

**FLUORIDE LEVELS AND ITS SAFETY IN TEA (*Camellia sinensis*) AND KHAT (*Catha edulis*)  
IMPORTED AND PRODUCED IN ETHIOPIA.**

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**Abstract**

The fluoride contents of sixteen(16) different packed tea leaves( *Camellia sinensis*) available in Ethiopian market and ten(10) samples of Khat leaves (*Catha edulis*) obtained from different localities were assessed using fluoride ion selective electrode. Amounts measured varies from 33.29 to 946 µg/gm on dried weight basis (DW) with an average value of 321.27±234.1 in tea samples and 13 to 20.01 µg/gm with a mean quantity of 14.94 ±1.45 in khat Samples. Based on the common practice of drinking tea three times a day by most people which is usually prepared using six grams of tea leaves and 100 to 200 gm chewing of khat leaves for those habitual users, possible daily intake of fluoride is calculated to be 1972.62 µg and 224.1-448.2 µg from tea and khat respectively based on the average fluoride quantity data from all the samples investigated. These values alone are within acceptable range of RDA (recommended daily allowance) of 2-4 mg fluorine by World Health Organization (WHO) except in one Ethiopian brand of tea where in its possible consumption the RDA limit was surpassed. Although dental problems were commonly reported in habitual khat chewers, the fluoride content of this plant is insignificant thus the chemical principle associated with such problems might be due to other ingredient(s) than fluorine. However the contribution of these plants to the overall dietary fluoride exposure should not be undermined and attention should be given to that of high values reported in tea leaves. Therefore drinking tea in areas with high fluoride levels from water sources should be cautioned to avoid the potential acute and chronic effects of fluoride.

**Key Words:** Fluoride, Tea, Khat, Ethiopia, Ion selective electrode

**Introduction**

Tea (*Camellia sinensis*) is a perennial shrub in which its leaves are used to prepare the most common beverage called tea (Fung *et al.*, 1999). Tea is the second most popular beverage in the world next to water (Harbowy and Balentine, 1997). About 50–60 billion cups of tea are consumed per annum in each of the USA and the UK although the habit of drinking it initially originates from China (Hope *et al.*, 2006). United Kingdom has a much higher per capita intake at about 3.5–4.0 cups/person/day although the consumption of tea is increasing in the USA. Black tea is by far the commonest form of tea that is taken in the world and the infusion is rich in certain nutrients, especially minerals (Hope *et al.*, 2006, Lu *et al.*, 2004). This plant is a known accumulator of fluoride (Ruan *et al.*, 2003). Fluoride is important to the body if it is in small amounts (0.20-0.35 g F<sup>-</sup> per kg body weight) otherwise it is very toxic (Tokalioglu *et al.*, 2004). The levels of fluoride in different black tea

commodities from different countries is investigated (Cao, *et al.*, 2006). These studies clearly demonstrate the presence of fluoride to the extent being harmful to human beings.

Overall tea production is estimated to be 3.2 million tonnes in 2004 with the main producing countries being Turkey, China, Kenya, Malawi and Srilanka (CTA, 2010). The figure rose to 3, 832, 650 tonnes in 2008 and the main producing countries were China, India, Kenya, Srilanka and Turkey (CTA, 2010). According to FAO the growth of consumption of tea is outpacing its production by 3.4 % in the years 2007-2009 (FAO, 2009). In Ethiopia tea is commercially grown on state farms (under Ethiopian Coffee and Tea Authority) at Gumaro and Wushwush localities in the South-Western part of Ethiopia. Private investors are also participating in this sector. The total cultivated area is currently estimated at about 1,300 to 1,500 hectares (EBDSN, 2006). Ethiopia was not a significant producer of tea previously, but nowadays investment in the sector is growing.

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Production of tea has rapidly expanded from 3 tonnes in 1974 to 2353 tonnes in 1992. Imports declined from a high of 2,793 metric tonnes in 1981 to 820 metric tonnes in 1990 (EBDSN, 2006). Ethiopia had already become net self-sufficient in tea. Tea is also another potential for production, processing and export. Ethiopia's tea is of an excellent quality as it is mainly organic. The total tea export for the year 2001/02 was 153 tonnes. In 2009 production reached 7000 tonnes outpacing annual national consumption of 5000 tonnes. Thus 30 % of the tea produced was exported to about twelve countries.

The favourable agro-climatic conditions in the country offer excellent opportunities for production and processing of tea for both export and domestic consumption (EBDSN, 2006, CTA, 2010, Ministry of Agriculture and rural development, 2009).

Khat is the common name for *Catha edulis*, a dicotyledonous evergreen shrub of the family Celastraceae (Al-hebishi and Skaug, 2005). It is also spelled and/or named by other names like Catha, Qaat, Qat, Kat, Chat, Quat, Tschat, Kuses-Salahin, Jaad, Kaad, Gat, Jimaa, Herari, Mirra, Tohai, African Tea, Abyssinian tea, Flower of paradise, bushman's tea and African salad (Dhaifalah & Santay, 2004, Abdusalam *et al.*, 2004). It is estimated that several million people are habitual khat users, living in geographical areas close to where khat is growing, particularly Yemen, Somalia, Ethiopia, Djibouti and Kenya (Kalix and Braenden, 1985). Because of the availability of road network and air transport and immigration, the habit has spread recently to western countries as far north as Finland (Nencini *et al.*, 1989; Browne, 1991; Tacke *et al.*, 1992).

The pharmacologically active constituents of khat are (-) - cathinone and, to a lesser extent, (+)-norpseudoephedrine. In humans, the main pharmacological actions of khat are euphoria, hyperactivity, logorrhoea, exaggerated cardiovascular response to physical effort, increased respiratory rate, mydriasis, hyperthermia, anorexia, mouth dryness, spermatorrhoea, impotence and insomnia (Kalix and Braenden, 1985).

The fact that khat leaves are chewed daily for several hours brings many questions to mind as to the possible dental and oral effects of this habit. Some studies associate khat chewing with

significant dental problems (Rosenzweig and Smith, 1966; Mengel *et al.*, 1996; Al-Bayatay *et al.*, 2011), while other studies show less association (Hill and Gibson, 1987; Jorgensen and Kaimenyi, 1990).

Khat business in Ethiopia is very significant in terms of local consumption and export. It is the second most foreign exchange earner next to Coffee till 2004/2005 and in the recent years till 2009/2010 it stands third, the second position taken by oilseeds (Gebremeskel, 2012). The revenue generated by export of khat amounted to US\$187.215 million (3426.037 million birr (Ethiopian currency) in 2009/2010 and the main export destinations are the neighbouring countries of Djibouti and Somalila (Gebremeskel, 2012; Belwal and Teshome, 2011).

Ethiopia is a country located in eastern Africa, where fluorosis commonly occurs in the rift valley region (Malde *et al.*, 2003). The geographic distribution of fluoride in surface and ground water in Ethiopia have been described as 24.2% of the analyzed wells contain a concentration of fluoride above the WHO recommended value of 1.5 mg/L (Teklehaimanot *et al.*, 2006). The rift valley area of Ethiopia records the highest amount above the WHO level (Reimann *et al.*, 2003 and Kloss *et al.*, 1999, Teklehaimanot *et al.*, 2006). Fluorosis problem in Ethiopia especially in the rift valley region is a well documented phenomenon in many studies (Reimann *et al.*, 2003 and Kloss *et al.*, 1999). Thus it is justifiable to study the level of fluoride in any supplemental food or other intakes by Ethiopians living in such critical environment for any control measures to counteract the above problem. Therefore, the aims of this study were to determine the fluoride level of the two plants namely; tea available in Ethiopian market (produced locally or imported) and khat produced in different parts of the country, evaluate the safety level of fluoride in these plants and test the hypothesis whether the prevalence of dental problems in habitual khat chewers was attributable to fluoride level or not.

## Materials and methods

### Samples

Packed tea leaves were purchased from the nearby supermarkets in Addis Ababa, Ethiopia in December, 2005. The details are described in table 1. To acquire representative sample, three packs

for each brand were uniformly mixed and then grinded to fine particle size using miller two. The powders were then stored in a clean polyethylene bags.

Khat plant materials were collected from different locations as shown in the table 2 in December, 2005 and brought to the laboratory wrapped in polyethylene bags. Respective herbarium specimens were deposited under "Ayenew Ashenef" with identification numbers AA01-AA10 in the National Herbarium, Department of Biology, Addis Ababa University,

Ethiopia. Verification was done by Mr. Melaku Wondafrash. In the laboratory, the khat leaves were washed with deionised water. The leaves were dried at room temperature. The dried samples were then grinded in two sequential millers; Miller one and Miller two to made the powder fine in size. The powders were then stored in clean polyethylene bags. To avoid cross contamination the stainless steel grinding system of the mill was washed with acetone after or before each grinding cycle.

Table 1 Detailed description of tea (*Camella sinensis*) samples analyzed

No	Sample name	Packaging/blender company and its address	Country	Batch No.(identification bar)	Manufacturing date	Expiry date	Quantity per pack (gm)
1	Addis tea	Ethio Agri safety PLC	Ethiopia	NM	04/2004*	04/2007*	100
2	Almeta Red label tea	NM	Ethiopia	NM	NM	NM**	100
3	Desta tea	Makobu Enterprise,	Ethiopia	NM	NM	NM	100
4	Ahadu tea	Ahadu PLC,	Ethiopia	NM	NM	NM	100
5	Evergreen tea	MOAB Pvt. Ltd. Co	Ethiopia	NM	NM	NM	100
6	Abbay red label tea, finest blend tea	Abbay Processing and packing PLC,	Ethiopia	(45200585312)	NM	NM	50
7	Haron Special tea	Haron Tea Enterprise	Ethiopia	NM	NM	NM	100
8	Abyssinia tea (red label quality tea)	Abyssinia Coffee and tea processing Enterprise	Ethiopia	NM	NM	NM	100
9	Qualitea Natural green tea	Qualitea Ceylon(Pvt) Limited	Srilanka	10605 (4791014002031)	02/2005	02/2008	100
10	Wushwush, highland grown Lemmon Twist	Tea Development and Marketing Enterprise R.twining and Company limited	Ethiopia	NM	NM	NM	100
11			United Kingdom	(07017707990)	NM	NM	30
12	Tea Dilmah Orange	MJF group	Srilanka	N1071323 (9312631123661) Item: 1236606	NM	06/2006	25X1.5

13	Tea Dilmah Lemmon	MJF group,	Srilanka	N1081323 (9312631123630) item:1236305	NM	05/2006	25X1.5
14	Tea Dimah Raspberry	MJF group	Srilanka	N1091333 (9312631123708) item: 1237006	NM	05/2006	25X1.5
15	Tea Dilmah Vanilla	MJF group	Srilanka	D1091393 (9312631125092) item: 1250905	NM	05/2006	25X1.5
16	Tea Black Lion	East Africa Industries and Comm (Eth) Ltd	Ethiopia	NM	NM	NM	1.5X50

NM; Not mentioned, \* Converted from Ethiopian calendar (08/97; 08/99) to GC \*\* simply labeled as two years.

Table 2 Description of the *Catha edulis* (Khat) samples assessed in this study

No	Collection site/town	Administrative zone	Region	Herbarium Deposit No.
1	Bahir Dar, south	Bahir Dar	Amhara	AA01
2	Bahir Dar, North	Bahir Dar	Amhara	AA02
3	Hirina	West Harerghe	Oromiya	AA03
4	Beleche	Sidama	Southern Peoples Nations and Nationalities (SPNNs)	AA04
5	Gelemso	West Harerghe	Oromiya	AA05
6	Wendo	Sidama	Southern Peoples Nations and Nationalities (SPNNs)	AA06
7	Indibir	Guraghe	Southern Peoples Nations and Nationalities (SPNNs)	AA07
8	Addis Ababa	Asko	Addis Ababa	AA08
9	Alemaya	West Harerghe	Oromiya	AA09
10	Beleche, Purchased from Addis market @	Arada	Addis Ababa	AA10

@ the shop address is given.

#### **Fluoride Determination**

Fluoride content of the khat and tea samples were determined by the procedure detailed in AOAC Official method 975.04 for fluoride determination in plants (AOAC, 1995).

#### **Data Analysis and Quality Assurance**

One way ANOVA was used for data analysis using Instat software. A cut of point of  $p < 0.05$  was employed to determine statistical significance. Standard addition method was employed to check the accuracy of the analytical method employed for fluoride measurement. Recoveries vary from

98 % -106 %. Hence reliable, validated method was employed for fluoride measurement.

## Results and Discussion

### Fluoride levels in tea and khat

After calibration which is linear with good correlation coefficient (-0.998) as shown in figure 1, the amount of fluoride in khat and tea samples were quantified. The results are given in table 3. Tea leaves accumulate a significant amount of fluoride. However, result obtained in the present study should be interpreted cautiously as the method quantifies only soluble fluorine while in capable of analyzing organic fluorine. The frequently observed dental problems associated with khat chewing may be attributed to other compounds in khat plant than fluorine as the level found is very small to cause skeletal/dental fluorosis.

Fluorosis is a symptom due to elevated intake of F<sup>-</sup> over prolonged periods of time. Skeletal fluorosis and dental fluorosis are the two main types. Skeletal fluorosis is the accumulation of F in skeletal tissues associated with pathological bone formation. Dental fluorosis is that the structural integrity of enamel is affected and small pits are left in teeth as it breaks away. The total daily intake of F that may cause fluorosis (chronic intake) for adults is over 13-14.5 mg/day (Cao *et al.*, 1997).

Tea consumption should be cautioned with respect to its fluorine level. Some case reports have already been documented for the development of skeletal fluorosis due to consumption of instant tea in a 52 year old Caucasian woman (Whyte *et al.*, 2005).

Highest values of fluoride in the tea samples was observed in Ahadu tea ( $929.33 \pm 16.77$  ug/gm) while the lowest value was observed in Lemmon twist tea ( $35 \pm 1.71$  ug/gm). The lowest value was observed in the latter case as it is a green tea variety.

Ethiopian teas are usually produced in Wushwush and Gumaro farms (Yemane *et al.*, 2008), the variation in fluoride level might be attributed to the fermentation procedure, handling facilities, as well as variation in the utilization of agricultural fertilizers and aids in each lot of the farm by each company. Besides the difference in plant type, quality of water used for irrigation and other

factors might contribute for such difference (Cao *et al.*, 2001). The values of fluoride in lemmon twist tea and tea dilmah vanilla are different from the other tea samples in a stastically significant manner ( $p < 0.001$ ).

As shown in the figure 2, Ethiopian teas contain higher levels of fluoride levels compared to England and Srilankan samples. The soil property of the country might be responsible for such outcome. The Rift valley region of Ethiopia highly prone to fluorosis problem, such high value of fluoride in the tea samples of Ethiopian origin is unwelcomed complication of the country's burden as locally produced teas are mostly consumed in such region. Ethiopia teas are of higher quality as they are organic and handled manually in weeding process because of abundance manpower rather than using herbicide chemicals. Even though the values of fluoride in Ethiopian teas are slightly higher, it is in the acceptable range with in the recommended daily allowance thus it should not be highly risky unless the consumer takes extra fluoride with the diet or water. But the fluoride content of Ahadu brand tea is very high making maximum possible exposure from drinking this brand of tea above the recommended daily allowance as shown in table 3 and 4. Besides there are reports of fluoride level up to 1 mg/gm in tea samples originated outside Ethiopia in the literature.

Khat samples of Ethiopia contain fluoride levels more or less in the same range in spite of the differences in the production localities. Highest value of fluoride was found in Khat sample cultivated in home garden at Addis Ababa. The justification for such finding might be attributed to the relative access of industrial contaminants in the garden of a metropolitan environment as compared to rural farms.

As shown in figure 2 and table 3, higher values compared to published reports (Atlabachew *et al.*, 2011 and Hattab and Angmar-Manson, 2000) of fluoride levels in khat in Yemen (2.1 ug/gm) and Ethiopia (3.4-7.1 ug/gm) was found in this study. The variation in the values might be attributed to the difference in the analytical methodologies employed as well as the sampling techniques. Generally AOAC methods are vigorously standardized and thus accurate. This is also cross checked by evaluating the recovery in the standard addition method. Moreover the accelerated drying

procedure by an oven employed in the methodology of Ethiopian khats analysis may facilitate evaporation of volatile complexes of fluoride than the natural air drying we employed in this study. In addition the difference in the

sources of the samples of Ethiopian khats analysed in this study and the other study done by Atlabachew *et al.*, (2011) should be noticed. Although the latter study is already published its actual laboratory execution is latter than this study.

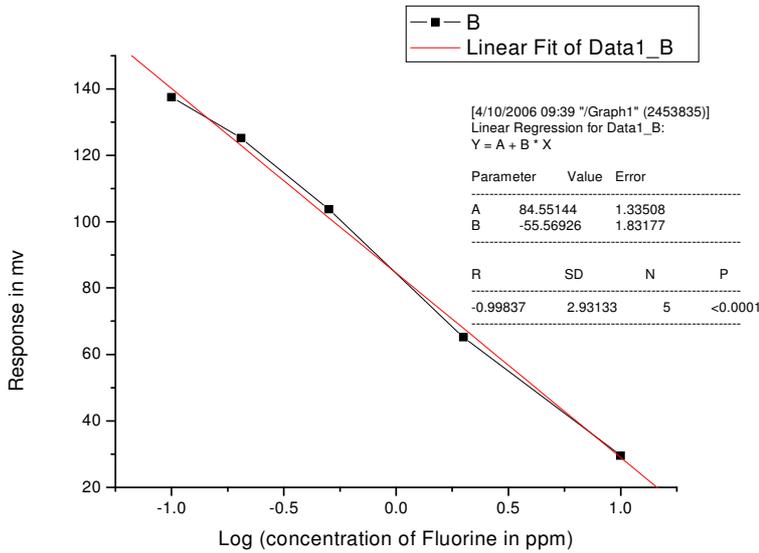


Figure 1 Calibration Curve used for Fluorine Determination

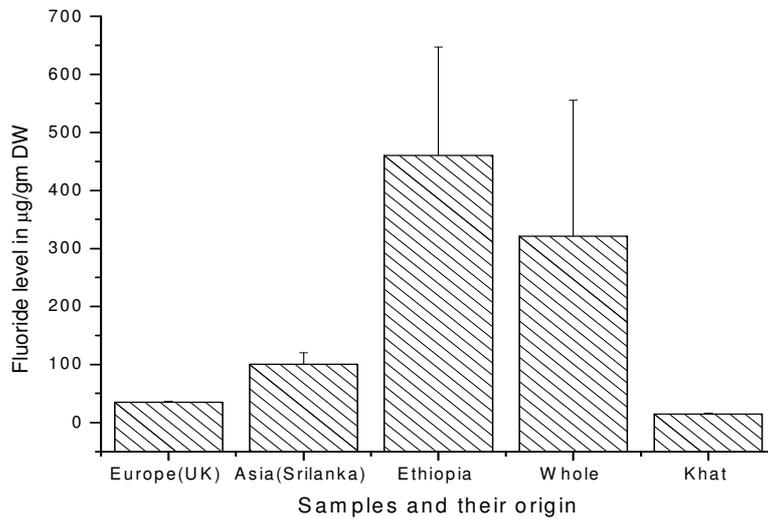


Figure 2 Average Fluoride Level as Mean ± SD in µg/gm Dried Weight (DW) Basis

Table 3 Fluoride levels in Khat and Tea Samples as  $\mu\text{g/gm}$  dried weight basis as Mean $\pm$  SD (%RSD) of triplicate measurements.

Tea Sample		Khat	
Brand name	Fluoride level	Khat type	Fluoride level
Abay red label	492 $\pm$ 8.72(1.77)	Addis Ababa, asko	18.53 $\pm$ 1.48(7.98) ***
Abyssinia	519.6 $\pm$ 26.84(5.17)	Alemaya	15.21 $\pm$ 0.4(2.66)
Addis	482 $\pm$ 6(1.24)	Beleche, Purchased in Addis	15.50 $\pm$ 0.31(2.00)
Ahadu	929.33 $\pm$ 16.77(1.8)	Bahir Dar south, Near air port	14.45 $\pm$ 0.49(3.38)
Almeta red label	322 $\pm$ 12.17(3.78)	Bahir Dar North	14.87 $\pm$ 0.42(2.8)
Black lion	199.93 $\pm$ 2(1)	Beleche, from the origin	13.77 $\pm$ 0.13(0.92)
Desta	460.67 $\pm$ 11.72(2.54)	Gelemso	15.25 $\pm$ 0.1(0.67)
Tea Dilmah Lemmon	90.53 $\pm$ 2.4(2.65)	Hirna	14.39 $\pm$ 1.02(7.07)
Tea Dilmah orange	98.6 $\pm$ 3.89(3.95)	Indibir	13.51 $\pm$ 0.12(0.89)
Tea Dilmah	98.67 $\pm$ 0.61(0.62)	Wendo	14.27 $\pm$ 0.33 (2.31)
Raspberry			
Tea Dilmah Vanilla	78.67 $\pm$ 3.41(4.33)*		
Ever green	461.33 $\pm$ 13.32(2.89)		
Haron special	429.33 $\pm$ 14.05(3.27)		
Lemmon twist	35 $\pm$ 1.71(4.88)***		
Qualitea Natural green	136 $\pm$ 1.04(0.76)		
Wushwush, Highland grown	306.67 $\pm$ 1.15(0.38)		

\*\*\*= Extreme significantly differ from other samples ( $P < 0.001$ ) while other pairs are non significant ( $P > 0.05$ )

\*: significantly differ from any pair

#### **Possible exposure to fluoride by intake of tea and khat chewing**

Calculations were performed based on the fact that an average chewer chews 100-200 gm Khat leaves per day on wet weight basis (Kalix, 1996) and the moisture content is 85 % hence 15-30 gm on dried weight basis while three cups of tea per day are common to most people (Ebadi *et al.*, 2005). To prepare tea two grams of tea leaves are included per cup making the average daily consumption 6 gm. The possible consumption exposure of the Fluoride without regarding the bioavailability factor is given in Table 4. All the samples of tea and khat possible exposure to the maximum scenario is below the RDA for adults except the Ahadu tea sample (Table 4 and 5). In the latter case at scenario of maximum bioavailability i.e. 80 % (Karak and Bhagat, 2010) to the tea infusions still exerts a considerable risk of fluoride exposure. Here the overall dietary contribution of fluoride from consuming tea and khat with other intakes should not be underestimated. The relatively high fluoride values

in tea samples should be noticed as possible fluorosis problem major contributor.

Dental problems associated with khat chewing have been widely documented in a number of case studies (Yarom *et al.*, 2010). The fluoride level of khat being very small as demonstrated in this study and others, the active ingredient(s) present in khat responsible for this harm effect should be further investigated. Although not scientifically proven, there is a suggestion of tannin(s) present in khat may be responsible for the negative dental effect observed in khat habitual users.

#### **Conclusion**

Ion selective electrode is used to measure the concentration of fluorine in Khat and tea samples. The concentration of fluoride found in tea samples varies from 35  $\pm$ 1.71 to 929.33 $\pm$ 16.77  $\mu\text{g/gm}$ . Ethiopian origin teas found to contain higher amounts of fluorine compared to the investigated teas originating elsewhere. In some brands of tea of Ethiopian origin the possible exposure of fluorine daily by consuming three times a day of tea surpasses the limit set by WHO and other regulatory bodies. The level of fluoride in khat

samples was found to be smaller which ranges from 13.51±0.12 µg/gm to 18.53 ±1.48 µg/gm. The possible daily exposure by khat chewers is far below the recommended daily allowance for fluoride intake by WHO and other regulatory agencies. Hence, the frequently dental problems seen in association with khat chewing (although not conclusive) might not be due to soluble fluorine as the level of fluorine is less in khat even compared to tea. The results in this work with respect to the level of fluorine in khat and tea of Ethiopia origin and market serves as a baseline

data to be used for dietary recommendations and toxicological monitoring.

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Table 4 Possible Daily intake of fluoride from consuming tea (three times per day) and Khat.(200 gm) in mg.

Tea Sample		Khat	
Brand name	Fluoride Exposure per day	Khat type	Fluoride exposure per day
Abay red label	2.952	Addis Ababa, asko	0.555
Abyssinia	3.117	Alemaya	0.456
Addis	2.892	Beleche, Purchased in Addis	0.465
Ahadu	5.575	Bahir Dar south, Near air port	0.433
Almeta red label	1.932	Bahir Dar North	0.446
Black lion	1.199	Beleche, from the origin	0.413
Desta	2.764	Gelemso	0.457
Tea Dilmah Lemmon	0.543	Hirna	0.432
Tea Dilmah orange	0.591	Indibir	0.405
Tea Dilmah Raspberry	0.592	Wendo	0.428
Tea Dilmah Vanilla	0.472		
Ever green	2.767		
Haron special	2.575		
Lemmon twist	0.21		
Qualitea Natural green	0.816		
Wushwush, Highland	1.840		

Table 5 Recommended daily fluoride level (mg/day)

People	WHO	USEPA	China	Poland
Children	2	2.5	2.4	1.5-2.5
Adult	2-4	4	3.5	1.5-4

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