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Relation between Traffic density, concentrations of Trace Metals in *Vernonia amygdalina* from Roadsides, and the Related Human Health Risks

Tochukwu, N. V.[1] and Ebong, G. A.[1*]
1Department of Chemistry, University of Uyo, Akwa Ibom State, Nigeria.

**E-mail: g_ebong@yahoo.com*

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Abstract: *Vernonia amygdalina* as one of the widely consumed vegetables and it has enormous nutritive and medicinal values however; when the plant is highly contaminated with toxic metals it becomes a source of health hazards to human. This research investigated the buildup of metals in *V. amygdalina* cultivated by roadsides with high traffic density namely: Mbierebe, Nwanaiba, Oron, and Ikot Ekpene Roads. *V. amygdalina* were also obtained from a road with low traffic density (Ikpa Road) and used as the Control. Samples obtained were treated using standard techniques, and the concentrations of Cd, Pb, Ni, Cu, and Zn in the samples and Control were obtained using Solar 969 atomic absorption spectrophotometer. The mean concentrations ($mgkg^{-1}$) of Cd, Pb, Ni, Cu, and Zn, obtained were 0.06±0.01, 0.10±0.02, 0.03±0.01, 1.07±0.09, and 1.11±0.16, respectively. The results revealed that, all the metals were within their safe limits except Pb. The mean concentrations of all the metals in the studied samples were higher than their levels in the Control. The daily intake rate of all the metals for the young and adult consumers were within their recommended oral reference doses. Principal component analysis identified vehicular emissions as the major source of the metals in the studied vegetable. The hazard index of metals via the consumption of *V. amygdalina* by the young ones was higher than one but less than one for the adult consumers. The mean total cancer risk values for the young and adult consumers of the studied vegetable were higher than the recommended limit. The young consumers were relatively more susceptible to both the cancer and non-cancer risks. The study revealed that, the consumption of vegetables from roadsides with high traffic density could be hazardous to the consumers. Consequently, the cultivation of vegetables by roadsides with high traffic density should be discouraged.

1. Introduction

Vegetables provide vitamins, protein, carbohydrate, minerals, essential elements, and water to the human body hence; they play significant role in human health (Ülger *et al*., 2018; Ebong *et al*., 2020; Pandey *et al*., 2022). *Vernonia amygdalina* is one of the vegetables widely consumed mostly in the southern part of Nigeria for both nutritious and medicinal values (Ojimelukwe and Amaechi, 2019; Degu *et al*., 2024; Umeh *et al*., 2024). *V. amygdalina* is a shrub that grows mostly in tropical Africa and is commonly called bitter leaf due to its intrinsic bitter taste (Ekam *et al*., 2010; Degu *et al*., 2024).

Contamination and subsequent pollution of vegetables with harmful substances including metals could have major negative effects on the health of their consumers (Gupta *et al*., 2021; Tongprung *et al*., 2024). It has been reported that, vegetables act as drivers for transporting contaminants from highly contaminated soils to human (Benson and Ebong, 2005; Boluspayeva *et al*., 2022). Thus, the cultivation of vegetables on contaminant-impacted soils can increase the levels of toxic substances in the plants and could be harmful to the consumers (Ebong *et al*., 2007; Nyiramigisha *et al*., 2021). Constant consumption of vegetables contaminated with elevated levels of metals may expose the consumers to both cancer and non-cancer risks (Alsafran *et al*., 2021; Boluspayeva *et al*., 2022; Onoyima *et al*., 2022).

Nevertheless; previous studies on the accumulation of metals by *V. amygdalina* concentrated mostly on wastes and manure–impacted soils (Ebong *et al*., 2007a; Ebong and Etuk, 2017; Etuk *et al*., 2022; Dan *et al*., 2023). However, reports have shown that vegetables by roadsides especially those with high traffic density have the tendency to accumulate high levels of toxic metals (Rahul and Jain, 2016; Onakpa *et al*., 2018; Bayissa and Gebeyehu, 2021). The quality of vegetables cultivated by roadsides with high traffic density are mostly affected by asphalt, tyres, combustion of fuel and oils, abrasion of brake linings and pads of vehicles (Ebong and Moses, 2016; Fussell *et al*., 2022; Skorbiłowicz *et al*., 2021; Ebong *et al*., 2023). Due to the high population of the consumers of *V. Amygdalina*, contamination of the vegetable can be very disastrous in Nigeria and Africa by extension (Igbakin and Oloyede, 2009; Mgbemena and Amako, 2020). The Crude oil activities in the area is another major source of contaminants to the soil and plants (Ebong and John, 2021; Adeniran *et al*., 2023; Ola *et al*., 2024). According to Bati *et al*. (2017), contamination of vegetables with high concentrations of metals is one of the major sources of human health problems globally. Thus, a regular evaluation of the quality of vegetables available for human consumption should be considered a necessary tool for maintaining good health and forestall health problems worldwide.

Consequently, this study was undertaken to evaluate the impacts of traffic density on metals loads in the widely consumed *V. amygdalina* harvested from roadsides. Accordingly, the suitability for human consumption or otherwise of the vegetable from roadsides with traffic density will be established. The real source of the metals in the vegetable was identified using principal component analysis. The research also assessed the carcinogenic and non-carcinogenic effects of the studied vegetable on the young and adult consumers. The results obtained indicated that, vehicular emissions have significant negative impact on the concentrations of metals along roadsides with high traffic density.

2.0 Materials and methods

2.1 Study Area

The research work was carried out in Uyo Urban where most people in the Akwa Ibom State live. Uyo is the capital of Akwa Ibom State, Nigeria. It lies approximately between latitudes 3° 05! - 5° 55! N and longitudes 7° 50! - 8° 02! E. Uyo locates within the South-eastern region of Nigeria with a land mass of about 314 km^2 (Akpan-Ebe *et al*., 2016). Uyo has temperature ranging between 25 °C and 28 °C with the coolest and hottest months in August and March, respectively. The average yearly rainfall varies from 2000 to 3000mm. Due to Crude Oil activities in the area, the population is high, and the number of vehicles on Uyo roads is also high. As a consequence of this, the rate of vehicular emissions in the area is very high thus, plant at roadsides may contain high levels of toxic sunstances including metals (Akpan *et al*., 2014). Uyo has two major seasons namely: dry and wet during March to December and April to November. A greater proportion of the land mass is used for the construction of houses due to urbanization. Consequently, cultivation of vegetables by the roadsides is common among subsistent farmers within the region.

2.2 Sample Collection and treatment

Leaves of *Vernonia amygdalina* commonly called bitter leaf were obtained at roadsides with high traffic density within Uyo Urban namely: Mbierebe, Nwanaiba, Oron, and Ikot Ekpene Roads, Nigeria (Akpan *et al*., 2014). Similar vegetable was also obtained from a road with relatively low traffic density (Ikpa Road) and used as the Control. The leaves of *V. amygdalina* were collected by the use of stainless steel knife to avoid contamination and taken to the laboratory in a cooler. In the laboratory, these samples were carefully washed with tap water to remove dirt and later with distilled water. The clean leaves were air-dried, cut into pieces and dried again in an oven at 60 ºC for 24 hours. The dried leaves were then ground into powdered form using mortar and pestle. The homogenized samples were then preserved in polyethene containers for digestion.

2.3 Digestion of samples

One gram of the sieved samples was weighed into a 100 mL beaker and 10 mL of 1:2 mixture of $HNO_3/HClO_4$ was added. The mixture was placed on a hot plate with constant stirring until the digestion process was completed. The digest was then filtered into a 250 mL clean volumetric flask and made to mark with distilled water. The concentrations of Cd, Pb, Ni, Cu and Zn in the samples were determined using atomic absorption spectrophotometer (solar 969 AAS thermo elemental UNICAM).

2.4 Health risks appraisal

The carcinogenic and non-carcinogenic health hazards of trace metals due to the consumption of *V. amygdalina* was done using the under mentioned parameters.

2.4.1 Daily intake rate of trace metals

The daily intake rate (DIR) of trace metals via the consumption of *V. amygdalina* impacted by vehicular emissions was determined using Eqn 1.

$$\text{DIR} = \frac{MC \ x \ IR}{BW} \qquad \textbf{Eqn.1}$$

MC indicates concentration of each trace metal in the vegetable, IR is the ingestion rate, and BW denotes the body weight. IR values used were 0.232 kg and 0.345 kg for the child and adult consumers, respectively (Gaurav *et al*., 2018; Ghalhari *et al*., 2024). The body weight of a child was 15 kg and 70 kg for an adult (USEPA 2011; Gruszecka-Kosowska, 2019).

2.4.2. Target hazard quotient (THQ) of trace metals

The THQ of trace metals through the consumption of *V. amygdalina* was calculated with Eqn. 2.

$$\text{THQ} = \frac{DIR}{RfD} \qquad \textbf{Eqn 2}$$

Where DIR signifies the daily intake rate of trace metals obtained from Eqn 1, RfD is the recommended oral reference dose of trace metals. RfD values used for Cd, Pb, Ni, Cu, and Zn used were 0.001, 0.0035, 0.02, 0.40, and 0.30 $mgkg^{-1}day^{-1}$, respectively (USEPA, 2021).

2.4.3. Hazard index (HI) of trace metals

The HI of trace metals through the consumption of studied *V. amygdalina* by the young and adult consumers was computed using Eqn 3.

$$HI = \Sigma THQ = THQCd + THQPb + THQNi + THQCu + THQZn \qquad \textbf{Eqn.3}$$

Where ΣTHQ symbolizes the sum of target hazard index for Cd, Pb, Ni, Cu, and Zn.

2.4.4. Incremental lifetime cancer risk (ILCR) of cancer-causing metals

The ILCR for the carcinogens (Cd, Pb, and Ni) via the consumption of *V. amygdalina* harvested from roadsides with high traffic density was estimated using Eqn 4.

$$ILCR = CSF \times DIR \qquad \textbf{Eqn. 4}$$

Where CSF indicates the cancer slope factor of the trace metals and DIR is the daily intake rate of Cd, Pb, and Ni. The CSF for Cd, Pb, and Ni used were 3.80E-01, 8.50E-03, and 1.7 $mgkg^{-1}day^{-1}$, respectively, (USEPA, 2000).

2.2.5. Total cancer risks (TCR) of the carcinogens

The TCR of Cd, Pb, and Ni through the consumption of *V. amygdalina* impacted by vehicular emissions was calculated using Eqn 5.

$$TCR = \Sigma ILCR = ILCRCd + ILCRPb + ILCRNi \qquad \textbf{Eqn. 5}$$

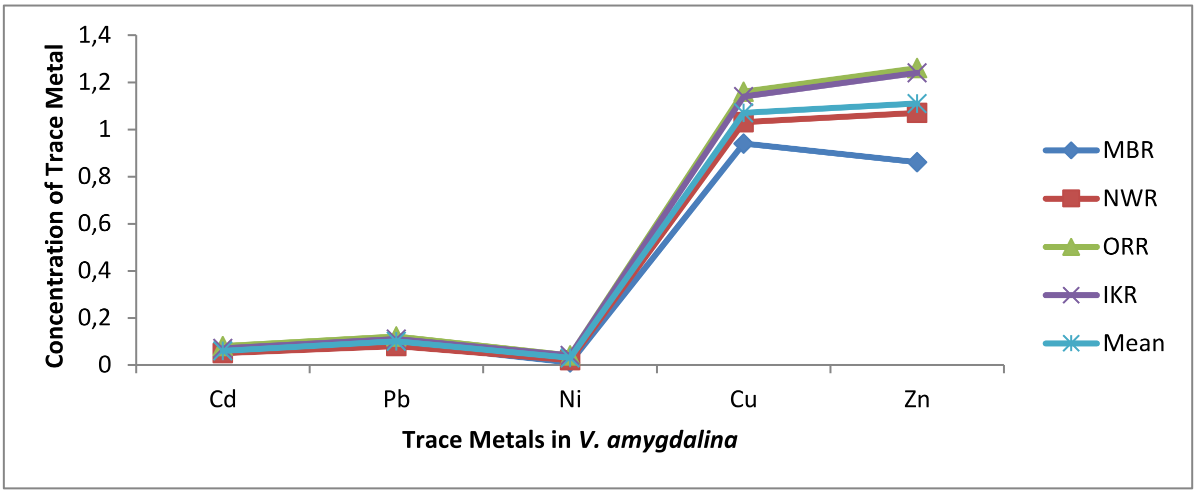
Where ΣILCR indicates the summation of incremental lifetime cancer risk for Cd, Pb, and Ni. The categories of TCR are as follows: 1.0E-01-1.0E-03 is the very high cancer risk class, 1.0E-04 belongs to the high cancer risk class, 1.0E-05 is in the medium cancer risk class, 1.0E-06 belongs to the low cancer risk class, and TCR less than 1.0E-06 belongs to the negligible cancer risk class, (USEPA, 1999).

3. Results and Discussion

Results of the concentrations of trace metals *V. amygdalina* from roadsides with high traffic density in Uyo, Nigeria are shown in Figure 1.

3.5 Analysis of Data

The study used IBM SPSS Statistic version 29.0.2.0 (20) Software for the treatment of data obtained. The average, standard deviation, maximum, and minimum values were acquired using the software. Principal component analysis (PCA) and Hierarchical cluster analysis (HCA) were performed through Varimax Factor analysis on the metals determined in the studied *V. amygdalina* and values from 0.878 and above were deemed significant. Dendrograms with linkages were employed for Hierarchical Cluster Analysis. The results of trace metals in *V. amygdalina* obtained from roadsides with high traffic density are shown in Figure 1.



MBR = Mbierebe Road, NWR = Nwanaiba Road, ORR = Oron Road, IKR = Ikot Ekpene, CTR = Control (Ikpa Road)

Figure 1: Concentrations (mgkg^{-1}) of Trace Metals in *Vernonia amygdalina* impacted by vehicular emissions.

The results in Figure 1 indicate the following ranges for Cd, Pb, and Ni: 0.05-0.08 mgkg^{-1}, 0.08-0.12 mgkg^{-1}, and 0.01-0.04 mgkg^{-1}, respectively. Cu and Zn in *V. amygdalina* varied from 0.94 to 1.16 mgkg^{-1} and 0.86 to 1.26 mgkg^{-1}, respectively. Based on the results in Figure 1, the essential metals had higher concentrations than the toxic ones. The mean concentrations of toxic metals (Cd, Pb, and Ni) were 0.06±0.01 mgkg^{-1}, 0.10±0.02 mgkg^{-1}, and 0.03±0.01 mgkg^{-1}, respectively (Figure 1). While the average values of the essential metals (Cu and Zn) were 1.07±0.09 mgkg^{-1} and 1.11±0.16 mgkg^{-1}, respectively (Figure 1). The mean concentrations of all the metals except Pb were within their acceptable limits by Gebeyehu and Bayissa (2020) & Gupta *et al*. (2022). The reported higher values of Pb in the studied *V. amygdalina* could be attributed to leaded fuel from vehicular emissions (Hui *et al*., 2017; Mielke *et al*., 2022). Consequently, the consumption of these vegetables may have adverse health problems on the consumers due to Pb toxicity as opined by Ebong *et al*. (2007b) and Okon *et al*. (2023). The mean concentrations of all the metals in the *V. amygdalina* from roadsides with high traffic density were higher than their concentrations in the control plot. This is consistent with the results obtained by Ebong *et al*. (2023) in a related study. This is to confirm the impacts of vehicular emissions on the metals loads of *V. amygdalina* at roadsides with high traffic density.

Table 1: Results of Principal Component analysis (PCA) of Trace Metals in the studied *V. amygdalina*

	V. amygdalina
	F1
Cd	0.972
Pb	0.911
Ni	0.988
Cu	0.983
Zn	0.954
Eigen value	4.63
% Variance	92.6

The principal component analysis (PCA) revealed one major factor responsible for the trace metals loads in the studied *V. amygdalina*. The factor has Eigen value of 4.63 and a total variance of 92.6%.

The factor identified strong positive loadings on all the trace metals determined in the vegetable (Table 1). This could be the influence of vehicular emissions on the metals loads of the studied *V. amygdalina* (Kuklová *et al*., 2022).

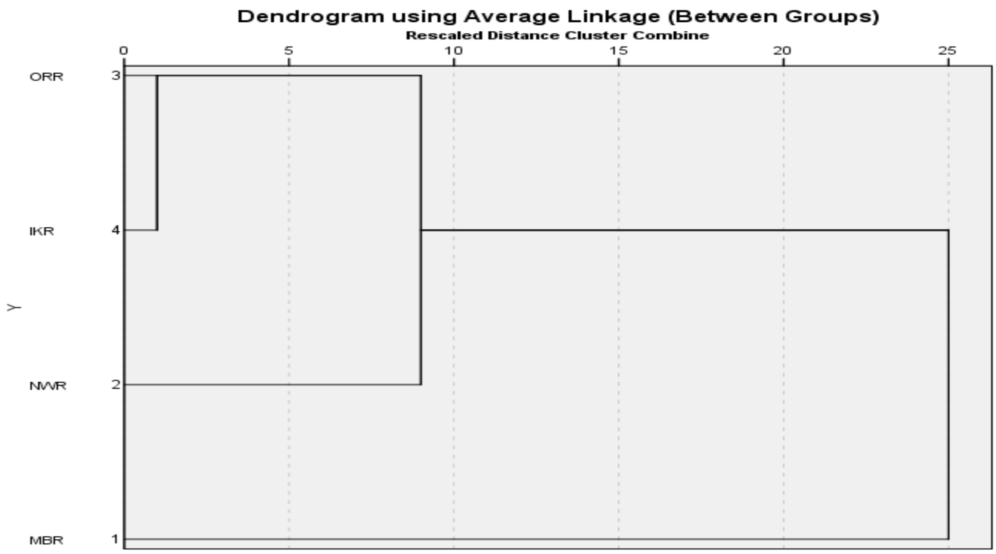


Figure 2: Hierarchical clusters of the Locations examined.

Hierarchical cluster analysis (HCA) for the site-to-site relationship is illustrated in Figure 2. The first cluster shows close association between Oron and Ikot Ekpene Roads. Hence, the levels of vehicular emissions along these Roads could have been similar. The second cluster links Nwanaiba Road only, while the third cluster connects Mbierebe Road alone. Consequently, levels of vehicular emissions at these locations were different from each other and dissimilar from those in cluster 1.

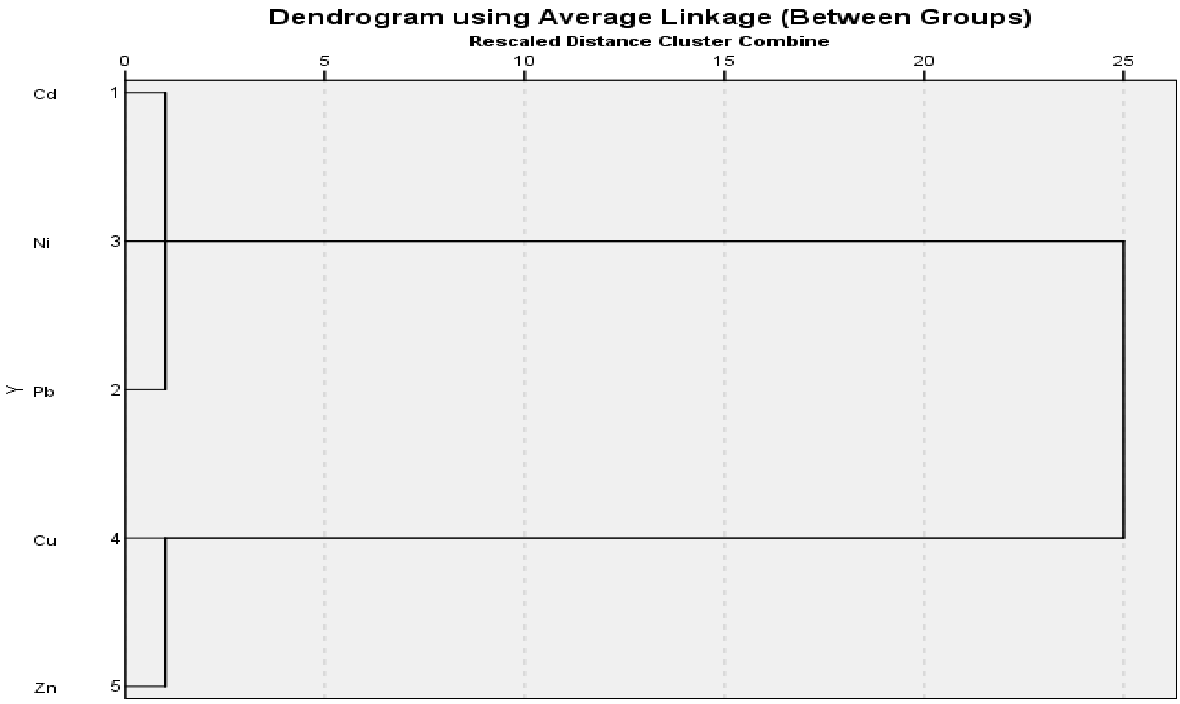


Figure 3: Hierarchical Cluster Analysis of Trace Metals in the studied *V. amygdalina*

The relationships among metallic contaminants emitted from vehicles are shown in Figure 3. The Figure shows two major clusters, cluster 1 connects the toxic metals namely: Cd, Ni, and Pb together. Cluster 2 links the essential elements Cu and Zn together. Thus, the toxic metals have common characteristics while essential elements also have similar properties.

Health risk assessment

Table 2: Mean values of the daily intake rate (DIR) of trace metals

	Cd	Pb	Ni	Cu	Zn
Young Consumers	9.28E-04	1.00E-03	4.64E-04	1.66E-02	1.72E-02
Adult Consumers	2.96E-04	4.93E-04	1.48E-04	5.27E-03	5.47E-03

The results of daily intake rate (DIR) of trace metals through the consumption of the studied *V. amygdalina* by the young and the elderly are shown in Table 2. The average DIR values of Cd, Pb, Ni, Cu, and Zn for the young consumers were 9.28E-04, 1.00E-03, 4.64E-04, 0.01.66E-02, and 1.72E-02 mgkg-1day^{-1}, respectively. The mean EDI values obtained for the elderly were 2.96E-04, 4.93E-04, 1.48E-04, 5.27E-03, and 5.47E-03 mgkg^{-1}day^{-1} for Cd, Pb, Ni, Cu, and Zn, respectively. The DIR values reported for the young and adult consumers of *V. amygdalina* were within their recommended oral reference doses by USEPA (2021). Hence, the consumption of *V. amygdalina* obtained from the studied locations may not have immediate health implications. However, the young consumers could be more exposed to the health problems associated with the consumption of the studied *V. amygdalina*. This is similar to the results obtained by Hussain and Qureshi (2020) & Ebong *et al*. (2024a).

Table 3: Mean values of non-cancer and cancer risks of trace metals

	Cd	Pb	Ni	Cu	Zn	**HI**
			THQ			
Young Consumers	9.28E-01	2.86E-01	2.32E-02	4.15E-02	5.73E-02	1.336
Adult Consumers	2.96E-01	1.41E-01	7.40E-03	1.32E-02	1.82E-02	4.76E-01
			ILCR			**TCR**
Young Consumers	3.53E-04	8.50E-06	7.89E-04	-	-	1.15E-03
Adult Consumers	1.13E-04	4.19E-06	2.52E-04	-	-	3.69E-04

The results of target hazard quotient (THQ) of trace metals through the consumption of *V. amygdalina* by the young and adult are shown in Table 3. The mean THQ values of Cd, Pb, Ni, Cu, and Zn for the young consumers were 9.28E-01, 2.86E-01, 2.32E-02, 4.15E-02, and 5.73E-02, respectively. For the adult consumers, the average THQ values obtained were 2.96E-01, 1.41E-01, 7.40E-03, 1.32E-02, and 1.82E-02 for Cd, Pb, Ni, Cu, and Zn, respectively. The mean THQ values of the trace metals for both categories of consumers were less than one as reported by Song *et al*. (2009) and Ametepey *et al*. (2018). However, the mean THQ values of trace metals recorded for the young consumers were higher. This is in agreement with the findings by Zhou *et al*. (2016) and Ebong *et al*. (2024b) in related studies.

The results of hazard index (HI) of trace metals through the consumption of *V. amygdalina* harvested from roadsides with high traffic density by the young and adult populations are indicated in Table 3. The HI values obtained for the young and adult consumers were 1.336 and 4.76E-01, respectively. Accordingly, the HI value of trace metals via the consumption of studied *V. amygdalina* by the young ones was higher than one as previously reported by Ogbo and Patrick-Iwuanyanwu (2019) & Wang *et al*. (2021). Consequently, the young consumers of *V. amygdalina* from the studied locations could be more exposed to the non-carcinogenic health risks than the adult (Hertzberg *et al*., 2024). It is therefore risky for the children class to consume *V. amygdalina* obtained from the studied locations constantly.

The carcinogenic risks of the metals associated with the consumption of *V. amygdalina* harvested from roadsides with high traffic density by the young and adult populations was assessed using incremental lifetime cancer risk (ILCR) and total cancer risk (TCR) (Usman *et al*., 2022; Bambara *et al*., 2023). The results of ILCR for Cd, Pb, and Ni (carcinogens) through the consumption of *V. amygdalina* by the young and adult populations are shown in Table 3. The mean ILCR values of Cd, Pb, and Ni via the consumption of studied *V. amygdalina* by the young ones were 3.53E-04, 8.50E-06, and 7.89E-04, respectively. Accordingly, the ILCR of Cd and Ni were in the high cancer risk class, while that of Pb belongs to the low cancer risk class (USEPA, 1999). The mean ILCR values of the metals via the consumption of the studied *V. amygdalina* by the adult class were 1.13E-04, 4.19E-06, and 2.52E-04 for Cd, Pb, and Ni, respectively. Thus, the cancer risk classes of these metals were similar to that of the children class. However, the average ILCR values of the metals were higher for the children than in adult class as obtained by Munene *et al*. (2023). However, the cancer risks of Cd and Ni for both categories of consumers were higher than the recommended safe range of 1.0E-06-1.0E-04 by USEPA (2010). Hence, the consumption of *V. amygdalina* from the studied locations may result in cancer and cancer-related ailments. Cu and Zn are not classified as carcinogens hence; there are no cancer slope values assigned to them. For this reason, there are no ILCR values reported for Cu and Zn in this study.

The results of total cancer risk (TCR) of trace metals via the consumption of *V. amygdalina* obtained from roadsides with high traffic density are indicated in Table 3. The mean TCR values recorded for the young and adult classes were 1.15E-03 and 3.69E-04, respectively. Thus, the TCR values for the young and adult belong to the very high and high cancer risk classes, respectively. However, higher TCR values were recorded for the young consumers as obtained by Ebong *et al*. (2020) but; inconsistent with the results reported by Orisakwe *et al*. (2018). These values are also higher than the acceptable range proposed by USEPA (2010). Consequently, the consumption of *V. amygdalina* cultivated by roadsides with high traffic density may results in cancer (Ashraf *et al*., 2021; Mizan *et al*., 2023).

Conclusion

This research assessed the impact of high traffic density on metals loads in *Vernonia amygdalina* cultivated by roadsides and the associated human health problems. The outcome indicated that, the mean concentration of Pb in the studied *V. amygdalina* was higher than the recommended safe limit. The principal component analysis revealed that, vehicular emissions was the major source of trace metals in the studied *V. amygdalina.* Consequently, consistent consumption of the vegetable from roadsides with high traffic density could be harmful to the consumers. The daily intake rate of all the trace metals were within their recommended oral reference doses however; the children class was more vulnerable to metal toxicity. The non-carcinogenic risks of the metals via the consumption of *V. amygdalina* by the young ones were higher than one. Hence, it can result in non-carcinogenic and related hazards in the young consumers' overtime. The total cancer risk of the metals for both the young and adult consumers of the studied *V. amygdalina* were higher than the permissible limit however; the young consumers were more susceptible to the risks. The study indicated that, cultivation of vegetables by roadsides with high traffic density could be hazardous to the consumers thus; it should be discouraged.

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