Scientific African 27 (2025) e02525

Contents lists available at ScienceDirect

Scientific African

journal homepage: www.elsevier.com/locate/sciaf

# Assessment of the impact of benzene, toluene, ethylbenzene, and xylene (BTEX) on soil microbial population in selected areas of Port Harcourt City, Nigeria

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ARTICLE INFO

Editor: DR B Gyampoh

*Keywords:*
Soil
BTEX
Fungi
Bacteria
Ecosystem integrity

ABSTRACT

Human activities are on the rise in these areas and consequently, the discharge of BTEX to the environment is on the rise. Benzene, toluene, ethylbenzene, and xylenes (BTEX), are known to cause cancer and mutagenesis. These elements are common in soil, water, and air samples from the environment, which raises the possibility of human exposure. The purpose of this study was to determine the concentrations of BTEX (benzene, toluene, ethylbenzene, and o-xylene) in soils, to determine the effects of BTEX concentrations on the population of fungi and bacteria in the soil, and to investigate the possible sources and spatial distribution of BTEX in the selected areas. The concentrations of BTEX were measured using a Gas Chromatograph (FID, ECD) in 9 contaminated and 3 control sites over 3 months. The spatial distribution of BTEX revealed that the highest concentrations were in the agricultural area (2.49±0.94 ppm) followed by industrial area (2.14 ±1.02 ppm) and the lowest in an urban area (1.32±0.44 ppm). BTEX assessment showed that the benzene concentration in all contaminated areas was above the recommended US EPA standard of 0.005 ppm (5 μg/kg). In addition, the mean concentrations of ethylbenzene were above the recommended U.S. EPA limit of 0.370 ppm in three areas; agricultural (0.76±0.61 ppm), industrial (0.89±0.68 ppm) and urban control (0.89±0.31 ppm). There was a significant difference in concentration of o-xylene between the study samples and control samples $F(11, 24) = 5.374$, $P < 0.000$. Pearson correlation showed a significant positive correlation between BTEX and total fungi (TF), $r = 0.351$. Pearson correlation also showed that o-xylene was significantly positively correlated with total fungi (TF), $r = 0.331$. The result showed a significant threat of benzene and ethylbenzene to soil health. Increased and regular monitoring is thus recommended to manage the increased concentrations of BTEX in future and reduce the adverse impacts of its effluence on soils and human health.

## Introduction

Soil pollution has a profound effect on soil ecosystem integrity and human health [32,89]. Over 2.3 million healthy lives were lost

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https://doi.org/10.1016/j.sciaf.2024.e02525
Received 24 March 2022; Received in revised form 17 September 2024; Accepted 24 December 2024
Available online 6 January 2025

due to ill health and disability caused by air pollution in Africa in the year 2019 [17]. In 2016, there were about 9 million premature deaths, which constitutes 16 % of global deaths due to pollution [30]. Globally, an estimated 3.2 million deaths annually, including over 237,000 deaths of children under the age of five, can be attributed to household air pollution in 2020 [37]. In addition, pollution brings economic strains to individuals and countries [82]. Further, food security is of global concern as soil pollution rises and reduces crop quality and yield. By 2050, 90 % of the world's topsoil will be in danger, according to FAO [28]. Food insecurity is already being made worse; according to the FAO 2023 SOFI report, one in five people in Africa is hungry [28]. There are various sources of pollutants to the environment and among them is the oil and gas industry, where the sources can be grouped as the upstream, midstream and downstream [75]. Oil spills and other effluents from the oil and gas industry are common in most oil-producing countries [66] and can occur upstream, midstream or downstream.

Nigeria is among the leading producers of crude oil in the world, with the highest population in Africa and is categorized as a lower-middle-income country [81,83]. Nigeria is especially vulnerable since the Niger Delta is home to a well-established, illegal artisanal oil refining industry that is well-known for contributing to pollution of the environment which frequently has dire consequences. The center of the Niger Delta region's production and exploration for oil and gas is Port Harcourt City, the capital of Rivers State [68]. Nigeria has over the past experienced inconsistent and ineffective enforcement of environmental protection standards [18]. Rivers State in the Niger Delta is a key area in crude oil production and has suffered immense environmental pollution [36,68]. Furthermore, Port Harcourt City is expeditiously growing and hence urbanization, industrialization, agricultural activities and exploitation of natural resources are increasing and are probable sources of pollutants to the environment [36,65]. Among the pollutants from oil are the volatile organic carbons (VOCs). Volatile organic carbons (VOCs) are carcinogenic to humans and their photochemical reactions produce secondary pollutants including; peroxyacetyl nitrate (PAN) and ozone ($O_3$), which compromise the ecosystem integrity [33], also, benzene [77]. Petroleum and petroleum products are essential for day to day running of activities in current society [9]. However, if they are not handled with caution, they contribute to environmental pollutants which compromise human health. Research on concentration of BTEX have been conducted in some parts of Nigeria [7,27,41,74] and the findings showed varied levels that fall within or above US EPA permitted standards.

Many hydrocarbons found in petroleum and its products have been detected in the environment [2,57,60,76,78]. However, a small fraction of the hydrocarbons has been characterized as toxic and carcinogenic [45,64]. For example, BTEX is among the VOCs that have recently attracted most attention due to its carcinogenic nature and relatively high presence in the environment. Benzene present in BTEX, has been grouped by the International Agency for Research on Cancer (IARC) as "Group 1″ carcinogenic substances to humans [22].

Research done on BTEX have concentrated on BTEX concentration in the atmosphere and bioremediation potentials [4,10,11,14, 20,21,34,50]. However, limited information is available on the effects of BTEX in soils so a need for periodic monitoring of the concentration of BTEX in soils [85]. Concentrations of BTEX are highly variable, and dependent on time and environmental conditions which necessitates continuous monitoring [13].

Soil microorganisms play an important role in the environment and their existence is affected by the presence of pollutants [16,87]. Some of the vital roles played by microorganisms in soil include; organic substance decomposition, nutrient cycling, and pollutant detoxification among others [40]. Microorganisms that play vital roles in the soil include; bacteria, fungi, algae, protozoa and micro-invertebrates. Mycorrhizal fungi form symbiotic relationships with plants, consequently increasing net primary production [42]. Fungi decompose organic substances to acquire energy and nutrients and then release $CO_2$ as a by-product. Fungi also yield their organic compounds which form residues in soils which remain in the environment for generations. They also mediate the cycles of nitrogen (N) and phosphorous (P) by discharging extracellular enzymes that convert organic N or P compounds into sub-products [71]. Depending on the biome, fungi dominate many soil communities, representing an average of 72 % of microbial biomass [46,79]. Therefore, their activities have significant effects on global biogeochemical cycles. The primary way nitrogen is available to plants is by bacteria such as *Rhizobium* fixing nitrogen and cyanobacteria such as *Nostoc, Spirulina* and *Anabaena*. Bacteria convert gaseous nitrogen to nitrate or nitrite and then release the products into the environment. Microorganisms can utilize BTEX for their nourishment [67] and have been recommended for bioremediation, for example *Pseudomonas* spp [41], *Anoxybacillus* spp*, Bacillus* spp and *Rhodococcus* spp [31,47]. Therefore, the primary drivers of essential ecological processes are the soil microorganisms (algae, actinomycetes, bacteria, fungi, protozoa, and viruses). Scientific work is the required tool to explore the possibilities for restoring damaged soils, fostering a stable microbial community and ensuring the integrity of soil ecosystems.

Situated in the Niger Delta, Port Harcourt City is an industrial city whose population is growing and whose pollution levels are rising. Consequently, it is unavoidable that BTEX compounds will be released, increasing urban environmental pollution and harming human health. To forecast the environmental quality in cities like Port Harcourt City, BTEX concentration monitoring is essential. To the best of our knowledge, there hasn't been much detailed reporting on the studies on BTEX's impact on Nigerian soil microorganisms. In Port Harcourt City, Nigeria, there is a need for frequent soil monitoring that will add to the information on soils for reference. Hence, this study aimed to determine the concentrations of BTEX (benzene, toluene, ethylbenzene and o-xylene) in the soils of selected areas, to determine the influences of BTEX concentrations in population of fungi and bacteria in the soil, and to investigate the probable sources and spatial distribution of BTEX in the selected areas.

## Material and methods

### *Study area*

The study was carried out in nine purposely selected sites in Port Harcourt City, Rivers State in Nigeria, with reference to their

economic activities (Fig. 1.1). The study sites were categorized into 3 broad areas; urban, industrial and agricultural. A control site was selected for each of the three broad areas 1 km away from the study areas and perceived virgin land. Urban area included; GRA phase 2, Diobu- Mile 1 and Mguoba. The control site for the urban area was in GRA Phase 3. The selected industrial areas include; Eleme- Onne- NNPC Refinery, Agbada and Trans-Amadi. The control site for the industrial area was Ogali - Eleme. For the agricultural area, the selected sites included; Aluu, Oquwi- Eleme and Emuoha- Eu, while the control site was in Emuoha- Eu. The economic activities of the study areas are listed in Table 1 below.

The following procedures (Fig. 1.2) were performed to determine the levels of BTEX in the soils of the study locations. The procedure included sampling of soil, extraction and detection of BTEX. BTEX levels in soil were determined using gas chromatography.

*Sample collection*

Composite samples were collected in triplicate from the topsoil within a depth of 0 −15 cm using a standard auger by simple random sampling from each of the 9 study sites and 3 control sites in the wet season (April, July and September 2018). To constitute the composite sample, five individual samples were collected following a zig-zag pattern around each study and control sites in order to ensure random sampling. The five individual samples were collected from each site and were thoroughly mixed by coning and quartering in a sterile container to attain a homogenous composite mixture. The study samples were coded as A1, A2, A3, I1, I2, I3 U1, U2 and U3, while the control samples were coded as CA, CI and CU (Table 1). For analysis of BTEX, the homogenized composite samples (400 g for each location) were packed in polyethylene bags and transported to the laboratory. For microbial analysis, the soil samples were collected using a stainless-steel autoclaved shovel and then placed in sterile plastic sample bags (3 M, USA). The soil samples were then transported to the laboratory within 4 h, and preserved at 4 °C until microbiological analysis had been completed. Sampling sites were identified using a GPS and the GPS readings were recorded (Table 1).

*Extraction and determination of benzene, toluene, ethyl-benzene, and o-xylene (BTEX)*

In order to extract BTEX from soils, soil suspensions in water were used. The samples were examined right away using a gas chromatograph after the vials were shaken for thirty to sixty minutes. Concentrations of BTEX in soil samples were determined using US EPA Method 8021 B/8260 B Quality control was observed following US EPA Method 8000B Ten grams of each soil sample was weighed separately into a 100 ml beaker using an analytical balance (Model: A & D FX-5000i). Fifty milliliters of Dichloromethane ($CH_2Cl_2$) [DCM] was added into each of the beakers and covered with a foil paper. The setup was then left to stand for 30 min after which the solutions were decanted separately into vial bottles for Gas Chromatography. Gas Chromatography was conducted for each

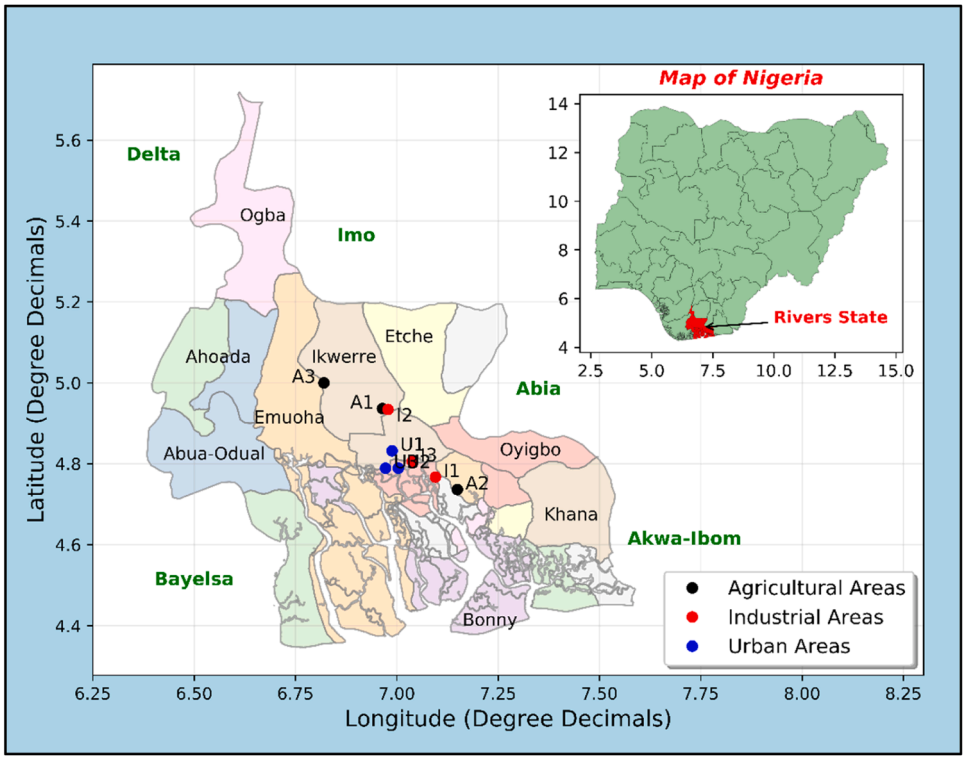


**Fig. 1.1.** Location of sampling sites in selected areas in Port Harcourt City, Rivers State, Nigeria.

**Table 1**
Study areas and their economic activities.

| No | Selected Study Sites | Study Site Coding | Coordinates<br>N latitude<br>E Longitude | Characteristic and main activities |
|---|---|---|---|---|
| | | **Agricultural Areas** | | |
| 1 | Aluu | A1 | 4° 56′ 11.160′<br>6° 57′ 52.248 | Flow station |
| 2 | Eleme | A2 | 4° 44′ 09.874′<br>7° 08′ 58.494′ | Village close to refinery Flow station |
| 3 | Emuoha | A3 | 5° 00′ 00.018′<br>6° 49′ 13.032′ | >1 km away from suspected areas |
| | Control | CA | 5° 00′ 21.384′<br>6° 49′ 00.000′ | |
| | | **Industrial Areas** | | |
| 1 | Onne | I1 | 4° 46′ 00.402′<br>7° 05′ 43.092′ | Hosts the NNPC Refinery |
| 2 | Agbada | I2 | 4° 56′ 03.444′<br>6° 58′ 42.060′ | Hosts SPDC- flow station in a rural setting |
| 3 | Trans-Amadi | I3 | 4° 48′ 20.455′<br>7° 02′ 17.646′ | Schlumberger/, Hallburton |
| | Control | CI | 4° 47′ 13.788′<br>7° 07′ 44.620′ | >1 km away from suspected areas |
| | | **Urban Areas** | | |
| 1 | GRA Phase 2 | U1 | 4° 49′ 53.574′<br>6° 59′ 45.552′ | Inhabited areas Perecuma street |
| 2 | Diobu-Mile 1 | U2 | 4° 47′ 20.382′<br>7° 00′ 13.164′ | Petroleum refinery |
| 3 | Mgbuoba | U3 | 4° 50′ 39.864′<br>6° 58′ 20.232′ | NTA |
| | Control | CU | 4° 49′ 17,040′<br>6° 59′ 24.168′ | >1 km away from suspected areas |

*Nigerian National Petroleum Corporation (NNPC)
Shell Petroleum Development Company of Nigeria Limited (SPDC)
Nigerian Television Authority (NTA). A residential area.

sample using Hewlett Packard HP 5890 Series II Gas Chromatograph (FID, ECD).

*Enumeration of fungi and bacteria*

*Enumeration of total heterotrophic bacteria and total fungi*

Total heterotrophic bacteria (THB) and TF were enumerated by modification of the American Public Health Association [APHA] [3] method (Pour plate method). One gram of soil sample was weighed separately into 9 ml sterile diluent (0.85 % NaCl) under an aseptic condition (laminar bench floor). Each mixture was then homogenized using a laboratory vortex mixer (Model: 10,101,001, IP42) and serially diluted. For the culture of bacteria, 0.1 ml aliquot of the inoculum was collected using a sterile pipette, inoculated on Nutrient Agar (NA) medium. The inoculum was then spread evenly using a sterile glass spreader. Plates were then incubated at 37 °C for 24 h. Thereafter, colonies were counted to obtain colony forming units (CFUs) value per gram of soil sample. For culture of fungi, 0.1 ml aliquot of inoculum was inoculated on Potato Dextrose Agar (PDA) acidified using 0.1 % lactic acid in order to inhibit the growth of bacteria. Inoculated plates were then incubated at ambient temperature (28±2 °C) for 5 to 7 days. For culture of Hydrocarbon Utilizing Bacteria (HUB), the above method was used. Afterwards, 0.1 ml aliquot of the inoculum was inoculated on Mineral Salt Agar (MSA) containing g/l of $MgSO_4{\cdot}7H_2O$ 0.42 g, KCl 0.29 g, $K_2HPO_4$ 1.25 g, $KH_2PO_4$ 0.83 g, $NaNO_3$ 0.42 g, NaCl 10 g and Agar Powder 18 g, using the spread technique. Sterile filter paper (Whatman 540) was soaked with autoclaved Bonny light crude oil and placed in the lid of Petri dish. The plates were incubated in an inverted position at room temperature (28±2 °C) for 7 days and CFUs were enumerated using a colony counter. For culture of fungi, the pour plate method was used. Afterwards, a sterile filter paper (Whatman 540) was soaked with autoclaved Bonny light crude oil and placed in the lid of the petri dish. The plates were incubated in an inverted position at room temperature (28±2 °C) for 14 days after which fungal CFUs were enumerated using a colony counter.

*Determination of % hydrocarbon utilizing fungi and bacteria*

Percent (%) hydrocarbon utilizing bacteria (HUB) and % hydrocarbon utilizing fungi (HUF) were expressed as a fraction of the total heterotrophic viable count using the formula;

$$\%\ \mathrm{HUB}\Big/\mathrm{HUF} = \frac{\textit{Total hydrocarbon utilizing bacteria/fungi}}{\textit{Total heterotrophic bacteria/fungi}} \times \frac{100}{1}$$

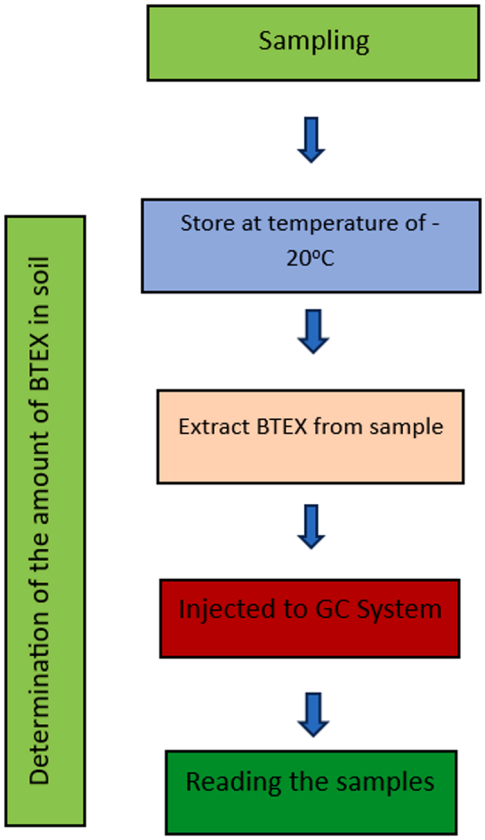


**Fig. 1.2.** Steps of sampling, extraction and detection of BTEX.

**Table 2**
Concentration assessment of benzene, toluene, ethylbenzene, o-xylene in soils of study sites.

| Study site | benzene | toluene | ethylbenzene | o-xylene | BTEX |
|---|---|---|---|---|---|
| A1 | 0.340 ± 0.24 $^{ab}$ | 0.490 ± 0.16 $^{ab}$ | 1.340 ± 0.10 $^{c}$ | 1.264 ± 0.19 $^{e}$ | 3.2404 ± 0.30 |
| A2 | 0.094 ± 0.01 $^{a}$ | 0.263±0.19 $^{a}$ | 0.491 ± 0.39$^{abc}$ | 0.513 ± 0.76$^{abc}$ | 1.761 ± 1.16 |
| A3 | 0.631 ± 0.46 $^{b}$ | 0.694 ± 0.43 $^{b}$ | 0.480 ± 0.83$^{abc}$ | 0.897 ± 0.20$^{cde}$ | 2.460 ± 0.68 |
| Average | **0.35 ± 0.35** | **0.48 ± 0.31** | **0.76 ± 0.61** | **0.89 ± 0.52** | **2.49 ± 0.94** |
| CA | 0.312 ± 0.12 $^{ab}$ | 0.168 ± 0.01 $^{a}$ | 0.274 ± 0.09 $^{a}$ | 0.178 ± 0.18 $^{ab}$ | 1.084 ± 0.23 |
| I1 | 0.124 ± 0.02 $^{a}$ | 0.385 ± 0.06 $^{ab}$ | 1.280 ± 1.08$^{bc}$ | 1.000 ± 0.08 $^{de}$ | 3.210 ± 1.05 |
| I2 | 0.278 ± 0.20 $^{ab}$ | 0.215 ± 0.21 $^{a}$ | 0.773 ± 0.52$^{abc}$ | 0.346 ± 0.16 $^{ab}$ | 1.729 ± 0.61 |
| I3 | 0.227 ± 0.06 $^{a}$ | 0.238 ± 0.01 $^{a}$ | 0.616 ± 0.19$^{abc}$ | 0.373 ± 0.18 $^{ab}$ | 1.475 ± 0.30 |
| Average | **0.21 ± 0.13** | **0.28 ± 0.13** | **0.89 ± 0.68** | **0.57 ± 0.34** | **2.14 ± 1.02** |
| CI | 0.177 ± 0.11 $^{a}$ | 0.296 ± 0.17 $^{a}$ | 0.254 ± 0.23 $^{a}$ | 0.355 ± 0.12 $^{ab}$ | 1.107 ± 0.06 |
| U1 | 0.325 ± 0.24 $^{ab}$ | 0.268 ± 0.00 $^{a}$ | 0.394 ± 0.37 $^{ab}$ | 0.632 ± 0.12$^{bcd}$ | 1.626 ± 0.01 |
| U2 | 0.249 ± 0.22 $^{a}$ | 0.399 ± 0.25 $^{ab}$ | 0.092 ± 0.01 $^{a}$ | 0.082 ± 0.03 $^{a}$ | 0.814 ± 0.42 |
| U3 | 0.221 ± 0.06 $^{a}$ | 0.377 ± 0.00 $^{ab}$ | 0.326 ± 0.21 $^{a}$ | 0.553 ± 0.00$^{abcd}$ | 1.507 ± 0.14 |
| Average | **0.27 ± 0.17** | **0.35 ± 0.14** | **0.27 ± 0.26** | **0.42 ± 0.26** | **1.32 ± 0.44** |
| CU | 0.274 ± 0.10 $^{ab}$ | 0.136 ± 0.03 $^{a}$ | 0.888 ± 0.31$^{abc}$ | 0.473 ± 0.15$^{abc}$ | 1.768 ± 0.09 |
| ANOVA | F(11,24)= 1.486P=0.201 | F(11,24)=2.186, $P$ = 0.053 | F(11,24)=2.093, $P$ = 0.063 | F(11,24)=5.374, $P$ < 0.000 | F(11,24)=5.881, $P$ < 0.000 |
| US EPA Std | 0.005mg/kg (ppm) | 0.400mg/kg (ppm) | 0.370mg/kg (ppm) | 10.00mg/kg (ppm) | |

Means for groups in homogeneous subsets are displayed.
Uses Harmonic Mean Sample Size = 3.000.
**Nb:** Different superscript letters (a, b, c and d) show that there is significant difference, while similar letters show that there is no significant difference.

*Statistical analysis*

Analysis of data was carried out by using SPSS (Version 24) program. Descriptive statistics were carried out. All concentrations of BTEX were found to be distributed in a normal manner. Analysis of Variance (ANOVA) was used to compare mean values of BTEX compounds and $LC_{50}$ between various sites and areas of study. Only p-values of 0.05 or lower were deemed significant. In addition, if the ANOVA test was significant, Tukey's post-hoc HSD analysis was performed to compare the variability of BTEX and $LC_{50}$ concentrations between the study sites, the study areas (urban, industrial and agricultural) and their control sites. Stepwise analysis was used to identify sites and areas of study that differed significantly from the control sites and also the $LC_{50}$ of soil microorganisms. Pearson's correlation analysis was used to measure the relationships between concentrations of BTEX and THB, HUB, % HUB, TF, HUF, and % HUF.

## Results and discussion

*Concentration assessment of benzene, toluene, ethylbenzene, o-xylene in soils from urban, agricultural and industrial areas*

The mean concentrations of BTEX in the study were 0.27±0.21, 0.33±0.21, 0.60±0.55 and 0.56±0.40 ppm for benzene, toluene, ethylbenzene and o-xylene respectively (Table 2). The means of BTEX in urban, industrial and agricultural areas were 1.32±0.44, 2.14 ±1.02 and 2.49±0.94 ppm respectively (Table 2). The mean BTEX concentrations in the industrial and agricultural sites were higher than their control sites (1.11±0.06 ppm and 1.08±0.23 ppm) respectively, while the mean concentration of BTEX of the urban study site was lower than its control site (1.77±0.09 ppm) (Table 2). The highest mean BTEX level was observed in the agriculture site with an average of 2.1364±1.03 ppm while the lowest BTEX level was observed in urban site with a mean value of 1.4287±0.43 ppm (Table 2). The concentration of Benzene in all the study sites were above the recommended US EPA standard of 0.005 ppm. The concentrations of toluene were within the US EPA limits except for the agricultural sites which had a mean concentration of 0.48±0.31 ppm against a required minimal concentration of 0.400 ppm. Concentrations of ethylbenzene were above the US EPA recommended levels of 0.370 ppm in three areas; agricultural, industrial and control urban area which had means of 0.76±0.61 ppm, 0.89±0.68 ppm and 0.89±0.31 ppm, respectively (Table 2).

*Concentration assessment of benzene, toluene, ethylbenzene, o-xylene in soils of study sites*

The varying amounts of BTEX were investigated over a period of time in study site soils. Table 2 shows how varied the means of BTEX were among the contaminated and control sites. There was no difference ($p > 0.05$) in levels of benzene between the control and the study sites (Table 2). The mean value of benzene ranged from a minimum of 0.094±0.01 ppm at A2 (Eleme) to a maximum of 0.631 ±0.46 ppm in A3 (Emuoha). There was a significant difference ($p < 0.05$) between the levels of toluene in the control and the contaminated sites (Table 2). The average toluene levels ranged between 0.136±0.03 ppm and 0.694±0.43 ppm. There was no

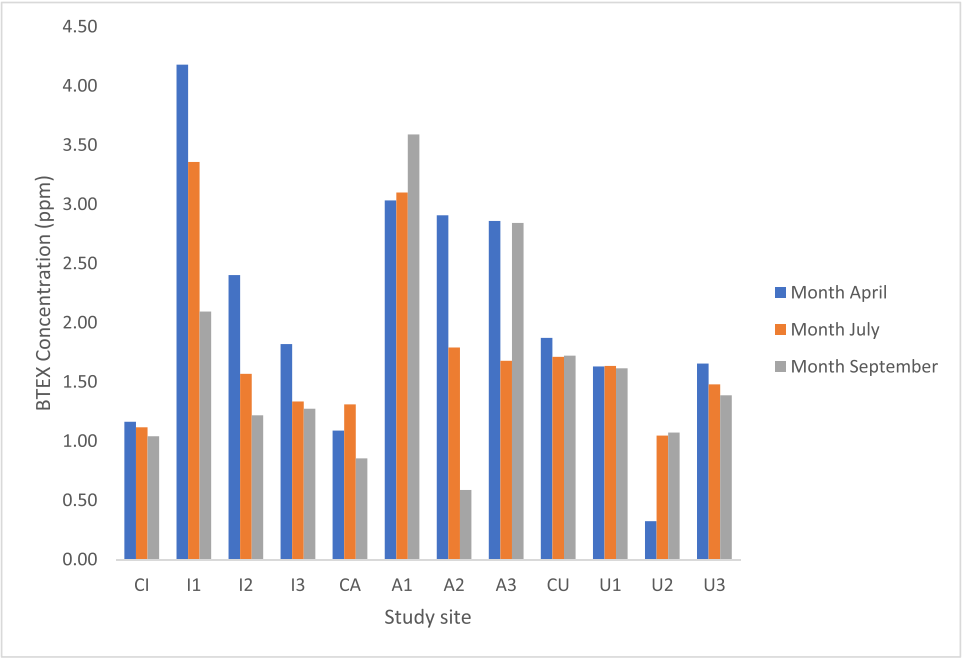


**Fig. 2.** Trend of BTEX in April, July and September (2018).

significant difference in levels of ethylbenzene between the control and the contaminated sites ($p = 0.063$) [Table 2] with mean values ranging between 0.092±0.01 ppm at U2 (Diobu-Mile 1) and 1.310±0.10 ppm in A1 (Aluu). The mean value of o-xylene varied between 0.082±0.03 ppm at CU and 1.264±0.19 ppm in A3 (Emuoha). There was a significant difference ($P < 0.000$) in the concentration of o-xylene between the contaminated and control sites (Table 2). The concentrations of benzene and toluene in A3 (Emuoha) were significantly different from other agricultural study sites. The concentration of ethylbenzene in A1 (Aluu) was significantly different from other agricultural contaminated sites. The concentration of o-xylene in A1 (Aluu) and U2 (Diobu-Mile 1) were significantly different from other agricultural and urban contaminated sites respectively as shown with different superscript letters (a, b, c and d) (Table 2).

*Seasonal variations in the average concentrations of BTEX in soil samples*

Reducing trends in the concentrations of BTEX were observed in CI (Control Industry), I1 (Onne), I2 (Agbada), I3 (Trans-Amadi), A2 (Eleme), CU (Control Urban), U1 (GRA Phase 2) and U3 (Mgbuoba) from April to September (Fig. 2). There was an increasing trend in the concentration of BTEX in A1 (Aluu) and U2 (Diobu-Mile 1) while there were fluctuating trends in CA (agricultural control site) and A3 (Emuoha). The highest concentration of BTEX was observed in I1 (Onne) followed by A1 (Aluu). The most stable concentration of BTEX in the three months of study was observed in U1 (GRA Phase 2) [Fig. 2].

*Populations of THB, HUB, % HUB, TF, HUF and % HUF in study sites*

Table 3 shows the populations of fungi and bacteria in various study and control sites. The populations include THB, HUB, % HUB, TF, HUF and % HUF. The lowest and highest THB populations were observed in CU (Control Urban) and CI (Control industrial) sites which were $5.17 \times 10^6 \pm 2.13 \times 10^6$ CFUs/g of soil and $3.92 \times 10^7 \pm 3.56 \times 10^7$ CFUs/g of soil respectively (Table 3). The HUB ranged between $1.05 \times 10^4 \pm 7.55 \times 10^3$ CFUs/g of soil in U1 (GRA Phase 2) to $3.59 \times 10^5 \pm 3.55 \times 10^5$ CFUs/g of soil in A2 (Eleme) (Table 3). Percent HUB ranged between 0.26 ± 0.15 % in I1 (Onne) to 4.10 ± 4.23 % in the CU (Control Urban) site. The lowest population of total fungi was observed in the CA (Control Agricultural) site and the highest population of TF was observed in I1 (Onne), where $4.59 \times 10^3 \pm 1.75 \times 10^3$ CFUs/g of soil and $3.29 \times 10^5 \pm 4.06 \times 10^5$ CFUs/g of soil were observed respectively (Table 3). The population of HUF was varied where it ranged between $1.25 \times 10^3 \pm 1.16 \times 10^3$ CFUs/g of in soil I2 (Agbada) and $2.30 \times 10^4 \pm 3.41 \times 10^4$ CFUs/g of soil (Table 4). Percent HUF was higher than % HUB with 39.88 ± 29.44 in CA (Control Agricultural), 33.61 ± 35.43 in U3 (Mgbuoba), 21.76 ± 17.47 in A3 (Emuoha), 16.46 ± 8.85 in U1 (GRA Phase 2) and 15.62 ± 30.93 in I1 (Onne) (Table 3).

*Pearson correlations between BTEX compounds and microorganism populations in soil*

Table 4 shows correlation between BTEX compounds and microorganism in soil. There was a significant positive correlation ($r = 0.351$) between BTEX and TF (Table 4). Further, Pearson correlation showed that o-xylene was significantly positively correlated with TF ($r = 0.331$), and % HUB was negatively correlated ($r = -0.396$) with THB (Table 4).

*Discussion*

Urbanization and industrialization have been linked to high levels of BTEX in the environment [13], but hardly have agricultural activities been linked to high levels of BTEX in the environment. Table 2 shows the mean BTEX levels in the soils in the current study areas. The concentration of BTEX in agricultural area was relatively high with a mean of 2.490 ± 0.94 ppm (Table 2), which can be attributed to the installation of pipelines and flow stations in agricultural areas. The highest concentrations of BTEX were observed in A1 (Aluu) with a mean of 3.240 ± 0.30 ppm which hosts flow stations and I1 (Onne) with a mean of 3.210 ± 1.05 ppm which hosts the NNPC Refinery (Table 2). This articulates the importance of the allocation of industries to sustain a healthy environment. Industries should be constructed away from agricultural areas. The mean concentrations of the total BTEX in the study areas were within US EPA

**Table 3**
Mean populations of THB, HUB % HUB, TF, HUF and % HUF in study sites.

| Study sites | THB | HUB | %HUB | TF | HUF | %HUF |
|---|---|---|---|---|---|---|
| A1 | $4.08 \times 10^6 \pm 3.09 \times 10^6$ | $1.53 \times 10^4 \pm 1.38 \times 10^3$ | 0.73 ± 0.58 | $8.70 \times 10^4 \pm 6.34 \times 10^4$ | $1.49 \times 10^3 \pm 1.46 \times 10^3$ | 1.34 ± 0.75 |
| A2 | $3.84 \times 10^7 \pm 3.55 \times 10^7$ | $3.59 \times 10^5 \pm 3.55 \times 10^5$ | 0.61 ± 0.31 | $1.80 \times 10^4 \pm 1.61 \times 10^4$ | $2.03 \times 10^3 \pm 2.17 \times 10^3$ | 11.09 ± 3.15 |
| A3 | $3.19 \times 10^7 \pm 2.79 \times 10^7$ | $4.65 \times 10^4 \pm 2.70 \times 10^4$ | 0.29 ± 0.27 | $6.47 \times 10^3 \pm 1.95 \times 10^3$ | $1.69 \times 10^3 \pm 1.56 \times 10^3$ | 21.76 ± 17.47 |
| CA | $3.10 \times 10^7 \pm 3.36 \times 10^7$ | $1.70 \times 10^4 \pm 3.02 \times 10^3$ | 2.17 ± 2.22 | $4.59 \times 10^3 \pm 1.75 \times 10^3$ | $2.25 \times 10^3 \pm 2.04 \times 10^3$ | 39.88 ± 29.44 |
| I1 | $1.01 \times 10^7 \pm 4.10 \times 10^6$ | $2.47 \times 10^4 \pm 1.28 \times 10^3$ | 0.26 ± 0.15 | $3.29 \times 10^5 \pm 4.06 \times 10^5$ | $3.50 \times 10^3 \pm 3.27 \times 10^3$ | 15.62 ± 30.93 |
| I2 | $6.80 \times 10^6 \pm 8.02 \times 10^5$ | $7.25 \times 10^4 \pm 5.70 \times 10^4$ | 0.75 ± 0.71 | $9.25 \times 10^3 \pm 4.38 \times 10^3$ | $1.25 \times 10^3 \pm 1.16 \times 10^3$ | 10.83 ± 7.96 |
| I3 | $1.71 \times 10^7 \pm 1.85 \times 10^7$ | $2.52 \times 10^4 \pm 1.59 \times 10^4$ | 1.28 ± 1.28 | $3.93 \times 10^4 \pm 3.91 \times 10^4$ | $3.49 \times 10^3 \pm 3.82 \times 10^3$ | 8.84 ± 2.20 |
| CI | $3.92 \times 10^7 \pm 3.56 \times 10^7$ | $3.11 \times 10^4 \pm 8.53 \times 10^3$ | 0.31 ± 0.15 | $2.87 \times 10^4 \pm 2.57 \times 10^4$ | $2.30 \times 10^4 \pm 3.41 \times 10^4$ | 7.03 ± 3.83 |
| U1 | $2.62 \times 10^7 \pm 3.60 \times 10^7$ | $1.05 \times 10^4 \pm 7.55 \times 10^3$ | 0.38 ± 0.52 | $8.98 \times 10^3 \pm 5.98 \times 10^3$ | $1.87 \times 10^3 \pm 1.82 \times 10^3$ | 16.46 ± 8.85 |
| U2 | $1.33 \times 10^7 \pm 8.04 \times 10^6$ | $3.16 \times 10^4 \pm 3.37 \times 10^3$ | 0.47 ± 0.30 | $3.58 \times 10^4 \pm 3.31 \times 10^4$ | $2.78 \times 10^3 \pm 2.66 \times 10^3$ | 7.16 ± 1.01 |
| U3 | $1.29 \times 10^7 \pm 7.63 \times 10^6$ | $4.38 \times 10^4 \pm 1.01 \times 10^4$ | 0.43 ± 0.20 | $3.32 \times 10^4 \pm 3.20 \times 10^4$ | $1.75 \times 10^4 \pm 2.42 \times 10^4$ | 33.61 ± 35.43 |
| CU | $5.17 \times 10^6 \pm 2.13 \times 10^6$ | $1.41 \times 10^5 \pm 1.32 \times 10^5$ | 4.10 ± 4.23 | $4.08 \times 10^4 \pm 3.97 \times 10^4$ | $1.61 \times 10^3 \pm 1.42 \times 10^3$ | 11.61 ± 15.20 |

**Table 4**
The influence of BTEX compounds on microorganism populations in soil.

| | | THB | HUB | % HUB | TF | HUF | % HUF |
|---|---|---|---|---|---|---|---|
| Benzene | Correlation | −0.065 | −0.227 | 0.011 | −0.199 | −0.05 | 0.206 |
| | Sig. (2-tailed) | 0.705 | 0.183 | 0.95 | 0.246 | 0.771 | 0.229 |
| Toluene | Correlation | 0.03 | −0.181 | −0.258 | 0.054 | 0.106 | −0.007 |
| | Sig. (2-tailed) | 0.861 | 0.292 | 0.129 | 0.755 | 0.539 | 0.969 |
| Ethylbenzene | Correlation | −0.327 | −0.009 | 0.198 | 0.149 | −0.29 | −0.317 |
| | Sig. (2-tailed) | 0.052 | 0.96 | 0.248 | 0.385 | 0.087 | 0.059 |
| o-xylene | Correlation | −0.231 | −0.074 | −0.004 | .331* | −0.143 | −0.058 |
| | Sig. (2-tailed) | 0.175 | 0.666 | 0.983 | 0.048 | 0.406 | 0.736 |
| Total BTEX | Correlation | −0.296 | −0.033 | 0.028 | .351* | −0.235 | −0.197 |
| | Sig. (2-tailed) | 0.08 | 0.847 | 0.872 | 0.036 | 0.168 | 0.249 |
| THB | Correlation | 1 | 0.253 | −0.396* | −0.285 | 0.213 | 0.13 |
| | Sig. (2-tailed) | | 0.137 | 0.017 | 0.092 | 0.213 | 0.45 |
| HUB | Correlation | 0.253 | 1 | 0.265 | −0.144 | −0.147 | −0.173 |
| | Sig. (2-tailed) | 0.137 | | 0.118 | 0.401 | 0.394 | 0.314 |
| % HUB | Correlation | −0.396* | 0.265 | 1 | −0.054 | −0.161 | −0.162 |
| | Sig. (2-tailed) | 0.017 | 0.118 | | 0.754 | 0.349 | 0.344 |
| TF | Correlation | −0.285 | −0.144 | −0.054 | 1 | −0.039 | 0.085 |
| | Sig. (2-tailed) | 0.092 | 0.401 | 0.754 | | 0.82 | 0.622 |
| HUF | Correlation | 0.213 | −0.147 | −0.161 | −0.039 | 1 | 0.192 |
| | Sig. (2-tailed) | 0.213 | 0.394 | 0.349 | 0.82 | | 0.261 |
| % HUF | Correlation | 0.13 | −0.173 | −0.162 | 0.085 | 0.192 | 1 |
| | Sig. (2-tailed) | 0.45 | 0.314 | 0.344 | 0.622 | 0.261 | |
| | N | 36 | 36 | 36 | 36 | 36 | 36 |

* Correlation is significant at the 0.05 level (2-tailed).

recommended limits (50 ppb). However, the assessment of the components of BTEX revealed that concentrations of benzene in all the study locations were above the US EPA recommended standard of 0.005 ppm (5 μg/Kg) with a mean of 0.270 ± 0.21 ppm. The concentrations of toluene in all the study sites were within US EPA limits of 0.370 mg/kg (ppm) except for A1 (Aluu) with a mean concentration of 0.490 ± 0.16 ppm, A3 (Emuoha) with a mean concentration of 0.694 ± 0.43 and U2 (Diobu-Mile 1) with a mean concentration of 0.400 ± 0.25 ppm. These study sites are hosts to petroleum refinery and petroleum flow stations. High BTEX concentrations have been observed closer to urban and petro-industrial sites [6], and are consistent with the results from this study. Benzene, toluene, ethylbenzene and xylene (BTEX) are found to be elevated in urban areas around the world [51] for instance areas of Shiraz (Iran) [21], in indoor microenvironments of Sakaka [Saudi Arabia] [24] and in semi-urban areas of Orleans, [France] [38]. These findings and the current findings all support that BTEX occurrence in the environment is influenced by anthropogenic activities in that particular area. Other sources of BTEX in the environment include; emissions [fossil fuels, incomplete combustion, industrial production, evaporation of solvents] [12,52]. Leakage from underground storage tanks, distribution facilities and industrial operations are also a major source of soil contamination with BTEX [36,73]. Benzene, toluene, ethylbenzene and xylene (BTEX) are classified as a priority because of their high mobility and toxicity [49]. The spatial distribution of toluene and o-xylene in this study are similar to findings by [39] who describe their distribution in soil as being less extensive as compared to benzene. Benzene is more volatile as compared to toluene and therefore the concentrations of the former are more likely to be affected by fluctuation in temperatures as compared to the latter [55,62]. This study was carried out in the wet season which is characterized by low temperatures and high soil moisture content. This could also be a cause of the observation of higher values of benzene as compared to toluene [26]. In the wet season, there is reduced evaporation of both benzene and toluene from the benzene-toluene system. The findings of this study are also similar to those of Baghani et al., [11] who observed that the concentrations of BTEX species in the atmosphere increase as a function of temperature, indicating that evaporation is a key source of BTEX species in ambient air and also loss of BTEX from soils [11,69]. Furthermore, the solubility of benzene in water is 1.79 g/L at 15 °C while that of toluene is 0.52 g/L at 20 °C. This can be a contributing factor to the higher distribution of benzene in the study areas as compared to toluene, where rain water was the distributing agent. Concentrations of o-xylene in all the study locations were within the US EPA recommended concentrations for clean-up [10.00mg/kg (ppm)]. Variation in the concentration of BTEX in the study areas can also be related to adsorption capacities [5]. For example, it has been observed in studies that different types of clay, lignite and polystyrene resins with varied organic cations have different adsorptive capacities of BTEX [1,43,44,72]. The presence of BTEX could be an indicator that the study soils were recently contaminated with petroleum hydrocarbons as BTEX is highly volatile and favourable temperatures facilitate quick volatilization.

Pearson correlation showed a significant positive correlation between BTEX and TF ($r = 0.351$). A decrease in the concentration of BTEX in soil can be associated with assimilation by microorganisms [25], evaporation, volatilization [26] and adsorption [5]. The correlation between BTEX concentration and soil biota is not exclusively researched, and there are many BTEX utilizing microorganisms yet to be discovered [23]. The finding of this study is comparable to other studies which show that fungi and bacteria have been associated with the biodegradation of BTEX [19,56], an example is *Cladophialophora* spp [61]. Fungi have the ability to utilize volatile aromatic hydrocarbons as source of carbon [48]. Other fungi that have demonstrated utilization of petroleum hydrocarbons as sources of energy include *Cladosporium sphaerospermum* and *P. variotii* [80]. Atakpa et al., [8] applied *Scedosporium* spp. ZYY and *Acinetobacter* spp. Y2 simultaneously for removal of total petroleum hydrocarbons (TPHs). Study of enzymes produced by *P. variotii* suggests the presence of enzymes can assimilate mono-aromatic compounds [80]. Two genes from the CYP52 family-*alkB* and

cytochrome P450 alkane hydroxylase are involved in different terminal TPHs oxidation processes in bacteria and fungus [88]. The primary components of crude oil, unbranched C5-C16 chain lengths, are broken down more easily by these enzymes. Bacteria can degrade toluene [15,59,63]. In the current study locations, levels of toluene were varied and can also be linked to utilization by microorganisms [25] as well as volatilization, leaching and adsorption among others. Another organism that has demonstrated degradation of BTEX and toluene is *Pseudomonus* spp. [35,54,58,84]. *Pseudomonus* spp. has been found to have a nonconjugative O-xylene-Degradative Plasmid (XYL) which is responsible for digestion of o-xylene [29]. *Aeromonas* spp. can degrade toluene and o-xylene [53], also, *Pseudomonas* spp. and *Rhodococcus* spp. have been shown for degrading benzene, toluene and styrene in sludge and sewage [70]. *Streptococcus* spp. has been found to degrade toluene and o-xylene [86]. Microorganisms are characterized with special genes. For example, hydrocarbon-degrading gene *bamA* amplification and metagenomic sequencing analyses revealed that f-Syntrophobacteraceae MAG116 may act as a toluene degrader in the non-electro-stimulated microbiota [90]. Therefore, the presence of biodegrading microorganisms in soil is a factor contributing to reducing concentrations of BTEX in soils and the population of microorganisms in soil.

## Conclusion

The levels of BTEX in soils in relation to microorganisms of selected sites in Port Harcourt City are reported for the first time in this study. These results provide a basis for monitoring the concentration and profile of BTEX compounds in soils of selected parts; urban, industrial and agricultural areas in Port Harcourt City over the wet season in April, July and September. The study determined the effect of the presence of BTEX in soils on populations of soil fungi and bacteria. The findings from this study are as follows:

1) The assessment of BTEX indicated that the mean concentrations of benzene was above the US EPA recommendation limits of 0.005 ppm (5 μg/Kg).
2) Distribution of BTEX indicated that Industrial and Agricultural areas had the highest concentrations of BTEX and which were reduced in urbanized areas.
3) The seasonal variation in concentration indicated highest concentrations in April and a reducing trend through to September.
4) Correlation analysis between BTEX and fungal populations indicate the influence of concentration of BTEX by microbial populations in soils of the study areas.

The results of this work have implications for environmental and human health. It demonstrates that soil microorganism community structures are disrupted by presence of deleterious hydrocarbons (BTEX). These can lead to the exposure of expansive human populations to carcinogenic emissions from highly volatile BTEX. This study recommends monitoring of the levels of BTEX in agricultural areas, human habited areas as well as industrialized areas. Also, the study recommends the identification of the sources of BTEX in all the study areas and a risk assessment is to be conducted in order to determine human health implications. The results emphasize the importance of soil fungi and bacteria on nutrient cycling and the processes of bioremediation.

## CRediT authorship contribution statement

**Paul Muyoma Wanjala:** Conceptualization, Methodology, Investigation, Resources, Data curation, Writing – original draft, Writing – review & editing, Visualization, Project administration, Funding acquisition. **Boadu Kwasi Opoku:** Conceptualization, Methodology, Investigation, Resources, Data curation, Funding acquisition, Supervision, Funding acquisition. **Etela Ibisime:** Methodology, Software, Validation, Formal analysis, Resources, Data curation, Writing – review & editing, Funding acquisition. **Eliud N Wafula:** Methodology, Software, Validation, Resources, Data curation, Writing – review & editing, Funding acquisition.

## Declaration of competing interest

Authors declare that no competing interests

## Acknowledgements

This work was carried out within the PhD Program of World Bank African Centre of Excellence for Oilfield Chemicals Research, in line with the World Bank's mandate for establishing the African Centre of Excellence in University of Port Harcourt in Nigeria. The authors further acknowledge the Regional Universities Forum for Capacity Building in Agriculture (RUFORUM) through Doctor Odogwu A. Blessing and Professor Ikechukwu O. Agbagwa of University of Port Harcourt for their continual mentorship and financial support under the Carnegie Post-Doctoral funding.

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