

# Investigation of Physicochemical Properties and Identification of Bacteria Present in a Diesel Oil Contaminated Soil in Southwest Nigeria

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**Abstract** – This work investigates and identifies the physicochemical properties and bacteria group present in the diesel oil contaminated soil from a mechanic workshop in IITA, Ibadan. In the laboratory analysis, the soil samples were air-dried, ground, sieved and analyzed to identify the following properties: pH, C, P, N, Ca, K, Na, Mg, Mn, Cu, Zn, Fe, and ECEC. The pH of the soil samples was determined by Black analytical method; Organic Carbon by complete oxidation method by Heanes; total Nitrogen (%) by Kjeldahl method; available phosphorous by Bray-1 method; Ca, K, Na, Mg, Mn, Cu, Zn, and Fe by Mehlich-3 extraction method. The bacteria group of the soil samples was identified by gram staining method. The results show that Organic Carbon, Nitrogen, Phosphorus, Calcium, Magnesium, and Effective Cation Exchangeable Capacity were higher at 5% level of significance ( $p < 0.05$ ) in the polluted soil. The results of the heavy metals such as Cu, Mn, Zn and Fe were found to be at increase in the diesel oil polluted site when compared to the non-polluted site; 4.11, 101.89, 64.23, 156.41 (contaminated) and 0.66, 32.88, 22.09, 115.2 (non-contaminated site) respectively. It was observed that there was a significant decrease at  $p < 0.05$  in the activities of these bacteria (*Bacillus*, *Streptococcus* and *Staphylococcus*) in the contaminated soil. These bacteria, although present in a minute quantity have been also found by other researchers to possess great potentials of feeding on the hydrocarbon compound of the diesel oil. Therefore, it is recommended that bioaugmentation and biostimulation can be applied to the contaminated area to boost and catalyze the activities of the facultative bacteria so as to adequately degrade the contaminants.

**Keywords** – Bacteria, Bioremediation, Diesel oil, Physicochemical Properties, Soil.

## I. INTRODUCTION

Diesel oil leakages from underground storage tanks, distribution facilities and various industrial operations represent an important source of soil and aquifer contamination. This fuel is a complex mixture of normal, branched and cyclic alkanes, and aromatic compounds obtained from the middle-distillate fraction during petroleum separation [1].

Soil organic matter comprises all living soil organisms and the remains of previous living organisms in their various degrees of decomposition [2]. Soil organic matter plays a major role in maintaining soil quality as it positively influences a wide range of soil properties such as the provision of nutrients, water retention and release, as well as reducing the risks of soil compaction, surface crusting and soil erosion. However, the adverse effect of diesel oil tends to reduce the organic matter content of the

soil unless appropriate management practices are implemented to reverse this trend. This decline though a gradual process can disrupt the physical, chemical and biological properties of the soil rendering the soil useless from generation to generation.

These soil contaminants have negative effects not only on plant growth, development, ecological system, soil fertility but also responsible for health problems such as cancers (arsenic, asbestos, dioxins), neurological damage, kidney diseases (lead, mercury, cadmium), and skeletal and bone diseases (lead, fluoride, cadmium) which are serious issues, costing several millions of lives.

Owing to the long effect of diesel oil in the soil, the process of replenishing soil organic matter is slow also, but can be achieved through adapted agricultural management practices and the addition of organic materials to the soil. One of the best approaches to restoring contaminated soil is to make use of microorganisms able to degrade the toxic compounds in bioremediation process. Strategies bioremediation include natural attenuation, biostimulation, bioventing, bioaugmentation, landfarming, composting, and phytoremediation [3]. Diesel oil bioremediation in soil can be promoted by stimulation of the indigenous microorganisms, by introducing nutrients and oxygen into the soil (biostimulation) [4] or through inoculation of an enriched microbial consortium into soil (bioaugmentation) [5] and [6].

In most field studies, enhancing biodegradation of petroleum hydrocarbons depends on the specific microbial population present. The composition of the microbial population is affected by the environmental conditions and the composition of the hydrocarbons [7].

For optimum biodegradation conditions, it is important to know the characteristics of the contaminated site before beginning treatments. Basic information such as residual oil concentration, physical and chemical properties of the soil, population density of the oil-degrading microorganisms and the biodegradation potential, are key factors to be considered for bioremediation of oil-polluted sites. Therefore, this work is aimed at investigating of physicochemical properties of the diesel oil contaminated soil and identifying the bacteria group present in the soil.

## II. METHODOLOGY

### A. Area of the Study

The soil samples were collected from a mechanic workshop (contaminated area) in International Institute of

Tropical Agriculture (IITA), Idi-Ose, Moniya, Ibadan, Oyo State. Ibadan is located on latitude  $7^{\circ}23'47''\text{N}$  and longitude  $3^{\circ}55'0''\text{E}$ . It is located in south-western Nigeria, 128 km inland northeast of Lagos and 530 km southwest of Abuja, the federal capital, and is a prominent transit point between the coastal region and the areas to the north.

### *B. Sample Collection*

Soil samples were collected in sterilized polyethylene bags using a cone agar by drilling and scooping method to about 0 – 15 cm (top soil) and 15 – 30 cm (subsurface) depth of the soil in the study area (contaminated and control area). The soil samples were air-dried for 24 hrs, grinded and sieved with 2 mm mesh [8]. 10 g of air-dried soil sample from the two different soil depths of both contaminated and control area was measured into sterilized white nylon bags and labeled appropriately. They were immediately transported to the IITA laboratory for analysis.

### *C. Soil Sample Preparation*

#### *Determination of Available Phosphorus (BRAY -1 Method)*

5 g air-dry soil (2 mm sieved) was weighed in extraction cup and 30 ml Bray-1 solution was added, stirred on a mechanical shaker for 5 mins and allowed for 2 mins and then centrifuged for 5mins at 3000 rpm. A Pipette of 1 ml of the clear supernatant (sample) was injected into a set of clean glass vials and mixed well with 6 ml of distilled water. 2 ml of color reagent was added and mixed well again followed by 1 ml of ascorbic acid solution and mixed thoroughly. After 6 min, the colour was measured at 650 nm on a colorimeter or visible range spectrophotometer [9].

#### *Total Nitrogen in Soil*

Grinded soil samples was passed through a 0.5 mm sieve and mixed uniformly. 0.5 g amount weighed poured into 50 ml digestion tubes (5 digestion tubes were without soil samples for the preparation of standards. The weight was recorded to nearest 0.001 g. 2.5 ml of sulphuric acid-selenium mixture was added into each tube and mixed with the acid on a vortex mixer. After which, 2X1ml of  $\text{H}_2\text{O}_2$  (hydrogen peroxide) was added into each tube and placed on a hotplate, preheated to  $300^{\circ}\text{C}$ . After 30 minutes of boiling, condensing bottles were placed over each digest tube and the temperature was increased to  $320^{\circ}\text{C}$  and allowed on hotplate until the digest was clear. Then the samples were cooled and diluted to 50 ml with distilled water.

#### *Determination of Organic Carbon (Complete Oxidation Method)*

The sample was passed through a 0.5 mm sieve before weighing and mixed thoroughly. 500 mg of the soil samples was weighed and added into a 50 ml digestion tubes. 1.00 ml of the 5 standard solutions was added into 5 digestion tubes. 5 ml of 1N  $\text{K}_2\text{Cr}_2\text{O}_7$  solution was added to samples and standards following the addition of 10 ml of concentrated  $\text{H}_2\text{SO}_4$ . Cap with a rubber stopper, and swirled on a vortex mixer until the soil sample was completely dispersed. Placed in a digestion block and preheated to  $150^{\circ}\text{C}$  for exactly 30 minutes allowed tubes to cool. After which it was diluted to 50 ml volume, mixed

using a vortex mixture, transferred standards and samples into 50 ml centrifuge tubes and centrifuged for 10mins at 3000 rpm. The absorbance of standards and samples was read on a spectrophotometer at a wavelength of 600 nm using a 1 cm cell. Absolute care was taken to avoid spills, as the solution is very acidic. The standards contain 0, 2.50, 5.00, 7.50, and 10.00 mg of C [10].

#### *Exchangeable Acidity (Al + H) and Aluminum (Titration Method)*

3 g was weighed of air-dried soil (grind to pass a 2 mm sieve) into folded filter paper placed on the extraction cups. 50 ml of 1.0 N KCL solution was gently poured through the soil in the filter paper and collected the leachate. And 5 drops of phenolphthalein indicator was added in the leachate. Then the leachate was titrated with 0.05 N NaOH to pink end point. The volume (V) (ml) of NaOH used was recorded [11].

#### *Analysis of Ca, Mg, K, P, Cu, Zn, Mn, Fe (Mehlich Method)*

Mehlich-3 extraction solution: In a 500 ml polythene bottle, 250 ml distilled (d/d) water was added. Then 69.45 g  $\text{NH}_4\text{F}$  and 36.75 g EDTA were dissolved, and diluted to 500 ml. To a 10 liter jug, 8 liters water and 200 g  $\text{NH}_4\text{NO}_3$ , 40 ml of the EDTA/ $\text{NH}_4\text{F}$  solution, 115 ml acetic acid, and 8.2 ml of 70% nitric acid were added and diluted to 10 liters. The pH was calibrated to  $2.5 \pm 0.1$ . Then 3.0 ml of soil was scooped in a 50 ml centrifuge tube, and recorded to the nearest 0.01 g. 5 blank samples for standard-sample matching and 5 soil check samples covering a range of Ca, Mg, K, and P concentrations including low, medium, and high values were included. 30 ml of Mehlich-3 extractant was later added to a batch of 24 samples, capped securely, and shook for 5minutes. And allowed stand for 10 minutes, then centrifuged for 5 minutes at 3000 rpm. After centrifugation, the samples were transferred extracts and blanks to tube to minimize soil contact after centrifugation [12].

#### *Cu, Zn, Mn, and Fe analysis of Mehlich-3 Extracts*

Zn-Cu-Mn-Fe stock solution: To a 250 ml volumetric flask was added 100 ml of 1000 ppm Mn, 100 ml 1000 ppm Fe, 1.00 ml of 1000 ppm Cu, and 1.00 ml of 1000 ppm Zn. This solution contains 400 ppm Mn and Fe and 8.00 ppm Cu and Zn. Standards: 30 ml of Mehlich-3 extractant was added into 50-ml centrifuge tubes. From the 5 respective tubes 0, 0.375, 0.750, 1.50, and 3.00 ml were removed. The samples were capped and shook for 5 minutes. These samples contained 0, 5.00, 10.00, 20.0, and 40.0 ppm Fe and Mn, and 0, 0.100, 0.200, 0.400, and 0.800 ppm Cu and Zn. Standards and undiluted sample extracts were read on the AAS [12].

#### *Determination of Soil pH*

10 g of sample was weighed into an extraction cup and added 10 ml of distilled water. Then allowed to stand for 15 minutes and shook on a mechanical shaker for 30 minutes at 150 rpm. It was allowed again to stand for another 10 minutes and the pH meter was calibrated using buffers 7.0 and 4.0 and the pH value was read on the pH meter.

#### D. Determination of Bacteria Group of the Soil

The bacteria group of diesel contaminated soil was determined using pour plate and gram staining method. The Yeast Mannitol Agar and Nutrient Agar were prepared and mixed vigorously until it dissolved. The media was autoclaved at 121<sup>0</sup>C for 15 minutes and put on water bath to cool it down to 47<sup>0</sup>C. A diluent was prepared by weighing out soil reagent and adding 250 ml of distilled water. The solution was stirred until it dissolved completely. 5 vials bottles of 20 ml were rinsed with distilled water and 9 ml of the diluents solution was dispensed into them and each vials was labeled from serial dilution of 10<sup>-1</sup> – 10<sup>-5</sup>. Then the solution was autoclaved at 121<sup>0</sup>C for 15 minutes and placed on a laminar flow wood to cool. After which, 1 g of the soil sample was weighed and added to 9 ml diluents to make it up to 10 ml. The vials were stirred properly and placed on sterile petri-dish in the laminar wood. 1ml was taken from the 10 ml into another 9 ml and labeled 10<sup>-1</sup> – 10<sup>-4</sup>. 1 ml was poured into plate following the addition of YMA into each plate and allowed to solidify for 30 mins. The plate sealed with paraffin and incubated for 48 hrs. The medium was observed for 24 hrs and 48 hrs and then sub-cultured the identified bacteria before gram staining.

#### Gram Staining

A fixed smear from the strains (bacteria) was prepared, stained with crystal violet solution for 1 min, rinsed with tap water and treated with iodine solution and rinsed with tap water again. The slide was decolorized with 96% ethanol until the solvent runs down colorless and rinsed with tap water. After which, the slide was counterstained with Satranin solution and rinsed with tap water and finally, the slide was dried and viewed under binocular microscope.

#### E. Data Analysis

Results were analyzed using SPSS Software to obtain the Analysis of Variance (ANOVA) and means were compared for significant at  $p < 0.05$  using Duncan's multiple range analysis.

### III. RESULTS AND DISCUSSIONS

The results obtained in the analysis of the physicochemical parameters are shown in table 1 below. There was a significant increase in at  $p < 0.05$  as the bottom soil samples were slightly more acidic than top soil samples in the contaminated area. The contaminated soil sites had the highest value of Organic Carbon, total Nitrogen, and Phosphorus ranging from 3.77 – 2.3, 0.36 – 0.16, and 1.1 – 0.68 respectively. Similarly, it was observed that there was significant variation between the Ca, K, Mg, Na, and ECEC values with the contaminated area having the highest figures ranged from 1.14 – 0.93, 0.6 – 0.49, 0.96 – 0.58, 0.99 – 0.78 and 2.09 – 1.51 respectively. Results of the physiochemical properties of soil in IITA affected by diesel oil spillage shown in table 1 indicated that available phosphorus and total nitrogen levels were elevated in the contaminated area compared to the non-contaminated area. The observed increases were greatest in the heavily polluted area. A similar trend was

reported by [13] that high concentrations of OC and Total Nitrogen are indicators of soil contaminated with diesel oil.

The concentrations of exchangeable cations (K<sup>+</sup>, Na<sup>+</sup>, Ca<sup>2+</sup>, Mg<sup>2+</sup>) increased with increased in pollutions. [14] reported such increase in exchangeable cation of soil from crude oil polluted soil in Ogoni land. Potassium (K<sup>+</sup>) and Calcium (Ca<sup>2+</sup>) values in contaminated area and non-contaminated soil samples were within the range for the low fertility class of Nigerian soils. The moisture content of the polluted soil samples reduced. This may be due to coating of the soil surface by hydrophobic hydrocarbon that reduces the water holding capacity of the soil and reduction in the binding property of clay soil. There were significant increases at ( $p < 0.05$ ) in organic carbon and organic matter, these may be attributed to the metabolic processes following oil spillage that facilitates agronomical addition of organic carbon from petroleum hydrocarbon by reducing the carbon mineralizing capacity of the microflora [15].

The table 2 below shows the heavy metal concentrations of soil samples in the study area. The following heavy metals were tested: Zn, Cu, Mn, and Fe. It is discernible from the table that the concentrations of heavy metals (Cr, Cu, Mn, Pb, Fe) in the contaminated soil samples was high at 5% level of significance and low in the non contaminated area. This is in agreement with the result of [16] that high heavy metals concentrations in a polluted soil may be due to hydrocarbon pollution. Also, it was observed that there a high level of Fe<sup>2+</sup> in the soils under the influence of diesel oil spillage and this may be attributed to the reduction of Fe<sup>3+</sup> to Fe<sup>2+</sup> due to favourable reducing condition provided by oil pollution. A similar report was recorded that a high concentration of Fe<sup>2+</sup> in the soil renders the soil toxic for plant growth, hence an overall pollution [14].

The results of bacteria activity and identification of the study area are shown in table 3. The gram staining identification method used was to identify the type of the bacteria inhabitant in the study area. The contaminated area has gram positive bacteria (Purple colour) cocci in both depths. In non-contaminated area, the depth 0 – 15cm has gram positive bacteria (Purple colour) while the depth 15 – 30cm has gram negative bacteria (pinkish colour). From the analysis, the contaminated area was observed to have some traces of Gram-positive bacteria which are a class of bacteria that take up the crystal violet stain used in the Gram staining method of bacterial differentiation. The thick peptidoglycan layer in the cell wall that encases their cell membrane retains the stain, making definitive identification possible [17]. The bacteria identified are *Streptococcus* and *Staphylococcus*, which are cocci (sphere-shaped). The remaining organisms are *bacilli* (rod-shaped) and can be subdivided based on their ability to form spores. The non-spore formers are *Corynebacterium* and *Listeria* (*a coccobacillus*), whereas *Bacillus* and *Clostridium* produce spores [18]. A similar trend was reported by [19], that the *bacillus*, *Streptococcus* and *Staphylococcus* are petroleum hydrocarbon utilizers which means that these bacteria (microorganisms) are capable of

degrading complex hydrocarbon compound although they are in minute quantities in contaminated soil site.

Table 1: Physicochemical Properties of Diesel oil contaminated Soil

	DC 1	DC 2	NDC 1	NDC 2
pH (Water)	6.2±0.30 <sup>c</sup>	5.93±0.21 <sup>c</sup>	7.03±0.15 <sup>b</sup>	7.8±0.10 <sup>a</sup>
OC (%)	3.77±0.54 <sup>a</sup>	2.3±0.66 <sup>b</sup>	0.95±0.02 <sup>c</sup>	0.89±0.02 <sup>c</sup>
Total N (%)	0.36±0.09 <sup>a</sup>	0.16±0.02 <sup>b</sup>	0.08±0.01 <sup>b</sup>	0.08±0.01 <sup>b</sup>
P (Cmol/kg Soil)	1.1±0.09 <sup>a</sup>	0.68±0.10 <sup>b</sup>	0.25±0.05 <sup>c</sup>	0.07±0.01 <sup>d</sup>
Ca (Cmol/kg Soil)	1.14±0.32 <sup>a</sup>	0.93±0.03 <sup>a</sup>	0.27±0.09 <sup>b</sup>	0.19±0.05 <sup>b</sup>
Mg (Cmol/kg Soil)	0.96±0.04 <sup>a</sup>	0.58±0.05 <sup>b</sup>	0.16±0.04 <sup>c</sup>	0.27±0.12 <sup>c</sup>
K (Cmol/kg Soil)	0.6±0.07 <sup>a</sup>	0.49±0.09 <sup>a</sup>	0.15±0.04 <sup>b</sup>	0.11±0.03 <sup>b</sup>
Na (Cmol/kg Soil)	0.99±0.13 <sup>a</sup>	0.78±0.19 <sup>a</sup>	0.42±0.08 <sup>b</sup>	0.35±0.18 <sup>b</sup>
ECEC (Cmol/kg Soil)	2.09±0.37 <sup>a</sup>	1.51±0.16 <sup>b</sup>	1.26±0.22 <sup>b</sup>	1.6±0.36 <sup>ab</sup>

Mean ± S.D in a row with different superscript are significantly different at 5% level (P<0.05). Mean separation done by Duncan multiple range test.

DC1 = Diesel Oil Contaminated (0 – 15cm) NDC1 = Non-diesel Oil Contaminated (0 – 15cm)

DC2 = Diesel Oil Contaminated (15 – 30cm) NDC1 = Non-diesel Oil Contaminated (15 – 30cm)

Table 2: Heavy Metals Concentrations of the Soil Sample

	Zn	Cu	Mn	Fe
DC 1	64.23±3.9 <sup>a</sup>	4.11±1.28 <sup>a</sup>	101.89±3.33 <sup>b</sup>	156.41±21.5 <sup>a</sup>
DC 2	50.99±10.3 <sup>a</sup>	2.69±0.45 <sup>b</sup>	125.31±12.64 <sup>a</sup>	129.85±13.47 <sup>ab</sup>
NDC 1	27.44±4.37 <sup>b</sup>	1.01±0.93 <sup>c</sup>	66.97±19.69 <sup>c</sup>	115.93±5.57 <sup>c</sup>
NDC 2	22.09±2.94 <sup>c</sup>	0.66±0.28 <sup>c</sup>	32.88±6.01 <sup>c</sup>	115.2±14.18 <sup>c</sup>

Mean ± S.D in a row with different superscript are significantly different at 5% level (P<0.05). Mean separation done by Duncan multiple range test.

Table 3: Gram Staining Bacteria Identification

Sample	Gram Staining Bacteria Identification
DC 1	Gram Positive bacteria ( Purple colour) cocci
DC 2	Gram positive bacteria ( Purple colour )
NDC 1	Gram Negative bacteria (Pinkish Red colour)
NDC 2	Gram Negative bacteria (Pinkish Red colour)

DC1 = Diesel Oil Contaminated (0 – 15cm) NDC1 = Non-diesel Oil Contaminated (0 – 15cm)

DC2 = Diesel Oil Contaminated (15 – 30cm) NDC1 = Non-diesel Oil Contaminated (15 – 30cm)

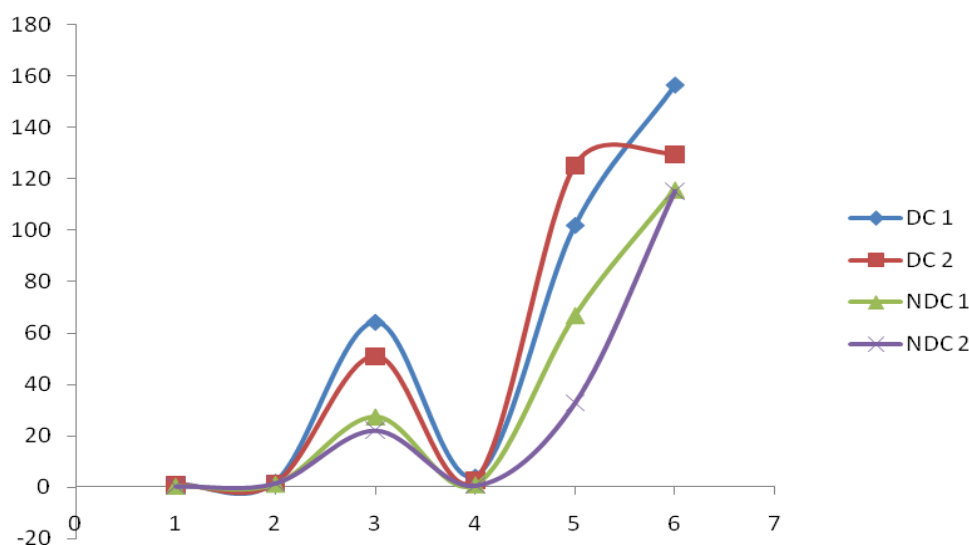


Fig.1. Concentrations of Heavy Metals of the Soil Samples

#### IV. CONCLUSION

The physicochemical properties and the bacteria groups of the diesel oil contaminated soil have been investigated and identified respectively. It was observed that the effects of this contaminant attributed to increase in pH value resulting in acidity of the soil, high increase in the OC and total Nitrogen percentage, high value of the Cu, Zn, Fe and Mn which makes it difficult for plant growth and development in that area. Also, from the result of this study, it was found that the diesel oil contaminated area has some traces of gram positive bacteria; *bacillus*, *Streptococcus* and *Staphylococcus* they have high potentials of degrading (digesting) the hydrocarbon compound of the diesel oil.

#### RECOMMENDATIONS

Since the group of bacteria present in the study area are petroleum hydrocarbon utilizers. Therefore, the hybrid of biostimulation and bioaugmentation is recommended as the best remedial measure for total recovery of the soil of this study.

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