

Research Article

Analysis of the Concentration of Heavy Metals in Khat Grown in Meru County and the Assessment of Their Associated Health Risks

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Contamination of farm produce by toxic heavy metals has become a serious global health concern. These metals can bioaccumulate in plant tissues and are precursors for major public health problems such as cancer and neural impairment. Khat (*Catha edulis*) also referred to as miraa has the potential to sequester and accumulate both micronutrients and potentially toxic heavy metals in its consumable parts—tender leaves and soft barks of young shoots which are known to possess psychoactive properties when consumed. Therefore, the motivation behind this contribution is to determine the levels of six heavy metals, namely, cadmium (Cd), copper (Cu), chromium (Cr), lead (Pb), iron (Fe), and nickel (Ni) in consumable Meru khat samples, compare these levels with the permissible limits of World Health Organization (WHO) in order to predict associated health risks, and to estimate the noncarcinogenic risks of these metals by total health quotient (THQ) and health index (HI) on khat consumers. 1.0 g of dry ground khat samples was digested in 0.05 M HCl and allowed to stand for 5 hours before being analyzed for heavy metals using inductively coupled plasma atomic emission spectroscopy (ICP-AES). The mean heavy metal concentrations (mg/kg) in dry khat samples of six toxic heavy metals were Cd (7.81 ± 1.56), Cr (15.98 ± 2.22), Cu (15.81 ± 2.84), Fe (97.35 ± 32.67), Ni (0.37 ± 0.02), and Pb (32.36 ± 9.95). Based on the results, the mean levels of Pb, Cd, and Cr exceeded WHO permissible limits. In addition, the Pb and Cd THQ values and the HI of the six heavy metals investigated in the khat samples exceeded the threshold value of 1.0. Furthermore, the THQ and HI values showed that Pb and Cd were potentially the major contributors to noncarcinogenic risks on regular khat consumers. This is a matter of concern on the excessive consumption of Meru khat-based products, which over time may cause a toxicological response. Based on the findings of this study, the use of agrochemicals should significantly be minimized in khat farming. Accordingly, the Meru khat farmers should be sensitized on alternative farming practices that do not potentially cause heavy metal contamination in khat.

1. Introduction

Khat farming and its related activities are a multimillion enterprise for many people in the khat-growing regions and also contribute immensely to the gross domestic product (GDP) for the countries where the plant is grown [1, 2]. In Kenya, Meru County is not only the main producer of khat for local and international markets but also the highest khat consumer [3, 4]. Nonetheless, the consumption of khat leaves for stimulative and psychoactive effects, for instance, is largely unregulated, and the evaluation and monitoring of contaminants such as heavy metals and pesticides in khat before consumption is rarely conducted. This leaves khat

consumers vulnerable to the health hazards that can result from contaminated khat. This study is therefore inspired by the potential toxic metals that are considered a major public health hazard due to their bioaccumulation in khat that may have the potential to cause severe etiological risks. Accordingly, the lack of awareness about the presence of heavy metals in food products especially khat has resulted in many health concerns that are detrimental to public well-being [5].

Periodic determination of heavy metals and other micronutrient elements in crops, soils, and water bodies is fundamental in monitoring their concentration levels and maintaining them within allowable limits [6, 7] in order to ensure good health and safety. It is notable that research on

the chemical composition of plant-based materials is expanding due to the need for sustainable developments in the agricultural sector, environmental heavy metal pollution, and natural activities including lightning and volcanic eruptions [8]. Therefore, enhanced food security should be aimed at preventing heavy metal contamination [9], potent pesticides, and other agricultural-based synthetic chemicals [10].

Clearly, trace and heavy metals are some of the major food chain contaminants which are precursors for deleterious health effects in humans even at low concentrations [5]. Despite much awareness about heavy metal contamination, anthropogenic activities throughout the world have increased significantly with soil as a sink for toxic metals, which are not only persistent but also accumulative in soil, and have the potential to increase the toxicity of soils after combining with inorganic and organic matters [11].

The khat plant can absorb both micronutrient elements responsible for its growth and macronutrient elements (heavy metals) into its tissues from contaminated soils such as landfills and industrial effluent [5]. This uptake is continuous and these elements biomagnify in the leafy part of the plant resulting in accumulations of varying concentrations at different plant parts [10, 12]. Important to note is that khat is susceptible to pests; consequently, the use of synthetic pesticides controls them and the application of fertilizers improves the production levels [1]. These practices are well known to introduce heavy metals into the soils which are then absorbed by the plant into its tissues [13]. Their bioaccumulation especially in plant tissues depends on the age and maturity of the plant during harvesting [10]. This agglomeration of toxic heavy metals makes consumable khat parts easily contaminated which in turn can cause serious health risks upon consumption [14].

Of late, heavy metal poisoning is a public health concern that calls for regular monitoring and assessment [10]. Thus, human risk assessment is critical as it estimates the risk of environmental contamination to human health through different modes of exposure such as inhalation, ingestion, and skin contact [15]. Towards this end, research reports from across the world have confirmed severe health problems induced by toxic heavy metals present in plant samples and possible threat to the lifespan of animals [6] and humans. This is due to the fact that these metals are non-biodegradable, have long-term duration in the environment, and are capable of causing serious public health problems such as cancer, nerve breakdown, and organ failure [13]. However, WHO in one of its reports affirmed that some heavy metals, especially Zn, Mn, Fe, and Cu, are not only essential but also necessary for healthy growth, provided their levels do not exceed acceptable limits beyond which they can cause diseases [6] and result in intoxication and chronic toxicity to humans [9].

The detection and quantification of heavy metals in various samples can be done using analytical techniques such as inductively coupled plasma atomic emission spectrometry (ICP-AES), atomic absorption spectrometry (AAS) [16], and inductively coupled plasma optical emission spectrometry (ICP-OES) [17]. To the best of our understanding, little

research has been conducted on heavy metals in the Meru khat. Therefore, the motivation underpinning this study is to determine the concentration profiles of heavy metals in leaf shoots of the Meru khat—Cd, Cr, Cu, Pb, Fe, and Ni—and to compare them with allowable limits of WHO in order to predict associated health risks on consumption of this khat. To assess the magnitude of the risks of these metals, the noncarcinogenic parameters THQ and HI have been estimated. This study provides a basis that will guide alternative agricultural practices in the growing of safer khat plants. Also, these results can enhance the understanding of the public health risks of toxic heavy metals in agricultural crops such as khat in the Meru region. This will provide insights into contamination control, with respect to human health risks and sustainable, human-friendly economic growth [18].

2. Study Area

A total of 11 khat samples used in this study were collected randomly from khat farmers in each of the selected regions—Kangeta, Maua, Laare, Kianjai, and Mutuati in Meru County, Kenya. Miraa referred to locally as *Miwee* among the Ameru people is a type of plant that is cultivated for local and export consumption. The sample collection sites are presented in Figure 1. The collected samples were then directly taken to the laboratory for further treatment and analysis.

Meru County, coordinates 0° 21' 21" N/37° 48' 32" E, is located in the central part of Kenya and covers a total land area of 7,006 km². As of the 2019 Kenya Population and Housing Census, the county's population was 1,545,714. The annual rainfall in the county ranges between 300 mm and 2500 mm where the long rains fall from March to May and short rains from October to December. The Meru region is situated close to the equator, making the summers difficult to define. The recorded annual range of temperature is from 12.92 to 23.45 °C characterized by coldest and warmest months. Khat farming is the main economic activity for inhabitants of this county who derive very good returns from this profitable agribusiness venture. This county is part of the country that not only consumes khat in large quantities but also exports it to neighboring countries such as Somalia, Madagascar and Uganda. Khat is an exceptional plant in the county that can tolerate climatic extremes, particularly drought conditions. The demand for domestic khat consumption and the international markets have caused the khat supply to be severely stressed in the past few years. Agriculture and animal husbandry are the major human activities in most of the study areas and fertilizers (animal manure and chemical fertilizers) and pesticides are widely used in these areas. Intercropping khat with other food crops system is mainly adopted by the khat farmers in this county.

2.1. Experimental

2.1.1. Reagents and Sample Preparation. All reagents used in this study were of analytical grade with % purity ≥ 99.9 . Materials and reagents including commercial 1000 ppm standard mixture solution containing 6 target heavy metal elements were purchased from Kobian chemical

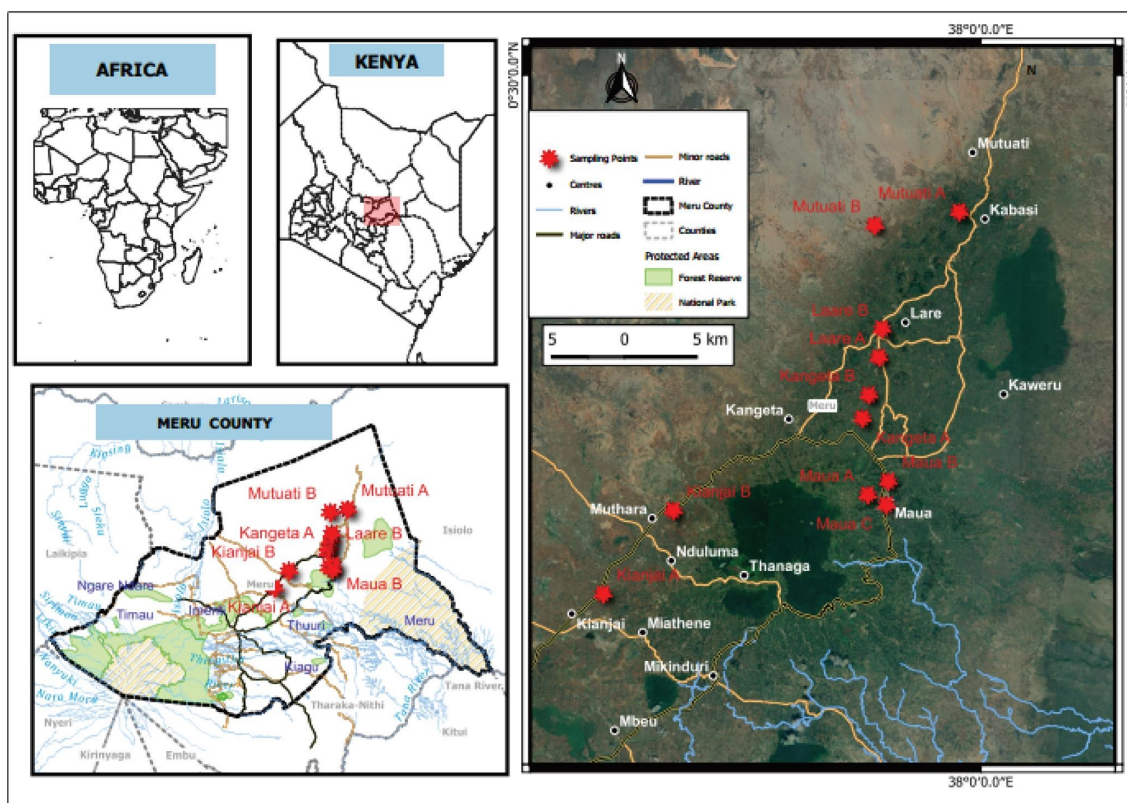


FIGURE 1: A map of Meru County showing khat sample collection sites.

Company—a subsidiary of Sigma-Aldrich Ltd., South Africa. Other reagents included 10% nitric (v) acid and 20 volumes of hydrogen peroxide (H_2O_2). Deionized water purchased from Sigma-Aldrich (Germany) was used throughout the study for rinsing, sample preparation, and dilution prior to the analysis.

Khat samples were air-dried in the laboratory for 1 week, followed by oven drying at 70°C for 72 hours to obtain constant mass. The dry samples were ground using a grinder to homogenize and reduce their sizes to 0.25 mm. The samples were then stored in sterilized plastic containers for analysis. The elemental concentrations of the digested khat samples were analyzed using an inductively coupled plasma atomic emission spectrometer (ICPE-9000).

To minimize contamination, stainless steel grinding systems were washed and thoroughly cleaned with acetone before and after each grinding cycle to avoid cross-contamination. The ground khat samples were stored in sterilized plastic containers. All the glassware used for analytical work was thoroughly rinsed using deionized water, followed by concentrated nitric acid wash. The disposable plastic gloves were worn when handling the khat samples during the sampling and analyzing stages. Finally, the digested khat solutions were kept in a refrigerator at all times until analysis was complete.

The digestion procedure of the khat samples was optimized for different parameters such as reagent volume, digestion time, volume ratio of the reagents, and digestion temperature. The trial method optimization was done by varying one parameter at a time and keeping other

parameters constant. The parameters that gave clear solutions at lower temperature and shorter reaction time and required minimum reagent volume and ratio were selected as an optimum procedure and were used for the digestion of khat samples. One gram (1.0 g) of finely ground dry khat sample was weighed into sample digestion specimen tubes. The sample was allowed to ash in a muffle furnace for 5 h at 450°C – 500°C before cooling to room temperature in a desiccator. The sample was then transferred into a 100 mL conical flask, followed by the addition of 1 mL mixture of $\text{H}_2\text{O}/\text{HCl}$ (1 : 1 v/v), $\text{H}_2\text{O}/\text{HNO}_3$ (1 : 1 v/v) and 10 cm³ of 20 volumes of hydrogen peroxide (H_2O_2), and the mixture evaporated to dryness on a hot plate. The mixture was reconstituted using 25 mL of 0.05 M HCl and allowed to stand for 5 hours awaiting metal analysis. The digests were used to determine the concentrations of Cd, Cu, Pb, Fe, and Ni by ICP-AES.

2.1.2. Analysis of Heavy Metals. Metal ion concentrations in khat plant samples were analyzed using an inductively coupled plasma atomic emission spectrometer (ICPE-9000). All the standard, blanks, and samples were prepared and analyzed in triplicate. An instrument quality control and subsequent tuning were performed using an instrument tuning solution at 0.1 mg/kg in hydrochloric acid. A nine-point calibration curve of multielement standard was drawn in absorbance mode and was used to determine the concentration of heavy metals present in the khat samples. The

ICP-AES method developed was validated for parameters such as accuracy, robustness, and precision. The instrumental parameters and working conditions for ICP-AES are given in Table 1. High-purity (99.99%) argon was used as a nebulizer, plasma, and auxiliary gas. The instrument was calibrated for various parameters before the experiments were conducted.

2.1.3. Evaluation of Method Accuracy and Precision. The precision and accuracy of the method were assessed by spiking 10 mL aliquot of 5 μ g of each analyte metal ion into 1 g khat sample, followed by the same optimized digestion procedure for the spiked and nonspiked samples. The levels of heavy metal ions in both samples were determined by ICP-AES, and the percentage recovery values were calculated using the following equation:

$$\text{Percentage recovery} = \frac{A_{\text{sks}} - A_{\text{ks}}}{A_a} \times 100, \quad (1)$$

where A_{sks} is the concentration in spiked khat sample, A_{ks} represents the concentration in nonspiked khat sample, and A_a is the amount added.

2.2. Target Hazard Quotient (THQ). The heavy metals' noncarcinogenic risk to humans due to regular ingestion of contaminated khat was determined by assessing the THQ [19]. The cumulative risks posed by exposure to all investigated heavy metals in consumable khat were assessed by calculating the HI. The THQ ratio of the investigated heavy metals was evaluated using equation (3) [19].

$$\text{THQ} = \frac{C^\circ \times \text{IR} \times \text{EF} \times \text{ED}}{\text{Bw} \times \text{RfD} \times \text{AT}} \times 10^{-3}. \quad (2)$$

Since $(\text{EF} \times \text{ED})/(\text{AT}) = 1$, then equation (2) becomes

$$\text{THQ} = \frac{C^\circ \times \text{IR}}{\text{Bw} \times \text{RfD}} \times 10^{-3}. \quad (3)$$

Here, Bw stands for adult body weight considered at 70 kg; C° denotes the average concentration of heavy metal in the khat (mg/kg dry weight); EF is the exposure frequency taken as 365 days/year; ED represents exposure duration taken as 70 years; AT denotes the average time for potential health risk taken as $\text{ED} \times 365$ days; and IR represents the ingestion rate of khat taken as 100 g/person/day as determined in previous studies [20].

The RfD (mg/kg/day) refers to the oral reference dose which is taken as 0.001 for Cd, 0.0035 for Pb, 0.02 for Ni, 0.04 for Cu, 1.5 for Cr, and 0.07 for Fe [19, 21–23]. The HI assumes that the magnitude of adverse effects on target organs and systems of humans will be proportional to the sum of the THQ of multiple heavy metal exposures in consumable khat. This is estimated using the following equation:

$$\begin{aligned} \text{HI} &= \sum \text{THQ} \\ &= \text{THQ}_{(\text{Pb})} + \text{THQ}_{(\text{Cu})} + \text{THQ}_{(\text{Cd})} + \text{THQ}_{(\text{Fe})} \\ &\quad + \text{THQ}_{(\text{Cr})} + \text{THQ}_{(\text{Ni})}. \end{aligned} \quad (4)$$

TABLE 1: Instrumental parameters and operating conditions for ICP-AES.

ICP-AES parameter	Value
Pressure of argon gas	450 \pm 10 kPa
Nebulizer flow	0.90 L/min
Plasma power	1300 W
Radio frequency generator	1.2 kW
Pump rate	25 rpm
Coolant flow rate	12.00 L/min
Vacuum pressure	>10 Pa
Exposure time	30.0 seconds
Carrier gas flow rate	0.70 L/min
Auxiliary gas flow	0.60 L/min
Plasma gas flow rate	10.0 L/min

If HI is less than 1.0, then the non-carcinogenic effect due to the heavy metal exposure is considered to be negligible. However, if the TH value exceeds 1.0, then these effects are detrimental [15, 24].

2.3. Statistical Analysis. All the statistical tests were carried out using Microsoft Excel spreadsheet and Statistical Package for Social Sciences (SPSS) IBM statistical software, version 25.0. The descriptive statistics such as the means, standard deviation, and relative standard deviation were done using Microsoft Excel. The variation in concentration of toxic heavy metals in khat plant collected from different farms was subjected to multivariate statistical analysis. Particularly, the Pearson correlation analysis was employed to analyze the correlation between variables (concentration of each heavy metal) with a view to determining data coherence and divergence. The principal component analysis (PCA) was applied to simplify the experimental data and conjecture on the sources of heavy metals in the Meru khat. PCA allows for the large datasets for dimension reduction while keeping most of the information.

2.4. Method Validation and Recovery Test. The calibration curve prepared using the pure standards of Fe, Cu, Cr, Ni, Pb, and Cd was found to be linear with correlation coefficient, $r \geq 0.998$. In order to assess the accuracy of the ICP-AES method, spiked khat samples were used. The relative recovery experiments at different concentrations were evaluated and the results are recorded in Table 2. The relative recovery values ranged between 91.7% and 109.1%, indicating that the method used exhibits commendable accuracy.

The sensitivity of the ICP-AES method was assessed by calculating limits of detection (LOD) and limits of quantification (LOQ). As a result, ten separate blank solutions were independently prepared and analyzed. The determination of LOD and LOQ was done based on the guidelines of the International Union of Pure and Applied Chemistry (IUPAC) [25], using the following equations:

$$\text{LOD} = 3 \left(\frac{\delta}{s} \right), \quad (5)$$

$$\text{LOQ} = 10 \left(\frac{\delta}{s} \right). \quad (6)$$

TABLE 2: Percentage recovery test results of heavy metals in khat samples.

Heavy metal	*Concentration in nonspiked khat sample (mg/kg)	Amount added (mg/kg)	**Concentration in spiked khat sample (mg/kg)	Percentage recovery (%) ^a
Cu	16.36	3.50	19.90 ± 0.08	101.1 ± 0.3
Cd	7.64	1.50	9.09 ± 0.11	96.7 ± 0.9
Pb	24.45	2.00	26.43 ± 0.07	99.0 ± 0.2
Fe	91.78	5.00	96.47 ± 0.80	93.8 ± 0.6
Ni	0.36	1.00	1.34 ± 0.01	98.0 ± 0.2
Cr	16.14	2.50	18.59 ± 0.20	98.0 ± 0.7

* Average value of three measurements; ** values are mean ± SD of triplicate analyses. ^aValues are mean ± SD of triplicate.

where, s is the slope of the calibration curve for each element and δ is the standard deviation of the measurements of the blank solution.

For the evaluation of method linearity, the calibration curves for each element were constructed by plotting the peak area of the optimum emission line to the concentration of the standard solution. Subsequently, least square linear regression analysis was used to evaluate the slope, intercept, and coefficient of determination.

2.5. Calibration. The selection of the optimum emission lines was based on the intensity and their sensitivity as well as the absence of spectral interferences. The selected emission lines for each element in this study were Cd (226.502 nm), Cu (324.752 nm), Ni (232.003 nm), Pb (217.000 nm), Fe (238.204 nm), and Cr (357.869 nm). The performance of the ICP-AES method was evaluated under the conditions described in Table 1. Table 3 shows the calibration curve, the regression lines, and the LOD and LOQ for all the examined heavy metals in khat samples. From Table 3, the coefficients of determination for all the heavy metals were good ($r^2 > 0.9800$) for the experimentally determined values. The LOD and LOQ of the ICP-AES method ranged between 0.072 and 1.193 mg/kg, and 0.220–3.616 mg/kg, respectively. The method applied demonstrated high sensitivity as shown by low values of LOD.

3. Results and Discussion

3.1. Concentration Profiles of Heavy Metals in Khat. In the present study, the concentrations of selected heavy metals, Cu, Cr, Ni, Pb, Cd, and Fe in khat samples collected from the Meru region are reported in Table 4. The results show a wide variation in the concentration of heavy metals in khat samples collected from different khat-growing farms of Kianjai, Laare, Kangeta, Mutuati, and Maua of Meru.

The average and RSDs percentages of all the heavy metals investigated were calculated and listed in Table 4. The contents of these metals show variations in concentrations for different khat samples. The RSDs percentages ranged from 4.2 to 33.6. These results showed that the most abundant metal in khat samples was Fe, followed by Pb, Cr, Cu, Cd, and Ni, respectively. Generally, iron being the most abundant metal in all khat samples has its concentration ranging from 72.10 ± 2.13 to 180.5 ± 3.02 mg/kg, with a mean of 97.35 ± 32.67 mg/kg as reported in Table 4. The highest

value was from the sample drawn from the Laare region (D), while the lowest value was from the Maua region (G). This could be attributed to the fact that khat is grown in different geographical locations with different soil types, different soil pH values, and the application of different agrochemicals. Comparatively, studies carried out on Ethiopian khat indicated that the levels of Fe were lower compared to those of the present study [13]. For instance, this result was lower than the iron content in khat drawn from some regions in Ethiopia which included the Amhara, Oromia, and Southern Nations, Nationalities and Peoples [12]. Nevertheless, the levels of Fe in this work are consistent with those reported from different areas in Ethiopia and Yemen [26].

High amount of organic matter in the soil is known to contribute to high levels of Fe, especially in the khat plant [26, 27]. More importantly, laying harvested maize stalks under khat trees not only prevents water loss from the ground but also decomposes over time and hence is reincarnated into soil composition which then enhances the levels of Fe in soil. It is estimated on average, that a khat user chews 50–200 g of young khat leaves and shoots per day on wet weight basis [12]. The calculations presented in Table 5 are based on this estimation, which are then compared with allowable limits in order to determine any associated risks. The results in Table 5 indicate that the THQ of iron calculated is less than 1.0; hence, there is no likelihood of causing health effects.

Therefore, in all the khat samples analyzed, the concentration of Fe was below the permissible limit (425.0 mg/kg) set by the WHO/FAO [21]. This shows that khat obtained from the regions of Meru could be a good supplement of Fe. Thus, its detection in khat is not only vital to khat users, but managing its levels below permissible limits helps in a range of metabolic processes such as oxygen and electron transport and synthesis of deoxyribonucleic acid (DNA) [28]. Besides, in the biological processes of the human body, Fe provides normal functioning of body cells, other vital organs, and generation of free body radicals which can be useful [29]. Copper is also an essential trace element responsible for healthy plants and animals. This study reports that the concentration of Cu in khat samples ranged from 12.08 ± 0.64 to 21.18 ± 0.61 mg/kg with a mean of 15.81 ± 2.84 mg/kg (Table 4). The lowest and highest concentrations of Cu were found in samples that were obtained from the Kangeta region (sample B) and Kianjai region (sample K), respectively (Table 4).

TABLE 3: Calibration curves, LODs, and LOQs of the developed ICP-AES method.

Element	Emission line (nm)	Equation	Slope	r^2	LOD (mg/kg)	LOQ (mg/kg)
Cd	226.502	$Y = 1.7402x - 371$	1.7402	0.9855	0.979	2.966
Ni	232.003	$Y = 1.1564x + 24$	1.1564	0.9994	0.195	0.296
Pb	217.000	$Y = 1.6231x - 199$	1.6231	0.9967	0.072	0.220
Cr	357.869	$Y = 1.3688x - 67$	1.3688	0.9953	0.552	1.673
Fe	238.204	$Y = 1.3819x - 74$	1.3819	0.9930	1.193	3.616
Cu	324.752	$Y = 1.4488x - 32$	1.4488	0.9958	0.924	2.801

TABLE 4: Concentration of heavy metal content in khat samples collected from Meru County.

Region in Meru County	Farm code	Metal concentration (mg/kg)					
		Cr	Cd	Cu	Pb	Fe	Ni
Kangeta	A	16.40 ± 0.23	5.51 ± 0.11	17.00 ± 0.42	24.14 ± 1.26	92.90 ± 3.12	0.378 ± 0.03
	B	19.10 ± 0.12	5.58 ± 0.10	21.18 ± 0.61	31.11 ± 1.52	139.0 ± 3.64	0.386 ± 0.02
Laare	C	13.90 ± 0.14	9.72 ± 0.08	16.79 ± 0.32	36.29 ± 1.02	83.30 ± 2.11	0.361 ± 0.03
	D	17.60 ± 0.25	8.88 ± 0.13	14.67 ± 0.35	42.55 ± 2.45	180.5 ± 3.02	0.392 ± 0.02
Mutuati	E	14.60 ± 0.18	5.48 ± 0.05	13.03 ± 0.26	15.53 ± 0.33	86.80 ± 2.50	0.373 ± 0.01
	F	19.00 ± 0.31	8.53 ± 0.06	13.70 ± 0.18	25.92 ± 1.92	83.40 ± 2.10	0.367 ± 0.02
Maua	G	12.30 ± 0.15	9.23 ± 0.05	17.23 ± 0.43	48.10 ± 2.82	72.10 ± 2.13	0.352 ± 0.02
	H	18.00 ± 0.27	8.74 ± 0.12	14.06 ± 0.32	$<0.001 \pm 0.00$	78.50 ± 2.13	0.399 ± 0.04
	I	15.60 ± 0.16	8.32 ± 0.23	14.43 ± 0.36	$<0.001 \pm 0.00$	88.50 ± 2.09	0.371 ± 0.03
Kianjai	J	15.00 ± 0.13	7.67 ± 0.04	19.70 ± 0.52	30.10 ± 2.21	80.40 ± 1.89	0.349 ± 0.02
	K	14.30 ± 0.11	8.28 ± 0.05	12.08 ± 0.64	37.47 ± 2.02	85.50 ± 2.05	0.365 ± 0.03
Mean \pm SD		15.98 ± 2.22	7.81 ± 1.56	15.81 ± 2.84	32.35 ± 9.95	97.35 ± 32.67	0.372 ± 0.016
RSD (%)		13.9	20.0	18.0	30.8	33.6	4.2

SD, standard deviation; RSD, relative standard deviation.

TABLE 5: The THQ and HI values of investigated heavy metals with respect to khat consumption.

Noncarcinogenic parameter	THQ						HI
Heavy metal	Cr	Cu	Ni	Fe	Cd	Pb	
Values	0.015	0.056	0.027	0.20	11.6	13.2	24.90

High levels of Cu could be attributed to the application of Cu-based pesticides meant to control and manage pests, although the khat plant could also absorb some copper from the soil [22]. The levels of Cu from this study are higher than those reported previously in some khat-growing regions from Ethiopia, whose levels were 5.11–9.55 mg/kg [12], but also lower than those obtained from khat-growing regions such as Aweday in Ethiopia with concentrations of 0.10–41.80 mg/kg [22]. Remarkably, the concentrations of Cu in this study are below the permissible limits for vegetables (73.0 mg/kg) set by WHO/FAO [21]. The THQ calculated and estimated in Table 5 shows that khat is not contaminated with Cu and does not pose adverse health challenges during khat consumption from the Meru region. It is notable that below acceptable limits this metal is a very important component of several enzymes such as catalase and metabolic reactions. It is also essential for neurological and hematological systems [30], as well as for some biological processes in living organisms [31].

Lead is a commonly known toxic heavy metal capable of causing environmental contamination and serious etiological risks [32]. The concentration of Pb in khat samples observed in this work ranged from 15.53 ± 0.33 mg/kg to

48.10 ± 2.82 mg/kg with a mean of 32.35 ± 9.95 mg/kg (Table 4). Lead in khat samples collected from sites H and I from farms in the Maua region was noted to be ≤ 0.001 mg/kg. This could be attributed to the low level of lead in the soil and the possible nonapplication of lead-based agrochemicals [33]. Contaminated rivers possibly with detergents and other effluents used for irrigation of khat plants may also be a contributing factor in observed levels of toxic heavy metals in general. A case in point is the high concentration of lead in sample G from the Maua region. Application of synthetic pesticides and fertilizers and use of compost manure also contribute to high levels of toxic heavy metals. Moreover, some studies have shown that as the pH decreases, the solubility of heavy metals increases [34], and this increases their absorption by plants. Motor vehicle exhausts containing trimethyl and tetramethyl lead may also contribute to Pb content in khat grown along busy highways [35]. Also, the concentration of Pb can potentially be increased in soils through the disposal of used batteries and waste paints into khat farms [36]. Nonetheless, the concentration of Pb metal in this study is lower than that obtained from some khat-growing regions in Ethiopia such as Aweday, Wendo, Haramaya, Indibir, and

Bole which ranged from 5.0 mg/kg to 119.0 mg/kg [22]. The THQ values calculated (Table 5) exceed 1.0, suggesting the likelihood of hazardous consequences of khat consumption. All the samples had a high concentration of lead as compared to the WHO/FAO permissible limit for vegetables (0.3 mg/kg) [22]. This shows that khat samples considered in this work were contaminated by lead metal. Therefore, there is a need to recommend safe khat and safe farming practices that would reduce the concentration levels of lead to be below WHO/FAO set threshold limits.

Cadmium, on the other hand, is a highly toxic element and a well-known potent human carcinogen [37]. Previous studies have reported that high Cd exposure may have significant consequences on skeletal damage, hypertension, and kidney dysfunction [38]. Results of this study show that the levels of Cd in khat samples ranged from 5.48 ± 0.05 to 9.72 ± 0.08 mg/kg with a mean of 7.81 ± 1.56 mg/kg. The highest concentration of Cd was recorded in khat samples collected from the Laare region (site C) and samples from site G collected from the Maua region, whereas the lowest concentration was observed in samples collected from sites A and B both from Kangeta region and those collected from site E from Mutuati region which recorded concentrations of 5.51 ± 0.11 , 5.58 ± 0.10 , and 5.48 ± 0.05 mg/kg, respectively. The presence of Cd in khat and their associated levels could be due to the regular use of phosphate fertilizers and manure especially animal manure from cows, chicken, and pigs that may contain traces of Cd [22]. Also, Cd could be traced to the disposal of used pigments and paints and some electronics discharged into the khat farms through surface run-offs, and which are later absorbed by the plant tissues [36]. Groundwater tapped through wells and boreholes mainly used to irrigate crops are potential candidates which contain Cd from soil sediments [39]. In this work, it is notable that mixed farming is common in Meru County, especially the intercropping of khat with maize and other food crops that necessitate the use of phosphate fertilizers during planting. Through this method of farming, khat may absorb fertilizers contaminated with Cd metal. The levels of Cd reported in this study, according to Table 4, were higher than 1.30–2.90 mg/kg reported in khat samples analyzed in Addis Ababa [40]. The possible reason for this could be due to the cultivation of different khat species in sample collection areas and existing soil types, in addition to agrochemicals applied during khat planting in the Meru region. This could also be due to the application of domestic animal manure containing traces of cadmium as a result of mixed farming. The cadmium levels in this study were lower than those reported from a study that involved vegetables collected from local farms in Kericho West Sub-County which ranged from 10.33 to 29.00 mg/kg [10]. This may be attributed to different agricultural practices, different soil types, and different agrochemicals applied during vegetable farming which differs markedly from khat husbandry. The WHO/FAO through its reports, recommended a standard limit of Cd of 0.2 mg/kg for vegetables [21]. Accordingly, the mean concentration of Cd metal shows exceeding values as well as THQ exceeded 1. This showed that analyzed khat in this study may be moderately hazardous to the consumers.

Nickel is a trace metal released to the environment through natural sources and anthropogenic activities such as steel and cement manufacture [41] and may therefore be present in all soil types and soil profiles [42]. In agricultural soils, it is needed in trace levels for normal plant growth and development otherwise in elevated levels it may be highly toxic to plants, animals, and humans [41, 43]. Nickel metal has shown evidence of carcinogenicity in humans and other mammals, and has been known to cause toxicity to the nervous system, lungs, liver, and reproductive tissues which may result in reduced fertility [44]. The nickel level in khat samples in the present study ranged from 0.349 ± 0.02 to 0.399 ± 0.04 mg/kg with a mean of 0.37 ± 0.02 mg/kg. The maximum concentration of Ni was recorded in sample D obtained from the Laare region and sample H from the Maua region, while the minimum concentration was recorded in sample J from the Kianjai region. This may be ascribed to its levels in different soil types and the farm chemicals applied during khat cultivation. The results of this study present low levels of Ni compared to those recorded in vegetables obtained from Dera Ghazi Khan District in Pakistan which ranged between 1.800 mg/kg and 5.050 mg/kg [21]. Evidently, this can be attributed to different abilities of crops to accumulate and sequester Ni from the environment. Table 4 shows that in all the khat samples, the Ni concentration is below permissible limit (10 mg/kg) set by the WHO [23]. Associated THQ affirms Ni intake through consumption of khat from all the selected regions has no adverse health risk. Especially when its levels are below WHO permissible limits, it serves as an essential element in proper growth and development of the plants such as the germination of seeds as well as in animal species and microorganisms [45]. In addition, it is an essential element for humans because it enhances hormonal activities, and use in lipid metabolic activities [46].

Another essential element in khat is Cr which is widely known to enhance biological functions such as blood sugar regulation, regulation of cholesterol levels, and fat synthesis in the liver [22]. Chromium levels in khat samples from this work ranged between 12.30 ± 0.15 and 19.10 ± 0.12 mg/kg with a mean of 15.98 ± 2.22 mg/kg (Table 4). High Cr levels in khat may be attributed to its high concentration levels in the soil and its chemical form in the soil, in addition to agrochemicals applied during khat cultivation [22]. In addition, soil redox potential and pH are important parameters that determine the characteristics of Cr in soil, where it has been reported that low soil pH boosts Cr levels in khat [47]. The Cr levels obtained in this work are higher than those obtained from khat samples in Addis Ababa which ranged from 3.10 to 6.76 mg/kg [40] and are fairly compared with khat cultivated in eastern Ethiopia, whose concentration ranged from 9.04 to 14.54 mg/kg [48]. This is due to different growing conditions and different agrochemicals applied during farming [40]. The standard limit for Cr set by the WHO is 2.30 mg/kg in vegetables [22]. The mean concentrations in this work exceeded the permissible limits and the THQ values, indicating that consumption of the Meru khat may cause severe etiological risks mainly through bioaccumulation. However, when maintained below acceptable limits, it is an essential element for biological functions in humans.

Most khat farmers in the Igembe region practice mixed farming such as planting maize, pumpkin, carrot, beans, potatoes, cassava, bananas, cowpeas, millet, and sugarcane in khat farms [3]. This study indicates that the application of fertilizers containing heavy metals to food crops indirectly become available for khat plants to sequester them into their consumable parts thus increasing its concentration levels.

3.2. Noncarcinogenic Health Risk Assessment of Khat Consumption. The THQ is the measure of possibility used to indicate the likelihood of developing non-carcinogenic health risks when exposed to heavy metal intake. Basically, the standard value of THQ for not developing adverse health problems is ≤ 1.0 ; otherwise, if exceeded, it may pose human non-carcinogenic risks [49–51]. This method in the present study considers the likelihood of exposure to heavy metals via consumption of khat but does not necessarily provide a reliable quantitative estimate on exposed population. The THQ values for Pb, Cd, Cu, Cr, Fe, and Ni due to khat consumption habits in the study area were determined and reported in Table 5. Clearly, THQ for lead and cadmium far much exceeded the threshold of 1.0 indicating their high hazardous potential. The THQ for all other heavy metals (cf. Table 5) in khat was found to be safe because they fall below 1.0. Generally, the THQ of heavy metals resulting from khat shoots and leaves intake was found to follow a decreasing order of $Pb > Cd > Fe > Cu > Ni > Cr$. This suggests that the potential health risks of Pb and Cd through khat consumption are significantly high.

The collective health risks of exposure to multiple metals were determined by taking the summation of the THQ of all heavy metals in khat in the calculation of HI which was used to predict the likelihood of adverse effects. In this study, the HI value recorded for all the analyzed heavy metals in the khat samples was 0.54 as indicated in Table 5. The present findings show that the major contributors to the total HI were Pb and Cd heavy metals. From Table 5, Pb and Cd contributed about 53.01% and 46.59%, respectively, while Fe, Cr, Cu, and Ni collectively contributed 0.4% to the HI.

3.3. Pearson Correlation Analysis. For Karl Pearson correlation analysis, correlation coefficient values and the data obtained are summarized in Table 6. The positive correlation coefficient values indicated a positive correlation among the concentrations of heavy metals, whereas the negative values showed a negative correlation. Correlation coefficient values close to zero (0) indicated non-significant correlation. Correlation coefficient values near 1 indicated a strong and significant correlation among the concentrations of two heavy metals. Fe exhibited the highest number of positive correlations possibly indicating multiple sources of contamination and origins. Iron, the most abundant heavy metal in all the khat samples showed positive correlations with Pb, Cr, Cu, and Ni. This could suggest that its source is uniquely different from those of other heavy metals. Heavy metals Cu, Cr, and Pb had the same number of positive correlations signaling possibly a shared source.

TABLE 6: Pearson correlation matrix obtained for five heavy metals.

	Ni	Cu	Fe	Cd	Pb	Cr
Ni	1					
Cu	−0.181	1				
Fe	0.565	0.168	1			
Cd	−0.221	−0.264	−0.131	1		
Pb	−0.411	0.293	0.294	0.216	1	
Cr	0.707	0.080	0.495	−0.258	−0.320	1

3.4. Principal Component Analysis. The PCA by varimax rotation method was applied to the dataset of six heavy metals to speculate the sources of these metals in the Meru khat. The data extracted from PCA is presented in Table 7 and consists of a component matrix, initial eigenvalues, a rotated component matrix, and rotation sums of square loadings. The first three principal components (PCs) with eigenvalues greater than 1 accounted for 83.81% of the total variability. Consequently, these PCs demonstrate that heavy metals in the Meru khat originate from three independent sources. The first PC (PC1) explicated 39.55% of the total variance with significantly high loadings of Ni (0.91), Cr (0.88), and Fe (0.65). The regular applications of fertilizers and pesticides might be responsible for Cd and Pb in the study area, signifying that these heavy metals exist as a result of human activities. The contribution of the second PC (PC2) was 25.44% of the total variance with high loadings of Cu (0.75%), Pb (0.78), and Fe (0.57). The result further shows that PC2 was negatively loaded with Ni and Cd. However, the data, especially as reported in Figure 2 further demonstrated that Cu may have a distinct source of origin in the study area. The third PC (PC3) explained 18.83% of the total variance with significant positive high loading of Cd (0.75). The PC3 had negative loading with Cu. The contamination by Fe could be a result of multiple contamination sources as predicted by significant loadings in PC1, PC2, and PC3. From the statistical analysis, significant correlations were found between heavy metals within the same principal components, demonstrating that the results of PCA and Pearson correlation analysis (cf. Table 6) were all important.

4. Pathophysiological Concerns of Toxic Heavy Metals

The means of human exposure to toxic heavy metals include skin contact, inhalation, ingestion, and direct contact [52]. It is increasingly noticeable that the nonbiodegradability and refractory nature of toxic heavy metals in the environment make them have long-lasting biotoxic consequences in biological systems; hence, there is a need to understand the mechanisms that make them detrimental to public health [36]. Moreover, heavy metals acutely reverse the normal functioning of biological structures and negatively affect biological system functionality which ultimately results in untimely death and other health complications [42]. For instance, heavy metals cause serious problems to the central nervous system such as mental breakdown, kidney damage, and lung activating pathways for disease manifestations [39]. Chromium toxicity in the exposed human population and

TABLE 7: The principal component analysis of heavy metals in Meru khat samples.

Metals	Principal components matrix			Metals	Rotated components matrix		
	PC1	PC2	PC3		PC1	PC2	PC3
Ni	0.91	-0.16	0.21	Ni	0.90	-0.30	-0.46
Cr	0.88	0.03	0.03	Cr	0.83	-0.20	0.19
Fe	0.65	0.57	0.41	Fe	0.82	0.48	0.07
Pb	-0.41	0.78	0.35	Pb	-0.16	0.93	-0.02
Cu	0.01	0.75	-0.49	Cd	-0.19	0.30	-0.81
Cd	-0.44	-0.14	0.75	Cu	-0.47	0.45	0.77

Component	Initial eigenvalues		
	Total	Variance (%)	Cumulative (%)
1	2.37	39.55	39.55
2	1.53	25.44	64.98
3	1.13	18.83	83.81
4	0.56	9.40	93.21
5	0.27	4.51	97.72
6	0.14	2.28	100.00

Component	Extraction sums of squared loadings			Component	Rotation sums of squared loadings		
	Total	Variance (%)	Cumulative (%)		Total	Variance (%)	Cumulative (%)
1	2.37	39.55	39.55	1	2.23	37.08	37.08
2	1.53	25.44	64.98	2	1.52	25.36	62.44
3	1.13	18.83	83.81	3	1.28	21.37	83.81

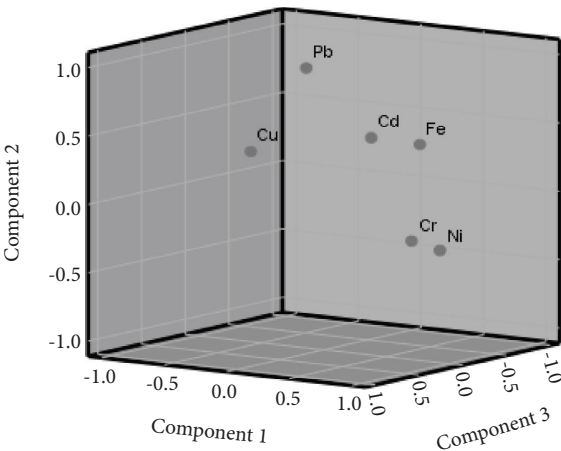


FIGURE 2: The component plot in rotated space for heavy metals in the Meru khat.

experimental animals also causes respiratory cancers [36]. Acute Ni toxicity causes symptoms such as vomiting, irritation, body weaknesses, constant cough, nausea, and visual disturbances in humans, while in animals such as rats, it causes renal damage, body weight loss, and instances of kidney failure [42]. On the other hand, chronic Ni poisoning affects the skin and pulmonary as well as cause cancer and dramatis [36], precipitates endocrine disruption in humans, and is a precursor for cardiovascular diseases as well as genotoxicity [42]. A study conducted in North California among children aged 4 to 5 years revealed that they are at a high risk of pediatric obesity due to high levels of Cd in prenatal blood samples [53]. Epidemiological studies showed that accumulation of Cd in body tissues causes development of musculoskeletal diseases such as osteoarthritis, osteoporosis, and kidney impairment [54].

Long-term Pb exposure decreases cognitive performance, causes anemia, hypertension, and causes miscarriages in pregnant women, damages mental faculties, and causes kidney damage [52]. It is recommended that metal chelation therapy, as applied in the treatment of chronic iron toxicity, be employed to minimize heavy metal poisoning arising from khat consumption and other food products [55].

5. Limitations of the Study

Only 11 khat samples were considered in this investigation for the evaluation of the potential health hazards resulting from khat consumption among the population. Considering the expansive nature of Meru County, the data collected may not be representative of the heavy metal toxicity in khat in the entire Meru region. Moreover, the non-carcinogenic adverse risks due to Pb and Cd cannot be adequately determined based on the small area of Meru County sampled. Basically, the estimation of THQ and HI values was based on the daily ingestion of khat; thus, there is a probability that the values obtained are overestimated. Heavy metal toxic lead was not conducted in this study but was necessary in order to reflect the adverse health effects on khat consumers. Furthermore, the fact that the data were collected during the rainy season cannot entirely be used to give a true picture of heavy metal contamination in the area of study especially during the dry season. Seasonal and temporal variations of heavy metal distribution in the khat-growing region of Meru were not carried out.

6. Conclusions

This study has found that edible parts of khat leaves and shoots collected from the Meru region were contaminated with high levels of lead and cadmium, which is considered

a serious public health concern among khat consumers. THQ for lead and cadmium far much exceeded the threshold of 1.0, indicating their high hazardous potential. The THQ for all other heavy metals in khat was found to be within the safe limit of below 1.0. Generally, the THQ for heavy metals resulting from khat shoots and leaves intake was found to follow a decreasing order of $Pb > Cd > Fe > Cu > Ni > Cr$. Nonetheless, the HI of all investigated heavy metals in khat exceeded the threshold value of 1.0, suggesting possible noncarcinogenic health risks to khat consumers. Statistical treatment of the data reported in this work showed that there were significant variations in the levels of Ni metal in the khat samples from different regions in Meru, although there were no significant variations among toxic selected heavy metals, Fe, Cu, Cd, and Pb. This observation could be attributed to different factors such as methods of farming, chemical composition of the soil, and application of various agricultural practices employed by different khat farmers. Also, principal components (PCs) with eigenvalues greater than 1 accounted for 83.81% of the total variability, therefore, demonstrating that heavy metals in the Meru khat originate from three independent sources. The first PC (PC1) explicated 39.55% of the total variance with significant high loadings of Ni (0.91), Cr (0.88), and Fe (0.65). Consequently, the regular application of agrochemicals including fertilizers and pesticides might be responsible for the observed high levels of Cd and Pb in the study area. On the other hand, Fe showed the highest number of positive correlations, possibly indicating multiple sources of contamination and origins. Iron exhibited positive correlations with Pb, Cr, and Cu but a moderate correlation with Ni and Cd. The main contribution of this study is to provide reliable information for the adoption of better farming practices where the use of agrochemicals should be minimized or avoided completely. Furthermore, Meru khat farmers should be sensitized on appropriate farming practices for safer khat products.

Data Availability

The data associated with the findings of this study are available from the corresponding author upon reasonable request.

Conflicts of Interest

The authors declare that they have no conflicts of interest.

Authors' Contributions

AMO analyzed, wrote, and edited the article. JKK contributed to the method development, edited the article, and provided supervision. JOA edited the article and performed supervision. All authors have read and approved the manuscript.

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