



(RESEARCH ARTICLE)



## Bacteriological and heavy metal assessment of a tropical crude oil- polluted soil, water and sediment

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### Abstract

Crude oil-polluted environment serves as a reservoir of microbial species, especially bacteria. Determining these bacterial genera is of great benefit to environmental assessment and recovery. This study evaluated the bacteria and selected heavy metals present in crude oil-polluted sites in K-dere community, Ogoniland. The study was carried out at the Department of Microbiology, University of Port Harcourt, Rivers State, Nigeria. Crude oil-polluted soil, water, and sediment samples were collected from K-dere community, Ogoniland. The pH and heavy metals composition of each sample was determined using pH meter and spectrophotometer, respectively, while isolation and biochemical characterization were done to evaluate the heterotrophic and hydrocarbon utilizing bacteria present in each sample. Results revealed that iron (Fe) had the highest concentration in the soil ( $898.77 \pm 0.022$  mg/kg) and sediment ( $1556.7 \pm 0.163$  mg/kg) samples, while lead (Pb) had the lowest overall concentration. The concentration of total petroleum hydrocarbon (TPH) ( $10410.5$  mg/kg) in soil sample exceeded the DPR standard limit of 5000 mg/kg for soil. The results of total culturable heterotrophic bacterial counts (TCHBC) and total culturable hydrocarbon utilizing bacterial counts (TCHUBC) showed that soil and sediment samples had the highest TCHBC and TCHUBC values of  $2.66 \pm 0.03 \times 10^8$  CFU/mL and  $4.7 \pm 0.14 \times 10^7$  CFU/mL respectively. Morphological and biochemical characterization of the isolates revealed the presence of *Pseudomonas* spp, *Bacillus* spp, *Acidiphilium* spp, *Mycobacterium* spp and *Leptospirillum* spp in the samples with *Pseudomonas* spp having the highest percentage occurrence. This study has revealed the presence of useful bacterial species in the sampled sites which can be harnessed for an *in situ* cleaning of crude oil- contaminated site, especially in the tropical region.

**Keywords:** Bacteria; Heavy metals; Hydrocarbon; Niger Delta; Pollution

### 1. Introduction

Crude oil comprises of hydrocarbon compounds which occur in nature as saturated alkanes and unsaturated alkenes and alkynes [1]. It is formed from the remains of plant and animals which have been buried very deep in the soil or water and has undergone heat pressure of the earth crust for a long duration, especially in the tropical region like Nigeria in West Africa. Crude oil when refined provides useful components such as gas, gasoline, kerosene, diesel, engine oil and naphthalene [2].

Crude oil pollution occurs when there is an introduction of crude oil into the soil and water, which interferes with the structure and texture of the soil, thereby affecting soil fertility, toxification of aquatic organisms, and general alteration in the natural characteristics of water bodies, which renders it unfit for man's use [1].

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Crude oil pollution affects microbial distribution in the soil and water. Microorganisms such as bacteria that inhabit soil and water bodies can either adapt and proliferate or become vulnerable and eliminated, when there is crude oil pollution. Those bacteria that are able to adjust to crude oil contamination by structural and physiological modification thrive due to their ability to assimilate hydrocarbon to obtain carbon and energy [3].

Research has also shown that heavy metals such copper, lead, chromium etc., are released during oil excavation. These heavy metals can be absorbed by plants via their roots. These heavy metals are very harmful to plants and animals. In plants, they can cause stunted growth and death while in animals; they are capable of causing genetic mutation and cancer [1].

Several researchers have studied the bacteriological assessment of a tropical crude oil-polluted soil [1] – [4] but more needs to be documented on the bacteriological assessment and heavy metals present in tropical crude oil-contaminated sites. Hence, this research is aimed at evaluating the bacterial genera and selected heavy metals present in a tropical crude oil-polluted soil, water and sediment. The result obtained from this study will help accelerate the remediation of crude oil-polluted environment.

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## **2. Materials and Methods**

### **2.1. Site Description**

Samples were collected from sites that have been contaminated due to crude oil spill during oil excavation by oil industry in K-dere community, Ogoni land. The land covers over 1000 km<sup>2</sup> in Rivers State, Southern Nigeria. The GPS co-ordinates of the sample sites are: K-dere I N 4° 41' 59" E 07° 14' 0" and K-dere II N 04° 14' 0" E 07° 14' 0".

### **2.2. Sample Collection**

Crude oil contaminated soil, water, and sediment were collected aseptically using suitable apparatus. Soil samples were taken at 0 – 30 cm depths using soil auger at different points and were brought together, forming complex samples, (homogenous mixture) then put into sterile black polyethylene bags. Sediment samples were also gotten, with the aid of an Eckman grab, surface water samples were taken against the route of the water flow into sterile screw cap bottles while ground water samples were collected at a depth of 200 m below the soil surface. All the samples were moved to the laboratory at 4 °C in an ice chest [5].

### **2.3. Physico-chemical Analysis of Sample**

The pH of the samples were evaluated using the method employed by Bates [6] with the aid of a pH meter (S-901).

### **2.4. Screening for Heavy Metal Concentration**

Heavy metal concentrations (Pb, Zn, Cu, Fe, Ni, Cr, Cd) were monitored according to the method employed by Singh [7]. 10 g of air dried sample was mixed with 0.2 M nitric acid solution for 60 minutes in a digestion flask under high temperature of about 80° C for the soil and sediment samples while for the water samples, 10 mL of the sample was mixed with the nitric acid solution and boiled. The digest was then filtered 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.

### **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 [4].

### **2.6. Enumeration of Culturable Bacterial Population**

The total culturable heterotrophic bacteria (TCHB) were evaluated by culturing on nutrient agar (Accumedia, Sweden) plates, respectively. The media preparation was done following the guidelines of the manufacturer. A serial diluted sample of 100 µL ranging from 10<sup>-3</sup> – 10<sup>-6</sup> dilutions of each sample was inoculated on the prepared agar media, followed by incubation at 30 °C for 24 h for TCHB. After incubation, the plates with distinct colonies ranging between 30– 300 were picked [8][9]. 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 [8]. Total viable cell (TVC) was enumerated and expressed in CFU/g and CFU/mL using the formula:

$$\text{TVC (CFU/g or CFU/mL)} = \frac{\text{Number of colonies} \times \text{dilution factor}}{\text{Volume of inoculum}} \text{ (Equation 1)}$$

The identification was authenticated with the aid of the Bergey's Manual of Determinative Bacteriology [10].

## 2.7. Statistical Analysis

The data obtained from this study are expressed in mean and presented using tables. The mean of the variables were subjected to one-way analysis of variance (ANOVA), followed by Turkey's multiple comparative test. The results were considered statistically significant at 95% confidence level ( $P < 0.05$ ). All data analysis were done with the GraphPad Prism software version 8.02.

## 3. Results

The result obtained from the pH analysis of the samples is presented in Table 1. From the results, sample B had the lowest pH (highly acidic) of  $2.1 \pm 0.08$ , followed by sample A ( $3.4 \pm 0.15$ ). Sample C had the highest pH value of  $6.0 \pm 0.75$  while sample D had a pH value of  $5.6 \pm 0.02$ .

**Table 1** PH analysis of the samples

Parameter	Sample A	Sample B	Sample C	Sample D
pH	$3.40 \pm 0.15$	$2.10 \pm 0.08$	$6.00 \pm 0.75$	$5.6 \pm 0.02$

Key: sample A: K-dere soil; sample B: K-dere sediment; sample C: K-dere surface; sample D: K-dere ground water.

The results of heavy metal analysis are presented in Table 2. From the results, sample A had the highest iron concentration of  $898.77 \pm 0.022$  mg/kg, followed by chromium ( $11.13 \pm 0.021$  mg/kg), nickel ( $4.55 \pm 0.037$  mg/kg) and zinc ( $4.52 \pm 0.02$  mg/kg). Other heavy metals monitored were present in low concentrations such as copper ( $1.39 \pm 0.024$  mg/kg), cadmium ( $1.94 \pm 0.024$  mg/kg) and lead ( $1.06 \pm 0.014$  mg/kg). For sample B, iron also had the highest heavy metal concentration of  $1556.70 \pm 0.163$  mg/kg, followed by chromium ( $10.10 \pm 0.082$  mg/kg), cadmium ( $6.40 \pm 0.141$  mg/kg) and nickel ( $3.34 \pm 0.028$  mg/kg). Others also occurred at negligible concentrations such as zinc ( $1.79 \pm 0.021$  mg/kg), copper ( $0.52 \pm 0.016$  mg/kg) and lead ( $< 0.001$  mg/kg). Overall, sample A had higher heavy metal concentration than the sample B except for iron and cadmium concentrations where sample B recorded higher values than sample A.

**Table 2** Heavy metal concentrations of sampled sites

Heavy metal	A (mg/kg)	B (mg/kg)	C ( $\mu\text{g/L}$ )	D ( $\mu\text{g/L}$ )
Lead (Pb)	$1.06 \pm 0.014$	$< 0.001$	$< 0.001$	$2.813 \pm 0.23$
Zinc (Zn)	$4.52 \pm 0.02$	$1.79 \pm 0.021$	$< 0.001$	$5.48 \pm 0.03$
Copper (Cu)	$1.39 \pm 0.024$	$0.52 \pm 0.016$	$< 0.001$	$0.33 \pm 0.001$
Iron (Fe)	$898.77 \pm 0.022$	$1556.7 \pm 0.163$	$< 0.001$	$4.12 \pm 0.02$
Nickel (Ni)	$4.55 \pm 0.037$	$3.34 \pm 0.028$	BDL	$0.85 \pm 0.02$
Chromium (Cr)	$11.13 \pm 0.021$	$10.1 \pm 0.082$	$0.02 \pm 0.00$	$7.52 \pm 0.21$
Cadmium (Cd)	$1.94 \pm 0.024$	$6.40 \pm 0.141$	$< 0.001$	$0.004 \pm 0.00$

BDL= below detection limit. Key: sample A: K-dere soil; sample B: K-dere sediment; sample C: K-dere surface water; sample D: K-dere ground water.

The heavy metals monitored in sample C and D recorded very low concentrations. Chromium had the highest value of heavy metal concentration of  $0.02 \pm 0.00 \mu\text{g/L}$  and  $7.5 \pm 0.21 \mu\text{g/L}$  for samples C and D respectively. lead, zinc, copper, iron and cadmium had concentration value of  $<0.001 \mu\text{g/L}$  while nickel was below detection limit (BDL) for sample C but in sample D, lead concentration was  $2.813 \pm 0.23 \mu\text{g/L}$ , zinc  $5.482 \pm 0.03 \mu\text{g/L}$ , copper  $0.33 \pm 0.001 \mu\text{g/L}$ , iron  $4.12 \pm 0.02 \mu\text{g/L}$ , nickel  $0.85 \pm 0.02 \mu\text{g/L}$  and cadmium  $0.004 \pm 0.00 \mu\text{g/L}$ . Overall, sample D had higher heavy metal concentrations compared to sample C.

The total petroleum hydrocarbon concentration (TPH) for all the samples is presented in Table 3. Results revealed that sample A had the highest TPH concentration of 10410.5 mg/kg, which exceeded the standard intervention limit specification by the Department of Petroleum Resources (DPR) [11], followed by sample B (546.66 mg/kg) and sample D (62.787 mg/L) while sample C had the lowest and negligible TPH concentration of 0.0837 mg/L.

**Table 3** Total petroleum hydrocarbon concentration of K-dere samples

Samples	TPH (mg/kg or mg/L)	DPR Limit (mg/kg or mg/L)
A	10410.5	5000
B	546.66	5000
C	0.0837	600
D	62.787	600

DPR = Department of petroleum resources. Key: sample A: K-dere soil; Sample B: K-dere sediment; sample C: K-dere surface water; Sample D: K-dere ground water.

### 3.1. Bacterial Isolates from the samples

The results obtained from total culturable heterotrophic bacterial counts (TCHBC) and total culturable hydrocarbon utilizing bacterial counts (TCHUBC) are presented in Tables 4 and 5. The result showed that sample A had the highest TCHBC of  $2.66 \pm 0.03 \times 10^8$  CFU/mL, samples B and C had similar TCHBC values of  $1.30 \pm 0.23 \times 10^8$  CFU/mL and  $1.30 \pm 0.30 \times 10^8$  CFU/mL respectively while sample D had TCHBC of  $1.22 \pm 0.02 \times 10^7$ . The result of total culturable hydrocarbon utilizing bacterial counts (TCHUBC) showed that sample B had the highest TCHUBC of  $4.7 \pm 0.14 \times 10^7$  CFU/mL, followed by sample A ( $3.8 \pm 0.54 \times 10^7$  CFU/mL), while samples C and D had TCHUBC of  $2.8 \pm 0.64 \times 10^7$  CFU/mL and  $1.64 \pm 0.51 \times 10^6$  CFU/mL respectively. The following bacterial genera were isolated and identified from the samples: *Pseudomonas* spp, *Bacillus* spp, *Acidiphilium* spp, *Mycobacterium* spp and *Leptospirium* spp. Two bacterial species out of the five identified isolates are well known hydrocarbon degraders (*Pseudomonas* spp, and *Bacillus* spp).

**Table 4** Total culturable heterotrophic bacterial counts of sampled sites

Samples	TCHBC (CFU/g or ml)	Log TCHBC
A	$2.66 \pm 0.03 \times 10^8$	8.424
B	$1.30 \pm 0.23 \times 10^8$	8.113
C	$1.30 \pm 0.30 \times 10^8$	8.113
D	$1.22 \pm 0.02 \times 10^7$	7.086

**Table 5** Total culturable hydrocarbon utilizing bacteria counts of sampled sites

Sample	TCHUBC (CFU/g or ml)	Log TCHUBC
A	$3.8 \pm 0.54 \times 10^7$	7.579
B	$4.7 \pm 0.14 \times 10^7$	7.672
C	$2.8 \pm 0.64 \times 10^7$	7.447
D	$1.64 \pm 0.51 \times 10^6$	6.215

Values represent the mean  $\pm$  standard deviation from three replicate counts  
Key: sample A: K-dere soil; sample B: K-dere sediment; Sample C: K-dere surface water; Sample D: K-dere ground water.

#### 4. Discussion

The pH values recorded during the analysis of the samples revealed a drastic decrease in pH in samples A and B (Table 1). This showed a highly acidic environment which could be attributed to the high crude oil contamination of the sampled sites. This observation agrees with the findings of Achife *et al.* [1], who investigated microbial population in soil and water around petroleum depot. The high acidity favors the dissolution of heavy metals such as cadmium, copper, mercury, lead, and zinc, which are highly detrimental to the growth and optimum yield of crops. The pH values of soil and sediment samples differ from the pH values obtained by Chikere *et al.* [2], who analyzed soil samples from Bodo community Ogoniland spill site, and obtained pH value of 7.8, which is slightly alkaline. The variation in pH values could be attributed to variation in the degree of oil contamination [1].

The presence of lead in the soil sample showed the high degree of toxicity, because lead is generally known as a toxicant. Though the amount is low, but can still impact negatively on the living organisms including microbes in the soil [12]. Report has shown that lead is retained in the soil for a long period of time due to its conversion to lead sulphate and its combination with organic matter in the soil to form inert compounds [12].

The concentration of zinc in soil sample is capable of causing serious threat to the soil structure and texture. It has also been reported that heavy metals such as zinc, cadmium, chromium, and nickel in their oxides and hydroxides forms may not cause serious harm to plants and other living organisms, but chemical reduction and oxidation due to alteration in soil pH can accelerate toxicity [13]. The presence of copper in soil and sediment samples indicated a strong attraction that exists between copper and organic carbons and oxides in soil, though the concentration is lower than the value obtained by Radulescu *et al.* [12], which could be due to variations in soil structure and texture. The high level of chromium in soil and sediment samples could be attributed to its ability to exist in the form of carbonate, which enhances accessibility to plants. The high concentration of iron in sediment and soil samples shows that the sites are highly acidic, because iron is known to persist in low pH environment. It is obvious that iron is required by plants in low concentration for optimum proliferation, but high concentration results in iron toxicity [12]. The high concentration of iron could cause manganese deficiency in plants, because the two micronutrients are highly competitive. Other heavy metals such as nickel, cadmium and chromium are well known toxic metals which are capable of causing stunted growth, yellowing and wilting of leaves, and inhibition of cell division, especially high concentration of nickel.

The presence of heavy metals in the ground water could be caused by leaching and the ability of the metals to be retained within the soil profile depth thus affecting the aquifer [14]. This will affect the water quality of the domestic water sources in K-dere community and can cause heavy metal poisoning to humans and aquatic lives when the water is consumed. The metals could also be absorbed and accumulate in plants through their root nodules which could also be transferred to humans and animals when the plants are consumed. The extremely low concentration of the heavy metals in the surface water sample may have been caused by dilution effect of the tidal water movement [15].

The heavy metals detected in this study could be traced to spillage due to oil excavation and their accumulation is enhanced by certain ecological factors like climate, soil texture and structure and also the biochemical reactions that occur in the environment. This could cause an ecological shift in the microbial composition of these environments and could also reduce the microbial abundance as a result of poor adaptation by some microorganisms.

The concentration of TPH (10410.5 mg/kg) in the soil sample exceeded the DPR standard limit of 5000 mg/kg [11]. This shows the presence of high hydrocarbon content that is yet to be degraded in that environment [16]. Research has shown that crude oil interferes with the pH and oxygen concentration in the soil, which could also cause a shift in the microbial species composition of the soil [4]. Bacterial species that are unable to modify their structure and functions are eliminated, while the fittest survives [3]. The high concentration of TPH above DPR standard limit [11] could also bring about nutrient deficiency in the affected regions, especially essential nutrients such as nitrogen and phosphorus, which plants must utilize for optimal productivity [3]. Low concentrations of nitrogen and phosphorus has great impact during bioremediation, because, the metabolism of the bacterial species is drastically reduced [3].

The high TCHBC and TCHUBC, particularly in soil and sediment samples showed that the sites contained diverse bacterial community [1]. The TCHBC in soil sample is higher than those found in the sediment and water samples. This could be attributed to the static nature of the soil environment, which tends to restrict leaching of nutrients required for the growth of bacterial species, unlike aquatic environment that permits leaching of nutrients. This agrees with the observation made by Achife *et al.* [1]. There was substantial number of TCHUBC which revealed that crude oil contamination has serious effect on the distribution of microorganisms in every ecosystem [16]. Among the bacteria isolated, *Pseudomonas* species dominated, which corresponds with the report of Achife *et al.* [1] and Ezekoye *et al.* [18], who reported that *Pseudomonas* species produce enzymes that are capable of neutralizing toxic effects of heavy metals

and also utilize hydrocarbons from crude oil. The TCHB counts recorded in the samples could be attributed to inability of the bacterial species to adapt and compete favorably in the crude oil-polluted environment. The low TCHB and TCHUB counts recorded in the ground water sample could be due to the sampling depth of the environment which may have encouraged anoxic conditions. These hydrocarbon utilizers can be harnessed, improved and applied in the cleanup of crude oil-impacted sites [18] [17].

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## 5. Conclusion

This study has revealed the presence of bacterial species and heavy metals in crude oil-impacted sites in K-dere community. Also, the presence of known hydrocarbon degrading bacterial species like *Pseudomonas* in this environment suggests that they harbor beneficial bacterial species that can secrete the required enzymes capable of degrading hydrocarbons and bioaccumulating heavy metals. These bacterial species can be harnessed, improved and applied in the bioremediation of crude oil derived hydrocarbon impacted ecosystem.

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## Compliance with ethical standards

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### *Disclosure of conflict of interest*

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

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