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Determination of Levels of Selected Essential and Non Essential Metals in Mango (*Mangifera indica* L.) Cultivated in Wolaita Zone, Southern Ethiopia

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ABSTRACTS

Background: Mango (*Mangifera indica* L.) is one of the most important tropical fruits commercialized and consumed worldwide fresh or processed, having an attractive color and distinct taste and aroma. The main objective of this study is to determine the level of selected essential and non-essential metals in Mango.

Methods: The levels of essential and non-essential metals were determined in mango (*Mangifera indica* L.) samples collected from four different sites (Boloso Bombe, Boloso Sore, Damot woyde and Humbo) in Wolaita Zone, Southern Ethiopia by flame atomic absorption spectrometry. A 0.5 g dried powdered mango sample was digested with 1 mL of nitric acid (HNO_3), 3 mL of perchloric acid ($HClO_4$) and 1 mL of hydrogen peroxide (H_2O_2) at 180°C for 2:00 hours. The study focused on two mango varieties, the Apple mango and the Keitt mango.

Result: The metal contents in the apple mango in mg/kg were found in the following order: Mg (20.04-0.34) > Ca (1.49-6.78) >Zn (2.32-4.23) > Fe (2.77-2.85) > K (2.03-2.41) > Na (1.72-1.97) > Mn (0.47-1.36) mg/kg; and that of the Keitt mango samples in mg/kg were in the order of: Mg (2o.08_20.34) > Ca (0.95_5.84) > Zn (2.69_3.66) > Fe (2.69_2.90) > K (1.91_2.77) > Na (1.79_1.93) > Mn (0.38_0.72) mg/kg, respectively. The levels of Cd and Pb were below method detection limit. Analysis of variance (ANOVA) at 95% confidence level indicated that there is significant difference in the mean concentration of all metals between the four sample sites. There was good correlation between the levels of some metals (Mg, Ca, Zn, Fe K, Na and Mn) in both varieties of mango samples. From the results of this study it can be concluded that the studied mango varieties grown in the study area are good source of essential metals and free from the toxic metals: Cd and Pb.

Key words: Apple mango, Flame atomic absorption spectrometry (FAAS), Keitt mango, Mango (*Mangifera indica* L.), Metals, Wet-digestion.

INTRODUCTION

Mango (*Mangifera indica* L.) is a fleshy stone fruit belonging to the panes Mangifera, consisting of numerous tropical fruiting trees in the flowering plant family *Anacardiaceae*. Mango is native to the south Asia from where it was distributed worldwide to become one of the most cultivated fruit in the tropics (Indu, 2017). Mango is produced in most frost free tropical and sub-tropical climates, more than 85 countries in the world cultivate mango. Mango is one of the most widely cultivated and globally traded tropical and subtropical fruit trees in the world (ibid). The total production area of mango in the world is around 3.69 million hectares (FAO, 2009).

Mango fruit is an excellent source of dietary antioxidants, such as ascorbic acid, carotenoids and especially phenolic compounds (Ma *et al*., 2011). In Sub-Saharan Africa (SSA), growing both domesticated and wild fruit species on farms diversifies the crop production options of small-scale farmers and can bring significant health, ecological and economic revenues (Weinberger and Lumpkin, 2005; Keatinge *et al*., 2010). As reported by Ayman *et al.* (2014) the level of Fe, Mn and Zn in Mango were in the ranges 3.17-17.86, 1.2-6.64 and 5.2-13.64 mg/k, respectively.

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Minerals play a vital role in regulation of cellular and physiological metabolism. Trace metals in the body maintain the pH, osmotic regularity and used as coenzyme which regularize the metabolic reactions (Ismail, 2011). The presence of essential metals like sodium, potassium, calcium, magnesium, iron copper, nickel and zinc are very useful for the healthy growth of the body though very high levels are intolerable, metals like lead and cadmium are toxic at very low concentrations (Adeyeye, 2005).

Plants, which are intermediate reservoirs of these metals, may take these metals from soil, water and air and accumulate them in or in their tissues due to their several

biochemical mechanisms developed during their evolution and course of life which enables them adapt and tolerate new or chemically imbalanced environment (Adeyeye, 2005). Generally, too low or too high concentration of trace elements in human diet can affect the quality of human life (Jung, 2008). The assessment of metal contents (essential and non-essential) in mango fruit was therefore necessary from the point of view of nutrition, toxicological, crop yield as well as many other applications (Nnorom *et al*., 2007). However, there is no study on the level of essential and non-essential metals in mango in Wolaita zone. Therefore, the objective of this study was to determine the level of essential (Na, K, Ca, Mg, Fe, Zn and Mn) and non-essential (Cd and Pb) metals in two mango varieties (Apple and Keitt) cultivated in Wolaita Zone.

MATERIALS AND METHODS

Description of the study area

The study was conducted on the four main mango growing districts of Wolaita Zone, namely, Boloso Bombe, Boloso Sore, Damot Woyde and Humbo in 2020. The zone is situated from 6′N-7.1′N latitude and 37.4′E-38.2′E longitude with an average altitude of 1750 meters and ranges between 501 and 3000 meters above sea level. It is bounded to the North and the North East by Kambata Tambaro Zone, to the West and South West by Dawaro zone, to the south by Gamo Gofa Zone and to the East by Sidama. There are 3 agro-ecological zones in the Zone out of which Dega accounts 9%, Weynadega 56% and Kolla 35% (WZFEDD, 2012).

Sample collection and preparation

Mature, healthy and fresh mango fruits were collected from the farmlands of four Districts of Wolaita Zone. These include Boloso Bombe, Boloso Sore, Damot Woyde and Humbo. The sampling sites were selected based on large-scale production area of mango in Wolaita Zone. To collect the representative sample composite samples were taken from each sampling site. Three composite samples of fresh mango were collected from each sample site and put in clean polyethylene plastic bags and brought to the laboratory for further pre-treatment (Desta and Ataklti, 2015).

The collected mango fruit was washed with a running tap water to remove adsorbed soil particulates and then rinsed with distilled water. The outer skin of mango sample was removed by Teflon knife and flesh part chopped in to pieces to facilitate drying. The sample was exposed to sun light for three days to reduce the moisture content and subsequently dried in the drying oven at 80°C for 24 hours to constant weight. The dried sample was powdered using electronic blender and sieved to prepare fine powder of mango for digestion (Desta and Ataklti, 2015) and all experiments were conducted in Wolaita Sodo University.

Optimization of digestion procedure

To select an optimum procedure for digestion parameters like digestion time, reagent volume, volume ratio of reagents and digestion temperature were optimized by varying one parameter at a time and keeping the others constant. Parameters giving clear solution at lower temperature, requiring minimum reagent volume and digestion time were selected as an optimum procedure for digestion of mango samples (AOAC, 1990). Among the tested procedures reagent mixture of 3 ml of $HClO_4$, 1 ml of HNO_3 and 1 ml of H_2O_2, digestion temperature 180°C and digestion time 120 minute were selected as optimum for digestion of 0.5 g of mango sample.

Applying the optimized wet digestion conditions, 0.5 g of prepared samples of mango were digested on a Kjeldahl digestion apparatus fitted with a reflux condenser till the clear solution was observed. The sample was digested by using mixture of HNO_3 and $HClO_4$. The clear solution was filtered into 100 mL volumetric flasks through Whatman paper. It was diluted with distilled water up to the mark and stored for further analysis (Huang *et al*., 2004; Gaudino *et al*., 2007; Wilson *et al*., 2005).

Instrument calibration

Calibration metal standard solutions were prepared for each of the metals from an intermediate standard solution containing 10 mg/L from the atomic absorption spectroscopy standard stock solutions that contain 1000 mg/L. These intermediate standards were diluted with distilled water to obtain four working standards for each metal of interest. Then, Na, K, Ca, Mg, Zn, Mn, Fe, Pb and Cd were analyzed with FAAS (Desta and Ataklti, 2015).

Three replicate determinations were carried out on each sample. All metals were analyzed by absorption/ concentration mode and the instrument readout was recorded for each solution manually. The same analytical procedure was employed for the determination of elements in digested blank solutions (Mebrahtu and Chandravanshi, 2016).

Analysis of metals in mango

The digested sample of mango was taken in triplicate and the selected metals (Na, K, Ca, Mg, Fe, Mn, Zn, Cd and Pb) were analyzed by using Flame atomic absorption spectrophotometer equipped with deuterium arc background corrector using air acetylene flame (Desta and Ataklti, 2015).

Method performance and method validation

To validate the analytical method, parameters such as; precision, accuracy (in terms of recovery), method detection limit, limit of quantitation (LOQ), analysis of laboratory control sample, matrix spike and matrix spike duplicate were carried out (Chauhan *et al*., 2015).

Method detection limits

The limit of detection is most commonly defined as the mass of analyte that gives a signal equal to three times the standard deviation on the blank (Butcher and Sneddon, 1998). In the present study, seven blank samples were digested and analyzed for metal concentrations of Na, K,

Ca, Mg, Fe, Zn, Mn, Pb and Cd in triplicate by flame atomic absorption spectrophotometer (FAAS). The standard deviation for each element was calculated from the seven blank measurements to determine method detection limit of the instrument. Then the method detection limit of each element was calculated as three times the standard deviation of the blank (3σblank, n = 7).

Recovery tests

To check the efficiency of developed optimized procedures, spiking experiments in which known volume and concentration of standard solutions were employed. Each sample was determined for their respective spiked metals by flame atomic absorption spectrophotometer (FAAS). The spiked and non-spiked samples were digested and analyzed in similar condition. Then the percentage recovery of the analyte was calculated as:

$$\% R = \frac{C \text{ in the spicked sample} - C \text{ in the non spiked sample}}{C \text{ added for spiking}} \times 100$$

Where,
C = Concentration of metal of interest.

Data analysis

All analyses were carried out in triplicates and the data was presented as means±standard deviations. Difference between treatment means were done by using analysis of variance (ANOVA) and for comparison of the means of the treatments, the Fisher's least significant difference (LSD) test was used at $P<0.05$ significance level. Pearson's correlation analysis was also applied to test the correlation between metals with in mango samples. All statistical analyses were done by SPSS and SAS software for windows.

RESULTS AND DISCUSSION

Instrument calibration

The calibrated instrumental operating conditions including its determined method detection limit are shown in Table 1.

The MDL was calculated by multiplying the pooled standard deviation of the reagent blank (Sblank) by three (MDL = 3 × Sblank, n = 7). Method detection limits of the metals of interest are given in Table 1. The smaller values for MDL indicate that the presence of trace amounts of metals of interest in the sample can be detected by the method.

Recovery test

The validity of the optimized procedure was assessed by spiking experiments. The spiked and non-spiked samples were digested and analyzed in similar condition using optimized procedure before for sample analysis. Then the percentage recovery of the analyte was calculated as:

$$\% R = \frac{C \text{ in the spicked sample} - C \text{ in the non spiked sample}}{C \text{ added for spiking}} \times 100$$

The results of recovery analysis are shown in Table 2 and the percentage recoveries lies within the range 97-103%.

Levels of metals in apple and Keitt mango

The metal contents in the apple and Keitt mango were found in the following decreasing order: Mg > Ca >Zn > Fe > K > Na > Mn (Table 3). Among the determined metals Mg and Ca were the most abundant essential elements in both apple and Apple and Keitt samples. This is because of metals such as Ca and Mg are mobile in to plant tissue (Marschner, 1995; Alemu *et al*., 2022). Another reason may be due to the plant having a greater affinity for Mg and Ca than other metals. The concentrations of the non-essential metals Pb and Cd were below method detection limit in both mango varieties. The distribution pattern of individual metals in both varieties of mango in all sampling sites was discussed in detail as follows.

Magnesium

The level of Mg was ranged from 20.04 to 20.34 mg/kg in apple mango whereas it ranged from 20.30 to 20.34 mg/kg in Keitt. The highest value observed in Damot Woyde and lowest value observed in Boloso Sore in apple mango. But in keitt highest value was observed in Boloso Sore and lowest in Boloso Bombe. Analysis of variance showed that the concentration of Mg significantly different in all except Damot Woyde and Boloso Bombe. Magnesium was the most accumulated metal in both varieties of mango. The reason why the Mg concentration was very high in all sample sites may be due to the presence of the limestone and gypsum in the soil. The marble and limestone could be the main sources of Mg in the soil from these sample areas. Moreover, Mg is amongst the major elements required by plants. In general level Mg in this study was lower than the result (1,6210 mg/kg) reported by (Tesfaye *et al*., 2015). Among the sample sites the level of Mg in apple mango in Damot woyde > Boloso Bombe > Humbo > Boloso Sore and the level of Mg in Keitt Mango in Boloso Sore > Boloso Bombe > Damot Woyde > Humbo.

Calcium

The level of Ca was ranged from 1.49 to 6.78 mg/kg in apple Mango whereas it ranged from 0.95 to 5.84 mg/kg in Keitt. The highest value was observed in Damot Woyde and the lowest value was observed in Boloso Sore in apple mango but in Keitt the highest value was observed in Humbo and the lowest value was observed in Damot Woyde. Analysis of variance showed that the mean concentration of calcium was significantly different in all sampling sites except Boloso Bombe and Humbo in apple and significantly different in all sampling sites in Keitt. The probable reason of the difference in value may be due difference in the pH of the soils, mineral composition of the soils and type of fertilizers used. The level of Ca obtained in this study was lower than with the result (75.10 mg/kg) reported by (Saeed *et al*., 2010).

Among the sampe sites the level of Ca in apple mango in Damot woyde > Boloso Bombe > Humbo > Boloso Sore and the level Ca in Keitt Mango in Humbo > Boloso Sore > Boloso Bombe > Damot Woyde.

Potassium

The level of K was ranged from 2.03 to 2.41 mg/kg in Apple whereas it is ranged from 1.91 to 2.77 mg/kg in Keitt. The highest value was observed in Boloso Bombe and the lowest value was observed in Boloso Sore in Apple Mango but in Keitt the highest value was observed in Boloso sore and the lowest value was observed in Humbo. Analysis of varience showed that the mean concentration of Potasium was significantly different in all sampling sites except Humbo and Damot Woyde. The probable reason due to difference in value may difference in soil nature (Feleke *et al*., 2023). The level of potasium obtained in this study was lower than the result (382.77 mg/kg) reported by (Saeed *et al*., 2010). Among the sample sites level of K in apple Mango in Boloso Bombe > Humbo > Damot Woyde > Boloso Sore and the level of K in Keitt in Boloso Sore > Boloso Bombe > Damot Woyde > Humbo.

Sodium

The level of Na was ranged from 1.72 to 1.97 mg/ kg in apple whereas it is ranged from 1.79 to 1.93 mg/ kg in Keitt. The highest value was observed in Damot Woyde and the lowest value was observed in Boloso Sore in Apple mango but in Keitt the highest value was observed in Humbo and the lowest value was observed in Damot Woyde. Analysis of varience showed that the mean concentration of Na was significantly different in all sampling sites in apple and significantly different in all sampling sites except Damot Woyde and Boloso Bombe in Keitt. The is reason due to different type of soil nutrients and nature of topography. The level of Na observed in this study was lower than the result (61.53 mg/kg) reported by (Saeed *et al*., 2010). Among the sample sites the level of Na in apple mango in Damot Woyde > Humbo > Boloso Bombe > Boloso Sore and the level of Na in Keitt Mango Humbo > Boloso Sore > Boloso Bombe > Damot Woyde.

Zinc

The level of Zn content was ranged from 2.32- 4.23 mg/ kg in Apple mango whereas it ranged from 2.69 to 3.66 mg/kg in Keitt. The highest value was observed in Boloso Sore and the lowest value was observed in Boloso Bombe in aplle Mango but in Keitt the highest value was observed in Humbo and the lowest value was observed in Boloso Sore. Analysis of varience showed that the mean concentration of zinc was significantly different in all sampling sites. This may be because of different back ground of soil character and having different temperature (Bunaka *et al*., 2023). The level of Zn observed in this study was higher than the result (2.76 mg/kg) reported by (Tesfaye *et al.,* 2015). Among the sample sites the level of Zn in apple Mango in Boloso Sore > damot Woyde > Humbo > Boloso Bombe and the level of

Table 1: The wavelength, method detection limit, correlation coefficient and calibration curve equation for determination of metals using FAAS.

Metals	Wavelength (nm)	Instrument detection limit	Method detection limit (mg g^{-1} dry weight)	Correlation coefficient	Calibration curve equation
Ca	422.7	0.01	0.01	0.998	y=0.080×-0.039
K	769.5	0.025	0.0024	0.999	Y=0.110×-0.081
Mg	285.2	0.005	0.006	0.998	Y=0.075×-0.231
Na	589	0.005	0.0024	0.998	Y=0.058×-0.013
Fe	248.3	0.05	0.008	0.998	Y=0.171×-0.040
Mn	279.2	0.03	0	0.999	Y=0.080×+0.006
Zn	213.9	0.005	0.004	0.999	Y=0.144×-0.272
Cd	228.9	0.01	ND	ND	ND
Pb	217	0.04	ND	ND	ND

ND: Not detected.

Table 2: Recovery test for the optimized procedure of mango sample.

Metals	Concentration of sample (mg kg^{-1})	Amount spiked (mg kg^{-1})	Concentration of spiked sample (mg kg^{-1})	Amount recovered (mg kg^{-1})	% Recovery
Ca	4.70	1.07	5.78	1.01	101
K	2.19	1.11	3.30	1.00	100
Mg	20.22	1.10	21.35	1.03	103
Na	1.86	1.15	2.99	0.98	98
Fe	2.81	1.00	3.80	0.99	99
Mn	0.70	1.16	1.82	0.97	97
Zn	3.05	1.06	4.10	0.99	99

Zn in Keitt mango Humbo > Damot Woyde > Boloso Bombe > Boloso Sore.

Iron

The level of Fe is ranged from 2.77 to 2.85 mg/ kg in apple Mango whereas it ranged from 2.69 to 2.90 mg/ kg in Keitt Mango. The highest value observed in Humbo and the lowest value was observed in Boloso Bombe in Apple but in Keitt the highest value observed in Damot Woyde and the lowest value was observed in Humbo. Analysis of variance showed that the mean concentration of Fe was significantly different in all sample sites except Boloso Sore and Damot Woyde in Keitt. Due to difference in mineral composition of soil and type of fertilizers used. The level of Fe observed in this study lower than maximum permissible limit of Fe the result (3.70 mg/kg) reported by (Saeed *et al*., 2010). Among the sample sites the level of Fe in apple Mango in Humbo > damot Woyde > Boloso Sore > Boloso Bombe and the level of Fe in Keitt Mango in Boloso Sore > Damot Woyde > Boloso Bombe > Humbo.

Manganese

The level of Mn ranged from 0.47 to 1.36 mg/ kg in apple whereas it ranged from 0.38 to 0.72 mg/ kg in Keitt Mango. The highest value observed in Boloso Bombe and the lowest value ovserved in damot Woyde in Apple but in Keitt highest Boloso sore and lowest in Humbo. Analysis of varience showed that the mean concentration of Mn was significantly different in all sampling sites. The probable reason due to different atmosphere condition and having different pH of soils. The level of Mn obtained in this study was lower than maximum permissible limit of Mn the result (30.1 mg/kg) reported by (Tesfaye *et al*., 2015). Among the sample sites, level of Mn in apple Mango in Boloso Bombe > Boloso Sore > Humbo> Damot Woyde and in keitt Mango Bolso Sore > Damot Woyde > Boloso Bombe > Hum Cadmium and Lead were below method detection limit in all sample sites in both Apple and Keitt varieties of Mango.

Comparison of metals with literature values and standards

The concentration of all metals recorded in this study, were lower than the level of metals reported by (Tesfaye *et al*, 2015) except Zn. The level of Zn obtained in this was higher than the level reported by (Tesfaye *et al*., 2015). The level of Fe (2.77_2.85) mg/kg obtained in this study was below the maximum permissible limit of fruits set by WHO which was 425 mg/kg. The level of Mn (0.47_1.36) mg/kg recorded in this study was less than the permissible limit of fruit established by WHO which was 500 mg/kg. The levels of Cd and Pb obtained in this study were below method detection limit. Thus the concentrations of all metals were fall below the established limit. So according to WHO the range was safe because all the concentration less than that of reported by (WHO/FAO, 2001).

Table 3: Concentration of metals in (mg/kg) (mean±SD) in apple and keitt mango in different sample sites.

Metal	Boloso sore		Bolos bombe		Damot woyde		Humbo		CV		LSD	
	Apple	Keitt	Apple	Keitt	Apple	Keitt	Apple	Keitt	Apple	Keitt	Apple	Keitt
Ca	1.49^{d}±0.24	5.40^{b}±0.04	6.25^{b}±0.20	4.81^{c}±0.02	6.78^{a}±0.01	0.95^{d}±0.01	6.07^{b}±0.14	5.84^{a}±0.08	3.35	1.09	0.33	0.09
K	2.03^{c}±0.03	2.77^{a}±0.02	2.41^{a}±0.03	2.05^{b}±0.01	2.21^{b}±0.02	1.95^{c}±0.01	2.22^{b}±0.01	1.91^{c}±0.01	1.03	0.66	0.04	0.03
Mg	20.04^{b}±0.07	20.34^{a}±0.03	20.07^{b}±0.06	20.3^{b}±0.02	20.34^{a}±0.01	20.22^{b}±0.01	20.29^{a}±0.06	20.08^{c}±0.09	0.27	0.25	0.10	0.09
Na	1.72^{c}±0.03	1.86^{b}±0.01	1.86^{d}±0.02	1.81^{c}±0.02	1.97^{b}±0.01	1.79^{c}±0.03	1.92^{a}±0.02	1.93^{a}±0.01	1.03	0.93	0.04	0.033
Fe	2.79^{b}±0.01	2.90^{a}±0.01	2.77^{b}±0.01	2.77^{b}±0.02	2.85^{a}±0.01	2.90^{a}±0.01	2.85^{a}±0.02	2.69^{c}±0.01	0.54	0.48	0.03	0.03
Mn	1.20^{a}±0.01	0.72^{a}±0.08	1.36^{b}±0.02	0.47^{b}±0.01	0.47^{c}±0.01	0.48^{b}±0.01	0.56^{d}±0.01	0.38^{c}±0.02	1.87	3.70	0.03	0.04
Zn	4.23^{c}±0.03	2.69^{d}±0.01	2.32^{a}±0.05	2.92^{c}±0.00399	2.86^{bc}±0.40	3.19^{b}± 0.05	2.54^{b}±0.03	3.66^{a} ±0.01	6.83	0.81	0.38	0.05
Pb	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
Cd	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND

ND: Not detected.

CONCLUSION

Based on the results of this study in both Mango varieties higher amounts of Mg and Ca were obtained in both Keitt and Apple mango variety. The Concentrations of determined metals decreased in the order: Mg > Ca > Zn > Fe > K > Na > Mn in both mango varieties. Non-essential metals Cd and Pb were found to be below detection limit. All the non-essential toxic metals analyzed in this study were below the permissible ranges presented by FAO/WHO standards revealing that the fruit is safe for dietary uses. All essential elements analyzed were below the optimum required level. Therefore, fruits under study should not supplement essential metals. The results of study suggest that this fruit is safe to be utilized as food, since the concentration of heavy metals is within the recommended limits.

Conflict of interest

All authors declared that there is no conflict of interest.

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