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An analysis of heavy metals contamination and estimating the daily intakes of vegetables from Uganda

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Abstract

Background: Environmental contamination with elevated levels of copper (Cu), cobalt (Co), iron (Fe), zinc (Zn), lead (Pb), chromium (Cr^{6+}), cadmium (Cd), and nickel (Ni)—all states of which are found in Uganda—raises health risk to the public. Pb, Cr^{6+}, Cd, and Ni for instance are generally considered nonessential to cellular functions, notwithstanding the importance of the oxidative state of the metals in bioavailability. As such, we aimed in this study (i) to evaluate heavy metal concentrations in four vegetables from a typical open-air market in Uganda, (ii) to assess the safety of consuming these vegetables against the World Health Organization (WHO) recommended limits of heavy metals consumption, and (iii) to formulate a model of estimated daily intake (EDI) among consumers in the country. **Methods:** This was a cross-sectional study conducted in five georeferenced markets of Bushenyi district in January 2020. Amaranthus, cabbages, scarlet

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eggplants, and tomatoes were collected from open markets, processed, and analyzed by atomic absorption spectrometry. Modeled EDI, principal component (PCA) and cluster analysis (CA) were conducted to identify relationships in the samples. **Results:** The levels of essential elements in the four vegetables were found to fall from Co > Cu > Fe > Zn. Those of non-essential metals were significantly higher and followed the pattern Cd > Cr > Pb > Ni. The highest EDI values were those of Cu in scarlet eggplants, Zn in amaranthus, Fe in amaranthus, Co in amaranthus, Pb in cabbages, total Cr in scarlet eggplant, Cd in cabbages and tomatoes, and Ni in cabbages. In comparison to international limits, EDIs for Zn, Cu, Co and Fe were low while Ni in cabbages were high. PCA showed high variations in scarlet eggplant and amaranthus. The study vegetables were found to be related with each other, not according to the location of the markets from where they were obtained, but according to their species by CA. **Conclusion:** The presence of non-essential elements above WHO limits raises policy challenges for the consumption and marketing of vegetables in the study area. Furthermore, low EDIs of essential elements in the vegetables create demand for nutritious foods to promote healthy communities.

Keywords

Food safety, heavy metals in vegetables, trade, vegetables, Uganda, vegetable consumption in Africa

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Introduction

In Uganda—the subject of this study, heavy metals have been identified in milk, beef, drinking water, and herbal medicines in southwestern Uganda.[1–3] Pb concentrations of between 4 and 18 ppm have been recorded in amaranthus within Uganda.[4] Vegetables grown along Lake Victoria (Uganda) were associated with high cadmium (Cd) and lead (Pb) at concentrations of 0,08–0.76 ppm and 0.003–5.06 ppm respectively above World Health Organization limits.[5] Globalization and increased population growth are associated with an increasing demand for vegetables,[6] and developing countries are no exception[7] demonstrating the importance of this study. Currently, dietary guidelines in 93% of countries (7 African countries in comparison to 17 in Asia and 33 in Europe) encourage the consumption of vegetables and fruits.[8] Vegetables not only contain essential nutrients necessary for maintaining the physiological function of somatic cells and tissues,[9,10] but extracts from various plants may have a role in refining disease management strategies through dietary patterns that may promote health. An amaranthus supplement at 25% and 50% w/w administered for 4 weeks, for example, was shown to mitigate type 2 diabetes mellitus in male Wistar rats,[11,12] with the possible application as a management adjunct in human pathology in diabetes.[13] Scarlet eggplant (*Solanum* spp) and cabbage (*Brasica* spp) extracts (i.e. 1 g in 30 ml of methanol) were shown to inhibit nitric oxide in macrophage cell cultures after 24 h of exposure[14] and to protect a rat cardiomyoblast cell line from oxidative stress i.e. methanol (1:8) extract at least 2 h, treated with 100, 200, and 300 μg/ml cabbage extract for 24 h,[15] thus suggesting the importance of these two vegetables in complementary medicine. This is important since *Brassica campestris* spp (administered at 50 mg/kg orally in mice) effects on obesity were associated with increased expression of lipolysis genes (i.e. *adipose TG lipase, adiponectin*, and *leptin*) and activation of cyclic AMP-dependent kinase.[16] The safety of consuming a plant-based diet, however, is threatened by the increasing presence of heavy metals in the environment from human activities and their subsequent bioaccumulation in vegetables and mutagenic health risks in humans. Furthermore, stress disrupts protein function, metabolic and gene expression.[17] Abiotic stress as a result of soil salinity, reduced rainfall, and high temperatures involve protein kinase activity which modulates energy consumption in the plants.[18] Heavy metal-induced stress has been associated with low growth rate, loss of chlorophyll, and plant death.[19,20]

Compounding the problem of heavy metal contamination are (i) the absence of a robust food safety policy and technical knowledge in most developing countries to carry out routine monitoring of the environment,[1] (ii) the nature of pollution reporting in those countries, particularly in Africa,[15] and (iii) not providing consumers information on the sources and the distribution of contaminants in food products. There was, therefore, a need not only to determine the nature and extent of food contamination by heavy metals in this region but also to encourage the development of technical skills needed to conduct such studies.

Minerals such as copper (Cu), iron (Fe), zinc (Zn), and cobalt (Co) are found naturally in vegetables and in small quantities are essential for cellular function. Copper in the Cu^{2+} state is also insoluble in water, but soluble as Cu^{+}, the form commonly present in naturally unstable copper sulfate. The importance of Cu^{2+}, Fe^{2+}, Zn^{2+}, and Co^{2+} in studies such as this one cannot, therefore, be overemphasized.[21] Fe is abundant on earth; it is insoluble in its Fe^{3+} oxidation state. Iron in oxidation state two (Fe^{2+}) is important to human and plant physiological function.[22] Chromium (Cr^{3+}) is essential in carbohydrate and lipid metabolism and its daily intake of 0.05–0.2 mg has been establisehd.[23,24] In addition, Cr^{3+} is considered safe following its wide safety range of 1 mg/day, although this cannot be said about Cr^{6+}.

Cr^{6+} is toxic to both humans and plants[25] and as such, toxicological evaluation of food products is placing increased emphasis on Cr^{6+}, especially following its documented public health relevance.[25–27] Nickel (Ni) is an essential metal for plants to complete their lifecycle, helps some leguminous plants for root nodule growth and hydrogenase activation.[28] In addition, many metalloenzymes such as mononuclear nickel in Ni-superoxide dismutase, glyoxylase I, and acireductone dioxygenase use Ni to conduct biological activities,[29] and its toxicity has been attributed to displacement, blocking, and modification of essential components in the biomolecules during enzyme function.[28]

Non-essential elements such as Pb, Cr^{6+}, Cd, and high Ni in vegetables are easily traced to at least the soils in[5,30,31] or the air particulates[32] proximate to vegetable cultivation. In soil, the natural pedogenetic processes of weathering constitute the main source of heavy metals.[33] This source is complemented by human activities such as agriculture.[34] The soil-to-plant transfer factor for heavy metals is known to vary with plant species.[35] Within species, the accumulation of heavy metals also varies with plant phytometry. For example, the accumulation of concentrations of total Zn and Cu in different plant tissues is species-specific[36] as has been illustrated by the differential bioaccumulation of heavy metals (Pb, Cu, and Zn) in *Brasica nigra* roots which was higher in the roots of *Colocasia esculentum*,[37] which in turn was found to be higher in *Raphanus sativus* roots.[38] Most studies agree that these observations are consequences of differential uptake and enrichment of metals among plant species[39,40] which are amplified in areas with naturally high levels of heavy metals in the soil.[41]

Heavy metal contamination in plants have been reported in *Vernonia amygdalina* i.e. total Cr at 121.8 ppm > Ni at 84.09 ppm > Zn at 53.87 ppm > Pb at 40.61 ppm > Cu at 28.75 ppm > Fe 14.15 ppm > Co at 7.923 ppm > Cd at 0.1163 ppm in Uganda.[3] In China, Cd was found to be above while Pb and Cr were below recommended limits in China.[42] Furthermore, Cu, Cd, and Pb have been identified in *Brassica oleracea*[43] and more heavy metals have been identified in several vegetables.[44] They may cause plant death by damaging roots, or by altering the physiological functions of plants overall.[45,46] In the latter, Cd, for example, inhibits photosynthesis and growth by preventing CO_2 fixation by the enzyme ribulose-1,5-bisphosphate carboxylase as was shown in mungbean,[47] and/or by suppressing efficient energy transformation in the photosynthetic electron transport processes, as was shown in durum wheat.[48] Cu, on the other hand, can modify chlorophyll degradation.[49] Both Cd and Cu at dosages of 7 mg/kg and 700 mg/kg after 20 days of exposure respectively induced DNA damage in *Pisum sativum* roots and leaves.[49]

Some plants have also been shown to bioaccumulate (a process associated with plant stress) Cu and Zn in the shoots more than in other parts of the plants.[46] In amaranthus, for example, Pb and Cd have been found above the recommended safe levels in the leaves and roots.[46,50] At high concentrations, non-essential metals such as Pb and Cd as well as essential metals Zn, Fe, and Cu may play a role in the pathogenesis of diseases such as Alzheimer's disease through oxidative stress mechanisms.[37–39] This is particularly important to African descendants who are genetically susceptible to this dementing process.[51] Disproportional deposition of metals in the body promotes the expression of secretases that disrupt organelle function, leading to impairment in function.[52,53] That being said, clinical studies directly linking heavy metals to neurological disease remain controversial.

To guide toxicological evaluation of vegetables, the World Health Organization/Food and Agriculture Organization (WHO/FAO) set these threshold limits at 40 ppm for Cu,[54] 425 ppm for Fe,[55] 60 ppm for Zn,[56] 50 ppm for Co,[55] 0.3 ppm for Pb,[54] 1.3 ppm for Cr,[55] 0.2 ppm for Cd,[54] and 0.2 ppm for Ni.[54] Ni has been reported as an important metalloenzyme and it helps some plants protect themselves against predatory insects,[57,58] and it's an established heavy metal whose concentrations in the environment have to be monitored.[59] Total Fe beyond the acceptable level and very low levels of Zn have been found in vegetables consumed in the Kumi district of eastern Uganda.[60] Deficiencies of Zn, Fe, and Cu are not unusual in African soils,[61] but micronutrient deficiencies in plants that raise the specter of nutritional deficiencies in humans are of public health concern.[22,62] The latter is the rationale for the present study. Here, the objective was (i) to determine the heavy metal concentrations in common vegetables consumed in the Bushenyi region of Uganda, a microcosm of a typical vulnerable community located in a developing country, (ii) to model the estimated daily intake that would define the health risks of consuming vegetables in the study population, and (iii) to test whether exploratory analysis could be utilized to classify vegetables broadly. Bushenyi was chosen for this study because of our previous experience in studying environmental safety.[3]

Methods

Study design

A cross-sectional survey was conducted in southwestern Uganda in major community markets supplying vegetables in Bushenyi district in January 2020. A total of five (5) georeferenced open-air markets (Bushenyi, Ishaka, Kanshenyi, Kizinda, and Nyakabirizi) were surveyed, and four (4) species of vegetable samples (cabbages, scarlet eggplants, tomatoes, and amaranthus)[63] were collected from each market ($N = 20$). The locations of these markets where we collected samples were then mapped using quantum geographical systems (qGIS®) 3.14 Cirona.

Mapping the study area

A Sentinel-2A satellite image file (L1C_T35MRV_A024957_20200402T082817, acquisition date: 2020/04/02) from the United States Geographical Surveys was imported

Table 1. The wavelengths and corresponding slit widths used to obtain instrumental linear calibration range for each metal.

Metal	λ, nm	Slit width, nm	Equation and coefficient[a]
Pb	217.0	1.0	$y = 0.0168x + 0.0082$, $R^2 = 0.9763$
Cr	357.9	0.2	$y = 0.0193x + 0.0067$, $R^2 = 0.9792$
Cu	324.9	0.5	$y = 0.1152x + 0.0034$, $R^2 = 0.9996$
Zn	213.9	1.0	$y = 0.2051x + 0.1166$, $R^2 = 0.9209$
Cd	228.8	0.5	$y = 0.2075x + 0.0884$, $R^2 = 0.9559$
Co	240.7	0.3	$y = 0.0332x + 0.011$, $R^2 = 0.9842$
Fe	248.3	0.2	$y = 0.0304x + 0.0112$, $R^2 = 0.9815$
Ni	323.0	0.2	$y = 0.0362x + 0.0125$, $R^2 = 0.9836$

[a]x: absorbance; y: concentration in ppm.

into qGIS® and divided into 4 levels: ≤66 units for lake Edward vegetation (blue), 113 units for heavy vegetation as seen in Queen Elizabeth National Park (green), 160 units for sparse vegetation (brown), and >160 for bare soil (red). Besides, a digital land elevation satellite image from SRTM1 Arc- Second Global was used (entity ID: SRTM1S01E030V3; publication date of 23-Sept-2014). Image file s01_e030_1arc_v3 was also divided into 4 illustrative levels, i.e. 909 units for sea level (blue), 1325 units for lowland level (green), 1740 units for midland level (brown), and 2156 units for highland level and hills.

Instrument calibration and heavy metal determination in vegetables

Standard stock solutions, prepared as previously described,[3] were used to obtain the linear calibration ranges of an atomic absorption spectrometer (AAS, Perkin Elmer 2380) for Pb, Cr, Cu, Zn, Cd, Co, Fe, and Ni, i.e. the metals of interest in this study. The preparation of sample extracts for analysis and the concentration of heavy metals in vegetables were carried out as described previously.[1,64] In brief, vegetable powder (1 g) was mixed with nitric acid (20 mL) and perchloric acid (4 mL) and digested on a hot plate until the total volume was 4 mL. The solution was cooled, filtered, and adjusted to a final volume of 50 mL with deionized water. The metal concentration in each 50 mL sample extract was then determined by the AAS at wavelengths and corresponding slit widths given in Table 1.

For public safety reasons, we preferred to determine the total amount of each elemental metal in samples, irrespective of their oxidation states. The choice to use the AAS technique in this study was made with this in mind since AAS first converts all ions of the same elements to free atoms (in the atomization step of the technique) before collecting analytical data.

Modeling of estimated daily intake of heavy metals in vegetables

The modeled estimated daily intake was calculated using the equation below i.e.

$EDI = \frac{C \times IR}{BW}$ where, EDI = estimated daily intake, C = concentration of total element, IR = ingestion rate of the vegetables. Following a scarcity of epidemiological surveys on vegetable consumption in Uganda, a general IR of 254.3 g/day was used.[65] Clinical guidelines from the Ugandan government were used to define children as those 6 years weighing 15 kg and adults were defined as those 30 years and above weighing 70 kg.[66]

Statistical analysis

Concentration data in MS Excel version 2019 were exported into GraphPad Prism Version 6 (GraphPad Software, La Jolla California USA). One-sample *t*-tests were conducted to compare the average values obtained in this study with the WHO reference values for vegetables (significance reported at $P < 0.05$). The estimated daily intake of heavy metals by children and adults alike were descriptively presented as boxplots. Where relevant, the standard error of the mean (SEM) was chosen here because it relates the variability of individual data value to the mean of a population. This is unlike the standard deviation (SD), which relates the same variability to the mean value of the sample instead. We believe that SEM is a better estimate of the likelihood of our sample means coming from the true population means understudy than SD.

For exploratory analysis, principal component analysis (PCA) and cluster analysis (CA) were performed with the aid of PAST software[67] were used to reduce data dimensionality in multivariate space and determine natural groupings of variables and cases, respectively. Data were scaled to have a mean of zero and a standard deviation of one before PCA and CA to make metal concentrations comparable across samples. The results of CA and PCA are presented here as a dendrogram and as scatter plots of relevant principal components, respectively. Ward's algorithm was used to measure dissimilarity between clusters in CA. The variance-covariance matrix and distance in eigenvalues were used for PCA, and the results are presented as a geometric plot of vectors (eigenvectors); the amount by which each vector is stretched from the origin is the eigenvalue.

Results

Description of the study area

The markets surveyed in Uganda for this study are located in commercial centers, all within a radius of approximately 6 miles (Figure 1). The centers are in hilly and midland level areas (Figure 1A) with moderate vegetation coverage (Figure 1B). The vegetation is densely green due to numerous tea-growing plantations and the cultivation of bananas for commercial and subsistence consumption. On the whole, the region is geographically characterized by deep valleys due to its proximity to the western rift valley of East Africa.

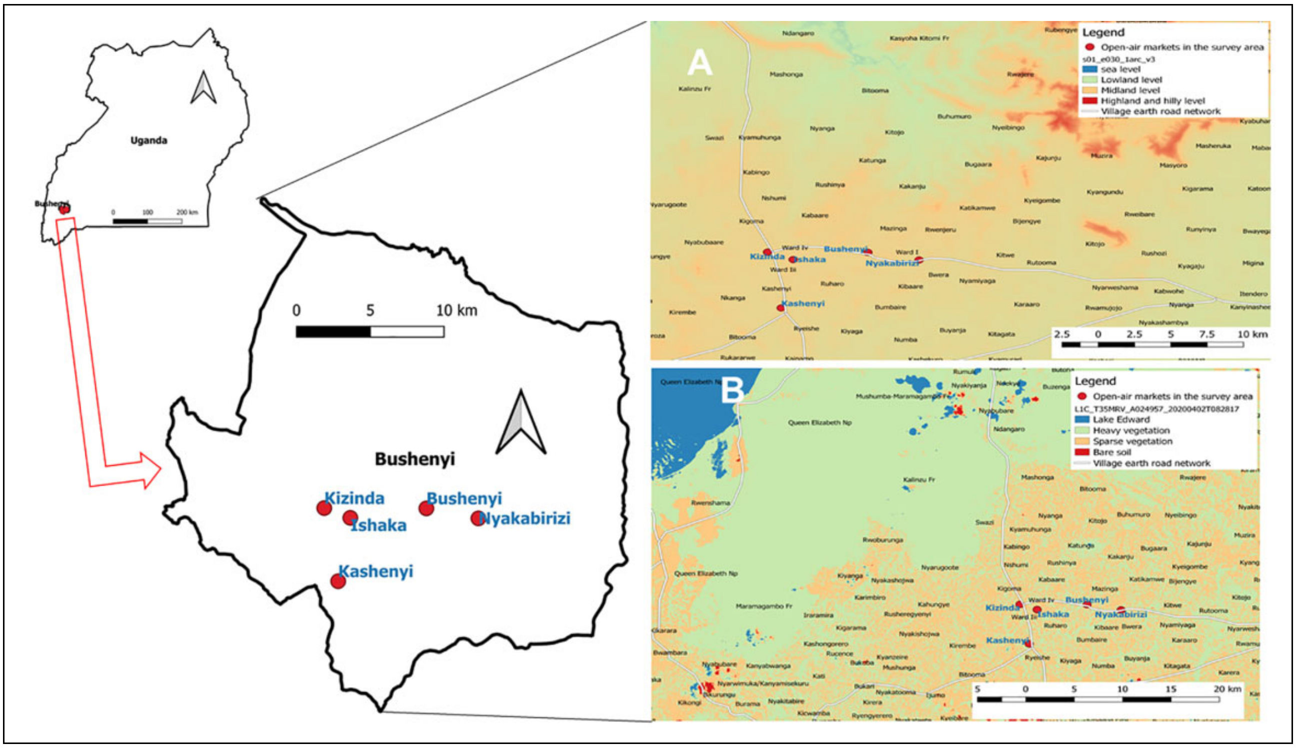

Figure 1. Map of the study area showing the relative location of the surveyed markets.

Descriptive analysis of heavy metal content of study vegetables

The concentrations of essential metals were in the order of Fe > Zn > Cu > Co, and non-essential metal concentrations were in the order of Pb > Cr > Ni > Cd. Concentrations of essential elements were significantly below WHO limits in the order Co > Cu > Fe > Zn; non-essential metals were significantly high compared to WHO limits in the order Cd > Cr > Pb > Ni in the vegetables. The combinations of vegetables and metals with levels exceeding the WHO limits were Zn in amaranthus, Pb in cabbage, scarlet eggplant, amaranthus and tomatoes (cabbage > scarlet eggplant > amaranthus > tomatoes), Cr in scarlet eggplant and amaranthus (scarlet eggplant > amaranthus), Cd in cabbage, tomatoes, amaranthus, and scarlet eggplant (cabbage > tomatoes > amaranthus > scarlet eggplant), and Ni in the order of amaranthus > cabbage > tomatoes > scarlet eggplant (Table 2). Note that $P > 0.05$, our chosen cutoff level for statistical significance, for Zn in amaranthus, Pb in tomatoes, Cr in amaranthus, cabbage, and scarlet eggplant, Cd in scarlet eggplant, and Ni in cabbage and scarlet eggplant. This means we cannot state with certainty that the levels of metals in these cases were significantly different from the WHO limits.

Comparing levels of heavy metals in study samples

The concentrations of Cu were lowest and statistically the same in amaranthus, tomatoes, scarlet eggplants, and cabbages ($P < 0.05$) (Figure 2A). Zn, Fe, Co concentrations were significantly higher in amaranthus than in all other vegetables (Figure 2B–D). Among non-essential elements, significantly higher concentrations of Pb were found in cabbages and scarlet eggplant than in amaranthus and tomatoes (Figure 2E). In addition, Cr was significantly highest in scarlet eggplant (Figure 2F), while Cd concentrations were significantly highest in tomatoes and cabbages, which were in turn higher than in amaranthus and scarlet eggplants in that order. Besides, amaranthus was found to contain significantly higher levels of Ni than other vegetables (Figure 2H) (Table 3).

Estimated daily intake of heavy metals in vegetables

The results of the risks associated with oral ingestion of the heavy metals studied were computed from the concentration values for each of the eight metals as indicated earlier here. The results, summarized in Figure 3, show that the risk of consuming Cu in eggplants was the highest of all metals for both adults and children (Figure 3A). The estimated daily intake (EDI) of Zn, Fe, and Co, on the other hand, was higher for adults than for children (Figure 3B–D). Additionally, the oral ingestion of Pb was higher in cabbages and scarlet eggplants in adults than in children (Figure 3E). The EDI for Cr was higher in scarlet eggplants for adults than for children (Figure 3F). Cabbages and tomatoes had higher Cd EDIs in adults than children, as shown in Figure 3.

Table 2. Description of heavy metal concentrations (ppm) in Ugandan vegetables and comparisons with WHO standards (one-sample t-tests).

Metals	N	Min	Q1	Median	Q3	Max	Mean	SEM	SD	WHO limits	Discrepancy	P-value
Cu												
Amaranthus	5	7.3	7.4	7.5	9.0	10.3	8.0	0.6	1.2	40.0	32.0	<0.0001
Tomatoes	5	1.8	3.2	5.1	6.3	7.3	4.8	0.9	2.0	40.0	35.2	<0.0001
Cabbage	5	0.5	0.5	0.9	1.4	1.4	0.9	0.2	0.4	40.0	39.1	<0.0001
Scarlet eggplant	4	1.5	3.1	8.3	11.1	12.0	7.5	2.2	4.4	40.0	32.5	0.0007
Fe												
Amaranthus	5	149.0	149.1	151.7	299.9	379.0	209.9	44.4	99.4	425.0	215.1	0.0084
Tomatoes	5	56.4	61.1	67.8	78.6	80.3	69.4	4.2	9.5	425.0	355.6	<0.0001
Cabbage	5	30.5	35.0	40.7	47.6	51.8	41.2	3.4	7.7	425.0	383.8	<0.0001
Scarlet eggplant	4	37.1	39.9	57.9	91.8	99.8	63.2	13.8	27.5	425.0	361.8	0.0001
Zn												
Amaranthus	5	38.8	46.2	57.4	79.9	84.0	61.9	8.1	18.1	60.0	−1.9	0.8231
Tomatoes	5	18.4	20.4	23.8	29.4	30.8	24.7	2.2	4.9	60.0	35.3	<0.0001
Cabbage	5	15.4	16.4	17.9	19.4	20.2	17.9	0.8	1.8	60.0	42.1	<0.0001
Scarlet eggplant	4	20.5	20.6	24.2	36.3	39.2	27.0	4.4	8.7	60.0	33.0	0.0048
Co												
Amaranthus	5	2.6	3.0	3.4	4.4	5.1	3.6	0.4	0.9	50.0	46.4	<0.0001
Tomatoes	5	0.7	0.9	1.6	1.8	1.8	1.4	0.2	0.5	50.0	48.6	<0.0001
Cabbage	5	0.3	0.4	0.6	0.8	1.0	0.6	0.1	0.2	50.0	49.4	<0.0001
Scarlet eggplant	4	0.6	0.6	0.9	1.6	1.7	1.0	0.3	0.5	50.0	49.0	<0.0001
Pb												
Amaranthus	5	2.0	2.9	4.3	5.7	5.7	4.3	0.7	1.6	0.3	−4.0	0.0046
Tomatoes	4	0.8	1.3	3.6	6.7	7.4	3.9	1.4	2.8	0.3	−3.6	0.0858
Cabbage	5	9.4	10.9	13.7	14.5	15.0	12.9	1.0	2.2	0.3	−12.6	0.0002
Scarlet eggplant	4	9.8	10.3	12.3	13.4	13.6	12.0	0.8	1.6	0.3	−11.7	0.0007
Cr												
Amaranthus	5	1.2	1.2	1.2	2.8	4.2	1.9	0.6	1.3	1.3	−0.6	0.3858
Tomatoes	4	0.3	0.4	0.5	0.7	0.7	0.5	0.1	0.2	1.3	0.8	0.0038
Cabbage	5	0.7	0.7	1.0	1.5	1.8	1.1	0.2	0.4	1.3	0.2	0.3084
Scarlet eggplant	4	1.2	2.9	8.4	9.4	9.7	6.9	1.9	3.8	1.3	−5.6	0.0618
Cd												
Amaranthus	5	1.3	1.3	1.5	1.9	1.9	1.6	0.1	0.3	0.2	−1.4	0.0004
Tomatoes	5	1.7	1.9	2.1	2.2	2.2	2.0	0.1	0.2	0.2	−1.8	<0.0001
Cabbage	5	2.0	2.0	2.1	2.2	2.3	2.1	0.1	0.1	0.2	−1.9	<0.0001
Scarlet eggplant	4	0.2	0.3	0.5	1.0	1.1	0.6	0.2	0.4	0.2	−0.4	0.1073
Ni												
Amaranthus	5	3.9	4.0	4.8	5.3	5.6	4.6	0.3	0.7	0.2	−4.4	0.0001
Tomatoes	5	0.7	0.9	1.4	1.7	2.0	1.3	0.2	0.5	0.2	−1.1	0.0063
Cabbage	5	0.4	1.2	3.9	6.5	8.7	3.8	1.4	3.1	0.2	−3.6	0.0602
Scarlet eggplant	4	0.5	0.5	0.8	1.5	1.7	0.9	0.3	0.5	0.2	−0.7	0.0721

KEY: N = number of samples, Min = minimum, Q1 = 25th percentile, Q3 = 75th percentile, Max = maximum, SD = Standard deviation, WHO = World Health Organization, ppm = parts per million.

Table 3. Tukey's multiple comparisons test showing adjusted P values for metal concentration comparisons in vegetables.

		Cu	Zn	Fe	Co	Pb	Cr	Cd	Ni
Multiple comparisons	N	Adjusted P values							
Amaranthus vs. Tomatoes	10	0.1660	0.0002	0.0040	0.0001	0.9873	0.7273	0.0358	0.0319
Amaranthus vs. Cabbage	10	0.0010	< 0.0001	0.0008	< 0.0001	< 0.0001	0.9145	0.0091	0.8690
Amaranthus vs. Scarlet eggplant	9	0.9862	0.0008	0.0045	< 0.0001	0.0004	0.0078	0.0002	0.0240
Tomatoes vs. Cabbage	10	0.0747	0.7355	0.8346	0.2339	< 0.0001	0.9718	0.8951	0.1295
Tomatoes vs. Scarlet eggplant	9	0.3368	0.9869	0.9980	0.8243	0.0004	0.0018	< 0.0001	0.9877
Cabbage vs. Scarlet eggplant	9	0.0034	0.5755	0.9250	0.7420	0.9240	0.0025	< 0.0001	0.0929

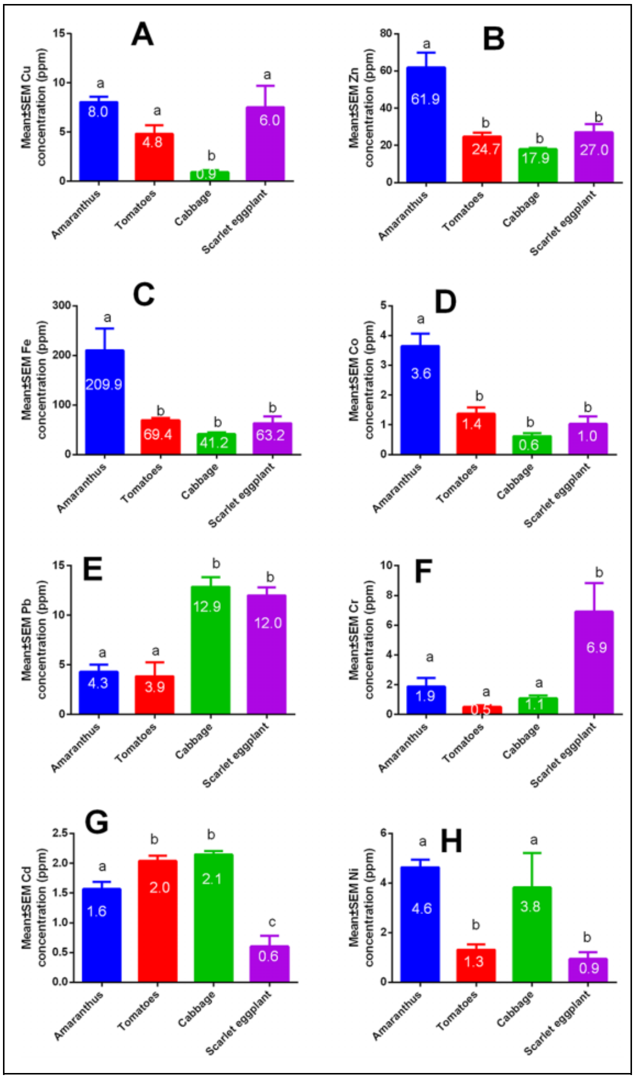

Figure 2. Mineral concentrations in vegetables from the study area. A = Cu, B = Zn, C = Fe, D = Co, E = Pb, F = Cr, G = Cd, H = Ni. Different superscripts indicate significant differences (P < 0.05). Amaranthus (n = 5), tomatoes (n = 5), cabbages (n = 5), scarlet eggplants (n =4).

To allow comparison on the same scale, the EDI data used in Figure 3 were standardized and replotted as shown in Supplementary material S1. The standardized plot showed that Cu oral ingestion was highest in scarlet eggplants, Zn in amaranthus, Fe in amaranthus, Co in amaranthus, Pb in cabbages, Cr in scarlet eggplant, and Cd in cabbages and tomatoes, while Ni EDI was highest in cabbages.

Exploratory analysis of estimated daily intake values

In an attempt to find additional information that might not be as apparent from the linear analyses reported above, PCA and CA were used to develop standardized EDI values determined in a multivariate space. The PCA results are displayed in Figure 4, in which PC1, PC2, and PC3 explained 44.3%, 23.9%, and 17.2% of the variance, respectively. Amaranthus scores positively along PC1, while scarlet eggplants and cabbage mainly score negatively along the same axis (Figure 4A). The EDI variation associated with tomatoes was not explained by PC1 (and indeed not that well by PC2 or PC3 either; Figures 4B and 4C). Along PC2, only scarlet eggplant scored highly and positively. Cabbage and tomatoes score low and negatively along this axis. PC2 did not explain variance in amaranthus EDI. Along PC3, no clear message is discernable from the vegetable scores, although the axis explained a substantial variance in EDI. Note that the 95% ellipses included in Figure 4 appear to suggest (i) a higher variation in scarlet eggplant and

The CA results, displayed in Figure 5, show that groupings are mostly by vegetable species (and not by the source market). This was expected from the dependence of heavy metal accumulation on plant species. One exception requiring further investigation is why eggplants and tomatoes from Kanshenyi and Ishaka appear to have intrinsically similar characteristics when the two markets are geographically far from each other. The conclusion here is that it is the uptake of metals by vegetables and not the regional source of vegetables that is more important in understanding the toxicity of vegetables in the study area.

Discussion

The present study demonstrated that heavy metals are indeed common in vegetables within Uganda. This was important since, previous studies have identified heavy metals at levels above WHO limits in beef, milk, and drinking water sources in the Bushenyi area.[1,2] In this study, high levels of two heavy metals, Pb and Cr, detected and above the WHO limits, were particularly worrisome. Essential elements were generally highest in amaranthus, while non-essential concentrations were highest in cabbages and scarlet eggplants. The differential concentration of heavy metals in vegetables has been reported previously in *Brasica nigra* and *Colocasia esculentum*,[36,37] demonstrating agreement with our study since all vegetables in their study had different concentrations of the elements. Furthermore, micronutrient deficiencies in African soils have been reported previously,[22,61,62] demonstrating the public health risks associated with the consumption of vegetables grown in poor soils. Since AAS measured the total element concentration, the importance of Cr^{6+}, Cr^{3+}, Fe^{2+}, Fe^{3+}, Cu^{+}, Cu^{2+} [21,25–29] were not investigated in this study.

In Africa, and Nigeria in particular, most vegetables and fruits consumed contain Zn, Pb, Fe, and Cu above WHO limits (see[68] and references therein for EDIs; see also[69]) for daily intake rate of metals in food crops and fruits, however, there is need to assess the entire diet. This is important since Zn deficiencies i.e. 60% in Ethiopia,[70] and in Uganda mineral consumptions were exceptionally high (≥80%) for Magnesium, Selenium, Zinc and Vitamins B2, B6, B9, C and E and lowest (≤50%) for Fe (30%), calcium (14.9%) in

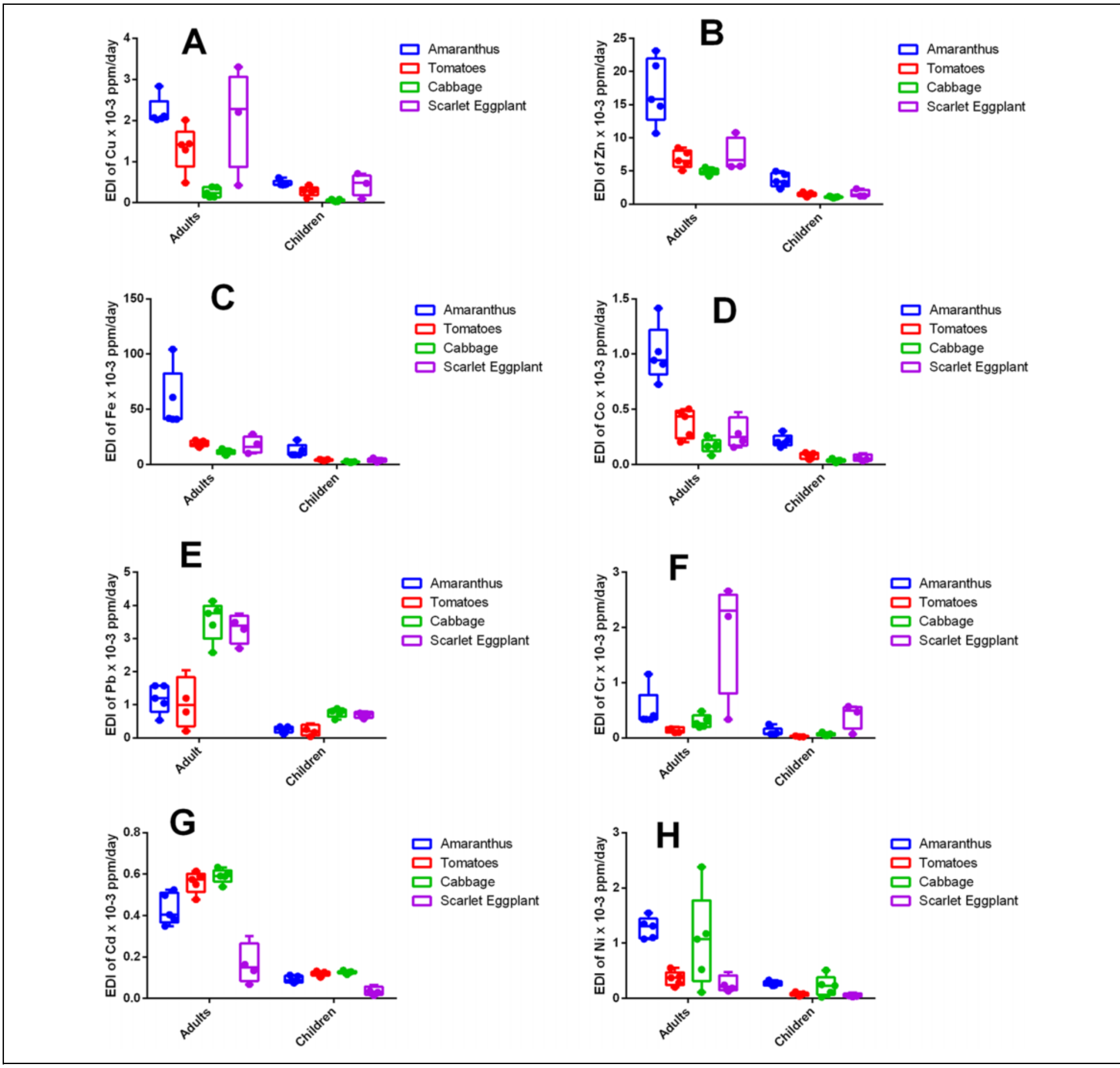

Figure 3. Estimated daily intake (EDI in ppm/day: mg/kg) of minerals in adults and children of Uganda from vegetables. A = Cu, B = Zn, C = Fe, D = Co, E = Pb, F = Cr, G = Cd, H = Ni. Amaranthus (n = 5), tomatoes (n = 5), cabbages (n = 5), scarlet eggplants (n = 4).

comparison to WHO limits.[71] In Kenya, spinach, kales, and coriander vegetables collected from the Makongeri market in Thika were found to contain higher levels of Cu, Zn, Cr, Ni, and Pb than other vegetables which were studied (Njuguna et al.[72]; see Pengpid and Peltzer[73]) although these were within WHO/FAO and the United States Environment Protection Agency (US EPA) allowable limits. Furthermore, Cu, Zn, and Pb have also been detected along with Cd in strawberries, cucumber, and spinach in Egypt.[74] This fact may suggest that vegetables in African markets can accumulate heavy metals contained within an ecosystem and perhaps serve as indicators of environmental pollution.[75,76] It is important to note though that detection of heavy metals in vegetables does not necessarily translate into biological importance if certain acceptable limits are not exceeded.

Heavy metals enter the food chain from the soils in which the roots are anchored,[30,31] the levels of which can be influenced by anthropogenic factors (not investigated in this study). Major human factors that propagate heavy metal contamination in vegetables include inappropriate usage of fertilizers, pesticides, biosolids, manure, and wastewater.[2,33] In the Bushenyi district, these are major anthropogenic factors identified in previous studies since this is a heavily agricultural area.[1,3] Vegetables in Bushenyi are grown through land cultivation, and no irrigation practices are practiced, although fertilizer application continues to be a routine practice. This was important since some plant

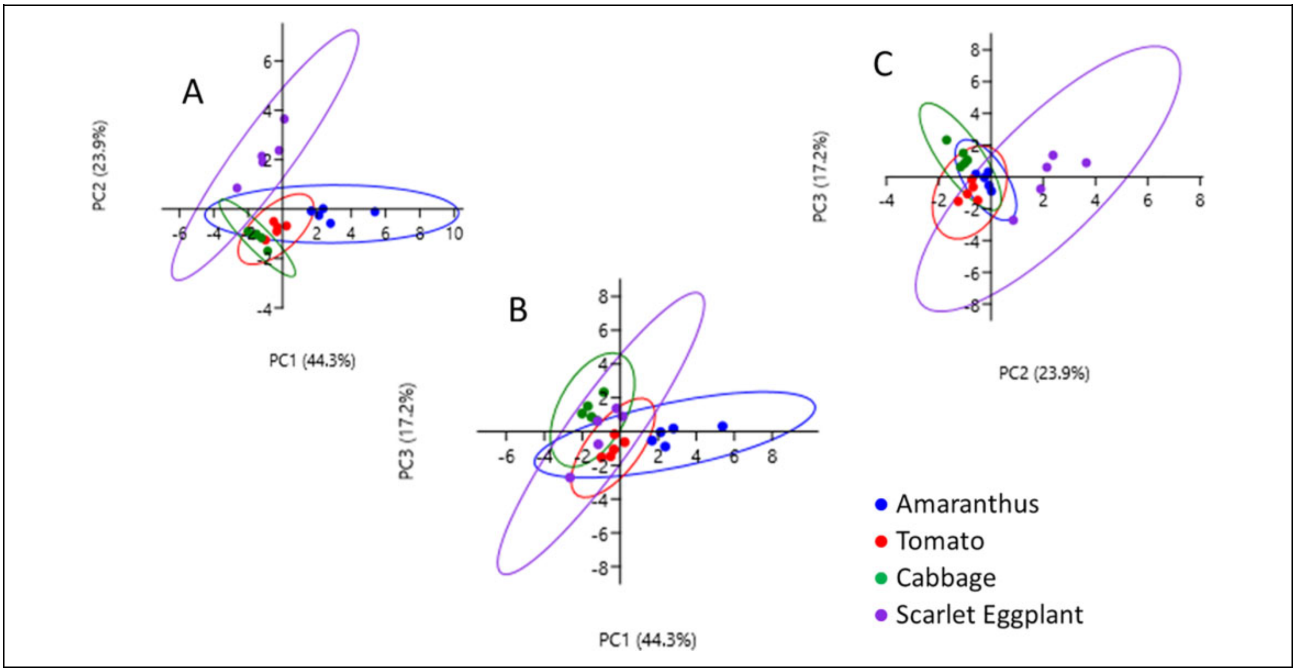

Figure 4. Principal component analysis of standardized estimated daily intakes of heavy metals in four study vegetables. The first three principal components used—PC1, PC2 and PC3—collectively explained 85.4% of the variance. Included are the 95% ellipses enclosing regions of confidence in the mean values for each vegetable. (A) A scatter plot of PC1 versus PC2, (B) PC1 versus PC3 and (C) PC2 versus PC3.

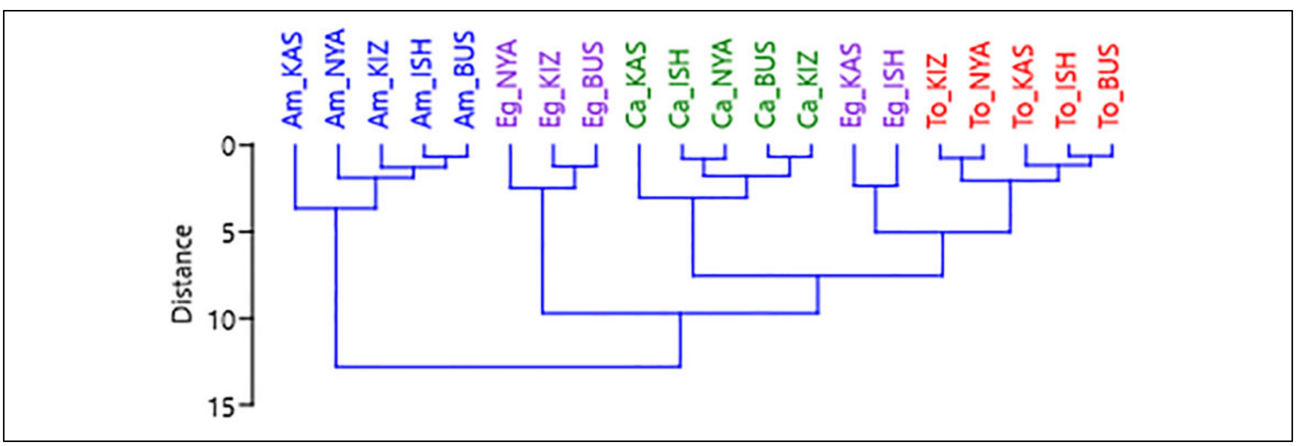

Figure 5. Cluster analysis. Am: amaranthus; Eg: scarlet eggplant; Ca: cabbage; To: tomato; KAS: Kashenyi; NYA: Nyakabirizi; KIZ: Kizinda; ISH: Ishaka; BUS: Bushenyi. Cophenetic correlation: 0.669; algorithm: Ward's method.

species appear to have higher enrichment preferences for particular elements.[40] In addition, the preference for some metals can be affected by the geographical distribution of the plants.[41] These metals in plants for human consumption raise public health concerns since the disruption of human physiology outweighs any dietary advantages associated with the vegetables.[3,11,12] Additionally, very high concentrations above WHO limits would predispose the community to neurological diseases.[51,52,77,78]

In the southern part of Africa, some heavy metals were above the maximum allowable concentrations in vegetables.[79] In Swaziland for example, Cr, Cd, and Pb at concentrations of 17.8 ppm, 6.5 ppm and 8.9 ppm respectively have been identified as a major public health risk, higher in children than adults,[80] demonstrating a need for local and regional periodic monitoring of vegetables in developing countries to generate more information to guide policy. This was shown by using the target hazard quotient although the estimated daily intake risk was low. Compounding this problem is contaminated water as the only source of drinking water in southern Africa.[81] Similar food safety challenges have been reported in western Africa where vegetable irrigation with a dye-polluted stream water raised the levels of heavy metals above the FAO/WHO/

Table 4. Estimated daily intake for Ni, Fe, Co, Zn and Cu in children and adults with different lifestyles.

Classification	Age category	EDI (mg/kg/day)	Element	Reference
Adults		0.001–0.0024	Ni	92
Children	0–6 months	9	Ni	92
Children	7–12 months	39	Ni	92
Children	1–3 years	82	Ni	92
Children	4–8 years	0.099	Ni	92
Adults	≥18 years	0.069–0.162	Ni	92*
Children	0–6 months	0.27	Fe	91
Children	7–12 months	11	Fe	91
Children	1–3 years	7	Fe	91
Children	4–8 years	10	Fe	91
Children	9–13 years	8	Fe	91
Children	Teen boys	11	Fe	91
Children	Teen girls	15	Fe	91
Adult men	19–50 years	8	Fe	91
Adult women	19–50 years	18	Fe	91
Elderly	≥51 years	8	Fe	91
Pregnant teens		27	Fe	91
Pregnant women		27	Fe	91
Breastfeeding teens		10	Fe	91
Breastfeeding women		9	Fe	91
Humans		0.6	Co	93
Food		0.005–0.04	Co	94
Males and females	0.6 months	2	Zn	95,96
Males and females	7 months to 3 years	3	Zn	95,96
Males and females	4–8 years	5	Zn	95,96
Males and females	9–13 years	8	Zn	95,96
Males	14–18 years	11	Zn	95,96
Females		9	Zn	95,96
Pregnancy		12	Zn	95,96
Lactation		13	Zn	95,96
Males	≥ 19 years	11	Zn	95,96
Females	≥ 19 years	8	Zn	95,96
Pregnancy		11	Zn	95,96
Lactation		12	Zn	95,96
Children	2–19 years	0.8–1	Cu	97*
Male (Adults)	≥20 years	1.4	Cu	97*
Women (Adults)	≥20 years	1.1	Cu	97*

Superscripts on reference indicates United States population recommended limits.

US-EPA limits in amaranth shoots.[45,46] The risks of bioaccumulation from the consumption of vegetables irrigated with wastewater are known as well.[82–84] Well water may also have high levels of heavy metals.[85,86] The information from developing countries though continues to be limited. The little that is known so far (in Nigeria) is that typical irrigation practices are associated with elevated levels of heavy metals in vegetables.[87]

This study found that the consumption of Cu, was higher in adults than in Ugandan children, perhaps due to their high consumption of scarlet eggplants.[68,72] Pb is a major contaminant compared with other heavy metals ($P < 0.05$) in cabbages and scarlet eggplants, and this was in agreement with an Egyptian study in which Pb was a major contaminant in cucumber and spinach.[74] Cabbages have been shown to have the highest metal pollution index,[40,88,89] demonstrating their importance as indicator species for environmental pollution. In tomatoes, Cd was identified as a major public health element in comparison to the other heavy metals. Pb and Cd have been identified as major pollutants in Ugandan vegetables[4,5] and this was similar to findings in the current study.

The study identifies important pollutants in vegetables used for household consumption,[75,76] thus raising major food safety concerns in Uganda. Furthermore, amaranthus was identified as a major source of Fe and Co in our vegetable samples. This was in agreement with a previous study in Uganda where concentrations of amaranthus were found above WHO limits.[61,90]

The EDI for Fe was less than 1 mg/kg/day for both adults and children demonstrating a need for Fe food supplementary feeding in children, adults, and the elderly since these vegetables were found to be deficient in comparison to international recommended levels (Table 4).[91] EDI for

Ni was highest in only cabbages in adults when compared against the United States limits,[92] showing that Ni EDI in scarlet eggplants, tomatoes, and amaranthus would be supplemented for human consumption. EDI for Co was so low (less than 1) as compared to internationally acceptable levels.[93,94] Similarly, low EDIs in Zn and Cu were also associated with these vegetables when compared to international limits.[95–97] Findings in this study justify the high malnutrition rates in the Bushenyi region despite its high agricultural activity.[98,99]

Exploratory analysis showed that the samples collected in this study clustered by vegetable species and not location. This pattern of clustering opens the door to comparing vegetable species from different locations of Uganda; the comparison could help improve our knowledge of food safety in Uganda. The WHO has established limits that would guide policy and the need for government ministries to partner and work in collaboration with research institutes has been strengthened by this study. This study also showed that Zn was the only essential element in amaranthus, while all non-essential elements were above WHO limits in other vegetables investigated.

The findings here, preliminary as they may be, when put together with a preponderance of evidence pointing to serious and sustained consumption of contaminated foods in Uganda,[3,82,90,100–104] justify a policy revisit by the responsible regulatory authorities in Uganda, i.e. National Drug Authority (NDA), Ministry of Health, and Ministry of Agriculture Animal Industry and Fisheries (MAAIF), Uganda National Environmental Authority (NEMA), to promote food safety and trade in vegetables. We suggest that the revisit be aimed at developing collaborative frameworks. On a broader scale, swamps in Uganda are used to grow vegetables and yet NEMA has not explicitly controlled the release of industrial waste into the swampy areas despite the risks this practice poses to public health.[45,46,81] Furthermore, the scarcity of information on the physiochemical composition of common vegetables consumed in developing countries generally compounds this problem.

Conclusion

We have shown in this study that vegetables from the southwestern part of Uganda contain both essential and nonessential elements. In this study, Fe was a major element, especially in amaranthus; the risk of Pb and Cd poisoning was highest in cabbages and scarlet eggplant, while tomatoes were associated with high Cd levels above WHO limits. Follow up studies to assess Pb sensitive biomarkers and plasma Pb levels in humans would help guide policy in the region. Studies to investigate bioavailability and biological implications of these heavy metals would help influence policy in the Ugandan food and drug industry. Additionally, studies with an emphasis on environmental remediation technologies to reverse heavy metal contamination in vegetables of Uganda would help guide policy and promote effective remediation strategies. A principal limitation in this study is the small number of samples that call into question the degree to which the results can be generalized. Further studies to screen more vegetables from various market centers in Uganda would help offer a much greater picture on the magnitude of the problem demonstrated in this basic study. It might also be valuable to survey discrete consumer populations and their dietary patterns across various age cohorts with an eye to better appreciating the regional and age-related clinical toxicology implications of heavy metals in Uganda.

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Author contributions

KIK conceptualized the study; KIK, AT designed the study; RM, MA acquired the samples; KIK, EOO, and OO conducted statistical analysis; HIN, GZ, GHM, FS, EDE, RM, JE, KM, BU, RK, SK, ETA, IMU, PE, SSO, RM, PCN, HM, GNK, GE-SB, OO interpreted the data. KIK prepared the initial draft while all authors revised, approved the final version and remained in agreement on the integrity of any part of the work.

Data availability statement

Data files can be accessed at https://figshare.com/s/272affa90fe3ed7f2641.

Declaration of conflicting interests

The author(s) declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

Ethical approval

Ethical approval was acquired from the Scientific and Ethics Review Committee of Kampala International University, Western Campus. Written consent was also acquired from the Bushenyi district local government before data collection.

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Supplemental material

Supplemental material for this article is available online.

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