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# Assessment of phytoremediation potential of cowpea (*Vigna unguiculata* L Walp) on used motor oil contaminated soil

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## Abstract

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Phytoremediation has been identified as an effective option for the clean-up of petroleum hydrocarbon contaminated soil. This study aims to assess the potential of Vigna unguiculata L. Walp (cowpea) in the remediation of used motor oilcontaminated soil. The germination and growth of cowpea on soil contaminated with 5% (w/w) of used motor oil were monitored for 28 days. One of the treatments received 15% (w/w) poultry manure seven days after pollution. The result of the physicochemical analysis of the soil and used motor oil shows that both have acidic pH values (5.90 for soil and 5.96 for used motor oil). Microbial degradation of the used motor oil was monitored by measuring the Total Heterotrophic Bacterial Count (THC), Hydrocarbon Utilizing Bacterial count (HUB), and gravimetric loss of used motor oil {Total Petroleum Hydrocarbon (TPH)} with time. The treatment options supplemented with poultry manure recorded higher THC and HUB counts  $(76.8 \times 10^{6} \text{ cfu/g and } 15.0 \times 10^{6} \text{ cfu/g respectively})$ . The result of the biodegradation studies shows a significant reduction of the concentration of the used motor oil with time. The highest reduction in TPH (83%) was observed in the contaminated soil with cowpea plant and poultry manure. It was also observed that the cowpea plant performed better in the presence of poultry manure, as reflected in the results of the agronomic studies carried out. This study has shown that cowpea (Vigna unguiculata) has great prospects as a tool for the clean- up of used oil-contaminated soil.

**Keywords:** Phytoremediation, Petroleum hydrocarbon, Biodegradation, Cowpea, Poultry manure

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## 1. Introduction

Soil contamination is a universal phenomenon. This menace is more prevalent in the developing nations of the world where there is no effective environmental protection policy (Onuoha *et al.*, 2011). The global use of lubricating oil has lead to an increase in the contamination of soil by used motor oil (Mandri and Lin, 2007). Motor lubricating oil is generally used in different types of automobile and industrial machinery (Abioye *et al.* 2012).

Apart from contaminating the air and freshwater, used motor oil is the source of polycyclic aromatic hydrocarbons (PAHs) in the soil. This is of public health concern because many PAHs are toxic, mutagenic,

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and carcinogenic (Njoku *et al.*, 2012). Long periods of exposure to significant oil concentrations may lead to the development of liver or kidney diseases, possible damage to the bone marrow, and increased risk of cancer (Lloyd and Cackette, 2001). Also, used motor oil contains heavy metals and PAHs that could contribute to chronic hazards including mutagenicity and carcinogenicity (Boonchan *et al.*, 2000). There is, therefore, the need for urgent and cost-effective measures for the remediation of the used motor oil-contaminated soils and possibly waterways in the Nigerian environment.

Phytoremediation is the use of plants and their association microorganisms to degrade, contain or render harmless, contaminants in soil or groundwater (Cummingham *et al.*, 1996; and Merkl, 2005). It is a cost-effective alternative for remediation of recalcitrant hydrocarbon contaminated soils (Salt *et al.*, 1998). Plants can provide a favourable environment for bioremediation and also reduce runoff leading from the contaminated site (Gandhi et al., 2002). Various plants with their associated microorganisms have been found to increase the removal of used motor oil (PAHs) from contaminated soil (Eskandary et al., 2017). This research investigated the use of cowpea (*Vigna unguiculata* L.) in the remediation of soil contaminated with used motor oil.

# 2. Materials and methods

## 2.1. Collection and processing of samples

The soil samples used for this study were collected randomly from the Federal University of Technology farmland using a hand-dug soil auger at the depth of 15 cm. The soil was thoroughly bulked together to form a composite sample and transported to the laboratory in a sack, air-dried and sieved through a 2 mm mesh.

Used (spent) motor oil was obtained from Orji mechanic village, Owerri, Imo State, Nigeria. The spent motor oil was unweathered, having been obtained fresh from an automobile engine and collected with sterile containers. Poultry manure was collected from a poultry farm located in Owerri Imo state Nigeria. Viable seeds of the leguminous crop, vegetable cowpea (*Vigna unguiculata*) was purchased at the Ekeonunwa market in Owerri, Imo State, and stored at room temperature (25 to 30 °C) for not more than 24 h.

## 2.2. Physicochemical analysis of soil and used motor oil

Soil organic matter content, total nitrogen, total phosphorus, total potassium, pH, particle size distribution and density were determined following standard methods from APHA (1998). Physicochemical characteristics of the used motor oil such as density, specific gravity, kinetic viscosity, moisture content, flash point, and pH of the spent motor oil were carried out using standard methods.

## 2.3. Soil preparation

Two kilograms (2 kg) of soil was filled in 15 polythene bags each (five different treatments with three replicates) were perforated at the sides and bases. The bags were arranged in a completely randomized design consisting of five replicated three times. A uniform concentration of 100 ml of spent motor oil was used for the treatments, which was poured evenly on the surface of the soil (surface pollution), using a well-calibrated sterile measuring cylinder. The spent motor oil was uniformly mixed with a hand trowel. The controls were not polluted with spent motor oil; the polluted soils were allowed to stay for one week to enable the volatile components present to vaporized following the procedure of Frank and Althoon (1985); 15% that is 300 g of poultry manure was added to only one treatment (contaminated soil + seed + organic manure) after seven days of contaminations and then allowed to incubate for 14 days before planting the cowpea seeds.

## 2.4. Remediation set up

The bags were kept in a plant screen house. Spacing between bags was 1.5 m following the procedure of Reminson *et al.* (1980). The moisture content was adjusted by distilled water addition ensuring that 60% of water holding capacity was maintained. There were four bags each pretreatment 300 g of poultry manure was added to a single treatment, the addition of poultry manure acts as an organic fertilizer to stimulate microbial and plant growth in the contaminated soil. The cowpea seeds were subjected to viability tests using the floatation technique. Five seeds of cowpea were planted in each bag. The plant screen house was regularly monitored to ensure that the experimental conditions were not altered. Periodic sampling from each treatment was done on day zero that is immediately after the contamination (pollution) then at one-week intervals for five weeks.

## 2.5. Enumeration of Total Heterotrophic Bacteria (THB)

Composite samples were obtained by mixing 5 g of soil collected from a different area of the microcosm. Serially diluted samples (0.1 ml) of appropriate dilution (dilution to produce colony counts of between 30-300 colonies) of soil suspension in distilled water on nutrient agar plates using the spread plate technique (Odokuma and Okpokwasili, 1993; and Odokuma and Ibor, 2002). Bacteria colonies were enumerated after 48hrs if incubation at 30 °C.

## 2.6. Enumeration of Hydrocarbon Utilizing Bacteria (HUB)

HUB in the soil samples were enumerated using a modified mineral salt medium of Milles *et al.* (1978)10.0 g NaCl, 0.42 g NaNO<sub>3</sub>, 0.29 g KCL 0.42 g MgSO<sub>4</sub>.7H<sub>2</sub>O, 1.25 g Na<sub>2</sub>HPO<sub>4</sub>). 83 g KH<sub>2</sub>PO<sub>4</sub> 15.0 g Agar, in 1000 ml distilled water, pH 7.4. the vapor phase transfer method (Amachukwu *et al.*, 1989) was used, a filter paper saturated with sterile spent motor oil was aseptically placed on the inside of the inverted Petri dishes and the culture plates were incubated at  $(28 \pm 2 \, ^{\circ}\text{C})$  for 7 days (Odokuma and Okpokwasili, 1993; and Odokuma and Ibor, 2002). Plates yielding 30-300 colonies were enumerated. Colonies of different hydrocarbon-utilizing bacteria were randomly subculturing on nutrient agar (Oxide). The bacteria isolates were characterized using microscopic techniques. The identities of the isolates were determined by comparing their characteristics with those of known taxa as described by Bergey's manual of determinative bacteriology (Buchanan and Gibbons, 1994).

## 2.7. Measurement of agronomic parameters

The following parameters; petiole length, plant height, number of leaves, leaf length, and width were measured according to the method of Agbogodi and Ofuoku (2005).

## 2.8. Determination of total hydrocarbon content of the soil

The residual hydrocarbon content in the oil-polluted soil during the study period was determined gravimetrically by chloroform cold extraction method of Adesodun and Mbagwu (2008). Soil samples (2 g) were weighed into a 120 ml plastic bottle and the content was made up to 50 ml by the addition of chloroform to extract the hydrocarbon in the soil by leaching. After shaking for 30 min, the mixture was allowed to stand for 24 h. The mixture was then filtered through Whatman No 1 filter paper. The liquid phase of the extract measured at 284 nm absorbance using a spectrophotometer (Model 6305 JENWAY). The THC in soil was estimated into a reference to standard curve derived from fresh spent motor oil diluted with chloroform.

## 2.9. Statistical analysis

Statistical analysis of data was carried out using Analysis of Variance (ANOVA), least significant difference. These were used to determine the relationship between the variables.

## 3. Results and discussion

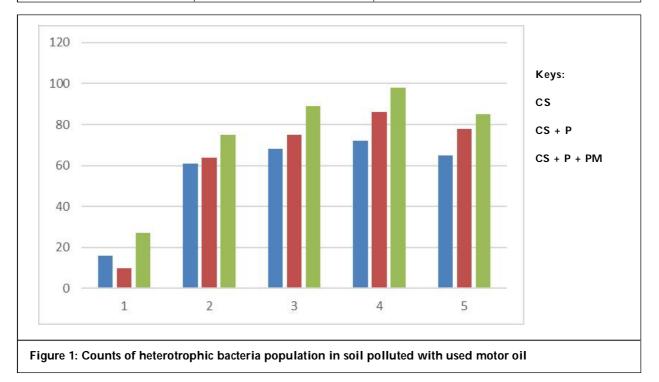
## 3.1. Physicochemical properties of soil and used motor oil

The physicochemical properties of soil and used motor oil used in this study are shown in Table 1. The soil was found to be acidic (pH 5.9). The nitrogen and organic carbon contents were 4.34 and 0.7222, respectively. The percentage of organic carbon of the soil is low, hence the need for the addition of organic manure to enhance the remediation process. The poultry manure also supplied additional nitrogen and phosphorus needed for bioremediation. Nitrogen is the most important limiting nutrient in bioremediation (Kim *et al.* 2005; and Frederic *et al.* 2005). The used motor oil has an acidic pH (5.96) and this may account for its toxicity.

## 3.2. Total Heterotrophic Bacteria (THB)

Figure 1 shows the aerobic THB count. The highest THB ((76.8 ×  $10^{\circ}$ cfu/g) was recorded in the polluted soil amended with poultry manure. This is followed by contaminated soil + plant ( $62.6 \times 10^{\circ}$ cfu/g), and then contaminated soil only ( $56.4 \times 10^{\circ}$ cfu/g). The treatments had a steady increase in THB up to the fourth week. However, towards the end of the study, the bacteria population dropped in all the treatments. Onuoha *et al.* (2014) observed that the drop in THB might be due to the decline in the readily metabolizable component of the hydrocarbon for the organisms. The high THB count in samples amended with poultry manure could be a

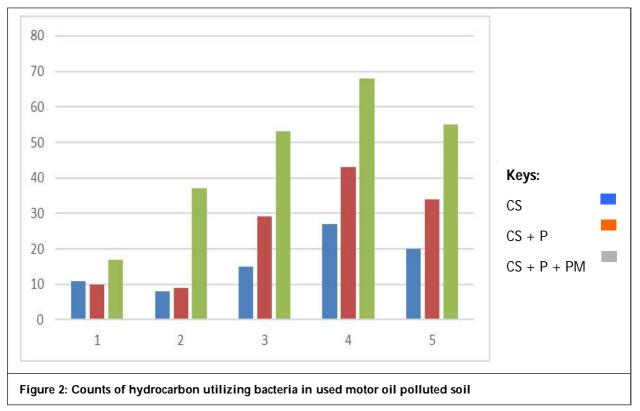
Table 1: Physicochemical properties of soil and used motor oil			
Parameter	Soil	Used motor oil	
рН	5.9	5.96 (at 27 °C)	
Nitrogen	4.34	-	
Organic Carbon	0.72222	-	
Sand %	86.24	-	
Clay %	5.82	-	
Silt %	7.88	-	
Potassium	0.02	-	
Specific gravity	-	0.9343	
Kinematic viscosity	-	1.46 × 10 <sup>-5</sup> at 76.14 s	
Flashpoint	-	220 °C	



result of high nitrogen content in the poultry manure which enhanced the proliferation of bacteria cells in the soil (ljah and Antai, 2003; Joo *et al.*, 2001, 2007; and Adesodun and Mbagwu, 2008). The low value of THB recorded in the contaminated soil without amendment could be due to the toxicity of the used motor on the soil bacteria (Odokuma and Dickson, 2003).

# 3.3 Total Hydrocarbon Utilizing Bacteria (HUB)

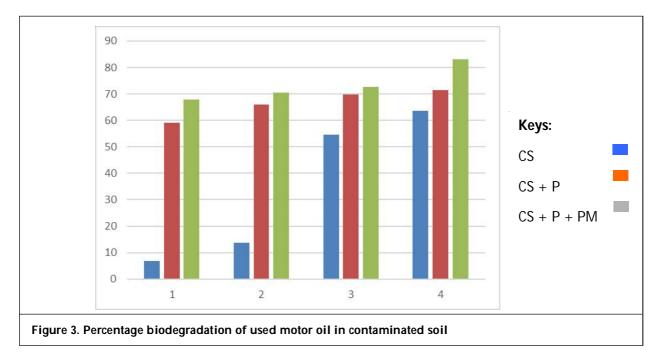
The total HUB count is shown in Figure 2. The result reveals that the total HUB in contaminated soil only (control) initially was  $11.2 \times 10^6$  cfu/g which later dropped to  $8.0 \times 10^6$  cfu/g in the second week and rose to  $15.0 \times 10^6$  cfu/g in the third week. The same phenomenon occurred in contaminated soil + plant while contaminated soil + plant + poultry manure had a steady increase. Generally, the highest HUB count was recorded in the contaminated soil containing poultry manure and cowpea plant. The higher HUB counts in



contaminated soil samples amended with poultry manure have been attributed to high nitrogen content in the organic waste (Adesodun and Mbagwu, 2008). Other authors have reported dramatic changes in microbial populations following the addition of different organic nutrients to hydrocarbon contaminated soil (Ting *et al*, 1999, and Vasudevan and Rajaram, 2001).

## 3.4. Biodegradation of used spent motor oil

Results of the biodegradation experiment reveal that there was a marked reduction in the total hydrocarbon content (THC) within the period of the study (Figure 3). It was also observed that the rate of biodegradation of used motor oil increased with time. The highest percentage of THC (83%) was observed in the contaminated soil with plant and poultry manure. The reduction of THC followed this order; CS < CS + P < CS + P + PM. Other researchers have also reported increases in biodegradation of used motor oil using organic manure



(Abioye et al., 2012; and Onuoha et al., 2011). Okolo et al. (2005) reported increased degradation of spent oil in soil augmented with poultry manure while Mbah et al. (2009) reported that the amendment of spent oil-contaminated soil with organic wastes led to improved soil physical properties and increased agronomic parameters of such soil.

Davies and Wilson (2005) reported that soil amendments improve the physical properties of such soil like water retention, water permeability, aeration and structure of the soil, this will lead to more degradation on remediating polluted soil. The addition of poultry manure helped to improve plant growth thereby enhancing the uptake of the hydrocarbon by the plant. Also, it is important to note that the observed reduction in spent oil or THC may not only be due to the biodegradation process induced by plant or nutrient addition, but other processes such as volatilization, adsorption to organic compounds, other abiotic factors are equally implicated in the reduction process. This is the case in the control sample where there was up to a 63.6% reduction in THC without any plant or organic waste amendment.

#### 3.5. Growth characteristics of Vigna unguiculata plant

There was a general improvement in the growth characteristics of the cowpea plant in the contaminated soil amended with poultry manure compared with the one without amendment. Results of the agronomic parameters (leaf length, leaf width, number of leaves, petiole length and plant height), show that the cowpea plant performed best in the uncontaminated soil, followed by the contaminated soil supplemented with poultry manure (Table 2). Mbah et al. (2009) reported that the amendment of spent oil-contaminated soil with organic wastes led to improved soil physical properties and increased agronomic parameters of such soil. The plant height significantly reduced in the plant in polluted soil without amendment. This could be attributed to nutrient deficiency needed to maintain the physiological processes involved in plant growth caused by nutrient stress due to the influence of the oil. The organic waste amendment would have reduced this effect according to (Onwusiri et al., 2017), who said that spent oil causes deficiency of available nutrients needed to maintain growth especially at apical regions of the crops if not amended. The poor growth observed in the plant in polluted soil without amendment when compared with the amended polluted soil and the control soil sample. This may be due to the inability of the plants in the polluted medium without organic manure to absorb the nutrient from the soil due to poor insulation and poor functioning of vascular bundles (phloem and xylem) (Edem et al., 2009). The unamended polluted soil had the least number of leaves which according to Jung (2008), may have been caused by the process of heavy metals in the spent oil which could distort within the plant tissues. The leaf is site photosynthesis, the spent oil caused also a significant reduction in leaf length and width in the amended and unamended polluted soils when compared within the control. The reduction in leaf length and width brings about a consequent reduction in surface area of leaf available for photosynthesis, hence reducing photosynthetic activities (Smith et al., 1989).

Growth parameter	Unpolluted soil + Plant	Polluted soil + Plant	Polluted soil + Plant + Poultry manure	
Leaf length	3.2	2.2	2.8	
Leaf width	2.4	1.7	2.1	
Number of leaves	9	6	8	
Petiole length	2.2	1.5	2	
Plant height	19	10	15	

Table 2: Effect of poultry manure on growth characteristics of *Vigna unguiculata* (cowpea) plant in used motor oil polluted soil

## 4. Conclusion

Phytoremediation has been known as a suitable alternative in the remediation of contaminated sites. The cowpea plant (*Vigna unguiculata*) has shown great potential in the degradation of used motor oil. Augmentation of soil with poultry manure was significantly beneficial in creating the optimum conditions for the plants to grow and microbial activities to thrive, thereby making phytoremediation a success. However, *Vigna unguiculata* 

was able to tolerate the spent motor oil in the soil without organic manure addition but the degradation was not up to that of the amended polluted soil. Thus, *Vigna unguiculata* with poultry manure amendment can be recommended for phytoremediation of spent motor oil-polluted sites.

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