RISKS OF EXPOSURE TO CONTAMINANT FLUORIDE AMONG LACTATING MOTHERS AND CHILDREN (0-24 MONTHS) IN NGARENANYUKI COMMUNITIES IN NORTHERN TANZANIA

Lucia Joseph Memba

A Dissertation Submitted in Partial Fulfillment of the Requirements for the Degree of Doctor of Philosophy in Life Sciences of the Nelson Mandela African Institution of Science and Technology

Arusha, Tanzania

July, 2025

ABSTRACT

This study revealed important information on fluoride contamination of agricultural produce, water, cow and human breast milk, and excretion through urine. Risks of exposure to contaminant fluoride among children and lactating mothers were investigated. Structured questionnaires and 24-hour dietary recall was used to estimate dietary intake. Samples were collected and analyzed for fluoride contents. In all samples, fluoride concentration was determined by using a fluoride ion-selective electrode. Univariate logistic regression analysis was used to correlate dietary fluoride exposure and urinary fluoride excretions in children and breast milk fluoride excretion in lactating mothers. Results identified maize (21.7%), African nightshade (18.4%), and East African highland banana (15.2%) as the most consumed food crops among lactating mothers; cow's milk (2.7%) for children aged 6 months, and maize for children aged 7-24 months (9.2%-22.5%). Fluoride mean concentration levels in maize were (0.03±0.01 mg/kg), nightshade (0.081±0.004 mg/kg), East African highland banana (0.025±0.002 mg/kg), domestic water (4.57±0.21 mg/L), public tap water (4.74±0.6 mg/L), cow's milk (0.34±0.18 mg/L), and human breast milk (0.077±0.03 mg/L). Both domestic water and human milk fluoride levels exceeded WHO safe standards of 1.5 mg/L and 0.002 mg/L, respectively. Dietary exposure among children was found ranged from 0.7±0.02 to 15.60±6.53 mg/kg bw/day, with food crops contributing 62.16%. Lactating mothers' exposure ranged from 0.621±0.09 to 88.12±25.2 mg/kg bw/day, with food crops contributing 60.3%. The studied population was exposed to dietary fluoride above the safe levels of 0.01 and 0.05 to 0.07 mg/kg/day by WHO for infants and adults, respectively. Biomarkers of fluoride exposure showed urinary fluoride excretion in children averaged 8.82±1.25 mg/L, with exposure increasing with age (p=0.003), and no significant gender differences (p=0.97). Positive correlations were observed between urinary fluoride excretion and consumption of cow's milk (ρ= 2.3, p<0.0000) and East African highland banana (ρ=-2.5, p<0.0001) in children, and between breast milk fluoride excretion and consumption of cow's milk (ρ=0.027, p=0.0001), maize (ρ=0.00022, p=0.02), and tap water (ρ=-0.0002, p=0.001) in lactating mothers. The study concludes with recommendations for comprehensive research across seasons, diverse food crops, other exposure routes, and detailed biomarker assessments to fully understand the risks associated with fluoride exposure.

DECLARATION

I, Lucia Joseph Memba, declare to the Senate that this thesis is my own original work. It is being submitted for the Doctor degree of Philosophy in Life Sciences, Nelson Mandela African Institution of Science and Technology. It has not been submitted for any degree or examination at any other University.

Lucia Joseph Memba Date

The above declaration is confirmed by:

Prof. Neema Kassim Date

Prof. Kelvin Mtei Date

Prof. Liliane Pasape Date

COPYRIGHT

This dissertation is copyright material protected under the Berne Convention, the Copyright Act of 1999 and other international and national enactments, in that behalf, on intellectual property. It must not be reproduced by any means, in full or in part, except for short extracts in fair dealing; for researcher private study, critical scholarly review or discourse with an acknowledgement, without the written permission of the office of Deputy Vice Chancellor (Academics, Research and Innovation), on behalf of both the author and the Nelson Mandela African Institution of Science and Technology.

CERTIFICATION

The undersigned certify that they have read and hereby recommend for acceptance by the Nelson Mandela African Institution of Science and Technology (NM-AIST) a dissertation *Entitled 'Risks of Exposure to Contaminant Fluoride Among Lactating Mothers and Young Children in Communities Around Mount Meru in Northern Tanzania'* in Partial fulfillment of the requirements for the degree of Doctor of Philosophy in Life Sciences of the Nelson Mandela African Institution of Science and Technology.

___

Prof. Neema Kassim   Date

___

Prof. Kelvin Mtei   Date

___

Prof. Liliane Pasape   Date

ACKNOWLEDGEMENTS

First, I thank God for good health and strength during the course of my study. I would like to express my appreciation to Prof. Neema Kassim, the main supervisor of my research work, for her willingness to give her time so generously, patient guidance, enthusiastic encouragement, and useful critiques during the planning and development of this research work. Great gratitude is extended to my co-supervisors, Prof. Kelvin Mtei and Prof. Liliane Pasape, for their genuine cooperation, generous advice, and constructive suggestions throughout my research work. I truly appreciate the assistance given by all academic and non-academic staff from the School of Life Sciences and Bioengineering. My special thanks to all technical officers of the School of Materials Energy, Water, and Environmental Sciences and the School of Life Sciences and Bioengineering for their help and cordial attitude during my study.

I truly appreciate the assistance given by technical officers and staff members of the Department of Food in the Tanzania Bureau of Standards for laboratory sample digestion (TBS). Thanks to all my colleagues from the School of Life Sciences and Bioengineering and other schools at NM-AIST for their cheerful encouragement, cooperation, and help during my study. Finally, how can I ever thank you My parents, Mr. Joseph Memba and Mrs. Anna Memba, I am so grateful to have a confidant and friend like you, Mother. Passion and fearlessness are my greatest examples. You push me and teach me to never doubt myself. Your love, prayer, and encouragement have sustained me throughout this work. My beloved husband, Dr. Kabanda Lugulu. His dedication to integrity, intellect, humor, and vitality never fails to brighten my days. He has tended to my well-being and happiness while I put in long days away from home. He has encouraged me through the 'lows,' cheered me through the 'highs, 'and he has been incredibly loving and patient throughout. He believes in me; I simply could not have done this work without my husband by my side. My beloved children Mercy Kabanda and Musa Kabanda, you're a wonderful gift from God and a blessing in our family. Also, thanks to the Deputy Vice Chancellors (ARI and PFA), Dean of Students, and all NM-Stians community. Financial assistance provided under the African Development Bank through NM-AIST is duly acknowledged.

'I can do all things through Christ who give me strength (Phil 4:13)'.

DEDICATION

This dissertation is dedicated to My parents: Mr. Joseph Memba and Mrs. Anna Memba, my beloved husband, Dr. Kabanda Lugulu and my beloved children Mercy Kabanda and Musa Kabanda, you're a wonderful gift from God and a blessing in our family.

TABLE OF CONTENTS

ABSTRACT..i

DECLARATION ii

COPYRIGHT... iii

CERTIFICATION iv

ACKNOWLEDGEMENTS v

DEDICATION..vi

TABLE OF CONTENTS..vii

LIST OF TABLES x

LIST OF FIGURES xi

LIST OF PLATES xiii

LIST OF APPENDICES...xiv

LIST OF ABBREVIATIONS AND SYMBOLS xv

CHAPTER ONE 1

INTRODUCTION 1

1.1 Background of the Problem 1

1.1.1 Fluoride and its Associated Health Risks 1

1.1.2 Fluoride Exposure 1

1.2 Statement of the Problem 4

1.3 Rationale of the Study...5

1.4 Research Objectives 5

1.4.1 General Objective 5

1.4.2 Specific Objectives 5

1.5 Research Questions 5

1.6 Significance of the Study 5

1.7 Delineation of the Study..6

CHAPTER TWO 8
LITERATURE REVIEW 8
2.1 Awareness of Fluoride Contamination Sources and Management Methods 8
2.2 Fluoride Contamination of Selected Food Crops, Milk and Domestic Water 9
2.2.1 Food Crops 9
2.2.2 Contaminated Milk 13
2.2.3 Contaminated Domestic Water 14
2.3 Dietary and Biomarkers of Fluoride Exposure 18
2.4 Fluoride Metabolism 19
2.5 Fluoride Health Effects 22
CHAPTER THREE 27
MATERIALS AND METHODS 27
3.1 Study Area 27
3.2 Research Design 30
3.3 Selection of Study Participants 30
3.4 Households Interviews and Sample Collection 30
3.5 Sample Preparation and Digestion 32
3.5.1 Determination of Fluoride 33
3.5.2 Assessments of Participants Dietary Intake 34
3.5.3 Fluoride Exposure Assessment 34
3.6 Statistical Analysis 34
3.7 Ethical Clearance 35
CHAPTER FOUR 36
RESULTS AND DISCUSION 36
4.1 Demographic Information and Locally Produced Food Crops 36
4.2 Awareness on Sources of Contaminant Fluoride and its Related Health Effects 40
4.3 Fluoride Contamination of Commonly Consumed Food Crops 43

4.4 Fluoride Levels in Domestic Water ... 47
4.5 Fluoride Contamination Levels in Cow's and Human Breast Milk ... 49
4.6 Consumption of Food Crops, Milk and Water ... 51
4.7 Dietary Fluoride Exposure ... 54
4.8 Biomarkers of Fluoride Exposure ... 60
4.8.1 Urinary Fluoride ... 60
4.8.2 Human Milk (Breast) ... 67
CHAPTER FIVE ... 74
CONCLUSION AND RECOMMENDATIONS ... 74
5.1 Conclusion ... 74
5.2 Recommendations ... 74
REFERENCES ... 76
APPENDICES ... 98
RESEARCH OUTPUTS ... 110

LIST OF TABLES

Table 1: Fluoride contamination levels in raw food crops as reported in the literatures .. 12

Table 2: Fluoride in groundwater and its possible causes as reported in the Literatures from 2000 to 2016 15

Table 3: Participant's demographic information at studied area (N=300) 36

Table 4: Community food consumption frequency at studied area (N=107) 39

Table 5: Children complimentary food consumption frequency (N=107) at studied area 40

Table 6: Participant’s awareness on fluoride exposure sources and its health effects 41

Table 7: Comparison of fluoride levels of locally grown and commonly consumed food crops from studied area to those reported in literatures (mg/kg) 45

Table 8: Comparison of fluoride levels in domestic water from studied area to those reported in literatures (mg/kg) 48

Table 9: Comparison of fluoride level in domestic water and human breast milk from studied area with those reported in the literatures (mg/L) 51

Table 10: Consumption of food crops, cow’s milk and drinking water among children of different age 52

Table 11: Consumption of locally grown and commonly consumed food crops, cows milk and drinking water among lactating mothers in the studied community 53

Table 12: Children exposure to fluoride through consumption of commonly consumed complimentary food, human milk and drinking water (mg/kgbw/day) 55

Table 13: Fluoride concentration in urine of children at studied area in comparison to those reported in the literatures (mg/kg) 64

Table 14: Association between dietary fluoride exposure and urinary biomarkers of fluoride exposure in children at studied area 64

Table 15: Association between dietary exposure to fluoride and human milk biomarkers of fluoride exposure in lactating mothers at studied area 67

LIST OF FIGURES

Figure 1: Distribution of community studies on fluoride (Idowu *et al.,* 2019)....................8

Figure 2: Fluoride in the groundwater above recommended safe limit of 1.5 mg/L by WHO (Amini *et al.,* 2008) 14

Figure 3: East African countries with high fluoride levels in its water sources (Malago *et al.,* 2017)....................16

Figure 4: Region with high fluoride levels in its water sources (Gumbo & Mkongo, 1995) 17

Figure 5: Flow chart for fluoride metabolism....................21

Figure 6: The study location 28

Figure 7: Fluoride levels of locally grown food crops (maize n=107; vegetables n=90; Cabbage n=30, Chinese n=30, Night shade n=30) (*P*= 0.001)....................44

Figure 8: Fluoride contamination of commonly consumed green banana varieties: Cavendish group (*Malindi*) (n=30) East African Highland banana (*Matoke*) (n=30); East African diploid banana (*Mchare*) (n=30) (*P* = 0.001)....................46

Figure 9: Fluoride contamination of water used for domestic purposes as sourced from both household and public taps (households n=107, public taps n=15) (*P*=0.035)....48

Figure 10: Fluoride levels of cow's milk (n=106) and human breast milk (n=101) (*P*= 0.001) 50

Figure 11: Sources of dietary fluoride exposure in children....................56

Figure 12: Fluoride exposure in lactating mothers through consumption of locally grown produce, cows milk and drinking water....................58

Figure 13: Sources of dietary fluoride exposure in lactating mothers 59

Figure 14: Fluoride concentration in urine from children at different age group (n=105) (*P*= 0.21)....................61

Figure 15: Relationship between urine fluoride level and age in children (n=106)............62

Figure 16: Fluoride concentration in urine from females and males children (n=107) (*P*= 0.97)....................63

Figure 17: Relationship between cows milk daily fluoride intake and 24h urinary fluoride levels in children ... 65

Figure 18: Relationship between E. A African highland banana daily fluoride intake and 24 h urinary fluoride levels in children ... 65

Figure 19: Relationship between daily fluoride intake due to drinking of water and 24h urinary fluoride levels in children ... 66

Figure 20: Relationship between daily fluoride intake due to maize and 24 h urinary fluoride levels in children ... 66

Figure 21: Relationship between daily fluoride intake due to human milk and 24 h urinary fluoride levels in children ... 67

Figure 22: Relationship between daily fluoride intake due to cows milk and human milk fluoride levels in lactating mothers ... 68

Figure 23: Relationship between daily fluoride intake due to maize and human milk fluoride levels in lactating mothers ... 68

Figure 24: Relationship between daily fluoride intake due to drinking water and human milk fluoride levels in lactating mothers ... 69

Figure 25: Relationship between daily fluoride intake due to nightshade and human milk fluoride levels in lactating mothers ... 70

Figure 26: Relationship between daily fluoride intake due to cabbage and human milk fluoride levels in lactating mothers ... 70

Figure 27: Relationship between daily fluoride intake due to chinese cabbage and human milk fluoride levels in lactating mothers ... 71

Figure 28: Relationship between daily fluoride intake due to East African highland banana and human milk fluoride levels in lactating mothers ... 71

Figure 29: Relationship between daily fluoride intake due to East African diploid banana and human milk fluoride levels in lactating mothers ... 72

Figure 30: Relationship between daily fluoride intake due to Cavendish banana and human milk fluoride levels in lactating mothers ... 72

LIST OF PLATES

Plate 1: Skeletal (A, B & C) and dental fluorosis (D) (Choubisa *et al.,* 2016; Shanmugam *et al.,* 2018; Torra *et al.,* 1998) 25

Plate 2: Ngarenanyuki River (A), Agricultural activities along the River (B) and (C) ... 29

Plate 3: Locally grown and commonly consumed (A) maize corn (B) flour 37

Plate 4: Commonly consumed leafy vegetables (C) Chinese cabbage (D) Cabbage (E) Night shade 37

Plate 5: Locally grown and commonly consumed green banana varrieties; Cvendish group (*malindi*) (F), East African highland banana (*matoke*) (G), East African diploid banana (*mchare*) (H) 38

LIST OF APPENDICES

Appendix 1: Ethical clearance 98

Appendix 2: Consent form.. 99

Appendix 3: Questionnaires guide for women of reproductive age of the community around mount Meru areas 100

Appendix 4: The 24-hour dietary recall.. 105

LIST OF ABBREVIATIONS AND SYMBOLS

AA (C20:4n-6)	Arachidonic acid
'AAA"	Cavendish group
AfDB	African Development Bank
Al	Aluminum
ANOVA	Analysis of Variance
C22:6*n*-3, DHA	Docosahexaenoic acid
Ca	Calcium
CaF_2	Calcium fluoride
CNS	Central nervous system
DED	District Executive Director
DMO	District Medical Officer
DRV	Dietary reference value
E.A.diploid banana-"AA"	East African diploid banana
E.A.highland banana-"AAA"	East African Highland triploid banana
EAI	Expandable Ion Analyzer
EDTA	Ethylenediaminetetraacetic acid
EFSA	European Food Safety Authority
F	Fluoride
Fe	Iron
FFQ	Food frequency questionnaire
H_2O_4	Hydroxyperoxide
H_2SO	Sulfuric acid
$HClO_4$	Perchloric acid
HMDS	Hexamethyldisiloxane
HSB	Honesty Significance differences
ISE	Ion-Selective Electrode
K	Potassium
Mg	Magnesium
$Na_3C_6H_5O_7$	Trisodium citrate
NaCl	Sodium chloride
NaOH	Sodium hydroxide
NIMR	National Institute for Medical Research

NM-AIST	Nelson Mandela African Institution of Science and Technology
SD	Standard Deviation
SOD	Superoxide dismutase
SPSS	Statistical Package for Social Sciences
TAG	Triglycerides
TBS	Tanzania Bureau of Standards
USDA	United States Department of Agriculture

CHAPTER ONE

INTRODUCTION

1.1 Background of the Problem

1.1.1 Fluoride and its Associated Health Risks

Fluoride, the 13th most electronegative element is widely dispersed in nature (WHO, 2015), highly reactive element that usually bound as an inorganic fluoride (WHO, 2014) and rarely found in its elementary nature. It occurs in the ionic forms of hydrogen fluoride when dissolving in water (Chen *et al.,* 2017; Moghaddam *et al.,* 2018; Slade *et al.,* 2018). It enhance mineral deposition in mineralized tissues such as bones and teeth (Mylonas *et al.,* 2018; Pereira *et al.,* 2018; Spencer *et al.,* 2018), especially when ingested during the period of tooth development; it makes the enamel more resistant to acid attacks at adult age (Viswanathan, 2018; Wei & Ekstrand, 2019). However, when above the minimum concentration, fluoride is known to be toxic in human as it binds calcium and interfere the activity of proteolytic and glycolytic enzymes (Dutta *et al.,* 2019; Kanduti *et al.,* 2016), leading to dental caries causing dental fluorosis (WHO, 2015) in both the primary and the permanent dentition.

The burden of the prevalence of fluorosis is associated with excessive exposure to contaminant fluoride when ingested from various contaminated sources during tooth development (Bhagavatula *et al.,* 2016; WHO, 2015). The severity of the condition depends on the dose, duration, and timing of fluoride exposure (Jiménez *et al.,* 2019; Spencer *et al.,* 2018; Yadav *et al.,* 2019). Fluoride contaminated diets, fluoridated supplements, toothpaste, infants formula, and drinking water are known as potential sources of fluoride exposure (Fallahzadeh *et al.,* 2018; Post *et al.,* 2017).

1.1.2 Fluoride Exposure

Globally, an approximately 200 million people are exposed to fluoride above safe limit of 1.5 mg/L in drinking water (WHO, 2015), the main causes of the burden of dental caries. Some Asian countries like Pakistan, Thailand, China, Iran, and Iraq (Mukherjee *et al.,* 2019; Paya & Bhatt, 2010; Perumal *et al.,* 2013), have been reported with high fluorosis due to excessively exposure to fluoride through consumption of contaminated diets including water. Also geological nature in most of countries along the Rift valley especially the West Rift Valley and the presences of minerals such as fluorspar (CaF_2), cryolite (Na_3AlF6), apatite ($Ca_5(PO_4)_3F$)

and hornblende [$(Ca, Na)_2(Mg, F, Al)_5(Si, Al)_8O_{22}(OH)_2$] (Thole, 2013), are known to contain high fluoride levels in water due to interaction through weathering of fluoride rich rocks and movement processes of water in soils and rocks (Adekola *et al.,* 2015; Kalpana *et al.,* 2019).

Fluoride exposures due to drinking water have been reported in adults, children and infants with a maximum of 0.08, 0.24 and 0.36 mg/kgbw/day, respectively (Guissouma *et al.,* 2017). While first symptoms of fluoride poisoning occurred at an estimated dose of 0.3 mg/kgbw/day (Akiniwa, 1997), long-term exposure to high-fluoride levels of up to 122 mg/kgbw per day have been reported to cause fluorosis (Gupta & Banerjee, 2011; Ruiz-Payan *et al.,* 2005), and more than 200 mg/kgbw leads to skeletal fluorosis (Bhagavatula *et al.,* 2016).

In Africa, high prevalence of fluorosis have been reported in the countries along the Great East African Rift Valley which extends from Jordan valley down through Sudan, Ethiopia, Uganda, Kenya and Tanzania (Addison *et al.,* 2020; Adekola *et al.,* 2015; Malago *et al.,* 2017). Amongst the countries, the highest fluoride levels were reported in Kenya, Tanzania and Ethiopia, with maximum levels of 800 690 and 250 mg/L, respectively in surface water bodies (Fadhullah *et al.,* 2019; Malago *et al.,* 2017). Ghana, Malawi, Nigeria, Algeria and the Republic of South Africa have also been reported to have high fluoride levels (Kanduti *et al.,* 2016; Thole, 2013).

In Tanzania, fluoride level in water sources has been reported since 1950 (Kaseva, 2006; Mjengera, 2001; Singano, 1991). The most affected regions include Arusha, Kilimanjaro, Mara, Manyara, Mwanza, Shinyanga, and Singida (Kanduti *et al.,* 2016; Malago *et al.,* 2017; Thole, 2013), with levels ranging from 13.57±64.16 to 7.44±13.26 mg/L (Malago *et al.,* 2017). Due to these high fluoride levels and low access to defluoridation technologies, the country has adopted a standard of 4 mg/L (TBS, 2005), for drinking water. This level is above the international recommendation safe level of 1.5 mg/L (WHO, 2011) which can also be a source for direct or indirect exposure to human.

In Arusha region, the highest fluoride level in its water source has been reported with a mean value of 13.57 ± 64.16 mg/L (Malago *et al.,* 2017). Being an agricultural zone, Arusha relies on its food production (Mkungu *et al.,* 2014), with very little sourced from other regions. This reliance on local and homegrown crops in the fluoride hotspots accelerates indirect human exposure to unacceptable levels of fluoride through food crops leaving alone the direct exposure through consumption of naturally fluoride water.

Maize (*Zea mays* L) is a primary source of dietary caloric intake for the majority of the population in developing countries especially in Tanzania (Kichana *et al.,* 2019). Maize has been reported with high fluoride levels in its outer and embryo parts of the grain ranging from 0.1 to 12.2 mg/kg (Brunson, 2014; Gautam *et al.,* 2010). Next to maize in the Northern zone is green banana, which are a good source of fibers, bioactive compounds such as phenolic compounds, and resistant starch (RS) (Chávez-Salazar *et al.,* 2017). It promotes health benefits by reducing glycaemia and consequently helping to prevent or treat type 2 diabetes (Riquette *et al.,* 2019), its popular dietary dish in Northern part of Tanzania. It has been reported with fluoride concentration levels of 0.02 mg/kg (USEPA, 2005).

Vegetables are good sources of vitamins such as A and B; minerals like magnesium, calcium and potassium (Gupta, 2019; Malago *et al.,* 2017), antioxidants like carotenoids and flavonoids, which has anticancer properties (Rouphael & Kyriacou, 2018). Vegetables have been reported with high fluoride levels of up to 11.30 mg/kg in cabbage (*Brassic*a *oleracea*) (Bhargava & Bhardwaj, 2009). Others vegetables including; tomato, brinjar and potato from fluoride endemic areas have been reported with fluoride contamination ranging from 2.6 to 6.8, 2.6 to 10.8 and 9.75 to 18.6 mg/kg, respectively (Devi & Sarma, 2016). The World Health Organization recommends a minimum intake of 0.4 mg/kgbw/day of vegetable and fruits, which can increase exposure to fluoride in places where fluorides are highly contaminated. Studies have shown that, excessive consumption of dietary with high fluoride concentration can increase fluoride level in the body (Gupta *et al.,* 2015; Leal *et al.,* 2020; Yin *et al.,* 2017).

Cow's milk used as family food, complimentary food for infants and in production of milk-based formula (Gribble & Fernandes, 2018; Viswanathan, 2018), is a good source of carbohydrates, fat, and protein, it contains high contents of water, vitamin A, Thiamine (B1), riboflavin (B2), vitamin B12 and vitamin D. It has minerals such as magnesium, potassium, and calcium, which are responsible for mineralized tissues development (USDA, 2009). Cow's milk was reported with fluoride contamination ranged from 0.09 to 0.8 mg/L (Gupta *et al.,* 2015; Kazi *et al.,* 2018).

Breast milk is the ideal food for infants; it provides all the nutrients and antibodies the baby needs (Martin *et al.,* 2016), with a complex matrix composition of water, fat, and protein (Aryeetey & Dykes, 2018; Karakochuk *et al.,* 2017). Breast milk from mothers with dental fluorosis was reported to contain up to 0.550 mg/L (Poureslami *et al.,* 2016), of fluoride, which was higher than recommended safe margin of 0.002 mg/L by World Health Organization

(WHO, 2002). A prolonged consumption of fluoridated water and food by lactating mothers, results in fluoridated breast milk, which implies transfer of the exposure to infants.

Sources of fluoride exposure in human are diverse (WHO, 1984b), suggesting fluoride biomarkers as an alternative method to monitor deficient or excessive intakes of bioavailable fluoride. Fluoride concentrations in urine are biological markers for assessment of present or very recent exposure to fluoride with established guideline by World Health Organization (WHO, 2014), and can rapidly detect any variations in fluoride exposure (Buzalaf *et al.,* 2012; Martínez-Mier, 2012; Rugg-Gunn *et al.,* 2011). Several studies attempted to investigate the ability of other biomarkers to predict fluoride exposure including; hairs, chipping nails and plasma (Buzalaf *et al.,* 2012; Joshi & Ajithkrishnan, 2018). However, to date there is no report on human exposure to fluoride in endemic areas of Tanzania.

1.2 Statement of the Problem

The primary human health risks associated with prolonged exposure to fluoride has been associated with drinking water (Adekola *et al.,* 2015; Buzalaf *et al.,* 2011; Malago *et al.,* 2017). Arumeru district is one of the fluoride endemic hotspots in Tanzania (Kaseva, 2006), it is also an agricultural zone for vegetables and other food crops (Mkungu *et al.,* 2014). Majority of farmers depend on the Ngarenayuki River as a source of water for domestic use when municipal water is scarce and irrigation during the dry seasons. Water from this river has been reported with high fluoride content of up to 26 mg/L (Ghiglieri *et al.,* 2010). The use of water from fluoride contaminated sources for agricultural production results in fluoride accumulation in both edible and non-edible parts of the plant (Colombani *et al.,* 2018; Lima-Arsati *et al.,* 2018; Rango *et al.,* 2017), resulting in unacceptable indirect human exposure to fluoride through consumption contaminated food crops. Despite this excessive fluoride and recurring fluorosis in such fluoride endemic areas (Kaseva, 2006), there is no literature on dietary and biomarkers of fluoride exposure in Tanzania. Thus, there is a need for exposure studies taking liability of these factors, which might be contributing to high prevalence of fluorosis in these endemic areas. Therefore, the current research estimated the risks of exposure to fluoride contamination among lactating mothers and young children in communities around mount Meru in northern Tanzania.

1.3 Rationale of the Study

The repeatedly reported high prevalence of dental fluorosis in the fluoride endemic areas (Kaseva, 2006; Malago *et al.,* 2017), incited discernments towards need to assess the potential of commonly consumed food crops grown in fluoride contaminated soil and/or irrigated with fluoridated water on human exposure to fluoride. Finding from this would guide management efforts to minimize fluorosis in the community.

1.4 Research Objectives

1.4.1 General Objective

The study aimed at investigating the risks of exposure to contaminant fluoride among lactating mothers and young children in communities around mount Meru, northern Tanzania.

1.4.2 Specific Objectives

(i) To assess households' awareness of fluoride potential sources and management methods.

(ii) To determine fluoride contamination of selected food crops, domestic water, and milk consumed by the community.

(iii) To assess fluoride exposure among young children and lactating mothers using urinary and breast milk biomarkers, respectively.

1.5 Research Questions

(i) What is the status of household awareness about fluoride contamination in locally grown food, domestic water, and what are the management methods?

(ii) What is the status of contaminant fluoride in locally grown and commonly consumed food crops, domestic water and milk?

(iii) What is the status of the fluoride exposure for the under-two years children and lactating mothers in the community?

1.6 Significance of the Study

The study findings will provide vital information about potential sources of contaminant fluoride, contamination of local and commonly consumed food crops, domestic water and milk, as well as community awareness and management options.

In addition, it will provide highlights on the risk of exposure to fluoride among young children and lactating mothers due to the consumption of locally grown food. The findings may be used by program planners/policymakers working on agriculture, food safety, and public health initiatives to develop targeted interventions aimed at reducing fluoride exposure in vulnerable populations. Furthermore, the study could serve as a basis for raising community awareness and fostering collaborative efforts to address the issue and nutrition interventions effectively. By highlighting the importance of targeted interventions and community awareness, the study emphasizes the need for a multifaceted approach to mitigate fluoride exposure risks. This collaborative effort can lead to more effective strategies that protect the health of young children and lactating mothers.

1.7 Delineation of the Study

The current research estimated the risks of exposure to fluoride contamination among lactating mothers and young children in communities around mount Meru in northern Tanzania. This study revealed important information on fluoride contamination of agricultural produce, water, cow and human breast milk, and excretion through urine. Risks of exposure to contaminant fluoride among children and lactating mothers were also investigated.

To date, this study is the first to assess dietary exposure to fluoride through locally grown and commonly consumed food crops, and urinary biomarkers of exposure in Tanzania considering within-individual variation. However, some limitations were; this was a cross-sectional study, the selection of the commonly consumed foods may have missed foods that are periodically consumed but have high concentrations of fluoride. Similarly, this study might have missed seasonal variations of fluoride in food crops, milk and water, which would affect both the contamination findings, dietary exposures estimate and the biomarkers. There might have been individual food consumption recall bias, which might cause underestimation or overestimation. Urine and breast milk fluoride biomarkers reflect a short-term exposure, and thus a longer-term biomarker might provide a better reflection of the exposure.

The objective of this dissertation is to improve understanding of fluoride contamination of food and domestic water. A selected vulnerable group of lactating mothers and children was used to assess whether consumption of locally grown and commonly consumed food crops is associated with fluoride exposure. The research was conducted in the community around Mount Meru areas, one of the fluoride-endemic areas in Tanzania. This is the first population-based study to provide an exposure profile of fluoride in children during early growing years and adults, specifically lactating mothers. Two different biomarkers, breast milk for lactating mothers and urine for infants, were used to examine whether consumption of contaminated diets may contribute to the actual exposure.

For children, the highest exposure was found due to the consumption of maize, followed by drinking water and East African highland banana, while human and cow’s milk showed a minimum exposure rate. For lactating mothers, all contaminated diets assessed showed a contribution to fluoride exposure, whereby maize and African nightshade were the important locally and commonly consumed food crops that accounted for more exposure rates; domestic water showed the highest contribution while cow's milk had the lowest contribution.

When we studied the association between dietary fluoride consumption and excretion, the study found that there was an association between the consumption of fluoride-contaminated diets and the burden of fluoride levels in the body for both children and lactating mothers. Additionally, the study found no measurable association between the consumption of fluoridated human milk and urinary fluoride exposure in children. Also, there was a significant increase in urinary fluoride excretion with the increase in the child’s age, while there was no significant difference in urinary fluoride excretion between female and male children. The increase in urinary fluoride excretion with a baby’s age underscores the fact that older babies are more exposed to family foods, which can bring multiple routes of exposure as compared to young ones.

CHAPTER TWO

LITERATURE REVIEW

2.1 Awareness of Fluoride Contamination Sources and Management Methods

Globally studies about fluoride has been reported from different areas, approximately 43 countries has been reported, mostly from India (n = 36), United States of America USA (n = 35) and China (n = 24) (Idowu *et al.,* 2019) (Fig. 1). The majority of the studies were carried out in either higher (79.1%) or upper-middle (56.3%) income economy countries (Das & Mondal, 2016). Most of studies reported were published (57%) after 1999: On average, five papers were published per year between 1999 and 2018 compared to two papers per year in the years up to 1999 (Idowu *et al.,* 2019), hence more recent studies are highly needed.

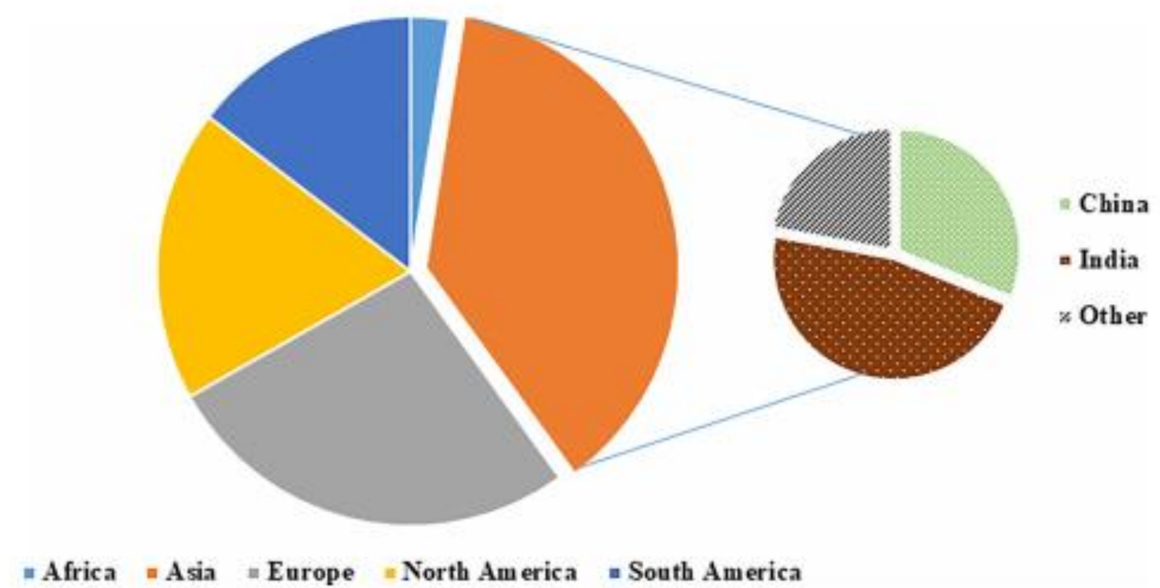

Figure 1: Distribution of community studies on fluoride (Idowu *et al.,* 2019)

Most of reported findings used water as a alternative for fluoride intake (Peters *et al.,* 2015; Zohoori *et al.,* 2019). While only few studies reported fluoride intake from other sources (n = 23; 11.2%) (Idowu *et al.,* 2019). Regardless of the various and increasing causes of fluoride exposure, and the intensification of fluoride through food and beverages daily intake (Zohoori & Maguire, 2018). There is insufficient studies which reported the community awareness on fluoride contamination sources (Villa *et al.,* 2010).

Fluoride management methods for fluoride removal in drinking water have been reported in Tanzania since 1986 (Isah *et al.,* 2014; Malago *et al.,* 2017; Thole, 2013; Mjengera, 2001; Singano, 1991). The reported methods including; bone char and calcinated magnesite (MgO)

as filter media which results in the formation of hydroxyapatite, with fluoride removed as sodium fluoride (Mjengera, 2001). Recently different technology has emerged (El-Said *et al.,* 2018), including Nano, regeneration of fluoride-saturated bone char (Hegde *et al.,* 2020). These methods relied on the drinking water, though no management methods reported at household's level in different fluoride areas in Tanzanian population.

Awareness of fluoride exposures through potential fluoride sources such as locally grown and commonly consumed food crops and its health risk associated in the community is insufficiently reported in the literature, though Arumeru District is reported with dental and skeletal fluorosis (Ghiglieri *et al.,* 2010; Kaseva, 2006), this possibly leads to excessively exposure to fluoride through various potential sources such as food crops, water and milk, and relevant to adverse related health effects in human.

2.2 Fluoride Contamination of Selected Food Crops, Milk and Domestic Water

2.2.1 Food Crops

Fluoride contamination levels in food crops ranged from 1 mg/kg to 0.3 mg/kg, as recommended by EPA, FAO and WHO (WHO, 1984a), which include fruits, vegetables and cereals. Plants derived fluoride ion from either contaminated air and/or soil, whereas, in the air enters the plant through the leaves, and in soil through the roots. Outer parts of food crops contain more fluoride ion than internal parts (Gupta & Banerjee, 2011). The fluoride enhancement in plants attributed to the increased rate of metabolism (and/or photosynthesis rate) in leafy/ shoots in comparison to seeds/grains or other storage organs (tubers) (Gurumurthy-Sastry *et al.,* 2010). Higher metabolic activity can be associated with higher intake of water resulting in increased fluoride concentrations in shoots/vegetables (Khandare & Rao, 2006).

Fluoride contamination reported in different food crops such as; cereals, vegetables, and infants/young children complimentary foods (Kanduti *et al.,* 2016; WHO, 2014; Yin *et al.,* 2017) (Table 1). Also, plants grown in either contaminated soil or fields irrigated with contaminated water have been reported with accumulation of fluoride in its edible and non-edible parts (Lima-Arsati *et al.,* 2018; Narsimha & Rajitha, 2018). Similarly, fluoride was reported to affect rivers, extending to agricultural areas during the dry season and resulting in a relevant issue for food insecurity (Firempong *et al.,* 2013).

Ngarenanyuki River is one of the rivers in the northern zone of Tanzania that has reported with fluoride up to 26 mg/L (Ghiglieri *et al.,* 2010). However, it has been used as a source of irrigation water for crop production during the dry seasons in the community as the only source of available and affordable water (Kaseva, 2006). Such high fluoride concentration may result into unacceptable fluoride contamination in food crops. Fluoride ion from boiling, processing or contamination water used for cooking was reported to add concentrations in food (Lakshmi, 2011). Cereals crops reported with minimum fluoride, with accumulation in the outer layer of the grain and in the embryo (Gautam *et al.,* 2010; Lakshmi, 2011).

Maize is the main source of calories dietary intake for most of the population in developing countries (Lyimo *et al.,* 2003), grown and consumed daily by the majority in Africa and particularly in Tanzania (Kichana *et al.,* 2019). Though, it has reported with fluoride contamination in its embryo and outer parts (Den-Besten & Li, 2011; Gautam *et al.,* 2010; Mustafa, 2014).

Similarly, maize thin porridge, known as *Uji* in Swahili made from sole maize flour added with milk, sugar, or lemon juice for taste used as a complementary food for children. This is due to availability and affordability for the majority in rural areas in Tanzania and being the major staple cereal food crop in a community. Although maize has been reported to accumulate fluoride (Chakrabarti & Bhattacharya, 2013), when grown in a contaminated environment. According to Buzalaf *et al.* (2011) the use of fluoridated water changes the fluoride concentration of infant formula. However, due to high fluoride levels in most of the water sources in Tanzania, the national recommended fluoride level is 4 mg/L for domestic purposes in rural areas Tanzania Bureau of Standards (TBS, 2005), which can subsequently lead to high fluoride level in the infants/children through both food consumption and drinking water.

Green banana is amongst of popular dietary dish in Tanzania and Africa in general with a good source of fibers, vitamins, bioactive compounds, and resistant starch (RS) (Falcomer *et al.,* 2019; Ghosh & Mondal, 2019; López-Guzmán *et al.,* 2019). Green banana reported with fluoride contamination (European Food Safety Authority [EFSA], 2013; United States Department of Agriculture [USDA], 2009), indicates a higher risk to the vulnerable population. Excessive consumption of dietary with high fluoride concentration reported to increase fluoride level in the body (Craig, 2015; Narsimha & Rajitha, 2018).

Additionally, banana porridge commonly known as *mtori* in Swahili made up of blended cooked green banana mixed with either meat or fish a common food for mothers on maternity

and complementary food for young children in Tanzania. Whereby East African Highland triploid green banana "AAA" (*matoke*), variety mostly preferred in making banana porridge due to its softness in nature. Although according to EFSA (2013) and USDA (2009) banana has been reported with varying degree of fluoride concentration level, hence consumption of such crops on a daily basis can exceed the dietary reference value (DRVs) and increases the prevalence of fluorosis in a vulnerable population.

High fluoride contamination in leafy vegetables irrigated with fluoride concentration of up to 9.46 mg/L to 12.28 mg/L was reported in bajra and moth such that 0. 276 mg/Kg and 0.13 mg/kg, respectively (Guissouma *et al.,* 2017). Similarly, it has been reported that, Raddish leaves (*Raphanus sativus*), Spinach leaves *(Spinacea oleoracea*) and mustard leaves (*Brassica compestris*) leafy vegetables were contaminated with fluoride of 0.15 mg/kg, 0.29 mg/kg and 0.14 mg/kg, respectively, when were irrigated with 3.54 mg/L to 11.82 mg/L fluoride concentration in water (Gautam *et al.,* 2010). It was reported that tomato has an ability to accumulate lower fluoride levels than leafy and root vegetables (Gautam *et al.,* 2010). Leafy vegetables are susceptible to air borne fluoride ion, this accounts for wide variations in the contents of vegetables grown in different areas (Kazi *et al.,* 2019).

Vegetables are good sources of antioxidants rich in vitamins and minerals (Rouphael & Kyriacou, 2018). The World Health Organization (WHO, 2015) recommends a minimum intake of 400 g per day of vegetables and fruits, but, if vegetables contaminated with fluoride such consumption rate can increase the prevalence of fluorosis.

Cabbage (*Brassic*a *oleracea*) and Chinese cabbage (*Brassic*a *rapa sbsp*) are an affordable vegetable commonly consumed by majority in the community and used as source of income, is a good source of minerals, vitamins (A and C) rich in compounds like antioxidants, ascorbic acid, tocopherols, carotenoids, isothiocyanates, indoles, and flavonoids which has anticancer properties (Gupta *et al.,* 2015). Though, it has been reported with fluoride contamination ranged between 0.022 to 0.047 mg/kg in Nigeria (Paul *et al.,* 2011). Whereby, in India it was reported with high fluoride contamination of up to 11.30 mg/kg. Fluoride contamination of 0.14 mg/kg in cabbage from high fluoride areas also was reported (Pal *et al.,* 2012). In Ethiopia fluoride levels in cabbage was found ranges between 2.12 mg/kg to 2.70 mg/kg (Dagnaw *et al.,* 2017). Fluoride contaminations in food crops as reported in literature are summarized in Table 1.

Table 1: Fluoride contamination levels in raw food crops as reported in the literatures

Food crops (Vegetable)	F (mg/kg)	Country	References
Cow peas (*Vigna Unguiculata*)	2.96	Kenya	Kahama *et al.* (1997)
Kale (*Brassica integrifolia*)	0.15	Kenya	Kahama *et al.* (1997)
Saget (*Gynadropsis gynandra*)	0.51	Kenya	Kahama *et al.* (1997)
Terere (*Amaranthus hybridus*)	0.59	Kenya	Kahama *et al.* (1997)
Muhika (*Asystasia Schimpen*)	0.37	Kenya	Kahama *et al.* (1997)
Togotia (*Erucastrum arabicum*)	0.097	Kenya	Kahama *et al.* (1997)
Pumpkin leaves (*Cucubita pepo*)	0.14	Kenya	Kahama *et al.* (1997)
Spinach (*Spinacia oleracea)*	0.04	India	Gautam *et al.* (2010)
Radish	0.11	India	Gautam *et al.* (2010)
Radish leaves	0.26	India	Gautam *et al.* (2010)
Sarso leaves	0.04	India	Gautam *et al.* (2010)
Methi	0.08	India	Gautam *et al.* (2010)
Mustard	0.014	India	Gautam *et al.* (2010)
Tomato	0.092	India	Gautam *et al.* (2010)
Beans	11.3	Tanzania	Masawe (2019)
Beans	15.3	India	Gupta and Banerjee (2011)
Chinese cabbage	0.078	India	Gupta and Banerjee (2011)
Cabbage	2.12	Ethiopia	Dagnaw *et al.* (2017)
Spinach leaf	5.34	Nigeria	Paul *et al.* (2011)
Coriander leaf	26.9	India	Gupta and Banerjee (2011)
Potato	4.01	India	Gupta and Banerjee (2011)
Tomato	8.7	India	Gupta and Banerjee (2011)
Eggplants	14.5	India	Gupta and Banerjee (2011)
Onion	9.2	India	Gupta and Banerjee (2011)
Spinach	11.37	India	Gupta and Banerjee (2011)
Cabbage (*brassica oleracea*)	0.054	Nigeria	Okibe *et al.* (2010)
Carrot (*daucus carota*)	0.035	Nigeria	Okibe *et al.* (2010)
Lettuce (*lactuca sativa*)	0.096	Nigeria	Okibe *et al.* (2010)
Banana	0.005	Ireland	EFSA (2013)
	0.01	USA	USDA (2009)
Cereals			
Maize			
	7.7	Tanzania	Masawe (2019)
	5.1	Burundi	Gautam *et al.* (2010)
	5.9	India	Sunitha and Reddy (2014)
	12.2	Ethiopia	Den-Besten and Li (2011)
	0.98	Ethiopia	Mustafa (2014)
	0.3	Tanzania	Gautam *et al.* (2010)
Sorghum	0.48	India	Gupta and Banerjee (2011)
Wheat	0.07	India	Gupta and Banerjee (92011)
Rice	0.56	India	Gautam *et al.* (2010)
	0.12	India	Gupta and Banerjee (2011)

2.2.2 Contaminated Milk

General milk is recognized as a complementary food for infants/children consumed either as a milk-based formula or as whole milk (Hojsak *et al.,* 2018; Viswanathan, 2018), with rich sources of vitamins, such as vitamin A, Thiamine B1, riboflavin B2, B12 and D (Kazi *et al.,* 2018), and minerals such as magnesium, potassium, and calcium (Dutta *et al.,* 2019; Gribble & Fernandes, 2018; USDA, 2009). Fluoride contamination levels in cow's milk has been reported ranged between 0.199 ± 0.082 to 0.983 ± 0. 21 mg/L, in Mathura City in India, this was an area with high fluoride levels in water of up to 20 mg/L (Gupta *et al.,* 2015; Kazi *et al.,* 2018). Similarly, in Kenya fluoride levels in cow's milk was found with contamination levels ranged from 0.04 mg/L to 0.34 mg/L, animal feeding system and fluoride concentration level in water, reported as causative factors in high fluoride contamination in milk (Lv *et al.,* 2018; O'Callaghan *et al.,* 2019; Senghor *et al.,* 2018). Children recommended daily milk intake is 200 ml/kg body weight (Opinya *et al.,* 1991) per day up to 6 months (NHS, 2012), 6 months up to 12 months, 600 ml per day. But such recommendations can be exceeded if milk will be contaminated with fluoride and be vital sources of exposure to fluoride.

The health benefits of breastfeeding as an ideal food for infants (WHO, 2002), it is globally recognized, with digestive enzymes, hormones and matrix compositions of water. Human milk contains different nutritional and biochemical factors essential for the metabolic and physiological process during embryonic and fetal development such as; Arachidonic acid (C20: 4*n*-6, AA), and docosahexaenoic acid (C22: 6*n*-3, DHA) (Aryeetey & Dykes, 2018; Karakochuk *et al.,* 2017) protein, and lactose. The World Health Organization recommends exclusively breastfed for the first six months of life (WHO, 2011).

Human milk was reported with fluoride contamination of up to 0.55 mg/L from women with dental fluorosis (Poureslami *et al.,* 2016). Contaminated drinking water and diets are reported as causative factors to increase fluoride levels in breast milk (O'Callaghan *et al.,* 2019; Senghor *et al.,* 2018). Maternal dietary intake determines macro and micronutrient contents provided by the mother to the fetus/infant and is directly associated with infant growth during breastfeeding (Barrera *et al.,* 2018). Overconsumption of such contaminated diets possibly can exceed the recommended safe level of <0.002 to 0.1 mg/L by World Health Organization in breast milk (WHO, 2011) and causes direct/or indirect fluoride exposure to infants /children during breastfeeding.

2.2.3 Contaminated Domestic Water

Globally nearly 26 countries reported with high fluoride contamination in groundwater (Fawell *et al.,* 2006) (Table 2), including, Africa, Middle-east Asia, South America, Australia and USA (Kut-KMK, 2016; Saxena & Sewak, 2003). These areas are found along Rift Valley, which extended from Syria through Egypt, Sudan, Kenya, Turkey, Iraq to north Thailand (Thole, 2013) (Fig. 2). Different factors has been reported as main sources of high fluoride in ground water such as industrial activities, agricultural and volcanic activity due to desorption of fluoride from fluoride-rich rocks (Currell, 2011).

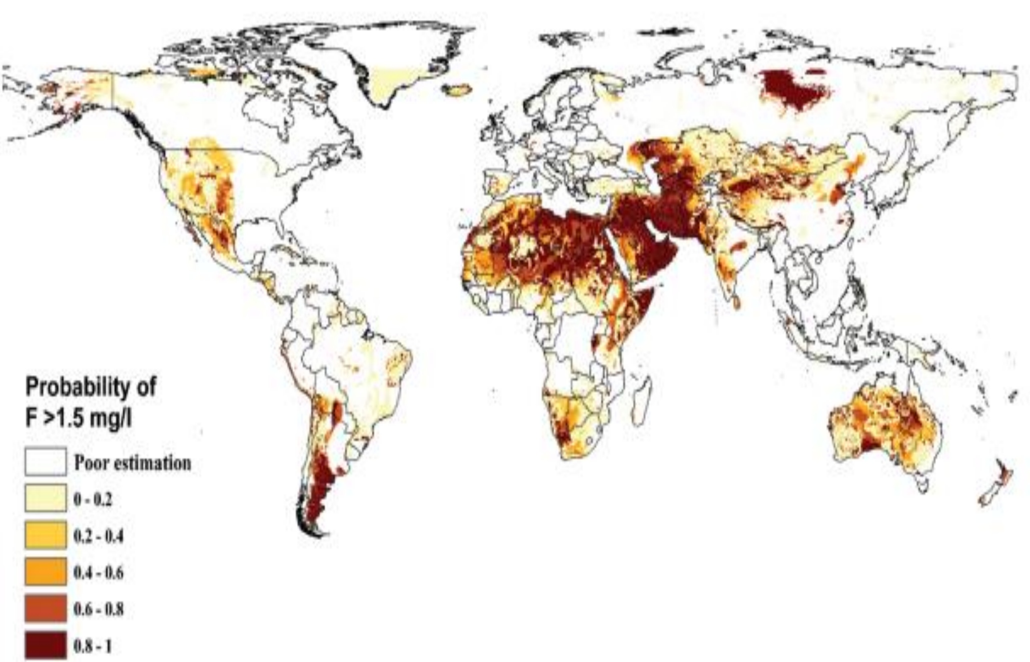

Figure 2: Fluoride in the groundwater above recommended safe limit of 1.5 mg/L by WHO (Amini *et al.,* 2008)

Fluoride contamination in domestic water sources has been reported as a critical health challenge (Adekola *et al.,* 2015; Moghaddam *et al.,* 2018) (Table 2). The World Health Organization (WHO, 2011), guidelines on safe water declared that, "all people, whatever their stage of development and their social and economic conditions, have the right to access to an adequate supply of safe drinking water." But challenges occur in most of developing countries to meet the recommended safe level of 1.5 mg/L (WHO, 2011), due to fluoride contamination in most of its water sources (Liu *et al.,* 2015).

Table 2: Fluoride in groundwater and its possible causes as reported in the Literatures from 2000 to 2016

Country	F (mg/L)	Causative factors
Argentina	0.7-2.2	Drilling activities
Brazil	3	Drilling activities weathering of rocks
Mexico	5.9-18.5	Weathering of rock
Canada	4.3-10	Runoff and infiltration of chemical fertilizers
USA	13.7	Drilling activities, liquid waste from industry
India	5-69.7	Weathering of rock
China	1.59-17	Industrial pollution and drilling activities
Pakistan	13.52	Industrial Pollution
Sri lanka	10	Drilling activities
Thailand	>10	Geothermal sources of water
Korea	7.53	Weathering of rock
Indonesia	14.2	Weathering of rock
Japan	0.4-7.8	Weathering of rock
German	88	Industrial pollution and drilling activities
Spain	4.59	Industrial pollution
Ukraine	20	Industrial contamination
Israel	3	Arid climate, weathering rock
Saudi Arabia	2.8	Arid climate, less rainfall
Sudan	3.2	Semi-arid climate
Kenya	2800	Volcanic activities and drilling activities
Nigeria	3.96	Volcanic activity
South Africa	57	Dissolution of minerals in bedrock and soil
Uganda	2.5	Metamorphic rock, volcanic activity
Tanzania	690	Volcanic activity
Senegal	7.4	Dissolution of minerals
Turkey	13.7	Dissolution of minerals
Norway	9.48	Drilling activities
Ethiopia	250	Volcanic activity

Kut KMK (2016), Saxena and Sewak (2003) and Thole (2013)

In Africa, like other continents which has been reported with high fluoride in its water sources (Isah *et al.,* 2014), high fluoride levels have been reported mostly in the countries which transverses by Great East African Rift Valley. This extends from Jordan valley through Sudan,

Ethiopia, Uganda, Kenya and Tanzania (Fawell *et al.,* 2006). Similarly, other countries reported with fluoride levels in its water sources includes: Ghana, Malawi, Nigeria, Algeria and the Republic of South Africa (Adekola *et al.,* 2015; Alfredo *et al.,* 2014).

In East Africa, the highest fluoride levels reported in Kenya, Tanzania, and Ethiopia (Fig. 4). In Kenya maximum fluoride of up to 800 mg/L was found in Lake Nakuru (Njenga, 2004), one of the Rift Valley Lakes in Kenya. Similarly, in Ethiopia high fluoride levels have been reported of up to 250 mg/L, in surface water body found in lake Chitu (Ayenew, 2008). Additionally, in Tanzania high fluoride level has been reported as high as 690 mg/L (Fawell *et al.,* 2006), in surface water body in Lake Momella in Arusha region, around Mount Meru areas (Fig. 3).

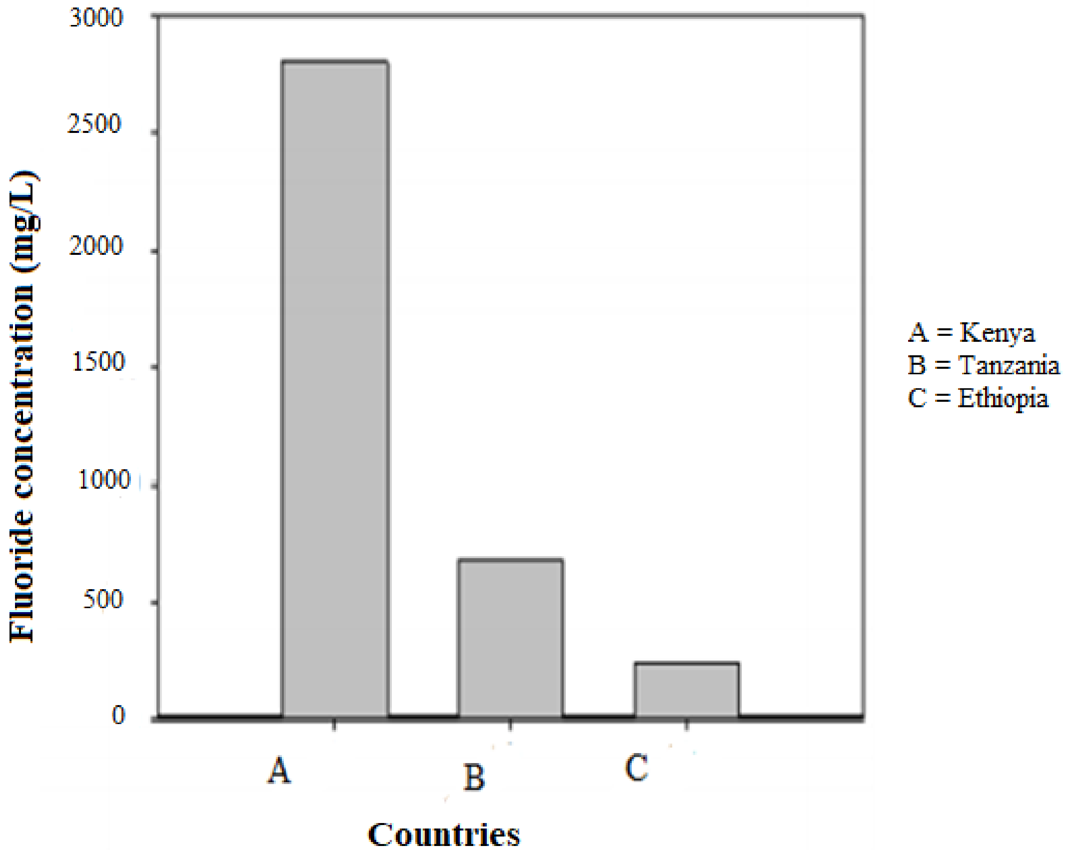

Figure 3: East African countries with high fluoride levels in its water sources (Malago *et al.*, 2017)

In Tanzania, fluoride concentration levels in water sources vary in different geographical areas (Fawell *et al.,* 2006; Malago *et al.,* 2017; Mkungu *et al.,* 2014). The most affected regions are those found along with the rift valley areas includes: Arusha, Kilimanjaro, Mara, Manyara, Mwanza, Shinyanga, and Singida (Malago *et al.,* 2017; Thole, 2013) (Fig. 4). Weathering associated with rock water interaction from soil and weathering of fluorine rich nephelinite and carbonatitic rocks has been reported as primary sources of fluoride contamination (Makoba,

2020). In areas around Mount Meru crater, gaseous expulsions through mineral springs and villiaumite (NaF) triggered by high temperatures coupled with high precipitation reported to increase fluoride concentrations (Komakech & Van der Zaag, 2011).

In boreholes fluoride concentration of up to 3.07 mg/L with the maximum level in Singida (Komakech & Van der Zaag, 2011) has been reported. Though was below the Tanzanian standard (4 mg/L) (TBS, 2005). it is above the WHO standard (1.5 mg/L) is likely to be a potential source for human exposure.

In springs fluoride concentration reported with a maximum concentration of 99 mg/L in Manyara (Komakech & Van der Zaag, 2011). For wells water sources maximum concentration mean value was 140.00 mg/L, while for surface water, rivers, and streams maximum value of 26 mg/L at Ngarenanyuki River (Ghiglieri *et al.,* 2010). The lakes/dams with high fluoride concentration include lake Momella (690 mg/L) (Ghiglieri *et al.,* 2010; Kaseva, 2006). Thus, it indicates that most of the water sources in Tanzania are contaminating with fluoride which can either attributing directly or indirectly human exposure to fluoride.

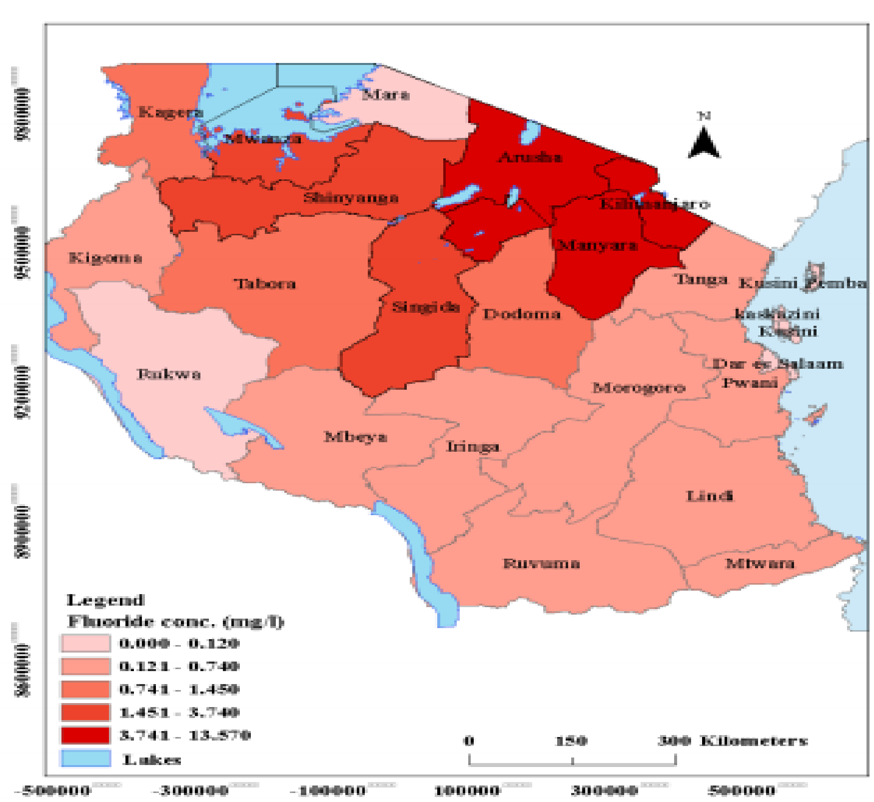

Figure 4: **Region with high fluoride levels in its water sources (Gumbo & Mkongo, 1995)**

2.3 Dietary and Biomarkers of Fluoride Exposure

Exposure to excessive fluoride through different fluoride contaminants sources such as, domestic water, fluoridated products like toothpaste, beverages, processed food and food crops which are grown in contaminated soil or irrigated with fluoride contaminated water reported as a primary sources of human exposure (Bashash *et al.,* 2018; WHO, 2015). Consumption of diverse diets with fluoride contamination can exceed the recommended dietary intake safe level of 0.01 mg /kg/d for infants up to 6 months and 0.05-0.07 mg /kg/d for all ages of above 6 months (EFSA, 2013; Rango *et al.,* 2014; WHO, 2003).

Fluoride is well known as a non-prescription toothpaste additive proven to prevent dental caries (Martin *et al.,* 2016). Sodium fluoride (NaF) acts as the most component for different dental products like toothpaste, gels, and mouth rinses (Akiniwa, 1997; Dutta *et al.,* 2019). Fluoridated products have been reported as potential sources for fluoride exposure ranged from 0.29 mg/L to 1.94 mg/L (Guissouma *et al.,* 2017) when unmanaged, initiation, and utilization for children (Moghaddam *et al.,* 2018). It was reported that, children who begin using fluoride toothpaste at age < 2 years are at higher risk for enamel fluorosis than children who starts later or who do not use fluoride toothpaste (Narsimha & Rajitha, 2018). The sixth year of life is the most crucial period for the development of dental fluorosis in late erupting permanent teeth (WHO, 2011), if fluoridated products excessively used (Bhagavatula *et al.,* 2016).

Fluoride levels of 0.5 mg/L in drinking water are recommended for prevention of dental cavities, with maximum limits of 1.5 mg/L (WHO, 2011). Though the daily intake of 122 mg/kgbw has been reported to cause fluorosis (Gupta & Banerjee, 2011; USEPA, 2005), while more than 200 mg/kgbw/day leads to skeletal fluorosis. A maximum of 0.08, 0.24 and 0.36 mg/kgbw/day, for adults, children and infant respectively was reported in areas with fluoride level of 2 mg/L (Colombani *et al.,* 2018). Whereas, the symptoms of acute fluoride poisoning reported at an estimated dose of 0.3 mg/kgbw/day (Idowu *et al.,* 2020).

Furthermore, it was reported that the use of trona leads to human exposure (Das & Mondal, 2016; Kaseva, 2006). Likewise, the majority of the population in the community of rural areas in Tanzania reported using trona (*Magadi* in Swahili) (Kaseva, 2006) for local food preparation such as those made of dehulled maize and beans (*makukuru)* to fasten the cooking process, possibly leads into human exposure.

Different factors accounted for susceptibility of individuals' exposure to fluoride such as bioavailability of ingested fluoride. This influenced by ionic or bound forms of fluoride solubility at a given pH, presence of other ions or complexion agents; and physiological factors like, food uptake which can influence the release and absorption of fluoride in foods eaten concurrently (Ranjan & Ranjan, 2015).

A fluoride biomarker is a factor used to assess the impact of fluoride in the body, whereby the recommended markers of present or recent fluoride intake include: blood, bone surface, milk, and urine. The concentrations in bone, teeth, nails, and hair are markers of historic fluoride intake (WHO, 2014). Dental fluorosis, whether minor or more noticeable, is the consequence of excessive fluoride intake (Rango *et al.,* 2017; Rugg-Gunn *et al.,* 2011).

The World Health Organization (WHO, 2011) recommended urine as "contemporary" biomarkers of fluoride exposure indicates the percentage of gastrointestinal absorption, retention in calcified tissue, and renal excretion (WHO, 2014). A proportion of ingested fluoride excreted in urine (Villa *et al.,* 2010). Whereas, daily urinary fluoride excretion is a useful biomarker of contemporary fluoride exposure (WHO, 2014), as an alternative method for estimation of fluoride exposure in populations and consequently as a basis for management of prevalence of fluorosis.

Moreover, human milk recommended as biomarkers of contemporary fluoride exposure which asses acute fluoride health effects (WHO, 2014); however, there are insufficient literature on the use and guideline of human milk as biomarkers of fluoride exposure. Thus there is a need of more studies to find an association between fluoride levels in human milk and food consumption to minimize the possibly route of human exposure to fluoride through dietary intake.

2.4 Fluoride Metabolism

Fluoride, absorbed in the body with a mean time of 20-30 minutes after ingested (Buzalaf & Whitford, 2011; Fawell *et al.,* 2006) about 90% absorbed in the gastrointestinal tract (Devi & Sarma, 2016), 25% in the stomach, and 77% in the proximal part of the small intestine (Buzalaf & Whitford, 2011) (Fig. 5). Its absorption, distribution, and excretion reported being independent of acidic nature, whereas, when the pH falls below the pKa of 3.4, 50 % of fluoride diffused in the undissipated form of hydrogen fluoride than as ionic form (Whitford *et al.,*

1999). At high pH (> 3.5) in the blood and saliva is absorbed in ionic form (Adekola *et al.,* 2015).

Adults retained approximately 36% of absorbed fluoride, whereas children almost 50% of fluoride; 99% of that contained in mineralized tissues such as bone and teeth, 1% in soft tissue (Buzalaf & Whitford, 2011). Fluoride which will not be absorbed in the stomach was reported to be in the proximal part of the small intestine in ionic form (Adekola *et al.,* 2015; Asif-Ul-Alam *et al.,* 2014; Buzalaf & Whitford, 2011). Lipid bilayer membranes are more permeable to hydrogen fluoride than to ionic form (F^-) (Buzalaf & Whitford, 2011). The presence of minerals such as magnesium, phosphorus, and aluminum has reported decreasing the absorption of fluoride in the body (EFSA, 2013), especially in food rich with Ca, this is due to the formation of fluoride complexes compounds (Cerklewski, 1997; Setnikar *et al.,* 1998). The absorption efficiency of fluoride from diverse dietary intake was reported of up to about 80-90 % of the ingested fluoride (EFSA, 2013).

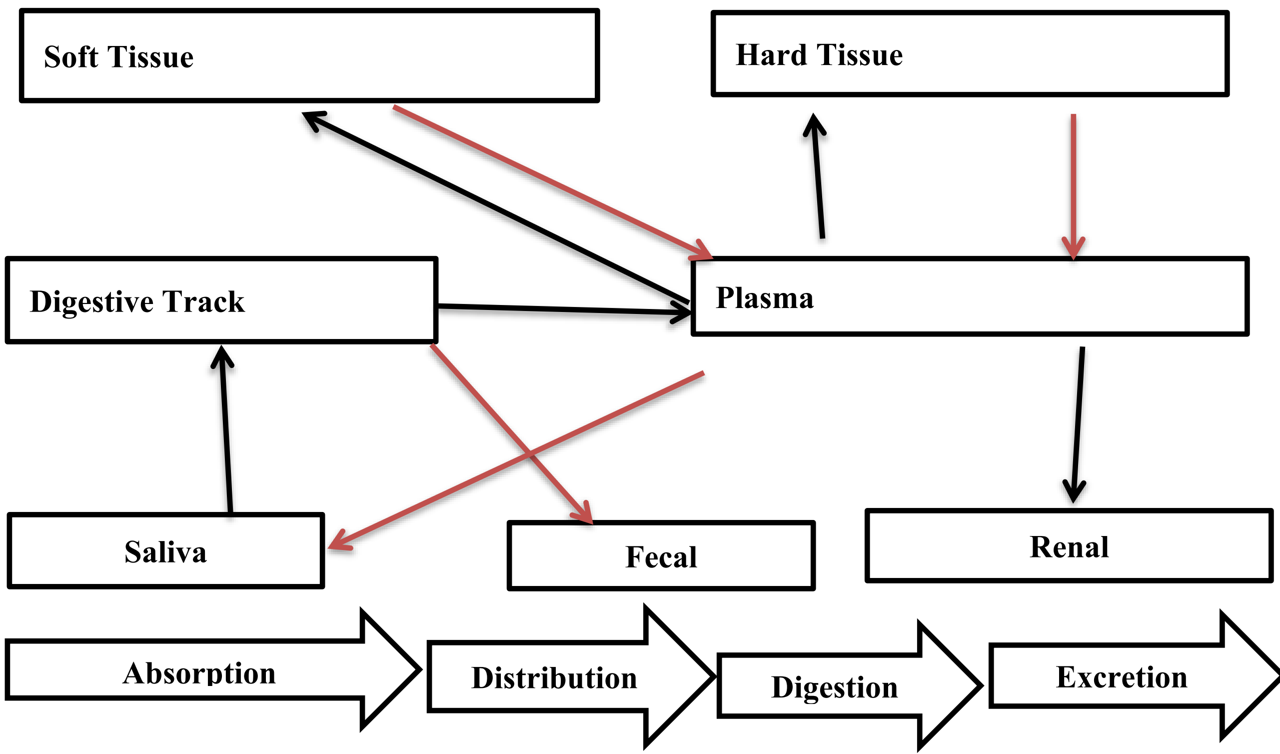

Figure 5: **Flow chart for fluoride metabolism**

Furthermore, it was reported that, when fluoride ingested with calcium in a meals, was associated with significantly delayed absorption and reduced peak plasma concentrations of fluoride (Buzalaf & Whitford, 2011; Villa *et al.,* 2000). In pregnant women, uptake of fluorides in the placenta is dependent on the fluoride concentration in the mother`s bloodstream (Addison *et al.,* 2020). When the concentration is low, fluoride is transmitting into the placenta (Gurumurthy *et al.,* 2010). On average, the fluoride is about 60% in the mother`s bloodstream (Gupta *et al.,* 1993). If the fluoride concentration increases over 0.4 ppm placenta works as a barrier, preventing the fluoride from passing through and thus protecting the fetus from a high fluoride concentration (Gupta *et al.,* 1993; Gurumurthy *et al.,* 2010).

Fluoride metabolism in the body is influenced by diets, acid-base disorders, physical activity, hormones, kidney function, genetic disorders (Narsimha & Rajitha, 2018). The remaining part of the absorbed fluoride excreted through the kidneys into the urine (Fawell *et al.,* 2006). Absorbed fluoride which is not deposited in calcified tissue is excreted via the kidney (Villa *et al.,* 2010), amount of absorbed fluoride excreted via the kidney in infants and/or children reported to be low up to the age of four months due to higher capacity of bone to accumulate fluoride (Buzalaf & Whitford, 2011). Dietary or other factors that change the acid-base balance of the body and decrease the pH value of the urine has been reported to reduce renal excretion of fluoride and lead to higher fluoride concentrations in the body (Chiniah, 2017). Also decrease of fluoride excretion has been reported to be associated with impaired renal function (Torra *et al.,* 1998) and with an age-related decrease of glomerular filtration (Jeandel *et al.,* 1992).

2.5 Fluoride Health Effects

Insufficient fluoride in the daily dietary intake has revealed no significant health effect during tooth development, though it has been reported to increase the vulnerability of enamel to acid occurrences after the eruption (Everett, 2011; Knuuti *et al.,* 1994). According to Bergmann (1995) in their findings on infants from an area with a low fluoride content of drinking water of about 0.25 mg/day, a normal growth was described. However, exposure to an excessive amount of fluoride from various contaminated sources over a lifetime (Grobler *et al.,* 2011; Ponikvar, 2008), leads to adverse health effects which can either be acute or chronic.

Generally identified acute health effects include death related to binding of fluorine with serum calcium and magnesium, vomiting, nausea, chronic convulsion, necrosis of the mucosa of the digestive tract and heart failure (Kabir *et al.,* 2020; Ullah *et al.,* 2017; Yadav *et al.,* 2019). The

symptoms of acute fluoride health effects depend on the type and chemical nature of the ingested compound, the age, duration, and doses of exposure range between 5 to 8 mg/kg body weight (Buzalaf *et al.,* 2012; Yousefi *et al.,* 2018). For example a soluble compound such as sodium fluoride (NaF) is more toxic than insoluble compounds calcium fluoride (CaF_2) because it releases more amounts of fluoride compared to calcium fluoride (Ponikvar, 2008). Other reported symptoms of acute fluoride health effects include, abdominal pain, diarrhea, weakness, hypocalcemia hypotension, bronchospasm, fixed and dilated pupils, and hyperkalemia, which may result in ventricular arrhythmias and cardiac arrest (Buzalaf, 2018).

Loss of body fluid contributes to an electrolyte imbalance, a state of hypovolemic shock, and decreased blood pressure (Bashash *et al.,* 2017), polyuria resembling diabetes insipidus, which may persist for days to months (Aydgan *et al.,* 2018). In a few instances, acute polyuric renal failure has been terminated fatally (Barrera *et al.,* 2018). A progressive, mixed metabolic and respiratory acidosis may develop because of the fail of renal and respiratory systems (Bashash *et al.,* 2018; WHO, 2014).

Chronic fluoride health effects described being more common than acute effects, which on several other factors such as nutritional status, renal function, and interactions with other trace elements (Jha *et al.,* 2011; Thompson, 2018). Commonly identified effects include increased likelihood of bone fractures, bone pain and tenderness in adults, increased chance of developing pits in tooth enamel for children under five years or younger, dental fluorosis (Štepec & Ponikvar-Svet, 2019; Wei & Ekstrand, 2019).

Scientific studies performed on animals suggest fluoride has toxic effects in the reproductive system, thyroid hormones, learning and memory abilities, growth, blood and feed efficiency (Rango *et al.,* 2017). Mullane *et al.* (2016), and Valdez-Jiménez *et al.* (2011), based on their studies; reported that fluoride has toxic effects in the reproductive system, growth.

Dental fluorosis is the most sensitive and the initial indicator of chronic fluoride toxicity (Kidd, 2005; Levine, 2010). The ingestion of fluoride more than 0.1 mg/kg regularly throughout the period of tooth development interfered with the process of enamel and dentin development, which leads into dental fluorosis (Das & Mondal, 2016; Kabir *et al.,* 2020; Limaleite *et al.,* 2015), lead to irregular distribution narrowing and disrupted of the lumina of the tubules (Den-Besten & Li, 2011; Kidd, 2005). It leads into the mild opaque white to brown mottling of enamel associated with pits and enamel fracture in both deciduous and permanent dentitions (García & Borgnino, 2015; Ponikvar, 2008). The severity of dental fluorosis not only depends

on excessive consumption of fluoride but also on the timing and duration of excessive fluoride consumption, the plasma concentration of fluoride, type of fluoride consumed, renal function, and genetic factors (Limaleite *et al.,* 2015).

Skeletal fluorosis is the condition characterized by an increase in bone mass and density, this is due to the excess deposition of fluoride within the bone matrix (Jha *et al.,* 2011). The skeletal fluorosis is associated with symptoms such as: Sporadic pain joints, stiffness due to fluoride deposition with resultant difficulty in mobility, kyphosis of the backbone, tingling sensation, muscle weakness, and fatigue (Guissouma *et al.,* 2017). The progressive phase of skeletal fluorosis had signs of arthritis and osteoporosis in long bones, spinal cord compression, and calcification of ligaments with resulting neurological defects and muscle wasting (Ranjan & Ranjan, 2015), reported to occur in fluoride doses greater than 4 mg/L (Mascarenhas, 2000; Torra *et al.,* 1998) (Plate 1).

The crippling skeletal fluorosis is associated with the intake of water with fluoride levels greater than 10 mg/L (Ranjan & Ranjan, 2015). Remarkable restraint of joint movements and deformities of major joints and spine, of which lead to neurological problems (Jha *et al.,* 2011; Tahir & Rasheed, 2013). The severity of skeletal fluorosis depends on the amount of water intake, quality of water, renal disease, and dietary factors such as calcium-rich diet, which has a protective effect and prevents the toxic effects of fluoride on bones (Rojas-Sanchez *et al.,* 1999).

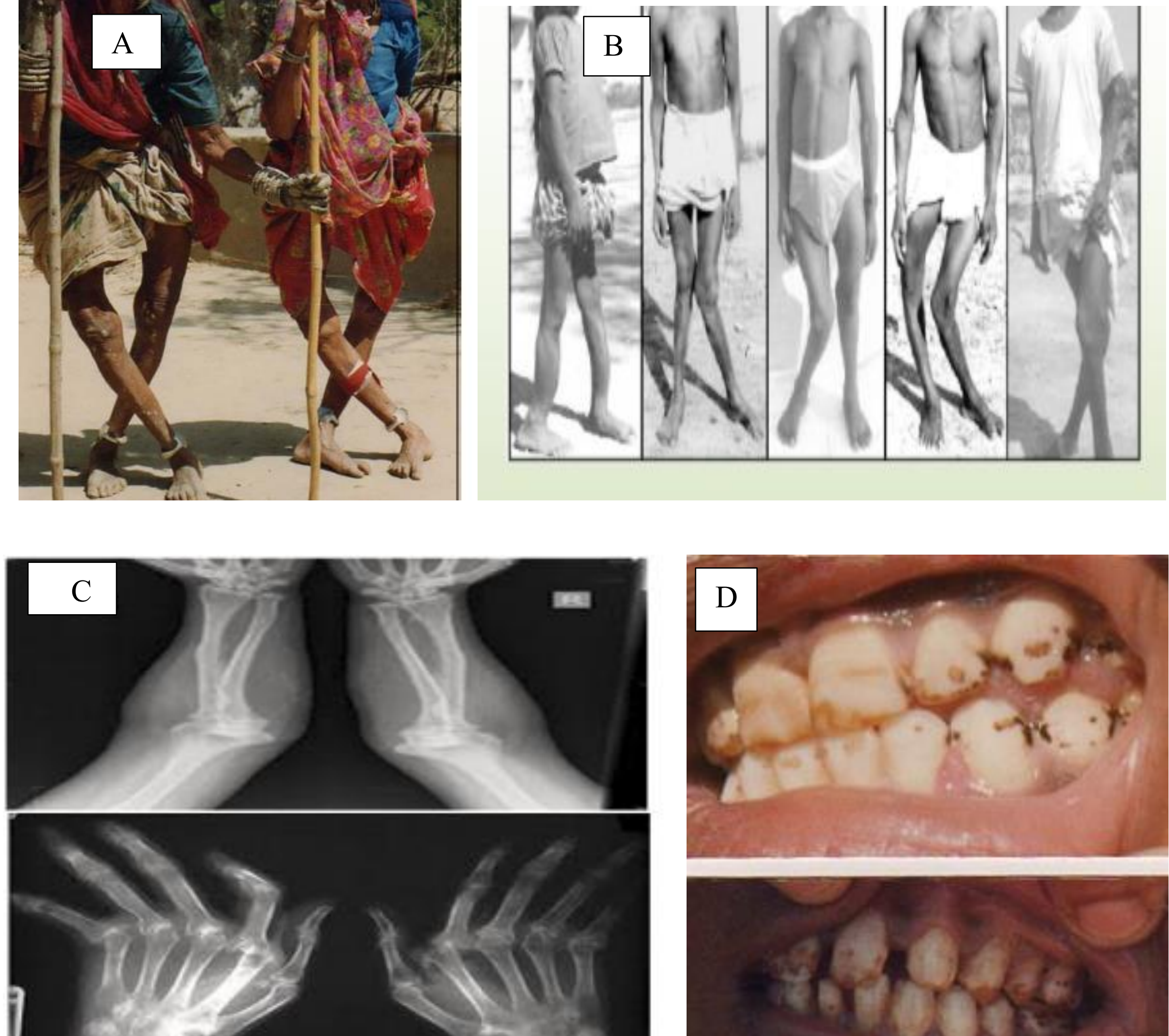

Plate 1: Skeletal (A, B & C) and dental fluorosis (D) (Choubisa *et al.,* 2016; Shanmugam *et al.,* 2018; Torra *et al.,* 1998)

The kidney is the most affected organ due to the uptake of fluoride within the kidney tubules results in the structural and functional changes (Jha *et al.,* 2011; Tylenda, 2011). Intake of water up to 8 mg/L leads to swelling, degeneration of tubular epithelium, fibrosis, atrophy of glomeruli, and tubular necrosis. All these structural changes result in increased serum creatinine and urea nitrogen (Limaleite *et al.,* 2015; Limón-Pacheco *et al.,* 2018). Fluoride reacts with gastric acid in the stomach and forms hydrogen fluoride, which irritates the gastric mucosa (Kaya *et al.,* 2015). Non-ulcer dyspeptic symptoms have been observed in populations consuming high fluoride concentration up to 3.2 mg/L (Kabir *et al.,* 2020). Animal studies reveal that fluoride has the potential to stimulate the secretion of gastric acids, diminish blood supply away from the stomach lining, and may result in the death of epithelial cells of GIT (Das & Mondal, 2016; Zhang *et al.,* 2016). Adverse GIT symptoms have been common reported in areas of fluorosis where nutrition is contaminated with fluoride (Ozsvath, 2009).

Fluoride has been reported to affect mental development, learning disorders, and decrease intelligence and hyperactivity in children when crossing the blood-brain barrier before birth (Mascarenhas, 2000; Reddy, 2016). The levels of the neurotransmitters and the number of receptors reported to decrease in areas with high fluoride in its water sources (Ranjan & Ranjan, 2015). Also, fluoride results in degenerative changes in neural tissues (Jetti *et al.,* 2016; Jiménez *et al.,* 2019). These changes might account for neurological alterations such as numbness, pain, and muscle spasm and decreased memory and learning ability of the experimental animals (Valdez-Jiménez *et al.,* 2011). Children ingesting high levels of fluoride (>2 mg/L) reported to score poorly on intelligence tests compared with children ingesting lower amounts of fluoride (<1 mg/L) (Ranjan & Ranjan, 2015). Besides, fluoride influences reaction times and visuospatial capabilities, hence lowering the IQ scores during the time-sensitive tests (Ozsvath, 2009).

Fluoride crosses the placental barrier and incorporates into the fetal tissues lead to disturbances in bone ossification (Buzalaf & Whitford, 2011; Firempong *et al.,* 2013). Fluoride affects the fetal brain tissues and results in significant neurological impairment, neuronal degeneration, and reduced secretion of neurotransmitters such as norepinephrine (Yadav *et al.,* 2019). Further, fluoride disrupts the secretion of neurotransmitters and nerve cell receptors and results in neural dysplasia (Buzalaf & Whitford, 2011; Mukherjee *et al.,* 2019; Petersen *et al.,* 2010).

According to Chabukdhara *et al.* (2017), it has been shown that fluoride concentration in drinking water is associated with decreased birth rates. Their study suggests sodium fluoride in drinking water of 2, 4, and 6 mg/L concentration for 6 months to male rats associated with testicular disorders and adversely affected their fertility and reproductive system. Also, Bashash *et al.* (2017) observed significant decreases in the serum-free thyroxin, free triiodothyronine levels, acetylcholine esterase activity in fluoride treated group. The same study suggests that change in levels of thyroid hormones might be due to inhibition of iodine absorption through fluoride interaction. The study concludes that fluoride exposure causes cumulative multigenerational effects resulting in decreased thyroid hormone which correlated to learning and memory impairments. Studies on toxicity of fluoride on animals suggest that biological responses of animals to fluoride are related to dosage and other factors that influence the animal's physiological and anatomical responses (Moghaddam *et al.,* 2018; Ranjan & Ranjan, 2015).

CHAPTER THREE

MATERIALS AND METHODS

3.1 Study Area

This study was carried out in the selected villages of Ngarenanyuki ward, of Arumeru District in the Arusha region. The areas are located at 3.2923° S, 36.8250° E around Mount Meru in the areas of northern Tanzania (Fig. 6). Ngarenayuki ward is a geographically diverse set of rural areas located approximately 55.7 km from Arusha town, an area characterized as an oceanic climate with mainland temperatures. The area's mean daily temperature ranges between 15°C to 30°C during the dry months of July and August and 12°C to 17°C in the cold months of December to June; the corresponding mean annual rainfall ranges between 950 mm to 100 mm (Mkungu *et al.,* 2014). This area was reported with high fluoride concentration in its water sources and soil (Craig, 2015; Malago *et al.,* 2017), due to its geological nature along the Rift Valley. Olkung'wado, Uwiro, and Ngabobo villages were purposively selected based on the involvement in agricultural activities along Ngarenayuki River which, supplies water for both agricultural production during the dry season and domestic use when the Municipal water supply system is scarce (Plate 2). The river reported to contain fluoride up to 26 mg/L (Ghiglieri *et al.,* 2010), and for this reason, areas served by this river identified with dental fluorosis (Kaseva, 2006), provided a suitable study location.

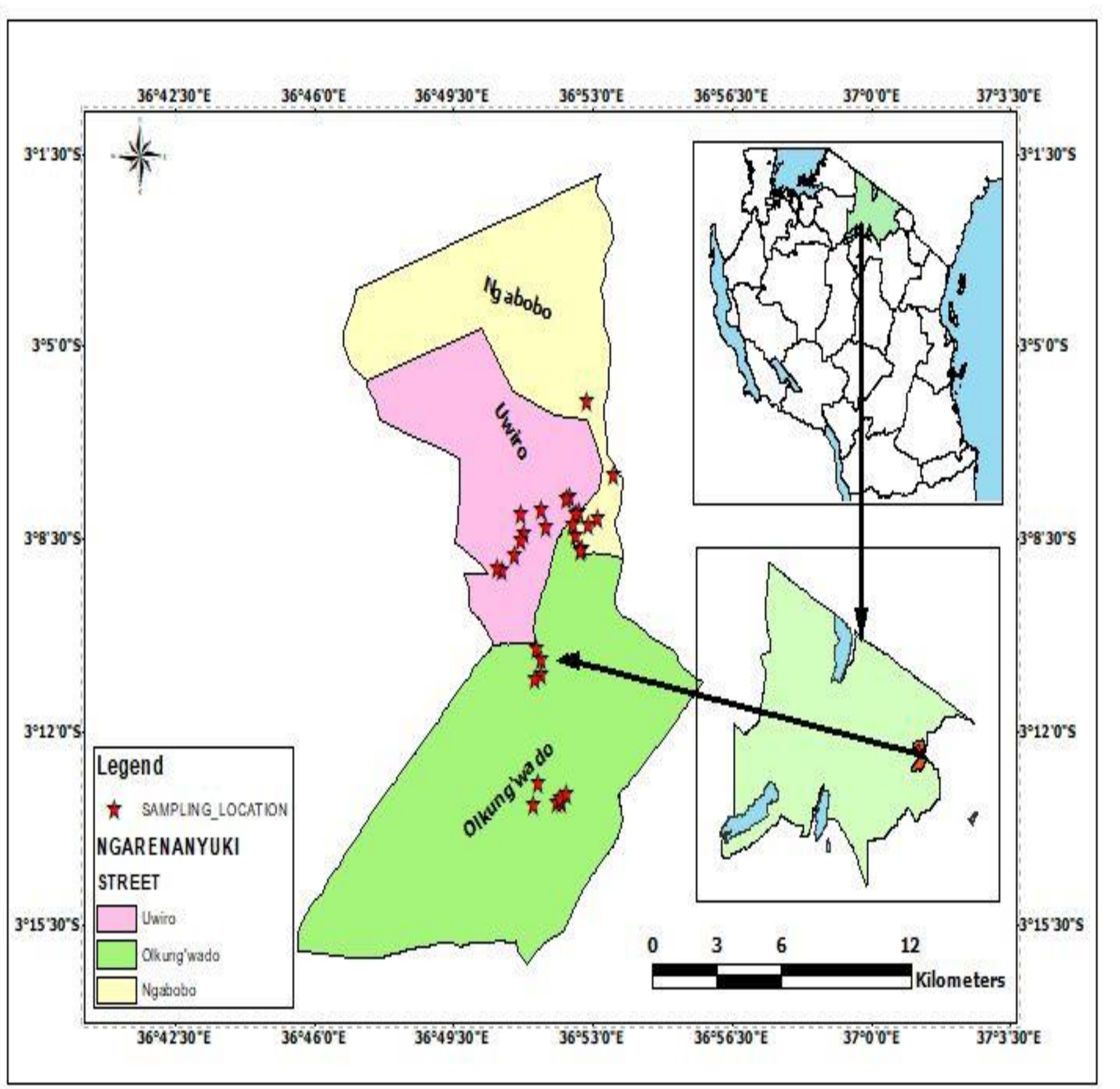

Figure 6: The study location

Plate 2: Ngarenanyuki River (A), Agricultural activities along the River (B) and (C)

3.2 Research Design

Mixed study designs with both cross-sectional and survey designs were used. Locally grown food crops and demographic information were obtained using a structured questionnaire, while a food frequency questionnaire and 24-hour diary recall, tools used to assess individual habitual dietary intake of foods and nutrients, were used to identify commonly consumed food crops and complementary food. Whereas experimental design was used to determine fluoride content in the identified commonly consumed food crops, water, milk, and urine.

3.3 Selection of Study Participants

(i) Sample Size

A total of 322 households were obtained using Slovene's formula (n=N/[1+Ne2]) from a population of 1650 households with infants and young children sourced from reproductive and child health clinics.

Where:

n= Sample size

N= Total population (1650)

e = Error tolerance =0.05

Therefore: n= 1650/(1+1650×0.052) = 322

(ii) Study Population

A total of 300 women of reproductive age (15-49 years) with children aged between 0 and 24 out of 322 were involved in this study. A random sampling technique was used to sub-sample 1/3 of participants from the total responses in order to get mother-baby pairs for fluoride exposure assessment. As a result, a total of 107 mother–baby pairs were selected. Women were only considered eligible to participate in the study if they were having a child aged between 0-24 months, had been breastfeeding, and had been introduced to complementary food during the survey. Their participation was anonymous and voluntary.

3.4 Households Interviews and Sample Collection

Mothers were interviewed to source information on demography, general awareness of fluoride sources and community management practices, locally grown and commonly consumed food types including milk, and sources of domestic water. In Tanzanian communities, women play

a key role in planning family meals, including complementary feeding and child caring; they take the lead in agricultural activities, including home gardening (Gudrun, 2012), though most of the farms are owned by men (Lyimo *et al.,* 2003).

Sampling of plant materials followed the methods described by Jones Jr (2001). The leaves of the vegetable were randomly selected from the four corners and center for each vegetable garden and thoroughly mixed to get representative samples. For cabbage *(Brassica oleracea)* and Chinese cabbage (*Brassica rapa sbsp),* 4-5 leaves from the center of the whorl prior to heading were sampled from 15-20 plants from each sampled garden. For African nightshade (*Solanum species*) vegetables, matured leaves from the top of the plant were sampled from 20-30 plants. Fresh plant tissues were placed on clean paper bags and partially air-dried.

Approximately 250 g of maize flour were collected from 107 households; samples were placed in food-grade plastic bags. For green banana varieties, East African highland banana (*matoke*), East African diploid banana (*mchare*) and Cavendish group (*malindi*) samples were randomly collected in four consecutive weeks from the local market during the market day, which takes place once a week. From a bunch of each cultivar, 10 fingers were randomly sampled from the second and third hands of each bunch (Asif-Ul-Alam *et al.,* 2014) to give a total of 30 samples for each cultivar.

Human breast milk sampling was done according to Mannel *et al.* (2012). Out of 107 women, 104 samples were collected from women who consented to participate in this study and were willing to give biological samples. Collection was carried out in the villages' health centers during clinic day. Women were instructed with the help of a health officer on how to collect milk samples, whereas the breast was swabbed with cotton wool and distilled water before milk collection, and then milk was hand-expressed to obtain about 15 mL of samples.

Similarly, approximately 200 mL of cow's milk were collected instantly during the normal milking schedule from 106 households. All milk samples were collected into sterile standard containers for biological sampling. Additionally, domestic water was sampled from 107 households in the communities while tap water was sampled from 15 public taps water from different location. About 500 mL of both domestic and taps water were collected into a standard sterile, sodium thiosulfate-free polyethylene bottles. All collected samples were placed in the cool box for less than six hours including the time for transportation to the NM-AIST laboratory.

Out of 107 children, 105 samples of urine (57 from boys and 48 from girls) were collected from children who met criteria, aged between 0-24 months, had been breastfeeding, and who had been introduced to complimentary food during the survey, and their parent's consent to participate in this study, and were willing to give biological samples. Sampling was carried out in the villages' health centers during the clinic day. Mothers were given labeled sterilized fluoride-free containers for biological sampling, guided by nurses on sampling procedures. Approximately 30 mL of urine from each child was collected and placed in a cool box. All collected samples were placed in the cool box for less than six hours, including the time for transportation to the NM-AIST laboratory.

3.5 Sample Preparation and Digestion

Samples were prepared according to the procedures described by Hue (2000). All collected leafy vegetable samples (cabbage, African nightshade, and Chinese cabbage) were washed with deionized water. On the other hand, green banana samples were peeled and sliced to 2 mm size. After that, each sample variety was placed on paper containers, followed by drying in a forced-air oven (Mmermet, Germany) at 70°C for 72 hours (Jones Jr, 2001). Dried plant and banana samples were ground (Waring blender, Thomas Scientific, Swedesboro, USA) to pass a 2-mm sieve (Thomas Scientific).

Ground samples of leafy vegetables, green banana, and maize flour samples were kept in a food-grade plastic bag and sent to the Tanzania Bureau of Standards (TBS) in Dar es Salaam for digestion as described by Gawalko *et al.* (1997). All the reagents used for sample digestion were of analytical grade (Merck, Darmstadt, Germany), whereby apparatus used for sample digestion, such as glassware, digestion flasks, and polythene bottles used for digestion and storage of samples, were pre-washed and soaked in detergent water for 2 hours. Properly washed and rinsed with deionized water and then soaked in 10% (v/v) HNO_3 for about 20 hours. Thereafter, they were rinsed with deionized water, followed by 0.5% (w/v) $KMnO_4$ solution, and finally rinsed with deionized water and allowed to air dry before use.

The sample was digested by using a mixture of nitric acid (Fishers Scientific UK Limited, Leicestershire, UK, perchloric acid) and sulfuric acid (Loba Chemie Pvt. Ltd., Mumbai, India); in the procedure, 0.5 g of sample was weighed (Analytical weighing balance (PA214) OHAUS, China) into a 50 ml graduated digestion flask, 1 mL of H_2O_4, 4 ml (1:1) HNO_4 (Loba Chemie Pvt. Ltd. Mumbai, India)–$HClO_4$, (SMiTH chemicals, India), and 5 ml of H_2SO_4 (RFCL Limited, Okhla, India) were added. Then samples were pre-digested overnight (16 h) in a class

100 clean fume hood at room temperature. A maximum of twelve vessels, including a reagent blank and a sensor, were sealed and digested using the following heating program: The 165°C within a ramp time of 10 min at full power (1000 W), hold for 20 min at 165°C under the recommended maximum working pressure of 13.8 bar (200 psi).

For milk samples, milk was brought to a constant temperature of 25ºC in a water bath, an equal volume of 0.01 M HCl (RFCL limited). Okhla, India, was added to the 3 mL of sample and left at 37ºC for 1 hour. Then, samples were centrifuged at 3000 × g (3K15 low-temperature and high-speed centrifuge (Sigma-Aldrich, Steinheim, Germany) for 5 min to obtain a supernatant, which was used for fluoride determination.

For urine samples, 3 M sulfuric acid (H_2SO_4) (Loba Chemie Pvt. Ltd., Mumbai, India) saturated with hexamethyldisiloxane (HMDS) was added to the sample for 20-24 hours to allow the fluoride to diffuse from the urine sample. The diffused fluoride was collected in the 0.05 M of sodium hydroxide (NaOH) (Loba Chemie Pvt. Ltd. Mumbai, India) on the petri dish cover, 0.25 M of acetic acid (Loba Chemie Pvt. Ltd. Mumbai, India) was added to the sodium hydroxide to neutralize the solution, and then the same procedures were carried out for fluoride analysis.

3.5.1 Determination of Fluoride

Fluoride-Ion-Selective Electrode (ISE) (Model 9609, Orion USA), a pH/ISE meter (Orion model, EA 940 Expandable Ion Analyzer, USA), was used for fluoride analysis in the samples and the standard solutions according to Ahmad *et al.,* 2015. Whereby, total ionic strength adjustment buffer (TISAB II) was prepared before analysis was conducted by dissolving 58 g sodium chloride (Fisher Scientific UK), 57 mL glacial acetic acid (100%, Sigma-Aldrich Laborchemikalien, Germany), 7 g of trisodium citrate (BDH Laboratory Supplies, Poole, England), and 2 g EDTA (Scharlau Chemie S.A., Barcelona, Spain) in 500 mL deionized water into a 1000 mL beaker, and its pH was adjusted to 5.5 with 5 M sodium hydroxide (NaOH).

A standard solution was prepared using serial dilution from a 0.1 M NaF stock solution. The instrument was calibrated with standard sodium fluoride solutions; the concentration of the unknown sample solution was directly read from the digital display of the meter. For plants and milk samples, calibration was done using two points with a solution of 0.01 and 0.1 mg/L. For drinking water and urine samples, calibration was done at 1 and 10 mg/L.

Briefly, a buffer solution is added to the standard and sample solution to minimize matrix interferences and ionic strength effects. Analysis of samples and fluoride standards was carried out in triplicate, where an equivalent amount of TISAB (5 mL of TISAB buffer) was added to a 5 mL sample solution in a 50 mL plastic beaker with continuous stirring.

3.5.2 Assessments of Participants Dietary Intake

A 24-hour' dietary recall assessment method for food intake was used; lactating mothers were asked to recall all food items and drinks consumed by themselves and their index child in the past 24 hours. Two non-consecutive visits were used to collect this information. Estimation of food was weighed by using a kitchen scale (CARMRY, model EK31). Whereby, household measures such as cups, bowls, spoons, and plates were used to estimate the quantity of food consumed. The weight of each participant, lactating mothers and children, was measured to the nearest 0.1 kg using a portable digital balance (Seca 803, Seca, Germany). The average daily intake (g/day) assessed during the two non-conservative visits from individual participant was used in the assessment of dietary fluoride exposure.

3.5.3 Fluoride Exposure Assessment

Fluoride exposure assessment for lactating mothers and children was calculated by combining food consumption data with the fluoride concentration obtained from the laboratory analysis of fluoride in collected samples per individual body weight, as shown in formula 1.

Formula 1: fluoride exposure (mg/kgbw/day) =

$$\frac{\text{Concentration of fluoride in food (mg/kg)} \times \text{Consumption (g/day)}}{\text{Body weight (kg)}}$$

3.6 Statistical Analysis

Information obtained through questionnaires was analyzed by using the Statistical Package for Social Sciences SPSS™ (Inc., Chicago, IL, USA) Version 20 (SPSS), where for each question, the percentage of respondents who gave similar responses was calculated. All the laboratory data on fluoride content were analyzed by R software (4.0) for Windows. One-way ANOVA was used to compare whether there was a significant difference in the mean levels of fluoride between plant samples. The significance level was set at 0.05, which means the value was separated by Tukey's HSD (Honesty Significance Differences).

3.7 Ethical Clearance

Ethical clearance obtained from the National Institute for Medical Research (NIMR), No. /HQ/R.8a/ Vol. IX/ 2786. The permission to work in the district was obtained from the District Executive Director (DED) and District Medical Officer (DMO) of Arumeru district. Simultaneously, permission to work in the villages and households was obtained from the Villages Health committees. Oral and written consent was obtained from each participant and confidentiality was assured.

CHAPTER FOUR

RESULTS AND DISCUSION

4.1 Demographic Information and Locally Produced Food Crops

The majority of the participants were housewives (88%) (Table 3), aged between twenty (22%) and thirty (58) years, and only two percent were above 45 years. Most of the participants had primary education (78%), whereby about 19% never went to school. About 58% of participants were gardeners, while 39% were farmers. Only 3% of the participants were leaders either in the community or ward levels.

Table 3: Participant's demographic information at studied area (N=300)

Ages (years)	N	Percentage (%)
15-24	66	22
25-34	174	58
35-44	54	18
>45	6	2
Education		
Never went to school	57	19
Primary	234	78
Secondary	6	2
College/university	3	1
Occupation		
Ward/Village officers	3	1
Community leader/elders	6	2
Gardeners	174	58
Farmers	117	39
Employment		
Employed	36	12
Home maker/housewife	254	88

Locally grown and commonly consumed food crops were identified, whereby among cereal crops, white maize was leading (Plate 3), while vegetables were cabbage (*Brassica oleracea*), African nightshade (*Solanum species*), and Chinese cabbage (*Brassica rappa subsp.*) (Plate 4). For indigenous green banana (*Musa acuminata*) varieties, East African Highland triploid banana "AAA" (*Matoke*), East African diploid banana "AA" (*Mchare*) and Cavendish group "AAA" (*Malindi*) (Plate 5) were identified.

Plate 3: **Locally grown and commonly consumed (A) maize corn (B) flour**

Plate 4: **Commonly consumed leafy vegetables (C) Chinese cabbage (D) Cabbage (E) Night shade**

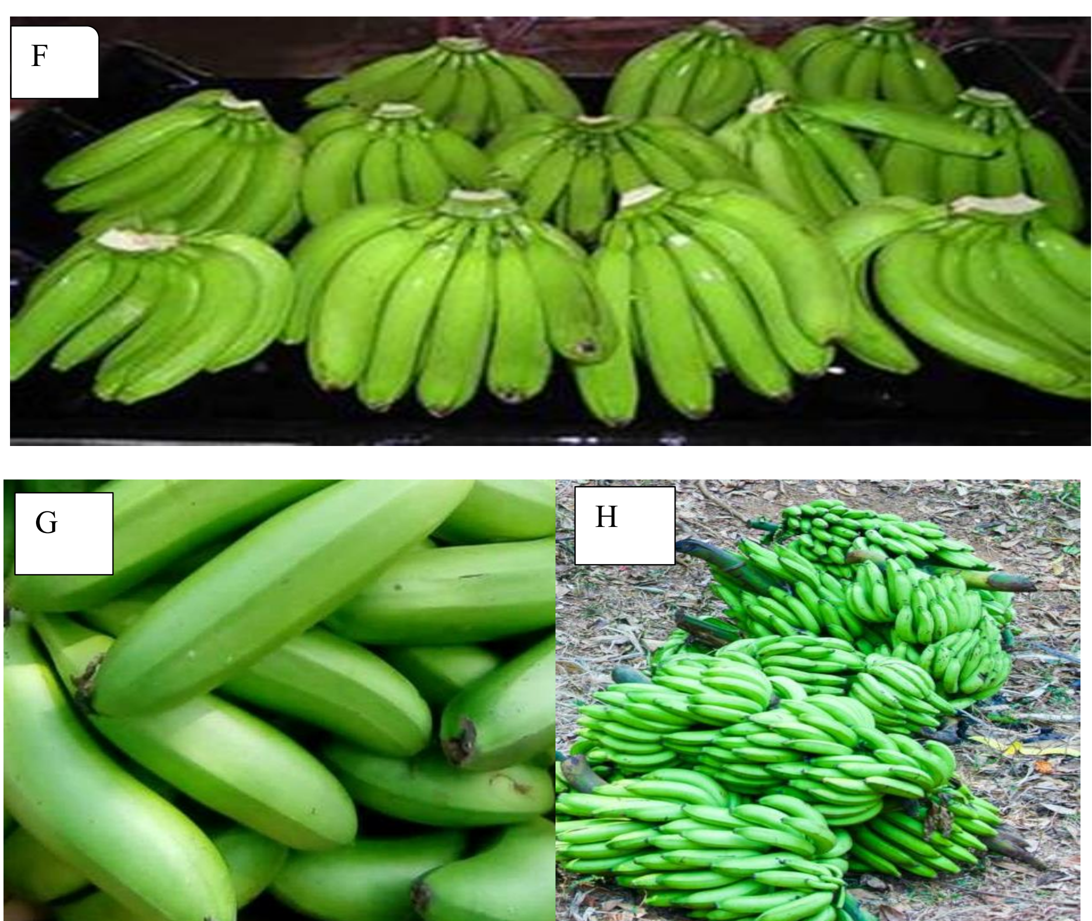

Plate 5: Locally grown and commonly consumed green banana varrieties; Cvendish group (*malindi*) (F), East African highland banana (*matoke*) (G), East African diploid banana (*mchare*) (H)

Results on community food consumption frequencies identified maize as the most consumed cereal (21.7%) by twofold that of rice (11.3%). Other cereals consumed in a very small proportion include wheat, millet, and sorghum. There was more diverse vegetable consumption, with a slightly higher frequency in African nightshade (18.4%), cabbage (15.2%), and Chinese cabbage (14.9%), followed by cowpea leaves, spinach, and pumpkin leaves. Other vegetables were consumed by fewer households. Among green banana varieties **(***Musa acuminata)***,** East African highland "AAA" (*Matoke***)** (15.4%) and East African diploid banana "AA" (*Mchare***)** (12.1%) were more consumed compared to Cavendish type, "AAA" (*Malindi**)*** (9.7%), and other varieties such as *Plantain AAB, Huti green, Muraru red, Ijihu inkundu, Njuru, Mlelembo* Mchare, and Kahuti were consumed, which were consumed by few, as shown in Table 4.

Table 4: Community food consumption frequency at studied area (N=107)

Locally grown crops	N	Consumption frequency
Cereals		
Maize	89	21.7
Rice	41	11.3
Wheat	1	0.2
Millet	4	0.3
Sorghum	3	0.2
Leafy vegetable		
Cabbage	63	15.2
Night shade	73	18.4
Chinese cabbage	62	14.9
Cowpea leaves	43	11.2
Pumpkin leaves	41	5.1
Spinach	51	9.2
Potato leaves	34	0.3
Okra	14	0.8
Amaranth	2	0.3
Beans leaves	9	0.1
Green banana (varieties)		
Cavendish (*malindi*)	45	9.7
E.A. diploid (*mchare*)	72	12.1
E.A. highland (*matoke*)	80	15.4
Plantain AAB	16	0.7
Huti green	16	0.1
Muraru red	2	0.1
Ijihu inkundu	9	0.3
Njuru	4	0.2
Mlelembo mchare	6	0.1
Kahuti	4	0.3

Consumption of food among children as part of complementary feeding was found to change with age, as shown in Table 5. For children aged 6 months, maximum consumption frequency was found in cow's milk (2.7%), while for age groups ranging from 7-12, 13-18, and 19-24 months, maize was consumed at a higher frequency of 9.2, 11.3, and 22.5%, respectively.

Table 5: Children complimentary food consumption frequency (N=107) at studied area

Age (month)	Variables	N	Percentage (%)
6	Cows milk	95	2.7
	Maize Porridge	100	2.1
	East African highland banana	89	0.6
7-12	Cows milk	100	7.0
	Maize	95	9.2
	East African highland banana	89	3.2
	Cows milk	105	8.13
13-18	Maize	107	11.3
	East African highland banana	105	9.8
	Cows milk	107	13.1
19-24	Maize	106	22.5
	East African highland banana	87	10.1

4.2 Awareness on Sources of Contaminant Fluoride and its Related Health Effects

Results showed that more than half (55.5%) (Table 6) of respondents were unaware of fluoride contamination. More than three quarters (77.5%) of the respondents were unaware of dietary fluoride exposure sources. The majority (66.7%) identified water as a potential source of fluoride, 9.1% and 24.1% identified soil, and other sources, respectively. Approximately 80% of participants, including children, were using fluoridated toothpaste. However, none of the respondents identified toothpaste as a source of fluoride. More than 90% of participants identified tap water as their primary source of domestic and drinking water. None of the participants mentioned any fluoride management methods or alternative domestic/drinking water sources to lower fluoride concentrations.

Table 6: Participant's awareness on fluoride exposure sources and its health effects

Attributes	N	Percentage
Fluoride contamination		
Aware	44	44
Not aware	55	55
Fluoride contamination sources		
Soil	9	9.1
Water	66	66.7
Other (s) (fresh fruits, juices, snacks)	24	24.2
Contaminant fluoride in food		
Aware	22	22.2
Not aware	77	77.8
Fluoride health effects		
Hard bended back	9	9.1
Large heads	7	7.1
Pits in tooth in children	79	79.8
Born fracture and pains	4	8
Domestic water sources		
River	2	2.02
Tap water	92	92.9
Bore hole	4	4.04
Fluoride reduction methods		
Not available	97	97.97
Available	2	2.02
Toothpastes usage		
Fluoridated	83	83.8
Non Fluoridated	16	16.16

Awareness about potential sources of fluoride contamination was insufficient in the community. Some participants identified water as the primary source of fluoride contamination. Even though the majority were unaware, they declared to have observed the presence of fluoride-like substances in domestic water. Since their ancient times, which were locally referred to as *'Magadi'*, they had been overused without knowledge for different purposes, such as feeding cows and in preparation of local food (*makukuru)* fasten the cooking process. However, there are no existing methods for the management of fluoride in domestic and drinking water at the household level. The use of *Magadi* was reported to cause excessive fluorosis (Das & Mondal, 2016; Kaseva, 2006), risks of fluoride accumulation, and severe health implications in children > 6 years of age when excessively ingested also was shown (Kanduti *et al.,* 2016). The study found participants were unaware of fluoride's recommended safe limit of 4 mg/L and 1.5 mg/L in drinking water for both national (TBS, 2005) and international (WHO, 2011) bodies, respectively.

The use of fluoride-contaminated water on a daily basis for drinking and cooking can contribute to direct and/or indirect exposure to fluoride and subsequently increase the prevalence of fluorosis. Economic status was mentioned as contributing to the inability to afford alternative water for domestic purposes. Also, insufficient awareness about potential fluoride contaminant sources like cereals crops/vegetables, dairy products, and fluoridated products might be causing excessive exposures to fluoride from other sources.

Likewise, the study indicated that the majority of participants were using fluoridated toothpaste since they were unaware that, especially in youngsters, fluoridated toothpaste could expose them to fluoride (Table 6). The fact that most mothers were not helping their kids wash their teeth might result in increased paste swallowing and higher fluoride absorption. As compared to children who start using fluoridated toothpaste later or do not use fluoridated toothpaste (Asawa, 2015; Rugg-Gunn *et al.,* 2011). Children who consume fluoridated toothpaste at levels higher than 0.7 to 0.5 ppm (Marín *et al.,* 2016; Sami *et al.,* 2016), are at risk for enamel fluorosis. Children who start using fluoridated toothpaste before the age of two years old are more likely to develop enamel fluorosis than those who start later or do not use fluoridated toothpaste (Asawa, 2015; Rugg-Gunn *et al.,* 2011). This is especially true for children who consume fluoridated toothpaste at levels higher than 0.7 to 0.5 ppm (Marín *et al.,* 2016; Sami *et al.,* 2016)

Similarly, fluoridated toothpaste has been reported to elevate fluoride concentration upon ingestion (Bhagavatula *et al.,* 2016; Duckworth & Morgan, 1991). Additionally, severe forms of this condition can occur when young children ingest excess fluoride during critical periods of tooth development (Cury & Tenuta, 2014; Narsimha & Rajitha, 2018), while use of a toothpaste with a conventional concentration of fluoride (between 1000 and 1500 µg/g) in a small quantity (0.1 to 0.3 g) (Cury & Tenuta, 2014), has been reported to reduce fluoride ingestion through toothpaste.

Furthermore, this study revealed that, the community awareness of the health effects caused by prolonged exposure to fluoride was insufficient. Majority of participants (79%) described red pits on tooth as a major fluoride health effects. Various scientific findings have reported that, fluoride exposure at high levels of up to 20 ppm sodium fluoride (NaF) cause loss of motor control, antioxidants (SOD), and oxidation of lipids in brain development (Jetti *et al.,* 2016; Reddy, 2016). It has been reported that, fluoride inhibits iodine absorption, leads to decrease

thyroid hormone causes learning and memory impairment (Bashash *et al.,* 2017; Mukherjee *et al.,* 2019; Valdez-Jiménez *et al.,* 2011).

Though, commonly known acute health effects include death related to the binding of fluorine with serum calcium and magnesium, vomiting, nausea, chronic convulsion, necrosis of the mucosa of the digestive tract, and heart failure (Kabir *et al.,* 2020; Liu *et al.,* 2015). Creating awareness could help the community understand health effects due to overconsumption of contaminated water and diets, and would possibly encourage the use of an alternative source of food and domestic water for drinking and food preparations particular for vulnerable populations like children.

4.3 Fluoride Contamination of Commonly Consumed Food Crops

The World Health Organization (WHO, 2002), has recommended fluoride safe intake of 0.01 mg/kgbw per day for infants of up to 6 months and 0.05 to 0.07 mg/kgbw per day for adults except in drinking water. In present study maize flour was found with mean fluoride levels at 0.03±0.01 mg/kg Fig. 8, which is similar to those of 0.1 to 0.29 mg/kg reported by EFSA (2013). On the other hand, the current mean fluoride level is lower than those reported in other studies summarized in Table 7. Maize, which is the commonly consumed staple cereal crops and primary source of calorie intake by most of the population in the study community, is consumed daily and can increase the prevalence of human exposure to fluoride.

Leafy vegetables mean fluoride level was found in the order of cabbage 0.022±0.01 mg/kg < Chinese cabbage 0.077±0.004 mg/kg < nightshade 0.081±0.004 mg/kg. This order may be caused by the capacity of the plants to accumulate fluoride and the amount of fluoride available for absorption. Vegetables are consumed daily by the majority as a side dish to maize-based stiff porridge, and this can accelerate human exposure to fluoride. This finding was similar to previous studies on fluoride contamination reported by EFSA (2013) for Chinese cabbage and (Paul *et al.,* 2011) for cabbage, but was lower than the means value reported by Bhargava and Bhardwaj (2009), Dagnaw *et al.* (2017) and Pal *et al.* (2012) (Fig. 7 and Table 7).

Similarly, study found that fluoride concentration in food crops were significant (p=0.001), suggesting the possibility of variation of fluoride levels in the soils in the study areas. This was in agreement with previous reported findings by Masawe (2019), identified different fluoride levels in the soil in the studied communities.

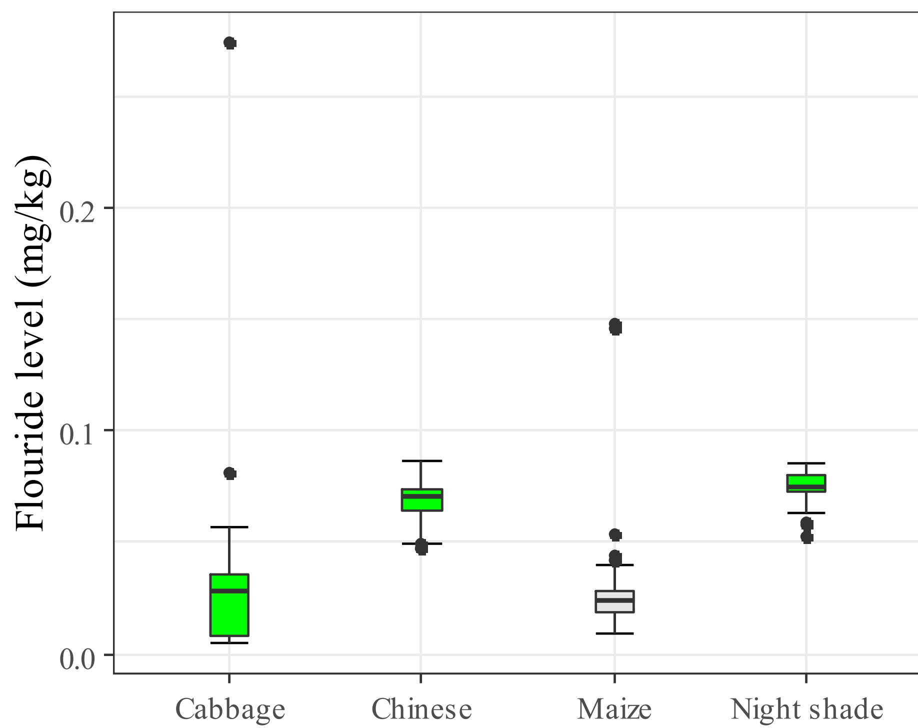

Figure 7: Fluoride levels of locally grown food crops (maize n=107; vegetables n=90; Cabbage n=30, Chinese n=30, Night shade n=30) (*P*= 0.001)

Table 7: Comparison of fluoride levels of locally grown and commonly consumed food crops from studied area to those reported in literatures (mg/kg)

Crops	Fluoride	Country	References
Chinese cabbage	0.077	Tanzania	Present study
	0.078	Ireland	EFSA (2013)
African Nightshade	0.081	Tanzania	Present study
Cabbage	0.02	Tanzania	Present study
	2.12	Ethiopia	Dagnaw *et al.* (2017)
	0.022	Nigeria	Paul *et al.* (2011)
	3.91	India	Bhargava and Bhardwaj (2009)
	1.25	India	Pal *et al.* (2012)
	0.05	Ireland	EFSA (2013)
Banana	0.005	Ireland	EFSA (2013)
	0.01	USA	USDA (2009)
Cavendish	0.018	Tanzania	Present study
E.A.diploid banana	0.022	Tanzania	Present study
E.A.Highland	0.025	Tanzania	Present study
Maize		Tanzania	Present study
	5.1	Burundi	Gautam *et al.* (2010)
	5.9	India	Sunitha and Reddy (2014)
	12.2	Ethiopia	Den-Besten and Li (2011)
	0.98	Ethiopia	Mustafa (2014)
	0.3	Tanzania	Gautam *et al.* (2010)
	0.03	Tanzania	Present study

Furthermore, for green banana varieties mean fluoride level was 0.018±0.006 mg/kg for Cavendish (*Malindi*) < 0.022±0.003 mg/kg East African diploid banana (*mchare*) < 0.025±0.002 mg/kg for East African highland banana (*matoke*) (Fig. 8) with significant correlation ($p = 0.001$). In Tanzania, East African highland triploid banana–"AAA" (*matoke*), green banana variety is preferred in making banana porridge, a common food for mothers on maternity and complementary food for young children. This study showed that the '*matoke*' variety had higher fluoride levels compared to other varieties, indicating a higher risk to the

vulnerable population. Similarly, the fluoride level in green banana found in this study was higher than those reported by EFSA (2013), but in line with those reported by USDA (2009).

While physical and chemical characteristics of agro-ecological zones of agricultural field and water used for crop irrigation was, reported as causative factors for variation in fluoride uptake by plant crops (Gupta, 2019; Rojas-Sanchez *et al.,* 1999). Food preparation methods such as: milling of cereals to flour reported to lower fluoride content (WHO, 1984b). Moreover, micro and/or macro contents of plant crops such as Fe, Zn, and Ca have shown to influence fluoride variation in the plant species (Dagnaw *et al.,* 2017; Yin *et al.,* 2017).

Additionally, the majority of the population in the study community grows maize during the rainy season, where the infiltrating water leaches the soil and replenishes the groundwater. This process has to reduce the concentration of fluoride in the groundwater soil, and subsequent the crops (Lakshmi, 2011; Oliveira *et al.,* 2018) reported that prolonged dietary exposure to fluoride increases the prevalence of fluorosis. In this study, the community reported using trona (*Magadi in Swahili*) for local food preparation such as those made from whole or dehulled maize and beans (*makukuru*) to fasten the cooking process. According to Kaseva (2006) and Das and Mondal (2016), the use of trona can increase fluorosis, as well as other severe health implications when ingested in excess (Kanduti *et al.,* 2016).

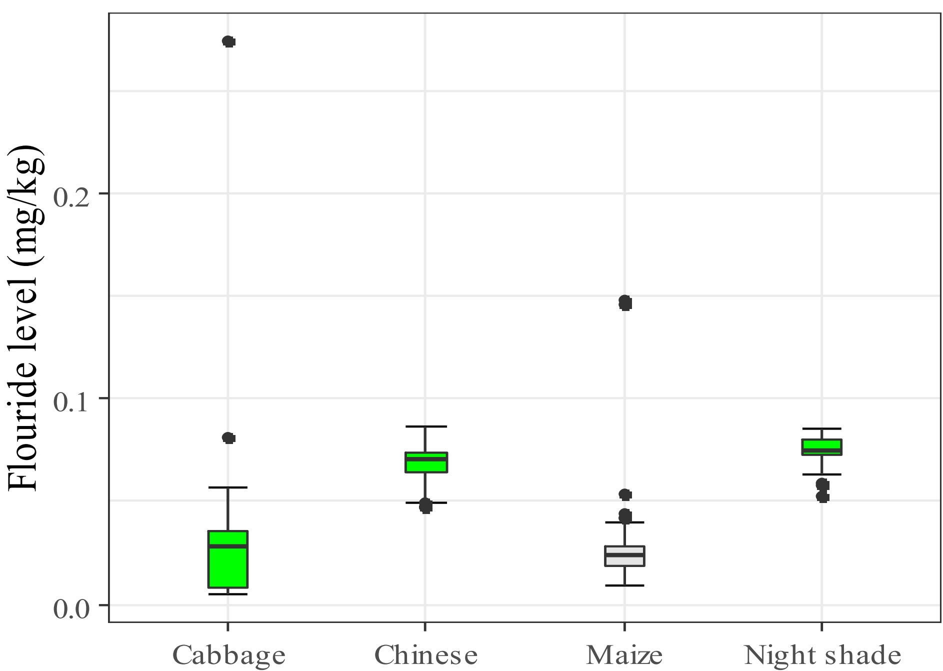

Figure 8: **Fluoride contamination of commonly consumed green banana varieties: Cavendish group (*Malindi*) (n=30) East African Highland banana (*Matoke*) (n=30); East African diploid banana (*Mchare*) (n=30) (*P* = 0.001)**

4.4 Fluoride Levels in Domestic Water

The current study found that mean fluoride contamination level in the domestic and public tape water used in the communities was 4.57±0.2 mg/L and 4.74 ±0.6 mg/L, respectively (Fig. 9), which was above international and national recommended safe level of 1.5 mg/L (WHO, 2002) and 4 mg/L (TBS, 2005), respectively. In all cases of domestic and public tap water, the study found no significant difference in the fluoride contamination levels regardless of their variation in location (p=0.035), implying that they are possibly originating from similar sources. Also, study found higher fluoride level in domestic water compared to those reported in the literatures (Table 9).

Previous studies in studied community had also reported high fluoride levels in domestic water (Kaseva, 2006; Malago *et al.,* 2017). Groundwater fluoride contamination is a critical health challenge (Adekola *et al.,* 2015). According to WHO (2011) on safe water, all people, whatever their stage of development and their social and economic conditions, have the right to have access to an adequate supply of safe drinking water. Due to water scarcity in the country and high fluoride concentration values of the ground and surfaces water sources in Tanzania (Malago *et al.,* 2017; Mkungu *et al.,* 2014) difficulties encountered in meeting national and international recommendation safe level.

Moreover, the use of fluoridated water above the recommended safe level in food preparation for both household and commercial purposes has been reported to increase fluoride content of the cooked food (Bashash *et al.,* 2018; Dagnaw *et al.,* 2017). This has been evidenced in the current study whereby due to insufficient awareness of fluoride contamination sources as reported in the Table 6, the community has been using fluoridated water of up to 4.6 mg/L for domestic purposes. However, due to high fluoride levels in most of the water sources in Tanzania, the national recommended fluoride level based on attainability is 4 mg/L for domestic purposes in rural areas (TBS, 2005), which can subsequently lead to high fluoride level in the body. Therefore, while locally grown food crops can be a vital route for human exposure to unacceptable levels of fluoride, food preparation can decrease or increase fluoride concentration of the food and the ultimate human exposure.

Table 8: Comparison of fluoride levels in domestic water from studied area to those reported in literatures (mg/kg)

Domestic water	Origin	References
<0.25	Jamaica	Warpeha and Marthaler (1995)
0.32–0.50	Jamaica	Warpeha and Marthaler (1995)
0.6	Chile	Villa *et al.* (2010)
0.94	USA	Drummond and Curzon (1985)
0.8–1.0	England	Rugg-Gunn *et al.* (2011)
0.9–1.1	Sri Lanka	Rugg-Gunn *et al.* (2011)
< 0.25	Jamaica	Warpeha and Marthaler (1995)
0.32–0.50	Jamaica	Warpeha and Marthaler (1995)
~ 4	Iran	Zohouri (1997)
1	Morocco	Abdennebi *et al.* (1995)
0.2	Spain	Torra *et al.* (1998)
0.95	India	Singh *et al.* (2007)
1.07	India	Jaganmohan *et al.* (2010)
0.05	India	Kumar *et al.* (2017)
4.56	Tanzania	Present study

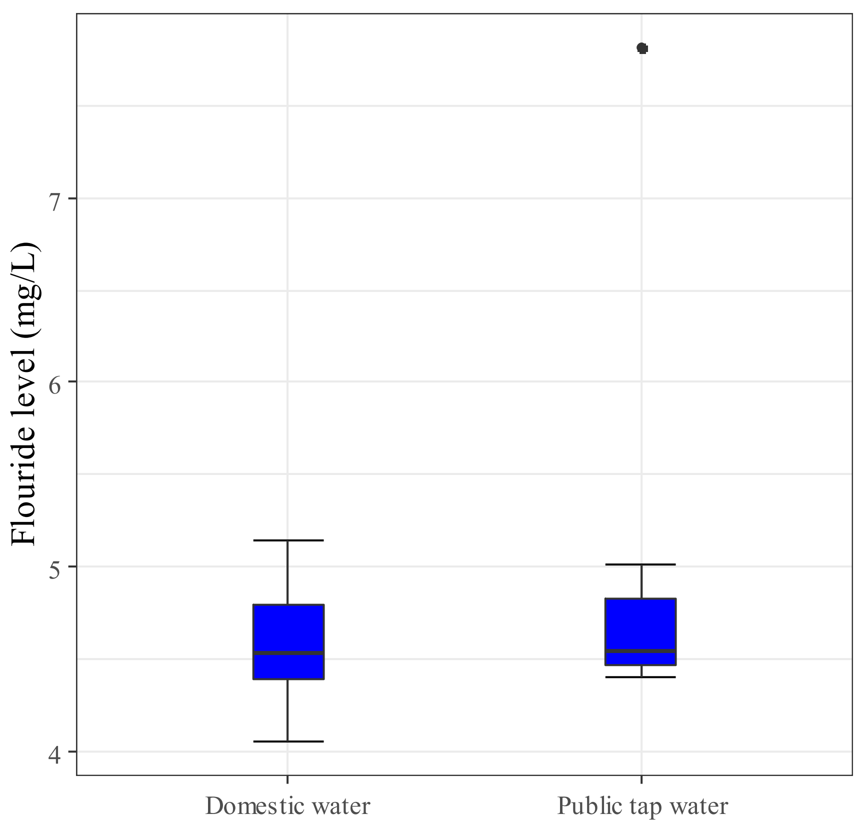

Figure 9: Fluoride contamination of water used for domestic purposes as sourced from both household and public taps (households n=107, public taps n=15) (*P*=0.035)

4.5 Fluoride Contamination Levels in Cow's and Human Breast Milk

Study found significant contamination of fluoride in both human and cow's milk ($p= 0.001$), whereby cow's milk showed a relatively higher contamination level than human breast milk (Fig. 10; 0.34 ±0.01 and 0.077 ± 0.03 mg/L, respectively). Fluoride contamination of cow's milk was higher than the findings reported by Lv *et al.* (2018), and O'Callaghan *et al.* (2019), Senghor *et al.* (2018). Though was in line with reported mean value by Kazi *et al.* (2019) an Spittle (2016). Higher fluoride means value found in this study might be due to the higher fluoride level in drinking water used by domesticated animals (cows). Ngarenanyuki River, with up to 26 mg/L Ghiglieri *et al.* (2010) was used as the source of drinking water for animals in the studied community. According to Barrera *et al.* (2018), use of fluoride contaminated water and feeding system to feed animals increase fluoride levels in cow's milk. Apart from being a primary source of protein dietary intake, cow's milk is well known as a complementary food for young children, consumed as a whole or mixed as an ingredient in maize-based thin porridge or a side dish for stiff porridge. Therefore prolonged consumption of fluoride contaminated diets can increase human exposure to unacceptable levels of fluoride and ultimate the prevalence of fluorosis.

Human breast milk, the mean fluoride level (Fig. 10; 0.077 ± 0.03 mg/L) were in line with other studies, as summarized in Table 9. Although was higher than those reported by Faraji (2014), and Şener *et al.* (2007) and the recommended safe level of 0.002 µg/ml by World Health Organization (WHO, 2011). According to the findings reported by Campus *et al.* (2014) and Poureslami *et al.* (2016), high fluoride level of up to 0.55 and 0.515 mg/L, respectively has been reported in the women with dental fluorosis.

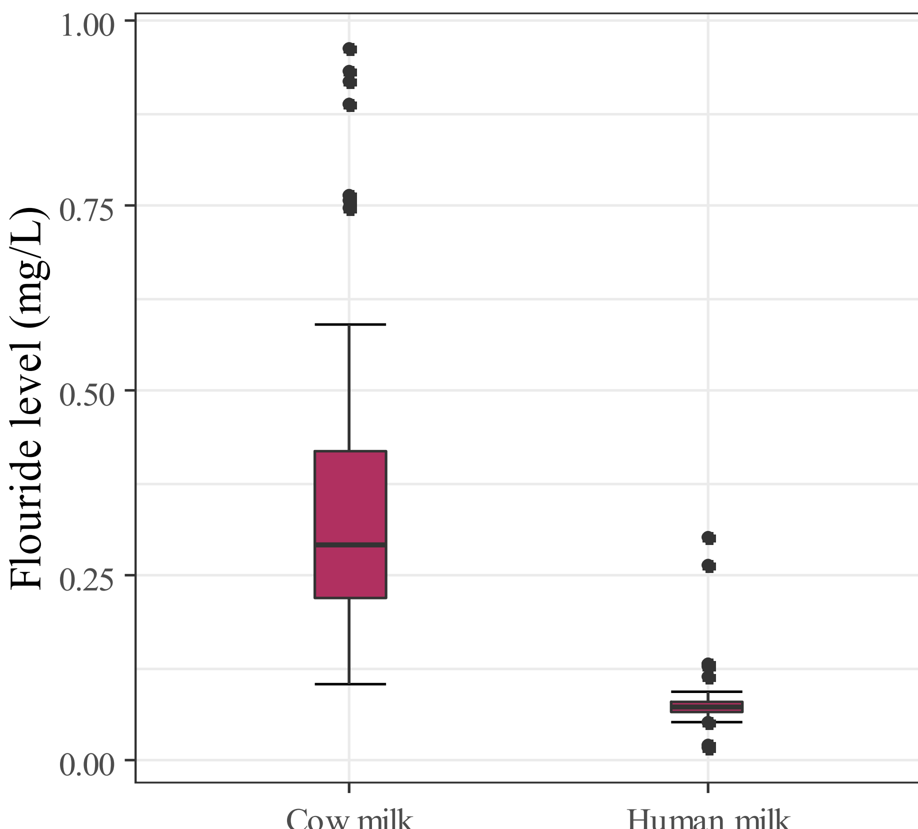

Figure 10: Fluoride levels of cow's milk (n=106) and human breast milk (n=101) (*P*= 0.001)

According to EPA's standards (NRC, 2007), it has been reported that human breast milk has a low fluoride concentration level. Of which was reflected in this study, whereby despite of the presence of high fluoride levels in studied areas, human breast milk was found with low fluoride mean value compared to cows milk. Furthermore, this result showed no correlation between fluoride concentrations mean values of breast milk and drinking water as shown in the Table 9. This is in agreement with a study of Ekstrand *et al.* (1984), who studied the correlation of the concentration of fluoride in human breast milk and those of the drinking water. Studies have reported that, fluoride contents in milk is associated with various factors such as diets, age, and genetic.

Table 9: Comparison of fluoride level in domestic water and human breast milk from studied area with those reported in the literatures (mg/L)

Domestic water	Human milk	References
	0.0053	Spak *et al.* (1983)
1	0.0068	Spak *et al.* (1983)
1	0.007	Spak *et al.* (1983)
	0.0044	Dabeka *et al.* (1986)
1	0.0098	Dabeka *et al.* (1986)
22.1	0.033	Opinya *et al.* (1991)
0.3	0.002–0.01	Hojsak *et al.* (2018)
	0.55	Poureslami *et al.* (2016)
	0.006	Şener *et al.* (2007)
	0.515	Campus *et al.* (2014)
	0.02	Ericsson and Ribelius (1971)
4.56	0.077	Present study

Furthermore, cows milk had been identified with higher mean fluoride level than human breast milk. Ruminant stomach contains microbial population that enhances the digestion and release of minerals contained in the animal diet such as calcium (Ca) (Colonetti *et al.,* 2018). Fluoride absorption is reduced by increased stomach pH and increases minerals such as calcium, magnesium, and aluminum which forms complex compounds with fluoride (Vale *et al.,* 2019) and varies as animal ages (Aydgan *et al.,* 2018). At low pH, more hydrogen fluoride is formed; promoting reabsorption, excretion of previously absorbed fluoride from the body (Buzalaf & Whitford, 2011; Yin *et al.,* 2017).

4.6 Consumption of Food Crops, Milk and Water

The study identified individual food consumption for young children (0-24 months) and lactating mothers. For children aged between 6 to 12, 13 to 18, and 19 to 24 months, respectively, consumption was found in the order of 4.59±0.28, 5.95±0.47, and 6.64±0.4 g for maize; 295.94±8.70, 344.21±14.54, and 376.58±21.22 mg, respectively, for African highland banana (*matoke*); 246.4±1.6, 292.3±1.07, and 300.3±1.56 ml, respectively, for cow’s milk; and 21.09±2.11, 25.04194, and 33.98±2.5 ml, respectively, for drinking water (Table 10).

Table 10: Consumption of food crops, cow's milk and drinking water among children of different age

Age (months)	Maize	E. A. H. Banana	Cows milk	Drinking water
6 to 12	4.59±0.28 g	295.94±8.70 mg	246.4±1.6 ml	21.09±2.11 ml
13 to 18	5.95±0.47 g	344.21±14.54 mg	292.3±1.07 ml	25.041.94 ml
19 to 24	6.64±0.4 g	376.58±21.22 mg	300.3±1.56 ml	33.98±2.5 ml

For lactating mothers, the highest food consumption was found in the maize (68.89 ± 0.67 g); for green banana varieties, the maximum amount was found in East African diploid banana (*mchare*) (58.99 ± 3.55 g); for leafy vegetables, nightshade showed the highest consumption (23.90 ± 1.82 g). Cow's milk and drinking water consumption was 0.13 ± 0.02 and 1.35 ± 0.04 L, respectively (Table 11).

Table 11: Consumption of locally grown and commonly consumed food crops, cows milk and drinking water among lactating mothers in the studied community

Maize	E.A.H. Banana	E.A.D banana	Cavendish	Cabbage	Chinese	Night shade	Cows milk	Drinking water
68.89±0.67 g	59.05±3.12 g	58.99±3.55 g	58.94±4.53 g	18.03±2.95 g	18.74±1.04 g	23.90±1.82 g	0.13±0.02 L	1.35±0.04 L

4.7 Dietary Fluoride Exposure

In the present study, young children aged between 6-12, 13-18, and 19-24 months with mean weights of 10.88, 12.12, and 13.26 kg, respectively, were involved in the estimation of fluoride exposure through locally grown and commonly consumed complementary food crops, domestic water, cows' and human milk. The maximum fluoride exposure was found in maize (13.09±0.80 for 6 to 12, 15.23±0.95 for 13-18, and 15.53±0.98 for 19-24 months, mg/kgbw/day, respectively), and minimum exposure was found in East African highland banana (0.68±0.02 for 6-12, 0.71±0.03 for 13-18, and 0.71±0.04 for 19-24 months, mg/kgbw/day, respectively).

For human breast milk, fluoride exposure assessment was estimated by using a recommended milk intake of 200 ml/kg for children aged up to 6 months and 600 ml/kg above 6 months for fluoride exposure assessment (NHS, 2012). The study revealed maximum exposure in children aged between 6-12 months (4.24±0.05 mg/kgbw/day), while minimum exposure was found in children aged between 19-24 months Table 12.

According to WHO (2002) and EFSA (2013), the recommended safe level of dietary fluoride intake per day is 0.05 and 0.1 mg/kgbw/day for children through all sources except in drinking water. Results from this study revealed that, children were exposed to fluoride above the recommended safe level in all assessed diets, include drinking water, compared to those recommended in the works of literatures, Table 10. Though it was lower in human milk and East African highland banana, this was in agreement with a previous study that reported a lower exposure in infants through breastfeeding but higher from other sources (Poureslami *et al.,* 2016).

Table 12: Children exposure to fluoride through consumption of commonly consumed complimentary food, human milk and drinking water (mg/kgbw/day)

Age (Months)	Maize	E. A. highland Banana	Cow's milk	Human breast milk	Drinking water
6 to 12	13.09±0.80	0.68±0.02	7.70±0.05	4.24±0.05	8.86±1.09
13 - 18	15.23±0.95	0.71±0.03	8.12±0.03	3.81±0.02	9.44±0.94
19 - 24	15.53±0.98	0.71±0.04	7.71±0.04	3.48±0.03	11.71±1.29

Maize which, is used for making thin porridge, a common complementary food for children in the studied community due to its availability and affordability, accounts for more than half (56.4%) of total fluoride exposure (Fig. 11). Being a staple crop that is consumed by more than half of the studied children and its high fluoride uptake, maize can be a major source of human exposure to dietary fluoride.

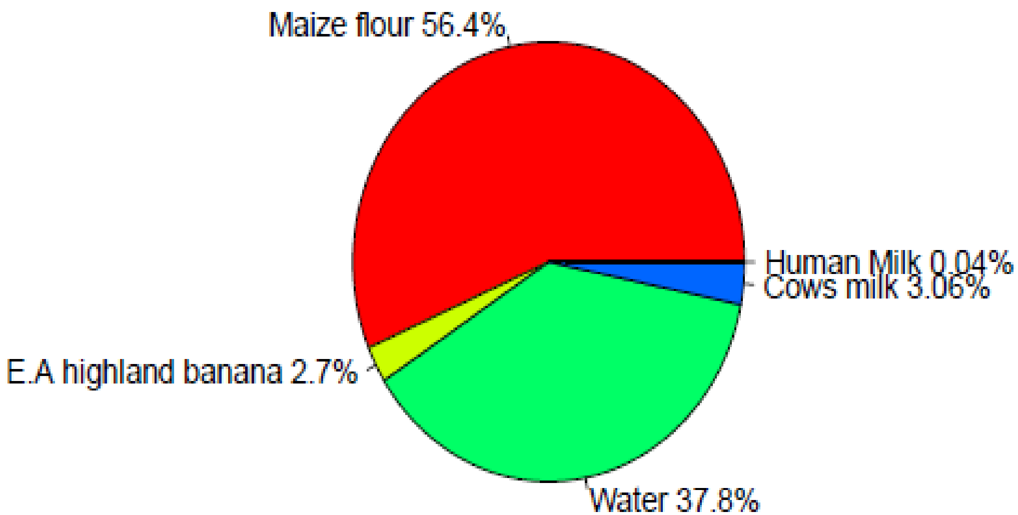

Figure 11: Sources of dietary fluoride exposure in children

According to Buzalaf and Whitford (2011), the use of fluoridated water increases fluoride levels in the food. Of which minimum fluoridated domestic water can reduce the fluoride contamination levels in the children's food and results less ingestion and exposure. The information obtained through the questionnaire in this study reported that 92.9 % (Table 6), of households in the community relied on tap water for domestic purposes, though identified with a high fluoride level up to 4.57±0.2 mg/L (Fig. 9). This was in line with previously reported findings by Ekstrand *et al.* (1984) and Barrera *et al.* (2018). In their studies, an association between exposure to fluoride and consumption of fluoride-contaminated diets, which was prepared with water contained high fluoride levels, was revealed. Also, was in agreement with the findings reported by Ibiyemi *et al.* (2018) about the effects of childhood exposure through drinking water, though the currently reported fluoride level in drinking water was higher than their reported mean values of 2±0.01 -3±0.02 mg/L.

Furthermore, the current study revealed that, though studied communities was reported with high fluoride levels in its water sources Kaseva (2006) and Malago *et al.* (2017), exposure rate through drinking water accounts for a lower in exposure (37.8%) compared with those in maize (56.4%). Climatic conditions can either increases or decrease the amount and frequency of

water intake, which in turn influence the exposure. Arusha is one of the regions with cool weather conditions especially during months of December to June (Mkungu *et al.,* 2014).

Next to maize, cooked East African highland banana (*matoke*) is another popular complementary food for young children in the Tanzanian population; this is due to its softness, easy mushing and good taste. The study found that children were exposed to fluoride through the consumption of maize almost ten times than the exposure through the consumption of contaminated banana. Though the fluoride contamination means value for maize and East African highland banana were close (0.03 and 0.025 mg/kg). This is because to the food consumption frequency reflects the dose of fluoride exposure. This was in agreement with findings reported by Oliveira *et al.* (2007), thus, the larger the quantity of food consumed by children, the higher the exposure to fluoride. Similarly, the finding from this study shows that maize was found with higher consumption frequency compared with other complimentary food (Table 4). This implies that children in the studied community are at high risks of exposure to fluoride through the consumption of these locally grown and commonly consumed food crops.

In this study, 101 lactating mothers aged between 15-49 years, with a mean weight of 67.9 kilograms, were involved in the fluoride exposure assessment through consumption of locally grown food crops, domestic water and cows milk. The study found lactating mothers were more exposure to fluoride through drinking water (Fig. 12; 88.12±25.2 mg/kgbw/day). For green banana varieties, maximum exposure was found in East African diploid banana (*mchare*) (20.70±1.15, mg/kgbw/day). For leafy vegetable, maximum exposure was found in African nightshade (27.78±1.54 mg/kgbw/day). Maize was found with a fluoride exposure of 30.64±1.52 mg/kgbw/day).

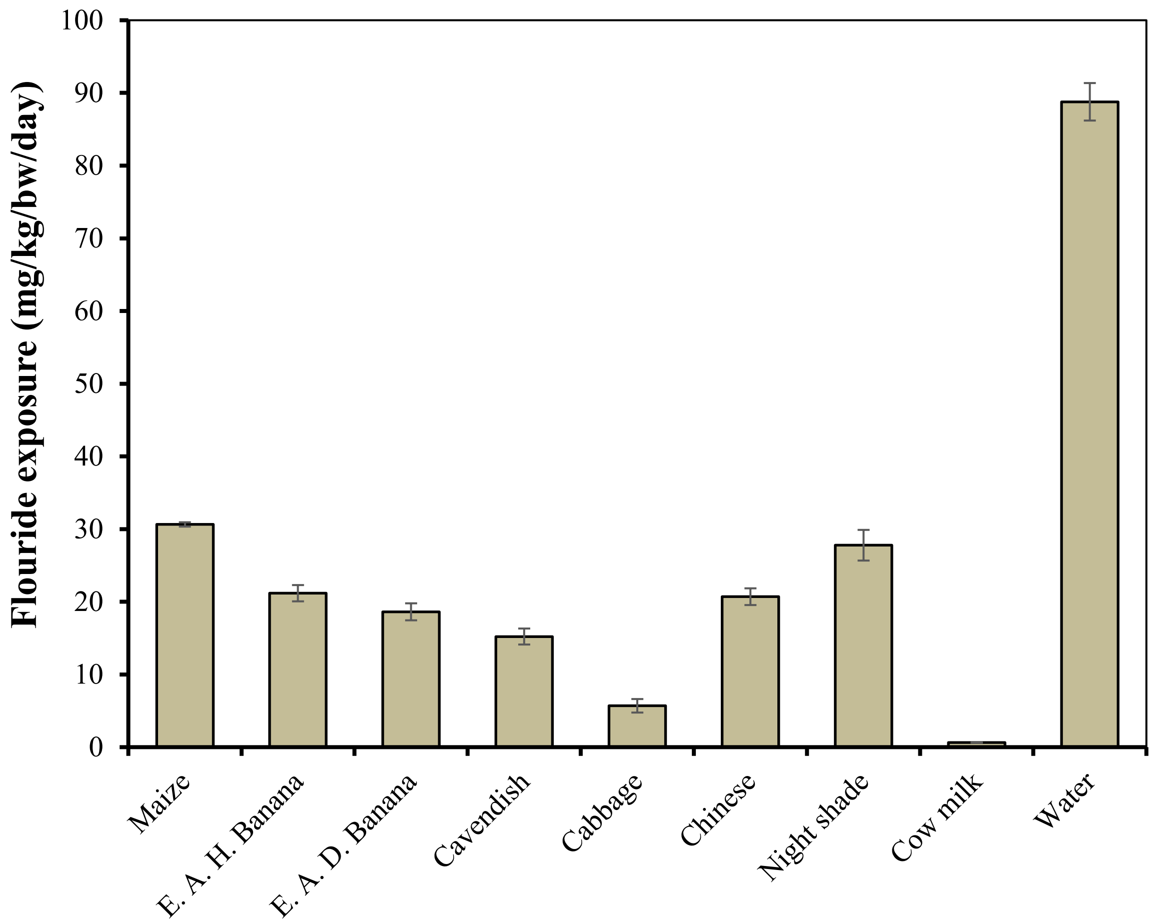

Figure 12: Fluoride exposure in lactating mothers through consumption of locally grown produce, cows milk and drinking water

Among the locally grown and commonly consumed food crops maize (13.4%) and African nightshade (12.3%) were the most important sources of dietary fluoride exposure among women, followed by East African highland banana (9.2%), Chinese cabbage (9%), East African diploid banana (8.1%), Cavendish group banana (6.6%), cabbage (2.5%), and cow's milk (0.3%). All leafy vegetables contributed about 23.6% of the total dietary exposure, while 23.3% was from cooked green banana varieties. Exposure rate through drinking water was the highest of all other dietary sources (Fig. 13; 38.7%).

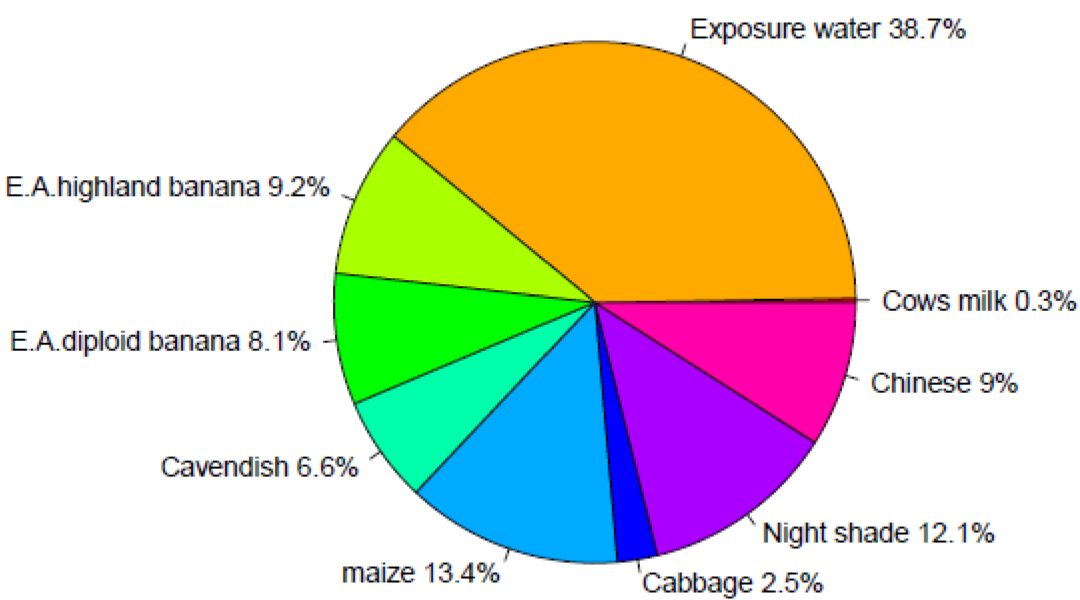

Figure 13: Sources of dietary fluoride exposure in lactating mothers

The study found that dietary exposure from all of the assessed sources exceeded the reference value of 0.05 to 0.07 mg/kgbw/day by the World Health Organization (WHO, 2002). This result was in agreement with previously reported findings by Barrera *et al.* (2018), who found an association between human exposure to fluoride through consumption of contaminated diets and drinking water.

The current study indicates that locally grown and commonly consumed food crops play a vital role in the fluoride exposure in the community, which subsequently leads to a high prevalence of fluorosis.

Study found that about sixty percent of total fluoride exposure was accounted for food crops (60.9%). Maize, which is a top cereal crop grown and commonly consumed daily as stiff porridge alongside leafy vegetables, has been found to account for the highest (13.4 %) fluoride exposure among other food crops. Additionally, cooked green banana is a popular dish in Tanzania, particularly in northern regions, commonly used as a complementary food for infants after exclusive breastfeeding and for mothers in maternity, and it accounts for almost 23 % of the exposure. This study revealed that, studied communities are exposed due to consumption of locally grown and commonly consumed crops. Exposure due to drinking water was found to contributed to more than one-third (1/3) of the total fluoride exposure (Fig.13). This is because more than 90% of the participants relied on domestic water with very high fluoride content (Table 7).

Also, the community consumed food prepared with water from the fluoridated water supply, which ultimately increase fluoride content in the food, increasing dietary exposure. This was in agreement with the findings of Casarin *et al.* (2007) who reported the reflection of the total concentration of fluoride in the water used to prepare food and fluoride in the food contents. According to Mkungu *et al.* (2014), Ngarenanyuki areas have been reported with favorable climatic conditions for vegetables and food crop production in the northern zones of Tanzania. Serves to improve community livelihood through income generation and reduce food insecurity both at the community and national levels. Unfortunately, the water used for irrigation is from the Ngarenanyuki River with the highest fluoride level up to 26 mg/L (Malago *et al.,* 2017), which leads into the accumulation of fluoride in the crops, resulting into humans and animal exposure through food consumption. Additionally, the exposure obtained in this study from water consumption were higher than those reported by Viswanathan *et al.* (2009), in the Dindigul district in Tamil Nadu in India, where the fluoride exposure for adults was recorded at 0.10 mg/kg/day. The greater exposure rate observed in the current study may be attributed to the relatively higher fluoride levels present in the drinking water used by this community.

4.8 Biomarkers of Fluoride Exposure

The study also quantified the biomarkers of fluoride exposure using urine in infants and breast milk in lactating mothers. Both urine and breast milk can be a suitable indicator for acute exposure to fluoride. Urine has been recommended, and guidelines have been given by WHO to be used as an indirect indicator for estimating 'optimum' or sub-optimal fluoride intakes (Marthaler, 1999). However, no guidelines have yet been established for the use of human milk as indicators of fluoride intake. Therefore, this subchapter aimed to assess the relationship between biomarkers of fluoride exposure in correlation with the consumption of fluoridated food crops and drinking water among children and lactating mothers.

4.8.1 Urinary Fluoride

A total of 105 infants and young children averagely 12.1 kilograms, 58 males and 47 females were involved in this study. Whereby 10.60 % was aged 6 months, 33.33% were 7-12 months, 28.78% were 13-18 months, and 25.76% were 19-24 months. Results showed that, mean fluoride concentration in urine was increasing with age in the order of; 7.39±1.78 for 6 months,

< 8.478 ±2.77 for 7-12 months, < 8.79±1.9 for 13-18 months, and < 9.369±1.23 for 19-24 months mg/L, respectively (*p*= 0.21; Fig.14).

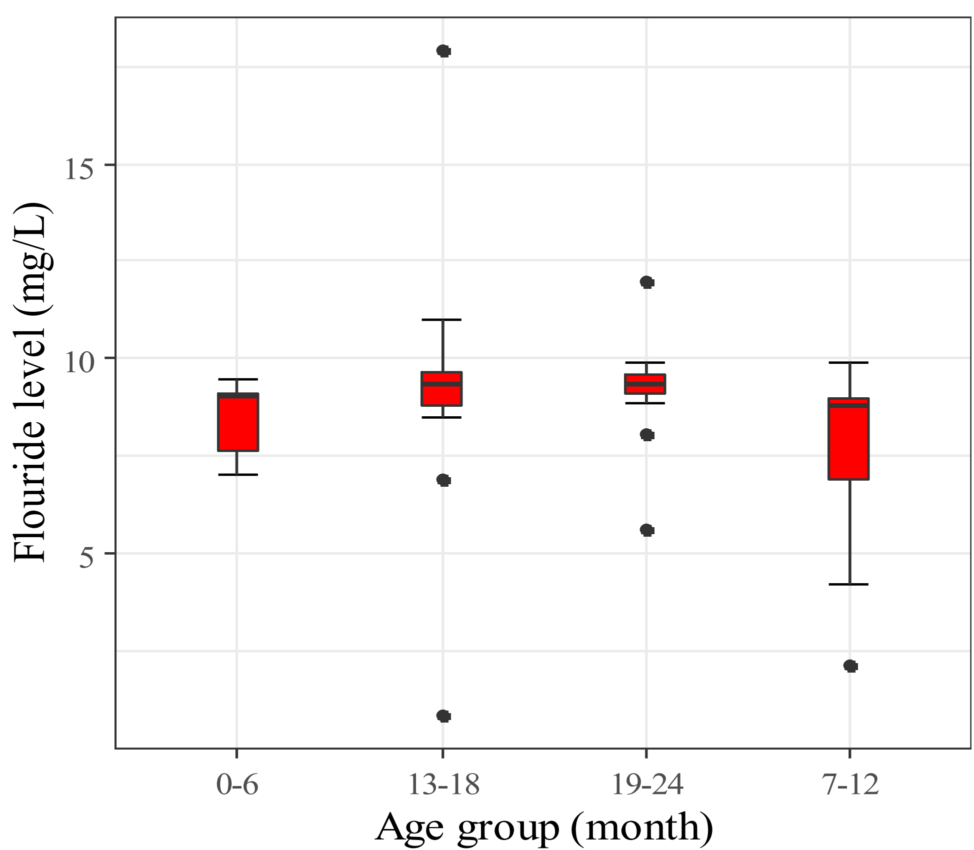

Figure 14: Fluoride concentration in urine from children at different age group (n=105) (*P*= 0.21)

According to the World Health Organization, it is recommended that children obtain food from other sources in addition to breast milk after first six months of exclusively breastfeeding (WHO, 2011). This recommendation is well regarded necessary to support child need for nutrients, physical and mental development. However it does not only open rood for child's need for growth, but also expose them into undesirable food related contaminants including excessive fluoride, which can cause fluorosis. This evident in this study, whereby higher fluoride level in urine observed as children's age and food intake increases (Fig. 15). This was in agreement with other studies, which suggested that, the first two years of life are the most important period for development of fluorosis in early-erupting teeth, whereas the first eight years of life reported to be the most important period for development of dental fluorosis in late-erupting permanent teeth (Molina-Frechero *et al.,* 2015).

Results on the univariate analyses showed that, the unit change in age led to the increase of fluoride level in urine by 0.094, thus, as age increases, fluoride level also increases (Fig. 15).

This indicates that children from the studied community are more exposed to fluoride from the time of introduction to complimentary food.

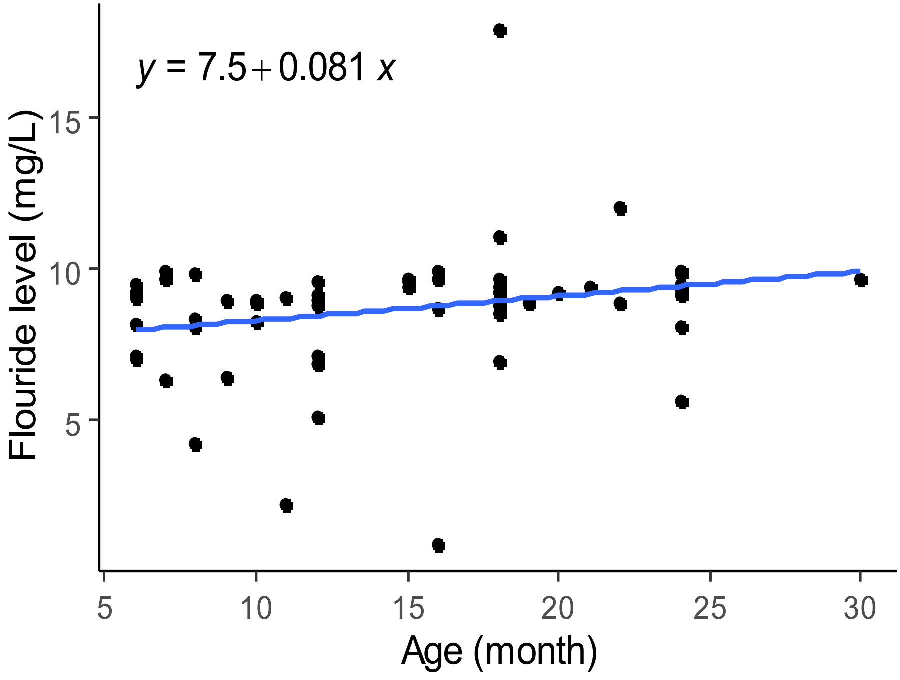

Figure 15: Relationship between urine fluoride level and age in children (n=106)

On the other hand, the study revealed no significant variations in mean fluoride concentration between female and males children (Fig. 16). These findings concur with those reported by Buzalaf (2018) that there was no correlation between fluoride intake and urinary fluoride excretion based on the gender, which was also in line with findings reported by Idowu *et al.* (2020).

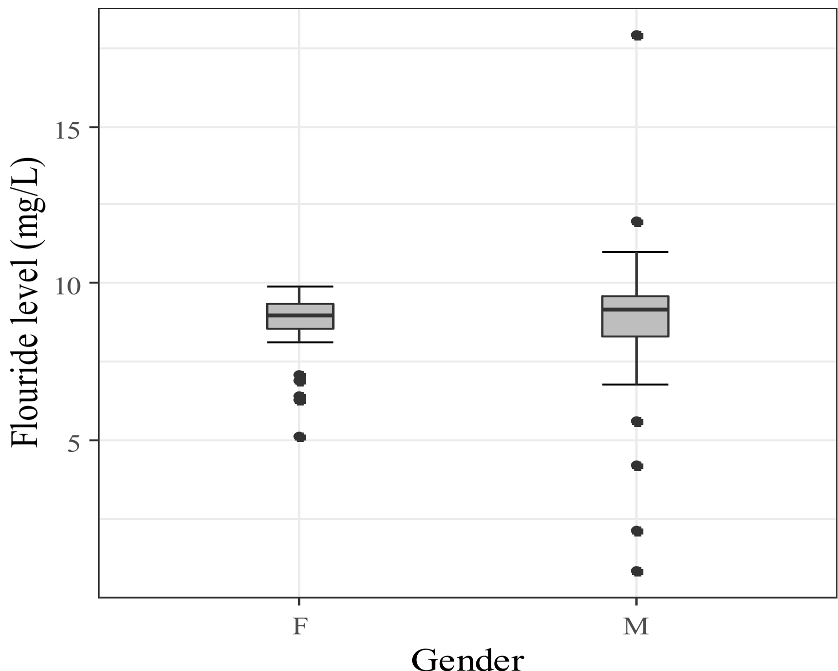

Figure 16: Fluoride concentration in urine from females and males children (n=107) (*P*= 0.97)

Furthermore, the current study found that the mean fluoride concentration in urine was almost twice the concentration in domestic water. These were in line with previous studies shown in Table 8, though was contradicted with reported findings by Idowu *et al.* (2020) who described that the concentration of fluoride in urine correlated with that in drinking water. This finding confirms the diversity of potential sources of fluoride including food crops and milk in addition to water.

The fluoride concentration means value in urine found in this study was higher than those reported in the literature (Table 13). This may be due to other factors as described by Buzalaf (2018). That different factor can influence and modify fluoride metabolism and consequently alter the relationship between fluoride intake and excretion, amongst the factors such as the composition of diets, dose, and form of fluoride, genetic, and nutrition status.

Table 13: Fluoride concentration in urine of children at studied area in comparison to those reported in the literatures (mg/kg)

Age	Urine	References
2–6 (years)	0.066	Warpeha and Marthaler (1995)
2–6	1.19	Warpeha and Marthaler (1995)
3–5	0.93	Villa *et al.* (2010)
3–6	0.61	Drummond and Curzon (1985)
4	1.02	Rugg-Gunn *et al.* (1993)
4	1.19	Rugg-Gunn *et al.* (1993)
2–6	0.77	Warpeha and Marthaler (1995)
2–6	1.41	Warpeha and Marthaler (1995)
4	5.88	Zohouri (1997)
-	0.17	Abdennebi *et al.* (1995)
-	0.159	Torra *et al.* (1998)
-	0.9	Singh *et al.* (2007)
-	2.37	Jaganmohan *et al.* (2010)
-	2.4	Kumar *et al.* (2017)
6 (Months)	7.39	Present study
7-12	8.47	Present study
13-18	8.79	Present study
19-24	9.37	Present study

The univariate analysis was carried out to find an association between daily dietary fluoride intake from identified commonly complimentary foods and urinary fluoride excretion in children as shown in Table 11.

Table 14: Association between dietary fluoride exposure and urinary biomarkers of fluoride exposure in children at studied area

Variable	Coefficient	SD	*P*-value
Cows milk	2.3	0.03	0.0000
E.A Highland banana	12.5	1.98	0.0001
Human milk	-410	23.43	0.92
Maize	-0.058	0.07	0.62
Water	0.0042	0.041	0.3

Results found a strong positive correlation between 24 hrs urinary excretion and fluoride daily intake though consumption of cows milk (Fig. 17; ρ= 2.3, p<0.0000). This shows that cows milk has an impact on the excessive fluoride level in children, as for the unit change in milk

led to the increase of fluoride level by 2.3 in the urine. It could potentially place a child at a greater risk of dental fluorosis.

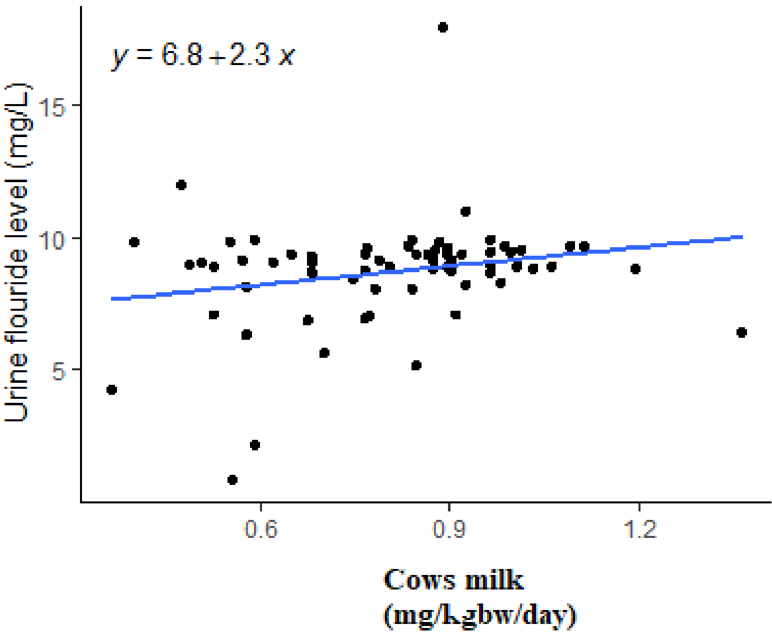

Figure 17: Relationship between cows milk daily fluoride intake and 24 h urinary fluoride levels in children

Similarly, study revealed strong positive significant correlation (Fig. 18; ρ= -2.5, p<0.0001) between East African highland banana and concentration of fluoride in urine. This study proves that East African highland banana used as complimentary food in the studied communities accounts for higher levels of fluoride exposure in children.

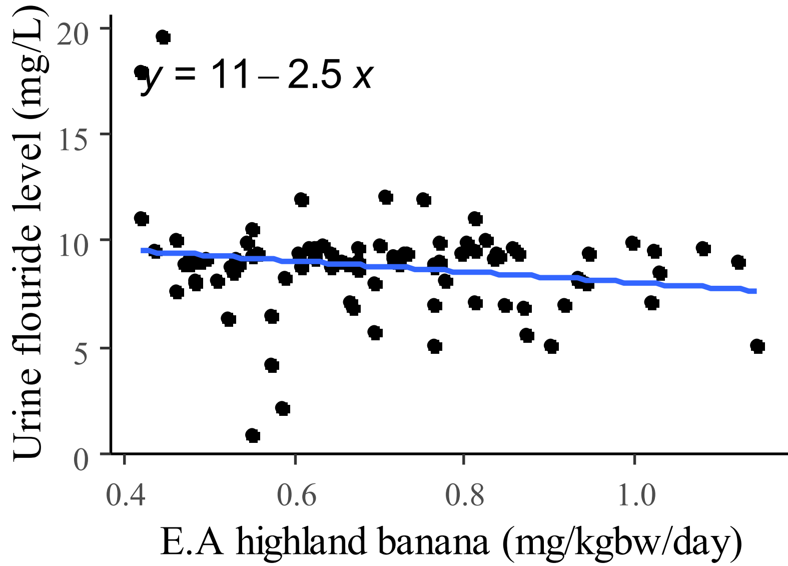

Figure 18: Relationship between E. A African highland banana daily fluoride intake and 24 h urinary fluoride levels in children

The observed strong correlation between fluoride exposure through consumption of locally grown food crops and urine in this study (Fig. 18), agreed with the reported findings by Villa *et al.* (2010), who reported the correlation of fluoride exposure through consumption of westernized diets. However, no significant correlation observed between daily fluoride intake and consumption of drinking water (Fig. 19; ρ = -0.0042, p=0.9), maize (Fig. 20; ρ= -0.058, p=0.6), and human milk (Fig. 21; ρ= -410, p=0.3). The lower correlation coefficient between fluoride intake and urinary fluoride excretion in this case may be due to factors that can influence fluoride concentrations in urine, such as urinary pH, glomerular filtration rate, and composition of the diet (Whitford *et al.,* 1999).

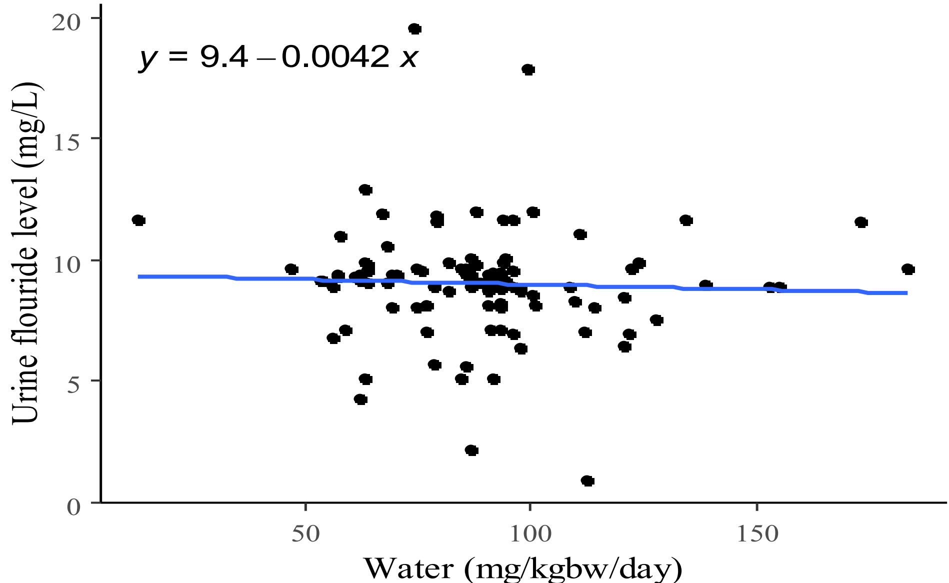

Figure 19: Relationship between daily fluoride intake due to drinking of water and 24 h urinary fluoride levels in children

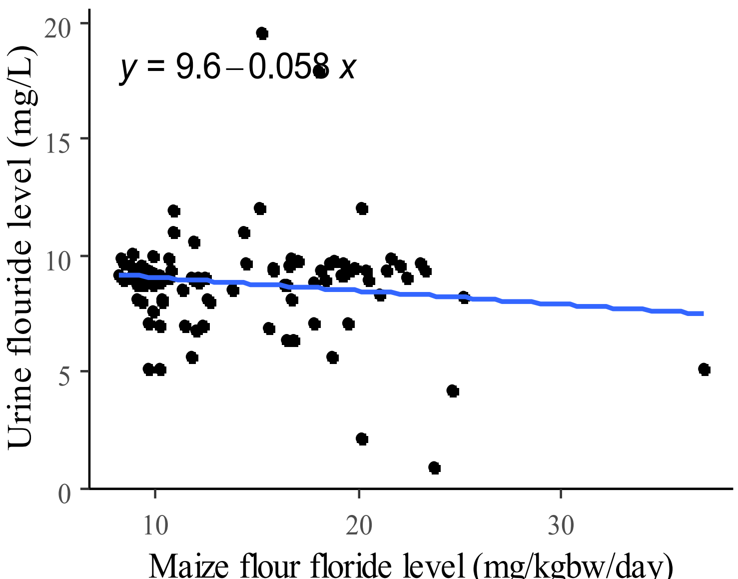

Figure 20: Relationship between daily fluoride intake due to maize and 24 h urinary fluoride levels in children

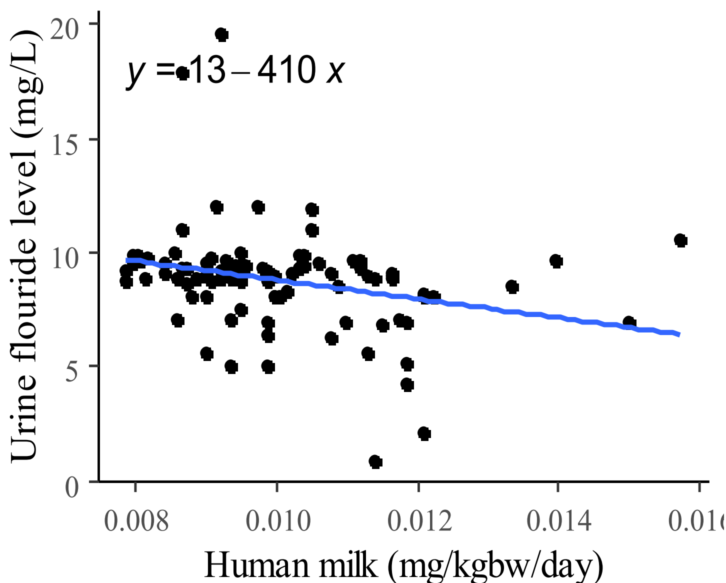

Figure 21: Relationship between daily fluoride intake due to human milk and 24 h urinary fluoride levels in children

Consequently gastric absorption and renal excretion of fluoride are pH-dependent; any diet-induced changes in the pH of the stomach as well as urinary pH could decrease or increase the concentration of fluoride in urine (Rango *et al.,* 2017; Rizzu *et al.,* 2020). For example, a mixed diet has been reported to reduce absorption of fluoride by 47%, whereby a vegetarian-based diet increases fluoride excretion (Chiniah, 2017; Rango *et al.,* 2017).

4.8.2 Human Milk (Breast)

Study quantified the amount of fluoride in breast milk in lactating mothers Fig. 10 and the findings were correlated to fluoride levels in commonly consumed food crops, domestic water and cows milk. In univariate regression analysis, association between fluoride dietary exposures and breast milk biomarkers was observed as summarized in Table 15.

Table 15: Association between dietary exposure to fluoride and human milk biomarkers of fluoride exposure in lactating mothers at studied area

Variables	Coefficients	SDS	*P* –value
E.A Highland banana	-0.0058	0.21	0.801
E.A diploid banana	-0.0066	0.32	0.27
Cavendish group	-0.0081	0.24	0.61
Cabbage	-0.022	0.2	0.07
Chinese cabbage	-0.0059	0.3	0.7
Nightshade	-0.0044	0.1	0.8
Domestic water	-0.00002	0.02	0.001
Maize	-0.00022	0.39	0.01
Cows milk	0.027	0.02	0.0001

A positive correlation was observed between human milk biomarker and consumption of cow's milk (Fig. 22, ρ=0.027, p=0.0001). For every unit change of cow's milk, an increase of mean fluoride concentration in the human milk by 0.027 was observed. This indicates that cows milk had significant impact in fluoride exposure among women.

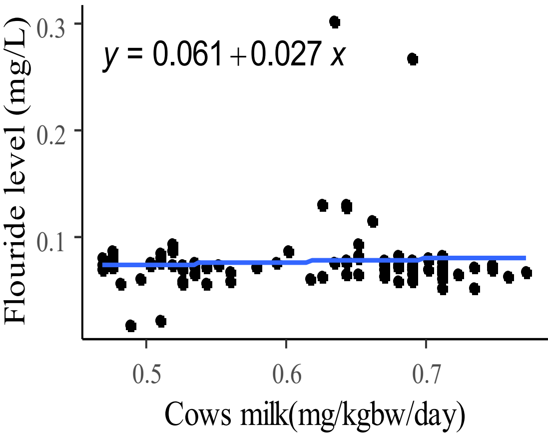

Figure 22: Relationship between daily fluoride intake due to cows milk and human milk fluoride levels in lactating mothers

Similarly, the study found a significant correlation between maize and fluoride concentration in human milk (Fig. 23, ρ=0.00002, p=0.01), in every unit change in maize fluoride intakes; human milk fluoride concentration was increased by 0.000022.

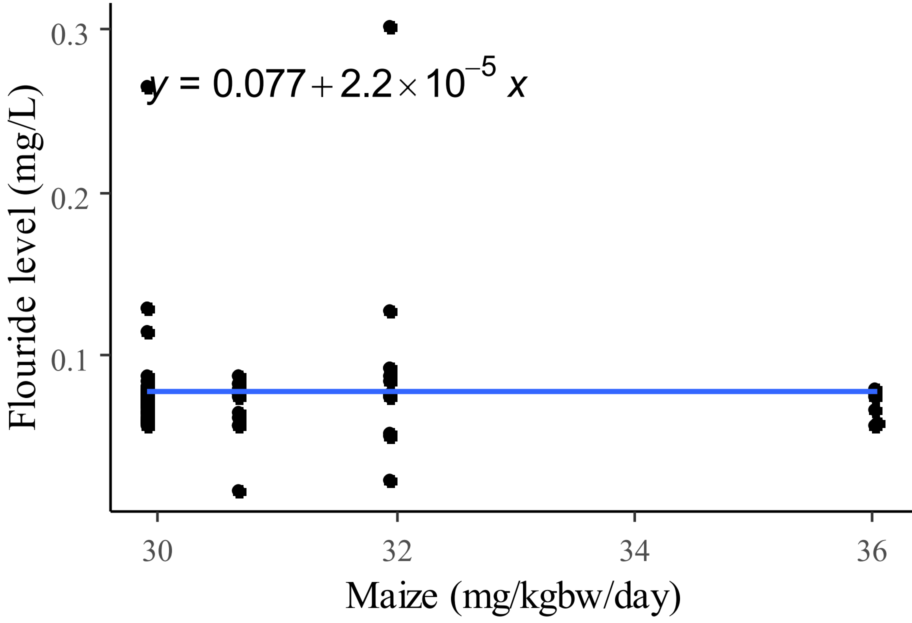

Figure 23: Relationship between daily fluoride intake due to maize and human milk fluoride levels in lactating mothers

Additionally, a significant correlation between water consumption and mean human milk fluoride concentration was obtained (Fig. 24, ρ= -0.00002, p=0.001) with an increase of 0.00002 in every unit change in water consumption.

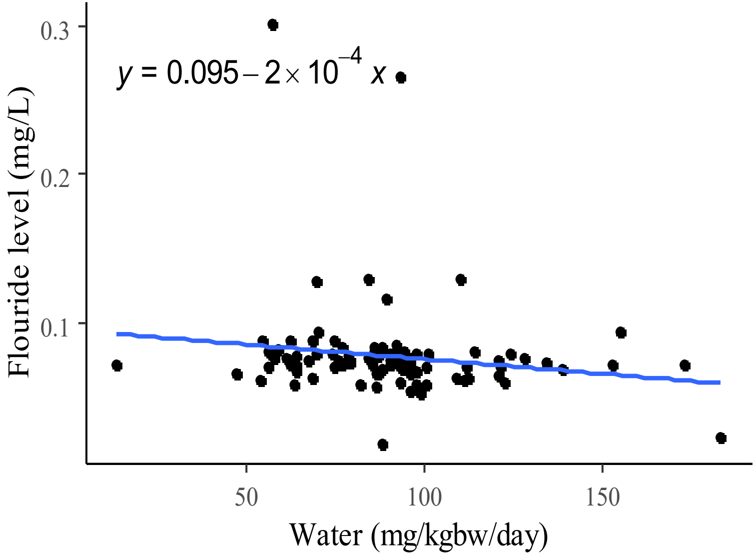

Figure 24: Relationship between daily fluoride intake due to drinking water and human milk fluoride levels in lactating mothers

In drinking water trace elements have tendencies of forming complexes compounds with fluoride, for example, concentration of aluminum (Al) of up to 0.4 mg/L in drinking water was reported (Priest, 1993). Whereby, two recognized sources has been identified, firstly, acid rain which escalates the leaching of Al from minerals into natural water, secondly through coagulant in water treatment to improve the watercolor (Flaten, 2001). This often reported to increase Al concentration in drinking water (Flaten, 2001; Ohman & Martin, 1994). Another potential source of Al is related to water fluoridation. Sodium fluoride, used in water fluoridation, usually comes as a by-product of Na_3AlF_6. Consistent with these early results, a more recent study on rabbits showed that fluoride accumulation in plasma, urine, incisors, and tibia decreased as aluminum concentration increased in drinking water (Ahn *et al.,* 1995).

In the present study, it was identified that the community relied on the public tap water for domestic purposes, whereby coagulants and other reported potential sources of traces elements might have been added and form complex compound with available fluoride. This might have been reduces the absorption of fluoride in the body for the studied population leading to poor correlation with biological fluids.

Moreover, in the present study, no significant correlation was observed between dietary intake of leafy vegetables: Nightshade (Fig. 25; ρ= - 0.0044, p= 0.8), Cabbage (Fig. 26; ρ= -0.002,

p=0.07), and Chinese cabbage (Fig. 27; ρ= -0.0059, p=0.7) and fluoride concentration in human milk.

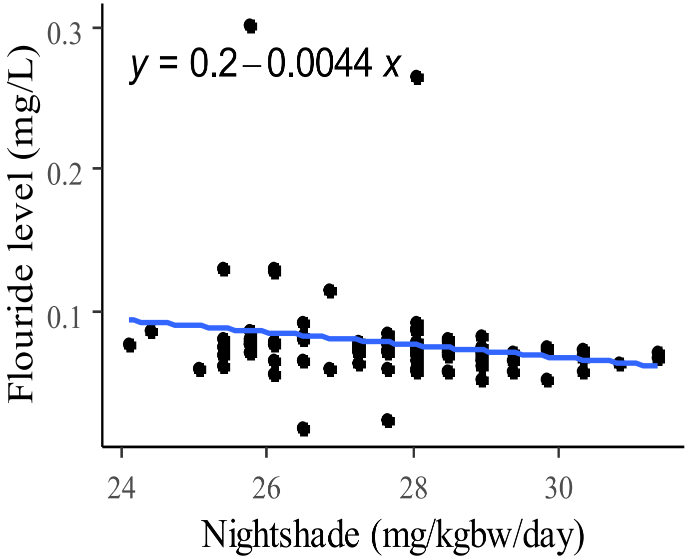

Figure 25: Relationship between daily fluoride intake due to nightshade and human milk fluoride levels in lactating mothers

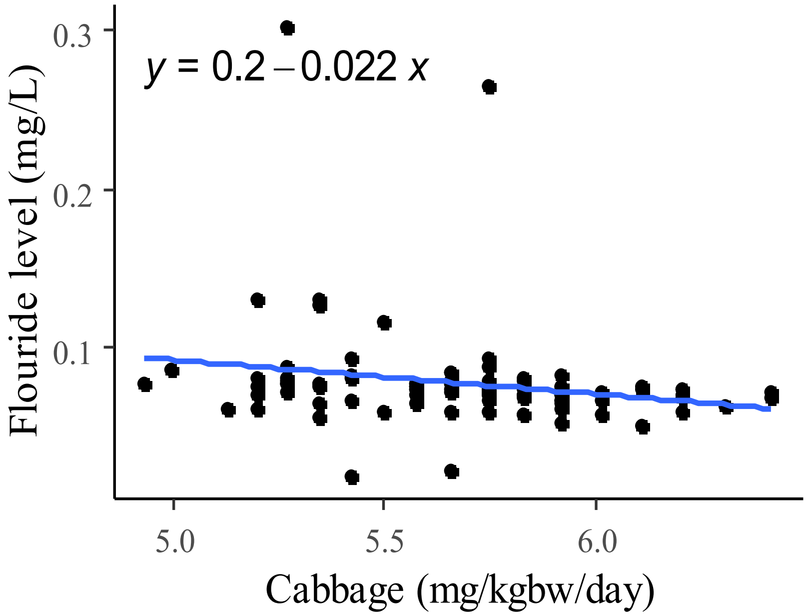

Figure 26: Relationship between daily fluoride intake due to cabbage and human milk fluoride levels in lactating mothers

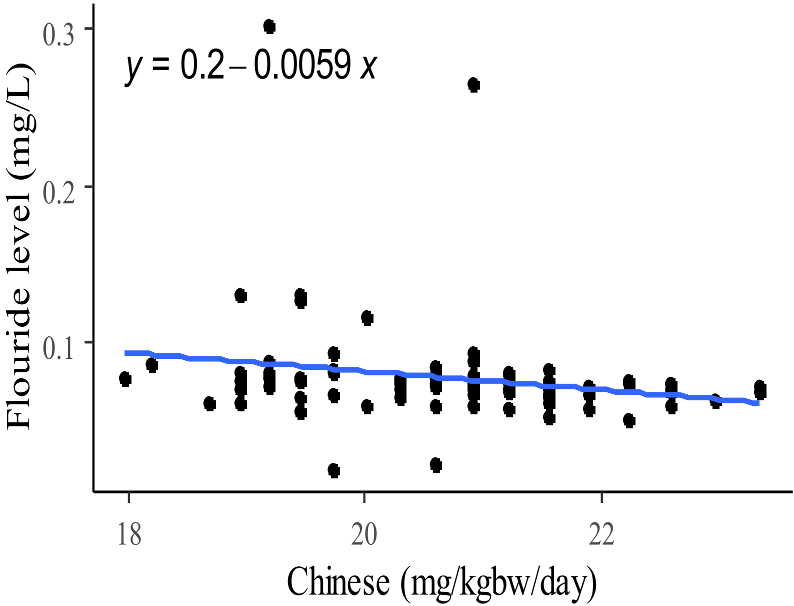

Figure 27: **Relationship between daily fluoride intake due to chinese cabbage and human milk fluoride levels in lactating mothers**

Similarly, no significant correlation was observed between dietary fluoride intake due to consumption of banana varieties: East African highland banana (Fig. 28; ρ= -0.0058, p=0.801), East African diploid banana (Fig. 29; ρ=-0.0.0066, p=0.27), Cavendish group (Fig. 30; ρ=0.0.0081, p=0.61) with fluoride concentration in human breast milk.

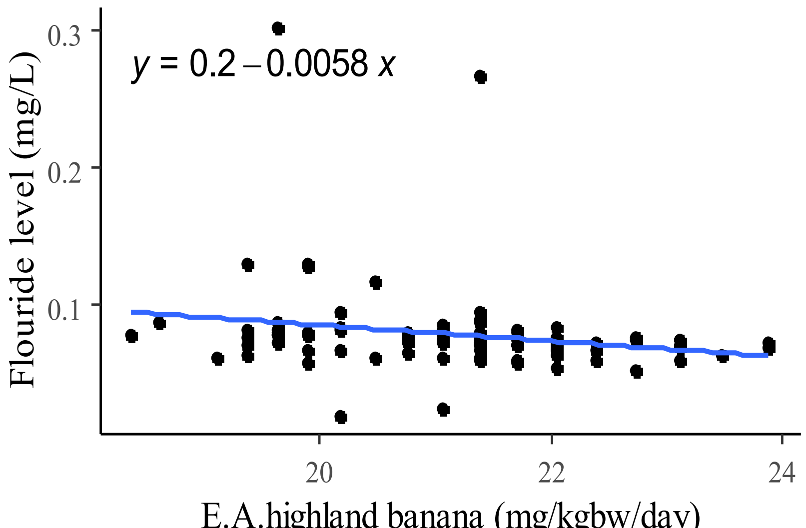

Figure 28: **Relationship between daily fluoride intake due to East African highland banana and human milk fluoride levels in lactating mothers**

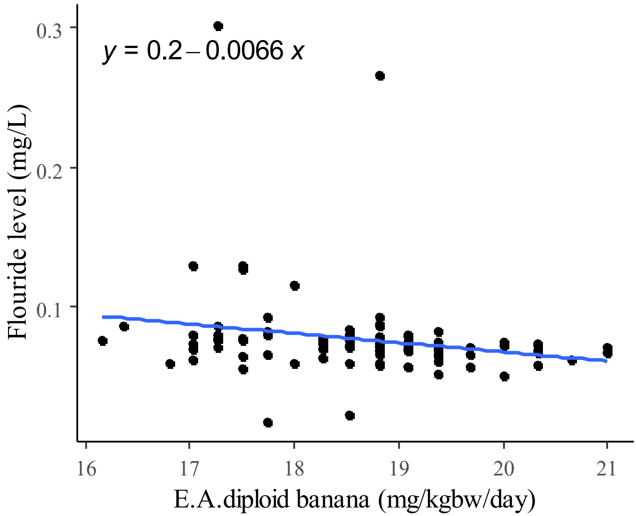

Figure 29: Relationship between daily fluoride intake due to East African diploid banana and human milk fluoride levels in lactating mothers

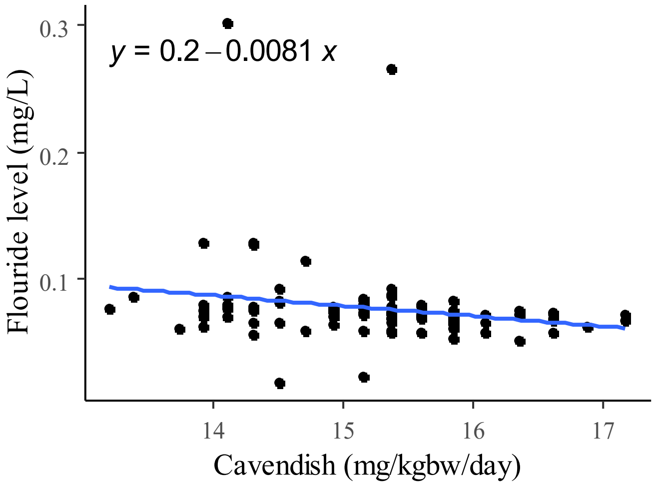

Figure 30: Relationship between daily fluoride intake due to Cavendish banana and human milk fluoride levels in lactating mothers

Studies reported that, availability of micro and/or macro contents of plant crops such as Fe, Zn, and Ca have shown to influence fluoride variation in the plant species (Barrera *et al.,* 2018; Gupta, 2019). This might be the reason for the variation observed between biomarkers and daily fluoride intake in this study. Some studies showed that aluminum ameliorates fluorosis in animals interfering with fluoride absorption (Becker *et al.,* 1950; Kessabi *et al.,* 1986), which reported the same case in human being which reported the same case in human being (Spencer & Kramer, 1985) due to ingestion of a relatively small dose of aluminum hydroxide (1.8 mg/day), regardless of the level of fluoride intake (from 4 to 50 mg/day), was associated with a significant increase in fecal fluoride excretion and a decrease in net fluoride absorption by 57%, the plasma fluoride level decreased by 41% (Spencer & Kramer, 1985).

According to Martin (1994) the structure and concentration of fluoride complexes formed with minerals in the body depend on both fluoride concentration and pH, in his findings reported that, in drinking water with 1 ppm, at pH 7.5, the predominant aluminum-containing species is Al(OH)4; at pH 4, the main species are AlF_2 and AlF_3. The more acidic the solution, the more free Al_3+ is available, the less the OH- group competes with Al_3+ in binding to F-, and the more Al-F complexes are formed (Martin, 1996; Powell & Thompson, 1993). This low pH is physiologically relevant since the pH of gastric juice, biological fluid, and most of the diets are around this level.

At a neutral pH, the main species are a mixture of AlF_3 and AlF_4. This is the condition in most of biochemical and cellular studies (Bodor *et al.,* 2000; Dutta *et al.,* 2019). The pH of diets can change the pH biological fluids (Remer & Manz, 1995), this might have caused variation in the fluoride concentration in breast milk and some locally grown food crops observed in this study.

In general, the study revealed that the correlation between daily dietary intake and biomarkers of fluoride exposure in children was positively correlated between cows milk and East African highland banana, whilst, no correlation was found due to consumption of human milk, maize, and water. For lactating mothers, strong correlation was found due to consumption of cow's milk, the minimum correlation was observed due to maize and water, while no correlation was found due to leafy vegetables and green banana varieties. However, all assessed diets for both children and lactating mothers, exposure rates were found above the recommended safe intake of 0.01 mg/kg per day for infants of up to 6 months and 0.05 to 0.07 mg/kg per day for adults (WHO, 2002). These results show that, the biochemical mechanism of fluoride and diet composition can influence the fluoride activities in the body and form stable complex compounds, which might alter the fluoride concentration in biological fluids in the body.

CHAPTER FIVE

CONCLUSION AND RECOMMENDATIONS

5.1 Conclusion

The present study found that the surveyed community had insufficient awareness about potential fluoride contaminant sources, and especially none of the respondents was aware of fluoride contamination of locally grown and commonly consumed food crops. Also, the majority of participants were unaware of the health effects associated with the consumption of excessive fluoride-contaminated diets. The use of locally made and affordable fluoride filters to filter drinking and cooking water might serve a long-term goal in solving the problem. Furthermore, this study has shown that locally grown and commonly consumed food crops were contaminated with fluoride to unacceptable levels, whereby leafy vegetables were more contaminated than maize flour and green bananas. Human breast milk and domestic water were contaminated above the WHO-recommended safe levels; this serves as an alarm to unacceptable fluoride exposures and the basic information to the dominant problem of fluorosis. Also, the present study revealed that studied populations were exposed to fluoride above the WHO recommended safe limit; whereby maize food crops showed a higher exposure rate for both children and lactating mothers. This study showed that, both fluoridated drinking water and locally grown and commonly consumed food crops play a significant role in human exposure to fluoride. This study can serve as baseline information for future research across seasons and with a broader food type.

5.2 Recommendations

As a result of the study findings, it can be recommended as follows:

(i) Awareness on fluoride contaminant sources and its related health: Raising awareness of fluoride contamination of various food materials, including water, its associated fluoride exposure, and potential health effects is necessary to gear up inclusive efforts in solving the problem. Appropriate interventions in the framework of nutrition are highly recommended to reduce exposure with the ultimate goal of lowering fluorosis in fluoride-endemic areas in Tanzania. In the short run, the community should be encouraged to use alternative water with minimum fluoride levels for drinking and food preparation, especially for infants and young children.

(ii) Fluoride levels in locally grown produce, domestic water and consumed milk: Though this was a cross-sectional study that is limited to seasonal variabilities, the findings call for short-term interventions alongside broader and detailed studies to quantify fluoride in a wide range of types of food crops and fresh produce, water, and food of animal origin across seasons as well as quantifying actual exposure from animal and human biomarkers. Infants should exclusively breastfeed for six months since human breast milk contains all essential nutrients for growth and development and has a very low fluoride concentration compared to cow's milk. Closer monitoring of the concentrations of fluoride added to water or salt, as well as defluoridation of drinking water and salts in the endemic communities. Toothpastes are generally added with fluoride to strengthen teeth; however, in fluoride-endemic areas, an exception should be made to consider fluoride-free toothpastes.

(iii) Dietary and Biomarkers of fluoride exposure: A relatively low exposure rate obtained through consumption of some locally grown leafy vegetables like cabbage proves that, some food crops may pose low fluoride uptake and /or accumulation ability. A detailed mapping of fluoride uptake and accumulation ability across food types may provide useful information to the communities in the endemic area to make a better use of less contaminated food choices. The community is also encouraged to diversify food sources grown from non-endemic areas for household's consumption rather than a total reliance on their locally grown produce.

REFERENCES

Abdennebi, E. H., Fandi, R., & Lamnaouer, D. (1995). Human fluorosis in Morocco: Analytical and clinical investigations. *Veterinary and Human Toxicology, 37*(5), 465-468.

Addison, M. J., Rivett, M. O., Robinson, H., Fraser, A., Miller, A. M., Phiri, P., Mleta, P., & Kalin, R. M. (2020). Fluoride occurrence in the lower East African Rift System, Southern Malawi. *Science of the Total Environment, 712*, 136260. https://doi.org/doi.org/10.1016/j.scitotenv.2019.136260

Adekola, O., Bashir, A., & Kasimu, A. M. (2015). Physico-chemical characteristics of borehole water quality in Gassol Taraba State, Nigeria. *African Journal of Environmental Science and Technology, 9*(2), 143-154. https://doi.org/10-20150223

Ahn, H. W., Fulton, B., Moxon, D., & Jeffery, E. H. (1995). Interactive effects of fluoride and aluminum uptake and accumulation in bones of rabbits administered both agents in their drinking water. *Journal of Toxicology and Environmental Health, Part A Current Issues, 44*(3), 337-350. https://doi.org/10.1080/15287399509531963

Akiniwa, K. (1997). Re-examination of acute toxicity of fluoride. *Fluoride, 30*(2), 89-104.

Alfredo, K. A., Lawler, D. F., & Katz, L. E. (2014). Fluoride contamination in the Bongo District of Ghana, West Africa: Geogenic contamination and cultural complexities. *Water International, 39*(4), 486-503.

Amini, M., Mueller, K., Abbaspour, K. C., Rosenberg, T., Afyuni, M., Møller, K. N., Sarr, M., & Johnson, C. A. (2008). Statistical modeling of global geogenic fluoride contamination in groundwaters. *Environmental Science & Technology, 42*(10), 3662-3668. https://doi.org/10.1021/es071958y

Aryeetey, R., & Dykes, F. (2018). Global implications of the new WHO and UNICEF implementation guidance on the revised Baby-Friendly Hospital Initiative. *Maternal & Child Nutrition, 14*(3), e12637. https://doi.org/10.1111/mcn.12637

Asawa, K. (2015). Association of temporomandibular joint signs & symptoms with dental fluorosis and skeletal manifestations in endemic fluoride areas of Dungarpur District, Rajasthan. *Clinical and Diagnostic Research, 9*, 18-21.

Asif-Ul-Alam, S., Islam, M. Z., Hoque, M. M., & Monalisa, K. (2014). Effects of drying on the physicochemical and functional properties of green banana (*Musa sapientum*) flour and development of baked product. *American Journal of Food Science and Technology, 2*(4), 128-133. https://doi.org/10.12691/ajfst-2-4-4

Aydgan, İ. Y., Yildirim, E., Kabakci, R., & Sümer, T. (2018). The Effect of Fluoride Toxication on Duodenum, Jejunum and Ileum of Broilers. *Tavukçuluk Araştırma Dergisi, 15*(2), 34-39.

Ayenew, T. (2008). The distribution and hydrogeological controls of fluoride in the groundwater of central Ethiopian rift and adjacent highlands. *Environmental Geology, 54*(6), 1313-1324.

Barrera, C., Valenzuela, R., Chamorro, R., Bascuñán, K., Sandoval, J., Sabag, N., Valenzuela, F., Valencia, M. P., Puigrredon, C., & Valenzuela, A. (2018). The impact of maternal diet during pregnancy and lactation on the fatty acid composition of erythrocytes and breast milk of Chilean women. *Nutrients, 10*(7), 839.

Bashash, M., Marchand, M., Hu, H., Till, C., Martinez-Mier, E. A., Sanchez, B. N., Basu, N., Peterson, K. E., Green, R., & Schnaas, L. (2018). Prenatal fluoride exposure and attention deficit hyperactivity disorder symptoms in children at 6–12 years of age in Mexico City. *Environment International, 121*, 658-666.

Bashash, M., Thomas, D., Hu, H., Angeles Martinez-Mier, E., Sanchez, B. N., Basu, N., Peterson, K. E., Ettinger, A. S., Wright, R., & Zhang, Z. (2017). Prenatal fluoride exposure and cognitive outcomes in children at 4 and 6–12 years of age in Mexico. *Environmental Health Perspectives, 125*(9), 097017.

Becker, D., Griffith, J., Hobbs, C., & MacIntire, W. (1950). The alleviation of fluorine toxicosis by means of certain aluminum compounds. *Journal of Animal Science,* 1950(9), 647.

Bergmann, R. (1995). *119 Fluoride Intake and Health RL Bergmann KE Bergmann. International Workshop Carbohydrates in Infant Nutrition and Dental Health: Maritim-Rhein-Main-Hotel, Darmstadt, November 17-18, 1994*. https://scholar.google.com

Bhagavatula, P., Levy, S. M., Broffitt, B., Weber-Gasparoni, K., & Warren, J. J. (2016). Timing of fluoride intake and dental fluorosis on late-erupting permanent teeth. *Community Dentistry and Oral Epidemiology, 44*(1), 32-45.

Bhargava, D., & Bhardwaj, N. (2009). Study of fluoride contribution through water and food to human population in fluorosis endemic villages of North-Eastern Rajasthan. *African Journal of Basic & Applied Sciences, 1*(3-4), 55-58.

Bodor, A., Tóth, I., Bányai, I., Szabo, Z., & Hefter, G. (2000). 19F NMR study of the equilibria and dynamics of the Al3+/F-system. *Inorganic Chemistry, 39*(12), 2530-2537.

Brunson, L. R. (2014). An Investigation of Sustainable Fluoride Water Treatment Technologies with a Focus on Ethiopia. *Science of the Total Enviroment, 488–489*, 580-587.

Buzalaf, M. (2018). Review of fluoride intake and appropriateness of current guidelines. *Advances in Dental Research, 29*(2), 157-166.

Buzalaf, M., Massaro, C., Rodrigues, M., Fukushima, R., Pessan, J. P., Whitford, G. M., & Sampaio, F. (2012). Validation of fingernail fluoride concentration as a predictor of risk for dental fluorosis. *Caries Research, 46*(4), 394-400.

Buzalaf, M., Rodrigues, M., Pessan, J., Leite, A., Arana, A., Villena, R., Forte, F., & Sampaio, F. (2011). Biomarkers of fluoride in children exposed to different sources of systemic fluoride. *Journal of Dental Research, 90*(2), 215-219.

Buzalaf, M. A. R., & Whitford, G. M. (2011). *Fluoride Metabolism. In Fluoride and the Oral Environment (Vol. 22, pp. 20-36)*. Karger Publishers. https://www.google.com

Campus, G., Congiu, G., Cocco, F., Sale, S., Cagetti, M. G., Sanna, G., Lingstroem, P., & Garcia-Godoy, F. (2014). Fluoride content in breast milk after the use of fluoridated food supplement. A randomized clinical trial. *American Journal of Dentistry, 27*(4), 199-202.

Casarin, R. C., Fernandes, D. R., Lima-Arsati, Y. B., & Cury, J. A. (2007). Fluoride concentrations in typical Brazilian foods and in infant foods. *Revista De Saude Publica, 41*, 549-556.

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

Chabukdhara, M., Gupta, S. K., Kotecha, Y., & Nema, A. K. (2017). Groundwater quality in Ghaziabad district, Uttar Pradesh, India: Multivariate and health risk assessment. *Chemosphere, 179*, 167-178.

Chakrabarti, S., & Bhattacharya, H. (2013). Inferring the hydro-geochemistry of fluoride contamination in Bankura district, West Bengal: A case study. *Journal of the Geological Society of India, 82*(4), 379-391.

Chávez-Salazar, A., Bello-Pérez, L., Agama-Acevedo, E., Castellanos-Galeano, F., Álvarez-Barreto, C., & Pacheco-Vargas, G. (2017). Isolation and partial characterization of starch from banana cultivars grown in Colombia. *International Journal of Biological Macromolecules, 98*, 240-246.

Chen, Y., Yan, W., & Hui, X. (2017). Treatment and prevention of skeletal fluorosis. *Biomedical and Environmental Sciences, 30*(2), 147-149.

Chiniah, J. P. R. (2017). *Exposure to Fluoride: Adverse Outcome and Toxicity in Review Universidade Fernando Pessoa].* Universidade Fernando Pessoa. https:// www. google. com

Choubisa, S. L., & Choubisa, D. (2016). Status of industrial fluoride pollution and its diverse adverse health effects in man and domestic animals in India. *Environmental Science and Pollution Research,* 23(8), 7244-7254.

Colombani, N., Di Giuseppe, D., Kebede, S., & Mastrocicco, M. (2018). Assessment of the anthropogenic fluoride export in Addis Ababa urban environment (Ethiopia). *Journal of Geochemical Exploration, 190*, 390-399.

Colonetti, K., Roesch, L. F., & Schwartz, I. V. D. (2018). The microbiome and inborn errors of metabolism: Why we should look carefully at their interplay? *Genetics and Molecular Biology, 41*(3), 515-532. https://doi.org/10.1590/1678-4685-gmb-2017-0235

Craig L, L. A., Berry K., & Yang W. (2015). Recommendations for fluoride limits in drinking water based on estimated daily fluoride intake in the Upper East Region, Ghana. *Science of the Total Environment Sciences, 53*(2), 127-137.

Currell, M., Cartwright, I., Raveggi, M., & Han, D. (2011). Controls on elevated fluoride and arsenic concentrations in groundwater from the Yuncheng Basin, China. *Applied Geochemistry*, 26(4), 540-552.

Cury, J. A., & Tenuta, L. M. A. (2014). Evidence-based recommendation on toothpaste use. *Brazilian Oral Research, 28*, 1-7.

Dabeka, R., Karpinski, K., McKenzie, A., & Bajdik, C. (1986). Survey of lead, cadmium and fluoride in human milk and correlation of levels with environmental and food factors. *Food and Chemical Toxicology, 24*(9), 913-921.

Dagnaw, L. A., Chandravanshi, B. S., & Zewge, F. (2017). Fluoride content of leafy vegetables, irrigation water, and farmland soil in the rift valley and in non-rift valley areas of Ethiopia. *Fluoride, 50*(4), 409-429.

Das, K., & Mondal, N. K. (2016). Dental fluorosis and urinary fluoride concentration as a reflection of fluoride exposure and its impact on IQ level and BMI of children of Laxmisagar, Simlapal Block of Bankura District, WB, India. *Environmental Monitoring and Assessment, 188*(4), 218. https://doi.org/10.1007/s10661-016-5219-1

Den-Besten, P., & Li, W. (2011). *Chronic Fluoride Toxicity: Dental Fluorosis. In Fluoride and the Oral Environment (Vol. 22, pp. 81-96)*.

Devi, G., & Sarma, H. P. (2016). Fluoride incorporation on selected vegetables in cultivated areas of Kamrup District of Assam. *Environment and Ecology, 2*(32), 34-45. https://doi.org/10.9790/2402-1404031217

Drummond, B., & Curzon, M. (1985). Urinary excretion of fluoride following ingestion of MFP toothpastes by infants aged two to six years. *Journal of Dental Research, 64*(9), 1145-1148. https://doi.org/10.1177/00220345850640091001

Duckworth, R., & Morgan, S. (1991). Oral fluoride retention after use of fluoride dentifrices. *Caries Research, 25*(2), 123-129. https://doi.org/10.1159/000261354

Dutta, V., Fatima, N., & Kumar, N. (2019). Excessive fluoride in groundwater of Central Ganga Alluvial Plain: A case study of Fatehpur, North India. *International Journal of Environmental Science and Technology, 16*(12), 7791-7798.

EFSA Panel on Dietetic Products, Nutrition, & Allergies (NDA). (2013). Scientific opinion on dietary reference values for fluoride. *EFSA Journal,* 11(8), 3332.

Ekstrand, J., Hardell, L., & Spak, C. J. (1984). Fluoride balance studies on infants in a 1-ppm-water-fluoride area. *Caries Research, 18*(1), 87-92.

El-Said, G. F., El-Sadaawy, M. M., & Aly-Eldeen, M. A. (2018). Adsorption isotherms and kinetic studies for the defluoridation from aqueous solution using eco-friendly raw marine green algae, Ulva lactuca. *Environmental Monitoring and Assessment, 190*(1), 14.

Ericsson, Y., & Ribelius, U. (1971). Wide variations of fluoride supply to infants and their effect. *Caries Research, 5*(1), 78-88. https://doi.org/10.1159/000259734

Everett, E. (2011). Fluoride's effects on the formation of teeth and bones, and the influence of genetics. *Journal of Dental Research, 90*(5), 552-560.

Fadhullah, W., Kamaruddin, M. A., Ismail, N., Sansuddin, N., & Abdullah, H. (2019). Characterization of Landfill Leachates and Groundwater and River Water Quality: A Case Lalang Waste Dumpsite, Kelantan. *Pertanika Journal of Science & Technology*, 27(2), 633.

Falcomer, A. L., Riquette, R. F. R., De Lima, B. R., Ginani, V. C., & Zandonadi, R. P. (2019). Health benefits of green banana consumption: A systematic review. *Nutrients, 11*(6), 1222. https://doi.org/10.3390/nu11061222

Fallahzadeh, R. A., Miri, M., Taghavi, M., Gholizadeh, A., Anbarani, R., Hosseini-Bandegharaei, A., Ferrante, M., & Conti, G. O. (2018). Spatial variation and probabilistic risk assessment of exposure to fluoride in drinking water. *Food and Chemical Toxicology, 113*, 314-321. https://doi.org/10.1016/j.fct.2018.02.001

Faraji, H., Mohammadi, A. A., Akbari-Adergani, B., Saatloo, N. V., Lashkarboloki, G., & Mahvi, A. H. (2014). Correlation between fluoride in drinking Water and its levels in breast milk in Golestan Province, Northern Iran. *Iranian Journal of Public Health*, 43(12), 1664.

Fawell, J., Bailey, K., Chilton, J., Dahi, E., & Magara, Y. (2006). *Fluoride in Drinking-Water*. IWA publishing. https://scholar.google.com/

Firempong, C., Nsiah, K., Awunyo-Vitor, D., & Dongsogo, J. (2013). Soluble fluoride levels in drinking water: A major risk factor of dental fluorosis among children in Bongo community of Ghana. *Ghana Medical Journal, 47*(1), 16-23.

Flaten, T. P. (2001). Aluminium as a risk factor in Alzheimer's disease, with emphasis on drinking water. *Brain Research Bulletin, 55*(2), 187-196.

García, M. G., & Borgnino, L. (2015). Fluoride in the context of the environment. *Chemistry, Analysis, Function and Effects*, 2015, 3-21.

Gautam, R., Bhardwaj, N., & Saini, Y. (2010). Fluoride accumulation by vegetables and crops grown in Nawa Tehsil of Nagaur district (Rajasthan, India). *Journal of Phytology*, 2(2), 80-85.

Gawalko, E. J., Nowicki, T. W., Babb, J., Tkachuk, R., & Wu, S. (1997). Comparison of closed-vessel and focused open-vessel microwave dissolution for determination of cadmium, copper, lead, and selenium in wheat, wheat products, corn bran, and rice flour by transverse-heated graphite furnace atomic absorption spectrometry. *Journal of AOAC International, 80*(2), 379-387.

Ghiglieri, G., Balia, R., Oggiano, G., & Pittalis, D. (2010). Prospecting for safe (low fluoride) groundwater in the Eastern African Rift: The Arumeru District (Northern Tanzania). *Hydrology and Earth System Sciences, 14*(6), 1081. https://doi.org/

Ghosh, S. B., & Mondal, N. K. (2019). Application of Taguchi method for optimizing the process parameters for the removal of fluoride by Al-impregnated Eucalyptus bark ash. *Environmental Nanotechnology, Monitoring & Management, 11*, 100206.

Gribble, K., & Fernandes, C. (2018). Considerations regarding the use of infant formula products in infant and young child feeding in emergencies (IYCF-E) programs. *World Nutrition, 9*(3), 261-283. https://doi.org/10.26596/wn.201893261-283

Grobler, S. R., Majeed, A., Moola, M. H., Rossouw, R. J., & Van Wyk Kotze, T. (2011). In vivo spectrophotometric assessment of the tooth whitening effectiveness of Nite White 10% with amorphous calcium phosphate, potassium nitrate and fluoride, over a 6-month period. *The Open Dentistry Journal, 5*, 18.

Keding, G. B., Msuya, J. M., Maass, B. L., & Krawinkel, M. B. (2012). Relating dietary diversity and food variety scores to vegetable production and socio-economic status of women in rural Tanzania. *Food Security*, 4, 129-140.

Guissouma, W., Hakami, O., Al-Rajab, A. J., & Tarhouni, J. (2017). Risk assessment of fluoride exposure in drinking water of Tunisia. *Chemosphere, 177*, 102-108. https://doi.org/10.1016/j.chemosphere.2017.03.011

Gumbo, F. J., & Mkongo, G. (1995). *Water Defluoridation for Rural Fluoride Affected Communities in Tanzania. In 1st International Workshop on Fluorosis Prevention and Defluoridation of Water: International Society for Fluoride Research Inc (pp. 109-114)*. https://www.google.com

Gupta. (2019). *Metabolism of Minerals. In Comprehensive Biochemistry for Dentistry* (pp. *473-493*). Springer. https://doi.org/10.1007/978-981-13-1035-5-18.

Gupta, P., Gupta, N., Meena, K., Moon, N. J., Kumar, P., & Kaur, R. (2015). Concentration of fluoride in cow's and buffalo's milk in relation to varying levels of fluoride concentration in drinking water of Mathura city in India: A pilot study. *Journal of Clinical and Diagnostic Research, 9*(5), LC05.

Gupta, S., & Banerjee, S. (2011). Fluoride accumulation in crops and vegetables and dietary intake in a fluoride-endemic area of West Bengal. *Fluoride, 44*(3), 153.

Gupta, S., Seth, A., Gupta, A., & Gavane, A. (1993). Transplacental passage of fluorides. *The Journal of Pediatrics, 123*(1), 139-141.

Gurumurthy-Sastry, M., Mohanty, S., Vyakaranam, S., Bhongir, A. V., & Rao, P. (2010). Transplacental Transport of Fluoride, Calcium and Magnesium. *National Journal of Intergrated in Medicine, 2*(3), 2230.

Hegde, R. M., Rego, R. M., Potla, K. M., Kurkuri, M. D., & Kigga, M. (2020). Bio-inspired materials for defluoridation of water: A review. *Chemosphere,* 253, 126657.

Hojsak, I., Bronsky, J., Campoy, C., Domellöf, M., Embleton, N., Fidler-Mis, N., Hulst, J., Indrio, F., Lapillonne, A., & Mølgaard, C. (2018). Young child formula: A position paper by the ESPGHAN Committee on Nutrition. *Journal of Pediatric Gastroenterology and Nutrition, 66*(1), 177-185.

Silva, J. A., & Uchida, R. S. (2000). *Plant Nutrient Management in Hawaii's soils: Approaches for Tropical and Subtropical Agriculture.* https://scholar.google.com

Ibiyemi, O., Zohoori, F., Valentine, R., & Maguire, A. (2018). Fluoride intake and urinary fluoride excretion in 4-and 8-year-old children living in urban and rural areas of Southwest Nigeria. *Community Dentistry and Oral Epidemiology, 46*(5), 482-491. https://doi.org/10.1111/cdoe.12396

Idowu, O. S., Azevedo, L. B., Valentine, R. A., Swan, J., Vasantavada, P. V., Maguire, A., & Zohoori, F. V. (2019). The use of urinary fluoride excretion to facilitate monitoring fluoride intake: A systematic scoping review. *PloS One, 14*(9), e0222260. https://doi.org/10.1371/journal.pone.0222260

Idowu, O. S., Duckworth, R. M., Valentine, R. A., & Zohoori, F. V. (2020). Biomarkers for the Assessment of Fluoride Exposure in Children. *Caries Research, 54*(2), 101-110. https://doi.org/10.1159/000504166

Isah, H. A., Mohammed, U. A., & Mohammed, A. A. (2014). Environmental distribution of fluoride in drinking waters of Kaltungo area, North-Eastern Nigeria. *American Journal of Environmental Protection, 3*(6-2), 19-24.

Jaganmohan, P., Narayana, S., & Sambasiva, R. (2010). Prevalence of high fluoride concentration in drinking water in Nellore district, Anadra Pradesh, India: A biochemical study to develop the relation to the rental failures. *World Journal of Medical Sciences, 5*, 45-48.

Jeandel, C., Lapicque, F., Netter, P., Bannwarth, B., Monot, C., Gillet, P., Payan, E., Guillaume, M., & Cuny, G. (1992). Effect of age on the disposition of sodium fluoride. *European Journal of Clinical Pharmacology, 43*(3), 295-297.

Jetti, R., Raghuveer, C., & Mallikarjuna, R. C. (2016). Protective effect of ascorbic acid and Ginkgo biloba against learning and memory deficits caused by fluoride. *Toxicology and Industrial Health, 32*(1), 183-187.

Jha, S. K., Mishra, V. K., Sharma, D. K., & Damodaran, T. (2011). Fluoride in the environment and its metabolism in humans. *Reviews of Environmental Contamination and Toxicology,* 211, 121-142.

Jiménez, L. V., Hernández, J. C., Atilano, R. I. C., Aguilar, S. Y. S., Torres, J. A. A., Salazar, R. C., & Amador, D. R. (2019). Level of exposure to fluorides by the consumption of different types of milk in residents from an area of Mexico with endemic hydrofluorosis. *Anales de Pediatría (English Edition), 90*(6), 342-348.

Jones Jr, J. B. (2001). *Laboratory Guide for Conducting Soil Tests and Plant Analysis*. CRC press. https://scholar.google.com

Joshi, N. A., & Ajithkrishnan, C. (2018). Scalp hair as biomarker for chronic fluoride exposure among fluoride endemic and low fluoride areas: A comparative study. *International Journal of Trichology, 10*(2), 71.

Kabir, H., Gupta, A. K., & Tripathy, S. (2020). Fluoride and human health: Systematic appraisal of sources, exposures, metabolism, and toxicity. *Critical Reviews in Environmental Science and Technology, 50*(11), 1116-1193.

Kalpana, L., Brindha, K., & Elango, L. (2019). FIMAR: A new Fluoride Index to mitigate geogenic contamination by Managed Aquifer Recharge. *Chemosphere, 220*, 381-390.

Kanduti, D., Sterbenk, P., & Artnik, B. (2016). Fluoride: A review of use and effects on health. *Materia Socio-medica, 28*(2), 133.

Karakochuk, C. D., Whitfield, K. C., Green, T. J., & Kraemer, K. (2017). *The biology of the First 1,000 Days*. CRC Press.

Kaseva, M. (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.

Akyuz, S., Yarat, A., Alturfan, E. E., & Kaya, S. (2015). *Fluoride in Saliva and Its Impact on Health*. https://scholar.google.com

Kazi, T. G., Brahman, K. D., Afridi, H. I., Shah, F., & Arain, M. B. (2018). Effects of high fluoride content in livestock drinking water on milk samples of different cattle in endemic area of Pakistan: Risk assessment for children. *Environmental Science and Pollution Research, 25*(13), 12909-12914.

Kazi, T. G., Brahman, K. D., Baig, J. A., & Afridi, H. I. (2019). Bioaccumulation of arsenic and fluoride in vegetables from growing media: Health risk assessment among different age groups. *Environmental Geochemistry and Health, 41*(3), 1223-1234.

Kessabi, M., Hamliri, A., & Braun, J. (1986). Experimental fluorosis in sheep: Alleviating effects of aluminum. *Veterinary and Human Toxicology, 28*(4), 300-304.

Khandare, A. L., & Rao, G. S. (2006). Uptake of fluoride, aluminum and molybdenum by some vegetables from irrigation water. *Journal of Human Ecology, 19*(4), 283-288.

Kichana, E., Minyila, S., & Quarcooc, G. (2019). The lethal effect of groundwater fluoride on the germination and growth of maize (Zea mays) and rice (Oryza sativa) seeds and plants. *Variations*, 285, 285.

Kidd, E. (2005). *Essentials of Dental Caries*. In. Oxford University Press. New York.

Knuuti, M. J., Yki-Järvinen, H., Voipio-Pulkki, L. M., Mäki, M., Ruotsalainen, U., Härkönen, R., Teräs, M., Haaparanta, M., Bergman, J., & Hartiala, J. (1994). Enhancement of myocardial [fluorine-18] fluorodeoxyglucose uptake by a nicotinic acid derivative. *Journal of Nuclear Medicine, 35*(6), 989-998.

Komakech, H. C., & Van der Zaag, P. (2011). Understanding the emergence and functioning of river committees in a catchment of the Pangani basin, Tanzania. *Water Alternatives, 4*(2), 197.

Kumar, S., Lata, S., Yadav, J., & Yadav, J. (2017). Relationship between water, urine and serum fluoride and fluorosis in school children of Jhajjar District, Haryana, India. *Applied Water Science, 7*(6), 3377-3384.

Kut, K. M. K., Sarswat, A., Srivastava, A., Pittman Jr, C. U., & Mohan, D. (2016). A review of fluoride in African groundwater and local remediation methods. *Groundwater for Sustainable Development*, 2, 190-212.

Lakshmi, D. V., Rao, K. J., Ramprakash, T., Reddy, A. P. K., & Kendra, K. V. (2017). Bioaccumulation of fluoride in different plant parts of food crops grown in Narkatpally Mandal of Nalgonda District, Telangana. *Environment & Ecology*, 35(3), 1753-1758.

Leal, J., Ferreira, R., Tabchoury, C., Lingström, P., & Vale, G. (2020). Dose-Response Effect of Fluoride Dentifrices on De-/Remineralization of Root Dentine in situ. *Caries Research*, 54(5-6), 502-508.

Levine, M. (2010). *Topics in Dental Biochemistry*. Springer Science & Business Media.

Lima-Arsati, Y. B. D. O., Gomes, A. R. L. F., Santos, H. K. A., Arsati, F., Oliveira, M. C., & Freitas, V. S. (2018). Exposure to fluoride of children during the critical age for dental fluorosis, in the semiarid region of Brazil. *Ciencia & Saude Coletiva, 23*, 1045-1054.

De Lima Leite, A., Buzalaf, C. P., & Rabelo Buzalaf, M. A. (2015). *Fluoride Intake in the Context of Dental Fluorosis.* https://scholar.google.com

Limón-Pacheco, J. H., Jiménez-Córdova, M. I., Cárdenas-González, M., Retana, I. M. S., Gonsebatt, M. E., & Del Razo, L. M. (2018). Potential co-exposure to arsenic and fluoride and biomonitoring equivalents for Mexican children. *Annals of Global Health, 84*(2), 257.

Liu, G., Ye, Q., Chen, W., Zhao, Z., Li, L., & Lin, P. (2015). Study of the relationship between the lifestyle of residents residing in fluorosis endemic areas and adult skeletal fluorosis. *Environmental Toxicology and Pharmacology, 40*(1), 326-332.

López-Guzmán, M., Alarcón-Herrera, M., Irigoyen-Campuzano, J., Torres-Castañón, L., & Reynoso-Cuevas, L. (2019). Simultaneous removal of fluoride and arsenic from well water by electrocoagulation. *Science of the Total Environment, 678*, 181-187.

Lv, Y., Yan, Z., Zhao, X., Gang, X., He, G., Sun, L., Li, Z., & Wang, G. (2018). The effects of gut microbiota on metabolic outcomes in pregnant women and their offspring. *Food & Function, 9*(9), 4537-4547.

Lyimo, M., Temu, R., & Mugula, J. (2003). Identification and nutrient composition of indigenous vegetables of Tanzania. *Plant Foods for Human Nutrition, 58*(1), 85-92.

Makoba, E. E. (2020). *Geological and Hydro-Geochemical Assessment of Fluorine Around Mount Meru, Northern Tanzania.* https://scholar.google.com

Malago, J., Makoba, E., & Muzuka, A. N. (2017). Fluoride levels in surface and groundwater in Africa: A review. *American Journal of Water Science and Engineering*, 3(1), 1-17.

Mannel, R., Martens, P. J., & Walker, M. (2012). *Core Curriculum for Lactation Consultant Practice*. Jones & Bartlett Publishers.

Marín, L. M., Cury, J. A., Tenuta, L. M. A., Castellanos, J. E., & Martignon, S. (2016). Higher fluorosis severity makes enamel less resistant demineralization. *Caries Research, 50*(4), 407-413. https://doi.org/10.1159/000447270

Marthaler, T. (1999). *Monitoring of Renal Fluoride Excretion in Community Preventive Programmes on Oral Health*. World Health Organization.

Martin, C. R., Ling, P. R., & Blackburn, G. L. (2016). Review of infant feeding: Key features of breast milk and infant formula. *Nutrients, 8*(5), 279.

Martin, R. B. (1994). Aluminum: A neurotoxic product of acid rain. *Accounts of Chemical Research, 27*(7), 204-210. https://doi.org/10.1016/S0010-8545(96)90008-9

Martin, R. B. (1996). Ternary complexes of $Al3^{+}$ and F^{-} with a third ligand. *Coordination Chemistry Reviews, 149*, 23-32. https://doi.org/10.1021/ar00043a004

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

Masawe, K., Mtei, K., & Machunda, R. (2019). Distribution of fluoride in beans and maize grown along the slope of Mount Meru, Tanzania. *Journal of Biodiversity and Environmental Sciences, 15*(5), 29-36.

Mascarenhas, A. K. (2000). Risk factors for dental fluorosis: A review of the recent literature. *Pediatric Dentistry, 22*(4), 269-277.

Mjengera, H. (2001). *Optimization of Bone Char Material for Defluoridation of Drinking Water* [University of Dar es Salaam]. University of Dar es Salaam. https://scholar.google.com

Mkungu, J., Machunda, R. L., & Muzuka, A. N. N. (2014). Application of soil composition for inferring fluoride variability in volcanic areas of Mt. Meru, Tanzania. *International Journal of Environmental Monitoring and Analysis, 2*(5), 231.

Moghaddam, V. K., Yousefi, M., Khosravi, A., Yaseri, M., Mahvi, A. H., Hadei, M., Mohammadi, A. A., Robati, Z., & Mokammel, A. (2018). High concentration of fluoride can be increased risk of abortion. *Biological Trace Element Research, 185*(2), 262-265.

Molina-Frechero, N., Gaona, E., Angulo, M., Pérez, L. S., González, R. G., Rascón, M. N., & Bologna-Molina, R. (2015). Fluoride exposure effects and dental fluorosis in children in Mexico City. *Medical Science Monitor: International Medical Journal of Experimental and Clinical Research, 21*, 3664.

Mukherjee, I., Singh, U. K., & Patra, P. K. (2019). Exploring a multi-exposure-pathway approach to assess human health risk associated with groundwater fluoride exposure in the semi-arid region of east India. *Chemosphere, 233*, 164-173.

Mustafa, K. (2014). Analyzing the attitudes of elite wrestlers related to the use of doping. *Türk Spor ve Egzersiz Dergisi, 16*(1), 128-134.

Mylonas, P., Austin, R., Moazzez, R., Joiner, A., & Bartlett, D. (2018). In vitro evaluation of the early erosive lesion in polished and natural human enamel. *Dental Materials, 34*(9), 1391-1400. https://doi.org/10.1016/j.dental.2018.06.018

Narsimha, A., & Rajitha, S. (2018). Spatial distribution and seasonal variation in fluoride enrichment in groundwater and its associated human health risk assessment in Telangana State, South India. *Human and Ecological Risk Assessment: An International Journal, 24*(8), 2119-2132.

NHS. (2012). *Pregnancy & Birth-National Resources.* https://scholar.google.com

Njenga, J. (2004). Comparative studies of water chemistry of four tropical lakes in Kenya and India. *Asian Journal of Water, Environment and Pollution, 1*(1, 2), 87-97.

NRC. (2007). *Fluoride In Drinking Water: A Scientific Review of EPA's Standards*. National Academies Press. https://scholar.google.com

O Mullane, D., Baez, R., Jones, S., Lennon, M., Petersen, P. E., Rugg-Gunn, A., Whelton, H., & Whitford, G. M. (2016). Fluoride and oral health. *Community Dental Health, 33*(2), 69-99.

O'Callaghan, T. F., Sugrue, I., Hill, C., Ross, R. P., & Stanton, C. (2019). *Nutritional Aspects of Raw Milk: A Beneficial or Hazardous Food Choice. In Raw Milk (pp. 127-148).* Elsevier. https://doi.org/10.1016/B978-0-12-810530-6.00007-9

Ohman, L. O., & Martin, R. B. (1994). Citrate as the main small molecule binding Al^{3+} in serum. *Clinical Chemistry, 40*(4), 598-601.

Okibe, F., Ekanem, E., Paul, E., Shallangwa, G., Ekwumemgbo, P., Sallau, M., & Abanka, O. (2010). Fluoride content of soil and vegetables from irrigation farms on the bank of river Galma, Zaria, Nigeria. *Australian Journal of Basic and Applied Sciences, 4*(5), 779-784.

Oliveira, M., Paiva, S., Martins, L., Ramos-Jorge, M., Lima, Y., & Cury, J. (2007). Fluoride intake by children at risk for the development of dental fluorosis: Comparison of regular dentifrices and flavoured dentifrices for children. *Caries Research, 41*(6), 460-466. https://doi.org/10.1159/000107933

Oliveira, P. F. T., Cury, J. A., Lima, C. V., Vale, G. C., Lima, M. D. D., Moura, L. D. F. A., & Moura, M. S. D. (2018). Is the fluoride intake by diet and toothpaste in children living in tropical semi-arid city safe? *Brazilian Oral Research,* 32, e26.

Opinya, G., Bwibo, N., Valderhaug, J., Birkeland, J., & Lökken, P. (1991). Intake of fluoride and excretion in mothers' milk in a high fluoride (9 ppm) area in Kenya. *European Journal of Clinical Nutrition, 45*(1), 37-41.

Ozsvath, D. L. (2009). Fluoride and environmental health: A review. *Reviews in Environmental Science and Bio/Technology, 8*(1), 59-79.

Pal, K. C., Mondal, N. K., Bhaumik, R., Banerjee, A., & Datta, J. K. (2012). Incorporation of fluoride in vegetation and associated biochemical changes due to fluoride contamination in water and soil: A comparative field study. *Annals of Environmental Science*, 2012(6), 123-139.

Paul, E., Gimba, C., Kagbu, J., Ndukwe, G., & Okibe, F. (2011). Spectrometric determination of fluoride in water, soil and vegetables from the precinct of river Basawa, Zaria, Nigeria. *Journal of Basic and Applied Chemistry, 1*(6), 33-38.

Paya, P., & Bhatt, S. (2010). Fluoride contamination in groundwater of Patan district, Gujarat, India. *International Journal of Engineering Studies, 2*(2), 171-177.

Pereira, D. L., Freitas, A. Z., Bachmann, L., Benetti, C., Zezell, D. M., & Ana, P. A. (2018). Variation on molecular structure, crystallinity, and optical properties of dentin due to Nd: YAG laser and fluoride aimed at tooth erosion prevention. *International Journal of Molecular Sciences, 19*(2), 433.

Perumal, E., Paul, V., Govindarajan, V., & Panneerselvam, L. (2013). A brief review on experimental fluorosis. *Toxicology Letters, 223*(2), 236-251.

Peters, M. D., Godfrey, C. M., Khalil, H., McInerney, P., Parker, D., & Soares, C. B. (2015). Guidance for conducting systematic scoping reviews. *International Journal of Evidence-Based Healthcare, 13*(3), 141-146.

Petersen, P. E., Kandelman, D., Arpin, S., & Ogawa, H. (2010). Global oral health of older people-call for public health action. *Community Dental Health, 27*(4), 257-267.

Ponikvar, M. (2008). *Exposure of Humans to Fluorine and its Assessment. In Fluorine and Health (pp. 487-549).* Elsevier. https://doi.org/10.1016/B978-0-444-53086-8.00012-6

Post, G. B., Gleason, J. A., & Cooper, K. R. (2017). Key scientific issues in developing drinking water guidelines for perfluoroalkyl acids: Contaminants of emerging concern. *PLoS Biology, 15*(12), e2002855.

Poureslami, H., Khazaeli, P., Mahvi, A. H., Poureslami, K., Poureslami, P., Haghani, J., & Aghaei, M. (2016). Fluoride level in the breast milk in Koohbanan: A city with endemic dental fluorosis. *Fluoride, 49*(4 Pt 2), 485-494.

Powell, J., & Thompson, R. (1993). The chemistry of aluminium in the gastrointestinal lumen and its uptake and absorption. *Proceedings of the Nutrition Society, 52*(1), 241-253. https://doi.org/10.1079/PNS19930056

Priest, N. D. (1993). The bioavailability and metabolism of aluminium compounds in man. *Proceedings of the Nutrition Society, 52*(1), 231-240.

Rango, T., Vengosh, A., Jeuland, M., Tekle-Haimanot, R., Weinthal, E., Kravchenko, J., Paul, C., & McCornick, P. (2014). Fluoride exposure from groundwater as reflected by urinary

fluoride and children's dental fluorosis in the Main Ethiopian Rift Valley. *Science of the Total Environment, 496*, 188-197.

Rango, T., Vengosh, A., Jeuland, M., Whitford, G. M., & Tekle-Haimanot, R. (2017). Biomarkers of chronic fluoride exposure in groundwater in a highly exposed population. *Science of the Total Environment, 596*, 1-11.

Ranjan, R., & Ranjan, A. (2015). *Fluoride Toxicity in Animals*. Springer.

Reddy, K. S. (2016). Global Burden of Disease Study 2015 provides GPS for global health 2030. *The Lancet, 388*(10053), 1448-1449. https://doi.org/10.1016/S0140-6736(16)31743-3

Remer, T., & Manz, F. (1995). Potential renal acid load of foods and its influence on urine pH. *Journal of the American Dietetic Association, 95*(7), 791-797.

Riquette, R. F. R., Ginani, V. C., Dos Santos Leandro, E., De Alencar, E. R., Maldonade, I. R., De Aguiar, L. A., De Souza Acácio, G. M., Mariano, D. R. H., & Zandonadi, R. P. (2019). Do production and storage affect the quality of green banana biomass? *Food Science and Technology, 111*, 190-203.

Rizzu, M., Tanda, A., Canu, L., Masawe, K., Mtei, K., Deroma, M. A., Roggero, P. P., & Seddaiu, G. (2020). Fluoride uptake and translocation in food crops grown in fluoride-rich soils. *Journal of the Science of Food and Agriculture, 100*(15), 5498-5509. https://doi.org/10.1002/jsfa.10601

Rojas-Sanchez, F., Kelly, S. A., Drake, K. M., Eckert, G. J., Stookey, G. K., & Dunipace, A. J. (1999). Fluoride intake from foods, beverages and dentifrice by young children in communities with negligibly and optimally fluoridated water: A pilot study. *Community Dentistry and Oral Epidemiology, 27*(4), 288-297.

Rouphael, Y., & Kyriacou, M. C. (2018). Quality and safety of fresh fruits and vegetables at harvest. *Scientia Horticulturae, 239*, 78-79.

Rugg-Gunn, A., Nunn, J., Ekanayake, L., Saparamadu, K., & Wright, W. (1993). Urinary fluoride excretion in 4-year-old children in Sri Lanka and England. *Caries Research, 27*(6), 478-483. https://doi.org/10.1159/000261584

Rugg-Gunn, A. J., Villa, A. E., & Buzalaf, M. R. A. (2011). *Contemporary Biological Markers of Exposure to Fluoride. In Fluoride And The Oral Environment (Vol. 22, Pp. 37-51)*. Karger Publishers.

Ruiz-Payan, A., Ortiz, M., & Duarte-Gardea, M. (2005). Determination of fluoride in drinking water and in urine of adolescents living in three counties in Northern Chihuahua Mexico using a fluoride ion selective electrode. *Microchemical Journal, 81*(1), 19-22. https://doi.org/10.1016/j.microc.2005.01.017

Sami, E., Vichayanrat, T., & Satitvipawee, P. (2016). Caries with Dental Fluorosis and Oral Health Behaviour Among 12-Year School Children in Moderate-Fluoride Drinking Water Community in Quetta, Pakistan. *Pakistan. Journal of the College of Physicians and Surgeons Pakistan, 26*(9), 744-747.

Saxena, K., & Sewak, R. (2003). Fluoride consumption in endemic villages of India and its remedial measures. *International Journal of Science and Engineering Invention, 4*(1), 58-73.

Şener, Y., Tosun, G., Kahvecioğlu, F., Gökalp, A., & Koc, H. (2007). Fluoride levels of human plasma and breast milk. *European Journal of Dentistry, 1*(1), 21.

Senghor, B., Sokhna, C., Ruimy, R., & Lagier, J. C. (2018). Gut microbiota diversity according to dietary habits and geographical provenance. *Human Microbiome Journal, 7*, 1-9.

Setnikar, I., Rovati, L., Schmid, K., Vens-Cappell, B., & Barkworth, M. (1998). Bioavailability and pharmacokinetic characteristics of two monofluorophosphate preparations with calcium supplement. *Arzneimittel-Forschung, 48*(12), 1172-1178.

Shanmugam, K., Siva, M., Ravi, S., Shanmugam, B., & VenkataSubbaiah, G. (2018). *A Review of Concentration of Fluoride in World and India: Effects of Fluoride on Human Body*. http://www.isca.co.in

Singano, J. (1991). *Defluoridation of Drinking Water by Using Magnesia and Polyaluminium Chloride.* https://scholar.google.com

Singh, B., Gaur, S., & Garg, V. (2007). Fluoride in drinking water and human urine in Southern Haryana, India. *Journal of Hazardous Materials, 144*(1-2), 147-151. https://doi.org/10.1016/j.jhazmat.2006.10.010

Slade, G., Grider, W., Maas, W., & Sanders, A. (2018). Water fluoridation and dental caries in US children and adolescents. *Journal of Dental Research, 97*(10), 1122-1128. https://doi.org/10.1177/0022034518774331

Spak, C., Hardell, L., & De Chateau, P. (1983). Fluoride in human milk. *Acta Pædiatrica, 72*(5), 699-701. https://doi.org/10.1111/j.1651-2227.1983.tb09796.x

Spencer, A., Do, L., Mueller, U., Baines, J., Foley, M., & Peres, M. (2018). Understanding optimum fluoride intake from population-level evidence. *Advances in Dental Research, 29*(2), 144-156. https://doi.org/10.1177/0022034517750592

Spencer, H., & Kramer, L. (1985). Osteoporosis: Calcium, fluoride, and aluminum interactions. *Journal of the American College of Nutrition, 4*(1), 121-128.

Spittle, B. (2016). Fluoride in human breast milk. *Fluoride, 49*(4), 471.

Štepec, D., & Ponikvar-Svet, M. (2019). Fluoride in human health and nutrition. *Acta Chimica Slovenica, 66*(2), 255-275. https://doi.org/10.17344/acsi.2019.4932

Sunitha, V., & Reddy, B. M. (2014). Determination of fluoride concentration in ground water by ion selective electrode. *International Journal of Current Research and Academic Review*, 2(8), 159-166.

Tahir, M. A., & Rasheed, H. (2013). Fluoride in the drinking water of Pakistan and the possible risk of crippling fluorosis. *Drinking Water Engineering and Science*, 6(1), 17-23.

TBS. (2005). *National Environmental Standards Compendium: Drinking (potable) Water Specification.* https://scholar.google.com

Thole, B. (2013). Ground water contamination with fluoride and potential fluoride removal technologies for East and Southern Africa. *Perspectives in Water Pollution*, 66-90. https://doi.org/10.5772/54985

Thompson, L. J. (2018). *Fluoride. In Veterinary Toxicology (pp. 429-431)*. Elsevier. https://doi.org/10.1016/B978-0-12-811410-0.00027-1

Torra, M., Rodamilans, M., & Corbella, J. (1998). Serum and urine ionic fluoride. *Biological Trace Element Research, 63*(1), 67-71. https://doi.org/10.1007/s10653-019-00444-x

Tylenda, C. A. (2011). *Toxicological Profile for Fluorides, Hydrogen Fluoride, and Fluorine (update)*. DIANE Publishing.

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.

USDA. (2009). *Agricultural Research Service, Nutrient Data Laboratory*. https:// scholar. google. com

USEPA. (2005). *Supplemental Guidance for Assessing Susceptibility from Early-Life Exposure to Carcinogens. Risk Assessment Forum*. https://scholar.google.com

Valdez-Jiménez, L., Fregozo, C. S., Beltrán, M. M., Coronado, O. G., & Vega, M. P. (2011). Effects of the fluoride on the central nervous system. *Neurología (English Edition), 26*(5), 297-300.

Vale, G., Simões, N., Santana, G., Mota, B., & Moura, M. (2019). Gastrointestinal Absorption and Renal Excretion of Fluoride After Ingestion of a High-Fluoride Dentifrice. *Biological Trace Element Research, 190*(1), 24-29.

Villa, A., Anabalón, M., & Cabezas, L. (2000). The fractional urinary fluoride excretion in young children under stable fluoride intake conditions. *Community Dentistry and Oral Epidemiology, 28*(5), 344-355.

Villa, A., Anabalon, M., Zohouri, V., Maguire, A., Franco, A., & Rugg-Gunn, A. (2010). Relationships between fluoride intake, urinary fluoride excretion and fluoride retention in children and adults: An analysis of available data. *Caries Research, 44*(1), 60-68. https://doi.org/10.1111/j.1600-0528.2006.00269.x

Viswanathan, G. (2018). *Contribution of Infant Formula and Tea on Daily Fluoride Intake and Prevalence of Fluorosis among Infants and Children. In Food Quality: Balancing Health and Disease (Pp. 339-363)*. Elsevier. https:// doi. org/ 10. 1016/ B978-0-12-811442-1.00011-0

Viswanathan, G., Jaswanth, A., & Gopalakrishnan, S. (2009). Mapping of fluoride endemic areas and assessment of fluoride exposure. *Science of the Total Environment, 407*(5), 1579-1587. https://doi.org/10.1016/j.scitotenv.2008.10.020

Warpeha, R. A., & Marthaler, T. (1995). Urinary fluoride excretion in Jamaica in relation to fluoridated salt. *Caries Research, 29*(1), 35-41.

Wei, S. H., & Ekstrand, J. (2019). *Dental caries: Resistance factors -Fluorides Systemized Prevention of Oral Disease: Theory and Practice*. https://scholar.google.com

Whitford, G. M., Thomas, J. E., & Adair, S. M. (1999). Fluoride in whole saliva, parotid ductal saliva and plasma in children. *Archives of Oral Biology, 44*(10), 785-788. https://doi.org/10.1016/S0003-9969(99)00083-7

WHO. (1984a). *Fluorine and Fluorides-Environmental Health Criteria 36*. https:// scholar. google. com

WHO. (1984b). *Guidelines for Drinking-Water Quality, Volume 1: Recommendations and Volume 2: Health Criteria and Other Supporting Information*. *Geneva, WHO*. https://scholar.google.com

WHO. (2002). *The World Health Report 2002: Reducing Risks, Promoting Healthy Life. World Health Organization*. https://scholar.google.com

WHO. (2003). *Implementing the Global Strategy for Infant and Young Child Feeding: Geneva,* https://scholar.google.com

WHO. (2011). *Nitrite in Drinking-Water Background Document for Development of WHO Guidelines for Drinking-Water Quality*. *Geneva: World Health Organization.* https://scholar.google.com

WHO. (2014). *Basic Methods for Assessing Renal Fluoride Excretion in Community Prevention Programmes for Oral Health*. World Health Organization. https://doi.org/www.who.int

WHO. (2015). *Guideline: Sugars Intake for Adults and Children*. World Health Organization. https://doi.org/www.who.int/nutrition

Yadav, K. K., Kumar, V., Gupta, N., Kumar, S., Rezania, S., & Singh, N. (2019). Human health risk assessment: Study of a population exposed to fluoride through groundwater of Agra city, India. *Regulatory Toxicology and Pharmacology, 106*, 68-80.

Yin, W., Yang, Y. M., Chen, H., Li, X., Wang, Z., Cheng, L., Yin, Q. D., Fang, H. Z., Fei, W., & Mi, F. L. (2017). Oral health status in Sichuan Province: Findings from the oral health survey of Sichuan, 2015–2016. *International Journal of Oral Science, 9*(1), 10-15.

Yousefi, M., Ghoochani, M., & Mahvi, A. H. (2018). Health risk assessment to fluoride in drinking water of rural residents living in the Poldasht city, Northwest of Iran. *Ecotoxicology and Environmental Safety, 148*, 426-430.

Zhang, J., Li, Z., Qie, M., Zheng, R., Shetty, J., & Wang, J. (2016). Sodium fluoride and sulfur dioxide affected male reproduction by disturbing blood-testis barrier in mice. *Food and Chemical Toxicology, 94*, 103-111.

Zohoori, F., & Maguire, A. (2018). Are there good reasons for fluoride labelling of food and drink? *British Dental Journal, 224*(4), 215-217.

Zohoori, F. V., Omid, N., Sanderson, R. A., Valentine, R. A., & Maguire, A. (2019). Fluoride retention in infants living in fluoridated and non-fluoridated areas: Effects of weaning. *British Journal of Nutrition, 121*(1), 74-81.

Zohouri, F. V. (1997). *Fluoride Intake and Excretion in 4 Year Old Iranian Children [Doctoral dissertation, University of Newcastle upon Tyne].* https://scholar.google.com

APPENDICES

Appendix 1: Ethical clearance

THE UNITED REPUBLIC OF TANZANIA

National Institute for Medical Research
3 Barack Obama Drive
P.O. Box 9653
11101 Dar es Salaam
Tel: 255 22 2121400
Fax: 255 22 2121360
E-mail: ethics@nimr.or.tz

Ministry of Health, Community Development, Gender, Elderly & Children
University of Dodoma, Faculty of Arts and Social Sciences
Building No. 11
P.O. Box 743
40478 Dodoma

NIMR/HQ/R.8a/Vol. IX/2786

22nd May 2018

Ms. Lucia Joseph
C/o Dr. Neema Kassim
Nelson Mandela African Institution for Science and Technology
P.O. Box 447
Tengeru

RE: ETHICAL CLEARANCE CERTIFICATE FOR CONDUCTING MEDICAL RESEARCH IN TANZANIA

This is to certify that the research entitled: Assessment of under two years children exposure to fluoride through food consumption: A case study of mount Meru slopes, Northern Tanzania (Joseph L. *et al.*) whose supervisor is Dr. Neema Kassim of Nelson Mandela African Institution for Science and Technology has been granted ethical clearance to be conducted in Tanzania.

The Principal Investigator of the study must ensure that the following conditions are fulfilled:

1. Progress report is submitted to the Ministry of Health, Community Development, Gender, Elderly & Children and the National Institute for Medical Research, Regional and District Medical Officers after every six months.
2. Permission to publish the results is obtained from National Institute for Medical Research.
3. Copies of final publications are made available to the Ministry of Health, Community Development, Gender, Elderly & Children and the National Institute for Medical Research.
4. Any researcher, who contravenes or fails to comply with these conditions, shall be guilty of an offence and shall be liable on conviction to a fine as per NIMR Act No. 23 of 1979, PART III Section 10(2).
5. Site: Arusha

Approval is valid for one year: 22nd May 2018 to 21st May 2019.

Name: Prof. Yunus Daud Mgaya

Signature
CHAIRPERSON
MEDICAL RESEARCH COORDINATING COMMITTEE

Name: Prof. Muhammad Bakari Kambi

Signature
CHIEF MEDICAL OFFICER
MINISTRY OF HEALTH, COMMUNITY DEVELOPMENT, GENDER, ELDERLY & CHILDREN

CC: RMO of Arusha.
DMOs/DEDs of selected districts.

Appendix 2: Consent form

Consent Form for Participation on: Risks of exposure to fluoride contamination among lactating mothers and young children in communities around mount Meru in northern Tanzania

Introduction---

Procedure: ---

Benefits: --

Precautions: --

Confidentiality---

Participant statement

I--have understood the above information explained to me by the investigator and I agree to take part in this study and I can withdraw without giving reason.

Participants : Name:………………………Signature………………… Date…………..

Investigators:Name :………………………Signature ………………..Date…………..

Appendix 3: Questionnaires guide for women of reproductive age of the community around mount Meru areas

Questionnaire on: Risks of exposure to fluoride contamination among lactating mothers and young children (0-24 months) in communities around mount Meru in northern Tanzania

S/N0	Section A: General information	Fill blanks/Tick where appropriate		
1	Interviewers name			
2	Center Name			
3	Name of respondent (Three names)			
4	Ward (Village)			
5	Street			
6	Date of interview			
7	Consent has been obtained	Yes	1	
		No	2	
8	Contact phone number of respondent			
9	Who is the owner of the phone number?	Yours	1	
		Husband	2	
		Someone else (Mention)	3	----
10	Name of Head of households			
11	Name of Local village/street leader		-----	
Section B: Demographic information				
12	What is your age or date of birth?	Date ----------------	Years	-----
13	What is the highest level of education you have attained?	Never went to school	1	
		Primary school	2	
		Secondary school	3	
		college/university	4	
14	What is your marital status	Never married	1	
		Married	2	
		Separated	3	
		Divorced	4	
		Widowed	5	

		Cohabiting	6	
		Refused to mention	99	
15	Which of the following describes your main work status?	Non-government employee	1	
		Self employed	2	
		Student	3	
		Home maker	4	
		Retired officer	5	
		Unemployed	7	
		Disabled	8	
		Others (mention)	9	----
16	What is your average income per month?	<250,000	1	
		250,000-450,000	2	
		500,000- 750,000	3	
		800,000-1,000,000	4	
		>1,000,000	5	
		Refused to mention	99	
Section C: Fluoride exposure awareness				
17	What is fluoride? Have you heard about fluoride exposure?	Yes	1	
		No	2	
		I don't know	88	
	If, Yes where have you heard?	School	1	
		Home	2	
		Nutritionist	3	
		Agricultural officers	4	
		Community	5	
		Refused to mention	99	
19	Do you know the sources of fluoride?		----	
		Yes	1	
		No	2	

		I don’t know	88	
21	What are the major sources of fluoride?	Soil	1	
		water	2	
		Other(Specify)	3	
		I don’t know	88	
22	Can fluoride contaminate with food?	Yes	1	
		No	2	
23	If, yes how?	Cooking	1	
		Meals preparations	2	
		In the soil	3	
		I don’t know	88	
24	Can fluoride cause effects in the human body?	Yes	1	
		No	2	
25	If yes, what are the effects?	Hard bended back	1	
		Large heads	2	
		Pits in tooth enamel in children	3	
26	Can fluoride contaminate in the vegetables?	Born fracture and pains	4	
26	Can fluoride contaminate in the vegetables?	Yes		
27	If yes what are types of vegetables can be easily contaminate?	Mention 1. 2. 3. 4.		
28	Is there possibility for cooked vegetables to contaminate with fluoride?	Yes No I don’t know	1 2 3	
29	Is there possibility of fruits to contaminate with fluoride?	Yes No	1 2	

30	What do you think are the major fruits which can be easily contaminate with fluoride?	Mention		
31	Does consumption of fluoridated drinking water increase the risk of fractures or other effects on the bones?	Yes	1	
		No	2	
		I don't know	88	
32	Does consumption of fluoridated drinking water impair children's cognitive development?	Yes	1	
		No	2	
		I don't know	88	
32	Does fluoride protect the teeth decay?	Yes	1	
		No	2	
		I don't know	88	
34	Why do some people object to fluoridation?	specify	1	
			2	
		I don't know	88	
35	Are there any methods used to reduce fluoride in domestic water?	Yes	1	
		No	2	
		I don't know	88	
36	If yes, how was it treated?	Specify	1	
			2	
37	Are you using the same meals with infants or prepare separate?	Yes	1	
		No	2	
			3	
38	If yes where do you get water used for preparing infant /baby meals?	River	1	
		Tap water	2	
		Bore holes	3	-----
39	Have you ever been used special drinking water for infant's meals preparations?	Yes	1	
		No	2	
		I don't know	88	
40	What toothpastes you're using for tooth brushing?	Fluoridated	--------	

		No-fluoridated	88	
		Other		
41	Are you helping children to brush their teeth or brush for themselves (1-2years children)?	Yes	1	
		No	2	
42	Your children are having pits in their tooth?	Yes	1	
		No	2	
		Refused to mention	99	
43	If yes what do you think are the causes?	Specify	-	

Appendix 4: The 24-hour dietary recall

Time	Food/drinks	Amount
Breakfast		
Snack		
Lunch		
Dinner		
Tool	**Unit of measure**	**Estimated amount in (mls/grams)**
Small plastic cup	Full	250
	Three quarter	187.5
	Half	125
	Quarter	62.5
Large plastic cup	Full	400
	Three quarter	300
	Half	200
	Quarter	100
Larger plastic cup with draft/squares	Full	450
	Three quarter	337.5
	Half	225
	Quarter	112.5
Largest plastic cup with circles	Full	550
	Three quarter	412.5
	Half	275
	Quarter	137.5
Glasi ya chuma/iron glass	Full	425
	Three quarter	318.75
	Half	212.5
	Quarter	106.25
Maziwa/uji		
Small plastic bowl	Full	350
	Three quarter	262.5
	Half	175
	Quarter	87.5
Small iron bowl	Full	250
	Three quarter	187.5
	Half	125
	Quarter	62.5
Bone bowl (bakuri la mfupa)	Full	450

Time	Food/drinks	Amount
	one-third	337.5
	Half	225
	Quarter	112.5
Various foods and spices		
Wali/Tambi		**grams**
Bone plate	Full	662
	Three quarter	
	Half	
	Quarter	
Plastic plate	Full	622
	Three quarter	
	Half	
	Quarter	
Plate with partition	large part	301
	small part	127
Small plastic bowl	Full	311
Small iron bowl	Full	236
Bone bowl (bakuri la mfupa)	Full	385
Serving spoon	Full	152
Table spoon	Full	22
Sugar		
Table spoon	Full	20
	Flat	10
Tea spoon	Full	7
	Flat	5
Salt		
Table spoon	Full	21
	Flat	10
Tea spoon	Full	9
	Flat	4
Ugali/stiff porridge		
Small plastic bowl	Full	400
Serving spoon/ngumi	Full	180-200
Vegetable		
Small plastic bowl	Cabbage	275
	Chinese cabbage	300
	nightshade	287
Small iron bowl	Cabbage	330
	Chinese cabbage	325
	nightshade	312
	Pumpkin leaves	283

Time	Food/drinks	Amount
Bone bowl (bakuri la mfupa)	Cabbage	440
	Chinese cabbage	399
	nightshade	330
Serving spoon	Matembele	220
	pumpkin leaves	173
	sukuma wiki	159
Table spoon	Matembele	65
	night shade plant vegetable/amaranthus	33
	pumpkin leaves	72
	sukuma wiki	42
Partioned plate	small part-Matembele	118
	pumpkin leaves	173
Makande(mixture of maize and beans)		
Potato	raw sweet potato-1	
	cooke sweet potato-1	
	irish/round -1	175
	cooked- 1	181
Yam	medium size 1-raw	183
	Cooked	188
Cocoyam	small size 1-raw	200
	Cooked	208
Ndizi		
Raw	Lain-medium size 1	105
Cooked	Lain-medium size 1	106
Raw	ngumu-medium size 1	119
Cooked	ngumu-medium size 1	130
Banana meat stew		
Banana fish stew	plastic plate- level	650
Legumes		
Beans/kidney beans	bone bowl-full	400
	plastic bowl-full	325
	iron bowl-full	245
	serving spoon	135
	table spoon	32
	partioned plate-small part-full	150
Green peas	bone bowl-full	388
	plastic bowl-full	332
	iron bowl-full	294
	serving spoon	109
	table spoon	29

Time	Food/drinks	Amount
	partioned plate-small part-full	123
Kunde	bone bowl-full	388
	plastic bowl-full	332
	iron bowl-full	294
	serving spoon	109
	table spoon	29
	partioned plate-small part-full	123
Choroko	bone bowl-full	388
	plastic bowl-full	332
	iron bowl-full	294
	serving spoon	109
	table spoon	29
	partioned plate-small part-full	123
Fruits		
Mango	Small	163.5
	Large	450
Watermelon	Small	1170
Tomato	large size	124
	medium size	73
	small size	55
Cucumber	medium size	230
Avocado	medium size	285
Ripe banana	medium size	108.6
Orange	medium size	118
Carrot	medium size	44
Egg plant	medium size	176
Okra	raw (1)	18
	cooked (1)	22
African egg plant	raw (1)	25
	cooked (1)	27
Apple	normal size	175
Onion	Medium	40
Mixed fruits salad	plastic plate/bone bowl	500/450
Meat/Fish/Poutry/		
Meat-beef/pork	large piece	35
	medium piece	20
	small piece	10
Chicken		
Fish	small piece-sold 2000/=	140
Dagaa	small plastic cup/iron bowl (sold 500/=)	57
Snacks/Vitafunywa		

Time	Food/drinks	Amount
Sausage		
Bread(white/brown)		
Cakes		
Maandazi		
Vitumbua vya mchele		
Chapati		
Samosas		

RESEARCH OUTPUTS

(i) Publications

Memba, L. J., Mtei, K., Pasape, L., & Kassim, N. (2021). Fluoride contamination of selected food crops, domestic water, and milk consumed by communities around mount Meru in Northern Tanzania. *Food Additives & Contaminants: Part B*, 14(2), 81-90.

Joseph, L., Mtei, K., Pasape, L., & Kassim, N. (2019). Community awareness on fluoride contaminant sources: A case study at Mount Meru slopes in Northern. *Tanzania.International Journal of Bioscience*, 2019, 254-265.

(ii) Poster presentation