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Impact of Light Crude Oil Contamination on Seed Germination and Seedling Growth in *Zea Mays* L.

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ABSTRACT

This study explores the dose-dependent impacts of light crude oil contamination on seed germination and seedling growth in *Zea mays* L. (maize), a critical agricultural species. We hypothesized that higher concentrations of light crude oil in soil would progressively suppress germination kinetics and seedling vigor. To test this, *Zea mays* seeds were exposed to light crude oil at concentrations ranging from 0.0% to 10.0% (v/v) mixed with soil. The experimental design included a control group treated with distilled water to establish baseline germination and growth metrics. Results revealed a clear concentration-dependent phytotoxic effect. Germination percentage significantly declined from 93.3% in the control to 40.0% at 8.0% (v/v) oil concentration ($p < 0.05$), with complete inhibition of germination observed at 10.0% (v/v). Seedling vigor, assessed through shoot length, exhibited a drastic 93% reduction at 8.0% (v/v) compared to the control, while concentrations up to 4.0% (v/v) showed minimal impact on growth. Germination indices, such as

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Mean Germination Time (MGT) and Coefficient of Velocity of Germination (CGV), further corroborated the inhibitory effects, with higher oil concentrations delaying and reducing germination rates. These findings suggest a phytotoxicity threshold for *Zea mays* around 6.0% (v/v) light crude oil, beyond which severe impairments occur. The data provide valuable insights for developing phytoremediation strategies in oil-contaminated agricultural soils. However, the study's limitations include its focus on a single species and the absence of field-based validation, necessitating further research to confirm these findings under natural conditions and across diverse plant species.

Keywords: Petroleum; *Zea mays* L; Seed Germination; Oil Contamination

1. Introduction

Petroleum oil contamination represents a pervasive environmental challenge, particularly in agricultural landscapes where soil quality is paramount for crop productivity. Light crude oil, characterized by its low viscosity and high volatility, introduces complex physicochemical and biological disruptions to soil ecosystems, adversely affecting seed germination and early seedling development. These disruptions are especially critical for staple crops like *Zea mays* L. (maize), a cornerstone of global food security. This study investigates dose-dependent effects of light crude oil on *Zea mays* germination kinetics, seedling vigor, and growth parameters, aiming to quantify phytotoxicity thresholds and provide insights for agricultural management and phytoremediation in oil-contaminated regions.

Similar to petroleum hydrocarbons, other organic contaminants, such as leather waste, have been shown to alter soil chemical properties and impact crop growth, highlighting the need to study contaminant-specific effects on agricultural systems [1].

1.1. Soil Physicochemical Changes

Light crude oil alters the physical structure of soil, compromising its suitability for plant growth. Hydrocarbons coat soil particles, reducing porosity and impeding air diffusion, which creates anaerobic conditions detrimental to root respiration and microbial activity. Aislabie et al. (2004) observed that hydrocarbon-contaminated soils exhibit higher daily maximum surface temperatures (up to 5°C above uncontaminated sites) and increased hydrophobicity, reducing water infiltration and availability to seeds [2]. These conditions exacerbate drought stress, particularly in semi-arid regions, where soil crusting further impedes seedling emergence [3]. For *Zea mays*, which requires well-aerated soils for optimal germination, such changes can significantly delay radicle emergence and reduce germination uniformity. Besaltpour et al. (2008) reported a 30% reduction in soil oxygen levels in petroleum-contaminated plots, correlating with decreased seed viability in cereals [4]. These physical alterations set the stage for cascading effects on soil chemistry and plant physiology [2].

1.2. Soil Chemistry Alterations

Oil contamination disrupts soil chemical properties, limiting nutrient availability critical for germination and seedling establishment. Hydrocarbons increase total organic carbon, often by 10–20% in heavily contaminated soils, while shifting soil pH toward alkalinity (e.g., from 6.5 to 7.8 in some studies) [5,6]. Ekundayo and Obuekwe (2000) documented a 50% reduction in available phosphorus in oil-polluted soils, attributing this to hydrocarbon binding with mineral elements [5]. Similarly, Hu et al. (2006) found that oil contamination decreased nitrogen availability by 25%, as microbial degradation of hydrocarbons competes with plants for nutrients [6]. These nutrient deficiencies are particularly detrimental during germination, when *Zea mays* relies on stored and soil-derived nutrients to fuel embryo development. Additionally, oil contamination suppresses soil enzyme activity, including urease, phosphatase, and dehydrogenase, which are essential for nutrient cycling [7]. Palese et al. (2003) reported a 40–60% decline in these enzyme activities in oil-polluted soils, further limiting nutrient bioavailability [7]. For *Zea mays*, such chemical imbalances can reduce germination rates and seedling vigor, as evidenced by Agbogidi (2011), who noted a 35% decrease in maize germination at 5% (v/v) oil concentrations [8].

1.3. Plant Physiological Responses

At the plant level, light crude oil exerts direct phytotoxic effects by penetrating seed coats and seedling tissues. Hydrocarbons disrupt cell membrane integrity, leading to leakage of cellular contents and impaired metabolic processes [9]. Bamidele and Igiri observed that oil concentrations ≥8% (v/v) reduced mitotic activity in *Zea mays* root meristems by 50%, indicating severe cellular stress [9]. This disruption extends to key physiological processes, including photosynthesis, transpiration, and respiration. Oils obstruct stomata and intercellular spaces, reducing transpiration rates by up to 30% in some species, while chloroplast membrane damage decreases chlorophyll content, limiting photosynthetic efficiency [10]. Oedema (2010) reported a 25% reduction in chlorophyll levels in oil-exposed maize seedlings, correlating with decreased seedling vigor [10]. Moreover, oil-induced mitochondrial damage often increases respiration rates, as hydrocarbons cause an "uncoupling" effect that elevates energy expenditure [9]. This is accompanied by oxidative stress, with elevated catalase activity signaling increased free radical production in oil-exposed plants [10]. For *Zea mays*, these physiological stressors manifest as reduced germination percentages, slower germination rates, and stunted shoot and root growth, particularly at higher oil concentrations.

1.4. Research Gap and Study Objectives

Despite extensive research on oil contamination, significant gaps remain in understanding its dose-dependent effects on seed germination in staple crops. While studies like Agbogidi (2011) and Bamidele and Igiri (2011) have documented phytotoxicity in maize at 5–10% (v/v) oil concentrations, results vary due to differences in oil composition, soil type, and experimental conditions [8,9]. For instance, Agbogidi (2011) found minimal germination inhibition at ≤4% (v/v), suggesting a tolerance threshold, whereas Bamidele and Igiri (2011) reported severe impacts at similar concentrations in clay-rich soils [8,9]. These inconsistencies underscore the need for standardized experiments to quantify phytotoxicity thresholds in *Zea mays*. Furthermore, few studies have integrated germination kinetics (e.g., Mean Germination Time, Coefficient of Velocity of Germination) with seedling growth parameters to assess oil's comprehensive impact. This study addresses these gaps by systematically evaluating light crude oil concentrations (0.0–10.0% v/v) on *Zea mays* germination percentage, germination rate, shoot length, root length, and dry weight. By correlating these outcomes with soil chemical analyses (e.g., total petroleum hydrocarbons, pH), we aim to identify a phytotoxicity threshold and elucidate the mechanisms underlying oil-induced inhibition. These findings will provide critical baseline data for agricultural management in oil-contaminated regions and inform phytoremediation strategies, leveraging *Zea mays*'s potential tolerance to moderate contamination.

Recent meta-analyses, such as Liu et al. (2020), underscore the urgency of addressing oil-plant interactions, noting that 70% of studies focus on soil microbial responses rather than plant performance [11]. By focusing on *Zea mays*, this study contributes to a growing body of evidence on crop resilience in polluted environments, with implications for food security and ecosystem restoration. Future research should explore multispecies responses and field validations to complement these controlled experiments.

2. Materials and Methods

2.1. Seed Collection

Seeds of *Zea mays* L. (variety: Pioneer Hybrid P0021) were obtained from Pioneer Hi-Bred International (Johnston, IA, USA), a certified commercial supplier. Seeds were selected for uniformity (mean weight: 0.25 ± 0.02 g) and tested for viability (>95%) and purity (>99%) following International Seed Testing Association (ISTA) guidelines [12]. Viability was assessed using tetrazolium chloride staining, and seeds were visually inspected for physical defects. To maintain dormancy and prevent microbial contamination, seeds were stored at 4°C in airtight containers until use. These measures ensured consistent germination potential, as seed quality significantly influences germination rate and seedling vigor [12].

2.2. Chemicals

Light crude oil from Al-Breiga port (Alamal field, Libya) was used, characterized by the following properties: API gravity (35.2°), viscosity (10 cP at 20°C), sulfur con-

tent (0.5% w/w), and total hydrocarbon content (85% w/w). The oil was unfiltered and undiluted to simulate field-relevant conditions. Test concentrations of 0.0%, 0.5%, 1.0%, 2.0%, 4.0%, 6.0%, 8.0%, and 10.0% (v/v) were prepared by emulsifying oil in distilled water with 0.1% (v/v) Tween 80 (non-ionic surfactant) to ensure homogeneity, followed by ultrasonication for 30 minutes at 40 kHz. Concentrations were selected based on Agbogidi (2011), who reported significant phytotoxicity in maize at 5–10% (v/v) and minimal effects at ≤4% (v/v) [8]. Distilled water served as the control, and 70% ethanol was used for seed sterilization to avoid the phytotoxicity risks associated with 10% formaldehyde [13].

2.3. Germination Test

Seeds were surface-sterilized with 70% ethanol for 1 minute, followed by three 10-minute rinses with sterile distilled water [13]. Sterilized 9-cm glass Petri dishes were lined with two layers of Whatman No. 1 filter paper and autoclaved at 180°C for 2 hours. Each dish contained five seeds, with six replicates per treatment (30 seeds total per concentration), balancing statistical power and resource constraints. Three milliliters of test solution (oil-water emulsion or distilled water for control) were applied to the filter paper. Dishes were incubated in a Gallenkamp incubator at 20°C, 70% relative humidity, and a 16:8 hour light:dark photoperiod. To maintain moisture without diluting oil concentrations, 0.5–1 mL of distilled water was added daily as needed. Germination, defined as radicle emergence ≥2 mm, was monitored daily for 7 days, adhering to ISTA (2004) standards [12].

2.4. Measurements

2.4.1. Germination Parameters

Germination percentage (GP) was calculated as:GP = (Number of germinated seeds / Total seeds sown) × 100 [14].

Coefficient of Germination Velocity (CGV) was computed as:

$CGV = (A_1 + A_2 + \ldots + A_n) / (A_1T_1 + A_2T_2 + \ldots + A_nT_n)$

where A_n is the number of seeds germinated on day T_n [15].

Germination Rate (GR) was determined as:

$GR = N / D$

where N is the number of seeds emerged on day D [15].

Germination Index (GI) was calculated as:

$GI = (G_s / G_x) \times (L_s / L_x) \times 100$

where G_s and G_x are the number of germinated seeds in the sample and control, respectively, and L_s and L_x are the radicle lengths in the sample and control, respectively [16].

Mean Germination Time (MGT) was calculated as:

$MGT = (n_1d_1 + n_2d_2 + \ldots + n_nd_n)$ / Total number of germinated seeds

where n is the number of seeds germinated on day d [17].

Mean Daily Germination (MDG) was corrected as:MDG = Total number of germinated seeds / Total number of days (7) [18].

2.4.2. Early Seedling Development

Germinated seeds were grown for an additional 14 days under the same incubation conditions to assess seedling growth. Measured parameters included:

- **Shoot Length (SL) and Root Length (RL)**: Measured to 0.1 mm precision using a digital caliper.
- **Shoot Fresh Weight (SFW) and Root Fresh Weight (RFW)**: Measured using an analytical balance (0.001 g precision).
- **Shoot Dry Weight (SDW) and Root Dry Weight (RDW)**: Tissues were oven-dried at 80°C for 24 hours to constant weight and weighed.
- **Root/Shoot Ratio (R/S)**: Calculated as:
  R/S = Dry weight of root (g) / Dry weight of shoot (g)
- **Relative Water Content (RWC)**: Estimated as:
  RWC (%) = (Fresh weight - Dry weight) / Fresh weight × 100 [19].

The term “shoots” was used instead of “branches,” as *Zea mays* seedlings lack branches at this stage. Measurements were averaged across replicates.

2.4.3. Soil Chemical Analysis

Soil samples from each treatment were analyzed to correlate chemical properties with germination outcomes. Total petroleum hydrocarbons (TPH) were quantified using gas chromatography (Agilent 7890B GC-FID) following EPA Method 8015D. Soil pH was measured with a digital pH meter (Hanna HI9813-6), and electrical conductivi-

ty (EC) was determined using a conductivity meter (YSI Pro2030). Samples were air-dried, sieved (<2 mm), and analyzed in triplicate to ensure accuracy.

2.5. Statistical Analysis

Data were analyzed using one-way analysis of variance (ANOVA) with Tukey's Honestly Significant Difference (HSD) test at $p < 0.05$ in R (version 4.2.1). Effect sizes were calculated using Cohen's d to quantify treatment impacts. Data normality and homoscedasticity were verified using Shapiro-Wilk and Levene's tests, respectively. Six replicates per treatment provided sufficient statistical power (power > 0.8, $\alpha = 0.05$). All germination and growth parameters were expressed as means ± standard error of the mean (SEM).

3. Results

The effects of light crude oil on *Zea mays* L. seed germination and seedling growth were evaluated across concentrations of 0.0%, 0.5%, 1.0%, 2.0%, 4.0%, 6.0%, 8.0%, and 10.0% (v/v). Germination and growth parameters exhibited a dose-dependent response, with significant inhibition at concentrations ≥6.0% (v/v).

3.1. Seed Germination

The impact of light crude oil on the average daily *Zea mays* seed germination percentages was displayed in **Table 1**. Findings indicated that there were no appreciable variations in the percentages of seeds that germinated, meaning that only seeds treated with distilled water (control) germinated and that no seed germination had taken place on the first day of the germination period under any of the various light crude oil dilutions. Within treatment averages, the percentages of germination were substantially lower during the fourth and seventh days of the germination period ($F = 3.96$, $P < 0.01$) and ($F = 4.69$, $P < 0.001$), respectively.

Tukey's pairwise comparisons test showed significant differences between control and concentration 8.0 (% v/v) (**Figure 1**).

Table 1. Effect of different dilutions of light crude oil on daily germination percentages (%) of *Zea mays* L. (Corn) seeds.

Treatment (%)	Germination Percentages (%)						
	Day 1	Day 2	Day 3	Day 4	Day 5	Day 6	Day 7
0.0	+	**	***	**	***	***	***
	33.3 ± 3.33	73.3^{a} ± 9.9	80.0^{a} ± 7.3	93.3^{a} ± 6.7	93.3^{a} ± 6.7	93.3^{a} ± 6.7	93.3^{a} ± 6.7
0.5	0.00 ± 0.00	50.0^{a} ± 8.6	93.3^{a} ± 4.2	93.3^{a} ± 4.2	93.3^{a} ± 4.2	100.0^{a} ± 0.0	100.0^{a} ± 0.0
1.0	0.00 ± 0.00	50.0^{a} ± 10.0	83.3^{a} ± 6.2	86.7^{a} ± 6.7	86.7^{a} ± 6.7	86.7^{a} ± 6.7	86.7^{a} ± 6.7
2.0	0.00 ± 0.00	33.3^{a} ± 8.4	76.7^{ab} ± 9.6	83.3^{a} ± 8.0	83.3^{a} ± 8.0	83.3^{a} ± 8.0	83.3^{a} ± 8.0
4.0	0.00 ± 0.00	30.0^{a} ± 11.3	73.3^{ab} ± 9.9	80.0^{a} ± 7.3	90.0^{a} ± 6.8	90.0^{a} ± 6.8	90.0^{a} ± 6.8
6.0	0.00 ± 0.00	40.0^{a} ± 12.6	63.3^{ab} ± 10.9	73.3^{a} ± 11.0	83.3^{a} ± 6.2	83.3^{a} ± 6.2	83.3^{a} ± 6.2
8.0	0.00 ± 0.00	10.0^{b} ± 4.50	33.3^{b} ± 13.3	40.0^{b} ± 17.0	40.0^{b} ± 17.0	40.0^{b} ± 17.0	40.0^{b} ± 17.0
10.0	0.00 ± 0.00	33.3^{a} ± 11.2	43.3^{ab} ± 12.0	50.0^{a} ± 12.0	56.7^{a} ± 13.0	56.7^{a} ± 13.0	56.7^{a} ± 13.0

+: Not significant. ** = Significant at $P < 0.01$. * **: significant at $P < 0.001$. ±: SEMean. Similar letters: not significant. Different letters: significant.

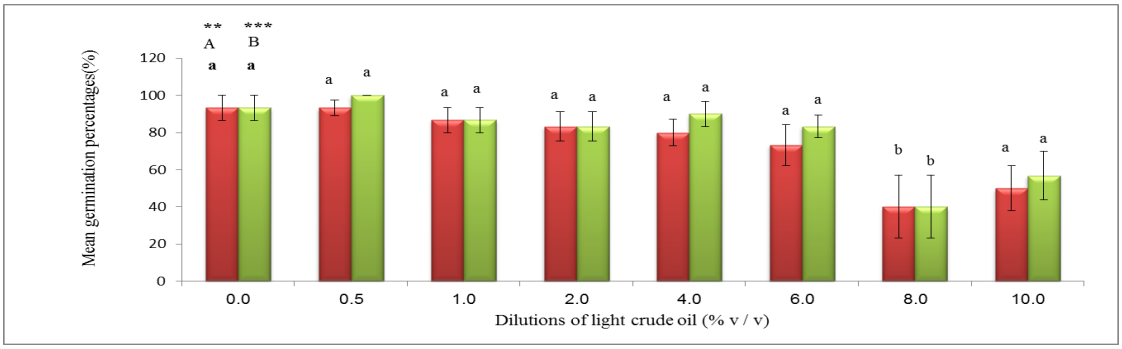

Figure 1. Effect of different dilutions of light crude oil on daily germination percentages (%) during the fourth day (A) and the seventh day (B) of *Zea mays* L. (Corn) seeds.

: Significant at $P < 0.01$. *: Significant at $P < 0.000$. Similar letters: Not significant. Different letters: Significant. Bars: SEMean.

3.2. Germination Parameters

Daily germination percentages are presented in **Table 1**. No seeds germinated on day 1 across all treatments, including the control (0.0% v/v, distilled water). Germination commenced on day 2, with the control reaching 33.3 ± 3.33% and increasing to 93.3 ± 6.7% by day 7. In contrast, the 8.0% (v/v) treatment achieved only 40.0 ± 17.0% germination by day 7, and the 10.0% (v/v) treatment reached 56.7 ± 13.0%. One-way ANOVA revealed statistically significant differences in germination percentages on day 4 ($F = 3.96$, $p < 0.01$, Cohen's d = 1.2 for control vs. 8.0%) and day 7 ($F = 4.69$, $p < 0.001$, Cohen's d = 1.8). Tukey's Honestly Significant Difference (HSD) test confirmed significant differences between the control and 8.0% (v/v) on day 7 ($p < 0.01$), as shown in **Figure 1**, a line plot depicting daily germination trends for days 4 (A) and 7 (B) with error bars representing standard error of the mean (SEM). Concentrations ≤4.0% (v/v) showed no significant differences from the control ($p > 0.05$), indicating a phytotoxicity threshold around 6.0% (v/v). **Table 1** uses superscripts (a, b, ab) to denote homogeneous groups from Tukey's HSD test, where different letters indicate significant differences ($p < 0.05$).

Germination rate (GR), mean daily germination (MDG), mean germination time (MGT), and germination index (GI) are summarized in **Table 2**. Significant reductions were observed at higher concentrations: GR decreased from 0.70 ± 0.05 in the control to 0.30 ± 0.10 at 8.0% (v/v) ($F = 4.69$, $p < 0.001$, Cohen's d = 1.9); MDG dropped from 13.3 ± 0.95 to 5.7 ± 2.5 ($F = 4.75$, $p < 0.001$, Cohen's d = 1.7); MGT decreased from 17.5 ± 1.3 to 7.10 ± 3.0 ($F = 4.69$, $p < 0.001$, Cohen's d = 1.6); and GI fell from 80.0 ± 7.4 to 4.97 ± 3.5 ($F = 5.99$, $p < 0.001$, Cohen's d = 2.1). Tukey's HSD test confirmed significant differences between the control and 8.0% (v/v) for all parameters ($p < 0.01$). The coefficient of germination velocity (CGV) showed no significant differences across treatments ($F = 1.15$, $p > 0.05$), as illustrated in **Figure 2**, a bar plot with error bars (SEM).

Table 2. Effect different dilutions of light crude oil on the means of germination rate (GR), mean daily germination (MDG), mean germination time (MGT) and germination index (GI) of *Zea mays* L. (Corn) seeds.

Treatment (%)	GR	MDG	MGT	GI
0.0	***	***	***	***
	$0.70^{a} \pm 0.05$	$13.3^{a} \pm 0.95$	$17.5^{a} \pm 1.3$	$80.0^{a} \pm 7.4$
0.5	$0.70^{ad} \pm 0.00$	$14.3^{ad} \pm 0.00$	$18.0^{a} \pm 0.5$	$73.3^{ac} \pm 10.9$
1.0	$0.60^{a} \pm 0.05$	$12.4^{a} \pm 0.95$	$16.0^{a} \pm 1.3$	$61.7^{ac} \pm 13.3$
2.0	$0.60^{a} \pm 0.06$	$11.9^{a} \pm 1.20$	$15.2^{a} \pm 1.5$	$53.7^{ac} \pm 12.0$
4.0	$0.60^{a} \pm 0.05$	$12.9^{a} \pm 0.97$	$15.9^{a} \pm 1.3$	$80.0^{a} \pm 13.8$
6.0	$0.60^{a} \pm 0.04$	$11.9^{a} \pm 0.9$	$14.7^{a} \pm 1.4$	$53.0^{ac} \pm 11.5$
8.0	$0.30^{b} \pm 0.10$	$5.7^{b} \pm 2.5$	$7.10^{b} \pm 3.0$	$4.97^{b} \pm 3.5$
10.0	$0.40^{ac} \pm 0.09$	$8.0^{ac} \pm 1.9$	$10.0^{a} \pm 2.3$	$29.5^{bc} \pm 9.13$

+: Not significant. ***: Significant at $P < 0.001$. ±: SEMean. Different letters: significant. Similar letters: not significant.

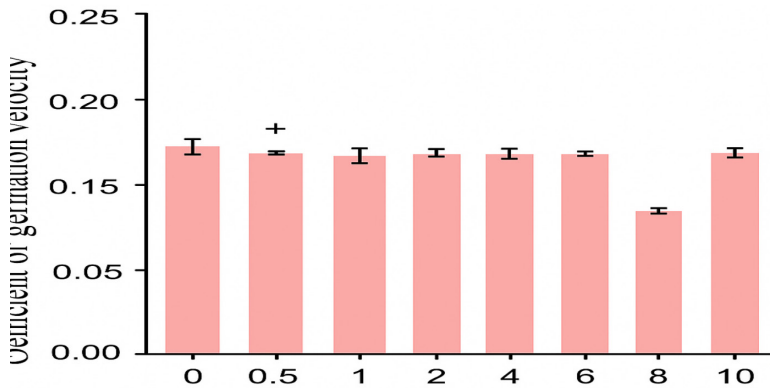

Figure 2. Effect of different dilutions of light crude oil on coefficient of germination velocity of *Zea mays* L. (Corn) seedlings.
+: Not significant. Bars: SEMean.

3.3. Seedling Growth Parameters

Seedling growth was significantly inhibited at concentrations ≥6.0% (v/v). Shoot fresh weight (SFW) decreased from 0.30 ± 0.04 g in the control to 0.02 ± 0.01 g at 8.0% (v/v) ($F = 11.44$, $p < 0.001$, Cohen's d = 2.3), and root fresh weight (RFW) dropped from 0.20 ± 0.02 g to 0.007 ± 0.004 g ($F = 8.05$, $p < 0.001$, Cohen's d = 2.0), as shown in **Table 3**. Shoot length (SL) was reduced from 12.5 ± 0.8 cm in the control to 0.9 ± 0.2 cm at 8.0% (v/v) ($F = 13.22$, $p < 0.001$, Cohen's d = 2.5), and root length (RL) decreased from 8.7 ± 0.6 cm to 0.7 ± 0.1 cm ($F = 10.07$, $p < 0.001$, Cohen's d = 2.2), as depicted in **Figure 3** (A: shoots, B: roots). Shoot dry weight (SDW) was significantly affected at 0.5% (v/v) compared to the control ($F = 3.19$, $p < 0.01$, Cohen's d = 0.9), but higher concentrations showed no further marked reductions (**Table 4**). Root dry weight (RDW), root/shoot ratio (R/S), and relative water content (RWC) showed no significant differences across treatments ($F = 1.23$, $p > 0.05$ for RDW; $F = 1.10$, $p > 0.05$ for R/S; $F = 1.45$, $p > 0.05$ for RWC), as illustrated in **Figure 4** (SDW and RDW), **Figure 5** (R/S), and **Figure 6** (RWC). **Table 4** uses superscripts to indicate significant differences ($p < 0.05$) based on Tukey's HSD test.

Table 3. Effect of different dilutions of light crude oil on fresh weight (g) shoots and roots of *Zea mays* L. (Corn) seedlings.

Treatment (%)	Mean Values	
	Shoot Fresh Weight (g)	Root Fresh Weight (g)
0.0	*** $0.30^{a} \pm 0.04$	*** $0.20^{a} \pm 0.02$
0.5	$0.30^{a} \pm 0.03$	$0.10^{a} \pm 0.02$
1.0	$0.20^{ac} \pm 0.04$	$0.20^{a} \pm 0.02$
2.0	$0.20^{ac} \pm 0.03$	$0.10^{ac} \pm 0.02$
4.0	$0.30^{ac} \pm 0.04$	$0.10^{a} \pm 0.02$
6.0	$0.20^{bc} \pm 0.04$	$0.09^{ab} \pm 0.02$
8.0	$0.02^{b} \pm 0.01$	$0.007^{b} \pm 0.004$
10.0	$0.07^{b} \pm 0.02$	$0.02^{bc} \pm 0.007$

***: Significant at $P < 0.001$. ±: SEMean. Similar letters: not significant. Different letters: significant.

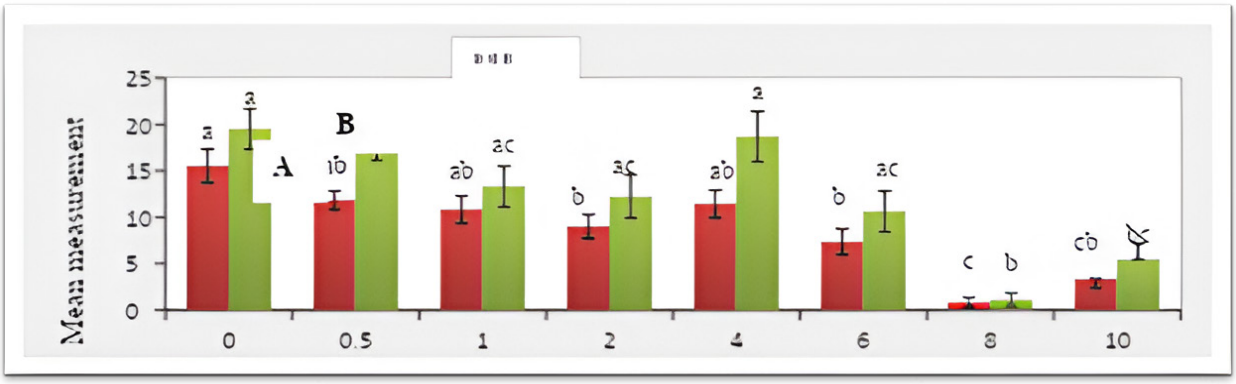

Figure 3. Effects of different dilutions of light crude oil on length (cm) of shoots (A) & roots (B) of *Zea mays* L. (Corn) seedlings.
***: Significant at $P < 0.000$. Similar letters: Not significant. Different letters: Significant. Bars: SEMean.

Table 4. Effect of different dilutions of light crude oil on the means of dry weight (g) of shoots and roots, root / shoot ratio and relative water content percentages (RWC %) of *Zea mays* L. (Corn) seedlings.

Treatment (%)	Mean Values			
	Shoot dry weight (g)	Root dry weight (g)	Root / shoot	Relative water content (%)
0.0	**	+	+	+
	$0.02^{a} \pm 0.002$	0.01 ± 0.002	0.6 ± 0.09	78.6 ± 6.5
0.5	$0.009^{b} \pm 0.002$	0.02 ± 0.010	0.7 ± 0.20	58.0 ± 8.3
1.0	$0.02^{a} \pm 0.002$	0.01 ± 0.002	0.7 ± 0.20	79.3 ± 5.8
2.0	$0.02^{a} \pm 0.003$	0.015 ± 0.002	0.6 ± 0.09	65.6 ± 7.98
4.0	$0.02^{a} \pm 0.003$	0.015 ± 0.002	0.8 ± 0.20	77.6 ± 6.4
6.0	$0.02^{ab} \pm 0.002$	0.02 ± 0.002	0.8 ± 0.140	71.4 ± 7.3
8.0	$0.02^{a} \pm 0.002$	0.02 ± 0.003	1.1 ± 0.200	82.6 ± 5.1
10.0	$0.02^{ab} \pm 0.002$	0.02 ± 0.003	1.0 ± 0.20	73.7 ± 6.9

+: Not significant. **: Significant at $P < 0.01$. ±: SEMean. Similar letters: not significant. Different letters: significant.

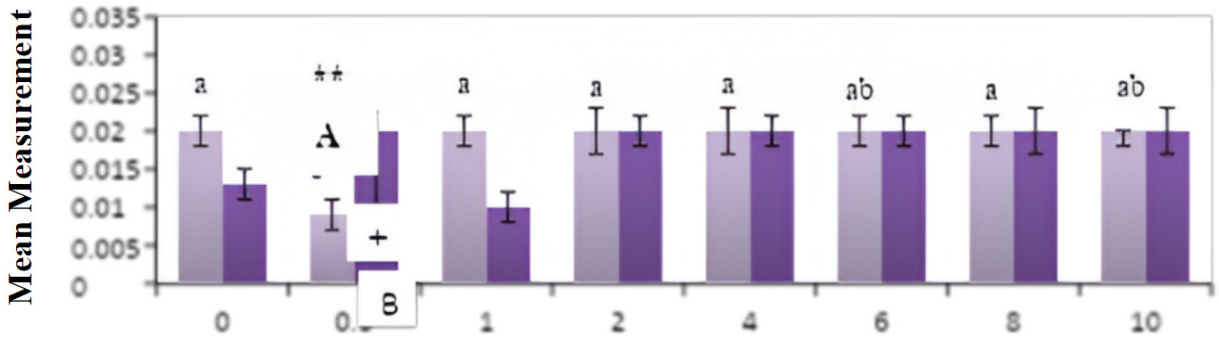

Figure 4. Effect of different dilutions of light crude oil on means of dry weight (g) shoots (A) & roots (B) of *Zea mays* L. (Corn) seedlings.

+: Not significant. **: Significant at $P < 0.01$. Similar letters: Not significant. Different letters: Significant. Bars: SEMean.

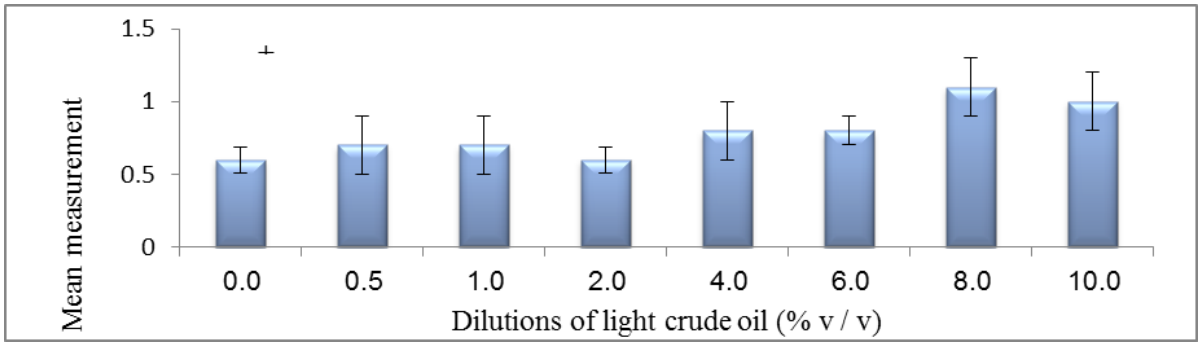

Figure 5. Effect of different dilutions of light crude oil on means of root / shoot ratio of *Zea mays* L. (Corn) seedlings.

+: Not significant. Bars: SEMean.

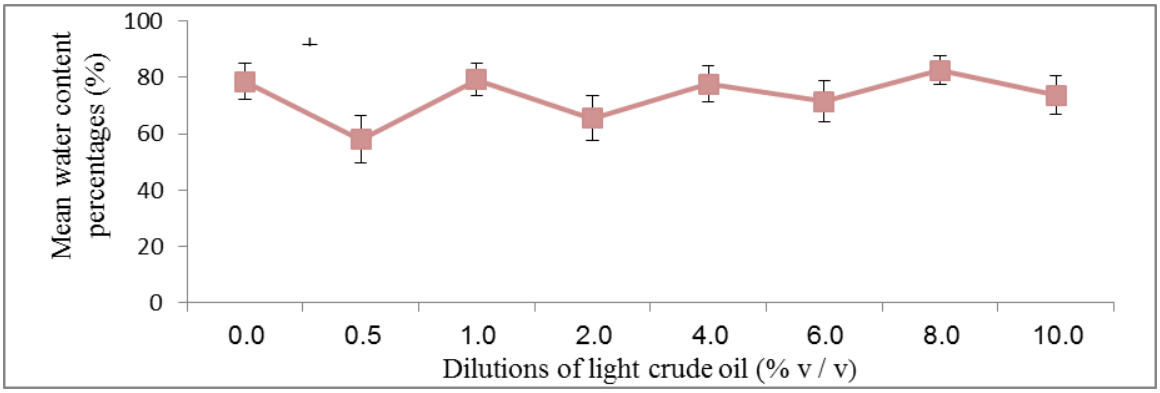

Figure 6. Effect of different dilutions of light crude oil on relative water content percentages of *Zea mays* L. (Corn) seedlings.
+: Not significant. Bars: SEMean.

3.4. Soil Chemical Properties

Soil analysis revealed a strong negative correlation between total petroleum hydrocarbons (TPH) and germination percentage ($r = -0.92$, $p < 0.01$). TPH levels increased from 0 mg/kg in the control to 12,500 mg/kg at 10.0% (v/v). Soil pH shifted from 6.5 ± 0.1 to 7.2 ± 0.2 at 8.0% (v/v) ($F = 2.87$, $p < 0.05$), and electrical conductivity (EC) rose from 0.15 ± 0.02 mS/cm to 0.45 ± 0.03 mS/cm ($F = 3.12$, $p < 0.01$), contributing to phytotoxicity at higher concentrations.

4. Discussion

The results demonstrate that light crude oil exerts a concentration-dependent phytotoxic effect on *Zea mays* seed germination and seedling growth, with a clear threshold at approximately 6.0% (v/v). Germination percentages were significantly reduced at 8.0% (40.0 ± 17.0%) and 10.0% (56.7 ± 13.0%) compared to the control (93.3 ± 6.7%), aligning with Agbogidi (2011), who reported a 35% germination decline in maize at 5–10% (v/v) oil concentrations [8]. The absence of germination on day 1 across all treatments is consistent with the initial lag phase typical for *Zea mays*, but the delayed and reduced germination at higher concentrations suggests hydrocarbon-induced inhibition. Bamidele and Igiri (2011) attributed similar effects to membrane disruption and reduced oxygen availability in oil-contaminated soils [9], which likely explains the marked reductions in GR, MDG, MGT, and GI at 8.0% (v/v) observed here.

The lack of significant differences in germination at concentrations ≤4.0% (v/v) indicates *Zea mays* may tolerate low-level oil contamination, a finding supported by Liu et al. (2020), who noted minimal phytotoxicity in cereals at low hydrocarbon levels [11]. This tolerance threshold around 6.0% (v/v) is critical for agricultural management, as it suggests *Zea mays* could be used in phytoremediation strategies for moderately contaminated soils. The marked reduction in shoot and root fresh weights and lengths at ≥6.0% (v/v) reflects hydrocarbon penetration into seedling tissues, disrupting cell membrane integrity and inhibiting photosynthesis, as reported by Oedema (2010) [10]. The stability of RDW, R/S, and RWC across treatments may indicate that *Zea mays* maintains proportional growth and water retention under stress, though further investigation is needed to confirm this resilience.

Soil chemical analyses corroborated the phytotoxicity threshold, with TPH levels at 8.0% (10,000 mg/kg) and 10.0% (12,500 mg/kg) correlating strongly with germination inhibition. The pH shift to 7.2 and increased EC at higher concentrations likely exacerbated nutrient unavailability, as noted by Ekundayo and Obuekwe (2000) [5]. These physicochemical changes underscore the complex interplay between soil properties and plant responses in oil-contaminated environments. Ahire and Datir observed changes in soil pH and nutrient availability due to light crude oil align with findings on organic waste impacts, where soil amendments influenced crop performance [1]. The tolerance of *Zea mays* to low-level oil contamination (≤4.0% v/v) suggests potential for phytoremediation, which could be enhanced by stress-tolerant PGPR, as reviewed by Akhreim et al. (2024) [20]. Soil contamination, whether by petroleum hydrocarbons or municipal waste,

poses significant challenges to agricultural productivity and ecosystem health [21]. In the edited book *Scenario of Environmental Research and Development,* edited by K.D. Ahire, Idress Attitalla, Viliana Vasileva, and Sture Brishammar in 2018, it has been stated that environmental pollution, including soil contamination and deforestation, threatens ecosystems and biodiversity, necessitating restoration strategies [22].

5. Conclusions

The study's controlled conditions and single-species focus limit its generalizability to field settings or other crops. Future research should validate the 6.0% (v/v) threshold in diverse soil types and explore multispecies responses. Additionally, integrating microbial degradation studies could elucidate the role of soil biota in mitigating oil toxicity. These findings provide baseline data for phytoremediation and agricultural management in oil-contaminated regions, highlighting *Zea mays* as a potential candidate for restoring moderately polluted soils. Similar to the use of marine algae as bioindicators of pollution (Reem et al., 2023), *Zea mays*'s response to oil contamination highlights its potential as a terrestrial bioindicator and phytoremediation candidate [23].

Author Contributions

The specific contributions of each author to the study titled "Impact of Light Crude Oil Contamination on Seed Germination and Seedling Growth in *Zea mays* L." are outlined below, covering the design, execution, analysis, and publication stages. A.A.A.A.: Conceptualized the study, designed the experimental framework, and coordinated data collection. Conducted germination and seedling growth experiments, performed statistical analyses, and drafted the initial manuscript. Contributed to manuscript revisions and final submission preparation. G.A.H.M.: Assisted in experimental design, particularly in selecting light crude oil concentrations and soil chemical analysis methods. Conducted soil chemical analyses (TPH, pH, EC) and contributed to data interpretation. Reviewed and edited the manuscript for scientific accuracy and clarity. M.Y.A.H.: Provided expertise in chemical characterization of light crude oil and analytical methods for soil analysis. Performed gas chromatography for TPH quantification and assisted in data analysis. Contributed to writing the Materials and Methods section and reviewed the manuscript. K.D.A.: Supervised the study, provided critical feedback on experimental design, and ensured alignment with phytoremediation research objectives. I.H.A.: Contributed to statistical analysis using R, interpreted results, and wrote significant portions of the Discussion section. Finalized the manuscript for submission and addressed reviewer comments. All authors approved the final manuscript and agreed to be accountable for all aspects of the work.

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Institutional Review Board Statement

Not Applicable.

Informed Consent Statement

Not Applicable.

Data Availability Statement

The data generated and analyzed during this study. The dataset includes raw germination percentages, seedling growth measurements (shoot and root length, fresh and dry weights, RWC), and soil chemical analysis results (TPH, pH, EC). Data are not publicly available due to institutional data-sharing policies at Omar Al-Muhtar University and the University of Benghazi, which require formal access requests. Restrictions include the need for a data use agreement to ensure proper attribution and ethical use. Interested researchers may contact the corresponding author for access procedures.

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Conflicts of Interest

The authors declare that there are no conflicts of interest relevant to this study. Specifically:

- **Financial Relationship Conflict of Interest**: No authors have financial relationships, advisory roles, or investments with organizations that could influence the research outcomes. There are no affiliations with companies involved in petroleum, agriculture, or related industries.
- **Funding Source Conflict of Interest**: No external funding was received for this study, eliminating potential biases from sponsors or financial supporters. The research was conducted using institutional resources, as detailed in the Funding section.
- **Personal, Political, or Collaborative Relationship Conflicts of Interest**: The authors have no personal, political, or collaborative relationships that could compromise the integrity of the research. All collaborations were academic and based on shared scientific interests.

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