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Assessment and health risk of fluoride from Northeast Indian tea (*Camellia sinensis* L.): Fixing up the maximum residue level of fluoride in tea

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ABSTRACT

The tea plant is considered a strong fluoride (F^-) hyperaccumulator plant. We have proposed the Maximum Residue Limit (MRL) of F^- in tea based on 321 tea samples collected from 8 tea-growing regions of Northeast India. Total F^- as well as water-soluble F^- content was analysed through the ion selective electrode with the recovery percentage of 100–108 to ascertain the risk associated with F^- towards human health. It has been observed that total F^- contents (mg kg^{-1}) in Cachar, Darjeeling, Dooars, North Bank, South Bank, Terai, Tripura, and Upper Assam ranged from 42.5 to 111.5, 16.0 to 168.0, 48.0 to 291.0, 36.5 to 314.0, 60.0 to 154.0, 26.5 to 233.5, 26 to 118 and 44 to 244, respectively. The F^- content in tea infusion varied between 1.08 mg L^{-1} and 2.43 mg L^{-1} across all eight regions with a mean of 1.90 mg L^{-1}. The average transfer of F^- from made tea (processed young shoot of tea plants comprising a bud and 2 to 3 leaves) to tea infusion was 100.46%. Fluoride content in infusion was decreased with the increment of infusion number but increased gradually with continuous infusion time. The non-carcinogenic health risk was assessed. Calculated Hazard Quotient values were far below 1. The calculated and rounded MRL (300 mg kg^{-1}) was recommended for F^- in tea in India.

1. Introduction

Fluorine (F) is the lightest halogen (means salt maker or salt former) and is present in group- XVII or group -VIIA in the periodic table along with four chemically related elements, viz. chlorine (Cl), bromine (Br), iodine (I), and astatine (At). Fluorine ranks 24th and 13th in universal abundance and terrestrial abundance (0.3 g kg^{-1}) of the earth's crust respectively (Prabhu et al., 2023, Yadav et al., 2007). Fluorine is a highly reactive halogen gas and rarely exists in its free form in the environment. Therefore, F is present in the environment in different minerals of F, viz. calcium fluorophosphate ($CaFO_3P$), cryolite (AlF_6Na_3, sodium hexafluoroaluminate), fluorapatite [$(Ca_5(PO_4)_3F$, pentacalcium fluoride triphosphate], fluorite (CaF_2, calcium fluoride), fluormica (for structural formula please refer to El-Meliegy and van Noort, 2012), gneisses (for structural formula please refer to Newhouse et al., 1949), granite {($C_{16}H_{14}F_5N_5O_5S$; 2-(2,2-difluoroethoxy)-N-(5,8-dimethoxy-[1,2,4]triazolo[1,5-*c*]pyrimidin-2-yl)− 6-(trifluoromethyl)benzenesulfonamide}, hornblende [$(Ca, Na)_{2-3}(Mg, Fe, Al)_5(Al, Si)_8O_{22}(OH, F)_2$], muscovite [$(KAl_2(AlSi_3O_{10})(F, OH)_2$, or $(KF)_2(Al_2O_3)_3(SiO_2)_6(H_2O)$], pegmatites, syenites and topaz ($Al_2SiO_4(F, OH)_2$), where F exists in its anionic form called fluoride (F^-) (Msonda et al., 2007). Fluoride is one of the important minerals for human health and plays a pivotal role in preventing tooth decay and the growth and strength of bones at a level of between 1.0 and 1.5 mg per day. For this reason, F^- addition in public drinking water supply is a common practice for one-third of Canadians and about three-fourths of Americans (Till and Green, 2021). Even though F^- contributes several health benefits, prolonged uptake of F^- at higher concentrations can lead to arthritis, damage to the tissues such as kidneys, liver, lungs, and testis, hard tissue deformities, hypertension, infertility, neurotoxicological effects, skeletal cancer, and skeletal fluorosis (Duggal and Sharma, 2022).

Out of the water, some commonly consumed foods and beverages, viz. fish, seafood like anchovies or pilchards, table salt, spinach, and beverages like tea, beers, and wines are the common sources of F^- (Essebbahi et al., 2023, Rodríguez et al., 2020). Tea, manufactured from the young shoots of the tea plant, is considered the most loved non-alcoholic medicinal beverage among the three widely consumed

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non-alcoholic beverages (cocoa, coffee, and tea) (Luo et al., 2021; Karak et al., 2017; Deka et al., 2021; Borgohain et al., 2022; Gogoi et al., 2022; Borgohain et al., 2023; Gogoi et al. 2023; Sarmah et al., 2023).

Chowdhury and Barooah (2020) reported that tea bioactive modulates innate immunity in the perception of the COVID-19 pandemic. The available evidence suggests that the tea polyphenols down-regulating NKG2A to induce NK cells can play an important role in the early stages of virus infection (Dhar and Mohanty, 2020). Another important aspect is "gut-lung" crosstalk, in recent times this phenomenon has gained a lot of attention and it is widely recognized that an ideal gut microenvironment is essential for a balanced immune response not only for the gastrointestinal tract but also for maintaining respiratory homeostasis.

On average, Indian people consumed 786 g of tea per capita (Kumar et al., 2021; Karak et al., 2016) making it an integral part of the Indian diet. Despite the enormous number of health benefits of tea, Miri et al. (2018) concluded that the high amount of F^- in tea can be harmful when F^- was estimated in 105 tea samples imported from Sri Lanka, Iran, Kenya, and Taksetare. Kakumanu and Rao (2013) reported that a 47 years old woman was affected by aching in the lower back, arms, legs, and hips as this woman consistently consumed a jug of tea made from 100 to 150 tea bags every day (estimated fluoride intake >20 mg day^{-1}). They concluded that the serum F^- concentration of this woman was exceptionally high amounting to 0.43 mg L^{-1} (23 μmol L^{-1}; normal concentration, < 0.10 mg L^{-1} [5 μmol L^{-1}]). Eventually, they concluded that the skeletal fluorosis of this woman was due to excessive tea drinking. So, non-carcinogenic human health risk assessment associated with the level of fluoride present in the NE Indian tea samples has been evaluated in this study.

A comprehensive review by Karak and Bhagat (2010) concluded that F^- concentration in tea infusion depends on the type of tea and F^- in infusions of black tea was higher than that in green tea. Zhang et al., (2023) reported F^- contents of tea types representing major cultivation areas in China e.g., Guangdong and Hunan Provinces of South China, Yunnan Province of Southwest China, Henan Province of North China and areas of Fujian, Jiangsu, and Zhejiang Provinces situated at the south of the Yangtze River to assess the possible health risk for the general population. They concluded that F^- content in 15.6% of samples of 218 tea leaves exceeded the Chinese maximum residue limit (200 mg kg$^{-1)}$. They also concluded that F^- level varied between producing regions and types of tea. However, risk assessment indicated no significant health risk via simply consuming tea.

Fluoride in made tea is an obvious fact as the tea plant is an F^--hyperaccumulator (Zhang et al., 2023). Luo et al. (2021) highlighted that tea plants can absorb an enormous amount of F^- from soil and 98% of total accumulated F^- by tea plants is present in tea leaves. Das et al. (2017) also reported that F^- in made tea exists in highly soluble form and 96–99% of total F in made can be extracted by water when 35 tea samples collected from 13 countries were analysed. This also confirms that tea is an F^- supplement for human beings. Furthermore, the digestive tract can straightforwardly absorb soluble F^- and the bioavailability of F^- from tea is close to 100% (Tokalıoğlu et al., 2004) which is similar to that from drinking water (Rao, 1984).

Presently, 48 countries in the world are cultivating tea. Among the tea-growing countries, India ranked second and contributed 10.45% of total global tea production in the year 2022. In India, tea cultivation is snowballing with their demand in the national and global markets (Parida et al., 2023). In India, major tea-growing states are Assam, Kerala, Tamil Nadu, Tripura, and West Bengal of which Northeast (NE) Indian states (Assam, Tripura, and West Bengal) have been commonly recognised for quality tea (Parida et al., 2023) and produce more than 80% of the total Indian tea production (Kanrar et al., 2022a). Traditionally Indian tea is grown on natural soils, however, most of the growers use phosphate fertilizers, which might lead to a significant increase of F^- in soil and its subsequent transfer from root to tea shoots (Yadav et al., 2012). Furthermore, an enormous accumulation of F^- by tea plants can not affect the usual physiological characteristics or any poisoning of tea plants due fluoride detoxification mechanism inside the plant (Yang, et al., 2020, Peng et al., 2021). Therefore, to understand the level of fluoride present in the NE Indian tea, a total of 321 tea samples from NE India have been analyzed for F^- content. Fluoride was analysed in total as well as in tea infusion.

Fluoride in biological samples can be determined by Ion Selective Electrode (ISE) (Yang, et al., 2020) and gas chromatographic (GC) (Peng et al., 2021) methods. Colorimetric methods for F^- estimation are also reported by Tokatlioğlu et al., (2004). Use of ISE technique for F^- estimation is widely being used due to the ease of sample processing and higher recovery rate (Yardımcı et al., 2022). A total-ionic strength adjustment buffer (TISAB), contains cyclo-hexylene-dinitrilotetra acetic acid and helps to remove Fe (III) and Al (III) interferences by forming chelate complexes (NIOSH, 1994). The direct determination of F^- using ICP-MS is challenging because of the very high ionization potential of fluorine.

Fluoride content in tea samples varied in different tea growing countries as well as different types of teas and also a substantial amount of F^- present in tea leaves is released during tea infusion which is to be absorbed by the body from tea infusions (Tokalıoğlu et al., 2004, Zazouli and Esfandiari, 2006, Lung et al., 2008, Yi et al., 2004). The comparison of F^- content in tea samples from China, Japan, Turkey, Sri Lanka, India, and Kenya has been reported by several researchers (Melissa et al., 2021, Emekli-Alturfan et al., 2009). So far there is no MRL for F^- in tea in India. In this study based on our research data, we have proposed MRL for F^- in tea in India. The origin of tea plants and the environment in which they grow appear to affect the amount of F^- that accumulates in them (Melissa et al., 2021). Tea leaves used to manufacture the made tea also affect the F^- content in the tea infusion as older leaves contain more F^- (Shih-Chun et al., 2003). The amount of F^- in the tea infusions is also influenced by the type of tea used (Pattaravisitsate et al., 2021), the quality of water used (Gupta and Sandesh, 2012), infusion time (Kalayci and Somer, 2003; Fung et al., 2003) and also the quality of milk and sugar added to it (Validandi et al., 2019). So, changes in F^- content with an increase in infusion time, and infusion number have also been assessed in this study.

Excessive consumption of tea beverages creates a risk for F^- toxicity but there is no MRL for F^- in tea in India. Keeping all these things in mind this study has been conducted to evaluate F^- content in Indian tea produced in NE India. Around 80% of Indian tea is produced in NE India (Kanrar et al., 2022a). A total of 321 tea samples from NE India have been analyzed for F^- content (total and in infusion). Tea infusion was prepared following the IS method (IS 3103) and estimated by an F^- ISE. Changes in F^- content with an increase in infusion time, and infusion number have also been assessed. Based on the generated data MRL for F^- in tea has been proposed and non-carcinogenic human health risk assessment has also been evaluated and presented in this manuscript.

2. Materials and methods

2.1. Chemicals and reagents

Fluoride (F^-) content was determined through the ion selective electrode (Make: Thermo Scientific, Model: Orion Star A214 Benchtop pH/ISE meter, Sl No: X67248). A total-ionic strength adjustment buffer (TISAB) was procured from Thermo Scientific, USA. Sodium hydroxide (NaOH) pellets (low chloride) for analysis (EMPARTA grade) and 37% Hydrochloric acid (ACS EMPARTA grade) were procured from Merck Life Science, India. Whatman No 1 and 40 filter paper was procured from GE Healthcare Life Science, UK.

2.2. Sample preparation

2.2.1. Sample collection and details about samples

Three hundred and twenty-one (321) drier mouth samples (these are samples drawn straight from the mouth of a drier, i.e. at the delivery end of the drier. Thus, a drier mouth sample means a tea sample that has undergone all the steps of manufacture and has finally been dried. This would also mean that the sample has not undergone the last steps of manufacture (viz. sorting, grading, and packing) and were collected from NE India mentioned in Fig. 1. The area of sampling consists of Darjeeling (32), Terai (44), Dooars (37), North Bank (34), South Bank (50), Upper Assam (62), Cachar (40), and Tripura (22). Samples were collected from June 2020 to April 2021. Tea samples were collected during the pre-monsoon, monsoon, and post-monsoon seasons. All the collected samples were properly marked, packed, and sent to TLabs, Tea Research Association, Kolkata for estimating F^-. TLabs is a network of TRA (Tea Research Association; research on all aspects of tea cultivation and processing is carried out at TRA, the oldest and the largest research station of its kind in the world) laboratories, comprising the ISO/IEC 17025:2017 accreditation.

2.2.2. Determination of total F^- in tea by ion-selective electrode

For total F^- content analysis, we followed the method published by Tsyr-Horng et al. (2009). As per this method, 0.25 g of homogenized tea was taken into a 150 mL quartz crucible. The crucible was put in an oven (150 °C) for 1.5–2.0 h after adding 6 mL 16.75 N NaOH solution. The crucible was kept inside the oven until NaOH was solidified. After that crucible was taken out and placed in a muffle furnace (600 °C) for 30 min. Then crucible was allowed to cool. Distilled water (10 mL) and 37% HCl solution (about 8 mL) were added slowly into the crucible to adjust the pH to 8–9. The solution was filtered through Whatman No. 40 filter paper in a 100 mL plastic volumetric flask and volume made up of distilled water. TISAB III (Thermo Scientific Orion, USA) of 200 μL was added to 2 mL of filtrate, and its F^- content was determined through the ion selective electrode. A blank control was prepared in parallel.

2.2.3. Preparation of tea infusion and determination of F^- by ion selective electrode

Tea infusion was prepared following the ISO standard (ISO, 3103, 2019). In brief, 3 g of tea was immersed in de-ionized boiling water (150 mL) for ~5 min. It was then filtered through a Whatman No.1 filter paper. The filtrate was then collected. TISAB III (Thermo Scientific Orion, USA) of 200 μL was added to 2 mL tea infusion solution, and the F^- content of tea infusion was determined following the protocol described in Section 2.2.

2.3. Method validation for F^- analysis

The limit of detection (LOD) and limit of quantitation (LOQ) were calculated based on the 10 unspiked sample readings of F^- in tea infusion of a tea sample following the Eurachem Guide (2014) guideline. The standard Deviation (SD) was multiplied by 3 and 10 to get the LOD and LOQ, respectively. The calculated LOD and LOQ were 0.03 and 0.099 mg L^{-1}, respectively.

The accuracy of the ISE method was also evaluated by the recovery percentage. Recoveries of F^- from tea infusions spiked in made tea with three different amounts of F^- (05, 10, and 20 mg kg^{-1}) were tested. As shown in Table 1, recoveries were 100–108% with RSD ranges of 3.06 to 9.02%. Every sample was analysed in triplicate in the F^- ion selective electrode (Make: Thermo Scientific, Model: Orion Star A214 Benchtop pH/ISE meter, Sl No: X67248) throughout the study. There was dilution (50 times) factors associated with each sample analysed after infusion. We have always maintained the analysis sequence by starting with blank solutions followed by calibration solutions and then test solutions. A standard curve for F^- concentrations determination was made using 0.1 to 10 mg L^{-1} standard F^- solution, average slope:− 59.4 mV (Fig S1). Fluoride concentration in an unknown solution was determined by adding TISAB III solution to the extract and then calculated from the standard curve. To authenticate the accuracy of the results, calibration solution and spiked recovery samples were studied at regular intervals.

2.4. Non-carcinogenic health risk assessment

The non-carcinogenic health risk associated with the F^- was assessed following the earlier published method (Kanrar et al., 2022b). The Estimated Daily Intake (EDI) of F^- on consuming five cups (10 g) of tea in μg per kg BW day^{-1} (BW stands for body weight in kg) was calculated by using the following formula

Table 1
The recovery test of fluoride content in tea infusions (n = 5).

Spiked concentration (mg kg^{-1})	unspiked sample (mg L^{-1})	Spiked sample (mg L^{-1})	Recovered concentration (mg kg^{-1})	Recovery (%)	RSD (%)
5.0	1.99	2.10	5.4	108.0	9.02
10.0	1.11	1.32	10.8	108.0	7.02
20.0	1.96	2.36	20.0	100.0	3.06

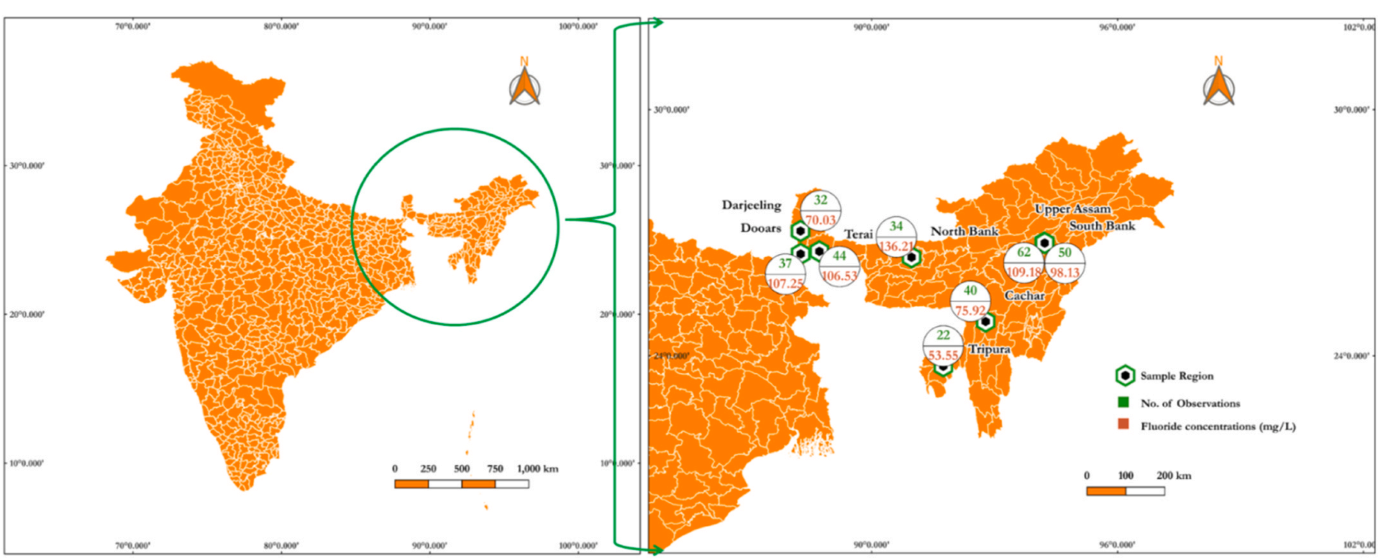

Fig. 1. Spatial representation of the study area along with the number of samples and mean total fluoride concentrations.

$$EDI = \frac{(C \times M \times T)}{(BW \times 100)} \ldots\ldots \quad (1)$$

where C is the mean concentration of the element in tea; M is the weight of tea consumed per day (10 g); T is the transfer rate of F^- in % and BW is the average body weight for Indian people i.e. 60 kg. The risk associated with the consumption of tea infusions with F^- content was assessed by determining the Hazard Quotient (HQ). The HQ is calculated by using the following formula:

$$\mathrm{HQ} = \mathrm{EDI}/\mathrm{ADI} \ldots\ldots \quad (2)$$

where ADI is Acceptable Daily Intake in µg per kg BW day^{-1} is the reference dose of the element in concern. The Adequate Intake (AI) dose for F^- was set by the European Food Safety Authority (EFSA, 2013).

2.5. Calculation and fixation of maximum residue limit (MRL)

The Maximum Residue Limit (MRL) of F^- in tea for the Indian population has been calculated and proposed as per the guidance document and standard operating procedures set by the Food Safety and Standards Authority of India, Government of India (FSSAI, 2021). For setting MRL, the Organisation for Economic Co-operation and Development (OECD) maximum residue calculator (EPA, 2022), is being used. The mean F^- data of each region were used to get the calculated and rounded MRL through the OECD calculator.

To characterize the risk, the National Estimated Daily Intake (NEDI) was calculated using the following equation:

$$\mathrm{NEDI} = \sum \mathrm{STMR} \times \frac{\mathrm{F_i}}{\mathrm{BW}} \ldots\ldots \quad (3)$$

where, STMR = Supervised Trial Median Residue; Fi = Per capita food consumption (kg day^{-1} per person); BW = Mean Body Weight.

Mean body weight was taken as 60 kg in the Indian context. This has been taken from the study conducted by the Indian Council of Medical Research (ICMR), National Institute of Nutrition (NIN), and Ministry of Health, Government of India across the age group 16 to 70 years (FSSAI, 2021). This BW was also taken for calculating Acceptable Daily Intake (ADI).

A comparison of NEDI and ADI per person was then assessed. If the NEDI is $\leq$ 80% ADI, the risk level is the lowest, and the calculated MRL is fixed as MRL.

2.6. Statistical Analysis

Most of the parametric statistical techniques are based on the assumption that the underlying distribution of the data-generating process is Gaussian. Therefore, it is important to check the underlying distribution of the study variable. Moreover, the best-fitted distribution of the study variable exhibits the pattern as well as a few statistical measures. An attempt was made to find statistical distributions for the total Fluoride concentrations in different regions as well as for different tea types. The study assessed the fit of various distributions by comparing the observed data with theoretical distributions using goodness of fit tests e.g., Kolmogorov-Smirnov (K-S) test and Anderson Darling (AD) test. These tests were chosen due to their ability to handle outliers and robustness. The null and alternative hypotheses of these tests are H_0: The data follow the specified distribution and H_A: The data do not follow the specified distribution.

It is based on the empirical cumulative distribution function (ECDF). Assume that we have a random sample from some continuous distribution with cumulative distribution function (CDF) as *F(x)*. The empirical CDF is denoted by

$$F_n(x) = \frac{1}{n}[Number of observations \leq x] \ldots\ldots \quad (4)$$

The K-S statistic (D) is based on the largest vertical difference between *F(x)* and $F_n(x)$. It is defined as

$$D_n = \sup_x |F_n(x) - F(x)| \quad \ldots\ldots \quad (5)$$

The AD statistic (AD) is given as

$$AD = -n - \frac{1}{n}\sum_{i=1}^{n}(2i-1)[\ln F(x_i) + \ln(1 - F(x_{n-i+1}))] \ldots\ldots \quad (6)$$

where, n is sample size; and i is i^{th} sample, calculated when the data is sorted in ascending order.

The hypothesis regarding the distributional form is rejected at the chosen significance level (*alpha*) if the test statistic, D or AD, is greater than its critical value.

The Kruskal-Wallis test followed by the Mann-Whitney U test with Bonferroni correction has been applied to test the difference in mean of study variable among the different regions as well as tea types. The Kruskal-Wallis test is a non-parametric statistical test used to compare the medians of two or more independent groups or samples when the assumptions for ANOVA are violated. The null hypothesis of this test states that the population medians of all groups are equal, while the alternative hypothesis states that at least one group differs. The test statistic is given as follows:

$$H = \frac{12}{N(N+1)} * \sum \frac{R_j^{\,2}}{n_j} - 3(N+1) \ldots\ldots \quad (7)$$

here, N is the total number of observations, R_j is the sum of ranks for group j, and n_j is the sample size for group j. Before applying this formula, the data should be combined from all groups and sorted in ascending order and observations are then assigned ranks. If the resulting test statistic exceeds the critical value, the null hypothesis is rejected, indicating significant differences between at least two groups. If the Kruskal-Wallis test demonstrates significant differences among the groups, post hoc tests such as the Mann-Whitney U test with Bonferroni correction can be employed to identify the specific groups that differ from one another.

Moreover, hierarchical cluster analysis was employed to form homogenous groups of different study regions. The objective of the technique is to group sample regions based on their similarities and relationships in Fluoride concentrations. The algorithm evaluated the increase in the error sum of squares criterion (ESS) as an indicator of information loss. Each possible pair of clusters is examined, and the two clusters with the smallest increase in ESS are combined. The resulting clusters are represented in a dendrogram, which visually depicts the clustering at different distances. The RStudio software (United States) with version 4.2.2 by CRAN has been used for the implementation of this statistical analysis.

3. Results and discussion

3.1. Total F content in made tea

Region-wise variations of total F^- content in made tea have been tabulated in Table S1. The range of total F^- content in made tea samples was found between 17.33 ± 1.15 mg kg^{-1} and 306.00 ± 8.0 mg kg^{-1}. The region-wise mean total F^- contents in made tea samples follows the following trend (Table 2): North Bank (136.21 ± 45.63 mg kg^{-1}) > Upper Assam (109.18 ± 40.78 mg kg^{-1}) > Dooars (107.25 ± 53.05 mg kg^{-1}) > Terai (106.53 ± 43.54 mg kg^{-1}) > South Bank (98.13 ± 20.85 mg kg^{-1}) > Cachar (75.92 ± 15.87 mg kg^{-1}) > Darjeeling (70.03 ± 30.62 mg kg^{-1}) > Tripura (53.55 ± 27.59 mg kg^{-1}).

Box plot along with density plot of total F^- concentration of different regions for the 321 made tea samples has been depicted in Fig. 2. The Interquartile Ranges (IQRs) of total F^- in made samples were found

Table 2
Region-wise mean total fluoride (F^-) contents, F^- contents in 2% (w/v) tea infusions, and transfer % of F^-.

Growing region	Parameters		
	F^- in tea infusion (mg L^{-1})	Total F^- (mg kg^{-1})	% of F^- infused
Cachar	1.49 ± 0.05[c]	75.9 ± 2.51[c]	98 ± 3.57[a]
Darjeeling	1.41 ± 0.11[c]	70 ± 5.41[c]	100 ± 4.04[a]
Dooars	2.18 ± 0.17[ab]	107 ± 8.72[ab]	102 ± 11.20[a]
North Bank	2.36 ± 0.10[a]	136 ± 7.83[a]	86.6 ± 4.66[b]
South Bank	2.05 ± 0.06[b]	98.1 ± 2.95[bc]	105 ± 2.75[a]
Terai	2.2 ± 0.13[ab]	107 ± 6.56[ab]	103 ± 6.62[a]
Tripura	1.08 ± 0.12[d]	53.6 ± 5.88[c]	101 ± 5.63[a]
Upper Assam	2.43 ± 0.11[a]	109 ± 5.18[ab]	111 ± 10.27[a]
Average	1.90	94.6	100.46

Note: Column wise different letters indicate the significance difference and same letters indicate no difference between the tea growing regions.

85.33–69.25 mg kg^{-1}, 86.50–48.50 mg kg^{-1}, 120.67–75 mg kg^{-1}, 159.33–114.75 mg kg^{-1}, 104–83.50 mg kg^{-1}, 118.67–87.33 mg kg^{-1}, 63.50–33.17 mg kg^{-1}, and 128.58–84.04 mg kg^{-1} for Cachar, Darjeeling, Dooars, North Bank, South Bank, Terai, Tripura, and Upper Assam, respectively.

3.2. Fluoride (F^-) content in tea infusion

The water-soluble F^- content was measured in tea infusion prepared as per ISO standards (ISO, 3103, 2019). The 2% tea to water ratio (w/v) along with the 3–5 min infusion time was allowed for all the total 321 tea infusion samples. The F^- content in tea infusion varied between 1.08 mg L^{-1} to 2.43 mg L^{-1} across all eight regions for 321 made tea samples with a mean of 1.90 mg L^{-1} (Table S2). The highest mean±SD concentration (2.43 ± 0.89 mg L^{-1}) of water-soluble F content was found in Upper Assam samples, followed by North bank (2.36 ± 0.58 mg L^{-1}), Terai (2.20 ± 0.86 mg L^{-1}), Dooars (2.18 ± 1.06 mg L^{-1}), South Bank (2.05 ± 0.44 mg L^{-1}), Cachar (1.49 ± 0.32 mg L^{-1}), Darjeeling (1.41 ± 0.60 mg L^{-1}) and Tripura (1.08 ± 0.55 mg L^{-1}) (Table 2). Fig. 3 represents the circular bar diagram of F^- concentration in tea infusion of 321 samples. The median and IQR data for Cachar, Darjeeling, Dooars, North Bank, South Bank, Terai, Tripura, and Upper Assam were 1.47, 1.69–1.35 mg L^{-1}; 1.25, 1.74–0.97 mg L^{-1}; 1.83, 2.47–1.50 mg L^{-1}; 2.47, 2.68–2.16 mg L^{-1}; 2.02, 2.30–1.78 mg L^{-1}; 2.07, 2.43–1.79 mg L^{-1}; 0.87, 1.29–0.67 mg L^{-1}, and 2.31, 3.07–1.75 mg L^{-1}, respectively.

The transfer of F^- from made tea to tea infusion was also studied zone-wise and presented in Table 2. Fluoride contents of these particular tea infusions ranged from 1.08 to 2.43 mg $L^{-1}$with a mean value of 1.90 mg L^{-1}, and the total F^- contents ranged from 53.55 to 136.21 mg kg^{-1} with a mean value of 94.60 mg $kg^{-1}$and the percentage of F^- infused ranged from 86.59 to 111.44%. The highest % F^- infused was observed in Upper Assam samples and the lowest was observed in North Bank region samples. The average % of F^- infused was 100.46.

The concentration of F^- in infusion was also studied in respect of varying continuous infusion times as well as differences in successive infusion numbers of the same sample and the data was presented in Fig. 4. From the figure, it transpired that F^- concentration decreased with the increment of infusion number. Specifically, it decreased drastically after the first infusion to the third infusion, and on the fourth and fifth infusions, it remains comparable at a lower rate. On the other hand in continuous infusion mode, the F^- concentration was increased gradually from 3 min to 30 min of infusion time.

3.3. Discussion

The per cent of F^- transfer rate at tea infusion for the NE Indian tea samples transpired that the F^- concentration in tea infusion if multiplied with the dilution factor, reproduces the total F^- content of the samples. The results also prove the usefulness of the methodology used in tea infusion preparation for this study to generate the data for F^- content in

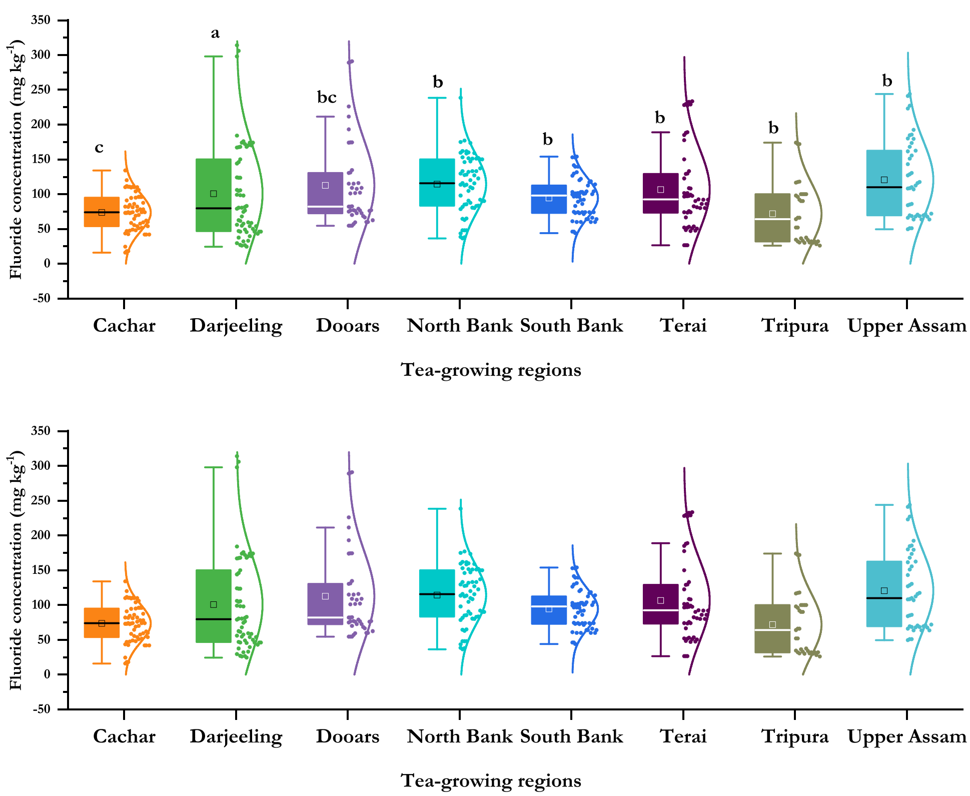

Fig. 2. Box plot with a density plot of total F^- concentration of different sample regions. Similar letters indicate no significant difference at a 5% level of significance.

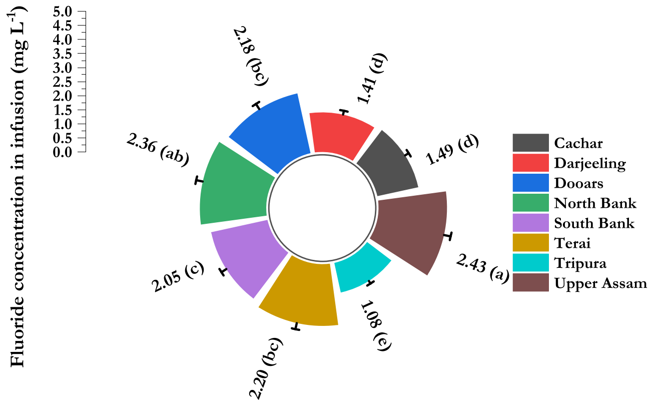

Fig. 3. Circular bar diagrams of F⁻ concentration in the infusion of different sample regions. Similar letters indicate no significant difference at a 5% level of significance.

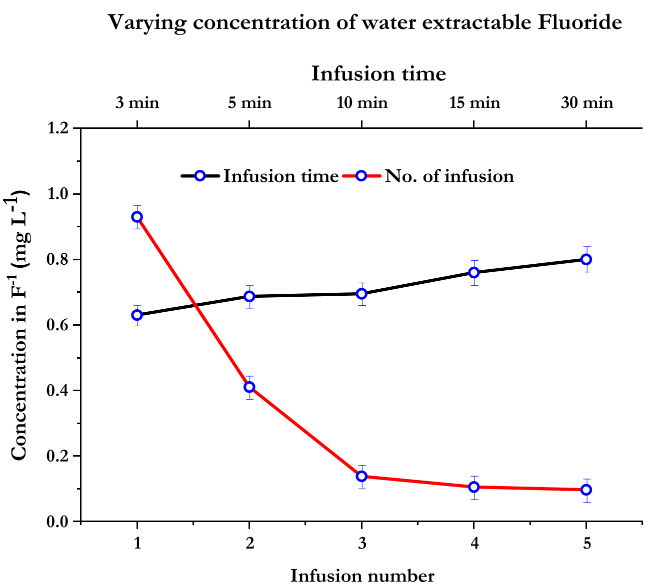

Fig. 4. Varying concentration of water extractable F⁻ concerning infusion time and the number of infusions.

Northeast Indian tea. The study result of F⁻ content in tea infusion, total F⁻ content as well as infusion percentage of F⁻ ion into the tea infusion corroborate the study result reported by Tokalioglu et al.(2001) of water-soluble F⁻ content of Turkish teas which was found to range from 55–127 mg L^{-1}. These levels correspond to 0.55–1.27 mg L^{-1} when converted into infusion concentrations (2 g tea infused in 200 mL of water). The content of total F⁻ has also been found to depend on the manufacturing process and our results also corroborate with the earlier published report by Fung et al. (1999, 2003) on the Chinese tea samples. Similar study results of incremental F⁻ content with the continuous infusion time were also reported by Malinowska et al. (2008). The pathways of F⁻ from acidic tea soil to tea plants are well established by Wong et al. (2003). The high content of AlF_x salt in the tea garden soil along with the acidic pH of the soil mostly contributed to the absorption of Al^{3+} along with F⁻ by tea root and translocated to the leaf (Ruan and Wong, 2001). Lu et al. (2004) also reported that the tea plant is an F⁻ accumulator. As a result, tea drinks invariably contain high levels of F⁻.

3.4. Non-carcinogenic health risk assessment

The human risk associated with the studied element (non-carcinogenic) through the consumption of 10 g of tea per kilogram body weight per day was estimated and presented in Table 3. The Estimated Daily Intake (EDI) values were 11.71, 19.65,12.40, 18.18, 20.28,17.12, 18.33, and 9.03 μg per kg BW day^{-1}, respectively for Darjeeling, North Bank, Cachar, Dooars, Upper Assam, South bank, Terai and Tripura. The average EDI of all the region samples was 15.99 μg per kg BW day^{-1}. The percentage of EDI with respect to Acceptable Daily Intake (ADI) set by EFSA (EFSA, 2013) varies between 18.07 (Tripura) - 40.56 (Upper Assam) with an average of 31.97%. For non-carcinogenic risk assessment, the Hazard Quotient (HQ) has been determined by comparing the EDI of F⁻ with their corresponding Acceptable Intake (AI) value set by EFSA (EFSA, 2013). The calculated HQ values ranging from 0.18 to 0.41 with an average of 0.32 were far below the 1 indicating the daily intake of 10 g of the Northeast (NE) Indian tea would not pose any health hazard. So, tea plays a vital role in the human diet system to supplement F⁻ requirements without posing any adverse health effects. This finding is also corroborating the study reported by Sofuoglu and Kavcar (2008).

3.5. Fixation of MRL

Table 4 displays the best-fitted distribution of total fluoride concentrations for different regions and tea types. In sample regions, Darjeeling, North Bank, Cachar, Dooars, Upper Assam, South Bank, Terai, and Tripura exhibit Weibull (3 P), Laplace, Normal, Frechet (3 P), Gamma (3 P), Log-Logistic, Log-Logistic, and Frechet (3 P) distribution respectively.

The results of pairwise comparison among different regions using the Kruskal-Wallis test and Mann-Whitney U test are presented in Fig. 3. The Fig. 3 revealed that among the different sample regions, the Upper Assam followed by the North Bank exhibited the highest total fluoride concentrations and Darjeeling and Cachar exhibited the lowest fluoride concentrations in infusion.

Fig. 5 displays the outcomes of hierarchical cluster analysis, to identify distinct and homogeneous groups of sample regions based on their F⁻ concentrations. The results led to three homogenous groups of regions: the first group comprised South Bank, Terai, and Dooars; the second group included Upper Assam and North Bank; and the remaining regions formed the third group.

Average values of total F⁻ content of all the eight regions samples (321) have been used to calculate the MRL using the OECD MRL calculator. The calculated and rounded MRL was 300 mg kg^{-1} for which risk has been assessed for fixation of MRL in India (Table 5). National Estimated Daily Intake (NEDI) (mg per person day^{-1}) was 0.05, taking 60 kg average body weight for the Indian Population, and the daily food factor for tea is 10 g. As per the EFSA (EFSA, 2013), the Adequate Intake (AI) for F⁻ is 0.05 mg kg^{-1} per BW day^{-1}. Based on risk assessment the

Table 3
Estimation of Risk associated with the F⁻ present in the Northeast Indian tea.

Region	Variables*							
	C	M	T	BW	EDI	ADI	EDI/ADI (%)	HQ
Cachar	75.9	10	98.0	60	12.4	50	24.8	0.25
Darjeeling	70.0	10	100	60	11.7	50	23.4	0.23
Dooars	107	10	102	60	18.2	50	36.4	0.36
North Bank	136	10	86.6	60	19.7	50	39.3	0.39
South Bank	98.1	10	105	60	17.1	50	34.3	0.34
Terai	107	10	103	60	18.3	50	36.7	0.37
Tripura	53.6	10	101	60	9.03	50	18.1	0.18
Upper Assam	109	10	111	60	20.3	50	40.6	0.41
Average	95.5	10	101	60	15.9	50	31.9	0.32

* : C = mean concentration of element present in 321 northeast Indian Tea (μg g^{-1}); M = Weight of the tea consumed per day (g); T = Transfer rate in infusion (%); BW = Average body weight of Indian population (kg); EDI = Estimated Daily Intake (μg kg^{-1} bw day^{-1}) = (C×M × T) / (BW × 100); ADI = (Acceptable Daily Intake in μg kg^{-1} bw day^{-1}): the Adequate Intake (AI) set by EFSA, 2013; HQ = Hazard Quotient = EDI/ADI.

Table 4
Fitted theoretical probability distribution of different sample regions for total F⁻ concentrations.

Region/Tea	Distributions	Probability density function	KS#	AD#
Different sample region				
Cachar	Normal	$f(x) = \frac{1}{20.34 * \sqrt{2\pi}} e^{-\frac{1}{2}\left(\frac{x-75.93}{20.34}\right)^2}$	0.08	0.59
Darjeeling	Weibull (3 P)	$f(x) = \frac{14.66}{60.80}\left(\frac{x-1.43}{60.80}\right)^{14.66-1} e^{-\left(\frac{x-1.43}{60.80}\right)^{14.66}}$	0.08	0.33
Dooars	Frechet (3 P)	$f(x) = \frac{19.31}{55.83}\left(\frac{55.83}{x-2.12}\right)^{19.31-1} e^{-\left(\frac{55.83}{x-2.12}\right)^{19.31}}$	0.08	0.29
North Bank	Laplace	$f(x) = \frac{1}{2 * 0.03} e^{-\left\lvert\frac{x-136.21}{0.03}\right\rvert}$	0.11	0.72
South Bank	Log-Logistic	$f(x) = \frac{94.47}{6.96 * x}\left(\frac{x}{6.96}\right)^{94.47-1} \frac{1}{\left(1+\left(\frac{x}{6.96}\right)^{94.47}\right)^2}$	0.10	0.56
Terai	Log-Logistic	$f(x) = \frac{90.41}{2.97 * x}\left(\frac{x}{2.97}\right)^{90.41-1} \frac{1}{\left(1+\left(\frac{x}{2.97}\right)^{90.41}\right)^2}$	0.10	0.73
Tripura	Frechet (3 P)	$f(x) = \frac{24.03}{8.39}\left(\frac{8.39}{x-1.07}\right)^{24.03-1} e^{-\left(\frac{8.39}{x-1.07}\right)^{24.03}}$	0.19	1.09
Upper Assam	Gamma (3 P)	$f(x) = \frac{(x-1.14)^{43.79-1}}{57.34^{115.51}\Gamma 43.79} e^{-\frac{x-1.14}{57.34}}$	0.11	0.77

#KS: Kolmogorov-Smirnov statistics #AD: Anderson Darling statistic

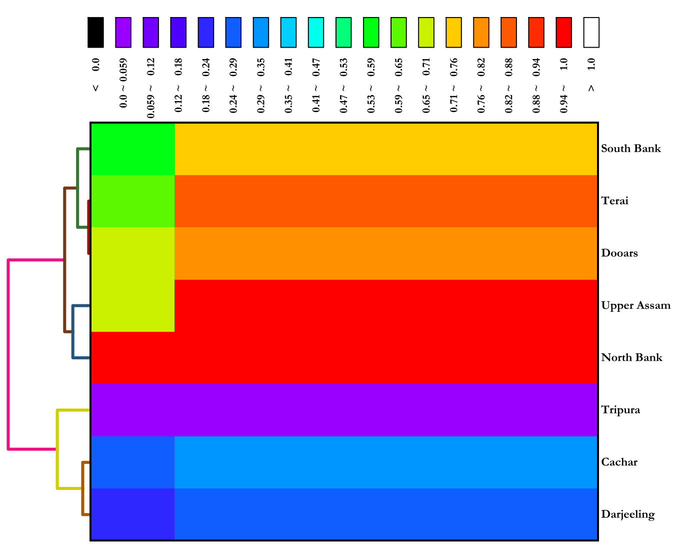

Fig. 5. Dendrogram with a heatmap of hierarchical cluster analysis of sample regions.

NEDI is 1.67% of ADI. Hence the calculated MRL of 300 mg kg^{-1} can be recommended for F⁻ MRL on tea in India. Currently, there is no MRL assigned to Tea in India. European Union has an MRL of 400 mg kg^{-1} on tea (European Commission, 2023).

4. Conclusion

The water-soluble F⁻ content was measured in tea infusion prepared as per the ISO 3103. The F⁻ content in tea infusion varied between 1.08 mg L^{-1}to 2.43 mg L^{-1}across all the eight regions for 321 made tea samples with a mean of 1.90 mg L^{-1}. The highest mean ± SD concentration) of water-soluble F content was found in Upper Assam samples, followed by North Bank, Terai, Dooars, South Bank, Cachar, Darjeeling and Tripura. Total F⁻ content also varies zone-wise. The highest mean total F⁻ content was found in North Bank (136.21 mg kg^{-1}) followed by Upper Assam (109.18 mg kg^{-1}), Dooars (107.25 mg kg^{-1}), Terai (106.53 mg kg^{-1}), South Bank (98.13 mg kg^{-1}), Cachar (75.92 mg kg^{-1}), Darjeeling (70.03 mg kg^{-1}) and the lowest in Tripura (53.55 mg kg^{-1}). The average transfer of F⁻ from made tea to tea infusion was 100.46%. The concentration of F⁻ in infusion was decreased with the increment of infusion number. On the other hand in continuous infusion mode, the F⁻ concentration was increased gradually from 3 min to 30 min of infusion time. The non-carcinogenic health risk was assessed in terms of Hazard Quotient (HQ) and the calculated HQ values were far below the 1 indicating the daily intake of 10 g NE Indian tea would not pose any health hazard. Average values of total F⁻ content of all the eight regions samples have been used to calculate the MRL using the OECD MRL calculator. The calculated and rounded MRL was 300 mg kg^{-1} for which risk has been assessed for recommendation of F⁻ MRL in tea in India. However, in this study, only 321 tea samples were analysed, but other factors such as the type of cultivar of the tea bush, the superiority of the plucked tea leaves, origin, and quality of the production processing they undergo need to be considered for future works. Furthermore, to the best of our learning, this is the first finding that represents data on F⁻ in tea of Northeast India and proposed MRL for F⁻.

Table 5
Calculation of MRL and Estimation of Risk associated with the recommended MRL on F⁻ element based on a total of 671 tea samples of northeast India.

Elements	F⁻
Calculated and rounded MRL on OECD MRL Calculator (mg kg^{-1})	300
NEDI (mg per person day^{-1})	0.05
ADI (mg per person day^{-1})	3.00
% ADI	1.67
Recommended MRL on tea (mg kg^{-1})	**300**

NEDI (National Estimated Daily Intake): NEDI = Σ STMR × Fi/bw (mg per person day^{-1})
where, NEDI = National Estimated Daily Intake (mg per kg body weight day^{-1}), STMR = Supervised trial median residue (mg kg^{-1}), Fi = Per capita food consumption (kg day^{-1} person^{-1}). MBW = Mean Body Weight (kg), ADI = (Acceptable Daily Intake in μg per kg body weight day^{-1}): the Adequate Intake (AI) set by EFSA, 2013% ADI: NEDI/ADI × 100.

CRediT authorship contribution statement

Kanrar Bappaditya: Conceptualization, Funding acquisition, Investigation, Project administration, Resources, Supervision, Writing – original draft. **Kundu Sangeeta:** Formal analysis, Investigation, Resources, Writing – original draft. **Sengupta Suparna:** Formal analysis, Resources, Writing – original draft. **Yeasin Md.:** Data curation, Formal analysis, Validation, Writing – original draft. **Paul Ranjit Kumar:** Data curation, Validation, Writing – original draft. **Karak Tanmoy:** Conceptualization, Data curation, Methodology, Writing – review & editing.

Declaration of Competing Interest

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests: Bappaditya Kanrar reports financial support was provided by National Tea Research Foundation.

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.

Data availability

Data will be made available on request.

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