ADDIS ABABA UNIVERSITY

SCHOOL OF GRADUATE STUDIES

COLLEGE OF NATURAL SCIENCES

DEPARTMENT OF CHEMISTRY

STUDIES ON THE LEVELS OF ESSENTIAL AND NON-ESSENTIAL METALS IN THE RAW AND PROCESSED FOOD (KOLO AND BREAD) OF MAIZE/CORN (*Zea mays* L.) CULTIVATED IN SELECTED AREAS OF ETHIOPIA

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JUNE, 2015

Studies on the levels of essential and non-essential metals in the raw and processed food (kolo and bread) of maize/corn (*Zea mays* L.) cultivated in selected areas of Ethiopia

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A thesis submitted to the school of graduate studies presented in partial fulfillment of the requirements for the degree of Master of Science in chemistry

Addis Ababa University

Addis Ababa, Ethiopia

June, 2015

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SCHOOL OF GRADUATE STUDIES

This is to certify that the thesis prepared by Adane Abebe, entitled: Studies on the levels of essential and non-essential metals in the raw and processed food (kolo and bread) of maize/corn (*Zea mays* L.) cultivated in selected areas of Ethiopia and submitted in partial fulfillment of the requirements for the degree of master of science in chemistry complies with the regulations of the University and meets the accepted standards with respect to originality and quality.

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ACKNOWLEDGEMENTS

First of all I would like to thank the son of St. Virgin Mary who is the creator and governor of everything in this universe and the owner of knowledge, peace and all other things. I thank him together with his mom for upholding me from the scratch of my life to this moment. Then I would like to express my deepest gratitude and appreciation to my research advisor Prof. B. S. Chandravanshi for his dedicated advices, closer help, friendly communications and warmest treatments besides the ideas, suggestions and comments he provided me. His support and encouragement from the beginning to the end of this study is highly appreciated. I have a special respect and appreciation to him, for his immediate responses and comments whenever he is requested.

I am thankful to Dr. Ahmed Mustafa, Head Department of Chemistry, for his unforgettable and fruitful advice he offered me while I was in dilemma. My sincere thanks go to my friends who are in the Ethiopian Conformity Assessment Enterprise, ECAE working as chemical analysts (Abel Anberbir, Belete Eshetu and Zerihun Abebe who helped me during sample preparation) and Mr. Gashaw Tesfaye, director general of ECAE for allowing me to use the chemical testing laboratory, ECAE during sample preparation, my colleagues Dagmawi Shiferaw and Biniam Tezera for their regular aid and moral support. Henok Mekonnen and Temesegen Aragaw for their kind and unreserved support in analyzing the samples and Dr. Nicolo Colombani (an Italian technician from University of Ferreira, Italy) for his strong aid and supervision during his stay here in AAU to provide me and my colleagues practical trainings on AAS, UV-Vis, IC and other equipments in the laboratories of school of Earth sciences, AAU. I would like to use this opportunity to thank Wro. Yewoyneshet, Wro. Kebebush, Ato Sahalemichael Dame and to those who are in the office of the department for their valuable cooperation in providing the necessary materials, chemicals and apparatus during my work.

I would like to acknowledge Mr. Ayalew Debebe for his positive approach in assisting me during sample preparation, optimizing the method and giving some technical advices in the long run of this research. I appreciate his kind support.

My deepest heart-felt gratitude goes to Wro. Bethelhem Tsige, who has been on my side since 2008. Beth! I thank you very much from the bottom most of my heart. Also I would like to thank my brothers Abebaw Amanu, Yilma Alemayehu and my sister Sefi Alemayehu for their unreserved supports. I am also grateful to Wro. Yigardu Jemberu and to my kids Bethelhem Adane and Biniam Adane for their love they are offering me.

Also I would like to acknowledge the School of Earth-Sciences, Addis Ababa University for allowing me to continue my education and to use all the resources available in the laboratories of the school. Really I thank the school and all the staff members there. Also the Department of Chemistry, Addis Ababa University for the financial support and giving me the chance to proceed my M.Sc. study. I am also thankful to Addis Ababa University at large for the sponsorship it offered me.

Finally I would like to thank my mother Wro. Ebstie Kebede, who is always on my side in my ups and downs and always pray to GOD for my success. I love you mom, you are the secret behind my success!!!

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Acronyms and abbreviations

Abbreviation	Expanded form/fully written as:
AAU	Addis Ababa University
AAS	Atomic Absorption Spectrometry
FAO	Food and Agricultural Organization
mg/kg	Milligram per kilogram
µg/kg	Microgram per kilogram
ha	Hectare
km	Kilometers
mg/day	Milligram per day
km^2	Square kilo meters
SD	Standard Deviation
^0C	Degree Celsius
SNNPR	South Nations Nationalities and Peoples Region
CSA	Central Statistics Agency
RATESP	Regional Agriculture Trade Expansion Support Program
KAT	Kembata Alaba Tembaro
IUPAC	International Union of Pure and Applied Chemistry
RDI	Recommended daily intake
UL	Upper limit

Abstract

Maize (*Zea mays* L.) is one of the most widely cultivated cereal crops in the world. It is among the staple food crops produced globally. It is also used for manufacturing of different industrial products. In this study the levels of essential and non-essential metals in raw maize seeds and its processed food (kolo and bread) were determined in samples collected from three selected areas (Shendi, Finote Selam and Debre Tabor), Ethiopia. After proper sample pretreatment, the volumes of reagents to be used, digestion temperature and digestion time were optimized. Then using the optimized conditions sample preparation was made and levels of metals were determined by flame atomic absorption spectrometry (for major metals) and graphite furnace atomic absorption spectrometry (for trace and heavy metals). The accuracy of the optimized procedure was evaluated by analyzing the digest of the spiked samples with standard solution and the percentage recoveries varied from 91.2% to 109%. The concentration determined (mg/kg dry weight) were in the ranges K (1589–2036), Na (125–195), Mg (304–411), Ca (66.5–178), Cr (0.17–1.72), Mn (0.52–3.98), Fe (16.5 –146), Co (0.34–0.76), Cu (0.04–2.72), Zn (59.2–116) and Pb (0.31–3.41) but the concentration of Cd and Ni were below the detection limit. K and Fe were with the highest concentration from major essential and trace metals, respectively. A statistical analysis of variance (ANOVA) at 95% confidence level indicated that there is significant difference in the levels of all metals among the three samples means except K, Mg and Pb. The results indicate that Ethiopian maize seed is a good source of essential metals and free from the toxic metal Cd but not from Pb, the concentration of Pb is higher than the WHO guideline (0.2 mg/kg).

Key words: Maize seed (*Zea mays* L.), essential metals, non-essential metals, flame atomic absorption spectrophotometry, graphite furnace atomic absorption spectrophotometry

1. INTRODUCTION

Maize/corn belongs to the family Poaceae, genus Zea and species *Zea mays* – corn. Maize/corn, is one of the most extensively cultivated and consumed cereal crops in the world (Fig. 1). It is the main cereal grain as measured by production but ranks third as a staple food, after wheat and rice. The reasons for this fact are varied, but some of them are related to cultural or social preferences (FAO, 1992).

Fig. 1. Maize plant and the various colors of its seeds

More maize is produced, by weight, than any other grain, and almost every country on the Earth cultivates maize commercially for a variety of uses. Currently, the United States, China, Brazil, Mexico, Argentina, India, France, Indonesia, South Africa, and Italy produce 79% of the world's maize production (Gwirtz and Garcia-Casal, 2014).

1.1. Background of the Study

Maize was the staple food for most pre-Columbian, Mesoamerican, South American and Caribbean cultures, whose life revolved around the milpa (cornfield), this was because maize was nutritious, easy to store and carry, adapted to diverse growing conditions and provided food and fuel. The crop is still associated with the Mesoamerican peoples' identity. Later it spread to North and South America and was brought by Columbus to Spain, from where it was further distributed throughout the world (McCann, 2005).

Maize has been introduced in Africa, and later in other tropical countries, mainly by Portuguese and Arab explorers in West and East Africa, from where it spread inland through the slave-trade routes, and later to Asia. Because of its wide climatic adaptability maize cultivation expanded rapidly and the grain became soon a part of the local diet as a diversification of traditional root crops (cassava, yams, sweet potatoes) and various small grains. Maize is now cultivated in almost all countries in the world except Antarctica (McCann, 2005).

The large and sudden rise in maize cultivation in some African countries both in terms of yield and in area planted since the 1980s followed the introduction of different new hybrids from the USA and South America. The expansion of maize on the African continent has several reasons. First, its taste has been easily accepted by the local population and, therefore, it could rapidly replace traditional starchy foods like sorghum and millets. It also became important when foodstuffs had to be transported to feed labor and populations which were not self-sufficient. The additional reasons for the rapid adoption and expansion of maize in Africa are: (i) it gives one of the highest yields per person/hour of labor spent on it; (ii) it provides nutrients in a compact form; it is easily transportable;

(iii) the husks give protection against birds and rain; (iv) it is easy to harvest and does not shatter; (v) it stores well if properly dried; (vi) it can be harvested over a long period, first as immature cobs, but can be left standing in the field at maturity before harvesting; (vii) cultivars with different maturing periods are available; (viii) in terms of taste, many people prefer maize to their local cereals (Purseglove, 1975; Pingali and Heisey, 1999). About two thirds of all African maize is produced in eastern and southern Africa. South Africa is the largest producer of maize with about 35% of the total regional production. Nigeria is the second most important producer (USDA, 2007).

1.2. Cultivation of maize

Corn is a summer crop that is best grown in a climate that offers warm weather and long sun-filled days. Corn is sensitive to frost, and if planted too early, an entire crop can be lost. The time for corn to grow to harvest can vary considerably among varieties of seeds (Belfield and Brown, 2008). The optimum temperature for maize growth and development is 18 to 32 °C, with temperatures of 35 °C and above considered inhibitory. The optimum soil temperatures for germination and early seedling growth are 12 °C or greater, and at tassel ling 21 to 30 °C is ideal. Maize can grow and yield with as little as 300 mm rainfall (40% to 60% yield decline compared to optimal conditions), but prefers 500 to 1200 mm as the optimal range. Maize is grown globally from 50°N to 40°S, and from sea level up to 4000 m altitude. Besides warm sunny weather, corn requires nutrient rich and moist soil. The ideal soil for growing corn is well-drained, preferably a sandy loam. Organic matter such as compost leaves and grass clippings can be added to soil to improve its overall quality and improve drainage, particularly for heavy clay soil. Like most vegetables, corn grows best in soil with a pH between 5.8 and 6.8. Corn needs soil with high levels of nitrogen for proper growth and development; thus, additional fertilizer can be added at planting or during the growing season. Yellowing leaves are a common sign of nitrogen deficiency. In the cases when the pH of the soil is outside the recommended limit, lime or sulfur can be added to adjust the pH to an ideal level for growing corn (Belfield and Brown, 2008).

1.3. Cultivation of maize in Ethiopia

Among cereals, maize accounts for the largest share in total production and the total number of farm holdings involved in the country. In 2010/11, maize accounted for 28% of the total cereal production, compared to 20% for teff and 22% for sorghum, the second and third most cultivated crops in the country. Maize is the largest and the most productive crop in Ethiopia.

According to the data of the Central Statistical Agency (CSA), in 2007/08, maize production was 3.75 million tons, this was 25% higher than teff and 41% higher than sorghum. At 2.5 tons per hectare, maize yield is the highest among cereal crops. Ethiopia is the fourth largest maize producing country in Africa next to South Africa, Nigeria and Egypt (USDA, 2007). The crop is widely cultivated at altitudes ranging from 1500-2200 meters above sea level of Western, Southwestern, and Southern parts of the country.

Currently, maize production in Ethiopia is exercised using both the traditional methods and extension package. The extension package is of a green revolution type characterized by the use of high yielding seed varieties, fertilizers and chemicals. According to World Bank (2006) farmers were only achieving on average 60% of their potential production. The potential for increasing maize production through extensive use of improved seeds is thus high in Ethiopia and is being increased today (Dawit et al., 2008).

Three regional states including Oromia, Amhara and SNNP contribute to 94% of the total annual production. Oromia region alone contributes to 60% of the country's maize production. West Gojam, East Showa, Jimma, East Welega and West Welega zones are major producing areas and together contribute to 60% of total production (World Bank, 2006).

Both organic and inorganic fertilizers are being used in the country. Organic fertilizers are farm-generated resources such as crop residues, farmyard manure and legumes. Inorganic (modern) fertilizer is a peculiar input the use of which requires improved varieties of maize seeds. Di-ammonium phosphate (DAP) and urea are the two most widely adopted inorganic fertilizers in Ethiopia (World Bank, 2006).

1.4. The types of maize

A number of maize types can be discerned on the basis of endosperm and kernel composition (Purseglove, 1972; Paliwal, 2000; Darrah et al., 2003). The great diversity that typifies maize germplasm is also evident in kernel characteristics. Kernels may be colorless (white) or solid yellow, red, blue or mottled in color. It can be grouped into various groups on the basis of the endosperm of kernels.

Flint maize kernels are characterized by their high percentage of hard endosperm around a small soft centre. Flint maize is grown predominantly in Latin America and Europe for food use. The color may be white or yellow. It is grown in Europe, Asia, Central America and South America as well it is a principal type of maize grown in India **(**www.starch.dk, 2015; www.corn.org, 2013).

Dent maize is the most commonly grown for grain and silage, and is the predominant type grown in the USA. Hard endosperm is present on the sides and base of the kernel. The remainder of the kernel is filled with soft starch; when the grain starts drying the soft starch at the top of the kernel contracts, producing the depression for which it is named (www.starch.dk, 2015; www.corn.org, 2013).

Field corn: In the USA it is used mainly to feed livestock, but in other countries it is used for human consumption as well **(**www.starch.dk, 2015; www.corn.org, 2013).

Sweet corn, also called sweet maize, this type of maize is most commonly eaten in the USA. It is a genetic variation that accumulates more sugar and less starch in the kernels; it is usually shorter than field corn. The developing grain of sweet maize is higher in sugar content due to one or more recessive mutations blocking conversion of sugar to starch **(**www.starch.dk, 2015; www.corn.org, 2013).

Baby corn, popularly used in Asian cuisine, is a variety of maize developed to produce many small ears, rather than a few larger ones. The ears are harvested very young while they are still immature, and are tender enough for the whole ear to be eaten. It is grown for young babies (cobs) to be used for vegetable soup and salad. Baby corn is rich in minerals and vitamins **(**www.starch.dk, 2015; www.corn.org, 2013).

Popcorn, the ability of maize kernels to “pop” and expand upon heating, was also discovered by the Native Americans. Maize is able to pop because, unlike other grains, its kernels have a hard moisture-sealing hull and a dense starchy filling. When heated, pressure builds inside the kernel until explosive “pop” results, and the starch expands and then hardens in the cooler air. Many maize varieties will pop, but some varieties have been specifically cultivated for this purpose. Pop maize is grown on a small scale compared to other types but popped kernels are consumed world-wide as a snack food (www.americanmaze.com, 2015; www.starch.dk, 2015; www.corn.org, 2013).

Indian corn was originally the term applied to what we now know as maize or corn, to differentiate it from the generic term of “corn” Europeans used for all grains at that time. Now, it usually refers to any corn that has different colored kernels. Usually it is dried and used for ornamental purposes **(**www.starch.dk, 2015; www.corn.org, 2013).

M**aize flour, or meal**, is made into a thick porridge in many cultures. The grains are composed of soft starch and have little or no dent. That means its endosperm is mainly composed of soft starch, making it easy to grind and process into foods. Maize meal is also used as a replacement for wheat flour, to make cornbread and other baked products. Flour corn is also known as soft corn. The kernels are soft and can be of all colors, but white and blue are the most common ones. Soft corn resembles to the flint corn in appearance and characteristics (www.starch.dk, 2015; www.corn.org, 2013).

1.5 . Uses of maize

Uses of maize as food source: In developing countries, up to 30% of the average maize produced is used for the direct human consumption. In sub-Saharan Africa, about 70% of the maize crop is used for human consumption. Green maize (fresh, on the cob) is an important food source early in the rainy season. Another common food use of maize in Nigeria, ‘Eko’, which is fermented, boiled and consumed with a spicy stew.

Industrial uses of maize: Maize is refined to generate a wide range of products including corn oil, sweetener, corn starch, and ethanol. New bio-products such as amino acids, antibiotics and degradable plastics are increasingly being synthesized using maize as a raw material. Maize is wet-milled to separate the grain into components (starch, oil, protein and fiber) which are then converted into higher value products Available at (http://www.corn.org/theprocess.htm, 2014.

New uses for maize: Scientists continually look for new uses of maize, especially environmentally friendly uses. One of these is as a bio-fuel. Another new use is as a biodegradable plastic. Polymers created from maize (specifically from the lactic acid that is generated during the fermentation process) are biodegradable and safer for the environment. Cornstarch, produced from the starch in the maize kernel, can also be used to make foam products similar to packing peanuts and package inserts. These maize-derived products are much better for the environment as they are biodegradable and made from renewable resources (Futon et al., 2011).

1.5.1. Uses of maize in Ethiopia

Six major staples: maize, teff, wheat, sorghum, barley and enset (false banana), dominate the national food basket in Ethiopia. Maize is the single most important cereal, accounting for 17% of the per capita calorie intake, followed by sorghum (14%) and teff (11%) (Rashid et al., 2010).

Maize dominates rural consumption baskets, more than that of the urban areas. Maize consumption has expanded mainly at the expense of teff. Maize is an important food security crop in Ethiopia, with the cheapest cost caloric source among all major cereals. Despite having the largest number of livestock in Africa, the use of maize grain as animal feed is very limited in Ethiopia (Rashid et al., 2010.

In Ethiopia, maize is mainly used for food and feed purpose. The stover is also used for construction and as a domestic fuel in the rural areas of the country, also as food for cattle and other animals. Though maize is mainly used for human consumption, its share in the total calorie intake in Ethiopia is lower when compared to other African countries (Reynolds 1999, Berhanu et al. 2007). In addition to its usage as food in different forms like bread, “kolo”, porridge, injera, and other forms, maize is used as the source of starch for traditional alcoholic beverages production like tella and katikala.

1.5.2. Health benefits and side effects of consuming maize (*Zea mays* L.)

The composition of maize endows it with many health benefits. The high fiber content is one characteristic linked to the nutritional benefits of maize. This condition makes it suitable for diets that are made to lose weight and those made with the aim of lowering cholesterol levels. The following can be mentioned as the major health benefits of maize: (i) Maize flour is used to make nutritious bread which is highly palatable, and is easily broken down in the body. When taken at intervals, bread helps to clean the colon and the dextrose produced is commonly used for medicinal purposes. (ii) Popcorn is a whole some staple food made by heating small grains. It is easily digested by the body. In addition, it is practically starch-free and not fattening, and is converted into intermediate carbohydrates and dextrin, which is easily absorbed in the body. It promotes peristalsis and is also beneficial in preventing constipation. (iii) Maize facilitates the removal of toxic food substances and also accelerates the passage of feces through the intestine. And, it protects the digestive tract thus promoting function of the gall-bladder and reducing stomach acidity. (iv) According to recent studies, the use of maize helps to combat the effects of certain cancers; this is because consuming maize reduces the development of cancer. (v) Maize is low in cholesterol and fat content. The fiber in whole grains helps to prevent the risk of heart diseases and diabetes, and all its nutrients boost the immune system (ISRN, 2013).

Although maize is not fattening, it is a starchy food crop, meaning it does contain carbohydrates. Consuming too much carbohydrates and overall calories can lead to weight and fat gain. So in contrary to the above benefits, consumption of corn has allergic effects on some adults and children. As corn is consumed by a large number of people, the number of corn allergy cases is increasing. Vomiting, migraine headache, rash, abdominal pain and bloating, nausea, itchy skin, swelling of tongue and mouth and fever are the minor symptoms of corn allergy. Diarrhea, urinary tract infection, allergic asthma, and arthritis are some of the severe symptoms of corn allergy. Anaphylaxis is the severest symptom of corn allergy. Although a rare condition, it can be life-threatening. It can lead to impairment of several bodily functions.

1.5.3. Maize nutritional value and chemical composition

Maize, being popular as a food item, is enjoyed by people in various forms, like, whole corn, corn flour, cornstarch, corn gluten, corn syrup, cornmeal, corn oil, popcorn, cornflakes, etc. Apart from satisfying the taste buds of its users, maize is also a good source of vitamins, minerals and dietary fiber.

The nutritional value of cornflakes is almost similar to that of cooked maize. One large ear of cooked yellow maize contains almost 4 g of protein, 3.5 g of dietary fiber, around 30 g of carbohydrates, 1.5 g of fat, 3.6 g of sugar, around 100 g of water, no cholesterol and amounts to 126 calories. The maize kernel is composed of four primary structures. They are endosperm, germ, pericarp, and tip cap, making up to 83%, 11%, 5%, and 1% of the maize kernel, respectively. The endosperm is primarily starch surrounded by a protein matrix (www.starch.dk, 2015; www.corn.org, 2013).

1.6. Popcorn and cornflake

1.6 1. Popcorn

Popcorn is scientifically known as *Zea mays everta*. It is a type of maize, or corn, and is a member of the grass family (Encyclopedia popcornica, 2015). Popcorn is a whole grain and is made up of three components: the germ, endosperm, and pericarp (or hull). Of the other types of corn it is only popcorn that pops! Popcorn differs from other types of corn in that its hull has just the right thickness to allow it to burst open. Each kernel of popcorn contains a small drop of water stored inside a circle of soft starch. Popcorn needs between 13.5-14% moisture to pop. The soft starch is surrounded by the kernel's hard outer surface. As the kernel heats up, the water begins to expand. With increasing temperature the water turns into steam and changes the starch inside each kernel into a superhot gelatinous goop. The pressure inside the grain increases, finally bursting the hull open. As it explodes, steam inside the kernel is released. The soft starch inside the popcorn becomes inflated and spills out, cooling immediately and forming into the odd shape we know and love (Fig. 2) (Encyclopedia popcornica, 2015).

Fig. 2. Unpopped and popped popcorn

Popcorn varieties are quite different from either sweet corn or field corn varieties. White, yellow, red, and black kernel varieties are available, but only white and yellow (both small and large kernels) are grown commercially. High-quality popcorn has a moisture content of 13.5%. Moisture content over or under these percentages greatly reduces popability (Encyclopedia popcornica, 2015).

The health benefits of popcorn are mainly derived from its impressive content of fiber, polyphenolic compounds, antioxidants, vitamin B complex, Mn and Mg. It avoids constipation, reduces the overall level of cholesterol from the body, its fiber regulates the release and management of blood sugar and insulin levels better than people with low levels of the fiber. Reducing these fluctuations in blood sugar is a major bonus for diabetic patients, for cancer prevention, used as anti-aging, and for weight loss, this is because popcorn has low caloric value. In addition to the above uses popcorn can be used for Christmas tree decoration and to produce biodegradable packing materials (http://en.wikipedia.org/wiki/popcorn, 2015).

Popcorn in Ethiopia: Ethiopians pop popcorn mostly during coffee ceremonies, especially in cities and towns. They are popping it at homes following a simple procedure. The extraneous matter from the popcorn is first removed and rubbed properly to make it free from dust and any other matter which is sticking to the surface of the popcorn. A small amount of oil or butter is added to the metal dish on a heating source. The unpopped corn kernels are then transferred to the dish. The dish with its content is covered with its lid and heating will be continued with frequent swirling by hand using heat resistant material, usually a piece of cloth. At the appropriate pressure and temperature the popcorn pops producing “POP! sound.

1.6. 2. Cornflakes

According to collinsdictionary.com (2015), cornflakes are defined as small flat pieces of maize that are eaten with milk as a breakfast cereal or toasted flakes made from the coarse meal of hulled corn for use as a breakfast cereal (Fig. 3). Cornflakes are good sources of vitamins, minerals, folate, dietary fiber, as well as proteins, and carbohydrates. Corn flakes are very rich in thiamine and have high iron content. Iron helps to keep the brain alert. Corn also contains a carotenoid called beta-cryptoxanthin, which is good for the health of the lungs and also prevents lung cancer. One of the benefits of corn is it provides lutein which the body cannot produce. Lutein is an important nutrient for eye health (http://www.organonutri.in/health, 2015).

The following are the health benefits of consuming cornflakes: (i) rich in complex carbohydrate and they offer energy for the body, (ii) are enriched in thiamine, riboflavin, iron, niacin and fiber that make it a complete food, (iii) avoids constipation problems, (iv) reduces blood pressure, (v) used to regulate weight, if one takes a bowl of cornflakes in the morning, his/her tummy gets filled with rich fiber and it satisfies his/her hunger, so that there will be no accumulation of excess food in the body**(**http://www.organonutri.in/health, 2015).

The following could be considered as limitations/side effects of consuming cornflakes: Commercially available cornflakes are rich in sugar, salt and fat which make them unhealthy if consumed in excess quantity. Grains are generally acidic in nature and causes acidic state in the blood and tissues. In case a person suffering from irritable bowel syndrome, the conditions may aggravate further with consumption of cornflake.

Fig. 3. A picture of cornflake

The major raw materials for the production of corn flakes are maize. Sugar is another raw material used for the manufacture of corn flakes other ingredients like flavors can also be included in the process. The milling process removes the corn kernels from the cobs and turns them into flaking 13 sized ‘grits’. Malted barley can be added to enhance the flavor of the cornflakes. The corn grits are cooked in steam pressure cookers, at temperatures exceeding 100 ^{0}C. This cooking process lasts for an hour and softens the hard grits. During cooking additional water is incorporated in the form of steam which condenses and the water content in the batch rises to 30-35%. Then the hot grits are transported from the cookers to large driers via the network of pipes. The grits spend several hours in the hot-air driers in order to reduce their moisture content. The corn grits are milled using rollers, which squeeze the grits flat (http://drying-machinery.com/process_description.html, 2015).

The flakes are then tumble toasted in huge cylindrical ovens. The air in the ovens is heated by 600 ^{0}C gas flames and the flakes are tossed around in a rotating drum. The drum is angled so that the flakes whirl around and pass through it quite quickly, and stops them spending too long in the fierce heat. The process of cornflake production can be shown as:

Corn → Cleaning → Peeling and degerming → Classifying → Cooking → Drying and Relending → Rolling, Flaking or Extruding → Toasting → Cooling → Packing → Finished Product (http://drying-machinery.com/process_description.html, 2015).

It is also possible to produce multigrain flakes by mixing cereals, such as wheat, rice, corn and other cereals.

Corn flakes in Ethiopia: The technology of cornflake production in Ethiopia is very new and most of the people in Ethiopia are not aware of this technology. As a result of this limitation the production and consumption of cornflake is very limited. In fact very few families in the cities are using this food purchasing from supermarkets which are imported from the abroad. Almost all cultivars of corn in the country have no ideas about cornflakes. It is in the form of bread, kolo, injera, and other forms that people in Ethiopia are consuming corn, but not as cornflakes.

In the recent time some private owners are promoting the technology and the production of corn flakes in the cities. In the near future the cornflake producing companies and the consumption rates will be increased.

1.7. Classification and uses of nutritional minerals

1.7.1. Macro-essential metals (macro minerals)

Life on the earth is built from a relatively small number of chemical elements. The most important ones include calcium, magnesium, sodium, potassium, sulfur, chlorine and phosphorus. These are sometimes called the macro-minerals. These are found in the large quantity in our bodies. Blood levels of these elements in human body remain fairly constant. If they vary even a little, especially for calcium, magnesium, sodium and potassium the person feels quite ill and it is a bad sign (www.powerhealth.org, 2015).

Calcium, the structural element, is found mainly in our bones. Most individuals are aware of the benefits of calcium supplementation. It is important for bone and teeth formation and structure. Calcium, also appears to play a role in maintaining normal blood pressure. In addition to this, it is required by the body for blood clotting, muscle contraction and nerve transmission. This is because, it (calcium) is a component of enzymes that contribute to blood clotting, muscle activity and nerve function. It also regulates cell membrane permeability to control nerve impulse transmission and muscle contraction. It regulates hormonal secretion and cell division. Calcium may play a role in triglyceride and cholesterol reduction due to its fat binding properties within the gastrointestinal tract. Good food sources of calcium are dairy products such as cheese and yogurt, fortified bread and flower, yoghurt, tofu, cereals and dark green vegetables, milk, sardines, egg yolks, almonds, sesame seeds, and seaweed (Wilson, 2012).

It is also known that over dose consumption of minerals may upset the normal functioning of the body. Toxicity of calcium occurs with increased intake of calcium. Calcium competes with a number of minerals for absorption therefore supplementation with a multi-mineral may be necessary to prevent decreased levels of other minerals due to calcium. Calcium supplements are usually the cause for the overdose. Bones and teeth are the main storage tissues of calcium in the body (Wilson, 2012).

Magnesium (Mg): It is a component of many coenzymes that regulate sugar metabolism, energy production, cell membrane permeability, and muscle and nerve conduction, Involved in thyroid/parathyroid production. It is an important factor of bone formation. As calcium is needed for muscle contraction, magnesium is needed for muscle relaxation. Too much calcium and not enough magnesium can cause pre-menstrual syndrome and a heart and other muscle cramps. Magnesium activates an enzyme located in all cell membranes. This enzyme controls the balance of sodium and potassium, keeping sodium in the fluid outside the cells and potassium inside the cells. Such a balance is essential for normal water balance, nerve cell activity and cellular energy production. Without sufficient cellular magnesium, potassium will be rapidly excreted from the body resulting in fatigue, heat exhaustion and weakness. Severe magnesium deficiency may cause muscle weakness, fatigue, abnormal heart rhythms, and depression (Howard et al., 1992).

Foods high in magnesium include milk, almonds, brazil nuts, cashews, whole soybeans, parsnips, wheat bran, whole grains, green vegetables, seafood, kelp, fish , nuts, beans, pumpkin seeds, spinach, potatoes and some bread, cereal products and molasses. Bone (60% of body stores), muscle, and liver are the storage sites of magnesium in the body. Most people need more magnesium than they are eating because food refining strips away magnesium (Wilson, 2012).

Potassium: Potassium, another solvent mineral also called a heart mineral regulates intracellular osmotic pressure, cell membrane potential and charge, thereby controlling nerve impulse, controls blood pressure by modifying smooth muscle activity and salt excretion. It is also essential for regulation of the heart beat, fluid balance and to maintain blood pressure. It is also needed for buffering the blood, and cell membrane effects including nerve transmission and muscular contraction. Too much/overdose consumption of K may lead to hyperkalemia in patients with cardiac irregularities and renal failure. Deficiency can cause cramps, fatigue and heart irregularities. Good sources of potassium are vegetables, fruits, meats, milk, prunes, beans, lentils, broccoli, potatoes, sardines, halibut, goose, most nuts and seeds, watercress, garlic, spinach, artichokes, lima beans, Swiss chard, avocados, buckwheat, wheat bran, molasses, and kelp (Wilson, 2012).

Sodium regulates extra-cellular electrolyte/cation ratio and osmotic homeostasis (fluid balance), thereby regulating blood volume and blood pressure, transport of carbon dioxide, and affects cell membrane permeability (essential for the transport of nutrients across the cell membranes) and other cell membrane functions. It is also important for the conversion of essential fatty acids to phospholipids. Sea salt, seafood, eggs, beet greens, Swiss chard, olives, peas, butter, haddock, milk, processed meats and fish, canned vegetables are good sources of Na. Table salt is a refined junk food for Na. People with strenuous physical activity; pregnancy; diuretic therapy; hypochlorhydria; chronic infections; hyper adrenalism; excessive weight loss; reproductive disorders are in exceptional needs of Na. Excessive intake of Na may contribute to hypertension. On the other hand prolonged periods of vomiting, diarrhea and diaphoresis; decreased calcium absorption; decreased vitamin "C" in the adrenals; hypochlorhydria; reproductive disorders, fatigue and fluid imbalances as well as low blood pressure are some of the results of Na deficiency. Bony skeleton and intra/extracellular spaces are the major storing tissues of Na (Wilson, 2012).

1.7.2. The trace elements (trace essential metals)

Though needed in small amounts, trace minerals are absolutely essential for life. They include Fe, Cu, Mn, Zn,Cr, Se, Li, Co, Si, B and probably a dozen of others. The trace essential (micro-essential) minerals considered in this particular study are Cr, Mn, Fe, Co, Ni, Cu and Zn. The daily allowances of these metals differ from person to person based upon the developmental levels, sex as well as the standards of the different countries they set. According to USA standards RDI of Cr, Mn, Fe, Cu and Zn are 0.03, 2.3, 8, 0.9 and 11 mg/day for matured adults, and 0.045, 2.6, 9, 1.3 and 13 mg/day for lactating females respectively (FNB, 2001). However, the value for Fe exceeds the listed values in pregnancy period and becomes 27 mg/day.

Cobalt, a vitamin B_{12} mineral is essential for life as part of the vitamin B_{12} molecule that means it is an intrinsic part of vitamin B_{12} (cyanocobalamin); required for the synthesis of methionine from homocysteine and the conversion of methylmalonic acid to succinyl CoA for fatty acid synthesis; necessary for folic acid synthesis. Vitamin B_{12} is required for the nervous system and blood formation. It is found in animal products such as kidney, beef, lamb, veal, poultry, ocean fish, milk, cheese and eggs. Bone, Muscle, and Kidney are the storage tissues of cobalt in the body. Increased intake of Co may cause hyperthyroidism and proliferation of other cancerous tissues; toxicity increased by excessive ethanol ingestion. Deficiency occurs mainly in strict vegetarians and in those with impaired digestion or any disorder of the stomach. It is commonly deficient to some degree in elderly people whose stomach just does not absorb it very well. It is very inexpensive insurance against the serious consequences of a vitamin B_{12} deficiency. People with hypertension; ethanol abuse; hypochlorhydria; vegetarian diets and pernicious anemia need cobalt in special order (Wilson, 2012).

Despite being a heavy metal, Cu meets the criteria of essentiality for plants and microorganisms, being classified as micronutrient (Lima et al.; 2006).

It is considered as a female element because it is needed more for certain functions in women. It is called the emotional mineral, because it tends to enhance all emotions when it is high in the body. It is extremely important for women's fertility and sexual function, and its levels often vary up and down with the level of estrogen. Copper is also required for healthy arteries, pigments in hair and skin, blood formation, as co-enzyme for energy metabolism, anti-inflammatory, maintains blood vessel wall integrity, important for iron absorption and utilization and other biological activities. Too much copper causes a wide variety of common symptoms for all human kind, especially for women. Depression, irritation of the gastrointestinal tract, mental/ physical fatigue, acne, migraine headaches, moodiness, autistic tendencies in babies and children, infertility and premenstrual tension are the most common ones. Liver, fish, soybeans, meats, seafood, nuts/seeds, beans whole grains, and legumes, chocolate are some of the dietary sources of copper. People with high tissue copper are often bright, young-looking, creative and emotional Liver, brain, and bile are the storage tissues of copper in the body. Excess copper is more common than deficiency in the body today, due to the use of copper water pipes, birth control pills, vegetarian diets and other causes (Wilson, 2012).

Iron is an oxygen carrier and energy mineral as well. It is required in hemoglobin for transporting oxygen in the blood and muscle tissue, for detoxification and energy production in the cells; it is involved in electron transport and oxidative phosphorylation; it also helps to improve muscle functions. People with pregnancy, menstruation, acute/chronic blood loss; vegetarian women; infancy, especially pre-maturity; bacterial infections; liver disease; cancer and estrogen therapy are exceptionally in need of high level of iron. Iron is found in lean meats, organ meats, shellfish, molasses, beans, whole-grain cereals, spinach, soybeans, dried legumes, clams, oysters, dried fruits, and egg yolk and dark green vegetables. Liver and spleen are the main storage tissues of iron in the body. Menstruating women and children on poor diets are most commonly low in iron. The intake of absorbable iron by people in the developing countries is often low, and a disease due to iron deficiency called anemia is common. Both oxidation states of Fe, ferric and ferrous, are absorbed well, providing that they are in an ionized form. Moreover, both forms are soluble at the pH found in the stomach. Ascorbic acid (vitamin C) markedly increases the absorption of non-heme Fe from various sources (Hallberg et al., 1989). Ascorbate functions to improve Fe absorption by converting ferric Fe to ferrous Fe which, as noted above, is more soluble. Enhancement of Fe absorption by ascorbic acid does not affect Mn absorption, and Mn absorption generally is not affected by many dietary factors that influence Fe absorption (Davidsson et al., 1991). In addition to ascorbate, other dietary organic acids, including citric acid, malic acid, tartaric acid and lactic acid, enhance Fe absorption (Bothwell et al., 1979). Vitamin A is another dietary factor that may affect Fe bioavailability.

In the human body, Mn displays a somewhat unique behavior with regard to its toxicity. It is relatively non-toxic to the adult organism except to the brain where it causes Parkinson-like symptoms when inhaled even at moderate amounts over longer periods of time. This element, when in large amounts, affect fertility in mammals and are toxic to the embryo and fetus, what implicates that pregnant women should not be exposed to Mn anytime. Mn is important for healthy tendons and ligaments, and for fat and sugar metabolism. High doses of manganese may cause hypertension and irreversible neurologic disorders; also may interfere with the metabolism of other metals/minerals. Manganese sources are nuts, especially walnuts, bran, corn, parsley, tea and wheat germ, whole grain cereals, leafy vegetables. Liver, kidney, pineal/pituitary glands and pancreas are the major storing tissues of Mn (Wilson, 2012).

Nickel metal and its compounds are used in multiple applications as stainless steel and other alloys, castings, catalysts, batteries, electronics, ceramics, pigments and even coins. Although not an element extensively released to the environment, Ni can present a risk to human health. The main danger of Ni to man besides the carcinogenicity is related to the ability to cause sensitivity reactions. The most common harmful health effect in the general population is allergic contact dermatitis elicited by prolonged skin contact of sensitized individuals with Ni. On the molecular level the toxic Ni species responsible for severe health effects, as allergic contact dermatitis and respiratory tract cancer, has been suggested to be caused by Ni^{2+}. Ni affects the following organ systems: cardiovascular (heart and blood vessels), dermal (skin), immunological (immune system), respiratory (from the nose to the lungs) (Wilson, 2012; Leonard et al., 1981).

Zinc, is a male mineral, so called because it is more essential for men than for women in some ways, although it is certainly essential for women as well. It is required as co-enzyme in several enzymes in the human body. These include the sense of taste and smell, vision, growth, sexual development, digestive enzyme production, male potency, prostate gland health, blood sugar regulation and processing of alcohol. Zinc is very important for the joints, the skin, wound healing, and to prevent birth defects. Zn is required for nucleic acid synthesis and carbon dioxide transport; essential for normal growth, healing, and immune function. Zn prevents diabetes and also helps to detoxify heavy metals. Adequate zinc has a calming effect and is needed to regenerate all body tissues. There are very few excellent sources of zinc today. Among the best are red meats, organ meats and some seafood. Poultry such as chicken and turkey, eggs, wheat, oatmeal, pumpkin and sunflower seeds, wheat germ and colostrums are the other sources but to a lesser amount. Three major factors are associated with the development of zinc deficiency in the body: dietary inadequacies; disease states which induce excessive losses or impair utilization of zinc; and physiological states that increase zinc requirements (Hotz and Brown, 2004). Of these, inadequate dietary intakes are the major factors that can be attributed to low intakes of zinc and to poor bioavailability. Vegetarians are in a high risk of zinc deficiency because they avoid red meats, in most cases. Low zinc, especially in vegetarians, tends to cause a worsening of copper toxicity. Excessive intake of Zn could result in impaired immune response, diaphoresis, ethanol intolerance, chronic nausea and vomiting. All organs, tissues and fluids in the mammalian body contain Zn. Muscles, bones, skin and liver have the largest pools of Zn in humans (Wastney et al., 1986) and in animals (House and Wastney, 1997). Prostate, skin, and retina are the storing tissues for Zn in the body.

1.7.3. Toxic metals (non-essential metals)

Toxic metals are among the major causes of health problems on earth today. They can cause every imaginable symptom. When the body is missing vital minerals in the diet such as calcium, magnesium, potassium and zinc, it absorbs toxic minerals from the environment to keep functioning. 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). The toxic metals include lead, mercury, cadmium, arsenic, aluminum, nickel, antimony, beryllium and others. These often function in enzymes to some extent, but not nearly as well as the physiological mineral. All toxic metals are neurotoxic.

Cadmium (Cd): It is one of the most toxic metals, presenting important harmful effects for biological activity on soil, plant metabolism, human and animal health. Cd has been mainly used in manufacturing Ni-Cd batteries, though, also has other applications as pigments, coatings, stabilizers for PVC (polyvinyl chloride) and alloys. In humans, Cd affects several organ systems, and can cause high blood pressure, heart disease, cancer, fatigue, arthritis, violence, infections, back pain and other problems. Tobacco smoke - whether cigarette, pipe smoke or other form of tobacco smoke contains Cd. 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 secondhand smoke. Inhalation of fumes and dust of Cd is extremely irritating to the lungs and can lead to such symptoms as headaches, chills, muscle aches, nausea, vomiting, and diarrhea. The human body can tolerate low levels of Cd but long-term chronic exposure can lead to serious health problems (ICdA, 2001).

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 (rhinitis); joint soreness; hair loss; dry, scaly skin; and loss of appetite. Cd toxicity threatens the health of the body by weakening the immune system. Because Cd is retained in the kidneys and liver (50 to 70 percent of accumulated Cd is deposited in these organs), excessive exposure can lead to kidney disease and serious liver damage (http://www.moondragon.org/health/ disorders/cadtox.html, 2015).

Chromium (Cr): Chromium, a blood sugar mineral is also called an energy mineral. It is essential for insulin metabolism. It can also help to lower cholesterol. It is Cr^{3+} which is important for the body but Cr^{6+} is very toxic. Skin, fat, adrenals, brain, spleen, and muscle are the storage tissues of chromium in the body. Meat products, cheese, whole grains, milk, eggs, brewer’s yeast, mushrooms, and peppers, liver, kidney, whole wheat bread, wheat germ and beets are some of the dietary sources of chromium. It can also be obtained from supplements, and this is usually the best way to make sure we get enough chromium for the body. As a heavy metal Cr, is one of the serious pollutants of air, soil and water (Frois et al, 2011; Wilson, 2012).

Lead (Pb): 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 industries, automobile exhausts, metal plating and finishing operations, fertilizers, pesticides and from additives in pigments and gasoline (Eick et al., 1999).

Among the toxic metals, Pb is present in larger amounts in the earth's crust, with an average concentration between 10 and 20 mg/kg Pb in soil is extremely stable and highly toxic to humans and animals. Most of the lead enters the human body through the respiratory and gastrointestinal routes, and after absorption, it can be found in the blood, soft and mineralized tissues.

Pb exposure can occur through multiple pathways, through inhalation of air, water, soil or dust, as it is emitted in the environment from vehicles and automobiles. It can also enter the food chain via plants (Wierzbicka and Antosiewiez, 1993). In plants, its accumulation has been reported in stem, leaves, roots and seeds that increase with increase in exogenous Pb levels (Singh et al., 1998).

1.8. Statement of the problem

Even though there are some reports about the metallic contents of maize (corn) from many countries in the world, no detail investigations were made as major, trace and toxic minerals (elements) for maize seeds cultivated in Ethiopia. Therefore the objective of this study was to study the levels of essential and non-essential metals in maize seed/corn (*Zea mays* L.) cultivated and consumed in Shendi, Finote Selam and Debre Tabor areas, Amhara region, Ethiopia.

1.9. Objectives of the study

1.9.1. General objective

The main objective of this thesis was to determine the levels of essential and non-essential metals in maize seed/corn (*Zea mays* L.) cultivated in selected areas of Ethiopia: Shendi, Finote Selam and Debre Tabor

1.9.2 Specific objectives

1. To develop an optimum working procedure for digestion of maize seeds/cereal samples to determine the level of essential and non-essential metals by AAS.
2. To determine the level of essential and non-essential metals in maize seeds/corn and its processed food (kolo and bread).
3. To compare the levels of the identified metals in maize seeds/corn from three different districts in Amhara region, Ethiopia, as well as with popcorn and cornflakes purchased from Addis Ababa supermarkets.
4. To compare the levels of the identified metals in maize seeds/corn in Ethiopia with the literature values.

1.10. Significance of the study

The major significance of this study is to provide information about the level of essential and non-essential metals in maize seeds cultivated in the selected areas of Ethiopia. This study is believed to give a clue for further studies in maize seeds cultivated in different parts of the country. Also it will give information for the concerned institutions and organizations to take some necessary actions for the wellbeing of the society.

2. EXPERIMENTAL

2.1. Equipments and reagents

2.1.1. Equipments and apparatuses

Polyethylene bags were used during sample collection and transportation. And glass bottles were used while preserving the grinded and homogenized samples before the actual laboratory experiments. Electronic blending device (FOSS KNIFETEC 1095, USA) was used for grinding and homogenizing the samples. Electronic series balance (OPTECH, A205EC, Italy) with precision of ±0.0001 g was used for weighing samples. 250 mL round bottom flasks fitted with reflux condenser were used with Kjeldahl (U.K.) apparatus hot plate to digest the dried and powdered maize seed samples. Filtration funnels (Kenutuf, England) and different sizes of filter papers such as Whatman® filter paper 150 mm (Whatman® 541) (Whatman international Ltd Maid-Stone, England) and filter paper 125 mm (from Schleicher & Schuell Micro Science GmbH, Germany) were used for filtration of sample solution after digestion during for both optimization and sample preparation processes. Volumetric flasks (50 and 100 mL) were used during dilution and preservation of samples and preparation of metals standard solutions. HTL pipettes with 0.01 mL division with pipette tips and micro pipettes (10-100 µL and 100-1000 µL) made of USA were used for measuring reagents used during optimization, sample preparation, preparation of standard solutions and spike solutions. Refrigerator (SANYO Electric Biomedical Co. Ltd, Japan) was used for sample preservation after digestion and before AAS analysis. Flame atomic absorption spectrophotometer (Analytikjena: Model ZEEnit700P, AAS VGP AAS, Germany) equipped with deuterium arc back ground connectors and hollow cathode lamps with air-acetylene flame was used for the analysis of the analyte metals. Graphite furnace atomic absorption spectrophotometer (PerkinElmer, AAnalyst 600, USA) was also used for this study.

2.1.2. Reagents and chemicals

H_2SO_4 98% (Research-Lab Fine Chem Industries, Mumbai, India) and $K_2Cr_2O_7$ (Remi-chem Ltd, Mumbai, India) were used for preparing chromate solution for soaking and washing condensers and other glass wares before starting digestion. HNO_3 69-72% (Scharlau Chemie S.A. European Union, Spain), $HClO_4$ 60% (BDH Laboratory Supplies AnalaR®, Poole, England) and extra pure H_2O_2 30% (Scharlau Chemie S.A., European Union, Spain) were used for digestion of maize seed samples. $La(NO_3)_3.6H_2O$ 98% (BDH Chemicals Ltd, Poole, England) was used to minimize the precipitation of Ca and Mg ions in the form of phosphates and sulfates. Stock standard solutions containing 1000 mg/L of the metals K, Na, Mg, Ca, Cr, Mn, Fe, Co, Ni, Cu, Zn, Cd and Pb (BDH Chemicals Ltd Spectrosol®, Poole, England) were used for preparation of calibration standards and for the spiking experiments. Deionized water was used for dilution of samples, intermediate and working metal standard solutions prior to analysis and for rinsing glass wares.

2.2. Sampling and sample sites description

2.2.1. Description of the study areas

2.2.1.1. Debre Tabor

Debre Tabor is a town and a woreda (district) in north central Ethiopia. It is located in the South Gondar zone of the Amhara region , about 100 km southeast of Gondar and 50 km east of Lake Tana and 103 km far from Bahir Dar, the capital of the region. According to the road transport authority of Ethiopia, Debre Tabor is about 666 km far from the capital city Addis Ababa and about 103 km far from Bahir Dar, the capital of the Amhara regional state. This town has a latitude and longitude of 11°51′N 38°1′E with an elevation of 2,706 meters above sea level (Fig. 4) ("Abyssinia" in the Encyclopedia Britannica, 1911).

2.2.1.2. Finote Selam (Jabi Tehnan)

Finote Selam is a town and separate woreda in western Ethiopia. It is located in the West Gojjam zone of Amhara region. This town has a latitude and longitude of 10°42′N and 37°16′E with an elevation of 1917 meters above sea level (Fig. 4) ("Local History in Ethiopia" The Nordic Africa Institute website, 2015). It is surrounded by Jabi Tehnan woreda. Currently Finote selam is the administrative center of West Gojjam administrative zone.

2.2.1.3. Shendi

Shendi (or Shendi Gebriel) is a town in western Ethiopia. It is located in the West Gojjam zone of the Amhara region. This town has a latitude and longitude of 10°38′N and 36°56′E with an elevation of 2,050 meters above sea level (Fig. 4) ("Local History in Ethiopia" The Nordic Africa Institute, 2015). Shendi is the administrative centre of Womberma woreda. Womberma is one of the woredas in the west Gojjam zone and is one of the surplus producers of crops in the Amhara region.

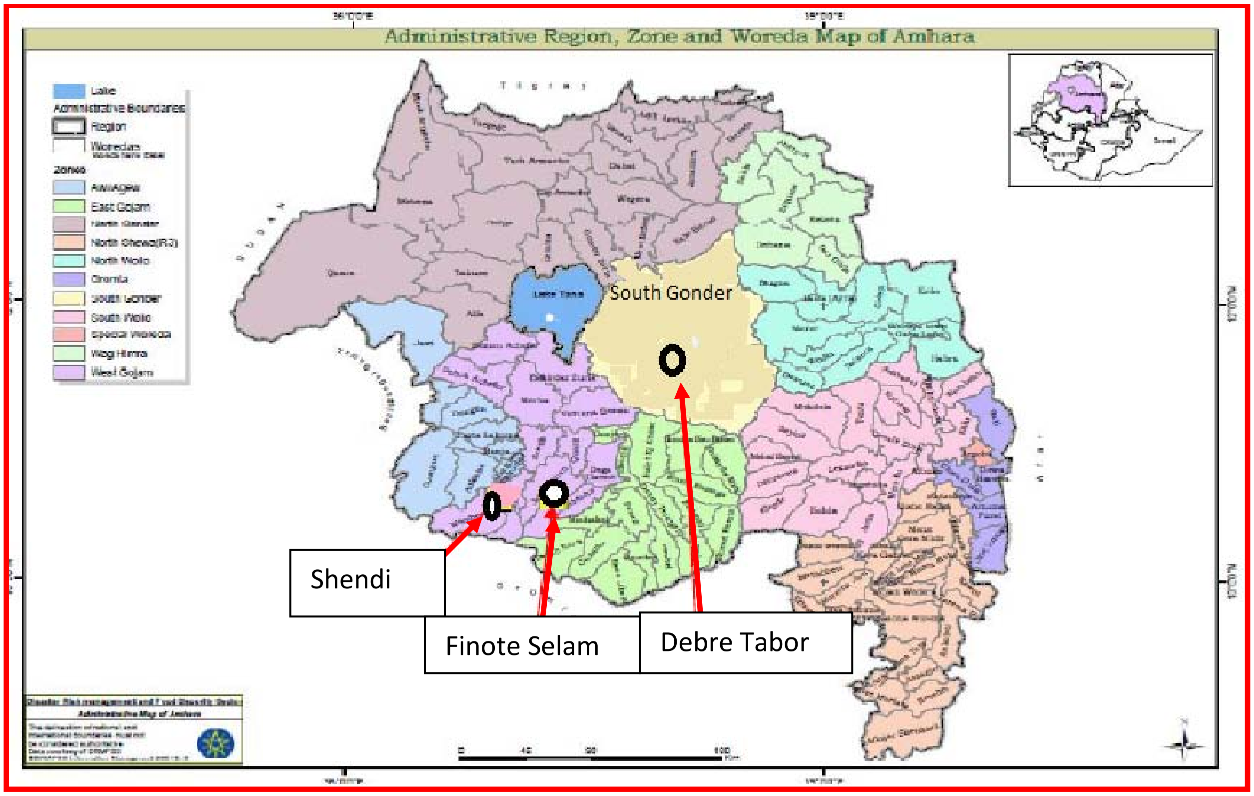


Fig. 4. The administrative map of Amhara regional state (www.dppc.gov.et, 2005).

The possible efforts were made to collect the representative samples of maize seeds from the study areas. Two of the areas selected for this study were from West Gojjam, the leading producer of maize in Ethiopia. West Gojjam is well known in maize cultivation and consumption. The other sample was collected from Debre Tabor, South Gonder. It is also one of the major maize producing zones in the region. Apart from the maize seeds the popcorn and cornflake samples were collected from super markets in Addis Ababa. There geographical locations of the sampling sites are described in Table 1.

Table 1: Geographical descriptions of sample collection sites

S. No	Sample site	Approximate geographical locations			
		Latitude	Longitude	Altitude above sea level (m)	Distance in kilometers and directions from Addis Ababa
1	Debre-Tabor	$11^0 51'$N	$38^0 1'$E	2,706	666 km North West
2	Finote-Selam	$10^0 42'$N	$37^0 16'$E	1,957	385 km North West
3	Shendi	$10^0 38'$N	$36^0 56'$E	2,050	427km North West

About 0.5 kg of samples was taken from ten farmers who were selling maize in the open markets from Shendi, Finote Selam and Debre Tabor towns. These ten half kilogram samples from each town were then mixed in to three separate polyethylene plastic bags to get about 5 kg of one bulk sample for each. Then only a representative portion of about 1.5 kg samples from each site were packed in the polyethylene plastic bags and were brought to Addis Ababa for analysis. The necessary information about the sample was properly labeled so that identification became easier. Also for popcorn and cornflake sampling was made properly. About 100 g of popcorn samples was taken/purchased from 5 shops, a total of 0.5 kg sample was collected. From this sample about 200 g was brought to the laboratory for further treatment/analysis.

Three packs of cornflake samples each with 250 g were purchased from three different supermarkets.

2.3. Working procedures

2.3.1. Cleaning the apparatuses

Condensers and digestion round bottom flasks of the digestion apparatus were soaked in 1% (w/v) chromate solution prepared from 1 g of $K_2Cr_2O_7$ and appropriate amount of 25% H_2SO_4 to remove metals and other contaminants left on the surface of the apparatuses. All glass wares, plastic containers/vials, pipette tips and all other material needed were properly soaked in the cleaning solution overnight. On the next day, by using a copious amount of tap water the cleaning solution was removed. Then all were rinsed by distilled water. The glass wares were then dried in hot air oven and stored in clean dry places free of contamination till use.

2.3.2. Sample preparation for elemental analysis

Sample holding glass bottles were soaked in the cleaning solution for 24 hours, washed and rinsed with de-ionized water and then dried in the oven for storing the prepared samples for digestion. After preparing sample containers clean, samples collected from the sampling areas were washed with tap water so that the adsorbed soil and other particulate matter were removed, then they were rinsed with deionized water. The samples were exposed to sun for drying until a constant weight was achieved.

2.3.2.1. Sample preparation for elemental analysis from raw maize seed

A portion of the dried maize seeds were ground using electronic blending device and the powder was transferred to the already prepared sample bottles.

2.3.2.2. Sample preparation for elemental analysis from kolo

Some portions of the samples were roasted using metal pans, get cooled and then got ground using the electronic blending device as it is traditionally done in Ethiopia. This roasted grain in the local term is called “kolo”. Then the powder of maize seed in the form of kolo and raw maize seed samples were kept in the properly washed and dried glass bottles until appropriate amounts of the samples were taken for digestion.

2.3.2.3. Sample preparation for elemental analysis from bread

The bread was prepared according to the traditional procedure used in Ethiopia. From the raw maize flour samples bread was made using clay pans. The flour was mixed well with distilled water. The metal pan was heated and then the prepared dough was placed on the pan. The pan with the dough was covered with lid, and after 10 min the bread was inverted upside down for uniform heat distribution. Then the bread made was taken off from the pan and allowed to cool and was cut in to pieces and exposed to sundry to complete dryness, i.e. removed the water added while baking repeatedly weighed until a constant weight was obtained. Then the dried bread sample was crushed in to powder form and made ready in the clean and dry glass bottle for digestion for analysis. Similar treatments were made for all the samples of the three sites.

2.3.2.4. Sample preparation for elemental analysis from cornflake

The three packs of cornflakes from Egypt purchased from Addis Ababa supermarkets were opened and thoroughly mixed in a single plastic bag. Then a portion of the mixture was crushed in to pieces by hand, and a representative portion of about 100 g was transferred in to the already cleaned and dried glass bottle till digestion.

2.3.2.5. Sample Preparation for elemental analysis from popcorn

The popcorn was prepared according to the traditional procedure used in Ethiopia. The popcorn from the shops was first rubbed with a piece of dry and clean cloth; this was to remove some extraneous matter adhering to the surfaces of the popcorn kernels. Then in a clean and dry metal dish about 5 mL of cooking oil was added. The dish containing the oil was transferred to a stove for heating. After about 3 min the unpopped popcorn was transferred in to the dish. The dish was covered with its lid. Heating the dish was resumed with frequent swirling by hand holding the dish with a piece of cloth. After the completion of popping, the popped popcorn then got was allowed to cool to room temperature. The cooled popped popcorn was then crushed in to pieces and finally transferred in to the already cleaned and dried sample bottle, ready for digestion.

2.4. Optimization of digestion procedure

In any scientific experiments especially in analytical chemistry creating an optimum working condition before starting analysis of the actual samples is a common practice. That means before preparing the samples for analysis the temperature, the volumes of reagents to be used and the duration of the preparation should be optimized. Wet acid digestion is one of the methods that are involved to get free metal ions in dissolved form from complex organic matrix based on changing different digestion parameters like volume ratio of reagents to be added, digestion temperature and duration of time. Kjeldahl apparatus is one of the wet acid digestion apparatuses by which organic components are assumed to be decomposed in the form of different gaseous forms and other metallic elements are left in the solution except those easily volatile metals like Hg. Hence the optimization procedures for the sample preparation for the determination of major, trace and toxic metal contents were made as shown in Tables 2-4.

Table 2: Optimization of volume ratio of the reagents for digestion

Optimization of reagent volumes for digestion						
S.No.	Sample mass (g)	Total volume (mL)	Ratio (mL) HNO_3: $HClO_4$:H_2O_2	T(^{0}C)	Time (h)	Observation during volume ratio optimization
1	2	6	5:0:1	60	2	Yellow solution with too much residue
2	2	7	6:0:1	60	2	Yellow solution with too much residue
3	2	8	7:0:1	60	2	Yellow solution with much residue
4	2	9	8:0:1	60	2	Yellow solution, but relatively lower residue
5	2	10	8:1:1	60	2	Yellow solution with too much residue
6	2	11	9:1:1	60	2	Yellow solution with some residue.
7	2	6	0:5:1	60	2	Dark colored cake like with little liquid
8	2	7	0:6:1	60	2	Dark and highly turbid solution
9	2	8	0:7:1	60	2	Dark and highly turbid solution
10	2	9	0:8:1	60	2	Dark and turbid solution
11	2	10	0:9:1	60	2	Dark and turbid solution
12	2	11	0:10:1	60	2	Dark but less turbid solution
13	2	7	5:2:0	60	2	Light yellow solution with little residue
14	2	8	6:2:0	60	2	Light yellow solution with very little residue
15	2	9	7:2:0	60	2	Light yellow solution with very little residue
16	2	10	8:2:0	60	2	Light yellow solution
17	2	11	9:2:0	60	2	Light yellow solution
18	2	12	10:2:0	60	2	Light yellow solution
19	2	8	5:2:1	60	2	Light yellow solution with little residue
20	2	9	6:2:1	60	2	Light yellow solution with very little residue
21	2	10	7:2:1	60	2	Light yellow solution with very little residue
22	2	11	8:2:1	60	2	Light yellow solution with very little residue

23	2	12	9:2:1	60	2	Light yellow solution with very little residue
24	2	13	10:2:1	60	2	Light yellow solution with very little residue
25	**2**	**10**	**6:2:2**	**60**	**2**	**A clear light yellow solution with negligible amount of residue on filtration**
26	2	10	5:2:3	60	2	Clear light yellow solution with some residue
27	2	10	6:1:3	60	2	Clear light solution with some residue
28	2	10	7:1:2	60	2	Clear light solution with some residue
29	2	10	4:4:2	60	2	Clear but brown solution

- The bold font shows the optimized volume ratio.

Table 3: Optimization of digestion temperature

Optimization of digestion Temperature						
S. No.	Mass of sample (g)	Total volume (mL)	Optimized volume ratio (mL)	T (^{0}C)	Time (h)	Observation
1	2	10	6:2:2	60	2	Some yellowish turbid matter
2	2	10	6:2:2	90	2	The turbid matter decreased
3	**2**	**10**	**6:2:2**	**120**	**2**	**Clear and light yellow color solution**
4	2	10	6:2:2	150	2	Clear yellow solution
5	2	10	6:2:2	180	2	Clear light yellow solution
6	2	10	6:2:2	210	2	The same as 4 and 5

- The bold font indicates optimum digestion temperature, T (^{0}C) = temperature

Table 4: Optimization of digestion time

Optimization of digestion Time						
S. No	Mass of sample (g)	Total volume (mL)	Optimized volume ratio (mL)	T (^{0}C)	Time (h)	Observation
1	2	10	6:2:2	120	1:00	Yellow solution with some residues
2	2	10	6:2:2	120	1:15	Yellow solution with some residue
3	2	10	6:2:2	120	1:30	Yellow solution with some residue
4	2	10	6:2:2	120	1:45	Yellow solution with some residue
5	2	10	6:2:2	120	2:00	Yellow solution with some residue
6	2	10	6:2:2	120	2:15	Yellow solution with some residue
7	2	10	6:2:2	120	2:30	Light yellow solution with very few residues
8	2	10	6:2:2	120	2:45	Light yellow solution with very few residues
9	**2**	**10**	**6:2:2**	**120**	**3:00**	**Clear light yellow solution**
10	2	10	6:2:2	120	3:15	Clear light yellow solution

- The bold font indicates optimum digestion time, T (^{0}C) = temperature

2.5. Digestion of the samples

Applying the optimized conditions (Tables 2-4), 2 g of homogenized dried and grounded maize samples were transferred into 250 mL round bottom flasks. Then 10 mL of the mixture of HNO_3 (69-72%), $HClO_4$ (60%) and H_2O_2 30% with a volume ratio of 6:2:2 (v/v) was added and the mixture was digested on a Kjeldahl digestion apparatus for the optimized period of time (3 hours) at the optimized temperature (120 ^{0}C). After 3 hours of digestion time the digested mixture was allowed to cool to room temperature for about 45 min without dismantling the condenser. Then at the time of dismantling the setup, about 10 mL of deionized water was added to the solution. This was done by rinsing the neck of the flask and the tip of the condenser which was in contact with the flask, to dissolve the precipitate formed on cooling and to minimize dissolution of filter paper by the digest residue while filtering with Whatman 541 filter paper into 50 mL volumetric flask. To a 50 mL volumetric flask containing about 0.67 g of $La(NO_3)_3.6H_2O$ the cooled solution was filled to the mark (50 mL) with deionized water. The round bottom flasks were rinsed subsequently with deionized water until the total volume reached to the 50 mL mark.

The main reason why $La(NO_3)_3.6H_2O$ was added was to prevent the precipitation of Ca^{+2} and Mg^{+2} with the SO_4^{-2} and PO_4^{-3} if they were present in the samples or the reagents used in the process. That means on the addition of $La(NO_3)_3.6H_2O$ to the sample solution the $SO_4^{-2}$and PO_4^{-3} were made precipitated so that the Ca^{+2} and Mg^{+2} ions became free for atomization. The reaction chemical equation can be represented as follows:

$$3MSO_4\downarrow + 2La(NO_3)_3 \rightarrow 3M(NO_3)_2 + La_2(SO_4)_3\downarrow$$

$$M_3(PO_4)_2\downarrow + 2La(NO_3)_3 \rightarrow 3M(NO_3)_2 + 2LaPO_4\downarrow$$

Where M = Ca and/or Mg

The digestion was carried out in triplicates for each bulk sample. Digestion of a reagent blank was also performed in parallel with the maize samples keeping all digestion parameters the same. The digested samples were kept in the refrigerator, until the level of all the metals in the sample solutions were determined. Each and every activity was done for the cornflakes and popcorn samples too.

2.6. Analysis of *Zea mays* L. samples for metal levels

For the analysis of the samples calibration of the instrument with the known concentration of standards were done for each metal of interest. First the intermediate (10 mg/L) standard solutions were prepared from the stock solutions which were 1000 mg/L in concentration. The working standards were prepared based on the sensitivity of the instrument towards the particular metals. All the standards used were AAS grade.

These secondary standards, the intermediate solutions were diluted with deionized water to obtain four working standards for each metal of interest. Then K, Na, Mg, Ca, Fe, Zn, Cu, Pb, Ni and Cr were analyzed with FAAS equipped with deuterium arc background corrector and standard air-acetylene flame system using external calibration curve after the parameters (burner and lamp alignment, slit width and wavelength adjustment) were optimized for maximum signal intensity of the instrument. Mn, Cd and Co concentrations were analyzed using GFAAS after the necessary optimizations and alignments were made. Three replicate determinations were carried out on each sample. Hallow cathode lamp for each metal operated at the manufacturer's recommended conditions were used at its respective primary source line. The acetylene and air flow rates were managed to ensure suitable flame conditions. Greater than 99.99% pure Argon gas was used in the case of GFAAS. All the thirteen metals were determined by absorption/concentration mode and the instrument readout was recorded for each sample and blank solution. The same analytical procedure was employed for the determination of elements in digested blank solutions. The operating conditions for FAAS employed for each analyte are given in Table 5.

Table 5: Instrumental operating conditions for determination of metals using FAAS

Metal	λ (nm)	SW (nm)	I (mA)	Energy (J)	LOD (mg/L)	Pmt (V)	t (s)
K	766.0	0.8	4.0	72.0	0.01	319	3
Na	589.0	0.8	3	67.2	0.002	276	3
Mg	284.2	0.7	1.5	67.1	0.001	236	3
Ca	422.7	1.2	3	67.1	0.025	238	5
Cr	357.9	0.2	4	67.2	0.05	338	5
Mn	279.5	0.5	3	67.3	0.01	341	3
Fe	248.3	0.2	4	68.2	0.03	389	3
Co	240.7	0.2	5	71.4	0.05	361	3
Ni	232.0	0.2	3	73.3	0.07	457	5
Cu	324.7	1.2	2	73.8	0.035	285	5
Zn	213.9	0.5	2	67.7	0.012	408	5
Cd	213.9	1.2	2	68.0	0.012	286	5
Pb	283.2	1.2	2	71.9	0.3	325	5

Pmt = photomultiplier tube, SW = slit width, λ = wave length, I = lamp current, LOD = detection limit, t = integrated time.

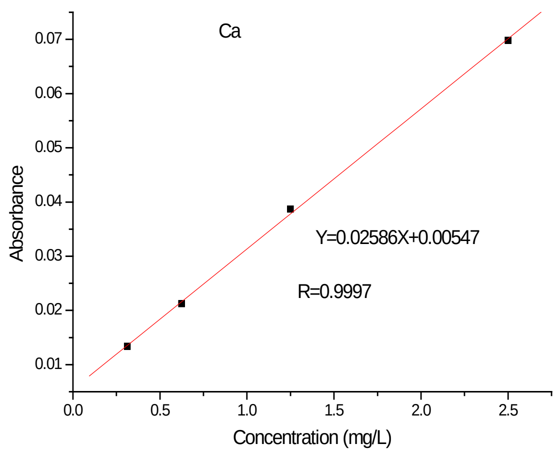
2.7. Instrument calibration

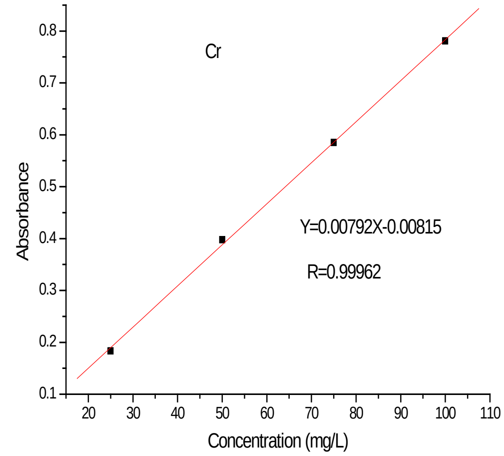
Both the FAAS and GFAAS were calibrated using four series of working standards for each metal of interest. The working standard solutions of each metal were prepared freshly by diluting the intermediate standard solutions. Concentrations of the intermediate standards, working standards, value of correlation coefficient of the calibration curve and equations for calibration curves for each metal are listed in Table 6 and their calibration curves are shown in Fig. 5.

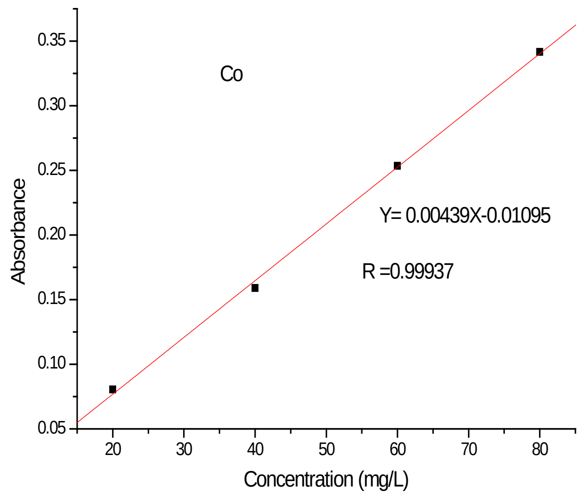
Table 6: Working standard concentration, correlation coefficient and equation of the calibration curves for determination of metals using FAAS and GFAAS

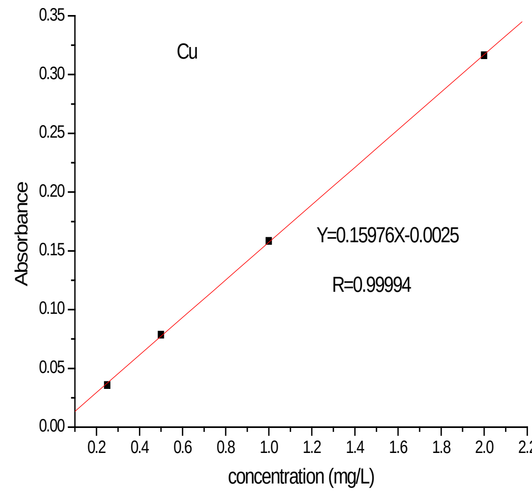
Metal	Concentration of working standards (mg/L)	Corr. coefficient (R)	Equation for calibration curves
K	0.5, 1.0, 2.0, 4.0	0.99955	A = 0.2136C + 0.0555
Na	0.25, 0.5, 1.0, 2.0	0.99972	A = 0.67698C + 0.00635
Mg	1.0, 2.0, 3.0, 4.0	0.99856	A = 0.48273C – 0.07642
Ca	0.3125, 0.625, 1.25, 2.5	0.9997	A = 0.02586C + 0.00547
Cr	0.025, 0.05, 0.075, 0.10	0.99962	A = 0.00792C – 0.00815
Mn	0.02, 0.04, 0.06, 0.08	0.99818	A = 0.00551C – 0.00785
Fe	1.0, 2.0, 3.0, 4.0	0.99832	A = 0.05583C + 0.01587
Co	0.02, 0.04, 0.06, 0.08	0.99937	A = 0.00439C – 0.01095
Ni	0.25, 0.5, 1.0, 2.0	0.9997	A = 0.00699C – 1.27 x 10^{-5}
Cu	0.25, 0.50, 1.0, 2.0	0.9994	A = 0.15976C – 0.0025
Zn	1.0, 2.0, 3.0, 4.0	0.99733	A = 0.16966C + 0.0024
Cd	0.25, 0.5, 1.0, 2.0	0.9999	A = 0.0671C + 6.27 x 10^{-4}
Pb	1.25, 2.5, 5.0, 10.0	0.99994	A = 0.01162C + 0.00256

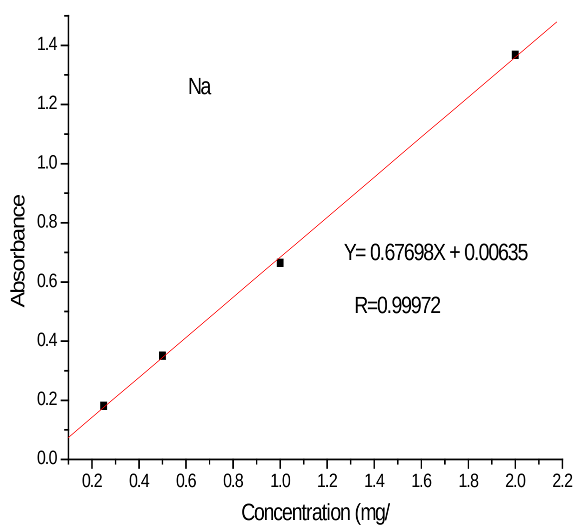
Corr. = correlation, A = Absorbance, C = Concentration

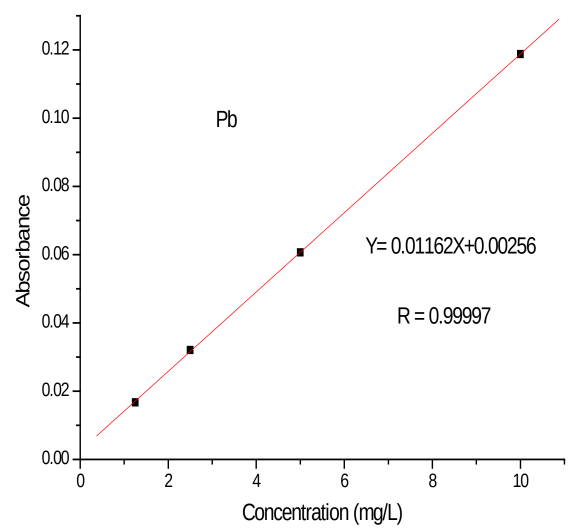

Ca
Y=0.02586X+0.00547
R=0.9997
Absorbance
Concentration (mg/L)

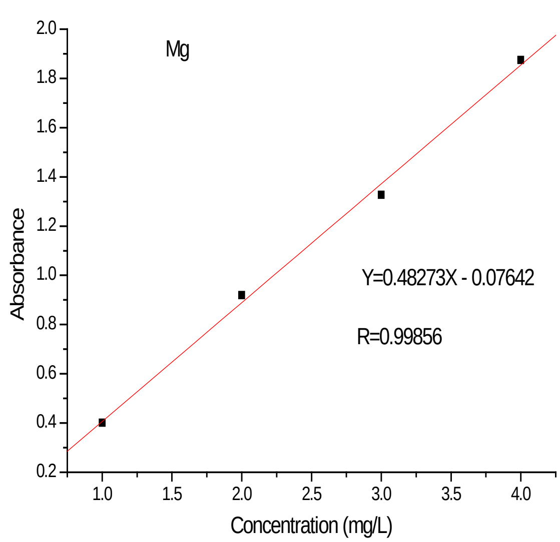

Cr
Y=0.00792X-0.00815
R=0.99962
Absorbance
Concentration (mg/L)

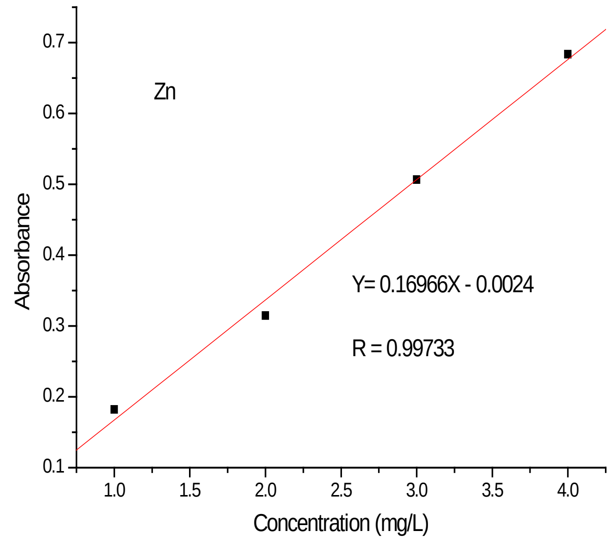

Co
Y= 0.00439X-0.01095
R =0.99937
Absorbance
Concentration (mg/L)

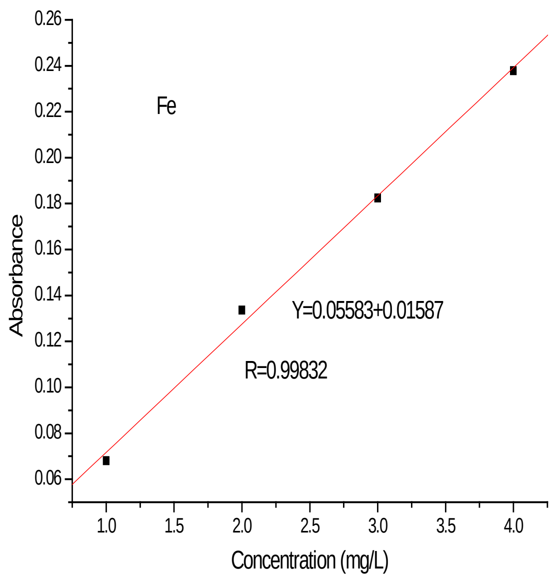

Cu
Y=0.15976X-0.0025
R=0.99994
Absorbance
concentration (mg/L)

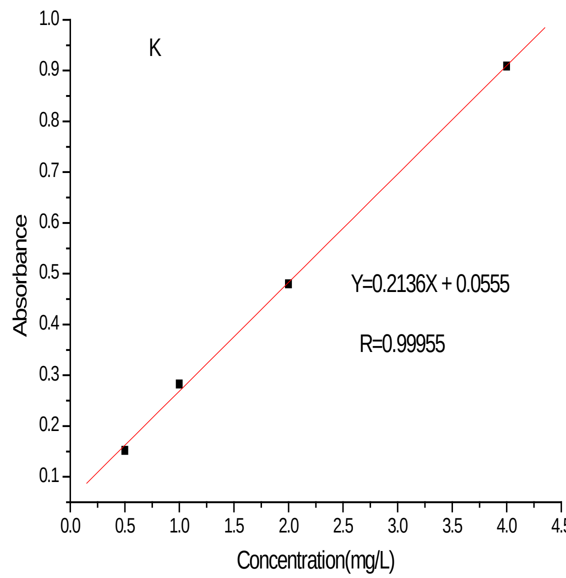

Na
Y= 0.67698X + 0.00635
R=0.99972
Absorbance
Concentration (mg/


Pb
Y= 0.01162X+0.00256
R = 0.99997
Absorbance
Concentration (mg/L)


Mg
Y=0.48273X - 0.07642
R=0.99856
Absorbance
Concentration (mg/L)


Zn
Y= 0.16966X - 0.0024
R = 0.99733
Absorbance
Concentration (mg/L)


Fe
Y=0.05583+0.01587
R=0.99832
Absorbance
Concentration (mg/L)


K
Y=0.2136X + 0.0555
R=0.99955
Absorbance
Concentration(mg/L)

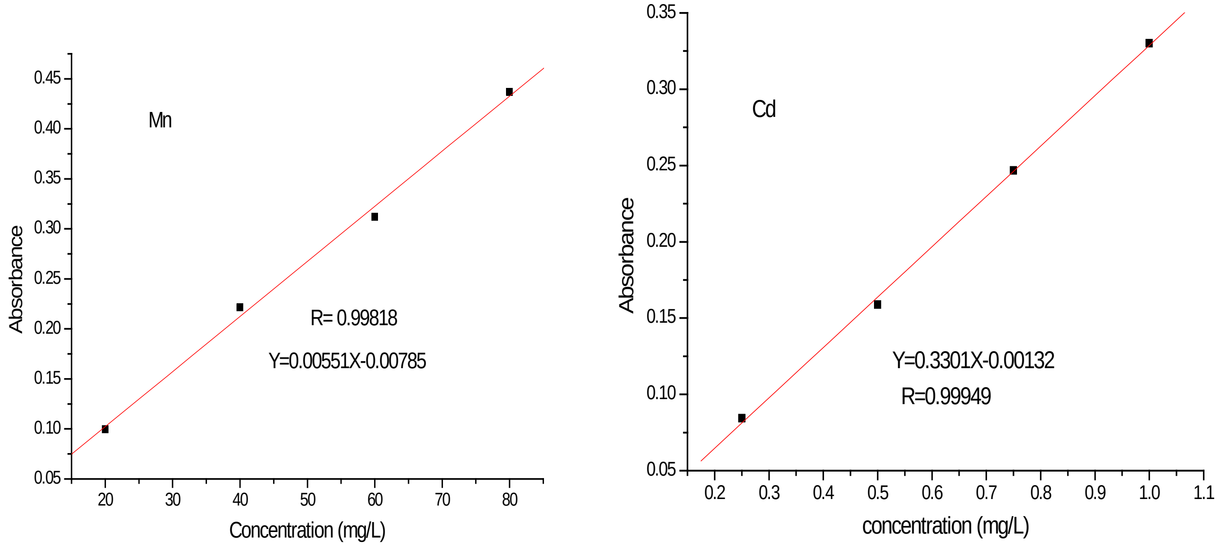


Fig. 5. Calibration curves of the standards of metals investigated

From the correlation coefficients in Table 6 and their corresponding calibration curves in Fig. 5 for each metal it is possible to say that, the change in absorbance with concentration is in good positive correlation and are linearly fit.

2.8. Method performance and method validation

2.8.1. Precision and accuracy

Accuracy and precision are the most common terms related to analytical quality procedures to express the extent of errors in analytical measurements. 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 the standard deviation, variance, coefficient of variance, relative standard deviation and range of series of measurements (Skoog et al., 1996). In this particular study the results of the measurements are expressed as the mean of the measurements together with the standard deviation of the triplicate samples with triplicate measurements of each sample.

2.8.2. Method detection limits

The detection limit (according to IUPAC) is the smallest concentration or absolute amount of analyte that has a signal significantly larger than the signal arising from a reagent blank (http://en.wikipedia.org/wiki/Detection_limit, 2015).

In atomic absorption spectrometry usually the detection limit is determined for a certain element by analyzing a diluted solution of this element and recording the corresponding absorbance. The experiment is repeated for 10 times. The 3SD (standard deviation) of the recorded absorbance signal can be considered as the detection limit for the specific element under the experimental conditions used wavelength, type of flame, instrument.

The method detection limit (MDL) 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 an analyte that can be reliably shown to be present or measured under defined conditions, and the limit of detection (LOD) is the lowest concentration that can be determined to be statistically different from a blank (99% confidence). The MDL/LOD is typically determined to be in the region where the signal-to-noise ratio is greater than 3 but not necessarily quantified as an exact value (Chen, 2007).

For a linear calibration curve, it is assumed that the instrument response y is linearly related to the standard concentration x for a limited range of concentration. It can be expressed using an equation

$y = a + bx$.

This equation is used to compute the sensitivity b and the LOD and LOQ. Therefore, the LOD and LOQ can be expressed as $LOD = 3S_a/b$, and $LOQ = 10S_a/b$, where S_a is the standard deviation of the response and b is the slope of the calibration curve (http://en.wikipedia.org/wiki/Detection_limit, 2015).

2.8.3. Method quantification limits (MQL)

It is the lowest concentration at which a measurement is quantitatively meaningful. It is also commonly defined as 10 times the signal/noise ratio. If the noise is approximated as the standard deviation of the blank (S_{blank}), then LOQ/MQL is 10 x S_{blank} (i.e., LOQ = 10 x S_{blank}, n = 18) (Mitra, 2003).

2.8.4. Validation of optimized procedure

The validity of the optimized procedure was assessed by spiking experiments. For this purpose standard solution of 1000 mg/L was used and intermediate standards of 100 mg/L and 10 mg/L were prepared. Thus, spiking was done by classifying the metals in to three groups. In the first group K, Na, Ca and Mg were grouped; Zn, Cu, Fe and Mn in the second and Co, Cr, Cd, Ni and Pb in the third group. Note that samples were taken from Shendi raw flour for the first group of metals, from Debre Tabor maize bread for the second group and from Finote Selam maize “kolo” for the the third group. This was to consider the forms and sources of samples.

The spiked and non-spiked samples were digested and analyzed in similar conditions using optimized procedure for sample analysis. Then, the percentage recoveries of the analytes were calculated by the use of the equation:

$$\% \, R = \frac{(C_M \text{ in the spiked sample}) - (C_M \text{ in the non-spiked sample})}{C_M \text{ added for spiking}} \times 100 \, \%$$

Where: C_M is concentration of metal of interest.

Or simply % R is the ratio of the amount recovered to the amount spiked multiplied by hundred.

The results of recovery analysis are shown in Table 7 and the percentage recoveries lay within the range 91.2–109%. The percentage recovery for maize seed samples were between 90 and 110% (100 ± 10), which were within the acceptable range for all metals.

Table 7: Recovery test for the optimized procedure of maize seed samples

Metal	Concentration of metal in unspiked sample	% spiked	Amt. spiked (amount added)	Concentration of metal in spiked sample	Amt. recovered	(% R)
K	1688±49	20	400.83	2072	383.90	95.8%
		10	200.42	1876	188.04	93.8%
Na	163±1	20	32.6	194	31.39	96.3%
		10	16.3	179	16.46	101%

Mg	392±4	20	78.4	473	81.3	104%
		10	39.2	434	41.8	107%
Ca	102±2	20	22.04	124	22.1	100.3%
		10	11.02	114	11.5	104%
Cr	0.34±0.004	50	0.17	0.49	0.155	91.2%
		25	0.085	0.42	0.08	94.1%
Mn	2.42±0.07	20	0.484	2.93	0.51	105%
		10	0.242	2.68	0.258	107%
Fe	45.95±0.68	50%	23.0	71.1	25.1	109%
		25%	11.5	57.8	11.8	103%
Co	0.58±0.08	50%	0.29	0.86	0.28	96.6%
		25%	0.14	0.73	0.15	107%
Ni	ND	-	2	2.21	2.11	105%
		-	1	0.95	0.95	95%
Cu	0.05±0.004	200%	10	10.8	10.8	108%
		100%	5	5.19	5.14	103%
Zn	112±3.65	25%	28	140	28.3	101%
		10%	11.2	124	11.5	103%
Cd	ND	-	0.5	0.48	0.48	96.0%
		-	0.25	0.23	0.23	92.0%
Pb	2.71±0.4	100%	2.71	5.6	2.89	107%
		50%	1.35	4.04	1.33	98.5%

ND = not detected, concentration is in mg/kg, Amt = Amount, % R = percent recovered

3. RESULTS AND DISCUSSIONS

3.1. Determination of levels of metals in maize seed samples

The accuracy and precision of the results were checked by the aid of different statistical methods after the determination of the levels of metals in the maize, popcorn and cornflake samples. The mean values were determined from triplicate analysis of each sample and triplicate samples were used from each sampling area. The mean values determined were triplicate of triplicate analysis for each metal and the results are reported in terms of mean values ($\overline{X}$) ± SD, for all the metals in this study). Among all the determined metals the concentration of Cd and Ni could not be expressed, because their amounts were below detection limits of the instrument. Results determined from each sampling sites are listed in terms of mean value and standard deviation of mg/kg in Table 8 and 9.

Table 8: Level of metals in mg/kg in maize seeds, kolo and bread from Shendi, Finote Selam and Debre Tabor

Metal	Concentration (mg/kg) ($\overline{X}$ ± SD) of metals in samples from :								
	Shendi			**Finote Selam**			**Debre Tabor**		
	Seed	**Kolo**	**Bread**	**Seed**	**Kolo**	**Bread**	**Seed**	**Kolo**	**Bread**
K	1688±49	1886±22	1748±50	1754±46	1925±83	1755±166	1784±151	1864±111	1936±100
Na	163±1	188±2	194±1	126±1	139±3	162±2	150±1	155±2	194±2
Mg	392±4	402±11	395±0.7	397±1	404±9	395±6	396±4	304±0.9	388±10
Ca	102±2	171±7	139±5	72.0±3	109±1	123±6	67.5±1	124±6	107±5
Cr	0.17±0.003	0.19±0.01	0.19±0.01	0.23±0.07	0.34±0.004	0.27±0.01	1.55±0.03	1.70±0.02	1.55±0.1
Mn	2.60±0.1	2.80±0.05	2.70±0.13	1.23±0.2	1.28±0.2	0.65±0.1	3.74±0.2	4.37±0.2	2.42±0.07
Fe	112±3	101±2	142±4	56.8±3	52.6±0.6	63.4±2	20.0±2	19.5±3	45.95±0.7
Co	0.48±0.01	0.70±0.06	0.44±0.07	0.43±0.2	0.58±0.08	0.48±0.02	0.46±0.02	0.66±0.10	0.69±0.06
Cu	1.32±0.31	1.95±0.77	2.87±0.25	0.08±0.01	0.05±0.002	0.05±0.004	0.04±0.001	0.05±0.003	0.05±0.004
Zn	66.7±5	61.4±2	77.6±4	72.7±5	75.1±8	77.5±3	69.9±8	73.3±2	112±4

Pb	1.21±0.9	1.20±1	2.60±0.8	1.50±0.5	2.71±0.4	2.02±0.6	2.11±0.5	1.25±0.03	2.04±0.5

Table 9: Range of metal concentrations in maize seed samples

Metal	Ranges of metal concentration (mg/kg)			Overall range (mg/kg)
	Seed	Kolo	Bread	
K	1633 – 1935	1753 – 2008	1589 – 2036	1589 – 2036
Na	125 – 164	137 – 190	160 – 195	125 – 195
Mg	388 – 400	304 – 411	378 – 401	303 – 413
Ca	66.5 – 104	118 – 178	102 – 144	66.5 – 178
Cr	0.17 – 1.58	0.18 – 1.72	0.18 – 1.65	0.17 – 1.72
Mn	1.04 – 3.98	1.09 – 4.60	0.52 – 2.83	0.52 – 3.98
Fe	18.0 – 115	16.5 – 103	45.3 – 146	16.5 – 146
Co	0.41 – 0.49	0.50 – 0.76	0.34 – 0.75	0.34 – 0.76
Cu	0.04 – 1.32	0.04 – 2.72	0.05 – 3.12	0.04 – 2.72
Zn	61.7 – 77.6	59.2 – 83.0	108 – 116	59.2 – 116
Pb	0.31 – 2.59	0.82 – 3.11	1.55 – 3.41	0.31 – 3.41

Table 10 Metal concentrations in popcorn and corn flakes from Addis Ababa supermarkets.

Metal	Concentration (mg/kg) ($\bar{X}$± SD)	
	Popcorn	Cornflake
K	1293±233	616±70
Na	148±3	410±5

Mg	387±11	323±11
Ca	97.9±4.2	196±99
Cr	0.68±0.09	0.30±0.07
Mn	6.17±0.18	3.0±0.1
Fe	9.5±2.1	5.5±0.74
Co	1.41 ±0.16	0.32 ±0.03
Cu	0.09 ±0.007	0.30 ±0.01
Zn	88.3±9.7	40.7±2.5
Pb	0.94±0.29	0.36±0.03

3.2. Distribution patterns of metals in the maize samples

The uptake of metals by plants is taking place through different and complex biochemical processes. These up taking processes vary based on the ability of the plants to absorb metals from the soil, the availability of the minerals in the soluble and usable forms, the abundance of particular minerals at the particular areas, the degree of contamination of the soil with heavy metals, etc. The differences in the levels of metals in soil arise mainly due to pollution of the biosphere resulting from the rapid industrialization and modern large scale agricultural activities, i.e. use of different types of fertilizers, pesticides, herbicides and other chemicals.

The use of sewage sludge, pesticides, herbicides, irrigation with polluted water and fertilizers on agricultural lands highly affect the quality of food products for humans and animals. The distribution and accumulation of metals in maize seeds are the reflections of the mineral composition of the soil and the degree of mineral pollution of the environment in which the maize plant grows. Therefore, the actual metal concentration of maize seeds vary considerably according to the geographic origin, the use of fertilizers with different chemical compositions and other characterizing features such as quality water for irrigation and also the storage conditions of the products.

3.2.1. Concentration of macro-essential (major) metals in maize seeds

Metals absorbed by plants from different sources are accumulated in different parts of the plant's body, like roots, stems, leaves, seeds and other parts. The amount of metals accumulated in the plants' body parts is variable, i.e. some of them are higher in the roots, some others in the seeds and others in stems and other parts of the plants. Since the focus of this study is on the level of metals in seeds of maize plant, the common edible part of the maize plant by human beings let us have a look on the contents of the metals analyzed in maize seeds, collected from different sample sites. There is a variation determined in the metal concentration of macro-essential metals among the sample areas. The concentration of K was the highest of all the major metals as shown in Table 8-9. It was also the highest of all the metals under consideration. It was within the range 1589–2036 mg/kg dry weight, followed by Mg, Na and Ca 303–413, 125–195 and 66.5–178 mg/kg, respectively. That means the concentration profile of macro-essential metals determined in maize seeds was K > Mg > Na > Ca.

Among the sample sites the highest concentration of K was determined in a sample from Debre Tabor (1633–2036 mg/kg dry weight) followed by Finote Selam (1589–2008 mg/kg) and Shendi is 1639–1908 mg/kg. In this study the concentration of K determined by sample sites decreased in the order Debre Tabor > Finote Selam > Shindi. The concentrations of Mg from Shendi and Finote Selam are about the same (388–413 and 389–413 mg/kg), but the value obtained from Debre Tabor is slightly lower than both sample sites (303–400 mg/kg). The amount of Na obtained in the samples from the three sites was as follows: Shendi 161–195 mg/kg, Finote Selam 125–164 mg/kg and Debre Tabor

149–195 mg/kg. This means that the level of Na in the sample from Shendi and Debre Tabor are nearly equal but both are higher than that of Finote Selam. The levels of Ca in the samples were recorded as 100–178 mg/kg, 69.0–129 mg/kg and 66.5–130 mg/kg from Shendi, Finote Selam and Debre Tabor samples, respectively. Shendi sample is higher in Ca than the samples from other two sites. The values of Ca in samples from Finote Selam and Debre Tabor are almost the same. These slight variations in the level of the above metals in maize seed by sample site may be resulted due to the availability of the minerals in the soluble and usable forms, the natural occurrence of these minerals in the areas, the degree of contamination of the soil by these metals, the use of fertilizers of different chemical compositions like NPK fertilizer, treatment of soil acidity: and other characterizing features such as water for irrigation could be the causes for the differences.

When we see the levels of metals in the raw seed, kolo and bread forms in Table 8 the values were in the order kolo > bread > raw seed samples for all metals except for Na. It is high in bread samples than kolo and seed for some unknown reason.

3.2.2. Concentration of micro-essential (trace) metals in maize seeds

From Tables 8 we can see that iron is the highest accumulated trace essential metal measured followed by Zn and Mn with concentration ranges 16.5–146 mg/kg , 59.2–116 mg/kg and 0.52–3.98 mg/kg, respectively. As it is clearly seen in Table 9, the concentration ranges of all the trace metals except Fe and Zn overlap each other among the sample sites. The highest concentration of Fe may be attributed to its higher levels in the soil (Mekassa and Chandravanshi, 2015; Wagesho and Chandravanshi, 2015).

Though most of the values are overlapping with each other the pattern of the overall concentration of trace metals in maize seeds collected from the three sample sites was in the order Fe > Zn > Mn > Pb > Cu > Co. And the order for each site was Fe > Zn > Mn > Cu > Pb > Co for Shendi, Zn > Fe > Pb > Mn > Co > Cu for Finote Selam and Zn > Fe > Mn > Pb > Co > Cu for the sample from Debre Tabor. If we compare the levels of individual metals by sample site the trend can be shown as follows: Shendi > Finote Selam > Debre Tabor in Fe content. Debre Tabor > Shendi > Finote Selam in Mn content. Shendi > Finote Selam≈ Debre Tabor in Cu content. DebreTabor > Shendi ≈ Finote Selam in Zn content. Debre Tabor≈ Shendi ≈ Finote Selam in the lev el of Co. The low concentration of minerals may be due to soil pH. Micronutrients are significantly affected by soil pH, decreasing with increasing soil pH. For example solubility of Fe decreases a thousand fold for each unit increase in soil pH in the range of 4 to 9 (Lindsay, 1979).

The variations in concentrations of Co and Mn are not that much significant and comparable to each other for the three sites. That means the distribution of these metals is invariant in comparison to other metals.

The variation of Fe by sample sites was the highest among the micro-essential metals and the variation for Co by sample sites is the least as shown in Table 9.

3.2.3. Concentration of non-essential (toxic) metals in maize seeds

According to World Health Organization the dietary exposure to Cd is estimated to be about 1.2 x 10^{-4} to 4.9 x 10^{-4} mg/kg of body weight daily. Intake of dietary Cd should not exceed 0.007 mg/kg of body weight, per week. However, the levels of Cd and Ni in this work were below method detection limits. Using maize seeds from these sites is safe for human consumption and safe from human health problems due to the accumulation of Cd and Ni in maize seeds.

Pb is a major chemical pollutant of the environment, and is highly toxic to man. The values determined for Pb level in this work are presented in Table 8 and the overall concentration range among different sample sites is shown in Table 9. As we can see from Tables 8 the variations among the sites are comparably small, but the amount of Pb determined from Finote Selam is slightly higher than both from Shendi and Debre Tabor. Shendi's is again slightly higher than that of Debre Tabor. The variation for Pb content in the maize seed by sample site may be attributed to agricultural inputs such as fertilizers herbicides and insecticides containing Pb as an ingredient. Exposure to contamination during storage and transportation by cultivators could be the other causes for the higher values (Eick et al., 1999).

The variation on the level of Cr between Shendi and Finote Selam is not that much significant, but the level in the sample from Debre Tabor is higher. We can compare the differences as Debre Tabor > Finote Selam > Shendi in Cr content.

3.2.4. Concentrations of metals from popcorn and cornflake samples

From Table 10 we can see that the concentration of K is the highest of all and Cu is the least in both popcorn and cornflakes. The order of concentration of the metals determined is K > Mg > Na > Ca > Zn > Fe > Mn > Co > Pb > Cr > Cu for the popcorn and K > Na > Mg > Ca > Zn > Fe > Mn > Co > Pb > Cr ≈ Cu for the cornflakes.

From the nutritional point of view cornflake samples are less preferable than both corn and pop corn. As it can be seen from Tables 9 and 10 all the levels of metals in the corn were higher than both the popcorn and the corn flake samples except for Na. The high value of Na may be resulted from the salt added in the manufacturing process for flavoring purpose. The lower levels of metals in the case of cornflakes are probably because of the leaching of the metals in the various steps of the manufacturing process:

Corn → Cleaning → Peeling and degerming → Classifying → Cooking → Drying and Relending → Rolling, Flaking or Extruding → Toasting → Cooling → Packing → Finished Product (http://drying-machinery.com/process_description.html, 2015).

3.3. Comparison of metal levels of the present study with literature values

Comparison of analytical data with reference material is a common practice in analytical chemistry to validate the results. However, there is no standard reference material to do so and the determined results should be compared with the investigations made in other countries by other investigators. Different researches were being made by different researchers in different countries on maize seed, but in the Ethiopian case no detail studies were made on the levels of major, trace and toxic metal composition on maize seeds/corn.

For the purpose of comparison the results of this study, and results from different literatures are shown in Table 11. As it is seen from the tables below, the levels of macro-essential metals determined in this work were in good agreement with other studies done in other countries. If we look the results in Table 11 the concentration of K is in the range 3.24–2662 mg/kg, Na in the range 75.8-3230 mg/kg, Mg in the range 48.94-1153.9 mg/kg and Ca 0.61-215 mg/kg dry weight. The results of this study (K 1589-2036, Na 125-178, Mg 303-413 and Ca 66.5-178 mg/kg) are all in the ranges. This tells us that the results of this study are in good agreement with that from the literatures.

Table 11: Comparison of macro-essential metals concentration, (mg/kg, dry weight basis) in maize seed samples with reported values

K	Na	Mg	Ca	Country	Reference
1701.1	535.8	1153.9	68.4	Turky	Özcan, 2006
2662	75.8	91.5	215.7	Turky	Hicsonmez et al., 2012
1620	2600	200	20	Brazil	Padovani et al., 2007
1950	3230	200	20	USA	Padovani et al., 2007
NR	NR	248.3-321	1.2-10.2	Nigeria	Olu et al., 2013
NR	NR	NR	NR	China	Hongxing and Yu-Kui, 2011
3.24	3.72	48.94	0.61	Nigeria	Wegwu et al., 2010
1589-2036	125-195	303-413	66.5-178	Ethiopia	This study

NR = not reported, ND = not detected

The levels of micro-essential metals in this study and from the literature are shown in Table 12 Also here the values from this study are in good agreement with those from literatures. If we look the values from the literatures (Özcan, 2006, Hicsonmez et al., 2012, Padovani et al., 2007, Olu et al., 2013, Hongxing and Yu-Kui, 2011, Wegwu et al., 2010) Fe is in the range 5.9–158.5 mg/kg, Cr 0.338–2.38 mg/kg, Mn ND to 8.4 mg/kg, Co ND to 0.8 mg/kg and Ni ND to 4.775 mg/kg. The levels of all the metals of this study are in the ranges from the literatures used, i.e. Fe 16.5–146 mg/kg, Cr 0.17–1.72 mg/kg, Mn 0.52–3.98 mg/kg, Co 0.34–0.76 mg/kg and Ni ND are all in the above ranges.

In fact the level of Fe in this study is in a higher range (16.5–146 mg/kg), preceded by only one literature (Hicsonmez et al., 2012) from Turkey 158.5 mg/kg. The high value of Fe in this study is most probably resulted from the high concentration of Fe in the soil. Researches in the recent years indicate that Ethiopian soil is high in Fe content, so plants can absorb Fe easily. This could be one reason behind the high value of Fe in Ethiopian maize. (Mekassa and Chandravanshi, 2015; Wagesho and Chandravanshi, 2015).

The determination for levels of toxic metals was also carried out in this study and Cd was found to be below detection limit of the instrument. The level of Pb was found as 0.31–3.41 mg/kg. When it is compared with the values in the literature it is lower than that mentioned by Olu et al. (2013) in Nigeria (62.5–150 mg/kg), but higher than most others.

Table 12: Comparison of micro-essential metals concentration, (mg/kg, dry weight basis) in maize seed samples with reported values

Cr	Mn	Fe	Co	Ni	Country	Reference
2.38	ND	37.89	NR	0.79	Turky	Özcan, 2006.
1.0	8.4	158.5	0.8	0.022	Turky	Hicsonmez et al., 2012
NR	NR	5.9	NR	NR	Brazil	Padovani et al., 2007
NR	NR	8.6	NR	NR	USA	Padovani et al., 2007
NR	NR	28.5-59.5	ND	1.87-4.775	Nigeria	Olu et al., 2013
338.75*	4.40	28.89	6.58*	123.34*	China	Hongxing and Yu-Kui, 2011
1.36	ND	8.66	ND	1.04	Nigeria	Wegwu et al., 2010
0.17-1.72	0.52-3.98	16.5-146	0.34-0.76	ND	Ethiopia	This study

NR = not reported, ND = not detected, * = concentration µg/kg, the rest are in mg/kg

Table 13: Comparison of Cu, Zn and heavy metals concentration, (mg/kg, dry weight basis) in maize samples with reported values

Cu	Zn	Cd	Pb	Country	Reference
2.85	33.55	NR	NR	Turky	Özcan , 2006
3.0	28.4	ND	1.5	Turky	Hicsonmez et.al.2012
0.46	5.0	NR	NR	Brazil	Padovani et al., 2007
0.58	3.9	NR	NR	USA	Padovani et al., 2007
2-10.7	NR	NR	62.5-150	Nigeria	Olu et al., 2013
2.86	15.03	32.31*	38.15*	China	Hongxing and Yu-Kui, 2011
2.05	6.69	ND	0.32	Nigeria	Wegwu et al., 2010
0.04-2.72	59.2-116	ND	0.31-3.41	Ethiopia	This study

NR = not reported, ND = not detected, * = Concentration in μg/kg

3.4. Statistical analysis

3.4.1. Analysis of variance (ANOVA)

The variations in sample means of the metals were tested by the help of ANOVA, whether the sources for variations were from experimental procedure or heterogeneity among the samples (i.e. difference in mineral contents of soil, pH of soil, water, atmosphere; variation in application of agrochemicals like fertilizers, pesticides, herbicides etc or other variations in cultivation procedures). The results in Table 14, shows the significance of the results between samples and within samples.

From the table one can see that, there is significant difference at 95 % confidence level in mean concentrations of all the metals except K, Mg and Pb in all the three sample forms and the means of Co concentration in flour and kolo as well as the means of Zn in the form of kolo are not significantly different. The source for this significant difference between sample means may be the difference in mineral compositions of the soil or pH of soil which predict the degree of mineral absorption by plants. As one can see from tables 14A to 14C, if the F- calculated exceeds the critical value, then the means are significantly different and no significant difference if the opposite occurs. It is based on this fact that we say the means of the above mentioned metals are not significantly different.

Table.14A: Analysis of variance (ANOVA) between and within maize seed samples at 95% confidence level

Metal	Comparison	SD (mg/kg)	Df	F_{cal}	F_{cr}	Remarks
K	Between samples	49.0_4	2		19.3	The means are not significantly different.
	Within samples	95.4	6	3.78		
Na	Between samples	18.5	2		5.14	There is significant difference among the sample means
	Within samples	1.27	6	212		
Mg	Between samples	2.67	2	1.81		The means are not significantly different.
	Within samples	3.59	6		19.3	
Ca	Between samples	18.9	2	86.2	5.14	There is significant difference among the sample means
	Within samples	2.04	6			
Cr	Between samples	0.79	2	521	5.14	Significant difference between sample means
	Within samples	0.03	6			
Mn	Between samples	0.72	2	13.7	5.14	There is significant difference among the sample means
	Within samples	0.20	6			
Fe	Between samples	56.6	2	617	5.14	There is significant difference among the sample means
	Within samples	2.28	6			
Co	Between samples	0.02_3	2	1.50	5.14	The means are not significantly different.
	Within samples	0.02	6			
Cu	Between samples	0.73	2	16.0	5.14	There is significant difference among the sample means
	Within samples	0.18	6			
Zn	Between samples	3.00	2	2.08	19.3	The means are not significantly different

	Within samples	6.11	6			
Pb	Between samples	0.46	2	2.05	19.3	The means are not significantly different.
	Within samples	0.65	6			

SD = standard deviation, Df = degree of freedom, F_{cal} = F calculated, F_{cr} = F critical

Table 14 B: Analysis of variance (ANOVA) between and within maize "kolo" samples at 95% confidence level

Metal	Comparison	SD (mg/kg)	Df	F_{cal}	F_{cr}	Remarks
K	Between samples	30.9	2	6.90	19.3	The means are not significantly different.
	Within samples	81.1	6			
Na	Between samples	24.9	2	126	5.14	There is significant difference among the sample means.
	Within samples	2.22	6			
Mg	Between samples	5.11	2	2.64	19.3	The means are not significantly different.
	Within samples	8.30	6			
Ca	Between samples	32.6	2	46.4	5.14	There is significant difference among the sample means.
	Within samples	4.78	6			
Cr	Between samples	0.84	2	4806	5.14	There is significant difference among the sample means.
	Within samples	0.01	6			
Mn	Between samples	1.55	2	76.2	5.14	There is significant difference among the sample means.
	Within samples	0.18	6			
Fe	Between samples	40.9	2	402	5.14	There is significant difference among the sample means.
	Within samples	2.04	6			
Co	Between samples	0.06	2	1.67	19.3	The means are not significantly different.
	Within samples	0.08	6			
Cu	Between samples	1.10	2	6.04	5.14	There is significant difference among the sample means
	Within samples	0.44	6			

Zn	Between samples	7.45	2	3.13	5.14	The means are not significantly different.
	Within samples	4.21	6			
Pb	Between samples	0.74	2	1.24	19.3	The means are not significantly different.
	Within samples	0.83	6			

SD = standard deviation, Df = degree of freedom, F_{cal} = F calculated, F_{cr} = F critical

Table14 C: Analysis of variance (ANOVA) between and within maize bread samples at 95% confidence level

Metal	Comparison	SD (mg/kg)	Df	F_{cal}	F_{cr}	Remarks
K	Between samples	109	2	1.13	19.3	The means are not significantly different.
	Within samples	116	6			
Na	Between samples	18.5	2	109	5.14	There is significant difference among the sample means
	Within samples	1.78	6			
Mg	Between samples	4.98	2	1.68	19.3	The means are not significantly different.
	Within samples	6.46	6			
Ca	Between samples	16.1	2	9.59	5.14	There is significant difference among the sample means
	Within samples	5.21	6			
Cr	Between samples	0.76	2	129	5.14	There is significant difference among the sample means
	Within samples	0.07	6			
Mn	Between samples	1.11	2	88.4	5.14	There is significant difference among the sample means
	Within samples	0.12	6			
Fe	Between samples	51.3	2	413	5.14	There is significant difference among the sample means.
	Within samples	2.53	6			
Co	Between samples	0.13	2	5.53	5.14	There is significant difference among the sample means.
	Within samples	0.06	6			
Cu	Between samples	1.63	2	125	5.14	There is significant difference among the sample means
	Within samples	0.14	6			

Zn	Between samples	19.9	2	31.6	5.14	There is significant difference among the sample means
	Within samples	3.54	6			
Pb	Between samples	0.33	2	2.92	19.3	The means are not significantly different.
	Within samples	0.56	6			

SD = standard deviation, Df = degree of freedom, F_{cal} = F calculated, F_{cr} = F critical

3.4.2. Pearson correlation of metals within maize seed samples

In this particular study, to correlate the effect of the concentration of one metal over the other metal, the Pearson correlation coefficients were employed. The relations for the three forms of the maize samples are shown in the following three tables, Table 15A–15C.

Table 15A: Pearson's correlation for maize seed samples

	K	Na	Mg	Ca	Cr	Mn	Fe	Co	Cu	Zn	Pb
K	1										
Na	0.54	1									
Mg	0.89	0.86	1								
Ca	0.98	0.68	0.96	1							
Cr	0.77	0.15	0.36	0.63	1						
Mn	0.25	0.69	0.21	0.67	0.82	1					
Fe	0.99	0.45	0.84	0.96	0.82	0.35	1				
Co	0.58	0.998	0.90	0.73	0.76	0.64	0.64	1			
Cu	0.96	0.75	0.98	0.996	0.56	0.25	0.93	0.25	1		
Zn	0.70	0.98	0.96	0.82	0.77	0.51	0.63	0.99	0.87	1	
Pb	0.91	0.15	0.82	0.61	0.96	0.62	0.95	0.20	0.77	0.35	1

Table 15B: Pearson's correlation for maize kolo samples

	K	Na	Mg	Ca	Cr	Mn	Fe	Co	Cu	Zn	Pb
K	1										
Na	0.45	1									
Mg	0.79	0.20	1								
Ca	0.38	0.998	0.27	1							
Cr	0.71	0.32	0.99	0.38	1						
Mn	0.99	0.29	0.88	0.22	0.82	1					
Fe	0.25	0.75	0.80	0.80	0.86	0.41	1				
Co	0.77	0.92	0.21	0.89	0.09	0.65	0.43	1			
Cu	0.16	0.95	0.48	0.97	0.58	0.01	0.92	0.76	1		
Zn	0.28	0.98	0.37	0.99	0.48	0.11	0.86	0.83	0.99	1	
Pb	0.94	0.13	0.94	0.06	0.90	0.99	0.56	0.51	0.12	0,05	1

Table 15C: Pearson's correlation for maize bread samples

	K	Na	Mg	Ca	Cr	Mn	Fe	Co	Cu	Zn	Pb
K	1										
Na	0.47	1									
Mg	0.999	0.50	1								
Ca	0.88	0.01	0.87	1							
Cr	1.00	0.45	0.999	0.89	1						
Mn	0.36	0.99	0.39	0.13	0.34	1					
Fe	0.67	0.34	0.64	0.94	0.68	0.46	1				
Co	0.99	0.36	0.99	0.93	0.99	0.24	0.75	1			
Cu	0.53	0.50	0.50	0.87	0.54	0.60	0.98	0.63	1		
Zn	0.50	0.50	1.00	0.86	0.998	0.39	0.64	0.99	0.50	1	
Pb	0.999	0.53	0.47	0.85	0.52	0.63	0.98	0.60	1.00	0.47	1

The values of Pearson correlation coefficient in Table 15A-15C revealed that, there is weak and/or moderate positive correlation between metals with each other except for some metals. The weak correlation indicating that the presence or absence of one metal affects the other metal in a lesser extent.

As we can see from the correlation tables there is a very high positive correlation of K with Fe in the case of seeds, with Mn in the case of kolo and with Cr, Pb and Mg in the case of bread. Na with Zn and Co both in flour and bread samples. Ca with Fe, Cr with Pb, Mn with Pb, Fe with Cu and Pb as well as Cu with Pb and Zn can be mentioned as the strong correlations seen from the table. These strong correlations may arise from common anthropogenic or natural sources as well as from similarity in chemical properties. Mn show week to medium correlation with most of the metals except the strong relations with K, Na and Pb.

Table16. Metal concentrations in maize (this study), the amount that an average adult man can get from 200 g maize per day, RDI and upper limit values of metals recommended by experts and agencies for a normal adult man

Metal	Concentration in maize (mg/kg)	Amount of metal a person can get from 200 g maize	Daily recommended intake (RDI)	Tolerable upper limit
Ca	66.5 – 178	13.3–35.6	1000–1200 mg	2500 mg/day
Mg	303 – 413	60.6–82.6	320–420 mg	750 mg/day
K	1589 – 2036	318–407	4700 mg	ND*
Na	125 – 195	25–39	1500 mg	2300 mg/day
Cr	0.17 – 1.72	0.03–0.25	25–35 μg	120 μg/day

Cu	0.04 – 2.72	0.01–0.54	0.9–2 mg	10 mg/day
Fe	16.5 – 146	3.3–29.2	10–15 mg	45 mg/day
Mn	0.52 – 3.98	0.104–0.796	1.8–2.3 mg	11 mg/day
Ni	ND	No	70–170 µg/kg*	1 mg/day
Zn	59.2 – 116	11.8–23.2	10–15mg	40 mg/day
Cd	ND	No	ND*	7 µg/kg bw/week
Pb	0.31 – 3.41	0.06–0.68	0.02–3 µg /kg bw	25 µg /kg bw/day
Co	0.34 – 0.76	0.07–0.15	5–40 µg /day*	0.25 mg/day

Indicates the estimated daily intake, bw = body weight, ND = not determined (not established)

Sources: FNB, Institute of Medicine, National Academies, 1997, USA (also from www.frantzmd.info, www.nap.edu, 2015 and www.sparkpeople.com.(minerals), 2015) for Ca, Mg, Na, K, Cr, Cu, Fe, Mn, Zn, Ni and Co, FAO/WHO 2011 for Cd and Pb

Assuming that an average adult person consumes 200 g maize per day on average the amount of mineral intake by the person is shown in Table 15. The amount of all major metals (Ca, Mg, K and Na) that a person can get is lower than the daily recommended values, this indicates that maize alone cannot be a good source of the major metals needed for the daily requirement for the major metals. Therefore the person must get supplementary Ca, Mg, K and Na from other sources. The amount of Mn and Cu that the man can get is also below the required amount. Hence supplementary diet is needed for these metals too. The amount of iron is very sufficient, for the sample from Shendi but below the required limit for those from the other two sites, so additional source of Fe is needed for those people in the two sites. The amount of Zn that a man can get from maize is in the range for those from Shendi and Finote Selam, the amount from Debre Tabor is higher than the RDI, but it is still below the maximum limit. The values for Co, Cr and Pb are all above the allowable limits. Maize in these areas has too much sources of these metals. The man must not consume foods from maize regularly. Since the level of Cd and Ni in samples from the three sites are below the detection limit. Hence, it is possible to conclude that, the man is free from the risks of Cd and Ni as a result of consuming maize.

4 CONCLUSSION AND RECOMMENDATIONS

An efficient digestion procedure for the determination of metals in the raw maize seed and its processed food (kolo, bread, popcorn and corn flake) samples was optimized and validated through spiking method and a good percentage recovery was obtained (100±10%) for all the metals of interest.

The levels of metals in maize seed determined in this particular study can be expressed in the following order: K (1589–2036 mg/kg) > Mg (303–413 mg/kg) > Na (125–195 mg/kg) > Ca (66.5–178 mg/kg) > Fe (16.5–146 mg/kg) > Zn (59.2–116 mg/kg) > Mn (0.52–3.98 mg/kg) > Pb (0.31–3.41 mg/kg) > Cu (0.04–2.72 mg/kg) > Cr (0.17–1.72 mg/kg) > Co (0.34–0.76mg/kg). The non-essential toxic heavy metal, Cd and the other trace metal, Ni were found to be below the detection limits the instrument. From the results of this work it is possible to conclude that maize seeds from the selected sites accumulated relatively larger amounts of K and Fe among the determined major and trace metals, respectively. And lower amount of Cu (e.g. 0.04–0.08 mg/kg from Finote Selam and Debre Tabor samples) is recorded. In soil, Cu is relatively immobile, since it binds strongly with organic matter and it seldom leaches, and its availability to plants strongly depends on the soil type, mainly on the organic matter content and pH (Kopsell and Kopsell, 2007; Burkhead et al., 2009). Uptake of Cu by plants is affected by many factors including the soil pH, the prevailing chemical species, and the concentration of Cu present in the soil (Barker and Pilbeam, 2007). Cu uptake in plants is among the lowest of all the essential elements (Kabata-Pendias and Pendias, 1992).

The ANOVA results at 95% confidence level suggests that there were significant difference in the mean concentration of all metals except for K, Mg and Pb among the sampling areas. These differences could be attributed to the difference in mineral contents of soil or pH of soil which predict the extent of mineral absorption by maize plants. Generally, the level of essential metals in maize seed determined in this study could be put in the following order K > Mg > Na > Ca > Fe > Zn. Mn, Co and Cu are in the ranges which are overlapped with each other. The non-essential heavy metal, Pb and Cr are also in the overlapping regions. The levels of Cd and Ni are below the detection limits of the instrument. Statistical analysis by using one way ANOVA indicates that there is significant difference in mean concentration of metals under investigation except K, Mg and Pb. This may be attributed to differences in soil composition, use of different fertilizers, pesticides, and may also be resulted from random and systematic errors in the experimental processes.

The concentrations of Pb determined in this work were relatively lower than most of the vales in the literatures mentioned. It is higher than the recommended values. This higher value may be due to the anthropogenic sources or from the ingredients of agricultural inputs, like fertilizers, insecticides and herbicides used. Therefore, further investigations should be made not only from maize seeds but also from other cereals and agricultural soils from other sites too using other alternative methods and analytical instruments so that the concerned authority can come up with firm decisions for the safety of the man and the environment in which he is living. Apart from this what we would like to recommend is that : cultivars, agricultural professionals, environmentalists, input suppliers and others should be aware on the ingredients of the inputs (fertilizers, herbicides, insecticides and others), and more care should be taken during storage and transportation of products to avoid contamination from Pb and other toxic metals.

5. REFERENCES

"Abyssinia" in the Encyclopedia Britannica, 11th ed., Vol. 1, **1911**.

Administrative atlas: Amhara region, available at (www.dppc.gov.et, **2005**) accessed on 21 May, 2015.

Africa development indicators, the World bank Washington, D.C., **2006**.

Barker, A.V.; Pilbeam, D.J. Handbook of Plant Nutrition. CRC Press, Boca Raton, FL, **2007**.

Berhanu Gebremedhin, Fernandez-Rivera S, Mohammed Hassena, Mwangi W and Seid Ahmed. Maize and livestock: Their inter-linked roles in meeting human needs in Ethiopia. Research Report 6. ILRI (International Livestock Research Institute), Nairobi, Kenya. **2007**; 103 pp.

Bothwell, T., Charlton, R., Cook, J. D. and Finch, C. Iron Metabolism in Man. Blackwell Scientific, Oxford, UK, **1979.**

Brown, W.; Darrah, L. Origin, adaptation, and types of corn: National Corn Handbook, Cooperative Extension Service, Iowa State University, Iowa, USA, **1985**.

Burkhead JL,; Gogolin Reynolds KA, Abdel-Ghany SE, Cohu CM, Pilon N. Copper homeostasis. New Phytol. **2009**; 182: 799-816.

Chen Y, Beaulieu N. Maximum likelihood estimation of SNR using digitally modulated signals. IEEE Trans Wirel Comm ; **2007**, 6(1), 210 – 219.

Christine Hotz and Kenneth H. Brown, International Zinc Nutrition Consultative Group (IZiNCG) Technical Document #1Assessment of the Risk of Zinc Deficiency in Populations and Options for its Control.**, 2004.**

Cimmyt Maize Program. Maize Diseases: A Guide for Field Identification, 4th ed. **2003**. Available at http://www.cimmyt.org/english/docs/field_guides/maize/diseases.htm#intro, accessed on Nov. 2014.

Corn Refiners Association Washington, D.C. www.corn.org, 12th ed. **2013.**

Corn Starch- Wikipedia, the free encyclopedia, 2015: "International **Starch**: Production of **corn starch**". **Starch**.**dk**. from "https://en.wikipedia.**org**. Retrieved **2013**- 10-31.

Crop Production in Ethiopia: Regional Patterns and Trends Ethiopia Strategy, available at http://www.fao.org ; **2011**, accessed on 23 Jan. 2015.

CSA, Central Statistic Authority. Crop Production Forecast Sample Survey, Report on area, production and yield of major crops for private holdings. Report number 439, Addis Ababa, Ethiopia, **2008**.

Das P, Samantary S, Rout GR; Studies on Cadmium toxicity in plants: a review **1997**, 98:29–36.

Davidsson, L., Cederblad, A., Lonnerdal, B., Sandstrom, B., The effect of individual dietary components on manganese absorption in humans. *Am. J. Clin. Nutr*. **1991**, 54, 1065–1070.

Dawit A, Wilfred M, Nigussie M, Spielman DJ . The maize seed system in Ethiopia: challenges and opportunities in drought prone areas. Afr. J. Agric. Res. **2008**, 3 (1), 305-314.

Eick, M.J.; Peak, J.D.; Brady, P.V.; Pesek, J.D. Kinetics of lead absorption/desorption on goethite: residence time effect. *Journal of Soil Science*, **1999**, 164, 28-39.

FAO Food and Nutrition Series, No. 25, (ISBN 92-5-103013-8, FAO code: 80 AGRIS: SO1), Maize in Human Nutrition, FAO, Rome (Italy), **1992.**

FAO World Agricultural Production. **2011**. Available at: http://faostat.fao.org/default.aspx. Accessed 14 April. 2015.

FAO/WHO (Joint FAO/WHO food standards programme codex committee on contaminants in foods) Fifth Session, The Hague, The Netherlands, 21 - 25 March**, 2011**

Food and Nutrition Board (FNB). Dietary Reference Intakes for Vitamin A, Vitamin K, Arsenic, Boron, Chromium, Copper, Iodine, Iron, Manganese, Molybdenum, Nickel, Silicon, Vanadium, and Zinc. Institute of Medicine, National Academic Press, Washington D.C., **2001**, 294-301.

Food and Nutrition Board, Institute of Medicine, National Academies, **1997**, USA.

Frois, S.R.; Grassi M.T.; Fernandes, T.C.; Barreto R.A.S.; Abate, G. Preconcentration of Cr(III) and speciation analysis of chromium employing montmorillonite saturated with potassium ions. *Química Nova*, **2011**, 34(3), 462-467.

Fulton, T.M.; Buckler, C.S.; Kissel, R.A. The Teacher-Friendly Guide to the Evolution of Maize. Paleontological Research Institution, Ithaca, NY, **2011**.

Gwirtz, J.A.; Garcia-Casal, M.N. Processing maize flour and corn meal food products. *New York Academy of Sciences*, **2014**, 1312, 66-75.

Hallberg L, Brune M, Rossander L. Iron absorption in man: ascorbic acid and dose-dependent inhibition by phytate. *Am J Clin Nutr* **1989**, 49:140-144.

Health benefits of cornflakes, available at http://www.organonutri.in/health) accessed on 21 Nov. **2014**

History of Popcorn, Available at http://www.popcorn.org/EncyclopediaPopcornica, accessed on 11 May **2015.**

Hongxing, Z.;Yu-Kui, R. Determination of trace elements, heavy metals and rare earth elements in corn seeds from Beijing by ICP-MS simultaneously; *E-Journal of Chemistry*, **2011**, 8(2) 782-786.

Hotz, C. and Brown, K.H. Assessment of the risk of zinc deficiency in populations and options for its control. Food Nutr Bull, **2004**, 25, 94-204.

House, W. A. & Wastney, M. E. Compartmental analysis of zinc kinetics in mature male rats. Am. J Physiol. **1997,** 273: 1117–1125.

Howard,J.M.; Davies S. Hunnisett A. Magnesium and chronic fatigue syndrome. Lancet **1992**, 340: 426.

ICdA. InternacionaL Cadmium Association, Revision of the Battery Directive: Public Consultation, **2001**.

Iqbal Hussain, Shamim Akhtar, Muhammad Arslan Ashraf, Rizwan Rasheed, Ejaz Hussain Siddiqi, and Muhammad Ibrahim, Response of maize seedlings to cadmium application after different time intervals. ISRN Agronomy Volume 2013 (**2013**), Article ID 169610, 9 pages available at http://dx.doi.org accessed on 2 November **2014.**

IUPAC, Compendium of Chemical Terminology, 2nd ed. (the "Gold Book") **1997**. Online corrected version: (2006) "detection limit, available at http://en.wikipedia.org/wiki/Detection_limit, accessed on 15 May, **2015**.

James C. McCann, Maize and Grace: Africas's Encounter with a New World crop, 1500-2000. Cambridge: Harvard University Press, **2005.**

Kabata-Pendias, A.; Pendias, H. Trace elements in soils and plants. 2nd ed. CRC Press, Boca Raton, Florida, **1992**.

Kopsell, D.E.; Kopsell, D.A. Copper. In: Barker AV, Pilbeam DJ (eds) Handbook of plant nutrition. Taylor and Francis Group, Boca Raton, Florida, **2007**; pp 293–328.

Leonard, A.; Gerber, P.; Jacquet, P. Carcinogenicity, mutagenicity and teratogenicity of nickel mutation. *Research/Reviews in Genetic Toxicology*, **1981**; 87(1), 1-15.

Lima AJB, Cardoso, MG, Guerreiro MC, Pimentel FA. Using activated carbon to remove copper from sugar cane spirit. *Quimica Nova* **2006**; 29(2) 247-250.

Lindsay WL , Chemical equilibria in soils. JohnWiley and Sons, New York, USA, **1979;** 449 p.

"Local History in Ethiopia" The Nordic Africa Institute website (accessed on 27 November **2015**)

Maize Crop profile, USDA **2007** available at http://www.ecea.gov.et accessed on 23 December 2014

Olu, M.; Olufade, O.I.; Adekoyeni, O.O.; Jimoh, M.O. Evaluation of heavy metal concentration in maize grown in selected industrial areas of Ogun State Nigeria and its effects on urban food security. *International Journal of Science, Technology and Society*, **2013**; 1(2), 48-56.

Mekassa, B.; Chandravanshi, B.S. Levels of selected essential and non-essential metals in seeds of korarima (*Aframomum corrorima*) cultivated in Ethiopia. *Brazilian Journal of Food Technology*, **2015**, in press.

Minerals for life, A basic introduction, Lawrence Wilson, MD. The center for development, August **2012**.

***Minerals* available at** www.powerhealth.org. **accessed on 16 November 2014.**

Mitra, S. Sample Preparation Techniques in Analytical Chemistry, John Wiley and sons, Inc., Hoboken, USA, **2003**.

M. Musa Özcan Determination of the mineral compositions of some selected oil-bearing seeds and kernels using Inductively Coupled Plasma Atomic Emission Spectrometry (ICP-AES), Grasas Y Aceites, 57 (2), AbriL-Junio, 211-218, **2006**.

National Research Council (US). Food and Nutrition Board. Recommended Dietary Allowances.10th edition. National Academy Press, Washington DC, **1989**.

Popcorn- Wikipedia, the free encyclopedia, available at http://en.wikipedia.org/wiki/popcorn accessed on 12 February, 2015.

Popcorn Promotion, Research and Consumer Information Order, Ag Marketing Service, USDA.

Popcorn, Field Crops: **2007** and **2002**, National Ag Statistics Service, USDA.

Prabhu L. Pingali and Paul W. Heisey. Cereal Crop Productivity in Developing Countries: Past Trends and Future Prospects, **1999.**

Purseglove J.W. Tropical crops. Monocotyledons Vol. 1 and 2 combined. Longmans, **1975**

Rashid S., K. Getnet and S. Lemma: Maize value chain potential in Ethiopia: Constraints and opportunities for enhancing the system, IFPRI, Working Paper, **2010.**

Renata M. Padovani, Dag M. Lima, Fernando A.B. Colugnati, De´ lia B, Rodriguez-Amaya : Comparison of proximate, mineral and vitamin composition of common Brazilian and US foods. Journal of Food Composition and Analysis **2007;** 20, 733–738, Brazil.

Reynolds L. Maize for food, feed and fertilizer. A literature review on multiple uses of maize in mixed farming systems in east and southern Africa. Consultant's Report to ILRI and SLP (System-wide Livestock Programme), **1999**.

"Real popcorn as packing material ". Abbey Newsletter. April **1992** available at http://en.wikipedia.org/wiki/popcorn accessed on 13 March **2015**.

Singh, R.P.; Dabas, S.; Chaudhary,A.; Maheshwari, R. Effect of lead on nitrate reductase activity and alleviation of lead toxicity by inorganic salts and 6-benzylaminopurine. *Journal of Plant Biology*, **1998**; 40, 399-404.

Skoog, D. A.; West, D. M.; Holler, F. J. Fundamentals of Analytical Chemistry, 7th ed., Saunders College Publishing: New York, **1996**

Stephanie Belfield and Christine Brown: Field Crop manual: Maize, A guide to upland production in Cambodia. January **2008.**

Technical memorandum on corn starch. International Starch Institute A/S, Agro Food Park 13, DK-8200 Aarhus N, Denmark. Available at http://www.starch.dk. Accessed July **2013**.

The amazing maize maze ; available at www.americanmaze.com and accessed on February **2015**

The production of corn starch, available at www.starch.dk accessed on 02 March **2015**

The Biology of Zea mays L. ssp mays (maize or corn), **2008**. (Purseglove, 1972; Paliwal, 2000; Darrah et al., **2003**) vilable at http://www.eolss.net accessed on 25 Dec. 2014.

Umran, Hicsonmez; Canan; Ozdemir; Semin Cam; Ali Ozdemir; F.Serap Erees. Major-minor element analysis in some plant seeds consumed as feed in Turkey. Journal of Natural sciences. **2012**; 4, 298-303. Available at: http://dx.doi.org/10.4236/ns.2012.45042. Accessed on May 22, 2015.

Wagesho and Chandravanshi. Levels of essential and non-essential metals in ginger (*Zingiber officinale)* cultivated in Ethiopia, Springer Plus (**2015**) 4:107

Verheye, W. Soils, Plant Growth and Crop Production, Vol. II, Growth and Production of Maize: Traditional Low-Input Cultivation, UNESCO-EOLSS Publishers, Oxford, UK, **2010**.

Wastney, M. E., Aamodt, R. L., Rumble, W. F. & Henkin, R. I. Kinetic analysis of zinc metabolism and its regulation in normal humans. *Am. J. Physiol*. **1986**; 251: 398 – 408.

Wegwu, M.O.; Omeodu, S.I. Trace metal contents of selected seeds and vegetables from oil producing areas of Nigeria. *Chemical Biodiversity*, **2010**; 7(7), 1737-1744.

World Bank. Ethiopia Accelerating Equitable Growth Country Economic Memorandum. Report No 38662-ET, Washington DC, **2007**.

Wierzbicka, M.; Antosiewiez, D. How lead can easily enter the food chain: A study of plant roots. *Science and Total Environmental Supply and Protection*, **1993**; 1, 423-429.