

Phytotoxic Effects of Crude Oil Contamination on Maize Growth Parameters

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ABSTRACT

Original research paper

This study evaluated the phytotoxic and biochemical effects of crude oil contamination on the growth and physiology of maize (*Zea mays* L.) cultivated in artificially polluted soil. Bonny Light crude oil was introduced at 0 mL/kg (control), 2 mL/kg, 3 mL/kg, and 4 mL/kg soil concentrations, and plants were grown for eight weeks in a greenhouse using a completely randomised design. Germination percentage declined progressively from 90% in the control to 60% at 4 mL/kg, while mean plant height reduced from 84.5 cm (control) to 46.7 cm in the highest treatment. Correspondingly, dry shoot biomass decreased from 6.42 g in the control to 2.85 g in the 4 mL/kg group, and the anthesis–silking interval extended from 2 days in the control to 6 days under maximum contamination. Chlorophyll a and b contents declined significantly ($p < 0.05$) from 1.32 mg/g and 0.94 mg/g in control plants to 0.71 mg/g and 0.46 mg/g, respectively, in the most polluted soil, indicating suppressed photosynthetic efficiency. In contrast, malondialdehyde (MDA) concentration increased from 0.42 $\mu\text{mol/g}$ in the control to 1.11 $\mu\text{mol/g}$ in the 4 mL/kg group, confirming membrane lipid peroxidation. Activities of superoxide dismutase (SOD), catalase (CAT), and glutathione peroxidase (GPx) increased by 1.6-, 2.1-, and 1.8-fold, respectively, suggesting a compensatory antioxidant response. These results demonstrate that crude oil stress impairs maize development by inducing oxidative damage and metabolic disruption, while enhanced antioxidant enzyme activities represent adaptive defence mechanisms against petroleum toxicity.

Keywords: Crude oil contamination, *Zea mays*, Germination, Chlorophyll, Malondialdehyde, Antioxidant enzymes, Oxidative stress.

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Introduction

Crude oil contamination of agricultural soils constitutes a profound ecological and agronomic concern, posing a serious impediment to crop productivity (Adeyemi and Otugboyega, 2025) and food security, particularly for staple cereals such as *Zea mays* (maize) (Nnaemeka, 2023). This form of environmental degradation—commonly arising from industrial discharges, pipeline ruptures, and poor waste management—introduces an array of hydrocarbon constituents that substantially modify the physicochemical properties of the soil (Adeyemi & Adeyemi, 2021; Adeyemi & Adeyemi, 2020). Such alterations affect nutrient dynamics,

water-holding capacity, and gaseous exchange within the rhizosphere, thereby creating suboptimal conditions for plant establishment and development (Adeyemi & Adeyemi, 2020; Ren et al., 2025).

The infiltration of petroleum hydrocarbons into soil systems impedes key physiological functions in plants, culminating in impaired photosynthetic activity and diminished biomass formation across various species, including maize (Fedeli et al., 2022). Hydrocarbon toxicity provokes a complex spectrum of phytotoxic manifestations characterised by disrupted metabolic processes, reduced chlorophyll concentration, and altered enzyme kinetics, all of which

compromise overall plant vigour and yield potential (He et al., 2022). Exposure to crude oil further stimulates oxidative stress through the generation of reactive oxygen species, which in turn damage vital cellular components such as chlorophyll molecules and lipid membranes (Bashir et al., 2024; Laurent et al., 2011). These perturbations in pigment integrity and membrane function severely constrain the plant's photosynthetic efficiency and energy utilisation capacity (Lassalle et al., 2019). Moreover, hydrocarbon residues frequently obstruct nutrient and water accessibility within the root zone, compounding the physiological strain on affected plants (Lassalle et al., 2019). The intricate interactions among plant tissues, soil minerals, pore fluids, and gaseous phases under the influence of non-aqueous phase liquids remain poorly elucidated, thereby complicating mitigation and remediation approaches (Oniosun et al., 2019). Addressing the adverse agricultural implications of petroleum contamination, therefore, necessitates a deeper comprehension of these multifaceted soil–plant–contaminant relationships and their specific repercussions on *Zea mays* physiology (Ali et al., 2021; Saeed et al., 2021).

Petroleum hydrocarbons are known to degrade chlorophyll pigments, retard germination, and interfere with plant–water relations and gaseous exchange. Among these, aliphatic, ethylenic, naphthenic, and aromatic fractions are particularly hazardous xenobiotics (Wyszkowski et al., 2020). Such compounds disrupt nutrient uptake and translocation, provoke oxidative imbalances, and induce morphological and physiological deformities that ultimately culminate in yield depression (Haider et al., 2021). The hydrophobicity of low molecular weight hydrocarbons produces an oily surface layer on the soil, thereby restricting infiltration and adversely influencing leaf expansion, seedling emergence, and root elongation (He et al., 2022). Petroleum pollutants can also cause cytological injury to root cells and tissues as hydrocarbons destabilise plasma membranes, impeding the absorption of water and mineral nutrients (Fitrisyaah et al., 2025). This nutrient deprivation often results in diminished root biomass and general stunting, as the plant reallocates limited resources to sustain essential metabolic functions under stress (Oniosun et al., 2019).

Furthermore, crude oil constituents such as polycyclic aromatic hydrocarbons (PAHs), total petroleum hydrocarbons (TPH), and trace metals may infiltrate plant tissues, influencing their water balance and photosynthetic competence. Low-carbon PAHs and C₅–C₁₀ TPH fractions are especially bioavailable and thus more toxic (Lassalle et al., 2018). Phytotoxicity assessments, including seed germination assays, have demonstrated that elevated TPH concentrations suppress germination and induce nutrient deficiency severe enough to preclude seedling establishment (Abdelhafeez et al., 2021). Such detrimental influences extend beyond the germination phase, with mature plants exposed to petroleum showing lowered photosynthetic efficiency and reduced stomatal conductance (Nendza et al., 2011). The intensity of

phytotoxicity is governed by factors such as hydrocarbon type, soil characteristics, and species tolerance—diesel typically exhibiting greater toxicity than petrol due to its lower volatility and more complex composition (Balseiro-Romero & Monterroso, 2015). Empirical evidence reveals that diesel exposure can reduce sorghum and pearl millet biomass by 67% and 74%, respectively, underscoring its pronounced inhibitory impact on plant growth (He et al., 2022).

Additionally, crude oil pollution diminishes soil biodiversity and disturbs the equilibrium of bioavailable nitrogen and phosphorus, thereby constraining nutrient uptake and leading to reduced macronutrient concentrations within plant organs (Wyszkowski et al., 2020). This pervasive toxicity highlights the necessity of comprehensive investigation into the physiological mechanisms underlying crude oil stress in *Zea mays* and the identification of viable mitigation approaches. Hence, the present study explores the phytotoxic consequences of crude oil contamination on maize by examining critical growth indices—seed germination, shoot and root elongation, biomass accumulation, and chlorophyll content (Fedeli et al., 2022; Gawryluk & Krzyszczyk, 2023). These metrics constitute essential indicators of plant health and will provide valuable insights into the extent of crude oil-induced growth inhibition across developmental stages of maize (Kaur et al., 2017).

Materials and Methods

Study Location and Experimental Design

The experiment was conducted under controlled greenhouse conditions at the Department of Environmental Management and Toxicology, Federal University of Petroleum Resources, Effurun (FUPRE), Delta State, Nigeria. The study employed a completely randomised design (CRD) comprising four treatment groups with five replicates each. The treatments consisted of crude oil concentrations of 0 mL/kg (control), 2 mL/kg, 3 mL/kg, and 4 mL/kg of soil. Bonny Light crude oil, representative of local petroleum sources, was obtained from the Warri Refining and Petrochemical Company (WRPC). The experimental duration spanned eight weeks, covering both vegetative and reproductive stages of *Zea mays* (maize) development.

Soil Preparation and Contamination Procedure

Topsoil (0–20 cm depth) was collected from uncontaminated farmland, air-dried, homogenised, and sieved through a 2 mm mesh to ensure uniform particle size. Measured volumes of crude oil (2, 3, and 4 mL/kg) were added to the soil on a weight-per-weight basis and thoroughly mixed to achieve homogeneous contamination. The contaminated soils were stabilised for seven days to simulate natural weathering before planting. The control soil (0 mL/kg) was similarly treated without crude oil addition.

Seed Selection and Planting

Viable *Zea mays* seeds were sourced from the Nigerian Institute of Agricultural Research, Ibadan. Uniform, healthy seeds were surface-sterilised with 1% sodium hypochlorite for 2 minutes, rinsed with distilled water, and air-dried. Five seeds were planted in each pot (25 cm diameter, 20 cm height) containing 5 kg of treated soil, later thinned to three vigorous seedlings after germination. Watering was performed daily with 200 mL of tap water to maintain adequate soil moisture without leaching the hydrocarbons.

Germination and Growth Assessment

Germination percentage (GP) was determined daily from the second to the sixth day after planting using the formula:

$$GP = \frac{\text{Number of germinated seeds}}{\text{Total seeds planted}} \times 100$$

Germination index (GI) and mean germination time (MGT) were calculated following the methods of Kaur et al. (2017). Emergence percentage was recorded on the sixth and eighth days. Growth parameters—plant height (cm) and stem girth (mm)—were measured fortnightly from week 2 to week 8 using a metre rule and Vernier caliper, respectively.

Biomass Determination

At harvest, plants were uprooted carefully, washed with distilled water, and separated into shoots and roots. Wet weights were recorded immediately, and samples were oven-dried at 80 °C for 48 hours to obtain dry biomass. Mean wet and dry shoot and root weights were expressed in grams per plant.

Reproductive Growth Evaluation

The reproductive stages—tasseling, anthesis, silking, and anthesis–silking interval (ASI)—were monitored daily. The number of days required for 50% and 100% of the plants in each treatment to exhibit these traits was recorded according to the phenological method of Waqas et al. (2021).

Biochemical and Oxidative Stress Analyses

Fresh leaf samples were homogenised in 80% acetone for pigment analysis. Chlorophyll a, chlorophyll b, and

carotenoids were quantified spectrophotometrically following Arnon's method (1949) with absorbance readings at 663 nm, 645 nm, and 480 nm, respectively. Total soluble protein was estimated using the Lowry method (1951) with bovine serum albumin as the standard. Lipid peroxidation was assessed via malondialdehyde (MDA) content using the thiobarbituric acid (TBA) reaction as described by Heath and Packer (1968).

Antioxidant enzyme activities were determined from fresh leaf extracts prepared in phosphate buffer (pH 7.0). Superoxide dismutase (SOD) activity was measured based on nitroblue tetrazolium inhibition; catalase (CAT) activity was assayed through hydrogen peroxide decomposition at 240 nm; ascorbate peroxidase (APx) activity followed Nakano and Asada (1981); glutathione reductase (GSH) and glutathione peroxidase (GPx) were evaluated following standard spectrophotometric procedures (Eluehike et al., 2019). Total phenolic content (TPC) was estimated using the Folin–Ciocalteu method, expressed as mg gallic acid equivalent (GAE) per gram of fresh weight.

Statistical Analysis

Data obtained from the various parameters were analysed using one-way analysis of variance (ANOVA) with SPSS version 27. Treatment means were separated using Duncan's Multiple Range Test (DMRT) at $p \leq 0.05$. Results are presented as means \pm standard error of the mean (SEM), and graphical representations were prepared using GraphPad Prism 10.

Results

Table 1a illustrates the impact of varying concentrations of crude oil on the germination percentage (%) of *Zea mays*. A clear trend was observed, with germination percentages decreasing as crude oil concentration increased: 0 ml > 2 ml > 3 ml > 4 ml. The highest germination rate was profiled in the control soil (0 ml/kg crude oil), where germination increased gradually from 80% on day 2 to 90% by day 6. In contrast, the lowest germination percentage occurred in the most heavily contaminated soil (4 ml/kg), ranging from 25% on day 2 to 60% on day 6.

Table 1: Effect of different concentration of crude oil on Germination percentage (%) of *Zea mays*

Treatment (ml/kg)	Day 2	Day 3	Day 4	Day 5	Day 6
0 ml (control)	80.00 \pm 0.1 ^a	80.00 \pm 0.5 ^a	82.50 \pm 0.5 ^a	85.00 \pm 0.5 ^a	90.00 \pm 0.3 ^a
2 ml	70.00 \pm 0.2 ^a	70.00 \pm 0.5 ^a	75.00 \pm 0.7 ^b	77.50 \pm 0.7 ^a	80.00 \pm 0.5 ^b
3 ml	42.50 \pm 0.3 ^c	42.50 \pm 0.7 ^c	62.50 \pm 0.5 ^c	65.00 \pm 0.8 ^b	70.00 \pm 0.7 ^c
4 ml	25.00 \pm 0.1 ^d	25.00 \pm 0.5 ^d	60.00 \pm 0.3 ^c	60.00 \pm 0.5 ^b	60.00 \pm 0.3 ^d

Different letters indicate significant differences ($p \leq 0.05$) as determined by DMRT

Table 1 shows the effect of different concentrations of crude oil on germination index (GI) and mean germination Time (MGT) of *Zea mays*. The control group exhibited the highest germination index (GI) compared to maize plants grown in crude oil-contaminated soils. Both the GI and mean germination time (MGT) of the control were significantly different from those examined in the polluted soils. GI values followed a decreasing trend with increasing contamination: 78% (0 ml/kg) > 70% (2 ml/kg) > 56% (3

ml/kg) > 46% (4 ml/kg), indicating that higher crude oil concentrations negatively affected seed vigor. Similarly, MGT increased with contamination level, reflecting a delay in germination as pollution intensified. The control soil (0 ml/kg) had the shortest MGT at an average of 2.25 days, whereas the most polluted soil (4 ml/kg) exhibited the greatest delay, with an average MGT of 3.31 days.

TABLE 2: Effect of different concentration of crude oil on germination index (GI) and mean germination time (MGT)

Treatment (ml/kg)	Germination index (GI)	Mean germination time (MGT)
0 ml	78±0.2 ^a	2.25±0.3 ^c
2 ml	70±0.1 ^b	2.29±0.2 ^c
3 ml	56±0.3 ^c	2.75±0.2 ^b
4 ml	46±0.2 ^d	3.31±0.1 ^a

Different letters indicate significant differences ($p \leq 0.05$) as determined by DMRT

Table 3 presents the mean emergence percentages of *Zea mays* in relation to crude oil concentration on the 6th and 8th days. The most heavily contaminated soil (4 ml/kg) showed the lowest emergence rates, with 22.5% on day 6 and 52.5% on day 8, whereas the uncontaminated control recorded 80% and 87%, respectively.

TABLE 3: The effect of different concentration of crude oil on the emergence percentage (%) of *Zea mays*

Treatment (ml/kg)	Mean Emergence% (Day 6)	Mean Emergence% (Day8)
0 ml	80.00±0.1 ^a	87.59±0.3 ^a
2 ml	47.50±0.3 ^b	67.50±0.2 ^b
3 ml	35.00±0.1 ^a	60.00±0.1 ^{bc}
4 ml	22.50±0.2 ^d	52.50±0.2 ^c

Different letters indicate significant differences ($p \leq 0.05$) as determined by DMRT.

Table 4 shows the effect of different concentrations of crude oil on the plant height (cm) of *Zea mays*. Crude oil contamination significantly inhibited plant height across the various concentrations tested. The control group exhibited mean heights of 20.24 cm and 163.00 cm in the 2nd and 8th weeks, respectively. Plant heights differed significantly ($P \leq 0.05$) among the different contamination levels.

Table 4: The effect of different concentration of crude oil on plant height of *Zea mays*

Treatment (ml/kg)	Week2	Week 4	Week 6	Week 8
0 ml	20.45±0.5 ^a	52.50±0.3 ^a	95.80±0.5 ^a	163.00±0.5 ^a
2 ml	17.10±0.3 ^b	43.38±0.5 ^b	72.63±0.3 ^b	154.00±0.7 ^b
3 ml	15.58±0.5 ^b	36.75±0.7 ^c	66.08±0.3 ^c	138.25±0.7 ^c
4 ml	11.83±0.5 ^c	28.75±0.4 ^d	47.83±0.7 ^d	129.75±0.4 ^d

Different letters are significantly different ($p \leq 0.05$) according to DMRT

Table 5 shows the effect of different concentration of crude oil on the stem girth (mm) of *Zea mays*. The results indicate that crude oil had a significant inhibitory effect on the stem girth of *Zea mays* from week 2 to week 6, compared to the control. Stem girth decreased progressively with increasing concentrations of crude oil. The control group recorded the highest mean stem girth of 17.73 mm, while the most contaminated soil (4 ml/kg) had the lowest mean stem girth of 11.78 mm.

Table 5: The effect of different concentrations of crude oil on the stem girth (mm) of *Zea mays*

Treatment (ml/kg)	Week 2	Week 4	Week 6	Week 8
0 ml	8.04±0.2 ^a	16.23±0.3 ^a	16.71±0.3 ^a	17.73±0.2 ^a
2 ml	6.12±0.3 ^b	13.86±0.5 ^b	14.25±0.5 ^b	15.25±0.2 ^b
3 ml	4.93±0.5 ^b	11.12±0.3 ^c	11.4±0.3 ^c	12.43±0.3 ^c
4 ml	3.38±0.3 ^c	10.22±0.3 ^c	10.78±0.5 ^c	11.78±0.5 ^c

Different letters indicate significant differences ($p \leq 0.05$) as determined by DMRT

Table 6 shows the effect of different concentration of crude oil on the wet shoot, wet root, dry shoot, and dry root (mm) of maize. The influence of crude oil concentrations on seedling biomass and height was noted to be notably significant. Statistically significant

differences ($p \leq 0.05$) were observed in both the wet and dry weights of shoots and roots. The highest biomass values (wet and dry) were recorded in the control group, while the lowest were observed at the 4 ml/kg treatment level.

Table 6: Effect of different concentration of crude oil on the wet shoot, wet root and dry shoot, dry root (mm) of *Zea Mays*

Treatment (ml/kg)	Wet Shoot (Wt)	Wet Root (Wt)	Dry Root (Wt)	Dry Root (Wt)
0 ml	192.58±0.7 ^a	35.45±0.5 ^a	58.98±0.8 ^a	5.95±1.0 ^a
2 ml	132.05±1.0 ^b	14.71±0.7 ^b	42.48±0.7 ^b	3.75±0.8 ^b
3 ml	116.86±0.8 ^c	11.67±0.7 ^b	39.98±1.0 ^b	3.58±0.9 ^b
4 ml	97.12±0.5 ^d	10.77±0.9 ^{bc}	32.70±1.0 ^c	3.39±1.0 ^b

Different letters indicate significant differences ($p \leq 0.05$) as determined by DMRT

Figures 1a, b and c describe the influence of crude-oil at varying concentrations on the tasseling, anthesis, silking and the anthesis-silking interval of *Zea mays* plant. As presented in the results, the higher the concentration of crude-oil, the longer the time for the emergence of developmental features. As shown in Figure 1a, significant difference ($P < 0.05$) was evident between the days taken for 50% of each treatment to tassell, produce anther and fruit. Likewise in Figure 1b, there is a significant difference ($P < 0.05$) between the days taken

for 100% of each treatment to tassell, produce anther and fruit. Maize planted in the uncontaminated soil (0 ml/kg) took the least days to tassell, devel anther and fruit, whereas, maize seeds planted in the most contaminated soil (4ml/ kg) took the longest days to tassell, develop anther and show signs of fruiting. Figure 1c presents the Anthesis-silking interval (ASI); number of days between anthesis and silking. The interval days increase with increasing concentration of crude-oil contamination.

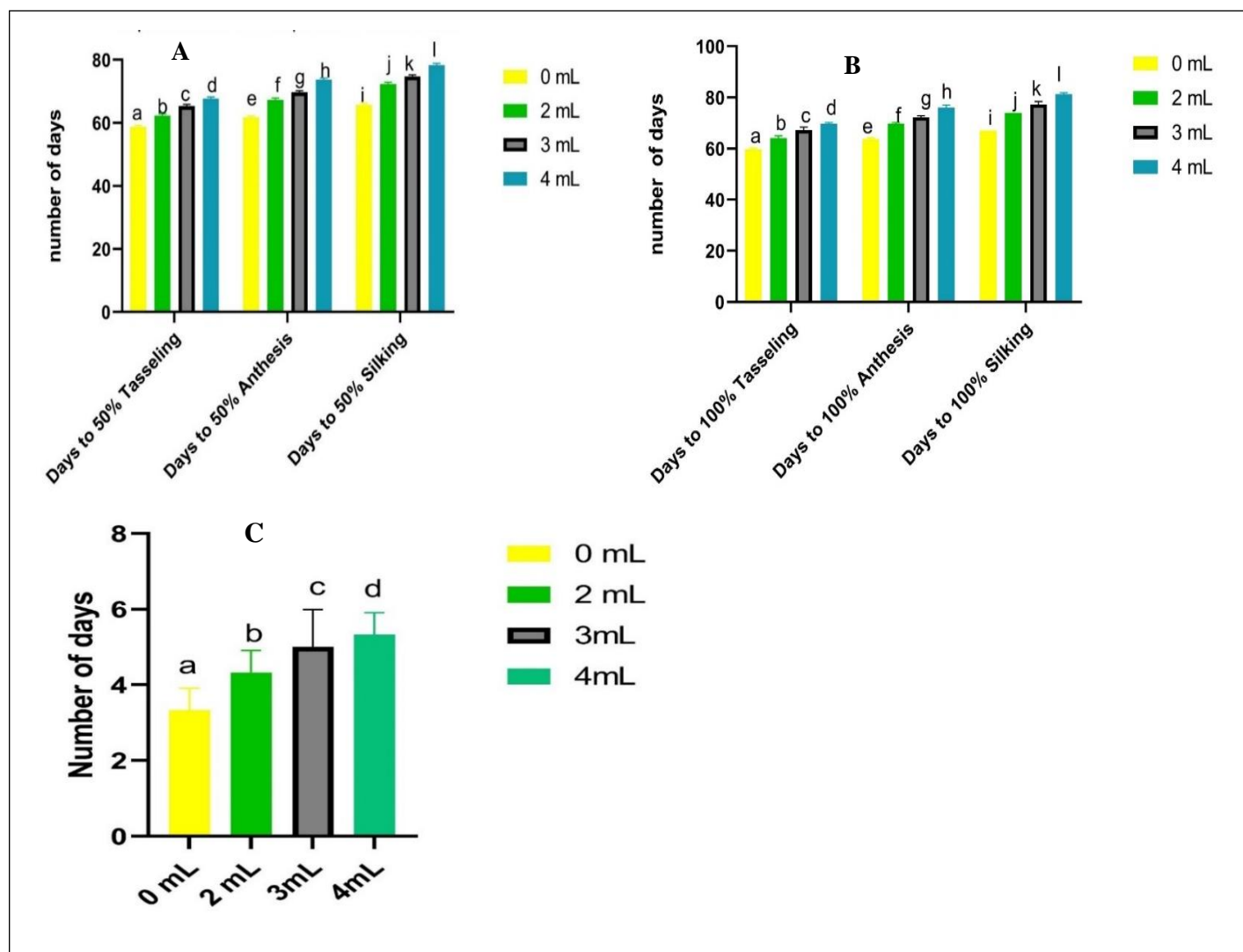


Figure 1: Effect of Crude-oil Concentration on (A) 'days to 50% tasseling, Anthesis and Silking of maize, (B) 'days to 100% Tasseling, Anthesis and Silking of *Zea mays*' and (C) 'days to Anthesis-Silking Interval of *Zea mays*' Bars with different letters represent significant differences ($p \leq 0.05$), using one-way Anova (Dunnnett's Multiple comparison test).

Figures 2a to e express the influence of crude oil contaminated soil on oxidative stress biomarkers in maize. As illustrated in Figure 2a, the chlorophyll *a* content of maize plants decreased with increasing contamination. The highest value was recorded in the control (27.61 mg/g), while the lowest was noted in the plants grown in the most contaminated soil (4 ml treatment: 5.27 mg/g). Intermediate values were recorded at 2 ml (10.00 mg/g) and 3 ml (6.93 mg/g) levels of contamination. A similar trend was observed for chlorophyll *b* (Figure 2b), with the control showing the highest value (10.98 mg/g) and the most contaminated sample recording the lowest (6.48 mg/g). Carotenoid content (Figure 2c) also declined progressively with increased crude oil concentration: control (6.79 mg/g) > 2 ml (3.13 mg/g) > 3 ml

(2.57 mg/g) > 4 ml (0.88 mg/g). Overall, all photosynthetic pigments in *Zea mays* leaves showed significant differences across the various crude oil concentrations ($P < 0.05$). Protein in Figure 2d, shows a decreasing trend with increasing crude-oil contamination. The control has the highest value of 46.40 mg/dl, while the least value of protein was in leaves of maize grown on the most contaminated soil; 36.04mg/dl.

Malondialdehyde (MDA), which is a product of lipid peroxidation, was also used as oxidative stress biomarker. A notable rise in MDA levels was documented in maize as crude-oil concentration increases, as expressed in Figure 2e, 0 ml, 2ml, 3ml, and 4ml yielded 0.913 $\mu\text{mol/ml}$, 0.96 $\mu\text{mol/ml}$, 0.98 $\mu\text{mol/ml}$ and 1.22 $\mu\text{mol/ml}$, respectively.

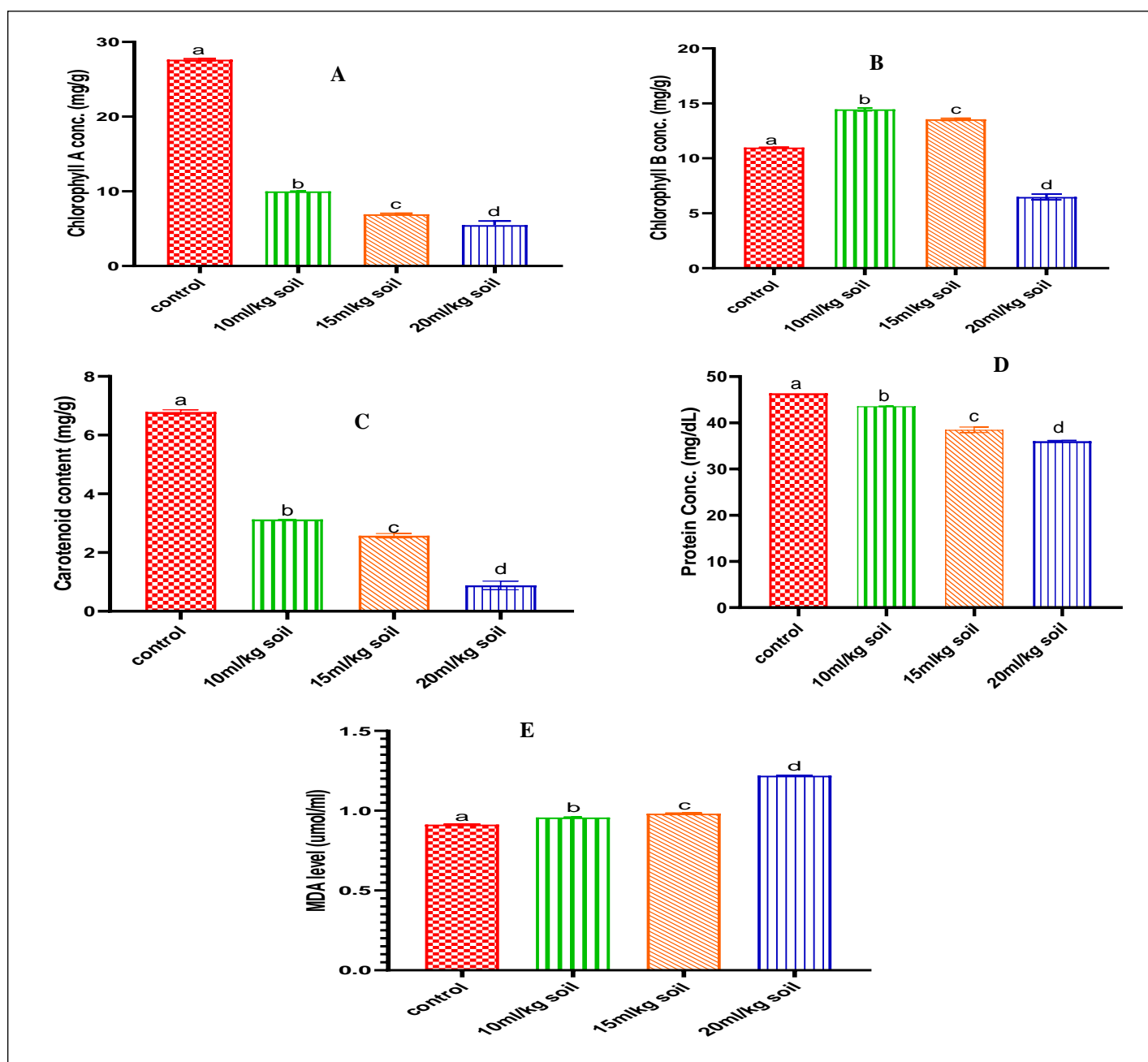


Figure 2: Effect of Crude-oil Concentration on (A) the Chlorophyll A Content of *Zea mays*, (B) the Chlorophyll B Content of *Zea mays*, (C) the Carotenoid Content of *Zea mays*, (D) the Protein Content of *Zea mays*, (E) the MDA level of *Zea mays*. Bars labeled with different letters represent significant differences ($p \leq 0.05$), using one-way ANOVA (Tukey's Multiple Comparison Test).

Figures 3a to f describe the effects of different concentrations of crude-oil on *Zea mays*. The activity of SOD (Figure 3a) increases with increasing crude-oil contamination. APx (Figure 3b) level increases with rising levels of crude-oil. Also, the level of GSH (Figure 3c) increases as concentration increases. Catalase activity (Figure 3d) increases with increasing concentration of crude-oil. The control had the lowest value (10.74 mmol/min/mg protein) while the most at 4ml/kg had the greatest value (15.08 mmol/min/mg protein). The GPx (Figure 3e) values also follows an upward trend,

with increasing values with increasing crude-oil concentration. 0 ml (4.10 mmol/min/mg protein) and 4ml (4.56mmol/min/mg protein).

The results of the effects of crude oil contamination on the total phenolic content (TPC) of maize plant are expressed in Figure 4.1.8f. The TPC in *Zea mays*, increases as the level of contamination increases. 0 ml (182 mg/GAE/g) < 2 ml (192 mg/GAE/g) < 3 ml (197 mg/GAE/g) < 4ml (252 mg/GAE/g).

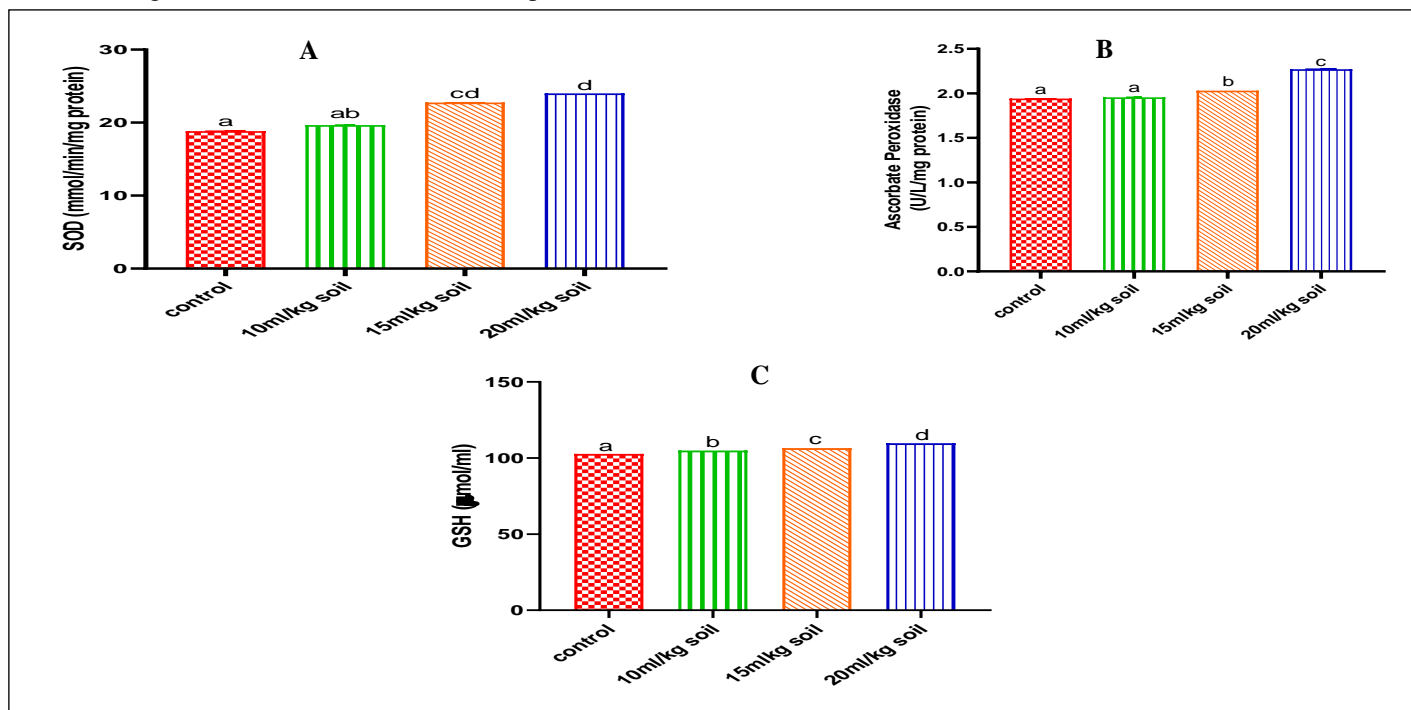


Figure 3: Effect of Crude-oil Concentration on (A) the SOD level, (B) the APx level, (C) the GSH level in maize. Bars labeled with different letters represent significant differences ($p \leq 0.05$), one-way ANOVA (Tukey's Multiple Comparison Test).

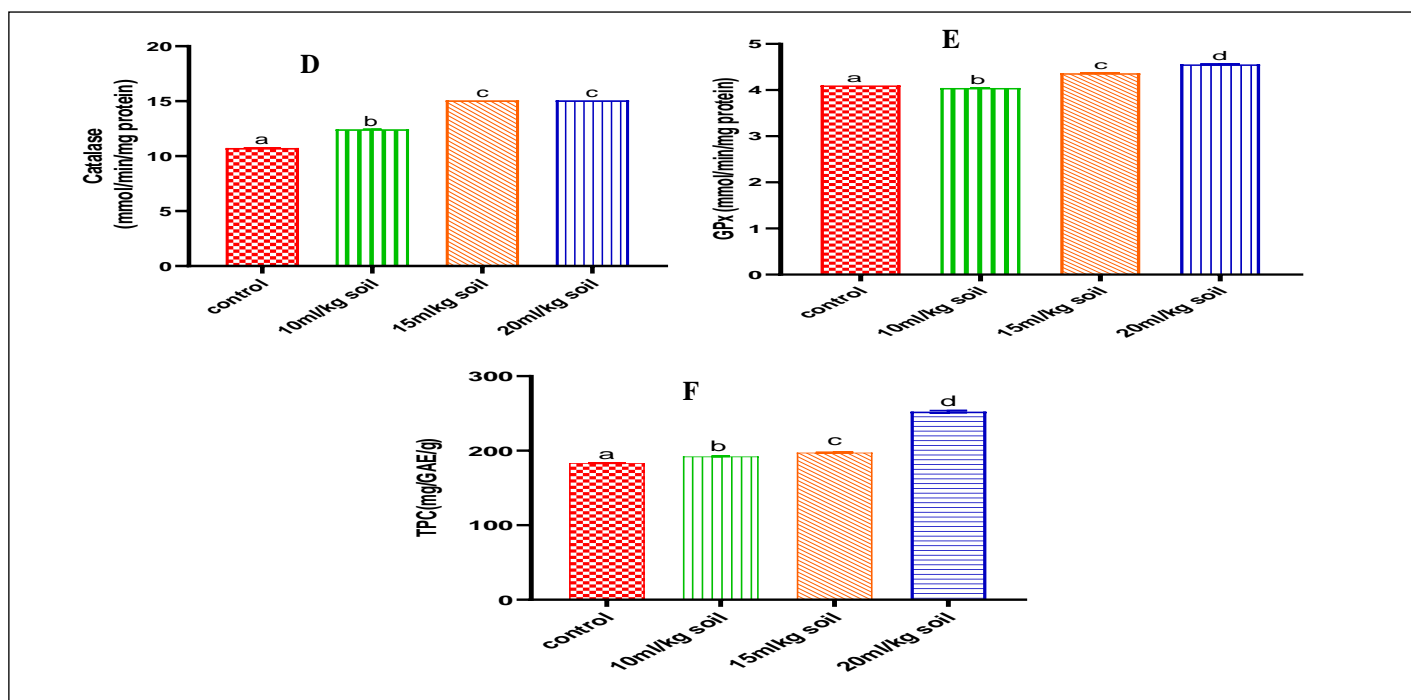


Figure 3: Effect of Crude-oil Concentration on (D) the Catalase level, (E) the GPx level and (F) the Total Phenolic Compounds' level in maize. Bars labeled with different letters represent significant differences ($p \leq 0.05$), using one-way ANOVA (Tukey's Multiple Comparison Test).

Discussion

Crude-oil contamination exhibited a pronounced phytotoxic influence on maize (*Zea mays*) germination dynamics. Germination percentage followed a distinct inverse trend with increasing crude-oil concentration (0 mL > 2 mL > 3 mL > 4 mL), demonstrating that oil presence in the rhizosphere substantially impedes early seed metabolic activation. The control soil (0 mL kg⁻¹) achieved the highest germination rate, increasing from 80 % on day 2 to 90 % by day 6, whereas seeds in the most contaminated soil (4 mL kg⁻¹) exhibited delayed and suppressed germination, progressing only from 25 % on day 2 to 60 % by day 6. These observations substantiate earlier findings that petroleum hydrocarbons prolong the lag phase before germination by interfering with water imbibition and enzymatic reactivation within the embryo (Mansour et al., 2021; da Silva Correa et al., 2022; Poddar et al., 2023). Hussein et al. (2022) and Luo et al. (2024) similarly noted that in hydrocarbon-polluted soils, the onset of maize germination can be delayed from 48 h to as much as 96 h, confirming a time-dependent suppression of physiological activity. Consistent with these patterns, Eze and Orjiakor (2020) documented significant reductions in mean germination time and overall percentage germination in crude-oil-impacted maize seeds.

The germination index (GI) and mean germination time (MGT) metrics further reflected the severity of oil stress. The control group exhibited the highest GI (78 %), which progressively declined to 70 %, 56 %, and 46 % in the 2, 3, and 4 mL kg⁻¹ treatments, respectively, denoting a concentration-dependent reduction in seed vigor. Conversely, MGT increased with contamination level, from 2.25 days in the control to 3.31 days in the 4 mL kg⁻¹ treatment, revealing prolonged germination latency under petrochemical stress. Comparable inverse associations between GI and pollutant load, and the corresponding elongation of MGT under environmental stressors, have been reported by Khan et al. (2023), Alzway et al. (2025), and Amin et al. (2024), who demonstrated that hydrocarbon toxicity diminishes seed vigor through inhibition of enzymatic hydrolysis, disruption of energy metabolism, and alteration of membrane permeability.

The decline in emergence percentage with increased crude-oil concentration—22.5 % on day 6 and 52.5 % on day 8 at 4 mL kg⁻¹, compared with 80 % and 87 % in the control—suggests that high biological oxygen demand (BOD) and reduced soil aeration impede gaseous exchange essential for embryonic respiration (Kwon et al., 2023). This aligns with Sagaya et al. (2023), who reported an oil-dose-dependent decline in germination rate and seedling emergence in similarly polluted soils. Collectively, these findings confirm that crude-oil contamination delays germination onset, reduces seed vigor, and inhibits early establishment of maize seedlings through multiple biochemical and biophysical constraints.

Crude-oil contamination exerted a statistically significant inhibitory effect on the morphological development of *Zea mays* ($p \leq 0.05$), particularly on plant height, stem girth, and biomass yield. The control group recorded mean plant heights of 20.24 cm (week 2) and 163.00 cm (week 8), while growth progressively declined with increasing crude-oil concentrations. This observation corresponds with the findings of Odiyi et al. (2020), who reported that maize cultivated in uncontaminated soils achieved superior height and vigor compared to those in hydrocarbon-polluted environments. The observed height reduction may be attributed to restricted aeration and diminished microbial respiration arising from the clogging of soil pores by oil films, which consequently limit oxygen diffusion, nutrient mineralisation, and water absorption. Fredrick et al. (2024) corroborated that toxic petroleum fractions interfere with root respiration and photosynthetic efficiency, leading to impaired nutrient translocation and slower biomass accumulation.

The negative impact of crude oil on stem girth followed a similar concentration-dependent pattern. Mean stem girth decreased progressively from 17.73 mm in the control to 11.78 mm in the 4 mL kg⁻¹ treatment. According to Igboke et al. (2025), such reductions are linked to elevated concentrations of petroleum-derived metals such as lead, nickel, and vanadium, which inhibit physiologically active enzymes essential for cell wall synthesis and elongation. Their study also revealed that enzymatic deactivation and oxidative damage compromise vascular integrity, culminating in reduced turgor pressure and stem robustness. Supporting this, Sagaya et al. (2023) demonstrated that hydrocarbons can accumulate in chloroplast membranes, disturbing the photosynthetic electron transport chain and thereby reducing chlorophyll synthesis. The resulting decline in autotrophic capacity hampers assimilate production and transport, leading to suppressed xylem and phloem function and reduced dry matter deposition in plant tissues.

In this study, significant differences ($p \leq 0.05$) were also observed in both wet and dry biomass of shoots and roots. The highest biomass was obtained in the control, while the lowest occurred under 4 mL kg⁻¹ contamination. The visual symptoms of oil toxicity—chlorosis, leaf suppression, stunting, necrosis, and stomatal irregularities—are consistent with the physiological stress responses described by da Silva Correa et al. (2022). These morphological degradations stem from the hydrophobic coating of soil particles by hydrocarbons, which restricts water and nutrient availability and creates hypoxic microzones in the rhizosphere. Fredrick et al. (2022) further emphasized that such oil-induced alterations reduce phosphorus and nitrogen availability, both of which are critical for nucleic acid and protein synthesis.

The findings of Balogun et al. (2022) support the proposition that crude-oil presence disrupts cell division, enlargement, and differentiation, thereby impeding physiological and anatomical development. The observed pattern in maize

height and biomass reduction parallels the results of Fredrick et al. (2024), who noted that *Monodora myristica* seedlings grown in oil-polluted soils suffered growth retardation due to limited gas exchange and poor nutrient mobility arising from occluded pore spaces. Similarly, Sagaya et al. (2023) documented progressive declines in *Crotalaria pallida* biomass with increasing contamination levels, indicating that hydrocarbon toxicity universally compromises plant energy assimilation and structural growth.

Cumulatively, these morphological responses substantiate that crude-oil contamination induces severe ecophysiological stress on *Zea mays*, manifesting as reduced plant height, stem girth, and biomass yield. The findings are consistent with earlier observations by Aruoren et al. (2022) and Igbokwe et al. (2025), which collectively affirm that crude-oil-polluted soils impair crop productivity by disrupting fundamental metabolic and structural processes. The present results therefore confirm that the phytotoxic response of maize is concentration-dependent and align closely with the observations of Skrypnik et al. (2021), who reported analogous reductions in chlorophyll content and biomass accumulation in petroleum-contaminated rye plants.

Crude-oil exposure markedly affected the reproductive performance of *Zea mays*, with higher contamination levels resulting in prolonged developmental stages and delayed emergence of reproductive structures. The number of days required for 50 % and 100 % of the plants to tassel, produce anthers, and fruit increased proportionally with oil concentration. Maize cultivated in uncontaminated soil (0 mL kg⁻¹) exhibited the shortest reproductive timeline, while plants in the 4 mL kg⁻¹ treatment required substantially longer durations for the same milestones. This delay highlights the physiological stress imposed by hydrocarbons on hormonal and metabolic regulation during floral initiation and development.

Maize is particularly sensitive to environmental stress during the tasseling stage, where disruptions can significantly impair pollination efficiency and yield potential. Hussain et al. (2019) demonstrated that adverse environmental conditions, including hydrocarbon toxicity, can reduce maize grain yield by up to 20 % when stress occurs during tasseling, while combined stresses such as drought and chemical exposure can cause kernel losses of 32–50 %. Similarly, Liu et al. (2023) confirmed that heat or pollutant-induced tassel degeneration reduces kernel number and overall yield. Waqas et al. (2021) attributed these reproductive impairments to diminished tassel length, volume, and pollen viability under environmental stress, leading to pollen abortion and reduced pollen tube growth. Liu et al. (2023) further corroborated this by showing that such morphological degradations of tassels result in yield losses exceeding 30 %.

Moreover, prolonged anthesis–silking intervals (ASI) were recorded with increasing levels of crude-oil contamination, indicating asynchronous development between pollen release

and silk receptivity. According to Waqas et al. (2021) and Odiyi et al. (2020), this asynchrony—often associated with oxidative and osmotic stress—can induce anther sterility and restrict fertilization success, resulting in poor kernel set. A shorter ASI is therefore a desirable indicator of synchronised reproductive processes and improved fertilization potential. Lima et al. (2023) emphasized that ASI serves as a reliable physiological index for predicting grain yield under both nutrient and environmental stress. In agreement, Bradford et al. (2021) and Monneveux et al. (2021) observed that prolonged ASIs under stress conditions are frequently associated with poor pollination efficiency, delayed silk emergence, and reduced kernel numbers per ear.

The results from the present study align with these reports, demonstrating that elevated crude-oil concentrations exacerbate reproductive asynchrony and hinder effective fertilization. The extended ASI observed may be due to impaired hormonal signaling and restricted assimilate translocation resulting from disrupted root function and reduced photosynthetic output. Gim et al. (2021) similarly reported that exposure to petroleum derivatives compromises root development and nutrient uptake, inducing systemic stress responses that mirror those observed under severe drought. Collectively, these findings suggest that crude-oil contamination not only delays reproductive development in maize but also reduces synchrony between male and female floral phases, thereby diminishing yield potential and reproductive efficiency.

Crude-oil exposure produced significant oxidative and photosynthetic stress responses in *Zea mays*, reflected by alterations in chlorophyll pigments, carotenoids, protein content, and malondialdehyde (MDA) accumulation. Chlorophyll *a* exhibited a steep concentration-dependent decline—from 27.61 mg g⁻¹ in the control to 5.27 mg g⁻¹ at 4 mL kg⁻¹—indicating severe disruption in the photosynthetic machinery. Intermediate values of 10.00 mg g⁻¹ and 6.93 mg g⁻¹ were recorded at 2 mL kg⁻¹ and 3 mL kg⁻¹, respectively. Chlorophyll *b* followed a similar trend, declining from 10.98 mg g⁻¹ in the control to 6.48 mg g⁻¹ under maximum contamination. Carotenoid concentrations also decreased progressively (6.79 mg g⁻¹ > 3.13 mg g⁻¹ > 2.57 mg g⁻¹ > 0.88 mg g⁻¹), confirming that crude oil interferes with pigment biosynthesis and stability. Comparable findings were reported by Fredrick et al. (2024), who documented significant reductions in chlorophyll levels of *Zea mays* under hydrocarbon stress, and by Skrypnik et al. (2021), who observed pigment loss in petroleum-contaminated rye varieties. These disruptions are primarily attributed to reduced nutrient uptake efficiency, oxygen limitation, and oxidative degradation of chloroplast structures (Fredrick et al., 2024).

Fredrick et al. (2024) further proposed that the decline in chlorophyll content may be linked to impaired absorption of micronutrients—especially boron, iron, magnesium, and

manganese—required for chlorophyll maturation, a hypothesis consistent with the biochemical insights of Cakmak and Engels (2024). The reduction in photosynthetic pigments, therefore, reflects not only the physical impediments caused by crude oil on root respiration but also metabolic dysregulation in pigment biosynthetic pathways.

Similarly, protein concentrations in maize leaves decreased as crude-oil levels increased. The control recorded the highest protein value (46.40 mg dL^{-1}), while the lowest value (36.04 mg dL^{-1}) was obtained at 4 mL kg^{-1} contamination. This trend corroborates the findings of Bunio and Tsvilynyuk (2021), who observed crude-oil-induced reductions in protein fractions of *Carex hirta*, and of Obi-Iyeke (2022), who reported analogous declines in *Zea mays* grown in polluted soils. Protein suppression likely arises from impaired nitrogen assimilation, enzyme inactivation, and oxidative modification of amino acid residues, all of which diminish the structural and functional integrity of cellular proteins.

The escalation in MDA levels across treatments—from $0.913 \text{ } \mu\text{mol mL}^{-1}$ in the control to $1.22 \text{ } \mu\text{mol mL}^{-1}$ at 4 mL kg^{-1} —reflects enhanced lipid peroxidation under hydrocarbon stress. This finding concurs with Eluehike et al. (2019), who reported that increased MDA levels in crude-oil-exposed maize signify oxidative membrane damage. Shafique and Ali (2020) also demonstrated elevated MDA accumulation in plants subjected to petroleum hydrocarbons, confirming MDA as a sensitive biomarker of oxidative injury.

Overall, the combined decline in chlorophylls, carotenoids, and proteins, together with the rise in MDA, underscores that crude-oil pollution provokes oxidative imbalance and metabolic dysfunction in *Zea mays*. Such alterations compromise photosynthetic efficiency, impair growth, and ultimately reduce biomass accumulation—effects that mirror those observed in other hydrocarbon-stressed plant systems (Fredrick et al., 2024; Skrypnik et al., 2021; Cakmak and Engels, 2024; Bunio and Tsvilynyuk, 2021; Obi-Iyeke, 2022; Eluehike et al., 2019; Shafique and Ali, 2020).

The enzymatic antioxidant system of *Zea mays* exhibited clear adaptive responses to crude-oil-induced oxidative stress. Key enzymatic markers, including superoxide dismutase (SOD), ascorbate peroxidase (APx), glutathione reductase (GSH), catalase (CAT), and glutathione peroxidase (GPx), showed progressive elevation with increasing contamination levels, reflecting the plant's intrinsic defense mechanisms against reactive oxygen species (ROS). The SOD activity rose consistently with crude-oil concentration, a finding corroborated by Eluehike et al. (2019), who linked elevated SOD levels to enzymatic detoxification of superoxide radicals during hydrocarbon exposure. Similar patterns have been reported in groundnut plants grown under petroleum stress, indicating a generalized plant response to oxidative challenges (Ugbeni & Okwu, 2022).

Likewise, APx activity increased proportionally with the degree of contamination, indicating enhanced conversion of hydrogen peroxide to water via the ascorbate–glutathione cycle. This trend aligns with Kovalchuk and Kovalchuk (2020), who identified APx induction as a key adaptation to oxidative stress in hydrocarbon-contaminated soils. GSH levels also exhibited concentration-dependent augmentation, consistent with the observations of Kovalchuk and Kovalchuk (2020), demonstrating that elevated glutathione synthesis helps maintain cellular redox equilibrium by regenerating oxidized antioxidants. Catalase activity increased from $10.74 \text{ mmol min}^{-1} \text{ mg}^{-1}$ protein in the control to $15.08 \text{ mmol min}^{-1} \text{ mg}^{-1}$ protein at 4 mL kg^{-1} , suggesting intensified peroxisomal degradation of hydrogen peroxide. These findings echo those of Eluehike et al. (2019), who reported similar catalase activation under petroleum toxicity. GPx activity also followed an upward trend (4.10 to $4.56 \text{ mmol min}^{-1} \text{ mg}^{-1}$ protein), paralleling the results of Shafique and Ali (2020), who established that GPx activity increases with rising hydrocarbon concentration as an essential safeguard against lipid peroxidation.

The cumulative increase in antioxidant enzyme activity underscores the dynamic modulation of redox homeostasis in *Zea mays* exposed to crude oil. Hydrocarbon contamination introduces xenobiotic compounds that elevate ROS generation, leading to oxidative perturbations in cellular metabolism. As highlighted by Rao et al. (2025), such abiotic stresses stimulate the synthesis of antioxidant enzymes to neutralize ROS, while Vicidomini et al. (2024) described these enzymatic cascades as pivotal in preserving membrane integrity and ensuring cellular viability under toxic conditions.

Furthermore, total phenolic compounds (TPC) in *Zea mays* leaves exhibited a progressive rise with increasing contamination— $182 \text{ mg GAE g}^{-1}$ (0 mL) $< 192 \text{ mg GAE g}^{-1}$ (2 mL) $< 197 \text{ mg GAE g}^{-1}$ (3 mL) $< 252 \text{ mg GAE g}^{-1}$ (4 mL). The increased phenolic content aligns with the reports of Goncharuk and Zagorskina (2023), who established that phenolic compounds act as potent ligands and radical inactivators under stress. Zoufan et al. (2020) observed similar elevation in phenolic metabolism during cadmium exposure, supporting the concept of phenolics as secondary antioxidants mitigating ROS-mediated injury. Ahlawat et al. (2024) elucidated that phenolic biosynthesis is tightly regulated by stress-responsive enzymes, and environmental stressors significantly enhance phenolic accumulation through activation of phenylpropanoid pathways.

Phenolic compounds perform multifaceted antioxidant functions, including lipid peroxidation inhibition, singlet oxygen quenching, and free radical neutralization (Hu et al., 2022). Their activity is largely attributed to their redox potential and hydrogen-donating ability. Additionally, polyphenols can chelate transition metals such as iron, thereby suppressing hydroxyl radical formation through

Fenton and Haber–Weiss reactions (Kim et al., 2023). Consequently, the increased TPC observed in this study signifies an essential biochemical adjustment contributing to oxidative stress mitigation and detoxification in oil-impacted maize.

Collectively, these findings affirm that crude-oil contamination induces pronounced oxidative challenges in *Zea mays*, triggering coordinated enzymatic and non-enzymatic antioxidant defenses. The observed biochemical modulations—elevated SOD, CAT, GPx, APx, GSH, and TPC—reflect a systemic adaptation to restore redox homeostasis and sustain cellular metabolism under hydrocarbon-induced stress.

Conclusion

The present study clearly demonstrates that crude oil contamination exerts significant phytotoxic and biochemical stress on maize (*Zea mays* L.), leading to impaired germination, stunted vegetative growth, and reduced reproductive performance. Progressive increases in crude oil concentration (2–4 mL/kg soil) resulted in marked declines in germination percentage, plant height, chlorophyll content, and total biomass, indicating severe disruption of photosynthetic and metabolic functions. The elevated levels of malondialdehyde (MDA) observed in contaminated plants reflect intensified lipid peroxidation and membrane destabilisation, which are hallmarks of oxidative stress. Conversely, the pronounced upregulation of antioxidant enzymes—superoxide dismutase (SOD), catalase (CAT), and glutathione peroxidase (GPx)—suggests an adaptive physiological response aimed at mitigating the deleterious effects of reactive oxygen species (ROS) generated under petroleum stress.

Overall, the results highlight the dual physiological impact of crude oil toxicity: suppression of growth processes and activation of antioxidative defences. The extended anthesis–silking interval (from 2 to 6 days) further underscores the detrimental influence of petroleum hydrocarbons on maize reproductive development and yield potential. These findings reaffirm that crude oil pollution compromises soil quality and crop productivity, posing ecological and food security risks in oil-producing regions such as the Niger Delta. Hence, the adoption of integrated remediation strategies—combining nano-, microbial-, and phytoremediation approaches—is recommended for sustainable restoration of contaminated farmlands and for safeguarding agricultural resilience in petroleum-impacted ecosystems.

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