Journal of Science and Inclusive Development Vol. 2, No. 1, DOI: 10.20372/jsid/2020-42

Determination of Essential and Non-essential Elements in *Moringa stenopetala* Leaves and Flowers Using Inductively Coupled Plasma-Optical Emission Spectroscopy (ICP-OES) in Dawuro Zone, Southern Ethiopia

Solomon Ragasa[1] and Kusse Gudishe[1]*

[1]Department of Physics, College of Natural and Computational Sciences, Wolaita Sodo University, P. O. Box 138, Wolaita, Ethiopia

*Correspondence Author: anaxorma@gmail.com; Phone: +251993900417

Received: 13 April 2020; Revised: 17 May 2020; Accepted: 26 May 2020; Published: 26 June 2020

Abstract

This study aimed at determining concentration levels of essential elements (K, Ca, Fe, Mn, Zn, Cu, Ni) and non-essential elements (Pb, and Cd) present in leaves and flowers of *Moringa stenopetala*. Dry ash digestion method was deployed. The absorption intensity of the elements in *Moringa stenopetala* leaves and flowers were recorded by inductively coupled plasma optical emission spectroscopy. Results indicate that the mean concentration of the elements in *Moringa stenopetala* leave samples are (in mg/kg): Ca (5,713.86 - 6621.14), K (27,587.10 - 28,315.40), Pb (0.73 - 0.81), Zn (41.17 - 47.00), Cd (0.05 - 0.83), Cu (2.41 - 4.74), Ni (0.54 - 0.80), Mn (26.75 - 26.92), Fe (61.05 - 65.30). Moreover, the mean concentration of the elements in *Moringa stenopetala* flower samples are (in mg/kg): Ca (2,796.97 - 3,028.67), K (28,358.30 - 36,008.60), Pb (0.54 - 0.68), Zn (24.48 - 30.66), Cd (ND - 0.03), Cu (1.83 - 4.69), Ni (0.53-0.61), Mn (12.37- 14.52) and Fe (108.97 - 114.03). Results show that the levels of elements are higher in the leaves than in the flowers, except K. The levels of most elements analyzed in *M. stenopetala* plant samples of this study were compared well with those reported for different medicinal plants from some other parts of the world and standards set by FAO/WHO. Leaves of *M. stenopetala*, commonly consumed part in Southern Ethiopia, showed appreciable mineral contents and thus are good source of essential nutrients while toxic elements are found less than permissible limits set by FAO/WHO and are safe for human consumption.

Keywords: Dry ash, ICP-OES, Essential element, *M. stenopetala*, Non-essential element

Introduction

Moringa plant has been the object of much research due to its multiple uses and well-known anti-bactericidal potential (Suarez et al., 2003). Leaves of this plant have immense nutritional value such as phytochemicals, vitamins, minerals, and amino acids (Khan et al., 2015; Busani et al., 2011). *Moringa* leave has been purported to be a good source of nutrition and a naturally organic health supplement that can be used in many therapeutic ways (Fahey, 2005; McBurney et al., 2004). Its utilization increases woman's milk production (Anwar et al., 2007; Siddhuraju and Becker, 2003). Flower parts of this plant are a good nectar source for honey; it can be cooked and eaten or dried and steeped to make tea (Armelle and Mellanie, 2010).

Moringa stenopetala is native to Ethiopia. Local communities in the Southern Ethiopia cook its leaves as cabbage and eat with their traditional food known as "Kurkurfa" (Ali and Masood, 2016). This plant is used for medicinal purposes by the local people. *Moringa stenopetala* leaves and flowers contain essential elements as well as trace elements used for human beings (Aberra and Kefyalew, 2013). Essential trace elements (Fe, Mn, Zn, Cu, Ni) are nutrients required in minute quantities in a number of physiological functions (Rajan et al. 2014). They play essential role in the formation of the active chemical constituents in medicinal plants (Ebrahim et al., 2012). When these elements are in high concentration, they are dangerous for consumers either for medical or for food. Heavy metals (Pb, As, Hg and Cd) are potential environmental contaminants with the capability of causing human health problems if present in excess in the food we eat or in the traditional medicines we use. They are given special attention throughout the world due to their toxic effects even at very low concentration (Pramond and Devendra, 2014). The elemental contents of these plants are very important and need to be screened for their dosage control in traditional usage. Knowledge about elements concentrations in medicinal plants is important from the point of their nutritional requirements as well as their control to avoid risks associated with consumption (Devi et al., 2015; Raju et al., 2013).

Lack of knowledge of local people on the effects of heavy metals requires additional studies in such medicinal and traditional plants. Different research scholars have tried to determine the concentration of different heavy and trace elements in different parts of this plant (Ebrahim et al., 2012; Annan et al., 2010; Maharia et al., 2010). Raghayendra et al. (2016) determined major and minor mineral composition of *Moringa stenopetala* leaves using ICP-OES technique. Similar study was conducted on determination of concentration of elements of *Moringa stenopetala*

Journal of Science and Inclusive Development Vol. 2, No. 1, DOI: 10.20372/jsid/2020-42

leaves using Atomic Absorption Spectroscopy by wet digestion method (Ali and Masood, 2016). Another study was conducted on determination of some heavy metals (Cu, Pb, Fe, Zn and Cr) in *Moringa stenopetala* tree leaves at three growing stages (young, matured and aged) using Flame Atomic Absorption Spectrometry (FAAS) in Southern Ethiopia (Yeshanew and Kusse, 2018). Determination of mineral compositions of *Moringa stenopetala* leaves cultivated in Arbaminch Zuria and Konso, Southern Ethiopia, was done on elements such as K, Na, Mg, Ca, Mn, Fe, Zn and reported range of their concentrations (Debebe and Eyobel, 2017).

Therefore, the determination of elemental compositions in traditional plants, food and related products is essential for understanding their nutritive importance (Khan et al., 2015). On other hand, the presence of some heavy metals over permissible limits in the body may have a toxic effect (Khan et al., 2015; Sharma et al., 2009; WHO, 2003). Thus, this study seeks to determine the concentration level of essential (K, Ca, Fe, Mn, Zn, Cu, Ni) and nonessential (Pb, and Cd) elements in *Moringa stenopetala* leaves and flowers that can be used by the communities in their daily consumptions.

Materials and methods

Description of the study area

The study was carried out in Dawuro Zone which is found in Southern Nations Nationalities and Peoples Regional State (SNNPRS). Geographically, it is situated at 7° 00′ 00″ N and 37° and 37° 09′ 60.00″ E.

Agro-ecologically, it consists of all the three traditional agro-ecological zones that cover 500-3200 m (Asnake et al., 2018) and comfortable growing condition for *M. stenopetala* (World Agroforestry, 2009). The selection of the study area was based on the productivity of the plant under investigation. Again with the same procedure two Woredas, namely Mareka and Tercha Zuria; with high productivity were selected for target sample study.

Sample collection

Fresh *Moringa stenopetala* leaves and flowers, each weighing 3 g, were collected by plastic bags in June 2019 from the two selected Woredas. The two selected Woredas were each represented by one leave and one flower samples. Totally, two samples of leaves and two samples of flowers

of *Moringa stenopetala* were collected. The samples were labeled as MS$L_{1,}$ MSF_{1} (for Mareka) and MS$L_{2,}$ MSF_{2} (for Tarcha site). The number denotes the sites where the samples were collected. The symbol 'L' stands for leaves and the symbol 'F' stands for flowers.

Sample preparation

The collected samples were washed with deionized water to remove surface contaminants. Both collected *M. stenopetala* leaves and flowers were dried in laboratory. The dried samples were protected from sunlight and weighed. Samples were cleaned and then subjected to dryness in hot oven. The temperature was set and controlled exactly at 65^{o}C. Samples were allowed to dry in the oven for 24 hours. The dried samples were then cooled for about 30 minutes before grinding to powder. Then, the dried samples were grounded and homogenized in to fine powder by the use of grinder. The powdered leaves' and flowers' samples were stored in a polyethylene bottles until used for digestion.

Apparatuses and instruments

Plastic bags were used for collections of samples from the selected sites. Volumetric flasks of different volumes were used during dilution and volume measurement of chemical solution. Beaker was used for holding of liquids in laboratory. Drying oven (Digit heat, J.P. Selecta, Spain) was used to dry desired samples. Whatman filter paper No 42 was used to separate solid substances from liquids during filtrations. Digital balance was used to measure mass of the samples. Ceramic pestle and mortar were used for grinding and homogenizing the samples to obtain powder. High form Porcelain crucible was used to dry the sample at muffle furnace. Inductively Coupled Plasma Optical Emission Spectroscopy (ICP-OES ARCOS FSH 12 MODEL, Germany) was used for recording absorption of the elements in the samples and standard solutions.

Chemicals and reagents

Concentrated HCl (Merck, Germany), H_2O_2 (Merck, Germany) and 0.5% of HNO_3 (Merck, Germany) were used for digestion. Distilled and deionized water was used throughout the experiment for sample preparation, dilution and rinsing of the apparatus. Stock standard solutions containing 1000 mg/L (Merck, Germany) were used for preparation of calibration curves. Reagents used in the analysis were all analytical grade.

Journal of Science and Inclusive Development Vol. 2, No. 1, DOI: 10.20372/jsid/2020-42

Digestion method

Dry ash reported by Asnake et al. (2018) was deployed in this work for digestion of samples. Approximately 1.25 g of each dried *Moringa Stenopetala* leave and flower powdered were weighed by a digital analytical balance into a porcelain crucible. The porcelain crucible was then heated in muffle furnace. The muffle furnace was programmed to raise the temperature starting from room temperature and gradually increased to 540˚C for 6 hours until the end of the process. White ash was obtained. This ash was digested with mixed 10ml of concentrated acid and 90ml of deionized water with the ratio of (HCl: H_2O = 1: 9) in a porcelain crucible and heated on hot plate until evaporated to near dryness. The residue was filtered through Whatman No 42 filter paper into volumetric flasks. Then, the samples were washed and diluted with deionized water and transferred into a 100 ml of volumetric flask. Finally, the concentrations of the elements in this dissolved solution were determined absorptions recorded by inductively coupled plasma optical emission spectrometry (ICP-OES) (Spectro Arcos FSH 12, Germany). The blank solution digestion procedures were also carried out in similar way.

Preparation of standard solutions

Intermediate standard solutions containing 10 mg/L were prepared in 100 ml volumetric flasks from the standard stock solutions that contained 1000 mg/L for each element. Nine working standards were freshly prepared for each element from the intermediate standard by proper dilution with deionized water in 0.5% of HNO_3 for calibration curve purpose. The spiked samples were prepared by adding a small known quantity of the element standard solutions.

Method of validation

Method validation is used to confirm the analytical procedures employed for specific test for its suitability in an intended use. Results from method validation can be used to judge the quality, reliability and consistency of the results. In order to validate the analytical method, the following method validation parameters such as recovery test, instrument detection limit, method detection limit, limit of quantification, precision and accuracy and linearity studies were carried out.

Recovery test

The recovery test of this work was obtained from spiking solutions as

$$\%\text{Recovery} = \frac{\text{Conc. after spike} - \text{Conc. before spike}}{\text{Conc. of added sample}} \times 100\% \qquad (1)$$

Recovery values within 80-120% range are acceptable methods [26].

Instrumental detection limit

The instrumental detection limits (IDL) were obtained from ICP-OES (ARCOS FSH 12 MODEL, Germany) manual which are encoded in the equipment itself.

Method detection limit

Method detection limit (MDL) 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, but it may not necessarily be quantified as an exact value. The method detection limit was calculated by multiplying the standard deviation of the blank concentration (SD) by 3. The MDL of each element was determined as per previous literatures (Yohannes et al., 2019; Rehman et al., 2008).

$$\text{MDL} = 3 \text{ x SD} \qquad (2)$$

Limit of quantification

Limit of quantification (LOQ) is the level above which quantitative results may be obtained with specific degree of confidence. The LOQ is equal to10 times the standard deviation (SD) of the results for a series of replicates. It is used to determine a justifiable limit of detection and obtained as

$$\text{LOQ} = 10 \text{ x SD} \qquad (3)$$

Precision and accuracy

Precision was evaluated regarding repeatability by calculating the relative standard deviation (RSD) of the recovery percentage for each spiked level. The relative standard deviation for replicate analyses of the same sample was obtained (Miller and Miller, 2010).

$$\%\text{RSD} = \frac{\text{SD}}{\text{Mean value}} \times 100\% \qquad (4)$$

Journal of Science and Inclusive Development Vol. 2, No. 1, DOI: 10.20372/jsid/2020-42

Linearity

The linearity of the experiment was confirmed from linear equation of calibration curves. Calibration curves for all the nine elements analyzed in this work were constructed from their standard solutions.

Statistical analysis

All the determinations of concentrations of the essential and nonessential elements were computed in triplicate and then the results were expressed as mean ± standard deviation (SD). The results of the minimum, maximum, standard deviation, coefficient of variation and least significant difference values of the measured concentrations of the metals were tabulated. The determined data were analyzed by analysis of variance (ANOVA) using SAS software version 9.1.3. SPSS software version 20 was used to determine Pearson's correlation among the concentrations of the elements in the samples.

Results and Discussion

Validation methods

Calibration curves for all the nine elements were constructed from their respective standard solutions. It was noted that all the calibration curves confirmed the linearity nature of the experiment. Figure (1) only displays the calibration curve for calcium standard solution. The validation methods LOD and LOQ were calculated and determined. Table (1) displays method validation results of all the nine elements studied in this work.

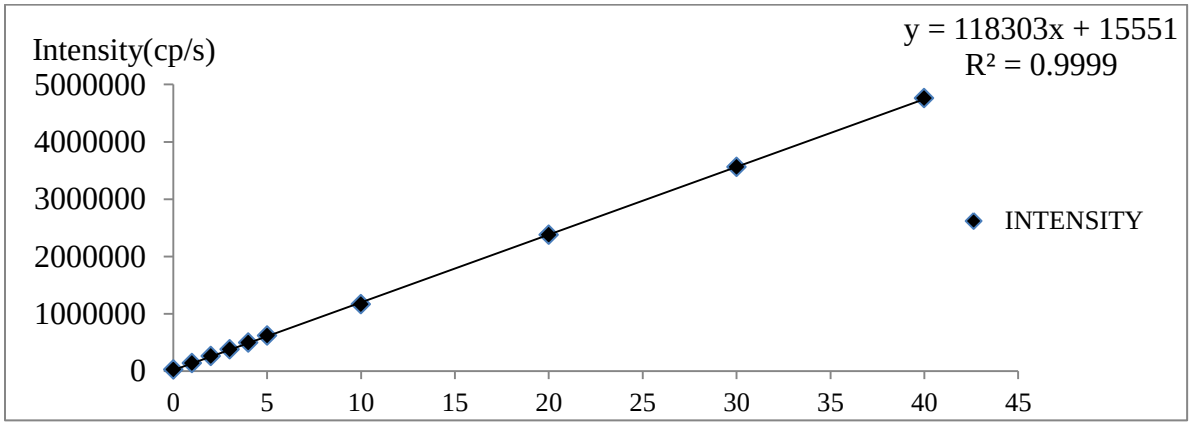


Figure 1. Calibration curve for Ca standard solution

Table 1. Spectral lines, Instrument detection limit, method detection limit and quantification limit for elements determined in this work

Elements	IDL(mg/kg)	Wavelength(nm)	LOD(mg/kg)	LOQ(mg/kg)
Cu	0.0025	214.44	0.06	0.6
Fe	0.0018	184.95	0.09	0.3
Mn	0.0002	324.75	0.03	0.3
Pb	0.0007	220.35	0.63	2.1
Ni	0.0007	259.94	0.07	0.25
Zn	0.0009	213.86	0.21	0.7
Cd	0.0001	231.60	0.02	0.07
K	0.0516	766.40	ND	ND
Ca	0.0023	317.93	ND	ND

IDL=Instrument Detection Limit, LOD=Limit of Detection, LOQ=limit of quantification, ND=Not detected

As can be seen from Table 2, the mean recoveries for the studied essential and nonessential elements in the spiked sample range between 80.4 and 116.86%. All the recovery values are within the acceptable range of 80-120% for elements analyzed in this work (Rehman et al., 2008). Figure (1) confirms the linearity (R^2 = 0.9999) of the experiments for Ca. The precision determined for each concentration level is less than 15% of the coefficient variation that fits in the acceptable range (Miller and Miller, 2010). The results indicate that the deployed method was linear, precise and accurate.

Journal of Science and Inclusive Development Vol. 2, No. 1, DOI: 10.20372/jsid/2020-42

Table 2. Recovery test for elements determined in this work

Elements	Mareka			Tercha			Added conc.
	Con. after spike (mg/kg)	Con. before spike (mg/kg)	RT (%)	Con. after spike (mg/kg)	Con. before spike (mg/kg)	RT (%)	
K	36074.7±288.81	27587.1±180.6	84.88	37337±131.07	28315.40±179.27	90.22	10000
Ca	11364.5±168.79	5713.86±65.19	113.01	12464.30±108.9	6621.14±52.89	116.86	5000
Fe	1808.58±77.96	61.049±2.65	87.38	1803.81±58.22	65.2960±2.25	86.93	2000
Zn	1811.05±85.19	47.003±2.36	88.20	1744.77±51.85	41.1660±1.21	85.18	2000
Mn	1670.18±69.07	26.92±1.41	82.16	1634.75±48.27	26.75±0.99	80.40	2000
Cu	462.19±15.34	2.411±0.133	91.96	467.29±9.97	4.7350±0.107	92.51	500
Ni	454.089±15.17	0.544±0.029	90.71	457.17±8.75	0.7963±0.065	91.27	500
Pb	486.531±25.96	0.7257±0.1588	97.16	482.54±26.61	0. 8090±0.1750	96.35	500
Cd	472.173 ±17.99	0.0487±0.0124	94.42	473.73±10.33	0.0520±0.0061	94.74	500

Concentration of elements

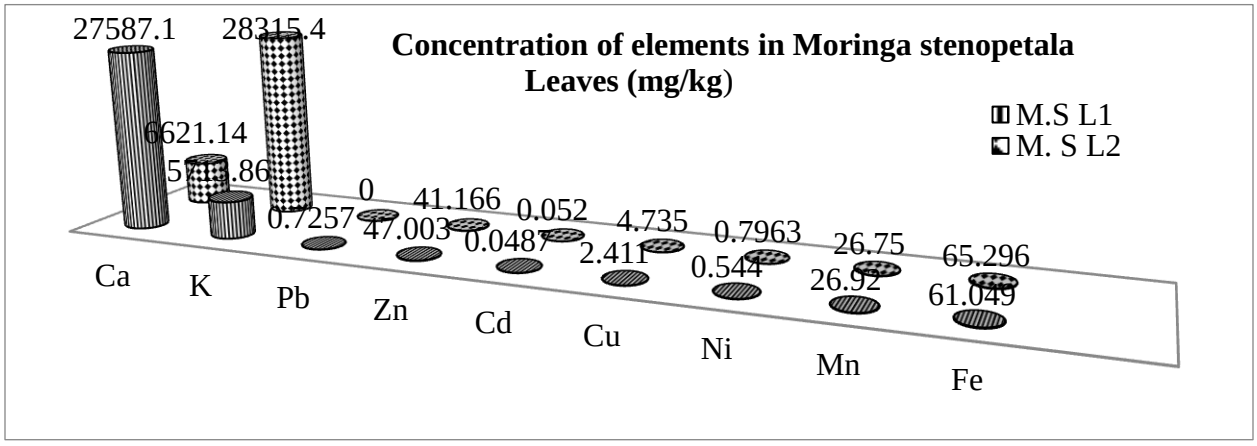
Concentration of elements in both types of the samples was observed. Only concentration of Cd in flowers of Maerka was seen to be below limit of detection of ICP-OES used in this work. Table (3) presents the mean concentration of the elemental analysis, LSD and CV of this work. The variations of content of the same elements in leaves and flowers were observed. It is very important to know trace and heavy elements differ in different parts of *Moringa stenopetala* tree in order to estimate their role as sources of human diet.

Table 3. Mean concentrations, LSD, CV of trace and heavy elements obtained in this work

Elements	Mareka		Tercha		LSD	CV
	Leaves	Flowers	Leaves	Flowers		
Ca	5713.86^{b}±65.19	2796.97^{d}±35.93	6621.14^{a}±52.89	3028.67^{c}±52.58	99.20	1.16
K	27587.1^{c}±180.46	28350.3^{b}±232.64	28315.4^{b}±179.27	36008.6^{a}±320.98	443.42	0.78
Pb	0.73^{ba}±0.16	0.54^{b}±0.05	0.81^{a}±0.18	0.68^{ab}±0.04	0.23	17.79
Zn	47.00^{a}±2.36	30.66^{c}±1.76	41.17^{b}±1.21	24.15^{d}±1.66	3.38	5.02
Cd	0.05^{a}±0.01	ND	0.05^{a}±0.06	0.025^{b}±0.03	0.01	22.32
Cu	2.41^{b}±0.13	1.83^{c}±0.17	4.74^{a}±0.11	4.69^{a}±0.23	0.31	4.84
Ni	0.54^{b}±0.08	0.61^{b}±0.06	0.796^{a}±0.07	0.53^{b}±0.05	0.12	10.18
Mn	26.91^{a}±1.41	14.52^{b}±0.79	26.75^{a}±0.99	12.37^{c}±0.57	1.87	4.93
Fe	60.74^{c}±2.54	106.18^{b}±5.82	65.30^{d}±2.23	114.03^{a}±3.79	7.28	4.47

Means with the same letter in a row are not statistically significantly different, ND = not detected

As can be seen from Figure (2), the concentration of K (36,008.60 ± 320.98 mg/kg) and Fe (114.03 ± 3.80 mg/kg) levels are high in flowers as compared to that of leaves in both sites. Concentration of Ca (5713.86 ± 65.19 mg/kg) is the highest values in Mareka (Figure 3) while concentration of K (36008.60 ± 320.98 mg/kg) showed the highest value at Tercha for leave samples. The levels of Ca concentration from the literature reports similar trends ranging from 15,100 mg/kg to 30,300 mg/kg (Fakankun et al., 2013). The difference between the nutritional compositions of *M. stenopetala* leaves and flowers from different locations could be due to the influence of soil characteristics, climate and environmental factors (Lamidi et al., 2017; Moyo et al., 2011).



Journal of Science and Inclusive Development Vol. 2, No. 1, DOI: 10.20372/jsid/2020-42

Figure 2. Concentration of both trace and heavy elements in *Moringa stenopetala* leaves

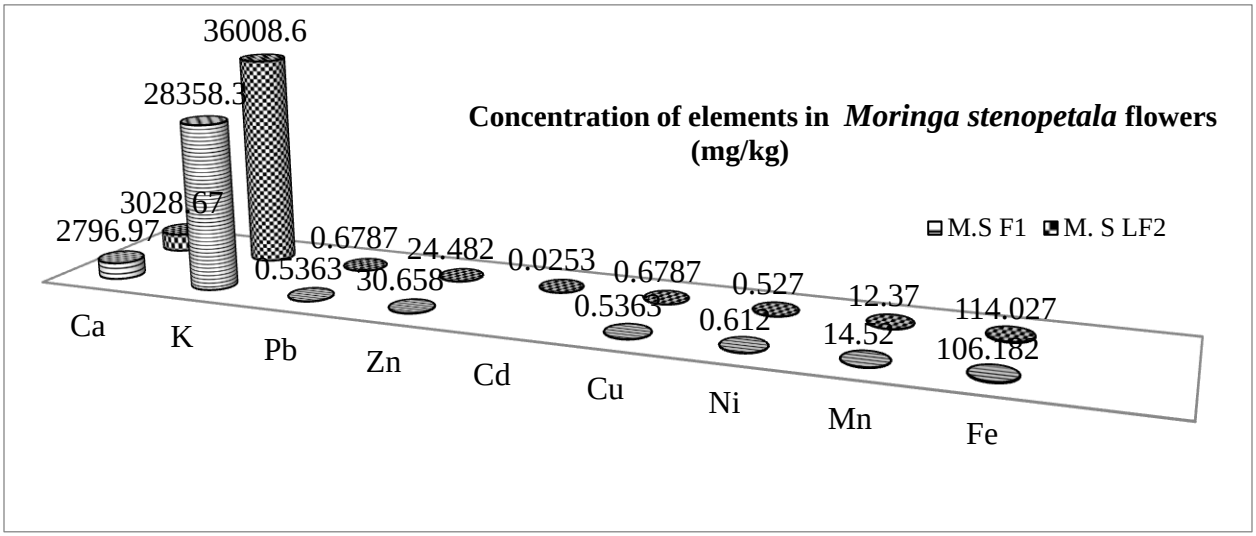


Figure 3. Concentration of trace and heavy elements in *Moringa stenopetala* flowers

A) Copper (Cu)

The concentrations of copper determined in this work in leaves samples are 2.41 ± 0.13 mg/kg and 4.74 ± 0.12 mg/kg while in flowers samples are 1.83 ± 0.17 mg/kg and 4.69 ± 0.23 mg/kg in Mareka and Tercha sites, respectively. More concentration of Cu is obtained in leaves and flowers samples of Tercha when compared to that of Mareka. The statistical analysis of one-way ANOVA shows that the mean concentration of Cu in Tercha has no significant variation ($P > 0.05$) between the tested leaves and flowers samples with CV of 4.84 and LSD of 0.31. The permissible limit set by FAO/WHO for Cu concentration in edible plants is 3.00 mg/kg (Jabeen et al., 2010). However, for medicinal plants, WHO (2005) limits has not yet been established for Cu, although, in medicinal plants, permissible limits for Cu set by China and Singapore are 20 mg/kg and 150 mg/kg, respectively (Khan et al., 2015). Results indicate that concentrations of copper in the two study areas in this work are below the limit set in China and Singapore. This indicates that using this plant as medicinal plant as well as food source is safe for health conditions.

B) Iron (Fe)

Iron is an important element as a nutrient for human beings. It is gained as a supplement from many plants which are rich in iron content. The iron concentration determined in this study is in

the range of 61.05 ± 2.65 - 65.30 ± 2.25 mg/kg. The result obtained from the present study shows that *Moringa Stenopetala* flowers have higher concentration of iron than that of leaves. A report presented by Debebe and Eyobel (2017) reveals that the iron concentration of Moringa stenopetala leaves range from 80.1 - 80.3 mg/kg. The results showed that the *Moringa stenopetala* leaves and flowers can be a good source of iron. The statistical analysis using one-way ANOVA showed that the mean concentration of Fe in the four samples showed significant variation ($P < 0.05$). The range of iron in the study of selective medicinal herbs in Egypt is between 261 to 1239 mg/kg (Jabeen et al., 2010). The permissible level set by WHO for iron in edible plants is 20 mg/kg (Saraf and Samant, 2013). The values are in accordance to recommended daily allowance of iron 100 to 130 mg/kg for children; 70 mg/kg for men and 120 to 160 mg/kg for women and breast feeding mothers (Ijarotimi et al., 2013). For both leaves and flowers of *Moringa stenopetala* studied in this work, the amount of iron accumulated is not less than the permissible level set by WHO (2005), however, is lower than similar reports. This is a caution for the need of identifying the source of the increment of this metal in the plant which could be soil properties of location where the plant grows (Anjorin et al., 2010) as the deficiency of Fe may result in anaemia.

C) Zinc (Zn)

Zinc is an important essential metal in protein synthesis, enzymes, energy production, and in maintaining membranes (Randjelovic et al., 2014). In this finding, the concentration of zinc analyzed in the *Moringa stenopetala* leaves and flowers samples are 47.00 ± 2.36 mg/kg and 30.66 ± 1.76 mg/kg in Mareka and 41.17 ± 1.21 mg/kg and 24.48 ± 1.08 mg/kg in Tercha, respectively. The concentration of zinc is higher in leaves as compared to its flower counterpart. Debebe and Eyobel (2017) reported that the concentration of zinc in *Moringa stenopetala* leaves falls in the range 21.3 - 57.6 mg/kg. A result obtained in this study is in good agreement with this work. One-way ANOVA analysis showed that the mean concentration of Zn in the four samples have statistically significant variation ($P < 0.05$) between the tested leave and flower samples among the sites with CV of 5.02 and LSD of 3.38. For medicinal plants, WHO (2005) limits has not yet been established for Zn. According to Jabeen et al. (2010), the range of Zn in agricultural products should be between 15 to 200 mg/kg and results in this work agree with this finding.

Journal of Science and Inclusive Development Vol. 2, No. 1, DOI: 10.20372/jsid/2020-42

D) Manganese (Mn)

Manganese is essential element for normal bone structure, reproduction and normal functioning of the Central Nervous System. Its deficiency causes reproductive failure both in males and in females (Lokhande et al., 2010). The concentrations of manganese in the *Moringa stenopetala* leaves samples analyzed in this work is 26.92 ± 1.41 mg/kg and 26.75 ± 0.99 mg /kg in Mareka and Tercha, respectively. The Mn concentration in the flowers' samples is found to be 14.52 ± 0.79 mg/kg and 12.37 ± 0.57 mg/kg in Mareka and Tercha, respectively. The obtained results show that Mn content is higher in leaves than in flower samples. One-way ANOVA analysis showed that the mean concentration of Mn showed statistically significant variation ($P < 0.05$) in flowers, but not in leaves. Literature from Debebe and Eyobel (2017) puts Mn concentration range as 72.4 to 87.5 mg/kg in *Moringa stenopetala* leaves which is far higher from results obtained in this work. However, this result of this study is an indication of identifying potential sources in the study area for the accumulation of manganese element in *M. stenopetala*.

The WHO acceptable limit for human consumption of manganese is 100 mg/kg (WHO, 2015). The permissible limit of edible plant proposed for manganese by WHO (2005) is 2 mg/kg. However, for medicinal plants, the WHO limits has not yet been established for Mn. The concentration of *Moringa stenopetala* in leaves and flower under the present study indicates that the concentration of Mn studied is around the normal range. The critical concentration of Mn is 300 to 500 ppm dry weight and the estimated safe and adequate daily dietary intake in adults is 11 mg/day (Khan et al., 2008).

E) Cadmium (Cd)

Cadmium is considered to be a highly toxic metal, able to cause many severe diseases (Batool and Khan, 2014). Cadmium poisoning in human beings could lead to anemia, renal damage, bone disorder and cancer of the lungs (Edward et al., 2013). The concentrations of cadmium are determined as 0.05 ± 0.01 mg/kg; 0.05 ± 0.01 mg/kg in leaves of Mareka and Tercha Zuria site, respectively. In flowers samples analyzed in this work, no Cadmium concentration is found in Mareka but is 0.03 ± 0.00 mg/kg was found in Tercha. The statistical analysis using one-way ANOVA showed that the mean concentration of Cd in the four samples showed no significant variation ($P > 0.05$) between the tested leave and flower samples among the areas with CV of

22.32 and LSD of 0.01. For medicinal plants, the permissible limit for Cd set by WHO, China and Thailand is 0.3 mg/kg. Similarly, permissible limits in medicinal plants for Cd set by Canada are 0.3 mg/kg in raw medicinal plant material and 0.006 mg/day in finished herbal products (WHO, 2005). The concentration of cadmium in this study is well below the limits set by standard setting organizations and some countries in the above literatures. As people of the study area use this plant for daily consumption, there could be no fear for health problems.

F) Potassium (K)

Among elements determined in this work, the highest mean concentration of potassium K is found in both leaves and flowers with the concentration of 27587.10 ± 180.46 - 36,008.60 ± 320.98 mg/kg. Results obtained in this work are higher than that of previous study of Debebe and Eyobel (2017) in *Moringa stenopetala* leaves. The statistical analysis of one-way ANOVA showed statistically significant variation ($P < 0.05$) between the tested leave and flower samples among the sites with CV of 0.78 and LSD of 443.42. WHO (2005) recommends an increase in potassium intake from food to reduce blood pressure and risk of cardiovascular disease, stroke and coronary heart disease in adults. This result indicates that *M. strenopetala* leaves and flowers could be good source for potassium.

G) Calcium (Ca)

The mean concentrations of calcium found in this work is the second highest next to K and falls in the range of 5,713.86 ± 65.19 - 6,621.14 ± 52.89 mg/kg in leaves samples and 2,796.97 ± 35.94 - 3,028.67±52.58 mg/kg for flowers samples. A result obtained by Debebe and Eyobel (2017) puts the concentration found in *Moringa stenopetala* leaves as 18,230-19,026 mg/kg which is higher than results found in current work. There is statically significant variation ($P < 0.05$) between results of leaves and flowers samples among the sites with CV of 1.16 and LSD of 99.198. High concentration of calcium is considered to be important in medicinal plants because of its role in bones, teeth, muscular system and heart functions (Muhammed et al., 2011) and consumption of this plant for food is a supplement.

H) Nickel (Ni)

Nickel plays some roles in body functions including enzyme functions. In very small amount, it may be beneficial to activate some enzyme systems, but its toxicity at higher levels is more

Journal of Science and Inclusive Development Vol. 2, No. 1, DOI: 10.20372/jsid/2020-42

evident. However, nickel toxicity is not very common in humans because nickel absorption is very low (Divrikli et al., 2006). In the findings of this study, the nickel concentration in the leaves is 0.05440 ± 0.0760 mg/kg in Mareka and 0.7963 ± 0.0652 mg/kg in Tercha and in flowers samples is 0.06120 ± 0.0570 mg/kg in Mareka and 0.5270 ± 0.0502 mg/kg in Tercha. It can be observed that Ni concentration in flowers is more than that of leaves. The permissible limit of Nickel set by WHO in edible plants is 1.63 mg/kg. However, for medicinal plants, the WHO limits has not yet been established for Ni (WHO, 2005). As can be seen from Table 3, the concentrations of Ni obtained in both leaves and flowers are below the permissible limit set by WHO and does not cause health problems.

I) Lead (Pb)

According to the finding of the current study**,** the concentration of lead in leaves' samples is found to be 0.7257 ± 0.1588 mg/kg in Mareka whereas 0.8090 ± 0.1750 mg/kg in Tercha. In the current work, the lead concentration in the flowers is 0.5363 ± 0.0465 mg/kg and 0.6787 ± 0.0404 mg/kg in Mareka and Tercha, respectively. From the statistical F-test, there are no significant differences ($P > 0.05$) in Pb concentration observed among the leaves' and flowers' samples analyzed. The content of lead analyzed in both leaves and flowers is below the permissible limit for medicinal plants set by China, Malaysia, Thailand and which is 10 mg/kg (WHO, 2005) and does not cause health problems for consumers.

J) Pearson's Correlation

The relationship between contents of different elements in leaves and flowers of *Moringa Stenopetala* were analyzed by Pearson's correlation coefficient. The correlation analysis is a bi-variant method which is applied to describe the relation between two different parameters. Table (4) presents Pearson correlation between the results.

Table 4. Pearson correlations of elements in Moringa St*enopetala* leaves and flowers in this work

	Ca	K	Pb	Zn	Cd	Cu	Ni	Mn	Fe
Ca	1.00								
K	-.546	1.00							
Pb	.614*	-.034	1.00						
Zn	.852**	-.782**	.401	1.00					
Cd	.884**	-.204	.704*	.696*	1.00				
Cu	.275	.576	.462	-.217	.445	1.00			
Ni	.547	-.403	.448	.319	.324	.331	1.00		
Mn	.961**	-.691*	.532	.962**	.821**	.033	.467	1.000	
Fe	-.942**	.698*	-.503	-.934**	-.798**	.025	-.382	-.970**	1.00

**Correlation is significant at 0.01 level (2-tailed). *Correlation is significant at 0.05 level (2-tailed).

Pearson's correlation at 0.01 and 0.05 significant level was calculated for finding relationship between content of elements in *Moringa Stenopetala* leaves and flowers. It was important to examine the correlations between the concentration of one element and other in *Moringa Stenopetala* leaves and flowers. Table (4) reveals that Ca is found strongly positively and significantly correlated with Zn (0.852), Cd (0.884) and Mn (0.961) while moderately correlated with Pb (0.614), but is strongly, significantly and negatively correlated with Fe (-0.942). K is significantly and negatively correlated with Zn (-0.782) and Mn (-0.691) and also positively and significantly correlated with Fe (0.698). It is found moderately and positively correlated with Cu (0.547). Zn is found positively and significantly correlated with Mn (0.962) and moderately and positively correlated with Cd (0.696); but significantly and negatively correlated with Fe (-0.934). Pb is found positively and significantly correlated with Cd (0.704) whereas moderately and positively correlated with Mn (0.532) and moderately and negatively correlated with Fe (-0.503).

As cane be seen from Table 4, Cd is significantly and negatively correlated with Fe (-0.798) but it is significantly and positively correlated with Mn (0.821), however, Mn is found negatively and significantly correlated with Fe (-0.970). On the other hand, there is insignificant correlation of Cu and Ni with other elements, except Ni has moderate and positive correlation with Ca (0.547) and Cu which is positively and moderately correlated with Pb (0.462) and K (0.576).

The high positive association between elements may arise from common anthropogenic or natural sources or the environment as well as from similarity in chemical properties (Birhanu and

Journal of Science and Inclusive Development Vol. 2, No. 1, DOI: 10.20372/jsid/2020-42

Chandravanshi, 2015; Wagesho and Chandravanshi, 2015; Zelalem and Chandravanshi, 2014). High and negative correlations between the elements indicate the large absorption of one may affect the absorption of the other metal (Yohannes et al., 2019; Wagesho and Chandravanshi, 2015). Elements with weak negative or positive correlations indicate that the presence or absence of one element affect in lesser extent to the other. This poor relationship might be due to environmental conditions and capacity of the plant to accumulate specific element (as well as associated with chemical properties like insoluble carbonates (Wagesho and Chandravanshi, 2015)

Trend analysis for the metal was carried out for leaves' and flowers' samples. The trends of results of elements undergone analysis in this work is displayed in Table (5). Leaves' and flowers' samples have similar trends of metal concentration. Potassium is seen to be high while cadmium is observed to be lower.

Table 5. Trends of elements in both *Moringa Stenopetala* leaves and flowers in this work

Moringa spetenopetala part	Trends of elements in concentration
Leaves	K > Ca > Fe >Zn > Mn > Cu > Pb > Ni >Cd
Flowers	K > Ca > Fe >Zn > Mn > Cu > Pb > Ni > Cd

Conclusion

The concentration levels of essential and nonessential elements from leaves and flowers of *Moringa stenopetala* were determined through dry ash digestion method using Inductively Coupled Plasma Optical Emission Spectroscopy (ICP- OES). Variation of the same element in leaves and flowers were observed. The variation of useful elements and trace element contents of *Moringa Stenopetala* leaves and flowers found in this study is more probably due to the environmental effects. The present study showed that *Moringa Stenopetala* leaves and flowers grown in the study areas are good sources of essential elements and minerals (Ca, K, Fe, Mn, and Cu) as appreciable contents of these elements are observed. On the other hand, harmful and heavy elements (Pb, Cd, Ni, and Zn) are found to be present below the toxic limits suggested by WHO and FAO and could not cause any health threat to the consuming population. Strong

correlations were observed in some elements. Further study has to be conducted to see the sources of the correlations and source of increments of some minerals.

References

Aberra Melesse, Kefyalew Berihun. 2013. Chemical and mineral compositions of pods of *Moringa stenopetala* and *Moringa Oleifera* cultivated in the lowland of Gamogofa Zone. J Environ Occup Sci. 2(1):33-38.

Ali MY, Masood AK. 2016. Atomic absorption spectrometric determination of some heavy metals from the leaves of *Moringa stenopetala* Grown in Gamo Gofa Zone, Ethiopia. British J App Sci Technol. 13:1-6.

Anjorin TS, Ikokoh P, Okolo S. 2010. Mineral composition of *Moringa oleifera* leaves, pods and seeds from two regions in Abuja, Nigeria. Int J Agric Biol. 12: 431–434.

Annan K, Kojo A, Asare C, Asare-Nkansah S, Bayor M. 2010. Profile of heavy metals in some medicinal plants from Ghana commonly used as components of herbal formulations. Pharmacognosy Res. 2: 41–44.

Anwar F, Latif S, Ashraf M, and Gilani AH. 2007. *Moringa oleifera*: A food plant with multiple Medicinal uses. Phytother Res. 21:17-25.

Armelle De SS, Mellanie B. 2010. Growing and processing of moringa Leaves. Moringa Association of Ghana. 27-29.

Asnake Boyana, Kefelegn Getahun, Ashenafi Melese Mathewos Agize. 2018. Suitability analysis for Enset (*Ensete ventricosum Cheesman* (*Welw*)) cultivation in Maraka District, Dawuro Zone, Southern Ethiopia. Int J Curr Res Aca Rev. 6: 43-53.

Batool S, Khan N. 2014. Estimation of heavy metal contamination and antioxidant potential of Pakistani condiments and spices. J Bio Env Sci. 5: 340-346.

Birhanu Mekassa B, Chandravanshi BS. 2015. Levels of selected essential and non-essential metals in seeds of korarima (*Aframomum corrorima*) cultivated in Ethiopia. Braz J Food Technol. 2: 102-111.

Busani M, Masika PJ, Hugo A, Muchenje V. 2011. Nutritional characterization of Moringa (*Moringa oleifera* Lam.) leaves. Afr J Biotechnol. 10:12925-12933.

Debebe Mikore, Eyobel Mulugeta. 2017. Determination of proximate and mineral compositions of *Moringa oleifera* and *Moringa stenopetala* leaves cultivated in Arbaminch Zuria and Konso, Ethiopia. Afr J Biotechnol. 16: 808-818.

Devi R, Sarma H, Sanjiv K. 2015. Investigation on trace and major elements in anti-asthmatic medicinal plants by PIXE and PIGE techniques. Nuclear Instruments and Methods in Physics Res. B: Beam Interactions of Materials and Atoms. 343: 163–166.

Divrikli U, Horzum N, Soylak M, Elci L. 2006. Trace heavy metal contents of some spices and herbal plants from Western Anatolia, Turkey. Int J Food Sci Technol. 41: 712-716.

Ebrahim AM, Eltayeb MH, Khalid H, Mohamed H, Abdalla W, Grill P, et al. 2012. Study on selected trace elements and heavy metals in some popular medicinal plants from Sudan. J Nat Med. 66: 671–679.

Edward JB, Idowu EO, Oso JA, Ibidapo OR. 2013. Determination of heavy metal concentration in fish samples, sediment and water from Odo-Ayo River in Ado-Ekiti, Ekiti-State, Nigeria. Int J Environ Monit Anal. 1: 27-33.

Fahey JW. 2005. A Review of the medical evidence for its nutritional, therapeutic, and prophylactic properties. Part 1. Trees for Life J. 1:5.

Fakankun OA, Babayemi JO, Utiaruk JJ. 2013. Variations in the mineral composition and heavy metals content of *Moringa oleifera*. Afr J Environ Sci Technol. 7: 372-379.

Ijarotimi OS, Adeoti OA, Ariyo O. 2013. Comparative study on nutrient composition, phytochemical, and functional characteristics of raw, germinated, and fermented *Moringa oleifera* seed flour. Food Sci Nutr. 1: 452-463.

Jabeen S, Shah MT, Khan S, Hayat MQ. 2010. Determination of major and trace elements in ten important folk therapeutic plants of Haripur basin, Pakistan. J Med Plant Res. 4:559-566.

Khan IA, Aziz A, Sattar M, Munawar HS, Manzoor Z, Raza MA, et al., 2015. Evaluation of wound healing potential of *Rumex Vesicarius* L. Leaf Extract and Fractions in Rabbit. Afr J Tradit Complement Altern Med. 12: 60-64.

Khan S, Waqas M, Ding F, Shamshad I, Arpd H. 2015. The influence of various biochars on the bio accessibility and bioaccumulation of PAHs and potentially toxic elements to turnips (Brassica rapa L). J Hazard Matter. 300: 243-253.

Khan ZR, Midega CAO, Amudavi DM, Hassanali A, Pickett JA. 2008. On-farm evaluation of the 'push–pull' technology for the control of stemborers and striga weed on maize in western Kenya. Field Crops Res. 106: 224–233.

Lamidi WA, Murtadha MA, Ojo DO. 2017. Effects of planting locations on the proximate compositions of *Moringa oleifera* leaves. J Appl Sci Environ Manage. 21:331-38331-338.

Lokhande R, Singare P, Andhele M, Acharya R. 2010. Study of some Indian medicinal plants by application of INAA and AAS techniques. J Nat Sci. 2: 26-32.

Maharia RS, Dutta RK, Acharya R, Reddy AVR. 2010. Pesticides, food contaminants and agricultural wastes. J Environ Sci Health, Part B. 45: 174–181.

McBurney RPH, Griffin C, Paul AA, Greenberg DC. 2004. The nutritional composition of African wild food plants: from compilation to utilization. J Food Comp Analysis. 17: 277-289.

Miller JN, Miller JC. 2010. Statistics and chemo metrics for analytical chemistry. 6th ed. Harlow: Pearson Education Limited Press; 34-142.

Moyo B, Masika PJ, Hugo A, Muchenje V. 2011. Nutritional characterization of Moringa (*Moringa oleifera* Lam.) leaves. African J Biotech. 10:12925-12933.

Muhammed A, Dangoggo SM, Tsafe AL, Itodo AU, Atiku FA. 2011. Proximate, mineral and anti- nutritional factors of Gardenia aqualla (Gauden dutse) fruit pulp. Pak J Nutr.: 10: 577-581.

Pramond HP, Devendra HJ. 2014. Determination of specific heavy metals in fruit juices using atomic absorption spectroscopy (AAS). Int J Res Chem Environ. 4: 163-168.

Raghavendra HL, Prashith KTR, Vijayananda BN, Dereje D, Tifsehit S. 2016. Nutritive composition and antimicrobial activity of *Moringa stenopetala* (Baker f.) Cufod. J Adv Med Pharm Sci. 10: 1-9.

Rajan J, Singh K, Kumar S, Mishra R. 2014. Trace elements content in the selected medicinal plants traditionally used for curing skin diseases by the natives of Mizoram, India.Asian Pac J Trop Med. 7: 410-414.

Raju N, Sarita P, Rao S, Rao B, Reddy B. 2013. Correlation of trace elemental content in selected anticancer medicinal plants with their curative ability using particle induced x-ray emission (PIXE). J Med Plants Res.; 7: 1081-1086.

Randjelovic SS, Kostic DA, Stojanovic GS, Mitic SS, Mitic MN, Arsic BB, Pavlovic AN. 2014. Metals content of soil, leaves and wild fruit from Serbia. Cent Eur J Chem. 12: 1144-1151.

Rehman S, Khan ZF, Maqbool T. 2008. Physical and spectroscopic characterization of Pakistan honey. Cien Inv Agr. 35: 199-204.

Saraf A, Samant A. 2013. Evaluation of some minerals and trace elements in *Achyranthes aspera* Linn. Int J Pharm Sci. 3: 229-233.

Sharma, Agrawal, Marshall. 2009. Heavy metals in vegetables collected from production and market sites of a tropical urban area of India. Food Chem Toxicol. 47: 583-591.

Siddhuraju P, Becker K. 2003. Antioxidant properties of various solvent extracts of total phenolic constituents from three different agro-climatic origins of drumstick tree (*Moringa oleifera* Lam.) leaves. J Agri Food Chem. 51:2144-55.

Suarez M, Entenza JM, Doerries C, Meyer E, Bourquin L, Sutherland J, et al. 2003. Expression of a plant-derived peptide harboring water-cleaning and antimicrobial activities. Biotechnol Bioeng.; 81: 13-20.

Wagesho Y, Chandravanshi BS. 2015. Levels of essential and non-essential metals in Ginger (Zingiber Officinale) cultivated in Ethiopia. Springer Plus. 4:107-120.

WHO (World Health Organization). 2005. Quality control methods for medicinal plant materials, revised, Geneva.

WHO (World Health Organization). 2015. Codex Alimentarius. General standard for contaminants and toxins in food and feed. Codex stan193-1995. Amended in 2015. Int Food Standards; 1-59.

World Agroforestry. 2009. Agroforestry Database 4.0.

Yeshanew Fikre, Kusse Gudishe. 2018. Determination of heavy metals in young, matured and aged leaves of *Moringa stenopetala* Tree Using Flame Atomic Absorption Spectroscopy in South Ethiopia. Phys Sci Int J. 20 (4): 1-8.

Yohannes Bogale, Alemu Lelago, Mesfin Bibiso. 2019. Determination of selected essential and non- essential metals of honey in Wolaita Zone, Southern Ethiopia. J Sci Incl Dev. 1(1): 16-33.

Zelalem KA, Chandravanshi BS. 2014. Levels of essential and non-essential elements in raw and processed *Lupinus albus* L. (White Lupin, Gibto) cultivated in Ethiopia. Afr J Food Agric Nutr Dev. 14: 2015-2035.