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ORIGINAL ARTICLE

HEALTH RISK ASSESSMENT AND DIETARY EXPOSURE TO POLYCYCLIC AROMATIC HYDROCARBONS (PAHs), LEAD AND CADMIUM FROM BREAD CONSUMED IN NIGERIA

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ABSTRACT

Objective. A risk assessment and dietary exposure to polycyclic aromatic hydrocarbons (PAHs), lead and cadmium from bread, a common food consumed in Nigeria.

Material and Methods. Sixty samples of bread were collected from different types of bakeries where the heat is generated by wood (42 samples) or by electricity (18 samples) from twenty bakeries located in Gusau Zamfara (B1-B14) and Port Harcourt Rivers States (B15-B20) in Nigeria. PAHs in bread were determined by gas chromatography. Lead and cadmium were determined using atomic absorption spectrophotometry.

Results. Non-carcinogenic PAHs pyrene (13.72 µg/kg) and genotoxic PAHs (PAH8), benzo[a]anthracene (9.13 µg/kg) were at the highest concentrations. Total benzo[a]pyrene concentration of 6.7 µg/kg was detected in 100% of tested samples. Dietary intake of total PAHs ranged between 0.004-0.063 µg/kg bw. day^{-1} (children), 0.002-0.028 µg/kg day^{-1} (adolescents), 0.01-0.017 µg/kg day^{-1} (male), 0.002-0.027 µg/kg day^{-1} (female), and 0.002-0.025 µg/kg day^{-1} (seniors). The Target Hazard Quotient (THQ) for Pb and Cd were below 1. Lead ranged from 0.01-0.071 mg/kg with 10.85 and 100% of bread samples violating the permissible limit set by USEPA, WHO and EU respectively. Cadmium ranged from 0.01-0.03 mg/kg, with all bread samples below the permissible limits as set by US EPA, JECFA and EU. The daily intake of Pb and Cd ranged from 0.03-0.23 µg/kg bw day^{-1} and 0.033-0.36 µg/kg bw day^{-1} respectively. Incremental lifetime cancer risk (ILCR) was 3.8 x 10^{-7}.

Conclusions. The levels of these contaminants in bread if not controlled might present a possible route of exposure to heavy metals and PAHs additional to the body burden from other sources.

Key words: *bread, polycyclic aromatic hydrocarbons, lead, cadmium, risk assessment, Nigeria*

INTRODUCTION

Food contamination by toxic chemicals has been the subject of extensive research in the last decades. Miscellaneous classes of chemical compounds from different sources such as polycyclic aromatic hydrocarbons (PAHs) and heavy metals have been detected in foods [29]. New toxicants residues (emerging contaminants) in foods are increasing as a consequence of new industrial processes, environmental pollution and climate changes [18].

Bread is an important constituent of the Nigerian diet in terms of the quantity consumed, where majority of the population eat bread on a daily basis. Chemical contamination of bread is thus likely to affect a large proportion of the population. Bread provides energy and nutrients like vitamins, proteins, lipids and minerals all of which are essential in human diet [18]. In Nigeria bread is produced in different types of bakeries where the heat is generated by wood or by electricity, and this is why nutritional concerns is supposed to be an important issue. Recently scientists have reported the concentration of some PAHs in bread using different fuels [12, 16, 39].

The European Union have stressed and recommended that levels of PAHs are to be measured in possibly a wide variety of food products in order to obtain data on the occurrence and specific concentrations in a wide variety of matrices [7]. Occurrence of PAHs in food results from environmental deposition, but the thermal treatment used in the preparation and manufacture of foods can also

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be a relevant contamination pathway [1]. Processing procedures such as smoking, roasting, grilling, baking and frying are documented as major causes of potentially high level of food contamination by PAHs [3, 21, 29]. According to recent studies on PAHs exposure, food is the main source of human exposure to PAHs and cereals constitute one of the major contributing sources [3]. The public interest in dietary exposure to PAHs has been increasing in recent years owing to the recognition of their toxic effects and presence in different categories of raw and processed food crops [23].

Specific nourishing demands of man are fulfilled through a combination of all types of macro and micro nutrients of which some are essential metals. Some authors have reported the presence of heavy metals in bread [30]. Lead and cadmium are very toxic heavy metals, and have been identified as health risks by the World Health Organization (WHO) [14]. Heavy metals are of great concern because of their toxic properties, though some metals are also essential for humans. Data on the PAHs and heavy metals (Pb and Cd) contamination of bread consumed in Nigeria is sparse. This study has employed the determination of the concentrations of lead, cadmium and PAHs, Estimated Daily Intake (EDI), Target Hazard Quotient (THQ), Hazard Index (HI) and also the Incremental Lifetime Cancer Risk (ILCR) of 16 EPA priority PAHs in an in depth risk assessment of wood and oven baked bread commonly consumed in Nigeria.

MATERIALS AND METHODS

Sampling and sampling preparations

In June 2015, freshly baked bread were purchased from twenty most popular bakeries located in Gusau Zamfara (B1-B14) and Port Harcourt Rivers States (B15-B20), Nigeria. Three loaves from each bakery, i.e sixty bread samples were collected (42 wood baked from Gusau Zamfara (B1-B14) and 18 electric oven baked breads from Port-Harcourt Rivers States (B15-B20). In order to avoid contamination after production, the oven fresh loaves were purchased directly from the bakeries and wrapped with clean aluminum foils (bread samples for PAHs analysis) and polythene bags (bread samples for Pb and Cd analyses). Samples of the three loaves from each bakery were cut into small pieces, homogenized and stored in the refrigerator in tightly sealed glass bottles prior to digestion, extraction and analysis.

Extraction and clean-up of PAHs

Glass wares were washed thoroughly with hot detergent solution followed by rinsing with purified water and acetone (analytical grade) respectively and heated in the oven at 100 °C overnight. To avoid contaminations of bread samples, different glass wares and syringes were used for standards and for solutions extracted from samples. Extraction of poly aromatic hydrocarbons from the bread samples was done with a sonicator (Ultrasonic bath-Elmsonic S40H) in accordance with US SW-846 Method 3550. Two grams of bread samples was extracted with a 50:50 mixture of acetone and methylene chloride spiked with 1 ml of PAH internal standard and shaken thoroughly before placing in an ultrasonic bath. The concentrations of 16 PAHs (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, dibenzo[a,h]anthracene, and benzo[g,h,i] perylene) were analyzed by gas chromatography (Gas Chromatograph (GC-FID) with GC recorder interfaced with a HP. The EPA-16 PAHs determination was conducted at Jaros Inspection Services Limited, Port Harcourt, Rivers State, Nigeria using Gas Chromatographic System (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 2.00 µl of extracts were injected into the GC port set at column conditions: HP-5 crosslinked PH-ME siloxane, length of 30 m, I.D: 0.25 mm, thickness of 1 µm with helium carrier gas set in the splitless, 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 analyzed compounds and results were less than 0.015 µg/kg dry weight (d.w.) for all analytes. The blank values of analytical procedure remained always below the quantification limit (LOQ) 0.05 µ g/kg d.w., estimated as 10 times. The potential for cancer effects was subsequently estimated by calculating the incremental probability that an individual will develop cancer over a lifetime as a result of chronic exposure to a particular substance (that is, above baseline lifetime risk).

Digestion and ashing of bread samples

For each sample of bread, 5 g was measured in a weighing balance using plastic materials to prevent contamination of metals. After that approximately 9 ml of 65% concentrated $HNO_3$and 3 ml perchloric acids were added in order to make a digestion prior to heating. The solution was then transferred to a hot plate where it was heated to a temperature of 120 ^{0}C for about 5 hours.

Afterwards, the sample was introduced into an oven under a temperature that was gradually increased in 10 °C every 60 minutes until the wished final temperature of 450 °C was reached 18 hours later, white ashes were obtained. Following this, samples were left to cool. The white ashes were then dissolved with 1.5% HNO_3 (5 ml) and a final volume of 25 ml was made by adding distilled water. The resulting solution was filtered. Pb and Cd concentration were determined using a Solar thermo Flame Atomic Absorption Spectrometer (S4 710).

Quality control

The instrument was recalibrated after every ten runs. The analytical procedure was checked using spike recovery method (SRM). A known standard of the metals was introduced into already analysed samples and re-analysed. The results of the recovery studies for Pb and Cd, were greater than 95%. The relative standard deviation between replicate analyses was less than 4%. The limit of detection (LOD) for Pb and Cd were 0.01 and 0.005 mg/kg respectively, with blank values reading as 0.00 mg/kg for all the metals in deionized water with electrical conductivity value of lower than 5 µS/cm. The limit of quantification LOQ for Pb was 0.01 and Cd was 0.004 mg/kg. Two-way analysis of variance (ANOVA) and a Student's t-test were used to determine whether the concentrations of the metals varied significantly, with values less than 0.05 ($p<0.05$) considered to be statistically significant. The statistical calculations were performed with Graph Pad Prism 5.0.

Lead and cadmium health risk assessment

Calculation of Estimated Daily Intake of metals (EDI)

The daily intake of metals depends on both the metal concentration in food and the daily food consumption. In addition, the body weight of the human can influence the tolerance of contaminants. The EDI was calculated based on the following formula [34].

$$EDI = \frac{C * D}{BW}$$

Where:

C - metal concentration in bread (mg/kg)

D - daily intake of bread (0.3 kg)

BW - average body weight in kg person^{-1}.

An average daily consumption of 0.3 kg [28] of bread was assumed. This method was adapted because bread is eaten as a traditional breakfast meal and as in-between meal snacks by majority of Nigerian population. Average adult body weight was considered to be 70 kg.

Calculation of Target Hazard Quotient (THQ)

Non carcinogenic risk due to lead and cadmium consumption was determined using THQ values. THQ is a ratio of the determined dose of a pollutant to a reference level considered not harmful. THQ values were determined based on the following formula (WHO 2001).

$$THQ = \frac{Efr \times ED \times FIR \times C}{RfDo \times B_{average\ weight} \times ATn \times 10^{-3}}$$

Where:

Efr - exposure frequency in 365 days year^{-1}

ED - exposure duration in 70 years (equivalent to an average lifetime) [34]

FIR - food ingestion rate in kg person^{-1}day^{-1},

C - concentration of metal in food sample, in mg/kg

RfDo - reference dose in mg/kg day^{-1}

ATn - average exposure time for non – carcinogens in days.

The following reference doses were used Pb = 4.0 x 10^{-3} and 1.0 x 10^{-3} (US EPA). THQs were calculated according to the methodology described by the Environmental Protection Agency (EPA) in the USA [4]. Doses were calculated using the standard assumption for an integrated risk analysis and an average adult body weight of 70 kg [4, 35]. In addition, based on EPA guidelines, it was assumed that ingested doses were equal to absorbed contaminant doses [4, 8, 38].

PAHs health risk assessment

Benzo[a]pyrene equivalent (B[a]p_{eq}) estimation

Benzo[a]pyrene has been characterized as the most potent carcinogenic PAH after dibenz[a,h]anthracene. Therefore the total PAH concentration is expressed as B[a]P_{eq} to illustrate the toxic potency [31]. The B[a]P_{eq} was calculated as the sum of the B[a]P_{eq} i i.e. value for individual PAHs. The b[a]p_{eq} value was calculated for each PAH from its concentration in the sample (cPAHi) multiplied by its toxic equivalency factor (TEF_{PAHi}) as proposed earlier by *Nisbet* and *LaGoy* [27] according to the formula below:

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

Estimation of dietary exposure

The daily intake of PAHs from foodstuffs was calculated by multiplying the respective concentration in each bread sample by the weight of bread consumed by an average individual from Zamfara or Rivers state. Total dietary intake of B[a]P, B[a]P_{eq} & PAH_8 (carcinogenic PAHs) were also calculated by multiplying concentrations for each bread sample with the Ingestion Rate (IR) which was assumed to be 0.3 kg. Consequently the daily dietary exposure dose level (E_D) was calculated with the equation below:

$$E_D PAH = \sum_i PAH_i \times IR$$

$$E_D PAH_8 = \sum_i PAH_8 \times IR$$

$$E_D B[a]P = B[a]P_i \times IR$$

$$E_D B[a]P_{EQ} = B[a]P_{eq} \times IR$$

The dietary exposure µg/kg body weight/day was calculated by dividing the dietary intake with the average body weight of 70 kg.

Cancer risk calculation

Incremental lifetime cancer risk (ILCR) for PAHs were calculated based on *Xia* et al. [40] method. The carcinogenic risk of a PAH mixture can be expressed by its total $B[a]P_{eq}$ concentration. The ILCR of population in Zamfara and Rivers states caused by PAHs dietary exposure in bread were calculated using the following equation;

$$ILCR = \frac{ED \times E_F \times E_D B[a]P_{eq} \times SF \times CF}{BW \times AT}$$

Where:
ED - the exposure duration (70 yrs),
E_F - the exposure frequency (365 days yr^{-1}),
$E_D B[a]P_{eq}$ - the exposure dose for B[a]P equivalent,
SF - the oral slope factor of benzo[a]pyrene (7.3 9 mg/kg $day^{-1})^{-1}$) [36]
CF - conversion factor (10^{-6} mg ng^{-1}),
BW - body weight (70 kg)
ATn - average life span (70 years i.e 25,550 days).

RESULTS

Lead and cadmium concentration

The concentration of Pb and Cd in bread samples collected from Zamfara and Rivers State respectively were within the following ranges: Pb (0.01 – 0.071 mg/kg) and Cd (0.01 – 0.03 mg/kg). The highest Pb concentration (0.071 mg/kg) was found in sample B14, this is followed by 0.062 mg/kg in sample B12. All other samples were below 0.05 mg/kg. The concentrations of Pb in wood baked bread samples ranged from 0.01 to 0.071 mg/kg, while that from electric baked bread was ranged from 0.01 to 0.042 mg/kg. In 45% of tested bread samples concentration of Cd was below the detection limit (<0.001). The highest concentration (0.031 mg/kg) was found in sample B20 followed by sample B10 (0.023 mg/kg), B1, B7, B15 (0.02 mg/kg), B2, B14 (0.011 mg/kg) and B8, B18 (0.01 mg/kg). The Pb levels found in bread (Figure 1) shows that B12 and B14 violated the permissible limit set by US EPA (0.05 mg/kg), B3, B16 and B20 fell within the limit set by WHO (0.01 mg/kg), while all the bread samples were in violation of the EU standard (0.002).The result of Cd levels found in bread (Figure 2), show that all the bread samples fell within the permissible limit as set by USEPA, WHO and EU (0.05 mg/kg).

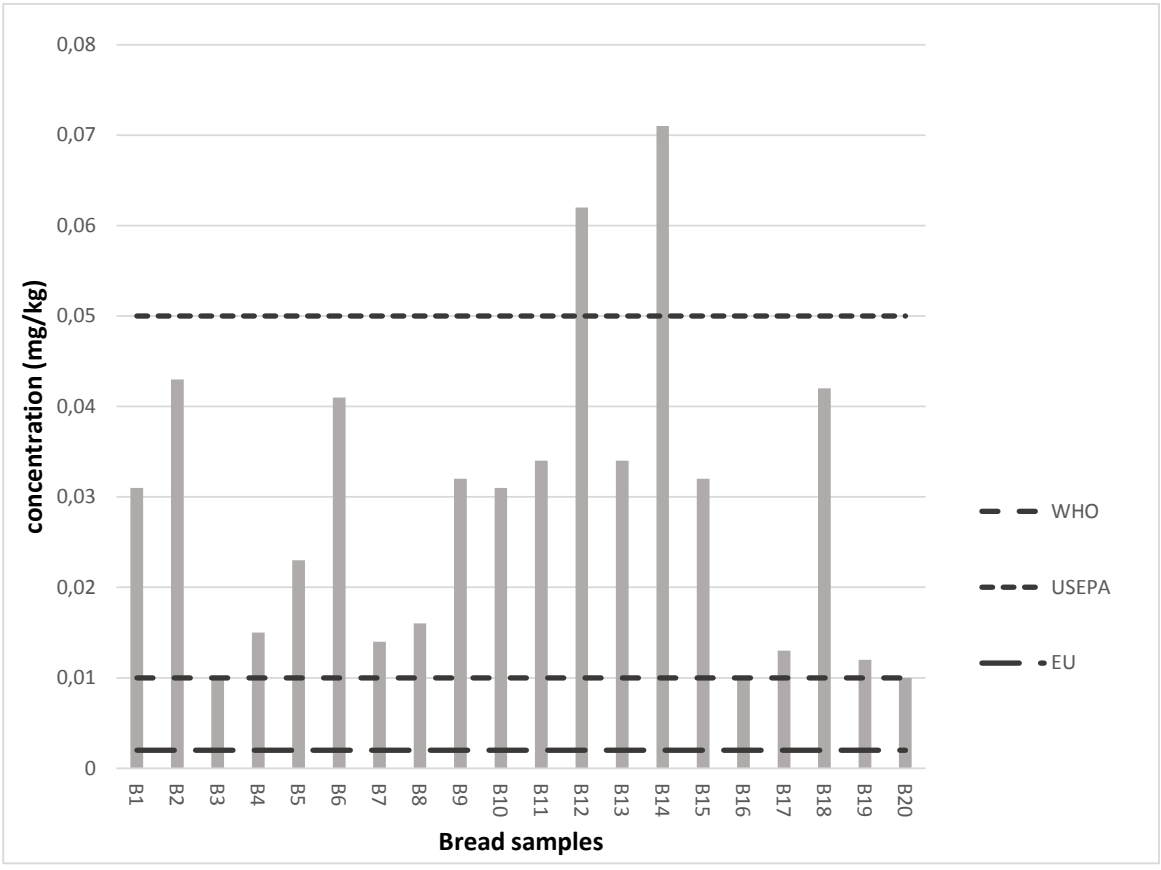

Figure 1. Lead mean concentration (mg/kg) in bread samples (B1 - B14 = wood baked bread from Zamfara and B15 - B20 = electric baked bread from Portharcourt).

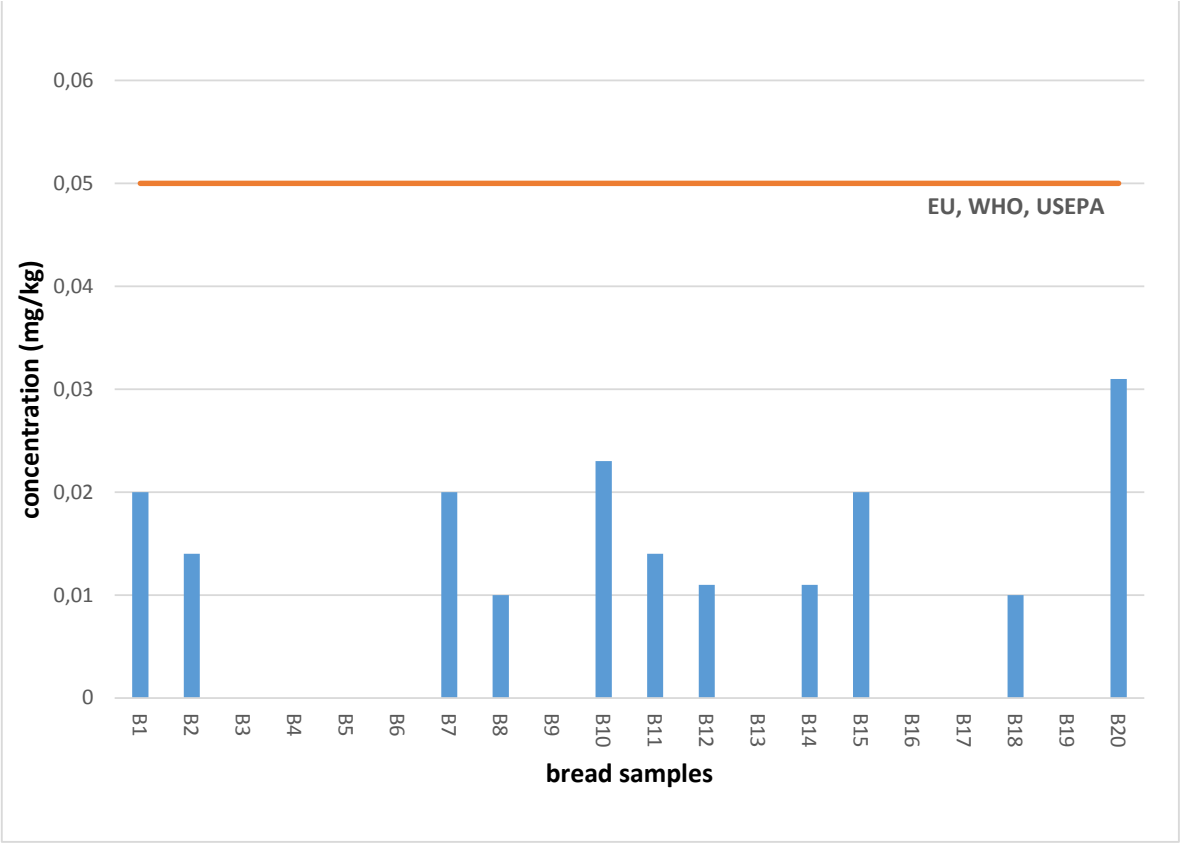

Figure 2. Cadmium mean concentration (mg/kg) in bread samples (B1- B14 = wood baked bread from Zamfara and B15 - B20 = electric baked bread from Portharcourt)

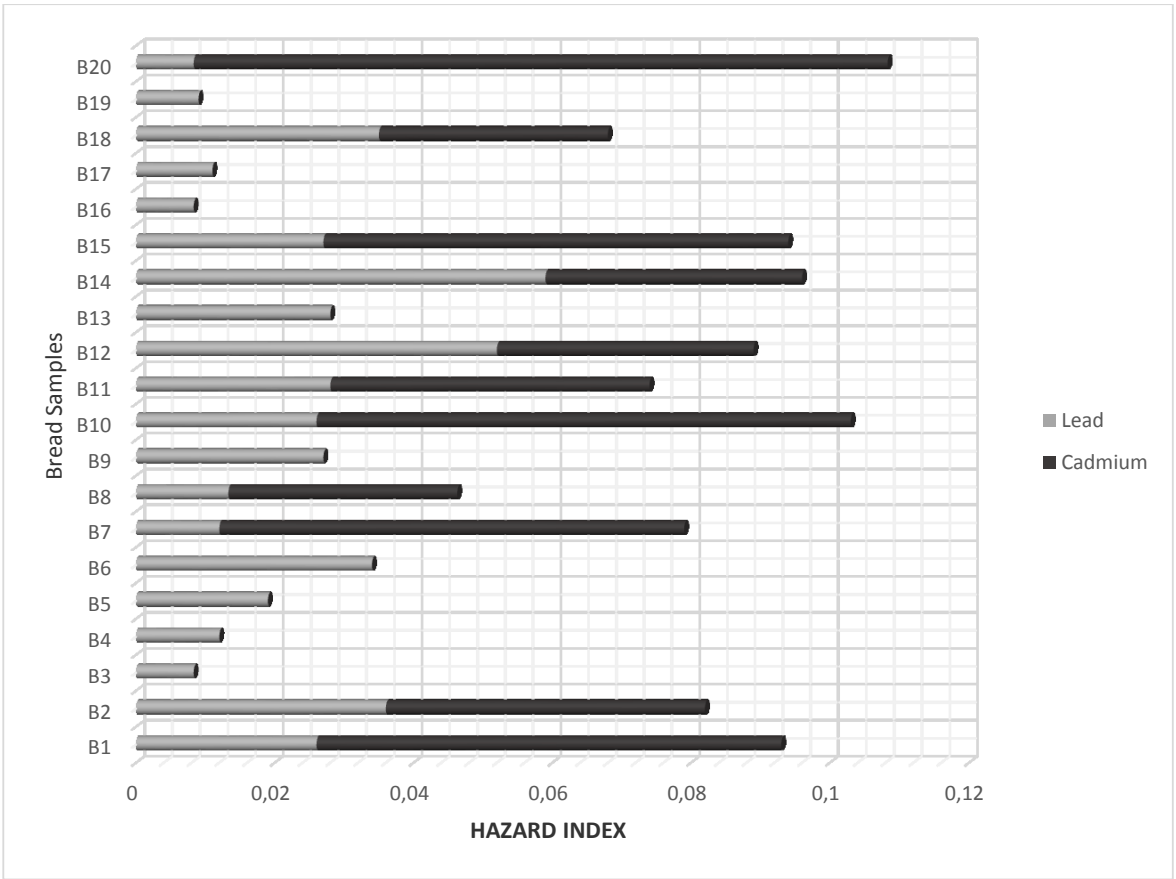

Figure 3. Hazard Index values of lead and cadmium through consumption of bread baked at bakery in Zamfara (samples B1-B14) and Portharcourt (samples B15- B20)

Table 1 shows the daily intake of Pb and Cd. The estimated daily intake (EDI) of Pb and Cd from the consumption of analysed bread showed large variations. The intake of Pb from bread consumption ranged from 0.00003 – 0.00023 mg/kg bw day^{-1}. The highest Pb intake was from consumption of B14 which was calculated from the highest concentration of Pb in wood baked bread from Zamfara state, which was lower than the TDI of lead (0.3 mg/kg bw day^{-1}) for a 60 kg individual. All tested samples were below the TDI. The estimated daily intake for Cd ranged from 0.000033 to 0.00036 mg/kg bw day^{-1}. Cd intake of in this study for all bread samples (wood or electric baked) were below the TDI.

Figures 3 show the estimated THQs from the consumption of bread (wood & electric baked). Estimated THQ values Pb and Cd ranged from 0.0083 to 0.052 and from 0.033 to 0.10 for Pb and Cd respectively. All 20 samples had THQ values less than 1.0.

Table 2 shows the distribution of individual PAHs analyzed in bread samples. The sum of the total mean levels of PAHs found in wood baked bread which ranged from 0.64 to 7.57 µg/kg were higher than in electric baked bread samples that ranged from 0.49 to 2.88 µg/kg. Among the single analyzed compounds there was a predominance of chrysene (3.3 µg/kg), benzo[a] pyrene (6.7 µg/kg) and dibenz[a,h]anthracene (7.42 µg/kg) which were found in all the samples. The highest total concentration of individual PAHs as shown in Table 2 was found to be pyrene (13.72 µg/kg). Other individual PAHs concentrations included benzo[a] anthracene (9.13 µg/kg), phenanthrene (8.25 µg/kg), benzo[g,h,i]pyrelene (6.1 µg/kg), anthracene (2.82 µg/kg) benzo[b,k]fluoranthene and indenol[1,2,3-cd] pyrene (2.28 µg/kg). The lowest total concentration for individual PAHs were those of fluoranthene (0.48 µg/kg), acenaphthalene (0.04 µg/kg) and fluorene (0.25 µg/kg). Finally naphthalene and acenaphthalene were not detected in any of the samples.

Table 1. Estimated Daily Intake of Pb and Cd (mg day^{-1} person^{-1}) with bread from bakeries for a person 60 kg b.w.

Samples	Lead	Cadmium
B1	0.0001	0.00006
B2	0.00014	0.000046
B3	0.000033	BDL
B4	0.00005	BDL
B5	0.000076	BDL
B6	0.00014	BDL
B7	0.000046	0.000066
B8	0.000053	0.000033
B9	0.00011	BDL
B10	0.0001	0.000076
B11	0.00011	0.000046
B12	0.0002	0.000036
B13	0.00011	BDL
B14	0.00023	0.00036
B15	0.00011	0.000066
B16	0.000033	BDL
B17	0.000043	BDL
B18	0.00014	0.000033
B19	0.00004	BDL
B20	0.000033	0.0001

Table 2. PAHs concentration (µg/kg) of 16 priority PAHs in tested bread samples

Samples	Nap	Ace	Acn	Fln	Phe	Ant	Flt	Pyr	B[a]A	Chr	B[bk]F	B[a]P	D[a,h]A	B[ghi]P	I[cd]P	∑PAHs
B1	ND	ND	ND	ND	ND	ND	ND	3.56	0.22	0.15	0.08	0.12	0.20	ND	0.01	4.34
B2	ND	ND	ND	ND	0.06	0.06	0.01	5.8	0.37	0.02	0.09	0.25	0.89	ND	0.02	7.57
B3	ND	ND	ND	ND	ND	ND	ND	0.52	0.89	0.33	0.19	0.77	0.88	1.13	0.22	4.95
B4	ND	ND	ND	0.11	2.04	0.68	0.07	0.13	0.02	0.33	0.05	0.19	0.22	0.28	0.06	4.18
B5	ND	ND	ND	ND	1.28	0.43	0.04	0.08	0.31	0.21	0.12	0.23	0.05	0.71	0.01	3.47
B6	ND	ND	ND	ND	ND	ND	0.05	0.44	0.06	0.24	0.14	0.55	0.63	0.81	0.16	3.08
B7	ND	ND	ND	0.05	1.02	0.14	0.04	0.06	0.25	0.17	0.09	0.39	0.04	ND	0.11	2.36
B8	ND	ND	ND	ND	ND	ND	0.03	0.22	0.37	0.14	0.08	0.32	0.37	0.47	0.09	2.09
B9	ND	ND	ND	ND	ND	ND	ND	0.18	0.06	0.04	0.05	0.21	0.11	ND	ND	0.64
B10	ND	ND	ND	ND	1.70	0.23	0.06	0.10	0.41	0.28	0.17	0.31	0.40	0.61	0.19	4.46
B11	ND	ND	ND	ND	0.16	0.14	0.02	0.69	3.66	0.02	0.38	0.17	0.68	ND	0.003	5.94
B12	ND	ND	ND	0.02	0.51	0.07	0.02	0.03	0.12	0.08	0.05	0.19	0.12	0.18	0.06	1.45
B13	ND	ND	ND	ND	ND	0.04	ND	0.05	0.36	0.02	0.03	0.05	0.17	ND	ND	0.73
B14	ND	ND	ND	ND	ND	0.85	0.09	0.66	1.12	0.41	0.25	0.97	1.10	1.42	0.28	7.13
B15	ND	ND	ND	ND	0.11	ND	ND	0.79	0.57	0.03	0.001	0.61	0.39	ND	0.33	2.83
B16	ND	ND	ND	0.04	0.73	0.09	0.03	0.04	0.03	0.12	0.07	0.13	0.17	0.26	0.08	1.80
B17	ND	ND	0.04	0.03	0.64	0.09	0.02	0.04	0.15	0.10	0.06	0.12	0.03	0.23	0.07	1.62
B18	ND	ND	ND	ND	ND	ND	ND	0.33	ND	0.03	0.02	0.07	0.06	ND	ND	0.49
B19	ND	ND	ND	ND	ND	ND	ND	ND	ND	0.57	ND	0.89	0.82	ND	ND	2.28
B20	ND	ND	ND	ND	ND	ND	ND	ND	0.16	0.03	0.39	0.16.	0.09	ND	0.59	2.88
TOTAL	ND	ND	0.04	0.25	8.25	2.82	0.48	13.7	9.13	3.32	2.31	6.7	7.42	6.1	2.28	

Keys: Naph=Naphthalene, Ace=Acenaphthalene, Acn=Acenaphthene, Fl=Flourene, Phe=Phenanthrene, Anth=Anthracene, Flo=Flouranthene, Pyr=Pyrene, BaA=Benzo[a]Anthracene, Chr=Chrysene, BbkF=Benzo[b,k]Flouranthene, BaP=Benzo[a]Pyrene, BghiP=Benzo[g,h,i]Pyrelene, DahA=Dibenzo[a,h]Anthracene, IcdP=Indeno[1,2,3,cd]Pyrene

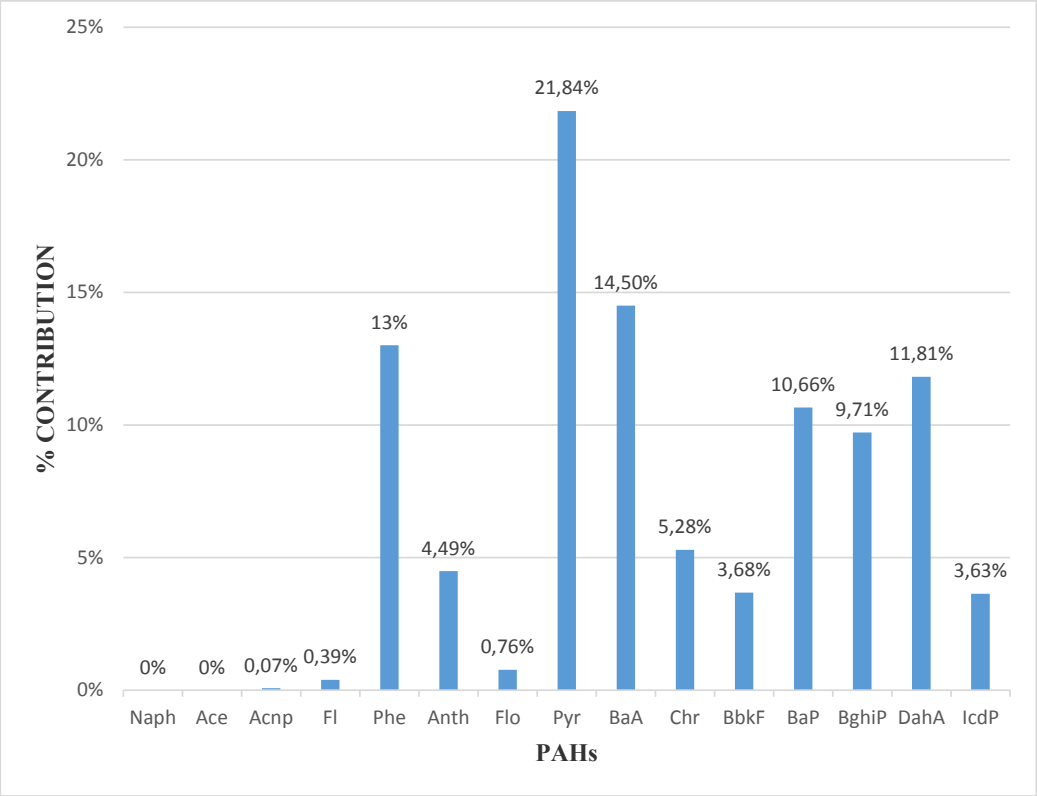

Figure 4. Relative percentage contribution of individual PAHs to the overall PAHs concentration in all bread samples

Dietary exposure to PAHs

Tables 3, 4 and 5 show the oral Exposure Doses (ED) to B[a]P, ∑B[a]Peq, ∑PAHs and ∑PAH8 with (children = 24 kg, adolescents = 54.5 kg, male = 70 kg, female = 55 kg, seniors = 62.5 kg) and without body weight adjustment. When the dietary intake of the sum of the ∑PAHs analyzed was calculated without body weight adjustment, the highest intake was from the consumption of B2 (1.51 µg/kg day^{-1}) and the least was from B9 (0.13 µg/kg day^{-1}). Carcinogenic dietary intake from B[a]P, PAH8 and B[a]Peq ranged from B13 (0.01 µg/kg day^{-1}) – B14 (0.19 µg/kg day^{-1}), B18 (0.03 µg/kg day^{-1}) – B14 (1.11 µg/kg day^{-1}) and B17,B18 (0.03 µg/kg day^{-1}) – B14 (0.45 µg/kg day^{-1}) respectively. However when the dietary intake of PAHs was expressed per kg of body weight it ranged from; Adults (0.0002 – 0.032 µg/kg day^{-1}, 0.001 – 0.019 µg/kg day^{-1} and 0.001 – 0.008 µg/kg day^{-1} for B[a]P, PAH8 and B[a]eq respectively). Children (0.004 – 0.063 µg/kg day^{-1}, 0.001 – 0.046 µg/kg day^{-1}, 0.0004 – 0.007 µg/kg day^{-1}, 0.001 – 0.019 µg/kg day^{-1} for ∑PAH, PAH8, B[a]P and B[a]P$_{eq}$). Adolescents (0.002 – 0.028 µg/kg day^{-1}, 0.0006 – 0.018 µg/kg day^{-1}, 0.0003 – 0.004 µg/kg day^{-1}, 0.0006 – 0.0083 µg/kg day^{-1} for ∑PAH, PAH8, B[a]P and B[a]P$_{eq}$). Males (0.001 – 0.017 µg/kg day^{-1}, 0.0004 – 0.016 µg/kg day^{-1}, 0.0002 – 0.003 µg/kg day^{-1}, 0.0004 – 0.006 µg/kg day^{-1} for ∑PAH, PAH8, B[a]P and B[a]P$_{eq}$). Females (0.002 – 0.027 µg/kg day^{-1}, 0.0005 – 0.02 µg/kg day^{-1}, 0.0002 – 0.004 µg/kg day^{-1}, 0.0005 – 0.008 µg/kg day^{-1} for ∑PAH, PAH8, B[a]P and B[a]P$_{eq}$).

Table 3. Estimated dietary intake of B[a]P, PAH8, ∑PAHs and ∑B[a]P$_{eq}$ from tested bread samples

		A*				B*			
Samples	Daily intake of bread	B[a]P	PAH8	∑PAHs	∑B[a]P$_{eq}$	B[a]P	PAH8	∑PAHs	∑B[a]P$_{eq}$
B1	0.3	0.02	0.05	0.87	0.07	0.0003	0.003	0.015	0.001
B2	0.3	0.05	0.34	1.51	0.24	0.0008	0.006	0.025	0.004
B3	0.3	0.15	0.89	0.99	0.36	0.0026	0.015	0.017	0.006
B4	0.3	0.04	0.23	0.84	0.09	0.0006	0.004	0.014	0.002
B5	0.3	0.05	0.33	0.69	0.07	0.0008	0.006	0.012	0.001
B6	0.3	0.11	0.52	0.62	0.25	0.0018	0.009	0.010	0.004
B7	0.3	0.08	0.21	0.47	0.09	0.0013	0.004	0.008	0.002
B8	0.3	0.06	0.37	0.42	0.15	0.0011	0.006	0.007	0.003
B9	0.3	0.04	0.09	0.13	0.07	0.0007	0.002	0.002	0.001
B10	0.3	0.06	0.47	0.89	0.16	0.001	0.008	0.015	0.003
B11	0.3	0.03	0.98	1.19	0.25	0.0006	0.016	0.020	0.004
B12	0.3	0.04	0.16	0.29	0.07	0.0006	0.003	0.005	0.001
B13	0.3	0.01	0.13	0.15	0.05	0.0002	0.002	0.003	0.001

		A*				B*			
Samples	Daily intake of bread	B[a]P	PAH8	∑PAHs	∑B[a]P$_{eq}$	B[a]P	PAH8	∑PAHs	∑B[a]P$_{eq}$
B14	0.3	0.19	1.11	1.43	0.45	0.0032	0.019	0.024	0.008
B15	0.3	0.12	0.39	0.57	0.22	0.002	0.007	0.009	0.004
B16	0.3	0.03	0.17	0.36	0.06	0.0004	0.003	0.006	0.001
B17	0.3	0.02	0.15	0.32	0.03	0.0004	0.003	0.005	0.001
B18	0.3	0.01	0.03	0.09	0.03	0.0002	0.001	0.002	0.001
B19	0.3	0.18	0.46	0.46	0.34	0.0029	0.008	0.008	0.006
B20	0.3	0.03	0.29	0.58	0.07	0.0005	0.005	0.010	0.001

A* - No b.w ; B* = Adults (male of 62.5 kg b.w.)

Table 4. Estimated dietary intake (mg/kg day^{-1} person^{-1}) of B[a]P, PAH8, ∑PAHs and ∑B[a]P$_{eq}$ for males (70 kg b.w.) and females (55 kg b.w.) from tested bread samples

		Males				Females	kg b.		
Samples	Daily intake of bread (in kg)	B[a]P	PAH8	∑PAHs	∑B[a]P$_{eq}$	B[a]P	PAH8	∑PAHs	∑B[a]P$_{eq}$
B1	0.3	3.0 E-04	2.0 E-03	1.2 E-02	1.0 E-03	4.0 E-04	3.0 E-03	1.6 E-02	1.0 E-03
B2	0.3	7.0 E-04	5.0 E-03	2.2 E-02	3.0 E-03	9.0 E-04	6.0 E-03	2.7 E-02	4.0 E-03
B3	0.3	2.0 E-03	1.3 E-02	1.4 E-02	5.0 E-03	3.0 E-03	1.6 E-02	1.8 E-02	7.0 E-03
B4	0.3	5.0 E-04	3.0 E-03	1.2 E-02	1.0 E-03	7.0 E-04	4.0 E-03	1.5 E-02	2.0 E-03
B5	0.3	7.0 E-04	5.0 E-03	9.0 E-03	1.0 E-03	8.0 E-04	6.0 E-03	1.3 E-02	1.0 E-03
B6	0.3	2.0 E-03	7.0 E-03	9.0 E-03	4.0 E-03	2.0 E-03	9.0 E-03	1.1 E-02	5.0 E-03
B7	0.3	1.0 E-03	3.0 E-03	7.0 E-03	1.0 E-03	1.0 E-03	4.0 E-03	9.0 E-03	2.0 E-03
B8	0.3	9.0 E-04	5.0 E-03	6.0 E-03	2.0 E-03	1.0 E-03	7.0 E-03	8.0 E-03	3.0 E-03
B9	0.3	6.0 E-04	1.0 E-03	2.0 E-03	1.0 E-03	8.0 E-04	2.0 E-03	2.0 E-03	1.0 E-03
B10	0.3	9.0 E-04	7.0 E-03	1.3 E-02	2.0 E-03	1.0 E-03	9.0 E-03	1.6 E-02	3.0 E-03
B11	0.3	5.0 E-04	1.4 E-02	1.7 E-02	4.0 E-03	6.0 E-04	1.8 E-02	2.2 E-02	5.0 E-03
B12	0.3	5.0 E-04	2.0 E-03	4.0 E-03	1.0 E-03	7.0 E-04	3.0 E-03	5.0 E-03	1.0 E-03
B13	0.3	1.0 E-04	2.0 E-03	2.0 E-03	7.0 E-04	2.0 E-04	2.0 E-03	3.0 E-03	9.0 E-04
B14	0.3	3.0 E-03	1.6 E-02	2.0 E-02	6.0 E-03	4.0 E-03	2.0 E-03	2.6 E-02	8.0 E-03
B15	0.3	2.0 E-03	6.0 E-03	8.0 E-03	3.0 E-03	2.0 E-03	7.0 E-03	1.0 E-02	4.0 E-03
B16	0.3	4.0 E-04	2.0 E-03	5.0 E-03	9.0 E-04	5.0 E-04	3.0 E-03	7.0 E-03	1.0 E-03
B17	0.3	3.0 E-04	2.0 E-03	5.0 E-03	4.0 E-04	4.0 E-04	3.0 E-03	6.0 E-03	5.0 E-04
B18	0.3	2.0 E-04	4.0 E-04	1.0 E-03	4.0 E-04	3.0 E-04	5.0 E-04	2.0 E-03	5.0 E-04
B19	0.3	3.0 E-03	7.0 E-03	7.0 E-03	5.0 E-03	3.0 E-03	8.0 E-03	8.0 E-03	6.0 E-03
B20	0.3.	5.0 E-04	4.0 E-03	8.0 E-03	1.0 E-03	6.0 E-04	5.0 E-03	1.1 E-02	1.0 E-03

Table 5. Estimated dietary intake (mg/kg day^{-1} person^{-1}) of B[a]P, PAH8, ∑PAHs and ∑B[a]P$_{eq}$ for children (24 kg b.w.) and adolescents (55 kg b.w.) from tested bread samples

		Children				Adolescents			
Samples	Qty of bread (in kg)	B[a]P	PAH8	∑PAHs	∑B[a]P$_{eq}$	B[a]P	PAH8	∑PAHs	∑B[a]P$_{eq}$
B1	0.3	1.0 E-03	7.0 E-03	3.6 E-02	3.0 E-03	4.0 E-04	3.0 E-03	1.5 E-02	1.3 E-03
B2	0.3	2.0 E-03	1.4 E-02	6.3 E-02	1.0 E-02	9.0 E-04	6.0 E-03	2.8 E-02	4.4 E-03
B3	0.3	6.0 E-03	3.7 E-02	4.1 E-02	1.5 E-02	2.8 E-03	1.6 E-02	1.8 E-02	6.6 E-03
B4	0.3	2.0 E-03	9.0 E-03	3.5 E-02	4.0 E-03	7.0 E-04	4.0 E-03	1.5 E-02	1.7 E-03
B5	0.3	2.0 E-03	1.4 E-02	2.9 E-02	3.0 E-03	8.0 E-04	6.0 E-03	1.3 E-02	1.3 E-03
B6	0.3	5.0 E-03	2.2 E-02	2.6 E-02	1.0 E-02	2.0 E-03	9.0 E-03	1.1 E-02	4.6 E-03
B7	0.3	3.0 E-03	9.0 E-03	1.9 E-02	4.0 E-03	1.4 E-03	4.0 E-03	9.0 E-03	1.7 E-03
B8	0.3	3.0 E-03	1.5 E-02	1.8 E-02	6.0 E-03	1.2 E-03	7.0 E-03	8.0 E-03	2.8 E-03
B9	0.3	2.0 E-03	4.0 E-03	5.0 E-03	3.0 E-03	8.0 E-04	2.0 E-03	2.0 E-03	1.3 E-03
B10	0.3	3.0 E-03	1.9 E-02	3.7 E-02	7.0 E-03	1.0 E-03	9.0 E-03	1.6 E-02	2.9 E-03
B11	0.3	1.0 E-03	4.1 E-02	4.9 E-02	1.0 E-02	6.0 E-04	1.8 E-02	2.2 E-02	4.6 E-03
B12	0.3	2.0 E-03	7.0 E-03	1.2 E-02	3.0 E-03	7.0 E-04	3.0 E-03	5.0 E-03	1.3 E-03

		Children				Adolescents			
Samples	Qty of bread (in kg)	B[a]P	PAH8	∑PAHs	∑B[a]P_{eq}	B[a]P	PAH8	∑PAHs	∑B[a]P_{eq}
B13	0.3	4.0 E-04	5.0 E-03	6.0 E-03	2.0 E-03	2.0 E-04	2.0 E-03	3.0 E-03	9.0 E-04
B14	0.3	8.0 E-03	4.6 E-02	5.9 E-02	1.9 E-02	4.0 E-03	2.0 E-02	2.6 E-02	8.3 E-03
B15	0.3	5.0 E-03	1.6 E-02	2.4 E-02	9.0 E-03	2.0 E-03	7.0 E-03	1.0 E-02	4.0 E-03
B16	0.3	1.0 E-03	7.0 E-03	1.5 E-02	3.0 E-03	5.0 E-04	3.0 E-03	7.0 E-03	1.0 E-03
B17	0.3	1.0 E-03	6.0 E-03	1.3 E-02	1.0 E-03	4.0 E-04	3.0 E-03	6.0 E-03	6.0 E-04
B18	0.3	6.0 E-04	1.0 E-03	4.0 E-03	1.0 E-03	3.0 E-04	6.0 E-04	2.0 E-03	6.0 E-04
B19	0.3	7.0 E-03	1.9 E-02	1.9 E-02	1.4 E-02	3.0 E-03	8.0 E-03	8.0 E-03	6.2 E-03
B20	0.3	1.0 E-03	1.2 E-02	2.4 E-02	3.0 E-03	6.0 E-04	5.0 E-03	1.1 E-02	1.3 E-03

DISCUSSION

Food consumption have been declared the major pathway for human exposure to environmental contaminants, accounting for over 90% of intake as compared to inhalation or dermal routes [21, 22]. The present study assessed the human health risk associated with exposure to lead, cadmium and polycyclic aromatic hydrocarbons through consumption of bread by the exposed population in Southern (Port-Harcourt) and Northern (Gusau) Nigeria.

There were higher levels of lead than cadmium in all the bread samples. The levels of these toxic metals ranged from 0.01- to 0.071 mg/kg and 0.01 – 0.03 mg/kg for Pb and Cd respectively. These values were lower than the levels found in bread in Spain (0.06 – 0.52 mg/kg and 0.1 – 0.22 mg/kg) [26]. The mean levels of Pb and Cd detected in bread sold in Baghdad was 0.33 and 0.07 mg/kg respectively [17]. The highest concentration of Pb in wood baked bread (0.071 mg/kg) was approximately two times higher than the highest level (0.032 mg/kg) found in electric baked bread. A similar study in Zaria, Nigeria [20] reported levels of Pb and Cd in bread ranging from 0.34 to 3.13 mg/kg and 0.013 – 0.098 mg/kg respectively, this finding is in agreement with our study which showed that the level of Pb were higher than Cd in bread.

In another study in southern Nigeria *Christhopher* et al. [6], reported a Cd concentration of 0.002 mg/kg in bread fluor suggesting that the bread fluor may also be a source of heavy metal contamination of bread in Nigeria prior to baking process. The non-violation of the permissible limit of Cd as set by WHO/FAO may indicate little or no risk of public health concern due to cadmium in the bread consuming. However the 100, 85 and 10% violation of the permissible limit of Pb set by EU, WHO/FAO and USEPA respectively may pose health risk. The degree of toxicity of heavy metal to humans depends on their daily intake from various sources of exposure which includes air, water and food. In order to assess the health risk of any pollutant, it is essential to estimate the level of exposure by quantifying the routes of exposure of a pollutant to the target organisms [41]. There are various possible exposure pathways of pollutants to humans but the food chain is one of the most important pathways. The Estimated Daily Intake (EDI) for Pb and Cd in our present study ranged from 0.00003 to 0.00023 mg/kg bw day^{-1} and 0.000033 – 0.00036 mg/kg bw day^{-1} respectively and are below the tolerable daily intake (TDI) for a 70 kg man. The estimated daily intake EDI of Pb and Cd from the consumption of bread samples showed large variations when compared with the Tolerable Daily Intakes (TDI) of Pb (0.005 mg/kg) and Cd (0.0004-0.002 mg/kg) as per FAO/WHO recommendations (JECFA 2014). In a similar study carried out in Iran an EDI of 0.0042 mg/kg bw day^{-1} from consumption of bread was reported by *Jawad* and *Allafaji* [17], this is higher than the concentration calculated in our present study which is lower. Generally the TDI is an estimate of daily exposure to the human population that is likely to be without an appreciable risk of deleterious effect during a lifetime (JECFA 2014).

This may be the first risk assessment on the level of PAHs in bread in Nigeria. The PAH8, B[a]P and B[a]P_{eq} ranged from 0.47 to 5.54 µg/kg, 0.05 – 0.97 µg/kg and 0.14 – 2.27 µg/kg respectively in this study. These levels are similar to the pattern of PAH levels reported by *Alomirah* et al. [1] and *Martí-Cid* et al. [21] in different types of bread. However, our result differ from the data of *Alomirah* et al. [1] that detected no B[a]P the surrogate marker of PAHs. *Kayali-Sayadi* et al. [18] reported concentration of B[a]P with ranges from 0.13 to 9.4 µg/kg in wood baked bread. This study with concentration of B[a]P ranging from 0.05 to 0.97 µg/kg is within the permissible limits (1 µg/kg) proposed by the European Union for processed cereal foods [37]. Also the PAH levels in wood baked bread in the study tend to agree with the work of European Commission [13] that reported a concentration of 2.2 µg/kg in cereal derived products. PAHs can formed as a result of certain food preparation such as baking, grilling and roasting.

High PAH concentrations have been reported in charcoal baked foods. When some foods, are cooked over an open flame, PAHs are formed usually by pyrolysis of the fats. Although the understanding of the exact

mechanism of formation of PAHs baked and roasted foods seem vague, three likely mechanisms have been suggested by *Alomirah* et al. [1] namely: the pyrolysis of organic matter (fat, protein and carbohydrates) at temperatures above 200 ºC (PAH formation is favored at a temperature range of 500-900 ºC), direct contact of lipids dripping at intense heat directly over the flame and lastly the incomplete combustion of charcoal, which can generate PAHs that are brought onto the surface of the food. Of these three postulated mechanisms, pyrolysis at high temperature and incomplete combustion of charcoal especially in wood baked bread may suffice as additional explanations for the presence of PAH in the studied bread samples from Nigeria.

Several European countries producing olive residual oil one of the ingredients used in making bread have set a maximum of 2 µg/kg for individual PAHs and 5 µg/kg for PAH8 [10]. The concentration of PAH8 were within the suggested permissible limit, although B14 with a concentration of 5.54 µg/kg showed a slight degree of violation. PAH8 represented absolutely 60% of the total contamination profile, with benzo[a]anthracene being the most concentrated compound. The total amount of carcinogenic PAHs as seen in wood baked and electric baked bread samples were 29.9 µg/kg and 6.01 µg/kg respectively which was higher than the range of PAH8 observed in other food products by *Moret* et al. [25], *Lorenzo* et al. [19] and *Djinovic* et al. [11].

The concentrations of PAHs in food and their daily intake have been studied in different parts of the world to assess the exposure of humans to this ubiquitous compound. Daily intake of $B[a]P_{eq}$ was calculated to indicate the daily consumption of carcinogenic PAHs in the sample based on the consumption of 0.3kg of bread day^{-1} and the range was between 0.07 – 0.45 µg/kg. This concentration is lower than those reported in 2010 by *Alomirah* et al [1]. The daily intake of B[a]P for a 60 kg individual ranged from 0.01 to 0.19 µg/kg. These levels were comparatively lower than those reported by *Kayali-Sayadi* et al. [18] and *Dost* and *Deli* [12].

The Agency for Toxic Substances and Disease Registry in 1995 [2, 32] established a Provisional Tolerable Weekly Intake (PTWI) of 7.3 µg/kg day^{-1} for B[a]P. In this study all bread samples are within the acceptable and the intake limit which may indicate that health risk associated with exposure to B[a]P may not be significant. There was greater presence of high molecular weight PAHs in all samples implicating combustion as a likely source [2, 33].

Although the concentration Pb and Cd in this study could be adjudged to be low notwithstanding that some samples were still in violation of some of the standard safety guidelines, the co-contamination of Pb, Cd and PAHs could likely be of significant public health importance. Co-exposure to metals and PAHs will lead to either additive or non-additive co-toxicity. In risk assessment, the non-additive effects are of greater concern since contaminants mixture toxicity is greater than the summed toxicity of each of its constituent [5]. Co-toxicity can arise from a variety of interactions, either directly among the co-occurring toxicants or indirectly through the effect of one toxicant on the various process involved in the transport, metabolism and detoxification of the co-occurring toxicant.

The estimated incremental lifetime cancer risk associated with the dietary intake of $B[a]P_{eq}$ from consumption of only bread which suggests that 4 out of every 10,000,000 individual from the population is likely to get cancer was lower than the acceptable risk level [15], but inclusion of PAHs exposure from other dietary and inhalation sources can increase this risk.

The THQ values of Pb and Cd were below 1, which indicates no health concern. The maximum acceptable concentration of 1 µg/kg for B[a]P set by European regulations for cereal processed foods was not exceeded in any of the samples. The contamination profile of PAHs, Pb and Cd in wood and electric baked bread showed no significant difference.

The major source of contaminants of bread consumed in Nigeria may be the raw materials and the different ingredients used in making the bread.

CONCLUSIONS

Results of this study suggest that the consumption of bread baked in wood or electric oven may pose no health risk for the consumers in Nigeria.

Conflict of interest

The authors declare no conflict of interest.

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