ADDIS ABABA UNIVERSITY

College of Natural and Computational Sciences

Department of Chemistry

Determination of selected essential and non essential metals in apple fruit cultivated from three different places in Ethiopia and from commercial market in Addis Ababa.

By Demisew Jemaneh

Advisor: Prof. B.S. Chandravanshi

February, 2020

Declaration

I, the undersigned, declare that this thesis in title “Determination of selected essential and non essential metals in apple fruit cultivated from three different places in Ethiopia and from commercial market in Addis Ababa” is my original work and has been submitted in partial fulfillment of the requirements for masters of Science in chemistry at Addis Ababa University.

All sources of materials used for this thesis have been duly acknowledged. This paper has never been submitted to and/or presented in any other university, college or institution.

Name: ____________________________

Signature: _____________________

Date: ________________

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By

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Approved by

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Acknowledgments

First and foremost, I want to thank God for helping me during my work and giving me the strength to do this thesis work.

Next, I would like to express my sincere gratitude to my advisor professor B.S. Chandravanshi for the continuous support of MSc study and research for his motivation, enthusiasm and immense knowledge. His guidance helped me during the time of research and writing of this thesis.

I would also like to express my deepest gratitude to Addis Ababa Educational Bureau for allowing me to carry out my higher education and providing sponsorship.

I would also like to thank Addis Ababa University for its provision of all resources necessary to accomplish my study from the very beginning to the end and I would like say thank you to the staff members of Chemistry Department.

Finally I would like to thank my family for their support and inspiration and also for all those directly or indirectly helped me to complete this paper.

Contents

List of Tables

List of Figures

List of Abbreviations and Acronyms

Abbreviation	Full form
ARA	Arbaminch red apple
AGA	Ankober green apple
EDTA	Ethylene demine tetra acetic acid
IRA	Israel red apple
LOD	Limit of detection
LOQ	Limit of quantification
MDL	Method detection limit
MP-AES	Microwave plasma-atomic emission spectroscopy
RDA	Recommended dietary allowance
RSD	Relative standard deviation
SAG	South Africa green apple
SD	Standard deviation
WGA	Wollega green apple
WRA	Wollega red apple
WHO/FAO	World Health Organization/Food and Agriculture Organization

Abstract

The aim of this study was to evaluate the levels of macro, micro essential elements and non essential elements in apple fruit samples collected from farmland in Wollega, Chencha, Ankober and from market in Addis Ababa which are imported to Ethiopia from South Africa and Israel. Levels of selected metals (Mg, Ca, Fe, Mn, Cu, Al, Zn, Cr, Co, Ni, Cd and Pb were determined by microwave plasma-atomic emission spectroscopy. Known weight of oven dried apple fruit samples were wet-digested using 3 mL HNO_3 and 1 mL $HClO_4$ for 2:15 hours and at temperature 210 ^{o} C. The validity of optimized procedure was evaluated by the analysis of spiked samples whose recoveries was 90.8-106%. The mean concentration range (mg/kg) of each metal in green apple and red apple fruit sample were Ca (1065-36275, 1013-36143), Mg (27-153, 78.3-139), Fe (103-276, 123-241), Mn (13.5-13.7, 11.5-16.3), Cu (5.13-6.56, 4.11-12.6), Al (77.8-129, 52.5-89.6), Zn (41.3-73.6, 47.4-54.4), Cr (6.15-10.04, 6.57-7.14), Co (6.57-7.14, 1.70-2.35), Ni (1.33-2.37, 1.43-7.66), respectively. Cd and Pb were not detected.

Green apple fruit has no significant difference ($p \geq 0.05$) for Mn and Cu. All the other metals have significance difference. Their p-value was $p \leq 0.05$. Red apple has no significance difference for Mn, Cu, Zn, Cr and Co ($p \geq 0.05$). The other metals have significance differences.

Pearson correlation coefficients of metals from green apple between Ca/Mg, Ca/Fe, Ca/Al, Ca/Zn, Ca/Cr, Ca/Ni, Mg/Fe, Mg/Al, Mg/Cr, Mg/Ni, Fe/Mn, Fe/Al, Fe/Zn, Fe/Co, Mn/Cu, Cu/Co, Al/Ni, Zn/Cr, Zn/Ni and Cr/Ni are in very strong correlation relationship. Green apple fruit sample showed strong, moderate and weak correlation. Ca/Cr, Mg/Fe, Mg/Zn, Mg/Ni, Fe/Zn, Fe/Ni, Mn/Cu, Mn/Al, Cu/Al, Zn/Ni have strong positive correlation relationships.Ca/Zn, Mg/Mn, Mg/Cu, Mg/Al, Fe/Mn, Fe/Cu, Fe/Al, Mn/Zn, Cu/Co, Cu/Ni, Al/Co, Al/Ni, Zn/Co have strong negative correlation relationships.

Keywords: Apple fruit, Digestion, Optimization, Microwave plasma-atomic emission spectroscopy, Essential metals, Non-essential metals

1. Introduction

1.1. Background of the study

Apple tree (*Malus pumila*) is a member of Rosaceae family. It is a deciduous tree (it loses its leaves seasonally). The trees can reach a height of 25-35 feet (7.5-10 m) or even more. Semi dwarf and dwarf trees reach a height of 6-20 feet (2-6 m). The blossoms are produced in spring. The fruits mature on shoots that are 2 or more years old. They mature in late summer or autumn depending on the variety. Apple is a king of all fruits. The fruit was originated in the Middle East just about 4000 years ago. It is one of the most favorite and popular fruits ever known. It is a sweet, edible fruit produced by an apple tree (*Malus domestica*). Apple trees are cultivated worldwide and are the most widely grown species in the genus *Malus*. The picture of apple tree and fruits are shown in Figure 1.

Figure 1. Picture of apple fruit and apple plant.

Fruit juices are essential part of modern diet in almost every society. Fruits and fruits product are known to be good source of nutrients such as minerals and vitamins (Nahar *et al.*, 1990). Fruit juices are available in most part of the world in bottles, cans, cups, laminated paper packs and almost every other form of packing in the diet, of most people, irrespective of age included significantly thus, it contribute to good health (Tasnim *et al.*, 2010). In countries having hot climate means that the intake of liquid must be high to compensate for the expected losses from respiration (Al Jedah and Robinson, 2002).

Fruit juices deserve special attention because of their important influence on human health. Intake of fruit juices has been constantly related with reduced risk of many cancer types (Brock *et al.*, 1988) and might be protective against stroke (Feldman, 2001) and hold up the beginning of Alzheimer's disease (Dai *et al.*, 2006). The widespread and growing intake of apples and apple juice/products and their rich phytochemical profile suggest their important potential to affect the health of the populations consuming them.

Apples are extremely rich in important antioxidants, flavonoids and dietary fiber. The phytonutrients and antioxidants in apples may help reduce the risk of developing cancer, improve neurological health, prevent dementia, reduces the risk of stroke, lowering levels of bad cholesterol, reducing risk of diabetes. Apple also contain pectin which helps bulk up to the stool to treat diarrhea and constipation and also contain some chemicals that seem to be able to kill bacteria, reduce swelling in the body and kill the cancer cell. Apple trees are large if grown from seed. Apple cultivators are propagated by grafting onto rootstock, which control the size of the resulting tree. There are more than 7,500 known cultivators of apple, resulting in a range of desired characteristics. Different cultivars are bred for various tastes and use, including cooking, eating raw and cider production. Trees and fruit are prone to a number of fungal, bacterial and pest problems, which can be controlled by a number of organic and non-organic means.

Apples are very vulnerable to the changing weather conditions, so special care has to be taken. The crucial part is not to grow apple trees but is the maintenance and sustainability of these trees until it reaches maturity. There are many factors why fertilizing apple trees are needed such as the soil pH and the presence of important elements and nutrients, nitrogen, phosphorus and potassium this has to be timely checked. NPK is the short form for nitrogen (N), phosphorus (P) and potassium (K) which are three most important elements in fertilizing apple trees in order to survive from the start to the end.

There are other important elements in fertilizing apple trees are sulfur, magnesium, calcium, boron, iron, zinc, copper and manganese which help in bringing the apple trees in its fullest potential. It is also highly recommended that the orchardist must purchase pre-packed fertilizers in fertilizing apple trees with their own fertilizers in order for the sufficient amounts to be distributed equally. In fertilizing apple trees, it is not only the nutrients are important, but also the amounts and proportions to be given. The distribution of the nutrients in appropriate proportions is a pivotal part in the successful growth of apple trees.

1.2. Statement of the problem

In Ethiopia consumption of apple is increasing from time to time. As a tradition in Ethiopia people feed fruits without knowing the permissible limit of metals in apple. Metals like cadmium, lead and arsenic have a toxic property even kill a person and some other metal also cause a disease when they are above permissible limit. Consequently there is an information gap about knowing content of metals in apple and maximum permissible limit for feeding of apple. To fill this gap this research were undertaken.

1.3. Objective of the study

1.3.1. General objective

The main objective of this study was to know the levels of macro (Mg and Ca), micro (Zn, Fe, Cu, Mn, Co, Cr, Ni) and toxic (Pb and Cd) metals in apple fruits from the plants cultivated in three selected area of Ethiopia and commercially available apple fruit in the market of Addis Ababa.

1.3.2. Specific objectives

- To develop reliable digestion procedure for the determination of essential and non-essential metals in apples fruit.
- To determine the metal contents in apple fruits from three selected area of Ethiopia.
- To compare the levels of metals in apple fruits of this study with WHO/FAO maximum permissible level.

1.4. Significance of the study

The importance of this study is to determine the presence of heavy metals in apple fruits that are daily consumed as fresh fruits or used for further processing. It is very important due to the possibility of permanent accumulation of heavy metals in the human organs and possible death. By estimating the heavy metals intake and metal content in apple fruit, it is possible to introduce continuous monitoring, which is important for human healthy.

All trace elements are toxic if consumed at sufficiently high levels for long enough periods. To adjust the daily intake of apple fruit this study is used.

2. Literature Review

2.1. Selected variety of apple

There are many variety of apples found across the world. In fact there are more than 7500 varieties of apples grown around the world. Some of the most popular varieties of apples include red delicious, Macintosh, Golden delicious and Fuji. But even though there are thousands of different varieties of apples they appear mainly in four different colors namely red, pink, yellow and green. The red color of an apple is due to the presence of chemical called anthocyanin.

Figure 2. Picture of red and green apple variety.

2.2.1. Green apple

Green apples have low sugar and calorie content to make them strong contender in the list of healthy super foods. From its benefits one is increasing the platelet count and weight management because of its antioxidant rich content and anti inflammatory nature (Kent, 2017).

2.2.2. Red apple

This apple contains the flavonoids pigment anthocyanins which gives it its red color. Red apples are more sweet and popular than green apples. Red delicious and Fuji are common examples of red apples (Kent, 2017).

2.3. Difference between green apple and red apple

Green apples have mostly sour taste, but red apples have sweeter taste. Green apple may contain slightly more fiber and less carbohydrates and sugar than red apples. By oxidants red apples is more preferable, because of it is rich in antioxidants, pectin, quercetin and flavonoids to protect cell from oxidative damage. The macronutrient balance of green apple is healthier than red apple.

2.4. Climate for apple growth

Apple tree varieties thrive in cold and wet climate where a cold winter is followed by cool spring and summer. Abundant sunlight is necessary as it significantly affects the color of the fruit. It succeeds best in regions where the trees experience uninterrupted rest in winter and abundant sunshine for good color development. It can be grown at an altitude of 1500-2700 m above the sea level. Well-distributed rainfall of 1000-1250 mm throughout the growing season is most favorable for optimum growth and fruitfulness of apple trees. The apple tree has higher needs in cold than most of other deciduous fruit trees. Most popular apple tree varieties thrive in regions where the temperature rarely increases above 32 ^{o}C.

2.5. The chemistry of soil for apple

Apples trees can grow in a wide range of soils from medium textured clays to gravelly stands. However poor soils produce poor results and the best crops are found on fertile sandy soils and loams. Soils should be well drained. Wet soils lead to poor aeration and increased incidence of crown root in apples. Rooting tends to be shallow and wet soils restrict development resulting in anchorage of the tree and reduced area of soil from which nutrients can be extracted. Soils with high organic matter contents are normally better structured and allow good rooting.

Apples prefer a slightly acidic to neutral soil (pH between 5.8 and 7.0) which is slightly acidic. For soil with a low pH, adding 5 pounds of a lime product for each 100 square feet of soil surface is needed. To lower the pH, adding either aluminum sulfate for immediate changes or sulfur for changes over a few months. The amount of these products varies depending on the soil, but generally, for each 10 square feet of soil with a high pH, needs 2 pounds of aluminum sulfate or 0.3 pounds of sulfur. The soil should be free from hard substrata and water-logged conditions. Soils with heavy clay or compact subsoil are to be avoided.

2.6. Human consumption of apple

All parts of the fruit, including the skin, except for the seeds, are suitable for human consumption. The core, from stem to bottom, containing the seeds, is usually not eaten and is discarded. Apples can be consumed various ways: juice, raw in salads, baked in pies, cooked into sauces and spreads like apple butter, and other baked dishes ("Apple Varietals" July 2001).

Several techniques are used to preserve apples and apple products. Apples can be canned, dried or frozen. Canned or frozen apples are eventually baked into pies or other cooked dishes. Apple juice or cider is also bottled. Apple juice is often concentrated and frozen.

2.7. Toxicity of apple fruit seeds

Apple seeds contain small amounts of amygdaline, a sugar and cyanide compound known as a cyanogenic glycoside. Ingesting small amounts of apple seeds causes no ill effects, but consumption of extremely large doses can cause adverse reactions. It may take several hours before the poison takes effect, as cyanogenic glycosides must be hydrolyzed before the cyanide ion is released. When taken by mouth: apples are likely safe for most people, as long as the seeds are not eaten. No side effects are generally known or expected to occur with apple fruit or apple juice. Taking a specific chemical found in apples, called apple polyphenols, is possibly safe when taken by mouth. Apple seeds, however, contain cyanide and are poisonous. Eating enough seeds (in one case, one cup of apple seeds) can cause death. The cyanide is released in the stomach as the seeds are digested, so it may take several hours for the symptoms of poisoning to appear. When applied to the skin: Specific chemicals found in apples, called apple polyphenols, are possibly safe when applied directly to the skin.

2.8. Phytochemical of apple

Apples are rich source of various including flavonoids (e.g., catechins, flavanols, and quercetin) and other phenolic compounds (e.g., epicatechin and procyanidins) found in the skin, core, and pulp of the apple they have unknown health value in humans. Phenolic compounds, such as polyphenol oxidase, are the main driving force behind browning in apples. Polyphenol oxidase catalyzes the reaction of phenolic compounds to O-quinones causing the pigment to turn darker and therefore brown.

Ideain (cyanidin 3-O-galactoside) is an anthocyanin, a type of pigment, which is found in some red apple cultivars. Phlorizin is a flavonoid that is found in apple trees, particularly in the leaves, and in only small amounts if at all in other plants, even other species of the genus *Malus* or related plants such as pear trees. Many apples grow readily from seeds. However, more than with most perennial fruits, apples must be propagated asexually by grafting to obtain the sweetness and other desirable characteristics of the parent. This is because seedling apples are an example of "extreme_heterozygote's", in that rather than inheriting genes from their parents to create a new apple with parental characteristics, they are instead significantly different from their parents, perhaps to compete with the many pests (John Lloyd and John Mitchinson, 2006).

2.9. Macro-essential metals

Some of the metals of interest in this study among macro-essential metals are Mg and Ca. The reference daily intake or recommended daily intake of these metals differs among standards of different countries as well as age and sex. According to USA standards reference daily intake of

Mg and Ca are 420 mg/day and 1200 mg/day for matured adults and 1300 mg/day for females, respectively (FNB, 1997).

2.9.1. Calcium

Calcium is a crucial mineral that is needed by the body for strengthen bones and teeth. There are many fruits that are quite high in Calcium which can aid in proper bone health of the body including these fruits in daily diet can prevent bone related issues like osteoporosis, muscle cramps, nervous disorders and abnormal heart functions. Calcium nutrition of an apple is best if fertilizers are applied through soil, i.e. the plants root system primarily for easier uptake of calcium from root surface and there are two basic ways in which calcium ions make contact with root surface for plant uptake root interception and mass low. The advantages of apple nutrition through the application of calcium needed are high and can be settled with difficulty solely by nutrition through the leaf (Von Bennewitze *et al.*, 2011).

2.9.2. Magnesium

The most well known role of magnesium in plants is its occurrence at the centre of the chlorophyll molecule. Usually 15-20% of the trees magnesium total is found in the chloroplast. It is also involved in various biochemical functions including activating enzymes involving phosphorrylation and protein synthesis. In addition to this function inadequate levels of magnesium can inhibit assimilation during photosynthesis. If there is magnesium deficiency in apple it makes leaf blotch of apples which appears as an edge burn or interveinal necrosis, together which in some varieties a yellow banding or mottling of the leaves, appearing First in the older leaves and associated with a low magnesium content of the leaves the scorched leaves usually containing less than 0.25 per cent magnesium. Seasonal variations in magnesium content of apples leaves agree with the seasonal differences in the incidence of leaf scorch and leaf blotch, respectively.

2.10. Trace essential elements

Some of the trace essential elements considered in this Study are Fe, M n, Cu, Al, Zn, Cr, Co and Ni. The daily consumption allowances of these metals also differ based up on the developmental levels and sex as well as the standards of the different Countries they set. Elements like Fe, Cu and Zn are found to be necessary in certain quantities in foods but these elements can cause ill effects when are injected in high amount. Al, Cr and Ni are metals which are not harmful when presented in amounts not exceeding 100 mg/kg. According USA standards of reference daily intake of Cr, Mn, Fe, Cu, Zn and Co are 0.03, 2.3, 8, 0.9, 11, 5-50 mg/day for matured adults and 0.045, 2.6, 9, 1.3, 13,5-50 mg/day for lactating females, respectively (FNB, 2001).

2.10.1. Iron

Iron is present in all body cells. As a component of hemoglobin and myoglobin, it functions as a carrier of oxygen in the blood and muscles. Because of iron losses during menstruation, women in their reproductive years require higher iron intakes than men. Therefore, the recommended dietary allowance (RDA) for women 11 to 50 years of age is 18 mg/day, but for men 19 years and older is only 10 mg/day. Women have difficulty achieving this high intake, because they generally have a relatively low caloric intake, and the usual U.S. diet provides only 6 to 7 mg of iron per 1,000 kcal. Since the need for iron is greater during periods of rapid growth, children from infancy through adolescence, as well as pregnant women, may fail to consume sufficient iron to meet their needs.

2.10.2. Manganese

Manganese is a necessary inorganic nutrient element, having an important role in many physiological procedures. Mn deficiency is more often observed in sandy soils, soils with high pH, organic soils with pH greater than 6, soils with high Fe, Zn, Ni, Cu concentration and tropical soils. Usually, it deteriorates under cool and liquid conditions. The application of Mn fertilizers through dispersal in the whole surface is not recommended due to the high absorption ability of most soils which have Mn deficiency. Instead, we prefer the placement of the fertilizer in rows. Foliar application of Mn is recommended when there are visible and testified symptoms of deficiency in the leaves, and when its application can be combined with random plant protection products in order to reduce the application cost. Foliar fertilizing of plants with Mn is usually more effective compared to the application of Mn in the soil, because during its foliar application, Mn not only bypasses the reactions of its immobilization in the ground, but it is also uniformly allocated in plant tissues. $MnSO_4$ has been established as the inorganic source of Mn. The inorganic forms of Mn that have been mainly tried in experiments of foliar fertilizing of random plant species are Mn-EDTA, Mn-HEDTA, Mn-citrate and Mn-glucoheptanate. Most common from the afro mentioned organic preparations is Mn-EDTA, which contains in its molecule 4 molecules of acetic acid.

2.10.3. Copper

Copper is essential to the growth of plants. Among other things it plays a part in several enzyme processes and is key to the formation of chlorophyll. Copper is one micronutrients needed in very small quantities by plants. The normal range in growth medium is 0.05-0.5 mg/kg, while in most tissues the normal range is between 3-10 mg/kg. In comparison the ideal range for iron in the tissue is 20 times higher than that of copper. Although copper deficiency or toxicities rarely occur it is best to avoid either extreme as both can have a negative impact on crop growth and quality. Copper activates some enzymes in plants which are involved in lignin synthesis and it is essential in several enzyme systems. It is also required in the process of photosynthesis is essential in plant respiration and assist in plant metabolism of carbohydrates and proteins. Copper also serves to intensify flavor in vegetables and color in flowers.

2.10.3. Aluminum

Aluminum is the 3rd most abundant element in earth's crust and is therefore a natural component of drinking water, food staffs and is component of many manufactured materials including drinking water, fruit juice, wine and beer. Increases aluminum exposure can be compensated for by excretion via intestine and normal healthy kidneys. Aluminum is one of the most common metals found in the environment and consequent. However aluminum levels have been increasing overtime due to acidification of the soils and anthropogenic activities. Al is known neurotoxin agent because this metal tends to accumulate in the brain and accumulation of Al may produce Alzheimer's disease. In case of short term dietary intake aluminum is scarcely harmful at all in case of elevated long term intake aluminum can however lead to brittle bones, anemia and brain damage in humans. In animal studies it was shown that low doses of the substance can already impair reproduction and the developing nervous system.

2.10.4. Zinc

Zinc, a constituent of more than 200 enzymes, plays an important role in nucleic acid metabolism, cell replication, tissue repair, and growth through its function in nucleic acid polymerases. These zinc-dependent enzymes include the potentially rate-limiting enzymes involved in DNA synthesis. Zinc also has many recognized and biologically important interactions with hormones and plays a role in production, storage, and secretion of individual hormones. Nevertheless, higher concentrations of Zn can be toxic to the organism (Rajkovic, *et al.*, 2008). The richest sources of zinc are shellfish (especially oysters), beef, and other red meats. Poultry, eggs, hard cheeses, milk, yogurt, legumes, nuts, and whole-grain cereals are also good sources. Many dietary factors, including other minerals, phytates, and dietary fiber, may adversely affect zinc absorption (Hambidge *et al*., 1986). Food sources of zinc have changed since the turn of the century. Zinc from animal sources appears to be better absorbed than that from plant sources.

2.10.5. Chromium

Chromium is an essential trace mineral that can improve insulin sensitivity and enhance protein, carbohydrate and lipid metabolism. People need by very small quantities. Chromium deficiency impaired glucose tolerance leading to reduced control of blood sugar in people with type 2 diabetes, less efficient control of cholesterol leading to a higher chance of atherosclerosis and heart disease. Cr is an essential micronutrient for animals and plants and is considered as a biological and pollution significant element (Jayana, 2009)

2.10.6. Cobalt

Cobalt is the 27th element of the periodic table, constitutes about 0.001% of the earth's crust where it is widely distributed usually in association with nickel and arsenic. It is relatively rare elements of the earth's crust which is essential to mammals in the form of cobalamin (vitamin B_{12}). Human dietary intake of cobalt varies between 5 and 50 µg per day. Co is a necessary cofactor for making the thyroid hormone thyroxin. Co has also been used in anemia treatment as it causes the red blood cells production. The toxicity of Co is quite low compared to that of many other metals (Song, *et al.*, 2003).

2.10.6. Nickel

The average daily exposure does not pose a threat to human health. Most of the nickel absorbed every day by humans is removed by the kidneys and passed out of the body through urine or is eliminated through the gastrointestinal tract without being absorbed. Nickel is not cumulative poison but larger doses or chronic inhalation exposure may be toxic even carcinogenic and constitute an occupational hazard (Buttice and Claudio, 2015).

2.11. Non essential metals

The problem of heavy metal pollution is increasing throughout the world. Their presence in the atmosphere, soil and water can cause serious problems to all organisms (Das *et al.*, 1997). Trace metals are present in food in amounts below 50 mg/kg and have some toxicological or nutritional significance. The metals like Pd, Cd, Hg, and As are found to cause deleterious effects even in low levels of 10-50 mg/kg.

2.11.1. Cadmium

Despite the importance of the type of a biotic stress, the effects of cadmium contamination have undergone little study in certain plants of agricultural interest. Cd is highly toxic and responsible for several cases of poisoning through food. Small quantities of Cd cause adverse changes in the arteries of human kidney. It replaces Zn biochemically and causes high blood pressures, kidney damage, and so forth (Rajappa *et al*., 2010). Dietary exposure to Cd is estimated to be about $1.2x10^{-4}$ mg to $4.9x10^{-4}$ mg/kg of body weight daily. Intake of dietary Cd should not exceed 0.007 mg/kg of body weight, per week, according to the World Health Organization. For a healthy male of about 80 kg of body weight that is only 0.56 mg per week, a very small amount indeed. Tobacco smoke weather cigarette or pipe smoke contains Cd and studies have shown that cigarette smokers have higher levels of Cd in their bodies than non-smokers. Smoking a pack of cigarettes can add between 0.002 and 0.004 mg of Cd to the body. Cd may also be accumulated from second hand smoke. Dangerous exposure to Cd usually occurs through inhalation of fumes and dust. Cd in this form is extremely irritating to the lungs and can lead to such symptoms as headaches, chills, muscle aches, nausea, vomiting and diarrhea. Cd is so hazardous that for long term workplace exposure to Cd contaminated dust and fumes, the level should be kept below 0.04 mg per cubic meter of air according to National Institute for Occupational Safety and Health Standards in USA. The human body can tolerate low levels of Cd but long term chronic exposure can lead to serious health problems. Elevated levels of Cd may result in hypertension (high blood pressure); a dulled sense of smell; anemia; yellow discoloration of the teeth; inflammation of mucous membrane of the nose; joint soreness; hair loss, dry, scaly skin and loss of appetite. Cd toxicity threatens the health of the body by weakening the immune system. It causes a decreased production of T lymphocytes.

2.11.2. Lead

Lead is one of the hazardous heavy metal pollutants of the environment that originates from various sources like mining and smelting of lead ores, burning of coal, effluents from storage battery industry, automobile exhausts, metal plating and finishing operations, fertilizers, pesticides and from additives in pigment and gasoline (Eick *et al*., 1999). It is extremely toxic metal whose effects on human health have been widely described (Juberg *et al*., 1997). Excessive lead exposure can cause mental retardation, behavioral disorder and its exposure can occur through multiple pathways, through inhalation of air, water, dust as it is emitted in the environment from vehicles and automobiles. It can also enter the food chain via plants (Wierzbicka and Antosiewiev, 1993)

3. Experimental

3.1. Equipment and reagents

3.1.1. Equipment

Polyethylene plastic bags were used to pack the harvested wild edible apple fruit samples. A drying oven (Digitheat, J. P. Selecta. S. a. Spain) was used to dry edible apple fruit samples. A digital analytical balance (Mettler Toledo, Model AG204, Switzerland) with ±0.0001 g precision was used to weigh apple fruits sample. 250 mL round bottom flasks fitted with reflux condensers were used in Kjeldahl (England) apparatus to digest the dried and powdered apple fruits. A refrigerator (Hitachi, Tokyo, Japan) was used to keep the digested sample until analysis. Agilent model 4200 (USA) microwave plasma-atomic emission spectrometer (MP-AES) was used for analysis of the metals (K, Mg, Ca, Fe, Mn, Zn, Cu, Co, Cr, Ni, Pb and Cd). A ceramic mortar and pestle (USA) was used for grinding the apple samples.

3.1.2. Reagents and chemicals

Reagents used in the analysis were all analytical grade. (69-72%) HNO_3 (Spectrosol, BDH, England) and 70% $HClO_4$ was used for digestion of apple plant fruit. Strontium nitrate (98%, Aldrich Muwaukee, USA) was used to avoid refractory interference (for releasing calcium and magnesium from their phosphates). Stock standard solutions containing 1000 mg/L, in 2% HNO_3, of the metals K, Mg, Ca, Fe, Mn, Zn, Cu, Co, Cr, Ni, Pb, Cd (Buck Scientific puro-Graphic^{tm} were used for the preparation of calibration standards and in the spiking experiments. Distilled water was used throughout the experiment for sample preparation, dilution and apparatus prior to analysis.

3.2. Procedures

Apparatus such as volumetric flasks, measuring cylinder and digestion flasks were washed with detergents and tap water and rinsed with distilled water and soaked in 50% nitric acid for two days. They were then rinsed with distilled water five times and dried in an oven and kept in dust free place until analysis begins.

3.3. Description of sampling sites

The sampling locations were chosen from farmland and commercial market. The samples were analyzed for 12 essential and non essential metals. Six apple samples were selected. Two different samples were selected from the market in Addis Ababa, which are imported from South Africa and Israel. One sample of red apple and other sample of green apple were collected from Wollega. One green sample was collected from Ankober around Debre birhan. One red sample was collected from Chencha Woreda in Arbaminch. The location of sample collected from Arbaminch is a place where there are high amount of sample especially from Chencha. Chencha Woreda is a place which is far from Addis Ababa by 374 km. Chencha's climate is classified as warm and temperate in which there is significant rainfall throughout the year. The average annual temperature of this place is 14 ^{o} C and the average rainfall is 1353 mm. The second sample is collected from Ankober a place which is far from Addis Ababa by 170 km and from Debrebirhan 42 km. It's annual rainfall range is 1000 to 1400 mm.

Table 1. Sample location for green apple and red apple

Sample site	Average temperature (^{o}C)	Rainfall precipitation (mm)
Ankober	22	1000-1400
Chencha	14	750-1000
Wollega	18.3	2080
South Africa	26	464
Israel	18	1254

3.4. Collection and preparation of apple fruits samples

The fruits of apple plants were collected manually using vinyl gloves for protecting hands. The bruised portions were removed and the remaining samples packed in the polyethylene bags for transporting to the analytical laboratory of Chemistry Department, Addis Ababa University. In the laboratory, collected fruit samples were washed with tap water and then with double distilled water to eliminate adsorbed dust and particulate matters. The fruit samples were then chopped into small pieces using a plastic knife in order to facilitate drying. The samples were then air dried for five to six days and further dried in a hot air oven at 50-60 ^{o} C for 24 h to remove moisture and maintain constant mass. The dried samples were ground into powder using acid washed commercial mortar and pestle and then sieved to 0.425 mm mesh size. The sieved samples were finally stored in the polyethylene bags and kept in desiccators until the time of digestion.

3.5. Optimization of the digestion procedure for fruit apple plant

Optimization procedure is the common procedure in scientific experiments of analytical chemistry in particular that optimum working conditions should be determined before carrying out any experimental activities for sample preparation before analysis.

For each of the apple fruit 0.5 g of powdered sample was weighted and transferred to 250 mL of round bottom flask. To do this optimization procedure different volumes of HNO_3 and $HClO_4$ at specified proportions (v/v) was added and digested at temperatures 120, 150, 180, 210, 240, 270 and at different duration of time which is 1:45, 2:00, 2:15, 2:30, 2:45, 3:00 hour. The optimized procedure was determined based on the formation of clear colorless solution. The digested solutions were cooled for 5 min on Kjeldal apparatus after it was switched off and cooled for 5 min outside of the apparatus. 5 mL of distilled water was added to dissolve the precipitate formed on cooling and gently shake and filtered into 50 mL volumetric flask through filter paper. Then the clear solution was diluted up to 50 mL with distilled water and stored for analysis by MP-AES.

3.6. Digestion of the fruit apple

For each of the apple fruit plant 0.5 g of powdered samples were weighted and transferred to 250 mL of round bottom flask. To do this 4 mL of 3:1 (v/v) HNO_3 and $HClO_4$ was added and digested at 210 ^{o}C for 2:15 h for all the apple sample digestion. The digested solution were allowed to cool and 5 mL distilled water was added to dissolve the precipitate formed on cooling and gently swirled and filter into 50 mL volumetric flask through filter paper. The clear solution obtained was diluted up to 50 mL with distilled water. Each apple fruit samples were digested in triplicate. Digestion of reagent blank was also performed in parallel with the samples. All the solutions were stored in tightly capped polyethylene bottles and stored in a refrigerator until analysis. The solutions were used to determine concentrations of Ca, Mg, Fe, Mn, Cu, Zn, Al, Cr, Co, Ni, Pb and Cd by microwave plasma-atomic emission spectroscopy (MP-AES).

3.7. Determination of standard solution formation for major, minor and trace metals

An intermediate 100 mg/L standard solution was prepared from microwave plasma-atomic emission spectroscopy (MP-AES) standard stock solutions tthat contained 1000 mg/L. These 100 mg/L standard solutions were diluted with distilled water to obtain four working standards for each metal . The metals Ca, Mg, Fe, Mn, Cu, Zn, Cr, Co, Ni, Pb and Cd were determined by microwave plasma-atomic emission spectroscopy (MP-AES). The operating conditions for MP-AES employed for each analyte are given in Table 2.

Table 2. The operating condition of MP-AES

Metal	Parameters											
	Wave-lengh (nm)	Back-ground correction	EGCM setting	Repli-cates	Pump speed (rpm)	Blank subtract-tion	Stabliz-ation time (s)	Sample up take time (s)	Sample uptake fast pump	Rinse time (s)	Read time (s)	Nebulizer flow (L/min)
Ca	422.7	Auto	High	3	15	On	15	27	On	10	3	0.6
Mg	279.553	Auto	High	3	15	On	21	28	On	10	3	0.9
Fe	371.99	Auto	High	3	15	On	19	30	On	10	3	0.65
Mn	259.372	Auto	High	3	15	On	16	40	On	12	3	0.9
Cu	327.395	Auto	High	3	15	On	16	40	On	12	3	0.7
Al	396.152	Auto	High	3	15	On	16	40	On	12	3	0.95
Zn	213.857	Auto	High	3	15	On	21	28	On	10	3	0.45
Cr	427.480	Auto	High	3	15	On	21	28	On	10	3	0.9
Co	340.512	Auto	High	3	15	On	21	28	On	10	3	0.75
Ni	305.081	Auto	High	3	15	On	16	40	On	12	3	0.7
Pb	368.346	Auto	High	3	15	On	21	28	On	10	3	0.75
Cd	226.502	Auto	High	3	15	On	16	40	On	12	3	0.5

3.8. Recovery test

Recovery is one of the most commonly used techniques utilized for validation of the analytical results and evaluating how far the method is acceptable for its intended purpose. It can be calculated as the percentage of the analyte response after sample workup compared to that of a solution containing the analyte at a concentration corresponding to 100% recovery. The efficiency of the optimized procedure was checked by adding the known concentration of each metal to 0.5 g sample for the one apple fruit.

3.9. Method Validation

Method validation is the process of defining an analytical requirement, and confirming that method under consideration has performance capabilities consistent with what the application requires.

3.9.1. Limit of detection

Limit of detection (LOD) is minimum concentration measurements of a substance by specific analytical protocol can be distinguished from measurements of a blank

The detection limit of an individual analytical procedure is the lowest amount of analyte in a sample which can be detected but not necessarily quantitated as an exact value. Based on the standard deviation of the response

Detection limit = 3 x standard deviation

3.9.2. Limit of quantification

The quantification limit of an individual analytical procedure is the lowest amount of analyte in a sample which can be quantitatively determined with suitable precision and accuracy.

Quantification of limit = 10 x standard deviation.

4. Results and Discussion

4.1. Optimization of the digestion procedure

Different digestion procedure for the apple plant were carried out using HNO_3, $HClO_4$ mixtures by varying volume of the acid mixture, digestion time and digestion temperature. This procedure was developed with some modification of the procedure in literature used to determine the metal content of apple plant fruits samples by FAAS (Boke *et al.*, 2015). Optimized procedures were selected based on the usage of lesser reagent volume, shorter digestion time and reasonable mild temperature for obtaining clear and colorless solutions of the resulting digests. In this work the apple fruit from the six different places were used for metal determination. The optimized parameters for digestion procedure were 3:1 of HNO_3 and $HClO_4$ at 210 ^{o}C and for 2:15 hours.

Table 3. Digestion results for 0.5 g apple fruits at different volume and constant temperature and time

Volume ratio (HNO_3 : $HClO_4$)	Digestion temperature (^{o}C)	Digestion time (h)	Observation

5:1	2700	3:00	Colorless
4:1	270	3:00	Colorless
3:1	**270**	**3:00**	**Clear colorless solution**
3:2	270	3:00	Colorless
2:2	270	3:00	Colorless
2:1	270	3:00	Colorless

From the optimization results the digestion of an apple which has the ratio of 3:1 volume HNO_3 to $HClO_4$ at 270 ^oC and 3 hours indicated the clear colorless solution. Even if, all the digestion procedure results were colorless, for cost minimizing the acid ratio of 3:1 was preferred.

Table 4. Digestion results of 0.5 g apple fruits at the same volume and time but different temperature

Volume ratio (HNO_3 : $HClO_4$)	Digestion temperature (^{o} C)	Digestion time (h)	Observations
3:1	120	3:00	Colorless
3:1	150	3:00	Colorless
3:1	180	3:00	Colorless
3:1	**210**	**3:00**	**Clear colorless solution**
3:1	240	3:00	Colorless
3:1	270	3:00	Colorless

From the optimization results of different temperature for digestion, a temperature of 210 ^oC was selected, because at the temperature of 210 ^oC and a volume of 3:1, the procedure gave clear solution.

Table 5. Digestion results of 0.5 g apple fruits at different digestion time and constant volume and temperature

Volume ratio (HNO_3 : $HClO_4$)	Digestion temperature (^{o}C)	Digestion time (h)	Observations
3:1	210	1:45	Colorless
3:1	210	2:00	Colorless
3:1	**210**	**2:15**	**Clear colorless solution**
3:1	210	2:30	Colorless
3:1	210	2:45	Colorless
3:1	210	3:00	Colorless

From the optimization results a volume of 3:1 and 210 ^{o}C the digestion obtained at a time 2:15 hour gave a clear solution. From tables 3-5 the digestion volume, temperature and time selected for digestion was 3:1 (4 mL), 210 ^{o}C and 2:15 h, respectively.

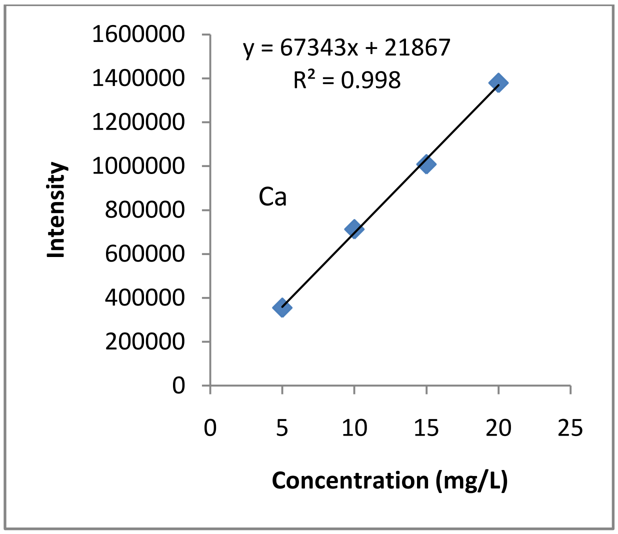
4.2. Calibration of the instrument

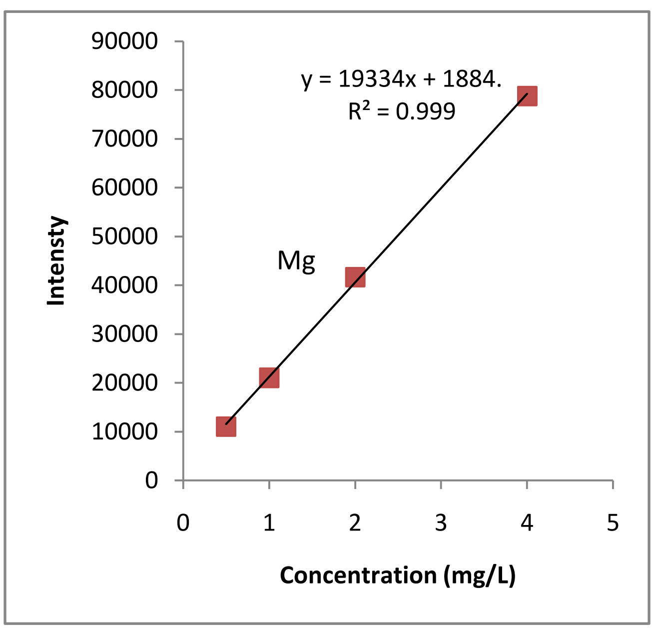
Calibration curves were prepared to determine the concentration of metals in the sample solution. The instrument was calibrated using series of working standards. Concentrations of the working standards, calibration equation and correlation coefficient value of the calibration curve for each of the metals are listed in Table 6. The calibration curve of each of the metals interest is shown in Figure 3.

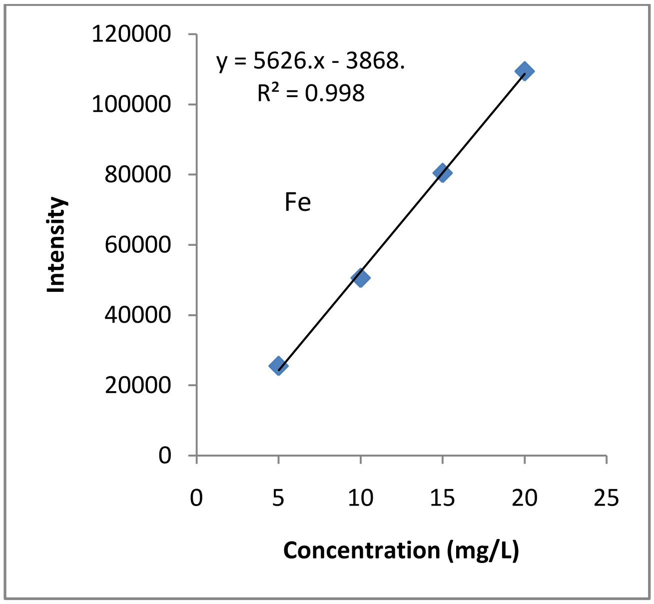
Table 6. Instrument calibration

Metals	Concentration of standards (mg/L)	Correlation coefficient	Calibration equation
Ca	5, 10, 15, 20	0.998	y = 67343x + 21867
Mg	5, 10, 15,20	0.999	y = 19334x + 1884
Fe	5, 10, 15, 20	0.998	y = 5626x – 3868
Mn	0.5, 1.0, 2.0, 4.0	0.999	y = 2977x + 206.3

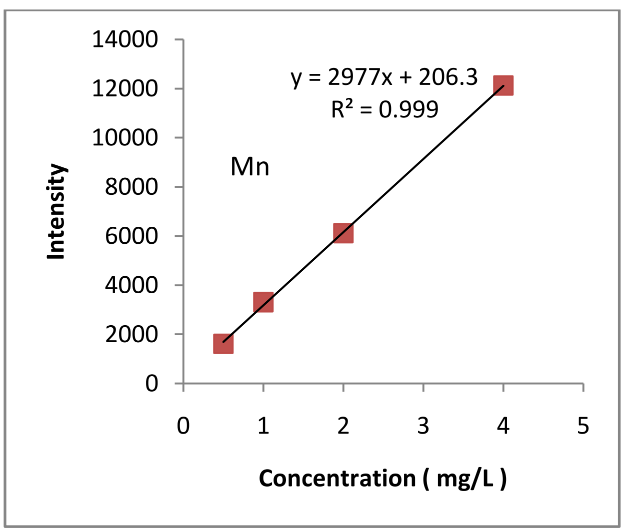
Cu	0.5, 1.0, 2.0, 4.0	0.999	y = 33562x + 525.3
Al	0.5, 1.0, 2.0, 4.0	0.999	y = 24298x + 759.3
Zn	0.5, 1.0, 2.0, 4.0	0.999	y = 4541x + 301.9
Cr	0.5, 1.0, 2.0, 4.0	0.994	y = 327.3x + 50.34
Co	0.5, 1.0, 2.0, 4.0	0.996	y = 5444.x + 178.5
Ni	0.5, 1.0, 2.0, 4.0	0.999	y = 4453x + 207.2
Pb	0.5, 1.0, 2.0, 4.0	0.999	y = 2616x + 108.9
Cd	0.5, 1.0, 2.0, 4.0	0.998	y = 660.3x + 118.9



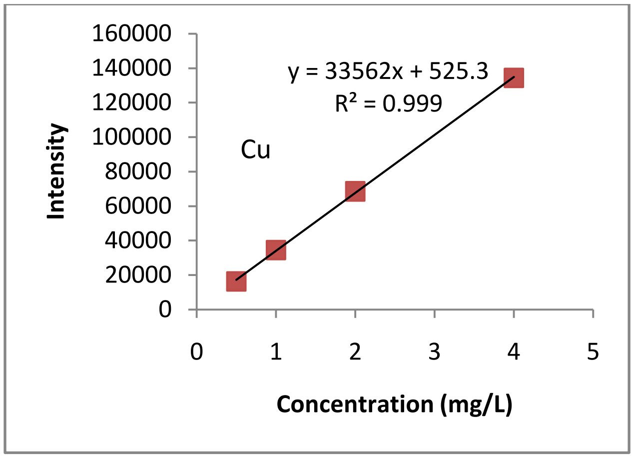


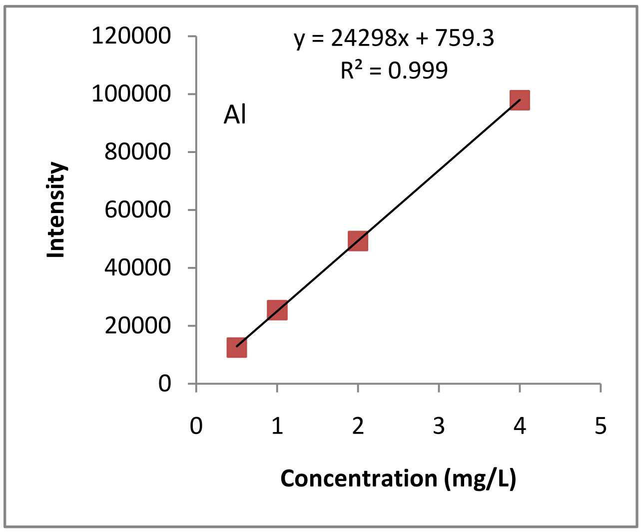


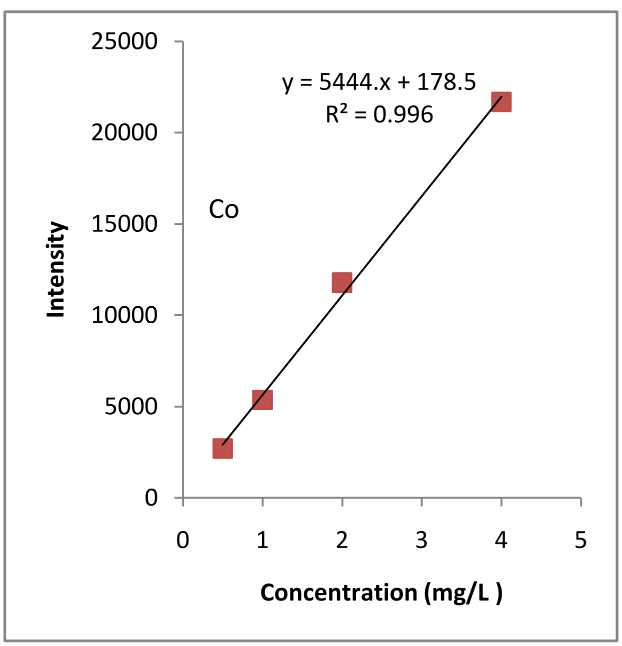
y = 5626.x - 3868.
R² = 0.998
Fe
Intensity
Concentration (mg/L)

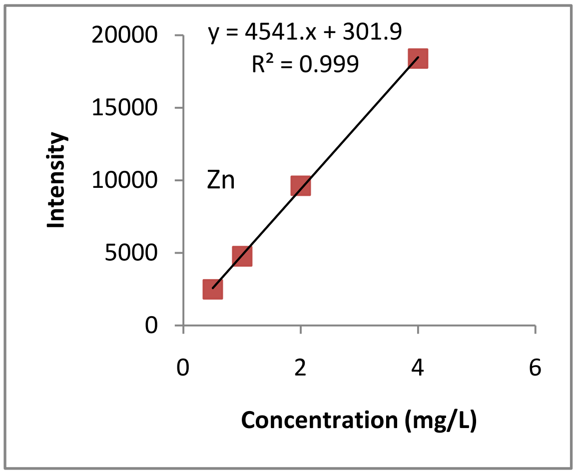


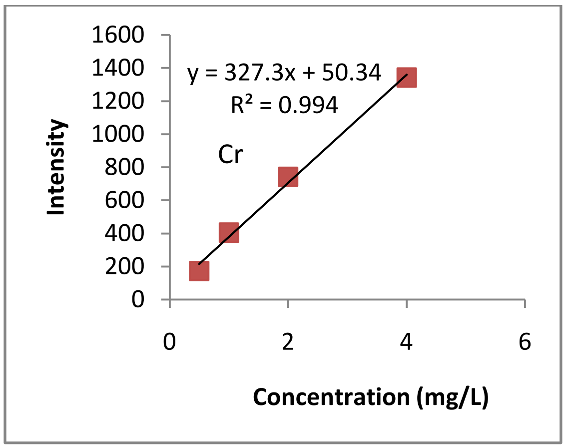
y = 2977x + 206.3
R² = 0.999
Mn
Intensity
Concentration (mg/L)

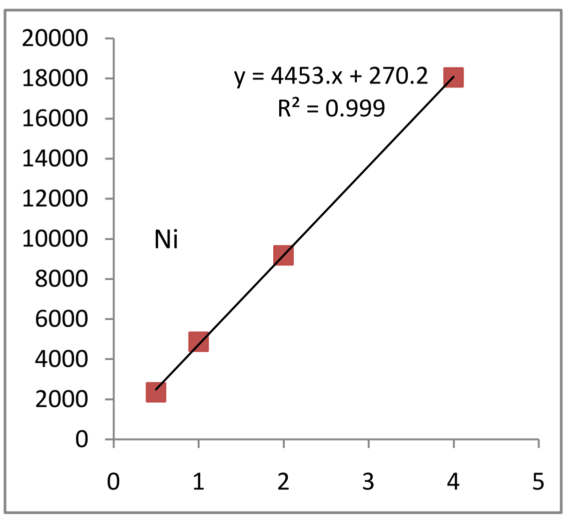

160000
140000
120000
100000
80000
60000
40000
20000
0
Intensity
y = 33562x + 525.3
R² = 0.999
Cu
0
1
2
3
4
5
Concentration (mg/L)

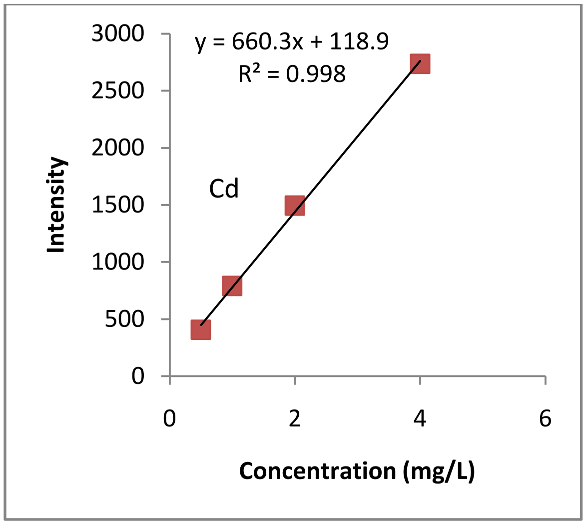

120000
100000
80000
60000
40000
20000
0
Intensity
y = 24298x + 759.3
R² = 0.999
Al
0
1
2
3
4
5
Concentration (mg/L)


25000
20000
15000
10000
5000
0
Intensity
y = 5444.x + 178.5
R² = 0.996
Co
0
1
2
3
4
5
Concentration (mg/L)


y = 4541.x + 301.9
R² = 0.999
Zn
Intensity
Concentration (mg/L)
20000
15000
10000
5000
0
0
2
4
6


y = 327.3x + 50.34
R² = 0.994
Cr
Intensity
Concentration (mg/L)
1600
1400
1200
1000
800
600
400
200
0
0
2
4
6


y = 4453.x + 270.2
R² = 0.999
Ni
20000
18000
16000
14000
12000
10000
8000
6000
4000
2000
0
0
1
2
3
4
5



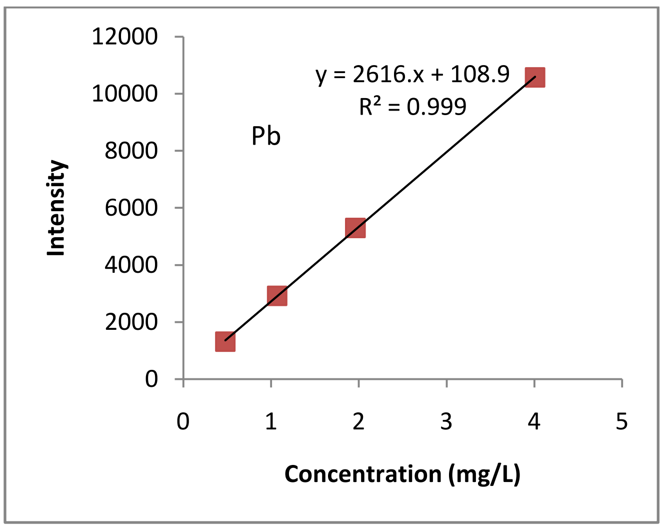


Figure 3. Calibration curves of metals for the standard solutions under optimum instrumental working condition

4.3. Precision and accuracy

Accuracy and precision are most common terms related to analytical quality procedures to express the extent of errors in a given analytical results. Analytical results must be evaluated to decide on the best values to report and attempt to establish the probable limits of errors of the values. Such qualities of data are performed by applying different statistical methods to analytical data. Most of the common statistical methods applied in analytical chemistry are standard deviation, variance, relative standard deviation and range of the series of measurements (Skoog *et al.*, 1996). In this study the precision of the results were evaluated by the pooled standard deviation and relative standard deviation of the triplicate measurements.

4.4. Limit of detection and Limit of quantification method analysis

The detection limit is the lowest concentration or weight of analyte that can be measured at a specific confidence level. Near the detection limit the signal generated approaches that from a blank and can be determined experimentally by running several blank samples to establish the mean and standard deviation of the blank (Boke *et al*., 2015). The limit of detection expressed as = 3 x sample blank standard deviation of the blank sample and limit of quantification is expressed as = 10 x sample blank standard deviation. In this study blank samples were digested and have been utilized for the apple fruit. The mean and standard deviations of the blanks were calculated to determine the method detection limit.

Table 7. Limit of detection and limit of quantification of metals in blank sample.

Metal	SD	LOD (mg/kg)	LOQ (mg/kg)
Ca	14.3	42.9	143
Mg	1.21	3.63	12.1
Fe	0.87	2.61	8.7
Mn	0.43	1.29	4.3
Cu	0.2	0.6	2
Al	1.46	4.38	14.6
Zn	0.74	2.22	7.4
Cr	0.17	0.51	1.7
Co	1.4	4.2	14
Ni	0.19	0.57	1.9
Pb	-	-	-
Cd	-	-	-

4.5. Validation of analytical method

Recovery is one of the most commonly used techniques utilized for validation of the analytical results and evaluation how far the method is acceptable for its intended purpose (Boke *et al.*, 2015). A recovery test was carried out by spiking pre-analyzed samples.

$$\% \text{ Recovery} = \frac{\text{Amount in spiked sample} - \text{amount in unspiked sample}}{\text{Added amount}} \times 100$$

The accepted recoveries ranged from 90% to 110%. The spiked apple fruit were digested in triplicate following the same procedure used for digestion of the fruit sample. The resulting digest spiked samples was analyzed for their respective metal contents using MP-AES and percent recoveries were calculated for the three fruit samples.

Table 8. Recovery test results for apple fruit

Metal	Conc. in sample (mg/kg)	% spiked	Amount added (mg/kg)	Conc. in the spiked sample (mg/kg)	Amount recovered (mg/kg)	(%) Recovery
Fe	129	20	25.5	156	27	106
Cu	7.94	80	6.35	14	20.1	95.4
Mn	15.3	70	10.7	25.9	10.6	99.1
Zn	47.4	45	21.3	67	19.6	91.8
Cr	7.14	75	5.35	12	4.86	90.8

From the standard solution amount of % spiked Fe(13 µL), Cu (3 µL), (5 µL), Zn (10.65 µL) and Cr (2.6µL) were calculated and measured .

A recovery test of the total analytical procedure was performed for all of the selected metals in selected apple fruits samples by spiking analyzed samples of metal standards are acceptable. Acceptable recoveries ranged from 90% to 110%. From the data in Table 8 acceptable recoveries in the range of 90.8-106% were obtained for the analyzed metals in red apple sample from Arbaminch, Chencha. The results indicated that the optimized procedure were accurate.

4.6. Comparison of metals concentration in green apple samples from different areas

The comparison of metal concentration in green apple samples from different areas is given in Table 9 and Figures 4-6.

Table 9. Mean concentration ± standard deviations (mg/kg) of metals in green apple

Element	Ankober		South Africa		Wollega	
	Mean ± SD	%RSD	Mean ± SD	%RSD	Mean ± SD	%RSD
Ca	1125±104	9.2	36275 ±3024	8.3	1065± 60	5.6
Mg	27.0 ± 1.7	6.2	153 ± 10	6.3	71.2 ± 6.5	9.2
Fe	162 ± 12	7.2	276 ± 10	3.7	103 ± 6	6.0
Mn	13.5+ 1.1	8.3	13.5 ± 0.9	6.5	13.7 ± 0.7	5.4
Cu	6.56±1.06	1.6	5.77 ± 0.46	8.1	5.13 ± 0.3	5.97
Al	88.1 ±8.1	9.1	129 ±10.15	7.8	77.8± 4.01	5.15
Zn	41.3±3.67	8.7	73.6 ± 2.52	3.4	49.7 ± 4.13	8.42
Cr	6.15±0.13	5.3	10.04 ± 0.79	7.9	8.57± 0.61	7.16
Co	2.48±0.13	6.5	2.48 ± 0.20	8.3	1.85 ± 0.10	5.76
Ni	1.33±0.07	5.3	2.37 ± 0.05	2.4	1.38± 0.11	8.54
Pb	ND	ND	ND	ND	ND	ND
Cd	ND	ND	ND	ND	ND	ND

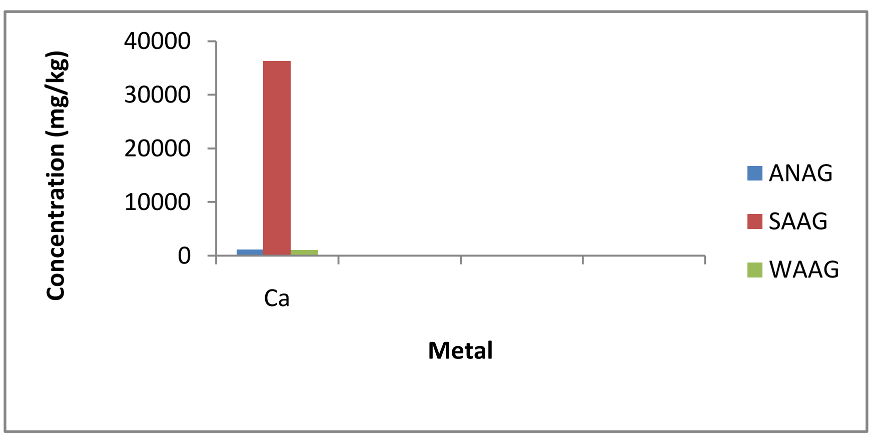
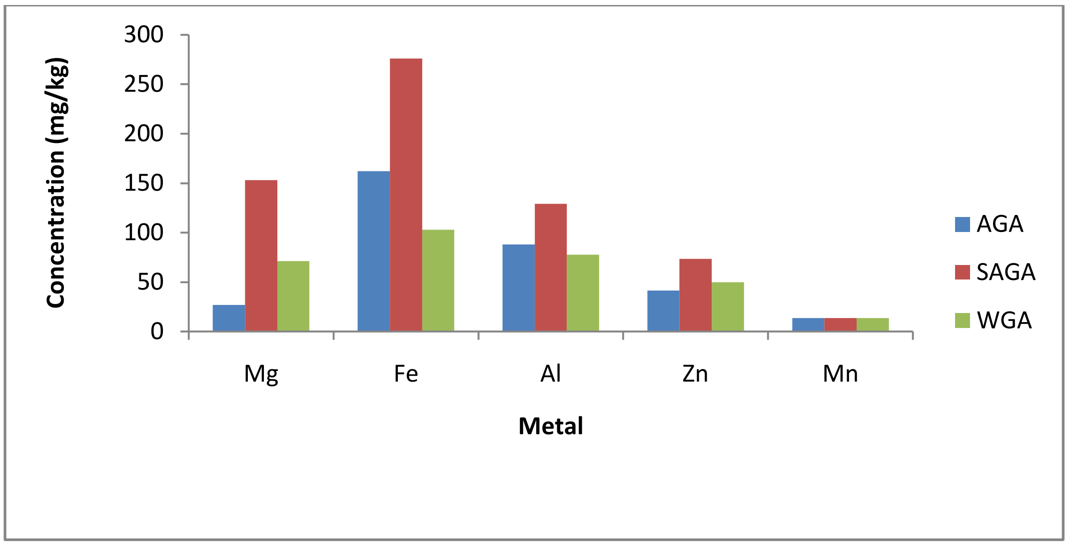


Figure 4. Comparison of calcium in green apple from three different places

The graph in figure 4 showed that the concentration of green apple fruit differs from each other. Concentration of metals in green apple which comes from South Africa is higher than the concentration of calcium in Ankober green apple and Wollega green apple. The mean concentration of calcium from South Africa green apple is higher than Ankober green apple metal concentration, which is also higher than Wollega green apple sample. The concentration in the green apple from South Africa, Ankober and Wollega were 36275, 1125, 1065 mg/kg, respectively.



AGA-Ankober green apple, SAGA-South Africa green apple, WGA-Wollega green apple

Figure 5. Comparison of Mg, Fe, Al, Zn and Mn content in green apple from Ankober, South Africa and Wollega areas

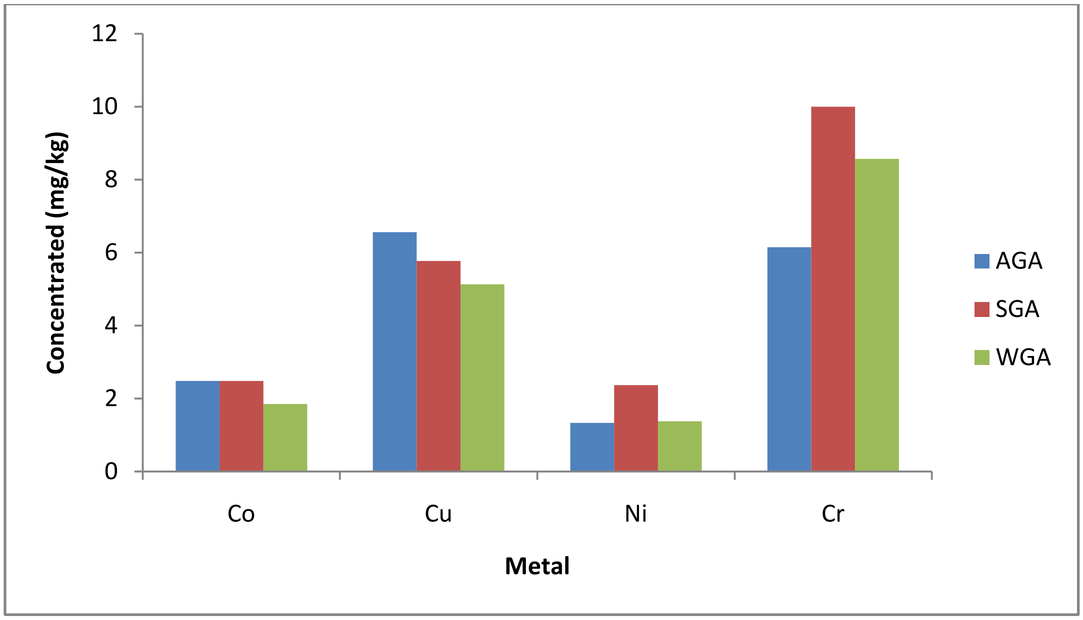
When the content of magnesium concentration in green apple from different sites is compared the concentration of magnesium in sample from South Africa is higher than magnesium concentration in green apple from Wollega. Magnesium concentration in green apple from Wollega is higher than Ankober green apple magnesium concentration. The mean concentration were 153, 71.2, 27 mg/kg respectively for South Africa, Wollega and Ankober.

When the concentration of iron in green apple sample from the three places is compared, the sample from South Africa is more concentrated than the other two places Ankober and Wollega. Ankober green apple is the second more concentrated and Wollega green apple sample have the least concentration of iron. The mean concentration of Fe were 276, 162, 103 mg/kg, respectively for green apple from South Africa, Ankober and Wollega.

The mean concentration of aluminum metal in green apple sample from South Africa is higher than in the sample from Ankober and the mean concentration of Al in the sample from Ankober green apple is higher than Wollega. Their mean concentration of Al were 129, 88.1, 77.8 mg/kg, respectively for the sample from South Africa, Ankober and Wollega.

Concentration of magnesium in green apple sample from South Africa is higher than in the samples from Wollega and Ankober. The second higher concentrated is in the sample from Wollega and the sample from Ankober was the least one. Their concentration were 73.6, 49.7, 41.3 mg/kg, respectively for samples from South Africa, Wollega and Ankober.

The concentration of manganese metal in green apple from the three different places was almost the same. Their concentration were 13.5, 13.5, 13.7 mg/kg, respectively for Ankober, South Africa and Wollega.



AGA-Ankober green apple, SGA-south Africa green apple, WGA-Wollega green apple

Figure 6. Comparison of metals Co, Cu, Ni and Cr content in green apple samples from Ankober, South Africa and Wollega Areas.

Concentration of cobalt in the apple samples from the two different places Ankober and South Africa are the same and Wollega green apple sample have less Co than in the samples from other two different places. Numerically sample from Ankober and South Africa showed 2.48 mg/kg Co for each and Co concentration in the sample from Wollega was 1.85 mg/kg. The content of copper metal obtained from the experiment in green apple sample from Ankober is higher than sample from South Africa and the sample from South Africa showed mean concentration higher than in the sample from Wollega. The mean concentration obtained in the sample from Ankober, South Africa and Wollega was 6.56, 5.77, 5.13 mg/kg as their order.

Nickel metal from South Africa green apple sample showed higher concentration than Ankober and Wollega. Their concentration were 2.37, 1.33, 1.38 mg/kg respectively in the samples of from Ankober, South Africa and Wollega.

Chromium metal concentration obtained from sample of South Africa > Wollega > Ankober. Their concentration were 10.04, 8.57, 6.15 mg/kg for sample from South Africa, Ankober and Wollega respectively.

The metal content obtained in Ankober green apple sample was as the decreasing order of Ca > Fe > Al > Zn > Mg > Cu > Cr > Co > Ni. South Africa green apple sample contain the metal content as the order of Ca > Fe > Mg > Al > Zn > Mn > Cr > Cu > Co > Ni and in Wollega green apple content of metal obtained was as the decreasing order of Ca > Fe > Al > Mg > Zn > Mn > Cr > Cu > Co > Ni.

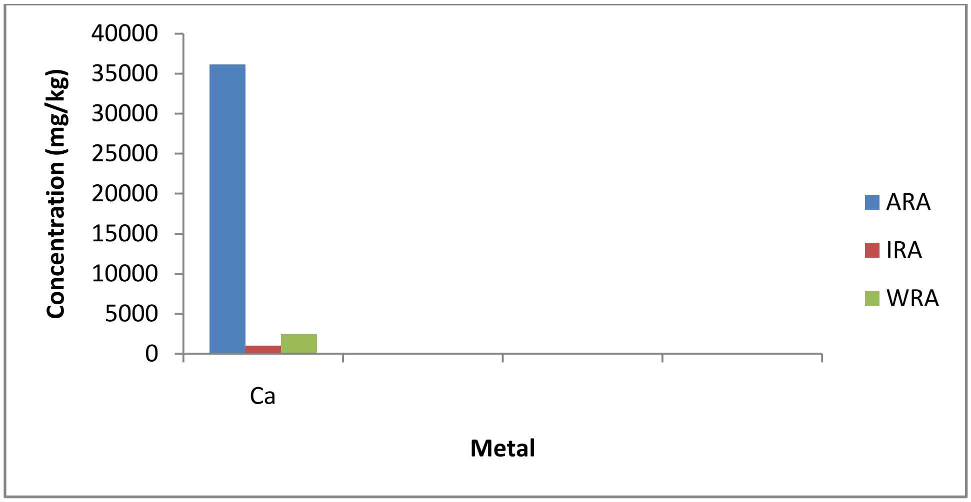
4.7. Comparison of metals concentration in red apple samples from different areas

The concentrations of metals in red apple samples from Arbaminch, Israel and Wollega are given in Table 10.

Table 10 . Mean concentration ± standard deviations (mg/kg) metals of red apple

Element	Arbaminch		Israel		Wollega	
	Mean ± SD	%RSD	Mean ± SD	%RSD	Mean ± SD	%RSD
Ca	36143± 3153	8.7	1013 ± 35.52	3.5	2444 ± 140	5.7
Mg	78.3 ± 4.75	6	139 ± 4.65	3.3	78.7 ±6.47	8.21
Fe	129 ± 9.94	7.7	241 ± 15.05	6.2	123 ± 5.19	4.2
Mn	15.3 ± 0.74	4.8	11.5 ± 1.04	9.09	16.3 ± 1.5	9.2
Cu	7.94 ± 0.23	2.9	4.11 ± 0.24	5.8	12.6 ± 1.13	9.01
Al	68.9 ± 2.11	3	52.5 ± 1.53	2.9	89.6 ± 8.15	9
Zn	47.4 ± 1.89	4	54.4 ± 4.51	8.29	51.3 ± 3.77	7.35
Cr	7.14 ± 0.18	2.5	6.57 ± 0.61	9.92	6.57 ± 0.41	5
Co	2.35 ± 0.17	7.5	2.28 ± 0.23	10	1.70 ± 0.04	2.77
Ni	1.43 ± 0.08	6.08	7.66 ± 0.51	6.69	1.78 ± 0.12	7.1
P b	ND	ND	ND	ND	ND	ND
C d	ND	ND	ND	ND	ND	ND

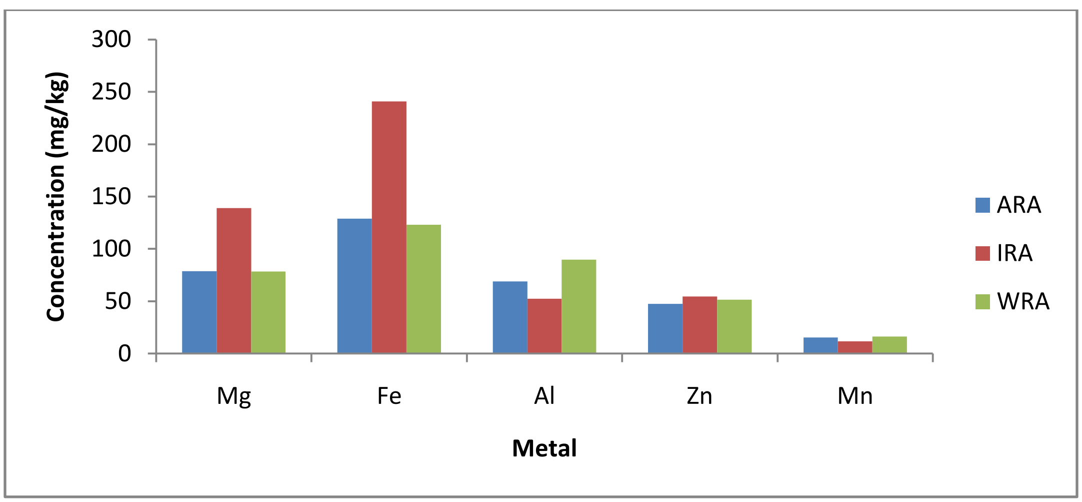
ND - Not detected.



ARA-Arbaminch red apple,   WRA-Wollega red apple, IRA-Israel red apple.

Figure 7 . Comparison of calcium metal content in red apple fruit between sample of Arbaminch, Israel and Wollega areas

Figure 7 shows that the content of calcium metal concentration in Arbaminch red apple is higher than Wollega red apple sample and Wollega red apple sample have higher concentration than Israel red apple. Their concentration were 36143, 2444, 1013 mg/kg, respectively.



ARA-Arbaminch red apple, IRA-Israel red apple, WRA-Wollega red apple

Figure 8. Comparison of Mg, Fe, Al, Zn and Mn metals content in red apple fruits sample from Arbaminch, Israel and Wollega areas.

Figure 9 shows the comparison of Mg, Fe, Al, Zn and Mn content in red apple fruits sample from Arbaminch, Israel and Wollega areas.

Red apple sample which was collected from Israel gave high magnesium concentration than Wollega red apple and Arbaminch red apple sample. Wollega and Arbaminch red apple sample showed almost the same concentration and were 78.7 and 78.3 mg/kg, respectively.

When concentration of iron in the red apple from the three sample places are compared with each other the mean concentration obtained in the red apple sample from Israel is higher than in the samples from Arbaminch and Wollega. The mean concentration were 241, 129, 123 mg/kg in the samples from Israel, Arbaminch and Wollega, respectively.

Concentration of aluminum in sample of red apple from Wollega is higher than sample of Arbaminch red apple and red apple sample from Israel. Their mean concentration were 89.6, 68.9, 52.5 mg/kg, respectively.

Zinc concentration for red apple from three place were 54.4, 51.3, 47.4 mg/kg, respectively, in the order of sample from Israel, Wollega and Arbaminch.

Concentration of Mn in the red apple sample from Wollega is higher than in the sample from Arbaminch and sample from Arbaminch have higher concentration than Israel red apple sample. The mean concentration order is 16.3, 15.3, 11.5 mg/kg, respectively.

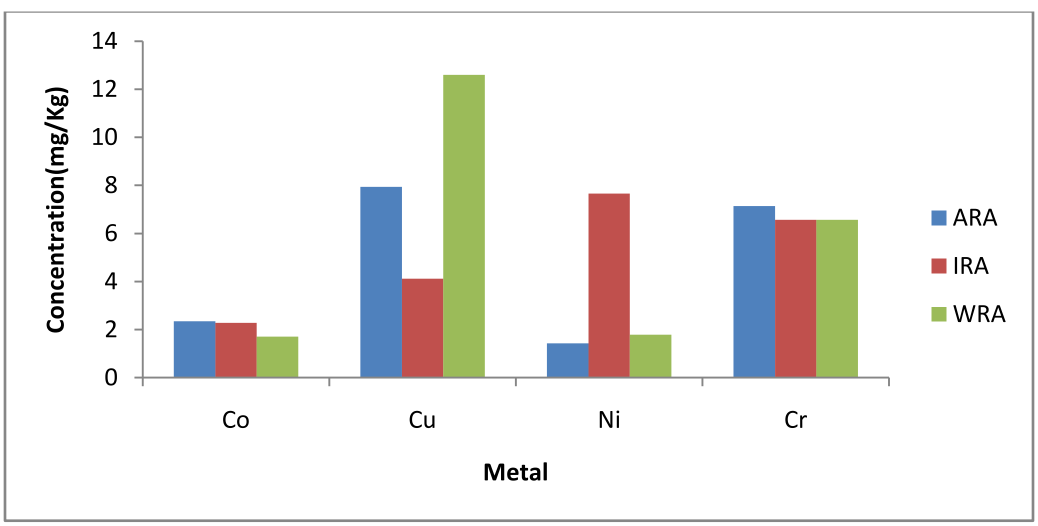


Figure 10. Comparison of Co, Cu, Ni and Cr metals content in red apple fruits sample from Arbaminch, Israel and Wollega area.

Cobalt metal content in red apple collected from three sample places were Arbaminch red Apple > Israel red apple > Wollega red apple. Their mean concentration were 2.28, 2.35, 1.70 mg/kg, respectively.

Copper content in the red apple of sample from three places were in the decreasing order of sample from Wollega, Arbaminch and Israel, respectively.

Mean concentration of nickel metal in the red apple samples from Israel is higher than sample from Wollega and Arbaminch and sample from Wollega red apple have higher concentration than sample of Arbaminch red apple. Their mean concentration is 7.66, 1.78, 1.43 mg/kg, respectively.

Mean concentration of chromium metal in the sample from Arbaminch red apple is higher than red apple sample from Israel and Wollega. Both samples of red apple from Israel and Wollega have almost the same concentration.

The mean concentration order of metal content in sample of Arbaminch red apple is Ca > Fe > Mg > Al > Zn > Mn > Cu > Cr > Co > Ni.
For the red apple sample from Israel the order of metal content is Ca > Fe > Mg > Zn > Al > Mn > Ni > Cr > Cu > Co and the order of metal content in Wollega red apple sample is Ca > Fe > Al > Mg > Zn > Mn > Cu > Cr > Ni > Co.

4.8. Comparison of overall metals concentration range in the green apple fruit and the red apple fruit from six different places.

Table 11. Metals concentration (mg/kg) for green apple and red apple fruit

Metal	Range of metal concentration (mg/kg)	
	Green apple	Red apple
Ca	1065-36275	1013-36143
Mg	27-153	78.3-139
Fe	103-276	123-241
Mn	13.5-13.7	11.5-16.3
Cu	5.13-6.56	4.11-12.6
Al	77.8-129	52.5-89.6
Zn	41.3-73.6	47.4-54.4
Cr	6.15-10.04	6.57-7.14
Co	1.85-2.48	1.70-2.35
Ni	1.33-2.37	1.43-7.66

The result in the Table 11 showed that concentration of calcium metal in green apple is slightly higher than red apple sample. In green apple its range is 1065-36275 concentration but in red apple its range is between 1013 and 36143 mean concentration. Green apple have higher aluminum mean concentration range than red apple that is, in green apple its range is between 77.8 and 129 mg/kg but in red its mean concentration range lie between 52.5 and 89.6 mg/kg. Green apple have lower nickel mean concentration range than red apple. All the others have varied concentration range.

4.9. The correlation coefficient of metals

A correlation coefficient is a statistical measure that calculates the strength of relation between the relative movements of two variables. The values range between -1.0 and 1.0. A correlation coefficient of -1 showed perfect negative correlation, while correlation of 1.0 shows perfect positive correlation. A correlation of 0.0 shows no relationship between the movements of the two variables (Akhilesh Ganti, 2019).

Pearson's correlation coefficient was used to investigate correlations between metal concentration in the green apple fruit and red apple fruit. A high correlation coefficient near +1 or -1 indicate good relationship between two variables and the concentration around zero means no relationship between them at a significant level of 0.05% level, it can be strongly correlated for r > 0.7, where as r values between 0.5 to 0.7 shows moderate correlation between two different parameters (Sharma, 2013).

Table 12. Pearson correlation coefficients between metals concentration in green apple

	Ca	Mg	Fe	M n	Cu	Al	Zn	Cr	Co	Ni
Ca	1.00									
Mg	0.93	1.00								
Fe	0.94	0.77	1.00							
M n	-0.5	0.17	0.76	1.00						
Cu	0.06	-0.4	0.27	0.83	1.00					
Al	0.98	0.86	0.98	-.66	0.13	1.00				
Zn	0.96	1	0.82	-0.2	031	0.9	1.00			
Cr	0.78	0.95	0.53	0.14	0.66	0.66	0.92	1.00		
Co	0.50	0.17	0.76	-1	0.83	0.66	0.27	0.14	1.00	
Ni	0.99	0.95	0.92	0.46	-0.1	0.97	0.98	0.81	0.46	1.00

The correlation coefficients between metals in green apple plants fruit reflected very good correlation, medium correlation and weak correlation (Table 12). Correlations of metals between the following Ca/Mg, Ca/Fe, Ca/Al, Ca/Zn, Ca/Cr, Ca/Ni, Mg/Fe, Mg/Al, Mg/Cr, Mg/Ni, Fe/Mn, Fe/Al, Fe/Zn, Fe/Co, Mn/Cu, Cu/Co, Al/Ni, Zn/Cr, Zn/Ni and Cr/Ni are very strong. Correlation between the metal of Ca/Cu, Ca/Co, Fe/Cr, Cu/Cr, Al/Cr and Al/Co are moderate. Ca/Mn, Mg/Cu, Mn/Zn and Cu/Ni have weak negative correlation relationships. Ca/Mn and

Mn/Al have moderate negative correlation relationships. Mg/Zn has perfect positive correlation relationships and Mn/Co have perfectively negative correlation relationships.

Table 13. Pearson correlation coefficients between metals concentration in red apple

	Ca	Mg	Fe	M n	Cu	Al	Zn	Cr	Co	Ni
Ca	1.00									
Mg	-0.53	1.00								
Fe	-0.49	0.99	1.00							
M n	0.35	-0.97	-0.98	1.00						
Cu	-0.02	-0.83	-0.86	0.92	1.00					
Al	-0.03	-0.82	-0.85	0.92	0.99	1.00				
Zn	-0.91	0.83	0.80	-0.70	-0.39	-0.38	1.00			
Cr	0.99	-0.50	-0.46	0.31	-0.05	-0.06	-0.89	1.00		
Co	0.55	0.40	0.45	-0.58	-0.84	-0.84	-0.16	0.58	1.00	
Ni	-0.57	0.99	0.99	-0.96	-0.80	-0.80	0.85	-0.54	0.36	1.00

From the Table 13, Pearson correlation coefficient shows strong correlation, moderate correlation and weak correlation relationships. Ca/Cr, Mg/Fe, Mg/Zn, Mg/Ni, Fe/Zn, Fe/Ni, Mn/Cu, Mn/Al, Cu/Al, Zn/Ni have strong positive correlation relationships. Ca/Zn, Mg/Mn, Mg/Cu, Mg/Al, Fe/Mn, Fe/Cu, Fe/Al, Mn/Zn, Cu/Co, Cu/Ni, Al/Co, Al/Ni, Zn/Co have strong negative correlation relationships. Ca/Co and Cr/Co have positive moderate correlation relationships. Ca/Mg, Mg/Cr, Mn/Co, Cu/Cr, Cr/Ni have negative moderate correlation relationships. The correlation relationship between Ca/M n, Mg/Co, Fe/Co, Mn/Cr and Co/Ni have weak positive relationship and Ca/Mn, Ca/Cu, Ca/Al, Fe/Cr, Cu/Zn, Al/Zn, Al/Cr and Zn/Co have weak negative correlation relationship.

4.10. Statistical analysis

Statistical analysis is concerned with the organization and interpretation of data according to well defined, systematic and mathematical procedures and rules. In statistical analysis data are represented by numbers. The value of numerical representation lies largely in the asserted clarity of numbers (Elizabeth Depoy *et al.*, 2016). In this study, samples of apple fruits were collected randomly from six different places. During these processes a number of random errors may be introduced in each aliquot and in each replicate measurement. Therefore depending upon the type and nature of results at hand, a statistical method is used to check whether there is contribution from these random errors for the difference in results of analysis or not. If there is differences statistical analysis it indicates whether the differences are significant or not at specified constant level.

The level of statistical significance is often expressed as a p value between 0 and 1. The smaller the p-value, is the stronger the evidence that should reject the null hypothesis. A p-value less than 0.05 is statistically significant. It indicates strong evidence against the null hypothesis, as there is less than 5% probability the null is correct. Therefore null hypothesis rejected and accept the alternatives. A p-value higher than 0.05 is not statistically significant and indicates weak against the null hypothesis. One way analysis of variance (ANOVA) is used to perform the statistical analysis with apple fruit as independent and concentration of the metals as dependent variable to test whether there are significant differences between means of each plant sample. Microsoft Excel was used to compare the statistical parameters and the result of the analysis is depicted in Table 14.

Table 14. Analysis of variance (ANOVA) within samples of the green apple

Parameters	Metals compared at 95% confidence level									
	Ca	Mg	Fe	Mn	Cu	Al	Zn	Cr	Co	Ni
$F_{critical}$	5.14	5.14	5.14	5.14	5.14	5.14	5.14	5.14	5.14	5.14
$F_{calculated}$	404	263	703	13.3	3.17	36	55.16	30.9	14.6	137
p-value	4×10^{-7}	1.4×10^{-6}	8×10^{-8}	6×10^{-3}	0.11	5×10^{-4}	1.4×1^{-4}	6.9×10^{-4}	4.9×10^{-3}	9.8×10^{-6}

From the results depicted in Table14 there is no significant difference $p \geq 0.05$ for Mn and Cu. All the other metals have significance difference. Their p-value was $p \leq 0.05$. This significance difference and insignificance difference arise from soil composition and environmental affect.

Table 15. Analysis of variance (ANOVA) within samples of red apple

Parameters	Metals Compared at 95% Confidence Level									
	Ca	Mg	Fe	Mn	Cu	Al	Zn	Cr	Co	Ni
$F_{critical}$	5.14	5.14	5.14	5.14	5.14	5.14	5.14	5.14	5.14	5.14
$F_{calculated}$	357	129	113	13.35	0.024	34.2	2.32	1.72	13.1	348
p-value	$6x10^{-7}$	$1.2x10^{-6}$	$2x10^{-5}$	$6 x10^{-3}$	0.97	$5x10^{-4}$	0.17	0.25	$6.4x10^{-3}$	$4.7x10^{-7}$

The result analysis in Table 15 shows there is a significance difference between the mean of metals in red apple. Mn, Cu, Zn, Cr and Co has no significance differences $p \geq 0.05$. The other metals have significance differences. This result may be arisen from the ability of variety of plant to accumulate different amount level of metals.

4.11. Comparison of metal concentration in apple fruit with other literature values

Comparison of metal concentration in apple fruit with other literature values is summarized in Table 16.

Table 16. Comparison of metal content in apple fruit of this study with other literature

	M106	M26	M9	AGA	SGA	WGA	ARA	IRA	WRA
Ele.	Mean±SD	Mean±SD	Mean±SD	Mean±SD	Mean±SD	Mean±SD	Mean±SD	Mean±SD	Mean± SD
Ca	-	-	-	1125 ±104	36275±04	1065± 60	36143±353	1013±35.5	2444± 140
Mg	-	-	-	27.0 ± 1.7	153 ± 10	71.2 ± 6.5	78.3± 4.75	139 ± 4.65	78.7 ±6.47
Fe	3.36 ±1.79	3.23 ±0.74	4.13±2.59	162 ± 12	276 ± 10	103 ±6	129± 9.94	241±15.05	123± 5.19
Mn	-	-	-	13.5+ 1.1	13.5 ± 0.9	13.7 ±0.7	15.3± 0.74	11.5± 1.04	16.3± 1.5
Cu	0.98 ±0.32	0.78 ±0.56	0.93 ±0.87	6.56 ±1.06	5.77 ±0.46	5.13 ± 0.3	7.94± 0.23	4.11± 0.24	12.6± 1.13
Al	-	-	-	88.1 ± 8.1	129±10.15	77.8± 4.01	68.9± 2.11	52.5± 1.53	89.6± 8.15
Zn	0.88 ±0.44	0.74±0.6	0.31± 0.13	41.3 ±3.67	73.6 ±2.52	49.7 ±4.13	47.4± 1.89	54.4± 4.51	51.3± 3.77
Cr	1.27± 0.63	0.99 ±0.58	0.49± 1.37	6.15 ±0.13	10 ±0.79	8.57± 0.61	7.14± 0.18	6.57± 0.61	6.57± 0.41
Co	-	-	-	2.48 ±0.13	2.48 ±0.20	1.85 ±0.10	2.35± 0.17	2.28± 0.23	1.70± 0.04
Ni	0.28± 0.07	0.18 ±0.17	0.19± 0.27	1.33 ±0.07	2.37 ±0.05	1.38± 0.11	1.43± 0.08	7.66± 0.51	1.78± 0.12
Pb	0.51± 0.34	0.38 ±0.26	0.31 ±0.21	ND	ND	ND	ND	ND	ND
Cd	0.009±11.1	0.08 ±0.58	0.003±3.3	ND	ND	ND	ND	ND	ND

Table 16 indicates this research mean concentration is compared with research done by Resmije Imeri; Endrit Kullaj and Lulzim Millaku. From the table a difference between mean concentrations of metals from this research and other literature is concluded.

Mean concentration of iron in the samples from different places is 162, 276, 103 mg/kg for green apple and 129, 241, 123 mg/kg for red apple respectively. But the study done by Resmije *et al.* (2 019) indicates 3.36, 3.23, 4.13 mg/kg.

For copper Table16 indicates concentration of 6.56, 5.77, 5.13 mg/kg for green apple and 7.94, 4.11 and 12.6 mg/kg for red apple sample but, from the study of Resmije *et al*. (2019) mean concentration is 0.98, 0.78, 0.93 mg/kg, respectively.

For zinc concentration in the green apple of this research is 41.3, 73.6, 49.7 mg/kg and in the red apple sample 47.4, 54.4, 51.3 mg/kg but the literature study by Rismije *et al*. (2019) indicates 0.88, 0.74, 0.31 mg/kg, respectively.

Chromium metal from green apple and red apple sample is higher than results from study of Rismije *et al.* (2019) which indicated in Table 16.

The mean concentration for nickel obtained in the green apple and red apple sample of this research is 1.33, 2.37, 1.38mg/kg and 1.43, 7.66, 1.78 mg/kg, respectively, but the literature study by Rismije *et al.* (2019) are 0.28, 0.18, 0.19 mg/kg, respectively.

Table 10 indicate non detected concentration for Pb and Cd, but Table 16 for the study of Rismije *et al.* (2019) indicates mean concentration of Pb 0.51, 0.38, 0.31 mg/kg and Cd 0.009, 0.08 and 0.003 mg/kg, respectively.

4.12. Comparison of results of the present study metals concentration with WHO/FAO joint CODEX, maximum permissible levels (1999)

Table13. Comparison of results of the present study metals concentration with WHO/FAO maximum permissible level of metal in fruit.

Parametres		Metals concentration by mg/kg					
		Cu	Zn	Co	Ni	Pb	Cd
WHO/FAO		40	11	5	0.116	0.3	0.2
Green	AGA	6.56	41.3	2.48	1.33	-	-
	SGA	5.77	73.6	2.48	2.37		-
	WGA	5.13	49.7	1.85	1.38	-	-
Red apple	ARA	7.94	47.4	2.35	1.43	-	-
	IRA	4.11	54.4	2.28	7.66	-	-
	WRA	12	51.3	1.70	1.78	-	-

Table 17 indicates that relationship between the metal content in apple fruit of present study with WHO/FAO report at 1999. The FAO/WHO have set a limit for the heavy metal intake based on body weight for an average adult, namely, 60 kg body weight. The contribution of heavy metal intake for human being from the apple fruit diet is Cu (40 *m*g /kg), Zn (11 mg/kg), Co(5 mg/kg) ,Ni (0.116 mg/kg) respectively. The result of this study indicates that concentration of Cu, Co, Pb and Cd metal were below the permissible level by WHO/FAO report.

5. Conclusion

This study determined two macro essential element (Mg and Ca), eight micro essential elements (Fe, Mn, Cu, Al, Zn, Cr, Co, Ni) and two toxic or non essential elements (Pb and Cd) in green and red apple fruit which were collected from different places. The determination of metals was performed using MP-AES. Pb and Cd were not detected. The optimized digestion method of red apple analysis was found efficient for metals selected. It was evaluated through the recovery experiment and a good percentage recovery was obtained (100±10).

The level of essential metal in apple fruit determined in this study were in the order: calcium (1065-36275) > Fe (103-276) > Al (77.8-129) > Zn (41.3-73.6) > Mg (27-153) > Mn (13.5-13.7) > Cr (6.15-10.04) > Cu (5.13-6.56) > Co (1.85-2.48) mg/kg in the green apple and Ca (1013-36143) > Fe (123-241) > Mg(78.3-139) > Al (52.5-89.6) > Zn (47.4-54.4) > Mn (11.5-16.3) > Cr (6.57-7.14) > Cu (4.11-12.6) > Co (1.70-2.35) > Ni (1.43-7.66) mg/kg in red apple. The non essential heavy metal cadmium and lead were not detected by MP-AES. Comparable results were found with some of the values reported in the literature which indicate some difference due to differences in soil properties and other environmental conditions.

Statistical analysis by using one way ANOVA indicates that there is a significance difference in mean concentration of metals under investigation. In green apple Mn and Cu have no significance differences. In red apple Mn, Cu, Zn, Cr and Co has no significance differences.

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