ADDIS ABABA UNIVERSITY

SCHOOL OF GRADUATE STUDIES

DEPARTMENT OF CHEMISTRY

Assessment of fluoride in the Ethiopian and imported; black and green tea (*Camellia sinensis*) infusions: Measurement and safety evaluation

By

Samuel Zerabruk

July 2009

DECLARATION

I the Undersigned, confirm that the results reported in this work were obtained by the research carried out by me under the supervision of my Advisors in the Faculty of Science, Department of Chemistry, Addis Ababa University in the academic year 2008-2009 G.C. All the sources of materials used for this study have been duly acknowledged.

Name: ______________________________

Signature: ___________ Date: __________

This project has been submitted to the examination with our approval as the university advisors.

Advisors:

Prof. B.S. Chandravanshi

Signature: ______________

Date: __________________

Dr. Feleke Zewge

Signature: ______________

Date: __________________

Place and date of submission: School of Graduate Studies
Addis Ababa University
July 2009.

Assessment of fluoride in the Ethiopian and imported; black and green tea (*Camellia sinensis*) infusions: Measurement and safety evaluation

Samuel Zerabruk

Graduate project submitted to

Department of chemistry

In Partial fulfillment of requirement for the Degree of Master of Science in Chemistry

July 2009

Dedication

To my sister, Hiwot Zerabruk

Acknowledgements

I am very grateful to my advisors Prof. B. S. Chandravanshi and Dr. Feleke Zewge for their endless constructive comments, editing, criticizing and encouraging to accomplish this work. I like to appreciate them for following up this work from the beginning to the end and for provision of useful reference materials in the preparation of the project. I have a special respect and appreciation to Prof. B. S. Chandravanshi for his fatherly advice in all aspects of life.

I would like to express my thanks to the Department of Chemistry, Addis Ababa University, for providing me with necessary knowledge, every assistance and facilities to conduct the project work; and Mekelle University for giving me full scholarship for my M.Sc. study.

My sincere thanks goes to Ato Yewendwsen Bruck, Head, Production section of TPPF in Ethio Agri CEFT P.L.C. for his kindly cooperation, friendly approach and explanation of tea cultivation, harvesting, processing and packing from his more than 20 years experience in the tea sector, during my visit to the factory in Addis Abeba.

I am deeply indebted to beloved Tlanesh Adane for her understanding, care and patience during my study.

Finally I am extremely grateful to my father, my mother and my sisters who encourage and support me to learn. I should not forget their care to me that brought me up to this success. I thank My Lord God and My Savior Jesus Christ for His unchanging love, amazing grace and giving me the opportunity to prepare this paper.

List of Acronyms

GTB Green Tea Bag

BTB Black Tea Bag

BTP Black Tea Powder/Leaf

ISE Ion Selective Electrode

DFI Daily Fluoride Intake

Table of Contents

List of Tables

List of Figures

Assessment of fluoride in the Ethiopian and imported black and green tea (*Camellia sinensis*) infusions: Measurement and safety evaluation

By Samuel Zerabruk

Advisors: Prof. B. S. Chandravanshi

Dr. Feleke Zewge

Abstract

The fluoride contents in the infusions of 21 commercially available Ethiopian and imported black and green tea brands; in leaf and bag forms was determined by fluoride ion-selective electrode. Of the samples analyzed 12 of them were products of Ethiopia. It is found that fluoride level increases with increasing brewing time (3, 5 and 10 min). The present study revealed wide differences in fluoride levels. Fluoride level of black tea leafs after 5 min brewing, which is the common time used to prepare tea infusions, was in the range of 117 – 682 mg/kg, for green tea bags it was between 111 – 190 mg/kg and that of black tea bags fluoride concentration was in the range of 141– 246 mg/kg. While the Ethiopian black tea leafs fluoride level ranges from 248 to 682 mg/kg with mean concentration of 458 mg/kg among ten brands. Of the imported products, teas of the same brand, the fluoride content was greatest in black tea bags, presumably because black tea bags are made from low cost older tea leaves. While the fluoride level in green tea was found lesser, as green teas are made from young shouts of the plant which contain less amount of fluoride. On the basis of the results obtained, the daily intake of fluoride provided from tea was estimated for an adult person and for children in comparison with the WHO guide line for daily fluoride in take thresholds.

Which is 2 mg for children and 4 mg for adults. Assuming that one consumes 4 cups of tea everyday (400 mL) and each cup uses 2.5 g of tea leaf or one tea bag, daily fluoride intake through taking the black tea leafs may be 1.11 – 6.82 mg, 1.00 - 1.38 mg through green tea bags and from the black tea bags it may range from 0.86 – 1.81 mg. from the Ethiopian black teas alone, the daily fluoride intake may range from 2.48 – 6.82 mg. Thus according to the WHO, 2002 recommendation for daily fluoride intake threshold and no or very less amount fluoride from other sources; all the black and green tea bags; and imported black tea leafs were safe for all age groups. From the 10 Ethiopian black tea leaf brands none of them were safe for children but 30% of them were safe for adults.

Thus, the control of tea quality is important to protect human against too high uptake of this element from black tea, which is the most popular beverage. Excessive intake of fluoride with black tea, especially in the regions with its high level in the drinking water, increases the risk of dental fluorosis in children during the years of tooth development. The long-term exposure to large amounts of fluoride can lead to potentially skeletal fluorosis.

Keywords: Black Tea Infusion, Green Tea Infusion, Fluoride, Brewing Time, Safety Evaluation, ISE

1. Introduction

1.1. The tea plant

Fig. 1: Picture of Tea plant (*Camellia sinensis*)

Kingdom:	Plantae
Division:	Magnoliophyta
Class:	Magnoliopsida
Order:	Ericales
Family:	Theaceae
Genus:	Camellia
Species:	*C. sinensis*
Binomial name:	*Camellia sinensis*

Camellia sinensis, the **tea plant**, is the species of plant whose leaves and leaf buds are used to produce tea. It is of the genus Camellia, a genus of flowering plants in the family Theaceae. White tea, green tea, oolong, pu-erh tea and black tea are all harvested from this species, but are processed differently to attain different levels of oxidation. Kukicha (twig tea) is also harvested from *Camellia sinensis*, but uses twigs and stems rather than leaves [1, 2]. The name *sinensis* means *Chinese* in Latin. *Camellia* is taken from the Latinized name of Rev. Georg Kamel, S.J. (1661-1706), a Czech-born Jesuit priest who became both a prominent botanist and a missionary to the Philippines. Though Kamel did not discover or name the plant, Carl Linnaeus, the creator of the system of taxonomy still used today, chose his name for the genus of this tree to honor Kamel's contributions to science. Older names for the tea plant include *Thea bohea*, *Thea sinensis* and *Thea viridis* [1, 2].

Camellia sinensis is native to mainland South and Southeast Asia, but today it is cultivated across the world in tropical and subtropical regions. It is an evergreen shrub or small tree that is usually trimmed to below two meters when cultivated for its leaves. It has a strong taproot. The flowers are yellow-white, 2.5–4 cm in diameter, with 7 to 8 petals.

The seeds of *Camellia sinensis* and *Camellia oleifera* can be pressed to yield tea oil, a sweetish seasoning and cooking oil that should not be confused with tea tree oil, an essential oil that is used for medical and cosmetical purposes and originates from the leaves of a different plant.

The leaves are 4–15 cm long and 2–5 cm broad. Fresh leaves contain about 4% caffeine. The young, light green leaves are preferably harvested for tea production; they have short white hairs on the underside. Older leaves are deeper green. Different leaf ages produce differing tea qualities, since their chemical compositions are different. Usually, the tip (bud) and the first two to three leaves are harvested for processing. This hand picking is repeated every one to two weeks.

1.2. Tea plant cultivation

Camellia sinensis is mainly cultivated in tropical and sub-tropical climates, in areas with at least 50 inches of rainfall a year. However, it is commercially cultivated from the equator to as far north as Cornwall on the UK mainland. Many high quality teas are grown at high elevations, up to 1500 meters (5,000 ft), as the plants grow more slowly and acquire a better flavor [1-3].

Tea plants will grow into a tree if left undisturbed, but cultivated plants are pruned to waist height for ease of plucking. Two principal varieties are used, the small-leaved Chinese variety plant (*C. sinensis sinensis*) and the large-leaved Assamese plant (*C. sinensis assamica*), which is mainly used for black tea preparation [3, 4].

1.3. Classification of tea

There is no agreement on the classification of tea. Tea can be classified by procedure, quality, preparation methods, and so on. The most common being the method of processing. More specifically, it is the way the leaves are processed-steamed, oxidized (often referred to as fermented, though no fermentation actually takes place), dried, or bruised-gives the tea the special characteristics of its category [1-4].

There are four main types of tea: green tea, black tea, oolong tea and white tea. All this varieties comes from the same plant. The way the leaves are processed after harvesting determines the type of tea that is created.

1.4. Tea Production

1.4.1. Black tea production

Black teas originated in China, where they are known as red tea, but now come from many growing regions throughout the world. The traditional method of processing black teas comprises four steps: withering, rolling, oxidizing and drying. First the leaves are spread out on racks of bamboo or woven straw to be wilted until soft enough to be rolled without tearing the leaf. Next, the withered leaf is rolled to release the chemicals in the leaf that will contribute to the tea's final color and flavor. Rolling will also determine the shape of the leaves and this will also impact the tea's flavor and pungency. The rolled leaves are spread out in cool and humid rooms and exposed to oxygen for several hours, which causes chemical changes in the leaves and turns them from green to coppery red. Finally, the completely oxidized leaves are fired (or dried) to stop oxidation. In China, the leaves are traditionally fired in hot woks. In other areas, the leaves may be baked in hot ovens [1-4].

1.4.2. Green tea production

The finest **green teas** are handmade during the spring season in China and Japan. Green teas are often referred to as non-fermented or unfermented teas. The intent is to preserve the healthy and natural elements of the fresh leaves. The traditional method of processing green teas involves withering (though not always), heating, rolling and drying. After picking, the fresh leaves are spread out on bamboo trays and exposed to sunlight or warm air for one to two hours. Then the leaves are heated to prevent oxidation and preserve freshness. Finally, the leaves are rolled into various shapes and then dried. The rolling also helps regulate the release of natural oils and flavor during steeping. In China, green teas are often pan fired in very large woks and then rolled by hand into various styles: twisted, flat, curly or balled. In Japan, the plucked leaves are quickly steamed on a bamboo tray over water or in a steaming machine, making them easier to shape. The leaves are then rolled by hand or machine before being dried [1-4].

1.4.3. Oolong tea production

The best **oolong tea** is picked by hand during the spring and winter months in southeast China and Taiwan. Oolong teas are partially oxidized teas and undergo the most difficult and time consuming processing method. Processed to be full-bodied teas, the leaves for oolong tea must not be picked too early but just when they reach their peak, and they must be processed immediately. First the leaves are withered in direct sunlight and then shaken gently in bamboo baskets to lightly bruise the edges of the leaves. Next the leaves are air-dried in the shade until the surface of the leaf turns slightly yellow. The process of shaking and drying the leaves is repeated several times. The oxidation period for oolong teas is less than that for black teas and depends on the type of oolong. This can vary from about 20% for a green oolong to 60% for a classic Formosa oolong. After the desired oxidation level is reached, the leaves are pan fired at high temperatures to prevent further oxidation. Due to the higher firing temperatures, oolong teas contain less moisture and have a longer shelf life than green teas [1-4].

1.4.4. White tea production

White tea is the most delicate of all teas. Produced mainly in China, primarily in Fujian province, white tea is made entirely from leaf buds that are covered with whitish hairs. The new buds are plucked before they open in early spring, then withered and dried slowly at low temperatures. Unlike other tea processing methods, the leaf buds are not rolled and only slightly oxidized. The result of this processing is a tea with a mild flavor and natural sweetness, with little of the grassy undertones sometimes associated with green tea [1-4].

1.4.5. Flow chart of various types of tea production

Here's how the four main varieties of tea that are produced from *Camellia sinensis* are treated differently.

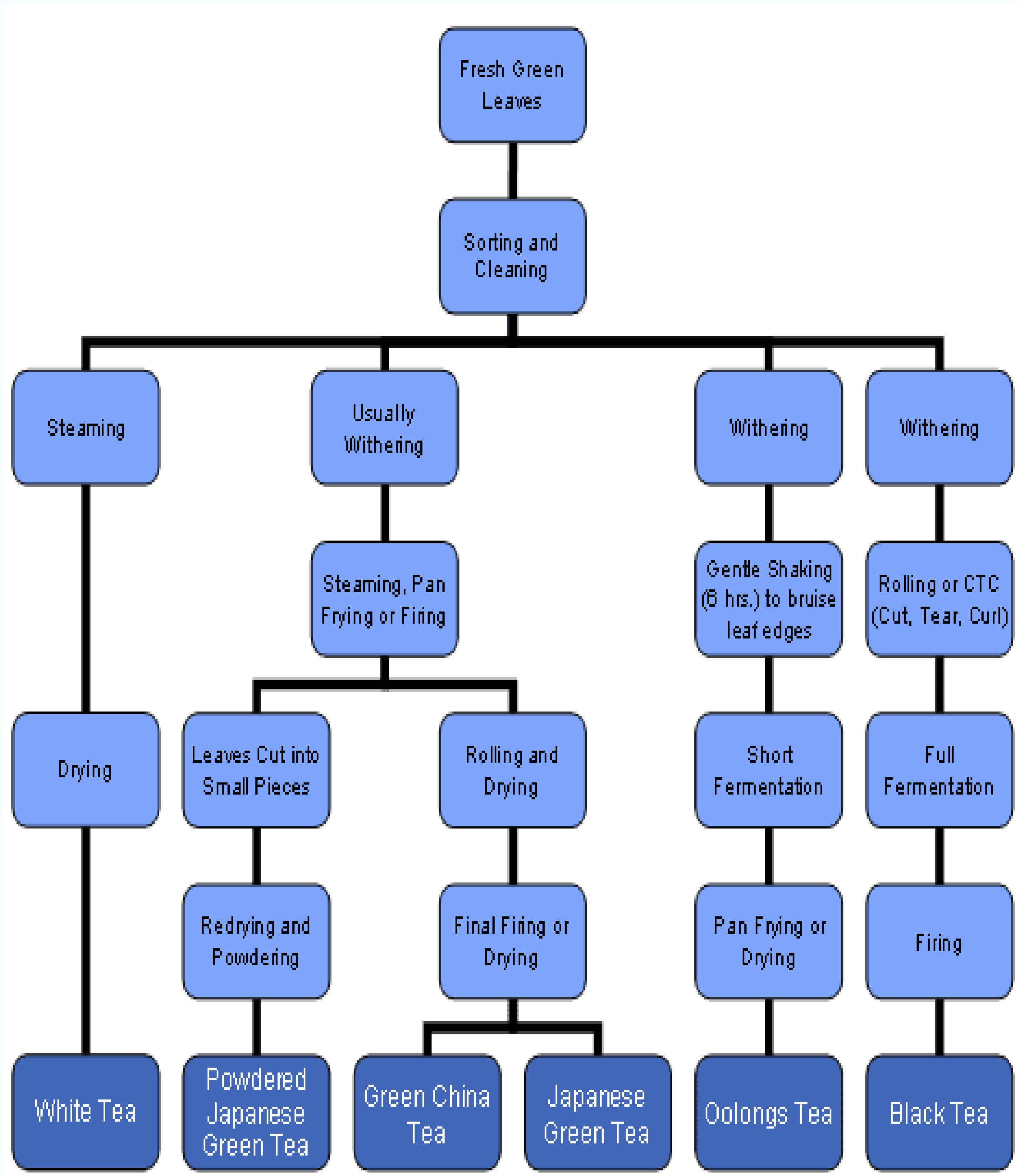

Fig. 2: Different Tea processing flow chart [2].

After the drying, the tea leaves then go on to the final stage of production: grading.

1.5. Medicinal use of tea plant

The tea plant is commonly used in Chinese herbalism, where it is considered to be one of the 50 fundamental herbs. Modern research has shown that there are many health benefits to drinking tea, including its ability to protect the drinker from certain heart diseases. It has also been shown that drinking tea can protect the teeth from decay, because of the fluoride naturally occurring in the tea. However, the tea also contains some tannin, which is suspected of being carcinogenic. The leaves are cardiotonic, diuretic, expectorant, stimulant and astringent. They exert a decided influence over the nervous system, giving a feeling of comfort and exhilaration, but also producing an unnatural wakefulness when taken in large doses. They are used internally in the treatment of diarrhoea, dysentery, hepatitis and gastro-enteritis. Tea is reportedly effective in clinical treatment of amoebic dysentery, bacterial dysentery, gastro-enteritis, and hepatitis. It has also been reported to have antiatherosclerotic effects and vitamin P activity. Excessive use, however, can lead to dizziness, constipation, indigestion, palpitations and insomnia. Externally, they are used as a poultice or wash to treat cuts, burns, bruises, insect bites, ophthalmia, swellings etc. Only the very young leaves and leaf buds are used, these can be harvested throughout the growing season from plants over three years old and are dried for later use [1, 2, 4, 5].

1.6. Other uses of tea plant

Besides its medicinal use the tea plant has also other uses, an essential oil is distilled from the fermented and dried leaves. It is used in perfumery and in commercial food flavoring. Non-drying oil is obtained from the seeds. Refined tea seed oil, made by removing the free fatty acids with caustic soda, then bleaching the oil with Fuller's earth and a sprinkling of bone black, makes an oil suitable for use in manufacture of sanctuary or signal oil for burning purposes, and in all respects is considered a favorable substitute for rapeseed, olive, or lard oils. The oil is different from cottonseed, corn, or sesame oils in that it is a non-drying oil and is not subject to oxidation changes, thus making it very suitable for use in the textile industry; it remains liquid below -18 ^{0}C. A grey dye is also obtained from the pink or red petals. The leaves contain 13 - 18% tannin. The leaves also contain quercetin, a dyestuff that, when found in other plants, is much used as a dye [1].

1.7. Tea production in Ethiopia

This part is obtained through personal communication of the Ethio Agri CEFT PLC workers.

The production of tea leaves in Ethiopia has got a history of more than half a century. Nevertheless, it is only three decades since the consolidated and organized development of tea production began.

So far the largest commercial tea plantations in Ethiopia are Wushwush and Gumaro owned by Ethio-Agri-CEFT PLC, one of the MIDROC Group Companies. This formerly state-owned plantations together with tea processing and packing factory were sold to Agri CEFT PLC by the Ethiopian privatization Agency in year 2000. Then the handover was completed in 2001.

Wushwush Tea Plantation counts with an ideal ecology for the production of good highland tea quality. Tea planting at Wushwush started as a trial in 1973. Then development on an expanded area started in 1981 till it reached its present size of 1249 hectares. As shown in the table below Wushwush plantation spreads over 1249 hectares of tea fields and 977 hectares of eucalyptus trees which serve as fuel for tea drying. In addition, Wushwush counts with modern tea processing facility.

Facts and figures

Table 1: Facts and figures of Wushwush and Gumaro tea plantations.

	Wushwush Tea Plantation	**Gumaro Tea Plantation**
Location	Kaffa Zone, Gimbo Wereda, SNNPR	Illubabor Zone, Ale Dido Wereda, Oromya region
Distance from Addis Ababa	460 km south west of Addis Ababa	637 km south west of Addis Ababa
Altitude	1900 m	1718 m
Annual Rainfall in mm	1820	2089
Temperature	Min 12 ^{o}C – Max 24 ^{o}C	Min 12 ^{o}C - max 27 ^{o}C
Area under tea,	1249 hectares	860 hectares
Soil	Fertile, good drainage, red-brown color, rich in organic matters	Fertile, good drainage, red-brown color, rich in organic matters
Eucalypts tree	977 hectares	761 hectares

Tea seed were introduced in Gumaro through Gore in 1928. It is believed that the British Council General in Gore (Operational during the time) obtained seeds from India and distributed them to local farmers. (Gumaro is 12 km far from Gore). Then in 1957, a Lebanese expatriate living in Gumaro, in cooperation with a Belgian, expanded his tea estate to 25 hectares for commercial concern. In 1981 the government started an expansion plan and developed it to its present size of 860 hectares.

Soil conservation is another important activity inbuilt in the tea plantation management practices. Both plantations have retained a few of the indigenous forest trees like albizzia seen scattered in the plantation particularly at the borders, river bottoms, hills and steep slopes, the trees, among other things, help to balance the ecology.

The interface between the tea plantation and the natural tropical dense forest is neat and there is no encroachment of the natural forest. There is a perfect integration between the two ecosystems. This together with the tea bushes insures a canopy cover close to 100% the dense primary tropical forest is intact, and the tea plantation that is a miniature forest itself with high canopy cover and contour line plantation methods applied everywhere jointly eliminates erosion.

This well integration of the plantation and the primary forest preserves the biodiversity for both plants and animals. In this regard, the Wushwush and Gumaro tea plantations can be described as a “tea planter’s dream”.

The tea factory in Addis Ababa plays the final sorting, blending, grading cup tasting and then packing. This is followed by marketing (domestic and export).

The cup tasting and blending ensures that each blend has good or enhanced flavor body. To this end, the cup tasting laboratory carries out several measurements and determination tests in line with ISO (International Standards Organization) parameters and decides on the best blending ratios. Blending is carried out in a huge blending machine. No artificial flavoring material is added to further enhance the flavor of tea.

1.8. Fluoride

Fluoride (F^-) is an important anion, present in various environmental, clinical and food samples. Small amounts of fluoride are vital for the human organism, but it is toxic in larger amounts. For adults the lethal dose is 0.20 – 0.35 g F^- per kg body weight. Fluoride is widely used in various branches of industry and some fluoride compounds are formed as by-products in certain processes. Excessive amounts of fluoride in the form of different compounds can inter the human body by means of polluted air, water and the food chain. An additional source of fluoride for humans is toothpastes containing 0.1% fluoride (NaF, SnF_2, Na_2PO_3F) and water fluoridation (adding fluoride in the form of NaF to drinking water). A small amount of fluoride is beneficial in the prevention of dental carries. Fluoride has also been used to treat osteoporosis. It is very characteristic that fluoride prevents tooth decay at about 1 mg/L but causes mottled teeth and bone damage at around 5 mg/L of fluoride over prolonged periods of time. Skeletal fluorosis and dental fluorosis are the 2 main types. In dental fluorosis the structural integrity of enamel is affected and small pits are left in teeth as it breaks away. Skeletal fluorosis is the accumulation of fluoride in skeletal tissues associated with pathological bone formation [40-42].

Rocks, soil, water, air, plants, animals and foodstuffs all contain fluoride in widely varied concentrations.

1.9. Fluoride in tea

Tea leaves are usually very rich in fluoride and the tea plant (*Camellia sinensis*) takes up fluoride from the soil and accumulates it in its leaves; it is considered a major source of fluoride. A substantial amount of fluoride is released during tea infusion and nearly all (about 94.9%) of the released fluoride is available to consumers. The common fluoride bearing minerals found in soil are fuorospar (CaF_2), cryolite (Na_3AlF_6) and chiolite ($Na_5Al_3F_{14}$). The mobility of fluoride in soil is determined by the amount of clay minerals present, the soil pH, the adsorption of positively charged complexes, and the concentrations of Ca, Fe, Al and P in soil. The high solubility of F^- in soil under acid conditions (pH < 6) corresponds to the presence cationic AlF_2^+ and AlF^{2+} complexes [5, 7].

Tea trees accumulate and store fluoride, absorbing it selectively from the air and soil. Up to 98% of the fluoride stored in tea trees is in the leaves [20, 21]. The fluoride content increases with the maturation stage of the leaves. Fluoride content of 100–430 mg/kg can be found in the delicate buds and young leaves, which are usually made into green tea or black tea, while fluoride levels of 530–2350 mg/kg can be found in the oldest leaves, which are not used as tea [19, 20, 30].

Currently, no international organization has set a standard for acceptable fluoride content in tea. However, there is an extremely large range of fluoride content in tea commodities and most consumers have no concept that common black or green teas may contain harmful levels of fluoride. Few test reports or safety evaluations have been published measuring fluoride content in a variety of black tea commodities [16-17].

Researchers at Washington University School of Medicine in St. Louis Missouri found that instant tea preparations contain 1 to as much as 6.5 mg/L of fluoride [3, 4]. The US Environmental Protection Agency allows 4 mg/L maximum in drinking water, based on their calculations that it takes at least 20 mg of fluoride a day everyday for 20 years to produce crippling skeletal fluorosis. But the Food and Drug Administration permits 2.4 mg/L in bottled water and beverages, while the Public Health Service allow 1.2 mg/L in drinking water [8, 10].

The World Health Organization recommends optimum levels of 1-1.2 mg/L, and an upper limit of 1.5 mg/L fluoride in drinking water. The 1 mg/L level of fluoride in UK water supplies deemed safe by the government is already 100 times that in mother's milk [8], and fluoride, like most toxins, is particularly harmful for infants.

Fluorosis resulting from the intake of tea was reported as early as 1966 in a British case that was originally misdiagnosed as rheumatoid arthritis. This victim of skeletal fluorosis had no other fluoride exposure except for his long time habits of drinking strong tea [19, 20, 30]. And more recently from a woman diagnosed with skeletal fluorosis in 1998 by Dr. Michael P. Whyte (bone specialist at Washington University) in the US.. The disease afflicts people in remote regions of Tibet, Mongolia and China too. In which fluoride replaces calcium in the bones. The bones become dense, weak and brittle, and sometimes the ligaments harden and changes bone structure, causing pain and crippling.

Studies in Tibet and other areas where people drink large amounts of brick tea have also shown that the beverage can be a significant source of fluoride. Brick tea is made from mature leaves, berries and twigs of the tea plant, which often contain high levels of fluoride absorbed from the soil, and instant tea tends to be made from brick tea.

The woman examined by whyte drank huge amounts of tea, one to two gallons of double-strength instant tea every day of her adult life. Whyte tested the woman's tea and found that her beverage added 26-52 mg of fluoride to her diet each day, besides the water she used to make the tea, which contained 2.8 mg/L. By Whyte's calculation, the woman drank a total of 37 - 74 mg of fluoride a day.

According to the researcher consumers should not be alarmed by the results, and that the amount of fluoride in tea fluctuates from batch to batch even from the same manufacturer. The woman drank to unusual excess and her symptoms improved over a five-year period once she stopped drinking tea and switched to lemonade [8].

Kleerekoper stated that tea drinkers have nothing to fear, and drinking tea within normal limits will probably not cause any health problems.

Tea can become very high in fluoride because tea leaves, especially older leave accumulate more fluoride from pollution of soil and air than any other edible plant. Fluoride content in tea has risen dramatically over the past 20 years due to industry contamination. Recent analyses have revealed a fluoride content of 17.25 mg per tea bag or cup in black tea and 22 g per tea bag or cup in green tea. Aluminum content was also high, over 8 mg. Normal steeping time is five minutes, and the longer a tea bag is steeped, the more fluoride and aluminum were released. After ten minutes, fluoride and aluminum almost doubled [13-15].

To get the maximum health benefits from tea, researchers proposed no fluoridation of water [9] while organic cultivation in non-polluted soils is essential. It is also recommended that Leaf tea, especially young leaves and shoots should be used rather than tea bags or instant tea. A requirement for routine analysis and listing of fluoride, aluminium and other heavy metals in tea labels would do much to protect consumers from harm while enjoying its health benefits [8].

1.10. Factors affecting the amount fluoride in tea

The quality of infused tea depends on the percent of extraction, which in turn is a function of type, strength, and duration of infusion. Boiling increases the fluoride extraction, but also affects the flavor of tea. Five minutes produces the best flavor with least extraction of unpleasant tasting tannin [31].

Recently several papers have been published on the fluoride content of tea leaves. However, the results obtained are often in poor agreement [11, 31]. The variation can be explained on the basis of the leaf age, maturity and genetics of the plant, rainfall, altitude, fertilizer, type of soil and water used for irrigation [13, 31]. The effect of tea grade, processing method, growing elevation and plunking season on the fluoride content of Turkish black teas was studied by Yunus *et al.* [6]. The results show that the poorest quality Grade 7 teas (dust tea) were found to contain higher concentration of fluoride because they contain older levels and pieces of steams. Old tea leafs are known to contain more fluoride, and therefore Grade 7 teas would be expected to contain more fluoride [18]. In regard to tea growing elevation, the result indicates that the elevation affect the fluoride content of teas with highland teas having a lower fluoride content than low land teas. Their difference may be due to fluoride contamination of air through evaporation of airborne sea water at lower elevations, or the soil being washed with rain water containing higher amounts of fluoride. But the comparison made among the two tea processing methods on their influence on fluoride content of tea levels showed no significant difference, thus indicating that processing method has no effect on fluoride content [6,18]. Considering plucking season, the leaves harvested in May were found to contain higher fluoride than those harvested on July and September.

In another study by Sukru *et al.* [9], extraction of fluoride from tea leaves to tea liquors was high with soft water than hard water. They have also seen the fluoride levels in tea liquors increases with the increase of brewing time. Effect of calcium level in the soil was seen by Jianyun *et al.* [26]. In this study, the reduced fluoride up to take following calcium application to the soil appeared to be not only due to simply the precipitation of CaF_2 in the soil or due to the complexation of Ca and F in roots but more likely due to the effect of calcium on the properties of cell wall or membrane permeability.

1.11. Methods of fluoride determination

In all cases fluoride is found at low levels and its determination demands very sensitive methods. The determination of fluoride in tea is usually carried out by direct potentiometric methods using an ion-selective electrode,ISE, replacing the expensive and time-consuming chromatographic methods. ISEs are easy to use and thus are suitable for continuous monitoring. They are cost-effective, as well as sufficiently sensitive, selective and accurate. The fluoride selective electrode is also used for the determination of fluoride in drinking water, industrial effluents, seawater, air, aerosols, flue gases, soils and minerals, urine, serum, plasma, plants, food, beverages, and other biological materials. If a sample contains water-soluble and/or suspended organic substances in addition to its metallic cations (e.g., Si^{4+}, Al^{3+}, Fe^{3+}, Mn^{3+}, Mn^{2+}, which forms stable complexes with CDTA), fluoride contents may be some what lower than the levels in real samples due to the adsorption and/or complexation of free fluoride. Thus samples should be analyzed for fluoride by mixing them with appropriate total ionic strength adjusting buffer, TISAB [7, 12].

The calculation of the results is obtained using a calibration curve, which shows that the electrode potential is linear to the logarithmic of the ionic activity. It is a simple procedure without any sample preparation for measuring aqueous samples [11].

1.12. Objectives of the present study

1.12.1. General objective

The main objective of this project is to determine the level of fluoride in tea infusions prepared from the commercially available Ethiopian and imported, black and green teas.

1.12.2. Specific objectives

1. To determine the fluoride level of tea infusions prepared from commercially available Ethiopian and imported, black and Green teas.
2. To see the effect of brewing time on the fluoride content of tea infusions.
3. To make safety evaluation and recommendations of consumptions.
4. To compare the fluoride level of Ethiopian Tea with those imported and sold abroad.

2. Experimental

2.1. Equipments and reagents

2.1.1. Equipments

Polypropylene containers were used to store the tea powder samples of different brands. A digital analytical balance (Mettler Toledo, Model AG204, Switzerland) with ±0.0001 g precision was used to weigh tea samples. A 250 mL beaker (Pyrex, grade A) was used to prepare the tea infusions with hot plate. A 10 and 5 mL graduated pipettes (Grade A, USA), 25, 50 and 250 measuring cylinders (Grade A, Germany) were used to measure volumes during standard solution and sample preparations for analysis. Plastic bottles were used to store tea infusions. 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/ION meter (WTW Inolab pH/ION Level 2, Germany) using unfilled pH glass electrode.

2.1.2. Reagents and chemicals

Reagents that were used in the analysis were all analytical grade. Anhydrous sodium fluoride (99.0% NaF, BDH Chemicals, England) was used to prepare the fluoride stock and calibration standard solutions. Glacial acetic acid (57 mL), sodium chloride (58 g), sodium citrate (7 g), EDTA (2 g) and sodium hydroxide (6 M) were used to prepare the total ionic strength adjustment buffer (TISAB). Deionized water was used throughout the experiment for sample preparation, dilution and rinsing apparatus prior to analysis.

2.2. Procedure

2.2.1. Sampling

A total of 21 tea brands which are commonly sold in Ethiopia were collected from different shops and supermarkets in Addis Ababa. From the total 21 tea brands three of them (14 %) were green tea and the rest 18 (86 %) were black teas.

Of the 18 black teas 5 of them were in bag form and the rest 13 were in powder form. From the 13 black tea powders 2 of them were in granular form and the rest 11 were in leaf form. All the green teas were in bag form. From the total 21 tea brands collected 13 of them (62 %) were Ethiopian and the rest 8 (38 %) were imported ones. The percent of the different teas sampled is shown in the pie chart below.

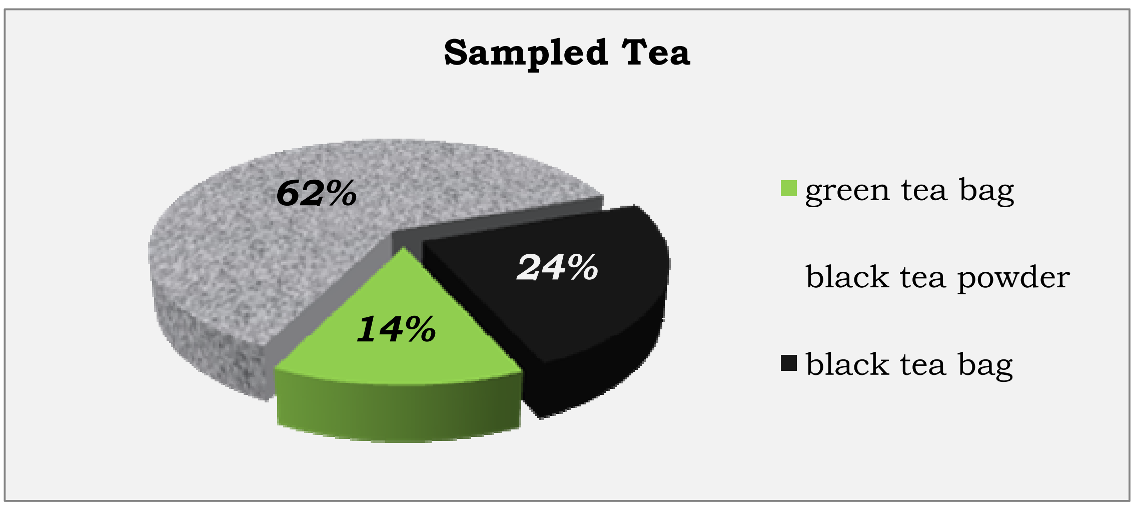

Fig. 3: Percentage distribution of sampled Teas

The details of the tea brands and the amount of samples taken are given in Table 2:

Table 2: List of sampled teas

No	Tea brands name	Form	Amount per box	Country of origin	Country packed in	Area grown	Sample taken, box
	Green tea bags						
1	Ethiopian green tea	Leaf	25 bags	Ethiopia	Ethiopia	W&GTP	3
2	Hyson green tea*	Leaf	25 bags	SriLanka	SriLanka	NA	2
3	Quality green tea*	Leaf	25 bags	SriLanka	SriLanka	NA	2
	Black tea powders						
4	Abay	Leaf	100 g	Ethiopia	Ethiopia	W&GTP	3
5	Abyssinia	Leaf	100 g	Ethiopia	Ethiopia	W&GTP	3
6	Addis	Granular	100 g	Ethiopia	Ethiopia	W&GTP	3
7	Ahadu	Leaf	100 g	Ethiopia	Ethiopia	W&GTP	3
8	Almeta	Leaf	100 g	Ethiopia and Kenya	Ethiopia	K,W&GTP	3
9	Anbessa	Leaf	100 g	Ethiopia	Ethiopia	CTP	3
10	Eirmon	Leaf	100 g	Ethiopia	Ethiopia	W&GTP	3
11	Gumaro	Leaf	100 g	Ethiopia	Ethiopia	GTP	3
12	Haron	Leaf	100 g	Ethiopia	Ethiopia	W&GTP	3
13	Hyson*	Leaf	200 g	SriLanka	SriLanka	NA	2
14	Lipton*	Leaf	250 g	China	Saudi Arabia	NA	2
15	Mohmood*	Granular	200 g	Kenya, India and SriLanka	SriLanka	NA	2
16	Wushwush	Leaf	100 g	Ethiopia	Ethiopia	WTP	3
17	Quality*	Leaf	150 g	SriLanka	SriLanka	NA	2
	Black tea bags						
18	Addis	Leaf	25 bags	Ethiopia	Ethiopia	W&GTP	3
19	Hyson*	Leaf	25 bags	SriLanka	SriLanka	NA	2
20	Lipton*	Leaf	25 bags	China	Saudi Arabia	NA	2
21	Lipton flavored black tea*	Leaf	25 bags	China	U.A.E	NA	2

*imported ones, W&GTP=Wushwush and Gumaro tea plantations, NA=not available,

K, W&GTP= Kenya, Wushwush and Gumaro tea plantations, CTP= Chewaka tea plantation, GTP=Gumaro tea plantation, WTP=Wushwush tea plantation.

A total of 54 samples were collected. No more than 1 sample of the same brand was collected from same shop or supermarket.

2.2.2. Tea infusion preparation

The common methods of tea infusion preparation in Ethiopia are basically three.

i. The tea powder is added to boiling water in teakettle and boiled together for an approximately 3-5 minutes and then poured in to a cup containing sugar.
ii. The tea powder is kept in a plastic filter and hot water is added to it, the extract is collected in a cup and adding hot water continues till the final diluted tea infusion give an attractive brick-red color.
iii. The other method is implemented when the tea powder is found in bag form which is commonly called *Tea bag*, here the tea bag is inserted to a boiled water found in cup and stayed there for an approximately 3-5 minutes till the color become brick-red and attractive to the eye.

Although the above mentioned tea infusion procedures are practiced in Ethiopia, the most common one is the first one, then the second and then the third. Thus in this study the first and third methods of tea infusion preparation were used for the powder and tea bag forms, respectively, according to the following procedure:

I. Tea powder

Adapting customary way of tea infusion preparation

i. Deionized water was allowed to boil in 3 different 100 mL beakers.

ii. 2.5 g of tea powder of the same brand was added to each of the 3 beakers and allowed to infuse for 3 minutes.

iii. After a 3 minute total infusion time, the tea was filtered using a plastic filter (mesh) and allowed to cool to room temperature.

iv. The infusion was filtered again using Whatman No.42 filter paper.

v. Volume of the infusion was checked and was made 100 mL again with deionized water to compensate for the loss during boiling.

vi. Finally the infusion was stored in plastic bottles.

II. <u>Tea bag</u>

Adapting again the customary way of tea infusion preparation

i. Deionized water was allowed to boil in 3 different 100 mL beakers.

ii. 3 different tea bags of the same brand were put in each of the three beakers and allowed to infuse for 3 minutes with no heating. After a 3 min infusion time the tea bags were taken away and the tea infusion was allowed to cool to room temperature.

iii. The infusions were filtered using a Whatman No. 42 filter paper.

iv. Volume of the infusions was checked and made 100 mL again with deionized water to compensate for the loss during boiling.

v. Finally the infusions were stored in 3 separate plastic bottles.

Using the same procedure, other tea infusions were prepared with brewing time of 5 and 10 minutes. Three infusions were prepared for each of the tea brands with equivalent brewing time.

2.2.3. Stock, standard and buffer solutions preparation

A 1000 mg/L fluoride stock solution was prepared by dissolving 2.21 g of anhydrous sodium fluoride (99.0% NaF, BDH Chemicals, England) in 1000 mL of deionized water in volumetric flask. All other standards and required fluoride concentrations were prepared by serial dilution of the stock solution.

The total ionic strength adjustment buffer (TISAB II) was prepared by mixing 57 mL of glacial acetic acid, 58 g of sodium chloride, 7 g of sodium citrate and 2 g of EDTA in volumetric flask and made to 500 mL with deionized water, its pH was adjusted to 5.3 with 6 M sodium hydroxide, and made up to 1000 mL in a volumetric flask with deionized water.

2.2.4. Calibration of the electrode

By subsequent dilution of the stock solution of 100, 20, 10, 5, 1, and 0.5 mg/L of F^- was prepared. A five point calibration curve was prepared using 10 mL of 20, 10, 5, 1, and 0.5 mg/L F^- solutions together with 10 mL of TISAB II.

2.2.5. Determination of fluoride in tea infusions

The fluoride content of the tea infusions prepared was measured using pH/ISE meter (Orion model, EA 940 Expandable Ion Analyzer, USA) equipped with combination fluoride selective electrode (Orion Model 96-09, USA) by taking 10 mL of the infusion and 10 mL of TISAB II.

2.2.6. Recovery test

In order to investigate the accuracy and precision of the method the tea samples were boiled in deionized water spiked with a fluoride standard solution and then they were analyzed by the described method. The accuracy expressed as percent recovery was obtained by comparing the results between the fluoride found and the fluoride added.

For this purpose three tea brands were taken, Ethiopian green tea, Addis black tea, and Lipton flavored black teas; to sample the three types of teas used in the analysis, i.e. green tea bag, black tea powder/leaf, and black tea bag. The procedure was as follows: 5 mL of 20 mg/L fluoride standard was added in to 95 mL deionized water and was allowed to boil, the green tea was then infused for 3 min. For the Addis tea, 10 ml of 20 mg/L fluoride standard was added into 90 mL of deionized water and was allowed to boil, the tea bag was then infused for 3 min. Similarly 5 mL of 20 mg/L fluoride standard was added into 95 mL of deionized water and was allowed to boil, Lipton flavored black tea bag was then infused for 3 min.

All infusions were prepared in triplicate and necessary volume adjustments due to loss during boiling was made by adding deionized water. The results were compared with the fluoride concentrations obtained in the infusions with brewing time of 3 min.

The amount of fluoride added per dry mass of the sample is shown in Table 3:

Table 3: Amount of fluoride added per dry mass of the sample

Tea brand	Mass of sample, g	Fluoride in the tea with 3 min infusion time, mg/kg	Fluoride added mg/kg
Ethiopian GTB	*1.858	145.621	40
Addis BTP	2.5	291.507	80
Lipton flavored BTB	*1.638	142.857	40

*Mean of five tea leafs found within tea bag, gm.

2.2.7. Method detection limit

Ten 100 mL deionized water samples were taken from the same source that was used for tea infusion preparation. The fluoride content of these blank samples was determined by taking 10 mL of blank sample and 10 mL of TISAB using the same procedure as that used for the tea infusion analysis. The standard deviation of the fluoride content of these blanks was used to determine the method detection limit which is defined as the least concentration of the analyte that can be detected with a predetermined level of confidence [50].

3. Results and Discussion

3.1. Calibration of the electrode

The quality of any analytical measurement critically depends on the calibration of the instrument and the standard solutions used for the calibration [50]. For this purpose a series of five fluoride standards (0.50. 1.00, 5.00, 10.00, and 20.00 mg/L) were prepared from freshly prepared 1000 mg/L fluoride stock solution for the calibration of the fluoride ISE. By converting the fluoride concentrations in mg/L to molar concentration and taking the negative log value of this; and from the potential readings, the following calibration curve was obtained. The slope of the curve found was 57.3 mV/Dec with E^0 and R^2 equal to134.4 mV and 0.9998, respectively.

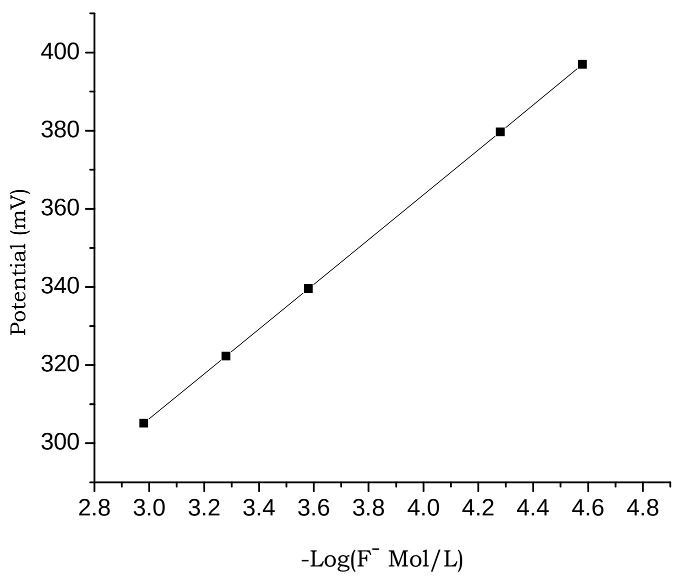

Fig. 4: Calibration curve of fluoride ISE.

3.2. Analytical method detection limit

The standard deviation of the analyte content of the ten blanks was used to determine the method detection limit which is defined as the least concentration of the analyte that can be detected within a predetermined level of confidence and is equal to three times the standard deviation of the blank. As a result the method detection limit found was 0.035 mg/L which is lower than the instrumental detection limit 0.02 mg/L.

3.3. Evaluation of analytical methods

As there is no any certified reference material used to compare the results with, the efficiency of the method used was assessed by spiking experiments. The precision of the method was determined as relative standard deviation and the accuracy of the method determined as percent recovery was calculated using the equation below.

$$\% \text{ Recovery} = (F^- \text{ in spiked sample} - F^- \text{ in non spiked sample}) / F^- \text{ added}$$

The recovery values obtained for the three brands of tea selected to assess the validity of the method are shown in the table below.

Table 4: The accuracy and precision of the method.

Tea brand	[a] F^- in tea	[b] F^- added	[c] F^- in spiked tea	Amount recovered	% Recovery	Precision (% RSD)
Ethiopian GTB	145.62	40	182.99 ± 2.92	37.37 ± 2.92	93.43 ± 7.3	7.82
Addis BTP	291.50	80	362.24 ± 3.68	70.73 ± 3.68	88.41 ± 4.6	5.2
Lipton flavored BTB	142.85	40	181.31 ± 2.83	38.46 ± 2.38	96.14 ± 5.9	6.19

[a] average value of 3 measurements (mg/kg).

[b] fluoride standard added to tea infusion (mg/kg).

[c] values are mean ± SD of triplicate analysis (mg/kg).

3.4. Amount of fluoride in tea infusions brewed in different time

The fluoride concentration of the infusions prepared from different tea brands collected from different shops and supermarkets in Addis after 3, 5 and 10 min brewing time are listed in the tables below. The fluoride concentration of the tea infusions ranges from 79.47 to 634 mg/kg for 3 min brewing time, 111.44 – 682.27 mg/kg for 5 min brewing time, and from 130.08 to 728.27 mg/kg after 10 min brewing time. The fluoride content of green tea bags ranges from 79.47 – 157.70 mg/kg for 3 min infusion, from 111.44 – 190.40 mg/kg for 5 min infusion, and 130.08 – 244.81 mg/kg for 10 min brewing time. For the black tea powders analyzed the fluoride concentration ranges from 82.13 - 634 mg/kg, 116.96 – 682.27 mg/kg, and 152.80 – 728.27 mg/kg for 3, 5, and 10 min brewing time respectively. Black tea bags fluoride content ranges from 110.08 – 188.84 mg/kg, 141.18 – 246.30 mg/kg, and 166.93 – 297.65 mg/kg for 3, 5, and 10 min brewing time, respectively.

The highest level of fluoride was found in the infusion of Abay black tea powder which is produced in Ethiopia with mean fluoride concentration 634, 682.27, and 728.27 mg/kg for 3, 5, and 10 min brewing time respectively. Where as the lowest fluoride concentration was found in the infusion prepared from Hyson green tea bag, which is imported from sirilanka with mean fluoride level of 79.47, 111.44, and 130.08 mg/kg for 3, 5, and 10 min brewing time, respectively.

Table 5: Fluoride level of the tea infusions for 3 min brewing time

Tea brands name	Range, mg F^-/kg	[a] Mean F^-, mg/kg	%RSD
Green tea bags			
Ethiopian green tea	144.83 - 146.60	145.62 ± 0.9	0.61
Hyson green tea	78.20 - 80.44	79.47 ± 1.14	1.44
Quality green tea	155.20 - 159.20	157.70 ± 2.18	1.38
Black tea powders			
Abay	631.60 - 636.80	634 ± 2.62	0.41
Abyssinia	547.20 – 554.00	550.93 ± 3.44	0.62
Addis	290.24 - 292.44	291.50 ± 1.13	0.39
Ahadu	546.00 - 549.60	547.73 ± 1.8	0.32
Almeta	192.00 – 192.80	192.27 ± 0.46	0.24
Anbessa	397.84 - 399.48	398.39 ± 0.94	0.23
Eirmon	355.36 - 359.04	356.8 ± 1.97	0.55
Gumaro	339.96 - 346.68	342.59 ± 3.59	1.04
Haron	348.12 - 352.36	349.53 ± 2.44	0.70
Hyson	80.24 - 84.95	82.13 ± 2.48	3.02
Lipton	88.44 - 93.12	91.04 ± 2.38	2.61
Mohmood	117.68 - 118.60	118.13 ± 0.46	0.39
Wushwush	517.60 - 524.40	520.53 ± 3.49	0.67
Quality	177.51 - 182.65	179.96 ± 2.58	1.43
Black tea bags			
Addis	184.34 - 191.49	188.84 ± 3.92	2.08
Hyson	122.88 - 127.44	124.55 ± 2.52	2.01
Lipton	108.23 - 112.56	110.08 ± 2.22	2.02
Lipton flavored black tea	139.80 - 145.54	142.86 ± 2.89	2.02

[a] values are mean ± SD (mg/kg), the mass of tea leafs in tea bags is calculated as mean of five tea bags.

Table 6: Fluoride level of the tea infusions for 5 min brewing time

Tea brands name	Range, mg F^-/kg	[a] Mean F^-, mg/kg	%RSD
Green tea bags			
Ethiopian green tea	159.09 - 160.92	160.26 ± 1.01	0.63
Hyson green tea	110.64 - 112.48	111.44 ± 0.94	0.84
Quality green tea	185.28 - 194.24	190.4 ± 4.61	2.42
Black tea powders			
Abay	678.40 - 687.60	682.27 ± 4.77	0.70
Abyssinia	559.20 - 563.20	562.4 ± 2.88	0.51
Addis	306.12 - 311.44	308.24 ± 2.82	0.91
Ahadu	572.80 - 580.00	576.8 ± 3.67	0.63
Almeta	245.60 - 249.96	247.70 ± 2.18	0.88
Anbessa	441.36 - 446.88	444.05 ± 2.76	0.62
Eirmon	421.44 - 426.56	424.77 ± 2.89	0.68
Gumaro	400.44 - 407.12	403.27 ± 3.46	0.86
Haron	360.08 - 361.36	360.64 ± 0.65	0.18
Hyson	115.46 - 118.21	116.96 ± 1.40	1.19
Lipton	116.16 - 120.24	118.2 ± 2.04	1.72
Mohmood	137.92 - 140.32	139.87 ± 1.76	1.26
Wushwush	570.40 - 572.40	571.47 ± 1.00	0.18
Quality	193.42 - 195.12	194.18 ± 0.86	0.44
Black tea bags			
Addis	242.60 - 248.15	246.30 ± 3.2	1.30
Hyson	139.83 - 142.43	141.18 ± 1.3	0.92
Lipton	142.17 - 146.60	144.07 ± 2.28	1.59
Lipton flavored black tea	186.39 - 193.96	191.04 ± 4.08	2.13

[a] values are mean ± SD (mg/kg), the mass of tea leafs in tea bags is calculated as mean of five tea bags.

Table 7: Fluoride level of the tea infusions for 10 min brewing time

Tea brands name	Range, mg F^-/kg	[a] Mean F^-, mg/kg	%RSD
Green tea bags			
Ethiopian green tea	238.33 - 245.18	242.73 ± 3.81	1.57
Hyson green tea	129.76 - 130.56	130.08 ± 0.42	0.32
Quality green tea	241.01 - 247.79	244.81 ± 3.47	1.41
Black tea powders			
Abay	725.60 - 731.20	728.27 ± 2.8	0.39
Abyssinia	612.8 - 619.20	615.73 ± 3.23	0.52
Addis	331.92 - 334.36	333.32 ± 1.26	0.38
Ahadu	658.00 - 663.20	660.13 ± 2.72	0.41
Almeta	301.46 - 302.62	302.01 ± 0.59	0.19
Anbessa	524.80 - 530.40	527.47 ± 2.8	0.53
Eirmon	477.60 - 483.20	480.8 ± 2.88	0.60
Gumaro	468.80 - 474.80	472.13 ± 3.06	0.64
Haron	451.52 - 455.20	453.52 ± 1.86	0.41
Hyson	150.72 - 155.44	152.8 ± 2.4	1.58
Lipton	153.42 - 159.47	157.25 ± 3.32	2.11
Mohmood	164.96 - 168.48	193.28 ± 0.54	0.28
Wushwush	621.60 - 625.20	623.33 ± 1.80	0.29
Quality	256.88 - 263.03	260.68 ± 3.32	1.27
Black tea bags			
Addis	295.52 - 299.60	297.65 ± 2.04	0.69
Hyson	192.86 - 193.89	166.93 ±1.80	1.08
Lipton	200.48 - 205.12	203.49 ± 2.61	1.28
Lipton flavored black tea	255.54 - 262.19	258.21 ± 3.51	1.36

[a] values are mean ± SD (mg/kg), the mass of tea leafs in tea bags is calculated as mean of five tea bags.

3.5. Comparison of fluoride concentration among the different tea brand infusions

The fluoride concentration of the tea infusions prepared from the 21 tea brands varies considerably from one brand to the other, this might be due to the differences in the tea plant type, area of cultivation of the plant which may include soil type, altitude, quality of water used for irrigation, etc., harvesting time and harvesting system, i.e. type of leaf harvested. But not due to the differences that exist in the processing of the teas [6, 9, 18, 26].

The fluoride concentration of the teas for 3 min brewing time from higher to lower follow the following scheme:

Abay BTP > Abyssinia BTP > Ahadu BTP > Wuswush BTP > Anbessa BTP > Eirmon BTP > Haron BTP > Gumerro BTP > Addis BTP (Gold label) > Almeta BTP > Addis BTB > Quality BTP > Quality GTB > Ethiopian GTB > Lipton flavored BTB > Hyson BTB > Mohmood BTP > Lipton BTB > Lipton BTP > Hyson BTP > Hyson GTP.

Except the Ethiopian green tea which is exceeded only by two imported tea brands; the top 53% are all produced in Ethiopia. This high fluoride content of the Ethiopian teas may be due to the high soil fluoride level, soil pH, low soil calcium level which is used to precipitate fluoride in the soil and roots of the plant. But most probably it may be due to the usage of the cheap and highly profitable steam parts, older and low grade dust tea leaves together with the younger shuts of the plant. The older and low grade dust tea leaves are expected to have higher level of fluoride than the young shuts [6, 9, 11, 18, 16].

After 5 and 10 min brewing time the fluoride content of the infusions follow similar hierarchy except for some interchanges with that brewed with in 3 min. This interchange may arise due to the difference that exist in the form of the fluoride found in the teas.

Thus for 5 and 10 min brewing time the fluoride level of the tea infusions follow the following order:

Abay BTB > Ahadu BTP > Wuswush BTP >Abyssinia BTP > Anbessa BTP > Eirmon BTP > Gumerro BTP >Haron BTP > Addis BTP (Gold label) > Almeta BTP > Addis BTB > Quality BTP > Lipton flavored BTB >Quality GTP > Ethiopian GTB > Lipton BTB > Mohmood BTP > Hyson BTB > Lipton BTP > Hyson BTP > Hyson GTB.

For the same tea brands the fluoride content of the tea bags was higher than the tea powders in the two imported black teas (Hyson and Lipton). This was expected and is inline with the results found in some publications. The reason for this is, in the general market tea bags are produced from very fine and older tea leafs which accumulate higher concentration of fluoride through time. While the tea powders or leafs are prepared from younger shoots of the plant which contain lesser fluoride than the leaf used to make the tea bag. But such comparison was not found in the Ethiopian black tea brand ADDIS, which has both powder/leaf and bag forms. The result for this brand shows the powder form contain higher fluoride concentration 291.5, 308.24, and 333.32 mg/kg for 3, 5, and 10 min brewing time respectively than the bag form which has 189, 246, and 302 mg/kg for 3, 5, and 10 min brewing time respectively. As it has been observed during our visit to the factory found in Addis Abeba, the factory (Ethio Agri CFET PLC) produces the bag form from a high quality leafs than the leaf used to produce the powder form which is in contrary to the general market. Although no effect was observed by the paper bag used to store the powder in tea bags on the fluoride concentration of the tea infusions for other brands studied [5], the paper bag used for this product may have its own effect in decreasing the amount of fluoride infused to the tea liquor too.

For the Hyson tea brand, which has GTB, BTP, and BTB forms the fluoride content follow BTB > BTP > GTB in all brewing time used. This is due to the green teas are prepared from younger leafs than used to produce the black tea [5, 11, 20].

The chart below shows the amount of fluoride in the three types of teas of the hyson brand and the effect of brewing time on the amount of fluoride infused to the tea liquor.

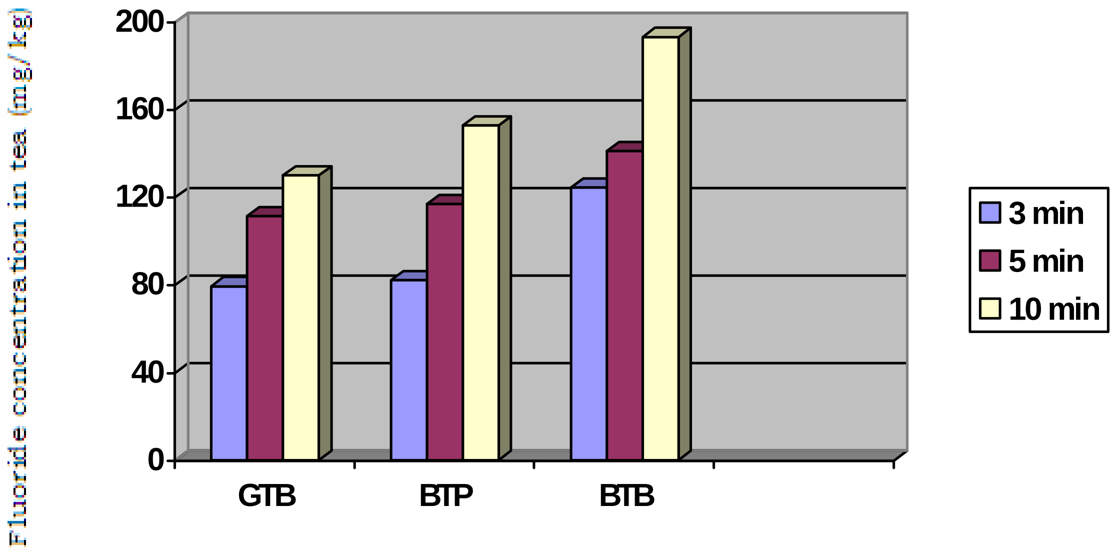

Fig. 5: Fluoride level of Hyson Teas at different brewing time

In this study two types of quality tea were taken, GTB and BTP. The fluoride concentration of the green one was lower than the black one with mean fluoride concentration 158, 190, and 245 mg/kg for the GTB and 180, 194, and 261 mg/kg for the BTP for 3, 5, and 10 min brewing time, respectively.

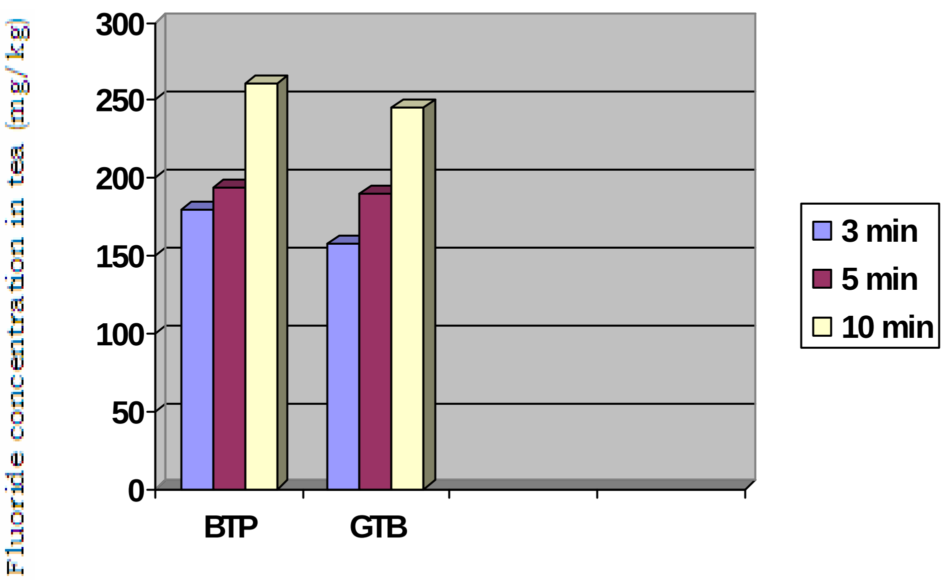

Fig. 6: Fluoride level of quality teas at different brewing time

For Lipton tea brand, its BTB form was found to contain higher fluoride than its powder form in all brewing times used.

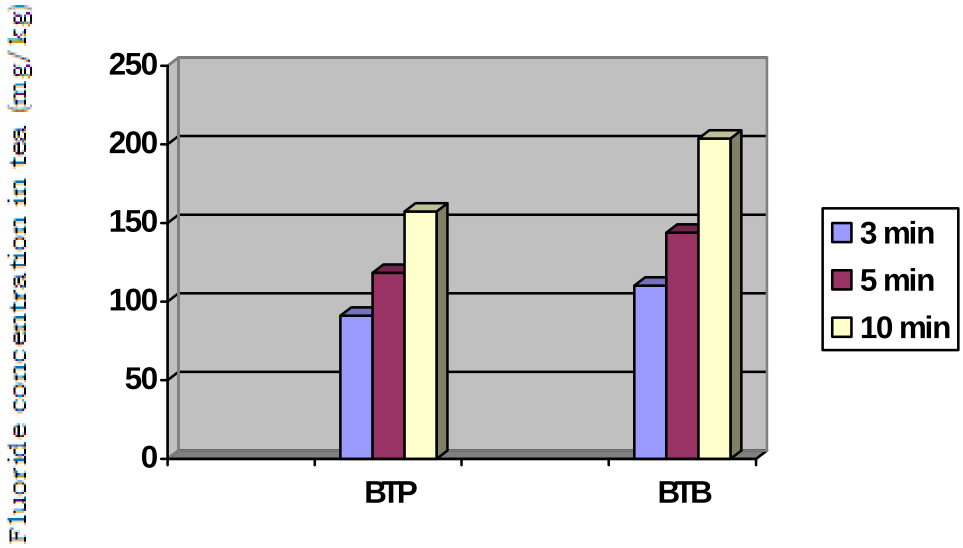

Fig. 7: Fluoride level of Lipton Teas at different brewing time

The Ethiopian green tea, Addis tea bag, and Addis tea powder (Gold label) which are all produced by Ethio Agri-CEFT P.L.C from tea leafs harvested from Wushwush and Gumerro tea farms has fluoride content in the order of BTP > BTB > GTB.

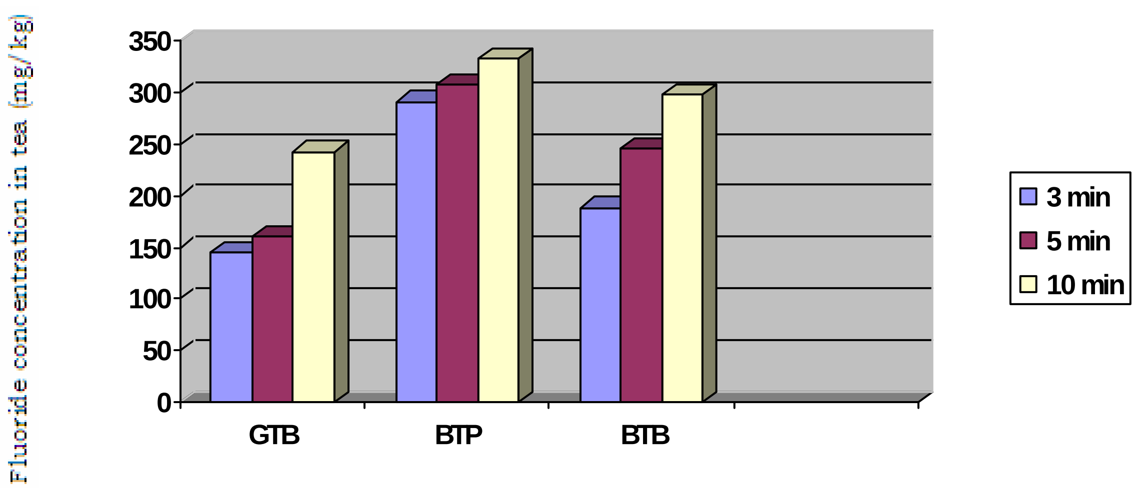

Fig. 8: Fluoride level of Ethiopian green tea bag, Addis black powder/leaf and bag at different brewing time.

3.6. Fluoride extraction in to tea infusions

The extraction of fluoride in to tea brew from tea leaves was different in different types of teas and tea brands. The amount of fluoride in the examined extracts increases after 5 and 10 min of brewing. The average increase of fluoride concentration released into BTP infusions after 5 min of brewing was 15.70% (in range from 2.08% for Abyssinia to 42.40% for Hyson) and 36.14% (in range from 11.76% for Abyssinia to 86.04% for Hyson) after 10 min in the GTB infusions the fluoride level increment after 5 min of brewing ranges from 10.05% to 40.22%, while after 10 min this was from 55.23% to 66.69%. The growth in fluoride level in BTB brews after 5 and 10 min of brewing ranged between 13.35% and 33.72%, and 55.18% - 84.86% respectively. Table below shows the average increase of fluoride content in tea liquors after 5 and 10 min of brewing.

Table 8: Average increase of fluoride content in tea liquors after 5 and 10 min of brewing.

Tea brands name	Mean % increment, 5 min	Mean % increment, 10 min
Green tea bags		
Ethiopian green tea	10.05 %	66.69 %
Hyson green tea	40.22 %	63.68 %
Quality green tea	20.73 %	55.23 %
Black tea powders		
Abay	7.61 %	14.87 %
Abyssinia	2.08 %	11.76 %
Addis	5.74 %	14.34 %
Ahadu	5.30 %	20.52 %
Almeta	28.82 %	57.08 %
Anbessa	11.46 %	32.40 %
Eirmon	19.04 %	34.75 %
Gumerro	17.71 %	37.81 %
Haron	3.18 %	29.75 %
Hyson	42.40 %	86.04 %
Lipton	29.83 %	72.72 %
Mohmood	18.40 %	41.31 %
Wushwush	9.79 %	19.74 %
Quality	7.90%	44.85%
Black tea bags		
Addis	30.42 %	57.62 %
Hyson	13.35%	55.18%
Lipton	30.88 %	84.86 %
Lipton flavored black tea	33.72 %	80.74 %

The concentration of fluoride in the tea infusions may indicate the tea quality. The tea leaves harvested in the season of early summer are superior in quality to those collected in lather season. Fung *et. al.* [6] reported that brick tea containing old leaves, fallen leaves and even branches, was characterized by the highest total fluoride contents than other brands made from young leaves. According to Cao *et. al.* [18] high fluoride content in brick tea depends on its content in raw material and is not caused by processing procedures.

3.7. Fluoride intake by human and safety evaluation of the teas

Fluorine is an essential element in human diet based upon its important role in bone and teeth mineralization, stimulatory and inhibitory effects on many soft tissue enzymes and dental caries resistance. The increased fluoride intake with water and food as well as on occupational exposure on fluoride dust could be a reason of the skeletal and dental fluorisis in humans [19, 20, 30, 47, 48, 49].

Fluoride intake threshold is a problem that should be worth more public attention. Several national and international organizations have already settled or recommended related standards, as listed in table below [19, 20, 38, 39, 47, 48, 49].

Table 9: Recommended daily fluoride intake (mg/d)

People	**WHO**	**USEPA**	**China**	**Poland**
Children	2 (WHO, 2002)	2.5 (USEPA, 1985)	2.4 (China ministry of health, 1997)	1.5 to 2.5
Adult	2-4 (WHO, 1984)	4 (USEPA, 1984)	3.5 (China ministry of health, 1997)	1.5 to 4

The present study revealed that fluoride level of BTP after 5 min brewing, which is the common time used to prepare tea infusions, was in the range of 117 – 682 mg/kg, for GTB it was between 111 – 190 mg/kg and that of BTB fluoride concentration was in the range of 141 – 246 mg/kg. While the Ethiopian black teas fluoride level ranges from 248 to 682 mg/kg with mean concentration of 458 mg/kg among ten brands.

Assuming that on average one consumes 4 cups of tea everyday (400 mL) and each cup uses 2.5 g of tea powder or one tea bag, daily fluoride intake through taking the BTPs may be 1.11 – 6.82 mg, 1- 1.38 mg through GTB and from the BTB it may range from 0.86 – 1.81 mg. from the Ethiopian black teas alone, the daily fluoride intake may range from 2.48 – 6.82 mg but assuming not brand loyal consumer, on average one may consume 4.58 mg fluoride daily from the infusions.

Thus according to the WHO recommendation for daily fluoride intake thresholds for different age groups, consumption of 4 cups of tea prepared from the green and black tea bags is safe for all age groups (children, adolescents and adults) considering other sources of daily fluoride intake are very minimum with respect to the fluoride found in the teas.

But in this study it is observed that consuming 4 cups of teas prepared from the Ethiopian black tea powders will exceed the recommended daily fluoride intake 4 mg by WHO even for adults. But some high quality teas produced in Ethiopia like Addis black tea powder (Gold label) which has the third minimum fluoride level next to Addis black tea bag and Almeta tea powder than any of the black teas produced in Ethiopia provide around 3.08 mg fluoride on consuming 4 cups of tea daily, which is safer for adults but higher for children and adolescents.

Thus according to the world health organization (WHO, 2002) recommendation for daily fluoride intake threshold which is 2 mg to children and adolescents, and 4 mg for adults and assuming 4 cups of tea daily consumption, no or very less amount fluoride from other sources; all the black and green tea bags, imported black tea powders were safe. From the 10 Ethiopian black tea powder brands none of them were safe for children but 30% of them were safe for adults. But if 2 cups of tea daily consumption is considered this increases to 100% and the former to 40% and further increases to 100% if one cup of tea daily consumption is considered. For 5 cups of tea daily consumption only 20% of the teas were safe for adults.

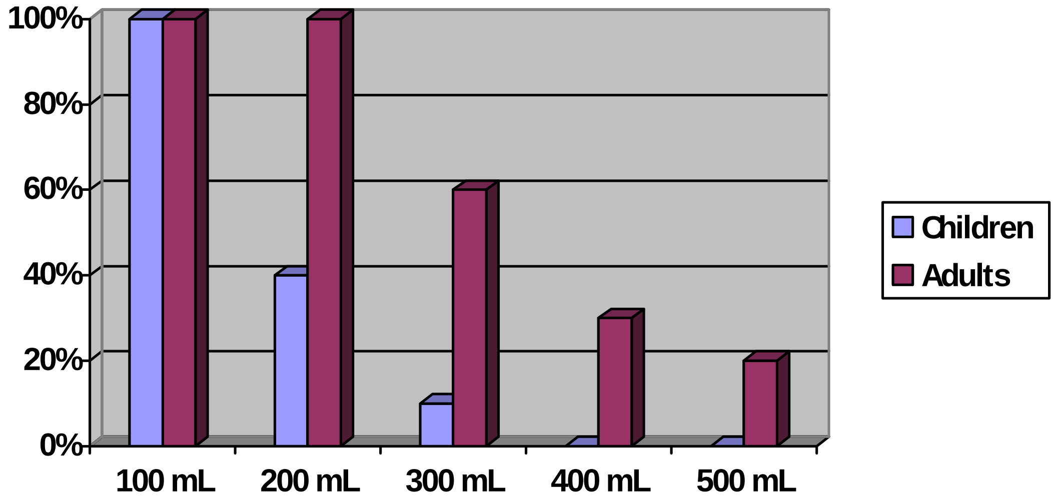

Fig. 9: Safety evaluation Ethiopian black teas at different consumption level.

The table below shows daily fluoride intake through consuming different cups of teas of different brand.

Table 10: Expected daily fluoride intake through consumption of different amount of teas from the studied brands.

Tea brands name	DFI, mg. 1 cup	DFI, mg. 2 cup	DFI, mg. 3 cup	DFI, mg. 4 cup	DFI, mg. 5 cup
Green tea bags					
Ethiopian green tea	0.298	0.595	0.892	1.19	1.49
Hyson green tea	0.217	0.433	0.649	0.866	1.08
Quality green tea	0.378	0.755	1.13	1.51	1.89
Black tea powders					
Abay	1.70	3.41	5.11	6.82	8.52
Abyssinia	1.40	2.81	4.21	5.62	7.02
Addis	0.77	1.54	2.31	3.08	3.85
Ahadu	1.44	2.89	4.32	5.77	7.21
Almeta	0.62	1.24	1.86	2.48	3.10
Anbessa	1.11	2.22	3.33	4.44	5.55
Eirmon	1.06	2.12	3.18	4.24	5.30
Gumerro	1.00	2.01	3.02	4.03	5.03
Haron	0.90	1.80	2.70	3.60	4.50
Hyson	0.278	0.555	0.832	1.11	1.39
Lipton	0.295	0.59	0.885	1.18	1.48
Mohmood	0.348	0.695	1.04	1.39	1.73
Wushwush	1.42	2.86	4.28	5.71	7.13
Quality	0.475	0.95	1.42	1.90	2.37
Black tea bags					
Addis	0.505	1.01	1.51	2.02	2.52
Hyson	0.229	0.457	0.686	0.914	1.14
Lipton	0.250	0.500	0.750	1.00	1.25
Lipton flavored black tea	0.312	0.625	0.938	1.25	1.56

*DFI = Daily fluoride intake, mg.

But in this study, as deionized water was used for the preparation of tea infusions the actual amount of fluoride taken from tea brewed with drinking water may be elevated.

The fluoride level of drinking water in many areas of the country including Addis Abeba is less than 1.30 mg/L [51] where as in areas of the Ethiopian rift valley it ranges from 1.2 – 36 mg/L with mean concentration of 10 mg/L [47]. Though, no water sample from any part of the country was analyzed for its fluoride level in this study, table 10 can be modified for teas brewed with drinking water of various fluoride level from different Part of the country with the help of the fluoride map of the Ethiopian drinking water shown in Fig. 10.

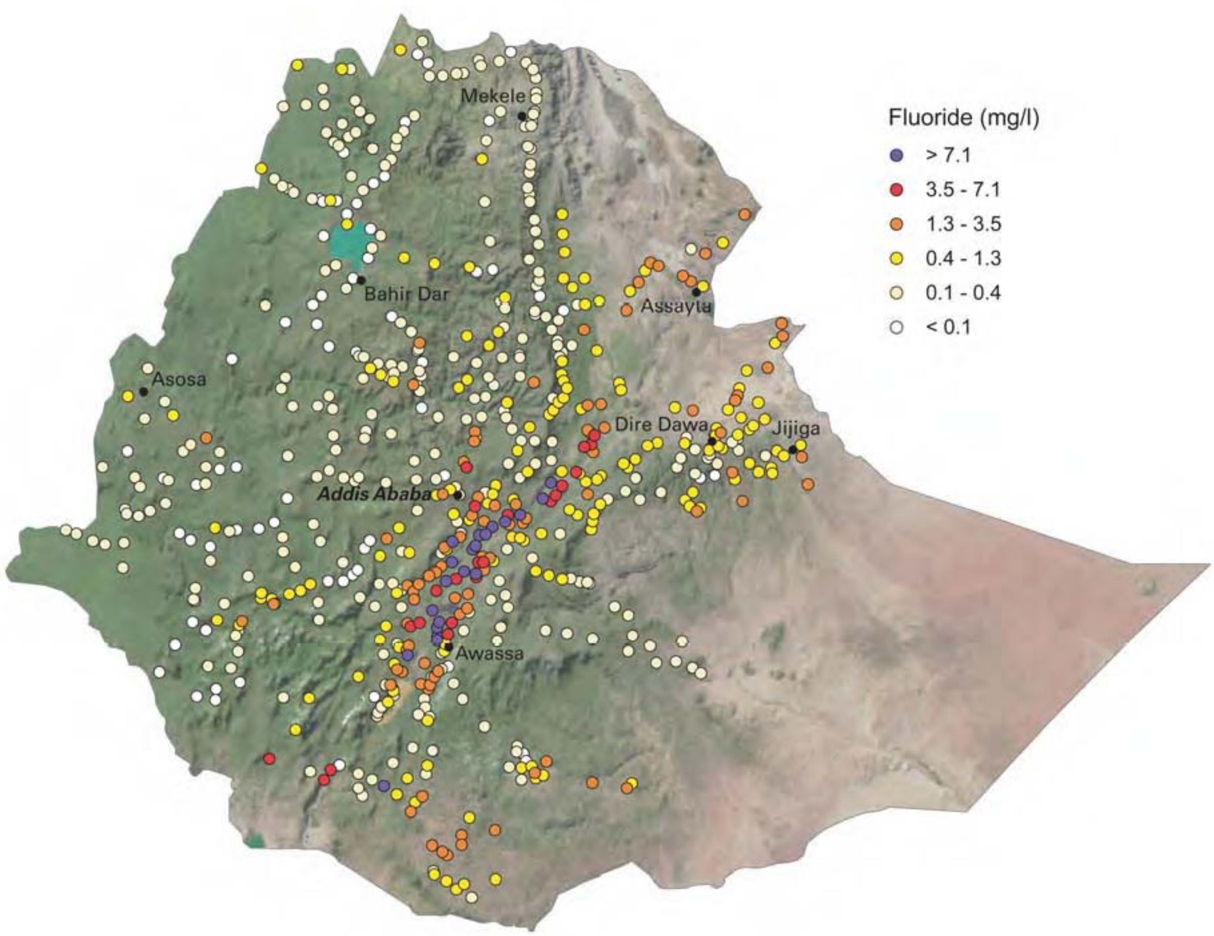

Fig. 10: Ethiopian drinking water fluoride map [51].

Table 11: Expected fluoride intake through consumption of 4 cups (400 mL) of the teas brewed with drinking water of various areas of the country with different level of fluoride.

Tea Brands Name	*AWDWFL <0.1 mg/L	AWDWFL 0.1 – 0.4 mg/L	AWDWFL 0.4 – 1.3 mg/L	AWDWFL 1.3 – 3.5 mg/L	AWDWFL 3.5 – 7.1 mg/L
Green tea bags					
Ethiopian green tea	<1.23	1.23 – 1.35	1.35 – 1.71	1.71 – 2.59	2.59 – 4.03
Hyson green tea	<0.90	0.90 – 1.02	1.02 – 1.39	1.39 – 2.27	2.27 – 3.70
Quality green tea	<1.55	1.55 – 1.67	1.67 – 2.03	2.03 – 2.91	2.91 – 4.35
Black tea powders					
Abay	<6.86	6.86 – 6.98	6.98 – 7.34	7.34 – 8.22	8.22 – 9.66
Abyssinia	<5.66	5.66 – 5.78	5.78 – 6.14	6.14 – 7.02	7.02 – 8.46
Addis	<3.12	3.12 – 3.24	3.24 – 3.60	3.60 – 4.48	4.48 – 5.92
Ahadu	<5.81	5.81 – 5.93	5.93 – 6.29	6.29 – 7.17	7.17 – 8.61
Almeta	<2.52	2.52 – 2.64	2.64 – 3.00	3.00 – 3.88	3.88 – 5.32
Anbessa	<4.48	4.48 – 4.60	4.60 – 4.96	4.96 – 5.84	5.84 – 7.28
Eirmon	<4.28	4.28 – 4.40	4.40 – 4.76	4.76 – 5.64	5.64 – 7.08
Gumaro	<4.07	4.07 – 4.19	4.19 – 4.55	4.55 – 5.43	5.43 – 6.87
Haron	<3.64	3.64 – 3.76	3.76 – 4.12	4.12 – 5.00	5.00 – 6.44
Hyson	<1.15	1.15 – 1.27	1.27 – 1.63	1.63 – 2.51	2.51 – 3.95
Lipton	<1.22	1.22 – 1.34	1.34 – 1.70	1.70 – 2.58	2.58 – 4.02
Mohmood	<1.43	1.43 – 1.55	1.55 – 1.91	1.91 – 2.79	2.79 – 4.23
Wushwush	<5.75	5.75 – 5.87	5.87 – 6.23	6.23 – 7.11	7.11 – 8.55
Quality	<1.94	1.94 – 2.06	2.06 – 2.42	2.42 – 3.30	3.30 – 4.74
Black tea bags					
Addis	<2.06	2.06 – 2.18	2.18 – 2.54	2.54 – 3.42	3.42 – 4.86
Hyson	<0.95	0.95 – 1.07	1.07 – 1.43	1.43 – 2.31	2.31 – 3.75
Lipton	<1.04	1.04 – 1.16	1.16 – 1.52	1.52 – 2.40	2.40 – 3.84
Lipton flavored black tea	<1.29	1.29 – 1.41	1.41 – 1.77	1.77 – 2.65	2.65 – 4.09

*AWDWFL= Areas with drinking water fluoride level.

3.8. Comparison of fluoride content in Ethiopian teas and other tea brands

Tea infusion is after water, the most consumed beverage in the world. The daily consumption of tea infusions may contribute to the dietary requirement of several essential elements such as potassium, magnesium and manganese [19]. Tea is also considered a major source of fluoride, since tea plants *Camellia sinesis* take up fluoride from the soil and accumulate in its leaves [25]. Especially acidic soils are conductive to take up the increased amount of fluoride by tea plants [9, 25]. Bearing in mind that fluoride is an essential element for human with a narrow margin between the requirement and toxicity, it is justifiable to control the concentration of this element in its rich food sources like teas.

During recent years several papers have been published on the fluoride content of tea infusions of different origin and type using ISE method. In this publications fluoride level of 352 – 576 mg/kg and even as high as 1175 mg/kg in Chinese brick tea [16], 2.10 – 123.40 mg/kg in Chinese green and black teas, 35 – 182 mg/kg in Iranian black teas [14], and fluoride level of 96.9 – 148 mg/kg in black tea powders/leaf and 139 – 223 mg/kg in black tea bags originated from United Kingdom, India, China, Japan, and Sri Lanka were reported [20, 19]. In this study it has also been shown that fluoride level in the imported teas ranges from 116 – 194 mg/kg for BTP, 141 – 191 mg/kg for BTB and 111 – 190 mg/kg for GTB. Which is lesser than the fluoride level found in the Ethiopian tea leaves. As a result the Ethiopian black teas were found to contain higher level of fluoride which is comparable with some of the black tea brands marketed in United States, United Kingdom, China and Hong Kong that were found to contain higher level of fluoride ranging from 311 – 604 mg/kg [20]. The tables below were collected from different publications and show level of fluoride in tea infusions with similar tea infusion preparation and method of analysis with that used in this study.

Table 12: The water extractable fluoride content of packed teas (mg/kg) [10].

Tea species	Sample No.	Nation where produced	Nation for source of tea	N (number of determinations)	Mean±SD
Black teas	1	UK	China	5	41.5±0.01
	2	UK	India	8	110.4±0.01
	3	Japan	Japan	5	159.6±0.02
	4	China	China	4	76.5±0.03
Green teas	1	Japan	Japan	4	144.5±0.03
	2	China	China	4	212.4±0.01
	3	China	China	4	165.7±0.02
Oolong teas	1	Taiwan of China	Taiwan of China	4	136.7±0.02
	2	China	China	4	155.8±0.02

Table 13: Estimated amount of fluoride (Mean ± SD; N = 72 and 16) in one bag of Brazilian and imported teas [15].

Brazilian black tea brands, N = 72	**Fluoride mg/bag**
Brand A	0.34 ± 0.032
Brand B	0.14 ± 0.027
Brand C	0.13 ± 0.026
Brand D	0.23 ± 0.026
Brand E	0.13 ± 0.034
Brand F	0.24 ± 0.05
Imported tea types, N = 16	
Black tea	0.33 ± 0.146
Green tea	0.08 ± 0.006
Oolong tea	0.33 ± 0.01

Table 14: Fluoride level in paper bag commodity (mg/kg) [5]

The manufacturer nation	Raw material originated from	n	Mean ± SD
UK	China	5	142 ± 12.5
Japan	Japan	3	168 ± 11.9
Sri Lanka	Sri Lanka	3	139 ± 13.4
UK	Darjeeling, India	3	173 ± 14.2
UK	Darjeeling, India	3	171 ± 10.8
UK	Sri Lanka	3	152 ± 9.7
UK	Qimeng, China	3	223 ± 13.1
China	Guangdong, China	3	146 ± 10.3

Table 15: Fluoride content in stick shaped/powdered black tea (mg/kg) [5].

Manufacturer	Raw material from	Mean ± SD; N = 3
UK Lipton	Unknown	148 ± 11.6
UK Twinings	Darjeeling, India	96.9 ± 9.8
Tea Institute of Qimeng, Anhui	Anhui, China	111 ± 11.3
Tongmu Tea Factory of Fujing	Fujing, China	148 ± 15.7
Dadugang Tea Factory of Yunnan	Yunnan, China	124 ± 14.2

Table 16: Fluoride level of black tea bags (mg/kg) [20].

Brand	Origin of raw material	Market	Fluoride level Mean ± SD
Food lion	Not available	US	604.20 ± 11.73
Twinings	China	US	501.68 ± 10.24
Twinings	China	UK	450.46 ± 6.96
Farmers	Not available	US	443.38 ± 13.24
Sir Winston	Not available	China	406.63 ± 16.70
Lipton	Not available	US	395.43 ± 18.96

Twinings	Not available	Hong Kong	389.75 ± 4.86
Twinings	China	UK	383.42 ± 5.92
Sir Winston Tea	Not available	China	311.32 ± 18.38
Melrose's	China	UK	292.44 ± 12.09
Sainsbury's	Not available	UK	265.33 ± 5.35
Hediard	Sri Lanka	Japan	248.36 ± 1.87
Bigelow	Not available	US	177.92 ± 2.71
Awake	India, Sri Lanka	US	117.97 ± 5.56

3.9. Influence of paper bag, milk and sugar on fluoride level in tea filtrates

Though it is not common in Ethiopia, in many areas tea consumers consume black tea with added milk and sugar supplements. To see whether adding milk and sugar supplement has an effect on the ionic fluoride level in the tea filtrate tests has been done by Cao *et al.* [5]. Cao has also seen whether the paper bag used to store the black tea leaves has an influence on the solubility of fluoride in the tea filtrate. His results, which are shown in the tables below reveals that neither addition of milk nor sugar has effect on the tea filtrate ionic fluoride level.

Cao *et al.* has also seen whether the paper bag used to store the black tea leaves has an influence on the solubility of the fluoride in the tea filtrate. His results indicated that the paper bag did not affect the fluoride levels with in the filtrate.

Table 17: Influence of milk and sugar on fluoride level in the tea filtrates (mg/kg)

Sample	N	With milk and sugar Mean ± SD	Without milk and sugar Mean ± SD	P
Black Tea No 1	3	96.8 ± 7.5	96.9 ± 7.6	> 0.5
Black Tea No 2	3	139 ± 9.8	139 ± 11.3	> 0.5
Black Tea No 3	5	42.1 ± 0.01	42.7 ± 0.02	> 0.5
Black Tea No 4	5	110.2 ± 0.01	110.4 ± 0.02	> 0.5

Table 18: Influence of paper bag on fluoride solubility in packed teas (mg/kg)

Sample	N	With paper bag Mean ± SD	Without paper bag Mean ± SD	P
Black Tea No 5	3	173 ± 8.9	174 ± 11.1	> 0.5
Black Tea No 6	3	146 ± 15.1	146 ± 9.9	> 0.5
Black Tea No 3	5	42.1 ± 0.01	42.4 ± 0.04	> 0.5
Black Tea No 4	5	110.8 ± 0.01	111.4 ± 0.02	> 0.5

The effect of acid on fluoride in the tea extract was also seen by Sukru *et al.* [9], the result show that the fluoride concentrations in the tea infusions were not changed by addition 1 M perchloric acid. Therefore, addition of lemon or lemon juice, which is a common way of drinking tea in some countries including Ethiopia, is not expected to affect the quantity of free fluoride in tea.

In another investigation the amount of fluoride released from powdered tea leaves treated with artificial gastric and intestinal juices was determined [34]. Direct eating of powdered tea leaves, which is a custom in Japan, was found in that study to increase the amount of fluoride intake.

4. Conclusion and Recommendations

Tea whether black or green is naturally rich in fluoride. Worldwide tea is one of the major teas used to prepare infusions for drinking tea. The tea plant (*Camellia Sinensis*) takes up fluoride from the soil and accumulates it in the leaves. Tea therefore can be a major source of human fluoride intake [14]. Other major sources of dietary fluoride intake are water, toothpaste, fish and wine [13, 17, 22, 28, 31, 36, 37, 38].

In this study 21 Ethiopian and imported; black and green teas were collected from different shops and supermarkets in Addis Abeba. The fluoride level of the tea infusions prepared from them were analyzed by fluoride ISE method at three different brewing times 3, 5 and 10 min. assuming 4 cups of tea daily consumption which is equivalent to 400 mL, safety of taking this teas was evaluated using the world health organization (WHO, 2002) guideline for daily fluoride intake for children and adults.

The method used for fluoride analysis in tea infusions was found efficient and it was evaluated through recovery experiment and a good percentage recovery was obtained.

The main findings of this study were:

- Increasing brewing time increases fluoride level in the tea liquors.

- The Ethiopian teas were found to contain higher level of fluoride than those imported ones and some other brands consumed abroad.
- For imported teas of the same brand the fluoride level was in the order of green tea < black tea leaf < black tea bag, this is due to: green teas are made from very young shuts of the tea plant than

those used for black tea and in the general world market tea bags are prepared from very fine older dust tea leafs which accumulate higher fluoride level through aging.

- From the safety evaluation results: Assuming no other source of dietary fluoride, consumption of 4 cups (5 min brewing time) of the black and green tea bags; and imported black tea leafs were safe for all age group according to the WHO guideline for daily fluoride intake thresholds. While daily consumption of 4 cups of any of the black Ethiopian teas analyzed were unsafe for children and only 30% were safe for adults.

Though the exact reason for this high level of fluoride in the Ethiopian tea is not known yet, it may be due to the high soil fluoride level, soil pH, low soil calcium level which is used to precipitate fluoride in the soil and roots of the plant. But most probably it may be due to the usage of the cheap and highly profitable steam parts, older and low grade dust tea leaves together with the younger shuts of the plant. Thus further analysis should be done on the soils of the main tea producing farms like Wushwush, Gumaro and Chewaka plantations and the plant genetics should also be studied as well.

To minimize the risk from consuming high fluoride levels from Ethiopian black teas either the amount of consumption of tea should be decreased or lesser brewing time should be used so that the fluoride infused to the tea brew will decrease.

In some literatures it is shown that increasing water pollution causes contamination of the entire food supply chain. Chemicals accumulating in aquatic organisms, particularly fish consumed by humans in large quantities are of special concern because a high retention of toxic substances in fish tissue may be detrimental to human health. Fluorine compounds readily accumulate in fish with a particular affinity of fluoride for their bone tissue [27]. And thus fish was also categorized as major source of fluoride intake for people where eating fish is very common. As a result determination of level of fluoride in fish from areas where ground waters are suspected to have high fluoride levels and fish is common food item like Zeway, Hwasa, and many others lakes around the Ethiopian rift valley can be area of research. Aquatic organisms would be expected to contain fluoride concentration proportional to those in their environment [21].

In other studies level of aluminum in tea infusions was directly correlated with level of fluoride in the tea infusions [15, 23, 24, 25] and thus the level of aluminum in the Ethiopian teas should be determined and safety evaluation should be made so as to control its adverse effects on human health like Alzheimer's disease which is related to high Al content in human brain [24].

5. References

1. Edible, Medicinal and Useful Plants for a Healthier World. **http://www.pfaf.org/** accessed on 3 February, **2009**.
2. Wikipedia, the free encyclopedia. **http://en.wikipedia.org/wiki/Camellia_sinensis** accessed on 2 February, **2009**.
3. Tea and oral health fact sheet http://www.springleaf.com.au/images/Tea_and_oral_health.pdf
4. Asian News, Feb 6-12, 2009, Volume 4 No 4, a magazine found online at **www.asiannews.net** accessed on February 8, **2009**.
5. Cao, J.; Luo, S.H.; Liu, J.W.; Li, Y. Safety evaluation on fluoride content in black tea. *J. Food Chem.* **2004**; 88: 233-236.
6. Hudaykuliyev, Y.; Tastekin, M.; Poyrazoglu, E.S.; Baspinar, E.; Velioglua, Y.S. Variables affecting fluoride in Turkish black tea. *Fluoride* **2005**; 38: 38-43.
7. Tokalioglu, S.; Kartal, S.; Sahin, U. Determination of fluoride in various samples and some infusions using a fluoride selective electrode. *Turk. J. Chem.* **2004**; 28: 204-211.
8. Fluoride in tea **http://www.i-sis.org.uk/full/fuloride_in_teafull**
9. Kalayci, S.; Somer, S. Factors affecting the extraction of fluoride from tea: Application to three tea samples. *Fluoride* **2003**; 36: 267-270; clarification: *Fluoride* **2004**; 37: 238 – 242.
10. Cao, J.; Liu, J.; Zhao, Y.; Qu, H.; Danzeng, S.; Da, W.; Guan, Y. Fluoride in Newer tea commodities. *Fluoride* **2004**; 37: 296-300.
11. Mochammad, Y. Determination of fluoride in black, green and herbal teas by ion selective electrode using a standard-addition method. *J. Dent.* **2005**; 38: 91-95.
12. Ji-Hyang, N.; Paul, C. Evaluation of the potentiometric determination of trace fluoride in natural and drinking water with a fluoride ion selective

electrode, ISE. South African research on fluoride, available online at **http://www.wrc.org.za**, accessed on February 14, **2009**.

13. Shin-Chun, C.L.; Pao-Kuel, H.; Kuang-Mao, C. Fluoride concentrations in three types of commercially packed tea drinks in Taiwan. *J. Exposure Analysis and Environmental Epidemiology* **2003**; 13: 66-73.

14. Amir Hussein, M.; Mohammad Ali, Z.; Masoud, Y.; Yahya, E. Fluoride content of Iranian black tea and tea liquor. *Fluoride* **2006**; 39: 266-268.

15. Mitsue, F.H.; Celso, S.Q.; Cinthia, P.M.T.; Jaime, A.C. Fluoride and Aluminum in teas and tea based beverages. *Rev. Saude Publica*, **2004**; 38: 100-105.

16. Cao, J.; Zhao, Y.; Liu, J. Fluoride in environment and brick tea-type fluorosis in Tibet. *J. Fluorine Chemistry* **2000**; 106: 93-97.

17. Messaitfa. A. J.; Safer. M.C. Fluoride Contents in Ground waters and main consumed foods (dates and tea) in northern Algeria Region. *J. Earth Science 1*. **2007**; 2: 70-75.

18. CaO, J.; Zho, Y.; Liu. J. Processing procedures of brick tea and their Influence on fluorine content. *J. Food and Chemical Toxicology* **2001**; 39: 959-962.

19. E. Malinocuska; I. Inkielewicz; W.Caarnoucuski P.Szefer. Assessment of fluoride concentration and daily intake by human from tea and herbal infusions. *J. Food and Chemical Toxicology* **2008**; 46: 1055-1061.

20. CaO, J.; Zhao, Y.; Li, Y.; Deng, H. J.; Yi, J.; Liu, J. W. Fluoride levels in various black Tea Commodities: Measurement and safety evolution. *J. Food and Chemical Toxicology* **2006**; 44: 1131-1137.

21. Sigler, W. F.; John, M. N. Fluoride intoxication in Fish. *J. Wild Life Science* **1972**; 252-254.

22. Anosuya, A.; Paranjope, P. K. Effect of parboiling on Fluoride content of Rice. *Fluoride* **1996**; 29: 193-201.

23. Jianyn, R.; Ming, H. W. Accumulation of Fluoride and Aluminum Related to Different Varieties of Tea Plant. *J. Environmental Geo-chemistry and Health* **2001**; 23: 53-63.

24. Xie, Z. J.; Che, Z.; Sun, W. J.; Wnag, J. Distribution of Aluminum and Fluoride in tea plant and soil of Tea Garden in Central and South West China. *J. Chinese Geographical Science* **2007**; 17(4): 376-382.

25. Arjun, L. K.; Shanker, R. G. Uptake of Flouride, Aluminium and Molybdenum by some vegetables from irrigation waters. *J. Hum. Ecol.* **2006**; 19(4): 283-288.

26. Jianyun, R.; Liteng, M. A.; Yunazhi, S.; Wenyau, H. The impact of pH and calcium on the uptake of Fluoride by Tea plants (*Camellia Sinesis* L.) *J. Annals Botany* **2004**; 93: 97-105.

27. Piotr, P.; Maria, J. M.; Jaunust, G. Fluoride in bone tissue of fish sampled from the old warta reservoirs Near Lubon and Radtew ice, Poland. *Fluoride* **2003**; 36(3): 185-188.

28. Jose, M. G.; Kelly, P. L; Vanessa, E. S. Fluoride content of several breakfast cereals and snacks found in Brazil. *J. Appl Oral Sci.* **2003**; 11(4): 306-310.

29. Huang, P. M.; Jackson, M. L. Fluorine Determinetion on the minerals and Rocks. *J. American Mineralogist* **1997**; 52: 1503-1507.

30. CaO, J.; Zhao, Y.; Liu, J. W. Safety Evaluation and Fluoride concentration of Pu'er brick Tea and Bianxao Brick Tea. *J. Food and Chemical Toxicology* **1988**; 36: 1061-1063.

31. Pires, M. A.; Dantas, E. S. K.; Munita, C. S. Fluoride Content of Some teas consumed in Sao Paulo. *Fluoride* **1996**; 29(3): 144-146.

32. History of Tea, A medicine that become a beverage. Taking Tea magazine Vol. 2, Issue 2. Ethio-Agri-CEFT PLC library.

33. Karimi, G.; Hasantadeh, M. K.; Nili, A.; Kheshey, Z.; Samiei, Z.; Nazari, F.; Teimury, M. Concentration and Health Risks of Heavy Metals in tea samples Marketed in Iran. *J. Pharmacology* **2008**; 3: 164-174.

34. Verme, R. J.; Trivedi, M. H.; Chinoy, J. J. Amelioration by Black Tea extracts of sodium Fluoride Induced Hemolysis of Human Red Blood Cell corpuscles. *Flouride* **2006**; 39(4): 261-265.

35. Negpal, D. I.; Damele, S. G. Comparison of salivary fluoride levels following use of dentifrices containing different concentrations of fluoride. *J. Indian Soc. Pedod. Prev. Dent.* **2007**: 20-22.

36. Sina, D.; Amir H. M.; Soheila, D. Fluoride content of bottled drinking water available in Iran. *Fluoride* **2008**; 41(1): 93-94.

37. Pehrsson, P. R.; Perry. C. R.; Cutrufelli, R. C. Sampling and initial findings fore study of fluoride in drinking water in the United States. *J. Food composition and Analysis.* **2006**; 19: 45-52.

38. Nancy, J. M. I.; Pamela, R. P.; Rena, L. C.; Joanne, M. H. Fluoride Content of Municipal water in the United States: what percentage is fluorinated? *J. Food Composition and Analysis.* **2003**; 16: 621-628.

39. Irene. R.; Roberto. H. C. G.; Marilia A. R. B. Evaluation of the fluoride concentration and consumption of mineral water. *Rev. Saude Publica.* **2004**; 38(3): 101-107.

40. Elise, B. B.; David. W. J.; Roger, B. D.; Marray, A. M. Age specific fluoride exposure in dirking water and osteosarcoma (United States). *J. Cancer causes Control.* **2006**; 17: 421-428.

41. David. M.; kvetoslava. N.; Irena. C. Fluoride determination, in urine with ion selective Electrode; with in laboratory Method and sample storage optimitation. *Chemica 43.* **2004**, 43: 1-7.

42. Dozol, S. R.; Herrera, M. T.; Cifuentes, E.; Barrata, A.; Rodriquez, J. P. L.; Sanin. L.H. Dental Fluorosis in rural communities of Chihuahua Mexico. *Fluoride* **2005**; 38(2): 143-150.

43. Daniel. W. Levels of Essential and Non-essential Metals in Commercially available Ethiopian Black Teas. M.Sc. Project, Addis Ababa University, Ethiopia, **2007**.

44. Wushwush and Gumaro Plantations. http://www.ethionet.et/ectpm/. Ethio Agri CEFT P.L.C.

45. Yam, A. A.; Kane. A. W.; Ciss, E. D. Traditional tea drinking in Senegal a Real Source of Fluoride in take for the population. *Odoutostometologie-Tropicale.* **1999**; 87: 25-28.

46. Fluoride in Drinking water. WHO Guideline, water quality series.

47. Fluoride in Drinking water: A scientific Review of EPA's Standards.

48. WHO Guidelines for drinking water Quality.

49. Fluorides, Environmental Health Criteria 227.

50. Miller, J. N.; Miller, J. C. *Statistics and Chemometrics for Analytical Chemistry*, Pearson Education Limited, England, **2000**, pp 107-147.

51. Research for Development **Fluoride** Mapping Poster **www.research4development.info**, accessed on July 3, 2009.

ADDIS ABABA UNIVERSITY

SCHOOL OF GRADUATE STUDIES

Assessment of fluoride in the Ethiopian and imported black and green tea (*Camellia sinensis*) infusions: Measurement and safety evaluation

By Samuel Zerabruk

Approved by Examining Committee	**Signature**
1. Prof. B.S. Chandravanshi (Advisor)	__________________
2. Dr. Feleke Zewge (Advisor)	__________________
3. Dr. Mesfin Redi (Examiner)	__________________
4. Dr. Ghirma Moges (Examiner)	__________________