Original Research Article

Heavy metal accumulation in vegetables grown in rock soils of Kilembe copper mine, Kasese, Western Uganda

.

ABSTRACT

Aim: Active mining in Kilembe copper mine, Kasese district, Uganda ended about four decades ago. However, various health problems are believed to be from the effect of mining activities that took place in the area. In this study therefore, we quantified the concentration of trace metals: chromium (Cr), cadmium (Cd), zinc (Zn), iron (Fe), lead (Pb), arsenic (As) and copper (Cu) in cabbages and tomatoes grown in Kilembe mining area and assessed the health risks from consumption of the vegetables after 40 days and 120 days of growth.
Study design: This study employed quantitative research design.
Place and Duration of Study: The experiments were done at Department of Chemistry, Mbarara University of Science and Technology, Uganda from 2018 to 2019.
Methodology: The vegetables were planted, and their roots, leaves, fruits and stems were sampled after 40 days and 120 days. Elemental analysis was performed using atomic absorption spectrophotometry. The estimated daily intake (EDI), target harzard quotient (THQ) and cancer risks were calculated to explore health risks that could arise from consumption of the vegetables.
Results: The levels of Fe, Cu, Zn and Cr did not exceed WHO/FAO permissible limits. The EDI of Fe 294.678 × 10^{-4} mg/kg/day) was the highest while the lowest was 0.052 × 10^{-4} mg/kg/day for Cr. The THQ ranged from 0.000004 to 1.3134. Cancer risk values ranged from 0.126 × 10^{-4} to 28650 × 10^{-4}.
Conclusion: There are discernable non-carcinogenic health risks (THQ >1) and cancer risks associated with consumption of cabbages and tomatoes by the local inhabitants. The risks are escalated in children who consume both vegetables after 40 days of growth. Arsenic and Cd poses the highest carcinogenic health risk while Pb poses the highest non-carcinogenic health risk. Consumption of the vegetables after 120 days of growth poses lower health risks.

Keywords: Trace metals, target hazard quotient, hazard index, estimated daily intake, carcinogenic risk.

1. INTRODUCTION

Deliberate and unintentional introduction of contaminants such as heavy metals (HMs), plastics, preservatives, agrochemicals, personal care products and pharmaceuticals into the environment has raised international sustainability concerns [1, 2]. This is specifically because they compromise the quality and safety of water, air, food and soils which are either ingested or taken up by animals, plants and other organisms. One group of such contaminants currently being monitored with keen attention are heavy metals (HMs)[3, 4].
In environmental toxicology, HMs are chemical elements with high molecular weights and specific gravity (five times greater than that of water or more) and elicit toxic effects at

concentrations above their permissible limits. Examples of HMs include lead (Pb), arsenic (As), cadmium (Cd), mercury (Hg), zinc (Zn), tin (Sn), chromium (Cr), cobalt (Co), copper (Cu), iron (Fe), molybdenum (Mo), nickel (Ni), strontium (Sr), titanium (Ti) and vanadium (V) [5]. Of these, Pb, Hg As, Cd and Cr are listed as priority HMs of public health significance due to their marked toxicity [6]. Though HMs are available naturally in the environment, their concentration is usually amplified by anthropogenic activities such as mining operations, urbanization, smelting and agricultural activities [7, 8].

HMs are known to be bioaccumulative and toxic to living organisms because they can react with biological systems by losing one or more electrons, forming cations that can ably bind with the nucleophilic sites of vital macromolecules. This impairs cell activities such as differentiation, growth and damage-repairing processes [6, 9]. The extent of toxicity elicited is however dependent on the exposure route, dose, chemical form, age, gender and nutritional status of the organism. In regards to regulations, there are set limits for each heavy metal in various matrices. However, monitoring of HMs concentration, and the assessment of their potential health effects is expensive and therefore not commonly done in East Africa.

Previous studies have hinted on the pollution of water, food crops and sediments due to the 1985-1995 Kilembe mining activities in Kasese district of Uganda [10-14]. However, a few of the foregoing studies evaluated the health risks from dermal contact and consumption of the matrices. In this study, we assessed the bioaccumulation of Zn, Cr, Cu, Fe, Pb, As and Cd in cabbages and tomatoes that form part of the commonly consumed vegetables in Kilembe mine, Kasese district, Western Uganda. The vegetables were sampled after 40 and 120 days of growth, representing the time period within which the local community start and stop consuming the leaves and fruits of the vegetables.

2. MATERIAL AND METHODS

2.1 Study area

The study was undertaken at Kilembe mine waste rock area located at the foot hills of Mount Rwenzori in Kasese district (**Figure 1**)[11, 13]. It is about 380 kilometres by road and west of Kampala (the capital city) of Uganda. Its coordinates are 0°12'30.0"N, 30°00'25.0"E, which is near River Nyamwamba where farming is done. The mine ceased active operations in 1978 but contaminants from the activities have continued to circulate in the environment and are carried into the Nyamwamba-Rukoki River which passes through Queen Elizabeth National Park and other wetlands into Lake George [12, 14].

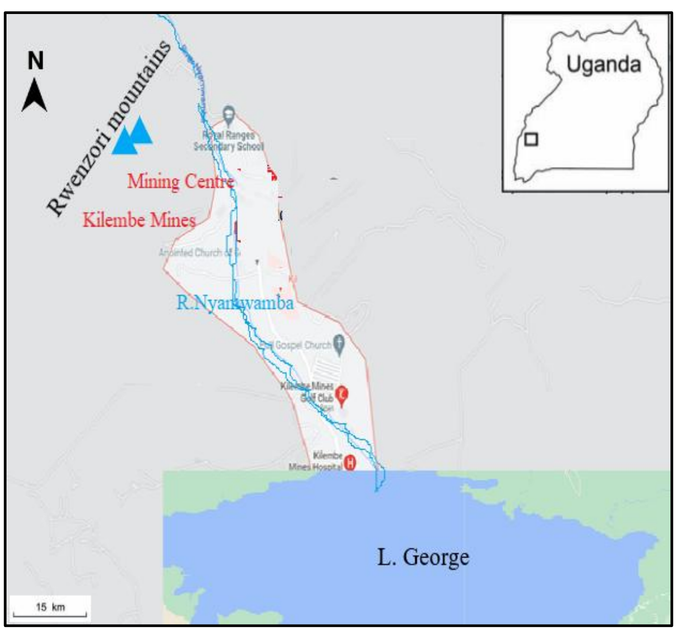

Fig. 1. Map showing the location of Kilembe mine, Kasese district, Western Uganda. Inset is the location of the mine in Uganda.

2.2 Reagents and chemicals

Analytical reagent grade chemicals and distilled water were used throughout the study. All glassware and plastic containers used in this work were washed with detergent solution followed by 20% (v/v) nitric acid and then rinsed with tap water and finally with distilled water.

2.3 Cultivation, sampling and preparation of the vegetables

The vegetables planted and sampled were cabbages (*Brassica oleracea* var. *cupitata*) and tomatoes (*Solanum lycopersicum* L.). They were chosen because they are fast growing plants and are commonly consumed by the local population. Certified seeds of the vegetables were purchased from Agricultural shops in Kasese district, Western Uganda. The vegetables were planted at Kilembe mines waste rock soils. The seedlings were watered daily to mimic the practices of local inhabitants when growing the vegetables for sale. After sprouting, the vegetables were allowed to grow naturally without any watering until they were ready for sampling at 40 days of growth.

Samples were manually collected after 40 days and 120 days of plant growth using purposive non-probability sampling. In the laboratory, each sample was washed with tap water and thereafter with distilled water, dried in an oven at 80°C. They were ashed in a muffle furnace at 600 ^{o}C. After cooling, they were ground into fine powder.

Measured 2.0 g of the sample was weighed out into a Kjeldahl flask mixed with 20 cm^3 of concentrated sulphuric acid, concentrated perchloric acid and concentrated nitric acid in the ratio 1: 4: 40 (v/v), respectively and left to stand overnight. Thereafter, the flask was heated at 70°C for 40 minutes and then heated to 120 °C. The mixture turned black after a while. The digestion was complete when the solution became clear and white fumes appeared. The digest was diluted with 20 cm^3 of distilled water and boiled for 15 min. This was then allowed to cool, transferred into 100 cm^3 volumetric flasks and diluted to the mark with distilled water. The sample solution was then filtered through Whatmann No. 42 filter paper and the digestion processes were performed in a laboratory fume hood in a screw capped polyethylene bottle.

An atomic absorption spectrophotometer (Analyst 400, Perkins Elmer) equipped with photomultiplier tube detector and a hollow cathode lamp was used for the determination of metal concentrations. Working standards were also prepared by further dilution of 1000 ppm stock solution of the nitrate salts of the metals and a calibration curve was constructed by plotting absorbance versus concentration. By interpolation, the concentrations of the metals in sample digests were determined.

2.4 Dietary intake and human health risk assessment due to ingestion of the trace metals

2.4.1 Non-carcinogenic health risks

The estimated daily intake (EDI) was computed to establish the daily HMs loading into the body system of a specified body weight of a consumer (adult/child) through consumption of contaminated vegetables (tomato fruits and cabbage leaves). The EDI in mg/kg/day was calculated using **Equation 1** [15].

$$\text{EDI} = \frac{E_F \times E_D \times F_{ir} \times C_v}{W_{ab} \times T_{aet}} \quad (1)$$

Where E_F = exposure frequency (365 days/year), E_D = exposure duration, the average lifetime (58.65 years for an adult Ugandan)[15, 16], Cv = heavy metal concentration in the vegetable sample (mg/kg), F_{ir} is the fresh food ingestion rate (kg/person/day) = 0.09 for tomatoes and 0.085 for cabbages (considering it as a vegetable) [17], W_{ab} = average body weight (considered to be 15 kg and 60 kg for children and adults, respectively), T_{aet} is the average exposure time for non-carcinogens = $E_{\text{f}} \times E_{\text{d}}$ [18].

2.4.2 Non-carcinogenic health risks (hazard quotient indices)

Health risk index was computed as target hazard quotient (THQ), the total risk of a non-carcinogenic element (**Equation 2**). For this assessment, THQ less than 1 indicate that the exposure is very unlikely to have adverse effects while THQ greater than 1 represent a possibility of non-carcinogenic effects [19].

$$\text{THQ} = \frac{EDI}{R_f D} \quad (2)$$

Where R_fD is the oral reference dose, which are 0.7, 1.5, 0.04, 0.03, 0.004, 0.0003 and 0.001 mg/kg for Fe, Cr, Cu, Zn, Pb, As and Cd respectively [20]. The reference dose refers to the maximum daily dose of a metal from a specific exposure pathway, that is believed not to lead to an appreciable risk of deleterious effects to sensitive individuals during a life time [21]. Thus, if the EDI is lower than the R_fD, THQ is less than 1 and adverse health effects are unlikely to appear. Otherwise, EDI more than R_fD implies that THQ is greater than 1 and adverse health effects are likely to appear. In this study, the target hazard quotient was calculated basing on a single pathway i.e. consumption of contaminated vegetables. The assumption made during the health risk calculations were that the ingested dose is equal to the dose absorbed into the body, since it is customary for local inhabitants to consume the vegetables raw [22].

Since exposure to more than a toxicant result in additive and/or interactive effects, the total THQ was treated as the sum of the individual metal THQs (**Equation 3**).

$$\text{TTHQ} = \text{THQ}_{\text{Pb}} + \text{THQ}_{\text{Zn}} + \text{THQ}_{\text{Cu}} + \text{THQ}_{\text{Cd}} + \text{THQ}_{\text{Cr}} + \text{THQ}_{\text{As}} \quad (3)$$

2.4.3 Cancer risk assessment

Cancer risk (CR), expressed as incremental lifetime cancer risk for the carcinogenic heavy metals (Pb, Cd, Cr and As) were computed as the product of EDI and the ingestion cancer slope factor (CSF) using **Equation 4** [8]. This was performed to establish the cumulative probability of developing cancer throughout a lifetime by exposure to a unit dose of a probable carcinogen. The total cancer risk (TCR) from intake of the HMs was calculated using **Equation 5** [23].

$$\text{CR} = \frac{E_F \times E_D \times F_{ir} \times C_v}{W_{ab} \times T_{aet}} \times \text{CSF} \quad (4)$$

$$\text{TCR} = \sum_{i=1}^{n} CR \quad (5)$$

2.5 Statistical analysis

Experimental data from triplicate analyses were entered into Excel and exported to SPSS where they were averaged. The means were subjected to one sample *t*-test to establish any significant variations in the HMs among the different parts for a particular metal. The analyses were executed at 95% confidence interval.

3. RESULTS AND DISCUSSION

3.1 Heavy metal content of the vegetables

The mean trace metal content of the vegetables (mg/kg fresh weight) after 40 days and 120 days of growth are given in **Table 1**. The highest concentration was 14.0826 mg/kg for Fe in cabbage leaves after 40 days of growth, but the concentration of the metals varied greatly among vegetable species and with the periods of growth (P<0.05). Leaves and roots tended to accumulate the trace metals after 40 days of growth, especially in cabbages. The levels of Cu, Zn and Cr recorded after 40 days and 120 days of growth did not exceed the WHO/FAO maximum permissible limits.

Table 1. Mean trace accumulation (mg/kg) in vegetables grown in soils at Kilembe mines, Uganda

Metal	Cabbages						Tomatoes						FAO/ WHO guidelines [24]
	40 days			120 days			40 days			120 days			
	Leaves	Stem	Roots	Leaves	Stem	Roots	Fruits	Stem	Roots	Fruits	Stem	Roots	
Fe	5.2002	0.71880	4.4827	2.7992	2.3211	14.0826	2.5616	21.4927	3.1297	3.2770	2.7992	2.1416	NE
Cu	0.2057	0.20571	1.6147	0.5407	0.3734	4.0439	1.2073	13.6234	2.6444	0.5391	0.8730	2.0563	40.0
Zn	4.9900	3.1200	3.0300	4.6100	1.1600	1.0400	0.0986	9.0260	4.3920	0.0986	0.0987	3.1831	20.0
Cr	0.0043	0.0094	0.0299	0.0037	0.0434	0.1484	0.0111	0.0130	0.0057	0.0148	0.0002	0.0002	2.3
Cd	0.0048	0.0194	**0.0729**	0.0238	0.0175	**0.3625**	**0.1335**	**0.1332**	**0.0888**	**0.0952**	**0.3735**	**0.3862**	0.05
As	0.0247	**0.5898**	0.0359	0.0337	**0.5783**	0.0245	0.0112	**0.3798**	**0.1333**	0.0048	0.0048	0.0920	0.10
Pb	**0.9271**	**0.9275**	**1.1282**	0.0999	**0.7206**	0.0565	0.0988	0.0986	0.0987	0.2025	0.100	0.0986	0.50

Note: Values in **bold** indicates exceedance of permissible limits. NE = No established limit.

3.2 Health risk assessment results

The EDI of iron from consumption of 40 days old cabbage leaves by children (294.678×10^{-4} mg/kg/day) was the highest while the lowest EDI (0.052×10^{-4} mg/kg/day) was for chromium consumed by adults in cabbages cultivated fro 120 days (**Table 2**). On the other hand, the THQ of the HMs ranged from 0.000004 for chromium in cabbages eaten by adults to 1.3134 for lead in cabbages consumed by children (**Table 3**). The main risk driver of non-carcinogenic health risks in this study was Pb, as its THQ in cabbages consumed by children and the total THQ exceeded 1.

Table 2. Estimated daily intake (mg/kg/day) from consumption of HMs contaminated vegetables (cabbage leaves and tomato fruits) from Kilembe mines, Uganda

Metal	Group	Cabbages ($\times 10^{-4}$)		Tomatoes ($\times 10^{-4}$)	
		40 days	120 days	40 days	120 days
Iron	Children	294.678	158.621	153.696	196.620
	Adults	73.669	39.655	38.424	49.155
Copper	Children	11.656	30.640	72.438	32.346
	Adults	2.914	7.660	18.110	8.087
Zinc	Children	28.277	261.233	5.916	5.916
	Adults	70.690	65.308	1.479	1.479
Chromium	Children	0.243	0.209	0.666	0.888
	Adults	0.061	0.052	0.167	0.222
Cadmium	Children	0.272	1.349	8.010	5.712
	Adults	0.068	0.337	2.003	1.428
Arsenic	Children	1.400	1.910	0.672	0.288
	Adults	0.350	0.477	0.168	0.072
Lead	Children	52.536	5.661	5.928	12.150
	Adults	13.134	1.415	1.482	3.038

Table 3. Hazard quotient from consumption of cabbage leaves and tomato fruits from Kilembe mines, Uganda

Metal	Group	Cabbages		Tomatoes	
		40 days	120 days	40 days	120 days
Fe	Children	0.0420	0.0226	0.0220	0.0280
	Adults	0.0105	0.0057	0.0055	0.0070
Cu	Children	0.0291	0.0766	0.1811	0.0808
	Adults	0.0073	0.0192	0.0453	0.0202
Zn	Children	0.0942	0.8707	0.0197	0.0197
	Adults	0.2356	0.2177	0.0049	0.0049
Cr	Children	0.00002	0.00001	0.00004	0.00006
	Adults	0.000004	0.00003	0.00001	0.00001
Cd	Children	0.0272	0.13490	0.8010	0.5712
	Adults	0.0068	0.03370	0.2003	0.1428
As	Children	0.4667	0.6367	0.2240	0.0960

Metal	Group	Cabbages		Tomatoes	
		40 days	120 days	40 days	120 days
	Adults	0.1167	0.1590	0.0560	0.0240
Pb	Children	**1.3134**	0.1415	0.1482	0.3038
	Adults	0.3284	0.0104	0.0371	0.0760
TTHQ	Children	**1.9481**	**1.8830**	**1.3960**	**1.0996**
	Adults	0.7053	0.44573	0.3491	0.2749

Note: TTHQ = Total target hazard quotient. Values in **bold** exceeds one.

In carcinogenic health risk assessment, the cancer risks was performed for both groups (**Table 4**). The values ranged from 0.126 × 10^{-4} for Pb consumed in tomatoes by adults to 28650 × 10^{-4} for arsenic in cabbages consumed by children. Overall, the risks were higher for cabbages than in tomatoes consumed after 40 days than those consumed after 120 days of growth. Cadmium and arsenic pose the highest carcinogenic health risk.

Table 4. Cancer risks through consumption of contaminated vegetables from Kilembe mines, Uganda

	Group	Cancer risk (× 10^{-4})								Total cancer risk (× 10^{-4})	
		Lead		Chromium		Cadmium		Arsenic			
		40 days	120 days	40 days	120 days	40 days	120 days	40 days	120 days	40 days	120 days
Cabbage leaves	Children	**4.466**	0.481	**1.215**	**1.045**	**1.034**	**5.126**	**21000**	**28650**	**21006.715**	**28656.652**
	Adults	**1.116**	0.120	0.305	0.260	0.340	**1.281**	**525**	**7155**	**526.761**	**7156.661**
Tomato fruits	Children	0.504	**1.033**	3.330	**4.440**	**40.05**	**21.706**	**10080**	**4320**	**10123.884**	**4347.179**
	Adults	0.126	0.258	0.835	**1.110**	**7.611**	**5.426**	**2520**	**1080**	**2528.572**	**1086.794**
Ingestion cancer slope factor (mg/kg/day)		8.50 × 10^{-6}		5.0 × 10^{-4}		3.80 × 10^{-4}		1.50 × 10^{0}		Not applicable	Not applicable

*Values in **bold** indicate exceedance of the US EPA cancer risk borderline (1 × 10^{-4} to 1 × 10^{-6}).

4. DISCUSSION

This study revealed that cabbages and tomatoes accumulated toxic heavy metals when grown in rock soils of Kilembe copper mine, Kasese district, Western Uganda. The results showed that Fe had the highest concentration in cabbage roots when compared to the other metals (**Table 1**). Such high Fe concentrations may be due to the geological composition of its soils [25]. Iron is an essential trace metal with no established permissible limit. In regards to the other HMs, studies elsewhere reported concentrations of 0.31 and 0.16 mg/kg, 0.51 and 0.37 mg/kg, 0.26 and 0.41 mg/kg, 38.0 and 50.0 mg/kg for As, Cd, Pb and Zn in cabbages and tomatoes grown in urban areas of Bangladesh [8]. The mean Cu content of the vegetables in this study were lower those reported in Bangladesh (8.63–27.94 mg/kg), India (15.66–34.49 mg/kg) and China (61.20 mg/kg)[26-28]. In Ecuador, the concentration of Cd and Pb in tomatoes were 0.009 to 0.058 mg/kg and 0.041 to 0.209 mg/kg [29]. Another report from Nigeria reported levels of 0.0069, 0.1699, 0.0036 and 0.0477 mg/kg for Fe, Pb, Cd and Cr which are comparable to the values found in this study [30]. While the concentrations of the HMs recorded were expected, the differences could be explained by the fact that several mechanisms mediate the bioaccumulation and phytoavailability of trace metals in vegetables. They are influenced by the physicochemical properties of the soil (for example pH, dissolved organic carbon, cation exchange capacity, biota community) and physiological properties of the plant [8, 15, 26, 31].

For daily intake, the EDI were lower than the R*f*D of the metals except for Pb in tomatoes consumed by children (294.678×10^{-4} mg/kg/day). The TTHQ all surpassed 1 for children, indicating that they are most likely to get non-carnogenic health effects. For cancercinogenic health risks, the range of cancer risks borderline given by US EPA is 1×10^{-4} to 1×10^{-6} and is considered unacceptable where the risk surpasses 1×10^{-4} [32]. Considering intake of the carcinogenic HMs, the cancer risks spanned from 0.126×10^{-4} to 28650×10^{-4}. These cancer risk values surpassed 1×10^{-4} by several folds especially for Cd and As, suggesting that there are potential cancer risks that could arise from consumption of the vegetables in Kilembe mines, Uganda. Taken together, the risks were higher for cabbages than in tomatoes consumed after 40 days than those consumed after 120 days of growth.

5. CONCLUSION

This study has substantiated previous reports, revealing that there are discernible non-carcinogenic health risks (THQ >1) and cancer risks associated with consumption of cabbages and tomatoes by the local community in Kilembe mines, Western Uganda. The risks are higher for children who consume both cabbages and tomatoes after 40 days of growth. Arsenic and cadmium pose the highest carcinogenic health risks while lead poses the highest non-carcinogenic health risk. The results showed that consumption of the vegetables after 120 days of growth poses lower health risks than when they are eaten after 40 days of growth. Future studies should examine relationships between the occurrence of trace metals in food stuffs with cancer, ulcers and other associated diseases in the area. Follow-up studies should also establish the dependence of the variations of HMs concentrations in the roots and leaves of the plants grown in other areas that are differently contaminated by the mine wastes.

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