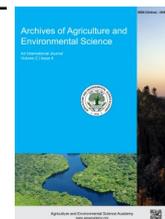




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ORIGINAL RESEARCH ARTICLE

Heavy metals and major nutrients accumulation pattern in spinach grown in farm and industrial contaminated soils and health risk assessment

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ABSTRACT

A pot experiment was conducted to study heavy metals and major nutrients accumulation pattern and to assess possible health risk for adult male and female human through consumption of spinach grown in farm and industrial contaminated soils. The concentrations of Fe, Mn, Cu, Zn, Cr and Pb in aqueous extracts of leaves and roots were determined by an atomic absorption spectrophotometer (AAS). The present study revealed that spinach grown in both soils accumulated higher amount of Cr, which could pose potential health concern to the local residents. On the contrary, it could be a good source of S, Ca and Mg for adult male and female human. Accumulation of heavy metals and major nutrients in leaves of spinach was in the sequence of Fe > Zn > Cr > Mn > Cu > Pb and K > S > Ca > Mg > P, respectively for industrial contaminated soil, while the order was Fe > Mn > Cr > Zn > Cu > Pb and S > K > Ca > Mg ≥ P, respectively for farm soil. The sequence of Zn, Mn, Ca, K and S accumulation in spinach was leaf > root. But in case of Fe, Cr and P the order of accumulation pattern was reverse. Among the metals, the calculated THQ value for Cr surpassed 1, and the values for male were 2.85 and 6.86 and for female were 4.47 and 10.75 due to consumption of spinach grown in farm and industrial contaminated soils, respectively. The study results inferred that Cr health risk through consumption of spinach is unsafe in industrial contaminated sites; and in both places female is more vulnerable than male.

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INTRODUCTION

Bangladesh is now on the way to be a middle income country and the number of industries increases rapidly over the last two decades. Industries are mainly found in urban and suburban areas of the country, and in some cases those are located near the agricultural fields. Most of the industries discharge industrial wastes without any treatment, which can easily disperse to agricultural lands and migrate to distant places through flooding and surface run off during monsoon. Those wastes containing heavy metals are great threat to surrounding environment, especially to soils, sediments and waters (Zakir *et al.*, 2017a;

2017b; Hossain *et al.*, 2017; Al Zabir *et al.*, 2016; Zakir *et al.*, 2016; Zakir and Hossain, 2016; Hossain *et al.*, 2015; Zakir *et al.*, 2015). Farmers of those places unconsciously grow cereals and different types of vegetables in such contaminated lands. Furthermore, they are irrigating the crops using untreated waste water/effluents. As a result, heavy metals are uptaken by the crops and vegetables, and finally accumulate into human body through consumption, which are associated with human health risk (Aysha *et al.*, 2017; Haque *et al.*, 2018). As a result, accumulation of heavy metals in human body through consumption of cereals and vegetables created growing concern nowadays. A number of health problems such as kidney trouble,

anaemia and blood disorders, stomach irritation, vomiting etc. can develop due to excessive dietary intake of heavy metals (Ahmed et al., 2012).

Vegetables are an important part of our daily diet and on an average 130 g vegetables are consumed by an adult per day in Bangladesh (Islam et al., 2005). Spinach (*Spinacia oleracea*) is an annual plant and may survive over winter in temperate regions. It is one among the most popular vegetables in winter in Bangladesh and leaves are the edible part of it. Spinach contains shallow root system and nutrients uptake by it varies with soil and climatic conditions. Transportation and accumulation of heavy metals in plants also depends on types of soil, soil pH, soil organic matter content, presence of other chemical and type of plant species. Two major impacts caused by heavy metal accumulation, one is its entrance into human diet and another is declining crop production due to inhibition of metabolic processes (Singh and Agrawal, 2008). Heavy metal contamination of the food items is one of the most important assessment parameters of food quality assurance (Wang et al., 2005; Khan et al., 2008). As a result, international and national regulations on food quality have lowered the maximum permissible levels of toxic metals in food items due to an increased awareness of the risk (Radwan and Salama, 2006). Considering the fact, the present study was planned to check the uptake patterns of different heavy metals along with major nutrients and to assess heavy metal health risk for human through consumption of spinach grown in both farm and industrial contaminated soils of Bangladesh.

MATERIALS AND METHODS

Experimental site

The pot experiment was carried out at the Net House, Department of Agricultural Chemistry, Faculty of Agriculture, Bangladesh Agricultural University (BAU), Mymensingh-2202, Bangladesh during the period from October 2015 to November 2016.

Collection of soils for experiment

Farm soil and industrial contaminated soil were used for the pot experiment. Among those, farm soil was collected from the field of Genetics and Plant Breeding Farm of BAU, Mymensingh-2202, Bangladesh. On the other hand, industrial contaminated soil was collected from the site near to Noman Composite Textile Ltd., Habirbari, Bhaluka of Mymensingh. Requisite amount of both the soils were brought to the Department of Agricultural Chemistry, BAU, Mymensingh and processed for pot experiment. After collection, both soil samples were analyzed by Haque et al. (2018) for available fraction of heavy metals (Fe, Mn, Cu, Zn, Pb and Cr) following standard method and analytical results are presented in Table 1.

Pot preparation for the experiment

The pots were prepared 15 days prior to sowing of the seeds of spinach. The collected soil was air dried first and then sun dried. Then both the soils were ground and subsequently sieved by using a 2 mm stainless steel sieve. All kinds of weeds, stubbles

and residues of crops and weeds were removed from the soil. After then 10 kg powered soil was poured in each plastic bucket and kept undisturbed upto sowing of the seeds of spinach. The experiment was laid out followed by completely randomized design (CRD) with four replications.

Test crops and intercultural operations

The experiment was conducted with the seeds of spinach (*Spinacia oleracea*) var. *Copipalong*, produced by Bangladesh Agricultural Development Corporation (BADC). Seeds were sealed in original packet which was procured from Mymensingh town. Before sowing, seeds were soaked in distilled water for 24 hours and then wrapped with a piece of blotting paper for 12 hours. Fertilizers applied in the pots as recommended for high yield goal and medium soil fertility status as described in Fertilizer Recommendation Guide (FRG, 2012). The recommended doses of nitrogen, phosphorus and potassium were 26, 8 and 8 kg ha⁻¹, and those were applied from urea, triple superphosphate (TSP) and muriate of potash (MoP) fertilizer, respectively. Intercultural operations viz. weeding, irrigation, disease and pest management were done using traditional methods as and when necessary.

Harvesting and processing of samples

Spinach was harvested on January 03, 2016. The plant samples were tagged and taken to the laboratory where the samples first air dried for 2-3 days followed by oven drying for 72 hours until a constant weight was noticed. After then, dried samples were ground and stored at room temperature for chemical analyses. Spinach root samples were also collected after harvesting of spinach, and processed on the same way as mentioned above.

Chemical analysis of plant samples

Powdered samples of spinach leaves and roots were used to prepare aqueous extract by wet oxidation method using di-acid mixture as described by Singh et al. (1999). The concentrations of different heavy metals (Cu, Zn, Pb, Cr, Fe and Mn) in aqueous extracts were measured by atomic absorption spectrophotometer (AAS) (AA-7000, Shimadzu, Japan). Mono element hollow cathode lamp was employed for the determination of each heavy metal of interest. At first the AAS was calibrated followed by the manufacturer's recommendation. Then the aqueous extract was diluted and/or run directly in AAS for the determination of metal in the sample.

Estimation of daily metal intakes (DMI)

To assess the health risk associated with heavy metal contamination in edible parts of spinach, the daily intake of different metal was calculated using the following formula:

$$DMI = (VIR \times C) / BW$$

Where, VIR is the vegetable ingestion rate (mg person⁻¹ day⁻¹), C is the individual metal concentration in edible parts of spinach samples (mg kg⁻¹, fresh weight), BW is the body weight assuming

70 kg for adult male and 50 kg for adult female in the present study (BBS, 2015).

Target hazard quotients (THQ)

THQ is calculated by the general formula established by the US EPA as follows:

$$\text{THQ} = (E_F \times F_D \times \text{DMI}) / (\text{RfD} \times W \times T)$$

Where, E_F is exposure frequency; F_D is the exposure duration; DMI is the daily metal ingestion ($\text{mg person}^{-1}\text{day}^{-1}$) and RfD is the oral reference dose ($\text{mg kg}^{-1}\text{day}^{-1}$); W is the average body weight (kg) and T is the average exposure time for noncarcinogens ($365\text{ days year}^{-1} \times \text{number of exposure years}$).

RESULTS AND DISCUSSION

Concentration of heavy metals in leaves and roots of spinach

The mean Cu content in spinach leaves was $36.85 \pm 1.10\ \mu\text{g g}^{-1}$ in the sample grown in farm soil while it was $35.87 \pm 1.09\ \mu\text{g g}^{-1}$ for industrial contaminated soil (Figure 1). On the other hand, the average concentration of Cu in spinach roots was $34.76 \pm 0.93\ \mu\text{g g}^{-1}$ in the sample grown in farm soil and $36.01 \pm 1.73\ \mu\text{g g}^{-1}$ for industrial contaminated soil (Figure 2). Copper content obtained by this study was more than twice as reported by Alam et al. (2003), who stated that leafy vegetables collected from Samta village of Jessore, Bangladesh contained $15.50\ \mu\text{g g}^{-1}\text{Cu}$. According to ATSDR (1998), vegetables and fruits that contain higher amount of chromium are tomato, spinach and broccoli, and a half cup of these vegetables contained $11.00\ \mu\text{g g}^{-1}\text{Cr}$. The average concentration of Cr in edible part i.e. leaves of spinach was $154.58 \pm 7.81\ \mu\text{g g}^{-1}$ for farm soil while it was $371.52 \pm 1.97\ \mu\text{g g}^{-1}$ for industrial contaminated soils (Figure 1). The mean concentrations of Cr in spinach roots were 175.32 ± 4.88 and $380.91 \pm 3.62\ \mu\text{g g}^{-1}$ for farm and industrial contaminated soils, respectively (Figure 2). According to Kabata-Pendias and Pendias (1992), both the Cu and Cr contents in leaves and roots of spinach grown in both farm soil and industrial contaminated soil exceeded the critical limit. This is might be due to presence of higher amount of available Cr in both farm and industrial contaminated soil (57.90 and $79.43\ \mu\text{g g}^{-1}$, respectively) (Table 1).

Concentration of Zn in both leaves and roots of spinach grown in industrial contaminated soil were found excessively higher than spinach grown in farm soil, which might be due to presence of >5 times of higher amount of available Zn ($66.34\ \mu\text{g g}^{-1}$) in industrial contaminated soil than farm soil ($13.23\ \mu\text{g g}^{-1}$) used in this study (Table 1). The mean Zn content in spinach leaves was $97.35 \pm 10.63\ \mu\text{g g}^{-1}$ in the sample grown in farm soil while it was $381.11 \pm 11.64\ \mu\text{g g}^{-1}$ for industrial contaminated soil (Figure 1). On the other hand, the average concentration of Zn in spinach roots was $33.82 \pm 5.17\ \mu\text{g g}^{-1}$ in the sample grown in farm soil and $272.79 \pm 22.38\ \mu\text{g g}^{-1}$ for industrial contaminated soil (Figure 2). Zn content in spinach leaves and roots grown in both farm and industrial contaminated soils was several times higher as reported by Sanyaolu et al. (2011). But Pb was found in trace

amount in both spinach leaves and roots samples. This might be due to presence of trace amount of available Pb content in both the soils used in the study (Table 1). Furthermore, it was reported by MacFarlane and Burchett (2002), that the accumulation of Zn reduced the accumulation of Pb in leaves and vice versa. The uptake pattern of Zn and Pb in Zn/Pb amended soil showed that both Zn and Pb affect the uptake of each other in an antagonistic way (Boedeker et al., 1993).

Iron is a major constituent of the cell redox systems. But after a certain limit Fe is regarded as toxic element for the plants. The safe limit of Fe in plants is $140\ \mu\text{g g}^{-1}$ (Misra and Mani, 1991). But all the spinach leaves and roots grown in both farm and industrial contaminated soils crossed that safe limit. Spinach root contained much higher Fe than the leaves. The mean Fe content in spinach leaves was $769.88 \pm 44.17\ \mu\text{g g}^{-1}$ in the sample grown in farm soil while it was $405.70 \pm 12.62\ \mu\text{g g}^{-1}$ for industrial contaminated soil (Figure 1). On the other hand, the average concentration of Fe in spinach roots was $1086.59 \pm 38.28\ \mu\text{g g}^{-1}$ in the sample grown in farm soil and $1518.63 \pm 34.60\ \mu\text{g g}^{-1}$ for industrial contaminated soil (Figure 2). On the contrary, the concentration of Mn in all spinach samples (both leaf and root) was found within the critical limit/ normal concentration of Mn ($20\text{-}300\ \mu\text{g g}^{-1}$) for plant as described by Kabata-Pendias and Pendias, (1992). The average concentration of Mn in spinach leaves was $217.20 \pm 9.71\ \mu\text{g g}^{-1}$ in the sample grown in farm soil and $247.82 \pm 7.92\ \mu\text{g g}^{-1}$ for industrial contaminated soil (Figure 1). But the mean concentration of Mn in spinach roots was $155.99 \pm 2.11\ \mu\text{g g}^{-1}$ in the sample grown in farm soil, while it was $238.08 \pm 6.43\ \mu\text{g g}^{-1}$ for industrial contaminated soil (Figure 1). It is evident from Figures 1 and 2 that average Mn concentration in both leaf and root samples was higher in spinach grown in industrial contaminated soil, because available Mn concentration in industrial contaminated soil ($25.50\ \mu\text{g g}^{-1}$) was more than twice than farm soil ($10.96\ \mu\text{g g}^{-1}$) (Table 1).

Concentration of major nutrients in leaves and roots of spinach

Among the essential macro nutrient elements, concentrations of Ca, Mg, P, K and S were measured by this study as dry weight basis. The mean Ca content in spinach leaves was $1.62 \pm 0.26\%$ in the samples grown in farm soil while it was $1.23 \pm 0.32\%$ for industrial contaminated soil (Figure 3). On the other hand, the average concentration of Ca in spinach roots was $1.04 \pm 0.32\%$ in the samples grown in farm soil and $0.91 \pm 0.09\%$ for industrial contaminated soil (Figure 4). It is evident from this study that edible part of spinach is a good source of Ca, although the recommended dietary calcium intakes for healthy men and women ranged between 800 and $1300\ \text{mg day}^{-1}$ (EFSA, 2006). The contents of Ca in plants differ widely depending on the plant species as well as plant parts and the range of Ca contents in plants varied from $0.2\text{-}1.0\%$ (Havlin et al., 2010). The average concentration of Mg in spinach leaves was $0.53 \pm 0.10\%$ in the samples grown in farm soil while it was $0.43 \pm 0.08\%$ for industrial contaminated soil (Figure 3). On the other hand, the mean concentration of Mg in spinach roots was $0.58 \pm 0.04\%$ in the

samples grown in farm soil and $0.42 \pm 0.07\%$ for industrial contaminated soil (Figure 4). According to EFSA (2006), the adult healthy body contains approximately 21-28 g (about 1 mole) of Mg; related to an average body weight of 70 kg or to 0.034% of body weight. It is the fourth most abundant cation in the mammalian body and the second most abundant cation in intracellular fluid. So, it can be inferred from the present study that spinach is also a good source of Mg for adult male and female. On the other hand, Havlin et al. (2010) stated that the concentration of Mg is higher in the di-cotyledons compared to monocotyledons and the range of Mg content in plants varied from 0.1-0.4%.

Phosphorus as phosphate is an essential nutrient involved in many physiological processes, such as the cell's energy cycle, regulation of the whole body acid-base balance, as a component of the cell structure (as phospholipids), in cell regulation and signaling, and in the mineralization of bones and teeth (EFSA, 2006). Phosphorus content was also found higher in both leaves and roots of spinach grown in farm soil than industrial contaminated soil. The mean P content in spinach leaves was $0.50 \pm 0.05\%$ in the samples grown in farm soil while it was $0.24 \pm 0.03\%$ for industrial contaminated soil (Figure 3). On the other hand, the average concentration of P in spinach roots was $0.43 \pm 0.01\%$ in the samples grown in farm soil and $0.41 \pm 0.04\%$ for industrial contaminated soil (Figure 4). According to EFSA (2006), normal healthy individuals can tolerate phosphorus (as phosphate) intakes up to at least 3000 mg day^{-1} without any adverse systemic effects. In some individuals, however, mild gastrointestinal symptoms have been reported if exposed to supplemental intakes $>750 \text{ mg P day}^{-1}$. Spinach leaves and roots grown in industrial contaminated soil contained higher amount of K than farm soil. The mean concentration of K in spinach leaves was $1.91 \pm 0.05\%$ grown in farm soil, while it was $2.66 \pm 0.15\%$ in industrial contaminated soil (Figure 3). On the other hand, the average concentration of K in spinach roots was $1.41 \pm 0.12\%$ in the samples grown in farm soil and $1.52 \pm 0.08\%$ for industrial contaminated soil (Figure 4). Potassium content is higher in shoot than in grain or seed and the typical concentration of K in shoot and seed ranged from 0.4-4.0% (Havlin et al., 2010), which is at par with this study result. Potassium is an essential nutrient involved in fluid, acid and electrolyte balance and is required for normal cellular function. Dietary deficiency of potassium is very uncommon due to the widespread occurrence of potassium in foods. The available data are insufficient to establish a safe upper intake level for potassium (EFSA, 2006). Edible part of spinach is a huge source of S and due to this it is known as thiol rich vegetable. The mean S content in spinach leaves was $2.56 \pm 0.16\%$ in the samples grown in farm soil while it was almost same $2.57 \pm 0.15\%$ for industrial contaminated soil (Figure 3). On the other hand, the average concentration of S in spinach roots was $2.27 \pm 0.17\%$ in the samples grown in farm soil and $1.94 \pm 0.11\%$ for industrial contaminated soil (Figure 4). The present study results obtained almost 4 times higher amount of S in spinach leaves as compared to the results published by Smatanova et al. (2004), and they reported that spinach leaves contained 0.20-0.58% S on dry matter basis.

Accumulation pattern of heavy metals in leaves and roots of spinach

Accumulation of heavy metals in the edible part (leaf) of spinach was in the sequence of $\text{Fe} > \text{Zn} > \text{Cr} > \text{Mn} > \text{Cu} > \text{Pb}$ for industrial contaminated soil, while the order was $\text{Fe} > \text{Mn} > \text{Cr} > \text{Zn} > \text{Cu} > \text{Pb}$ for farm soil (Figure 1). On the other hand, spinach roots uptake different heavy metal in the sequence of $\text{Fe} > \text{Cr} > \text{Zn} > \text{Mn} > \text{Cu} > \text{Pb}$ for industrial contaminated soil and the order for farm soil was $\text{Fe} > \text{Cr} > \text{Mn} > \text{Cu} \geq \text{Zn} > \text{Pb}$ (Figure 2). It is evident from both the Figures 1 and 2 that spinach leaf and root accumulate very little amount of Pb than other heavy metals, which might be due to absence of available Pb content in both the soils used for this study. On other hand, available concentrations of Zn in both the farm and industrial contaminated soils were 13.23 and $66.34 \mu\text{g g}^{-1}$, respectively (Table 1). There is a report that characteristically Pb and Zn interact with each other negatively (MacFarlane and Burchett, 2002). So it can be inferred that both of Pb and Zn antagonistically affected the accumulation rate of each other in both leaf and root of spinach. According to Boedeker et al. (1993) the uptake pattern of Zn and Pb in Zn/Pb amended soil showed that both Zn and Pb affect the uptake of each other in an antagonistic way. The sequence of Zn and Mn accumulation in spinach was leaf > root. But in case of Fe and Cr the order of accumulation pattern was reverse i.e. root > leaf. On the other hand, Cu accumulation was almost same in both leaf and root of spinach (Figures 1-2). The accumulation pattern of different heavy metals revealed by the present study was almost at par with the results observed by Haque et al. (2018) and Ngayila et al. (2009) for growth of *Solanum lycopersicum* L. and *Brassica juncea*, respectively.

Accumulation pattern of major nutrients in leaves and roots of spinach

Spinach leaf accumulate major nutrients in the sequence of $\text{S} > \text{K} > \text{Ca} > \text{Mg} \geq \text{P}$ for farm soil, while the order was $\text{K} > \text{S} > \text{Ca} > \text{Mg} > \text{P}$ for industrial contaminated soil (Figure 3). On the other hand, spinach roots uptake major nutrient elements in the sequence of $\text{S} > \text{K} > \text{Ca} > \text{Mg} \geq \text{P}$ for industrial contaminated soil and the order for farm soil was $\text{S} > \text{K} > \text{Ca} > \text{Mg} > \text{P}$ (Figure 4). It is evident from the Figures 3 and 4 that both leaf and root of spinach accumulate a significant amount of sulphur. Furthermore, spinach is also categorized as one among the sulphur rich leafy vegetables, which is also reflected by the obtained study results. Except sulphur, the obtained sequence for other macro elements was almost similar as reported by Ghani et al. (2012) of few medicinal plants. But Azarmi et al. (2011) analyzed tomato seedling shoot and root for major elements and the observed order was $\text{Ca} > \text{Mg} > \text{P} > \text{K}$. However, the sequence of Ca, K and S accumulation pattern in spinach was leaf > root. But in case of P the order of accumulation pattern was reverse i.e. root > leaf. On the other hand, Mg accumulation was almost same in both leaf and root of spinach (Figures 3-4).

Estimation of daily metals intakes (DMI)

To appraise the health risk connected with heavy metal contam-

ination of spinach, the daily intake of metals was calculated. The food chain is the most important one among the different possible pathways of exposure of toxic heavy metals to humans. The daily intake of toxic metals was calculated on the basis of the average vegetable consumption rate for both adults male and female. A survey was conducted in March 2016 by using a prepared questionnaire of 30 family heads at industrial contaminated sites of Habirbari area of Bhaluka Upazila and 50 family heads at Sutiakhali area of Mymensingh Sadar Upazila. Thus a total of 80 families faced the interview and in total 270 persons were effectively interviewed from two study areas (Aysha et al., 2017). This survey data were used to calculate an average consumption rate of vegetable per person per day. The survey results revealed that 0.010 kg of spinach as typical serving for a day for male and 0.008 kg for female (Aysha et al., 2017). The daily metals intakes estimate of Fe, Mn, Zn, Cu, Cr and Pb from spinach were calculated by multiplying the daily intake (from survey results) by the metals concentrations determined in this study. The calculated DMI for Zn, Cr and Mn were higher for both male and female due to consumption of spinach grown in industrial contaminated soils, but DMI for Fe was higher due to consumption of spinach grown in farm soils (Table 2). The DMI were compared with the upper tolerable daily intakes for metals. It is also evident from Table 2 that daily metal intakes for Cr and Mn were several times higher than that of oral reference doses, but any heavy metal did not cross the tolerable upper intake level.

Target hazard quotients (THQ)

Target hazard quotients (THQ) are reported as a complex parameter used for the estimation of possible health risks connected with long term exposure to chemical pollutants (Khan et al., 2009; Petroczi and Naughton, 2009; Harmanescu et al., 2011). The THQ < 1 means the exposed population is assumed to be safe, $1 < \text{THQ} < 5$ means that the exposed population is in a level of concern interval and THQ > 5 means the exposed population is in health risk. THQ is an index without any dimension and generally its values are additive, but not multiplicative. It is worth mentioning that usually THQ is not a measure of risk but it indicates a level of concern. THQ was measured considering DMI of people, average body weight [for male: 70 kg and female: 50 kg as mentioned by Guyton and Hall (2006)] and average life expectancy of people in Bangladesh [for male: 70.6 and female: 73.1 as found in BBS (2015)]. THQ values for investigated heavy metals due to dietary intake of spinach are presented in Table 3. It is apparent from Table 3 that there was only one individual THQ value that surpassed 1, and the metal was Cr. The values for male were 2.852 and 6.856 and for female were 4.473 and 10.750 for farm and industrial contaminated soils, respectively. So it can be inferred that the exposed populations of the industrial contaminated sites are in health risk through the food chain via consumption of spinach and peoples of the farm sites are in a level of concern interval; and in both places female is more vulnerable than male. The computed combined target hazard quotients (CTHQ) values for industrial contaminated soils were also exceeded the safe limit (THQ > 5) for both male and female (7.106 and 11.142, respectively) (Table 3).

Table 1. Morphological characteristics and available heavy metal contents present in the soils used for the study (after Haque et al., 2018).

Name of soil	Agro-ecological zone (AEZ)	Land type	Soil colour	Concentration of available heavy metal ($\mu\text{g g}^{-1}$)					
				Cu	Zn	Pb	Cr	Fe	Mn
Industrial contaminated soils	AEZ-9 (Old Brahmaputra Floodplain)	Medium high land	Light brown	9.05	66.34	Trace	79.43	14.99	25.50
Farm soils	AEZ-9 (Old Brahmaputra Floodplain)	Medium high land	Dark grey	8.87	13.23	Trace	57.90	16.56	10.96

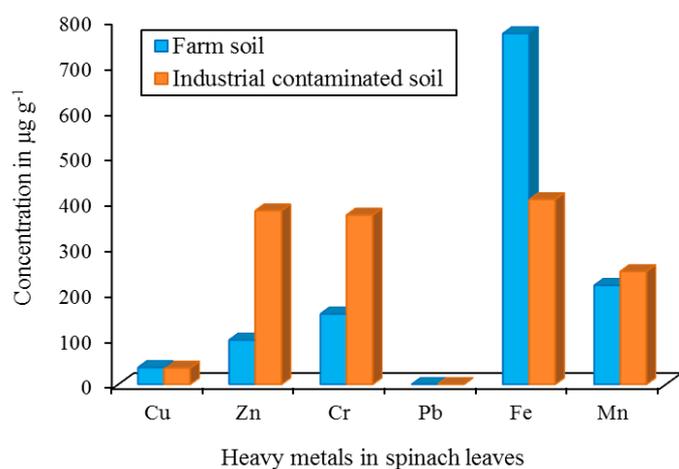
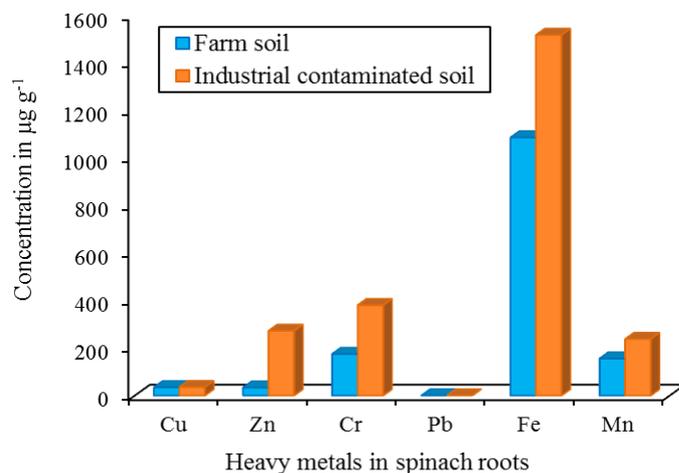
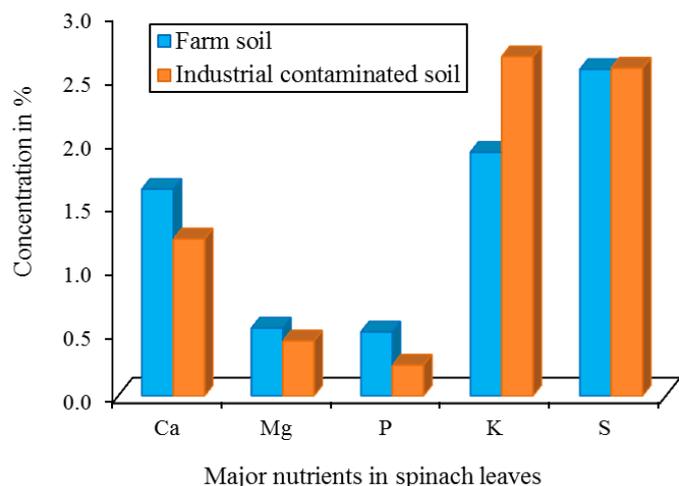
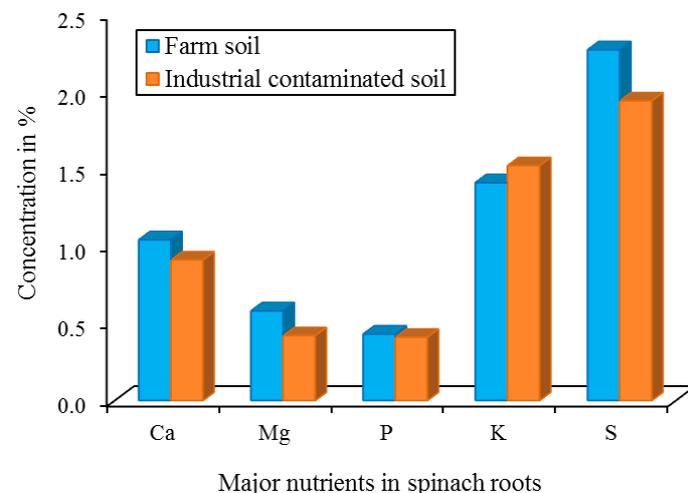
Table 2. Daily intakes of heavy metals (DMI) from spinach for both male and female at farm and industrial contaminated soils of the study areas.

Heavy metals	DMI from tomato grown in farm soils ($\text{mg day}^{-1} \text{person}^{-1}$)		DMI from tomato grown in industrial contaminated soils ($\text{mg day}^{-1} \text{person}^{-1}$)		Oral reference doses (RfD) ($\text{mg kg}^{-1} \text{day}^{-1}$)	Tolerable upper intake level (UL) ($\text{mg day}^{-1} \text{person}^{-1}$)
	Male	Female	Male	Female		
Cu	0.579	0.649	0.564	0.631	0.040 ^a	10.00 ^d
Zn	1.530	1.714	5.989	6.708	0.300 ^a	40.00 ^d
Cr	2.429	2.721	5.839	6.539	0.003 ^b	NE ^d
Pb	0	0	0	0	0.004 ^c	0.24 ^e
Fe	12.099	13.551	6.376	7.141	0.700 ^a	45.00 ^d
Mn	3.413	3.823	3.895	4.362	0.014 ^a	11.00 ^d

NE= Not established; ^a = US EPA (2010); ^b = IRIS (1987); ^c = Khan et al. (2008); ^d = FDA (2001) and ^e = Garcia-Rico et al. (2007).

Table 3. Target hazard quotients (THQ) and combined target hazard quotient (CTHQ) of heavy metals for both male and female due to consumption of spinach.

		Target Hazard Quotients (THQ)						CTHQ
		Cu	Zn	Cr	Pb	Fe	Mn	
Industrial contaminated soils of the study area	Male	0.050	0.070	6.856	0	0.032	0.098	7.106
	Female	0.078	0.110	10.750	0	0.050	0.154	11.142
Farm soils of the study area	Male	0.051	0.018	2.852	0	0.061	0.086	3.068
	Female	0.080	0.028	4.473	0	0.095	0.135	4.811

**Figure 1.** Heavy metals concentration ($\mu\text{g g}^{-1}$) in spinach leaves grown in both industrial contaminated and normal farm soils.**Figure 2.** Heavy metals concentration ($\mu\text{g g}^{-1}$) in spinach roots grown in both industrial contaminated and normal farm soils.**Figure 3.** Major nutrients concentration (%) in spinach leaves grown in both industrial contaminated and normal farm soils.**Figure 4.** Major nutrients concentration (%) in spinach roots grown in both industrial contaminated and normal farm soils.

Conclusion

Accumulation of heavy metals and major nutrients in leaves of spinach was in the sequence of $\text{Fe} > \text{Zn} > \text{Cr} > \text{Mn} > \text{Cu} > \text{Pb}$ and $\text{K} > \text{S} > \text{Ca} > \text{Mg} > \text{P}$, respectively for industrial contaminated soil, while the order was $\text{Fe} > \text{Mn} > \text{Cr} > \text{Zn} > \text{Cu} > \text{Pb}$ and $\text{S} > \text{K} > \text{Ca} > \text{Mg} \geq \text{P}$, respectively for farm soil. The sequence of Zn, Mn, Ca, K and S accumulation in spinach was leaf > root. But in case of Fe, Cr and P the order of accumulation pattern was reverse i.e. root > leaf. On the other hand, Cu and Mg accumulation was almost same in both leaf and root of spinach. The present study revealed that spinach grown in both soils accumulated higher amount of Cr, which could pose a potential health concern to the local residents. On the contrary, spinach grown in both soils

could be a good source of S, Ca and Mg for adult male and female human. Among the heavy metals studied, only the concentration of Cr in edible part of spinach had individual THQ value that surpassed 1 (2.852 and 6.856 for male, and 4.473 and 10.750 for female in farm and industrial contaminated soils, respectively). Thus the study results inferred that the exposed populations of the industrial contaminated sites are in health risk through the food chain via consumption of spinach and people of the farm sites are in a level of concern interval; and in both places female is more vulnerable than male. In conclusion, further investigation is recommended by incorporating the contribution of other vegetables, cereals, processed food items and water that may represent further contamination sources to the population subjected.

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