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## Preliminary Assessment of Water Quality in Some Hydrocarbon-imparted Ogoni Communities in River State, Nigeria

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#### Authors' contributions

This work was carried out in collaboration among all authors. Author CKW designed the study. Author SDA wrote the protocol, and wrote the first draft of the manuscript. Authors CBG and EGW managed the analyses of the study. Author CBG also managed the literature searches. All authors read and approved the final manuscript.

#### Article Information

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#### **ABSTRACT**

**Aim:** This study aimed to assess the quality of drinking water in some hydrocarbon-impacted Ogoni communities.

Study Design: The study employ a cross-sectional and analytical design using stratified sampling method

**Place and Duration of Study:** Department of Medical Laboratory Science of Rivers State University, Giolee Global Resource Limited and Environmental Consultancy Services between March 2020 and March 2021.

**Methodology:** Water samples were collected from (20 hydrocarbon- impacted communities) in the 4 Local Government Area (LGAs) of Ogoni land. These water samples were analyzed to determine the physicochemical, bacteriological, heavy metal and total petroleum hydrocarbon (TPH) parameters using standard methods and operational procedures. The data obtained were subjected to descriptive statistical analysis. The general linearized model (GIG) was used to

generate analysis of variance (ANOVA) mean and standard error and arrange, statistically significant was set as *p*-value of .05 (95% confidence limit). Pearson correlation test was used to calculate the correlation between TPH, Heavy metal, and physicochemical parameters in hydrocarbon and non-hydrocarbon impacted communities. All statistical analyses were performed using GraphPad Prism (Version 8).

Results: The results obtained for physicochemical parameters were pH 4.3±0.8 mg/l, EC 0.03±0.05 mg/l, DO 5.5±1.6 mg/l, Temp 25.0±0.0 mg/l, Mv 0.32±0.27 mg/l, Nitrite 0.0058±013 mg/l, Nitrate 0.1530±158 mg/l. These results were all below the recommended standard for Nigeria standard of drinking water quality (NSDWQ) and World Health Organization (WHO). The bacteriological analyses were carried out using multiple tubes technique (Most Probable Number), total coliform count, (TC), total heterotrophic count (THBC) and total fungal count (TFC). The results obtained were 0.941±2.397 cfu/ml, 89.3±176.6 cfu/ml, 297.8±144.4 cfu/ml, and 0.32±0.84 cfu/ml respectively. The p-values for TFC (<0.0002) were statistically significant. Heavy metal profiling was: Cr 0.194±0.320 mg/l, Cd 0.469±0.569 mg/l, Cu 0.211±0.348 mg/l, Pb 0.0336±0.20 mg/l, Fe 0.705±1.244 mg/l, Zn 0.258±0.249 mg/l, respectively. Generally, the concentration of heavy metal increased more than the standard recommended by NSDWQ and WHO except for Zn 0.255±0.249 mg/l, and Cu 0.56±0.50 mg/l, that is slightly lower than the acceptable limit recommended by WHO and NSWWQ. The sequences of heavy metal concentration were in Cd > Cr> Pb>Fe> Cu.>Zn. The statistical significance values for Pb p=.003 and for Zn p=.009 were statistically significant. The concentration of TPH were (349.9 ppm/ml) higher than the recommended values for NSDWQ and WHO.

**Conclusion:** The findings in this research reviewed a worrisome level of TPH and Pb, and which could have devastating impact on bacterial biodiversity.

Keywords: Petroleum hydrocarbon; bacteriological analysis; hydrocarbon-impacted; ogoni communities; water contamination.

#### 1. INTRODUCTION

Access to safe water is an essential basic human right and a component of effective policy for protection [1]. Water plays indispensable role in the sustainability of life and also a powerful environmental determinant of health [2]. The term water quality is used to express the suitability of water to sustain various uses or processes [3]. The United Nation General Assembly in 2010 recognized the human right to safe water and sanitation and advocated for a right for sufficient, continuous, safe, accessible and affordable water for everybody [4]. Although water plays an essential role in supporting life. Man approaches toward water usage has been unsustainable, it is reported that 4.6% of global Disability Adjusted Life Years (DALYs) and 3.3% of global death is related to water quality [5].

Some studies in the Niger Delta region of Nigeria have linked water contamination with flowing rivers that has been contaminated with heavy metals and unsuitable physico-chemical character from industrial waste [6]. Water also has great potential in transmitting diseases when contaminated [7]. Over the last 5 decades, oil exploration activities have introduced about 3

million barrels of oil into the environment in Niger Delta [8]. Crude oil is known to contain heavy metal, given the high spill in the Niger Delta, there are possibility for substantial heavy metal pollution, heavy metal is known to drive significant metal into the environment, and some of this heavy metal had the potential for bioaccumulation in tissue and cell of vulnerable animal [9]. However, the quality of water available and accessible to most community has been tremendous polluted and therefore has great impacts on their standard and wellbeing of the people essentially the four LGAs in Ogoni [10].

Population growth coupled with industrialization and agricultural activities generate lots of waste materials which get into water bodies thereby polluting the water tables. Pathogens such as bacterial, fungi, viruses and protozoa can get into the water tables through wash up from industrial activities, floods, open dumps, fecal excreter, etc. Some infectious pathogens can be transmitted from contaminated water. Contaminated water remained a health challenge globally causing disease outbreaks ranges from Cholera, Diarrheal, and Dysentery etc. [11] five million death of children occurred annually and make it 1/6 of the world population due to diseases from

water contamination. In 2017 the WHO estimated that Diarrhea is the most widely known disease linked to water and food contamination. Some researchers estimated that water borne pathogens infect about 250 million people annually with mortality rate of about 20 million [12]. Water can be polluted with toxic inorganic substances which can causes major damage to human health ranges from acute to chronic diseases. Chronic effect may include various system disorder, ranging from cancers, organ damage, and birth defects. Acute effects include skin irritation, nausea, dizziness, and vomiting and lung irritation [13]. Ogoni land had experienced a deplorable state in their environment for many decades due to oil exploration [14].

UNEP 2011, reported that hydrocarbon contamination in Ogoni land is wide spread and has impacted on several component of the environment, UNEP reviewed the presence of PAH and BTEX in ground water and in air, this group of hydrocarbons are known to have carcinogenic and mutagenic effects on the people. A pathetic scenario was seen in Nsisioken Ogale in Eleme LGA where benzene was detected in drinking water 900 times above World Health Organization (WHO) guideline for

drinking water, and in other cases where hydrocarbon was detected in ground water adjacent to polluted sites 1,000 times above Nigeria standard for drinking water 3 µg/l. [14]Several other studies around the region have reviewed higher level of hydrocarbon pollution than that of Environmental Guideline and Standard for Petroleum Industry in Nigeria. (EGASPIN) and Federal Environment Protection Agency (FEPA) especially the studies evaluating the impact of hydrocarbon in Bodo community in Gokana LGAs [15]. This study seeks to assess microbial analysis, heavy metal, physicochemical parameters and total petroleum hydrocarbon of water samples in some hydrocarbon-impacted Ogoni communities.

#### 2. MATERIALS AND METHODS

#### 2.1 Study Area

Ogoni land covers an area of 1,000 km² and is located in the eastern part of River State in Nigeria. It is located at a latitude 4°40′5″N and 4°43′19.5″N and longitude 7°22′53.7″E and 7°27′9.8″E. 11-13. It extends across four Local Government Areas (LGAs) namely Gokana, Khana, Tai and Eleme.

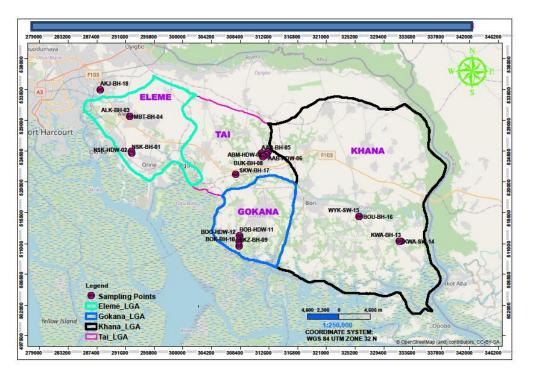


Fig. 1. Map of Ogoni showing Sample Location

#### 2.2 Sample Collection

Water samples were collected from the 4 LGAs of Ogoni from hydrocarbon impacted Ogoni communities: 5 samples of three different water sources (borehole water, well water, surface water) were collected from each LGAs from different hydrocarbon impacted communities. each sample point was referenced using the geographical position system (GPS). Control samples were collected from Ikenga Ogidi community in Idemili LGAs in Anambra State. Bole hole samples were collected using the 250 ml sample containers prior to collection, the tap was allowed to flow to 1 to 2 minutes to ensure sterility is achieved. The sample bottle was filled onto 200 ml leaving some space to allow shaking before analysis. Samples were then transferred into cooling box containing ice pack and it was transported to the laboratory. All samples were stored at 4 °C and analyzed within 48 hrs.

Surface water samples were collected using sterile 2-liter plastic sampling container, during the sampling, the container was rinsed three times with the sample before filling the container. Midstream samples were achieved by dipping the container at 30 cm below the water surface projecting the mouth of the container against the direction of the water flow. Sample were packed into cooling box containing ice pack and transported to the laboratory prior to analysis. Well water was collected using a sterile baler, the water samples were then transferred to 250 ml sample container and pack into a coiling box and sent to laboratory analysis.

#### 2.3 Physicochemical Parameter Analyses

Water samples were analyzed temperature, salinity, total dissolved solid. electrical conductivity. dissolved oxidation and reduction potential using Hanna multimeter HI 989. Heavy metals analysis: hundred millilitres (100 ml) of the water sample were measured into a conical flask, 5 ml nitric acid was then added; the sample was then transferred into the electric hotplate, and heated till about 50 ml was left. It was allowed to cool, filtered into a 100 ml standard flask; and madeup with 1 M nitric acid to the 100 ml mark. The digest was aspirated into the equipment; while the individual metals (Cu, Cr, Cd, Zn, Pb and Fe) were quantitated using Varian SpectroAA 200 atomic absorption spectrophotometer after proper calibration of dilution from pure stock solution of 1000 mg/l [16]. Brucine method was used for nitrate concentration to determination in the sample. The reaction between nitrate and brucine usually produces a yellow colour that is used for colorimetric estimation of nitrate. The intensity of the nitrate concentration and measured at a wavelength of 41nm using 25mm cell. To a 50ml sample, 2 ml of colour reagent (sulphanilamide and (1-naphthl) ethylenediamine chloride) was added. The mixture was allowed to stand for colour development for 15 minutes, therefore, absorbance was read at 543 nm using the spectrophotometer. [16].

#### 2.4 Gas Chromatography Analyses

The residual total petroleum hydrocarbon (TPH) was extracted from the sample and the concentrates were analytically determined using gas chromatography (Varian GC CP3800 coupled with flame ionization detection), using a HP-5 fused silica capillary column (30 m  $\times$  0.32 mm  $\times$  0.25  $\mu m$  film thickness), injecting 1  $\mu l$  sample in split less mode at 300 °C. The carrier gas was hydrogen at flow rate of 1.2 mL/min, average velocity of 20.47 cm/s and the detector temperature [16].

#### 2.5 Microbial Analyses

#### 2.5.1 Bacteriological analysis

The bacteriological analyses of the water samples were carried out using the most probable number (the multiple tubes fermentation technique) for the estimation of total coliform count. MacConkey bile broth alongside with sterilized Durham tubes were used. Serial dilution of the water sample was made and incubated into the MacConkey broth medium, samples were then incubated at 37 °C for 48 hrs for the presumptive test. Positive samples were further transfer to Brilliant green lactose bile and incubated for 48 hrs at 35 °C. Gas production in the medium confirmed the presence of coliform [17].

# 2.6 Total Heterotrophic Bacterial and Fungal Count

The total heterotrophic bacterial and fungal count were enumerated using the spread plate method on nutrient agar (NA) and sabouraud dextrose agar (SDA) respectively. Ten-fold serial dilution of the water samples was aseptically dispensed on the agar plates and spread evenly with the aid of a sterile glass rod, it was incubated at 27 °C

for 24hrs and 28 °C for 1 and 7 days for bacterial and fungal respectively. The colonies were counted and reported as colony forming unit per ml [17].

#### 2.7 Statistical Analyses

The data obtained were subjected to descriptive statistical analysis. (95% confidence limit) The general linearized model (GIG) was used to generate analysis of variance (ANOVA) Mean Standard error and arrange, statistically significant was set as *p*-value of 0.05. Pearson correlation test was used to calculate the correlation between TPH, Heavy metal, and physicochemical parameters in hydrocarbon and non-hydrocarbon impacted communities. All statistical analyses were performed using GraphPad Prism (Version 8).

#### 3. RESULTS AND DISCUSSION

The results for the physicochemical parameter of water samples obtained in our study were lower than the set standard for WHO and NSDWQ. This is in agreement with [14] that the crude oil alter pollution significantly can physicochemical properties of the receiving environment. Water temperature is an important factor because it determines if water is safe for human consumption and for usage, temperature has a noticeable influence on the chemical and biochemical reactions. the higher temperature, the toxicity of heavy metal and also decreased the sensitivities of microbe to toxic substance [18]. The values for temperature (25.0±0.0) °C observed in our study do not have any negative implication on the obtained result because it is within room temperature. This is similar to the work done [15] which affirmed that the recorded temperature ranges from 15.25-24.75 °C did not poses any threat to the homeostatic balance of the river. The pH is the measure of the activity of the hydrogen ion (H+) and is reported as the reciprocal of the logarithm of the hydrogen ion activity. The pH of the water samples was in the range of 4.3±0.8 mg/l, it is imperative to note that pH value for all water samples evaluated across the 4 LGAs in some hydrocarbon impacted Ogoni communities had an acidic pH, this does not comply with the standard recommended by WHO and NSDWQ (6.5 to 8.5). A lower pH value as seen in Table 1 was also observed in our study. Although pH values do not have effect on human domestic usage, its indirect impact on the physiological process cannot be over emphasized when

consumed [19]. Total dissolved substance (TDS) in water is the term use to describe the inorganic and small amount of organic matter present in water solution, the principal constituent are usually sodium, magnesium, calcium, potassium, cation and carbonate, hydrogen-carbonate, nitrate, anions, chloride, sulfate. It has been reported by [3] that high level of TDS in water can affect the taste of water while extremely low level of TDS unacceptable because of its flat dull taste.

The TDS obtained in our study (8.0±1.2 to 43.0±60.1) was extremely lower than the set standard recommended by NSDWQ (500) and (500-1500). WHO standard Electrical Conductivity (EC) in our study was quite low (16.3±2.1-35.0±19) when compared to the Standard recommended by NSDWQ (1000). This seems not to agree with the work done by [23] that demonstrate EC exhibits negative but significant with correlation with turbidity. The pvalue for DO (0.0119) was statistically significance, the result obtained in Eleme (3.7± 2.2) were below the standard for NSDWQ (5.0 mg/l) while those obtained from Gokana (6.5±0.5) were slightly higher the normal ranged. The concentration of heavy metal obtained in our study exceeded the standard recommend by NSDWQ and WHO, except for Copper and Zinc that had a lower value of 0.211±0.348 and 0.255±0.249 respectively. recommended standard set by WHO for Cu (2.00 mg/l) and Zn (3.00 mg/l) while for NSDWQ, for Cu is (1.00 mg/l) and for Zn (5.00 mg/l)respectively. We observed an elevated value in Cd (0.469±0.569 mg/l) about 156 time higher than when compared with the recommended set standard by NSDWQ (0.003 mg/l).

The result for heavy metal concentrations were in the sequences of Cd > Cr> Pb>Fe> Cu>Zn. Crude oil is known to contain heavy metal, given the high spill in the Niger Delta, there are possibility for substantial heavy metal pollution. The concentration of lead (Pb) in our study was 0.0336±0.207 which is 3-times higher than the standard recommended by NSDWQ and the P value for Pb was statistically significant (0.0033) when compare with the control. This agreed with the work done by [15] they observed a higher level of Pb pollution from sediment in Bodo community. The health implication of lead is enormous, high level of lead can damage the kidney in both children and adult, this can pose a great danger to the people that consume these water sources. In addition, exposure to Cadmium

at an extended level causes kidney damage and exert toxic effect on the skeletal and respiratory system [4].

According to the UNEP [14]. The people living in Ogoni drink these waters because there is no alternative source of water for consumption. Our studies review a higher level of TPH (349.9 mg/l)

than the intervention value EGASPIN (50 mg/l). This study confirms the report by the UNEP [14] that reviewed a worrisome level of TPH in the environment. Total Coliform are used to access the general quality of water [21]. Fecal Coliform may represent pathogenic microorganism that could transmits water borne diseases.

Table 1. Physicochemical Properties based on Water bodies in Hydrocarbon and Nonhydrocarbon Impacted Communities

Parameters	Exposed	Control	<i>p-</i> value		
pH	4.3±0.8	5.5±0.3	0.0044		
Salinity	0.03±0.05	26.0±23.8	<0.0001		
DO	5.5±1.6	2.5±2.1	0.0025		
Temp	25.0±0.0	28.9±1.3	<0.0001		
Mv	0.32±0.27	47.6±40.7	0.0189		
Nitrite	0.0058±0.013	0.001±0.00	0.4540		
Nitrate	0.1530±0.158	0.0218±0.009	0.0805		

Key: DO: dissolved oxygen

Results were analyzed using t-test. Statistical significance was considered at p-value less than 0.05

Table 2. Physicochemical Parameter of Waterbodies in Different LGA in Hydrocarbon Impacted Communities

LG A	рН	Ec	TDS	Salinity	DO	Temp	Mv	Nitrate	Nitrite
Ele	4.8±1 .1	26.4±20.4	43.0±60 .1	0.01±0. 06	3.7±2.2 abc	28.1±1 .0	40.4±57 .9	0.211±0. 26	0.001±0. 00
Gok	4.2±0 .8	143.8±14 4.1	72.0±72 .1	0.07±0. 07	6.5±0.5	30.0±1 .3	52.6±48 .4	0.142±0. 14	0.013±0. 00
Kha	4.2±0 .5	16.3±2.1	8.0±1.2	0.01±0. 01	6.3±0.5	29.5±1 .8	47.6±31 .8	0.081±0. 07	0.001±0. 00
Tai	4.1±0 .7	35.0±19.4	17.6±9. 9	0.01±0. 01	6.0±0.4	28.4±0 .6	49.7±39 .1	0.178±0. 12	0.008±0. 01
SO N	6.5- 8.5	1000	500	-	5	40	-	-	-
<i>P</i> ₋ valu	0.145 9	0.0610	0.2460	0.2768	0.0119	0.0910	0.9824	0.6316	0.3531
е						50 "		5 "	

Keys: Ec; electrical conductivity, TDS; total dissolved substance DO; dissolved oxygen. Results were analyzed using ANOVA and post analyses. Statistical significance was considered and represented in alphabet in superscript when the p-value is less than 0.05

Table 3. Comparative Analyses of the Levels of Heavy Metals from Water Sources in Hydrocarbon and Non-hydrocarbon Impacted Communities

Heavy Metals	Exposed	Control	<i>p-</i> value	NSDWQ	WHO
Cr	0.194±0.320	0.007±0.002	0.2122	0.05	0.05
Cd	0.469±0.569	0.001±0.000	0.8290	0.003	0.005
Cu	0.211±0.348	0.174±0.221	0.8215	1	2
Pb	0.0336±0.207	0.264±0.118	0.0033	0.01	0.01
Fe	0.705±1.244	0.642±0.647	0.9156	0.3	0.3
Zn	0.255±0.249	1.465±1.978	0.0095	3.0	5.0

Keys: Cr: Chromium, Cd: Cadmium, Cu: Copper, Pb: Lead, Fe: Iron, Zn: Zinc. NSDWQ: Nigeria Standard for Drinking Water Quality, WHO: World Health Organization

Table 4. Comparative Analyses of the Levels of Heavy Metals from Water Sources in Hydrocarbon and Non-hydrocarbon Impacted Communities

	Tai	Eleme	Gokana	Khana	<i>p</i> -value	WHO	NSDWQ
Cr	0.45±0.43	0.23±0.40	0.03±0.03	0.06±0.02	0.1415	50.00	0.05
Cd	1.00±0.85 <sup>b</sup>	0.57±0.38	0.08±0.05 <sup>b</sup>	0.23±0.19	0.0360	5.00	0.003
Cu	0.56±0.50	0.17±0.32	0.04±0.01	0.07±0.08	0.0512	2.00	1.00
Pb	0.32±0.15	0.41±0.38	0.35±0.05	0.26±0.13	0.7281	0.01	0.01
Fe	0.75±0.76	0.75±1.05	1.27±2.19	0.05±0.02	0.5274	0.30	0.30
Zn	0.22±0.18	0.36±0.45	0.22±0.15	0.22±0.15	0.7984	3.00	5.00

Keys: Cr: Chromium, Cd: Cadmium, Cu: Copper, Pb: Lead, Fe: Iron, Zn: Zinc. Post-analysis: Tai vs. Eleme (a), Tai vs. Gokana (b) Tai vs. Khana (c) Eleme vs. Gokana (d) Eleme vs. Khana (e) and Gokana vs. Khana (f)

Table 5. Pearson Correlation Matrix TPH, Heavy Metals and Physicochemical Parameters in the Hydrocarbon-exposed group

	TPH	Cr	Cd	Cu	Pb	Fe	Zn	рН	Sal	DO	Temp	Μv
TPH	1.00											
Cr	-0.11	1.00										
Cd	0.05	0.69	1.00									
Cu	-0.09	0.89	0.89	1.00								
Pb	0.35	0.26	-0.10	0.04	1.00							
Fe	0.25	0.31	0.20	0.30	0.11	1.00						
Zn	0.33	0.54	0.29	0.39	0.42	0.35	1.00					
рН	0.11	0.43	0.11	0.29	0.08	0.41	0.53	1.00				
Sal	0.42	0.22	0.08	0.13	0.50	0.01	0.42	0.00	1.00			
Do	-0.38	-0.15	-0.09	0.02	-0.22	-0.06	-0.54	-0.53	-0.34	1.00		
Tem	-0.14	0.30	0.04	0.21	0.06	0.46	0.35	0.07	0.07	0.20	1.00	
Μv	-0.03	0.11	-0.12	-0.10	0.24	0.02	-0.04	-0.19	-0.09	-0.06	-0.05	1.00

Keys: TPH: total petroleum hydrocarbon, Cr. Chromium, Cd. Cadmium, Cu. Copper, Pb. Lead, Fe. Iron, Zn. Zinc. SON: Standard Organisation of Nigeria, WHO: World Health Organisation

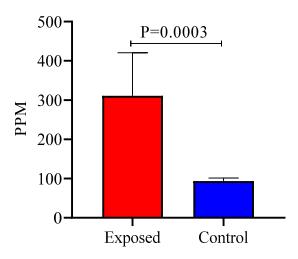


Fig. 2. Level of Total Petroleum Hydrocarbon in Subjects Living in in Hydrocarbon and non-hydrocarbon Impacted Communities. Exposed (n=20), Control (n=5). Exposed (311.0±109.7), Control (93.4±7.9)

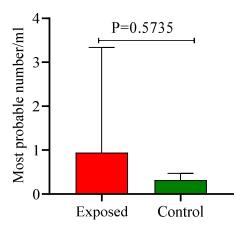


Fig. 3. Estimation of Faecal Coliform in Water Sources Collected from Different Hydrocarbon and Non-hydrocarbon Impacted Communities. Exposed (n=20), Control (n=5)

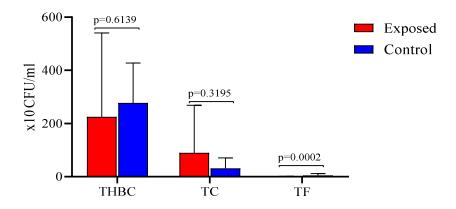


Fig. 4. Level of Microbial Load in Subjects Living in in Hydrocarbon and Non-hydrocarbon Impacted Communities. THBC: total heterotrophic bacterial count, TC: total fungal, and TF: total fungal. Exposed (n=20), Control (n=5)

The high values of Coliform count observed in our studies can be attributed to faecal contamination from non-point sources [11]. Water quality in our studies fail to meet up with the recommended standard for Coliform by NSDWQ 10 cfu/100 ml, and 0 cfu/100 ml by WHO. Usage of water from un-wash tank or untreated tank can also lead to deterioration of the water quality, linkage and corrosion within the pipe can serve as point of entry by some water borne pathogen and causes serious outback of diseases such as cholera, gastroenteritis, typhoid fever, salmonellosis and guinea worm infection, hepatitis A, amoebic dysentery, shigellosis schistosomiasis [22]. This can pose severe health impact both to animal and human that

depend on this water for consumption, the water samples in this study had poor microbial quality.

This study recorded a higher TPH values above the standard for WHO and NSDWQ, a higher value in heavy metal except for copper and zinc that fall below the recommended standard for both WHO and NSDWQ. The sequences of heavy metal concentration were in Cd > Cr> Pb>Fe> Cu>Zn. An elevated value in Cadmium (0.469±0.569 mg/l), the *P*-valves for Pb (0.0033) was statistically significant when compared with the control results. Hydrocarbon has previously been demonstrated to induce resistance to antibiotics to some opportunistic pathogens such as Escherichia coli and Staphylococcus aureus

[23]. Another study also demonstrated that both acidic and basic pH have the ability to induce virulence such as growth, biofilm production and also production of some secretory molecules. These point to the fact that the studied physicochemical variables have the potential of enhancing microbial pathogenesis.

A lower value in physicochemical properties of the water below the recommended set standard by WHO and NSDWQ and an acidic pH in the water quality across the 4 LGAs in Ogoni land from some hydrocarbon impacted communities and a higher coliform count in the water sampled analyzed.

#### 4. CONCLUSION

Total petroleum hydrocarbon (TPH) concentration and heavy metals such as Pb, Cd, and Fe was observed to be higher in hydrocarbon impacted communities and physicochemical parameters were lower than the recommended standard for world health organization and standard organization of Nigeria.

#### **CONSENT AND ETHICAL APPROVAL**

Prior to the commencement of this study, an ethical approval was obtained from Rivers State Ministry of Health with the number MH/PRS/391/VOL.2/624 on 10th February 2020. Oral informed and written consent were obtained before samples were collected.

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#### **COMPETING INTERESTS**

Authors have declared that no competing interests exist.

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