

Determination of Total Petroleum Hydrocarbon Detection in Hydrocarbon Contaminated Surface Waters in the Niger Delta; Using ELISA as an Analytical Technique

Dr. Alaye T. T.¹; Kanda R.¹; Dr. Chaudhary A. J.¹; Otuku I. E.²

¹Professor

¹College of Health, Medicine and Life Sciences (CHMLS), Department of Life Sciences,
Division of Environmental Sciences, Brunel University London

²Seats Research Centre, Port Harcourt, Rivers State.

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Abstract: The Niger Delta region of Nigeria has experienced persistent hydrocarbon contamination due to extensive oil and gas exploration activities. This study determined of total petroleum hydrocarbon (TPH) detection in hydrocarbon contaminated surface waters in the Niger Delta; using ELISA as an analytical technique. Ninety (90) surface water samples were collected from six (6) sampling locations, including a control site, and analyzed using the ELISA technique. Results revealed that TPH concentrations in surface water ranged from 0.033 to 10.32 mg/L, with an average of 3.49 ± 2.64 mg/L. These concentrations exceeded the Nigerian Standard for Drinking Water Quality (NSDWQ) limit of 0.003 mg/L and the Environmental Guidelines and Standards for the Petroleum Industry in Nigeria (EGASPIN) limit of 0.05 mg/L, indicating severe hydrocarbon pollution. The study concluded that ELISA method demonstrated high accuracy, sensitivity, and reproducibility, validating its efficiency as an alternative to conventional chromatographic methods for environmental hydrocarbon analysis.

Keywords: Hydrocarbon Pollution, TPH Concentration Determination, ELISA Technique, ERT Technique, Niger Delta Region, Nigeria.

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I. INTRODUCTION

Environmental pollution refers to the introduction of harmful substances or energy into the natural environment, resulting in adverse ecological and health impacts. Pollutants may be chemical, physical, or biological in nature—ranging from crude oil and pesticides to heat or noise—and can originate from both natural and anthropogenic sources. In Nigeria, particularly in the Niger Delta region, hydrocarbon pollution has become one of the most pervasive forms of environmental degradation (Kadafa, 2012; Anejionu et al., 2015).

Over the past five decades, petroleum exploration, production, and transportation have intensified across the Niger Delta, generating substantial economic benefits while simultaneously posing severe environmental challenges (Johnson et al., 2022). The release of petroleum hydrocarbons through spills, leakages, and operational discharges into

rivers and creeks has severely compromised water quality and ecosystem health. Hydrocarbon contamination of surface waters not only threatens aquatic biodiversity but also endangers human populations that rely on these water bodies for domestic, agricultural, and industrial purposes (Ite et al., 2018; Ahiamammunnah, 2010).

The Niger Delta region remains the hub of Nigeria's oil and gas industry, hosting over 600 oil fields, with approximately 360 located onshore and 246 offshore (NNPC, 2020). Since the discovery of oil in commercial quantity at Oloibiri in 1956, petroleum has dominated Nigeria's economy, contributing about 95% of foreign exchange earnings and over 60% of government revenue (CIA World Factbook, 2020). However, this dependence has come at great environmental cost. Frequent oil spills and leakages, coupled with illegal refining and pipeline vandalization, have led to persistent contamination of rivers, mangroves, and wetlands (Idoga et al., 2025; Egwu, 2012).

The environmental impacts of these pollutants are extensive—ranging from oxygen depletion in aquatic systems to bioaccumulation of toxic compounds in fish and other aquatic organisms (Mustafa et al., 2024; Nriagu et al., 2016). Hydrocarbon pollution also affects livelihoods by disrupting fishing and farming activities in local communities. According to the National Oil Spill Detection and Response Agency (NOSDRA, 2019), over 9,000 oil spill incidents have been recorded in the past decade, underscoring the magnitude of this ecological crisis.

Monitoring and assessing the extent of hydrocarbon pollution in surface waters are essential for environmental management. Traditionally, analytical techniques such as Gas Chromatography (GC) and Infrared Spectrophotometry (IR) have been employed to determine Total Petroleum Hydrocarbon (TPH) concentrations in environmental samples. However, these methods are often expensive, time-consuming, and require sophisticated laboratory facilities (Ezeani et al., 2022; Adeniji et al., 2017).

In contrast, the Enzyme-Linked Immunosorbent Assay (ELISA) offers a rapid, cost-effective, and sensitive analytical approach for detecting hydrocarbons in water. ELISA operates on the principle of antigen-antibody specificity, allowing for accurate quantification of petroleum hydrocarbon residues even at trace concentrations (USEPA, 2014; Ghosh et al., 2019). The technique's simplicity and

reproducibility make it particularly suitable for large-scale monitoring in resource-limited settings such as the Niger Delta (Okparanma & Mouazen, 2013).

Given the persistent hydrocarbon contamination in surface waters and the need for efficient analytical tools, this study focuses on evaluating the effectiveness of the ELISA technique in detecting Total Petroleum Hydrocarbons (TPH) in hydrocarbon-contaminated surface waters of the Niger Delta. The research provides an empirical basis for adopting ELISA as an alternative analytical technique to enhance environmental monitoring and management in oil-impacted ecosystems.

II. MATERIALS AND METHODS

➤ *Geology and Hydrogeology of the Study Area*

The study area lies within the Niger Delta region of Nigeria, characterized by a flat to gently undulating topography with an average elevation of about 50 m above sea level. The region experiences high annual rainfall ranging between 1,450 and 2,400 mm, mostly occurring from April to October, which provides significant groundwater recharge (Udom et al., 1998). The drainage system is dense, consisting of numerous perennial rivers and creeks that discharge into the Atlantic Ocean, creating marshy lowlands and floodplains.

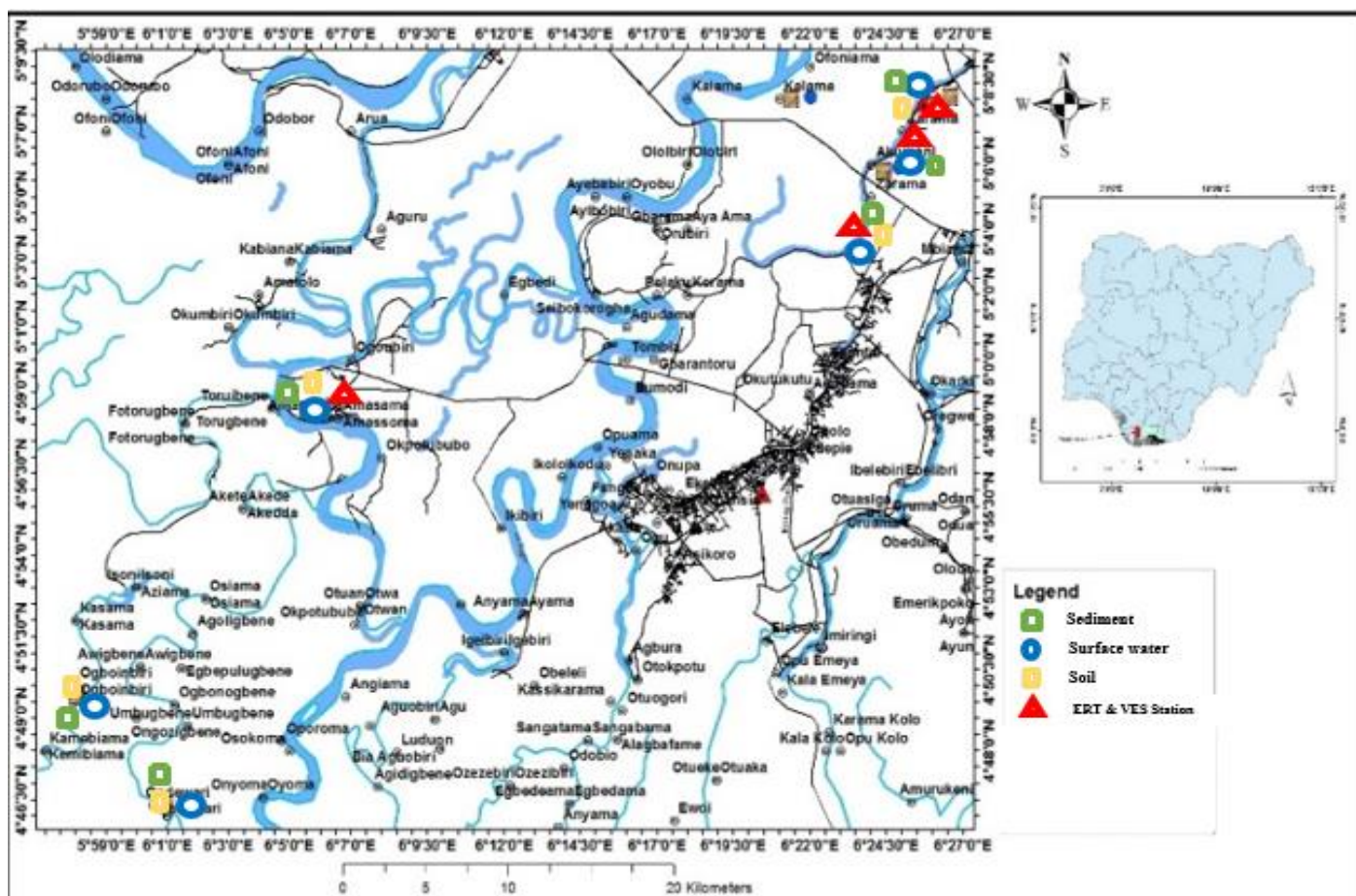


Fig 1 Map Showing the Study Area and Sampling Location in Upstream and Downstream Areas.

Source; www.bayelsagis.gov.ng (Accessed, 12/07/2019)

Geologically, the area is underlain by the Benin Formation, a component of the Niger Delta sedimentary basin, composed of unconsolidated sands, silts, and gravel with clay intercalations (Nwozor et al., 2025; Wali et al., 2021). This formation overlies the Agbada and Akata Formations of Eocene and Paleocene ages, respectively. The hydrogeological system supports shallow aquifers with borehole depths typically between 15 and 50 m (Nwankwoala et al., 2014), recharged primarily through rainfall infiltration (Eyankware et al., 2021).

➤ Climate

The Niger Delta lies within the sub-equatorial climatic zone and experiences high humidity (average 75%) and temperatures ranging from 20°C to 33°C throughout the year. The region is marked by two main seasons: the wet season (April–October) and the dry season (November–March). The wet season is characterized by heavy rainfall, peaking in June and July, interrupted briefly by the “August break,” a short dry spell. Annual rainfall exceeds 3,000 mm (Ofoezie et al., 2022), while the dry season is influenced by the northeast trade winds that bring harmattan conditions.

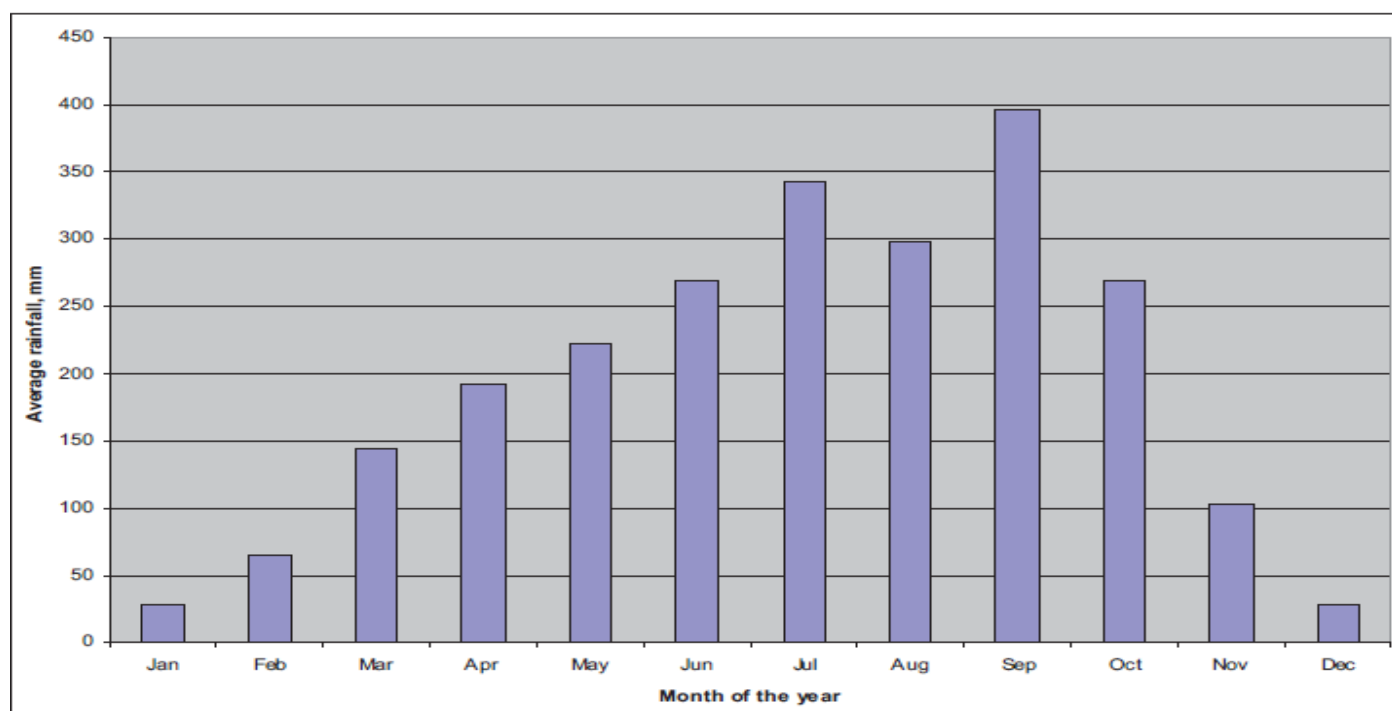


Fig 2 Mean Monthly Rain Fall in the Study Area (UNEP, 2011)

➤ Vegetation

The vegetation is dominated by tropical rainforest, freshwater swamp forest, and mangrove ecosystems. The freshwater swamp forest is rich in raffia palms and African mango, while the tropical rainforest supports dense tree growth of high economic value for timber and industrial use. The vegetation provides a supportive ecological environment but is increasingly threatened by hydrocarbon contamination from oil exploration and production.

➤ Sampling Locations

Surface water samples were collected from six major rivers across Bayelsa State in the central Niger Delta. These include Atumatu Creek (Tein-Biseni), Oya Creek (Ikarama), Taylor Creek (Kilama), Ogboinbiri/Ossiam River, Okpotuwari/Ondewari Creek, and Amassoma River (control site). The sampling sites were strategically selected to represent hydrocarbon-impacted and control areas. Each location has a history of petroleum operations or spill incidents, except Amassoma, which served as the uncontaminated control station. In the polluted sites, oil spills from facilities operated by Shell Petroleum Development Company (SPDC) and Nigeria Agip Oil Company (NAOC)

have been recurrent, leading to varying degrees of hydrocarbon contamination in surface waters.

A random sampling approach was adopted to ensure unbiased representation of the study area (Makwana et al., 2023). At each river, 15 sampling points were established along the watercourse, spaced approximately 15–20 m apart. This method aligns with previous environmental sampling studies (Micheal & Chukwu, 2023; Alilou et al., 2019).

➤ Surface Water Sample Collection

Surface water samples were collected using a locally fabricated stainless-steel scoop attached to a 25 m pole to ensure safe collection from the river center. Approximately 1–2 litres of surface water were collected into pre-cleaned amber glass bottles at each sampling point. The containers were rinsed thrice with the sample water before collection to avoid contamination. Samples were labelled, stored in ice boxes, and transported to the laboratory for analysis within 24 hours.

➤ Analytical Methodology

This research adopted an integrated analytical approach for the detection and quantification of Total Petroleum

Hydrocarbons (TPH) in surface waters using the Enzyme-Linked Immunosorbent Assay (ELISA) method. The analysis was carried out using the ModernWater RaPID Assay Kit (Serial No: A00162), designed for in-field and laboratory detection of petroleum hydrocarbon compounds.

➤ Calibration and Standard Preparation

Calibration standards were prepared using diesel as a reference hydrocarbon. Six calibration concentrations (0.00, 0.42, 0.84, 1.62, 2.80, and 4.20 mg/L) were used to generate a standard curve for quantification. These concentrations were selected based on the linear range of the assay kit as recommended in the manufacturer's manual. The standards were prepared using deionized water and analyzed before field testing to verify accuracy and reproducibility.

➤ Principle of the ELISA RaPID Assay

The ELISA technique operates on antigen-antibody specificity. In the RaPID Assay, samples and enzyme conjugates are combined with magnetic particles coated with BTEX-specific antibodies. Both the target hydrocarbon and enzyme-labelled analog compete for antibody binding sites. After incubation, a magnetic field isolates the bound particles, and unbound reagents are removed. A colorimetric substrate (3,3',5,5'-tetramethylbenzidine) and hydrogen peroxide are added to produce a color change. The intensity of the color, measured photometrically at 450 nm, is inversely proportional to the hydrocarbon concentration. Lighter color indicates higher hydrocarbon levels, while darker color indicates lower concentrations (USEPA, 2014).

➤ Dilution of Samples

Samples with TPH concentrations exceeding the assay's upper detection limit were diluted using deionized water. For surface water samples, 1 mL of the original sample was

diluted to 10 mL total volume. All dilutions were performed in duplicate to ensure analytical precision and consistency.

➤ Data Analysis

Analytical results were processed using R Studio (v3.2.3), ProUCL (v5.0), and Microsoft Excel (2013). R Studio was used for graphical visualization, histogram plots, and one-way ANOVA. ProUCL was applied for computing mean, standard deviation, and one-sample t-tests, as well as normality assessments. Excel was used to plot calibration curves and regression analyses for TPH concentration determinations. Statistical significance was evaluated at $p < 0.05$.

In all, this methodology provided a systematic framework for evaluating the efficacy of ELISA in detecting Total Petroleum Hydrocarbons (TPH) in hydrocarbon-contaminated surface waters of the Niger Delta. The study's design ensured representativeness of both impacted and control sites, while the analytical process offered a reliable, cost-effective, and field-deployable alternative to conventional chromatographic techniques.

III. RESULTS

➤ Analysed Surface Water Samples

The results of the surface water samples analysed are shown in Table 1. For each sampling station, after the calibration of the Rapid Assay test equipment with the required prepared standard solution that were prepared according to the linear range of the equipment, each surface water sample was analysed three times to determine the random variability. From the normality test carried out, showed that results obtained are normally distributed, hence, a parametric test procedures were followed for the various data analysis.

Table 1 Descriptive Summary Statistics of Surface Water Samples that were Analysed Three Times Each for TPH Concentration from the Six Various Locations and their Sampling Points of the Study Area with the Standard Deviation as the Error Bar.

Location	Analysed samples results from the various surface water sampling stations (mg/L)					
	Amassoma river (control)	Oya-Creek	Atumatu-Creek	Ogboinbiri/Ossiamma river	Okpotuwari/Ondewari River	Taylor creek
Sample point 1	0.096±0.004	6.412±0.041	4.21±0.003	0.354±0.015	2.015±0.001	2.064±0.003
Sample point 2	0.124±0.003	2.285±0.015	2.163±0.002	2.017±0.003	0.266±0.004	9.167±0.005
Sample point 3	0.033±0.002	10.08±0.057	1.637±0.002	1.220±0.009	7.236±0.024	6.186±0.005
Sample point 4	0.186±0.002	8.807±0.009	3.262±0.001	0.319±0.005	0.372±0.002	0.24±0.007
Sample point 5	0.091±0.001	0.663±0.018	4.216±0.002	5.134±0.005	0.416±0.047	6.12±0.006
Sample point 6	0.272±0.003	4.534±0.026	1.164±0.003	2.622±0.010	1.145±0.002	2.153±0.180
Sample point 7	0.084±0.002	8.147±0.006	4.213±0.004	2.415±0.015	2.163±0.002	3.225±0.004
Sample point 8	0.095±0.004	7.401±0.009	3.341±0.002	4.230±0.006	1.916±0.004	1.237±0.003
Sample point 9	0.133±0.003	10.32±0.015	3.113±0.008	0.177±0.014	4.809±0.007	2.346±0.020
Sample point 10	0.209±0.007	6.441±0.009	2.142±0.003	0.626±0.001	0.629±0.007	5.315±0.016
Sample point 11	0.083±0.002	0.124±0.002	3.137±0.002	5.220±0.001	5.421±0.009	8.143±0.006
Sample point 12	0.099±0.004	2.16±0.001	6.224±0.002	1.317±0.003	7.122±0.010	4.127±0.012
Sample point 13	0.035±0.003	1.22±0.008	2.551±0.001	0.152±0.002	1.177±0.003	2.252±0.061

Sample point 14	0.049±0.002	3.06±0.009	3.143±0.003	6.263±0.002	3.523±0.010	8.191±0.051
Sample point 15	0.213±0.002	2.17±0.006	4.223±0.002	2.174±0.003	4.614±0.002	1.238±0.014
Ranges	0.033-0.272	0.124-10.32	1.164-6.224	0.152-6.263	0.266-7.236	0.240-9.167
Mean	0.120	4.922	3.249	2.283	2.855	4.134
SD	0.070	3.511	1.329	2.339	2.434	2.868
Skewness	0.828	0.198	0.439	0.530	0.699	0.488

➤ *Surface Water Regulatory Standard for TPH Intervention and Target Values*

Results in Table 3 presents the calculated one sample t-test parameters of surface water TPH concentration obtained from the study area against the NSDQW (NSDQW, 2018). Table 2 shows the summary statistics of the total mean

concentration and pooled standard deviation for surface water samples TPH concentration analysed from the six sites including the control site in the study area. Table 4 shows the Environmental Quality Standard for drinking water between Nigerian and WHO.

Table 2 One Sample T-Test Parameters Calculated for the NSDQW Standard (0.003 mg/L)
Comparison for Surface Water Samples TPH Total Mean Concentration

Parameters	AM	OY	AT	OO	OD	TC
Average mean	0.120	4.922	3.249	2.283	2.855	4.134
Test statistics	6.453	5.426	9.917	4.339	4.539	5.579
Critical value	1.761	1.761	1.761	1.761	1.761	1.761
df	14	14	14	14	14	14
p-value	0.006	0.005	0.008	0.004	0.004	0.005

For AM, the $H_0; \mu_{AM} < \text{NSDQW}$ permissible limit of 0.003 mg/L against the alternative $H_1; \mu_{AM} > \text{NSDQW}$ permissible limit was determined with a ProUCL software. For AM surface water with total mean of 0.120±0.070 mg/L analysed against the NSDQW 0.003 mg/L as limit for drinking water shows that, AM surface water mean of 0.120±0.70 mg/L lies within the rejection region (critical value-1.761, df-14 one sided and a p-value-0.006). Hence the null hypothesis is rejected and concluded that the total mean concentration of TPH for AM surface water of 0.120 mg/L is greater than the NSDQW permissible limit of 0.003 mg/L for a drinking water.

From the data analyses, OY surface water with TPH total mean concentration of 4.922 mg/L with a standard deviation of 3.511 with a calculated t-value of 5.426 lies

within the rejection region (critical value-1.761, df-14 one sided and a p-value-0.005). Thus, the null hypothesis is rejected and concluded that the total mean concentration of TPH for OY surface water samples of 4.922 mg/L is greater than the NSDQW permissible limit of 0.003 mg/L for a drinking water. For surface water intervention value, Table 2.5 shows the EGASPIN intervention and target values for TPH in groundwater. Table 4.8 shows the various calculated one sampling t-test values of surface water TPH total mean against the NSDQW limit for drinking water, while Table 4.9 and 4.10 shows the calculated one sample t-test parameters for surface water TPH concentration obtained from the study area against the EGASPIN target and intervention values for drinking water sources.

Table 3 One Sample T-Test Parameters Calculated for the EGASPIN Target Values Standard (0.05 mg/L)
Comparison for Surface Water Samples TPH Total Mean Concentration.

Parameters	AM	OY	AT	OO	OD	TC
Average mean	0.120	4.922	3.249	2.283	2.855	4.134
Test statistics	3.864	5.374	9.774	4.250	4.464	5.515
Critical value	1.761	1.761	1.761	1.761	1.761	1.761
df	14	14	14	14	14	14
p-value	0.0008	0.0005	0.0008	0.0004	0.0002	0.0004

For AM, the $H_0; \mu_{AM} < \text{EGASPIN}$ target value of 0.05 mg/L against the alternative $H_1; \mu_{AM} > \text{EGASPIN}$ target value was determined with a ProUCL software. Table 4.16 shows the analysed t-value, critical value, df and p-value of the various sites. From the data analyses, AM surface water with total TPH mean concentration of 0.120 mg/L with a standard deviation of 0.070 with a calculated t-value of 3.864 lies within the rejection region (critical value-1.761, df-14 one sided and a p-value-0.0008). Hence the null hypothesis is

rejected and concluded that the total TPH mean concentration for AM surface water of 0.120 mg/L is greater than the EGASPIN target value of 0.05 mg/L for a drinking water sources. For OY, the $H_0; \mu_{OY} < \text{EGASPIN}$ target value of 0.05 mg/L against the alternative $H_1; \mu_{OY} > \text{EGASPIN}$ target value was determined with a ProUCL. From the data analyses, OY surface water with TPH total mean concentration of 4.922 mg/L with a standard deviation of 3.511 with a calculated t-value of 5.374 lies within the rejection region (critical value-

1.761, df-14 one sided and a p-value-0.005). Thus, the null hypothesis is rejected and concluded that the total TPH mean concentration for OY surface water samples of 4.922 mg/L is

greater than the EGASPIN target value of 0.05 mg/L for a drinking water sources.

Table 4 Comparative Environmental Standards for Drinking Water Between Nigerian Standard for Drinking Water Quality (NSDQW) and WHO

Substance	Nigeria Drinking water Standard ($\mu\text{g/L}$)	WHO guideline ($\mu\text{g/L}$)
Benzene	No standards set	10
Toluene	No standards set	700
Ethyl benzene	No standards set	300
PAHs	7	No standards set
Mineral oil	3	No standards set

Source; SON, (2007). Chrome-Extension://efaidnbmnnnibpcajpcglclefindmkaj/viewer.html

Table 5 One Sample T-Test Parameters Calculated for the EGASPIN Intervention Value Standard (0.6 mg/L) Comparison for Surface Water Samples TPH Total Mean Concentration

Parameters	AM	OY	AT	OO	OD	TC
Average mean	0.120	4.922	3.249	2.283	2.855	4.134
Test statistics	-26.44	4.768	8.093	3.203	3.588	4.772
Critical value	1.761	1.761	1.761	1.761	1.761	1.761
df	14	14	14	14	14	14
p-value	1	0.005	0.007	0.0032	0.005	0.004

For AM, the H_0 ; $\mu_{AM} < \text{EGASPIN intervention value of } 0.6 \text{ mg/L}$ against the alternative H_1 ; $\mu_{AM} > \text{EGASPIN intervention value}$ was determined with a ProUCL software. From the data analyses, AM surface water with total TPH mean concentration of 0.120 mg/L with a standard deviation of 0.070 with a calculated t-value of -26.44 lies within the retention region (critical value-1.761, df-14 one sided and a p-value of 1). Hence the null hypothesis is accepted and concluded that the total TPH mean concentration for AM surface water of 0.120 mg/L is less than the EGASPIN intervention value of 0.6 mg/L for a drinking water sources.

For OY, the H_0 ; $\mu_{OY} < \text{EGASPIN intervention value of } 0.6 \text{ mg/L}$ against the alternative H_1 ; $\mu_{OY} > \text{EGASPIN intervention value}$ was determined with a ProUCL. From the data analyses, OY surface water with TPH total mean concentration of 4.922 mg/L with a standard deviation of

3.511 with a calculated t-value of 4.768 lies within the rejection region (critical value-1.761, df-14 one sided and a p-value-0.0005). Thus, the null hypothesis is rejected and concluded that the total TPH mean concentration for OY surface water samples of 4.922 mg/L is greater than the EGASPIN intervention value of 0.6 mg/L for a drinking water sources.

➤ Histogram Plot of Surface Water Sampling Sites

The histogram plot of the various surface water sampling locations are shown in Figure 4.9 - 4.14. From the plots, the concentration frequency and the concentration ranges of the analysed samples are indicated pictorially. The various histogram plots enable the inspection of data set for its underlying frequency distribution (normalcy distribution, outliers, skewness, etc). TPH concentration ranges where divided into series of interval.

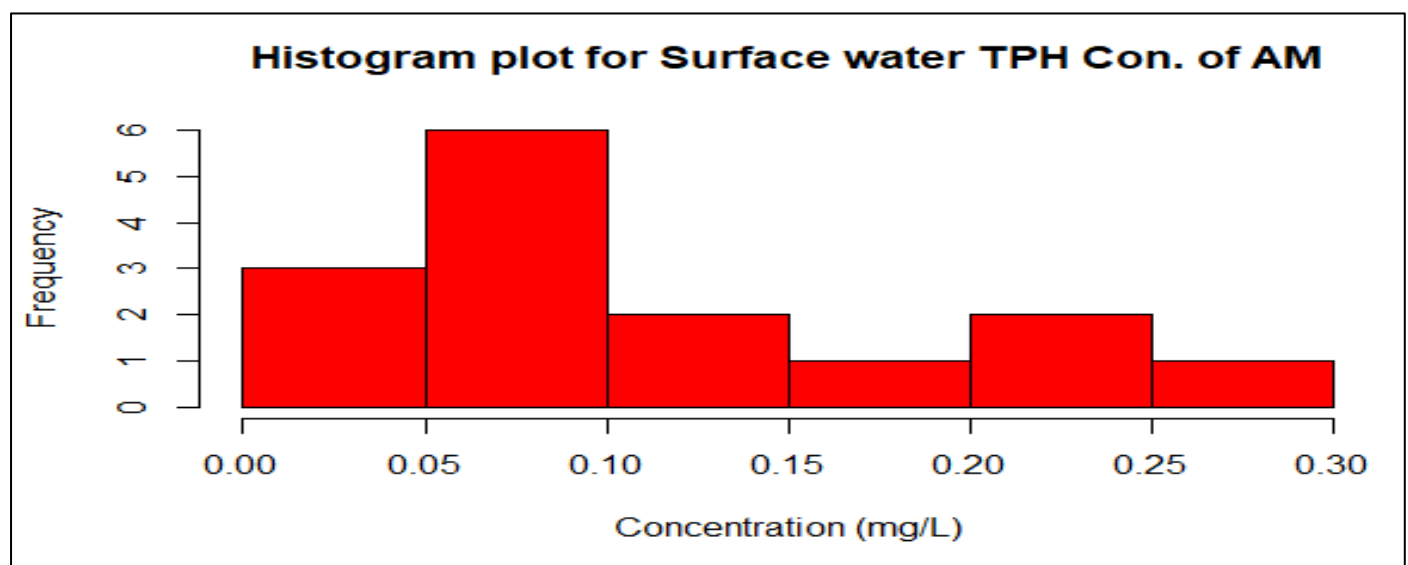


Fig 3 Histogram Plot of Surface Water TPH Concentration of Amassoma River (Control Site)

The data in Figure 3 illustrate the concentration frequency of TPH in surface water samples in Amassoma River which serves as the control in the study area. From the histogram plot, TPH concentrations ranges from 0.033 to 0.272 mg/L. The surface water TPH concentration values of 0.00 to 0.04 mg/L comprises of 20% of the total percentage, while the concentration values of 0.05 to 0.09 mg/L comprises of 40% of the total percentage and is more

dominant with 6 samples falling within the range out of the 15. The concentration values of 0.10 to 0.14 mg/L comprises of 13.3%, 0.15 to 0.19 mg/L comprises of 6.7%, 0.20 to 0.24 mg/L comprises of 13.3% and finally 0.25 to 0.29 mg/L comprises of 6.7% respectively. The histogram frequency plot was obtained with the application of R/Studio package (R version 3.1.3)

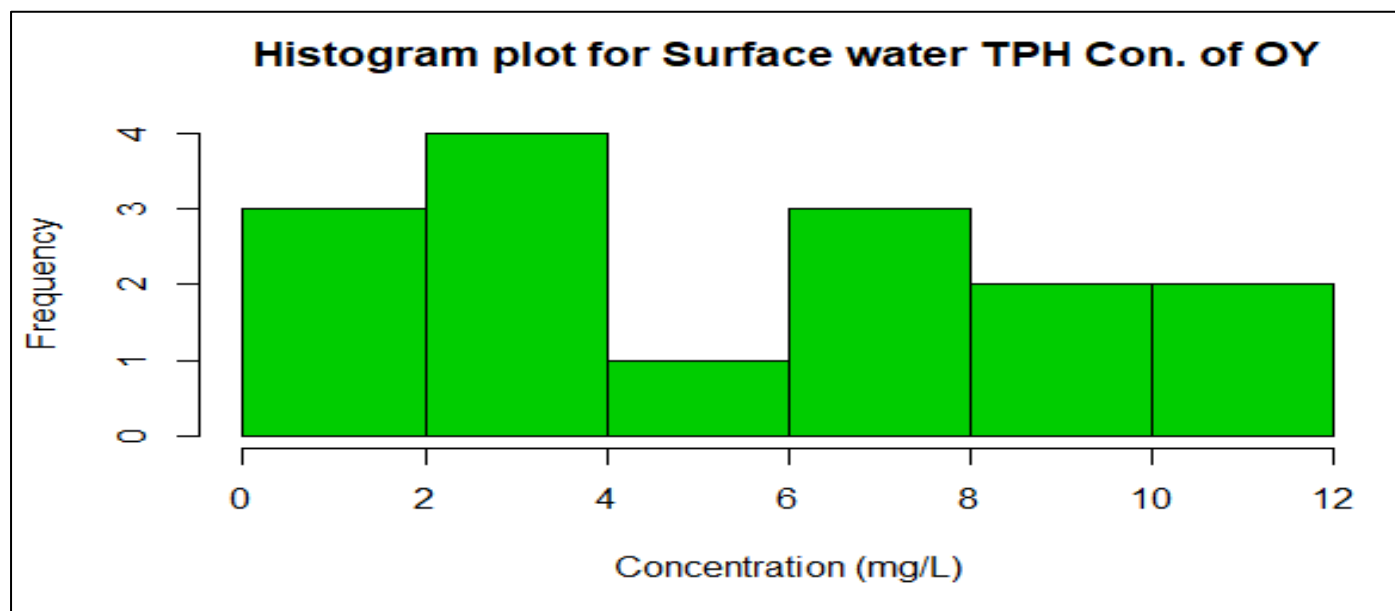


Fig 4 Histogram Plot of Surface Water TPH Concentration of Oya Creek (Ikarama Community)

The data in Figure 4 illustrate the TPH concentration frequency of analysed surface water samples in Oya- Creek (Ikarama community) in the study area. From the analysed data of the surface water samples, the TPH concentrations in Oya-creek ranges from 0.124 to 10.32 mg/L. From the histogram plot, TPH concentration range from 0.124 to 1.9 mg/L comprises of 20% out of the total percentage, while concentration range from 2.0 to 3.9 mg/L comprises of 26.7%

and is more dominant within the 15 analysed surface water samples in the river. TPH concentration range of 4.0 to 5.9 mg/L comprises of 6.7%, 6.0 to 7.9 mg/L comprises of 20%, 8.0 to 9.9 mg/L comprises of 13.3% while 10.0 to 11.9 mg/L also comprises of 13.3% out of the 15 samples analysed for TPH in Oya-creek respectively. The plot was obtained with the application of R/Studio package (R version 3.1.3).

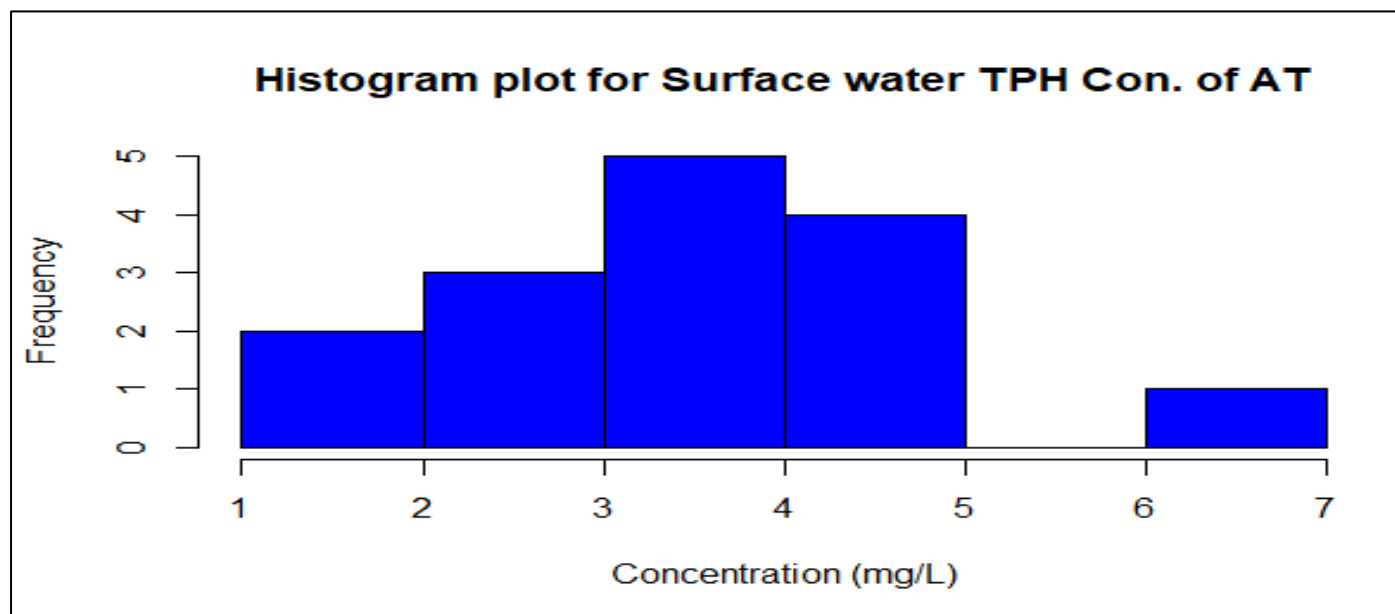


Fig 5 Histogram Plot of Surface Water TPH concentration of Atumatu Creek (Tein-Biseni Community).

The data in Figure 5 illustrated the TPH concentration frequency of analysed surface water samples of Amumatu Creek (Tein-Biseni Community) of the study area. From the data, TPH concentration ranges from 1.164 to 6.224 mg/L. From the histogram plot, TPH concentration values between 1.164 to 1.9 mg/L comprises of 13.3% of the total analysed samples, while TPH concentration values between 2.0 to 2.9

mg/L comprises of 20% of the total analysed samples. TPH concentration range between 3.0 to 3.9 mg/L comprises of 33.3%, while 4.0 to 4.9 mg/L comprises of 26.7% respectively. TPH concentration values from 5.0 to 6.224 mg/L comprises of 6.7% of the total analysed surface water samples of Atumatu creek. Histogram plot was obtained with the R/Studio package.

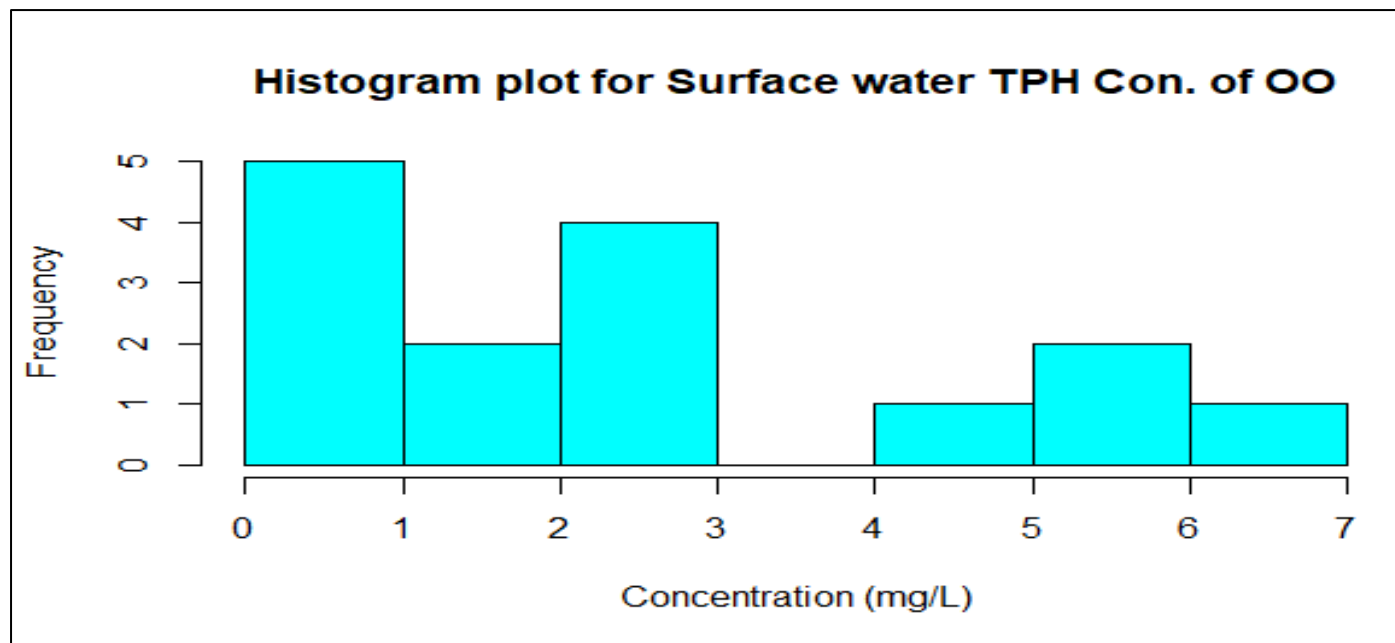


Fig 6 Histogram Plot of Surface Water TPH Concentration of Ogboinbiri/Ossiamia River

The data in Figure 6 illustrated the TPH concentration frequency of analysed surface water samples of Ogboinbiri/Ossiamia River of the study area. From the data, TPH concentration ranges from 0.126 to 6.263 mg/L. From the histogram plot, TPH concentration values between 0.126 to 0.9 mg/L comprises of 33.3% of the total analysed samples, while TPH concentration values between 1.0 to 1.9 mg/L comprises of 13.3% of the total analysed samples. TPH

concentration range between 2.0 to 2.9 mg/L comprises of 26.7%, while 3.0 to 4.9 mg/L comprises of 6.7 % respectively. TPH concentration values from 5.0 to 5.9 mg/L comprises of 13.3% and 6.0 to 6.9 mg/L comprises of 6.7% of the total analysed surface water samples of Ogboinbiri/Ossiamia River. Histogram plot was obtained with the R/Studio package.

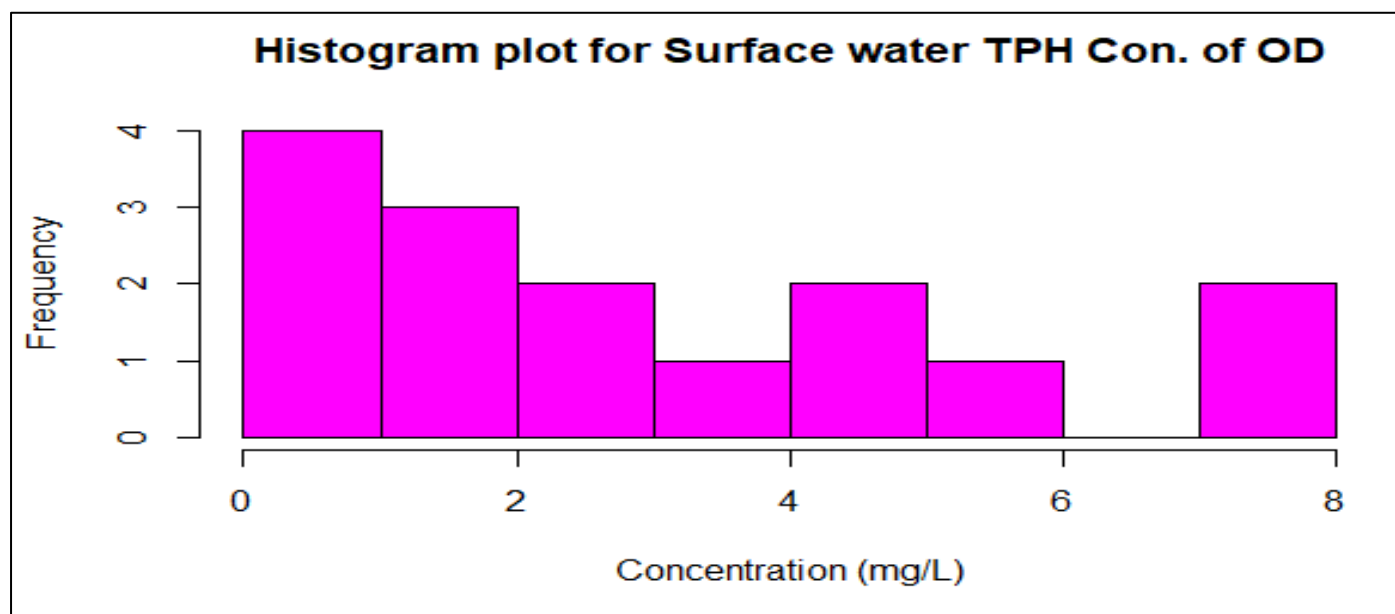


Fig 7 Histogram Plot of Surface Water TPH Concentration of Okpotuwari/Ondewari River.

The data in Figure 7 illustrated the TPH concentration frequency of analysed surface water samples of Okpotuwari/Ondewari River of the study area. From the data, TPH concentration ranges from 0.266 to 7.236 mg/L. From the histogram plot, TPH concentration range between 0.266 to 1.9 mg/L comprises of 46.7% of the total analysed samples, while TPH concentration values between 2.0 to 3.9 mg/L

comprises of 20% of the total analysed samples. Also TPH concentration value between 4.0 to 5.9 mg/L comprises of 26.7%, while 6.0 to 7.9 mg/L comprises of 13.3% respectively of the total analysed surface water samples of Okpotuwari/Ondewari River. Histogram plot was obtained with the R/Studio package.

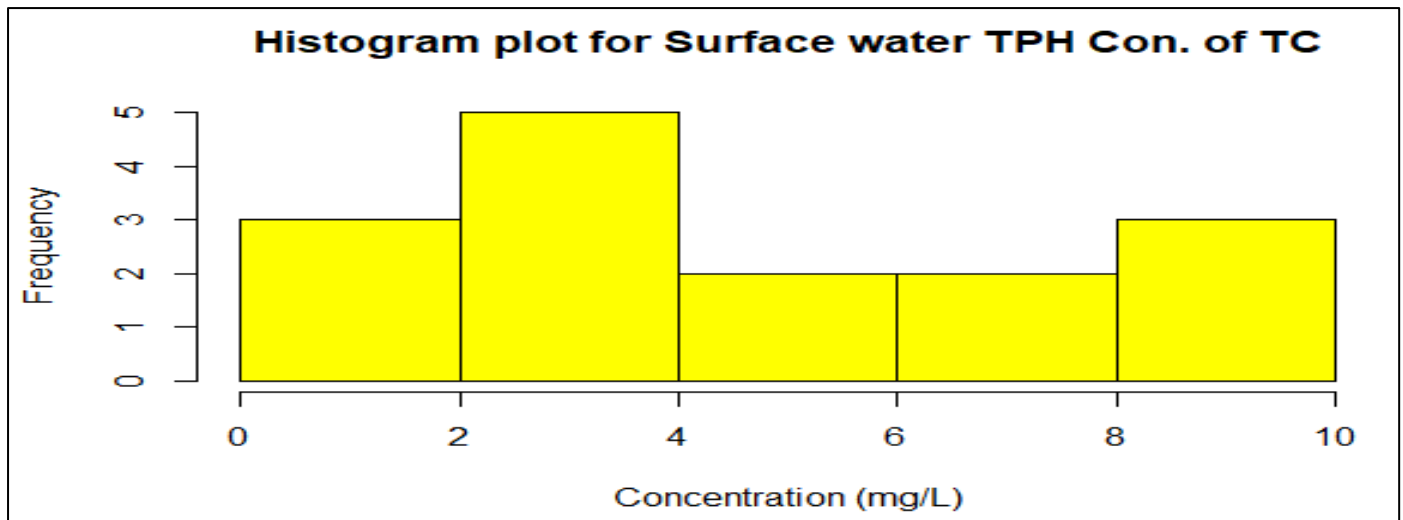


Fig 8 Histogram Plot of Surface Water TPH Concentration of Taylor Creek (Kilama Community).

The data in Figure 8 illustrated the TPH concentration frequency of analysed surface water samples of Taylor Creek (Kilama Community) of the study area. From the data, TPH concentration ranges from 0.240 to 9.167 mg/L. From the histogram plot, TPH concentration range between 0.266 to 1.9 mg/L comprises of 20% of the total analysed samples, while TPH concentration values between 2.0 to 3.9 mg/L comprises of 33.3% of the total analysed samples. Also TPH concentration value between 4.0 to 7.9 mg/L comprises of 13.3%, while 6.0 to 9.9 mg/L comprises of 26.7% respectively of the total analysed surface water samples of Taylor Creek. Histogram plot was obtained with the R/Studio package.

➤ Surface Water TPH Concentration Post-hoc-Test and Boxplot Test

From the one-way analysis of variance (ANOVA) test which indicated that there is difference between the means of the six groups with a F-calculated value 7.743 which is greater than the F-table value 2.34, (df(B)5, df(W)84 at 95% upper critical value) with a pooled standard deviation of 2.317 and p-value 0.00005. Hence, the values obtained from the ANOVA test shows that, there is a significant difference among the locations of samples. Therefore, the null hypothesis is rejected, while the alternative hypothesis is accepted.

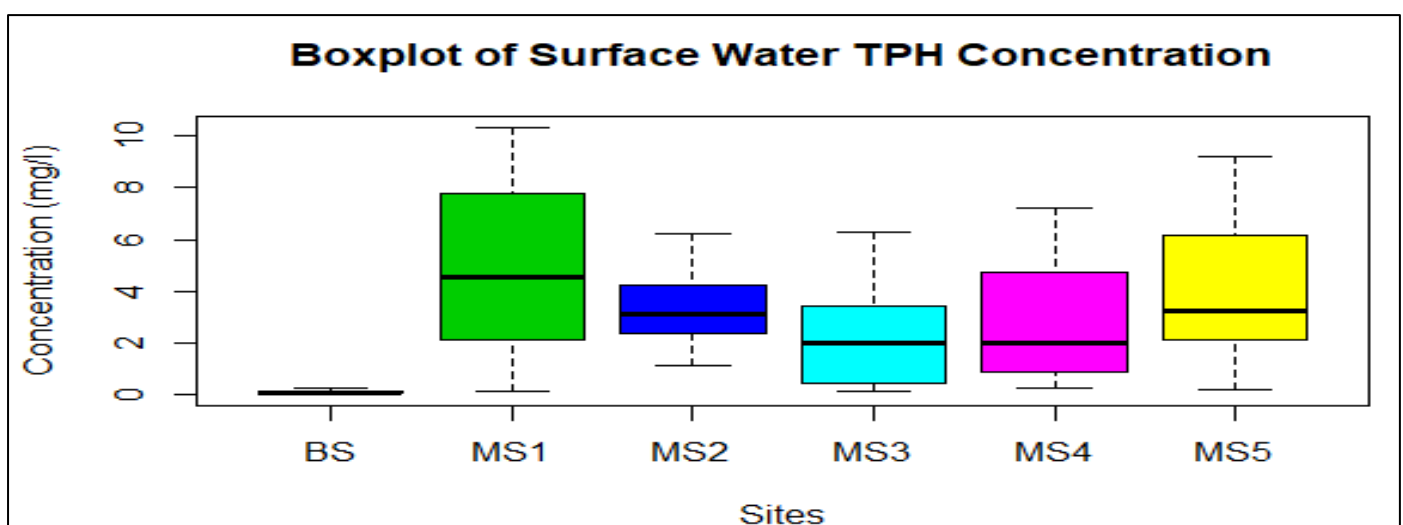


Fig 9 The Boxplot Test of Surface Water TPH Sample Concentration for the Six Locations in the Study area (BS represent the AM sampling site, MS1 represent OY sampling site, MS2 represent AT sampling site, MS3 represent OO sampling site, while MS4 represent OD sampling site and MS5 representing sampling site TC).

The data in Figure 9 illustrated the Boxplot analysis of the six sampling sites of the surface water samples for TPH concentration of the study area. It shows a pictorial view of the significant differences that exist between the means of the six sampling sites of the study area. Boxplot was obtained with the R/Studio package.

IV. DISCUSSION

The analysis of surface water samples using the Enzyme-Linked Immunosorbent Assay (ELISA) technique in this study demonstrated the method's reliability, repeatability, and sensitivity for Total Petroleum Hydrocarbon (TPH) detection in hydrocarbon-contaminated environments. Results from calibration curve analyses and field sample determinations confirmed that ELISA provided consistent and reproducible measurements of TPH concentration across all examined surface water matrices.

The precision of ELISA in quantifying hydrocarbon contamination highlights its utility as a rapid, affordable, and field-deployable alternative to conventional laboratory-based methods such as Gas Chromatography–Mass Spectrometry (GC–MS). The findings revealed that ELISA can offer fast, accurate, and cost-effective quantitative measurement of TPH levels in environmental samples. This outcome aligns with previous research by Douglas et al. (2017) and Wang et al., (2021) who independently reported ELISA as a rapid and cost-effective in-situ analytical technique for petroleum hydrocarbon detection.

The comparative advantage of ELISA lies not only in its analytical speed but also in its affordability and portability. While GC–MS remains the benchmark analytical method for hydrocarbon quantification, it requires complex instrumentation, skilled technicians, and substantial financial investment. During the course of this study, the planned GC–MS validation phase could not be completed due to restrictions imposed by the COVID-19 lockdown, which limited laboratory access and sample transport. Nonetheless, the ELISA assay successfully provided quantitative and reproducible results under field conditions, emphasizing its robustness and adaptability as an alternative tool for environmental hydrocarbon monitoring.

A supporting study by Lourenço et al. (2021), which evaluated several petroleum hydrocarbon field test kits, indicated that immunoassay (IMA) analytical methods, including ELISA, are reliable for field-level measurement of hydrocarbons in environmental media. However, Lourenço et al. (2021) noted that although IMA results are not exact replicas of those obtained from GC–MS, they show strong correlation trends and provide sufficiently accurate estimates for field monitoring and rapid decision-making. Similarly, Francioni et al. (2002) evaluated commercially available ELISA kits and confirmed their suitability as dependable in-field diagnostic tools for assessing hydrocarbon pollution in aquatic systems.

Furthermore, the U.S. Environmental Protection Agency (EPA, 2014) also recognizes immunoassay-based

field kits as credible methods for delineating petroleum hydrocarbon contamination. These evaluations collectively validate ELISA as a dependable and efficient analytical approach, capable of providing immediate contamination assessment without the logistical and financial constraints of conventional chromatographic techniques.

From an economic standpoint, the use of ELISA is particularly advantageous in developing nations where environmental monitoring is often constrained by limited resources. According to Lourenço et al. (2021) and Okparanma and Mouazen (2013), the cost of conducting a single GC–MS hydrocarbon analysis typically ranges around one hundred pounds sterling (£100) per sample. In contrast, the present study found that ELISA analysis costs approximately fourteen pounds (£14) per sample, representing nearly an 86% reduction in analytical expenditure. This affordability enhances the feasibility of routine monitoring across multiple sites and encourages more frequent environmental assessments, particularly in resource-limited settings such as the Niger Delta.

Given Nigeria's economic context—where governmental and industrial priorities often emphasize revenue generation over environmental sustainability (Kadafa, 2012; Sam et al., 2017)—the use of affordable and efficient techniques like ELISA can substantially improve the capacity for environmental surveillance. Its low cost, simplicity, and adaptability make it a practical choice for national and local environmental protection agencies to implement periodic hydrocarbon pollution assessments and strengthen early warning systems for ecological degradation.

Moreover, the ELISA technique offers additional benefits, including minimal sample preparation, reduced solvent usage, and field portability, enabling rapid analysis directly at contamination sites. The ability to generate results within hours rather than days allows for immediate response to pollution events, such as oil spills, which are frequent in the Niger Delta. This operational advantage is particularly critical in regions where delayed laboratory analyses can result in widespread environmental damage before intervention measures are implemented.

In summary, findings from this research affirm that ELISA provides a reliable, cost-effective, and reproducible approach for determining TPH concentrations in surface waters affected by hydrocarbon pollution. While GC–MS remains indispensable for confirmatory analysis, ELISA serves as an efficient screening and monitoring tool, especially under conditions where speed, cost, and accessibility are crucial. The research further underscores the necessity for environmental authorities in Nigeria to integrate ELISA-based assays into their standard monitoring protocols. Doing so will enable more comprehensive hydrocarbon contamination mapping, enhance regulatory compliance, and support remediation planning in oil-producing regions of the Niger Delta.

Therefore, the adoption of ELISA as a rapid field analytical technique could revolutionize environmental

monitoring in developing economies by making large-scale, continuous assessment of petroleum pollution feasible, affordable, and scientifically robust. Its demonstrated accuracy and reproducibility in this study support its broader application for sustainable environmental management and pollution control in the Niger Delta and similar hydrocarbon-impacted ecosystems worldwide.

V. CONCLUSION

Total Petroleum Hydrocarbon (TPH) concentrations in surface waters of the Niger Delta region and evaluated the efficiency of the Enzyme-Linked Immunosorbent Assay (ELISA) as a rapid, cost-effective analytical technique for hydrocarbon detection. The Niger Delta, a hub of crude oil exploration and production, has suffered extensive hydrocarbon pollution from spills, leaks, and improper remediation practices (Anejionu et al., 2015). Despite numerous environmental regulations, weak enforcement, poor funding, and inadequate technical capacity among regulatory bodies have perpetuated the region's ecological degradation (Lindén & Pålsson, 2013). Surface water samples from six locations were analyzed using ELISA test kits. Results revealed high TPH concentrations across all sites, ranging between 0.033 and 10.32 mg/L, exceeding both the Nigerian Standard for Drinking Water Quality (0.003 mg/L) and EGASPIN limit (0.05 mg/L). These elevated values indicate that surface waters in the study area are heavily contaminated and unsuitable for domestic use. The findings confirm the persistence of hydrocarbon pollution, largely due to ineffective remediation and weak regulatory enforcement (UNEP, 2011; Kadafa et al., 2012). ELISA proved to be a reliable, affordable, and rapid alternative to the conventional GC/MS method (Lourenço et al. 2021; Okparanma & Mouazen, 2013). Its accuracy, reproducibility, and low operational cost make it an efficient tool for routine environmental monitoring and hydrocarbon pollution assessment in developing regions like the Niger Delta.

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APPENDIXES



Fig 10 Showing Talyor Creek in the study area as crude oil sheen covered the entire surface of the river body, with farmers applying the route to their various farms. (ERA/FoEN, Bayelsa centre, unpublished document. 2019).



Fig 11 Showing Crude Oil Contamination on the Shores of the Coastal Rivers and Visible Hydrocarbon Sheen in Surface Water in the Study Area. (UNEP Ogoni Report, 2011).