ADDIS ABABA UNIVERSITY
COLLEGE OF NATURAL AND COMPUTATIONAL SCIENCES
DEPARTMENT OF CHEMISTRY

MSc Thesis

Determination of selected metals in traditional toothbrush from common privet (*Ligustrum vulgare*), wild date palm (*Phoenix reclinata*), and African wild olive (*Olea africana*) commonly used in Addis Ababa, Ethiopia.

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Advisor: Prof. B.S. Chandravanshi

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A thesis submitted to the college of natural and computational science Addis Ababa University in partial fulfillment of the requirements for the degree of Master of Science in chemistry.

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List of abbreviations

AAS	Atomic absorption spectrometry
ACS	American Chemical Society
ANOVA	Analysis of variance
BDL	Below detection limit
CV	Coefficient of variance
EGCM	Extra gas control module
FAAS	Flame atomic absorption spectrometry
IDL	Instrument detection limit
ICP-MS	Inductively coupled plasma-mass spectrometry
ISO	International Organization of Standardization
LOD	Limit of detection
LV	*Ligustrum vulgare*
MDL	Method detection limit
MP-AES	Micro plasma-atomic emission spectrometry
NTU	Nephelometric turbidity units
ND	Not detected
OA	*Olea Africana*
PR	*Phoenix reclinata*
PTFE	Polytetrafluoroethylene
PVC	Polyvinylchloride
R^2	Correlation coefficient
RSD	Relative standard deviation
SD	Standard deviation
µs	Microsiemens
WHO	World Health Organization

Abstract

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. Traditional toothbrush is 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 are useful in decreasing dental caries. The main purpose of this study was to determine the essential and non essential metals in traditional toothbrushes which are extensively used in the cleaning of teeth and take care of the oral health.

Essential and non essential metals like Ca, Fe, Mg, Al, Cu, Cr, Ni, Zn, Mn, Pb, and Cd were quantified in selected traditional toothbrushes from plants including *Ligustrum vulgare*, *Phoenix reclinata* and *Olea africana* samples collected from three different areas of Addis Ababa (Kotebe, Arat Kilo and Ferensay) by using microwave plasma-atomic emission spectroscopy (MP-AES) after wet digestion. The volume of reagents used, digestion temperature and digestion time was optimized for each plant type using the mixtures of HNO_3 (69.5%) and $HClO_4$ (70%). The optimized conditions were found to be a volume ratio of 4:1 (v/v), 4:1 (v/v), and 3:1 (v/v) and temperature 270 ^oC, 240 ^oC and 210 ^oC for 2:15, 2:45 and 2:00 hours for *Olean africana*, *Ligustrum vulgare* and *Phoenix reclinata*, respectively. The accuracy of the optimized procedure was evaluated by analyzing the digest of the spiked samples with standard solution. Recoveries of the 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 (46–122) > Zn (27.2–175) > Mn (20.1–29) > Cu (6.6–20.3) > Cr (6.7–8.9) > Ni (2.6–7.9). The results indicate that the selected three traditional toothbrushes are good sources of essential metals and the selected toothbrushes were free from toxic metals such as Pb and Cd. A statistical analysis of variance (ANOVA) at 95% confidence level indicated that there were significant differences ($p < 0.05$) in the mean values of Cu, Zn, Mn, Cr, Al, Ca, and Fe in the *Olea africana* sample collected from the three sites. Whereas, there was no significant difference ($p > 0.05$) in the mean values of Mg and Ni in the samples collected from the three sites. There were significant differences ($p < 0.05$) for Cu, Zn, Ni, Mg, Cr, and Fe in the *Ligustrum vulgare* sample collected from the three sites whereas, there was no significant difference ($p > 0.05$) in the mean values of Ca, Mn and Al, the samples collected from the three sites. There were significant differences ($p < 0.05$) for Cu, Zn, Mn, Ni, Mg, Al, Ca, and Fe in the *phoenix Reclinata* sample collected from the three sites whereas, there was no significant difference ($p > 0.05$) in the mean values of Cr the samples collected from the three sites. There were significant differences ($p < 0.05$) in the mean values of for Zn, Mg, Al, and Mn between *Ligustrum vulgare*, *Phoenix reclinata* and *Olea africana* samples whereas, there was no significant difference ($p > 0.05$) in the mean values of Ca, Cr, Cu, Fe and Ni between the three samples. The Pearson correlation was used to predict the dependence of metal levels on one another. The correlation done for selected metals in *Ligustrum vulgare*, *Phoenix reclinata* and *Olea africana* and between the three plants mostly showed strongly positive correlation between some metals while strongly negative correlation between other metals.

Key word: Traditional toothbrush, *Ligustrum vulgare*, *Phoenix reclinata* and *Olea Africana*, Essential metals, Non-essential metals, Microwave plasma-atomic emission spectroscopy

1. Introduction

1.1. Background of the study

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 (WHO, 2004).

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 (Bone, 2005). One of the important things 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 toothbrush which are traditional and synthetic toothbrush. 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 (Kassu, 1999). 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. 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.

In mostly Muslim Ethiopian people say there is religious precedent for the use of the chewing sticks. In holy Islamic writings known as the Hadith, the Prophet Mohammed recommended chewing sticks use as part of cleaning rituals that are an essential element of daily prayers. Although commercially made toothbrushes from leading international brands are available in Addis Ababa supermarkets and pharmacies, many people say they prefer the chewing sticks. It is better because it is natural. Kassu *et al.* (1999) have reported on the antibacterial activities of 20 plants that are used commonly as toothbrush sticks in Ethiopia.

The present study is based on the three most widely used plants in Addis Ababa Ethiopia. These are Mefaqia (Amharic) or Common privet (English) or *Ligustrum vulgare* (scientific name), Zembaba (Amharic) or Wild date palm (English) or *Phoenix reclinata* (scientific name), Weira (Amharic) or African wild olive (English) or *Olea Africana* (scientific name). The pictures of the three plants are shown in Figure 1.

Phoenix reclinata plant *Olea africana* plant *Ligustrum vulgare* plants

Branches of three traditional toothbrush plants

Figure 1.The pictures of the three plants and branches used for toothbrush.

Ligustrum vulgare also sometimes known as common privetor European privetisa species of *Ligustrum* native to central and southern Europe, north Africa and southwestern Asia, from Ireland and southwestern Sweden south to Morocco, and east to Poland and northwestern Iran (Bailey, 1949). *Ligustrum 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.

Phoenix reclinata the wild date palm or Senegal date palm, is a species of flowering plant in the palm family native to tropical Africa, the Arabian Peninsula, Madagascar and the Comoro Islands. It is also reportedly naturalized in Florida, Puerto Rico, Bermuda and the Lee Ward Islands (Govaerts and Faden, 2013). *Phoenix reclinata* is cultivated in Ethiopia in different area specially urban areas and hot regions like Amhara region, Bahir Dar, Foneteselam, Debre Markos, Addis Ababa, Hawasa, etc. for evergreen the road and compounds in addition to use as toothbrush.

The African wild olive, previously known as *Olea 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 (Long *et al.*, 2010). It is geographically distributed throughout the Southern Africa and Northwards through East Tropical African into Eritrea. It has a variety of habitats, from forest, riverside, open grassveld, bush, mountain kloops and rocky ledges (Nilsson, 1988). *Olea africana* is cultivated in Ethiopia in all regions both rural and urban area specially hot regions for most of the time 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 remedy of which substitution of synthetic plastic brush and paste used for caries oral health and teeth.

Most of 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 *Ligustrum vulgare*, *Phoenix reclinata*, and *Olea africana*. Since those plants take essential and non essential metals from the soil and water to grow and the source toxic metals may be from polluted air, water and soils. The source pollutants may be smog of cars, industries are increased by day to day in Ethiopia specially Addis Ababa.

The aim of this study is to determine the level of metal contents in *Ligustrum vulgare*, *Phoenix reclinata*, and *Olea africana*, because there is no any study that shows the content of heavy metals in the branch part of three plants up to now 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 *Ligustrum vulgare*, *Phoenix reclinata*, and *Olea africana*.

1.2. Objective of the study

1.2.1. General objective

The main objective of this study is to determine selected metal contents in *Ligustrum vulgare*, *Phoenix reclinata*, and *Olea africana* branches in Addis Ababa, Ethiopia.

1.2.2. Specific objectives

1. To develop an optimum working procedure for digestion to determine selected metal contents in *Ligustrum vulgare*, *Phoenix reclinata*, and *Olea africana* by microwave plasma-atomic emission spectroscopy (MP-AES).

2. To determine the selected trace metal contents found in *Ligustrum vulgare*, *Phoenix reclinata*, and *Olea africana* by microwave plasma-atomic emission spectroscopy (MP-AES).
3. To compare the levels of the identified metals in *Ligustrum vulgare*, *Phoenix reclinata*, and *Olea africana* cultivated in three different areas in Addis Ababa.
4. To compare the concentration of each metal in the sample with other medicinal plants and commercial toothpastes reported in the literature.

1.3. Statement of the problem

African toothbrush sticks have been used for centuries for the maintenance of oral hygiene. The chewing sticks, termed as such due to the need for the user to chew the stick prior to brushing, have been used throughout the Greek, Roman, Jewish and Islamic Empires (Almas, 2001). Even though many people have abandoned the traditional toothbrush sticks and adapted to the commercial tooth brushing method, some societies still make use of chewing sticks as a daily ritual to maintain oral hygiene. This is especially true in developing countries where economics, customs, religion and the availability of oral hygiene tools play a role in their continued use (Kemoli *et al.*, 2001). Often, chewing sticks are used by the majority of the population, as is the case of Ethiopians (Fadulu, 1975; Olsson, 1978).

Although the World Health Organization has promoted the use of toothbrush sticks (Wu *et al.*, 2001) and has encouraged further research of their efficacy, few studies have been undertaken on the potential antimicrobial properties of chewing sticks (Al-hebshi *et al.*, 2006). Kassu *et al.* (1999) assessed the most common toothbrush sticks used by rural and urban Ethiopians. From the most common used three are *Ligustrum vulgare*, *Phoenix reclinata*, and *Olea africana* are important traditional toothbrush used by most Ethiopian peoples. These traditional toothbrushes have high importance in removing part of food left from swallowing between teeth in addition to their antibacterial property, since synthetic tooth paste prepared from industry are known their metal contents which are important for our health but there is no any research studies which show the metal content of traditional toothbrushes obtained from the plants.

In the world especially in Ethiopia all traditional toothbrush users have no information about metal contents in each different types of traditional toothbrush. So it is necessary to create awareness about the metal content of each traditional toothbrush in the world especially developing countries like Ethiopia. Moreover this study aimed to give general information and to determine the selected metals of *Ligustrum vulgare*, *Phoenix reclinata*, and *Olea africana* by using microwave plasma-atomic emission spectroscopy.

1.4. Significance of the study

The output of this study will have different benefits when viewed in different perspectives. The first one is the determination of heavy metals that helps us to differentiate one from the other by the content of toxic metals present when using *Ligustrum vulgare*, *Phoenix reclinata*, and *Olea africana* as toothbrush. The second is the determination of essential metals that helps to compare the synthetic tooth pastes which have appropriate content for our health. The third is the developed analytical method, generated analytical data, and information on the chemistry of the traditional toothbrush can be used as a baseline information for the future researcher in other types of traditional toothbrush in the world and in various fields related to *Ligustrum vulgare*, *Phoenix reclinata* and *Olea africana*.

2. Literature Review

2.1. General overview of *Ligustrum vulgare, Phoenix reclinata*, and *Olea africana*

Ligustrum vulgare belongs to the genus *Ligustrum* L. (*Oleaceae*), which contains, according to different classifications, between 37 and 50 species, divided into five sections. Most of these species are native to Asia and only a few species are present in Europe, Australia, North America or North Africa (Qin, 2009, Starr and Starr, *et.al* 2003). It is also known as common privet or European privet (Nesom, 2009). *Ligustrum vulgare* is a deciduous shrub species which can reach heights up to 4 or 5 m (Chesnoiu *et al.*, 2009).

Phoenix reclinata is a species of flowering plant in the palm family native to tropical Africa, the Arabian Peninsula, Madagascar and the Comoro Islands. It is also reportedly naturalized in Florida, Puerto Rico, Bermuda and the Leeward Islands (Govaerts, and Faden, 2013).

Olea africana belongs to *Oleaceae* family comprising 30 genera with 600 species (Muzzalupo *et al.* 2014). *Olea africana* is a small, ever green tree, averaging 6cm or more in height. It has many thin branches with opposite branch lets and shortly-stalked, opposite, lance late leaves about 5 cm long, acute, entire and smooth, pale green above and silvery below. The bark is pale grey.

2.2. Culinary and medicinal uses of *Ligustrum vulgare, Olea europaea* and *Phoenix reclinata*

Leaves of *Ligustrum vulgare* have been used for treatment of oropharyngeal inflammations or as antirheumatic, diuretic, and hypotensive agents in folk medicine in southern Europe (Stolarczyk, *et.al*, 2013). The leaves are astringent, bitter, detergent, vulnerary. Internal use of *Ligustrum vulgare* plant should be avoided since it can produce allergic symptoms. Externally it is a safe and effective treatment.

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 (Somova, *et al.* 2003). The leaves of the tree were reported to be potent for the treatment of malaria in 1854 (Altinyay *et al.*, 2011).

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. Oil of seeds is taken orally as a laxative and also applied externally as a balm for inflammation (Al-Khalil, 1995). 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 (Bellakhdar *et al.*, 1991). Continuous application of olive oil is also useful to prevent hair loss. In Canary Islands, the infusion prepared from olive leaves is taken orally as a hypotensive while it is administered through rectum for hemorrhoids (Darias *et al.*, 1996; Darias *et al.*, 1986). In East-Africa the infusion of the bark of olive tree is taken for tapeworm infestation after soaking for whole night (Kokwaro, 2009). In Greece hot water extract of olive leaves is taken orally to treat high blood pressure (Lawrendiadis, 1961). In Italy, the extract of the essential oil of fruit is taken orally to treat renal lithiasis. It is applied externally to treat burns, ores, and rheumatism and to promote circulation (De Feo *et al.*, 1992). Infusion of fresh leaves is also taken as anti-inflammatory. Tincture of olive leaves is taken in Italy as a febrifuge (Pieroni *et al.*, 1996) and applied externally as an emolument for ingrown nails and a restorer of epithelium (De Feo and Senatore, 1993). In Japan olive leaves are taken orally for stomach and intestinal diseases and their essential oil is used orally for constipation and liver pain (Bellakhdar *et al.*, 1991).

2.3. Essential and non essential metals

Because dietary habits are among the major concerns of man, and are increasing in importance in almost all countries, ingestion, both in amount and quality of the nutrients for good functioning of the human organism is fundamental (Carvalho *et al.*, 2005).

Essential metals are considered potentially non toxic, have metabolic function in the living organism in very small quantity (generally less than 100 mg/day) including Fe, Cu. Ca, Zn, Mn, etc. (Ražić *et al.*, 2003). Non essential metals are those which are considered potentially toxic, and have no metabolic functions in the living organisms, including Cd, Pb, Al, As, Sr, Hg, etc. (Davis and Greger, 1992).

2.4. Classification of minerals

Minerals are usually classified into two main groups on the basis of their relative amounts in the body. One of the groups is macro-elements or macro-minerals occurring in relatively large amounts and needed in quantities of 100 mg or more per day which include Ca, Mg, Na and K. Minerals occurring small amounts and needed in quantities of a few milligrams or less per day are called microelements or trace elements, which includes Fe, Zn, Cu, Mn, Co, Ni, Cr and B. Other trace metals like Al, Pb, Hg and As are till now recognized as potentially harmful. Actually all essential elements may also be toxic to animals and humans if ingested at sufficiently high levels and for a long enough period (WHO, 1996; Sager and Hoesch, 2005). The physiological role of Ca, Mg, Fe, Cu, Zn, Mn, Cr, Ni, Cd, and Pb are briefly described below.

Calcium (Ca)

Calcium is required for the normal development and maintenance of the skeleton. It is stored in the teeth and bones, where it provides structure and strength (National Health and Medical Research Council, 2005). Calcium is also important as a positive ion (Ca^{2+}) in blood clotting, muscle contraction, and nerve impulse transmission. Excessive intakes of Ca induce constipation and place up to half of otherwise healthy hypercalciuric males at increased risk of urinary stone formation. A high Ca intake may inhibit the intestinal absorption of Fe, Zn, and other essential minerals (Food and Nutrition Board, 1989; National Research Council, 1997). Its deficiency is found to result in stunted growth, rickets, and osteoporosis.

Magnesium (Mg)

Soft tissue magnesium functions as a co-factor of many enzymes involved in energy metabolism, protein synthesis, RNA and DNA synthesis, and maintenance of the electrical potential of nervous tissues and cell membranes (WHO, 2004). Excessive intakes of magnesium are harmful to people with impaired renal function resulting in magnesium retention is often associated with hypomagnesaemia. Early symptoms of hypermagnesemia include nausea, vomiting, and hypotension. Its depletion results gastrointestinal tract abnormalities associated with mal-absorption or excessive fluid and electrolyte losses; renal dysfunction with defects in cation re-absorption; and general malnutrition and alcoholism (Food and Nutrition Board, 1989; National Research Council, 1997).

Iron (Fe)

Iron is a constituent of hemoglobin, myoglobin, and a number of enzymes and, therefore, is an essential nutrient for humans (Food and Nutrition Board, 1989; National Research Council, 1997). Iron has several vital functions in the body. It serves as a carrier of oxygen to the tissues from the lungs by red blood cell hemoglobin, as a transport medium for electrons within cells, and as an integrated part of important enzyme systems in various tissues (WHO, 2004). Deficiency of iron results in anemia which is recognized by its symptom such as low blood iron level, small and red blood cells and low blood hemoglobin values. Functional indicators of iron deficiency may include reduced physical work capacity, delayed psychomotor development in infants, impaired cognitive function, impaired immunity and adverse pregnancy outcomes (National Health and Medical Research Council, 2005). Iron toxicity usually results from a generic disorder called hemochromatosis. This disease causes over absorption and accumulations of iron, which can result in severe liver and heart damage (Food and Nutrition Board, 1989; National Research Council, 1997).

Copper (Cu)

Copper is an essential trace metal for animals and human beings and have diverse functions in plants. It is required in the formation of erythrocytes and hemoglobin as well as some enzymes like tyrosinase. Copper is also involved in bone and elastic tissue development (Bertini *et al.*, 1994). The deficiency of copper results anemia related to defective iron metabolism, skeletal defects, affect the central nervous system and the immune and cardiovascular systems notably in infants, defects in pigmentation and structure of hair or wool, reproductive failure, and decreased arterial elasticity (Food and Nutrition Board, 1989; National Research Council, 1997; National Health and Medical Research Council, 2005).

Zinc (Zn)

Zinc is an essential component of a large number of enzymes participating in the synthesis and degradation of carbohydrates, lipids, proteins, and nucleic acids as well as in the metabolism of other micronutrients (Godt *et al.*, 2006). Severe zinc deficiency in humans are growth retardation, delayed sexual and bone maturation, eye and skin lesions, diarrhoea, alopecia, impaired appetite, increased susceptibility to infections mediated via defects in the immune system, and the appearance of behavioral changes (Food and Nutrition Board, 1989; National Research Council, 1997; National Health and Medical Research Council, 2005).The toxicity signs are nausea, vomiting, diarrhoea, fever, and lethargy and have been observed after ingestion of 4–8 g zinc. Long-term zinc intakes higher than the requirements could, however, interact with the metabolism of other trace elements. Copper seems to be especially sensitive to high zinc doses. A zinc intake of 50 mg/day affects copper status index in erythrocytes (Food and Nutrition Board, 1989; National Research Council, 1997; WHO, 2004).

Manganese (Mn)

Manganese is an essential element to both plants and animals. It is necessary for normal bone metabolism and important enzyme reactions. It also helps to maintain normal nerve, brain and thyroid function (National Health and Medical Research Council, 2005). Signs of Mn deficiency include poor reproductive performance, growth retardation, congenital malformations in the offspring, abnormal formation of bone and cartilage, changes in carbohydrate and lipid metabolism, and impaired glucose tolerance. It also interferes with skeletal development (Food and Nutrition Board, 1989; National Research Council, 1997; National Health and Medical Research Council, 2005). Exposure to high level of Mn can cause both mental and emotional disturbance, along with increased slowness and clumsiness of the body movements. This disease is called manganism. Any brain injury due to the accumulation of Mn in the brain is permanent (Agency of Toxic Substances and Disease Registry (ATSDR), 2001).

Chromium (Cr)

Chromium(III) is an essential element in humans. Trivalent chromium is required for maintaining normal glucose metabolism; it acts as a cofactor for insulin. Chromium deficiency has been induced in several animal species, resulting in impaired glucose tolerance in the presence of normal concentrations of circulating insulin and, in severe cases, in a diabetes-like syndrome (Food and Nutrition Board, 1989; National Research Council, 1997). Chromium(III) is much less toxic than chromium(VI). The respiratory tract is the major target organ for Chromium(VI) toxicity, for acute (short-term) and chronic (long-term) inhalation exposures. Shortness of breath, coughing, and wheezing were reported from a case of acute exposure to chromium(VI), while perforations and ulcerations of the septum, bronchitis, decreased pulmonary function, pneumonia, and other respiratory effects have been noted from chronic exposure. Inhaled chromium(VI) is a human carcinogen, resulting in an increased risk of lung cancer (US EPA, 2010).

Nickel (Ni)

Nickel is believed to play a role in physiological processes as a co-factor in the absorption of iron from the intestine. Nickel increased the absorption of iron from the diet in iron deficiency but only when dietary iron was in the unavailable ferric form (Das *et al.*, 2008). Contact with nickel compounds can cause a variety of adverse effects on human health, such as nickel allergy in the form of contact dermatitis, lung fibrosis, cardiovascular and kidney diseases and cancer of the respiratory tract. Acute health effects of Ni manifest as a variety of clinical symptoms (nausea, vomiting, abdominal discomfort, diarrhea, visual disturbance, headache, giddiness, and cough). The most common type of reaction to nickel exposure is a skin rash at the site of contact (Duda-Chodak and Blaszczyk, 2008).

Lead (Pb)

Lead serves no useful purpose in the human body, and its presence in the body can lead to toxic effects, regardless of exposure pathway. Lead toxicity influences brain, heart, kidneys, liver, nervous system, and pancreas. It may cause many signs and symptoms such as abdominal pain, anemia, anorexia, anxiety, bone pain, brain damage, confusion, constipation, convulsions, dizziness, drowsiness, fatigue, headaches and hypertension. It also diminishes IQ in children (Ali, 2003).

Cadmium (Cd)

Cadmium has no known nutritional value, and it has been considered an extremely significant pollutant affecting all life forms because of its high toxicity and high solubility in soil and water and easy accumulation in roots of most plant tissues. Excessive Cd exposure may give rise to renal, pulmonary, hepatic, skeletal, reproductive effects, and cancer (Nordberg, 2003). Intake of cadmium-contaminated food causes acute gastrointestinal effects, such as vomiting and diarrhoea (Godt *et al.*, 2006).

3. Experimental

3.1. Equipment and reagents

3.1.1. Equipments

Chopping board (PTFE, China) and Teflon (PTFE) knife were used to cut the branch of selected plants and to change size in to pieces. A drying oven (Griffin and George Ltd, Britain) was used to dry *Ligustrum vulgare*, *Olea africana*, and *Phoenix reclinata* sample. Mortar and pestle was used to grind sample. Analytical balance (Larko, LA114, 110 g/0.1 g) with precision of ± 0.0001 g was used to weigh the *Ligustrum vulgare*, *Olea Africana* and *Phoenix reclinata* 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 (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 *Ligustrum vulgare*, *Olea africana* and *Phoenix reclinata* samples.

3.1.2. Reagents and chemicals

Reagents that were used in the analysis were all analytical grade. HNO_3 (69.5% reagent grade, ACS, ISO, European Union) and $HClO_4$ (70%, 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 products of Perkin Elmer (Boston, USA) were used for preparation of calibration standards and in the spiking experiments. Deionized water (chemically pure with conductivity $\leq$ 1.5 μs/cm was used for dilution of sample and intermediate metal standard solutions prior to analysis and rinsing glassware and sample bottles.

3.2. Instrumentation

All measurements were performed using an Agilent 4200 MP-AES, with nitrogen supplied from an Agilent 4107 Nitrogen Generator. The sample introduction system consisted of a Micro Mist nebulizer, double pass glass cyclonic spray chamber and solvent resistant sample tubing. An external gas control module (EGCM) accessory and auto sampler were used. The MP-AES was controlled using the intuitive MP Expert software, which recommends wavelengths for the selected elements and automatically sets the nebulizer flow rate and EGCM settings. Auto background correction was used to resolve the element emission line from the organic matrix. Instrument operating conditions are given in Table 1. The sample introduction system consists of PVC peristaltic pump tubing, a single-pass glass cyclonic spray chamber and the one nebulizer. The Agilent MP Expert software was used to automatically subtract the background signal from the analytical signal.

Table 1. Agilent 4200 MP-AES operating conditions**.**

Metals	Parameter									
	Back ground correction	Replicates	Pump speed	Blank subtraction	Stabilization time (s)	Sample uptake time (s)	Sample uptake fast pump	Rinse time (s)	Read time (s)	Nebulizer flow (mL/min)
Zn	Auto	3	15	on	21	28	On	10	3	0.45
Pb	Auto	3	15	on	21	28	On	10	3	0.75
Mg	Auto	3	15	on	21	28	On	10	3	0.9
Cr	Auto	3	15	on	21	28	On	10	3	0.9
Cu	Auto	3	15	on	16	40	On	12	3	0.7
Cd	Auto	3	15	on	16	40	On	12	3	0.5
Mn	Auto	3	15	On	16	40	On	12	3	0.9
Ni	Auto	3	15	on	16	40	On	12	3	0.7
Al	Auto	3	15	on	16	40	On	12	3	0.95
Ca	Auto	3	15	on	15	27	On	10	3	0.6
Fe	Auto	3	15s	on	22	30	On	10	3	0.65

3.3. Descriptions 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 (Köppen, 2015) with precipitation varying considerably by the month. Addis Ababa is provided with on balance 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 ^{o}C, 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 ^{o}C in mean temperatures. The highest temperature on record was 30.6 ^{o}C on 26 February 2019, while the lowest temperature on record was 0 ^{o}C recorded on multiple occasions. The three types of traditional toothbrush were collected from three different part of Addis Ababa, Ethiopia namely: Kotebe, Ferensay and Arat Kilo AAU campus where the three plants are more cultivated in Addis Ababa. Ferensay and Kotebe are located in Yeka sub city and Arat Kilo campus is located in Arada sub city.

3.4. Sample decomposition

The purpose of sample decomposition is for converting all the species in which a given element is present in such a way that it becomes present in one defined form, eliminating interfering substances from the matrix, and obtaining the element in a homogeneous and easily accessible matrix (Quevauviller, 1995). The digestion methods could be classified in to wet digestion, acid digestion, dry ashing, and microwave digestion. In this study wet digestion method was used.

3.5. Wet digestion

Samples to be analyzed for elemental metal content are usually prepared by digesting the matrix in a strong acid. In the case of organic matrices, an oxidizing mixture is used to destroy the entire organic matrix and solubilize the sample. This yields a clear solution containing the metals for analysis by such techniques as AAS, ICP-MS, or MP-AES. Nitric acid is commonly used, because there is no chance of forming insoluble salts as might happen with $HClO_4$, HCl or H_2SO_4. Hydrogen peroxide may be added to increase the oxidizing power of the digestion solution (Quevauviller, 1995; Mitra, 2004).

3.6. Nitric and perchloric acid digestions

Nitric acid is rarely used alone. It is best used in combination with sulfuric and/or perchloric acids for organic sample digestion. For samples that are not highly aromatic and/or contain a high -OH functionality, nitric acid is preferred followed by perchloric acid. The only element that may be lost from a nitric/perchloric digestion is Hg. Care should be exercised and the literature consulted before attempting to use nitric acid in combination with other acids for organic sample digestions.

HNO_3 is added to samples for two purposes. One, it reduces pH < 2 and below pH 2, precipitation, adsorption to container wall and microbial degradation are minimized. For this purpose any acid will serve the purpose, HNO_3 is preferred because of its oxidizing nature. Adding HNO_3 converts metal ions into their nitrate salts, which are highly soluble. Secondly, sample digestion is required before analysis. The purpose is to destroy the matrix, which otherwise interfere during atomization. Also digestion converts all form of metal in to a single oxidation state. Though different acids, i.e. HNO_3, H_2SO_4, $HClO_4$ in different combinations are used, HNO_3 suits very well for water and waste water samples. The choice of acid mixture depends on the organic matter content and turbidity of the sample. Water having turbidity < 1 NTU can be analyzed without digestion (Syed Hadi, 2019).

3.7. 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. The basic requirements for sample preparation for analysis are to get an optimum condition for digestion. The optimum condition 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 that is suitable for the analysis using MP-AES, for each of the traditional toothbrush sample, 0.5 g of powdered and homogenized samples was weighed and transferred to 250 mL of a round bottom flask (24/29). To this, different volumes of nitric acid and perchloric acid at specified proportions (v/v) were added and digested at different temperature and different time (Tables 2-4).

Table 2. Different conditions tested for optimization of digestion procedure for 0.5 g of *Olea africana.*

	Trial	Reagents used	Reagent volume ratio (mL)	Temperature (^{o}C)	Digestion time (h)	Observation
Optimization for reagent volumes	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 temprature	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

Table 3. Different conditions tested for optimization of digestion procedure for 0.5 g of *Ligustrum vulgare L.*

	Trial	Reagents used	Reagent volume ratio (mL)	Temperature (^oC)	Digestion time (h)	Observation
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 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 Temprature	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

Table 4. Different conditions tested for optimization of digestion procedure for 0.5 g of *Phoenix reclinata*

	Trial	Reagents used	Reagent volume ratio (mL)	Temperature (^oC)	Digestion time (h)	Observation
Optimization for reagent volume s	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 temprature	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

3.8. Digestion of *Ligustrum vulgare, Phoenix reclinata* and *Olea Africana*

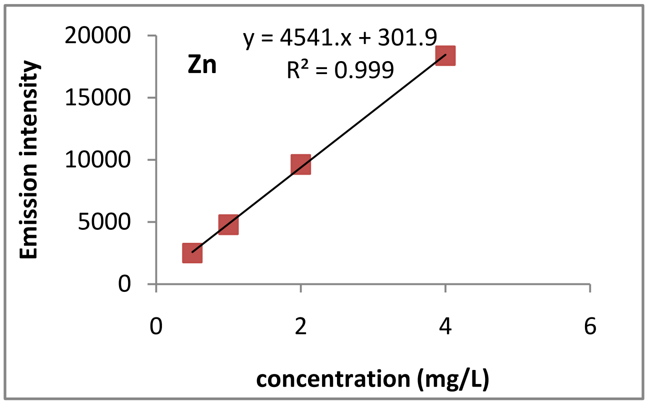
By applying the optimized conditions (Tables 2-4), 0.5 g of the dried and homogenized samples were transferred to 250 mL round bottom flask, a total 5 mL of the mixture of nitric acid (69.5%) and perchloric acid (70%) with a volume ratio of 4:1 (v/v) for *Olea africana* and 4:1 (v/v) for *Ligustrum vulgare* 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 *Phoenix reclinata* for each 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. In similar manner after the digestion, the digest was allowed to cool to room temperature for 10 min without dismantling the condenser from the flask and 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 rinsed subsequently with 5 mL deionized water until the total volume reached around 45 mL and the solution slowly filled to the mark show 50 mL.

3.9. Calibration of the instrument in the determination of selected metals by MP-AES

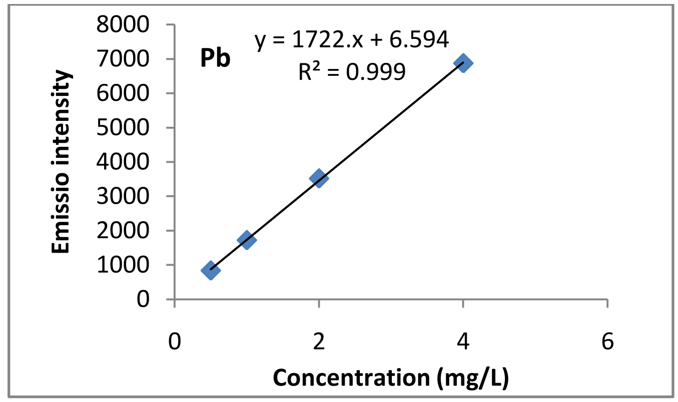
Calibration metal standard solutions were prepared for each of the metals from an intermediate standard solution containing 10 mg/L which was prepared from the atomic absorption spectroscopy standard stock solutions that contained 1000 mg/L. These secondary standards were diluted with deionized water to obtain three working standards for each metal of interest. The digest from three different traditional toothbrush samples from three different areas were determined three times Al, Ca, Mg, Cu, Zn, Mn, Ni, Fe, Cr, Pb and Cd by MP-AES. The optimum operating conditions of MP-AES are given in Table 1.

3.10. Calibration curves of working standard concentrations of intensity versus concentration

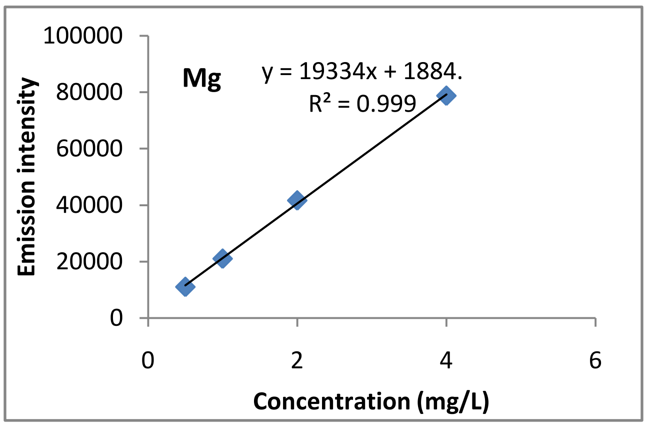
The calibration curves for the studied elements are shown in Figure 1.



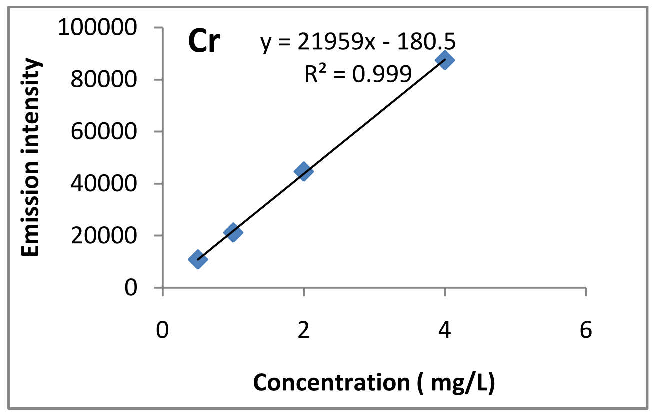
(a)



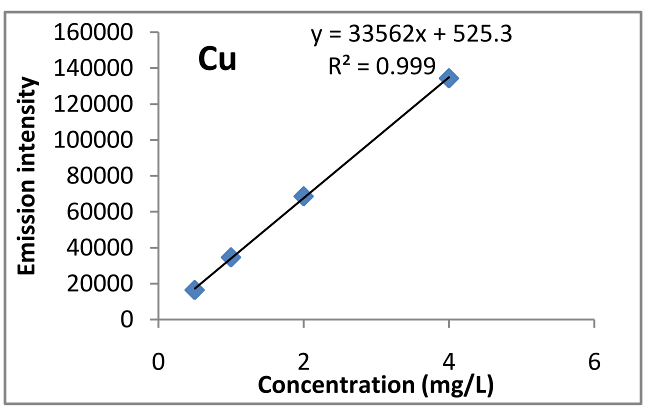
(b)



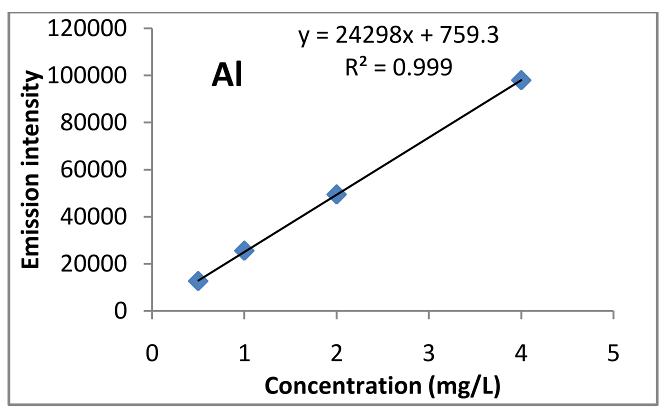
(c)



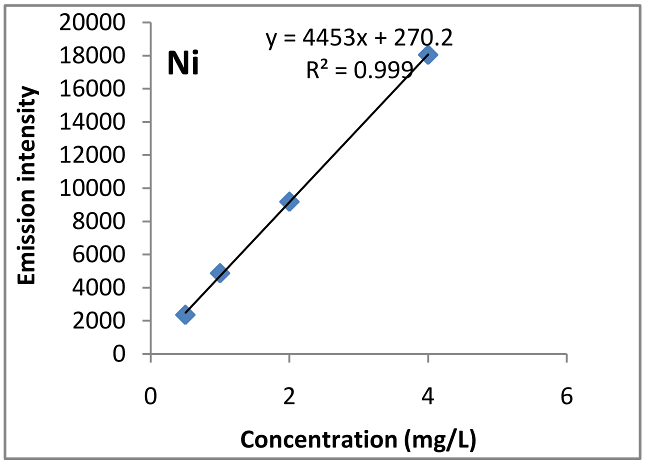
(d)

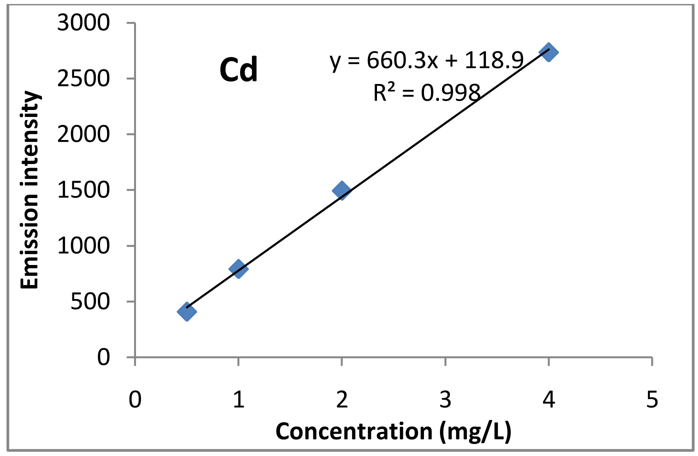


(e)



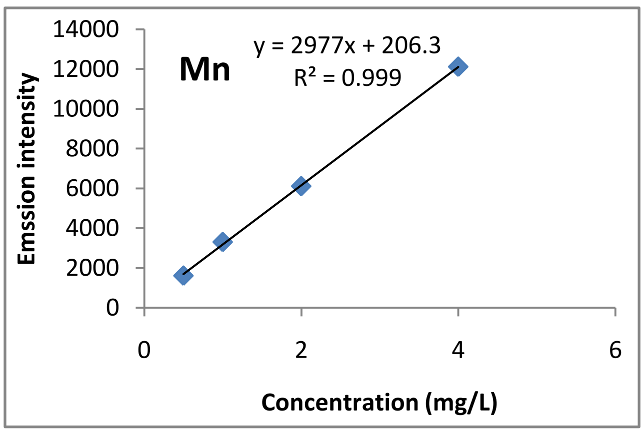
(f)

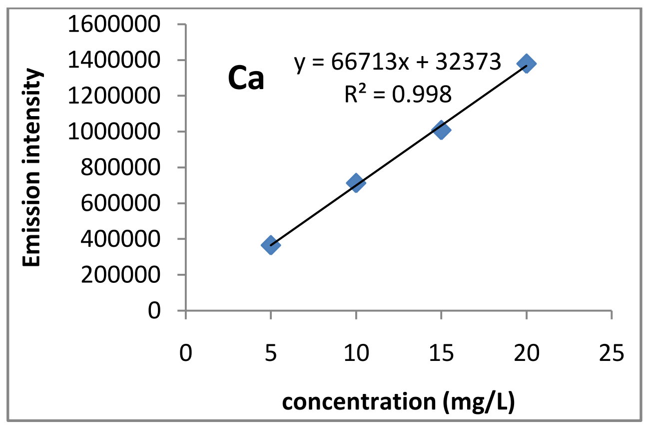




(g)

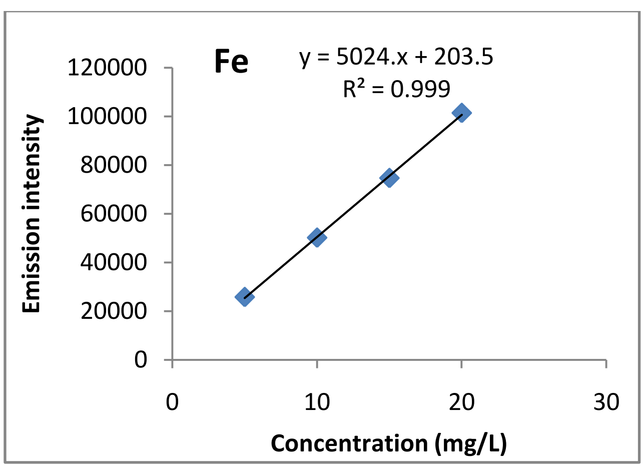
(h)





(i)

(j)



(k)

Figure 2. (a-k) the calibration curves for the studied elements.

3.11. Precision

The precision of an analytical procedure expresses the closeness or agreement between a replicate measurements obtained from multiple sampling of the same homogeneous sample under the prescribed conditions (repeatability or reproducibility). The common term used to measure variability is the coefficient of variation (CV) or relative standard deviation (RSD), which may also be expressed as a percentage and it is the parameter of choice for expressing precision in analytical sciences. In this study, the precision of the results were evaluated by the pooled standard deviation, and relative standard deviation of the results of nine measurements for a given sample (i.e. three samples (n = 3) and triplicate readings for each sample).

3.12. The method detection limit (MDL)

It is the minimum concentration that can be detected by the analytical method with a given certainty. It is also the smallest concentration or amount of analyte that can be reliably shown to be present measured under specific confidence level. Limit of detection can calculated by multiplying the standard deviation of the blank (SD_b) three times ($LOD = 3SD_b$).

The detection limit (with all steps of the analysis included) is called the MDL. The practical method for determining the MDL is to analyze 9 samples of concentration near the expected limit of detection. The standard deviation is then determined. The one-sided t-distribution is determined and multiplied versus the determined standard deviation. For nine samples (with eight degrees of freedom) the t value for a 99% confidence level is 3.14. Rather than performing the complete analysis of seven identical samples, if the instrument detection limit is known, the MDL may be estimated by multiplying the instrument detection limit or lower level of detection by the dilution prior to analyzing the sample solution on the instrument. This estimation, however, ignores any uncertainty that arises from performing the sample preparation and will therefore probably underestimate the true 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 time of the standard deviation. The wavelength, method detection limit correlation coefficient and calibration curve equations are given in Table 5.

Table 5. The wavelength, method detection limit correlation coefficient and calibration curve equations.

Metal	Wavelength (nm)	IDL for (mg/kg)	MDL for (mg/kg)	MQL^2 (mg/kg)	Correlation coefficient	Calibration curve Equation
Ca	422.673	0.1	0.285	0.95	0.998	y = 66713c + 32373
Mg	279.553	0.1	0.364	1.21	0.999	y = 19334 c + 1884
Cu	327.395	0.1	0.21	0.699	0.999	y = 33562c+ 525.3
Zn	213.857	0.1	0.163	0.543	0.999	y = 4541c + 301.9
Mn	259.372	0.1	0.188	0.626	0.999	y = 2977c + 206.3
Ni	305.081	0.1	0.96	3.195	0.999	y = 4453c + 270.2
Fe	371.993	0.1	0.39	1.298	0.999	y = 5024c + 203.5
Cr	427.480	0.1	0.77	3.85	0.999	y = 21959c – 180.5
Cd	226.502	0.01	-	-	0.998	y = 660.3 c+ 118.9
Pb	368.346	0.01	-	-	0.999	y = 1722c + 6.594
Al	396.152	0.1	0.31	1.03	0.999	y = 24298c +753.3

Where c is the concentration, MDL = method detection limit, IDL = Instrument detection limit, MQL^2 = method quantification limit.

3.13 Checking of the validation of digestion procedure

The validity of the digestion procedures were assured by spiking or recovery experiment of the samples with a standard solution of known concentration of target analaytes. The need of this recovery experiment is because there is no any certified reference material for traditional toothbrush samples in the laboratory. For this purpose standard solution of 1000 mg/L of each analayte was used. Thus spiking was done for one of the three samples that is *Olea africana* branch sample in three 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 *Olea africana* sample. The spiked and non spiked samples were digested and analyzed in similar manner using the optimized procedure that is 4:1 (v/v) ratio of HNO_3 and $HClO_4$, respectively with 270 ^oC for 2:45 hours. As shown in Table 6 seven metals were analyzed in triplicate standard metal solutions to evaluate the efficiency of the procedure and the percentage recoveries lies within the range from 91 to 107% and were calculated using the following formula.

$$\%R = \frac{spiked\ sample - non - spiked\ sample}{amont\, added\ for\ spiking} x100\%$$

where, R = recovery.

Table 6. Analytical results for recovery test of *Olea 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

4. Results and Discussion

The concentrations of metals shown in Tables 7-9 were determined by using MP-AES and precision of the results was assessed as relative standard deviation. The relative standard deviations of the results are less or equal to 10% which is within the recommended range. But in the results of Ni in Kotebe sample %RSD values were slightly higher than 10, which may be caused due to contamination with other materials. Mean values were determined from triplicate analysis of each sample and triplicate samples were used for each sample site.

All the results obtained from the MP-AES that are expressed in terms of (mg/L) were converted into (mg/kg) using the following equation:
mg/kg = C x V x D/W where; C = concentration in mg/L, L = volume in liter, W = weight of samples in kilogram and D = dilution factor (considered as unity).

4.1. Concentration of metals in *Ligustrum vulgare* samples

Table 7. Concentration of metals (mg/kg, mean ± SD, n = 3) and %RSD in traditional toothbrush obtained from *Ligustrum vulgare*.

Metals	Kotebe		Arat Kilo		Ferensay	
	mean ± SD	%RSD	mean ± SD	%RSD	mean ± SD	%RSD
Zn	175 ± 11.9	6.8	134 ± 1.2	0.9	145 ± 2.4	1.6
Cr	8.9 ± 0.8	9	8.8 ± 0.1	1.7	6.3 ± 0.7	10
Cu	12.6 ± 0.4	2.9	10.4 ± 0.7	6.5	10.2 ± 0.8	7.9
Ni	4.8 ± 0.4	7.8	4.7 ± 0.5	10.9	3.9 ± 0.2	5.8
Mn	24.9 ± 1.2	4.9	26.7 ± 2.6	9.9	22.6 ± 0.6	2.6
Mg	102 ± 8	7.8	94 ± 2	2.1	89 ± 5	5.6
Al	143 ± 13.1	9.2	136 ± 12.0	8.8	126 ± 5.2	4.1
Ca	6143 ± 535	8.7	5619 ± 554	9.9	6061 ± 293	4.8
Fe	318 ± 34.4	10	235 ± 14.1	6	216 ± 8.2	3.8
Pb	BDL	BDL	BDL	BDL	BDL	BDL
Cd	BDL	BDL	BDL	BDL	BDL	BDL

Where, BDL is below detection limit.

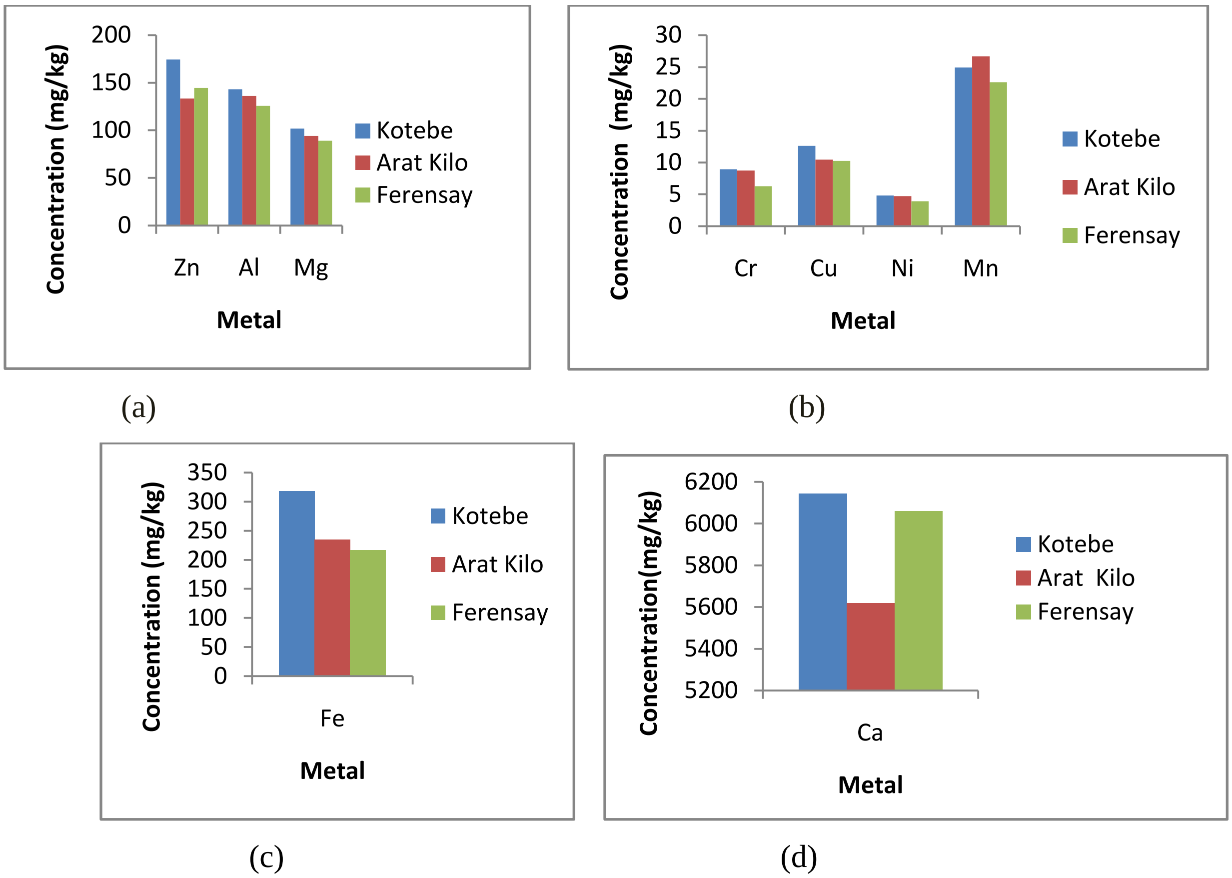


Figure 3. (a-d) bar graph of mean concentration of metals (mg/kg) in *Ligustrum vulgare* samples collected from three different sampling areas.

As shown in Table 7 and Figure 3 the maximum overall mean concentration levels in the *Ligustrum vulgare* samples 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. Similarly the minimum overall mean concentration levels in *Ligustrum vulgare* samples for 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. As shown in Table 7 the results of concentrations of metals showed that the *Ligustrum 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 on *Ligustrum vulgare* samples from other sample sites. But the concentrations of Mn in samples collected from Arat Kilo sample site is higher than samples collected Kotebe and Ferensay sample sites. The concentrations of Cd and Pb from

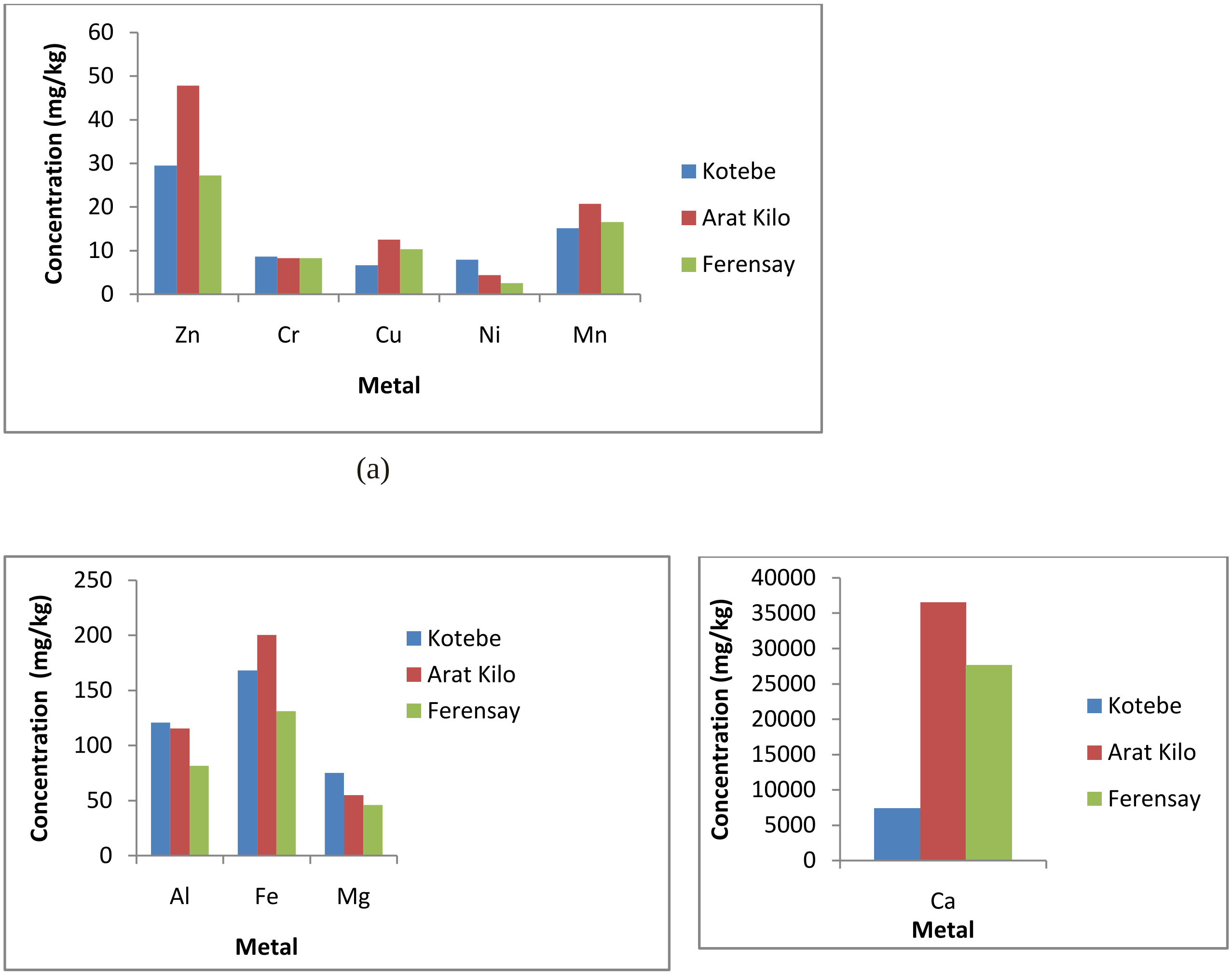
Ligustrum vulgare samples collected from three different sample sites are below detection limit. From the selected metals mean concentrations in *Ligustrum vulgare* samples from three different sampling sites, the concentrations Ca are higher than other metals mean concentrations and the mean concentrations of Ni are lowest as compared to other metals concentrations in each sample sites.

4.2. Concentration of metals in *Phoenix reclinata* samples

Table 8. Concentration of metals (mg/kg, mean ± SD, n = 3) and %RSD in traditional toothbrush from *Phoenix reclinata*.

Metals	Kotebe		Arat Kilo		Ferensay	
	mean ± SD	%RSD	mean ± SD	%RSD	mean ± SD	%RSD
Zn	29.5 ± 0.6	2.2	47.8 ± 2.4	5	27.2 ± 1.3	4.7
Cr	8.6 ± 0.1	1.7	8.3 ± 0.3	3.9	8.2 ± 0.7	8
Cu	6.6 ± 0.4	6.4	12.5 ± 0.5	4.2	10.3 ± 1.0	9.8
Ni	7.9 ± 1.1	13	4.4 ± 0.3	6.8	2.6 ± 0.2	9.6
Mn	15.1 ± 0.9	5.8	20.1 ± 1.1	5.6	16.6 ± 0.5	2.9
Mg	75 ± 1	1.3	55 ± 5	9.1	46 ± 4	8.7
Al	121 ± 3.1	2.6	115 ± 9.4	8.2	81.6 ± 0.7	0.87
Ca	7397 ± 593	8	36514 ± 2991	8.2	27676 ± 2800	10.1
Fe	168 ± 12	7.2	200 ± 6.9	3.4	131 ± 4.5	3.4
Pb	BDL	BDL	BDL	BDL	BDL	BDL
Cd	BDL	BDL	BDL	BDL	BDL	BDL

Where BDL is below detection limit.



(b) (c)

Figure 4. (a-c) bar graph of mean concentration of metals (mg/kg) in *Phoenix reclinata* samples collected from three different sampling areas.

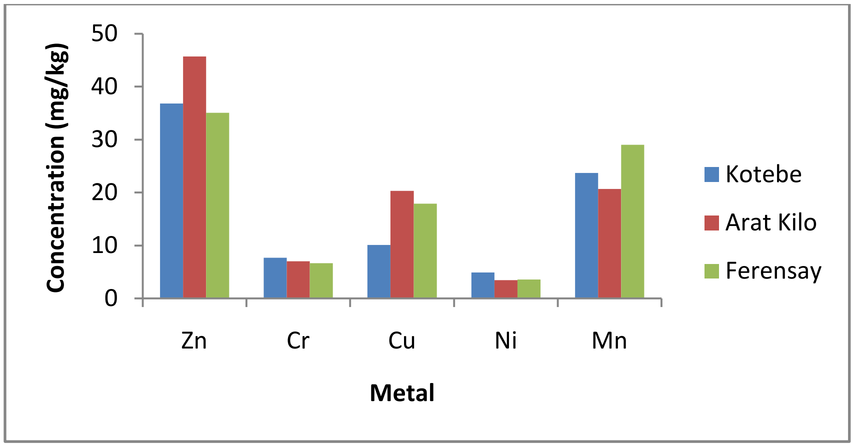
As shown in Table 8 and Figure 4 the maximum overall mean concentration levels in the *Phoenix reclinata* samples of elements Ca, Fe, Al, Mg, Zn, Mn, Cu, Cr, and Ni are 36514, 200, 121, 75, 47.8, 20.1, 12.5, 8.6, and 7.9 mg/kg, respectively. Similarly the minimum overall mean concentration levels in *Phoenix reclinata* samples 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. As shown Table 8 the results of concentrations of metals showed that the *Phoenix reclinata* samples collected from Arat Kilo site have higher amounts of Zn, Cu, Mn, Ca, and Fe compared to the concentration of those metals on *Phoenix reclinata* samples from other sample sites. But the concentrations of Cr, Ni, Mg, and Al in *Phoenix 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 *Phoenix reclinata* samples collected from three different sample sites are below detection limit. From the selected metals mean concentrations in *Ligustrum vulgare* samples from three different sample sites, the concentrations Ca are highest than other metals mean concentrations and the mean concentrations of Ni are lowest as compared to other metals concentrations in each sample sites.

4.3. Concentration of metals in *Olea afiricana* samples

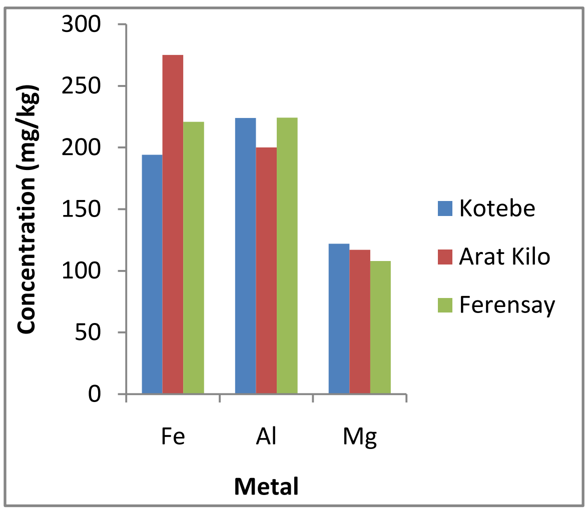
Table 9. Concentration of metals (mg/kg), (mean ± SD, n = 3) and %RSD in traditional toothbrush from *Olea afiricana*.

Metals	Kotebe		Arat Kilo		Ferensay	
	mean ± SD	%RSD	mean ± SD	%RSD	mean ± SD	%RSD
Zn	36.8 ± 1.2	3.4	45.7 ± 4.1	9	35.1 ± 0.1	0.4
Cr	7.66 ± 0.5	5.9	7.0 ± 0.5	6.9	6.7 ± 0.1	1.8
Cu	10.1 ± 0.9	8.9	20.3 ± 0.6	3	17.9 ± 1.7	9.5
Ni	4.9 ±1.1	8.1	3.5 ± 0.2	6.6	3.6 ±0.2	6.9
Mn	23.7 ± 1	7.4	20.7 ± 0.5	2.4	29.0 ± 1.1	3.6
Mg	122 ± 4	3.3	117 ± 4	3.4	108 ± 8	7.4
Al	224 ± 12.1	5.4	200 ± 6.1	3	224 ± 5.2	2.3
Ca	4267 ± 368	8.6	23414 ± 699	3	28178 ± 2142	7.6
Fe	194 ± 14	9.1	275 ± 9.9	3.6	221 ± 19.9	9
Pb	BDL	BDL	BDL	BDL	BDL	BDL
Cd	BDL	BDL	BDL	BDL	BDL	BDL

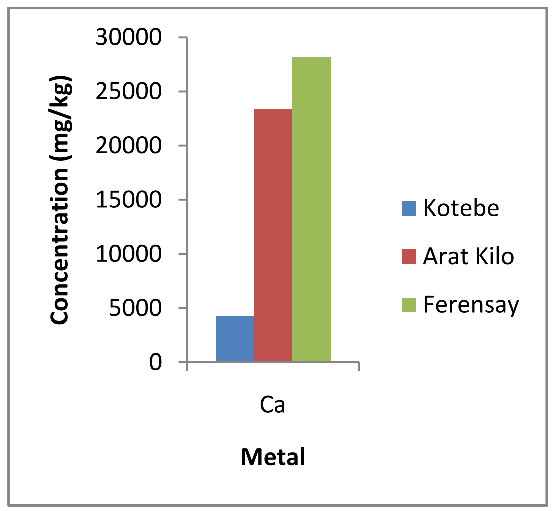
Where BDL is below detection limit.



(a)



(b)



(c)

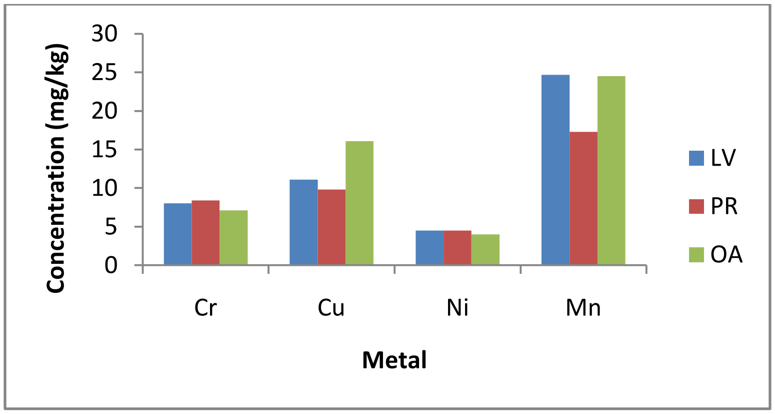
Figure 5. (a-c) bar graph of mean concentration of metals (mg/kg) in *Olea afiricana* samples collected from three different sampling areas.

As shown in Table 9 and Figure 5 the maximum overall mean concentration levels in the *Olea afiricana* samples of elements 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. Similarly the minimum overall mean concentration levels in *Olea africana* samples for elements Ca 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. As shown in Table 9 the results of concentrations of metals showed that the *Olea africana* samples collected from Ferensay site have higher amounts of Mn, Al, and Ca, and *Olea africana* samples collected from Arat kilo site have higher amounts of Fe, Zn, Cu, and *Olea africana* samples collected from Kotebe site have higher amounts of Cr, Ni, and Mg as compared to the concentration of those metals on *Olea africana* samples from other sample sites. But the concentrations of Cd and Pb from *Olea africana* samples collected from three different sample sites are below detection limit. From the selected metals mean concentrations in *Olea africana* samples from three different sample sites, the concentrations Ca are highest compared to other metals mean concentrations and the mean concentrations of Ni are lowest as compared to other metals concentrations in each sample sites.

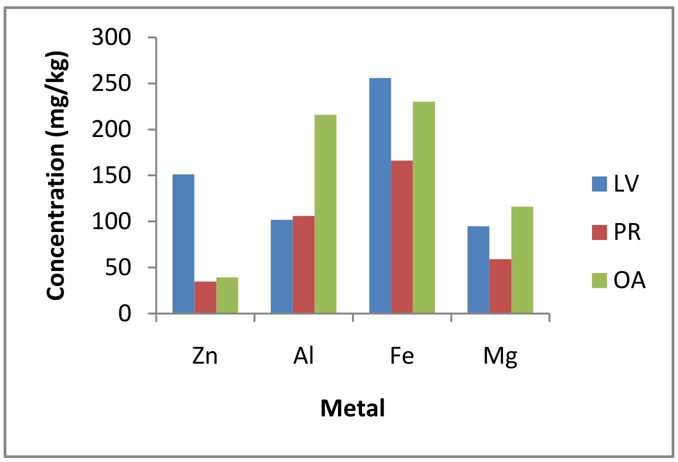
4.4. Comparison of average metal concentrations (mg/kg) in *Ligustrum vulgare*, *Phoenix reclinata*, and *Olea africana* plant branch samples

Table 10. Comparison of average metal concentrations (mg/kg) of *Ligustrum vulgare*, *Phoenix reclinata*, and *Olea africana* plant branch samples.

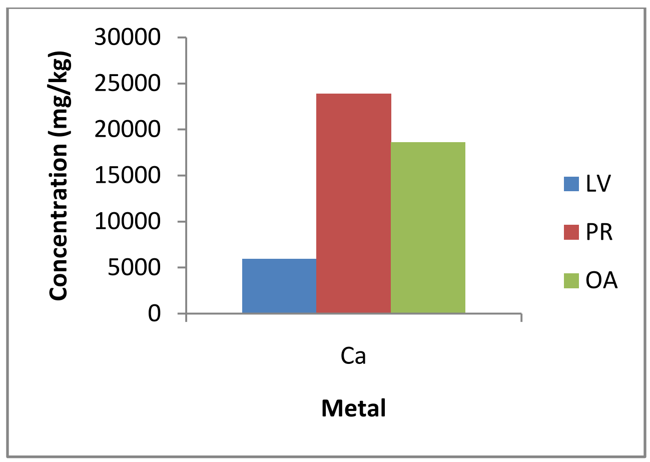
metals	*Ligustrum vulgare*	*Phoenix reclinata*	*Olea Africana*
	Mean of the mean	Mean of the mean	Mean of the mean
Zn	151	34.8	39.2
Cr	8.0	8.4	7.12
Cu	11.1	9.8	16.1
Ni	4.5	4.5	4
Mn	24.7	17.3	24.5
Mg	95	59	116
Al	102	106	216
Ca	5941	23862	18620
Fe	256	166	230



(a)



(b)



(c)

Where LV = *Ligustrum vulgare*, PR = *Phoenix reclinata*, OA = *Olea africana*

Figure 6. (a-c) comparison of average metals concentration (A) Cr, Cu, Ni, and Mn and (B) Zn, Mg, Al, and Fe and (C) Ca in *Ligustrum vulgare*, *Phoenix reclinata*, and *Olea africana* plant branch samples.

The results of this study showed that the metal contents of traditional toothbrush plant branches varied between the traditional toothbrush plant branches varieties for some metals (Table 10 and Figure 6). This variation may be genetic factors. The highest mean concentration of Ca (23862 mg/kg) was observed in *Phoenix reclinata* plant branch samples as compared to mean concentration of Ca (5941 mg/kg, 18620 mg/kg) in *Ligustrum vulgare* and *Olea africana* plant samples, respectively, and relatively the mean concentration of Ni was the lowest in the three traditional toothbrush samples as compared to the mean concentrations of other selected metals in the three traditional toothbrush samples. The highest mean concentration of Ca, Fe, Al, Mg and Zn are observed in the three traditional toothbrush samples and relatively lower amounts Cr, Cu, Ni and Mn are observed compared to mean concentration of Ca, Fe, Al, Mg and Zn.

4.5. Comparison of metals concentration (mg/kg, dry mass) of the three traditional toothbrush plants branch with other medicinal plants branch metals concentrations reported in literature

Table 11. Comparison of metals concentration (mg/kg, dry mass) of the three traditional toothbrush plants branch with other medicinal plants metals concentrations reported in the literature.

Plant types	Plant parts	Country	Method	Metals					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	Gebre, and Chandravanshi, 2012
Zingiber Officinale	Root	Ethiopia	FAAS	BDL	0.38-0.97	1.1-4.78	38.5-55.2	41.8-89	Wagesho and Chandravanshi, 2015
Phoenix dactylifera	Fruit		ICP AES	0.78 -5.83	0.04-0.18	0.92-1.53	0.23-0.95	120-315	Taha and Al Ghtani, 2015
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	Metals						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	Gebre, and Chandravanshi, 2012
Zingiber Officinal	Stem	Ethiopia	FAAS	6.02-10.8	200-2543	2700-4094	184-401	5.46-8.4	ND	Wagesho and Chandravanshi, 2015
Phoenix dactylifera	Fruit	Saudi	ICP AES	ND	110-200	ND	31.8-89.3	8-11.75	13.6-74.9	Taha and Al Ghtani, 2015
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

Where ND = not detected , BDL = below detection limit .

As shown in Table 11, the Ca contents determined in the three traditional toothbrush by MP-AES are higher than Ca contents in other medicinal plants from *Rhamnus Prinoides* (Afewerk Gebre 2012) and *Zingiber Officinal* (Yohannes Wagesho 2015). The Mg contents determined in this study is lower compared with Mg contents determined from other medicinal plants like *Rhamnus Prinoides* and *Zingiber Officinal* reported by Afewerk Gebre (2012) and Yohannes Wagesho (2015) by FAAS, the concentrations of Fe and Al in this study are higher than concentrations of Fe and Al from other medicinal plants reported but the contents of Pb and Cd in this study were below detection limit, the concentrations of other metals such as Ni, Cr, Zn and Cu in this study and the reported value from the medicinal plants are closed to each other.

4.6. Comparison of metals concentration (mg/kg) of the three traditional toothbrush plants branch with toothpastes metals concentrations reported in the literature

Table 12. Comparison of metals concentration (mg/kg) of the three traditional toothbrush plants branch with other toothpastes metals concentrations reported in the literature.

Type of toothpaste	Country	Method	Metals (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	Vella and Attard, 2019
Local toothpastes manufactured in Nigeria	Nigeria	FAAS	0.001-1.284	0.001 – 5.97	ND	5.472-18.63	4.514 -23.575	Oriskwe, *et al.*, 2016
Toothpastes imported to Nigeria	Nigeria	FAAS	0.001-2.49	0.001-10.85	ND	8.975-18.535	6.329-18.092	Oriskwe *et al.*, 2016
Commercial toothpastes	India	AAS	<0.031	-	-	-	0.84-1.58	Rao and Rao, 2014
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	Metals			Reference
			Fe	Mn	Zn	
Commercial toothpastes	Malta	MP-AES	1.76-17.7	0.2-2.02	0.00-2417	Vella and Attard, 2019
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

As shown Table 12 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 the commercial toothpastes is closely comparable to this study, the concentrations of Fe, Cu and Mn in this study are higher as compared to concentrations in commercial toothpastes but the concentrations of Cr in the local toothpastes used in Nigeria and toothpastes imported to Nigeria are comparable to 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 in this study was below detection limit, which is preferable.

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

Table 13. Comparison of heavy metal contents (mg/kg) in the traditional toothbrushes samples with available international standards in cosmetics and toothpastes.

International standards	Pb	Cd	Ni	Cr	Reference
European Union	0.1	0.05	-	-	EU, 2006
World Health Organization	2	2	-	-	FAO/WHO, 2001
Canada	10	3	-	-	Chan, 2012
Germany	0.5	0.1	-	-	Whitehouse, 2017
US Food and Drug Administration	10	-	-	50	US FDA, 2018
Ethiopian traditional toothbrushes	BDL	BDL	2.6 – 7.9	6.3 – 8.6	This study

Where BDL is below detection limit

As shown in Table 13 the maximum heavy metal contents in Ethiopian traditional toothbrush samples are compared with available international standards in toothpastes. The Pb, Ni, Cd and Cr contents in Ethiopian traditional toothbrush samples are within the range for and hence safe for human use.

4.8. Daily intake of metals from traditional toothbrush

Daily intake of metals from the three traditional toothbrushes has been calculated based on the assumption that an average traditional toothbrush exposed to mouth is about 5 g per day. The amounts of mineral intake by the traditional toothbrush users (three times per day from the three different types of plant branches) are given in Table 14.

Table 14. Comparison of daily intake of metals from traditional toothbrushes with recommended daily intake and tolerable upper limit of daily intake of metals (Joint FAO/WHO 2011; Food and nutrition board USA, 1997).

Metal	Concentrations (mg/kg) in			Amount of metals per 5 g LV,PR and OA each consumed			Recommended daily intake	Tolerable upper limit
	LV	PR	OA	LV	PR	OA		
Zn	134-175	27.2-47.8	35.1-45.7	0.67-0.875	0.136-0.239	0.176-0.229	10-15 mg	40 mg/day
Cr	6.3-8.9	8.2-8.6	6.7-7.66	0.032-0.0445	0.041-0.043	0.034-0.038	25-35 µg	120 µg/day
Cu	10.2-12.6	6.6-12.5	10.1-17.9	0.051-0.063	0.033-0.063	0.051-0.09	0.9-2 mg	10 mg
Ni	3.9-4.8	2.6-7.9	3.5-4.9	0.02-0.024	0.013-0.040	0.018-0.025	70-170 µg	1 mg/ Day
Mn	22.6-26.7	15.1-20.1	20.7-29	0.113-0.134	0.076-0.101	0.104-0.145	1.8-2.3 mg	11 mg/day
Mg	89-102	46-75	108-122	0.45-0.51	0.23-0.38	0.54-0.61	320-420 mg	750 mg/day
Al	126 - 143	81.6 -121	200-224	0.630-0.715	0.408-0.605	1.0-1.12	NA	NA
Ca	5619-6143	7397- 36514	4267-28178	28.1-30.7	37-183	21.3-141	1000-1200 mg	2500 mg
Fe	216-318	131-200	194-275	1.08-1.59	0.655-1.0	0.97-1.375	10-15 mg	45 mg/day
Pb	BLD	BLD	BLD	_	_	_	0.02 3 µg/kg body wt	25 µg/kg body wt/week
Cd	BLD	BLD	BLD	_	_	_	ND	7 µg/kg body wt/week

Where NA = data not available, ND = not detected, BDL = below detection limit, LV = *Ligustrum vulgare*, PR = *Phoenix reclinata* and OA = *Olea africana*.

The amount of all the metals determined in this study are safe (not harmful) for a person who uses these three traditional toothbrushes, means amounts of all the metals are below the recommended daily intake values for human.

4.9. Statistical analysis

The analysis of variances (ANOVA) and the relationships (correlation) among metals were assessed by Pearson correlation methods and the graphical expression was done using Microsoft excel 7 in addition to arrangements of different data sets.

4.10. Analysis of variance (ANOVA)

Analysis of variance (ANOVA) is a parametric statistical technique used to compare data sets. Analysis of variance (ANOVA) is a statistical method used to test differences between two or more means. When we are comparing more than three groups based on one factor variable, then it said to be one way analysis of variance. ANOVA use the F statistic to compare whether the difference between sample means is significant or not (Hepp *et al.*, 2014). If the calculated value of F (the ratio of SD between samples to SD within samples) is greater than $F_{critical}$ (the value obtained from the table at specified confidence level and degree of freedom), in sample means are significant.

In this study, traditional toothbrush samples were collected from three different areas and the metal levels of each sample was 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).

Table 15. Analysis of variance (ANOVA) between and within *Ligustrum vulgare* samples at 95% confidence level.

Metal	$F_{Calculated}$	$F_{Critical}$	p-value	Remark
Zn	269.5	5.14	1.33×10^{-6}	Significant difference among the sample means
Cr	13.8	5.14	0.0057	Significant difference among the sample means
Cu	180.8	5.14	4.36×10^{-6}	Significant difference among the sample means
Ni	61.3	5.14	0.000102	Significant difference among the sample means
Mn	4.2	5.14	0.07	No significant difference among the sample means
Mg	12.96	5.14	0.0063	Significant difference among the sample means
Al	2.06	5.14	0.208	No significant difference among the sample means
Ca	1.055	5.14	0.405	No significant difference among the sample means
Fe	70	5.14	6.67×10^{-5}	Significant difference among the sample means

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

Table 16. Analysis of variance (ANOVA) between and within *Phoenix reclinata* samples at 95% confidence level.

Metals	$F_{Calculated}$	$F_{Critical}$	p-value	Remark
Zn	113.8	5.14	1.7×10^{-5}	Significant difference among the sample means
Cr	0.15	5.14	0.86	No Significant difference among the sample means
Cu	103.4	5.14	2.24×10^{-5}	Significant difference among the sample means
Ni	41.47	5.14	0.000307	Significant difference among the sample means
Mn	121.3	5.14	1.4×10^{-5}	Significant difference among the sample means
Mg	51.5	5.14	0.000167	Significant difference among the sample means
Al	540.9	5.14	1.689×10^{-7}	Significant difference among the sample means
Ca	117.0	5.14	1.56×10^{-5}	Significant difference among the sample means
Fe	250.1	5.14	1.67×10^{-6}	Significant difference among the sample means

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

Table 17. Analysis of variance (ANOVA) between and within *Olea africana* samples at 95% confidence level**.**

Metals	$F_{Calculated}$	$F_{Critical}$	p-value	Remark
Zn	23.4	5.14	0.0015	Significant difference among the sample means
Cr	61	5.14	9.99×10^{-5}	Significant difference among the sample means
Cu	81	5.14	4.45×10^{-5}	Significant difference among the sample means
Ni	4.12	5.14	0.075	No significant difference among the sample means
Mn	222.28	5.14	2.36×10^{-6}	Significant difference among the sample means
Mg	4.86	5.14	0.0555	No significant difference among the sample means
Al	143.5	5.14	8.59×10^{-6}	Significant difference among the sample means
Ca	439.2	5.14	3.11×10^{-7}	Significant difference among the sample means
Fe	74.32	5.14	5.84×10^{-5}	Significant difference among the sample means

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

Table 18. Analysis of variance (ANOVA) between and within Ligustrum vulgare, Phoenix reclinata and Olea africana samples at 95% confidence level**.**

Metals	$F_{Calculated}$	$F_{Critical}$	p-value	Remark
Zn	62.8	5.14	$9.48x10^{-5}$	Significant difference among the sample means
Cr	1.505	5.14	0.295	No significant difference among the sample means
Cu	2.5587	5.14	0.1574	No significant difference among the sample means
Ni	0.259	5.14	0.7799	No significant difference among the sample means
Mn	5.67	5.14	0.041	Significant difference among the sample means
Mg	23.89	5.14	0.001388	Significant difference among the sample means
Al	40.95	5.14	0.00032	Significant difference among the sample means
Ca	1.99	5.14	0.2167	No significant difference among the sample means
Fe	3.266	5.14	0.1097	No significant difference among the sample means

Results of variance analysis showed that there were significant differences ($p < 0.05$) in the mean values of for Zn, Mg, Al and Mn between *Ligustrum vulgare, Phoenix reclinata* and *Olea africana* samples whereas, there was no significant difference ($p > 0.05$) in the mean values of Ca, Cr, Cu, Fe and Ni between the three samples.

4.11. Pearson correlation of metals

The Pearson correlation coefficient (r) is used to measure the strength of linear association between two variables (e.g. concentration of metal A and B). Where the value $r = 1$ means a perfect positive correlation. A positive value indicates large values of A tend to be associated with large values of B and small value of A tend to be associated with small values of B and the value $r = -1$ means a perfect negative correlation. A negative value indicates large values of A tend to be associated with small values of B and small values of A tend to be associated with large values of B. In this study, to correlate the effect of one metal concentration on the concentration of the other metal, the Pearson correlation matrices using correlation coefficient (r) for the samples were used and presented in Tables 17-19. 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 *Ligustrum vulgare*, *Phoenix reclinata* and *Olea africana* samples and are summarized in Table 19-21, respectively.

Table 19. Pearson correlation coefficients between metals concentrations in *Ligustrum vulgare* plant branch samples.

	Zn	Cr	Cu	Ni	Mn	Mg	Al	Ca	Fe
Zn	1.0000								
Cr	0.2911	1.0000							
Cu	0.9436	0.5913	1.0000						
Ni	0.3550	0.9977	0.6444	1.0000					
Mn	-0.1907	0.8836	0.1449	0.8500	1.0000				
Mg	0.8069	0.8000	0.9569	0.8387	0.4259	1.0000			
Al	0.6315	0.9256	0.8525	0.9491	0.6407	0.9676	1.0000		
Ca	0.8027	-0.3369	0.5600	-0.2756	-0.7386	0.2954	0.0444	1.0000	
Fe	0.9055	0.6695	0.9949	0.7182	0.2438	0.9813	0.9008	0.4737	1.0000

According to Table 19 the correlation coefficients between metals in *Ligustrum vulgare* plants branch 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.

Table 20. Pearson correlation coefficients between metals concentrations in Phoenix reclinata plant branch samples**.**

	Zn	Cr	Cu	Ni	Mn	Mg	Al	Ca	Fe
Zn	1.0000								
Cr	-0.1780	1.0000							
Cu	0.7169	-0.8136	1.0000						
Ni	-0.0810	0.99SS52	-0.7529	1.0000					
Mn	0.9216	-0.5461	0.9313	-0.4616	1.0000				
Mg	-0.1056	0.9973	-0.7689	0.9997	-0.4833	1.0000			
Al	0.4652	0.7882	-0.2836	0.8446	0.0851	0.8312	1.0000		
Ca	0.9751	-0.3918	0.8537	-0.3000	0.9847	-0.3235	0.2573	1.0000	
Fe	0.8946	0.2806	0.3298	0.3731	0.6509	0.3500	0.8118	0.7732	1.0000

According to Table 20 the correlation coefficients between metals in *Phoenix reclinata* plants branch 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.

Table 21. Pearson correlation coefficients between metals concentrations in Olea africana plant branch samples.

	Zn	Cr	Cu	Ni	Mn	Mg	Al	Ca	Fe
Zn	1.0000								
Cr	-0.0633	1.0000							
Cu	0.5652	-0.8591	1.0000						
Ni	-0.6090	0.8302	-0.9985	1.0000					
Mn	-0.8616	-0.4520	-0.0682	0.1221	1.0000				
Mg	0.3082	0.9299	-0.6106	0.5669	-0.7485	1.0000			
Al	-0.9888	0.2116	-0.6821	0.7206	0.7762	0.1628	1.0000		
Ca	-0.7461	-0.6173	0.1276	-0.0738	0.9808	0.8634	0.6383	1.0000	
Fe	0.8854	-0.5199	0.8839	-0.9078	-0.5270	0.1692	-0.9449	0.3512	1.0000

According to Table 21 the correlation coefficients between metals in *Olea africana* plants branch 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.

Table 22. Pearson correlation coefficients between metals average concentrations in *Ligustrum vulgar*; *Phoenix reclinata* and *Olea africana* plant branch samples**.**

	Zn	Cr	Cu	Ni	Mn	Mg	Al	Ca	Fe
Zn	1.0000								
Cr	0.1788	1.0000							
Cu	-0.2893	-0.9935	1.0000						
Ni	0.4708	0.9522	-0.9807	1.0000					
Mn	0.5486	-0.7245	0.6416	0.4793	1.0000				
Mg	0.1832	-0.9345	0.8881	0.7811	0.9224	1.0000			
Al	-0.4978	-0.9423	0.9742	0.9995	0.4520	0.7614	1.0000		
Ca	-0.9677	0.0752	0.0385	-0.2330	-0.7418	-0.4252	0.2630	1.0000	
Fe	0.7456	-0.5223	0.4221	0.2368	0.9662	0.7916	0.2067	0.8896	1.0000

Table 22 shows the correlation coefficients between metals in *Ligustrum vulgare*, *Phoenix reclinata* and *Olea 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.

5. Conclusion

An efficient digestion procedure for the determination of essential and non essential metals content in traditional toothbrush samples such as *Ligustrum vulgare*, *Phoenix reclinata* and *Olea africana* was optimized and validated through spiking method and a good percentage recovery was obtained for the metals of interest. The mean concentration levels of the metals in the three types of traditional toothbrush samples collected from the three sampling areas indicated that higher concentrations of Ca, Fe and Al were found in the three samples collected from Kotebe, Arat Kilo, and Ferensay relatively from other selected metal concentrations and the concentrations of Cr and Ni in *Ligustrum vulgare*, *Phoenix reclinata* and *Olea africana* were minimum. 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 *Phoenix reclinata* and *Olea africana* were much higher as compared to the concentrations of Ca in *Ligustrum vulgare*, and the concentrations of Zn in *Ligustrum vulgare* was higher than the concentrations of Zn in *Phoenix reclinata* and *Olea africana*, the concentrations of Mg in *Ligustrum vulgare* and *Olea asfricana* were higher than the concentrations of Mg in *Phoenix reclinata.* But the concentrations of other metals were almost comparable.

The toxic metals Pb and Cd were not detected in any of three traditional toothbrushes studied. Therefore, the three traditional toothbrushes are safe for human use.

The present study gives information about the essential and non essential metal contents of commonly used three traditional toothbrush plants from the selected areas of Addis Ababa. But to have complete and general information further research should focus on these and other traditional toothbrush plants from another parts of Addis Ababa and Ethiopia and the mineral status of the soil and the water used for irrigation or other sources of water and soil used for tradition toothbrush plants during the growth of the plant conducted are recommended for further study.

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