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Assessment of Hydrocarbon and Microbial Contamination of Groundwater Facilities Located Around Selected Petroleum Product Tank Farms in Delta State

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ABSTRACT

The storage and distribution of refined Petroleum products involves the use of above the ground storage tanks built in clusters, called Tank Farm. Storage tanks could corrode with time and seep hydrocarbon into the ground which later contaminates groundwater. This research was aimed at assessing the physicochemical parameters, hydrocarbon utilizing bacteria (HUB) and corrosion causing bacteria in groundwater in the vicinities of tank farms in three different locations (Oghara, Koko, and Warri) and in Asaba without a Tank farm (Control) all located in Delta State, Nigeria. Physicochemical and microbiological parameters were analysed using standard methods. Results showed the following ranges pH, 6.2-6.9; Total Dissolved Solids, 30-37mg/l; Biological oxygen Demand, 0.03-1.01mg/l; and Total Petroleum Hydrocarbon (TPH), 0.87-8.00mg/l. Heavy metal ranges were, Copper (Cu), 0.05–0.13mg/l; Zinc (Zn), 0.07–0.27mg/l; Iron (Fe), 0.08–0.25mg/l; and Lead (Pb), <0.00–0.01mg/l. There was significant difference ($p<0.05$) across the means of TPH of the different sampled locations. Generally, TPH exceeded the Department of Petroleum Resources (DPR) target and Intervention limits and Iron was above Federal Ministry of Environment (FMEnv) limit in some points sampled. All other parameters analysed fell below regulatory standard of W.H.O, FMEnv and DPR. Hydrocarbon utilizing bacterial counts in the groundwater samples ranged from 1.0 x 10^5- 2.5 x 10^5 CFU/ml. HUB was not detected in the control samples. Molecularly identified hydrocarbon utilizing isolates were *Acinetobacter Junii, Lysinibacillus macrolides, Acinetobacter nocomialis, Morganella morgani.* Corrosion-causing bacteria were not detected in the groundwater samples analysed. The presence of potential pathogens and pathogenic *Acinetobacter nocomialis* which is a serious global threat and TPH that bioaccumulates in organs and its carcinogenic effects in the groundwater samples from the tank farm locations poses a public health concern. Government is advised to provide potable water for the inhabitants of communities around these tank farms to forestall health conditions and diseases that could arise from consumption of these groundwaters.

Keywords*:* Petroleum tank farm, groundwater, contamination, hydrocarbon utilizing bacteria, *Acinetobacter*.

Introduction

Pollution is the introduction of a contaminant into the environment (Owa, 2017; Ercumen *et al.*, 2017; Ahmed *et al*., 2018). Three main types of pollution include; land pollution, air pollution and water pollution. Water pollution can be described as the contamination of marine and freshwater bodies (such as lakes, rivers, oceans, aquifers and groundwater) with domestic sewage, agricultural pesticides and fertilizers, oils and oil dispersants, metals, solid wastes, industrial effluents among others. Groundwater contamination, most frequently with heavy metals and petroleum products, is a widespread problem in Nigeria. Consequently, pollution caused by petroleum hydrocarbons is one of the most common environmental issues (Adebiyi *et al*., 2015; Hassan *et al.*, 2018). The extent of groundwater contamination depends on some factors such as, rainfall pattern, depth of water table, and distance from the source of contamination and soil properties.

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Contaminants, such as toxic metals, hydrocarbons, trace organic contaminants, pesticides, nanoparticles, microplastics, and other emerging contaminants, are a threat to human health, ecological services, and sustainable socioeconomic development (Li *et al*., 2015). Hydrocarbon contamination of groundwater may arise from the following sources; Landfill, storage tank, septic system, hydrocarbon exploration and exploitation. Storage tanks are used to conserve gasoline, oil, and other chemicals, etc. It is estimated that over ten million underground storage tanks are buried in the USA alone. However, fatigue, rusting and leakages may occur as a result of extended usage, thus allowing contaminants to escape and contaminate the groundwater aquifer (Wood *et al.*, 2016).

The quality of water is described by its physical, chemical and biological contents (Olukanni *et al.*, 2018). The effects of water contamination may be aesthetic (with respect to colour, taste and odour), cosmetic (causing skin or tooth discoloration) or technical (causing damage to water equipment or hampering treatment for other contaminants). These contaminants pose health hazards to consumers of the water from the underground sources (Soladoye *et al*., 2014). Contamination of ground water can result in poor drinking water quality, loss of water supply, degraded surface water systems, high cleanup costs, and high costs for alternative water supplies, and/or potential health problems. The consequences of contaminated groundwater or degraded surface water are often serious. Mandal *et al.* (2012) isolated 324 bacteria belonging to 110 different species from oil contaminated soils and crude oily sludge and these were found to efficiently degrade different fractions of total petroleum hydrocarbons. Bacteria genera *Pseudomonas, Stenotrophomonas, Ochrabactrum, Bacilllus, Agrobacterium, Brevundimonas, Gordonia, Acinetobacter, Achromobacter, Microbacterium, Sphingobium, Kocuria, Rhodococcus, Luteibacter,* and *Novosphingobium* were isolated from oil contaminated environments have been reported (Mahjoubi*et et al*., 2013). Bacterial genera *Gordonia, Burkholderia, Aeromicrobium, Mycobacterium, Dietzia,* and *Brevibacterium* were also isolated from petroleum contaminated soil (Dindar, 2013).

Scientific literature on the physicochemical and microbiological quality of groundwater in the vicinities of tank farms in Delta State is scarce. This present study was therefore aimed at assessing the physicochemical parameters, and hydrocarbon utilizing bacteria (HUB), and corrosion causing bacteria in groundwater in the vicinities of tank farms in three different locations (Oghara, Koko, and Warri) and in Asaba without a Tank farm (Control) all located in Delta State, Nigeria.

Materials and Methods

Study Area

The study was conducted in three communities within the vicinity of petroleum product tank farms where there are visible activities. The study communities are Koko, (5°58'01.9"N 5°33'07.0"E; 5°58'01.4"N 5°33'04.2"E 5°57'54.6"N 5°33'07.9"E); Warri (5°34'15.9"N 5°43'26.7"E; 5°34'14.9"N 5°43'27.2"E; 5°34'16.8"N 5°43'28.6"E; 5°34'07.5"N 5°47'38.1"E) and Oghara (5°56'34.9"N 5°38'58.2"E; 5°56'37.0"N 5°38'58.6"E; 5°56'32.2"N 5°39'00.5"E; 5°56'05.5"N 5°40'34.2"E) and Asaba (control) (6°13'02.8"N 6°41'41.2"E 6°13'02.0"N 6°41'35.4"E 6°13'03.4"N 6°41'30.7"E, 6°13'03.6"N 6°41'32.9"E) without a visible tank farm far from the other three locations are all located in Delta State, Nigeria. The GERMIN GPS (USA) was used to obtain all the coordinates of the various sampling points in the study sites. A map of the study area showing the sampling points was generated using ArcGis Software and is as shown in Figure 1.

Collection of Groundwater Samples

Four groundwater samples were collected from four boreholes at a distance of 50m, 100m, 150m and 500m and designated as A, B, C, and D respectively from the tank farms in Koko, Oghara and Warri. Groundwater samples from four boreholes at Asaba were collected and used as control. Samples were collected in triplicates. Water samples for physicochemical parameters analysis were collected using 500ml plastic containers, filled and tightly corked and preserved in ice pack at 4^{o}C. Water samples for bacterial analysis were collected in special Sterilin® (USA) sample bottles preserved in ice pack at 4^{o}C. Water samples for heavy metals were sampled in 1 litre plastic cans and preserved with 2ml of 1:1 v/v trioxonitrate (v) acid to pH <2.0, while water samples for Total Petroleum Hydrocarbon (TPH) were collected in 1Litre glass bottles and preserved with 2ml of 1:1 v/v tetraoxosulphate (vi) acid to pH <2.0. All the water samples were then transported to the Biology/Microbiology Laboratory of Petroleum Training Institute, Effurun, Delta State where they were assayed within 24 hours of sampling.

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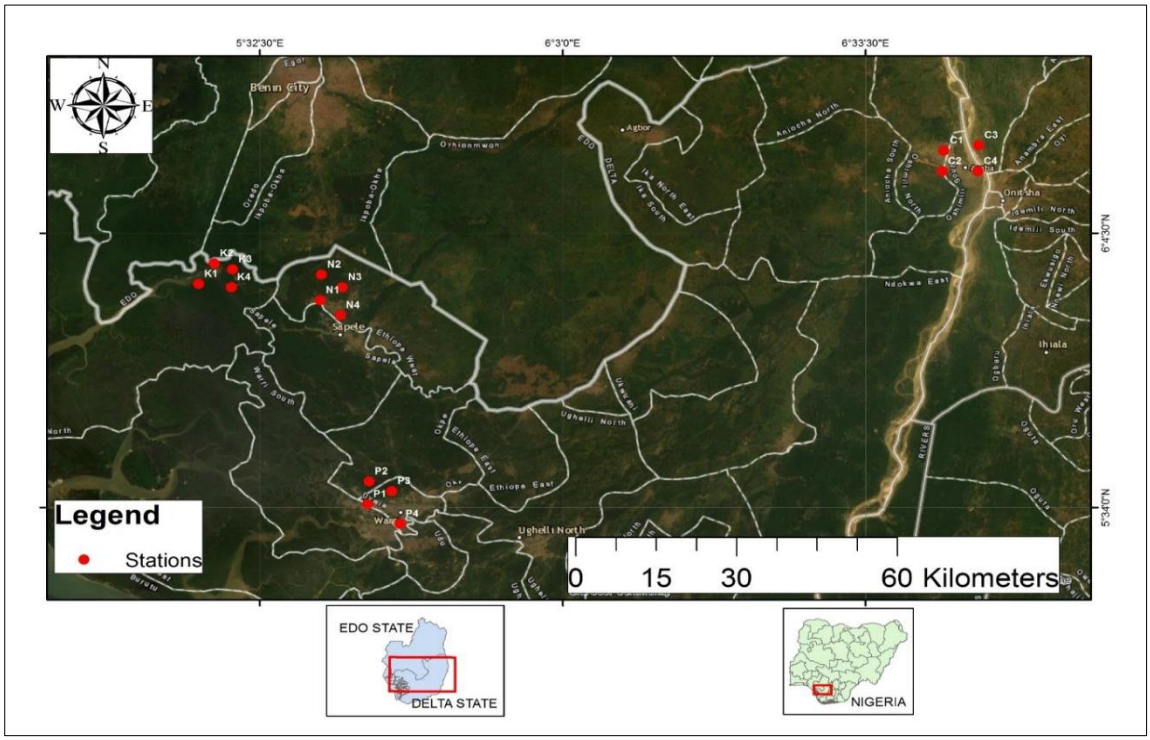

Fig. 1: **Map of the Study Area showing the Sampling Points**

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Determination of pH, Electrical Conductivity and Total Dissolved Solids (TDS)

These *In situ* parameters were assessed on site using the following procedures; pH (APHA 4500-H^{+} B using Hanna pH electronic meter), temperature (APHA 2550 - B laboratory and field methods), electrical conductivity and total dissolved solids (APHA 2510-B using Hanna desktop conductivity meter) and dissolved oxygen (DO) (APHA 4500-O C by azide modification method).

Laboratory Analysis of Physicochemical Parameters

Biochemical oxygen demand (BOD) was analysed by APHA 5210 B, by 5-Day test method)**,** TPH was measured using the method specified by**,** APHA 2017, 5520B. This method utilized the gas chromatography (GC) in addition to Flame Ionization Detector (FID) for TPH analysis in the oil extracts.

This method measured C_{9} to C_{36} range of hydrocarbons (Environmental Research Institute, 1999). Heavy metals were analysed in the method as described by APHA 2017, 3030D and the metals were analysed using Atomic Absorption Spectrophotometer, AAS (Pinnacle 900T Perkin Elmer).

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Microbiological Analyses

Enumeration of Heterotrophic Bacteria counts

Nutrient agar was used for the analysis of isolation and enumeration of total heterotrophic bacterial (THB) population. It was prepared according to manufacturer's instruction and sterilized by autoclaving at 121°C for 15 minutes at 15psi. Ten-fold serial dilutions of the samples were made. An aliquot (1ml) of the dilutions was put into sterile Petri dishes and 15ml- 20ml of sterilized and cooled molten agar was poured into each of the plates. The culture plates were swirled for homogenization, allowed to solidify and incubated at 28 ± 2°C for 18-24 hours (bacteria) After incubation, colonies which developed were counted and computed as Colony forming units (CFU) per milliliter (ml); Calculated using the formula:

$$\text{CFU/ml} = \frac{\textit{No of colonies counted}}{\textit{Volume of sampled used (ml)}} \times \frac{1}{\textit{dilution factor}}$$

Enumeration of hydrocarbon utilizing Bacteria

Bushnell-Haas agar with the following composition in 990.0ml was used: Agar (15.0g), KH_2PO_4 (1.0g), K_2HPO_4 (1.0g), NH_4NO3 (1.0g), $MgSO_4 \cdot 7H_2O$ (0.2g), $FeCl_3$ (0.05g), $CaCl_2 \cdot 2H_2O$ (0.02g). All components were added to distilled/deionized water and the volume was brought to 1 Litre. The mixture was then mixed thoroughly, gently heated and brought to boil. It was there after autoclave for 15 min at 15 psi pressure at 121°C. 1% filter sterilized crude oil (v/v) was added as the sole carbon source to isolate the hydrocarbon utilizing degrading bacteria from the water samples. The medium was then dispensed into Petri dishesand then allowed to cool and solidify. 0.1ml from the dilutions (10^{-3} – 10^{-5}) was plated onto the sterile nutrient medium using the spread plate method. The plates were inverted and incubated at 28 ± 2ºC for 3-7days. The colonies were computed and discrete colonies were thereafter subcultured unto nutrient agar plates by streaking to isolate pure cultures. The pure cultures were identification using cultural, morphological and molecular biology techniques.

Enumeration of Corrosion-causing Bacteria

i) **Detection of Sulphate Reducing Bacteria**

Sulphate API Broth was used with the following composition: NaCl (10.0g), Sodium lactate (5.2g), Yeast extract (1.0g), $MgSO_4 \cdot 7H2O$ (0.2g), Ascorbic acid (0.1g), $Fe(NH_4)_2(SO4)_2 \cdot 6H2O$ (0.1g), K_2HPO_4 (0.01g),was used for the detection of Sulphate reducing bacteria. The components were added to distilled/ deionized water and volume was brought to 1.0Litre.pH was adjusted to 7.5 at 25°C This was then mixed thoroughly until dissolved and distributed into tubes in 9.0mL volumes.

Thereafter the medium was autoclaved for 15 min at 15 psi pressure and at 121°C. 1 ml of the water sample was injected immediately after collection into each sterilized and cooled tube using sterile syringe and left for 7days. Colour changes were observed (Atlas, 2010).

ii) Detection of Acid Producing Bacteria

Phenol Red Dextrose Broth was used for the detection of Acid producing bacteria in the water samples. The broth is composed of the following: Proteose peptone (10.0g), NaCl (5.0g), Glucose (5.0g), Beef extract (1.0g), Phenol Red (0.018g), pH 7.4 ± 0.2 at 25°C. The components were added to distilled/deionized water and brought to 1.0L. This was then thoroughly mixed and distributed into tubes with inverted Durham tubes. It was then autoclaved for 15 min at 15 psi pressure and at 121°C. 1 ml of the water sample was injected immediately after collection into each tubes using sterile syringe and left for 7 days and colour changes were noted (Briggs, *et al*., 2019).

Identification of hydrocarbon utilizing Bacteria (HUB) isolates

i)Morphological Identification HUB isolates

Morphological identification of HUB isolates was done by Gram staining according to the method of Tortora *et al.* (2014).

ii)Molecular Identification of Bacteria Isolates

Extraction and Purification of Bacterial DNA

The Genomic DNA was extracted from the cultures using the Quick-DNATM Fungal/Bacterial Miniprep Kit (Zymo research, Catalogue NO D6006). The 16s target region was amplified using OneTaq® Quick-Load® 2x Master Mix (NEB catalogue No. M0486) with the primers are 16s-27F sequence 5'AGAGTTTGATCMTGGCTCAG-3'and 16S-1492R sequence 5'- CGGTTACCTTGTTACGACTT-3'. The PCR products were run on a gel and gel extracted with the Zymoclean™ Gel DNA Recovery kit (Zymo Research, Catalogue No D4001)

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16S rRNA Amplification and Sequencing

The extracted fragments were sequenced in the forward and reverse direction (Nimangen, BrilliantDye™ Terminator Cycle sequencing kit V3.1, BRD3-100/1000) and purified (Zymo Research, ZR-96 DNA sequencing clean-up kit™, Catalogue NO. D4050). The purified fragments were analyzed on the ABI 3500xl Genetic anayzer (Apllied Biosystems, ThermoFisher Scientific) for each reaction for every sample as listed in section 1. CLC Bio Main Workbench v7.6 was used to analyze the .ab1 files generated by the ABI 3500XL genetic Analyzer and results were obtained by a BLAST search (NCBI).

Results

The result of the mean values of pH of the ground water samples in the vicinity of the tank farms, and of the control is shown in Figure 2 below. The pH values ranged from 6.33±0.15 - 6.87± 0.06. The highest mean pH value was recorded in borehole D (500m) in all the sampling locations (Warri, Koko, Oghara and Asaba), while the lowest value was recorded in boreholes A (50m) in (Warri, Koko and Oghara). The minimum value was below the standard limit of 6.5. All the groundwater samples were slightly acidic.

The result of the range of mean values of constituents of the groundwater samples in the vicinity of the petroleum tank farms and in the control locations are presented in Table 1.

The mean values of conductivity ranged from 62.13±2.05-75.05±0.90 µs/cm. The highest mean value of conductivity was observed in water from borehole C (150m) in (Oghara, Warri, Koko) and least in water from borehole B (100m) in (Oghara, Warri, Koko). The conductivity values obtained were above WHO and FMENv limits of 40 and 3.0mg/l respectively, but below NSDWQ limit of 1000mg/l. The values of total dissolved solids in all the water samples ranged from 30.0 - 37.2 mg/l. The highest value was observed in water from borehole D in Warri tank farm and least in sampling location in Koko tank farm. The concentrations of TDS in the water samples in all the stations sampled were within the recommended limits of all compared regulatory limits. The values of dissolved oxygen in the groundwater samples ranged from 2.27±0.23-3.03mgl/l in all the sampling locations. The minimum recorded value of dissolved oxygen was observed in the water from borehole B in Oghara and Koko and maximum in Oghara borehole B. All values were within recommended limits (Table 1). Considerable fluctuations were found in all the four locations throughout the study period. The values of BOD from the sampling stations ranged from 0.89±0.03- 1.01±0.02 mgl/l. The lowest value was recorded in water from borehole B in Warri and Koko, while the highest was mean value was recorded in water from borehole D of warri, Oghara and Koko tank farms. The maximum value was above WHO and NSDQW limits of 1 and 0.0mg/l respectively.

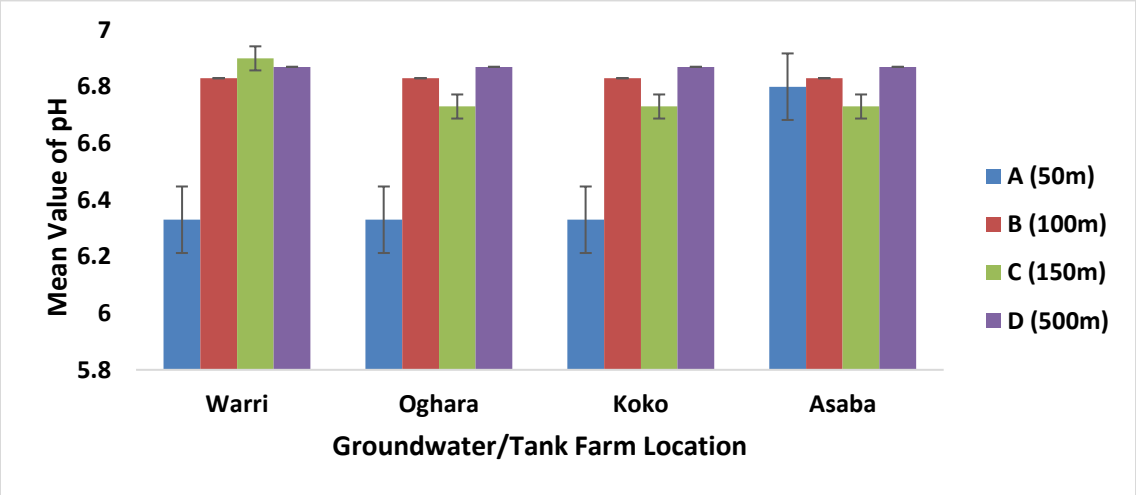

Fig. 2: Mean values of pH of groundwater samples around petroleum tank farms
Legend: A, B, C & D are borehole distances in metres (m) from each tank farm location.

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Table 1: Physicochemical constituents of groundwater (Borehole) samples in the vicinity of tank farms in Delta State, Nigeria

Parameter	Groundwater Samples Around Warri Tank Farm				WHO (2019) Standard	FMEnv (2003) Standard limits	NSDWQ
	A (50m)	B (100m)	C (150m)	D (500m)			
pH	6.33 ±0.15	6.83± 0.12	6.73± 0.15	6.87± 0.06	6.5-8.5	6-9	6.5-8.5
EC (µs/cm)	64.57± 1.58	62.13 ±2.05	75.07 ±0.90	72.13± 2.10	40	3.0	1000
TDS (mg/l)	31.67± 1.53	35.00± 1.00	34.07± 0.90	35.33 ±2.08	500	2000	500
DO (mg/l)	2.27 ±0.23	3.03 ±0.15	2.73± 0.31	2.30 ±0.26	2	NA	7.5
BOD (mg/l)	0.96 ±0.05	0.89± 0.03	1.00 ±0.01	1.01± 0.02	1	NA	0
	Groundwater Samples Around Koko Tank Farm						
	A (50m)	B (100m)	C (150m)	D (500m)			
pH	6.33 ±0.15	6.83± 0.12	6.73± 0.15	6.87± 0.06	6.5-8.5	6-9	6.5-8.5
EC (µs/cm)	64.57± 1.58	62.13 ±2.05	75.07 ±0.90	72.13± 2.10	40	3.0	1000
TDS (mg/l)	31.67± 1.53	35.00± 1.00	34.07± 0.90	35.33 ±2.08	500	2000	500
DO (mg/l)	2.27 ±0.23	3.03 ±0.15	2.73± 0.31	2.30 ±0.26	2	NS	7.5
BOD (mg/l)	0.96 ±0.05	0.89± 0.03	1.00 ±0.01	1.01± 0.02	1	NS	0
	Groundwater Samples Around Oghara Tank Farm						
	A (50m)	B (100m)	C (150m)	D (500m)			
pH	6.33± 0.15	6.83 ±0.12	6.73± 0.15	6.87± 0.06	6.5-8.5	6-9	6.5-8.5
EC (µs/cm)	64.33± 0.58	62.43±2.11	75.07± 0.90	72.13± 2.10	40	3.0	1000
TDS (mg/l)	32.33± 2.52	34.00± 2.00	33.67± 1.15	35.33 ±2.08	500	2000	500
DO (mg/l)	2.27± 0.23	3.03± 0.15	2.73 ±0.31	2.30 ±0.26	2	NS	7.5
BOD (mg/l)	0.95 ±0.06	0.90 ±0.02	0.99± 0.01	1.01 ±0.02	1	NS	0
	Groundwater Samples in Asaba Location Without Tank Farm (Control)						
	A (50m)	B (100m)	C (150m)	D (500m)			
pH	6.80± 0.26	6.83 ±0.12	6.73± 0.15	6.87± 0.06	6.5-8.5	6-9	6.5-8.5
EC (µs/cm)	49.67± 0.58	53.00 ±1.73	53.67 ±1.15	53.00 ±1.00	40	3.0	1000
TDS (mg/l)	32.47± 2.47	34.53 ±2.04	34.07 ±0.90	35.40± 2.16	500	2000	500
DO (mg/l)	2.27± 0.23	3.63± 0.15	2.73 ±0.31	2.30± 0.26	2	NA	7.5
BOD (mg/l)	0.68 ±0.53	1.39± 0.47	0.89 ±0.21	1.01 ±0.01	1	NA	0

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The result of the range of mean values of total petroleum hydrocarbon (TPH) in groundwater samples in the vicinity of the petroleum tank farms and in the control locations in this study is as shown in Table 2. While concentration of total petroleum hydrocarbon (TPH) in groundwater samples in comparison with DPR limits is shown in Figure 3. The value of total petroleum hydrocarbon (TPH) ranged from 4.94 ±0.04 - 8.00 ± 0.20 mg/l. There was significant difference ($p<0.05$) across the means of the different sampled locations. All the ground water from the boreholes sampled from Warri, Koko and Oghara showed a free phase of hydrocarbon while the maximum recorded value of hydrocarbon was observed in water from borehole A in Warri. The groundwater samples from Asaba (control) had no hydrocarbon sheen. WHO, NSDQW and FMNEv does not have available limits for TPH. However, the TPH concentrations in the groundwater samples in all the three tank farms locations were non-compliant when compared with DPR target and intervention values of 50 and 600µg/l respectively as indicated in Figure 3 below. This is a strong evidence of groundwater contamination by hydrocarbon leaks in the studied areas.

Table 2: Mean values of total petroleum hydrocarbon (TPH) concentrations of groundwater samples in the vicinity of tank farms in Delta State, Nigeria

Sampling Location (Distance)	TPH concentration (mg/l) of Groundwater Samples and Tank Farm Location			Asaba (Control)	DPR Target Value (µg/l)	DPR Intervention Value (µg/l)
	Warri	Koko	Oghara			
A (50m)	8.00 ± 0.20	4.80 ± 0.29	4.97 ± 0.04	<1.00 ±0.00	50	600
B (100m)	7.66 ± 0.00	4.96 ± 0.05	4.96 ± 0.04	<1.00 ±0.00	50	600
C (150m)	7.32 ± 0.02	4.96 ± 0.06	4.99 ± 0.01	<1.00± 0.01	50	600
D (500m)	7.33 ± 0.03	4.94 ± 0.03	4.97 ± 0.04	<1.00 ±0.01	50	600

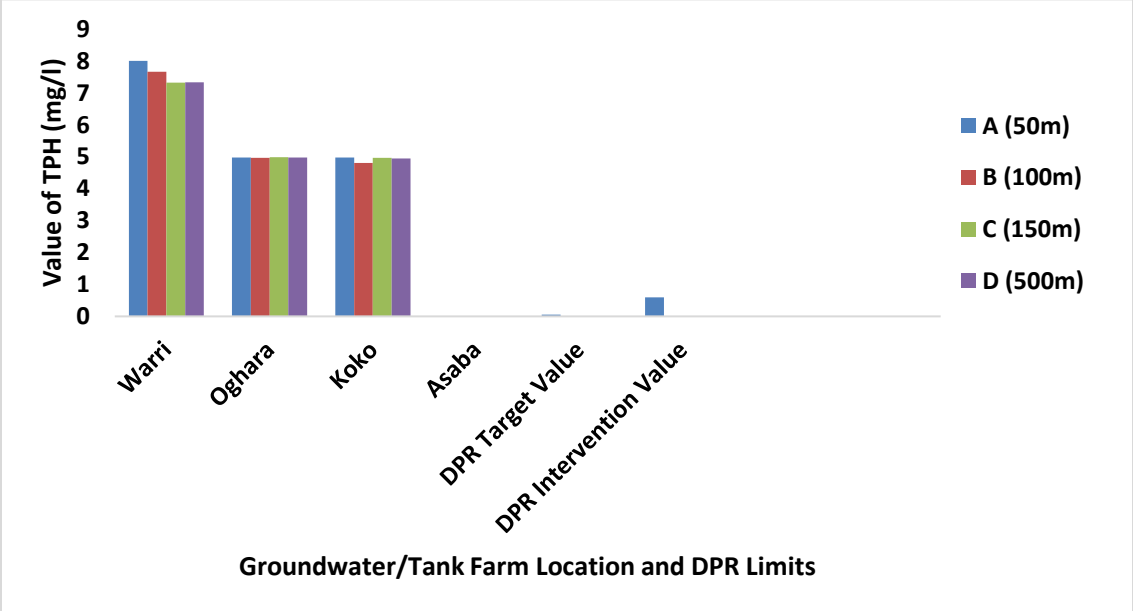

Fig. 3: Concentration of total petroleum hydrocarbon (TPH) in groundwater samples in comparison with DPR regulatory limits

Legend: A, B, C & D are borehole distances in metres (m) from each tank farm location.

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The range of heavy metal concentration and of iron (Fe) in the four locations sampled is as presented and compared with the guideline values of regulatory bodies in Table 3 and Figure 4 respectively. Results showed that, the heavy metal concentrations were low in relation to the regulatory limits. Copper (Cu) was detected in all groundwater samples at concentrations within the range of 0.067 – 0.117 mg/l. The control point recorded Copper (Cu) concentration of 0.067-0.087mg/l. Lead ranged from <0.001-0.01mg/l. Zinc concentrations ranged from 0.07mg/l (both Koko and Oghara, samples D -150m) - 0.273mg/l (Koko sample A and Warri sample A). The concentrations of Iron (Fe) in the groundwater from the four locations sampled, ranged from 0.08-0.25mg/l. All the recorded concentrations were found to exceed FMENV limit (0.05mg/l) but below WHO limits and DPR intervention limit of 0.30mg/l (Fig. 3).

Table 3: Comparison of heavy metal concentration of groundwater around petroleum tank farms with guideline values of regulatory bodies

Parameter	Range of concentration (mgl^{-1}) of heavy metals in ground water around tank farms	WHO	NSDWQ	FMEnv	DPR Target Value	DPR Intervention value
Copper (Cu)	0.05 – 0.13	2.0	1	-	0.075	1.3
Zinc (Zn)	0.07 – 0.27	5.0	3	5.0	0.800	5.0
Iron (Fe)	0.08 – 0.25	0.3	0.3	0.05	NA	0.3
Lead (Pb)	0.00 – 0.01	0.01	0.01	0.01	0.075	0.015

Key: WHO = World Health Organization, FMEv = Federal Ministry of Environment. NSDWQ = Nigerian Standard for Drinking Water Quality

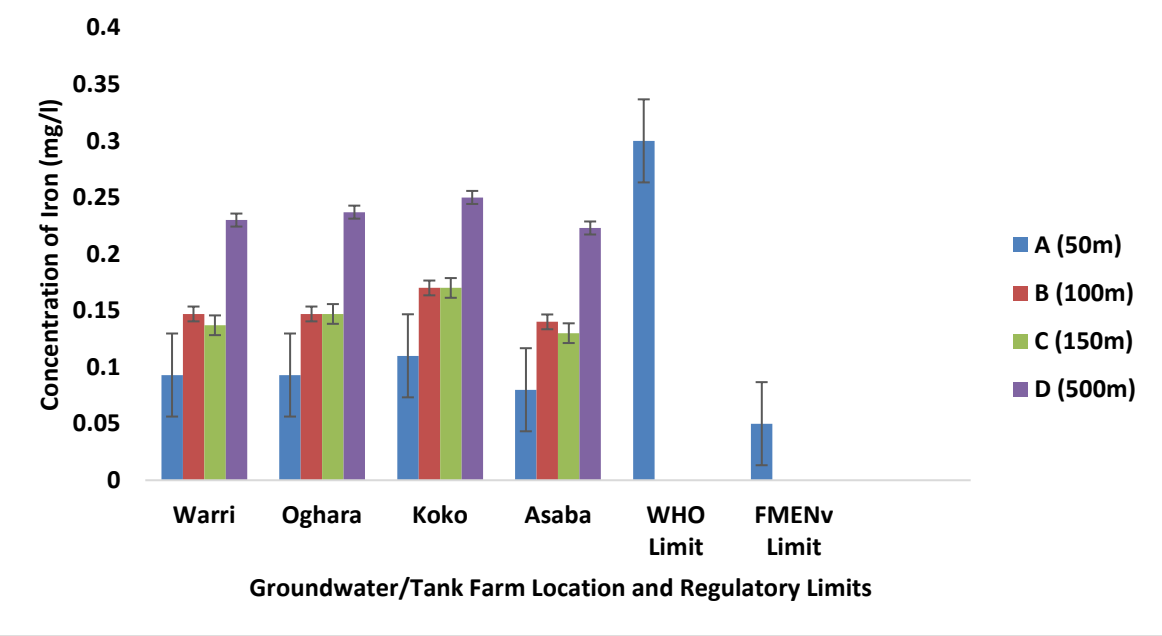

Figure 4: Concentration of Iron (Fe) in groundwater samples in comparison with standard limits
Legend: A, B, C & D are borehole distances in metres (m) from each tank farm location.

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The bacteria load (counts x 10^5) of the groundwater samples from the vicinity of the tank farms and control are presented in Table 4. The concentration of bacteria ranged from 1.0 x 10^5 to 2.5 x 10^5cfu/ml. Heterotrophic bacteria densities were high in the water from borehole A in Warri and all sampled boreholes in Asaba. Bacteria load were slightly high in the water from borehole C in Oghara.

The counts of the control groundwater samples (Asaba) ranged from 2.50 – 3.20 CFU/ml and generally higher than counts of all the groundwater samples in the vicinity of the petroleum tank farms.

The Hydrocarbon utilizing bacteria count (Log10 CFU/ml) in groundwater samples in the vicinity of the Tank farm and control locations is shown in Figure 5. The mean counts of Hydrocarbon utilizing bacteria (HUB) ranged from 1.0 x 10^5 - 2.5 x 10^5cfu/ml. The lowest was recorded at Koko boreholes A (50m) and C (150m) and Oghara boreholes A (50m), B (100m) and C (150m) while the highest was recorded at Warri borehole A (50m). The control samples ranged from 2.50-3.20 CFU/ml, and were higher than the counts obtained from the groundwater samples from the vicinity of the tank farms. HUB was not detected in the control groundwater samples (Asaba).

Table 4: Mean values of total heterotrophic bacteria counts of groundwater samples in the vicinity of tank farms in Delta State, Nigeria

Sampling Location (Distance)	Total heterotrophic bacteria count (CFU/ml) of groundwater Samples in the vicinity of tank farm Locations			Asaba (Control)
	Warri	Koko	Oghara	
A (50m)	2.5 x 10^5	1.0 x 10^5	1.0 x 10^5	3.0 x 10^5
B (100m)	2.0 x 10^5	2.0 x 10^5	1.0 x 10^5	2.5 x 10^5
C (150m)	1.0 x 10^5	1.0 x 10^5	1.0 x 10^5	2.5 x 10^5
D (500m)	2.0 x 10^5	2.0 x 10^5	2.0 x 10^5	3.2 x 10^5

Key: CFU/ml= Colony Forming Unit per milliliter of the sample

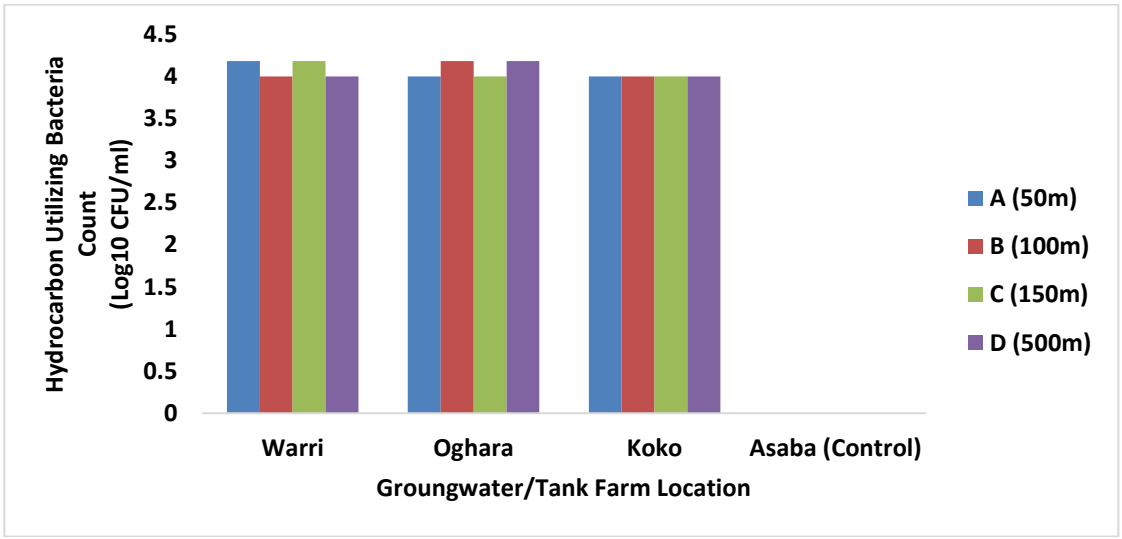

Fig 5: Hydrocarbon utilizing bacteria count (Log10 CFU/ml) in groundwater samples around Tank farm locations

Legend: A, B, C & D are borehole distances in metres (m) from each tank farm location.

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The results obtained from the broth tests for SRB and APB in the ground water samples studied are shown in Table 5 below. All the samples tested were negative for both SRB and APB, indicating the absence of SRB and APB in all of the ground water samples.collected for all the tank farm locations including Asaba (control).

The results obtained for the colonial and morphological (Gram reaction) characterization as well as molecular identification of HUB isolated from the groundwater samples are presented in Table 6 below. Molecular characterization based on 16S rDNA and NCBI BLAST search was used to confirm the identity of the isolated HUBs from the groundwater samples. Of the eight (8) HUB isolated, only four isolates were identified, due to isolation of colonies with similar characteristics. The four identified are: *Acinetobacter Junii, Lysinibacillus macroide, Acinetobacter nocomiali and Morganella morgani*.

Table 5: Colour Change obtained in test broth for SRB and APB after seven days

	Groundwater/Tank Farm Sampling Location			
Medium	**Warri**	**Koko**	**Oghara**	**Asaba (Control)**
Sulphate API Broth (SRB)	Colourless	Colourless	Colourless	Colorless
Phenol Red Dextrose Broth (APB)	Colourless	Colourless	Colourless	Colourless
Result	Negative	Negative	Negative	Negative

Table 6: Gram reaction, colonial characteristics, Blast result and GenBank Accession Numbers of the Hydrocarbon Degrading Bacterial Isolates

Isolate No.	Gram Reaction	Colonial Characteristics	Blast Result	GenBank Accession
1	Negative, cocci	Non pigmented, mucoid	*Acinetobacter Junii*	MT613873.1
2	Positive, Rod	Cream, dull	*Lysinibacillus macroides*	MT613873.1
3	Negative, cocci	Cream, mucoid	*Acinetobacter nocomialis*	MT540255.1
4	Negative	Cream,	Undetermined	-
5	Negative, cocci	Non pigmented, mucoid	*Acinetobacter Junii*	KJ806388.1
6	Negative, cocci	Cream, mucoid	*Acinetobacter nocomialis*	MK729019.2
7	Negative, Rod	Cream, small, shinny	*Morganella morgani*	CP048275.1
8	Negative, rod	Yellow, shinny	Undetermined	-

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Discussion

Groundwater is generally believed to be relatively free of microorganisms and thus fit for consumption (Jurado *et al.*, 2012). However, increase in human population in urban areas has exerted enormous pressure on the provision of safe drinking water. Consequently, the provision of high-quality water as well as protecting and conserving this scarce water resources is therefore one of the challenges facing national and regional governments (Sorensen *et al.*, 2015).

The hydrocarbon utilizing bacteria isolated from the borehole water samples were identified from the gene ascension bank as; *Acinetobacter Junii*, *Lysinnibacillusmacroides, Acinetobacter nocomialis, Morganella morgani*are. The isolates also have pathogenic tendencies as they are capable of causing infections in man (Lasarte-Monterrubio *et. a*l., 2022).

The pH values recorded in all the samples tested and the control were slighty acidic, thus the acidic effect could not be attributed to the tank farm activities. pH is one of the most important operational water quality parameters and it usually has no direct impact on consumers, but could lead to corroding of infrastuctures, especially metalic in nautre, such as pipelines, the acidic particles corrode metal and cause paint and stone to deteriorate more quickly. They also dirty the surfaces of buildings and other structures such as monuments (USEPA, 2023). Besides TPH, all the physicochemical parameters analysed in all the sampling locations were not significantly different from results obtained for the control groundwater samples and were within the stipulated regulatory limits. TPH results in all the locations sampled except the control were higher than the DPR target and intervention values of 50 and 600µg/l respectively. Organic contaminants have been widely detected in drinking water, and many of these compounds are regarded as human carcinogens or endocrine disrupting chemicals (Prideaux, 2016). In this present study, four (4) metals contaminants were detected in the ground water at different levels and stations, and this in agreement with findings of (Jurado *et al.* 2012; Lapworth *et al.* 2012; Sorensen *et al.* 2015; Peiyue *et al.*, 2021) which reported that toxic metals and metalloids are a risk factor for the health of both human populations and for the natural environment in ground water. Exposures at high concentrations can lead to severe poisoning, although some of these elements are essential micronutrients at lower doses (Hashim *et al*. 2011).

Results of this present study showed that, the heavy metal concentrations were low in relation to the regulatory limits. Copper (Cu) was detected in all groundwater samples collected from the study locations at concentrations within the range of 0.067 – 0.117 mg/l (Table 3). The control point recorded Copper (Cu) concentration of 0.067- 0.087mg/l. These values are below the safe limits of WHO (2017) stipulated guideline of 2.0 mg/l for drinking water. The USEPA guideline value for drinking water is 1.3 mg/l, while FMENV have no fixed limits. High intake of Copper can cause liver and kidney damage which may eventually lead to death. It also causes stomach ache, dizziness, vomiting and diarrhea (Baby *et a*l., 2020). Zinc concentrations ranged from 0.07mg/l (Koko, sample D and Oghara, sample D) - 0.273mg/l (Koko sample A and Warri sample A). These values were higher than that of the control point which ranged from 0.027 - 0.087mg/l. In relation to the stipulated regulatory limits used in the study, all the concentrations were within the recommended limits. The concentrations of Iron (Fe) in the groundwater from the four locations sampled, ranged from 0.08-0.25mg/l. All the recorded concentrations were found to exceed FMENV limit (0.05mg/l) but below WHO limits and DPR intervention limit of 0.30mg/l (Fig. 3). Iron concentrations however do not pose potential health risk as they fell well within the recommended daily dietary allowance (7mg – 18mg) (Hurrell and Egli, 2010). Water with high iron concentrations may discolour and stain washed clothing. Lead metal concentration obtained from the samples tested ranged from <0.001-0.01mg/l. Pb was not detected in most of the water samples tested. Groundwater samples with Pb detected were within the stipulated regulatory limits. Lead has many toxic effects on human health with children being the most vulnerable population (Mandal *et al.*, 2022).

Though, this study revealed that the values of the TPH, physico-chemical properties and heavy metals analysed were within/below the WHO and DPR standards and corrosion-causing microorganisms were also not detected, members of the Warri, Koko and Oghara communities must be sensitized on the potential health hazards associated with the use of ground water close to the tank farms due to its variable density and diversity of potential pathogenic microorganisms, metals and TPH. Though the

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presence of these parameters are currently low but chronic exposure to TPH and heavy metals could lead to deleterious health effects such as cancer and failure of sensitive organs (Mandal *et al.*, 2022). The presence of potential pathogens and pathogenic *Acinetobacter nocomialis* which is a serious global threat and TPH that bioaccumulates in organs and its carcinogenic effects in the groundwater samples from the tank farm locations poses a public health concern. Government is advised to provide potable water for the inhabitants of communities around these tank farms to forestall health conditions and diseases that could arise from consumption of these groundwaters.

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