አዲስ አበባ ዩኒቨርሲቲ
ADDIS ABABA UNIVERSITY
ENQUIRY. MASTERY. SERVICE.

College of Natural and Computational Sciences
Center for Food Science and Nutrition

The association between dietary fluoride and calcium intake of school-age children to the level of dental and skeletal fluorosis in Halaba, Southern Ethiopia

By: Nahom Tefera

Advisors: Dr. Kaleab Baye
Prof. Susan Whiting

MSC Thesis submitted to the School of Graduate Studies of Addis Ababa University in Partial Fulfillment of the requirements for Degree of Master of Science in Food Science and Nutrition

September 2019

ADDIS ABABA UNIVERSITY

SCHOOL OF GRADUATE STUDIES

CENTER FOR FOOD SCIENCE AND NUTRITION

This is to declare that the thesis entitled "Dietary fluoride and calcium intake, dental and skeletal fluorosis level and associated factors among school-age children in Halaba, Southern Ethiopia" submitted in partial fulfillment of the requirements of MSc. degree in food science and Nutrition to the school of Graduate studies food science and nutrition program. Food Science and nutrition center is a record of original research carried out by Nahom Tefera Demiss under my supervision and no part of the thesis has been submitted for any other degree or diploma. The assistance and help received during this study have been properly acknowledged. Therefore, I recommend that it be accepted as fulfilling the thesis requirements.

The Thesis has been approved for submission by:

Dr. Kaleab Baye (PhD)	______________	**October, 2019**
Name of advisor	Signature	Date
Prof. Susan Whiting	______________	**October, 2019**
Name of advisor	Signature	Date

SCHOOL OF GRADUATE STUDIES

FINAL THESIS APPROVAL

As participants of the Board of Examiners of the final MSc. open defense, we declare that we have read and evaluated the thesis prepared by Nahom Tefera under the title allowed "Dietary fluoride and calcium intake, dental and skeletal fluorosis level and associated factors among school-age children in Halaba, Southern Ethiopia" and recommend for the degree of Master of Science in Food Science and Nutrition.

Signed by the Examining Committee:

Dawd Gashu	[signature]	Nov 7, 2019
Name of Internal Examiner	Signature	Date
EYOBEL MULUGETA (PhD)	[signature]	Nov 7, 2019
Name of External Examiner	Signature	Date
Mesered Ayem	[signature]	7/10/2019
Name of Chairperson	Signature	Date

Acknowledgments

First I would like to thank my God for everything happened in my life. My Advisor Dr. Kaleab Baye, thank you very much for your supportive comments, I have learned more from your technical support in every research process. Next, I would like to extend my gratitude to my Advisor Prof. Susan Whiting, thank you a lot Professor, you helped me more from the beginning, by covering the research fund and giving timely technical support.

Masresha Tessema (Ph.D. candidate), I thank you very much for your support in every moment of the thesis work. I would like to thank also Demmelash Mulualem (Ph.D. candidate) for your constructive comments and suggestions in my thesis work. Finally. My gratitude goes to Dr. Aweke Kebede for your commitment and valuable comments.

My thanks go to Ethiopian Public Health Institute for the laboratory access and covering some field costs. I thank SNNPR and Halaba Zone health offices for their positive acceptance of the thesis research work.

Finally I thanks my parents, EPHI and AAU Center for Food Science and Nutrition staff, and my dear classmates for your considerable contributions.

Table of contents

List of figures

List of Tables

List of Acronyms and Abbreviations

AAU- Addis Ababa University

AI- Adequate Intake

AOAC- Associations of Official Analytical Chemists

CF-Complementary Food

CNPP- Center for Nutrition Policy and Promotion

CSA- Central Statistical Agency

DE- Design Effect

DF- Dental Fluorosis

DRI- Dietary Reference Intakes

EAR - Estimated Average Requirement

EPHI- Ethiopian Public Health Institute

ERV- Ethiopian Rift Valley

ES: Ethiopian Standard

FAO- Food and Agricultural Organization

FFQ- Food Frequency Questionnaire

HDL- High Density Lipoprotein

HHs- Households

ISE- Ion-Selective Electrode

ISO- International Standards Organization

LDL- Low Density Lipoprotein

RDA- Recommended Dietary Allowance

RECC- Research and Ethical Clearance Committee

RiPPLE- Research-inspired Policy and Practice Learning in Ethiopia

SA- School-age Children

SF- Skeletal Fluorosis

SNNPR- Southern Nations Nationality Peoples Republic

TISAB- Total Ionic Strength Adjustment Buffer

UL- Tolerable upper Intake Level

USDA- United States Department of Agriculture

WHO- World Health Organization

Abstract

Fluorosis, which is the toxic effect of excess fluoride intake, is a major public health problem in Ethiopian Rift Valleys. This study is aimed to investigate the relationships between dietary fluoride and calcium intakes of school age (6-13 years) children to the levels of dental and skeletal fluorosis, in Halaba, Southern Ethiopia. A study designed in environment assessment and cross-sectional analysis was undertaken in which 127 children were selected using cluster and simple random sampling techniques. The dietary calcium and fluoride intake data were collected by using 24 hour and food frequency questionnaire methods. Dental and skeletal fluorosis assessment was conducted by a qualified dentist and physiotherapist respectively.

The average diet diversity score was 3.13 from seven food groups. The mean dietary calcium and fluoride intakes were 520 mg/day and 70 mg/day respectively. At all age categories, the average calcium intake was too low compared to the RDA for calcium and the fluoride intake was very high compared to the tolerable upper intake level of fluoride. Among 127 children 70.9% were prevalent to dental fluorosis (from very mild to severe symptoms), whereas 27.6- 44.9% were prevalent to skeletal fluorosis.

The calcium and fluoride intakes were significantly associated with dental and skeletal fluorosis levels ($P < 0.05$). Improving the consumption of calcium-rich foods like dairy products, Ethiopian kale, millet, and enset are recommended.

Key words: Calcium intake, Fluoride intake, Dental fluorosis, Skeletal fluorosis, Rift Valley, Ethiopia

1. Introduction

1.1 Background

Fluorine is the lightest member of the halogen family and the most electronegative among all elements of the periodic table. Fluoride is the ionic and physiologically available form of fluorine (Institute of Medicine, 1997). Fluoride ions play an important role in human physiology (Hussain, Sharma, & Hussain, 2004). During the mineralization of bones and teeth, calcium and phosphorus form crystals called hydroxyapatite. Fluoride replaces the hydroxyl (OH) portions of the hydroxyapatite crystal, forming fluorapatite, which makes the bone stronger and the teeth more resistant to decay (Ellie et al., 2008). At low concentration fluoride is beneficial, but at higher concentrations (i.e. above 1.5 mg/L) it adversely affects human health. Thus excess fluoride intake can initiate an erratic Calcium metabolism which results in deformed bones and mottling of teeth. Prolonged ingestion of fluoride into the body system above the recommended level leads to a dreaded, crippling disease called Fluorosis which includes dental and skeletal fluorosis apart from thyroid problems, growth retardation, kidney damage, heart attacks, etc. (Panda et al., 2015).

Humans consume fluoride through water and food. The significant source of ingested fluoride is considered to be fluoride in drinking water. Foods like fish bones, fruit juices, tea leaves, dietary fluoride supplements, etc. are other considerable sources of fluorides (Levy et al., 2003; Wondwossen et al., 2006). More than 200 million people worldwide rely on drinking water with fluoride concentrations exceeding the present WHO guideline of 1.5 mg/L (WHO, 2011). Excess consumption of fluoride is associated with different diseases. Globally, the prevalence of fluorosis-related health problems has been reported to be about 32 %. Especially in the Great Rift Valley that extends from northern Syria to central Mozambique in East Africa, dental and skeletal fluorosis are important clinical and public health problems. (Mella et al., 1994).

In Ethiopia, 8 million people are exposed to a high level of naturally occurring fluoride. In the Ethiopian rift valley, 41.2% of the drinking water has fluoride content above the recommended level (1.5 mg/l). The regions that are facing excessive fluoride intake from groundwater and food are Afar, Oromia, and SNNPR. (Tekle-Haimanot et al., 2006). In Halaba, Southern Nation's Nationalities and People Regional State (SNNPR), 60% of children are consuming fluoride above the upper level of 10 mg/day (Kebede et al., 2016a). The higher prevalence of excess fluoride intake leads to fluorosis. That study in Ethiopia Rift Valley shows (especially in Fentale, Halaba, and Adamitulu) 2.1-20%, 12.5-45%, 25-40%, 17- 39.6%, and 3-10.4% of the study populations are affected by questionable, very mild, mild, moderate, and severe dental fluorosis respectively. In these areas, skeletal fluorosis has a significant effect on young children based on the criteria of clinical symptoms and the ability to do physical exercises (Kebede et al., 2016 a).

The severity of dental and skeletal fluorosis is dependent on different factors. Dietary fluoride obtained from food and water is efficiently absorbed from both the stomach and small intestine without regulation (Zuo et al., 2018). However, the effect of fluoride that causes severe fluorosis is associated with factors like fluoride dose, length of exposure, altitude, and individual differences including health and nutritional status (Akosu & Zoakah, 2008; Alvarez et al., 2009). A study performed using an animal model reported that calcium-magnesium salts or a plant source of these minerals effectively reduced apparent fluoride absorption indicating proper diet may reduce fluorosis development (Kebede et al., 2016 b).

In Ethiopia, there is no enough data on the level of dental and skeletal fluorosis and its related factors, dietary fluoride intake, and dietary calcium intake. The level of dental and skeletal fluorosis levels has been assessed in previous studies in the study area. This study provided further information about dental and skeletal fluorosis levels by assessed data by dentists they specialize in this area. Additionally, no one has reported about the association between dietary calcium intake to the level of dental and skeletal fluorosis in school age children, this study answered these such kinds of questions. Therefore researching these areas could contribute to the reduction of adverse health effects related to excess fluoride intake.

1.2 Objectives

1.2.1. General Objective

- To investigate the association between dietary fluoride and calcium intake of school-age (6-13 years) children to the level of dental and skeletal fluorosis in Halaba, Southern Ethiopia

1.2.2. Specific Objectives

- To determine dietary fluoride and calcium intake of school children
- To assess dental, skeletal and non-skeletal fluorosis level among school children
- To assess the associations between dietary calcium and fluoride intakes of children to the level of dental and skeletal fluorosis

2. Literature review

2.1 Fluoride

Fluoride is the ionic form of fluorine, a halogen and the most electronegative of the elements of the periodic table. It is a negatively charged non-metallic halogen. Both organic and inorganic forms of fluoride are frequently found in a variety of natural environments. It is ubiquitous. Consumption of fluoride is necessary for human and animal health as it plays an important role in maintaining the structure and physiological function of bones and teeth (Institute of Medicine, 1997; Zhang et al., 2013). Owing to its high affinity for calcium, fluoride is mainly associated with calcified tissues. Its ability to inhibit, and even reverse, the initiation and progression of dental caries is well known. It also has the unique ability to stimulate new bone formation, and as such, it has been used as an experimental drug for the treatment of osteoporosis (Institute of Medicine, 1997). At low concentration fluoride is beneficial, however, at higher concentrations (i.e. above 1.5 ppm) it adversely affects human health (Panda et al., 2015). Long-term excessive fluoride intake is known to be toxic and can damage a variety of organs and tissues in the human body (Zhang et al., 2013).

2.2 Health effects of fluoride

Fluoride (F) is an important micronutrient that accumulates within mineralized tissues such as teeth and bone. Fluoride is essential in the diet and is thought to be required for normal dental and skeletal growth (John et al., 1983). Even though fluoride is very important for human health, it adversely affects health when adults and children are exceeding the agreed limits (0.5-1.5ppm), contributing to a rapid rise in dental fluorosis—the first sign of fluoride toxicity (Erdal & Buchanan, 2005). The most obvious and widespread impact of fluoride is dental fluorosis. In some cases where fluoride levels are very high or where there is prolonged ingestion at 2 ppm or higher, cases of skeletal fluorosis have been reported. Skeletal fluorosis is a chronic metabolic bone disease caused by ingestion or inhalation of large amounts of fluoride. In regions with water fluoride concentrations over 2 ppm, or among workers constantly exposed to fluoride in aluminum or fertilizer industries, skeletal fluorosis is common (>20% prevalence) and manifested as joint pain in both upper and lower limbs, numbing and tingling of the extremities, back pains, and knock-knees. Vertebral osteosclerosis may result in spinal cord compression. Besides, an increase in bone mass due to fluoride ingestion or treatment (for osteoporosis) does not translate into improved bone strength, and high doses of sodium fluoride for osteoporosis treatment may increase the risk of vertebral fractures (Riggs et al., 1990; Yunzhao et al ., 1994).

Fluoride has benefits in terms of reduction of dental caries but also significant costs with cognitive impairment, hypothyroidism, dental and skeletal fluorosis, enzyme and electrolyte derangement, and uterine cancer. Dental fluorosis is associated with high fluoride intake during the critical time of tooth development, whereas skeletal fluorosis is associated with prolonged high intake of fluoride over many years. Therefore fluoride in low doses is beneficial for teeth and bone health as it strengthens the apatite matrix of skeletal tissues and teeth (Barbier et al., 2010). The WHO's permissible limit for fluoride in drinking water is 1.5 mg/ L (WHO, 2011) whereas adequate fluoride intake from all sources, including water, beverages, and diet, is at 0.05 mg/d/kg body weight(Institute of Medicine, 1997). The beneficial and harmful effects of fluoride are summarized as in Figure 1 (Hussain et al., 2004):

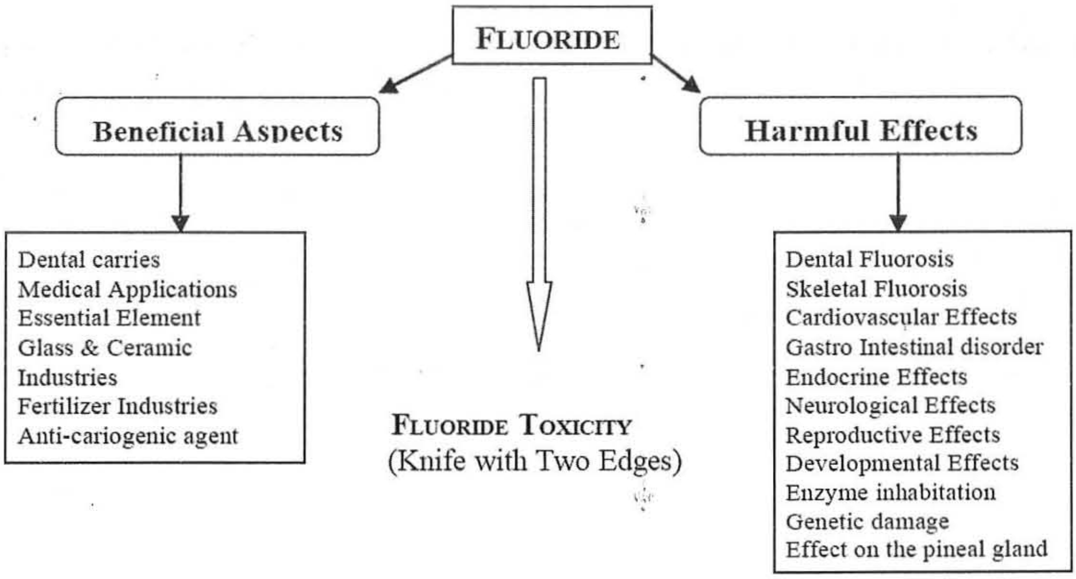


Figure 1: Some beneficial and harmful effects of fluoride (Hussain et al., 2004)

The possible mechanisms of fluoride toxicity are (Martínez-Mier, 2012):

(a) As the fluoride comes in contact with moisture this results in the formation of hydrofluoric acid and this acid formation results in the burning of tissues due to low pH.

(b) Inhibition of nerve impulse or nerve function is because calcium forms chemical complexes with fluoride leading to hypocalcemia and ultimately results in inhibition of physiological nerve functioning.

(c) Cellular poisoning results due to the inhibition of enzymes required for the physiological functioning of cells.

(d) Hypocalcemia and hyperkalemia result in electrolyte imbalance and eventually result in disturbances in cardiac rhythm.

(e) Fluoride is one of the most reactive elements. In the case of a toxic amount of fluoride in the body, fluoride attacks oxygen and disrupts the metabolism resulting in the production of hydrogen peroxide as a product. Also, fluoride results in excessive production of free radicles that disrupt the antioxidant formation (Yang & Liang, 2011).

The toxicity of fluoride due to excessive ingestion is classified into acute toxic effects and chronic effects (Figure 2).

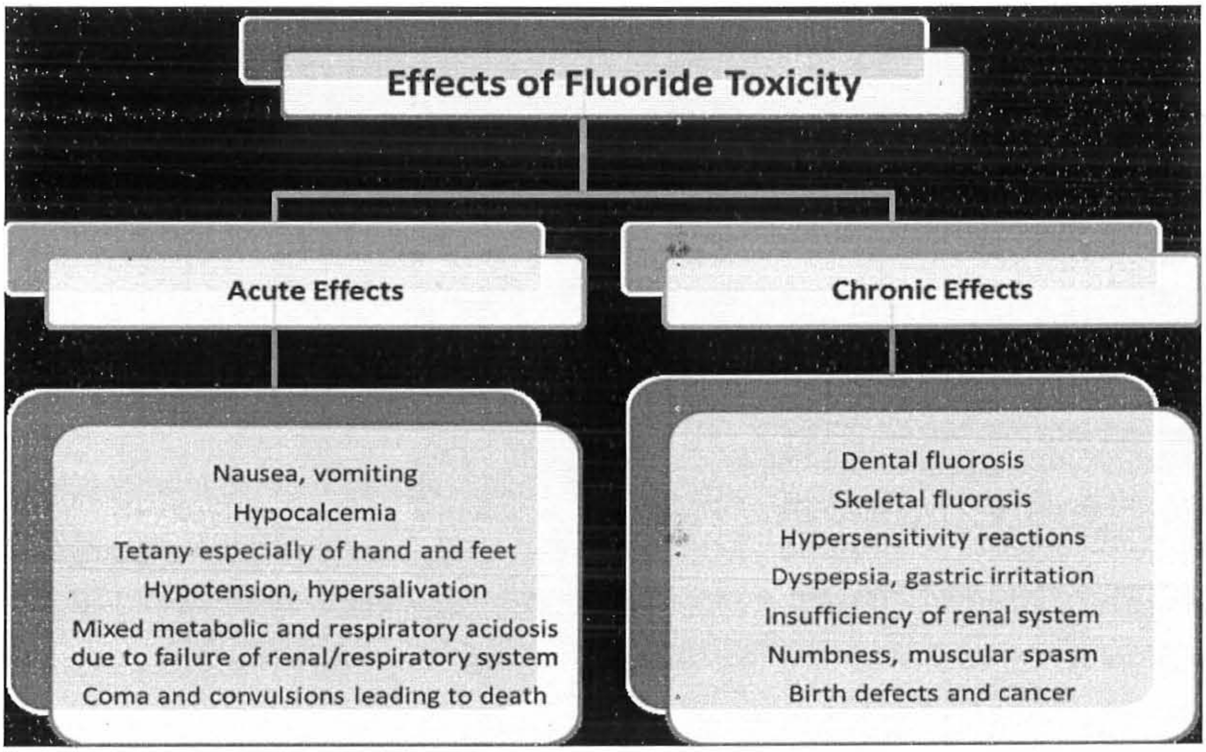


Figure 2: Classification of toxic effects due to excessive ingestion of fluoride (Ullah, Zafar, & Shahani, 2017)

2.3 Sources of fluoride intake

Living organisms are exposed to fluoride in the forms of fluoride compounds like hydrogen fluoride, calcium fluoride, sodium fluoride, sulfur hexafluoride, and silico-fluorides. These compounds are considered to be the most relevant of the inorganic fluorides based on quantities released to the environment, environmental concentrations and toxicological effects on living organisms. Fluorides are released into the environment naturally through the weathering and dissolution of minerals, in emissions from volcanoes and marine aerosols. Fluorides are also released into the environment via coal combustion and from various industrial processes, including steel manufacture, primary aluminum, copper and nickel production, phosphate ore processing, phosphate fertilizer production and use, glass, brick and ceramic manufacturing, and glue and adhesive production. The use of fluoride-containing pesticides, as well as the controlled fluoridation of drinking-water supplies, also contributes to the release of fluoride from anthropogenic sources (WHO, 2002).

Fluoride can be ingested by human beings from different sources including intentional sources such as dietary fluoride supplements and foods and beverages, and unintentional sources of ingested fluoride such as ingestion from fluoride dentifrices, mouth rinses, and other fluoride-containing products (Pendrys, 1990). However, the fluoride from water remains as the primary source of ingested fluoride. This increased availability of fluorides has led to an increase in the prevalence of fluorosis. In general, Dietary fluorides, fluoride supplements, and dentifrices and Oral Hygiene Products are the significant sources of fluoride.

2.3.1 Dietary sources

Humans consume fluoride from numerous sources. The main source of ingested fluoride is considered to be fluoride in drinking water. For instance, in the United States, about 150 million people received fluoridated water at concentrations of 0.7 to 1.2 parts per million (ppm) (Horowitz, 2003). All foodstuffs contain at least trace amounts of fluoride. Beverages that are manufactured in regions with fluoridated water and foods that are cooked in fluoridated water also contain fluoride. Seafood and fruit juices are significant sources of fluorides. Fruit juices, especially grape juice, can be high in fluorides. Tea leaves contain considerable amounts of fluoride and high consumption of tea is known to increase the risk of fluorosis. The bone of fish contains a large amount of fluoride and excessive consumption of fish with bone during tooth mineralization can cause fluorosis (Wondwossen & Bjorvatn, 2006).

2.3.2 Fluoride supplements

Dietary fluoride supplements are prescription medications that are intended for use by children living in non-fluoridated communities (Nourjah & Horowitz, 1994). Additionally, in infant formula (baby foods), fluoride is mixed especially in low fluoride areas. Therefore it has a contribution to excess fluoride intakes cumulated with other sources (Levy S et al., 1995).

2.3.3 Dentifrices and oral hygiene products

Nowadays using fluoride-containing dentifrices, mouth rinses and home-use gels has become very common in the world people. The use of dentifrices flavored for children increases the quantity of toothpaste used and can increase the risk of swallowing the toothpaste. Fluoride mouth rinses can also be a significant source of fluoride ingestion, especially in younger children. Toothpaste ingestion remains the main source of fluoride toxicity followed by fluoride-containing mouthwashes and supplements. Currently, mouth rinses are not recommended for use by children under school age because of the risk of swallowing (Steven et al., 1997). These toxicities are since the swallowing reflex in children is not completely developed and fluoride toothpaste is flavored, which results in voluntary toothpaste swallowing (WHO, 2014).

2.4 Fluoride distribution in Ethiopia

The great African Rift Valley extends from Syria and Jordan in the Middle East to Mozambique. It is associated with high fluoride levels in groundwater. The main drinking sources of fluoride in the semiarid areas of the Rift Valley regions are deep wells. There are significant variations in the fluoride levels of the deep wells within the Rift Valley and even in the same area. The Ethiopian part of the African Rift Valley bisects the country in a southwesterly direction (Figure: 3) (Tekle-Haimanot et al., 2006). The region of the Ethiopian rift valley has an elevation that varies between 500 and 1800 meters above sea level. These areas are characterized by a hot and dry climate with an average temperature of 23 ^{0}C (ranges from 15 ^{0}C to 38 ^{0}C). In most of these regions, groundwater contains very high concentrations of naturally occurring fluorides beyond the WHO guideline value for drinking water (Tekle-Haimanot et al., 1987).

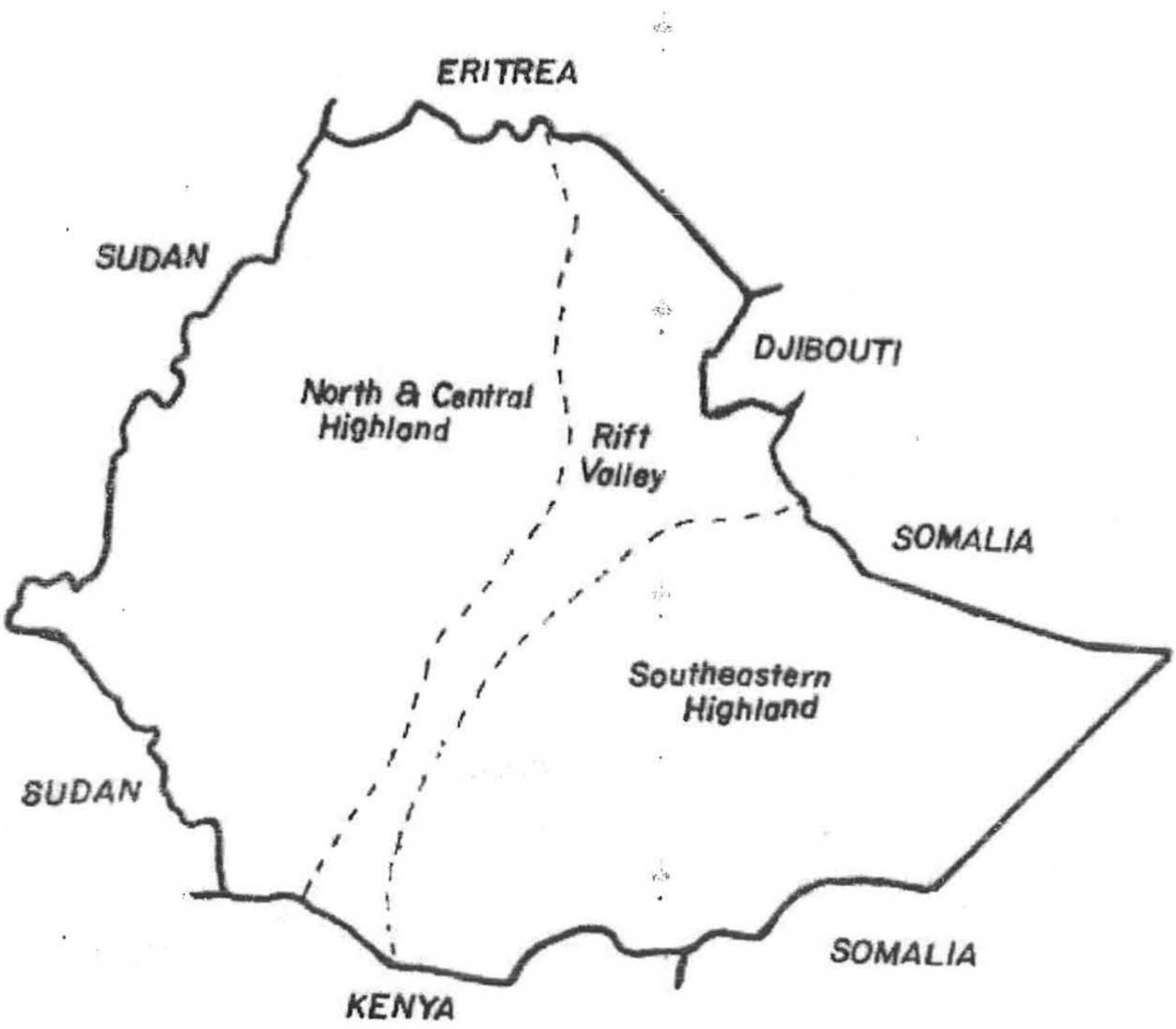


Figure 3: Map of Ethiopia showing the highland regions and the Rift Valley (Rift Valley escarpments in dotted lines) (Tekle-Haimanot et al., 2006)

In Ethiopia, the regions that are facing excessive fluoride in groundwater are Afar, Oromia and the Southern Nations and Nationalities Regional State (SNNPR). These regions are located in the Ethiopian Rift Valley part of the Great African Rift Valley. As the Ethiopian part of the African Rift Valley bisect the country, it is characterized by volcanic and basaltic rocks, which are more likely to release a high concentration of fluoride. The study area of this project Halaba is part of the Ethiopian Central Rift Valley (ECRV), which is found in SNNPR (Figure:4) (RiPPLE, 2009).

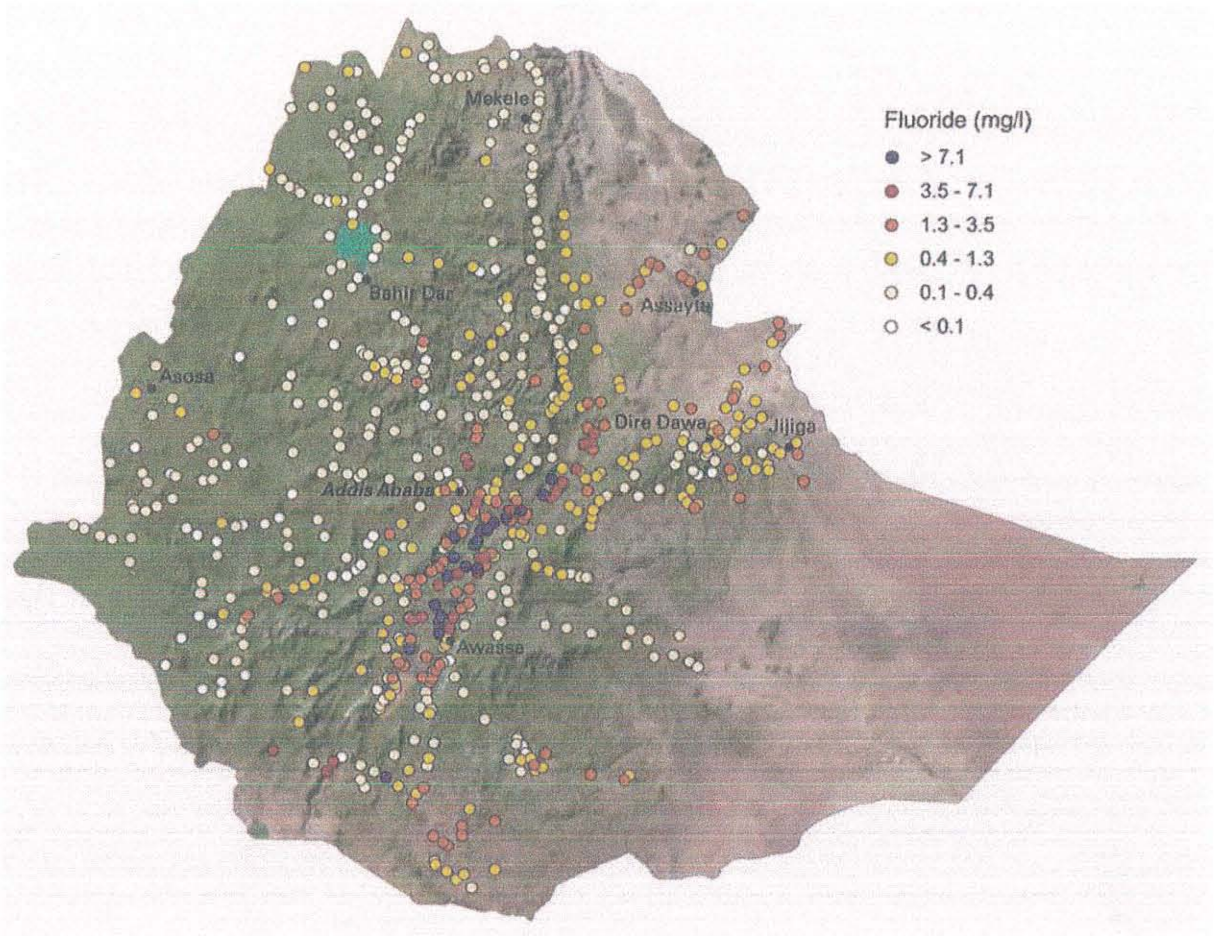


Figure 4: Fluoride concentrations in groundwater across Ethiopia (RiPPLE, 2009)

2.5 Fluorosis level in Ethiopia

Fluoride intake is often regarded as a double-edged sword. When consumed in inadequate quantities (less than 0.5 ppm), F causes health problems (e.g., dental caries, lack of formation of dental enamel, and deficiency of mineralization of bones), especially among children. In contrast, if fluoride is consumed or used in excess (more than 1.0 ppm), it can cause health problems in the young, old, or both. The various forms of fluorosis that may arise from excessive intake of fluoride through drinking water are summarized in Table 1(Whitacre, 2011).

Table 1: Effects of fluoride ingestion on human health (Whitacre, 2011)

Fluoride Concentration (mg/L)	Effects
< 0.5	Conductive to dental caries
0.5-1.5	Promotes the development of strong bones and teeth
1.5-4.0	Promotes dental fluorosis in children
>4	Promotes dental and skeletal fluorosis
>10	Crippling skeletal fluorosis, possibility cancer

Chronic toxicity of fluoride is more common than acute toxicity. The effects of chronic ingestion of fluoride depend not only on the duration and dose but also on several other factors such as nutritional status, renal function, and interactions with other trace elements (Whitacre, 2011). In regions of Ethiopia that have high fluoride consumptions, fluorosis is an endemic health problem (Tekele-Haimanot et al., 1987).

2.5.1 Dental fluorosis

The association between excessive ingestion of fluoride and dental mottling (fluorosis) was initially discovered over a century ago by Frederick Sumner McKay a practicing dentist in Colorado Springs area and G. V. Black (Levine, 2011). Dental fluorosis is the most sensitive and the earliest indicator of chronic fluoride toxicity (WHO 1996). Although fluoride is an important element for caries prevention, the chronic intake of fluoride greater than 1 mg/l or 0.1 mg/kg daily during the period of tooth development interferes with the process of enamel and dentin formation and leads to dental fluorosis (WHO 1996, Limaleite AD 2015).

According to Kloos H and Tekle-Haimanot R (1999) finding dental fluorosis in some highland communities, where the water is abstracted from volcanic rocks. Regionally 42% of groundwater sources tested in Oroma, 30% in SNNPR and 12% in the Afar region have excessive fluoride concentrations in water as long as 2 mg/L (Tekle-Haimanot et al., 1987).

Figure 5: Dental fluorosis pictures in the study area

2.5.2 Skeletal fluorosis

In Ethiopia, the regions which are susceptible to excess fluoride exposure are Afar, Oromia, and the Southern Nations, Nationalities and Peoples Regions (SNNPR). As a result of the long-term use of high-fluoride drinking water, both dental and skeletal fluorosis are common in these regions. The severe cases of skeletal fluorosis (32%) were reported from the study at Wonji (Tekle-Haimanot et al., 1987). The same study reported that the maximum prevalence is seen in the 10-14 years old age-group.

Figure 6: Skeletal fluorosis in Ethiopia (Tekele-Haimanot, 2005) as cited by *Kebede A. (2014)*

2.6 Fluoride intake assessment

Studies of fluoride ingestion were originally driven by efforts to better understand fluoride's role in pre-eruptive caries prevention, as it was believed that "the addition of fluoride to children's diets during the first seven or eight years of life offers great promise of reducing the prevalence of dental caries". In more recent times, studies of fluoride ingestion have focused on the timing of ingestion relative to the development of dental fluorosis(Levy et al., 2003). : The "optimal" intake of fluoride is between 0.05 and 0.07 mg fluoride per kilogram of body weight (mg F/kg bw) (Warren et al., 2009).

2.7 Analytical methods in determination of fluoride

The most common procedure used to quantify free fluoride anion is the fluoride ion-selective electrode. Micro diffusion techniques are considered to be the most accurate methods of sample preparation (i.e., the liberation of free ionic fluoride from organic and inorganic complexes) (WHO, 2002). Ethiopian standards have a method for the determination of fluoride from drinking water using a fluoride ion-selective electrode. This method is identical to ISO 10359-1:1992 as it reaffirmed in 2013 (ES, 2013). The alkali fusion method is well suited for analyzing fluoride in food using a fluoride ion-selective electrode. However, the neutralization process is time consuming and procedures to simplify this step should be encouraged (Malde & Bjorvatn, 2001).

2.8 Calcium intake and health

2.8.1 Sources of calcium

In many countries such as the USA, milk and milk product covers two-thirds of dietary calcium intakes (Heimbach & Mill, 1994). Indeed, calcium intake is usually associated with the intake of dairy products such as milk, yogurt, and cheese, as they are rich sources of calcium. Calcium-rich foods are dairy products, especially hard cheese that can provide 1 g of calcium per 100 g, whereas milk and yogurt can provide between 100 mg to 180 mg per 100 g. Cereals usually have around only 30 mg per 100 g. Nuts and seeds are also rich in calcium, especially almonds, sesame and chia that can provide between 250 to 600 mg per 100 g. Vegetables rich in calcium are kale, broccoli, and watercress, which provide between 100 and 150 mg per 100 g USDA (2016) as cited (Gabriela Cormick, 2019). Other data in the US reported that 73 percent of calcium intake is from milk products, 9 percent is from fruits and vegetables, 5 percent is from grain products, and the remaining 12 percent is from all other sources CNPP (1996) as cited by (Institute of Medicine, 1997). In Ethiopian diet, millet, enset (false banana), moringa, Ethiopian kale, and dairy products are the good sources of calcium (EPHI, 2013b; Kebede, et al., 2016; Tezera, et al, 2017). Supplements are also a dietary source of calcium for some populations. Some calcium supplements, available with no prescription, have up to 500-600 mg of calcium per tablet, which represents the nutritional requirements for most adults. However, the use of supplements also varies between countries (Gabriela Cormick, 2019).

2.8.2 Calcium intake and effect on health

Calcium accounts for 1 to 2 percent of adult human body weight. Over 99 percent of total body calcium is found in teeth and bones (Institute of Medicine, 1997). Adequate calcium intake has many health benefits besides its favorable effects on bone health. Enough consumption of calcium is associated with the reduction of the risk of high blood pressure, the risk of recurrent colorectal adenomas, low-density lipoprotein (LDL) cholesterol and it is associated with increasing high-density lipoprotein (HDL) cholesterol (Gabriela Cormick, 2019). Chronic calcium deficiency resulting from inadequate intake or poor intestinal absorption is one of several important causes of reduced bone mass and osteoporosis. Osteoporosis is characterized by reduced bone mass, increased bone fragility, and increased risk of fracture (Institute of Medicine, 1997). When calcium intakes are so low that plasma calcium ion concentrations cannot be maintained, calcium is mobilized from the skeleton (Theobald, 2005). Regarding the achievement of calcium recommendations at the population level, long-term beneficial effects can be expected, in the improvement of health (Gabriela Cormick, 2019).

2.9 Mitigation of dental and skeletal fluorosis

The major cause of fluorosis is the elevated level of fluoride in drinking water, nutrition, and health status, and deficiency of calcium and vitamins are important contributory risk factors for developing skeletal and dental fluorosis (Kaseva, 2006). The primary preferred option is to find a supply of safe drinking-water with safe fluoride levels. Where access to safe water is already limited, de-fluoridation may be sought as a solution. But the removal of excessive fluoride from drinking-water is difficult and expensive (Kebede et al., 2016b). Some studies have indicated that an increased intake of calcium (through for instance milk, yogurt and cheese), vitamins C (orange, carrots, lemons and tomatoes) D and E (nuts, beans etc.) and antioxidants (garlic, ginger, pumpkins) help reduce the fixation of fluoride to the apatite in the human bones (Susheela & Bhatnagar, 2002).

3. Materials and Methods

3.1. Study area

The study has been carried out in the Halaba zone, 85 km southwest of Southern Nation's Nationalities and People Regional State Capital, Hawassa. It is situated on an average altitude of 1800m above sea level with an average 750 mm annual rainfall which is classified as dry midland; in the Ethiopian Rift Valley. The total population is estimated to be 305,555, of which 85% of the population lives in 79 rural and the remaining in 5 urban Kebeles (small administrative units) (CSA, 2007). The livelihood of the people mainly depends on rain-fed agriculture producing crops like maize, sorghum, teff, millet, wheat, Haricot bean, red pepper, and potato. The major livestock species in the district are cattle, goats, sheep, donkeys, and poultry. Rainfall is the major limiting factor of agricultural production in the area. Due to lack or poor distribution of rainfall, Halaba is one of the districts in southern Ethiopia where drought occurs and affects many households.

Halaba has one district hospital and 79 health posts functioning in each rural Kebeles supported by 10 satellite health centers. There are currently 10,271 women health development armies (1,672 development team leaders and 8,599 leaders of 1 to 5 network) working in the district to support the HEP by mobilizing the community, passing health and nutrition messages, and positively influencing households to exercise proper health and nutrition care particularly for women and children. In Halaba woreda, the water fluoride level is above the recommended limit, which is 5 mg/l (Kebede et al., 2016 a).

Figure 7: The study area for dietary fluoride and calcium intakes and fluorosis assessment among school age children

3.2. Study design and subject

The study design of the thesis project was cross-sectional analysis. It was part of a larger project that was a randomized control trial. We used cross-sectional school children data from baseline-collected in January 2018. We have further collected the dietary data from the same subject in January 2019. The overarching project from which this subproject is derived has three study populations: young children, school children, and their mothers. Cluster sampling was used to determine one kebelle (Lay Arsho) which is participated in the study from the Halaba zone. From this kebelle (Lay Arsho), the households were selected randomly by the lottery method. The households had their eligible children. From each household, fluoride and calcium intakes were assessed by 24-hour dietary recall method and food frequency questionnaire (FFQ) for long term exposure.

3.3. Study period

The study was conducted in several stages. Dental, skeletal, and non-skeletal fluorosis assessment data was collected in January 2018. The dietary intake data, food, and water samples were collected in January 2019. Fluoride levels of food and water samples were analyzed within April and May 2019.

3.4. Population and sampling

3.4.1. Source population

The source populations for this study were all school-age children (6-13 years) who lived in Lay Arsho kebelle, Halaba SNNPR, Ethiopia.

3.4.2. Target population

The study population was all eligible school-age children. This age group was selected based on two reasons: First, the dietary intake status during this period is responsible for the health of the future life of adult health. Secondly, in this age group, dental fluorosis can be clearly identified.

3.4.3. Inclusion and exclusion criteria

The inclusion criteria of the subjects were:-

- School-age children (6-13 years),
- Being in good health (have no informed sickness)

The exclusion criteria were:-

- Sickness or illness

3.4.4. Sample size determination

The total sample size of the project was calculated by G*power software version 3.1.9.2 by considering the design effect (*DE* = 2) for cluster randomization, power (1- β) 100% to be 80% (Z_2=0.84) at 95% level of confidence (Z_1=1.96), medium effect size, d = 0.5 and 10% of loss to follow up (Table 2). Based on these assumptions, the total sample size becomes 126 households. From each HHs there are three population groups. These are young children, school-age children, and mothers. This study was taken the data of 127 school-age children for fluoride and calcium intake, and fluorosis assessments.

Table 2: Summary of assumptions used to determine sample size using G*Power software

Group	Confidence level	Power	Effect size (d)	The loss to follow up	Design effect	Sample size
Kebelle	95%	80%	0.5	10%	2	127

3.4.5. Sampling technique and randomization

From the Halaba zone, one kebelle (Lay Arsho) was selected by cluster sampling, from that kebelle, the required numbers of households were assigned randomly by a lottery system. Then from all selected households, dental and skeletal fluorosis levels of school-age children were assessed in January 2018. The dietary intake data of school-age children were collected by food frequency questionnaire (FFQ) and 24 hr- dietary recall method. Water and food samples were collected for fluoride level analysis.

During the dietary intake data collection, 20 types of food items consumed by the HHs were identified. From each category of identified food items, 1kg of food sample was collected from some of HHs by composite sampling method. At the study area, the community has used tap, ground, and spring water sources. One liter of water sample was collected from each source.

3.5. Study variables

3.5.1. Dependent variables

- Dental fluorosis
- Skeletal fluorosis
- Non-skeletal fluorosis

3.5.2. Independent variables

- Fluoride intake
- Calcium intake
- Age
- Sex
- Socioeconomic characteristics

3.6. Data collection

3.6.1. Data collection tools

- Questionnaires
- Weighing balance
- Measuring cylinder
- Soft paper
- Plastic bag
- Bowl
- Plate
- Plastic bottles
- Play dough
- Refrigerator

3.6.2. Dietary assessment procedures by FFQ and 24 hr. method

The type and amount of food consumed by the study participants were collected by 24-hour dietary recall method using a pre-structured questionnaire (annex II) developed based on internationally recognized multiple-pass method (Rosalind S. Gibson, 2008). The recall was repeated on a different day of the week in 20 samples to check their usual food intake. Additionally, the dietary intake data for children was collected by pre-structured FFQ using Wu et al., (2009) method for the estimation of long term exposure of fluoride and calcium. Bowls and plates were provided to mothers for use on the recall days to help them visualize and serve the amount of each staple food consumed by their child. The Ethiopia food composition table was used to estimate the dietary calcium intake of study subjects. Fluoride intake was estimated by using food and water fluoride content analyzed in this study, Dr. Aweke Kebede's findings 2015, and USDA data. We used the Willett (2013) procedure for the quantification of FFQ.data.

3.6.3. Dental and skeletal fluorosis assessment procedures

School-age children were assessed for dental fluorosis based on the Dean index (Dean, 1934). The study participants were told to clean their teeth thoroughly before the assessment. During the assessment, the level of dental fluorosis of the school-age children was classified into 6 categories (annex III). These are normal, questionable, very mild, mild, moderate, and severe fluorosis. Based on these criteria's the level of dental fluorosis was assessed by a qualified dentist in the study area.

Descriptions to score the level of dental fluorosis:

- **Normal condition (0)** - the apparent white spots depict "high lights" and are not mottled enamel. The surface is smooth, glossy, and usually of a pale creamy white color.
- **Questionable condition (0.5)** - the enamel discloses slight aberrations from the translucency of normal enamel, ranging from a few white flecks to occasional white spots. This classification is utilized in those instances where a definite diagnosis of the mildest form of fluorosis is not warranted and a classification of 'normal' is not justified
- **Very mild condition (1)** - Small opaque, paper white areas scattered irregularly over the tooth but not involving as much as25% of the tooth surface. Frequently included in this classification are teeth showing no more than about 1–2 mm of white opacity at the tip of the summit of the cusps of the bicuspids or second molars.
- **Mild condition (2)** - White opaque areas in the enamel of the teeth are more extensive but do not involve as much as 50% of the tooth.
- **Moderate condition (3)** - all enamel surfaces of the teeth are affected, and the surfaces subject to attrition, show wear. Brown stain is frequently a disfiguring feature.
- **Severe condition (4)** - includes teeth formerly classified as 'moderately severe and severe.' All enamel surfaces are affected and hypoplasia is so marked that the general form of the tooth may be affected. The major diagnostic sign of this classification is discrete or confluent pitting.

Skeletal fluorosis (excessive deposition of fluoride in the bone) rates among school-age children were assessed by a qualified physiotherapist in the study area using clinical symptoms and physical exercises (Annex VI) as developed by Susheela and Shashi(Shashi et al., 2008; Susheela & Bhatnagar, 2002). According to Susheela *et al* (2002), individuals who could not perform the physical exercise in the endemic areas were categorized as having skeletal fluorosis. In the study participants, stiffness of the back and neck muscles, unable to bend forward and to stand straight are some of the indicators of skeletal fluorosis; a pathological condition that is by far the most important aspect of chronic exposure to elevated levels of fluoride, either by inhalation or by ingestion (Shashi *et al*., 2008).

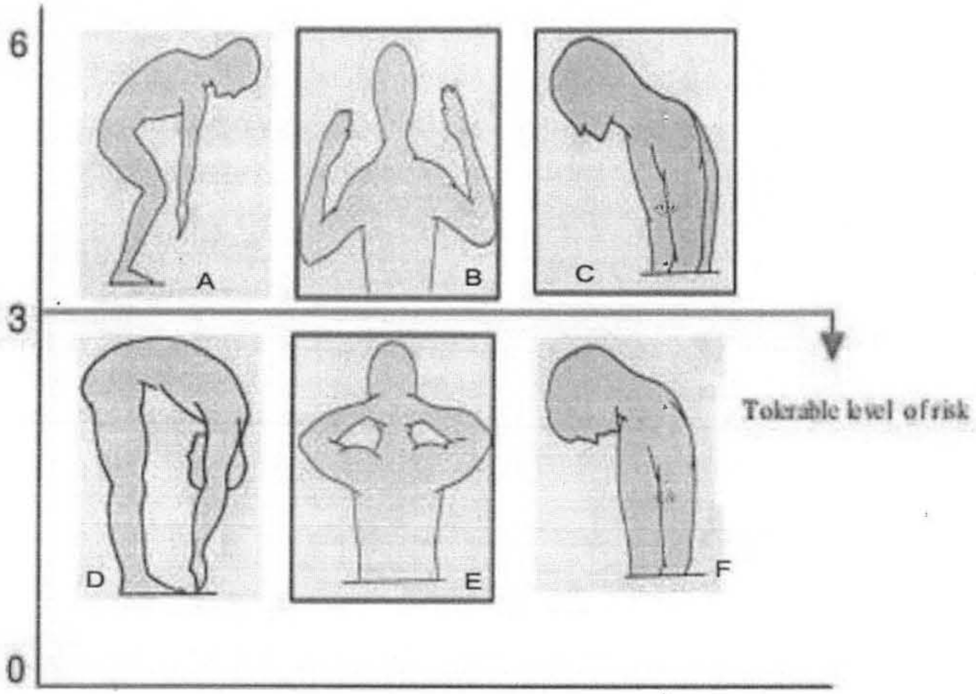


Figure 8: Physical exercises used to identify skeletal fluorosis and tolerable level of risk

Fluoride toxicity manifestation (Exercises):

A = Unable to bend without folding knees (SF1)

B = Unable stretch hands, fold arms and touchback of head (SF2)

C = Unable to bend neck-touching chest with chin not possible (SF3)

Normal healthy individual (Exercise):

D = Can bend the body and touch the floor/toes

E = Can stretch hands, fold arms and touchback of head

F = Can touch the chest with chin

It has to be noted that the dental and skeletal fluorosis level assessment was carried out by recruited qualified dentist and physiotherapist, respectively.

3.7. Food and water samples preparation and analysis

The food samples which are collected from different HHs were dried, ground, and homogenized using drying oven, miller, and homogenizer respectively for fluoride analysis.

3.7.1 Moisture content determination of food sample

The moisture content of food samples was determined by using the AOAC 925.10, 2016 method. A clean box on its inverted lid was dried in the drying oven at 100 °C for 30 minutes. The boxes were covered with their lid and cooled with desiccator for 30 minutes, then the weight was recorded. 2 g of well-mixed test portion was accurately weighed and transferred to the dried box. The samples were dried for 1 hour at 130 °C. The drying boxes were transferred to the desiccator and stayed there for 30 minutes to reduce the temperature to room temperature and weighed. The losses in weight were taken as the moisture content of food samples.

3.7.2 Determination of fluoride from water and food samples

Water fluoride was determined by using ES ISO 10359-1: 2001 method, whereas the food fluoride level was measured by the food fluoride test method (Malde & Bjorvatn, 2001).

3.7.2.1 Reagent preparation

A. 5 M Sodium hydroxide (NaOH): 100 g of NaOH was dissolved in distilled water within a 500 ml volumetric flask.

B. 8 M Sodium hydroxide (NaOH): 160 g of NaOH was dissolved in distilled water within a 500 ml volumetric flask.

C. TISAB (Total ionic strength adjustment buffer) solution: 58 g sodium chloride, 2 g EDTA, 7 g tri-sodium acetate, 57 mL glacial acetic acid, and 500 mL distilled water were dissolved. The pH of the solution was adjusted to 5.0 to 5.5 by using 5 M NaOH solution and the volume was adjusted to 1000 ml with distilled water.

D. Standard solutions: Stock standard (1000ppm) was prepared by dissolving 2.21g of sodium fluoride (NaF) with 1 liter of distilled water. 100ppm, 10ppm, 5ppm, 1ppm, and 0.1ppm working standards were prepared from the stock standard by using consecutive dilutions.

3.7.2.2 Water fluoride analysis

5 ml of buffer solution was pipetted followed by 5 ml of the water sample, into a measuring cell. An equal amount of buffer solution was also added for working standards. The electrode was calibrated by working standards starting from most diluted to concentrated standard. The concentration of the solutions was directly measured in ppm by using a fluoride ion-selective electrode.

3.7.2.3 Food fluoride analysis

0.5g samples were weighed to the nearest 0.0001 g directly into nickel crucibles. The samples were covered with 5.0 ml of 8 M sodium hydroxide solution. The sample and sodium hydroxide solution was carefully mixed. The crucibles were put on a hot plate for evaporation to dryness before they were covered and put into the muffle furnace for combustion. The temperature program for the muffle furnace was set at 200 °C for approximately 16 h after which the temperature was increased to 525 °C and kept there for 3 h. The crucibles were cooled, 15 ml distilled water was added, and the crucibles were put on a hot plate to aid the dissolution of the fusion cake. After approximately 2 h, the sample solutions were transferred to 50 ml capped plastic tubes. The sample solutions were neutralized using concentrated and then diluted hydrochloric acid. Concentrated hydrochloric acid was added drop-wise until the pH decreased from 12.0-13.0 to 8.0-8.5. Diluted hydrochloric acid was added drop-wise until the pH decreases from 8.0-8.5 to 7.2-7.5. pH meter was calibrated by pH buffers 7.0 and 10.0. The sample solution was diluted to 50 ml solution with distilled water. The solutions were stored in 50 ml air-tight plastic tubes until analyzed. Aliquots of 5 ml were taken out for analysis after the ash had settled and the solutions were clear. Care was taken to avoid the settled ash. Before analyzing, 0.5 ml TISABIII was added to obtain a pH of 5.2-5.4, which is the optimal pH-range for fluoride determination. Reagent blanks were always prepared together with the samples and were brought through the whole procedure. The fluoride level of standard solutions and sample solutions were measured in ppm using a fluoride ion-selective electrode. For all food samples, 1ml of 100ppm fluoride standard was spiked for the verification of the accuracy of the method in terms of % recovery. Recovery (%) was calculated as the following formula (Patnaik, 1997):-

$$\% \text{ Recovery} = \frac{\text{Measured concentration}}{\text{Theoretical concentration}} * 100 \text{ ---------------------- Equation 1}$$

Theoretical concentration $=\frac{(Cu*Vu)}{(Vu+Vs)}+\frac{(Cs*Vs)}{(Vu+Vs)}$ -------------------------Equation 2

Where:-

- C_U = measured concentration of the unknown sample,
- C_S = concentration of the standard,
- V_U = volume of the unknown sample and
- V_S = volume of the standard.

3.8. Data analysis

Nutri-survey, Microsoft excel, and SPSS version 20 software were used for dietary and fluorosis data analysis. 24-hour data was estimated by Nutri-survey software whereas Microsoft excel was used for FFQ data estimation. The descriptive and significance test analysis were performed by SPSS version 20.

3.9. Ethical clearance

Ethical approval was obtained from Addis Ababa University (AAU). A letter of support was]obtained from the regional health offices of SNNPR. The study was conducted according to rules and guidelines of the Research and Ethical Clearance committee (RECC) of Addis Ababa University which is called CNS-IRB. Woreda (district) health, Kebele (subdistrict) administrators and health extension workers have briefed the objective of the study. The HHS was requested for willingness to provide information for dietary data collection and their willingness for dental and skeletal fluorosis assessment. The study participants were informed about the objective of the study. The households who participated in the study were compensated by soap for time wasted for responding to the questionnaire. They were told about the right to withdraw from the study.

4. Results

4.1 Socio-demographic characteristics

The main source of income for all households included in this study was farming. Most (92.5%) of the households were Muslim by religion. For the majority (67.7%) of households, motor pump groundwater was the source of drinking. The mean age (SD) of school children was 7.8 (1.9) years. About one-third (34.6%) of school-age children had not attended formal school education. The average family size (number of parents and children living together) was approximately 7 ± 2 (Table 3).

Table 3: Socio-demographic characteristics of school-age children (n=127) aged 6-13 years from selected one kebelles, Halaba zone, SNNPR Ethiopia, 2019

Variables	**Frequency (%) /mean ±SD**
Occupation	
Farming	127 (100)
Religion	
Orthodox Christian	2 (1.6)
Muslim	118 (92.9)
Protestant Christian	7 (5.5)
Educational status	
Educating	83 (65.4)
Non-educating	44 (34.6)
Age (years)	7.82 (1.925)
Sex	
Male	68 (53.5)
Agricultural land size	
</= ½ hectare	108 (85)
> ½ hectare	19 (15)
Family size	7.13 ± 1.55
No. of males	3.36± 1.349
No of Females	3.72 ± 1.384

Sources of drinking water	
Motor pump groundwater	86 (67.7)
Springwater	36 (28.3)
Tap water	5 (3.9)

4.2 Biochemical results

4.2.1 Water fluoride

The contents of fluoride for tap, motor pump groundwater, and spring water were 4.73, 6.21, and 3.33 mg/l respectively, which exceeds WHO guidelines for drinking water 1.5 mg/l. The mean water fluoride level at lay Lay arsho woreda was 4.76 mg/l.

Table 4: Fluoride levels of water collected from different sources from Halaba SNNPR, Ethiopia, 2019

Water type	Fluoride level in mg/L	Average
Tap water	4.73	4.76
Motor pump ground water	6.21	
Spring water	3.33	

4.2.2 Moisture and fluoride levels of foods and beverages

The fluoride content of selected food items varied between 0.83 – 13.61 mg/kg as shown in Table 5. Food items that were prepared from maize and millet had a high concentration of fluoride. These types of food items are the staple foods for the community in the study area. The contribution of these types of staple foods is crucial for increasing excess fluoride consumption as their fluoride level is high. However, vegetable sources such as cabbage and potato had a low concentration of fluoride. For all food items, % recovery was varied between 90.4 - 107.89%, which falls on an acceptable range of % recovery.

Table 5: Moisture content and fluoride levels of foods and beverages collected from Halaba zone SNNPR Ethiopia, 2019

Sample Type	Moisture (%)	Fluoride (mg/kg)	Recovery (%)
Injera (unknown ingredients)	49.5	10.61	100.64
Unleavened bread (unknown ingredients)	36.7	13.61	97.25
Unleavened bread, maize	39.5	12.51	102.48
Taro, Colacasia antiquorum: boiled	72.1	4.61	96.19
Injera (Maize, Millet (1:1))	54.4	8.28	102.54
Unleavened bread, millet	30.2	4.89	93.70
Boiled cabbage	92.8	0.83	103.18
Boiled potato and carrot without pepper	81.2	1.6	92.21
Stew without tomato	77.9	3.87	103.60
Banana	73.7	5.26	107.89
Boiled beetroot	83.6	3.2	102.68
Cabbage and potato stew	81.4	2.42	95.92
Rice with carrot	74.6	3.18	103.64
Injera (Sorghum, maize, millet (1:1:1))	52.9	4.95	95.36
Injera (millet)	55.2	7.84	105.68
Bread (maize)	41.7	10.49	99.02
Coffee (prepared from coffee leaf)	-	3.5	104.45

4.3 Dietary diversity and dietary intakes

4.3.1 Diet diversity score

Out of seven food groups, the mean diet diversity score was 3.13. The study showed that their diet was predominantly cereal-based. Legumes and nut products were consumed by 30.7% of children, whereas 9.4% of children consumed dairy products. Poultry products, meat, and fish products were not consumed at all children. Vitamin A-rich fruits and vegetables were consumed by 84.3 % of children, whereas 89.8% of children consumed other fruits and vegetables.

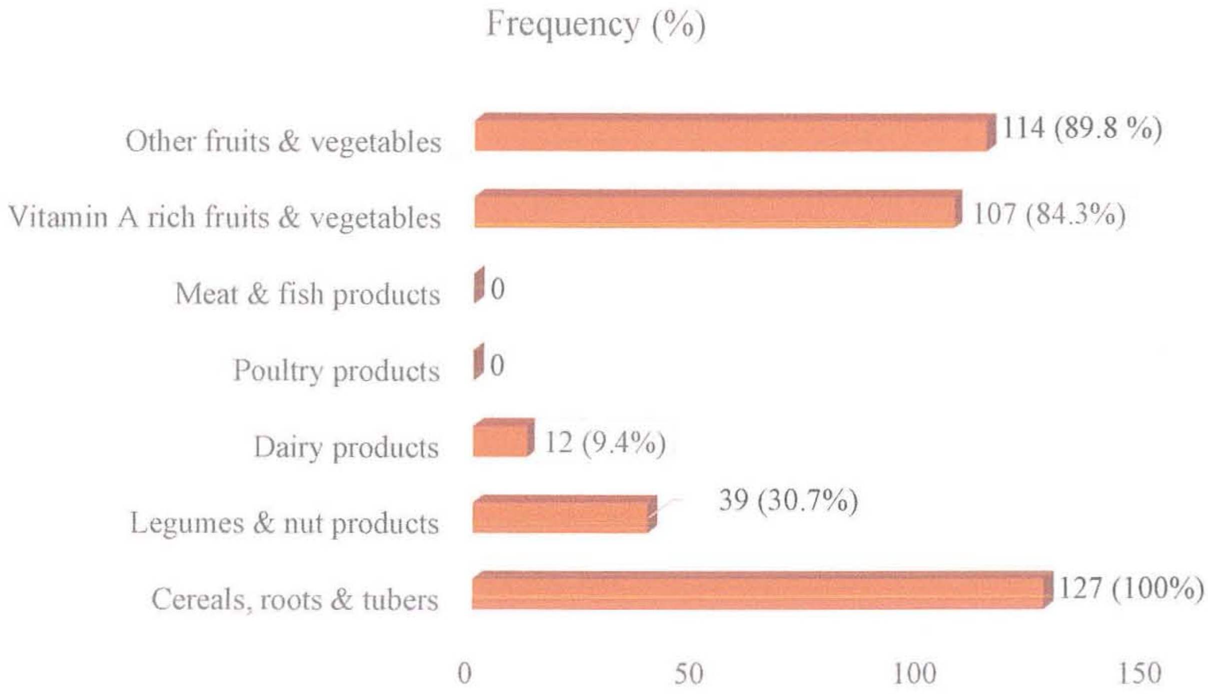


Figure 9: Consumption (%) of seven food groups in Halaba zone, SNNPR Ethiopia

4.3.2 Energy and nutrient intakes from 24-hour data

The average energy intake was 1640 kcal/day, which less than the recommended intake. However, the average protein intake (42 g/day) fulfills the recommended intake. As the study showed, vitamin A intake 581 μg/day fulfilled an estimated intake of WHO. At the age of 4-6 years, vitamin B1 and B2 intakes were above estimated needed whereas at the age of 7-13 years vitamin B1 and B2 intakes were below the recommended intakes. The average intake of vitamin C was 23.4 mg/day which was below the recommended intake. Calcium intake of school children was too low (on average 520 mg/day) compared to the recommended intake at all age categories. The mean fluoride intake was 70 mg/day, which much exceeds the recommended intake of fluoride for school children. There was enough consumption of iron and zinc-based on WHO and FAO guidelines. Estimated needed to be determined by estimated energy requirement value for energy and recommended dietary allowance value for protein (Ellie Whitney, 2008), recommended nutrient intake for minerals and vitamins (FAO/WHO, 1998), recommended dietary allowance for calcium and adequate intake, tolerable upper intake level, and reference weight for fluoride (Institute of Medicine, 2011). The values of energy and nutrients are summarized in table 7, Figures 10 and 11.

Table 6: Mean vitamins and minerals intake of school children (6-13years) in comparison with estimated needs from 24-hour data, Halaba Zone selected kebelle, SNNPR, Ethiopia (2019)

Energy and nutrients (/day)	**All age** (N=127)	**Age 4-6 years** (N=10)	**Age 7-9 years** (N=79)	**Age 10-13 years** (N=38)
Vitamin A (μg)	581.4	598.3	533.5	676.4
Estimated needed		450	500	600
Vitamin B1 (mg)	0.71	0.71	0.72	0.68
Estimated needed		0.6	0.9	1.1-1.2
Vitamin B2 (mg)	0.82	0.80	0.84	0.79
Estimated needed		0.6	0.9	1.0-1.3
Vitamin C (mg)	23.4	16.3	20.8	30.6
Estimated needed		30	35	40
Iron (mg)	78.28	74.95	78.02	79.7
Estimated needed		12.6L, 6.3M	17.8L,8.9M	29.2L, 14.6M
Zinc (mg)	9.41	9.94	9.1	9.91
Estimated needed		4.8	5.6	7.2

L- Low bioavailability, M- High bioavailability

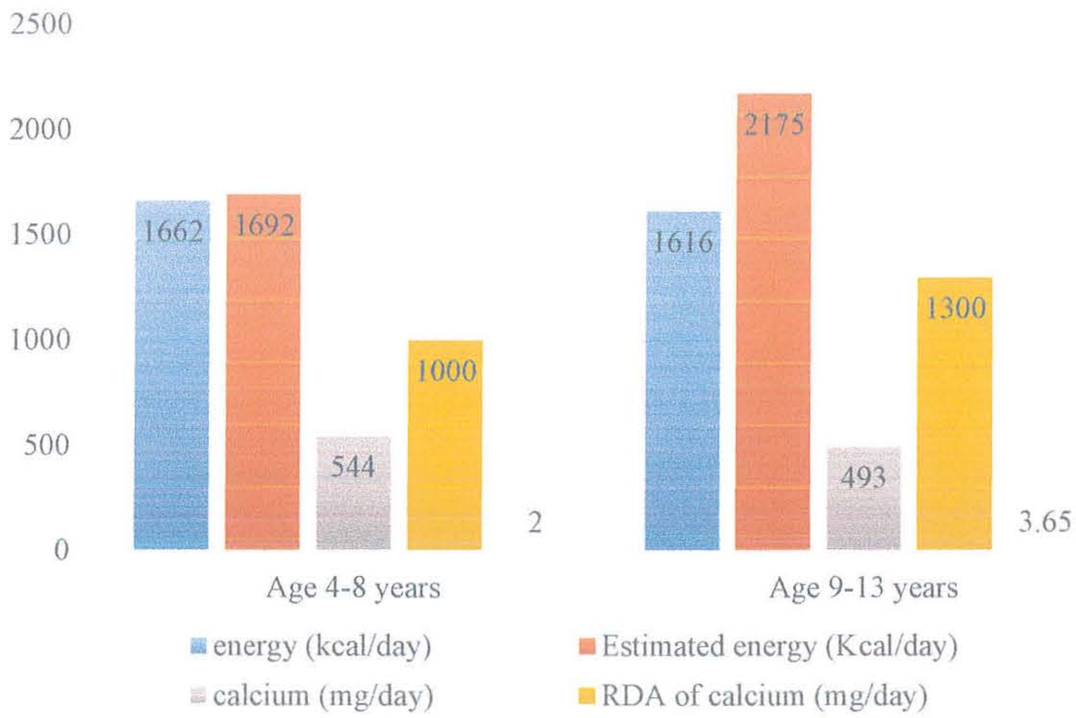


Figure 10: Mean energy and calcium intake of school children 6-13years (N=127) in comparison with estimated needs from 24-hour data, Halaba Zone selected kebelle, SNNPR, Ethiopia (2019)

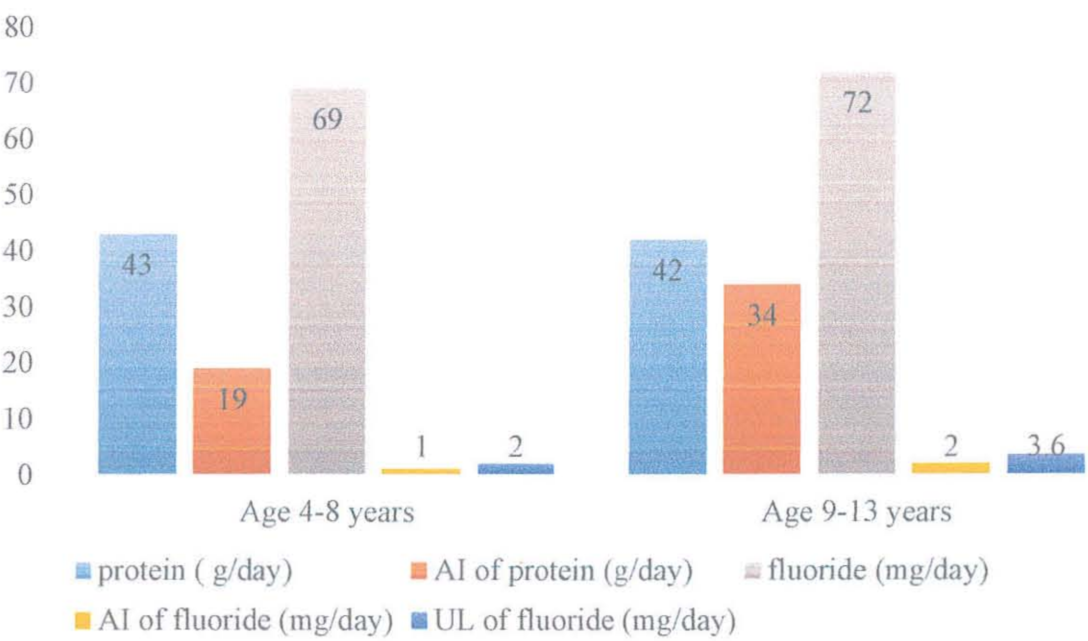


Figure 11: Mean protein and Fluoride intake of school children 6-13years (N=127) in comparison with estimated needs from 24-hour data, Halaba Zone selected kebelle, SNNPR, Ethiopia (2019

4.3.3 Calcium and Fluoride intakes from FFQ data

The average calcium intake of school children was 452.2 mg/day which is less than the requirements. There was a high consumption of dietary fluoride 9.02 mg/ day, which exceeds the recommended limit.

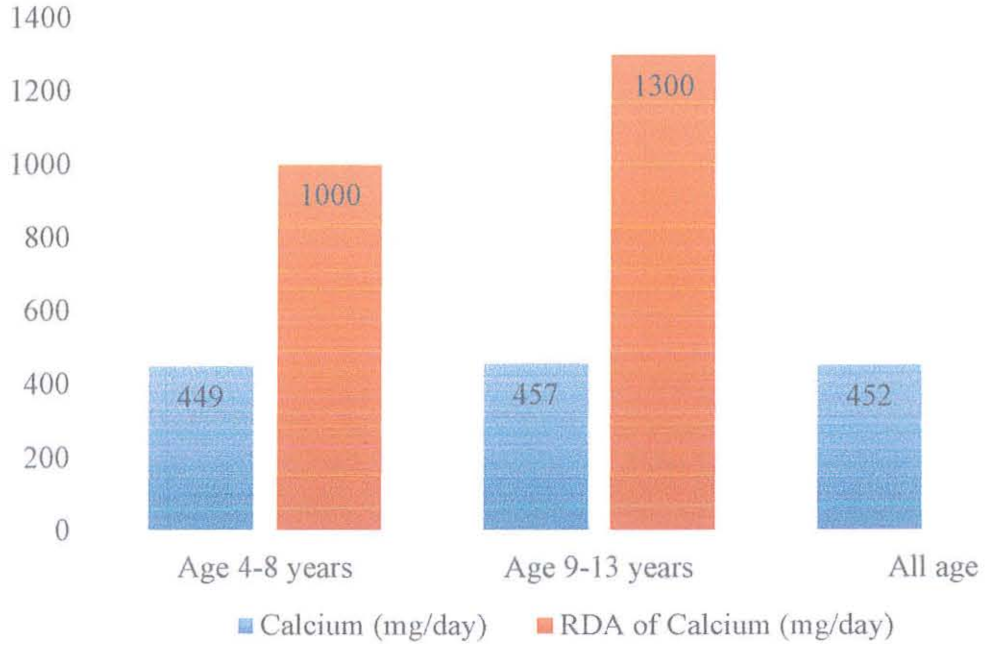


Figure 12: Calcium intake of school children 6-13 years (N=127) in comparison with RDA from FFQ data, Halaba Zone selected kebelle, SNNPR, Ethiopia (2019)

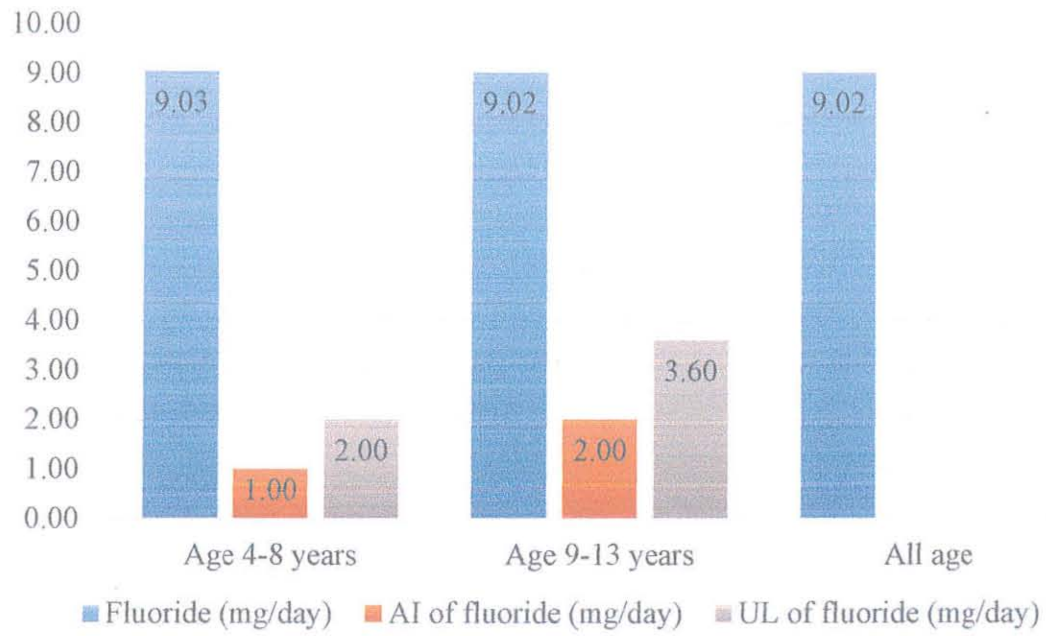


Figure 13: Fluoride intake of school children 6-13 years (N=127) in comparison with estimated needs from FFQ data, Halaba Zone selected kebelle, SNNPR, Ethiopia (2019)

4.4 Dental, skeletal, and non-skeletal fluorosis level

4.4.1 Dental fluorosis

The overall prevalence of dental fluorosis (from very mild to severe symptoms) was around 70.9%. The prevalence of severe dental fluorosis was 5.5% (Figure 14).

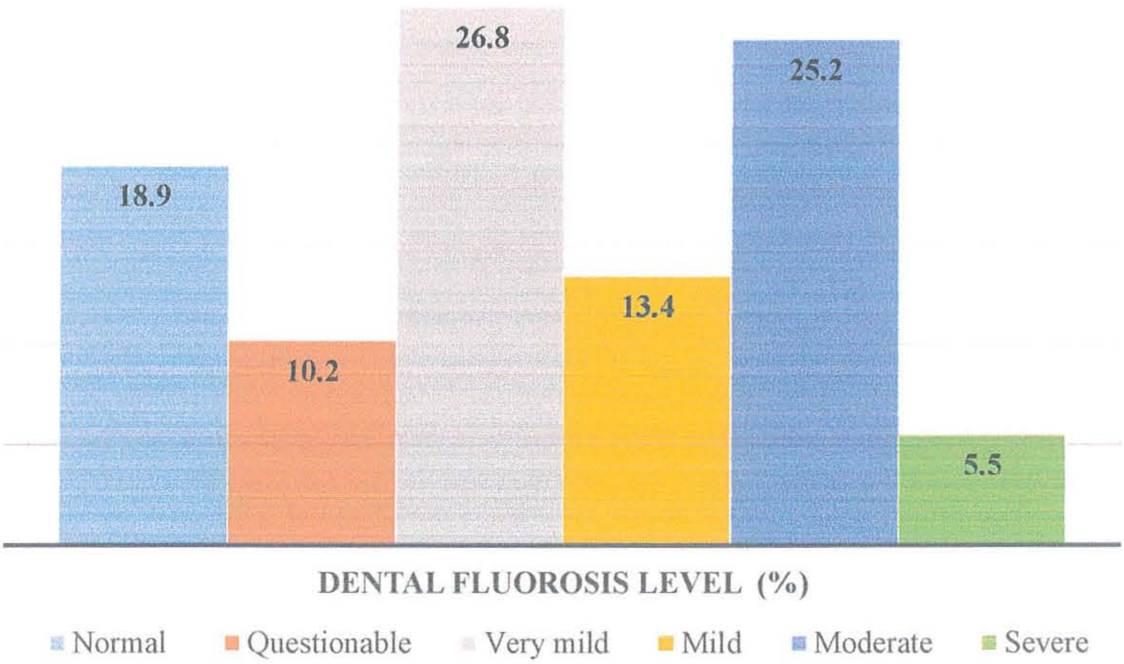


Figure 14: levels of dental fluorosis among school children (N=127) in Halaba, SNNPR Ethiopia, 2019

4.4.2 Skeletal fluorosis

The prevalence of skeletal fluorosis was varied between 27.6- 44.9%. The majority (44.9%) of the children were unable to bend body touch floor or toe and 27.6 % of children were unable to stretch and fold arms to touch back of the head.

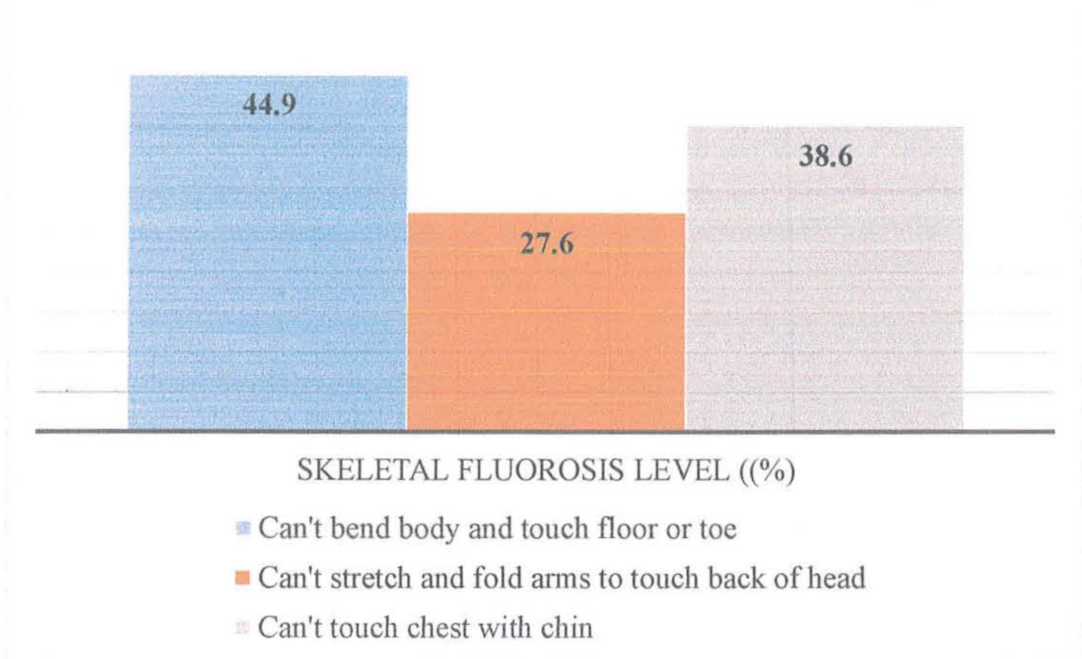


Figure 15: Skeletal fluorosis level among school children (N=127) in Halaba SNNPR, Ethiopia 2019

4.4.3 Non-skeletal fluorosis

The prevalence of non-skeletal fluorosis symptoms varied between 7-70% as shown in Table 7.

Table 7: Prevalence of non-skeletal fluorosis levels among school children SNNPR, Ethiopia 2019

Non- skeletal fluorosis Signs	Frequency	Fluorosis prevalence (%)
Feel lower back pain	47	37
Feel leg pain, joints	12	9.4
Feel arm pain, joints	7	5.5
Feel the tingling sensation in hands and feet	10	7.9
Feel neck pain with movement	62	48.8
Feel muscle weakness	39	30.7
Feel the loss of appetite	36	28.3

Have nausea	47	37
Have abdominal pain	69	54.3
Have bloating in the stomach	39	30.7
Experience polydipsia (excessive thirst)	12	9.4
Experience polyuria (excess urine volume)	24	18.9
Experience constipation	70	55.1

4.5 Associations between calcium and fluoride intakes and fluorosis

4.5.1 Associations with dental fluorosis

The dietary fluoride intake of children was significantly associated ($P < 0.01$) with dental fluorosis level. The dietary calcium intake of children also significantly associated ($P < 0.01$) with dental fluorosis level. The mean dietary calcium intake was 520 mg/d which is much lower than the daily recommended intake by children.

Table 8: Predictors of fluorosis symptoms (Dental fluorosis) among school children from 24-hour dietary data: logistic regression model, Halaba zone, SNNPR Ethiopia, 2019

Variable (n=270)	**Dental fluorosis[1]**	
	COR (95%CI)	**AOR (95%CI)**
Dietary fluoride	1.042 (1.019, 1.067)**	1.033 (1.006, 1.060)*
Dietary calcium	0.994 (0.992, 0.997)**	0.995 (0.992, 0.998)**
Age (4-8 years)	1.262 (0.586, 2.715)	1.898 (0.739, 4.870)
Sex (Female)	0.883 (0.410, 1.901)	0.730 (0.287, 1.854)

1. The six symptoms of dental fluorosis were categorized in to categorical variable (present=1 or absent =0) regression analysis. The first group was DF absent (normal and questionable symptoms) and the second group was DF present (very mild, mild, moderate, and severe symptoms).

*There was significant associations at $P < 0.05$, **There was significant associations at $P < 0.01$.

4.5.2 Associations with skeletal fluorosis

The dietary fluoride intake of children was significantly associated (at $P < 0.05$) with their skeletal fluorosis level of each physical activity test, but hadn't association when different factors were considered. Calcium intake and SF2 had an association (at $P < 0.05$) without considering other factors, but not associated with the presence of other factors. SF1 and SF3 levels of children hadn't association (at $P < 0.05$) with their dietary calcium intake per day with and without considering the other factors, however, SF1 is associated (at $P < 0.05$) with calcium intake when other factors were considered. The age of children and SF2 are highly associated ($P < 0.05$ and 0.01) in the presence and absence of factors, but SF1 had not an association in both conditions. SF3 was associated (at $P < 0.05$) with age in the presence of the other factors, but not associated with the absence of the other factors.

Table 9: Predictors of fluorosis symptoms (Skeletal fluorosis) among school children from 24-hour dietary data: logistic regression model, Halaba zone, SNNPR Ethiopia, 2019

'ariable	SF Level 1[a]		SF Level 2[b]		SF Level 3[c]	
n= 127)	COR (95%CI)	AOR (95%CI)	COR (95%CI)	AOR (95%CI)	COR (95%CI)	AOR (95%CI)
ietary luoride	0.985 (0.971, 1.000)*	0.991 (0.975, 1.007)	0.980 (0.964, 0.996)*	0.985 (0.968, 1.003)	0.984 (0.969, 0.999)*	0.986 (0.971, 1.003)
ietary alcium	1.002 (1.001, 1.004)	1.002 (1,000, 1.004)*	1.003 (1.000, 1,005)*	1.002 (0.999, 1.004)	1.002 (1.000, 1.004)	1.001 (0.999, 1.003)
ge	0.934 (0.777, 1.123)	0.946 (0.781, 1.148)	0.761 (0.619, 0.935)**	0.765 (0.616, 0.950)*	1.187 (0.972, 1.449)	1.235 (1.001, 1.524)*
ex	0.823 (0.408, 1.660)	0.823 (0.395, 1.715)	1.042 (0.477, 2.276)	1.000 (0.430, 2.328)	1.108 (0.541, 2.269)	1.236 (0.582, 2.623)

*There was significant associations at $P < 0.05$. **There was significant associations at $P < 0.01$.

a. SF1 is symptoms of skeletal fluorosis with physical exercise "can't bend body touch floor or toe"

b. SF2 is symptoms of skeletal fluorosis with physical exercise "can't stretch and fold arms to touch back of the head"

c. SF3 is symptoms of skeletal fluorosis with physical exercise "can't touch the chest with the chin"

5. Discussion

In this study high prevalence of dental fluorosis among school-age children has been shown. Besides dental fluorosis, a high prevalence of skeletal and non-skeletal fluorosis was seen in the study area. The dietary fluoride intake of school children much exceeds the upper tolerable intake level at this age level. However, the dietary calcium intake was below the recommended limit. Our findings showed that dietary fluoride and calcium intakes were significantly associated with dental and with some aspects of skeletal fluorosis. This showed that excess dietary fluoride intake and low consumption of dietary calcium increases the risk of dental and skeletal fluorosis in school-age children.

Energy, calcium, and other micronutrients (except fluoride) were below the recommended limit. The result supported by the national food consumption survey, which confirmed that low diet diversity results decrease in nutrient intake below the recommended level (EPHI, 2013a). However, protein intake meets the recommended level for school children. It is expected that from the staple foods in the study area, like millet, maize, sorghum, teff, and bean have enough amount of protein. The total fluoride intake by children was above the recommended upper tolerable intake level of 0.1 mg/kg/body wt.(Institute of Medicine, 1997). Water is the major source of excess fluoride intake in that community. In addition to water, food and beverages have a significant contribution to excess fluoride intakes by children. This finding supports previous studies result in Ethiopia (Kebede, et al., 2016; Malde, et al., 2011). In tropical areas, the high fluid intake increases the exposure to fluoride consequently to fluorosis risk (Murray, 1986).

Dental fluorosis is a dose-response effect caused by excess fluoride ingestion during the pre-eruptive development of teeth (Institute of Medicine, 1997). According to the result obtained in this study, the prevalence of dental fluorosis was very high. From this prevalence, 25.2 % of children showed moderate dental fluorosis (all surfaces affected, with some brown spots and marked wear on surfaces subject to attrition) and 5.5 % of them affected by severe dental fluorosis (widespread brown stains and pitting). This figure shows an increase in the risk of dental fluorosis compared to a previous study at Halaba 17 % (moderate) and 3 % (severe) (Kebede, et al., 2016a). As the intakes of fluoride at the study site have been above the tolerable upper intake level (0.1 mg/kg/body weight), the maximum prevalence in dental fluorosis is expected.

The result of skeletal fluorosis found in this study conducted using physical exercise showed, 44.9 % of children can't bend the body and touch floor or toe, 27.6 % of them can't stretch and fold arms to touch back of the head, and 38.6 % of children can't touch the chest with chin. This result was supported by the study conducted in Ethiopia, which found that linear relationships were observed between the development of skeletal fluorosis, fluoride concentration of drinking water and period of exposure to it (Tekle-Haimanot R, et al., 1987). The development of skeletal fluorosis and its severity is directly related to the level and duration of exposure to excess fluoride. Most epidemiological research has indicated that an intake of at least 10 mg/day for 10 or more years is needed to produce clinical signs of the milder forms of the condition (Institute of Medicine, 1997). According to the dietary fluoride intake result found in this study, most children exceed 10mg/day. This might be the cause of the presence of a high prevalence of skeletal fluorosis together with other factors in children. Another study conducted in the animal model also supports that, highly fluoridated water decreased bone strength when given to rats for a prolonged period (Turner et al., 1995).

In addition to adverse health effects on calcified tissues (bone and teeth), fluoride has an acute toxic effect which is called non-skeletal fluorosis. In acute poisoning of fluoride, death is a likely result within 2-4 hours when an average adult consumes about 5 grams of sodium fluoride. Nausea, vomiting, and abdominal cramping are some of the early symptoms followed by excess fluoride intake (Cerklewski, 1997). Among the thirteen clinical symptoms of non-skeletal fluorosis, we found that the prevalence was varied between 5.5-55.1% of children. It is expected result because dietary fluoride intakes of children exceeded the recommended limit.

The multivariate analysis showed that dietary calcium and fluoride intakes had a strong significant relationship with dental and skeletal fluorosis levels of children. According to Cerklewski (1997) report calcium form insoluble complexes with fluoride which significantly decreases fluoride absorption and its uptake into bone and teeth. The other study supported that when fluoride is ingested together with calcium-rich foods, the bioavailability decreases by 40% (Murray, 1986). Previous studies report and the result in this study showed a low level of calcium intake below the recommended limit, which was highly associated with an increase in fluoride absorption. The study conducted in Ethiopia found that age, sex, and milk consumption were significantly associated with DF severity (Kravchenko et al., 2014). Besides its positive effect on human health, fluoride can become toxic when the quantity ingested exceeds the body's homeostatic mechanism to adequately respond to the challenge (Cerklewski, 1997). The dietary fluoride intake of children much exceeded the upper tolerable intake level and has strong relationships with a high prevalence of dental and skeletal fluorosis. Based on this evidence and our result, it can be said that excess fluoride intake has a significant effect on both the dental and skeletal fluorosis risk of children.

According to the result obtained the age of children has an association in SF2 and SF3, however, it has no relationship with their SF1 and dental fluorosis status. Different reports support age has a relationship with fluorosis (Evans, 1989; Thylstrup & Larsen, 1977).

Different limitations need to be considered when interpreting the result obtained. First, the cross-sectional nature of dietary assessment does not allow inferences to be made on causality and rather indicate associations with chronic effects (fluorosis). In addition to that, the symptoms for skeletal fluorosis assessment are not specific signs. Dietary data were collected in one season, which may not indicate the usual intake of school-age children. Furthermore, some food composition data for fluoride were taken from USDA, which may greatly varied from the Ethiopian diet. We used the average fluoride result from different water sources.

6. Conclusions and recommendations

The risk of dental, skeletal, and non-skeletal fluorosis was the major health concern in the community. The dietary fluoride intake was much higher than the upper tolerable intake limit of 0.1mg/kg/body weight recommended by the Institute of Medicine. Water is the major source of fluoride, followed by food and beverage sources. The water fluoride level (4.76 mg/L) consumed by the community exceeds the WHO guideline (1.5 mg/L) for drinking water. The dietary calcium intake of children is below the recommended intake level. Excess fluoride intake and low consumption of dietary calcium were the causes of dental and skeletal fluorosis risks of children.

To mitigate the risk of fluorosis results from excess fluoride intake, improving the dietary intake of calcium is recommended. Further, water de-fluoridation may also be needed as immediate strategies. Hence, intervention to improve calcium statuses such as consumption of calcium-rich foods like Ethiopian kale, millet, and enset is needed in the study area. Further studies on the effect of fluoride on improving the awareness of the community about fluoride toxicity and mitigation of fluorosis using calcium supplementation are needed.

7. References

Akosu, T. J., & Zoakah, A. I. (2008). Risk factors associated with dental fluorosis in Central Plateau State, Nigeria. *Community Dentistry and Oral Epidemiology*, *36*(2), 144–148. https://doi.org/10.1111/j.1600-0528.2007.00387.x

Alvarez, J. A., Rezende, K. M. P. C., Marocho, S. M. S., Alves, F. B. T., Celiberti, P., & Ciamponi, A. L. (2009). Dental fluorosis: Exposure, prevention and management. *Medicina Oral, Patologia Oral y Cirugia Bucal*, *14*(2).

Barbier, O., Arreola-Mendoza, L., & Del Razo, L. M. (2010). Molecular mechanisms of fluoride toxicity. *Chemico-Biological Interactions*, *188*(2), 319–333. https://doi.org/10.1016/j.cbi.2010.07.011

Cerklewski, F. L. (1997). Fluoride bioavailability - Nutritional and clinical aspects. *Nutrition Research*, *17*(5), 907–929. https://doi.org/10.1016/S0271-5317(97)00057-2

CSA, 2007, Population and housing census report SNNPR part I: population size and characteristics, *CSA*, page 1-442.

Dean, H. T. (1934). Classification of Mottled Enamel Diagnosis. *The Journal of the American Dental Association*, *21*(8), 1421–1426. https://doi.org/10.14219/jada.archive.1934.0220

Ellie, W., & Rolfes, S. R. (2008). Understanding Nutrition (11th edition).

EPHI. (2013a). Ethiopia National Food Consumption Survey. *Ethiopian Public Health Institute*, *3*, 54–67.

EPHI. (2013b). Food composition table for for use in Ethiopia.

Erdal, S., & Buchanan, S. N. (2005). A quantitative look at fluorosis, fluoride exposure, and intake in children using a health risk assessment approach. *Environmental Health Perspectives*, *113*(1), 111–117. https://doi.org/10.1289/ehp.7077

ES. (2013). Water quality - Determination of fluoride - ES ISO 10359-1:2001. *Ethiopian Standards Agency*.

Evans, R. W. (1989). Changes in dental fluorosis following an adjustment to the fluoride concentration of HONG KONG ' S water supplies. *Adv Dent Res*, *3*(2), 154–160.

Gabriela Cormick, J. M. B. (2019). Calcium Intake and Health. *Nutrients*, 1–16.

Gibson, RS and Ferguson, E.L.. (2008). An interactive 24-hour recall for assessing the adequacy of iron and zinc intakes in developing countries. *Intereconomics* (Vol. 8). https://doi.org/10.1007/BF02927624

Heimbach, T., & Mill, T. F. (1994). Consumption of Calcium in the U. S.: Food Sources and Intake Levels. *American Institute of Nutrition*, *94*, 1426–1430.

Horowitz, H. S. (2003). The 2001 CDC Recommendations for Using Fluoride to Prevent and Control Dental Caries in the United States. *Journal of Public Health Dentistry*, *63*(1), 4–9.

Hussain, J., Sharma, K. C., & Hussain, A. (2004). Fluoride in Drinking Water in Rajasthan and Its Ill Ef- Fects on Human Health. *Journal of Tissue Research*, *4*(2), 263–273. Retrieved from http://www.tcrjournals.com/uploads/9621216._Hussain.pdf

Institute of Medicine. (1997). "Dietary Reference Intakes for Calcium, Phosphorus, Magnesium, Vitamin D, and Fluoride. " *National Academic Press Washington, D.C. Copyright* (Vol. 55). https://doi.org/10.1111/j.1753-4887.1997.tb0162.x

Institute of Medicine. (2011). Dietary reference intakes for calcium and vitamin D. *The National Academic Press Washington, D.C.*

John R. Farley, J. E. W. and D. J. B. (1983). Fluoride Directly Stimulates Proliferation and Alkaline Phosphatase Activity of Bone-Forming Cells Author (s): John R . Farley , Jon E . Wergedal and David J . Baylink. *Science*, *222*(4621), 330–332.

Kaseva, M. E. (2006). Contribution of trona (magadi) into excessive fluorosis-a case study in Maji ya Chai ward, northern Tanzania. *Science of the Total Environment*, *366*(1), 92–100. https://doi.org/10.1016/j.scitotenv.2005.08.049

Kebede A, (2014) Assessment of Fluoride Intake in Endemic Areas of Ethiopia and Potential use of Calcium Rich Foods in Mitigating Ingested Fluoride, PhD Dissertation in Food Science and Nutrition, *Addis ababa University*, page 1-195.

Kebede, A., Retta, N., Abuye, C., Whiting, S. J., Kassaw, M., Zeru, T., … Kjellevold, M. (2016a). Dietary fluoride intake and associated skeletal and dental fluorosis in school age children in rural Ethiopian Rift Valley. *International Journal of Environmental Research and Public Health*, *13*(8), 1–10. https://doi.org/10.3390/ijerph13080756

Kebede, A., Retta, N., Abuye, C., Whiting, S. J., Kassaw, M., Zeru, T., & Woldeyohannes, M. (2016b). Minimizing Bioavailability of Fluoride through Addition of Calcium-magnesium Citrate or a Calcium and Magnesium-containing Vegetable to the Diets of Growing Rats. *International Journal of Biochemistry Research & Review*, 10(2), 1–8. https://doi.org/10.9734/IJBCRR/2016/23693

Kloos H and Tekle-Haimanot R (1999) Distribution of fluoride and fluorosis in Ethiopia and prospects for control, *Tropical Medicine and International Health.* 4(5): 355-364.

Kravchenko, J., Rango, T., Akushevich, I., Atlaw, B., McCornick, P. G., Merola, R. B., … Jeuland, M. (2014). The effect of non-fluoride factors on risk of dental fluorosis: Evidence from rural populations of the Main Ethiopian Rift. *Science of the Total Environment*, *488–489*(1), 595–606. https://doi.org/10.1016/j.scitotenv.2013.12.087

Levine, M. (2011). *Topics in Dental Biochemistry. Springer* (Vol. 11).

Levy S, Kohout F , Mary Kiritsy, Judy Heilman, J. W. (1995). Infants' fluoride ingestion from water, supplements and dentifrice. Journal of the American Dental Association (1939), 126(12), 1625–1632. https://doi.org/10.14219/jada.archive.1995.0110

Levy, S. M., Warren, J. J., & Broffitt, B. (2003). Patterns of Fluoride Intake from 36 to 72 Months of Age. *Journal of Public Health Dentistry*, *63*(4), 211–220.

Limaleite AD, Buzalaf CP, Buzalaf M. (2015) Fluoride intake in the context of dental fluorosis. Fluorine: Chemistry, Analysis, Function and Effects;. P. 22-38.

Malde, M. K., & Bjorvatn, K. (2001). Determination of fluoride in food by the use of alkali fusion and fluoride ion-selective electrode. *Food Chemistry*, *73*, 373–379.

Malde, M. K., Scheidegger, R., Julshamn, K., & Bader, H. P. (2011). Substance flow analysis: A case study of fluoride exposure through food and beverages in young children living in Ethiopia. *Environmental Health Perspectives*, *119*(4), 579–584. https://doi.org/10.1289/ehp.1002365

Martínez-Mier, E. A. (2012). Fluoride: Its metabolism, toxicity, and role in dental health. *Journal of Evidence-Based Complementary and Alternative Medicine*, *17*(1), 28–32. https://doi.org/10.1177/2156587211428076

Mella, S., Molina, X., & Atalah, E. (1994). Prevalence of endemic dental fluorosis and its relation with fluoride content of public drinking water. *Revista medica de Chile*, *122*(11), 1263-1270.

Murray, J. J. (1986). Appropriate use of fluorides for human health. *World Health Organization, Geneva*.

Nourjah, P., & Horowitz, A. M. (1994). Factors Associated with the Use of Fluoride Supplements and Fluoride Dentifrice by Infants and Toddlers. *Journal of Public Health Dentistry*, *54*(1), 47–54.

Panda, L., Kar, B. B., & Patra, B. B. (2015). Fluaoride and Its Health Impacts-A Critical Review. *IOSR Journal of Electronics and Communication Engineering*, *10*(4), 79–91. https://doi.org/10.9790/2834-10417991

Patnaik, P. (1997). *Handbook of Environmental Analysis*.

Pendrys, D. (1990). The Fluorosis Risk Index : a Method for Investigating. *Journal of Public Health Dentistry*, *50*(5), 291–298.

RiPPLE. (2009). Mapping for water supply and sanitation (WSS) in Ethiopia. *Research-Inspired Policy and Practice Learning in Ethiopia and the Nile Region (RiPPLE)*, 1–33. https://doi.org/10.3389/fneur.2012.00087 [doi]

Shashi, A., Kumar, M., & Bhardwaj, M. (2008). Incidence of skeletal deformities in endemic fluorosis. *Tropical Doctor*, *38*(4), 231–233. https://doi.org/10.1258/td.2008.070379

Steven Adair, William Piscitelli, C. M.-H. (1997). Steven M. Adair, DDS, MS William P. Piscitelli, DDS Carole McKnight-Hanes, DMD. *American Academy of Pediatric Dentis*, *19*(2), 99–103.

Susheela, A. K., & Bhatnagar, M. (2002). Reversal of fluoride induced cell injury through elimination of fluoride and consumption of diet rich in essential nutrients and antioxidants. *Molecular and Cellular Biochemistry*, *234–235*, 335–340.

https://doi.org/10.1023/A:1015986410685

Tekle-Haimanot R., Fekadu, A., & Bushra, B. (1987). Endemic fluorosis in the Ethiopian Rift Valley. *Tropical and geographical medicine*, *39*(3), 209-217.

Tekle-Haimanot, R., Melaku, Z., Kloos, H., Reimann, C., Fantaye, W., Zerihun, L., & Bjorvatn, K. (2006). The geographic distribution of fluoride in surface and groundwater in Ethiopia with an emphasis on the Rift Valley. *Science of the Total Environment*, *367*(1), 182–190. https://doi.org/10.1016/j.scitotenv.2005.11.003

Tezera F, Whiting SJ, and S. G. (2017). Dietary calcium intake and sunlight exposure among children aged 6-23 months in Dale woreda, Southern Ethiopia. *African Journal of Food, Agriculture, Nutrition and Development*, *17*, 12451–12464.

Theobald, H. E. (2005). Dietary calcium and health. *British Nutrition Foundation Nutrition Bulletin*, *30*, 237–277.

Thylstrup, A., & Larsen, M. J. (1977). Clinical and structural features and possible pathogenic mechanisms of dental fluorosis Letter from the Editor. *Scnnd J Denl Res*, 510–534.

Turner, C. H., Hasegawa, K., Zhang, W., Wilson, M., li, Y., & Dunipace, A. J. (1995). Fluoride Reduces Bone Strength in Older Rats. *Journal of Dental Research*, *74*(8), 1475–1481.

Ullah, R., Zafar, M. S., & Shahani, N. (2017). Potential fluoride toxicity from oral medicaments: A review. *Iranian Journal of Basic Medical Sciences*, *20*(8), 841–848. https://doi.org/10.22038/ijbms.2017.9104

Warren, J. J., Levy, S. M., Broffitt, B., Cavanaugh, J. E., Kanellis, M. J., & Weber-Gasparoni, K. (2009). Considerations on optimal fluoride intake using dental fluorosis and dental caries outcomes - A longitudinal study. *Journal of Public Health Dentistry*, *69*(2), 111–115. https://doi.org/10.1111/j.1752-7325.2008.00108.x

Whitacre, D. (2011). Reviews of Environmental Contamination and Toxicology. *Springer* (Vol. 211).

WHO (1996) Trace elements in human nutrition and health; *Geneva* page, 1-343

WHO. (2002). Environmental Health Criteria 227. *WHO Geneva*, 1–290.

WHO. (2011). Guidelines for drinking-water quality - Fourth edition. *Water Sanitation Health*, 1-564.

WHO. (2014). Basic methods for assessment of renal fl uoride excretion in community prevention programmes for oral health. *WHO France*, 1–91.

Willett, W. (2013). Nutritional Epidemiology Third Edition. *Oxford University Press*.

Wondwossen, F., & Bjorvatn, K. (2006). Sociodemographic and behavioural correlates of severe dental fluorosis. *International Journal of Paediatric Dentistry*, *16*, 95–103.

Wu, H., Gozdzik, A., Lynn, J., Wagner, D., Cole, D. E., Vieth, R., … Whiting, S. J. (2009). The development and evaluation of a food frequency questionnaire used in assessing vitamin D intake in a sample of healthy young Canadian adults of diverse ancestry ☆. Nutrition Research, 29(4), 255–261. https://doi.org/10.1016/j.nutres.2009.03.006

Yang, K., & Liang, X. (2011). Fluoride in Drinking Water: Effect on Liver and Kidney Function. *Encyclopedia of Environmental Health*, 769–775. https://doi.org/10.1016/B978-0-444-52272-6.00418-9

Yunzhao Wang, Yuming Yin, Louis Gilula, A. W. (1994). Endemic Skeleton : Patients Fluorosis Radiographic of the Features. *American Roentgen Ray Society*, *162*, 93–98.

Zhang, S., Jiang, C., Liu, H., Guan, Z., Zeng, Q., Zhang, C., … Wang, A. (2013). Fluoride-elicited developmental testicular toxicity in rats: Roles of endoplasmic reticulum stress and inflammatory response. *Toxicology and Applied Pharmacology*, *271*(2), 206–215. https://doi.org/10.1016/j.taap.2013.04.033

Zuo, H., Chen, L., Kong, M., Qiu, L., Lü, P., Wu, P., … Chen, K. (2018). Toxic effects of fluoride on organisms. *Life Sciences*, *198*(2017), 18–24. https://doi.org/10.1016/j.lfs.2018.02.001

8. Annexs

8.1 English version questionnaire

I. Consent form

My name is------------------. I came from Addis Ababa University. The institution is working to improve the health of the community by providing solutions for different problems based on tangiable researches. At this time we came here to research the association of dietary intakes and fluorosis. It enables us to provides understanding for the community to protect the spreading of dental and skeletal fluorosis. Your parents are coosen for the study participant. I would like to express my good feeling about that. We are going to conduct 24-hour dietary recall assessment, dental fluorosis assessment, and skeletal fluorosis assessment. We will give you soap for your time killing with us and cooperation. So, are you volunteer to continue with us?

Please give your permission._____

1. yes
2. No

If you don't want to participate please give your reason__________________________________

____

Data collector: Name___________, Signature__________, Date________________.

II. Study subject's detail information

ubject category: ☐ 1. School age child 2. Mother			
Participant's code: ☐☐☐☐	2. Date of interview ☐☐ Day ☐☐ Month ☐☐ Year	3. Location ☐☐☐☐	4.Field worker code: ☐☐
Subject's name: ______ First name ______ Middle name ______ Last name			
Birth day ☐☐ Day ☐☐ Month ☐☐ Year		7. Income ______	
. Religion ______	9. Educational status ______	10. Where did you born? ______	
1. Age in year ☐☐	12. Sex (1= M, 2 = F) ☐	13. Is the water source the same over dwelled years in the area? ______	
4. Marital Status ______	15. How many years the subject live in the study area?______	NOTE: if the study participants are on health defects, do not apply the survey	
6. Yesterday, was it a holiday in the community? (0 = No, 1 = Yes) ☐		17. Yesterday, was there a celebration in the family ☐ (0 = No, 1 = Yes)	
3. Is there any heath defects yesterday? (0 = No, 1 = Yes) ☐		19. Last two week, was the participant sick? ☐ (0 = No, 1 = yes)	

III. 24 Hour recall form

Interviewer: Scale no.: Day food eaten:	Location: Subject ID: Subject name:	Sex: Age:

Time	Place eaten	Food or drink	Description and cooking method	Raw (1) Cooked. (2)	Amount served (g)	Amount left (g)	Amount eaten (g)	Food code

Probe for alcohol: Yes ☐ No ☐	Probe for sickness Yes ☐ No ☐ If yes, did sickness affect appetite? If yes, how? Increase ☐ Decrease ☐
Was food intake unusual? Yes ☐ No ☐ If yes, how was it unusual?	Probe for tablets Yes ☐ No ☐ Iron ☐ Vitamins ☐ Other supplements ☐ Anti-malaria ☐
Was it a feast day? Yes ☐ No ☐ Was it a market day? Yes ☐ No ☐ Was it a fasting day? Yes ☐ No ☐	Name of supplement (Record this from the label, if available)

IV Food frequency questionnaire

ood frequency questionnaire answered by the mothers

nstruction: For each food item listed below, indicate with a checkmark (√) the category that best describes the frequency with which the child usually eat that particular food item.
hinking about the last three months, how frequently did your child eat the following food items within per week during the last one months and one year

ood item	Once or more than once per day	3-6 times per week	Once or twice per week	Twice per month or less	never	Food item	Once or more than once per day	3-6 times per week	Once or twice per week	Twice per month or less	never
range						Avocado					
anana						Carrot					
lango						Pumpkin					
omato						Sweet potato					
otato						Maize					
eet root						Teff					
thiopian kale						Milk					
apaya						Cheese					
entils						Butter					
hickpeas						Whey					
ettuce						Yogurt					
aricot beans						Chicken					

Oats						Egg					
Bean						Fish with bone					
Wheat						Fish without bone					
Barley						Beef					
Pea						Goat meat					
Sorghum						Sheep meat					
Vetch						Peanut					
Moringa						Sesame					
False banana						Niger					
Green pepper						Rice					
Cassava						Millet					

V. A dental fluorosis assessment form

Dean's Index filled by a qualified dentist

Dental Fluorosis Category #	Rating score	Notes
Normal		
Questionable		
Very Mild: opaque white areas covering 25% of the tooth surface		
Mild: white areas covering 25%–50% of the tooth surface		
Moderate: all surfaces affected and some browning		
Severe: widespread brown stains and pitting		

VI. A skeletal fluorosis assessment form

A. Physical exercise (able= 1 , unable= 2) based on figure 2

	Exercise 1	Exercise 2	Exercise 3	Exercise 4	Exercise 5	Exercise 6
Subject						

B. Symptoms of skeletal and non-skeletal fluorosis

To be filled by Physiotherapist or trained nurse or other health care professional

S. No	Skeletal and Non-Skeletal Symptoms #	1 = Yes; 0 = No		Notes
		Mother	Child	
1	Cannot bend the body and touch floor or toe			
2	Cannot touch the chest with chin			
3	Cannot stretch and fold arms to touch back of the head			

4	Feel lower back pain			
5	Feel leg pain, joints			
6	Feel arm pain, joints			
7	Feel tingling sensation			
8	Feel neck pain with movement			
9	Feel muscle weakness			
10	Feel the loss of appetite			
11	Have nausea			
12	Have abdominal pain			
13	Have to bloat			
14	Experience polydipsia (excessive thirst)			
15	Experience polyuria (excess urine volume)			
16	Experience constipation			

8.2 Questionnaire (Amharic version)

ሀ፡ የፈቃደኝነት መግለጫ

እኔ --- እባላለሁ፡፡ የመጣሁት ከኢትዮጵያ የሕብረተሰብ ጤና ኢንስቲትዩት ነው፡፡ ተቋሙ ተጨባጭ የሆኑ የምርምር ሥራዎችን በመስራት እንዲሁም የመፍትሄ ሀሳቦችን በመጠቀም የሕብረተሰቡን ጤና ለማሻሻል እየሰራ የሚገኝ ተቋም ነዉ፡፡ እዚህ የመጣነዉ ጥራቱን ያልጠበቀ ውሀ እና ምግብ በመውሰድ የሚያመጣውን የጤና ጉዳት መጠንና ስፋት ለማጥናትና የመፍትሔ ሀሳብ ለመጠቆም ምርምር ለማካሄድ ነው፡፡ የእርሶ ቤተሰብ ለዚህ ጥናት ተመርጧል፡፡ የእርሶም ቤተሰብ የዚህ ጥናት አባል ሆነው በመመረጠ ደስ ይለናል፡፡ የ ልጃችሁን የ24 ሰዓት የአመጋገብ ሁኔታ እና የድግግሞሽ መጠን የሚገልፅ መረጃ እንሰበስባለን፡፡ እርሶ የሚሰጡን መረጃዎች በሙሉ ሚስጥራዊነታቸው የተጠበቀ ነው፡ ፡ ሊቃለ መጠይቁ ከአምስት እስከ አስራ አራት ዓመት የሆነ ልጅና እናቱን እንፈልጋለን፡፡ በተጨማሪም የምግብ እና ዉሃ ናሙና እንወስዳለን፡፡ በቅድሚያ ለምታደርጉልን ቀና ትብብር እናመሰግናለን፡፡ ስለዚህ በዚህ ምርምር ላይ ለመሳተፍ ፈቃደኛ ነዎት?

እባክዎ ስምምነትዎን ይግለጹ-------------------

(1, አዎ   2= አይደለም)

መሳተፍ ካልፈለጉ እባክዎ ምክንያቶን ይግለጹ --

---.

የመረጃ ሰብሳቢዉ: ስም ____________, ፊርማ___________, ቀን _________________.

ለ፡ የ ጥናቱ ተሳታፊ ግላዊ ዝርዝር መረጃ

ናቱ ተሳታፊ: ☐ 1. ልጅ 2. እናት			
የተሳታፊዉ መለያ ቁጥር: ☐☐☐☐	2. የቃለመጠይቁ ቀን ☐☐ ቀን ☐☐ ወር ☐☐ ዓመት	3. ቦታ ________	4.የመስክ ሰራተኛዉ መለያ : ☐☐
የተሳታፊዉ ስም: ________ መጠሪያ ስም	________ የአባት ስም		________ የአያት ስም
የትዉልድ ቀን ☐☐ ቀን ☐☐ ወር ☐☐ ዓመት		7. የገቢ ምንጭ ________	
ሀይማኖት ________	9. የትምህርት ሁኔታ ________	10. የተወለዱበት ቦታ? ________	
1. ዕድሜ (በዓመት) ☐☐	12. ፆታ (1= ወ, 2 = ሴ) ☐	13. ለብዙ ዓመታት ተመሳሳይ የዉሃ ምንጭ ነዉ የተጠቀሙት? ________	
. የጋብቻ ሁኔታ________	15. በጥናቱ ቦታ ለምን ያክል ጊዜ ኖሩ? ________	**ማስታወሻ፡ የጤና እክል ያለበትን ተሳታፊ አይመለከትም**	
6. ትላንት ዓመታዊ በዓል ነበር? (0 = አልነበረም, 1 = አዎ) ☐		17. ትላንት በቤተሰብ ዉስጥ ድግስ ነበር? (0 = አልነበረም, 1 = አዎ) ☐	
. ትላንት የጤና እክል አጋጥሞት ነበር? (0 = አላጋጠመኝም, 1 = አዎ) ☐		19. ላለፉት ሁለት ሳምንታት ታመዉ ነበር? (0 = አልታመምኩም, 1 = አዎ) ☐	

ሐ. የ 24 ሰዓት የአመጋገብ ሁኔታ

ጠያቂዉ: Scale no.: ምግቡን የተመገቡበት ቀን:____________				ቦታ: የተሳታፊዉ መለያ: የተሳታፊዉ ስም:			ፆታ: ዕድሜ:	
የተመገቡበት ሰዓት	የተመገቡበት ቦታ	የምግብ ወይም መጠጥ ስም	የምግቡ ዝርዝር መግለጫ እና የአበሳሰል ዘዴ	ጥሬ (1) የበሰለ(2)	የቀረበዉ መጠን (g)	የተረፈዉ መጠን (g)	የተመገቡት መጠን (g)	የምግቡ መለያ

አልኮል ይጠጣሉ?፡ አዎ ☐ አልጠጣም ☐	ታመዉ ነበር? አዎ ☐ አልታመምኩም ☐ አዎ ካሉ አመጋገብዎ ላይ ተፅዕኖ ነበረዉ?__________ አዎ ካሉ እንዴት? ጨመረ ☐ ቀነሰ ☐
አመጋገብዎ ያልተለመደ ነበር? አዎ ☐ አልነበረም ☐ አዎ ካሉ እንዴት?	መድሃኒት ይወስዳሉ? አዎ ☐ አልወስድም ☐ Iron ☐ Vitamins ☐ Other supplements ☐ Anti-malaria ☐
የበዓል ቀን ነበር? አዎ ☐ አልነበረም ☐ የገበያ ቀን ነበር? አዎ ☐ አልነበረም ☐ የፆም ቀን ነበር? አዎ ☐ አልነበረም ☐	Name of supplement (Record this from the label, if available)

መ. የምግብ ድግግሞሽ መጠይቅ

ኢናቶች የሚመለስ የምግብ ድግግሞች መጠይቅ

መሪያ: ከስር ለተዘረዘሩት የምግብ ዓይነቶች የህፃኑን የሁልጊዜ አመጋገብ የሚገልፀዉን የምግብ ድግግሞሽ ክፍል በ (√) ምልክት ያመልክቱ። ያለፉትን ሦስት ወራት ሲያስታዉሱት ልጅዎ የሚከተሉትን የምግብ ዓይነቶች በምን ያክል ግግሞሽ ይመገባል

ምግቡ ዓይነት	በቀን አንድ ጊዜ ወይም ከዚያ በላይ	በሳምንት ከ 3-6 ጊዜ	በሳምንት 1 ወይም 2 ጊዜ	በወር 2 ወይም ከዚያ በታች	በፍፁም	የምግቡ ዓይነት	በቀን አንድ ጊዜ ወይም ከዚያ በላይ	በሳምንት ከ 3-6 ጊዜ	በሳምንት 1 ወይም 2 ጊዜ	በወር 2 ወይም ከዚያ በታች	በፍፁም
ርቱካን						አቮካዶ					
ዝ						ካሮት					
ንጎ						ዱባ					
ማቲም						ስካር ድንች					
ንች						ቦቆሎ					
ደስር						ጤፍ					
ገሻ ጎመን						ወተት					
ፓያ						አይብ					
ስር						ቅቤ					
ንብራ						አጓት					
ጣ						እርጎ					
ሎቄ						የዶሮ ስጋ					

ጃ						እንቁላል					
ቈላ						አሳ አጥንት ያለዉ					
ንዴ						አሳ አጥንት የሌለዉ					
በስ						የበሬ ስጋ					
ተር						የፍየል ስጋ					
ሸላ						የበግ ስጋ					
ያ						ለዉዝ					
ሪንጋ						ሰሊጥ					
ነሰት						ኑግ					
ሪያ						ሩዝ					
ሳቫ						ዳጉሳ					

8.3 Ethical Approval Letter

College of Natural & Computational Sciences
Addis Ababa University

Office of the Dean

Ref. No. CNSDO/204 /11/2018
Date December 20, 2018

To Whom It may Concern

The College of Natural & Computational Science Institutional Review Board (CNS-IRB) Committee in its meeting held on 25/11/2018 Minute No. IRB/035/2018 has examined the project proposal entitled **"Dietary fluoride and calcium intake, dental and skeletal fluorosis level and associated factors among school age children in Alaba SNNPR Ethiopia"**, by **Nahom Tefera** from the Addis Ababa University.

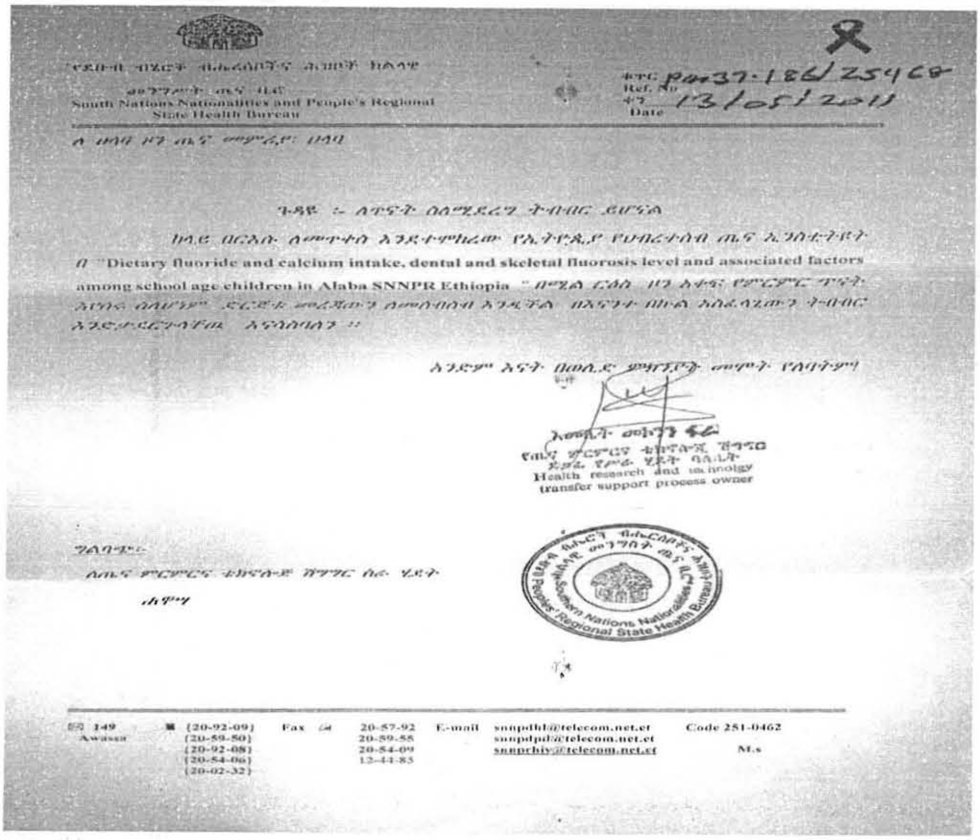
The proposal is approved for implementation.

With regards,

Shibru Temesgen /Dr./
Dean , College of Natural & Computational Science

Tel. +251-11-123-94-72
Fax: +251-11-123-94-69
POBox 1176 Addis Ababa, Ethiopia
E-mail: dean.cns@aau.edu.et
Please Quote our reference number in you correspondence
"Examine all things, hold fast that which is good"

8.4 Regional Acceptance Letter



South Nations Nationalities and People's Regional State Health Bureau

Ref. No

Date 13/05/2011

" Dietary fluoride and calcium intake, dental and skeletal fluorosis level and associated factors among school age children in Alaba SNNPR Ethiopia "

Health research and technolgy transfer support process owner

Southern Nations Nationalities and Peoples' Regional State Health Bureau

149 Awassa	(20-92-09) (20-59-50) (20-92-08) (20-54-06) (20-02-32)	Fax	20-57-92 20-59-55 20-54-09 12-44-85	E-mail	snnpdhl@telecom.net.et snnpdpd@telecom.net.et snnprhiv@telecom.net.et	Code 251-0462 M.s