Inorganic pollutants in edible grasshoppers (*Ruspolia nitidula*) of Uganda and their major public health implications

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Abstract

Background: Inorganic contamination of food products is associated with adverse health effects, however, information on grasshoppers in Africa is sparse. The objective of the study was to determine antioxidant, heavy metal and food safety status of edible grasshoppers of Uganda.

Methods: A cross-sectional study was conducted in central and southwestern Uganda, in which a questionnaire was administered to grasshopper harvesters. Grasshopper samples were collected from each harvesting point and analyzed in the laboratory for antioxidant and heavy metal content i.e. Lead (Pb), Chromium (Cr), Zinc (Zn) and Cadmium (Cd) using atomic absorbance spectrometric (AAS) method on the heads and abdomen of the insects.

Results: Major antioxidants were Catalase > Glutathione > Glutathione peroxidase. In addition concentrations of heavy metals were in the order of Pb > Cr > Zn > Cd in the heads and abdomens of the grasshoppers. Pb concentrations were found to be higher in the heads than the abdomens and the carcinogenic potential of the grasshoppers was over 10 times over the recommended levels. Grasshoppers were found not to be safe especially in children due to their small body weight in comparison to adults.

Conclusion: Pb poisoning in the Ugandan children would be propagated through contaminated grasshoppers.

Keywords: "Ecotoxicology," Food Safety," "Metals in Insects," "Nutritional Toxicology, Pb poisoning, Uganda.

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Introduction

Environmental pollution is a major public health threat that is currently affecting developing countries the most, thus creating a lot of strain on the already crippled healthcare system in a majority of these countries[1,2]. This has been a result of the rapid economic and agricultural growth, leading to an increased usage of inorganiclements of industrial origin that had never been used before. For example, the use of herbicides, acaricides, and insecticides is associated with modern methods of farming although their safety continues to be a subject of major intellectual debate[3–5]. The herbicides and pesticides that are commonly used by farmers contain several elements such as lead (Pb), zinc (Zn), cadmium (Cd), iron (Fe), chromium (Cr) and manganese (Mn)[6]. Heavy metal contamination of soils as a result of human activity is a real threat due to the ability of the plants to absorb these molecules,

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the heavy metals can then enter the food chain[6,7]. This level of contamination is a result of poor usage or lack of clear policies to monitor usage and disposal of farm waste.

In Africa, heavy metal contamination of major water bodies has been demonstrated to be a threat[8], although the mechanism through which such contamination is propagated has not been fully investigated. In Uganda, population exposure to heavy metals as a result of human activities in mining and agricultural sites has been demonstrated[9]. In addition, attention has been placed on fish associated with the fresh water lakes of Uganda[10,11]. Heavy metal load in livestock products of Uganda have been shown to have higher than the recommended levels, thus posing a major public health risk[12–14]. This means that animal products from Uganda still have a hurdle in accessing international markets, and this continues to be complicated by a lack of continuous surveillance data on major food products. Among these products, are grasshoppers, whose commercialization has been recommended by several scientists[15,16]. Eating of insects acts as an alternative cheap source of food and reduces environmental pollution following biomass transformation in man[17]. In Uganda, grasshoppers are a source of economic livelihood[15] and a recent study in Uganda showed that the edible grasshoppers (*Ruspolia nitidula*) are nutritious[16], although information on chemical hazard food safety is sparse. This would be highly important since the high antioxidant activity, accumulated from the forage eaten can be affected by levels of heavy metals in the tissues[18–20]. Bearing in mind that grasshoppers have been shown to have the ability to concentrate heavy metals in their exoskeleton especially the heads[21,22], questions on food safety need to be addressed before they can be branded as safe foods for national and international consumption. Bioaccumulation of heavy metals especially of Pb, Zn, Cd, and Cr have been shown to a major threat that would ultimately disrupt physiological processes in humans, just as it does in the insects[23,24]. The objective of the current study was to determine antioxidant activity, heavy metal load, and investigate food safety of grasshoppers that are eaten in Uganda.

Materials and methods

Study design

This was a cross-sectional study conducted in insect feeding communities of Central and Southwestern Uganda. Raw grasshoppers (*Ruspolia nitidula*) were collected purposively and by convenience method during November to December 2016 in southwestern (Bushenyi, Sheema and Mbarara districts) and Central (Wakiso, Kampala and Mukono districts) regions of Uganda as shown in Figure 1. Sampling was done to ensure that both rural (N =30) and urban (N = 30) settings contributed to the homogenized sample pool in each district. Convenience and purposive sampling were conducted and samples were acquired following harvesting by the community in each major center in both peri-urban (towns) and rural (villages) areas. These were then transported to the Department of Physiology laboratory using a cool box with ice packs and subsequently stored at -20^{o}C for laboratory analysis. In the communities, a semi-structured questionnaire was administered to 239 grasshopper gatherers who were chosen randomly. They provided responses on their experience in the grasshopper business, history of professional training, health complications acquired during their stay in this occupation, quantity of grasshoppers eaten by their homesteads, education background, hygiene status, and biosafety measures implemented while at work.
In the laboratory, each grasshopper was separated into the head and abdomen segments. These were subsequently homogenized for biochemical and heavy metal analysis. Samples were separated according to districts, 1 gram of each sample was then weighed and placed into a homogenizer with 0.1M phosphate buffer saline. The mixture was then centrifuged at 15000 rpm for 5 minutes and the supernatant was placed in sterile Eppendorfs for analysis.

Antioxidant determination

Glutathione peroxidase (GPx) was determined according to standard methods 25. Glutathione (GSH) was determined according to standard methods 26 and absorbance was read at 405 nm. Catalase (CAT) was determined according to standard methods 27 since these were small animal samples and absorbance was read at 580 nm, after which standard curves were made.

Heavy metal determination

Following homogenization, approximately 1g of the head and abdomen from each sample was subjected to heavy metal analysis i.e. Lead (Pb), Cadmium (Cd), Chromium (Cr) and Zinc (Zn) by using standard spectrometric methods[28]. Wet digestion of the samples i.e. heads and abdomens were done using 30 ml of nitric acid at 150^{o}C for 45 minutes. 2 ml of hydrogen peroxide were added to further the digestion after the solution reduced to about 10 ml. The entire solution was then made up to 30 ml with deionized water and transferred to a plastic bottle ready for analysis. The sample solution was analyzed with an atomic absorption spectrophotometer (AAS), model Perkin Elmer 2380 (Artisan Group Company, Champaign, IL, USA). Working standards of 0.2 ppm, 0.5 ppm, 1 ppm, 2 ppm, and 5 ppm were prepared from a stock solution of 1000 ppm (Pb, Cd, Cr, and Zn). Standard curves were generated as described previously[14], and these were used to determine the concentration of the inorganic compounds in each sample.

Food safety analysis

This was done using methods as previously described[14] where the Estimated Daily Intake (EDI) was calculated using the equation below:

EDI = (C x IR) / BW where, C = concentration of the metal (mg/kg), IR = ingestion (g/kg) rate for grasshoppers in each region were got after calculating quantity eaten each day. The IR was calculated using the questionnaire from which amount eaten per day was calculated since there was no available epidemiological data on this in Uganda. The mean weight (BW) for adults and children in Uganda of 60.7 kg and 20.5 kg respectively were used in line with global projections[29].

Cancer risk

In food products, the US EPA cancer risk regulatory acceptable levels are in the range of 1 x 10-6 to 1 x 10-4. Incremental lifetime cancer risk (ILCR) was obtained using the oral Cancer Slope Factor (CSF), and the US EPA reference CSF values[30] were used due to limited reference values from Africa. The CSF was defined as the risk produced by a lifetime average dose of 1 ppm/BW/day and its contaminant specific. For Pb, Zn, Cr, and Cd, the corresponding CSFs used are 0.0085; 0.0001; 41 and 6.3 ppm/day, thus the ILCR was calculated for each metal using this equation:

ILCR = CDI * CSF where CDI is the chronic daily intake of chemical (mg/kg/day) and it represents the lifetime average daily dose of exposure to a chemical[14].

CDI = (EDI * EFr * EDtot)/AT where, EDI was the estimated daily intake of a metal; EFr was exposure frequency (365 days/year); EDtot was the exposure duration 58.65 years (lifetime average for Ugandans; and AT = was the period of exposure for non-carcinogenic effects, and 70 year lifetime for carcinogenic effects (i.e. 70years *365 days/year)[14]. The cancer risk was taken to be a summation of exposure to different inorganic pollutants in the grasshoppers[14].

Non-cancer risks

These are assumed to exhibit a threshold below which no adverse effects are expected to be observed. As such, non-carcinogenic health hazards are evaluated by the target hazard quotient (THQ) using the equation below:

THQ = CDI/RfD Where; CDI = exposure dose obtained and RfD is the oral reference dose of the contaminant[14]. The RfD was an estimation of the maximum permissible risk on the human population through daily exposure, taking into consideration a sensitive group during a lifetime. The RfD values for Pb, Cd, Cr and Zn that were used are 0.004, 0.001, 1.5 and 0.3 respectively. Exposure to multiple contaminants results in additive and interactive effects therefore to evaluate the effect of multiple exposures, the chronic hazard index which was the sum of all the hazard ratios (THQ) was calculated for individual contaminants for oral ingestion. The HI is assumed safe in a population when the HI < 1 and it's a measure of concern when the 1< HI <5.

Statistical analysis

Qualitative data from the questionnaire were coded and entered into SPSS Version 20 for analysis, subjected to normality testing, and parametric tests were chosen. Chi-square, Fisher's test, and 95% confidence intervals were determined. In addition, quantitative data from laboratory analysis of the samples was analyzed with Graph Pad Prism Version 6. Turkey's multiple comparison tests was conducted on metal concentrations and information was displayed inform of means ± SEM. In addition, a one-sample t-test was conducted during food safety analysis and significance was set at 95% confidence and superscripts were used to indicate significant differences. Inferences were made in comparison to international reference standards to demonstrate the public health risk for each contaminant.

Results

The mean (±SEM) age of those involved in the grasshopper industry was found to be 30.96 (±0.62) and 31.93 (±0.80) years from both the peri-urban and rural study population. The population also showed a low level of experience in any food industry and levels of grasshoppers eaten were relatively the same in both peri-urban and rural settings. No significant differences were seen ($P > 0.05$) amongst age, experience in the food processing business and amount of grasshoppers consumed in the study population that would be associated with the different regions in Uganda as shown in Table 1.

Table 1 Mean age, experience and amount of grasshoppers eaten in peri-urban and urban Uganda

Independent Variable	Dependent Variable	N	Mean ±SEM	P-value
Age (Yrs.)	Peri-urban	122	30.96±0.62	.340
	Rural	117	31.93±0.80	
Experience in Food Industry	Peri-urban	122	1.36±0.14	.837
(yrs.)	Rural	117	1.31±0.19	
Amount consumed in study	Peri-urban	122	345.86±28.33	.
population (g)	Rural	117	310.69±29.90	.404

KEY: T-test conducted.

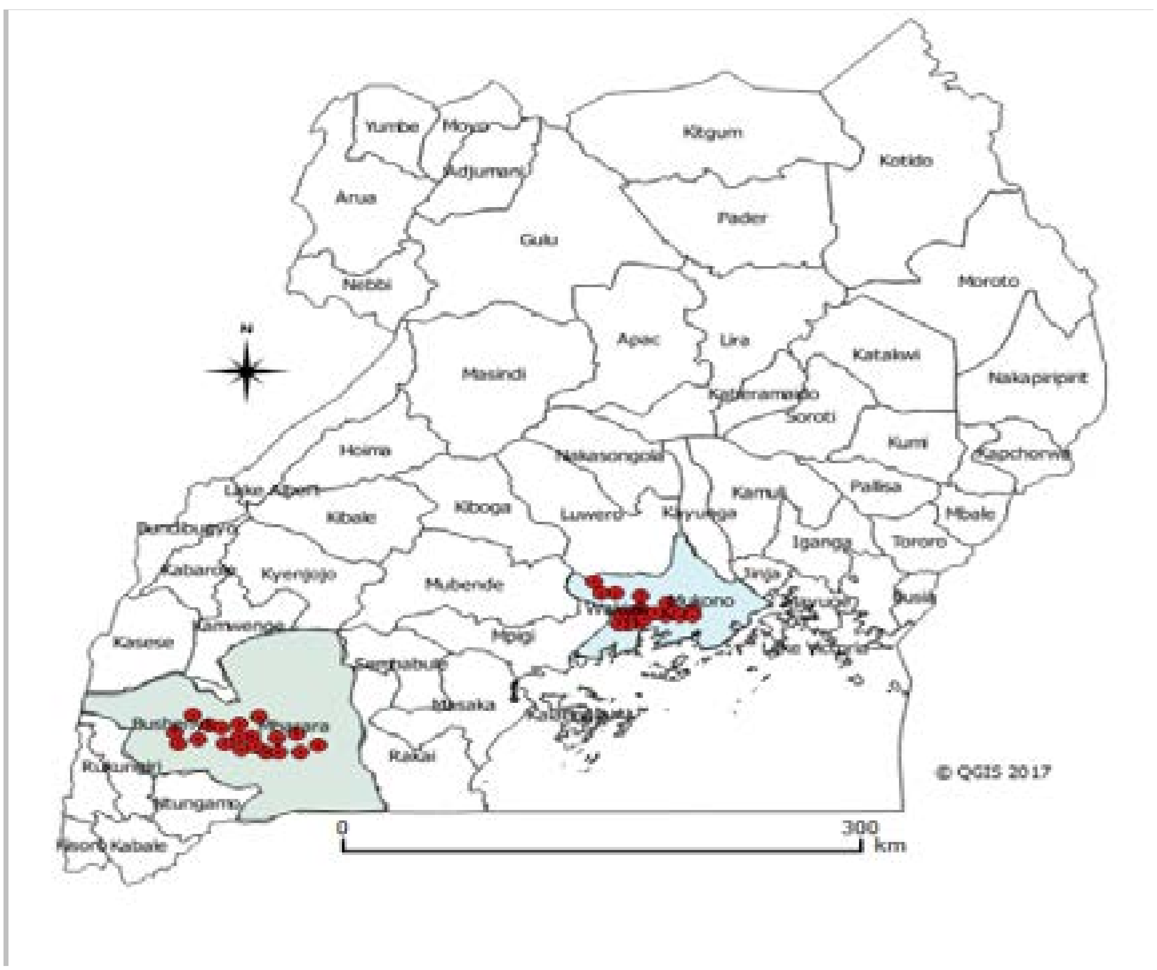

Figure 1: Map of Uganda showing study districts where the edible grasshoppers (*Ruspolia nitidula*) were collected

The study population composed of mainly the male gender (170/239) and were in favor of the male population showing that the business is dominated by men from Central Uganda (101/120). In the population, a majority of the population (136/239) didn't have a basic level of education and of these 97/120 were from central Uganda with only a primary education, demonstrating that a major part of the population in the industry are illiterate. During grasshopper harvesting, a majority (128/239) did not use appropriate materials for collecting the grasshoppers, irregularly cleaned the work items (158/239) of which 104/120 were from central Uganda. The community didn't process the grasshoppers effectively (176/239) and a majority of these were from central Uganda and lacked facilities to control the temperatures (179/239). In addition, very high significant differences were seen ($P < 0.05$) in the population from central and southwestern Uganda as shown in Table 2.

Table 2 Frequency (percentage) of major socio-economic and food safety measures amongst grasshopper handlers in Uganda

Variable		Frequency (%) of participants response from each region				P-value
		Central	Southwestern	Total		
				Value	95% CI: LL; UL	
Sex	Female	19 (15.83)	50(42.02)	69(28.87)	23.21; 35.06	0.00[a]
	Male	101(84.17)	69(57.98)	170(71.13)	64.94; 76.79	
Education level	≤ Primary	97(80.83)	39(32.77)	136(56.90)	50.36; 63.27	0.00[a]
	≥ Secondary	23(19.17)	80(67.23)	103(43.10)	36.73; 49.64	
Harvesting Material	Curved	26(21.67)	85(71.43)	111(46.44)	39.99; 52.79	0.00[a]
	Tampering	94(78.33)	34(28.57)	128(53.56)	47.01; 60.01	
Cleaning Done	Regular	16(13.33)	65(54.62)	81(33.89)	27.91; 40.27	0.00[a]
	Irregular	104(86.67)	54(45.38)	158(66.11)	59.73; 72.09	
Processing after Harvest	Cold Treatment	1(0.83)	62(52.10)	63(26.36)	20.89; 32.43	0.00[b]
	Open Air	119(99.17)	57(47.90)	176(73.64)	67.57; 79.11	
Biosafety Gear	Harvesting clothing	68(56.67)	91(76.47)	159(66.53)	60.16; 72.48	0.00[a]
	Casual clothing	52(43.33)	28(23.53)	80(33.47)	27.52; 39.84	
Physiological status	Infection from work	8(6.67)	66(55.46)	74(30.96)	25.16; 37.24	0.00[a]
	Healthy	112(93.33)	53(44.54)	165(69.04)	62.76; 74.84	
Temperature and Hygiene Control	Facilities present	0(0.00)	58(48.74)	58(24.47)	19.14; 30.46	0.00[b]
	No Facilities	120(100)	59(51.26)	179(75.53)	69.54; 80.86	
Traceability of Products	Documentation	0(0.00)	60(50.42)	60(25.10)	19.74; 31.10	0.00[b]
	Impossible	120(100)	59(49.58)	179(74.90)	68.90; 80.26	
Locality setting	Peri-urban	63(52.50)	59(49.58)	122(51.05)	44.52; 57.55	0.65[a]
	Rural	57(47.50)	60(50.42)	117(48.95)	42.45; 55.48	

KEY: Superscripts; a = Chi square test; b = Fisher's Exact test; CI = confidence interval, LL = lower limit and UL = upper limit.

Self-reported infections (36.0%) that had developed due to the grasshopper harvesting period were found to be most prevalent in rural workers. Moreover, the same population that was facing infections was found to have a low work experience in the business as compared to the more senior population as shown in Figure 2. The mean age in both the employee and employer population was found to be equally the same, although major variations existed in the employer population as shown in Figure 3.

Figure 2: Grasshoppers physiological effects on workers from different locations and their experience in handling grasshoppers.

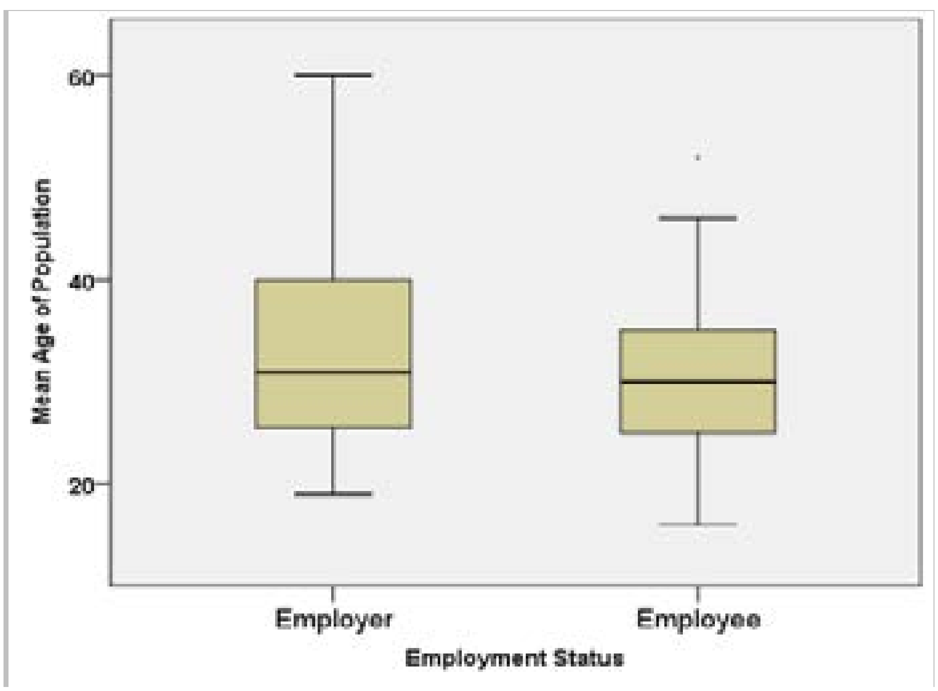

Figure 3: Age distribution and employment status in grasshopper business community.

Antioxidants in grasshoppers of Uganda

Laboratory analysis showed high levels of catalase in the heads and these concentrations were significantly highest in rural isolates from the Central region ($P < 0.05$) as shown in Table 3. Grasshopper catalase concentrations were highest in head samples from peri-urban areas. In addition, significant differences ($P < 0.05$) were found in catalase concentrations in both the heads and abdomen from both isolation centers.

Table 3 Antioxidants concentrations in grasshoppers from different locations of Uganda.

Antioxidant ((µM/g))	N	Peri-Urban		Rural	
		Head	Abdomen	Head	Abdomen
		Concentrations ± SEM			
Central Uganda					
CAT	3	379.85±138.77[a]	173.87±42.06[b]	561.93±20.99[a]	235.99±49.19[b]
GPx	3	0.65±0.05[a]	0.38±0.06[a]	1.52±0.18[a]	0.40±0.12[a]
GSH	3	92.40±36.92[a]	32.05±14.95[a]	61.14±23.83[a]	15.89±2.06[a]
Southwestern Uganda					
CAT	3	374.17±6.97[a]	149.51±40.57[b]	361.18±74.76[a]	118.34±29.69[b]
GPx	3	0.40±0.04[a]	0.13±0.03[a]	0.30±0.12[a]	0.07±0.03[a]
GSH	3	32.81±12.54[a]	10.61±3.91[a]	20.50±2.34[a]	8.50±2.06[a]

KEY: CAT = Catalase; GPx = Glutathione peroxidase; GSH = Glutathione; N = Number of districts sampled. Superscripts of different letters (a, b) indicate significant differences.

Heavy metals in grasshoppers of Uganda

Significantly high concentrations of Pb ($P < 0.05$) were found to be common in grasshopper samples from the rural than peri-urban areas of Central Uganda. Similarly, there was a significant difference in the mean Pb concentrations in the heads compared to the abdomen of these grasshoppers from the same locality. Furthermore, concentrations of Zn were found to be elevated although no statistical differences were seen from the different body parts in the samples from the different locations. In southwestern Uganda, significantly high concentrations of Pb ($P < 0.05$) were found primarily in the heads of grasshoppers collected from both peri-urban and rural settings, while low concentrations of Pb were found in the abdomens. In addition, high concentrations of Zn and Cr were observed in the head grasshopper samples from rural communities as shown in Table 4. The study also showed that very high concentrations of all the heavy metals especially Pb are concentrated in the heads while low levels are concentrated in the abdomens of the grasshoppers. Concentrations of Cr and Zn in all grasshopper samples from both central and southwestern Uganda were low as shown in Table 4.

Table 4: Heavy metal concentrations in grasshoppers from different locations of Uganda

Heavy Metals	N	Peri-urban		Rural	
		Heads	Abdomen	Heads	Abdomen
		Concentrations ±SEM (ppm)			
Central Uganda					
Pb*	3	307.11±62.07[a]	150.95±38.91[a]	455.55±90.30[a]	140.81±93.76[b]
Zn	3	137.06±22.62[a]	117.87±17.95[a]	134.63±16.93[a]	87.89±8.96[a]
Cr	3	166.93±33.94[a]	53.97±6.69[a]	190.50±54.24[a]	90.01±22.67[a]
Cd	3	0.12±0.12[a]	0.07±0.04[a]	0.16±0.05[a]	0.02±0.02[a]
Southwestern Uganda					
Pb	3	150.56±97.56[a]	39.10±39.10[a]	102.88±102.88[a]	78.62±78.62[a]
Zn	3	92.72±21.[82a]	85.01±32.51[a]	113.95±21.83[a]	91.07±19.67[a]
Cr	3	105.76±28.30[a]	38.13±22.91[a]	112.51±39.95[a]	55.18±9.91[a]
Cd	3	0.03±0.03[a]	0.02±0.01[a]	0.06±0.03[a]	0.06±0.03[a]

KEY: Heavy metals; Pb = Lead, Cr = Chromium, Zn = zinc and Cd = cadmium. Significant differences seen in Pb concentrations (*$P < 0.05$). Different letters signify significant differences in concentrations of lead. Differences were found in heads from peri-urban and abdomen from rural centers (a). In the rural area, differences were found in heads and abdomen Pb concentrations. N = number of districts from which samples were collected.

Estimated daily Intake (EDI) for grasshoppers in Uganda

The EDI showed that lead ingested was beyond the recommended levels in food products. In particular, lead ingestion levels are higher in grasshoppers from the Central region than the southwestern region of Uganda. Also, significant variations existed in EDI of Pb in the heads and abdomen, showing that the risk possessed by consumption of heads of grasshoppers was two to three times greater than that from ingestion of the abdomen as shown in Table 5. Furthermore, EDI in children was three fold higher than that in adults, showing that children are more at risk of Pb toxicity.

Table 5 Estimated Daily Intake of Heavy Metals in Grasshoppers of Uganda.

Heavy Metals Consumed	TDI	Peri-urban		Rural	
		Heads	Abdomen	Heads	Abdomen
		Concentration in mg/kg/day			
Central Uganda					
Pb	3.57	67.01[a]*	32.93[a]*	99.39[a]*	30.72[a]*
Zn	1000	29.89[b]	25.72[b]	29.37[b]	19.18[b]
Cr	150	36.42[b]	11.78[b]	41.56[b]	19.64[b]
Cd	1	0.026[b]	0.015[b]	0.035[b]	0.0044[b]
Southwestern Uganda					
Pb	3.57	22.81[a]*	5.92[a]*	15.59[a]*	11.91[a]*
Zn	1000	14.05[b]	12.88[b]	17.26[b]	13.80[b]
Cr	150b	16.02[b]	5.78[b]	17.04[b]	8.34[b]
Cd	1	0.0045[b]	0.003[b]	0.0091[b]	0.0091[b]

KEY: TDI = Tolerable Daily Intake. EDI = Estimated daily intake for an average in adult in Central Uganda. Asterisks indicate one sample t-test significant differences and superscripts (a, b) indicate risk level; a = above; b = below, while * = $P < 0.05$, ** = $P < 0.005$.

Cancer risk assessment in grasshopper feeding community of Uganda

The ILCR in all the aforesaid samples were at least 100 times greater in a majority of the samples collected from either the central or southwestern regions of Uganda. In addition, the ILCR was generally two to three times higher in the head than the abdomen with lead, and chromium from each region as shown in Table 6.

Table 6: Incremental lifetime cancer risks for adults in Uganda feeding Grasshoppers.

Contaminant	Peri-urban		Rural	
	Heads	Abdomen	Heads	Abdomen
	Mean ILCR ($\times 10^{-4}$)			
	Central Region			
Adult				
Pb	4.772[a]	2.346[a]	7.079[a]	2.188[a]
Zn	2.129[a]	5.423[a]	2.092[a]	1.366[a]
Cr	2.594[a]	2.483[a]	2.960[a]	1.399[a]
Cd	0.002[b]	0.003[b]	0.002[b]	0.000[b]
SM	9.497[a]	10.255[a]	12.133[a]	4.953[a]
Child				
Pb	14.130[a]	6.945[a]	20.960[a]	6.479[a]
Zn	6.303[a]	5.423[a]	6.194[a]	4.044[a]
Cr	7.680[a]	2.483[a]	8.765[a]	4.141[a]
Cd	0.006[b]	0.003[b]	0.007[b]	0.001[b]
SUM	28.119[a]	14.855[a]	35.926[a]	14.665[a]
	Southwestern Region			
Adult				
Pb	1.62[a]	0.422[b]	1.110[b]	0.848[b]
Zn	1.000[b]	0.917[b]	1.229[b]	0.983[b]
Cr	1.141[b]	0.411[b]	1.214[b]	0.595[b]
Cd	0.000[b]	0.000[b]	0.001[b]	0.001[b]
SUM	3.766[a]	1.751[a]	3.554[a]	2.427[a]
Child				
Pb	6.927[a]	1.799[a]	4.733[a]	3.617[a]
Zn	4.266[a]	3.911[a]	5.243[a]	4.190[a]
Cr	4.866[a]	1.754[a]	5.177[a]	2.539[a]
Cd	0.001[b]	0.001[b]	0.003[b]	0.003[b]
SUM	16.061[a]	7.465[a]	15.156[a]	10.349[a]

KEY: ILCR = Incremental lifetime cancer risks; Superscripts (a, b) indicate cancer risk, a = cancer risk present, b = cancer risk absent.

Non-cancer risks in grasshopper eating communities of Uganda

The target hazard quotient (THQ) showed that levels of the metals are generally above 1 thus raising major health concerns. In central Uganda, the hazard index (HI) was 100 times greater than the recommended levels which were primarily due to the THQ of Pb, however, the THQ for Cr and Cd was low levels, demonstrating the role of Pb contamination in grasshoppers. In southwestern Uganda, the THQ Zn, Cr, and Cd were all below one (THQ < 1) demonstrating differences in the risk posed by grasshoppers from the two regions in Uganda investigated in this study.Furthermore, the HI was mainly elevated due to Pb concentrations, as shown in Table 7.

Table 7: Target Hazard Quotients for Central Uganda.

Contaminant	Peri-urban		Rural	
	Heads	Abdomen	Heads	Abdomen
	Mean THQ (ppm/day)			
	Central region			
Adult				
Pb	16.7516[a]	8.2337[a]	24.8484[a]	7.6806[a]
Zn	0.0996[b]	0.2538[b]	0.0979[b]	0.0639[b]
Cr	0.0243[b]	0.0232[b]	0.0277[b]	0.0131[b]
Cd	0.0262[b]	0.0452[b]	0.0349[b]	0.0044[b]
HI	16.9017[a]	8.5560[a]	25.0090[a]	7.7620[a]
Child				
Pb	49.6012[a]	24.3799[a]	73.5756[a]	22.7422[a]
Zn	0.2950[b]	0.2538[b]	0.2899[b]	0.1893[a]
Cr	0.0719[b]	0.0232[b]	0.0820[b]	0.0388[b]
Cd	0.0755[b]	0.0452[b]	0.1034[b]	0.0129[b]
HI	50.0456[a]	24.7022[a]	74.0510[a]	22.9831[a]
	Southwestern Region			
Adult				
Pb	5.7020[a]	1.4808[a]	3.8963[a]	2.9775[a]
Zn	0.0486[b]	0.0429[b]	0.0575[b]	0.0460[b]
Cr	0.0107[b]	0.0039[b]	0.0114[b]	0.0056[b]
Cd	0.0045[b]	0.0030[b]	0.0091[b]	0.0091[b]
HI	5.7640[a]	1.5306[a]	3.9743[a]	3.0381[a]
Child				
Pb	24.3169[a]	6.3150[a]	16.6161[a]	12.6979[a]
Zn	0.1997[b]	0.1831[b]	0.2454[b]	0.1961[b]
Cr	0.0455[b]	0.0164[b]	0.0485[b]	0.0238[b]
Cd	0.0194[b]	0.0129[b]	0.0388[b]	0.0388[b]
HI	24.5815[a]	6.5274[a]	16.9487[a]	12.9565[a]

KEY: HI = Hazard index (summation of individual target hazard quotients for different contaminants. Superscripts (a, b) indicate non-carcinogenic effects i.e. a = level of health concern, b = no health concern.

Discussion

The grasshopper business was mainly dominated by adult men with little to no experience in the food handling business (Table 1). These findings re-emphasize the popularity of edible grasshoppers in Uganda that efforts to commercialize these as food products are in advanced stages[15], although information on the food safety status of grasshoppers has not been fully evaluated in previous studies in Uganda[14,16]. As demonstrated in the current study, processing, storage and effective handling are major challenges that the communities face, and work on addressing these limitations would be challenged by the increased liberalization of food products[16]. Despite advances in food science research in Uganda, the ability of the local Ugandan population to process grasshoppers effectively has not been exploited fully, showing key challenges in the translation of academic research into field work probably due to limited funding and infrastructure support. In the current study, we demonstrated that grasshoppers are commonly eaten in sub-Saharan Uganda (Figure 1 and Table 2) and this was in agreement with previous reports which had only placed emphasis on central Uganda[15,16]. The eating of grasshoppers, just like other insects[17] sheds light on the food safety challenges facing a majority of developing countries, since health risks accumulated following chronic exposure to chemical contaminants would ultimately affect human health (Figure 2). This would inevitably create unnecessary strain on an already struggling healthcare system[1,2], demonstrating the wisdom in establishing strong food safety systems for routine screening, safety evaluation of food products in Uganda.

The study showed that catalase was the most abundant antioxidant in the grasshoppers of Uganda and this was found to be highly concentrated in the heads of the grasshoppers (Table 3). This results from the highly abundant forage in the study area, coupled with plenty of water since Uganda lies in the great lakes region. The forage was highly succulent during this study period and the pro-oxidants accumulated were acquired from the plants the insects feed on[31,32]. In Uganda, the Central region has a very high land cover has compared to southwestern Uganda[33] due to variations in the major farming practices and these are responsible for the changes in antioxidant status observed in grasshoppers from each region. This was important since high levels of antioxidants accumulated in the tissues of the grasshoppers are important in offering protection against oxidative stress[18]. Catalase works in synergy to other pro-oxidant enzymes to reduce on the effects of reactive oxygen species, thus justifying the common practice that grasshoppers are cheaper sources of protein and nutrients in Ugandan communities as compared to livestock protein[16,17]. Increased reliance on grasshopper protein by local communities would subsequently be of nutritional benefit, thus promoting nutrient recycling within the ecosystem[34].

The major environmental pollutant identified in the grasshoppers was Pb, although a majority of inorganic compounds were above international recommended levels in foods of animal origin (Table 4). Its apparent that a majority of animal protein foods in Uganda are contaminated with Pb since a recent study has demonstrated high Pb concentrations in milk and beef[14], demonstrating the importance of promoting ecosystem protective measures to promote food safety. Accumulation of high Zn levels in the heads of grasshoppers had been demonstrated earlier by other researchers[35], however, this was the first study in which high levels of Pb which carries a higher carcinogenic risk than zinc have been detected in grasshoppers which are consumed by the general population in the East African community[16]. Observations in the study showed that grasshoppers in central Uganda are heavily contaminated with Pb, probably due to high bioaccumulation in the grasshopper tissues following a heavy feeding season due to plenty of forage in the region[33]. Vegetation in Uganda often gets exposed to Pb through the on-going irrational usage of pesticides showing that findings in this study are of public health importance since central Uganda is home to major business centers in the country due to an increased risk of Pb contamination in the Ugandan population following consumption of grasshoppers. Bearing in mind that heavy metals have already been isolated from a majority of commercial pesticides[3–6], government environmental protection strategies[6,7] which promote ecosystem health would be promoted to reverse the current trend of events in Uganda. A linear relationship was established in the antioxidant and heavy metal concentrations in grasshoppers of central Uganda demonstrating the vicious cycle created in the tissues following a buildup of reactive oxygen species due to high inorganic concentrations in the tissues. In addition, the study used wild grasshoppers as indicators of environmental contamination since these are common in several Ugandan communities[31]. The grasshopper heads have been shown to have high levels of nutritious oils[16], however, the current study demonstrates that these are major sites for Pb accumulation, and the threat of bioaccumulation in man is a real threat for all communities which feed on them[16,17,22].

Food safety analysis was mainly affected by the high EDI Pb concentrations (Table 5). Increased human consumption of Pb through the grasshoppers would subsequently predispose the Ugandan community to cancer[14]. The incremental lifetime cancer risk (ILCR) for a Ugandan adult population showed was 100 times above the acceptable limits demonstrating the health hazard of grasshopper consumption (Table 6). Consumption of grasshopper heads would triple the risk than eating abdomens, showing that grasshoppers carry a long-term carcinogenic potential and this risk was higher in children than in adults. These findings show that cancer in Uganda would be on the increase in grasshopper eating communities showing a need to strengthen food safety systems in Uganda for the promotion of human health[23,24]. The improved policy would subsequently promote international trade following the commercialization of healthy grasshoppers for the local and global markets[15,16]. Finally, the study showed that the HI was high (HI > 5) showing that the contaminants in Ugandan grasshoppers are extremely high (Table 7), necessitating the authorities to intervene as concentrations are not safe. The pollutants identified in this basic study would be responsible for the health concerns raised by the communities (Figure 2), showing a need for further studies with an emphasis on Pb in other major food products of Uganda. Observations in this study demonstrate a need to spearhead commercial production of grasshoppers against the seasonal harvesting of wild grasshoppers which might be carriers of chemical carcinogens.

Conclusion

Grasshoppers are an important source of livelihood to the Ugandan community, however, high concentrations of Pb outweigh their beneficial antioxidant activity. In addition, the heads of grasshoppers were found to concentrate all inorganic compounds higher than the abdomens, demonstrating the wisdom of avoiding grasshopper heads in the general population. Strategies to increase food safety screening in Uganda would provide more information which would guide consumers against the consumption of hazardous foods. This would also guide policymakers and commercial development partners on prospective investment options in the sector.

Competing interests

Authors declare no competing interests

Author contributions

All authors contributed equally and approved the manuscript for publication.

Author contributions

Authors contributed equally and approved the manuscript for publication. K.I.K designed the study. C.N and E.B collected the data. K.I.K, A.O, C.A, S.M, and J.K conducted data analysis and interpretation. K.I.K prepared initial draft while C.N, E.B, C.A, A.O, S.M, and J.K read and approved final version for publication.

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