International Journal of Scientific & Engineering Research, Volume 11, Issue 7, July -2020.
ISSN 2229-5518

# Elemental Analysis of Medicinal Plant Moringa Stenopetala leaves by Atomic Absorption Spectroscopy

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## *Abstract:*

*The purpose of the current study is to analyze the concentration levels of selected trace metals: Ca, Cu, Fe, K and Zn in medicinal plant Moringa stenopetala grown in SNNPR, Ethiopia. A wet digestion procedure is used. Thus, the result showed that the concentrations of Ca, Cu, Fe, K and Zn in Moringa stenopetala grown in Gamo Gofa (Arbaminch) were;2.80±0.36, 0.866±0.134, 7.98±1.23, 3.02±0.63 and 84±0.92 mg/Kg, in Wolaita 3.28±0.28, 0.94±0.16, 7.13±1.32, 7.13±1.32 and 0.61±0.11mg/Kg and in Konso were; 2.97±0.67, 0.77±0.098, 6.79±0.73, 2.39±0.23 and 0.37±0.006mg/Kg respectively. The results indicate that the distribution pattern of metals in Moringa stenopetala grown in Arbaminchi was found to be in the order of: Iron > Potassium> Calcium >Copper > Zinc; in Wolaita: potassium>Calcium > Copper > Iron > Zinc and in Konso: Calcium > potassium Copper > Iron > Zinc. The concentration levels of all five trace metals in all areas of the study were tolerable because they are below the upper permissible limits for heavy metals in plant leaves/vegetables as it is recommended by World Health Organization.*

**Keywords:** Analysis, Moringa stenopetala, Atomic Absorption Spectroscopy.

## 1. Introduction

Ethiopia is an agricultural country where large majority of people are engaged in cultivation of food crops and rearing of livestock. Crops are produced for subsistence (Yisehak *et al*., 2010; Yisehak, 2008).

In parts of southern Ethiopia, the consumption of wild food plants appeared to be one of the most important local survival strategies and to have intensified due to the reaped climatic shocks hampering agricultural production thus leading to food shortage (Guinad and Lemessa, 2000; Feyssa *et al*., 2011). *Moringa* species is one of the world's most useful plants; it is a fast-growing, much more drought-tolerant and multi-purpose tree that it has been described as a 'miracle tree' (Fuglie, 2003; Yisehak *et al.*, 2011; Ashfaq *et al.*, 2012). Among the wide range of uses it provides are human food, fuel wood, livestock forage, medicine, dye, water purification, soil and water conservation, quality of cooking oil, green manure and the tree is used as source of income for Moringa growers (Demeulenaere, 2001; Palada and Jiru *et al.*, 2006; ECHO, 2009; Morey, 2010; Melesse *et al*., 2011).

In Ethiopia it is grown as a backyard crop in the southern parts of the Rift Valley and adjoining lowlands for its edible leaves, flowers and tender pods. Moringa has attracted enormous attention of ethno botanists and plant genetic resource conservationists due to its widespread use in agriculture and medicine.

The cultivation of *Moringa* in Ethiopia occurs mainly in the Zones and Special districts of Southern Nations, Nationalities and People Regional State (SNNPR); such as South Omo, Gamo Gofa, Kaffa, Sheka, Bench Maji, Wolaita, Dawaro, Bale, Borena, Sidama, Burji, Amaro, Konso and Derashe (Edwards *et al*., 2000). The leaves are one of the best vegetable foods that can be found in the locality. All parts of the tree except the wood are edible, providing a highly nutritious food for both humans and animals. The edible parts are exceptionally nutritious (Jiru *et al*., 2006Ram, 2004). It is very popular vegetable in Southern Nations and Nationalities and Peoples

International Journal of Scientific & Engineering Research, Volume 11, Issue 7, July -2020.
ISSN 2229-5518

Regional State of Ethiopia and valued for its special flavor.

The mankind uses different medicinal plants in many respects, as a food for nutritional purpose, medicine for treatment of infections, and constituent of cosmetics for maintenance of healthy skin and as it is summarized in short in figure1.1.below.

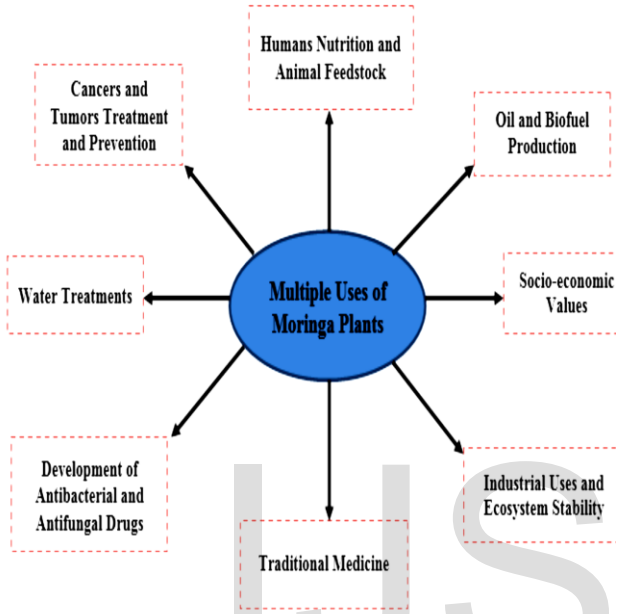



**Figure1.1.Summary on Multiple Uses of Moringa Trees**

*Moringa* is a multi-purpose miracle tree with tremendous potential uses such as food for human beings, feed for livestock, dye, perfume, skin lotion, lubricant and water purification (Agena, 2009). The leaves of *Moringa stenopetala* are nutritionally rich and an excellent source of concentrated proteins, vitamins and minerals (Armelle and Melanie, 2010). Due to its appreciable contribution to human consumption, the interest of the society is ever increasing from time to time.

## Statement of the Problem

People in SNNPR, who cultivate and utilize *Moringa stenopetala*, face several challenges/problems such as:diseases, lack of improved varieties, lack of wide opportunities to sell and announce their product for consumers for the socio-economic development. Thusthey use it only for the fulfillment of mineral requirements for their families and livestock, especially during the dry season when other vegetables and forage crops are scarce(Locket, Stein Müller, N. Sonder, Kubitzki, Kroschel, J., Manama *et al*, 2015).

Because of its multiple uses and easy of propagation and ability to thrive under harsh environments, its acreage as a cultivated crop is on the increase, as is the demand for its products (Tenaye *et al*., 2009).

Although *Moringa* is fast growing, drought tolerant and easily adapted to poor soil and arid conditions, it has not received significant research attention like *Moringa oleifera* to select and develop potential ecotypes that might be valuable both as horticultural and medicinal crops.Despite its nutritional and chemical composition, there has been no scientific study conducted on the variation of the metallic/ elemental compositions of *Moringa stenopetala* leaves among different agro-ecological Zones (Gamo Gofa, Konso, Derashe) in Southern Ethiopia and even no scientific study was conducted in Wolaita Zone related to either nutritional or metallic composition of the plant.

Moreover, there is lack of clearly studied concentration level of heavy metals in these plants to attain the permissible limit for heavy metals in plant leaves as recommended by World Health Organization (WHO).

## Objective of the Study

The general objective of the study is to analyze the elemental composition of medicinal plant *Moringa stenopetala* by using Atomic Absorption spectroscopy in the case of SNNPR (Arbaminch, Wolaita, and Konso), Ethiopia.

## Specific Objectives

The specific objectives of the current study are:

- To analyze the level of elemental concentration of some trace metals especially; Calcium (Ca), Copper (Cu), Iron (Fe), Potassium (K) and Zinc (Zn) in the leaves of *Moringa stenopetala in* Arbaminch, Wolaita and Konso Zone, by Atomic Absorption Spectroscopy (AAS).
- To put a scientific base line data (information) on the concentration level of these five metals in the leaves of *Moringa stenopetala* in Wolaita Zone.
- To compare theconcentration levels of different metals in *Moringa* leavesin different areas (Arbaminch, Wolaita and

International Journal of Scientific & Engineering Research, Volume 11, Issue 7, July -2020.
ISSN 2229-5518

Konso) with each other and their upper permissible limit for plants /vegetables as recommended by World Health Organization (WHO).

### Scope of the Study

In this study focus was given on districts (sites) where there has been more agro- ecological practice of *Moringa Stenopetala* tree cultivation and utilization. The current study aimed at solving problems related to consumption levels of *Moringa* leaves by analyzing the elemental composition of only five heavy metals in the tree. So study covers three districts (zones) of the Southern Ethiopia, especially Konso, Arbaminch and Wolaita.

Because of time constraint, financial limitation and other related factors, it is difficult to identify the overall elemental composition of *Moringa*tissues in the study area. It doesn't also measure and quantify actual environmental impacts and soil composition. Thus, the study emphasized on concentration level of five trace metals, namely Calcium (Ca), Copper (Cu), Iron (Fe), Potassium (K) and Zinc (Zn) in *Moringa Stenopetala* leaves under controlled experimental working conditions and procedures in the study area by Flame Atomic Absorption Spectroscopy (FAAS).

### Significance of the Study

This study is important for the society ; as it provide the reliable information on the level of concentration of heavy metals in the leaves of *Moringa Stenopetala* . Because the deficiency or excess in- take thesefiveelements namely calcium, copper, iron, potassium and zinc can highly disturb metabolic levels in the human body and can cause chronic disease as they are toxic. As the study is new in this field, it may serve as the base line data (especially in Wolaita) and for comparative study on level of heavy metals in *Moringa stenopetala* leaves in the further scientific study in the field.

By raising and creating community–wide awareness on multipurpose uses of *Moringa stenopetala*, the study can increase the market demand, social interaction and maximize resource utilization in the society.

## 2. Materials and Methods

**2.1 Description of the study area**:The studywas conducted in three major moringa growing agro-ecological Zones in the Southern Ethiopia, SNNPR, namely: Gamo Gofa Zone (Arbaminch Zuria), Konso, and Wolaita. Arbaminch Zuria district is in Gamo Gofa Zone, Derashe and Konso districts are in Segen zone and Wolaita district is in Wolaita Zone of SNNPR, Ethiopia.

Arbaminch Zuria district is located at 6°01'59" N and 37° 32'59" E, altitude of1269m.a.s.l and 505 km away from the capital city, Addis Ababa. Konso district is located at 5°15'00" N and 37°28'59" E and altitude of 1031 m.a.s.l.Wolaita zone is found in Southern NationsNationalitiesand Peoples'Regional State (SNNPRS), at South Central Ethiopia between $6.4^0$-$6.9^0$Nlatitude and $37.4^0$-$37.8^0$E longitude and is located at 390 km south of Addis Ababa and 160km from Hawassa, the capital of the Regional State. The study area encloses three districts, each containing four sub-sites. That is; total of 12 sub-sites are selected purposely due to their potential multipurpose cultivation and utilization *Moringa Stenopetala*.

### 2.2 Experimental design, instruments (apparatus) and procedures

Stainless steel axe and Teflon(SSAT) knife were used to cut the plant pieces while air-circulating universal oven were for drying the samples placed on porcelain.Blending device, ceramicpestle and mortal were used for grinding and homogenizing the samples.Digital analytical balance was used for weighing the samples.Round bottom flasks with grounded glass(100mL) fitted with reflux condenser were employed in digesting the sample on Kjeldahl heating apparatus(Gallenhamp,England). Borosilicate volumetric flasks (50,100 and 250mL) were used during dilution of sample and preparation of metal standard and in fusion solutions. Measuring cylinders, pipettes, micropipettes (Dragonmed,1-10µL,100-1000µL) were used during measuring different quantities of volumes of sample solution,acid reagents and metal standard solutions. Determination of metal concentration was done by Flame Atomic Absorption spectroscopy (FAAS) equipped with deuterium back ground corrector and hollow cathode lamps with air-acetylene flame.The HCL which is a tube filled withan inert gas such as Neon (Ne) or Argon (Ar) at pressure of 1-5 torr.

### 2.3 Experimental design and basic process occurring during working with FAAS.

The research design used is controlled laboratory based study by using Flame Atomic Absorption spectroscopy (FAAS).In FAAS the light source (to be more precise that part of the light which is not absorbed) gets directly to the light resolving unit, the monochromator (the light source, the flame and the monochromator are arranged in such a way that they are in one line). In newer instruments the light resolving unit (monochromator) is the diffraction

International Journal of Scientific & Engineering Research, Volume 11, Issue 7, July -2020.
ISSN 2229-5518

grid, the detection of the light is attributed to the photomultiplier. The signal processing/displaying unit of the photometer's can be very different (analogue/digital displays, graphical recorders), but the modern spectrometers are equipped with computers.

### 2.4 Sample introduction

The sample introduction: is to introduce samples (mostly liquids) to the flame with good reproducibility and with high efficiency so that the interfering effects should remain minimal (the liquid must be introduced as fine aerosol). The sample introduction is done most often by spraying the liquid. The small droplets formed in the nebulization transform gradually while going through the high temperature zones of the flame. It is an important aspect that the particle size of the droplets (<5µm) should be possibly in similar size ranges in order the particles to be desolvated and to be further transformed in the same region of the flame.

For the nebulization of solutions the indirect pneumatic nebulization is the most frequently used method which has sample introduction efficiency about 10%.The sample gets into the nebulizer through a plastic capillary and the sample leaving the capillary is surrounded by the gas (air) feeding the flame. After this high speed gas comes out through the tight emergent hole of the nozzle pressure decrease is established in the capillary which results the take up of the liquid. By the way the high speed gas breaks the up taken liquid into small drops thus aerosol is formed.In this type of spectroscopy Meker burners were used.These burners are actually such tubes that are covered from one end with a plate containing several holes. The application of this burner is appropriate for flames with slow burning speed. Nowadays burners with slots are the most frequently used burner types.

The 10 cm long slot is favourable especially for the absorption measurements in air/acetylene flame. In the design of slotted burners the width and the length of the slot, and the heat conducting property of the material of the burner is of great importance. The temperature of the most commonly used burners is in the range of 2200-3300 °C In this temperature range only the spectrum lines having higher excitation energy than 4.5 eV can be excited.

### 2.5 Processes occurring in flame

Three steps are involved in turning a liquid sample into an atomic gas.They are: Desolvation (the liquid solvent is evaporated, and the dry sample remains); Vaporization (the solid sample vaporizes to a gas); and Volatilization (the compounds that compose the sample are broken into free atoms).

From the liquid drop the solvent evaporates first, and solid aerosol particles (micro sized crystals of salts) are formed, in the next step it loses its 'crystalline water (if possessed) then the crystals melt and evaporate that is molecule vapour forms. In the higher regions of the flame the thermal dissociation of the molecules occurs: ground state atoms are formed (for atomic absorption measurements only these particles are useful). Of course, when the temperature of the flame makes it possible, the thermal processes go further, by the side of the ground state atoms excited atoms, even ions will be present in the flame

### 2.6 The resolution of light

The light leaving the flame is led to the detecting unit with a proper optical device. Due to the background radiation of the flame and the emission of the other metals present in the flame with direct measurements we cannot get proper results. By the help of an optical device (prism) we try to eliminate the interfering radiations and we let only the almost monochromatic, to the investigated metal characteristic light to the detector. While formerly this function was tried to be fulfilled with colour filters, nowadays nearly there are only monochromators in use. The monochromators are capable for the separation of small wavelength ranges with 0.01-1 nm width in a wide (190-800 nm) spectrum. The desired wavelength is set by rotating the prism or diffraction grid. Although by increasing the size of the slit, the intensity of the light increases, however, the purity of the spectrum decreases.

### 2.7 Cleaning apparatus

Apparatus such as glass ware,plastic containers and polyethylene bags were washed with tap water using detergent followed by rinsing with deionized water.The appaatuses were then be soaked in about10%(v/v) nitric acid for 24hr. followedby rinsing with deionized water several times. Then, the apparatus was dried in oven and kept in dust free place until further use.

### 2.8 Optimization of the working procedures

Itisimportant to develop an optimum working procedure in order to get a reliable result froman analytical experiment. Thus, to prepare a clear and colorless sample solution that issuitable for the analysis using AAS, different working procedures for the digestion of plantsamples were assessed

International Journal of Scientific & Engineering Research, Volume 11, Issue 7, July -2020.
ISSN 2229-5518

using mixtures of $HNO_3$ and $HClO_4$acids by varying parameters suchas volume of the acids mixture, digestion time and digestion temperature( Debebe Mikore and Eyobel Mulugeta, 2017). By examining thenature of the final digests obtained by varying the above parameters, the optimized procedurewas selected depending up on the clearness of the digests, less digestion time, less reagentvolume consumption and simplicity.

## 3. Sample Collection

**3.1 Sample Preparation:** Samples of fresh *Moringa* leaves were collected from matured (age >15 years) and immature (age 3-15years) tree. From each zone four sub-sites are selected by using both random and cluster sampling method. In Gamo Gofa zone; Lante, Lasho, Shara and Sikela are selected. In Wolaita zone; Bale, Badesa, Humbo and Offa are selected. In Konso and Derashe; Karat, Dera, Derashe and Gato are selected. Twenty four households(Eight from each sub-sites) were randomly selected and total of 24 trees; eight from each sampling sub-site selected .For each sub-site atotal of about500g bulk fresh *Moringa stenopetala* leavessamples were collectedrandomly and placed in polyethylene plastic bags, labeled and taken toArba Minch University, College of Natural science, Department of Chemistry for further treatment and analysis.

**Figure3. 1***Moringa Stenopetala* sample collection picture from Arbaminch (Gamo Gofa)

The *Moringa* leaf sampleswere air-dried followed by the oven at 70°C for 72 h (until constant weight is gained) and ground by a grinder blender to homogenize and reduce the size of theparticles to pass through a 0.5-1 mm sieve and stored inpolyethylene bags prior to analysis.

### 3.2 Sample digestion

Precisely 0.5 g of the crushed, powdered and sieved portion of the plant samples were accurately weighed on a digital analytical balance and quantitatively transferred into digestion tubes. An optimized amount of freshly prepared mixture of 72%(v/v) of conc. $HNO_3$, 30% of conc.$H_2O_2$and 70% of conc. $HClO_4$ were added to each plant samples according to optimized digestion procedures mentioned in Appendix 1, for plant samples. The digested solutions were allowed to cool for 30 minutes. To the cooled solutions, two 5 mL portions of distilled de ionized water were added to dissolve the precipitate formed on cooling and gently swirled. The resulting solutions were filtered into a 50mL volumetric flask with a Watchman filter paper number 41 to remove any suspended and turbid matter. Subsequent rinsing of the filtrate with 5 mL distilled deionized water was followed until the volume reached the mark(Debebe Mikore and Eyobel Mulugeta, 2017). For each bulk sample, triplicate digestions were carried out. The digested and diluted sample solutions were stored in volumetric flask and were kept in refrigerator until analysis time (Table 4.1).

### 3.3. Chemical Analysis

#### 3.3.1 Operating conditions

Intermediate standard solutions were prepared from the atomic absorption spectroscopy standard stock solutions containing 1000 mg/L. These intermediate standards were diluted with distilled water to obtain working standards for each metal of interest. Parameters (burner and lamp alignment, slit width and wavelength adjustment) were optimized for maximum signal intensity of the instrument based on the instrument instruction(Muhammad Akhyar Farrakhan, 2011). Three replicate determinations were carried out on each plant sample. Hallow cathode lamp for each metal operated at the manufacturer's recommended conditions were used at its respective primary line source. The acetylene and air flow rates were managed to ensure suitable flame conditions. All the five metals (Ca, Cu, Fe, K, and Zn) were analyzed by the absorption mode of the instrument. Three readings were recorded for each digestion by optimizing the different operating conditions for FAAS shown in Table to give the maximum signal intensity (Table 4.2).

### 3.4. Instrument calibration

Calibration curves were prepared to determine the concentration of each metal in the sample solutions. The instrument was calibrated using series of working standards(Muhammad Akhyar Farrakhan, 2011).

International Journal of Scientific & Engineering Research, Volume 11, Issue 7, July -2020.
ISSN 2229-5518

The working standard solutions of each metal were prepared from intermediate standard solutions of the respective metals.

Different parameters such as; wavelengths, concentration of the intermediate standards, working standard solutions and the correlation coefficients of the calibration curves of each metal for the plant samples are presented (Table 4.2 and 4.3).

### 3.5. Method Validation

Methodvalidation is the process of providing that analytical method is acceptable for its intended purpose. Because of the absence of certified reference material for the samples in the laboratory, the validity of the optimized digestion procedure was assured by spiking thesamples with a standard of known concentration of the analyte metals. Thus, the efficiency of the optimized procedure is checked. The spiked samples were digested in triplicate following the same digestion procedure developed previously for plant samples(Debebe Mikore and Eyobel Mulugeta, 2017). The digested spiked samples were analyzed for their respective metals using FAAS. Finally, % recovery was determined by using recovery formula:

$$\%Recovery = \frac{amount\ of\ analyte\ recovered}{amount\ of\ ion\ added}\ x\ 100 \quad ..3.1$$

### 3.6. Method Detection Limit

Method detection limit is defined as the minimum concentration of analyte that can be measured and reported with 99% confidence that the analyte concentration is greater than zero. In other words, it is the lowest analyte concentration that can be distinguished from statistical fluctuations in a blank, which usually correspond to the signal of blank and three times the standard deviation of the blank (Limit of detection(LOD) = YB + 3SB), where SB = standard deviation of the blanks (Miller and Miller, 2005).

Five blank samples were digested following the same procedure as the samples and each of the blank samples were analyzed for metal concentrations of Ca, Cu, Fe, K and Zn by FAAS.

### 3.7. Statistical Analysis

Each experimental analysis was done in triplicate. Data obtained from experiments were analyzed by one way ANOVA (Analysis of Variance) using SPSS version 20 and Origin version 6; for calculation of mean, standard deviation, Pearson correlation value and significance level.

The analysis was used to compare the elemental composition of *Moringa stenopetala* leaf samples collected from three different agro-ecological zones.

Data entry management and preliminary summaries were done on Microsoft Excel spread sheet. The means, standard deviations and significance level of the result/data collected were determined using Microsoft Excel and origin software. Experimental treatment significant differences ($p < 0.05$) were determined for equality of means and the linear correlations were determined using the Pearson matrices product-moment correlation.

Calibration curve for five metals namely calcium, copper, iron, potassium and zinc in standard solutions were drawn by using both Microsoft Excel spread sheet and Origin 6 software.

## 4. Result and Discussion

The accuracy and precision of the methods were tested by spiking the samples with a standardof known concentration of the analyte metals. The results indicated that the concentrations ofelements determined are in agreement (100 ± 10%) within theacceptable range for all metals(Miller and Miller, 2005).The following lists of tablesare results from optimized laboratory methods for analysis ofmetals using Flame atomic absorption spectroscopy.

One of the basic requirements for sample preparation for analysis is to get an optimumcondition for digestion. The optimum condition is the one which leads: Minimum reagent volume consumption, Minimum digestion time, Minimum residue (clear solution) and ease of simplicity. Optimizing of the digestion procedure involved some changes of parameters such as reagentvolume, digestion temperature and digestion time. Accordingly, procedures were tested in triplicate for digestion of *Moringa* (Table 4.1)

International Journal of Scientific & Engineering Research, Volume 11, Issue 7, July -2020.
ISSN 2229-5518

**Table 4. 1** Procedures tested during optimization of method for digestion of *Moringa*

| S.№ | Sample Size (g) | Reagents added (v/v) | Initial temp. (°C) | Final Temp. (°C) | Digestion Time (hr.) | Nature of the digested sample After filtration |
|---|---|---|---|---|---|---|
| 1 | 0.5 | 3ml $HNO_3$(72%) 1ml $H_2O_2$(30%) 1ml $HClO_4$(70%) | 60 | 215 | 3:15 | Clear and yellowish color |
| 2 | 0.5 | 3ml $HNO_3$(72%) 1ml $H_2O_2$(30%) 1ml $HClO_4$(70%) | 60 | 220 | 3:25 | Clear brown and turbid color |
| 3 | 0.5 | 2.5 ml $HNO_3$ (72%) 1ml $HClO_4$(70%) 1 ml $H_2O_2$ (30%) | 60* | 210* | 3:00* | Clear and colorless * |

Optimum digestion conditions*

Based on the above listed criteria, the optimal digestion procedure chosen was the one that fulfilled the selected criteria for complete digestion of 0.5 g of the dry sample powders, with 2.5 mL of $HNO_3$ (72%), 1 mL $HClO_4$ ( 70 %) and 1 mL $H_2O_2$ (30 %) for a total of 3 hours. The mixture was digested smoothly by setting the temperature first to 60 $^0$C for 30 min and then increased to 210 $^0$C for the next 2 hr and 15 min then after the digested solution was allowed to cool for 5 min without dismantling the condenser from the flask and for 10 min after removing the condenser. The procedures that required higher reagent volume longer digestion time and colored digest solution were rejected (Taylor and Francis, 2012).

**Table 4. 2**: Instrumental parameters for the analysis of metals in *Moringa stenopetala*

| No | Element | Wave Length (nm) | Lamp current (mA) | Slit width (nm) | Ionization Energy (eV) | IDL* (mg/kg) | Flame type |
|---|---|---|---|---|---|---|---|
| 1 | Ca | 422.7 | 5.8 | 0.7 | 4.513 | 0.01 | Air-acetylene |
| 2 | Cu | 324.7 | 5.8 | 0.7 | 3.342 | 0.02 | Air- acetylene |
| 3 | Fe | 248.3 | 6.4 | 0.2 | 3.256 | 0.03 | Air- acetylene |
| 4 | K | 769.9 | 6.0 | 0.2 | 4.345 | 0.030 | Air- acetylene |
| 5 | Zn | 213.9 | 5.0 | 0.7 | 3.047 | 0.005 | Air- acetylene |

IDL*(mg/kg): Instrumental Detection Limit

In practical analysis; all the upper parameters, have an influence on the sensitivity and almost fulfilled completely. Some points might be realized, but the majority will deviate from the optimum (Marks, J.Y.and Welcher, and G.G., 2007). It is necessary to evaluate carefully during method development which compromises in the selection of instrumental parameters, in sample preparation and in the analytical procedure in order to obtain optimum conditions for a given task.

**Table 4. 3.** Concentrations of standard solutions, samples, absorbance and correlation

| Metal | Conc. of working standard (mg/L) | Absorbance | Correlation (r) value |
|---|---|---|---|
| Ca | 1,2,3,4,5, samples | 0.089,0.178,0.272,0.377,0.487, and 0.52* respectively | 0.998 |
| Cu | 0.5,1,1.5,2,2.5,samples | 0.098,0.181,0.271,0.367,0.471 and 0.36* respectively | 0.9981 |
| Fe | 0.15,0.25,0.35,0.45,0.55,sample | 0.003,0.009,0.02,0.027,0.03 and 0.47* respectively | 0.9982 |
| K | 1,2,3,4,5,samples | 0.106,0.224,0.348,0.464,0.592 and 0.55* respectively | 0.998 |
| Zn | 0.25,0.5,0.75,1,1.25,samples | 0.0102,0.024,0.41,0.058,0.074 and 0.072 * respectively | 0.9988 |

* Mean Absorbance of the analytes in *Moringa stenopetala* samples.

The calibration graphs of these five metals were drawn by Origin 6, based on the result, using the experimental and standard solution data obtained from this table. It is related that the instrument response (Absorbance), *Y* is linearly related to the standard concentration *X* for a limited range of concentration and can be expressed in a form of equation: y = mx + b., where, m is the slope and b is the intercept/ absorptivity constant (Ferris, A.P., *et al.*, 2000).They have the slope equation of a linear calibration curve as shown below.

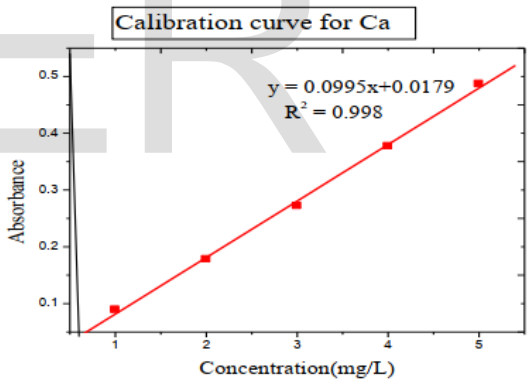
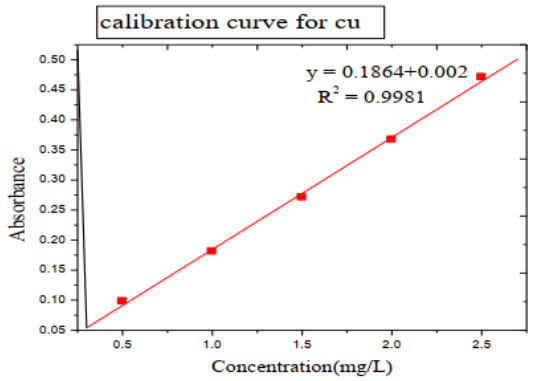
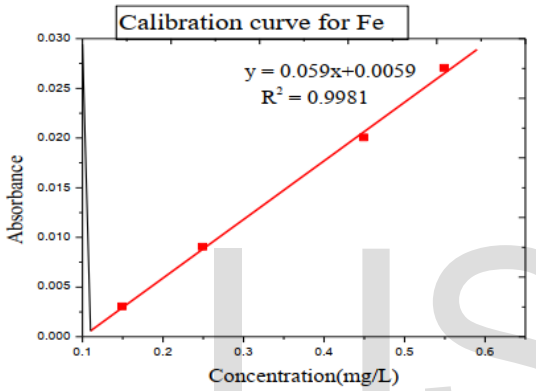



**Figure4. 1** Calibration curve of Calcium standard solution

International Journal of Scientific & Engineering Research, Volume 11, Issue 7, July -2020.
ISSN 2229-5518



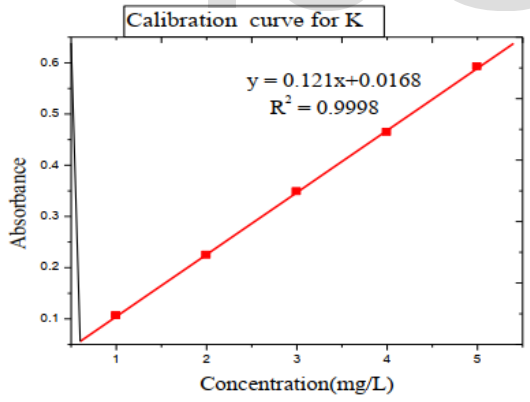




**Figure4. 4** Calibration curve of Potassium standard solution

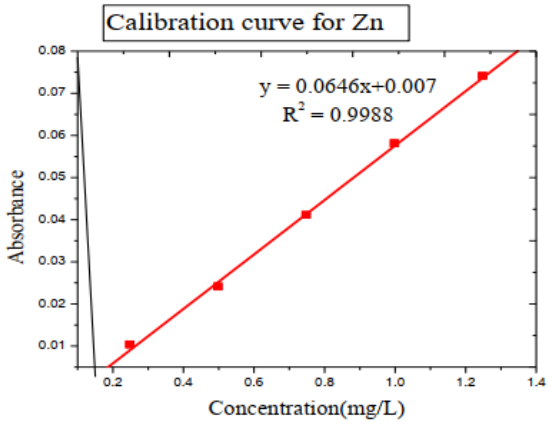



**Figure4. 5** Calibration curve of Zinc standard solution

A correlation coefficient measures the strength and direction of a linear association between two variables. It ranges from -1 to +1. The closer the absolute value is to 1, the stronger the relationship.

A correlation of zero indicates that there is no linear relationship between the variables. The coefficient can be either negative or positive (Ferris, A.P., *et al.*, 2000).

**Table 4. 4.**Analytical recovery results for the validation of the optimized samples

| Metal | Conc.in sample (µg/g) | Amount added (µg/g) | MDL* (mg/kg) | Conc. in spiked sample (µg/g) | Recovery (%) |
|---|---|---|---|---|---|
| Ca | 2.45 | 0.2 | 0.283 | 2.60± 0.03 | 95± 0.01 |
| Fe | 7.47 | 0.2 | 0.278 | 7.59± 0.02 | 88± 0.05 |
| Zn | 0.74 | 0.15 | 0.93 | 0.8± 0.01 | 94± 0.015 |
| Cu | 0.63 | 0.2 | 0.349 | 0.8± 0.03 | 83± 0.06 |
| K | 2.13 | 0.1 | 0.121 | 2.2± 0.08 | 93±0.02 |

MDL*: Method detection Limit (mg/kg)

The proportion of analyte (incurred or added) remaining at the point of the final determination from the analytical portion of the sample measured is the value of recovery (Taylor & Francis 2012). Usually recovery is expressed as a percentage and it acceptable when it is above 80%.

## 4.1. Elemental analysis of *Moringa stenopetala* leaves by FAAS

The result showed that, the concentration of the five heavy metals, (Ca, Cu Fe, K, and Zn) in the medicinal plant *Moringa stenopetala* leaves from Arbaminch, Wolaita and Konso were determined.

International Journal of Scientific & Engineering Research, Volume 11, Issue 7, July -2020.
ISSN 2229-5518

The mean absorbance and mean concentration value (mean ± Stdev) of the each analyte/metal in *Moringa* samples of each area of study were calculated (Table4.5 to Table 4.8).

**Table 4. 5** The mean value each five metal in *Moringa* of Gamo Gofa Zone

| Metals | Mean | Stdev | Min | max | Range | Sum | N |
|---|---|---|---|---|---|---|---|
| Ca(abs) | 0.2612 | 0.03628 | 0.226 | 0.321 | 0.095 | 1.306 | 5 |
| Ca(mg/L) | 2.80503 | 0.36462 | 2.45126 | 3.40603 | 0.95477 | 14.02513 | 5 |
| Fe(abs) | 0.4652 | 0.07233 | 0.393 | 0.581 | 0.188 | 2.326 | 5 |
| Fe(mg/L) | **7.98475**** | 1.22588 | 6.76102 | 9.94746 | 3.18644 | 39.92373 | 5 |
| Zn(abs) | 0.0472 | 0.00597 | 0.041 | 0.055 | 0.014 | 0.236 | 5 |
| Zn(mg/L) | **0.83901*** | 0.09249 | 0.74303 | 0.95975 | 0.21672 | 4.19505 | 5 |
| k(abs) | 0.3494 | 0.07605 | 0.242 | 0.422 | 0.18 | 1.747 | 5 |
| k(mg/L) | 3.02145 | 0.62751 | 2.13531 | 3.62046 | 1.48515 | 15.10726 | 5 |
| Cu(abs) | 0.1594 | 0.02489 | 0.116 | 0.177 | 0.061 | 0.797 | 5 |
| Cu(mg/L) | 0.86588 | 0.13351 | 0.63305 | 0.9603 | 0.32725 | 4.3294 | 5 |

**Highest concentration level (mg/L),* Lowest concentration level (mg/L).

**Table 4. 6** The mean value each five metal in *Moringa* of Wolaita Zone

| Metals | Mean | Stdev | Min | max | Range | Sum | N |
|---|---|---|---|---|---|---|---|
| Ca(abs) | 0.3082 | 0.02778 | 0.276 | 0.346 | 0.07 | 1.541 | 5 |
| Ca(mg/L) | 3.27739 | 0.27919 | 2.95377 | 3.65729 | 0.70352 | 16.38693 | 5 |
| Fe(abs) | 0.4148 | 0.07817 | 0.301 | 0.499 | 0.198 | 2.074 | 5 |
| Fe(mg/L) | **7.13051**** | 1.32488 | 5.20169 | 8.55763 | 3.35593 | 35.65254 | 5 |
| Zn(abs) | 0.0322 | 0.00683 | 0.021 | 0.039 | 0.018 | 0.161 | 5 |
| Zn(mg/L) | **0.60681*** | 0.10579 | 0.43344 | 0.71207 | 0.27864 | 3.03406 | 5 |
| K(abs) | 0.408 | 0.04906 | 0.344 | 0.461 | 0.117 | 2.04 | 5 |
| K(mg/L) | 3.50495 | 0.40475 | 2.9769 | 3.94224 | 0.96535 | 17.52475 | 5 |
| Cu(abs) | 0.1734 | 0.03017 | 0.123 | 0.203 | 0.08 | 0.867 | 5 |
| Cu(mg/L) | 0.94099 | 0.16186 | 0.6706 | 1.09979 | 0.42918 | 4.70494 | 5 |

**Highest concentration level (mg/L),* Lowest concentration level (mg/L)

**Table 4. 7** The mean value each five metal in *Moringa* of Konso Zone

| Metals | Mean | Stdev | Min | max | Range | Sum | N |
|---|---|---|---|---|---|---|---|
| Ca(abs) | 0.2674 | 0.06688 | 0.199 | 0.355 | 0.156 | 1.337 | 5 |
| Ca(mg/L) | 2.96734 | 0.67219 | 2.1799 | 3.78774 | 1.56784 | 14.93668 | 5 |
| Fe(abs) | 0.3946 | 0.04323 | 0.322 | 0.434 | 0.112 | 1.973 | 5 |
| Fe(mg/L) | **6.78814**** | 0.73271 | 5.55763 | 7.45593 | 1.89831 | 33.94068 | 5 |
| Zn(abs) | 0.01678 | 0.00433 | 0.0111 | 0.0231 | 0.012 | 0.0839 | 5 |
| Zn(mg/L) | **0.36811*** | 0.06706 | 0.28019 | 0.46594 | 0.18576 | 1.84056 | 5 |
| K(abs) | 0.346 | 0.02786 | 0.299 | 0.367 | 0.068 | 1.73 | 5 |
| k(mg/L) | 2.3934 | 0.22984 | 2.60561 | 3.16667 | 0.56106 | 14.967 | 5 |
| Cu(abs) | 0.1422 | 0.01821 | 0.122 | 0.169 | 0.047 | 0.711 | 5 |
| Cu(mg/L) | 0.77361 | 0.09771 | 0.66524 | 0.91738 | 0.25215 | 3.86803 | 5 |

**Highest concentration level (mg/L),* Lowest concentration level (mg/L).

The results indicated that the samples had variable composition of each analyte metals with different concentration ranges among different study areas. The variations in mineral micronutrients/trace metals content in *Moringa* of reported values may be due to different ages of trees, and possibly due to different stages of maturity (Yang *et al*., 2006).

There were significant differences at ($P<0.05$) among the mean concentrations of metals in the *Moringa* leaf samples. These differences could probably be the result of plant nutrition, climate and soil conditions (Hamurcu *et al*., 2010).

**Table 4. 8** The mean concentration (X ± SD, n = 5, mg/L) of five metals in *Moringa*

| Metals | *Moringa stenopetala* in: Arbaminch | Wolaita | Konso | MPL* (WHO/FAO) |
|---|---|---|---|---|
| Ca | 2.80±0.36 | 3.28±0.28 | 2.97±0.67 | 100* |
| Cu | 0.866±0.134 | 0.94±0.16 | 0.77±0.098 | 10* |
| Fe | 7.98±1.23[a] | 7.13±1.32 | 6.79±0.73 | 15* |
| K | 3.02±0.63 | 3.50±0.40 | 2.39±0.23 | 350* |
| Zn | 0.84±0.92 | 0.61±0.11 | 0.37±0.006[b] | 20* |

MPL*: Maximum permissible limit of metals in (µg/g) in plant leaves,
*Source:* FAO/WHO (2001). Highest concentration of all metals [a], lowest concentration [b].

## Distribution pattern (Levels) of some trace heavy metals in Moringa stenopetala

The concentrations of five trace elements (Ca, Cu Fe, K and Zn) in the digested and diluted solutions of *Moringa* were identified with AAS.

The unknown concentrations of all the metals were determined from the standard calibration graph. The level of total metals content in *Moringa* samples show that *Moringa* were rich source of mineral nutrients.

The results showed that out of the five analyzed trace metals Fe was found in the large amount compared to other with the concentration of 7.98±1.23 mg/kg, but Zn was the smallest one with the concentration of 0.37±0.006 mg/kg.

The amount of the analyzed metals from Arbaminch moringa samples were arranged in an increasing order of their concentration Zn < Cu < Ca < K < Fe and the concentration of these metals is less than the permissible limit of metals for plants that is recommended by WHO (Table 4.4). Accordingly, the results of *Moringa stenopetala* leaves showed that; in

International Journal of Scientific & Engineering Research, Volume 11, Issue 7, July -2020.
ISSN 2229-5518

Arbaminch the metal Fe has the highest concentration followed by K, Ca, Cu and Zn respectively. The mean concentrations of the metals are Fe 7.98±1.23 mg/kg, K 3.02±0.63 mg/kg, Ca 2.80±0.36 mg/kg, Cu 0.866±0.134 mg/kg and Zn 0.84±0.92 mg/kg.

In Wolaita Fe has the highest concentration followed by K, Ca, Cu and Zn respectively. The mean concentrations of the metals are Fe 7.13±1.32 mg/kg, K 3.50±0.40 mg/kg, Ca 3.28±0.28 mg/kg, Cu 0.94±0.16 mg/kg, and Zn 0.61±0.11 mg/kg.

In Konso Fe has the highest concentration followed by Ca, K, Cu and Zn respectively. The mean concentrations of the metals are Fe 6.79±0.73 mg/kg, Ca 2.97±0.67mg/kg, K 2.39±0.23 mg/kg, Cu 0.77±0.098mg/kg and Zn 0.37±0.006 mg/kg. These differences could probably be the result of climate and soil conditions (Hamurcu *et al*., 2010).

## 4.2 Comparison of metals in *Moringa stenopetala* of different sampling area

### 4.2.1 Calcium status

As shown in Table 4.4, the concentration of Calcium in *Moringa* plant for the studied areas ranged from 2.80±0.36mg/kg to 3.28±0.28 mg/kg. The concentration pattern of Ca in *M*oringa of studied areas was in the order: Wolaita > Konso>Arbaminch.

The concentration of calcium was relatively higher in Wolaita than other areas under study. According to the study conducted in SNNPR of Ethiopia, the levels of Ca reported to be 79.28mg/100g, which exist in significant concentrations (Abuye *et al*., 2003).

However, the result of this study showed that the concentration of calcium was below the above literature values.

There were significant differences at (P <0.05) among the mean concentrations of Ca metal in the leaf samples. These differences could probably be the result of plant nutrition, climate and soil conditions (Hamurcu *et al*., 2010).

The results indicated that the concentrations of Calcium in *Moringa* plant of all areas were below the permissible limit set by FAO/WHO, which is 100mg/kg.

### 4.2.2 Copper status

The highest concentration of Copper in the *Moringa* leaves was detected in Wolaita (0.945±0.16 mg/Kg) followed by Arbaminch and Konso with concentrations of 0.866±0.134 and 0.77±0.098 mg/ kg, respectively.The concentration of copper ranges from 0.77±0.098 mg/ kg to 0.945±0.16 mg/Kg (Table4.4).

According to previous study conducted the level of Cu was recorded to be 1.10 mg/kg (Abuye *et al*., 2003).

The result in this study showed that the level of Cu was in agreement with the literature values. There were significant differences at (P <0.001) among the mean concentrations of Cu metal in leaf samples from three agro–ecological areas under this study.

The results indicated that the concentration of copper in *Moringa* plant of all areas were below the maximum permissible limit set by FAO/WHO ;100mg/kg, (WHO, 2005).

### 4.2.3 Iron status

The highest concentration of Iron in leaves *Moringa* sample was detected in Arbaminch (7.98±1.23mg/Kg) followed by Wolaita and Konso with concentrations of 7.13±1.32, 6.79±0.73 mg/ kg respectively. The concentration of iron ranges from 6.79±0.73 mg/ kg to 7.98±1.23mg/Kg, (Table 4.4).

One-way analysis of variance showed that the mean concentration of Iron was significantly different among plant species in three agro-ecological zones, p <0.05. The concentration of iron in Wolaita was found to be significantly lower than the concentration of iron in Arbaminch and it was significantly higher than that of Konso. The mean concentration of iron in Arbaminch was significantly higher than mean concentration of iron and all other metals in all areas under the study.

Although the limit for iron in medicinal plants have yet not been established (WHO, 2005), the permissible limit set by FAO/WHO in edible plants was 20 ppm (FAO/WHO, 2004). Comparing the level of iron in this study with the above values, it was lower than permissible level of iron in edible plants.

### 4.2.4 Potassium Status

The higher concentration of potassium was observed in Wolaita (3.50±0.40 mg/Kg) followed by Arbaminch and Konso with concentrations of 3.02±0.63, and 2.39±0.23mg/kg respectively. The concentration of potassium ranges from 2.39±0.23mg/kg to 3.50±0.40 mg/Kg. Analysis of variance revealed that the mean concentration of potassium was significantly different among plant species in three areas of study, at p < 0.001.

International Journal of Scientific & Engineering Research, Volume 11, Issue 7, July -2020.
ISSN 2229-5518

The permissible limit set by FAO/WHO (2001), for potassium in edible plants was 350µg/g. After comparison, the metal concentration in this medicinal plant with those proposed by FAO/WHO, was found to be that K is below this limit for *Moringa* plants in studied area.

### 4.2.5 Zinc Status

The higher concentration of zinc was observed in Arbaminch (0.84±0.92 mg/Kg) followed by Wolaita and Konso with concentration of 0.61±0.11 and 0.37±0.006 mg/kg respectively.

Analysis of variance was revealed that the mean concentration of zinc was significantly different among plant species in study areas, at $p < 0.001$.

The mean concentration value of zinc in this study is in- line with the previous study; 0.53±0.08, conducted by Abuye *et.al* (2003).

The permissible zinc limit set by FAO/WHO in edible plants was 27.4 mg/kg. After comparison, metal limit in the studied medicinal plants with those proposed by FAO/WHO, was found that Zn is below this limit for edible plants. The WHO (2005) limits has not yet been established for zinc. The data/result of the present study matches well with the concentrations of zinc; 0.82 mg/kg, in other medicinal plants reported (Rashed, 1995; Shad *et al*., 2008; Amare *et al*., 2010; Muahammad *et al*., 2010).

The variations in mineral micronutrients, crude lipid and protein contents of the reported values may be due to different ages of trees, and possibly due to different stages of maturity (Yang *et al*., 2006).

## 4.3 Pearson correlation

The Pearson correlation matrices using correlation coefficient (r) from Tables 4.3 for the plant samples were shown in (Tables 4.6) for medicinal plant studied.

In plant samples for the metals analyzed, the correlation was very high (close to ± 1) for some metals like Fe and Cu, moderate (around ± 0.5) for some others like Ca and weak (< ± 0.5) for the remaining. The poor relationship might be due to the fact that different soil types, environmental conditions and capacity of the plant to accumulate specific metals were considered (H.D. White, 2003).

The values of Pearson correlation coefficient revealed that there was weak and/or moderate positive or negative correlation of metals with each other except for some metals. The weak negative or positive correlation indicates that the presence or absence of one metal affect in lesser extent to the other (EGGHE, Leo, Leydesdorff, L, 2009).

**Table 4. 9.** The Pearson correlation matrices between metals in Moringa samples

| | Cu | Zn | Ca | K | Fe |
|---|---|---|---|---|---|
| Cu | 1 | | | | |
| Zn | 0.37 | 1 | | | |
| Ca | 0.40 | 0.93** | 1 | | |
| K | -0.79** | -0.30 | -0.40 | 1 | |
| Fe | -0.15 | 0.01 | -0.20 | 0.21 | 1 |

**. Correlation is significant at the 0.01 level (2-tailed).

*. Correlation is significant at the 0.05 level (2-tailed).

Strong positive correlations were seen between; zinc and calcium in *Moringa stenopetala* plant leaves. There was positive correlation between calcium, copper and Zinc. There was strong negative relation between potassium and copper. There is negative relation between calcium, potassium and iron.

# 5. Conclusion

## 5.1 Conclusion

Based on the findings of this study the following conclusions were made.

The level of five trace metals namely; Calcium, Copper, Iron, potassium and Zinc in medicinal plant *Moringa stenopetala* were analyzed by flame atomic absorption Spectroscopy (FAAS).

The optimized procedures were followed for wet digestions method, in a digester heater block for the digestion of the medicinal plant samples powder.The optimized wet digestion method for *Moringa* analysis was found to be effective for all the minerals/metals and the effectiveness of digestion methods was revealed by the excellent recoveries obtained which were found within the acceptable range for metals analyzed.

In medicinal plant under the study, the concentration of Iron is the highest of all other elements in all areas (Arbaminch, Wolaita and Konso). However, the concentration level of Zinc is the smallest value recorded in three areas of study.

International Journal of Scientific & Engineering Research, Volume 11, Issue 7, July -2020.
ISSN 2229-5518

The distribution pattern of metals in *Moringa stenopetala* grown in Arbaminch was found to be in the order of; Iron > Potassium> Calcium >Copper > Zinc.

The concentration levels of all five trace metals in all areas of the study are below the upper permissible limits (UPL) for heavy metals in plant leaves/vegetables as recommended by WHO.

The results of current study suggest that the *Moringa* plant are safe to be utilized as both nutritional cabbage tree and medicinal plant, since the concentration of all trace metals are below/within the recommended/permissible limits.

Therefore, according to this study consuming of *Moringa stenopetala* leaves as food supplement and medicinal plant is recommendable and cannot lead health problems on consumers, because it has less concentration levels of these trace metals in the studied area when compared to WHO/FAO upper permissible limit for heavy metals in plants and vegetables.

## 5.2 Acknowledgements

This work was supported in part by a grant from ET Learns.

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