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# Bacteriological and heavy metal evaluation of abandoned crude oil–contaminated sites in Gio community, Ogoniland, Nigeria

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## Abstract

The environmental pollution in the Niger Delta has been a course of concern. Microorganisms such as bacteria have proved to be of great benefit in the degradation of petroleum derived hydrocarbons. This study evaluated the bacteriological and heavy metal concentration of abandoned crude oil–contaminated sites in Gio community, Ogoniland, Nigeria. Soil, water, and sediment samples were collected from the sites. pH and selected heavy metals in the samples were monitored. Isolation and biochemical characterization were done to determine the heterotrophic and hydrocarbon utilizing bacteria present in the samples. Soil and sediment samples had pH values of 4.80±0.04 and 4.8±0.07 respectively while the surface and ground water samples had pH values of 6.40±0.216 and 6.50±0.01. Iron had the highest heavy metal concentration in all the samples, especially the sediment (1000.80±0.01 mg/kg) while copper and lead had the lowest concentration of < 0.001mg/kg in all the samples except sediment sample. The total petroleum hydrocarbon in the soil (9114.86±0.036 mg/kg), exceeded DPR intervention limit while sediment (1034.46±0.022 mg/kg), surface water (2.515±0.003 µg/L) and ground water sample (32.38±0.99 µg/L) were below DPR's limit. The soil sample had the highest total culturable heterotrophic bacterial counts and total culturable hydrocarbon utilizing bacterial counts of 5.20 ± 0.21 X $10^8$ CFU/g and 4.00 ± 0.11 X $10^7$ CFU/g, respectively. The following heterotrophic bacteria were isolated and identified from the samples; *Pseudomonas* spp, *Bacillus* spp, *Acidiphilium* spp, *Acidibrevibacterium* spp and *Leptospirillum* spp. This study has shown the presence of indigenous resident bacteria which possess the ability to degrade hydrocarbons. These bacteria can be improved through bioaugmentation and bio stimulation for the bioremediation of these sites.

**Keywords:** Bacteria; Contamination; Crude oil; Hydrocarbons; Heavy metals

## 1. Introduction

Environmental contamination has been a huge threat to both aquatic and terrestrial organisms [1]. Crude oil is one of the contaminants that enter the environment through the activities of man during oil exploration and oil spill during transportation process [2].

Nigeria is one of the major oil producers in Africa. When crude oil is released into the environment, the components are deposited in the soil and surrounding water bodies, thereby altering the normal composition of both biotic and abiotic components of the affected ecosystem [3].

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Contamination of soil and water reduces land available for agriculture thus affecting crop yield and also aquatic lives in the water bodies. In some cases where agricultural activities are performed on contaminated soil, the plants become toxic and the health of the animals in that environment is at risk [1].

Crude oil-contamination also leads to variation in the composition of resident microorganisms in an ecosystem. When crude oil contamination occurs, microbes in such habitat respond to the stimulus [1]. The response could either be positive or negative. In positive response, the microorganisms especially bacteria maintain their ecological niche due to their ability to withstand the introduced stress. This adaptive measure enables the organisms to source their nutrients from the components of the crude oil. When the response is negative, the bacterial species are sensitive to the components of the crude oil, so they cannot withstand the stress, which may result to their complete elimination from the habitat [4].

Crude oil contamination drastically enhances heavy metal concentration in soil and water bodies [4]. Heavy metals such as zinc, chromium, nickel, mercury, iron and copper are components of crude oil, though in low concentrations. It has been revealed that heavy metals accumulate in the soil, especially when there is an oil spillage. The absorption of these heavy metals is facilitated by low soil pH, which can be accelerated by bacteria products of metabolism and organic matter [4].

Several researchers have reported on the bacteriological assessment of crude oil-contaminated soil such as Ali *et al*. [1]; Erdogan and Karaca [2]; and Cocarta *et al*. [3], but little is known about the bacteriological and heavy metal evaluation of abandoned crude oil-contaminated sites in Gio community, Ogoniland, Nigeria. Hence, this study is aimed at evaluating the bacteriological and heavy metal composition of abandoned crude oil-contaminated site in Gio community, Ogoniland, Nigeria. The outcome of this study would provide vital information on bacterial species which can survive in the presence of high hydrocarbon and heavy metal concentration.

## 2. Material and methods

### 2.1. Study Site Description

Samples were collected from abandoned crude oil-polluted sites from oil pipelines in Gio community, Ogoni land. The area covers over about 900 $km^2$ in Rivers State, Southern Nigeria. The co-ordinates of the sampling points evaluated with the Global Positioning System (GPS) are; Gio 1 N $04^0$ $38^1$ $45^{11}$ E $07^0$ $14^1$ $11^{11}$ and Gio II N $04^0$ $38^1$ $45^{11}$ E $07^0$ $14^1$ $13^{11}$, for soil, water and sediment samples.

### 2.2. Sample Collection

The collection of soil, water, and sediment samples were done aseptically using the appropriate apparatus. Soil samples were collected at 0 – 30 cm depth using soil auger from four different points of the site, made into a composite sample and put into sterile black polyethylene bags. Sediment samples were also collected using an Eckman grab, ground water sample was collected at about 220 m depth below the soil surface from four different points and mixed together while surface water sample was taken against the route of the water flow into sterile screw cap bottles. All samples were then conveyed to the laboratory at 4 °C in an ice chest [5].

### 2.3. Physico - chemical Analysis of Sample

The pH of the samples was analyzed using the method employed by Bates [6] with the aid of pH meter S-901.

### 2.4. Screening for Heavy Metal Concentration

Heavy metal concentrations for lead, zinc, copper, iron, nickel, chromium and cadmium were monitored in each sample.

#### *2.4.1. Soil and sediment sample extraction for heavy metal analysis*

This was done using the method of Singh [7]. 10 g of each air-dried sample was mixed with 0.2M nitric acid solution for 60 minutes in a digestion flask under high temperature of about $80^0$ C. The digests were then filtered separately through a filter paper and the volume made up to 100 mL by adding distilled water [7]. The filtrates were analyzed using the atomic absorption spectrophotometer (AAS) GBC 908PBMT, Australia. The spectrophotometer operational setting was done in compliance with the manufacturer's instructions and was calibrated with analytical grade metal standard stock solutions (1 $mg/dm^3$) in triplicates.

*2.4.2. Water sample extraction for heavy metal analysis*

This was done according to the method employed by Alinnor and Nwachukwu [5]. 500 mL of each water sample was transferred into a 1litre separating flask. 30 µg/mL of surrogate was mixed in 1 mL of dichloromethane (DCM) and then added into the flask containing the sample then an extra 20 mL of DCM was added into the mixture. The mixture was swirled vigorously and the built-in pressure was released gradually then allowed to settle for few minutes resulting in the formation of two separate layers in the flask. The lower layer which is the extract of the sample was collected into a beaker using a filter paper. The filtrate was then allowed to concentrate to 1 mL by evaporation in a fume cupboard [8]. The concentrated filtrates were then analyzed using the atomic absorption spectrophotometer (AAS) GBC 908PBMT, Australia.

### 2.5. Screening for Total Petroleum Hydrocarbon (TPH)

The screening was carried out using 1 g/ 1 mL of the samples, dissolved in 10 mL of hexane and mixed for ten minutes with the aid of a rotary shaker, then filtered with a Whatman no 4-filter paper. 1 mL of the filtrate was added into 50 mL of hexane and the absorbance measured using a HACH DR/2010 Spectrophotometer at 460 nm while hexane without the sample was used as blank [9].

### 2.6. Enumeration of Culturable Bacterial Population

The total culturable heterotrophic bacteria (TCHB) were evaluated by culturing on nutrient agar (Accumedia, Sweden) plates. The media preparation was done following the guidelines of the manufacturer. A serial diluted sample of 100 µL ranging from $10^{-3}$ – $10^{-6}$ dilutions of each sample was inoculated on the prepared agar media, followed by incubation at 30 $^0$C for 24 h for TCHB. After incubation, the plates with distinct colonies ranging between 30– 300 were picked [10] [11]. Total viable cell (TVC) was enumerated and expressed in CFU/g and CFU/mL.

Also, total cultural hydrocarbon-utilizing bacteria (TCHUB) were counted using Bushnell Haas Agar (with 1 % v/v Bonny light crude oil as sole carbon source) modified with 0.01 % w/v nystatin [10]. Total viable cell (TVC) was enumerated and expressed in CFU/g and CFU/mL using the formula:

$$\text{TVC}\left(\frac{\text{CFU}}{\text{g}}\text{ or }\frac{\text{CFU}}{\text{mL}}\right) = \frac{\text{Number of colonies x dilution factor}}{\text{Volume of inoculum}} \quad \text{(Equation 1)}$$

The identification was authenticated with the aid of Bergey's Manual of Determinative Bacteriology [12].

### 2.7. Statistical Analysis

The data gotten from this study were expressed in mean then presented using tables. The mean of the variables was subjected to one-way analysis of variance (ANOVA). The results were considered statistically significant at 95% confidence interval ($\alpha = 0.05$) All data analyses were done using the GraphPad Prism software version 8.02.

## 3. Results

The result gotten from the pH analysis of all the samples is presented in Table 1. From the result, samples A and B had pH value of 4.80±0.004 and 4.80±0.07 respectively; sample C had pH value of 6.40±0.216 while sample D had pH value of 6.50±0.01. This showed that samples A and B were more acidic than samples C and D which can be described as being slightly acidic.

**Table 1** pH analysis of the samples

| Sample | pH value |
|---|---|
| A | 4.80±0.04 |
| B | 4.80±0.07 |
| C | 6.40±0.216 |
| D | 6.50±0.01 |

Key: sample A: Gio soil; sample B: Gio sediment; sample C: Gio surface water; sample D: Gio ground water.

The results of the heavy metal analysis for all the samples are presented in Table 2. Sample A had the highest iron concentration of 254.61±0.02 mg/kg, followed by chromium concentration (13.43±0.022 mg/kg), nickel (3.39±0.008 mg/kg), cadmium (1.87±0.016 mg/kg) and zinc (1.66±0.016 mg/kg). The concentration of copper and lead were <0.001 mg/kg each which is negligible. Iron also had the highest concentration of 1000.80±0.01 mg/kg in sample B, followed by chromium (8.46±0.00 mg/kg), zinc (3.45±0.029 mg/kg), cadmium (3.24±0.029 mg/kg), nickel (2.58±0.014 mg/kg), copper (0.79±0.014 mg/kg) while lead was <0.001 mg/kg. For sample C, iron also recorded the highest concentration of 247.68±0.014 µg/L while the rest of heavy metals had negligible concentration such as zinc (0.88±0.01 µg/L), chromium (0.32±0.016 µg/L), nickel (0.09±0.00 µg/L), lead (< 0.001 µg/L), copper (< 0.001 µg/L) and the concentration of cadmium was below detection limit (BDL). Sample D recorded <0.001 µg/L for all the heavy metals except zinc and iron concentrations which had 0.79±0.002 µg/L and 0.42±0.001 µg/L respectively.

**Table 2** Heavy metal concentrations of samples

| Heavy metal | Sample A (mg/kg) | Sample B (mg/kg) | Sample C (µg/L) | Sample D (µg/L) |
|---|---|---|---|---|
| Pb (Lead) | <0.001 | <0.001 | <0.001 | <0.001 |
| Zn (Zinc) | 1.66±0.016 | 3.45±0.029 | 0.88±0.01 | 0.79±0.002 |
| Cu (Copper) | <0.001 | 0.79±0.014 | <0.001 | <0.001 |
| Fe (Iron) | 254.61±0.02 | 1000.8±0.01 | 247.68±0.014 | 0.42±0.001 |
| Ni (Nickel) | 3.39±0.008 | 2.58±0.014 | 0.09±0.00 | <0.001 |
| Cr (Chromium) | 13.43±0.022 | 8.46±0.00 | 0.32±0.016 | <0.001 |
| Cd (Cadmium) | 1.87±0.016 | 3.24±0.029 | BDL | <0.001 |

BDL= Below detection limit. Key: sample A: Gio soil; sample B: Gio sediment; sample C: Gio surface water; sample D: Gio ground water.

The result of total petroleum hydrocarbon concentration (TPH) in all the samples is presented in Table 3. From the result, sample A had the highest TPH concentration of 9114.86±0.036 mg/kg which exceeded the DPR intervention limits, followed by sample B (1034.46±0.022 mg/kg), then sample D which had TPH concentration of 32.38±0.99 µg/L while sample C had the lowest TPH concentration of 2.515±0.003 µg/L.

**Table 3** Total petroleum hydrocarbon concentration of Gio samples

| Sample | TPH (mg/kg or µg/L) | DPR Intervention Limit (mg/kg or µg/L) |
|---|---|---|
| A | 9114.86±0.036 | 5000 |
| B | 1034.46±0.022 | 5000 |
| C | 2.515.00±0.003 | 600 |
| D | 32.38±0.99 | 600 |

TPH=Total petroleum hydrocarbon, DPR=Department of petroleum resources
Key: sample A: Gio soil; sample B: Gio sediment; sample C: Gio surface water; sample D: Gio ground water.

The results obtained from total culturable heterotrophic bacterial counts and total culturable hydrocarbon utilizing bacterial counts are presented in Tables 4 and 5. Sample A had the highest TCHBC of 5.20 ±0.21 X $10^8$ CFU/g, sample B had TCHBC of 2.20±0.34 X $10^8$ CFU/g, sample C had TCHBC of 1.60±0.16 X $10^8$ CFU/mL while sample D had the lowest TCHBC of 1.31±0.022 X $10^8$ CFU/mL. The result of total culturable hydrocarbon utilizing bacterial counts (TCHUBC) revealed that sample A had the highest TCHUBC value of 4.00±0.11 X $10^7$ CFU/g, followed by sample C (3.7±0.17 X $10^7$ CFU/mL) and sample D (2.8±0.43 X $10^7$ CFU/mL) while sample B had the lowest TCHUBC of 1.10 ± 0.14 X $10^7$ CFU/g. The following heterotrophic bacterial genera were isolated and identified in the samples; *Pseudomonas* spp, *Bacillus* spp, *Acidiphilium* spp, *Brevibacterium* spp and *Leptospirillum* spp, with *Pseudomonas* spp having the highest percentage occurrence followed by *Acidiphilium* spp. *Pseudomonas* and *Bacillus* are well hydrocarbon degrading bacteria [13].

**Table 4** Total culturable heterotrophic bacterial counts of samples

| Samples | TCHBC (CFU/g or CFU/mL) |
|---|---|
| A | $5.20 \pm 0.21 \times 10^8$ |
| B | $2.2 \pm 0.34 \times 10^8$ |
| C | $1.60 \pm 0.16 \times 10^8$ |
| D | $1.31 \pm 0.022 \times 10^8$ |

Values represent the mean ± standard deviation from three replicate counts
Key: sample A: Gio soil; sample B: Gio sediment; sample C: Gio surface water; sample D: Gio ground water

**Table 5** Total culturable hydrocarbon utilizing bacterial counts of samples

| Sample | TCHUBC (CFU/g or mL) |
|---|---|
| A | $4.00 \pm 0.11 \times 10^7$ |
| B | $1.10 \pm 0.14 \times 10^7$ |
| C | $3.7 \pm 0.17 \times 10^7$ |
| D | $2.8 \pm 0.43 \times 10^7$ |

Values represent the mean±standard deviation from three replicate counts
Key: sample A: Gio soil; sample B: Gio sediment; sample C: Gio surface water; sample D: Gio ground water

## 4. Discussion

The result of pH analysis showed that samples A and B were moderately acidic while samples C and D were slightly acidic. This could be due to the by-products of bacteria metabolism and the presence of organic substances in the environment as reported by Ogbo and Okhuoya [14]. Meanwhile, the pH values obtained from this study were lower than the values reported by Ekperusi *et al* [4], who analyzed crude oil-contaminated soil and obtained pH of 5.39. The slight variation in pH could be linked to the soil structure and texture as explained by Ekperusi *et al*. [4]. The shift in the normal pH of water from neutral to slightly acidic could also be as a result of hydrocarbon contamination [4]. This acidic pH in the soil can affect the resident bacterial community composition in that environment. For the aquatic environment, a slightly acidic pH could also cause the death or migration of aquatic lives. The slightly acidic pH of the ground water can affect the quality of water available for drinking and domestic use in Gio community negatively.

Most of the heavy metals detected in this study have been described as vital for optimum functioning of soil microbes [1] and plants species except for cadmium and lead which are known to be toxic even at low concentrations as explained by Ekperusi *et al* [4]. These heavy metals have serious influence on the microbial distribution and fertility of the ecosystem [15]. Though some heavy metals play a significant role in the proliferation of both micro and macro-organisms in the soil, research has revealed their ability to pose threat to the existence of microbial life at high concentration according to Mustafa *et al*. [16], who also reported that crude oil contamination enhances the concentration of heavy metals.

The mobility of these heavy metals in the soil is enhanced by low pH. This shows that the heavy metals detected in this study are available for absorption by plants and assimilation by microorganisms [4]. These heavy metals can also be transferred to man through the food chain, when aquatic organisms and plants are consumed [3]. The presence of zinc and iron at low concentrations in the ground water could be due to the leaching process which is responsible for transporting compounds from the top soil to the subsoil then down to the aquifer.

The high concentration of TPH present in sample A shows a high degree of crude oil contamination which exceeds the DPR intervention limit for soil. This high concentration is therefore unfriendly to the ecosystem, and is capable of altering soil fertility and productivity [17]. The low TPH concentration in sample B and C could be as a result of dilution of the aquatic environment while that of sample D could be due to poor leaching in the soil [15].

The total culturable heterotrophic bacterial count (TCHBC) and total culturable hydrocarbon bacterial count (TCHUBC) was highest in sample A. This proves the ability of the soil to harbor diverse and high population of bacteria despite the presence of physical forces or changes in chemical composition as a result of crude oil-contamination [18]. The TCHBC of sample B was higher than that obtained from samples C and D. This could be attributed to the sedimentation of nutrients which encourages microorganisms to settle and thrive at the bottom of aquatic systems more than the surface water which experiences dilution as a result of continuous tidal movements and runoffs. The difference in the TCHBC and TCHUBC of each sample was due to the uniqueness of the various environments of sample collection and the different physical and chemical compositions of these ecosystems. The changes in TCHUBC of the different samples can also be attributed to the variation in the degree of crude oil-contamination experienced by these sample sites and the duration of pollution [13]. The indigenous bacterial genera isolated from these sites are; *Pseudomonas, Bacillus, Acidiphilium, Brevibacterium,* and *Leptospirillum.* Similar bacteria were isolated by Ekhaise and Nkwelle [19] from hydrocarbon contaminated workshop soil in Benin, Nigeria. The ability of these genera of bacteria to proliferate in these sites could be ascribed to their adaptive features, which enabled them to survive despite the toxic effects of the hydrocarbons and heavy metals present in these environments [19].

## 5. Conclusion

This study has revealed that crude oil contamination has tremendous impact on the pH, soil structure, heavy metal compositions and microbial distribution in every environment. The bacteria which are capable of utilizing hydrocarbons as energy and carbon source secrete enzymes that help them to adapt to the environments. However, further study is necessary to determine the biodegradative potentials of the bacterial isolates with the aim of applying them for use in the bioremediation of these impacted sites.

## Compliance with ethical standards

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### *Disclosure of conflict of interest*

The authors declare that they do not have any competing interest with regards to this study.

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