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# Organic Manure Enhanced Phytoremediation of Crude Oil Contaminated Soils in The Niger Delta: The Potentials of Cowpea (*Vigna Unguiculata*)

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**ABSTRACT:** *In spite of frequent oil pollution, a cost effective, environmentally friendly and sustainable means of remediating polluted soils is yet to be discovered in the Niger Delta. This study, examined the effect of two rates of poultry manure (0 and 20 t/ha) remediating 0 and 5% oil in a 2x2x3 factorial, using cowpea (Vigna unguiculata) as possible phytoremediation plant. Two weeks after contamination, Total Petroleum Hydrocarbon (TPH) increased significantly (P<0.05) from 0.02 mgkg$^{-1}$ to 125.1 mg/kg$^{-1}$ and at eight weeks after remediation, TPH reduced from125.1 mgkg$^{-1}$ to 91.86 mgkg$^{-1}$ in the unamended contaminated soil and the poultry manure amended contaminated soil decreased significantly (P<0.05) to 73.08 mgkg$^{-1}$. Soil pH increased significantly (P<0.05) from 5.34 to 5.80, two weeks after contamination and after eight-week of remediation, pH in the unamended contaminated soil increased to 5.85 and the amended contaminated soil increased to 5.97. Organic carbon increased significantly (P<0.05) from 1.15% to 2.32% after two weeks of contamination and eight weeks after remediation, increased to 2.45% in the unamended contaminated soil, and to 2.58% in the amended contaminated soil. Though oil contamination slowed germination, cowpea germination in the unamended contaminated soil recorded 60% while poultry manure amendment increased germination to only 66% indicating cowpea has phytoremediation qualities. However, poultry manure amendment increased cowpea germination energy. The study recorded no significant difference in cowpea germination percentage and growth parameters between the unamended contaminated soil and remediated oil contaminated soil indicating poultry manure application rate need to be increased.*

**KEYWORDS:** Phytoremediation, cowpea, organic manure, crude oil, pollution.

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## INTRODUCTION

Petroleum Hydrocarbon contamination caused by crude oil is the most prevalent problem in the environment due to the widespread occurrences of spills and the risks it poses to human life and the environment. As a result of yearly exploration, production and refining of crude oil, thousands of barrels of crude oil are spilled annually into the environment through pipelines and tanks (Enegide and Chukuma, 2018). These spills occur as a result of lack of regular maintenance of pipelines or deliberate vandalism (Nwilo and Badejo, 2006). Introduction of crude oil into the soil results in an alteration of soil physical, chemical, and biological properties (Agbogidi and Ejemete, 2005). This ultimately leads to inhibition of seed germination (Pena-Castro et al. 2006) and plant growth (Bamidele and Igiri, 2011).

Governments, private companies, and oil companies operating in the region find it difficult to effectively clean up these soils as most of the existing conventional remediation methods such as soil washing, vacuum extraction, and chemical oxidation among others are expensive and require high technical expertise. These methods also move the pollution to a secondary phase such as air pollution, thereby causing further disturbance to the already damaged environment, especially if the remediation is done ex-situ (Khalid et al, 2017).

Phytoremediation is an emerging technology which uses plants to clean up contaminants in the environment. It is a natural, environment friendly method and is also more cost effective than other existing techniques. Using phytoremediation as a clean-up option may not only degrade contaminants but could also enhance the recovery of habitat through the stimulation of vigorous vegetative plant growth (Lee et al. 2001). Several studies have consistently demonstrated that some plant species especially grasses and legumes have the potential to degrade petroleum hydrocarbons in soil. (Godwin and Peter, 2014; Akpokodje et al. 2019). However, only few phytoremediation plants are known, and the effect of organic manure on the phytoremediation abilities of these plants has not been widely studied.

The objective of this study was to determine if cowpea (*Vigna unguiculata)* possesses any phytoremediation ability and to evaluate the effect of organic manure on the crop performance and phytoremediation ability.

## MATERIALS AND METHODS

**Experimental site:**
The experiment was carried out in the screenhouse of the Niger Delta University Teaching and Research Farm located in Amassoma community of Southern Ijaw Local government in Bayelsa state, Nigeria which lies between latitude 4°58'52.8" and longitude N.6°06'27.2"E

Five planting bags of size 20 cm x 15 cm x 13 cm (Height x Top diameter x Bottom diameter) were filled with 2 kg of soil and labelled as follows: (A) Soil two weeks after contamination, (B) Contaminated soil + Cowpea, (C) Contaminated soil + Poultry Manure + Cowpea, (D) Uncontaminated soil + cowpea, (E)Uncontaminated soil + Poultry Manure + cowpea.

A simple 2x2x3 factorial experiment fitted into Randomized Complete Block Design (RCBD) was used in the study. The factors were crude oil contamination at 0 and 5% levels and poultry manure at 0 t/ha and 20 t/ha replicated three (3) times with cowpea as the phytoremediation plant. The bags labelled B to E were arranged at random and each planted with 10 seeds of cowpea. Blocking was achieved by changing the position of the bags to ensure that they all receive sunlight. The percent germination was recorded and after germination was completed, seedlings were thinned to three seedlings per bag to avoid overcrowding. The soils were watered at 3-day intervals and plant growth measurements were taken for eight weeks, after which the plants were carefully harvested, oven dried, and weighed for biomass weight. Soil samples were collected from each bag and air dried, crushed with a mortar and pestle, and sieved using a 2mm mesh sieve after which they were taken to the laboratory for various physical and chemical analysis.

**Soil Analyses:**
Particle Size Analysis was carried out using the Bouyoucous hydrometer method (Gee & Or, 2002), while soil pH was determined using 1:1 ratio using…….. Soil organic carbon content was determined using the Walkey-Black wet oxidation method as reported by Udo et al. (2009), while soil organic matter (SOM) was determined by adopting the Van Bem-melen's factor of multiplying organic carbon values by 1.724 (Pribyl, 2010). Total Nitrogen (N) was determined by the Kjeldahl method, as described by Udo et al. (2009). Available phosphorus (P) was determined by use of Bray P-1 method of Bray and Kurtz (1945) as described by (Udo et al., 2009). Exchangeable bases were extracted using 1NH4OAc, Ca++ and Mg++ were analyzed using PGI 990 Atomic Absorption Spectrophotometer (PG Instruments Ltd, UK) while K+ and Na+ were determined using ATS 200S Flame Photometer (ATS-Technology, Cyprus). Exchangeable Acidity was determined by leaching the soil with 1N KCl solution and titrating the extract with standard NaOH solution (Udo et al., 2009). The Effective cation exchange capacity (ECEC) was determined by summing up the exchangeable cations plus exchangeable acidity. Total petroleum Hydrocarbon (TPH) was determined using a spectrophotometer according to the method described by (Osuji et al., 2006).

**Statistical Analysis:**
Analysis of variance (ANOVA) was carried out on the data collected and significant means were separated using tukey test at 5% level of probability. Results were presented as mean ± SD using Charts and Tables.

## RESULTS AND DISCUSSION

The physico-chemical properties of the soil sample are as presented in Table 1.

**Table 1:** Physico-chemical properties of the soil used in the study.

| Parameters | Unit | Value |
|---|---|---|
| Total Petroleum Hydrocarbon (TPH) | mg/kg | 0.02 |
| pH ($H_2O$) | | 5.34 |
| Organic Carbon (C) | % | 1.15 |
| Nitrogen (N) | % | 0.05 |
| C/N | | 22.11 |
| Available Phosphorus | mg/kg | 5.69 |
| Calcium (Ca) | cmol/kg | 0.72 |
| Magnesium (Mg) | cmol/kg | 0.63 |
| Potassium (K) | cmol/kg | 0.56 |
| Sodium (Na) | cmol/kg | 0.05 |
| Exchangeable Acidity | cmol/kg | 1.61 |

| | | |
|---|---|---|
| Total Exchangeable Bases | cmol/kg | 1.96 |
| ECEC | cmol/kg | 3.56 |
| Base Saturation | % | 55.73 |
| Sand | % | 24.33 |
| Silt | % | 9.95 |
| Clay | % | 65.72 |
| Textural Class | | Clay |

**Impact of organic manure enhanced phytoremediation of crude oil contamination on soil chemical properties**

A drastic increase in Total Petroleum Hydrocarbon (TPH) concentration, two weeks after contamination was observed which was attributed to the presence of hydrocarbon compounds in crude oil as previously reported by Udeh et al. (2013) and Godwin and Peter (2014). On the addition of poultry manure (PM), the TPH removal efficiency in the soil amended with PM was higher (41.5%) compared to the unamended soils' 26.5% (Figure .1). This was due to enhanced availability of nutrients for plants and microorganisms and improvement of other soil conditions. The reduction could also be as a result of acclimatization of microorganisms to the crude oil contaminants which enabled them to utilize hydrocarbon compounds.

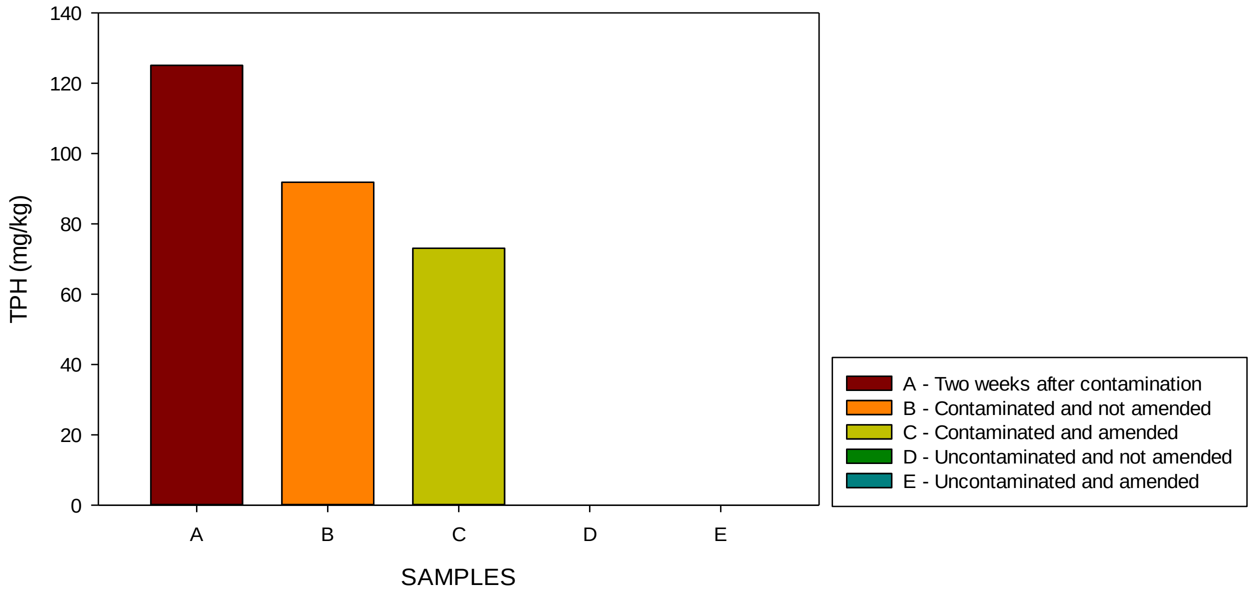


**Figure 1:** Total Petroleum Hydrocarbon content of treatments

The soil pH increased significantly ($P<0.05$) after contamination with crude oil. The pH of the soil treated with PM was higher than the untreated after the eight-week remediation period (Table 2). In the explanation of Kayode et al. (2009) who recorded similar result, sulphur and vanadium compounds present in crude oil in trace amounts could increase the rate of chemical oxidation and alter redox potentials of contaminated soils, resulting in increase in the pH. Uquetan et al. (2017), on the other hand, attributed the increase to increase in the accumulation of exchangeable bases in the oil polluted soils which further affects the ionic stability in the soil, nutrient availability and uptake by plants.

The percentage organic carbon in the soil also increased markedly as TPH concentrations increased (Table 2). This agreed with the findings of Wang et al. (2013) who recorded higher organic carbon content in marsh soils contaminated by crude oil when compared to the control soil. Nitrogen on the other hand, was reduced by almost 40% in the contaminated soils (Table 2), resulting in a high C/N ratio (78%) in the soil which implied reduced degradation of organic matter (Aislabie et al. 2004). Organic carbon increased in the remediated treatments when compared to initial values at two weeks after remediation. However, the soil treated with poultry manure had higher organic carbon values due to additional carbon supplied by the poultry manure. In the uncontaminated soil, organic carbon increased in both treatments and was higher in the amended soil.

Nitrogen values were higher after remediation, increasing to the control level in the untreated soil and surpassing it in the treated soils. Nitrogen concentration in the uncontaminated soils were even higher because the soil N was not depressed by crude oil, and the cowpea plant was able to fix nitrogen. The increasing trend of nitrogen helped to reduce the C/N ratio, and C/N was lower in the treated soil (35:1) but was still higher than the recommended ratio of 24:1 for agricultural soils. This means there was degradation of organic matter but organic matter was much higher than that of the soil that was not treated with manure. In the uncontaminated soils which had lower carbon content but higher nitrogen, the C/N ratio fell slightly below the recommended value (Table 2).

The concentration of available P in the oil contaminated samples decreased by 50.7% as total petroleum hydrocarbon (TPH) concentration and the soil pH increased (Table 2). Kayode et al. (2009) reported that the decrease in available P may be due to the conversion of $H_2PO_4^-$ (most available form of phosphorus) to $HPO_4^{2-}$ (less available form for plants uptake) and then to $PO_4^{3-}$ as the soil pH increased. Eneje et al. 2012 similarly recorded a decrease in available P concentration by as much as 66% as a crude oil concentration of 30mg/kg. When measured after remediation, available P was higher in the treated soils due to the input from PM.

**Table 2:** Soil nutrient and chemical properties before and after remediation

| Treatments | pH (H2O) | Org C (%) | N (%) | C/N | Avail. P (mg/kg) |
|---|---|---|---|---|---|
| A | 5.80a | 2.32abc | 0.03c | 77.44a | 2.80a |
| B | 5.85a | 2.43ab | 0.05bc | 48.60a | 3.97a |
| C | 5.77ab | 2.58a | 0.07bc | 36.86b | 4.28a |
| D | 5.32c | 1.31c | 0.08ab | 16.41b | 5.65a |
| E | 5.39bc | 1.53bc | 0.11a | 13.94b | 5.81a |

**KEY:** A – Two weeks after contamination, B – Contaminated but not amended, C – Contaminated and amended. D – Uncontaminated but not amended, E – Uncontaminated and amended

The initial calcium content of the soil was low (0.72cmol/kg) when compared with FAO, (2006) ratings for Ca++, potassium was medium (0.56cmol/kg) while magnesium content (0.069cmol/kg) fell within the medium range when compared to the critical value of 0.3-0.1 cmol/kg by Esu, (1991). The availability of all exchangeable bases in the soil further reduced after the soil was contaminated with crude oil (Table. 3). Calcium became even lower at 0.39 cmol/kg, potassium dropped from medium to low (0.23cmol/kg), magnesium remained at a medium level although it was barely above the critical limit at 0.039cmol/kg. Uquetan et al. (2017) also recorded a similar pattern of decrease in exchangeable bases.

Addition of poultry manure increased the concentration of exchangeable bases as compared to the untreated soil but was not able to raise it to the control level. The rating of potassium however increased from medium to high following the FAO (2006) ratings while all others remained in the former rating. In the uncontaminated treatments, exchangeable bases increased more in concentration in both the treated and untreated soils (Table. 3). The potassium rating increased from medium to high following the FAO (2006) ratings for $K^+$ while all others remained the same but recorded higher values.

**Table 3:** Exchangeable bases before and after remediation

**Table 3:** Exchangeable bases before and after remediation

| Treatments | Ca (cmol/kg) | Mg (cmol/kg) | Na (cmol/kg) | K (cmol/kg) |
|---|---|---|---|---|
| A | 0.33b | 0.39c | 0.03b | 0.27b |
| B | 0.38b | 0.42b | 0.04b | 0.3b |
| C | 0.47b | 0.49bc | 0.05ab | 0.39b |
| D | 0.76a | 0.69ab | 0.05ab | 0.65a |
| E | 0.87a | 0.71a | 0.06a | 0.69a |

**KEY:** A – Two weeks after contamination, B – Contaminated but not amended, C – Contaminated and amended. D – Uncontaminated but not amended, E – Uncontaminated and amended

**Impact of organic manure enhanced phytoremediation of crude oil contamination on Seed Germination and Plant Growth Parameters**

Oil contamination significantly reduced (P<0.05) the rate of germination of cowpea seedlings in the contaminated samples as compared to the uncontaminated samples (Table 4) in line with the report of Bamidele and Igiri, (2011) who also recorded significant reduction in seed germination and biomass production due to crude oil contamination. In this case, the rate of germination of the seeds in the contaminated soil amended with poultry droppings was higher than that of the soil that was contaminated but not amended, implying that the addition of organic manure boosted seedling germination in the contaminated soil. Germination energy was measured four days after planting to determine the percentage of fast germinating seeds (Marek et al. 2019). When compared with the uncontaminated samples, it was observed that crude oil contamination greatly delayed germination (Table 4). However, germination of cowpea (Table 4) showed that cowpea has potentials as a plant for phytoremediation of oil contaminated soils as cowpea germination in the contaminated soil without remediation recorded 60% as compared to 66% in the oil contaminated but remediated soil. It is possible that increasing the poultry manure level in the contaminated soil could improve cowpeas' seedling germination and its' potential in phytoremediation.

Oil contamination also reduced plant height, root length, leaf area, number of leaves, and biomass (Table 4). A similar trend was reported by Lale et al. (2014), who recorded reduced plant height, laminar leaf area, number of leaves and shoot dry weight of cowpea plant cultivated in a soil contaminated by spent lubricating oil. All measurements in the contaminated treatment were significantly reduced ($P<0.05$) due to reduced nitrogen and oxygen levels and limited water availability to plants caused by crude oil contamination. This probably led to the death of most of the plants in the contaminated soils after the eight-week remediation period.
However, higher mean values were recorded in the soils amended with poultry droppings in both contaminated and uncontaminated soils. This is supported by the reports of Hou et al. (2012) and Steiner et al. (2007) who reported that addition of organic manure improved nutrient availability in the soil which in turn improved plant growth. Improved crop growth in the soils contaminated and amended with poultry manure was also responsible for the higher TPH removal efficiency.

**Table 4:** Plant Germination and growth in the different treatments

| **Treatments** | **Germination (%)** | **GE** | **Shoot Height (cm)** | **Root Length (cm)** | **Leaf Area (cm2)** | **Number of Leaves** | **Biomass** |
|---|---|---|---|---|---|---|---|
| B | 60.00b | 0.00b | 14.83b | 9.06c | 10.50c | 14.30b | 3.42c |
| C | 66.67b | 13.33b | 17.84b | 10.60c | 13.19c | 16.77b | 4.60b |
| D | 100.00a | 76.67a | 30.73a | 16.45b | 27.69b | 29.80a | 7.70a |
| E | 100.00a | 80.00a | 35.47a | 20.35a | 41.74a | 36.90a | 8.63a |

**KEY:** B – Contaminated but not amended, C – Contaminated and amended. D – Uncontaminated but not amended, E – Uncontaminated and amended

## Potentials of cowpea in phytoremediation

The fact there was no significant difference in the cowpea shoot length, root length, plant height, leaf area and number of leaves in the contaminated soil without remediation and in the remediated oil contaminated soil, indicated that cowpea has potential in the phytoremediation of oil contaminated soils. It might be necessary to increase the poultry manure dosage to improve cowpea performance in phytoremediation.

## CONCLUSION

The results showed that contamination of soil by crude oil has adverse effects on soil properties and plant growth. Crude oil contamination astronomically raised the TPH content of the soil, which led to increase in soil pH. It further led to increase in organic matter content, decrease in nitrogen fixation, and decrease in phosphorus availability. Exchangeable cations were also found to be less available after contamination by crude oil. Crude oil contamination also had adverse effect on all plant parameters measured; germination, shoot height, leaf area, number of leave produced, and plant biomass were all significantly reduced when compared with control levels after contamination.

Planting of cowpea in the contaminated soil contributed to reduction of TPH levels which was further enhanced by addition of poultry manure. The crops assisted with poultry manure recorded better germination and cowpea growth parameters. This implies that the phytoremediation potential of cowpea increased when it is aided by poultry manure. Since there was no significant difference between cowpea germination percentage, shoot length, root length, plant height, leaf area and number of leaves in the contaminated soil without remediation and in the remediated oil contaminated soil, the quantity of poultry manure applied is too low and need to be increased.

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