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Factors influencing the relationship between fluoride in drinking water and dental fluorosis: a ten-year systematic review and meta-analysis

M. H. Akuno, G. Nocella, E. P. Milia and L. Gutierrez

ABSTRACT

The relationship between naturally fluoridated groundwater and dental fluorosis has received large attention from researchers around the world. Despite recognition that several factors influence this relationship, there is a lack of systematic studies analyzing the heterogeneity of these results. To fill such a gap, this study performs a systematic review and meta-analysis to understand which factors influence this relationship and how. Selected studies were sampled between 2007 and 2017 from Web of Science, PubMed, Google Scholar and Scopus using keywords and Boolean operators. Results of the systematic review show that dental fluorosis affects individuals of all ages, with the highest prevalence below 11, while the impact of other factors (gender, environmental conditions, diet and dental caries) was inconclusive. Meta-regression analysis, based on information collected through systematic review, indicates that both fluoride in drinking water and temperature influence dental fluorosis significantly and that these studies might be affected by publication bias. Findings show that fluoride negatively affects people's health in less developed countries. The conclusions discuss policy tools and technological innovations that could reduce fluoride levels below that of the WHO (<1.5 mg/L).

Key words | children, contaminated drinking water, dental fluorosis, fluoride, meta-analysis, systematic review

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INTRODUCTION

Fluorine ranks 13th in the world for abundance and constitutes 0.08% of the earth's crust (Sananda & Biplab 2016). Fluorine, a natural element, is easily soluble in water, soil and air, and being one of the most reactive chemical elements, does not exist on its own in the natural environment but rather as fluoride. Fluoride represents compounds that have ion F⁻ (Pitzer 1975). Surface water is normally low in fluoride, with values lower than (1.5 mg/L), while groundwater can contain higher concentrations of

fluoride depending on geological conditions (Sananda & Biplab 2016). Starting from the first water fluoridation studies in the USA in the late 1940s, fluoride has had a tremendous impact on the oral health of millions of adults and children (Blinkhorn & Mekertichian 2013). The reduction in dental caries ranged from 20% to 40% (Downer & Blinkhorn 2007). However, epidemiological evidence suggests that higher fluoride concentrations in drinking water above (1.5 mg/L) increases the risk of dental fluorosis, while progressively higher concentrations lead to increasing the risks of skeletal fluorosis (World Health Organization (WHO) 2011). Skeletal fluorosis is a slow, progressive, crippling condition, such as bone fracture resulting in health

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complications with overlapping manifestations in several other diseases (Gopalan *et al.* 2008). In early stages, dental fluorosis in humans is mainly identified through dental defects (Dhar & Bhatnagar 2009). Other conditions and diseases associated with excess fluoride intake include paralysis, respiratory complications and low blood pressure (Andezhath & Gosh 1999), while after chronic exposure, symptoms such as weight loss, anorexia, anemia and cachexia are very common (Ibrahim *et al.* 2011). In most circumstances, dental fluorosis occurs during tooth formation and influences dental enamel formation or mineralization and its structure (Blinkhorn & Mekertichian 2013). It can result in esthetic and functional problems depending on the severity of the condition (Sananda & Biplab 2016).

Dental and skeletal fluorosis has now become a global problem, with more than 200 million individuals in nearly 25 countries ostensibly affected (WHO 2011). Fluorosis, as well as being affected by climate conditions, is also said to be caused by beverage, food and livelihood habits (e.g. brick tea, tobacco, 'magadi' use in the Rift Valley and burning coal) (Galan 1957; United Nations Children's Fund (UNICEF) 1999; Kaseva 2006; Mandinic 2009; Malde 2011; Rango *et al.* 2012; O'Mullane *et al.* 2016). According to WHO (2011) and Mohanta & Mohanty (2018), drinking water containing high concentrations of fluoride is one of the main sources of fluorosis.

For this study, we evaluated the impact on dental fluorosis from naturally fluoridated water while controlling for other possible factors related to this condition. Epidemiological studies that analyze the relationship between intentional water fluoridation and dental fluorosis are not the focus of this paper. We believe that it is useful to keep these two phenomena separated because of their diverse causes and different policies to reduce the risk of dental fluorosis.

Regarding the factors that may influence dental fluorosis, we investigated the influence of age on the severity of dental fluorosis in children exposed to drinking water, as well as other factors such as local temperature, rainfall and altitude indicated as important factors in determining the total concentration of fluoride in drinking water. Higher temperatures can increase the level of water intake above that usually consumed (Galan 1957; Firempong 2013). On the other hand, the use of collected rainwater

can reduce the use of unsafe groundwater (Susheela 1999; Edmunds & Smedley 2005). Furthermore, it has been reported (Manji *et al.* 1986; Rwenyonyi *et al.* 1999) that altitude causes an increase in the prevalence and severity of dental fluorosis.

To understand the relationships between naturally fluoridated water and fluorosis, we proposed to answer the following research questions:

- What is the relationship between naturally occurring water fluoride concentrations and the prevalence of dental fluorosis?
- Is this relationship the same in different geographic areas of the world where, according to the systematic review, this disease is more prevalent?
- What are the impacts of other factors, such as age, climate and altitude on the prevalence of dental fluorosis?

The remainder of this article is organized as follows. The methodology section will explain both the criteria used to select studies for the systematic review and the statistical approach used to perform the meta-analysis. The results section will discuss the qualitative and quantitative findings of the systematic review and meta-analysis, respectively. Finally, in the conclusions, we will discuss possible policy implications that emerged from the study.

METHODS

It is widely recognized that systematic reviews and meta-analyses have become more important in many disciplines because they allow researchers and practitioners to be up to date on advances in the field, to identify research questions based on the results obtained in different contexts and to justify grants for further research (Cooper 1998; Littell 2006). To answer the research questions presented above and to achieve the objective of this study, we conducted both a systematic review and a meta-analysis. A systematic review is a process that uses systematic and explicit methods to identify, select and critically appraise relevant research. It collects and analyzes data from the studies included in the review (Moher *et al.* 2009; Gough *et al.* 2012; Mallett *et al.* 2012). The well-defined process of inclusion and exclusion of suitable articles is based on the identification of *ad hoc*

criteria and promotes a confident approach to analyzing data not only qualitatively but also quantitatively bringing to fore empirically robust results via meta-analysis.

Search strategy and identification criteria

We searched the Web of Science (www.webofknowledge.com), PubMed (www.ncbi.nlm.nih.gov/pubmed), Google Scholar (scholar.google.it) and Scopus (www.scopus.com) databases for publications spanning ten years and dated between 2007 and 2017. The following terms were searched using the Boolean operators ‘and’ and ‘or’: ‘dental fluorosis’, ‘fluorosis’, ‘Dean Index’, ‘Thylstrup and Fejerskov Index’ and ‘drinking water’. This search focused only on human beings and papers published in the English language. During the systematic review, we detected papers written in Chinese, Spanish and Arabic languages. We do not consider these articles following the suggestions of Cochrane Handbook, Higgins & Green (2011). They noted that the extent and effects of language bias have diminished because of the shift toward the publication of studies in English. Same results are reported in Morrison *et al.* (2012).

The motive for including the Dean and Thylstrup-Fejerskov indexes in the Boolean search is linked to the fact that many studies reporting information about dental fluorosis widely used the two indexes in their analyses. The choice of the proposed time span was motivated by the fact that fluoride concentration and variation can be related to climate, rainfall and temperature, and thus enlarging the time-period can introduce not only additional heterogeneity, but also information that does not necessarily reflect the current situation related to climatic changes. Table 1 shows the criteria that were used to select studies included in this systematic review and meta-analysis. One of the most important criteria for the initial selection of studies was the evaluation of the relationship between natural fluoride content in drinking water and the prevalence of dental fluorosis.

Sampling of studies and data extraction

Figure 1 shows that initially, a total of 2,580 peer-reviewed papers were identified from the four databases as follows: Web of Science (970), Scopus (883), Pub Med (577) and Google Scholar (149), and one study was obtained by

Table 1 | Inclusion and exclusion criteria

Inclusion criteria of studies	Exclusion criteria of studies
<ul style="list-style-type: none"> Articles in the English language. Articles focusing on humans. Empirical, theoretical, review, clinical, academic papers and reports providing information from all countries around the world. Articles that focused on connections between natural fluoride content in drinking water and prevalence of dental fluorosis. Articles that focused on population that are long-term inhabitants. Articles providing information on fluoride content. Articles providing information on the sample size. Articles providing information on the number of people affected by fluorosis. Articles providing information on the age of participants. Articles published between April 2007 and May 2017 	<ul style="list-style-type: none"> Articles in other languages other than English. Duplicated articles. Misclassified articles. Articles on intentional water fluoridation. Articles on water de-fluoridation technologies. Articles that focused on animal dental fluorosis both livestock and laboratory research animals. Articles providing information on dental fluorosis caused by fluoride from soil and air. Articles providing information on dental fluorosis caused by industrial pollution. Articles providing information on dental fluorosis linked to other health issues e.g. children IQ, cancer, etc.

requesting it from the author. Information gathered at this stage on all articles was stored in Endnote. The first major elimination of duplicated copies was carried out on Endnote, where 796 studies were eliminated. For the remaining stages, two reviewers (MA and LG) analyzed the remaining 1,784 studies and independently applied the inclusion and exclusion criteria as indicated in Table 1. Disagreements were resolved through discussion and consensus with a third author (GN). At the end of the screening stage, 39 articles were included in this study. All 39 articles underwent a content check and qualified for systematic review. However, only 21 of the 39 articles qualified for meta-analysis (see Table 2), since 18 articles did not contain information on key variables, such as fluoride level in drinking water or the number of people affected by dental fluorosis.

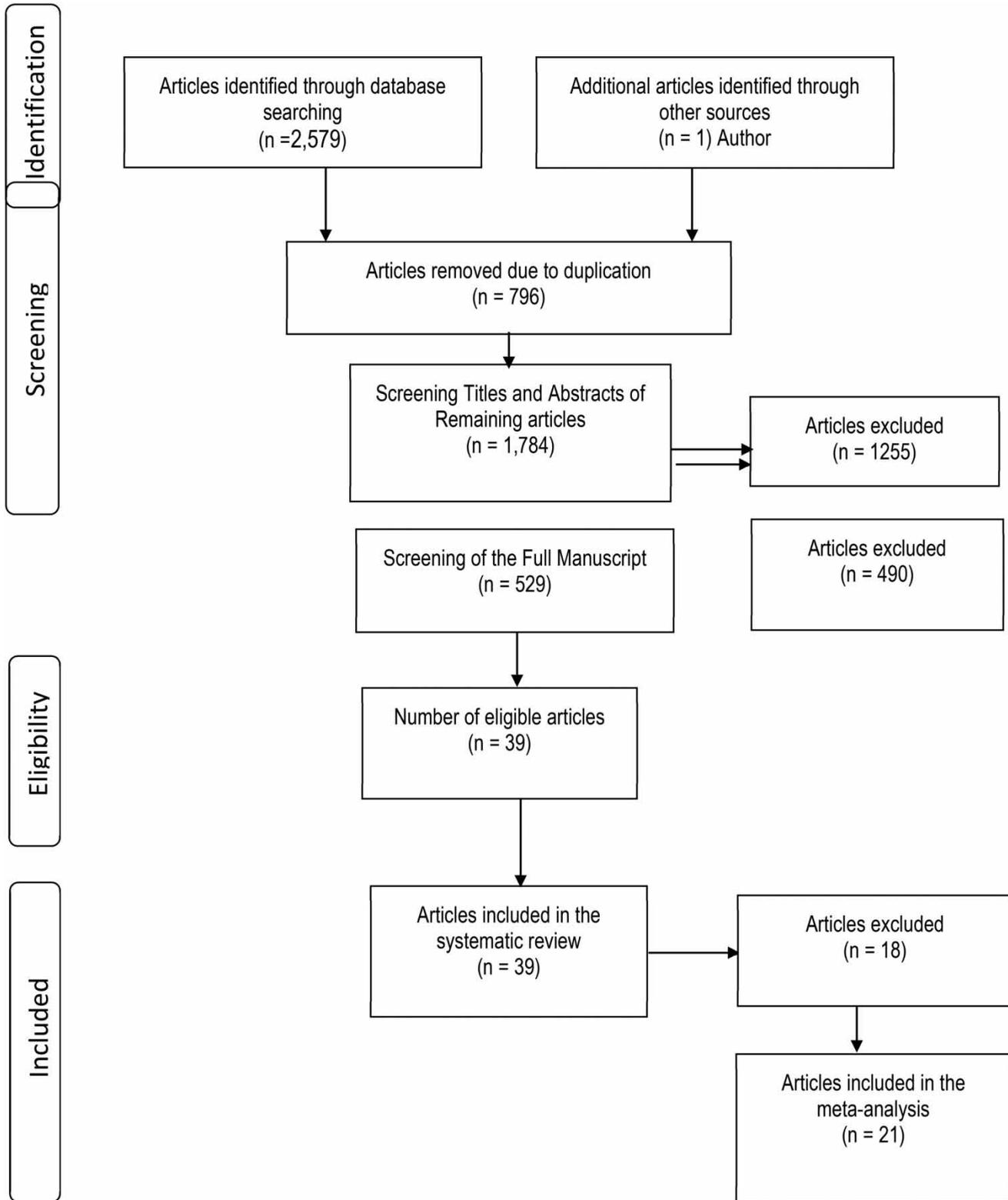


Figure 1 | Flow diagram on the identification of eligible studies to final inclusion.

Table 2 | Summary of the characteristics of studies included in the meta-analysis

N	Author	Continent	Country	Zone	No of Subjects	No of people affected by fluorosis	Avg. Prevalence rate	Avg. Age (years)	Avg. Fluoride Content (mg/L)	H-index Journal	Citations per year	Excessive Temperature ratio	Altitude (m)	Rainfall (mm)
1	Almerich-Silla <i>et al.</i> (2008)	Africa	Algeria	Tindouf, South Algeria, Refugee camp	572	206	36.01%	8.5	1.905	77	3.778	1.4005	400	55.0
2	Arif <i>et al.</i> (2013)	Asia	India	Didwana Tehsil, Nagaur district	1136	788	69.37%	28.0	3.608	11	4.250	1.2483	342	434.0
3	Bagh <i>et al.</i> (2012)	Asia	India	Birbhum district	54,546	5468	10.02%	26.0	2.900	5	0.000	1.2496	60	373.0
4	Bhalla <i>et al.</i> (2015)	Asia	India	Kampur city, Uttar Pradesh	1343	243	18.09%	12.0	2.250	0	0.500	1.2041	48	886.0
5	Firempong <i>et al.</i> (2013)	Africa	Ghana	Bongo district	200	126	63.00%	15.0	1.543	11	4.750	1.1178	0	1118.0
6	Vilasrao <i>et al.</i> (2014)	Asia	India	Chhattisgarh State	3390	725	21.39%	12.0	2.000	0	2.000	1.2756	330	1319.0
7	Ruan <i>et al.</i> (2007)	Asia	China	Haoping, Shaanxi Province	596	419	70.30%	10.0	1.017	53	1.200	1.6768	103	591.0
8	Isaac <i>et al.</i> (2009)	Asia	India	Kaiwara, Karnataka State	416	100	24.04%	10.0	2.082	12	1.250	1.1698	919	582.0
9	Asawa <i>et al.</i> (2015)	Asia	India	Dad, Bokersal & Deotalab villages, Dungarpur District	750	395	52.67%	40.0	2.750	12	5.000	1.2235	100	701.0
10	Keçeci <i>et al.</i> (2014)	Asia	Turkey	Deregümü, Isparta	293	276	94.20%	40.0	1.830	37	0.667	1.6580	1035	555.0
11	Kotecha <i>et al.</i> (2012)	Asia	India	Vadodara district, Gujarat	6093	2960	48.58%	29.0	1.601	68	11.400	1.1870	129	1044.0
12	Garg <i>et al.</i> (2013)	Asia	India	Gurgaon district, Haryana State	650	517	79.54%	10.0	2.366	11	1.000	1.2732	229	634.0
13	Malde <i>et al.</i> (2011)	Africa	Ethiopia	Wonji Shoa Sugar Estate Nazareth, (Adama)	28	28	100.00%	3.5	6.100	227	6.167	1.0968	1540	1654.0
14	Marya <i>et al.</i> (2010)	Asia	India	Gurgaon and Hisar districts, Haryana State	3007	1636	54.41%	14.0	1.510	5	1.286	1.2682	215	734.0
15	Medina-Solis <i>et al.</i> (2008)	America	Mexico	Tula de Allende, Hidalgo State	1538	1184	76.98%	13.6	1.957	52	3.111	1.1244	2020	648.0
16	Sebastian <i>et al.</i> (2016)	Asia	India	Mysore district, Karnataka	405	169	41.73%	11.0	1.200	28	8.000	1.1564	758	567.0
17	Shitumbanuma <i>et al.</i> (2007)	Africa	Zambia	Choma district	37	37	100.00%	3.5	6.986	53	2.400	1.1710	1208	804.0

(continued)

Table 2 | continued

N	Author	Continent	Country	Zone	No of Subjects	No of people affected by fluorosis	Avg. Prevalence rate	Avg. Age (years)	Avg. Fluoride Content (mg/L)	H-Index	Citations per year	Excessive Temperature ratio	Altitude (m)	Rainfall (mm)
18	Rango <i>et al.</i> (2012)	Africa	Ethiopia	Central Main Ethiopian Rift	200	200	100.00%	13.5	10.338	137	17.600	1.0889	1600	1422.0
19	Vuhahula <i>et al.</i> (2009)	Africa	Tanzania	Rift Valley in Northern Tanzania	2912	2804	96.29%	14.7	4.600	128	2.125	1.0931	1400	837.0
20	Yadav <i>et al.</i> (2009)	Asia	India	Jhajjar District, Haryana State	9667	5052	52.26%	11.0	1.809	53	6.250	1.2720	220	600.0
21	Akosu <i>et al.</i> (2008)	Africa	Nigeria	Central Plateau	1022	124	12.13%	13.5	0.6812	82	1.778	1.1178	1280	1177.0

For the 21 articles selected for meta-analysis data of maximum and average annual temperature, annual rainfall and local altitude were additionally sourced. When not included in the studies, data were collected from <https://www.weather2visit.com/>.

Statistical analysis

Meta-analysis refers to the use of statistical techniques in a systematic review to integrate the results of included studies. Data from the 21 studies selected for the quantitative analysis showed dental fluorosis heterogeneity. We focused on random-effects meta-analysis of the dental fluorosis prevalence rate defined as the proportion of people affected by dental fluorosis. Binomial exact confidence intervals were used to contrast and summarize the results. The use of the random effect model is justified by the expectation that observed differences among the prevalence rates cannot be entirely attributed to sampling error, but also to other factors such as differences in the population under observation, publication bias etc. (Egger *et al.* 2001). Heterogeneity is quantified using the I^2 statistic that represents the percentage of total variation across all studies due to between-study heterogeneity (Higgins & Thompson 2002). Usually, an I^2 value greater than 50% indicates significant heterogeneity. We also performed an independent meta-regression analysis to estimate the contribution of different study characteristics to heterogeneity within the selected sample of studies worldwide. A p -value <0.05 was considered statistically significant, unless otherwise specified. Statistical analysis was conducted using Stata 15.0 employing the metaprop (Nyaga *et al.* 2014) and metareg (Harbord & Higgins 2008) packages.

The prevalence rate of dental fluorosis was investigated by pooling the sample of studies in subgroups given by the level of fluoride in drinking water and the age of people surveyed in the studies. With respect to the level of fluoride, the prevalence rates were sub-grouped as follows: one group for studies with values of fluoride in drinking water (<1.5 mg/L), a second group for values which range between 1.5 and 3.0 mg/L, which is the limit allowed in some countries such as Tanzania and one final group for values >3.0 mg/L. Data categorization was also conducted by subgrouping the prevalence rate by age, i.e. splitting the sample of

people surveyed into those below the age of 11, those with ages ranging from 11 to 15 and the final group with age >15. Forest plots were produced for the two categorical variables of fluoride level in drinking water and age.

Finally, following Murtaugh (2002), we propose a simple analysis for detecting possible publication bias in our meta-analysis. This method is based on the relationship between the strength of the results in published studies and different indexes representing the quality of the studies. The idea is that if the magnitude of an estimated effect influences the likelihood that a study's results will be published, authors of papers describing the studies which report a low effect size could find it difficult to submit and to have them accepted by high-quality ranking journals, and/or to receive attention in the literature. To examine this hypothesis, we regress the prevalence rate of dental fluorosis reported in our studies both over the h-index of each journal and over the yearly average number of citations of the study. These two measures were chosen because they will allow us to explore possible bias in the review process (h-index) and within the scientific community (yearly average citation number of the study). The h-index values were obtained from SCIMAGO (<https://www.scimagojr.com/>), while the average number of citations per year were sourced from Google Scholar (<https://scholar.google.it/>).

RESULTS

Systematic review results

All the studies included in this systematic review confirmed the higher prevalence of dental fluorosis with long-term consumption of elevated fluoride levels (>1.5 mg/L) in drinking water. One article confirms lower dental fluorosis prevalence rate with a lower level of fluoride in drinking water (Malde 2011). Fluoride levels in drinking water (>6 mg/L) were associated with substantially higher prevalence of all three forms of fluorosis i.e. dental, skeletal and non-skeletal fluorosis among populations under observation. On the other hand, concentration of fluoride (<6 mg/L) finds dental fluorosis to be more prevalent than skeletal and non-skeletal fluorosis. Other studies gauged confounding factors, such as climate, altitude, age, gender and dental

caries on the prevalence of dental fluorosis. These studies investigated naturally fluoridated water to explore the link between high fluoride content in water and the prevalence of dental fluorosis (Akosu & Zoakah 2008; Sudhir *et al.* 2009; Yadav *et al.* 2009; Bagh *et al.* 2012; Goodarzi *et al.* 2016).

Besides the impact of naturally occurring fluoride content on dental fluorosis, the analysis of these studies shows that this condition is influenced by several factors that have been categorized into the following five relationships:

1. Age and prevalence of dental fluorosis;
2. Gender and prevalence of dental fluorosis;
3. Temperature, rainfall and altitude and prevalence of dental fluorosis;
4. Dental caries and prevalence of dental fluorosis and
5. Other dietary intake and prevalence of dental fluorosis.

Age and prevalence of dental fluorosis

In 37 out of 39 papers selected for this systematic review, age was considered by all authors to be a key variable that is related to the prevalence of dental fluorosis. In the studies included here, dental fluorosis emanating from water use is the most endemic condition where most of the school children under observation present different stages of dental fluorosis and high levels of fluoride in urine (Shitumbanuma *et al.* 2007; Medina-Solis *et al.* 2008; Marya *et al.* 2010). Prevalence of dental fluorosis, in most studies, display a positive linear relationship with greater age. Most of these studies were conducted in schools, and the age of children does not necessarily refer explicitly to a specific age, but rather to diverse age brackets ranging from 3 to 18 years of age.

Children in the teeth and body tissue growth phase (0–5 years) are more susceptible to dental fluorosis due to tissue, bone and teeth mineralization. This situation increases the susceptibility of the enamel to many forms of attack leading to discolored or mottled teeth.

Dental fluorosis in children also seems to be more prevalent and evident in permanent teeth than in primary teeth, further suggesting that in children over ten years of age, dental fluorosis is more visible and prevalent than those below the age of eight (Shitumbanuma *et al.* 2007; Almerich-Silla *et al.* 2008; Medina-Solis *et al.* 2008; Isaac

et al. 2009; Vuhahula *et al.* 2009; Marya *et al.* 2010; Bagh *et al.* 2012; Chauhan *et al.* 2012; Kotecha 2012; Rango *et al.* 2012; Yadav 2012; Firempong *et al.* 2013; Garg & Singh 2013; Keçeci *et al.* 2014; Vilasrao *et al.* 2014; Bhalla 2015; Goodarzi *et al.* 2016; Sebastian *et al.* 2016). On the other hand, one study focused on dental fluorosis reduction or slow down by eliminating fluoride in drinking and cooking water for children (Malde *et al.* 2011). It highlights that a complete removal of fluoride from water could reduce the incidence of dental fluorosis in children by 50% (Malde *et al.* 2011). This study further recommends the investigation of other potential sources of fluoride, such as food and beverage. Interestingly, a study by Sudhir *et al.* (2009) shows only a minimal correlation between dental fluorosis and age.

For people above 18 years, both dental and skeletal fluorosis are present, accompanied by other non-skeletal conditions. In this age bracket, skeletal fluorosis is more prevalent leading to health deterioration with time, while dental fluorosis stagnates. The literature shows that those under observation between the age of 18 and 34 exhibit higher skeletal fluorosis when compared to dental fluorosis than to those below 18. This group also presents low fluoride levels in urine samples when compared to those above 35, although their urine fluoride levels are still higher than those below 18 years of age. Although the importance of the prevalence and severity of dental fluorosis are widely noted to increase with age, health deterioration in those people over 35 years of age tends to be faster toward skeletal fluorosis. In fact, several studies find that those people above 35 years of age start to present partial or total loss of teeth due to dental fluorosis. This group also returns the highest fluoride level in tested urine samples. In the oldest group of individuals (above 35 years of age), even though dental fluorosis appears to stagnate, problems such as difficulty in eating or chewing, gastrointestinal fluorosis or even death are experienced. For these people, nutritional deficiencies, bad lifestyle habits and environmental factors appear to be the common phenomena among those highly affected (Kotoky *et al.* 2008; Li *et al.* 2009; Mandinic *et al.* 2009; Yadav *et al.* 2009; Marya *et al.* 2010; Malde *et al.* 2011; Bagh *et al.* 2012; Chauhan *et al.* 2012; Yadav *et al.* 2012; Garg & Singh 2013; Tahir & Rasheed 2013; Keçeci *et al.* 2014; Asawa *et al.* 2015).

Gender and prevalence of dental fluorosis

Analysis of studies focusing on gender showed mixed results. While some studies show that this factor does not have any relationship with dental fluorosis, other studies indicate significant differences between males and females but without a clear direction. For example, some studies show that the prevalence of dental fluorosis is higher in females than males, and this might be due to poor nutrition among girls (Arvind *et al.* 2012; Chauhan *et al.* 2012; Keçeci *et al.* 2014; Asawa *et al.* 2015). On the other hand, other studies find that males are more affected by dental fluorosis than females. In males, the literature indicates that the highest prevalence of dental fluorosis is exhibited between the ages of 12 and 24. Furthermore, those males above 35 years old seem to be more affected where their condition deteriorates more rapidly compared to that of their female counterparts. In the oldest group, the male fluorosis prevalence rate is also found to be consistently higher than that of females, but these differences are not significant both for dental fluorosis and for urine samples tested in the observed population (Marya *et al.* 2010; Kotecha *et al.* 2012; Yadav *et al.* 2012; Arif *et al.* 2013; Firempong *et al.* 2013; Garg & Singh 2013; Vilasrao *et al.* 2014; Asawa *et al.* 2015; Bhalla *et al.* 2015).

The higher prevalence rate of dental fluorosis that is observed in males could be explained by the fact that males might be away from home and involved in activities that require them to consume more and different types of water. It could also be explained through fluoride retention in the body. Fluoride retention in the body can also be influenced by nutritional and climatic conditions away from home. Finally, these differences could also be explained by different biological reactions to fluoride and individual susceptibility to the duration of exposure (Li *et al.* 2009; Sudhir *et al.* 2009; Chauhan *et al.* 2012).

Temperature, rainfall, altitude and prevalence of dental fluorosis

Part of the difficulty and complexity of dealing with exposure to fluoride contamination arise from the impact of factors, such as temperature, altitude and rainfall because

they can influence the dissolution of fluoride into the water, consumption of fluoride contaminated water and body retention of fluoride. In 1984, WHO advised that in regions with warm weather, a concentration of fluoride in drinking water should be below 1 mg/L, while in cooler climates it could be up to 1.2 mg/L (Yadav *et al.* 2009; WHO 2011; Sebastian *et al.* 2016).

Studies show that average annual temperature, average maximum daily temperature, rainfall, altitude and the depth of wells are determinants and confounding factors that can influence the prevalence rate of dental fluorosis. These factors are not decisive but represent a major challenge to preventive efforts for dental fluorosis. Literature indicates that fluoride intake varies with the variation in temperatures, i.e. the higher the temperature, the higher the fluoride intake because hot conditions might trigger the higher consumption of water creating a higher risk of acquiring dental fluorosis condition. This pattern has been confirmed in high-temperature zones, which show the highest prevalence rates of dental fluorosis even when fluoride levels were under the optimal concentrations in drinking water as suggested by the WHO (2011). On the other hand, in cold temperature zones, dental fluorosis seems to affect the people utilizing contaminated coal for heating their homes and cooking which predispose many people to effects of airborne fluorosis (Almerich-Silla *et al.* 2008; Sudhir *et al.* 2009; Vuhahula *et al.* 2009; Chauhan *et al.* 2012; Firempong *et al.* 2013; Garg & Singh 2013; Sebastian *et al.* 2016).

Altitude is increasingly studied in the literature to define its role in the prevalence rate of dental fluorosis, but its direction is not clear. Some studies on higher altitudes have shown that urine becomes acidic influencing fluoride ions to last longer in the body and thus altitude stimulates faster absorption and spread of fluoride ions in the intestine. These conditions are known to quicken fluoride retention in the bones and muscles that have been proved to influence fluoride mineralization in tissues causing dental fluorosis (Shitumbanuma *et al.* 2007; Kotoky *et al.* 2008; Vuhahula *et al.* 2009; Yadav *et al.* 2009; Liu *et al.* 2015; Fan *et al.* 2016; Taghipour *et al.* 2016). In contrast, another study finds that both lower and higher altitudes have similar effects on the prevalence rates of fluorosis (Akosu & Zoakah 2008).

Dental caries and prevalence of dental fluorosis

Even though adequate levels of fluoride ions (0.6 mg/L) are needed to inhibit dental caries and develop stronger bone mass and teeth, when fluoride is above this limit it is not clear how fluorosis influences dental caries. Some authors agree that a higher dosage of fluoride reduces dental caries because they find that in regions with a high prevalence of dental fluorosis, there is a lower prevalence of dental caries. These results suggest the role of fluoride as a protective element against dental caries (Li *et al.* 2009; Mandinic *et al.* 2009; Vuhahula *et al.* 2009; Bagh *et al.* 2012; Kotecha *et al.* 2012; Tellez *et al.* 2012; Firempong *et al.* 2013; Fan *et al.* 2016; Sebastian *et al.* 2016).

An interesting concern is that some severe dental fluorosis patients also exhibit higher levels of caries, which may be explained by the loss of protective enamel in pitting with severe fluorosis. Finally, a few studies show no relationship between dental fluorosis and dental caries. However, additional research should be conducted to understand how many factors (e.g. caries developing resistance to fluoride, effect of concentration of fluoride on personal oral hygiene, prevalence of cariogenic bacterial strains in the mouth and socio-economic status of individuals) influence the relationship between dental caries and dental fluorosis (Almerich-Silla *et al.* 2008; Sudhir *et al.* 2009; Keçeci *et al.* 2014; Taghipour *et al.* 2016; Fan *et al.* 2016).

Other dietary intake and prevalence of dental fluorosis

Also, for dietary consumption, findings show that the relationship between foodstuffs and dental fluorosis is not clear. Several studies indicate that dietary foods positively influence dental fluorosis. Food, beverages (brick tea, tea and butter tea) infant formula, fish, beans, potatoes, wheat, animal and plant proteins and 'magadi' popularly utilized as tuber softener have questionable fluoride content and thus impacting on the fluorosis prevalence rates (Almerich-Silla *et al.* 2008; Li *et al.* 2009; Vuhahula *et al.* 2009; Malde *et al.* 2011; Kotecha *et al.* 2012; Abuhaloob & Abed 2013; Fan *et al.* 2016). However, other studies that also focused on tea, animal and plant proteins found no difference in fluorosis prevalence rates associated with the products (Abuhaloob & Abed 2013; Firempong *et al.* 2013).

Meta-analysis results

Table 2 presents information in relation to the following variables for each study: the number of participants, the number of people affected by dental fluorosis, the average content of fluoride in drinking water, the average age of participants, the extreme temperature, rainfall, altitude, h-index and the number of citations per year of the studies used to perform the meta-analysis. It shows that 13 studies are from Asia, 7 from Africa and 1 from Central America. Table 2 allows us to explore some differences between Asian and African studies. For example, comparing the averages of prevalence rate of dental fluorosis and content of fluoride in drinking water, we find that for African studies, these values are higher than those conducted in Asia. However, differences are significant to the independent sample *t*-test only for content of fluoride ($\bar{X}_{\text{diff}} = 2.53$; $p = 0.0001$; $df = 18$). For age of participants, we observe the opposite, i.e. the average age of participants for Asia ($\bar{X} = 19.46$; $s = 11.56$) is higher than that for Africa ($\bar{X} = 10.32$; $s = 5.12$). Also, this difference was significant to the independent sample *t*-test ($\bar{X}_{\text{diff}} = 9.15$; $p = 0.001$; $df = 18$). With regard to climatic variables, we do not observe significant differences between African and Asian studies, while for the h-index we observe that African studies are published in more prestigious journals. This difference was significant to the independent sample *t*-test ($\bar{X}_{\text{diff}} = 79.45$; $p = 0.001$; $df = 18$).

The meta-proportion results

The meta-analysis of the 21 selected studies starts by proposing in Figures 2 and 3 the forest plots related to the impact of age and fluoride level in drinking water on the prevalence of dental fluorosis. The forest plots display the results from each study as a square and a horizontal line, representing the intervention effect estimate together with its confidence interval. The analysis highlights a substantial level of heterogeneity for all the variables.

In Figure 2, the forest plot of the fluoride content in drinking water is presented. Most of the studies showed a level of fluoride between 1.5 and 3.0 mg/L. The forest plot also shows that the overall prevalence rate is 60% (0.60; 95% CI = 0.44–0.75) and that increasing levels of fluoride in drinking water affect the higher occurrence of dental

fluorosis. For values below 1.5 mg/L, which is the recommended value by Taghipour *et al.* (2016), the prevalence rate was 41% (0.41; 95% CI: 0.03–0.80). For values higher than 3.0 mg/L, the percentage of people affected by the dental fluorosis condition was statistically 92% (0.92; 95% CI: 0.84–0.99). However, the heterogeneity was significant ($p < 0.001$, $I^2 = 99.93\%$).

With respect to age, Figure 3 presents the pooled prevalence rates of the studies divided into three subgroups, under 11, between 11 and 15 and older than 15 years. The results indicate a higher prevalence rate for the first group. However, in this case the heterogeneity is relevant ($p < 0.001$, $I^2 = 99.93\%$) as well.

The meta-regression results

Considering the relatively high heterogeneity shown in these studies, meta-regression analysis was conducted to explore the possible sources of this heterogeneity further. Table 3 shows the results of two meta-regression analyses, where the dependent variable is the prevalence rate of dental fluorosis of the 21 studies reported in Table 2.

Meta-regression 1 included only the fluoride level (FLUOR) in drinking water while meta-regression 2 includes also other regressors: two dummy variables DAFRICA and DAMERICA, respectively, with 1 if the observation is in a group of countries in Africa or America and zero otherwise. The two dummies were included due to the possible differences among the three group of studies (Africa, America and Asia) which emerged from the preliminary analysis. In meta-regression 2, we also included the TEMP indicator calculated as a ratio between the maximum and average annual local temperature through which we want to consider possible increases in water consumption due to warmer temperatures. Temperature data were retrieved from the studies and when not available, from <https://www.weather2visit.com/>. Other models were estimated that included the rainfall and altitude variables. For brevity, these results were not reported due to the non-significance of the two variables.

Table 3 shows that FLUOR is significant ($p < 0.05$) in both models and the TEMP variable in model 2 ($p < 0.05$) is also statically significant while the two dummies are not statistically significant. These results are extremely

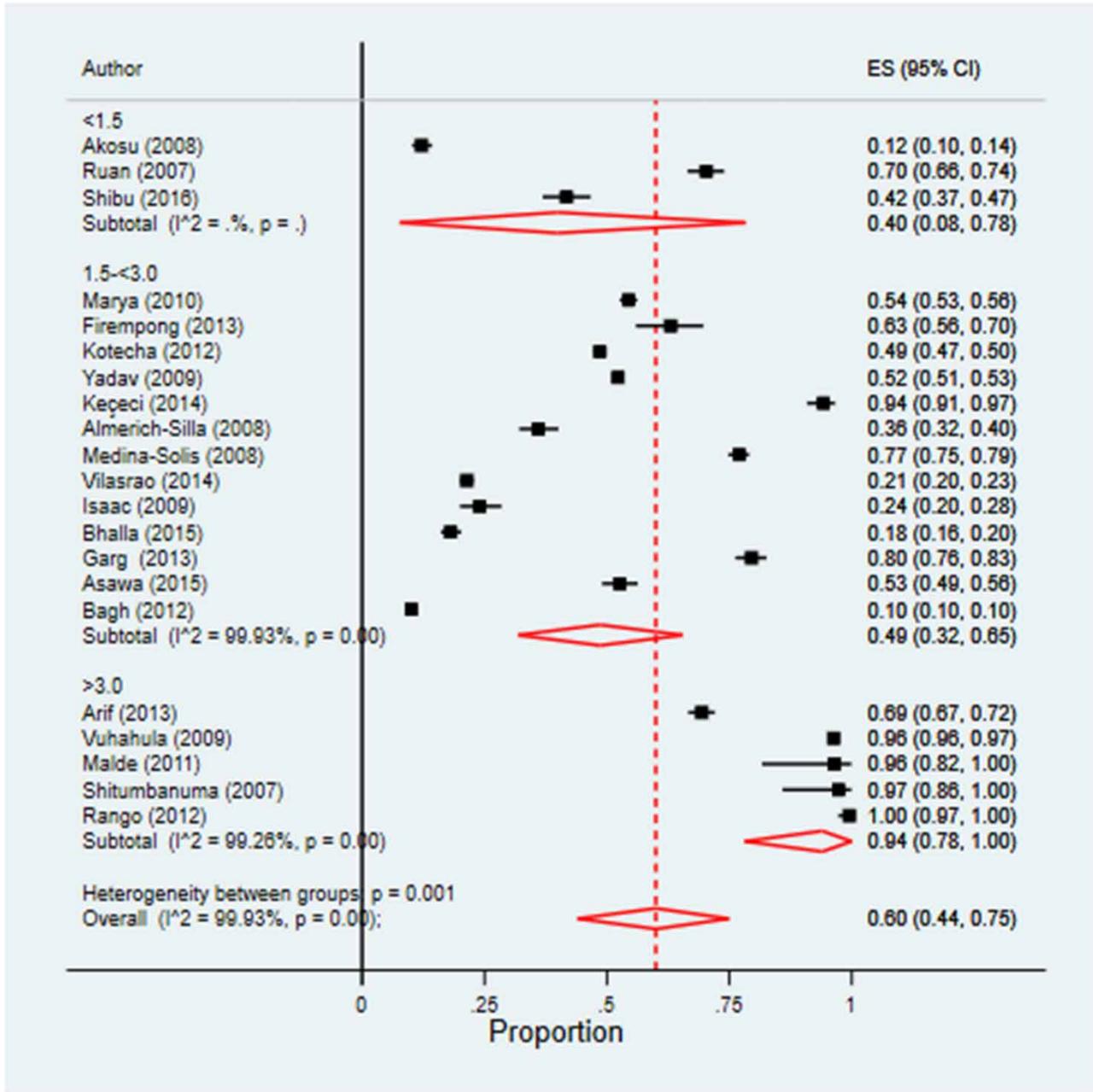


Figure 2 | Forest plot of the prevalence of dental fluorosis for different levels of fluoride in drinking water.

important because they allow us to work out the marginal effect of FLUOR from a large number of studies. In both meta-regressions, the FLUOR beta parameter is positive, i.e. the higher FLUOR (concentration in water) is, the higher we may expect the dental fluorosis prevalence rate will be. In particular, this is important for policy makers because *ceteris paribus* if FLUOR is reduced by 1 mg/L

this will reduce the number of people affected by about 7.6% (95% CI: 2.6%–12.6%) using the beta parameter in model 1 or 8.8% (95% CI: 3.1%–14.4%) in model 2. By the same token, our results indicate that *ceteris paribus* an increase of one degree Celsius above the average temperature would affect the prevalence of dental fluorosis by an estimated 2%.

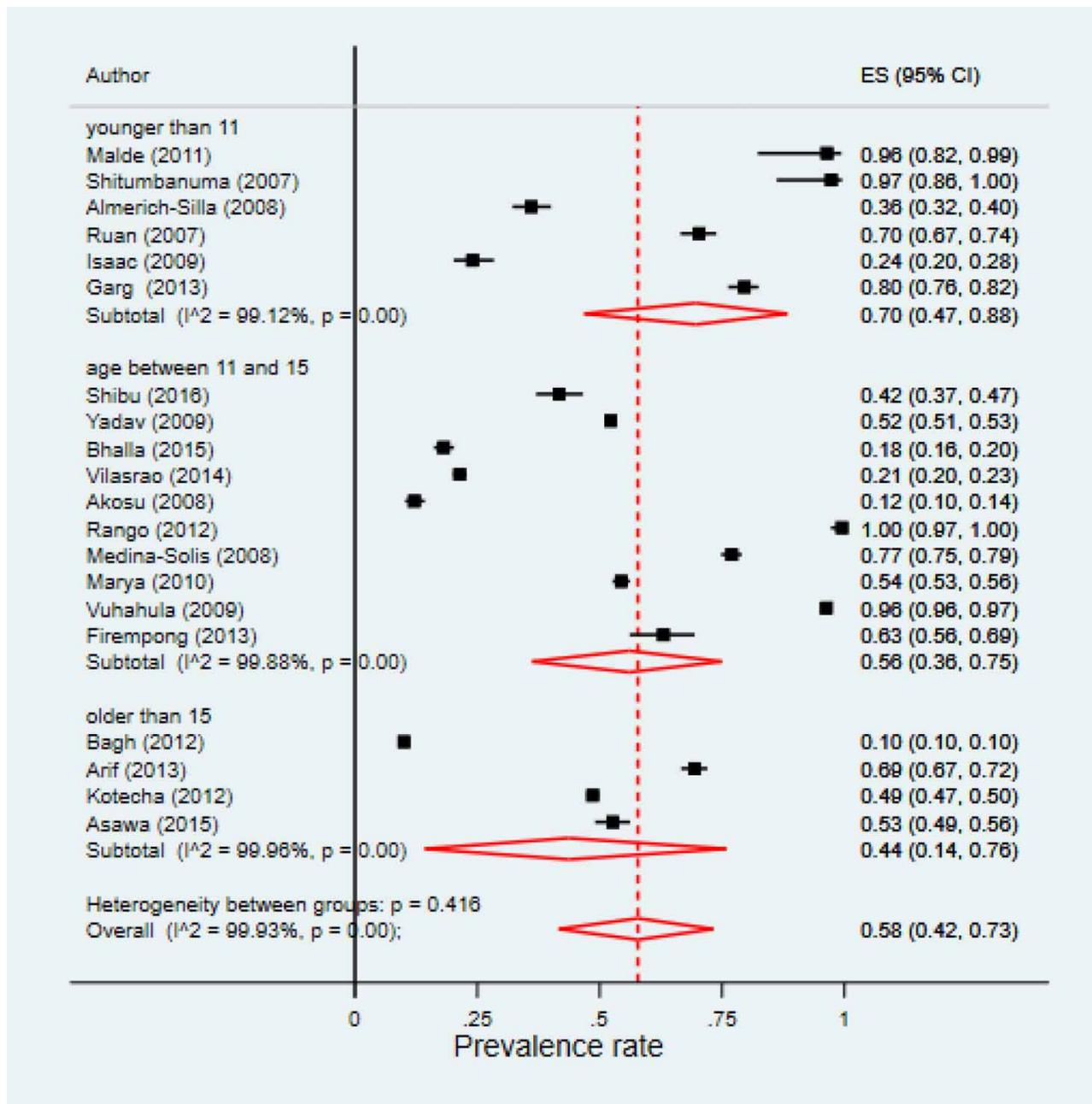


Figure 3 | Forest plot of the prevalence of dental fluorosis for different age groups.

Publication bias

Results presented in Table 4 suggest that the publication process of these articles and the scientific community seem to value information regarding the prevalence rate of fluorosis differently. In model 1, the beta parameter of the h-index is positive and statistically significant ($p <$

0.05); therefore, it is likely that the higher prevalence rate of dental fluorosis seems to influence more the acceptance rate of papers managed by high-profile journals. On the other hand, model 2 shows that the beta parameter of the number of citations is not significant, and thus this does not influence the citation behavior of the scientific community.

Table 3 | Factors influencing the prevalence rate of dental fluorosis

Variables	Meta-regression 1	Meta-regression 2
FLUOR (mg/L)	0.076 (0.024) {0.005,0.019} [95% CI: 0.026–0.126]	0.088 (0.0287) {0.005,0.015} [95% CI: 0.031–0.144]
DAFRICA	–	0.114 (0.132) {0.398,0.43} [95% CI: –0.165–0.394]
DAMERICA	–	0.425 (0.248) {0.106,0.146} [95% CI: –0.101–0.951]
TEMP	–	0.780 (0.367) {0.050,0.084} [95% CI: 0.001–1.555]
Constant	0.357 (0.088) {0.001} [95% CI: 0.172–0.541]	–0.703 (0.496) {0.175} [95% CI: –1.756–0.348]
N. obs	21	21
T^2	0.0610	0.0532
I^2	99.97%	99.94%
Adj. R^2	31.49%	40.85%
F test and p -value	–	4.44
Joint covariate test		{0.013}

In parentheses () the standard errors, in parentheses {} the p -values of the t -statistics. The first is p -value under the t -statistic distribution, the second one is the p -value computed using a permutation test proposed in Higgins and Thompson (2004) to simulate the data under the null-hypothesis. Twenty thousand replications were used to compute the p -values. The T^2 is the estimate of the between-study variance and I^2 is the percent of residual variation due to heterogeneity.

DISCUSSION AND CONCLUSIONS

In this study, we present the results of a systematic review and meta-analysis aimed at understanding how environmental, age and geographic area influence the prevalence of dental fluorosis caused by fluoride contained mainly in groundwater. The systematic review is based on 39 studies identified in selected scientific databases using key words and Boolean operators. Our findings indicate that while

Table 4 | Assessing publication bias

Variables	Meta-regression 1	Meta-regression 2
h-index	0.002 (0.001) {0.018,0.003} [95% CI: 0.000–0.004]	–
Citations per year	–	0.021 (0.016) {0.194,0.183} [95% CI: –0.011–0.054]
Constant	0.441 (0.078) {0.000} [95% CI: 0.276–0.604]	.493 (0.089) {0.000} [95% CI: 0.304–0.601]
N. obs	21	21
T^2	0.070	0.064
I^2	99.86%	99.93%
Adj. R^2	22.24%	28.24%

In parentheses () the standard errors, in parentheses {} the p -values of the t -statistics. The first is p -value under the t -statistic distribution, the second one is the p -value computed using a permutation test proposed in Tahir and Rasheed (2013) and Higgins and Thompson (2004) to simulate the data under the null-hypothesis. Twenty thousand replications were used to compute the p -values. The T^2 is the estimate of the between-study variance and I^2 is the percent of residual variation due to heterogeneity.

dental fluorosis affects all age brackets, factors such as gender, temperature, altitude, rainfall, diet and dental caries's influence on this association is not clear. Concerning age, dental fluorosis affects all groups of children, but the highest prevalence is found in those below 11 years of age, with a prevalence rate very close to 100%. There is a need to conduct more research on children younger than 11 years old and especially those living in small villages, where there is a lack of alternatives to groundwater sources that are rich in fluoride (Edmunds & Smedley 2005). Children affected by fluorosis are perceived by their unaffected peers as, not intelligent, unattractive, unhappy and unsmiling, with poor hygiene and lack interactive social skills. All these beliefs greatly harm children's development and call for high urgency in information sharing, training and health education programs addressing causes and negative impacts of dental fluorosis (Akosu & Zoakah 2008; Isaac *et al.* 2009; Yadav *et al.* 2009; Kotecha *et al.* 2012; Yadav *et al.* 2012; Firempong *et al.* 2013).

For individuals older than 18, dental fluorosis is also associated with skeletal and non-skeletal fluorosis, and for those older than 35 years of age, there is also the loss of teeth, nutritional deficiencies and bad lifestyle habits. In this case, policy makers could devise information for nutritional campaigns aimed at increasing the consumption of fruit, vitamins A and C, thiamine, folic acid, milk, period of breast-feeding, calcium and promoting remedies, proper storage of water in ceramic tanks, clay pots for storing water and elimination of tea in children's diets. Evidence-based nutritional interventions are strongly recommended in different contexts especially for children (Almerich-Silla *et al.* 2008; Kotecha *et al.* 2012; Rango *et al.* 2012; Yadav *et al.* 2012; Arif *et al.* 2013; Liu *et al.* 2015; Taghipour *et al.* 2016). However, for the other confounding factors, more research should be conducted to understand how they interact with age and dental fluorosis.

Furthermore, to the best of our knowledge, this is the first meta-analysis exploring the effects of naturally fluoridated water on dental fluorosis by pooling together 21 studies collecting information from 88,508 participants in different countries around the world. Forest plots show high levels of heterogeneity of dental fluorosis both for the content of fluoride in drinking water and the age of participants. However, meta-regression analyses conducted using a random effect model provides evidence that fluoride exposure from drinking water and temperature significantly affect dental fluorosis. Other predictors such as age of participants, rainfall and altitude were not statistically significant. The beta parameter of fluoride content indicates that *ceteris paribus* a reduction of 1 mg/L would reduce the probability of being affected by dental fluorosis by an estimated 7.6% (95% CI: 2.6% – 12.6%) in model 1 and 8.8% (95% CI: 3.1% – 14.4%) in model 2. This result can be extremely important to African Rift Valley countries where the average level of fluoride in drinking water ranges from 6 to 10 mg/L. Technological innovation capable of reducing fluoride below WHO recommended levels (<1.5 mg/L) can provide health benefits to thousands of children in the Rift Valley. For example, in Tanzania and Ethiopia, an abatement of between 5 and 9 mg/L of fluoride in drinking water may reduce dental fluorosis in their populations between 38% and 80%. Furthermore, the significant positive

relationship between temperature and fluoride in drinking water suggests that the use of innovative technologies cannot be neglected, because if temperature increases as a consequence of long-term climatic changes, the problem of dental fluorosis in these geographic areas will be exacerbated.

The significant positive relationship between temperature and fluoride in drinking water suggests that the use of innovative technologies cannot be neglected, because if temperature increases as a consequence of long-term climatic changes, the problem of dental fluorosis in these geographic areas will be exacerbated. Rainwater harvesting systems, as for example the use of rainwater collection tanks, can help in reducing the consumption of unsafe groundwater (Susheela 1999; Edmunds & Smedley 2005). However, recent research (Haque *et al.* 2016) showed that the performance of these systems can be negatively impacted by climate change conditions. Such situations mainly involve an expected worsening of drought periods in the future decades.

Meta-analysis also seems to indicate publication bias, and thus more attention should be paid to the publishing process to include in high h-index-ranked journals more studies reporting low and medium prevalence rates of dental fluorosis disease.

The negative impact of dental fluorosis on people's health is well recognized by many governments but not very much is done to tackle this issue affecting the life of millions of people in developing countries. The inadequate capacity of poor people to find alternative water sources is a challenge leading to high exposure to dental fluorosis in fluoride-affected regions. Improved water management systems can efficiently respond to naturally fluoride water impacts, but this solution is often overlooked in villages where people struggle to find better sources of water. Governments should invest in modern social water policy programs that can supply filtrated or clean water in remote villages where lack of alternatives has forced residents to utilize springs and other unconventional sources of water. However, to achieve such results, actions such as the development of simple and low-cost de-fluoridation devices for use at the household or village level represent promising solutions to tackle this problem in many areas of the world (Akosu & Zoakah 2008; Isaac *et al.* 2009;

Chauhan et al. 2012; Kotecha et al. 2012; Tellez et al. 2012; Keçeci et al. 2014).

The development of innovative technologies on behalf of locals, by national or international industry must be supported by technical and socio-economic research. Technical innovation can provide solutions to abate the level of fluoride in drinking water, but more efficient and powerful filters cannot be transferred and adopted in these countries without understanding the consumption habits and attitudes of drinking water, as well as preferences toward the adoption of the new technology. For example, Flowered (www.floweredproject.org) an EU H2020 project is working in this direction to develop sustainable water management systems capable of reducing levels of fluoride in drinking water in the Rift Valley countries. To achieve this objective, the Flowered project employs a scientific interdisciplinary approach that develops and disseminates innovative solutions obtained, considering not only technical and scientific aspects but also social challenges and public engagement.

Prevention via innovative technologies and implementation of these policies appear to be a promising strategy to improve the quality of life of both children who are reported to undergo socio-psychological distress due to mottling and damage of teeth and adults who also face dental skeletal fluorosis. However, considering that the complexity of dental fluorosis is determined by the content of fluoride in drinking water, preventive results can only be achieved with more international cooperation and interdisciplinary research to tackle this problem from different angles.

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