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Evaluation of the potentials of *Pennisetium glaucum* and *Vigna unguiculata* for phytoremediation of Spent-engine oil contaminated soil

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Abstract
One promising technique for cleaning up soil polluted by spent engine oil is phytoremediation. Particularly in the tropics, where resources can be scarce and the climate is favorable for plant development. Finding plant species that will work well with this technology was the aim of this investigation. The ability of a cereal (Pennisetum glaucum) and a legume (Vigna unguiculata) to remediate soil contaminated with 1%, 2%, 3%, 4%, and 5% (W/V) of spent engine oil was evaluated. After 150 days of incubation, the Total Petroleum Hydrocarbon (TPH) loss in greenhouse studies was calculated. The total petroleum hydrocarbon content of soil that was vegetated with P. glaucum and V. unguiculata was substantially lower than that of non-vegetated soil. Furthermore, a positive association was discovered between the initial TPH and the total petroleum hydrocarbon loss caused by planting P. glaucum. It was also discovered that P. glaucum outperformed V. unguiculata in remediation capacity. These findings suggest that P. glaucum be investigated further, which might lead to a better understanding of how to apply phytoremediation to soils contaminated with spent-engine oil.

Keywords: Bioremediation, Carcinogenic, Contamination , Spent-Oil, Toxic,

INTRODUCTION
Contamination of soil occurs globally due to a lack of efficient environmental protection policies, which is more common in underdeveloped countries worldwide (Ezeji and Chukwudi, 2021). Spent engine oil is the source of polycyclic aromatic hydrocarbons (PAHs) in the soil in addition to damaging the air and waterways. Given that many PAHs are poisonous, mutagenic, and carcinogenic, this is a public health concern (Njoku *et al.,* 2012). Remedial action is under pressure as a result of the effects of crude oil exploration in Nigeria, gaining attention on a national and worldwide level (Adesipo *et al.,* 2020). The most promising method of reclaiming soil is bioremediation, and it's exciting to see how attempts are being made to incorporate microbial and plant activity into the process. On a laboratory and field

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scale, plants—especially legumes and grasses—have been found to be crucial in the remediation of oil-polluted soil (Ismail *et al.,* 2014). It has been determined that phytoremediation is a successful method for cleaning up soil contaminated with petroleum hydrocarbons (Ezeji and Chukwudi, 2021). In Nigeria, it is customary for motor mechanics in particular to dispose of spent engine oil (SEO) into fields, open unoccupied plots, gutters, and water drainages (Ayotammo *et al.,* 2006). This oil, also known as waste engine oil or spent lubricant, is typically collected after car and generator engines have been serviced and drained; the majority of this oil is then dumped into the ground (Anoliefo and Vwioko, 2001). According to Achuba and Peretiemo-Clarke (2008), spent oil considerably reduced soil catalase and dehydrogenase activities. The ecosystem is constantly at risk of oil contamination due to ongoing production, processing of spent oil, and transportation (Agbogidi *et al.,* 2005). Agricultural areas are negatively impacted by oil pollution, whether it is acute or chronic, and this has a major impact on plant development (Agbogidi *et al.,* 2005).According to this research, a wide variety of plants, including freshwater wetland plants like reeds and grasses, leguminous plants, and agricultural crops, have the potential to remove a wide range of toxins. The motivation for this work is the severity of spent lubricating oil pollution in the environment and the potential for bioremediation to eliminate different forms of oil pollution. This research investigate the use of cowpea (*Vigna unguiculata*) and millet (*Pennisetum glaucum*) in the remediation of soil contaminated with spent engine oil.

MATERIALS AND METHODS

Collection of Materials
The sandy loam soil used for the study was obtained from the Botanical Garden, University of Lagos. Akoka. The used (spent) engine oil was obtained from the roadside automobile mechanic. workshop in Yaba area of Lagos. Seeds of the plants *Vigna unguiculata* (Cowpea) and *Pennisetum Glaucum* (Millet) were obtained from the genebank section of the National Institute for Horticulture (NIHORT) Ibadan using their ascension numbers. Buckets for the planting of the seeds were of two different sizes: the smaller (2.5 liters) for planting cowpea, while the bigger buckets (10 liters) were used for the planting of millet.

Preparation of Materials/Pollution of Soil
A 1 mm mesh screen was used to separate plant fragments and detritus from the soil. Each of the thirty buckets used for planting cowpea and millet was filled with 3 kg and 7 kg of soil respectively, by weighing the dirt after which the 30 buckets were divided into ten (10) replicates, three (3) for each of the treated and control groups. This was carried out by using Njoku *et al.*'s methodology (2009a). Using Adenipekun and Kassim (2006) method, the soil was contaminated with spent engine oil to achieve 1%, 2%, 3%, 4%, and 5% (w/v) respectively. Following the contamination of the soil, it was soaked with water and allowed to stand for three days prior to the millet and cowpea seeds were planted in separate buckets.

Design of the Experiment
Randomized complete block design was used in this study. The sources of variation are due to concentration of spent engine oil used, size of bucket and species of plant used (different species form a block) and treatment is the spent engine oil. The appropriate model for the design is given below:
Yij = +bi +tj +eij
Where;
Yij = individual observation
bi = Block effect

tj = Treatment effect
eij = Experimental error which may assumed to be normally independently identically distributed.

Planting of Seeds
Seeds were planted into groups where various concentration of spent engine oil had previously been added. Using the procedure outlined by Ayatamuno *et al.* (2006), a total of five Cowpea and ten Millet seeds were planted into each of their respective buckets and thoroughly sprinkled with water (manually).

Collection of Samples
Soil samples were obtained before planting the test plants (Millet and Cowpea) and after the plant growth. Samples were collected using aseptic technique in which all materials were made sterile through the use of methylated spirit. This was done to avoid any interference of undesired parameter with parameter of interest. It was ensured that, materials used for collecting the soil samples were sterilized before use. Also, it was ensured that soil samples in buckets which seeds were planted were collected from the root region of the plant (Njoku *et al.*, 2009b).

Soil Extraction
In this experiment, an automated Brinkmann Büchi 461 extraction apparatus was used for Soxhlet extraction. The U.S. EPA (1996) and ASTM (Annual Book of ASTM Standard, 2005)-approved method for removing semi-volatile and non-volatile organic pollutants from solid materials, such as soil, was chosen as the extraction technique for this investigation.

The 10 g lots of homogenized soil were weighed into Soxhlet thimbles and then placed within Soxhlet extraction tubes above the 300 ml dicloromethane (DCM)-filled two-neck round-bottom flasks. With a water condenser running, the entire Soxhlet assembly was submerged in a bath of boiling water. Ten cycles per hour of Soxhlet extraction were performed for a duration of 16 ± 2 hours. The solution was decanted into a Kudema Danish evaporator after the conclusion of the extraction phase, and the volume was lowered to about 100 ml. Prior to Gas chromatography-flame ionization detection (GC-FID) analysis, the solution was quantitatively transferred into a 100 ml volumetric flask and shaken to achieve homogenization after the volume was adjusted to the calibration mark with DCM.

Sample Clean-up
Before the samples were subjected to a GC column analysis, they were cleaned up to remove any contaminants, moisture, and polar hydrocarbons. This was accomplished by passing the extract through a dual-layer, 6-milliliter glass Florisil®/Na2SO4 SPE tube (2 g) while applying pressure. Natural hydrocarbons were efficiently eliminated during the cleanup process, and the amount of petroleum hydrocarbons remaining was not significantly impacted.

Determination of Total Petroleum Hydrocarbon content of the Soil (GC-FID Analysis)
GC-FID analysis was used to determine the total petroleum hydrocarbon (TPH). The GC-FID Fisson MD-800 equipment was utilized for the analysis of the oil extracts. A Chrompack WCOT column CP-Sil 5 CB/FID column (30m, 0.32mm ID) with a helium (He) flowrate of 1.1 ml/min was fitted to the GC. The on-column injection technique was utilized to avoid discrimination because of the large range of molecular weights of the components of interest (128 for naphthalene to 276 for benzo(a)pyrene). The temperature of the oven was set

to rise from 50 °C to 65°C at 15 °C/minute, from 65 °C to 150°C at 8 °C/minute, and from 150 °C to 300°C at 3°C/minute.

The samples were introduced into a 100 ppm hexane solution, with n-dodecane serving as the internal standard. At 70 eV, the ion source was activated. The temperature settings for the source and the transfer line were 250 °C and 300 °C, respectively.
Data handling was done with Agilent Chemstation chromatography software (version 10). This was carried out in accordance with standard methods (EPA, 1998).

Data and Statistical Analysis
The remediation due to natural attenuation, TPH loss due to plant growth and the percentage remediative ability of the test plants were calculated as follows:
(a) Remediation due to Natural Attenuation =
Quantity of TPH at the beginning – Quantity of TPH in the soil without plant at the end of the study period
(b) % TPH lost =

$$\frac{\text{Quantity of TPH at the beginning} - \text{Quantity of TPH in the soil at the end}}{\text{Quantity of TPH lost at the beginning}} \times 100$$

(c) TPH loss due to plant growth =
Quantity of TPH at the beginning - Quantity of TPH in the soil vegetated with such plant atthe end of the test period
(d) % Remediative ability of plant=

$$\frac{\text{\% lost from soil with plant} - \text{\% lost from soil without plant}}{\text{\% lost from soil with plant}} \times 100$$

Data analyses involved the use of Spearman correlation as contained in Graphpad 5.0 prism software.

RESULTS

Remediation by Natural Attenuation
The amount of total petroleum hydrocarbon (TPH) at the start of the test period was higher than the total TPH in all the soil samples at the end of the test period i.e150 days (Table 1). The correlation between the amount of TPH at the beginning of the test period and the total petroleum hydrocarbon removed by natural attenuation (without vegetation) was positive ($p = 0.0354$). However, there was a higher percentage loss of TPH as a result of natural attenuation in 1% and 2% when compared to 3%, 4%, and 5%.

Total Petroleum Hydrocarbon Loss due to Plant Growth
Soils containing plants lost more total petroleum hydrocarbon (TPH) than soils without plants (Table 2). Compared to *Vigna unguiculata*, *Pennisetum gluacum* growth resulted in a higher loss of total petroleum hydrocarbon from the soil. There was a higher percentage loss of total petroleum hydrocarbons by both *Pennisetum gluacum* and *Vigna unguiculata* as the concentration increased from 1% to 2%, but a gradual decline in the loss of TPH was noticed in 3% and 4%. The test plants, *Pennisetum gluacum* and *Vigna unguiculata*, show a tendency to remove more total petroleum hydrocarbon, as there was a higher percentage loss of TPH in soils vegetated with the two plants at 5% compared to 3% and 4%. There was a negative correlation between the TPH loss from the soil vegetated with *Vigna unguiculata* and the amount of TPH at the beginning of the test period; this shows there was no significant correlation between the two ($p = 0.1026$). Although there was a positive correlation between

TPH loss from soil vegetated with *Pennisetum gluacum* and the amount of TPH at the beginning (p = 0.0238),

Pennisetum gluacum **and** ***Vigna unguiculata*** **to Remediate Spent Engine Oil Contaminated Soil**

The soil without vegetation, where there was the least amount of total petroleum hydrocarbon (TPH) loss from the soil (Table 3) has the lowest percentage of remediation for each concentration. Although *Pennisetum gluacum* has a greater remedial ability than *Vigna unguiculata* (Table 4), as the concentration increased, there was no statistically significant difference in the quantity of TPH lost from the soils containing *Pennisetum gluacum* compared to *Vigna unguiculata* ($P > 0.05$).

Table 1: Remediation of Total Petroleum Hydrocarbon (TPH) by Natural Attenuation

Concentration (%)	TPH level (ppm)			% Loss by Natural Attenuation
	Initial	Final	TPH Loss by Natural Attenuation (ppm)	
1	224.25	206.87	17.38	7.78
2	475.14	435.95	39.19	8.25
3	558.02	525.02	33.00	5.91
4	678.90	645.75	33.15	4.88
5	932.28	876.90	55.38	5.98

(Ppm= part per million; TPH= Total petroleum hydrocarbon)

Table 2: Total Petroleum Hydrocarbon (TPH) Loss Due to Growth of *Pennisetum gluacum* and *Vigna unguiculata*

		TPH Loss (ppm)		% TPH Loss	
Conc. %	Initial TPH (ppm)	*Vigna unguiculata*	*Pennisetum gluacum*	*Vigna unguiculata*	*Pennisetum gluacum*
1	224.25	45.08	82.77	20.10	36.91
2	475.14	191.08	241.94	40.21	50.92
3	558.02	206.99	275.80	37.10	49.43
4	678.90	133.91	236.67	19.73	34.86
5	932.28	249.32	362.86	26.74	38.92

Table 3: Remediative Ability of *Pennisetum gluacum* and *Vigna unguiculata*

	% Remediative Ability	
Conc.%	*Vigna unguiculata*	*Pennisetum gluacum*
1	72.17	82.65
2	82.98	86.06
3	86.26	89.32
4	80.17	87.72
5	81.82	86.76

DISCUSSION

The results of this research indicated that the soils with vegetation (*Pennisetum gluacum* and *Vigna unguiculata)* as well as the soil without vegetation (control) had reduced TPH levels at the conclusion of the study. This suggested that TPH in the soils decreased over time. The decrease in TPH level of soil without vegetation (control) suggested that natural attenuation could help remove total petroleum hydrocarbons (TPH) from soil contaminated by spent engine oil. The current result is consistent with the research conducted by Ebuchi *et al.*(2005), who found that enhanced natural attenuation techniques reduced the TPH concentration of sandy soil after eight weeks. Fingas (2004); Hejazi and Husain (2004) reported a similar outcome, noting that hydrocarbons in soil can weather and disperse abiotically based on their properties and the local climate.

Plants utilized in this study can improve the remediation of spent engine oil polluted soil, as seen by the reduced TPH levels in vegetated soils at the end of the study compared to non-vegetated soil. This is consistent with studies by Njoku *et al.* (2009), Wang *et al.* (2008), and Merkl *et al.*(2005) who found that plants improve the remediation of crude oil. The two plants' varying capacities to purify soil contaminated by spent engine oil are demonstrated by the variations in TPH levels in the soil containing the various plants. Similar findings were reported by Njoku *et al.* (2012) in their investigation of the relative benefits of *Corchorus olitorius* and *Abelmoschus esculentus* on petroleum product-contaminated soil. Of the two plants utilized in this study, *Pennisetum glaucum* typically demonstrated more capacity than *Vigna unguiculata* to repair soil contaminated by spent engine oil.

The possible reason for the disparity in *Pennisetum glaucum* and *Vigna unguiculata*'s remedial efficacy could be attributed to their distinct botanical characteristics. According to Akinola and Njoku (2007), variations in an organism's genetic composition can affect how it reacts to pollution. The impact of organisms on contaminated or polluted soils can be considered an extension of this concept. Therefore, the genetic variations between *P. glaucum* and *V. unguiculata* may be the cause of their different remediative capacities.

The differential ability of the test plants to remediate soil contaminated with spent engine oilI could be that *P. glaucum* is a deep-rooted crop while *V. unguiculata* is a shallow-rooted crop. The deep rootedness of *P. glaucum* must have made the plant able to remove more TPH than *V. unguiculata*. This assertion was supported by Odiyi and Bamidele (2013), who found that the main advantage of grass species is their extensive fibrous root systems, which have significantly greater root surfaces area (per m^3 of soil) than do other species and may penetrate the soil to a depth of up to 3m.

However, it was observed that all treatments showed a significant reduction in TPH content after the growth of *P. glaucum* and *V. unguiculata*. This is in correlation with the report by Diab (2008) that plants were able to remove petroleum hydrocarbons from the soil. Also, Osuji and Onajake (2007) reported the same trend in the TPH content of soil samples. It may also be a result of the influence of the plant on the physiochemical properties of the soil (texture, pH, density, organic carbon), which makes the biodegradation of contaminants more effective, as suggested by Kafle *et al.* (2022). Other mechanisms suggested include the biodegradation of contaminants by microbes in the soil.This was suggested by McCutcheon and Schnoor (2003), who stated that plants influence the microbial load of the soil. They asserted that plants may help increase the microbial load of the soil, which in turn increases the rate of contaminant biodegradation.

The degradation of the spent engine oil in the contaminated soil by *P. glaucum* and *V. unguiculata* may have occurred due to one of the many mechanisms of phytoremediation, which include; phytodegradation, phytovolatilization, phytotransformation, phytostabilization, and rhizofiltration (Gao and Zhu, 2003). This suggests that the ability of *P. glaucum* and *V. unguiculata* to effect phytoremediation of the hydrocarbon-contaminated soil is likely due to its capacity to enhance microbial activity in the rhizosphere (Anderson *et al.*, 1993; Muratova *et al.*, 2003) and the activities of the detoxifying enzymes of the plant themselves (Newman and Reynolds, 2004). The high density of degrading microorganisms and their degradative activities in the rhizosphere of plants are frequently linked to the efficacy of this process (Muratova *et al.,* 2003). Especially since nitrogen fixed in the soil by legumes (e.g., *V. unguiculata*) tends to reduce plant/microbe competition for nitrogen and boost plant growth exudates production, increasing the ability of the plants to improve the degradation of the contaminants (Njoku *et al.,* 2009b). The production of root exudates and plant materials serve as sources of carbon, nitrogen, and phosphorus for petroleum degrading microbes (Alexander, 1997; Bisht *et al*., 2015).

CONCLUSION

The study's findings demonstrated that there was significantly more TPH in the soils at the start than at the end of the investigation. Additionally, at the completion of the research, the TPH levels in the plant-filled soils were much lower than those in the plant-free soils. This suggested that the two plants have the capacity to clean up soil contaminated by spent engine oil, with *P. glaucum* having the highest capability.

It is therefore recommended that more comprehensive researches be done to further uncover the feasibility of the application of plants for practical and economically viable remediation of soils polluted with petroleum hydrocarbons.

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