and Oberly (2001).

Phenolic and Flavonoid Content Estimation: The phenolic content of the maize leaves was measured by Folin-Ciocalteu (FC) spectrophotometric method reported by Rehman *et al*. (2026). Briefly, Plant leaf was mixed with 100µL of FC reagent, followed by addition of 1.16mL of water and 200 µL of Na_2CO_3 (20 percent). This mixture was incubated at 40ºC for 30 minutes and absorbance of the reaction mixture was measured at 700nm. Phenolic content was reported as mg gallic acid equivalent per gram using gallic acid standard curve

Total flavonoid content of maize leaf was measured using the Aluminum chloride method. Briefly, 100 µL of plant leaf was mixed with 250 µL of $AlCl_2$ (10 percent) and 200 µL of deionized water. After 6 minutes of incubation 250 mL of 1M NaOH was added and incubated for another 15 minutes. Reaction mixture was diluted by adding 2.5 mL of dH_2O and absorbance was measured at 500nm. TFC of plant leaves was reported as µg catechin equivalent per gram using catechin as a standard.

DPPH Activity Assay: Radical scavenging activity of the maize leaves was measured using the 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical inhibition assay as described by Siddiqui *et al*. (2020). In this assay, 10 µL of maize leaf extract and 190 µL of freshly prepared DPPH solution was mixed and incubated in dark for 15 minutes at room temperature. Absorbance of the reaction mixture was measured at 520nm and percentage DPPH inhibition activity was calculated.

Statistical Analysis: A two-way analysis of variance (factorial under CRD) was employed for statistical analysis of the acquired data in order to assess the impacts of treatments and their interactions. OriginPro 2024, CoStat, and Statistix 8.1 were used for the analysis. Tukey's Honestly Significant Difference test was used to compare treatment means at the 5% probability level (Gomez and Gomez, 1984). Correlation analysis was conducted to assess the relationships among the studied traits. Graphical illustrations, such as the graphical abstract and graphs, were created using Canva and Origin Pro.

RESULTS

Emergence Parameters: Compared with the control group, Pb toxicity negatively affected the germination characteristics of maize seeds. This was indicated by increased mean germination time, prolonged emergence durations (including time to 50% germination and time to initial germination), lowered final germination percentage and a reduced emergence index (Figure 2). In contrast, VC amendment alleviated these negative effects induced by Pb. Specifically, applying 8 tons ha^{-1} VC resulted in the greatest improvement: it increased germination rate, reduced mean germination time, enhanced the germination index, and shortened emergence durations. Applying 6 tons ha^{-1} VC increased the final emergence percentage of maize seedlings.

Photosynthetic Indices: Mean comparison (Tukey test, $\alpha = 0.05$) between treatments and stress showed that the efficacy of photosynthetic pigments was significantly affected by both Pb dosage and organic amendment rate. As shown in Figure 3, Chl. a, Chl. b, carotenoids, and total Chl. were decreased by 28.16%, 9.19%, 24.72%, and 8.66%, respectively compared to the control group of plants. These reductions are indicative of Pb toxicity ($100<200<300$ mg kg^{-1} soil). It was also observed that increasing the VC rate to 8 tons ha^{-1} improved the efficiency of photosynthetic traits. This enhancement can be attributed to the ameliorative effects of organic amendments on soil health, which improve nutrient availability and reduce oxidative stress induced by Pb toxicity. As shown in Figure 3, Chl. a, Chl. b, carotenoids, and total Chl. contents increased by 13.52%, 19.08%, 15.55%, and 14.59%, respectively.

Relative Water Content: Substantial variations ($p \leq 0.001$) were seen in our results for relative water content under the individual effect of both factors. Compared with control group of maize plants, Pb showed negative effects on RWC (13.41% reduction) (Figure 4). However, harmful effects of Pb stress were mitigated with the VC application playing a positive role. Among the various treatment levels, 8 tons ha^{-1} VC application had the greatest impact, increasing the relative water content to 17.73%.

Oxidative Stress Determinants and Cell Membrane Parameters: Tukey test ($\alpha = 0.05$) revealed that Pb toxicity (mainly 300 mg kg^{-1} soil level) generally increased EL, MDA, and H_2O_2 content, while decreasing MSI. The evidence from this study is shown in Table 3. Pb stress (300 mg kg^{-1} soil) had higher EL, H_2O_2, and MDA contents by 113.81%, 1.97%, and 268.15% sequentially, and the membrane stability index decreased by 14.43%. The application of VC (8 tons ha^{-1}) reduced the adverse consequences of Pb stress compared to the other treatment levels. It decreased EL (61.56%), MDA (55.75%), and H_2O_2 (1.8%). Additionally, it increased the MSI by 16.48%. Indeed, the application of VC scavenged the toxicity of the Pb and increased the growth of the maize crop.

Organic Osmolytes: Pb toxicity (dose dependent impact) negatively affected total soluble sugar content (37.66% reduction) compared with the control group, whereas it positively affected total protein content, increasing concentration by 36.92% (Figure 5). However, harmful effects of Pb stress were mitigated with the VC application playing a positive role. Among the various treatment levels, the 8 tons ha^{-1} application showed the greatest impact, increasing total soluble sugar content by 54.39%. It had increased soluble protein content by 40.67%.

Enzymatic Antioxidants: The mean comparison test reveals that Pb levels, especially at 300 mg kg^{-1} soil, significantly increased the activity of antioxidant enzymes, likely by regulating their catalytic efficiency to combat stress. In this experiment, SOD, POD, and CAT increased by 47.65%, 127.55%, and 193.26%, respectively, under Pb stress compared with the non-stressed group. While the organic amendment, VC at 8 tons ha^{-1}, enhanced the activities of SOD, POD, and CAT by 36.09%, 37.88%, and 83.94%, respectively (Figure 6). Through VC application, the activity of enzymatic antioxidants further increased, thereby ameliorating the toxic effects of Pb stress.

Non-enzymatic Antioxidants: Pb toxicity influences the non-enzymatic antioxidants and triggers their catalytic activity to prevent maize plants from oxidative damage. The TPC, TFC, and DPPH increased by 27.48%, 41.28%, and 21.63%, respectively, under Pb stress (300 mg kg^{-1} soil). However, the addition of VC reduced the negative effects of Pb stress and upregulated non-enzymatic antioxidants. Figure 6 clearly shows that VC application at 8 tons ha^{-1} mitigated the harmful effects of Pb stress and further increased TPC, TFC, and DPPH by 29.65%, 40.86%, and 22.25%, respectively.

Yield Traits: VC application produced a significant effect ($P \leq 0.05$) on maize yield traits under varying levels of Pb stress (100, 200, and 300 mg kg^{-1} soil). Pb exposure reduced maize productivity, as indicated by decreases in plant height, cob length, cob diameter, number of grains per cob, 100-grain weight, and biological yield by 22.09%, 20.97%, 25.27%, 19.04%, 34.60%, and 16.06%, respectively. Table 4 demonstrates the substantial positive impact of VC, with yields surpassing those of the control group. VC application at 8 tons ha^{-1} level increased maize productivity by 46.15% (plant height), 74.41% (cob length), 34.69% (cob diameter), 153.84% (number of grains per cob), 75.25% (100-grain weight), and 22.65% (biological yield).

Correlation Analysis: Pearson correlation analysis revealed a strong association between photosynthetic pigments and yield traits, as well as between the membrane stability index and total soluble sugar in the maize crop (Figure 7). A very strong positive correlation was observed among all the studied parameters as represented by a violet-blue colour, whereas the chocolate-brown colour indicated a negative association. Furthermore, stress oxidants, electrolyte leakage, and enzymatic antioxidants exhibited negative correlations with the majority of the understudied variables, such as chlorophyll content, TSS, and yield parameters, but showed associations among themselves. On the other hand, TSP, TPC, TFC, and DPPH indicated no association with all remaining plant parameters but indicated a weak negative association with each other.

Table 1 | Physical characteristics of the soil used for this study

Parameters	**Characteristics**
Soil texture	Sandy loam
Sand (%)	72
Silt (%)	16
Clay (%)	12
Depth (cm)	0-15

Table 2 | Characteristics of Vermicompost

Studied Parameters	**Values**
Organic Matter (%)	69.42
Total C (%)	37
Nitrogen (%)	2.87
Moisture (%)	45.23
Phosphorous (%)	1.39
Potassium (%)	0.82
Calcium (ppm)	1320
Magnesium (ppm)	419
Fe (ppm)	170
Zinc (ppm)	125

Table 3 | The impact of vermicompost application on the stress oxidants and membrane indices of maize under Pb toxicity.

Pb	**Treatment**	**H_2O_2 (µ mol/g FW)**	**MDA (µ mol/g FW)**	**MSI (%)**	**EL (%)**
	T0	$0.0301{\pm}5.09{\times}10^{-5}$ c-e	$3.49{\pm}0.09$ fg	$82{\pm}2.89$ a-c	$15.46{\pm}0.23$ d
	T1	$0.0300{\pm}6.69{\times}10^{-5}$ c-f	$2.74{\pm}0.05$ g-i	$86.06{\pm}2.00$ ab	$9.53{\pm}0.21$ h
S0	**T2**	$0.0298{\pm}6.94{\times}10^{-5}$ ef	$2.36{\pm}0.06$ hi	$87.66{\pm}3.17$ ab	$7.76{\pm}0.23$ hi
	T3	$0.0297{\pm}4.17{\times}10^{-5}$ f	$1.99{\pm}0.05$ i	$89.16{\pm}2.35$ a	$6.03{\pm}0.12$ i
	T0	$0.0303{\pm}7.86{\times}10^{-5}$ bc	$5.36{\pm}0.15$ de	$78.43{\pm}2.47$ a-c	$20.46{\pm}0.78$ c
	T1	$0.0301{\pm}7.18{\times}10^{-5}$ c-e	$3.53{\pm}0.07$ fg	$83{\pm}1.52$ a-c	$12.13{\pm}0.31$ fg
S1	**T2**	$0.0300{\pm}5.41{\times}10^{-5}$ d-f	$2.96{\pm}0.11$ f-h	$84.86{\pm}3.07$ ab	$9.76{\pm}0.38$ gh
	T3	$0.0298{\pm}6.15{\times}10^{-5}$ ef	$2.39{\pm}0.03$ hi	$86.63{\pm}2.18$ ab	$7.86{\pm}0.29$ hi
	T0	$0.0306{\pm}9.62{\times}10^{-5}$ ab	$8.56{\pm}0.23$ c	$74.8{\pm}1.67$ bc	$24.93{\pm}0.71$ b
	T1	$0.0303{\pm}5.41{\times}10^{-5}$ b-d	$5.98{\pm}0.10$ d	$80.46{\pm}2.92$ a-c	$15.06{\pm}0.59$ de
S2	**T2**	$0.0302{\pm}5.41{\times}10^{-5}$ c-e	$4.86{\pm}0.15$ e	$82.6{\pm}2.76$ a-c	$12.46{\pm}0.54$ f
	T3	$0.0300{\pm}7.18{\times}10^{-5}$ c-f	$3.78{\pm}0.10$ f	$84.4{\pm}2.39$ ab	$9.93{\pm}0.24$ gh
	T0	$0.0307{\pm}5.41{\times}10^{-5}$ a	$12.87{\pm}0.37$ a	$70.16{\pm}2.33$ c	$30.13{\pm}0.63$ a
	T1	$0.0305{\pm}8.39{\times}10^{-5}$ ab	$9.91{\pm}0.16$ b	$77.03{\pm}2.49$ a-c	$18.93{\pm}0.55$ c
S3	**T2**	$0.0303{\pm}6.29{\times}10^{-5}$ bc	$7.99{\pm}0.20$ c	$79.36{\pm}1.85$ a-c	$15.56{\pm}0.24$ d
	T3	$0.0302{\pm}8.08{\times}10^{-5}$ b-d	$5.95{\pm}0.17$ d	$81.73{\pm}2.89$ a-c	$12.9{\pm}0.46$ ef

Data are presented as mean ± standard error of three replicates. Tukey's test (α = 0.05) was used for mean comparison, and values sharing the same letters do not differ significantly. Treatment VC; vermicompost = [T0: 0, T1: 4, T2 6, and T3: 8 tons ha^{-1}]; Pb Toxicity = [S0: 0, S1: 100, S2: 200, and S3: 300 mg kg^{-1} soil].

Table 4 | The impact of vermicompost application on the yield traits of maize under Pb toxicity.

Pb	Treatment	PH (cm)	CL (cm)	CD (cm)	GRNC	100 GRW (g)	BY (g)
	T0	44±1.52^{fg}	10.66±0.33^{f-h}	3.06±0.07^{c-e}	168±4.93^{ef}	22.39±0.70^{e-i}	101.66±2.18^{a-e}
	T1	49±1.52^{c-f}	13.46±0.33^{c-e}	3.12±0.093^{b-e}	243.33±7.52^{d}	22.83±0.64^{d-f}	107±2.64^{ab}
S0	**T2**	54±1.73^{a-d}	15.36±0.44^{bc}	3.48±0.064^{a-c}	352±8.55^{a-c}	26.46±0.88^{a-c}	108±3.79^{ab}
	T3	60.33±1.20^{a}	18.43±0.31^{a}	3.88±0.151^{a}	384.33±12.73^{a}	28.83±1.05^{a}	113±2.31^{a}
	T0	39±1.52^{gh}	10±0.57^{gh}	2.99±0.076^{c-f}	156±5.51^{f}	17.36±0.37^{h-j}	95±3.46^{b-f}
	T1	45±1.00^{e-g}	12.56±0.29^{d-f}	3.02±0.115^{c-e}	230.66±5.49^{d}	20.83±0.52^{e-h}	102±2.31^{a-e}
S1	**T2**	51.66±1.20^{b-e}	14.16±0.58^{b-e}	3.28±0.114^{b-d}	345±11.69^{a-c}	25.43±0.93^{a-d}	106±2.64^{a-c}
	T3	57±2.08^{ab}	16.26±0.57^{ab}	3.58±0.099^{ab}	377±12.78^{a}	28.4±0.163^{ab}	112±2.31^{a}
	T0	39±1.52^{gh}	8.6±0.25^{h}	2.69±0.105^{e-g}	143±4.04^{f}	15.36±0.46^{jk}	88.66±2.73^{ef}
	T1	42.33±1.45^{f-h}	12.26±0.49^{d-g}	2.99±0.131^{c-e}	219±7.55^{d}	18.83±0.48^{g-j}	93±2.31^{c-f}
S2	**T2**	48±1.52^{d-f}	13.9±0.61^{b-e}	3.18±0.069^{b-e}	330±11.80^{bc}	22.43±0.80^{d-g}	98±3.05^{b-f}
	T3	56±1.52^{a-c}	15±0.57^{bc}	3.38±0.069^{bc}	363±8.09^{ab}	24.86±0.58^{b-d}	105±2.51^{a-d}
	T0	36±0.57^{h}	8.46±0.16^{h}	2.29±0.069^{g}	136.66±3.51^{f}	13.33±0.49^{k}	85.33±3.18^{f}
	T1	41.66±1.20^{f-h}	12±0.57^{e-g}	2.49±0.038^{fg}	205.66±7.54^{de}	16.86±0.46^{i-k}	92±1.52^{d-f}
S3	**T2**	43±1.52^{f-h}	13.66±0.18^{c-e}	2.88±0.109^{d-f}	314±6.66^{c}	19.43±0.68^{f-i}	95±2.64^{b-f}
	T3	47±1.52^{d-f}	14.56±0.54^{b-d}	3.08±0.048^{c-e}	341.66±10.75^{a-c}	23.66±0.75^{c-e}	104.66±2.18^{a-d}

Data are presented as mean ± standard error of three replicates. Tukey's test ($\alpha = 0.05$) was used for mean comparison, and values sharing the same letters do not differ significantly. Treatment VC; vermicompost = [T0: 0, T1: 4, T2 6, and T3: 8 tons ha^{-1}]; Pb Toxicity = [S0: 0, S1: 100, S2: 200, and S3: 300 mg kg^{-1} soil]. Abbreviations; PH: Plant height, CL: Cob length, CD: Cob diameter, GRNC: Number of grains/cob, 100 GW: 100 grains weight, BY: Biological yield

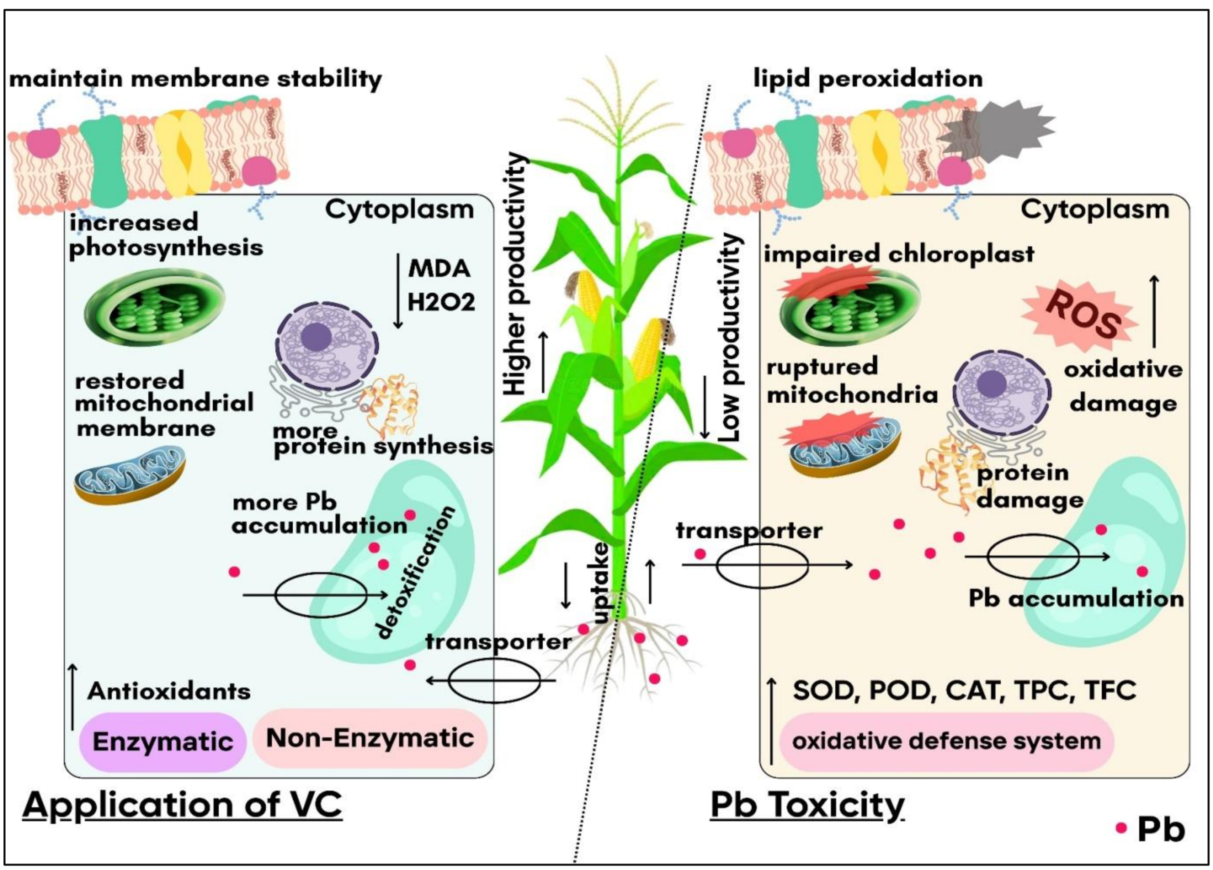

Figure 1: Graphical Abstract for experiment.

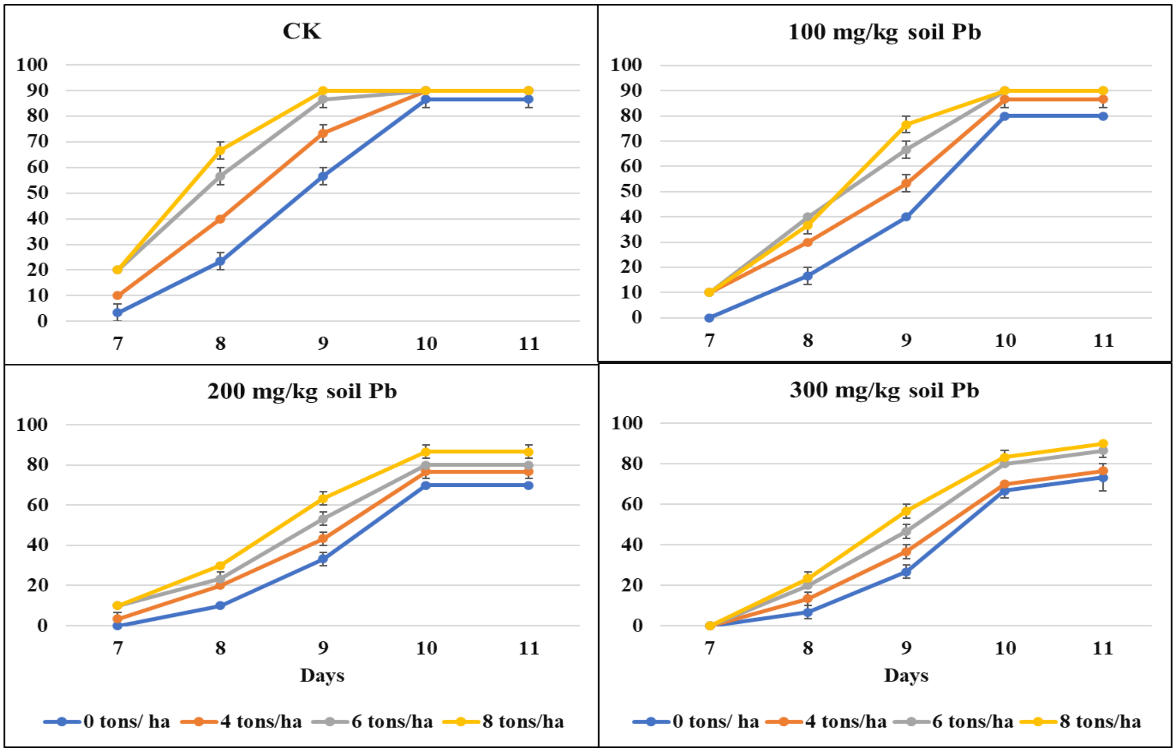

Figure 2: Response of maize seedling emergence to vermicompost application under Pb-induced stress. The Y-axis indicates the percentage of final emergence.

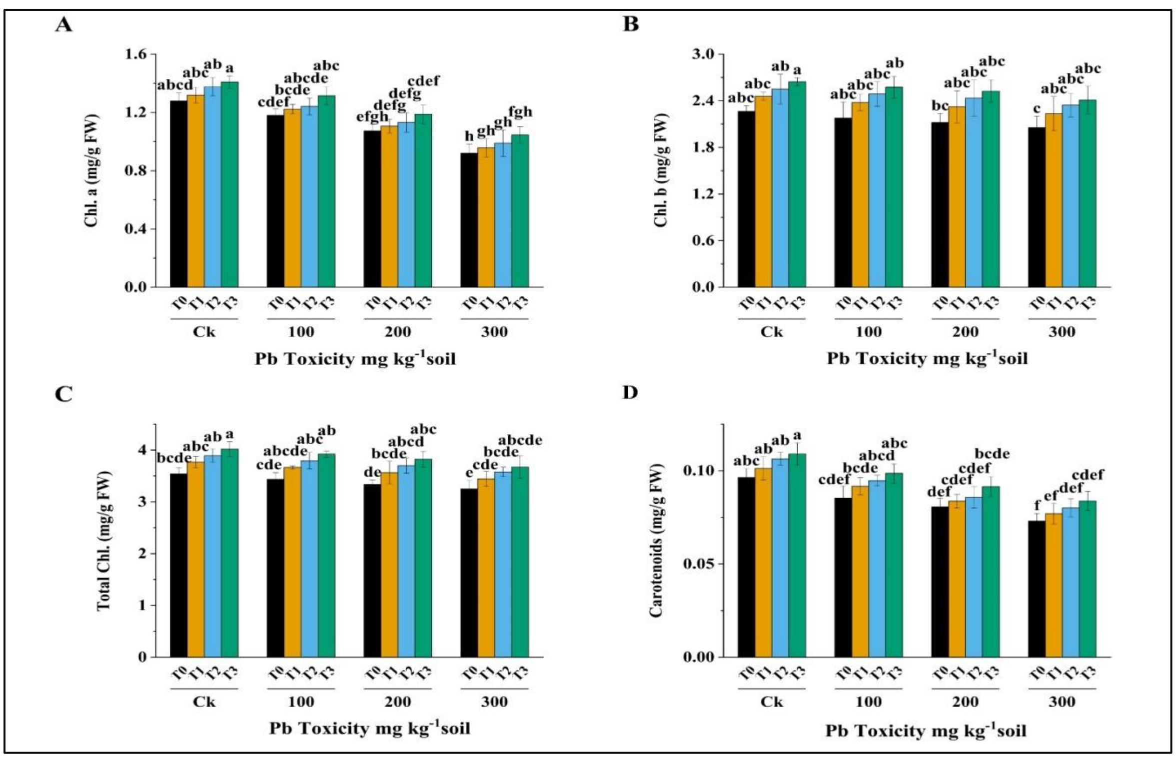

Figure 3: Impact of vermicompost application; [T0: 0, T1: 4, T2 6, and T3: 8 tons ha^{-1}] on Chl. a (A), Chl. b (B), Tot. Chl. (C), and carotenoid contents (D) in maize plants to various levels of Pb Toxicity Ck (control). Data are presented as mean ± S.E. of three independent measurements. Bars with the same letters indicate no significant difference.

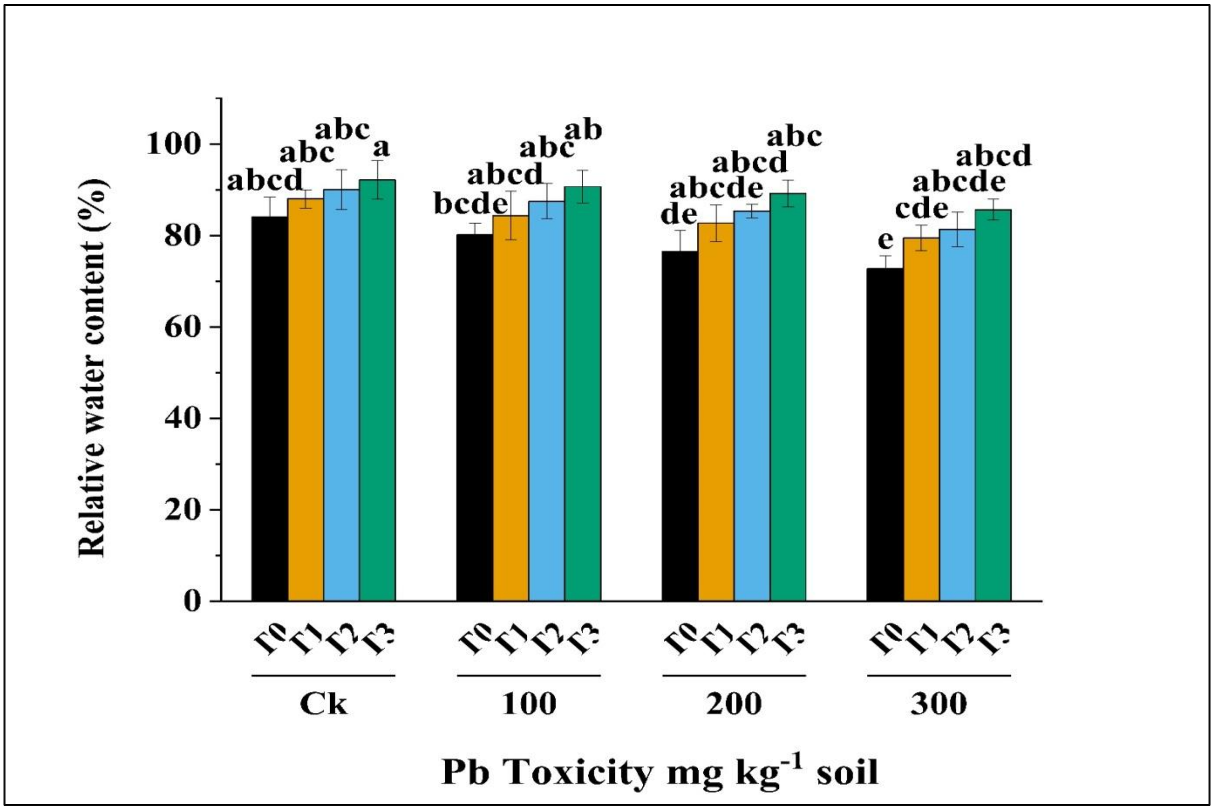

Figure 4: Impact of vermicompost application; [T0: 0, T1: 4, T2 6, and T3: 8 tons ha^{-1}] on relative water content of maize plant under Pb toxicity.

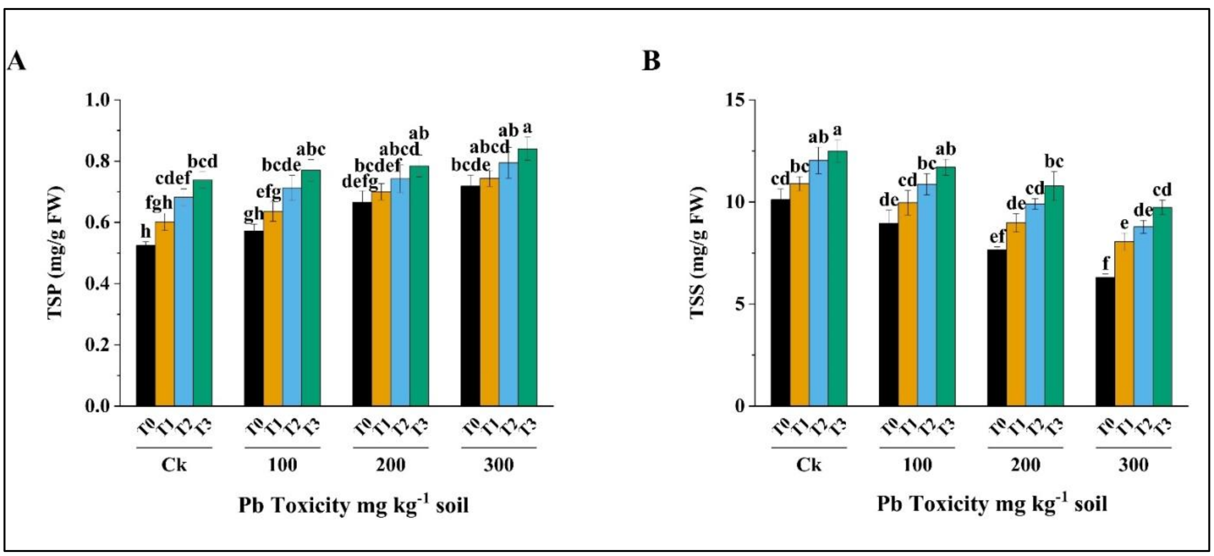

Figure 5: Impact of vermicompost application; [T0: 0, T1: 4, T2 6, and T3: 8 tons ha^{-1}] on total soluble protein (A), and total soluble sugar contents (B) in maize plants to various levels of Pb Toxicity Ck (control). Data are presented as mean ± S.E. of three independent measurements. Bars with the same letters indicate no significant difference.

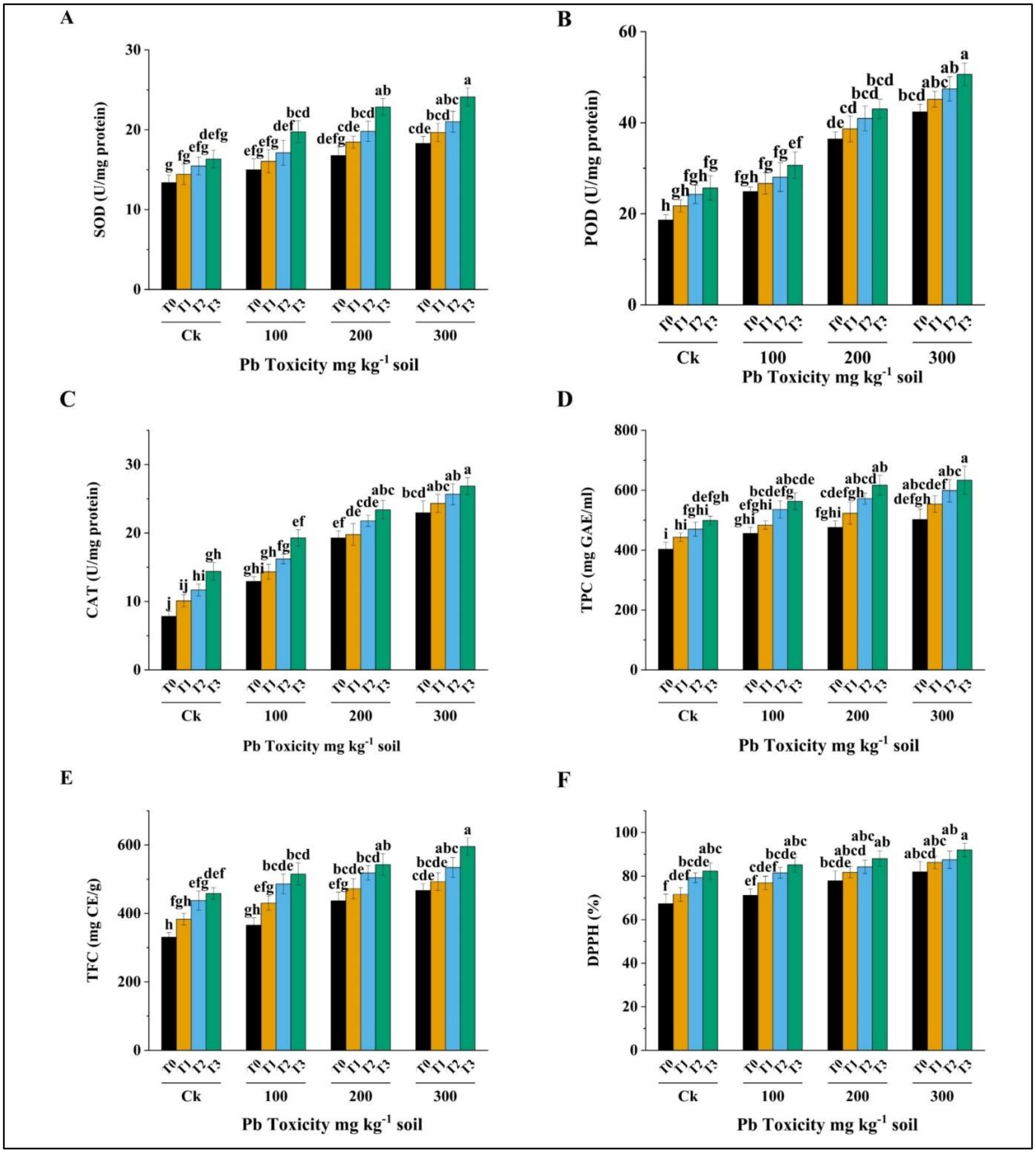

Figure 6: Impact of vermicompost application; [T0: 0, T1: 4, T2 6, and T3: 8 tons ha^{-1}] on SOD (A), POD (B), CAT (C), TPC (D), TFC (E), and DPPH (F) of maize plants to various levels of Pb Toxicity Ck (control). Data are presented as mean ± S.E. of three independent measurements. Bars with the same letters indicate no significant difference.

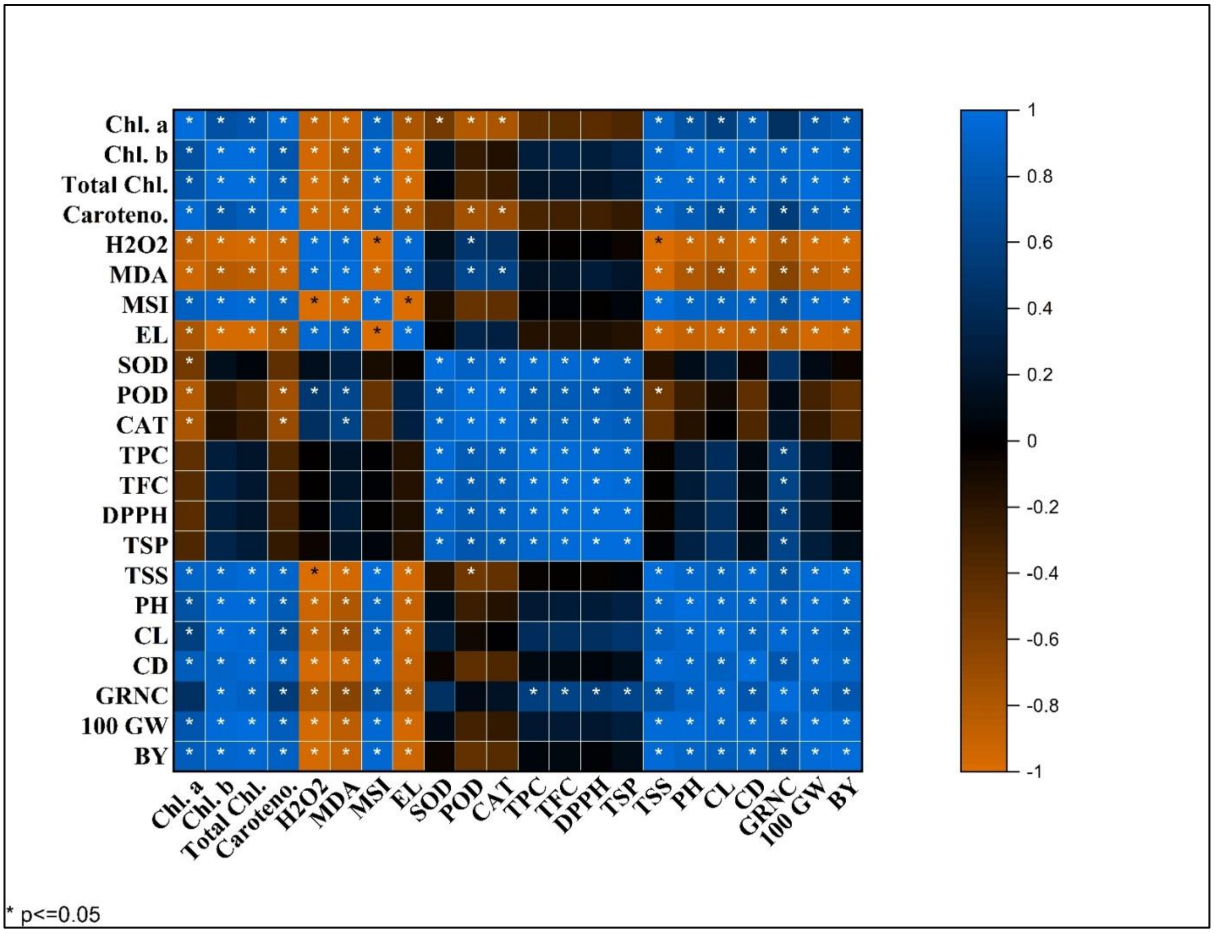

Figure 7: Correlation matrix between photosynthetic contents, stress oxidants, antioxidants, RWC, and yield attributes.

DISCUSSION

The accumulation of hazardous elements degrades soil structural quality and negatively affects the overall ecosystem. This degradation inhibits plant growth and disrupts soil microbial activity. The application of organic matter amendments for soil enrichment facilitates the immobilization of toxic elements, such as Pb, Ni, Cd, As, and Co. These amendments also enhance plant growth rates during phytoremediation, thereby potentially increasing the efficacy of contaminant removal (Wang *et al*., 2022). This study highlights the remedial effect of crop residue-based VC to reduce Pb stress by improving the growth of maize seedlings. VC has a fine texture and peat-like structure, which enhances its water-retention and aeration properties (Belliturk *et al*., 2015). These characteristics increase soil cation exchange capacity, facilitating the absorption of positively charged ions, including toxic elements (Aransiola *et al*., 2023).

The two developmental stages most susceptible to Pb contamination are germination and stand establishment (Kabir *et al*., 2008) germination is delayed because Pb accumulates in the seed embryo during the late stage of imbibition (Lamhamdi *et al*., 2011). Furthermore, the present findings are consistent with previous studies showing that Pb toxicity delays germination and reduces the final germination percentage. Similar findings have been reported in heavy metal-safflower studies, where germination was inhibited only at the highest metal combination dose (180 mg kg^{-1} soil). However, it was further observed that auxin degradation reduced plant growth, and that oxidative damage, ion and heavy metal toxicity (Mathur *et al*., 2022). These effects are primarily responsible for the low germination rate. The findings of this study indicated that VC application considerably improved the emergence parameters under Pb toxicity (Figure 2). The application of VC enhances seed germination by providing water-soluble bioactive compounds, including humic acids, phytohormones, and microbial metabolites, which stimulate metabolic activities in germinating seeds. These compounds facilitate enzymatic activation, increase nutrient availability, and regulate hormonal balance, thereby accelerating embryo growth, radicle emergence, and early seedling development. This process ultimately leads to higher and more rapid germination rates (Arancon *et al*., 2012).

Pb is known to cause phytotoxicity in maize plants and can decrease the rate of photosynthesis process. At the same time, when maize plants were treated with VC along with Pb, the photosynthetic contents were increased compared to control plants (Figure 3). Similarly, the findings regarding carotenoid content and photosynthetic rate in tomato leaves are consistent with previous reports that observed increased attributes following VC application. In addition, these microorganisms synthesize growth-promoting substances that stimulate physiological processes, leading to improved photosynthetic activity, enhanced gas exchange characteristics, and increased accumulation of carotenoids, which collectively support better plant growth (Aslam *et al*., 2023). One important factor influencing the association among water and plant physiology is relative water content. Pb toxicity reduced RWC content (Figure 4). Although the RWC uses stomatal conductance to track fluctuations in plant water status, it plays a crucial role in plants under adversity (Ma *et al*., 2023). RWC is decreased by Pb stress in plants via a number of metabolic pathways. In addition, Pb poisoning impairs aquaporin function, which impacts the movement of water along cell membranes (Bakhtiari *et al*., 2023). However, it was improved by the use of VC as demonstrated in the present experiment. Our outcomes are similar with Aslam *et al*. (2023) who also explained that inputting VC to the soil greatly boosted RWC in tomato leaves. Applying VC improves soil by improving its structure, adding more organic matter, and boosting its ability to hold water and exchange nutrients. These improvements encourage greater microbial activity and make nutrients more available to plant roots. As a result, plants can take up nutrients more easily.

Our results showed that maize-stressed plants had oxidative damage from harmful Pb metals, as seen by lower MSI and higher H_2O_2, MDA, and EL levels (Table 3). These results indicate that Pb stress leads to elevated production of H_2O_2 and MDA (cause plants to produce reactive oxygen species). In plant cells, ROS harm a variety of biomolecules, including lipids, proteins, and DNA (Akeel and Jaleel, 2023). The results of the current investigation showed a notable rise in the MSI of maize, decreased H_2O_2, MDA, and EL levels plants due to the application of VC by alleviating Pb stress. Remarkably, under stress, plants showed a notable decline in H_2O_2 and MDA cumulation when treated with VC. Reduced MDA and H_2O_2 generation may explain the observed increase in MSI and decrease in EL values. These results are consistent with those of Ghaffari *et al*. (2022), who reported that that VC treatment in stressed plants enhances antioxidant defense mechanisms that regulate the accumulation of reactive oxygen species. This process reduces lipid peroxidation and membrane damage, leading to an increased membrane stability index and decreased levels of MDA, H_2O_2, and EL. Organic osmolytes showed different responses to Pb pollution (Figure 5). TSS and TSP content play roles in osmoregulation, energy production, and stress resistance. This is due to that abiotic stress like heavy metal stress can cause a plant's water status to become unbalanced, which affects the process of osmotic adjustment, and eventually causes a higher accumulation of suitable osmolytes (TSP) in crops (Mashabela *et al*., 2023). In contrast to this, the level of TSS was lowered as also found in previous research in cowpeas (Padhi *et al*., 2022). The soil conditioning with VC, in addition to heavy metals, showed a higher level of organic osmolytes in the plant tissues than plants treated with Pb alone. Similarly, Jankauskienė *et al*. (2022) showed that the addition of VC considerably raised the amounts of soluble protein and soluble sugar in Chinese cabbage leaves as well.

Under metallic stress, the antioxidant enzyme system is crucial for minimizing oxidative harm to plants. Antioxidants are capable of controlling ROS metabolism (Zhou *et al*., 2021). Pb toxicity causes oxidative stress in cells to produce superoxide free radicals. The disproportionation of superoxide free radicals can be catalyzed by the SOD to produce H_2O_2 and O_2 followed by up regulated POD and CAT activity. The current study shows that as a result of Pb stress, the concentrations of antioxidants increase to remove ROS (Figure 6). However, application of VC enhances the antioxidant capacity of plants by stimulating antioxidant defense systems. This process improves the scavenging of reactive oxygen species, which protects cellular components from oxidative damage and supports normal physiological functions. The oxidation-reduction characteristics of phenolic molecules are linked to their decomposition of peroxidases, absorption and neutralization of free radicals, and suppression of ROS (Khosropour *et al*., 2022). The present work shows that the addition of VC reduces Pb stress by improving the water and nutrient properties of maize plants, which in turn increased the synthesis of non- enzymatic antioxidants (Figure 6). Consistent with the findings by Babashpour-Asl *et al*. (2022), coriander plants have been shown to have the greatest levels of DPPH, TPC, and TFC under Cd toxicity. VC is a formidable antioxidant that can both reduce and absorb reactive oxygen species (Aslam *et al*., 2021b). Moreover, the abundance of Zn, Fe, Mn, and Cu in VC may promote the activation of antioxidant enzymes (Talaat and Abdel-Salam, 2024).

The results of this study indicate that exposure to Pb levels reduced both physio-biochemical parameters and yield traits in maize (Table 4). These reductions in yield traits are consistent with previous findings by Sun *et al*. (2025). The observed decline in growth and yield can be attributed to the impact of toxic metals on stomatal conductance and iron transport, which together limit plant development (Mousavi *et al*., 2020). The increase in crop yield is closely related to the type, quantity, and combination of fertilizers to a certain extent. Fertilizer, as the main source of crop nutrients, directly participates in or regulates crop nutrient metabolism and cycling (Zhang *et al*., 2025). In current research, the use of VC increased the productivity of maize. According to Singh *et al*. (2008), VC amendment resulted in greater plant growth and higher marketable yield compared to inorganic fertilizer. VC is reported to promote plant growth through biologically mediated mechanisms, including the production of bioactive substances that enhance nutrient availability. Improvements in photosynthetic efficiency, assimilate production, and grain storage capacity led to increases in grain size, grain yield, and harvest index. These findings are consistent with the results reported by Ansari *et al*. (2025). While VC substantially mitigated the negative impacts of Pb toxicity on maize germination and physio-biochemical responses, crops cultivated in Pb-contaminated environments necessitate appropriate management. In accordance with food safety guidelines established by FAO/WHO, plant material produced in contaminated soils must be assessed prior to consideration for food or feed use. Effective management strategies may involve Phyto management techniques, utilization for non-food purposes, or controlled disposal to reduce the risk of Pb entering the food chain.

Conclusions: The study showed that Pb toxicity interfered with important plant functions and led to serious damage in plant tissues. On the other hand, using VC reduced lead toxicity in maize by regulating its physiological and biochemical mechanisms (Figure 1). The amendment improved plant growth, increased photosynthetic pigment levels, and made cell membranes more stable. Using VC also boosted antioxidant enzyme activity, which helped reduce oxidative stress by removing ROS. These findings support the hypothesis that organic amendments reduce Pb stress. VC demonstrates potential for remediating Pb-contaminated soils and improving crop safety. Additional field studies are required to confirm consistency and feasibility for large-scale use in various agro-climatic zones.

Author Contributions: CRediT

Hafeez ur Rehman: Writing - original draft, Software, Methodology, Formal analysis. **Athar Mahmood:** Writing - review & editing, Validation, Supervision, Conceptualization. **Muhammad Shahbaz:** Supervision, Methodology, Conceptualization. **Zubair Aslam:** Supervision, Conceptualization.

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