



Concentration of Polycyclic Aromatic Hydrocarbons in Water Sources of High and Low Crude Oil-Producing Communities in Rivers State, Nigeria.

Anwuri Luke^{1*}, Charles Tobin-West², Vitalis Obisike Ofuru³, Golden Owhonda¹, Tondor Cleopatra Uzosike¹, Bright Owhonda Ogbondah¹, Pabara Newton Igwele⁴

¹Department of Community Medicine, College of Medical Sciences, Rivers State University, Nkpolu-Oroworukwo, Port Harcourt, Rivers State, Nigeria;

²Africa Centre of Excellence Centre for Public Health and Toxicological Research (ACE-PUTOR), University of Port Harcourt, Rivers State, Nigeria.

³Department of Surgery, College of Medical Sciences, Rivers State University, Nkpolu-Oroworukwo, Port Harcourt, Rivers State, Nigeria;

⁴Ministry of Health, Yenagoa, Bayelsa State.

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*Corresponding Author

Dr Luke, Anwuri (MBBS, MPH, PhD, FWACP)

E-mail: luke.anwuri@ust.edu.ng

Phone: +2348032773870

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ABSTRACT

Background: Polycyclic aromatic hydrocarbons (PAHs) are toxic, highly lipophilic, endocrine-disrupting and extensive contaminants with low solubility in water sources. The emission of these toxic pollutants into the environment during crude oil-refining activities may result in harmful health consequences.

Method: This cross-sectional comparative community-based study employed the multistage sampling technique to analyse the concentration of PAHs in the drinking water of high and low oil-producing communities in Rivers State. The water sample points were geo-referenced, collected from 28 sources in 10 communities and analyzed using a gas chromatograph and mass spectrometer. Data were analysed using the Statistical Package for the Social Sciences (SPSS) version 26 and summarised as means and standard deviations. The test of association was measured using the independent t-test and Analysis of Variance at a significance level of $p < 0.05$ and a 95% confidence level.

Result: The mean concentrations of TPH (0.35), TOG (0.78), and PAH (0.05) were significantly above the recommended WHO permissible limit in the high compared to the low oil-producing sites. In descending order, six identified PAH metabolites: Naphthalene ($p=0.001$), Acenaphthene ($p=0.004$), Fluorene ($p=0.021$), Phenanthrene ($p=0.001$), Anthracene ($p=0.012$), and Fluoranthene ($p=0.031$) were significantly lower than the standard limits in the high than in the low oil-producing site.

Conclusion: The sampled water sources were significantly contaminated by PAH and its metabolites in the high oil-producing site, instead of the low site. Therefore, it is recommended that municipal water treatment and strict enforcement of laws guiding the control of oil spills be implemented to improve the portability of the water supply.

INTRODUCTION

The increasing demand for primary energy in the BRICS (Brazil, Russia, India, China, and South Africa) countries with similar stages of rapidly advancing economic development and toxic pollution from crude oil-rich countries such as Saudi Arabia, Dubai, Nigeria, etc., has consistently degraded the environment (Sasana & Ghazali, 2017; Wang et al, 2022). These toxic pollutants expelled into the environment (atmosphere, water sources, and soil) during exploration and exploitation activities may result in pollution associated with health consequences. However, long-term exposure to these pollutants is seen as a major contributor to the rising burden of non-communicable diseases in oil-producing countries like Nigeria (Ibeawuchi, 2016; Nriagu et al, 2016; Ohanmu et al, 2018). Exposure to these pollutants occurs when oil spills or liquid form of crude and their products are released into the atmosphere, water sources or/ land during conventional or/ artisanal refining processes such as drilling rigs and wells; oily refuse (waste oil) from artisanal refining or pipeline vandalism; or/and storage, transportation, and distribution of refined petroleum products (gasoline, diesel) in large ships (bunker fuel) or tankers. Despite, the huge economic benefits of the oil and gas industry, the majority of the effluents from crude oil production processes are

accidentally or intentionally spilt into the environment during; domestic, industrial, or commercial activities leading to harmful effects on the terrestrial ecosystem; water sources, soil macronutrients, aquatic bodies (Cordes et al, 2019; Bello & Amadi, 2019; Adeola et al, 2022).

Furthermore, hydrocarbons and their metabolites have been implicated as one of the major pollutants in oil-producing areas as they are extensively emitted into the surrounding environments during the combustion of petrol and diesel with resultant contamination of the atmosphere, water sources, and soil, including food crops (Abdel-Shafy & Mansour, 2016; Truskewycz et al, 2019; Pandolfo et al, 2023). Hydrocarbons are a range of chemical compounds derived from crude oil and can be transmitted into the human body through inhalation, ingestion, and dermal contact (Banton et al, 2019). The number of hydrocarbons trapped in the human body following exposure is referred to as the *mean concentration of higher molecular weight hydrocarbons* (Indiketi et al, 2022). Depending on their chemical properties, these compounds can be differentiated from the original combination through evaporation into various forms and dissolved into soil particles or groundwater sources. The commonest contaminants found in the environments of crude oil-producing regions of developing countries and

associated with health-related consequences are *Total petroleum hydrocarbons (TPH)* and *polycyclic aromatic hydrocarbons (PAHs)*, as well as *oil and grease measured as TOG (total oil and grease)*. Total petroleum polyaromatic hydrocarbons are the mean concentration of toxic oil components of crude oil with associated carcinogenic potentials. While polycyclic aromatic hydrocarbons are groups of highly lipophilic, toxic, endocrine-disrupting and extensive environmental contaminants with low solubility in soil, surface and groundwater sources (Saunders et al, 2022; Liu et al, 2020). They can be isolated in the soil, sediments and air but are emitted into the environment through natural and anthropogenic sources. These sources originate from either pyrogenic PAHs from incomplete combustion of municipal wastes, biomass, and fossil fuels or petrogenic PAHs spilt from by-products of petroleum and organic matter in oxygen-deprived environments (Abbasi & Keshavarzi, 2019; Patel et al, 2020). The impact of oil and gas industrial activities on the ecosystem, aside from predisposing the residents of host communities to long-term health consequences, may result in consequent poverty thriving amid wealth (Madeen & Williams, 2017; Nkem et al, 2022). Some of these pollutants in humans have been observed in reproductive, gastrointestinal, and cardiovascular problems apart from their carcinogenic predispositions. These deleterious effects may occur due to the ability of hydrocarbons to travel in large proportions into the atmosphere, water sources, or food crops grown in polluted soils (Igwe et al, 2021).

A portable water source is safe, wholesome, free from pathogens, devoid of other impurities (heavy metals, hydrocarbons), suitable for human consumption and essential for the sustainability of health and environmental development (Ukah et al, 2018). However, in crude-oil-producing regions, the water sources have been reported to largely contain impurities such as heavy metals (barium, uranium, cadmium, chromium, strontium and lead), total petroleum hydrocarbon (TPH), the polycyclic aromatic hydrocarbon (PAH), volatile aromatics like benzene, toluene, ethyl benzene and xylene (BTEX) and other effluents which sip into the water sources from conventional or artisanal refining processes (Inyang et al, 2019). These contaminants are mainly inorganic compounds compared to their organic counterparts. The inorganic compounds found in the mineral form of heavy metal poisoning include (Pb), which is known to delay the physical and mental growth in infants, while Arsenic (As), Selenium (Sn) and mercury (Hg) are responsible for cardiovascular diseases, kidney-related problems, neurocognitive diseases, and cancers associated with skin, kidney, and liver pathology (Muhammad et al, 2020).

According to the World Health Organization (WHO) and the Environmental Protection Agency of the United States of America (USEPA), PAHs are a group of more than 100 organic compounds, mostly colourless but whitish or yellowish in a few. However, sixteen of them have been identified as priority PAHs due

to their toxicity to the human body and require a regulatory framework. These PAHs are; Acenaphthene (ACE), Acenaphthylene (ACY), Anthracene (ANTH), Benzo(a)anthracene, Benzo(a)pyrene, Benzo(b)fluoranthene, Benzo(k)fluoranthene, Benzo(g,h,i)perylene, Chrysene (CHRY), Dibenz(a,h)anthracene (D[ah]A), Fluoranthene (FLTH), Fluorene (FLU), Indeno(1,2,3-c,d)pyrene, Phenanthrene (PHEN), Pyrene (PYR) and Naphthalene (NAP). Of these, seven have also been identified as toxic and capable of causing carcinomas in humans, viz: benzo(a)anthracene, benzo(a)pyrene, indeno(1,2,3-c)pyrene, dibenzo(ah)anthracene, benzo(b)fluoranthene, benzo(k)fluoranthene, and chrysene. In evaluating for quality of drinking water, six PAHs (fluoranthene, phenanthrene, pyrene, anthracene, and fluoranthene) have shown the highest concentrations, but only fluoranthene has been found at significant levels. Also, among PAHs with carcinogenic effects, BaP is one of the most extensively studied, though others like dibenzo[a,e]pyrene, dibenzo[a,h]pyrene, and chrysene have been found in relatively low concentrations in drinking water sources of oil-producing regions (Abdel-Shafy & Mansour, 2016; Patel et al, 2020; Sun et al, 2018).

It is worth noting that the concentration of hydrocarbons and their corresponding effects on the ecosystem and the health of humans determine the extent of remediation intervention to be instituted, especially in crude oil-producing regions (Ukah et al, 2018; Adipah et al, 2018; Abadi et al, 2018). Hence, this study analysed the concentration of polyaromatic hydrocarbons in the drinking water sources of high and low crude oil-producing communities in Rivers State, Nigeria.

MATERIALS AND METHODS

Study Area

Rivers State is a major oil-producing area in the Niger Delta region, with Port Harcourt city as its capital. It is administratively split into 319 political wards and 23 Local Government Areas: Five urban (Port Harcourt City, Obio/Akpor, Ikwerre, Oyiibo, and Eleme) and eighteen rural. It is bordered to the south by the Atlantic Ocean, to the north by Anambra, Imo, and Abia State, to the east by Akwa-Ibom State, and to the west by Bayelsa and Delta States. The State's coastline extends from Bonny in the south to Ndoni in the north, as the lowland stretches. While its tropical rainforest is found within the inland part, surrounded by the typical Niger Delta environmental features of mangrove swamps. It is the sixth most populous with a total area of 11,077 km² and a density of 635.89 people per km² and is estimated at 7,326,630 with an annual growth rate of 4.99%. It ranked as the second-largest industrial city in 2007 with a gross domestic product (GDP) of \$21.07 billion and a per capita income of \$3,965, due to the numerous notable oil-

producing firms (the Nigeria Liquefied Natural Gas Complex, Shell Petroleum Development Company, Chevron, Agip, Total, Elf, etc.) located particularly in Port Harcourt Metropolis (Port Harcourt City and Obio/Akpor). Other natural resources such as silica, glass, and clay sand are found within its borders. (Uriah et al., 2014). The State is multi-cultural and multi-tribal, having Ijaw, Ikwerre, Etche, Ogoni, and Ogba/Egbema as the leading ethnic groups, though most of the indigenes speak British English and Pidgin English (Cookey, 2018; World Bank & United Nations, 2021)

Study design

This research is a cross-sectional, comparative study that assessed the concentration of polycyclic aromatic hydrocarbons in water sources of high and low-crude-oil-producing communities in the Niger Delta region of Nigeria.

Definition of terms

The high crude oil-producing rural communities.

These communities are defined as places where exploitation and exploration activities have occurred unabated over the last ten years and beyond (≥ 10) and located within at least 500 meters (approximately 0.3 miles) of gas flaring plants, flow stations and where people live (Maduka & Tobin-West, 2017; World Bank, 2022).

Low oil-producing rural communities

These are communities with little or no history of exposure to exploitation and exploration activities that occurred over the last ten years (≤ 10) and situated beyond 500 meters (>0.3 miles) from gas flaring plants, flow stations and where people live (Maduka & Tobin-West, 2017; World Bank, 2022).

Sampling technique

A multi-stage sampling technique was used to select high and low-crude-oil-producing communities where exploration/exploitation activities occurred in the last 10 years.

Rivers State was stratified into the three existing Senatorial districts.

- i. Rivers East: Emohua, Etche, Ikwerre, Obio/Akpor, Ogu/Bolo, Okrika, Omuma, Port Harcourt.
- ii. Rivers South-East; Andoni, Eleme, Gokana, Khana, Opobo/Nkoro, Oyigbo, Tai.
- iii. Rivers West: Abua/Odual, Ahoada-east, Ahoada-west, Akuku-Toru, Asari-Toru, Bonny, Degema, Ogba/Egbema/Ndoni.

Stage 1: Two out of the three existing Senatorial districts were selected by balloting:

- i. Rivers East; Emohua, Etche, Ikwerre, Ogu/Bolo, Okrika, Omuma LGAs.
- ii. Rivers West: Abua/Odual, Ahoada-East, Ahoada-West, Akuku-Toru, Asari-Toru, Bonny, Degema, Ogba/Egbema/Ndoni LGAs.

The LGAs of the two selected senatorial districts were stratified into 2 (high and low) crude-oil-producing communities.

- i. High-crude-oil-producing: Akuku-Toru, Asari-Toru, Bonny, Degema, Obio/Akpor, Ogba/Egbema/Ndoni, Port Harcourt.
- ii. Low-crude-oil-producing: Abua/Odual, Ahoada-east, Ahoada-west, Emohua, Etche, Ikwerre, Ogu/Bolo, Okrika, Omuma.

Stage 2: At least one LGA was selected by balloting from a list of the eight high and low-crude-oil-producing communities to reflect conventional and artisanal operations.

- i. High-oil-producing: Ogba/Egbema/Ndoni (Conventional flow stations/Artisanal), Emohua.
- ii. Low-oil-producing (Artisanal); Ikwerre

Stage 3: 5 communities were selected, each from a list of the high and low-crude-oil-producing LGAs by balloting.

- i. High-oil-producing; Ebocha, Ibaa, Obrikom, Ohali/Usumini/Idu, Okwuzi,
- ii. Low oil-producing: Ipo, Omuanwa, Omerelu, Ozuoha, Ubima

Selection of sampling points

The water sampling points were geo-referenced. The criteria for selecting sampling points were based on the population density, areas of industry, and the river catchment areas. Water samples were collected from three major water sources (borehole/deep well, river, commercial) in five locations from each site: High-crude-oil-producing communities, Ebocha, Ibaa, Obrikom, Okwuzi; Low-crude-oil-producing, Ipo, Omuanwa, Omerelu, Ozuoha, Ubima.

The water samples from the borehole pumps were collected midstream, while the samples for the surface water (rivers/streams) were collected from points where members of the communities commonly fetched their water.

Sample collection, storage, and transportation

The drinking water sources of each of the study communities were collected. A total of 28 water samples, 20 boreholes, 2 rivers, and recycled water (2 bottles and 4 sachets) were collected from the 10 communities (5 high sites; 5 low sites). A 2-litre sterile non-reactive plastic bottle was rinsed before collecting water samples from each source, leaving a margin of 2.5cm to allow the mixing of particles by shaking. Each water sample was labelled appropriately, stored in an ice-lined cooler (geo-style boxes), and transported to the laboratory within 24 hours for analysis, using the American Public Health Association (APHA) recommended guidelines (WHO, 2017).

Chemical analysis of water samples

A comprehensive chemical analysis was conducted on the sampled water sources collected from the georeferenced point in the selected communities. The chemical parameters assessed include: Total Petroleum Hydrocarbons (TPH), Total Oil and Gas (TOG), Polycyclic Aromatic Hydrocarbons (PAH) and its metabolites (Acenaphthylene, Acenaphthene, Anthracene, Benz[a]anthracene, Benzo[b]fluoranthene, Benzo[k]fluoranthene, Benzo[g,h,i]perylene, Benzo[a]pyrene, Chrysene, Dibenz[a,h]anthracene, Fluoranthene, Fluorene, Indeno[1,2,3-cd]pyrene, Naphthalene, Phenanthrene, Pyrene). The outcome of each parameter was compared with the standard World Health Organization (WHO) for drinking water quality.

Chemical characterization of water sources

Four litres of each water source were collected from each selected high and low crude oil-producing site. The water samples were stored at 4°C and sent to a reference laboratory for analysis within 24 hours. Each water sample was subjected to instrumental analysis to assess the concentration of the selected PAHs:

Instrumental analysis

Samples were analyzed using a GC2010 gas chromatograph coupled with a QP2010 mass spectrometer (GC-MS, Shimadzu, Japan). Aliquots of sample extracts (1 μ L) were added by the splitless injection at 280°C. Rtx-5MS capillary column (length 30.0 m, i.d. 0.25 mm, film 0.25 μ m, Shimadzu, Japan)

and employed to complete Chromatographic separation of PAHs over 57 min using a triple ramp oven programme (initial temperature 60°C; 20°C/min to 160°C; 3°C/min to 280°C, held for 6 min; 20°C/min to 300°C, held for 5 min). Also, to ensure sustained flow (1 mL/min) of ultrapure helium carrier gas (99.999%), mass spectra were collected between 50-500 m/z at a transfer line and ion source temperatures of 2200 and 2500°C at electron ionization of 70 eV. Then, a full scan of the mixture standard comprising each hydrocarbon (1 μ g/mL) was performed at its peaks based on its retention times and mass spectra. However, to guarantee the reproducibility of retention time hydrocarbon, retention time locking with an internal standard was applied, followed by data acquisition, requiring identification and distinct fragment ions for each hydrocarbon within an allotted time window (Liu et al, 2020).

Statistical analysis

The independent descriptive variables identified as the concentration of TPH, PAH, TOG, and the above-selected sixteen polycyclic aromatic hydrocarbons were summarized as mean and standard deviation such as. In the bivariate analysis, the significance of the association was tested using the independent t-test and Analysis of Variance (ANOVA). At the multivariate level, the significant variables were set at the probability value ($p < 0.05$) and 95% confidence interval and compared with the standard WHO guideline for drinking water quality.

RESULTS

Table 1 shows that the mean concentration of TPH (0.35), TOG (0.78), and PAH (0.05) in the high oil-producing communities was found above the recommended WHO permissible limits for water quality. However, all three parameters were significantly higher in the high oil-producing site compared to the low sites.

Table 2 shows that twelve of the sixteen analyzed PAH metabolites were detected in the water sources of high and/or low-oil-producing sites. However, of these twelve metabolites, only five were observed significantly higher in the high-oil-producing site as opposed to the low site: Naphthalene ($p=0.001$), Acenaphthene ($p=0.004$), Fluorene ($p=0.021$) and Phenanthrene ($p=0.001$), Anthracene ($p=0.012$) and Fluoranthene ($p=0.031$). However, all the identified PAH compounds were below the recommended WHO limit.

Table 1: Comparison of PAH in water sources in high & low oil-producing sites

Variable	High site		low site		t	P-value	WHO Limit
	Mean	Std	Mean	Std			
TPH (mg/L)	0.35	0.28	0.03	0.05	3.985	0.001*	0.001
PAH (mg/L)	0.05	0.03	0.00	0.01	5.376	<0.001*	0.002
TOG (mg/L)	0.78	0.49	0.19	0.30	3.728	0.001*	0.2
*Significant							

Table 2: Comparison of PAH metabolites in water sources of high and low oil-producing sites in Rivers State.

Variable	Main sites		Control sites		t	P-value	WHO LOD ($\mu\text{g}/\text{kg}$)
	Mean	Std	Mean	Std			
Naphthalene	0.012	0.008	0.002	0.006	3.783	0.001*	0.01
Acenaphthylene	0.006	0.009	0.001	0.004	1.732	0.095	0.0073
Acenaphthene	0.008	0.008	0.001	0.003	3.162	0.004*	0.073
Fluorene	0.005	0.008	0.000	0.000	2.463	0.021*	0.01
Phenanthrene	0.011	0.009	0.001	0.003	3.918	0.001*	0.01
Anthracene	0.004	0.005	0.000	0.000	2.687	0.012*	0.01
Fluoranthene	0.003	0.005	0.000	0.000	2.280	0.031*	0.073
Pyrene	0.002	0.004	0.000	0.000	1.883	0.071	0.73
Benz[a]anthracene	0.002	0.006	0.000	0.000	1.385	0.178	0.73
Chrysene	0.003	0.006	0.000	0.000	1.749	0.092	0.0073
Benzo[b]fluoranthene	0.001	0.004	0.000	0.000	1.472	0.153	0.73
Benzo[k]fluoranthene	0.001	0.003	0.000	0.000	1.000	0.327	0.0073
Benzo[a]pyrene	0.000	0.000	0.000	0.000	-	-	-
Indeno[1,2,3-cd] pyrene	0.000	0.000	0.000	0.000	-	-	-
Dibenz[a,h]anthracene	0.000	0.000	0.000	0.000	-	-	-
Benzo(g,h,i)perylene	0.000	0.000	0.000	0.000	-	-	-

*Significant

DISCUSSION

The findings from this study revealed that the mean concentration of TPH, PAH, and TOG was significantly above the recommended WHO acceptable limit of detection for water quality in the high oil-producing site compared to the low site. This observation is consistent with previous studies carried out in Rivers State (Igwe et al, 2021; Simeon, 2020), Bayelsa (Amolo et al, 2023; Ighariemu et al, 2019; Imasuen & Egai, 2013), and the Niger Delta (Okechukwu et al, 2021) with reported varying degrees of hydrocarbon pollution above the regulatory standards. However, these previous studies were only carried out in high oil-producing communities. On the other hand, an earlier study conducted in three Niger Delta States, Bayelsa, Delta, and Rivers, observed high concentrations of TOG and TPH above the recommended limits. However, there was no significant difference in the values observed between the gas-flaring and non-gas-flaring communities (Maduka & Ephraim-Emmanuel, 2019). On the contrary, studies done in Akwa-Ibom (Inyang et al, 2019) and Bayelsa State (Seiyaboh & Angaye, 2018) showed that the mean concentration of TPH and TOG was within the recommended regulatory limits.

The above outcome from the present study is perhaps due to the continuous occurrence of oil spills from pipeline vandalism, artisanal, and conventional oil-refining activities carried out in the high oil-producing sites as opposed to the low sites, where mainly storage, transportation, and distribution of crude products are carried out. This implies that the drinking water sources of the high oil-producing communities contain more pollutants, thus predisposing them to a greater risk of

deleterious health consequences than those in the low oil-producing sites (Ibeawuchi, 2016; Uzorka et al, 2023).

Furthermore, six identified PAH metabolites (Naphthalene, Acenaphthene, Fluorene, Phenanthrene, Anthracene, and Fluoranthene) were significantly lower than the recommended WHO permissible limits in the high oil-producing site compared to the low site. This is in line with a previous study carried out in Rivers and Bayelsa State with reported mean concentrations below the recommended standards (Okechukwu et al, 2021). On the contrary, a study conducted in three different flow stations in Delta State observed significantly elevated mean concentrations of PAH compounds beyond the WHO-recommended safety limits. However, benzo[k]fluoranthene and benzo[b]fluoranthene had the highest concentrations, while the lowest was benzo[a]anthracene and anthracene (Uche, 2015). The presence of higher concentrations of the observed polycyclic aromatic hydrocarbon compounds in the analysed water sources of the high oil-producing communities may predispose the community members to a higher risk of carcinomas than those living in the low sites. However, this may be attributed to the fact that communities hosting oil and gas industries still engage in artisanal refining in addition to conventional gas-flaring activities despite the ongoing remediation processes. Whereas their counterparts in the low oil-producing sites mainly indulge in the storing, transporting, and distributing of refined crude products.

Limitations

Even though this study was conducted in equal proportions of five study sites each in both the low and high oil-producing communities, potential biases, like;

sampling, interviewer, recall, and information biases, were anticipated during the study. Also, the problem of insecurity with associated incessant cases of abduction due to the peculiarity of the study sites may have posed a major challenge in accessing some of the sampled communities. However, this was circumvented with the use of a reputable community representative who served as a tour guide during the period of data collection.

CONCLUSION

This study observed that the sampled drinking water sources (boreholes and surface water) in the high and low-oil-producing sites were contaminated. However, the high site where both conventional and artisanal refining activities are practised showed higher concentrations of hydrocarbons (PAHs) as opposed to the low sites where mainly storage, transportation, and distribution of petroleum products are carried out. Therefore, these contaminated water sources are unfit for human consumption. To guarantee the provision of a sustainable potable water supply in oil-producing communities, the responsible agencies should ensure that municipal water sources undergo conventional treatment processes. Also, the existing policies and laws guiding the control of oil spills during refining processes should be strictly enforced. This will ensure that the reported ongoing surveillance and remediation activities are successful in reducing the effects of these pollutants on the ecosystems and the well-being of community members.

What is already known on this topic

1. Polycyclic aromatic hydrocarbons and their metabolites have been implicated as one of the major pollutants in oil-producing areas, as they are widely released into the air, water sources, and food crops during exploration and exploitation activities with associated health consequences.
2. The water sources in crude oil-producing communities have been reported to largely contain impurities such as heavy metals, polycyclic aromatic hydrocarbons, and other effluents due to conventional or artisanal refining processes.
3. The concentration of polycyclic aromatic hydrocarbons and their corresponding effects on the ecosystem and the health of humans determine the extent of remediation intervention to be instituted, especially in crude oil-producing regions.

What this study adds

Findings from this study will enable the responsible government authorities/agencies to:

1. Provide a sustainable potable water supply to the affected oil-producing communities
2. Periodic conventional treatment processes of all municipal water sources in the affected communities

3. Enforce existing policies and laws guiding the control of oil spills during refining processes
4. Ensure that the reported ongoing surveillance and remediation activities are successful in reducing the effects of these pollutants on the ecosystems and the well-being of community members

Competing interest

The authors declare no conflict of interest

Authors' contribution

Authors AL conceptualized the study, and the study design and also monitored the data collection, data analysis, and interpretation, and wrote the manuscript. All authors reviewed made corrections where necessary and read and agreed on the submission of the final manuscript.

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