

## Determination of Arsenic Uptake Potential In an Edible Plant Species (*Trigonella Foenum- Granecum*) and Assessment of Human Health Risk

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### Abstract

Arsenic is a carcinogenic and toxic element that possesses a high health risk from its presence in crops, water, and soil. The present study has been conducted by fenugreek (*Trigonella foenum-granecum*) seeds which is a very common spice used for cooking, especially in India. An equal number of seeds have been germinated in laboratory conditions. Three concentrations e.g. 1, 2, and, 3 mg/L of arsenite ( $As^{+3}$ ) and arsenate ( $As^{+5}$ ) salt solutions were used throughout the experiments for the treatment of plants. After 10 days of germination, the concentration of the arsenic accumulated into the plant edible parts was estimated and health risk was assessed. Effects of arsenic concentration were observed through estimating the total chlorophyll (a, b, c), carotenoid content, and taking the fresh weight and dry weight of both the control and treatment plants. The results of the biochemical analysis revealed that chlorophyll and carotenoid contents were decreased than that of control plants. Moreover, fresh weight and dry weight results also showed lower values in treatments than in controls. The bioaccumulation factor results demonstrated that an increased level of soil arsenic doesn't certainly result in high arsenic uptake by the Fenugreek plants. From the concentration estimated in the plant body, the health risk was assessed in adults and children and found that both adults and children having a potential health risk upon consumption of fenugreek. Moreover, Incremental Life Time Cancer Risk was found high which indicates the presence of potential cancer risk. Hence, it is possible to conclude from the present study that fenugreek can bio-accumulate arsenic and it may be used as an indicator plant for arsenic-contaminated areas.



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## Introduction

Natural and anthropogenic sources are the two major areas from where arsenic (As) may contaminate the normal environment.<sup>1</sup> Contamination of arsenic can be toxic and fatal if it will enter the food web via water and soil routes. Previous researchers also observed the contamination of groundwater and foods via arsenic in different parts of the world.<sup>2,3</sup> Contamination of soil by arsenic may result from agricultural activities, various anthropogenic activities, etc. According to Huang *et al.*, (2006) inorganic-As is the most predominant toxic form presents in soil and water.<sup>4</sup> However, few previous studies reported about As- methylation in the soil-paddy system, where they found that the inorganic form of arsenic may be converted from organic arsenic by microorganisms.<sup>5</sup> The uptake of arsenic by a plant species depends on many factors like soil properties, the concentration of arsenic present in the soil, and most importantly depends on the plant species itself.<sup>6</sup> Physical characteristics of plant species is an important aspect which is evident from few previous studies that found the concentration

of arsenic was estimated high in root parts followed by leaves, stems, fruits, and seeds.<sup>7,8</sup> However, Farid *et al.*, (2003) have found aerial parts of leafy vegetables can accumulate high concentration of arsenic than any other plant body parts.<sup>9</sup> So, it is clear that the bioavailability of arsenic and ability to translocate these two factors were primary which determine the arsenic distribution in the plant body.<sup>10</sup> Studies on arsenic availability in the plant body are important as they can bioaccumulate in human tissue through the food chain.<sup>11</sup> The effects of chronic arsenic exposure on humans can cause serious health effects like keratosis, hepatomegaly, pigmentation, diabetes mellitus, cardiovascular and cerebrovascular problems, peripheral neuropathy, kidney, bladder, and skin, etc.<sup>12, 13</sup>

Keeping the facts in mind, this study urge to explore the sensitivity and tolerance against the arsenic toxicity in a common plant species, which is widely used as a food ingredient in most of the part of India, and it also will assess the health risk among the population consuming this edible plant species.

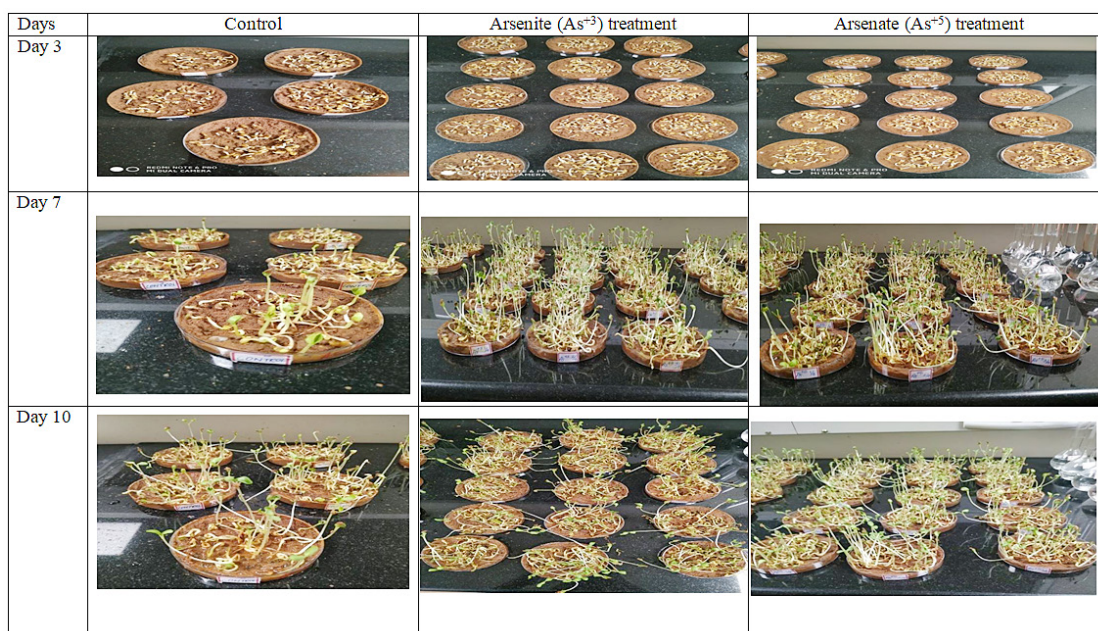


Fig.1: Growth of control and arsenic-treated fenugreek plants in laboratory condition

## Materials and Methods

### Experimental Methodology

Seeds of fenugreek (*Trigonella foenum-graecum*) were procured from the grocery stores. Generally, fenugreek leaves were used as vegetables, and

seeds were used as common Indian spices. The entire experiment was performed in the laboratory condition. Three different concentrations of sodium arsenite (NaAsO<sub>2</sub>) and sodium arsenate (Na<sub>2</sub>HAsO<sub>4</sub>, 7H<sub>2</sub>O) (dissolved in distilled water only) solutions

were used for this study. The sterilization process of the experimental seeds was carried out with 1% sodium hypochlorite (NaOCl) solution for a minute and then washed with double distilled water (DDW).<sup>14</sup> The process of seeds germination and growth was made in the soil collected from the institute campus using Petri dishes. Petri dishes were covered with lids and were incubated at room temperature (30°C). Stock solutions of the As<sup>+3</sup> and As<sup>+5</sup> were prepared by the required amount of arsenic salts in DDW to make 100 ppm solution for each As<sup>+3</sup> and As<sup>+5</sup>. Required dilutions were made to get the working solutions i.e. 1 mg/L, 2 mg/L, and 3 mg/L. The experimental study was performed using respective arsenic solutions. The Petri dishes were periodically nourished and moisturized during the entire experimental period with 10 ml of the solution once a day. Control sets of experiments were set up by using only DDW. Three replicates were taken for each treatment and control. Germination and growth of the seeds were observed for 10 days. Estimation of chlorophyll, Carotenoid contents, dry and wet weight was recorded after 10 days of the experiment (Fig. 1).

#### Determination of Fresh and Dry Weights

A total of 10 fully grown seedlings with almost uniform size were taken from each dish (both from control and treatments) and were thoroughly washed with DDW to remove the excess soil. Tissue papers were used to soak the excess water. Fresh weights were taken after the seedling was room dried and dry weights were taken after the seedlings were dried at 70°C for 48 hr in an oven.<sup>14</sup>

#### Estimation of Chlorophyll and Carotenoid

After 10 days of the experiment, fenugreek leaves were taken for the estimation of chlorophyll and carotenoid. Standard methodologies were followed for the estimation of chlorophyll and carotenoid.<sup>15</sup>

#### Total Arsenic Estimation in Plants

For estimation of total arsenic, plants were weighed to measure their mass. Two grams of dry plant sample was digested in the tri-acid mixture (HCl-HNO<sub>3</sub>-HClO<sub>4</sub>) until the mixture became a muddy-like texture. The digestion mixture was diluted with DDW to make up the volume to 50 ml and then it was filtered with filter papers. Finally, the filtrate was used for the estimation of arsenic in Atomic Absorption Spectroscopy (Bruker, ARTAX).

#### Bioaccumulation Factor Calculation

Bioaccumulation factors (BAF) were calculated by taking the ratio of concentrations found in plants to the soil arsenic concentration Eq. 1.

$$BAF = \frac{C_{As-plant}}{C_{As-soil}} \quad \dots(1)$$

Where  $C_{As-plant}$  is the concentration of arsenic in the plant body and  $C_{As-Soil}$  is the concentration of arsenic in the soil

#### Chronic Intake Dose (CDI) Estimation

CDI is calculated taking the arsenic concentration found in the fenugreek plant and following the formula Eq. 2:

$$CDI = \frac{C_{As} \times CF \times IR_{veg}}{BW} \quad \dots(2)$$

Where,  $C_{As}$  = As-concentration (mg/kg),  $IR_{veg}$  = Intake rate of contaminated plant (mg/day),  $CF$  = Conversion factor,  $BW$  = Body weight

#### Risk Assessment

The mean value of total arsenic concentrations was used in the estimated daily dose (EDD) calculation Eq. 3.<sup>16,17</sup>

$$EDD = \frac{C_{As} \times IR_{veg} \times CF \times EF \times ED}{LE \times BW} \quad \dots(3)$$

Where,  $C_{As}$  = As-concentration (mg/kg),  $IR_{veg}$  = Intake rate of contaminated plant (mg/day),  $CF$  = Conversion factor,  $EF$  = Exposure factor,  $ED$  = Exposure duration (years),  $LE$  = Lifetime expectancy,  $BW$  = Body weight

#### Determination of Hazard Quotient and Hazard Index

A Hazard Quotient (HQ) is the proportion between potential exposures to the safe level of exposure. Hazard Quotient less than 1 predicted to be the safest level. The reference dose (RfD) is the limit above which any chemicals may pose a toxic effect on the health of the human. HQ was estimated by following Eq. 4. The hazard index (HI) Eq. 5 was calculated as the sum of average HQ values.<sup>18</sup>

$$HQ = \frac{EDD}{RfD} \quad \dots(4)$$

$$HI = \sum HQ \quad \dots(5)$$

**Incremental Life Time Cancer Risk Assessment**

For assessment of the incremental lifetime cancer risk (ILTCR), the established formula Eq. 6 was used.<sup>18</sup> According to USEPA (2010), ILTCR value > 10<sup>-4</sup> indicates high-potential cancer risk, and < 10<sup>-4</sup> indicates probable health risk.

Where,  $C_{As}$  = As-concentration (mg/kg),  $IR_{veg}$  = Intake rate of contaminated plant (mg/day), CF = Conversion factor, EF = Exposure factor, ED = Exposure duration (years), LE = Lifetime expectancy, BW = Body weight, CFS = cancer slope factor (per mg/kg/day)

$$ILTCR = \frac{C_{As} \times IR_{veg} \times CF \times EF \times ED}{LE \times BW} \times CFS \quad \dots(6)$$

**Table 1: Fresh and dry weight of fenugreek plants**

		Fresh weight (g)	Dry weight (g)
Arsenite (mg/L) (As <sup>+3</sup> )	Control/ Treatments	5.551 ± 0.124	0.689 ± 0.177
	1	5.127 ± 0.826	0.686 ± 0.089
	2	5.106 ± 0.867	0.652 ± 0.171
Arsenate (mg/L) (As <sup>+5</sup> )	3	4.899 ± 0.700	0.612 ± 0.030
	1	4.481 ± 0.339	0.655 ± 0.069
	2	5.030 ± 0.541	0.630 ± 0.047
	3	4.558 ± 0.088	0.497 ± 0.039

**Table 2: Chlorophyll and Carotenoid content of the control, As<sup>+3</sup>, and As<sup>+5</sup> treated fenugreek plants**

		Chl a (mg/g, Mean ± SD)	Chl b (mg/g, Mean ± SD)	Total Chl (mg/g, Mean ± SD)	Carotenoid (mg/g, Mean ± SD)
<b>Control/Treatments</b>		0.559 ± 0.024	0.712 ± 0.467	1.042 ± 0.184	0.226 ± 0.022
Arsenite (mg/L) (As <sup>+3</sup> )	1	0.535 ± 0.125	0.17 ± 0.141	0.695 ± 0.318	0.128 ± 0.080
	2	0.519 ± 0.147	0.224 ± 0.067	0.792 ± 0.224	0.159 ± 0.065
	3	0.413 ± 0.093	0.163 ± 0.028	0.609 ± 0.124	0.109 ± 0.030
Arsenate (mg/L) (As <sup>+5</sup> )	1	0.422 ± 0.135	0.175 ± 0.058	0.633 ± 0.202	0.091 ± 0.026
	2	0.612 ± 0.088	0.230 ± 0.032	0.881 ± 0.122	0.178 ± 0.032
	3	0.532 ± 0.064	0.2 ± 0.038	0.768 ± 0.109	0.145 ± 0.035

**Table 3: Concentration of Arsenic in soils and in fenugreek plants and respective bioaccumulation factors**

		Treatments	Soil (mg kg <sup>-1</sup> )	Plant (mg kg <sup>-1</sup> )	BAF
Arsenite (mg/L) (As <sup>+3</sup> )	1		0.238 ± 0.002	0.155 ± 0.002	0.651
	2		0.236 ± 0.001	0.243 ± 0.007	1.028
	3		0.259 ± 0.001	0.223 ± 0.001	0.857
Arsenate (mg/L) (As <sup>+5</sup> )	1		0.536 ± 0.004	0.159 ± 0.002	0.297
	2		0.159 ± 0.001	0.116 ± 0.003	0.724
	3		0.133 ± 0.004	0.209 ± 0.002	1.573

BAF: Bioaccumulation factor

## Results and Discussion

Table 1 represents the fresh and dry weight of the control and treatment plant samples. After the experimental period was over, it was observed that control plants showed higher weight than that of treatments. Among all the treatments, 3 mg/L treatments in both As<sup>+3</sup> and As<sup>+5</sup> showed the lowest wet weight ( $4.899 \pm 0.700$  g; and  $4.558 \pm 0.088$  g) as well as dry weight ( $0.612 \pm 0.030$  g; and  $0.497 \pm 0.039$  g), respectively. Chlorophyll and carotenoid contents were estimated in both the control and treatment groups. It was observed from table 2 that chlorophyll and carotenoid contents were decreased irrespective of treatments than that of the control group. However, results found that with the increasing concentrations of arsenic the chlorophyll and carotenoid contents were decreased in both the treatment groups. Results revealed that in 3 mg/L treatment of As<sup>+3</sup>, the chlorophyll a, chlorophyll b, total chlorophyll, and carotenoid were found  $0.413 \pm 0.093$  mg/g,  $0.163 \pm 0.028$  mg/g,  $0.609 \pm 0.124$  mg/g, and  $0.109 \pm 0.030$  mg/g, respectively. This decrease in concentration indicates that the chlorophyll synthesis system and chlorophyllase activities might be affected by the exposure to a high level of toxic metal concentrations.<sup>5</sup> The same trends have been observed in 3 mg/L of As<sup>+5</sup> treatment ( $0.532 \pm 0.064$ ,  $0.2 \pm 0.038$ ,  $0.768 \pm 0.109$ , and  $0.145 \pm 0.035$  in case of chlorophyll a, chlorophyll b, total chlorophyll and carotenoid, respectively. Although there was no previous report on the effect of arsenic concentration on the chlorophyll content in the fenugreek plant, however, Miteva and Merakchyska (2002) reported that arsenic may put an effect on the photosynthetic process in bean plants.<sup>19</sup> The uptake potential of arsenic by a crop depends on the concentration of arsenic in water, soil and it also depends on soil and crop's physiological properties.<sup>3</sup> The concentration of soil arsenic and in the plant shoot portion in each treatment were represented in Table 3. Probably these higher arsenic contents might have resulted from the direct absorption of arsenic from the soil environment. Similar results have been observed by previous researchers, who reported that the concentrations of arsenic in the plant's body might increase more or less linearly with the total concentration of arsenic available in the soil.<sup>2,1,20</sup> Moreover, it was also reported by previous researchers that the high concentration of arsenic in the soil might result in a high concentration of arsenic in the plant body.<sup>4</sup>

On the other hand, soil arsenic concentration was also estimated to find out the ability of arsenic accumulation by fenugreek plants. However, bioaccumulation factor (BAF) analysis results showed that an independent relationship with soil arsenic concentration (Table 3). The highest bioaccumulation factor was found in 2 mg/L As<sup>+3</sup> treatments (1.028) and 3 mg/L As<sup>+5</sup> treatments (1.573). The study results showed that the increased concentration of soil Arsenic may not certainly represent higher arsenic accumulation by the fenugreek plants. This result may differ in different plants due to the difference in physiological characteristics. Moreover, our present study results were very similar to the study reported by Mandal and Suzuki (2002).<sup>21</sup> On the other hand, Das *et al.*, (2004) was also observed that the concentration of arsenic in potato, vegetable leaves, rice, spinach, the gourd was in the range between 0.02 to 3.99 mg kg<sup>-1</sup> while estimated from an arsenic-contaminated field.<sup>22</sup> Pendergrass and Butcher (2006) were also have found the bioaccumulation of arsenic in the range of 1.5 to 178 mg kg<sup>-1</sup> in plants.<sup>23</sup> Correlation study between soil and plant arsenic concentration with pigment contents were represented in Table 4. The study result showed that soil and plant arsenic concentration had a negative correlation with all estimated pigment contents i.e., chlorophyll a: -0.699; chlorophyll b: -0.474; total chlorophyll: -0.638; carotenoid: -0.783 and chlorophyll a: -0.445; chlorophyll b: -0.081; total chlorophyll: -0.277; carotenoid: -0.124. It was clear from the study results that pigments contents were affected by the arsenic concentrations which invariably means that fenugreek is not an arsenic resistant species but it can be used as an indicator plant variety in arsenic contaminated areas. It is important to measure the level of toxicity by quantifying the arsenic exposure pathways to determine the human health risk. To estimate the potential human health risk associated with fenugreek consumption, chronic daily intake (CDI) was estimated. Table 5 summarized the CDI and HQ for adults and children upon consumption. The results revealed that the CDI values for adults and children were relatively high in both arsenic (As<sup>+3</sup> and As<sup>+5</sup>) concentrations. However, all CDI values are < 1. HQ is used often to evaluate a possible non-cancerous health risk from toxic pollutants. It was observed that most of the HQ values for children were high than the adults irrespective of treatments. It might be due to higher



intake rates and lower body weights for the children. Some previous community-level studies reported that children and teenagers might pose a high risk due to the consumption of arsenic-contaminated vegetables.<sup>24</sup> The hazard index (HI) for adults and child considering exposure to all As<sup>+3</sup> treatments were estimated as 2.85E-01 and 4.16E-01,

respectively. The same for As<sup>+5</sup> were 2.23E-01, and 3.25E-01 for adult and child, respectively. Individual HQs and cumulative HI for both adults and children were found to be less than unity in the observed study. Hence, exposure to As<sup>+3</sup> and As<sup>+5</sup> due to consumption of fenugreek should not cause any non-cancer health hazard in either adults or children.

**Table 4: Correlation between plant Chlorophyll and Carotenoid contents, Plant and Soil arsenic concentration**

	Chl a	Chl b	Total Chl	Carotenoid	Soil As
Chl b	0.749				
Total Chl	0.926**	0.940**			
Carotenoid	0.906*	0.894*	.963**		
Soil As	-0.699	-0.474	-0.638	-0.783	
Plant As	-0.445	-0.081	-0.277	-0.124	-0.116

\*\* Significant at the 0.01 level (2-tailed)

\* Significant at the 0.05 level (2-tailed)

**Table 5: Calculated CDI and HQ values from the concentration estimated from fenugreek in different treatments**

	Concentration/ Treatments	CDI (adult)	CDI (children)	HQ (adult)	HQ (children)	
Arsenite (mg/L) (As <sup>+3</sup> )	1	2.26E-02	3.30E-02	7.13E-02	1.04E-01	
	2	3.54E-02	5.16E-02	1.12E-01	1.63E-01	
	3	3.25E-02	4.73E-02	1.02E-01	1.49E-01	
HI				2.85E-01	4.16E-01	
	Arsenate (mg/L) (As <sup>+5</sup> )	1	2.32E-02	3.39E-02	7.32E-02	1.07E-01
		2	1.69E-02	2.46E-02	5.32E-02	7.76E-02
3		3.06E-02	4.46E-02	9.64E-02	1.41E-01	
HI				2.23E-01	3.25E-01	

**Table 6: Calculated EDD and ILTCR values from the concentration estimated from fenugreek in different treatments**

	Concentration/ Treatments	EDD (adult)	EDD children)	ILTCR (adult)	ILTCR (children)
Arsenite (mg/L) (As <sup>+3</sup> )	1	2.26E-02	3.30E-02	7.13E-02	1.04E-01
	2	3.54E-02	5.16E-02	1.12E-01	1.63E-01
	3	3.25E-02	4.73E-02	1.02E-01	1.49E-01
Arsenate (mg/L) (As <sup>+5</sup> )	1	2.32E-02	3.39E-02	7.32E-02	1.07E-01
	2	1.69E-02	2.46E-02	5.32E-02	7.76E-02
	3	3.06E-02	4.46E-02	9.64E-02	1.41E-01

EDD and ILTCR were estimated to determine the cancer risks associated with arsenic-contaminated fenugreek shoot ingestion by the common people (Table 6). The EDD values were found greater than the acceptable limits for daily exposure of total arsenic. The study results revealed that the potential cancer risk was found from the estimation of ILTCR for both adults and children as the values found to be > 10<sup>-4</sup>.

### Conclusion

Findings from this research will add some new information on arsenic toxicity assessment in an edible vegetable plant. The present study showed that the pigment content decreased in fenugreek with the increasing concentration of both As<sup>+3</sup> and As<sup>+5</sup> treatments. This study also concluded that a high concentration of soil arsenic content does not necessarily mean a higher accumulation rate in plant body parts and it may depend on the plant physiological properties. Results also indicated that fenugreek grown in arsenic-contaminated areas may

pose a potential health risk upon consumption as a vegetable by adults and children. This experimental study results concluded that fenugreek may be used as an indicator plant species in arsenic-contaminated areas which will be helpful for easy health risk assessment.

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### Conflict of Interest

The authors do not have any conflict of interest.

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