INCORPORATION OF FLUORIDE IN VEGETATION AND ASSOCIATED BIOCHEMICAL CHANGES DUE TO FLUORIDE CONTAMINATION IN WATER AND SOIL: A COMPARATIVE FIELD STUDY

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ABSTRACT

High fluoride concentrations in natural groundwater of Nowapara and Junidpur villages of the Birbhum District in India has recently been highlighted as a serious environmental concern. A study has been conducted to estimate fluoride concentrations and water quality along with the translocation of fluoride into vegetables through soil and the stress effect of fluoride on some biochemical parameters in this area. The result has been compared with a non-contaminated area of Burdwan University farm of the Burdwan District as a control zone by collecting equal numbers and types of samples. The results showed a positive correlation of fluoride concentration with depth, indicating higher concentrations of fluoride in drinking water drawn from deep tubewells in this semi-arid region. A high bioconcentration factor (BCF) of fluoride in vegetables imposes a high health risk due to fluoride intake both from water and vegetation. Probable exposure to the inhabitants of these villages is speculated due to changed bio-chemical parameters like chlorophyll, sugar, amino acid, ascorbic acid and protein in the vegetables as a result of fluoride stress. In the future, ground water monitoring to supply safe drinking water may be an effective way against the negative impact of fluoride on the inhabitants.

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Keywords: Fluoride; birbhum district; vegetables; chlorophyll; ascorbic acid; amino acid

1. INTRODUCTION

Water-related problems have become a modern day challenge and a worldwide health problem is caused by the consumption of drinking water containing high fluoride [1]. There is a risk of endemic fluorosis where the fluoride level is more than 1.0 mg L^{-1} in drinking water [2]. Fluorosis is endemic in 20 states of India, affecting more than 65 million people, including 6 million children [3]. The villages of the Birbhum District of West Bengal, India, are under threat of fluorosis [4]. Harmful effects of fluoride are more relevant in the context of India compared to most Western countries where fluoridation of water is recommended to help prevent dental caries [5]. In nature, fluoride mainly occurs in ground water [6]. Enhanced fluoride intake through drinking water coupled with dietary intake could significantly substantiate total fluoride accumulation in body tissue [7].

Lithology and soil are the main factors that control the quality of water [8].The amount of fluoride occurring naturally in groundwater is governed principally by climate, composition of the host rock and hydrogeology. Some anthropogenic activities such as use of phosphate fertilizers, pesticides, sewage and sludges for agriculture, depletion of groundwater, etc., are also implicated as causes of increased fluoride concentration in groundwater [9,10]. These activities may take decades to increase the fluoride level in water by exceeding the adsorption capacity of soil [11]. Weathering of rocks and leaching of fluoride-bearing minerals are the major reasons of elevated concentrations of fluoride in groundwater [12,13]. Evaporation is another important phenomenon that concentrates the fluoride in arid regions [14]. When groundwater is used in irrigation, the vegetables grown also incorporate fluoride. Fluoride is absorbed by plant roots [15,16] and then transported via xylematic flow to the transpiratory organs, mainly leaves, where it can be accumulated with adverse effects. Certain physiological processes are known to be markedly affected by fluoride, including decreased plant growth, chlorosis, leaf tip burn and leaf necrosis [17-19]. This fluoride may affect the biochemical ratio of the plant body [20]. The toxic effect of fluoride on pigments like chlorophyll and some secondary metabolites like sugar, ascorbic acid, amino acids and proteins are well documented [18-24]. Fluoride causes reduction in photosynthetic pigment concentration [25], inhibition of photosynthesis [26] and changes in carbohydrate metabolism [27].

An inventory of fluoride concentration in drinking groundwater and its effect on plant physiology is thus an important step toward curbing the spread of fluorosis. The ability of plants to uptake and accumulate the non-essential element fluorine makes a potential threat to human health through its entrance into the food chain.

In the present study, we concentrate on the semi-arid zone of Birbhum District. Its groundwater is known to have elevated level of fluoride as reported by the School of Environment Studies, Jadavpur University, Kolkata. In this district, substantial amounts of different vegetables are cultivated with fluoride contaminated groundwater. As a result, fluoride accumulation in different parts of vegetables grown there is likely leading to fluoride contamination in the food chain. Therefore, in continuation of the authors' previous study by sampling the water, soil and vegetable, an attempt was made to assess the water quality, the transport of fluoride from soil to vegetation by examining the bioconcentration factor (BCF) and the effect of fluoride on biochemical constituents of leaves and fruits of the fluoride-contaminated area in comparison with the control area.

2. MATERIALS AND METHODS

2.1. Study Area

Two areas are chosen for the present study: one is a highly endemic fluoride region and the other is the control region where fluoride pollution is not reported so far. The polluted regions Nowapara (24°6'20.7"N and 77°47'3.1"E) and Junidpur (24°6'1.4"N and 77°46'53.5"E) are located in the Birbhum District of West Bengal, India (Fig. 1). The most interesting part of the geology of this area stems from the gradient of red soil. A kind of sandy hard red soil of the alfisoil type and latterite soil of this area give rise to potential aquifers at depth. Bore wells and open wells are the main source of water for domestic and agricultural purposes in this arid region. The Burdwan University seed multiplication farm and the nearby locality (23°15'12"N and 77°50'51"E) of the Burdwan District were chosen as the control area for the study (Fig. 1). Different types of soil are encountered in different topographical, biological and hydrological sites as well as geological conditions within the Burdwan District. In the study area, alluvial soil attains an enormous thickness. This alluvial soil is formed of alluvium brought down by the Damodar and numerous other rivers. These soils are sandy, well drained and slightly acidic in nature. Here also bore wells is the main source of drinking water; water of shallow pumps and river water is used for irrigation purposes.

2.2. Sampling

Water from ponds (S4, S9); tube wells (S1, S2, S3, S5, S6, S7, S8, S10, S11) having depths of 10 ft,12 ft, 100 ft, 65 ft, 65 ft, 97 ft, 67 ft, 60 ft, 60 ft, 75 ft, 90 ft, respectively, and shallow (S12, S13) (which is used for irrigation) having depths of 75ft and 70ft, respectively, were collected from the polluted zone during the winter season and stored in pre-cleaned and sterilized polythene bottles of one-liter capacity following standard protocols. The water samples were immediately refrigerated after collection. Vegetable samples were also collected from the polluted area, refrigerated immediately after collection and analysis was done as quickly as possible. Thirty-three soil samples were collected from the vegetable fields near the plants (three samples near each plant) in the polluted area and stored in zippered polyethene packets. Pond water (C1, C2); tube well water (C3, C4, C5, C6, C7, C8, C9, C10, C11) and shallow water (C12, C13) having depths of 11 ft,12 ft, 110 ft, 60 ft, 45 ft, 110 ft, 60 ft, 60 ft, 60 ft, 90 ft, 116 ft, 70 ft and 60 ft, respectively, along with vegetables and fruits similar to the polluted zone were collected from the control area.

2.3. Analysis of Water Quality Parameters

Some of water quality parameters such as pH , bicarbonate (HCO_3^-), calcium (Ca), magnesium (Mg), iron (Fe), nitrate (NO_3^-), phosphate (PO_4^{3-}), chloride (Cl^-), electrical conductivity (EC), sodium (Na), potassium (K), sulfate (SO_4^{2-}) values of the water samples were measured quantitatively using Standard methods of examination of water and waste water [28].

2.4. Analysis of Fluoride in Water and Soil

After adding 25 mL TISAB (4g 1,2-cyclohexanediamine-N,N,N',N'-tetraacetic acid (CDTA) + 57 g NaCl and 57 g glacial CH_3COOH in 1 L of distilled water adjusted to pH 5-5.5 with 6 N NaOH) to 25 mL of a water sample, fluoride concentration was measured with a fluoride ion selective electrode using an ORION 5 star ion analyzer. For the determination of water soluble fluoride in soil [29], an extract was made (1:1) using distilled water. Then the same procedure was followed as used for the water samples using 25 mL of the extract in the place of 25mL water sample. The limit of detection (LOD) of the method was 0.02 mg/L. All of the water parameters were analyzed following standard methods [28]. Bio-chemical parameters such as sugar [30], chlorophyll [31], ascorbic acid [32], protein [33] and amino acid [34] were studied from leaves and fruits of different vegetables.

2.5. Analysis of Fluoride in Vegetable Samples

Fluoride concentrations in vegetables and leaves were measured by the following method. After thorough washing with water, the fresh vegetables/leaves harvested from the contaminated and control area were dried at 105°C and crushed into powder so as to pass through a 40 mesh sieve. About 0.5 g each of the powdered samples was transferred into a 150-mL nickel crucible and moistened with a small amount of de-ionized water. Six mL of 16.8 N NaOH was added and the crucible was placed in an oven (150°C) for 1.5-2.0 hr until NaOH was solidified. The crucible was placed in a muffle furnace set at 300°C, then raised to 600°C and kept at 600°C for 30 min in order to fuse the sample in the crucible. The crucible was placed in a hood and allowed to cool, and 10 mL distilled water was added. Then, 37% HCl solution (about 7 mL) was added slowly to adjust the pH to 7-9. The sample solution was transferred to a 100 mL plastic volumetric flask, made up to volume with distilled water and filtered through a Whatman No. 40 filter paper [35]. The filtrate was used for analysis of fluoride with the same procedure and the same ion selective electrode used for water analysis.

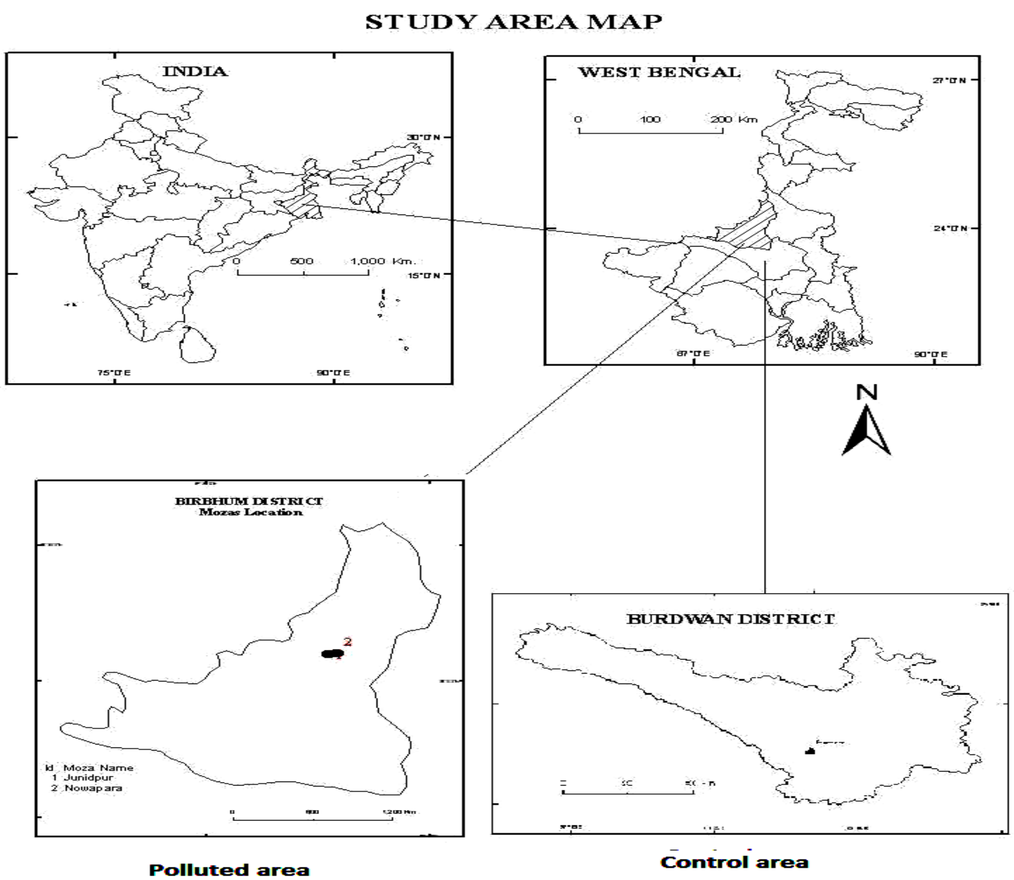

Figure 1 Study area

2.6. Bioconcentration Factor (BCF)

For estimating fluoride concentrations in vegetables the common parameter is the Bioconcentration Factor (BCF). BCF is the ratio of F concentration in the vegetable and F concentration in soil, i.e.,

$$BCF = \frac{\text{F concentration in vegetable (mg /kg of vegetable)}}{\text{F concentration in soil (mg/Kg soil)}}$$

2.7. Statistical Analysis

The data were expressed as mean ± standard deviation. A correlation study between the water fluoride level and the other water quality parameters was performed with SPSS statistical version 16.0.

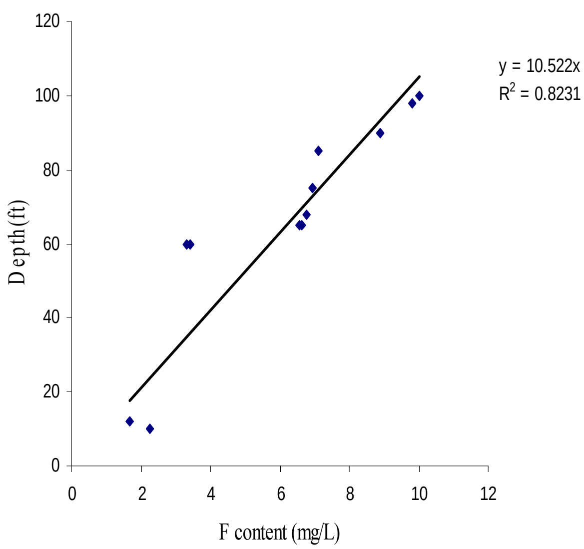

Figure 2 Correlation of depth of source with fluoride concentration in selected ground water samples in the polluted zone

3. RESULTS

3.1. Water Quality Parameters

The pond and shallow water samples were acidic (pH <7.0) and the water samples from the tube-wells were alkaline with pH values ranging from 7.1 to 8.7 in the polluted area. All the samples from different sources (pond, tube-well, shallow) were non-saline (EC <1.0 mS/cm) and soft in nature as the values of Ca and Mg were below the permissible limit of 75 mg/L and 150 mg/L, respectively [36]. Nitrate, chloride and iron contents of all the water samples from different sources (pond, tube-well, shallow) were below the permissible limit of 45 mg/L, 500mg/L and 1 mg/L, respectively [36]. The phosphate content of 23.07% water sources was above the limit of 0.1mg/L permitted by USPH [37]. The phosphate content of shallow water samples were well below the permissible limit.

The concentration of fluoride in the water from different source types ranged from 3.05 mg/L to 10.2 mg/L for tube wells, 1.54 to 2.30 mg/L for ponds and 0.560 to 0.612 mg/L for shallow samples (Table 1). All the water samples from tube wells had fluoride concentration above the permissible limit of 1.5 mg/L [36]. The water samples from the control zone were non-fluorinated (F^- <1.5mg/L), slightly alkaline (pH >7), non-saline (EC <1mS/cm). The Cl^-, total iron, Ca and Mg contents, of this water was well below the permissible limit [36] (Table 2).

3.2. Depth Variation of Fluoride

The approximate depths of sources of the samples showed a positive correlation with the fluoride concentration in the polluted area (Fig. 2). But this correlation is not so significant (Fig. 3) in the control area as the control area contains low level of fluoride. The highest mean values of fluoride were found to be 10.0 mg/L in groundwater having the highest depth (100 ft) in the fluoride-contaminated area. The amounts of fluoride in surface water were lower in comparison to groundwater (Table 1).

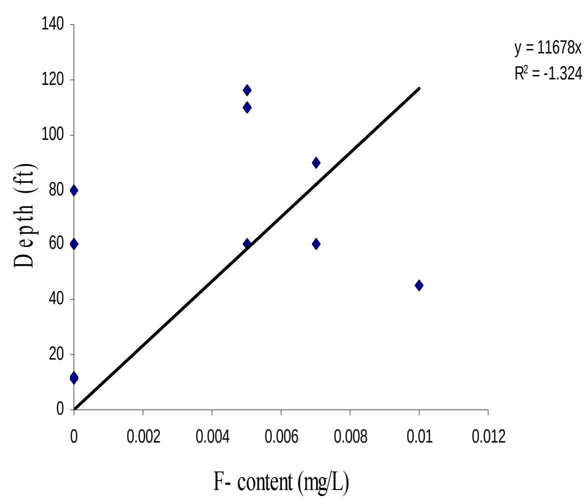

Figure 3 Correlation of depth of source with fluoride concentration in selected ground water samples in the control zone.

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Table 1 Analytical data of selected water samples of Nowapara and Junidpur in the Birbhum District

ample (with depth)	F^- (mg/L)	pH	HCO_3^- (mg/L)	Ca (mg/L)	Mg (mg/L)	Fe (mg/L)	NO_3^- (mg/L)	PO_4^{3-} (mg/L)	Cl^- (mg/L)	EC (mS/cm)	Na (mg/L)	K (mg/L)	SO_4^{2-} (mg/L)
S1(100ft)	10.00± 0.27	8.4± 0.2	200.60 ±4.69	40.0±5.3	4.7± 1.2	0.153± 0.013	1.093± 0.092	0.048± 0.005	31.48± 0.81	0.3± 0.0	116.30 ±2.92	19.93±0.23	426.13 ±6.12
S2(65ft)	6.56± 0.35	7.7± 0.1	144.17 ± 1.90	2.7±1.2	3.3± 1.2	0.218± 0.004	0.759± 0.035	0.152± 0.003	44.07± 0.40	0.6± 0.1	68.57± 2.64	32.97±0.57	216.43 ±4.06
S3(65ft)	6.62± 0.29	8.1± 0.2	71.5± 1.9	4.7±1.2	8.0± 2.0	0.135± 0.003	2.151± 0.069	0.219± 0.013	195.17 ± 0.38	0.5± 0.1	80.23± 1.22	45.13±0.57	129.93 ±2.89
S4(10ft)	2.24± 0.10	6.9± 0.4	81.67± 3.13	4.7±1.2	24.0± 3.5	0.200± 0.007	1.622± 2.105	0.084± 0.006	56.66± 0.63	0.4± 0.1	51.67± 1.26	53.10±0.44	173.27 ±8.28
S5(98ft)	9.80± 0.18	7.9± 0.2	32.8± 2.6	2.0±2.0	230.0± 7.2	0.620± 0.036	14.145 ±0.403	0.000± 0.00	119.62 ± 0.52	0.8± 0.1	135.00 ±2.55	23.30±1.01	309.27 ±6.50
S6(68ft)	6.78± 0.17	7.8± 0.3	28.13± 0.47	2.7±1.2	44.0± 5.3	0.480± 0.015	2.668± 0.161	0.010± 0.009	44.07± 0.25	0.4± 0.0	98.23± 2.61	56.40±0.66	103.87 ±7.83
S7(60ft)	3.31± 0.23	7.3± 0.3	59.50± 2.36	3.3±1.2	64.0± 6.9	0.190± 0.020	1.380± 0.322	0.114± 0.008	37.78± 0.70	0.5± 0.0	234.1± 1.3	107.33±0.9 3	144.53 ±4.87
S8(60ft)	3.42± 0.92	8.5± 0.3	135.77 ± 2.16	36.0±5.3	132.0± 5.3	0.174± 0.005	2.001± 0.127	0.030± 0.014	188.87 ± 0.61	0.7± 0.1	147.93 ±2.47	31.26±1.03	329.70 ±7.13
S9(12ft)	1.67± 0.11	6.7± 0.2	19.33± 1.85	4.0±2.0	56.0± 5.3	0.246± 0.219	1.909± 0.053	0.012± 0.002	62.96± 0.06	0.4± 0.0	199.07 ±3.09	33.40±0.70	227.63 ±5.48
S10(85ft)	7.10± 0.13	8.5± 0.1	110.33 ±2.15	2.0±2.0	50.0± 6.0	0.709± 0.004	1.714± 0.127	0.015± 0.003	44.07± 0.14	0.8± 0.1	64.30± 1.45	87.17±0.83	213.77 ±3.96
S11(90ft)	8.88± 0.23	8.2± 0.2	70.80± 6.12	24.0±4.0	132.0± 10.6	0.700± 0.006	0.667± 0.064	0.010± 0.002	62.96± 0.29	0.9± 0.0	135.33 ±2.01	61.67±0.71	401.10 ±8.78
S12(75ft)	6.94± 0.12	6.6± 0.2	50.03± 1.99	36.0±3.5	76.0± 5.3	0.632± 0.008	1.576± 0.058	0.029± 0.004	94.44± 0.43	0.7± 0.0	90.30± 1.45	71.40±0.46	214.90 ±8.69
S13(70ft)	0.58± 0.03	6.2± 0.1	64.83± 1.40	28.0±4.0	128.0± 10.6	0.290± 0.004	0.334± 0.040	0.031± 0.005	44.07± 0.03	0.4± 0.0	76.80± 1.45	92.57±0.71	137.63 ±7.07

Table 2 Analytical data of selected water samples of control area of the Burdwan District

Sample (with depth)	F^- (mg/L)	pH	HCO_3^- (mg/L)	Ca (mg/L)	Mg (mg/L)	Fe (mg/L)	NO_3^- (mg/L)	PO_4^{3-} (mg/L)	Cl^- (mg/L)	EC (mS/cm)	Na (mg/L)	K (mg/L)	SO_4^{2-} (mg/L)
C1(11ft)	0.00± 0.00	8.6± 0.1	16.27± 0.50	34.1± 2.2	14.1± 2.2	0.053± 0.013	0.22± 0.03	0.082± 0.004	63.01± 0.06	0.8±0.1	35.53± 0.70	11.47± 0.57	130.04±2.39
C2(12ft)	0.00± 0.00	8.5± 0.2	40.37± 0.07	14.0± 2.2	36.2± 4.2	0.061± 0.004	0.25± 0.07	0.066± 0.011	44.11± 0.41	0.6±0.0	24.30± 0.75	8.93± 0.25	144.87±3.55
C3(110ft)	0.00± 0.06	7.8± 0.2	34.70± 0.92	22.2± 2.0	44.0± 5.3	0.124± 0.003	0.10± 0.02	0.121± 0.010	63.10± 0.29	0.7±0.0	43.47± 0.03	15.57± 0.38	92.80±2.51
C4(60ft)	0.01± 0.06	8.2± 0.1	46.73± 0.50	36.0± 4.2	18.1± 2.0	0.082± 0.007	0.334± 0.08	0.154± 0.004	37.81± 0.71	0.4±0.0	52.47± 0.76	21.33± 0.87	47.03±3.85
C5(45ft)	0.01± 0.11	8.1± 0.1	51.67± 0.63	10.3± 2.0	20.9± 2.1	0.061± 0.004	0.121± 0.01	0.100± 0.015	47.12± 2.01	0.5±0.0	49.37± 0.70	12.43± 0.21	198.83±5.40
C6(110ft)	0.01± 0.06	7.8± 0.2	36.27± 0.41	56.2± 5.3	24.2± 4.0	0.174± 0.005	1.33± 0.11	0.265± 0.004	44.07± 0.25	0.7±0.1	60.13± 1.20	13.47± 0.61	173.2±2.82
C7(60ft)	0.00± 0.00	8.0± 0.2	27.77± 0.38	50.4± 6.0	22.1± 2.0	0.290± 0.004	1.02± 0.21	0.351± 0.016	45.80± 0.71	0.4±0.0	21.90± 0.36	16.67± 0.45	221.27±3.33
C8(60ft)	0.00± 0.00	7.9± 0.1	23.43± 0.70	18.1± 2.0	36.1± 4.2	0.120± 0.008	0.226± 0.01	0.045± 0.002	56.66± 0.63	0.4±0.1	14.47± 0.94	10.20± 0.56	83.30±2.69
C9(60ft)	0.00± 0.00	7.8± 0.1	13.83± 0.35	21.0± 2.1	22.3± 2.0	0.051± 0.011	0.76± 0.04	0.115± 0.009	52.92± 0.78	0.8±0.1	12.40± 0.66	8.93± 0.25	152.73±3.23
C10(90ft)	0.01± 0.06	7.7± 0.1	43.87± 0.31	36.0± 4.2	10.0± 2.0	0.075± 0.010	0.25± 0.08	0.282± 0.075	44.07± 0.14	0.3±0.0	17.87± 0.65	17.30± 0.44	232.1±3.72
C11(116ft)	0.01± 0.06	8.1± 0.1	17.73± 0.50	24.2± 3.5	36.0± 5.3	0.133± 0.012	0.12± 0.01	0.131± 0.007	75.52± 1.15	0.6±0.1	15.93± 0.35	25.93± 0.25	187.13±5.04
C12(80ft)	0.00± 0.00	7.7± 0.1	25.40± 0.78	56.1± 4.2	40.2± 2.0	0.095± 0.012	0.67± 0.07	0.057± 0.005	30.22± 3.20	0.5±0.1	24.77± 0.55	32.37± 0.42	78.10±4.03
C13(60ft)	0.01± 0.06	8.0± 0.2	54.57± 1.81	40.1± 5.3	10.3± 2.0	0.046± 0.008	0.10± 0.02	0.081± 0.004	44.07± 0.03	0.4±0.0	31.83± 1.23	20.03± 0.40	210.33±2.15

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Table 3 Correlation matrix of different parameters in ground water of a fluoride polluted area of Birbhum

	F^-	pH	HCO_3^-	Ca	Mg	Fe	NO_3^-	PO_4^{3-}	Cl^-	EC	Na	K
pH	0.756**											
HCO_3^-	0.271	0.494*										
Ca	0.088	0.169	0.446									
Mg	0.081	-0.017	-0.38	0.145								
Fe	0.482*	0.264	-0.357	-0.039	0.475							
NO_3^-	0.391	0.140	-0.335	-0.301	0.665**	0.326						
PO_4^{3-}	-0.076	0.011	0.221	-0.289	-0.571*	-0.604*	-0.255					
Cl^-	0.043	0.353	-0.057	0.128	0.265	-0.159	0.290	0.287				
EC	0.429	0.501*	-0.088	0.01	0.565*	0.727**	0.330	-0.260	0.288			
Na	-0.164	-0.074	-0.247	-0.045	0.270	-0.175	0.125	-0.157	-0.002	-0.019		
K	-0.388	-0.319	-0.295	-0.075	0.009	0.230	-0.377	-0.015	-0.335	0.081	0.059	
SO_4^{2-}	0.536*	0.527*	0.513*	0.549*	0.303	0.175	0.156	-0.379	0.054	0.369	0.189	-0.511*

* Correlation is significant at the 0.05 level   ** Correlation is significant at the 0.01 level

Table 4 Correlation matrix of different parameters in ground water of the control area of Burdwan

	F^-	pH	HCO_3^-	Ca	Mg	Fe	NO_3^-	PO_4^{3-}	Cl^-	EC	Na	K
pH	-0.156											
HCO_3^-	-0.279	0.509*										
Ca	-0.149	-0.333	-0.351									
Mg	-0.309	-0.152	0.265	-0.214								
Fe	-0.184	-0.241	-0.198	0.454	0.212							
NO_3^-	-0.301	-0.348	-0.134	0.664**	-0.039	0.598*						
PO_4^{3-}	0.192	-0.299	-0.236	0.499*	-0.361	0.700**	0.586*					
Cl^-	-0.033	0.235	-0.15	-0.481*	0.229	0.044	-0.378	-0.146				
EC	-0.303	0.247	0.090	-0.160	0.244	-0.160	0.182	-0.254	0.456			
Na	0.558*	0.126	-0.123	0.199	-0.143	0.005	0.156	0.112	-0.235	0.152		
K	0.147	-0.309	-0.326	0.528*	0.176	0.116	-0.023	0.006	-0.224	-0.343	-0.036	
SO_4^{2-}	0.256	-0.081	-0.023	0.06	-0.501*	0.2	0.117	0.533	0.09	-0.178	-0.187	0.256

* Correlation is significant at the 0.05 level ** Correlation is significant at the 0.01 level

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Table 5 Variation of biochemical constituents in leaves of different vegetable plants

Name of vegetables	Sugar		Chlorophyll Chl-a		Chl-b		Total Chl		Ascorbic acid		Protein		Amino acid	
	Control	Polluted	Control	Polluted	Control	Polluted	Control	Polluted	Control	Polluted	Control	Polluted	Control	Polluted
Cabbage (*Brassica oleracea*)	578.9±8.3	566±4.07**	0.11±0.01	0.10±0.02a	0.42±0.01	0.25±0.001*	0.41±0.02	0.36±0.01*	0.30±0.01	0.241±0.01*	560.1±3.2	555.41±3.51a	6.13±0.14	6.03±0.08a
Onion (*Allium cepa*)	718.1±5.3	703.6±3.4*	0.08±0.001	0.05±0.003*	0.43±0.008	0.28±0.01*	0.66±0.01	0.53±0.03*	0.24±0.01	0.22±0.01a	192.4±3.3	188.6±3.8a	4.18±0.04	3.54±0.09*
Spinach (*Spinacia oleracea*)	762.45±3.1	751.84±3.61*	0.20±0.006	0.18±0.01**	0.59±0.016	0.54±0.01*	1.00±0.003	0.89±0.01*	0.18±0.03	0.17±0.01a	895.5±4.6	892.48±7.54a	1.28±0.04	0.32±0.01*
Radish (*Raphanus sativus*)	559.2±8.1	554.2±13.9a	0.34±0.004	0.23±0.01*	0.66±0.01	0.15±0.01*	1.02±0.15	0.57±0.01*	0.08±0.01	0.06±0.08a	492.8±3.5	491.8±5.9a	4.18±0.16	2.89±0.07*
Potato (*Solanum tuberosum*)	891.4±12.5	883.3±7.3a	0.96±0.047	0.13±0.01*	1.48±0.04	0.24±0.01*	1.02±0.05	0.38±0.003*	0.25±0.01	0.19±0.01*	418±1.9	411±4.1**	2.25±0.11	1.29±0.03*
Cauliflower (*Brassica oleracea* var. *botrytis*)	952.4±3.4	951.4±19.2a	0.33±0.006	0.21±0.01*	0.37±0.008	0.29±0.02*	0.73±0.02	0.60±0.01*	0.21±0.02	0.16±0.01*	742.9±6.4	739.9±4.9a	2.25±0.02	1.93±0.13*

* Change is significant at 1% level, ** Change is significant at 5% level, a Change is not significant. All the biochemical constituents are measured in terms of mg/100g leaf

Table 6 Variation of biochemical constituents in different vegetables

Name of the sample	Sugar		Ascorbic acid		Protein		Amino acid	
	Control	Polluted	Control	Polluted	Control	Polluted	Control	Polluted
Papaya (*Carica papaya*)	154.68±4.30	148.48±5.24 [a]	1.012±0.019	1.12±0.10[a]	766.22±6.11	763.17±7.06[a]	5.99±0.19	6.79±0.91[a]
Kundri (*Coccinia grandis*)	107.92±7.80	107.81±2.49[a]	0.290±0.100	0.38±0.04[a]	425.33±4.46	423.98±6.14[a]	18.12±2.75	19.3±3.01[a]
Radish (*Raphanus sativus*)	290.49±8.66	287.49±6.89[a]	0.480±0.119	0.57±0.06[a]	806.44±8.92	805.56±5.00[a]	15.44±3.03	16.08±2.83[a]
Potato plant (*Solanum tuberosum*)	307.46±8.81	307.36±7.89[a]	0.364±0.061	0.44±0.04[a]	580.42±5.03	572.37±8.01[a]	21.75±3.51	22.5±3.06[a]
Brinjal (*Solanum melongena*)	153.33±2.94	152.26±5.70[a]	0.278±0.022	0.32±0.01[**]	300.11±8.10	296.79±6.57[a]	33.21±3.75	35.37±3.72[a]
Seem (*Dolichos lablob*)	554.41±8.43	549.45±7.97[a]	0.243±0.002	0.25±0.02[a]	311.68±9.56	301.58±6.83[a]	21.49±4.00	28.94±1.49 [**]
Korola (*Momordica charantia*)	98.11±8.03	96.80±6.78[a]	0.052±0.012	0.06±0.01[a]	496.66±4.49	487.57±7.86[a]	14.00±1.15	14.27±2.53[a]

[*] Change is significant at 1% level, [**] Change is significant at 5% level, [a] Change is not significant. All the biochemical constituents are measured in terms of of mg/100g vegetable

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3.3. Correlation between Water Parameters

An attempt was made to correlate the fluoride with various parameters for both the polluted and control zones, as indicated in Tables 3 and 4, respectively. All the parameters showed positive correlation with fluoride except phosphate, sodium and potassium in the polluted area. The positive correlation between bicarbonate and fluoride along with the significant positive correlation between pH and fluoride was found in the fluoride-contaminated area.

Calcium and magnesium showed positive correlation with fluoride as found by Alagumuthu and Rajan [38]. However, the correlations between fluoride and other water parameters are not as significant as the fluoride level is very low in the control area (Table 4).

3.4. Piper Diagrams

Figures 4 and 5 show Piper diagrams taking the mean values of the parameters of the water samples collected from the study and polluted areas to illustrate the chemical analyses. On the basis of Walton's classification [39], in 92% of the water samples of the polluted area, the non-carbonate hardness (secondary salinity) exceeds 50%, i.e., by alkaline earths and weak acid.15.4% of the water samples showed an excess of alkalis with respect to alkaline earths and 7.7% showed an excess of weak acid with respect to strong acid in the polluted area.

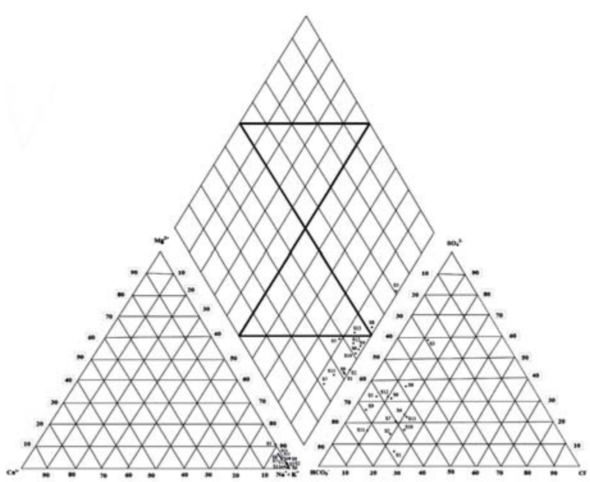

Figure 4 Piper diagram of water parameters of Nowpara and Junidpur

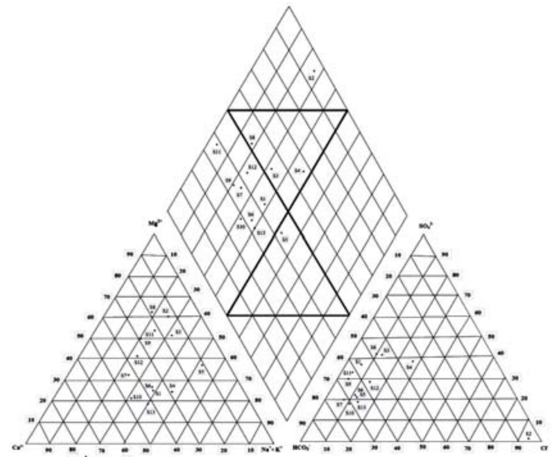

Figure 5 Piper diagram of water parameters of the control area

In the control area 69.2% of the water samples showed an excess of alkaline earths with respect to alkalis, 7.7% showed an excess of weak acid with respect to strong acid and 23.1% showed an excess of strong acid with respect to weak acid.

3.5. Fluoride in Soil

Fluoride concentrations in the soil samples were measured to access the translocation of fluoride from soil to plant body by calculating the BCF. The fluoride content was found to be highest (4.5 mg/kg soil) in the soil of a radish field (Fig. 6). High fluoride concentrations in soil are indicated by high mean concentrations of fluoride in radish (4.21 mg/kg vegetables) and its leaves (3.21 mg/kg vegetables).

3.6. Biochemical Parameters

Along with the water study, biochemical parameter such as chlorophyll, amino acids, proteins, soluble sugars, ascorbic acid in some vegetables and vegetable leaves were studied and are listed in Tables 5 and 6. In Table 5, we see that the reducing sugar contents of the leaves decreased monotonically with respect to the control area and the reduction was maximum in case of cabbage (2.15%) and minimum in case of cauliflower (0.11%). The chl. a, chl. b and total chlorophyll of leaves in the fluoride-affected area were reduced considerably comprised to the control area. For potatoes, maximum reduction of 86.1%, 84.1%, 62.7% of chl a, chl b and total chl content, respectively were obtained, indicating the susceptibility, of potato plants to fluoride.

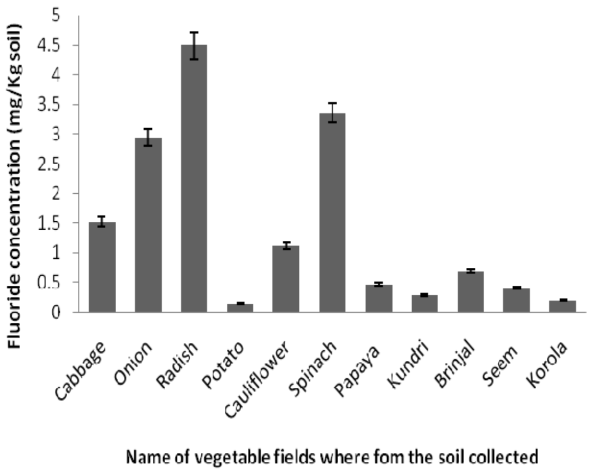

Figure 6 Fluoride concentration in soil where vegetables are cultivated. Values are means ± SE N(3) from two independent experiments.

Lesser amounts of ascorbic acid were found in leaves of different vegetables in the fluoride-affected area than in the control area. Reduction caused by fluoride was found to be highest in cauliflower (26.2%) and lowest in spinach (6.5%). The change of total protein content in leaves of different vegetables of the polluted site due to the stress of fluoride was not as significant but showed a decreasing trend with respect to the control site. The highest reduction was found in onion (1.9%) and lowest in radish (0.2%).

The free amino acid contents in the leaves and vegetables of different species under fluoride stress showed significant reduction in comparison to the control area. For spinach the maximum reduction was 78.8% of ascorbic acid was found and minimum reduction was found in cabbage (1.5%).

For vegetables, sugar and protein levels showed a decreasing tendency, whereas ascorbic acid and amino acid showed increasing tendency due to the fluoride stress in the affected area in comparison to the control area. The highest and lowest reductions of sugar were found in papaya (4.0%) and potato (0.03%) respectively. The reduction of protein was highest in seem (3.2%) and lowest in radish (0.10%). Ascorbic acid and amino acid contents in the vegetables were significantly higher in the fluoride affected area with respect to the control area. Enhancement of ascorbic acid content due to fluoride stress was found to be the highest in kundri (31.0%) and lowest in seem (4.5%). Seem showed the highest degree of augmentation of amino acid (34.7%) and korola showed the lowest (1.9%).

3.7. Bioconcentration Factor

Fluoride concentration along with mean BCF values of F in vegetable leaves and vegetables are presented in Tables 7 and 8. The fluoride concentration and mean BCF value of F was found to be highest in leaves and vegetable of *Raphanus sativus* (radish). Higher concentrations of fluoride in soil where the vegetable is cultivated may be the reason for higher BCF. Other than radish, leafy vegetables like spinach, cabbage and cauliflower leaves had BCF values of F greater than 1.

Table 7 Mean fluoride concentration levels of different types of vegetable leaf samples and BCF

Sl.No.	vegetable	Polluted zone (F^- mg.kg^{-1})	control zone (F^- mg.kg^{-1})	Mean BCF in polluted zone
1.	Cabbage (*Brassica oleracea*) (N = 3)	1.25± 0.07	ND	1.22
2.	Onion (*Allium cepa*) (N = 3)	3.19± 0.07	ND	0.92
3.	Radish (*Raphanus sativus*) (N = 3)	3.21± 0.04	0.009±0.001	1.40
4.	Potato (*Solanum tuberosum*) (N = 3)	0.65±0.07	0.004±0.001	0.23
5.	Cauliflower (*Brassica oleracea* var. *botrytis*) (N = 3)	1.11±0.12	ND	1.01
6.	Spinach (*Spinacia oleracea*) (N = 3)	2.56±0.09	0.010±.001	1.31

ND: Not Detectable

Table 8 Mean fluoride concentration levels of different types of vegetable samples and BCF

Sl.No.	vegetable	Polluted zone (F^- mg.kg^{-1})	control zone (F^- mg.kg^{-1})	Mean BCF in polluted zone
1.	Papaya (*Carica papaya*) (N = 3)	0.58±0.05	ND	0.82
2.	Kundri (*Coccinia grandis*) (N = 3)	0.80±0.03	ND	0.36
3.	Radish (*Raphanus sativus*) (N = 3)	4.21±0.02	0.006±0.001	1.06
4.	Potato (*Solanum tuberosum*) (N = 3)	1.47±0.03	0.005±0.001	1.1
5.	Brinjal (*Solanum melongena*) (N = 3)	1.35±0.03	ND	0.51
6.	Seem (*Dolichos lablob*) (N = 3)	0.65±0.03	ND	0.62
7.	Korola (*Momordica charantia*) (N = 3)	0.40±0.03	ND	0.51

ND: Not Detectable

4. DISCUSSION

4.1. Variation of Fluoride with Depth

A positive correlation between fluoride concentration and depth of sampling indicates that fluoride is present in the form of fluorite minerals in the Precambrian granite or granitic-gneiss of the underground basement [40,41]. The process of weathering of rock releases fluoride in soil and groundwater. With <1.0 µg/L of F in drinking water as the optimum level for warmer climates as suggested by WHO [42], people living in the villages Nowapara and Junidpur in the Birbhum District are under threat from potential F toxicity from groundwater as well as contamination from surface water sources. Depth-wise variation in F concentration may be attributed to the lithological homogeneity of the area. Trace amounts of F found in surface water and shallow water may be attributed to long time application of phosphate fertilizers and subsequent leaching as agricultural runoff into the surface water-body sources [24].

4.2. Correlation of F with other Water Parameters

Water quality parameters such as alkalinity, pH and hardness have high impact on water fluoride levels due to the release of fluoride from fluoride-containing minerals by carbonates and dissolved solids [43]. The positive correlation between bicarbonate and fluoride indicates that high alkalinity water promotes leaching of fluoride and thus affects the concentration of fluoride in groundwater [44]. The ionic radius of fluoride (0.136 nm) is the same as that of hydroxyl ion, which can be easily substituted from water at high pH [45,46]. Alkaline pH where bicarbonate activity is high, promotes the fluoride dissolution represented as: $CaF_2 + 2NaHCO_3 = CaCO_3 + 2Na^+ + 2F^- + H_2O + CO_2$ [44]. Calcium and magnesium showed positive correlation with fluoride [38]. The positive correlation with calcium observed may be attributed to the presence of limestone in those areas [40]. There was a positive correlation between chloride and fluoride [47,48]. Iron and sulfate had a significant positive correlation with fluoride [49]. However, the correlation between fluoride and other water parameters are not so significant as the fluoride level is very low in the control area.

4.3. Biochemical Parameters

Chlorophyll. It is reported that tuberous vegetables such as potato appear to accumulate relatively higher to levels of fluoride [50] and a high stress effect is observed. Reduction in the chl a, chl b and total chl content may be due to the breakdown of chlorophyll under stress or due to inhibition of chlorophyll biosynthesis [51]. Magnesium is a central component of chlorophyll; it traps fluoride as MgF_2 in a detoxification mechanism [52] and this may be cause decrease in the chlorophyll content in the plant body. Decrease in chlorophyll content may also be due to the disruption of chloroplast membranes as described by Horvath et al. [21]. Earlier studies confirm that fluoride causes a reduction in the chlorophyll content of foliage [19]. The biochemical basis of this effect may be a consequence of inhibition by fluoride of incorporation of γ-aminolevulinic acid into chlorophyll synthetic pathway [53].

Sugar. The sugar levels in plants are directly related to stress factors [22]. The reducing sugar of both leaves and vegetables of the fluoride-affected area were significantly decreased considerably in comparison to the control area. This may be due to a lower level of photosynthesis leading to lower accumulation of photo assimilate in leaves and fruits under fluoride stress, decreasing the sensitivity of different crop plants [18]. Since formation of reducing sugars such as glucose, fructose, and mannose in leaves is thought to be inhibited by F, the tendency of plants exposed to F to decrease the concentrations of such sugars in their leaves indicates the possible conversion of these sugars to non-reducing sugars, such as sucrose and raffinose or sugar alcohols. Under these conditions, increased levels of non-reducing sugars in tissues might be a mechanism adopted by plants to reduce F toxicity [23]

Ascorbic acid. Ascorbic acid as an antioxidant plays an important role in protection against physiological stress [54]. Ascorbic acid content in leaves of different vegetables of the fluoride-affected area decreased due to inhibition of ascorbic acid synthesis under fluoride stress [20]. In vegetables, the ascorbic acid content of the affected area showed an increasing trend over the controlled area, which may be attributed to binding of fluoride with ascorbic acid oxidase enzyme thereby inhibiting the breakdown of ascorbic acid in the plant system [51].

Protein. Chang [55] stated that fluoride decreased the number of ribosomes and destroyed the structure of ribosomal proteins, which negatively affected the entire protein synthesis. We can reach the same inference with our results. Protein content in the leaves and vegetables of different species under fluoride stress showed significant reduction in comparison to the control area due to the reduced rate of amino acid synthesis under fluoride stress [20].

Amino acid. The total free amino acid content in leaves of different vegetables of the polluted site showed a decreasing trend with respect to the control site. This may be due to less degradation of storage protein, amino acid synthesis and amino acid utilization for protein synthesis and for respiration under fluoride stress [24]. The increased amino acid the content in vegetables in the affected area in comparison to control area may be attributed to increased rates of dark CO_2 fixation under fluoride stress [20].

4.4. BCF of Fluoride

BCF has been used as an indicator of affinity for the accumulation of F in plants and because of its simple application, it is widely used [56,57]. Earlier investigations found increased fluoride translocation in plants where metabolism is higher [58]. Leafy vegetables like radish, spinach, cabbage and cauliflower leaves had BCF values of F greater than 1, which indicates a higher rate of photosynthesis in leafy vegetables associated with higher intake of water resulting in a higher BCF value of F. However, Swartjes et al. [59] reported that BCF values are not always constant in specific vegetables and are largely affected by soil properties like soil pH, clay content, organic matter and fluoride concentration in soil and plant factors like the type of plant and growth rate.

5. CONCLUSION

Keeping in view the ground water quality that is the source of drinking water, the residents of this locality of the Birbhum District are in an alarming position. A positive correlation was found with the depth of water sample, which imposes a greater risk in these fluoride-affected villages. Because the drinking water level got down deep due to dryness in this area, the fluoride levels were higher in the drinking water. Vegetable plants are also under stressed conditions. It is presumable that this kind of stress effect on plant metabolism could produce disturbances in natural biosynthetic turnover. Working on cell tissue culture, Diesendorf and Sutton [60] reported that in the presence of fluoride, DNA molecules may be damaged and genetic malformations may be induced. Genetic malformation can produce any type of physiological or biochemical change in a plant body, imposing harmful effect on plant species and the agricultural system. Beside this fact, by estimating the BCF it may be speculated that the people in this area are in chronic toxic exposure to organo-fluourine compounds which can be synthesized by crops and vegetable plants after transportation of fluoride from water and soil. Future mitigation attempts should consider alternative fluoride-free water sources for drinking and irrigation purposes.

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