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## Research article

## Assessment of heavy metals accumulation by vegetables irrigated with different stages of textile wastewater for evaluation of food and health risk

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## ABSTRACT

Wastewater irrigation for vegetable cultivation is greatly concerned about the presence of toxic metals in irrigated soil and vegetables which causes possible threats to human health. This study aimed to ascertain the accumulation of heavy metals (HMs) in edible parts of vegetables irrigated with different stages of textile dyeing wastewater (TDW). Bio-concentration factor (BCF), Estimated daily intake (EDI), and target hazard quotient (THQ) were computed to estimate human health risks and speculate the hazard index (HI) of adults and children with the consumption of HMs contaminated vegetables at recommended doses. Five vegetables (red amaranth, Indian spinach, cauliflower, tomato, and radish) in a pot experiment were irrigated with groundwater (T1) and seven stages of TDW (T2–T8) following a randomized complete block design (RCBD) with three replications. Among the TDW stages, T8, T7, T4, and T5 exhibited elevated BCF, EDI, THQ, and HI due to a rising trend in the accumulation of Pb, Cd, Cr, and Ni heavy metals in the edible portion of the red amaranth, followed by radish, Indian spinach, cauliflower, and tomato. The general patterns of heavy metal (HM) accumulation, regarded as vital nutrients for plants, were detected in the following sequence: Zn > Mn/Cu > Fe. Conversely, toxic metals were found to be Cd/Cr > Ni > Pb, regardless of the type of vegetables. Principal Component Analysis (PCA) identified T8, T7, and T4 of TDW as the primary contributors to the accumulation of heavy metals in the vegetables examined. Furthermore, the analysis of the heavy metals revealed that the BCF, THQ, and HI values for all studied metals were below 1, except for Pb. This suggests that the present consumption rates of different leafy and non-leafy vegetables, whether consumed individually or together, provide a low risk in terms of heavy metal exposure. Nevertheless, the consumption of T8, T7, and T4 irrigated vegetables, specifically Indian spinach alone or in combination with red amaranth and radish, by both adults and children, at the recommended rate, was found to pose potential health risks. On the other hand, T2, T3, and T6 irrigated vegetables were deemed safe for

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consumption. These findings indicated that the practice of irrigating the vegetables with T8, T7, and T4 stages of TDW has resulted in a significant buildup of heavy metals in the soils and edible parts of vegetables which are posing health risks to adults and children. Hence, it is imperative to discharge the T8, T7, and T4 stages of TDW after ETP to prevent the contamination of vegetables and mitigate potential health risks.

## 1. Introduction

Textile industries are now recognized as the driving force of the global economy as it is earning about 1 trillion dollars which accounts for 7 % of the total world export business and creating employment opportunities for approximately 35 million people worldwide (Desore and Narula, 2018). Despite its significant contribution to the world economy, this sector is further recognized as the largest environmental polluter (Lellis et al., 2019; Bhatia, 2017; Kumar et al., 2020a,b). In addition, an enormous quantity of fresh water along with dyes, chemicals, and energy is used for textile wet processing (Hossain et al., 2018). Apart from this, the textile dyeing industry emits nitrogen and sulfur dioxides into the air and non-biodegradable organic compounds derivatives into the water bodies from the used dyes (Orts et al., 2018; Wang, 2016). Meanwhile, 1 kg of cotton fabrics required reactive dye colors, 80–200 L of water, 0.6–1.0 kg of common salt, 40 g of reactive color (organic and synthetic compounds), caustic soda, and dyeing auxiliary substances for processing (Allegre et al., 2006). Bangladesh Textile Industries are producing ca. 2.9 million metric tons of fabric and generated about 349 million m<sup>3</sup> of wastewater in 2021 (Hassan et al., 2022).

There are 17 steps involved in each dyeing process where fabrics are vigorously washed or rinsed with clean water to remove residual chemicals (Hassan et al., 2022) (Supplementary Figure 1). In many cases, this wastewater is discharged into nearby sewers, drains, and agricultural land after each dyeing procedure without proper treatment (Khan et al., 2011; Hassan et al., 2022; Kubra et al., 2021, 2021a, 2023). However, different types of wastewater treatments are suggested to ensure a sustainable environment for the forthcoming generation using effluent treatment plants (ETP) (Setiadi et al., 2006; Eswaramoorthi, 2011; Jordão et al., 2018; Orts et al., 2018). However, due to the high operational and utility costs, these are not cost-feasible. As a result, many developing countries, such as Bangladesh, operate most of their textile dyeing plants without an ETP. The untreated wastewater is discharged into the surrounding areas and utilized for crop cultivation by the local farmers with easy access to irrigation without any cost. Although, it has some positive effect on the growth and yield of the plants due to possessing some necessary micro and macronutrients of plants but also contains some heavy metals that might get transferred from the cultivated plants to the human body through the food chain if those are consumed (Hassan et al., 2022; Faysal et al., 2021; Islam et al., 2021; Neha et al., 2021).

Excess amounts of such trace metals in irrigated soil cause metal uptake by cultivating crops, affecting food quality (Khan et al., 2008; Tuzen et al., 2020, 2022; Nemati et al., 2023; Khoubnasabjafari et al., 2023; Ullah et al., 2023). Trace element (TE) accumulation is higher in edible parts of leafy vegetables than in grain or fruit crops (Arora et al., 2008). Different orders of TEs concentration and accumulation patterns also may vary from vegetable to vegetable and even among different edible parts (root, stem, leaf) of vegetables (Letshwenyo and Mokokwe, 2020; Islam et al., 2021, 2021a, 2023; Kabir et al., 2021; Saha et al., 2017; Teo et al., 2022). Consequently, toxic metal-polluted vegetables pose a danger to human health and inhibit countries from meeting the United Nations' Sustainable Development Goals 2 (Zero Hunger), Goal 3 (Good health and well-being), and Goal 12 (Responsible Consumption and Production) (Resolution A/RES/70/1, 2015).

Previous studies found that excessive consumption of heavy metal-contaminated vegetables may cause carcinogenic, mutagenic, neurotoxic, and teratogenic effects on human health, leading to serious health

risks like Alzheimer's in adults, intellectual disability in children, DNS (deviated nasal septum), insomnia, kidney, and liver diseases, and more (Emamverdian et al., 2015; Jan et al., 2011; Khatri et al., 2018; Altunay et al., 2022; Salman et al., 2021, 2021a, 2023, 2023a). In this case, an effective method for utilizing textile effluents in agriculture irrigation is required in order to reduce aquatic environmental contamination, depletion of groundwater resources for farming, and expenses associated with wastewater treatment (Hassan et al., 2022; Hasan et al., 2021, 2013; Thamaraiselvi et al., 2023; Pradip et al., 2019; Shiv et al., 2021).

However, numerous studies have been conducted to investigate the physicochemical properties of textile wastewater and their effects on plant growth, crop yield, nutritional value, accumulation of toxic metals in soil and crops, and the assessment of associated health risks. These studies specifically focused on analyzing the final discharged effluents as the research material (Rahman et al., 2020, 2022, 2020; Rai et al., 2019; Rind et al., 2023; Khandaker et al., 2018). During the process of dyeing knit fabric, there are multiple stages in which none of the effluent is substantially contaminated. Some dyeing procedures employ both chemicals and fresh water, while others solely rely on fresh water for cleansing textile goods following chemical treatment. Haque (2008) stated that around half of the effluent from the dyeing process is polluted and requires treatment, but the other wastewater can be released as is or with minimal treatment. Hence, there is ample opportunity for enhancement in the several phases of reusing wastewater from textile dyeing. Based on our previous research, we found that the wastewater from three stages of a dyeing process can safely be used for agriculture, depending on its impact on seed germination yield and growth of red amaranth, country bean, and tomato plants through considering the factors such as the enrichment of heavy metals, sodium absorption ratio, and pollution load index (Hassan et al., in 2013 and 2022; Khandaker et al., in 2013 and 2017a,b; Sandeep et al., 2019; Neha et al., 2019). Nevertheless, there are persistent concerns that necessitate thorough investigation into the potential human health hazards associated with the ingestion of different crops irrigated with effluent from textile dyeing.

This feature allows for an additional opportunity to work with certain stages of the dyeing process for safe irrigation in agricultural land, as well as suitable laws by policymakers and industry owners addressing the reusing of textile wastewater in agriculture. As a consequence of this, it has been suggested that several phases of textile dyeing effluent may differ physiochemically and accumulate heavy metals in different edible sections of cultivated crops. In this research, several phases of textile knit-dyeing effluent for irrigation were upgraded, with a focus on heavy metal accumulation in various edible sections of several vegetables, as well as health risk assessment in adults and children. Therefore, this study was designed with the following specific objectives: (i) To evaluate the accumulation pattern of heavy metals (Fe, Cu, Zn, Mn, Ni, Cr, Cd, and Pb) in different vegetables watered with effluent from several phases of textile dyeing; (ii) To examine the health hazards of the toxic metals contaminated vegetable consumption and their sources of contamination; (iii) To speculate the potential danger of the toxic metals in consumable leaf and non-leaf vegetables frequently consumed by the adult and children.

## 2. Materials and methods

### 2.1. Experimental design

The study used five different kinds of vegetables as test crops: a root

crop (radish), a stem-leaf crop (Indian spinach), a leaf crop (red amaranth), a flower crop (cauliflower), and a fruit crop (tomato) at the Bangabandhu Sheikh Mujibur Rahman Agricultural University's (BSMRAU) Horticultural Research Farm and Soil Science Laboratory in Bangladesh. We chose different kinds of vegetables because people want to eat different kinds of vegetables every day. Moreover, these veggies also grow in net houses, commercial fields, and home gardens. The selected vegetables have specific growing duration of about three months for tomatoes and cauliflower, two months for radishes, one and a half months for Indian spinach, and one month for red amaranth (BARI, 2020). Multiple stages of wastewater from the textile dyeing process were used as irrigation water for vegetable production and groundwater was used as a control (Supplementary Fig. 1). Physicochemical parameters of vegetable-growing soil and water were examined before irrigation. No pesticides or fertilizers were used on the crops so that there wouldn't be any other different nutrients interaction in the present investigation. To prevent rainwater from contaminating the used specimens, the plants were raised in earthen pots in a white polythene-covered net house. After that, the accumulation of toxic metals in the investigated vegetables was assessed at the optimal harvesting maturity. After harvesting, the cultivated soils of the pots were further investigated to assess the metal residual content. Lastly, health hazards were evaluated utilizing internationally recognized measures such as BCF (bio-concentration factor), EDI (estimated daily intake), and HI (hazard index).

## 2.2. Physicochemical characteristic of textile dyeing wastewater (TDW)

The six (6) stages of textile dyeing wastewater (TDW) out of seventeen (17) processing stages of a fabric dyeing process and one (1) mixed effluent from the equalization tank just before purifying it at the ETP were collected from the Tex Euro BD Ltd factory having 20-ton knit fabric dyeing capacity (Supplementary Fig. 1). The wastewater samples (T2–T8) and ground water (T1) were collected in an individual 120 L plastic container and stored in a refrigerator at 4 °C to analyze the various physico-chemical features. The physicochemical parameters such as pH, temperature (T), color, electrical conductivity (EC), dissolved oxygen (DO), chemical oxygen demand (COD), biochemical oxygen demand (BOD), total suspended solids (TSS), total dissolved solids (TDS), and nitrate ( $\text{NO}_3^-$ ), phosphate ( $\text{PO}_4^{3-}$ ), sulfate ( $\text{SO}_4^{2-}$ ), chloride ( $\text{Cl}^-$ ) and seven heavy metals were tested at Environment Research Laboratory, Atomic Energy Research Establishment, Savar, Dhaka, Bangladesh. During the wastewater collecting process, the temperature was monitored with a portable thermometer. pH was tested using a portable HACH pH meter. Other parameters such as color, nitrate ( $\text{NO}_3^-$ ), sulfate ( $\text{SO}_4^{2-}$ ), and phosphate ( $\text{PO}_4^{3-}$ ) were determined in the lab using a DR-2800TM spectrophotometer. The conductivity meter (EC150, HACH) was used to determine the electrical conductivity. Biochemical oxygen demand (BOD) was determined using the dilution technique (Baird et al., 2012). The dichromate digestion technique was employed to calculate the chemical oxygen demand (COD). Mohr's silver-nitrate method was used to calculate chloride. Total solids (TS) were computed by adding SS and TDS, which were measured gravimetrically. Heavy metals (copper, cadmium, lead, iron, zinc, nickel, and chromium) were measured using an Atomic Absorption Spectrophotometer (PerkinElmer AAnalyst 300, USA).

## 2.3. Physicochemical characteristics of soil

Soil samples (0–30 cm depth) were collected from each pot after harvesting and preserved in sealed polythene bags with treatment labels for further analysis to identify the soil composition before and after irrigation with TDW. There were three replicates of the data, each based on samples taken from three locations of each pot. Soil samples were air-dried, ground, and passed through a sieve with a mesh size of 2 mm. The samples were then tested using established procedures to determine the

soil parameters (Hassan et al., 2022).

## 2.4. Pot preparation for vegetable cultivation

All of the vegetables used in the study were produced from seeds obtained from the nursery maintained by the Department of Horticulture. To avoid genetic resource claims, the plants were kept by scientific ethics. In clay pots, the seeds of vegetables were grown in a randomized complete block design (RCBD) with three replicates. The 72 pots (diameter 37 cm x height 50 cm) were loaded with 10 kg of farming soil. In each of the vegetables under investigation, two plants were planted in each of the three pots used for each treatment. All vegetables were harvested at optimal maturity, and the experiment was extended for 90 days for tomato and cauliflower, 60 days for radish, 45 days for Indian spinach, and 30 days for red amaranth. Vegetables were watered with GW (control) and TDW (7 treatments) at 6 six-day intervals throughout the full growing period of each vegetable. We tried to avoid leaching and waterlogging by using the same quantity of water for all treatments. After planting, irrigation was done every 4 days for 4 weeks, then every 6 days for 7 weeks. Finally, for the remaining two weeks of growth, irrigation was done at 8-day intervals, revealing an average of 6-day intervals. Tomatoes, cauliflower, and radishes were each watered with 15 L of experimental water, whereas radishes only received 10 L. The water needed to irrigate red amaranth and Indian spinach was 10 L and 9 L, respectively. For data collection and analysis, edible plant samples (morphological and fruit) were taken from three replications, each with three experiment pots. To control rainfall during cultivation, the experiment was conducted under the shed house.

## 2.5. Accumulation of heavy metals in vegetables

The consumable portion of plant samples (red amaranth, Indian spinach, cauliflower, tomato, and radish) watered with TDW was pre-cleaned and stored into sanitized polyethylene bags with correct tagging to estimate heavy metals concentration. About 1 kg of each vegetable was separately collected in triplicate from six randomly selected subsampling locations (two locations from each pot per treatment in each replication) and stored in isolation as per the TDW treatment. The samples were treated with acid (0.01 % HCl) and extensively rinsed with tap and distilled water to eliminate soil and particle matter in the lab. The cleaned samples were cut up with a plastic knife to dry faster. The samples were then air-dried in a hot air oven at 70 °C for 24 h to remove moisture and maintain a constant weight. The dried materials were crushed into a fine powder and sieved through a 2 mm mesh and stored in air-tight polyethylene bags and kept in desiccators until digestion and analysis. 0.5 g of dried vegetable sample was digested with a Tri-Acid combination ( $\text{HNO}_3$ :  $\text{H}_2\text{SO}_4$ :  $\text{HClO}_4$  = 5:1:1) until visible fumes were produced (Ahmad and Goni, 2010). Samples were cooled and filtered using Whatman filter paper no. 1. A total of 50 ml was obtained by adding double-distilled water to the mixture. The heavy metal content of samples was analyzed using Atomic Absorption Spectrophotometer (PerkinElmer AAnalyst 300, USA).

## 2.6. Quality control and quality assurance of data

The precision and analytical accuracy of the estimated data were checked by analysis of standard reference material (SRM) from the National Institute of Science and Technology (NIST), NISM-SRM 2709 for soil, NIST-SRM 1570 for water and NIST-SRM 1573 for plant samples. Each sample was analyzed in triplicate to assess the precision and accuracy of the AAS method. The results were found to be within 2 % of certified values for every heavy metal. The limits of detection of Cu, Zn, Fe, Pb, Cd, Ni and Cr are 0.006, 0.005, 0.05, 0.04, 0.016, 0.015 and 0.01  $\mu\text{g/L}$  respectively. If there is any data with value 0 indicates below the limits of detection.

## 2.7. Health risk assessment

The impact of TDW-irrigated crops on human health risks was assessed according to the following indices:

### 2.7.1. Bio-concentration factor (BCF) of heavy metals

The BCF of metals from cultivated soils to vegetables was calculated from the ratio between the concentration of each metal in the edible portion of the plants and the concentration of corresponding heavy metals in the respective soil. If the BCF is lower than 1, then fewer heavy metals are being transferred from the soil to the vegetables. In contrast, a BCF value greater than one suggests a greater absorption of heavy metals from the soil by the veggies that were examined (Zhang et al., 2017; Khandaker et al., 2018). It was evaluated by the subsequent formula (Samsuri et al., 2019):

$$BCF = \frac{C_{veg}}{C_{soil}} \quad (i)$$

Where  $C_{veg}$  represents the metal content in vegetables and  $C_{soil}$  represents the metal content in soil samples (mg/kg) expressed as a dry weight (DW) quantity.

### 2.7.2. Estimated daily intake (EDI)

The regular consumption of vegetables is computed by following the formula given below (Affum et al., 2020):

$$EDI = C_{veg} \times IR_{veg} \quad (ii)$$

Where EDI is the expected daily intake,  $C_{veg}$  signifies the metal concentration (mg/kg of fresh weight) in edible portions of the vegetables, and  $IR_{veg}$  represents the consumption of vegetables.

The estimated EDI was used to determine the target hazard quotient (THQ). The THQ was considered to calculate the potential danger to human health from eating heavy metal-contaminated plants (Xu et al., 2013; US EPA, 2007a,b). THQ is the ratio of the CDI-revealed dosage of a contaminant to a reference dose (RfD) (Xu et al., 2013). It is assessed by using the below equation (Affum et al., 2020):

$$THQ = \frac{CDI}{RfD} = \frac{C_{veg} \times IR_{veg} \times EF_{veg} \times ED \times 10^{-3}}{BW \times AT \times RfD} \quad (iii)$$

Where  $EF_{veg}$  indicates the exposure frequency of vegetable consumption. The  $EF_{veg}$  values for leafy (red amaranth, Indian spinach) and non-leafy vegetables (tomato, cauliflower, radish) are 100 and 50 days/year, respectively (Bangladesh Demographic and Health Survey, 2004). On the other hand,  $C_{veg}$  is the concentration of heavy metals in edible vegetable tissue (mg/kg, fresh weight). The  $IR_{veg}$  is the daily consumption of veggies, and the respective values for leafy and non-leafy vegetables are 89 and 23 g/day, respectively (Bangladesh Demographic and Health Survey, 2004). The ED is the length of exposure (73 years for adults) and BW is the body weight (52 kg for adults 25 years of age) (Bangladesh Demographic and Health Survey, 2004). AT represents the averaging time for non-carcinogens (ED 365 days per year). The RfD is the oral reference dose for each metal. It is an estimate of how much the human population is exposed to every day without a noticeable risk of health issues for life (US EPA, 2007a,b). While the chronic daily intake (CDI) (mg/kg/day) represents the population's lifelong exposure. RfD values for Zn, Fe, Cd/Pd, Ni, Cr, and Cu are respectively 0.3, 0.7, 0.001, 0.02, 0.003, and 0.3 mg/kg/day (Latif et al., 2018). If the THQ value is greater than one, there is a chance of a noncarcinogenic effect related to values. If THQ is less than one, it will be assumed to be safe for noncarcinogenic effects (Antoine et al., 2017).

### 2.7.3. Hazard index (HI)

The human health risk from metals in the diet is measured by a number called the hazard index (HI), which is the total of the target hazard quotients (THQs) calculated for various metals. (Alsafran et al.,

2021; Maurya et al., 2023). It is estimated as below:

$$HI = \sum_{i=1}^n THQ_i, i = 1, 2, 3, \dots, n \quad (iv)$$

where HI is the sum of different poisonous metal hazards. Here,  $HI < 1$  has no evident health consequence.  $HI > 1.0$  suggests a health risk.  $HI > 10$  causes significant chronic health problems.

In the US EPA Integrated Risk Information System database (US EPA, 2007a,b), there was a lacking of information on the cancer slope factor for toxic metals to identify the cancer risk. For monitoring reasons, the standard or accepted cancer risk is set between 10<sup>-6</sup> and 10<sup>-4</sup> per million (US EPA, 2007a,b). Therefore, in this study, an assumption was verified about the ultimate consequences of the health risk if one could take the standard doses of vegetables throughout their lifetime. We have reported the probable consequences of the health hazard index for adults and children after taking into consideration the recommended vegetable consumption rate for adults and children are 280 g/day (220 g non-leafy + 60 g leafy vegetables) and 232 g/day (184 g non-leafy + 48 leafy vegetables) respectively (WHO, 2007).

## 2.8. Statistical analyses

Mean values and standard errors were calculated after analyzing the data statistically using three separate repetitions of each variable. A two-way analysis of variance (ANOVA) was conducted at a significance level of 1 % to evaluate the hypothesis. The HSD Tukey mean comparison post hoc test was employed to compare the mean values of dependent variables among irrigation treatments. Further, a correlation matrix, cluster analysis, and principal component analysis (PCA) were used to identify the important contributing qualities for TDW irrigation in consideration of the health risks posed by the consumption of heavy metals-contaminated vegetables. The research data was analyzed using the R-program (version 4.1.2) with the help of the packages of "ggplot2", "agricolae", "factominer", "corrplot", and "factoextra".

## 3. Results

### 3.1. Accumulation of heavy metals in TDW

#### 3.1.1. Bioconcentration factor (BCF) in different vegetables irrigated with TDW

The BCF of heavy metals varied in relation to the vegetables irrigated with different stages of TDW (Supplementary Table 1). It has been observed that the BCF of various heavy metals in red amaranth were in decreasing order of Ni < Cr < Cd < Fe < Pb < Mn < Cu < Zn; for Indian spinach Ni < Cr < Cd < Fe < Pb < Mn < Cu < Zn; for cauliflower Cd < Cr < Ni < Pb < Fe < Mn < Cu < Zn; for tomato Cd < Cr < Ni < Pb < Fe < Mn < Cu < Zn and for radish Ni < Cr < Cd < Fe < Pb < Mn < Cu < Zn.

Meanwhile, among the irrigation water, the BCF of heavy metals (Fe, Cu, Zn, Mn, Pb, Cd, Cr, Ni) for red amaranth was found the highest in the mixed effluent (T8) followed by 2nd wash water after the dye bath drain (T4) > fixing chemical treated wastewater (T7) > neutralization treatment process wastewater (T5) > enzyme chemical treated wastewater (T3) > 2<sup>nd</sup> wash after scouring and bleaching treatment (T2) > 2nd wash after soaping treatment (T6) > groundwater (T1); for Indian spinach it was in the order of T8 > T4 > T7 > T5 > T6 > T3 > T2 > T1; for cauliflower T8 > T4 > T7 > T5 > T3 > T6 > T2 > T1; for tomato T8 > T4 > T7 > T5 > T6 > T3 > T2 > T1; for radish T8 > T4 > T7 > T5 > T3 > T6 > T2 > T1.

As we observed from the findings of the BCF of toxic metals in the TDW irrigated vegetables, Cd, Cr, and Ni were shown a consistent decreasing trend in all the studied five types of vegetables with some exceptions in Pb and Fe. While BCF of Pb was higher in radish, red amaranth, and Indian spinach and Fe was in tomato and cauliflower. Interestingly, a similar decreasing trend of the rest of the metals like Mn, Cu, and Zn was found in all the studied vegetables. Meanwhile, among

the TDW, T8, T4, T7, and T5 followed a similar trend of increasing order in all the vegetables whereas T3 and T6 were exchanged according to the vegetables and T2 remained in the lowest position followed by T1 in all cases.

### 3.2. Correlation among the accumulated heavy metals

Fig. 1(A) shows Pearson's correlation matrix for heavy metal buildup in the five varieties of vegetables irrigated with TDW. The current observation reveals that most of the collected heavy metals have positive correlations with one another, except for Cd and Ni, which have a correlation that is -41 % in the other direction. Among the estimated heavy metals, a strong positive correlation between plant toxic compounds of Cd-Pb (79 %), Pb-Cr (64 %), Cr-Ni (50 %), and Cd-Cr (48 %) was detected, whereas plant essential elements like Zn-Fe, Zn-Cu, Zn-Mn, and Cu-Fe shown a strong positive correlation with each other at the rate of 80 %, 77 %, 75 %, and 74 %, respectively (Fig. 1(A)).

### 3.3. Principal component analysis (PCA) for BCF in TDW irrigated vegetables

The first two principal components (PC1, PC2) are sufficient to account for 69.20 % of the pattern variation, as shown in Fig. 1(B) and 2 (A) where PC1 explained 46.80 % and 22.40 % variation was explained by PC2. PCA findings revealed that the highest positive loadings were recorded for Zn (90 %), Mn (80.63 %), Cr (73.05 %), Cu (72.33 %), and Fe (69.78 %) on PC1 (Dim1). Meanwhile, PC2 (Dim2) exhibited higher positive scores for Cd (80.87 %), Pb (64.1 %), and negative scores for Ni (-67.09 %). However, Cd and Pb also contributed positive scores in the PC1 axis, revealing their importance on both dimensions (with the explanation of 69.2 % of variance). Furthermore, in this model among the studied TDW stages irrigation water, T8>T4>T7>T5 contributed higher with respect to the PC1 and PC2 which were positioned in distinct than others. On the other side, T1<T2<T6<T3 contributed lower in the PC1 and PC2 which overlapped with each other (Fig. 1 B). In the case of the studied vegetables, radish was found in distinct positions with positive scores while red amaranth and Indian spinach were positioned overlapped towards the positive scores in PC1 and PC2 directions. However, tomato and cauliflower were negatively scored and positioned on opposite sides closer to the circle of correlations than that of the other vegetables (Fig. 2 A). This specified leafy, stem, and root vegetables with short duration had higher affinity to accumulation of heavy metals compared to others such as cauliflower > tomato.

### 3.4. Dendrogram of hierarchical cluster analysis for BCF in TDW irrigated vegetables

Fig. 2(B) depicts the source profile of heavy metal accumulation in vegetables from the studied TDW and the clarification of vegetables from hierarchal cluster analysis. The dendrogram displays two main clusters with several subclusters, separated by the abundance of heavy metals in vegetables grown with TDW agriculture. Broadly, grouped in cluster one (low BCF), irrigation stages of T1, T2, T3, and T6 were closely related to each other in the case of radish, Indian spinach, tomato, and cauliflower apart from red amaranth. In contrary group two (high BCF), TDW stages of T8, T4, and T7 were closely related to each other and commonly observed in all the studied 5 vegetables. However, the TDW stages such as T2, T3, T5, and T6 were also found to correlate with each other and belong to the same cluster with respect to red amaranth, Indian spinach, tomato, and cauliflower. Nevertheless, radish and red amaranth formed a distinct cluster in relation to the TDW stages of T8, T4, and T7 which are closely correlated with each other. These clusters distinguish confirmed the higher sensitivity in the order of red amaranth > radish > Indian spinach to accumulate heavy metals while irrigated with T8 (mixed wastewater) > T4 (2nd wash after dye bath drain) > T7 (fixing chemical treated wastewater).

### 3.5. Human health risk assessment

#### 3.5.1. Target hazard index (THQ), hazard index (HI), and estimated daily intake (EDI)

The EDI and non-carcinogenic risks such as THQ and HI from edible parts (23 g for leafy and 89 g for non-leafy) of TDW irrigated different vegetables are presented in Supplementary Table 2 and Table 3. The EDI of metals in the consumable parts of red amaranth (except the root), Indian spinach (leaf-stem), cauliflower (curd), tomato (fruit), and modified radish (root) in Bangladesh was compared to the acceptable upper dietary intake level (UL) of the Food and Nutrition Board of the Academy of Science, USA (Food and Nutrition Board, Institute of Medicine, 2001), the World Health Organization's guideline for Europe (WHO, 2007), and Kumar et al. (2020a,b). The EDI of the toxic metals species was higher in the order of Zn > Mn/Cu > Fe and was lower in the order of Cd/Cr > Ni > Pb in TDW irrigated vegetables of red amaranth, Indian spinach, radish, and cauliflower while exception was observed in tomato where the order was Ni > Cr > Cd > Pb. Among the TDW irrigation water, the EDI was found to be highest in the T8 treatment, followed by T7, T4 and T5 while the lowest EDI was seen in the order of T3>T2/T6>T1. Meanwhile, the order of preference for the vegetables

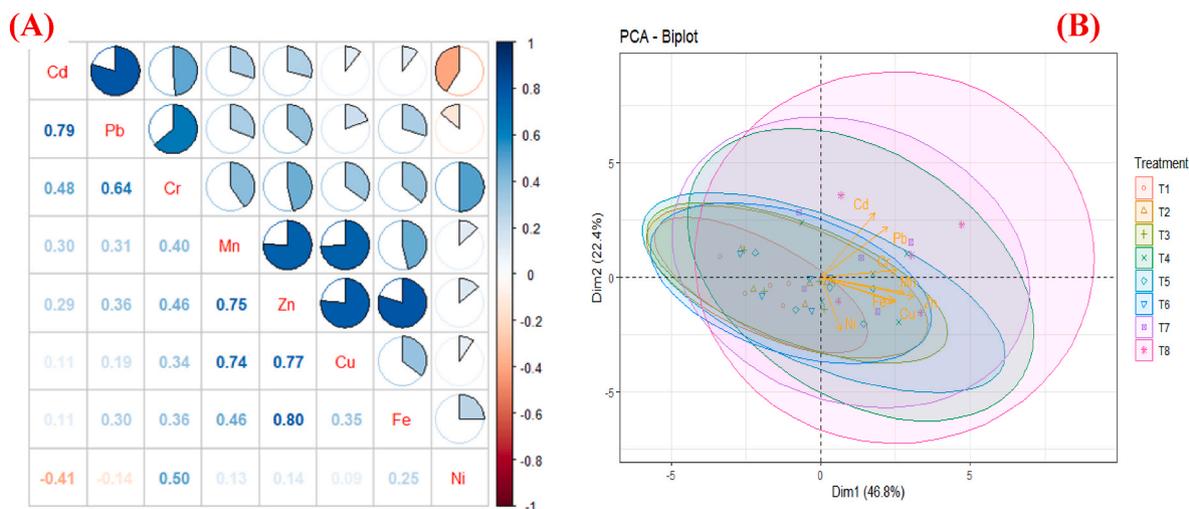
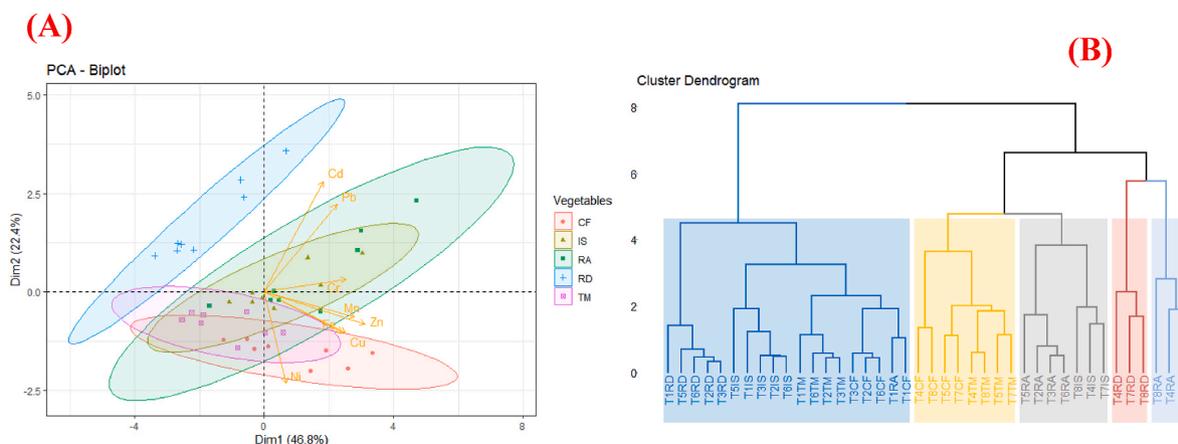


Fig. 1. Correlation among the accumulated heavy metals (BCF) in TDW irrigated vegetables (A) and Biplot of PC1 and PC2 for BCF of heavy metals in textile dyeing wastewater (TDW) stages (B).



**Fig. 2.** Biplot of PC1 and PC2 for BCF of heavy metals in TDW irrigated vegetables (A) and Dendrogram results of hierarchical cluster analysis for BCF in TDW irrigated vegetables (B).

with the highest EDI of heavy metals mentioned as follows: Indian spinach > radish > red amaranth > cauliflower > tomato. As Indian spinach was regarded to be both a leafy and non-leafy vegetable, its consumption was higher (112 g), indicating a larger EDI, whereas tomato, as a fruit vegetable, accumulated fewer heavy metals, resulting in a lower EDI. The estimated EDI ( $\mu\text{g/day/person}$ ) of the HMs accumulated in the vegetables were ranged at 712–14336, 356–8064, 506–3808, 4.90–71.91, 0–20.47, 0–21.27, 0–8.19, 0–7.65 for Zn, Mn, Cu, Fe, Cd, Cr, Ni, and Pb respectively (Supplementary Table 2).

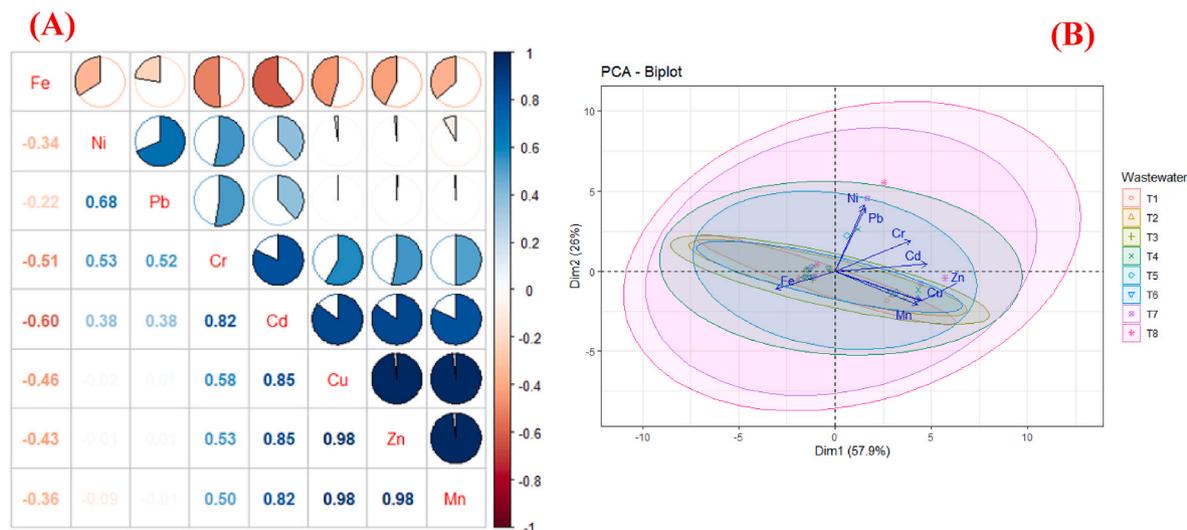
Almost similar to EDI, the trend of THQ and HI in respect of higher accumulation of metals was observed in the order of T8>T7/T4>T5, while T3>T2/T6>T1 was detected as lower in different vegetables (Supplementary Table 3). Whereas metals accumulation in different studied vegetables was also in the order of Indian spinach > radish/red amaranth > cauliflower > tomato. Interestingly, THQ for different metals slightly changed from EDI and revealed higher in the order of Mn/Zn > Cu/Cd > Cr and was lower in the order of Pb > Fe/Ni in TDW irrigated vegetables but indicated <1 that indicated within the low health risk limit. Likewise, decreased order of HI was noticed in Indian spinach (0.529–0.709) > radish (0.094–0.201) > red amaranth (0.079–0.134) > cauliflower (0.070–0.083) > tomato (0.023–0.055) and was also within the safe limit of <1.

**3.5.2. Pearson correlation for heavy metals in the THQ of TDW irrigated vegetables**

Fig. 3(A) depicts the correlation matrix of the estimated THQ of heavy metals in the TDW irrigated vegetables. The values range from negative to positive, with a preponderance of positive values, implying that most toxic metals were positively correlated. An increase in one will lead to an increase in the other, and vice versa. The higher the value, the stronger the correlation between the metals under evaluation (Shahat et al., 2023). As observed, Mn, Zn, and Cu were strongly positively correlated with each other (98%), while other metals were correlated as Cu with Cd (85%), Cd with Cr (82%), and Pb with Ni (68%). Reversely, all of these were negatively correlated with Fe, where more specifically Cd and Cr were shown a highly negative relation with Fe (−60% and −51% accordingly).

**3.5.3. Principal component analysis for metals in the THQ of TDW irrigated vegetables**

From the PCA eigenvalues, it can be seen, the first two PCs were sufficient to account for 83.9% of the total pattern of variations. In precise, 57.9% were explained by PC1 and 26% by PC2 which proved to be suitable for the present dataset. It has been observed that Cd, Cu, Zn, Mn, and Cr had the highest positive loadings on PC1 (80.1–97.7%) and only Ni and Pb (81 and 85%) had the highest positive loading on PC2,



**Fig. 3.** Correlation matrix for heavy metals in the THQ of TDW irrigated vegetables (A) and PCA-Biplot for THQ of heavy metals from the textile dyeing wastewater (TDW) stages (B).

while Fe was negative in both the PCs (Fig. 3(B)). Considering the ellipse formation analysis, positively correlated variables of the toxic metals of Ni, Pb, Cr, Cd, Zn, Cu, and Mn were grouped on the factor map, whereas the negative loading variable (Fe) was positioned on opposite sides of the plot origin (Fig. 3(B) and 4(A)). Loading of Fe was not so higher as other metals, therefore, implying a quasi-independent behavior within the group compared to other metals (Shahat et al., 2021; Al-Hazmi et al., 2023; Hasan et al., 2021;2013; Krishna et al., 2018). Furthermore, among the TDW stages irrigation water, T8>T7>T4>T5 contributed higher and formed ellipse with wider distribution, however, T1<T6<T2<T3 contributed lower represented as the shorter ellipse indicated higher to lower contributors towards health risk (Fig. 3(B)). Indian spinach and radish contributed most to positive scores, showing a higher probability with a distinct ellipse position that could affect human health through metal accumulation in edible parts (Fig. 4(A)).

3.5.4. Correlation matrix between BCF and THQ about heavy metals

About the earlier findings of the PCA for BCF (Fig. 1(B) and 2(A)) where Zn, Cd, Pb, and Ni were the most contributors among the studied metals in the accumulation of the TDW irrigated vegetables. Fig. 4(B) shows the results of the heavy metal correlation matrix between BCF (B) and THQ (T). The positive figures were dominant which indicated that the majority of the toxic metals were positively related to each other. Heavy metal BZn was positively related in chronological order with TFe, TMn, Zn, and TCu, while BCD was related to TCr, TPb, TNi, TCd, and BPb was related to TPb, TCr, TCd, and TNi. Interestingly, negative relation was detected between BMn and TNi, as well as BNi with TCd, TCu, TZn, and TMn. An increase in BCF of Mn and Ni caused a decrease in the THQ of Cd, Cu, Ni, Zn, and Mn in the edible part of the TDW irrigated vegetables (Fig. 4(B)).

3.5.5. PCA of BCF and THQ for TDW irrigated vegetables

From the PCA eigenvalues, it revealed that the first two PCs were enough to explain 60.4 % of the total variations of the dependent variables where 33.9 % being explained by PC1 and 26.5 % by PC2 (Fig. 5 (A and B)). The factor map gathered positively associated variables, whereas the negative loading variable was on opposite sides of the plot origin and showed a similar expression pattern as BCF and THQ. However, considering the irrigation of the TDW stages, mixed effluent(T8) > fixing treatment (T7)>2<sup>nd</sup>wash after bath drain (T4) > neutralization treatment (T5) showed a higher contribution in the total variations indicated higher risk towards health. Simultaneously, 2<sup>nd</sup>wash after soaping (T6), enzyme-treated water (T3), 2<sup>nd</sup>wash after scouring and bleaching (T2), and Groundwater (T1) were indicated as lower health risk contributors (Fig. 5(A)). Again, among the studied vegetables, Indian spinach, and radish contributed major towards positive scores and depicted in distinct positions, while red amaranth contributed to some extent which

exhibited a higher probability to affect the human body through the uptake of metals in their edible portion (Fig. 5(B)).

3.6. Predicting health risks from TDW-irrigated vegetables

Previous results on the HI and THQ were determined based on the individual average vegetable consumption at the presently available rate. Afterward, this study also illustrated the possibilities of the HI after considering the vegetable types (leafy and non-leafy) and individual types of adults and children. Here, adults (>25 years) and children (<17 years) were supposed to ingest leafy (23 g) (red amaranth), non-leafy vegetables (89 g) (radish, tomato, and cauliflower) and Indian spinach (112 g as both categories) grown through TDW and contaminated with heavy metals then the health risk would be predicted. The probable health risk was calculated based on the current average consumption rate (112 g/day), the recommended rate for adults (280 g/day) and children (232 g/day) of the vegetables grown with TDW irrigation incorporating both non-leafy (89, 220, 184 g/day respectively) and leafy (23, 60, 48 g/day respectively) types (WHO, 2007; BDHS, 2004). However, out of five studied vegetables, Indian spinach was considered as both leafy and non-leafy category vegetable. Three combinations of non-leafy and leafy vegetables (NL1, NL2, NL3) along with Indian spinach alone as 4th combination (NL4) were considered to estimate the health hazard index according to the present (PHI) and standard (SHI) rate for adults as well as child (CHI) hazard index on the standard rate which is depicted in Supplementary Table 4.

The findings revealed that the frequency of the hazard index increased in the order of NL1<NL2<NL3<NL4. This specified that HI may increase when a leafy vegetable (red amaranth) is taken with non-leafy vegetables in the order of fruit < flower < root, while Indian spinach (NL4) might threaten more human health alone as compared to the other studied vegetables. Although current leafy and non-leafy consumption did not exceed the safe limit (HI < 1.01) unless Indian spinach (NL4) even within the recommended dose would not consume that might be a threat for both adults and children with exceeding the safe limit (HI > 1.01) (Supplementary Table 4). The vegetables that were irrigated with the mixed effluent (T8) resulted in the highest health risk index followed by fixing treatment (T7) or 2<sup>nd</sup>wash after bath drain (T4) than that of the other TDWs. In addition, neutralization treatment (T5) irrigated vegetables indicated moderate risk with some fluctuations towards lower-higher health risk through their consumption. However, 2<sup>nd</sup>wash after soaping (T6), enzyme-treated water (T3), 2<sup>nd</sup> wash after scouring and bleaching (T2) expressed the safe position towards health risk of adults and children along with groundwater (T1) irrigated vegetable consumption.

Principal component analysis (PCA) was accomplished to reveal the confirmation of the above-mentioned health risk factors (Fig. 6(A and B)).

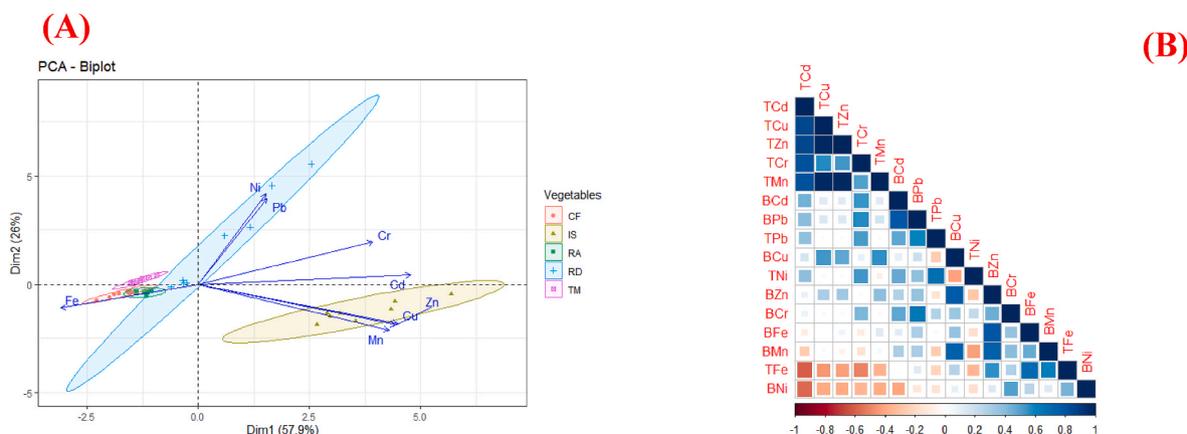


Fig. 4. PCA-Biplot for THQ of heavy metals in the TDW irrigated vegetables (A) and Correlation matrix between BCF and THQ in relation to heavy metals (B).



amaranth > Indian spinach. This indicated that leafy and stem vegetables tended to accumulate more HMs (Pb, Cd, Cr, Ni) than flower, fruit, and root vegetables due to their high translocation, high transpiration rate, and fast growth rate. Besides, the rest of the studied vegetables (radish, tomato, cauliflower) had a considerable accumulation of zinc, copper, and manganese, but a low accumulation of lead and iron, and no accumulation of any other metals. BCF values of 1–10 indicate hyper-accumulator, BCF > 0.1 to 1 indicates moderate accumulator, BCF 0.01 to 0.1 indicates low accumulator and BCF < 0.01 indicates no accumulator plant (Netty et al., 2013). Based on our BCF findings, among the studied irrigation sources of TDW, a significant amount of metal uptake was noticed in T8, T4, T7, and T5 considered as a major contributing factor for higher accumulation of heavy metals in the soil and cultivated vegetables. On the contrary, TDW stages of T2, T3, and T6 denoted comparatively less risk of accumulation and uptake of metals in the studied soils and plants and T1 was revealed as no accumulator. This accumulation pattern was corroborated by Kfle et al. (2020) in *Corchorus olerius*. Fe is less soluble than Ni, but its absorption is controlled because of its importance to plant growth (Hamilton et al., 2007). The low BCF of Cr and Ni in all vegetables was also demonstrated by Affum et al. (2020), who noted that plants have self-defense mechanisms to limit the uptake of hazardous chemicals.

The correlation matrix indicated that metals especially Zn positively correlated with Fe, Cu, and Mn, while Cd with Pb and Cr were positive and Cd with Ni was negative but Pb with Cr, Ni was positive. Principal component analysis indicated Zn, Cd, Pb, and Ni as major contributors to exploring the heavy metals accumulation in the studied vegetables. An almost similar suggestion was also announced by Gergen and Harnanescu, 2012 (Zn, Cd, Pb, and Cu) in pollution assessment. The accumulation of heavy metals in the root vegetables exhibited an increasing tendency compared to the leafy vegetables, following the order of radish < tomato < cauliflower < red amaranth < Indian spinach. But PCA indicated the order of red amaranth > radish > Indian spinach as major contributors towards positive scores. This defined those leafy plants acquire more metals (45 % of the examined metals) than others (Kfle et al., 2020; Subaihi and Shahat, 2023; Alluhaybi et al., 2023; El-Bindary et al., 2023a). Vegetables may have specialized mechanisms for absorbing and translocating metals to their leaves and roots, respectively (Affum et al., 2020). Cluster dendrogram revealed that mixed effluent (T8), fixing treatment water (T7), and second wash after bath drain (T4) showed a propensity for greater BCF with these three veggies, with red amaranth revealing the most significant effect (Fig. 2 (B)). Gary et al. (2014) reported that in Haryana State, India, leafy vegetables absorb more heavy metals than root vegetables. Leafy vegetables are vulnerable to soil dust and splashed water because of their rapid translocation, transpiration, and growth (Mochuweti et al., 2006).

The EDI, THQ, and HI of the uptake metals species followed almost a similar pattern as that of the BCF of Zn > Mn/Cu > Fe but there was a little bit changed observed in the order of Cd/Cr > Ni > Pb where Pb stands for the lowest health risk in TDW irrigated vegetables. The EDI of Zn (712–14,336), Cd (0–20.47), Ni (0–8.19), and Pb (0–7.65 µg/day/person) were lower than the recommended limit (WHO, 2007; Food and Nutrition Board, Institute of Medicine, 2001; Kumar et al., 2020a,b). Leafy vegetables, excluding cauliflower, have higher Fe accumulation than other edible parts of non-leafy vegetables, making the leaf a crucial Fe sink (Connorton et al., 2017). Moreover, due to its low solubility and chelated oxidized form (Fe<sup>2+</sup> and Fe<sup>3+</sup>), Fe, which is plentiful in soil and essential for plant development, is inaccessible to roots. Hamilton et al. (2007) found that Cd<sup>2+</sup> is very soluble in soil solution, allowing it to compete with divalent elements Ca<sup>2+</sup>, Mg<sup>2+</sup>, and Fe<sup>2+</sup> while being conveyed through crop cation transporters' membrane and active affinity. Although Singh et al. (2012) revealed no specific absorption mechanism for Cr, it is possible that the decreased accumulation of Cr, Ni, and Pb in crops' edible sections is attributable to their toxicity (Affum et al., 2020; Althumayri et al., 2023; Mohamed et al., 2021). The amounts of various metals in the different portions of vegetables were

significantly lower than the values reported by multiple authors from different regions of the world (Samuel et al., 2012; Amin et al., 2013; Huang et al., 2013; Alvarenga et al., 2014; Akoto et al., 2015).

Moreover, all metals studied had THQ values below 1 indicating a safe limit (Naughton and Petroczi, 2008). The decreased order of HI in Indian spinach (0.529–0.709) > radish (0.094–0.201) > red amaranth (0.079–0.134) > cauliflower (0.070–0.083) > tomato (0.023–0.055), were < 1 and expressed as safe for human ingestion (Pan et al., 2014). The EDI and THQ were computed based on edible parts and Indian spinach was considered both leafy and non-leafy categories. This consideration of Indian spinach (leaf and stem) increased daily ingestion rate 23 (leafy) or 89 (non-leafy) to 112 g and exposure frequency 50 (leafy) or 100 (non-leafy) to 150 days. Consequently, increased EDI and THQ as well as HI than other vegetables. BCF of radish was the lowest, while THQ of radish was ranked 2nd indicating the higher tendency of accumulating heavy metals in the root part. In advance, BCF of Zn, Pb, Cr, and Ni were sturdily correlated with all the metal concentrations of THQ in edible parts. In most of the cases, there was positive relation, while BCF of Zn was negatively correlated with Cu and Cd of THQ. PCA also exhibited the logical confirmation of the above-mentioned discussion. It also expressed that T8, T7, T4, and T5 TDW stages were comparatively more contributors to other stages.

Therefore, individual uptake of different TDW irrigated vegetables was not too much threatened for human health. The current average vegetable consumption in Bangladesh is 112 g daily (23 g leafy and 89 g non-leafy) and recommended rate for an adult is 280 g (60 g leafy and 220 g non-leafy) and 232 g (48 g leafy and 184 g non-leafy) for children. Human health risks from eating TDW irrigated vegetables at both the maximum safe dosage (PHI) and the safe doses for an adult and children (SHI and CHI) were calculated, and the results showed that PHI was under the safe limit (< 1.01) but SHI crossed the limit for NL4 (Indian spinach alone) under all TDW stages even though including ground-water. Non-leafy and leafy combination, NL3 (radish and red amaranth) indicated the next higher risk for adult health. An almost similar risk was detected for the child from both the combinations of vegetables NL3 and Indian spinach alone NL4 consumption. Comparatively, a higher risk was revealed from the irrigation sources of T8, T7, and T4, while the other TDW stages have a moderate to low lifetime risk of exposure. Nonetheless, the bioaccumulation of heavy metals in human tissues and organs raises concerns for human health (Horiguchi et al., 2004). The present study was based on only some representative vegetable consumption, however, all other sources of food (cereal, pulse, fruit, animal, etc.) from other diversified contaminated sources should be carried out in the future.

## 5. Conclusions

This study showed that the vegetables irrigated with TDW were contaminated with heavy metals, where truly toxic metals accumulated in lower concentrations in the order Cd/Cr > Ni > Pb, and metals deemed necessary for plant growth accumulated in higher concentrations at various edible parts of vegetables in the sequence Zn > Cu > Mn > Fe. Conversely, the levels of heavy metals in the edible portions of different vegetables differed, with leafy plants (like red amaranth) showing a greater tendency to accumulate heavy metals than non-leafy vegetables (including radish, Indian spinach, tomato, and cauliflower). The analyzed vegetables' BCF, THQ, and HI values for the specific hazardous metals (Cd, Cr, and Ni) were all less than one, suggesting that the plants took in the metals but did not accumulate them. As a result, eating at the current rate poses no damage to human health. However, among the TDW irrigation sources, T8, T4, T7, and T5 showed a higher uptake of heavy metals than T2, T3, and T6, which indicated a relatively lower danger of metal accumulation and uptake in the irrigated vegetable plants. Consuming vegetables irrigated with T8, T7, and T4 poses a greater risk to children than adults. This risk is heightened when both leafy (red amaranth) and non-leafy vegetables (radish, tomato,

cauliflower) are consumed together, or when only non-leafy vegetables (like Indian spinach) are consumed. These findings indicate that it is not advisable to reuse the T8, T7, and T4 stages of TDW without ETP for vegetable cultivation. Instead, it is recommended to use the T2, T3, and T6 stages TDW without ETP in order to ensure the production of safe food and promote excellent human health.

#### CRedit authorship contribution statement

**Jahidul Hassan:** Formal analysis, Investigation, Validation, Writing – original draft. **Md. Mijanur Rahman Rajib:** Data curation, Methodology, Resources. **Md. Noor-E-Azam Khan:** Data curation, Methodology. **Shahjalal Khandaker:** Methodology, Validation. **Md. Zubayer:** Formal analysis, Resources. **Kazi Raghieb Ashab:** Methodology, Validation. **Takahiro Kuba:** Project administration, Resources, Software. **Hadi M. Marwani:** Investigation, Project administration, Visualization. **Abdullah M. Asiri:** Project administration. **Md. Munjur Hasan:** Methodology, Resources, Software. **Aminul Islam:** Formal analysis, Investigation, Methodology. **Mohammed M. Rahman:** Funding acquisition, Resources. **Md. Rabiul Awual:** Conceptualization, Supervision, Writing – review & editing, Funding acquisition, Writing – original draft.

#### Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

#### Data availability

The data that has been used is confidential.

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#### Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.jenvman.2024.120206>.

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