

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

4
5 **ABSTRACT**

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

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

17 **1. INTRODUCTION**

18 *Moringa* tree is a multi-purpose miracle tree with tremendous for food and medical potential [1].
19 *Moringa* is the genus of family of Moringaceae. It requires an annual rainfall of between 250 and
20 3000 mm. It is drought resistant tree. It grows best at altitudes up to 600 m but it still grows at
21 altitudes of 1000 m. Worldwide, some 14 species of the *Moringa* tree have been reported.

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

26 Its parts have different potential medicinal and nutritional uses for human as well as animals. The
27 *Moringa* leaves are nutritionally rich and excellent source of concentrated proteins,

28 vitamins and minerals [3]. Studies indicate that the leaves have immense nutritional value such
29 as phytochemicals, vitamins, minerals, and amino acids [4]. The edible leaves are eaten
30 throughout East Africa and parts of Asia.

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

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

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

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

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

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

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

70 2. MATERIALS AND METHODS

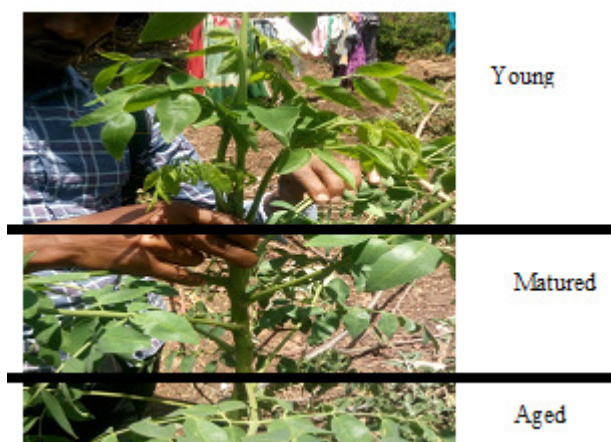
71 2.1. Description of the Study Area

72 The study was conduct in Gamo-Gofa and SegenArea Peoples (SAP) Zones. Konso-Karat,
73 Konso-Dara and Derashe from Segen Area Zone and Shara and Lante from Gamo-Gofa Zone
74 were considered in this work. Arba Minch Zuria, capital city of Gamo-Gofa Zone, is located at
75 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,
76 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
77 536 km far from Addis Ababa.

78 2.2. Sampling Protocol

79 Fresh leaves of *Maringa Setnopetala* tree were collected from the selected study areas. The study
80 areas were selected purposefully based on the productivity and regular *Maringa Setnopetala*
81 leaves consumption habits of the people in the study areas. However, Woredas were randomly
82 selected. Samples were based on three growing stages of leaves of the same as young, matured
83 and aged (See figure 1). Young leaves are very emerging soft leaves with yellowish color and of
84 2.48 cm height and 1.38 cm width. The matured leaves are next to young leaves on the same

85 branch. They are green in color. Matured leaves are 5.48 cm high and 2.9 cm wide in average.
86 Aged leaves are the ones relatively aged. At this stage the color changes from very green to
87 yellowish and are relatively hard in structure. They are on average 4.78 cm and 2.46 cm wide.
88 Leaves were picked from the same main vein and 500 g of the samples were collected from each
89 place and placed in pre-cleaned plastic bags, labeled and was transported to the laboratory for
90 further treatment. Total of 15 samples were collected and analyzed according to their growing
91 ages. For data interpretation, we have made designations: young – A, matured – B and aged - C.



92

93 Fig 1. *Moringa Stenopetala* leave sample from Konso-Karat.

94 2.3. Sample Preparation

95 The *Moringa Stenopetela* leaves samples were washed with deionized water to remove dust
96 materials and were air dried in a drying oven at 70⁰C for 12 hours ensuring their greenish
97 coloration and maintaining nutritional values. The samples were sieved through 2 mm sieve to
98 remove coarse particles. The powders were package in pre-cleaned bags, labeled and stored at
99 room temperature 24-26⁰C. One gram of sieved samples were weighed and kept in acid washed
100 glass beaker. Then the samples were digested by the addition of 20 cm³ of aquaregia (mixture of
101 HCl and HNO₃, ratio 3:1) and 10 ml of 30% H₂O₂. The H₂O₂ was added in small portions to
102 avoid any possible overflow leading to loss of material from the 100 ml conical flask. The analyt
103 was digested for 2 hr in 100 ml conical flask covered with watch glass, and reflex over a hot
104 plate at 90⁰C. The conical flask wall and watch glass was washed with distilled water and the
105 samples were filtered out to separate the insoluble solid from the supernatant liquid. The volume

106 was adjusted to 100 ml with distilled water. Blank solution was handled as detailed for the
107 samples.

108 **2.4. Experimental Setup**

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

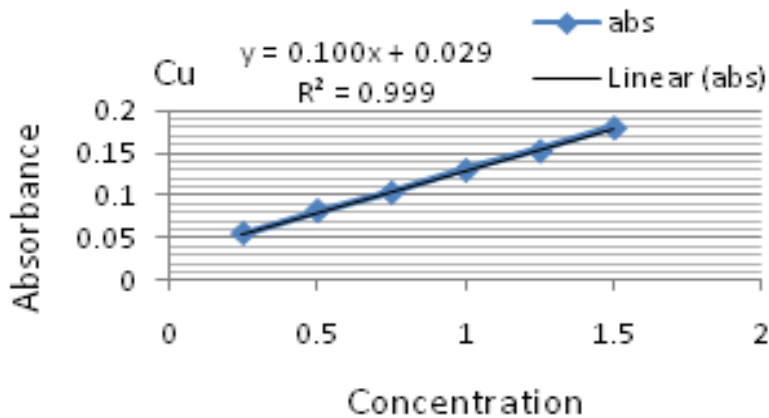
114 **2.5. Statistical Analysis**

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

120 **3. RESULTS AND DISCUSSIONS**

121 **3.1. Results and Analysis**

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



131

132 Fig 2. Calibration curve for Cu.

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

140 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

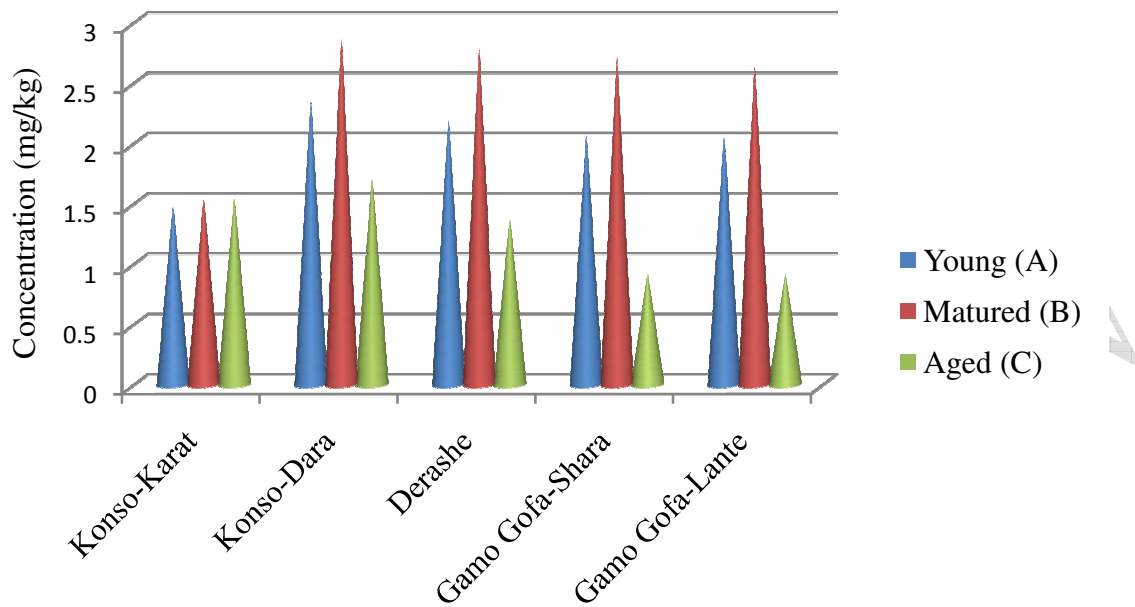
8	Derashe B	ND	2.7836 ^A ±0.0125	3.7234 ^{ED} ±0.1397	0.5734 ^{FG} ±0.2451	ND
9	Derashe C	ND	1.3612 ^E ±0.0111	4.4397 ^A ±0.1769	0.5232 ^H ±0.1280	ND
10	Gamo-Gofa1 A	ND	2.0625 ^C ±0.0184	1.8511 ^J ±0.1606	0.5381 ^{HG} ±0.1951	ND
11	Gamo-Gofa1 B	ND	2.7139 ^A ±0.0199	3.5319 ^{EF} ±0.1739	0.4609 ^I ±0.0854	ND
12	Gamo-Gofa1 C	ND	0.9093 ^F ±0.0185	4.1844 ^{BA} ±0.2235	0.3587 ^J ±0.0818	ND
13	Gamo-Gofa2 A	ND	2.0492 ^C ±0.0525	1.8014 ^J ±0.2623	0.7602 ^D ±0.1717	ND
14	Gamo-Gofa2 B	ND	2.6341 ^{BA} ±0.0302	3.3404 ^{GF} ±0.1124	0.5093 ^H ±0.1253	ND
15	Gamo-Gofa2 C	ND	0.9093 ^F ±0.0073	3.8227 ^{ECD} ±0.1722	0.6115 ^{FE} ±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	

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

143
144 The mean concentrations of elements detected in this work were generated with respect to ages
145 of the leaves of the same main vein in the study areas.

146 **Copper (Cu)**

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

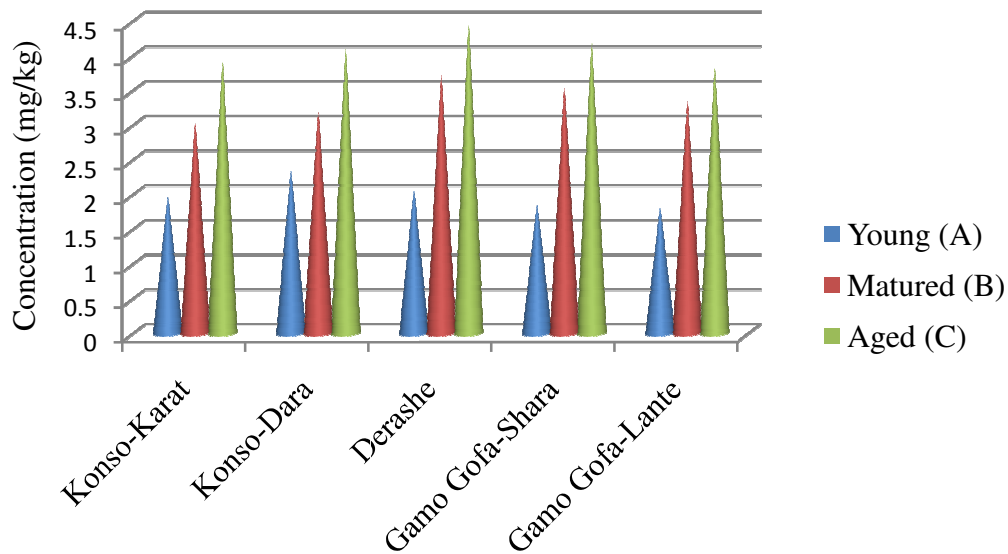


156 Fig 3. Copper concentration at different growing ages.

157 It can be observed from Figure 3 that in comparisons between different sites (Konso, Derashe and
 158 Gamo Gofa) at the same growing age, matured leaves (B) in Konso-Karat sample
 159 (1.5307 ± 0.0981 mg/kg) had less concentration than other sites. As can be seen from Table 2, the
 160 average concentration of copper in all sites at different growing stages showed statistically
 161 significant different value, except Karat sample. Furthermore, concentrations of copper in Karat
 162 sample in young leaves (A) (1.4676 ± 0.017 mg/kg) had less value than that of other site. The
 163 concentration of copper in aged leaves (C) is significantly similar in Gamo-Gofa areas and
 164 approximately similar in Konso and Derashe sites. The concentration of copper is greater in
 165 matured leaves and followed by intermediate value in young leaves and less in aged leaves in all
 166 sample sites (i.e $B > A > C$) (See Figure 3).

167 Iron (Fe)

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

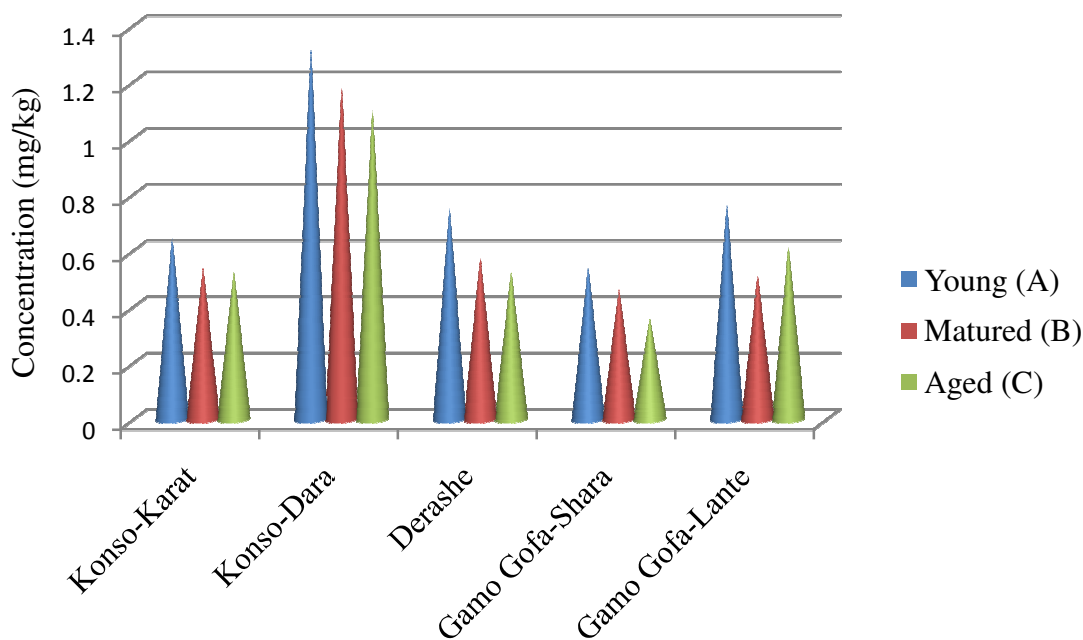


181 Fig 4. Concentration of iron

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

188 Zinc (Zn)

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



195 Fig 5. Concentration of Zinc

196

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

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

206 3.2. Discussions

207 Table 2 displays the WHO limit and permissible range in heavy metals traced in this study. The
 208 concentration of copper falls in the range of 0.91-2.86 mg/kg in the study areas. As can be seen
 209 from the Table 2 and comparing with the values obtained in this study, the copper content in

210 young and matured leaves lie in the permissible range. Thus, the one who wants more copper in
211 his/her diet can take young and matured leaves than the aged leaves.

212 Table 2. WHO limits, concentration of permissible ranges (ppm) of heavy metals in plants [12,
213 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

214
215 Research conducted in Arba Minch area, Gamo-Gofa administrative zone, determined that
216 concentration of copper metal in *Moringa Stenopetala* leaves was 0.67 mg/kg [14]. However,
217 results obtained in this work in Gamo-Gofa area showed more presence of concentration of
218 copper than the one revealed in the research of Ali *et al.* Kassa Belay and his coworkers have
219 found that the average concentration of copper metal in *Moringa Oleifera* leaves collected from
220 Wukro was 2.866 ± 0.0436 mg/kg [15]. This result agrees with the result of this work.

221 Ali and his co-researchers determined that the concentration of iron metal in *Moringa*
222 *Stenopetala* leave collected from Arba Minch area, Gamo-Gofa administrative zone, was 1.18
223 mg/kg [14]. This is very close to the result found in this research in Gamo-Gofa (Lante) area.
224 The concentration of iron in this research was found to be in the range of 1.8014 ± 0.2623 -
225 4.4397 ± 0.1769 mg/kg, which is more than that of Ali and his coworkers' result. As can be seen
226 from Table 2, the concentration level of iron found in this work is below the toxic limit set by
227 WHO [12, 13].

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

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

237 **4. CONCLUSIONS**

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

242 Results showed that elements had showed difference in concentration as the age of the leaves
243 progress in all sites. Zinc concentration showed decrement as the age of the leaves increased (i.e
244 $A > B > C$). On the opposite side, averagely greater, intermediate and less concentration of iron
245 was observed in aged, matured and young leaves, respectively, in all sample sites (i.e $C > B$
246 $> A$). The concentration of copper is greater in matured leaves and followed by intermediate
247 value in young leaves and less in aged leaves in all sample sites (i.e $B > A > C$).

248 **COMPETING INTERESTS**

249 There is no competing interest.

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