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Human Health Risk Assessment of Heavy Metals and Hydrocarbons in *Murraya koenigii* in Rivers State, Nigeria

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ABSTRACT

Background and Objective: The entry of contaminants into the environment due to human and natural actions is one of the major problems facing the ecosystem today. This study evaluated the human health risks and selected heavy metals and hydrocarbon composition of *Murraya koenigii* commonly called curry leaves, harvested from selected communities in Eleme LGA. **Materials and Methods:** Fresh curry leaves were harvested in Aleto, Ogale, Akpajo and Alesa in Eleme Local Government Area of Rivers State, Nigeria and Rivers State University as a control sample. The curry leaves were ovum-dried for about two weeks, ground into powder and stored before analysis of heavy metals using an Atomic Absorption Spectrophotometer. Human health risk assessment from the consumption of the plant was computed using the standard formula as described by United States Environmental Protection Agency. **Results:** The heavy metal concentration in the samples was below the permissible limit of WHO except for Fe. Ogale had the highest concentration (961.768 μg/kg) of PAH while RSU recorded the lowest value (41.916 μg/kg). Indeno[1,2,3-cd]pyrene in the study areas was above WHO permissible limit. Human health risk assessment revealed that Ogale had the highest CF while RSU had the lowest. The EDI for all the heavy metals was within the USEPA permissible limits except Fe. The THQ had all its detected PAHs below the permissible limit of 1 except Benzo(a)pyrene. The cancer risk in all study locations of Eleme LGA exceeded the USEPA limit of 1×10^{-4}. **Conclusion:** The study suggests that consumption of the leaves of study poses both potential non-cancer health risks and cancer risks, thus, there should be regular monitoring of activities that lead to the presence of heavy metal and hydrocarbon pollution in the environment.

KEYWORDS

Heavy metals, contamination factor (CF), pollution load index (PLI), estimated daily intake (EDI), target hazard quotient (THQ), hazard index (HI), cancer risk assessment (CR), *Murraya koenigii*

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INTRODUCTION

The advent of Industrial activities has been of great economic benefits, however, the production of a variety of compounds and chemicals has posed great threats to both humans and the ecosystem at large[1]. Heavy metals have harmful effects on human health and exposure to these metals has been increased by industrial and anthropogenic activities. The pollution of water and air by toxic metals is a significant environmental issue, impacting hundreds of millions of people globally[2]. Heavy metals are metallic elements with a relatively high density compared to water. Due to their weight and toxicity, this category also includes metalloids like arsenic, which can cause toxicity even at low levels of exposure[3]. While heavy metals are naturally occurring elements present in the Earth's crust, most environmental contamination and human exposure stem from human activities. These include mining and smelting operations, industrial production and use, as well as the domestic and agricultural application of metals and metal-containing compounds, which can exacerbate pollution[3].

Polycyclic aromatic hydrocarbons (PAHs) are chemical compounds characterized by two or more fused aromatic rings. They are widespread in air, water and soil and are classified as general environmental pollutants that are harmful to the ecosystem[4]. They are ubiquitous and are environmentally persistent with various structures and varied toxicity. They have toxic effects on organisms through various actions. Generally, PAHs enter the environment through various routes and are usually found as a mixture containing two or more of these compounds[5].

Curry leaf (*Murraya koenigii*) is part of the Rutaceae family, which includes 150 genera and around 1,600 species[6]. Curry is a small, deciduous aromatic shrub that grows to a height of about 6 to 9 m and thrives at elevations of 1,500 m above sea level. It is native to South Asia, especially in India, Sri Lanka and Bangladesh[7]. The leaves are a popular spice and are commonly used to enhance the flavour of various dishes. The leaves are used for both medicinal and culinary applications. They are highly aromatic and have a unique flavour[8]. Curry leaves are a popular culinary herb in Eleme LGA, Nigeria. They are used in a variety of dishes, including soups and stews. Eleme LGA is a major industrial and agricultural hub and this can lead to the contamination of soil and water with heavy metals and other pollutants, curry leaves grown in contaminated soil and water can also become contaminated.

Various studies have reported the bioaccumulation potency of heavy metals in the body over time when consumed from food sources and the environment. Heavy metal exposure can cause a variety of health problems, including cancer, neurological disorders and reproductive problems, hence, there is a need to intermittently evaluate the concentration of these toxicants in the environment and food sources and assess their human health risk. This present study thus carried out the human health risk assessment, heavy metal and polycyclic aromatic hydrocarbon concentrations in curry leaves grown in selected communities in Eleme LGA of Rivers State, an area prone to numerous industrial activities and also a major habitation where agricultural activities are at its peak.

MATERIALS AND METHODS

Study duration: This study was carried out from September, 2023 to February, 2024.

Sample collection and preparation: Fresh curry leaves (1000 g) were harvested from the University Farm, Rivers State University, Port Harcourt, Aleto, Ogale, Akpajo and Alesa in Eleme Local Government Area of Rivers State, Nigeria. Only mature leaves without signs of lesion were used. The curry leaves were oven-dried for a specific period of time and ground into powder. The powdered samples were stored until the sample was needed for analysis.

Heavy metal analysis of plant leaves samples
Method (APHA 3111C)

Samples pre-treatment: Prior to the digestion of the leave samples, each sample was washed with distilled water to remove any trace of dirt such as sand and debris. Samples were oven-dried at 80-105°C to constant weight and pulverized to fine powder using the laboratory grinder. Ground samples were passed through 2 mm sieve to obtain the portion that will be used for acid digestion.

Digestion procedure for heavy metals in leaves samples: The ground sample (1 g) was weighed accurately and passed through (2 mm Sieve) using the weighing balance (Shenzhen City Feiya Weighing Ltd., China, ISO9001:2015) and was transferred to a 250 mL conical flask. It was transferred into 20 mL volume of well-mixed digestion mixture-Perchloric acid, nitric acid and sulfuric acid in the ratio of 1:2:2 into the flask containing the sample. Some amount of Anti bumps or henger granules was added to minimize spatter. The sample was placed in the fume hood (Terra Universal. Inc., USA, California) and heated for about (15-30 min) in the hot plate (Corning 6795-400D, Merck KGaA, Darmstadt, Germany) until a white fume was observed. Digestion was completed when a light-coloured, clear solution was observed. The sample was removed from the hood and allowed to cool off. The beaker wall was washed down with a small amount of distilled water and was filtered into a 100 mL volumetric flask using Whatman 42 filter paper. The filtrate was made to 100 mL mark on the volumetric flask and was transferred to the final 100 mL plastic can for AAS analysis. Using the same digestion procedure, the blank digest was carried out without the sample.

Analysis of digested sample (AAS)

Measuring condition: The AAS was adjusted in accordance with the operating procedure and the response was optimized using air-acetylene gas flame.

Preparation of standard calibration curve: A series of appropriate calibration standards were prepared (e.g 0.5, 1.0, 1.5 and 2.0 ppm) by diluting the standard stock solution with 5% nitric acid. The absorbance of each calibration standard was measured. The standard curve was prepared by plotting the absorbance against the calibration standards and the regression equation was obtained.

Measurement of test sample: The absorbance of the test sample was measured under the same conditions as the calibration standards. An aliquot of the test sample was digested with water (5% nitric acid) to obtain an absorbance in the linear part of the calibration curve.

Calculation: The absorbance was used to calculate the concentration of the metal in the digested sample from the regression equation. Finally, the heavy metal concentration in the sample (mg/kg) was calculated using the formula[9]:

$$\text{Concentration} = \frac{(A \times B)}{Z\ \text{sample}}$$

Where:

A = Concentration of metal in digested solution
B = Final volume of digested solution (dilution factor)
Z = Sample weighed

Statistical analysis: Data were analyzed using SPSS (Statistical Package for Social Sciences) version 25.0. Statistical evaluations of the difference between the group mean values were tested by One-way Analysis of Variance (ANOVA) and the Tukey's *post hoc* test for multiple comparisons. The results were expressed as Mean±Standard Deviation and statistical significance was considered at $p \leq 0.05$.

Hydrocarbon content

Method No: EPA 418.1/413.2 and EPA 1664: The 10 g of the sample was measured out into an extraction bottle and 10 mL of n-hexane was added into the extraction bottle. Each bottle was placed in the sonicator (Scientz Ltd., Zhejiang, China) and allowed to extract for approximately 10 min. The organic solvent was siphoned into a clean and dry beaker. Additional portion of 10 mL n-hexane was added to the sample again and allowed to sonicate for about 5 min. A repeated process of siphoning the organic solvent into the clean beaker was carried out to avoid residues. The solvent layer was drained through a funnel containing a filter paper and 10 g of Na_2SO_4 (Sodium Sulfate salt) was added to the filter paper to avoid water contamination of the samples. A teaspoon of silica gel was added to the organic solvent before reading in absorbance mode. Each Organic solvent or sample was placed in the cell. The cell containing the organic solvent was then in the cell holder of the instrument and read in ABS Mode (absorbance mode). The result was calculated in mg/L using the Regression Equation prepared from the external standards.

Human health risk assessment

Contamination factor (CF):

$$CF_m = \frac{Cm_{sample}}{Cm_{refernce}} \quad (1)$$

$$CF_{PAH} = \frac{CPAH_{sample}}{CPAH_{reference}} \quad (2)$$

Where:

Cm_{sample} = Concentration of metal in the sample (from Eleme LGA)
$Cm_{reference}$ = Concentration of metal in comparison site (Rivers State University)
$CPAH_{sample}$ = Concentration of PAH in the sample (from Eleme LGA)
$CPAH_{reference}$ = Concentration of PAH in comparison site (Rivers State University)

The contamination factor measures the site-specific level of heavy metal contamination. The CF evaluation based on the level of contamination is segmented into low ($C_{factor}<1$), moderate ($1<C_{factor}<3$), considerable ($3<C_{factor}<6$) and very high ($C_{factor}>6$)[10]:

$$\text{Pollution load index (PLI)} = (CF1xCF2xCF3xCF4......CFn)^{1/n} \quad (3)$$

Where:

CF = Contamination factor
N = Number of study metals

Estimated daily intake (EDI): The estimated amount of heavy metal ingested daily through consumption of *Murraya koenigi* was calculated in mg/kg b.wt./day using the formula below:

$$EDI = \frac{C \times IR \times ER \times ED}{BW \times LT} \quad (4)$$

Where:

C = Concentration of the contaminant in *Murraya koenigii* (mg/kg)
IR = Ingestion rate of the *Murraya koenigii* (kg b.wt./day/person)
EF = Exposure frequency (days/year)
ED = Exposure duration (365 days/year)
BW = Body weight (kg)
LT = Lifetime

For carcinogenic risk, AT = LT×356, where LT is the lifetime. Life expectancy at birth in Nigeria as of 2021 was estimated to be 63.4 years[11]. The average age average body weight for adult Nigerians is 65 kg[12,13]. A study focusing on the dietary intake of vegetables in Southwestern Nigeria reported that the average daily intake of leafy vegetables was around 0.083 kg/person/day[14]. Refinery activities in Eleme have been ongoing since at least 1965 with the operation of both PH I and PH II[15]. This equates to an estimated ED of 59 years as of July, 2024.

Target hazard quotient (THQ):

$$\text{THQ} = \frac{\text{EDI}}{\text{RFD}} \quad (5)$$

Where:
RfD = Reference doze for contaminants (mg/kg/day)

The RfD of heavy metals in mg/kg/day: Hg, Zn, Mn, Cu, Co, Ni = 0.0003, 0.3, 0.14, 0.04, 0.00003, 0.02, respectively[16].

The RfD for PAHs in mg/kg/day: Naphthalene, Acenaphthene, Fluorene, Anthracene, Benzo(b)fluoranthene, Benzo(a)pyrene = 0.02, 0.06, 0.04, 0.3, 0.00003[17]. The unavailability of certain RfDs for some PAHs indicates that either the data is not established or the EPA has not yet provided a specific RfDs value for those substances.

Hazard Index (HI):

$$\text{HI} = \sum \text{THQ (sum of THQs for all analyzed metals and PAHs)} \quad (6)$$

The hazard index value determines the level of health risk, lower health risk (THQ<1), potential health risk with the need for further monitoring ($1 \leq \text{THQ} \leq 10$) and higher health risk (THQ)[18].

Cancer risk assessment: The cancer risk assessment estimates the probability of developing cancer over a lifetime due to exposure. The Pb and Ni, are the carcinogenic metals of study, although Hg raises primary concerns about the neurological effect of long-term exposure and Chrysene, Benzo(b)fluoranthene, Benzo(k)fluoranthene, Benzo(a)pyrene, Indeno(1,2,3-c,d)pyrene, Dibenz(a,h)anthracene are the carcinogenic PAHs of study[19,20]:

$$\text{Cancer risk (CR)} = \text{EDI} \times \text{CSF} \quad (7)$$

Where:
CSF = Cancer slope factor in $(\text{mg/kg/day})^{-1}$

Cancer slope factor for carcinogenic heavy metals of study Pb, Ni (1.5, 0.84, respectively) and PAHs Benzo(b)fluoranthene, Benzo(k)fluoranthene, Benzo(a)pyrene, Indeno(1,2,3-c,d)pyrene, Dibenz(a,h)anthracene (0.25, 0.25, 7.3, 0.73, 7.3, respectively)[21]. Chrysene has no specified cancer slope factor provided by the EPA, but it is classified as a possible human carcinogen (Group 2B)[22]. If CR values are above 10^{-4}, it is not safe for human consumption, while values between 10^{-6} and 10^{-4} are free from cancer risk[23].

RESULTS AND DISCUSSION

Table 1 shows the concentration of selected heavy metals in curry leaves harvested from four communities (polluted samples) in Eleme LGA and Rivers State University (control sample). From the result obtained, it was observed that heavy metal concentrations in the samples were below the permissible limit of WHO except for Iron. It was also observed that Aleto recorded the highest concentration of zinc, phosphorus and copper. The same concentration of mercury (<0.0005), cobalt (<0.001) and nickel (<0.001) were recorded in samples from all locations.

Table 2 shows the concentration of polycyclic aromatic hydrocarbon (PAH) in curry leaves harvested from four communities (polluted samples) in Eleme LGA and Rivers State University (control sample). From the result obtained, it was observed that the concentration of PAHs in Ogale, Aleto, Akpajo and Alesa was higher than that of RSU sample that served as the control. Ogale recorded the highest concentration (961.768 μg/kg), followed by Akpajo (485.619 μg/kg), Aleto (135.111 μg/kg), Alesa (97.596 μg/kg) while RSU recorded the least value (41.916 μg/kg).

Table 3 reveals the contamination factor, pollution load index and estimated daily intake of heavy metals through consumption of *Murraya koenigii*. Leaves harvested in Ogale community had the highest CF values for Pb, indicating a high level of Pb contamination in that area, as compared to the reference site, Rivers State University (RSU). Alesa had the lowest CF values for all analyzed metals, while Ogale and Akpajo had the highest CF values. However, all CF values fell under the low level (CF<1)[9]. The estimated daily intake for all metals in all study sites was within the permissible limits provided[24], except for Fe, whose values exceeded the adequate intake for infants, as provided[25].

Table 1: Heavy metals concentrations of *Murraya koenigii* from Eleme LGA, Rivers State

Heavy metals (mg/kg)	Ogale	Aleto	Akpajo	Alesa	RSU	WHO limit
Mercury	<0.0005	<0.0005	<0.0005	<0.0005	<0.0005	0.01
Zinc	32.198^{v}	$50.328\ 0.195^{w}$	$25.4920.249^{x}$	$26.2100.182^{y}$	$38.1560.174^{z}$	99.4
Manganese	44.110^{v}	$27.3860.23^{wy}$	$25.2520.09^{x}$	$27.8570.13^{yw}$	$50.1400.274^{z}$	500
Iran	911.561±0.53	315.025 ± 0.421^{w}	281.875 ± 0.750^{x}	196.7 ± 0.0320^{y}	243.900 ± 0.62^{z}	0.03
Lead	0.080±0.003	<0.001	0.023±0.010	0.001	0.001	425
Phosphorous	8.505 ± 0.206^{wxyz}	12.438 ± 0.23^{vxyz}	11.216 ± 0.203^{vwxyz}	7.185 ± 0.535^{vwxy}	6.377 ± 0.223^{vwxy}	NA
Copper	8.343 ± 0.684^{v}	11.471 ± 0.426^{w}	10.610 ± 0.325^{x}	10.824 ± 0.083^{xy}	7.774 ± 0.210^{vz}	73.3
Cobalt	<0.001	<0.001	<0.001	<0.001	<0.001	0.1
Nickel	<0.001	<0.001	<0.001	<0.001	<0.001	67.9

Values in the table are expressed as Mean±Standard Deviation, values with the same superscript are not significant at p<0.05, values with different superscripts are significant at p<0.05 all are significant down the column and Ogale, Aleto, Akpajo, Alesa are communities in Eleme LGA and RSU: Rivers State University

Table 2: Polycyclic aromatic hydrocarbon (PAH) concentration in *Murraya koenigii* from Eleme LGA, Rivers State

PAH components (μg/kg)	Ogale	Aleto	Akpajo	Alesa	RSU	WHO/FAO limits
Naphthalene	34.353	ND	45.553	7.998	4.110	-
Acenaphthylene	64.302	2.879	18.171	2.737	5.389	-
Acenaphthene	61.356	4.061	15.752	3.110	4.154	-
Fluorene	177.020	5.258	31.966	7.278	3.627	-
Phenanthrene	39.483	7.666	12.393	3.363	0.979	-
Anthracene	ND	ND	ND	ND	ND	-
Fluoranthene	38.268	9.202	12.206	4.165	ND	-
Pyrene	107.013	33.851	84.420	14.359	1.986	-
Benz(a)anthracene	156.391	36.771	139.999	26.074	10.349	-
Chrysene	ND	ND	ND	ND	ND	100
Benzo(b)fluoranthene	ND	ND	ND	ND	ND	10
Benzo(k)fluoranthene	111.505	18.000	65.366	11.232	7.944	20
Benzo(a)pyrene	37.281	7.944	25.373	6.671	1.231	10
Indeno(1,2,3-c,d)pyrene	87.569	9.480	20.026	6.768	ND	1
Dibenz(a,h)anthracene	ND	ND	ND	ND	ND	0.1
Benzo(g,h,i)perylene	47.228	ND	14.395	3.840	2.147	-
Total (μg/kg)	961.768	135.111	485.619	97.596	41.916	

ND: Not detected

Table 3: Human health risk assessment of heavy metals through consumption of *Murraya koenigii*

	Ogale (mg/kg b.wt./day)		Aleto (mg/kg b.wt./day)		Akpajo (mg/kg b.wt./day)		Alesa (mg/kg b.wt./day)		RSU (mg/kg b.wt./day)
Heavy metals	CF	EDI	CF	EDI	CF	EDI	CF	EDI	EDI
Mercury	1.00	<0.0001	1.00	<0.0001	1.00	<0.0001	1.00	<0.0001	<0.0001
Zinc	0.84	0.0007	1.32	0.0011	0.67	0.0006	0.69	0.0006	0.0008
Manganese	0.88	0.0009	0.55	0.0006	0.50	0.0006	0.56	0.0011	0.0011
Iron	3.74	0.0203	1.29	0.0070	1.16	0.0063	0.81	0.0044	0.0054
Lead	80	<0.0001	1.0	<0.0001	23	<0.0001	1.0	<0.0001	0.0000
Phosphorus	1.33	0.0002	1.95	0.0003	1.76	0.0002	1.13	0.0001	0.0001
Copper	1.07	0.0003	1.48	0.0002	1.36	0.0002	1.39	0.0002	0.0002
Cobalt	1.00	<0.0001	1.00	<0.0001	1.00	<0.0001	1.00	<0.0001	0.0000
Nickel	1.00	<0.0001	1.00	<0.0001	1.00	<0.0001	1.00	<0.0001	0.0000
PLI	1.8947		1.0609		1.4054		0.9247		-

CF: Contamination factor, PLI: Pollution load index and EDI: Estimated daily intake

Table 4: Non-carcinogenic risk assessment of heavy metals through consumption of *Murraya koenigii*

Heavy metals (mg/kg)	Ogale	Aleto	Akpajo	Alesa
THQ for mercury	0.3333	0.3333	0.3333	0.3333
THQ for zinc	0.0023	0.0037	0.0020	0.0020
THQ for manganese	0.0064	0.0043	0.0043	0.0079
THQ for iron	-	-	-	-
THQ for lead	-	-	-	-
THQ for phosphorus	-	-	-	-
THQ for copper	0.0075	0.0050	0.0050	0.0050
THQ for cobalt	0.3333	0.3333	0.3333	0.3333
THQ for nickel	0.3333	0.3333	0.3333	0.3333
HI = THQ_{metals}	1.0161	1.0129	1.0112	1.0148

THQ: Total hazard quotient and HI: Hazard index

Table 5: Human health risk assessment of PAH through consumption of *Murraya koenigii*

	Ogale		Aleto		Akpajo		Alesa		RSU
PAH components (µg/kg)	CF	EDI	CF	EDI	CF	EDI	CF	EDI	EDI
Naphthalene	8.36	0.0008	-	-	11.08	0.0010	1.95	0.0002	0.0001
Acenaphthylene	11.93	0.0014	0.53	0.0001	3.37	0.00040	0.51	0.0001	0.0001
Acenaphthene	14.77	0.0014	0.98	0.0001	3.79	0.0004	0.75	0.0001	0.0001
Fluorene	48.81	0.0039	1.45	0.0001	8.81	0.0007	2.01	0.0002	0.0001
Phenanthrene	40.33	0.0009	7.83	0.0002	12.66	0.0003	3.44	0.0001	<0.0001
Anthracene	-	-	-	-	-	-	-	-	-
Fluoranthene	382680	0.0009	92020	0.0002	122060	0.0003	41650	0.0001	-
Pyrene	53.88	0.0024	17.04	0.0008	42.51	0.0019	7.23	0.0003	<0.0001
Benzo(a)anthracene	15.11	0.0035	3.55	0.0008	13.53	0.0031	2.52	0.0006	0.0002
Chrysene	-	-	-	-	-	-	-	-	-
Benzo(b)fluoranthene	-	-	-	-	-	-	-	-	-
Benzo(k)fluoranthene	14.04	0.0025	2.27	0.0004	8.23	0.0015	1.41	0.0002	0.0001
Benzo(a)pyrene	30.29	0.0008	6.45	0.0002	20.61	0.0006	5.42	0.0001	<0.0001
Indeno(1,2,3-c,d)pyrene	175.138	0.0019	18.96	0.0002	40.052	0.0004	13.536	0.0002	-
Dibenz(a,h)anthracene	-	-	-	-	-	-	-	-	-
Benzo(g,h,i)perylene	22	0.0011	-	-	6.70	0.0003	1.79	0.0001	<0.0001
PLI	58.1028		1.9075		24.6545		5.4745		0.0005

CF: Contamination factor, PLI: Pollution load index and EDI: Estimated daily intake

Table 4 shows the THQ for heavy metals, with Hg having the highest THQ values in all study sites, followed by Cu and Mn. Ogale and Akpajo had the highest THQ concentrations for most heavy metals. All THQ values were less than 1, however overall Hazard Index shows values greater than 1 (HI>1), indicating potential non-cancer health risk.

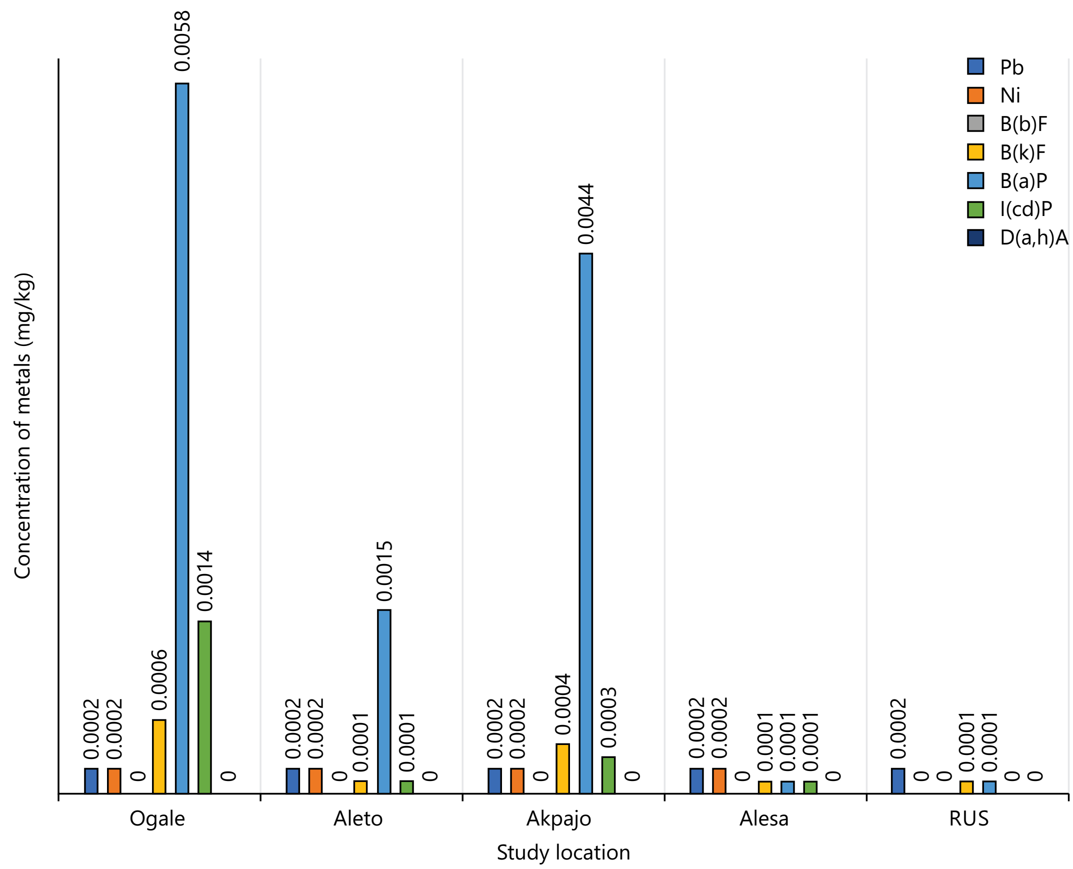

Fig. 1: Cancer risk (CR) of selected carcinogenic heavy metals and PAHs

Table 6: Non-carcinogenic risk assessment of PAH through consumption of *Murraya koenigii*

PAH components (μg/kg)	Ogale	Aleto	Akpajo	Alesa	RSU
THQ for naphthalene	0.0400	0.0000	0.0500	0.0100	0.005
THQ for acenaphthylene	-	-	-	-	-
THQ for acenaphthene	0.0233	0.0017	0.0067	0.0017	0.0017
THQ for fluorene	0.0975	0.0025	0.0175	0.0050	0.0025
THQ for phenanthrene	-	-	-	-	-
THQ for anthracene	0.0000	0.0000	0.0000	0.0000	0.0000
THQ for fluoranthene	-	-	-	-	-
THQ for pyrene	-	-	-	-	-
THQ for Benzo(a)anthracene	-	-	-	-	-
THQ for chrysene	-	-	-	-	-
THQ for Benzo(b)fluoranthene	0.0000	0.0000	0.0000	0.0000	0.0000
THQ for Benzo(k)fluoranthene	-	-	-	-	-
THQ for Benzo(a)pyrene	26.6667	6.6667	20.0000	3.3333	<3.3333
THQ for Indeno(1,2,3-c,d)pyrene	-	-	-	-	-
THQ for Dibenz(a,h)anthracene	-	-	-	-	-
THQ for Benzo(g,h,i)perylene	-	-	-	-	-
HI = THQ_{PAH}	26.8275	6.6709	20.0742	3.3450	3.3425

THQ: Total hazard quotient and HI: Hazard index

Table 5 shows the contamination factor, pollution load index and estimated daily intake of detected PAHs in *Murraya koenigii* harvested in Eleme communities and RSU. The estimated daily intake for Benz(a)anthracene, Benzo(k)fluoranthene, Benzo(a)pyrene and Indeno(1,2,3-cd)pyrene were higher than their permissible limit of 0.0001 mg/kg bw/day for all communities in Eleme. Acenaphthylene, Acenaphthene, Fluorene, Phenanthrene and Benzo(g,h,i)perylene have no set permissible limit to compare them with, however, these PAHs in *Murraya koenigii* from Ogale and Akpajo had higher EDI values when compared to RSU.

Table 6 displayed the THQ values for all detected PAHs and the Hazard Index (HI). The THQ values for all detected PAHs were below the permissible limit of 1 (HI<1) except for Benzo(a)pyrene (26.6667, 6.6667, 20.0 and 3.3333 at Ogale, Aleto, Akpajo and Alesa, respectively).

Cancer risk (CR) of selected heavy metals and PAH: Figure 1 shows the cancer risk for both heavy metals and PAHs in *Murraya koenigii* leaves from all four communities within Eleme LGA. The values significantly exceeded the acceptable limit of 1×10^{-4}. The Pb and Ni had values of 0.0002 for *Murray koenigii* from all four communities in Eleme. Benzo(a)pyrene in Ogale and Akpajo recorded the highest values of 0.0058 and 0.0044, respectively. Benzo(b)fluoranthene and Dibenz(a,h)anthracene were not detected. Benzo(k)fluoranthene had CR>10^{-4} values in Ogale and Akpajo (0.0006, 0.0004, respectively).

DISCUSSION

Table 1 shows the concentration of selected heavy metals in curry leaves harvested from four communities (polluted samples) in Eleme LGA and Rivers State University (control sample). From the result obtained, it was observed that heavy metal concentrations in the samples were below the permissible limit of WHO except Iron. The concentration of phosphorus and copper was higher in polluted samples than in control samples while the concentration of manganese was higher in control samples than in polluted samples. It was also observed that Aleto recorded the highest concentration of zinc, phosphorus and copper. The same concentration of mercury (<0.0005), cobalt (<0.001) and nickel (<0.001) were recorded in samples from all locations.

Mercury is a toxic metal found naturally in the environment and its pollution is a global environmental issue. Mercury exposure can result in a range of health problems, particularly affecting the nervous system. It can also lead to cardiovascular, gastrointestinal and other health issues. The result obtained in this study revealed that the level of mercury was below 0.0005 in the curry leaves obtained from all locations. It was also evident that the concentration of mercury in the samples was below the permissible limit and this indicates safety from the metal. However, some other studies have indicated the presence of Hg in various study locations in India and they were above the permissible limits[26].

Zinc is an essential mineral for humans and is often used as a dietary supplement to support immune function, tissue repair, proper growth and development in children and wound healing and growth. Zinc deficiency can lead to health problems including impaired immune function, growth retardation and delayed wound healing. The result obtained in this study showed that the concentration of zinc in curry leaves cultivated from all locations was lower than the WHO permissible limit. The highest concentration of zinc was seen in the curry leaves obtained from Aleto followed by that of Rivers State University. The lowest value was recorded in the sample obtained from Akpajo. The high level of zinc found in the sample obtained from Aleto could be attributed to the high industrial activities taking place in the area. In the research of Ihoma *et al.*[27] it was explained that factories, refineries and manufacturing plants often use or produce zinc-containing materials and pollutants from these sources can lead to increased zinc levels in the vicinity. The level of zinc was also high in sample obtained from Rivers State University and this could be attributed to the vehicular movement of the area[28]. In the findings of Ogundele *et al.*[29] it was reported urban areas with higher human activity tend to have more sources of zinc pollution including transportation and industrial emissions. From the study, it was also observed that the concentration of zinc in the analyzed samples was within permissible limits and that confirms the safety of the metal. The result obtained in this study is in tandem with the findings of Prashanth *et al.*[30] who reported that the concentration of zinc found in leaves of different vegetables grown in Ojo, Lagos was below WHO permissible limit.

Manganese is a vital nutrient necessary for various biochemical processes. It plays a crucial role in bone development, wound healing, reproduction and the proper functioning of the central nervous system[31], reproductive failure occurs in both males and females and can also lead to stunted growth[32].

According to the result obtained in this study, it was observed that the concentration of manganese was higher in control sample than polluted sample. The concentrations were in the order: RSU>Ogale>Rukpokwu>Aleto>Akpajo. The high level of manganese observed in a sample obtained from RSU could be attributed to the fact that there was improper disposal of manganese-containing materials and waste such as batteries, electronic devices or industrial waste. Lasocki *et al.*[32] also stated that pollutants including manganese particles can be transported through the air and water over long distances and when they settle in the environment, they can contribute to higher manganese levels. The concentration of manganese obtained in this study was all below the permissible limit. The result obtained in this study is in tandem with the findings of some researchers who reported that the concentration of heavy metals such as manganese found in leaves of different vegetables grown in Ojo, Lagos was below WHO permissible limit[32].

Iron is a vital mineral for life and is important in diets. It plays a key role in the production of hemoglobin and is involved in oxygen and electron transfer within the human body[33]. The results of this study indicated that the iron concentration in the samples exceeded the permissible limit set by the WHO. Additionally, the iron content in curry leaves was found to be higher in polluted samples compared to the control sample, except the Alesa sample.

Copper is an essential micronutrient, it aids the body in eliminating free radicals, producing red blood cells, protecting nerve cells and preventing damage to cell structures. The result obtained in this study showed that the concentration of copper in curry leaves harvested from all locations was lower than the WHO permissible limit. It was also evident that the copper content of polluted samples was higher than that of the control sample. The highest value was found in the sample from Aleto (11.471±0.426) μg/mg followed by that of Alesa (10.824±0.083) μg/mg. The elevated concentrations at certain sites may be attributed to burned vehicles along major roads, as copper is often present in electrical wiring[34].

Polycyclic aromatic hydrocarbons are organic pollutants and are widespread across the globe mainly due to long-term anthropogenic sources of pollution[35]. Table 2 shows the concentration of polycyclic aromatic hydrocarbon (PAHs) in curry leaves harvested from four communities (polluted samples) in Eleme LGA and Rivers State University (control sample). From the result obtained, it was observed that a total of 12 PAHs were detected in curry leaf samples. The result revealed that Aleto and River State University Farm recorded 10 recognized PAH while locations Ogale, Akpajo and Alesa detected 12 PAH concentrations except for Anthracene, Chrysene, Benzo[b] flouranthene and Dibenz[a,h] anthracene. Sample Aleto detected 10 PAHs as anthracene, Chrysene, Benzo(b) flouranthene, Dibenz[a,h]anthracene, Naphthalene and Benzo[g,h,I] perylene were found undetected. Sample RSU detected a total of 10 PAH components. The result also revealed that the concentration of PAHs in polluted samples was higher than that of unpolluted samples. Ogale recorded the highest concentration (961.768 μg/kg), followed by Akpajo (485.619 μg/kg), Aleto (135.111 μg/kg) and Alesa (97.596 μg/kg) while RSU recorded the least value (41.916 μg/kg). It was also observed that Anthracene, Chrysene, Benzo[b]fluoranthene and Dibenz[a,b]anthracene were not detected in the samples. Indeno[1,2,3-cd] pyrene was higher in polluted samples than WHO permissible limit. Benzo[k]fluoranthene and Benzo[a]pyrene were higher in curry leaves harvested from Ogale and Akpajo. The high concentration of PAH found in curry leaves harvested in Ogale can be attributed to the high industrial activities ongoing in the area. PAHs are released from sources such as vehicle exhaust, industrial emissions and residential heating[28].

Table 3 shows the estimated daily intake of heavy metals from *Murraya koenigii* from Eleme LGA and a reference site, RSU. The estimated daily intake for the selected metals was below the permissible limits for the EDI for the selected heavy metals. The Hg, Zn, Mn, Pb, P, Cu, Co and Ni, have permissible EDI values of 0.0001, 0.3, 0.14, 0.0036, 4, 0.001 and 0.5, respectively[21]. However, the estimated daily intake for Fe in Ogale was seen to be slightly higher than the adequate intake (AI) provided[25] for infants.

In Table 4, the THQ for detected metals at the respective sites were all within the range of lower health risk (THQ<1), but the hazard index had values slightly above 1 (HI>1), indicating possible accumulated adverse impact and potential non-cancer health risk, with a need for further monitoring[18].

Table 5 shows the contamination factor, pollution load index and estimated daily intake of detected PAHs in *Murraya koenigii* harvested from Eleme communities and RSU, while Fig. 1 revealed the cancer risk assessment of detected PAH and heavy metals in the study locations. Anthracene, Chrysene, Benzo(b)fluoranthene and Dibenzene(a,h)anthracene were not detected. The estimated daily intake for Benz(a)anthracene (0.0035), Benzo(k)fluoranthene (0.0025), Benzo(a)pyrene (0.0008) and Indeno(1,2,3-cd)pyrene (0.0019) at all sites excluding RSU, were higher than their permissible limits of 0.0001 mg/kg b.wt/day[36]. This poses significant threats to human health through the consumption of *Murraya koenigii* harvested from Eleme community. Other PAHs assessed (Acenaphthylene, Acenaphthene, Fluorene, Phenanthrene and Benzo(g,h,i)perylene) have no permissible EDI limits provided because they are not classified as to human carcinogenicity[36], however, possible toxicity could exist for Acenaphthylene, Acenaphthene, Flourene, Phenanthrene and Benzo(g,h,i)perylene from Ogale and Akpajo community, as their EDI values are significantly higher than the estimated daily intake from *Murraya koenigii* harvested from RSU.

Table 6 shows the THQ values for all detected PAHs and the Hazard Index (HI) for *Murraya koenigii* harvested in the respective sites of study. At all sites, THQ values for all detected PAHs were below the permissible limit of 1 (HI<1)[37], except for Benzo(a)pyrene, who's THQ values exceeded the permissible limit, with values of 26.6667, 6.6667, 20.0 and 3.3333 at Ogale, Aleto, Akpajo and Alesa, respectively. The high Target Hazard Quotient (THQ) for Benzo(a)pyrene (BaP) in *Murraya koenigii* leaves harvested from an industrial area suggests a potential non-carcinogenic health risk associated with its consumption[37]. Industrial areas are known sources of PAH contamination due to various activities like fuel combustion and industrial processes[38]. The BaP is a potent PAH classified as a probable human carcinogen (International Agency for Research on Cancer). Plants grown in contaminated soil or exposed to airborne PAHs can accumulate in their tissues[39].

Figure 1 shows that the total cancer risk from heavy metals and PAHs in *Murraya koenigii* leaves from all four communities within Eleme LGA significantly exceeds the acceptable limit of 1×10^{-4} [39] with values ranging from 0.0002 to 0.0058. This suggests that consumption of these leaves poses a potential health risk, as chronic exposure to heavy metals and PAHs has been linked to an increased risk of various cancers[37]. Notably, the concentrations of Benzo(a)pyrene (BaP), Benzo(k)fluoranthene (BkF) and Indeno(1,2,3-cd)pyrene (Indeno(1,2,3-cd)P) in Akpajo were particularly concerning, exceeding the 1×10^{-4} threshold. While Benzo(b)fluoranthene and Dibenz(a,h)anthracene were not detected in the samples, the overall risk from the identified contaminants indicates that *Murraya koenigii* leaves from these communities are unsafe for human consumption. This finding aligns with previous studies highlighting the potential for PAHs to accumulate in leafy vegetables grown in contaminated environments[39].

CONCLUSION

Heavy metals and PAHs are alarming environmental toxins. The concentrations of the heavy metals in the *Murraya koenigii* of study are not an assurance of safety, as heavy metals can be toxic to humans even in trace amounts. Additionally, the elevated PAH concentrations above WHO limits raise serious concerns about environmental pollution and public health risks. The human health risk assessment points towards potential non-cancer health risk and cancer risks, suggesting that the vegetables in the study were unsafe for consumption. In light of the above findings, regular monitoring in these areas is recommended.

SIGNIFICANCE STATEMENT

Heavy metals and PAHs are alarming environmental toxins. The concentrations of these pollutants raise serious concerns about the environment and public health risks. This study revealed that the content of heavy metals in the curry leaves from different locations was below WHO permissible limits, implying they were of good quality and safe for consumption. Although, the content of heavy metals in *Murraya koenigii* from study locations was below WHO permissible limits, consumption of *Murraya koenigii* from these locations could also be unsafe since heavy metals can be toxic to humans even in trace amounts. The PAH concentrations in the study areas revealed that the concentration of Indeno[1,2,3-cd]pyrene in the Eleme sample was above WHO permissible limit, hence a need for regular monitoring by the regulatory bodies.

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