

Bioaccumulation of arsenic and fluoride in vegetables from growing media: health risk assessment among different age groups

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Abstract The current study was conducted to evaluate the arsenic (As) and fluoride (F^-) concentrations in growing media (stored rainwater and soil), of district Tharparkar, Pakistan. The bioaccumulation/transportation of As and F from growing media to different types of vegetables (wild cucumis, Indian squish and cluster bean) was evaluated. Total concentrations of As and F^- in stored rainwater samples were observed up to 585 $\mu\text{g/L}$ and 32.4 mg/L , respectively, exceeding many folds higher than WHO provisional guideline values. The As and F^- contents in soil samples of nine agricultural sites were found in the range of 121–254 mg/kg and 115–478 mg/kg , respectively. The highest contents of As and F^- were observed in wild cucumis as compared to Indian squish and cluster bean ($p < 0.05$), grown in the same agricultural field. The bioaccumulation factors of As and F^- were to be > 4.00 , indicating the high rate of transportation of As and F^- from growing media to vegetables. A significant positive correlation of As and F^- in vegetables with their concentrations in soil and water was observed ($r > 0.60$ with $p < 0.05$). The risk assessment elucidated that the population of different age group consuming local vegetables and drinking

water contaminated with As and F^- may have adverse health effects.

Keywords Arsenic · Fluoride · Water · Soil · Vegetable · Bioaccumulation · Estimated daily intake

Introduction

The high concentrations of arsenic (As) and fluoride (F^-) in the soil, water and biota can be created health risks to human and animals (Martínez-Acuña et al. 2016). Uptake of different elements by plants depends on their levels in growing media (soil and irrigation water) and the nature of plant species (Kazi et al. 2009). It was reported that As-contaminated water used for agricultural purposes adversely influences the yield of crop, height and growth of the plant (Abedin et al. 2002).

Arsenic even at low concentration has adverse impacts on the skin and other physiological disorders in human (Tsuji et al. 2014). Arsenic is a potent carcinogen and also created problems in the normal functioning of cardiovascular and nervous systems (Kurzius-Spencer et al. 2016). High levels of As in groundwater ($> 10 \mu\text{g/L}$) and soils ($> 5 \text{mg/kg}$) have been reported in various parts of the world, including Argentina, Chile, Bangladesh, China, Hungary, Mexico, Taiwan, Vietnam and Africa (Bhattacharya et al. 2006; Ferreccio and Sancha 2006; Milton et al. 2005;

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Guo et al. 2006; Börzsönyi et al. 1992; Carrillo-Rivera et al. 2002a, b; Yang et al. 2003; Berg et al. 2007).

Fluoride (F^-) is an essential element for the population of every age, to make stronger teeth and skeletal tissues (Das et al. 2016), whereas excessive consumption of F^- causes different physiological problems, such as renal and neurological disorder, myopathy, especially dental and skeletal fluorosis (Ayoob and Gupta 2006). The prevalence of fluorosis is common in different parts of the world, such as in Mexico (Carrillo-Rivera et al. 2002a, b), China (Guo and Wang 2005) and India (Das et al. 2016) and Africa (Fantong et al. 2010, 2013). The elevated concentrations of F^- in groundwater are found especially in those regions where geologically enriched fluorine-bearing minerals are present (Naseem et al. 2010; Fantong et al. 2010; Brahman et al. 2013a). The sources of fluoride in natural waters are fluoride-bearing minerals such as fluorite, fluorapatite, cryolite and apophyllite (Rafique et al. 2009). Geochemically, fluoride has the same charge and nearly the same radius as hydroxide ions, thereby facilitating the replacement of each other, e.g., in the ferromagnesium silicates and clay minerals (Dey et al. 2012). The different weather conditions erode the rocks, result in discharging of fluoride from minerals, which are the main causes of high F^- levels in groundwater (Shah and Danishwar 2003; González-Horta et al. 2015).

A number of investigations indicated that F^- can be frequently accumulated in plants (Ruan et al. 2004; Gao et al. 2014). Ruan et al. (2004) studied that the uptake of F^- by different plants especially in tea was considerably higher at low pH of soil (pH 5.5). It was further reported that to enhance the pH of soil up to the neutral (≥ 6.55), generally by liming, consequently reduces the F^- accumulation in tea plant. (Ruan et al. 2004; Gao et al. 2014). The high uptake of F^- by different plants indicates a noticeable injury of leave and fruits, whereas the yields of crops were also altered (Ando et al. 1998). Uptake of F^- via roots of different vegetations is mostly depended on its concentration in the agricultural system (Yadav et al. 2012). The accumulation of F^- in human body might be owing to its elevated concentrations in both drinking water and food crops, cultivated with contaminated irrigation water (Poureslami et al. 2008; Khan et al. 2015; Yadav et al. 2017).

The concentration of dissolved arsenic (As) and fluoride (F^-) in natural water depends on the

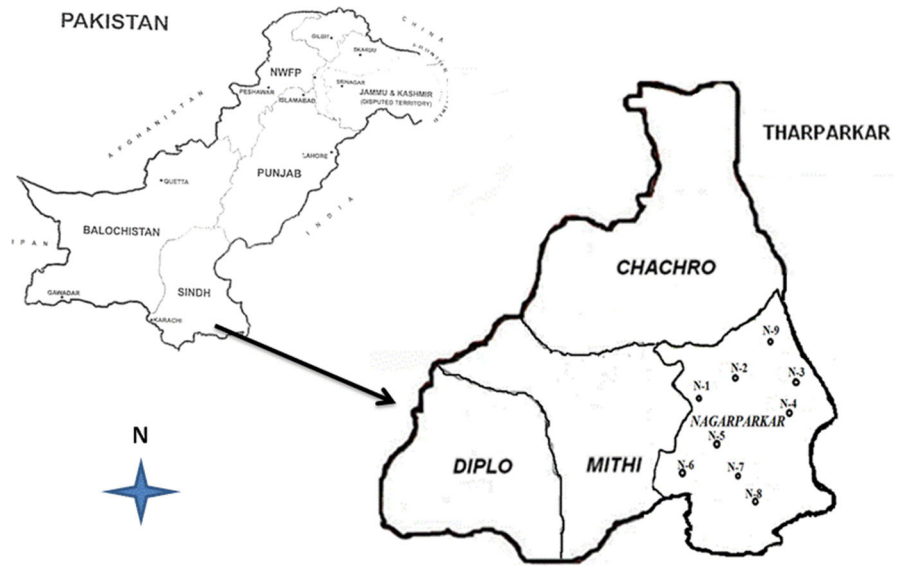
geological and geochemical composition of natural sources (Bhattacharya et al. 2006; Bundschuh et al. 2004; Gómez et al. 2009). Arsenic distribution is also affected by anthropogenic activities, such as mining coal-fired combustion, use of agricultural herbicides, pesticides and medicinal products (Orloff et al. 2009), but the major source of As, in general, is chemical weathering of rocks (Bhattacharya et al. 2007).

The serious adverse health effects due to contaminated water and food crops with As and F^- were reported in Argentina (Bustingorri and Lavado 2014), Inner Mongolia (Smedley and Kinniburgh 2002), Southern China (Finkelman et al. 2002) and Pakistan (Farooqi et al. 2007; Brahman et al. 2013a). Different areas of Pakistan are facing As-related severe public health crises like neighboring country (Patel et al. 2005; Yadav et al. 2012). In various parts of Pakistan, the surface and groundwater are highly contaminated with As and are not suitable for consumption (Shrestha 2002; Farooqi et al. 2007; Brahman et al. 2013a, b). It was reported that drinking water of Thar Desert, Pakistan, has very high levels of As and F^- (Rafique et al. 2008; Naseem et al. 2010; Brahman et al. 2013a). The Pakistan Council of Research in Water Resources and UNICEF pointed out that groundwater of different parts of Punjab province, Pakistan, is contaminated with As up to 200 $\mu\text{g/L}$ (Tahir 2000; Nickson et al. 2005; Farooqi et al. 2007), whereas in Sindh province 16–36%, population have been adversely effected by the consumption of As and F^- contaminated water (Baig et al. 2009; Brahman et al. 2013a). Therefore, it is necessary to evaluate the As and F^- in water used for drinking and irrigation purpose in contaminated endemic areas (Lu et al. 2000; Brahman et al. 2014).

The main route of exposure for both elements was considered as ingestion of food commodities (Bustingorri and Lavado 2014). Concentrations of As and F^- especially in drinking water were selected to estimate the possible health risk including average daily dose and hazard quotient (Jha et al. 2013). The adverse impacts of high intake of F^- , especially in children suffered from the weakened immune system and decline the intellectual quotient (Lu et al. 2000). Whereas, the chronic exposure to As can create the different intensity of arsenicosis (WHO 2008).

The objective of present study was to evaluate the As and F^- concentrations in surface water (stored rain water), soil (total and extractable) and vegetables grown on agricultural lands of sub-district

Fig. 1 Map of study area



Nagarparkar (Tharparkar, Pakistan). The translocation capability of As and F⁻ from the growing media to the edible part of vegetables termed as bioaccumulation factor (BAF) was calculated. Moreover, the correlation of As and F⁻ in vegetables (locally grown) with their concentration in soil and water was also calculated. In addition, estimated daily intake (EDI) and hazardous index values of As and F⁻ in the population of three age groups (7–15, 16–25 and 26–50 years).

Materials and methods

Study area

The Thar Desert is located in the Tharparkar district, southeastern part of Sindh, Pakistan, which is administratively divided into four sub-districts (Fig. 1). Nagarparkar sub-district is situated in the southeast extremity of the Thar Desert and lie in between latitude 24°21.734'–24°36.952'N and longitude 70°39.490'–70°45.192'E (Brahman et al. 2013b). The Tharparkar region especially selected area (Nagarparkar) is rich in mineral resources like granite, coal, kaolin and other salts which might increase the release of elements and enhance the salinity of groundwater, or stored rainwater (Rafique et al. 2008; Naseem et al. 2010; Brahman et al. 2013b). During the storage of rainwater in ponds for a long period, there is a chance of leaching of mineral

constituents in soil. The population of the study area consumes groundwater and stored rainwater for drinking and agriculture purposes (Brahman et al. 2014; Rafique et al. 2008). The Kharif crops are the main agricultural products of Thar region, which grow before monsoon season (June and July). The vegetables include Indian squash (*Praecitrullus fistulosus*), wild cucumis (*Cucumis pubescens*) and cluster beans (*Cyamopsis tetragonoloba*).

Sampling and pretreatment

Water sampling and analysis

The surface water (stored rain water) was collected from nine locations ($n = 10$ of each) of Tharparkar (N-1 to N-9, as shown in Fig. 1). The pH and conductance of surface water were measured at sampling sites. The water samples were placed in an insulated ice cooler and transported on the same day to laboratory. The collected surface water was filtered using membrane filter (0.45 μm) and made two subsamples of each. HNO₃ (0.2% v/v) was added to one subsample and was kept for the determination of total As concentrations, whereas other portion was used for F⁻ determination. All water samples were reserved in previously cleaned bottles with 10% HNO₃ and then rinsed thoroughly with ultrapure water.

For the determination of total As in surface water, the samples were diluted 25–50 times with deionized

water and analyzed by atomic absorption spectrometer (Perkin Elmer, Norwalk, CT, USA) with a background correction coupled with hydride generation system (HGAAS) and detail was given in our earlier works (Brahman et al. 2013a, 2014). The concentration of F^- was determined in surface water samples by an ion selective electrode using ion meter (Metrohm, Herisau, Switzerland). The TISAB buffer was added in the ratio of 1:1 to the standard and sample solutions to achieve sufficient ion energy and optimal pH to the proper functioning of the electrode. TISAB buffer contains 1 mol/L NaCl, 0.001 mol/L $NaNO_3$, 0.25 mol/L CH_3COOH , 0.75 mol/L CH_3COONa , and ionic strength 1.75 mol/L. The concentration of F^- based on the measurement of the voltage is obtained from the standard curve (Brahman et al. 2013a, b).

Total As in soil and vegetables

Vegetable and adjoining agricultural soil (0–25 cm) were simultaneously collected from nine agricultural sites ($n = 10$ of each site). The collected soil samples were transferred to the laboratory on same days and air-dried at room temperature for 8 days in a fume cupboard. The soil was removed from the vegetable and thoroughly washed with distilled water, then again washed three times with ultrapure water. The separated edible parts of vegetable were air-dried for 48–72 h at 60 °C in an electric oven, to avoid effects on volatile characteristics of organo-arsenical compounds. The dried vegetables and soil samples were ground in an agate mortar to homogenize and sieve from nylon sieve with mesh size < 100 μm . Then, all samples were kept in labeled polypropylene containers at ambient temperature till further analysis.

To determine the pH of the soil, added deionized water to the soil at a ratio of 1:2.5, and shaking at 24 rpm in an electric shaker for 12 h then filtered and determined the pH using 781-pH meter, Metrohm (Jamali et al. 2008; Baig et al. 2009). Total As contents in soil and vegetables samples were measured after decomposition of organic matrices in a microwave oven (Baig et al. 2011).

Weighed 0.1 g of each soil and 0.2 g of dried vegetables (duplicate of each) in PTFE flasks, separately. Then, 3 mL of an acid mixture (HCl/HNO_3) (3:1) and H_2O_2/HNO_3 (1:2) was added in soil and vegetable samples, respectively. The flasks were

placed in a PTFE container separately (for both samples) then heated following a one-stage digestion programme (80% of total power 900 W), 5 min for soil and 3 min for vegetables. After cooling, the extra acid has been evaporated to obtain a semidried mass. The contents of flasks were dissolved in 0.1 mol/L of HNO_3 (10 mL), and filtered, further added ultrapure water and made volume up to 25 mL in volumetric flasks. The blank were prepared (devoid of standards and samples) following the same procedure of the samples and analyzed by HGAAS and detail is mentioned in our previous work (Brahman et al. 2013a; Baig et al. 2009).

Total fluoride contents in soil and vegetable samples

Weighed 1.0 g of composite soil and vegetable samples of each site ($n = 3$ of each) in covered ceramic crucibles, after that treated with 5 mL of 5 mol/L of NaOH solutions, and heated on a electric hot plate (150 °C). The slurries were heated to semidried mass; then, again one pellet of NaOH was added. After that, the samples were placed in an electric furnace (560 °C) for 2 h, afterward kept at room temperature to cool the contents of crucibles, then added ultrapure water and subjected to heating on a temperature-controlled electric hot plate (100 °C) to dissolve the fusion cake. The pH of sample solutions was adjusted at 5.5, with a buffer solution prior to determination of F^- using an ion selective electrode.

Water-soluble As and F^- contents in soil

Triplicate of each composite soil samples (2.0 g) was weighed into the conical flasks and added 20 mL of deionized water. The contents of flasks were shaken for one hour at 120 rpm, then allowed to stand for 3 h, then filtered through a Whatman 42 filter paper. Then, As and F^- were determined as given above (Baig et al. 2011; Brahman et al. 2014).

Nutritional survey

A nutritional survey was conducted among five households resided in nine sites/villages (N1–N9), based on the type of food and drinking water. The food habits, consuming different vegetables such as Indian squash (IS), wild cucumis (WC) and cluster beans (CB) by three different age groups, adolescents

(7–15 years), adults of two age groups (16–25 years) and (25–50 years) with body weights 25, 50 and 60 kg, respectively, were assessed. The assessment was based on a survey, whereas biomedical research is not involved to administer medicine, injection or chemicals to human subjects, to evaluate the adverse health effects. All parameters including intake of different vegetables and drinking water by selected population in study areas were calculated as average values. However, the obtained facts may vary due to different socioeconomic conditions of the study population.

Estimation of daily intake

The risk assessment for human health was quantitatively estimated by evaluating the As and F⁻ exposure doses, owing to utilization of drinking water and vegetables (locally grown). The estimated daily intake (EDI) of both elements was determined as reported elsewhere (Jha et al. 2011).

The EDI was calculated as:

$$EDI = [(C \times IR \times EF \times ED \times AF \times CF)] / (BW \times AT)$$

where EDI is the estimated daily intake (mg/kg/day), ‘C’ the concentration of As/F⁻ in vegetables (mg/kg) and drinking water (mg/L or µg/L), ‘IR’ the ingestion or intake rate for vegetables (g/day) and drinking water (L/day), exposure frequency (EF) denoted as d year⁻¹, the exposure duration in year (ED), the absorption factor (AF) is unit less, conversion factor denoted as CF (in the case of vegetable 10⁻⁶ mg/kg), body weight (BW) in kg and average time in days (AT). The absorption factor was selected as 75%, and the average body weights of study population were divided into age groups (7–15 years), (16–25 years) and (26–50 years) corresponding to 25, 50 and 60 kg, respectively. The quantity of drinking water was consumed, and body weight was assessed, based on survey data. The drinking water container/glass commonly had the same size (250 mL) and style. It was assumed that consuming locally grown vegetable in addition to drinking stored rainwater would enhance the EDI of both elements (As/F⁻), which can create adverse effects on health. The cumulative EDI was also estimated in the study area as:

$$EDI_{Cumulative} = EDI_{vegetable} + EDI_{drinking\ water}$$

The health risk is assessed owing to exposure dose of both As and F⁻ in study population of different age groups as hazard index (HI), that is characterized as the ratio of cumulative EDI to the reference dose (R_fD), corresponds to entire As/F⁻ exposure risk:

$$HI = EDI_{Cumulative} / R_fD$$

R_fD is the reference dosage (reference value for oral toxicity). R_fD values for F⁻ and As are 0.06 and 3.04E-04 mg/kg/day, respectively.

Quality control

The calibration curve of working standards solutions of As prepared from certified stock standard (1000 mg/L), from the quantification limit up to 100 µg/L by successive dilution. The calibration curves based on peak area measurements of standards and calculated by equation (*n* = 5), using HGAAS as given below:

$$Y = (0.188 \pm 0.0084)[As] + (4.6 \times 10^{-3} \pm 0.0001)$$

where Y is integrated absorbance and [As] is the As concentration in µg/L. The limit of detection (LOD) stated as 3 × s/m, where ‘s’ being the standard deviation related to 10 injections of a blank solution and ‘m’ the slope of the calibration curve. The quantification limit (LOQ) defined as 10 × s/m. The LOD and LOQ were found at 0.126 and 0.421 µg/L of As, respectively. The calibration graph for F⁻ was obtained by ion selective electrode in the dynamic range of 1.0–10.0 mg/L; obtained slope value ‘m’ was 60.3. The LOD and LOQ of F⁻ were calculated as 0.006 and 0.020 mg/L, respectively.

Result and discussion

Fluoride and arsenic in surface water

The resulted data of stored rainwater termed as surface water of nine sampling sites, during July to September in 2011–2013. The pH of collected surface water samples was observed in the range of 7.53–8.65, which is within the WHO recommended values (6.5–8.5) for drinking water. As and F⁻ concentrations in surface water samples were found from 345 to 585 µg/L and 18.8 to 32.4 mg/L, respectively (Table 1). These concentrations were 34–58 and

12.5–22.6 fold higher than the WHO recommended limits for As and F⁻ for drinking water, respectively. The mean values of As and F⁻ in studied surface water samples were higher than reported in the literature for other areas (Mukherjee et al. 2005). The intake of As-contaminated drinking water in high range for a few years is vulnerable to multiple health hazards (Yoshida et al. 2004). The positive correlation ($r = 0.640\text{--}0.671$) was observed among total As with F⁻ in water samples collected from nine sites of selected study areas, which supports the hypothesis of a common source which is consisted with other studies (Buchhamer et al. 2012; Gómez et al. 2009).

It was reported in the literature that the concentration of F⁻ higher than allowable limit in drinking water (1.5 mg/L) can create the risk of dental fluorosis in persons of all age groups, whereas the skeletal fluorosis in elders is also occurred (Ruan et al. 2004). Fluorosis creates damage to the structure and functions of the non-skeletal systems, such as liver and kidney (Farooqi et al. 2007; Xiong et al. 2007). The serious adverse impact of As and F on health of living beings, especially in Southern China (Finkelman et al. 2002; Zheng et al. 1996) and Inner Mongolia (Wang et al. 1999; Smedley and Kinniburgh 2002), might be due to coal mining and combustion.

Total and extractable arsenic and fluoride in soil

The pH values of soil samples were obtained from 7.65 to 8.83. The mean values of arsenic and fluoride concentrations in soil samples (composite) of nine different areas were detected from 121 to 254 mg/kg and 115 to 478 mg/kg, respectively (Table 2). The tolerable/permmissible limit for As in soils of different countries was 0.10–40.0 mg/kg. A risk assessment predicted that up to 4.0 mg/kg of As contents in the soil could not create hazardous effects to living beings. Thus, US EPA set a recommended value of 2–5 mg/kg for As in soil (Smedley and Kinniburgh 2002). The soil samples irrigated with surface water at different agricultural lands of Nagarparkar significantly ($p = 0.001$) exceeded the recommended values of As in soil (Table 2).

The bioavailable or water-soluble contents of As in soil provided information regarding the possible threat to all living beings. The available contents of (water soluble/extractable) As and F⁻ in soil were correspondingly observed in the range of 3.78–8.23 and

3.86–17.2 mg/kg, respectively. The total and water extractable As in soil is calculated on the dried weight basis as indicated in Table 2. Most of the F⁻ in soil is fixed or in combined form, as a result, it is not generally assimilated by plants. The capability of plant to absorb As and F⁻ from the soil also depends on the plant species in addition to their accessible forms, e.g., water extractable (WHO 2008). It investigated about the extraction methods for evaluating the available fraction of elements in growing media to translocate in plants (Smedley and Kinniburgh 2002). The determination of bioavailable/water extractable elements in soil is used to evaluate the deficiency or toxicity of elements to crops and their consumers (Tsuji et al. 2014).

Total As and F⁻ in vegetables

The As and F⁻ concentrations in selected vegetables (IS, WC and CB) are determined and presented in Table 3. The concentration of As and F⁻ in IS, WC and CB was found in the range of (4.54–11.6, 10.1–12.9 and 6.33–11.6) and (85.3–113.2, 158–186 and 61.4–68.6) mg/kg, respectively. The minimum concentration of As and F⁻ in selected vegetables was observed at sites N-1, while both elements are highly accumulated in those vegetables grown at N-9 and N-7. The contents of As and F⁻ were elevated in WC as compared to those values observed for IS and CB, although they were grown in the same agricultural sites (Table 3). The values of As were found to be higher in selected vegetables than in literature-reported values (Martínez-Acuña et al. 2016), whereas lower than those vegetables (20.9–21.2 mg/kg) grown

Table 1 pH, total arsenic and fluoride in surface water samples of Nagarparkar, Sindh, Pakistan

Sample I.D.	pH	As (μg/L)	F ⁻ (mg/L)
N-1	7.53 ± 0.24	345 ± 10.2	18.8 ± 1.12
N-2	8.43 ± 0.35	504 ± 16.4	26.8 ± 1.45
N-3	8.18 ± 0.42	398 ± 9.8	23.6 ± 1.34
N-4	8.24 ± 0.45	421 ± 12.4	25.4 ± 1.82
N-5	7.87 ± 0.38	362 ± 6.68	19.8 ± 1.42
N-6	8.72 ± 0.43	552 ± 16.4	30.5 ± 1.42
N-7	8.38 ± 0.37	412 ± 13.2	22.6 ± 1.75
N-8	7.84 ± 0.37	392 ± 13.4	20.1 ± 1.85
N-9	8.65 ± 0.44	585 ± 11.6	32.4 ± 1.27

Table 2 pH, As, water extractable As, total F⁻ and water extractable F⁻ in soil of Nagarparkar, Sindh, Pakistan (mg/kg)

Sample I.D.	pH	Arsenic		Fluoride	
		Total	Water extractable	Total	Water extractable
N1	7.65 ± 0.45	121 ± 5.34	3.78 ± 0.18	115 ± 4.56	3.86 ± 0.32
N2	7.67 ± 0.45	178 ± 5.32	5.28 ± 0.32	283 ± 5.62	4.76 ± 0.42
N3	7.82 ± 0.41	195 ± 4.86	5.82 ± 0.41	231 ± 7.23	5.12 ± 0.47
N4	7.81 ± 0.31	220 ± 6.36	6.59 ± 0.36	178 ± 4.43	5.82 ± 0.46
N5	8.32 ± 0.54	158 ± 6.25	4.44 ± 0.28	164 ± 3.87	8.15 ± 0.58
N6	7.83 ± 0.34	236 ± 6.43	6.46 ± 0.34	263 ± 7.45	5.86 ± 0.64
N7	7.56 ± 0.38	208 ± 5.67	7.25 ± 0.47	466 ± 21.5	12.8 ± 0.65
N8	7.75 ± 0.46	178 ± 4.52	5.41 ± 0.33	143 ± 5.37	9.26 ± 0.81
N9	7.85 ± 0.42	254 ± 7.49	8.23 ± 0.37	478 ± 11.4	17.2 ± 1.14

in arsenic affected areas of Murshidabad district, West Bengal (Roychowdhury et al. 2003). The resulted data indicated that the accumulation of As and F⁻ in plants depends on the their levels in soil and irrigated water, consisting with those findings reported elsewhere (Bae et al. 2002; Duxbury et al. 2003; Khandare and Rao 2006; Okibe et al. 2010; Baig et al. 2011; Martínez-Acuña et al. 2016). The contents of As in vegetables grown on agricultural soil were significantly correlated with its total and water extractable amount

in the soil and irrigated water ($r = 0.700\text{--}0.996$; $p < 0.05$). The F⁻ concentrations in IS, WC and CB have positively correlated with total F⁻ in water ($r = 0.613\text{--}0.884$; $p < 0.05$), whereas its water extractable contents in agricultural soil were not significantly correlated ($r = 0.256\text{--}0.372$; $p > 0.05$) with those values of F⁻ obtained for IS, WC and CB.

Table 3 As and F⁻ in local vegetables grown in different agriculture plots (mean ± standard deviation) and bioaccumulation factor (BAF)

Elements	Sample I.D.	IS*	BAF (%)	WC**	BAF (%)	CB***	BAF (%)
As	N-1	4.54 ± 0.40	4.12	10.1 ± 0.32	9.18	6.33 ± 0.26	5.76
	N-2	8.04 ± 0.54	4.40	11.2 ± 0.45	6.13	8.06 ± 0.54	4.40
	N-3	8.91 ± 0.62	4.50	11.4 ± 0.41	5.76	8.97 ± 0.67	4.53
	N-4	10.4 ± 0.57	5.22	11.9 ± 0.47	5.94	9.44 ± 0.42	4.72
	N-5	6.17 ± 0.49	4.03	10.8 ± 0.38	7.08	7.45 ± 0.56	4.87
	N-6	10.6 ± 0.49	5.31	12.0 ± 0.52	6.00	9.84 ± 0.44	4.92
	N-7	10.8 ± 0.38	5.25	12.2 ± 0.57	5.95	10.0 ± 0.48	4.89
	N-8	7.93 ± 0.25	4.66	11.1 ± 0.47	6.56	7.67 ± 0.35	4.51
	N-9	11.6 ± 0.45	4.36	12.9 ± 0.51	4.84	11.6 ± 0.41	4.37
F	N-1	85.3 ± 3.47	68.2	158 ± 6.48	126.5	61.4 ± 2.21	49.1
	N-2	104 ± 4.75	37.6	178 ± 7.65	64.0	67.0 ± 3.16	24.1
	N-3	98.4 ± 4.42	44.3	177 ± 8.52	79.7	64.2 ± 2.08	28.9
	N-4	96.6 ± 3.68	56.2	176 ± 8.81	102.4	63.0 ± 3.99	36.6
	N-5	95.9 ± 5.93	63.1	173 ± 6.93	114.0	62.0 ± 2.84	40.8
	N-6	103 ± 6.29	41.1	177 ± 7.22	70.7	66.7 ± 4.19	26.6
	N-7	113 ± 6.87	20.0	186 ± 6.98	32.9	68.5 ± 3.37	12.1
	N-8	92.7 ± 4.11	68.6	161 ± 5.83	119.6	61.7 ± 3.62	45.7
	N-9	106 ± 6.16	21.1	180 ± 5.66	35.9	67.7 ± 4.25	13.5

*Indian squish, **wild cucumis, ***cluster bean

Bioaccumulation and correlation of As and F among vegetables and growing media

The bioaccumulation factor (BAF) of As ranged from 4.03 to 5.13, 4.84 to 9.18 and 4.37 to 5.76 for IS, WC and CB, respectively. The WC sample of N-1 showed high BAF (9.18), and the minimum value (4.03) was predicted in IS sample of site N-5 (Table 3). The estimated BAF of As is higher than 4.00 in all samples of IS, WC and CB. The resulted data also indicated significant higher BAF values of F⁻ in WC samples of all sites as shown in Table 3. The minimum value of BAF of F⁻ was observed in a CB sample of N-7 (12.1). However, the BAF values of F⁻ were significantly higher in all vegetables ($p < 0.05$). It was observed that the BAF gradually declined with the increase in As and F⁻ concentrations in the media, which may imply the restriction in soil–root transfer at higher concentrations of them, these findings consisted with reported data in the literature (Tsuji et al. 2014; Yadav et al. 2012; Baig and Kazi 2012). On the other hand, it was observed that BAF values for specific crops are not all the time regular, whereas it affected the different parameters of soil such as pH, organic matter, clay content, total and bioavailable contents of elements as well as botanical factors, type of plant, species and growth rate (Tsuji et al. 2014). The possibility of the presence of organic or tightly bound As and F⁻ in food materials remains to be verified (Bustingorri and Lavado 2014).

Estimated daily intake of vegetables and drinking water

The survey among the study groups of three age groups (7–15 years), (16–25 years) and (26–50 years), for the nutritional status was carried out. The mean intake of all three vegetables (IS, WC and CB) for the age group of 7–15 was 9.70, 4.74 and 3.28 g/day, respectively, while in the age groups 16–25 and 26–50 years, it was calculated as 19.4, 9.48 and 6.57 g/day, respectively. The mean intake of drinking water in age group 7–15 years was 1.5 L/day and for the elder groups (16–25 and 26–50 years) are > 2.5 L/day, which may be due to the hot weather in understudied area. There was a large difference in the diet pattern to use different vegetables and drinking water among the selected population.

The major cause of arsenicosis and fluorosis is believed so far to be the ingestion of As and F enriched water resources, whereas food item grown in contaminated growing media enhanced the intake of both elements (Das et al. 2004). Therefore, the estimated daily intake (EDI) of As and F⁻ through vegetables and drinking water was calculated to assess the exposure of both elements in different age groups. The mean concentration of As and F⁻ in vegetables and drinking water was used to calculate EDI. All parameters used for the calculation of individual and cumulative EDI of vegetables and drinking water in three age groups are given in Table 4.

The individual EDI of IS, WC, CB and drinking water indicated that the age group (7–15 years) had high level of EDI as compared to other age groups. The individual EDI of vegetables according to age group decreased in the approximate order as: 7–15 years < 16–25 years < 26–50 years. The cumulative EDI values of As were 0.045, 0.0405 and 0.0337 while for F⁻ 1.706, 1.457 and 1.215 mg/kg/day for the age group 7–15, 16–25 and 26–50 years, respectively. The cumulative EDI values of As and F⁻ in age group 7–15 years were found to be higher as compared to the other age groups ($p \leq 0.05$); this might be due to low body weights compared to higher age groups (Table 4).

Toxic risk assessment

The risk assessment was evaluated as hazard index (HI), for three age groups resided in different places as shown in Table 4. It was found that HI of As and F⁻ in the case of age group (7–15 years) was 148 and 28.4, respectively. Since these findings, the rural population in the study area consuming studied vegetables, IS, WC, and CB in addition to drinking water were at the risk of severe toxicity of As and F⁻, because HI values found to be > 1 (Sofuoglu and Kavcar 2008; Nguyen et al. 2009). The first cases of arsenicosis and fluorosis were already reported in 2002 at the study area (Tharparkar) (Brahman et al. 2013a; Rafique et al. 2008).

To evaluate the risk of exposure via consumption of As- and F⁻-contaminated drinking water on population based on three age groups creates confronts on their health. The different physiological disorders such as dental fluorosis and hyperkeratosis are based on the reference dose of fluoride and arsenic, although it is

Table 4 Pearson coefficient (*r*) and *p* value of As and F⁻ in growing media (soils and irrigation water) with As and F⁻ in vegetables

	As in soil	Water extractable As in soil	As in water
As in IS			
<i>r</i> value	0.926	0.972	0.700
<i>p</i> value	0.001	0.001	0.036
As in WC			
<i>r</i> value	0.966	0.996	0.780
<i>p</i> value	0.001	0.001	0.013
As in CB			
<i>r</i> value	0.964	0.992	0.795
<i>p</i> value	0.001	0.001	0.010
	F ⁻ in soil	Water extractable F ⁻ in soil	F ⁻ in water
F ⁻ in IS			
<i>r</i> value	0.881	0.491	0.642
<i>p</i> value	0.002	0.180	0.062
F ⁻ in WC			
<i>r</i> value	0.766	0.372	0.613
<i>p</i> value	0.016	0.324	0.079
F ⁻ in CB			
<i>r</i> value	0.884	0.443	0.767
<i>p</i> value	0.002	0.232	0.016

recognized that As and F⁻ also created different physiological effects on the central nervous system. Whereas for the adverse effects of both elements, their reference dose does not exist, especially in the children exposed to mixture of both elements via consuming ground and surface water (Martínez-Acuña et al. 2016). Exposure to F⁻ was calculated and compared with the adequate intake of minimal safe level exposure dose of 0.05 mg/kg/day, and it was noted that population of every age group may be at high risk of fluorosis. Chronic exposure to high As and F⁻ levels in this population represents a concern due to possible adverse health effects attributed to these elements. Recently it was reported that if drinking water has F⁻ level < 0.7 mg/L, consumed for long time have lower risk for moderate or severe fluorosis, whereas above than 1.5 mg/L created more severe fluorosis (Irigoyen-Camacho et al. 2016). The population of study area consumed As- and F⁻-contaminated drinking water and other food items from early age such as during development (pregnancy) then infancy period to adolescence, and up to childhood, so the adverse effects associated with elevated exposure of As and F⁻ are increased (Rager et al. 2014). The

population of understudy areas has poor economic and mainly depends upon groundwater and locally grown vegetables. However, they have no adequate information on the nature of As and F⁻ in food materials. These circumstances lead to motivate the researchers and scientists to work on health risk associated with utilization of As-contaminated food and drinking water (Patel et al. 2005; Juhasz et al. 2006; Madeira et al. 2012). In addition to high levels of As and F⁻ in food and water other problems especially illiteracy, poverty, malnutrition, little medical facilities and existence of additional toxicant in food and drinking water, could have synergistic effects on the health of people. Our assumption is also consistent with other studies (Roychowdhury et al. 2003; Das et al. 2004).

Conclusion

The survey about the contamination of surface water, soil and vegetables with As and F⁻ in a district Tharparkar, Pakistan, has been conducted to assess the adverse health effects to local population. The As and F⁻ in stored rainwater were significantly higher than

WHO permissible limit of both elements for drinking water. The As and F⁻ levels in soil and vegetables were also found to be high than recommended values. The translocation of As and F⁻ was estimated by bioaccumulation factor. The BAF values of As and F⁻ were found to be > 4.00, indicating the high rate of transportation of As and F⁻ from growing media to vegetables. The hazardous index values was observed to be > 1.0, predicting that the rural population of Nagarparkar, Sindh, Pakistan, are exposed to severe chronic toxicity of As and F which adversely effects their health. The exposure doses of As and F⁻ obtained by the consumption of selected vegetables and drinking water were found to be elevated in lower age group (7–15 years) as compared to elder groups. It indicated that children may have high risk of arsenicosis and fluorosis. It is recommended to the local public health agencies and organizations of Pakistan to focus on exposure of As and F⁻ due to the consumption of the contaminated water and food.

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