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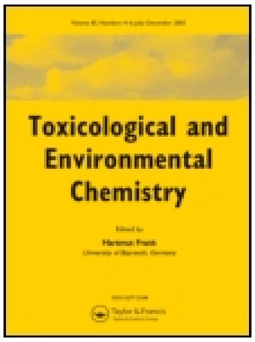

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Bio-concentration of fluoride in Lady's finger (*Abelmoschus esculentus*) grown in contaminated alkaline soil and evaluation of exposure risk in human

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A pot culture study was conducted to evaluate the bio-accumulation of fluoride (F) in terms of bio-concentration factor (BCF) in Lady's finger (*Abelmoschus esculentus*) when grown in sodium F (NaF) contaminated alkaline soil. Toxicological exposure risk on humans in terms of estimated daily intake was assessed. It was found that the maximal F accumulation took place in roots (16.64–106.2 mg kg^{-1}), whereas in the edible part (fruit), it varied between 39.3 to 48.51 mg kg^{-1} in the treatment range of 0–600 mg NaF kg^{-1} soil. The order of F accumulation in plant tissues followed root > leaf > fruit > shoot. The BCF values in the fruit showed a decreasing trend (6.74–5.17 mg kg_{dwt}^{-1}.plant mg^{-1} kg_{dwt}^{-1} soil), whereas in root, it increased (6.69–12.27 mg kg_{dwt}^{-1} of plant per mg kg_{dwt}^{-1} soil) with the rise in added F (100 mg F per kg soil to 600 mg F per kg soil). The risk of F exposure due to the consumption of fruit was estimated for an adult (18–70 years), which were found to be 0.001 mg kg^{-1} day^{-1} at the minimum F concentration of 39.3 mg kg^{-1} (dwt.) and 0.0013 mg kg^{-1} day^{-1} at the maximum F concentration of 48.51 mg kg^{-1} (dwt.) of fruit.

Keywords: bio-accumulation; fluoride; alkaline; exposure; risk

Introduction

Fluorosis is a major public health problem in 20 out of 32 constituent states in India (Choubisa 2001) affecting approximately 62 million people including six million children from dental, skeletal, and/or non-skeletal endemic fluorosis (Carton 2006) and the causes mainly ascribed to the consumption of fluoride (F) contaminated ground water. However, the consumption of tea, wheat, spinach, cabbage, carrots and other foods also contribute significantly to the total F intake (Susheela 2003). It is well known that industrial installations such as brick kilns, phosphate fertilizers, glass, coal-fired power stations, and aluminum smelters are the important sources of gaseous and particulate F pollution (Cronin et al. 2000). The gaseous F are absorbed mainly through leaf stomata, transported to various tissue parts in the plants and upon accumulation produce physiological, biochemical, and structural damage. F accumulation in soil and vegetation in the vicinity of brick fields was previously reported (Jha et al. 2008). Besides, the direct uptake through

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the leaf stomata, plants incorporate F from contaminated soils (Domingos et al. 2003). The information regarding the identity and biological toxicity of ionic species of F which are taken up by plant roots are not adequate (MacLean, Hansen, and Schneider 1992; Sikora et al. 1992), as F uptake by different species of plant differs significantly based on their generic features. Therefore, the adverse effects of F in different crops may also vary significantly. F in soil or more specifically the phyto-availability of F is predominantly governed by the types of soil in which the crop is grown. In soil solution of neutral to alkaline pH, F exists predominantly as free F. At slightly acidic soil (pH 5.5–6.5), much of F is adsorbed to the soil and at $pH < 5.5$, the F forms a complex with Al (Barrow and Ellis 1986; Wenzel and Blum 1992). The greater solubility of F under acidic condition was attributed to formation of AlF_x complexes, whereas under alkaline conditions by desorption of free F there results a repulsion of negatively charged surfaces (Wenzel and Blum 1992). The F thus absorbed by roots from soil is translocated to various parts of the plant and accumulated there. The F uptake by plants from contaminated soil through root was previously reported (Singh 1990; Jha, Nayak, and Sharma 2009). The consumption of edible parts of the plants containing higher F content may lead to a higher dietary F intake. In absence of any stringent threshold limit of F in plants and soils, the higher dietary F intake may be detrimental to human health. The World Health Organization (1984) has however, indicated the maximum permissible limit of F in drinking water is $1.5\,mg\,L^{-1}$.

Abelmoschus esculentus is an annual vegetable crop grown in tropical and subtropical regions. In India, the state of Uttar Pradesh is one of the major known growers of this vegetable. As the role of dietary intake is also responsible for fluorosis, the present study basically aimed to (1) evaluate the translocation of F and its partitioning in Lady's finger (*Abelmoschus esculentus*) when grown in contaminated alkaline soil and (2) assess the risk of F exposure in humans due to its ingestion.

Methods

Treatment design

A pot experiment was conducted with alkaline soil collected from the upper part of soil (0–15 cm) classified as Typic Natraustalf, from the research farm of Central Soil Salinity Research Institute located at central Indo-Gangetic plains of Uttar Pradesh. The soil was mixed, dried, ground and sieved through 2 mm sieve. The initial characteristics of the soil such as pH, electrical conductivity (EC), sand, silt, clay, organic carbon (OC), total fluoride (TF), soluble F ($CaCl_2$ extractable F, F_{Ca}) were determined. Thoroughly mixed 8 kg soil was filled into earthen pots lined with polythene sheet. A mixture of nutrients was added to each pot; 300 mg N as urea and 150 mg P as $KH_2PO_4 \times H_2O$. The soils were contaminated with graded concentration from 0, 100, 200, 300, 400, 500, or 600 mg NaF per kg by adding NaF salt to the pots, thoroughly mixed and incubated for 2 weeks before sowing. Each treatment was replicated thrice. Eight Nos. of seeds of Lady's Finger (*Abelmoschus esculentus*) cv. "Parvani Kranti" were sown. Two Nos. of plants were maintained in each pot. Another 300 mg N as urea was applied at 20 days after sowing as top dress. The irrigation was applied with de-ionized water. All plants were harvested at 60 days. Each plant was segregated into shoots, roots, fruits and leaves. Each plant was then dried, weighed and milled to pass through 0.2 mm sieve and kept for F determination in various tissues. Similarly, soil samples collected from each pot after the harvest were subjected to analysis of pH and soluble ($CaCl_2$ extractable) F (F_{Ca}).

Soil analysis

pH (1:2) and EC (1:2) of the initial soil was determined by using ORION ion Analyser (5-Star series). Textural analysis (Sand, Silt, and Clay) of the soil was carried out by International Pipette Method (Klute 2002), OC by Walkley Black Method (Nelson and Sommers 1996), soluble F, F_{Ca} (0.01 M $CaCl_2$ extractable) by the method (Larsen and Widdowson 1971). The TF in soil was determined by alkali fusion method using ion selective electrode technique (McQuaker and Gurney 1977).

Fluoride determination in fruit, leave, shoot and root

F in fruit, leave, shoot, and root was determined by extracting the dried, grinded, and sieved samples with 0.1 N perchloric acid (Villa 1979). The average recoveries based on the spiked samples at two different levels of F were $98 \pm 6\%$.

Moisture % determination in fruit

The edible part (fruit) of *Abelmoschus esculentus* plant (about 40 g) were chopped into pieces, left to dry in air for 2 days and then kept in hot air oven at 70°C for 3 days until a constant weight attained. The moisture % was calculated by using formula given below:

$$\text{Moisture\%} = \frac{(W1 - W2)}{W1} \times 100$$

where $W1$ = fresh weight of vegetable; $W2$ = weight of vegetable after drying at 70°C. The values of the moisture content of *Abelmoschus esculentus* were required (on dry weight basis) for determination of exposure doses of F in humans.

Bio-concentration factor determination

Bio-concentration factor (BCF) is a common parameter for estimating the F concentration in vegetables and subsequently human exposure through consumption of vegetables which is defined as the ratio between the concentration of F in the edible part of the vegetable and F concentration in soil (Jha, Nayak, and Sharma 2011).

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

Where

BCF BCF of F (mg kg^{-1}_{dwt} of plant per mg kg^{-1}_{dwt} soil)
F vegetable F concentration in the edible part of vegetable (mg kg^{-1}_{dwt} of plant)
F soil F concentration in soil (mg kg^{-1}_{dwt} of soil)

Exposure dose of F from fruit

The exposure doses of F in terms of estimated daily intake (EDI) in adult (18–70 years) due to the consumption of edible part (fruit) of *Abelmoschus esculentus* were calculated on minimum and maximum F contents found in fruit during this study by using generic equation (USEPA 1992).

$$\mathrm{EDI} = \frac{\mathrm{C} \times \mathrm{IR} \times \mathrm{EF} \times \mathrm{ED} \times \mathrm{AF} \times \mathrm{CF}}{\mathrm{BW} \times \mathrm{AT}}$$

EDI	($\mathrm{mg\,kg^{-1}\,day^{-1}}$)
C	Concentration of F in vegetables ($\mathrm{mg\,kg^{-1}}$)
IR	Ingestion or Intake Rate ($\mathrm{mg\,day^{-1}}$)
EF	Exposure frequency (days per year)
ED	Exposure duration (year)
AF	Absorption Factor (unitless)
CF	Conversion Factor ($10^{-6}\,\mathrm{kg\,mg^{-1}}$)
BW	Body weight (kg)
AT	Averaging time (days)

Statistical analysis

Treatment means were compared using ANOVA and the level of significance considered was $p > 0.01$ (Gomez and Gomez 1984).

Results

Initial characteristics of the soil

The soil characteristics such as pH and EC of initial soil used for the pot experiment was determined and found to be 8.42 and 0.65 $\mathrm{dSm^{-1}}$, respectively, in which the organic carbon was 0.41%. The textural analysis revealed % sand, silt, and clay at 49.1%, 18.5%, and 32.5%, respectively. The soluble F (F_{Ca}) and TF were 6.01 $\mathrm{mg\,kg^{-1}}$ and 311 $\mathrm{mg\,kg^{-1}}$, respectively.

Soluble F in soil

The soluble F (F_{Ca}) was determined in the soils of different treatments. Soluble F varied between 2.51 and 8.65 $\mathrm{mg\,kg^{-1}}$ in the treatment range of 0–600 mg NaF $\mathrm{kg^{-1}}$ soil. The variation of soluble F in the soil with the added F is presented in Figure 1. There was a marked increase of soluble F up to the added F of 100 mg NaF $\mathrm{kg^{-1}}$ soil, whereas between 200 and 600 mg NaF $\mathrm{kg^{-1}}$ soil there was a steady rise with the added F.

Fluoride concentration in the plant tissues

The F accumulated in various tissue parts of *Abelmoschus esculentus* are presented in Figure 2. The order of F accumulation was as follows: root > leaf > fruit > shoot. The maximal F accumulation was found in roots, which varied from 16.64 to 106.2 $\mathrm{mg\,kg^{-1}}$. An apparent ion transport mechanism was not obvious in this plant on the basis of F accumulation in tissues. However, F accumulation in root at 0 mg NaF $\mathrm{kg^{-1}}$ soil (control) was found to be minimal compared to other tissue parts, whereas with the increase of added F, roots accumulated F at the higher concentration and uptake rate was markedly greater than all other plant parts. This indicated that the plant might actively

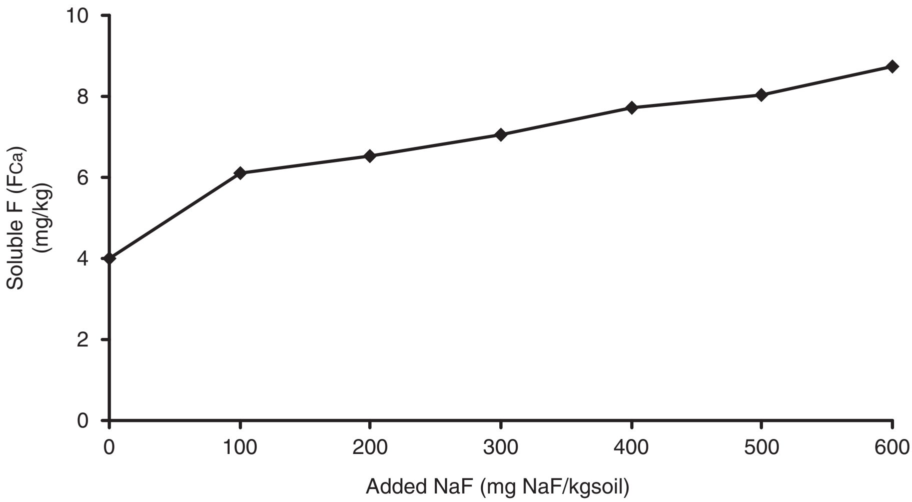

Figure 1. Effect of added NaF on Soluble F (F_{Ca}).

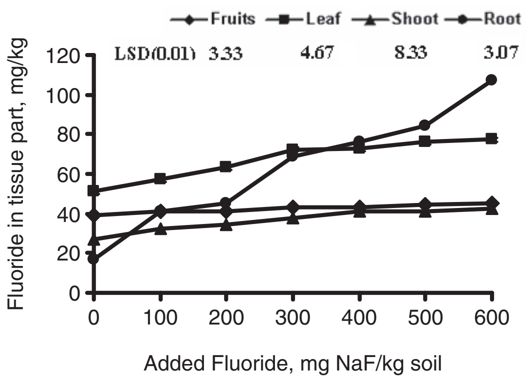

Figure 2. F accumulation in tissue parts of *Abelmoschus esculentus* with added NaF.

exclude F at the root at high pH for this plant species. F accumulation in fruits varied between 39.3 and 48.51 mg kg^{-1} in the treatment range of 0–600 mg NaF kg^{-1} soil.

Bio-mass yield

The visible phyto-toxic symptoms due to F were not observed in the form of marginal necrosis (tip-burn, scorching, or lesions) or death of plants in the treatment range of 0–600 mg NaF kg^{-1} soil. However, the bio-mass per plant (dry wt.) revealed a significant decrease at 200 mg NaF kg^{-1} soil and higher compared to control (Figure 3). The fall in biomass was 4.5%, 17.9%, 23.9%, 26.9%, and 29.1% at added F of 200, 300, 400, 500, and 600 mg NaF kg^{-1} soil.

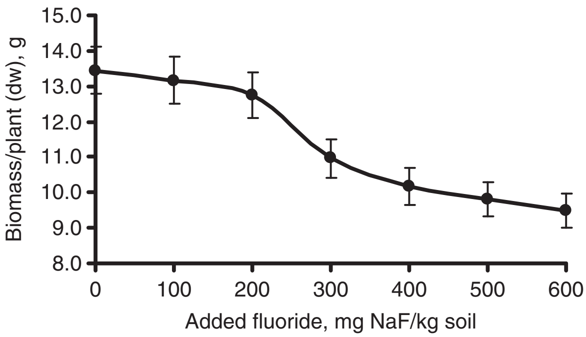

Figure 3. Effect of added NaF on bio-mass yield per plant of *Abelmoschus esculentus*.

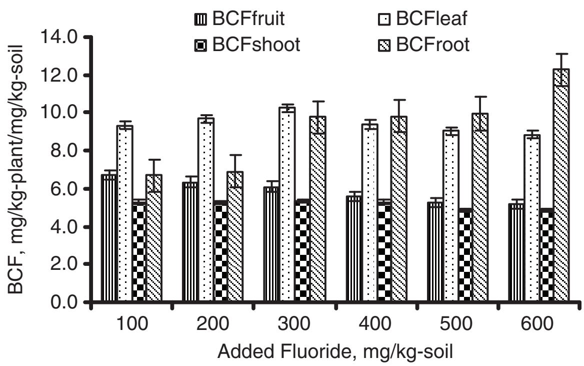

Figure 4. BCF values of F in tissue parts of *Abelmoschus esculentus* plant with added F.

BCF of F in plant tissues

The BCF was determined for all tissue parts of the plant which is defined as the relative uptake of F ion by plants with respect to presence of F in soil solution. The BCF values in the edible part (fruit) showed a decreasing trend (6.74–5.17 mg kg^{-1}_{dwt} of plant per mg kg^{-1}_{dwt} soil) with increase in added F (100 mg F per kg soil to 600 mg F per kg soil) while the values showed an increasing trend in root 6.69–12.27 mg kg^{-1}_{dwt} plant per mg kg^{-1}_{dwt} soil (Figure 4). The shoot and leaf did not show any consistent pattern. The BCF values in the leaf rose with elevation of added F up to 300 mg F per kg soil but presented a decreasing trend from 400 mg F per kg soil to 600 mg F per kg soil. In shoot, no significant differences were observed in the BCF values with addition of F in soil up to 600 mg F per kg soil.

Risk assessment in human

The exposure risk due to F ingestion through fruit (the edible part) of this plant was estimated for adults (18–70 years) by considering 70 kg as the average weight of an adult. As per the stipulated dietary guidelines (ICMR 2009) under vegetable intake, average consumption of 100 g fresh weight per day was taken. The moisture content of 85.9% was calculated and the dry weight (14.1 g per day) equivalent to the fresh weight of fruit was

Table 1. EDI of F due to the ingestion of Lady's finger (*Abelmoschus esculentus*) at the minimum and maximum concentration of the added F.

Concentration of F in fruit ($mg\,kg^{-1}$)	EDI ($mg\,kg^{-1}\,day^{-1}$) for adults (18–70 years)
39.30	0.0010
48.51	0.0013

taken for the estimation of exposure dose in terms of EDI of F. The frequency of intake was presumed 8 months (twice a week), i.e. 64 days per year on the basis of a general survey conducted earlier (Jha, Nayak, and Sharma 2011). It was found that the exposure dose in terms of EDI for an adult (18–70 yrs.) was $0.001\,mg\,kg^{-1}\,day^{-1}$ at minimum F concentration of $39.3\,mg\,kg^{-1}$ in fruits whereas at the maximum concentration of $48.51\,mg\,kg^{-1}$, the EDI was found to be $0.0013\,mg\,kg^{-1}\,day^{-1}$ (Table 1).

Discussion/conclusions

Initial characteristics of the soil

The bio-availability of F to plants is influenced by several factors such as presence of metal ions forming complexes, precipitation, and pH variations. Brewer (1965) reported that the availability of soil F to plants is controlled by pH, soil type and clay content of soil. The determination of pH of the initial soil indicated that the soil used was alkaline in nature. The textural analysis of the soil revealed comparatively higher clay content. Clay plays an important role in retaining F in soil in the pH range of 5.5–6.5 whereas at pH > 6.5, desorption of F takes place due to repulsion of negatively charged surfaces (Stevens et al. 2000). Robinson and Edington (1946) noted that most soil contain $20–500\,mg\,kg^{-1}$ F derived from minerals such as fluoroapatite, fluorospar, and cryolite but some micaceous clays contains several thousands of $mg\,kg^{-1}$ F. The average TF content of world wide soil was reported to be 320 ppm (Kabata-Pendias and Pendias 1984), which agrees with the concentration of $311\,mg\,kg^{-1}$ found in the soil taken in the present pot trial study. However, the soluble F content in soil varied between 0.3 and 16.1 ppm (Rodriguez, Rodriguez, and Marcos 2001) and is independent of TF content of soil.

Soluble F in soil

The soil TF may not be a reliable indicator of the amount of F bio-available to plants because in soils, the solubility of F is controlled mainly through F adsorption by the inorganic constituents present in the soil (Loganathan et al. 2003). It is only F in solution or easily desorbable F that is taken up by plants (Brewer 1965; Cooke et al. 1976). Therefore, for all soil it is the soluble F content that is biologically important to plants and animals (Davison 1983). Hence, soluble F (F_{Ca}) was determined in soils following different treatments. The sharp increase of soluble F up to the added F of 100 mg NaF per kg soil might be due to the release of OH ion during the adsorption process (Bower and Hatcher 1967). Between 200 and 600 mg NaF per kg soil, the steady rise may be due to increase in pH of soil solution (Figure 5), which is in agreement with earlier findings (Barrow and Ellis 1986; Wenzel and Blum 1992), according to which if a high concentration of F is added to

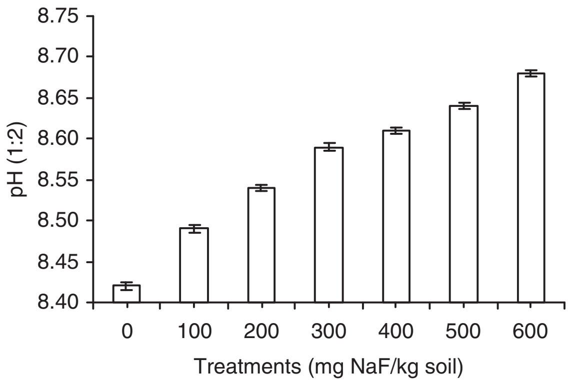

Figure 5. Variation of pH with added F.

soil or soil solution, pH becomes more alkaline and F might rise in soil solution and more F ion would be potentially available for the uptake by the plant root.

Fluoride concentration in the plant tissues

Maximal accumulation of F was found in the roots in the present experiment, in agreement with findings of Jha, Nayak, and Sharma (2009) and Patel and Vyas (2004). Kabata-Pendias and Pendias (1984) suggested that soluble F fraction in soil is taken up passively by roots and easily transported in plants. The transport of most of the F across the roots remains in the cell wall and intercellular space (apoplast) rather than through the cell membrane because permeability of cell membrane to F ion is low, which limits diffusion into cells (Takmaz-Nisaneiouglu and Davison 1988). The F concentration in the root was found to be higher than leaves, fruits, and shoots probably due to relatively low permeability through endodermis (Weinstein and Davison 2004; Gupta and Banerjee 2009). As the experiment was carried out in alkaline soil (pH = 8.42), this meant a higher bioavailability of F to plants mainly in the form of F^- ion which is taken up by plants easily through root system. However, Cooke, Johnson, and Davison (1978) noted that positively charged AlF_x complex are more easily taken up by the roots than free F^- ion due to anion exclusion by the negatively charged cell walls, particularly in the case of acidic soil where F exists as AlF_x complex (Elrashidi and Lindsay 1986). Stevens, McLaughlin, and Alston (1997) also reported that F was taken up by the plant in the form of AlF_3 complex from solution culture containing Al and F.

Biomass yield

No phyto-toxic symptoms were noted in the plant with added F which might be due to F exclusion at the root or detoxifying F at cellular level in plant (Jha, Nayak, and Sharma 2008). However, the occurrence of phytotoxic symptoms due to F varies greatly with plant species and cultivars which are in agreement with findings of Khandare and Rao (2006).

BCF of F in the tissues

BCF is an important parameter that has been used widely (Alonso et al. 2003; Tome, Rodriquez, and Lozano 2003) for the assessment of contamination in the soil. Jha, Nayak, and Sharma (2011) used BCF as an indicator of affinity for the accumulation of F in plants. Basically BCF is a relative uptake of F ion by plants with respect to presence of F ion in solution. A ratio greater than 1 indicates hyper-accumulation of F in plants while less than 1 is a hypo-accumulation (Gupta and Banerjee 2009). It was observed that the root retained much of the F and restricted its translocation to different tissue parts with increase of added F in soil. Generally the crops genetic characteristics are also responsible for the differential accumulation (Peris et al. 2007). However, Swartjes et al (2007) also reported that BCF values are not always constant in specific vegetables and are largely dependent upon the types of soil and its characteristics on which the vegetables are grown as well as plant factors like type of plant and growth rate.

Risk assessment for humans

In absence of any stringent national guidelines with respect to the dose effect relationship, a dose of 0.06 mg F day^{-1} kg^{-1} body weight as stipulated by USEPA (1992) was taken for comparing the risk of F on humans due to the ingestion of the fruit (edible part). The EDI values were found to be 0.001 mg kg^{-1} day^{-1} at the minimal F concentration of 39.3 mg kg^{-1} (dry wt.) and 0.0013 mg kg^{-1} day^{-1} at maximal F concentration of 48.5 mg kg^{-1} day^{-1} (dry wt.) for an adult. These values were also found to be less than the prescribed limits (Hargreaves 1990; IOM 1999) for adults which are 1.5–4 mg F day^{-1} or 0.0214–0.0571 mg kg^{-1} day^{-1}. As the values obtained in the study were only the dietary intake through the consumption of Lady's finger (*Abelmoschus esculentus*) fruits and did not account for the F intake through other sources, it revealed that the vegetable alone does not contribute appreciably to exposure doses of F in humans. The F content of prepared foodstuffs mainly depends on F content of food, the concentration and amount of water used and retained in the food during its preparation need to be considered (Malde et al. 2004).

The change in pH due to higher F treatments to soil was found to contribute in F uptake by *Abelmoschus esculentus*. Under such soil conditions, F became potentially bioavailable for plant's uptake through root system. The F accumulation in various tissue parts of the plant followed the order root > leaf > fruit > shoot. As dietary intake through consumption of fruits of Lady's finger (*Abelmoschus esculentus*) is quite low, its contribution to exposure in form of EDI in human (adult) was within the prescribed limit when F ingestion through this vegetable alone was considered.

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