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Determination of selected major and trace metals in lemongrass (*Cymbopogon citratus*) by microwave plasma-atomic emission spectrometry

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ABSTRACT: *Cymbopogon citratus* is very important and of great interest due to its commercially valuable essential oils, its common use in food and beverage industries as well as in traditional folk medicine. The objective of the present study was to determine the levels of selected major (K, Mg, Ca) and trace (Mn, Fe, Cu, Zn, and Pb) metals in lemongrass (*Cymbopogon citratus*) samples collected from Addis Ababa, Ankober, Finote Selam (Gojjam), and Wondogenet by microwave plasma-atomic emission spectrometry. The optimized wet acid digestion method for the analysis of lemongrass was found efficient for all the metals and it was evaluated through the recovery test and good percentage recovery of 86.9 to 106% was obtained for all the metals identified. The metals K, Ca, Mg, Fe, Mn, Cu, Zn and Pb were with a concentration of 743.8-1020, 123.1-129.3, 23.9-36.3, 10.35-22.3, 10.0-12.7, 1.48-2.53, 0.59-1.07, 0.13-0.20 mg/kg, respectively. The results of the Pearson correlation showed that there was a weak and strong positive correlation between the concentrations of the metals analyzed. Statistical analysis by using one-way ANOVA indicated that there were significant differences in the mean concentrations of all the metals (except Ca and Mn) across each sampling points. The results also showed that *Cymbopogon citratus* are beneficial sources of essential metals. The levels of the metals in the analyzed samples were within the WHO maximum permissible limits and thus safe for human consumption.

Keywords/phrases: Major and trace metals, *Cymbopogon citratus*, wet digestion, microwave plasma-atomic emission spectroscopy

INTRODUCTION

Cymbopogon citratus popularly known as lemongrass is one of the aromatic plants which are used to make herbal medicine (Wilson *et al.*, 2012; Christopher *et al.*, 2014; Singh, 2015). The lemon-like odour of the plant could be ascribed to the existence of a cyclic monoterpene (Manvitha and Bidya, 2014).

The genus *Cymbopogon* belongs to the family of Gramineae (syn. Poaceae), which are herbs known around the world for their high yield of essential oil. The herb is grown in a wide range of soil and climatic conditions. It requires a hot and humid climate with high rainfall and long sunlight (Aftab *et al.*, 2011; Thorat *et al.*, 2017). The plant grows in dense clumps up to two meters in diameter and has leaves up to a height of one meter (Aftab *et al.*, 2011). It is widely distributed and cultivated in tropical and subtropical countries of Asia, Africa,

and America (Shah, 2011; Anal, 2014; Avoseh *et al.* 2015).

Most of the population in Africa and other developing countries rely on herbal medicine to meet their primary health care needs for the maintenance of good health. (Falkenberg, 2002; Singh, 2015; Nkansah *et al.*, 2016).

Cymbopogon citratus is of great interest due to its commercially valuable essential oils, its common use in food industries as well as in traditional medicine (Manvitha and Bidya, 2014). The plant is broadly used as flavoring agent in the food and beverage industries. Moreover, its applications have been reported in pharmaceutical, cosmetics, soap, and detergent industries. Some studies (Francisco *et al.*, 2011; Ajayi *et al.*, 2016; Ekpenyong *et al.*, 2015; Avoseh *et al.*, 2015) have reported the pharmacological activities of the plant such as antibacterial, antifungal, anti-inflammatory, anticancer, analgesic, antiseptic, etc. Thus, the plant is used commonly as a remedy in the treatment of illness from a toothache, swollen

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gums, cough, rheumatism, malaria, bladder, and digestive problems (Shah, 2011; Balakrishnan *et al.*, 2014; Ahmad and Viljoen, 2015; Babarinde *et al.*, 2016).

Human beings require minerals in varying proportions for proper growth, health maintenance, and general wellbeing. For example, the major metal magnesium is essential to the enzymatic function of all cellular life (Moomaw and Maguire, 2008). Potassium is important for its diuretic nature and helps in the proper function of the brain as well as nerves and participates actively in the maintenance of cardiac rhythm, thereby preventing stroke (Silva *et al.*, 2005; He and MacGregor, 2008). Calcium is a multifunctional nutrient essential to the body's metabolism and the formation of bones and teeth (Silva *et al.*, 2005). The trace metals are the components of the structures of different active bio-compounds and are necessary for the physiological and biological function of the human body. For instance, zinc, copper, and manganese are essential components of many enzymes, also important for proper growth, bone and connective tissues development, elimination of free radicals, reproductive and the central nervous system. Iron has various functions in the human body including oxygen supply, energy production, and immunity (Devi and Sarma, 2013; Dghaim *et al.*, 2015; Yohannes *et al.*, 2018). However, too low or excessive intake of minerals beyond permissible limits in human diets can result in adverse effects to human health as they can cause various disorders of the body function.

Some studies have been carried out on the levels of major and trace metals of lemongrass by different methods in different countries. For instance, Anal (2014) has reported the metal content in lemongrass in India by graphite furnace-atomic absorption spectroscopy, Aftab *et al.* (2011) studied the mineral content in lemongrass in Pakistan by X-ray fluorescence spectroscopy, Nkansah *et al.* (2016) has reported metal content in lemongrass in Ghana by atomic absorption spectrometry and Salami *et al.* (2007) investigated mineral content in lemongrass in Nigeria by atomic absorption spectrometry.

Recent studies have also been conducted on the levels of essential and non-essential metals in some spices and medicinal plants cultivated in Ethiopia (Endalamaw and Chandravanshi, 2015; Dubale *et al.*, 2015; Hagos and Chandravanshi, 2016; Abdella

et al., 2018; Admasu *et al.*, 2018). In this study, MP-AES was used because the technique is faster, cost-efficient, sensitive, better linear dynamic range, and has lower detection limit to sub ppb level than conventional flame atomic absorption spectroscopy (FAAS) (Vysetti *et al.*, 2014; Zamanian and Kashanaki, 2018; Ramos and Lamorena, 2021). Unlike FAAS techniques, MP-AES runs on nitrogen that can be extracted directly from air instead of using hazardous and flammable gases like nitrous oxide and acetylene (Zamanian and Kashanaki, 2018).

Addis Ababa, Ankober, Gojjam and Wondogenet are some of the areas in which lemongrass is growing abundantly in Ethiopia. However, the levels of the major and trace heavy metals in the plant have not been studied yet in the country. Lemongrass can help Ethiopian people to balance the essential metals within the body when it is used as folk medicine, flavoring agent in food and beverage industries, herbal tea, etc. Therefore, the main objective of the study was to detect the presence and determine the levels of selected major and trace metals, namely, K, Mg, Ca, Mn, Fe, Cu, Zn, and Pb in lemongrass samples from the different regions of the country by using simple, sensitive and reliable method microwave plasma-atomic emission spectroscopy (MP-AES). The specific objectives of the study were: i) to develop and optimize digestion procedure for the determination of selected metals in lemongrass for their subsequent determination by MP-AES, ii) to compare the levels of the metals in lemongrass from the sampling areas, iii) to compare the results of the study with recommended values from WHO and other reported values from the literature.

MATERIALS AND METHODS

Collection of samples

Lemongrass samples (1 kg from each site) were collected by using a random sampling technique from four different regions of Ethiopia (Addis Ababa, Ankober, Gojjam, and Wondogenet). All the samples collected from sampling areas were stored in clean plastic bags. The selection of the sample collection areas was based on more accessibility for sampling. The geographical locations (latitude, longitude, and elevation) of sampling sites are given in Table 1.

Chemicals, reagents, and standard solutions

For digestion of lemongrass samples, HNO₃ (69.5%) (Sigma Aldrich Steinheim, Germany), HClO₄ (70%) (Sigma Aldrich Steinheim, Germany), HCl (37%) (India) were used. Deionized water was used for the preparation of standard solutions, dilution and for cleaning purposes. 1000 mg/L stock standard solutions of the metals (Sigma Aldrich Steinheim, Germany) Ca, Cu, Zn, Mg, K, Mn, Fe, and Pb were prepared for each element in 2% HNO₃ and used for the preparation of calibration curves for the determination of metals in the samples.

Table 1. Geographical location, elevation of sampling sites

Sample Sites	Latitude	Longitude	Altitude (m)
Addis Ababa	9°01'29" N	38°44'48" E	2405
Ankober	9° 35' 46N	39° 43' 57E	2896
Gojjam	11°36'N 36°57'E	11°36'N 36°57'E	2072
Wondogenet	7°1'N38°35'E	7°7'1'N38°35'E	1723

Instrumentation and procedures

An electrical grinder was used for grinding lemongrass leaves. Digital analytical balance (Model 22ADAMT, Switzerland) with ±0.0001g precision was used for weighing and plastic bags for drying the samples at room temperature. Kjeldahl apparatus (Gallenkamp, UK) was used to digest the lemongrass samples, blank solution, and spiked samples. A refrigerator was used to keep the samples until analysis. Pipettes and micropipettes (Dragonmed, Shanghai, China) were used for measuring different amounts of acids and standard solutions and for spiking of standard solutions for the recovery test. 50 mL volumetric flasks were used to dilute sample solutions and prepare standard solutions. The Agilent 4200 microwave plasma-atomic emission spectrometer (MP-AES, USA) was used for the analysis of the metals.

Lemongrass sample preparation for elemental analysis

The lemongrass samples were washed first by tap water and rinsed with distilled water to remove earthy impurities. Then, lemongrass samples were cut into small pieces by stainless scissors, allowed to dry in air for ten days, chopped, and ground to

powder with an electrical grinder (Figure 1). Then the powdered samples were sieved to 0.425 mm mesh size and stored in dry and clean polyethylene plastic bags until digestion. Finally, 0.5 g of the sample was taken from each sample for digestion, and a solution for final metal determination was prepared.

Optimization of digestion procedure

To select an optimum procedure for digestion, parameters like, volume ratio of reagents, digestion temperature, and digestion time were optimized by varying one parameter at a time and keeping the others constant. Parameters giving clear and colorless solution at lower temperature, at minimum reagent volume and digestion time and maximum concentration of metals were selected as an optimum procedure for digestion of lemongrass sample. Finally, the optimum procedure was chosen on the basis of these criteria requiring 3.0 mL of 69.5% HNO₃, 2 mL of 37% HCl and 2.0 mL 70% HClO₄ for complete digestion of 0.5 g lemongrass samples for a time of one and a half hours at a temperature of 270 °C (Table 2).

Digestion of leaves of lemongrass samples

A 0.5 g of powdered lemongrass sample was weighed and placed in a 250 mL round bottom flask. Then, a total volume 7 mL of mixture of the acids (3 mL HNO₃, 2 mL HCl, and 2 mL of HClO₄) was added. The round bottom flask was fitted to a reflux condenser and heated on a Kjeldahl apparatus hot plate for one and half hours at a temperature of 270 °C. The digested samples were allowed to cool for 10 min without separating the condenser and then further cooled to room temperature for 10 min by separating the condenser. The mixture was diluted with 20 mL of deionized water and filtered with Whatman filter paper No. 42 (Germany) into a 50 mL volumetric flask. The round bottom flask was further rinsed with 10 mL of deionized water and added to the filtrate. Then, the flask was filled to the mark with deionized water. For each sample the digestion was done in triplicates. Triplicates of blank samples were digested following the same procedure as the samples. Finally, all the digests were kept in the refrigerator until analysis using MP-AES.

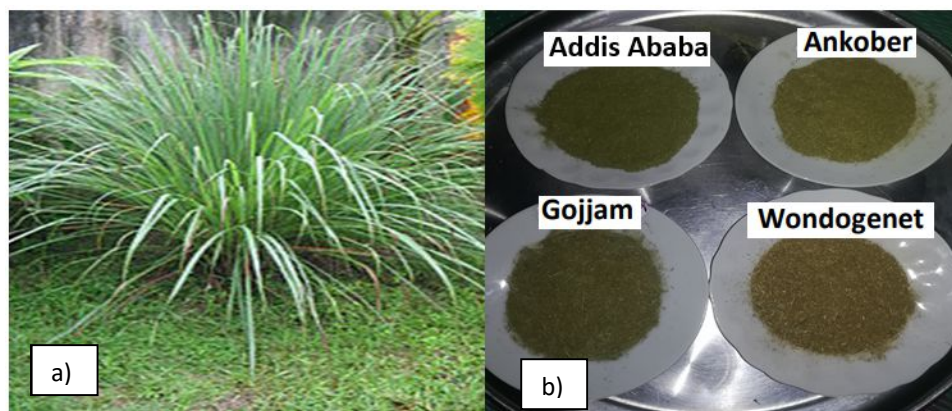


Figure 1. Lemongrass plant a) and powdered lemongrass samples b).

Table 2. Digestion procedures for lemongrass samples.

Volume ratio of reagents	Digestion temperature (°C)	Digestion time (h)	Observation
3:1 (HNO ₃ :HClO ₄)			Yellow clear
3:2 (HNO ₃ :HClO ₄)			Yellow clear
2:1:1(HNO ₃ :HCl:HClO ₄)	270	2	Light yellow clear
3:1:1(HNO ₃ :HCl:HClO ₄)			Light yellow clear
3:1:2(HNO ₃ :HCl:HClO ₄)			Very Light yellow clear
3:2:2(HNO₃:HCl:HClO₄)			Colorless clear solution
	180		Yellow clear solution
3:2:2(HNO ₃ :HCl:HClO ₄)	210	2	Light yellow clear
	240		Light yellow clear
	270		Colorless clear solution
		½	Yellow clear
3:2:2(HNO ₃ :HCl:HClO ₄)	270	1	Light yellow clear
		1:30	Colorless clear solution

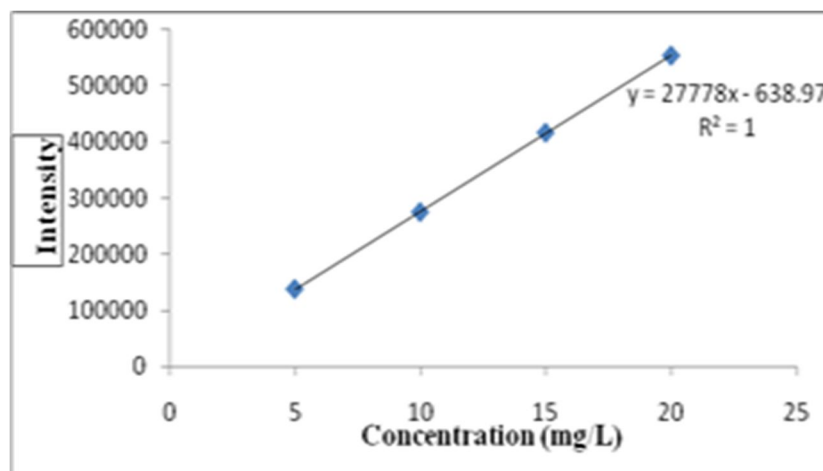


Figure 2. Calibration curve for manganese determination.

Calibration of the instrument and determination of the metals by MP-AES

The 10 mg/L intermediate standard solutions of metals of interest were prepared from the standard stock solutions that contained 1000 mg/L. These secondary standards were diluted with deionized water to obtain working standards (5, 10, 15, 20 mg/L) of each metal, i.e., Ca, K, Mg, Mn, Fe, Cu, Zn, and Pb. The Emission intensities of the working standard solutions were measured and the calibration curves for each of the analyte metals were constructed. The calibration curve of Mn has been shown as a representative example (Figure 2). The concentrations of each metal in the samples were determined by MP-AES after the instrumental operating conditions were optimized for the maximum signal intensity of the instrument. Triplicate determinations were carried out on each sample. The same analytical procedure was also employed for the determination of elements in the digested blank solutions.

Validation of the optimized procedure

The results from a method validation procedure can be used to judge the quality, reliability, and consistency of analytical results. Spiking experiments were performed to validate the optimized procedure. For this purpose, standard solutions of 1000 mg/L of the metals (Ca, K, Cu, Fe, Mg, Mn, Zn and Pb) were used. For recovery measurement, spiking was done for the metals whose concentrations were determined in triplicates. Thus, 2550, 323, 6, 57, 56, 9, 0.13 and 0.02 µL of 1000 mg/L standard solution for K, Ca, Cu, Mn, Fe, Mg, Zn and Pb, respectively, were spiked into round bottomed-flasks containing 0.5 g sample and 7 mL of acid mixture (3 mL HNO₃, 2 mL HCl, and 2 mL of HClO₄). Then, the round bottomed flasks containing the mixtures were fitted with the condenser and digested simultaneously with un-spiked samples on a hot

plate Kjeldahl apparatus by applying the optimized digestion procedure used in sample analysis. Then, the percentage recoveries of the analytes were calculated by using the equation:

$$\%R = \frac{(C_M \text{ of spiked sample}) - (C_M \text{ of unspiked sample})}{C_M \text{ added for spiking}} \times 100$$

where C_M is the concentration of metal and %R is the percent of the recovery. The results of the recovery analysis are presented in Table 3. The percentage recoveries lie within the range 86.9-106, which is within the acceptable range for all the metals.

Table 3. Recovery test for lemongrass samples.

Metal	Conc. of metal in unspiked sample (mg/L)	Amount spiked (mg/L)	Conc. of metal in spiked sample (mg/L)	% R ± SD
K	5.100	2.5500	7.800	106 ± 0.5
Ca	0.647	0.3233	0.928	86.9 ± 0.7
Mg	0.181	0.0900	0.272	101 ± 0.1
Mn	0.113	0.0570	0.167	94.7 ± 1.5
Fe	0.115	0.0580	0.174	102 ± 0.9
Cu	0.013	0.0063	0.019	95.2 ± 0.6
Zn	0.00535	0.00268	0.008	98.9 ± 0.4
Pb	0.00075	0.000375	0.00112	93.3 ± 0.5

RESULTS AND DISCUSSION

Levels of metals in the leaves of lemongrass samples

The MP-AES method was applied for the determination of the levels of eight metals (Ca, K, Mg, Cu, Zn, Mn, Fe, and Pb) in lemongrass samples. Results obtained for each sample in terms of mg/kg with values for the mean, standard deviation, and ranges of metal concentration are shown in Table 4.

Table 4. Metal concentration in different lemongrass samples (mean ± SD mg/kg).

Metal	Sample sites, conc. (mean ± SD) in mg/kg				Range of metal conc. (mg/kg)
	Addis Ababa	Ankober	Gojjam	Wondogenet	
K	913.5 ± 0.85	1020 ± 0.2	743.8 ± 0.5	879.3 ± 1.4	743.8-1020
Ca	125.1 ± 0.1	129.3 ± 0.5	123.1 ± 0.1	126.3 ± 0.9	123.1-129.3
Mg	26.0 ± 1.8	36.3 ± 2.7	23.9 ± 1.7	33.0 ± 3.4	23.9-36.3
Fe	18.5 ± 0.7	22.3 ± 1.2	18.5 ± 0.9	10.35 ± 1.4	10.35-22.3
Mn	12.6 ± 4.7	12.7 ± 4.3	10.0 ± 1.3	12.5 ± 2.8	10.0-12.7
Cu	2.35 ± 1.2	2.5 ± 0.8	1.48 ± 1.3	1.94 ± 1.9	1.48-2.5
Zn	0.69 ± 0.5	1.07 ± 0.05	0.6 ± 0.1	0.59 ± 1.0	0.59-1.07
Pb	0.15 ± 0.3	0.20 ± 0.1	0.13 ± 0.4	0.17 ± 0.3	0.13-0.20

As indicated in Table 4, the metals Ca, K, Cu, Fe, Mn, Mg, Zn and Pb are found in all the samples of lemongrass. A relatively higher amount of K in the sample from all areas could be due to its higher natural abundance in the soil and the nature of the lemongrass. This result is in good agreement with the report from Godwin and his coworkers (Godwin, 2002).

In comparison to other sampling areas, Ankober is more exposed to human activity and the farms may have been used for a long time which could have a significant contribution to a gradual accumulation of metals in lemongrass farms through agricultural activities. The result showed that the metal contents of lemongrass varied with the geographical origin in which the lemongrass grows. The types of soil, climatic condition, humidity, the natural weathering of rocks, agricultural activities could contribute to the different concentrations of metals determined in lemongrass.

Concentration of metals in lemongrass collected from different sample sites

The concentrations of metals in lemongrass collected from different sample sites are given as follows: In Addis Ababa, K is found in the highest amount in the samples with concentration of 913.5 mg/kg followed by Ca (125.1 mg/kg), Mg (26.0 mg/kg), Fe (18.5 mg/kg), Mn (12.7 mg/kg), Cu (2.35 mg/kg), Zn (0.69 mg/kg) and Pb (0.15 mg/kg). In Ankober area, K (1020 mg/kg), Ca (129.5 mg/kg), Mg (36.3 mg/kg), Fe (22.3 mg/kg), Mn (12.7 mg/kg), Cu (2.53 mg/kg), Zn (1.1 mg/kg) and Pb (0.20 mg/kg). In Gojjam, K (743.7 mg/kg), Ca (123.1 mg/kg), Mg (23.9 mg/kg), Fe (18.5 mg/kg), Mn (10.0), Cu (1.48 mg/kg), Zn (0.6 mg/kg) and Pb (0.13 mg/kg) and in Wondogenet, K (879.4 mg/kg), Ca (126.3 mg/kg), Mg (33.0 mg/kg), Fe (10.35 mg/kg), Mn (12.5), Cu (1.94 mg/kg), Zn (0.59 mg/kg) and Pb (0.17 mg/kg). The comparison of metals in all sample sites is indicated in Figure 3.

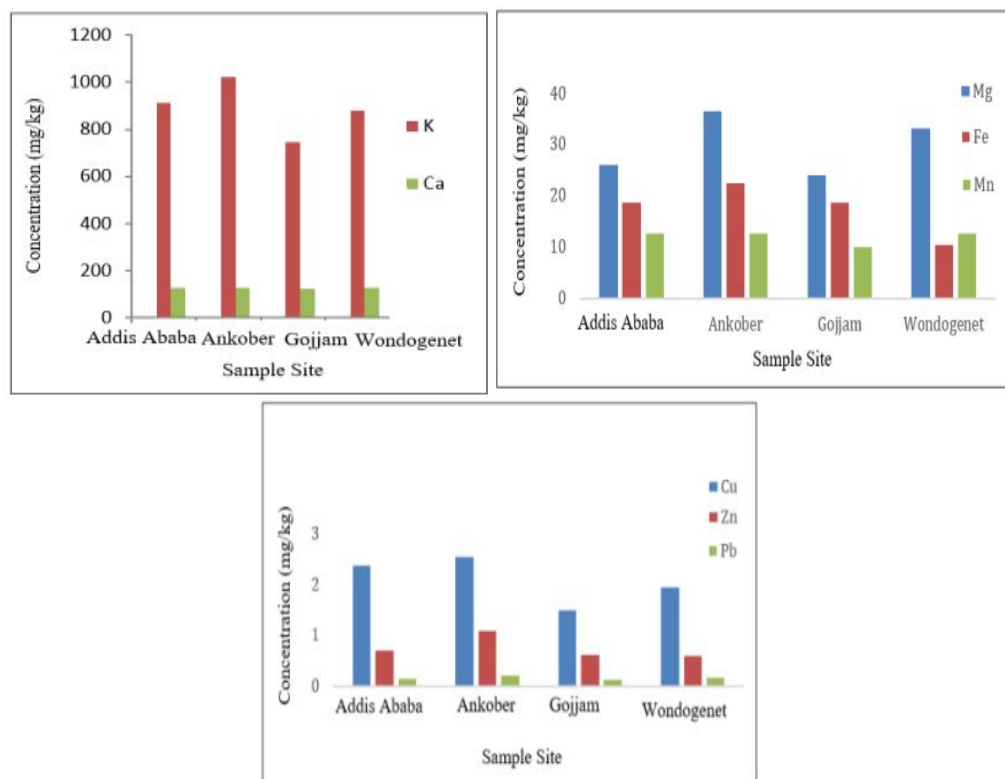


Figure 3. The concentration of metals in lemongrass from different sample sites

In general, it can be summarized that the lemongrass sample from the Ankober area contains the highest amount of the metals studied while the lowest concentration is in the Gojjam sampling area (Figure 3). This could be attributed to the fact that the sample taken from Gojjam is in a very rural area with no industrialization and thus fewer anthropogenic sources for the metals in this sample site.

Analysis of variance (ANOVA)

In this study, the variation in sample means of the analytes was tested whether they have significant difference or not by using one way ANOVA at 95% confidence level. Accordingly, there is no significant difference among the sample means for Ca and Mn. While there is significant difference among the sample means for K, Mg, Fe, and Cu, Zn, and Pb (Table 5). This may be due to variations in factors other than the experimental procedures. The source for this significant difference between sample means could be possibly attributed to the differences in soil composition, usage of different fertilizers, pesticides and industrial activities.

Table 5. Analysis of variance (ANOVA) between and within lemongrass samples at 95% confidence level

Metal	K	Ca	Mg	Fe	Mn	Cu	Zn	Pb
F _{cal}	10.5	2.10	11.2	6.40	0.25	4.50	3984	5.30
F _{crit}	5.27	3.29	5.10	5.64	0.35	1.23	3816	4.29

Pearson correlation of metals within lemongrass samples

A Pearson correlation is a number between -1 and 1 that indicates the extent to which two variables are linearly related. If the correlation coefficient approaches to positive one there is a strong positive relationship among the two variables. If the correlation coefficient is -1, the two variables have a strong negative relationship. If there is no relationship between the two variables

the correlation coefficient equal to zero (Bhan *et al.*, 2005).

In this study, to correlate the effect of one metal concentration on the concentration of other metals, Pearson correlation matrices for the samples were applied for the metals determined in the samples (Table 6). The results of the Pearson correlation show positive correlations in the case of K, Ca, Mg, Fe, Mn, Cu, Zn and Pb with each other. Besides, the metal K with (Fe and Mn), Ca with (Mn and Cu), Mg with (Fe, Mn with K, Ca, Cu, Zn, and Pb), Fe with (K, Ca, Mg, Cu and Pd), Cu with (Ca, Fe, Zn, and Pb), Zn with (Mn and Cu) and Pb with (Fe, Mn, and Cu) were observed to have a positive weak correlation.

The weak correlations indicate that one metal affecting the other metal to a lesser extent and strong correlations may arise from common natural or anthropogenic sources as well as from similarity in chemical properties. A good positive correlation is observed between K and Ca and also between Cu and Mn. In contrast, a weak correlation is observed for Mn with K and Pb.

Table 6. Pearson correlation matrix for metals in leaves of lemongrass samples.

Parameters	K	Ca	Mg	Fe	Mn	Cu	Zn	Pb
K	1.00							
Ca	0.75	1.00						
Mg	0.51	0.69	1.00					
Fe	0.27	0.26	0.13	1.00				
Mn	0.04	0.27	0.52	0.63	1.00			
Cu	0.59	0.35	0.55	0.43	0.76	1.00		
Zn	0.66	0.61	0.55	0.70	0.14	0.42	1.00	
Pb	0.66	0.71	0.67	0.31	0.028	0.34	0.65	1.00

Comparison of the concentrations of the metals in lemongrass sample obtained in the present study with literature and acceptable range of medicinal plant by WHO

The findings of the present study were compared to that of other authors from different countries and an acceptable range of medicinal plant by WHO. The comparative levels of metals in the studied samples with the levels reported in the literature are indicated in Table 7.

Table 7. Comparison of metal concentrations of lemongrass samples of this study with reported literature and an acceptable level in medicinal plants by WHO.

Metal	Conc. of metals in lemongrass sample (mg/kg)			Conc. of metals in medicinal plant (mg/kg). WHO
	Ethiopia	Pakistan (XRF)	India (AAS)	
K	743.8-1020	723	-	-
Ca	123.1-129.3	65	5.2-6.0	-
Mg	23.9-36.3	60	0.76-0.79	2000
Fe	10.35-22.3	8.7	1.5-1.98	261-1239
Mn	10.0-12.7	5.2	0.19-0.27	200
Cu	1.48-2.5	0.266	0.05-0.07	20-150
Zn	0.59-1.07	2.23	0.11-0.12	50
Pb	0.13-0.20	-	0.14	10
Reference	This study	(Aftab <i>et al.</i> , 2011)	(Anal <i>et al.</i> , 2014)	(WHO,1998; Nkuba and Mohammed, 2017)

As shown in Table 7, the concentrations of all the above listed metals are greater in Ethiopia when compared to India and in good agreement with the results from Pakistan. The variations observed might be due to differences in soil fertility, climatic conditions, usage of fertilizers, etc.

The level of most of the metals found in the lemongrass samples collected from four selected areas (Addis Ababa, Ankober, Gojam, and Wondogenet) were found comparable to the maximum tolerable limits recommended by WHO.

CONCLUSION

The determination of the level of the metals K, Ca, Mg, Fe, Mn, Cu, Zn, and Pb in *Cymbopogon citratus* samples was carried out by microwave plasma-atomic emission spectrometry. The result was evaluated through the recovery test and good percentage recovery of 86.9 to 106% was obtained for all the metals identified. The concentration of the metals in the samples can be expressed in the following order: K > Ca > Mg > Fe > Mn > Cu > Zn > Pb.

Statistical analysis by using one-way ANOVA at a 95% confidence level indicated that there were no significant differences among the sample mean concentrations for the metals Ca and Mn while there were significant differences in the mean concentration of K, Mg, Fe, Cu, Zn, and Pb in the samples. This could be attributed to the differences in soil composition, usage of different fertilizers, pesticides, and industrial activities. The results of the Pearson correlation showed that there was a weak and strong positive correlation between the

metals. The levels of metals in the samples were found to be comparable with the results reported from different countries and within the permissible limits of WHO.

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