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

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Authors' contributions

This work was carried out in collaboration between both authors. Both authors read and approved the final manuscript.

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ABSTRACT

This study was aimed at concentration determination of some heavy metals (Cu, Pb, Fe, Zn and Cr) in *Moringa stenopetala* 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.

Keywords: FAAS; Moringa stenopetala; heavy metals; concentration.

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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.

Studied in Africa have tried to cover in determination of heavy and trace elements in different parts of *Moringa oleifera* tree. In Nigeria researches observed presence of Cu, As, Pb, Cd, Cr, Mn and Zn elements [5]. Another study in the same country reported elements like As, Cd, Co, Cr, Cu, Fe, Pb, Mn, Ni and Zn [6]. Study in South Africa indicated that presence of major and trace nutrient elements in leaves nd flowers of *Moringa oleifer*a tree [7].

Limmatvapirat et al. resented comparisons of eleven heavy metals in various products of *Moringa oleifera* were analyzed to determine eleven heavy metals (Al, As, Cd, Cr, Cu, Fe, Pb, Mn, Hg, Ni, and Zn) using Inductively Coupled Plasma-Mass Spectrometry [8]. Jaya and Amit studied concentration of trace metals in the leaves of *Moringa oleifera* [9]. In the assessment carried by Eva et al., indicated existence of both major and minor elements in *Moringa oleifera* tree leaves [10]. Abdulkadir et al. assessed heavy metals (Fe, Cu, Pb, Ni and Zn) in root, bark and leaves of medicinal plant Moringa oeifera [11]. Other research assesses nutritional quality and saft of heavy metals in *Moringa oleifera* [12].

For people in the areas covered in this research (Konso, Derashe and Aamo Gofa), *Moringa* leaves are the common item of food per day. They consume it frequently. “Kurkofa”, local food from maize and sorgum, is prepared with *Moringa* leaves. As Korkufa is a daily based food for those people the consumption of some heavy as well as trace elements is direct because it is consumed directly after immediate harvesting. It needs testing of the heavy metals concentrations in green leaves innorder to know amount of heavy metals people consume through leaves of *Moringa stenopetala*. On top of that People of Konso, Derashe and Aamo Gofa rely on the matured growing age of the leaves to consume. Therefore, investigation of heavy metals as the age of the leaves progresses has to draw attention of researchers in this regard.

As can be seen from the literature, most of the studies tilt more of *Moringa oleifera,* 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 heavy metals in the leaves of species s*tenopetala* at three growing ages 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 stenopetala* tree were collected from the selected study areas. The study areas were selected purposefully based on the productivity and regular *Maringa stenopetala* 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 Fig. 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.

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 dried leaves grounded to get powder out of it. The powder samples were sieved through 2 mm sieve to remove coarse particles. Sieved 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 (FAAS) (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.

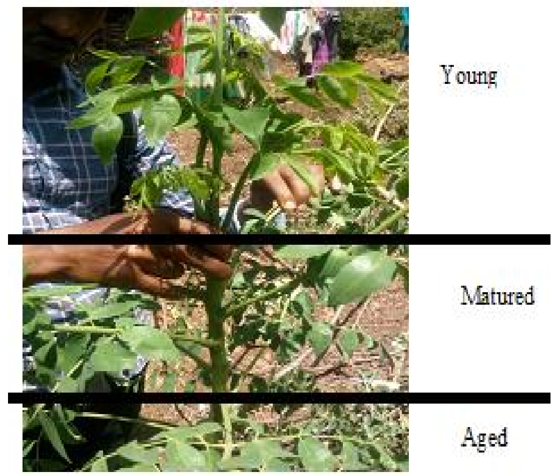

Fig. 1. *Moringa stenopetala* leave sample from Konso-Karat

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 DISCUSSION

3.1 Results

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 (Fig. 2 for Cu only).

Results obtained in this work are displayed in Table 1. Concentrations were reported in (mean ± standard deviation).

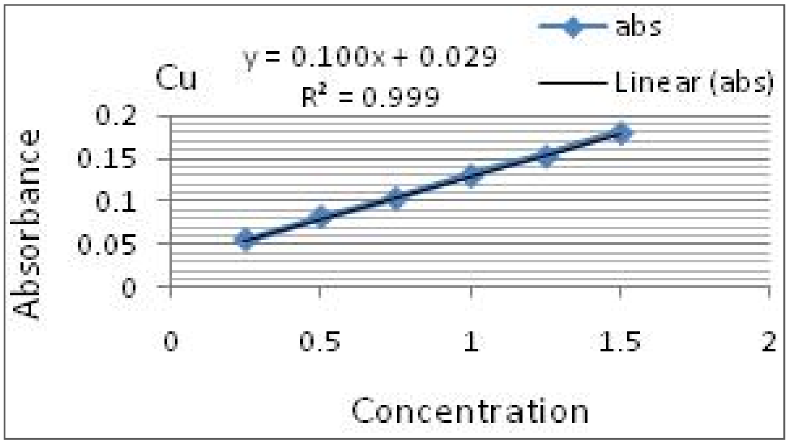

Fig. 2. Calibration curve for copper metal

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	1.47^{ED} ± 0.02	1.96^{J} ±0.25	0.64^{E} ±0.02	ND
2	Konso Karat B	ND	1.53^{ED}±0.10	3.02^{H} ± 0.03	0.54^{HG} ±0.02	ND
3	Konso Karat C	ND	1.54^{ED}±0.04	3.91^{BCD}±0.09	0.52^{H} ±0.60	ND
4	Konso Daraa A	ND	2.35^{BC}±0.06	2.34^{I} ±0.16	1.32^{A} ±0.02	ND
5	Konso Daraa B	ND	2.86^{A} ±0.01	3.18^{GH} ±0.11	1.18^{B} ±0.12	ND
6	Konso Daraa C	ND	1.50^{D} ±0.01	4.10^{BC} ±0.19	1.10^{C} ±0.18	ND
7	Derashe A	ND	2.19^{C} ±0.01	2.05^{JI} ±0.19	0.75^{D} ±0.06	ND
8	Derashe B	ND	2.78^{A} ±0.01	3.72^{ED} ±0.14	0.57^{FG} ±0.25	ND
9	Derashe C	ND	1.36^{E} ±0.01	4.44^{A} ±0.18	0.52^{H} ±0.13	ND
10	Gamo-Gofa1 A	ND	2.06^{C}±0.02	1.85^{J} ±0.16	0.54^{HG} ±0.20	ND
11	Gamo-Gofa1 B	ND	2.71^{A}±0.02	3.53^{EF} ±0.17	0.46^{I} ±0.09	ND
12	Gamo-Gofa1 C	ND	0.91^{F} ±0.02	4.18^{BA} ±0.22	0.36^{J} ±0.08	ND
13	Gamo-Gofa2 A	ND	2.05^{C} ±0.05	1.80^{J} ±0.26	0.76^{D} ±0.17	ND
14	Gamo-Gofa2 B	ND	2.63^{BA}±0.03	3.34^{GF} ±0.11	0.51^{H} ±0.13	ND
15	Gamo-Gofa2 C	ND	0.91^{F} ±0.01	3.82^{ECD}±0.17	0.61^{FE} ±0.13	ND
LSD			0.33	0.29	0.04	
CV			10.25	5.61	3.54	
F value			31.49	82.06	402.17	
Error			0.04	0.03	0.00	

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

3.2 Copper (Cu)

One-way analysis of variance showed that the average concentration of copper in *Moringa stenopetala* 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 (10.25). 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.

It can be observed from Fig. 3 that in comparisons between different sites (Konso, Dirashe and Gamo Gofa) at the same growing age, matured leaves (B) in Konso-Karat sample (1.53±0.10 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) (1.47 ±0.02 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 Fig. 3).

3.3 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 (1.96 ±0.25 mg/kg) and Derashe (2.05 ±0.19 mg/kg) sample site. On the other side, the concentrations of iron in aged leaves have got high concentration in all sample sites.

As can be seen from Fig. 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 (4.44 ±0.18 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 Fig. 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).

3.4 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 (3.54) value and high F value (402.17).

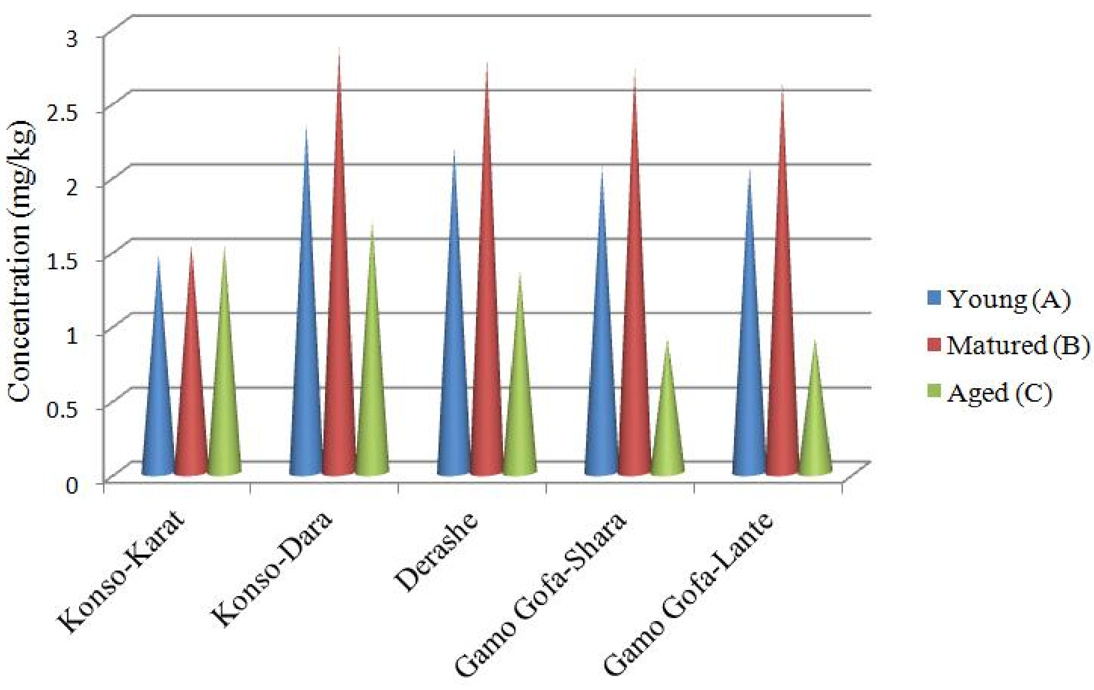

Fig. 3. Copper concentration at different growing ages

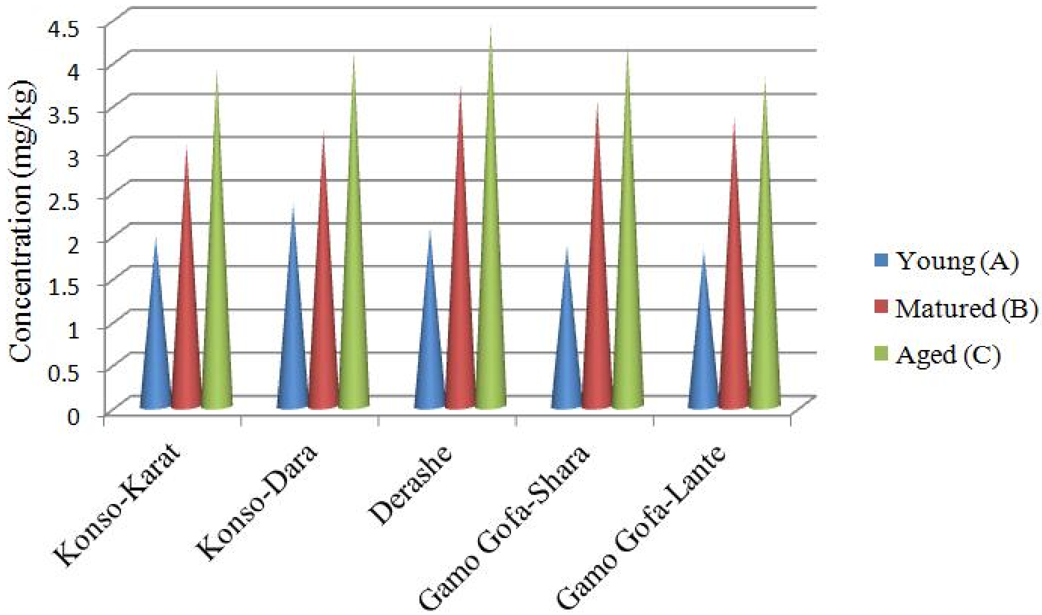

Fig. 4. Concentration of iron

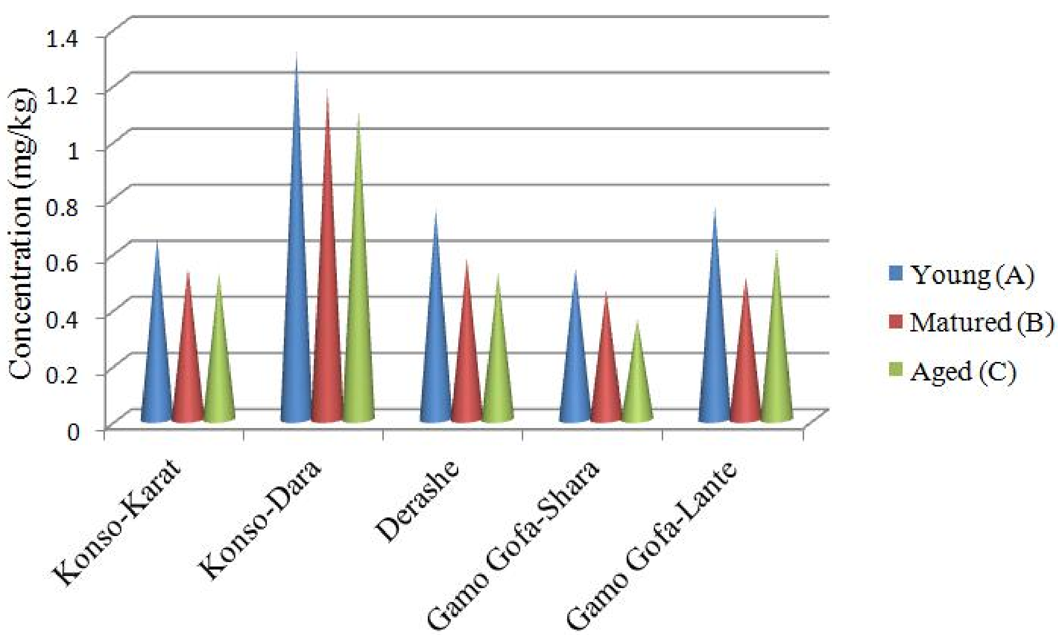

Fig. 5. Concentration of zinc

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 Fig. 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. It could be unambiguously overcome by taking more quantity of samples. 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.

3.5 Discussion

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 [13,14]

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 [15]. However, results obtained in this work in Gamo-Gofa area showed more presence of concentration of copper than the one revealed in other research [15]. Kassa Belay and his coworkers have found that the average concentration of copper metal in *Moringa Oleifera* leaves collected from Wukro was 2.87±0.04 mg/kg [16]. 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 [16]. 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 1.80±0.26-4.44±0.18 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 [13,14].

The concentration of zinc in the *Moringa stenopetala* tree leaves considered in this research is determined to be between 0.36±0.08 - 1.32±0.02 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 [17]. 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 [13,14]. As the people in the study areas rely on consuming the matured level of the leaves, the benefit from getting more copper and iron, which are very crucial in photosynthesis and respiration system of plants.

4. CONCLUSION

We report concentrations of heavy metals from the leaves of *Moringa stenopetala* tree at different growing stage using (FAAS) in deploying wet digestion method. The investigation helps to know content of elements present in Moring tree leaves and to further identify which are in the limit of permissible range for human health. Results showed that elements had showed difference in concentration as the age of the leaves progress. Zinc concentration showed decrement while iron concentration showed increment through increasing age of the leaves. Copper concentration has got high value in matured ages of the leaves when people of the research area traditionally rely on for food consumption. However, the average concentrations of the three elements detected in this work were below the limit set by WHO. As a result, according to this work, consuming leaves of *Moring stenopetala* could be recommended as less than permissible limit were detected that could have damaged health if found in excess amount.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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