Original Research Article

Determination of Heavy Metals in Young, Matured and Aged Leaves of *Moringa Spetenopetala* Tree Using Flame Atomic Absorption Spectroscopy in South Ethiopia

ABSTRACT

This study was aimed at concentration determination of some heavy metals (Cu, Pb, Fe, Zn and Cr) in *Moringa Spetenopetala* tree leaves at three growing stages (young, matured and aged). Determination was made on samples collected from Southern part of Ethiopia using flame atomic absorption spectrometry (FAAS) with acidic digestive method deployed. In the results, three of five metals (Cu, Fe and Zn) are detected but Pb and Cr was not detected by the technique. Results indicated that presence of the metals in all the three growing stages (young, matured and aged) varied. It was observed that mean concentration of iron content increases as the age of the leave increases while mean concentration of zinc decreases as the age of the leave increases. Mean copper concentration was found to be higher in matured and lower in aged leaves. However, the heavy metals lead and chromium were not detected in this experiment.

Key words: FAAS, *Moringa Stenopetala*, Heavy metals, Concentration

1. INTRODUCTION

Moringa tree is a multi-purpose miracle tree with tremendous for food and medical potential [1]. *Moringa* is the genus of family of Moringaceae. It requires an annual rainfall of between 250 and 3000 mm. It is drought resistant tree. It grows best at altitudes up to 600 m but it still grows at altitudes of 1000 m. Worldwide, some 14 species of the *Moringa* tree have been reported.

Among these, the best studied with regard to potential medicinal uses and the identification of compounds of potential therapeutic importance, is *Moringa Oleifera*, which is native to the Indian subcontinent. *Moringa Stenopetala* species is endemic to East Africa [2] and grows widely in southern parts of Ethiopia.

Its parts have different potential medicinal and nutritional uses for human as well as animals. The *Moringa* leaves are nutritionally rich and excellent source of concentrated proteins, vitamins and minerals [3]. Studies indicate that the leaves have immense nutritional value such as phytochemicals, vitamins, minerals, and amino acids [4]. The edible leaves are eaten throughout East Africa and parts of Asia.

The root bark is used to kill different kinds of intestinal worms, increases food appetite, protect abdominal constipation, cure for different kinds of respiratory diseases such as bronchitis and influenza and the stem bark is being used to treat eye diseases, intestinal worms, and to decrease or neutralize the venom power of snake, bee and scorpion [5]. The bark is sometimes used to make mats and rope. A blue dye is also made from the wood in Senegal and Jamaica. The young pods of this tree are eaten much like green beans. The flowers can be eaten or used to make a tea. In Haiti, tea from the flowers is drunk for colds. The flowers provide good amounts of calcium and potassium [6].

Seeds can be extracted and eaten as "peas" (boiled or fried) when still green. The mature seed is about 40% oil. *Moringa* oil is of excellent quality (73% oleic acid, similar to olive oil) for cooking. It is used in cooking and perfumes and has been used as watch lubrication [6]. The Romans, Greeks, and Egyptians extracted edible oil from the seeds and used it for perfume and as a skin lotion. People in Indian subcontinent have long used *Moringa* pods for food.

Moringa Oleifera contains several elements which are the basic building block of matter. Some of the elements are calcium, magnesium, potassium, sodium and the minor elements are iron, zinc, copper and manganese [7]. In Africa, many studies have indicated that a vast number of indigenous wild plants play a significant role in the diet of the population [8, 9]. Vegetables are the cheapest and the most available sources of important nutrients, supplying the body with minerals, salts, vitamins and certain hormone precursors, protein, energy and essential amino acids [10]. *Moringa Stenopetala* is one of the most frequently cultivated indigenous species for its palatable leaves in the semiarid areas of Konso, Derashe and Arbaminch areas and locally called as Shiferaw (Amharic), Halako (GamoGofa), Shelkata (Konso), Haleko (Derashe) and Cabbage Tree (English) among local communities in southern Ethiopia [5].

In Ethiopian crops, *Moringa Stenopetala* tree leaves contained the highest median concentrations of all elements except Cu and Zn, which were greater in Enset (*false banana*). The median concentration of Se in *Moringa Stenopetala* leaves is 7-fold, 10-fold, 23-fold, 117-fold and 147-

fold more than that in amaranth leaves, baobab fruits, sorghum grain and maize grain, respectively. The median Se concentration is 78-fold and 98-fold greater in *Moringa Oleifera* in seeds than in sorghum and maize grain, respectively [11].

For people in the areas covered in this research, Moringa leaves are the common item of food per day. They consume it frequently. “Kurkofa”, local food from maize and sorgum, is prepared with Moring leaves. As Korkufa is a daily based food for those people the consumption of some heavy as well as trace elements is direct.

As can be seen from the literature, most of the studies tilt more of *Moringa Olivera,* which is more common in Asia. It can be believed that the common species in Ethiopia, *Moringa Stenopetala*, could has been evaluated in a similar manner where is more applicable in a more drought attacked area, such as Konso, Gamo Gofa. And this research tries to determine concentration of trace elements in the species *Stenopetala* in some areas of Southern part of Ethiopia.

2. MATERIALS AND METHODS

2.1. Description of the Study Area

The study was conduct in Gamo-Gofa and SegenArea Peoples (SAP) Zones. Konso-Karat, Konso-Dara and Derashe from Segen Area Zone and Shara and Lante from Gamo-Gofa Zone were considered in this work. Arba Minch Zuria, capital city of Gamo-Gofa Zone, is located at 6° 01’59” N and 37° 32’59” E, at altitude of 1269 m.a.s.l and 505 km away from the capital city, Addis Ababa. Konso is located at 5°15’00” N and 37°28’59” E and altitude of 1031 m.a.s.l. It is 536 km far from Addis Ababa.

2.2. Sampling Protocol

Fresh leaves of *Maringa Setnopetala* tree were collected from the selected study areas. The study areas were selected purposefully based on the productivity and regular *Maringa Setnopetala* leaves consumption habits of the people in the study areas. However, Woredas were randomly selected. Samples were based on three growing stages of leaves of the same as young, matured and aged (See figure 1). Young leaves are very emerging soft leaves with yellowish color and of 2.48 cm height and 1.38 cm width. The matured leaves are next to young leaves on the same branch. They are green in color. Matured leaves are 5.48 cm high and 2.9 cm wide in average. Aged leaves are the ones relatively aged. At this stage the color changes from very green to yellowish and are relatively hard in structure. They are on average 4.78 cm and 2.46 cm wide. Leaves were picked from the same main vein and 500 g of the samples were collected from each place and placed in pre-cleaned plastic bags, labeled and was transported to the laboratory for further treatment. Total of 15 samples were collected and analyzed according to their growing ages. For data interpretation, we have made designations: young – A, matured – B and aged - C.

Fig 1. *Moringa Stenepetala* leave sample from Konso-Karat.

2.3. Sample Preparation

The *Moringa Stenopetela* leaves samples were washed with deionized water to remove dust materials and were air dried in a drying oven at 70^0C for 12 hours ensuring their greenish coloration and maintaining nutritional values. The samples were sieved through 2 mm sieve to remove coarse particles. The powders were package in pre-cleaned bags, labeled and stored at room temperature 24-26^0C. One gram of sieved samples were weighed and kept in acid washed glass beaker. Then the samples were digested by the addition of 20 cm3 of aquaregia (mixture of HCl and HNO_3, ratio 3:1) and 10 ml of 30% H_2O_2. The H_2O_2 was added in small portions to avoid any possible overflow leading to loss of material from the 100 ml conical flask. The analyt was digested for 2 hr in 100 ml conical flask covered with watch glass, and reflex over a hot plate at 90°C. The conical flask wall and watch glass was washed with distilled water and the samples were filtered out to separate the insoluble solid from the supernatant liquid. The volume was adjusted to 100 ml with distilled water. Blank solution was handled as detailed for the samples.

2.4. Experimental Setup

Flame atomic absorption spectrophotometer (Model: 210-VGP, USA) was used for absorbance recordings of Pb, Cu, Fe, Zn and Cr. Working standard solutions of all metals were prepared from stock standard solution (1000 ppm) and absorbance was noted from standard solution of each element. Signal of each radiation for specific element was detected and were converted into concentration information for the analyts from calibration curves of each element.

2.5. Statistical Analysis

All measurements were done in triplicates and expressed as mean ± standard deviations. Data were analyzed using one-way analysis of variance (ANOVA) at probability level of 5% ($P \leq 0.05$) followed by least significant difference Post Hoc test in Microsoft Excel for the determination of statistical significance of a given metal across the samples. Data were further manipulated with ASA and SPSS 20 as well as Origin pro 8 software.

3. RESULTS AND DISCUSSIONS

3.1. Results ~~and Analysis~~

To know how much of the concentration of the element out of the quantity taken in measurement, it is of high importance to know first the standard concentration of a given element. The working standard solutions of each metal were prepared from 100 mg/l standard solutions of their respective metals. The calibration graphs of standard solutions of the three metals detected in this work were drawn using the standard solution data and unknown concentrations of each metal was determined using the slope equation from the calibration graph. While calibrations curves were constructed for all the three metals, graph of copper standard solution is shown in Figure 2 from the concentration and its respective absorbance of standard solutions.

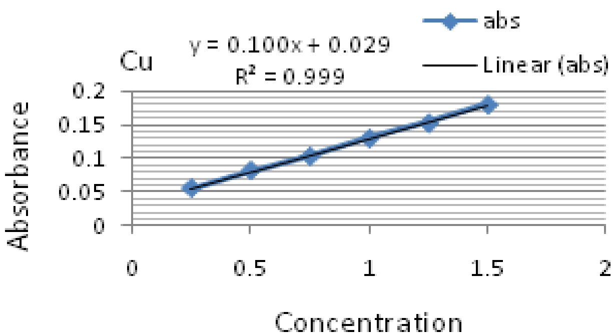

Fig 2. Calibration curve for Cu.

Comment [a2]: This figure should be included in the Material and Method chapter

In order to maximize the reproducibility of the experiment, data have been taken three times for each sample at all sites. Then average of the triplicate data is tabulated in Table 2 with help of SSPS and ASA data software manipulations. One-way analysis of variance (ANOVA) for heavy metals concentration in mg/kg at different sites was deployed to see statistical significance in the concentration of a single element in the same area along all the three growing stages of the leaves. Table 1 presents the average concentration and results from SSPS and ASA software analysis outcome.

Comment [a3]: This information should be included in the Material and Method chapter

Table 1. Mean concentration (mg/kg) of heavy metals in this work

S.N	Sample site	Concentration				
		Pb	Cu	Fe	Zn	Cr
1	Konso Karat A	ND	1.4676^{ED} ± 0.017	1.9645^{J} ±0.253	0.6440^{E} ±0.020	ND
2	Konso Karat B	ND	1.5307^{ED}±0.0981	3.0248^{H} ± 0.0251	0.5381^{HG} ±0.0225	ND
3	Konso Karat C	ND	1.5374^{ED}±0.0407	3.9078^{BCD}±0.085	0.5232^{H} ±0.6033	ND
4	Konso Daraa A	ND	2.3450^{BC}±0.0624	2.3404^{I} ±0.1610	1.3161^{A} ±0.0238	ND
5	Konso Daraa B	ND	2.8601^{A} ±0.0113	3.1844^{GH} ±0.1053	1.1785^{B} ±0.1195	ND
6	Konso Daraa C	ND	1.6996^{D} ±0.0140	4.0993^{BC} ±0.1919	1.0986^{C} ±0.1818	ND
7	Derashe A	ND	2.1928^{C} ±0.0073	2.0496^{JI} ±0.1928	0.7509^{D} ±0.0635	ND
8	Derashe B	ND	2.7836^{A} ±0.0125	3.7234^{ED} ±0.1397	0.5734^{FG} ±0.2451	ND

9	Derashe C	ND	$1.3612^{E} \pm 0.0111$	$4.4397^{A} \pm 0.1769$	$0.5232^{H} \pm 0.1280$	ND
10	Gamo-Gofa1 A	ND	$2.0625^{C} \pm 0.0184$	$1.8511^{J} \pm 0.1606$	$0.5381^{HG} \pm 0.1951$	ND
11	Gamo-Gofa1 B	ND	$2.7139^{A} \pm 0.0199$	$3.5319^{EF} \pm 0.1739$	$0.4609^{I} \pm 0.0854$	ND
12	Gamo-Gofa1 C	ND	$0.9093^{F} \pm 0.0185$	$4.1844^{BA} \pm 0.2235$	$0.3587^{J} \pm 0.0818$	ND
13	Gamo-Gofa2 A	ND	$2.0492^{C} \pm 0.0525$	$1.8014^{J} \pm 0.2623$	$0.7602^{D} \pm 0.1717$	ND
14	Gamo-Gofa2 B	ND	$2.6341^{BA} \pm 0.0302$	$3.3404^{GF} \pm 0.1124$	$0.5093^{H} \pm 0.1253$	ND
15	Gamo-Gofa2 C	ND	$0.9093^{F} \pm 0.0073$	$3.8227^{ECD} \pm 0.1722$	$0.6115^{FE} \pm 0.1253$	ND
LSD			0.332	0.2948	0.0408	
CV			10.25395	5.609276	3.535863	
F Value			31.49	82.06	402.17	
Error			0.03964241	0.03124591	0.00059930	

ND - not detected, Gamo-Gofa 1-Shara, Gamo-Gofa 2-Lante, CV- Coefficient variance, Means with the same letters are not statistically significantly different

The mean concentrations of elements detected in this work were generated with respect to ages of the leaves of the same main vein in the study areas.

Comment [a4]: This information should be included in the Material and Method chapter

Copper (Cu)

One-way analysis of variance showed that the average concentration of copper in *Moringa Stenepetala* leaves has showed significant difference (33 %) as its age progresses, except Karat sample where there is no significant difference between the average concentrations of copper in young, matured and aged leaves. This significant variance was confirmed with higher value of coefficient variance (10.25395). Aged leaves (C) of the *Moringa Stenopetala* have got less concentration of copper whereas matured leaves (B) contained high average concentration of copper. Moreover, young (A) leaves have intermediate copper concentration between the aged and matured ones.

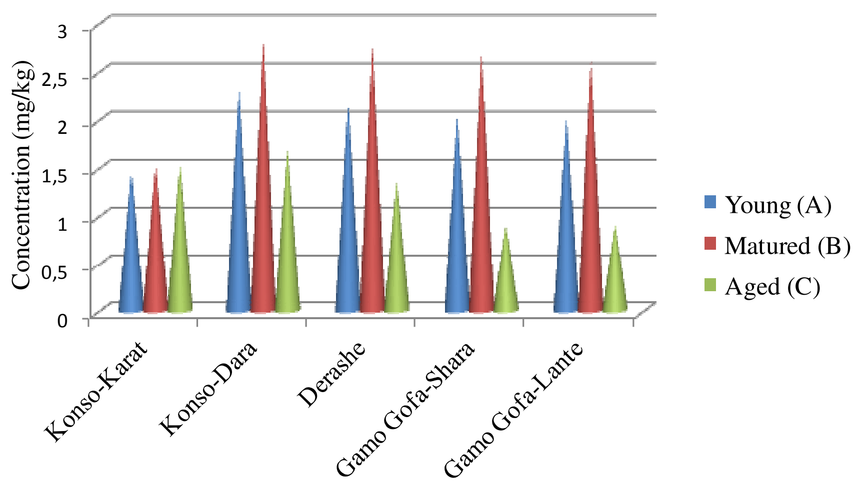

Fig 3. Copper concentration at different growing ages.

It can be observed from Figure 3 that in comparisons between different sites (Konso, Dirashe and Gamo Gofa) at the same growing age, matured leaves (B) in Konso-Karat sample (1.5307±0.0981 mg/kg) had less concentration than other sites. As can be seen from Table 2, the average concentration of copper in all sites at different growing stages showed statistically significant different value, except Karat sample. Furthermore, concentrations of copper in Karat sample in young leaves (A) (1.4676 ±0.017 mg/kg) had less value than that of other site. The concentration of copper in aged leaves (C) is significantly similar in Gamo-Gofa areas and approximately similar in Konso and Derashe sites. The concentration of copper is greater in matured leaves and followed by intermediate value in young leaves and less in aged leaves in all sample sites (i.e B > A > C) (See Figure 3).

Iron (Fe)

The analysis of one-way analysis of variance (ANOVA) showed that the concentration of iron is significantly different among sampled sites. The concentration of iron in young leaves is significantly similar in all sample sites but slightly greater in Konso-Darra (1.9645 ±0.253 mg/kg) and Derashe (2.0496 ±0.1928 mg/kg) sample site. On the other side, the concentrations of iron in aged leaves have got high concentration in all sample sites.

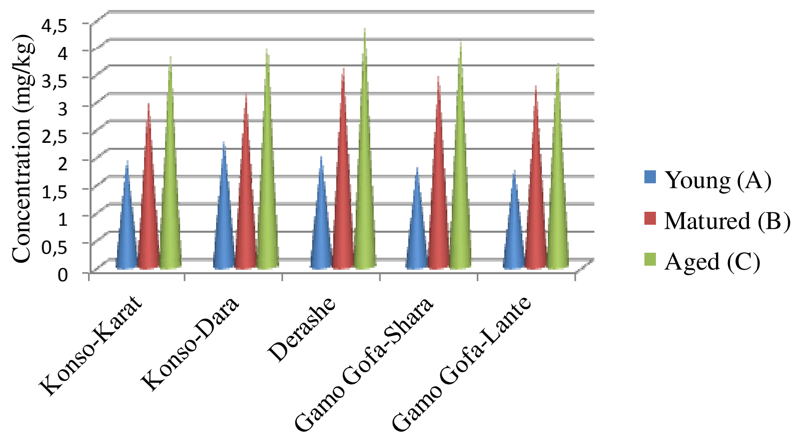

Fig 4. Concentration of iron

As can be seen from Figure 4, it can be said that, unlike copper, the concentration of iron increase as the age of the leaves increase. The average concentration of aged leaves in Derashe area (4.4397 ±0.1769 mg/kg) is higher than all the other areas while in Gamo Gofa it was lower than other areas of study in this work. As can be seen from Table 2 and Figure 4, ~~it can be noticed that~~ averagely greater, intermediate and less concentration was observed in aged, matured and young leaves, respectively, in all sample sites (i.e C > B >A).

Zinc (Zn)

Moreover, one-way analysis of variance showed that the concentration of zinc is significantly different among sampled sites. The concentration of zinc averagely and comparatively is higher in Konso-Darra study area. On the other hand, it has got less concentration in Gamo Gofa (Shara) area averagely as its age progresses. The concentration levels of young leaves (A) were significantly similar in Gamo-Gofa and Derashe samples. Less (4%) significant difference was observed in zinc and is confirmed with less CV (3.535863) value and high F value (402.17).

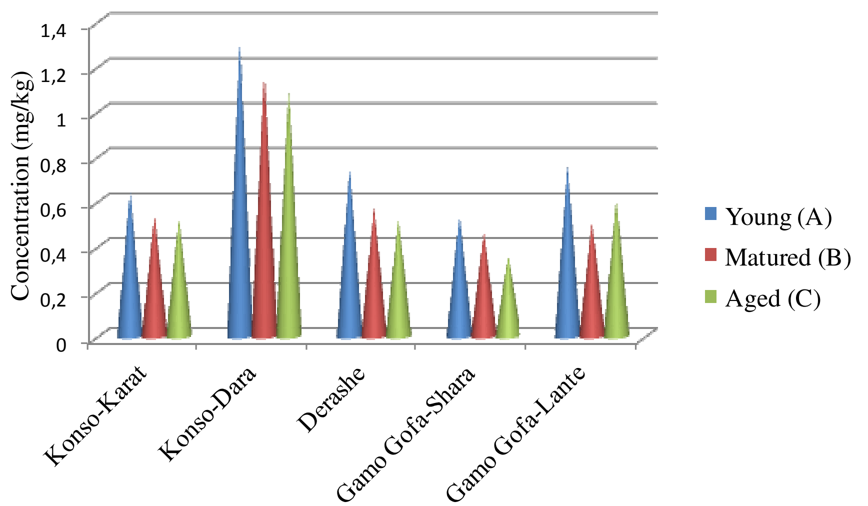

Fig 5. Concentration of Zinc

On top of that, it can be seen that the concentration level of zinc in all study areas covered in this work decrease as the ages of the leaves increases. As can be seen from Figure 5, the zinc concentration is greater in young leaves (A) in all sample sites and less in aged leaves of *Moringa Stenopetala* tree leaves in the study areas. (i.e A >B >C).

The concentration of lead and chromium elements in all sites covered under this study were not to the level of detection of spectroscopic technique deployed in this experiment and thus were not detected by the lamp. In general, it can be observed that iron presents in more amounts and zinc with less amount whereas cooper takes the in-between place in value of concentration of the analyzed metals in this work.

3.2. Discussions

Table 2 displays the WHO limit and permissible range in heavy metals traced in this study. The concentration of copper falls in the range of 0.91-2.86 mg/kg in the study areas. As can be seen from the Table 2 and comparing with the values obtained in this study, the copper content in young and matured leaves lie in the permissible range. Thus, the one who wants more copper in his/her diet can take young and matured leaves than the aged leaves.

Table 2. WHO limits, concentration of permissible ranges (ppm) of heavy metals in plants [12, 13]

Heavy metals	Concentration		Permissible range
	Normal	Toxic	
Cu	3-15	20	2-5
Pb	1-5	20	0.50-30
Zn	15-150	200	20-100
Fe	50-250	>500	400-500

Research conducted in Arba Minch area, Gamo-Gofa administrative zone, determined that concentration of copper metal in *Moringa Stenopetala* leaves was 0.67 mg/kg [14]. However, results obtained in this work in Gamo-Gofa area showed more presence of concentration of copper than the one revealed in the research of Ali *et al*. Kassa Belay and his coworkers have found that the average concentration of copper metal in *Moringa Oleifera* leaves collected from Wukro was 2.866±0.0436 mg/kg [15]. This result agrees with the result of this work.

Ali and his co-researchers determined that the concentration of iron metal in *Moringa Stenopetala* leave collected from Arba Minch area, Gamo-Gofa administrative zone, was 1.18 mg/kg [14]. This is very close to the result found in this research in Gamo-Gofa (Lante) area. The concentration of iron in this research was found to be in the range of 1.8014±0.2623-4.4397±0.1769 mg/kg, which is more than that of Ali and his coworkers' result. As can be seen from Table 2, the concentration level of iron found in this work is below the toxic limit set by WHO [12, 13].

The concentration of zinc in the *Moringa Stenopetala* tree leaves considered in this research is determined to be between 0.3587±0.0818 - 1.3161±0.0238 mg/kg on average. Limmatvapirat and other researchers recorded that the concentration of zinc in *Moringa Oleifera* leaves in rural garden in Thailand using ICP-MS was 1.1 mg/kg [16]. This is in the range of the average of the concentration of *Moringa Stenopetala* found in this research.

It can be observed that the amount of the analyzed metals in the *Moringa Stenopetala* leaves can be arranged in an increasing order of their concentration as Fe < Cu < Zn and the concentration of these metals is less than the permissible limit of metals for plants recommended by WHO [12, 13].

4. CONCLUSIONS

The analysis and identification of heavy metals from the leaves of *Moringa Stenopetala*tree at different growing stage using flame atomic absorption spectroscopy was determined in wet digestion method. The optimized wet digestion routine for analysis was found effective for three of the trace heavy metals.

Results showed that elements had showed difference in concentration as the age of the leaves progress in all sites. Zinc concentration showed decrement as the age of the leaves increased (i.e A >B >C). On the opposite side, averagely greater, intermediate and less concentration of iron was observed in aged, matured and young leaves, respectively, in all sample sites (i.e C > B >A). The concentration of copper is greater in matured leaves and followed by intermediate value in young leaves and less in aged leaves in all sample sites (i.e B > A > C).

Comment [a5]: This conclusion should speak more to relevance of results presently this is just repeating results.

COMPETING INTERESTS

There is no competing interest.

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