ISSN No:-2456-2165

Polycyclic Aromatic Hydrocarbons, PAHs Contamination Levels in Nigerian Staple Grains

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Abstract:- Polycyclic aromatic hydrocarbons (PAHs) are persistent environmental pollutants known to be carcinogenic, mutagenic and teratogenic to humans and other organisms. These PAHs enter the environment by combustion and fossil fuel sources and can enter food chain through plant up take from contaminated soils. In this study, PAHs contamination levels of locally produced grains in Nigeria have been assessed. Samples of various types of grains which were rice, beans, maize, guinea corn, wheat, soya beans, pigeon pea, bambara nut were purchased from various markets in Eastern Nigeria. The gram samples were extracted by sonication. The concentration levels of sixteen priority PAHs in the grains were determined using gas chromatography coupled with flame ionization detector, GC-FID. The average PAHs concentrations ($\times 10^{-2}$ µg/kg) in various rice samples ranged from 0.04 to 4.68, from 0.03 to 5.27 in maize, 0.03 to 5.64 in wheat, 0.02 to 6.55 in guinea corn, 0.03 to 4.75 in beans, 0.06 to 4.46 in soya beans, 0.03 to 5.91 in pigeon peas and 0.02 to 6.31 in bambara nut. The PAHs were wide spread being determined in all the grains. Benzo[a]pyrene showed the highest concentration in all the analyzed grains at average of 5.29 while naphthalene showed the lowest concentration at the average of 0.26. All the values were too low, at least to two orders of magnitude less than the permissible limit established by European Food Safety Authority, EFSA which is 1.0 µg/kg. These results are indicating wholesomeness of grain samples from PAHs contaminations.

***Keywords**:- Polycyclic Aromatic Hydrocarbons, Determination, Grains, Staple Foods.*

I. INTRODUCTION

Polycyclic aromatic hydrocarbons or polyaromatic hydrocarbons (PAHs) come from petroleum and fossil fuels, they enter the environment from both natural and anthropogenic sources. PAHs are neutral, non polar molecules having 2-6 aromatic rings; produced generally as a result of incomplete combustion of organic matter such as in engines, incinerators and when biomass burns in forest fires. They occur at high levels in foods like meat cooked at high temperatures over open flame or partially burnt meat (Ogbonna and Nwaocha, 2015; Miculis et al., 2011). PAHs have been identified as pollutants of serious concern because of their carcinogenicity, mutagenicity and toxicity for humans and other organisms (Crone and Lolstoy, 2010). They belong to the persistent, bioaccumulative and toxic, (PBT) substances, which if released can no longer be removed from the environment due to the fact that they accumulate in plants, animals and ultimately humans.

Studies have shown that several of them damage DNA and cause mutations which in some cases may result to cancer (Phillips 1999). Grains can be contaminated by PAHs in the environment especially through contaminated soil. PAHs can enter the food chain by deposition from air or by deposition and transfer from soil and water. Bishnoi et al., (2006) reported that leafy vegetables were found to be more contaminated with higher concentrations of PAHs when compared to underground vegetables due to their greater surface area being responsible of trapping higher concentration of PAHs from polluted air. This food may be contaminated by PAHs present in the environment or during processing such as drying, smoking and cooking like grilling, roasting and frying. Cereal and legume grains constitute major staple foods for most developing countries and serve as important sources of energy, protein, vitamin B and some mineral elements. There are many species and varieties of grains which are commonly grown and consumed in Nigeria, which form most of staple foodstuff.

As staple foods, contamination of grains with PAHs is of public health concern due to their carcinogenic and bioaccumulation properties. Both the United States Environmental Protection Agency, USEPA and International Agency for Research on Cancer, IARC listed 16 PAHs as priority pollutants. These are naphthalene (Naph), acenaphthylene (Acylene), acenaphthene (Acenaph), fluorene (Fl), phenanthrene (Phe), anthracene (Ant), fluoranthene (Fla), pyrene (Pyr), benzo[a]anthracene (B[a]A), chrysene (Chr), benzo[b]fluoranthene (B[b]F), benzo[k]fluoranthene (B[k]F), benzo[a]pyrene (B[a]P), indeno[1,2,3-cd]perylene (I[cd]P), dibenzo[a, h]anthracene (D[a, h]A) and benzo[g, h ,i]perylene (B[g, h, i]P) (USEPA, 1990; IARC, 1983). So it is necessary to determine the contamination levels of PAHs in grains grown in Nigeria. This is necessary since no such study has been carried in Eastern part of Nigeria and in order to provide data with respect to the quality of the grains.

II. MATERIALS AND METHODS

A. Sampling and Sample Preparation

Different varieties of grain samples were purchased from five major markets in the region. Varieties of each grain were pooled into composite samples as follows: beans(13), soya beans(4), pigeon peas(6), bambara nut(6), rice(6), corn(9), guinea corn(4). Altogether, a total of 48 composite were prepared for extraction of PAHs.

ISSN No:-2456-2165

B. Materials and Reagents

All reagents and solvents were of analytical grade and were purchased from Sigma Aldrich U S A. These included hexane, dichloromethane, activated alumina as well as four deuterated (surrogate) standard namely acenaphthalene d_{10}, chrysene d_{12}, phenanthrene d_{10} and perylene d_{12} .

C. Extraction of Samples

Recovery experiments to optimize PAH extraction from grain samples were carried out. Three mixed standard solutions of concentrations 100, 500 and 1000 µg/mL were prepared using four deuterated PAHs (d-PAHs). These were used to spike three 5 g portions of ground grain samples which were extracted by sonication using 3:1 dichloromethane-hexane mixture as solvent. The extracts were cleaned-up in an alumina column using the same solvent mixture.

D. Determination of PAHs

PAHs concentrations were determined with a gas chromatography equipped with flame ionization detector, GC-FID, (HP 6890). Following recoveries of 94.0 to 99.2%, the grain samples were extracted and PAHs determined by the same procedure.

E. Statistical Analysis

Analysis of variance and Pearson Correlation Coefficient at 95% confidence level were carried out using SPSS version 16.00 on the data obtained.

F. Calculation of PAH Diagnostic Ratios

The diagnostic ratios were calculated using the expression, Ant/(Phe + Ant) for LMW PAHs, B[a]A/(B[a]A + Chr) and Fla/(Pyr + Fla) for MMW PAHs then I[cd]P/(I[cd]P + B[ghi]P) for HMW PAHs (Tobiszewski and Namie´snik, 2012). The PAHs involved in each ratio have close molar mass, so it is assumed they have similar physicochemical properties based on the PAH isomer ratios in source identification compiled by Yunker et al., (2002).

G. Calculation of Total Toxicity Equivalent Concentrations (TTEC)

The total toxicity equivalent concentrations were calculated using the expression:-

$TTEC = \sum C_n \times TEF_n$; where,

TTEC = Total Toxicity Equivalent Concentration,

C_n = Concentration of the individual congener or carcinogenic PAH (cPAH) in the mixture and

TEF_n = Toxic equivalency factor of individual congener or cPAH associated with its respective mixture.

III. RESULTS

Average recoveries of PAHs ranged from 94.0 to 99.2% indicating high efficiency of the extraction procedure. Limits of detection and quantification were (0.03 - 0.09) x 10^{-3} µg/l and (0.09 - 0.25) x 10^{-3} µg/l respectively showing high efficiency of the determination procedure.

Concentrations of sixteen PAHs in various Nigerian cereal and legume grain samples are shown in Tables 1 and 2 respectively.

PAH	Rice	Maize	Wheat	Guinea Corn
Naphthalene	0.04	0.03	0.03	0.03
Acenaphthylene	0.05	0.03	0.04	0.04
Acenaphthene	1.50	1.31	2.56	0.18
Fluorene	0.55	0.06	1.89	0.02
Phenanthrene	2.33	1.81	2.10	1.86
Anthracene	5.01	4.83	5.46	4.88
Fluoranthene	1.11	0.72	0.72	0.64
Pyrene	3.13	4.91	3.93	6.14
Benzo[a]anthracene	3.51	4.28	3.27	4.48
Chrysene	1.75	1.58	3.62	0.26
Benzo[b]fluoranthene	0.50	0.45	0.34	0.45
Benzo[k]fluoranthene	0.52	0.41	0.39	0.46
Benzo[a]pyrene	4.83	5.27	5.16	6.55
Indeno[1,2,3-cd]pyrene	0.08	0.06	0.09	0.02
Dibenzo[a,h]anthracene	0.05	0.07	0.13	0.03
Benzo[g,h,i]perylene	0.42	0.22	0.25	0.29
TOTAL	25.38	26.01	31.00	26.32
Mean Total PAHs	1.59 ± 1.65	1.63 ± 1.99	1.94 ± 1.91	1.65 ± 2.39

Table 1:- Average Concentrations of PAHs (× 10^{-2} µg/kg) in Nigerian Cereal Grains

PAH	Beans	Soya beans	Pigeon pea	Bambara nut
Naphthalene	0.03	0.06	0.03	0.03
Acenaphthylene	0.10	0.13	0.08	0.05
Acenaphthene	1.30	0.63	0.94	1.03
Fluorene	0.14	0.11	0.13	0.14
Phenanthrene	4.75	2.00	3.56	3.34
Anthracene	4.35	3.37	4.77	5.52
Fluoranthene	1.08	0.93	0.78	1.08
Pyrene	3.34	3.44	6.29	6.31
Benzo[a]anthracene	2.94	3.22	5.29	5.32
Chrysene	1.75	1.48	1.61	2.58
Benzo[b]fluoranthene	0.52	0.54	0.42	0.42
Benzo[k]fluoranthene	0.42	0.32	0.43	0.47
Benzo[a]pyrene	4.69	4.46	5.91	6.01
Indeno[1,2,3-cd]pyrene	0.07	0.16	0.07	0.09
Dibenzo[a,h]anthracene	0.05	0.06	0.17	0.16
Benzo[g,h,i]perylene	0.26	0.63	0.29	0.34
Total	25.77	21.52	30.76	32.90
Mean Total PAHs	1.61 ± 1.79	1.35 ± 1.48	1.92 ± 2.36	2.06 ± 2.42

Table 2:- The Average PAHs Concentrations ($\times 10^{-2}$) µg/kg in Nigerian Legume Grains

SAMPLES	Ant/Ant+Phe	Fla/Fla+Pyr	I[cd]P/I[cd]P+B[ghi]P	B[a]A/B[a]A+Chr
Rice	0.57	0.34	0.21	0.61
Maize	0.73	0.13	0.20	0.73
Wheat	0.73	0.29	0.27	0.48
Guinea corn	0.73	0.10	0.07	0.95
Beans	0.54	0.25	0.21	0.63
Soya beans	0.63	0.21	0.20	0.68
Pigeon pea	0.57	0.11	0.19	0.77
Bambara nut	0.62	0.17	0.2	0.67
Total	5.12	1.59	1.55	5.51
Average	0.64	0.20	0.19	0.69

Table 3:- PAH Diagnostic Ratios

Grain sample	TTEC
Rice	4.75
Corn	5.82
Wheat	5.62
Guinea corn	7.31
Beans	5.11
Soya bean	4.9
Pigeon pea	6.57
Bambara nut	6.69

Table 4:- Total Toxicity Equivalent Concentration, TTEC ($\times 10^{-2}$ µg/kg)

ISSN No:-2456-2165

The sixteen PAHs were detected in all the samples. In the cereal grains (Table 1), the total concentrations (× 10^{-2} µg/kg) of two to three- ring PAHs (Naph + Acylene + Acenaph + Fl + Phe + Ant) ranged from7.01 in guinea corn to 12.26 in wheat which was 26.6 to 39.5% of total PAHs content. Anthracene was the dominant PAH among these having total concentration of 20.36 × 10^{-2} µg/kg. The total concentration of four- ring PAHs (Fla + Pyr + B[a]A + Chr) in the cereal grains varied from 9.5 in rice to 12.40 in wheat making up 37.4 to 44.2% of the total PAHs. Pyrene had the highest concentration among these four-ring PAHs with total concentration of 18.11. The five-ring PAHs (B[b]F + B[k]F + B[a]P + D[a,h]A) showed concentration levels of 5.9 in rice to 7.49 in guinea corn representing 19.4 to 28.5% of the sixteen PAHs in the cereal grains. Benzo[a]pyrene was the most abundant with total concentration of 21.81. The six-ring PAHs (I[c,d]P + B[g,h,i]P) varied from 0.28 in maize to 0.50 in rice representing just 1.1 to 2.0%. The most prominent PAHs in the cereal grains were anthracene, pyrene, benzo[a]anthracene and benzo[a]pyrene. Among these, B[a]A and B[a]P are probable human carcinogens.

Among legume samples, (Table 2), total concentrations(× 10^{-2} µg/kg) of two to three-ring PAHs varied from 6.3 in soya beans to 10.67 in beans constituting 29.3 to 41.4% of the total PAHs content. As with cereals, anthracene was dominant among the two and three ring PAHs. It has a total concentration of 18.01. The four-ring PAHs showed concentrations in the range of 9.07 in soya beans to 15.29 in bambara nut constituting 35.4 to 46.5% of total PAHs. Pyrene like in the cereals was the dominant four-ring PAH with total concentration of 19.38. The five-ring PAHs ranged from 5.38 in soya beans to 7.06× 10^{-2} µg/kg in bambara nut representing 21.5 to 25.0%. Benzo[a]pyrene was most abundant with total value of 21.07. The six-ring PAHs showed concentrations ranging from 0.33 in beans to 0.79 in soya beans, forming 1.2 to 3.7% of total PAHs content. The most dominant PAHs in the legume grains were phenanthrene, anthracene, pyrene, benzo[a]anthracene, benzo[a]pyrene. The probable human carcinogens among these dominant PAHs are B[a]P and B[a]A. The four-ring PAHs also recorded the most abundant in the legume samples and the six- ring PAHs recorded the least.

The concentrations of PAHs determined in this study are at least two orders of magnitude lower than the permissible limit (1.0 µg/kg) established by European Food Safety Authority, EFSA for cereals and cereal-based products. The EFSA established the PAHs indicators which are PAH2 (Chr + B[a]P), PAH4 (Chr + B[a]P + B[b]F + B[a]A) and PAH8 (Chr + B[a]P + B[b]F + B[a]A + B[k]F + D[a,h]A, I[c,d]P, B[g,h,i]P) as the best indicators for occurrence and effects of PAHs in foods instead of using only B[a]P as indicator. In the analyzed cereal grains, PAH2 concentrations (× 10^{-2} µg/kg) varied from 6.58 in rice to 8.78 in wheat, PAH4 from 10.59 in rice to 12.39 in wheat and PAH8 varied from 11.66 in rice to 13.25 in wheat samples. The PAH2, PAH4 and PAH8 respectively constituted 25.9 to 28.3%, 40.0 to 44.5% and 42.7 to 47.6% of total PAHs determined. The highest concentrations of PAH2, PAH4 and PAH8 occurred in bambara nut samples. The concentrations (× 10^{-2} µg/kg) of PAH2 in the analyzed legume samples ranged from 5.94 in soya beans to 8.59 in bambara nut, PAH4 from 9.92 in beans to 14.33 in bambara nut and PAH8 ranged from 10.72 in beans to 15.39 in bambara nut. The PAH2, PAH4 and PAH8 respectively formed 24.4% to 27.6%, 38.5% to 45.1% and 41.6% to 50.5% of the sixteen PAHs. The highest concentrations of PAH2, PAH4 and PAH8 were found in bambara nut.

From analysis of variance, the analyzed grain samples showed similar PAHs concentrations values, $p > 0.05$. Table 3 shows diagnostic ratios for eight PAHs. Ant/Ant + Phe ratio ranged from 0.54 to 0.73, being > 0.1 indicates combustion source. Fla/Fla + Pyr showed the range of 0.10 to 0.34, being < 0.4 shows petrogenic source. I[c,d]P/I[c,d]P + B[g,h,i]P varied from 0.07 to 0.27, this shows both petrogenic and fuel combustion sources(> 0.2 and lying between 0.2 -0.5). B[a]A/B[a]A + Chr varied from 0.48 to 0.95, this shows fuel combustion source being > 0.35.

From Table 4, total toxic equivalence concentrations for cPAH of the analyzed grains ranged from (4.75 to 7.31) × 10^{-5} mg/kg which are five orders of magnitude lower in comparison to method B clean-up level of the reference chemical (B[a]P) given as 0.137 mg/kg.

IV. DISCUSSION

PAHs concentration determined in rice (1.65 x 10^{-2} µg/kg) was two orders of magnitude less than many reported works. Xiaoxing Liu and Takashi (2001) reported the mean concentration levels of 19 ± 2.6 µg/kg dry weight for nine PAHs in polished rice. Essumang et al., (2011) reported the total PAHs concentration of 3.6 µg/kg. However the PAHs concentrations obtained in this study were below the permissible limit (1.0 µg/kg) established by EFSA for cereals and cereal products.

Generally, the average concentrations of the PAHs determined in the grains were more than two orders of magnitude less than the values of reported work the literature. Embbey et al., (2015) reported average total PAHs concentrations ranging from 45.1 to 441.3 µg/kg in canned maize, which is comparably too far above what was detected in this work also above the permissible limit. Olabemiwo (2013) reported detections of high molecular weight polycyclic aromatic hydrocarbons, HMW-PAHs in roasted corn (probable human carcinogen with exception of chrysene) with total concentration ranging from (3.70 to 5.88) × 10^{-2} µg/kg and mean value of 4.73 ± 0.82. In this study, the total concentration of HMW-PAHs was 0.17 × 10^{-2} µg/kg being comparably lower than that reported by Olabemiwo on grilled maize. Also Olabemiwo et al., (2013) in their study on PAHs determination in roasted pop corn (guguru), obtained the total 16 PAHs concentration levels of 3.84 µg/kg, being far above the value obtained in this study. The average total PAHs concentrations (in 10^{-2} µg/kg) detected in guinea corn, bambara nut, beans, soya beans and pigeon peas were respectively 26.32, 32.90, 25.77, 21.52 and 30.76. These values were too far below the values

ISSN No:-2456-2165

detected by Abou-Arab et al., (2014) in vegetables- 8.98 µg/kg in spinach and 6.20 µg/kg in potatoes. Also very high PAHs concentrations were reported by Bishnoi et al., (2006) in both leafy and underground vegetables- values ranging from 59.8 ug/kg in potatoes to 195.0 µg/kg in spinach. The PAH concentrations in bambara nut was highest showing high content of gluten protein in bambara nut (Mohammed et al., 2012). Thus the concentration of PAHs increases with gluten content. Soya bean showed the lowest average PAH concentrations among the grain samples.

Analysis of variance showed $p > 0.05$ indicating no significant difference between the PAHs concentration of the analyzed grains. The Pearson correlation coefficient analysis indicated that the PAHs concentrations of all the analyzed grains showed strong positive correlation indicating that they are from the same source. From the Table 3, based on the PAH isomer ratios in source identification complied by Yunker et al, Ant/Ant + Phe ratio being > 0.1 for all the samples indicating fuel combustion emission source. Fla/ Fla + Pyr ratio obtained was < 0.4 each in all samples indicating petrogenic emission source. While B[a]A/B[a]A + Chr for all the samples were > 0.35 indicating fuel combustion source. Then for I[c,d]P/I[c,d]P + B[g,h,i]P ratio, with exception of guinea corn and pigeon pea which were < 0.2 indicating petrogenic source, every other analyzed sample lies between 0.2 to 0.5 showing fuel combustion emission. So the two sources of PAH emission in the analyzed samples were petrogenic and combustion, combustion being the primary emission source (Yunker et al., 2002). The TTEC for the cPAHs of the analyzed grains compared very low with that of reference chemical, B[a]P indicating non-toxicity of the analyzed grains with respect to PAHs.

V. CONCLUSION

All the sixteen priority PAHs were detected in the analyzed grain samples. Most of the PAHs get into them either through plant absorption of contaminated soil or during food processing or even through vehicle emission and fuel combustion during transportation. The diagnostic ratio showed that the source is mainly combustion, even though diagnostic ratio suggested possibility of petrogenic source in addition. However these PAHs can get into human system by ingesting contaminated food, inhaling contaminated air or dermal contact with contaminated soil.

Although the PAHs concentrations obtained in this study were below the safety limit recommended by EFSA for cereals and cereal based products, these PAHs at certain significant concentration level can be very dangerous to human health. This study has provided base values for future monitoring of contamination values of the grains. Also there is assurance of high quality and safety of Nigerian grains with respect to PAHs contamination levels. All the environmental substances such as foods, soil, water and air should always be on regular analysis to ensure that they are not contaminated by PAHs beyond the safety limit.

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