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The Impact of Lead (Pb) Pollution on Cellular Structure of Wheat (*Triticum aestivum* L.): Ultrastructural and Biochemical Evidence

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Abstract—This Lead (Pb) is a long-lasting environmental pollutant that can accumulate in agricultural soils via industrial emissions, traffic deposition, wastewater irrigation, and Pb-laden fertilizers. Wheat (Triticum aestivum L.) is a key food crop that is highly relevant to Pb toxicity studies owing to its significance in food security and human health. The current study examines Pb-induced changes in wheat cellular ultrastructure employing cutting-edge microscopy (TEM/SEM), fluorescence-based oxidative stress imaging, and biochemical markers of membrane integrity and antioxidant systems. Wheat seedlings were treated with varying concentrations of Pb and assessed for ultrastructural injury to chloroplasts, mitochondria, membranes, and nuclei, in addition to chlorophyll, lipid peroxidation (MDA), electrolyte leakage, and enzyme activities (SOD, CAT, POD). Pb treatment is anticipated to induce thylakoid membrane disorganization, plastoglobuli accumulation, mitochondrial swelling, nuclear changes, and ROS accumulation, in accordance with oxidative stress mechanisms. The holistic approach bridges Pb uptake and transport with cellular injury, offering evidence for environmental remediation and mitigation in wheat-based agroeco systems.

Keywords—Wheat, Lead (Pb), TEM, SEM, ROS, Chloroplast ultrastructure, Oxidative stress.

I. INTRODUCTION

Lead (Pb) pollution in the environment is still a concern worldwide due to the non-biodegradable, accumulative, and toxic nature of Pb to living organisms[1]. In plants, Pb can cause growth inhibition, photosynthesis disruption, and oxidative stress, which can be manifested at the cellular level by membrane damage and organelle ultrastructural changes[2]. One of the major mechanisms of Pb toxicity is the overproduction of ROS, resulting in lipid peroxidation, protein oxidation, and DNA damage, which is manifested by changes in chloroplast and mitochondrial integrity[3].

Wheat (Triticum aestivum L.) is a major crop that can be exposed to Pb from contaminated soil and irrigation water. Ultrastructural analysis (TEM) and other biochemical indices provide a robust basis for understanding the impact of Pb stress on cellular damage[5]. Previous studies have shown that Pb can inhibit wheat root growth and is linked to oxidative damage and ultrastructural changes in membranes and organelles[6][7].

Aim: To evaluate the impact of Pb pollution on wheat cellular structure by combining:

1. Pb uptake/translocation (ICP-OES/AAS),
2. ultrastructural microscopy (TEM/SEM),
3. ROS imaging and biochemical stress markers[10].

II. ADVANCED LABORATORY METHODOLOGY

A. Plant Material and Growth Conditions

- Plant: Wheat (*Triticum aestivum* L.) seeds (uniform size, high viability).
- Seed sterilization: 1–2% sodium hypochlorite for 5 min, rinse 4–5× with sterile distilled water.
- Germination: on moist filter paper (2–3 days), then transplant to pots.
- Growth conditions (controlled):
  - 25 ± 2°C, 16/8 h light/dark
  - 50–70% RH
  - Light intensity: ~150–250 µmol m^{-2} s^{-1}

B. Experimental Design

Completely randomized design (CRD), 4 groups, **n ≥ 5** biological replicates:

- C0: Control (0 Pb)
- Pb1: Low Pb
- Pb2: Medium Pb
- Pb3: High Pb

Figure 3. Stylized TEM schematic of chloroplast ultrastructure (control vs Pb stress)

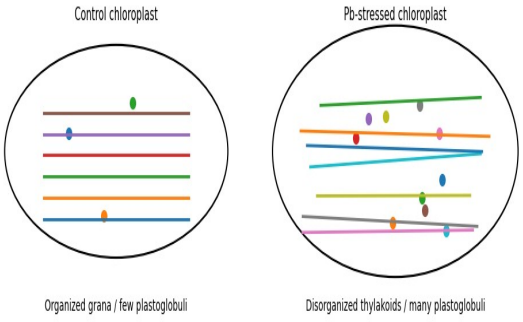

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Figure 1. Experimental workflow for Pb stress assessment in wheat

C. Pb Treatment

- Use $Pb(NO_3)_2$ solution to spike soil to:
  - 0, 250, 500, 1000 mg Pb/kg dry soil
- Maintain soil moisture at ~60–70% field capacity for 21 days.

D. Sampling

At day 14 and/or day 21:

- Leaves (young fully expanded) and root tips (2–3 mm) harvested.
- Wash gently with distilled water (avoid tissue damage).
- Immediately proceed to fixation (TEM/SEM) or freeze at −80°C (biochemistry).

III. Ultrastructure & Cytology

A. Transmission Electron Microscopy (TEM)

Purpose: detect organelle-level damage (chloroplasts, mitochondria, nucleus, membranes) [9].

Fixation and processing

1. Primary fixation: 2.5% glutaraldehyde in 0.1 M phosphate buffer (pH 7.2), 2–4 h at 4°C.
2. Buffer rinse: 3× (10 min each).
3. Post-fixation: 1% OsO_4, 1–2 h at 4°C.
4. Dehydration: ethanol series (30→50→70→90→100%).
5. Embedding: epoxy resin.
6. Ultrathin sections: 60–90 nm.
7. Staining: uranyl acetate + lead citrate.
8. TEM imaging: standardized magnifications for comparison.

B. Ultrastructural endpoints

- Chloroplast: thylakoid/grana disorganization, plastoglobuli number, envelope integrity.
- Mitochondria: swelling, cristae disruption.
- Nucleus: chromatin condensation, nuclear envelope irregularity.
- Plasma membrane: separation from cell wall (plasmolysis), discontinuity.

C. Quantification

- Chloroplast area (µm²), grana density, plastoglobuli count
- Mitochondrial area (µm²), cristae density (semi-quantitative scoring)

Pb is known to induce ultrastructural changes and oxidative damage-related injury patterns in plants; similar mechanisms are reported in wheat and other species.

IV. Scanning Electron Microscopy (SEM) **Purpose:** assess leaf surface, stomatal behavior, and deposition-related injury.

Procedure

1. Fixation: 2.5% glutaraldehyde (buffered).
2. Dehydration: ethanol series.
3. Drying: critical point drying (or HMDS).
4. Sputter coating: Au/Pd.
5. SEM imaging: leaf epidermis, stomata.

Metrics

- Stomatal density (#/mm²)
- Stomatal pore length/width (µm)

A. Confocal/Fluorescence Imaging (ROS & Membrane Integrity)

- ROS probe: DCFH-DA (general ROS, fluorescence intensity).
- H_2O_2 localization: DAB staining.
- Superoxide localization: NBT staining.
- Membrane damage (roots): Propidium iodide (PI). Quantify fluorescence intensity (a.u.) under identical exposure settings.

V. Biochemical and Physiological Assays

A. Chlorophyll a, b, Total Chlorophyll

Extract pigments with 80% acetone; read absorbance at 663 and 645 nm.

Arnon-type equations (commonly used):

- Chlorophyll a (µg/mL) = 12.7·A663 − 2.69·A645
- Chlorophyll b (µg/mL) = 22.9·A645 − 4.68·A663
- Total chlorophyll (µg/mL) = 20.2·A645 + 8.02·A663

Convert to mg/g FW:

$$\text{Chl (mg/g FW)} = \frac{C(\text{mg/L}) \times V(\text{L})}{W(\text{g})}$$

B. Lipid Peroxidation (MDA; TBARS Method)

Read A532 and A600 to correct non-specific turbidity.

$$\text{MDA (M)} = \frac{(A_{532} - A_{600})}{\varepsilon \cdot l}$$

where $\varepsilon \approx 155{,}000\, M^{-1} cm^{-1}$ and $l = 1\, cm$. Arpi +1

Convert to nmol/g FW:

$$\text{MDA (nmol/g FW)} = \frac{C(\text{mol/L}) \times V(\text{L}) \times 10^9}{W(\text{g})}$$

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C. Electrolyte Leakage (EL%)

$$EL(\%) = \frac{C_1}{C_2} \times 100$$

where C1C_1C1 is initial conductivity, C2C_2C2 is conductivity after boiling[8].

D. Antioxidant Enzymes (SOD, CAT, POD)

Normalize activities per mg protein (Bradford assay).

CAT (H_2O_2 decomposition at 240 nm):

$$CAT = \frac{\Delta A_{240}/min \times V_{tot}}{\varepsilon \times l \times V_{enz} \times \text{protein}}$$

(Use suitable ε\varepsilonε for H_2O_2 at 240 nm based on your protocol.)

SOD: based on % inhibition of NBT photoreduction (1 U often = 50% inhibition).

VI. Pb Uptake and Translocation (ICP-OES/AAS)

A. Pb Concentration in Roots and Shoots

- Dry tissues (60–70°C), grind, acid digest (HNO_3/H_2O_2), analyze Pb via ICP-OES or AAS.

Bioaccumulation Factor (BAF):

$$BAF = \frac{C_{plant}}{C_{soil}}$$

Translocation Factor (TF):

$$TF = \frac{C_{shoot}}{C_{root}}$$

These indices are widely used to evaluate uptake and movement of metals in plants.

B. Statistical Analysis

- Normality: Shapiro–Wilk; homogeneity: Levene.
- One-way ANOVA + Tukey HSD ($p < 0.05$).
- Pearson correlation between Pb accumulation (root/shoot) and (MDA, chlorophyll, ultrastructure scores).

VII. Results

A. Chlorophyll and Oxidative Damage

- Chlorophyll a, b, total chlorophyll decrease with Pb.
- MDA and EL% increase with Pb (membrane lipid peroxidation).
- Antioxidant enzymes often increase at low/moderate Pb then may decline at high Pb due to severe damage.

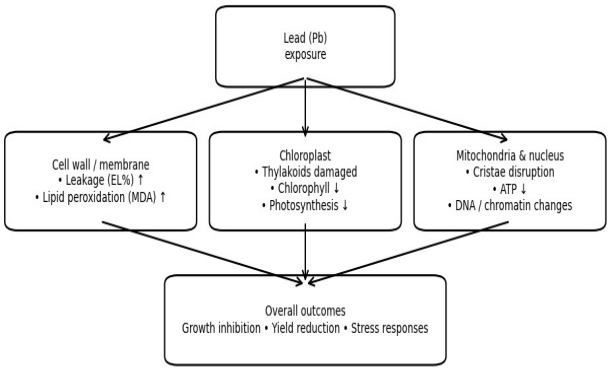
B. TEM Findings (Ultrastructural)

Expected trends (dose-dependent):

- Chloroplast: thylakoid disorganization, swollen chloroplasts, increased plastoglobuli.
- Mitochondria: swelling and cristae disruption.
- Nucleus: chromatin condensation / irregular envelope.

These patterns are consistent with Pb-induced oxidative injury mechanisms reported in plant ultrastructure studies and wheat evidence.

Figure 2. Conceptual model of Pb-induced cellular injury in wheat



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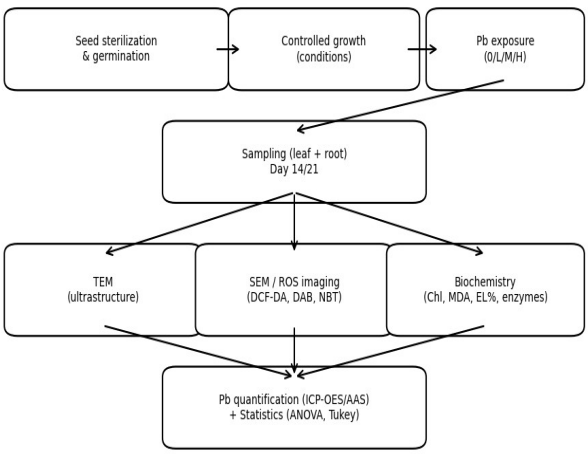

Figure 3. Stylized TEM schematic of chloroplast ultrastructure (control vs Pb stress)

VIII. Discussion

Pb stress affects wheat cellular structure mainly via oxidative stress mechanisms. ROS overproduction may peroxidize membrane lipids, as evidenced by the accumulation of MDA

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and electrolyte leakage, and may affect chloroplast membranes, leading to a reduction in photosynthetic pigments and thylakoids. Ultrastructural alterations, such as plastoglobuli accumulation, are often indicative of thylakoid membrane stress and lipid alterations. Mitochondrial swelling and cristae disruption indicate impaired respiration and ATP synthesis, consistent with a systemic energy deficiency.

In wheat, there is evidence of Pb-induced oxidative injury and ultrastructural changes in membranes and organelles, which support the conclusion that cellular damage is a key contributing factor to growth inhibition induced by Pb pollution.

IX. CONCLUSION

This study shows that lead (Pb) contamination has a strong impact on wheat (Triticum aestivum L).) at the cellular, ultrastructural, and biochemical levels, showing why it matters as an environmental stressor in farming systems.As Pb concentrations went up, the cells showed clear signs of damage. The cell membranes lost their integrity, there was more electrolyte leakage, and lipid peroxidation went up, which we could see from the higher malondialdehyde (MDA) levels.These changes show serious oxidative stress and a breakdown in membrane stability caused by Pb pollution.Looking closely at the organelles, we saw clear damage to the chloroplast thylakoid membranes. This came with a drop in chlorophyll levels and a loss in the plant's ability to photosynthesize properly.Mitochondrial swelling and messed-up cristae show that the cell's energy system is off, which might lower ATP production and slow down plant growth.Nuclear chromatin changes seen under Pb stress suggest that genetic regulation and stress-response signaling pathways are being affected.From an ecological point of view, these changes in cells and their parts act as early-warning signs of environmental pollution.Since wheat is a main producer in farming systems, lead-caused cell damage might end up lowering how much plants produce, hurting food quality, and upsetting the balance of the whole ecosystem.The buildup of Pb in plant tissues raises worries about it moving up the food chain, which could be risky for animals higher up, including humans.To sum up, combining ultrastructural microscopy, biochemical stress indicators, and metal accumulation analysis offers a solid way to assess how heavy metal pollution affects the environment.Wheat cells reacting to Pb stress can act as clear signals when checking soil contamination and the overall health of the environment.Future research should look into long-term field studies, how different pollutants mix and affect each other, and finding wheat types that can handle Pb. This would help keep farming sustainable and manage environmental risks better.

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