Polycyclic Aromatic Hydrocarbons In Edible Mushrooms from Niger Delta, Nigeria: Carcinogenic and Non-Carcinogenic Health Risk Assessment

Sorbari Igbiri¹, Nnaemeka Arinze Udowelle¹, Osazua Clinton Ekhatr°, Rose Ngozi Asomugha², Zelinjo Nkeiruka Igweze³, Orish Ebere Orisakwe¹*

Abstract

In the oil-rich Niger Delta, hydrocarbon pollution and oil spillages, gas flaring and sundry anthropogenic activities constitute sources of polycyclic aromatic hydrocarbons (PAHs), with food contamination playing a major role in human exposure. In this study we assessed PAH levels in wild and cultivated edible mushroom species consumed by the general population from the oil producing Niger Delta, Nigeria. The concentrations of USEPA-16 PAHs were determined by gas chromatography and carcinogenic and non-carcinogenic health risks were calculated. The concentrations of USEPA-16 PAHs ranged from 0.02 mg/kg – 3.37 mg/kg. The dietary intake of carcinogenic and non-carcinogenic USEPA-16 PAHs (Naphthalene, Acenaphthylene, Acenaphthene, Anthracene, Phenanthrene, Fluorene, Fluoranthene, Pyrene, Benzo[a]Anthracene, Chrysene, Benzo[a]Pyrene, Benzo[b]Fluoranthene, Benzo[K]Flouranthene, Benzo[g,h,i]Perylene, Dibenzo[a,h]Anthracene and Ideno[1,2,3-cd]Pyrene) for adults, adolescents and seniors ranged from 0.00 – 0.05 mg/kg/day, 0.00 – 0.06 mg/kg/day and 0.00 – 0.07 mg/kg/day. The BaPeq ranged from 0.02 – 2.76 with margin of exposure MOE values of BaP ranging from 3,500,000 to 700,000, 3,500,000 and 3,500,000 to 7,000,000 for adults, adolescents and seniors indicating very insignificant health risk. The incremental lifetime cancer risk was within the safe range of 1.56x10⁻⁸ – 1.73x10⁻⁶ with the highest calculated risk found for wild Pleurotus ostreatus mushroom species from the study area.

Keywords: Superfoods- farm produce- organic pollutants- risk assessments- public health

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Introduction

Edible mushrooms (macrofungi) comparable with eggs, milk and meat are major sources of proteins and other essential nutrients (Kulshreshtha et al., 2014), have been appreciated as source of both food nutrients and pharmacologically important compounds useful in medicine, nutrition and survival: a reason why Nigerians consume these mushroom either as food or medicine (Oyetayo, 2011). Edible mushrooms possess high quantities of fibers, few sugars and low cholesterol and a high quantity of the amino acids such as phenylalanine, threonine and tyrosine with low cholesterol levels. Of the 8-10 different species of mushroom consumed in Nigeria (Akpaja et al., 2003), some are known to accumulate environmental toxicants (including PAHs (Kalac et al., 2004).

Contamination of the environment by PAHs is becoming a rising environmental concern (Anyakora and Coker, 2011). Edible mushrooms can enter the food chain by deposition from air or by deposition and transfer from soil and water. Food can be contaminated from environmental sources (natural and mostly anthropogenic), from industrial food processing, and from some domestic cooking practices (Zelinkova and Wenzl, 2015) and the PAHs may accumulate in organisms due to their low solubility and high octanol-water partition coefficient (Yang et al., 2014).

The carcinogenic property of PAHs has been identified as a public health concern worldwide (Orisakwe et al., 2015). This property is associated with the complexity of the molecule (i.e. increasing number of benzenoid rings). According to the United States Environmental Protection Agency (USEPA), there are at least 11 carcinogenic or mutagenic PAHs. The International Agency for Research on Cancer (IARC) lists the following PAHs as human carcinogens or potential carcinogens:benz[a]anthracene,benzo[b]fluoranthene,benzo [j] fluoranthene, BaP,benzo[a,h]anthracene,7H-dibenzo [c,g] carbazole, dibenzo[a,h]pyrene, dibenzo [a,i] pyrene, indeno [1,2,3-cd]pyrene, benzo [k] fluoranthene, dibenzo [a,e]
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collected in triplicates from different locations in Port Harcourt, Rivers State, Nigeria. Decaying wood matter and growing wood plant, soil and spent mushroom substrate (SMS) samples where the mushroom samples were harvested were also collected in triplicates and stored in glass petri dishes. Mushroom samples were collected using stainless steel forceps while the substrate, soil and decaying organic wood materials was collected using stainless steel knife and spoon into Pyrex glass petri dishes rinsed in deionized water. Samples were then covered, labeled and transported to the laboratory. The coordinates of the sampling sites are shown on Table 1.

Samples S1, S2 and S3 were oyster mushroom samples (Pleurotus ostreatus) cultivated samples by the University of Port Harcourt (UNIPORT) Mushroom unit and Dilomats farm at the Rivers State University of Science and Technology (RSUST). S4 and S5 were species of Auricularia auricular-judae commonly called ear mushroom, mushroom sample S5, S7 and S8 were species of Pleurotus tuber-regium and mushroom sample S9 and S10 was wild Pleurotus ostreatus. Samples S4 – S10 were all obtained from the wild in forest around Alakakia, Port Harcourt (Samples S6, S7, S8, S9 and S10) and forest in Igruita, Port Harcourt. Spent mushroom substrate (SMS), decaying wood material, soil samples and even growing plant with dead part consisted the background where the mushrooms were harvested.

Reagents. All chemicals and reagents used were of analytical grade. Hexane (purity 99.8%) was from Riedel-de Haën (Seelze, Germany), while dichloromethane (LC grade), anhydrous sodium sulfate (purity 99%), alumina, and silica gel were obtained from BDH (Poole, UK). A PAH standard mixture containing the 16 priority PAHs, namely, naphthalene (Nap; 1,000 mg/ml), acenaphthylene (Acy; 2,000 mg/ml), acenaphthene (Ace; 1,000 mg/ml), fluorine (Flu; 199.9 mg/ml), phenanthrene (Phe; 99.8 mg/ml), anthracene (Ant; 100.0 mg/ml), fluoranthene (Flt; 200.1 mg/ml), pyrene (Pyr; 99.9 mg/ml), benzo[a]anthracene (BaA; 100.1 mg/ml), chrysene (Chy; 100.0 mg/ml), benzo[b] fluoranthene (Bbf; 200.2 mg/ml), benzo[k]fluoranthene (Bkf; 99.9 mg/ml), benzo[a]pyrene (BaP; 100.0 mg/ml), dibenz [ah] anthracene (DahA; 200.0 mg/ml), indeno[1,2,3-c,d]pyrene (IndP; 100.1 mg/ml), and benzo[g,h,i]perylene (BghiP; 100.0 mg/ml), was purchased from Supelco (Bellefonte, PA). Working mixed standard solutions containing all the PAHs were prepared by dilution of the stock solution with acetone and stored at 24°C in darkness to avoid volatilization and photodegradation.

Sample preparation, Extraction and Clean-up.

The mushroom were cut into small pieces and kept in the refrigerator in the tightly sealed glass petri dish for extraction, digestion and analysis. The PAHs in mushroom samples were analysed by adapting the procedure in use in our laboratory (Orisakwe et al., 2015). Glass wares were washed thoroughly with hot detergent solution followed by rinsing with purified water and acetone (analytical grade) respectively. These were finally baked in the oven at 100 °C overnight. To avoid contaminations of mushroom samples, different glass wares and syringes were used for standards and for solutions extracted from samples.

Materials and Methods

Sample collection. In October 2015 ten mushroom samples (wild and cultivated mushroom species) were collected in triplicates from different locations in Port Harcourt, Rivers State, Nigeria including the Niger Delta region characterized by extensive environmental degradation. The present study was conducted to examine the levels of PAHs in edible mushrooms (wild and cultivated) and to determine the carcinogenic and non-carcinogenic health risk associated with its consumption.

pyrene, dibenzo [a,l] pyrene, and 5-methylchrysene (IARC, 2010). BaP, a potent carcinogen and its derivatives have the capacity to enter redox cycles and induce the production of reactive oxygen species (ROS), thereby causing oxidative stress (An, et al., 2011). If the rate of ROS generation is greater than their removal it is likely that more DNA damage will result (Ali, et al., 2011). Basically, the margin of exposure (MOE) is a tool used by risk assessors to consider possible safety concerns arising from the presence in food and feed of substances which are both genotoxic (they may damage DNA) and carcinogetic. MOE of 10,000 or higher, if it is based on the ‘Benchmark-dose lower bound’ (BMDL) would be of low concern from a public health point of view while a lower MOE will indicate significant Public Health Concern from PAHs exposure (EFSA, 2012). In Nigeria, at least 102,100 and 71600 people are diagnosed of new cancer and die from cancer annually respectively (Cancer Index, 2015). Elderly who have declining organ function, young children with immature and developing organs, smokers (and therefore inhale PAHs), people with history of excessive sun exposure (enhanced skin cancer response if simultaneously exposed to PAHs via skin), people who have liver and skin diseases, women of child bearing age and unborn foetus are recognized as high risk individuals to PAHs induced toxic effects (SA Health, 2012). Breast, lungs, skin, bladder and gastrointestinal cancers with other mild symptoms like eye irritation, nausea and vomiting, diarrhea and confusion have been reported as side effects of acute and chronic PAHs exposure (Breast Cancer Fund, 2016; Thamaraiselvan et al., 2015; Shen et al., 2014).

Activities associated with petroleum exploration, development and production have local detrimental and significant impacts on the atmosphere, soils and sediments, surface and groundwater, marine environment and terrestrial ecosystems in the Niger Delta (Nduka and Igruita, Port Harcourt, Rivers State, Nigeria. Decaying wood matter, soil samples and even growing plant with dead part consisted the background where the mushrooms were harvested.

There is a dearth of risk assessment data on the PAHs for standards and for solutions extracted from samples. These were finally baked in the oven at 100 °C overnight. To avoid contaminations of mushroom samples, different glass wares and syringes were used for standards and for solutions extracted from samples.

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Spent mushroom substrates (SMS), soil samples and decaying wood organic matter were ground into powder in a ceramic mortar and demagnetized with a magnetic rod and kept in sealed in a refrigerator at 4°C. Extraction of PAHs from mushroom and background was done with a sonicator (Ultrasonic bath-Emlsonic S40H) in accordance with US SW-846 Method 3,550. Two grams of each of the mushroom and background samples was extracted with a 50:50 mixture of acetone and methylene chloride spiked with 1 ml of PAH internal standard and shaken thoroughly for proper mixing before placing in an ultrasonic bath.

**Chemical Analysis**

The EPA-16 PAHs (naphthalene, acenaphthylene, acenaphthene, fluorene, phenanthrene, anthracene, fluoranthene, pyrene, benzo[a]anthracene, chrysene, benzo[b]fluoranthene, benzo[k]fluoranthene, benzo[a]pyrene, indeno[1,2,3-c,d]pyrene, dibenz[a,h]anthracene, and benzo[g,h,i]perylen) determination was conducted at Jaros Inspection Services Limited, Port Harcourt, Rivers State, Nigeria using Gas Chromatography (6890 series and 6890 plus) equipped with a dual detector (FID-ECD), dual column and TriPlus AS auto-sampler with helium carrier gas and a quadrupole Mass Spectrometer (Agilent 5975 MSD) based on USEPA method 8100(EPA 1984). A 2.00μl of extracts were injected into the GC port set at column conditions: HP-5 cross linked PH-ME siloxane, length of 30 m, i.d: 0.25 mm, thickness of 1 μm with helium carrier gas set in the spitless, constant flow mode with 1.2 ml/min flow rate. Other GC and MS operating set-up were done according to the instrument’s method development as specified in the operating instruction manual. Identification and quantification of individual PAHs was based on internal calibration standard containing known concentrations of the 16 PAHs (EPA-16). The specificity of the 16 PAHs sought for in the samples was confirmed by the presence of transition ions (quantifier and qualifier) as shown by their retention times which corresponded to those of their respective standards. The measured peak area ratios of precursor to quantifier ion were in close agreement with those of the standards.

The detection limit (LOD), estimated as three times the background noise (IUPAC criterion), was similar for all analysed compounds and results were less than 0.015 μg/kg d.w. for all analytes. The blank values of analytical procedure remained always below the quantification limit (LOQ): 0.05 μg/kg d.w., estimated as 10 times.

Quality control and quality assurance. All solvents used (e.g. dichloromethane) were picograde quality. External calibrations were obtained with PAH solutions at five concentration levels. To evaluate the matrix effect was evaluated by spiking the fish sample with a concentration range used for calibration and then comparing the correlation coefficient and slope of the spiked standard to the matrix with the original standard calibration curve. No matrix effect was observed.

Statistical analysis. Two-way analysis of variance (ANOVA) and a Student’s t-test were used to determine whether the concentrations of the PAHs varied significantly between each mushroom, with p-values less than 0.05 considered to be significant. The statistical calculations were performed with Graph Pad Prism 5.0.

**PAHs Health Risk Assessment**

**Benzo[a]Pyrene equivalent (B[a]Peq) estimation**

Benzo[a]Pyrene has been characterized as the most potent carcinogenic PAH after Dibenzo[a,h]Anthracene. Therefore the total PAH concentration is expressed as B[a]Peq to illustrate the toxic potency (Perugini et al., 2007). The B[a]Peq was calculated as the sum of the B[a]Peq i.e. value for individual PAHs. The B[a]Peqi value was calculated for each PAH from its concentration in the sample (cPAHi) multiplied by its toxic equivalency factor (TEFPAHi) as proposed earlier by (Nisbet & Lagoy, 1992).

\[
B[a]Peq = \sum(B[a]Peqi) = \sum(cPAHi \times TEFPAHi)
\]

**Estimates of dietary exposure**

The daily intake of PAHs from mushroom was calculated by multiplying the respective PAHs concentrations in each mushroom by the weight of the mushroom consumed by an average individual. Total dietary intake of B[a]P, B[a]Peq & PAH8 (carcinogenic PAHs) was also calculated by multiplying concentrations for each for each sample with the Ingestion Rate (IR) of 0.5kg (Fang et al., 2014). Consequently the daily dietary Exposure Dose level (ED) was calculated with the equation below;

\[
EDPAH= \Sigma_i PAHi \times IR \\
EDPAH8= \Sigma_iPAH8 \times IR \\
EDB[a]P= B[a]Pi \times IR \\
EDB[a]PEQ= B[a]Peq \times IR
\]

Therefore, the estimated daily intake of the different PAHs group was calculated my multiplying the Ingestion rate of mushroom (0.5kg) according to Fang et al., (2014) by the mean concentration of particular PAH group in each mushroom sample. For oral exposure of the population to mushroom, the estimated daily intake was calculated for adults, adolescents and seniors using models adopted by Falcó et al., (2003).

**Carcinogenic risk**

Incremental lifetime cancer risk (ILCR) for PAHs was calculated based on (Xia et al., 2010) method. The carcinogenic risk of a PAH mixture can be expressed by its total B[a]Peq concentration. The ILCR of population in Port Harcourt, Nigeria caused by PAHs dietary exposure in mushroom sample was calculated using the following equation;

\[
ILCR = \frac{ED \times SF \times EDB[a]Peq \times SF \times CF}{BW \times AT}
\]

Where Ef is the exposure frequency (365 days yr-1), ED is the exposure duration (70yrs), EDB[a]Peq is the exposure dose for B[a]P equivalent, SF is the oral slope factor of benzo[a]pyrene (7.3 (mg/kg day-1)-1) USEPA
2001, CF is a conversion factor (10^6 mg ng^-1), BW is the body weight (70kg) and AT is the average life span (70 years i.e 25,550 days).

Toxic equivalency factor. Toxic equivalency factor for individual PAH (PAHi) was used in the calculation of BaPeq. However, The use of the toxic equivalency factor approach for risk characterization of PAH mixtures in food was judged not scientifically valid, because of the lack of data from oral carcinogenicity studies on individual PAHs, their mode of action, and the evidence of poor predictability of the carcinogenic potency of PAH mixtures based on the current toxic equivalency factor value (EFSA, 2008; SCF, 2002). The European Food Safety Authority CONTAM Panel suggested the use of the margin of exposure (MOE) approach for risk assessment.

Margin of exposure (MOE). The MOE is the ratio of the benchmark dose lower limit (BMDL10) to the estimated human intake of the compound. The BMDL10 is the lower bound of a 95% confidence interval on the Benchmark Dose corresponding to a 10% tumor incidence. This was chosen as a reference point in the dose-response curve ((EFSA, 2008; Larsen, 2006)). The calculated BMDL10 values were 0.07, 0.17, 0.34, and 0.49 mg/kg of body weight (bw) per day for BaP, PAH2, PAH4, and PAH8, respectively. The MOE from each mushroom sample consumption was estimated using the following equation:

\[ \text{Margin of exposure (MOE)} = \frac{\text{BMDL}^{10}}{\text{Daily Intake}} \times 10^6 \]

**Results**

The concentrations of sixteen (16) priority PAHs namely Naphthalene, Acenaphthylene, Acenaphthene, Anthracene, Phenanthrene, Fluorene, Fluoranthene, Pyrene, Benzo[a]Anthracene, Chrysene, Benzo[a] Pyrene, Benzo[b]Fluoranthene, Benzo[K]Fluoranthene, Benzo[g,h,i]Perylene, Dibenzo[a,h]Anthracene and Indeno[1,2,3-cd]Pyrene were all analysed for their presence in mushroom samples obtained from ten different locations in Niger-Delta, Nigeria and their individual background/substrate concentrations is presented in Table 2. In the present study, the PAHs content of Mushroom ranged from 0.02 mg/kg to 3.37 mg/kg for Fluoranthene in Pleurotus tuber-regium and pleotus ostreatus mushroom samples. Both mushroom samples with highest and least concentrations were of wild sources. For ∑PAHs in the mushroom samples, the least was observed in cultivated specie of Pleurotus ostreatus while the highest concentration was in wild Pleurotus tuber-regium mushroom sample from Alakahia Forest. In all mushrooms, benzo[a]pyrene, benzo[a]pyrene and indeno[1,2,3-cd]pyrene were abundant. Food consumption is a significant route of exposure to PAHs. Food can be contaminated with PAHs from environmental sources, industrial food processing and from certain home cooking practices (EFSA, 2008). Mushroom and other farm produce may add to the body burden of PAHs in Nigerian population. In Nigeria, a total PAHs of 10 mg/kg was reported in oyster mushroom while individual PAHs namely Fluoranthene (0.06 mg/kg), Acenaphthylene (0.22 mg/kg), 2-Methylnaphtalene (0.26 mg/kg), Phenanthrene (0.32 mg/kg), Benzo (k) fluoranthene (0.37 mg/kg), Benzo (a) anthracene (0.42 mg/kg), Benzo (b) fluoranthene (0.46 mg/kg), Benzo (a) pyrene (0.54 mg/kg) and chrysene (5.30 mg/kg) was reported in oyster mushroom found on polluted soils by Adedokun (2015). Food crops like spinach (8.977 μg/kg), potatoes (6.196 μg/kg), apple (2.867 μg/kg) and guava (2.334 μg/kg) were reported with PAHs content in Egypt (Abou-Arab, 2014). Elsewhere in Brazil, the mean levels of total PAHs were 13.53 μg/kg in lettuce, 9.50 μg/kg in tomatto, 8.86 μg/kg in cabbage, 4.05 μg/kg in apple, 3.77 μg/kg in grape and 3.87 μg/kg in pear (Rojo Camargo and Toledo, 2003).Another study also found that the highest concentration of PAHs was in the leafy vegetable, followed by melon and fruit vegetable, while the lowest concentration was found in the rhizome vegetable (Shen et al., 2007). Higher PAHs levels were detected in some of the mushroom samples in the present study compared to PAHs levels in vegetables found in Egypt (Abou-Arab, 2014) and Brazil (Rojo Camargo and Toledo, 2003). The elevated levels observed in the PAHs may be due to contamination from soil, food, substrate, rain water and packaging materials (WHO, 2005). In all mushroom and background (soil/substrate) samples, benzo[ghi]perylene, benzo[a]pyrene and pyrene were most abundant while naphthalene, acenaphthylene, acenaphthene, dibenz[a,h]anthracene and benzo[k]fluoranthene were not detected.

As shown in Table 2, the concentration of individual PAHs in mushroom soil, substrate and wood material was within the range of 0.08 mg/kg – 14.85 mg/kg for benzo[a]anthracene and Pyrene. The ∑PAHs was 0.10 mg/kg – 16.08 mg/kg with maximum concentration in mushroom soil/substrate sample 7. This is considerably lower than the result of a study carried out in two communities namely Alakahia and Eleme of Niger – Delta region of Nigeria, where Okereke et al., (2016) found PAHs range of 0.00 mg/kg – 47.45 mg/kg. Jiang et al., (2011) found PAHs concentration range of 140.7 to 2,370.8 μg/kg for 21 PAHs and 92.2 to 2,062.7 μg/kg for 16 priority PAHs in agricultural soil of Shanghai, China. The PAH levels in our study was however lower than 33.7 to 350 μg/kg in agricultural soils from Shunde, Guangdong, China, as reported by Li et al., (2008). Adetunde et al., (2014) found that the concentration of the sum of PAHs ranged from 0.2 to 254 μg/g in agricultural soils in Lagos, Nigeria. Soil is the primary steady reservoir and sinks for PAHs in the terrestrial environment, because PAHs are readily absorbed by organic matter in soil and difficult to degrade (Wild and Jones, 1995). The accumulation of PAHs in soil may lead to contamination of food chains, which could cause a potential risk to human health (Jiang et al., 2011).

The Benzo[a]Pyrene equivalence (BaPeq) for individual PAHs in the ten mushroom samples and the total Benzo[a]Pyrene equivalence (∑BaPeq) from each mushroom samples is presented in the Table 3. B[a]Peq convert the concentrations of each of the targeted PAHs in the mushroom to an equivalent amount of the reference carcinogen benzo[a]pyrene (i.e., BaP equivalents), and calculates the total quantity of BaP equivalents in the material as the sum of the contributions from each
Table 1. Mushroom Sample Type and Sampling Sites

| S/N | Mushroom Type | Type of Background | Sampling Site | Latitude  | Longitude | Botanical Name/Mushroom Type |
|-----|---------------|--------------------|---------------|-----------|-----------|-------------------------------|
| 1   | S1            | Spent Mushroom Substrate (SMS) | UNIPORT mushroom Unit, Choba - Port Harcourt | 4.8939146 | 6.9107407 | Pleurotus ostreatus Cultivated |
| 2   | S2            | Spent Mushroom Substrate (SMS) | Dilomats farm, RSUST-Port Harcourt | 4.8062535 | 6.9801697 | Pleurotus ostreatus Cultivated |
| 3   | S3            | Spent Mushroom Substrate (SMS) | Dilomats farm, RSUST-Port Harcourt | 4.8062535 | 6.9801697 | Pleurotus ostreatus Cultivated |
| 4   | S4            | Soil sample containing organic matter | Igruita Forest, Rivers State. | 4.9414167 | 7.0249455 | Auricularia auricula-judae Wild |
| 5   | S5            | Wood material        | Igruita Forest, Rivers State. | 4.9429702 | 7.0266296 | Pleurotus tuber-regium Wild |
| 6   | S6            | Decaying wood material | Forest in Alakahia – Port Harcourt | 4.8787787 | 6.9249225 | Auricularia auricula-judae Wild |
| 7   | S7            | Wood material        | Forest in Alakahia – Port Harcourt | 4.8810387 | 6.9199152 | Pleurotus tuber-regium Wild |
| 8   | S8            | Soil sample          | Forest in Alakahia – Port Harcourt | 4.8797916 | 6.9195182 | Pleurotus tuber-regium Wild |
| 9   | S9            | Decaying wood material | Forest in Alakahia – Port Harcourt | 4.8809635 | 6.919649 | Pleurotus ostreatus Wild |
| 10  | S10           | Growing plant with dead parts | Forest in Alakahia – Port Harcourt | 4.8801795 | 6.9200456 | Pleurotus ostreatus Wild |

Figure 1. Map Showing the Study Area and the Samples Collected from Sampling Site.
targeted, carcinogenic. PAH (Lemieux et al., 2015). In the present study, BaPeq was calculated by the sum of B[a]Peq for each PAH using toxicity equivalent factors (Wickramasinghe et al., 2012). The B[a]Peq ranged from 0.00 in Pluerotus tuber-regium mushroom from Alakahia Forest to 2.46 in same mushroom specie. The ∑B[a]Peq ranged from 0.02 to 2.76 in mushroom 2 and mushroom 7. Benzo[a]pyrene (BAP) is the most completely studied of the PAHS, and data, while problematic, are sufficient for calculation of quantitative estimates of carcinogenic potency (Schoeny & Poirier, 2016). In Table 4, the mean concentration of PAH groups in individual mushrooms were presented. B[a]P ranged between 0.09 – 2.46 mg/kg. The range of mean concentrations for PAH4, PAH8 and PAH 16 were 0.08 – 4.29 mg/kg, 0.20 – 4.29 mg/kg and 0.20 – 8.32 mg/kg respectively. Benzo[a]pyrene,
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Table 3. BaPeq and ∑BaPeq of PAHs in Mushroom Samples

| Pahs                        | Mushroom | PAH4 | PAH8 | PAH16 |
|-----------------------------|----------|------|------|-------|
| Napthaleine                 | ND       | 1.89 | 1.89 | 4.83  |
| Acenaphthylene              | ND       | ND   | 0.2  | ND    |
| Acenaphthene                | 0.22     | 2.48 | 3.35 | 3.35  |
| Fluorene                    | 0.09     | 0.09 | 0.48 | 0.48  |
| Phenanthrene                | 0.86     | ND   | 0.26 | ND    |
| Anthracene                  | ND       | ND   | ND   | ND    |
| Fluoranthe      | 2.46     | 4.3  | 7.29 | 8.32  |
| Pyrene                      | 0.44     | 0.44 | 0.97 | 0.97  |
| Benzo[Al]Anthracene        | 2.31     | 2.31 | 5.03 | 7.95  |
| Chrysenne                   | ND       | ND   | ND   | ND    |
| Benzo[B]Fluoranthe      | ND       | ND   | ND   | ND    |
| Benzo[K]Fluoranthe        | ND       | ND   | ND   | ND    |
| Benzo[A]Pyrene              | 1.89     | 0.22 | 0.09 | 0.09  |
| Dibenzo[A,H]Anthracene     | ND       | ND   | ND   | ND    |
| Benzo[Ghi]Perylene          | 0.29     | 0.02 | 0.09 | 0.04  |
| Indeno[1,2,3 C,D]Pyrene    | 0.97     | 0.02 | 0.31 | 0.13  |
| Total                       | 2.18     | 0.02 | 0.31 | 0.13  |

Table 4. ∑PAHs Groups in Mushroom Samples (mg/kg)

| Mushroom | B[a]P | PAH4 | PAH8 | PAH16 |
|----------|-------|------|------|-------|
| S1       | 1.89  | 1.89 | 4.83 | 4.83  |
| S2       | ND    | ND   | 0.2  | ND    |
| S3       | 0.22  | 2.48 | 3.35 | 3.35  |
| S4       | 0.09  | 0.09 | 0.48 | 0.48  |
| S5       | 0.86  | ND   | 0.26 | ND    |
| S6       | ND    | ND   | ND   | ND    |
| S7       | 2.46  | 4.3  | 7.29 | 8.32  |
| S8       | 0.44  | 0.44 | 0.97 | 0.97  |
| S9       | 2.31  | 2.31 | 5.03 | 7.95  |
| S10      | ND    | ND   | ND   | ND    |

B[a]P, Benzo[a]pyrene; PAH8, Genotoxic 8 PAHs include the sum of benz[a]anthracene, chrysene, benzo[b]fluoranthe, benzo[k]fluoranthe, benzo[a]pyrene, indeno[1,2,3-cd]pyrene, dibenzo[a,h]anthracene, and benzo[g,h,i]perylene; B[a]Peq, Benzo[a]pyrene equivalent estimation; PAH4, benz[a]anthracene, chrysene, benzo[a]pyrene, benzo[b]fluoranthe, PAH 16, naphthalene, acenapthylene, acenaphthene, fluorene, phenanthrene, anthracene, fluoranthene, pyrene, benzo[a]anthracene, chrysene, benzo[b]fluoranthe, benzo[k]fluoranthe, benzo[a]pyrene, indeno[1,2,3-cd]pyrene, dibenzo[a,h]anthracene, and benzo[g,h,i]perylene.

The estimated daily intake data as captured in Table 5 shows that the daily intakes of B[a]P were 0.00 – 0.02 mg/kg/day, for adults, adolescents and seniors respectively. The highest daily intake of B[a]P from our present study was from mushroom sample S1, S& and S9. For PAH4, the estimated daily intake was 0.00 – 0.03 mg/kg/day, 0.00 – 0.04 mg/kg/day and 0.00 – 0.03 mg/kg/day for adults, adolescents and seniors. The highest daily intake of PAH4 was from mushroom sample S7 and by adults. The estimated daily intake range of values for PAH8 and PAH16 were 0.00 – 0.05 mg/kg/day and 0.00 – 0.06 mg/kg/day for adults, adolescents and seniors. The highest daily intake of PAH4 was from mushroom sample S7 and by adults. The estimated daily intake range of values for PAH8 and PAH16 was 0.00 – 0.05 mg/kg/day and 0.00 – 0.06 mg/kg/day for adults, adolescents and seniors. The highest daily intake of PAH4 was from mushroom sample S7 and by adults.

The estimated daily intake was 0.00 – 0.03 mg/kg/day, was from mushroom sample S7 and by adults. The estimated daily intake was 0.00 – 0.03 mg/kg/day, for adults, adolescents and seniors respectively. Same mushroom sample S7 provided the highest daily intake of PAH8 and PAH16. In Greece, the dietary intake of PAHs via vegetables was recently found to range from 1.6 to 4.5 mg per person per day (Voutsas & Samra, 1998). In another study by Ibáñez et al., (2005), the mean intake of B[a]P in the Spanish population was 0.14 μg/day, and the mean intake of total PAHs was 8.57 μg/day. Duan et al., (2016) estimated the daily dietary intake due to PAHs exposure and found that benzo[a]pyrene ranged from 0.06 μg per day to 13.5 μg per day with a median of 0.69 μg per day. The estimated mean dietary intake for a standard male adult (70-kg body weight) was 6.72 μg/day was also noted in the consumption of Food by populations in Catalonia, Spain (Martorell, et al., 2010).

The margin of exposure (MOE) of B[a]P, PAH4 and PAH8 are captured in table 6. MOE of B[a]P ranged from 3,500,000.0 to 700,000.00, 3,500,000.0 and 3500000.0 to 7,000,000.0 for adults, adolescents and seniors. The MOE for PAH4 ranged from 11,333,333.0 to 34,000,000.0, 8,500,000.0 to 17,000,000.0 and 11,333,333.0 to 17,000,000.0 for adults, adolescents and seniors. For PAH8, the range of MOE was 980,000,000 to 49,000,000.0, 7,000,000.0 to 49,000,000.0 and 0.00 – 0.06 mg/kg/day for adults, adolescents and seniors respectively.
Table 7. Estimated Daily Intake of PAHs Groups in for Adults, Adolescents and Seniors (mg/kg/day)

| Mushroom | B[a]P | PAH4 | PAH8 | PAH16 |
|----------|-------|------|------|-------|
| S1       | 0.01  | 0.02 | 0.02 | 0.01  |
| S2       | ND    | ND   | ND   | ND    |
| S3       | 0.00  | 0.00 | 0.00 | 0.02  |
| S4       | 0.00  | 0.00 | 0.00 | 0.00  |
| S5       | 0.01  | 0.02 | 0.02 | 0.02  |
| S6       | ND    | ND   | ND   | ND    |
| S7       | 0.02  | 0.02 | 0.03 | 0.04  |
| S8       | 0.00  | 0.00 | 0.00 | 0.01  |
| S9       | 0.02  | 0.02 | 0.02 | 0.02  |
| S10      | ND    | ND   | ND   | ND    |

AD, Adults (70 kg); ADL, Adolescents (54.5 kg); SEN, Seniors (62.5 kg); B[a]P, Benzo[a]pyrene; PAH8, Genotoxic 8 PAHs include the sum of benz[a]anthracene, chrysene, benzo[b]fluoranthene, benzo[k]fluoranthene, benzo[a]pyrene, indeno[1,2,3-c,d]pyrene, dibenz[a,h]anthracene, and benzo[ghi]perylene; B[a]Peq, Benzo[a]pyrene equivalent estimation; PAH4, benz[a]anthracene, chrysene, benzo[a]pyrene, benzo[b]fluoranthene; PAH 16, naphthalene, acenaphtylene, acenaphtene, fluorene, phenanthrene, anthracene, fluoranthene, pyrene, benzo[a]anthracene, chrysene, benzo[b]fluoranthene, benzo[k]fluoranthene, benzo[a]pyrene, indeno[1,2,3-c,d]pyrene, dibenz[a,h]anthracene, and benzo[ghi]perylene.

Table 6. Margin of Exposure (MOE) of PAHs Groups for Adults, Adolescents and Seniors.

| Mushroom | B[a]P | PAH4 | PAH8 |
|----------|-------|------|------|
| S1       | 7,000,000 | 3,500,000 | 17,000,000 |
| S2       | ND    | ND   | ND   |
| S3       | ND    | ND   | ND   |
| S4       | ND    | ND   | ND   |
| S5       | 7,000,000 | 3,500,000 | 17,000,000 |
| S6       | ND    | ND   | ND   |
| S7       | 3,500,000 | 3,500,000 | 8,500,000  |
| S8       | ND    | ND   | ND   |
| S9       | 3,500,000 | 3,500,000 | 17,000,000 |
| S10      | ND    | ND   | ND   |

AD, Adults (70 kg); ADL, Adolescents (54.5 kg); SEN, Seniors (62.5 kg); B[a]P, Benzo[a]pyrene; PAH8, Genotoxic 8 PAHs include the sum of benz[a]anthracene, chrysene, benzo[b]fluoranthene, benzo[k]fluoranthene, benzo[a]pyrene, indeno[1,2,3-c,d]pyrene, dibenz[a,h]anthracene, and benzo[ghi]perylene; B[a]Peq, Benzo[a]pyrene equivalent estimation; PAH4, benz[a]anthracene, chrysene, benzo[a]pyrene, benzo[b]fluoranthene.

Table 7. Incremental Lifetime Cancer Risk

| Mushroom | ILCR  |
|----------|-------|
| S1       | 1.36E-06 |
| S2       | 1.25E-08 |
| S3       | 1.93E-07 |
| S4       | 7.95E-08 |
| S5       | 1.56E-08 |
| S6       | ND    |
| S7       | 1.73E-06 |
| S8       | 3.10E-07 |
| S9       | 1.59E-06 |
| S10      | ND    |

81,666,667.00 to 49,000,000.00. In this study, 100% of mushrooms with detected PAHs concentrations had MOEs above 10,000. This indicated that the consumption of mushroom from the region will be of relatively low public health concern. Benzo[a]pyrene recorded the least MOE values. EFSA’s Scientific Committee expressed the view that in general a margin of exposure of 10,000 or higher, if it is based on the ‘Benchmark-dose lower bound’ (BMDL) from an animal study, would be of low concern from a public health point of view (EFSA, 2012). In this study, all values were above the critical limit of 10000 by EFSA indicating very low public health risk concern. This is different from the study by Minmin et al., (2016) who reported that MOE of PAH4 in vegetables from Nanjing is different from the study by Minmin et al., (2016) who reported that MOE of PAH4 in vegetables from Nanjing was less than 10,000 for similar groups of adults and seniors calculated in our study.

Table 7 shows the incremental lifetime cancer risk. The incremental lifetime cancer risk ranged from 1.56x10−8 to 1.73x10−6 with highest cancer risk in wild Pleurotus tuber-regium from Alakahia forest and the least cancer risk in same wild species of mushroom from Igruita. Risks values exceeding 1x10−4 are regarded as intolerable, risks less than 1x10−6 are not regarded to cause significant health effects, and risks lying between 1x10−4 and 1x10−6 are regarded generally as satisfactory range (Hu et al., 2012). One in a million (1 x 10−6) cancer risk means that if a million people are exposed, one additional cancer case
would be expected. From our study, the life time cancer risk was within safe limits.

B[a]P (benzo[a]pyrene) has been used as a prototype carcinogenic PAH since its isolation from coal tar in the 1930’s (Rubin, 2001; Hardonnière et al., 2016). The most common mechanism of carcinogenesis induced by PAHs is DNA damage through the formation of adducts. The presence of reactive oxidative species also precipitate DNA damage. When PAHs are metabolized, reactive diol epoxide enantiomers are generated. Diol epoxides, BPDE-2, is considered its ultimate carcinogen on the basis of its binding to DNA, mutagenicity and extreme pulmonary carcinogenicity (Rubin, 2001).

These enantiomers form DNA adducts with different structures, motifs and biological activities. DNA adducts of diverse conformations are excised by DNA repair enzymes at different rates. PAH diol epoxides (PAHDEs) bind covalently to exocyclic amino groups of guanine and adenine, forming stable adducts within DNA (Lin, et al., 2001). BaP derivatives have the capacity to enter redox cycles and induce the production of reactive oxygen species (ROS), thereby causing oxidative stress (An, et al., 2011). BaP radical-cations are precursors for 6-OH-BaP. Auto-oxidation of this derivative may result in the formation of BaP quinones such as 6, 12-, 1, 6- and 3,6-BaP dione (Briede, et al., 2004). If the rate of ROS generation is greater than their removal it is likely that more DNA damage will result. PAHs may absorb light energy in UVR (280–400 nm) region and may induce DNA damage by production of ROS. For example, chrysene, induces apoptosis and DNA damage in human keratinocytes by generating ROS in response to UVB radiation (Ali, et al., 2011).

Discussion

The most important mechanism of carcinogenesis is a deficient DNA repair system in key genes involved in cell cycle control. The failure of repair mechanisms and constant exposure to PAHs induce mutagenesis in cells. These mutations are present in multiple genes including those that participate in cell survival. In particular, p53 mutations are associated with risk of carcinogenesis in PAH exposed individuals. Since the p53 protein is a transcription factor that regulates cell proliferation, differentiation, apoptosis, and DNA repair, mutations induced in this important protein could lead to severe damage in cells and genes. Some studies have associated p53 mutations to PAH exposure (Mordukhovich, et al., 2010, Yoon, et al., 2003). The assessment of damage caused by PAHs exposure is carried out by measuring specific metabolites like 1-hydroxypyrene, DNA adducts, pyrene in primary compound detection, CYP1A1 for polymorphism analysis and CYP1B1 for gene expressions (Muñoz and Albores, 2012).

Food consumption is an important pathway for human exposure to environmental contaminants. Food consumption was shown to be the main source of PAH intake and thus highlighted the importance of research on PAHs in food and the development of mitigation strategies to reduce their contents in food (SCF, 2002). PAHs can enter the food chain by deposition from air, or by deposition and transfer from soil and water (Howsam and Jones, 1998). Vegetables are important constituents of the human diet across the world and Nigeria both in terms of quantities consumed and nutritional value. PAHs have been detected in various vegetables. The principal pathway for contamination of vegetables with PAHs is gas and particle deposition, while in some cases a significant uptake of PAHs from soil was observed (Rojo et al., 2003).

In the Niger Delta region characterized by the petro-chemical industries, gasses emission and flaring, indiscriminate burning of bush, wastes and other polymer based compounds can contribute to the PAHs burden in the environment which can be deposited on plants, mushrooms and soil while others can be carried by running water. The long heavy tropical rainfall (March-October) of the Niger Delta give rise to increased run-off water known to be a factor in the PAHs deposition and transport to farm lands and other agricultural sites.

While mushroom from this study seem relatively safe for consumption, there is need to carry out more robust human health risk assessment study on the PAHs concentration in other agricultural produce within the region with a view to determining the extent of contamination and the risk associated with these natural products consumption by the general population.

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