



## **Microbiological and Physicochemical Assessment of Sediment in Bodo Community, Rivers State, Niger Delta**

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### **Authors' contributions**

This work was carried out in collaboration among all authors. Authors EEE and ICE designed the study. Authors EEE, UOE, ENM, GEU, VOE and IUB managed different aspects of the bench work, performed the statistical analysis, wrote the protocol and first draft of the manuscript. Authors EEE, UOE and IUB managed the literature searches. All authors read and approved the final manuscript.

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### **ABSTRACT**

Crude oil pollution is common place in the Niger Delta despite well known adverse effects on aquatic and terrestrial biodiversities. Triplicate sediment samples were obtained from Bodo community in Ogoni Land, Niger Delta and made into a composite sample. The sample was then evaluated for physicochemical parameters, heavy metals, total petroleum hydrocarbon (TPH), polyaromatic aromatic hydrocarbon (PAH), total heterotrophic bacteria (THBC), and fungi (THFC) using standards methodologies. Concentration profile of heavy metals was iron (Fe) > chromium

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(Cr) > zinc (Zn) > nickel (Ni) > vanadium (V) in decreasing order. Furthermore, the concentrations of total nitrogen, total phosphorus, nitrate, sulphate were 884.53, 12.32, 34.45, and 26.76 mg/kg, respectively. TPH and PAH concentrations were 30,797 and 52.12 mg/kg, respectively. Nitrate, iron, chromium, nickel, TPH, and PAH all failed to meet regulatory standards. TPH failed to meet Environmental Guidelines and Standards for the Petroleum Industry in Nigeria (EGASPIN) target and intervention values of 50 and 5,000 mg/kg, respectively. Total heterotrophic bacteria and fungi counts were observed to decrease with days of incubation in crude oil. The findings in the study reveal worrisome levels of TPH, PAH, iron, chromium, and nickel, and impact on microbial diversity. Thus, there is a need for periodic monitoring of sediments and water bodies in the Niger Delta.

*Keywords: Petroleum hydrocarbon; heavy metals; crude oil, Niger delta; sediment.*

## 1. INTRODUCTION

The Niger Delta ecosystem is one of the most fragile ecosystems in the world according to a United Nation Report [1]. The region has an abundance of crude oil deposits whose exploration has been the main stay of the Nigerian economy [2]. Over 50 years of improperly managed exploration of this crude oil has made the region the most polluted of all oil-producing regions of the world [1].

Crude oil hydrocarbon is a well known environmental pollutant [1,2]. In the last five decades, oil exploration activities have introduced at least 3.1 million barrels of crude oil into various ecosystems in the Niger Delta [3]. Crude oil spillages are known to drive significant metal pollution in addition to increasing the amounts of total petroleum hydrocarbon and total polyaromatic hydrocarbon [3]. Some of these metals such as Cr, Pb and Ni apart from Fe are known to be toxic and have the potential to bioaccumulate in tissues and cells of certain vulnerable organisms [4]. Furthermore, these metals, TPH and PAH can bioaccumulate in sea foods and sediments altering the function and diversity of such sediments [5].

Crude oil spillage is common place in Bodo community, Ogoni Land [1,6]. Ogoni land has been plagued with the consequences of incessant crude oil spillages for over 30 years. Thus, it was the epicenter of the UNEP report of 2011. In 2008 and 2009, major spills were reported in addition to several minor and even more recent oil spillages. The creeks of this community support the daily livelihoods of its inhabitants such as the provision of domestic water, fishing, farming, processing of cassava, transportation and waste disposal [6]. Studies have shown that crude oil pollution can significantly alter the physicochemical properties

of the receiving environment [1,7,8]. Several studies around the Niger Delta have revealed crude oil hydrocarbon levels higher than those of Federal Environmental Protection Agency (FEPA), the Environmental Guidelines and Standards for the Petroleum Industry in Nigeria (EGASPIN) and that of Nigerian Department of Petroleum Resources (DPR) standards [3-7]. Studies evaluating the impact of crude oil in Bodo community that is well known for frequent crude oil spillages are very few. Thus, this study aimed to evaluate the microbiological, physicochemistry and hydrocarbon pollution status of Bodo community.

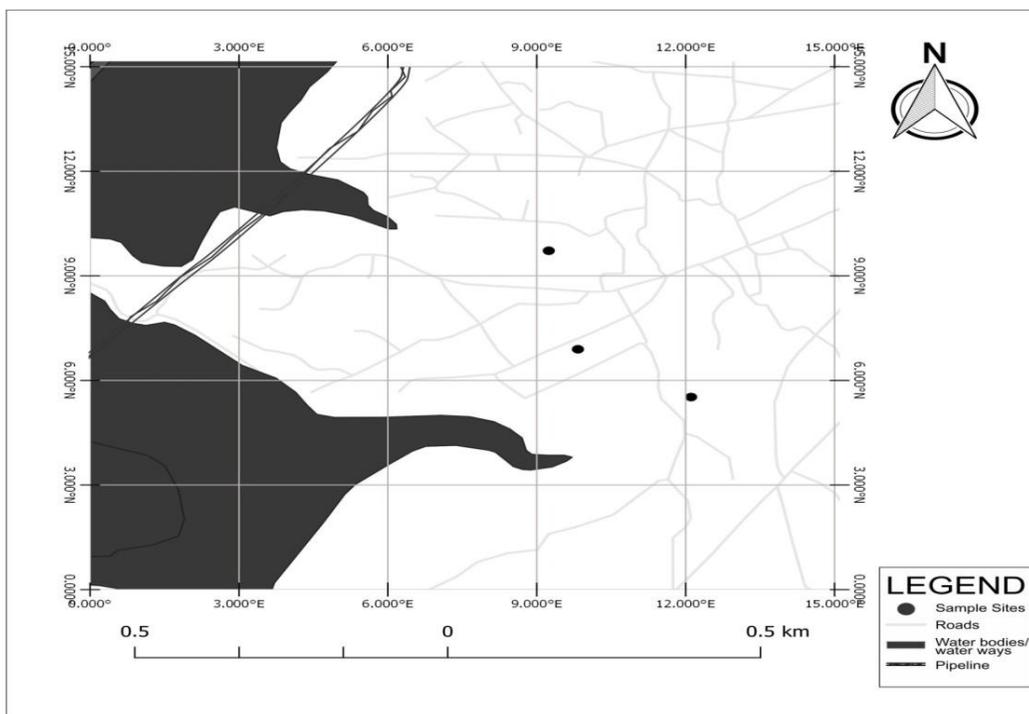
## 2. MATERIALS AND METHODS

### 2.1 Study Site

Sediment samples used for this study were collected from the estuary water of Bodo Creek. Bodo is a community in Ogoni land, Gokana Local Government Area of River State, Niger Delta, Nigeria. Ogoniland covers a total land mass of 1,000 km<sup>2</sup>. It has been a site of oil and gas industry operations since the 1950s. The hydrology and structure are well studied and it is characterized by tidal flats, semi-diurnal tidal regimes, and dry and wet seasons [6]. As at the time of sampling, there were visible crude oil spills on the estuary water. The sampling points are as shown in the map.

### 2.2 Sample Collection and Preprocessing

Sampling point was geo-referenced as previously reported using geographical positioning system (GPS) [6] the coordinates of the sampling point was 7°16.1"E and 4°37.239"N. From this coordinate, three samples each weighing 1 kg each were collected and transferred into sterile stainless basins. The benthic sediment samples were collected using a mud-grab from a depth of



**Fig. 1. Map of Bodo community showing the sampling points**

10 m as previously described [5]. The samples were then sieved using a 2 mm pore sieve and then made into one composite sample. After homogenization of samples, pH was evaluated in-situ using a hybrid meter (HM Digital PH-80 Hydrotester, USA) and then transferred into an amber coloured sterile bottle. The sample was then transported in an ice pack to the laboratory for microbiological and physicochemical properties analyses.

### 2.3 Physicochemical Analysis

Physicochemical parameters including pH, nitrate, phosphate, total organic carbon, total nitrogen, total phosphate, electrical conductivity, vanadium, iron, chromium, nickel, lead and zinc, were determined as previously reported [6,9].

### 2.4 Gas Chromatograph

Residual total petroleum hydrocarbons (TPH) and polyaromatic aromatic hydrocarbon (PAH) were extracted from the sample and quantified using gas chromatograph-flame ionization detector (GC-FID) according to methods of ASTM 3921 and USEPA 8270B as previously reported [10] and compared with standards [11, 12].

### 2.5 THBC and THFC

The total heterotrophic bacterial and fungal counts in the sediment sample was determined using the spread plate method on plate count agar (PCA) and Sabouraud's dextrose agar (SDA), respectively. Exactly 1gram of the sample was homogenized in 0.85% of normal saline. Decimal dilutions (10-fold) of the suspension were plated out on PCA and SDA and incubated at 30°C for 24 hrs and 28°C for 1 and 7 days for bacteria and fungi, respectively. The colonies were reported as colony-forming units per ml. These were repeated every 7 days for 35 days.

### 2.6 Statistical Analysis

Replicate readings for the physicochemical parameters were analyzed using one-way analysis of variance (ANOVA). Significance was set at  $p < 0.05$ . Total heterotrophic bacteria and fungi counts were analyzed using a semi-log plot.

## 3. RESULTS

The result of the physicochemical parameters evaluated and that of Department of Petroleum Resources (DPR) standards are presented in Table 1. The pH of the sediment sample was

near neutral at 6.72 and this was within the lower limit for the DPR standard of 6-9. There are no equivalent standards available for electrical conductivity ( $\mu\text{S}/\text{cm}$ ), total nitrogen ( $\text{mg}/\text{kg}$ ), phosphate ( $\text{mg}/\text{kg}$ ), and total organic carbon (%). However, our values for these parameters were 7340, 884.53, 12.32 and 6.43  $\text{mg}/\text{kg}$ , respectively. The concentrations of nitrate and sulphate were 34.45 and 26.76  $\text{mg}/\text{kg}$ . Compared to the DPR standards, nitrate level in our sample was higher than of DPR which is 20  $\text{mg}/\text{kg}$ . However, the concentration of sulphate was lower than that of the standard 50-200  $\text{mg}/\text{kg}$  value. The results of the concentrations of heavy metals, total petroleum and polyaromatic hydrocarbons are presented in Table 2. Iron had the highest concentration (3,242  $\text{mg}/\text{kg}$ ) and was more 162 times higher than that of the DPR and FEPA standards. The concentrations of chromium and nickel were 54.20 and 35.53  $\text{mg}/\text{kg}$ , respectively and these were consistently higher than those of FEPA and DPR standards by at least a factor of 50 except for zinc that was lower than the concentration of the standards. Lead was within range of the DPR and FEPA standards. Furthermore, the concentration of TPH was 30,797  $\text{mg}/\text{kg}$  and this was higher than the Environmental Guidelines and Standards for the Petroleum Industry in Nigeria (EGASPIN)

target (or the baseline levels) and intervention values of 50 and 5,000  $\text{mg}/\text{kg}$ , respectively. PAH was also higher than the maximum allowable range of 0.005 to 3.00  $\text{mg}/\text{kg}$ .

Fig. 2 shows the semi-log plot of the total heterotrophic bacteria (THBC) and fungi (THFC) count over a 35 days period. Both counts showed a time dependent decrease in the counts; bacteria counts were consistently higher than those of fungi. The ranges of the counts were 5.2 to 4.5 log units for THBC and 3.5 to 0.70 log units for THFC.

#### 4. DISCUSSION

The neutral pH value reported in our study is within range for the DPR standard of 6 to 9. In an earlier study, higher pH values that ranged from 8.8 to 8.9 were reported for sediment samples obtained from Bodo community [6]. Our findings were more consistent with those of Kolo Creek in the Niger Delta with reported pH values of 5.90 to 6.80. The nitrate level observed in our study was higher than the DPR set standard of 20  $\text{mg}/\text{kg}$ . Sediment and coastal zones can act as a sink for riverine and atmospheric nitrogen [13]. Nitrate in water or sediment is largely due to anthropogenic sources such as organic and

**Table 1. Physicochemical parameters**

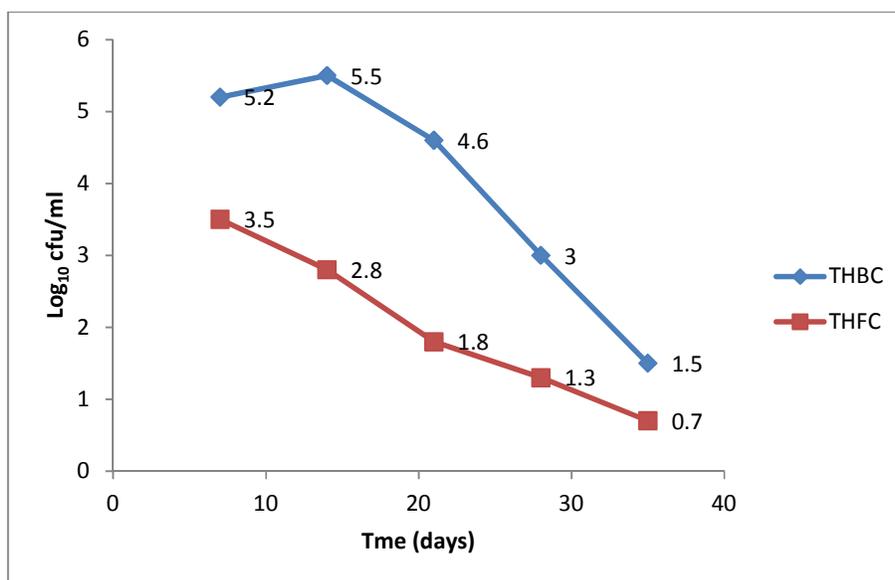
Parameters	Composite sediment sample	DPR standard (2002)
pH	6.72 $\pm$ 0.21 <sup>a</sup>	6-9
Electricity conductivity ( $\mu\text{S}/\text{cm}$ )	7340 $\pm$ 341	-
Total Nitrogen ( $\text{mg}/\text{kg}$ )	884.53 $\pm$ 23	-
Total phosphorus ( $\text{mg}/\text{kg}$ )	12.32 $\pm$ 0.47	-
Nitrate ( $\text{mg}/\text{kg}$ )	34.45 $\pm$ 2.14	20
Sulphate ( $\text{mg}/\text{kg}$ )	26.76 $\pm$ 1.8	50-200
Total organic carbon (%)	6.43 $\pm$ 0.18	-

*Superscript indicates significant ANOVA ( $p < 0.05$ ) for each row/parameters. DPR= Department of Petroleum Resources*

**Table 2. Heavy metal, TPH and PAH concentrations**

Heavy metal	Concentration ( $\text{mg}/\text{kg}$ )	DPR (2002) and FEPA (1999) standards
Vanadium (V)	0.002 $\pm$ 0.00 <sup>a</sup>	-
Iron (Fe)	3,242 $\pm$ 104	20
Chromium (Cr)	54.20 $\pm$ 2.14	0.5
Nickel (Ni)	35.53 $\pm$ 0.57	0.80
Lead (Pb)	17.43 $\pm$ 0.91	2-20
Zinc (Zn)	37.56 $\pm$ 0.87	50-300
TPH	30,797 $\pm$ 589	50/5,000
PAH	52.12 $\pm$ 4.11	0.005-3.00

*Key: FEPA = Federal Environmental Protection Agency (1999); DPR= Department of Petroleum Resources. PAH = polyaromatic aromatic hydrocarbon and TPH = Total petroleum hydrocarbon. Superscript indicates significant ANOVA ( $p < 0.05$ ) for each row/parameters*



**Fig. 2. Semi-log plot of total heterotrophic bacteria and fungi counts over 35 days**

inorganic fertilizers, septic tanks, feedlots and poultry farm wastes [14]. The high level of nitrogen and nitrate in this study are not surprising as a vibrant human community is located in our study site and they utilize the estuary water for domestic purposes and also disposal of sewage and human excreta. At concentrations above 10 mg/L, nitrate has been shown to cause health challenges in human, lower water quality and can contribute to nutrient enrichment of lakes [14]. In a recent study, the levels of phosphate were much lower than our reported concentration [6]. Phosphorus is an important nutrient like nitrate for the growth and metabolism of aquatic organisms and together with nitrate can result in eutrophication [15]. In an earlier study, the concentration of phosphate in sediment ranged from 544.76 to 932.11 mg/kg in sediment samples obtained from China [15]. The observed difference could be due to the higher anthropogenic influence in their study site compared to our study site.

Crude oil is known to contain heavy metals and given the incessant crude oil spillages in the Niger Delta, there is a tendency for significant heavy metal pollution [2-5]. In our study, the least abundant heavy metal was vanadium while the most abundant was chromium with concentrations of 0.002 and 54.20 mg/kg, respectively. Lower Cr levels were reported in an earlier study in Bodo community, Niger Delta [6]. Similarly, the levels of Ni, Pb, Fe and Zn were consistently higher in our study than reported

previously [6]. Fe, Cr and Ni were consistently higher in our study than earlier reported in the same study site, and those of DPR and FEPA standards [6]. In an earlier study, a higher level of Pb, Cr and Ni were reported for Koko Creek in the Niger Delta [7]. The sequence of metal abundance was Fe > Cr > Zn > Ni > V. The concentration of Fe although very high in our study site is not associated with any health hazard. As a metal, iron is the fourth most abundant metal. This high abundance together with anthropogenic sources explains the high abundance of the metal. Compared to previous studies, the levels of Ni and Pb in our study were higher than those reported in the Niger Delta estuary and sediments [10,16]. The studied heavy metals apart from iron and zinc are known to be toxic even at low concentrations and they are capable of bioaccumulation in lower organisms used as food such as periwinkles and higher organisms such as human, poisoning their tissues [4].

Hydrocarbon contamination is common place in the Niger Delta region. The most comprehensive hydrocarbon contamination study carried out in the Niger Delta remains the UNEP study [1]. The report revealed gross hydrocarbon contamination across Ogoni land. Our total petroleum hydrocarbon (TPH) value was higher than EGASPIN target and intervention values of 50 and 50,000 mg/kg. This conforms with the UNEP study that reported higher values of TPH and PAH than those of EGASPIN and is a call for

concern. Much lower levels of TPH and PAH were reported in Eastern Obolo, an oil-producing community in the Niger Delta [2]. Crude oil hydrocarbon is a well-known toxicant that is capable of upsetting microbial diversity and as well as function and also capable of selecting those isolates capable of utilizing crude oil hydrocarbons as carbon sources [10]. PAH as a class of hydrocarbon in crude oil is very toxic to organisms including microbial degraders at high concentration and also recalcitrant [17]. Like PAH and TPH, other pollutants are capable of selecting and altering microbial community structure [8,17,18].

In earlier studies culture independent technique (metagenomics) revealed reduction in microbial communities in contaminated environments while enriching taxa capable of utilizing the carbon source [5,8,10,16]. The low microbial load observed in our study is lower than those previously reported for non-crude oil impacted [19] and crude oil impacted environments [10]. This finding confirms the toxic nature of crude oil hydrocarbons evaluated in this study. Microorganisms play various beneficial roles in their environment and the presence of pollutants such as PAH and TPH is capable of altering these beneficial roles such as biogeochemical cycling [8]. However the microbial load in our study is higher than the total heterotrophic bacteria counts of  $\leq 100$  CFU/ml allowable by the World Health Organization in drinking water [20-22]. The selected microbes and their abundance coupled with favorable a pH value of 6.72 is an indication that bioremediation is on-going in the study site by the selected autochthonous hydrocarbon utilizers as shown by the THB and THF counts. However, the gradual reduction in the number of THB and THF counts attests to the toxic nature of crude oil even amongst the utilizers indicating that toxicity of crude is concentration-dependent [23].

## 5. CONCLUSION

The findings in this study indicate significant anthropogenic influence on the study site and as expected some of the evaluated parameters failed to meet regulatory standards. These parameters were nitrate, chromium, nickel, polyaromatic aromatic hydrocarbon and total petroleum hydrocarbon. The sequence of heavy metal concentration was  $Fe > Cr > Zn > Ni > V$ . The findings in this study is a call for concern and highlight the need for periodic monitoring of sediments and water bodies not just the Bodo

community but in all the oil-producing communities in the Niger Delta.

## COMPETING INTERESTS

Authors have declared that no competing interests exist.

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