eISSN: 2455-1716
Udotong *et al.*, 2018
DOI:10.21276/ijlssr.2018.4.4.12

Research Article

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Microbial, Hydrobiological Indicators and Physicochemical Characteristics of a Remote Aviation Fuel-Contaminated Lentic System in Ibeno, Nigeria

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Received: 15 Feb 2018/ Revised: 30 April 2018/ Accepted: 22 June 2018

ABSTRACT

This research paper presented the microbial and hydrobiological indicators and the physicochemical quality of water samples from a lentic ecosystem in Ibeno LGA, Nigeria, after sixteen (16) years of an aviation fuel spill. Using culture-dependent methodologies, the hydrocarbonoclastic bacterial and fungal counts (HBC & HFC) ranged from 3.4 x 10^4 to 1.2 x 10^5cfu/l and 4.7 x 10^3 to 1.8 x 10^4 cfu/l, respectively with the ratios of total heterotrophic bacterial counts to HBC and total fungal counts to HFC ranging from 8 to 12% and 15 to 22%, respectively. Predominant bacterial indicators included *Pseudomonas aeruginosa, Bacillus subtilis, Micrococcus varians* and *Enterobacter aerogenes* while predominant fungal indicators included *Aspergillus niger, A. terreus, Candida* sp, *Saccharomyces* sp, *Phoma* sp. and *Botrytis* sp. Predominant zooplanktons in the sampled area were rotatoria while the least were nematodes and followed the trend: Rotatoria > Copepoda > Cladocera > Nematoda. Water samples from the area showed evidence of oil sheen when disturbed; with pH values (6.2 to 7.8) tending generally towards neutral. Total petroleum hydrocarbon (TPH) from this aviation fuel-contaminated lentic system ranged from 81.5 mg/l to 505.2 mg/l. Dissolved oxygen (DO) were generally low with high BOD and COD of 46.3 mg/l and 321.1mg/l, respectively. Other physicochemical parameters were typical of lentic ecosystems in the Niger Delta region, Nigeria. The impact of this and many other spells were enormous. This confirmed that it takes a long time for recovery once the environment was polluted.

Key-words: Aquatic pollution, Aviation fuel, Hydrobiological parameters, Hydrocarbonoclastic Microorganisms, Lentic ecosystem, Physicochemical characteristics

INTRODUCTION

Oil spills occur in the Niger Delta environment almost on a daily basis as a result of a number of causes including well blow-out, pipeline rupture, sabotage, equipment failure, etc. According to Udotong [1], Leonardi *et al*. [2], and Udotong *et al.* [3] there are therefore a number of oil-contaminated sites in the region.

How to cite this article

Udotong JIR, Udotong IR, Udoudo UP. Microbial, Hydrobiological Indicators and Physicochemical Characteristics of a Remote Aviation Fuel-Contaminated Lentic System in Ibeno, Nigeria. Int. J. Life Sci. Scienti. Res., 2018; 4(4): 1940-1945.

Access this article online

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On 8th August 2001, the 30-year-old 4" QIT-Jetty Aviation fuel pipeline ruptured resulting in the spillage of about 1,000 barrels of Aviation fuel into the environment of Inua Eyet Ikot in Ibeno local government area of Akwa Ibom State, Nigeria. The aviation fuel spillage continued at high pressure for more than 3 hours before it was stopped. The spilled aviation fuel drained into Akpauchat swamp, a lentic ecosystem in Ibeno in the Niger Delta region of Nigeria. The clean-up effort embarked upon by the company involved the use of dispersants, after which no other remediation method has been attempted. This article presents the microbial and hydrobiological indicators and the physicochemical characteristics of water samples from this lentic system contaminated by aviation fuel after sixteen (16) years of the spill.

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eISSN: 2455-1716
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DOI:10.21276/ijlssr.2018.4.4.12

Water samples from this contaminated lentic system in Ibeno in the Niger Delta region of Nigeria were characterized using standard methods, sixteen years after aviation fuel spill; with a view to documenting predominant microbial and hydrobiological indicators and physicochemical characteristics of such remote Aviation fuel-contaminated site.

MATERIALS AND METHODS

Description of study area and sample collection- The aviation fuel-contaminated site is located at No 65^{A} Qua Iboe Terminal (QIT) road at Inua Eyet Ikot village in Ibeno LGA of Akwa Ibom State, Nigeria (Fig. 1). The spill site lies within Longitude 7^{o}30' to 8^{o}00'E and Latitude 4^{o}30' to 4^{0}45'N on the South Eastern Nigerian Coastline. The study site (Fig. 2) is a swamp and thus a lentic system.

Field sampling for this study was embarked upon from 25th April 2016 to 2nd August 2016. Water samples were collected at three (3) locations from this aviation fuel-contaminated site using sterile plastic containers for microbiological, hydrobiological and physicochemical analyses. The samples for microbiological analyses were stored in ice-packed coolers and were transported to the Microbiology Laboratory for analyses. These and other samples for physicochemical and hydrobiological analyses were transported to the Fisheries and Hydrobiology Laboratory of University of Uyo, Uyo, Nigeria for analyses.

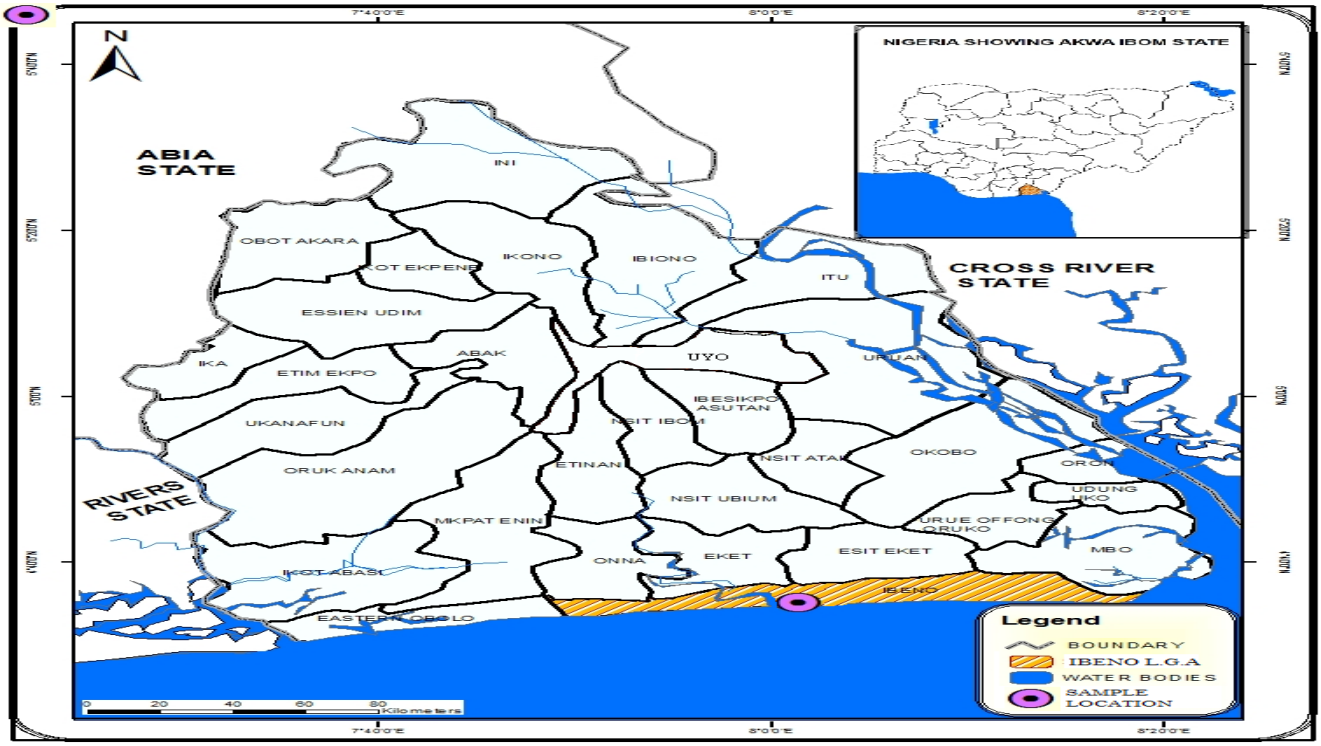

Fig. 1: Map of Akwa Ibom State showing Sampling location at Ibeno

Microbiological Analysis

Ten-fold serial dilution of samples, inoculation, and incubation- Serial ten-fold dilutions of each of the samples were prepared according to the methods of Collins and Lyne [4] and Harrigan and McCance [5]. Appropriate dilutions were inoculated onto appropriate nutrient media and were incubated. Hydrocarbonoclastic microorganisms were determined using the mineral salt medium (MSM) as described by Okpokwasili and Okorie [6]. After incubation, discrete microbial colonies were counted and multiplied by the reciprocal of the dilution factor and expressed in colony forming units per millilitre (cfu/l) and their respective cultural morphologies was observed and recorded.

Purification and maintenance of pure culture of isolates- Discrete colonies were purified by repeated sub-culture onto appropriate nutrient media. Pure cultures were preserved on nutrient agar slants and stored in the refrigerator for further characterization and identification.

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Fig. 2: The aviation fuel-contaminated site

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 (Cruickshank *et al*. [7]). Identification of the bacterial isolates was accomplished by comparing the characteristics of the cultures with that of known taxa as in Holt *et al.* [8]. Characterization and identification of fungal isolate were carried out as Domsch *et al.* [9] and Hunter [10].

Analyses for hydrobiological indicators- Characterization of the limnological indicators of this system was carried out. The periphyton removed from substrata were fixed and preserved in 0.5% acetic lugol, except for samples for determination of diatoms, which were preserved in 4% formalin. Techniques of oxidization and preparation of permanent slides of diatoms followed the method of Simonsen [11]. After this taxonomic analysis, organisms were quantified using an inverted microscope at 400X, according to methods of Utermöhl [12]. Additional counts were made on permanent slides (100 individuals) to differentiate very similar diatom species as recommended by Biggs [13].

The classification system used to be that of Round [14]. The systematic arrangement and generic diagnoses followed Bourrelly [15], except for the classes Cyanophyceae and Bacillariophyceae, which were classified according to Gleitler [16] and Krammer & Lange-Bertalot [17], respectively. Counting of phytoplankton was done by the direct census method of Jhingran *et al.* [18]. The rates of primary production of the surface water were estimated as per the standard "Light and dark bottle method" (Gaarder and Gran) [19].

Physicochemical analysis of water samples- Water quality parameters *viz.*, temperature, suspended particulate matter (SPM), transparency, salinity, pH, carbon dioxide, alkalinity, dissolved oxygen (DO), chemical oxygen demand (COD) and nutrients (nitrate, ammonia, phosphate and silicate) were determined following standard methods (APHA; FWPCA; Philbert; Strickland and Parson) [20-23]. Extinction coefficient (Kt) was estimated from the formula, Kt = 1.44/Secchi depth in meter Holmes [24]. For the collection of phytoplankton, five liters of water were collected in a plastic bottle and fixed with Lugol's iodine.

RESULTS

Microbiological Indicators- The results of total heterotrophic bacterial and fungal counts (THBC & TFC) ranged from 3.4 x 10^4 to 1.2 x 10^5 cfu/l and 4.7 x 10^3 to 1.3 x 10^4 cfu/l, respectively with the ratios of hydrocarbonoclastic bacterial counts to THBC (HBC /THBC) and Hydrocarbonoclastic fungal counts to total fungal counts (HFC/TFC) ranging from 8% to 12% and 15% to 22%, respectively (Table 1).

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Table 1: Microbial Counts of Remote Aviation fuel-contaminated lentic system

Sample Code	THBC (cfu/ml)	HBC (cfu/ml)	HBC/THBC	TFC (cfu/ml)	HFC (cfu/ml)	HFC/TFC
AFW – 1	5.8×10^4	5.8×10^3	10%	6.4×10^3	1.2×10^3	19%
AFW – 2	1.2×10^5	1.4×10^4	12%	1.3×10^4	2.9×10^3	22%
AFW – 3	3.4×10^4	2.7×10^3	8%	4.7×10^3	7.1×10^2	15%

Legend: THBC- Total Heterotrophic Bacterial count; HBC- Hydrocarbonoclastic Bacterial Count; TFC- Total Fungal Count; HFC- Hydrocarbonoclastic Fungal Count

Predominant Microbial Isolates- Predominant bacterial indicators isolated from this remote aviation fuel-contaminated lentic system were *Pseudomonas aeruginosa, Bacillus subtilis, Micrococcus varians, Enterobacter aerogenes* and *Aeromonas* sp while predominant fungal indicators isolated were *Aspergillus niger, A. terreus, Candida* sp, *Saccharomyces* sp, *Phoma* sp and *Botrytis* sp.

Hydrobiological Indicators- Diatoms were the most abundant phytoplankton group in this aviation fuel-contaminated lentic system followed by the dinoflagellates and Blue green algae. In terms of species richness, the periphyton groups identified at this site followed the sequence Bacillariophyceae> Cyanophyceae> Chlorophyceae> Euglenophyceae. Predominant zooplanktons in this aviation fuel-contaminated lentic system were in the sequence of Rotatoria > Copepoda > Cladocera > Nematoda.

Physicochemical Characteristics of water samples- Most of the water samples from the study area were colorless and clear, but with evidence of oil sheen when disturbed; with pH values (6.2 to 7.8) tending generally towards neutral. Total petroleum hydrocarbon (TPH) in water samples from this aviation fuel-contaminated lentic system ranged from 81.5 mg/l to 505.2 mg/l. Dissolved oxygen (DO) was low with high BOD and COD of 46.3 mg/l and 321.1 mg/l, respectively (Table 2).

Table 2: Physicochemical Characteristics of water samples

Parameters	Sample Code		
	AFW – 1	**AFW – 2**	**AFW – 3**
Colour	Colourless / Clear	Colourless / Colour	Colourless / Colour
pH	7.8	6.2	7.0
TPH (mg/l)	276.1mg/l	505.2mg/l	81.5mg/l
DO (mg/l)	7.0	12.0	8.0
BOD (mg/l)	43.0	46.3	44.2
COD (mg/l)	302.0	321.1	311.1
Heavy metals			
Fe (mg/l)	10.5	8.3	12.0
Pb (mg/l)	0.02	0.01	0.03
Cu (mg/l)	0.01	0.02	0.01
Cd (mg/l)	0.01	0.01	ND
Zn (mg/l)	0.1	0.1	0.1
Mg (mg/l)	0.01	0.01	0.01
Mn (mg/l)	0.01	0.01	ND
Co (mg/l)	0.01	ND	0.01

ND – Not detected

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DOI:10.21276/ijlssr.2018.4.4.12

DISCUSSION

These ratios of hydrocarbonoclastic bacteria count to THBC in excess of 1% are indicative of petroleum hydrocarbon input into this lentic system. All the microbial isolates listed are known crude oil-degrading microorganisms (Ijah [25]; Udotong [1]; Udotong *et al.* [26]).

The dominance of diatoms in this contaminated site may be due to the fact that food stored in diatoms takes the form of oil droplets and it is thought that petroleum deposits may represent the pre-historic accumulation of an incredible number of such droplets from Diatoms and other groups of plankton. Specific phyto- & zoo-plankton indicators at this aviation fuel-contaminated lentic system are listed in Akpan and Akpan [27]. The observed dominance of these indicators in terms of species richness has been a common feature at hydrocarbon-contaminated lentic systems and is typical of a biologically productive freshwater ecosystem in the Niger Delta region, Nigeria (Akpan and Akpan [27]).

About 80% of all water samples from this study area had some heavy metals (Fe, Pb and Cu) concentrations in excess of WHO maximum permissible levels for drinking water. All other physicochemical parameters of this aviation fuel-contaminated lentic system were typical of lentic ecosystems in the Niger Delta region, Nigeria (RPI) [28].

CONCLUSIONS

About sixteen years after the spill, the lentic system was seen to be contaminated. Although natural processes are ongoing at this aviation fuel-contaminated lentic system in the past sixteen years, there is need for enhanced decontamination (bioremediation) of this and many other such sites that exist in the Niger Delta region in Nigeria. Integrated bioremediation including phyto-remediation techniques have been advocated.

There are on-going researches by these authors to monitor microbial (eubacteria, archaea, and eukarya) diversity at this site using metagenomics and other omics approaches with a view to identifying efficient hydrocarbonoclastic microorganisms that can be used for effective bioremediations of this and other such polluted sites in the Niger Delta Region. This research is one of the pioneering efforts at bioremediation of petroleum hydrocarbon polluted sites in Nigeria.

CONTRIBUTION OF AUTHORS

Justina Ime Rufus Udotong- Literature search, Sample collection and samples/ data analysis and interpretation and writing of article for Physicochemical Characteristics, critical review and final approval

Ime Rufus Udotong- Literature search, Sample collection and samples/ data analysis and interpretation, writing of article for Microbial characteristics and article review.

Unyime Patrick Udoudo- Literature search, Sample collection and samples/ data analysis and interpretation, writing of article for Hydrobiological characteristics.

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