*J. Mater. Environ. Sci. 6 (11) (2015) 3134-3142*
*ISSN : 2028-2508*
*CODEN: JMESCN*



# Influence of Fluoride Contaminated Irrigation Water on Biochemical Constituents of Different Crops and Vegetables with an Implication to Human Risk through Diet

**D. Mondal[1] and S. Gupta[1*]**
[1]*Department f Environmental Science; The University of Burdwan; Burdwan, West Bengal, India*

*Received 24 Jan 2015, Revised 15 Oct 2015, Accepted 18 Oct 2015*
**Corresponding author: E-mail: srimantagupta@yahoo.co.in*

## Abstract
This study was attempted to find out the effect of fluoride (F) contaminated irrigation water on soil, crops and vegetables in the context of F accumulation and biochemical constituents in crops/vegetables as well to quantify its intake through diet. In comparison to pre harvest control soil, application of F contaminated irrigation water shows significant accumulation of $F_{Total}$ (164 mg/kg) and $F_{H2O}$ (9.41 mg/kg) in post harvest treated soil. Study reveals that in treated condition $F_{Total}$ accumulation in vegetables is in the sequence of spinach (55 mg/kg)> celeriac leaf (42 mg/kg)> onion leaf (31.6 mg/kg)> cabbage (29.8 mg/kg)> garlic (27.4 mg/kg)> pea (27 mg/kg)> onion (26.2 mg/kg)> carrot (23.8 mg/kg)> beet (20.6 mg/kg)> cucumber (18.6 mg/kg), whereas in cultivated crops it is in the sequence of mustard (43.6 mg/kg)> wheat (28.4 mg/kg)> lentil (25.2 mg/kg)> paddy (17.4 mg/kg). In comparison to control condition, most of the crops and vegetables cultivated in the treated condition show decrease in chlorophyll and sugar and increase in ascorbic acid and prolin content. Leafy vegetables show lower protein content in stressed condition. Nutritional survey reveals that villagers of the study area are exposed to 35% more F by consuming food grains and vegetables cultivated by F contaminated (4.8 mg/L) irrigation water.

*Keywords:* Fluoride contaminated irrigation water, Fluoride accumulation, Crops/vegetables, Biochemical response, Human risk through diet.

## 1. Introduction
The principal source of fluoride (F) that causes fluorosis in humans is believed to be the sources of drinking waters. But in many parts of the world, elevated levels of F contaminated groundwater, often used for irrigation purposes, can have considerable adverse effects on the crops [1, 2]. Intake of F ion into roots is largely dependent on types of soil also. Fluoride is more soluble in acid soils due to which its uptake by plants is enhanced [3]. The toxic effect of F on pigments like chlorophyll and some secondary metabolites like sugar, ascorbic acid, amino acids and proteins are well documented [4 - 6].

Fluoride ingestion through food is comparatively less than through water. However, it cannot be neglected in the endemic areas because it will increase the F burden in addition to water. Fluoride of food items depends upon the F contents of soil and water used for irrigation. There is no stringent threshold limit of F in soil and plants above which the ingestion may be detrimental to human health.

In India, there are only limited studies available in the literature on fluoride content of raw foods and it became clear that fluorosis varies within the population. Factors responsible for these variations could be fluoride intake by drinking water, dietary intake, especially intake of food grown in soil or irrigated with water rich in fluoride [7]. So far no research work has been done have to study the effect of F on biochemical signature of crops/vegetables cultivated in actual field condition under the experimental design of control and treated condition and that to comparative assessment of human risk from F exposure through consumption of those cultivated crops/vegetables as dietary intake.

Junidpur village (24º06′07.5″N and 87º46′54.7″E) of Rampurhat block of the Birbhum district, West Bengal (Figure 1), where fluorosis has been known to be prevalent for some 6 – 8 years, was selected as one of the study areas for conducting the present research. The total population of Junidpur is about 400. Junidpur is selected as an appropriate area for conducting this research because people of this village are not only consuming F through drinking water but also consume through crops/vegetables cultivated in their own agricultural fields with the aid of F- contaminated irrigation water.

With these backgrounds the aims of the present research work are (i) to study the F accumulation in agricultural soils and crops/vegetables cultivated in control and treated condition (ii) to study the effect of F on biochemical parameters and (iii) to assess the risk of F exposure through diet in control and treated condition.

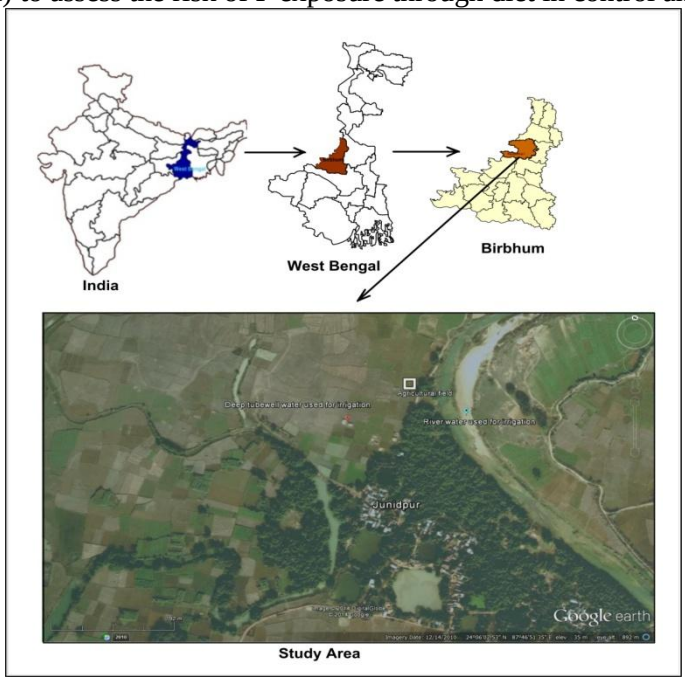


**Figure 1:** Study area location (Source: Google Earth)

## 2. Material and methods

*2.1. Layout of experimental plots*

Total experimental area of 66 $m^2$ was equally subdivided into 24 $m^2$ (excluding irrigation channel) plot along with irrigation channel for each for control and treated area. Each plot was then again subdivided into eight (8) small subplots (3 $m^2$). Each sub-plot was tilled with hand tractor and the soil was treated with 900 g of NPK (10:26:26) fertilizer and 100 g of urea before showing the seeds.

Different kinds of crops and vegetables *viz*., wheat, rice, mustard, onion, garlic, carrot, beet, peapod, cucumber, spinach, celeriac, lentil and cabbage were sown (Figure 2) in each subplots during winter season (Nov. 2012 to Feb. 2013) having atmospheric temperature of 13 – 18ºC. Another dose of 100 g nitrogenous fertilizer was applied at the middle session of the cultivation. Both control and treated plots were irrigated with river and deep bored submersible water having F concentration of 0.25 and 4.8 mg/L respectively for five times during entire cultivation times.

*2.2. Collection and analysis of soil and crop/vegetable samples*

Altogether thirty-two (32) pre and post harvest soil samples (grab samples) were collected from 16 subplots of control and treated condition. In case of crop and vegetable, four replicates were collected for each species and from each sub-plot. The collected vegetables were washed with distilled water. Their edible part were then separated and further dried, chopped into pieces, and blended thoroughly.

Next, 100 g samples of edible part were air-dried at 80ºC, ground, and passed through a 40-mesh sieve. The un-sieved material was sealed in polythene plastic bottles for further use. Similar kind of procedure was also

followed for the husked rice, wheat and mustard. Various biochemical parameters such as leaf extracts pH [8], relative water content (RWC) [9], total chlorophyll [10], ascorbic acid [11], soluble sugar [12] and protein [13] were measured from the processed edible samples.

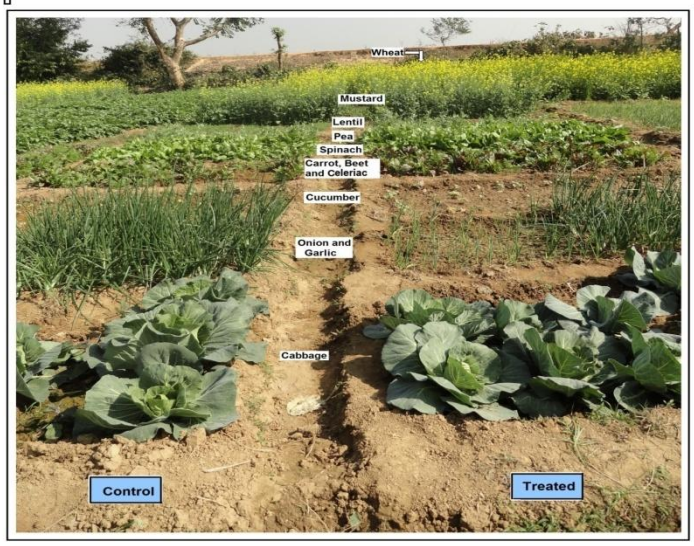


**Figure 2:** Layout of experimental plot layout

Apart from this, water-soluble F ($F_{H2O}$) in crops and vegetables samples was determined by water-extractable (1:1) method [14]. In this method, $F_{H2O}$ of the plant samples was estimated by calcium sulphate extraction method (10 g air-dried plant sample added to 0.1 g $CaSO_4$ in 200 ml of deionized water). The total F ($F_{Total}$) in samples was determined by using alkali fusion-Ion selective technique [15]. Approximately 0.25 g of the dried (105°C) grounded sample was taken into a 50 ml Ni crucible and 3 ml of 17N NaOH was added to it. The crucible was tapped slightly to mix the content and placed in oven atm 150°C for 1 h. The crucible was then placed inside a muffle furnace and slowly raised the temperature to 600°C for 30 min. Then the crucible was removed from the furnace and cooled, and 10 ml of de-ionized water was added and heated slightly to dissolve NaOH cake. After cooling, about 8 ml of concentrated HCl was added and pH of solution was adjusted to 8 – 9 to precipitate the interfering ions such as Fe and Al. The content was then transferred to 100 ml volumetric flask, diluted to the volume and filtered through Whattman 40 filter paper. 5 ml of TISAB-III was added to 5 ml of filtrate and fluoride measurement was done by Orion ion-selective electrode.

*2.3. Bio-Concentration Factor (BCF) determination*

Bio-concentration factor (BCF) is a common parameter for estimating the $F^-$ concentration in vegetables and subsequently human exposure through consumption of vegetables [16], which is defined as the ratio of between the concentrations of $F^-$ in the edible part of the vegetable to $F^-$ concentration in soil:

$$BCF = \frac{\text{F vegetable}}{\text{F soil}}$$

where, BCF is the bio-concentration factor of $F^-$ (mg/kg $d.wt._{plant}$/mg/kg $d.wt._{soil}$).

*2.4. Nutritional survey*

The nutritional survey among the adults (18 -70 yrs) was conducted in both the endemic areas of Junidpur and Nowapara. Survey revealed that on an average everyday each person takes 200 g of rice, 200 g of grains (wheat: 140 g; mustard: 30 g and lentil: 30 g) and 250 g vegetable as diet.

*2.5. Estimation of total F intake (TFI)*

Total F intake (TFI) is the summation of the daily F intake through entire diet sources. Daily F intake (DFI) from the particular diet source [7] was calculated by multiplying the F concentration of the respective item with total quantity of the particular item consumed per day.

$$TFI = \sum(DFI) \; ; \; DFI = FC \times QD$$

Where, FC is the F concentration in the diet source and QD is the quantity of the diet intake per day.

### *2.6. Statistical analysis*

#### *2.6.1. Pearson co-relation co-efficient*

The degree of association or the strength of a linear relationship among the biochemical variables and fluoride concentration in experimental crops and vegetables was evaluated by calculating the Person's coefficient of correlation (r).

$$r = \frac{\sum_{i-1}^{n}(X_{i-}\bar{X})(Y_{i-}\bar{Y})}{(n-1)S_X S_Y}$$

where, X and Y are two variables, with means $\bar{X}$ and $\bar{Y}$ respectively and standard deviations $S_X$ and $S_Y$.

#### *2.6.2. Matched pair data analysis*

Matched pair data analysis techniques is applied for biochemical parameters under control and treated condition, which can be analysed using the t-test to assess the significance of mean difference.

$t = \frac{\bar{D}}{SE}$ ; where $\bar{D}$ = mean difference between n pairs of values; Standard error (SE) of mean difference = $\frac{SD}{\sqrt{n-1}}$ ; where standard deviation (SD) = $\sqrt{\sum(D-\bar{D})^2/n}$

### *2.7. Quality control assurance for biochemical analysis*

All the chemical and reagents are used for the present research work of analytical grades. Sulfuric acid ($H_2SO_4$), hydrochloric acid (HCl), sodium hydroxide (NaOH) was purchased from Merk (Darmstadt, Germany). TISAB III (Total Ionic Strengh Adjustment Buffer) concentrates with CDTA were purchased from Thermo Fisher Scientific, USA. Calibration standard were prepared for certified traceable to National institute of standards and technology (NIST) standard reference material solution of $F^-$ 100 mg/L, Thermo (USA). The pH of the sample solution was adjusted with 0.1 M HCl/ 0.1 M NaOH. All the glassware were kept overnight in 5 M $HNO_3$, rinsed with deionized water before used.

## 3. Results and discussion

### *3.1. Fluoride in soil*

Soluble F content in soil is biologically important to plant and animals [17]. The solubility of F in soil is controlled mainly through F adsorption by inorganic constituents of the soil and soil pH [18] In normal soil the F is strongly adsorbed to the soil and hence plant uptake of F is generally minimal [19, 20]. In comparison to pre harvest control soil, application of F-contaminated irrigation water shows significant ($r = 0.55$; $p < 0.05$) accumulation of $F_{Total}$ (164 mg/kg) and $F_{H2O}$ (9.41 mg/kg) in post harvest treated soil (Table 1). Treated soil is slightly acidic in nature which may also suggest greater solubility of F due to the formation of $AlF_x$ complexes.

**Table 1:** Statistical summary of pre and post harvest soil pH, $F_{Total}$ and $F_{H2O}$ under control and treated condition (n=32)

| Descriptive Statistics | Pre harvest | | | | | | Post harvest | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Control | | | Treated | | | Control | | | Treated | | |
| | pH | $F_{Total}$ | $F_{H2O}$ | pH | $F_{Total}$ | $F_{H2O}$ | pH | $F_{Total}$ | $F_{H2O}$ | pH | $F_{Total}$ | $F_{H2O}$ |
| **Range** | 5.75 – 6.10 | 153.20–174.00 | 10.90 – 17.20 | 5.55 – 6.12 | 153.60 – 178.80 | 13.8 – 17.35 | 5.10 – 5.49 | 146.40 – 169.80 | 6.25 – 10.30 | 5.18 – 5.67 | 154.00 – 171.20 | 7.75 – 13.10 |
| **Mean** | 5.95 | 160.42 | 13.98 | 5.87 | 164.38 | 15.57 | 5.33 | 154.18 | 8.22 | 5.41 | 164.73 | 9.41 |
| **Standard Deviation** | 0.12 | 6.42 | 2.35 | 0.12 | 8.37 | 1.40 | 0.21 | 8.22 | 1.27 | 0.16 | 5.71 | 1.80 |

*3.2. Fluoride in crops and vegetables*

In case of treated plot total F accumulation in vegetables is in the sequence of spinach> celeriac leaf > onion leaf > cabbage > garlic> pea> onion> carrot> beet> cucumber, whereas in cultivated crops it is found in the sequence of mustard> wheat> lentil> paddy. Similar kind of accumulation trend is noticed in case of water soluble F also (Table 2). Among vegetables a distinct kind of accumulation trend is noticed *i.e.* underground rooted vegetables accumulate less F than leafy vegetables [21]. Higher accumulation of $F^-$ can also be explained by differential source, sink and translocation system of plant organs along with differential absorption potential of $F^-$ due to active and passive transport of $F^-$ along with water. Again strong transpiration pool theory can explain higher $F^-$ accumulation in the foliage parts of plant due to efficacious $F^-$ transport as a result of creation of transpiration pool mediated by rapid loss of water through stomata. In our experiment maximum $F^-$ accumulation in leaf might be due to the active transport of water along with $F^-$ as well as its accumulation in the foliage leaf.

*3.3. Effect of F on biochemical constituents*

Analytical results of biochemical parameters are represented in Table 2. The Relative Water Content (RWC) indicates change in leaf matrix hydration condition and will generate higher acidic condition when RWC is low. In our study area similar kind of observations is also noticed among the treated vegetables.

Experimental results indicate that except onion and pea, chlorophyll content in most of the vegetables has decreased in treated condition. Maximum decrease is found in cabbage (36%) followed by spinach (27%), onion leaf (23%), cucumber (11%) and celeriac (5%). One of the early symptoms of F damage in plants is the loss of chlorophyll, which seems to be related to the destruction of chloroplasts. Reduction in chlorophyll concentration is due to inhibition of incorporation of δ - aminolevulinic acid into chlorophyll synthetic pathway [22, 23]. According to Pearson Correlation Co-efficient chlorophyll has a significant ($p< 0.05$) negative correlation with total F in onion leaf ($r = -0.678$) and celeriac ($r = - 0.945$).

The sugar level in plants is directly related to stress factors [24]. Like ascorbic acid the reducing sugar content initially has decreased then increased with increasing concentration of F [6]. In this study sugar concentration has decreased under treated condition. Maximum decrease in sugar content is noticed in onion (41%). Soluble sugar has a significant positive ($p< 0.05$) correlation Co-efficient with total F in case of treated vegetables *viz.*, onion leaf ($r = -0.654$), carrot ($r = -0.777$), garlic ($r = -0.703$), pea ($r = 0.849$), cucumber ($r = -0.568$) and crops like wheat ($r = -0.613$) and rice ($r = -0.881$).

In the present study protein shows a mixed up signature of decrease and increase in content among treated cops and vegetables. Among vegetables, maximum decrease (63%) is found in cabbage whereas in crop it is found in lentil (23%). Reported work shows gradual decrease of protein content in leaves of seedlings due to F stress condition [25]. According to some researchers [26] decrease of protein content may be attributed to the ability of F to modify the ratio free nucleotides and RNA. Protein has significant negative ($p< 0.05$) correlation with total F in case of treated spinach ($r = 0.903$), cabbage ($r = 0.852$) and rice ($r = 0.875$).

Ascorbic acid, an anti-oxidant, plays an important role against physiological stress [27]. In the present investigation treated vegetables have higher ascorbic acid content which may be due to degradation of soluble protein. Maximum ascorbic acid content is found in cabbage (76%) followed by garlic (41%), onion (39%), pea (35%) and spinach (16%). The binding of F with ascorbic acid oxidised enzyme inhibits the breakdown of ascorbic acid which may responsible for the increase in ascorbic acid content at the highest F concentration [6].

Proline also stabilizes cellular structure and acts as a free radical scavenger [28]. A characteristic feature of the present investigation is the higher accumulation of proline content in crops/vegetables under the influence of F-contaminated irrigation water. In many crops and vegetables level of free proline is known to rise under stress condition [29]. The results of the present investigations are also in accordance with the findings. Maximum increase in proline content has been found in cabbage where as minimum one is noticed in beet.

Ascorbic acid and proline have significant ($p< 0.05$) positive correlation with total F in case of treated spinach ($r = 0.830$ and 0.700 respectively) and treated onion leaf ($r = 0.528$ and 0.765).

**Table 2:** Biochemical analysis [except pH and RWC (%) all the parameters are expressed in mg/100g-f.w.] of crops and vegetables[*]([†]n=4) along with total fluoride and water soluble fluoride (mg/kg)

| Crops/Vegetables | Scientific name | pH | RWC | Total Chlorophyll | Soluble Sugar | Protein | Ascorbic acid | Proline | $F_{H2O}$ | $F_{Total}$ |
|---|---|---|---|---|---|---|---|---|---|---|
| ‖Spinach C | *Spinacia oleracea* | 6.18±0.026 | 78.68±0.095 | 3.1±0.068 | 0.368±0.023 | 11.047±0.354 | 1.242±0.045 | 0.104 ± | 8.3±.359 | 47±2.160 |
| ‖Spinach T | | 6.38±0.026‡ | 80.95±0.242‡ | 2.26±0.026‡ | 0.427±0.021‡ | 9.520±0.393‡ | 1.437±0.137‡ | 0.143±0.04 | 10.3±.27 | 55±2.944‡ |
| Onion C | *Allium cepa* | 5.26±0.029 | 87.94±0.346 | 0.037±0.002 | 8.548±0.177 | 1.414±0.139 | 14.580±0.180 | 0.613±0.19 | 1.08±.01 | 24±2.582 |
| Onion T | | 5.61±0.051§ | 89.92±0.202‡ | 0.066±.003‡ | 5.086±0.207‡ | 2.043±0.175‡ | 20.242±0.167‡ | 0.681±0.17 | 1.19±.02 | 26.2±1.867‡ |
| Onion (leaf)C | | 6.15±0.034 | 86.14±0.036 | 2.12±0.034 | 3.497±0.166 | 6.355±0.163 | 4.451±0.101 | 0.095±0.04 | 1.39±.02 | 20.8±1.992 |
| Onion T | | 6.18±0.029‡ | 87.1±0.026‡ | 1.62±0.026‡ | 4.006±0.234‡ | 5.761±0.166‡ | 4.840±0.115‡ | 0.162±0.04 | 1.73±.01 | 31.6±.627‡ |
| Cabbage C | *Brassica oleracea* | 6.45±0.032 | 87.27±0.289 | 2.32±0.026 | 2.064±0.169 | 6.027±0.174 | 2.284±0.143 | 0.186±0.03 | 0.86±.01 | 22.6±2.570 |
| Cabbage T | *Capitata* | 6.52±0.026‡ | 83.46±0.316‡ | 1.48±0.107‡ | 3.474±0.163‡ | 2.235±0.161‡ | 4.027±0.189‡ | 0.705±0.16 | 0.868±.0 | 29.8±.787‡ |
| Beet C | *Beta vulgaris* | 5.88±0.034 | 86.7±0.183 | nm** | 3.140±0.189 | 0.860±0.159 | 13.311±0.388 | 0.291±0.01 | 1.002±.0 | 17.36±.084 |
| Beet T | | 5.89±0.032 | 89.98±0.041‡ | nm | 2.979±0.222§ | 1.263±0.178‡ | 14.070±0.235‡ | 0.308±0.02 | 1.178±.0 | 20.6±.594‡ |
| Celeriac C | *Apium graveolens* | 6.05±0.026 | 79.32±0.148 | 3.59±0.041 | 2.106±0.252 | 6.068±0.200 | 15.407±0.174 | 0.301±0.05 | 4.04±.02 | 29.6±.529 |
| Celeriac T | *rapaceum* | 6.52±0.026‡ | 83.91±0.156‡ | 3.39±0.041‡ | 1.414±0.194‡ | 6.696±0.185‡ | 15.985±0.050‡ | 0.340±0.04 | 4.52±.03 | 42±2.160‡ |
| Carrot C | *Daucus carota* | 5.29±0.032 | 88.44±0.034 | nm | 7.762±0.160 | 1.076±0.240 | 6.300±0.024 | 0.078±0.03 | 1.39±.02 | 20±2.582 |
| Carrot T | *sativus* | 5.56±0.036‡ | 89.83±0.039‡ | nm | 4.824±0.120‡ | 1.032±0.201 | 6.564±0.003‡ | 0.084±0.03 | 1.46±.02 | 23.8±.258‡ |
| Garlic C | *Allium sativum* | 4.92±0.032 | 88.64±0.195 | nm | 7.060±0.189 | 0.769±0.126 | 26.109±0.161 | 0.680±0.10 | 0.392±.0 | 8.26±.196 |
| Garlic T | | 5.6±0.316‡ | 85.86±0.419‡ | nm | 6.244±0.153‡ | 0.850±0.117‡ | 36.912±0.130‡ | 1.656±0.22 | 0.554±.0 | 27.4±1.866‡ |
| Pea C | *Pisum sativum* | 4.92±0.026 | 87.76±0.252 | 0.128±0.003 | 8.593±0.230 | 3.786±0.141 | 6.527±0.576 | 0.330±0.13 | 1.106±.0 | 25.4±0.497 |
| Pea T | | 5.69±0.034‡ | 92.35±0.190‡ | 0.188±0.003‡ | 6.314±0.165‡ | 4.006±0.229‡ | 8.779±0.774‡ | 0.630±0.17 | 1.21±.03 | 27±1.826§ |
| Cucumber C | *Cucumis sativa* | 5.19±0.028 | 90.56±0.214 | 0.686±0.025 | 4.576±0.181 | 2.644±0.144 | 5.618±0.119 | 0.364±0.02 | 1.342±.0 | 12.52±0.108 |
| Cucumber T | | 5.26±0.032‡ | 88.54±0.032‡ | 0.606±0.034‡ | 4.166±0.166‡ | 1.314±0.118‡ | 5.904±0.003‡ | 0.402±0.01 | 1.562±.0 | 18.64±0.360 |
| Lentil C | *Lens culinaris* | 6.21±0.029 | 36.87±0.295 | nm | 6.811±0.116 | 6.398±0.157 | 21.562±0.236 | 0.576±0.20 | 1.9±.059 | 21.4±0.294 |
| Lentil T | | 6.29±0.037‡ | 34.59±0.248‡ | nm | 5.163±0.084‡ | 4.916±0.193‡ | 22.325±0.249‡ | 0.867±0.14 | 2.38±.02 | 25.2±0.337‡ |
| WheatC | *Triticum sp* | 5.79±0.037 | 37.06±0.290 | nm | 5.972±0.166 | 1.292±0.126 | 7.891±0.262 | 0.217±0.09 | 0.902±.0 | 15.06±0.127 |
| Wheat T | | 5.91±0.037‡ | 26.29±0.192‡ | nm | 5.655±0.206‡ | 1.307±0.121‡ | 8.911±0.168‡ | 0.157±0.08 | 1.946±.1 | 28.4±0.497‡ |
| Rice C | *Oryza sativa* | 6.35±0.029 | 43.57±0.212 | nm | 4.319±0.096 | 0.131±0.027 | 2.882±0.194 | 0.122±0.01 | 0.888±.0 | 14.9±0.294 |
| Rice T | | 6.47±0.018‡ | 32.63±0.283‡ | nm | 3.304±0.117‡ | 0.374±0.094‡ | 2.684±0.179 | 0.144±0.02 | 1.49±.05 | 17.44±0.029 |
| Masturd C | *Brassica juncea* | 6.28±0.026 | 19.35±0.032 | nm | 4.213±0.095 | 11.779±0.305 | 57.191±0.026 | 1.392±0.15 | 2.24±.04 | 20.8±0.572 |
| Mastard T | | 6.66±0.026‡ | 18.81±0.022‡ | nm | 5.012±0.181‡ | 13.400±0.114 | 42.420±0.385‡ | 1.759±0.16 | 2.28±.03 | 43.6±0.572‡ |

[*]Data aremean±SD; [†]n=4 meaning 4 samples of each species was analysed; ‡p<.01; §p<.05; ‖C, T: indicates control and treated; ** nm: not measure

*3.4. Bio-Concentration Factor (BCF) of fluoride in vegetable and cereal crops*

Bio-concentration factor has been used as an indicator of affinity for the accumulation of F in plants. Because of its simple application, it is widely used [30]. In case of leafy vegetables BCFs in both the control and treated area are in the sequence of spinach> radish leaf> celeriac> onion leaf > cauliflower > cabbage but in case of fruity and underground rooted vegetables the sequences are tomato> cucumber> pea and radish> carrot> beet> potato> garlic respectively both in control and treated areas (Table 3). Regarding crops wheat shows marginally higher BCF than rice in both the areas. In control soil areas, the BCF values are found to be quite less in most of the vegetables and cereal crops. It is observed that the BCF increased with the increase of F concentrations in the treated soil. These findings are consistent with those reported in literature of [21]. The higher BCF values in spinach, tomato and radish indicated that these plants have high affinity for the accumulation of F under treated condition. However, Swartjes et al., [31] had reported that BCF values are not always constant in specific crops and vegetables and is largely affected by soil properties like soil pH, clay content, organic matter and F concentration in soil and also plant factors like type of plant and growth rate.

**Table 3:** Bio-concentration factor of different crops and vegetables

| Types of crops/vegetables | Crops/ vegetables | Scientific name | Control | | Treated | |
|---|---|---|---|---|---|---|
| | | | Water soluble fluoride (*$F_{H2O}$) | Bio-concentration factor (BCF) | Water soluble fluoride ($F_{H2O}$) | Bio-concentration factor (BCF) |
| **Leafy vegetables** | Spinach | *Spinacia oleracea* | 8.30 | 0.708 | 10.30 | 0.769 |
| | Cabbage | *Brassica oleracea Capitata* | 0.86 | 0.073 | 0.87 | 0.065 |
| | Cauliflower | *Brassica oleracea* | 0.86 | 0.073 | 0.92 | 0.069 |
| | Celeriac | *Apium graveolens rapaceum* | 4.04 | 0.345 | 4.52 | 0.337 |
| | Onion leaf | *Allium cepa* | 1.08 | 0.092 | 1.19 | 0.089 |
| | Radish leaf | *Raphanus sativus* | 4.06 | 0.346 | 4.88 | 0.364 |
| **Fruity vegetables** | Cucumber | *Cucumis sativa* | 1.34 | 0.114 | 1.56 | 0.116 |
| | Pea | *Pisum sativum* | 1.11 | 0.095 | 1.21 | 0.090 |
| | Tomato | *Solanum lycopersicum* | 2.02 | 0.172 | 2.54 | 0.190 |
| **Underground rooted vegetables** | Carrot | *Daucus carota sativus* | 1.39 | 0.119 | 1.46 | 0.109 |
| | Potato | *Solanum tuberosum* | 0.59 | 0.050 | 1.05 | 0.078 |
| | Beet | *Beta vulgaris* | 1.00 | 0.085 | 1.18 | 0.088 |
| | Onion | *Allium cepa* | 1.08 | 0.092 | 1.19 | 0.089 |
| | Garlic | *Allium sativum* | 0.39 | 0.033 | 0.55 | 0.041 |
| | Radish | *Raphanus sativus* | 3.02 | 0.258 | 3.56 | 0.263 |
| **Cereal crops** | Lentil | *Lens culinaris* | 1.90 | 0.162 | 2.38 | 0.178 |
| | Mustard | *Brassica juncea* | 2.24 | 0.191 | 2.28 | 0.170 |
| | Wheat | *Triticum sp* | 0.90 | 0.077 | 1.95 | 0.146 |
| | Rice | *Oryza sativa* | 0.89 | 0.076 | 1.49 | 0.111 |

** Water soluble fluoride ($F_{H2O}$) represents in mg/kg*

*3.5. Dietary intake of fluoride through meals*

Daily intake of F through rice, grains and vegetables (total quantity 650 g fwt) from control condition are 0.253, 0.290 and 0.625 mg whereas in treated condition intakes are 0.296, 0.512 and 0.772 mg fwt. With respect to total F intake these are 1.80 and 2.437 mg/kg fwt in control and treated condition respectively (Table 4). This observation very much corroborates to our earlier observation [2]. Outcome of this study reveals that study area is people 35% more exposed to F through dietary intake consisting of treated area crops and vegetables.

**Table 4:** Daily intake of F (mg/kg) through diet

| | | | Control | | Treated | | |
|---|---|---|---|---|---|---|---|
| **Vegetables** | **Daily intake (g fwt)** | **$F_{Total}$ (mg/kg dwt)** | **#$F_{Total}$ (mg/kg fwt)** | **Daily intake of F (mg)** | **$F_{Total}$ (mg/kg dwt)** | **$F_{Total}$ (mg/kg fwt)** | **Daily intake of F (mg)** |
| Spinach | 55 | 47 | 4.00 | 0.220 | 55 | 4.68 | 0.257 |
| Onion | 30 | 24 | 2.04 | 0.061 | 26.2 | 2.23 | 0.067 |
| Onion (leaf) | 25 | 20.8 | 1.77 | 0.044 | 31.6 | 2.69 | 0.067 |
| Cabbage | 52 | 22.6 | 1.92 | 0.100 | 29.8 | 2.53 | 0.132 |
| Beet | 15 | 17.36 | 1.48 | 0.022 | 20.6 | 1.75 | 0.026 |
| Celeriac | 12 | 29.6 | 2.52 | 0.030 | 42 | 3.57 | 0.043 |
| Carrot | 20 | 20 | 1.70 | 0.034 | 23.8 | 2.02 | 0.040 |
| Pea | 32 | 25.4 | 2.16 | 0.069 | 27 | 2.30 | 0.073 |
| Cucumber | 42 | 12.52 | 1.06 | 0.045 | 18.64 | 1.58 | 0.067 |
| Total F Intake | | | | 0.625 | | | 0.772 |
| Total F intake/250g of vegetable | | | | 0.552 | | | 0.682 |
| **Grains** | **Daily intake (g dwt)** | **$F_{Total}$ (mg/kg dwt)** | **$F_{Total}$ (mg/kg fwt)** | **Daily intake of F (mg)** | **$F_{Total}$ (mg/kg dwt)** | **$F_{Total}$ (mg/kg fwt)** | **Daily intake of F (mg)** |
| Mustard | 30 | 20.8 | 1.768 | 0.053 | 43.6 | 3.706 | 0.111 |
| Lentil | 30 | 21.4 | 1.819 | 0.055 | 25.2 | 2.142 | 0.064 |
| Wheat | 140 | 15.06 | 1.2801 | 0.179 | 28.4 | 2.414 | 0.338 |
| **Rice** | **Daily intake (g dwt)** | **$F_{Total}$ (mg/kg dwt)** | **$F_{Total}$ (mg/kg fwt)** | **Daily intake of F (mg)** | **$F_{Total}$ (mg/kg dwt)** | **$F_{Total}$ (mg/kg fwt)** | **Daily intake of F (mg)** |
| Paddy | 200 | 14.9 | 1.267 | 0.253 | 17.440 | 1.482 | 0.296 |
| **Diet intake/day/person** | **Rice (200g)** | **Grains (200g)** | | **Vegetables (250 g)** | **Total (mg/650g)** | | **Total Intake of F(mg/kg)** |
| Control | 0.253 | 0.29 | | 0.625 | 1.168 | | 1.80 |
| Treated | 0.296 | 0.512 | | 0.772 | 1.580 | | 2.43 |

# fwt to dwt conversion factor=0.085 [32]

## Conclusion

An inventory of fluoride concentration in irrigation water and its effect on plant biochemical constituents is thus an important step toward curbing the spread of fluorosis. As seen here, the F content of leafy parts of vegetables is much higher than that of fruits and tubers. In comparison to vegetables F accumulation in seed is low. In order to reduce the risk of human exposure to F, the use of F contaminated irrigation water should be reduced as much as possible. It is advisable to grow crops with relatively low capabilities to enrich F, such as those with seeds or tubers as the main edible part.

**Acknowledgements-** Financial support from the Department of Science & Technology (SERC division; Sanction order no. SR/S4/ES-547/2010), Government of India is gratefully acknowledged. The authors also wish to thank all the inhabitants of Junidpur village, Rampurhat – II block, Birbhum district for their sincere support in conducting the experiment as well as nutritional survey.

## References

1. Dissanayake C.B., Chandrajith, R., Freiwald A., series editor. Berlin Heidelberg: Springer, Springer Science Business Media, Springer-Verlag Berlin Heidelberg, (2009).
2. Gupta S., Banerjee, S., *Fluoride*, 44 (2011) 153.
3. Yadav R.K., Sharma, S., Bansal, M.,, Singh, A., Panday, V., Maheshwari, R., *Adv. Biores.*, 3 (2012) 14.
4. Singh V., Sharma, R.K., Arya, K.P.S., *Int. J. Plant Sci.*, 4 (2009) 79.
5. Chakraborty S., Patra, P.K., Mandal, B., Mahato, D., *Fluoride*, 45 (2012) 257.
6. Gupta S., Banerjee, S., Mondal, S., *Fluoride*, 42 (2009)142.
7. Amalraj A., Anitha Pius, A., *Food Sci. Human Wellness***,** 2 (2013) 75.
8. Singh SK., Rao, DN., Proceedings Symposium on Air Pollution Control, (Indian Association for Air Pollution Control, New Delhi, India), 1(1983) 218.
9. Sen D.N., Bhandari, M.C., *Indian Arid Zone, A.M. (Ed.). Environ. Physiol. Ecol. Plants*, (1978) 203.
10. Arnon D.I., *Plant Physiol*., 24 (1949) 1.
11. Keller T., Schwager, H., *Eur. J. Forestry Pathol*., 7 (1977) 338.
12. Sadashivam S., Manikam, A., 2nd edn. New Delhi: New Age international (p) limited, (1996).
13. Lowry O.H., Rosenbrugh, N.J., Fan, A.L., Randal, R.J., *J. Biol. Chem*., 193 (1951) 265.
14. Jezierska-Madziar M. Pinskwar, P., *Fluoride*, 36 (2003) 21.
15. McQuaker N.R., Gurney, M., *Anal. Chem*., 49 (1977) 53.
16. Gupta S., Mondal, D., Food and Nutritional Components in Focus No. 6, Royal Society of Chemistry, United Kingdom, Chapter 7 (2015)117.
17. Davison A.W., *Uptake, transport and accumulation of soil and airborne fluorides by vegetation. Eds. J. L. Shupe, H. B. Peterson and N.C. Leone, Paragon Press*, (1982) 61.
18. Loganathan P., Hedley, M.J., Grace, N.D., Lee, J., Cronin, S.J., Bola, N.S., Zanders, J.M., *Aust. J. Soil Res*., 41(2003) 501.
19. Barrow N.J., *J. Soil Sci.*, 37(1986) 267.
20. Singh B.R., *Norw. J. Agric. Sci*., 4 (1990) 23
21. Jha S.K., Nayak A.K., Sharma, Y.K., *Ecotoxicol Environ. Saf.*, 74 (2011) 940.
22. Wallis W.J., Miller, G.W., Psenak, M., Shieh, J., *Fluoride*, 7 (1974) 69.
23. Bhargava D., Bhardwaj, N., *J. Phytol*., 4 (2010) 41.
24. Verma S., Dubey, R.S., *Biologia. Plantarum.*, 44 (2001) 117.
25. Singh G., Kaur, P., Sharma, R., *Plant Physiol. Biochem*., 12 (1985) 104.
26. Baunthiyal M., Ranghar, S., *Fluoride*, 47 (2014) 287.
27. Abbasi M., Faghani, E., *J. Bio. Env. Sci.*, 6 (2015) 107.
28. Alia M.P., Matysik, J., *Amino Acids*, 21 (2001) 191.
29. Choudhary S., Bohra, S.P., *Bot. Bull. Academia sinica*., 30 (1989) 9.
30. Tome F.V., Rodriquez, M.P.B., Lozano, J.C., *J. Environ. Radioactivity*, 65 (2003) 161.
31. Swartjes F.A., Dirven-Van Breemen, E.M., Otte, P.F., Van Beelen, P., Rikken, M.G.J., Uinstra, J., Spijker, J., Lijzen J.P.A., Bilthoven, Netherlands, RIVM Report 711701040 (2007).
32. Rattan R.K., Datta, Sp., Chhonkkar, P.K., Suribabu, K., Singh, A.K., *Agric. Ecosys. Environ*., 109 (2005) 310.