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Delineation of Oil – Polluted Sites in Ibeno LGA, Nigeria, Using Microbiological and Physicochemical Characterization

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***Abstract*—**Mobil Producing Nigeria Unlimited (MPNU), a subsidiary of ExxonMobil and the highest crude oil & condensate producer in Nigeria has its operational base and an oil terminal, the Qua Iboe terminal (QIT) located at Ibeno, Nigeria. Other oil companies like Network Exploration and Production Nigeria Ltd, Frontier Oil Ltd; Shell Petroleum Development Company Ltd; Elf Petroleum Nigeria Ltd and Nigerian Agip Energy, a subsidiary of the Italian ENI E&P operate onshore, on the continental shelf and in deep offshore of the Atlantic Ocean, respectively with the coastal waters of Ibeno, Nigeria as the nearest shoreline. This study was designed to delineate the oil-polluted sites in Ibeno, Nigeria using microbiological and physico-chemical characterization of soils, sediments and ground and surface water samples from the study area. Results obtained revealed that there have been significant recent hydrocarbon inputs into this environment as observed from the high counts of hydrocarbonoclastic microorganisms in excess of 1% at all the stations sampled. Moreover, high concentrations of THC, BTEX and heavy metals contents in all the samples analyzed corroborate the high recent crude oil input into the study area. The results also showed that the pollution of the different environmental media sampled were of varying degrees, following the trend: ground water > surface water > sediments > soils.

***Keywords*—**Microbiological characterization, oil-polluted sites, physico-chemical analyses, total hydrocarbon content.

I. Introduction

IBENO Local Government Area (LGA) is one of the thirty-one (31) LGAs in Akwa Ibom State, Nigeria. It is the location of massive oil deposits, which have been extracted for decades by Mobil Producing Nigeria Unlimited (MPNU), a subsidiary of ExxonMobil Corporation and some marginal oilfield operators like Network Exploration and Production Nigeria Ltd and Frontier Oil Ltd. Over the past 6 decades, crude oil discharged into the environment of Ibeno LGA, including oil in process water, oil discharges from tanker washing, hydrocarbon in gas flares, oil spills from pipeline ruptures and many other sources as well as spills during the Nigerian civil war of 1967-70, when many oil installations were either bombed or sabotaged is quite enormous [1]. Oil spills, oily sludge / wastes dumping and gas flaring are endemic in the Niger Delta.

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The National Oil Spill Detection and Response Agency (NOSDRA) [2], which was established in 2006 has identified all crude oil-polluted sites needing remediation in the Niger Delta. As of April 2008, it had identified approximately 2,000 sites. The majority of these sites were apparently SPDC sites [3]. Several methods can be adopted in delineating oil-polluted sites [4]. This research project on the delineation of oil-polluted sites in Ibeno LGA using microbiological and physico-chemical characterization of the soil, riverbed sediment and surface and ground water samples was designed as the first step to addressing the environmental pollution problems in the study area.

Other multinational oil companies like Shell Petroleum Development Company Ltd (SPDC), Elf Petroleum Nigeria Ltd (EPNL) and Nigerian Agip Energy (NAE), a subsidiary of ENI E&P operate onshore, on the continental shelf and in deep offshore Akwa Ibom State coastal waters, respectively. In case of major oil spills, they end up in Ibeno LGA and on Akwa Ibom State shoreline, leaving the entire shoreline an oil-polluted site [1].

II. Materials and Methods

A. Study Area

The study area (Ibeno LGA) is located in the coastal area of Akwa Ibom State, Nigeria. It lies between Latitude 4º 30'N and 4º 36'N and Longitude 7º 48'E and 8º 18'E.

Ibeno LGA hosts Mobil Producing Nigeria (MPN) Unlimited, a subsidiary of ExxonMobil and currently the highest oil & condensate producing company in Nigeria. MPN's operational base and its crude oil loading terminal (the Qua Iboe Terminal, QIT) are located at the mouth of the Qua Iboe River by the Atlantic Ocean. Also facilities of Network Exploration and Production Nigeria Ltd and Frontier Oil Ltd Gas Plant are located in Ibeno LGA.

B. Sample Collection

1. Soil Samples

At each observation point along the established grids, soil samples spatially distributed around the points, were taken at depths of 0-15, 15-50, 50-100 and 100-150cm using a hand-held steel auger as in [5]. The sampling stations are presented in Table I. The samples were composited, mixed and sub-sampled and packed in polyethylene bags and preserved for onward transmission to the laboratory for microbiological and physico-chemical analyses.

TABLE I
SOIL SAMPLING STATIONS FOR STUDY AREA

LOCATION	COORDINATES (UTM)		ELEVATION (masl)
	Easting	Northing	
Itak Abasi Shore	0387598	0502105	2.0
Okoroutip	0382698	0503681	2.0
Uton-Iwuo-Achang	0385453	0504614	3.0
NTA Ikang	0394797	0502146	3.0
Ntafre	0373027	0501324	2.0
New Baracks	0406804	0502298	6.0
Itak Ibang/Okposo I	0418743	0501353	9.0
Itak Ibang/Okposo II	0418732	0501379	6.0

2. Water and Sediment Samples

The sample locations and corresponding coordinates for water and sediment samples taken in the study area are as presented on Table II. The methods of sample collection, handling and subsequent laboratory analysis were those specified in DPR Guidelines [6] and other international analytical standards [7] as presented in Table III.

C. Laboratory Analyses

1. Microbiological Analyses

a. Serial Dilution

Ten-fold serial dilutions of the soils, sediments and water samples were made as in [7], [8].

b. Inoculation and Incubation

One milliliter of appropriate ten-fold serial dilutions of the samples were inoculated onto Nutrient agar (Oxoid CM 314), Malt Extract Agar (Oxoid) and Sabouraud Dextrose Agar plates in triplicates using pour plate methods [7], [8] and spread plates methods [9]. The hydrocarbon utilizing bacterial counts (HUB) were enumerated by the spread plate technique using oil-mineral salt medium (MSM) [10]. The media were supplemented with cycloheximide (100µg/m1 and benomyl (50µg/m1) to prevent fungal growth [11]. The crude oil used was sterilized by filtering through Millipore filter (0.45µm pore size) and stored in sterile bottles. Inoculated plates were incubated at 28±2°C for 18-24 hours and 48-72 hours for the enumeration of total heterotrophic bacteria and fungi, respectively. Visible discrete colonies in incubated plates were counted and expressed as colony forming unit per gram (cfu/g) of soil or sediment samples or colony forming unit per litre (cfu/l) of surface or underground water samples. Only nutrient media plates with population densities of between 30-300 colonies were counted.

c. Maintenance of Pure Culture

Discrete colonies were purified by repeated sub-culture unto appropriate agar media. Pure cultures were preserved on nutrient agar slants and stored in the refrigerator (40°C±20°C) and at ambient temperature (28°C ± 2°C) for further tests.

TABLE II
SAMPLING STATIONS FOR WATER AND SEDIMENT IN THE STUDY AREA

LOCATION	COORDINATES, UTM		SEDIMENT	SURFACE WATER	BORE-HOLE
	Easting	Northing			
Upenekang Beach	0385983	0504844			✓
Ntafre	0373027	0501324			✓
Inua Eyet Ikot	0389052	0502205			✓
Itak Abasi Backshore	0387544	0502101			✓
Iwuo-Achang Union Beach	0385983	0504844			✓
Upenekang Beach	0386416	0504822	✓		
Inua Eyet Ikot	0389052	0502205	✓		
Itak Abasi Backshore	0387531	0502143	✓	✓	
NTA Ikang	0394797	0502146		✓	
New Barracks	0406804	0502298		✓	
Uton-Iwuo-Achang	0385453	0504614		✓	

TABLE III
METHODOLOGY FOR DETERMINATION OF PHYSICOCHEMICAL PROPERTIES OF WATER SAMPLES

Parameter	Unit	Methodology/equipment used	Reference
Conductivity	μScm^{-1}	Conductivity/TDS meter (Hach Co.150)	APHA-209C
TDS	mg/l	Conductivity/TDS meter (Hach Co.150)	APHA-209C
TSS	mg/l	Gravimetric	APHA-209D
DO	mg/l	Titrimetric (Winkler's)	APHA-422B
BOD_5	mg/l	Titrimetric (Dichromate reflux)	APHA-507
COD	mg/l	Titrimetric (Dichromate reflux)	APHA-508
Turbidity	NTU	Nephelometric	APHA-214
Oil and Grease	mg/l	Spectrophotometric	API-RP 45
Heavy Metals	mg/l	AAS (Pye Unicam SP. 190)	API-RP 45
Bicarbonate (HCO_3)	mg/l	Titrimetric (mixed indicator)	API-RP 45
Salinity (CL^-)	mg/l	Colorimetric (Auto-Analyzer)	API-RP 45
Ammonium (NH_4)	mg/l	Colorimetric (Auto-Analyzer)	API-RP 45
Sulpahte (SO_4^{2-})	mg/l	Colorimetric (Auto-Analyzer)	APHA-427C
Nitrate (NO_3^-)	mg/l	Colorimetric (Auto-Analyzer)	ASTDMD 3867

d. Characterization and Identification of Microbial Isolates

Pure cultures of microbial isolates were identified based on cultural parameters, microscopic techniques and biochemical tests including carbohydrate utilization [12]. Identification of the bacterial isolates was accomplished by comparing the characteristics of the cultures with that of known taxa as in [13]. Characterization and identification of fungal isolates was carried out as in [14], [15].

2. Physicochemical Analyses

a. Surface and Ground Water Analyses

Physicochemical properties of surface and ground water samples collected during the field studies were analyzed as described in Table III.

b. Soil and Sediment Analysis

Prior to laboratory analysis, soil/sediment samples were air dried, gently crushed with pestle in agate mortar and passed through 2mm sieve. The less than 2mm fraction was retained for analyses.

The soil samples were subsequently analyzed in the laboratory for physicochemical parameters, THC and BTEX according to standard methods [7]. Particle size analysis was done using the Bouyoucous Hydrometer method [16]. The pH of soil and sediment samples was determined as in [17].

Electrical Conductivity of the soil sample was determined as in [18] and exchangeable cations were determined as in [18], [19]. Total nitrogen in the soil sample was determined by Microkjedahl digestion and distillation methods as in [18] and available phosphorus was determined by the Bray No. 1 method [20] and Blue Molybdocolometric method [21]. Effective cations exchange capacity (ECEC) was determined as in [7]. Total organic matter contents was determined as in [22], while the micro-nutrients (heavy metals) of the soil was determined using the atomic absorption spectrophotometer, AAS (UNICAM AA 919 mode) [19]. Total hydrocarbon content (THC) and BTEX were determined using standard methods.

D. Statistical Analyses

The statistical analyses employed in this work were simple descriptive and relational statistics as well as standard deviations [23], [7].

III. Results and Discussion

A. Microbiological Characteristics

1. Surface and Ground Water

The results of the microbiological characteristics of the surface and ground water samples of the study area are as presented in Tables IV and V.

The ratio of hydrocarbonoclastic bacteria (HUB) to the total heterotrophic bacterial counts (THBC) in all the surface water sample locations were in excess of 1%, with the highest value at Itak Abasi followed by New Barracks, indicating recent significant crude oil input into the study area [24]. The same trend was observed for the river bed sediment samples; the ratio of HUB to THBC in all the sediment samples were in excess of 1% at Itak Abasi followed by Uton Iwuo-Achang and Nta Ikang. The values were very low and less than 1% for underground water samples in the study area, indicating that the underground water did not experience the recent crude oil pollution.

TABLE IV
MICROBIOLOGICAL RESULTS OF SURFACE WATER

LOCATION	THBC (cfu/l)	TFC (cfu/l)	HUB (cfu/l)	% HUB / THBC
Itak Abasi Backshore	7.0×10^3	3.8×10^2	1.2×10^2	1.7
Nta Ikang	7.3×10^3	3.2×10^2	1.0×10^2	1.4
New Barracks	7.5×10^3	2.7×10^2	1.1×10^2	1.5
Uton-Iwuo-Achang	7.3×10^3	3.4×10^2	1.0×10^2	1.4

TABLE V
MICROBIOLOGICAL RESULTS OF RIVER BED SEDIMENT

LOCATION	THBC (cfu/l)	TFC (cfu/l)	HUB (cfu/l)	% HUB / THBC
Itak Abasi Backshore	2.7×10^3	2.5×10^3	2.3×10^2	8.6
Nta Ikang	2.5×10^3	2.2×10^3	1.8×10^2	7.2
New Barracks	2.3×10^3	2.0×10^3	1.6×10^2	7.0
Uton-Iwuo-Achang	2.9×10^3	2.4×10^3	2.1×10^2	7.2

2. Soil/Sediment

In general, high bacteria counts with comparatively lower fungal counts were recorded for most of the soils and sediment samples. The soils showed average heterotrophic bacterial and heterotrophic fungal loads of 4.44×10^6 and 1.05×10^3 cfu/g, respectively. Fungal densities were much lower than those of bacteria, especially under the anoxic conditions of the soils; fungi being strict aerobes cannot proliferate in the absence of oxygen. The prominent isolates were *Pseudomonas aeruginosa*, *P. pseudomallei*, *Klebsiella edwardsii* and *Klebsiella pneumoniae* and Yeasts sp.

Some crude oil degraders were isolated from the soil samples including *Pseudomonas aeruginosa, Klebsiella pneumoniae* and members of other well known genera, earlier isolated in the Niger Delta [25] also occurred within the flora.

They include species of Alkaligenes and other Pseudomonas. The population of hydrocarbon degraders ranged from 0 to 6.85x104for bacteria and from 0 to 1.4x102 for fungi. While the populations of heterotrophic bacteria and fungi were average in soil, that of hydrocarbon degraders was relatively high at some locations, indicative of an already existing hydrocarbon impacted environment. Petroleum degraders mainly utilize hydrocarbons as nutrient sources and are normally most abundant in areas where contamination by hydrocarbons had taken place. The wide range of population of hydrocarbon degraders thus confirm past hydrocarbon impact of the soil.

B. Physicochemical Characteristics

1. Surface and Ground Water

The physicochemical data obtained during the survey revealed that the surface waters were acidic to moderately alkaline (pH 6.2 – 7.3) with high conductivity (180-

1983µs/cm) and high levels of dissolved oxygen (DO = 2.85-4.22mg/l), low mineral contents (Ca, K and Na). The levels of chloride (255-50,764 ppm) were generally high, reflecting sea influence. The waters were very low in sulphate, nitrate, and heavy metals (Fe, Mn, Zn, Cd, Pb, Ni, V and Cr) were within environmental limits. The THC contents ranged from 3.23ppm to 112.25 ppm most of which were above permissible limit of 10mg/l [6] at some locations, thus revealing that there was oil contamination of surface water bodies in the study area. The THC and BTEX values of underground water were within DPR regulatory limits [6], indicating that the underground water did not receive recent hydrocarbon input.

2. Soil/Sediment

The soils samples were sandy in texture with 73%-97% sand and 1%-9% clay contents. They were generally acidic (pH 4.6-6.3) except at some upland locations with pH of 7.7-8.2. The soil samples had high electrical conductivity (1.81mS/cm to 5.85mS/cm) that was indicative of saline conditions but with low organic carbon contents (0.18% and 2.61%), low total N (below the critical 0.2% level) and high available P values. According to [24]-[26], such soils are considered to be of low fertility status as most nutrients were below critical levels.

The high concentrations of phosphate (28.82-48.11ppm), sulphate (68.92-115.05ppm) and chloride (283.6-453.76) ions in soil profile samples may be due to contamination and/or prevailing anoxic acid soil conditions. Chloride salinity was below DPR limit of 6000mg/l. The average soil contents of exchangeable cations Ca, Mg, K and Na in the profiles were low. Soil levels of total hydrocarbon concentrations (THC) vary widely and ranged from 9.78ppm to 324.0ppm in locations. Values at all of the locations except one were beyond the 30ppm THC soil DPR regulatory limit. The distribution of heavy metals (Fe, Mn, Pb, Zn, Cd, Ni, V and Cr) were higher in these oil-polluted soils than in normal (unpolluted) soils [26], indicating the source of some of these heavy metals accumulation (especially Pb, Ni, V, and Cr) at some locations. Fe was generally high due to acid solubilization under the prevailing acidic conditions of the soil [27]. BTEX values for all the soil samples were found to be above DPR limits [6].

The bottom sediments were found to be sandy (>85% sand content) with admixture of organic remains (1.13-1.64% organic carbon) at different stages of decomposition with pH range of 5.9-7.9 and EC range of 0.17-7.21mS/cm. Phosphate (40.24-48.75ppm), sulphate (95.14-115.48ppm) and chloride (155.98-595.56ppm) concentrations were found to be high. In most of the sediments samples, the contents of basic cations (Ca, Mg, Na, K) were found to be low (<1Cmol/kg) while Base saturation were generally high (78-89%). The concentrations of Pb, Ni, V and Cr showed little or no accumulation of these heavy metals in the sediments but THC and BTEX values were above regulatory limits.

IV. Conclusion

The results of microbiological analyses as well as results of the THC and BTEX of the soil, water and sediment samples of the study area show that there were significant recent hydrocarbon contaminations of the study area. The high counts of crude oil degrading microorganisms (hydrocarbonoclastic microorganisms) in excess of 1% are indicative of recent exposure of the soil environment to crude oil contamination [28]. While it is believed that some previously impacted sites had virtually fully recovered especially in the top soil layers, others were still heavily impacted with crude oil from recent spills. By comparison with reported soil THC values (245-774ppm) for fresh oil spills, the levels of contamination observed (9-324ppm) in this study are indicative of high levels of crude oil contamination.

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