Science of the Total Environment 407 (2009) 5298–5307

Contents lists available at ScienceDirect

Science of the Total Environment

journal homepage: www.elsevier.com/locate/scitotenv

Determining the optimal fluoride concentration in drinking water for fluoride endemic regions in South India

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ARTICLE INFO

Article history:
Received 22 February 2009
Received in revised form 22 June 2009
Accepted 23 June 2009
Available online 26 July 2009

Keywords:
Fluorosis
Optimal fluoride level
Total fluoride intake
Bone fluoride
Community fluorosis index
Regression analysis

ABSTRACT

Fluoride ion in drinking water is known for both beneficial and detrimental effects on health. The prevalence of fluorosis is mainly due to the intake of large quantities of fluoride through drinking water owing to more than 90% bioavailability. The objective of this study is to predict optimal fluoride level in drinking water for fluoride endemic regions by comprising the levels of fluoride and other water quality parameters in drinking water, prevalence of fluorosis, fluoride intake through water, food and beverages such as tea and coffee and also considering the progressive accumulation of fluoride in animal bones, by comparing with non fluoride endemic areas comprise of the same geological features with the aid of regression analysis. Result of this study shows that increase of fluoride level above 1.33 mg/l in drinking water increases the community fluorosis index (CFI) value more than 0.6, an optimum index value above which fluorosis is considered to be a public health problem. Regression plot between water fluoride and bone fluoride levels indicates that, every increase of 0.5 mg/l unit of water fluoride level increases the bone fluoride level of 52 mg/kg unit within 2 to 3 years. Furthermore, the consumption of drinking water containing more than 0.65 mg/l of fluoride can raise the total fluoride intake per day more than 4 mg, which is the optimum fluoride dose level recommended for adults by the Agency for Toxic Substances and Disease Registry. From the result, the people in fluoride endemic areas in South India are advised to consume drinking water with fluoride level within the limit of 0.5 to 0.65 mg/l to avoid further fluorosis risk.

1. Introduction

Access of drinking water is measured by the number of people who has a reasonable means of getting an adequate amount of water that is safe for drinking. There is a substantial shortfall in the availability of potable water in less developed countries, primarily arising from contamination and pollution (Shiklomanov, 2000; Rao Nagendra, 2003). About 80% of the diseases in the world are due to poor quality of drinking water, and the fluoride contamination in drinking water is responsible for 65% of endemic fluorosis in the world (WHO, 1984a; Felsenfeld and Robert, 1991; WHO, 2002). Furthermore 50% of the ground water sources in India have been contaminated by fluoride and more than 90% of rural drinking water supply programmes are based on ground water. Sporadic incidence of high fluoride contents in drinking water has been reported from India, China, Sri Lanka, West Indies, Spain, Holland, Italy, Mexico, and North and South American countries (Suttie, 1969; Mella et al., 1994; Grimaldo et al., 1995; Li et al., 1995). The prevalence of fluorosis in man is reported from 22 states of India, affecting more than 40 million people (Siddiqui, 1955; Jolly et al., 1973; Teotia and Teotia, 1991; Chinoy, 1991; Srikanth et al., 1994; Susheela et al., 1993, Susheela, 2000; Karthikeyan et al., 1996, 2005). Fluoride concentration in water depends on several contributing factors such as pH, total dissolved solids, alkalinity and hardness (Karthikeyan and Shanmugasundarraj, 2000, Subba Rao, 2003). Many of the previous studies, from various parts of the world reported the development of dental fluorosis even if the people consume drinking water with fluoride less than 1.0 mg/l (Brouwer et al., 1988; Riordan, 1993; Clark, 1994; Ibrahim et al., 1995; Karthikeyan et al., 1996; Heller, 1997), which implies that the optimal fluoride dose level in drinking water may vary with various features like local climatic conditions (Galagan and Vermillion, 1957; Galagan et al., 1957; Khan et al., 2004), methods of food processing and cooking (Martin, 1951; Grimaldo et al., 1995), amount of food and water intake and its fluoride and other nutrients level (WHO, 1984b; Kahama et al., 1997; Karthikeyan et al.,

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doi:10.1016/j.scitotenv.2009.06.028

2005; Viswanathan et al., 2009) and dietary habits of the community (Danielsen and Gaarder, 1955; Cao et al., 1996; Kaseva, 2006). So the prediction of optimum fluoride level in drinking water is needful for individual areas by considering the factors which influence fluoride consumption or fluorosis prevalence. In this context, regression analysis is the suitable aid for identifying a community specific safe level of fluoride by comparing it with nearby non fluorotic areas with similar climatic conditions, food processing and dietary habits. The prevalence of fluorosis was determined through clinical survey and expressed as percentage of fluorosis incidence and the quantitative assessment of severity of dental fluorosis was done by calculating the community fluorosis index (CFI) using Dean's classification (Dean and Elvove, 1935; Dean, 1942). Many of the previous studies expressed the degree of fluorosis prevalence using CFI measurement (Karthikeyan et al., 1996; Kaseva, 2006). Samples of drinking water, diet and beverages normally consumed by the village people were collected and analyzed for fluoride to find out the total fluoride intake. In order to understand the rate of fluoride accumulation in the bone with respect to the consumption of drinking water containing different fluoride levels, goat bone samples were collected from fluorotic and non fluorotic (control) areas and analyzed for fluoride. Goat bones were used because, they are mammalian and suitable for testing human implants and materials as they are considered to have metabolic rate and bone remodeling rate similar to that of humans (Spaargaren, 1994; Anderson et al., 1999). The numerical relationships were calculated with the aid of regression analysis and the correlations were estimated through correlation coefficient based on the method of least squares.

2. Materials and methods

2.1. Collection of water sample and selection of study areas

A total of 320 drinking water samples were collected from 64 villages in Nilakottai and neighboring blocks such as Dindigul and Athoor for fluoride analysis. Each sample collected from a particular village is identified by a different symbol and stored in polyethylene bottles and kept in a refrigerator below 10 °C before analysis. One control and two fluoride endemic areas were selected for this study. The criteria for the selection of control and fluoride endemic areas were discussed below.

2.2. Selection of control area (C)

The criteria for the selection of control are the state of absence of fluorosis in any of its form in that particular area and the drinking water fluoride level should be within 1 mg/l.

As per these criteria, ten villages that belong to the Athoor and Dindigul block of Dindigul District were selected as control areas (C) as indicated in Fig. 1.

2.3. Selection of fluoride endemic area 1 (F1)

As per the clinical survey and drinking water fluoride level, ten villages that belong to Nilakottai block of Dindigul District were selected as fluoride endemic areas F1 (Fig. 1), nearly 50% of the villagers were affected by different forms of fluorosis and the water fluoride level was between 1 to 2 mg/l.

2.4. Selection of fluoride endemic area 2 (F2)

Ten villages that belong to Nilakottai block of Dindigul District were selected as fluoride endemic area 2 (Fig. 1). In this area, water fluoride level was more than 2 mg/l and more than 50% of the people were affected by fluorosis and some of the villagers were affected severely.

2.5. Clinical survey and community fluorosis index (CFI)

Clinical survey was conducted among the people of different age groups and gender living in the selected areas. Community fluorosis index was assessed on the basis of dental fluorosis symptoms, which are classified into seven categories according to Dean's classification (Dean and Elvove, 1935; Dean, 1942) viz., normal, questionable, very mild, mild, moderate, moderately severe and severe and each of these seven classifications were given a numerical weight such as 0, 0.5, 1, 1.5, 2, 3 and 4 respectively as shown in Fig. 2. People with symptoms of dental fluorosis were identified and classified in each category and the number of people in each category was multiplied by the corresponding numerical weight, the products thus obtained for the various categories

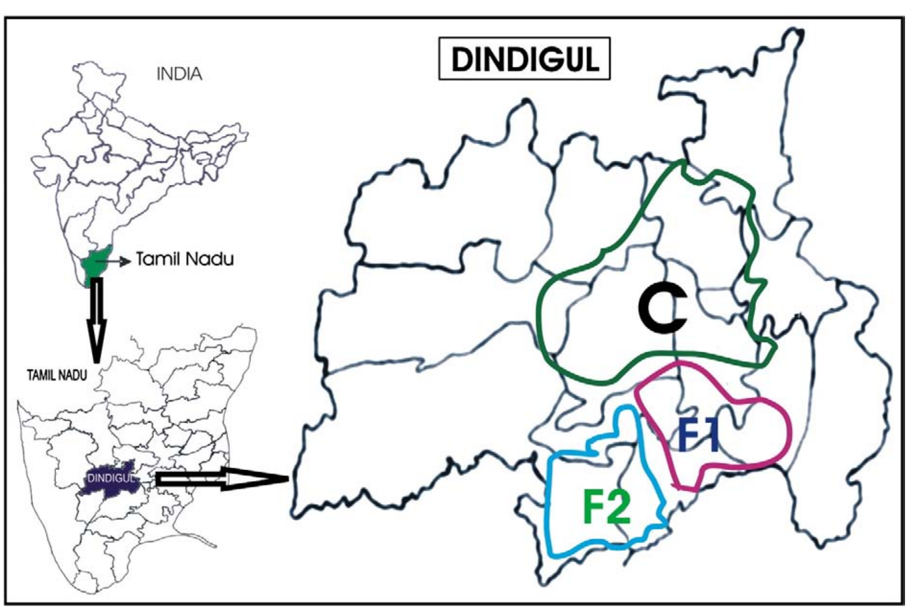

Fig. 1. Illustration of control and fluoride endemic areas in Dindigul district, C — control area, F1 — fluoride endemic area 1, F2-fluoride endemic area 2.

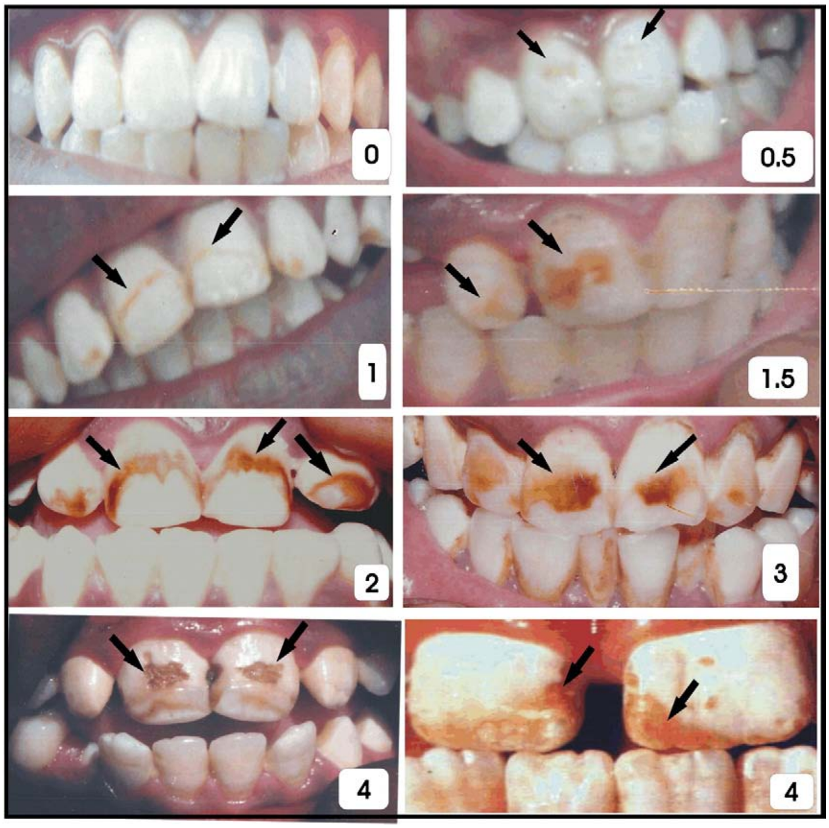

Fig. 2. Illustration of various degrees of dental fluorosis as Dean's classification.

were added up and the sum total divided by the total number of people surveyed, gives the community fluorosis index. Only when the community fluorosis index value exceeds 0.6, fluorosis is considered to be a public health problem in that area.

$$\mathrm{CFI} = \sum \frac{(\text{Number of people} \times \text{Dean's numerical weight})}{\text{Total number of people examined}}$$

The percentage incidence of fluorosis was calculated from the number of people affected by fluorosis from the respective area with total number of people surveyed. A total of 559 boys and 520 girls aged between 8 and 15 years and 3108 males and 2994 females within the age group of 16–66 were examined for this study. Persons with dental fluorosis were identified and characterized with the help of two practicing dentists.

2.6. Estimation of water quality parameters

Quantitative estimation of some of the water quality parameters such as pH, Electrical conductivity, Total Dissolved Solids, Total Alkalinity, Total Hardness, Chloride and Sulphate in drinking water samples were performed by using standard methods described as in Standard methods of the examination of water and waste water (APHA, 2005).

2.7. Estimation of fluoride

2.7.1. Estimation of fluoride in drinking water

Three drinking water samples were collected from ground water sources of each village of the selected area and stored in precleaned, high density polyethylene bottles at 4 °C before being analyzed. Fluoride level was measured by using fluoride ion selective electrode Orion 9609 with expandable ion analyzer EA 940 diluted with 1:1 ratio of Total Ionic Strength Adjustment Buffer (TISAB II). The instrument was calibrated with standard fluoride solutions so chosen that the concentration of one was ten times the concentration of the other and also that the concentration of the unknown falls between those standards. Then the concentration of the unknown was directly read from the digital display of the meter (Fluoride electrode manual, 1991).

2.7.2. Estimation of fluoride level in food

Food samples were collected from the study areas which actually consumed per day by 90 adult human subjects having average body weight of 70 kg and the food fluoride concentration was estimated on the basis of the method described by Villa (1979).

2.7.3. Estimation of fluoride level in beverages

Beverage samples were collected and carefully packed in polyethylene bottles. Fluoride concentration was measured by diluting the samples with TISAB (II) buffer in the ratio of 1:1 and the contents were stirred for 5 min on a magnetic stirrer and analyzed using fluoride ion selective electrode Orion 9609 with expandable ion analyzer EA 940.

2.7.4. Estimation of total fluoride intake per day

Daily fluoride intake from drinking water, food and beverages was calculated as described by Biego et al. (1998), by multiplying the fluoride concentration of the respective items with total quantity of the particular item consumed,

$$\text{Fluoride intake} = (\mathrm{Qi} \times \mathrm{Ci}) \tag{1}$$

where Qi — quantity of the diet intake per day, Ci — concentration of fluoride in diet source.

Table 1
Community fluorosis index and % of dental fluorosis prevalence.

Category of areas	Groups	No of people classified according to Dean's classification							Total	Community fluorosis index	% of fluorosis incidence
		0	0.5	1	1.5	2	3	4			
C	Boys	207	0	0	0	0	0	0	207	0	0
	Girls	182	0	0	0	0	0	0	182	0	0
	Male	963	0	0	0	0	0	0	963	0	0
	Female	948	0	0	0	0	0	0	948	0	0
F1	Boys	94	18	16	14	19	13	12	186	0.92	49
	Girls	66	24	20	17	24	15	14	180	1.15	63
	Male	494	126	102	88	108	74	66	1058	0.94	53
	Female	418	117	94	84	131	90	73	1007	1.09	58
F2	Boys	53	21	23	24	18	14	13	166	1.20	68
	Girls	39	24	19	22	16	21	17	158	1.44	75
	Male	372	144	138	141	108	96	88	1087	1.18	66
	Female	287	125	142	159	117	108	101	1039	1.35	72

Table 2
Correlation between water fluoride level (X) and related variables (Y).

Variables	Regression equation	Correlation coefficient (r)	Coefficient of determination ($R2$)	Significant level	F ratio
% incidence of fluorosis (Y)	Y = − 16.1720 + 36.7 X	0.9767	0.9540	$p = 0.138$	20.73
Community Fluorosis Index (Y)	Y = − 0.3026 + 0.680 X	0.9784	0.9573	$p = 0.133$	22.40
Total fluoride intake (Y)	Y = 0.0346 + 6.1231 X	0.9998	0.9995	$p = 0.014$	2035
Bone fluoride level (Y)	Y = 89.5962 + 104.04 X	0.7146	0.5107	$p = 0.493$	1.04

From household survey, it was observed that, normally 2.5 l of drinking water, 1.6 kg of food, 250 ml of tea and 150 ml of coffee were consumed by the villagers residing in the selected regions.

2.7.5. Estimation of fluoride contribution from various dietary sources

The percentage of contribution from each source on daily fluoride intake was calculated by dividing the amount of fluoride intake from

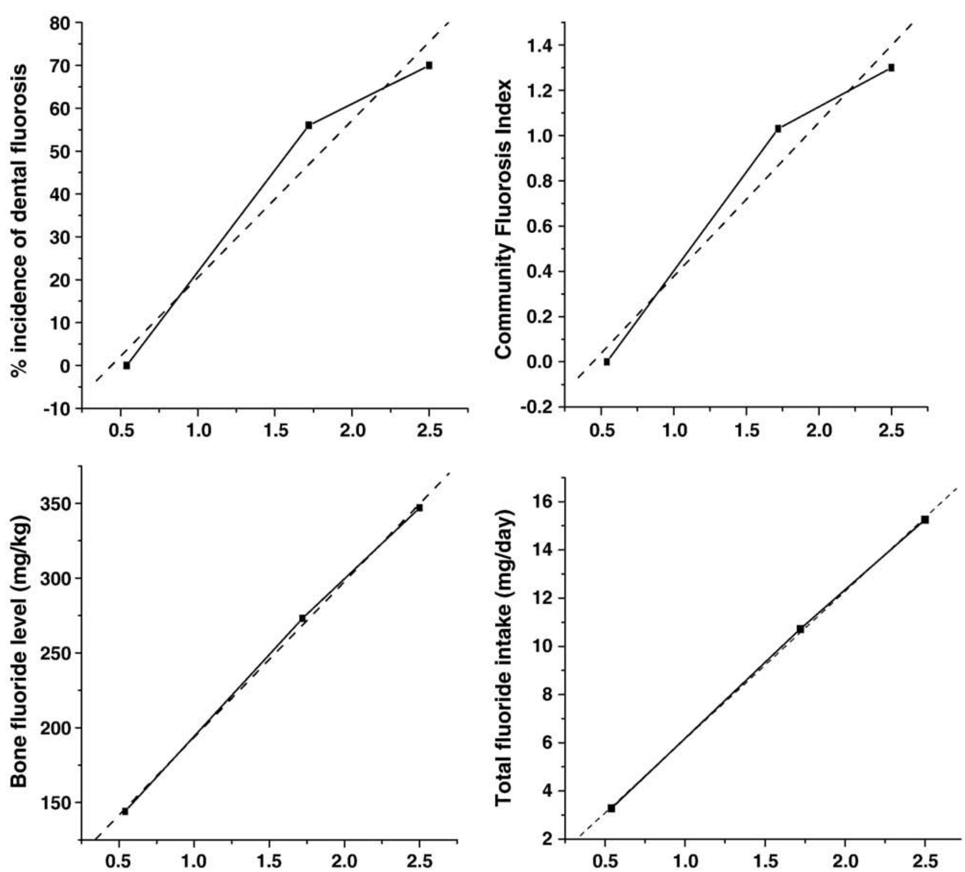

Fig. 3. The dashed lines and straight lines indicate regression plots and original plots of variables with respect to water fluoride level (mg/l) in *x*-axis respectively.

Table 3
Impact of water quality parameters (Y) on drinking water fluoride level (X).

Water quality parameter	Regression equation	Correlation coefficient (r)	Coefficient of determination (R2)	Significant level	95% confidence interval	ANOVA	F-ratio
pH (Y)	Y = 6.384 + 0.318 X	0.624	0.389	$p<0.0001$	0.4784 to 0.7357	$p<0.001$	56.02
EC (Y)	Y = 0.112 + 0.039 X	0.478	0.228	$p<0.0001$	0.3003 to 0.6231	$p<0.001$	26.01
TDS (Y)	Y = 526 + 291.64 X	0.644	0.414	$p<0.0001$	0.5036 to 0.7506	$p<0.001$	62.22
TH (Y)	Y = 274 + 146.16 X	0.792	0.627	$p<0.0001$	0.6991 to 0.8580	$p<0.001$	147.65
TA (Y)	Y = 9.22 + 106 X	0.832	0.692	$p<0.0001$	0.7547 to 0.8862	$p<0.001$	197.55
Chloride (Y)	Y = 264.7 + 38.69 X	0.215	0.046	$p<0.0001$	0.0081 to 0.4039	$p=0.042$	4.26
Sulphate (Y)	Y = 56.31 + 12.32 X	0.243	0.059	$p=0.0212$	0.0375 to 0.4283	$p=0.021$	5.51

the particular source with total fluoride intake from all selected sources of the respective area, and then multiplying with 100 as shown by the following equation (Eq. (2)).

$$\%FCi = (FIs \div FIt) \times 100 \quad (2)$$

where, % FCi — percentage of fluoride contribution from each source per day, FIs — amount of fluoride intake from particular source, FIt— total fluoride intake from all selected sources.

2.7.6. Estimation of bone fluoride

Tibias of goat bone were collected from five goats in the slaughter house located in selected regions. The animals were selected according to their dental age group between 2 to 3 years. The samples were carefully packed in polyethylene papers and kept in a freezer below −10 °C. Aliquots of fat free, cleaned bones were accurately weighed and then ashed for 3 h at 600 °C in a muffle furnace. About 10 mg of accurately weighed powdered bone ash sample was carefully transferred into a 100 ml standard measuring flask and dissolved in

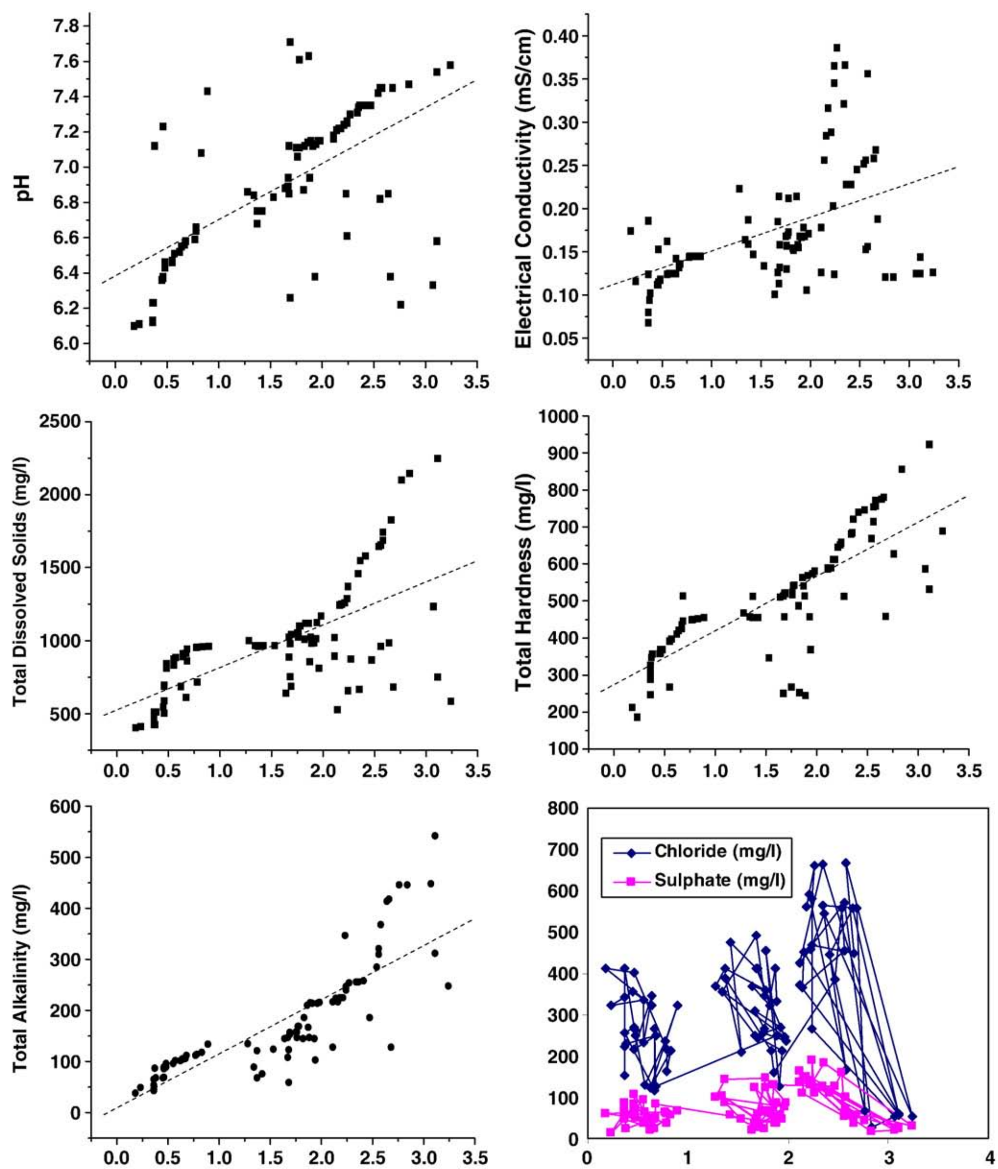

Fig. 4. Influence of water quality parameters (*y*-axis) on water fluoride level (*x*-axis).

3 ml of 40% perchloric acid and the acidity is neutralized by using 0.5 M NaOH, then the volume of the contents was made up to 100 ml using double distilled water, then 10 ml of the aliquot is mixed with 10 ml of TISAB II buffer, the contents were analyzed using specific ion selective electrode Orion 9609 with expandable ion analyzer EA 940 as described by Wharton, 1962.

2.8. Statistical analysis

The values were expressed as mean ± standard deviation. Statistical comparisons between the groups were performed using one way analysis of variance (ANOVA) and F-ratio. The degree of linearity and correlation is assessed by correlation coefficient (r) and coefficient of determination (R^2). The quantitative numerical relationship between the water fluoride level and other water quality parameters is done by regression analysis. Statistical analyses were performed by using SPSS statistical version 16.0. A p value < 0.05 was considered statistically significant.

3. Results

3.1. Magnitude of fluorosis prevalence

The percentage of fluorosis prevalence and community fluorosis index (CFI) from all the selected regions were shown in Table 1. There was no incidence of fluorosis in all age groups of people in control area. The percentage prevalence of dental fluorosis in fluoride endemic areas 1(F1) among the boys and girls were 49% and 63% and community fluorosis indices were about 0.92 and 1.15 respectively. In fluoride endemic areas 2 (F2), higher prevalence of fluorosis is observed from girls and females that are 75% and 72% respectively with 1.44 and 1.35 of CFI values, whereas 68% and 66% of the incidence of fluorosis are estimated from the boys and males respectively with 1.20 and 1.18 of CFI values. The rate of fluorosis prevalence gradually increased (p < 0.138) as water fluoride level increased (r = 0.977, R^2 = 0.954). Nearly 30% of the people in the selected fluoride endemic areas was affected with more than mild degree of fluorosis that is, the CFI value is higher than the numerical score of 2. The quantitative numerical relationship between the water fluoride level with percentage of fluorosis prevalence and community fluorosis indices was mentioned in Table 2 and shown in Fig. 3. The relationship between water fluoride levels and community fluorosis indices of the study areas indicates that, if the water fluoride level exceeds 1.33 mg/l, then fluorosis can be a public health problem in the selected regions.

3.2. Relationship of water quality parameters on fluoride ion concentration in drinking water

Normally the drinking water samples from fluoride endemic areas show slightly higher pH level (r = 0.624, p < 0.0001) than from non fluoride endemic area water samples. Total dissolved solids are also positively correlated with respect to fluoride level in water (r = 0.644, p < 0.0001). Drinking water samples from fluoride endemic areas (F1 & F2) acquire more total alkalinity (r = 0.832, p < 0.0001) and total hardness level (r = 0.792, p < 0.0001) than samples from non fluoride endemic area (C), these results were comparable with previous studies (Karthikeyan et al., 1996; Karthikeyan and Shanmugasundarraj, 2000, Subba Rao, 2003). The electrical conductivity values are slightly higher in fluoride endemic area water samples with limited dependence (r = 0.478, p < 0.0001) than in control area water samples. There was no significant relationship of chloride and sulphate level on fluoride ion concentration in drinking water as observed and the results were comparable with a previous study (Rajmohan, 2003). From the observed F-ratio mentioned in Table 3 it indicates that, the total alkalinity in drinking water has more influence on water fluoride level than the other water quality parameters. The derived relationships of water quality

Table 4
Levels of water quality parameters in villages of control area (C).

Site identity	pH	EC (mS/cm)	TDS (mg/l)	TH (mg/l)	TA (mg/l)	F^- (mg/l)	Cl^- (mg/l)	$SO4^{2-}$ (mg/l)
A1	6.43	0.117	814	369	89	0.48	263	49
A2	6.46	0.118	845	369	96	0.48	248	56
A3	6.13	0.186	506	310	46	0.36	413	48
B1	6.23	0.08	512	327	65	0.36	153	87
B2	6.23	0.094	426	348	87	0.37	221	68
B3	7.12	0.102	512	357	68	0.38	228	23
C1	6.58	0.136	945	513	112	0.68	249	51
C2	6.38	0.112	689	364	86	0.46	215	84
C3	6.55	0.142	912	421	105	0.64	345	33
D1	6.47	0.124	834	392	96	0.55	234	38
D2	6.46	0.162	875	268	96	0.55	335	94
D3	6.11	0.116	412	186	49	0.23	321	13
E1	6.13	0.124	459	289	43	0.36	341	64
E2	6.36	0.112	548	358	68	0.45	356	56
E3	6.55	0.125	890	420	104	0.64	321	45
F1	6.10	0.174	405	213	38	0.18	412	61
F2	6.37	0.153	588	359	69	0.46	401	64
F3	6.64	0.145	716	451	114	0.78	216	37
G1	6.66	0.145	957	451	114	0.78	162	61
G2	7.08	0.145	960	453	118	0.83	212	58
G3	6.52	0.125	687	411	102	0.62	124	21
H1	7.43	0.145	962	456	134	0.89	321	68
H2	6.59	0.144	954	450	112	0.77	237	64
H3	6.51	0.125	887	398	102	0.57	129	62
I1	6.38	0.153	698	368	89	0.46	265	105
I2	7.23	0.115	505	369	89	0.46	268	67
I3	6.12	0.068	426	247	54	0.36	256	45
J1	6.56	0.132	612	426	106	0.67	115	23
J2	6.56	0.135	914	436	108	0.67	265	46
J3	6.58	0.136	861	447	112	0.68	127	84

EC — electrical conductivity, TA — total alkalinity, TH — total hardness, TDS — total dissolved solids.

Table 5
Levels of water quality parameters in villages of fluorotic area (F1).

Site identity	pH	EC (mS/cm)	TDS (mg/l)	TH (mg/l)	TA (mg/l)	F^- (mg/l)	Cl^- (mg/l)	$SO4^{2-}$ (mg/l)
A1	6.94	0.155	1025	513	147	1.88	248	38
A2	7.11	0.169	1102	541	169	1.77	456	123
A3	7.12	0.167	986	568	214	1.91	125	45
B1	7.13	0.168	1125	369	103	1.94	214	46
B2	6.94	0.126	889	513	147	1.67	248	38
B3	6.38	0.178	1015	458	145	1.93	269	56
C1	6.89	0.185	1025	251	108	1.67	310	124
C2	7.11	0.130	1056	523	158	1.76	265	68
C3	7.63	0.158	856	540	167	1.87	410	36
D1	7.15	0.168	983	245	215	1.89	332	87
D2	7.06	0.157	1025	516	147	1.76	346	23
D3	7.12	0.214	756	520	123	1.68	492	45
E1	6.84	0.164	969	458	89	1.34	354	102
E2	7.71	0.158	1045	521	157	1.69	241	48
E3	7.11	0.168	1056	268	158	1.75	254	64
F1	7.15	0.171	1171	580	216	1.98	237	86
F2	7.11	0.212	1102	542	169	1.78	359	147
F3	6.26	0.132	689	520	153	1.69	412	56
G1	6.87	0.154	1011	487	145	1.82	321	68
G2	6.88	0.101	642	510	145	1.64	368	21
G3	7.61	0.173	1102	542	169	1.78	359	147
H1	6.75	0.187	967	456	68	1.37	412	144
H2	6.86	0.223	1003	468	135	1.28	369	101
H3	7.15	0.106	812	573	214	1.96	244	78
I1	6.85	0.113	980	458	59	1.68	412	26
I2	6.75	0.147	968	456	76	1.42	475	58
I3	6.83	0.134	968	347	124	1.53	210	45
J1	6.68	0.159	963	512	121	1.37	387	87
J2	7.12	0.152	1120	253	186	1.83	214	61
J3	7.14	0.214	1120	563	210	1.86	158	128

EC — electrical conductivity, TA — total alkalinity, TH — total hardness, TDS — total dissolved solids.

parameters on fluoride ion concentration are shown in Table 3 and Fig. 4 and the levels of fluoride ion and other water quality parameters in drinking water from all the study areas were mentioned in Tables 4, 5 and 6.

3.3. Influence of water fluoride level on total fluoride intake

Daily fluoride intake registered a significant increase from 3.28 mg to 15.25 mg per day ($r = 0.9998$, $p < 0.015$), if the water fluoride level

Table 6
Levels of water quality parameters in villages of fluorotic area (F2).

Site identity	pH	EC (mS/cm)	TDS (mg/l)	TH (mg/l)	TA (mg/l)	F^- (mg/l)	Cl^- (mg/l)	$SO4^{2-}$ (mg/l)
A1	7.35	0.245	869	746	186	2.47	386	126
A2	7.42	0.252	1645	668	285	2.54	557	159
A3	6.82	0.153	963	714	310	2.56	455	54
B1	7.21	0.256	529	589	224	2.14	364	110
B2	7.22	0.284	1245	612	217	2.16	451	151
B3	6.58	0.125	752	923	542	3.11	61	24
C1	7.34	0.366	668	684	256	2.35	664	127
C2	7.18	0.178	896	589	128	2.11	426	136
C3	6.85	0.203	1288	651	347	2.23	458	128
D1	7.35	0.228	1548	721	256	2.36	545	184
D2	7.58	0.126	586	689	248	3.24	54	31
D3	7.45	0.356	1687	756	368	2.58	668	69
E1	6.38	0.268	1827	780	418	2.66	447	37
E2	7.25	0.365	659	655	240	2.24	467	131
E3	7.45	0.256	1654	754	321	2.56	571	59
F1	7.47	0.121	2145	856	446	2.84	25	15
F2	6.33	0.125	1233	586	448	3.07	54	21
F3	6.61	0.124	1368	658	243	2.24	267	129
G1	7.26	0.345	1369	658	247	2.24	580	190
G2	7.3	0.386	875	512	254	2.27	660	110
G3	7.22	0.316	1250	612	225	2.18	560	148
H1	7.24	0.288	1257	645	225	2.21	590	138
H2	7.45	0.156	1742	772	368	2.58	165	98
H3	7.31	0.321	1458	681	256	2.34	564	124
I1	7.45	0.188	683	459	128	2.68	557	57
I2	6.22	0.121	2102	627	446	2.76	65	44
I3	7.35	0.228	1578	740	258	2.41	446	108
J1	6.85	0.258	986	775	414	2.64	557	56
J2	7.54	0.144	2248	531	312	3.11	55	28
J3	7.16	0.126	1023	586	217	2.11	373	162

EC — electrical conductivity, TA — total alkalinity, TH — total hardness, TDS — total dissolved solids.

Table 7
Contribution of various sources on total fluoride intake in selected regions.

Source of fluoride	Quantity intake per day	Fluoride concentration (mean ± S.D)			Amount of fluoride intake (mg/day)		
		C	F1	F2	C	F1	F2
Drinking water	2.5 L	0.54* ± 0.18	1.72* ± 0.17	2.50* ± 0.33	1.35 ± 0.45	4.31 ± 0.42	6.25 ± 0.81
Food	1.6 kg	0.65@ ± 0.11	3.20@ ± 0.35	4.54@ ± 0.43	1.04 ± 0.17	5.11 ± 0.56	7.27 ± 0.69
Tea	250 ml	2.60* ± 0.67	3.80* ± 0.72	5.18* ± 0.82	0.65 ± 0.17	0.95 ± 0.18	1.30 ± 0.20
Coffee	150 ml	1.57* ± 0.76	2.34* ± 1.03	2.85* ± 0.72	0.24 ± 0.11	0.35 ± 0.15	0.43 ± 0.11
Total fluoride intake (mg/day)		3.28	10.72	15.25			

(mg/l)*, (mg/kg) @.

increased from 0.54 to 2.5 mg/l. Contributions of fluoride through water in the control areas, fluorotic areas F1 and F2 were 41%, 40% and 41% respectively, whereas through food were 32%, 48% and 47% respectively. Consumption of tea contributes substantial fluoride to the daily fluoride intake and it was 20% in control area and 9% in both fluorotic areas F1 and F2. Coffee samples also afford considerable fluoride to daily fluoride intake; the values are shown in Table 7. The relationship between water fluoride levels with daily fluoride intake is mentioned in Table 2 and shown in Fig. 3.

3.4. Impact of fluoride ion concentration in drinking water on bone fluoride level

Mean fluoride level in control areas' goat bone was 144 ± 26.5 mg/kg, whereas fluorotic areas F1 and F2 registered more than two fold higher fluoride level in the bone (Table 8). The linear relationship between bone fluoride level and water fluoride level is established by the correlation coefficient value ($r = 0.715$, $p = 0.493$). Regression plots between water fluoride levels and bone fluoride levels elucidate that the increase of every 0.5 mg/l unit of fluoride in drinking water may progressively increase the bone fluoride level by 52 mg/kg unit within 2 to 3 years. The limitation of this study is that the quantity of drinking water consumed by the goats on daily basis from the selected areas may not be known. The numerical relationship between the bone fluoride levels with water fluoride levels in the respective areas were mentioned in Table 2 and Fig. 3.

4. Discussion

4.1. Effect of fluoride level in drinking water and other dietary sources on fluorosis prevalence

The drinking water fluoride level has significant influence on fluorosis prevalence even though it contributes only 41% of the total fluoride intake per day, excluding the water used for cooking and food processing. This may due to nearly 100% bioavailability of soluble fluoride in drinking water (Ekstrand et al., 1984; Whitford, 1996; Maguire et al., 2005) and it get absorbed readily through the gastrointestinal tract without much intervention of interfering elements such as Ca, Mg and Al in the form of HF (Ekstrand et al., 1978; Ekstrand and Ehrnebo, 1979; Jackson et al., 2002). Inside the stomach, low pH gastric acid favors the formation of the HF^o complex, which comprises over 90% of the total fluoride at pH 2 (Doull et al., 2006). HF^o is readily absorbed from both the stomach and small intestine by a process of simple diffusion, and once it enters the less acidic mucosa, it dissociates

Table 8
Mean of various parameters in selected regions.

Areas	Water fluoride level (mg/l)	% of fluorosis incidence	Community fluorosis index (CFI)	Total fluoride intake (mg/day)	Bone fluoride level (mg/kg)
C	0.54	0	0	3.28	144 ± 27
F1	1.72	56	1.03	10.72	273 ± 39
F2	2.5	70	1.30	15.25	347 ± 32

to release fluoride (Whitford and Pashley, 1984; Nopakun and Messer, 1990; Whitford, 1994; Whitford, 1996) but, the absorption of less soluble inorganic and organic fluorides from food and beverages is more complicated, and a variety of dietary factors can either increase or decrease the amount that is absorbed (Ericsson, 1968; Cremer and Buttner, 1970; Whitford, 1994; Cerklewski, 1997; Ozsvath, 2009). Water used for cooking food or for preparation of tea or coffee infusion has to be boiled, upon boiling, the fluoride level increase proportionally due to loss of volume by evaporation on heating (Grimaldo et al., 1995); this ultimately enhances the fluoride intake through food and beverages. This study indicates that through quantitative numerical relationship between water fluoride level and total fluoride intake, if water used for drinking or food processing has fluoride level more than 0.65 mg/l it enhances the total fluoride intake more than the standard limit of 4 mg/day for normal adults recommended by the Agency for Toxic Substance and Disease Registry (Agency for Toxic Substances and Disease Registry, 1993) and the National research council, USA (NCR, 2001). Murray (1986) also reports that fluoride intake per individual per day ought to range from 0.2 mg in infants to 5.0 mg in adults. Tea consumption enhances the daily fluoride intake level which may be due to the characteristic biological behavior of the tea plants of selectively absorbing more fluoride from the soil and progressive fluoride accumulation and liberation and concentration of more fluoride through boiling of water for preparing tea infusion as reported in an earlier study (Cao et al., 2000).

4.2. Effect of water quality parameters on fluoride ion concentration in drinking water

Fluoride is present in the water as fluoride ion, due to the almost complete dissociation of the parent fluoride compounds. The undissociated proportion is between 10^{-18} and 10^{-30} of the dissociated fluoride ions over the pH range of 6 to 9. The solubility of CaF_2 that is present in minerals increases with the increase in total alkalinity in the ground water according to the following reactions (Ramamohana Rao et al., 1993; Saxena and Ahmed, 2001):

$$CaF_2 + CO_3^{2-} \rightarrow CaCO_3 + 2F^- \quad (3)$$

$$CaF_2 + 2HCO_3^- \rightarrow CaCO_3 + 2F^- + H_2O + CO_2 \quad (4)$$

The carbonate and bicarbonates are also hardness producing ions. A positive correlation of total alkalinity with fluoride ($r = 0.83$) elucidates leaching of fluoride from fluoride bearing minerals into groundwater at alkaline environment or in other words increase of pH of the water enhances the leaching of fluoride from the minerals containing fluoride. Even though alkalinity and pH are the important factors that has influence on fluoride ion concentration in drinking water, the correlation between pH and fluoride is weak, because increase of alkalinity is due to the increase of carbonate and bicarbonate ions, these ions not having direct influence on pH level like hydroxyl ion, so the increase of alkalinity does not increase the pH level linearly. This is also suggested by Wodeyar and Sreenivasan

(1996), Subba Rao et al., 1998 and 2003. A higher level of total dissolved solids improves the ionic strength, leading to increase the solubility of CaF_2 in groundwater (Perel'man, 1977). Normally fluoride endemic area water samples show slightly higher values of some of the water quality parameters such as alkalinity, hardness and pH, increase of these parameters more than the prescribed limit may affect the quality of the drinking water. In general many of the drinking water samples from non fluoride endemic areas contain fluoride level from 0.4 to 1.0 mg/l showing safe limit of other water quality parameters such as total alkalinity, pH and hardness and acquiring quality to drink. So drinking water having fluoride ion concentration more than 1.0 mg/l in fluoride endemic areas needs further attention about the level of the other water quality parameters for maintaining the good quality of the drinking water.

4.3. Effect of fluoride ion concentration in drinking water on bone fluoride level

The chronic intake of fluoride-rich water in endemic areas F1 and F2 leads to a significant increase of bone fluoride level that leads to enhance the risk of skeletal fluorosis. The threshold level of fluoride ingestion needed to cause skeletal fluororis varies depending on water intake, water quality, and other dietary factors (Jolly et al., 1969; Raja Reddy et al., 1985). Normally the fluoride exposure dose level from drinking water decreases with increase of age (Viswanathan et al., 2009). Approximately 99% of the fluoride in the body is associated with skeletal tissues (Whitford, 1994; Urbanska et al., 2001) and nearly 50% of the absorbed fluoride in each day becomes associated with calcified tissues within 24 h and the remainder is excreted in the urine (Neuman and Neuman, 1958; Whitford, 1996; Cerklewski, 1997) but, the uptake of fluoride by the skeleton is most efficient in children and decreases with age (Whitford, 1999; Ozsvath, 2009) and this process may discontinue after the age of 55 (Rao, 2003). In order to maintain the safe fluoride level in the bone and avoid further fluorosis risk due to the excess fluoride accumulation in the bone through fluoride absorption from water and food stuffs, the drinking water fluoride level should be maintained below 1.0 mg/l.

4.4. Prediction of optimal fluoride level

It is generally believed that organisms, including human beings receive fluoride mainly from drinking water sources and that the total daily intake of fluoride by individuals from country to country depends on the amount of fluoride present in the water and in other sources including foodstuffs. Many of the previous studies from various countries indicated that the generalized guidelines for optimal fluoride level cannot be applied universally due to the various diverse factors that influence fluoride exposure. A fluoride level of 0.7 to 1 ppm, which is considered optimal for Austria (Nell and Sperr, 1994), may be considered to be too high for Bophuthatswana (Zietsman, 1991) and Sudan (Ibrahim et al., 1995), where fluorosis has been observed at 0.5 ppm. The upper limits of fluoride in drinking water of Senegal have been recommended at 0.6 ppm (Brouwer et al., 1988). From this study, we identified that most of the villages in Nilakottai block of Dindigul District in Tamil Nadu comprise more than the recommended upper safe fluoride limit of 1.5 mg/l in drinking water prescribed by the World Health Organization (WHO, 1986), in order to avoid further risk of fluorosis and control its prevalence; the safe fluoride level in drinking water for the selected regions should be maintained in the level of 0.5 mg/l. By considering the cumulative progress on bone fluoride level in fluoride endemic areas' animal bone, it is necessary to maintain the fluoride level in drinking water not more than 0.5 mg/l to avoid the risk of fluorosis. The observation through clinical survey indicated that in both fluoride endemic areas, dental fluorosis was accompanied by considerable brownish discoloration of the teeth, which constitutes a serious aesthetic and social problem, because the people use some hard materials to scrap off the strain, thus aggravating fluorotic damage to the teeth.

5. Conclusion

Water quality parameters such as alkalinity, pH and hardness have high impact on water fluoride level due to the promotion of fluoride from fluoride containing minerals by carbonates and dissolved solids. Fluoride in drinking water considerably contributes to the daily fluoride intake, but more than 50% of the fluoride in total fluoride intake per day is derived from food and beverages such as tea and coffee. The cumulative progress of bone fluoride level that depends on water fluoride content, suggests that the bone fluoride level may be useful as biomarker of fluoride. In order to maintain the safe level of total fluoride intake, to get assured about the good quality of drinking water and bone health, the optimal fluoride level in drinking water in the selected fluoride endemic areas in Dindigul district in Tamil Nadu, South India is set to be in the range between 0.5 and 0.65 mg/l. Regression analysis is a successful tool for predicting the optimal safe fluoride level in drinking water for fluoride endemic areas by comparing it with nearby non endemic areas. This type of approach for assessing the optimal fluoride level in drinking water in the selected regions is more appropriate and is much helpful for governmental and non governmental organizations in supplying drinking water with appropriate low fluoride content.

Acknowledgement

We are very much thankful to our beloved Professor Dr.G. Karthikeyan for his exceptional guidance and blessings and we extend our thanks to Dr.P.Bothi raja, Post Doctorate Fellow, University Sains Malaysia for his excellent technical assistance.

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