ADDIS ABABA UNIVERSITY

SCHOOL OF GRADUATE STUDIES

LEVELS OF FLUORIDE IN STAPLE CEREALS AND LEGUMES PRODUCED IN SELECTED AREAS OF ETHIOPIA

BY

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DEDICATION

To My Mother Khedija Ahmed, to My Father Mustofa Esleman

and

to All My Brothers and Sisters

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LIST OF ACRONYMS AND ABBREVIATIONS

ANOVA	Analysis of Variance
CDC	Centers for Disease Control and Prevention
DPPC	Department for Disaster Prevention and Preparedness Commission
EATA	Ethiopian Agricultural Transformation Agency
ESID	Ecologically Sustainable Industrial Development
FAO	Food and Agriculture Organization
FNBIM	Fluoride and Nutritional Board of the Institute of Medicine
ISE	Ion Selective Electrode
LOD	Limit of Detection
MDL	Method Detection Limit
MOA	Ministry of Agriculture
SD	Standard Deviation
SNNPR	Southern Nations, Nationalities and Peoples Region
SCHER	Scientific Committee on Health and Environmental Risks
TISAB	Total Ionic Strength Adjustment Buffer

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By: Seid Mustofa

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ABSTRACT

Fluorosis is a public health problem in certain part of Ethiopia, especially in rift valley areas. Fluoride is widely present throughout the food supply. The general population can be exposed to fluoride through drinking water and food consumption. Foods are the major contributor of fluoride exposure to human being, next to water. Staple cereals (tef, wheat, corn and barley) and legumes (peas, lentils and chickpea) are important food crops in Ethiopia. The objective of this study was to determine level of fluoride in staple cereal and legume grains. The cereals were collected from Arsi Negele, Wonji, Adola and Gonder while legumes from Ada, Dessie, Burrie and Gonder. These samples were dried, powdered, sieved and 0.5 g from each types of the sample was taken for fluoride determination. Alkaline fusion was used for sample preparation for fluoride determination by FISE. Validation of the procedure was evaluated using spiking method and an acceptable percentage recovery was obtained. In this study, cereals from Arsi Negele and Wonji were found to contain higher level of fluoride than from Adola and Gonder. Legumes from Ada were also found to contain higher level of fluoride than from Gonder, Burrie and Dessie. One way ANOVA at 95% confidence level indicated, significant difference between the means of fluoride in each types of cereals and legumes from four sample sites. Fluoride level in cereals varied between (0.98±0.06 mg/kg) in corn from Adola to (10.98±0.66 mg kg) in tef from Wonji. Fluoride levels in cereals from Arsi Negele and Wonji were found to be higher: tef (10.98±0.66 mg/kg) from Wonji, wheat (10.30± 0.49 mg/kg) and tef (8.00±0.56 mg/kg) from Arsi Negele. Legumes from Ada had higher fluoride level: Chickpea (8.25±1.22), pea (11.07±0.66), lentil (6.83±0.31) in mg/kg. Fluoride levels in legumes were found between (1.52±0.09mg/kg) in chickpea to (11.07±0.66 mg/kg) in pea from Ada.

1. INTRODUCTION

1.1 Background

Food is a fundamental part of our lives. A staple food, sometimes simply referred to as a staple, is generally defined as a food that is eaten regularly (dominant part of the diet) and supplies a major proportion of energy and nutrient needs. Staple foods vary by location but typically include grains, tubers, legumes, or seeds (WB, 2012). For example, *injera* is the staple diet in many area of Ethiopia, corn in other areas, and *kocho* in the southern part of the country. Generally, Ethiopian diet consists of grains (tef, corn, wheat and barley) and legumes (peas, lentils, chickpea and broad beans). An Ethiopian meal usually consists of a stew (wot) of meat, vegetables and pulses and sauce including spices and is served with flat bread (injera) (Common Wealth of Australia, 2006). Legume wot (stew) and grain breads and injera are major diets in large part of the Ethiopian populations. In Ethiopia, food is mostly served in a regular sequence of three main meals time: morning meal (*qurs*), mid-day meal (*misa*), and evening meal (*erat*) (Abbebe Kifleyesus, 2013).

1.1.1 Cereals and Legumes

Grains such as tef, corn (*boqqolo*), wheat (*sinde*), barley (*gabs*) and sorghum (*zengadea*) are the basis of Ethiopian indigenous diet. Most of Ethiopian main meals consist of breads (injera, qitta and dabbo varieties) made of cereal grains (tef, wheat, barley, corn and sorghum) (Abbebe Kifleyesus, 2013). The legume seeds or pulses, sometimes termed ‘grain legumes’, belong to the family *Leguminosae.* Grain legumes are edible seeds of annual legumes and are produced throughout the world (Janzen *et al*., 2006). They are the next important food crop after cereals. Legumes include plants such as field peas, lentils, dry edible beans, chickpeas, soybeans, fenugreek, fababeans and other minor plants. Food legumes play an important and diverse role in the diets of poor people around the world (Janzen *et al*., 2006; Akibode and Maredia, 2011). They have high protein and essential amino acid content and play an important role in the diets of poor people around the world. In most countries of the world they are considered as secondary crops. Legumes are multipurpose crops and are consumed either directly as food or in various processed forms. In Ethiopia, pulse crops contribute 15% of per capital total protein intake (Akibode and Maredia, 2011). Legumes can be supplemented with cereals (Janzen *et al*., 2006). In Ethiopia, Some of these legumes are also eaten fresh (*Ishet*) around September and October (Abbebe Kifleyesus, 2013).

The most commonly consumed legumes in Ethiopia are peas, lentils as well as some dried beans and chickpeas. Legumes such as peas (atar—*Pisum sativum*), beans (baqela—*Vicia faba*), and lentil (missir—*Lens culinaris*) are used to make thin or thick stews called shiro or kik. Shiro or kik wot (stew) can be made from peas, lentil, chickpea or beans, and various spices including green and red peppers. It is the commonest popular dish throughout Ethiopia (Abbebe Kifleyesus, 2013).

1.2 Production and Consumption of Cereals and Legumes in Ethiopia

1.2.1 Tef

Tef (*Eragrostis tef*) is one of the major staple food crops of Ethiopia. It provides over two thirds of the human nutrition in the country. Ethiopia is not only the origin of tef, but it is also the center of diversity (Ministry of Agriculture and Rural Development, 2009; Melese Temesgen, 2013). Tef is an indigenous cereal to Ethiopia when compared with other food crops grown in the country. It is primarily grown to prepare *injera* (Ethiopian bread), porridge and some native alcoholic drinks (Ministry of Agriculture and Rural Development, 2009; Melese Temesgen, 2013; Hailu Tefera and Seyfu Ketema, 2001).

There are three types of tef varieties in Ethiopia: white, red and mixed. White tef is the most preferred and expensive one. Red tef is becoming more popular related to its increased iron content (Doris Piccinin, 2007). Considering the country as a whole, tef is produced in seven regions to varying degrees. Amhara (East Gojam, West Gojam, North and South Gonder, North Shewa and South Wello) and Oromiya (North Shewa, West and East Shewa, East and West Welega and Jimma) is the largest producer of tef, followed by Southern Nations Nationalities and Peoples Region (SNNPR) and Tigray. The crop contains iron, and has high amount of calcium, potassium and other essential minerals found in an equal amount than in other cereal grains (Melese Temesgen, 2013).

1.2.2 Wheat

Wheat is one of the various cereal crops largely grown in the highlands of Ethiopia. It is produced largely in the southeast, central and northwest parts of the country. Small amount is produced in the rest of the north and south regions (Ministry of Agriculture and Rural Development, 2009). According to Vavilov, the diversity in Ethiopian wheats comprises six wheat species: *T. durum subsp*, *Abyssinicum*; *T. turgidum subsp. abyssinicum*; *T. dicoccum*; *T. aestivum*; *T. polonicum and T. compactum.* Currently, the five Vavilov's tetraploid species listed above are classified under *Triticum turgidum* (Janzen *et al*., 2006). Durum wheat (*Triticum durum)* is tetraploid wheat species and traditionally grown heavily in the highlands between 1800-2800 meters above sea level (Common Wealth of Australia, 2006). Durum wheat is used to make spaghetti, macaroni and other pastas (Ministry of Agriculture and Rural Development, 2009).

1.2.3 Corn

Corn (*Zea mays* L.) is widely produced in the country. In Ethiopia, corn is also used as staple food. It is consumed as injera, porridge, bread, *Nifro* or *kollo* (roasted). In addition to the above it is also used to prepare *Tella* and *Arekie* (Ministry of Agriculture and Rural Development, 2009). Corn is largely produced in Western, Central, Southern and Eastern regions of Ethiopia.

1.2.4 Barley

Barley (*Hordeum vulgare* L.) is one of the most important staple food crops in the high lands of Ethiopia. In Ethiopia barley is the fifth most important cereal crop next to corn, tef, sorghum and wheat (Melese Temesgen, 2013). Both food and malting barley are produced in the country (Ministry of Agriculture and Rural Development, 2009).

Barley is the most preferred grain, after tef, for making the traditional bread called *Injera*, which can be used either solely or in combination with tef flour or other cereal flours. It is used to prepare various types of foods (*kollo*, *besso*, *kitta*) and local drinks (*tella*, *araki*, *borde* and *buqri*) and industrial beverages (beer) (Ministry of Agriculture and Rural Development, 2009; Melese Temesgen, 2013).

Some recipes, such as *Besso* (fine flour of well-roasted barley grain moistened with water, butter or oil), *Zurbegonie* (same type of flour used for *Besso* dissolved in cold water with sugar) and *Chiko* (*besso* soaked with butter and spice), which have long shelf life, can only be prepared from barley grain. *Genfo* (thick porridge), *Kollo* (roasted barley grain), *Kinche* (thick cooked) are most popular when made from barley grain, but can be prepared from other cereals also. Other recipes, such as *Dabbo* (bread), *Kitta* (thin, unleavened, dry bread) and *Atmit* (gruel) can be prepared with barley or blended with other cereal flours. Local beverages *Tella* and *Borde* can be made from barley grain. Barley is also traditionally used in the preparation of gruel utilized as weaning food. Barley is mainly used as a source of carbohydrates, although the protein content is also important (Melese Temesgen, 2013).

1.2.5 Pea

In Ethiopia, pea (*Pisum sativum*) is a highly consumed pulse in the daily diet of the society in urban and rural areas (Ethiopian Export Promotion, 2004). It is an important pulse crop next to haricot beans and chickpeas. Pea plant is grown well at 2300-3000 mt and 1800-2300 masl in high and mid altitude areas, respectively (Ethiopian Export Promotion, 2004). A rain falls of 800-1100 mm and 700-900 mm in suitable for high and mid altitude pea growing areas with maximum temperature 20–25 ^{o}C. In Ethiopia, peas are usually sown from mid June to first week of July when there is sufficient moisture. Average yield of pea (productivity) in Ethiopia ranges from 0.5 to 0.6 tone/ha under rain feed (Ethiopian Export Promotion, 2004). Pea is eaten whole, split or milled usually fresh, fried, boiled or mixed with other cereals to make various types of stews, soups (Ethiopian Export Promotion, 2004). It is widely used for food because of its highest protein contents in the form of *Shiro*, *Kik wot*, *Nifro* and Soup (Ethiopian Export Promotion, 2004).

1.2.6 Lentil

Lentils (*Lens culinarus*) are relatively tolerant to drought and grown throughout the world (Akibode and Maredia, 2011). It is one of the important legume crops heavily consumed in Ethiopia (Ethiopian Export Promotion, 2004). It is largely produced in the highland and semi-highland regions of the country mainly on clay soil (Ministry of Agriculture and Rural Development, 2009). Lentil is grown as winter crop in Ethiopia and particularly important in Oromiya and Amhara regions (Ethiopian Export Promotion, 2004). In Ethiopia, out of the total area under cultivation, 94,946 hectares of land is covered with lentil and about 947,734 quintals are produced currently (Ministry of Agriculture and Rural Development, 2009). Lentil types comprise a wide variety, with colors that include yellow, red-orange, green, brown, red, white and black. It is a popular ingredient of every day diet in the majority of household. Lentil is widely used as whole, split in stews, soups and various forms of sandwiches (Ethiopian Export Promotion, 2004). Lentil in Ethiopia is sown from end of June to mid July on red soils (Ethiopian Export Promotion, 2004). Its local price is also higher than most legume crops. It is usually consumed as fried, roasted and boiled whole or split in the form of wot (stews), vegetable soups mixed with other bean. It is also ground to powder to prepare *shiro wot* (stew).

1.2.7 Chickpea

Chick pea belongs to *Leguminoseae* family. It is grown all over the world in about 57 countries under varied environmental conditions. Average chickpea yield in the world is about 1 tons per hectare. India is the single largest producer of chickpea in the world accounting for 68% (Steenbergen *et al*., 2013).

There are two types of chickpea: Desi (*Cicer arientinum* L.), grown near the equator predominates in South Asia and East Africa, and Kabuli (*Cicer kabulium*) which is a large seeded type suited to the more temperate climates (Steenbergen *et al*., 2013). Major producers of the Kabuli types are Turkey, Syria, Iran, Mexico, Morocco and Ethiopia (Janzen *et. al*., 2006).

Chickpeas are important crop in Ethiopia; they are the fourth most produced and cultivated pulse after faba, field and haricot beans (EATA and MOA, 2012). Ethiopia is the sixth largest global chickpea producer, accounting for 3% of production, and is the largest producer of chickpeas in Africa, accounting for 60% of the continent's production in 2010. There are two main varieties of chickpeas in Ethiopia: Kabuli or White Gram and Desi or Brown Gram. Although Ethiopia primarily consumes most of its chickpea production domestically, it is also the 7th largest global exporter, contributing 3.7% of global exports in 2010 (Ethiopian Export Promotion, 2004; EATA and MOA, 2012). Much of the chickpea produced is traded within the domestic markets (Steenbergen *et al*., 2013). Chickpeas are grown across the highlands and semi-arid regions, mostly in the Oromiya and Amhara regions, which account for 90% of chickpea production nation-wide (Steenbergen *et al*., 2013). The major growing areas in the country include Eastern Showa, Western Showa, Gondar, Gojam, and Wello.

The bulk of chickpea variety in Ethiopia is dominated by Desi Type, and the Kabuli type chickpeas are also grown in limited areas (EATA and MOA, 2012; Ethiopian Export Promotion, 2004). Ethiopian domestic markets and exports are dominated by the small-seeded and golden-to-brown colored Desi types (Steenbergen *et al*., 2013). Chickpea is widely used for food for its high protein content (Ministry of Agriculture and Rural Development, 2009). Chickpeas provide eleven times more protein than meats. Chickpeas are an excellent source of protein, fiber, complex carbohydrates, vitamins and minerals. It is also ground in to flour to make baby feed mixed with other cereals, soup, and bread and sweat meat (Ethiopian Export Promotion, 2004). Chickpeas are also the main ingredient in *shiro* (CDC, 200; EATA and MOA, 2012).

Processed chickpeas are provided to the market in processed forms. There are two major types of processors of chickpeas in Ethiopia: (1) large industrial food processing companies, such as Fafa Health Food, Green Star and East Africa which produce various value-added products using Chickpeas; (2) small-scale food processors, such as *Selam Baltina*, *Hilina* and traditionally known as *Baltinas*. These processors prefer the high quality Desi. Small processors target Kabuli products to high-value urban markets (e.g.

hotels and supermarkets) while they may continue to cater the Desi types to their traditional buyers (Steenbergen *et al*., 2013).

1.3 Fluoride

Fluorine is seventh in the order of frequency of occurrence, accounting for 0.06-0.09% of the earth's crust (SCHER, 2010). Some elements are essential in trace amount for human being while higher concentration of the same can cause toxic effects (Tewari and Dubey, 2009); fluoride is one of them (Tewari and Dubey, 2009). Fluorides are defined as binary compounds or salts of fluorine and another element. Fluoride is not considered to be essential for human growth and development but it is considered to be beneficial in the prevention of dental caries (tooth decay) and skeletal fluorosis (SCHER, 2010).

1.3.1 Occurrence of Fluoride in the Environment

Fluoride is a natural component of the earth's crust and soil (USDHS, 2003). Fluorides are released into the environment through a combination of natural and anthropogenic processes (Australian Government, 2007). Variety of inorganic and organic fluorides occur in varying concentrations in rocks, soil, water, air, plants and animals both naturally and as a consequence of human activity such as agricultural or industrial processes. Human exposure may be through any or all of these sources (Ahokas *et al*., 1999). Fluoride is found in drinking water, in air and in foodstuffs (Tamiru Alemayehu, 2006). The use of fluoride containing pesticide and controlled fluoridation of drinking-water supplies also contribute to the release of fluoride from anthropogenic sources (WHO, 2002).

1.3.2 Fluoride in the Soil and Water

Fluoride is a component of most types of soil, with total fluoride concentrations ranging from 20 to 1000 mg/kg in areas without natural phosphate or fluoride deposits and up to several thousand micrograms per gram in mineral soils with deposits of fluoride. The clay and organic carbon content as well as the pH of soil are primarily responsible for the retention of fluoride in soils. In general, fluoride bound to soil is relatively resistant to leaching and it is the soluble content that is most important to terrestrial animals and plants. Factors known to influence water fluoride levels include the presence of natural rock rich in fluoride (such as granites and gneisses and sediment of marine origin. Additionally, elevated inorganic fluoride levels are often seen in regions where there is geothermal or volcanic activity (Australian Government, 2007).

Fluoride exists abundantly in the earth's crust and can enter groundwater by natural processes (Hailu Tefera and Seyfu Ketema, 2001). Fluoride beyond desirable amount (0.5-1.5 ppm) in ground water is a major problem in many parts of the world (Yadav *et al.*, 2013). Fluorides in soils are transported to surface waters through leaching or runoff of particulate-bound fluorides (CDC, 2001). Naturally fluoridated waters contain the fluoride ion derived from sparingly soluble minerals such as fluorapatite or fluorite (Ahokas *et al.*, 1999). Clay and organic carbon content of the soil are also responsible for the retention of fluoride in soils (WHO, 2002). Recommended limited value for fluoride in drinking water by World Health Organisation (WHO) and European Union (EU) is 1.5 ppm (Rajković and Novaković, 2007).

1.3.3 Fluoride in Plant

Fluoride is strongly retained by soil, forming complexes with soil components (CDC, 2001). The amount of fluoride taken up by plants depends on the type of plant, the nature of the soil, and the amount and form of fluoride in the soil (WHO, 2002). Availability of fluoride from soil depends on the solubility of the compound, the acidity of the soil and the presence of water (SCHER, 2010). Low levels of calcium in water supplies may also lead to higher levels of fluoride solubility (Australian Government, 2007). In water, fluorides associate with various elements present in the water, mainly with aluminum in freshwater and calcium and magnesium in seawater, and settle into the sediment where they are strongly attached to sediment particles (USDHS, 2003). The transport and transformation of fluoride in soil are influenced by pH and the formation of predominantly aluminum and calcium complexes. For all soils, it is the soluble fluoride content that is biologically important to plants and animals (WHO, 2002).

Most of the fluoride in the soil is insoluble and therefore, less available to plants; However, high soil fluoride concentrations or low pH, clay and/or organic matter can increase fluoride levels in soil solution, increasing uptake via the plant root. Fluoride in soil is mainly bound in complexes. The maximum adsorption of fluoride to soil was reported to occur at pH 5.5. Fluoride in alkaline soils at pH 6.5 and above is almost completely fixed in soils as calcium fluoride, if sufficient calcium carbonate is available. In sandy acidic soils, fluoride tends to be present in water-soluble forms (WHO, 2002).

The concentration of fluorides in soils is usually between 200 and 300 mg/kg (USDHS, 2003). Plants accumulate fluoride in different extents, depending on the type of the plant and of the soil where they grow (WHO, 2002). Among beverages, tea, coffee, coconut water, beer and wine, etc. contain higher levels of fluoride. Among beverages tea has exceptionally high fluoride content (Mahapatra, 2007).

Tea plants, the most known, accumulate fluorides, and sea foods are also known to accumulate high level of fluoride. The amount of fluoride in brewed tea is dependent upon the concentration of soluble fluoride in the tea leaves, the level of fluoride in the water used in its preparation and the length of the brewing period (WHO, 2002; USDHS, 2003). Some foodstuffs such as vegetables and fruits normally contain fluoride through at low concentration (0.1 mg/kg–0.4 mg/kg) and thus contribute to fluoride intake by human (Yadav *et al*., 2013).

1.3.4 Fluoride in Food

Fluoride exposure is not limited to its presence in fluoridated drinking water (Ahokas *et al*., 1999). Fluoride ingestion through food is comparatively less than through water (Bhargavi *et al*., 2004). Food generally contains low levels of fluoride (USDHS, 2003); however, food grown in areas where soils have high amounts of fluorides or where phosphate fertilizers are used may have higher levels of fluorides (CDC, 2001). The use of water containing relatively low (<3.1 mg/L) levels of fluoride for crop irrigation generally does not increase fluoride concentrations in foodstuffs. However, this is dependent on plant species and fluoride concentrations in soil and water (WHO, 2002).

Food grown in areas where soils have high amounts of fluorides or where phosphate fertilizers are used may have higher levels of fluorides (USDHS, 2003). Besides water, food items especially agricultural crops are heavily contaminated with fluoride bearing rocks. Fluoride level in the food depends on mainly: fluoride level in the soil, fluoride level in the atmosphere, use of fertilizers and pesticides and other source of contamination (Sunitha and Reddy, 2008).

The general population can be exposed to fluoride through the consumption of fluoridated drinking water, food, and dentifrices. The average dietary intake (including water) of fluoride ranges between 1.4 and 3.4 mg/day (0.02–0.048 mg/kg/day) for adults living in areas with 1.0 mg/L fluoride in the water. In areas with < 0.3 mg/L fluoride in water, the adult dietary intakes ranged from 0.3 to 1.0 mg/day (0.004–0.014 mg/kg/day). In children, the dietary intakes ranged from 0.03 to 0.06 mg/kg/day in areas with fluoridated water and from 0.01 to 0.04 mg/kg/day in areas without fluoridated water. The Food and Nutrition Board of the Institute of Medicine has developed adequate intakes (AIs) for fluoride. The AI is the "estimated fluoride intake that has been shown to reduce the occurrence of dental caries maximally in a population without causing unwanted side effects including moderate dental fluorosis." The AIs for each age group are presented in Table 1 (CDC, 2001).

Table 1. Adequate levels of fluoride for different age groups.

Age Group	Sex Type	age	Adequate Level
Children	Both	0-6 months	0.01 mg/day
		7-12 months	0,5 mg/day
Boys/Girls	Both	9-13 years	2 mg/day
		14-18 years	3 mg/day
Male	Male	19 and above	4 mg/day
Female	Female	19 and above	3 mg/day

Source: (CDC, 2001)

1.3.5 Fluoride in Other Supplements

Fluoride, either naturally present or intentionally added to water, food, consumer and medical products, is considered beneficial to prevent dental caries (tooth decay) (SCHER, 2010; Ahokas *et al*., 1999). There is a range of alternatives for provision of fluoride to the community including fluoridated drinking water; fluoridated toothpastes; supplementary tablets or drops; fluoridated salt, sugar or milk (Ministry of Agriculture and Rural Development, 2009). The amount of fluoride intake depends on the final concentration of the product and the conditions under which food is processed (Casarin *et al*., 2007). The fluoride content of milk-based powders ranged from 0.23 to 3.71 g/kg and soybased powders ranged from 1.08 to 2.86 g/kg (Australian Government, 2007).

Human being may also be exposed to fluoride in dental products, such as toothpastes, fluoride gels, and fluoride rinses. Dental products used in the home such as toothpastes, rinses, and topically applied gels contain high concentrations of fluoride (230–12,300 mg/kg) (CDC, 2001). The most commonly used dental products (toothpastes) contain 900–1,100 mg/kg fluoride, most often as sodium fluoride (CDC, 2001).

1.3.6 Fluoride Intake of Human

Total exposure to fluoride depends on contributions from other sources, such as drinking water, water based beverages, food, food supplements, use of toothpaste and to a lesser extent from other environmental sources (SCHER, 2010). The major factor which determines the extent of adverse effect depends on the fluoride content of natural drinking-water, the total amount ingested daily, the duration of ingestion and the efficiencies of intestinal absorption and renal excretion (WHO, 1996; Tamiru Alemayehu, 2006). The total intake of adults is usually within the range 0.2–2.0 mg of fluoride/day but higher intakes are common where the fluoride content of drinking-water is high (WHO, 1996). People who drink large quantities of tea may be exposed to high levels of fluoride in their diets (USDHS, 2003). The total ingestion of fluoride from all sources is taken into consideration when safe levels are derived (WHO, 1996). Low intake of Ca in the diet leads to greater retention of fluoride in the bones (Bhargavi *et al*., 2004).

1.3.6.1 Metabolism of Fluoride

Generally, most of the fluoride in food or water after consumption enters bloodstream quickly through the digestive tract. Biological interactions between fluoride and calcium are known to occur. Most of the fluoride ion that stays in the body is stored in bones and teeth (WHO, 1996; CDC, 2001).

However, the amount of fluoride that enters bloodstream also depends on factors such as amount of the fluoride consumed, the solubility of fluoride in water and types foods to be eaten or drunk (CDC, 2001). After absorption, fluoride ion is quickly distributed throughout the body, easily crossing the membranes and going into tissues. It accumulates in body due to high reactivity of fluoride ion with calcium of teeth and bones. It forms calcium fluorophosphates (fluorapatite) crystal and leaves unbound calcium in the same tissue, which gets calcified and in turn results in stiffness of tissues and joints. This finally leads to skeletal fluorosis in later stage. That's why fluoride is called as bone seeking mineral and bones as sink for fluoride. About 90% of the fluoride retrieved in body is associated with calcified tissues (Tewari and Dubey, 2009). Particularly, severe clinical forms of fluoride toxicity are reported among population groups whose calcium nutritional status is poor (WHO, 1996).

Absorption of dietary fluoride varies from region to region and is influenced by dietary composition. While exposure to toxic levels of fluoride produces osteosclerotic forms of fluorosis in those whose dietary intakes of calcium are adequate, osteomalacia develops in subjects exposed to fluoride whose calcium intake is low (WHO, 1996). In general, the more soluble the fluoride, the more that can be absorbed by oral ingestion, and the more toxic it is (CDC, 2001). All organisms are exposed to fluoride from natural and/or anthropogenic sources (WHO, 2002). There are multiple effects of fluoride on human health. Dental fluorosis and effects on bones are the two well documented adverse effects of fluoride intake (SCHER, 2010). Bone and tooth having highest amount of calcium in the body attracts the maximum amount of fluoride and is deposited as calcium fluoroapatite crystals (Tewari and Dubey, 2009). Fluoride being an electronegative element and having a negative charge is attracted by positively charged ions like calcium (Ca^{2+}) (Tewari and Dubey, 2009). Fluoride accumulates in the teeth and bone resulting in beneficial and detrimental alterations in the structure (CDC, 2001). Fluorosis is a result of abnormal deposition of fluoride in hard tissues (Mahapatra, 2007). Fluorosis is seen as mild moderate and severe depending on the amount of fluoride ingested during the stages of formation of the teeth (Tewari and Dubey, 2009).

1.3.7 Fluoride Effect on Health

Both beneficial and detrimental dental and skeletal effects have been observed in humans (CDC, 2001). Adverse health effects of fluoride, particularly dental and skeletal fluorosis, caused by chronic exposure to excessive fluoride, continue to be major health problems in many parts of the world (The International Society for Fluoride Research, 2012). Many countries are identified for the problem of fluorosis, such as Pakistan, Bangladesh, Argentina, USA, Morocco, and Middle East countries, Japan, South Africa, New Zealand and Thailand (Sunitha and Reddy, 2008). Fluorosis is a public health problem in certain part of Ethiopia, especially in rift valley areas (Dagnew Tadesse, Assefa Desta, Aberra Geyid, Woldemariam Girm, 2010).

1.3.7.1 Skeletal Fluorosis

Skeletal fluorosis can be caused by eating, drinking, or breathing very large amounts of fluorides (USDHS, 2003). Excessive quantity of fluoride deposited in the skeleton (Tewari and Dubey, 2009). Fluoride ions are incorporated into bone substituting hydroxyl groups in the carbonate-apatite structure to produce fluorohydroxyapatite, thus altering the mineral structure of the bone (SCHER, 2010). Signs of skeletal fluorosis range from increased bone density to severe deformity, known as crippling skeletal fluorosis. In the more severe cases of crippling fluorosis, complete rigidity of the spine can occur. Reported cases are found almost exclusively in developing countries, particularly India and China, and are often associated with malnutrition (CDC, 2001). This disease only occurs after long-term exposures and can cause denser bones, joint pain, and a limited range of joint movement. In the most severe cases, the spine is completely rigid (USDHS, 2003). The fluoride content of human bones varies from 300 to 7000 pg/g of dry tissue depending on total fluoride exposure (WHO, 1996). Skeletal fluorosis may occur when fluoride concentrations in drinking water exceed 4–8 mg/L(Sunitha and Reddy, 2008).

1.3.7.2 Dental Fluorosis

Dental fluorosis is the loss of luster and shine of the dental enamel. Dullness of the teeth and loss of shine with developed white and yellow spots are the symptoms of dental fluorosis (Tewari and Dubey, 2009). Teeth commonly affected by fluorosis are central incisors, lateral incisors and the molars of the permanent dentition. It affects both the inner and the outer surfaces of teeth (Tewari and Dubey, 2009). The severity and prevalence of dental fluorosis is related to the fluoride concentration in drinking water, however it is the daily total fluoride intake over a prolonged period of time during the developmental phase of the teeth that results in fluorosis (SCHER, 2010). The minimal daily intake of fluoride that can cause very mild or mild fluorosis is estimated to be about 0.1 mg/kg body weight (Tewari and Dubey, 2009). Clinical data indicate that adequate calcium intake is clearly associated with a reduced risk of dental fluorosis. Vitamin C may also safeguard against the risk (Tewari and Dubey, 2009). The above studies have also suggested that the amount of fluoride absorbed from the diet depends not only on the absolute quantity of the element present but also on the dietary calcium and magnesium (Bhargavi *et al*., 2004).

1.4 Fluoride Distribution in Ethiopia

Fluorosis is endemic in at least 25 countries around the world with India, China and Africa being the countries with the highest prevalence rates (Mahapatra, 2007). Dental fluorosis is widespread in eastern part of Africa (Malde *et al*., 1997). In certain parts of Ethiopia, especially rift valley areas, fluorosis has been observed to be a public health problem (Dagnew Tadesse, Assefa Desta, Aberra Geyid, Woldemariam Girma, Solomon Fisseha, Oliver Schmoll, 2010). Fluorosis is assessed as a risk for a population of close to

8.5 Million in Ethiopia. The population at risk lives mainly in the Rift Valley—spread over different regional states: Afar, Amhara, Oromyia and SNNPR (Steenbergen *et al*., 2011).

The moderately high fluoride concentrations (1.5–5.0 mg/L) are found in the highlands of Ethiopia, indicating that fluorosis is not confined to the Rift Valley (Tamiru Alemayehu, 2006). In the Rift Valley region of Ethiopia, the problem of high fluoride concentrations in natural waters, especially groundwater, is severe and widespread (Dagnew Tadesse, Assefa Desta, Aberra Geyid, Woldemariam Girma, Solomon Fisseha, Oliver Schmoll, 2010). There is wide difference in fluoride among different water bodies. Fluoride varies from 1.9 to 250 mg/L for lakes 2 to 150 mg/L for hot spring, from non detectable to 6.4 mg/L for boreholes and 2 to 68 mg/L for geothermal wells (Tamiru Alemayehu, 2006). In Halaba, part of rift valley, the area is rich in fluoride. Fluoride levels of drinking water systems in Halaba are high, ranged between 1.3 to 13.1 mg/L (Steenbergen *et al*., 2011).

The Wonji area and its environments contain high fluoride in the groundwater attributed to the high thermal activity (Tamiru Alemayehu, 2006). Workers of Wonji sugar factory were the first to be diagnosed of fluorosis in the mid 1970's. In addition to the Rift Valley region, groundwater resources in Oromiya region have been found to contain significant fluoride concentrations (Ministry of Agriculture and Rural Development, 2009).

The fluoride level in dry legume and cereal grains has been reported in different parts of the countries. Study by Sunitha and Reddy (2008) reported that cereal and legume crops grown in fluoride endemic areas of India had fluoride level in their grains: wheat (4.6 mg/kg), corn (5.9 mg/kg), soybean (4.0 mg/kg) (Sunitha and Reddy, 2008).

Recently, Meseret Dessalegne (2011) and Tesfaw Ashagrie (2011) determined fluoride levels in cereals and legume based foods consumed in fluoride rift valley area (Bidara Fuka and Dibibisa kebele, located in rural part rift valley), and Dura Woreda (East Shewa Zone of Oromiya) of Ethiopia: bread prepared from cereal mixture (wheat and corn) and (corn and sorghum) is 3.54±0.004 mg/kg and 5.33±0.76 mg/kg, respectively; bread prepared from corn only is 3.91±0.015 mg/kg; bread prepared from Wheat only is

2.47±0.01 mg/kg (Tesfaw Ashagrie, 2011). Meseret Dessalegne (2011) determined the level of fluoride in corn flour (12.2 mg/kg) and tef flour (15.1 mg/kg), which are food ingredients used for preparation of food and beverages consumed in population living in Dugda Woreda.

Injera in Bidara Fuka Kebele is prepared from single or mixture of cereals (corn, sorghum and tef) and daily food intake of young children (10 to 14 years old) living in Bidara Fuka and Dibibisa kebeles of SNNPR and Oromiya region, a rural part of Ethiopian rift valley (Tesfaw Ashagrie, 2011). In Bidara Fuka and Dibibisa kebeles, the average daily fluoride intake of intake of young children was 9.29 mg/day (0.310 mg/kg/body weight) and 7.71 mg/day (0.256 mg/kg body weight), respectively (Tesfaw Ashagrie, 2011).

In Ethiopian, tradition stew (wot) is prepared from different types of legumes. In Bidara Fuka and Dibibisa kebeles, like in other areas, wot is prepared from lentil, chickpea, guaya (grass pea), pea and beans. Tesfaw Ashagrie (2012) found that Fluoride level in wot consumed by population living in Bidara Fuka and Dibibisa was (4.17±0.85) mg/kg, which is prepared from mixture lentil and guaya; (3.62±0.004), (3.69±0.002) and (5.33±0.007) mg/kg, which is prepared from lentil, pea and beans, respectively.

In India, Yadav *et al.* (2012) studied fluoride levels in wheat crops (3.24-14.30 mg/kg), which were cultivated in Dausa District (Rajasthan, India) where fluoridated water (5.6-14.7ppm) was used for irrigating those crops.

Both Meseret Dessalegne (2011) and Tesfaw Ashagrie (2011) confirmed that cereal and legume based foods consumed in the population living in rift valley areas (Bidara Fuka and Dibibisa Kebele, and Dura Woreda) has high fluoride content. Recently, level of fluoride in the raw rice (0.1-5.5 mg/kg) and was reported by Bisrat Tegegne, B.S Chandravanishi and Feleke Zewge (2013). *Enset* is a staple food in southern part of Ethiopia. Study by Gautam *et al*. (2005) showed that fluoride level in *Enset* (*bulla*) is 2.5 mg/kg.

Dental as well as skeletal fluorosis is endemic in Ethiopian, especially in rift valley areas. Cereals and legumes are cultivated both in rift valley and outside rift valley areas. Most of fluoride related reports in Ethiopia were from the rift valley areas. Tesfaw Ashagrie (2011) and Meseret Dessalegne (2011), for example, focused determination of fluoride content on foods prepared from cereals and legumes which are consumed rift valley areas (Bidara Fuka and Dibibisa Kebele and Dura Woreda). However, this research focused on cereal and legume grains produced in selected area of Ethiopia, both in rift valley and outside rift valley. Therefore, it was important to determine levels of fluoride in staple cereals and legumes, because food ingredients can accumulate fluoride in their edible parts, which may thus affect human health.

1.5 Objectives of the Study

1.5.1 General Objective

The main objective of this thesis is to determine the levels of fluoride in the grain of cereals and legumes produced in selected areas of Ethiopia.

1.5.2 Specific Objectives

- To determine the levels of fluoride in different varieties of cereal and legume grains produced in selected area of Ethiopia;
- To compare levels of fluoride in cereals and legumes with the other similar crops;
- To compare the levels of fluoride in cereal with respect to the cultivation site; and
- To compare level fluoride of cereals and legumes grains of this study with values reported in other literatures.

1.6 Significance of the Study

Determination of fluoride level in the food ingredients is important step in prevention of dental and skeletal fluorosis, considering that food consumption is also a mechanism of human fluoride exposure. This study provides total fluoride content of staple cereals and legume grains which are produced in selected areas of Ethiopia. It helps to determine whether these staple grains contribute fluoride in food consumption. Generally, the results of this study is used as base-line information for further studies, show the direction in setting guideline values for health related chemicals in consumption of cereal and legume based types of foods.

2. EXPERIMENTAL

2.1 Apparatus, Instrumentation, Chemicals and Reagents

2.1.1 Apparatus

Polyethylene plastic bags were used for collecting cereal and legume samples. Electronic blending device (Moulinex, France) was used for grinding and homogenizing the sample. Electronic balance (Adam Equipment, Model WL 3000, U.K.), with precision of 0.0001 g was used for weighing of both cereal and legume samples. A pH/ISE meter (Orion model, EA 940 Expandable Ion Analyzer, USA) equipped with combination fluoride selective electrode (Orion Model 96-09, USA) was employed for the determination of fluoride in the samples and standard solutions. The pH was measured with pH meter (HANNA instrument, HI 9025, Singapore) using pH glass electrode. Borosilicate volumetric flasks (50, 100, 500 and 1000 mL) were used for preparation of both 8 M NaOH solution and 1000 mg/L NaF stock solution. Ni crucibles (50 and 70 mL) were used for fusion of cereal and legume samples for total fluoride determination. Muffle furnace (Audiotronics, Wagtech International Ltd., UK) were used for the fusion of cereal and legume samples. Hot plate with magnetic stirrer was used for dissolution of the sample and fusion cake. Measuring cylinders (Duran, Germany), pipettes (Pyrex, USA); micropipettes (Dragonmed, 1–10 µL, 100–1000 µL, Shangai, China) were made use during measuring different quantities of volumes of sample solution and fluoride standard solutions.

2.1.2 Chemicals, Reagents and Standard Solutions

All reagents used in this work were analytical grade. Distilled-deionized water was used for all dilutions. Sodium fluoride (99.0% NaF, BDH Chemicals Ltd, Poole, England) was used to prepare fluoride stock standard solution. Glacial acetic acid (99.5%, BDH limited, Poole, England), sodium chloride (Scharlau, European Union), sodium citrate (Research-Lab Fine Chem. Industries Mumbai 400 002, India) and EDTA (Reagent grade, Spain) to prepare total ionic strength adjustment buffer (TISAB) solution, sodium hydroxide (Scharlau, European Union) solution to adjust pH of TISAB solution and hydrochloric acid (Scharlau, European Union) to adjust pH of the sample solution.

1000 mg/L fluoride stock solution was prepared by dissolving 2.210 g of NaF in 1000 mL of distilled-deionized water. This was stored in a plastic volumetric flask. The TISAB (Total Ionic Strength Adjustment Buffer) was prepared by dissolving 57 mL of glacial acetic, 58 g of NaC1, 7 g of sodium citrate and 2 g EDTA in 500 mL distilled water and finally diluted to 1000 mL in a volumetric flask with distilled-deionized water. The solution was then adjusted to pH 5–5.5 with a 5 M NaOH solution (PASCO Scientific, 1997).

2.1.3 Instrumentation

Orion fluoride ion selective electrode was used for routine determination of fluoride ion in cereals and legume samples. A pH/ISE meter (Orion Model, EA 940 Expandable Ion Analyzer) equipped with combination fluoride-selective electrode (Orion Model 96-09) was employed. The pH was measured with pH/ion meter (WTW Inolab pH/ION Level 2, Germany) using unfilled pH glass electrode.

2.2.4 Description of the Sample Area

For this study, four types of cereals (tef, wheat, corn and barley) and three types of legume (pea, lentil and chickpea) from selected areas of Ethiopia were collected. The samples were collected from both from rift valley and non-rift valley areas of Ethiopia, i.e. from Amhara and Oromiya regional states: Arsi Negele, Adola, Wonji, Ada, Gonder, Burrie and Dessie. Both types of samples were collected from local accessible market in the area where they are cultivated in large amount. From Amhara region, the cereals are collected from Gonder, and legumes from Gonder, Burrie and Wollo. From Oromiya regions, cereals were collected from Arsi Negele, Wonji and Adola and legumes were collected from Ada. Wonji, Arsi Negele and Ada are located in the rift valley areas. Sampling of cereals and legumes sample were took place in February and September 2012, respectively. A total of 28 samples, i.e. 4 types of cereals from four sample areas and 3 types of legumes from four sample areas, were collected.

The Amhara Region is located in the northwestern part of Ethiopia between 9°20’ and 14°20’ north latitude and 36°20’ and 40°20’ east longitude. Out of all area under grain, tef, barley and sorghum took up 26.03%, 11.36% and 13.56%, yielding 21.01%, 9.95% and 15.58% of the regional grain production, respectively (CSA, 2003). Gojam is located in Amhara regional state and it is located at an elevation of 2,792 meters above sea level. In Gojam, both legumes (horse beans, chickpea) and cereals (tef, wheat, corn, barley and sorghum) are cultivated (CSA, 2003). In Gonder, both legumes (Horse beans, chick peas and field peas) and cereals are produced (Steenbergen *et al*., 2013; CSA, 2003).

Burrie

It is one of the 15 *Woredas* in West Gojam administrative Zone. The mean annual rain fall of the *Woreda* is 1000–1500 mm. The altitude of the district ranges from 713 to 2600 meters above sea level, and its annual rainfall varies from 1386 to 1757 mm. The average minimum and maximum temperature of the district is 14 ^{0}C and 24 ^{0}C, respectively.

Gonder

Gonder is located in Amhara regional state 728 km away from Addis Ababa. The mean annual maximum and minimum temperature ranges from 22–30.7 ^{o}C and 12.3–17.7 ^{o}C, respectively, with an annual average temperature of 19.9 ^{0}C. It is located at an altitude of 2,220 meter above sea level and the area receives a mean annual rain fall of 1172 mm.

Dessie

Wollo is located in Amhara regional state. It has two classification, north and south Wollo. Dessie is the capital city of South Wollo Administrative Zone. The town is situated at 400 km from Addis Abeba, Ethiopia. The South Wollo Belg livelihood zone is totally located the in the South Wollo Administrative Zone.There are three seasons mainly known in the area. These are: Belg (short rains (January-June)), Kiremt (long rains (July-September)) and Meher (harvest (October-December)). Legumes (lentil, faba beans, field pea, and chickpea) and cereal (barley, domestic oats (adja) and wild oats) are the most produced crops in Wollo. In Wollo, the legumes mentioned above are used to prepare *Wot* to accompany the injera (www.feg-consulting.com/, 2007).

Wonji

Wonji is located in Oromiya regional state, about 100 km away from Addis Ababa city. The altitude of this area lies from 1500-1700 masl. The average annual rainfall of the area is about 688 mm and its mean maximum and minimum temperatures are 27.2 ^{0}C and 14.4 ^{0}C, respectively.

Ada Liben

Ada'a Liben is one of 180 woredas in the Oromiya National Regional State of Ethiopia, found 47 km South East of Addis Ababa. About 90% of the woreda belongs to the sub tropical climatic zone. The altitude of the Woreda ranges from 1500 m to over 2000 m, and the mean annual rainfall, mean min. and mean maximum temperatures are 851 mm, 11 ^{o}C and 29 ^{o}C, respectively (Kassahun Melesse , 2008). Important grain legumes are: chick pea (*Cicer arietinum*), horse beans (*Vicia faba*), field peas (*Pisum sativum*), rough peas (*Lathyrus satvus*) and lentils (*Lens esculanta*). Ada Liben can be roughly subdivided in to Rift valley agro-ecology and a mid/high altitude area. The rift valley zone ranges from 1500-1800 m. The highland zone covers at an elevation of 1800-2000 m. Ada is bordered on the south by Dugda Bora, on the west by the Mirab Shewa Zone, on the northwest by Akaki, on the northeast by Gimbichu, and on the east by Lome. Part of the Misraq Shewa Zone located in the Great Rift Valley.

Arsi Negele

Arsi Negele is located in central rift valley, at a distance of 225 km from Addis Ababa. Mean annual temperature is 25 ^{0}C. Corn, tef and wheat are produced large amount, including vegetables and fruits. The majority of the producers were also dependent on subsistence farming. The monthly mean maximum temperature in Arsi Negele varies between 24.9 and 29.9 degrees Celsius. Monthly mean minimum temperatures are between 6.7 and 13.8 ^{0}C. Arsi Negele is located in rift valley area of Ethiopia.

Adola

Adola is located in Oromiya regional state. Adola town is located between 5^{o}44’10”–6^{o}12’38” northing latitudes and 38^{o}45’10”–39^{o}12’37” easting longitudes. Adola town district is situated at a distance of 475 km from Addis Ababa. Adola town has an area of about 5.75 square kilo meters. The area is characterized by three agro-climatic zones, namely Dega, Woina Dega and Kola (locally known as ‘Bada or Dega’) which starts in early October up to march and Woina Dega (locally known as ‘Bada Dare’) which starts late April up to reaches beginning of June and Kola or ‘Gamoji’ which starts May up to June. The mean annual rain fall is about 900 mm and the annual temperature of the district 25 ^{0}C. Wheat, barley, corn, sorghum, fruits and vegetables are grown.

2.2.5 Sample Preparation for Fluoride Analysis

The determination of fluoride in cereal and legume using fluoride ion selective electrode was done by slightly modifying reported methods (Malde *et al*., 1997). The cereals and legume grains were separately washed by tap water and then distilled water. After washing, they were sun dried. Then, they were ground using a blender device in the laboratory and sieved with about 0.457 mm sieve. 0.5 g powdered sample from each samples was accurately weighed and placed in to 50 mL of nickel crucibles. 5 mL of 8 M NaOH was added. Then, the samples and sodium hydroxide solutions were carefully mixed. The crucibles were placed on a hot plate for evaporation until the mixed NaOH and sample was solidified. The crucible was then placed in a muffle furnace and set at 200 ^{o}C for 2 h, after which the temperature was increased to 525 ^{o}C and kept there for 3 h in muffle furnace in order to fuse the sample in the crucible. The crucible was placed in a hood and allowed to cool. 10 mL distilled water was added and placed on hot plate to facilitate the dissolution of the fusion cake. After 2 h, the sample solution was transferred to plastic volumetric flask, made up to volume with distilled water and filtered through a Whatman No. 40 filter paper. The pH of the filtrate was adjusted to pH 7.2–7.5 using concentrated HCl or 5 M NaOH. The sample solution was transferred into 50 mL volumetric flask and made up to the mark. Then, these digested samples were kept in the refrigerator until the analysis. All the analysis was made in triplicate. The fluoride content of cereals and legumes was then determined based on the calibration curve plotted using standard solutions.

2.2.6 Fluoride Determination in the Samples

There are different techniques of fluoride measurements: fluoride ion selective electrode, chromatography, atomic and molecular spectroscopy, etc. Fluoride ion selective electrode is the most successful ion sensitive electrode, and has received wide acceptance to stage where it is now uncommon to see alternatives reported (Jeffrey Ripp, 1996). The advantage of ion selective electrode usage for determination of fluoride ion is in a wider range of linearity, short time of response, non-destructivity of samples and there is no contamination of samples (Akibode and Maredia, 2011). Potentiometric analysis of fluoride content (as F^{-} ion) in solutions by using fluoride ion-selective electrode is simple, reliable and cheap. Very small concentrations of fluoride-ions (to 10^{-6} M) can be determined by fluoride selective electrode, with regulation of ion strength of a solution and control of concentration of hydroxide ions and interfering ions of metals (Malde *et al.*, 1997). FISE should be a method of choice for those with little experience in analytical chemistry. It is easy to operate, relatively rapid and require little technical skill (Campbell, 1987). In direct potentiometry method, concentration can be determined with a single measurement (Bratovčić *et al.*, 2009). In this research, fluoride levels of cereals and legumes were determined by the method of direct potentiometry, and in the case of very low contents, by standard addition method. The detection limit of the fluoride ion selective electrode potentiometry used was 0.02 mg F/L.

2.2.7 Instrument Calibration

2.2.7.1 Calibration of the Fluoride Electrode

With appropriate instrumentation and sample preparation, FISE is capable of providing very reliable (accurate and precise) results, using either a standard calibration curve for reference or a known addition procedure to eliminate matrix effects on the analysis (SCHER, 2010). The standard curve of fluoride reflects the ratio between the content of fluoride in a solution and the resulting measurement response.

Standard solution was prepared by dissolving 2.21 g of NaF in 1000 mL deionised water. Five standard solutions (0.5, 1.0, 5.0, 10.0 and 20.0 mg/L) were prepared by serial dilution from 1000 mg/L standard solution. The concentration of the samples was determined by constructing a calibration curve from these standards. A typical calibration curve is shown in Figure 1. At room temperature, the ISE was placed in a beaker containing 5 mL of standard solution, along with 5 mL of TISAB (1:1). Adding of TISAB to standards and to samples breaks fluoride complexes of Fe and Al, providing a constant ionic strength (Buck and Cosofret, 1993). When Calibrating, it is assumed that the added TISAB has no effect on the standard concentration (Buck and Cosofret, 1993).

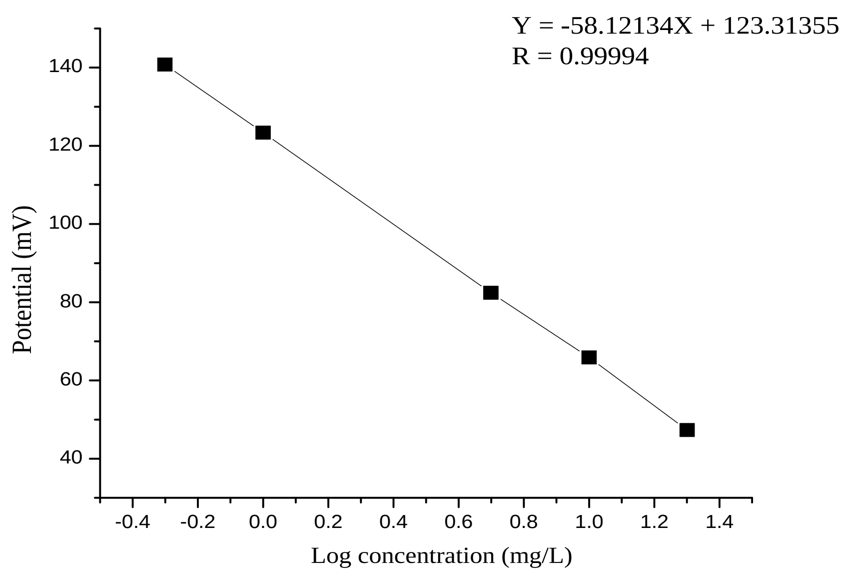

Figure 1. Calibration curve for fluoride determination.

The operation of ion selective electrodes is based on the fact that there is a linear relationship between the electrical potential developed between an ion-selective electrode (ISE) and a reference electrode immersed in the same solution, and the logarithm of the concentration of the ions in the solution. This relationship is described by the Nernst equation:

$$E = E_o \pm (2.303RT/zF) \times \text{Log } C$$

where E = the total potential (in mV) developed between the sensing and reference electrodes, E_o = constant characteristic of the particular ISE/reference pair, (it is the sum of all the liquid junction potentials in the electrochemical cell), 2.303 = the conversion factor from natural to base 10 logarithm, R = the gas constant (8.314 Joules/degree/mole), T = the absolute temperature (K), z = absolute value of the charge on the ion, F = the Faraday constant (96,500 coulombs per mole), Log C = the logarithm of the concentration of the measured ion. The slope of electrode response is ± 2.303RT/zF (which shows plot of E (mV) against Log C is a straight line within the concentration range within linear range of the electrode response. This is an important diagnostic characteristic of an ISE. Because all of the solutions are at a constant ionic strength, Equation 1 can be rewritten as follows: E = constant - 0.059 log [F^-]. The calibration curve equation clearly indicates that the slope of calibration curve was 58.1, which is very close to the Nernstian slope of 59.2 mV. The calibration curve equation also showed that the correlation coefficient was 0.99994 indicating very good correlation between potential and concentration of fluoride.

2.2 Method Validation

A method of analysis is characterized by its performance parameters, which have to be assessed if they are to provide the correct performance values. These performance values must be in accordance with previously defined requirements that the method of analysis should satisfy (Gumustas, 2011). Parameters such as accuracy, precision, specificity, limit of detection, limit of quantization, etc are used for method validation.

2.2.1 Precision and Accuracy

Precision is a measure of the random error associated with a series of repeated measurements of the same parameter within a sample. Precision describes the closeness with which multiple analyses of a given sample agree with each other, and is sometimes referred to as reproducibility. Precision is determined by the absolute standard deviation, relative standard deviation, variance, coefficient of variation, relative percent difference, or the absolute range of a series of measurements. The accuracy of an analytical method is defined as the degree to which the determined value of analyte in a sample corresponds to the true value. Accuracy may be measured in different ways and the method should be appropriate to the matrix (Jeffrey Ripp, 1996). Repeatability expresses the closeness or agreement between a series of measurements obtained from multiple sampling and sample analysis (Jeffrey Ripp, 1996). It is often expressed as the standard deviation or relative standard deviation of replicate measurements.

2.2.2 Detection Limits

There are several different types of detection limits depending what is being defined. However, method detection limit (MDL) is the only one designed to be determined in laboratory using chemicals, equipment, and technicians. The other detection limits are either determined by the instrument manufacturer or are calculated from the MDL (Tim Loftus, 2003).

Limit of detection (LOD) or detection limit, is the lowest concentration level that can be determined to be statistically different from a blank (99% confidence) (Jeffrey Ripp, 1996). The generally accepted and common definition of method detection limit is the concentration that gives a signal three times the standard deviation of the blank or background signal. In this study, the precision of the results were evaluated by standard deviation of the results of nine measurements triplicate sample preparation and triplicate readings. Instrument detection limit is 0.02 mg/L.

2.2.3 Validation of the Procedure

Analytical method can be validated using different techniques: Validation by using alternative method, validation by using proficiency testing schemes or by using certified reference materials (Ndlovu, 2005). In this study, the procedure was validated by analyzing fluoride concentration sample prepared by alkali fusion and spiked with a small amount of fluoride. Validation of the method used was checked by performing recovery tests. The percentage recovery of method was evaluated by calculating percentage recovery (% R). Recovery is defined as the ratio of the observed result to the expected result expressed as a percentage (Jeffrey Ripp, 1996).

The spiked samples were prepared by adding a small known quantity of fluoride standard solution to cereal and legume sample by applying similar procedure to prepare the sample and analyzed for the levels of fluoride to calculate the recovery percent. Each cereals and legume samples were spiked with fluoride solution of which the fluoride content was equivalent to 25%, 50%, or 100% of the fluoride content of the original (unspiked) samples. Thus, percent recovery was obtained by comparing the results between the fluoride found and the fluoride added. Percentage recovery of added element is calculated as follow:

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

where: C_M= Concentration of fluoride. The recovery of fluoride in this study, in cereals and legumes grain, is summarized in Table 2 and 3.

3. RESULTS AND DISCUSSION

3.1 Evaluation of Analytical Results

Method validation is the process of providing information regarding the acceptance of a given analytical method for its intended purpose. Since there is no certified reference material in our laboratory, the validity of the procedure for fluoride determination was checked by carrying out recovery test.

3.2 Recovery Results of Fluoride Determination

Percent recovery was calculated for each of cereal and legume sample. Recovery results of fluoride determination in cereal and legume samples were presented in Table 2 and 3. Recoveries for cereals was ranged between (93±2) and (102±5)%, and between (93±7) to (102±5)% for legumes. Therefore, recoveries were within the acceptable range.

Table 2. Recovery test for cereal samples.

Type of grain	Amount added		Concentration in unspiked 0.5 g sample (mg/kg)	Concentration in spiked 0.5 g sample (mg/kg)	Recovery (%)
	Percentage of F in unspiked sample (%)	mg/kg of F in unspiked sample			
Tef (Gonder)	25	1.06	3.84±0.28	4.91±0.06	101±6
	50	2.13	3.84±0.28	6.01±0.11	102±5
	100	4.23	3.84±0.28	7.96±0.3	93±7
Wheat (Wonji)	25	1.80	7.51±0.60	9.27±0.15	98±8
	50	3.60	7.51±0.60	11.01±0.19	97±6
	100	7.20	7.51±0.60	14.30±0.56	93±7
Corn(Arsi Negele)	25	1.13	4.20±0.14	5.26±0.08	93±7
	50	2.27	4.20±0.14	6.33±0.11	94±6
	100	4.53	4.20±0.14	8.39±0.08	93±2
Barley	25	1.03	4.23±0.34	5.25±0.07	99±5

(Wonji)	50	2.06	4.23±0.34	6.33±0.13	102±6
	100	4.11	4.23±0.34	8.13±0.13	96±4

Table 3. Recovery test for legume samples.

Type of grain	Amount added		Concentration in unspiked 0.5 g sample (mg/kg)	Concentration in spiked 0.5 g sample (mg/kg)	Recovery (%)
	Percentage of F in unspiked sample (%)	mg/kg of F in unspiked sample			
Chickpea (Ada)	25	2.07	8.10±0.48	10.18±0.14	101±8
	50	4.13	8.10±0.48	12.08±0.23	96±6
	100	8.25	8.10±0.48	15.71±0.54	93±7
Pea (Gonder)	25	1.33	5.02±0.25	6.28±0.10	95±8
	50	2.67	5.02±0.25	7.55±0.07	94±3
	100	5.33	5.02±0.25	10.36±0.46	100±9
Lentil (Ada)	25	1.71	6.65±0.47	8.39±0.09	102±5
	50	3.42	6.65±0.47	9.93±0.20	96±6
	100	6.83	6.65±0.47	13.07±3	94±2

3.3 Analytical Precision

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. The precision of an analytical procedure is usually expressed in terms of variance, standard deviation, and relative standard deviation. For this study, the precision of the analytical method was evaluated in terms of pooled standard deviations limit and relative standard deviation (% RSD) of the triplicate measurements of each sample (n = 9). The % RSD were < 10%, which are within the acceptable range indicating that the results are precise.

3.4 Level of Fluoride in Cereal and Legume Grains

Consumption of fluoride above the recommended level poses a risk on human health, such as skeletal and dental fluorosis. All food items contain fluoride in different level. Most of Ethiopian staple daily foods are prepared from the cereals and legumes.

Level of total fluoride in cereal and legume grain sample was determined by fluoride ion selective electrode. The instrument was first calibrated with standard fluoride solution, and then amount of fluoride present in the sample was determined. The average levels of fluoride were expressed as (mean ± standard deviation, SD) in Tables 4 and 5.

Fluoride levels found in all sample of this study is presented in Tables 4 and 5, with both the highest and lowest was found in pea. In this study, fluoride levels found in cereals and legumes ranged from (11.07 mg/kg) to (1.52 mg/kg) both in the pea from Ada. Fluoride levels in cereals were found to be ranged between (0.98 mg/kg) in corn from Adola and (10.98 mg/kg) in tef from Wonji.

Table 4. Average levels (mean ± SD, n = 9, mg/kg dry weight) of fluoride in cereals grains (tef, wheat, corn and barley) from four sample sites.

Type of grains	Sample site	Fluoride level, mean (mg/kg) ± SD
Tef	Arsi Negele*	8.00 ± 0.56
	Wonji*	10.98 ± 0.66
	Gonder	3.20 ± 0.56
	Adola	2.20 ± 0.18
Wheat	Arsi Negele*	10.30 ± 0.49
	Wonji*	7.20 ± 0.47
	Gonder	1.76 ± 0.34
	Adola	5.60 ± 0.36
Corn	Arsi Negele*	4.53 ± 0.28
	Wonji*	3.70 ± 0.22
	Gonder	2.54 ± 0.10
	Adola	0.98 ± 0.06
Barley	Arsi Negele*	6.07 ± 0.25
	Wonji*	4.11 ± 0.20
	Gonder	3.83 ± 0.23
	Adola	3.68 ± 0.09

* Areas located in rift valley

Table 5. Average levels (mean ± SD, n = 9, mg/kg dry weight) of fluoride in legume grains (pea, lentil and chickpea) from four sample sites.

Type of grains	Sample site	Fluoride level, mean (mg/kg) ± SD
Chickpea	Ada*	8.25 ± 1.22
	Gonder	4.99 ± 0.43
	Burrie	2.31 ± 0.16
	Dessie	1.52 ± 0.09
Pea	Ada*	11.07 ± 0.66
	Gonder	5.33 ± 0.31
	Burrie	1.52 ± 0.06
	Dessie	2.65 ± 0.19
Lentil	Ada*	6.83 ± 0.31
	Gonder	6.32 ± 0.92
	Burrie	2.63 ± 0.13
	Dessie	2.89 ± 0.06

*Areas located in rift valley area

Table 6. Range of fluoride level in cereals and legumes samples found in this study.

Type of grains	Fluoride level range (mg/kg)
Barley	3.68 – 6.07
Corn	0.98 – 4.53
Tef	2.20 – 10.98
Wheat	1.76 – 10.30
Chickpea	1.52 – 8.25
Pea	1.52 – 11.07
Lentil	2.63 – 6.83

3.4.1 Distribution Pattern of Fluoride in Cereal Samples

Table 4 shows the levels of fluoride in cereal grains from Arsi Negele, Wonji, Gonder and Adola. Comparison of fluoride level among cereals is presented in Figure 2. The values in cereals were ranged between (1.76 mg/kg) in wheat from Gonder and (10.98 mg/kg) in tef from Wonji. Fluoride levels in cereals from rift valley areas were higher than those from outside rift valley areas. Generally, cereal samples from Arsi Negele and Wonji were found to contain higher levels of fluoride, ranging from (3.70 mg/kg) in corn from Wonji to (10.98 mg/kg) in tef from Wonji. Fluoride levels in tef and wheat from Arsi Negele and Wonji were found to be higher, while similar cereals from other areas were found to be lower (Table 4). It was found that fluoride levels in cereals from Arsi Negele were found in the order of: wheat (10.30 mg/kg) > tef (8.00 mg/kg) > barley (6.07 mg/kg) > corn (4.53 mg/kg).

The fluoride levels of cereals from outside rift valley areas were between (0.98 mg/kg) in corn from Adola to (5.60 mg/kg) in wheat from Adola. In cereals from rift valley areas, fluoride levels ranged between (3.70 mg/kg) in corn from Wonji and (10.98 mg/kg) in tef from Wonji. Wheat from Gonder was the sample site with the lowest (1.76 mg/kg) levels of all cereals, and Arsi Negele with the highest fluoride level (10.30 mg/kg) of all cereal samples. From all corn samples, corn (4.53 mg/kg) from Arsi Negele had the highest fluoride level, while corn (0.98 mg/kg) from Adola was the lowest.

Generally, the order of fluoride level obtained in four types cereals was tef (Wonji) > wheat (Arsi Negele) > tef (Arsi Negele) > wheat (Wonji) > barley (Arsi Negele) > corn (Arsi Negele) > barley (Wonji) > barley (Gonder) > corn (Wonji) > barley (Adola) > tef (Gonder) > corn (Gonder) > tef (Adola) > wheat (Gonder) > corn (Adola).

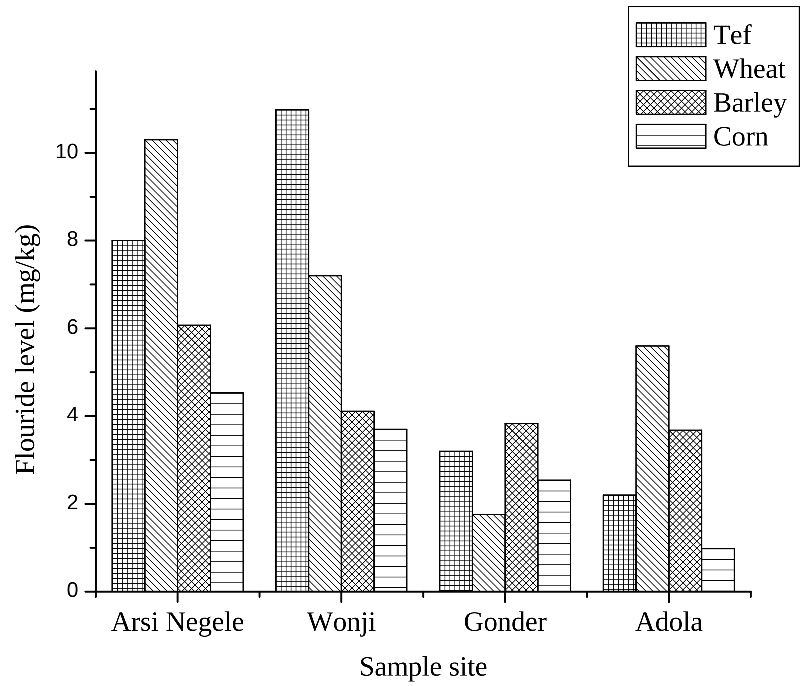

Figure 2. Comparison of levels of fluoride in cereal samples.

3.4.2 Distribution Pattern of Fluoride in Legume Samples

Table 5 shows the fluoride levels of legume grains from Ada, Burrie, Gonder and Dessie. The comparison of fluoride level in legumes is shown in Figure 3. The highest level of fluoride was found in the pea (11.07 mg/kg) from Ada, and the lowest level in chickpea (1.52 mg/kg) from Dessie and in pea (1.52 mg/kg) from Burrie (Table 6). Fluoride content of legume samples from rift valley areas (Ada) were found to have higher than those from outside rift valley sample sites (Gonder, Burrie and Dessie) (Table 5). When chickpea from four sample sites are compared, the highest value (8.25 mg/kg) was found from Ada, followed by a value (4.99 mg/kg) from Gonder and (2.31 mg/kg) from Burrie.

Generally, there is significance difference ($p < 0.05$) in the fluoride levels between legumes from Ada and from Burrie or Dessie. Although lentil (6.32 mg/kg) from Gonder was from outside rift valley area, it was found to contain almost similar fluoride level as lentil (6.83 mg/kg) from Ada. Lentil (2.63 mg/kg) from Burrie and lentil (2.89 mg/kg) from Dessie were almost similar in their value. Fluoride level in chickpea (5.33 mg/kg) was about two times that of the pea (2.65 mg/kg) from Dessie. Fluoride level in legumes from Ada sample sites ranged from 6.83 to 11.07 mg/kg, both the highest in pea and the lowest in lentil (Table 5).

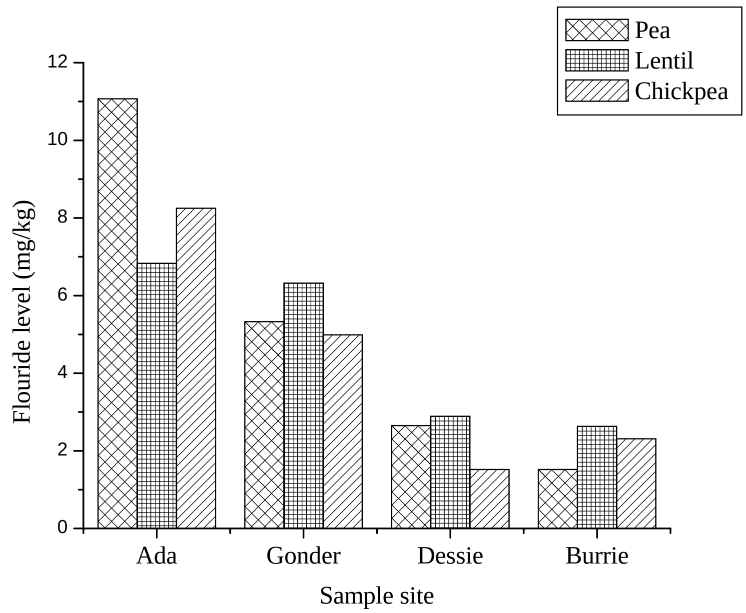

Figure 3. Comparison of levels of fluoride in legume samples.

Cereal and legume based diets make large contribution to the diets of majority of the people in Ethiopia. A study conducted by Meseret Dessalegne (2011) showed that cereal food ingredients such as corn and tef had higher levels of fluoride. In general in this study, legumes were found to contain lower level of fluoride when compared to cereals: corn (0.98-4.53 mg/kg), barley (3.68 -6.07 mg/kg), wheat (1.76–10.30 mg/kg), tef (2.20–10.98 mg/kg), lentil (2.63–6.83 mg/kg), chickpea (1.52–8.25 mg/kg) and pea (1.52–11.07 mg/kg) (Table 4 and 5).

The fluoride levels in most of the cereals and legumes in this study (Table 4 and 5) were found to be above the ranges reported in other studies. Fluoride levels in cereals might be increased from irrigation of crops by fluoridated water (Sunitha and Reddy, 2008; Gautam *et al*., 2005). Both cereal and legume samples from rift valley areas were found to be higher in fluoride levels than those from outside rift valley areas. This is consistent with the work of Gautam *et al*. (2005), who determined the levels of fluoride in grains cultivated in fluoride endemic area of India, and they found that barley grains cultivated from those areas had (3.84 mg/kg) and (5.66 mg/kg), which were irrigated by the water having 10.32 mg/L and 14.62 mg/L, respectively (Gautam *et al*, 2005).

Fluoride may be added to soils from agricultural inputs such as fertilizers and pesticides. Crops subjected to fertilizers and pesticides that contain fluoride might have higher levels in their edible parts. Generally, cereal samples from outside rift valley area were lower in their fluoride values. However, cereal samples from outside rift valley sites (Adola and Gonder) were found to contain higher fluoride than that of rift valley sites (Arsi Negele and Wonji). This suggests that higher level fluoride in the cereals from Adola and Gonder might be due to fertilizers applied to the soil where crops were grown (Ahokas *et al*., 1999; CDC, 2001; WHO, 2002; USDHS, 2003; Gautam *et a*l., 2005). Similarly, the fluoride level of legumes from Ada (fluoride endemic areas) was higher than other sites, which suggests that fluoride in the soil and water is likely to be available to the plant (WHO, 2002; Sunitha and Reddy, 2008; Australian Government, 2007).

Phosphetic fertilizers especially the superphosphates are the most important sources of fluoride in agricultural lands (Gautam *et a*l., 2005). Some cereals from outside rift valley areas were found to contain higher level of fluoride: wheat (5.60 mg/kg) from Adola, barley (3.83 mg/kg) from Gonder, Barley (3.68) from Adola. This might be due to the higher agricultural fertilizer and pesticides in the soil where crops are cultivated (CDC, 2001; USDHS, 2003).

In this study, levels of fluoride variation in the same types of cereals but from different sample site might be due to physical and chemical characteristics of the soil (WHO, 2002; Australian Government, 2007). The fluoride level in wheat (10.30 mg/kg) from Arsi Negele was about five times higher than in wheat (1.76 mg/kg) from Gonder. However, wheat from Adola was found to contain higher level of fluoride, this may be due to content of fluoride in the soil where crop were grown or due to fertilizer application on the farmland (CDC, 2001; WHO, 2002; Gautam *et al*., 2005; Tamiru Alemayehu, 2006; Australian Government, 2007).

Several factors can influence the availability of fluoride in soil, such as pH, content of clay, organic matter, Al and Fe oxides/hydroxides (USDHS, 2003; WHO, 2002). Fluoride from insoluble or sparingly soluble substances is less efficiently absorbed. Some cereals such as corn (4.53 mg/kg) from Arsi Negele and barley (4.11 mg/kg) and corn (3.70 mg/kg) from Wonji were found to contain lower levels of fluoride than other cereals of the same site.

In the soil containing higher levels metals such as Ca, Mg and Al, fluoride ion can form a complex with those metals, thus reducing fluoride uptake by plant. Some cereals and legumes from rift valley areas were found to contain lower level of fluoride. This might be due to the soil containing higher level of metals such as Ca, Mg and Al form complex, thus reducing up-take of fluoride by crops from the soil (WHO, 2002; Australian Government, 2007).

High fluoride concentration is often found in food items rich in minerals and trace elements. Tef, for example, is a positive food item because of its high content of Fe, Zn, and Ca (Malde *et al*., 1997). From all samples, tef from the Wonji was found to contain the highest level of fluoride (10.98 mg/kg). The higher fluoride levels in tef might be due higher level metals such Fe Zn and Ca in tef, but requires further research.

Generally, the order of fluoride level obtained in three types legumes was pea (Ada) > chickpea (Ada) > lentil (Ada) > lentil (Gonder) > pea (Gonder) > chickpea (Gonder) > lentil (Dessie) > pea (Dessie) > lentil (Burrie) > chickpea (Burrie) > chickpea (Dessie) ≈ pea (Burrie).

3.5 Comparison of Results

3.5.1 Comparison of Fluoride Levels of This Study with Literature Values

The comparative studies of fluoride levels in the samples of in this study and those reported in literature are presented in Table 7. When this study is compared to other studies, levels of fluoride in wheat (0.51-14.30 mg/kg) from India and corn (5.1 mg/kg) from Burundi (Gautam1 *et al*., 2005; Sunitha and Reddy, 2008; Bhargava and Bhardwaj, 2009; Yadav et al., 2012) have comparable value with this study (Table 7). Fluoride level in corn (0.3 mg/kg) reported by Gautam *et al*. (2005) in Tanzania contains lower level than the level found in this study. Fluoride level in barley (3.84 mg/kg) reported by Gautam1 *et al*. (2005) is comparable to the level found in this study, except barley (6.07 mg/kg) from Arsi Negele.

Processes such as milling of cereals to flour result in lowering of residue levels (WHO, 1997). Traditionally, cereal grains can be milled in to flour ingredients, with or without washing. Fluoride levels in corn powder (12.2 mg/kg) and tef powder (15.1 mg/kg) reported by Meseret Dessalegne (2011) was higher value than this study (Table 8): The possible reasons of higher level of fluoride in tef and corn might be milling of unwashed cereal grains into flour. This might contribute fluoride levels in fluoride determination. However, in this study samples were washed by distilled water and dried, befor they were changed into powder samples.

Table 7. Comparison of fluoride levels in cereals and legumes with reported values.

Grain type		Concentration in mg/kg dry wt	Origin	Reference
Cereals	Tef	2.20-10.98	Ethiopia	Present study
	Red tef (powder)	15.1	Ethiopia	(Meseret Dessalegne, 2011)
	Tef (red) flour	6.9	Ethiopia	(Malde *et al*., 1997)
	Wheat	3.24 -14.3	India	(Yadav *et al*., 2012)
		4.6	India	(Sunitha and Reddy, 2008)
		0.51-5.98	India	(Bhargava and Bhardwaj, 2009)
		1.76-10.30	Ethiopia	Present study
		6.96	India	(Gautam1 *et al*., 2005)
	Corn	5.1	Burundi	(Gautam1 *et al*., 2005)
		5.9	India	(Sunitha and Reddy, 2008)
		0.3	Tanzania	(Gautam *et al*., 2005)
	Corn (powder)	12.2	Ethiopia	(Meseret Dessalegne, 2011)
	Corn	0.98-4.53	Ethiopia	Present study
	Barley	3.84	India	(Gautam *et al*., 2005)
		0.45-3.65	India	(Bhargava and Bhardwaj, 2009)
		3.68-6.07	Ethiopia	Present study
Legumes	Chickpea	1.52-8.25	Ethiopia	Present study
	Pea	8.34	India	(Gautam1 *et al*., 2005)
		0.042-0.086	Brazil	(Gautam1 *et al*., 2005)
	Pea (yellow)	1.6	Tanzania	(Malde *et al*., 1997)
	Pea	1.52-11.07	Ethiopia	Present study
	Lentil	2.63-6.83	Ethiopia	Present study
	Beans (yellow)	1.1	Tanzania	(Malde *et al*., 1997)
	Beans (red)	4.4		
	Beans	1.0	Burundi	
		0.015	India	(Sunitha and Reddy, 2008)
	Gram	2.5	India	(Sunitha and Reddy, 2008)

Generally, most of fluoride level of legumes in this study were found to contain higher values than other values reported (Table 8), except pea (1.52 mg/kg) from Burrie. With the Exception of pea (11.07 mg/kg) from Ada, fluoride levels in pea from this study were found to have lower value than pea (8.34 mg/kg) reported by Gautam *et al*. (2005) in India. Fluoride level in pea (1.52 mg/kg) from Gonder was found to contain the lowest value when compared to all legumes in this study. The content of fluoride in pea (0.042-0.086 mg/kg) reported by Casarin *et al*. (2007) in Brazil is much lower than values reported in this study (Table 7). Table 5 and 7 shows that fluoride level in pea (11.07 mg/kg) from Ada was higher than that of pea (8.34 mg/kg) reported by Gautam1 *et al*. (2005) in India. Fluoride level of legumes in this study was found to contain higher fluoride level than values of yellow beans (1.1 mg/kg) and red bean (4.4 mg/kg) in Tanzania, which are reported by Malde *et al*. (1997).

In this study, mean fluoride level in pea from outside rift valley area was found to be in the range of 1.52 to 5.33 mg/kg, which is lower than the values in pea (8.34 mg/kg) reported by Gautam *et al*. (2005) in India. Fluoride level in pea (1.52 mg/kg) from Burrie was almost similar to the values reported in yellow pea (1.6 mg/kg) (Malde *et al*., 1997) from Tanzania. Fluoride level in pea from Ada was found to be 11.07 mg/kg, which is above the values (8.34 mg/kg) reported by Gautam *et al*. (2005) in India.

3.6 Statistical Analysis

3.6.1 Analysis of Variance

Variation in the mean levels of fluoride between the samples were tested whether it was from a random error or treatment (difference in fluoride contents of soil, water, atmosphere; variation in application of agrochemicals like fertilizers, pesticides, herbicides, or other variations in cultivation procedures). Analyses of variance (ANOVA) are widely used statistical methods to compare group whether the source for variation was from sampling or heterogeneity among the samples. One way ANOVA was used to compare whether there was difference in the mean levels of fluoride among samples. The statistic analysis in this study was made by SPSS 15.0 Window Evaluation Version program.

There is significance difference ($p<0.05$) in the mean levels of fluoride among cereals and among legumes collected from four sites. This implies that variation in fluoride level among sample site is not due to random errors introduced during the sampling to analysis rather than it is real. The factors that contribute to such variation are likely to be a difference in the physical and chemical characteristics of fluoride content of the soil and variety of crops. Similarly, statistical analysis revealed that there are significant differences ($p<0.05$) observed among legume grain seeds in their fluoride level.

4. CONCLUOSION

The study showed that fluoride levels in cereals from Adola and Gonder (both are out of rift valley) were found to lower than samples from Arsi Negele and Wonji (both from rift valley region). In general, the levels of fluoride in the cereals and legumes from the places away from rift valley region of Ethiopia are comparable to the similar grains from other countries. While the levels of fluoride in the cereals and legumes from the rift valley region of Ethiopia were found to be higher than similar grains from other countries. Analysis of variance showed that there was significant difference at 95% confidence level in the mean fluoride level in the samples.

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