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PII: S2772-4166(23)00083-9
DOI: https://doi.org/10.1016/j.hazadv.2023.100312
Reference: HAZADV 100312

To appear in: *Journal of Hazardous Materials Advances*

Received date: 18 March 2023
Revised date: 2 May 2023
Accepted date: 4 May 2023

Please cite this article as: Kamal Kishor , Jonathan S. Singsit , Charles U. Pittman Jr. , Dinesh Mohan , Demographic study of fluoride excretion vs intake: Influence of variables, *Journal of Hazardous Materials Advances* (2023), doi: https://doi.org/10.1016/j.hazadv.2023.100312

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Highlights:

- Mean urinary fluoride (U_F) excreted values (2.21-4.50 mg/L) were higher than mean water fluoride (W_F) intake (2.31–2.45 mg/L).
- Percent of mean urinary fluoride exceeding mean water fluoride increased from 5.63, 48.05 to 83.67 % in Son (S), Father (F) and Grandfather (GF) respectively.
- Age and its interplay with different habits is a significant factor influencing fluoride excretion.
- Overall, vegetarians, alcohol consumers, smokers and regular brushers excrete more fluoride than their counterpart.

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Demographic study of fluoride excretion vs intake: Influence of variables

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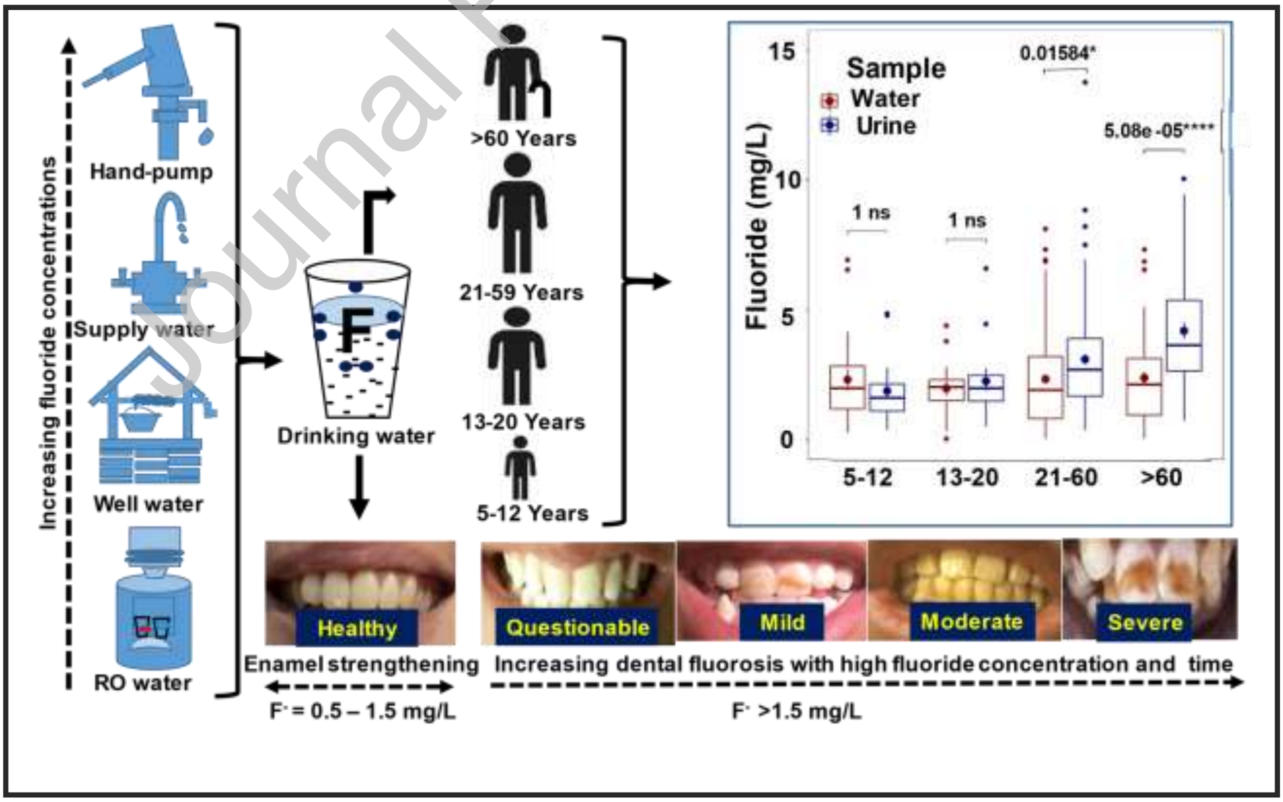
Graphical abstract



Abstract

Urinary fluoride is a widely used biomarker in public health and epidemiological studies, globally. The present study investigates fluoride exposure and compares the effect of different variables on the sampled population of the Rohtak district, Haryana, India. The relation between fluoride intake, excretion, and retention of fluoride across different age groups is unveiled. Cross-sectional study of urine samples of 207 inhabitants between the ages 5- to 96years and of 83 drinking water samples were analyzed to determine fluoride using an ion selective fluoride electrode (potentiometric method). Drinking water was sourced from supply water (n = 28), hand-pumped water (n = 45), well water (n = 3) and RO water (n = 7). Urine samples were categorized based on three different generations: Grandfather (GF), Father (F), and Son (S) from the 71 villages covered in the study. Mean urinary fluoride excretion was significantly correlated to age with values 4.50 ± 2.56, 3.42 ± 2.00 and 2.44 ± 1.30 mg/L, in declining order of these age groups, respectively. The percent increase in fluoride excretion in the urine over that consumed in the intake water was 83.67, 48.05 and 5.62% for GF, F and S respectively, indicating that other sources of fluoride intake were responsible for the increment. The eigenvalues of PCA are 1.36, 1.25, 1.19 and 1.18 for PC1, PC2, PC3 and PC4, respectively. In all plots p-values show statistical significance ($p<0.05$), except for those abstaining from alcohol in the father (FA) category. Sons in the smoking category (R^2 = 0.30, $P<0.001$) exhibited higher fluoride excretion than non-smokers. The group GF and F group participant regression results were found to be the opposite. Linear regression, PCA, Tukey's test, and radar chart methods were used to examine the relation between fluoride exposure and other variables like the water source (hand-pump, well, supply,

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reverse osmosis), diet (vegetarian/non-vegetarian), habits (smokers/non-smokers, alcohol consumers/non-consumers, regular/irregular brushing). Study concluded that age is the most significant variable which influences the retention and excretion of fluoride.

Keywords: Urinary fluoride, demographic effect, habitual influences, health, principal component analysis.

1. Introduction

Globally, 200 million people live in fluoride prone areas and are at risk of adverse health effects (Edmunds & Smedley, 2013; Rahman et al., 2020; Su et al., 2021). A narrow range of fluoride drinking water concentrations from 0.5 to1.5 mg/L F^- promotes hard tissue development, while lower and higher concentrations can cause dental caries and dental/skeletal fluorosis, respectively (Ding et al., 2011; Li et al., 2009; Mohan et al., 2012; Rahman et al., 2020; Rango et al., 2017; Rugg-Gunn et al., 2011). Fluorosis occurs when too much fluoride is ingested during teeth development leading to discoloration and teeth weakening. Skeletal fluorosis occurs when fluoride accumulates in the bones and causes them to harden and become weaker and more brittle. This can lead to joint pain, stiffness, and limited mobility. These conditions typically occur with high fluoride intake, often in areas with naturally high fluoride levels in water (Rahman et al., 2020). A permissible limit of 1.5 mg/L was prescribed by the Bureau of Indian Standards (BIS) and WHO (BIS, 2012) (WHO, 2011). Fluoride contamination and related health issues were reviewed (Amini et al., 2008; Kashyap et al., 2021; Kut et al., 2016; Maity et al., 2021; Podgorski et al., 2018; Solanki et al., 2022; Wu et al., 2022).

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Soft tissue contains a meager 1% of the body burden of fluoride, while the remaining fluoride is calcified in the hard tissues (Buzalaf & Whitford, 2011; Rugg-Gunn et al., 2011; Whitford, 1996). Accumulating excessive fluoride disrupts plant and animal physiological functions (Ghosh et al., 2013). Prolonged exposure to high fluoride levels leads to such health problems as: fluorosis (Rahman et al., 2020; Revelo-Mejía et al., 2021; Srivastava & Flora, 2020; Yadav et al., 2019), lower children IQ (Ali et al., 2023; Choi et al., 2012; Grandjean, 2019; Miranda et al., 2021; Wang et al., 2020), attention deficit hyperactivity disorder (ADHD) (Bashash et al., 2018; Malin & Till, 2015) and hypothyroidism (Reddy & Deme, 1979; Singh et al., 2014; Wang et al., 2020). The younger populace is more prone to enamel fluorosis when exposed to high fluoride levels (Saeed et al., 2020). Ingested fluoride takes the form of hydrofluoric acid (HF) in the stomach.

Fluoride exposure can be determined by fluorine uptake in teeth, bone, plasma, urine, nails, hair, sweat, milk and saliva. However, urinary fluoride is the best indicator of fluoride exposure and is the most widely used biomarker (Rugg-Gunn et al., 2011; Villa et al., 2010; Whitford, 2005).

Habits like smoking (Koç et al., 2018; Kuo et al., 2008; Laisalmi et al., 2003; Schwarz et al., 2020), alcohol consumption (Prystupa et al., 2021) and intake through dietary preference (Idowu et al., 2019; Spak et al., 1989), and brushing with toothpaste (Bentley et al., 1999) can contribute to fluoride intake (Rahman et al., 2020). Tobacco smoking is a worldwide prevalent habit and its detrimental impacts are widely acknowledged. The constituents present in tobacco smoke can cause harmful biochemical reactions in the body. Smoking may impair the excretion of fluoride (F)

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leading to high fluoride serum concentrations. This can be a concern as high levels of fluoride can be toxic to the body (Koç et al., 2018). Previous urinary fluoride studies have been performed in the state of Haryana (Haritash et al., 2018; Kumar et al., 2017; Singh et al., 2007; Yadav et al., 2006; Yadav et al., 2007; Yadav & Lata, 2003). However, they are often unidimensional and confined to a particular age group (Haritash et al., 2018; Kumar et al., 2017), consumption habit (Yadav et al., 2007) or limited to a groundwater quality survey (Garg & Malik, 2004; Gupta & Misra, 2018; Singh et al., 2007). This current study is more comprehensive, covering dietary preferences and lifestyle habits across all age-groups. It seeks to examine the association between urinary and drinking water fluoride and unravel the influence of different variables. This research focuses on the quantitative assessment of the association between variables (age group, weight, height, BMI, food and drinking habits) on fluoride intake and excretion.

2. Materials and Methods

2.1 Site specifications and sampling

The study area is situated in Rohtak district, Haryana, India located between latitudes 28 ° 40.4 6 N and 29 ° 06.0 8 N and longitudes between 76 ° 12.4 0 E and 76 ° 52.0 0 E. Study maps were plotted by using Arc GIS 10.5 tool **(Figure 1)**. Urine (n = 207) and drinking water (n = 83) samples were collected from 71 villages during the month of December, 2017. Drinking water samples were collected from four sources viz; supply water (SW), hand pump water (HP), well water (WW) and reverse osmosis treated water (RO) **(Figure 2, Table 1SM)**. Drinking water samples were collected from households of the participants. Participants in this study were selected using a stratified

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and multilevel random sampling approach. Urine (n = 207) samples were collected early mornings in 100 mL high density polyethylene bottles. The samples were collected from the males falling within the age group 5-96 years. Sample collection and laboratory analysis of fluoride in water and urine were done by standard recommended methods (Li et al., 2009; Rango et al., 2017). Ethics approval was received from the Institutional Ethics Review Board (IERB), Jawaharlal Nehru University, New Delhi, India (reference no: 2022/Ph.D. Student/298)

2.2 Selection of participants

Out of 269 samples, only 207 were selected due to mismatch in urine samples between members of the same family. Only those individuals who were long-term settlers in the villages (> 10 years) were sampled. At the time of sample collection, at least two generations were selected from each household in order to ensure representation from different age groups. The subjects in this study are broadly divided as: grandfather (n = 48), father (n = 78) and son (n = 81). A detailed schematic flow chart covering the variables and sample sizes is given in Figure 1.

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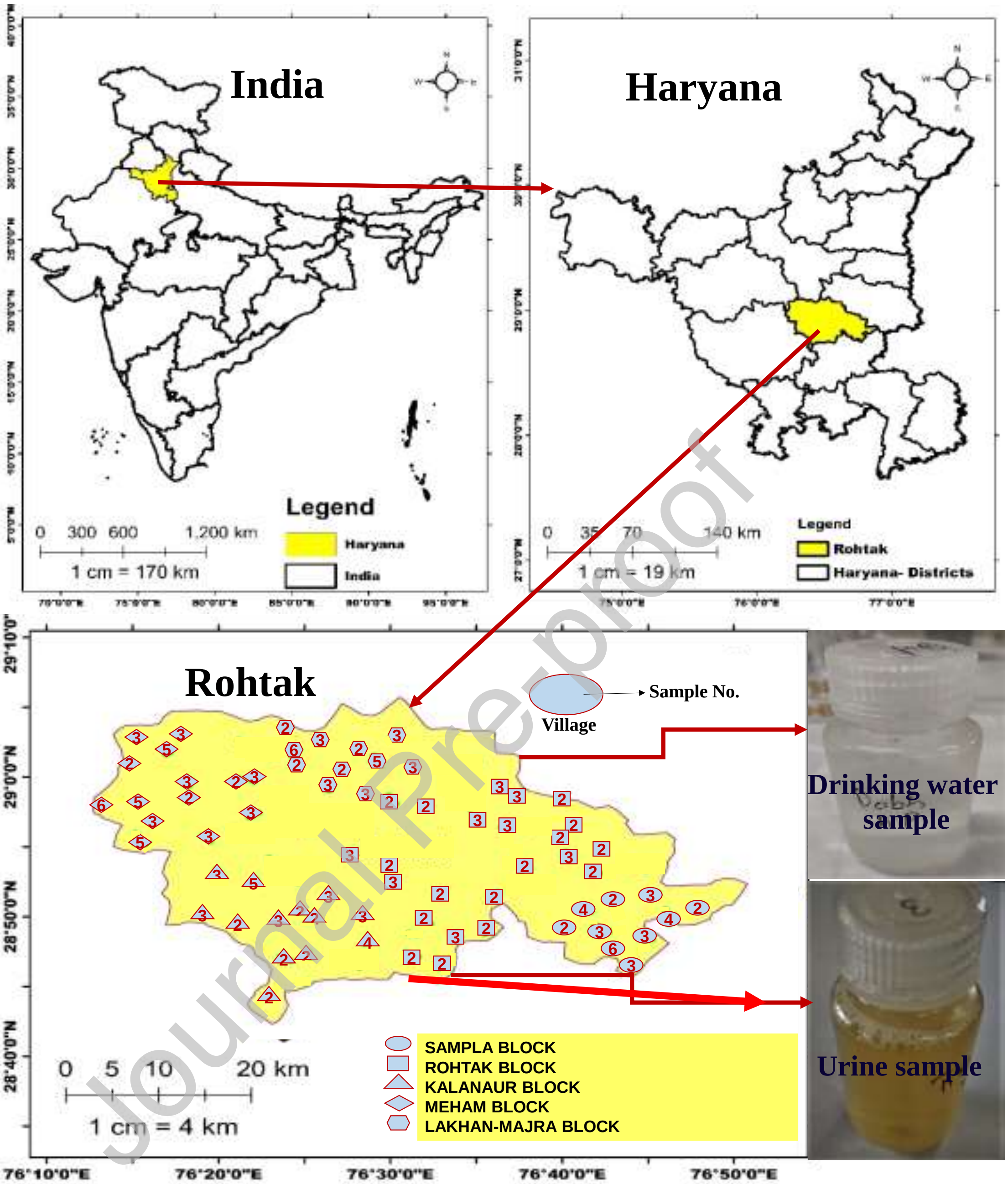

Figure 1. Study area map showing Rohtak district, Haryana, India, where urine and water samples were collected.

2.3 General data collection

The demographic details were collected by face-to-face interviews with the respondents and providing them with detailed questionnaires. Questions were orally

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translated in the local language whenever required in order to ensure clarity. Demographic data was collected from the questionnaires and summarized in Table SM1. The detailed questionnaire given in the supplementary section, (Table SM1) contains information on age, height, weight, BMI, sex, education, occupation, tooth count (dental carries), dietary preference and habits (smoking, drinking, brushing).

- **Age:** Urine samples were taken from people in the age groups from 5 to 96 years. One or a maximum of two families were selected from each village, in which at least two or maximum three generations of people were categorized as: grandfather (GF), father (F) and son (S).
- **BMI (Body mass Index):** BMI is calculated by using body weight and height of the person. Height of each participant (barefoot) was measured with a measuring tape. Weight was measured with a portable digital balance while ensuring that participants were barefoot and lightly clad. Some exceptions were made for the older people who could not stand upright where their height and weight were enquired based on an earlier measurement; care was taken to ensure that there was no overestimation. Body mass index, BMI is calculated as,

$$\text{BMI} = \frac{\textbf{Weight (Kg)}}{(\textbf{Height})^2\ (\textbf{m})^2} \qquad (1)$$

- **Habits:** Habits like smoking, drinking alcohol, brushing and dietary preferences were a part of the questionnaire. From a total of 207 participants, 150 of them drank alcohol, 120 were smokers, 123 vegetarians and 115 brushed regularly representing 72.46, 57.97, 59.42 and 55.55 % of total participants **(Table SM1, Figure 2)**. Brushing habits are further classified into regular brushing, irregular

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brushing and non-brushing which occupy a proportion of 55.55, 26.57 and 17.87 % respectively.

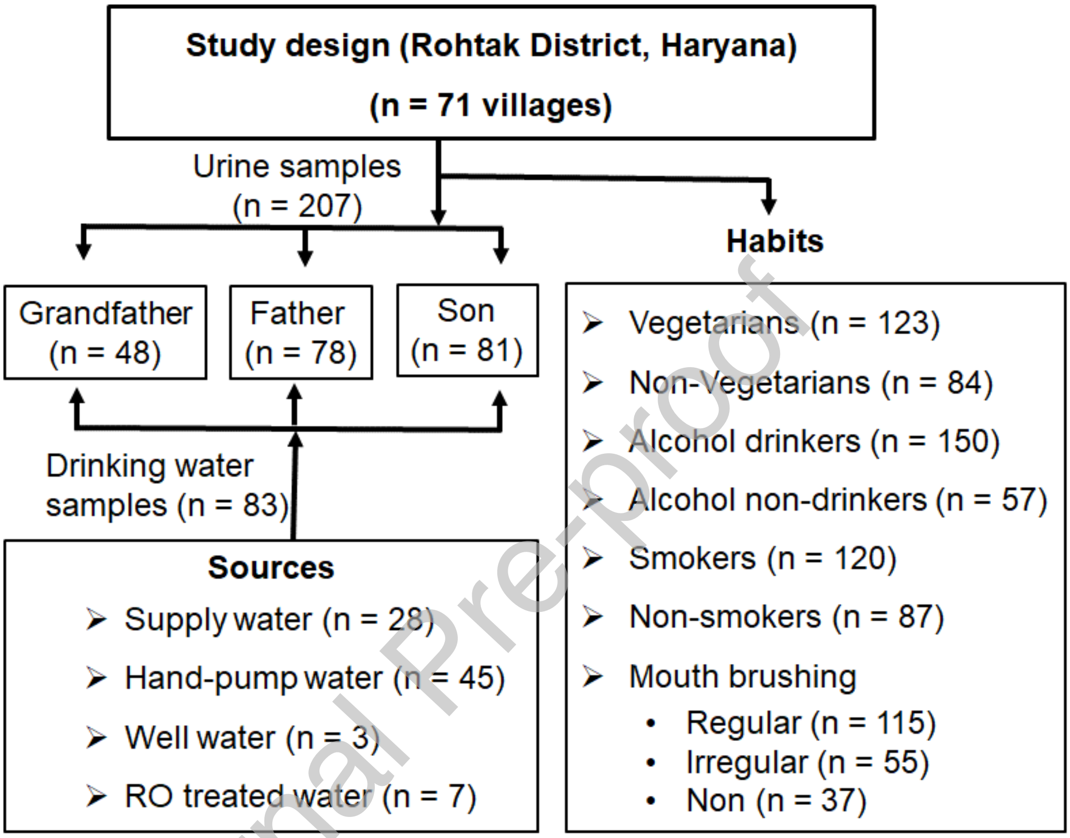

Figure 2. Schematic diagram showing the study design in Rohtak district, Haryana.

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2.4 Analytical procedure

Fluoride concentrations were analyzed in groundwater and urine samples by using a multi parameter ion meter (model Orion 5 star, Thermo Scientific). Standard fluoride solutions with concentrations: 0.25, 0.5, 1.0, 2.5 and 5 mg/L were prepared by serial dilution using a stock of 1000 mg/L. Wet anhydrous NaF (0.221 g) was dissolved in 1000 mL of deionized water to prepare the stock solution. Analytical Reagent (AR)

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grade chemicals were used throughout the analysis and the reagents were prepared in double distilled water (DDW). A fluoride selective electrode was calibrated using freshly prepared fluoride standards. Water samples were analyzed straight forward; however, the urine samples were diluted with DDW in a 1:1 ratio in a beaker (10 ml of urine sample and 10 ml of DDW) to give a combined diluted solution. Samples with high fluoride content were further diluted after which 2 ml of TISAB III (Total ionic strength adjustment buffer III) was added to 20 mL of the combined diluted solution in a 1:10 ratio before analysis. To check the reliability and precision of fluoride analysis, 30 samples were randomly re-analyzed. Electrode was immersed in known standards in between readings to ensure authenticity of the readings. The precision and accuracy for analytical work strictly followed quality assurance (Rasool et al.) and quality control (QC) described by (Rango et al., 2017).

2.5 Statistical approach

Data from different variables were presented as mean ± standard deviation (Gaur et al.) of fluoride in water and urine samples (Table 1). Statistical analysis including regression analysis, principal component analysis (PCA) and visualization using radar diagrams were performed in R-software version 3.6.3 (R core team 2020) and Microsoft Excel 2019. Radar diagrams of different variables were plotted categorically to show the percent increase in fluoride output from intake (drinking water). Regression analysis was applied to assess the relation between urinary and water fluoride with respect to lifestyle variables: smoking, alcohol consumption, food habits and drinking water sources. Furthermore, regression plots were applied to detect significant associations between different variables and urinary fluoride concentration. ArcGIS (ver.10.5)

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software was used for mapping of study area. One way ANOVA was also performed between different age groups with the dependent (urinary fluoride) and independent variables (water fluoride). The hypothesis testing employed two-tailed tests, thus a p value < 0.05 was considered statistically significant.

3. Results

3.1 Descriptive statistics: Urinary/water fluoride and characteristics of all variables

The fluoride concentration of 207 urine samples and 81 drinking water samples are presented in Table 1 and Table SM1. The mean ± SD of the various variables, water and urine fluoride concentration are shown in detail in Table 1. The mean ± SD of body mass index (BMI) in GF, F and S are revealed 20.91 ± 5.47, 24.97 ± 4.12 and 24.27 ± 5.00 respectively (Table 1). The mean BMI values for all three generations are within the normal weight range of 18.5 - 24.9 kg/m^2, with few exceptions in the father and son category(Table 1). Damage and loss of teeth amongst the older participants were documented during the interview and sample collection. The mean ± SD of number of teeth are: GF (10.56±10.13), F (24.7±5.06) and S (28.5±1.72). The study clearly identifies dental decay in those whose drinking water had elevated F^- levels and were older individuals. Age is an important variable as fluoride fluctuations in ingestion and excretion were observed in the dataset. Therefore, the age of the participants was categorized generation wise (GF, F & S) (Table 1) and range wise (<12, 13-20, 21-59, >60) (Table 2). The mean ± SD of the ages of GF, F and S are 71.85±11.58, 47.94±11.22 and 20.53±9.49 respectively. Mean urinary fluoride (U_F) value ranges from 2.21-4.5 mg/L F^-, which is higher than mean water fluoride (W_F) of 2.31–2.45 mg/L F^-. Similar trends where $U_F > W_F$ were reported which implies other fluoride sources existed apart from drinking water (Czarnowski et al., 1996; Li et al., 2009). In Tables 1 & 2, the percent increase denoted by *X* gives the percent of mean urinary fluoride excreted the mean water fluoride ingested (equation 3). Generation-wise, the urinary fluoride increases from 5.63, 48.05 to 83.67 % in S, F and GF, respectively (Table 1). Range-wise, the *X* value also increases from 9.31, 23.71, 32.18 and 75.62 in childhood (0-12 years), adolescence (13-20 years), adulthood (21-59 years) and elderly (>60 years), respectively (Tables 1 and 2) The drastic increment of *X* with age indicates low accumulation of fluoride amongst the older age group. Previous studies that support this study demonstrate higher urinary fluoride concentration occurs with age (Li et al., 2009). Children are more prone to fluoride related issues than adults because of higher retention and lower excretion (Li et al., 2009). The age variable exerts the most influence on the *X* value, with a tendency to increase with age irrespective of the habits (Table. 1).

$$X = \frac{U_F - W_F}{W_F} * 100 \qquad (3)$$

X = Percent of mean urinary fluoride exceeding the mean water fluoride ingested

U_F = mean of urinary fluoride concentration

W_F = mean of drinking water fluoride concentration

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Table 1. Urinary fluoride excretion compared to water fluoride affected by lifestyle habits and demographic variables when data is categorized generation wise.

	Grand Father (n = 48)[a]				Father (n = 78)[a]				Son (n = 81)[a]			
Demographic Variables	**(Variable)[a]**	**W_F (mg/L)[a]**	**U_F (mg/L)[a]**	***X* %**	**(Variable)[a]**	**W_F (mg/L)[a]**	**U_F (mg/L)[a]**	***X* %**	**(Variable)[a]**	**W_F (mg/L)[a]**	**U_F (mg/L)[a]**	***X* %**
Age (Years)	71.85±11.58	2.45±1.84	4.50±2.56	83.67	47.94±11.22	2.31±1.80	3.42±2.00	48.05	20.53±9.49	2.31±1.83	2.44±1.30	5.62
Weight (Kg)	59±16.01	2.45±1.84	4.50±2.56	83.67	73±11.82	2.31±1.80	3.42±2.00	48.05	62.6±26.06	2.31±1.83	2.44±1.30	5.62
Height (McMahon et al.)	169±5.97	2.45±1.84	4.50±2.56	83.67	171±7.18	2.31±1.80	3.42±2.00	48.05	152±36.60	2.31±1.83	2.44±1.30	5.62
BMI (Kg/m^2)	20.91±5.47	2.45±1.84	4.50±2.56	83.67	24.97±4.12	2.31±1.80	3.42±2.00	48.05	24.27±5.00	2.31±1.83	2.44±1.30	5.62
Number of tooth	10.56±10.13	2.45±1.84	4.50±2.56	83.67	24.7±5.06	2.31±1.81	3.42±2.00	48.05	28.5±1.72	2.31±1.83	2.44±1.30	5.62
Lifestyle Variables	**(Variable)[n]**	**W_F (mg/L)[a]**	**U_F (mg/L)[a]**	***X* %**	**(Variable)[n]**	**W_F (mg/L)[a]**	**U_F (mg/L)[a]**	***X* %**	**(Variable)[n]**	**W_F (mg/L)[a]**	**U_F (mg/L)[a]**	***X* %**
(Vegetarian)	28	2.53±1.80	4.93±2.86	94.86	44	2.03±1.50	3.42±1.94	68.47	51	1.82±0.98	2.06±1.48	13.19
(Non-Vegetarian)	20	2.34±1.94	3.91±2.00	67.09	34	1.88±1.65	3.36±2.08	78.72	30	2.01±1.60	2.15±0.96	6.96
(Smoking)	30	2.63±2.00	4.45±2.96	69.20	55	2.45±1.96	3.55±2.08	44.90	35	2.65±2.08	2.69±1.30	1.51
(Non-smoking)	18	2.15±1.54	4.10±1.79	90.69	23	1.97±1.32	3.09±1.75	56.85	46	2.06±1.60	2.16±1.21	4.85
(Alcoholic)	27	2.42±1.94	4.30±2.41	77.68	65	2.30±1.65	3.50±2.03	52.17	58	2.41±1.57	2.48±1.54	2.9
(Non-alcoholic)	21	2.49±1.75	4.76±2.79	91.16	13	2.36±2.33	3.14±1.92	33.05	23	2.30±1.90	2.15±1.24	-
Brushing (Regular)	8	2.75±1.58	3.43±2.01	24.72	54	2.15±1.50	3.18±2.32	47.91	53	2.42±1.71	2.44±1.65	0.8
Brushing (Irregular)	40	2.24±1.78	3.21±1.98	43.30	20	2.71±1.58	3.34±2.63	23.24	32	2.30±0.95	2.41±1.20	4.8

[n] = number of individuals, [a] = mean ± SD, W_{F} = fluoride in water, U_{F} = fluoride in urine, BMI = body mass index, *X* = Percent of mean urinary fluoride exceeding mean water fluoride.

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Table 2: Percent of mean urinary fluoride exceeding the mean water fluoride

Variables	Percent increase, X (%)[a]
Grandfather (GF)	83.7
Father (F)	48.0
Son (S)	5.5
Childhood (0-12 years)	9.3
Adolescence (13-20 years)	23.7
Adulthood (21-59 years)	32.2
Old (>60 years)	75.6
Regular brushing (RB)	27.3
Irregular brushing (IRB)	22.6
Alcoholic (A)	48.9
Non-alcoholic (NA)	23.5
Smokers (S)	37.2
Non-smokers (NS)	34.5
Vegetarian (V)	53.5
Non-vegetarian NV	18.5
RO treated water (RO)	98.2
Well water (WW)	95.1
Supply water (SW)	64.4
Hand pump water (HP)	22.3

[a]***X* (%) =** Percent of mean urinary fluoride exceeding the mean water fluoride (eqn 3)

3.2 Comparison between percent of fluoride output and other variables

Figure 3 is a radar graph representing the categorical data generation wise: grandfather (GF), father (F) son (S); age range: <12, 13-20, 21-59, >60; habits: regular brushing (RB), irregular brushing (IRB), alcoholic (A), non-alcoholic (NA), smokers (S) and non-smokers (NS); diet: vegetarian (V) and non-vegetarian (Kishor et al.); water source: hand-pump (HP), supply water (SW), reverse osmosis (RO) and well water (WW). The percent output of fluoride (in urine) compared to intake water fluoride concentrations are given for each category in Figure 3. Trends can now be visualized. Age-wise increment in fluoride output has been observed **(Figure 3a)**. Interesting habit category trends illustrate those irregular teeth brushers, non-alcoholics, non-smokers and non-vegetarian individuals all registered lower fluoride excretion from the body

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(Figure 3b). Percent mean urinary fluoride exceeding mean water fluoride increases from hand pump, supply water, well water and RO water **(Figure 3c)**.

3.3 Regression analysis

The regression analysis provides the mathematical relation between the independent variable (water fluoride) and the degree of influence it exerts on the value of the dependent variable (urinary fluoride). Within the generational category: “grandfather”, “father” and “son”, habit-wise regression analysis was performed and presented **(Figure 4 a, b, c)**. The age is the most influential factor for fluoride excretion therefore, we separated the habits generation-wise**.** They are abbreviated by the prefixes GF = grandfather, F = father, S = son in combination with one of the following suffixes for habit: V = vegetarian NV = non-vegetarian; A = alcoholic, NA = non-alcoholic, S = smokers, NS = non-smokers. Figure d is based on water source. There is a positive correlation between the independent and the dependent variable in all the habits studied.

3.3.1. Association between vegetarian and non-vegetarian diet

Figure 4a gives the relation based on diet (vegetarian vs non-vegetarian). In the category GF (R^2 = 0.42, p=0.005) and S (R^2 = 0.56, p<0.001) GFNV (R^2 = 0.35, p<0.001) showed a higher correlation with higher fluoride output per mg/L of F^- intake (**Figure 4a**). p values were significant (<0.05) among all categories except for vegetarian fathers (FV), **(Table SM2)**.

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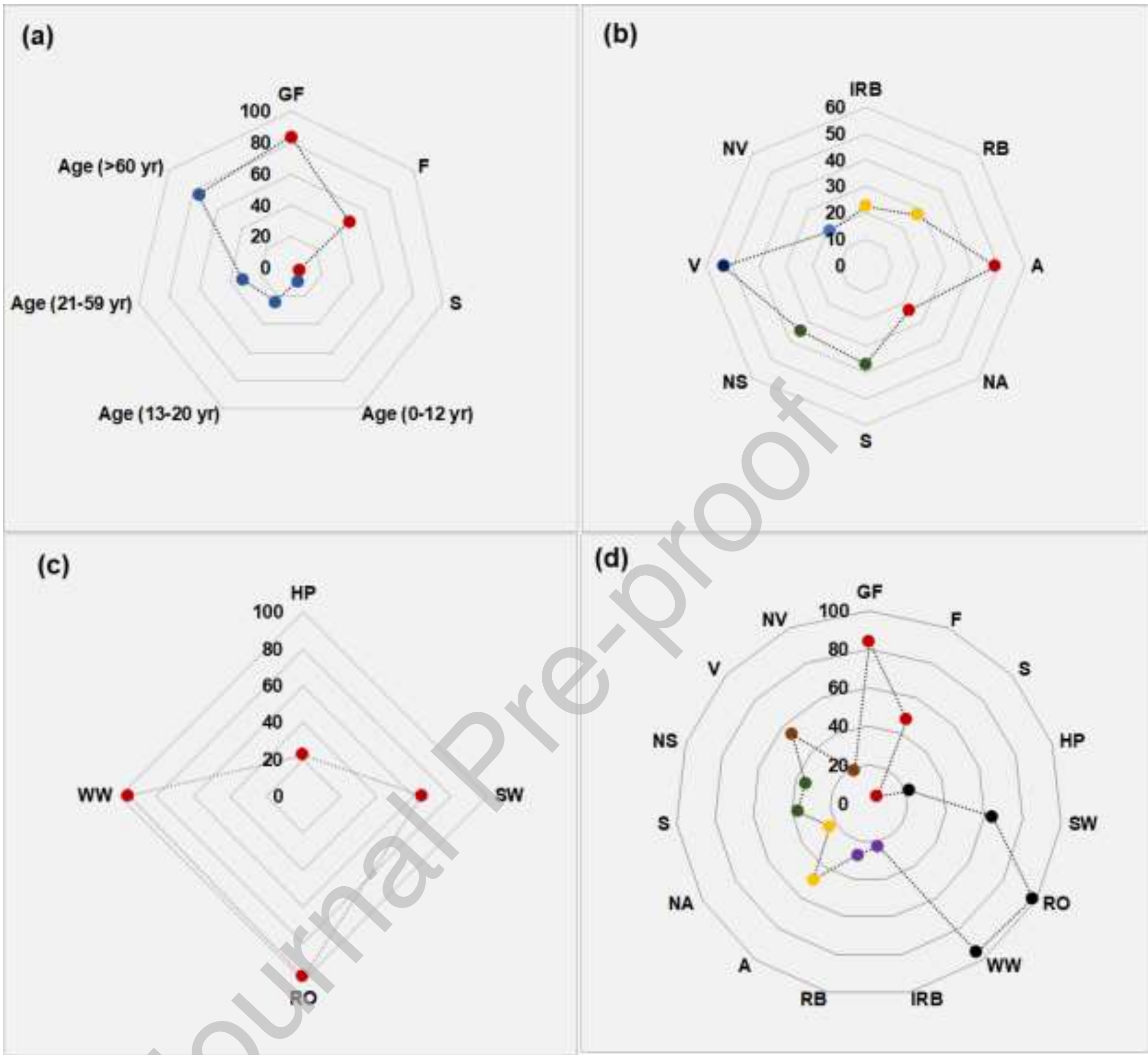

Figure 3: Radar graph showing the percent increase in fluoride output compared to intake based on different parameters. a) age, b) habits, c) water source d) aggregated data of all parameters.

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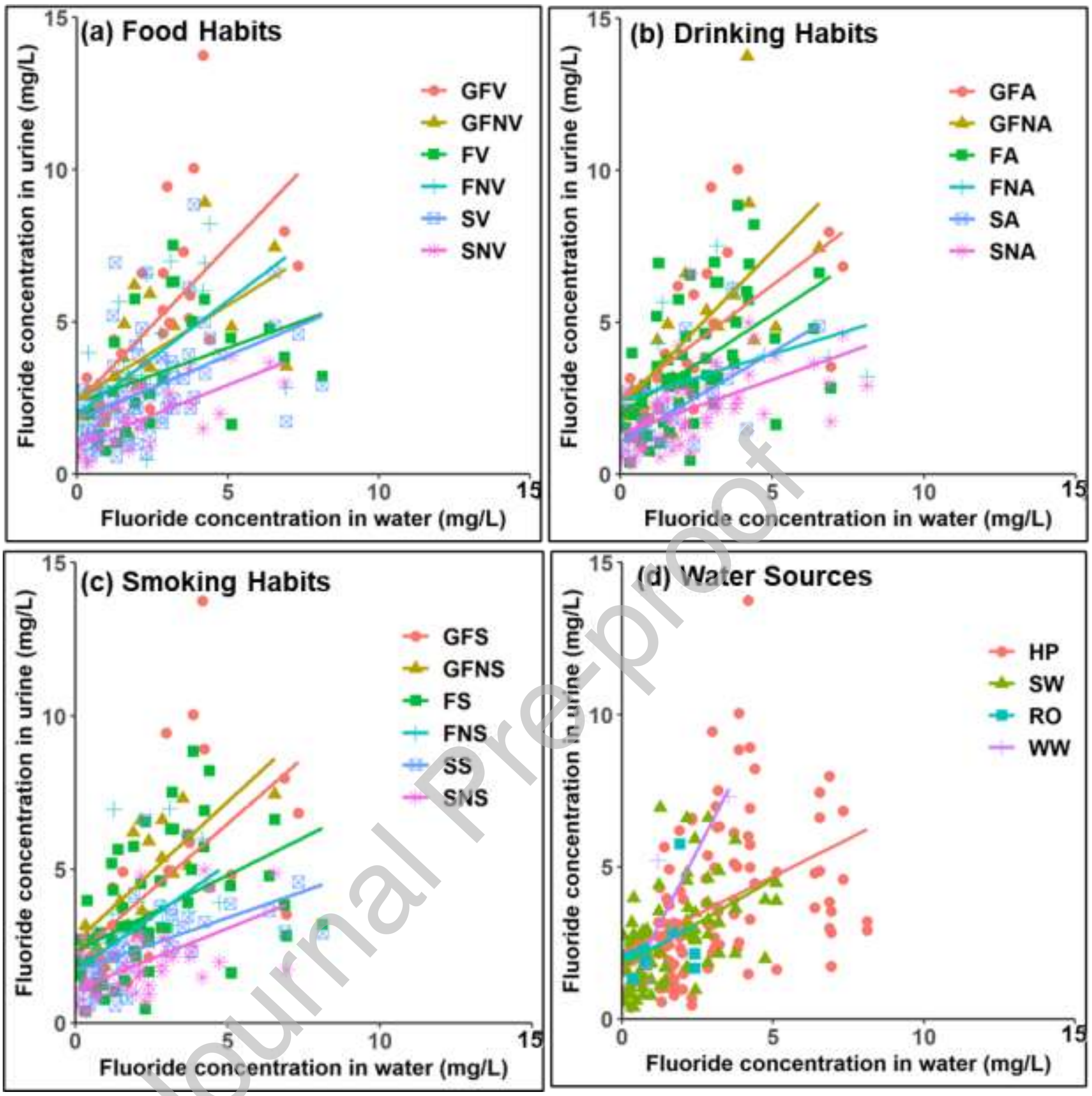

Figure 4: Regression graph showing correlation between the independent variable (Fluoride concentration in water) and the dependent variable (Fluoride concentration in urine). (a) Food habits; (b) drinking habits; (c) smoking habits and d) water source.

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3.3.2. Association between alcohol drinkers and alcohol abstainers

In Figure 4b, the alcohol consumers in the father (R^2 = 0.27, p<0.001) and son (R^2 = 0.32, p = 0.028) category shows a higher correlation with higher fluoride output per mg/L of F^- intake. Whereas those abstaining from alcohol in the GF category had a higher correlation coefficient (R^2 = 0.41, p = 0.0016). In all plots p-values show statistical significance (p<0.05), except for those abstaining from alcohol in the father category (Table SM2).

3.3.3. Association between tobacco smokers and non-smokers

Figure 4c represents the smokers and non-smokers. In the GF (R^2 = 0.62, p<0.001) and F^- (R^2 = 0.27, p=0.013) categories, (R^2 = 0.30, p<0.001) the non-smokers have a higher urinary fluoride output per mg/L of aqueous F^- intake. The p-values in all the plots are statistically significant. **(Table SM2)**.

3.3.4. Association between different drinking water sources

The hand pump water and supply water showed good correlation with a significant p value (<0.001), whereas fewer RO water and well water samples were a variable and therefore difficult to interpret statistical interpretation difficult (p value > 0.14). SW source showed a more significant correlation (R^2 = 0.25, p<0.001) than others **(Figure 4d)**.

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3.4 Principal Component Analysis

PCA bi-plot **(Figure 5)** exhibits a total of 10 variables, including age groups (5-12 years, 13-20 years, 21-59 years and >60 years) and habits (vegetarians/non-vegetarians, alcohol drinkers/alcohol abstainers, smokers/non-smokers). The symbols used for the different variables are A (alcohol drinkers), NA (alcohol abstainers), S (smokers), NS (non-smokers), V (vegetarians), NV (non-vegetarians). The variables toward origin point and away from origin define minimum and maximum variability on the basis of contribution of variables in totals (Nieto-Librero et al., 2017; Saeed et al., 2021). In addition, the angles of variables close to each other show good correlations whereas at 90° and 180° defined none and negative correlations, respectively (Nieto-Librero et al., 2017; Saeed et al., 2021).

Principal component analysis (PCA) revealed that first two principal components (PC1 and PC2) contributed 16.5% and 13.1% respectively of the total variance in the obtained data (Figure 5). PCA generated two different clusters, one representing fluoride in water and others for fluoride in Urine samples (Figure 5). First two PCs accounted for 29.6% of the standardized variance.

The distribution and association of fluoride in water and urine samples are represented in PCA bi-plot (Figure 5). Fluoride in urine (yellow color cluster) showed high variability in the data compared to fluoride in water (blue color cluster) samples. The age group of 5-12 years highly contributed in data variability and indicated strong negative correlation with the 13-20, 21-59 and >60 age group, whereas 13-20, 21-59 and >60 age group peoples showed strong positive correlation with each other no correlation(Figure 5).In 5–12 years age group, vector arrow inclined toward the water fluoride circle indicating

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less excretion and high accumulation compared to other age group variables (13-20, 21-59 and >60 years) **(Figure 5)**.However, among the habits, NA and NS showed high contribution PC1 and positively associated to each other. A and NV have less contribution in data variability but show positive correlation. NS and NA showed with the V and A.

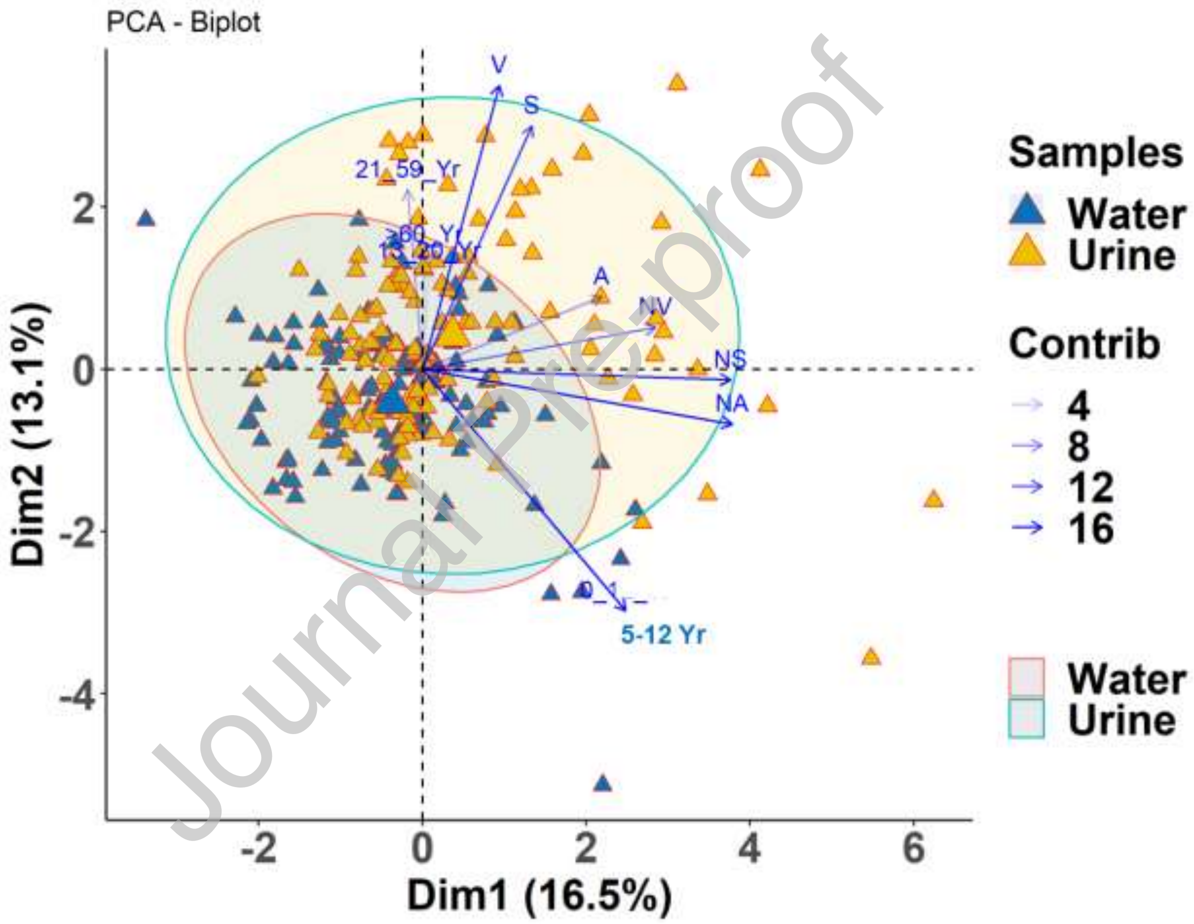

Figure 5. PCA bi-plot diagram of different variables with urinary fluoride and aqueous fluoride concentrations. Participant age (5-12, 13–20, 21-59, and >60 years), V = vegetarians, NV = non-vegetarians, A= alcohol drinkers, NA = alcohol non-drinkers, S = smokers and NS = non-smokers.

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4. Discussion:

Fluoride in the urine samples exceeds the drinking water levels in the majority of cases **(Table SM1)**. This indicates that additional fluoride is ingested from solid/liquid diet and inhalation of dust contaminated with fluoride (Czarnowski et al., 1996). Fluoride metabolism is affected by skeletal growth, renal condition, diet and genetics (Whitford, 1994). Factors acidifying the urine is known to increase fluoride retention (Whitford, 1994). About half the fluoride consumed associates with calcified tissues, while the remaining half is excreted in the urine. However, there is a greater retention within infants and children compared to adults (Whitford, 1994). Clearance of fluoride from the plasma occurs mainly through renal and skeletal means. Our significant findings showed fluoride excretion increases in older individuals, which make sense as skeletal growth subsides with age. (Tables 1 & 2, Figure 6). The (X) values of the sons was only 5.48 % indicating more retention than fathers and grandfathers (Table 2, Figure 3b) . Therefore, fluoride has a higher impact on younger males in agreement with previous studies (Ding et al., 2011; Pendrys, 1999).

Cigarettes can contribute about 0.01 mg F^{-}/kg body weight daily in regular smokers (Schwarz et al., 2020). Tobacco contains fluoride in the range 0.68-6.60 mg/L and cigarette filters from 0.07-0.09 mg/L (Going et al., 1980). Schwarz et al., showed no correlation between urinary fluoride content associated with smoking (Schwarz et al., 2020). This conclusion is opposite to ours where there is an increase in urinary fluoride amongst smokers **(Figure 6)**. Overall, smokers excrete a high mean percent of fluoride through urine **(Figure 3b, Table 2)**. Kuo and coworkers reported a significantly higher urinary fluoride excretion in smokers over a sample of 300 students (Kuo et al., 2008).

Smoking increased the serum fluoride concentration in patients treated with the fluorine-containing anesthetic enflurane (Laisalmi et al., 2003). Regular smoking was associated with a significant increase in the serum F concentration following enflurane anesthesia. The increase may be due to the fact that enflurane is primarily metabolized to inorganic F in the liver via the cytochrome P450 2E1 isoform and smoking may increase the activity of this enzyme, resulting in an increased transformation of enflurane to inorganic F^- (Koç et al., 2018; Laisalmi et al., 2003). Thus, smokers experience increased occurrences of dental and skeletal fluorosis, as well as elevated fluoride in their urine (Khandare et al., 2010; Koç et al., 2018; Riddell et al., 2021).

Alcoholic beverages contain fluoride in the range of 0.08 to 2.02 mg/L (Styburski et al., 2017). Alcohol disrupts organs like the intestine (Chauhan et al., 2013) and liver (Prystupa et al., 2021). Oxidative stress induced on the intestine of female Sprague Dawley rats augmented by the presence of ethanol causes fluoride toxicity (Styburski et al., 2017).

Serum fluoride concentration is known to increase as cirrhosis progresses (Prystupa et al., 2021). High alcoholic content beverages like vodka, rum, whisky, gin have lower fluoride values (9 mg/100g) than lower alcoholic content beverages like beer and lager (44, 45 mg/100g), while red and white wines (202 mg/100g) have a much higher fluoride contents (Goschorska et al., 2016). Our dataset shows that overall, there is a higher fluoride excretion among alcohol consumers **(Table 2, Figure 3b & d, Figure 6)**.

The rates of gastric absorption occurring in the stomach and the small intestine is directly related to the acidity (Whitford, 1994). Cations and fluoride are incorporated in biological apatites (enamel, bone, dentin) (LeGeros et al., 1988). The presence of both

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M^{2+} and F^{-} gives rise to the formation of F^{-}-containing apatite which results acid dissolution. The negative effects of the cations on the apatite properties were greatly reduced when F^{-} are present simultaneously. (LeGeros et al., 1988). Elevating both plasma fluoride and calcium in the diet increases the fecal excretion of fluoride (Whitford, 1994). This could explain the trend in Figure 3c where those individuals drinking from RO water had maximum urinary fluoride. Although water sourced from hand pump has the highest fluoride content (i.e., two times the permissible limit), the additional presence of cations could have resulted in the formation of apatite, limiting its urinary excretion.

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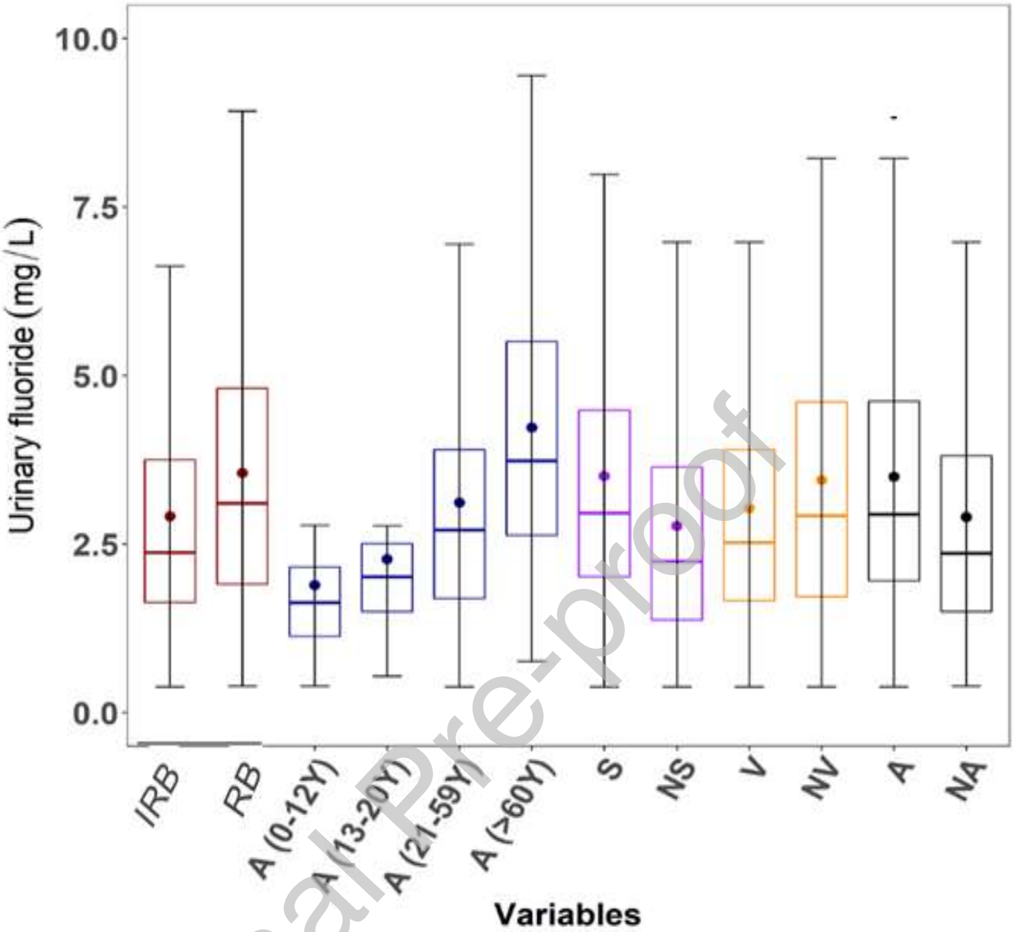

Figure 6: Box-plot showing urinary fluoride concentration (mg/L) between different categories (IRB = irregular brushing, RB = regular brushing, A = age, S = smoking, NS = non-smokers, V = Vegetarian, NV = non-vegetarian, AD = alcohol drinkable, AND = alcohol non-drinkable)

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Spak and coworkers suggested that food in the stomach provides a physical impediment for the fluoride absorption by the mucosal membranes (Spak et al., 1989). Vegetarian diets increase fluoride excretion (Idowu et al., 2019). Our study had a higher percent of fluoride increase amongst vegetarians (Table 1, 2 and Figure 3b). Regular brushers (RB) exhibited high *X* values due to toothpaste having additional fluoride. Toothpaste ingestion may be considered as one of the fluoride sources. In the

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sampled population, fluoride containing toothpaste in the range of 600-797 mg/L was used. Swallowing of toothpaste has been documented previously where about 72% of toothpaste is retained in the mouth (Bentley et al., 1999). This is consistent with our results in Table 1, 2 and Figure 3b & 4a. Here, regular brushing participants (RB) exhibited high *X* values due to toothpaste having additional fluoride. Children accumulate more fluoride as they are prone to swallowing toothpaste. Significant difference between fluoride intake and urinary fluoride was recorded between the ages of 21–59 ($p = 0.015$*) and >60 years ($p = 0.00005$****) , indicating low retention in older groups. This gives rise to concerns about various fluoride associated ailments in the young group ages 5-12 years. **(Figure 7)**. The abundance of dental caries in old age group ages >60 years resulting in teeth loss. This indicates the chronic fluoride exposure effect (**Table 2)**.

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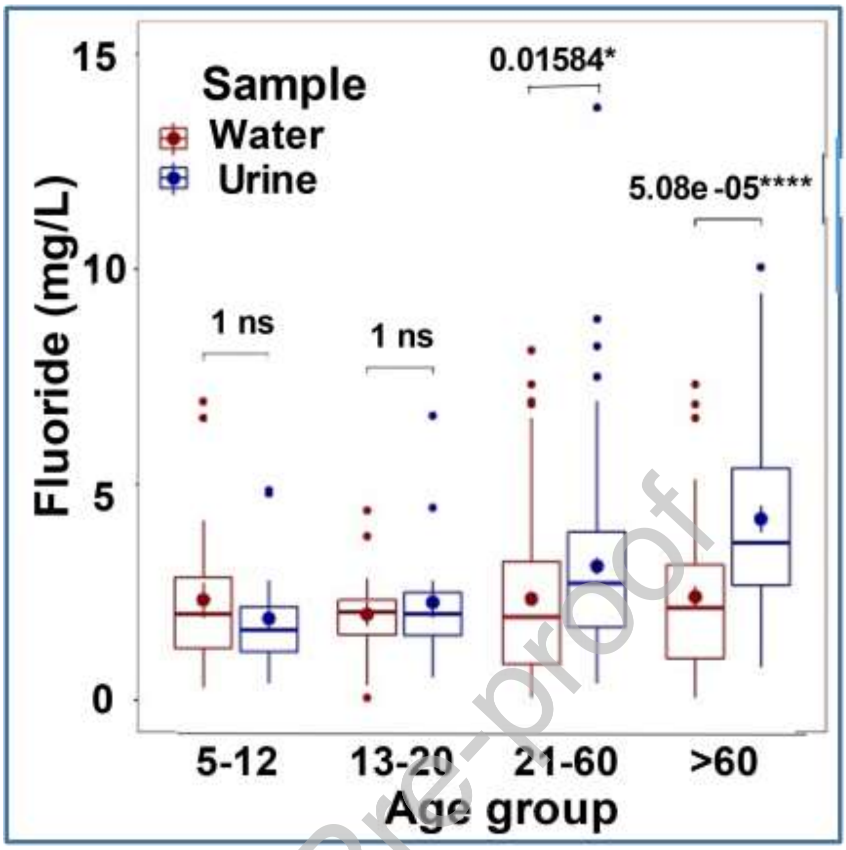

Figure 7. Box plot between different age groups (5-12, 13-20. 21-59 and >60 years) plotted against with water and urinary fluoride concentration (mg/L).

5.0 Conclusions

The current study goes beyond the uni-dimensionality of considering drinking water sources alone and attempts to draw inferences from the lifestyle habits and age on fluoride retention. Age posed a significant effect on the correlation between fluoride intake and urinary excretion. Lower-age (5-12 years) participants accumulated higher fluoride compared to the older age-groups. Other variables (food habits, drinking habits and smoking habits) also reflect fluoride ingestion pathways. The higher mean U_F values over W_F indicate other intake sources in addition to drinking water. Older age participants suffered tooth loss which can be attributed to high amounts of fluoride in

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drinking water. Accumulation of effects from smoking and alcohol consumption could have contributed. However, long-term documenting of the subject and fluoride ingestion and excretion will strengthen this claim. A matter of concern is the fact that children are prone to swallowing toothpaste which can aggravate various fluoride associated ailments. Safe levels of fluoride within the BIS recommended limits (1.5 mg/L) and proper dental hygiene must be achieved in order to circumvent the detrimental health risks. Meanwhile, alternative, and low-cost methods to limit fluoride in drinking water must be devised.

To further investigate the difference between the Schwarz's lack of excess urinary fluoride content from smokers and our higher urinary content, further studies were suggested by a reviewer. A biochemical and mutagenicity evaluation of blood in smokers vs non-smokers should help in explaining the elevated fluoride secretion of smokers. It would provide higher precision that greater fluoride absorption occurred in the gastro-intestinal system, via simple diffusion through bloodstream. This might help affirm the hypothesis of toxicity discussed in previous studies mentioned in the introduction.

Acknowledgement

KK is thankful to University Grants Commission for providing Senior Research Fellowship. DM is thankful to PSA, GOI for financial assistance under the project "Delhi Cluster-Delhi Research Implementation and Innovation (DRRIV).

Conflicts of interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Funding information

KK is thankful to University Grants Commission for providing Senior Research Fellowship. DM is thankful to PSA, GOI for financial assistance under the project “Delhi Cluster-Delhi Research Implementation and Innovation (DRRIV).

Declaration of Interest

No *conflict of interest* exists. We wish to confirm that there are no known conflicts of *interest* associated with this publication and there has been no significant financial support for this work that could have influenced its outcome.

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