



Blood Plasma Concentration of Polycyclic Aromatic Hydrocarbons in Under Five Children in Niger Delta, Nigeria

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Abstract	Article History
<p>PAHs are known carcinogens associated with immune system suppression, skin irritation, allergic reactions, respiratory problems, mutagenic issues and are generally toxic to humans. In this study, we assessed the presence and proportions of PAHs in the blood plasma of under-five children living in crude oil-polluted environment in Ogoniland, Niger Delta region of Nigeria. Communities resident in close proximity to crude oil exploration sites are constantly faced with environmental, socio-economic and health consequences of crude oil pollution. People can be exposed to PAHs through multiple routes including inhalation, ingestion and dermal contact. Ethical clearance for this study was obtained from the Ethics Committee, University of Port Harcourt. A total of 78 children were recruited from Gokana (male =17, female= 22) and Ideato (Male=15, female=24) using multi-clustered sampling method. Standard procedures were observed in this study. Results obtained were compared with under five children living in non-oil polluted environments within the Niger Delta province. The overall average PAHs was 0.331±0.383 mg/kg for Gokana males and 0.058±0.057mg/kg for Ideato males; overall average PAHs was 0.514±0.373 mg/kg for Gokana females and 0.100±0.049 mg/kg for Ideato female; overall average PAHs was 0.423±0.383 mg/kg Gokana and 0.081±0.057 mg/kg for Ideato. Under-five children in Ogoniland showed positive blood plasma PAHs higher than the comparison group in Ideato. However, carcinogenic PAHs were below levels of concern in the study population. The male gender showed higher positive PAHs than the female gender. Further studies on inflammation, stress factors and overall health status are recommended considering the vulnerability of the study population.</p> <p>Keywords: <i>Crude oil pollution, under-five children, polycyclic aromatic hydrocarbons, Niger Delta</i></p>	<p>Received: 21 Aug 2023 Accepted: 28 Aug 2023 Published: 01 Sept 2023</p> <p>Scan QR code to view*</p>  <p>License: CC BY 4.0*</p>  <p>Open Access article.</p>
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Introduction

Crude oil pollution remains a subject of global concern. The term "oil pollution" refers to the process through which crude oil, refined petroleum products, or the by-products of these processes are released into the environment. The United Nations Environment Programme (UNEP) claims that when crude oil, refined petroleum products, or their by-products are released into the environment, it has a negative impact on the air, water, soil, and living beings (UNEP, 2019). The detrimental impacts that oil pollution has on people's health are a major source of concern, and this is especially the case for communities that are situated in close proximity to oil exploration and production sites (Gorovtsov *et al.*, 2018). Gokana in Ogoniland, Niger Delta region, Nigeria has

not been spared from the devastating health and socio-economic consequences of crude oil exploitation activities (Bodo, 2018). Furthermore, crude oil pollution affects soil nutrients in Ogoniland impacting on plant yield (Otaiku, 2019). This pollution also contaminates soil water bodies, which ultimately leads in the death of aquatic animals, the ruin of marine and coastal ecosystems, and the disruption of food chains (Tripathi *et al.*, 2019).

Polycyclic aromatic hydrocarbons (PAHs) are persistent organic pollutants that have been linked to severe global health consequences (Joshua *et al.*, 2016). PAHs are products of incomplete combustion of fossil fuels and organic substance containing carbon and hydrogen (Onyidinma *et al.*, 2021), with

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very low solubility and high thermostability. They can persist in the environment for a protracted period of time, causing damage to flora and fauna throughout the course of a longer period of time. The US environmental protection agency has listed 16 PAHs (Naphthalene, Acenaphthylene, Acenaphthene, Fluorene, Phenanthrene, Anthracene, Fluoranthene, Pyrene, Benz[a]anthracene, Chrysene, Benzo[b]fluoranthene, Benzo[k]fluoranthene, Benzo[a]pyrene, Indeno[1,2,3-cd] pyrene, Dibenzo[a,h]anthracene and Benzo[ghi]perylene) as top toxicological concern out of more than 100 of the existing compounds (Patel *et al.*, 2020; Ajiboye *et al.*, 2021).

Exposure to potentially harmful compounds, such as PAHs and heavy metals, may also result through the consumption of contaminated seafood and crops. Consuming food crops that have been tainted with crude oil and chemicals carries with it the additional risk of undesirable impacts on one's health as a result of the consumption of these crops (Dai *et al.*, 2019). Ouro-Sama *et al.* (2023) and Ajiboye *et al.* (2021), further added that these substances are known to have a negative impact on human health and create problems such as genotoxicity, carcinogenicity, and organ damage.

The default heterocyclic aromatic ring structure of PAHs, thermostability, hydrophobicity, causes them less degradable and highly persistence in the environment (Patel *et al.*, 2020). PAHs causes a number of health concerns, including respiratory difficulties, skin disorders, and an increased risk of cancer (Patel *et al.*, 2020; Ajiboye *et al.*, 2021).

According to the findings of studies that was carried out by Ouro-Sama *et al.* (2023) and Dai *et al.* (2019), oil contamination of food crops leads to increased levels of PAHs. PAHs are compounds that, when consumed, have the potential to cause genotoxicity and carcinogenicity. There have been reports of an accumulation of heavy metals in food crops as a result of oil pollution, as stated by Ajiboye *et al.* (2021).

The facilitation of PAHs uptake through inhalation is attributed to their heightened solubility in fats. BaP is the PAH that exhibits the highest degree of lipid solubility. The aforementioned compound has the potential to interact with lipid distribution molecules, including chylomicrons and other lipoproteins. This interaction may result in the compound permeating various systems that are involved in lipid absorption and distribution. As a consequence, the compound may accumulate in tissues and organs that play an active role in these processes, such as the liver and small intestine. This assertion was made by Ifegwu and Anyakora (2015).

According to Ouro-Sama *et al.* (2023), PAHs have been found to cause mutations in chromosomes, disrupt fusion and junction processes, and have the potential to induce chromosomal breaks. As a result, the genetic material contained within these cells exhibits instability. In the absence of DNA repair mechanisms, the genotoxic agent will cause a permanent impact on the DNA of the cell during the initiation phase of transcription. This will lead to an unavoidable mutation that can result in the formation of a pre-neoplastic cell, as reported by Choudhuri *et al.* (2018).

According to Tiwari and colleagues (2019), certain sensors responsible for detecting DNA damage, such as ataxia telangiectasia mutated and ataxia telangiectasia Rad3-related, are capable of identifying alterations in the genetic sequence and activating appropriate repair mechanisms. In addition to instigating reparative mechanisms, these kinase enzymes impede the progression of the cell cycle by catalyzing intermolecular phosphorylation of substrates. According to Chen *et al.* (2017), it has been observed that they also trigger the activation of the tumor suppressor gene p53, which can impact other genes responsible

for regulating cells and inducing DNA repair, while also promoting apoptosis.

According to Schirmer (2016), the complete progression of pre-neoplastic cells can occur due to the failure of one or more suppression systems or persistent and excessive exposure to genotoxic agents. The aforementioned cells exhibit resistance towards apoptosis and, upon the stimulation of clonal expansion, give rise to benign tumors that possess a discernible cellular mass. Following this, the cells gain independent growth potential for an indeterminate duration, leading to the stimulation of angiogenesis and influencing the metastatic potential of the tumor. Thus, it is possible for a cancerous tumor to develop that has the ability to alter metabolic pathways to facilitate its growth and evade immune cell responses, as suggested by Choudhuri *et al.* (2018).

According to Tong *et al.* (2018), surpassing the acceptable threshold (10⁻⁶) for PAH exposure carries a 45% likelihood of carcinogenic risk. The high lipophilicity of PAHs is responsible for their substantial accumulation and bioavailability in internal organs that are abundant in adipose tissue subsequent to exposure, as noted by Lee and Vu (2010) and Abdel-Shafy and Mansour (2016).

Rengarajan *et al.* (2015) reported the occurrence of embryo toxicity in experimental animals as a result of exposure to naphthalene, benzo(a)anthracene, and benzo(a)pyrene. The study conducted by Bolden *et al.* (2017) exhibited the potential of these compounds to function as anti-estrogens and/or anti-androgens through their direct interaction with estrogen and androgen receptors. Bolden and colleagues (2017) reported various non-cancerous health effects related to the reproductive system in both males and females resulting from exposure to PAHs. These effects include alterations in sperm quality, testicular function, and egg viability, as well as DNA damage in oocytes, ovarian damages, and other reproductive diseases.

Statement of the problem

The characteristics of crude oil has made it a major source environmental pollutant. Surpassing the acceptable carcinogenic risk threshold of 45% for PAHs. The presence and proportion of PAHs in under-five children living in crude oil polluted Ogoniland, Rivers state is unknown.

Aim and Objectives

This study aims to ascertain the proportion of polycyclic aromatic hydrocarbons in the blood plasma of under-five children living in crude oil polluted environment in Niger Delta of Nigeria.

However, the study was guided by the following specific objectives:

1. Ascertain the presence of polycyclic aromatic hydrocarbons in the blood plasma of under five children living in Niger delta region of Nigeria.
2. To determine the proportion of PAHs (if found) in the blood plasma of under five children living in crude oil polluted environment in Niger Delta of Nigeria.

Research questions

1. Are there PAHs in the blood plasma of under five children living in crude oil polluted environment in Niger Delta of Nigeria.
2. What are the proportion of PAHs (if found) in the blood plasma of under five children living in crude oil polluted environment in Niger Delta of Nigeria.

Methodology

This cross sectional study with comparative group was conducted in 3 communities in Gokana, Rivers state (B. Derek, K. Dere, Bomu) labelled the case study region and 3 non-crude oil exploration communities in Ideato North LGA, Imo state (Umukegwu, Umuezeaga, Owerre-Akokwa) assigned control, both states are in Niger Delta region of Nigeria. Gokana is one of the 4 Local government in Ogoniland heavily polluted by crude oil located along : latitude $4^{\circ} 40' 5''$ N and $4^{\circ} 43' 19.5''$ N and longitude $7^{\circ} 22' 53.7''$ E and $7^{\circ} 27' 9.8''$ E (Nkpaa *et al.*, 2017). Ideato North North is located along Latitude: 5.88528, Longitude: $7.13139 5^{\circ} 53' 7''$ North, $7^{\circ} 7' 53''$ East (Nwosu *et al.*, 2020). The data collection period was in December 2022. Ethical approval was obtained from University of Port Harcourt Ethics Committee. A community based cross-sectional comparative study was carried out among under five children from households in the region. Parents and guardians of children signed consent forms provided before participating in the study. Seventy-eight (78) children were recruited for the study through a multi stage sampling method. Two milliliter (2ml) of the whole blood was collected in a well labelled ethylenediaminetetraacetic acid (K_3EDTA) bottle gently inverter to mix with the anticoagulant and store at $4^{\circ}C$ throughout before centrifuge. The samples were transferred into labelled tubes and loaded into the centrifuge adhering to all safety protocols and manufacturers guidelines.

Extraction and Analysis of Sample

Liquid-liquid extraction (LLE) separation technique used for the separation finds wide application in analytical chemistry and sample preparation for transfer of solutes from one liquid phase (blood plasma) to another immiscible liquid phase (the chloroform) (Mahmoudpour *et al.*, 2017). Chloroform serves as non-polar organic solvent that selectively extract the hydrophobic PAHs from the aqueous blood plasma phase. One milliliter (1ml) serum was pipette into a clean separatory flask, to this was added 10ml Chloroform and the flask was corked with a glass stopper, mixed using a vortex mixer to form an immiscible liquid phase. The flask was kept to separate the content on a ringed stand by letting into the flask about 25ml distilled water. The organic phase which is the down was ran into a clean sample bottle (vial) and corked tightly to avoid volume reduction by evaporation. The Organic extract was passed through an extract column packed with glass wool, silica gel and anhydrous Sodium Sulphate.

NB: It should be noted that the Silica gel aids the clean-up of the extract from debris and impurities of other compounds that are not PAHs. Anhydrous Sodium sulphate was used to concentrate and dehydrates water droplets in the extract.

Gas Chromatography-mass spectrophotometer (GC-MS) technique was adopted for analysis of the samples. The samples were analysed in duplicates and result obtained enhanced with integrator.

Results and Discussion

The results from Table 1 shows a significant difference in PAHs between Gokana and Ideato for the males, since the calculated t-test statistic of 2.725 with p-value 0.005 is less the critical 5% value. For Gokana and Ideato for the females, a significant difference was found since the calculated t-test statistic of 5.403 with p-value 0.000 is less the critical 5% value. Similarly, the result shows a significant difference between Gokana and Ideato for both males and females, since the calculated T test statistics of 5.817 with p-value 0.000 is less the critical 5% value. This

indicated statistically that the individual from Gokana and Ideato differ, since only Naphthalene (mg/l) and Acenaphthylene (mg/l) of the measure Polycyclic Aromatic Hydrocarbons are not presents in both sample and control individual.

The results of concentration of polycyclic aromatic hydrocarbon illustrated in Figure 1 shows that 15 PAHs were found in trace quantity in the male gender; 14 were found in female gender of Gokana except Naphthalene, Acenaphthylene absent in male and Naphthalene, 2-methyl naphthalene, Acenaphthylene not found in Gokana female

In Ideato, 12 PAHs was present in little quantities in the male category of Ideato, excluding Naphthalene, 2-methyl naphthalene, Acenaphthylene, Acenaphthene & Fluoranthene while Naphthalene, 2-methyl naphthalene, Fluorene & acenaphthene were not found. The study found traces of PAHs, including 2-methyl naphthalene, Fluorene, Acenaphthene, Phenanthrene, Anthracene, Fluoranthene, Pyrene, Benzo(a) anthracene, Chrysene, Benzo(b) fluoranthene, Benzo(k) fluoranthene, Benzo(a) pyrene, Benzo(a,h) anthracene, Indeno(1,2,3,cd)pyrene, and Benzo(g,h,i)perylene in the plasma of Gokana children.

Proportion of PAHs in the blood plasma of under-five children living in crude oil polluted environment

The significant finding of this study regarding PAHs proportion in the plasma levels of the under-5 children showed that all PAHs, apart from Naphthalene and Acenaphthylene, were present in some quantities in the Plasma levels of under-5 children in Gokana, which showed a significant difference compared with the control group of under-5 children in Ideato. The average for males in mg/l or kg was 0.331 ± 0.383 , 0.514 ± 0.373 for females, and 0.423 ± 0.383 for both male and female groups. The traces of these PAHs identified in the plasma of these children suggests that PAHs are significant substances from crude oil.

Ephraim-Emmanuel and Ordinioha (2021) and Okoye *et al.* (2021) agree with the current finding, as they reported that the PAHs are significant toxic components of crude oil prevalent in the Niger Delta region.

Howard *et al.* (2021) finding is related to the current fining. Howard *et al.* (2021) investigated riverbed sediments in the Niger Delta region and reported significant PAHs contaminated sediments of the 16 priority PAHs excluding Naphthalene, Acenaphthylene, and three other PAHs. Therefore, the finding by Howard *et al.* (2021) corresponds with the current study's findings, as Naphthalene and Acenaphthylene were not present in the identified traces of the PAHs in the plasma of the children in this study. However, the current study disagrees with Howard *et al.* (2021) findings, as it identified all the significant PAHs in the plasma of the under-5 children in Gokana in the Niger Delta region, excluding Naphthalene and Acenaphthylene. Regarding the European classification of PAHs pollution in soil, heavily contaminated PAHs are >1.0 mg/kg (Howard *et al.*, 2021). Based on the above classification, the proportions of PAHs in the plasma of under-5 children may not be highly contaminated. In contrast, the sediments investigated by Howard *et al.* (2021) indicated heavily contaminated PAHs.

Table 1: Blood plasma concentration of PAHs for Gokana (sample) and Ideato North (control) LGA

Gender	Parameters (mg/l)	Mean \pm SD Gokana (Case)	Mean \pm SD Ideato (Control)
Male	Naphthalene	ND	ND
Male	2-methyl naphthalene	0.105 \pm 0	ND
Male	Acenaphthylene	ND	ND
Male	Fluorene	0.001 \pm 0.001	0.019 \pm 0.033
Male	Acenaphthene	0.001 \pm 0.003	ND
Male	Phenanthrene	0.007 \pm 0.013	0.003 \pm 0.004
Male	Anthracene	0.004 \pm 0.011	0.001 \pm 0.001
Male	Fluoranthene	0.006 \pm 0.018	0
Male	Pyrene	0.008 \pm 0.021	0.001 \pm 0.002
Male	Benzo(a) anthracene	0.003 \pm 0.006	0.012 \pm 0.034
Male	Chrysene	0.07 \pm 0.084	0.006 \pm 0.006
Male	Benzo(b) fluoranthene	0.013 \pm 0.014	0.002 \pm 0.004
Male	Benzo(k) fluoranthene	0.057 \pm 0.085	0.009 \pm 0.012
Male	Benzo(a) pyrene	0.037 \pm 0.099	0.006 \pm 0.012
Male	Benzo(a,h) anthracene	0.065 \pm 0.077	0.013 \pm 0.017
Male	Indeno (1,2,3,cd) pyrene	0.037 \pm 0.042	0.018 \pm 0.015
Male	Benzo (g,h,I) perylene	0.038 \pm 0.085	0.006 \pm 0.005
	Overall Average PAHS	0.331\pm0.383	0.058\pm0.057
Female	Naphthalene	ND	ND
Female	2-methyl naphthalene	ND	ND
Female	Acenaphthylene	ND	0.003 \pm 0.004
Female	Fluorene	0.007 \pm 0.011	ND
Female	Acenaphthene	0.003 \pm 0.005	ND
Female	Phenanthrene	0.051 \pm 0.084	0.002 \pm 0.003
Female	Anthracene	0.002 \pm 0.004	0.001 \pm 0.002
Female	Fluoranthene	0.002 \pm 0.004	0.001 \pm 0.001
Female	Pyrene	0.003 \pm 0.006	0.001 \pm 0.002
Female	Benzo(a) anthracene	0.003 \pm 0.009	0.001 \pm 0.001
Female	Chrysene	0.082 \pm 0.127	0.017 \pm 0.027
Female	Benzo(b) fluoranthene	0.0034 \pm 0.0027	0.003 \pm 0.002
Female	Benzo(k) fluoranthene	0.067 \pm 0.086	0.01 \pm 0.01
Female	Benzo(a) pyrene	0.076 \pm 0.178	0.009 \pm 0.016
Female	Benzo(a,h) anthracene	0.075 \pm 0.13	0.023 \pm 0.021
Female	Indeno (1,2,3,cd) pyrene	0.104 \pm 0.121	0.022 \pm 0.021
Female	Benzo (g,h,I) perylene	0.042 \pm 0.035	0.012 \pm 0.009
	Overall Average PAHS	0.514\pm0.373	0.1\pm0.049
Both	Naphthalene	ND	ND
Both	2-methyl naphthalene	0.21 \pm 0.458	ND
Both	Acenaphthylene	ND	0.003 \pm 0.004
Both	Fluorene	0.003 \pm 0.007	0.011 \pm 0.026
Both	Acenaphthene	0.003 \pm 0.004	0
Both	Phenanthrene	0.029 \pm 0.063	0.003 \pm 0.003
Both	Anthracene	0.003 \pm 0.008	0.001 \pm 0.002
Both	Fluoranthene	0.004 \pm 0.014	0.001 \pm 0.001
Both	Pyrene	0.005 \pm 0.014	0.001 \pm 0.002
Both	Benzo(a) anthracene	0.003 \pm 0.007	0.006 \pm 0.023
Both	Chrysene	0.077 \pm 0.109	0.013 \pm 0.022
Both	Benzo(b) fluoranthene	0.0031 \pm 0.0016	0.002 \pm 0.003
Both	Benzo(k) fluoranthene	0.063 \pm 0.084	0.01 \pm 0.011
Both	Benzo(a) pyrene	0.058 \pm 0.146	0.008 \pm 0.014
Both	Benzo(a,h) anthracene	0.071 \pm 0.109	0.02 \pm 0.02
Both	Indeno (1,2,3,cd) pyrene	0.075 \pm 0.1	0.021 \pm 0.019
Both	Benzo (g,h,I) perylene	0.041 \pm 0.061	0.01 \pm 0.008
	Overall Average PAHS	0.423\pm0.383	0.081\pm0.057

*ND-Not detected.

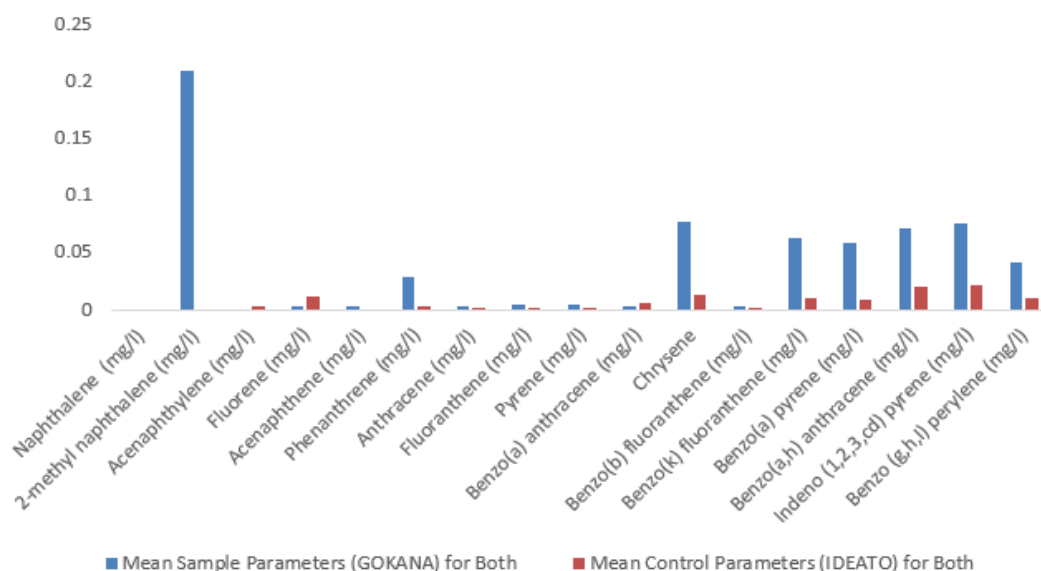


Figure 1: Levels of polycyclic aromatic hydrocarbons for Gokana (sample) and Ideato North (control) LGA (Male)

Toxicological Concerns

Studies have shown that hydrophobic PAHs with high thermostability do not decompose rather they bio-accumulate over time posing serious negative health consequences (Onyidinma *et al.*, 2021). The differences in the levels of PAHs in blood plasma of under-five children in Gokana suggest greater exposure of the environmental pollutant crude oil pollution (Bodo, 2018) than control group in Ideato.

The difference in the two study groups agrees with Igwe *et al.* 2012 that human population exposed to the environmental pollutant as in Gokana (under-5 children) are at greater risk of the contaminant and suffers as a result of exploitation of the region endowed natural resources (Dai *et al.*, 2019 and Bodo, 2018). In addition, Dai *et al.* (2019) indicated that PAHs readily enter the body through consumption of contaminated plant and sea food in the geographical location (Bodo, 2018) which may suggest the reason for the lower levels of PAHs in the proportion of the plasma of the under-5 children in Ideato. Therefore, the current finding on the proportion of PHAs in the plasma of under-5 children in Gokana may not be high but may accumulate and result in toxicity in the children in the future.

Conclusion

This study has shown that under-5 children living in Gokana in the Niger Delta region, a region known to be polluted by the crude oil, resulting from crude oil exploitation, and oil spillage have proportions of Poly Acyclic Hydrocarbons in their blood plasma. The amount of PAHs present in their blood plasma were assumed to be of high positive levels compared with other under-5 children in Ideato. However, the current study showed that PAHs were found in the blood plasma of the children excluding Naphthalene and Acenaphthylene, which are regarded as not being heavily contaminated when compared to the standard level of PAHs heavily contamination levels of >1 mg/kg.

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