A **Research Paper**

Assessment of the Physico-Chemical Properties of Crude Oil Contaminated Soils From Iyede-Owhe, Delta State Nigeria, With Reference to Genomics of Soil Microbiota for Bioremediation

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Abstract

Soil pollution by Crude oil spillage either deliberately or accidental is a threatening menace of the environment flora, fauna and means of livelihood of oil producing communities in the Nigeria Delta region of Southern Nigeria. Therefore this study aimed at assessing the Physico-chemical properties of Crude oil contaminated soils from Iyede-owheOil and Gas field 9 extension in Isoko North Local Government Area of Delta state Nigeria. The soils samples were collected at a depth of 5cm, poured into pre-labelled sterilized Bama bottles and transported within 24 hours to Federal College of Land and Agricultural Resources Technology (FECOLART) Kuru, Plateau State Nigeria for analysis, following standard laboratory procedures. The chemical results of the soil samples tagged A, B, C corresponding to Crude oil contaminated soils and control respectively were as follows. The pH of the soil was acidic, ranging from 5.53+.078- 5.91+ 0.19, showing no significant difference since p < 0.644 was higher than p < 0.05. The temperature range of the soils was 24 + $2.09\degree$ ⁰C - $25.5 + 2.55\degree$ C also showing no significant difference since $p < 0.85$ was greater than $p > 0.05$. *Organic matter (14.63 + 0.76, 14.92 + 0.01, 0.47 + 0.04), Active Carbon (0.03 + 0.02, 0.02 + 0.01, 0.04 + 0.01), Nitrate (82.25 + 0.25, 83.03 +0.11, 84.10 + 0.10),Phosphorus (0.08 + 0.01. 0.07 + 0.03, 0.18 + 0.03). Potassium (2.60 + 0.60, 258.71 + 0.72, 870.05 + 0.05),Sulphate(1.14 + 0.01, 1.18+ 0.02, 1.33 + 0.02). Calcium (0.88 + 0.03, 0.091 + 0.04, 0.75 + 0.05), Magnesium (0.53 + 0.03,0.61 +0.02, 0.23 + 0.03). There was no significant difference in Phosphorus, Active Carbon and Calcium contents of the soils since their p- values were higher than p < 0.05, while organic matter, Nitrate, Potassium, Sulphateand Magnesium contents showed significant differences because their p – values were less than p <0.05. The physical parameters results of the soils samples were; Moisture (64.78 + 0.32, 66.31 + 0.41, 41.77 + 0.44), Bulk density (1.89 + 0.05, 1.99 + 0.4, 1.54 + 0.01), Sand (59.91 + 0.10. 61 .21 +0.10, 76 .96 + 0.24), Clay (23.25 + 0.25, 23.02 + 0.20. 22.50 +0.50), Silt (17.39 + 0.11, 18.04 + 0.21, 1.29 + 0.01). The textural class of the soils was: Sandy-Clay- Loam. There was significant difference in the physical parameters, since their p- values were greater than p < 0.05, with the exception of Silt with p- value (0.312), which was higher than p < 0.05. From the foregoing it was concluded that crude oil contamination alters the affected soils Physico-chemical properties, hence it was recommended that; the effected communities be adequately compensated by the Federal Government and Oil companies operating in the affected communities, and the funding of research into the possible application of genomics of soil Microbiota for the remediation of crude oil polluted soils*

Key words: Crude Oil Contamination,Physico-Chemical, Genomics, Soil Microbiota, Bioremediation

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I. INTRODUCTION

"*Soil*" which is the top mostlayer of the earth surface wherehumans live (Singh*et al*., 2017) not only support soil Microbiota, but also nourishes good plant growth yield and is home to diverse fauna and flora. Soil also store and purifies water useful to man, animals and plants. Pollution of the soil by petroleum otherwise referred to as Crude oil (Andrew *et al*., 2014) is a global concern, especially in communities where it is being explored, and frequently spilled on the soil. Spillage is causedby either human sabotage or leakages from faulty pipeline caused by lack of maintenance and/or during transportation (ImehandEkpo2012; Ohamu*et al*., 2018). Soil contaminationby Petroleum hydrocarbons clumps soil particles together, blocking air diffusion in the soil pores filling the pores with oil, expelling waters which causing flooding and excessive increasein soil moisture. It also depletes soil minerals making them not available to plants, alters soil pH to acidic level and deteriorating of soil structure (Wang *et al*., 2013, Onojakeand Osuji 2012;Devatha*et al*., 2019). These factors affect plant growth, giving them a burnt appearance. Apart from its deleterious effects on plants, the hydrocarbon contents of crude oil have been implicated to belong to Carcinogenic, Mutagenic, Tetratogenic, Nephrotoxic, Hepatotoxic to animals and humans found in such areas via trophic transfer in the food web (Obi *et al*.,2016; Chioma*et al*., 2020). The constant spills of Crude oil on the soils and its deleterious effects is a worrisome sight found in Iyede-OwheOil and Gas pipeline field 9, its extension, located in Isoko North local government area of delta state Nigeria. Isoko North with geographical co-ordinates; Latitude 5^0 350⁰N and Longitude 6^0 100⁰E, is one of the rich oil producing area in the oil rich Niger-Delta region of Southern Nigeria, hosting lots of Oil wells, contributing to Nigerians main economy based on Crude oil derived revenue. The threat of constant oil spillage is not only depleting the ecosystem, but it is gradually corroding away arable fertile farmlands, fishing grounds, depriving many animals of their natural habitats and also affecting the means of livelihood and economic activities of the indigenes who are mainly farmers and fishermen, either immediately or with time. To combat the deleterious effects of Crude oil spillage, soil microbiota can aid environmental restoration either by Oxidizing, Binding,Mobilizing or transformation of the hydrocarbon components of the Crude oil benign forms. They are able to carry out these activities because of their inherent genetic makeup domicile in their genome. Thus the use of the genetic capability of organism such as soil microbiota to interact with their environment is termed Genomics (Kaur, 2013). The technique of using soil microbiota, their genes, gene products in environmental restoration is known as "Bioremediation".

Thus this study was carried out to assess the physico-chemical properties of crude oil contaminated soils from Iyede-Owhe Oil and Gas field 9 and extension, located in Isoko Local Government Area of Delta State, Nigeria, with the view of future exploration of the genomic capabilities of the indigenous soil Microbiota for the bioremediation of crude oil polluted soils.

II. MATERIALS AND METHODS

Physico-chemical Parameters

The various physical properties, like colour, soil particles, soil texture, soil moisture and bulk density were analyzed for the soil samples. The chemical properties such as pH, Temperature, Active carbon, total Organic matter, available Phosphorus, Nitrate, Potassium, Calcium, Magnesium were also analyzed according to standard laboratory procedures.

Soil sample collection

Contaminated Soil samples were collected from Iyede-OwheOil and Gas field 9 and extension, while the uncontaminated soils was collected from an open filed along Olomorow road. The soil samples were collected according to method described by (Allamin*et al*., 2014) into a pre labelled sterilizedBama bottles in September 2019, during the rainy season. The samples were transported within 24hours in a tight zipped bag to Federal College of Agriculture and Land Resources Technology (FECOLART)Kuru in Jos, Plateau State, for Physico-chemical analysis.

Soil Sample processing

200gm of the soil samples were air dried for 2days, crushed and sieved with a 2mm sieve as described by (Oyen, and Oyen, 2013). The sieved soils were poured into tight zip-locked sterile labelled polythene bags for further analysis.

pH and Temperature determination of the Soils Samples

1gm of soil samples each were weighed with a top loading digital weighing balance (Model: 572, Kern, United Kingdom) and poured into pre labeled 20ml conical flask. 10 ml of distilled water was dispensed into the conical flasks, and the soil samples were shaken vigorously to dislodge the soil into the distilled water to form 1:10 (w/v) Soil/water suspension. The pH of the soil sample suspension was read using a digital pH meter (Model: 3510 Genlab, England), results recorded in triplicates.

The temperature of the soil samples suspension was done using a thermometer (Genlab, England) and temperature readings taken in triplicates.

Determination of Nitrate in 0.01m CaCl² Soil Filtrate

The Nitrate LAGUA meter (Model: 743x, Horiba Scientific, Japan) was calibrated using standard 200ppm Nitrate.This was followed by adding distilled water on the bowl of the LAGUA Nitrate meter until the meter read less than 10ppm, after which the sensor bowl of the meter was carefully cleaned by using the small 10mm x 10mm tissue wipes. Another clean piece of tissue was carefullydropped into the sensor bowl of the meter and two drops of the soil filtrate was added into the meter and reading taken when a smiley face showed on then LCD of the meter.

Determination of Potassium (K) in 0.01 CaCl2Soil Filtrate

The calibrated Potassium LAGUA twin K-meter (model: B-731, HORIB Japan) was used to measure available Potassium of the soil filtrate in 0.01 m CaCl₂, by placing athin fresh 10 mm X 10mm rectangular tissue wipe on the bowl of the sensor and carefully dropping two drops of the soil filtrate over the tissue. Reading in partsper million (ppm) was taken when a smiley face showed on the LCD screen of the Potassium LAGUA meter.

Determination of Phosphorus (p) in 0.01m CaCl2, using Molybdate Blue Phosphate Reagent.

8 mls of 0.01 MCalcium Chloride (CaCl₂) was poured into well labeled vials, while 2mls of filtrate were added followed by adding powdercolour developer (HANNA Phosphate H1 reagents catalog number: 736-25) to the respective vials. The vials were each shaken vigorously for 5minutes until the powder completely dissolved to form a solution. The vials were allowed to stand for 30minutes for colour development after which the Phosphate meter (Hanna, Japan) meter was used to take readings of the blank and soil filtrate in parts per million (ppm)

Determination of Sulphate (SO4) in 0.01 ml CaCL2.using Barium Chloride.(BaCl2)

4ml of 0.01mCacL₂ and 4ml of distilled water were poured into a vial to make blank solution while 4ml of soil filtrate and 4ml of distilled water were poured into labeled vials. Barium Chloride-dihydrate (BaCl2H2O) was added to the vials. 5 drops of Sulfate solution was added to the blank and reading was taken in order to calibrate the colorimeter, after which the test soil filtrates were each poured into a cuvete, that was inserted into the colorimeter and the readings recorded in part per million (ppm)

Determination of Active Carbon (AC) In 0.02 M Potassium Permanganante (KMnO4)

2.5gm of dried soil samples were weighed using a digital weighing balance (Model: JT 1601N: Germany) and the sand poured into amber bottles. 18 ml of distilled water was added and the bottles shaken vigorously and allowed to settle for 2 minutes. 2 ml of 0.02M KMnO⁴ was used as blank and its titer value of 1.0 ppm was recorded. Using a dropping pipette 2ml of 0.2MKmO₄ and 0.2ml of soil filtrates was poured intocuvete and the reading recorded. The value of active carbon was calculated using the formula; Blank-X-Y0.1995 Where:

 $Blank = 1.00$ ppm $Y =$ Titre correcting factor (0.1995) $X =$ filtrate value

Calcium and Magnesium Determination

Calcium and Magnesium were determined by pouring 20 ml of soil suspension into 250 ml conical flask (Duran Germany) and 100 ml of distilled water added. This was followed by adding 15 ml of concentrated Ammonia solution (NH₃) as buffer. 10 drops of 5% Hydroxyl Ammonium Chloride (OHNH₃Cl) was added, followed by the addition of 5 drops of Eriochrome Black T indicator. Titration with 0.01 M Ethyl diamine tetra acetic acid (EDTA) was done until colour change from wine red to deep blue. Calcium and Magnesium was calculated using the formula;

T X M X VIX 100 V2 W

Where:

T= Titre value of EDTA M= Molarity of EDTA VI= Volume of distilled water

V2= Volume of soil suspension

W= Weight of soil

Calcium alone was determined by titration using a pinch of Calcine as an indicator. The same steps mentioned above was followed until colour change from blue to wine red. Calcium was calculated using the same formula $Ca = T$ X M X VIX 100 V2 W

Magnesium value was determined by subtraction of Calcium value from the Calcium and Magnesium value indicated as;

 $Mg = (Ca + Mg) - Ca$

Moisture Content of the soil

Moisture contents of the soil samples was determined according to (Singh *et al*., 2017 modified). Moisture content of the soilsamples were done by weighing 395.6gm of wet soil (W2), oven dry them at 105° C for 24 hours. After 24 hours the samples were weighed again (W3). The moisture content was calculated by subtracting the final weight (Oven dried soil) from the initial weight of the soil (W2-W3)

W

While density was calculated by diving the final weight of the soils by the volume of the core sampler. The soil colours were determined by visual method. The soil texture was done according to standard laboratory procedures by suspending 50gm of each soil samples in 100 ml of Calgon solution, followed by addition of 500mls of distilled water. The specific gravity and temperature of the soil suspension were measured using hydrometer and thermometer respectively. The percentage of Silt, Clay and sand were calculated using the formula;

R= 40Sec- Ra X Rc X 100

Weight of soil Where:

Ra= 40sec. blank hydrometer reading

Rb = 2hrs blank hydrometer reading

 $Rc = 40$ sec correction factor (Temperature x 0.360)

Rd= 2hrs correction factor (Temperature x 0.36)

Total organic matter using the soil samples were analyzed using the procedure described by (Onojake and Osuji 2012)

Statistical Analysis

The statistical tool used was one way analysis of variance (ANOVA). Using graph pad prism 7. Data were expressed as mean 4 standard error of mean (SEM,) and value at p< 0.05 were considered significant.

Note: Values are expressed as mean \pm SEM; NS= Not significant, LOS= Level of Significance, $* =$ significant at P<0.05

KEY

A= Crude oil contaminated soil samples from Iyede-Owhe Oil well field 9

B= Crude oil contaminated soil samples from Iyede-Owhe Oil and Gas field 9 extension

C= Uncontaminated Soil Sample from an open field in Olomoro road

Table 2

Note: Values are expressed as mean \pm SEM; NS= Not significant, LOS= Level of Significance, $*$ = significant at P<0.05

KEY

TABLE 3 Physical Parameters of Hydrocarbon Polluted Soil and N Sample

Note: Values are expressed as mean \pm SEM; NS= Not significant, LOS= Level of Significance, $*$ = significant at P<0.05

KEY

A= Crude oil contaminated Soil from Iyede-owheOil and Gas field 9

B= Crude oil contaminated Soil from iyede-owhe Oil and Gas field 9 extension

C = Uncontaminated Soil from open field in Olomorow road

The soil chemical analysis results are presented in Tables 1 and 2, while the physical parameters results are presented in table 3.

pH which is defined as hydrogen ion concentration is an important chemical soil features that affects availability of nutrients needful for plant growth and survival of soil Microbiota, encouraging soil microbes activities in breaking down organic materials, freeing nutrients contained for plant use. The soil pH were in the acidic range 5.53 \pm 0. 23- 5.91 \pm 0.19, showing no significant difference since the p- value (0.644) was greater than p <0.05. The acidic pH of the soils may be connected to the, heavy rainfall, leaching of the top soils, crude oil contaminants presence, whose hydrocarbon contents reacts with the soil salts and mineral, changing the alkaline minerals in the soil to become acidic. This findings agrees with the report of (Oyem*et al*., 2013) and deviated from (Devatha*et al*., 2019, Ohamu*et al*., 2018 and Yuniati., 2018) who proposed a pH range 6-9 for optimal bacteria and plant growth in crude oil contaminated soils.

Soil temperature is the ratio of energy absorbed by the soil to the energy given out by the soil. The temperature range of the soil samples was $24 \pm 2.09^{\circ}$ C – 25.5 $\pm 2.55^{\circ}$ C. There was no statistical significance difference in the soil samples Temperature because the $p -$ value (0.859) was greater than $p \le 0.05$. This temperature range supports Mesophilicsoil Microbiotaandplants found in tropical rain forest biomes. This low temperature range in this study can be attributed to the climatic weather condition of IsokoNorth Local Government Area of Delta State, who experience all most year round heavy rainfall, causing most of the lands to be sub-merged in fresh water. This findings corroborates with the research of Preetbyand Nilanjana, 2010) giving a Temperature of 20 $^{0}C - 30$ ^{0}C , for soil Microbiotagrowth in fresh water habitat.

The crude oil contaminated soil had higher organic matter than the uncontaminated soil hence the significant difference where the p- value (0.001) was lesser than ($p \le 0.05$). The increase observed in the contaminated oil may be linked to the increase Crude oil spills on the soils, clumping the soils with the viscous oils. This factor will cause the selective genomic adaptation of soil microbes to increase their mineralization activities of the hydrocarbon contaminants for their energy source and growth. Also the viscosity of the crude oil starve plants of nutrients, making the plants to have a burnt fire like appearance. Even, such soils does not support the presence of animals which may likely die from the viscous Crude oil. The increase in organic matter agrees with (Singh *et al*., 2017), where he reported increase in organic matter in oil impacted soils. There was no significant difference of active Carbon (which is the microbiologically available energy sources) in the soil samples since the (p-value \leq 0.698), was higher than (p \leq 0.05). The low active Carbon observed may be likely due to increase mineralization of the carbon components of the crude oil by the soil microbiota. The findings in this work agrees with (Devatha*et al*., 2019) but disagrees with (Singh *et al*., 2017) who results showed higher carbon concentration in oil contaminated soil. The Nitrate $(NO³)$ levels in the soil samples had significant difference since the value of $(p < 0.021)$ was lower than $p < 0.05$, the low Nitrate level may be married to the increased in soil microbial mineralization activities in using the Carbon content of Crude oil as energy sources for their growth, leading to the concomitants demand for more Nitrogen. Also the Carbon in the Crude oil reacts with the little available nitrogen to form nitrate ions (NO-³) which gets evaporated further leading to low levels of nitrate in such soils. In addition such soils may not support the growth of Nitrogen fixing bacteria and consequently plant growth and may affect the quick Bioremediation of such soils because Nitrogen is a limiting factor in Bioremediation. This current findings is sin line with (Devatha*et al*., 2019) who reported similar findings of low Nitrate concentrations in oil polluted soils. The available phosphorus (AP) in the soil samples showed no significant difference since (P= \leq 0.072) was higher than (p \leq 0.05). This decrease available phosphorus may be due to the activities of soil Microbiotautilizing considerable amount of available Phosphorus to degrade the crude oil hydrocarbons contaminants in the soils, findings that agrees with (Devatha*et al*., 2019). The Potassium (K^+) concentration in the soil showed significant difference since the(p- value of 0.001) was far lower than $(p < 0.05)$. This low level of Potassium in the Crude oil polluted soil may be due to the viscous Crude oil clumping of the soil and low aeration, thus preventing the release of Potassium into the soil to be taken up by plants. This current work is in line with (Singh *et al*., 2019) who also reported very trace amount of Potassium in crude oil polluted soils.

Sulphate in the soil is the easily absorbable form of organic sulphur important in the formation of sulphur containing Amino acids like Methanione, Cystine which are in co-operated into proteins. The sulphate concentration in the soil samples showed a significant difference since $p \le 0.05$. the Sulphatevalues observed in this work which was below the thresh hold value of 10ppm is attributed to crude oil spills on the soils which causes lack of oxidation of organic Sulphurinto Sulphate. Also low levels of Sulphatemay be attributed to the activities of soilMicrobiotain using the available Sulphatefor their protein synthesis, whereas the plants are deprived the use of organic Sulphatefor growth. In this work there was higher build-up of Calcium and Magnesium levels in the crude oil contaminated soils than in the uncontaminated soils probably due to the increase activities of soil Microbiotamineralization of the Crude oil contaminants to benign forms, thus leading to the release of calcium and magnesium ions into the affected soils. This report favours the findings of (Akpogidi*et al*., 2007) who reported increase of calcium and magnesium ions in crude oil contaminated soil. Although the level of these ions were high, due to the highly viscous nature of the crude oil, these mineral may not be available to support plants growth.

The physical analysis results of the soil samples (Table 3) revealed varying degrees of significant difference in their p- values. The soil colours though not captured in Table 3 was; theCrude oil contaminated soil had very dark oily colour, due to the presence of the crude oil, while the non- contaminated soil had a whitish oily colour. The oil may be due to the washing off of crude oil from contaminated nearby fields to pristine out laying lands. The moisture contents of the soils showed significant difference since $(p < 0.001)$ was higher than $(p < 0.05)$. The higher moisture content observed in the crude oil contaminated soils is linked to the displacement of air in the oil spilled soils by the viscous crude oil,causing water to flood the top soil without draining into the soil. In addition the high moisture contents may also be connected to the almost all year round rainfall, synonymous to the climate of Delta state. This high moisture contents may cause plant roots rot and also disturb the activities of animals found in such places. This findings favours (Onojake*et al*., 2012) who reported similar finding. Morealso, increased bulk density of the crude oil contaminated soils in this study as higher than the uncontaminated soil causing a significant difference since $(p < 0.021)$ was lower than $(p < 0.05)$. The increased bulk density of the Crude oil contaminate soils may be linked to the settlement of the viscous crude oil into the soil pores, increasing the soil wet weight and liquid contents, a situation that results in increased bulk density of the soil. This situation is unfavourable for plant growth, whereas soil Microbiotaadapts to this challenges via their genetic modification in their genomes. Oyen*et al*., (2013) reported similar findings. The textural class of all the soil samples was Sandy-Clay- Loam (SCL). In the soil particles size, all the soils samples showed no significant difference in the Clay composition since ($p \le 0.312$) was greater than ($p \le 0.05$). The Sand and Silt composition of the soil had statistical difference because ($p < 0.0001$) was far lesser than ($p <$ 0.05). The greater sand composition and average Clay and Silt component of the uncontaminated soil will not only favour plants growth but will also encourage the presence of animals in the area.Whereas the higher Clay, Silt and lower composition of the crude oil contaminated soils may not favour plants growth and the presence of animals due to poor aeration, higher moisture and bulk density, unavailability of nutrient and the viscous toxic nature of the crude oil contents. Even though this condition is worrisome nature's own little Chemist (soil Microbiota) have been linked to possess the genetic capability in thriving in such environment, hence can be useful in the Bioremediation of such Oil polluted soils.

IV. CONCLUSION

From the results of the Physico-Chemical analysis of the soil samples, it can be deduced that Crude oil contamination of the soils as seen in the Iyede-owheOil and Gas field 9 and extension, can alter soils, physical and chemical contents which may havehazardous consequences on the environment,Fauna, Flora of such host communities and may also affect the means of livelihood of the indigenes either immediately or with time

V. RECOMMENDATION

The revelation from the successful physic-chemical analysis of the Crude oil contaminated soils from Iyede-owheOil and Gas field 9 and extension, which is an oil producing community, makes it necessary to put forward the following recommendations.

Firstly, the Federal Government and operating oil companies in the area should review upward any existing compensation package to enable the host community to be adequately compensated.

Secondly the Federal Government and other stake holders should give meaningful financial intervention into Academic research in the area of applying the Genomics of soil Microbiota for the Bioremediation of Crude oil polluted soils

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