



# Capability of Vetiver (*Vetiveria Zizanioides*), Guinea Grass (*Panicum Maximum*) and Organic Manures to Remove Total Hydrocarbon and Heavy Metals from Crude Oil Contaminated Soil in Port Harcourt

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## **Authors' contributions**

*This work was carried out in collaboration with another author. Author JAC designed the study, performed the statistical analysis, wrote the protocol, carried out the analysis, wrote the first draft of the manuscript and bulk of the literature searches. Author OJK wrote part of the literature searches and part of the manuscript. All authors read and approved the final manuscript.*

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## **ABSTRACT**

In a field study conducted at the Teaching and Research Farm of Rivers State University PortHarcourt, Nigeria, to examine the ability of vetiver and guinea grasses amended with organic manures in remediation of crude oil polluted soil; soil polluted with Bonny light crude oil at 0 and 2% v/w was subjected to a remediation processes using vetiver (*Vetiveria zizanioides*) and Guinea grass (*Panicum maximum*) amended with organic manures for a period of twelve months. Two weeks after pollution, poultry and rabbit manures were applied at 0, 10, 20 and 30 tons per hectare respectively. Vetiver and guinea grasses were planted two weeks later. It was fitted in factorial split plots randomized block design. Results of the study revealed that remediation of the soil with grasses degraded the Total hydrocarbon content (THC) to 23 and 21.2% for vetiver and guinea grass respectively, while the quantity of heavy metals removed from the soil were 28.4% and 25.9% for iron, 37.3 and 32.8% for zinc, 35.8 and 30.8% for lead (Pb) and 23.1 and 38.5% cadmium for vetiver and guinea grass respectively. Amendment of the soil with 30 tons' /ha organic manures

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increased the quantity of THC degraded to 70.6% and 67.9% for vetiver and guinea grass respectively while the concentration/quantity of heavy metals removed from the soil increased to 58.4 and 54.7% for Fe, 66.9 and 65.1% for Zn, 69.4 and 66.6% for Pb and 80.8 and 71.2%, in Cd for vetiver and guinea grass respectively. The quantity and concentration of THC and heavy metals degraded in the soil was higher in vetiver than guinea grass plots and in vetiver amended with poultry manure than in guinea grass amended with rabbit manure. There was a significant ( $P < 0.05$ ) difference in uptake of THC and heavy metals in plant tissues between vetiver and guinea grass. Vetiver accumulated more of the contaminants in their tissues than guinea grass. Amendment of the soil with organic manures reduced the uptake of the contaminants (THC and heavy metals) from the plant tissues. Generally, both grasses have the capability to remediate crude oil contaminated soil. Augmentation of the two grasses with organic manures enhanced their capability.

*Keywords: Crude oil; THC; heavy metals; Vetiver; guinea grass; poultry; rabbit; manures.*

## 1. INTRODUCTION

The high demand of crude oil as raw material and source of energy has led to an increase in production, refining and transportation in petroleum industries. Production and transportation of this oil has brought about hardship and negative effects due to environmental pollution as a result of oil spills and gas flaring [1,2]. These impacts are seen in soil, plants, micro-organisms and human beings dwelling within the ecosystem [3].

Port Harcourt in Rivers State is situated at the heart of Niger Delta Region of Nigeria where intense petroleum activities are taking place and is regarded as one of the richest part of Nigeria in terms of natural resources which include large deposit of oil and gas as well as extensive forest, good agricultural land and abundant fishes.

Despite these numerous natural resources, the region is threatened by diverse environmental challenges of which crude oil pollution is major. From extraction to arrival at refinery, there are a number of potential pollution outlets and resultant contamination of environment, impairment of human health, pollution of water bodies and marine organisms [4].

It is important to note that crude oil contamination on soil is not itself toxic to plants rather has an indirect effect as it creates a condition that makes essential nutrients for plant growth unavailable and at the same time makes some nutrients that are toxic to plants available [5,6], inferred that spills from crude oil extraction contaminate soil ambient air, surface/ground water and crops with hydrocarbons and other known carcinogen like polycyclic aromatic hydrocarbon and benzo (a) pyrene naturally radioactive materials.

Petroleum hydrocarbon creates conditions which lead to unavailability of essential plant nutrients such as nitrogen and availability of some toxic elements such as heavy metals- arsenic, lead and iron to plants [7]. This makes the soil remain unsuitable for crop growth for months or years until the soil is degraded to a tolerable level [8].

When oil spill occurs either as a result of oil well blowout, pipeline leakages, spent drilling, mud or effluent water discharges, heavy metals are released into soil and mud water bodies. These heavy metals may have harmful effects on soil, crop and human health [9]. It is therefore necessary to remediate the soil to restore it back to its original or near original status.

Phytoremediation technique (use of Vetiver and Guinea grasses) amended with organic manures were adopted to remove, contain or render harmless environmental contaminants from the soil in this study [10,11]. Vetiver has the ability to grow well even in harsh condition as it establishes strong symbiotic association with wide range soil microbes in the rhizosphere that provide nutrients for plant development [11,12]. Guinea grass on the other hand is widely grown in most part of the country even under harsh condition. Both grasses have the ability to remediate polluted soils. They are environmentally friendly, are naturally occurring materials, cost effective [13], high biomass with enhanced metal uptake, very high metal accumulation capacity and fast growing.

There is very limited study with the use of these grasses amended with organic manures in Niger Delta Region of Nigeria. Therefore, the use of these grasses amended with organic manure in reducing the heavy metal contamination in crude oil and total hydrocarbon content from the soil and plants needs to be explored.

The objective of this study is (i) to examine the uptake capacity of these grasses to absorb and accumulate heavy metals especially lead (Pb), cadmium (Cd), zinc (Zn), and iron (Fe) and total hydrocarbon content in their tissues (ii) To reduce the concentration of total hydrocarbon content and that of heavy metals from crude oil contaminated soil.

## 2. MATERIALS AND METHODS

The study was carried out at Rivers State University, Nkpolu, PortHarcourt teaching and research farm. The site is situated at latitude  $4^{\circ} 51' N$  and longitude  $7^{\circ} 01' E$  with an elevation of 18m above sea level [14]. Mean annual rainfall ranges from 3000 to 4000mm [14]. Annual temperature varies between 22 to  $31^{\circ}C$  [15], while the relative humidity (RH) is between 35 to 90% depending on the particular period of the year.

### 2.1 Soil of the Study Site

The soil was from coastal plain sands geomorphic region. It is typically sandy loam (typic paleudult) formed over sedimentary rocks and belongs to the ultisol order of the United State Soil Taxonomy [16].

### 2.2 Sources of Crude Oil

Nigerian Bonny light crude oil (fresh) obtained from shell Petroleum Company Nigeria limited, Bayelsa State flow station was used and a concentration of 0 and 2% v/w was used in the studied area. Each of the experimental plots (3 x 4m) with exception of the control plot was treated with crude oil from a watering can; evenly sprayed and worked into the soil with garden fork.

### 2.3 Amendment Materials

Organic manures (Poultry and rabbit) were used as amendment materials. Poultry wastes are very common and rich in nutrients, while Rabbit wastes is also rich; the farming is growing and becoming popular in this area. Both (poultry and rabbit) wastes have immense potentials as soil amendment materials. These materials (organic manures) were applied unto the soils with the exception of control plots two weeks after contaminating the soil with crude oil. They were broadcast and worked into the soils at the rate of 0, 10, 20 and 30 tons per hectare, respectively.

### 2.4 Preparation and Planting of Plant Materials

The site of the study has been under continuous cultivation with different crops, the last being cassava and maize.

### 2.5 Experimental Design

The area was ploughed, harrowed with tractor, marked and pegged. Vetiver grass (*Vetiveria zizanioides*) and guinea grass (*Panicum maximum*) collected from National Root Crops Research Institute Umudike, Abia State and Rivers State University Teaching and Research Farm, respectively were planted two weeks after amendment materials were added at a spacing of 20 by 30cm and 30 by 30cm for vetiver and guinea grasses respectively. A total of 24 treatment combinations (Table 1) above were laid out in a factorial fitted into a split plot randomized complete block design with contaminated and uncontaminated as the main plots, other factors served as sub plots. All the treatments were replicated three times making a total of 72 plots.

### 2.6 Collection and Preparation of Soil Sample for Laboratory Analysis

Soil samples were randomly collected from a depth of 0-20cm from each of the plots with bucket auger. The soil samples were crushed with hands, composited and left to air-dry at a room temperature in the laboratory. The samples were later pulverized with mortar and pestle, sieved in a 2mm mesh screen and sent to the laboratory for the determination of the following parameters; zinc (Zn), iron (Fe), lead (Pb), Cd and total hydrocarbon (THC). The samples were collected before and after the experiment.

### 2.7 Laboratory Analysis

Soil sample for heavy metals (Zn, Fe, Pb and Cd) were extracted by wet digestion method of (17), 0.5g of soil samples were weighed into 50mls conical flask, 10mls of nitric acid ( $HNO_3$ ) was added and heated to dampness in fume cupboard, the samples were removed and allowed to cool to  $25^{\circ}C$  room temperature before 10mls of perchloric acid ( $HClO_4$ ) was added to it. The samples were then removed from the fume cupboard, allowed to cool and made up to 50ml mark with distilled water.

**Table 1. Treatment combinations are as follows**

| Treatments | Code | Key   |
|------------|------|---|
| C0P0       | T1   | Control, planted with vetiver grass, no organic manures, no crude oil             |
| C0P2       | T2   | No crude oil, 20 tons/hectare poultry manure, vetiver planted                     |
| C0R0       | T3   | No crude oil, no rabbit manure, vetiver planted                                   |
| C0R2       | T4   | No crude oil, 20 tons per hectare rabbit manure, vetiver planted                  |
| C1P0       | T5   | Contaminated with crude oil, no poultry manure, vetiver planted                   |
| C1P1       | T6   | Contaminated with crude oil, amended with 10tons/ha poultry, vetiver planted      |
| C1P2       | T7   | Contaminated with crude oil, amended with 20tons/ha poultry, vetiver planted      |
| C1P3       | T8   | Contaminated with crude oil, amended with 30tons/ha poultry, vetiver planted      |
| C1R0       | T9   | Contaminated with crude oil, no rabbit manure, vetiver planted                    |
| C1R1       | T10  | Contaminated with crude oil, amended with 10tons/ha rabbit, vetiver planted       |
| C1R2       | T11  | Contaminated with crude oil, amended with 20tons/ha rabbit, vetiver planted       |
| C1R3       | T12  | Contaminated with crude oil, amended with 30tons/ha rabbit, vetiver planted       |
| C0P0       | T13  | No crude oil, no poultry manure, guinea grass planted                             |
| C0P2       | T14  | No crude oil, amended with 20tons/ha poultry, guinea grass planted                |
| C0R0       | T15  | No crude oil, no rabbit manure, planted with guinea grass                         |
| C0R2       | T16  | No crude oil, amended with 20tons/ha rabbit manure, guinea grass planted          |
| C1P0       | T17  | Contaminated with crude oil, no poultry manure, guinea grass planted              |
| C1P1       | T18  | Contaminated with crude oil, amended with 10tons/ha poultry, guinea grass planted |
| C1P2       | T19  | Crude oil contaminated, amended with 20tons/ha poultry, guinea grass planted      |
| C1P3       | T20  | Crude oil contaminated, amended with 30tons/ha poultry, guinea grass planted      |
| C1R0       | T21  | Crude oil contaminated, no rabbit manure, guinea grass planted                    |
| C1R1       | T22  | Crude oil contaminated, amended with 10tons/ha rabbit, guinea grass planted       |
| C1R2       | T23  | Crude oil contaminated, amended with 20tons/ha rabbit, guinea grass planted       |
| C1R3       | T24  | Crude oil contaminated, amended with 30tons/ha rabbit, guinea grass planted.      |

The digest was filtered with No 42 whatman filter paper into transparent plastic containers for Atomic Absorption Spectrophotometer (AAS) elemental reading. AAS variance spectr AA 220FS model was used to read the concentration values of (Zn, Fe, and Pb) in the soil digest following Horneck and Hanson method in [17]. The data generated were subjected to statistical analysis using Analysis of Variance (ANOVA). Duncan test was used to test the data.

The THC was estimated using the method of [18]. 10g portion of the soil sample was shaken with 10mls of carbon-tetrachloride. The total hydrocarbon content was extracted and determined by the absorbance of the extract at

420nm spectrophotometer. Standard curve of the absorbance of different known concentrations of equal amount of crude oil in the extract was first drawn after reading from the spectrometer.

### 3. RESULT AND DISCUSSION

Total hydrocarbon content (THC) of the soils is as presented in Table 2 below, THC of the soils ranged from 0.017 to 5472.2µg/g with 5472.6µg/g as initial value (after contamination without treatments), in both vetiver and guinea grass plots while the lowest values were obtained in uncontaminated plots. Similar trend in results were obtained in the four heavy metals (Fe, Zn, Pb and Cd) investigated.

**Table 2. Mean THC ( $\mu\text{g/g}$ ) and heavy metals content ( $\text{mg/kg}$ ) in soil remediated with *Vetiver* & *Guinea grass***

| Para Meter | Initial value | Grasses   | Treat ments |       |       |        |       |        |        |        |       |        |       |        |
|------------|---------------|-----------|-------------|-------|-------|--------|-------|--------|--------|--------|-------|--------|-------|--------|
|            |               |           | COP0        | COP2  | COR0  | COR2   | C1P0  | C1P1   | C1P2   | C1P3   | C1R0  | C1R1   | C1R2  | C1R3   |
| THC        | 5472.6        | Vetiver   | 0.034       | 0.017 | 0.032 | 0.018  | 4220  | 2593   | 2124.4 | 1610.3 | 4221  | 2830   | 2521  | 2227.9 |
|            | 5472.6        | Guinea Gr | 0.038       | 0.03  | 0.034 | 0.03   | 4232  | 2717   | 2300   | 1754.8 | 4224  | 2851.8 | 2612  | 2312.4 |
| Fe         | 362.34        | Vetiver   | 138.9       | 113.6 | 136   | 118.27 | 259.5 | 200.18 | 179    | 148.9  | 260.1 | 205.6  | 192.3 | 156.4  |
|            |               | Guinea Gr | 140.6       | 120.6 | 139.1 | 120.3  | 265   | 214.22 | 197.2  | 162    | 265   | 210.5  | 196.7 | 160.2  |
| Zn         | 29.1          | Vetiver   | 9.14        | 5.25  | 9.16  | 4.94   | 18.25 | 13.9   | 12.1   | 9.64   | 18.15 | 13.1   | 12.12 | 10.1   |
|            |               | Guinea Gr | 9.7         | 5.75  | 9.13  | 5.2    | 18.7  | 14.5   | 12.5   | 10.1   | 19.2  | 13.6   | 12.5  | 10.4   |
| Cd         | 0.52          | Vetiver   | 0.15        | 0.08  | 0.14  | 0.08   | 0.4   | 0.17   | 0.12   | 0.10   | 0.41  | 0.16   | 0.12  | 0.088  |
|            |               | Guinea Gr | 0.15        | 0.083 | 0.13  | 0.07   | 0.43  | 0.20   | 0.12   | 0.10   | 0.40  | 0.18   | 0.13  | 0.090  |
| Pb         | 19.88         | Vetiver   | 5.68        | 3.4   | 5.65  | 3.4    | 12.76 | 10.03  | 8.0    | 7.6    | 12.92 | 9.9    | 7.1   | 6.2    |
|            | 19.88         | Guinea Gr | 6.0         | 3.70  | 6.1   | 3.55   | 14.45 | 9.7    | 8.35   | 7.8    | 14.8  | 9.8    | 7.2   | 6.35   |

The variation of Total hydrocarbon content (THC) due to treatments is shown in Table 3 below. A significant ( $P < 0.05$ ) difference was observed between the treatments and the two grasses (vetiver and guinea) with vetiver remediated plots amended with 30 tons/hectare (C1P3) having the highest mean of ( $3882.3 \pm 0.06^a$ ) in contrast to guinea grass remediated plot with the same treatment mean of ( $3173.11 \pm 0.06^b$ ); thus indicating that vetiver grass has higher capability of degrading crude oil contaminated soil than guinea grass. This agrees with the reports of [19,20,21] who noted that plants enhanced crude oil remediation.

Significant ( $P < 0.05$ ) difference was observed between poultry and rabbit manures with vetiver remediated plots amended with 20 tons' poultry manure (C1P2) having a mean value of ( $3348.2 \pm 4.72^a$ ) against a mean value of  $2978.13 \pm 3.72^b$  observed in vetiver remediated plots amended with same level of rabbit manure. This showed that hydrocarbon degradation was higher in vetiver planted plots amended with poultry manure than those amended with rabbit manure.

Amendment of the soil with organic manures (poultry and rabbit) enhanced the ability of vetiver and guinea grasses to degrade the contaminants. This is in line with the observation of [22] that fertilization is necessary for enhanced hydrocarbon degradation rates in polluted soils. The reason being that adequate fertilization helped to reduce competition between plants and micro-organisms.

The percentage of THC degraded ranged from 23 to 70.6% from contaminated unamended (C1P0) sample to contaminated amended with 30 tons/hectare of poultry manure in vetiver remediated plots and 21.2% from contaminated unamended (C1P0) to 67.9% on contaminated amended with 30 tons /ha of poultry manure respectively in guinea grass remediated plots (Table 4).

The high rate of degradation of THC in soil as observed from the study could be due to the presence of plants (vetiver and guinea grasses) and organic manures which enhanced their ability and the degrading action of micro-organisms that utilizes hydrocarbon as energy source in their tissues over a given time. This observation agrees with the report of [23,24] that

recognized in their studies the relevance of plants in crude oil degradation in soil. [25] Reported a decrease in crude oil and an increase in soil recovery after a given time with the help of crude oil degrading organisms.

There was no significant ( $P > 0.05$ ) difference between vetiver and guinea grasses in control (C0P0) plots in Total Hydrocarbon Content (THC) uptake in plant tissues as shown in Table 5. While a significant ( $P < 0.05$ ) difference were observed in contaminated unamended (C1P0) plots with mean value of  $878.33 \pm 0.41^a$  in vetiver planted plots against the contaminated unamended (C1P0) plots with a mean value of  $846.10 \pm 0.06^b$  in guinea grass plots. This result showed that the uptake of THC in vetiver tissues was higher than that of guinea grass.

The increase in the rate of absorption of THC in plant tissues could probably be due to the fact that addition of vegetation to contaminated system influenced the rate of contaminants removal. This corroborates with the findings of [26,24] who reported that hydrocarbon depending on their chemical properties may be absorbed by plant roots and shoots and accumulate in plant tissues, volatilize or metabolize by plants. [24] Observed that contaminant dissipation is enhanced by plant roots.

Result of the study revealed that the concentration of iron in the soil increased from control C0P0 (138.9) and C0P0 140.6kg/mg) to C1P0 (259.5) and 265.0kg/mg) for contaminated unamended with poultry manure in vetiver and guinea grass cultivated plots respectively, thus implying that contamination of the soil with crude oil increased the concentration of iron in the soil. This observation tallies with those of [27,28] who reported an increase in iron content in a crude oil contaminated soil.

A significant ( $P < 0.05$ ) increase was observed between vetiver remediated plots amended with 20 tons of poultry manure C1P2 ( $182.4 \pm 1.10^a$ ) against C1P2 ( $165.3 \pm 1.90^b$ ) of guinea grass indicating that vetiver grass has higher capability to reduce concentration of iron in crude oil contaminated soil than guinea grass.

Similarly, a significant ( $P < 0.05$ ) difference was recorded between the treatments and uptake of iron in the tissues of the two grasses with vetiver having higher uptake values  $51.2 \pm 0.69^i$  in control (C0P0) and  $151.30 \pm 0.17^a$  in contaminated

**Table 3. Quantity of THC (µg/g) degraded and heavy metals (mg/kg) removed in the soil during the period of the study**

|                  |      | <b>THC (ug/g)</b>             | <b>Fe</b>                   | <b>Zn</b>                 | <b>Pb</b>                   | <b>Cd</b>                  |
|------------------|------|-------------------------------|-----------------------------|---------------------------|-----------------------------|----------------------------|
| <i>Vetiver</i>   | C0P0 | 0.034±0.21 <sup>e</sup>       | 128.9±2.02 <sup>c</sup>     | 8.0±1.01 <sup>d</sup>     | 4.68±0.20 <sup>d</sup>      | 0.15±0.12 <sup>d</sup>     |
|                  | CIPO | 1252.0 ± 1.16 <sup>d</sup>    | 102.8 ± 1.16 <sup>c</sup>   | 10.9 ± 1.00 <sup>c</sup>  | 7.1 ± 2.01 <sup>c</sup>     | 0.12 ± 0.01 <sup>c</sup>   |
|                  | CIPI | 2879.2 ± 5.78 <sup>b</sup>    | 162.2 ± 1.16 <sup>b</sup>   | 15.2 ± 0.00 <sup>b</sup>  | 10.2 ± 0.12 <sup>b</sup>    | 0.35 ± 0.03 <sup>b</sup>   |
|                  | CIP2 | 3348.2 ± 4.72 <sup>a</sup>    | 182.4 ± 1.10 <sup>a</sup>   | 17.0 ± 0.01 <sup>b</sup>  | 11.9 ± 0.33 <sup>a</sup>    | 0.40 ± 0.02 <sup>a</sup>   |
|                  | CIP3 | 3882.3 ± 1.16 <sup>a</sup>    | 213.4 ± 0.12 <sup>a</sup>   | 19.5 ± 0.01 <sup>a</sup>  | 12.3 ± 0.02 <sup>a</sup>    | 0.42 ± 0.01 <sup>a</sup>   |
|                  | CIR1 | 2721.0 ± 5.77 <sup>c</sup>    | 156.9 ± 1.73 <sup>b</sup>   | 16.0 ± 0.10 <sup>b</sup>  | 9.9 ± 1.16 <sup>b</sup>     | 0.36 ± 0.02 <sup>b</sup>   |
|                  | CIR2 | 2978.13 ± 264.72 <sup>b</sup> | 170.0 ± 5.77 <sup>b</sup>   | 15.9 ± 0.20 <sup>b</sup>  | 11.8 ± 1.56 <sup>a</sup>    | 0.40 ± 0.03 <sup>a</sup>   |
|                  | CIR3 | 3533.7 ± 0.12 <sup>a</sup>    | 205.4 ± 12.70 <sup>a</sup>  | 19.5 ± 1.07 <sup>a</sup>  | 12.0 ± 1.73 <sup>a</sup>    | 0.43 ± 0.02 <sup>a</sup>   |
|                  | C0P0 | 0.038.0±0.10 <sup>e</sup>     | 130.2±0.12 <sup>c</sup>     | 7.5±0.021 <sup>d</sup>    | 4.00±0.15 <sup>d</sup>      | 0.15±0.01 <sup>c</sup>     |
| <i>Guinea G.</i> | CIPO | 1157.6 ± 0.12 <sup>d</sup>    | 94.1 ± 0.06 <sup>c</sup>    | 9.55± 0.03 <sup>c</sup>   | 6.12 ± 0.01 <sup>c</sup>    | 0.20 ± 0.06 <sup>c</sup>   |
|                  | CIP1 | 2755.4 ± 1.67 <sup>c</sup>    | 143.5 ± 1.16 <sup>b</sup>   | 14.45 ± 0.07 <sup>b</sup> | 9.85 ± 0.03 <sup>b</sup>    | 0.33 ± 0.02 <sup>b</sup>   |
|                  | CIP2 | 2717.25 ± 1.00 <sup>c</sup>   | 165.3 ± 1.90 <sup>b</sup>   | 16.36 ± 0.01 <sup>b</sup> | 11.5.07 ± 0.01 <sup>b</sup> | 0.35 ± 0.01 <sup>b</sup>   |
|                  | CIP3 | 3173.1 ± 0.06 <sup>b</sup>    | 194.767 ± 3.79 <sup>a</sup> | 18.95 ± 0.01 <sup>a</sup> | 12.1 ± 0.02 <sup>a</sup>    | 0.37 ± 0.01 <sup>b</sup>   |
|                  | CIR1 | 2620.9 ± 0.00 <sup>c</sup>    | 148.0 ± 0.17 <sup>b</sup>   | 15.7 ± 1.16 <sup>b</sup>  | 9.9 ± 0.40 <sup>b</sup>     | 0.32 ± 0.01 <sup>c</sup>   |
|                  | CIR2 | 2861.0 ± 2.03 <sup>b</sup>    | 165.8 ± 0.11 <sup>b</sup>   | 15.6 ± 0.10 <sup>b</sup>  | 11.6 ± 2.01 <sup>a</sup>    | 0.25±0.01 <sup>d</sup>     |
|                  | CIR3 | 3120.2 ± 0.01 <sup>b</sup>    | 202.1 ± 1.09 <sup>a</sup>   | 19.0 ± 0.02 <sup>a</sup>  | 12.0 ± 1.03 <sup>a</sup>    | 0.21.0 ± 0.02 <sup>d</sup> |

a, b, c, d, etc: means with the same superscripts are not significantly (P<0.05) different

**Table 4. Percentage of THC degraded and heavy metals removed in the soil during the period of the study for vetiver grass**

| <b>Parameter</b> | <b>CIP0</b>   | <b>CIP1</b> | <b>CIP2</b> | <b>CIP3</b> | <b>CIR1</b> | <b>CIR2</b> | <b>CIR3</b> | <b>CIP0</b>  | <b>CIP1</b> | <b>CIP2</b> | <b>CIP3</b> | <b>CIR1</b> | <b>CIR2</b> | <b>CIR3</b> |
|------------------|---------------|-------------|-------------|-------------|-------------|-------------|-------------|--------------|-------------|-------------|-------------|-------------|-------------|-------------|
| THC              | 1252.3        | 2879.6      | 3348.2      | 3862.3      | 2721.0      | 3244.8      | 3533.7      | 1157.5       | 2755.4      | 3173.1      | 2717.85     | 2620.9      | 2860.6      | 3160.2      |
|                  | 23%           | 52.6%       | 61.2%       | 70.6%       | 49.7%       | 59.3%       | 64.6%       | 21.2%        | 50.3%       | 57.9%       | 67.9%       | 47.9%       | 52.3%       | 57.7%       |
| Fe               | 102.8         | 162.2       | 182.4       | 213.4       | 156.9       | 170.0       | 205.4       | 94.1         | 143.5       | 165.3       | 198.1       | 148.2       | 165.64      | 202.14      |
|                  | 28.4%         | 44.8%       | 50.4%       | 58.4%       | 43.3%       | 46.9%       | 56.8%       | 25.9%        | 39.6%       | 45.6%       | 54.7%       | 40.9%       | 30.3%       | 55.8%       |
| Zn               | 10.9          | 15.2        | 16.9        | 19.5        | 16.0        | 17.0        | 19.0        | 9.55         | 14.52       | 16.36       | 18.95       | 15.7        | 16.6        | 18.7        |
|                  | 37.3%         | 52.2%       | 58.2%       | 66.9%       | 54.9%       | 58.3%       | 65.3%       | 32.8%        | 49.9%       | 56.2%       | 65.1%       | 53%         | 57.0%       | 64.3%       |
| Pb               | 7.12          | 10.06       | 12.21       | 13.8        | 10.72       | 12.74       | 13.6        | 6.12         | 9.85        | 12.07       | 13.23       | 10.37       | 0.39        | 0.43        |
|                  | 35.8%         | 50.6%       | 61.4%       | 69.4%       | 53.9%       | 64.1%       | 68.5%       | 30.8%        | 49.5%       | 60.7%       | 66.6%       | 52.2%       | 75%         | 82.7%       |
| Cd               | 0.12          | 0.35        | 0.40        | 0.42        | 0.36        | 0.4         | 0.43        | 0.2          | 0.33        | 0.35        | 0.37        | 0.32        | 12.68       | 13.53       |
|                  | 23.1%         | 67%         | 76.9%       | 80.8%       | 69.2%       | 76.9%       | 83.1%       | 38.5%        | 63.5%       | 67.3%       | 71.2%       | 61.5%       | 63.8%       | 68.1%       |
|                  | Vetiver grass |             |             |             |             |             |             | Guinea grass |             |             |             |             |             |             |

**Table 5. Concentration of THC ( $\mu\text{g/g}$ ) and Heavy Metal (mg/kg Uptake in Vetiver and Guinea Grasses**

| Treatment | <b>Vetiver</b>                 |                                |                              |                               |                               |
|-----------|--------------------------------|--------------------------------|------------------------------|-------------------------------|-------------------------------|
|           | THC( $\mu\text{g/g}$ )         | Fe(mg/kg)                      | Zn(mg/kg)                    | Pb(mg/kg)                     | Cd(mg/kg)                     |
|           | <b>Vetiver Grass</b>           |                                |                              |                               |                               |
| COP0      | 0.041 $\pm$ 0.00 <sup>k</sup>  | 51.20 $\pm$ 0.69 <sup>i</sup>  | 1.10 $\pm$ 0.06 <sup>g</sup> | 1.28 $\pm$ 0.068 <sup>h</sup> | 0.028 $\pm$ 0.01 <sup>e</sup> |
| CIP0      | 878.33 $\pm$ 4.41 <sup>a</sup> | 151.30 $\pm$ 0.17 <sup>a</sup> | 6.20 $\pm$ 0.12 <sup>a</sup> | 5.45 $\pm$ 0.029 <sup>a</sup> | 0.357 $\pm$ 0.07 <sup>a</sup> |
| CIP1      | 832.60 $\pm$ 1.16 <sup>c</sup> | 133.30 $\pm$ 1.04 <sup>b</sup> | 3.94 $\pm$ 0.00 <sup>c</sup> | 3.43 $\pm$ 0.06 <sup>c</sup>  | 0.19 $\pm$ 0.00 <sup>c</sup>  |
| CIP2      | 668.40 $\pm$ 0.23 <sup>g</sup> | 122.90 $\pm$ 1.16 <sup>b</sup> | 3.50 $\pm$ 0.12 <sup>e</sup> | 2.93 $\pm$ 0.17 <sup>e</sup>  | 0.10 $\pm$ 0.00 <sup>d</sup>  |
| CIP3      | 578.00 $\pm$ 1.16 <sup>j</sup> | 108.30 $\pm$ 0.17 <sup>e</sup> | 3.00 $\pm$ 0.06 <sup>f</sup> | 2.25 $\pm$ 0.02 <sup>g</sup>  | 0.083 $\pm$ 0.00 <sup>e</sup> |
| CIR1      | 812.00 $\pm$ 1.16 <sup>d</sup> | 130.90 $\pm$ 0.58 <sup>b</sup> | 3.71 $\pm$ 0.12 <sup>d</sup> | 3.41 $\pm$ 0.06 <sup>c</sup>  | 0.221 $\pm$ 0.00 <sup>b</sup> |
| CIR2      | 698.40 $\pm$ 0.23 <sup>f</sup> | 120.40 $\pm$ 0.23 <sup>c</sup> | 3.30 $\pm$ 0.12 <sup>e</sup> | 3.20 $\pm$ 0.12 <sup>d</sup>  | 0.185 $\pm$ 0.00 <sup>c</sup> |
| CIR3      | 575.90 $\pm$ 0.58 <sup>j</sup> | 109.40 $\pm$ 0.23 <sup>d</sup> | 2.80 $\pm$ 0.06 <sup>f</sup> | 2.50 $\pm$ 0.12 <sup>f</sup>  | 0.080 $\pm$ 0.00 <sup>e</sup> |
|           | <b>Guinea Grass</b>            |                                |                              |                               |                               |
| COP0      | 0.041 $\pm$ 0.00 <sup>k</sup>  | 49.16 $\pm$ 0.33 <sup>j</sup>  | 1.10 $\pm$ 0.06 <sup>g</sup> | 1.30 $\pm$ 0.17 <sup>i</sup>  | 0.030 $\pm$ 0.00 <sup>e</sup> |
| CIP0      | 846.10 $\pm$ 0.06 <sup>b</sup> | 104.90 $\pm$ 1.16 <sup>e</sup> | 5.30 $\pm$ 0.16 <sup>b</sup> | 4.80 $\pm$ 0.06 <sup>b</sup>  | 0.330 $\pm$ 0.02 <sup>a</sup> |
| CIP1      | 760.20 $\pm$ 0.12 <sup>e</sup> | 95.40 $\pm$ 1.73 <sup>f</sup>  | 3.30 $\pm$ 0.17 <sup>e</sup> | 3.00 $\pm$ 0.12 <sup>e</sup>  | 0.23 $\pm$ 0.06 <sup>b</sup>  |
| CIP2      | 644.03 $\pm$ 3.51 <sup>g</sup> | 84.80 $\pm$ 0.06 <sup>g</sup>  | 2.90 $\pm$ 0.06 <sup>f</sup> | 2.90 $\pm$ 0.12 <sup>e</sup>  | 0.130 $\pm$ 0.02 <sup>d</sup> |
| CIP3      | 525.80 $\pm$ 1.65 <sup>j</sup> | 77.20 $\pm$ 0.16 <sup>h</sup>  | 1.20 $\pm$ 0.12 <sup>g</sup> | 2.33 $\pm$ 0.01 <sup>g</sup>  | 0.080 $\pm$ 0.01 <sup>e</sup> |
| CIR1      | 605.38 $\pm$ 0.91 <sup>h</sup> | 90.10 $\pm$ 0.06 <sup>g</sup>  | 2.90 $\pm$ 0.00 <sup>f</sup> | 2.23 $\pm$ 0.05 <sup>g</sup>  | 0.180 $\pm$ 0.01 <sup>c</sup> |
| C1R2      | 582.1 $\pm$ 0.12 <sup>i</sup>  | 118.0 $\pm$ 0.03 <sup>c</sup>  | 2.52 $\pm$ 1.10 <sup>f</sup> | 1.72 $\pm$ 1.20 <sup>h</sup>  | 0.121 $\pm$ 0.02 <sup>d</sup> |
| C1R3      | 564.2 $\pm$ 1.11 <sup>j</sup>  | 105.2 $\pm$ 0.01 <sup>d</sup>  | 1.43 $\pm$ 0.02 <sup>g</sup> | 1.50 $\pm$ 2.04 <sup>i</sup>  | 0.065 $\pm$ 1.13 <sup>e</sup> |

*a, b, c, d, etc: mean with the same superscripts are not significantly ( $P < 0.05$ ) different*



unamended (C1P0) with poultry manures in vetiver against a mean value of  $49.16 \pm 0.33^i$  and  $104.90 \pm 1.16^e$  for the same treatment in guinea grass plots. This result revealed that both grasses have high ability to accumulate iron in their tissues and that vetiver has high uptake potential than guinea grass. This is in line with the work of [29,30,31] that in their various studies cleaned up oil polluted and heavy metals contaminated soils with vetiver and other grasses.

Amendment of the soil with organic manures (poultry and rabbit) decreased the ability of the plants to up take iron. In vetiver remediated plots, the plant was able to uptake  $151.30 \pm 0.17^a$  in contaminated unamended (C1P0) plots, but after amendment with 30 tons/ha poultry manure (C1P3), the uptake was reduced to  $108.30 \pm 0.17^e$ . Similarly, in guinea grass, the uptake rate for C1P0 was  $104.90 \pm 1.16^e$ , but after amendment with poultry manure C1P3, the uptake rate was decreased to  $77.20 \pm 0.16^h$  in contaminated amended with 30 tons' poultry manure. This may possibly mean that organic manures alleviated the toxicity of iron to plants, hence the decrease. The above observation agrees with the findings of [32,33]. The decrease was more in vetiver than guinea grass and on poultry than rabbit manures.

There was significant ( $P < 0.05$ ) increase in the content of zinc in both vetiver and guinea grasses remediated plots in contaminated unamended C1P0 ( $10.6 \pm 0.00^b$  and  $9.55 \pm 0.03^b$ ) as against control plots ( $8.0 \pm 1.01^d$ ) and ( $7.50 \pm 0.02^d$ ) respectively. This agrees with the finding of [29] who reported an increase in the concentration of zinc in crude oil contaminated soil over control.

The increase could be attributed to the fact that contamination of soil with crude oil leads to unavailability of essential nutrients and bring about availability of some heavy metals in plants. The results showed that the quantity of zinc removed from the soil ranged from 37.3% in contaminated unamended (C1P0) to 66.9% in contaminated amended with 30 tons (C1P3) of poultry manure in vetiver remediated plots as against 32.8 and 65.1% removed from the soil in guinea grass plots with same treatments (Table 3); implying that vetiver has stronger capability to remove crude oil contaminants from the soil than guinea grass.

The result also revealed a significant ( $P < 0.05$ ) difference in uptake of zinc, between the

treatments and the two grasses with vetiver having a mean value of ( $6.20 \pm 0.12^a$ ) in C1P0 as against guinea grass with ( $5.30 \pm 0.16^b$ ) in C1P0. The two grasses had the ability to accumulate high concentrations of zinc in their tissues. This tally with the observations of [34,35, 36]. The significant ( $P < 0.05$ ) increase in zinc uptake in vetiver over guinea grass (Table 5) indicates that vetiver was able to accumulate more zinc ions in their tissues than guinea grass.

Application of organic manures (poultry and rabbit) reduced the uptake of zinc ions in both grasses ( $6.20 \pm 0.12^a$ ) for C1P0 (contaminated unamended to ( $3.0 \pm 0.06^f$ ) for C1P3 contaminated and amended with 30 tons of poultry manure in vetiver plots and and ( $5.30 \pm 0.16^b$ ) for C1P0 to ( $1.20 \pm 0.12^g$ ) for C1P3 for guinea grass. Similar trend was observed in the two grasses amended with rabbit manure. The decrease in zinc intake as observed in the study agrees with the report of [37] that organic manures reduced accumulation of zinc ion in plant tissues. The decrease was more in poultry than in rabbit manure.

The percentage of lead (Pb) removed from the soil increased from 35.8% in contaminated unamended (C1P0) to 69.4% in contaminated augmented with 30 tons (poultry manure (C1P3) in contrast to 30.8% in C1P0 for contaminated unamended and 66.6% for contaminated amended with 30 tons (C1P3) in guinea grass plots. Statistical analysis of the result showed there was significant ( $P < 0.05$ ) reduction C1P0 ( $7.12 \pm 0.01^b$ ) and C1P3 ( $13.80 \pm 0.12^a$ ) in the concentration Pb in vetiver remediated plots compared to C1P0 ( $6.12 \pm 0.01^e$ ) and C1P3 ( $11.37 \pm 0.00^b$ ) for guinea grass plots amended with poultry manure (Table 4), indicating that vetiver has strong ability to remove the contaminants from the soil than guinea grass.

There was significant increase ( $P < 0.05$ ) in the uptake of Pb in plant tissue C0P0 ( $1.28 \pm 0.068^h$ ) to  $5.45 \pm 0.029^a$  in C1P0 for vetiver plots and  $1.30 \pm 0.17^f$  in control (C0P0) to  $4.80 \pm 0.06^b$  in C1P0 (contaminated unamended plots) in guinea grass. This shows that the two grasses have the capability to accumulate high concentration of Pb in their tissues. The finding corroborates with the report of [34,35,36].

Addition of organic manure in the soil reduced the extractable Pb in plant tissues as the result showed a decrease in Pb uptake in both vetiver

and guinea grasses. This agrees with the observation of [38, 37,39]. The decrease was more in vetiver than guinea grass and on poultry than rabbit manure. Organic manures in this study enhanced the effectiveness of vetiver and guinea grasses in reducing Pb bioavailability, thus lowering Pb uptake in the plants. This agrees with [32]. The result also implied that vetiver and guinea grasses have the capability to retain Pb in their tissues and so remove Pb from the soil thereby reducing the chance of spread of the contaminants.

Cadmium (Cd) is one of the largest groups of elements with differing characteristics in the biosphere. It is a non-essential plant nutrient that is highly toxic to plant [40]. The study revealed that the concentration of Cd removed from the soil increased from 23.1% in contaminated unamended (C1P0) to 80.8% in contaminated amended with poultry manure (C1P3) in vetiver remediated plots as against 38.5% and 71.2% in contaminated unamended (C1P0) and contaminated amended with poultry manure for guinea grass plot respectively.

The significant ( $P < 0.05$ ) increase from control C0P0 ( $0.15 \pm 0.01^c$ ) to contaminated unamended C1P0 ( $0.40 \pm 0.01^a$ ) in vetiver and ( $0.15 \pm 0.01^c$ ) in control (C0P0) to  $0.30 \pm 0.06^b$  in contaminated unamended (C1P0) for guinea grass plots (table 4) as observed in the two grasses indicate that crude oil contamination increase the concentration of Cd in soil. There was no significant ( $P > 0.05$ ) difference in Cd uptake in control and contaminated unamended plots between vetiver and guinea grass, however significant increase was observed on contaminated augmented with manures between vetiver and guinea grasses. Amendment of the soil with manures decreased Cd uptake in the plant tissues. This is in contrast to the observation of [37] who reported that addition of organic manures did not decrease Cd uptake in vetiver grass.

Physico-chemical properties of the soil have been reported earlier [41] in their study observed higher concentration of %TOC, %TN and C: N ratio and a decrease in available P and exchangeable cations in both grasses after contamination with crude oil and an increase in total nitrogen, total organic carbon, available phosphorus, exchangeable cations and a decrease in C: N ration after amendment with organic manure.

#### 4. CONCLUSION

The study revealed that vetiver and guinea grass have the capability of degrading total hydrocarbon content and reducing the quantity of heavy metals ions from crude oil polluted soil. Amendment of the soil with poultry and organic manures enhanced their capability. Vetiver was more effective than guinea grass. Amendment of the soil with poultry manure was more effective than rabbit manure. The two grasses have higher potentials to up taking these contaminants in their tissues.

#### COMPETING INTERESTS

Authors have declared that no competing interests exist.

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