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Assessment of Polycyclic Aromatic Hydrocarbons in Popularly Known Commercial Brands of Soft drinks Sold in different Cities in Nigeria

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Abstract: *Concentrations and profiles of 13 polycyclic aromatic hydrocarbons were determined in popular known commercial soft drinks sold in different cities (Aba, Abakiliki, Enugu, Onitsha, Owerri, and Porthacourt) in Nigeria using Hexane for Extraction and gas chromatography equipped with flame ionization detector for quantitative analysis. Physiochemical parameters such as pH was determined as a measure to assess the acidic strength of these drinks , the result obtained revealed the softdrink samples to be acidic with pH values of 2.40 in Onitsha to 2.48 in Abakiliki. Concentrations of the 13 Polycyclic aromatic hydrocarbons (PAHs) in these drinks ranged between 0.76- 31.26, 0.92-38.11, 9.82-14.54, 0.05-28.10, 0.045- 28.29, 9.63-21.49 mg/mL for Aba, Abakiliki, Enugu, Onitsha, Owerri and Porthacourt respectively. The polycyclic aromatic hydrocarbon profiles indicate the dominance of four and five rings PAHs in these drinks.. The study further accessed the effect of the pH values for soft drinks samples and the result obtained revealed that acidic pH of the softdrink samples increased the levels of PAHs. Six (6) out of eight (8) carcinogenic polycyclic Aromatic hydrocarbon as classified by the European Food Safety Authority (EFSA) was present in these drinks with concentration ranged from nd-28.29, nd-0.16, nd-11.85, nd- 26.62 nd-13.19, 19.93-31.96, for benzo(a)anthracene, chrysene, benzo(a)pyrene, benzo(b)fluoranthrene, dibenz(a_h)anthracene, benzo(g_h_i)perylene respectively. With the exception of dibenzi(a_h)anthrancene, benzo(a)pyrene and chrysene, the concentration values for the carcinogenic PAHs were higher than the maximum limits of 22.10 set by USEPA for water in highly industrialized area in Nigeria. The carcinogenic risk assessment code(CRAC) was accessed in these drinks, based on the tenets of CRAC benzo(g_h_i)perylene was of high risk in soft drink samples purchased from Abakilikiwhich show that people living is such city who consumes this product on daily basis would be exposed to high risk of cancer.*

Keywords: *Polycyclic aromatic hydrocarbons (PAHs), Soft drinks, Beverages, Carcinogenic, cancer, GC-FID and Nigeria.*

1. INTRODUCTION

Polycyclic aromatic hydrocarbon (PAHs) represent a class of organic compounds with carbon and hydrogen structure, having 2 or more fussed ring.[1,2] recently the environmental protection agency (USEPA) included sixteen of these PAHs compounds in its list of persistent organic Pollutants (POPs). [3,4]PAHs compounds are of public interest because it has a unique characteristic of being carcinogenic, mutagenic and tetratogenic[5] and to this effect contributes to $3.1x10^{-5}$ incremental life time of cancer risk which is major cause to the increased cancer death in Nigeria. The most widely known carcinogenic PAHs is Benzo(a)Pyrene which is considered as a suitable biomarker for it's occurrence in food and other environmental samples.[6]But recently, the European Food Safety Authority (EFSA) suggest that benzo(a) pyrene alone should not be the most suitable biomarker of carcinogenic PAHs but rather a total number of 8 heavy molecular weight PAHs namely;benzo(a)anthracene, chrysene, benzo(a)pyrene, benzo(b)fluoranthrene, benzo(k)fluoranthrene, dibenz(a,h)anthracene, benzo(g,h,i) perelyne, and indeno(1,2,3-cd) pyrene was considered as a more suitable indicator for its occurrence and effect of PAH in food.[7,8]

Globally the estimated average intake of PAHs ranged from 0.02 to 3.6 µg/per day/ per person while in Nigeria ranged from 6.0 µg/ per day/per person. [9]Besides 2-12 % inhalation exposures to PAHs, diet contributes 88-98 % of PAHs exposure especially in the case of non-smoking population.[10] soft drinks may contain large deposits of PAHs incurred through various stages of production such as boiling, heating given that levels of PAHs concentrations are highly temperature dependent. [11] These compounds can also transported through masses of air and soil into water used for industrial production of soft drinks. [12-14]The use of plastic containers for caning also constitute a major source of PAHs deposit or formation in these drinks. [15]

Highly industrialized or densely populated areas like Aba, Abakiliki, Enugu Onitsha, Owerri and Porthacourt in Nigeria is often associated with vast introduction of PAHs this is as a result of their high level of anthropogenic activities such as increased heating and boiling, expels from heavy power or gas plant, exhaust of generators, pasteurizing, smoking, urban surface run of from roads and fields, waste incineration and most importantly through emissions from motor vehicles, and other industrial processes.[16] [17]

As a general rule the lower PAHs which contains not more than four (4) tends to be more soluble, volatile and relatively mobile in the environment, [18-20] while heavy PAHs which are compounds with 5 or more aromatic rings are considered toxic and stable [21] to this effect PAHs differ in their physiochemical characteristics and ecotoxicologicaleffect on human body and its environment.

Recent studiesdone by Chukwujinduet *al*., 2015revealed the presence 16 PAH spresentin 20 samples of commercial brands of tea, coffee, cocoa based food drinks sold in Delta state (Nigeria).[22]Experiments from Olufumilayo et *al.*, 2018also revealed a total of 11 PAHs in water samples from Atlas cove Lagos, Nigeria ranged from 46-507µg/L. [23]

To the best of our knowledge there is no or inadequate data or reports of PAHs deposit in soft drinks and dietary exposure through soft drinks is more dangerous than exposure through other routes this is because, to the exception to water, soft drinks is largest consumed beverage, it's consumption was rated at 159.85g/person/day in 2007. [24]Therefore it is of utmost importance to carry out this research using a more sensitive and selective analytical method, although many analytical techniques has been widely used lately in determining these PAHs in beverages, and among the few considered were HPLC-Fluorescence [25] and GC/MS,[26]But GC-FID due to it's exceptional recovery during routine evaluation of PAHs in soft drinks is considered.[27]The result obtained from this research will be of great importance to future researchers, soft drinks manufacturers and other agencies in Nigeria like IARC, NAFDAC, UNEP, EPA by providing them with the required information given a more scientific proven advice and recommendation on the consumption of soft drink contaminated with PAHs thereby helping in solving problems of nutrition and health.

2. MATERIALS AND METHODS

The concentrations of 13 individual PAHs were measured in samples of commercially sold brand of soft drink samples. Sample extraction was done at chemistry Laboratory Imo State University while GC-FID quantitative analysis was done at Springboard laboratory opposite i7 udoka housing estate Awka, Anambra State.

2.1. Materials

Seperatory funnel, Whatman filter paper, Spatula, Weighing balance, n-Hexane (C_6H_{14}), Sodium chloride (NaCl), Sodium Hydroxide (NaOH), Distilled water, Buffer 7 and buffer 4 Tablet, Glass beaker (0-500) Ml, Conical flask (0-500) mL, 250 mL Glass measuring cylinder, Volumetric flask, Universal bottles, Oven, dessicator, Anhydrous sodium sulphate(NaSO4),Florisil(Magnesium silicate), glass wool, Pastuerpippette, Stop clock, pH meter (JENWAY 3510), AID Agilent technology 200 GC -FID Spectroscopy**.**

2.2. Sample Collection

Six (6) plastics of commercially sold soft drinks were purchased from six (6) different cities namely; Aba, Enugu, Abakiliki, Port Harcourt, Onitsha and Owerri in Nigeria during the month of July-October 2019. The purchased samples were properly stored at room temperature (25^{o}C) in a cool dry place. All necessary chemicals and reagents used were of high analytical grade and were purchased from chemi science lab Owerri, Nigeria.

2.3. pH

Jenway 3510 pH meter was used to measure the pH of the soft drink samples. The pH meter was calibrated using buffer 4 and buffer 7 by dissolving one capsule each in 100 mL of distilled water, then the electrode was introduced into each of the soft drink samples until the reading was stabilized, measurements were then taken.

2.4. Sample Extraction from Soft Drink Samples

Soft drinks samples were properly agitated and filtered through a filter paper, pH of the samples were checked and it was found to be acidic, pH of all the samples were adjusted to neutral by using 1mL each of 0.1 M NaOH. After filtration 25 mL of each Samples were taken into a seperatory funnel and 5 mL of saturated NaCl solution was added, 10 mL of n-Hexane solvent was added to extract the samples and the mixture was shaken vigorously for about 2-3minute, after proper agitation the mixture was allowed to settle, the layers were separated. The soft drinks solution formed at the upper layer while the extracted solvent were seen at lower layer, the cork of the separating funnel was opened to gradually decant the extracted solvent which was then taken for florisil clean-up.

2.5. Florisil Clean of Extracted Samples

Florisil (Magnesium silicate) was heated in an oven at 130^0 C overnight (ca.15h) and transferred to a 250ml size beaker and placed in a desicator, anhydrous $NaSO_4$ was added to 1.0g of activated flosiril (magnesium silicate) (60 – 100nm mesh) on an 8ml column plugged with glass wool. Packed column filled with 5ml n – Hexane was used for conditioning. Stopcock was opened to allow n – Hexane run out until it reached the top of sodium sulphate into a receiving vessel whilst top of the column was tapped gently the till the florisil settled well in the column. The Extract was transferred on to the column with disposable Pasteur pipette from an evaporating flaskeach evaporating flask was rinsed twice with 1ml portions of n – hexane and added to the column. Eluate was collected into an evaporating flask and rotary evaporated to dryness, Dry eluate was dissolved in 1ml n – hexane for PAH Chromatographic analysis.

2.6. GC-FID Analysis

The quantitative analysis for PAHs in the extracted solvent were done using AID Agilent Technology 200 GC-FID with A RESTEK 15METER MXT-1 column and the LC is usually estimated by integrating the areas of the resolved and unresolved, Helium was the carrier gas and a pressure 5 PSI was used for column elution. Sample injection was carried out using a syringe. The GC oven temperature was programmed first from 50ºC (hold 1 min), ramped at 10ºC to a final temperature of 110ºC (1 min).

3. RESULTS

Table 1.0.*pH of the tested soft drinks*

Locations	pH
Aba	2.42±0.12
Abakiliki	2.48±0.10
Enugu	2.46±0.10
Porthacourt	2.43±0.09
Onitsha	2.40±0.10
Owerri	2.44±0.11

Table1.1. S*howing percentage concentrations of PAHs in the soft drinks samples from their respective location.*

PAHs COMPOUDS	A% of AB	A% of AK	A% of EN	A% of ON	A% of OW	A% of PH
Acenapthene	0.76	0.92	ND	ND	ND	ND
Fluorene	34.20	3.74	16.4	11.88	11.99	16.11
Anthracene	31.26	38.11	14.54	13.19	13.30	14.28
Fluoranthene	ND	ND	9.82	ND	ND	9.63
Benzo(a)pyrene	5.78	7.21	11.85	ND	0.045	11.69
Benzo(g_h_i) perylene	26.24	31.96	21.92	19.93	20.13	21.49
Dibenzl(a_h) anthracene	ND	ND	13.19	0.05	ND	12.93
1_2 Benzathracene	0.15	ND	ND	28.10	28.29	ND

Benzo(k) fluoranthene	0.10	ND	ND	ND	ND	ND
Pyrene	14.88	18.04	ND	0.34	0.22	ND
Chrysene	ND	ND	ND	ND	0.16	ND
Benzo(b) fluoranthene	ND	ND	ND	26.62	25.87	ND
Acenapthylene	ND	ND	12.31	ND	ND	13.96

ND= Not detected, A% = percentage concentrations of the individual PAHs compounds present in the soft drink samples, AB= Aba, AK= Abakiliki, EN= Enugu, ON= Onitsha, OW = Owerri, PH =Porthacourt.

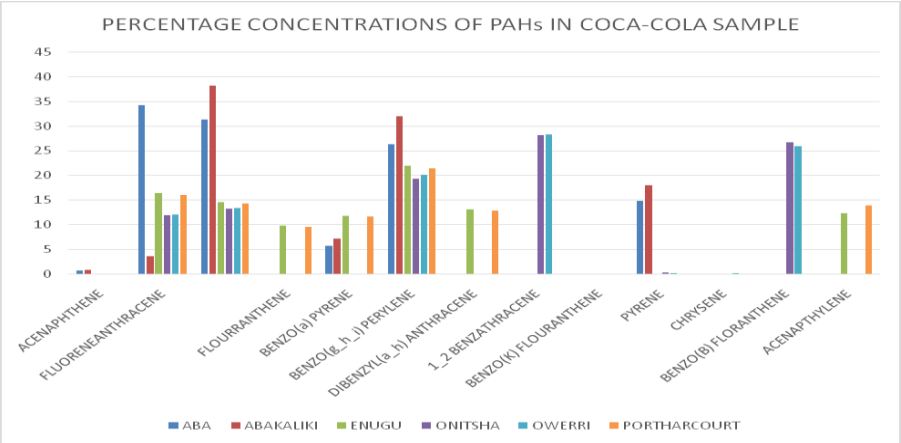

Fig1.0. *showing the percentage concentrations of PAHs in soft drink samples purchased from each locations*

A Graphical Representation of Percentage Concentration of Pahs Against pH

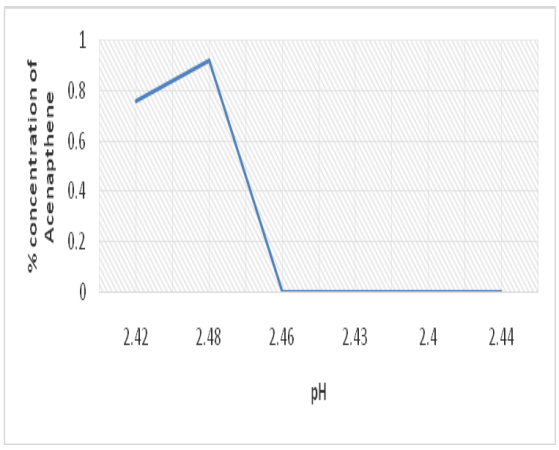

Fig 1.1. *Graphical representation of acenapthene percentage concentrations against pH*

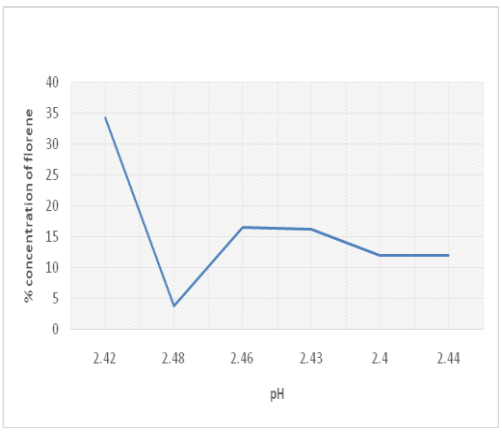

Fig 1.2. *Graphical representation of fluorene percentage concentrations against pH*

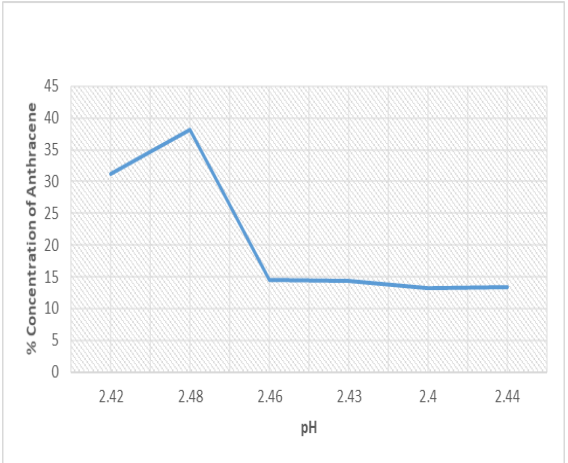

Fig1.3. *Graphical representation of anthracene percentage concentrations against pH*

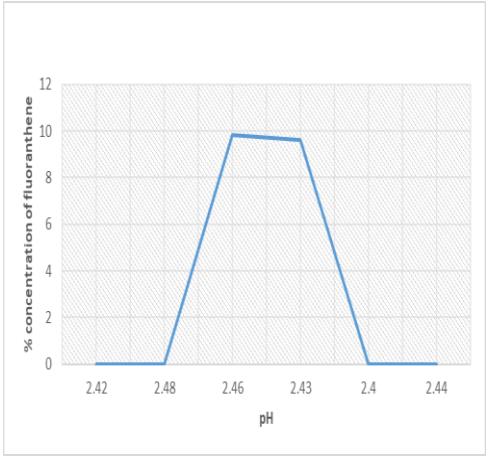

Fig1.4. *Graphical representation of fluoranthene percentage concentrations against pH*

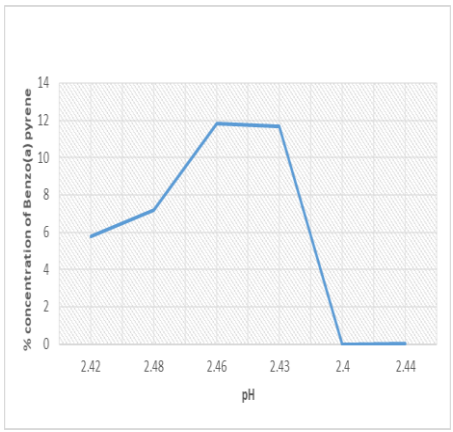

Fig1.5. *Graphical representation of benzo(a)pyrene percentage concentrations against pH*

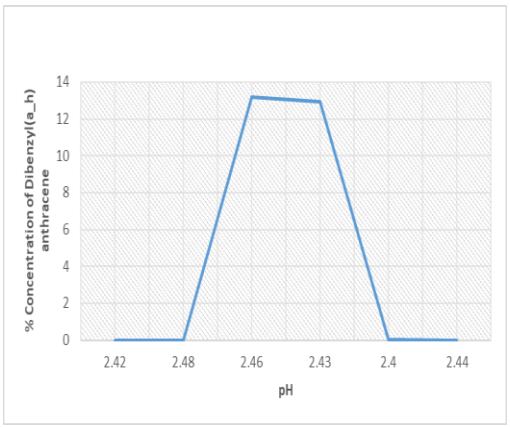

Fig 1.6. *Graphical representation of dibezyl (a_h) anthracene percentage concentrations against pH*

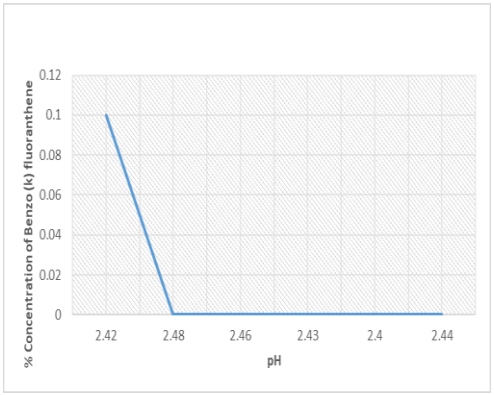

Fig. 1.7. *Graphical representation of bezo(k) fluoranthene percentage concentrations against pH*

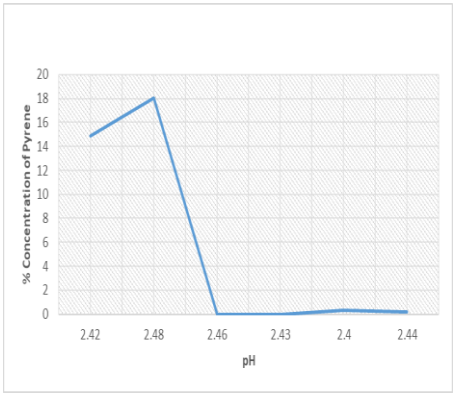

Fig1.8. *Graphical representation of pyrene percentage concentrations against pH*

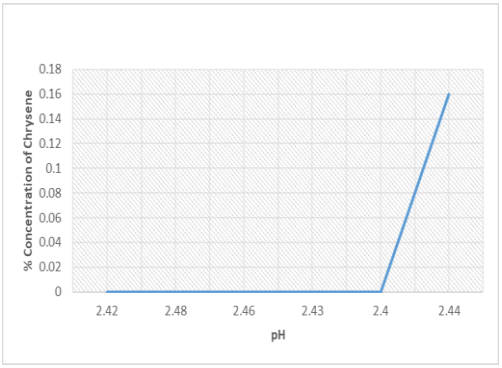

Fig1.9.*Graphical representation of chrysene percentage concentrations against pH*

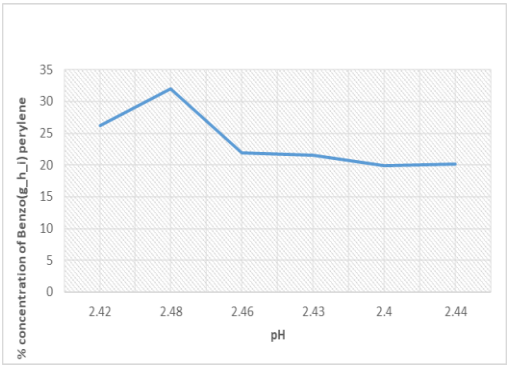

Fig1.10. *Graphical representation of benzo(g_h_I) perylene percentage concentrations against pH*

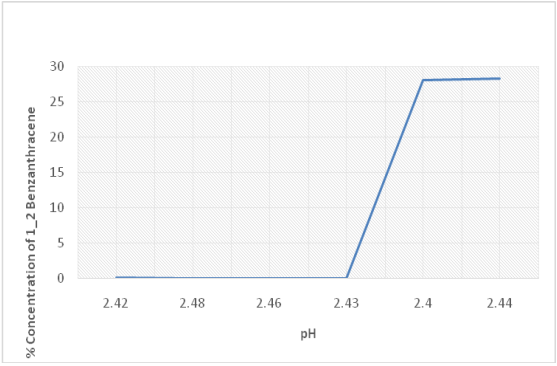

Fig1.11. *Graphical representation of 1_2 benzan thracene percentage concentration against pH*

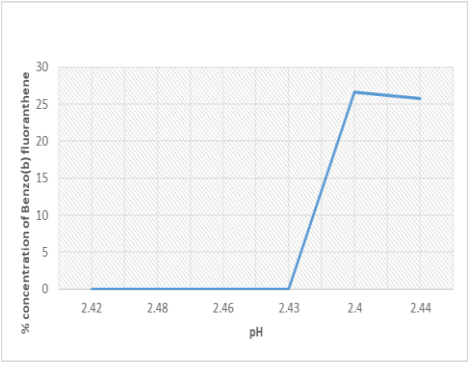

Fig 1.12. *Graphical representation of benzo(b) fluoranthene percentage concentrations against pH*

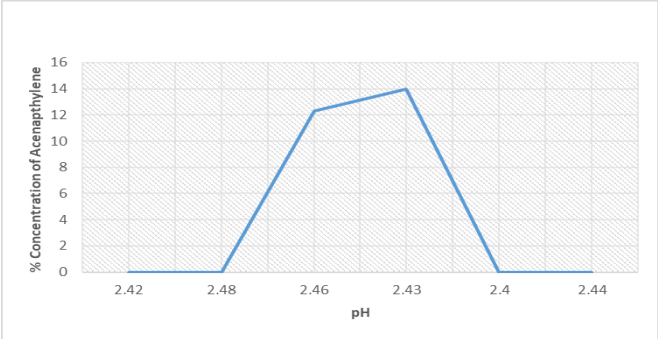

Fig 1.13. *Graphical representation of acenapthylene percentage concentrations against pH*

Table1.2. *Shows the sum of Carcinogenicity and Non-Carcinogenicity.*

Carcinogenic PAHs	Sum of carcinogenic PAHs	Non carcinogenic PAHs	Sum of non carcinogenic PAHs
1_2 Benzanthracene	56.54	Acenahthene	1.68
Chrysene	0.16	Fluorene	94.32
Benzo(a)pyrene	36.58	Anthracene	124.68
Benzo(b) fluoranthene	52.49	Fluoranthene	19.45
Benzo(k) fluoranthene	0.10	Pyrene	33.48
Dibenzl(a_h) anthracene	26.17	Acenapthylene	26.27
Benzo(g_h_i) perylene	141.67		
	∑c= 313.71		∑nc = 299.88

Carcinogenicity ratio = 1.0, % Carcinogenicity =51 %, % Non Carcinogenicity= 49 %

Table1.3. *Carcinogenic Risk assessment code (CRAC) for the soft drinks in each state*

Carcinogenic PAHs	AB	AK	EN	ON	OW	PH
1_2 Benzathracene	0.13	0	0	28.10	28.29	0
Chrysene	0	0	0	0	0.16	0
Benzo(a)pyrene	5.09	7.21	11.85	0	0.045	11.69
Benzo(b) fluoranthene	0	0	0	26.62	25.87	0
Benzo(k) fluoranthene	0.08	0	0	0	0	0
Dibenzyl(a_h) anthracene	0	0	13.19	0.05	0	12.93
Benzo(g_h_i) perylene	23.14	31.96	21.92	19.93	20.13	21.49

4. DISCUSSIONS

4.1. pH

pH is an indication of the acidic strength of the nutrient solutions in the soft drink samples.[28] From Table 1.0 the pH values of the soft drinks samples ranged from 2.40 in Onitsha to 2.48 in Abakiliki with an average mean pH of 2.44 which is in correlation with mean pH values of 2.46and 2.45 carried by Hara et al[29] and Lussi et al.[30]thus the samples were highly acidic (i.e pH <4). The acidity of the soft drink samples could be deduced as result of the presence of Phosphoric acid (H_3PO_4) added as one of the major components to balance the sweetness of sugar present and also with the carbonic acid (HCO_3) formed due to CO_2 dissociated in water (H_2O).[31]

Basically from **fig 1.1-1.13** the graphical representation of the percentage concentration against pH it can be deduced that the pH of the soft drinks samples increases as PAHs concentrations increases and vice versa. Aba with higher acidic pH of 2.42 has a higher number of PAHs deposit than the remaining samples, works from Efe et Al reveals that soils or water bodies within such industrialized area is majorly acidic owing to their improper emision from hydrocarbon production or sources[32]and the results revealed from studies carried out on formation of PAHs in water and sediment by (Abiodun, *et al*) [33] proves that acidic pH greatly influences PAHs deposition or formation.

4.2. Percentage Concentrations of PAHs

The result obtained for the percentage concentrations of PAHs in the soft drink samples from each state is represented in table 1.2 as shown above. A total number of 13 PAHs was observed in these soft drinks with percentage concentrations ranged between 0.76- 31.26 %, 0.92-38.11 %, 9.82-14.54

%, 0.05-28.10 %, 0.045- 28.29 %, 9.63-21.49 % for Aba, Abakiliki, Enugu, Onitsha, Owerri and Porthacourtre spectively. Acenapthene was present in samples from Aba and Abakiliki respectively with concentrations ranged between 0.76-0.92 mg/mL, Flourene, anthraceneand Benzo(g_h_i)) perylene was detected in all the samples with percentage concentrations ranged between 3.74-34.20, 13.19-38.11 and 19.93-31.96 respectively, works from Obini et al [15] reveals that anthracenedeposit in soil samples at auto mechanic village Abakiliki is due tomajor use of plastic containers and other petroluem products. Fluoranthene was present in samples from Enugu and Porthacourt. Benzo(a) pyrene was detected in all samples with the exception to Onitsha at percentage concentration ranged between 0.045-11.85mg/mL. The polycyclic aromatic hydrocarbon profiles indicate the dominance of four and five rings PAHs in these drinks, The lighter PAH were about9which includes namely; Acenahthene, acenapthylene, Fluorene, anthracene, Dibenzl (a_h) anthracene, chrysene, pyrene, fluoranthene, could be associated with PAHs deposits from while the heavy PAHs where about 4 namely; Benzo(a)pyrene, Benzo(k) fluoranthene, Benzo(g_h_i)perylene, Dibenzyl(a_h) anthracene.

From Table 1.2 Six(6) carcinogenic PAHs namely: Benzo(a) anthracene, chrysene, Benzo(a) pyrenebenzo (b) fluoranthene, dibenzy(a_h) anthracene, Benzo (g_h_l) perylene were identified in these sample with % concentration values ranged between nd-28.29, nd-0.16, nd-11.85, nd- 26.62 nd-13.19, 19.93-31.96, respectively. fall within the category of PAHs with the highest risk especially at prolonged exposure as reported by AATSDR[34]. With the exception of dibenzi (a_h) anthrancene, benzo(a)pyrene and chrysene, the concentration values for the carcinogenic PAHs were higher than the maximum limits of 22.10 set by USEPA for water in highly industrialized area in Nigeria. [35]

4.3. Carcinogenic Risk Assessment Code (CRAC) in the Soft Drink Samples

The model assumes that the most bioavailable fraction in the soft drink samples are likely to be absorbed in the body system hence; it is considered carcinogenic to humans. Therefore in the basic tenets of CRAC five categories are employed in the interpretation of the model result obtained in table **3.12** as follows; No Risk CRAC > 1, low Risk 1 < CRAC > 10, Medium Risk 11 < CRAC > 30, High Risk 30 < CRAC > 50, Very high Risk CRAC < 50. This is to show that in the soft drinks samples purchased from Aba all PAHs detected were of low Risk except Benzo (g_h_i) perylene which was of medium risk, same also applies to Abakiliki but Benzo (g_h_i) perylene was of a very high Risk. In considering soft drinks samples from Enugu Benzo (a) pyrene, benzo(g_h_i) perylene and Dibenzyl(a_h) anthracene were all of medium risk. Onitsha had PAHs detected to be of medium risk too same applies to Owerri and Porthcourt respectively .Benzo (g_h_i) perylene which is known to be carcinogenic was present in all soft drinks samples analyzed in this study (table 1.1) considering the tenets of CRAC in (table 1.3) Benzo (g_h_i) was of high or medium risk and it's contribution to carcinogenicity was significant. Therefore it is suggested that most people living within these cities most especially Abakiliki would be exposed to high risk of cancer. The deposition of these carcinogenic PAHs into these drinks could be attributed to ability of PAHs in the environment to have leached into water used industrially for production or would be as a result of various Anthropogenic activities in these cities such as emissions from, petroleum, coal heavy gas plants, automobile, improper waste incineration emissions from exhaust generators which is a common practice these cities.[36] Thus their accumulative effect over time through soft drinks could be detrimental to human health and may cause some of life threatening diseases such as cancer associated with presence of PAHs. [37]

5. Conclusion

The results revealed soft drinks contained significant amounts of six carcinogenic PAHs namely: benzo(a) anthracene Chrysene benzo (a) pyrenebenzo (b) fluoranthenedibenzy (a_h)anthracene and benzo(g_h_l) perylene which could pose a serious hazard to consumers who take these products on a regular basis. The PAH profiles indicate the dominance of 4-5-ring PAHs. Considering the carcinogenic Risk assessment code (CRAC) benzo(g_h_i) perylene was of a high Risk in soft drink samples from Abakiliki, Thus this study stressed the need to alleviate anthropogenic activities carried by man in these cities and also ensure good manufacturing practice among soft drinks manufacturers and also relevant agencies such as Consumers Protect Rights, National Agency for Food, Drug Administration and Control (NAFDAC) and Standard Organization of Nigeria (SON) should enforce strict compliance to the desired levels of these contaminants in beverages and Hazard Analysis Critical Control Point (HACCP) in all beverage production.

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