J Bangladesh Agril Univ 19(2): 168–177, 2021 https://doi.org/10.5455/JBAU.55279

ISSN 1810-3030 (Print) 2408-8684 (Online)

Journal of Bangladesh Agricultural University

Journal home page: http://baures.bau.edu.bd/jbau

Mineral Nutritional Status and Health Risk Assessment of Red Amaranth (*Amaranthus cruentus*) Collected from a Water-Logged Area of Bangladesh

Popy Saha[1], Md. Arifur Rahman[2], Md. Saljar Rahman Chowdhury[1], Kamrun Nahar[1], Istiaq Ahmed[1,3], K. M. Mohiuddin[1]✉

[1]Department of Agricultural Chemistry, Bangladesh Agricultural University, Mymensingh 2202, Bangladesh
[2]Department of Agricultural Chemistry, Khulna Agricultural University, Khulna 9100, Bangladesh
[3]School of Biological Sciences, University of Aberdeen, Aberdeen, United Kingdom

ARTICLE INFO

Article history
Received: 12 Feb 2021
Accepted: 08 Apr 2021
Published: 30 Jun 2021

Keywords
Bhabadah,
DMI,
Heavy metal accumulation,
Mineral nutrient,
Red amaranth,
THQ

Correspondence
K.M. Mohiuddin
✉: mohiuddin@bau.edu.bd

Abstract

Red amaranth (*Amaranthus cruentus*) is an excellent source of essential nutrients, vitamins, and minerals that are crucial for human nutrition. The present study was carried out to assess the mineral and heavy metal concentration and human health risk through the intake of red amaranth collected from Bhabadah water-logging area of Khulna Division in Bangladesh. A total of 24 red amaranth samples were collected from 24 different locations. Average concentrations of phosphorus, potassium, sulphur, calcium, magnesium and sodium were 0.90 ± 0.46%, 0.24 ± 0.06%, 0.56 ± 0.14%, 2.22 ± 0.75%, 1.39 ± 0.51% and 0.11 ± 0.10%, respectively. Phosphorus and calcium concentration were the highest in Damukhali area. The concentration ranges of zinc, lead, cadmium and copper were 26.48 - 172.24, trace - 46.41, trace - 0.63 and 7.74 - 32.58 µg g^{-1}, respectively. Chromium was detected in red amaranth samples collected from only two locations. The most studied essential nutrients were at the optimum level in the collected samples for human nutrition. The survey data and the analytical data expressed that the daily metal intake (DMI) of both male and female were higher than that of upper permissible limit indicating that serious adverse effects that might be linked to the intake of red amaranth of the study area. Incremental lifetime cancer risk (ILCR) data showed Pb in an alarming amount to cause carcinogenesis. Target hazard quotients (THQ) of lead (Pb) was 6.550 for female (THQ> 5.00) indicating that the exposed population is unsafe and for male was 4.179 (1 < THQ < 5) indicating that the exposed population is at a level of concern interval. Food chain contamination by heavy metals through an elevated level of Pb in red amaranth of Bhabadah area is evident through this research and immediate action should be taken to ensure food safety.

Introduction

Water-logging in Bhabadah is a national problem which affects the south-western parts of Bangladesh. The total area of Bhabadah spreads over 478 square kilometers covering 200 villages in two Upazila under Khulna district and three Upazila under Jashore district (The Daily Star, 2017; Hossain *et al.,* 2020). In the early 1980s Bhabadah water-logging problem began and for these last three decades, the Bhabadah crisis is known to people due to the manufacturing of a sluice gate in Bangladesh resulted silt deposition on the river-beds of Mukteshwari, Teka, Sree and Hari as well as the bottoms of a number of canals and water bodies, locally known as *beels* (The Daily Star, 2017 & 2018; Hossain *et al.,* 2020). This region is primarily low-lying land and characterized by numerous morphological active tidal rivers and creeks provided drainage networks for the system of embanked hydrological units which allows entering the saline water (The Daily Star, 2018). In the winter season, farmers grow vegetables and other cereal crops using that stagnant water.

Vegetables are essential sources of nutrients such as minerals, carotene, protein, and vitamins necessary for human health, considering them as part of daily diets (Jimoh and Oladiji, 2005). They are also considered a cheap source of energy compared to other food alternatives. They are good source of natural antioxidants and help to neutralize acidic substances produced during digestion (Andrés-Bello *et al.,* 2013; Thomson and Kelly, 1990). Inorganic mineral substances in vegetables are necessary for maintaining different physicochemical processes and activities of the body

Cite This Article
Saha, P., Rahman, M.A., Chowdhury, M.S.R., Nahar, K., Ahmed, I., Mohiuddin, K.M. 2021. Mineral Nutritional Status and Health Risk Assessment of Red Amaranth (*Amaranthus cruentus*) Collected from a Water-Logged Area of Bangladesh. *Journal of Bangladesh Agricultural University*, 19(2): 168–177. https://doi.org/10.5455/JBAU.55279

(Soetan *et al.,* 2010; Eruvbetine, 2003; Hays and Swenson, 1985). Red amaranth (*Amaranthus tricolor* L.) is one of the most commonly cultivated vegetables and total production in 2017-2018 was 12,912 thousand metric tons on a land area of 4,83,194 hectares in Bangladesh (BBS, 2020). It is also well known as comparatively higher metal accumulator (Alexander *et al*., 2006; Yang *et al*., 2010); but contamination of vegetables with heavy metals such as Cd, Hg, Co, Ni, Pb etc. are a serious threat because of their toxicity, environmental persistence, bioaccumulation and biomagnifications in the food chain (Rajaei *et al.,* 2012; Ali *et al.,* 2019). Because of the non-biodegradable nature and long biological half-life of heavy metals, and their accumulation potentiality in different body parts, they become very harmful. Most of the heavy metals are ubiquitous, extremely toxic. However, their presence is in a minute concentration because their solubility in water having harmful effects to man and animals for the bioaccumulation and wastewater contains substantial amounts heavy metals that create problems (Chen *et al*., 2013). Irrigation of crops with waterlogged wastewater is a widespread practice in Bhabadah area.

Vegetables are contaminated with heavy metals through irrigation water, industrial emissions, the harvesting process, storage and/or at the point of sale and industrial emissions might be the primary pollution pathway in the developing countries (Jiang *et al*., 2006). Once the heavy metals are dispersed into water, soil, and air, they can be accumulated by crops (Hao *et al*., 2009; Hernández-Martínez and Navarro-Blasco, 2012). Luo *et al.* (2011) observed that high level of Cd (0.79 mg kg^{-1}) in broccoli and Pb (0.38 mg kg^{-1}) in lettuce growing in the contaminated soil from electronic waste processing site of China. Gupta *et al.* (2008) found 17.79 mg kg^{-1} Cd and 57. 63 mg kg^{-1} Pb in radish, which was irrigated by wastewater in Titagarh suburban area, India. High level of Cd is responsible for kidney stones, while excess Pb affects brain activity in children (Toplan *et al*., 2004). The high exposure of these metals certainly has negative effects to human health such as cancers and damage nervous system (Karalliedde and Brooke, 2012; Pan *et al*., 2013). The uptake of different heavy metals (Pb, Zn, Cd, Cr, Ni and Cu) by the vegetables grown in polluted water is of great importance as they might be hazardous to human health when consumed in excess of dietary intake. Therefore, the hypothesis is that the intake of vegetables containing heavy metals has potential health risk to consumers. Thus, the present study was conducted with an aim to assess the level of mineral nutrients and heavy metals in red amaranth from three Upazila of Bhabadah water-logging area of Khulna Division and their effects on human health due to intake of these vegetables.

Materials and Methods

Sampling

Total 24 red amaranth samples at the seedling growth stage were collected from 24 different locations of two Upazila of Jashore district and one Upazila of Khulna district of Bhabadah water-logging area Bangladesh. The information regarding red amaranth sampling has been reported in Table 1. Samples were put into an individual polythene bag with definite marking and tagging and then brought to the laboratory of Department of Agricultural Chemistry, Bangladesh Agricultural University, Mymensingh for chemical analysis.

Sample preparation and analyses

The collected amaranth samples were dried in an oven at 65°C and ground by a mechanical grinder after cooling. The prepared samples were then kept into plastic bottles until extract preparation. The plant extract was prepared by wet oxidation method using di-acid mixture (HNO_3: $HClO_4$= 2:1) following Singh *et al*. (1999).

Determination of mineral nutrient

Calcium and Mg concentrations in samples were analyzed by titrimetric method (Page *et al*., 1982; Gupta, 2013). The concentrations of K and Na in samples were determined by flame photometric method and the levels of PO_4 and SO_4 in samples were determined by spectrophotometric method (Page *et al*., 1982, Tandon, 1995; APHA, 2012; Gupta, 2013).

Determination of heavy metals

The concentrations of Cd, Pb, Cr, Zn and Cu ions in red amaranth samples were analyzed by atomic absorption spectrophotometer (AAS) (Model: SHIMADZU AA-7000) at the wavelengths of 228.8, 283.3, 357.9, 213.9 and 324.8 nm, respectively as described by APHA (2012). At first, AAS was calibrated followed by the manufacturer recommendation. Then the waste sample extract was diluted (if required) and/or run directly in AAS to determine heavy metal in the sample. A standard curve was prepared by plotting the absorbance reading on Y-axis versus the concentration of each standard solution of metal on X-axis. Then, the concentration of metal was calculated in the samples of interest by plotting AAS reading on the standard curve.

Table 1. Detailed information of sampling sites

Sample No.	Name of sampling areas	Locations	Sample No.	Name of sampling areas	Locations
1	Shorkhola	23°00'54.7"N 89°22'35.3"E	13	Balidha	22°57'46.6"N 89°20'26.9"E
2	Andha	23°00'30.7"N 89°22'28.4"E	14	Kalibari	24°42'57.4"N 90°10'11.2"E
3	Cholisia	23°00'34.0"N 89°22'18.5"E	15	Lokhaidanga	22°58'28.2"N 89°20'42.4"E
4	Baghdha	22°59'53.5"N 89°23'50.8"E	16	Mohonpur	24°32'47.1"N 88°38'54.1"E
5	Khota	22°59'38.1"N 89°21'41.3"E	17	Nehalpur	22°58'09.0"N 89°19'21.3"E
6	Dighalia	22°58'40.3"N 89°22'48.2"E	18	Sujatpur	22°59'40.1"N 89°21'27.4"E
7	Fakirhat	22°57'43.7"N 89°22'23.2"E	19	Kultia	22°59'47.8"N 89°18'51.8"E
8	Tekerhat	23°14'00.7"N 90°01'27.4"E	20	Hatgasha	23°00'15.4"N 89°21'02.2"E
9	Bhabadah	22°55'58.4"N 89°21'56.1"E	21	Bolarabad	23°00'33.0"N 89°22'01.6"E
10	Damukhali	22°56'31.5"N 89°22'36.9"E	22	Bedvita	22°59'45.5"N 89°21'47.5"E
11	Bhabadah	22°55'58.5"N 89°21'56.7"E	23	Dumurtola	23°11'18.5"N 89°30'06.9"E
12	Panchakari	22°57'17.5"N 89°21'20.4"E	24	Bhuikara	23°01'01.4"N 89°23'39.9"E

Estimation of Daily metal intake (DMI)

Daily metal intake (DMI) through consumption of red amaranth was calculated with the following formula:

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

Where, FIR is the amaranth ingestion rate (g person^{-1} day^{-1}), C is the metal concentration in amaranth 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. The average consumption rate of red amaranth person^{-1} day^{-1} was assessed as 10.0 and 8.0 g for male and female, respectively from a survey conducted in the study area on 60 respondents.

Estimation of Incremental Lifetime Cancer Risk (ILCR)

Incremental lifetime cancer risk (ILCR) is the expression of potential cancer risks associated with exposure to a measured dose of heavy metal or chemical contaminant. One in a million (1×10^{-6}) cancer risk means that one additional cancer case would be expected if a million people are exposed. Incremental lifetime cancer risk is obtained using the Cancer Slope Factor (CSF), which is the risk produced by a lifetime average dose of 1 mg kg^{-1} BWday^{-1} and is contaminant specific. Incremental lifetime cancer risk (ILCR) was calculated by using following equation (Liu *et al.,* 2013).

$$ILCR = CDI \times CSF$$

Here, CDI (chronic daily intake of chemical, mgkg^{-1} BWday^{-1}) represents the lifetime average daily dose of exposure to the respective heavy metal. The CDI value was calculated on the basis of the equation (Liu *et al.,* 2013).

$$CDI = DMI \times EF_r \times ED_{tot} / AT$$

Where, DMI is daily metal intake; EF_r is exposure frequency (365 days/year); ED_{tot} is the exposure duration 73 years, average lifetime for Bangladesh (Macrotrend, 2021); the AT is the period of exposure for non-carcinogenic effects (equal to $EF_r \times ED_{tot}$), and 73-year life time for carcinogenic effects (i.e., 73 years $\times$ 365 days/year) (Liu *et al.,* 2013).

Estimation of Target hazard quotients (THQ)

Target hazard quotients (THQ) was calculated by the general formula established by the USEPA (2010) as follows:

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

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

Statistical analysis

Statistical analyses of analytical results obtained from red amaranth samples were performed (Gomez and Gomez, 1984) by SPSS 17.0 and results were expressed as mean ± SD. The potential health risk for adult male and female was also studied by calculating the daily heavy metal intakes and target hazard quotients.

Results and Discussion

Mineral nutrient concentrations

Phosphorus

Maximum phosphorus concentration (2.10%) was at the Damukhali and minimum concentration (0.19%) was at the Bedvita with a mean value of 0.90% (Fig. 1 and Fig. 2). Out of 24 samples, 12 samples were above the mean value and the rest 12 samples were below the average value. The reference value of the concentration of P in green leafy vegetables is from 0.20 to 0.50% (Epstein, 1965; Ward *et al*., 1973; Campbell, 2000).

So, P concentration of Bhabadah water-logging area was above the range of reference value. Therefore, red amaranth is considered as good source of P and daily intake 100 g of this vegetable to meet almost 100% daily requirement of an adult where the daily requirement for an adult is 0.7 g day^{-1} (NAS, 2004).

Potassium

The highest concentration of potassium (0.38%) was at the Balidha and the lowest concentration (0.09%) was at the Bedvita with an average value of 0.24% (Fig. 1 and Fig. 2). Out of 24 samples, 14 samples were above the mean value and the rest 10 samples were below the average value. Reference value of the concentration of K in green leafy vegetables is from 1.50 to 4.50% (Epstein, 1965; Ward *et al*., 1973; Campbell, 2000). So, K concentration in red amaranth of Bhabadah water-logging area was below the range of reference value. The human body requires at least 100 mg of K daily to influence vital processes. Considering the daily requirement of 4.7 g day^{-1} for an adult and 3.0 g/100 g for a child of 1 - 3 years old (NAS, 2004), red amaranth is not good enough to supply K to the people of Bhabadah. A high potassium intake reduces the risk of overall mortality by 20%. Aykroyd *et al.* (1963) found that K concentration in edible portion of red amaranth was 341 mg l00 g^{-1}.

Sulphur

The status of sulphur (0.79%) in red amaranth samples was maximum at the Kalibari and minimum (0.21%) was at the Bedvita with a mean value of 0.56% (Fig. 1 and Fig. 2). Out of 24 samples, 12 samples were above the mean value and the rest 12 samples were below the average value. The reference value of the concentration of S in green leafy vegetables is from 0.15 to 0.65% (Epstein, 1965; Ward *et al*., 1973; Campbell, 2000). So, S concentration in red amaranth of Bhabadah water-logging area was within the range of reference value.

Calcium

Maximum calcium (Ca) concentration (3.85%) was at the Damukhali and minimum concentration (0.80%) was at the Lokhadanga with a mean value of 2.22% (Fig. 1 and Fig. 2). Out of 24 samples, 13 samples were above the mean value and the rest 11 samples were below the average value. Reference value of the concentration of Ca in green leafy vegetables is from 0.20 - 1.0% (Epstein, 1965; Ward *et al*., 1973; Campbell, 2000). So, Ca concentration in red amaranth of Bhabadah water-logging area was above the range of reference value. Considering the daily requirement of 1.3 g day^{-1} for an adult as recommended by NAS (2004), red amaranth is treated as a good source of Ca as 100 g of that vegetable consumed daily is adequate to provide well above 100 % of daily requirement for an adult. Begum (2006) reported 374 mg Ca 100 g^{-1} of the edible portion of red amaranth.

Magnesium

Magnesium concentraiton in red amaranth samples was maximum (3.85%) at the Bhuikara and minimum concentration (0.34%) was at the Damukhali with a mean value of 1.39% (Fig. 1 and Fig. 2). Out of 24 samples, 10 samples were above the mean value, and 14 samples were below the average value. Reference value of the concentration of Mg in green leafy vegetables is from 0.14 to 1.0% Mg (Epstein, 1965; Jones *et al*., 1991; Campbell, 2000; Barker and David, 2007). So, Mg concentration in red amaranth of Bhabadah area was above the range of reference value. So, red amaranth is considered as a good source of Mg as 100 g of that vegetable consumed daily is capable of providing about 2 g of Mg, which is well above the daily requirement 0.42 g day^{-1} (NAS, 2004) for an adult. Aykroyd *et al.* (1963) observed Mg concentration as 247 mg 100 g^{-1} in the edible portion of red amaranth.

Sodium

Maximum concentration of sulphur (0.33%) in red amaranth was at Sujatpur and the minimum concentration (0.01%) was at the Bedvita with a mean value 0.11% (Fig. 1 and Fig. 2). Out of 24 samples, 8 samples were above the mean value and the rest 16 samples were below the average value. Reference value of the concentration of Na in green leafy vegetables is from 0.43 to 1.5% (Epstein, 1965; Ward *et al*., 1973; Campbell, 2000). So, Na concentration in red amaranth of Bhabadah water-logging area was below the range of reference value.

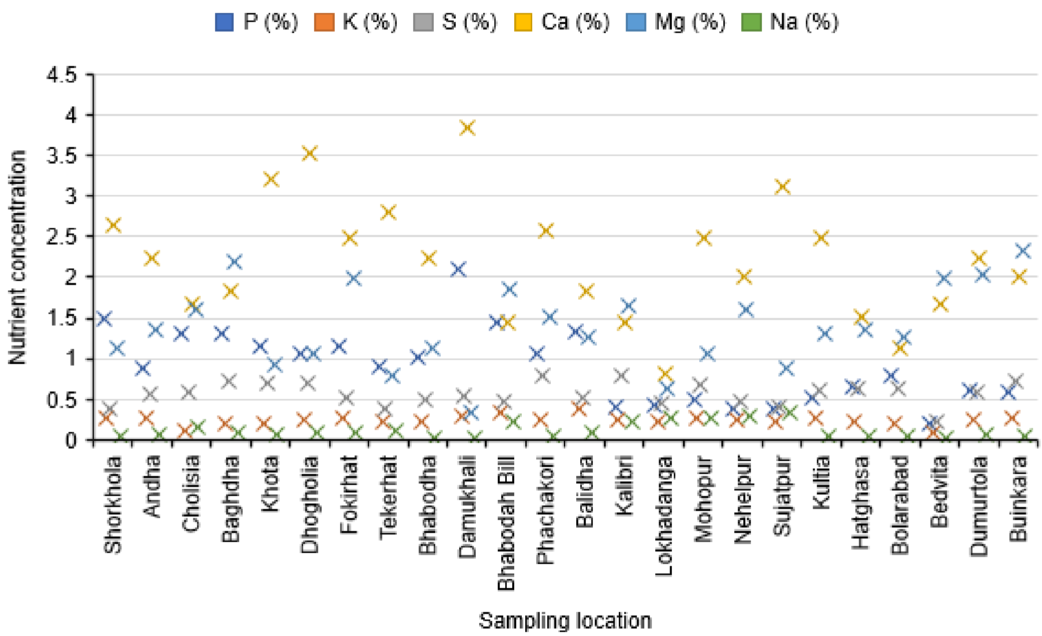

Figure 1. Boxplot showing mineral nutrient distribution of red amaranth in different sampling locations

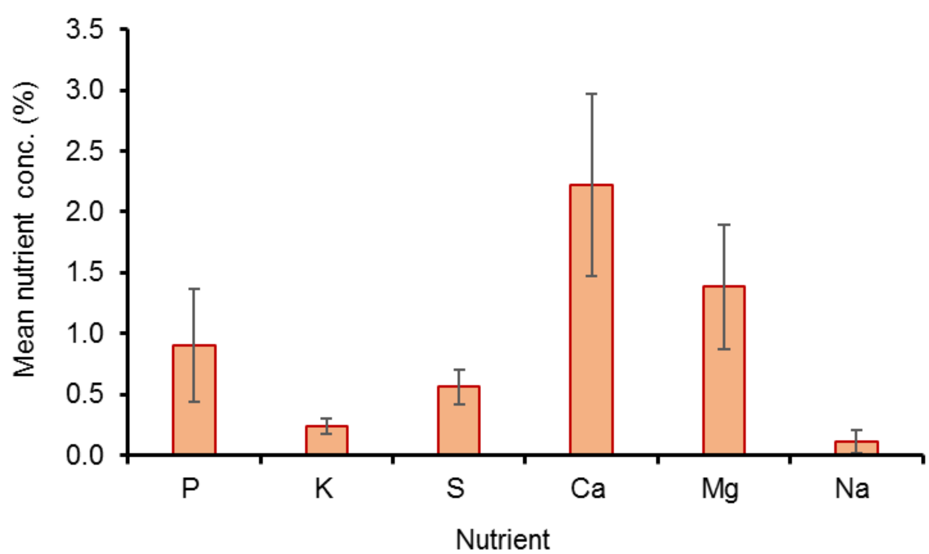

Figure 2. Mean mineral nutrient concentration of red amaranth in different sampling locations

Heavy metal concentration

Zinc

Zinc status (172.24 µg g^{-1}) in red amaranth was maximum at the Bhabadah and minimum concentration (26.48 µg g^{-1}) was at the Bedvita with a mean value of 88.64 µg g^{-1} (Table 2). Out of 24 samples, 12 samples were above the mean value and the rest 12 samples were below the average value. Zinc concentration in the collected amaranth samples might vary with the sampling location, resulting from the presence of added fertilizer or other sources. This statement was supported by Kacholi and Sahu (2018). Reference value of the concentration of Zn in green leafy vegetables is from 20 to70 µg g^{-1} (Reuter and Robinson, 1986; Ward *et al*., 1973; Campbell, 2000). So, Zn concentration in red amaranth of Bhabadah water-logging area was above the range of reference value. The value of Zn found in this study was far higher than 0.3 mg kg^{-1} (FAO/WHO, 1993). Maximum permissible level of Zn in vegetables is 100 µg g^{-1} as reported by CAC (2001).

Lead

Maximum concentration of lead (46.41 µg g^{-1}) in red amaranth was at the Khota and trace amount was detected in Shorkhola, Andha, Dhogolia, Fakirhat, Tekerhat, Bhabodha and Damukhali areas with a mean value of 8.20 µg g^{-1} (Table 2). Out of 24 samples, 10 samples were above the mean value, and 14 samples were below the average value. This mean value is higher than the safe limit (0.3 mg kg^{-1}) recommended by FAO/WHO (1993) and Bangladesh Food Safety (Contaminants, Toxins and Harmful Residues) Regulations (2017). The concentration of Pb exceeding maximum permissible limits of 0.03 mg 100 g^{-1} (CAC, 2001) in human body, which can damage the nervous system, bones, liver, pancreases, lungs, teeth and gum and causes blood diseases (Rahman *et al*., 2013; Kacholi and Sahu, 2018).

The high level of Pb in this study might be attributed to industrial waste dumped in the river at the upstream level, which is subsequently used to irrigate the farmlands in downstream areas. Atmospheric deposition of Pb might be a reason for increased concentration in the amaranth samples of the respective areas of higher contamination. This result is in line with the previous reports (Rahman *et al*., 2020; Rahman *et. al*., 2018) that observed the higher Pb concentrations in red amaranth which might be due to the high metal accumulation capacity of red amaranth (Yang *et al*. 2010). This metal also could be disposed from traffic activities, which can contaminate roadside grown amaranth (Kacholi and Sahu, 2018).

Cadmium

The highest Cd concentration (0.63 µg g^{-1}) in red amaranth was at the Sujatpur and trace amount was recorded in Shorkhola, Cholisia, Baghdha, Dighalia, Fakirhat, Tekerhat, Bhabodah and Panchakari areas were with a mean value of 0.18 µg g^{-1} (Table 2). So, Cd concentration in red amaranth of Bhabadah water-logging area was within the range of reference value. However, World Health Organization has established a provisional tolerable weekly intake of 7 µg g^{-1} Cd for body weight (WHO, 1992). The value of Cd found in this study was far higher than the safe value 0.2 mg kg^{-1} (FAO/WHO, 1993) and Bangladesh Food Safety (Contaminants, Toxins and Harmful Residues) Regulations (2017).

Chromium

Out of 24 red amaranth samples, only Dumurtola and Bhuikara area contained chromium (Table 2). In recent years, contamination of the environment by Cr has become a significant concern and exposure to Cr may occur through breathing air, drinking water, or eating food containing Cr or even through skin contact. In human beings and animals, it is considered to be an essential metal for carbohydrates and lipid metabolism within a certain range of concentrations (up to 200 µg day^{-1}). The mean value of Cr found in this study is higher than 2.3 mg kg^{-1} (FAO/WHO, 1993) and 0.02 mg kg^{-1} as reported by Bangladesh Food Safety (Contaminants, Toxins and Harmful Residues) Regulations (2017). The concentration of Cr in vegetables was found 6.4 µg g^{-1} in a previous report (Salvatore *et al*., 2009).

Copper

Among the red amaranth samples, maximum concentration of copper (32.58 µg g^{-1}) was at the Bhuikara area and minimum concentration (7.74 µg g^{-1}) was at the Andha area with an average value 18.43 µg g^{-1} (Table 2). Out of 24 samples, 14 samples were above the mean value and the rest 10 samples were below the average value. Reference value of the concentration of Cu in green leafy vegetables is from 4.5 to 15 µg g^{-1} Cu (Epstein, 1965; Campbell, 2000). So, Cu concentration of Bhabadah water-logging area was within the range of reference value in seven areas and out of range in the rest areas. The addition of agricultural products like fertilizers and other industrial sources for water pollution might be responsible for the contamination of Cu in red amaranth samples of those areas (Kacholi and Sahu, 2018). The value of Cu found in this study was lower than 40 mg kg^{-1} (FAO/WHO, 1993).

Table 2. Status of trace and heavy metals in red amaranth

Sl. No.	Location name	Zn (μg g^{-1})	Pb (μg g^{-1})	Cd (μg g^{-1})	Cr (μg g^{-1})	Cu (μg g^{-1})
1.	Shorkhola	106.41	Trace	Trace	Trace	12.78
2.	Andha	76.46	Trace	0.055	Trace	7.74
3.	Cholisia	89.69	10.06	Trace	Trace	10.78
4.	Baghdha	104.89	28.82	Trace	Trace	12.26
5.	Khota	63.02	46.41	0.055	Trace	9.22
6.	Dighalia	103.96	Trace	Trace	Trace	18.56
7.	Fakirhat	94.10	Trace	Trace	Trace	15.90
8.	Tekerhat	46.86	Trace	Trace	Trace	11.00
9.	Bhabodha	59.30	Trace	0.065	Trace	10.85
10.	Damukhali	46.78	Trace	0.045	Trace	18.56
11.	Bhabodah Bill	172.24	0.62	Trace	Trace	20.42
12.	Panchakari	149.84	2.22	Trace	Trace	19.97
13.	Balidha	144.48	3.76	0.085	Trace	19.08
14.	Kalibari	49.74	3.51	0.215	Trace	16.49
15.	Lokhadanga	79.10	7.86	0.305	Trace	21.53
16.	Mohonpur	110.34	6.39	0.345	Trace	21.83
17.	Nehalpur	73.10	8.32	0.345	Trace	19.68
18.	Sujatpur	125.68	11.31	0.625	Trace	26.35
19.	Kultia	68.38	9.21	0.285	Trace	21.46
20.	Hatghasa	91.14	6.57	0.275	Trace	26.28
21.	Bolarabad	68.56	9.42	0.425	Trace	20.34
22.	Bedvita	26.48	9.47	0.235	Trace	17.16
23.	Dumurtola	97.78	17.56	0.475	1.30	31.61
24.	Bhuikara	78.92	15.36	0.405	1.60	32.58
Range		26.48 -172.24	0.0 - 46.41	0.0 - 0.63	0.0 - 1.60	7.74 - 32.58
Average±SD		88.64±35.15	8.20±10.74	0.18±0.19	0.12±0.41	18.43±6.57
Reference values		20-70 μg g^{-1} [1,2,3]; 0.3 mg kg^{-1} [4]	0.3 mg kg^{-1} [4]	0.2 mg kg^{-1} [4]	2.3 mg kg^{-1} [4]; 0.002 μg g^{-1} [5]	40 mg kg^{-1} [4]

[1]Reuter and Robinson (1986); [2]Campbell (2000); [3]Ward *et al*. (1973); [4]FAO/WHO (1993); [5]Bose and Bhattacharyya (2008). Trace means the amount of below detectable level of flame AAS, where the detection limit is 0.05 mg L^{-1} for Pb; 0.01 mg L^{-1} for Cr and <0.01 mg L^{-1} for Cd.

Daily metals intake (DMI)

Daily metal intake was calculated according to the average vegetable consumption for both adults male and female. The daily metals intakes of Zn, Pb, Cd, Cr and Cu from red amaranth samples were calculated by multiplying the daily intake by metal concentrations determined in this study. Average consumption rate of red amaranth day^{-1} person $^{-1}$ assumed 10.0 and 8.0 g as typical serving for a day for male and female, respectively (Aysha *et al*., 2017). Previously, the average daily intake of vegetable for adults and children were considered to be 0.345 and 0.232 kg $person^{-1}$ day^{-1}, respectively (Wang *et al*., 2005). DMI were compared with the upper tolerable daily intakes for metals. It was evident from Table 3 that Zn and Cu values for both male and female were lower than upper tolerable intake level. On the other hand, Pb and Cd were higher for both males and females than those of upper tolerable intake level, indicating that serious adverse effects have been associated with the intake of red amaranth in Bhabadah water-logging. Sampling location might be the factors of the presence of higher metal sources in the red amaranth for water contamination for use of agrochemicals or industrial wastes disposal into the agricultural fields (Kacholi and Sahu 2018). The present study results in accordance with the results of previous studies on heavy metal contamination in the edible parts of vegetables grown with wastewater at different sites (Liu *et al*., 2005; Khan *et al*., 2008).

Incremental Lifetime Cancer Risk (ILCR)

Incremental lifetime cancer risk was calculated for both the adults male and female according to the average vegetable consumption in the study area. The risk of carcinogenesis of Pb, Cd and Cr were presented in Table 3, where Zn and Cu tended to zero (0), since cancer slope factor (CSF) of them are zero (0). The maximum carcinogenic risk levels for male of Pb, Cd and Cr were 9.95E-03, 1.14E-02and 1.00E-02, respectively for ingestion exposure whereas 1.11E-02, 1.14E-02 and 1.00E-02, respectively were found for female. Only Pb in male is at very near to carcinogenic risk but the people of the study area are marginally at risk through the ingestion of Cd and Cr which does not constitute threat been below the regulatory range of 1×10^{-6} to 1×10^{-4}, which can be eliminated easily by using proper means (Liu *et al.,* 2013).

Target hazard quotients (THQ)

Target hazard quotients (THQ) is a complex parameter used for the estimation of potential health risks associated with long term exposure to chemical pollutants (Khan *et al*., 2009; Harmanescu *et al*., 2011). THQ<1 means the exposed population is assumed to be safe; <THQ<5 means that the exposed population is in a level of concern interval and THQ>5 means exposed population is unsafe. As a dimension less index, THQ values are additive, not multiplicative and indicates their concern level but not a measure of risk. Target hazard quotients was calculated considering DMI of people, average body weight (male: 70 kg and female: 50 kg) and average life expectancy (male: 70.6 and female: 73.1) (BBS, 2015). Values of this parameter (THQ) based on consumption of red amaranth available in the Bhabadah water-logging area for the investigated metals have been presented in Fig. 3. THQ value for Pb surpassed 5.0 for female (6.550) indicating that the exposed population is unsafe and surpassed 1.0 for male (4.179) indicating that the exposed population is in a level of concern interval. THQ value for Cu also surpassed 1.0 for female (1.475) but not for male (0.939). The rest of the THQ values of Zn, Cd, and Cr for both males and females were below 1.0 indicating that the exposed population is assumed to be safe. From point of view of human health, THQ values of Cu were >1 of studied vegetables reported by Shaheen *et al*. (2016), which could pose health hazard due to exposure to the heavy metals.

Table 3. Calculated daily metals intakes (DMI) and incremental lifetime cancer risk (ILCR) for red amaranth collected from the Bhabadah water-logging area along with the recommended upper tolerable intake level (UTIL), oral reference doses (RfD) and cancer slope factor (CSF)

		Zn	Pb	Cd	Cr	Cu
UTIL (mg day^{-1} person $^{-1}$)		40.00[a]	0.24[b]	0.064[b]	NE	10.00[a]
RfD (mg kg^{-1} day^{-1})		0.300[c]	0.004[e]	0.001[c]	0.003[d]	0.040[c]
CSF (mg kg^{-1} day^{-1}) $^{-1}$		-	0.0085[f]	0.38[f]	0.50[f]	-
Daily Metal Intake (mg day^{-1} person^{-1})	Male	12.66	1.17	0.03	0.02	2.63
	Female	14.18	1.31	0.03	0.02	2.95
Incremental Lifetime Cancer Risk (ILCR)	Male	-	9.95E-03	1.14E-02	1.00E-02	-
	Female	-	1.11E-02	1.14E-02	1.00E-02	-

NE= Not established; a= FDA (2001); b= Garcia-Rico *et al.* (2007); c= USEPA (2010); d= (1987) and e= Khan *et al.* (2008), f= USDOE (2011).

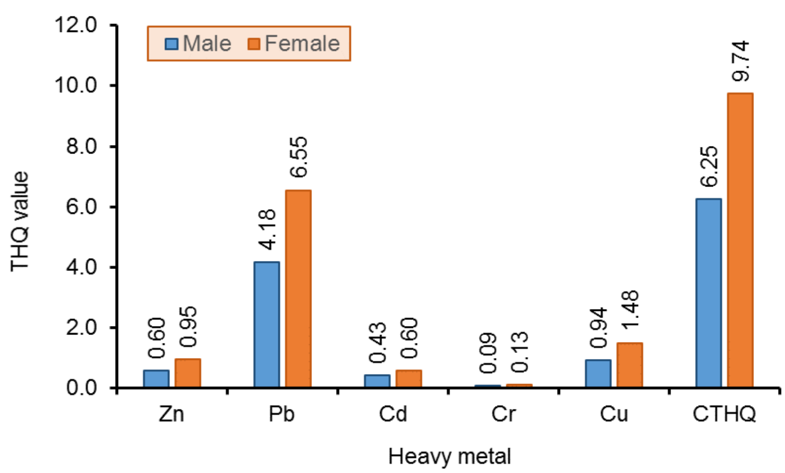

Figure 3. Target hazard quotients (THQ) and combined target hazard quotient (CTHQ) of heavy metals in red amaranth.

Conclusion

It may be concluded that red amaranth is a good source of minerals and trace elements. In all red amaranth samples, heavy metals were below FAO/WHO recommended safe permissible level except Pb. Daily metal intake and cancer risk results showed that Pb concentration in the collected samples was in alarming situation. The information generated from this study could be used as a baseline data for developing food composition database for Bangladesh. The most of essential nutrients were in optimum level for human nutrition. Overall result of this study suggests that food chain contamination by heavy metals through elevated level of Pb concentration in red amaranth samples of Bhabadah area is evident through this research and it is recommended that the government and its related organizations or authority could take emergency initiatives to develop specific awareness on heavy metals level of vegetables.

Acknowledgements

The authors gratefully acknowledge the authority of Bangladesh Agricultural University Research System (Project no.: 2017/219/AU-GC) for financial support of this research.

Conflict of Interests

The authors declare that there is no conflict of interests regarding the publication of this paper.

References

Alexander, P.D., Alloway, B.J. and Dourado, A.M. 2006. Genotypic variations in the accumulation of Cd, Cu, Pb and Zn exhibited by six commonly grown vegetables. *Environmental Pollution*, 144(3): 736-745. https://dx.doi.org/10.1016/j.envpol.2006.03.001.

Ali, H., Khan, E. and Ilahi, I. 2019. Environmental chemistry and ecotoxicology of hazardous heavy metals: environmental persistence, toxicity, and bioaccumulation. *Journal of chemistry*, Article ID 6730305, 1-14. https://doi.org/10.1155/2019/6730305

Andrés-Bello, A., Barreto-Palacios, V. I. V. I. A. N., García-Segovia, P., Mir-Bel, J., & Martínez-Monzó, J. 2013. Effect of pH on color and texture of food products. *Food Engineering Reviews*, 5(3): 158-170. https://doi.org/10.1007/s12393-013-9067-2.

APHA. 2012. Standard Methods for the Examination of Water and Wastewater, 22nd Edn. American Public Health Association, Washington.

Aykroyd, W.R., Gopalan, C. and Balansubramanian, S.C. 1963. The nutritional value of Indian foods and planning of satisfactory diets. Special Report Series No. 42, I.C.M.R., New Delhi.

Aysha, M.I.J., Zakir, H.M., Haque, R., Quadir, Q.F., Choudhury, T.R., Quraishi, S.B. and Mollah, M.Z.I. 2017. Health risk assessment for population via consumption of vegetables grown in soils artificially contaminated with arsenic. *Archives of Current Research International,* 10(3): 1-12. https://doi.org/10.9734/ACRI/2017/37612.

Barker, A.V. and David, J. 2007. Handbook of Plant Nutrition. Taylor & Francis Group 6000 Broken Sound Parkway NW, Suite 300 Boca Raton, p. 104.

BBS. 2020. Statistical Yearbook Bangladesh 2019. 39th Edn. Statistics and Informatics Division (SID), Bangladesh Bureau of Statistics, Ministry of Planning, Government of the People's Republic of Bangladesh. pp. 138-155.

BBS. 2015. Health and Morbidity Status Survey. Bangladesh Bureau of Statistics. p. 81.

Bangladesh Food Safety (Contaminants, Toxins and Harmful Residues) Regulations, 2017. Bangladesh Safe Food Authority, Ministry of Food, People's Republic of Bangladesh. http://www.bfsa.gov.bd/sites/default/files/files/bfsa.portal.gov.bd/law/81690675_9a69_483b_8915_1fe8f6b2ddd0/Food-Safety-(Contaminants,-Toxins-and-Harmful-Residues)-Regulations,-2017.pdf (Access date: 11 April, 2021).

Begum, R.A. 2006. Assessment of water and soil pollution and its effect on rice and red amaranth. Ph.D. Thesis, Department of Agricultural Chemistry, Bangladesh Agricultural University, Mymensingh.

Bose, S. and Bhattacharyya, A.K. 2008. Heavy metal accumulation in wheat plant grown in soil amended with industrial sludge. *Chemosphere,* 70:1264–1272. https://doi.org/10.1016/j.chemosphere.2007.07.062.

Campbell, C.R. 2000. Reference sufficiency ranges for plant analysis in the southern region of the United States. *Southern Cooperative Series Bulletin*, 394: 29-32.

CAC (Codex Alimentarius Commission). 2001. Joint FAO/WHO Food additives and contaminants. Joint FAO/WHO food Standards program, ALINORM 01/12A. pp. 1-289.

Chen, S., Lin, Y., Kao, Y. and Shih, Y. 2013. Trace elements and heavy metals in poultry and livestock meat in Taiwan. *Food Additives and Contaminants* Part B. 6(4): 231-236. https://doi.org/10.1080/19393210.2013.804884.

Epstein, E. 1965. Mineral metabolism. In: J. Bonner and J.E. Varner (Eds), Plant biochemistry, p. 438. New York: Academic Press.

Eruvbetine, D. 2003. Canine Nutrition and Health. A paper presented at the seminar organized by Kensington Pharmaceuticals Nig. Ltd., Lagos.

FAO/WHO (Food and Agriculture Organization/World Health Organization). 1993. Food Additives and Contaminants. Joints FAO/WHO Food Standard Programme, ALINORM 01/12A.

FDA (Food and Drug Administration). 2001. Dietary reference intakes for vitamin A, vitamin K, arsenic, boron, chromium, copper, iodine, iron, manganese, molybdenum, nickel, silicon, vanadium, and zinc. Report of the panel on micronutrients, National Academy Press, Washington, DC, Dietary supplements, Center for Food Safety and Applied Nutrition.

Garcia-Rico, L., Leyva-Perez, J. and Jara-Marini, M.E. 2007. Content and daily intake of copper, zinc, lead, cadmium, and mercury from dietary supplements in Mexico. *Food and Chemical Toxicology*, 45(9): 1599-1605. https://doi.org/10.1016/j.fct.2007.02.027.

Gomez, K.A. and Gomez, A.A. 1984. Statistical Procedures for Agricultural Research. 2nd Edn. A Wiley-Interscience Publication, New York, USA. pp. 442-443.

Gupta, N., Khan, D.K. and Santra, S.C. 2008. An assessment of heavy metal contamination in vegetables grown in wastewater-irrigated areas of Titagarh, West Bengal, India. *Bulletin of Environmental Contamination and Toxicology,* 80(2): 115-118. https://doi.org/10.1007/s00128-007-9327-z.

Gupta, P.K. 2013. Soil, Plant, Water and Fertilizer Analysis, 2nd Edn. Agrobios Jodhpur, India. pp. 254-263.

Hao, X.Z., Zhou, D.M., Huang, D.Q., Cang, L., Zhang, H.L. and Wang, H. 2009. Heavy metal transfer from soil to vegetable in southern Jiangsu Province, China. *Pedosphere,* 19(3): 305-311. https://doi.org/10.1016/S1002-0160(09)60121-1.

Harmanescu, M., Alda, L.M., Bordean, D.M., Gogoasa, I. and Gergen, I. 2011. Heavy metals health risk assessment for population via consumption of vegetables grown in old mining area; a case study: Banat County, Romania. *Chemistry Central Journal*, 5(1): 1-10. https://doi.org/10.1186/1752-153X-5-64.

Hays, V.W. and Swenson, M.J. 1985. Minerals and bones. Dukes' Physiology of Domestic Animals, 10th Edn. pp. 449-466. Cornell University Press, London, UK.

Hernández-Martínez, R. and Navarro-Blasco, I. 2012. Estimation of dietary intake and content of lead and cadmium in infant cereals marketed in Spain. *Food Control*, 26(1): 6-14. https://doi.org/10.1016/j.foodcont.2011.12.024.

Hossain, M. T., Rahman, M. A., Nahar, K., & Mohiuddin, K. M. (2020). Assessment of Water Quality of Bhabadah Water-logging Area of Khulna Division. *Journal of Bangladesh Agricultural University*, 18(S1): 831-838. https://doi.org/10.5455/JBAU.18787

Jiang, G.B., Shi, J.B. and Feng, X.B. 2006. Mercury pollution in China. *Environmental Science and Technology*, 40(12): 3672-3678.

Jimoh, F.O. and Oladiji, A.T. 2005. Preliminary studies on Piliostigma thonningii seeds: proximate analysis, mineral composition and phytochemical screening. *African Journal of Biotechnology*, 4(12): 1439-1442.

Jones Jr., J.B., Wolf, B. and Mills, H.A. 1991. Plant Analysis Handbook: A Practical Sampling, Preparation, Analysis, and Interpretation Guide. Micro-Macro Publishing, Athens.

Kacholi, D.S. and Sahu, M. 2018. Levels and health risk assessment of heavy metals in soil, water, and vegetables of Dar es Salaam, Tanzania. *Journal of Chemistry,* Article ID 1402674. https://doi.org/10.1155/2018/1402674.

Karalliede, L. and Brooke, N. 2012. Toxicity of heavy metals and trace elements. In Essentials of toxicology for health protection: A handbook for field professionals. Oxford: Oxford University Press, UK.

Khan, S., Cao, Q., Zheng, Y.M, Huang, Y.Z. and Zhu, Y.G. 2008. Health risks of heavy metals in contaminated soils and food crops irrigated with wastewater in Beijing, China. *Environmental Pollution*, 152: 686-692. https://doi.org/10.1016/j.envpol.2007.06.056.

Khan, S., Farooq, R., Shahbaz, S., Khan, M. A., and Sadique, M. 2009. Health risk assessment of heavy metals for population via consumption of vegetables. *World Applied Sciences Journal*, 6(12): 1602-1606.

Liu, W.H., Zhao, J.Z., Ouyang, Z.Y., Söderlund, L., and Liu, G.H. 2005. Impacts of sewage irrigation on heavy metal distribution and contamination in Beijing, China. *Environment International*, 31(6): 805-812. https://doi.org/10.1016/j.envint.2005.05.042.

Liu, X., Song, Q., Tang, Y., Li, W., Xu, J., Wu, J., Wang, F. and Brookes, P.C. 2013. Human health risk assessment of heavy metals in soil–vegetable system: a multi-medium analysis. *Science of the Total Environment*, 463: 530-540. https://doi.org/10.1016/j.scitotenv.2013.06.064.

Luo, C., Liu, C., Wang, Y., Liu, X., Li, F., Zhang, G. and Li, X. 2011. Heavy metal contamination in soils and vegetables near an e-waste processing site, south China. *Journal of Hazardous Materials*, 186(1): 481-490. https://doi.org/10.1016/j.jhazmat.2010.11.024.

Macrotrend, 2021. https://www.macrotrends.net/countries/BGD/bangladesh/life-expectancy. (Access date: 14 April, 2021)

NAS (National Academy of Sciences). 2004. Dietary References Intake (DRIs) Recommended Intakes for Individuals, Elements. Food and Nutrition Board, Institute of Medicine, National Academies.

Page, A.L., Miller, R.H. and Kenny, D.R. (Eds) 1982. Methods of Soil Analysis Part- 2. Chemical and Microbiological Properties, 2nd Edn. American Society of Agronomy, Soil Science Society of America, Inc. Publishers, Madison, Wisconsin, USA.

Pan, X.D., Tang, J., Chen, Q., Wu, P.G. and Han, J.L. 2013. Evaluation of direct sampling method for trace elements analysis in Chinese rice wine by ICP–OES. *European Food Research and Technology*, 236(3): 531-535. https://doi.org/10.1007/s00217-012-1888-3.

Rahman, M.A., Saha, B.K., Chowdhury, M.A.H., Chowdhury, M.A.K. and Mohiuddin, K.M. 2020. Public perception and health implication of loom-dye effluent irrigation on growth of rice (*Oryza sativa* L.) and red amaranth (*Amaranthus tricolor* L.) seedlings. *Environmental Science and Pollution Research*, 27: 19410-19427. https://doi.org/10.1007/s11356-020-08377-0.

Rahman, M.M., Asaduzzaman, M. and Naidu, R. 2013. Consumption of arsenic and other elements from vegetables and drinking water from an arsenic-contaminated area of Bangladesh. *Journal of Hazardous Materials*, 262: 1056-1063. https://doi.org/10.1016/j.jhazmat.2012.06.045.

Rahman, M.A., Rayhan, M.Y.H., Chowdhury, M.A.H., Mohiuddin, K.M. and Chowdhury, M.A.K. 2018. Phytotoxic effect of synthetic dye effluents on seed germination and early growth of red amaranth. *Fundamental and Applied Agriculture*, 3(2): 480-490. https://doi.org/10.5455/faa.299239.

Rajaei, G., Mansouri, B., Jahantigh, H. and Hamidian, A.H. 2012. Metal concentrations in the water of Chah nimeh reservoirs in Zabol, Iran. *Bulletin of environmental contamination and toxicology*, 89(3): 495-500. https://doi.org/10.1007/s00128-012-0738-0

Reuter, D.J. and Robinson, J.B. 1986. Plant Analysis: An Interpretation Manual. 1st Edn. Inkata Press, Melbourne, Australia.

Salvatore, M.D., Carratù, G., and Carafa, A.M. 2009. Assessment of heavy metals transfer from a moderately polluted soil into the edible parts of vegetables. *Journal of Food, Agriculture and Environment*, 7(2): 683-688.

Shaheen, N., Irfan, N.M., Khan, I.N., Islam, S., Islam, M.S. and Ahmed, M.K. 2016. Presence of heavy metals in fruits and vegetables: Health risk implications in Bangladesh. *Chemosphere*, 152: 431-438. https://doi.org/10.1016/j.chemosphere.2016.02.060.

Singh, D., Chhonkar, P.K. and Pandey, R.N. 1999. Soil Plant Water Analysis: A Methods Manual. IARI, New Delhi, India. pp. 72-86.

Soetan, K.O., Olaiya, C.O. and Oyewole, O.E. 2010. The importance of mineral elements for humans, domestic animals and plants-A review. *African journal of food science*, 4(5): 200-222.

Tandon, H.L.S. (Eds) 1995. Methods of Analysis of Soils, Plants, Waters and Fertilizers, Fertilizer Development and Consultation Organization. New Delhi, India. pp. 84-90.

The Daily Star 2017. Bhabadaha: A tale of sorrow. August 05, 2017. https://www.thedailystar.net/frontpage/bhabadaha-tale-sorrow-1443832 (Access date: 12 April 2021).

The Daily Star 2018. Save waterlogged Bhabadah, Water Mitigation Movement Committee urges govt. August 21, 2018. https://www.thedailystar.net/news/country/save-waterlogged-bhabadah-1623568 (Access date: 12 April 2021).

Thomson, H.C. and Kelly, W.C. 1990. Vegetable Crops.: McGraw Hill Publishing Company, New Delhi, India.

Toplan, S., Özcelik, D., Gulyasar, T. and Akyolcu, M. C. 2004. Changes in hemorheological parameters due to lead exposure in female rats. *Journal of Trace Elements in Medicine and Biology*, 18(2): 179-182. https://doi.org/10.1016/j.jtemb.2004.02.006.

USDOE. 2011. U.S. Department of Energy's Oak Ridge Operations Office (ORO). The Risk Assessment Information System (RAI). United States of America.

USEPA (United States Environmental Proctction Agency). 2010. Human Health Risk Assessment: Risk-Based Concentration Table. http://www.epa.gov/reg3hwmd/risk/human/rbconcentration_table/Generic_Tables/index.htm.

Wang, X., Sato, T., Xing, B. and Tao, S. 2005. Health risks of heavy metals to the general public in Tianjin, China via consumption of vegetables and fish. *Science of the Total Environment*, 350(1-3): 28-37. https://doi.org/10.1016/j.scitotenv.2004.09.044.

Ward, R.C., Whitney, D.A. and Westfall, D.G. 1973. Plant analysis as an aid in fertilizing small grains. In: L.M. Walsh and J.D. Beaton (Eds), Soil Testing and Plant Analysis. SSSA, Madison, WI. pp. 329-348.

WHO. 1992. Guidelines for Drinking Water Quality, 2nd Edn. World Health Organization, Geneva.

Yang, J., Guo, H., Ma, Y., Wang, L., Wei, D. and Hua, L. 2010. Genotypic variations in the accumulation of Cd exhibited by different vegetables. *Journal of Environmental Sciences*, 22(8): 1246-1252. http://doi.org/10.1016/S1001-0742(09)60245-X.