International Journal of Innovative Biochemistry & Microbiology Research 12(4):16-24, Oct-Dec., 2024

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doi:10.5281/zenodo.14163658

Lipid Profile Alterations in Women Due to Chronic Gas Flare Exposure

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ABSTRACT

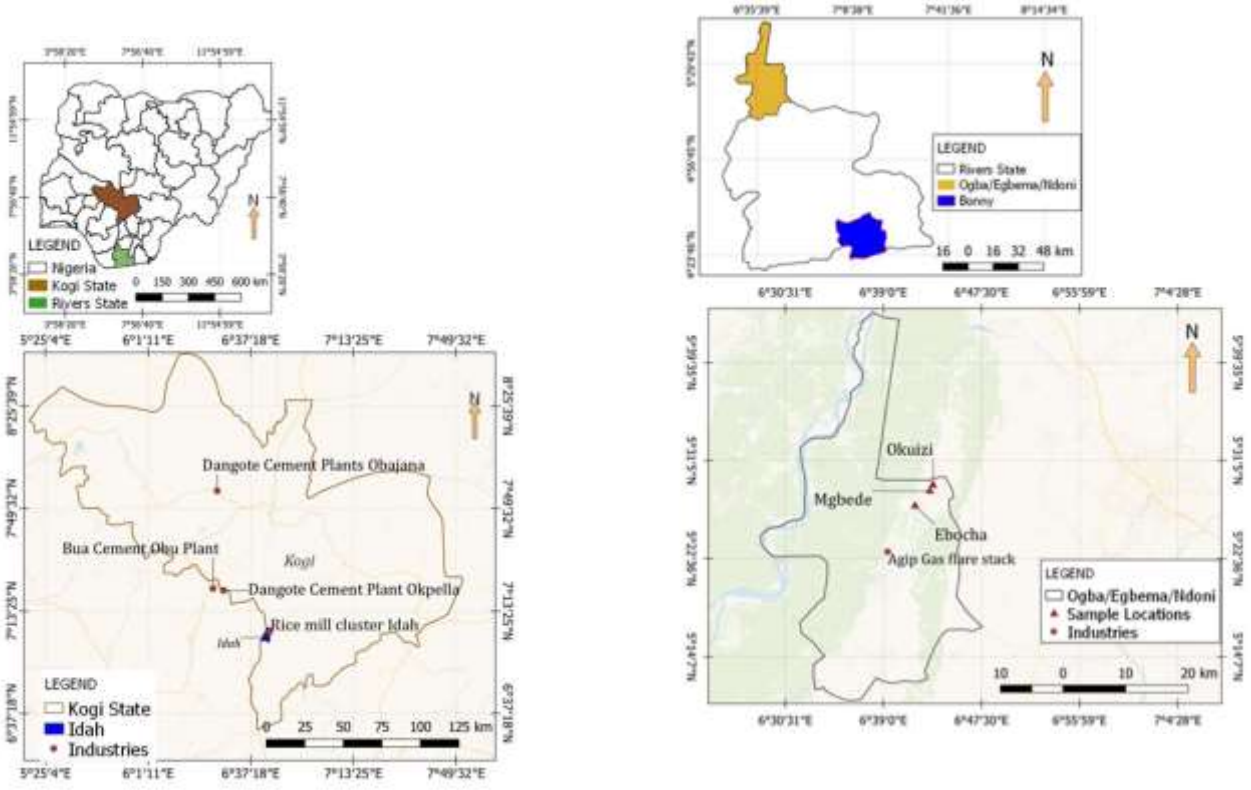
Effect of long term exposure to gas flare on the lipid profile of women (pregnant and non-pregnant), living in two communities (Bonny and Omoku) in the Niger Delta Regions of Nigeria was studied, with Idah, Kogi State as control. Blood samples were collected from volunteers, with plasma extracted after centrifugation, and analysed. The Cholesterol (CHOL), High Density Lipoproteins (HDL), Low Density Lipoproteins (LDL) and Triglycerides (TG) concentrations of plasma samples from study participants were determined. Analyses were done using standard analytical methods. Results for all parameters tested in mmol/L (CHOL, HDL, LDL and TG) were all within the reference ranges except the cholesterol level for non-pregnant women of Bonny, which was lower. The recorded values for LDL for non-pregnant women of Idah and Bonny were significantly lower ($p \leq 0.05$) than the corresponding values for the pregnant women of Idah and Bonny respectively. The highest recorded values for CHOL (mmol/L), HDL (mmol/L), LDL (mmol/L), TG (mmol/L) were; 5.72±1.35, 1.08±0.03, 3.13±0.13 and 1.01±0.06, recorded for pregnant women of Bonny, non-pregnant and pregnant women of Idah, respectively. The recorded LDL values for pregnant women from Idah and pregnant women from Bonny were significantly higher ($p \leq 0.05$), than those from the non-pregnant women of the same locations. However, though the results showed no specific consistent pattern, the values recorded for samples from the control subjects were generally lower than those from the test subjects. The study indicates that exposure to gas flare may have affected the lipid profile of inhabitants of the gas flare host communities.

Keywords: Gas Flaring, Triglycerides, Cholesterol, High Density Lipoprotein, Low Density Lipoprotein

INTRODUCTION

Crude oil extraction in Nigeria started in 1956 when Shell Darcy identified commercially viable oil reserves in Oloibiri, within the Niger Delta region. Revenue from the country's petroleum sector expanded in the 1960s and soared during the oil boom of the early 1970s. (Ahmadu and Egbodion, 2013; Mokelu *et. al* 2020). Following the discovery of crude oil in Nigeria's Niger Delta region and the subsequent initiation of gas flaring from associated gases, flaring operations have steadily intensified in the area. Approximately 17.2 billion cubic meters of natural gas are burned off annually in Nigeria, predominantly in the Niger Delta (GGFRI, 2000). Environmental pollution, affecting air, soil, and water through gas flares, as well as frequent oil blowouts and spills, is a common issue in the region. (Njeze, 1983; Adienbo, and Nwafor, (2010).

Gas flaring presents a major threat to the health of exposed communities. It contaminates the air, warms the atmosphere, and emits greenhouse gases. (WHO, 2003; Ite and Ibok, 2013; Maduka and Tobin-West, 2017). Despite Nigeria's commitment to ending gas flaring and imposing penalties on oil exploration companies that continue the practice, gas flaring persists in the country. (Vidal, 2012). Many of these companies concede that burning off associated and non-associated gas is detrimental and inefficient. Nevertheless, they maintain that Nigeria's oil and gas sector is burdened by infrastructure and technical limitations that prevent the proper utilization of natural gas. Consequently, Nigeria is the 10th largest emitter of flared natural gas globally, with flaring volumes reaching 426 billion cubic feet (bcf) in 2013, representing 10% of global gas flaring. (Vidal, 2012; USEIA, 2015)). Gas flaring leads to the release of greenhouse gases (GHGs) and other airborne contaminants, including carbon dioxide (CO_2), methane (CH_4), ethane, propane, butane, hydrogen sulfide (H_2S), and nitrous oxide (NO_2). Greenhouse gases have been widely linked to global warming and climate change. (WHO, 2003). Another major component of natural gas is volatile organic compounds (VOCs), a category of organic contaminants whose extensive presence has garnered significant interest in environmental science. Benzene, toluene, ethylbenzene, and xylenes (ortho-, meta-, and para-)—collectively known as BTEX—are the primary examples of VOCs. These substances possess carcinogenic and mutagenic properties. Exposure to BTEX is linked to negative impacts on the liver, nervous system, cardiovascular system, kidneys, and elevates the risk of developing non-lymphocytic leukemia. There is evidence showing the detrimental impact of heat-trapping gases on surface temperatures. (WHO, 2003; Ajugwo, O., 2013). In addition to their impact on atmospheric temperature, the gases released from flaring act as pollutants to both air and water. Greenhouse gases also contribute to the creation of acid rain. These factors result in adverse health effects for individuals residing and working in areas affected by gas-flaring activities. Health issues include chronic and recurring respiratory conditions, abnormal blood indices, heightened risk of blood disorders, skin diseases, and cancers, among others. There is also a recognized link between global warming and the spread of diseases. (WHO, 2003; Ite and Ibok, 2013; Maduka and Tobin, 2017).



A B

Maps: A, Map of Nigeria showing the study sites, with a close up map of Kogi State showing the control site. B, Map of Rivers state showing the test locations

The test locations, Bonny and Omoku, are situated in Rivers State, at the core of Nigeria's Niger Delta region. Bonny is trapezoidal with coordinates, Northwest Latitude 4˚33'N and Longitude 7˚08'E, Northeast Latitude 4˚30'N and Longitude 7˚20'E, Southwest Latitude 4˚22'N and Longitude 7˚20'E and Southeast Latitude 4˚22'N and Longitude 7˚08'E. She hosts several oil and gas facilities, including Nigeria Liquefied Natural Gas (NLNG), Mobil oil and gas operations, and Shell Petroleum, among others. (Jumbo and Ihuah 2024). Omoku the second test site is situated at approximately Latitude 4° 51' 29.16" N and Longitude 6° 55' 15.24" E (Oweisana et al., 2021). Oil companies that operate there include Shell Petroleum Development Company, Total Exploration & Production Nigeria, and Nigerian Agip Oil Company (SPDC, 2023). The control site, Idah, is situated in the southwest of Kogi State at Longitude 5°45' East and Latitude 7°5' North. Industrial activities in Idah include several small-scale industries, such as bakeries, palm oil milling, block making, sachet water production, and soap manufacturing. (Tokula, A. E., 2019).

The test sites for this research have been plagued with gas flaring and its associated environmental degenerations and destructions for over twenty years. Bonny Island particularly, and her surrounding areas have been criticized by numerous environmental scientists from Nigeria, the United Kingdom and the United States, as being among the most polluted regions globally. A significant factor contributing to this severe environmental condition has been pinpointed as the continuous discharge of associated gases during oil extraction (Odo *et. al*. 2019). Various researchers have suggested a connection between air pollution and non-communicable diseases. Some include Maduka and Tobin-West, 2017, who, from their research, found that, after adjusting for the participants' age, hypertension was more prevalent among individuals at both extremes of adult age living in gas-flaring host communities compared to those in non-gas-flaring areas. Gas flaring was also reported by Ajugwo, 2013, to have detrimental effect on human health. According to Ajugwo's findings, inhaling hazardous air pollutants released during the incomplete combustion of flared gases harms human health, leading to cancer, as well as neurological, developmental, and reproductive issues (Ito and Ugbome, 2017). Ovuakporaye et al., 2012 also reported deformities in children, ranging from lung damage and skin problems (Ito and Ugbome, 2017). Additionally, incomplete combustion of hydrocarbons has been found to negatively affect hematological parameters, causing harmful changes to blood and blood-forming cells (Ajugwo, 2013), which can result in conditions such as aplastic anemia and pancytopenia (Kindzierski 2000; Ito and Ugbome, 2017). Further to this, Egwurugwu *et al*., 2013, reported a significant rise in the Total Cholesterol (TC), Triglycerides (TG), Low Density Lipoprotein (LDL), TC/HDL, and LDL/HDL from the blood samples of individuals (both men and women) from the Niger Delta region of Nigeria in comparison to the control (Nwaogu, et. al., 2021). Similarly, Nwaogu, *et. al*., 2021, demonstrated that prolonged exposure to gas flaring significantly ($p<0.05$) lowered the concentrations of serum triglyceride, total cholesterol and high-density lipoprotein cholesterol (HDL-c) but significantly ($p<0.05$) increased serum low-density lipoprotein cholesterol. The aim of this study was to investigation the possible associated alterations of chronic gas flare exposure on the Lipid Profile of women in the host communities.

MATERIALS AND METHODS

Sample collection:

Human sampling was done using convenience sampling method. The inclusion criteria were women between the ages of 18 and 50, willing participants who had resided permanently in the host communities for 10 years or more and visited the local health centers during the period the research was conducted. The sample size was 180, and constituted of 60 women from each study location, 30 out of which were pregnant and the other 30 non-pregnant.

Air particulates were collected using Air volume sampler active sampling. The filter papers were extracted after the exercise and sent to the laboratory for analysis. PAHs and Btex were analysed using gas chromatography. The criteria air pollutants were determined using real time monitoring, using the aeroqual series 500 and attaching the relevant sensors for detecting of specific gases. Results are shown in tables 1, 2, and 3

Consent forms and questionnaires were administered to volunteers, after which blood samples were collected. Three mls of blood was collected per volunteer and transported to the lab for laboratory analysis. In the laboratory, the samples were centrifuged and subsequently imputed into the COBAS C III for analysis. Results obtained are displayed in table 4

RESULTS

Table 1. Criteria Air Pollutant Analysis for the study sites [Bonny (Test 1), Omoku (Test 2) and Idah (Control site)]. Results are presented as mean ± Standard Error Mean (S.E.M).

PARAMETERS	IDAH	BONNY	OMOKU	PERMISSIBLE LIMIT
PM2.5 µg	0.01 ± 0.0^{a}	$0.01\pm0.00^{a,c}$	$0.02\pm0.00^{a,c}$	
PM10 µg	0.08 ± 0.03^{a}	$0.06\pm0.03^{a,c}$	$0.07\pm0.02^{a,c}$	
CO_2 ppm	769.89 ± 6.04^{a}	$63.11\pm1.61^{b,c}$	$647.44\pm48.60^{b,d}$	
CO ppm	0.81 ± 0.47^{a}	$2.81\pm1.73^{a,c}$	$0.00\pm0.00^{a,c}$	100 mg/m^3 (90 ppm) for 15 minutes (WHO, 2000)
SO_2 ppm	0.16 ± 0.10^{a}	$0.00\pm0.00^{a,c}$	$0.00\pm0.00^{a,c}$	
NO_2 ppm	0.07 ± 0.00^{a}	$0.07\pm0.00^{a,c}$	$0.07\pm0.00^{a,c}$	0.5 – 1.0 ppm (WHO, 1963)
VOC ppm	0.13 ± 0.09^{a}	$0.51\pm0.29^{a,c}$	$0.10\pm0.07^{a,c}$	
CH_4 ppm	76.89 ± 30.79^{a}	$0.00\pm0.00^{b,c}$	$20.22\pm4.25^{a,c}$	
NH_3 ppm	0.21 ± 0.13^{a}	$0.01\pm0.01^{a,c}$	$0.08\pm0.08^{a,c}$	
H_2S ppm	0.00 ± 0.00^{a}	$0.01\pm0.01^{a,c}$	$0.00\pm0.00^{a,c}$	0.1 – 0.2ppm (WHO,1963)
O_3 ppm	0.00 ± 0.00^{a}	$0.00\pm0.00^{a,c}$	$0.15\pm0.01^{a,c}$	
RH %RH	63.12 ± 3.05^{a}	$70.80\pm1.33^{a,c}$	$46.41\pm3.37^{b,d}$	
WS m/s	0.97 ± 0.31^{a}	$9.78\pm7.14^{a,c}$	$0.78\pm0.19^{a,c}$	
Temp ^{O}C	33.82 ± 1.28^{a}	$30.90\pm0.65^{a,c}$	$37.16\pm1.32^{a,d}$	
Noise dB	56.50 ± 2.50^{a}	$52.69\pm2.44^{a,c}$	$62.20\pm2.53^{a,d}$	

Values are mean ± Standard Error Mean (S.E.M.). Values with different superscript in the same row are significantly different at the 0.05 levels ($p\leq0.05$). Superscript (a,b) compares Bonny and Omoku to Idah (1st letters) while superscript (c,d) compares Omoku to Bonny (2nd letters) across the row. $PM_{2.5}$ and PM_{10} are measured in micrograms (µg), CO_2, CO, SO_2, NO_2, VOC, CH_4, NH_3, H_2S, and O_3 were measured in parts per million (ppm), RH, WS, Temp, and Noise were measured in %, m/s, ^{0}C and dB respectively.

Table 2. Polycyclic Aromatic Hydrocarbon (PAHs) of Air from study sites [Bonny (Test 1), Omoku (Test 2) and Idah (Control site)]. Results are presented as mean ± Standard Error Mean (S.E.M).

POLYCYCLIC AROMATIC HYDROCARBONS (ppm)	IDAH	BONNY	OMOKU	PERMISSIBLE LIMITS (No safe levels))
Naphthalene	203.86±25.40^{a}	151.34±7.90^{a,c}	133.66±11.84^{b,c}	
Acenaphthylene	124.71±32.69^{a}	213.58±5.10^{b,c}	242.21±4.29^{b,c}	
Acenaphthene	163.92±28.00^{a}	167.56±27.89^{a,c}	133.47±2.21^{a,c}	
Flourene	0.00±0.00^{a}	0.00±0.00^{a,c}	38.87±19.44^{a,c}	
Phenanthrene	219.88±37.98^{a}	166.70±28.85^{a,c}	318.30±12.51^{a,d}	
Anthracene	41±31.02^{a}	149.68±45.17^{a,c}	104.05±52.03^{a,c}	
Flouranthene	115.42±29.15^{a}	90.72±23.88^{a,c}	108.13±29.99^{a,c}	
Pyrene	71.06±35.53^{a}	97.60±27.06^{a,c}	180.63±11.24^{b,c}	
Benz(a)anthracene	44.07±22.04^{a}	126.48±32.90^{a,c}	108.47±27.15^{a,c}	
Chrysene	168.94±47.28^{a}	143.55±42.73^{a,c}	187.44±29.89^{a,c}	
Benzo(b)flouranthene	0.00±0.00^{a}	0.00±0.00^{a,c}	0.00±0.00^{a,c}	
Benzo(k)flouranthene	0.00±0.00^{a}	0.00±0.00^{a,c}	0.00±0.00^{a,c}	
Benzo(a)pyrene	0.00±0.00^{a}	53.07±26.54^{a,c}	0.00±0.00	
Indeno(1,2,3-cd)pyrene	0.00±0.00^{a}	0.00±0.00^{a,c}	0.00±0.00^{a,c}	
Dibenz(a,h)anthracene	0.00±0.00^{a}	0.00±0.00^{a,c}	0.00±0.00^{a,c}	
Benzo(g,h,i)perylene	0.00±0.00^{a}	0.00±0.00^{a,c}	0.00±0.00^{a,c}	
Total PAH	1307.27±180.12^{a}	1360.28±159.10^{a,c}	1608.18±82.03^{a,c}	

Values are mean ± Standard Error Mean (S.E.M.). Values with different superscript in the same row are significantly different at the 0.05 levels ($p \leq 0.05$). Superscript (a,b) compares Bonny and Omoku to Idah (1st letters) while superscript (c,d) compares Omoku to Bonny (2nd letters) across the row. PAHs were measured in parts per million (ppm).

Table 3. Benzene, Toluene, Ethylbenzene and Xylene (BTEX) of Air from study sites [Bonny (Test 1), Omoku (Test 2) and Idah (Control site)]. Results are presented as mean ± Standard Error Mean (S.E.M).

BTEX (ppm)	IDAH	BONNY	OMOKU	PERMISSIBLE LIMITS
Benzene	9.95 ± 0.80^{a}	$4.37\pm1.29^{b,c}$	$4.81\pm1.22^{b,c}$	No safe levels (WHO,2000)
Toluene	1.76 ± 0.88^{a}	$2.36\pm0.92^{a,c}$	$7.45\pm1.89^{b,d}$	0.26 mg/m^3 (WHO, 2000)
o-xylene	6.44 ± 1.91^{a}	$3.25\pm0.96^{a,c}$	$2.90\pm0.98^{a,c}$	
m-xylene	2.26 ± 0.67^{a}	$4.49\pm1.08^{a,c}$	$6.12\pm0.91^{b,c}$	
p-xylene	11.04 ± 0.99^{a}	$5.22\pm1.43^{b,c}$	$5.61\pm1.50^{b,c}$	
Ethylbenzene	7.93 ± 2.64^{a}	$3.92\pm1.36^{a,c}$	$4.11\pm1.18^{a,c}$	
Total BTEX	39.37 ± 1.38^{a}	$23.62\pm1.00^{b,c}$	$31.00\pm0.76^{b,d}$	

Values are mean ± Standard Error Mean (S.E.M.). Values with different superscript in the same row are significantly different at the 0.05 levels ($p\leq0.05$). Superscript (a,b) compares Bonny and Omoku to Idah (1st letters) while superscript (c,d) compares Omoku to Bonny (2nd letters) across the row. BTEX were measured in parts per million (ppm)

Table 4. Lipid profile [Cholesterol (CHOL), High Density Lipoproteins (HDL), Low Density Lipoproteins (LDL) and Triglycerides (TG)] of plasma samples collected from study participants [Pregnant women (PW) and Non-Pregnant women (NPW)] from study sites [Bonny (test site 1), Omoku (test site 2) and Idah (control site)]. Results are presented as mean ± Standard Error Mean (S.E.M).

STUDY SITE	CHOL (mmol/L)		HDL (mmol/L)		LDL (mmol/L)		TG (mmol/L)	
	NPW	PW	NPW	PW	NPW	PW	NPW	PW
Kogi	$3.96\pm0.10^{a,,e}$	$4.56\pm0.11^{a,,e}$	$1.08\pm0.03^{a,,e}$	$1.00\pm0.03^{a,,e}$	$2.51\pm0.12^{a,,e}$	$3.13\pm0.13^{a,,f}$	$0.95\pm0.04^{a,,e}$	$1.01\pm0.06^{a,,e}$
Bonny	$4.05\pm0.09^{a,c,g}$	$5.72\pm1.35^{a,c,g}$	$1.04\pm0.03^{a,c,g}$	$0.97\pm0.03^{a,c,g}$	$2.44\pm0.12^{a,c,g}$	$2.95\pm0.12^{a,c,h}$	$0.97\pm0.06^{a,c,g}$	$1.00\pm0.05^{a,c,g}$
Omoku	$4.18\pm0.07^{a,c,i}$	$4.25\pm0.08^{a,c,i}$	$1.00\pm0.03^{a,c,i}$	$1.02\pm0.03^{a,c,i}$	$2.73\pm0.10^{a,c,i}$	$2.78\pm0.12^{a,c,i}$	$1.00\pm0.05^{a,c,i}$	$0.98\pm0.05^{a,c,i}$
Reference Ranges	4.2 – 5.8		0.8 -1.8		1.8 – 4.0		0.68 – 1.58	

Values are mean ± Standard Error Mean (S.E.M.). For 1st and 2nd letter comparisons, comparisons are vertical, i.e., values with different superscript in the same column are significantly different at the 0.05 levels ($p\leq0.05$). Superscript (a,b) compares Pregnant and Non-Pregnant Women of Bonny and Omoku to Pregnant and Non-Pregnant Women of Idah (1st letters), superscript (c,d) compares Pregnant and Non-Pregnant Women of Omoku to Pregnant and Non-Pregnant Women of Bonny (2nd letters). For 3rd letter comparisons, i.e., e,f; g,h and i,j, comparisons are horizontal (along the rows), and values with different superscript in the same rows are significantly different at the 0.05 levels ($p< 0.05$). Superscript (e,f) compares Pregnant Women of Idah to Non-Pregnant Women of Idah, superscript (g,h) compares Pregnant Women of Bonny to Non-Pregnant Women of Bonny, while superscript (i,j) compares Pregnant Women of Omoku to Non-Pregnant Women of Omoku (3rd letters). CHOL, HDL, LDL and TG were all measured in millimole per liter (mmol/L).

DISCUSSION

Criteria Air pollutant assessment results from Idah showed relatively higher CO_2 and CH_4 levels, moderate SO_2, and NH_3 presence, in comparison to the other study locations, indicating that though there is no gas flare emission, there is emission from other industrial and human activities. The noise levels were moderate, and particulate matter concentrations were low, suggesting relatively good air quality but with specific localized pollutants. The results from Bonny on the other hand was characterized by

elevated CO and VOC levels, likely related to gas flaring and industrial activities. The high relative humidity and wind speed may have helped mitigate the dispersion of pollutants, but the site still faces significant air quality challenges. Noise levels in Bonny were the lowest among the three study sites. While the criteria air quality assessment of Omoku displayed the highest temperature and O_3 levels, with moderately high methane and noise levels. The combination of low wind speed and low relative humidity could have led to poor air dispersion, exacerbating air quality issues in this area. These findings suggest that each site has unique environmental factors influencing air quality, with potential implications for public health and environmental management. The analysis of Polycyclic Aromatic Hydrocarbons (PAHs) revealed that Omoku had the highest total PAH concentration at 1608.18 ppm, and reflected substantial contamination, particularly for compounds such as phenanthrene, acenaphthylene, and pyrene. Bonny was close behind, with a total PAH concentration of 1360.28 ppm and significant levels of acenaphthylene and benzo(a)pyrene. Although Idah, the control site, exhibited the lowest contamination, it still showed relatively elevated levels of naphthalene, anthracene, and phenanthrene, with a total PAH concentration of 1307.27 ppm. Both Omoku and Bonny, impacted by gas flaring, showed substantial pollution, especially in Omoku, where the presence of toluene and m-xylene suggests strong flaring effects. The elevated BTEX levels observed at the control site, Idah, however, despite the absence of flaring, imply that other sources of pollution—potentially from industrial operations or transportation—are contributing to environmental degradation.

These results underscore the complexity of distinguishing the effects of gas flaring, as numerous factors influence BTEX concentrations in the environment. The study, further highlights the necessity of thoroughly evaluating all possible pollution sources, even in control areas, to fully grasp the environmental consequences of gas flaring.

Except for cholesterol levels for Bonny, which was above the reference range, the lipid profile results fell within the reference ranges across study sites. It is notable that the general observation showed relatively higher values for the test sites when compared to the control site, except for High Density Lipoproteins, which was higher for the control site than the test site. This result corroborated the research results as reported by Egwurugwu et al. (2013) who reported a significant increase in the following lipid profiles namely TC, TG, LDL, TC/HDL, LDL/HDL from the blood samples of individuals (both men and women) from the Niger Delta region, Nigeria when compared to the control in that there was an increase in the same parameters for the test sites in comparison to the control site, though in the case of our research, this increase didn't record a statistical significance. It however was not quite consistent with the results reported by Nwaogu, *et. al*., 2021, except for the fact that the HDL concentration for the test site show a reduction in comparison to the control site, indicating that chronic exposure to gas flaring significantly ($p<0.05$) reduced the concentration of HDL.

The results of this study show that plasma samples from the test locations exhibited higher concentrations of TG, LDL, and C, and lower HDL, when compared to the control site, with Bonny showing more significant deviations in lipid profiles, particularly in pregnant women. These alterations could be associated with gas flaring activities.

CONCLUSION

The air quality assessment across the study sites of Idah, Bonny, and Omoku highlights significant environmental disparities likely influenced by localized industrial activities and natural gas emissions. Idah, while exhibiting relatively good overall air quality, shows elevated CO_2 and CH_4 levels, indicative of specific localized pollution sources. Bonny on the other hand, faces considerable air quality challenges, with elevated levels of CO and VOCs, likely due to gas flaring and industrial emissions, though its higher wind speed and humidity appears to partially mitigate pollutant dispersion. While Omoku presents the most concerning conditions, with the highest recorded temperatures and ozone levels, coupled with poor air dispersion due to low wind speed and humidity, suggesting a higher potential for air quality degradation.

Though the lipid profile results were all within the reference ranges, except for Cholesterol in Bonny which was above, all results were reflective of the air quality results in respective test sites, as indicated by the general observation of relatively higher values for the test sites when compared to the control site. Exposure to gas flare may have adversely affected the lipid profile in the women from gas flare host communities compared to the control site. These findings underscore the need for targeted environmental management strategies to address the unique pollution profiles of each site, particularly in regions where industrial activities and gas flaring contribute to significant atmospheric contamination. The data also highlight the importance of ongoing monitoring and intervention to protect public health and mitigate environmental impacts in these areas.

DECLARATION

Data Availability Statement: The data used to support the findings of this study are available from the corresponding author upon request.
Funding: This research was fully sponsored by the Bonny Kingdom Education Trust Fund BKETF
Ethical Approval: Ethical approval was obtained from the University of Port Harcourt.
Conflict of Interest: No conflict of interest.

AUTHORS' CONTRIBUTIONS

Conceptualization, AAJ., MOM. and LCC.; methodology, AAJ. and LCC.; validation, AAJ. and MOM.; formal analysis, AAJ.; investigation, AAJ.; resources, AAJ.; data curation, AAJ., MOM. and LCC.; writing—original draft preparation, AAJ.; writing—review and editing, MOM. And LCC.; supervision, MOM. and LCC.; project administration, AAJ.; funding acquisition, LCC. and AAJ. All authors have read and agreed to the published version of the manuscript.

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