

Determination of Some Selected Heavy Metals in Chewable Khat Leaf from Selected District of Gurage, Southern Nation and Nationalities of Peoples Regional State, Ethiopia

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Citation: Worku A, Belete T, Hailemariam T, Hailu T (2024) The Impacts of English Language Proficiency on Employment Opportunities. American J Sci Edu Re: AJSER-184.

Received Date: April 10, 2024; Accepted Date: April 27, 2024; Published Date: May 03, 2024

Abstract

Fresh chewable part of khat leaf samples were collected from three farms namely Cheha, Ezha, and Enamor ena ener districts of Gurage Zone, Ethiopia and transported to laboratory using PE plastics. It was air dried and grinded to powder size. Then the powdered khat samples were digested by the optimized microwave digestion method using HNO_3 and H_2O_2 . The concentration of five heavy metals (Mn, Zn, Pb, Cd, and Cr) in the digested and diluted solutions of khat was analyzed with FAAS and GFAAS. Mn and Zn were the most abundant heavy metals in khat among the tested heavy metals. Moreover, the concentrations of toxic metals Cu, Pb and Cd in the studied Khat plant leaves were too low to be detected. The order of metal mean concentration in mg/kg is $\text{Mn} > \text{Zn} > \text{Cu} = \text{Cr} = \text{Cd}$. Thus, the concentration of Mn (21.55 to 23.78 mg/kg), Zn (19.93 to 323.26 mg/kg), Cd, Cr and Pb were not detected. Finally, the overall results of this study imply that, there were no significant variations in the level of Mn between the Khat samples, but there was significance difference between the khat samples of the three districts for Zn metal. The variation may be attributed due to different factors such as age of the harvested Khat, geographical and climatic variation, difference in physicochemical nature of the soil, and different agricultural practices among Khat cultivars.

Keywords: Chewable part of khat leaf, FAAS, GFAAS, heavy metals, microwave digestion.

1. Introduction

Studies on chemical composition of plant materials are growing because of ongoing developments in agriculture, nutrition, environmental studies, biogeochemical surveying, and mineral prospects. It is globally accepted that some heavy metals such as Fe, Zn, Cu, and Mn; hereinafter referred to as microelements; are essential for healthy growth and development within certain permissible limits (Anonymous *et al*, 1999, Hagos *et al*, 2010, Deribachew *et al*, 2015). Researchers have discussed the potential health implications of trace metals in various plants and fruits such as Cd, Pb, Ni, and Al, which are known as toxic heavy metals and may pose a serious threat to plant, animal, human, and environmental health. This is why because, they are not degradable by bioprocess and remain in environment and passes to food chain (Ayar and Akin, Qin *et al*, 2009, Khan *et al*, 2013, Tassew *et al*, 2014, Ali and Masood, 2015).

It is apparent that heavy metal contamination of the food chain is becoming a serious issue, globally due to various factors related to human activities like rapid industrial growth, the widespread use of chemicals in agriculture, and increasing urbanization. The toxicity of heavy metals are attributed to the role of metals play in biological processes as well as the way they interact with essential elements both at intestinal and organ level once absorbed in the body (WHO, 1993). They are able to remove electrons from the amino acids or bases of DNA that cause a reaction, which disrupts the ability of the cell to carry out their biological functions. These modified biological molecules lose their ability to function properly and result in

malfunction or death of the affected cells (Jamnicka *et al.*, 2007). Nowak and Chmielnicka, (2000) observed that in some cases toxic metals displace chemically related metal ions that are required for important biological functions. The alteration of these structures leads to toxic consequences that have an array of disorders.

Further, heavy metals are non-biodegradable, thermo stable and persistent (Sharma *et al.*, 2007) and undergo bioaccumulation and bio magnification up the food chain when ingested. The cumulative effects occur after along exposure to low levels of heavy metals. Exposure of consumers to health risks are usually expressed as provisional maximum tolerable daily intake (PMTDI) or provisional maximum tolerable weekly intake (PMTWI) reference values established by the FAO/WHO (FAO/WHO, 1999). The symptoms are periodically progressive resulting into gradual and severe problems (Sailus, 2014).

Zinc chloride fumes have caused injury to mucous membranes and pale gray cyanosis. Ingestion of soluble salts may cause nausea, vomiting and purging. Breathing large amounts of zinc (as dust or fumes) can cause a specific short-term disease called metal fume fever. This is believed to be an immune response affecting the lungs and body temperature (ATSDR, 2005). Long term exposure to copper dust can irritate your nose, mouth and eyes, and cause headaches, dizziness, nausea and diarrhea. Higher level of copper in water may experience vomiting, diarrhea, stomach cramps and nausea (Molla Tefera, 2009). Lead; is toxic to the blood and the nervous, urinary, gastric and

genital systems and has a number of toxic effects including inhibition of the central and peripheral nervous system and the kidneys (Tassew et al, 2014). Cadmium accumulated within the kidney and liver over long time. Cadmium adversely affects several important enzymes; it can also cause painful osteomalacia (bone disease) and kidney damage. Acute effects of cadmium occur by breathing high levels dust or fumes, may cause throat dryness, cough, headache, vomiting, chest pain, extreme restlessness and irritability, pneumonitis, possibly bronchopneumonia, and can cause death due to severe lung damage (Mukesh *et al*, 2008, Enb *at al*, 2009 & Dawd, Gashu, 2010). Based on the effect of heavy metals on consumers, different organizations have proposed maximum permissible limits of the metals in edible vegetation, wastewater, and soils. However, concerning heavy metal accumulation in khat leaves, there is rareness of reported literatures (Hussein et al, 2010).

Khat (*Catha edulis* Forsk) is an evergreen perennial shrub plant that belongs to the *Celastraceae* family. It is widely cultivated in East Africa and Arabian Peninsula more specifically in Yemen. A young leaves and shoots of a flowering evergreen tree or shrub that grows at high altitudes in the region extending from eastern to southern Africa, as well as in the Arabian Peninsula (Tilahun, 2009, Lamina, 2010; Sikiru, 2012, Hailu et al, 2016). The most favored part of the plant is leaves, particularly the young shoots near the top of the plant. However, leaves and stems at the middle and lower sections are also used. Khat is chewed by millions of people on a daily basis for its stimulating property due to the presence of the phenylpropylamino alkaloids: cathinone, cathine, and norephedrine (Hailu et al, 2016). In Ethiopia, depending upon geographical location, various chemotypes of khat are cultivated and these chemo types differ in color, size, height and size of the young shoots of the leaves and the plant as a whole and marketed under different names such as Gelemso, Gurage, Beleche, Wendo, Sebeta, Bahir Dar, Kuto, Berdaye, Anferara, Awedi, Abo Mismar, Colombia, Debo, Gurbicho, Magna (Atlabachew et al., 2011).

A wet mineralization procedure using high pressure (and high temperature), based on microwave decomposition with acid in hermetic Teflon containers, has recently been proposed. Sample heating is carried out in special microwave ovens that currently exist in the market. In 1975, microwaves were first used as rapid-heating sources for wet, open-vessel digestions. They were used to heat acids rapidly, in Erlenmeyer flasks, to digest biological matrices, and the conventional sample digestion times were reduced from 1 – 2 hours to 5 – 15 min. In the beginning, microwave sample preparation made use of common laboratory glassware and open Teflon vessels to digest matrices at the boiling point of the acids in commercial microwave ovens (Ojeda et al, 2005). Open-vessel and closed vessel microwave digestion are all well documented in the literature. Although open vessel microwave digestion is popular because of its simplicity, such methods suffer from the same drawbacks as conventional methods: loss of volatile elements, risk of sample contamination, release of acid gases, and maximum digestion temperatures limited by the boiling point of the acid mixture. In contrast, samples digested in closed-vessel environments are protected from the atmosphere and undergo accelerated decomposition reactions because of increased pressure and temperature. In addition, the elevated temperature in a closed-vessel procedure increases the oxidizing power of mineral acid and achieves decomposition of matrices components that would not be possible in an open vessel or other conventional

environments. Microwaves only heat the liquid phase, while vapors do not absorb microwave energy. The temperature of the vapor phase is therefore lower than the temperature of the liquid phase and vapor condensation on cool vessel walls takes place. As a result, the actual vapor pressure is lower than the predicted vapor pressure (Li et al, 2005).

2. Materials and Methods

2.1 Description of Study Area

Gurage zone was the sample areas where large population of khat is cultivated. The study area is around 170 Km far from the capital city of Ethiopia, Addis Ababa to southwest direction. Geographically, Gurage Zone is located between 7.8^o – 8.5^o North latitude and 37.5^oC – 38.7^o East longitude of the equator. Gurage zone has a total area 5932 km². It has 13 woredas with a total population estimated about 1343246 (CSA, 2011). The zone comprises altitudes ranging from 1,001 to 3,500 meters above sea level (m.a.s.l). It is classified into three agro-climatic zones: Dega (high altitude) covers 28.3% of the area and ranges between 2,500-3662 m.a.s.l, Woinadega (mid-altitude) at 1,500-2,500 m.a.s.l, encompasses about 64.9% of the area, and Kolla (lowland) at 1,000-1,500 m.a.s.l covers 6.8% of the area. The mean annual temperature of the zone ranges between 13 – 30^oc and the mean annual rainfall ranges 600- 1600 mm. The rainfall pattern in the Gurage Zone is bimodal in which 80% of rain falls in the Kreamt period of June to August whereas 20 % in the Belg period of February to May. According to the land utilization data of the region 298,369 ha cultivated land, 67,678 ha forest, bushes and shrub covered land, 70,249.31ha grazing land, and 14,234 ha of land is covered by others (GZADD, 2011).

2.2 Materials and Apparatus

Ceramic pestle and mortar were used to ground and homogenized the khat sample. A drying oven was used to dry chat samples. A digital analytical balance with 0.0001g precision was used to weigh chat samples. A microwave digestion had applied based on the method described by the Speed Wave 4 microwave digestion system manufacturer to digest the dried and powdered khat samples. Flame Atomic absorption spectrophotometer and Graphite Furnace Atomic Absorption Spectrometer were used for the determination of the metals in the samples.

2.3 Instruments Used

Apparatus such as volumetric flasks, measuring cylinder and Teflon digestion vessels were washed with detergents and tap water, rinse with deionized water soaked in 8% nitric acid for 24 hrs, rinsed with deionized water five times, dried in oven and keep in dust free place until analysis begins. Instruments such as microwave digestion, Flame Atomic Absorption Spectrometer, FAAS (model 240FS AA manufactured by Agilent technology and Graphite Furnace Atomic Absorption Spectrometer, GFAAS (Model 240Z AA manufactured by Agilent technology) were used to detect the metals in the samples.

2.4 Chemical and Reagents used

All reagents that were used in the analysis are analytical grade. Buffered acetonitrile (1% HAc) anhydrous magnesium sulfate (MgSO₄), primary secondary amine (PSA) and analytical standards Chromatographic grade obtained from sigma Aldrich were used for extraction of pesticide residue. And (72%) HNO₃, (70 %) HClO₄ and (30%) H₂O₂ were used for the digestion of chat samples for mineral analysis. Stock standard solutions containing 1000 mg/L of the metals Mn, Cr, Cd, Zn and Pb were

used for the preparation of serious calibration standards and in the spiking experiments. Deionized water was used throughout the experiment for sample preparation, dilution, and rinsing apparatus prior to analysis.

2.5 Preparation of reagents

Working solutions are prepared by further dilution of all the reagents and solvents of analytical reagent grade. There are two stock standard solutions prepared for the determination of metal analysis from the samples. These are primary and secondary stock solutions. The stock solutions are prepared for each metal determination by the dilution of the metal piece and its salt in deionized water and nitric acid. When making mixed calibration standards there may be convenient to prepare secondary stock standard solutions containing 1000 mg/ml of the element of interest.

2.6 Cleaning of glassware and sample containers

All glassware were cleaned with detergent and hot water, rinsed several times with tap water, and then soaked for 12 hours in 10% analytical grade nitric acid solution. Finally, they were rinsed with distilled de-ionised water and dried in the oven at 105 °C. The plastic containers were cleaned with detergent and tap water, soaked in 1:1 nitric acid and water over night and rinsed thoroughly with distilled de-ionized water. They were then dried in an open rack and stored safely in a locked dust free storage area.

2.7 Sample Collection

The samples were gathered from Gurage zone of different sites, three different dominant Woreda such as, Endbir (Cheha Woreda), Ambagenet (Ezha woreda), Aroda (Enemor na Ener woreda), because most of the khat sold in Gurage zone was distributed from these places. Chewable parts of khat were bought from the local farms of dominant Woreda in each zone. From each Woreda again three sites were selected. From each sites 10 samples were selected randomly and homogenized to represent the bulk sample. The collected sample was placed in plastic materials until sample preparation and analysis. Khat samples analyzed for mineral analysis. The total selected samples for analysis was 120 khat samples.

2.8 Preparation of Khat sample for metal analysis

A portion of the sample was digested using nitric acid (HNO₃) and hydrogen peroxide (H₂O₂) in a microwave digestion apparatus until complete digestion is achieved. The samples were then diluted in the appropriate manner and analyzed using the Flame and Graphite Furnace Atomic Absorption Spectrometer as appropriate. Viewing this, a study was done by taking 250 mg of each sample and digested with 4 mL of HNO₃ (65%) and 2 mL of H₂O₂ (35%). Conditions were set as

recommended by the manufacturer. All sample solutions were clear before determination of the elements of interest using GFAA Kingston (1986). Accordingly, in our study, a mixture of HNO₃ (70 %) and H₂O₂ (30 %) were used for digestion of khat samples. Powdered khat samples (0.5g) from each sites and each of the three dried powdered khat samples were directly transferred in to DPA-60K Teflon digestion vessels followed by the addition of HNO₃ (3 ml) and H₂O₂ (3 ml). The mixture was then shaken carefully until the solid material dissolved. The vessels were placed in a fume-hood for 15 min for pre-digestion and placed inside the turntable of the microwave system and heated in the microwave. After completion of the program, the power was put off and the digestion vessels kept in the fume hood. The digestion vessels were cooled to room temperature to avoid foaming and splashing. Then, the digestion vessels were opened carefully in a fume hood. The resulting clear and colorless solutions were cooled at room temperature and transferred quantitatively to 10 mL volumetric flasks and made up to the mark with de-ionized water. Each khat samples were digested in triplicate. The digested and diluted sample solutions were then be stored in tightly capped polyethylene bottles and kept in refrigerator for further analysis by flame atomic absorption spectroscopy and graphite furnace atomic absorption spectroscopy.

2.9 Digestion of the Blank Samples

Digestion of reagent blank was also performed in parallel with each of the khat samples keeping all digestion parameters the same. For the analysis of each khat samples, six reagents blank were prepared. All the digested samples were stored in refrigerator until the analysis.

2.10 Method Detection Limit (MDL)

Method detection limit is the minimum concentration of analyte that can be identified, measured and reported with 99 % confidence that the analyte concentration is greater than zero. In the present study, to know the method detection limit of each metal, six blank for each were digested and analyzed along with khat samples. Then the mean concentration of the blank and the standard deviation of the six blank samples were calculated for each metal. Finally, the detection limits were obtained by mean concentration of the blank plus three times of the standard deviation of the reagent blank.

$$MDL = \bar{X}b + 3Sb$$

Where, MDL is method detection limit, $\bar{X}b$ is mean concentration of the reagent blank and S_b is the standard deviation of the reagent blank. As shown in Table 3.2, the method detection limit of each element is above the instrument detection limit.

Table 2.1: Method Detection Limit for Khat Leaf (n=6).

Metals	Mn	Zn	Cr	Cd	Pd
IDL	0.03	0.01	0.06	0.02	0.04
MDL	0.031	0.08	0.09	0.03	0.05

Values are mean plus 3×standard deviation of six blank determinations each measured three times. IDL: instrument detection limit and MDL: Method Detection Limit

2.11 Instrument operating conditions and Calibration for metal analysis

Khat samples were analyzed for heavy metals using FAAS and GFAAS. In the use of FAAS and GFAAS for trace metal analysis optimization of the operating conditions is very critical. Wavelength, energy, lamp and burner alignment and slit width

were optimized for Cr, Cd, Mn, Pb and Zn analysis (table 3.1). Standards used in establishing the analytical curve for trace metal determinations were prepared from 1000 mg/L standard stock solutions (Agilent's calibration standards, UK). The working solutions (10 mg/L) of each metal were then freshly prepared by diluting the stock solution in 100 mL volumetric flask with deionized water. Acceptability of linearity data is often judged by examining the correlation coefficients and the y intercept of the linear regression line of the elements which were obtained from prepared working standard concentration versus their corresponding absorbance.

Flame Atomic Absorption Spectrophotometer (Model 240FS AA manufactured by Agilent Technologies) equipped with deuterium arc background corrector and standard air-acetylene burner system and Graphite Furnace Atomic Absorption Spectrophotometer (Model 240Z AA manufactured by Agilent technologies) were used. A hollow cathode lamp for each metal (Mn, Zn, Cd, Cr, and Pb) operated at the manufacturer's recommended conditions were used (Table 3.1). The acetylene and air flow rates were managed to ensure suitable flame conditions. The burner height was adjusted for optimum sensitivity and the nebulizer uptake rate was optimized (6-7 mL/min) to provide optimum absorbance signal in conventional sample aspiration.

Table-2.2: Instrumental operation conditions used for sample digestion.

Parameter	Steps				
	1	2	3	4	5
Temperature (°C)	120	135	160	50	50
Pressure (bar)	40	40	40	0	0
Power (%)	30	40	50	0	0
Ramp (min)	2	2	2	1	1
Time (m)	5	5	12	1	1

Table 2.3: Instrumental operating conditions for the determination of heavy metals by flame atomic absorption spectrometer.

Element	Wave length (nm)	Energy (KJ)	Slit width (nm)	Lamp current (mA)	Detection limit (mg/kg)	Oxidant/ Fuel
Mn	279.5	3.531	0.2	5.0	0.03	Air-C ₂ H ₂
Zn	213.9	3.369	1.0	5.0	0.01	Air-C ₂ H ₂
Cr	357.9	3.913	0.2	7.0	0.06	Air-C ₂ H ₂
Pb	217.0	2.525	1.0	5.0	0.04	Air-C ₂ H ₂
Cd	326.1	3.228	0.5	4.0	0.02	Air-C ₂ H ₂

2.12 Analysis of trace metals

The analytical technique applied in this research for the determination of metals in khat plant was the flame atomic absorption spectrometer and Graphite furnace Atomic Absorption Spectroscopy [FAAS and/or GFAAS]. Samples were collected from the stated areas on the spot following proper sampling procedure. Proper extraction (wet digestion procedure) was devised for quantitative extraction of metals from khat leaves. Then correlation analysis of each metal in plant was done. Furthermore, contribution of khat chewing for daily intake of metals was estimated. In this work, an attempt was made on trace metal analysis composition using FAAS and GFAAS thereby different chemometric techniques (multivariate based techniques to develop an authentication system and marker element in all the sample studied. As the first step in chemometric study, display techniques such as principal component analysis and cluster analysis were applied in order to demonstrate that FAAS and GFAAS contained useful information to develop a pattern recognition classification system to authenticate samples with different pattern recognition techniques.

2.13 Determination of the concentration of metals in the khat sample solution

Secondary standard solutions containing 10 mg/L were prepared from standard stock solutions that contained 1000 mg/L. These secondary standards were diluted with deionized water to obtain

four working standards for each metal of interest. Cd and Pb were analyzed by GFAAS (Model 240Z AA manufactured by Agilent technology). And Cr, Mn and, Zn were analyzed with FAAS (BUCK SCIENTIFIC MODEL 210GP) equipped with deuterium arc background corrector and standard air-acetylene flame system using external calibration curve after the parameters (burner and lamp alignment, slit width and wavelength adjustment) were optimized for maximum signal intensity of the instrument. Three replicate determinations were carried out on each sample. Hollow cathode lamp for each metal operated at the manufacturer is recommended conditions were used at its respective primary source line. The acetylene and airflow rates were managed to ensure suitable flame conditions. The four elements were determined by absorption/concentration mode. The same analytical procedure was employed for the determination of elements in six-digested blank solutions.

2.14 Method Validation

The ability to provide timely accurate and reliable data is central in analytical chemistry. In order to see the validation of the method (accuracy), spiked sample analysis was performed using standard solutions. Method validation is the process of proving that analytical method is acceptable for its intended purpose. Therefore, analysts increasingly encouraged to validate analytical procedures and to estimate uncertainty associated to the results. Since there is no certified reference material (khat) in our laboratory, the accuracy and validity of the measurement

were determined by analyzing spiked samples using standard solutions. Testing was done on one of sample type. Recovery values in range 16.0 ± 0.6 mg/kg to $26.30\text{mg} \pm 1.1\text{kg}$ are acceptable for environmental investigations and the digestion procedure for Mn and Zn [AOAC 999.11]. The main causes of losses of recovery could be attributed during the digestion process and could include systematic and random sources of uncertainty during analysis using FAAS and GFAAS.

2.15 Method of Data Analysis

The statistical analysis of the results was done using the statistical software like excel to analyze the generated data during experimentation. The statistical analysis was conducted using statistical package of microcal origin 6.1. One-way ANOVA was made to check whether there is significant difference or not between means at 95% confidence interval. Calibration graphs and bar graphs were drawn using Microsoft Office Excel 2007 and microcal origin 6.1. Methods such as D-

PLS Discriminant Partial Least Squares regression; SIMCA: Soft independent modeling of class analogy; KNN: K-nearest neighbors; and MLF-NN: Multilayer feed forward neural networks) to derive diverse models for the samples class with the objective of detecting possible falsification of these high-quality samples.

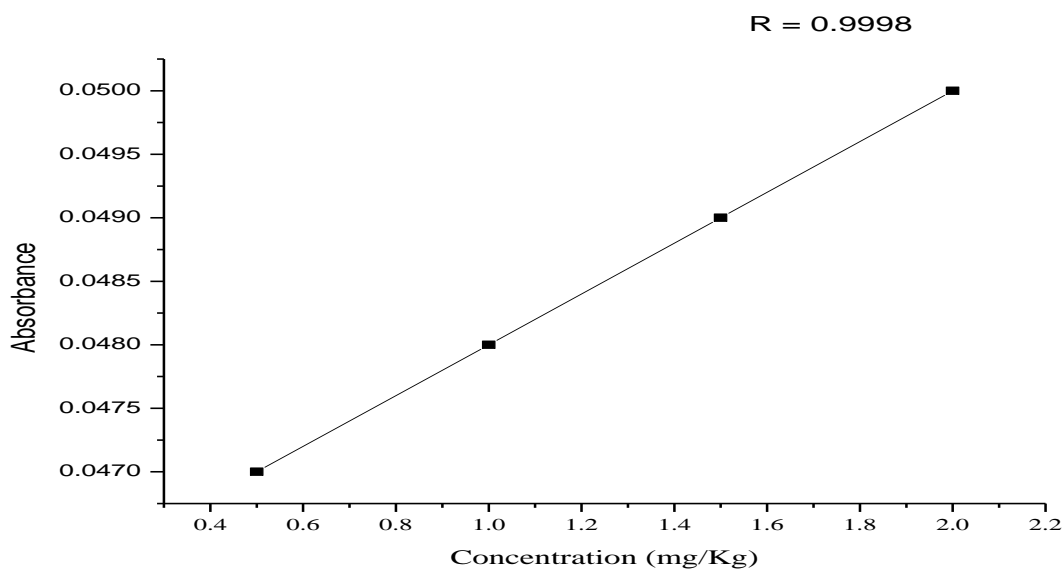
3. Result and Discussion

3.1 Calibration Curve of the Instrument

The qualities of results obtained for trace metals analysis using FAAS and GFAAS are seriously affected by the calibration and standard solution preparations procedures. The instrument was calibrated using four series of working standards. The working standard solutions of each metal were prepared freshly by diluting the intermediated standard solutions mentioned under the table below. Concentrations of the intermediate standards, working standards and value of correlation coefficient of the calibration graph for each of the metals are listed in Table 3.1.

Table 3. 1. Working Standards and Correlation Coefficients of the Calibration Curves for Determination of Metals in Khat using FAAS and GFAAS.

Element	Concentration of Intermediate standards solution in mg/L	Concentration of working standard solutions in mg/L	Correlation coefficient of calibration curves
Mn,	10	0.5, 1, 1.5, 2	0.9998
Cd	10	0.015, 0.04, 0.06, 0.09	0.9998
Pb	10	0.005, 0.05, 0.1, 0.2	0.9999
Zn,	10	0.05, 0.5, 1, 3	0.9998
Cr	10	0.05, 0.1, 0.5, 0.75	0.9996



3.2 Analytical Procedures for method validation

3.2.1 Method validation for metal analysis

The mean \pm SD recoveries for these metallic elements were obtained in the ranges of 97.20 ± 2.44 to 108.89 ± 2.83 which shows the procedure had good accuracy; hence the digestion method is valid. Percent recovery (% R) for each metal analyte was calculated using equation:

$$\% R = \frac{C_s - C}{S} \times 100$$

Where: %R = percent recovered,

Cs = fortified sample concentration (Conc. of spiked + sample (mg/kg)),

C = sample background concentration (Conc. of sample (mg/kg)) and

S = concentration of analyte added to fortify the sample (Amount added (mg/kg)).

Table 3. 2. Recovery Test for Khat Samples.

Metals	Conc. of sample (mg/kg) ^a	Amount added (mg/kg)	Conc. of spiked sample (mg/kg) ^b	% Recovery ^a
Mn	23.00	12	36.067 ± 1.416	108.89 ± 2.835
Zn	21.41	10	31.13 ± 0.05	97.20 ± 2.444

^a Average value of nine measurements (µg/g), ^b Values are mean ± SD of triplicate readings of triplicate analyses, ^c Values are mean ± SD of triplicate readings of triplication.

3.3 Determination of Heavy Metals concentration

The concentration of five metals (Mn, Zn, Pb, Cd, and Cr) in the digested and diluted solutions of khat were analyzed with FAAS and GFAAS. Mn and Zn were the most abundant heavy metals in khat among the rest tested heavy metals. The remaining metals were below the detection limit of the instrument.

The levels of Mn, Zn, Pb, Cd, and Cr khat samples obtained from farms in the three main khat growing woredas of Gurage zone were determined in triplicates using computerized Flame Atomic Absorption spectrophotometer and Graphite Furnace Atomic Absorption Spectrophotometer. The validity of these spectrophotometers result was assessed by spiking of samples with standards of known levels and calculating percentage recoveries.

Table 3.3: Mean Concentration (X±SD, n=9, mg/kg dry weight) and Relative Standard Deviation (% RSD) of trace metals in Khat Samples.

Metals	Khat sample					
	Arode (mean)	%RSD	Emdibir (Mean)	%RSD	Ambagenet (Mean)	%RSD
Mn	23.78±0.28	1.18	21.55±0.49	2.27	23.69±0.20	0.85
Zn	19.93±0.33	1.67	21.04±0.25	1.19	23.26±0.32	1.37
Cr	ND	ND	ND	ND	ND	ND
Cd	ND	ND	ND	ND	ND	ND
Pd	ND	ND	ND	ND	ND	ND

Where, ND = Not Detected (concentration of the tested metal below the method detection limit)

3.4 Levels of heavy metals in khat

In the present study the concentration of five heavy metals (Mn, Cu, Cr, Pb and Cd) in the three Khat leave samples varieties (Arode, Emdibir, and Ambagenet) were analyzed and their concentration is presented in table 4.1 above and in figure 4.1

below. This figure shows how the metals vary between the three-khat sampling sites. The order of metal mean concentration is Mn > Zn > Cu = Cr=Cd. Thus the concentration of Mn (21.55 to 23.78mg/kg), Zn (19.93 to 323.26 mg/kg), Cd, Cr and Pd were not detected.

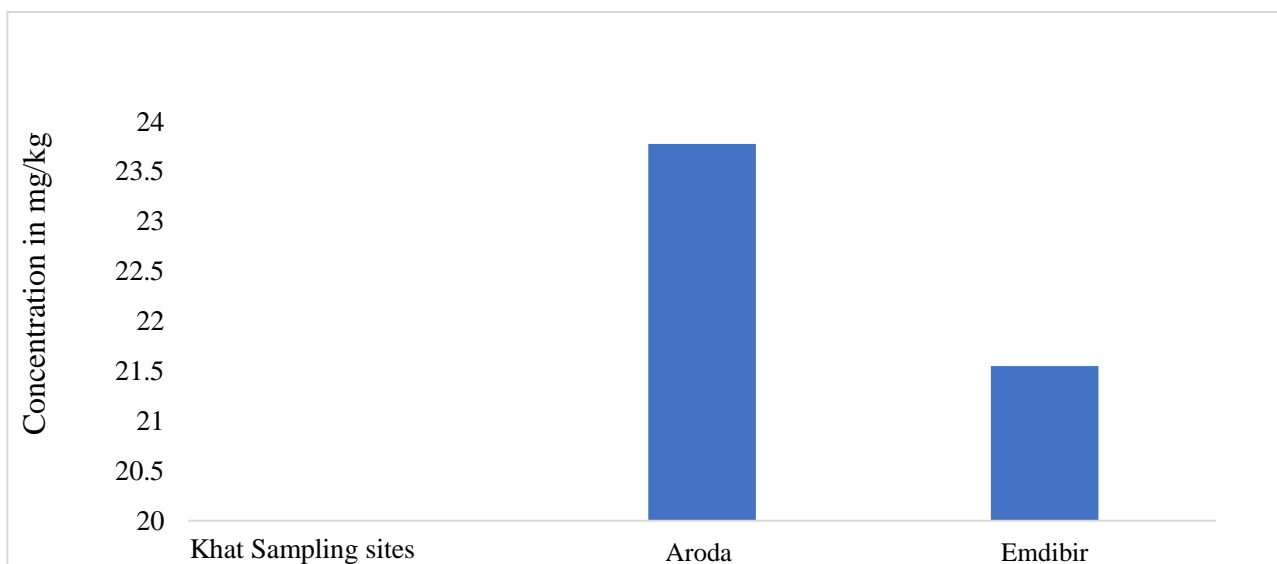


Figure 3.1. The Mean Concentration of Mn in Khat samples at different Sampling sites.

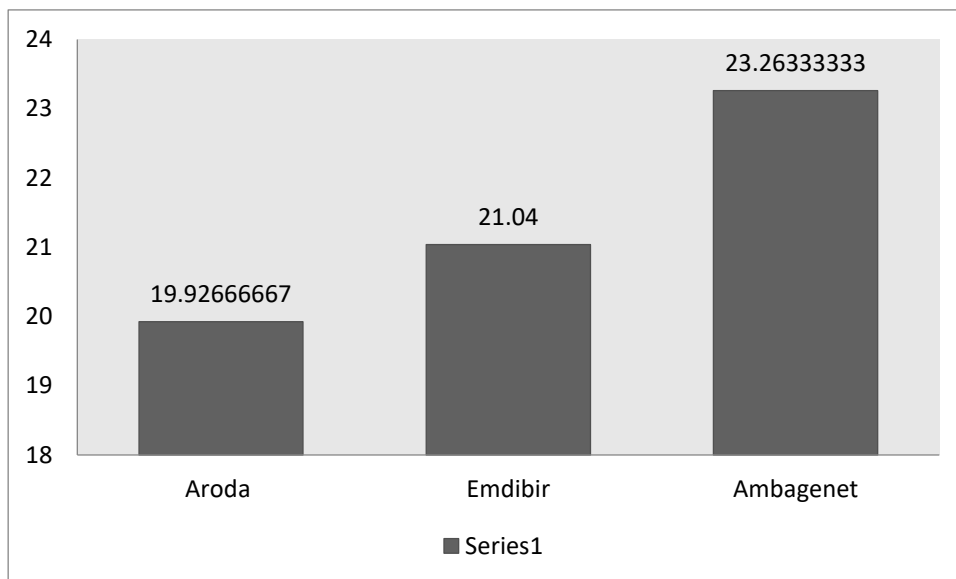


Figure 3.2. The Mean Concentration of Zn in Khat samples at different Sampling sites.

Various factors affect the concentration of metals in the plant. These are nature of chemical and physical property of the soil, climatic condition of the region, and application of fertilizers and pesticides. Increase in population and industrialization results pollution to water, air, and soil, which in turn causes in unexpected concentration of trace metals in the plant. Particularly agricultural activities such as use of fertilizers, pesticides, and irrigation with contaminated sewage are the major source of contaminations. As can be seen from tables 3.1, there is a wide variation in concentration of heavy metals within and among the khat samples. The pattern of concentration of elements in most of khat samples analyzed were in the order of Mn (21.55 to 23.78 mg/Kg) > Zn (19.93 to 323.26 mg/kg), while Cr, Cd and Pb were below the method detection limit of the analysis. Adane et al (2015) investigated the concentration of trace metals in khat samples around Arbaminch site and reported the concentration of Mn to be 36.02, 7.206, 85.29 mg/ Kg and Zn to be Zn 167.21, 202.64, 36.423 in khat samples of Gidole, Koyra and Konso respectively, which are relatively higher than the results of the present study.

Ali Mohammed et al (2015) reported the concentration of chromium to be 3 mg/ Kg where as in the present study it was not detected and the other metals which were analyzed in the present study were not reported. Moreover, the concentrations of toxic metals Pb and Cd in the studied Khat plant leaves were too low to be detected. This may be due to the fact that there are insufficient factories in the studied areas to cause substantial pollution.

Sileshi et al (2015), Adaneet al (2015) and Minaleshewa (2010) has reported the ranges for essential and non-essential metals of different Ethiopian Khatsamples. In addition, Ireri S.M. (2014) has reported the nutrient concentrations in the Kenyan khat leaves. The pattern of concentration of elements in most of khat samples obtained by Minaleshewa (2010), were in the order: Ca>Mg>Fe>Mn>Zn>Cu>Cr>Co, while Cd and Pb were below the method detection limit of his analysis. According to the report of Sailus (2014), the metal concentrations was; K (10.3-30.3 mg/g), Ca(9.44-33.2 mg/g) and Mg(1.14-7.19 mg/g).

Table 3.3. Comparison of Levels of some Elements with the Available Data in Literature.

Country	Location	Mn	Zn	Cr	Cd	Pb	References
Ethiopia (mg/Kg)	Gurage	21.55	23.78	ND	ND	ND	Present study
	Arba minch			3.00			Ali et al, 2015
	Gidole	36.02	167.21	2.75	ND	ND	Adane et al, 2015
	Konso	85.29	36.423	4.09	2.12	ND	
	Koyra	7.206	202.64	1.52	ND	ND	
	-	NR	49.9-131.3	NR	NR	NR	Atilebachew, 2011
Addis Ababa	NR	NR	3.1 - 6.76	NR	1.3-2.9	Tilahun et al, 2009	
Kenya (mg/Kg)	-	NR	NR	18.62-1.48	0.15-0.90	5.00 - 119.00	Sailus, 2007
Yemeni (blood of khat chewer)	-		0.4	NR	NR	NR	Ekram et al, 2012

3.5 Comparison of analyzed heavy metals in khat with recommended limit for plant

The results in this study indicates that the concentration of the permissible limit of Chromium, Cadmium and Lead in plants, recommended by WHO, is 1.30 µg/g , 0.02 µg/g and 2µg/g respectively, but in this study the concentration of chromium, Cadmium, and Lead were not detected. Zinc is one of the important trace elements that play a vital role in the physiological and metabolic process of many organisms and at high level, Zinc is neurotoxin. The WHO's recommended limit of Zinc in plant is 50µ g/g and in this study its value 23.78 µg/g which is below the permissible limit. So Zn does not possess potential harm to the plant users.

The concentration of Mn in the present study is 21.55 µg/g. There are a number of minerals which contain Mn in sufficient quantity namely, pyrolusite (MnO₂), hausmannite (Mn₃O₄), manganite (MnOOH), rhodochrosite (MnCO₃). It is the second highest amount among the micronutrients in the studied soil next to Fe. Exposure to high concentration of manganese is associated with impaired neurological and neuromuscular control, mental and emotional disturbances (muscle stiffness, and lack of coordination). Exposure to very high doses result in impaired male fertility, birth defects, and impaired bone development. However, the recommended daily allowance is 200 µg/day. Generally, based on the WHO recommended limit for plant Cr, Pb and Cd were not found to cause any risk to the people by consuming the khat plants grown in the area where the khat is planted.

4. Conclusion

Khat is clearly an important cash crop in Ethiopia and well known for its foreign currency earnings. Nonetheless, it is little understood and given no development and research attention yet. The study would help to underline the considerable level of sophistication that characterizes the cultivation and marketing of Khat in Southern Ethiopia. In this study the levels of metal in edible portion of Khat leaves collected from southern region of Ethiopia (Gurage Zone) were analyzed for their contents of Mn, Zn, Cr, Pb and Cd using flame and graphite furnace atomic absorption spectrometer. The optimized microwave digestion method for the analysis of samples was efficient for all the metals evaluated through the recovery experiment and a good percentage recovery of 97.20 to 108.89% were obtained for the metals analyzed.

Based on the results of this study, heavy metals Mn and Zn had found to be higher levels in the three-khat samples. The levels of Cd and Pb in all Khat samples were too low to be detected by the method used in this study. However, their absence in the studied Khat plants of the farms may not necessarily guarantee the nonexistence of these toxic metals in the different commercial Khat plants available in markets. The content of metal in all Khat samples were in the order of: Mn>Zn >Cd=Cr=Pd. Finally, the overall results of this study suggest that, there was no significant variation in the level of Mn metals between the Khat from different sites. However, there is there is a significance variation in the level of Zn metal between the sites which may be attributed due to different factors such as age of the khat harvested, geographical and climatical variation, deference in physicochemical nature of the soil, and deferent agricultural practices among Khat cultivars.

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