# Polycyclic Aromatic Hydrocarbon Levels In Wistar Rats Exposed To Ambient Air Of Port Harcourt, Nigeria: An Indicator For Tissue Toxicity

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**Research**

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# Abstract

**Background:** Over the years, the ambient air quality of Port Harcourt metropolis has deteriorated largely because of the petrochemical and hydrocarbon activities. This study, therefore, investigated the PAH levels in wistar rats exposed to ambient air of Port Harcourt metropolis.

**Method:** Using an animal model, this study evaluated the blood PAH concentrations as an indicator of toxicity in living tissues exposed to ambient air polluted with particulate matter in Port Harcourt metropolis. Twenty (20) Wistar rats imported from a non-polluted city (Enugu) were exposed to both indoor and outdoor air for 90 days. Following the IACUC regulation, baseline data were obtained from 4 randomly selected animals, while the remaining 16 rats (8 each for indoor and outdoor) were left till day 90. Blood samples from the animals were obtained by cardiac puncture, and the PAHs concentrations were determined using Gas Chromatography Flame-Ionization Detector (GC-FID). GraphPad Prism (version 8.0.2) Sidak's (for multiple data set) and unpaired t-tests (for two data sets) were used to evaluate the differences in group means at 95% confidence level.

**Result:** Seven (7) of the PAHs found in indoor and outdoor rats were absent in blood tissues of baseline rats. The total mean concentrations of PAH were (82%), (13%), and (5%) in outdoor, indoor, and baseline animal groups respectively. Additionally, Dibenz(a,h)anthracene, Indeno(1,2,3-c,d)pyrene, Pyrene, 2-methyl, and other carcinogenic PAHs were all significantly higher ($P<0.05$) in outdoor samples when compared to the indoor and baseline samples.

**Conclusion:** The results demonstrated that the ambient air of Port Harcourt was highly concentrated with PAHs. Vulnerable groups such as outdoor workers, pregnant women, children and infants, and terminally ill are the most susceptible to the health risks posed by these pollutants. Therefore, urgent environmental and public health measures are necessary to mitigate the looming danger.


# Background

Globally, ambient air pollution is a threat to health and the climate, as it is responsible for the majority of recorded mortality and morbidity [1, 2]. The urban airborne particulate matter remains an important air quality indicator and a major pollutant of public health concern accountable for tissue toxicity and high disease burden [1–3]. According to WHO's IARC, soot, petroleum refining, and other industrial exposures and the content's pollutants are classified as mixtures with health effects of carcinogenic (Group 1) and probable carcinogenicity (Group 2A) [1, 4, 5] respectively. Airborne particulate matter pollution results from incomplete combustion of organic substances, especially petroleum derivatives compounds [5–7]. Particulate matter contains both organic and inorganic compounds including carcinogenic substances like polycyclic aromatic hydrocarbons (PAHs) and heavy metals [6, 7]. PAHs are the most common subclass of polycyclic organic matter and contain only carbon and hydrogen [8]. They are made up of two or more fused aromatic rings. PAHs with lower molecular weights and having two to three rings are predominantly found in the atmosphere in vapor form, while those with higher molecular weights, four

rings, and above are particle-bonded and are said to be more toxic to human health [9–11]. These particulate-bonded PAHs environmental pollutants have been found to have low solubility in water and penetrate tissue membranes through hydrophobic attraction and afterward its bio-concentrate in tissues [9, 12].

In the body cells, these pollutants accumulate and undergo toxicokinetics and metabolism that result in rapid disadvantageous cellular changes; contributing to acute and chronic morbidities and mortality in the general population, especially the vulnerable individuals relating to age, sex, pregnancy, and routine occupation [13–16]. The ability of PAHs traveling over long distances, even to very remotes areas has been reported [4]. Most PAHs are potent carcinogens and are typically attached to particles in the air. Exposure to carcinogenic PAHs occurs primarily in the air via inhalation of particles and no guideline limit can appropriately be estimated as exposure levels remain relevant to public health risk [16]. Assessment of bioaccumulation of toxic and hazardous compounds in tissues is vital in predicting the health effects and risks of environmental compounds on human health, especially PAHs known for high carcinogenic implications [17]. The WHO IARC and several investigations have implicated polycyclic aromatic hydrocarbons as the major causative factor of cancers, cardiovascular, renal, and respiratory dysfunctions [1, 18–20].

Unlike places such as Abuja where relative clean air have been noted [21, 22], the ambient air quality of the crude oil-rich city of Port Harcourt has been reported to be polluted with particulate matter of petroleum origin, especially $PM_{10}$ (averaged 494 μg/m3) and $PM_{2.5}$ (averaged 155 μg/m3), and its constituent compounds including PAHs [23–26]. Using secondary data from hospital records, a significant increase in trends of respiratory morbidity, and mortality in Port Harcourt over the years, have been documented [20, 23]. While Particulate matter containing PAHs results from incomplete combustion of hydrocarbons, inhabitants are exposed to PAHs through air inhalation and re-suspended soil and dust, consumption of food and water, and dermal contact with soil and dust [10, 17, 27]. Air inhalation occurs in both indoor and outdoor exposures, while food and water consumption relate mostly to indoor exposures, contact with the skin generally occurs in outdoors. Except for certain occupations and social defects, people spent 80–93% of their time indoors, and hence inhalation route would result mostly from indoor air [17, 28]. In America and most developed States, food ingestion is likely the largest route of PAHs exposure as against inhalation. While drinking-water and soil are generally minor sources of PAHs [17, 27], in developing nations, biomass and fossil fuel are generally used for both industrial and domestic combustions and ambient environmental tobacco smoke (ETS), remains dominant, with no flue and microenvironments fitted with non-airtight; the contribution of inhalation to PAHS exposure could be very high. Hence, inhalation would be the main contributor to the total daily intake of PAHs [17, 23, 25]. The kinetics, metabolism, and toxicity of PAHs from different routes of exposure have been well demonstrated in published documents [10, 29]. Indoor and outdoor Inhalation remains of critical discussion, especially in airborne particulate matter polluted ambient, such as the atmosphere of Port Harcourt [23–26].

The toxicokinetic of the individual PAHs differ widely due to differences in physicochemical properties and molecular weight. Upon inhalation into the respiratory pathway, the fate of the PAH is determined by the structure of the PAH, the dimensions, and the chemical nature of the particulate. The PAHs may dissolve from particles and rapidly absorbed from the solutions following biphasic absorption kinetics in the lungs, while the remainder in particles is likely removed through bronchial mucociliary clearance (swallowed), or the PAHs in particles most probably persist in the lungs at long durations [17, 30]. The absorption kinetics is said to be dependent on the site of the respiratory tract that the PAHs deposits. It is more rapidly desorbed and absorbed into circulation through type I epithelial cells in the alveolar region [17, 30, 31] and systemically it is rapidly metabolized [31] as against PAHs deposits through the tracheobronchial region where it slowly absorbs into circulation and intensely metabolized locally [17]. Dose-dependent absorption kinetics has also been demonstrated in lungs of perfused rats at low exposure concentrations, absorption of PAHs in the mucosa follows the first-order kinetics with substantial local metabolism, while at high exposure doses, the capacity of epithelium to dissolve and metabolize PAHs becomes saturated and the absorption rate turns zero-order kinetics [17, 30–33]. In the indoor environment, human exposure likely to follow the low-dose, while first-order kinetics likely to be observed in ambient air inhalation where concentrations are largely higher [30–32]. PAHs are rapidly distributed in the body, as lipophilic compounds which penetrate biological membranes easily [17, 30]. Hence, the detectable concentration of PAHs could be noted in most tissues between a few minutes and hours after exposure, irrespective of the exposure route [17, 30, 34]. PAHs do not accumulate in the body but are found more in adipose tissues [17] however in the lungs, PAHs are not well correlated [35]. PAHs are generally demonstrable in most human tissues and risk of toxicity and susceptibility differs across population demographics and tissue sites [17, 36]. Respiratory tract metabolism is significant to the toxicity of inhaled particles containing PAHs. Macrophages actively metabolize and engulfs particulates containing PAHs in the lungs, transports it to the bronchi and reactive carcinogenic metabolites which bounds covalently to proteins, and nucleic acids are released, resulting to toxicity and carcinogenicity [10, 37–39]. Three principal pathways have been known to activate PAHs for toxic intermediates and further metabolism which includes; (dihydro)diol-epoxide formation, radical cation formation, and the o-quinone pathway. Also, cascades of enzymes interplays in PAHs metabolism, especially CYPs (cytochrome P450s) and epoxide hydrolase. Studies have also found PAHs to cross the placental barrier easily [30, 40, and 41].

Exposure to hazardous compounds such as heavy metals and PAHs from urban airborne particulate matter (PM) pollution inducing tissue toxicity through oxidative stress and mutilation of vascular endothelial cells have been identified as the likely causes of the rise in morbidity and mortality of inhabitants in hydrocarbon polluted environment [42–44]. Depending on the dose and duration of exposure, in vivo and in vitro animal model experiments using rodents and mammals (including cultured human and placenta) tissues, found that both isolated and or combined PAHs induces and promote skin irritation, and inflammation, carcinogenicity, immune suppression, genotoxicity and mutagenicity and teratogenicity leading to embryotoxic effects [43–47]. The compounds prompt disease through oxidative

stress and change in the genome; thus, altering DNA methylation and expression of the specific gene [5, 48].

Unfortunately, there have been limited in vitro and or in vivo studies [30] using animal tissues to assess the potential health effects of indoor and outdoor particulate matter pollution in Port Harcourt metropolis. To further make this complicated, findings from studies using secondary hospital data [20, 23], air quality index measurements and satellite determination of particulate load [24–26] are contradictory as there might be confounding factors other than particulate matter pollution and its constituents responsible for the observed change in trends of morbidity. Conversely, etiology from hospital records is neither reliable for lack of data quality and accuracy [49–52] and is not specifically linked to environmental pollutants including particulate matter and PAHs. Though they could show changes for scientific speculations, but cannot give explanations [30]. However, some studies used the Lichen plant as a bioindicator for air pollution in Port Harcourt [53], while other studies used electrical turbines powered by petroleum fuel to generate controlled fumes for exposed Wistar rat's inhalation. Others also use intratracheal instillation [30, 54]. These studies incorporate designs and method that weakens possible casualty or indicator for toxicity. The lipophilic and hydrophobic toxicokinetic and metabolism in animal tissues [10, 17, 29] and plant metabolism [53] differ widely. Also, humans are not physiological designed for controlled and episodic fumes inhalation, which is likely to can kill human cells within the controlled inhalation time, through carboxyhemoglobin pathogenesis resulting from carbon monoxide poisoning [55, 56] as against normal ambient air [33]. Consequently, simulated findings from such study methods most probably do not correlate relatively on animal health or indicator for toxicity on human tissues; as against studies that expose experimental animals (from hydrocarbon and petrochemical pollution-free environment) to normal atmospheric ambient air accustomed to human respiration [33, 54]. To this end, using an animal model [57], this study evaluated the concentrations of PAHs in blood tissues of Wistar rats imported from a non-petroleum industrial environment (see Fig. 1 below) and those exposed to normal ambient air polluted with particulate matter in Port Harcourt, as an indicator of toxicity to human health and population susceptibility.

# Materials And Methods

### Study design

Animal Experimental model study design was used for the study as shown in Fig. 1 below.

**Animal Housing and Care**: Twenty (20) Wistar rats (all ≤ 1-month-old, weighing between 25-30grams) were imported from a non-air polluted (free of petroleum-based activity) location, the University of Nigeria, Nsukka (Geo-Coordinate: 6.8429N, 7.3722E; Altitude: Temp: ~37.6˚C; Relative humidity: 45.9; $PM_{2.5}$; 1–12 μg/$m^3$ and $PM_{10}$; 4–25 μg/$m^3$) to Anatomy animal house, College of Medicine, Rivers State University, Port Harcourt (Geo-Coordinate: Temp ~ 28 ˚C; Relative humidity; ~ 90; $PM_{2.5}$: 32–158 μg/$m^3$ and $PM_{10}$: 33–467 μg/$m^3$). The rats were housed in two separate wooden cages with the body made of thin barb-wire (for adequate ventilation) under a controlled environment (room of 12 m height, with double

windows and day/night cycle). Food and water were provided and were PAHs-free (through food and water product content description). The rats were made to acclimatize to the animal house for 2 weeks before the commencement of the experiment. Handheld (China Way CW-HAT200) SPM optical meters were used to monitor the daily (at mornings and evenings) particulate matter ($PM_{2.5}$ and $PM_{10}$), relative humidity, and ambient temperature.

### Animal Exposure/Treatment

Following the IACUC regulation [58], four (4) randomly selected animals were euthanized following administration of the AGTE on arrival (baseline) and the remaining 16 rats (8 each for indoor and outdoor) were randomly allocated into wooden cages and left for Ninety (90) days. While the indoor animals remained inside the room all through the experiment, the outdoor animals were brought out to the open ambient for 8 hours (8 am to 4 pm) every day and then sent indoors.

### Sample Collection

At the end of the 90 days-experiment, three rats (each at a time) from the respective experimental groups were euthanized using diethyl ether in a desiccator. After 2 minutes, the rats (each at a time) were dissected and 4 ml of blood collected through cardiac puncture.

### Analyte extraction and sample preparation

The PAHs were extracted using a liquid-liquid extraction process. This was done by adding 20 ml of Dichloromethane (DCM) to 4 ml of the blood sample. The extractant was added in the vials, capped and vortexed in the centrifuged set at 300 r.p.m for 20seconds. The organic layer was separated using a pipette attached with a pipette filter into a thermally treated and cleaned amber glass bottle. Silica gel fractionation was done to clean-up the samples for the analyte to be eluted. The eluted samples were then concentrated by nitrogen blow-down and transferred into GC vial for analysis.

### GC-FID analysis

The reconstituted, cleanly extracted samples were analyzed at Analytical Concept LTD Poultry Rd. By 2nd Railway, Odani Green City, Elelenwo Port Harcourt, Rivers State, Nigeria. Total PAHs and the individual PAHs concentrations were determined using gas chromatography (GC) equipped with Flame-Ionization Detector (FID). The peaks were identified using the GC retention time for each PAH in the analyte sample. Calibration curves were prepared for each PAHs investigated using GC output signal responses (shown by the peak area in the screen) at a specific retention time against the concentration of the analyte injected into the GC through an injection pot The blood sample extracted analyte were analyzed using an HP 5890 series II Gas Chromatography Flame Ionization Detector equipped with an HP-1 (Methyl Silicone Gum) Capillary column (Agilent, 30 m x 0.32 mm x 0.00025 mm film thickness) operating with Helium as carrier gas at 2 µL per mL. The injection volume was 2 µL, using a splitless inlet mode. The GC temperature program was set according to an established method [36] with slight modification. The Oven was

configured to 60ºC and held for 1 minute, ramped to 320 ºC at the rate of 9 ºC $min^{-1}$ and held for 5 minutes. The injector and the FID were held at 275 ºC and 325 ºC respectively. The solvent's (in the solution used in extraction and storage) peak were first noted in the GC-FID screen. Afterward, spikes of the lightest compounds in the PAHs to the heaviest and their respective concentrations were detected and total PAHs recorded. The retention time (from injection to detection) of the GC-FID was 40 minutes.

### Statistical Analysis

The animal experimental data were analyzed using GraphPad Prism (version 8.0.2) software manufactured by GraphPad Software Inc (San Diego, USA). The data were summarized as mean (± S.D) and inferential statistics were used to test the level of significance (at 95%, confidence level) difference in the mean parameters. Sidak's multiple comparison test was used to evaluate the differences in the three groups, while Student's t-test was used when only two groups were available.

# Quality assurance and quality control:

First, the Wistar rats were imported from a non-petroleum industrial environment (Nsukka, Enugu State) of about 233KM away from Port Harcourt (see Fig. 1), to minimize the presence of PAHs and particulate matter other than petrogenic and petrochemical sources.

During sample preparation and preservation, possible coagulation of the collected blood samples was prevented by the use of a heparinized tube for onward storage, preservation, and centrifugation. PAHs are known to be sensitive to UV light, and to prevent its damage, the plasma was instantly transferred to an amber bottle. Before using the GC-FID for analysis, the instrument was left on for one hour, to heat the system and limit the availability of unwanted compounds inside the instrument. To further clean and clearing the GC's column, blank organic solvent (DCM) was injected twice into the column before the actual analyte sample and twice after the analysis. These were done to prevent possible interference on the investigated samples by unwanted compounds from carry-over from the previous analysis. The detection was 0.00 mg per gram.

Water (Eva bottled water, with NAFDAC No. 01-0492) and palette animal feeds (TopFeed animal feeds brand, NAFDAC No. A9-0317) free of PAHs were administered to the animals to ensure experimental parameters been examined were not re-ingested outside the route (air) being investigated.

### Limitations

Quantitative cancer risk estimations and forensic investigation for Alkylhomologue abundance involving methylation standard and quantification were beyond the scope of this study.

# Results

The daily indoor and outdoor particulate matter levels were recorded within the ranges of 32 μg/$m^3$ to 467 μg/$m^3$ ($PM_{2.5}$: 32–158 μg/$m^3$ and $PM_{10}$: 33–467 μg/$m^3$). There were observable changes and differences in skin color and weight in the rats from the indoor and outdoor. The outdoor rat's skins were pale and weighed less (43–74 g) compared to the indoor rats that weighed between 55–82 g with brighter white skin. Seven (outdoor) rats, mostly in the second month of exposure and died before the end of the study, and the deaths mostly occurred at nights.

The mean concentrations of individual PAHs in the respective animal groups are presented in Tables 1. When they were undetected in the animal group, it was stated as N/F. The graphical presentation of the test of mean differences in the PAHs concentrations in the animal groups is presented in Figs. 5–11.

In baseline animals, some PAHs were undetected. The mean concentrations of Benz(a)anthracene, Benzo(b)fluoranthene, Benzo(k)fluoranthene, Fluorene, Chrysene and Phenanthrene were not detected in the baseline animal groups. Benzo(a)pyrene B[a]P, a major PAHs carcinogenic maker was detected with mean concentrations of $1.58 \times 10^{-4} \pm 1.55 \times 10^{-5}$ .The mean concentrations of 2-methyl Naphthalene, Acenaphthene, Acenaphthylene, Anthracene were observed to be $8.78 \times 10^{-4} \pm 2.00 \times 10^{-5}$, $3.85 \times 10^{-5} \pm 1.56 \times 10^{-6}$,$1.25 \times 10^{-4} \pm 7.02 \times 10^{-6}$, $2.82 \times 10^{-4} \pm 3.70 \times 10^{-5}$ respectively. While those of Dibenz(a,h)anthracene, Indeno(1,2,3-c,d)pyrene and Pyrene were noted as $6.12 \times 10^{-4} \pm 1.30 \times 10^{-5}$, $2.22 \times 10^{-4} \pm 1.86 \times 10^{-5}$ and $3.94 \times 10^{-4} \pm 1.80 \times 10^{-5}$ respectively.

In the indoor animals, the mean concentrations of 2-methyl Naphthalene, Acenaphthylene and B[a]P were not detected. While the mean concentrations of Acenaphthene, Anthracene, Benz(a)anthracene, Benzo(b)fluoranthene, Benzo(b)fluoranthene, Chrysene were $6.99 \times 10^{-5} \pm 4.16 \times 10^{-6}$, $4.62 \times 10^{-4} \pm 4.00 \times 10^{-5}$, $4.57 \times 10^{-5} \pm 2.47 \times 10^{-6}$, $6.84 \times 10^{-4} \pm 4.97 \times 10^{-4}$, $1.89 \times 10^{-3} \pm 1.51 \times 10^{-4}$, and $4.04 \times 10^{-3} \pm 2.26 \times 10^{-3}$ respectively and those of Dibenz(a,h)anthracene, Fluoranthene, Fluorene, Indeno(1,2,3-c,d)pyrene, Phenanthrene and Pyrene were found to be respectively $1.90 \times 10^{-5} \pm 1.05 \times 10^{-4}$, $6.83 \times 10^{-5} \pm 2.35 \times 10^{-6}$, $1.69 \times 10^{-4} \pm 1.47 \times 10^{-5}$, $3.08 \times 10^{-3} \pm 1.06 \times 10^{-4}$, $3.16 \times 10^{-5} \pm 2.37 \times 10^{-6}$, and $7.06 \times 10^{-4} \pm 1.19 \times 10^{-5}$.

In the outdoor animals, B[a]P and Fluoranthene were not detected in the outdoor animal group. The mean concentrations of 2-methyl Naphthalene, Acenaphthene, Acenaphthylene, Anthracene, Benz(a)anthracene, Benzo(b)fluoranthene, Benzo(k)fluoranthene were noted to be $9.71 \times 10^{-4} \pm 5.16 \times 10^{-5}$, $8.18 \times 10^{-4} \pm 2.10 \times 10^{-5}$, $2.30 \times 10^{-4} \pm 2.06 \times 10^{-5}$, $1.28 \times 10^{-3} \pm 1.29 \times 10^{-4}$, $8.15 \times 10^{-5} \pm 2.66 \times 10^{-6}$, $2.93 \times 10^{-3} \pm 2.37 \times 10^{-4}$, and $2.89 \times 10^{-3} \pm 1.17 \times 10^{-4}$ respectively. While those of Chrysene, Dibenz(a,h)anthracene, Fluorene, Indeno(1,2,3-c,d)pyrene, Phenanthrene, Pyrene had mean concentration of $8.66 \times 10^{-3} \pm 4.50 \times 10^{-4}$, $2.08 \times 10^{-2} \pm 1.86 \times 10^{-3}$, $1.87 \times 10^{-4} \pm 2.93 \times 10^{-5}$, $1.42 \times 10^{-2} \pm 7.00 \times 10^{-4}$, $4.74 \times 10^{-5} \pm 4.29 \times 10^{-6}$, and respectively $5.22 \times 10^{-3} \pm 1.88 \times 10^{-4}$.

Table 1
The mean(± S.D) concentrations of PAHs in Baseline, Indoor and outdoor experimental animal groups

| | Experimental Animal Groups | | |
|---|---|---|---|
| | **Baseline** | **Indoor** | **Outdoor** |
| **2-methyl Naphthalene** | $8.78 \times 10^{-4} \pm 2.00 \times 10^{-5}$ | N/F | $9.71 \times 10^{-4} \pm 5.16 \times 10^{-5}$ |
| **Acenaphthene** | $3.85 \times 10^{-5} \pm 1.56 \times 10^{-6}$ | $6.99 \times 10^{-5} \pm 4.16 \times 10^{-6}$ | $8.18 \times 10^{-4} \pm 2.10 \times 10^{-5}$ |
| **Acenaphthylene** | $1.25 \times 10^{-4} \pm 7.02 \times 10^{-6}$ | N/F | $2.30 \times 10^{-4} \pm 2.06 \times 10^{-5}$ |
| **Anthracene** | $2.82 \times 10^{-4} \pm 3.70 \times 10^{-5}$ | $4.62 \times 10^{-4} \pm 4.00 \times 10^{-5}$ | $1.28 \times 10^{-3} \pm 1.29 \times 10^{-4}$ |
| **Benz(a)anthracene** | N/F | $4.57 \times 10^{-5} \pm 2.47 \times 10^{-6}$ | $8.15 \times 10^{-5} \pm 2.66 \times 10^{-6}$ |
| **Benzo(a)pyrene** | $1.58 \times 10^{-4} \pm 1.55 \times 10^{-5}$ | N/F | N/F |
| **Benzo(b)fluoranthene** | N/F | $6.84 \times 10^{-4} \pm 4.97 \times 10^{-4}$ | $2.93 \times 10^{-3} \pm 2.37 \times 10^{-4}$ |
| **Benzo(k)fluoranthene** | N/F | $1.89 \times 10^{-3} \pm 1.51 \times 10^{-4}$ | $2.89 \times 10^{-3} \pm 1.17 \times 10^{-4}$ |
| **Chrysene** | N/F | $4.04 \times 10^{-3} \pm 2.26 \times 10^{-3}$ | $8.66 \times 10^{-3} \pm 4.50 \times 10^{-4}$ |
| **Dibenz(a,h)anthracene** | $6.12 \times 10^{-4} \pm 1.30 \times 10^{-5}$ | $1.90 \times 10^{-5} \pm 1.05 \times 10^{-4}$ | $2.08 \times 10^{-2} \pm 1.86 \times 10^{-3}$ |
| **Fluoranthene** | N/F | $6.83 \times 10^{-5} \pm 2.35 \times 10^{-6}$ | N/F |
| **Fluorene** | N/F | $1.69 \times 10^{-4} \pm 1.47 \times 10^{-5}$ | $1.87 \times 10^{-4} \pm 2.93 \times 10^{-5}$ |

Note: *N/F connotes not found*

From Fig. 3, the total mean concentration of PAHs in the outdoor animal group (82%) was far higher than those of indoor (13%) and baseline (5%), while at the individual PAHs levels, Dibenz(a,h)anthracene and Indeno(1,2,3-c,d)pyrene had the highest mean concentrations noted in the outdoor animal group (Fig. 4).

| | Experimental Animal Groups | | |
|---|---|---|---|
| | **Baseline** | **Indoor** | **Outdoor** |
| **Indeno(1,2,3-c,d)pyrene** | $2.22 \times 10^{-4} \pm 1.86 \times 10^{-5}$ | $3.08 \times 10^{-3} \pm 1.06 \times 10^{-4}$ | $1.42 \times 10^{-2} \pm 7.00 \times 10^{-4}$ |
| **Phenanthrene** | N/F | $3.16 \times 10^{-5} \pm 2.37 \times 10^{-6}$ | $4.74 \times 10^{-5} \pm 4.29 \times 10^{-6}$ |
| **Pyrene** | $3.94 \times 10^{-4} \pm 1.80 \times 10^{-5}$ | $7.06 \times 10^{-4} \pm 1.19 \times 10^{-5}$ | $5.22 \times 10^{-3} \pm 1.88 \times 10^{-4}$ |
| **Total** | $\mathbf{1.2 \times 10^{-3} \pm 1.31 \times 10^{-4}}$ | $\mathbf{3.27 \times 10^{-3} \pm 3.2 \times 10^{-3}}$ | $\mathbf{2.07 \times 10^{-2} \pm 3.81 \times 10^{-3}}$ |
| Note: *N/F connotes not found* | | | |
| From Fig. 3, the total mean concentration of PAHs in the outdoor animal group (82%) was far higher than those of indoor (13%) and baseline (5%), while at the individual PAHs levels, Dibenz(a,h)anthracene and Indeno(1,2,3-c,d)pyrene had the highest mean concentrations noted in the outdoor animal group (Fig. 4). | | | |

Figures 5–11 represents the differences in the baseline, indoor, and outdoor PAHS concentrations in tissues of experimental animal groups. While the outdoor animals had significantly higher mean values for both 2-methyl Naphthalene ($P = 0.043$) and Acenaphthylene ($P < 0.0011$) relative to the baseline (Fig. 5A and 6A). The highest concentration of Acenaphthene and Anthracene was observed in outdoor animals which were significantly greater than the levels in the outdoor ($P < 0.0001$) and baseline ($P < 0.0001$) animals; however, the differences in the Acenaphthene ($P = 0.061$) and Anthracene ($P = 0.098$) levels in baseline and outdoor animals were not significant (Fig. 5B and 6B).

The detected levels of Benz(a)anthracene and Benzo(b)fluoranthene in Fig. 6 - A & B, the levels remained significantly higher in the outdoor (Benz[a]anthracene; $P = 0.043$ and Benzo[b]fluoranthene; $P = 0.002$), while it was undetected in the baseline. Similarly, Benzo(k)fluoranthene ($P = 0.0008$) and Chrysene ($P = 0.026$) levels were significantly higher in the outdoor animals when compared to the indoor animals (Fig. 8 - A and B).

In Fig. 9A, the difference in the mean Dibenz(a,h)anthracene concentrations of baseline and indoor animals was not significant ($P = 0.471$); however, the outdoor was significantly higher than the baseline ($P < 0.0001$) and indoor ($P < 0.0001$). Fluoranthene was undetected in the baseline animals, but the detected levels in indoor and outdoor animals were not significant ($P = 0.380$) (Fig. 10). The mean Pyrene levels in all animals were significantly different ($P < 0.05$), with significantly higher values observed for outdoor when compared with the baseline and indoor animals ($P < 0.0001$). The indoor animals also had significantly greater mean concentrations than the baseline ($P = 0.038$) (Fig. 11).

# Discussion

The World Health Organization's International Agency for Research on Cancer (IARC) established that outdoor air pollution is carcinogenic to humans, as the association between increased incidence of cancer and particulate matter have been established [59]. The presence of daily $PM_{2.5}$ at 32–158 μg/$m^3$ and PM10 at 33–467 μg/m3 beyond WHO acceptable limits, confirms the likelihood of Port Harcourt residents being exposed to hazardous compounds [60, 61]. High amounts of fine particulate matter in indoor and outdoor environs noted in the study were similar to those found in highly petroleum-based industrial areas [23–27, 62–64]. The observed increase in particulate matter is most likely responsible for the relative upsurge in trends of morbidity and mortality recorded over the years in Port Harcourt, confirming reports from previous studies [19, 20, 62], as toxicity and mortality, cancers, upsurge in respiratory, cardiovascular, and renal dysfunctions resulting from exposure to particulate matter bonded with PAHs have been reported in a plethora of epidemiological studies [47, 65, 44]. Although the ration of $PM_{10}$ and $PM_{2.5}$ have not been studied for atmospheric particulate matter of Port Harcourt, studies have implicated infants and children being at risk of fine aerodynamic particulates [22, 66]. The study findings also aggress with earlier reports of weight loss and skin toxicity as observed on the outdoor experimental rats, due to exposure to particulate matter and effects of PAHs [43, 67]. Over 500 PAHs and its related compounds have been found in the air [10] and the carcinogenic PAHs such as benz[a]anthracene, benzo[a]pyrene, benzo[b]fluoranthene, benzo[k]fluoranthene, chrysene, dibenzo[a, h]anthracene, benzo[ghi]perylene and indeno[1,2,3-cd]pyrene [10, 28]. Most of the PAHs found in the study were mostly of the carcinogenic class. There is strong evidence that injuries to the lung tissues and DNA damage could result from sub-chronic exposure to low amounts of particles, even below the WHO's 24hours threshold [33].

Five established carcinogenic PAHs such as benz[a]anthracene, benzo[b]fluoranthene, benzo[k]fluoranthene, chrysene, benzo[ghi]perylene and indeno[1,2,3-cd]pyrene [5, 23] including Phenanthrene were not found in samples of the baseline animal group as against those of indoor and outdoor animal groups (see Table 1). Also, of the mean concentrations noted, the indoor exposed animal group constituted a mere 5% (see Figs. 3 and 4) implicating a relatively clean air in the University of Nigeria Nsukka, where the experimental animals were imported from for the study assessment. This suggests that people in non-hydrocarbon and petroleum-free environments are at lower risk of cancers and general tissue toxicity compared to residents inhaling air polluted with PAHs bonded particulate matter due to petroleum and hydrocarbon related industrial activities [61–67]. Also, the continuous inhalation of PAHs bounded particulate increases the concentrations of these compounds in the tissues, enabling toxicity through oxidative stress, inflammation, and DNA methylation [5, 48].

Nsukka is located in Enugu, south-east Nigeria. It is an agricultural town, with the University of Nigeria (UNN) situated in it and having minimal commercial and industrial activities that are likely to generate pollutants such as PAHs [68]. As seen in Fig. 2 above, the experimental animals were imported from this non-petroleum and hydrocarbon pollution-free environment (UNN) which should ideally record no concentrations of PAHs. Although in lesser amounts, the study found some concentrations of the

individual PAHs, including B[a]P in blood samples of the baseline animal group. This is likely to result from pyrogenic sources, including domestic combustion and most probably not from hydrocarbon or petrogenic events [36, 69, 70]. This calls for a further forensic investigation of the presence of abundant alkyl homologs in such an environment.

The indoor air concentration constitutes about 13% of PAHs levels investigated. Though absent in the baseline animal group, the presence of fluoranthene in the ambient of the indoor animal group indicates toxicity (see Table 1), as fluoranthene is said to be a complementary indicator to B[a]P [5]. Studies have demonstrated the burning of biomass, fossil fuel, and solid fuel as important sources of airborne particulates and its component pollutants, including PAHs, which are emitted into indoor environs through unvented or flueless combustion [70, 71]. Reports have shown that low molecule (less than for rings) PAHs predominantly concentrates indoor air [72], thus the high concentrations of large-molecule PAHs (including dibenz[a,h]anthracene, benzo[b]fluoranthene, benzo[k]fluoranthene, and indeno[1,2,3-cd]pyrene ) know for high carcinogenicity and toxicity noted in the indoor animal groups as against those of baseline, suggests intrusion of outdoor air [5, 73]. Cooking activities and indoor combustion sources such as fossil fuels and biofuel [74] and indoor environments impacted by ETS [10] have been reported. Suggesting a higher concentration of some PAHs in indoor exposures likely to occur during the cooking period [17, 74]. In as much as outdoor air influences the concentrations of PAHs in indoors, the type of cooking fuel used impacts greatly on indoor PAHs levels in descending order of dung cake > dung cake/wood mixture > wood > coal > kerosene > LPG [5, 17, 74]. Also cooking temperature has been found to influences the production of most indoor PAHs as increasing temperatures affects evaporation of PAHs into the air and pyrolysis from partially cracked organic compounds [17].

Higher indoor concentrations of 12 PAHs (naphthalene, acenaphthene, fluorene, phenanthrene, anthracene, fluoranthene, pyrene, benz[a]anthracene, chrysene, benzo[k]fluoranthene, benzo[e]pyrene and benzo[a] pyrene) have been recorded across homes with an unvented fireplace, lacking airtight stoves and windows, homes and public places ( such as restaurants, discotheques, and clubs ) with ETS, while lower levels were noted across offices, hospitals, schools, libraries and coffee shops [17, 75–80] and children were noted to be adversely affected [81]. Conducting the study during the dry season could also have influenced the increased PAHs concentration in the ambient. Though seasonal variations were not investigated, earlier reports have established seasonal differences in PAHs concentrations, with higher levels in rainy (winter) than in dry or summer seasons [72]. Thus, outdoor air, indoor combustion sources, cooking activities, cooking temperatures, indoor ETS, ventilation characteristics of the living house, climatic factors, and seasonal variations all affect indoor PAHs concentrations. This implicates women; especially in Africa have a higher chance of being affected by indoor air pollutants such as PAHs [79] as their activities are observed to be predominantly indoors.

As seen in Fig. 2 above, 82% of total mean concentrations of PAHs noted, were from the outdoor experimental animal group. This indicates high toxicity of ambient air inhaled by residents and implicates finding of the previous study that used lichen as a bioindicator for air pollution [53]. Combustion in energy and transformation industries, nonindustrial combustion plants, combustion in the manufacturing

industry, production processes, traffic road transport, ETS, other mobile sources, waste incineration, agriculture and forestry, natural sources were all found to contributes significantly to outdoor PAHs concentrations in industrialized nations with the variety of sources and emission factor across the countries [82, 83]. High toxicity was predicted for pregnant women in these studies [82]. While in developing countries, pyrogenic and petrogenic combustions for industrial, commercial, and domestic uses involving biomass, fossil fuel and solid fuel, including petroleum and hydrocarbon refining sources contribute to outdoor particulates matter containing PAHs [70, 71]. Although no empirical emission inventory has been conducted for Port Harcourt, places with petrochemical economic and industrial ecology have been reported to have ambient air largely polluted by petroleum and hydrocarbon [23–26, 62, 70, 84, 85] most probably responsible for the increase in trends of morbidity and mortality [20, 23] noted in the metropolis.

With outdoor animal group recording higher concentrations of total PAHs (see Figs. 3 and 4), especially the larger-molecule including dibenz[a,h]anthracene, benzo[b]fluoranthene, benzo[k]fluoranthene, and indeno[1,2,3-cd]pyrene and the recorded experimental dead Wistar rats notably from the outdoor group, continues inhalation of ambient air of Port Harcourt suggests higher chances of tissue toxicity. Especially inhabitants engaged in outdoor activities such as road sweepers, street hawkers, traffic wardens, building, and road constructors. This could be responsible for the clinician's hematological findings of rare toxins in blood samples of some young patients at tertiary hospitals in Port Harcourt [23]. Hence the medical advice to residents to avoid places where crude oil is cooked, as management of neoplasms and myelofibrosis conditions resulting from crude oil pollution remains expensive, especially for low-income people [23].

From the study result of outdoor PAHs concentration, it is most probable that the indoor air conditions were influenced by outdoor air [5, 73] This might be further worsened by ETS at open spaces adjacent to night clubs. Unlike risk to indoor air PAHs concentration, men in Africa are highly at risk of PAHs concentration in outdoor air environs [86] as they carry out more of outdoor occupations. Ambient air in densely populated and metropolitan locations have been demonstrated to have higher PAHs concentrations than less dense, rural, forest, and agricultural areas because of the numerous sources of fossil fuel combustions [27]. Carcinogenic PAHs such as dibenzo[a, h]anthracene, benzo[ghi]perylene, indeno[1,2,3-cd]pyrene and pyrene had the predominant concentrations of PAHs in the study. This agrees with previous studies in which toxicity resulting from ambient exposure in petroleum industrial places was demonstrated [23, 62, 84].

While levels of the PAHs especially Benz(a)anthracene, Benzo(b)fluoranthene, and Fluoranthene remained significantly higher in the outdoor, they were often undetected in the baseline animals. This suggests that, unlike residents in an environment free from petroleum-based ambient air pollution, inhabitants of Port Harcourt metropolis are inhaling into her body system, air poisoned with PAHs due to petroleum activities. Figures 3 and 4 also implicatively show that residents of Port Harcourt are more predisposed to morbidity and mortality associated with PAHs [19, 38, 43, 87, 88], unlike those in hydrocarbon pollution-free environment [54]. This could explain hematologists' sudden notice of

myelofibrosis in some young residents of Port Harcourt involved in artisanal petroleum refining [23]. The clinician observation negates empirical evidence of myelofibrosis found to be common in the older person of 60 + years [23, 89, and 90]. Studies by the United Nations Environmental Programme have shown that substandard oil explorations and artisanal petroleum refining significantly added to the effect of hydrocarbon pollutions which affect both the environment and health [91]. Adversely, exposure to PAHs poses dangers to residents of Port Harcourt, as continuous exposures to outdoor and indoor air polluted with PAHs resulting from petroleum industrial activities likely to increase cases of cancers [59, 62, 92] and deformities, especially in pregnant women, infants and children and obese persons [20, 62]. Adverse birth outcomes such as preterm birth, early pregnancy fetal death, low birth weight likely to be common, as well as intrauterine growth retardation with the capacity to distort the academic performance of children in the future when compared to cohort children from the non-polluted environment [19, 91–93].

Unlike studies were both indoor and outdoor PAHs concentrations were observed to be similar [94], the PAH levels found in this study were significantly higher in the outdoor animals (82%) when compared to the indoor animals (13%) as shown in Fig. 3 and the mean Pyrene levels in all animals were significantly different. With significantly higher values observed for outdoor animals (Figs. 3 and 4). These findings implicate exposure to the variation of doses of PAHs [95]. Suggesting that when compared to indoor workers, outdoor workers such as street traders, beggars, market sellers, road construction workers, and children in open schools within Port Harcourt metropolis are more at risk of adverse health effects associated with PAHs induced by petroleum-based particulate matter pollution [16, 18]. The notable negation of study results from similar studies [96–98] in which higher levels of indoor PAHs were observed, is likely to result from the difference in geographic location, seasonal variations, and source of particulate matter inducing the PAHs [72, 75]. Deaths recorded from the outdoor experimental exposed rats are likely to result from tissue toxicity beyond the bearable limit and immune suppression prompted by oxidative stress and alteration of DNA methylation and expression of specific genes [44, 65, 94, 95, 99–102], as caution was taken prevent physical stress and trauma on the rats. The study findings explain and implicate previous reports on increased trends of selected mortality and morbidity associated with particulate matter pollution in Port Harcourt between 2016 and 2018 as well as an upsurge in health burden [20, 23]. Similarly, the result also proves that the risk of toxicity, carcinogenicity, and mutagenicity due to high concentrations of PAHs are likely higher in inhabitants living in hydrocarbon industrial polluted environments than those in non-hydrocarbon industrial areas [5, 44, 54, 62, 84, 102–104].

The high levels of carcinogenic PAHs such as dibenzo[a, h]anthracene, benzo[ghi]perylene, indeno[1,2,3-cd]pyrene and pyrene detected in the study, indicate the likelihood of tissue toxicity of exposed inhabitants [17, 23]. Toxicity resulting in high incidences of deaths and cancers of several types and at different tissue locations have been documented in places with exposure to carcinogenic PAHs in petroleum industrial ecology [17, 62, 105–109]. The death of seven outdoor experimental rats before the end of 90days study period, loss of weight, and change in skin color of exposed animals noted in the study remain a concern and the likelihood of such effects on human inhabitants is most probable [17, 23, 62, 107, 109]. And interactions of PAHs with tissues have been found to results in cancers and mortality,

[109]. Animal model studies have demonstrates lymphoreticular system tumours associated with exposures to dibenzo[a, h]anthracene, benzo[ghi]perylene, indeno[1,2,3-cd]pyrene [135, 136]. Implicatively, this suggests, that exposed inhabitants were most probably susceptible to the toxicity of PAHs [137–141], due to its suppression of immunosurveillance and immunocompetence of body [140].

# Conclusion And Recommendation

The findings demonstrate that the PAHs level in the atmosphere of Port Harcourt metropolis is notably high, which indicates a high probability of toxicity on human tissues and health. Unlike inhabitants in non-hydrocarbon and petrochemical free environments, continuous inhalation of air polluted with toxic particulate matter and high concentrations of PAHs in petroleum refining places such as Port Harcourt could exacerbate the already high prevalence of morbidity and health burden associated with airborne particulate matter pollution. With a continuous rise in PHAHs level, the risk of chronic diseases such as cancers, respiratory cardiopulmonary dysfunctions, kidney, and other systemic failure and mortality remains most probably high in no distant time. Surveillance framework is instituted for clinicians and laboratory experts' prompt identification of rare diseases such as myelofibrosis, birth defects, and others. Outdoor workers, especially those involved in petroleum refining, road sweepers, traffic managers, constructions work, infants and children, pregnant mothers, obese persons as well as the elderly are more at risk of toxicity, morbidity, and mortality associated with high PAHs concentrations in the ambient air. Systematic emission inventory and routine air quality monitoring are necessary for highly polluted hydrocarbon industrial places, to enhance the detection of indoor and outdoor sources of airborne particulates. There are strong shreds of evidence suggesting PAHs are associated with injuries to the lungs tissues, DNA damage, neurologic disorders, resulting from sub-chronic exposure to a low amount of particulate matter and PAHs concentrations, even below the WHO's 24hours threshold [17, 33, 36, 102–104]. While the control and elimination of emission sources airborne particulates are strongly recommended, there is an urgent need for appropriate public health screenings for carcinogenicity, nutritional therapy, and environmental interventions to control the potential effects of PAHs and concentrations of carcinogenic compounds in the ambient air of Port Harcourt.

# Abbreviations

ETS: Environmental tobacco smoke

NAFDAC National agency for food and drug administration and control

UPTH: University of Port Harcourt Teaching Hospital

RSU: Rivers State University

RSMEN: Rivers State Ministry of Environment

WHO: World Health Organization

# Declarations

**Ethics approval:** Approval to keep the animals in the Histology laboratory was obtained from the Provost, College of Medical Sciences, RSU. All protocols and procedures were conducted in line, in compliance and authorization of the Rivers State Ministry of Health (MH/PRS/391/VOL.2/632) ethical approval. For the use of animals for experimentation, the animals were maintained in line with the national guidelines and protocol of the Institutional Animal Care and Use Committee (IACUC) [58].

**Consent for publication:** Not applicable

**Availability of data and materials:** All data generated or analyzed during this study are included in this published article. Data were deposited at the Harvard Dataverse repository, available at https://doi.org/10.7910/DVN/APXZAP.

**Competing interests:** The authors declare that they have no competing interests

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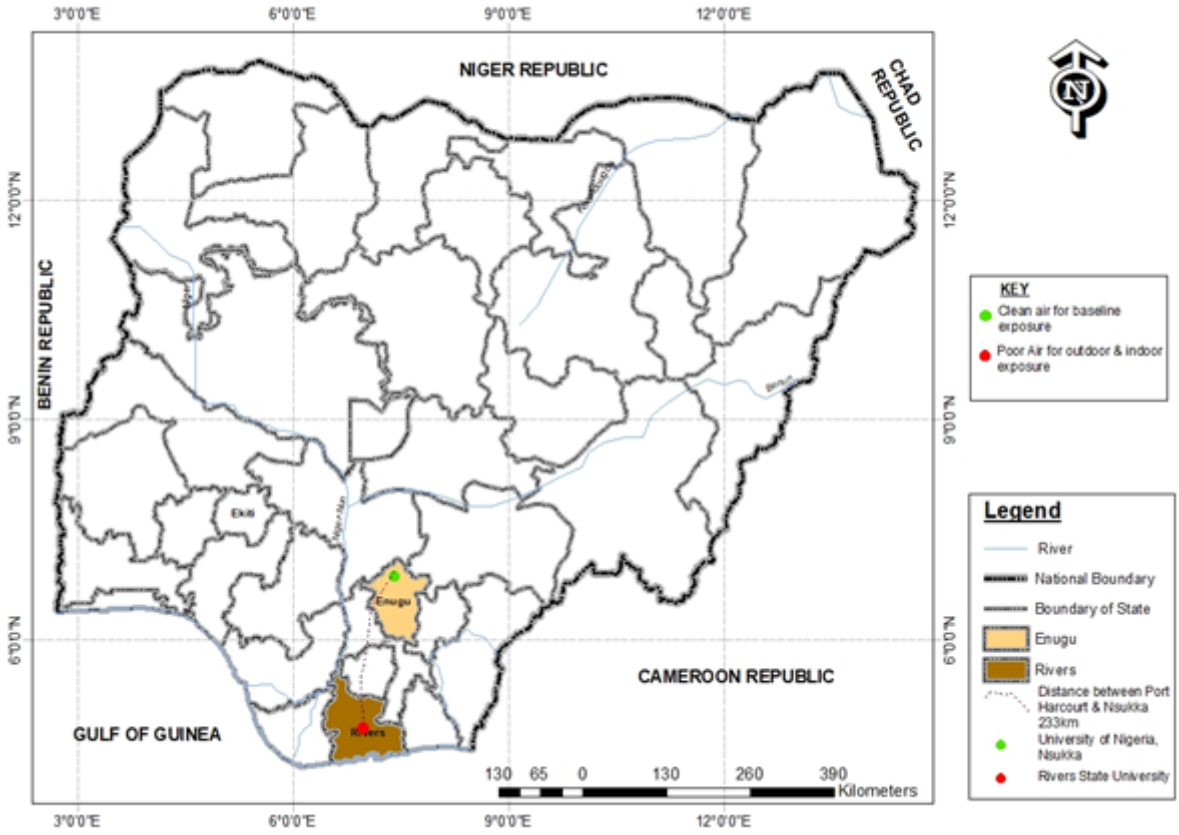
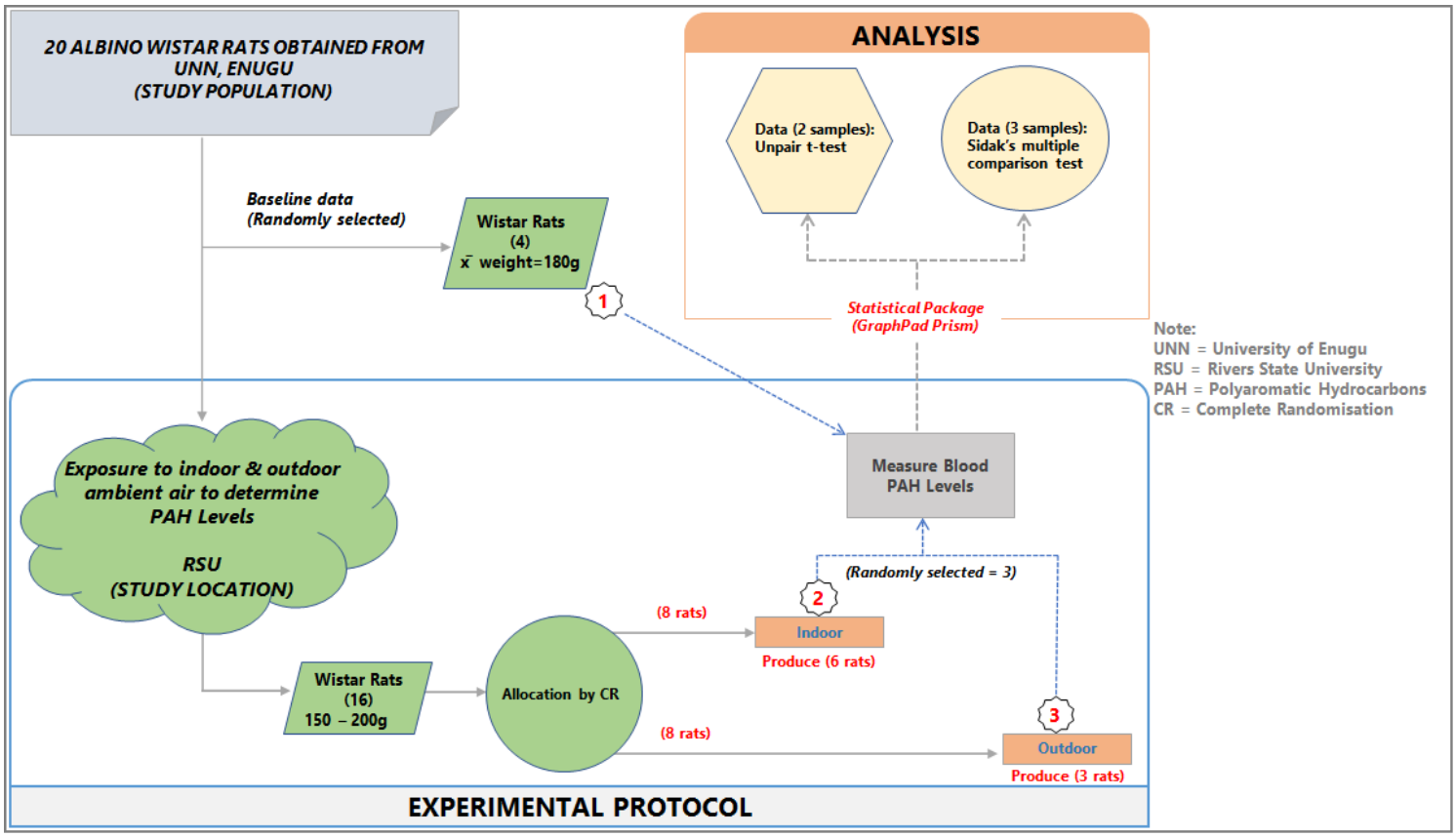
# Figures



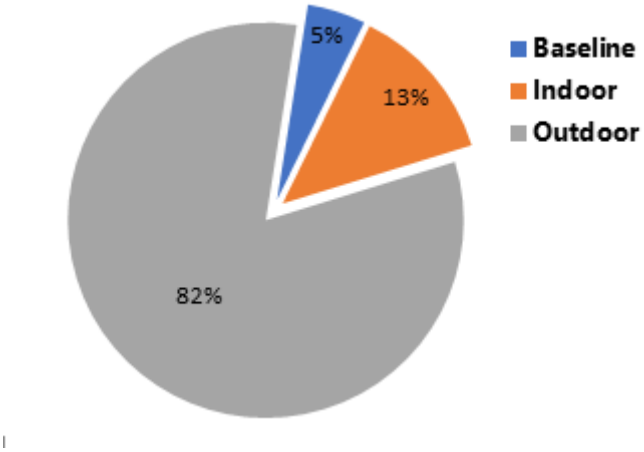

## Figure 1

Study Area



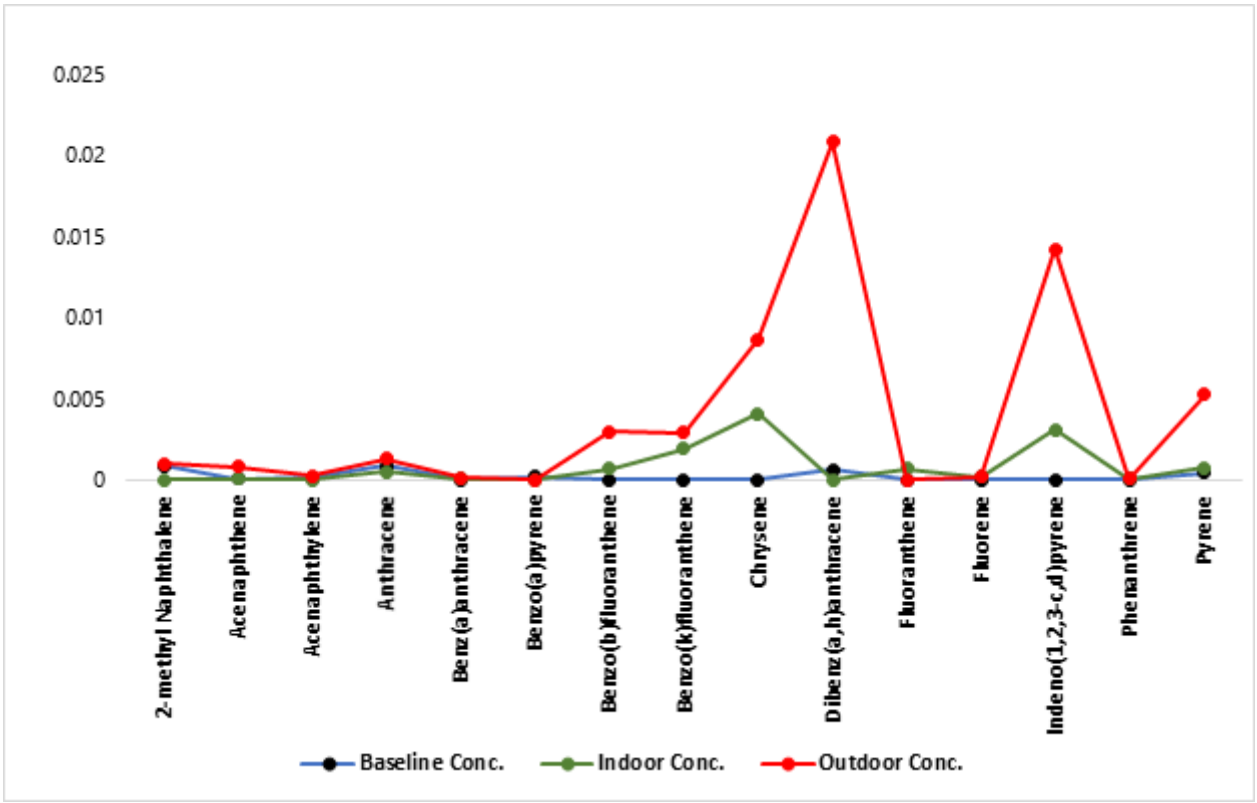
## Figure 2

Research Design



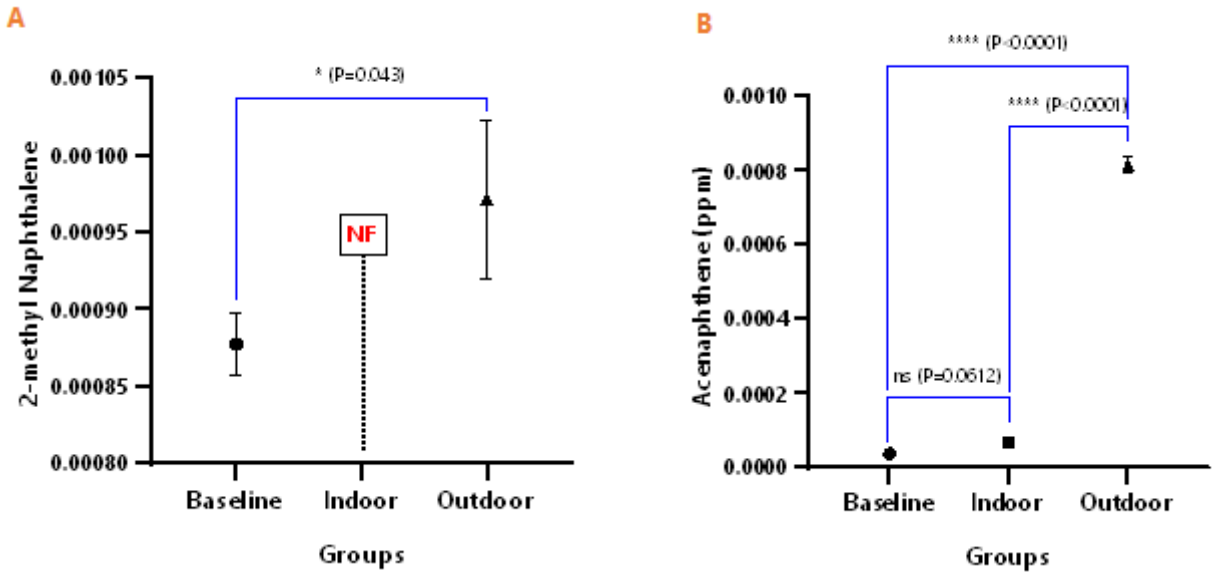

## Figure 3

Total mean concentrations of PAHs



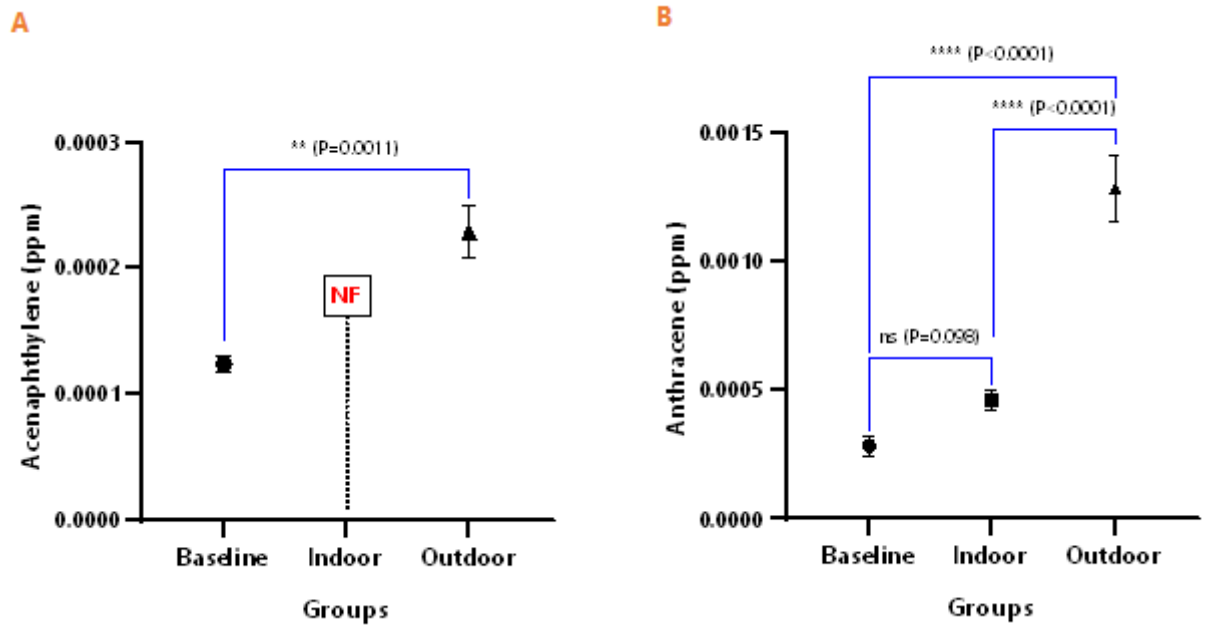

## Figure 4

The mean PAH concentrations in ppm



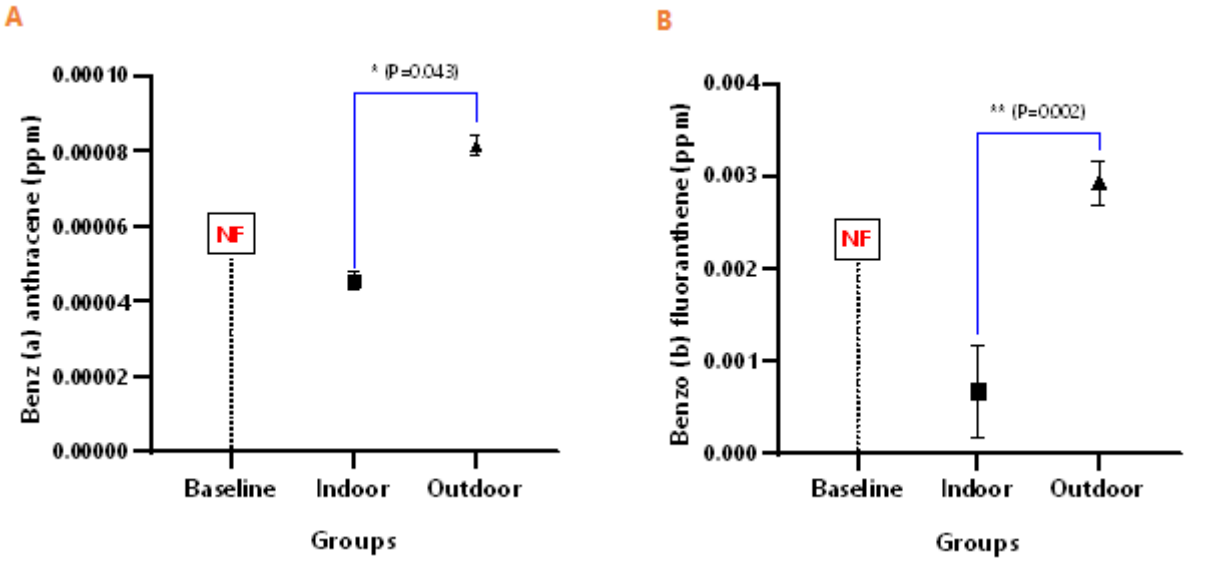

## Figure 5

methyl Na ph thalene and Acena phthene



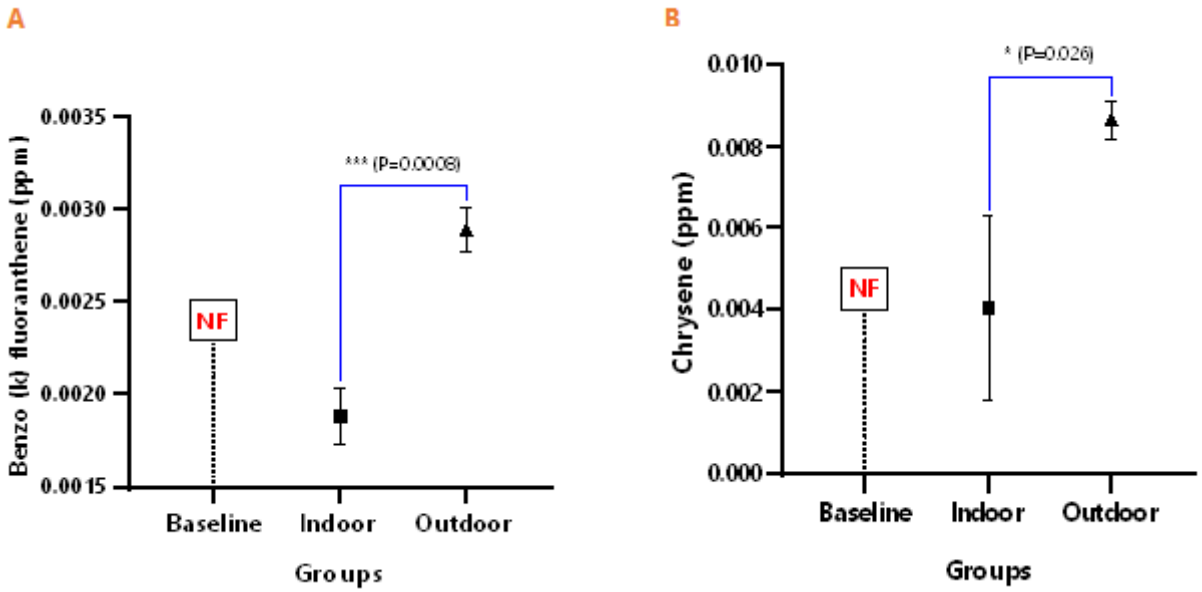

## Figure 6

Acenaphthylene and Anthracene



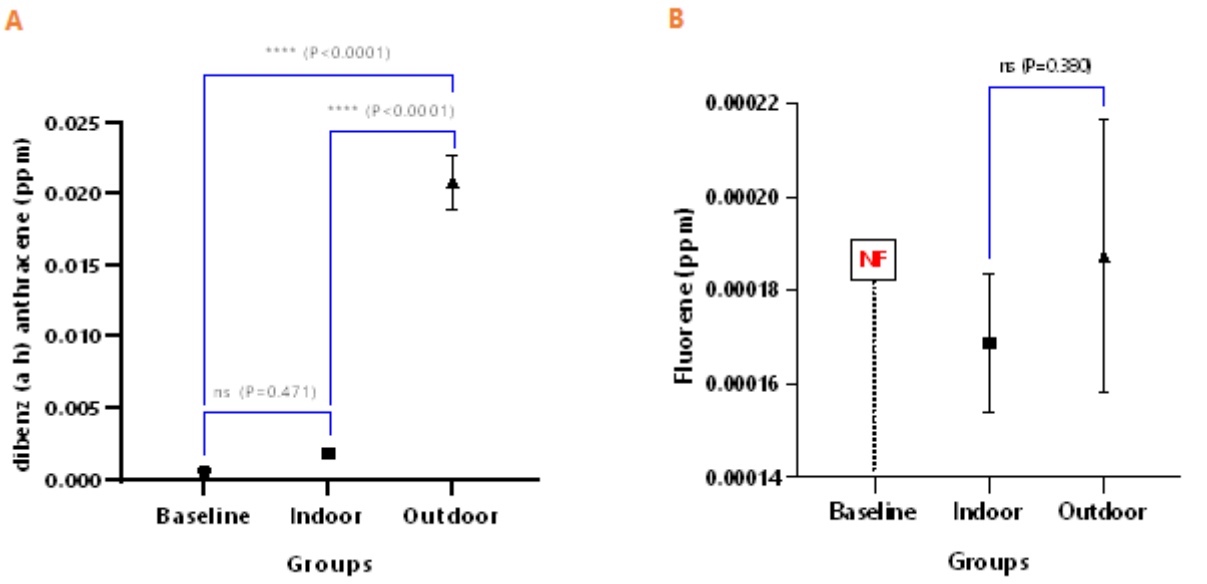

## Figure 7

Benzaanthr acene and B Benzob fluoran thene




## Figure 8

Benzok fluoran the ne and Chrysene




## Figure 9

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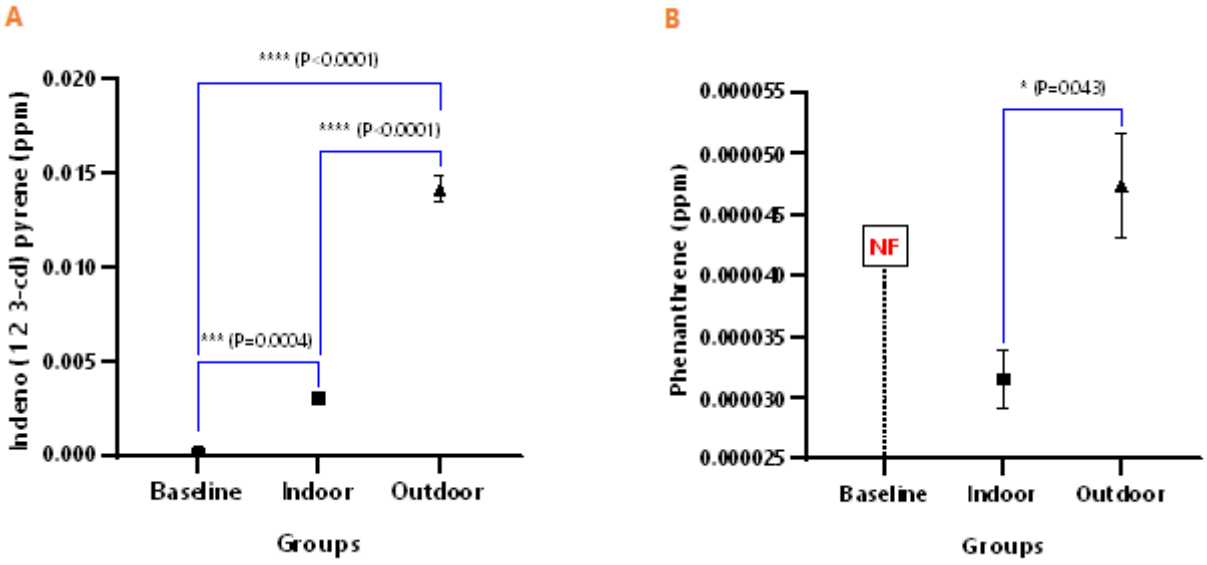


## Figure 10

Indeno 123 cd pyrene and Phenanthrene

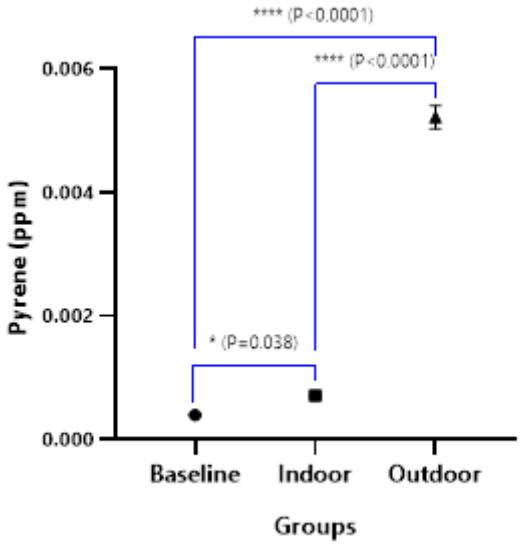


**Figure 11**

Pyrene

## Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- PAHs.pzfx