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CONCENTRATION OF POLYCYCLIC AROMATIC HYDROCARBONS, MICRO AND MACRO NUTRIENTS IN *Amaranthus hybridus* (GREEN LEAF) SOLD IN SELECTED MARKETS ACROSS DELTA STATE, NIGERIA

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ABSTRACT

The concentrations of US EPA 16 PAHs were determined in green leaf obtained from four (4) different markets in Delta State with a view to evaluating the extent of associated risk when exposed to the PAHs found vegetable samples. The PAHs were extracted using an ultrasonic method with n-hexane and dichloromethane, and the amounts were determined using gas chromatography-mass spectrometry. The benzo[a]pyrene carcinogenic and mutagenic equivalency factors were used to assess the health risk that PAHs in the samples posed. The sample's overall PAHs value varies from 595 to 2992 µg/kg. The compositional patterns of PAHs assayed in these samples followed the order: 5-rings>6-rings>4-rings>3-rings>2-rings. Higher total PAHs values were found in sample 4.The benzo[a]pyrene carcinogenic (BaPTEQ) equivalent factor ranges from 80.37 to 340.27 µg/kg with a mean value of 221.525 ug/kg and mutagenic (BaPMEQ) equivalency factor ranged from 122.79 to 669.89 µg/kg with a mean of 352.255 µg/kg. All the samples except sample 3 recorded both BaPTEQ and BaPMEQ values higher than the target value of 100 µg/kg. This indicates a heavy contamination with PAHs in three of the samples and there is need for urgent attention.

Keywords: PAHs, green leaf (Amaranthus hybridus), Asaba, Ughelli, Warri and Sapele

INTRODUCTION

Amaranthus hybridus (green leaf) is commonly used in numerous Nigerian dishes due to its rich nutritional and health benefits. Known as African spinach, it contains nutrients that may support chemotherapy, act as nutraceuticals, and aid in disease prevention and management. It promotes better digestion and vision, supports healthy bones and metabolism, and is a good source of zinc with cardio-protective properties (Baraniak and Kania-Dobrowolska, 2022). Additionally, it helps prevent anemia and cancer (Peter and Gandhi, 2017; Baraniak and Kania-Dobrowolska, 2022).

Amaranthus extracts have traditionally been utilized in ancient medicinal practices across India, Nepal, China, and Thailand to address various ailments such as urinary infections, gynecological issues, diarrhea, pain, respiratory problems, diabetes, and as a diuretic (Agral et al., 2008; Baral et al., 2011).

In India, the root extract of A. spinosus is used as a vermifuge by the Santhali and Paharia tribes in eastern Bihar, while a water-based decoction of the plant is employed to treat chronic diarrhea in southern Orissa (Zeashan et al., 2009). Some tribal groups use A. spinosus to induce abortion. Among the tribals in Kerala, the juice of A. spinosus is applied to reduce stomach swelling, and the leaves, boiled without salt and consumed for 2–3 days, are used to treat jaundice (Peter and Gandhi, 2017).

Polycyclic aromatic hydrocarbons (PAHs) are a group of organic compounds made up of multiple aromatic rings (Arora et al., 2015). They are generated through the incomplete combustion of organic materials and can be found wherever burning occurs and smoke is produced. A. hybridus can absorb PAHs from contaminated soils via absorption or adsorption, as well as from markets where products are openly displayed (Sun et al., 2016). Additionally, exposure can happen near sites where refuse is burned or from vehicle exhaust smoke (Duan et al., 2015).

Eating vegetables contaminated with PAHs may lead to harmful chronic health effects due to their accumulation in the body, which can also reduce the nutritional and medicinal value of the plants. PAHs are recognized as potentially carcinogenic since they can cause genetic mutations (Soceanu et al., 2014; Arora et al., 2015). Prolonged human exposure may also trigger autoimmune reactions, leading to worsening health issues (Tao et al., 2014; Wu et al., 2014). Therefore, this study aims to evaluate the levels of PAHs in A. hybridus and assess the health risks associated with their consumption, considering the plant's high content of micro and macronutrients.

MATERIAL AND METHOD

Sample Collection

Samples of *Amaranthi hybridus* (green leaves) were collected from 4 different food markets located at Warri, Ughelli, Asaba and Sapele in Delta State Nigeria. The collected samples were openly displayed for sale at the market.

Sample Preparation

The collected samples were thoroughly washed under running tap water and oven dried at 40°C until a constant weight is obtained. The dried sample was then pulverized and extracted using 60 mL mixture of n-hexane and dichloromethane in a ratio of 1:1 by ultra-sonication at 60°C for 60 minutes. The contents were filtered and the process was repeated 4 times by sonication of the residue in a fresh quantity of n-hexane and dichloromethane mixture. The extract was combined and passed through a column containing Na_2SO_4 using 60 mL of 1:1 volume mixture of dichloromethane and n-hexane (Ossai *et al*., 2015), the eluted fraction was then evaporated to 2 mL by using a rotary evaporator. Subsequently, the PAHs were eluted using 20 mL of n-hexane and dichloromethane in a volume ratio 4:1 using a mode of steam of nitrogen, the volume was reduced to about 0.5 mL after the eluted fractions were mixed. The concentrated extract was transferred into a vial bottle using a pipette, labelled appropriately and store at 4°C.

Macro and Micronutrient Evaluation Sample

Samples Preparation

The washed and oven dried samples was ground to powder using a mortar.

Sample Digestion

One gram of each powdered sample was first dissolved in 8 mL of nitric acid (HNO_3) and allowed to stand for 10hours and then heated to 120°C until the reddish-brown fumes of the acids disappeared. 3 mL of perchloric was carefully added to the digestion flask, and heated for 4minutes; then 8 mL of aqua regia was added and heated to near dryness. The digested sample was dissolved in 50 mL of deionized water and filtered using Whattman filter paper. The volume of the filtrate was then made up to 200 mL using deionized water (Rio *et al*., 2002).

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Atomic Absorption Spectrophotometer (AAS) Analysis of Samples

The macro and micronutrient evaluation of samples was determined following the method described by Onyedikach et al. (2018) using Perkin Elmer A analyst 700 flame atomic absorption spectroscopy for K, Mg, Ca, Zn, Cu, Ni, CO, Mn, Fe.

Chemical Analysis

Samples were analyzed for 16 PAHs by high resolution gas chromatography (Hewlett Packard 6890) which was equipped with flame ionization detector (FID), HP5 (cross linked PHME Siloxane) (0.020 um film thickness x 0.20 µm x 30m). 0.5 MI of extract was injected with a syringe. The temperature of injector was maintained at 250°C.The temperature was first set at 60ºC for 1 minute and then 20℃ for another 1minute ramping at 300°C for 10 minutes. The helium carrier gas was at flow rate of 1.36 mL/min (Wenceslao et al., 2011; Portella et al., 2015).

Quality Control

Estimation of the efficiency of the extraction of the PAHs compounds recovery study was carried out by spiking selected already analyzed samples with known concentration of the individual PAHs Compound. The percent recovery ranged from 89% to 90%. The reagents used were of analytic grade.

RESULTS AND DISCUSSION

The result of the study on the presence of polycyclic aromatic hydrocarbons, micro and macronutrients in *A. hybridus* (green leaf) sold in selected markets across Delta state Nigeria are presented in Tables 1 to 4.;

Table 1: Mean concentration of micro and macro nutrient ± standard deviation (n=4) (mg/kg)

Site	K	Ca	Mg	Fe	Mn	Zn	Cu	Co	Ni
Asaba	186570± 0.112	3104± 251.01	6670.41 ±211.22	623.24± 124.03	181.51± 101.50	24.10± 3.13	921± 1.12	6.17± 0.31	6.93± 1.13
Warri	18870.23 ±0.121	3071± 213.13	6924.02± 123.31	644.13± 113.10	162.31± 114.04	21.05± 52.21	9.03± 1.26	5.98± 0.15	7.01± 122
Ughelli	18451.40 ±0.11	2998± 126.15	6723.18± 351.42	617.41± 234.01	153.21± 003.21	19.87± 214.13	8.87± 1.03	6.23± 2.11	7.13± 103
Sapele	1813.53± 1.13	3212± 223.2	6663.73± 132.0	614.78± 131.23	159.10± 136.20	24.23± 235.31	9.32± 1.13	5.87± 1.1	6.83± 110
FAO/WHO	470-490	800-1000	375-400	425.5	67.9	99.4	73.3	0.2	67.9

Table 2: Concentrations (µg/kg) of PAHs in the Green Leaf Samples

Site	2 rings			3 rings				4 rings					5 rings	6rings	5rings	6rings	Total µg/kg	Mean
	NAP	ACY	ACE	FLU	ANT	PHE	FLT	PYR	BaA	CHRY	BbF	BkP	BaP	IndP	DahA	BghiP		
S1 Asaba	0.002	0.004	0.001	0.002	0.016	0.14	0.06	0.004	0.004	0.006	0.018	0.033	0.298	ND	ND	ND	0.584	0.0365
S2 Ughelli	0.168	0.06	ND	ND	ND	ND	0.002	0.166	0.126	0.028	0.260	0.132	0.080	0.096	0.126	0.102	1.346	0.0841
S3 Sapele	0.008	0.002	0.00	0.00	0.002	0.002	0.108	0.00	0.04	0.014	0.088	0.036	0.046	0.106	0.106	0.05	0.606	0.0379
S4 Warri	0.11	ND	ND	ND	ND	ND	ND	ND	0.014	0.228	0.676	0.324	0.17	0.33	0.648	0.492	2.992	0.187

Table 3: BaPTEQ and BaPMEQ (µg/kg) of PAHs in the Green Leaf Samples

Site	BaA	Chry	BbF	BkF	BaP	IndP	DahA	BaPTEQ	BaA	Chry	BbF	BkF	BaP	IndP	DahA	BaPMEQ
S1	0.004	0.001	0.018	0.33	0.298	0.0	0.00	0.651	0.033	0.010	0.045	0.036	0.298	0.00	0.00	0.422
S2	0.012	0.003	0.026	0.001	0.08	0.01	0.013	0.799	0.01	0.048	0.065	0.15	0.08	0.30	0.37	1.445
S3	0.004	0.001	0.009	0.036	0.046	0.011	0.011	0.118	0.003	0.024	0.022	0.004	0.046	0.033	0.015	0.193
S4	0.001	0.023	0.068	0.003	0.170	0.033	0.65	0.948	0.001	0.004	0.169	0.036	0.17	0.102	0.188	0.670

Table 4: Compositional Pattern of PAHs in the Green Leaf Samples

	Sites				Total	Mean
	S1 Asaba	S2 Ughelli	S3 Sapele	S4 Warri		
2Rings (%)	0.009	0.228	0.01	0.11	0.357	0.089
3Rings (%)	0.158	0	0.004	0	0.162	0.041
4Rings (%)	0.092	0.582	0.250	0.918	1.842	0.461
5Rings (%)	0.331	0.338	0.388	1.142	2.199	0.550
6Rings (%)	0	0.198	0.156	0.822	1.176	0.294

The compositional pattern of PAHs was 5rings > 4 rings > 6 rings > 2 rings > 3 rings

Potassium (K)

The mean values of K obtained varies from 18870.23 – 1813.53 (mg/kg). This stage difference in value in the sample size may be the result of varying uptake from the soil. Potassium is essential to normal cardiac function cell growth and development with daily requirement of 3000 mg for men and 2600 mg for women and 2500 mg for children, green leaf is suitable for all age. The study revealed was above the FAO/WHO permissible limit of potassium in leafy vegetable (WHO/FAO, 2022), although potassium is generally beneficial but when consumed in high proportion can cause detrimental effects on human health such as lower blood pressure and hyperkalemia (Kanda et al., 2022; D'Elia, 2024).

Calcium (Ca)

The average concentration of calcium in the study sample varies from 3212 ± 223.2 in Sapele to 2998 ± 126.15 (mg/kg) in Ughelli. Calcium is a constituent of bone and teeth and used in the management of hypokalemia with a minimum daily requirement of 1300 mg in children and 1000 mg in adult green leaves may not be a non-source of calcium. The finding from this study revealed a high concentration of calcium in the plant sample while were quite higher than the FAO/WHO permissible limit of calcium in leafy vegetables, while calcium has beneficial effect on human bones and teeth, excess calcium in the blood can pose some potential harm to human when ingested into the blood stream such osteoporosis, hypercalcemia, kidney stones etc (Fan et al., 2016).

Magnesium (Mg)

The magnesium content in studied sample ranges from 6924.02 ± 123.31 (mg/kg) in Warri to 6663.73 ± 132.09 (mg/kg) in Sapele. Magnesium is a cofactor in a cascade enzyme system reaction involving phosphate transfer neutron transmission and muscle contractibility with a daily requirement of 420 mg in older men and 360 mg in children. Green leaf is a rich source of magnesium in all the study sample. The FAO/WHO permissible limit of magnesium in leafy vegetable is 375-400 mg/kg, the study shows that A. hybridus was higher than this value, this can lead to gastrointestinal issues such as diarrhea and nausea when ingested and in severe cases muscles weakness, low blood pressure and even cardiac arrest (Sarafidis et al., 2010; Rude, 2012)

Iron (Fe)

Iron is basically used in management of iron deficiency anemia. In the study sample, the mean iron concentration ranges from 644.13±113.10 (mg/kg) in Warri to 614.78±131.23 (mg/kg) in Sapele. The permissible limit for iron established by FAO/WHO (2022) in edible plants is 425.5 mg/kg. Iron is a heavy metal and the concentration found in this study is well above the tolerable limit. This may result from harmful agricultural practices and high absorption from soil and ground water. There are numerous studies that confirms the positive correlation of Zn in plant tissues and Zn in surrounding habitat (de Figueiredo et al., 2017; Roboredo et al., 2019; Drava et al., 2019).

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An experimental exposure of 5 mM and 10 mM of Zn to common bean, Phaseolus vulgaris, has revealed a positive Zn accumulation in consequence of the exposure (de Figueiredo et al., 2017). Another study that samples common purslane Portulaca oleracea in Costa da caparica, Portugal, also revealed similar pattern of Zn accumulation. However, due to high Zn contamination in two of their sites, the P. oleracea from those sites were highly contaminated and consumption were deemed unsafe (Roboredo et al., 2019).

Manganese (Mn)

Manganese is an essential mineral required by the body, the permissible limit determined by FAO/WHO (2022) in leafy vegetables is 67.9 mg/kg. Its deficiency produces severe skeletal and reproductive abnormalities in mammals. High concentration of manganese (Mn) causes hazardous effects on lungs and brains of humans (Mohammed et al., 2022). In the study, the average concentration of manganese varies from 181.51 ±101.50 (mg/kg) in Asaba to 153.27±003.21 (mg/kg) for samples in Ughelli which was higher than FAO/WHO permissible limit of Mn in leafy vegetables. Manganese toxicity is a relatively common problem compared to other micronutrient toxicity. It is normally associated with soils of pH 5.5 or lower, but can occur whenever the soil pH is below 6.0. Possible symptoms include chlorosis and necrotic lesions on old leaves.

Zinc (Zn)

Zinc mean concentration found in the study ranges from 24.23±235.31 (mg/kg) for samples collected in Sapele to 19.87±214.13 in Ughelli. This is within the permissible of 99.4 mg/kg by FAO/WHO (2022). Zinc is one of the most essential metals for normal growth and development in humans (Mohammed et al., 2022). Zinc deficiency is of growing concern in developing countries. Zinc deficiencies have been attributed to the large consumption of vegetables. Excess Zinc can also be harmful, and cause Zinc toxicity. Zinc (Zn) accumulation in high amounts can cause eminent health problems, such as stomach cramp, skin irritation, vomiting, nausea and anemia (Mohammed et al., 2022).

Copper (Cu)

Copper is a cofactor in numerous enzymes reactions and helps the body utilize injected iron. The highest mean concentration of 9.32±1.13 mg/kg was found in samples collected from Sapele with the lowest average concentration of 8.87±1.03 mg/kg in Ughelli. The permissible limit for copper established by FAO/WHO (2022) for edible plants was 73.3 mg/kg daily. This value is well below the concentration observed in this study which with long term accumulation in the body may be hazardous to the kidney and live.

Cobalt (Co)

Cobalt concentration found in this study ranges from 6.23± 2.11 mg/kg for samples collected in Ughelli to 5.87± 1.11 mg/kg for those in Sapele. Cobalt forms part of vitamin B12 and is essential for cell function, protein synthesis, fat and oil, carbohydrate metabolism. The concentration of cobalt found in this study is significantly higher than the permissible limit of cobalt outlined by FAO/WHO (2022). Health effects that area result of the uptake of high concentrations of cobalt are; Vomiting, nausea, Vision problems, Heart problems Thyroid damage etc (Mohammed et al., 2022). Comparably Ashraf et al. (2021) reported a higher value of Co 7.56 mg/kg in soil and lower value of 0.036 mg/kg than the values in Tomato and Onion in this study.

Nickel (Ni)

Nickel is an essential micro-nutrient in the body. It is a constituent of DNA and is found in every cell which may be required for lipid metabolism with no established RDA. Nickel is also used for preventing nickel levels in the blood from getting too low (nickel deficiency). It is also used for low levels of healthy red blood cells (anemia) due to iron deficiency and treating weak and brittle bones (osteoporosis). The mean content of Nickel found in this study ranges from 7.13±103 mg/kg in Ughelli to 6.85±110 mg/kg in Sapele. The concentrations of Ni in this study were found to be higher than

the reported value of 2.97 mg/kg in vegetable by Ismail et al. (2020) and also a reported value of Ni (3.54 mg/kg) in vegetable by Akan et al. (2013). Notwithstanding, the levels of Ni determine in A. hybridus sample was observed to be lower the FAO/WHO acceptable limit of 67.9 mg/kg in leafy vegetable as specified by FAO/WHO (2022).

PAHs Concentration in Green Leaf (*A. hybridus*) Samples

The concentrations of PAHs are presented in Table 2. The result, revealed the total value range of 0.002 to 0.648 µg/kg. It was observed that Indeno[1,2,3-cd]perylene, Dibenz[a,h]anthracene and Benzo[ghi]perylene were not detected in sample 1. While acenaphthylene, acenaphthene, fluorene, anthracene, Phenanthrene, Fluoranthene and Pyrene were not detected in green leaf samples from sample 4. Also, acenaphthylene, acenaphthene, fluorene, anthracene, Phenanthrene were not detected in sample 2. With respect to Benzo[a]anthracene, Chrysene, Benzo[b|fluoranthene, benzo(k)fluorancene and Benzo[a]pyrene, data revealed that they were detected in sample 1, 2, 3 and 4. The data presented in this study were found to be lower than those in the report of Nsonwu-Anyanwu et al. (2024) who reported the concentration of PAHs in food, water and vegetable in Southern Nigeria. Additionally, their incorporation into the food chain raised a significant threat to the human health with nearly 30% of human cancer cases linked to low-level PAH exposure through dietary intake (Onyedikachi et al., 2019; Al-Nasir et al., 2022).

Data showed that PAHs levels in the samples collected are quite variable in the green leaf samples. The concentrations of 16 PAHs analysed in green leaf samples are presented in Table 1: the concentration of the summation of 16 PAHs ranged from 0.002 to 0.648 µg/kg. This concentration in green leaf are relatively higher compared with previously reported concentration ranges reported in different varieties of vegetables from Brazil and some European countries (Ledesma et al., 2016; Silva et al., 2018; Sampaio et al., 2021). The high concentration and composition of PAHs in these samples is related to the high levels oil contamination concentration of PAHs in this part of the Niger Delta, through oil exploration, gas flare and bush burning which contribute about 89% PAHs Concentration fraction in soil, vegetables absorb this contaminant from the contaminated soil.

The concentrations of PAHs in different samples collected under investigation are quite variable. The results revealed that the compositional patterns of PAHs followed the order 5-rings > 4-rings > 6-rings > 2-rings > 3-rings. The compositional pattern of PAHs is presented in Table 4. It was observed that the 5rings PAHs dominates in all samples collected with a mean value of 0.550 µg/kg. Sample 4 from Warri markets showed the highest percentage of 5 rings with concentration of PAHs 1.142% this relates to high dense environment, rapid use of fossil fuels and released of environmental contaminant from car exhaust in high populated city. The 6 rings PAHs concentration valued 0.822, which was found dominant in sample 4 from related high dense region. Sample 4 had the highest value of 4 rings PAHs (0.918%), the individual total PAHs concentration of the vegetable samples from various cities of Delta State ranging within the respective limit of 2.0 µg/kg and 25 µg/kg prescribed by the German society for fat science (Payanan et al., 2013).

The values of BaPTEo and BaPMEQ of PAHs in the green leaf vegetable samples are presented in Table 3. It varies from 0.118 – 0.948 µg/kg and 0.193 – 1.445 µgkg with a mean of (0.629) and (0.683) respectively. The BaPrEQ and BaPMEQ concentration pattern of the vegetable samples followed this order S4>S2>S1>S3 were the vegetable samples from Warri (S4) have the highest concentration level of BaPTEQ and BaPMEQ than those from Asaba (S2) > Warri (S4) > Ughelli (S1) > Sapele (S3). BbF, BaP, IndP and DAhP are the major contributors to mutagenic potency factor in these samples. BbF, BaP and DahA contributed significantly to the BaP TEQ

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factor in samples 4 while BaP, IndP and DahP have no impact on the BaP MEQ values. The concentration levels of the BaPTEQ and BaP MEQ are high. From the result there is high risk of consumers of green leaf in developing cancer. Consumers of green leafy vegetables are exposed to great health risk due to high carcinogenic and mutagenic potency factors of PAHs (Montano et al., 2025). The results indicate that there are 5 to 669 chances in a million population of the risk of developing cancer.

CONCLUSION

This study reveals that green leaf vegetable samples (*A. hybridus*) collected from Warri, Ughelli, Asaba, and Sapele markets in Delta State Nigeria are rich sources of macro and micro nutrients such as K, Ca, Mg, Fe, Mn, Zn, Cu, Co, Ni which are essential minerals required for normal cell and enzymatic body functions. The content of calcium and copper found in the study are lower than the RDA set by FAO/WHO (2005), the concentration of iron and copper are well above their permissible limit, which may not be entirely unwholesome. The study also shows that the concentration of Mg, Zn, K are within normal limit. However, there was no preponderance of all macro and micro nutrients studied in one sample area over another.

The study revealed that the vegetable contained significant concentrations of PAHs, especially high molecular weight compounds. The compositional pattern of PAHs followed the order 5 rings > 4 rings > 6 rings> 2 rings > 3 rings. The total cancer risk observed in this study suggest that consumption as much as 15 g of green vegetable per day could pose a considerable cancer risk. The carcinogenic PAHs that contributed majorly are BaP and BaF and also influenced by handling of vegetables as well as the cultivated soils. It is therefore necessary for consumers to wash this green vegetable carefully and ask of their source before consuming to reduce risk of exposure.

Authors' Declaration

The authors hereby declare that the work presented in this article is original and that any liability for claims relating to the content of this article will be borne by them.

Conflict of Interest

The authors has no conflict of interest.

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