



Groundwater quality and its health impact: An assessment of dental fluorosis in rural inhabitants of the Main Ethiopian Rift

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ARTICLE INFO

Article history:

Received 23 October 2011

Accepted 6 March 2012

Available online xxxx

Keywords:

Fluoride

Dental fluorosis

Thylstrup and Fejerskov index

Milk consumption

Main Ethiopian Rift

ABSTRACT

This study aims to assess the link between fluoride content in groundwater and its impact on dental health in rural communities of the Ethiopian Rift. A total of 148 water samples were collected from two drainage basins within the Main Ethiopian Rift (MER). In the Ziway–Shala basin in particular, wells had high fluoride levels (mean: 9.4 ± 10.5 mg/L; range: 1.1 to 68 mg/L), with 48 of 50 exceeding the WHO drinking water guideline limit of 1.5 mg/L. Total average daily intake of fluoride from drinking groundwater (calculated per weight unit) was also found to be six times higher than the No-Observed-Adverse-Effects-Level (NOAEL) value of 0.06 mg/kg/day. The highest fluoride levels were found in highly-alkaline (pH of 7 to 8.9) groundwater characterized by high salinity; high concentrations of sodium (Na^+), bicarbonate (HCO_3^-), and silica (SiO_2); and low concentrations of calcium (Ca^{2+}). A progressive Ca^{2+} decrease along the groundwater flow path is associated with an increase of fluoride in the groundwater. The groundwater quality problem is also coupled with the presence of other toxic elements, such as arsenic (As) and uranium (U). The health impact of fluoride was evaluated based on clinical examination of dental fluorosis (DF) among local residents using the Thylstrup and Fejerskov index (TFI). In total, 200 rural inhabitants between the ages of 7 and 40 years old using water from 12 wells of fluoride range of 7.8–18 mg/L were examined. Signs of DF (TF score of ≥ 1) were observed in all individuals. Most of the teeth (52%) recorded TF scores of 5 and 6, followed by TF scores of 3 and 4 (30%), and 8.4% had TF scores of 7 or higher. Sixty percent of the teeth exhibited loss of the outermost enamel. Within the range of fluoride contents, we did not find any correlation between fluoride content and DF. Finally, preliminary data suggest that milk intake has contributed to reducing the severity of DF. The study highlights the apparent positive role of milk on DF, and emphasizes the importance of nutrition in management efforts to mitigate DF in the MER and other parts of the world.

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1. Introduction

Previous studies suggest that up to 8 million people living in the Ethiopian Rift Valley (primarily in the Main Ethiopian Rift, or MER) are at risk from regular exposure to high levels of naturally occurring fluoride in the groundwater they consume (Ayenew, 1998; Gizaw, 1996; Gossa, 2006; Rango et al., 2009, 2010a, 2010b; Reimann et al., 2003). The high fluoride concentrations in water are linked to the geology of the MER; which is composed of young volcanic materials and fluvio-lacustrine sediments that release several toxic elements into

the environment including fluoride. Previous studies have also provided evidence of high prevalence of dental and skeletal fluorosis in the region (Gossa, 2006; Kloos and Tekle-Haimanot, 1999; Tekle-Haimanot et al., 1987).

Optimum fluoride intake plays an essential role in the development of tooth enamel, but excessive fluoride consumption interferes with the normal formation of tooth enamel and bones (Erdal and Buchanan, 2005; Fejerskov et al., 1994). The WHO standard for fluoride in drinking water is 1.5 mg/L (WHO, 2006); the recommended level to achieve maximum protection from dental caries is considered to be 0.5–1 mg/L (Dissanayaka, 1991; WHO, 2002). Fluoride exposure above the guideline level during enamel formation may increase risk of dental fluorosis (DF) (Dissanayaka, 1991; Grobler et al., 2001). It is generally assumed that the principal source of fluoride intake is drinking water, but other sources, such as fluoride-rich beverages and agricultural products and foods prepared with fluoride-rich

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water, could also be significant source of exposure (Kaseva, 2006; Malinowska et al., 2008; Mandinic et al., 2009; Martinez-Mier et al., 2003; Viswanathan et al., 2009, 2010). Several recent studies in African countries, including Tanzania, Sudan, and Nigeria, have found a high prevalence of DF even among populations that consume drinking water with relatively low fluoride content (<0.5 mg/L) (El-Nadeef and Honkala, 1998; Ibrahim et al., 1995; Van Palenstein Helderman et al., 1997). This was partially attributed to fluoride intake from dietary sources, such as the consumption of tea (Opinya et al., 1991) and the use of fluoride-containing trona (Awadia et al., 2000; Mabelya et al., 1997).

In contrast, increased intake of calcium may reduce the severity of fluorosis, by interfering with the rate of fluoride absorption. Some foods, such as milk, are known to be an excellent source of calcium, and have been found to diminish fluoride availability in the gastrointestinal tract by 20% to 50% (Ekstrand and Ehrnebo, 1979; Spak et al., 1983; Trautner and Sibert, 1986; Whitford, 1996). Milk is also rich in fats, which increase the lag time of food and beverages in the stomach (Trautner and Sibert, 1986; Whitford, 1996). Animal studies have further elucidated the relationships between calcium bioavailability and DF, revealing that calcium plays a key role in the formation of enamel by increasing the secretion of amelogenin, which neutralizes proton obstruction during the growth phase of enamel (Bronckers et al., 2006, 2009; Chen et al., 2009). Chen et al. (1997) reported that the occurrence of fluorosis among milk-consuming children was lower than that of non-milk-consuming children. Similarly, studies in India have shown an association between vitamin D and nutritional deficiencies and some of the clinical features of fluorosis (Misra et al., 1992), and have documented reductions in DF among populations receiving calcium and vitamin D supplements (Gupta et al., 1994).

Thus, while it is generally thought that the prevalence and severity of DF increases with higher fluoride intake (Fejerskov et al., 1977; Thylstrup and Fejerskov, 1978), it seems that other nutritional factors may also play an important role. This hypothesis is supported by several studies from the field. For example, they do not appear to be significant differences in the prevalence and severity of DF among inhabitants of northern parts of Tanzania, where drinking-water fluoride concentrations are low (0.2 mg/L), and those among residents from neighboring areas with drinking water exposures of 3.6 mg/L (Awadia et al., 1999, 2000; Thylstrup and Fejerskov, 1978; Van Palenstein Helderman et al., 1997; Yoder et al., 1998).

The precise threshold for fluoride concentration and DF risk has not been established (Sohn et al., 2009). For the US, an approximate population threshold for severe DF was evaluated at 2 mg/L (Selwitz et al., 1998), while other studies in Ethiopia have not shown evidences for a clear threshold (Haimanot et al., 1987). Differences in the thresholds could be, at least in part, due to differences in susceptibility to fluoride exposure, and country-/population-specific varying amounts and sources of drinking water and other dietary sources of fluoride (Acharya, 2005; Chandrashekar and Anuradha, 2004; EPA, 2006).

Dental fluorosis has several stages. At first, the teeth become chalky and opaque as a result of subsurface hypomineralization. As DF progresses, the teeth lose enamel and increasingly develop pits and grooves. The severity of DF was first measured using the four-level Dean's index (DI), developed in 1934 (Dean, 1934). Subsequently, the Thylstrup and Fejerskov index (TFI) was created to allow for an extended range of scores from '0' to '9' (Thylstrup and Fejerskov, 1978). For high fluoride exposures (for example when groundwater concentrations exceed 5 mg/L of fluoride), TFI is a better choice than DI because of additional sensitivity in the measurement of DF severity (Fejerskov et al., 1988; Rozier, 1994).

It is likely that different types of teeth have different sensitivity to fluoride exposure: more sensitive permanent maxillary central incisors have the highest risk period during the first three years of life, while for other teeth which appear to be less sensitive and the period

at highest risk is at 6–8 years (Franzman et al., 2006; Levy et al., 2002). However, the severity of DF depends not only on the tooth-specific critical periods for tooth development; cumulative exposure throughout the entire maturation stage is also important in determining the extent of DF (DenBesten, 1999).

Here in this study we hypothesize that long-term consumption of fluoride-rich groundwater results in DF and a dose-response relationship may differ for the fluoride content of drinking water and certain dietary patterns (such as cow milk consumption) may notably influence a severity of DF. In order test this hypothesis we conducted a systematic study that evaluates the relationships between the groundwater quality in rural areas of the MER and the DF. Based on numerous studies (see review in Vengosh, 2004) we assume that the groundwater quality is stable over time and fluoride fluctuations are negligible. The main objectives of the study are (1) to evaluate the occurrence of contaminants, with emphasis on fluoride, in groundwater that is used as the principal water supply for drinking and cooking by the rural population of the MER; (2) to identify and map high-fluoride "hot spots" that present high health risks and to determine the prevalence and severity of DF among populations of these areas using the TFI; and (3) to investigate the role of dietary factors (i.e., milk consumption) in the severity of DF observed among individuals exposed to high levels of fluoride in their drinking water.

2. Study area and regional setting

The study area comprises two large basins; the Ziway–Shala and Abaya–Chamo basins, and a small catchment (Awasa) located in the central sector of the Main Ethiopian Rift (MER) valley. The MER is characterized by a chain of lakes (Ziway–Langano–Abijata–Shala–Awasa–Abaya–Chamo) that lie at an average altitude of 1600 m above sea level (m.a.s.l.). These lakes receive surface inflow from rivers and springs that drain western and eastern highlands (elevation above 2500 m.a.s.l. on average) bordering the MER.

The climatic conditions characterizing the highlands, the escarpment, and the Rift valley differ greatly. Mean annual rainfall in the highlands ranges from about 800 mm to over 2400 mm, while the Rift valley is semi-arid to arid, with rainfall varying from 300 mm to 800 mm (Ethiopian Mapping Authority, 1988). The mean annual temperature in the highlands is less than 15 °C and evaporation less than 1000 mm; on the Rift floor, mean temperature is greater than 20 °C and evaporation exceeds 2500 mm (Le Turdu et al., 1999). Rainfall in the Rift is concentrated during the summer months from June to September, with additional modest rains coming from March to May. During the long, dry period between October and February, water availability is low. As evapotranspiration significantly exceeds rainfall, water quality in the Rift valley, particularly in its lakes, is negatively affected by evaporative enrichment, which increases concentrations of fluoride and other naturally occurring elements.

Despite widespread awareness of the fluoride problem among local water agencies in the MER, rural communities still rely primarily on groundwater wells for drinking and domestic uses. This is largely due to a lack of economic development and infrastructure that provides affordable fluoride-free water supply alternatives. In addition, groundwater is also used by the region's small-scale agro-industries, commercial irrigation, and floriculture farms. In contrast, most of the major towns in the MER use treated water from the rivers and high-discharge fresh water springs that emerge within the rift and along the rift margin.

3. Materials and methods

3.1. Water sampling and analysis

Two field studies were carried out in April–May 2010 and March 2011, during which a total of 148 water samples were collected

from various sources (112 groundwater wells, 8 cold springs, 19 geo-thermal springs, and 9 lakes; Fig. 1). The groundwater samples were collected typically from active pumping wells, allowing the water to flow for a few minutes from the sources prior to sampling. Water from springs and lakes was collected at the mouth of the source and 50–100 m away from the shore, respectively. Fifty samples were collected in the Ziway–Shala basin during the first field study, and 49 were taken in the Abaya–Chamo basin. Subsequently, 13 of the original groundwater wells in Ziway–Shala basin were resampled during a second field visit that was also organized for the purpose of conducting DF examinations.

Concentrations of major cations of calcium (Ca^{2+}), magnesium (Mg^{2+}), sodium (Na^{2+}), and silica (SiO_2) were measured using a direct-current plasma spectrometer (DCP) calibrated using solutions prepared from plasma-grade single-element standards. Major anions of chloride (Cl^-), sulfate (SO_4^{2-}), and nitrate (NO_3^-) were analyzed using an ion chromatograph (IC). Fluoride content was determined by ion-selective electrode (ISE). Samples were mixed at a 1:1 volume

ratio with a total ionic strength adjustment buffer (TISAB) of pH 5–5.5, which allows optimum analyses of fluoride in the aqueous solution. Alkalinity (as HCO_3^-) was measured using titration techniques to pH 4.5. Trace elements (As, U, B, Mo and V) were analyzed via a Perkin-Elmer Elan 5000 inductively coupled plasma-mass spectrometer (ICP-MS) calibrated to the National Institute of Standards and Technology (NIST) 1643e standard.

3.2. Survey questionnaire and examination of dental fluorosis

A total of 73 randomly selected inhabitants consuming well water ($n=50$) in the Ziway–Shala basin were interviewed during the first field visit. During these interviews, data were collected on subject gender, age, ethnicity, tobacco use, amount and source of drinking water consumption, dietary patterns, (in particular related to the frequency of milk consumption), and severity of DF. DF severity was identified based on visual interpretation of individuals' teeth (confirmed by digital images).

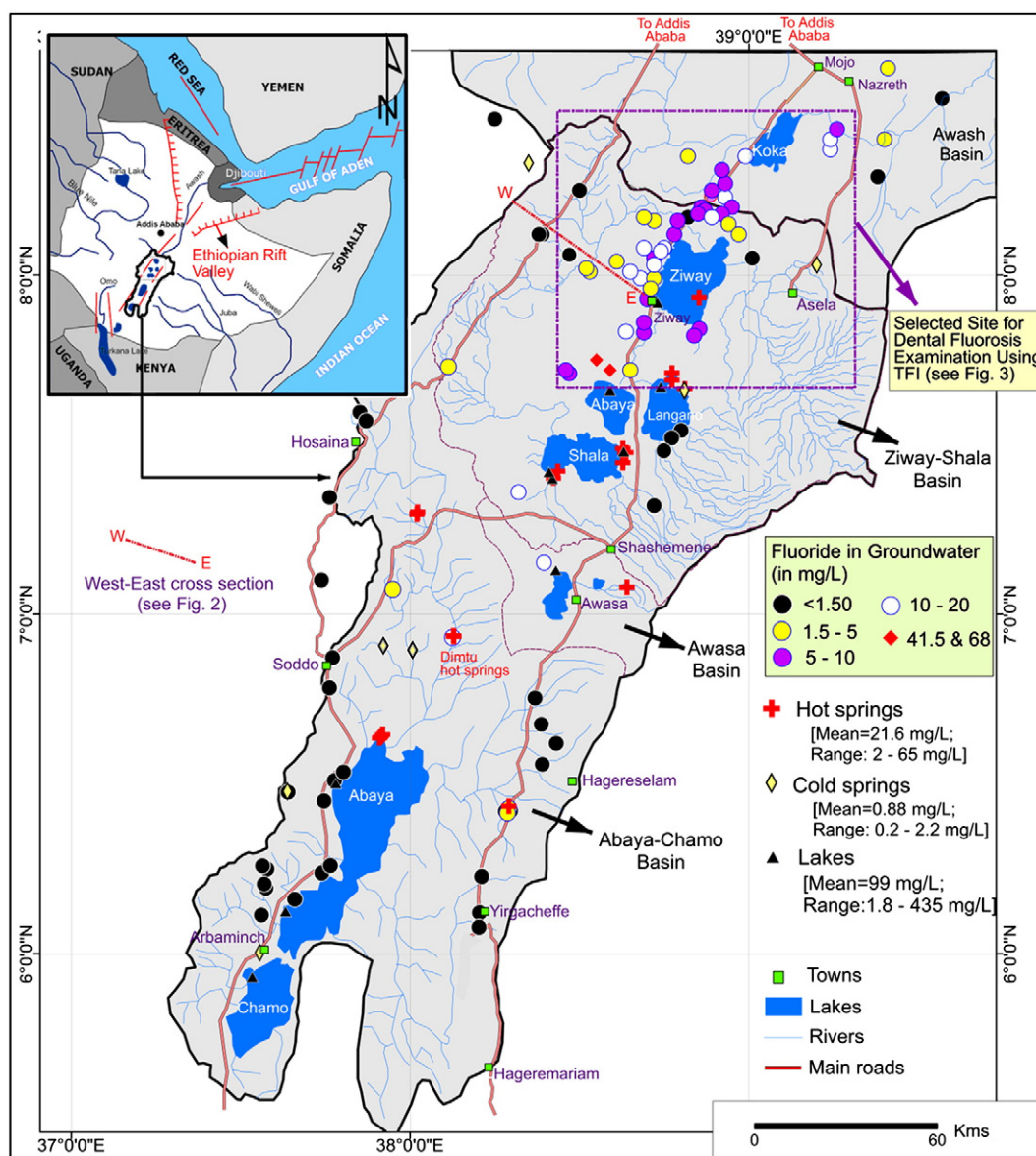


Fig. 1. Distribution and identification of water sampling sites in this study according to type (groundwater, hot and cold springs, and lakes) and range of fluoride levels. Groundwater concentrations are color-coded. Note that the squared black represents the site selected for examination of DF. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

Subsequently, rural villages in and around the localities of Wonji, Alemtena, Meki, and Ziway, were targeted for follow-up investigation during the second study visit (Fig. 1). The selected communities were characterized by use of high-fluoride groundwater sources (7.8–18 mg/L; $n = 12$) and indications of high DF (based on results obtained during the first survey). A 13th groundwater well with relatively low fluoride (1.1 mg/L) was also included in this second survey visit for the purpose of comparison. During these revisits, a second set of water samples was collected in order to confirm the stability of fluoride concentrations measured during the first visit; fluoride levels in the two field samplings were found to be highly correlated ($R^2 = 0.98$). At the same time, survey data were collected for 200 individuals born and raised near the 13 selected wells. These data included all information collected during the first set of surveys plus additional measurements of height, weight and TFI-based measures of DF.

All dental examinations were conducted at convenient locations such as well sites, local community centers, and schools. After cleaning and drying the vestibular (buccal) surfaces of each study participant's teeth with sterile gauze, DF was evaluated in natural light using the TFI (based on Thylstrup and Fejerskov, 1978). A TFI score of '0' indicates that the tooth enamel has normal translucency (absence of fluorosis). Increasing values of the index denote an increase in the severity of fluorosis; scores of '1' to '4' correspond to increasing degrees of opacity with no loss of enamel, and scores of '5' or more denote increasing degrees of enamel loss (pitting of teeth).

Only buccal surfaces were examined, as prevalence studies have shown that no extra information is gained by including other surfaces (Thylstrup and Fejerskov, 1978). To improve the quality of the TFI field examination, each study participant was examined by two independent and qualified experts. The two experts carried out a preliminary discussion and TFI scoring calibration exercises prior to the actual examination.

Teeth with cavities or any sign of dental caries were excluded from the examination and marked accordingly. Overall, a total of 5226 teeth were classified by the examiners, and the assigned scores were then discussed and compared to obtain the final recorded measurements. The reliability of the TF scores was later reassessed using photos (of both maxillary and mandibular teeth); this quality control measure yielded scores generally consistent with those of the field examiners ($R^2 = 0.7$). Generally, the scores based on interpretation of photos were lower than those given in the field, probably due to the lower visibility of teeth in the photos.

The survey questionnaire design and study were conducted after ethical approval (Protocol No. A0045) by the Duke University Institutional Review Board (IRB). Additional permission to carry out the survey was also obtained from the Addis Ababa University and local institutes in the studied region (schools, water bureaus, hospitals) after an explanation of the objectives and the method of study. The anonymity of all investigated subjects has been maintained.

3.3. Statistics

A spreadsheet-based statistical package (Microsoft Excel 2010 and IBM SPSS Statistics 19) was used for data collection and analysis. Bivariate analyses were performed using *t*-tests. A multivariate regression analysis was conducted using Stata SE version 11 to further consider the associations between fluoride concentration in drinking water, milk consumption, BMI and socio-economic variables, on the one hand, and the severity of dental damage (with the TFI value as a main outcome), on the other.

4. Results and discussion

4.1. Water quality

The data generated in this study confirm previous reports of high concentrations of fluoride in groundwater, predominantly within the basin of the MER, i.e. the lowlands

of the surveyed basins (Fig. 1). The highest fluoride concentrations measured for different types of waters in the basin were: 435 mg/L in Lake Abijata; 68 mg/L for groundwater sampled from a well north of Lake Abijata; and 65 mg/L at a hot spring east of Lake Shala. All sampled cold springs in the basins had much lower fluoride concentrations (0.2 to 2.2 mg/L).

As this study is mainly focused on the effect of fluoride-rich groundwater on dental tissue, emphasis is given to the detailed distribution of fluoride concentrations in the local groundwater. The data show wide variations in fluoride content across the studied basins; wells in the Abaya–Chamo basin in the southern MER had lower fluoride levels than wells in the Ziway–Shala basin in the north (Fig. 1). All groundwater wells in Abaya–Chamo had fluoride concentrations below 3 mg/L (mean: 0.7 ± 0.87 mg/L) except one (13 mg/L). The inhabitants of the Abaya–Chamo basin also consume surface water from low-fluoride rivers and cold springs originating in the highlands. However, a few communities in this area have no access to groundwater resources or have malfunctioning wells. For example, the residents of Dimitu village utilize high-fluoride hot springs (13 mg/L; $n = 2$) for drinking, cooking, and other household activities (Fig. 1). Likewise Lake Awasa (fluoride level of 8.3 mg/L) is used by nearby communities for domestic purposes.

Most high-fluoride zones within our sampling frame were found in the Ziway–Shala basin in the central part of the MER, with fluoride concentrations ranging from 1.1 to 68 mg/L (mean: 9.4 ± 10.5 mg/L). In this area, 48 of the 50 (96%) sampled wells were found to have fluoride concentrations exceeding the guideline value of 1.5 mg/L for drinking water recommended by the World Health Organization (WHO). In addition, the data reveal that a large fraction of the wells in basin have concentrations of arsenic (As) (27 wells, 54%), uranium (U) (29 wells, 62%), boron (B) (10 wells, 20%), and molybdenum (Mo) (4 wells, 8%) exceeding the WHO drinking water guideline values of 10, 15, 500, and 70 $\mu\text{g/L}$, respectively. The As concentrations in groundwater ranged from 0.58 to 190 $\mu\text{g/L}$ (mean 20.4 ± 33.5 $\mu\text{g/L}$), B ranged from 14.8 to 2100 $\mu\text{g/L}$ (mean 310 ± 353 $\mu\text{g/L}$), U ranged from 0.06 to 69 $\mu\text{g/L}$ (mean 10.4 ± 14.3 $\mu\text{g/L}$), and Mo ranged from 1.53 to 128 $\mu\text{g/L}$ (mean 24.3 ± 30.2 $\mu\text{g/L}$). Overall, about 45% of the wells in the Ziway–Shala basin exceeded the WHO recommended limits for combined fluoride and arsenic. These results suggest that groundwater quality problems extend to naturally-occurring contaminants other than fluoride.

4.2. Groundwater geochemistry: mobilization of major elements and relationships with fluoride abundance

The MER is composed of pyroclastic volcanic materials and reworked fluvio-lacustrine sediments (Fig. 2a). Previous studies have suggested that dissolution of glass phases of these volcanic materials releases fluoride to the groundwater system (Rango et al., 2009). This glass dissolution produces water with high alkalinity (pH up to 8.9) and high sodium (Na^+), bicarbonate (HCO_3^-), and silica (SiO_2) content, with the latter ranging from 57 to 111 mg/L (mean $= 86 \pm 11.3$ mg/L; $n = 50$). The rise of Na^+ concentration and salinity is associated with a progressive decrease of Ca^{2+} content (Fig. 2b). This relationship is explained by a process of cation-exchange that is associated with weathered clay-rich volcanic rocks, in which Ca^{2+} uptake by the aquifer matrix is balanced by the release of Na^+ into groundwater.

The results presented in this study thus confirm previous findings that fluoride content in groundwater is positively correlated with salinity, Na^+ , and HCO_3^- (Rango et al., 2009). Fluoride levels in groundwater are also negatively correlated with calcium concentrations (Fig. 2b,c). In fact, the groundwater sample with the highest measured fluoride level in this study (68.5 mg/L) also had the lowest Ca^{2+} content (0.93 mg/L). Furthermore, solubility measurements indicate that low Ca^{2+} concentrations in the water limit the precipitation of fluoride in the form of calcium fluoride (CaF_2) (mean saturation index: -0.4 ± 0.5 ; $n = 48$); fluoride therefore mobilizes readily and becomes a stable soluble species in Ca-depleted groundwater. The gradual decrease of Ca^{2+} is associated with an increase of the overall salinity and fluoride contents along a cross-section through the Rift Valley.

4.3. Socio-demographic information, dietary patterns, and tobacco use

Table 1 presents the water quality characteristics for the samples taken from the 13 wells in the Ziway–Shala basin selected for the DF examinations. The specific geographic locations of these wells are shown in Fig. 3. Demographic data for the 200 individuals living near these wells and examined for signs of DF are summarized in Table 2. The investigated population was equally represented by males and females, with a mean age of 13.6 ± 5 years old (93.5% are 7 to 20 years old). The three dominant ethnicities in the sample are Oromo (62%), Kembata (19%), and Amhara (10%). The predominant ethnic group in nine of the sites is Oromo; the other locations are characterized by a mix of two or more ethnic groups. This is especially the case in the communities of the Wonji Shoa Sugar Estate (WSSE), which are comprised of diverse ethnicities (Kembata, Amhara, Oromo and Hadya).

The socio-economic status and dietary habits of individuals in the study area were similar. The diet is primarily cereal-based (maize, teff and wheat), with occasional intake of vegetables and fruits. Meat is rarely consumed: the average frequency of meat intake (from cows and other sources) is about 11 times per year.

Most of the Oromo practice farming, and many also raise livestock. As a result, 37.5% of the investigated individuals regularly consume milk, with an average frequency of 5.6 times per week (ranging from 2 to 7 times per week), while the rest of the investigated individuals have no intake of milk.

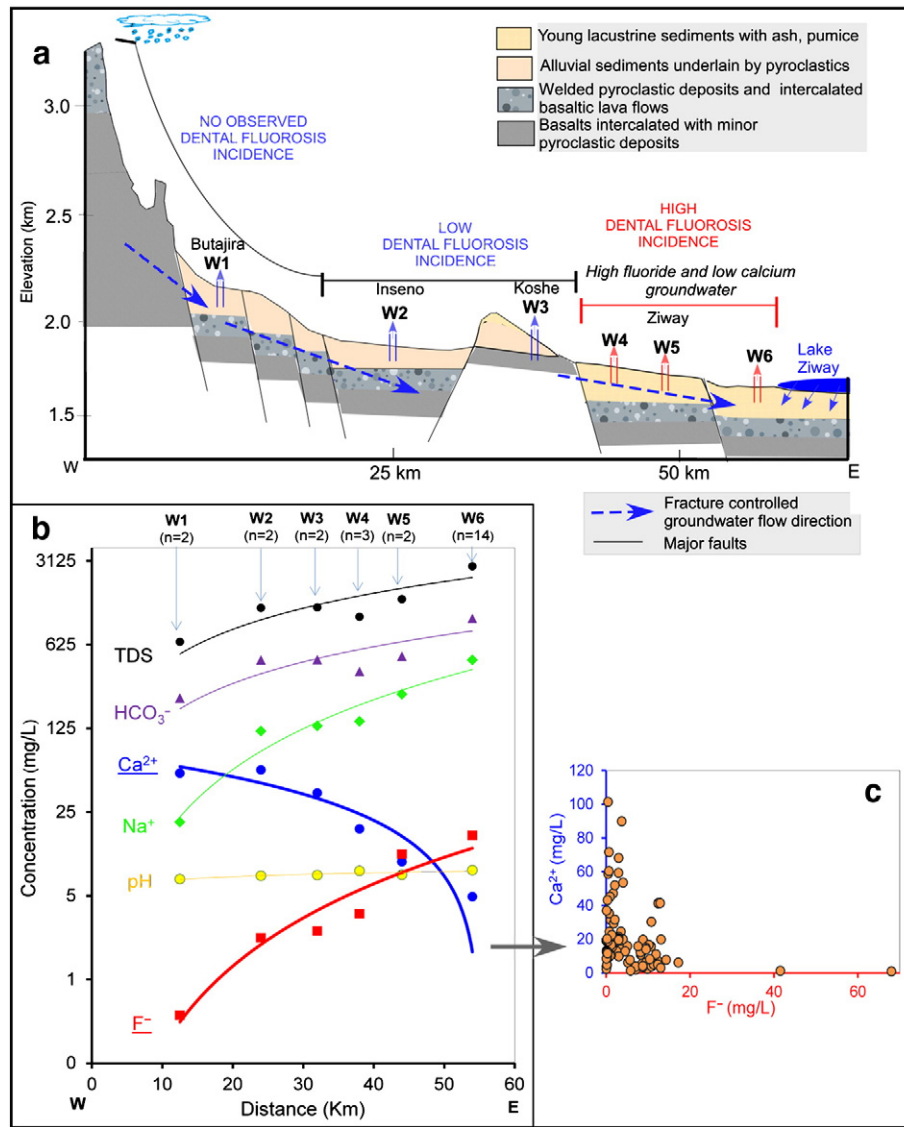


Fig. 2. a) Geological and topographic cross-sections (west of Lake Ziway) with zones of DF occurrences, b) changing groundwater chemistry as a function of distance from escarpment to the rift, and c) inverse relationship between Ca^{2+} and F^- in sampled groundwater wells.

Table 1

Concentration of fluoride, major ions (represented as EC and TDS) and trace elements (As, U, B, Mo, and V) in high-salinity groundwater wells where examinations for DF were conducted. In addition, the elemental concentrations of the wells were compared with WHO standards, where nm: not mentioned (WHO, 2006).

Village name	Salinity		Fluoride F^- mg/L	Calcium Ca^{2+}	Silica SiO_2	Trace elements				
	EC	TDS				As	U	B	Mo	V
	$\mu\text{S}/\text{cm}$	mg/L				$\mu\text{g}/\text{L}$				
Oda	470	480	1.1	31.4	57	2.6	1.7	32	1.5	0.7
Tejitu	943	891	7.8	11.0	84	5.6	1.7	202	10.0	9.1
Berta site	1041	–	8.0	2.5	–	4.9	1.2	223	9.2	5.2
Tuchigabriel	2847	2705	8.7	4.1	76	42.0	68.6	797	58.1	26.7
Aneno	1148	1025	8.8	3.4	85	22.8	4.1	294	12.5	38.9
Wonji-camp 3	1268	1161	9.7	15.0	90	3.2	0.6	557	27.8	0.4
Tuchigrabona	1940	1761	10.7	15.6	74	15.1	32.8	253	10.6	2.7
Wulumbula	1984	1868	10.8	3.9	82	73.4	26.6	606	19.5	71.3
Woyogabriel	1784	1759	11.3	5.0	85	44.7	18.9	524	8.4	63.9
Wonji-camp 7	1409	1229	13.0	41.2	84	10.3	2.0	497	46.5	0.8
Wegea	2397	1832	13.2	3.0	86	67.0	35.6	512	94.0	81.2
Wonji-camp 9	1301	1215	13.3	41.3	84	12.3	2.2	573	49.3	0.7
Chelelki	1850	1786	18.0	6.2	94	55.1	25.5	650	17.9	46.0
Mean \pm SD	1568	1476	10 ± 4	14 ± 14.5	82 ± 9.6	28 ± 25.6	17 ± 20.5	440 ± 219	28 ± 26.7	27 ± 30
WHO drinking water quality standards			1.5	nm	nm	10	15	500	70	nm
Number of wells exceeding the standards (in %)			92%	–	–	69%	46%	54%	7.7%	–

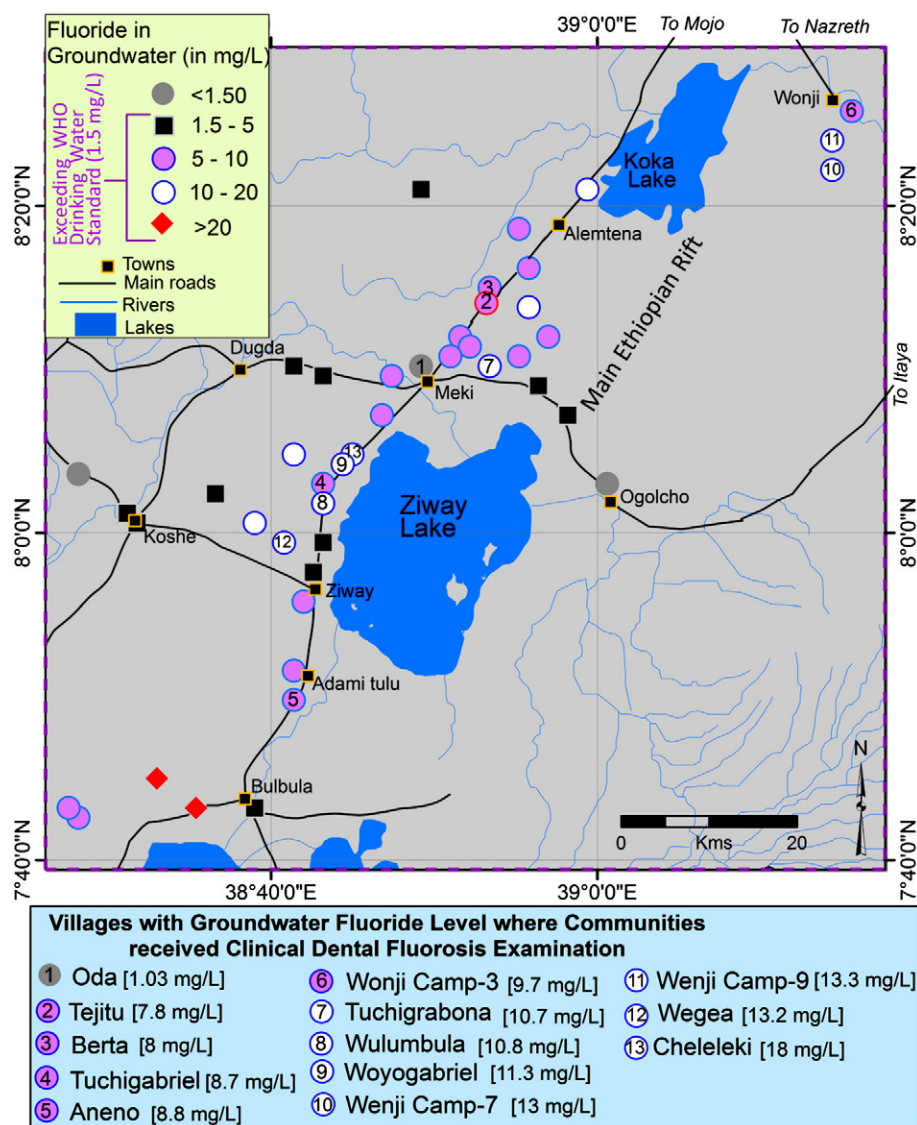


Fig. 3. Location and fluoride concentrations in wells selected for DF analysis using the Thylstrup and Fejerskov Index (TFI).

None of the individuals examined in the sample smoke tobacco, which rules out the option of fluoride intake through tobacco consumption documented in some studies (Sengupta and Pal, 1971).

4.4. Fluoride intake through drinking groundwater

Total daily fluid intake through drinking water was evaluated taking into account the individuals' body weight (bw). The data from the survey indicated that each inhabitant consumes between 0.5 and 3 L of water per day from groundwater sources (on average 1.2 L of water per day). These values were used to calculate the amount of daily fluoride intake among four age groups (Table 3a). The results were compared with the No-Observed-Adverse-Effects-Level (NOAEL) value for fluoride (0.06 mg/kg bw/day) published by US EPA (2002), as well as the "optimal" range of fluoride intake for children, which is considered to be 0.05–0.07 mg/kg bw/day (Casarin et al., 2007; Ketley and Lennon, 2001).

The comparison of the measured values with the NOAEL value of 0.06 mg/kg bw/day shows that individuals' fluoride exposure is on average six times higher than the NOAEL value. In terms of per-person exposure from drinking water sources, the residents in the high-fluoride area are exposed to fluoride concentrations ranging from 4 to 54 mg/person/day (Table 3b). These levels are much higher than the required total fluoride intake levels (0.2 mg/person/day in infants and 5.0 mg/person/day in adults) proposed by Murray (1986).

Table 3c also shows the calculated body mass indices (BMI, expressed as weight (in kg)/height (in m²)) by age group. The BMI values range from 11.1 to 26.6 kg/m² and increase with the examined individuals' age.

4.5. Prevalence and severity of dental fluorosis

The prevalence of DF (TF score of ≥ 1) on maxillary and mandibular teeth among inhabitants consuming water from the 12 wells with high fluoride levels was 100%. More than half of the teeth examined (51.7%) had TF scores of 5 and 6, followed by 29.7% with TF scores of 3 and 4, 10.2% with TF scores of 1 and 2, 7.6% with a TF score of 7, and 0.8% with TF scores of 8 and 9 (see Fig. 4 for the frequency distribution of TF scores for maxillary teeth). The maxillary teeth show a higher frequency of severe DF (TF score of ≥ 5 ; 67.7% vs. 52.2% for mandibular teeth, $p < 0.05$), whereas the mandibular teeth show high frequency of mild to moderate DF (TF score of ≤ 4 ; 47.7%). In these high-fluoride areas, it was observed that even partially erupted permanent teeth appeared to be fully chalky white (TF score of 4), followed by more severe cases of pitting and discoloration, and finally loss of enamel (TF score of ≥ 5). Our data show that even the low fluoride site (Oda; fluoride level of 1.1 mg/L), more than 60% of maxillary teeth and about 35% of mandibular teeth had signs of DF (TF scores ranging from 1 to 3).

The frequencies of TF scores (i.e., severity of DF) do not seem to vary directly with drinking-water fluoride levels. The fluoride impact is predominantly reflected in TF scores of 5 and 6 in all investigated wells with fluoride levels greater than 7.8 mg/L. The lack of a linear dose–response relationship at the fluoride range of 7.8–18 mg/L has important implications for the need to establish a threshold level of fluoride at which DF is minimal (such as a TF score of less than 4) for the Rift valley population in Ethiopia. This threshold could be used as the fluoride level goal to which defluoridation efforts may be targeted.

In accordance with their mineralization and age of eruption, all examined teeth were categorized as either early-erupting (i.e., incisors and first molars) or late-

Table 2

Demographic characteristics of the individuals who participated in the DF examinations. N = number of individuals, n = number of counts, S.D. – standard deviation.

Villages	F ⁻	Age groups		Age	Gender		Water intake/person/day (in liter)			Milk access	
	(mg/L)	(n)		(years)	(n)					(n)	
		7–20	20–40	Mean ± S.D.	M	F	0.5–1	> 1–2	Mean ± S.D.	Yes	No
Oda (N = 5)	1.1	4	1	16 ± 6.2	4	1	2	3	1.6 ± 0.5	3	2
Tejitu (N = 10)	7.8	9	1	15 ± 4.8	4	6	10	0	1 ± 0	2	8
Berta (N = 19)	8	19	0	12.9 ± 1.9	8	11	16	3	1.1 ± 0.3	9	10
Tuchigabriel (N = 11)	8.7	10	1	13 ± 4.9	4	7	7	4	1.23 ± 0.56	1	10
Aneno (N = 11)	8.8	11	0	11.3 ± 3.5	8	3	7	4	1.23 ± 0.56	7	4
Wonji-3 (N = 10)	9.7	10	0	12.4 ± 3.4	5	5	9	1	1 ± 0.41	3	7
Tuchigrabona (N = 16)	10.7	16	0	12.4 ± 3.5	8	8	13	3	1.13 ± 0.51	4	12
Wulumbula (N = 10)	10.8	10	0	13.4 ± 3.2	4	6	9	1	1.1 ± 0.32	8	2
Woyogabriel (N = 21)	11.3	20	1	12.1 ± 3.9	7	14	16	5	1.14 ± 0.42	10	11
Wonji-7 (N = 11)	13	11	0	14.3 ± 3.3	2	9	8	2	1.14 ± 0.4	3	8
Wegea (N = 18)	13.2	18	0	12.8 ± 2.1	9	9	15	3	1.1 ± 0.2	13	5
Wonji-9 (N = 43)	13.3	34	9	16 ± 8	29	14	26	17	1.35 ± 0.68	10	33
Chelelki (N = 15)	18	15	0	12.5 ± 2.7	6	9	10	5	1.33 ± 0.65	2	13
Total	–	187	13	–	98	102	122	34	–	75	125

Table 3

Summary of daily fluoride exposure through drinking groundwater expressed in terms of body weight and per person.

Age group	Fluoride intake								BMI c) in kg/m ²
	a) in mg/kg bw/day				b) in mg/person/day				
	Min	Max	Mean ± SD	N	Min	Max	Mean ± SD	N	
7 to 10	0.15	1.07	0.41 ± 0.21	50	4.4	26.6	11.0 ± 4.9	50	16.2 ± 2.4
11 to 15	0.10	0.93	0.34 ± 0.16	101	4.0	36.0	13.3 ± 6.3	103	18.0 ± 2.7
16 to 20	0.09	1.02	0.32 ± 0.20	30	4.8	54.0	17.5 ± 10.5	30	19.2 ± 2.8
> 20	0.13	0.60	0.37 ± 0.15	12	7.8	39.9	21.9 ± 9.2	12	19.6 ± 3.3

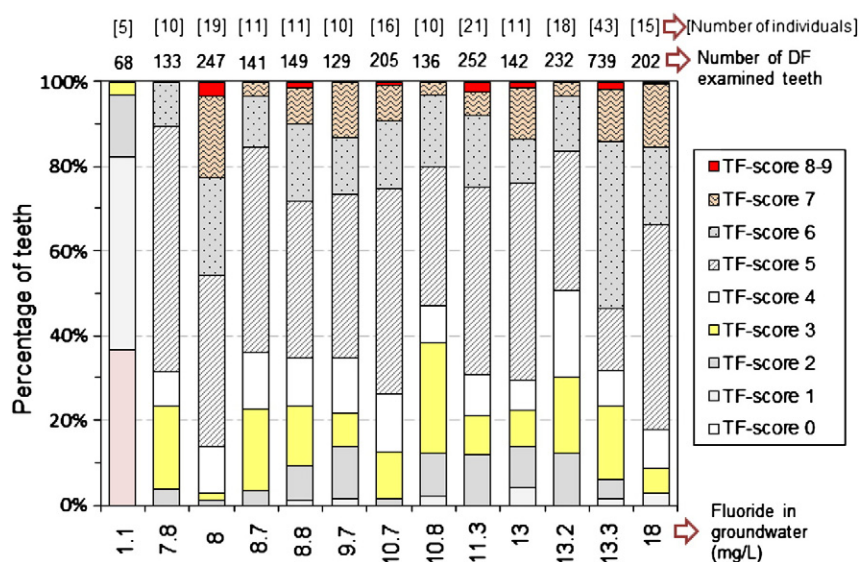
**Fig. 4.** Percentage distribution of TF-scores in examined individuals' maxillary teeth at each of the 13 sampled wells.

Table 4
Mean TF-scores of each tooth type and group of teeth (early, late and all teeth) for all DF examined individuals (n = 195) in high fluoride groundwater villages. Note: S.D. – standard deviation; † – significant difference between maxillary and mandibular teeth, (* – p < 0.05 and ** – p > 0.05).

	Teeth types					Teeth groups		
	Incisors*	Canines**	Premolars*	First molars**	Second molars*	Early erupting teeth*	Late erupting teeth**	All teeth*
Maxillary TF-scores [mean ± S.D.]	4.6 ± 0.56†	4.2 ± 0.53	4.6 ± 0.34†	5.5 ± 0.46	5.3 ± 0.33†	4.9 ± 0.46†	4.6 ± 0.37	4.7 ± 0.37†
Mandibular TF-scores [mean ± S.D.]	3.8 ± 0.47	4.1 ± 0.48	4.4 ± 0.4	5.6 ± 0.5	5.6 ± 0.51	4.3 ± 0.41	4.5 ± 0.39	4.4 ± 0.35

erupting (i.e., canines, premolars, and second molars). The severity of fluorosis for categories of teeth was expressed as the mean TF score, with TF score of ≥ 5 categorized as severe fluorosis. Severity of DF was significantly higher in maxillary teeth (relative to mandibular ones), with average TF scores of 4.66 ± 1.24 and 4.41 ± 1.2 , respectively (p < 0.001, N = 200). The incisors of the maxillary teeth were also more severely damaged than the mandibular (p < 0.001).

DF in early-erupting teeth was also more severe in maxillary than in mandibular teeth (see Table 4). First molars and second molars were more affected than other

teeth types (p < 0.05). These results are broadly consistent with findings of previous studies on DF (Latham and Grech, 1967; Manji et al., 1986; Thylstrup and Fejerskov, 1978).

4.6. The effect of milk consumption on severity of dental fluorosis

To study the associations between dietary calcium intake and DF, the frequency of regular cow's milk consumption per week was evaluated in individuals with and

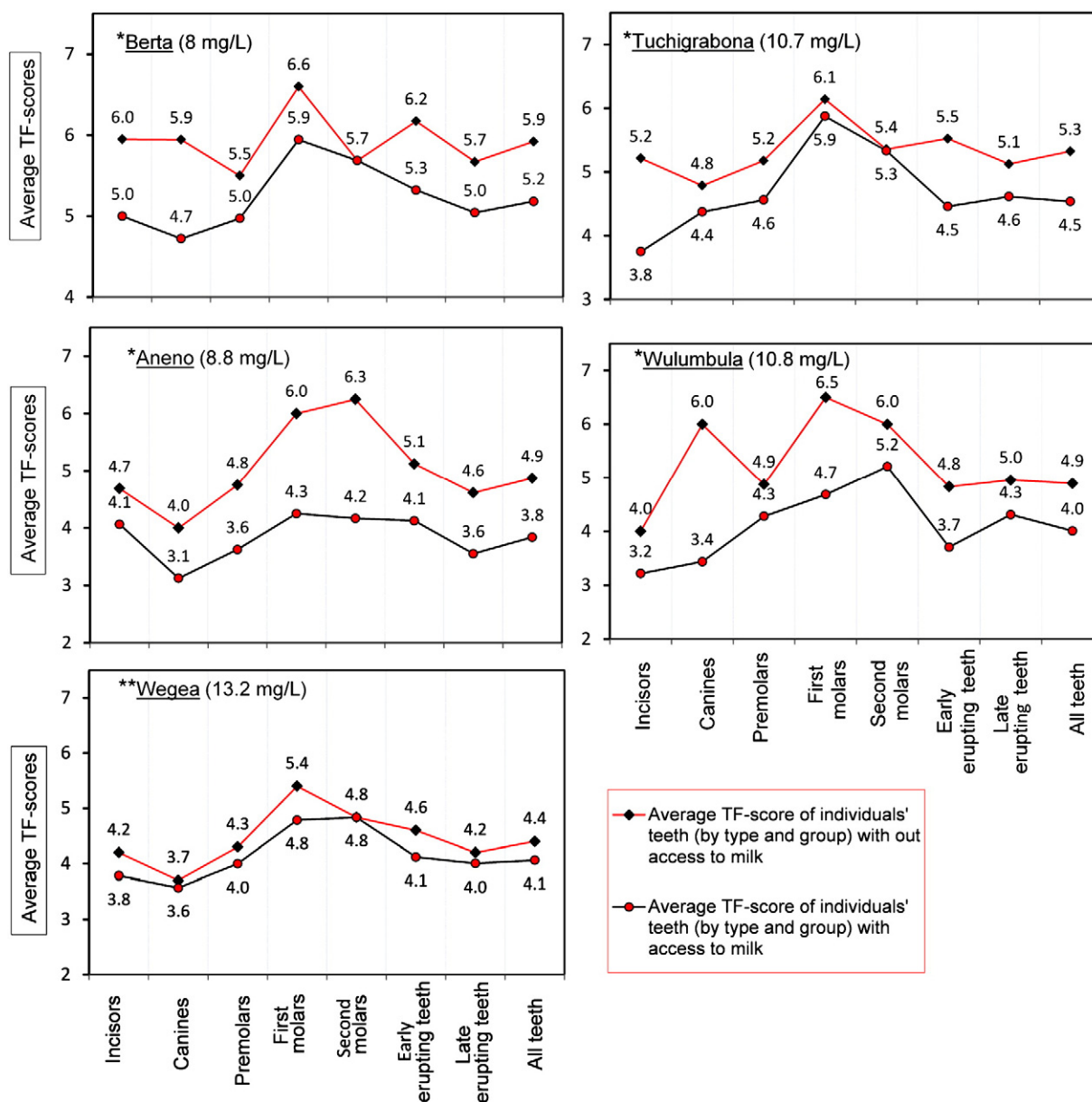


Fig. 5. Line diagram displaying higher average TF scores on individuals' who have no access to milk (red line) versus those with access to milk (black line) at 5 selected villages. Considering all the teeth of individuals in the 5-villages, an estimated 15% average reduction in the dental fluorosis is observed. Note that: similar ages were considered in the comparison. (* – p < 0.05 and ** – p > 0.05) are statistical significance of the comparison. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

without access to milk. Five villages at Berta, Aneno, Tuchigrabona, Wulumbula, and Wegea were selected because of the relatively higher availability of milk. The percentage of individuals who have access to milk at these villages was 47% at Berta, 64% at Aneno, 25% at Tuchigrabona, 80% at Wulumbula, and 72% at Wegea. In these villages, TF scores (except at Wegea, which is not statistically significant) were higher for those who did not consume milk (Fig. 5). This analysis suggests that milk consumption, which provides calcium, may reduce the effects of fluoride toxicity.

4.7. Multivariate analysis of associations between fluoride level and dental fluorosis

The association of fluoride levels with TF scores was further explored using multivariate regression analysis controlling for other factors including age, gender, ethnicity, and other water quality measures, BMI, and milk consumption (Table 5). Estimates were obtained for a limited number of samples; however, it was not possible to adjust for a number of potential confounding factors to fluoride exposures such as from diet.

Several alternative specifications suggest that there is a positive association between fluoride content in drinking water and TF scores (columns a–c). The models (column a and c) suggest that higher fluoride concentration in drinking water is associated with more severe DF. An individual's maximum TF score is somewhat more strongly correlated with fluoride levels than his/her average TF scores (column b). However these positive associations are due only to the differences in TF scores between individuals using the well with low fluoride compared to all other high fluoride wells. Excluding the low-exposure community, the data show that there is no significant association between average TF score and fluoride (column d), which is consistent with the lack of dose–response function found in the analyses described previously.

The models also reveal negative associations between age and TF score, and positive associations between the BMI and TF score, but these effects are inconsistent across models and require further investigation, with larger samples and additional nutritional data and controls. Interestingly, a positive association was found between the BMI and TF score ($p < 0.05$). This phenomenon was observed in both females and males (the estimate value 0.004 ± 0.019 , $p < 0.05$, and 0.009 ± 0.004 , $p < 0.05$, respectively). This may be partly explained by the fact that BMI is highly correlated with age and, consequently, to the prolonged exposure to fluoride from drinking water (see Table 3), as well as increased consumption of certain foods which accumulate fluoride.

To further investigate the correlations between milk consumption and TF scores, multivariate analysis controlling for milk consumption was also conducted (Table 5, column e). A dummy variable for any milk consumption is strongly negatively associated with average TF score, reducing it by 3.7 points ($p = 0.016$). The effect of increasing fluoride consumption can however be seen in the positive coefficient for the interaction between the dummy for milk consumption and the fluoride concentration ($+0.36$, $p = 0.015$). This suggests that the protective effect of milk may decrease as fluoride concentrations increase. One may also interpret this interaction as indicating the effect of increasing fluoride concentration in groundwater, which by itself is insignificant in this model. The interaction of fluoride levels and the frequency of milk consumption is weakly and negatively associated with TF score ($p = 0.092$), suggesting that higher levels of milk consumption may be protective when exposure to fluoride levels increases. It should be noted that these results are generally consistent across outcome measures (average vs. maximum TF scores) and also when applied to data from restricted age groups (results not shown), though statistical power in the restricted number of samples is limited. Also, as shown in, these results do not change qualitatively with exclusion of the low fluoride community (column f).

5. Integration

As shown above, this study found widely varying fluoride concentrations across the groundwater, lakes, and springs (hot and cold) of the MER region. These variations appear to be driven by the complex geology and hydrology of the MER. Since groundwater is the principal source of drinking water for the rural communities of the MER region, long-term ingestion of high-fluoride groundwater through drinking and cooking has caused widespread DF problems in some locations. The literature shows that fluoride intake depends on the amount of water consumed, which is partly influenced by climatic conditions; it is particularly high in semi-arid and arid climatic conditions. Taking account of the annual average maximum air temperatures in the MER (which exceed 20°C), the optimal and maximum allowable concentrations of fluoride in drinking water should be 0.9 mg/L and 1.8 mg/L, respectively, according to guidelines issued by the USPHS (US Public Health Service, 1962). Yet the results in this study indicate that fluoride in MER drinking water is significantly higher than both these and the WHO guideline levels, resulting in exposures between 4 and 54 mg/person/day (Table 3).

This study's examination of DF among populations consuming water from high-fluoride groundwater in the MER region revealed 100% prevalence of fluorosis, most of it severe (TF scores of 5 and 6). These results are consistent with previous studies in Ethiopia (Wondwossen et al., 2004). About 60% of all teeth examined in the present study had TF scores of ≥ 5 , indicating the fracture and loss of enamel, cosmetic defects and causing pain as well that can have a significant negative impact on quality of life. DF also adversely affects food choices and chewing efficiency. Field observations have indicated that a few children remove the brown, stained enamel from their front teeth (incisors) by scraping with hard glasses, which further negatively affects the strength of the teeth. Mottling of teeth also has psychological effects; some children are reluctant to speak and smile. Other studies have shown that children with high fluorosis frequency have diminished learning ability (Chen et al., 2008; Trivedi et al., 2007; Zhao et al., 1996).

It is generally assumed that drinking water is the predominant source of total daily fluoride intake, but exposure also depends on the relative amounts of fluoride in food sources. Symptoms of fluorosis have been reported in places where fluoride concentrations are below the WHO upper limit for drinking water sources (Brouwer et al., 1988). Our study also shows mild forms of fluorosis (TF scores mostly ≤ 2) in villages utilizing groundwater with relatively low

Table 5
Regression analysis of associations between TF scores and water chemistry, and milk consumption and BMI.

Outcome Variable	a: Basic model		b: Basic model		c: Other elements		d: Excluding low fluoride community		e: Milk		f: Milk, excluding low fluoride community	
	Average TF score		Max TF score		Average TF score		Average TF score		Average TF score		Average TF score	
	Coef.	St. err.	Coef.	St. err.	Coef.	St. err.	Coef.	St. err.	Coef.	St. err.	Coef.	St. err.
Fluoride	0.097***	0.037	0.11***	0.040	0.14***	0.041	−0.004	0.026	−0.04	0.097	−0.037	0.11
Fluoride milk									0.36*	0.15	0.32*	0.18
Calcium					−0.0163**	0.0069			−0.0081	0.0066	0.0008	0.0059
Age	−0.023	0.02	−0.064***	0.021			−0.014	0.020	−0.12	0.079	−0.049	0.11
Gender	−0.28*	−0.17	0.022	0.18			−0.19	0.16	−0.27*	0.16	−0.21	0.15
BMI	0.092***	0.030	0.046	0.033			0.089***	0.029	0.099***	0.030	0.093***	0.029
Milk consumption									−3.7**	1.5	−3.2*	1.8
Frequency of milk consumption (daysmilk)									0.45	0.30	0.47*	0.28
Fluoride daysmilk									−0.048	0.028	−0.048*	0.028
Constant	2.37***	0.71	5.15***	0.71	4.03***	0.49	3.51***	0.633	4.07***	1.20	3.98***	1.31
N	199		199		200		194		199		194	
R ²	0.131		0.156		0.092		0.070		0.195		0.087	

*** Significant at the 1% level.

** Significant at the 5% level.

* Significant at the 10% level.

fluoride levels (1.1 mg/L). This suggests that fluoride intake may also come from food sources. In this region, the diet is mostly composed of cereals such as maize, teff, and wheat; consumption of dairy products (such as cow's milk) and meat is limited. This limited, cereal-based diet includes little dietary calcium and vitamin D, which may partly limit the negative impacts of high fluoride consumption. Indeed, BMI values among study subjects indicated that some were at risk of malnutrition, which is plausibly indicative of a low-protein and vitamin-deficient diet that potentially aggravates the fluorosis problem. Similarly, Rugg-Gunn et al. (1997) reported a high prevalence of dental fluorosis in children suffering from malnutrition.

The high fluoride levels found in the drinking water are also associated with high salinity (TDS), as well as high levels of silicon (Si), uranium (U), arsenic (As), vanadium (V), molybdenum (Mo), and boron (B). These other contaminants may aggravate fluoride toxicity, and potentially increase the risk of kidney failure, as documented in studies in India (Dissanayake et al., 2010; Reddy, 1985). Other studies (Lantz et al., 1987; Xiong et al., 2007) have also shown that fluoride intoxication can be damaging to kidneys, as high fluoride intake by itself leads to changes in kidney structure, function, and metabolism, which inhibit efficient removal of fluoride in the urine and further aggravate the fluoride problem. Findings on combined effects of arsenic and fluoride are contradictory and most are based on animal models (Chouhan and Flora, 2010; Yao and Wang, 1988). While no direct deposit of arsenic has been found in dentine of rats, some researchers hypothesize that the toxicity of fluoride may be increased due to arsenic's role in damaging normal kidney function (Chouhan and Flora, 2010; Kavr, 1986).

6. Conclusions

This study integrated geochemical data of groundwater resources with a detailed investigation of fluorosis patterns in rural communities of the MER Valley, Ethiopia. The results show that the distribution of fluoride (7.8–18 mg/L) in the local groundwater is associated with high levels of fluorosis. The ingestion of fluoride from drinking water by the local population is far above the WHO drinking guideline (1.5 mg/L) and the NOAEL value (0.06 mg/kg bw/day). The effect of this consumption is manifest in the very high observed prevalence of advanced dental fluorosis (DF), as shown by our clinical examination of the teeth of 200 individuals (using the TFI) consuming water from high fluoride wells. The prevalence of dental fluorosis (TF score of ≥ 1) on both the maxillary and mandibular teeth of those who consume groundwater from the high-fluoride wells in our survey was 100%. Most of the teeth examined, 51.7%, had TF scores of 5 and 6, followed by 29.7% with TF scores of 3 and 4, 10.2% with TF scores of 1 and 2, 7.6% with a TF score of 7, and 0.8% with TF scores of 8 and 9. Of all examined teeth, 60% had a TF score of ≥ 5 (loss of outermost enamel), which is indicative of severe DF; 40% of them could be characterized as mild to moderate DF (various levels of chalky white appearance without loss of enamel).

In our sample, no direct correlation was found between the TFI scores and the actual fluoride concentrations in our groundwater samples (which ranged from 7.8 to 18 mg/L). At these fluoride concentrations, and without controlling for possible confounding factors such as diet, we cannot establish a dose–response relationship. Further work is therefore necessary to establish the critical fluoride level at which DF is minimal, and to evaluate the mechanisms that control DF occurrence in the rural communities of the MER. Some indications of the effect of diet on DF were shown in this study, as subjects who consume cow's milk appeared to have reduced severity of DF. The lack of significant DF variations along a fluoride range of 7.8 to 18 mg/L in drinking water implies that mitigation attempts based on only water treatment may not be sufficient given the partial removal of fluoride in water treatment. Future mitigation attempts should also

consider changes in the diet and nutrition, and possibly increased accessibility to milk, particularly for children.

Acknowledgments

The authors would like to express sincere gratitude to the Duke Global Health Institute and the Nicholas Institute for Environmental Policy Solution at Duke University for supporting the study. Special thanks are extended to the children, parents, school authorities, and water bureaus in the Rift Valley of Ethiopia for their kindly welcome and active participation during the field mission. We thank Dr. Gary Dwyer, Laura Raul, and Nathaniel Warner from the Division of Earth and Ocean Sciences, Nicholas School of Environment, Duke University for laboratory analytical assistance of water samples. We also thank the anonymous reviewers for providing valuable comments that improved the manuscript.

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