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## Investigating vegetable contamination in indigent communities by heavy metals: a case of food safety in Bushenyi, Uganda

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### ABSTRACT

Food contamination by heavy metals is a health burden in sub-Saharan Africa. Here, we illustrate this burden by quantifying levels of Cd, Cr, Ni, Co, Pb, Fe, Cu and Zn in vegetables from Bushenyi District (Uganda). Results show that cabbage, scarlet eggplant, tomato and amaranth sold in Bushenyi, Ishaka, Kashenyi, Kizinda and Nyakabirizi open markets contain high levels of Zn and Fe. The uptake of metals overall appeared to be species-specific. Amaranth, for example, had more metals than scarlet eggplant, which in turn had more metals than tomato or cabbage. Within a species, cabbage from Ishaka and Kashenyi presented a combinatorial set of characteristics quite distinct from cabbage from other areas. Such differences arose perhaps from differential capacities to uptake/retain metals from soil or atmospheric particulates. More studies are needed to pinpoint sources of vegetable contamination in Bushenyi. Perhaps then remedial measures can be proposed.

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## Introduction

The mobilization of heavy metals into the environment by anthropogenic activities [1,2], part of which end up on dining tables [3–5], is increasingly having a negative impact on the health of indigents communities in the sub-Saharan Africa [6,7]. These communities usually have few alternatives to safe sources of food because of unregulated solid waste disposal [8–10], application of wastewater for irrigation [11], emission from industries, the widespread uses of fertilizers, Pb-containing paints and gasoline [12], and above all, poor land management [13].

Vegetables are known to accumulate heavy metals at times to levels unacceptable [14–19]. The rate at which vegetables accumulate metals has been shown to be dependent on both the metal (as confirmed here) and on the species as well reviewed by [20]. The former is illustrated by a much higher bioaccumulation rate of Cd vis-à-vis Pb and Cr in vegetables [21]. The latter is illustrated by the differential bioaccumulation rate of heavy metals in cabbage which tends to be much higher than in tomato [16]. Tomato itself, the berry of the plant *Solanum lycopersicum*, accumulates Cu and, to some extent, Zn at a higher rate than most metals.

That being said, comparing bioaccumulation rates of heavy metals in plants on the basis of their phytotomomy may be more importance to public health than

a comparison across species. This is because vegetables are the main source of protein and vitamins in most sub-African communities and such comparison could inform the selection of vegetable parts to consume and when. Pb for instance accumulates sixfold in the roots of some plants and four times more in leaves of others when compared to levels in the shoots [22]. Specifically, the roots of eggplant (*Solanum melongena* L.) grown in sewage sludge amended soil was found to accumulate more heavy metals than the shoots [19], the shoots in turn was shown to accumulate more heavy metals than the fruit. In the same study, the fruit of the eggplant on the other hand was shown to accumulate Pb, Cd and Ni selectively (Pb > Cd > Ni). In this illustrative case of eggplant then, consumers would be better off avoiding the roots and shoots of eggplant in their diet.

In this study, we investigate not only the extent to which vegetables are contaminated by Cd, Cr, Ni, Co, Pb, Fe, Cu and Zn in small-scale farming outlets located in a collection of poor communities in Uganda but also use the associations among contaminants themselves to estimate potential common sources of the heavy metals. In a similar fashion, we use the associations among sources of vegetables to estimate whether vegetables in the study area are coming from a single source or a diversity of suppliers. In the process, we develop a set of portable

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empirical techniques for investigating the nature and the extent of heavy metals contamination in vegetable elsewhere in Africa. The country we have chosen for convenience is Uganda, a typical sub-African country, one in which most elements conducive to food poisoning are in place. Uganda is also convenient in complementary terms because results from similar studies on foods and drinks are available [8,23–25]. The communities studied are Bushenyi, Ishaka, Kashenyi, Kizinda and Nyakabirizi, all in the Bushenyi District of Uganda. These communities were chosen because they have two things in common: (i) they are located in highly populated areas which are also experiencing rapid (and perhaps uncontrolled) industrialization and modernization, and (ii) they are communities in which environmental narratives developed 90 years ago during the colonial period are still continuing to inform policies despite ample evidence suggesting otherwise [26]. From the communities, we attempt to identify factors associated with, and the potential risk of, consuming heavy metals in vegetables sold in the open markets by harnessing the potentials of multivariate statistics.

The four vegetables sampled from an open market in each community were cabbage, scarlet eggplant, tomatoes and amaranth (a leafy vegetable locally known as *doodo* and herein referred to at times as such). Tomato, domesticated by the Aztecs and Incas around the 700 AD, is a major source of income and food security for small-scale farmers across Uganda [27]. Edible cabbage, a descendent of *Brassica oleracea* var. *oleracea* [28] and a good source of dietary fiber, vitamins K and C, is widely consumed in the country. *Doodo*, indigenous to Africa [29], is rich in dietary minerals, Ca, Mg, P, and K [30]. Studies on *doodo* in Nigeria have shown extensive bioaccumulation of heavy metals in this vegetable, at times to levels above safety recommendation [14,15,31]. Scarlet eggplant, a cultivar group of *Solanum aethiopicum*, is cultivated in East, West and Central Africa for its fruits and leaves [32], and references therein.

## Materials and methods

### Sampling, samples preparation and instrumental analysis

All vegetables sampled for this study are assumed to have come from small local farmers who supplied the four targeted open markets. This assumption was necessary for interpreting results since information on the primary sources of the vegetables was not available at collection. Tomato, cabbage, and *dodo* samples were collected from all five targeted open markets (Bushenyi, Ishaka, Kashenyi, Kizinda and Nyakabirizi). Scarlet eggplant was collected only from Ishaka, Kashenyi, Kizinda and Nyakabirizi open markets due to logistical difficulties.

To prepare samples for analysis, metals were freed from the solid matrix by first drying in an oven and grinding in a mortar until free-flowing. The powdery vegetable (1 g) was then digested with nitric acid (20 mL) and perchloric acid (4 mL) on a hot plate in a fume hood until the total volume was 4 mL. The solution was cooled, filtered, and adjusted to a final volume of 50 mL with deionized water. Traditionally, a certified reference material would be processed and analyzed alongside field samples to assess accuracy of the analytical process. However, this option was not available at the time of this study for logistical reasons. Furthermore, resource constraints prevented spiking the vegetable matrices in analytical batches to verify the efficiency of vegetable pretreatment on metal analysis.

To determine metal contents of sample digests, standard concentration curves were first generated separately from the absorbance of Cd, Cr, Ni, Co, Pb, Fe, Cu and Zn in reagent water using an atomic absorption spectrometry (AAS, PerkinElmer 2380). The absorbance of each metal in extracts from the same amount of vegetable samples (after nitric acid and perchloric acid digestion) was then converted to concentration using the linear range of the concentration curve.

### Statistical analysis

Principal component analysis (PCA), a powerful tool for reducing dimensions of multivariate datasets without much loss of information, was used to remove redundancy in data. In contemporary multivariate analysis, PCA is popular with 'cleaning' data for cluster analysis (CA); here we use PCA to verify CA results instead.

Variables were mostly scaled to make concentrations comparable across samples. Scaling was done by standardizing values to have a mean of zero and a standard deviation of one. Variance-covariance matrix and distance in eigenvalues were used for PCA. A tree-based format was used to display CA results. To measure dissimilarity between any two clusters of the CA tree, the Ward's minimum variance method was applied to log-transformed data. The Ward's method minimizes the total within-cluster variance.

PAST software [33] was used for PCA and for assessing concentration distribution by boxplots. R software [34] was used for CA and assessing correlation among variables and cases by the Pearson's *r* measure.

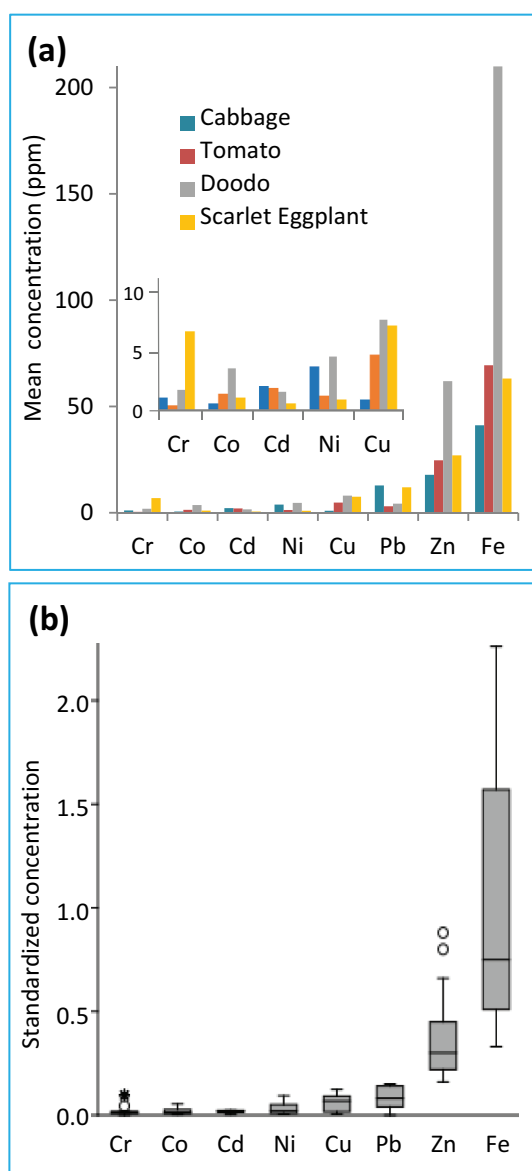
## Results and discussion

### Descriptive analysis

To aid in a deeper understanding of the nature and the extent of vegetable contamination by Cr, Co, Cd, Ni, Cu, Pb, Zn and Fe in Uganda, we interrogated exhaustively our data for their empirical as well as predictive

values using both descriptive and exploratory statistical tools. The description of the data is presented in Figures 1–3 and the results of probing the internal structure of the data are displayed in Figures 4–6.

The histogram in Figure 1(a) displays the average heavy metal loads for each vegetable species. It can be seen that Fe and Zn were overrepresented in our samples, followed closely to some extent by Pb and Cu. The least represented were Co, Cd, Ni and Cr. The histogram also shows (i) that *doodo* was the most contaminated vegetable, mostly by Fe and Zn; it also contained the most amount of Co, (ii) that scarlet eggplant together with cabbage contained the most amount of Pb (0–10 ppm level scale insert in Figure 1



**Figure 1.** Overall distribution of heavy metals in the four vegetables studied. (a) Histogram showing the nature and extent of contamination by Cr, Co, Cd, Ni, Cu, Pb, Zn and Fe. (b) Boxplots showing the overall spread of concentration data across the four vegetables. Values are standardized (mean = 0, standard deviation = 1); one high value for Fe (3.98) is excluded. Outlier values are shown open circles (Cr and Zn) and asterisk (Cr) in (b).

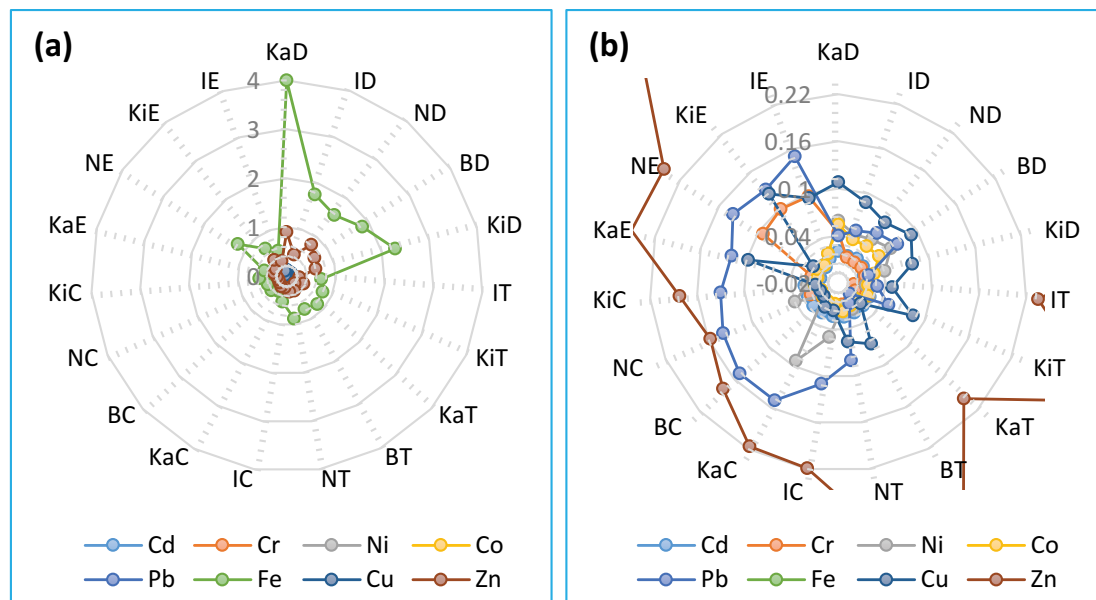
(a)), (iii) that scarlet eggplant contained the most amount of Cr, and (iv) that cabbage and *doodo* together had the most amount of Ni.

By proportion, *doodo* had the most contamination overall at 49% of the total amount of heavy metals detected in this study. This was followed by scarlet eggplant at 20%, tomato at 18% and cabbage at 13%. *Doodo* being the most contaminated should not be surprising since the plant grows almost anywhere in Uganda, especially on household rubbish dump, and near open urinals and such places. Others have also shown that leafy vegetables such as *doodo* accumulate heavy metals to a greater extent than, say, fruity tomato [16].

The results, empirical as they may be, also point to variability in bioaccumulation rate across and within plant species. For example, the decreasing proportions of contamination from *doodo* to cabbage could be signifying a decreasing capacity (i) to absorb heavy metals from soil, (ii) to bioaccumulate the metals in part of the plants harvested for food, and/or (ii) to retain atmospheric particulates containing the metals (from polluted air rampant in this area of Uganda).

One way of visualizing the spread of data in Figure 1(a) is by comparing side-by-side boxplots of the standardizing concentrations (to account for the high levels of Fe encountered) as shown in Figure 1(b). Descriptively, the Pb, Cu, Ni, Cd, Co and Cr boxes are comparatively shorter with median values closer to zero for Cd, Co and Cr. The Zn and Fe boxes on the other hand are not only taller but more so for Fe than for Zn (which had two outlier values shown as open circles). Furthermore, the long upper whisker and the unevenly divided middle 50% of the Fe box means the level of Fe in the four species of Bushenyi vegetables are highly varied amongst the most contaminated vegetables. Explaining this high level of Fe and its variability may require factoring in the *acric ferralsols* characteristics of soil upon which we assume the samples were grown in Bushenyi [13]. Such soil contains high levels of  $\text{Fe}_2\text{O}_3$ , an easy source of Fe contamination in plants. The observed large difference in Fe distribution may then be explained in part as a reflection of this soil type.

A radar plot of metal concentrations as a function of the type of vegetables and source markets is displayed in Figure 2. This display is intended to amplify results not obvious from the boxplots distribution in Figure 1(b): that for the same amount of samples (by mass), Fe was the dominant contaminant in the vegetables studied, most of which came from just one vegetable collected from one market: *doodo* (last letter 'D' in market identification code) from Kashenyi market (KaD) (Figure 2a). On the lower end of concentrations (Figure 2b), Pb was more, and appeared to have been accumulated at the same rates, in cabbage ( $0.14 \pm 0.02$  ppm) and scarlet eggplant



**Figure 2.** 3-D plots showing the extent of contamination by each metal in each vegetable sample from each of the four different open markets in Bushenyi District, Uganda. (a) Low resolution; (b) high resolution showing the same relationship but between low concentrations of heavy metals and vegetable types and source markets otherwise incomprehensible in (a). Market codes: first one or two letters B, I, Ka, Ki, and N stand for Bushenyi, Ishaka, Kashenyi, Kizinda and Nyakabirizi, respectively; the last letters C, D, E and T stands for Cabbage, *Doodo*, Scarlet eggplant and Tomato, respectively.

( $0.14 \pm 0.01$  ppm). This particular observation is important because Pb is a known carcinogen [35–38] and no amount of carcinogen in food is safe.

To check for concentration correlations, a generalized pairs plot was also generated using the standardized levels of contamination. This was meant to point to common sources of these metals in the study communities. The acceptance criteria for likelihood of co-occurrence were based on linear  $r$  (Pearson's) closeness to 1. The results of bivariate combinations of levels, displayed in Figure 3, show a strong and a positive three-way correlation of Fe, Co and Zn concentrations (red rectangles) in vegetables in the study area. This suggests common sources for the three metals. Such information is helpful in narrowing down target sources for further investigation. The results also show a moderate correlation between Zn and Cu, and between Cu and Co levels (color coded green in Figure 3). The latter would suggest adding Cu to the potential common sources of the triad Fe, Co, and Zn. However, no correlation was detected between the levels of Cu and Fe to necessitate the inclusion.

### Exploratory analysis

To extract information beyond those acquired through linear modeling, CA was used to interrogate the standardized concentration data as well. This was done purposely to determine potential samples clustering (Q-mode) and to check for systematic concentration variations that may exist within the dataset (R-mode).

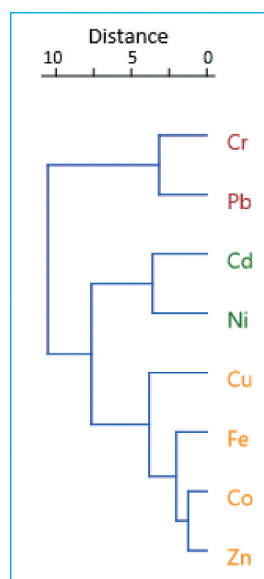
The results, displayed as dendrograms in Figures 4 and 5, respectively, can be interpreted in two ways. Reading Figure 4 from left to right or Figure 5 from inside outward identifies large-scale groupings. Reading the dendrograms from right to left (Figure 4) or outside inward (Figure 5) identifies the metals or the vegetables which are most similar to each other on the basis of the first clades to join them together.

In reading Figure 4 from left to right (i.e. 0 being equivalent to identically similar), two large groupings can be recognized at a distance of 10. In one grouping, only Cr and Pb are present as members (identified by the two-leaved bifolious clade representing them far removed from the rest of the clades). In the second and the largest grouping, six elements are members, but with a within-group sub-division separating Cd and Ni from Cu, Fe, Co and Zn. The basis of this subdivision is the similarity between the concentration characteristics of Cd and Ni on one hand and those of Cu, Fe, Co and Zn on the other hand. Having said that CA groupings should not be construed as evidence of concentration correlation. For example, whereas Cd and Ni, or Cr and Pb are grouped together in Figure 4, their concentrations are actually not correlated (Figure 3). Further investigation of these Cd and Ni, and Cr and Pb dichotomous relationships in vegetables from the study area may be warranted given the bonafide status of Cd, Cr, Ni and Pb as carcinogens [35–38].

In reading the dendrogram in Figure 4 from right to left, it can be seen that Co and Zn – two metals whose concentrations were found to be strongly and



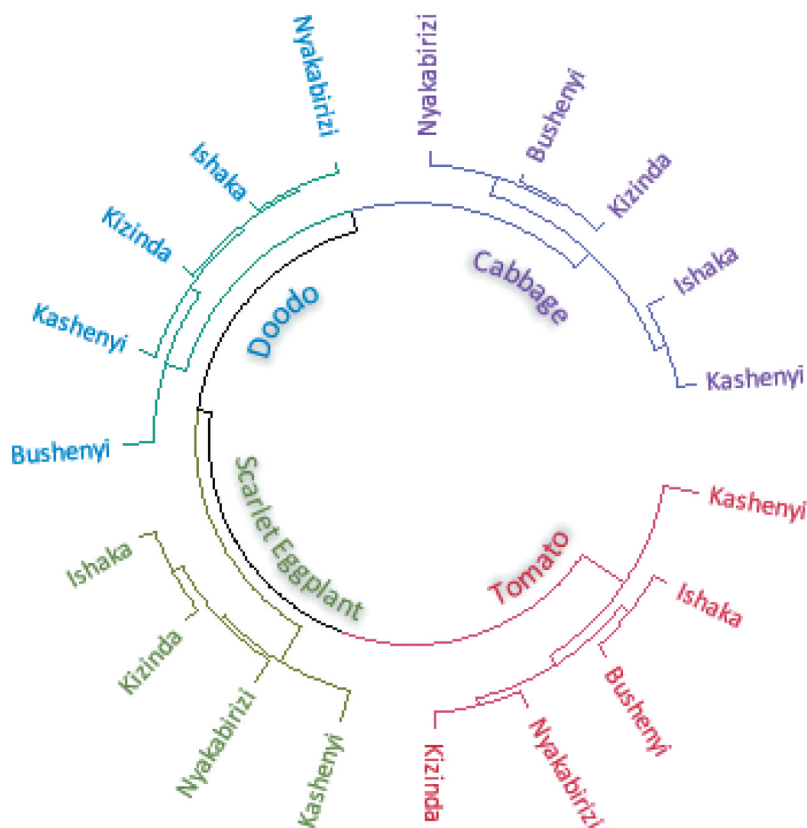
**Figure 3.** A generalized pairs plot displaying paired combinations of standardized levels of Cd, Cr, Ni, Co, Pb, Fe, Cu and Zn in cabbage, *dodo*, scarlet eggplant and tomato samples collected from open markets in Bushenyi, Ishaka, Kashenyi, Kizinda and Nyakabirizi (Bushenyi District, Uganda). Strong and positive correlation between Fe and Co, Zn and Co, and between Zn and Fe are color coded red; moderately positive correlation between Zn and Cu, and between Cu and Co are color coded green.



**Figure 4.** A dendrogram representing similarity among levels of metal contaminants in vegetables from four Bushenyi open markets overall (0: identical, 10: dissimilar; algorithm: Ward's method; cophenetic correlation: 0.841).

positively correlated – do have intrinsically similar concentration characteristics as well. This is strong evidence linking Co and Zn in the vegetables studied to a single source. No such certainty of narrowing down the sources of the remaining members of the groupings (as read) can be made (see the cases of Cr/Pb or the Cd/Ni discussed above).

Reading the fan dendrogram in Figure 5 from inside outward determines the vegetables that were most similar to each other. In doing so, a separation of vegetables into four large clusters by species is clear. This very distinct separation can be interpreted as a reflection of the differential bioaccumulation rates of heavy metal by different species of vegetables. In reading the fan dendrogram from outside inward, one can see that tomatoes and scarlet eggplants from Kashenyi were unique sets of vegetables. It can also be seen that cabbages from the study area can be categorised into two major classes: those from Ishaka and Kashenyi, and those from Bushenyi, Kizinda and Nyakabirizi. *Dodo* from Bushenyi is distinctively local.



**Figure 5.** Fan dendrogram representing similarity relationships among cabbage, *dodo*, scarlet eggplant and tomato from Bushenyi, Ishaka, Kashenyi Kizinda and Nyakabirizi open markets (Bushenyi, Uganda) on a collective the basis of Cr, Pb, Cd, Ni, Cu, Fe, Co and Zn contamination in vegetables (algorithm: Ward's method; cophenetic correlation: 0.747). Standardized values used.

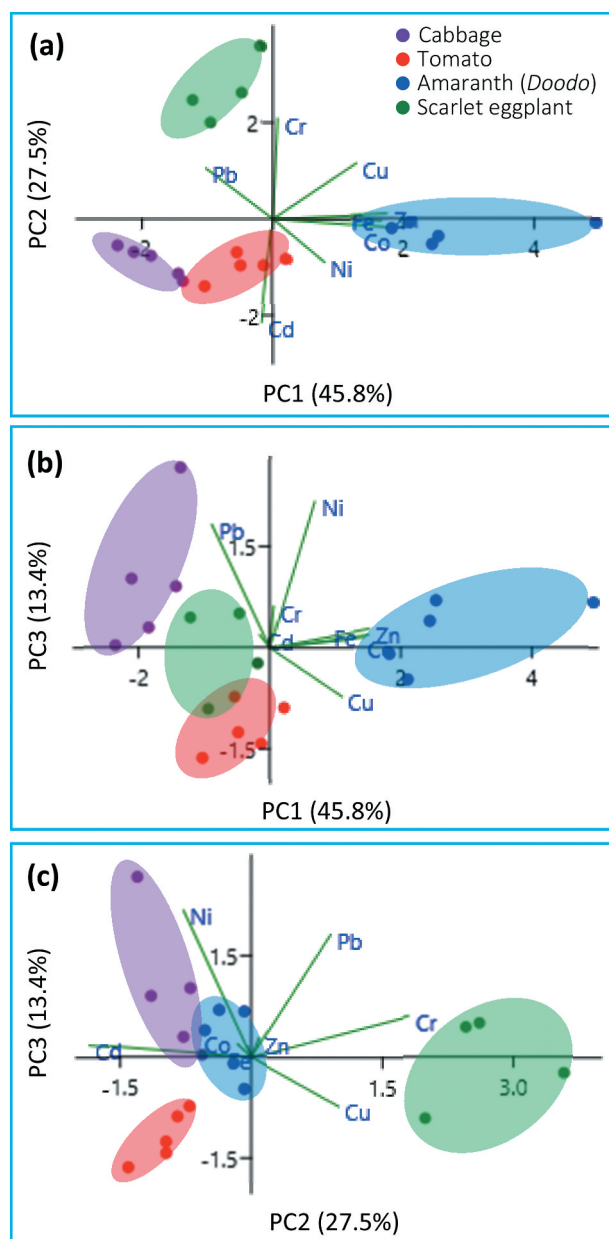
A PCA evaluation of the dendrogram groupings of the vegetables and the variance among the measured concentrations in Figures 4 and 5 confirms the general results. Shown in Figure 6 are all the two-dimensional combinations of the first three and most important PCA principal component axes. The cumulative variance for the three axes (PC1, PC2 and PC3) explained nearly 87% of the variations in the bioaccumulation data. The axis PC1 separates *dodo* from the rest of the vegetables (Figures 6a and b); PC2 separates scarlet eggplants similarly. Scarlet eggplant scored strongly and positively along this second axis. Scoring negatively along PC2 on the other hand were tomato and cabbage (Figures 6a and c) which were only separated along the third axis, PC3 (Figures 6b and c).

To identify metals driving these separations along PCA axes, loading plots are also included as vectors in Figure 6 (green lines). A loading that is far removed from 0 on any axis indicates that the metals involved strongly influenced vegetable scores along the axis. In that context, PC1 can be seen to explain a dominant Cu, Zn, Ni, Fe and Pb concentration gradient along the axis. While Cu, Zn, Ni, Fe loaded positively, Pb loaded negatively along PC1. This pattern of loading suggests that PC1 represents the ease with which heavy metals are available to plants for uptake from the soil, Pb usually being the least available during plant growth [39,40]. The fact that *dodo* scores highly and

positively along PC1 means *dodo* is one vegetable with relatively lower capacity to extract Pb from the soil (assuming all four vegetables studied were grown locally in the same *acric ferralsols* soil). The four metals that loaded positively on PC1 (Cu, Zn, Ni and Fe) were correlated to some extent as shown in Figure 3 (except Cu and Ni, a distinction which appears to be explained by the different loadings on the second and third axes, PC2 and PC3 in Figure 6).

The second axis, PC2, described a gradient between vegetables that differ in concentration properties not exhibited by all metals (and unknown to us at this time). Having said that, the positive loadings on this axis is clearly associated with high levels of Pb, Cr and Cu while negative loadings should be associated with high levels of Ni and Cd. In that context, the strong and positive scoring by scarlet eggplant along this axis was then viewed essentially as driven by the concentration of Cr. Similarly, the negative scoring of tomato along this axis was driven by the Cd vector. The third axis explained what is common to Pb and Ni as opposed to Cu, the basis of which we cannot provide at this time.

Overall, PCA confirms CA results: that each study vegetable was unique in its own right. PCA results also suggest that the combined levels of Cu, Ni, Zn and Fe drove the separation of *dodo* from the rest of the vegetables. Likewise, levels of Pb, Cr and Cu collectively drove the separation of scarlet eggplant from the



**Figure 6.** Point-vector plots of vegetable groupings resulting from variance among levels of heavy metals in Bushenyi vegetables. Vectors include Cr, Pb, Cd, Ni, Cu, Fe, Co and Zn levels. The first three PCA axes are shown; each plot showing a different combination of the three axes: (a) axes PC1 and PC2, (b) axes PC1 and PC3, (c) axes PC2 and PC3. Vegetable scores and an approximate area covered by the scores are similarly color-coded (ovals): cabbage, purple; tomato, red; *doodo*, blue; scarlet eggplant, green.

rest of the vegetable. Lack of Pb or Cu appears to have been the driving force behind the separation of cabbage or tomato from the rest of the vegetables, respectively.

### Study limitations

This study lacked source documentation which would have provided details such as soil on which samples were cultivated. This would have in turn help perhaps pinpoint sources and mechanism by which the metals

entered the food chain. Consequently, it should be taken as an exploratory step towards understanding the nature of food quality in Uganda.

### Conclusion

We have shown in this study that cabbage, scarlet eggplant, tomatoes and amaranth (*doodo*) from Bushenyi, Ishaka, Kashenyi, Kizinda and Nyakabirizi in Bushenyi District of Uganda collectively contain high levels of Zn and Fe, and that Zn, Fe and Co contamination could potentially be traced to the same sources in the communities. Carcinogens Cd, Cr, Ni and Pb were also found in these vegetables which constitute the main dietary source of protein for this indigent community in Uganda. Carcinogens have *no* safe levels; their presence is enough to cause cancer.

Broadly, our study confirms the notion that plant species vary in their uptake rate of the eight metals studied. We believe that the observed differential capacities emanate from varying capacities (i) to absorb heavy metals from soil, (ii) to bioaccumulate heavy metals in different part of plants, or (iii) to retain atmospheric particulates containing the metals from polluted air.

For public safety, further studies are needed to determine sources of these metals in Bushenyi vegetables. Elsewhere, we advise increased application of CA and PCA, and similar exploratory techniques in understanding food safety in the sub-Saharan Africa.

### Declarations

### Disclosure statement

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### Author contributions

KIK: study concept and KIK, GZ, KM instrumental analysis; EOO: statistical analysis and manuscript drafting; OO: statistical analysis, interpretation, and manuscript drafting. All

authors reviewed and approved the manuscript for publication and remain in agreement on all aspects of the work.

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