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ASSESSMENT OF METALS CONTENT OF WIDELY USED TRADITIONAL TOOTHBRUSHES IN ADDIS ABABA, ETHIOPIA

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ABSTRACT. Traditional toothbrushes are used by the vast majority of people who cannot afford to buy the commercial toothbrush and toothpaste. The traditional toothbrushes are generally obtained from any slim woody part of a toothbrush tree. The main purpose of this study was to determine selected metals (Ca, Fe, Mg, Al, Cu, Cr, Ni, Zn, Mn, Pb, and Cd) in traditional toothbrushes obtained from three plants including *Ligustrum vulgare* L., *Phoenix reclinata* and *Olea africana*, which are extensively used in Addis Ababa, Ethiopia, by using microwave plasma-atomic emission spectroscopy (MP-AES) after wet digestion. Recoveries of the metals in spiked samples varied from 90.4–107%. The overall mean concentrations determined (mg/kg, dry weight) were in the ranges of Ca (4267–36514) > Fe (131–318) > Al (81.6 –224) > Mg (45.6–122) > Zn (27.2–175) > Mn (20.1–29) > Cu (6.6–20.3) > Cr (6.7–8.9) > Ni (2.6–7.9). Analysis of variance at 95% indicated significant differences in the metals' contents of three toothbrushes. The results indicated that the selected traditional toothbrushes are good sources of essential metals and free from Pb and Cd. Therefore, the investigated Ethiopian traditional toothbrushes are found to be safe for human use.

KEY WORDS: Metal contents, Traditional toothbrushes, Wild privet (*Ligustrum vulgare*), Wild date palm (*Phoenix reclinata*), African wild olive (*Olea africana*), Ethiopia

INTRODUCTION

Oral health is part of total health and essential to the quality of life. The World Health Organization puts oral diseases among the top five causes of burden in 'lost healthy years' worldwide [1]. The major cause of such oral diseases is due to dental caries which are mainly a result of poor hygiene. Moreover, systemic health may be affected as a result of problems in oral hygiene [2]. One of the important ways to decrease oral health problem is the use of toothbrush. The toothbrush is an oral hygiene instrument used to clean the teeth and gums that consists of a head of tightly clustered bristles mounted on a handle, which facilitates the cleansing of hard-to-reach areas of the mouth. In the world people use two broad types of toothbrushes which are traditional and synthetic toothbrushes. Traditional toothbrushes are used by the vast majority of people who cannot afford to buy the commercial toothbrush and toothpaste. The traditional toothbrushes are important for the oral and dental hygiene of the users and hence may be useful in decreasing dental caries [3]. The traditional toothbrush is generally obtained from any slim woody part of the tree. Mostly it is harvested from branches although harvest from woody roots is also known. Long twig sections of 50 or 100 cm are cut and transported to the market before being cut to retail sizes. The thickness of these toothbrushes is dependent on the type of tree and the part of branch harvested while the length is more dependent on the retailer [4]. The World Health Organization has encouraged the use of chewing sticks (traditional toothbrushes) as an alternative source of oral hygiene in poor countries where many people cannot afford commercial dental products.

Although commercially made toothbrushes from leading international brands are available in Addis Ababa supermarkets and pharmacies, many people say they prefer the chewing sticks.

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It is better because it is natural. The present study is based on the three most widely used plants in Addis Ababa, Ethiopia. These are wild privet (*Ligustrum vulgare*), wild date palm (*Phoenix reclinata*) and African wild olive (*Olea africana*). *L. vulgare* also sometimes known as common privet is native to central and southern Europe, North Africa and southwestern Asia [5]. *L. vulgare* is cultivated in Ethiopia in different area specially urban areas in Harar, Bahir Dar, Addis Ababa, Hawasa, etc. for evergreen the campus compound (in the green field) and parks in addition to use as toothbrush.

P. reclinata the wild date palm is a species of flowering plant in the palm family native to tropical Africa, the Arabian Peninsula, Madagascar and the Comoro Islands [6]. *P. reclinata* is cultivated in Ethiopia in different area especially urban areas and hot regions like Amhara region, Bahir Dar, Finote Selam, Debre Markos, Addis Ababa, Hawasa, etc. for ever greening the road and compounds in addition to use as toothbrush.

The African wild olive, previously known as *O. africana* subspecies *cuspidata* is now regarded as *O. europaea* subspecies *africana*. The names depend on which taxonomy and nomenclature are followed. Its common name is African wild olive. The plant belongs to the family Oleaceae [7]. It is geographically distributed throughout the Southern Africa and Northwards through East Tropical Africa into Eritrea [8]. *O. africana* is cultivated in Ethiopia in all regions both rural and urban and especially in hot regions, for most of the time it is cultivated evergreen for traditional uses, e.g. for its smoke for coffee ceremony in addition to use as toothbrush. The *O. europaea* subspecies *africana* plant branches are used in traditional toothbrush as a substitute of synthetic plastic brush and paste used for oral health and prevention of dental.

Leaves of *L. vulgare* have been used for treatment of oropharyngeal inflammations or as an anti-rheumatic, diuretic, and hypotensive agents in folk medicine in southern Europe [9]. The *O. europaea* subspecies *africana* plant leaves are used in folk medicine as a remedy for eye infections, sore throat, urinary tract infections, kidney problems and backaches or headaches. It is also used as a hypotensive, emollient, febrifuge and styptic [10]. The leaves of the tree were reported to be potent for the treatment of malaria [11]. In vitro antioxidant activity of olive leaf extract (*Olea europaea* L.) and its protective effect on oxidative damage in human erythrocytes have been reported [12]. *O. europaea* has a number of traditional and contemporary uses in medicine. *O. europaea* is extensively used in traditional medicine for a wide range of ailments in various countries. Its bark, fruits, leaves, wood, seeds, and oil are used in different forms, alone or sometimes in combination with other herbs. The oil of the seed is taken orally as a laxative and also applied externally as a balm for inflammation [13]. Decoctions of dried leaves and fruit are used orally to treat diarrhea, respiratory and urinary tract infections, stomach and intestinal diseases, and as mouth cleanser [14]. Continuous application of olive oil is also useful to prevent hair loss. In East-Africa the infusion of the bark of the olive tree is taken for tapeworm infestation [15].

Although the World Health Organization has promoted the use of toothbrush sticks [16] and has encouraged further research of their efficacy, few studies have been undertaken on the potential antimicrobial properties of chewing sticks [17]. These traditional toothbrushes have high importance in removing parts of food left on the teeth, in addition to their antibacterial property. Synthetic toothpaste contain known metal content which are important for oral health, however, there exists no current studies which evaluate the metal content of traditional toothbrushes obtained from the plants. Because dietary habits are among the major concerns of human health, and are of increasing importance in almost all countries of essential nutrients are fundamental [18].

Most Ethiopian people use traditional toothbrush especially in Addis Ababa. Besides many essential metals required for human health some toxic metals may also present in toothbrushes from *L. vulgare*, *P. reclinata*, and *O. africana*. Since these plants take essential and non-essential metals from the soil and water to grow, the source of toxic metals may originate from polluted air, water and soils, or may also be naturally occurring. In the world especially in Ethiopia all traditional toothbrush users have no information about metal content in different types of traditional toothbrushes. Therefore, it is necessary to create awareness about the metal content of each traditional toothbrush in the world especially developing countries like Ethiopia.

The literature survey revealed that there are no studies on the content of heavy metals in the branch part of three plants namely *L. vulgare*, *P. reclinata*, and *O. africana* commonly used as traditional toothbrushes in Ethiopia. Therefore, this study is focused on the determination of eleven selected metals such as Ca, Mg, Al, Fe, Cu, Zn, Mn, Pb, Cr, Ni and Cd in *L. vulgare*, *P. reclinata*, and *O. africana* by microwave plasma-atomic emission spectroscopy (MP-AES). The study aims to compare the levels of the identified metals in the three plants cultivated in three different areas in Addis Ababa to the concentration of each metal in other medicinal plants and commercial toothpastes reported in the literature.

EXPERIMENTAL

Apparatus and equipments

Chopping board (PTFE, China) and Teflon (PTFE, China) knife were used to cut the branch of selected plants and to reduce the size into small pieces. A drying oven (Griffin and George Ltd, Britain) was used to dry the plant samples. Mortar and pestle were used to ground the samples. Analytical balance (Larko, LA114, 110 g/0.1 g) with precision of ± 0.0001 g was used to weigh the samples. A 250 mL round bottomed flasks (24/29) fitted with reflux condensers were used in Kjeldahl apparatus hot plate to digest the dried and powdered samples. Borosilicate volumetric flasks (50 mL) were used during dilution of sample and preparation of metal standard solutions. Measuring cylinders (Duran, Germany), micro-pipettes (1-20 µL and 10-100 µL, Pyrex, USA), were used during measuring different quantities of volumes of sample solution, acid reagents and metal standard solutions. Microwave plasma-atomic emission spectrometer (Agilent 4200 MP AES, USA) was used for the determination of the analyte metals (Al, Ca, Mg, Cu, Zn, Mn, Ni, Fe, Co, Cr, Pb and Cd in the *L. vulgare*, *O. africana* and *P. reclinata* samples.

Reagents and chemicals

Reagents used in the analysis were all analytical grade. HNO_3 (69.5% reagent grade, ACS, ISO, European Union) and $HClO_4$ (70%, Fine-Chem, Mumbai, India) were used for digestion of samples. Stock standard solutions containing 1000 mg/L of the metals Al, Ca, Mg, Cu, Zn, Mn, Ni, Fe, Co, Cr, Pb and Cd (Perkin Elmer, Boston, USA) were used for preparation of calibration standards and in the spiking experiments. Deionized water (chemically pure with conductivity ≤ 1.5 µs/cm) was used for dilution of samples and standards.

Description of the study area

Addis Ababa is the capital city of Ethiopia. The area of Addis Ababa covers about 540 km^2 of which 18 km^2 are rural. Addis Ababa has a subtropical highland climate with precipitation varying considerably by the month. Addis Ababa is provided with an average 1089 mm of rainfall per year, or 90.8 mm per month (2009-2017). The city has a complex mix of highland climate zones, with temperature differences of up to 10 ^oC, depending on elevation and prevailing wind patterns. The high elevation moderates temperatures year-round, and the city's position near the equator means that temperatures are very constant from month to month. As such as no month is above 22 ^oC in mean temperatures. The highest temperature on record was 30.6 ^oC on 26 February 2019, while the lowest temperature on record was 0 ^oC recorded on multiple occasions. The three types of traditional toothbrushes were collected from three different parts of Addis Ababa, Ethiopia namely: Kotebe, Ferensay and Arat Kilo Addis Ababa University campus where the three plants are more cultivated in Addis Ababa. Kotebe is located in Yeka sub-city, Ferensay is located in Gulele sub-city, and Arat Kilo, Addis Ababa University campus is located in Arada sub-city. The location map of Addis Ababa showing the three sub-cities is shown in Figure 1.

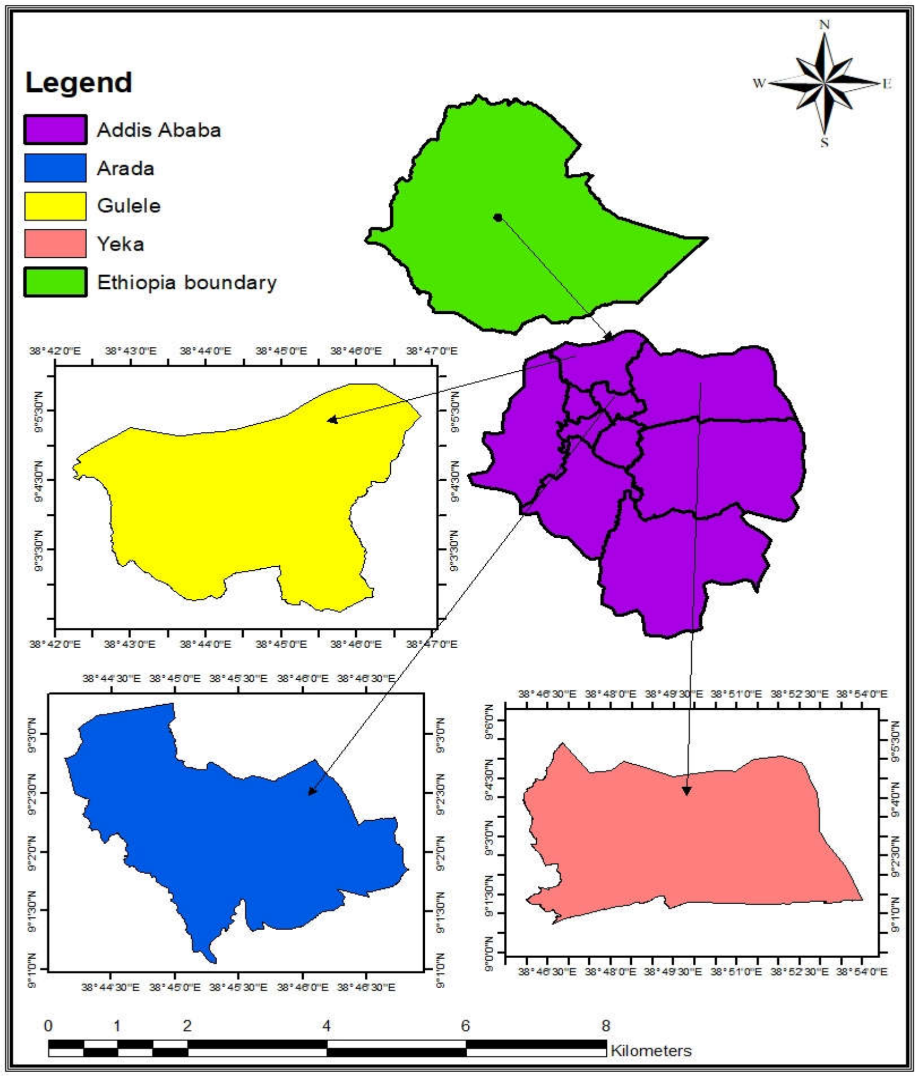


Figure 1. The location map of Addis Ababa showing the three sub-cities.

Optimization of the digestion procedure for selected traditional toothbrush samples

It is common practice in any scientific experiments in general and in analytical chemistry in particular that the optimum working conditions should be determined before carrying out any experimental activities for sample preparation before analysis [19]. The basic requirements for sample preparation for analysis are to establish an optimum condition for the digestion. The optimum condition in the wet digestion is the one which required minimum reagent volume consumption, minimum reflux time, clarity of digests, and ease of simplicity. In this study, to prepare a clear colorless sample solution for the analysis using MP-AES, 0.5 g of powdered and homogenized traditional toothbrush sample was weighed and transferred to 250 mL of a round bottom flask. To this, different volumes of nitric acid and perchloric acid at specified proportions (v/v) were added and digested at different temperature and for different time (Table 1). During the optimization, the total volume was kept constant while studying the volume ratio of the two acids. Similarly, the volume ratio of the two acids was kept constant while varying the total volume. Different volumes of nitric and perchloric acids are commonly used for the decomposition of plant materials [20-26]. Therefore different volumes of nitric and perchloric acids were used for the decomposition of traditional toothbrushes in this study.

Table 1. Different conditions tested for optimization of digestion procedure for 0.5 g of *O. africana*, *L. vulgare* and *P. reclinata*.

Parameter	Trial	Reagents used	Reagent volume ratio (mL)	Temperature (°C)	Digestion time (h)	Observation
O. africana						
Optimization for reagent volume	1	HNO_3:$HClO_4$	3:3	300	3:00	Light yellow
	2	HNO_3:$HClO_4$	4:2	300	3:00	Deep yellow
	3	HNO_3:$HClO_4$	3:2	300	3:00	Yellow
	4	**HNO_3:$HClO_4$**	**4:1***	**300**	**3:00**	**Clear and colorless**
	5	HNO_3:$HClO_4$	2:2	300	3:00	Clear light yellow
	6	HNO_3:$HClO_4$	3:1	300	3:00	Deep yellow
Optimization for temperature	1	HNO_3:$HClO_4$	4:1	150	3:00	Deep yellow
	2	HNO_3:$HClO_4$	4:1	180	3:00	Clear light yellow
	3	HNO_3:$HClO_4$	4:1	210	3:00	Clear light yellow
	4	HNO_3:$HClO_4$	4:1	240	3:00	Clear and light yellow
	5	**HNO_3:$HClO_4$**	**4:1**	**270***	**3:00**	**Clear and colorless**
	6	HNO_3:$HClO_4$	4:1	300	3:00	Clear and colorless
Optimization for time	1	HNO_3:$HClO_4$	4:1	270	1;45	Deep yellow
	2	HNO_3:$HClO_4$	4:1	270	2:00	Clear and light yellow
	3	**HNO_3:$HClO_4$**	**4:1**	**270**	**2:15***	**Clear and colorless**
	4	HNO_3:$HClO_4$	4:1	270	2:30	Clear and colorless
	5	HNO_3:$HClO_4$	4:1	270	2:45	Clear and colorless
	6	HNO_3:$HClO_4$	4:1	270	3:00	Clear and colorless
L. vulgare						
Optimiza	1	HNO_3:$HClO_4$	3:3	300	3:00	Deep yellow
	2	HNO_3:$HClO_4$	4:2	300	3:00	Deep yellow

	3	HNO_3:$HClO_4$	3:2	300	3:00	Clear and light Yellow
	4	**HNO_3:$HClO_4$**	**4:1***	**300**	**3:00**	**Clear and colorless**
	5	HNO_3:$HClO_4$	2:2	300	3:00	Clear and light yellow
	6	HNO_3:$HClO_4$	3:1	300	3:00	Deep yellow
Optimization for Temperature	1	HNO_3:$HClO_4$	4:1	150	3:00	Deep yellow
	2	HNO_3:$HClO_4$	4:1	180	3:00	Deep yellow
	3	HNO_3:$HClO_4$	4:1	210	3:00	Clear and light yellow
	4	**HNO_3:$HClO_4$**	**4:1**	**240***	**3:00**	**Clear and colorless**
	5	HNO_3:$HClO_4$	4:1	270	3:00	Clear and colorless
	6	HNO_3:$HClO_4$	4:1	300	3:00	Clear and colorless
Optimization for time	1	HNO_3:$HClO_4$	4:1	240	1;45	Deep yellow
	2	HNO_3:$HClO_4$	4:1	240	2:00	Clear and light yellow
	3	HNO_3:$HClO_4$	4:1	240	2:15	Clear and light yellow
	4	HNO_3:$HClO_4$	4:1	240	2:30	Clear and light yellow
	5	**HNO_3:$HClO_4$**	**4:1**	**240**	**2:45***	**Clear and colorless**
	6	HNO_3:$HClO_4$	4:1	240	3:00	Clear and colorless
P. reclinata						
Optimization for reagent volume	1	HNO_3:$HClO_4$	3:3	300	3:00	Deep yellow
	2	HNO_3:$HClO_4$	4:2	300	3:00	Deep yellow
	3	HNO_3:$HClO_4$	3:2	300	3:00	Clear and light yellow
	4	HNO_3:$HClO_4$	4:1	300	3:00	Clear and light yellow
	5	HNO_3:$HClO_4$	2:2	300	3:00	Clear and light yellow
	6	**HNO_3:$HClO_4$**	**3:1***	**300**	**3:00**	**Clear and colorless**
Optimization for Temperature	1	HNO_3:$HClO_4$	3:1	150	3:00	Deep yellow
	2	HNO_3:$HClO_4$	3:1	180	3:00	Deep yellow
	3	**HNO_3:$HClO_4$**	**3:1**	**210***	**3:00**	**Clear and colorless**
	4	HNO_3:$HClO_4$	3:1	240	3:00	Clear and colorless
	5	HNO_3:$HClO_4$	3:1	270	3:00	Clear and colorless
	6	HNO_3:$HClO_4$	3:1	300	3:00	Clear and colorless
Optimization for time	1	HNO_3:$HClO_4$	3:1	210	1:45	Deep yellow
	2	**HNO_3:$HClO_4$**	**3:1**	**210**	**2:00***	**Clear and colorless**
	3	HNO_3:$HClO_4$	3:1	210	2:15	Clear and colorless
	4	HNO_3:$HClO_4$	3:1	210	2:30	Clear and colorless
	5	HNO_3:$HClO_4$	3:1	210	2:45	Clear and colorless
	6	HNO_3:$HClO_4$	3:1	210	3:00	Clear and colorless

*The bold font indicates the optimum condition.

Digestion of traditional toothbrushes samples

A 0.5 g of each dried and homogenized samples were transferred to individual 250 mL round bottom flask, and a total of 5 mL of the mixture of nitric acid (69.5%) and perchloric acid (70%) with a volume ratio of 4:1 (v/v) for *O. africana* and 4:1 (v/v) for *L. vulgare* and total 4 mL of the mixture of nitric acid (69.5%) and perchloric acid (70%) with a volume ratio of 3:1 (v/v) for *P. reclinata* sample was added and the mixture was digested on a Kjeldahl digestion apparatus fitting the flask to a reflux condenser by setting the temperature at 270 ^{o}C, 240 ^{o}C, and 210 ^{o}C for 2:15, 2:45, and 2:00 hours, respectively. The digest was allowed to cool to room temperature for 10 min without dismantling the condenser from the flask and a further 10 min after removing the condenser. To the cooled solution, 20 mL of deionized water was added to dissolve the precipitate formed on cooling and to minimize dissolution of filter paper by the digest residue while filtering with Whatman (110 mm diameter) filter paper in to 50 mL volumetric flask. The round bottom flask was rinsed subsequently with 5 mL deionized water until the total volume was reached around 45 mL and finally the solution was diluted to 50 mL.

Calibration of MP-AES instrument

Working standard solutions were prepared for each of the metals from an intermediate standard solution containing 10 mg/L, which was prepared from the atomic absorption spectroscopy stock standard solutions that contained 1000 mg/L. These standards were diluted with deionized water to obtain four working standards for each metal of interest. The selected metals (Al, Ca, Mg, Cu, Zn, Mn, Ni, Fe, Cr, Pb and Cd) in the digest from three different traditional toothbrush samples from three different areas were determined three times by MP-AES.

It should be noted that even though the MP-AES is multi-element technique, working standard solution of each metal individually is required for the construction of calibration curve of individual metal.

Table 2. The wavelength, method detection limit correlation coefficient and calibration curve equations.

Metal	Wavelength (nm)	MDL (mg/kg)	MQL (mg/kg)	Correlation coefficient	Calibration curve Equation
Ca	422.673	0.285	0.95	0.998	y = 66713c + 32373
Mg	279.553	0.364	1.21	0.999	y = 19334c + 1884
Cu	327.395	0.21	0.699	0.999	y = 33562c+ 525.3
Zn	213.857	0.163	0.543	0.999	y = 4541c + 301.9
Mn	259.372	0.188	0.626	0.999	y = 2977c + 206.3
Ni	305.081	0.96	3.195	0.999	y = 4453c + 270.2
Fe	371.993	0.39	1.298	0.999	y = 5024c + 203.5
Cr	427.480	0.77	3.85	0.999	y = 21959c – 180.5
Cd	226.502	-	-	0.998	y = 660.3c+ 118.9
Pb	368.346	-	-	0.999	y = 1722c + 6.594
Al	396.152	0.31	1.03	0.999	y = 24298c +753.3

Method detection limit (MDL)

Limit of detection (LOD) is the smallest amount or concentration that can be detected by the analytical method with a given certainty. Limit of detection can be calculated by multiplying the standard deviation of the blank (SD_b) by three ($LOD = 3SD_b$). The detection limit (with all steps of the analysis included) is called the MDL. The limit of quantification (LOQ) is the smallest quantity of analyte that can be measured with acceptable accuracy and precision and it is described as 10 times the standard deviation of the blank. The wavelength, method detection limit (MDL), method quantification limit (MQL), correlation coefficient and calibration curve equations are given in Table 2.

The wavelengths for the selected metals listed in Table 2 are apparently looks in contradiction to reported wavelengths. This is because MP-AES is an optical emission spectrometer. The wavelengths were selected according to the manual of the instrument provided by the manufacturer and the instrument was operated in emission mode.

Validation of digestion procedure

The validity of the digestion procedures was assured by spiking the samples with a standard solution of known concentration of target analaytes. For this purpose a stock standard solution of 1000 mg/L of each analayte was used. Thus, spiking was done for one of the three samples that is *O. africana* branch sample in triplicates. From the stock solution of 1000 mg/L, 4.5 µL of Zn, 1.5 µL of Cr, 3.6 µL of Cu, 1 µL of Ni, 4.4 µL of Mn, and 27.7 µL of Fe were added to 0.5 g of *O. africana* sample. These small volumes of solutions were measured using a micropipette of the volume range 1-20 µL. The spiked and unspiked samples were digested and analyzed in a similar manner. As shown in Table 3, selected metals were analyzed in triplicate to evaluate the efficiency of the procedure and the percentage recoveries lies within the range from 91 to 107%.

The spiking experiment was performed only for *O. africana*. This is because the digestion procedures for the three plants are not much different and the analysis procedure is the same for all the three plants.

Table 3. Analytical results for recovery test of *O. africana* sample.

Metals	Concentration of unspike sample (mg/kg)	% spiking	Amount added (mg/kg)	Spiked sample (mg/kg)	% Recovery
Zn	35.1 ± 0.1	25	8.78	43.1 ± 3.0	91.0
Cr	6.68 ± 0.1	45	3.0	9.49 ± 0.74	93.7
Cu	17.9 ± 1.7	40	7.16	24.9 ± 0.72	97.8
Ni	3.6 ± 0.2	50	1.8	5.43 ± 0.36	102
Mn	29.0 ± 1.1	30	8.7	37.1 ± 4.9	93.0
Fe	221 ± 19.9	25	55.3	280.3 ± 1.2	107

Statistical analysis

The analysis of variances (ANOVA) and the correlation among metals were assessed by Pearson correlation methods and the graphical expression was done using Microsoft excel 7.

RESULTS AND DISCUSSION

The concentrations of metals shown in Table 4 were determined by using MP-AES and precision of the results were assessed as relative standard deviation. The relative standard deviation of the results with a few exception were less than or equal to 10% which is within the recommended range. Mean values were determined from triplicate analysis of each sample and triplicate samples were used for each sample site.

Concentration of metals in L. vulgare samples

As shown in Table 4, the maximum concentration of elements Ca, Fe, Zn, Al, Mg, Mn, Cu, Cr, and Ni are 6143, 318, 175, 143, 102 , 26.7, 12.6, 8.9 and 4.8 mg/kg, respectively, in the *L. vulgare* samples. Similarly, the minimum concentration of elements Ca, Fe, Zn, Al, Mg, Mn, Cu, Cr and Ni are 5619, 216, 134, 126, 89, 22.6, 10.2, 6.3 and 3.9 mg/kg, respectively, in *L. vulgare* samples. The results showed that the *L. vulgare* samples collected from Kotebe site have higher amounts of Zn, Cr, Cu, Ni, Mg, Al, Ca, and Fe compared to the concentrations of those metals in *L. vulgare* samples from other sample sites. But the concentrations of Mn in samples collected from Arat Kilo sample site was higher than samples collected at Kotebe and Ferensay sample sites. The concentrations of Cd and Pb in the *L. vulgare* samples collected from the three different sample sites were below detection limit. The concentrations of Ca are higher than other metals and the concentrations of Ni are lower as compared to other metals in *L. vulgare* samples collected from the sites.

Table 4. Concentration of metals (mg/kg, mean ± SD, n = 3) in traditional toothbrushes obtained from *L. vulgare*, *P. reclinata* and *O. afiricana*

Metals	Concentration of metals (mg/kg, mean ± SD, n = 3)								
	L. vulgare			*P. reclinata*			*O. afiricana*		
	Kotebe	Arat Kilo	Ferensay	Kotebe	Arat Kilo	Ferensay	Kotebe	Arat Kilo	Ferensay
Zn	175±11.9	134 ± 1.2	145 ± 2.4	29.5 ± 0.6	47.8 ± 2.4	27.2 ± 1.3	36.8 ± 1.2	45.7 ± 4.1	35.1 ± 0.1
Cr	8.9 ± 0.8	8.8 ± 0.1	6.3 ± 0.7	8.6 ± 0.1	8.3 ± 0.3	8.2 ± 0.7	7.66 ± 0.5	7.0 ± 0.5	6.7 ± 0.1
Cu	12.6 ± 0.4	10.4 ± 0.7	10.2 ± 0.8	6.6 ± 0.4	12.5 ± 0.5	10.3 ± 1.0	10.1 ± 0.9	20.3 ± 0.6	17.9 ± 1.7
Ni	4.8 ± 0.4	4.7 ± 0.5	3.9 ± 0.2	7.9 ± 1.1	4.4 ± 0.3	2.6 ± 0.2	4.9 ±1.1	3.5 ± 0.2	3.6 ±0.2
Mn	24.9 ± 1.2	26.7 ± 2.6	22.6 ± 0.6	15.1 ± 0.9	20.1 ± 1.1	16.6 ± 0.5	23.7 ± 1	20.7 ± 0.5	29.0 ± 1.1
Mg	102 ± 8	94 ± 2	89 ± 5	75 ± 1	55 ± 5	46 ± 4	122 ± 4	117 ± 4	108 ± 8
Al	143± 13.1	136 ± 12.0	126 ± 5.2	121 ± 3.1	115 ± 9.4	81.6 ± 0.7	224 ± 12.1	200 ± 6.1	224 ± 5.2
Ca	6143±535	5619± 554	6061± 293	7397± 593	36514±2991	27676±2800	4267± 368	23414±699	28178±2142
Fe	318± 34.4	235 ± 14.1	216 ± 8.2	168 ± 12	200 ± 6.9	131 ± 4.5	194 ± 14	275 ± 9.9	221 ± 19.9
Pb	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL
Cd	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL

BDL = below detection limit.

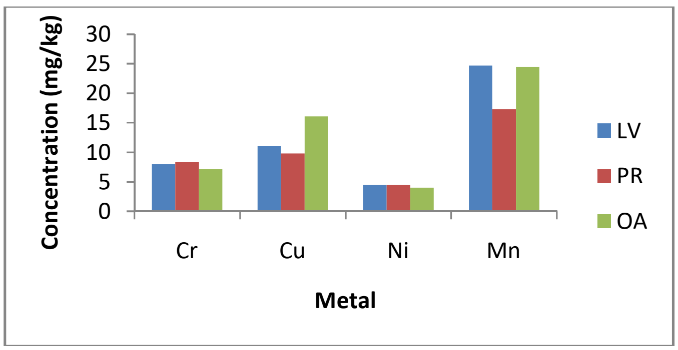
Concentration of metals in P. reclinata samples

As shown in Table 4, the maximum concentration of metals Ca, Fe, Al, Mg, Zn, Mn, Cu, Cr, and Ni are 36514, 200, 121, 75, 47.8, 20.1, 12.5, 8.6, and 7.9 mg/kg, respectively, in the *P. reclinata* samples. Similarly, the minimum concentration for elements Ca, Fe, Al, Zn, Mg, Mn, Cu, Cr, and Ni are 7397, 131, 81.6, 46, 27.2, 15.1, 6.6, 8.2, and 2.6 mg/kg, respectively, in *P. reclinata* samples. The results showed that the *P. reclinata* samples collected from Arat Kilo site have higher amounts of Zn, Cu, Mn, Ca, and Fe compared to the concentration of the metals in *P. reclinata* samples from other sample sites. But the concentrations of Cr, Ni, Mg, and Al in *P. reclinata* samples collected from Kotebe sample site are higher than samples collected Arat Kilo and Ferensay sample sites. The concentrations of Cd and Pb in *P. reclinata* samples collected from three different sample sites are below detection limit. The concentration of Ca was higher than other metals and the concentration of Ni was lower than other metals in *P. reclinata* samples collected from three different sample sites.

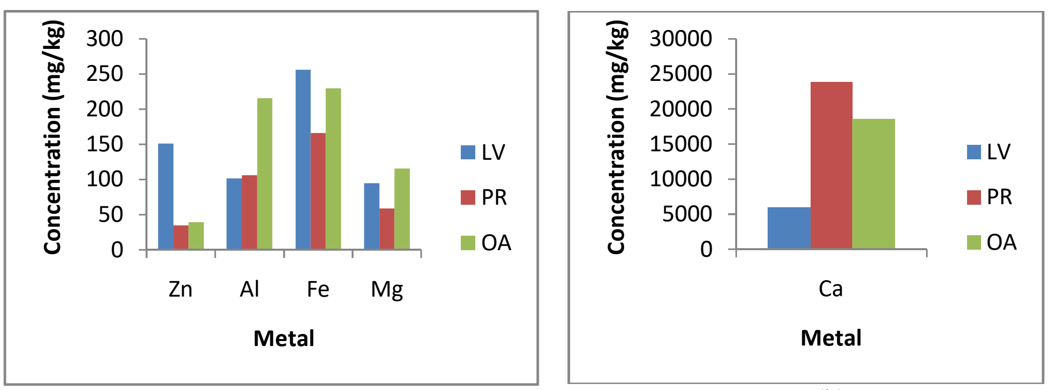
Concentration of metals in O. africana samples

As shown in Table 4, the maximum concentration of metals Ca, Fe, Al, Mg, Zn, Mn, Cu, Cr, and Ni are 28178, 275, 224, 122, 45.7, 29.0, 20.3, 7.66, and 4.9 mg/kg, respectively, in the *O. africana* samples. Similarly, the minimum concentration levels for elements Ca, Fe, Al, Mg, Zn, Mn, Cu, Cr, and Ni are 4267, 194, 200, 108, 35.1, 20.7, 10.1, 6.7 and 3.5 mg/kg, respectively, in *O. africana* samples. The results of concentrations of metals showed that the *O. africana* samples collected from Ferensay site have higher amounts of Mn, Al, and Ca, and *O. africana* samples collected from Arat kilo site have higher amounts of Fe, Zn, and Cu, and *O. africana* samples collected from Kotebe site have higher amounts of Cr, Ni, and Mg as compared to the concentration of metals in *O. africana* samples from other sample sites. The concentrations of Cd and Pb in *O. africana* samples collected from three different sample sites were below detection limit. The concentration of Ca was the highest compared to other metals and the concentration of Ni was the lowest compared to other metals in in *O. africana* samples collected from the three different sample sites.

Comparison of metal concentrations (mg/kg) in L. vulgare, P. reclinata, and O. africana samples



(a)



(b)

Figure 2. Comparison of metals concentration (A) Cr, Cu, Ni, and Mn and (B) Zn, Mg, Al, and Fe and (C) Ca in *L. vulgare* (LV), *P. reclinata* (PR), and *O. africana* (OA) samples.

Comparison of metal concentrations (mg/kg) in the traditional toothbrushes from *L. vulgare*, *P. reclinata*, and *O. africana* are shown in Figure 2. The metals are grouped according to their concentrations and not according to their classification as essential, trace or toxic metals. The results of this study showed that there is a variation in the metal contents of the three traditional toothbrushes. This variation may be due to genetic factors and can also be due to environmental factors as well. The highest concentration of Ca (23862 mg/kg) was observed in *P. reclinata* samples as compared to concentration of Ca in *L. vulgare* (5941 mg/kg) and *O. africana* (18620 mg/kg) samples, respectively. The concentration of Ni was the lowest in the three traditional toothbrushes as compared to the concentrations of other selected metals. The highest concentration of Ca, Fe, Al, Mg and Zn were observed in the three traditional toothbrush samples and relatively lower amounts of Cr, Cu, Ni and Mn were observed compared to concentration of Ca, Fe, Al, Mg and Zn.

Comparison of metals concentration (mg/kg, dry mass) of the three traditional toothbrushes with other medicinal plants reported in literature

The metal concentrations (mg/kg, dry mass) in the three Ethiopian traditional toothbrushes are compared with other medicinal plants reported in literature. The results are summarized in Table 5. The Ca contents determined in the three traditional toothbrushes are higher than Ca contents in other medicinal plants: *Rhamnus prinoides* [27] and *Zingiber officinal* [28]. The Mg contents determined in this study are lower compared with Mg contents determined from other medicinal plants like *Rhamnus prinoides* and *Zingiber officinal* reported by Gebre and Chandravanshi [27] and Wagesho and Chandravanshi [28]. The concentrations of Fe and Al in this study are higher than concentrations of Fe and Al from other medicinal plants reported. The concentrations of other metals such as Ni, Cr, Zn and Cu in this study and the reported values from the medicinal plants are comparable. The concentrations of Pb and Cd in this study were below detection limit.

Table 5. Comparison of metals concentration (mg/kg, dry mass) of the three traditional toothbrushes with other medicinal plants reported in the literature.

Plant types	Plant parts	Country	Method	Metal concentration (mg/kg)					Reference
				Pb	Cd	Cu	Zn	Fe	
Rhamnus prinoides	Branch	Ethiopia	FAAS	ND	1.0-1.26	16.8-233	17.4-28.2	22-124	[27]
Zingiber officinale	Root	Ethiopia	FAAS	BDL	0.38-0.97	1.1-4.78	38.5-55.2	41.8-89	[28]
Phoenix dactylifera	Fruit		ICP-AES	0.78-5.83	0.04-0.18	0.92-1.53	0.23-0.95	120-315	[29]
Phoenix reclinata	Branch	Ethiopia	MP-AES	BDL	BDL	6.6-12.5	27.2-47.8	131-200	This study
Ligustrum valgare	Branch	Ethiopia	MP-AES	BDL	BDL	10.2-12.6	134-175	216-318	This study
Olea africana	Branch	Ethiopia	MP-AES	BDL	BDL	10.1-20.3	31.5-45.7	194-275	This study

Plant types	Parts	Country	Method	Metal concentration (mg/kg)						References
				Cr	Ca	Mg	Mn	Ni	Al	
Rhamnus prinoides	Branch	Ethiopia	FAAS	5.42-17.4	360-5750	2635-5528	2.16-3.98	9.68	ND	[27]
Zingiber officinal	Stem	Ethiopia	FAAS	6.02-10.8	200-2543	2700-4094	184-401	5.46-8.4	ND	[28]
Phoenix dactylifera	Fruit	Saudi	ICP-AES	ND	110-200	ND	31.8-89.3	8-11.75	13.6-74.9	[29]
Phoenix reclinata	Branch	Ethiopia	MP-AES	8.2-8.6	7397-36514	89-102	15-20.1	2.6-7.9	81.6-121	This study
Ligustrum valgare	Branch	Ethiopia	MP-AES	6.3-8.9	5619-6143	46-75	22.6-26.7	3.9-4.8	126-143	This study
Olea africana	Branch	Ethiopia	MP-AES	6.7-7.66	4267-28178	108-122	20.7-29	3.5-4.9	200 -224	This study

ND = not detected, BDL = below detection limit.

Comparison of metals concentration (mg/kg) of the three traditional toothbrushes with toothpastes metals concentrations reported in the literature

The metal concentrations (mg/kg) in the three traditional toothbrushes are compared with the metals concentrations in commercial toothpastes reported in the literature. The results are summarized in Table 6. The concentrations of Ni are higher in local toothpastes used in Nigeria and toothpastes imported to Nigeria as compared to this study, but the concentration of Ni in other commercial toothpastes is comparable to this study. The concentrations of Fe, Cu and Mn in this study are higher as compared to concentrations in commercial toothpastes. The concentrations of Cr in the local toothpastes used in Nigeria and toothpastes imported to Nigeria are comparable to the values of this study. The concentration of Zn in commercial toothpastes is higher than in present study. The toxic metals Pb and Cd concentrations in commercial toothpastes in Malta, local toothpastes in Nigeria, toothpastes imported to Nigeria are present in appreciable amount, but were below detection limit in the three Ethiopian traditional toothbrushes, which is preferable.

Table 6. Comparison of metals concentration (mg/kg) of the three traditional toothbrushes with other toothpastes metals concentrations reported in the literature.

Type of toothpaste	Country	Method	Metal concentration (mg/kg)					Reference
			Cd	Cr	Cu	Ni	Pb	
Commercial toothpastes	Malta	MP-AES	-	0.28-7.35	0.73-3.68	0.43-2.54	2.23-12.04	[30]
Local toothpastes	Nigeria	FAAS	0.001-1.284	0.001-5.97	ND	5.472-18.63	4.514 - 23.575	[31]
Commercial toothpastes	Nigeria	FAAS	0.001-2.49	0.001-10.85	ND	8.975-18.535	6.329-18.092	[31]
Commercial toothpastes	India	AAS	<0.031	-	-	-	0.84-1.58	[32]
Ligustrum vulgare	Ethiopia	MP-AES	BDL	6.3-8.9	10.2-12.6	3.9-4.8	BDL	This study
Olea africana	Ethiopia	MP-AES	BDL	6.7-7.66	10.1-20.3	3.5-4.9	BDL	This study
Phoenix reclinata	Ethiopia	MP-AES	BDL	8.2-8.6	6.6-12.5	2.6-7.9	BDL	This study

Type of toothpaste	Country	Method	Metal concentration (mg/kg)			Reference
			Fe	Mn	Zn	
Commercial toothpastes	Malta	MP-AES	1.76-17.7	0.2-2.02	0.00-2417	[30]
Ligustrum vulgare	Ethiopia	MP-AES	216-318	22.6- 26.7	134-175	This study
Olea africana	Ethiopia	MP-AES	194- 275	20.7- 29	31.5-45.7	This study
Phoenix reclinata	Ethiopia	MP-AES	131- 200	15.1-20.1	27.2-47.8	This study

Comparison of maximum heavy metal contents in the traditional toothbrush samples with the international standards in cosmetics and toothpastes

The maximum heavy metal contents in Ethiopian traditional toothbrush samples are compared with available international standards in toothpastes (Table 7). The toxic heavy metals Pb and Cd were not detected in Ethiopian traditional toothbrush samples while Ni and Cr are within the range of international standards for toothpastes. Therefore, the Ethiopian traditional toothbrushes are found to be safe for human use.

Table 7. Comparison of heavy metal contents (mg/kg) in the traditional toothbrush samples with available international standards in cosmetics and toothpastes.

International standards	Pb	Cd	Ni	Cr	Reference
European Union	0.1	0.05	-	-	[33]
World Health Organization	2	2	-	-	[34]
Canada	10	3	-	-	[35]
Germany	0.5	0.1	-	-	[33]
Ethiopian traditional toothbrushes	BDL	BDL	2.6–7.9	6.3–8.6	This study

BDL = below detection limit.

Analysis of variance (ANOVA)

Analysis of variance (ANOVA) is a statistical method used to test differences between two or more means. ANOVA use the F statistic to compare whether the difference between sample means is significant or not [36]. In this study, traditional toothbrush samples were collected from three different areas and the metal levels of each sample were analysed by MP-AES. During the processes of sample preparation and analysis a number of random errors may be introduced in each aliquot and in each replicate measurement. The variation in sample mean of the analyte was tested by using ANOVA, whether the source for variation was from experimental procedure or heterogeneity among the samples (i.e., difference in mineral contents of soil, water, atmosphere; variation in application of agrochemicals like fertilizers, pesticides, herbicides etc or other variations in cultivation procedures). The results are summarized in Table 8.

Table 8. Analysis of variance (ANOVA) between *Ligustrum vulgare*, *Phoenix reclinata* and *Olea africana* samples at 95% confidence level.

Metal	*Ligustrum vulgare*			*Phoenix reclinata*			*Olea africana*		
	$F_{Calculated}$	$F_{Critical}$	p-value	$F_{Calculated}$	$F_{Critical}$	p-value	$F_{Calculated}$	$F_{Critical}$	p-value
Zn	270	5.14	$1.33x10^{-6}$	114	5.14	$1.7x10^{-5}$	23.4	5.14	0.0015
Cr	13.8	5.14	0.0057	0.15	5.14	0.86	61	5.14	$9.99x10^{-5}$
Cu	181	5.14	$4.36x10^{-6}$	103	5.14	$2.24x10^{-5}$	81	5.14	$4.45x10^{-5}$
Ni	61.3	5.14	0.000102	41.5	5.14	0.000307	4.12	5.14	0.075
Mn	4.2	5.14	0.07	121	5.14	$1.4x10^{-5}$	222	5.14	$2.36x10^{-6}$
Mg	13.0	5.14	0.0063	51.5	5.14	0.000167	4.86	5.14	0.0555
Al	2.06	5.14	0.208	541	5.14	$1.689x10^{-7}$	144	5.14	$8.59x10^{-6}$
Ca	1.06	5.14	0.405	117	5.14	$1.56x10^{-5}$	439	5.14	$3.11x10^{-7}$
Fe	70	5.14	$6.67x10^{-5}$	250	5.14	$1.67x10^{-6}$	74.3	5.14	$5.84x10^{-5}$

Results of ANOVA showed that there were significant differences ($p < 0.05$) in the mean values of Cu, Zn, Ni, Mg, Cr, and Fe between *L. vulgare* samples while there was no significant difference ($p > 0.05$) in the mean values of Ca, Mn, and Al between the samples.

Results of ANOVA showed that there were significant differences ($p < 0.05$) for Cu, Zn, Mn, Ni, Mg, Al, Ca, and Fe between *P. reclinata* samples while there was no significant difference ($p > 0.05$) for Cr between these parameters.

Results of ANOVA showed that there were significant differences ($p < 0.05$) in the mean values of for Cu, Zn, Mn, Cr, Al, Ca, and Fe between *O. africana* samples while there was no significant difference ($p > 0.05$) in the mean values of Mg and Ni between the three samples

Results of ANOVA showed that there were significant differences ($p < 0.05$) in the mean values of for Zn, Mg, Al and Mn between *L. vulgare*, *P. reclinata* and *O. africana* samples while there was no significant difference ($p > 0.05$) in the mean values of Ca, Cr, Cu, Fe and Ni between the three samples.

Pearson correlation of metals

The Pearson correlation coefficient (r) is used to measure the strength of linear association between two variables [37]. In this study, to correlate the effect of one metal on the other metal, the Pearson correlation matrices using correlation coefficient (r) for the samples were used. The correlation values are categorized as no correlation (r = 0.00–0.19), weak correlation (r = 0.20–0.5), medium correlation (r = 0.50–0.75) and strong correlation (r = 0.76–0.99). Linear regression correlations tests were performed to investigate the correlations between metal concentrations in the *L. vulgare*, *P. reclinata* and *O. africana* samples.

The correlation coefficients between metals in *L. vulgare* reflected strongly positive correlation between Zn/Cu, Zn/Mg Cr/Ni, Cr/Mg, Cu/Al, Cu/Mg, Ni/Mg, Zn/Ca, Zn/Fe, Cr/Mn, Ni/Mn, Ni/Al, Cr/Al, Mg/Al, Al/Fe, Cu/Fe, Mg/Fe, moderately positive correlation between Zn/Al, Cu/Ni, Ni/Fe, Mn/Al, Cr/Fe, Cr/Ca, Cu/Ca, moderately negative correlation between Mn/Ca, weakly positive correlation between, Mn/Mg, Zn/Al, Ca/Fe, Mn/Fe, Mg/Ca, Zn/Cr, Zn/Ni, weakly negative correlation between Cr/Ca, Ni/Ca, and no correlation between Zn/Mn, Cu/Mn, and Al/Ca.

The correlation coefficients between metals in *P. reclinata* reflected strongly positive correlation between Zn/Mn, Cr/Ni, Cr/Mg, Cu/Mn, Ni/Mg, Zn/Ca, Zn/Fe, Cu/Ca, Ni/Al, Mn/Ca, Mg/Al, Al/Fe, Cr/Al, Ca/Fe and strongly negative correlation between Cr/Cu, Cu/Ni, Cu/Mg, moderately positive correlation between Zn/Cu, Mn/Fe, and moderately negative correlation between Cr/Mn, weakly positive correlation between, Zn/Al, Cu/Fe, Ni/Fe, Cr/Fe, Mg/Fe, Al/Ca, weakly negative correlation between Mn/Mg, Ni/Mn, Ni/Ca, Mg/Ca, Cr/Ca, Cu/Al, and no correlation between Zn/Cr, Zn/Ni, Mn/Al and Zn/Mg.

The correlation coefficients between metals in *O. africana* reflected strongly positive correlation between Zn/Fe, Cr/Mg, Cr/Ni, Cu/Fe, Mn/Ca, Mg/Ca, Mn/Al, strongly negative correlation between Zn/Mn, Zn/Al, Cr/Cu, Cu/Ni, Ni/Fe, Al/Fe, moderately positive correlation between Ni/Al, Al/Ca, Zn/Cu, Ni/Mg, moderately negative correlation between Zn/Ni, Zn/Ca, Cr/Ca, Cu/Mg, Cu/Al, Mn/Mg, Cr/Fe, Mn/Fe, weakly positive correlation between, Zn/Mg, Cr/Al, Ca/Fe, weakly negative correlation between Cr/Mn, and no correlation between Zn/Cr, Cu/Mn, Cu/Ca, Ni/Mn, Ni/Ca, Mg/Al, and Mg/Fe.

The correlation coefficients between metals in *L. vulgare, P. reclinata* and *O. africana* plants branch reflected strongly positive correlation between, Cr/Ni, Cu/Mg, Ni/Mg, Mn/Mg, Cu/Al, Ni/Al, Mg/Al, Mn/Fe, Mg/Fe and Ca/Fe, strongly negative correlation between Cr/Cu, Cu/Ni, Cr/Mg, Cr/Al and Zn/Ca, moderately positive correlation between Zn/Mn, Cu/Mn and Zn/Fe moderately negative correlation between Cr/Mn, Mn/Ca, and Cr/Fe, weakly positive correlation between, Zn/Ni, Ni/Mn, Mn/Al, Al/Ca, Cu/Fe, Ni/Fe and Al/Fe weakly negative correlation between Zn/Cu, Zn/Al, Ni/Ca and Mg/Ca and no correlation between Zn/Cr, Zn/Mg, Cr/Ca and Cu/Ca.

CONCLUSION

The levels of the metals in the three types of traditional toothbrushes from the plants namely *L. vulgare*, *P. reclinata* and *O. africana* collected from the three sampling areas indicated that there were higher concentrations of Ca, Fe and Al than the other selected metals while Cr and Ni were lower in all the three samples. The overall mean concentrations determined (mg/kg, dry weight) were in the ranges of Ca (4267–36514) > Fe (131–318) > Al (81.6–224) > Mg (46–122) > Zn (27.2–175) > Mn (20.1–29.0) > Cu (6.6–20.3) > Cr (6.7–8.9) > Ni (2.6–7.9). The mean concentrations of Ca in *P. reclinata* and *O. africana* were much higher as compared to the concentrations of Ca in *L. vulgare*, and the concentrations of Zn in *L. vulgare* was higher than the concentrations of Zn in *P. reclinata* and *O. africana*, the concentrations of Mg in *L. vulgare* and *O. africana* were higher than the concentrations of Mg in *P. reclinata.* But the concentrations of other metals were almost comparable. The toxic metals Pb and Cd were not detected in any of the three traditional toothbrushes studied. Therefore, the three traditional toothbrushes are safe for human use.

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REFERENCES

1. World Health Organization. *International statistical classification of diseases and related health problems*, 10th revision, 5th ed., World Health Organization: Geneva; **2016**.
2. Bone, K. Phytotherapy for periodontal disease and improved oral hygiene. *Townsend Letter for Doctors and Patients* **2005**, 263, 38-41.
3. Kassu, A.; Dagne, E.; Abate, D.; Castro, A.; Van, B.W. Ethnomedical aspects of the commonly used toothbrush sticks in Ethiopia. *East Afr. Med. J.* **1999**, 76, 651-653.
4. Entele, W.; Chandravanshi, B.S. Metal contents in traditional chewing sticks commonly used in Ethiopia: A comparative analysis. *Chem. Int.* **2021**, 7, 163-171.
5. Bailey, L.H. *Manual of Cultivated Plants*, Rev. ed., Macmillan: U.K.; **1949**.
6. Govaerts, R.H.A.; Faden, R.B. *World Checklist of Selected Plant Families*, Royal Botanic Gardens: Kew; **2013**.
7. Long, H.S.; Tilney, P.M.; Van Wyk, B.E. The ethnobotany and pharmacognosy of *Olea europaea* subsp. *africana* (Oleaceae). *South Afr. J. Bot.* **2010**, 76, 324-331.
8. Nilsson, S. A survey of the pollen morphology of *Olea* with particular reference to *Olea europaea* Sens. Lat. *Kew Bull.* **1988**, 43, 303-315.
9. Stolarczyk, M.; Naruszewicz, M.; Kiss, A.K. Extracts from *E. pilobium* sp. herbs induce apoptosis in human hormone-dependent prostate cancer cells by activating the mitochondrial pathway. *J. Pharm. Pharmacol.* **2013**, 65, 1044-1054.
10. Somova, L.I.; Shode, F.O.; Ramnanan, P.; Nadar, A. Antihypertensive, antiatherosclerotic and antioxidant activity of triterpenoids isolated from *Olea europaea*, subspecies *africana* leaves. *J. Ethnopharmacol.* **2003**, 84, 299-305.
11. Altinyay, Ç.; Güvenç, A.; Altun, M.L. Antioxidant activities of *Oleuropein* and the aqueous extracts of *Olea Europaea* L. varieties growing in Turkey. *Turk. J. Pharm. Sci.* **2011**, 8, 23-30.
12. Lins, P.G.; Pugine, S.M.P.; Scatolini, A.M.; de Melo, M.P. In vitro antioxidant activity of olive leaf extract (*Olea europaea* L.) and its protective effect on oxidative damage in human erythrocytes. *Heliyon* **2018**, 4, e00805.
13. Al-Khalil, S. A survey of plants used in Jordanian traditional medicine. *Int. J. Pharmacogn.* **1995**, 33, 317-323.
14. Bellakhdar, J.; Claisse, R.; Fleurentin, J. and Younos, C. Repertory of standard herbal drugs in the Moroccan pharmacopoea. *J. Ethnopharmacol.* **1991**, 35, 123-143.
15. Kokwaro, J.O. *Medicinal Plants of East Africa*, University of Nairobi Press: Nairobi; **2009**.
16. Wu, C.D.; Darout, I.A.; Skaug, N. Chewing sticks: timeless natural toothbrushes for oral cleansing. *J. Periodontal Res.* **2001**, 36, 275-284.
17. Al-hebshi, N.; Al-haroni, M. and Skaug, N. In vitro antimicrobial and resistance-modifying activities of aqueous crude khat extracts against oral microorganisms. *Arch. Oral Biol.* **2006**, 51, 183-188.
18. Carvalho, M.L.; Santiago, S.; Nunes, M.L. Assessment of the essential element and heavy metal content of edible fish muscle. *Anal. Bioanal. Chem.* **2005**, 382, 426-432.

19. Quevauviller, P. *Quality Assurance in Environmental Monitoring*. Wiley-VCH Verlag GmbH: Germany; **1995**.
20. Debebe, A.; Chandravanshi, B.S.; Wondimu, T. Metallic nutrients in enset (*Ensete ventricosum*) corm cultivated in Wolliso and Wolkite towns in Ethiopia. *SINET: Ethiop. J. Sci.* **2012**, 35, 71-80.
21. Dubale, A.A.; Chandravanshi, B.S.; Gebremariam, K.F. Levels of major and trace metals in the leaves and infusions of *Croton macrostachyus*. *Bull. Chem. Soc. Ethiop.* **2015**, 29, 11-26.
22. Abdella, A.; Chandravanshi, B.S.; Yohannes, W. Levels of selected metals in coriander (*Coriandrum sativum* L.) leaves cultivated in four different areas of Ethiopia. *Chem. Int.* **2018**, 4, 189-197.
23. Admasu, S.; Chandravanshi, B.S.; Yohannes, W. Levels of selected metals in the leaves of *Ruta chalepensis* L. (Rue) collected from four different areas of Ethiopia. *Bull. Chem. Soc. Ethiop.* **2018**, 32, 185-197.
24. Gebregewergis, A.; Chandravanshi, B.S.; Redi-Abshiro, M. Levels of selected metals in teff grain samples collected from three different areas of Ethiopia by using microwave plasma-atomic emission spectroscopy. *Bull. Chem. Soc. Ethiop*. **2020**, 34, 449-462.
25. Jemaneh, D.; Chandravanshi, B.S. Mineral contents of Ethiopian red and green apple fruits: A comparison with WHO/FAO standards. *Chem. Int.* **2021**, 7, 112-122.
26. Pawlos, Z.; Chandravanshi, B.S.; Yohannes, W.; Embiale, A. Levels of selected metals in *Ficus sur* Forssk fruit and soil of the plant grown in different parts of Ethiopia. *SINET: Ethiop. J. Sci.* **2021**, 44, 1-12.
27. Gebre, A.; Chandravanshi, B.S. Levels of essential and non-essential metals in *Rhamnus prinoides* (Gesho) cultivated in Ethiopia. *Bull. Chem. Soc. Ethiop.* **2012**, 26, 329-342.
28. Wagesho, Y.; Chandravanshi, B.S. Levels of essential and non-essential metals in ginger (*Zingiber officinale*) cultivated in Ethiopia. *Springer Plus* **2015**, 4, 107.
29. Taha, K.K.; Al Ghtani, F.M. Determination of the elemental contents of date palm (*Phoenix dactylifera* L.) from Kharj Saudi Arabia. *World Sci. News* **2015**, 6, 125-135.
30. Vella, A.; Attard, E. Analysis of heavy metal content in conventional and herbal toothpastes available at Maltese pharmacies. *Cosmetics* **2019**, 6, 28.
31. Orisakwe, O.E.; Okolo, K.O.; Igweze, Z.N.; Ajaezi, G.C.; Udowelle, N.A. Potential hazards of toxic metals found in toothpastes commonly used in Nigeria. *Roczniki Państwowego Zakładu Higieny* **2016**, 67(2), 197-204.
32. Rao, R.N. and Rao, T.N. Determination of heavy metals in toothpastes containing tin as an active ingredient. *Indian J. Chem. Technol.* **2014**, 21, 238-243.
33. European Commission. Regulation (EC) No 1881/2006 of 19 December 2006 on Setting Maximum Levels for Certain Contaminants in Foodstuffs. 2006. *Official J. Eur. Union* **2006**, 364, 5-24.
34. Codex Alimentarius Commission. Joint FAO/WHO Food Standards Programme, Codex committee on contaminants in foods. Fifth Session, The Hague, The Netherlands, 21-25 March **2011**.
35. Chan, E. Health Canada Published Guidelines on Heavy Metal Impurities in Cosmetics. Sparkle Volume 632. 2012. Available online: http://www.intertek.co.th/uploadedFiles/Intertek/Divisions/Consumer_Goods/
36. Hepp, N.; Mindak, W.R.; Gasper, J.W.; Thompson, C.B.; Barrows, J. Survey of cosmetics for arsenic, cadmium, chromium, cobalt, lead, mercury, and nickel content. *J. Cosmet. Sci.* **2014**, 65, 125-145.
37. Miller, J.N.; Miller, J.C. *Statistics and Chemometrics for Analytical Chemistry*, 5th ed., Pearson Practice Hall: England; **2005**.