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Determination of concentration of some essential and heavy metals in roots of *Moringa stenopetala* using flame atomic absorption spectroscopy

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This study is aimed at determination of concentration of essential (Ca, K, Na and Mg) and heavy (Cd, Cr, Pb, Hg, Cu and Zn) metals using Flame Atomic Absorption Spectroscopy (FAAS) of samples taken from fresh roots of *Moringa stenopetala*. A wet digestion method involving use of mixture of (HCl and HNO_3, ratio 3:1) and 10 ml of 30% H_2O_2 at an optimum temperature and time duration was deployed during sample preparations. Results show that concentration of essential elements Mg, Ca, Na, and K range from 10.12 to 10.99 mg/kg, 2.6 to 5.64 mg/kg, 4.3 to 5.26 mg/kg, and 1.26 to 1.77 mg/kg, respectively. Among the heavy metals, concentration of Cu fall in the range of 0.81-1.44 mg/kg while that of zinc fall in the range of 0.37-2.34 mg/kg, both lying below toxic level. The levels of toxic metals (Cd, Cr, Pb and, and Hg) were not detected. The results of the study indicate that the concentration of the entire essential and heavy metals are below the range of WHO/FAO limits.

Key words: *Moringa stenopetala*, essential metals, heavy metals, roots, flame atomic absorption spectroscopy (FAAS)

INTRODUCTION

Moringa tree is a multi-purpose miracle tree with tremendous potential for food and medical uses (Agena, 2009). It belongs to the genus of family *Moringaceae*. *Moringa* requires an annual rainfall of 250-3000 mm to grow. It is drought resistant tree that grows best at altitude up to 600 m but still grows at altitude of 1000 m. Worldwide, some 14 species of the *Moringa* tree have been reported (Diriba et al., 2017). Among these, the best studied species with regard to potential nutritive, 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 (Bosch, 2004) and grows widely in southern parts of Ethiopia.

Almost all parts of *Moringa* tree can be used as a source of nutrition for human and livestock as well as for other purposes (Bosch, 2004). *Moringa* tree is a deciduous plant with tuber-like root at the earlier stage. *Moringa oleifera* roots are important agents of healing and nourishment. The roots are used to make medicines, perfumes, natural pesticides, fertilizers, cleaning agents, animal feeding and many other important products. When

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Moringa oleifera seedlings are 60 cm tall or shorter, their roots can be used to make special sauce that can serve medicinal purposes. Individuals suffering from malnutrition are encouraged to consume sauce made from the *Moringa oleifera* roots sauce as it contains high levels of fiber, protein, vitamins and minerals, which are known to bring about a quick recovery (Fahey, 2005).

Moringa roots are popular in medicine both traditionally and in modern life. Traditionally, the restorative and healthful benefits of the *Moringa* root have been used by Ayurvedic practitioners in India for centuries to treat a wide variety of ailments and in controlling disorders of the circulatory system including minor cardiovascular complaints. In small doses, *Moringa* tree roots can be used to stimulate appetite and improve function of digestive tract, making it useful for individuals with gastric upset and irritable bowel syndrome. Additionally, roots of this tree have been used in controlled doses to treat impotence, sexual dysfunction, female reproductive tract issues and to bring on menstruation. In poultice form, *Moringa* roots are used for cramps and arthritis pains (Karuna and Rajni, 2014).

In modern medical uses, *Moringa* tree roots play great role. As stated in Bosch (2004) and Karuna and Rajni (2014), *Moringa* tree roots contain elements that can combat epithelial ovarian cancer and provide new hope for cancer sufferers. Roots extracts of *Moringa* tree can help to reduce or eliminate kidney stones by allowing body to flush calcium and phosphates from the kidneys more efficiently. This tree root is also used as anti-inflammatory agents with solid results in laboratory rats showing reduced swelling and improved healing in Edema and other artificially induced inflammations. The analgesic and soporific effects of *Moringa* tree root compounds have undergone rigorous scientific testing and have been found to be useful in supplementing pharmaceutical remedies, allowing patients to experience longer, less interrupted sleep when taking pain medications.

Metal concentration in human diets are classified into essential like Na, K, Cu, Zn, Ca, Mg, Fe and Mn and non-essential or toxic such as Hg, Cd, Pb and Cr metals (Longhurst, 2010). Low intake of essential metals produces deficiencies, while higher consumption may cause toxicity. However, non-essential metals are lethal and toxic even at low concentrations to human and environment. Non-essential metals are ranked among the most hazardous toxic substances owing to their persistence in the environment and absorption in food chain (Khan et al., 2013; Muhammad et al., 2013). Toxic effects of metals include vomiting, diarrhea, headache, irritability, hypertension, heart, lung, kidney, liver and intellectual problems and cancer (Shah, 2012).

The term heavy metal refers to any metallic element that has a relatively high density and is toxic or poisonous at low concentration. Heavy metals include Cadmium (Cd), Copper (Cu), Lead (Pb), Zinc (Zn), Mercury (Hg), Arsenic (As), Silver (Ag), Chromium (Cr), Iron (Fe) and Platinum. Metal elements like copper and zinc are essential trace elements for living organisms at low concentration (< 10 mg/L). The characteristics of heavy metals are described by Loannidou et al., (2005) and Wang et al. (2005). Toxicity that can last for long time in nature cannot be degraded including bio-treatment and are very toxic even at low concentration (1.0 - 10.0 mg/L). With respect to their toxicity, heavy metals can be divided into two groups: micronutrients like Fe, Mn, Mo, Cu, Cr, Ni and Zn that are essential in small amounts and the only toxic ones are As, Cd, Hg and Pb without any known biological importance. Toxic metals are extremely persistence in the environment even at low concentrations and have been reported to produce damaging effects on human and animals because there is no good mechanism for their elimination from the body (Loannidou et al., 2005; Adah et al., 2013).

As deduced from different studies, *Moringa* tree have got different essential and non-essential, heavy as well as trace metals at different levels. According to Fagbohun et al. (2014), the following minerals were analyzed from *Moringa oleifera* tree roots in Nigeria using spectroscopic techniques FTIR and XRD. The research found zinc (Zn), iron (Fe), chromium (Cr) and copper (Cu) with concentration values (in ppm) 10.00, 21,510.00 and 20.00, 2.755, respectively.

Karuna and Rajni (2014) have analyzed *M. oleifera* tree roots using spectroscopic techniques FTIR and XRD for its elemental composition in India and found zinc (Zn), iron (Fe), calcium (Ca), potassium (K), sodium (Na) and magnesium (Mg) with concentration values (in ppm) 47.84, 5.04, 286.07, 860.59, 17.17 and 43.79, respectively. In addition to the nutritionally important minerals abovementioned, the study conducted by Karuna and Rajni (2014) had analyzed heavy metals such as Pb, Se, Hg and As, however, only Pb was detected (0.19 ppm) from the root part of *Moringa oleifera* using inductively coupled plasma atomic emission spectroscopy. Studies conducted in Nigeria have assessed concentration of heavy metals in *Moringa oleifera* and found lead, iron, copper and zinc with mean (mg/kg) of 0.9471±0.0173, 55.60±0.012, 0.1762±0.0230 and 3.225±0.022, respectively though atomic absorption spectrometer analysis (Abdulkadir et al., 2016).

Most of the data reported in research account for *M. oleifera*. However, all the plant parts of *M. stenopetala,* which is highly cultivated in southern part of Ethiopia, have not been analyzed for their metal concentration and nutritive values. The present study showcases a comprehensive investigation on the elemental constitutes of *M. stenopetala* tree roots. People in the study areas, especially Konso people, use *M. stenopetala* tree roots for abdomen pain relief just by digesting it and swallowing water component only. The usage is without any scientific dosage and presence of toxic and heavy metals could result in cause of health problems. Thus, the purpose of

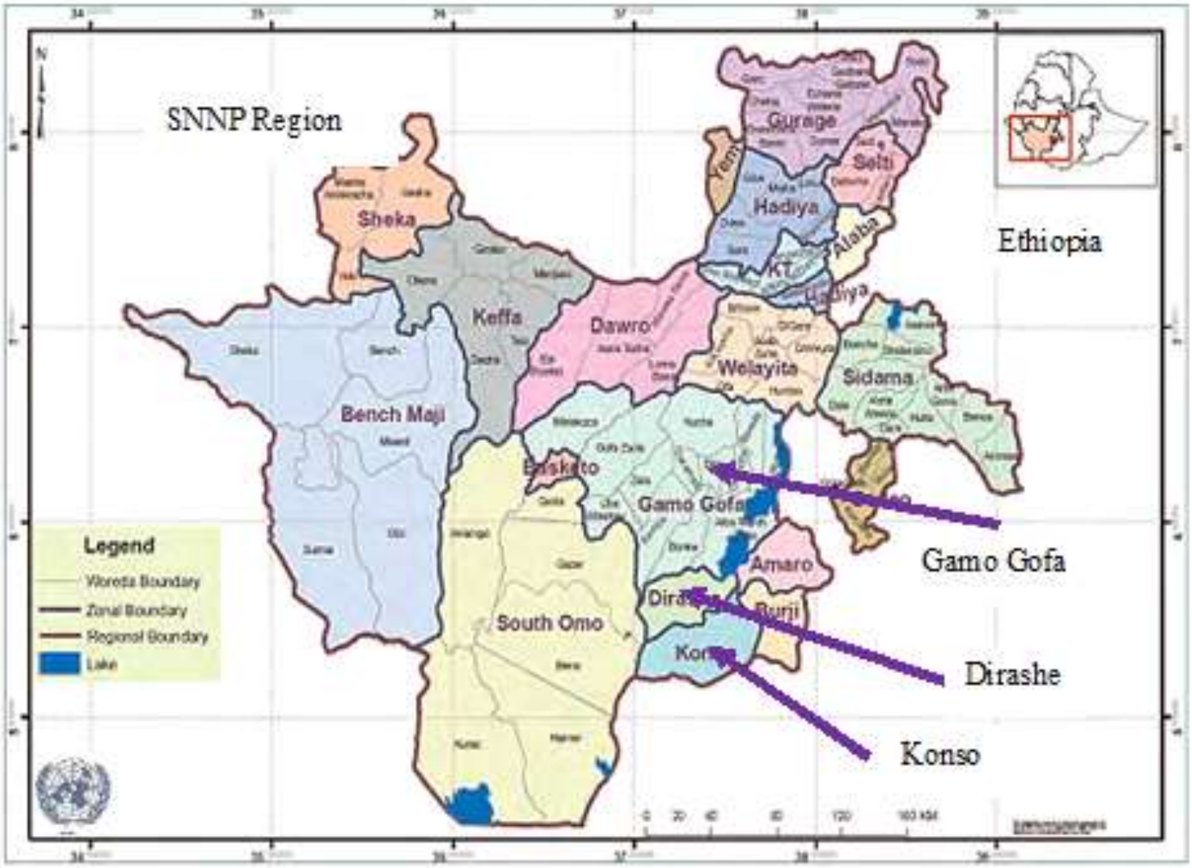

Figure 1. Map of study area (retrieved at: www.rippleethiopia.org/page/snnpr).

this study was to determine the concentrations level of six heavy and four essential metals in *M. stenopetala* roots that are cultivated in urban and rural gardens in Southern part of Ethiopia.

MATERIALS AND METHODS

Description of the study area

The study was conducted in South Nation Nationalities and Peoples Region of Ethiopia. Three Woredas were selected: Gamo-Gofa Zuria, Konso and Derashe. Specifically, two places were taken: Karat and Dara from Konso, Gidole and Gato from Dirashe and Shara and Lante from Gamo-Gofa. Figure 1 displays study areas covered in the survey of this work.

Sampling protocol

Matured healthy and fresh *M. stenopetala* tree roots were collected from the farmlands of three areas mentioned in the section of study area; namely Gamo Gofa, Konso and Dirashe. The study areas were selected purposefully based on the productivity and traditional use of roots parts of *M. stenopetala* for medicinal purpose. To collect the representative sample from each sampling sites, two subsamples were taken from each site. In total, six samples were collected and put in clean polyethylene plastic bags, labeled and brought to laboratory for further pre-treatment.

Sample preparations

Roots samples collected from farmlands were washed with a running tap water to remove absorbed soil particulates and then rinsed with deionized water. The samples were chopped into pieces by stainless steel axe Teflon knife and Chopping board to facilitate drying and subsequently dried in drying oven at 70°C for constant weight. The dried samples were powdered using electronic blender and the powder was sieved through 2 mm sieve to prepare fine powder for digestion.

Acid digestion procedure

A gram of sieved powder of the samples was weighed out into acid washed glass beaker. Thereafter, the powder was digested with addition of 20 cm^3 of aquaregia (mixture of HCl and HNO_4, ratio 3:1). 10 ml of 30% H_2O_2 was added to avoid any possible overflow leading to loss of material from the 100-ml conical flask. Hydrogen peroxide is also used to enhance the reaction speed and to ensure complete digestion. The analyte was digested for 2 h in 100-ml conical flask covered with watch glass and reflex over a hot plate at 90°C for 2 h. The volume was adjusted to 100-ml with distilled water. Blank solution was handled as detailed for the samples. Working standard solutions of all metals were prepared from stock

Table 1. Mean concentration of essential metals calculated in this work.

SN	Sample sites	Mean concentration (mg/kg)			
		Ca	K	Na	Mg
1	Dirashe Gato	5.64^{a}±0.25	1.77^{a}±0.26	5.26^{a}±0.13	10.99^{a}±0.01
2	Dirashe Gidole	$4.67^{ba\pm}$0.26	1.53^{b}±0.17	4.74^{a}±0.00	10.43^{d}±0.13
3	Konso Karat	3.64^{bc}±1.59	1.51^{b}±0.00	5.26^{a}±0.09	10.78^{b}±0.00
4	Konso Dara	2.60^{c}±0.13	1.26^{d}±0.06	4.33^{a}±0.80	10.53^{c}±0.05
5	Gamo Gofa-Shara	3.36^{c}±0.01	1.26^{d}±0.00	5.31^{a}±0.28	10.43^{d}±0.01
6	Gamo Gofa-Lante	3.47^{c}±1.25	1.38^{c}±0.20	4.92^{a}±0.51	10.12^{e}±0.22

*Means with the same letter in a given column are not statistically significantly different.

Table 2. Mean concentration of heavy metals calculated in this work.

SN	Sample Sites	Mean concentration (mg/kg)					
		Cu	Zn	Cr	Hg	Pb	Cd
1	Derashe Gato	0.81^{b}±0.01	0.45^{b}±0.01	ND	ND	ND	ND
2	Derashe Gidole	1.42^{a}±0.74	1.88^{a}±1.22	ND	ND	ND	ND
3	Konso Karat	1.44^{a}±0.35	2.34^{a}±0.01	ND	ND	ND	ND
4	Konso Dara	1.43^{a}±0.01	0.38^{b}±0.01	ND	ND	ND	ND
5	Gamo Gofa shara	1.34^{a}±0.02	0.39^{b}±0.00	ND	ND	ND	ND
6	Gamo Gofa lante	1.35^{a}±0.28	0.37^{b}±0.90	ND	ND	ND	ND

*ND -Not Detected, *means with the same letters in a column are not significantly different.

standard solution (1000 ppm).

Experimental setup

Flame atomic absorption spectrophotometer (FAAS) (Model: 210-VGP, USA) was used for absorbance recordings of the elements. Signal of each radiation for specific element was detected and were converted into concentration information for the analytics from calibration curves of each element.

Statistical analysis

Data entry management and preliminary summaries were done on Microsoft Office Excel spread sheet. Means and standard deviations of data collected were determined. All analyses were carried out in triplicates and data presented as means ± standard deviations. One-way analysis of variance (ANOVA) at $p < 0.05$ was used to determine statistically significant differences in the mean concentrations of metals among groups of root samples from different study areas. Data were further manipulated with ASA and SPSS 20.

RESULTS AND DISCUSSION

Concentration of four essential and two heavy metals were obtained form absorption intensity. Average results of the elemental analysis of the metals observed in this work are displayed in Tables 1 and 2. It is to be noted that each result is an average of three independent triplicate measurements (n = 3). Results show that essential (Ca, K, Na, Mg) and heavy (Cu, Zn) metals were detected and the heavy metals Pb, Cd, Cr and Hg were not found to be to the level of detection limit of the technique used in this experiment. Results indicate that samples have variable composition of each analyte metals with different concentration ranges among different sample sites. Table 1 presents concentration of essential elements while Table 2 presents mean concentration of heavy metals.

Essential metals

Calcium (Ca)

One-way analysis of variance (ANOVA) shows that mean concentration of calcium is statistically insignificant in all the areas of investigation, except the two Dirashe areas. The average concentration of calcium in the studied sites ranges from 2.60±0.13 to 5.64±0.25 mg/kg. Relatively high concentration was observed in Dirashe areas (Table 1). The pattern of concentration of calcium in the studied sample sites is in the order; Derashe Gato > Derashe Gidole > Konso Karat > Gamo Gofa Lante > Gamo Gofa Shara > Konso Dara.

Potassium (K)

As can be seen from Figure 2, mean concentration of

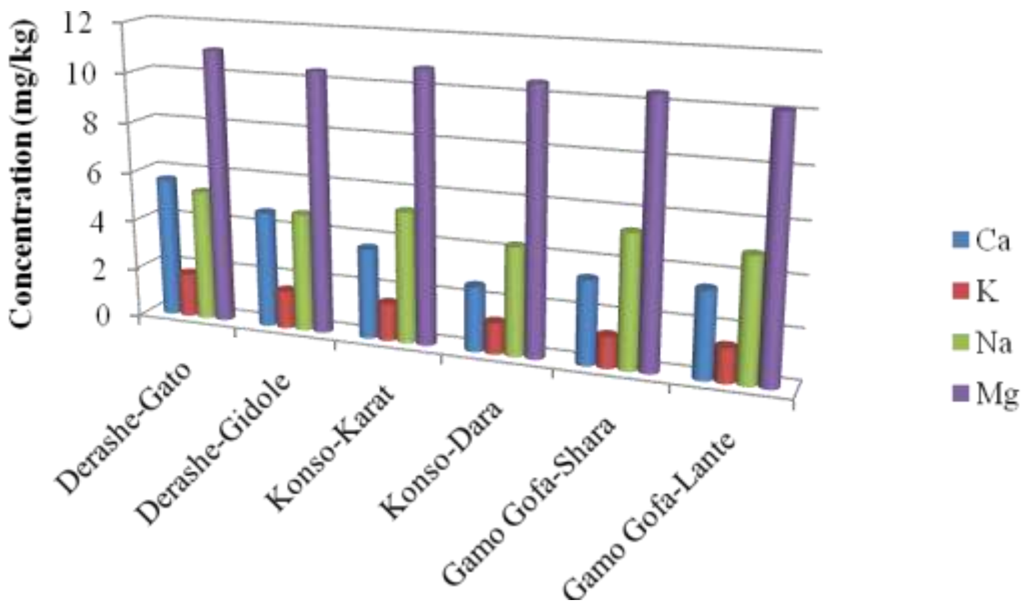

Figure 2. Concentration of essential metals.

potassium in all areas is found to be lower. When sites are taken into consideration, there is statistical significant difference in the content of potassium concentration. The highest concentration of potassium is observed in Derashe-Gato (1.77±0.26 mg/kg). While relatively the lowest concentration is obtained in Gamo Gofa-Shara (1.26±0.00 mg/kg). Depending on the potassium levels in the studied samples, the order of the studied areas is Derashe-Gato > Derashe-Gidole > Konso-Karat > Gamo Gofa-Lante > Konso-Dara, Gamo Gofa-Shara.

Sodium (Na)

The concentration of sodium found in this work range from 4.33±0.80 to 5.31±0.28 mg/kg in studied root samples. As can be observed from Table 1, the mean concentration of sodium is next to that of magnesium, except, in Dirashe. The pattern of sodium level in the samples is in the order of; Gamo Gofa-Shara > Derashe-Gato > Konso-Karat > Gamo Gofa-Lante > Derash-Gidole > Konso-Dara. One-way analysis of variance shows that the mean concentration of sodium does not show statistically significant difference among the six sample sites.

Magnesium (Mg)

As can be observed from Figure 1, in comparison to other elements, magnesium concentration is observed to be high in all areas covered in this work. Moreover, as can be seen from Table 1, its average is greater or equal to 10 mg/kg, which is far higher in content than all the metals determined in this work, in all the three study Woredas. Relatively, the concentration of magnesium is found to be higher in Derashe Gato (10.99±0.01 mg/kg) and followed by other sample sites in the order of 10.78±0.00 mg/kg, 10.53±0.05 mg/kg, 10.43±0.13 mg/kg, 10.43±0.01 and 10.12±0.22 mg/kg for Konso-Karat, Konso-Dara, Derashe-Gidole, Gamo Gofa-Shara and Gamo Gofa-Lante, respectively. Analysis of variance reveals that the mean concentration of magnesium is significantly different among the six sample sites.

Heavy metals

Copper (Cu) and Zinc (Zn)

Of the heavy metals considered in this work, copper and zinc are to the limit of detection level of the spectroscopic technique deployed in this experiment. The level of the heavy metals copper and zinc in this study samples are in the range of 0.81±0.01 – 1.44±0.35 mg/kg and 0.37±0.90 – 2.34±0.01 mg/kg, respectively. One-way analysis of variance shows that the concentration of copper has shown significantly no difference among sampled sites, except Dirashe-Gato. The Derashe Gato (0.81±0.01 mg/kg) copper concentration is significantly lower than other sample sites while Konso-Karat relatively records more (1.44±0.35 mg/kg) mean concentration. One-way analysis of variance shows that the concentration of zinc is significantly different among sample sites. Zinc concentration is observed to be higher than copper in Derashe and Konso-Karat. On the other

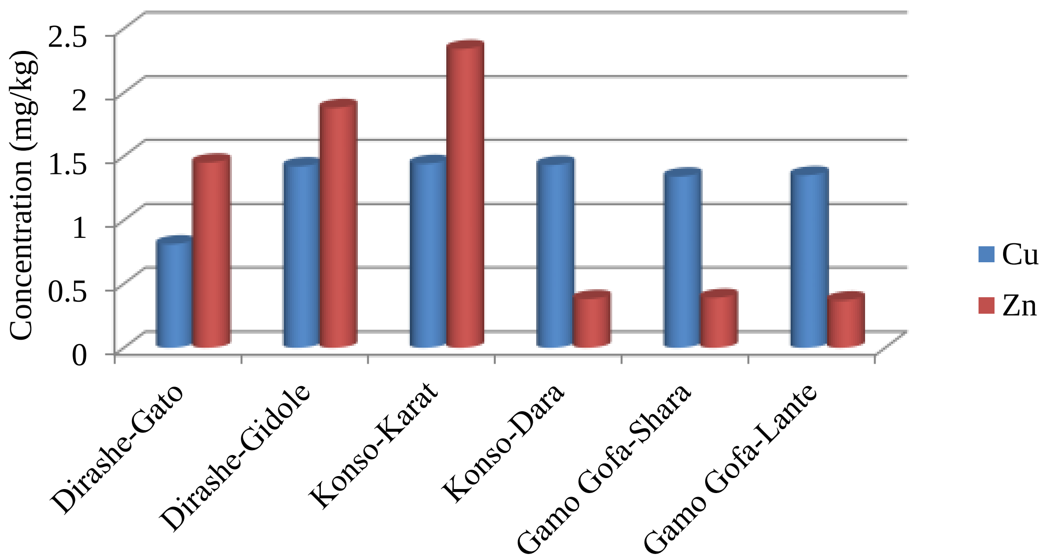

Figure 3. Concentration of heavy metals.

hand, it shows upper hand in Konso-Dara and Gamo Gofa areas, even with statistically significance figures. The concentration of zinc is in the range of 0.37-2.34 mg/kg. As can be observed from Figure 3, the lowest zinc content is obtained from Gamo Gofa Lante (0.37±0.90 mg/kg) and the highest one is from Konso-Karat (2.34±0.01 mg/kg) areas.

DISCUSSION

Average concentration of calcium in this study ranges from 2.6±0.13 to 5.64±0.25 mg/kg for *Moringa stenopetala* root. As cited in Kokou et al. (2001), concentration of calcium in *M. oleifera* root in Togo is found to be significantly higher than the concentration of calcium in *Moringa stenopetala* in this work. According to the studies conducted in Nigeria, the level of calcium concentration is reported (Fagbohun et al., 2014) to be 286.07 ppm in *M. oleifera* and 24.86 mg/g in medicinal plants (Oloyede, 2005). Research conducted in India for the determination of calcium in *M. oleifera* roots is 286.07 ppm (Karuna and Rajni, 2014). Result of this study show that the concentration of calcium in *M. stenopetala* root is below the aforementioned literature values. High concentration of calcium is important because of its role in bones, teeth, muscle system and heart functions (WHO, 2004).

The concentration of sodium and potassium in *M. stenopetala* root in this work is found to be 1.26-1.77 and 4.33-5.26 mg/kg, respectively. As reported by Karuna and Rajni (2014) in India, concentration (ppm) of sodium and potassium in *M. oleifera* roots were found to be 17.17 and 860.59, respectively. According to the study in Pakistan, the level of sodium ranges from 113.49 to 2174.38 mg/kg in different traditional medicinal plants. This indicates that the sodium and potassium concentration levels in Moringa stenopetala roots in this work is less than the concentration observed in the medicinal plants in Pakistan (Shazia et al., 2010). The highest concentration of potassium measured in this work is 1.77±0.26 mg/kg. Potassium is necessary for proper functioning of our body system. It maintains the electrolyte balance, manages blood pressure, keeps heart functioning properly and enhances muscle control, growth and health of the body cells. Its deficiencies can lead to varieties of mental and physical problems. The obtained data in this study indicated that the *M. stenopetala* roots are not deficient in potassium. Therefore, it is useful to be used as a food source, rich in K, for humans as it might help in the case of potassium deficiency. Results indicate that the concentration of potassium in all sample sites is found in the recommended daily intake level and also found below values obtained by Shazia et al. (2010).

The concentration of magnesium found in this work is observed to be 10.22-10.99 mg/kg. As studied by (Karuna and Rajni 2014), Mg is found to be 43.79 ppm in *M. oleifera* roots. Study conducted in Pakistan showed that content of Mg ranged from 2241.88 to 6350.63 mg/kg in different medicinal plants. As can be seen from the reports, the level of Mg in this study is below literature values and as a macronutrient it is below recommended level for plants. The level of the heavy metals Cu and Zn in this study are in the range of 0.811±0.01–1.44±0.35 mg/kg and 0.37±0.9–2.34±0.01 mg/kg, respectively. Reports in Nigeria indicate that the concentration of Cu and Zn in *M. oleifera* roots are found to be 2.755 mg/kg (Fagbohun et al., 2014) and 0.1762±0.023 mg/kg (Abdulkadir et al., 2016) for copper and 10.00

mg/kg (Fagbohun et al., 2014) and 3.225±0.022 (Abdulkadir et al., 2016) for zinc.

Results indicate that the concentration of copper in all sample sites is below the permissible limit set by World Health Organization (WHO). Highest mean concentration of zinc in this study is 1.44 mg/kg which is much lower than the standard limit set by WHO (WHO, 2004; Kabata-Pendias, 2011; WHO, 2002) which is 10 to 160 mg/kg. The permissible limit of zinc set by WHO in edible plants is 27.4 mg/kg. On the other hand, the finding of this research differs from literature value of (Karuna and Rajni 2014) which is 47.84 mg/kg. The recommended limit of Zinc in medicinal plants by WHO is 50 mg/kg and its intake in food is 11 mg/day. In general, results of the heavy metals analyzed in this work show that the concentration level are below the standard guide lines for maximum limit proposed for medicinal plants by WHO.

Conclusions

Concentration of four essential (K, Ca, Na and Mg) metals and two heavy metals (Cu and Zn) in *M. stenopetala* tree roots are detected using FAAS with acid digestive method. Results indicate that *M. stenopetala* root contains low concentration of K, Ca, Na and Mg metals as compared to cited literatures, but optimum amount for consumption, and the concentration level of the toxic heavy elements are very low and could not cause any health threat to the consuming population. All essential elements analyzed are at the optimum required level and consumption of the *M. stenopetala* root could supplement essential metals required for human health. On the contrary, non-essential toxic metals analyzed in this study are below the permissible ranges presented by WHO standards revealing that the *M. stenopetala* root in the study area is safe for dietary as well as medicinal uses.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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