Addis Ababa University School of Graduate Studies

Determination of Fluoride in the Ethiopian and Imported Black Tea *(Camellia sinensis*) Infusions Prepared in Tap and Fluoride Rich Water

Master's Thesis (Chem.750)

In Partial Fulfillment of the Requirement for the Degree of Masters of Science in Chemistry

By

Asamene Embiale Taye

Advisors: Prof. B.S. Chandravanshi and Dr. Feleke Zewge

June 1, 2011

Addis Ababa University School of Graduate Studies

Determination of Fluoride in the Ethiopian and Imported Black Tea *(Camellia sinensis*) Infusions Prepared in Tap and Fluoride Rich Water

By

Asamene Embiale Taye

Department of Chemistry
Faculty of Chemical and Physical Sciences

Approved by examining board signature

Prof. B.S. Chandravanshi ______________
Advisor

Dr. Feleke Zewge ______________
Advisor

Dr. Ghirma Moges ______________
Examiner

Contents

DECLARATION

I, the undersigned, confirm that the results reported in this thesis were my original work under the supervision of my advisor in Faculty of Chemical and Physical Sciences, Department of Chemistry, Addis Ababa University in the academic year 2010-2011 and all sources of materials used for the thesis work has been duly acknowledged.

	Signature
Asamene Embiale	___________________

This thesis has been submitted for examination with my approval as university advisor.

Advisor name	Signature
Prof. B.S. Chandravanshi	______________________
Dr. Feleke Zewge	________________________

Place and date of submission: School of Graduate Studies

Addis Ababa University

June 2011

List of Figures

Figures **page**

List of Tables

List of Acronyms and Abbreviations

USA	United Sates of America
UK	United Kingdom
HVA	Hogeschool van Amsterdam
NRC	National Research Council
CDTA	1,2-Cyclohexanediaminetetracetic
TISAB	Total ionic strength adjustment buffer
EDTA	Ethylene diamine tetra-acetate
TDS	Total dissolved substance
ISE	Ion selective electrode
ABTP	Abyssinia black tea powder
BBTP	Black lion black tea powder
LBTP	Lipton black tea powder
ABTB	Addis black tea bag
SBTB	Strawberry black tea bag
FBTB	Flavoured black tea bag
EGTB	Ethiopian black tea bag
HGTB	Hyson green tea bag
QGTB	Quality green tea bag
DIW	Deionized water
TAW	Tape water

MEW	Mekie water
ABW	Abonsa water
ALW	Alemtena water
WOW	Wonji shoa water
KOW	Koka water
DIF	Daily intake of fluoride
RDIF	Recommended intake of fluoride
FNBIM	Food and Nutrition Board of the Institute of Medicine
AIs	Adequate Intakes

Acknowledgements

I am extremely gratefully to my research advisor Prof. B.S. Chandavanshi and Dr. Feleke Zewge for their continuous supervision, encouragement and guidance during the course of this Thesis.

I would like to acknowledge Ministry of Education for giving me the opportunity in my postgraduate program.

I would also like to thank Addis Ababa University for giving material and finical support during the whole study.

Abstract

Tea leaves are very rich in fluoride, since the tea plant take up fluoride from the soil and accumulate in its leaves. Depending upon the fluoride content of the water, dried tea leaves are also able to absorb fluoride from water with high fluoride level. In this study, the possible effect of original fluoride concentration in natural water on the fluoride release from tea has been investigated and also conductivity, salinity, TDS and pH of naturally fluoride rich water and the tea infusions prepared in deionized water, tap water and fluoride rich water have been determined. Moreover, the possible capacity of tea leaves (commercially available tea) to absorb fluoride from naturally high-fluoride water which were collected from Ethiopia Rift Valley where the fluoride level of water is high has been investigated.

Infused fluoride content, conductivity, salinity, TDS and pH of black (either powder or bag form) and green (bag form) teas and different water (sampled from Ethiopia Rift Valley) were assayed. Fluoride ion selective electrode, conductivity meter and pH meter were used to determine the fluoride content, conductivity, salinity, TDS and pH of the six naturally different types of water, nine brands of tea prepared in deionized water, tap water and naturally fluoride rich water whose origin from Ethiopia, Sri Lanka and China. The fluoride content, conductivity, salinity, TDS and pH of six naturally different water ranged from 0.254-30.2 mg/L, 2.27-1706 µs, 0-0.9 %o, 48.7-838 mg/L and 7.73-8.59, respectively, infused fluoride, conductivity, salinity, TDS and pH in nine brands of tea ranged from 50.8-2057 mg/kg, 414-2377 µs, 0.2-1.2 %o, 196-1180 mg/L and 4.83-8.52, respectively, which were prepared in naturally different types of water in different brewing time (3, 5 and 10 min) infusion. Even though, the fluoride level of tea prepared in naturally fluoride rich water is high, one can reduce the daily intake of fluoride level by taking tea rather than taking the naturally fluoride rich water itself.

Keywords: naturally fluoride rich water, fluoride, tea infusion, brewing time, ISE

1. Introduction

1.1 History of tea

The history of tea as a beverage is traced by the Chinese to about 2700 BC at the time of Emperor Shen Nung. From China, the tradition of tea drinking came to Japan in the 6th century. It was then used by the ‘privileged society, and became popular for all only about 700 years ago. Later, tea use was introduced into what is now known as Indonesia and from there through the Dutch colonials into Holland. In the middle of the 17th century the English played a major role in merchandising and popularizing tea. By tradition, people in the Orient prefer green tea or oolong tea. In North Africa green tea is also used. In much of the rest of world, black tea is the customary beverage. In the United Kingdom, Ireland, and in Canada, black tea is taken with milk and often sugar. In most other countries, black tea is consumed sweet or with lemon. Green tea is usually drunk neat. In many countries, but particularly in the Orient, it is customary to offer a cup of tea to a guest during a social or business call. In Japan, a diversity of beverages, but especially tea is sold from vending machines located along the street [1].

1.2 Botany of tea plant

Tea is in genus of flowering plants in the Kingdom: Plantae, in Division: Magnoliophyta, in Class: Magnoliopsida, in Order: Ericales, Family: Theaceae in Genus: Camellia and in Species: *Camellia sinensis*. White tea, green tea, oolong, and black tea are all harvested from this species, but are processed differently to attain different levels of oxidation. Common names include tea plant, tea tree, and tea shrub [2].

Figure 1. The tea plant with leaves [57].

Chinese *Camellia sinensis* is native to mainland China and South and Southeast Asia, but it is today 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 (six feet) 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 [3]. 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 [3].

1.3 Historical background of tea plantation in Ethiopia

Tea, as a beverage and plantation, is a recent introduction to Ethiopia. Arab and Indian traders could well be credited for its introduction in the urban areas of Ethiopia. Whatever its origin, however, tea has made considerable headway in establishing itself as an alternative hot beverage at least in the urban areas. The annual national tea consumption is about 3,500 tons, a far cry from that of coffee with an annual estimated domestic consumption of about 100,000 tons [4].

Ethiopia's favorable conditions for tea plantation were not unknown until the beginning of the last century. A Lebanese investor is known to have first tried to grow tea in Gumaro in the early fifties in the Illubbabor Zone of Oromiya. But the credit for establishing large-scale tea plantations goes to the government, which had launched the Gumaro and Wush Wush projects in the 1980s. The long season of cloudy sky and outbreaks of rain and drizzle, which characterizes the climate of that part of the country, are significant factors for a viable tea plantation [4].

The first factory at Wush Wush in the highlands of Ethiopia was constructed in 1986-1988 with engineering assistance provided by Bohea. A tea agronomist introduced new clonal tea varieties raised by tissue culture in the UK [5].

Since 1997 HVA (Hogeschool van Amsterdam) International has provided technical assistance and training in the field of tea marketing, tea factory engineering and tea making for the Wush Wush II Tea Project in Ethiopia, financed by the African Development Bank. The assignment includes consulting engineering for the equipping and commissioning of a second tea factory in the area [6].

So far the largest commercial tea plantations in Ethiopia are Wush Wush and Gumaro which are located in South Western Ethiopia. These formerly state-owned plantations together with the Tea Processing and Packing Factory were bought by Ethio Agri-CEFT in 2000 [6].

Currently the total annual production of the two plantations is about 5000 tons. But, through improved management and processing, both productivity and quality will grow. As a result, the annual total production is expected to reach about 7000 tons in a few years time [6].

Both Gumaro and Wush Wush plantations produce black tea. For this, Wush Wush has two and Gumaro has one tea factory. The fresh leaves undergo withering, cut, tear and curl, fermentation, drying, and cup tasting. Each step determines the tea quality before it is transported to Addis Ababa for the final grading and packaging [6].

1.4 Types and production of tea

All tea comes from the evergreen tea bush (*Camellia sinensis*). The following terms only describe tea leaves after they are harvested from the tea bush and processed for consumption [7].

1.4.1 Green tea

Oxidization is a chemical reaction that takes place when tea leaves are picked and begin to wither and die. Green tea is not allowed to oxidize and is quickly dried, pan-fried or oven fired to dehydrate the tea leaves for storage. This process retains many of the polyphenol, catechins, and flavonoids that are associated with the health benefits of drinking green tea [7].

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 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 [8].

1.4.2 Black tea

Black tea is allowed to oxidize which "ripens" the tea and creates a deep, rich, robust flavor with uniqueness based on the tea grower's knowledge and skill. The oxidation process is commonly referred to as fermentation [7].

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 oven straw to be wilted until soft enough to be rolled without tearing the leaf. Next, the withered leaves are rolled to release the chemicals in the leaves that will contribute to the tea's final colour 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 [9].

1.4.3 Oolong tea

Oolong tea falls somewhere between green tea and black tea in the amount of time the tea leaves are allowed to oxidize. Two terms often used to describe oolong tea are “green” and “amber” style. The “amber” styles are allowed to oxidize slightly more than the “green style” oolong tea [7].

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 [10].

1.4.4 White tea

White tea is picked before the leaf buds fully open and are still covered with fine silky hairs. The delicate buds are quickly air dried to produce some of the rarest and most expensive tea available. White tea is said to have three times more antioxidants than green or black tea. The polyphenols in white tea have been shown to be very effective in mopping up free radicals that can lead to aging, and wrinkles, and sagging skin [7].

The new white tea buds are plucked before they open in early spring, then withered and dried slowly at low temperatures. White tea is not rolled and is only slightly oxidized. The result is a tea with a mild, fresh flavor and natural sweetness [11].

1.5 Chemical composition of tea

The chemical composition of tea is complex: polyphenols, catechins, caffeine, amino acids, carbohydrates, protein, chlorophyll, volatile compounds, fluoride, minerals, and other undefined compounds. Among these, the polyphenols and catechins constitute the most interesting group of tea leaf components: epigallocatechin gallate, epicatechin gallate, epigallocatechin, epicatechin, gallocatechin, and catechins. The oxidized polyphenols are often called *tannins*, which is very different chemically from the commercial *tannic acid* or the plant tannins. It is estimated that a cup of green tea (2.5 g of green tea leaves/200 mL of water) may contain 90 mg of epigallocatechin gallate. Black tea has many more components than green tea, including bisflavanols, theaflavins, theaflagallins, epiflavic acids, and thearubigens. Tea is also a dietary source of antioxidant nutrients such as carotenoids, tocopherols, ascorbic acid (vitamin C), and non-nutrients phytochemicals generally classified as flavonoids [12].

1.6 Medicinal and health use of tea

Tea has about 50 mg per cup or only 40-50% of the caffeine content of coffee. On many people, caffeine has a pleasant, stimulating action. People with difficulty sleeping may use a decaffeinated tea. This is produced under mild conditions, extracting specifically caffeine, and leaving the tea polyphenols. Caffeine has had some cancer-preventing action, but not as extensive as that of the tea polyphenols [1]. More recently, detailed research has explored through epidemiologic and marker studies the health-promoting actions of tea. ‘Tea drinkers seem to have a lower risk of heart disease and stroke, and several types of cancer [1]. Experimental research in laboratory animals has demonstrated inhibition of carcinogenesis at a number of organ sites: of special interest are tissues such as lung, stomach and esophagus, associated with tobacco carcinogens or salt [1]. Other investigations noted inhibition at organs related to nutritional carcinogenesis. Such as colon or mammary gland [1]. These laboratory approaches provide tools to explore the underlying mechanisms. Inhibition through detoxifying enzyme induction [1] and also antioxidant effects has been documented [1], although in some cases activation reactions may also be stimulated. This could be a matter of dose-response controlling the ratio biochemical activation/detoxification [1].

According to studies, green and white teas are said to be more effective in preventing cancer, than black tea, Clinical studies indicate that drinking green tea increases the metabolism rate and hastens fat oxidation. Beneficial compounds called catechin polyphenols in green tea also increase the rate at which calories are burnt. Studies suggest that drinking green tea plays a role in preventing the onset of diabetes. The amino acid L-theanine in tea leaves helps to increase mental awareness by influencing the brain's attention networks. Theanine is also believed to strengthen the immune system of the body. Besides, the oxalates found in tea are said to help the immune system in fighting infections. A medical study suggests that drinking tea lowers the likelihood of cognitive impairment. Clinical trials indicate that drinking black tea helps to reduce stress levels. Tea drinking is also surmised to have a role to play in reducing the risk of heart attacks and cardiovascular diseases [13].

The polyphenols may contribute to defenses against oxidative damages. Numerous studies have demonstrated that aqueous extract or the major polyphenols of green tea possess antimutagenic, antidiabetic, antioxidant, antibacterial, anti-inflammatory, antitumor, hypocholesterolemic, and above all, cancer-preventive activities in a variety of experimental animal models system. Oral diseases including dental caries, periodontal disease, and tooth loss may significantly impact a person’s overall health. Among these, dental caries is a multifactorial infectious disease in which diet; nutrition, microbial infection, and host response play important roles. Earlier reports suggested that tea consumption reduces dental caries in humans and experimental animals [12]. In vivo animal studies have shown that specific pathogen-free rats infected with *Streptococcus mutans* and then fed a cariogenic diet containing green tea polyphenols have significantly lower caries scores [12]. Supplementing drinking water of rats with 0.1% green tea polyphenols along with a cariogenic diet also significantly reduced total fissure caries lesions [12]. Animal studies using oolong tea produced similar results. Cariostatic activity of oolong tea extract was effective even after the establishment of *Streptococcus sobrinus* in the oral cavity and was more effective in drinking water than in the diet [12]. Drinking tea (without added sugar) has been associated with lower caries levels in humans [12]. Caries among children who drank a cup of tea immediately after lunch were found to be significantly lower. The data suggested that tea polyphenols may be responsible for the noted effects [12]. Rinsing with 0.2% Chinese green tea while brushing has also been found to reduce plaque and gingival index significantly [12]. Zhang and Kashket reported that tea extracts inhibits human salivary amylase and that tea consumption may reduce the cariogenic potential of starch-containing foods such as crackers and cakes because tea may reduce the tendency for these foods to serve as slow-release sources of fermentable carbohydrate [12]. It is likely that the cariogenic challenge in a cariogenic diet is reduced by the simultaneous presence of green tea in the diet. Although tea is a source of fluoride in addition to many other dietary trace elements, recent studies have demonstrated that tea polyphenols rather than fluoride contribute to the anticariogenic potential [12]. Several mechanisms have been proposed for the observed anticariogenic properties of teas. These include tea's inhibitory effect on bacterial growth, bacterial viability, glucosyltransferase, and salivary amylase activities [12]. Thus far, most research on antimicrobial activity and oral health benefits of teas has focused on green tea or oolong tea [12]; less attention has been directed to the fully fermented black tea. Worldwide, 80% of the tea consumed is black tea, which is also the more popular drink in Europe and Northern American countries. Recent studies in Wu's laboratory have demonstrated that black tea and its polyphenols inhibit growth, acid production, metabolism, and glucosyltransferase enzyme activity of *S. mutans* and dental plaque bacteria [12]. Adults rinsing with black tea 10 times a day for 7 days had a significantly less pronounced pH fall and numerically fewer *S. mutans* and total oral *Streptococci* in plaque but not in saliva. Fluoride concentrations in plaque and saliva increased, reaching a maximum at day 7 [12]. It is evident that black tea and its polyphenols also benefit human oral health by inhibition of dental plaque, its acidity, and its cariogenic microflora.

Even though tea has the above medicinal use, there are some medical disadvantages attributed to drinking tea: some medical experts surmise that the fluoride in tea leaves may reduce its anti-cancer properties or even cause cancer. The caffeine in tea has been linked to sleep disorders. The oxalates found in tea could affect the kidneys, especially if tea is consumed in excess. A study suggests that having tea with milk reduces its ability to prevent cardiovascular disease [13].

1.7 Fluoride

Fluoride is the anion F^-, the reduced form of fluorine. Compounds containing fluoride anions and in many cases those containing covalent bonds to fluorine are called fluorides. Fluoride, like other halides, is a monovalent ion (−1 charge). Its compounds often have properties that are distinct relative to other halides. Structurally, and to some extent chemically, the fluoride ion resembles the hydroxide ion. Fluorine-containing compounds range from potent toxins such as sarin ($C_4H_{10}FO_2P$) to life-saving pharmaceuticals such as Efavirenz, and from inert materials such as calcium fluoride to the highly reactive sulfur tetrafluoride. The range of fluorine-containing compounds is considerable as fluorine is capable of forming compounds with all the elements except helium and neon [14].

Solutions of inorganic fluorides in water contain F^- and bifluoride HF_2^- [14]. Few inorganic fluorides are soluble in water without undergoing significant hydrolysis. Examples of inorganic fluorides include hydrofluoric acid (HF), sodium fluoride (NaF), and uranium hexafluoride (UF_6). In terms of its reactivity, fluoride differs significantly from chloride and other halides, and is more strongly solvated due to its smaller radius/charge ratio. Its closest chemical relative is hydroxide. The Si-F linkage is one of the strongest single bonds. In contrast, other silyl halides are easily hydrolysed [14].

Many fluoride minerals are known, but paramount in commercial importance are fluorite and fluorapatite. Fluoride is found naturally in low concentration in drinking water and foods. Water with underground sources is more likely to have higher levels of fluoride, whereas the concentration in seawater averages 1.3 mg/L [15]. Fresh water supplies generally contain between 0.01–0.3 mg/L, while the ocean contains between 1.2 and 1.5 mg/L [16].

The common fluoride bearing minerals found in soil are fluorospar (CaF_2), cryolite (Na_3AlF_6) and chiolite ($Na_5Al_3F_{14}$) [46]. 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 [42]. The high solubility of F^- in soil under acid conditions (pH < 6) corresponds to the presence of cationic AlF^{+2} and AlF^{3+} complexes [42].

1.8 Fluoride in tea

Tea (*Camellia sinensis*) is a perennial plant capable of taking up fluoride from soil and accumulating it in leaves [18-22]. Excess fluoride in tea leaves causes necrosis in leaf margins and tips [17]. Fluoride in tea leaves may be released into tea infusions and contributes to total fluoride intake in humans [18, 22]. Tea drinks are one of the main fluoride sources from ingestion. Adequate fluoride ingestion is helpful to avoid caries, but over-ingestion induces dental and skeletal fluorosis, which may result in malfunction of the bone and joint system [23, 24]. It has been suggested that daily fluoride intake should not exceed 10 mg per person [23]. Prolonged ingestion of high levels of fluoride, leads to corroded, pitted rusty brown teeth. The upper limit for clinically acceptable dental fluorosis is not well known, but the value of 0.05-0.07 mg F/kg/day is generally accepted as a reference [27].

Fluoride content in tea has risen dramatically over the last 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 a whopping 22 mg of soluble fluoride ions per teabag or cup in green tea [17].

Pro-fluoridation infant medical group states that a cup of black tea contains 7.8 mg of fluoride which is the equivalent amount of fluoride from 7.8 liters of water in an area fluoridated at 1 mg/L. Some British and African studies from the 1990s showed a daily fluoride intake of between 5.8 mg and 9 mg a day from tea alone. [17].

Leaves of tea contain nearly 98% of total fluoride content of the whole plant. Old leaves, harvested in the summer or autumn, contain a higher level of fluoride compared to young tender shoots harvested in the spring [28]. The tea plant absorbs fluoride from acidic soil through passive diffusion, which is then accumulated in the leaves during the plant’s life span [29, 30]. In the past, tea was grown in natural soil but nowadays fertilizers are used to boost production. It is also known that plants can take up more fluoride when the mineral fertilized soil is used [31].

Dr. Gary Whitford, Regents Professor of oral biology in the School of Dentistry, notes that the increased risk appears to be associated with people who drink one or more gallons of black tea daily for a prolonged period of time. Previous studies have indicated that black tea contains 1 to 5 mg of fluoride per liter, but new research indicates that one liter may contain as much as 9 mg. People who ingest about 20 mg daily for more than 10 years face a significant risk to bone health [32].

The Fluoride Action Network website notes several other risks associated with ingestion of fluoride. According to the National Research Council (NRC), animal studies have shown that fluoride can damage the brain, resulting in dementia-like effects in mice at the same concentration used to fluoridate water (1 mg/L). Studies in humans have shown that 1.8 mg/L in children can have a negative impact on IQ [32].

The NRC also notes that fluoride in drinking water may reduce thyroid function in people who have low iodine intake. Individuals who have kidney disease are highly susceptible to fluoride toxicity, largely because they have a reduced ability to eliminate it from the body. Therefore the toxin can accumulate in the bones and cause or exacerbate a painful bone disease called renal osteodystrophy [32].

Whitford points out that “the additional fluoride from drinking two to four cups of tea a day won’t harm anyone; it’s the very heavy tea drinkers who could get in trouble” [32].

Whitford discovered that the method used to identify the levels of fluoride in black tea was not accurate because it does not account for the amount that combines with aluminium to form aluminum fluoride, which is not detectable using the conventional testing method. Tea leaves accumulate large concentrations of fluoride and aluminium, and when the teas are brewed, some of these minerals leach into the beverage [32].

Whitford used another testing method called diffusion, which breaks the bond between aluminium and fluoride. This allows all the fluoride in tea samples to be extracted and measured. When he used this approach using seven brands of black tea purchased in stores, he found that the amount of fluoride was 1.4 to 3.3 times greater than that detected using the traditional method [43].

The recommended daily dose of fluoride through fluorinated drinking water, toothpaste and food is 2 to 3 mg a day [33].

Tea plants accumulate fluoride in their leaves. In general, the oldest tea leaves contain the most fluoride [34]. Most high quality teas are made from the bud or the first two to four leaves, the youngest leaves on the plant. Brick tea, a lower quality tea, is made from the oldest tea leaves and is often very high in fluoride. Symptoms of fluoride excess (i.e., dental and skeletal fluorosis) have been observed in Tibetan children and adults who consume large amounts of brick tea [35, 36]. Unlike brick tea, fluoride levels in green, oolong, and black teas are generally comparable to those recommended for the prevention of dental caries (cavities). Thus, daily consumption of up to one liter of green, oolong, or black tea would be unlikely to result in fluoride intakes higher than those recommended for dental health [37, 38]. The fluoride content of white tea is likely to be less than other teas, since white teas are made from the buds and youngest leaves of the tea plant.

1.9 Factors affecting fluoride extraction in tea infusion

The quality of infused tea depends on the percent of extraction, which in turn is a function of type, strength, and duration of infusion [40]. Boiling increases the fluoride extraction, but also affects the flavour of tea [39]. Five minutes produces the best flavour with least extraction of unpleasant-tasting tannin [41].

1.10 Fluoride intake by human and safety evaluation of the teas

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 enter 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) [42]. It is very characteristic that fluoride prevents tooth decay at about 1 mg L^{-1} but causes mottled teeth and bone damage at around 5 mg L^{-1} when it is present in water. Fluorosis is caused by elevated intake 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 [42].

One of the suggested causes of enamel fluorosis is an increase in dietary fluoride intake by children [44]. This has been attributed to accidental ingestion of fluoride dentifrices, topical gels, foods and beverages prepared with fluoridated water, and foods and beverages with high natural fluoride content [44]. In 1994, the Council on Dental Therapeutics of the American Dental Association adopted a new schedule for dietary supplementation for US children which lowered previous recommendations [45]. This was done in an attempt to reverse or stop the increase in fluorosis indicated by epidemiological studies [12]. A previous study by Chan and Koh reported that excessive tea consumption during early childhood "could result in excessive fluoride intake and lead to enamel fluorosis [46]." The study reported that depending on infusion time, fluoride concentrations of infusions of tea ranged from 0.84-5.12 mg/L [46]. Significant differences in fluoride concentrations were found among the three types of tea: caffeinated (mean = 1.50 mg/L), decaffeinated (mean = 3.19 mg/L), and herbal (mean = 0.05 mg/L). The high level of fluoride in tea has been known for years and has been suggested to have a potential impact on total dietary fluoride intake [47, 48].

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 which can replace the expensive and time-consuming chromatographic methods. The other official method for fluoride determination is spectrophotometer using sodium 2-(parasulfophenylazo)-1,8-dihydroxy-3,6-naphthalenedisulfonate (SPADNS) as calorimetric method [60]. 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 somewhat lower than the levels in real samples due to the adsorption and/or complexation of free fluoride [37, 38].

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 [43].

1.12 Fluoride contents in tea infusions

The tea plant (*Camellia sinensis*) is known to accumulate fluoride from soil. As the fluoride is easily released during infusion (WHO, 1994), tea is considered a major source of fluoride among tea drinkers [35].

A substantial amount of fluoride is released during tea infusion and nearly all (about 94.9%) of the released fluoride is available to consumers [42].

The fluoride concentration of several brands of tea powders and tea bags available in Ethiopian supermarkets and their tea infusions in tap water was studied by Zerabruk and coworkers [58] which were found to be in the range of 80–634 mg/kg for 3 min brewing time, 111–682 mg/kg for 5 min brewing time, and from 130–728 mg/kg after 10 min brewing time. The fluoride content of green tea bags was in the range of 80–158 mg/kg for 3 min infusion, 111–190 mg/kg for 5 min infusion, and 130–245 mg/kg for 10 min brewing time. For the black tea leaves analyzed the fluoride concentration was in range of 82–634 mg/kg, 117–682 mg/kg, and 153–728 mg/kg for 3, 5, and 10 min brewing time, respectively. Black tea bags fluoride content was found to be in the range of 110–189 mg/kg, 141–246 mg/kg, and 167–298 mg/kg for 3, 5, and 10 min brewing time, respectively [58].

Fluoride contents of 12 brands of commercial tea leaves purchased in Minjian Township of Nantou County in Taiwan were determined to be 100-451 mg/kg dry weight in tea leaves and

0.39-1.21 mg/L in tea infusions is prepared by boiling water for 5 min with 1% of tea leaves to boiling water ratio. Percent of fluoride infused from tea leaves depends on methods of infusion and can be up to 83% of total fluoride contents of tea leaves [55].

Natural fluoride from drinking water and food, especially tea infusions and high fluoride salts are the main sources of the total fluoride intake of the population. In Ethiopia the concentration of fluoride in surface water is generally below 1.5 mg/L in highland areas except in some locations, whereas in lowlands mainly in the Rift Valley Regions, the fluoride content of drinking water from ground water is as high as 33 mg/L. Fluorosis resulting from the intake of tea was reported in different parts of the world [59].

Even though, the fluoride concentration of different types of tea, there infusion in deionized water and the fluoride absorbing capacity from fluoride added deionized water (20 mg/L) were done, the significance of the present study were: to determine the daily intake fluoride from tea infusion prepared by naturally fluoride rich water which were sampled from Ethiopian Rift Valley, since naturally fluoride rich water has a high concentration of different metal ions which may increase or decrease the fluoride absorbing capacity of tea leaves from naturally fluoride rich water by forming fluoride metal complex. So to determine the real amount fluoride absorbing capacity of tea leaves from naturally fluoride rich water and to determine amount of fluoride increase or decrease in daily intake for consuming of tea prepared by naturally fluoride rich water.

The absorbing capacity of the teas seems to be independent of the fluoride concentration of the tea leaves. Thus, both the high-fluoride tea and low-fluoride tea reduced the fluoride concentration of the infusion below the initial water fluoride concentration. This may indicate that factors other than the fluoride content of the tealeaves are of importance for fluoride release and absorption [50].

1.13 Objective of present study

1.13.1 General objective

To investigate the fluoride (F^-) content of various commercial brands of tea (*Camellia sinensis*) marketed in Ethiopia and to compare the release of F^- from them in their infusion prepared in deionized water, tap water and naturally fluoride rich water.

1.13.2 Specific objectives

- ❖ To determine the daily intake of fluoride from drinking tea and compare it with the safe threshold value.
- ❖ To compare the fluoride content of Ethiopia black tea infusion in deionized water, tap water and naturally fluoride rich water.
- ❖ To compare the fluoride content of Ethiopian black tea infusions with other imported ones and those reported in other countries.
- ❖ To determine the pH, conductivity, total dissolved substance and salinity of tea liquor prepared in different naturally fluoride rich water.

2 Experimental

2.1 Equipment

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. A digital analytical balance (Adam Equipment, Model WL3000, U.K.) with ±0.001 g precision was used to weigh tea samples. The electrical conductivity (EC), salinity and total dissolved solid (TDS) of infused tea and water sample were measured using Thermo Orion EC meter, model 145Aplus (USA). A 250 mL beaker (Pyrex, USA) was used to prepare the tea infusions with hot plate. A 10 and 5 mL graduated pipettes (Pyrex, USA), 25, 50 and 100 mL measuring cylinders (Germany) were used to measure volumes during standard solution and sample preparations for analysis. Plastic beakers were used to store tea infusions and sampling water. Apparatus such as volumetric flasks, measuring cylinder, plastic beaker and polyethylene zerican were washed with detergents and tap water, rinsed with deionized water.

2.2 Reagents and chemicals

Fluoride stock solution (1000 mgL^{-1}) was prepared from sodium fluoride (99.0% NaF, BDH Chemicals, England) and stored in 1 L volumetric flask. Total ionic strength adjustment buffer (TISAB) solution containing 58 g of sodium chloride, 57 mL of glacial acetic acid, 2 g of ethylene diamine tetra-acetate (EDTA, Spin), 7 g of sodium citrate and approximately 100 mL of 6 M NaOH in a volume of 1000 mL (to adjust pH, 5.0-5.5) and 0.01 M KCl was used for calibration of conductivity meter. The TISAB solutions regulates the ionic strength of samples, standard solutions and adjust the pH, and also avoids interferences by polyvalent cations such as Al(III), Fe(III) and Si(IV); which are able to complex or precipitate with fluoride and reduce the free fluoride concentration in the solution. EDTA forms stable complexes with polyvalent metal cations (e.g., Al(III), Fe(III) and Si(IV)), which are more stable than metal-fluoride complexes (AlF^{3-}, FeF^{3-}, etc.) in solution. The EDTA preferentially complexes with polyvalent cations present in water and/or aqueous solution (e.g., Si^{4+}, Al^{3+} and Fe^{3+}). There are 6 complexing groups in EDTA and it forms metal-EDTA complexes in a metal-ligand ratio of 1:1, freeing the fluoride ion from its complexes with the cations.

2.3 Sampling area and sample collection description

2.3.1 Sampling area description

Naturally fluoride rich water samples were collected from five different places in Ethiopia Rift Valley namely Koka, Wonji Shoa, Alemtena, Abonsa and Mekie by 10 L polyethylene zerican. They are located in East Shoa, Oromia region, and south west of Addis Ababa. These sampling areas have water with fluoride content ranged from 1.5 mg/L to 36 mg/L [57] which were used to prepare infusion of tea with low, medium and high fluoride content water that have been used to determine the fluoride binding capacity of tea leaves with different level of naturally fluoride rich water and also people who live in this area are affected skeletal fluorosis and dental fluorosis, this might be caused by taking of high dose of fluoride from different source and the tea samples were collected from different supermarkets in Addis Ababa.

2.3.2 Sample collection description

2.3.2.1 Water sample collection

Naturally fluoride rich water sample was collected from ground water source used for drinking, washing and making food. From each five sub-sites about four liter of water were collected, mixed and formed one bulk sample in a pre-cleaned, dried and rinsed with the sample water solution in the five liter plastic polyethylene container (zerican) and brought to the laboratory for immediate measurement of fluoride concentration and other parameters like pH, conductivity, salinity and total dissolved solid without any pre-treatment.

2.3.2.2 Tea sample collection

A total of nine different brands of tea which are either imported or produced in Ethiopia were collected from different supermarkets in Addis Ababa. From nine brands three were green tea bags, three were black tea powder and the other three were black tea bags. For each of the nine brands three packets of the same brand were randomly collected, mixed well and put in plastic bags. A total of 27 samples were collected, no more than one sample was collected from one supermarket. List of sampled tea brands and their details are given in the Table 1.

Table 1. List of sampled teas and the details.

No	Tea brand name	Form	Amount per box	Country of origin	Country of packing	Number of sample taken, box
Green tea bag						
1	Ethiopian green tea	Leaf	25 bags	Ethiopia	Ethiopia	3
2	Hyson green tea	Leaf	25 bags	Sri Lanka	Sri Lanka	3
3	Quality green tea	Leaf	25 bags	Sri Lanka	Sri Lanka	3

Black tea powder						
4	Abyssinia	Leaf	80 g	Ethiopia	Ethiopia	3
5	Black lion	Leaf	80 g	Ethiopia	Ethiopia	3
6	Hyson	Leaf	200 g	Sri Lanka	Saudi Arabia	3
Black tea bag						
7	Addis	Leaf	50 g	Ethiopia	Ethiopia	3
8	Mervin strawberry	Leaf	50 g	Sri Lanka	Sri Lanka	3
9	Lipton flavoured	Leaf	50 g	China	U.A.E.	3

2.4 Sample preparation

2.4.1 Tea infusion preparation

The tea infusion was made from each of the tea types. Though there is no universal agreement on how to prepare tea beverage, it is usually in Ethiopia to prepare it by first boiling the water, adding the required amount of tea powder and continue boiling but put the bag to the boiled water for bag form of tea and infused for certain time depending up on personal interests, mostly 3-5 min. For this study tea infusion was prepared as follows- simulating home tea preparation: 100 mL of water (deionized water, tap water or naturally fluoride rich water) was added to three different beakers and was brought to boil, the heater was turned off and one tea bag was added to each of the three beakers. After 3, 5 and 10 min of infusion, the tea bag was taken out. When the solution reached room temperature, it was filtered by Whatman No. 70 filter paper and water was added in a volumetric flask to make up a 100 mL volume and finally the liquor was stored in plastic beaker, but for powder form one gram tea was added to the boiled water and boiling was continue for different infusion time of 3, 5 and 10 min, after their infusion time it was filtered using plastic mesh. When the tea reached room temperature, it was filtered by Whatman No. 70 filter paper and water was added in a volumetric flask to make up a 100 mL volume and finally the liquor was stored in a plastic beaker. Ready-to-drink teas were analysed by taking samples directly from plastic beaker.

2.5 Fluoride determination in tea infusion, tap water and fluoride rich water

The quality of any analytical measurement critically depends on the calibration of the instrument and the standard solutions used for the calibration.

Calibration of the fluoride ion selective electrode for fluoride measurement of tea infusion and naturally fluoride rich water were done by preparing a series of 1, 5, 10, 20 and 30 mg/L standards solutions from stock solution of 1000 mg/L, then 10 mL of standard solution from each series was taken and mixed to 10 mL of TISAB to each of 50 mL plastic beaker, the mixture were stirred (uniformly) thoroughly using the magnetic stirrer, the fluoride selective electrode was immersed in each beaker and the calibration was done. But the calibration of the electrode for measuring of fluoride concentration of tap water was done by preparing a series of 0.1, 0.5, 1, 5 and 10 mg/L standard solutions.

After calibration of the fluoride ion selective electrode within the selected working range (1 to 30 mg/L), the fluoride concentration of tea infusion and naturally fluoride rich water were determined. Thus, F^- concentration in tea infusion was measured by mixing equal volumes (10 mL) of tea infusion and 10mL of TISAB in a 50 mL plastic beaker and the mixture was stirred (uniformly) thoroughly using the magnetic stirrer. The combination fluoride selective electrode was immersed in the solution and the fluoride concentration of the tea infusion was determined from a direct readout. Similarly the fluoride concentration of tap water was also done by this procedure, except the working range of standard solutions for calibration were change to 0.1, 0.5, 1, 5 and 10. All measurements were made in triplicates.

Figures 2 and 3 show the calibration curves for determination of fluoride in tap water and naturally fluoride rich water having the slope and correlation coefficient (r) of the curve found were -57.6 mV/dec and -0.9999 for tap water, -57 mV/dec and -0.9997 for fluoride determination in naturally fluoride rich water and tea infusion respectively.

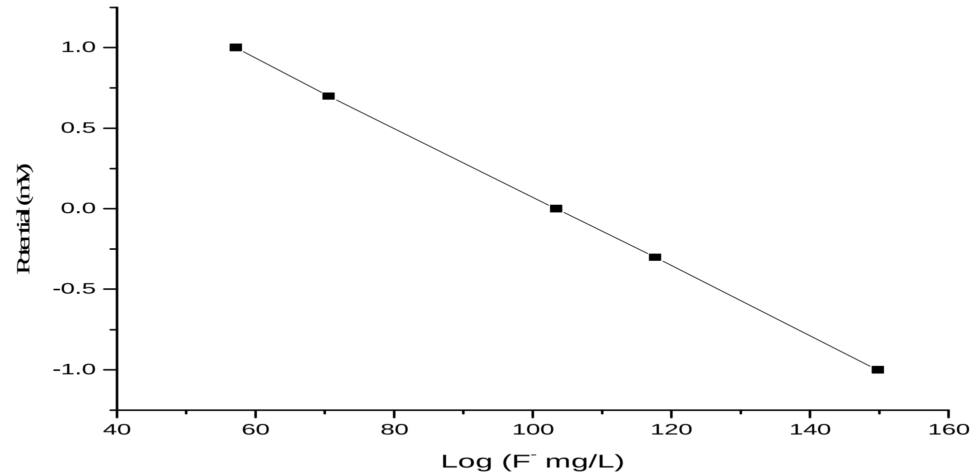

Figure 2. Calibration curve for fluoride determination in tap water by ISE.

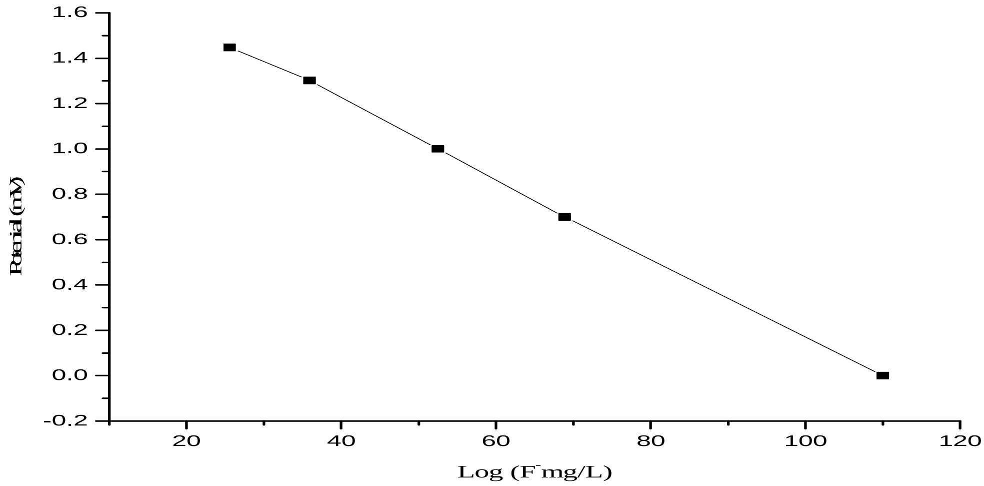

Figure 3. Calibration curve for fluoride determination in naturally fluoride rich water and tea infusion by ISE.

2.6 Determination of pH, conductivity, salinity, TDS in tea infusion, tap water and naturally fluoride rich water

The conductivity meter was calibrated with 0.01 M KCl solution (which was made by adding 0.0746 mg of KCl to 100 mL volumetric flask), after the conductivity meter was calibrated, 30 mL of tap water was added to 100 mL plastic beaker and was stirred (slightly) by magnetic stirrer. Finally conductivity probe was immersed to tap water and the conductivity, salinity and TDS were determined. By similarly manner conductivity, salinity and TDS of tea infusion and naturally fluoride rich water were determined.

The pH meter was calibrated by a buffer solution of pH of 4 and pH of 10, after the pH meter was calibrated; 30 mL of tap water was added to 100 mL plastic beaker and was stirred (slightly) by magnetic stirrer. Finally pH meter probe was immersed to tap water and the pH of tap water was determined. By similarly manner pH of tea infusion and naturally fluoride rich water were determined. All measurements were made triplicates.

3 Results and Discussions

3.1 Method validation for fluoride analysis

The analyst will be concerned with the question of precision (repeatability of results), that is, the agreement between set of results in terms of standard deviation, variance, relative standard deviation and the range of series of measurements. In this study, the precision of the results were evaluated by the standard deviation of the results of three readings of sample determination and validity were evaluated by calculating percent recovery. Thus, percent recovery was obtained by comparing the results between the fluoride found and the fluoride added.

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

where F^{-}total the sum of spiked standard and the original sample

In this study the recovery values were obtained from the nine brands of tea in tap water and deionized water to assess the validity of the method.

The procedure was as follows: 0.5 mL of 100 mg/L fluoride standard was added in to 100 mL deionized or tap water and was allowed to boil; 1 g of BBTP and 1bag of ABTB (separately) were then added. For the SBTB and LBTP, 0.6 mL of 100 mg/L fluoride standard was added into

100 mL of deionized water or tap water and was allowed to boil. But also for HGTB and FBTB, 0.7 mL of 100 mg/L standard was added into 100 mL of deionized or tap water was allowed to boil. Similarly 0.4, 0.8 and 0.9 mL of 100 mg/L fluoride standard was added into 100 mL of deionized or tap water for ABTP, EGTB and QGTB teas respectively and were allowed to boil. All teas were then infused for 3 min.

All infusions were prepared in triplicate and necessary volume adjustments due to loss during boiling were made by adding deionized water. Thus Table 2 and Table 3 shows the percent recovery were ranged from 92.5-112 and 92.9-105 respectively which indicate that the method valid.

Table 2. The recovery of the spiked standard and its precision for the method using deionized water for tea preparation.

Types of tea	[a] F^- added	[b] F^- in un-spiked tea	[c] F^- in spiked tea	% Recovery
ABTP	40	630 ± 2	667 ± 6	92.5 ± 4.6
BBTP	50	469 ± 4	520 ± 3	102 ± 2
LBTP	60	109 ± 4	165 ± 6	93.3 ± 5.5
HGTB	35	94.9 ± 1.3	134 ± 4	112 ± 8
EGTB	40	79.0 ± 4.0	118 ± 4	97.5 ± 2.5
QGTB	45	64.5 ± 2.5	112 ± 4	106 ± 6
ABTB	25	276 ± 2	302 ± 1	104 ± 2
FBTB	35	61.5 ± 8.5	96.5 ± 1.5	100 ± 4
SBTB	30	47.9 ± 8.5	80.0 ± 1.5	107 ± 7

[a] fluoride standard added to tea infusion (µg/g).

[b] average value sample of 3 measurements (µg/g) before standard addition.

[c] average value sample of 3 measurements (µg/g) after standard addition.

Table 3. The recovery of the spiked standard and its precision for the method using tap water for tea preparation.

Types of tea	[a] F^- added	[b] F^- in un-spiked tea	[c] F^- in spiked tea	% Recovery
ABTP	40	685 ± 16	724 ± 12	96.7 ± 26.7
BBTP	50	479 ± 29	532 ± 16	105 ± 25
LBTP	60	123 ± 10	178 ± 14	92.9 ± 16.4
HGTB	35	102 ± 8	138 ± 12	103 ± 18
EGTB	40	84.5 ± 3.8	125 ± 2	101 ± 8
QGTB	45	65.5 ± 7.0	110 ± 2	98.9 ± 9.7
ABTB	25	290 ± 13	314 ± 2	96.0 ± 20.1
FBTB	35	69.5 ± 8.0	105 ± 1	101 ± 5
SBTB	30	51.5 ± 8.0	81.5 ± 1	100 ± 3

[a] fluoride standard added to tea infusion (μg/g).

[b] average value sample of 3 measurements (μg/g) before standard addition.

[c] average value sample of 3 measurements (μg/g) after standard addition.

3.2 Amount of fluoride, conductivity, pH, salinity and TDS in tea infusions brewed for 3, 5 and 10 min in different water

The fluoride concentration of the tea liquor prepared in all types of water used in this study ranged from 44-2057 mg/kg, 49.1-2040 mg/kg, and 50.9-1807 mg/kg, for 3, 5, and 10 min brewing time, respectively. The conductivity, pH, salinity and total dissolved substance ranged, 384-2360 μs, 4.84-8.52, 0.2-1.2 %o and 182-1180 mg/L for 3 min brewing time, 402-2377 μs, 4.94-8.3, 0.2-1.2 %o and 190-1180 mg/L for 5 min brewing time and 396-2140 μs, 4.9-8.3, 0.2-1.2 %o and 186-1142 mg/L for 10 min brewing time, respectively. The ranges included all brands infused in all type of naturally fluoride rich water including deionized water used in this study.

The highest level of fluoride was found in Black Lion tea powder with mean fluoride concentration 2057 mg/kg and 2020 mg/kg for 3 and 5 min infusion, respectively, while for 10 min infusion Abyssinia black tea powder was the highest with mean fluoride concentration 1807 mg/kg in which Koka water was used for tea liquor preparation in both tea brands. Similarly the highest level of TDS, conductivity and salinity were found in Strawberry black tea bag with mean TDS (1179 mg/L, 1180 mg/L and 1142 mg/L), conductivity (2360 µs, 2377 µs and 2140 µs) and salinity (1.2 %o, 1.2 %o, and 1.2 %o) for 3, 5, and 10 min infusion in which Abonsa water was used for tea liquor preparation. Whereas the lowest concentration of fluoride was found in Strawberry black tea bag with mean fluoride 44 mg/kg, 49.1 mg/kg and 50.6 mg/kg for 3, 5, and 10 min infusion in which deionized water was used for tea liquor preparation. But also the lowest conductivity, TDS were found in LBTP with mean value (182 µs, 186 µs and 184 µs) and (384 mg/L, 413 mg/L and 396 mg/L) using DIW for tea liquor preparation for 3, 5, and 10 min, respectively. Table 4 shows details about the ranges of fluoride concentration in (mg/kg), conductivity (µs), pH, salinity (%o) and total dissolved substance (mg/L) of all brands of tea in each different naturally fluoride rich water for 3, 5, 10 min brewing time. The variations in fluoride concentration can be explained on the basis of the leaf age, maturity and genetics of the plant, rainfall, altitude, fertilizer, and type of soi1 [50, 51].

Table 4. The ranges of fluoride concentration (mg/kg), conductivity (µs), pH, salinity (%o) and TDS (mg/L) from all brands of tea in each type of water for 3, 5, 10 min brewing time.

	Infusion time (min)	Type of water used						
		DIW	TAW	MEW	ABW	ALW	WOW	KOW
Fluoride (mg/kg)	3	44.0-629	50.8-683	92.7-603	175-692	247-840	320-881	783-2057
	5	49.1-632	52.3-745	94.7-583	176-663	248-812	330-875	775-2020
	10	50.6-681	59.2-792	96.7-559	181-662	254-721	331-782	757-1807
EC (µs)	3	384-857	438-903	907-1629	1508-2360	620-1356	956-1869	1023-1869
	5	402-880	436-906	791-1688	1477-2377	715-1335	1109-1911	1039-1738

	10	396-915	414-957	880-1638	1445-2140	654-1367	1063-1886	954-1772
pH	3	4.84-5.72	4.83-5.97	5.87-7.13	6.29-8.52	6.35-8.23	6.04-7.57	6.46-7.63
	5	5.00-5.73	4.94-5.65	6.17-7.24	6.46-8.30	5.67-7.95	6.17-7.55	6.25-7.48
	10	4.9-6.13	5.04-5.70	5.60-7.33	5.70-8.30	5.89-7.77	5.80-7.53	5.90-7.56
Salinity (%o)	3	0.2-0.4	0.2-0.4	0.5-0.8	0.8-1.2	0.4-0.7	0.5-0.9	0.5-0.9
	5	0.2-0.4	0.2-0.4	0.4-0.8	0.7-1.2	0.3-0.7	0.5-1.0	0.5–0.9
	10	0.2-0.4	0.2-0.5	0.4-0.8	0.7-1.2	0.3-0.7	0.5-0.9	0.5-0.9
TDS (mg/L)	3	182-416	208-438	436-799	108-1179	298-660	456-921	490-866
	5	190-424	207-417	380-829	110-1180	341-651	522-944	500-853
	10	186-442	196-455	426-803	106-1142	314-667	515-932	459-872

The fluoride, conductivity, pH, salinity and total dissolved substance of the infusions prepared from different tea brands in deionized water, tap water and naturally fluoride rich water after 3, 5 and 10 min brewing time are listed in Tables 5-9.

Table 5. Fluoride level (mg/kg x ± SD) of tea infusion prepared in deionized water, tap water and different naturally fluoride rich water for 3, 5, and 10 min infusion.

Type of tea	Infusion time (min)	DIW (F^- = not measurable)	TAW (F^- = 0.254 mg/L)	MEW (F^- = 2.46 mg/L)	ABW (F^- = 6.00 mg/L)	ALW (F^- = 10.1 mg/L)	WOW (F^- = 12.5 mg/L)	KOW (F^- = 30.2 mg/L)
ABTP	3	629 ± 12	683 ± 15	603 ± 4	692 ± 10	840 ± 9	881 ± 9	2040 ± 20
	5	632 ± 8	745 ± 27	583 ± 12	663 ± 13	812 ± 24	875 ± 13	2017 ± 15
	10	681 ± 22	792 ± 60	559 ± 8	662 ± 5	720 ± 14	782 ± 11	1807 ± 12
BBTP	3	469 ± 9	476 ± 11	481 ± 5	679 ± 10	799 ± 4	857 ± 32	2057 ± 10
	5	517 ± 22	568 ± 19	479 ± 5	572 ± 10	783 ± 11	840 ± 10	2020 ± 31
	10	522 ± 14	577 ± 10	459 ± 4	568 ± 8	721 ± 11	770 ± 20	1770 ± 10
LBTP	3	108 ± 6	122 ± 1	170 ± 2	333 ± 8	531 ± 9	617 ± 10	1690 ± 20
	5	117 ± 1	126 ± 4	167 ± 3	327 ± 6	522 ± 16	616 ± 6	1667 ± 15

	10	124 ± 1	146 ± 1	146 ± 2	308 ± 5	491 ± 10	564 ± 8	1570 ± 17
HG TB	3	94.7 ± 3.0	101 ± 3	100 ± 1	206 ± 3	260 ± 4	388 ± 5	855 ± 10
	5	98.0 ± 3.0	105 ± 6	119 ± 1	207 ± 2	266 ± 4	381± 3	822 ± 10
	10	99.0 ± 1.0	105 ± 4	126 ± 1	215 ± 3	278 ± 2	359 ± 3	778 ± 6
QG TB	3	62.0 ± 2.0	65.0 ± 1.0	138 ± 2	248 ± 3	297 ± 2	393 ± 2	887± 16
	5	64.0 ±1.0	75.0 ± 2.0	144 ± 2	262 ± 4	331 ± 3	359 ± 3	857 ± 4
	10	69.0 ± 1.0	76.0 ± 1.0	157 ± 3	271 ± 3	336 ± 3	340 ± 5	827 ± 15
EGT B	3	78.0 ± 1.0	84.0 ± 2.0	144 ± 2	222 ± 2	319 ± 4	385 ± 5	905 ± 5
	5	82.5 ± 0.9	88.3 ± 2.3	152 ± 2	224 ± 2	320 ± 5	376 ± 2	890 ± 11
	10	86.5 ± 0.9	92.2 ± 1.3	168 ± 2	231 ± 7	325 ± 3	372 ± 6	887 ± 10
AB TB	3	274 ± 1	288 ± 6	277 ± 4	342 ± 4	424 ± 4	434 ± 7	1067 ± 6
	5	283 ± 8	292 ± 8	264 ± 5	338 ± 3	471 ± 5	473 ± 9	1018 ± 3
	10	284 ± 6	294 ± 7	264 ± 4	339 ± 3	486 ± 5	489 ± 6	1002 ± 24
FBT B	3	61.0 ± 1.5	68.5 ± 1.5	92.7 ± 2.4	175 ± 3	247 ± 3	320 ± 4	783 ± 8
	5	61.5 ± 0.7	70.0 ± 1.8	94.7 ± 1.8	176 ± 2	248 ± 2	330 ± 4	775 ± 10
	10	61.0 ± 0.9	73.3 ± 1.3	96.7 ± 1.8	181 ± 4	254 ± 3	331 ± 4	757 ± 10
SBT B	3	44.0 ± 0.4	50.8 ± 1.5	120 ± 4	220 ± 3	304 ± 4	361 ± 3	875 ± 20
	5	49.1 ± 0.6	52.3 ± 2.3	128 ± 2	231 ± 2	314 ± 3	378 ± 3	872 ± 10
	10	50.6 ± 2.5	59.2 ± 1.8	130 ± 3	238 ± 5	327 ± 3	380 ± 2	872 ± 12

Table 6. Total dissolved solids (TDS), (mg/L x ± SD) of tea infusion prepared in deionized water, tap water and different naturally fluoride rich water for 3, 5 and 10 min infusion.

Type of tea	Infusion time (min)	DIW (2 mg/L)	TAW (48.7 mg/L)	MEW (478 mg/L)	ABW (838 mg/L)	ALW (273 mg/L)	WOW (616 mg/L)	KOW (480 mg/L)
ABTP	3	190 ± 4	212 ± 4	453 ± 3	795 ± 5	354 ± 5	516 ± 7	645 ± 6
	5	196 ± 5	208 ± 5	380 ± 3	733 ± 3	342 ± 4	562 ± 7	667 ± 15
	10	202 ± 5	198 ± 5	438 ± 3	692 ± 7	336 ± 5	515 ± 7	639 ± 6

BBTP	3	211 ± 11	208 ± 6	454 ± 4	758 ± 6	324 ± 4	456 ± 11	490 ± 5
	5	204 ± 4	207 ± 4	434 ± 5	723 ± 5	341 ± 3	537 ± 5	544 ± 4
	10	196 ± 5	203 ± 3	431 ± 5	696 ± 6	314 ± 4	568 ± 8	459 ± 6
LBTP	3	182 ± 3	211 ± 5	436 ± 6	738 ± 6	298 ± 4	462 ± 5	513 ± 3
	5	184 ± 3	213 ± 4	450 ± 5	733 ± 7	357 ± 5	522 ± 6	518 ± 4
	10	186 ± 6	196 ± 5	424 ± 4	722 ± 5	351 ± 4	579 ± 5	481 ± 2
HGTB	3	262 ± 5	241 ± 5	525 ± 5	827 ± 13	402 ± 2	655 ± 11	542 ± 2
	5	253 ± 4	249 ± 6	566 ± 7	847 ± 4	407 ± 1	666 ± 8	512 ± 6
	10	236 ± 5	246 ± 9	521 ± 4	838 ± 6	403 ± 1	646 ± 7	554 ± 12
QGTB	3	396 ± 6	408 ± 4	568 ± 8	1087 ± 6	568 ± 8	780 ± 6	859 ± 21
	5	393 ± 8	417 ± 7	629 ± 7	1108 ± 19	630 ± 7	797 ± 7	866 ± 8
	10	420 ± 4	406 ± 7	601 ± 8	1063 ± 6	601 ± 8	884 ± 10	872 ± 9
EGTB	3	372 ± 5	387 ± 6	569 ± 3	808 ± 5	515 ± 2	671 ± 4	553 ± 3
	5	386 ± 6	380 ± 5	601 ± 4	835 ± 9	481 ± 7	672 ± 2	627 ± 6
	10	403 ± 6	371 ± 7	644 ± 6	877 ± 3	474 ± 6	730 ± 2	636 ± 5
ABB	3	408 ± 8	390 ± 5	615 ± 5	865 ± 6	517 ± 2	690 ± 1	656 ± 1
	5	405 ± 7	439 ± 9	627 ± 7	952 ± 1	546 ± 1	747 ± 2	500 ± 7
	10	380 ± 5	344 ± 6	632 ± 5	944 ± 16	542 ± 2	722 ± 2	459 ± 4
FBTB	3	336 ± 6	354 ± 8	615 ± 5	876 ± 6	455 ± 5	662 ± 9	640 ± 5
	5	329 ± 5	308 ± 6	580 ± 5	854 ± 5	472 ± 1	675 ± 1	608 ± 9
	10	335 ± 5	341 ± 6	598 ± 8	873 ± 4	467 ± 6	687 ± 9	590 ± 6
SBTB	3	416 ± 6	435 ± 5	799 ± 9	1179 ± 17	660 ± 4	921 ± 11	866 ± 9
	5	424 ± 4	417 ± 5	829 ± 9	1180 ± 10	651 ± 7	944 ± 10	853 ± 7
	10	442 ± 7	455 ± 7	803 ± 11	1142 ± 8	667 ± 5	932 ± 11	861 ± 10

Table 7. pH (x ± SD) of tea infusion prepared in deionized water, tap water and different naturally fluoride rich water for 3, and 10 min infusion.

Type of tea	Infusion time (min)	DIW (5.96)	TAW (7.73)	MEW (8.42)	ABW (8.59)	ALW (8.34)	WOW (8.18)	KOW (8.42)
AB TP	3	5.53 ± 0.03	5.22 ± 0.03	7.05 ± 0.01	7.89 ± 0.03	7.22 ± 0.04	7.43 ± 0.03	7.10 ± 0.05
	5	5.50 ± 0.02	5.17 ± 0.02	6.91 ± 0.01	7.95 ± 0.02	7.03 ± 0.05	7.55 ± 0.05	6.82 ± 0.08
	10	5.44 ± 0.18	5.15 ± 0.03	7.33 ± 0.06	8.06 ± 0.04	7.70 ± 0.04	7.18 ± 0.03	7.56 ± 0.06
BB TP	3	5.72 ± 0.04	5.42 ± 0.05	6.32 ± 0.05	8.52 ± 0.02	6.97 ± 0.03	7.24 ± 0.02	6.46 ± 0.03
	5	5.73 ± 0.05	5.32 ± 00.4	6.18 ± 0.03	8.30 ± 0.02	6.81 ± 0.02	7.31 ± 0.03	6.89 ± 0.06
	10	5.42 ± 0.38	5.06 ± 0.05	6.61 ± 0.01	8.20 ± 0.03	6.67 ± 0.03	7.53 ± 0.05	7.01 ± 0.02
LB TP	3	4.92 ± 0.01	5.54 ± 0.04	7.03 ± 0.07	8.20 ± 0.03	7.10 ± 0.02	6.75 ± 0.05	7.63 ± 0.03
	5	5.00 ± 0.01	5.32 ± 0.03	7.24 ± 0.06	8.10 ± 0.04	7.19 ± 0.01	7.24 ± 0.03	7.48 ± 0.03
	10	4.90 ± 0.03	5.45 ± 0.08	6.97 ± 0.02	8.30 ± 0.01	6.61 ± 0.01	7.34 ± 0.04	7.29 ± 0.05
H GT B	3	5.32 ± 0.04	5.53 ± 0.04	6.86 ± 0.03	8.01 ± 0.06	6.62 ± 0.06	7.57 ± 0.07	7.47 ± 0.06
	5	5.21 ± 0.03	5.29 ± 0.04	7.06 ± 0.01	7.95 ± 0.03	6.41 ± 0.04	7.37 ±0.07	7.46 ± 0.02
	10	5.24 ± 0.04	5.70 ± 0.02	7.13 ± 0.01	7.93 ± 0.02	6.35 ± 0.05	7.33 ± 0.02	7.14 ± 0.06
Q GT B	3	5.04 ± 0.03	5.11 ± 0.02	6.31 ± 0.43	7.88 ± 0.08	6.50 ± 0.02	6.07 ± 0.05	6.01 ± 0.02
	5	5.25 ± 0.12	4.94 ± 0.04	6.38 ± 3.80	6.46 ± 0.06	5.67 ± 0.04	6.17 ± 0.04	6.25 ± 0.06
	10	5.10 ± 0.03	5.04 ± 0.04	6.36 ± 0.16	7.76 ± 0.02	5.89 ±0.05	5.80 ± 0.04	5.90 ± 0.03
EG TB	3	5.20± 0.01	5.97 ± 0.02	6.68 ± 0.02	7.95 ± 0.02	6.39 ± 0.05	7.35 ± 0.06	7.39 ± 0.03
	5	5.25 ± 0.12	5.54 ± 0.02	6.28 ± 0.01	7.57 ± 0.27	6.55 ± 0.05	7.19 ± 0.04	7.33 ± 0.03
	10	6.13 ± 0.01	5.63 ± 0.03	7.07 ± 0.02	7.46 ± 0.34	6.69 ± 0.03	6.29 ± 0.05	7.23 ± 0.03
AB TB	3	4.84 ± 0.06	5.20 ± 0.04	5.87 ± 0.01	6.29 ± 0.03	8.23 ± 0.03	7.17 ± 0.08	7.09 ± 0.03
	5	5.04 ± 0.04	5.22 ± 0.05	6.58 ± 0.01	5.89 ± 0.04	7.95 ± 0.06	6.90 ± 0.06	7.40 ± 0.05

	10	5.00 ± 0.03	5.10 ± 0.04	5.60 ± 0.02	5.70 ± 0.01	7.77 ± 0.08	7.02 ± 0.03	7.60 ± 0.03
FB TB	3	5.20 ± 0.02	5.20 ± 0.03	6.23 ± 0.01	7.57 ± 0.03	7.20 ± 0.05	6.59 ± 0.05	6.84 ± 0.04
	5	5.38 ± 0.03	5.43 ± 0.04	6.67 ± 0.02	7.62 ± 0.01	7.22 ± 0.07	6.21 ± 0.04	6.86 ± 0.03
	10	5.04 ± 0.04	5.10 ± 0.04	6.20 ± 0.03	7.43 ± 0.02	7.14 ± 0.07	7.03 ± 0.07	6.74 ± 0.03
SB TB	3	4.95 ± 0.05	4.83 ± 0.06	6.07 ± 0.05	7.05 ± 0.08	6.21 ± 0.04	6.04 ± 0.04	7.13 ± 0.02
	5	5.68 ± 0.87	5.65 ± 0.07	6.17 ± 0.04	7.37 ± 0.06	6.12 ± 0.02	6.38 ± 0.38	7.03 ± 0.04
	10	5.55 ± 0.05	5.44 ± 0.04	5.80 ± 0.04	7.81 ± 0.02	6.24 ± 0.03	6.14 ± 0.03	7.24 ± 0.04

Table 8. Conductivity (μs/cm x ± SD) of tea infusion prepared in deionized water, tap water and different naturally fluoride rich water for 3, 5 and 10 min infusion.

Type of tea	Infusion time (min)	DIW (2.27 μs/cm)	TAW (102 μs/cm)	MEW (987 μs/cm)	ABW (1706 μs/cm)	ALW (573 μs/cm)	WOW (1264 μs/cm)	KOW (992 μs/cm)
ABTP	3	402 ± 1	438 ± 29	940 ± 4	1623 ± 8	741 ± 5	1070 ± 10	1322 ± 12
	5	413 ± 9	438 ± 7	791 ± 6	1497 ± 6	733 ± 30	1158 ± 14	1361 ± 28
	10	426 ± 6	418 ± 6	904 ± 7	1415 ± 14	702 ± 4	1063 ± 12	1312 ± 9
BBTP	3	432 ± 6	443 ± 6	938 ± 8	1547 ± 7	676 ± 6	959 ± 9	1023 ± 5
	5	430 ± 5	436 ± 6	902 ± 2	1477 ± 10	715 ± 5	1109 ± 8	1124 ± 3
	10	407 ± 9	433 ± 7	893 ± 8	1425 ± 9	654 ± 6	1172 ± 11	954 ± 4
LBTP	3	384 ± 8	445 ± 4	907 ± 6	1508 ± 8	620 ± 11	956 ± 9	1059 ± 7
	5	388 ± 4	446 ± 4	933 ± 5	1499 ± 12	745 ± 6	1093 ± 12	1068 ± 11
	10	396 ± 1	414 ± 6	880 ± 7	1477 ± 9	732 ± 7	1195 ± 15	999 ± 2
HGTB	3	552 ± 1	507 ± 6	1082 ± 6	1684 ± 24	836 ± 3	1354 ± 13	1118 ± 12
	5	531 ± 4	526 ± 8	1168 ± 10	1726 ± 7	837 ± 3	1372 ± 8	1121 ±1 3
	10	496 ± 6	520 ± 13	1074 ± 9	1712 ± 4	835 ± 1	1319 ± 10	1145 ± 18
QGTB	3	829 ± 5	845 ± 6	1173 ± 11	2180 ± 20	1173 ± 11	1591 ± 11	1760 ± 22

	5	819 ± 10	863 ± 13	1288 ± 13	2233 ± 35	1288 ± 13	1624 ± 12	1762 ± 15
	10	869 ± 7	847 ± 7	1237 ± 12	2140 ± 10	1237 ± 12	1797 ± 20	1772 ± 18
EGTB	3	841 ± 6	802 ± 8	1171 ± 11	1650 ± 3	1063 ± 6	1371 ± 4	1143 ± 3
	5	802 ± 9	794 ± 4	1250 ± 3	1694 ± 6	1028 ± 15	1379 ± 3	1290 ± 5
	10	774 ± 6	775 ± 14	1322 ± 11	1775 ± 3	981 ± 10	1494 ± 2	1313 ± 15
ABTB	3	850 ± 10	810 ± 9	1165 ± 7	1763 ± 5	1067 ± 1	1414 ± 3	1146 ± 12
	5	841 ± 11	906 ± 9	1291 ± 6	1924 ± 4	1125 ± 1	1533 ± 17	1039 ± 14
	10	792 ± 7	716 ± 8	1298 ± 7	1936 ± 16	1117 ± 6	1477 ± 3	950 ± 7
FBTB	3	705 ± 5	739 ± 6	1262 ± 8	1781 ± 7	940 ± 8	1404 ± 6	1316 ± 62
	5	688 ± 7	711 ± 12	1194 ± 7	1765 ± 6	983 ±10	1382 ± 17	1252 ± 17
	10	698 ± 8	720 ± 9	1230 ± 11	1780 ± 4	966 ± 13	1427 ± 38	1217 ± 15
SBTB	3	857 ± 12	903 ± 7	1629 ± 19	2360 ± 20	1356 ± 16	1869 ± 21	1869 ± 21
	5	880 ± 8	861 ± 10	1688 ± 19	2377 ± 25	1335 ± 14	1911 ± 24	1738 ± 15
	10	915 ± 13	957 ± 11	1638 ± 21	2140 ± 10	1367 ± 11	1886 ± 16	1754 ± 19

Table 9.Salinity (% o) of tea infusion prepared in deionized water, tap water and different naturally fluoride rich water for 3, 5 and 10 min infusion (*).

Type of tea	Infusion time (min)	DIW (0 % o)	TAW (0 % o)	MEW (0.5 % o)	ABW (0.9 % o)	ALW (0.3 % o)	WOW (0.6 % o)	KOW (0.5 % o)
ABTP	3	0.2	0.2	0.5	0.8	0.4	0.5	0.7
	5	0.2	0.2	0.4	0.7	0.4	0.6	0.7
	10	0.2	0.2	0.5	0.7	0.4	0.5	0.7
BBTP	3	0.2	0.2	0.5	0.8	0.3	0.5	0.5
	5	0.2	0.2	0.4	0.7	0.3	0.5	0.6
	10	0.2	0.2	0.4	0.7	0.3	0.6	0.5

LBTP	3	0.2	0.2	0.5	0.8	0.3	0.5	0.5
	5	0.2	0.2	0.5	0.8	0.4	0.5	0.5
	10	0.2	0.2	0.4	0.7	0.4	0.6	0.5
HGTB	3	0.3	0.3	0.5	0.8	0.4	0.7	0.5
	5	0.3	0.3	0.6	0.9	0.4	0.7	0.5
	10	0.3	0.3	0.5	0.9	0.4	0.7	0.5
QGTB	3	0.4	0.4	0.6	1.1	0.6	0.8	0.9
	5	0.4	0.4	0.6	1.2	0.6	0.8	0.9
	10	0.4	0.4	0.6	1.1	0.6	0.9	0.9
EGTB	3	0.4	0.4	0.6	0.8	0.5	0.7	0.6
	5	0.4	0.4	0.6	0.8	0.5	0.7	0.6
	10	0.4	0.4	0.7	0.9	0.5	0.7	0.6
ABTB	3	0.4	0.4	0.6	0.9	0.5	0.7	0.6
	5	0.4	0.4	0.6	1.0	0.5	0.8	0.5
	10	0.4	0.3	0.6	1.0	0.5	0.7	0.5
FBTB	3	0.3	0.4	0.6	0.9	0.5	0.7	0.6
	5	0.3	0.4	0.6	0.9	0.5	0.7	0.6
	10	0.3	0.4	0.6	0.9	0.5	0.7	0.6
SBTB	3	0.4	0.4	0.8	1.2	0.7	0.9	0.9
	5	0.4	0.4	0.8	1.2	0.7	1.0	0.9
	10	0.4	0.5	0.8	1.2	0.7	0.9	0.9

(*) SD is very small (close to zero).

3.3 Comparison of fluoride concentration among the different tea brand infusions in different types of water

The fluoride concentrations of nine different brands of tea varied one brand to the other; this might be due to type of tea plant, fertilizer, soil type, age of leaves, rain fall and quality of water for irrigation and harvesting time [28-31, 48, 49].

The fluoride concentration of teas for 3, 5, and 10 min brewing time from higher to lower follow the following scheme in deionized water, tap water, Mekie, Abonsa, Alemtena, Wonji shoa and Koka water.

ABTP > BBTP > ABTB > LBTP > HGTB > EGTB > QGTB > FBTB > SBTB in DIW.

ABTP > BBTP > ABTB > LBTP > HGTB > EGTB > FBTB > QGTB > SBTB in TAW.

ABTP > BBTP > ABTB > LBTP > EGTB > QGTB > SBTP > HGBTB > FBTB in MEW. ABTP > BBTP > ABTB > LBTP > QGTB > EGTB > SBTB > HGTB > FBTB in ABW.

ABTP > BBTP > ABTB > LBTP > EGTB > SBTB > QGTB > HGTB > FBTB in ALW.

ABTP > BBTP > ABTB > LBTP > QGTB > HGTB > EGTB > SBTB > FBTB in WOW and BBTP > ABTP > LBTP > ABTB > EGTB > QGTB > SBTB > HGTB >FBTB in KOW for 3 min brewing time.

ABTP > BBTP > ABTB > LBTP > HGTB > EGTB > QGTB > FBTB > SBTB in DEW.

ABTP > BBTP > ABTB > LBTP > HGTB > EGTB > QGTB > FBTB > SBTB in tap water. ABTP > BBTP > ABTB > LBTP > EGTB > QGTB > SBTB > HGTB > FBTB in Mekie water. ABTP > BBTP > ABTB > LBTP > QGTB > SBTB > EGTB > HGTB > FBTB in Abonsa water. ABTP > BBTP > LBTP > ABTB > QGTB > EGTB > HGTB > SBTB > FBTB in Alemtena water.

ABTP > BBTP > LBTP > ABTB > HGTB > SBTB > EGTB > QGTB > FBTB in Wonji shoa and BBTP > ABTP > LBTP > ABTB > EGTB > SBTB > QGTB > HGTB > FBTB in Koka water for 5 min brewing time.

ABTP > BBTP > ABTB > LBTP > HGTB > EGTB > QGTB > FBTB > SBTB in deionized water.

ABTP > BBTP > ABTB > LBTP > HGTB > EGTB > QGTB > FBTB > SBTB in tap water. ABTP > BBTP > ABTB > EGTB > QGTB > LBTP > SBTB > HGTB > FBTB in Mekie water. ABTP > BBTP > ABTB > LBTP > QGTB > SBTB > EGTB > HGTB > FBTB in Abonsa water. BBTP > ABTP > LBTP > ABTB > QGTB > SBTB > EGTB > HGTB > FBTB in Alemtena water.

ABTP > BBTP > LBTP > ABTB > SBTB > EGTB > HGTB > QGTB > FBTB in Wonji shoa water and ABTP > BBTP > LBTP > ABTB > EGTB > SBTB > QGTB > HGTB > FBTB in Koka water for 10 min brewing time.

But generally the fluoride concentration of each brand of tea increased as the fluoride concentration of the water used for tea preparation was increased. Figures 3, 4 and 5 show the amount of fluoride concentration of different brands of tea in different types of naturally fluoride rich water for 3, 5, and 10 min brewing time separately.

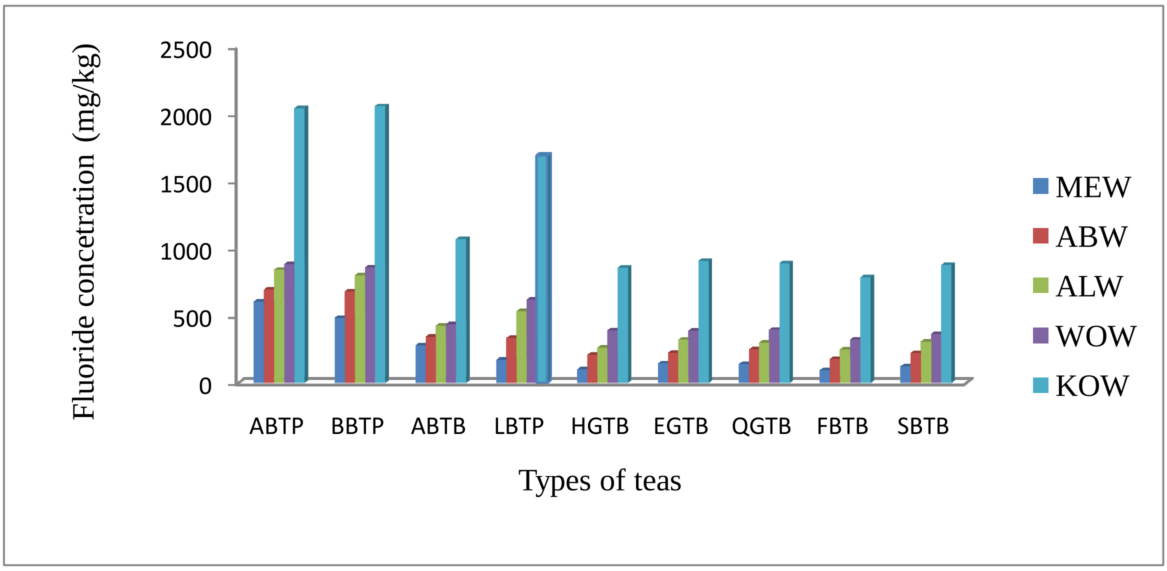

Figure 4. Fluoride level of ABTP, BBTP, ABTB, LBTP, HGTB, EGTB, QGTB, FBTB and SBTB in different types of naturally fluoride rich water for 3 min infusion.

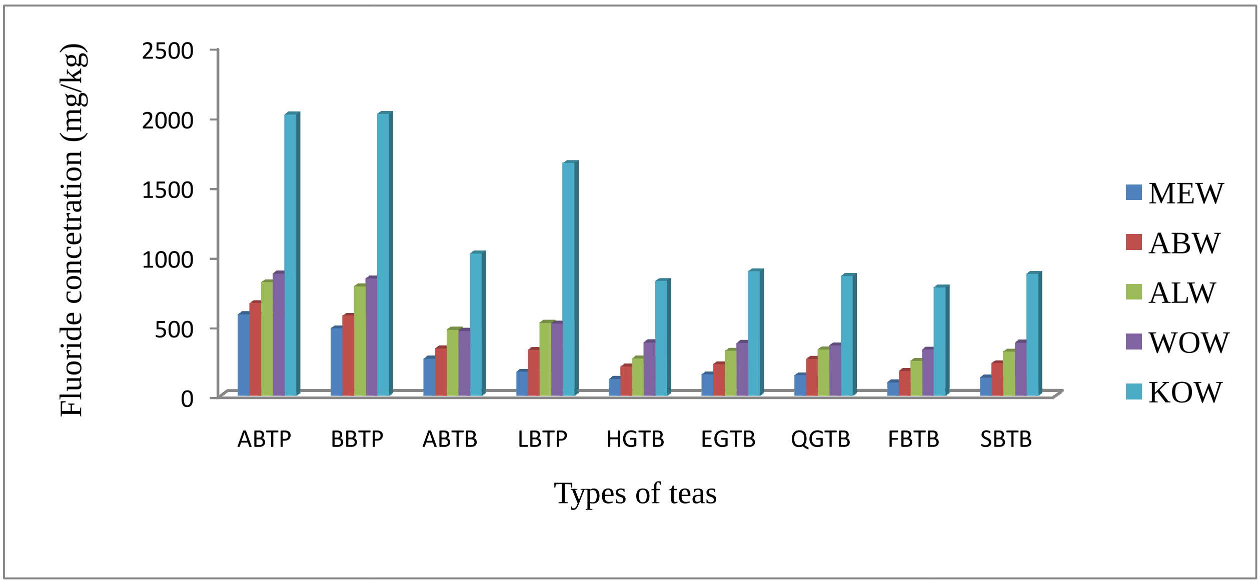

Figure 5. Fluoride level of ABTP, BBTP, ABTB, LBTP, HGTB, EGTB, QGTB, FBTB and SBTB in different types of naturally fluoride rich water for 5 min infusion.

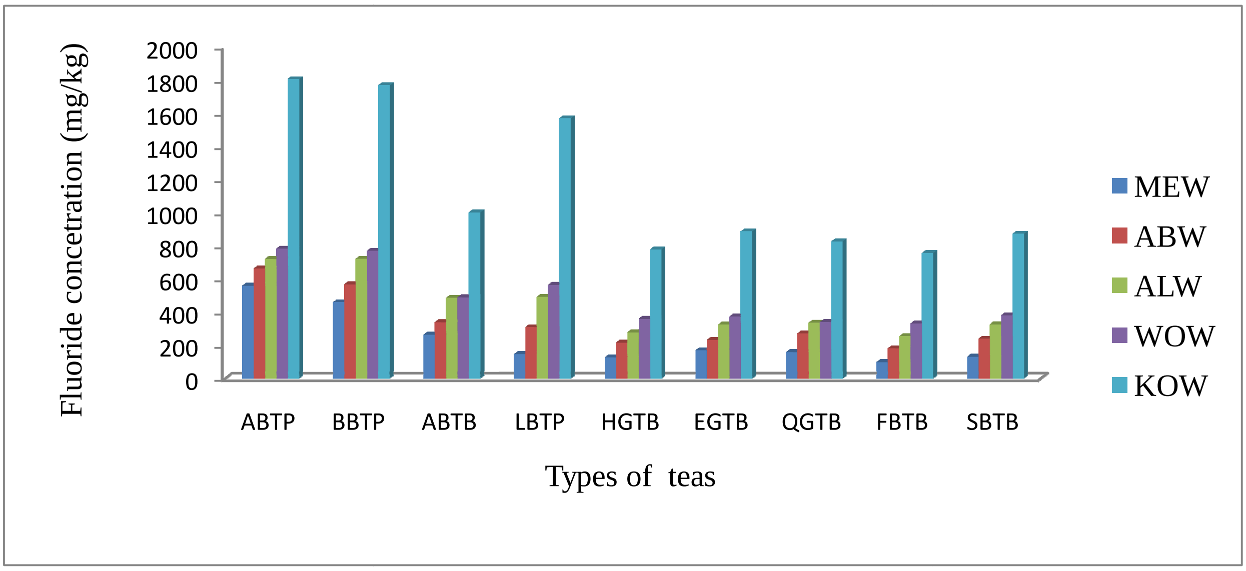

Figure 6. Fluoride level of ABTP, BBTP, ABTB, LBTP, HGTB, EGTB, QGTB, FBTB and SBTB in different types of naturally fluoride rich water for 10 min infusion.

3.4 Conductivity, TDS and salinity of different types of water

Total dissolved solids (TDS): this is also referred to as “filterable residue” and is the concentration of dissolved substances in water, and includes mineral and organic matter, whether or not this is in ionic form [59]. This term therefore also includes SiO_2 and is expressed in mg/L. Conductivity: this is the ability of a solution to conduct an electric current. It is not only dependent on the concentration of dissociated salts and dissolved gases [59], but also on colloidal suspensions. Consequently, conductivity is affected by temperature, pressure and rate of flow, but it is not affected by dissolved silica or undissociated ions such as H_2CO_3 which do not carry an electric charge. Conductivity for most natural waters is measured in $mScm^{-1}$. Total dissolved ions (TDI) are the total number of ions in solution, whether they are dissociated or not. It is defined by [59] as the sum of the major ions in the water expressed in $mgL^{-1.}$ In most surface waters these include the cations Na^+, K^+, Ca^{2+}, and Mg^{2+}, and the anions Cl^- and $HCO_3{}^-$, with $SO_4{}^{2-}$ occasionally being significant, Total Soluble Salts (TSS) can be considered the same as TDI [59].

According to WHO, there is no health based limit for TDS in drinking water, as TDS occurs in drinking water at concentrations well below toxic effects may occur, but the palatability of water with TDS level of less than 500 mg/L is generally considered to be good. Drinking water becomes significantly and increasingly unpalatable at TDS levels greater than about 1000 mg/L. TDS greater than 1200 mg/L may be objectionable to consumers and could have impacts for those who need to limit their daily salt intake, e.g. Severely hypertensive, diabetic, and renal dialysis patients [59].

Electrical conductivity (EC) which is a measure of water’s ability to conduct an electric current is related to the amount of dissolved minerals in water, but it does not give an indication of which element is present but higher value of EC is a good indicator of the presence of ions such as sodium, potassium, chloride or sulfate [59].

Abonsa water was found to have the highest conductivity, salinity and TDS. As expected the lowest conductivity, salinity and TDS were measured in deionized water. However, some brands of tea did not followed any defined trend in the conductivity, salinity and TDS of different brands of tea prepared in deionized water, tap water and different naturally fluoride rich water. This may be due to tea leaves may absorb or release metal ions which leads to conductivity, salinity and TDS of tea decrease or increase.

The conductivity, salinity and TDS of different water sampled from different places in Ethiopian Rift Valley are shown in Figures 5 and 6.

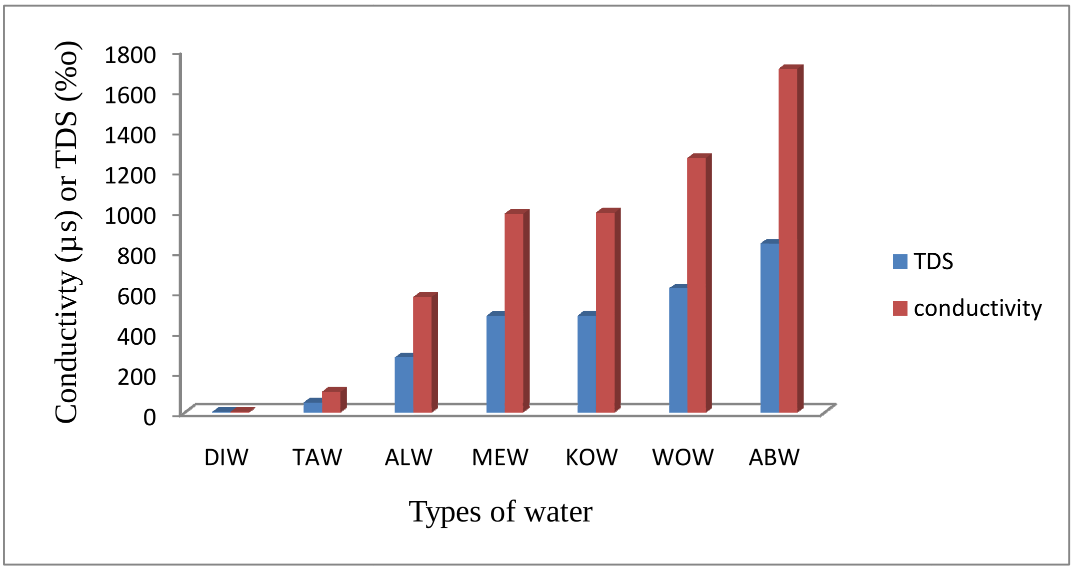

Figure 7. Conductivity and TDS of different water.

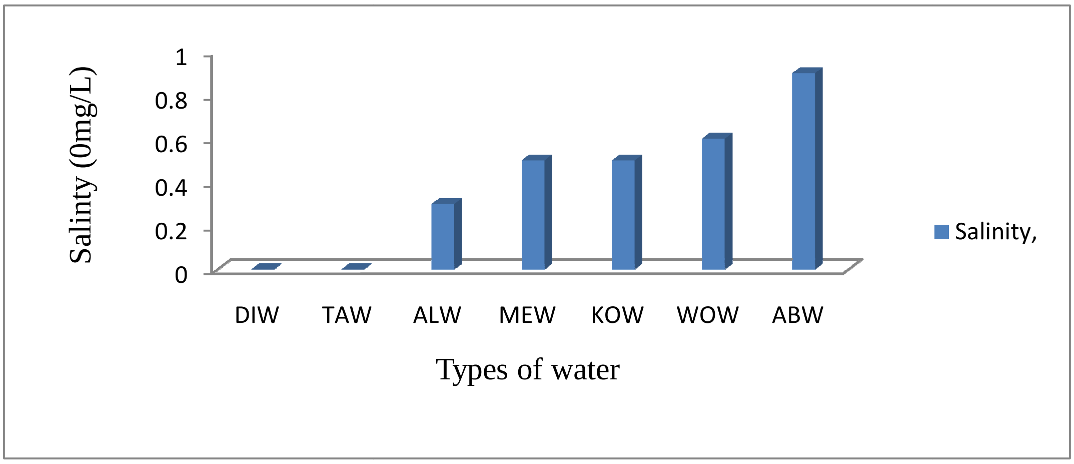

Figure 8. Salinity of different water.

3.5 Percentage of fluoride absorbed from naturally fluoride rich water by tea leaves

In low-fluoride water, fluoride is easily released from tea leaves. Depending upon the fluoride content of the water, dried tea leaves are also reported to absorb fluoride from fluoride rich water [52].

Tables 10 and 11 show the percentage of fluoride absorbed from different naturally fluoride rich water by different brands of tea in which their fluoride concentration varies. The percentages of absorbed were calculated by considering deionized water and tap water as reference.

A. When deionized water is used as reference:

$$\% \text{ Fluoride absorbed from water by tea} = \frac{\text{F total} - \text{Ft}}{\text{F total}} \text{ x } 100$$

where F total = fluoride in tea liquor prepared in deionized water + fluoride in water used for tea preparation, Ft = fluoride in tea liquor prepared in fluoride rich water.

Table 10. Percentage (% X ± SD) of fluoride absorbed from different fluoride rich water by different brand of tea leaves in which tea liquor is prepared in deionized water as a reference.

Type of tea	Infusion time (min)	% of fluoride absorbed from MEW by tea leaves	% of fluoride absorbed from ABW by tea leaves	% of fluoride absorbed from ALW by tea leaves	% of fluoride absorbed from WOW by tea leaves	% of fluoride absorbed from KOW by tea leaves
ABTP	3	31.6 ± 1.3	43.7 ± 0.8	48.7 ± 0.6	53.1 ± 0.7	44.1 ± 0.5
	5	33.8 ± 1.7	46.2 ± 0.6	50.5 ± 2.7	53.5 ± 1.1	44.8 ± 0.4
	10	39.3 ± 1.0	48.3 ± 0.3	57.4 ± 0.6	59.5 ± 0.8	51.2 ± 0.3
BBTP	3	32.6 ± 1.0	35.3 ± 1.5	49.4 ± 0.6	50.1 ± 1.5	41.0 ± 0.4
	5	37.3 ± 1.3	48.8 ± 1.1	48.7 ± 0.3	52.5 ± 0.5	42.8 ± 0.1
	10	50.2 ± 0.1	49.4 ± 0.5	52.9 ± 1.0	56.5 ± 0.9	52.1 ± 0.3
LBTP	3	51.9 ± 2.9	53.0 ± 0.7	52.5 ± 0.6	54.6 ± 0.6	46.0 ± 0.3
	5	56.0 ± 2.9	54.4 ± 2.2	53.7 ± 1.5	54.9 ± 1.2	46.9 ± 0.8

	10	84.4 ± 0.3	57.5 ± 0.3	56.7 ± 0.5	59.0 ± 0.3	50.1 ± 0.5
HGTB	3	70.7 ± 0.8	70.4 ± 0.2	76.5 ± 1.5	71.2 ± 0.3	72.6 ± 0.3
	5	65.4 ± 0.9	70.3 ± 0.2	76.0 ± 0.2	71.7 ± 0.2	73.6 ± 0.3
	10	63.3 ± 1.2	69.2 ± 0.5	74.9 ± 0.1	73.4 ± 0.2	75.0 ± 0.1
QGTB	3	55.2 ± 1.1	62.5 ± 0.1	72.3 ± 0.1	70.0 ± 1.0	71.2 ± 0.2
	5	53.5 ± 1.2	60.5 ± 0.4	69.2 ± 0.2	72.7 ± 0.1	72.2 ± 0.7
	10	50.2 ± 1.0	59.5 ± 0.3	68.9 ± 0.2	74.2 ± 0.1	73.2 ± 0.4
EGTB	3	55.5 ± 1.1	67.3 ± 0.20	70.7 ± 0.5	71.0 ± 0.6	70.8 ± 0.2
	5	53.7 ± 0.9	67.0 ± 0.2	70.7 ± 0.2	71.8 ± 0.2	71.3 ± 0.3
	10	49.3 ± 1.2	66.4 ± 0.3	70.4 ± 0.2	72.2 ± 0.3	71.5 ± 0.2
ABTB	3	46.6 ± 0.8	60.4 ± 0.6	67.0 ± 0.1	71.5 ± 0.7	67.6 ± 0.4
	5	50.1 ± 0.9	61.7 ± 0.2	63.6 ± 0.1	69.1 ± 0.2	69.2 ± 0.3
	10	50.3 ± 0.5	61.6 ± 0.3	62.1 ± 0.3	68.1 ± 0.2	69.7 ± 0.3
FBTB	3	69.7 ± 0.4	73.5 ± 0.3	76.9 ± 0.2	75.6 ± 0.7	74.6 ± 1.8
	5	69.2 ± 0.7	73.4 ± 0.3	76.9 ± 0.4	74.8 ± 0.3	74.9 ± 0.2
	10	68.7 ± 0.6	72.3 ± 0.1	76.3 ± 0.2	74.8 ±0.3	75.4 ± 0.3
SBTB	3	58.7 ± 0.4	65.8 ± 0.2	71.4 ± 0.8	72.1 ± 0.2	71.6 ± 0.4
	5	57.2 ± 1.0	64.5 ± 0.2	70.3 ± 0.2	70.9 ± 0.5	71.6 ± 0.2
	10	56.9 ± 0.7	63.5 ± 0.1	69.2 ± 0.2	70.8 ± 0.3	71.5 ± 1.8

The percentage of fluoride absorbed from naturally fluoride rich water from Mekie (2.46 mg/L), Abonsa (6.0 mg/L), Alemtena (10.1 mg/L), Wonji shoa (12.5 mg/L) and Koka (30.2 mg/L) by ABTP, BBTP and LBTP were increased with increasing brewing time to 3, 5, and 10 min. But also from Koka water (30.2 mg/L) by HGTB, EGTB, QGTB, ABTB, SBTB, FBTB teas, from Wonji shoa (12.5 mg/L) by HGTB. EGTB and QGTB were also showed similar increment. Whereas the percentage of fluoride absorbed from naturally fluoride rich water (Mekie, Abonsa and Alemtena) by HGTB. EGTB, QGTB ABTB, SBTB and FBB decreased when brewing time was increased to 3, 5, and 10 min. This is may be due to presence of higher concentrations of fluoride compelaxing metals (Fe, Al, Ca, ...) in some brands of tea leaves and infused to the tea liquor in high amount which resulting the percentage of fluoride absorbed from fluoride rich water by tea leaves decreased as brewing time increased to 3, 5, and 10 min by forming complex to free fluoride in naturally fluoride rich water. But when the fluoride concentration is so high in Koka water fluoride absorbed by tea leave increased as brewing time increased to 3, 5, and 10 min, even though fluoride complexing metals infused from tea leave to water reduce free fluoride in Koka water there is enough free fluoride absorbed by tea leaves during each brewing time. From the Table 10 and Table 11 the percentage of fluoride absorbed from naturally fluoride rich water by different brand tea leaves were differ this is may be, each brand of tea has its own maximum fluoride binding capacity depending upon presence of fluoride compelaxing metal in tea or in naturally fluoride rich water.

B. When tap water is used as reference:

$$\text{\% Fluoride absorbed from water by tea} = \frac{\text{F total} - \text{Ft}}{\text{F total}} \text{ x } 100$$

where F total = fluoride in tea liquor prepared in tap water + fluoride in water used for tea preparation, Ft = fluoride in tea liquor prepared in fluoride rich water.

Table 11. Percentage (% X ± SD) of fluoride absorbed from different naturally fluoride rich water by different brand of tea leaves in which tea liquor is prepared in tap water as a reference.

Type of tea	Infusion time (min)	% of F^- absorbed from MEW by tea leaves	% of F^- absorbed from ABW by tea leaves	% of F^- absorbed ALW by tea leaves	% of F^- absorbed WOW by tea leaves	% of F^- absorbed KOW by tea leaves
ABTP	3	35.1 ± 0.8	46.1 ± 0.1	50.4 ± 0.3	54.4 ± 0.4	44.9 ± 0.3
	5	41.2 ± 1.2	50.5 ± 1.6	53.7 ± 2.5	56.1 ± 0.7	46.2 ± 0.3
	10	46.1 ± 3.4	52.4 ± 1.8	59.8 ± 0.8	61.7 ± 1.2	52.5 ± 0.5
BBTP	3	33.4 ± 1.2	36.9 ± 0.7	46.6 ± 1.0	50.3 ± 1.6	42.2 ± 1.6
	5	41.2 ± 2.2	51.0 ± 1.3	50.4 ± 0.8	53.8 ± 0.9	42.7 ± 1.6
	10	44.2 ± 1.0	51.7 ± 0.1	54.6 ± 0.3	57.9 ± 0.7	50.8 ± 0.5

LBTP	3	53.8 ± 08	53.9 ± 0.7	53.1 ± 0.8	55.0 ± 0.4	46.2 ± 0.4
	5	55.1 ± 0.9	55.0 ± 0.3	54.0 ± 0.5	55.2 ± 0.3	47.0 ± 0.4
	10	62.8 ± 0.9	58.7 ± 0.3	57.5 ± 0.6	59.6 ± 0.4	50.7 ± 0.4
HGTB	3	71.2 ± 0.8	70.6 ± 0.2	76.6 ± 0.2	71.3 ± 0.3	71.7 ± 0.2
	5	66.1 ± 0.2	70.6 ± 0.1	76.1 ± 0.1	71.9 ± 0.2	72.8 ± 0.3
	10	64.1 ± 1.4	69.5 ± 0.6	75.1 ± 0.1	73.5 ± 0.2	74.3 ± 0.1
QGTB	3	55.6 ± 0.8	62.7 ± 0.1	72.4 ± 0.2	70.1 ± 1.1	71.2 ± 0.2
	5	55.1 ± 0.4	61.2 ± 0.3	69.5 ± 0.2	72.9 ± 0.1	72.3 ± 0.7
	10	51.2 ± 0.9	59.9 ± 0.2	69.1 ± 0.2	75.5 ± 0.1	73.3 ± 0.4
EGTB	3	56.4 ± 0.9	67.5 ± 0.2	70.8 ± 0.2	71.1 ± 0.6	70.8 ± 0.2
	5	54.5 ± 0.2	67.4 ± 0.4	64.5 ± 0.2	71.9 ± 0.1	71.4 ± 0.3
	10	50.3 ± 1.3	66.6 ± 0.4	70.5 ± 0.1	75.6 ± 0.3	71.5 ± 0.2
ABTB	3	48.1 ± 0.9	61.4 ± 0.1	67.3 ± 0.6	71.8 ± 0.7	67.7 ± 0.4
	5	50.9 ± 0.2	62.1 ± 0.2	63.9 ± 0.2	69.3 ± 0.2	69.3 ± 0.3
	10	51.1 ± 0.7	62.1 ± 0.1	62.7 ± 0.2	68.3 ± 0.2	69.8 ± 0.1
FBTB	3	70.5 ± 0.5	73.8 ± 0.2	77.1 ± 0.2	75.5 ± 0.8	74.7 ± 0.2
	5	69.9 ± 0.1	73.7 ± 0.3	77.0 ± 0.2	75.0 ± 0.3	74.9 ± 0.2
	10	69.6 ± 0.5	73.1 ± 0.1	76.5 ± 0.2	75.0 ± 0.3	75.5 ± 0.3
SBTB	3	59.6 ± 0.6	62.2 ± 0.4	71.3 ± 0.1	72.3 ± 0.2	71.5 ± 0.4
	5	57.0 ± 0.3	64.6 ± 0.3	70.4 ± 0.2	71.0 ± 0.5	71.6 ± 0.3
	10	57.4 ± 0.9	63.9 ± 0.2	69.4 ± 0.1	71.0 ± 0.3	71.7 ± 0.3

Generally the amount of fluoride absorbed from naturally fluoride rich water by different brands of tea leaves follow similar trend in the above case in which tap water was used as reference.

3.6 Fluoride intake by human and safety evaluation of the teas

Fluoride (F) is a recognized substance used worldwide to control dental caries. However, ingestion of high levels of fluoride during tooth formation and mineralization is responsible for dental fluorosis. Prolonged ingestion of high levels of fluoride, leads to corroded, pitted rusty brown teeth. The upper limit for clinically acceptable dental fluorosis is not well known, but the value of 0.05-0.07 mg F/kg/day is generally accepted as a reference [27].

Surplus of fluorides in organism can provoke teeth and skeleton fluorose. Fluorides inhibit many enzymes. Affected enzyme contains metal ion which unites with fluoride and creates metal-fluoride complex. Fluor in organism has its optimal, security-tolerant and toxic dose, which depends of person's age, weight and health. In the first year of life the optimal content of fluor is 0.045 mg/kg of body mass, tolerant 0.073 mg/kg, chronically toxic 0.150 mg/kg. Optimal dosage of fluor for adults is 0.020-0.025 mg/kg of body mass [57].

As taking of fluorides, according to World Health Organization recommendation (WHO), is limited in the range from 2 to 4 mg per day, it is necessary to give the content of fluorides on all products that are used in human consumption [57].

The Food and Nutrition Board of the Institute of Medicine (USA) 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 12 [50].

Table 12. Adequate intake levels for fluoride.

Age range	Adequate intake level (mg/day)	Adequate intake level (mg/kg/day)
0 – 6 month	0.01	0.0014
7 – 12 month	0.5	0.056
1 – 3 years	0.7	0.054
4 – 8 years	1	0.045
9 – 13 years (females or males)	2	0.05

14 – 18 years (males)	3	0.046
14 -18 years (females)	3	0.053
>18 years (males)	4	0.052
>18 years (Females)	3	0.049

In this study the fluoride level of different brands of tea in different fluoride rich water (ignoring, deionized water) ranges from 50.8-2057 mg/kg in 3 min brewing time which is mostly practiced in Ethiopia. By assuming on the average one consume two cups (200 mL) of tea every day, the daily fluoride intake only from tea without conceding other source of fluoride including water ranges from 0.10-4.11 mg. Thus according to the Food and Nutrition Board of the Institute of Medicine (USA) recommended daily intake of fluoride, consuming of two cups of tea from all brands (except ABTP, LBTP, ABTB and BBTP which are prepared in KOW) are safe for all population (> 9 years old) in which the tea is prepared in all different naturally fluoride rich water. But all brands of tea prepared in all different types of water were not safe for all population (0-6 months) age. In this study it is also observed that consuming two cups of tea of ABTP (in all types of water), all brands of tea in KOW, BBTP (in ABW, ALW and WOW), LBTP (in ALW and WOW) will exceed the recommended daily intake 1 mg by FNBIM for all population below 8 years. Except FBTB in ALW, BBTP and ABTB (in all types of water), all brands of tea (in WOW) and EGTB and ABTB (in ALW) are also exceeding the recommended daily intake 0.7 mg by FNBIM for all population below 3 years.

Table 13. Expected daily fluoride intake from different brands of teas prepared by different types of water one consuming one cup (100 mL) of tea in mg.

Types of tea	DIW (F^- = not measurable)	TAW (F^- = 0.254 mg/L)	MEW (F^- = 2.46 mg/L)	ABW (F^- = 6.00 mg/L)	ALW (F^- = 10.1 mg/L)	WOW (F^- = 12.5 mg/L)	KOW (F^- = 30.2 mg/L)
ABTP	0.63	0.68	0.60	0.69	0.84	0.88	2.04
BBTP	0.47	0.48	0.48	0.68	0.80	0.86	2.06
LBTP	0.11	0.12	0.17	0.33	0.53	0.62	1.69
HGTB	0.10	0.10	0.10	0.21	0.26	0.34	0.86

EGTB	0.078	0.084	0.14	0.22	0.32	0.39	0.91
QGTB	0.062	0.065	0.14	0.25	0.30	0.39	0.89
ABTB	0.27	0.29	0.28	0.34	0.42	0.43	1.07
FBTB	0.061	0.069	0.093	0.18	0.25	0.32	0.78
SBTB	0.044	0.051	0.12	0.22	0.30	0.36	0.88

Table 14. Expected daily fluoride intake from different brands of teas prepared by different types of water one consuming two cups (200 mL) of tea in mg.

Type of tea	DIW (F^- = not measurable)	TAW (F^- = 0.254 mg/L)	MEW (F^- = 2.46 mg/L)	ABW (F^- = 6.00 mg/L)	ALW (F^- = 10.1 mg/L)	WOW (F^- = 12.5 mg/L)	KOW (F^- = 30.2 mg/L)
ABTP	1.26	1.37	1.21	1.38	1.68	1.76	4.08
BBTP	0.94	0.95	0.96	1.36	1.60	1.71	4.11
LBTP	0.22	0.24	0.34	0.67	1.06	1.23	3.38
HGTB	0.19	0.20	0.20	0.41	0.52	1.23	1.71
EGTB	0.16	0.17	0.29	0.44	0.64	0.77	1.81
QGTB	0.12	0.13	0.28	0.50	0.59	0.79	1.77
ABTB	0.55	0.58	0.55	0.68	0.85	0.87	2.13
FBTB	0.12	0.14	0.19	0.35	0.49	0.64	1.57
SBTB	0.088	0.10	0.24	0.44	0.61	0.72	1.75

The data in the Table 15 showed that fluoride ingestion in consuming two cups tea was ranges from 4.4% (in deionized water) to 206% (in KOW) of recommended daily intake for a person 40 kg weight. A person who consumes two cups (ABTP, BBTP, LBTP and ABTB) of tea prepared using KOW was ingested even above the recommended daily intake from all sources.

$$\% \text{ RDIF} = \frac{DIF}{RDIF} X\ 100$$

where %RDIF = Percent of recommended intake of fluoride/kg/ day. DIF = Daily intake of fluoride from any source. RDIF = Recommended intake of fluoride/kg/ day (0.05 mg/ kg/day).

Table 15. Percent of recommended fluoride intake (0.05 mg/kg/day) for different brands of tea prepared in different types of water for a person (40 kg weight) consuming two cups of tea.

Type of tea	DIW (F^- = not measurable)	TAW (F^- = 0.254 mg/L)	ABW (F^- = 2.46 mg/L)	MEW (F^- = 6 mg /L)	ALW (F^- = 10.1 mg/L)	WOW (F^- = 12.5 mg/L)	KOW (F^- = 30.2 mg/L)
ABTP	62.9	68.3	60.3	69.2	84.0	88.1	204
BBTP	46.9	47.6	48.1	67.9	79.9	85.7	206
LBTP	10.8	12.2	17.0	33.3	53.1	61.7	169
HGTB	9.50	10.1	10.0	20.6	26.0	38.8	85.5
EGTB	7.8 0	8.40	14.4	22.2	31.9	38.5	90.5
QGTB	6.20	6.50	13.8	24.8	29.7	39.3	88.7
ABTB	27.4	28.8	27.7	34.2	42.4	43.4	106.7
FBTB	6.1 0	6.90	9.30	17.5	24.7	32.0	78.3
SBTB	4.4 0	5.10	12.0	22.0	30.4	36.1	87.5

3.7 Comparison of fluoride content in Ethiopian teas and other tea brands

Determination of fluoride concentration in tea infusion has received considerable attention due to its toxicity effect on health. Many researchers have reported the concentration of fluoride in tea infusion in different brands of tea. Jin Cao *et al*. [52] determined the fluoride content in 13 tea samples, in various black tea commodities, namely stick-shaped and granular, originally produced in India, Sri Lanka, China, Japan and UK. The fluoride content of stick-shaped black teas was 96.9–148 mg/kg, and that of granular black tea was 139–223 mg/kg, total fluoride contents of tea leaves were 133-376 mg/kg in distilled water for Chin-Shin Oolong teas, 100-193 mg/kg in distilled water for Tzuiyu tea, 178-451 mg/kg in distilled water for Shy-Jih-Chue tea, 184-196 mg/kg in distilled water for Shy-Jih-Chue tea, and 225 mg/kg in distilled water for Jasmine green tea, whereas fluoride contents of tea infusions were 0.41-0.84 mg/L for Oolong tea, 0.39-0.73 mg/L for Tzuiyu tea, 0.57-1.21 mg/L for Shy-Jih-Chue tea, 0.74-0.80 mg/L for Shy-Jih-Chue tea, and 1.14 mg/L for Jasmine green tea [55]. The content of fluoride in black tea extracts ranged from 0.32 to 4.54 mg/l, 0.02–0.09 mg/L for herbal tea 0.59 to 1.83 mg/L for green tea, the contents of fluoride in oolong and pu-erh tea infusions were between 0.39-1.68 mg/L and 0.37-0.54 mg/L for white tea extract after 5 min of brewing [54]. In this study also the fluoride concentration of black tea powder ranges from 108-629 mg/kg, 117-632 mg/kg and 124-681 mg/kg for green tea bag; 62.0-94.7 mg/kg, 64-98 mg/kg and 69-99 mg/kg, for black tea bag; 44-274 mg/kg, 49.1-283 mg /kg and 50.6-284 mg/kg 3, 5 and 10 min brewing time respectively, using deionized water for tea preparation. Tables 16-18 present literature data collected from different publications which show the fluoride content of different brands originated from different places and the infused fluoride concentration during tea preparation.

Table 16. Fluoride concentration (mg/L) in black tea samples for 5 min infusion.

Type of tea/brand	Origin	Shape	Fluoride (mg/L)	Ref.
Black, Butcem[a]	Turkey, Sri Lanka, Kenya	Bags	1.71	[51]
Black, Deren[a]	Turkey	Bags	1.63	[51]
Earl Grey, Deren[a]	Turkey	Bags	1.33	[51]
Earl Grey, Carrefour[a]	Turkey	Granular	2.51	[51]
Black, Carrefour[a]	Turkey, Sri Lanka, Kenya	Bags	2.04	[51]
Black Earl Grey, Migros[a]	Turkey, Sri Lanka, Kenya	Bags	2.20	[51]
Black, Migros[a]	Turkey, Sri Lanka, Kenya	Bags	2.82	[51]
Black, Caykur[a]	Turkey	Bags	1.57	[51]
English Breakfast, Lipton[a]	NA	Bags	3.53	[51]

Black Earl Grey, Lipton[a]	NA	Bags	2.27	[51]
Black, Lipton[a]	NA	Bags	2.75	[51]
Black, Dogus[a]	Turkey	Bags	1.99	[51]
Black, Dogus[a]	Turkey, Sri Lanka, Kenya, India	Bags	1.40	[51]
Black Earl Grey, Dia[a]	Turkey	Bags	3.72	[51]
Black, Muma[a]	Turkey	Granular	1.51	[51]
Black, Oba[a]	Turkey	Granular	2.09	[51]
Black, Dia[a]	Turkey	Bags	1.63	[51]
Black, Organic Ozcay[a]	Turkey	Granular	2.00	[51]
Black, Ozcay[a]	Turkey	Granular	2.00	[51]
Black Darjeeling, Twinnings[a]	Darjeeling, India	Bags	1.59	[51]
Black Earl Grey, Twinnings[a]	NA	Stick	1.41	[51]
Black, Ahmad[a]	Sri Lanka	Granular	0.73	[51]
Black, Ceylon Istıkan[a]	Sri Lanka	Stick	0.57	[51]
Black, Ceylon Gun powder[a]	Sri Lanka	Granular	0.92	[51]
Black, Ceylon Pekoe[a]	Sri Lanka	Stick	0.94	[51]
Black, Ceylon OP[a]	Sri Lanka	Stick	0.85	[51]
Black, Abyssinia[b]	Ethiopia	Leaves	6.32	Present study
Black, Black Lion[b]	Ethiopia	Leaves	5.17	Present study
Black, Lipton[b]	China	Leaves	1.17	Present study
Black, Addis[b]	Ethiopia	Bags	2.84	Present study
Black, Flavored[b]	Sri Lanka	Bags	0.61	Present study
Black, Strawberry[b]	Sri Lanka	Bags	0.49	Present study

NA : not available.

([a]) One tea bag sample was added to 100 mL boiled water for five minutes of infusion for past study[53].

([b]) Tea infusions were prepared using 1g of tea powder leaves and 2 g bag tea leaves with 100 mL deionized water for present study.

Table 17. Fluoride concentration (mg/L) in tea infusions.

Type of tea and name	Origin/brand	Time of brewing (min)		Ref.
		5	10	
Black Yunnan Gold[a]	China	0.32	0.53	[54]
Black Assam[a]	India	0.79	1.01	[54]
Black Darjeeling[a]	India	0.57	0.65	[54]
Black Assam FOP[a]	India	1.15	1.36	[54]
Black High Grown[a]	Sri Lanka	0.72	0.91	[54]
Black Maloom[a]	Nepal	0.51	0.57	[54]
Black Golden Tippea[a]	Kenya	0.93	1.39	[54]
Black[a]	Georgia	0.89	0.99	[54]
Black Ceylon Gold[a]	Dilmah	1.08	1.28	[54]
Black[a]	Telety	2.26	2.49	[54]
Black Earl Grey[a]	Telety	2.63	3.03	[54]
Black[a]	Posti	2.25	2.32	[54]
Black Assam[a]	Ahmad Tea	1.43	1.59	[54]
Black[a]	Lipton	2.76	3.28	[54]
Black Earl Grey[a]	Lipton	1.22	1.44	[54]
Black[a]	Dilmah	2.13	2.29	[54]
Black Yunnan[a]	ZAS-Pol	1.09	1.37	[54]

Black Vanilla[a]	Dilmah	2.03	2.32	[54]
Black Ygara[a]	Astra	4.56	6.13	[54]
Black Darjeeling Himalaya[a]	Astra	0.82	0.91	[54]
Black, Abyssinia[b]	Ethiopia	6.32	6.84	present study
Black, Black Lion[b]	Ethiopia	5.17	5.22	present study
Black, Lipton[b]	China	1.17	1.24	present study
Black, Addis[b]	Ethiopia	2.74	2.84	present study
Black, Flavored[b]	Sri Lanka	0.61	0.61	present study
Black, Strawberry[b]	Sri Lanka	0.49	0.5	present study
Green Gunpowder[a]	China	0.59	1.11	[54]
Green Bancha[a]	Japan	1.06	1.36	[54]
Green Sencha Lemon[a]	Japan	1.25	1.59	[54]
Green[a]	Vietnam	0.84	1.12	[54]
Green[a]	Java	1.83	2.14	[54]
Green Haichao[a]	China	0.71	0.79	[54]
Green[a]	Ahmad Tea	1.12	1.26	[54]
Green[a] Gunpowder[a]	Bio-Active	1.46	1.74	[54]
Green[a]	Teekane	1.34	1.56	[54]
Green Large leaf [a]	Dilmah	1.62	1.79	[54]
Green Yunan[a]	Posti	0.79	0.88	[54]
Green, Hyson[b]	Sri Lanka	0.98	0.99	present study
Green, Ethiopia[b]	Ethiopia	0.83	0.87	present study
Green, Quality[b]	Sri Lanka	0.64	0.69	present study

([a])Tea infusions were prepared using 2 g of tea leaves, granules and blocks with 100 ml of drinking water for past study [56].

([b])Tea infusions were prepared using 1g of tea leaves and 2 g bag tea leaves with 100 mL deionized water for present study.

Table 18. Fluoride content in tea infusions, n = 2, for 5 min brewing time.

Type of tea	Mass of fluoride (mg)	Type of tea	Mass of fluoride (mg)	Ref.
Mate Canela[b]	0.011	Mate[b]	0.035	[56]
Yerba Mate[b]	0.017	English Breakfast[b]	0.078	[56]
Boldo do Chile[b]	0.021	Ceylon[b]	0.175	[56]
Capim Cidreira[b]	0.021	Royal Ceylon[b]	0.205	[56]
Mate Limão[b]	0.029	Yellow Label[b]	0.215	[56]
Flores & Frutas[b]	0.033	Royal Blend[b]	0.230	[56]
Abyssinia Black[a]	0.632	Black Lion[a]	0.517	Present study
Lipton Black[a]	0.117	Addis Black[a]	0.274	Present study
Flavored Black[a]	0.061	Strawberry Black[a]	0.049	Present study
Hyson Green[a]	0.098	Ethiopian Green[a]	0.083	Present study
Quality Green[a]	0.064			Present study

([b])Infusion prepared from one tea bag (1.7-2.0 g of tea) per 100 mL of boiling water for past study [58].

([a]) Infusion prepared from one tea bag (2 g) and 1 g of tea powder per 100 mL of boiling water for present study.

3.8 Comparison of EC, pH, TDS and salinity of different types of water from limit set by different national and international organizations

Table 19. Drinking water contaminants and maximum admissible limit set by different national and international organizations and present study values.

Limit set by different national and international organizations				Ref.	Type of water	Present study values		
	EC (µS/cm)	TDS (mg/L)	pH			EC (µS/cm)	TDS (mg/L)	pH
USEPA, 2008	NM*	500	6.5-8.5	[59]	DIW	2.3	2.0	5.96
EU, 1998	2500	NM	6.5-9.5	[59]	TAW	102	48.7	7.73
WHO, 2008	250	NGL**	NGL**	[59]	MEW	987	478	8.42
Iranian, 1997	NM	500	6.5-8.5	[59]	ABW	1706	838	8.59
Australian, 1996	NM	500	6.5-8.5	[59]	ALW	573	273	8.34
Indian, 2005	NM	1500	6.5-9.2	[59]	WOW	1264	616	8.18
New Zealand, 2008	NM	1000	7.0-8.5	[59]	KOW	992	480	8.42

*NM = Not mentioned,

** NGL= No Guideline, because it occurs in drinking-water at concentrations well below those at which toxic effects may occur,

Table 20. Classification of the drinking water samples on the basis of salinity Rabinove *et al*. [59].

Description of the drinking water	Salinity (g/L)
Non-saline	< 1
Slightly saline	1-3
Very saline	> 10

The present study showed that all types of water used for tea preparation have been a salinity value below 1 mg/L which indicates they are non-saline.

4. Conclusion

The fluoride concentration of tea infused in different water and the fluoride concentration of different water in Ethiopian Rift Valley were assessed in this study. The sample preparation and efficiency of the instrument were tested by assessing relative standard deviation and conducting recovery tests. The fluoride concentration in water samples were ranged from 0.254 mg/L (Addis Ababa) to 30.2 mg/L (KOW). The fluoride concentration of tea infused ranges from 44 mg/kg (deionized water) to 2057 mg/kg (KOW) including all brands of tea in all types of water.

The main findings of this study are the following:

- ❖ A person who consumes high fluoride level water can reduce the daily fluoride intake by addition of tea leaves to fluoride rich water, especially tea leaves with low fluoride level have a high potential to reduce the fluoride level of water.
- ❖ Depending upon the brands of tea or fluoride rich water used for tea preparation, increasing of brewing time can increase or decrease the amount of fluoride infused from fluoride rich water to tea leaves, which may be due to the fluoride binding capacity of tea, fluoride compelaxing metals present in ether tea or water used for tea preparation.
- ❖ Ethiopian black tea powder and black tea bag have high levels of fluoride than imported ones. From imported ones HGTB has high level fluoride than EGTB which is Ethiopian green tea. This variation 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 or the tea may made from old leaf which cause tea high fluoride level.
- ❖ According to WHO, (Table 20) the maximum limit of conductivity of water is 250 µs/cm. But the conductivity of ABW, ALW, WOW, MEW and KOW have a value > 250 µs/cm which may possibly contain high level of metal ions that may cause health problem on humans.
- ❖ All type of water used for tea preparation are not saline property with values < 1 mg/L [59].

- ❖ All tea infusions prepared in all types of water were increased the conductivity and TDS of water except: ABTP, LBTP and BBTP for 3, 5 and 10 min infusion in MEW, ABW and WOW, BBTP in KOW for 10 min infusion. But also EGTB and HGTB tea infusion for 3 min only are not increase the conductivity and TDS of water.

- All brands of tea infusion prepared in DIW, TAW, MEW, ALW, ABW, WOW and KOW decreased the pH of water. Thus may be due to infusion of amino acids and tannic acid present in tea to water.

5. References

1. J. H. Weisburger. Tea and health: a historical perspective. *Cancer Letters*, **1997**, 114, 315-317.

2. *Camellia sinensis*- Wikipedia the free encyclopedia. Available on line at http://en.wikipedia.org/wiki/Camellia_sinensis. Accessed on September **2010**.

3. *Camellia sinensis*. Available on line at http://www.hort.purdue.edu/newcrop/duke_energy/Camellia_sinensis.html. Accessed on September **2010**.

4. Historical background of tea plantation in Ethiopia page. Available on line at http://www.telecom.net.et/~ectpm/TeaHistory.htm. Accessed on October **2010**.

5. Tea production – HVA international. Available on line at http://www.hvainternational.nl/tea.htm. Accessed on October **2010**.

6. Tea plantations and processing factories of the company. Available on line at http://www.ethioagriceft.com/pages/teaplantation.htm. Accessed on October **2010**.

7. The production of tea. Available on line at http://www.imperialteagarden.com/teas.html. Accessed on October **2010**.

8. The production green tea. Available on line at http://www.thefragrantleaf.com/greentea.html. Accessed on October **2010**.

9. The production black tea. Available on line at http://www.thefragrantleaf.com/blacktea.html. Accessed on October **2010**.

10. Preparation-tea information from planet tea. Available on line at http://www.thefragrantleaf.com/oolongtea.html. Accessed on October **2010**.

11. The production whitenof tea- the fragrant leaf. Available on line at http://www.thefragrantleaf.com/whitetea1.html. Accessed on October **2010**.

12. D.W. Christine and G.X. Wei. Tea as a functional food for oral health. *Nutrition and Oral health*, **2002**, 18, 443– 444.

13. The truth about tea. Available on line at http://www.indiaparenting.com/health/325_2074/the-truth-about-tea.html. Accessed on October **2010**.

14. Fluoride-Wikipedia the free encyclopedia. Available on line at http://en.wikipedia.org/wiki/Fluoride. Accessed on October **2010**.

15. WHO *Fluoride in Drinking-water: Background document for development of WHO Guidelines for Drinking-water Quality*. World Health Organization, Geneva, **2004**, p 2.

16. WHO *Environmental Health Criteria 227: Fluorides*, World Health Organization, **2002**, p 38.

17. Fluoride in tea. Available on line at http://100777.com/node/1798. Accessed on October **2010**.

18. S.C. Duckworth and R. Duckworth. The ingestion of fluoride in tea. *British. Dental of Journal*, **1978**, 145, 368-370.

19. K.F. Fung, Z.Q. Zhang, J.W.C. Wong and M.H. Wong. Fluoride contents in tea and soil from tea plantations and the release of fluoride into tea liquor during infusion. *Environmental Pollution*, **1999**, 104, 197-205.

20. Y.H. Lee, T.H. Shyu and M.Y. Chiang. Fluoride accumulation and leaf injury of tea and weeds in the vicinity of a ceramics factory. *Taiwanese Journal of Agriculture Chemistry and Food Sciences*, **2003**, 41, 87-94.

21. L.H. Weinstein and A.W. Davison. Uptake, transport and accumulation of inorganic fluorides by plants and animals. *In "Fluoride in the Environment"*. CABI Publishing. Wallingford, U.K. **2004**, pp 21-56.

22. Institute of Medicine Fluoride. In *Dietary reference intakes for calcium, phosphorus, magnesium, vitamin D, and fluoride*. National Academy Press. Washington, D.C., U.S.A. **1997**, pp 288-313.

23. WHO *Fluorides*, World Health Organization (Environmental Health Criteria 227); **2002**.

24. N.R. Mc Quaker and M. Gurney. Determination of total fluoride in soil and vegetation using an alkali fusion-selective ion electrode technique. *Analytical Chemistry*, **1977**, 49, 47-53.

25. AOAC. *Fluoride in plants-potentiometric method*. Official Methods of analysis. 16th ed., AOAC, Gaithersburg. Maryland, U.S.A. **1997**.

26. B.A. Burt. The changing patterns of systemic fluoride intake. *Journal of Dental Research*, **1992**, 71, 1228-1237.

27. Lu.Y. Guo and X.Q Yang. Fluoride content in tea and its relationship with tea quality. *Journal of Agriculture and Food Chemistry*, **2004**, 52, 4472-4476.

28. Y.Z. Han, J.Q. Zhang, X.Y. Liu and L.Z. Zhang. High fluoride content of food and endemic fluorosis. *Fluoride*, **1995**, 28, 201-202.

29. J.Y. Ruan and M.H. Wong. Accumulation of fluoride and aluminum related to different varieties of tea plant. *Environmental Geochemistry Health*, **2001**, 23, 53-63.

30. D. Hillman, D. Bolenbaugh and E.M. Convey. Fluorosis from phosphate mineral supplements in Michigan dairy cattle. *Fluoride*, **1979**, 12, 100-102.

31. Black tea can pose threat to bones. Available on line at http://www.knowabouthealth.com/black-tea-can-pose-threat-to-bones/4209/. Accessed on October **2010**.

32. Fluoride in black tea may pose health risks. Available on line at http://www.emaxhealth.com/1275/fluoride-black-tea-may-pose-health-risks. Accessed on September **2010**.

33. J. Cao, X. Bai, Y. Zhao, J. Liu, D. Zhou, S. Fang, M. Jia and J. Wu. The relationship of fluorosis and brick tea drinking in Chinese Tibetans. *Environmental Health Perspect*, **1996**, 104, 1340-1343.

34. J. Cao, Y. Zhao, J. Liu, D. Zhou, S. Fang, M. Jia and J. Wu. Brick tea fluoride as a main source of adult fluorosis. *Food Chemistry Toxicology*, **2003**, 41, 535-542.

35. K,F. Fung, Z.Q. Zhang, J.W.C. Wong and M.H. Wong. Fluoride contents in tea and soil from tea plantations and the release of fluoride into tea liquor during infusion. *Environmental Pollution*, **1999**, 104, 197-205.

36. J. Cao, S.F. Luo, J.W. Liu and Y.H. Liu. Safety evaluation on fluoride content in black tea. *Food Chemistry*, **2004**, 88, 233-236.

37. S. Tokalioglu, S. Kartal and U. Sahin. Determination of fluoride in various samples and some infusions using a fluoride selective electrode. *Turkish Journal of Chemistry*, **2004**, 28, 204-211.

38. N. Ji-Hyang and C. Paul. 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 **2009**.

39. H. Farsam and N. Ahmadi. Fluorine content of teas consumed in Iran. *Journal of Food Science*, **1978**, 43, 274-275**.**

40. K.R. Koch, M.A.B. Pougnet and S.D. Villiers. Determination of aluminium levels in tea and coffee by inductively coupled plasma optical emission spectrometry and graphite furnace atomic absorption spectrometry. *Analyst*, **1989**, 114, 911-913.

41. M.F. Harrison. Fluorine content of teas consumed in New Zealand. *British Journal of Nutrition*, **1949**, 3, 162-166.

42. S.Tokalioglu, S. Kartal and U. Sahin. Determination of fluoride in various samples and some infusions using a fluoride selective electrode. *Journal of Chemistry*, **2004**, 28, 203-211.

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

44. G.M. Whitford. Acute and chronic fluoride toxicity. *Journal of Dental Research*, **1992**, 71, 1249-1254.

45. J. Jakush. New fluoride schedule adopted. ADA News. May **1994**, p 14.

46. J.T. Chan and S.H. Koh. Fluoride content in caffeinated, decaffeinated and herbal teas. *Caries Research,* **1996**, 30, 88-92.

47. Farsam, H. and Ahmadi, N. Fluorine content of teas consumed in Iran. *Journal of Food Science*, **1978**, 43, 274-275.

48. H. Schmidt-Hebel, I. Pennacchiotti and Truhaut. Content of fluorine in some foods and beverages consumed in Chile. *Fluoride,* **1972**, 5, 82-84.

49. Background and environmental exposures to fluorides, hydrogen fluoride, and fluorine in the United States. Available on line at http://www.atsdr.cdc.gov/ToxProfiles/tp11-c2.pdf. Accessed on March **2011**.

50. M. K. Malde, R.G. Simonsen, K. Julshamn and K. Bjorvatn. Tea leaves may release or absorb fluoride, depending on the fluoride content of water. *Science of the Total Environment*, **2006**, 366, 915–917.

51. J. Cao, S. F. Luo, J. W. Liu and Y. Liu. Safety evaluation on fluoride content in black tea. *Food Chemistry*, **2004**, 88, 233–236.

52. E.A. Ebru, A. Yarat and S. Akyuz. Fluoride levels in various black teas, herbal and fruit infusions consumed in Turkey. *Food and Chemical Toxicology*, **2009**, 47, 1495–1498.

53. E. Malinowska, I. Inkielewicz, W. Czarnowski and P. Szefer. Assessment of fluoride concentration and daily intake by human from tea and herbal infusions. *Food and Chemical Toxicology*, **2008**, 46, 1055–1061.

54. T.H Shyu, J.H. Chen and Y.H. Lee. Determination of fluoride in tea leaves and tea infusions by ion selective electrode. *Journal of Food and Drug Analysis*, **2009**, 17, 22-27.

55. M.A.F. Pires, E.S.K. Dantas and C.S. Munita. Fluoride content of some teas consumed in Sao Paulo. *Fluoride*, **1996**, 29, 144-146.

56. M.B. Rajkovic and I.D. Novakovic. Determination of fluoride content in drinking water and tea infusions using fluoride ion selective electrode. *Journal of Agricultural Sciences*, **2007**, 52, 155-168.

57. The tea plant. Available on line at http://www.dadotea.com/page/Tea-Plant.aspx Accessed on September **2010**.

58. S. Zerabruk, B.S. Chandravanshi and F. Zewge. Fluoride in black and green tea (*Camellia sinensis*) infusions in Ethiopia: measurement and safety evaluation. *Bulletin Chemical Society of Ethiopia*, **2010**, 24, 327-338.

59. Concentration of heavy metals in drinking water from urban areas of the Tigray Region, Northern Ethiopia. Available on line at http://www.mu.edu.et/mejs/pdfs/v3n1/Gebrekidan&Samuel.pdf. Accessed on September **2010**.

60. N. T. Crosby, A**.** L. Dennis and J. G. Stevens. An evaluation of some methods for the determination of fluoride in potable waters and other aqueous solutions. *Analyst*, **1968**, 93, 643-652