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PHYTOREMEDIATION POTENTIALS OF *GLYCINE MAX* (L) (SOYBEAN) SEEDLINGS ON DIESEL AND SPENT ENGINE OIL CONTAMINATED SOIL

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ABSTRACT

The primary aim of this study was to investigate the phytoremediation potential of *Glycine max* (L.) seedlings in soils contaminated with diesel and spent engine oil. The experiment was conducted at the back of the Faculty of Science Complex, Benue State University Makurdi, Benue State Nigeria. *Glycine max* seedlings were grown in soils contaminated with mixed varying concentrations (100 ml, 200 ml, 300 ml, 400 ml, 500 ml) of diesel and spent engine oil. The experiment spanned 18 days, with germination count, number of leaves, plant height, and stem width measured at intervals of 4, 8, 12 and 18 days. Additionally, the concentrations of heavy metals such as Lead (Pb), Cadmium (Cd) and Nickel (Ni) in both contaminated and uncontaminated soils were analyzed before experimentation. The germination count, number of leaves, height, and stem width of *Glycine max* were adversely affected by increasing concentrations of contaminants. Higher concentrations (400 ml and 500 ml) significantly ($P<0.05$) reduced plant growth parameters compared to lower concentrations (100 ml and 200 ml). Specifically, germination was notably lower at higher contamination levels (300ml and above), showing stunted growth, reduced number of leaves and short stems with a mean germination count of 0.00 at 400 ml and 500 ml on day 4. Plant height and stem width also showed a similar trend, with the control group exhibiting the highest growth. These results indicate that *Glycine max (L)* seedlings are negatively impacted by high levels of diesel and spent engine oil contamination, affecting their growth and development. Despite these challenges, the soybean plants exhibited some tolerance at lower contamination levels, suggesting potential for phytoremediation if contaminant concentrations are managed. *Glycine max* shows potential for phytoremediation of soils contaminated with diesel and spent engine oil, particularly at lower concentrations. Future research should focus on optimizing conditions for phytoremediation and understanding the mechanisms of contaminant uptake and degradation by *Glycine max*.

KEYWORDS*:* Contamination; engine oil; germination; heavy metals; phytoremediation.

INTRODUCTION

Environmental pollution due to diesel and spent engine oil is a significant concern. These hydrocarbons are prevalent contaminants that adversely affect soil health, plant growth, and groundwater quality. Phytoremediation, a sustainable and cost-effective green technology, utilizes plants to degrade, extract, contain, or immobilize contaminants from soil and water (Kumar et al.,2025). Soil contamination with petroleum and petroleum-based hydrocarbons has caused critical environmental and health defects (Njoku et al.,2022).

The release of petroleum-based hydrocarbons into environment through various activities such as municipal wastes, sewage release, dumping off oil and fuel, offshore and onshore petroleum industrial activities including drilling, exploration, storage, transportation, processing, refining, unplugging of oil wells as well as accidental oil spills, cause petroleum hydrocarbons pollution and contaminate soil, groundwater as well as ocean in large scale (Ahmed et al., 2023). The toxicity of petroleum hydrocarbons is well established, affecting our ecosystem, agricultural practice and human health.

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The persistent nature of petroleum hydrocarbons negatively impacts soil and water ecosystems. It alters soil biological properties by affecting microbial diversity and enzymatic activities (Ahmed et al., 2023). They also have phytotoxic impact on seed germination and plant growth due to inhibiting the uptake of minerals and water, primarily reducing the root length and leaf area of plants (Haider et al., 2021).
The common techniques involved in the cleaning up of soil contaminated sites are the physical, chemical and thermal processes. These techniques however, have some adverse effects on the environment and are also expensive. Some of the techniques are costly while some are not environmentally friendly, leaving recalcitrant by-products in the environment. Recently, biological techniques have been evaluated for the remediation of sites contaminated with petroleum. *Glycine max* (soybean) was selected for this phytoremediation study due to its high biomass production, deep root system, and nitrogen-fixing ability, which enhance its capacity to tolerate and extract contaminants from soil. Compared to *Helianthus annuus* (sunflower), which is effective in heavy metal uptake but more susceptible to drought and requires high water input, *G. max* was reported more adaptable to diverse soil conditions. (Njoku et al., 2022; Okparanma et al., 2022; Ahmed et al., 2023). Leguminous plants have gained attention in the field of phytoremediation due to their unique ability to improve soil quality and remove or mitigate various environmental contaminants (Gopamma et al.,2024). In view of this, the primary aim of this study was to investigate the phytoremediation potential of *Glycine max (L.)* (Soybean) and seedlings in soils contaminated with diesel and spent engine oil.

MATERIALS AND METHODS

The experiment was conducted at the back of the Faculty of Science Complex, Benue State University Makurdi, and Benue State Nigeria. Benue State University is located near the Southern bridgehead of the Benue River. It occupies a 6 square kilometers piece of land between Gboko Road and the River Benue. Makurdi is the capital of Benue State, and the city is situated within Benue and situated at the heart end of the guinea savanna vegetation zone of Nigeria (middle Belt). The town has a monthly temperature range of 27-37°C with mean annual rainfall ranging from 15-18 mm. The rainy season occurs from the month of April - October and is more prolonged than the dry season which is usually from November – March.

Collection of Experimental Materials

The diesel and Spent Engine oil were collected at pooled used engine oil from Mechanic workshops in Benue links, Kanshio mechanic village and North bank Market, all within Makurdi, Benue State and were taken to Benue State University for further studies. The topsoil used for the study was dug at a depth of 0-8cm, with the use of a shovel. The experimental plant of interest (*Glycine Max* L) seeds was obtained from the Abintse local market along the Gboko-Makurdi road, and polythene bags were obtained from the Wurukum market in Makurdi.

Experimental Design and Layout

The experiment involves, the phytoextracting plant *Glycine Max* (L) (Soybean) and soil having one (1) control and 5 different levels of Diesel and Spent Engine oil treatment with three (3) replications giving a total of 18 experimental units. The entire experiment was executed in a Randomized Complete Block Design (RCBD) as adopted by Oluwanisola and Abdulrahman (2018).

Treatment Application

The collected topsoil was air-dried for one week in the Laboratory, crushed and sieved with a 2mm steel sieve. The soil sample was divided into 5 portions, weighed 4kg each, and were further subjected to 5 different levels of Diesel and Spent Engine oil and contamination (treatments): 100ml, 200ml, 300ml, 400ml, 500ml and 0ml/kg as control following the method of Oluwanisola and Abdulramann (2018). The soil samples were thoroughly mixed with Diesel and Spent Engine Oil and were allowed to stay for two (2) weeks for proper absorption of the Diesel and spent engine oil, 4kg each of the sample was put in each of the perforated polythene bag and watered to field capacity 3 times per week for two weeks, the seeds of soybean were sown into 18 perforated polyethene bags each at the rate of 6 seeds per bag at the depth of 3 cm and were labeled accordingly.

Agronomic Data

Viability of the seeds and growth experiment

The seed viability was determined by the simple floatation method before 6 viable seeds were planted into the contaminated and uncontaminated soil. After planting, the experimental setup was watered 2 times a day (morning and evening) to field capacity and monitored for 4 weeks after planting. The experiment was done under a shady environment to minimize evapotranspiration parameters such as seed germination count, the rate of growth (in terms of number of leaves, height and width of the plant) (Manga et al.,2020).

Phytoextraction and soil analysis

At the end of the 7th week of experimentation, each plant was carefully removed from its respective polythene bag, gently washed under running tap water and separated into the shoots and roots. The harvested samples were immediately air dried for four days and milled into powder form in preparation for digestion and heavy metal analysis.
Both the shoots and roots samples of the plant on both contaminated and uncontaminated soil were digested separately after which the digested samples were

determined for Pb, Cd and Ni on an Atomic absorption spectrometer (Bello et al*.,*2021).
The Indices used for the Phytoremediation potential of the experimental plant are (a) Enrichment Coefficient (EC) =Metal concentration in the roots/shoots/ Metal concentration at the site .... (Kisku et al*.,*2000).

Data Analysis

All the data collected were used to calculate their respective means with standard deviation (SD) and the mean values were separated using Fisher's Least Significant Difference (FLSD) in order to determine which of the means differ at a 5% level of significance in the Statiscal Package for Social Science (SPSS) Version 23.

RESULTS AND DISCUSSION

Phytoremediation, the use of plants to remove, stabilize, or destroy contaminants in soil and groundwater, is a promising method for mitigating pollution from hydrocarbons such as diesel and spent engine oil. This study investigates the potential of *Glycine max* (soybean) seedlings in remediating soils contaminated with varying concentrations of diesel and spent engine oil. The focus is on evaluating the impact of contamination on seed germination, leaf production, plant height, and stem width, as well as the concentration of heavy metals in the soil.

Effect of Different Concentration Levels of Diesel and Spent Engine Oil on the Germination Count

Table 1 shows the effect of different concentration levels of diesel and spent engine oil contaminated soil on the germination count and number of surviving seedlings. At Day 4 of the observation, only 2 seedlings in each of the 100ml, 200ml and 300ml treated soil germinated, while no seedling emerged in the 400ml, 500ml and the control groups but only at the early days of planting may be due their inability to break-off from their dormancy. The results also indicate a notable impact of diesel and spent engine oil contamination on the germination of *G. max* seeds. The control group showed the highest germination count by Day 18, while the highest contaminated group (500 ml) demonstrated significantly ($P<0.05$) reduced germination rates throughout the observation period. Notably, seeds in the 100 ml and 200 ml treatments managed to sustain some level of germination, albeit much lower than the control. These results suggest that even at lower concentrations, diesel and spent engine oil negatively affect the germination process, likely due to the toxic effects of hydrocarbons which impair seed metabolic activities and cellular respiration (Alvarez & Illman, 2006; Dib & Sadoudi, 2020 and Almed et al*.*,2023).

Effects of Different Concentration Levels of Diesel and Spent Engine Oil on the Number of Leaves

The effect of different concentration levels of diesel and spent engine oil on the number of leaves of soybean is presented in Table 2. The Result showed no significant difference ($P>0.05$) in the number of leaves of the plants in the control and the plants in the diesel and engine oil contaminated soil throughout the period of observation. The number of leaves produced by the soybean plants also decreased with increasing contamination levels. The control plants consistently produced more leaves than the contaminated ones, with no significant ($P>0.05$) leaf production observed in the highest contamination levels (400 ml and 500 ml) on Days 4 and 8. By Day 18, although there was some recovery in leaf production in contaminated soils, it remained lower than in the control. This indicates that while *Glycine max* (L) can tolerate some level of contamination and continue to grow, the efficiency of leaf production is significantly compromised in highly contaminated soils. This can be attributed to impaired photosynthesis and nutrient uptake (Adam & Duncan, 2002; Haider et al*.*, 2021).

Table 1. Effects of different concentration levels of diesel and spent engine oil on the germination count

Concentration (ml)	Mean Germination Count			
	Day 4	Day 8	Day 12	Day 18
100	$2.00±0.00^{f}$	3.00±0.00	$2.00±0.00^{b}$	$3.00±1.41^{e}$
200	$2.00±1.41^{f}$	3.00±1.41	$3.00±0.00^{c}$	$3.00±0.00^{e}$
300	$2.00±0.00^{f}$	3.00±0.00	$5.00±1.41^{d}$	$3.00±0.00^{e}$
400	$0.00±0.00^{g}$	2.00±0.00	$3.00±0.00^{ac}$	$2.00±0.00^{e}$
500	$0.00±0.00^{g}$	2.00±0.00	$2.00±0.00^{b}$	$3.00±0.00^{e}$
Control	$0.00±0.00^{g}$	2.00±0.00	$4.00±0.00^{cd}$	6.00±1.41
FLSD (0.05)	2.500	NS	1.250	1.49

Values are Mean± Standard deviation in triplicate; Means with the same superscripts are not significant NS = Not Significant

Table 2. Effect of different concentration levels of diesel and spent engine oil on the number of soybean leaves

Concentration (ml)	Mean Number of Leaves			
	Day 4	**Day 8**	**Day 12**	**Day 18**
100	0.67±1.15	2.00±1.73	3.33±2.89	3.67±3.21
200	0.00±0.00	1.67±1.53	1.67±1.53	3.00±2.64
300	0.00±0.00	1.00±1.73	1.67±1.53	3.33±3.06
400	0.00±0.00	0.00±0.00	2.00±1.73	4.00±3.46
500	0.00±0.00	0.00±0.00	1.33±1.54	2.67±2.31
Control	0.00±0.00	2.00±0.00	3.00±0.00	3.00±0.00
FLSD (0.05)	NS	NS	NS	NS

Values are Mean± Standard deviation in triplicate.
Mean values with same superscripts are not significant NS = Not Significant.

Table 3. Effect of different concentration levels of diesel and spent engine oil on the height (cm) of soybean

Concentration (ml)	Mean Height (cm)			
	Day 4	**Day 8**	**Day 12**	**Day 18**
100	$1.00±1.73^{a}$	3.17±2.75	5.17±4.48	$6.00±5.22^{cd}$
200	$1.83±1.67^{a}$	2.50±2.18	4.17±3.62	$5.17±4.54^{cd}$
300	$0.93±0.81^{a}$	1.17±1.04	1.40±1.22	$1.77±1.57^{d}$
400	$0.00±0.00^{b}$	0.93±0.83	1.23±1.07	$1.80±1.56^{d}$
500	$0.00±0.00^{b}$	0.76±0.68	1.17±1.04	$1.73±1.55^{d}$
Control	$0.00±0.00^{b}$	1.67±1.53	5.17±4.54	$9.83±8.52^{c}$
FLSD (0.05)	0.501	NS	NS	3.90

Values are Mean± Standard deviation in triplicate.
Means with same superscripts are not significant NS = Not Significant.

Table 4. Effect of different concentration levels of diesel and spent engine oil on stem width (cm)

Concentration (ml)	Mean Stem Width (cm)			
	Day 4	**Day 8**	**Day 12**	**Day 18**
100	$0.07±0.12^{a}$	0.10±0.17	0.17±0.29	3.23±0.40
200	$0.07±0.12^{a}$	0.23±0.21	0.30±0.26	0.37±0.32
300	$2.00±0.20^{a}$	0.37±0.32	0.40±0.35	0.40±0.35
400	$0.00±0.00^{b}$	0.33±0.31	0.50±0.44	0.70±0.62
500	$0.00±0.00^{b}$	0.53±0.68	0.57±0.67	0.67±0.76
Control	$0.00±0.00^{b}$	0.40±0.53	0.77±0.75	1.03±0.91
FLSD (0.05)	1.05	NS	NS	NS

Values are Mean± Standard deviation in triplicate.
Mean values with same superscripts are not significant NS = Not Significant.

Table 5a. Concentration of some heavy metals (Pb, Cd, Ni) in contaminated and uncontaminated soils before experimentation

Samples	Weight (g)	Metals (Mg/g)		
		Pb	**Cd**	**Ni**
CS	1.38	BD	1.20	1.57
US	1.63	BD	0.9570	3.52

KEY: BD-Below Detection Limit; CS = Contaminated Soil, US = Uncontaminated Soil

Table 5b. Phytoaccumulation of heavy metals (Pb, Cd, Ni) in *Glycine max* (*L.*) root and shoot grown in contaminated and uncontaminated soil after experimentation

Soil-plant Samples	Weight (g)	Metals (Mg/g)		
		Pb	Cd	Ni
US	1.23	BD	0.36	3.02
RUS	0.72	BD	BD	3.56
SUS	1.15	BD	1.07	2.97
CS	1.08	BD	0.55	1.17
RCS	0.39	BD	BD	5.81
SCS	0.98	BD	1.01	2.99

KEY:BD-Below Detection Limit: US-Uncontaminated Soil; RUS-Root from Uncontaminated Soil SUS-Shoot from Uncontaminated Soil; CS-Contaminated Soil; RCS-Root from Contaminated Soil; SCS-Shoot from Contaminated Soil

Effect of Different Concentration Levels of Diesel and Spent Engine Oil on the Height of Soybean

Table 3 shows the effect of different concentration levels of diesel and spent engine oil on the height of soybean. From Day 4 to 12, a significant difference (P>0.05) in height was observed between the treatment and the control. At day 18, the highest was observed in the control group, which was significantly higher (P<0.0.5) compared to the spent engine oil contaminated soil groups, except at 100ml. Plant height, another critical indicator of health and vigor, was significantly affected by soil contamination. The control plants reached the highest average height by Day 18, whereas the plants in the 500 ml contamination level averaged the least. This drastic reduction shows the severe impact of diesel and spent engine oil on plant growth. The presence of toxic compounds such as polycyclic aromatic hydrocarbons (PAHs) and heavy metals in the contaminated soil likely inhibits cellular elongation and division, leading to stunted growth as similarly reported by Franco et al., (2004), Bello et al., (2021) and Rehman et al., (2025).

Effects of Different Concentration Levels of Diesel and Spent Engine Oil on Stem Width

The effect of different concentration levels of diesel and spent engine oil on the stem width of soybean. The result showed no significant difference (P<0.05) in stem width between the treatments and the control throughout the period of the study. The stem width data further corroborate the adverse effects of contamination. The control group consistently showed greater stem width compared to the contaminated groups. Notably, by Day 18, the stem width in the control group increased, whereas the stem width in the 500 ml group (Table 4). The reduced stem width in contaminated soils suggests weakened structural integrity and mechanical strength, which can be attributed to hindered lignin biosynthesis due to toxic stress (Okparanma et al., 2022

Concentration of Some Heavy Metals (Pb, Cd, Ni) in Contaminated and Uncontaminated Soil before Experimentation

The concentration of some Heavy Metals (Pb, Cd, Ni) in contaminated and Uncontaminated Soil before Experimentation using an Atomic Absorption Spectrometer (AAS) as presented in Table 5a, revealed that all the metals were present in both contaminated and uncontaminated soil samples except Lead (Pb), which was below detection.

Phytoaccumulation of some Heavy Metals (Pb, Cd, Ni) in Soybean Roots and Shoots Grown in Contaminated and Uncontaminated Soil after Experimentation

Table 5b shows phytoaccumulation of some heavy metals (Pb, Cd, Ni) in soybean roots and shoots grown in contaminated and uncontaminated Soil after experimentation using Atomic Absorption Spectrometer (AAS) and it revealed that Cadmium (Cd) and Nickel (Ni) were present in the Contaminated Soil (CS), Root from Contaminated Soil (RCS), Contaminated Shoot (SCS), Root from Uncontaminated Soil (RUS) and Uncontrolled Soil (US) but Lead (Pb) was below detection in all the parameters. The result of heavy metal analysis provides insight into the potential mechanisms of toxicity in contaminated soils. The presence of nickel (Ni) and cadmium (Cd) in both contaminated (CS) and uncontaminated soils (US) indicates an intrinsic level of these metals, but their concentrations were generally higher in the uncontaminated soils before experimentation. The absence of lead in soils after contamination with used diesel engine oil may indicate either a lack of lead in the oil, soil conditions unfavorable to lead mobility, or analytical limitations. While this result might suggest minimal lead related risk, it doesn't rule out the presence or risk of other toxic metals or organic pollutants in the oil, (Polyanska et al., 2024). However, the observed differences in heavy metal concentrations can still contribute to overall soil toxicity, affecting plant growth and development (Khan et al., 2000; Osman et al.,2024).

CONCLUSION

This study demonstrates that Glycine max (L) seedlings are significantly affected by soil contamination with diesel and spent engine oil, impacting germination rates, leaf production, plant height, and stem width. These findings highlight the sensitivity of soybean plants to hydrocarbon pollution, which disrupts physiological and biochemical processes essential for growth. Despite some levels of tolerance and adaptation, the overall growth performance of Glycine max in contaminated soils is considerably lower than in uncontaminated soils. This underlines the challenges of phytoremediation in highly contaminated environments but also suggests that with lower contamination levels, Glycine max can still play a role in remediation efforts.

RECOMMENDATION

Implementing phytoremediation in conjunction with other remediation techniques, such as bioremediation with microbial consortia, can enhance the degradation of hydrocarbons and improve soil health. Combining physical, chemical, and biological methods can yield more effective results.

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COMPETING INTERESTS

Author has declared that no competing interests exist.

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