Ngweme GN, et al., J Food Sci Nutr 2021, 7: 087
DOI: 10.24966/FSN-1076/100087

HSOA Journal of
Food Science and Nutrition

Research Article

Contamination Levels of Toxic Metals in Marketed Vegetable (*Amaranthus Viridis*) at Kinshasa, Democratic Republic of the Congo

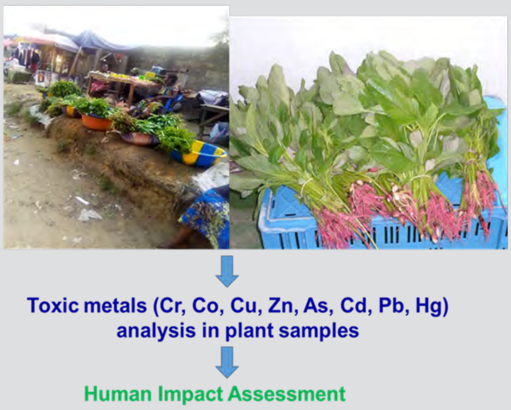
Georgette N Ngweme[1], Joël NN Konde[1], Amandine Laffite[2], Guillaume M Kiyombo[1], Crispin K Mulaji[3] and John W Poté[2,3]*

[1]School of Public Health, Faculty of Medicine, University of Kinshasa, Kinshasa XI, Democratic Republic of the Congo

[2]Faculty of Science, F.-A. Forel Institute and Institute of Environmental Sciences, University of Geneva, Switzerland, 66 Boulevard Carl-Vogt, Geneva, Switzerland

[3]Faculty of Science, Department of Chemistry, University of Kinshasa, Kinshasa XI, Democratic Republic of the Congo

Graphical Abstract



***Corresponding author:** John W Poté, Faculty of Science, F.-A. Forel Institute and Institute of Environmental Sciences, University of Geneva, Switzerland, 66 Boulevard Carl-Vogt, Geneva, Switzerland; Faculty of Science, Department of Chemistry, University of Kinshasa, Kinshasa XI, Democratic Republic of the Congo, Tel: +41 223790321; E-mail: john.pote@unige.ch

Citation: Ngweme GN, Konde JNN, Laffite A, Kiyombo GM, Mulaji CK, et al. (2021) Contamination Levels of Toxic Metals in Marketed Vegetable (*Amaranthus Viridis*) at Kinshasa, Democratic Republic of the Congo. J Food Sci Nutr 7: 087.

Received: January 16, 2021; **Accepted:** January 27, 2021; **Published:** February 08, 2021

Abstract

The contamination of food chain by toxic metals constitutes a major concern worldwide because of human health potential risks. *Amaranthus viridis* (*A. viridis*) is one of the most consumed vegetables in South Asian and Sub-Saharan African countries. However, the accumulation of pollutants, such as toxic metals in this plant is still little investigated. Consequently, this research investigates the level of toxic metals in *A. viridis* collected from four major markets of Kinshasa, Capital City of the Democratic Republic of the Congo, according to the seasonal variation in order to determine potential health risk for the consumers. The results revealed high metal concentrations in edible leaf vegetables during both dry and wet seasons. Metal concentration reaching the values (in mg kg^{-1}) of 3.6(Cr), 1.5(Co), 29.7(Cu), 348.2(Zn), 1.7(As), 1.5(Cd), 18.3(Pb) and 0.2(Hg). Except for Cu, the analyzed metal concentrations in leafy vegetables exceeded the permissible levels set by Food and Agriculture Organization/World Health Organization (FAO/WHO) for human consumption. Given the level of toxic metals in *A. viridis* leaf according to the regulation of FAO/WHO, human health consequences are likely to occur on continuous vegetable consumption. Our results recommend implementation of measures and efforts to improve the quality of this most consumed vegetable in order to minimize potential health risk for the consumers.

Keywords: *Amaranthus viridis*; Food analysis; Food consumption; Food quality; Human health risk assessment; Toxic metals; Vegetable contamination

Introduction

The vegetable is an essential part of the healthy human diet considered as a source of many vital nutrients to maintain normal physiological functions, antioxidants, dietary fiber metabolites and prevention of several diseases [1-3]. Vegetables also attract a wide range of pests and are affected by diseases, and therefore need intensive pest management [4-6]. Toxic metals can be accumulated in vegetables and transferred to humans through food consumption, leading to potential human-associated health risks. Therefore, the impact of contamination of vegetable by toxic metals has been intensively and worldly investigated by several researches. Many studies have been performed to assess the accumulation of organic and inorganic pollutants in different edible vegetable species because of their importance in the human diet for the prevention of consumer risks [7-11]. Toxic metal accumulation occurs mostly in edible parts of plants such as leaf and root, which may finally result in a reduction of crop quality and present a threat for human and animal health. Consumption of foodstuffs contaminated by toxic metals leads to their accumulation in the human body. It can cause several diseases including cancer, anaemia, infertility to males, cardiovascular, nervous, kidney and lung disease [5,6,12,13]. Even at low concentration, the consumption of vegetable contaminated by toxic metals can originate potentially health-threatening effects [6,14]. It is therefore crucial and recommended to consume non-contaminated vegetable by organic pollutants and toxic metals to avoid the potential human health risks.

Amaranthus sp. have significant colorific value, contain high mineral levels and important sources of copper, iron, zinc phosphorus, manganese, magnesium, and vitamin C [15,16]. The plants are mainly cultivated in peri-urban market gardening under tropical conditions (south of Asian and sub-Saharan African countries) in the field potentially contaminated by heavy metals [3,17-21]. Many of these studies demonstrated the potential of toxic metal accumulation in *Amaranthus* sp. and recommended further studies to assess the toxic metal levels linked to the consumption of part of the plant and over vegetables cultivated in urban areas of developing countries under tropical conditions to prevent potential health exposures to consumers. For instance, due to inadequate infrastructure in wastewater treatment facilities in developing countries and the use of urban wastewaters for vegetables growth in urban regions, toxic metal concentrations in urban vegetables are generally higher than WHO recommended levels [6,22].

Kinshasa is the capital and largest city of the Democratic Republic of the Congo, with an estimated population of about 13 million inhabitants and covering 9’965 km².For such high population density, the urban agriculture plays a central economic and socioeconomic development role in the daily life of the population and assures more than 70% of consumed fresh foodstuffs supply of the city. After cassava leaf, *A. viridis* has been identified to be a second most consumed leaf vegetable in the DRC [2].The agriculture is mainly performed in the urban municipalities in many cases along the rivers (watersheds) and near the heavily busy roads [19]. Worryingly, the use of urban wastewaters in irrigation is common in the vicinity of Kinshasa. Contaminated agricultural soil and atmospheric depositions significantly contribute to the accumulation of toxic metals in vegetables [9,23-26].To our best knowledge, studies on toxic metal contamination of vegetables commercialized in different Kinshasa markets has not been reported so far. Consequently, the aim of the present work is to determine the levels of toxic metals including Cr, Co, Cu, Zn, As, Cd, Pb and Hg in *A. viridus* leaves from 4 main vegetable markets of Kinshasa City. The evaluation of human health risks was performed by comparing the metal concentrations in *A. viridis* with the permissible levels for human consumption set by Food and Agriculture Organization and World Health Organization [27].

Materials and Methods

Study site and sampling procedure

This study was carried out in four main vegetable markets located in different municipalities of Kinshasa, the capital city of the Democratic Republic of the Congo (Figure 1). These randomly chosen markets, arbitrary named A, B, C and D, are major central distribution points (Figure 2 (A,B)) of vegetables produced in Kinshasa and surrounding provinces (Kongo Central, Kwilu, Kwango and Mai-Ndombe). The *A. viridis* sold in these markets are mainly produced in several gardening markets located in peri-urban areas of great towns.

The sampling took place in February 2019 (wet season) and August 2019 (dry season). For each market and during each season, samples were taken in triplicate from 3 randomly selected sellers (e.g. Figure 2 (C,D)). Each replicate consisted in about 500g of leafy vegetables homogenized to form a composite sample. After collection, samples were stored in an icebox and transported to the laboratory within 24h. After preliminary treatments (washing with deionized water and weighted), samples were frozen and sent to the Department F.-A. Forel (University of Geneva, Switzerland) for analysis.

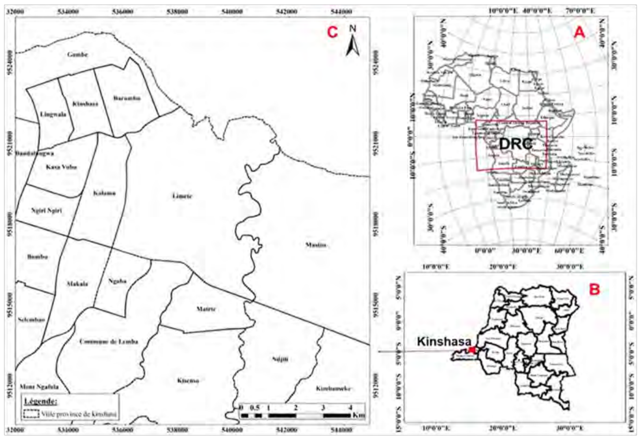


Figure 1: Adapted map showing: A: Location of the Democratic Republic of the Congo (DRC) in Africa continental map; B: Location of Kinshasa City in the Democratic Republic of the Congo and C: The municipalities of Kinshasa (which are located investigated markets).

Figure 2: Photos taken by Georgette Ngweme in February 2019, indicating; 2 (A,B): *Amaranthus viridis* in sale at Market B, 2 (B,C): *Amaranthus viridis* samples from Markets A and B, respectively.

Digestion of plant samples

The digestion of leafy vegetables was performed as described by Larras et al. [28]. Briefly, plant samples were washed with deionized water, lyophilized for 12 hours (water content was calculated) weighted and ground with liquid nitrogen. About 0.5g of ground plant samples were digested with 8mL of HNO_3 (65%, Suprapur®, Merck KGaA, Darmstadt Germany) and 2mL of H_2O_2 (30%, Merck KGaA, Darmstadt Germany) for 16h at 105°C. The supernatant was centrifuged at 20°C during 15 minutes at 4000rpm anddiluted 50 times with 1% HNO_3 (Suprapur®, Merck KGaA, Darmstadt Germany) for ICP-MS analysis.

Metals analysis in plant samples by ICP-MS

The concentration of toxic metals(Cr, Co, Ni, Cu, Zn, As, Cd, and Pb) in digested plant samples were measured by ICP-MS (Agilent, model 7700 series, Santa Clara, CA, USA). A collision/reaction cell and interference equations were performed for the correction of spectral interferences. The ICP multi-element standard solution Merck IV, 1000mgL^{-1} (Merck IV, KGaA, Darmstadt Germany) was used for the preparation of calibration solution at different concentrations (0, 0.2, 1, 5, 10, 20, 50 and 100µgL^{-1}). The Arsenic (As) was prepared from a mono element solution 1000mgL^{-1} (Merck KGaA, Darmstadt Germany). Metal concentrations in plant samples were expressed in mgkg^{-1} dry weight.

Mercury analysis in plant samples

The analysis of Hg in plant samples was performed using the Atomic Absorption Spectrophotometer for mercury determination (Advanced Mercury Analyser (AMA 254, Altecs.r.l.) Czech Rep.) as previously described by Larras et al. [28] and Roos-Barraclough et al. [29]. The limit of detection (3 x SD blank) was 0.005mgkg^{-1} and the reproducibility better than 3%. The Hg concentration is expressed in mgkg^{-1} of dry weight.

Quality control and statistical analysis

The accuracy of the method was checked by analysis of the certified reference material (CRM-482, EU Commission-JRC, Geel, Belgium), prepared and analyzed in the same conditions as the plant samples, for both ICP-MS and AMA analyses. Statistical treatment of data (ANOVA followed to Bartlett's Test and in case of non-applicability, Kruskal-Wallis test**)** has been realized using Epi Info version 3.5.4 in order to compare the averages concentrations between metal species. The Pearson correlation was performed using SPSS Statistics version 20.

Results and Discussion

Quality control and certified reference material values of metal concentrations

For ICP-MS analysis, the Limit of Detection (LOD) was calculated as 3 times standard deviation of the blanks and was less than 0.001µgL^{-1} for all analyzed elements. The total variation coefficients of triplicate sample measurements were below 2% and chemical blanks for the procedure were less than 1% of the sample signals. The obtained values of analyzed metals by ICP-MS and AMA for the reference material CRM-482 were in the certified range. The results are reported in table 1. The recovery values ranged from 91.1 to 98.3% for ICP-MS analysis and 95.8% for Hg analysis by AMA. The good recoveries of metal concentrations from certified reference material CRM-482 demonstrated the precision of the used protocol for leafy vegetables analysis.

Metal concentration in leafy vegetables

The toxic metals (Cr, Co, Cu, Zn, As, Cd, Pb and Hg) concentrations in *A. viridis* are shown in table 2. Metal levels in *A. viridis* were compared with international regulation for human consumption set by the Food and Agriculture Organization and World Health Organization [27], in order to evaluate consumer human health risks (Figure 3). Except for Cu, metal concentrations from all investigated markets exceeded the maximum level recommended by Joint FAO/WHO Expert Committee on Food Additives (Figure 2 (A,B)) [27]. The toxic metals concentration in leafy vegetables was found to be varied significantly according to sampling sites and seasonal variation ($p<0.05$). According to the PCA, season were responsible of quantity variations (Figure 4A) whereas sampling sites, where responsible of quality variations of leafy metal contamination (Figure 4B). These differences in *A.virdis* contamination could be attributed to the continuous use of untreated urban wastewaters in irrigation from nearby canals and drains and their intrinsic characteristics .In general, metal levels in leaves during the dry season were higher than during the wet season. Taking all markets and season together, the relative order of average concentrations of analyzed toxic metals in *A. viridis* leaves was: Zn>Cu>Pb>Cr>As>Cd>Co>Hg. Similarly, in a previous study the same tendency was observed in *Amaranthus Hybridusin* [18]. Maximum concentration for all metals was observed in the leafy vegetables from Market D during the dry season, with values (in mgkg^{-1}) of 348.2 (Zn), 29.7 (Cu), 18.3 (Pb), 3.4 (Cr), 1.7 (As), 1.6 (Cd), 1.5 (Co) and 0.19 (Hg). According to our short survey, more than 80% of *A. viridis* sold in this market are produced in two main gardening markets located near heavily trafficked roads and use many pesticides, fertilizers and highly contaminated water for irrigation to produce *A. viridis* [30,31]. Emissions from the heavy traffic on roads contain toxic metals including Pb, Cd, Zn and Ni which are present in the fuel as anti-knock agents and susceptible to contaminate the vegetables [32,33]. The use of wastewater, pesticide and micronutrient fertilizers in agriculture can be considered as one of the main sources of vegetable contamination by toxic metals [8,9,34,35].

Metal	Certified Value	Measured Value (n=3)	Recovery (%)
Cr	4.12±0.15	3.85±0.30	93.44
Ni	2.47±0.07	2.26±0.51	91.49
Cu	7.03±0.19	6.91±1.04	98.29
Zn	100.6±2.0	96.80±4.63	96.22
As	0.85±0.07	0.78±0.09	91.76
Cd	0.56±0.02	0.51±0.06	91.07
Pb	40.9±1.40	38.7±0.34	94.62
Hg	0.48±0.06	0.46±0.03	95.83

Table 1: Certified and observed values of metal concentrations of reference material CRM-482 (in mgkg^{-1} dry weight).

Significant variation ($p<0.05$) of Zn, Pb, Cd and As concentrations were observed in leafy vegetables with the values (in mgkg^{-1}) ranged between 88.6-348.2, 1.8-18.3, 0.24-1.7 and 0.4-16 for Zn, Pb, As and Cd, respectively. These values are about 1.5 to 5.8, 3.0 to 61.0, 2.4 to 17 and 2-8 higher than that of the FAO/WHO maximum permissible level for Zn (60 mgkg^{-1}), Pb (0.3 mgkg^{-1}), As (0.1) and Cd (0.2), respectively (Figure 3). The values of Zn observed in this study were higher than the values of 78.45±7.48mgkg^{-1} reported by Islam et al. [20], in *A. viridis* produced in Dhaka city, Bangladesh. However, the concentration of Pb and Cd obtained in leafy vegetables of this research was lower than 68 to 152mgkg^{-1} and 0.5 to 4.9, respectively reported by Atayese et al. [33], in *A. viridis* produced along major highways in Lagos, Nigeria. Concerning Pb, As and Cd, concentrations where higher than those found by Islam et al. [20], who reported the values of 2.54±0.56, 0.19±0.04, 0.15±0.03mgkg^{-1} for Pb, As and Cd, respectively in *A. viridis* but lower than those reported by Azi et al. [3], in *A. viridis* grown in Abakaliki, Nigeria (i.e. 8.2-12.0, 6.4-8.5 and 0.8-0.9 for Pb, As and Cd, respectively).

		Cr		Co		Cu		Zn		As		Cd		Pb		Hg	
		ppm	±SD	ppm	±SD	ppm	±SD	ppm	±SD	ppm	±SD	ppm	±SD	ppm	±SD	ppm	±SD
Market A	A1	2.12	0.71	1.01	0.30	9.23	3.91	133.23	29.31	0.62	0.09	0.64	0.08	3.34	0.70	0.03	0.01
	A2	0.38	0.13	0.22	0.05	8.35	2.67	165.58	36.43	0.89	0.13	0.45	0.06	6.34	1.33	0.05	0.02
	A3	1.93	0.55	0.18	0.04	1.46	0.31	99.57	21.91	0.37	0.06	0.55	0.07	1.76	0.58	0.03	0.01
	A4	3.57	0.91	1.32	0.07	9.98	2.55	216.32	47.59	1.03	0.15	0.86	0.11	9.28	1.95	0.08	0.03
	A5	2.44	0.84	1.28	0.06	11.5	3.68	194.48	42.79	0.98	0.15	0.92	0.13	8.15	1.71	0.06	0.02
	A6	3.17	0.65	1.3	0.08	12.3	2.50	186.21	34.20	1.07	0.11	0.78	0.17	11.04	2.03	0.09	0.02
Market B	B1	0.77	0.48	0.19	0.04	7.76	2.48	163.87	36.05	0.24	0.11	0.37	0.05	4.98	1.05	0.02	0.01
	B2	2.41	0.65	0.17	0.25	12.38	3.96	88.56	19.48	1.22	0.18	0.44	0.05	7.14	1.50	0.04	0.01
	B3	0.96	0.26	0.33	0.08	13.42	4.29	154.56	34.00	0.85	0.13	0.76	0.09	2.68	0.56	0.01	0.00
	B4	2.82	0.49	1.27	0.06	15.75	2.80	209.61	46.11	1.33	0.20	1.04	0.13	12.34	2.59	0.07	0.02
	B5	2.53	0.68	1.35	0.08	14.73	4.71	215.45	47.40	1.41	0.21	0.94	0.12	11.26	2.36	0.06	0.02
	B6	2.64	0.78	1.33	0.04	15.28	3.16	241.62	38.74	1.28	0.15	1.12	0.14	10.75	1.95	0.09	0.03
Market C	C1	1.34	0.36	0.42	0.10	6.44	2.06	123.74	27.22	0.76	0.11	0.35	0.04	6.22	1.31	0.03	0.01
	C2	0.23	0.11	0.24	0.06	9.75	3.12	174.27	38.34	1.17	0.18	0.46	0.06	2.74	1.21	0.01	0.00
	C3	0.86	0.23	0.51	0.12	10.27	3.29	154.42	33.97	0.92	0.14	0.42	0.05	3.91	0.82	0.04	0.01
	C4	2.11	0.57	0.56	0.06	13.67	4.37	234.61	51.61	1.23	0.18	0.94	0.12	15.22	3.20	0.09	0.03
	C5	2.47	0.40	0.67	0.11	19.41	3.01	255.17	56.14	1.42	0.21	0.78	0.10	8.23	1.94	0.07	0.02
	C6	2.41	0.28	0.59	0.05	14.36	2.32	196.28	32.82	1.33	0.18	0.84	0.16	14.77	2.38	0.09	0.02
Market D	D1	2.12	0.84	0.32	0.07	24.57	7.86	135.32	29.77	1.25	0.19	0.87	0.11	7.88	1.65	0.08	0.03
	D2	1.41	0.92	1.16	0.40	15.22	3.99	221.45	48.72	0.96	0.14	0.72	0.09	3.87	1.07	0.06	0.02
	D3	2.22	0.60	1.13	0.26	25.17	8.05	222.46	48.94	1.34	0.20	0.88	0.11	8.52	1.79	0.04	0.01
	D4	3.43	0.39	1.25	0.06	29.73	6.31	348.23	76.61	1.58	0.24	1.56	0.19	14.37	3.02	0.12	0.04
	D5	2.37	0.64	1.49	0.11	25.29	4.89	316.62	69.66	1.74	0.26	1.35	0.17	18.28	3.84	0.09	0.03
	D6	2.61	0.85	1.38	0.28	27.63	3.73	258.47	34.83	1.62	0.33	1.45	0.65	17.24	2.68	0.19	0.09

Table 2: Toxic metal concentrations (ppm: mgkg-1 dry weight) in A. viridis leaves from 4 markets.

Note: A1-A3 and A4-A6: Sampling from market A during the wet and dry season respectively.
B1-B3 and B4-B6: Sampling from market B during the wet and dry season respectively.
C1-C3 and C4-C6: Sampling from market C during the wet and dry season respectively.
D1-D3 and D4-D6: Sampling from market D during the wet and dry season respectively.

The concentration of Cr in leafy vegetables from investigated markets exceeded the permissible levels for human consumption set by FAO/WHO of 1.3mgkg^{-1} during the dry season. Except for the market D, the Cr values during wet season were within the recommended maximum acceptable levels proposed by FAO/WHO. The values (in mgkg^{-1}) ranged from 0.2-2.2 and 2.1-3.4 in the wet and dry season, respectively. The level of Cr in this study was higher than 0.1mgkg^{-1} reported by Azi et al. [3] and similar to that reported by Islam et al. [20], with the value of 2.28±0.6mgkg^{-1}.

Even at low concentration, Hg is very dangerous to humans because of its extreme toxicity and ability for biomagnification and bioaccumulation in food chains [36,37]. The Hg concentration obtained in this investigation ranged between 0.01 and 0.19mgkg^{-1}. These values exceeded 10 to 190 times than the permissible levels for human consumption set by FAO/WHO of 0.001mgkg^{-1} (1µgkg^{-1}). Despite, the Hg levels are lower than 3.8-14.0mgkg^{-1} in *A. viridis* leaves as reported by Azi et al. [3]. Our present results were concordant with the range values of 0.003-0.046mgkg^{-1} obtained by Li et al. [37], in *Amaranth* leaves.

The concentration of Cu in *A. viridis* leaves for all samples ranged between 1.5 and 29.7mgkg^{-1}. These values are under the permissible levels for human consumption set by FAO/WHO of 40mgkg^{-1} (Figure 3). The Cu values observed in this study is similar to that observed by Islam et al. [20] and lower than 123.5mgkg^{-1} as reported by Chabukdhara et al. [25]. The Co concentration ranged from 0.18-1.5mgkg^{-1}. These values are under the permissible levels for human consumption, according to the value of 50mgkg^{-1}, reported by Chiroma et al. [38].

The cultivation of *A. viridis* in DRC is mainly performed in the peri-urban municipalities, in many cases alongside rivers and/or heavily trafficked roads [19]. The agricultural activities including, frequent use of pesticides, fungicides fertilizers which contain metals as a natural impurity [35,39]. Additionally, in many cases, urban agriculture is using the contaminated water for irrigation [31,40-43]. These aspects can be considered as main sources of leafy vegetables contamination by toxic metals.

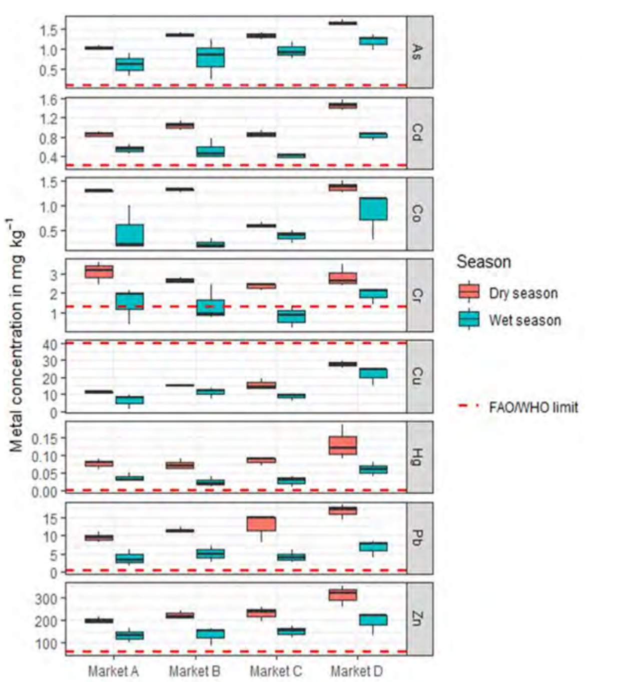


Figure 3: The concentration of analyzed toxic metals in *Amaranthus viridis* from four investigated markets comparing to regulation for human consumption set by the Food and Agriculture Organization (FAO) and World Health Organization (WHO).

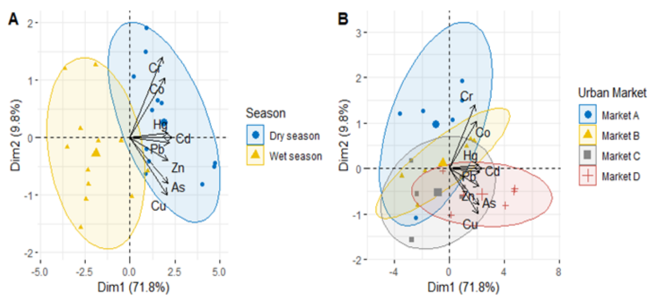


Figure 4: PCA analysis of analyzed metals in *Amaranthus viridis* according to season (4A) and urban markets (4B).

Metal correlation in leafy vegetables

Metal correlation in leafy vegetables was analyzed by PCA (Figure 4). Pearson correlation analysis showed a strong and positive correlation between As and Cu (r=0.81, $p<0.001$) and Hg, Cd and Pb (r>0.80, $p<0.001$), indicating their common source and transport pathway from agricultural practices and road traffic deposition respectively.

In leafy samples from the market D, where peri-urban gardens are known to be affected by heavily trafficked roads. The concentrations of Cr, Cu, Cd and Pb were significantly correlated, indicating the impact of traffic. Cd had a stronger positive correlation with Pb (r=0.92, p < 0.001) (Table 3), implying road traffic deposition on the surrounding environment and subsequent accumulation on plants. There was a significant correlation observed between Co and Zn, suggesting that that application of chemical fertilizers and its impact on the leafy vegetable.

	Cr	Co	Cu	Zn	As	Cd	Pb	Hg
Cr	**1.00**	**0.26**	**0.92***	**0.66**	**0.75**	**0.86***	**0.69**	**0.54**
Co		1.00	0.13	0.82*	0.51	0.56	0.58	0.33
Cu			1.00	0.44	0.82*	0.79	0.74	0.54
Zn				1.00	0.67	0.79	0.68	0.35
As					1.00	0.90*	0.98**	0.58
Cd						1.00	0.92**	0.77
Pb							1.00	0.69
Hg								1.00

Table 3: Pearson correlation matrix of toxic metals content in A. viridis leaves from market A.

Note: *Correlation is significant at the 0.05 level.
**Correlation is significant at the 0.01 level.

Conclusion

The present research constitutes the first assessment of toxic metals in *A. Viridus* selling in Kinshasa markets. The leafy vegetables contain the high values of most investigated toxic metals. Our results demonstrated that, except Cu, the toxic metals including Cr, Co, Zn, As, Cd, Pb and Hg present the values which were several folds higher than the international regulation for human consumption set by the Food and Agriculture Organization (FAO) and World Health Organization (WHO). Such elevated levels of toxic metals in *A. virdis* could cause a potential impact on human health on regular consumption. The accumulation of toxic metals could be due to the use of contaminated irrigation water, chemicals used to combat and prevent vegetable diseases, and the vehicle and motorcycle emissions around crop fields. Therefore, this study warrants proper control measures to prevent the sources of the metals into the irrigation water. Correlation analysis indicated that combined sources, including wastewater from channels, fertilizer application, and atmospheric deposition, are identified as sources of toxic metals. Finally, our research heavily recommends further studies for the assessment of toxic metals and other micro pollutants in vegetables, fruits and cereals selling in different markets of Kinshasa, identify the sources of contamination and comprehensive evaluation of consumer human health risks.

Acknowledgment

We are grateful to financial support from the Forel Department to Georgette Ngweme during her training at University of Geneva and the Open Access Publication Funds of University of Geneva.

Compliance with Ethical Standards

We confirm that the field studies did not involve endangered and protected species. The funders had no role in study design, data collection and analysis, decision to publish, or preparation of the manuscript.

Conflict of Interest

The authors declare no conflict of interest.

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