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Integrated analysis of the impacts of used engine oil on seed germination, growth, and nutrient dynamics in ryegrass

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ABSTRACT

Polycyclic aromatic hydrocarbons (PAHs), represent an environmental hazard affecting soil quality and plant growth through both toxic and nutrient imbalance pathways. This study investigated the effects of used engine oil (UEO) contamination on germination, growth and nutrient uptake by ryegrass (*Lolium multiflorum* L.) under a controlled pot experiment. The contamination level with UEO ranged from 1 to 30 mg/kg soil, for the V2–V5 treatments in comparison to the control one, V1. The results showed a contamination dose-response with negative effect on seed germination potential, growth and yield reduction of the plant. In the V5 treatment, a complete inhibition of the plant growth indicators was obtained. Total nutrient uptake analysis revealed that phosphorus (P) had the strongest influence on plant yield followed by potassium (K) and nitrogen (N), while carbon (C) and hydrogen (H) also contributed significantly. UEO stress confirmed a restructured nutrient-yield relationship, significantly evidenced on contaminated treatments compared to the control one. Furthermore, these findings suggest that increased UEO toxicity resulted not only from high hydrocarbon levels, but also from reduced nutrient bioavailability and disrupted soil–plant interactions. Ryegrass, due to its moderate tolerance and lower nutrient needs, can be used as a potential phytoremediation technique for the UEO contaminated soils.

Keywords: used engine oil, germination, plant inhibition, nutrient imbalance, ryegrass.

INTRODUCTION

Polycyclic aromatic hydrocarbons (PAHs), which significantly contribute to environmental pollution, are widely distributed in air, water and soil. They are known for their harmful effects on ecosystems and public health (Berríos-Rolón, et al. 2025). Their toxic, mutagenic and carcinogenic properties, coupled with their tendency to be accumulated in biological tissues, by rising substantial concerns about their long-term environmental footprint (Dominguez and Pichtel, 2003; Srogi, 2007). PAHs impairs seed germination, disrupt nutrient uptake and plant metabolism, by causing oxidative stress and tissue necrosis, reducing plant growth and yield (Mukome et al., 2020; Haider et al., 2021). In a petroleum contaminated soil, PAHs such as phenanthrene, anthracene, fluorene and dibenzothiophene were detected from microbial degradation tests from 1 g/L to 10 g/L per individual PAH compounds under controlled conditions (Kirk et al., 2005). Other studies reported that the soil contaminated with mixture of PAHs up to 10.57% by weight, has significantly exceeded ecological safety thresholds by impacting soil biota quality and growth (Tang et al., 2011). PAH presence in an agriculture soil near the oil refinery process in Patos Marinza, Albania, was found to be 4–90 µg/ m^3 (SEPA, 2025). One of the most hazardous forms of the PAHs in the environmental include residues from used engine oils (UEO), which contain hydrocarbons with carbon chains ranging from C15 to C50 and are composed of both aromatic and aliphatic compounds (Armioni et al., 2024). It was found that UEO in concentration 2 g/kg significantly decreased the seed germination for maize, mung bean, tomato and potato by

10–75% and also reduced the plant growth from 33.2 to 66.7% (Ngozi et al., 2017). Also, PAH concentrations above 0.5% and contamination with compounds like anthracene or UEO, significantly reduce seed germination in crops as rice, maize and wheat by up to 98% in some cases (Somtrakoon and Chouychai, 2013; Xie et al., 2020). Previous studies have demonstrated that UEO contains complex mixtures of polycyclic aromatic hydrocarbons (PAHs), aliphatic hydrocarbons, and trace heavy metals, which are responsible for phytotoxic effects in contaminated soils (Szyszlak-Bargłowicz et al., 2021; Armioni et al., 2024). In addition to hydrocarbons, UEO may also carry heavy metals, which contribute to increase their toxicity and environmental persistence (Szyszlak-Bargłowicz et al., 2021). The combination of these components makes used motor oils a particularly dangerous form of PAH pollution, highlighting the urgent need for effective remediation strategies (Dominguez and Pichtel, 2003). Due to their low water solubility and high chemical stability, UEO accumulate in soils and sediments, posing long-term environmental risks and presenting substantial challenges to remedial tin efforts (Vázquez-Duhalt and Vélez, 1989; Hajabbasi, 2016). UEO contamination has showed significant reduction of seed germination in crops such as: *Z. mays* L., *P. vulgaris, S. lycpersium* and *S. saccharatum,* where in higher concentration (2%) complete germination inhibition and severe stunting of seedling growth process were observed (Ezenwa et al., 2017). In the soils contaminated by UEO, essential plant nutrients such as nitrogen (N), phosphorus (P) and potassium (K), are often adversely affected, including the decrease in P and K availability, disrupted N cycle and soil carbon content alteration, posing a significant risk for plant growth and soil health (Zaborowska and Rodzik, 2022). It was also reported that the critical roles of C, H, N, P and K in plant responses are due to UEO contamination (Zhan *et al.,* 2015). This nutrient is fundamental to plant metabolic system and their productivity, but these contaminants can lead to nutrient deficiencies, compromised physiological function and impaired growth (Hafiz *et al.,* 2022). The pressure on this nutrient directly correlates with the extent of plant inhibition, as it was observed in species like *Lolium multiflorum* L., which is commonly used in phytoremediation experiments due to its tolerance to adverse soil conditions and its ability to influence microbial activity (Hajabbasi, 2016; Gawryluk and Krzyszczak, 2024). In light to the discussion above, this study was carried out to assess the seed germination and plant yield for *Lolium multiflorum* L., along with their interaction with C, H, N, P and K content in plant under different level of UEO toxicity. The determination of the toxicity level that led to complete inhibition of the plan growth and nutrient uptake was also another scope of this study.

EXPERIMENT

Experiment design

The topsoil (0–20 cm) for the experimental setup was collected from the garden of the Agricultural University of Tirana (AUT); then, it was air-dried, homogenized and prepared for contamination experiments as well as seedling trials. Prior to the start of the experiments, the physical and chemical properties of soil were analyzed at the Agro-Environment and Ecology Laboratory of AUT, using standardized analytical methods. The obtained data of the soil properties are presented in Table 1.

The experiments were conducted under greenhouse conditions in a completely randomized block design in triplicate for each treatment. Five treatments were tested with varying concentrations of UEO: V1 (control, no UEO), V2 (1 g/kg), V3 (5 g/kg), V4 (10 g/kg) and V5 (30 g/kg) as it is shown in Figure 1.

The used engine oil was taken from a car service after being collected from different cars. For better homogenization, UEO was first applied to an aliquot of 200 g of sieved air-dried soil (2 mm mesh) in a 500 ml beaker containing 1500 g sieved, air-dried soil (5 mm mesh) and thoroughly mixed dried soil (2 mm mesh) in a 500 ml beaker and carefully mixed with a spatula. The treated portion was then transferred to a 2000 ml beaker containing 1500 g of sieved, air-dried soil (5 mm mesh) and thoroughly mixed. Finally, the homogenized soil sample was transferred to the plastic pot. In total, 15 experimental pots were used, each containing1700 g of soil in a volume pot of about 2500 cm^3 with a diameter of upper area of 16 cm. The experimental pots were irrigated with distilled water and kept for 10 days prior to seedling, in order to activate soil microflora. Ryegrass (*Lolium multiflorum* L., was used to assess the potential toxicity of UEO on seed germination and plant growth. A total of 100 seeds of *Lolium*

Table 1. The soil physical and chemical properties before experiment setup

	pH-water	pH-salt	Total – N (mgkg^{-1})	OM %	W%	Exchangable P (mgkg^{-1})	Exchangable K (mgkg^{-1})	Oil and grease (mgkg^{-1})	CEC (cmol$_c$kg^{-1})	Texture (%)		
										Sand	Clay	Silt
Soil	5.82	5.72	780.5	1.36	1.42	18.5	41.44	0.82	0.89	67.04	7.61	25.36

Figure 1. Pots of experiment in the greenhouse before first harvest was performed

multiflorum L., were sown in each pot. To maintain a uniform nutrient availability, a balanced NPK fertilizer (20:20:20) was applied at a rate of 1.5 g per pot before seedling. After seedling, the pots were irrigated to maintain 70% of water holding capacity (WHC) throughout the 76 days experimental period, as determined by the gravimetric method based on Richards (1949). The seed germination process was observed weekly and data was recorded each week until 21 days were completed. Meanwhile, for the purpose of the study, two harvests were applied in all the treatments including the control (V1). The first harvest was performed 50 days and the second one 76 days after seedling. After each harvesting, plant biomass yield for each treatment was weighed fresh, then dried in the thermostat at 60 °C and milled with IKA A 11 with 250 000 rpm, as required for chemical analysis with EPA 3050B method. For each harvest the following parameters were determined: Water content (%) with gravimetric method (sample dried at the thermostat at 60 °C), Total K and Total P by using AAS Flame spectrometry (Analytic Jena) and CHN (%) by using EuroVector Elemental Analyser. The data were processed using JMP 11 (SAS Institute, Cary, NC, USA). UEO treatments were compared through analysis of variance (ANOVA) and significant effects ($p < 0.05$) were separated using the LSD post hoc test. The relationships among nutrient contents (C, H, N, P, K) and plant growth were estimated using Pearson correlation and multiple regression models in order to identify the most influential predictors. To reduce dimensionality and highlight interdependence among variables, multivariate patterns (MP) were also performed. JMP graphical tools (scatterplots, biplots, residual analysis) supported validation of model assumptions and improved interpretation of treatment effects.

Results and discussion

The effects of used engine oil (UEO) contamination *Lolium multiflorum* L., were evaluated in terms of germination, biomass yield and nutrient uptake.

Impact of UEO on seed germination

After 21 days seedling of *Lolium multiflorum*, germination was complete for all experimental variants, and the results are presented in Figure 2. The control treatment (V1) showed a stable, linear increase in germinated seeds on 8th day (44 seeds) to 21st day (75.66 seeds), corresponding to a cumulative germination of 76 % of the total seed number. In treatment V2, where the lowest UEO contamination was applied, reached 67 % of total germination, with seed numbers rising from 39.33 seeds in 8th day to 67 seeds by 21st day. The highest level of UEO contamination (V5) showed the strongest inhibition, with only 24 germinated seeds in the first week and a final total of 71 seeds after three weeks, representing the lowest overall germination efficiency. At day 21, although recovery was observed, control (V1) still maintained higher rates of germination, with V5 being most suppressed.

Although slight differences are noticed among treatments, the statistical test in Table 2 shows consistent germination results at the end of the germination process. This confirms that even high contamination levels did not significantly affect the germination capacity of *Lolium multiflorum*, demonstrating its strong tolerance to polluted environments (Tang et al., 2011; Azorji et al., 2021). These results are similar to the previous studies which showed that UEO has adverse effects on seed germination by interfering with soil aeration, oxygen and water transport (Odejegba and Sadiq, 2002).

Impact of UEO on plant growth and development

The following results present the yield data obtained after two consecutive harvests conducted 50 and 75 days after seedling. They also reflect the total cumulative yield obtained under different levels of UEO contamination. Table 3 also presents the results of the test, one-way ANOVA revealed a significant effect between treatments (V1-V5) of UEO concentration on plant yield in the first cut, 50 days after seedling, (F (4,10 degrees of freedom) =15.10, $p = 0.0003$). The control treatment (V1) showed the highest mean yield with 24.21 g, while the highest concentration of UEO contamination (V5) drastically reduced yield to 5.45 g/pot. Meanwhile in treatment (V2, V3, V4) moderate yields (18.35–19.49 g/pot), which were significantly higher than V5 but lower than the control were observed. Pairwise comparisons confirmed that V5 differed significantly from all other treatments ($p<0.001$). These results suggest a dose-dependent negative effect of UEO at higher levels, whereas low to moderate concentrations do not strongly impact yield. Maintaining UEO at ≤ 10 g/kg appears optimal for sustaining plant productivity in this system (Ngozi et al., 2017). Similar findings have been reported, where elevated levels of alternative oils can manifest phytotoxic effects, reducing biomass production (Cheng et al., 2017; Scavo et. al., 2019).

The pairwise comparison (Table 4) of plant yield across treatments with varying used engine

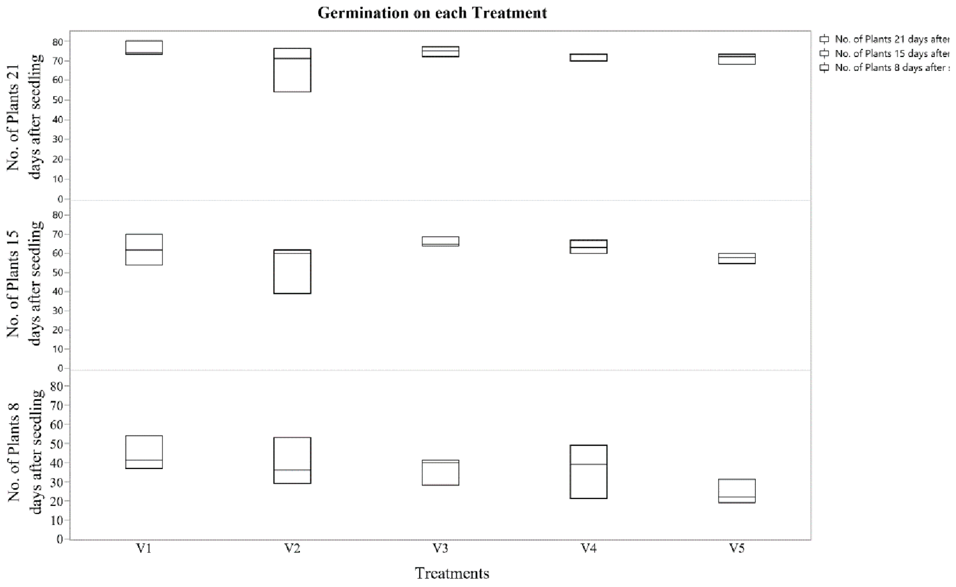

Figure 2. UEO effect for each treatment [V1 (control), V2, V3, V4, V5] on germination rates in successive counting on 8th, 15th, 21st day after planting

Table 2. Least-Squares means of germinated seeds per pot at 8, 15, and 21 days after sowing by treatment (V1–V5).

Treatments	Differences Student`s t about number of germinated seeds in three different period		
	Least Sq Mean + St Error		
	8 days after seedling	15 days after seedling	21 days after seedling
V1	44.00^{a}	62.00^{a}	75.66^{a}
V2	39.33^{ab}	53.66^{a}	67.00^{a}
V3	36.33^{ab}	66.00^{a}	74.66^{a}
V4	36.33^{ab}	63.33^{a}	72.00^{a}
V5	24.00^{b}	57.66^{a}	71.00^{a}

Note: Letter groupings indicate significant differences between treatments within each time period.

Table 3. Mean plant yield (g) under different concentrations of used engine oil (UEO) on the first cut

Treatments	UEO g/kg	n	Yield (Mean ± SD, g/pot)	Grouping	Lower 95%	Upper 95%
V1	0	3	24.21 ± 1.59[1]	a[2]	20.19	28.23
V2	1	3	18.35 ± 3.03	a	14.33	22.37
V3	5	3	19.49 ± 4.61	a	15.47	23.51
V4	10	3	19.01 ± 3.05	a	14.99	23.03
V5	30	3	5.46 ± 2.54	b	1.43	9.47
Source	DF	Sum of squares	Mean square	F Ratio	Prob > F	
UEO g/Kg	4	590.94	147.73	15.10	0.0003*	

Note: [1]Values represent mean ± standard deviation (SD), [2]Different letters indicate significant differences among treatments according to Tukey's HSD test at $p < 0.05$, marked with an asterisk (*).

oil (UEO) concentrations revealed a clear dose-dependent response, with all comparisons. Treatment V5 showed highly significant differences ($p < 0.01$) compared to lower treatment results (V1–V4). In contrast, comparisons among the lower UEO levels exhibited no statistically significant differences ($p > 0.05$) in Table 4 and Figure 3, defining that plant productivity remains stable up to approximately 10 g/kg UEO contamination. These findings indicate a threshold effect beyond which UEO contamination becomes phytotoxic, likely due to the accumulation of polycyclic aromatic hydrocarbons (PAHs) and heavy metals that impair root respiration and nutrient uptake (Azorji et al., 2021; Tang et al., 2011).

The obtained results in the second cut (Figure 4) (75 days after seedling) demonstrated that UEO concentration had a strong and statistically

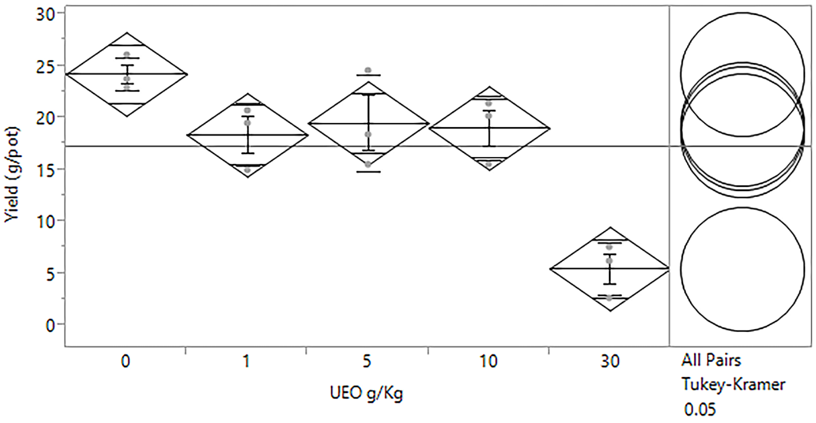

Figure 3. One-way ANOVA means plot of plant yield (g/pot) across different UEO (g/kg) concentrations in First Cut
Error bars represent standard errors of the mean

Table 4. Pairwise comparisons of plant yield between treatments on the first cut

Treatment's comparison		UEO g/kg	UEO g/kg	p-Value[1]
V1	V5	0	30	0.0002*
V3	V5	5	30	0.0019*
V4	V5	10	30	0.0025*
V2	V5	1	30	0.0035*
V1	V2	0	1	0.2231
V1	V4	0	10	0.3158
V1	V3	0	5	0.4001
V3	V2	5	1	0.9905
V4	V1	10	1	0.9989
V3	V4	5	10	0.9997

Note: [1]p-values are based on Tukey's HSD test; significant differences at $p < 0.05$ are marked with an asterisk (*).

significant effect on plant yield (F(4,10) = 99.75, p<0.0001) in the V5 treatment. The control (V1) and the lowest UEO concentration (V2) showed the highest yields (51.57 g/pot and 53.66 g/pot, respectively), with no significant difference between them. In treatment V3, the yield decreased to 43.92 g/pot, whereas in V4, a marked inhibition of plant growth was observed, resulting in a yield of only 26.80 g/pot (Table 5). The highest concentration (V5) resulted phytotoxic, by reducing the yield to only 5.60 g/pot.

Pairwise comparisons in Table 6, confirmed that V5 differed significantly (p<0.0001) from all other treatments, while moderate differences were also observed between V1/V2 and V3/V4. These results highlight a clear dose-response relationship, where low UEO doses maintain productivity, but increasing concentrations strongly inhibit plant growth (Figure 4). Similar inhibitory effects of UEO at high concentrations on biomass productivity have been reported in prior studies (Cheng et al., 2017).

The obtained data on plant biomass yield for all treatments are presented in Table 7 and 8. Total biomass shoot part, demonstrated that UEO concentration had a strong and statistically significant effect on plant yield (F(4,10) = 99.75, p<0.0001). The control (V1) and the lowest concentration (V2) reveal the highest yields (75.79 g/pot and 72.02 g/pot, respectively), with no significant difference between them. Moderate concentrations (V3) reduced yield to 63.42 g/pot, while higher levels in V4 showed plant inhibition to only 45.82 g/pot. The highest concentration V5 was phytotoxic, reducing yield to only 11.06 g/pot.

Pairwise comparisons in Table 8 revealed that the treatment with the highest UEO concentration (V5) differed significantly from V1, V2, and V3 (p = 0.0018, 0.0034, and 0.0139, respectively), while its difference from V4 was not statistically significant (p = 0.1709). No significant variations were found among the lower UEO levels (V1–V4; p > 0.05). These results indicate that plant yield remains stable from V1 to V4 treatments,

Table 5. Mean plant yield (g) under different concentrations of UEO on the second cut

Treatments	UEO g/kg	n	Yield (Mean ± SD, g/pot)	Grouping	Lower 95%	Upper 95%
V1	0	3	51.57 ± 3.35[1]	a[2]	43.24	59.90
V2	1	3	53.66 ± 4.03	ab	43.64	63.68
V3	5	3	43.92 ± 3.50	b	35.20	52.64
V4	10	3	26.80 ± 4.51	c	15.59	38.01
V5	30	3	5.60 ± 0.95	d	3.24	7.96
Source	DF	Sum of squares	Mean square	F Ratio	Prob > F	
UEO g/Kg	4	4876.96	1219.24	99.75	<.0001*	

Note: [1]Values represent mean ± standard deviation (SD), [2]Different letters indicate significant differences among treatments according to Tukey's HSD test at p < 0.05, marked with an asterisk (*).

Table 6. Pairwise comparisons of plant yield between treatments on the second cut

Treatment's comparison		UEO g/kg	UEO g/kg	p-Value[1]
V2	V5	1	30	**<.0001***
V1	V5	0	30	**<.0001***
V3	V5	5	30	**<.0001***
V2	V4	1	10	**<.0001***
V1	V3	0	10	**<.0001***
V3	V5	10	30	**0.0002***
V2	V4	5	10	**0.0010***
V2	V3	1	5	**0.0414***
V1	V3	0	5	0.1278
V2	V1	1	0	0.9436

Note: [1] p-values are based on Tukey's HSD test; significant differences at $p < 0.05$ are marked with an asterisk (*).

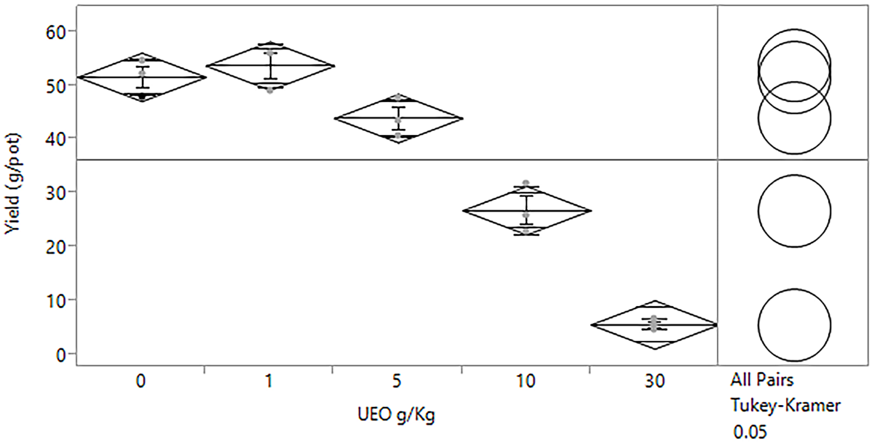

Figure 4. One-way ANOVA means plot of plant yield (g/pot) across different UEO (g/kg) concentrationin second cut. Error bars represent standard errors of the mean

but in V5 the productivity declines, leading to a phytotoxic threshold. Comparable dose–response effects have been observed in earlier studies involving organic extract toxicity on plant biomass (Scavo et al., 2019).

The decline from V1/V2 → V3 → V4 → V5 was consistent with phytotoxic responses to UEO (Figure 5), where the increased level of hydrocarbons inhibited the plant yield by underlying the findings of the previous studies about toxicity in

Table 7. Mean plant yield (g) under different concentrations of used engine oil (UEO) on the total biomass shoot part

Treatments	UEO g/kg	n	Yield (Sum ± SD, g/pot)	Grouping	
V1	0	6	75.79 ± 4.84[1]	a[2]	
V2	1	6	72.02 ± 7.03	a	
V3	5	6	63.42 ± 6.30	a	
V4	10	6	45.82 ± 7.34	ab	
V5	30	6	11.06 ± 3.29	b	
Source	DF	Sum of squares	Mean square	F Ratio	Prob > F
UEO g/Kg	4	51882.97	2875.39	6.24	0.0013*

Note: [1]Values represent mean ± standard deviation (SD), [2]Different letters indicate significant differences among treatments according to Tukey's HSD test at $p < 0.05$, marked with an asterisk (*).

Table 8. Pairwise comparisons of plant yield between treatments on the total biomass shoot part

Treatment's comparison		UEO g/kg	UEO g/kg	p-Value
V1	V5	0	30	**0.0018***
V2	V5	1	30	**0.0034***
V3	V5	5	30	**0.0139***
V4	V5	10	30	0.1709
V1	V4	0	10	0.2938
V2	V4	1	10	0.4229
V3	V4	5	10	0.7645
V1	V3	0	5	0.9199
V2	V3	1	5	0.9776
V1	V2	0	1	0.9991

Note: [1]p-values are based on Tukey's HSD test; significant differences at $p < 0.05$ are marked with an asterisk (*).

cowpea and maize (Azorji et al., 2023; Gawryluk and Krzyszczak, 2024).

Impacts of UEO on plant nutrient uptake and elemental composition

The results obtained on elemental analysis of all treatments presented in Table 9 are analyzed on plant tissues further highlighted visible alterations in C, H, N, P and K concentrations, emphasizing the results of earlier studies that UEO has had a prohibitory behavior on nutrient cycling and soil fertility (Dominguez and Pichtel, 2003; Hajabbasi, 2016). To better understand the relationships between treatments a correlation analysis and multivariate patterns (MP) were conducted by showing that phosphorus (P) and potassium (K) were primarily determinant elements of productivity of the plants, under UEO stress.

The nutrient composition in plant as it is shown in Table 9, varied significantly across treatments, reflecting the impact of UEO contamination on nutrient plant absorption and distribution. The nitrogen (N) levels were relatively stable in all the treatments, ranging from 8.81% (V4) to 9.60% (V5), though higher maximum was found in V3 with 14.6% evidencing variability linked to differential root uptake under stress. The highest content of carbon (C) was found in V5 with 49.8% and the lowest in V4 43.0%, indicating some compensation in absorption processes where the contamination was lower. The hydrogen (H) levels were consistent (4.96–5.88%), with the highest values in V5, indicating the partial maintenance of tissues hydration due to low water infiltration created on the soil with the high UEO concentration. The data obtained for potassium (K) showed high reduction in V5 with 2.04% compared to control V1 with 2.50%, highlighting the UEO interaction in cation exchange and membrane transport. High sensitivity was observed also in the phosphorus (P) content on plant tissues with the lowest level found in

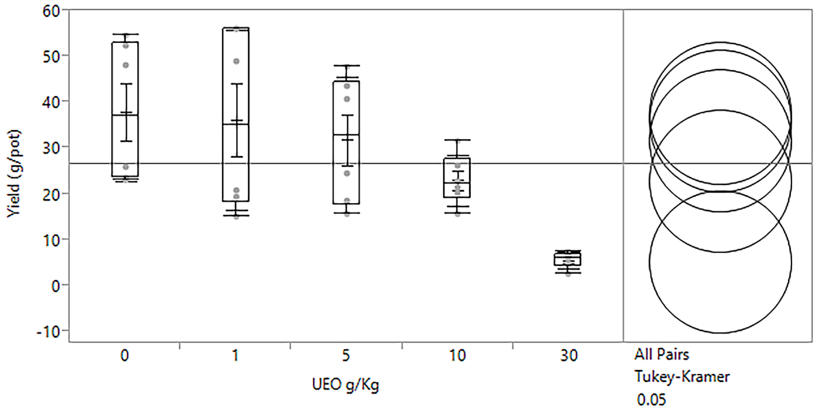

Figure 5. One-way ANOVA means plot of plant yield (g/pot) across different UEO (g/kg) concentration on the total biomass shoot part. Error bars represent standard errors of the mean

Table 9. The effect of UEOs on the content (%) of nitrogen (N), carbon (C), hydrogen (H), potassium (K) and phosphorus (P) in the biomass of *Lolium multiflorum* L.

Parameter	Treatments																								
	V1					V2					V3					V4					V5				
	N (%)	C (%)	H (%)	K (%)	P (%)	N (%)	C (%)	H (%)	K (%)	P (%)	N (%)	C (%)	H (%)	K (%)	P (%)	N (%)	C (%)	H (%)	K (%)	P (%)	N (%)	C (%)	H (%)	K (%)	P (%)
Mean	8.93	45.38	5.37	2.50	0.16	8.60	42.20	5.01	2.70	0.15	9.26	44.05	5.45	2.78	0.14	8.81	42.96	4.96	2.91	0.17	9.60	49.76	5.88	2.04	0.18
Std Dev	±0.63	±5.72	±0.76	±2.04	±0.04	±2.09	±7.48	±0.84	±2.00	±0.05	±3.10	±11.69	±1.49	±1.36	±0.03	±1.65	±8.60	±1.00	±1.07	±0.04	±1.08	±4.74	±0.72	±1.05	0.05
Max.	9.80	56.10	6.70	4.72	0.21	11.30	51.20	5.90	4.95	0.22	14.60	61.90	7.60	4.75	0.19	10.90	54.50	6.20	4.30	0.23	11.30	57.10	6.80	2.91	0.25
Min.	8.00	40.00	4.70	0.00	0.10	5.70	28.50	3.50	0.04	0.09	5.60	27.90	3.40	1.38	0.12	6.50	31.30	3.60	1.83	0.13	8.10	42.80	4.80	0.00	0.12
n	6	6	6	6	6	6	6	6	6	6	6	6	6	6	6	6	6	6	6	6	6	6	6	6	6

V2 (0.15%) and highest in V5 (0.18%). These findings were found also in other studies were UEO decreased the microbial activity by enhancing the mineralization process under anaerobic conditions (Kaimi et al., 2007; Razafimbelo et al., 2013; Carvalho et al., 2015). The results confirm that UEO dose used in V5 has affected the nutrients absorption dynamics especially for K and P, both elements are critical for physiological processes. This decrease was due to blocking films crated on root system that reduces nutrient uptake and lower soil microbial activity (Ekpo et al., 2012; Odjegba and Bamgbose, 2012).

The relation between UEO content and C levels analyzed in plant tissues are shown in Figure 6. The findings evidenced a moderate but consistent correlation between yield (g/pot) and C content (%). The higher level of C was found in V3 with 61.9% meanwhile in V5 the content of C was 34.3%, related to yield reduction. This indicates that hydrocarbon stress reduces photosynthetic assimilation and carbohydrate accumulation, especially at higher UEO doses. Similar findings were observed by Rusin et al., (2015), where oil pollution has reduced the chlorophyll and impaired carbon assimilation through oil film

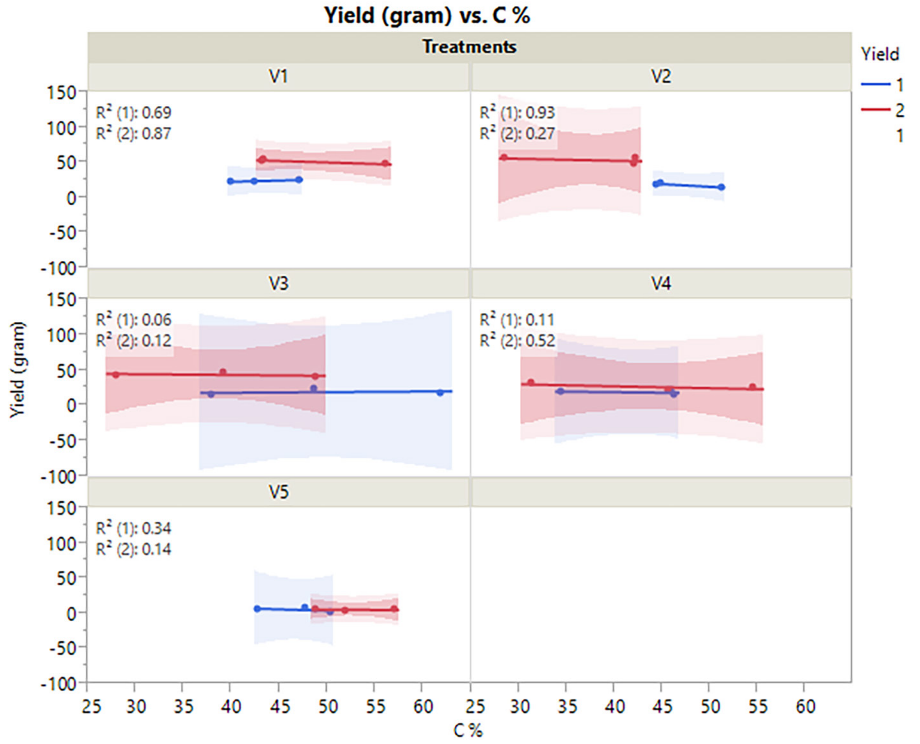

Figure 6. Relationship between plant tissue carbon (%) content and yield (g/pot) across treatments

cover of root surface that inhibited light and gas exchange. Consistent with this, even though carbon itself is not a soil-limiting factor, UEO reduces the ability of plant to assimilate and transport of C. Also, the highest contamination treatment (V5) showed both the highest carbon percentage (49.8%) and the lowest biomass production, indicating that carbon accumulation did not correspond to increased plant productivity. Similar responses have been reported in the plants exposed to petroleum derived pollutants, where stress induced metabolic changes alter carbon assimilation and allocation (Rusin et al., 2015).

UEO showed a different effect between nitrogen (N) plant uptake concentration and yield obtained in each treatment. The result of higher yield was obtained in V3 were the N (%) also was higher, while the lower N content with a correlation to yield decrease was found in V5 (Figure 7). This trend indicates that higher UEO concentrations may affect soil nitrogen mineralization process by inhibiting microbial activity, root nodulation and N uptake with direct impact on protein synthesis and plant growth Anoliefo and Vwioko (1995). Likewise, as it was observed for the C content, the N content uptake by plant followed the same decreasing trend, which implicates this element as a key limiting factor in hydrocarbon stress.

The hydrogen data has not shown a strong correlation with biomass yield (Figure 8). Furthermore, lower levels of the H content in biomass (V2 second cut, 3.5%; V6, 3.6%) were related to lower yield, and higher H content (V5, 6.8%) to relative biomass increase. This is a suggestion that hydrocarbon stress disrupts plant water balance and organic synthesis, both closely related to the hydrogen content. UEO pollution increases soil hydrophobicity and reduces water absorption, lowering relative water content and biomass hydration (Anoliefo and Vwioko, 1995). Thus, reduced H content reflects lower metabolic activity and turgor pressure in plants. Although not nearly as strong a predictor as N or P, H still reflects physiological stress, confirming that UEO suppresses biomass indirectly via impaired water uptake and hydrogen metabolism.

Significant relationship between phosphorus (P %) in plant and plant yield (g/pot) across the treatment were observed in Figure 9. In the control (V1), this relation has strong positive correlation ($R^2 = 0.79 – 0.88$) by indicating that higher P availability can increase biomass production. A similar, but weaker trend was observed in V2 and V4, by indicating partial nutrient efficiency despite low level of UEO used. In contrast, V3 and V5 showed weak correlation ($R^2 <0.10$), reflecting the substantial linkage between higher UEO contamination

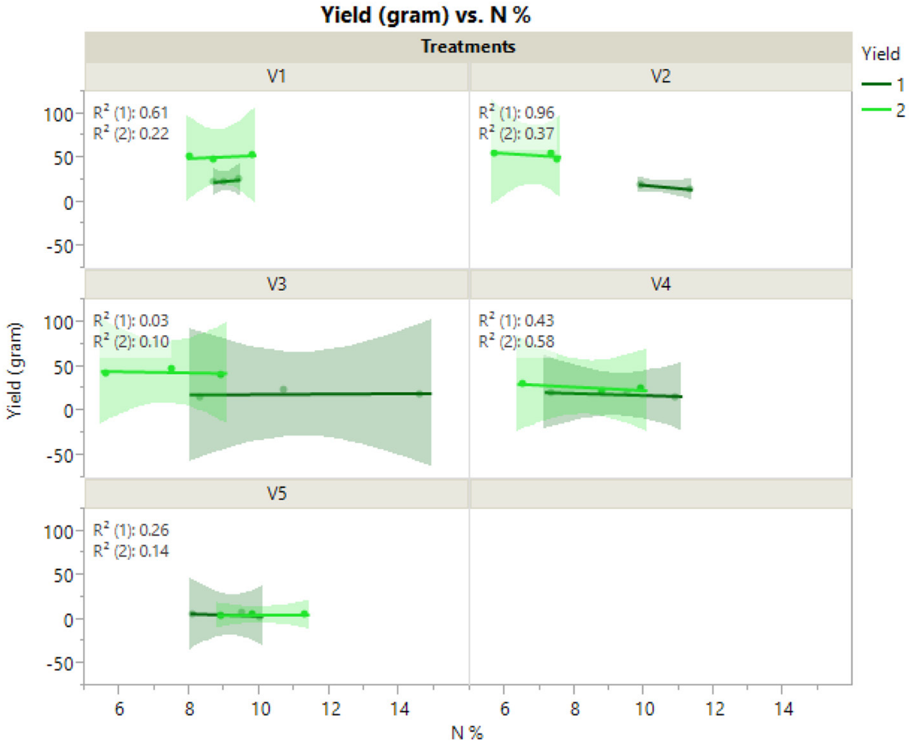

Figure 7. Relationship between plant tissue nitrogen (%) content and yield (g/pot) across treatments

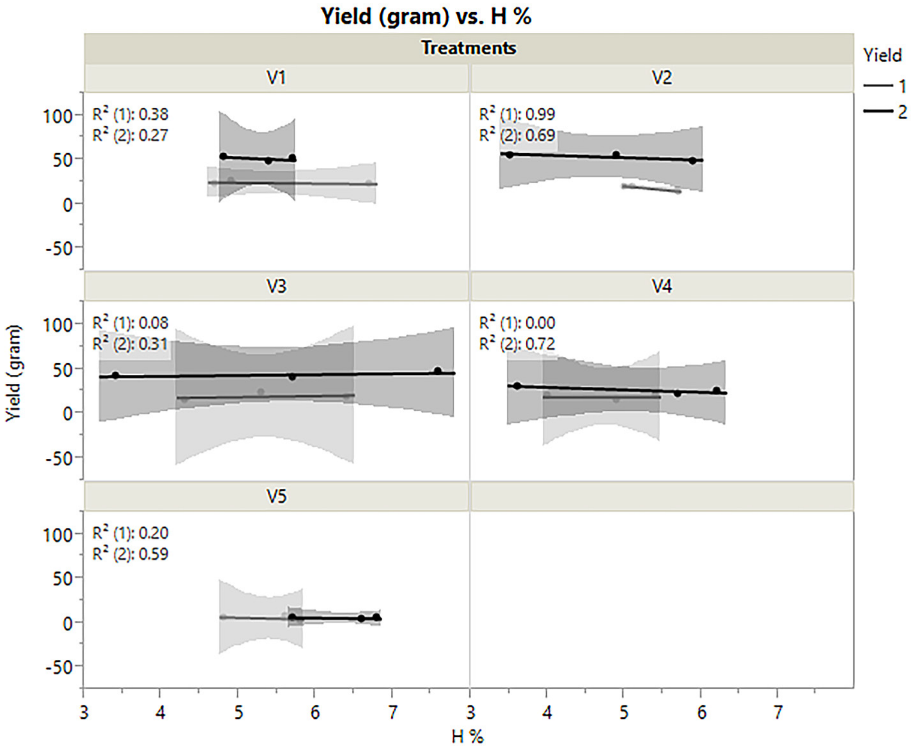

Figure 8. Relationship between plant tissue hydrogen (%) content and yield (g/pot) across treatments

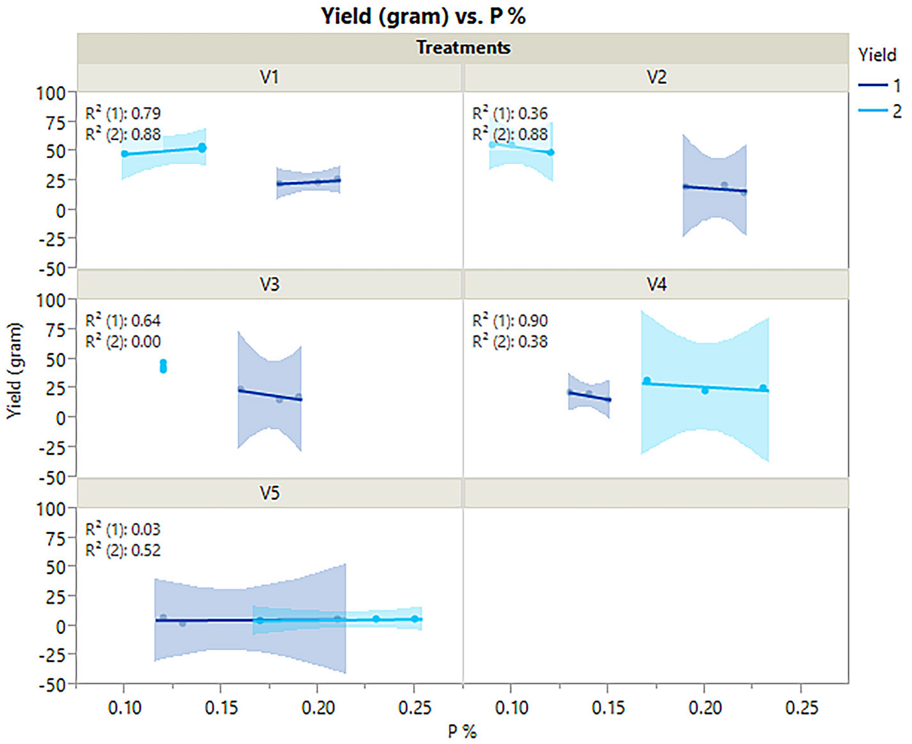

Figure 9. Relationship between plant tissue phosphorus (%) content and yield (g/pot) across treatments

and P uptake by a plant. Thus, phosphorus status is the key yield determinant in hydrocarbon stress. In conclusion, this implies that between the elevated levels of UEO and the disruption of phosphorus uptake, assimilation has occurred due to impaired root activity and microbial P mineralization, ultimately reducing the plant nutrient use efficiency and productivity (Ekpo et al., 2012).

Potassium (K) also responded positively to yield, though less than phosphorus, as it is shown in Figure 10. In treatment V3, with higher dose of UEO, the content of K = 4.75%, was responsible for higher yield, while the opposite behavior between yield and K content as observed on the V5 treatment with just 2.36–2.91% K uptake. The same trend like in P indicate that UEO inhibits the uptake of potassium, by altering the soil cation exchange and degrading the root membranes as it was observed previously in okra (Odjegba and Bamgbose, 2012). UEO has suppressed the absorption of K, leading to poor stomatal control, enzyme activation and carbohydrate translocation. The present results further support the findings described by Odjegba and Bamgbose (2012). While the P content remains the limiting nutrient, the K content is pivotal to resistance to UEO stress, and therefore fertilization with K is crucial to correct UEO contamination.

Table 10 presents the findings about Linkelihood-Ratio Chi-Square tests, beginning with germination responses, followed by nutrient-yield relationships and MP.

The MP likelihood-ratio test showed that variation in tissues metabolic elemental composition is tightly linked to yield under UEO stress. Phosphorus showed the strongest association (χ^2= 25.72), followed by K and N, with C and H also being significant. This finding suggests that P availability is a key limiting factor under UEO stress conditions, likely due to impaired root activity and reduced microbial mineralization processes in contaminated soils. Importantly, each nutrient displays a significant interaction with yield, suggesting that biomass reductions are not simple due to toxicity effects of UEO but it result from the disrupted nutrient uptake mechanisms. Significant terms were found as follows: C% (χ^2=8.98, p=0.0027), H% (6.94, p=0.0084), N% (9.49, p=0.0021), P% (25.72, p<0.0001), K% (14.59, p=0.0001) yield with Nutrient interaction were from C (46.18, p<0.0001) to N (4.16, p≤0.0316). This is consistent with the mechanisms where UEO reduces soil aeration and water flow, disrupting root physiology and rhizosphere microbiology, thereby limiting P and K availability and affecting N cycle (Kayode et al., 2009; Moses et al., 2023). Therefore, the threshold identified in this study should be considered a preliminary indicator of phytotoxic risk rather than a universal field value. Nevertheless, the experimental findings provide valuable insight into the dose-response relationship between UEO

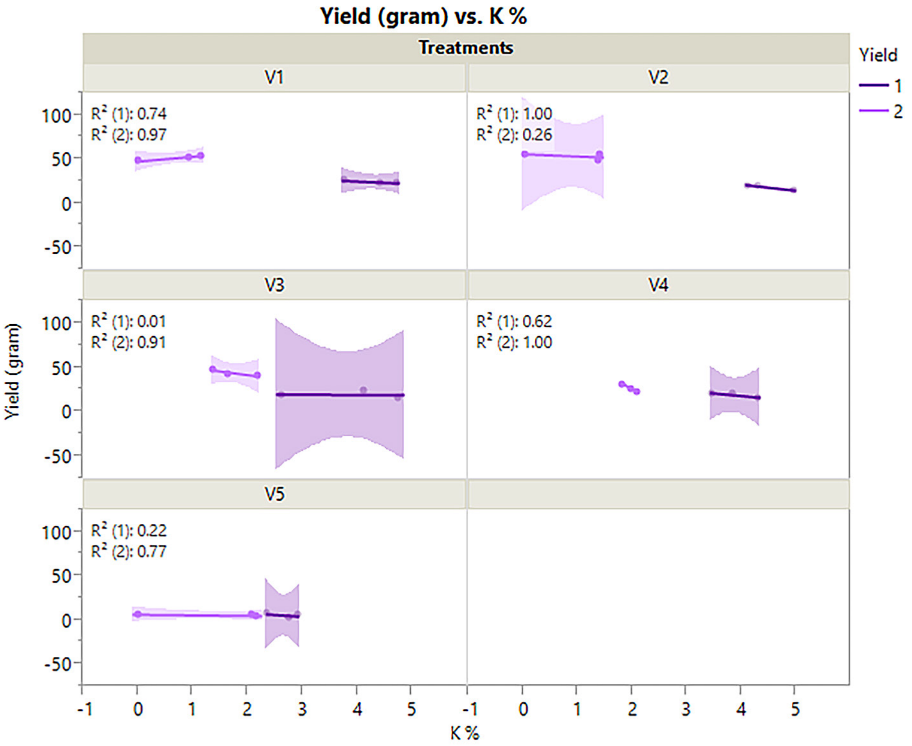

Figure 10. Relationship between plant tissue potassium (%) content and yield (g/pot) across treatments

contamination and plant productivity, supporting previous research indicating that high concentrations of petroleum hydrocarbons can lead to severe inhibition of plant growth (Tang et al., 2011; Haider et al., 2021).

Table 10. Likelihood-Ratio Chi-Square tests for main effects of plant C, H, N, P, K (%) and their interactions with yield (g/pot)

Parameter	L-R ChiSquare	Prob>ChiSq
C %	8.98	0.0027*
H %	6.94	0.0084*
N %	9.49	0.0021*
P %	25.72	<.0001*
K %	14.59	0.0001*
Yield (g/pot)	46.18	<.0001*
C %*Yield (g/pot)	14.69	0.0001*
H %*Yield (g/pot)	14.02	0.0002*
N %*Yield (g/pot)	4.61	0.0316*
P %*Yield (g/pot)	7.89	0.0050*
K %*Yield (g/pot)	13.52	0.0002*

CONCLUSIONS

This study demonstrated that the UEO, as a source of pollution has serious phytotoxic effect on *Lolium multiflorum* L., in which germination, plant growth and nutrient uptake were significantly inhibited with the increase of its confrontation from 1 to 30 g/kg soil. A clear dose – response effect was observed in the reduction of the seedling process, suppressed biomass yield and altered nutrient uptake, with higher level of UEO (30 g/kg soil). Reverse effect was obtained in low-level of UEO treatment, such as V2 with minimal metabolic effects and moderate in treatments V3 and V4 with respectively 5 and 10 mg UEO/kg soil. Nutrient dynamics played a crucial role in plant response. Phosphorus was the determinant nutrient of yield under the pressure of UEO, followed by potassium and nitrogen, by emphasizing that hydrocarbons induced nutrient imbalance with crucial role to growth inhibition. Carbon and hydrogen also showed a significant role through secondary associations with performance corresponding to UEO disruption in photosynthesis and water relations. Multivariate Patterns results emphasize that UEO stress redistributes nutrient – yield relations, distinctive contaminated treatments from the control one. The findings of this study align with previous research result on the possibility that a site contaminated with UEO can lead to complete plant inhibition due to suppressed microbial activity and the decreases of metabolic nutrient availability responsible also for plan stress tolerance and enzymatic function. The main results on dual effect that UEO has shown in direct phytotoxicity and indirect to nutrient imbalance makes this study effective to be continued in future were the special mechanisms of how plant continue to grow even with non-maximum yield under these polluted environmental conditions. The moderate tolerance observed though this study in *Lolium multiflorum* L. indicates its potential application in phytoremediation practices for the UEO-polluted soils.

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