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Health risk assessment for heavy metal accumulation in leafy vegetables grown on tannery effluent contaminated soil

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ABSTRACT

Accumulation of metals (Cr, Zn, Ni, Cd, and Cu) in leafy vegetables cultivated on tannery effluent contaminated soil and agricultural land soil were determined with an Atomic Absorption Spectrophotometer (AAS). The values of risk factors for the human population were studied, where metals were transferred from tannery effluent to plants via effluent contaminated soil and finally, transmitted to human body through the consumption of these metal accumulated leafy vegetables. Leafy vegetables, namely Stem amaranths (*Amaranthus lividus*), Spinach (*Spinacia oleracea*), Red amaranths (*Amaranthus gangeticus*), Jute mallows (*Corchorus capsularis*), Water spinach (*Ipomoea aquatica*), and Malabar spinach (*Basella alba*) were cultivated on the soils collected from downstream of Hazaribagh tannery area and Keraniganj agricultural land. The study revealed that the metal contents in contaminated soil exceeded the permissible limits recommended by WHO/DoE. Tannery effluent contaminated soil was found more polluted than the agricultural land soil. Metal contents in leafy vegetables cultivated on contaminated soil were higher than that of agricultural soil and exceeded the permissible limit, particularly in the case of Cr (125.50–168.99 mg/kg Dw) and Cd (0.19–0.83 mg/kg Dw). Metal content order was found as Cr>Zn>Ni>Cu>Cd for contaminated soil and Zn>Cr>Cu>Ni>Cd for agricultural land soil. The metal accumulation and translocation were found in vegetables in the order of Spinach>Water spinach>Malabar spinach>Jute mallows>Red amaranths>Stem amaranths. The analyses also revealed that the metal translocation rate in the plants of contaminated soil was higher than that of non-contaminated agricultural soil. The values of each risk index exceeded 1 in case of vegetables cultivated in contaminated soil. Therefore, the possible threat of chronic and carcinogenic diseases emerged if those polluted vegetables would be consuming as daily diet.

1. Introduction

The desire for a luxurious life with the emerging benefits from industrialization might bring comfort in our daily lifestyle, however it has led the environment towards successive deterioration. Heavy metals discharged from various industries play a critical role in this context and bring about a serious threat to public health [1,2]. Tanneries have been recognized as a possible source of producing heavy metal load to the environment even though having a significant contribution to the economic development and employment in many countries [3,4]. Leather processing from animal hides/skins includes a series of mechanical and chemical operations such as soaking, liming/unhairing, de-liming, bating, pickling, skin degreasing, tanning, post-tanning, and dyeing of raw hides/skins using different types of chemicals e.g., sodium hydroxide, sodium hypochlorite, enzymes, lime, chlorides, sulfuric acid, formic acid, ammonium salts, different metallic salts, and organic chemicals, etc. [5]. A large amount of chromium (Cr) salt named basic chromium sulfate (BCS) is used in the tanning process to provide better hydrothermal stability and some unique properties to the tanned leathers [6]. Heterocomplex iron (Fe) salts are used instead of basic chromium sulfate (BCS) to achieve sufficient waterproofing effect and strengthen the loose fibers through higher exhaustion of tannage within a short tanning span [7]. Furthermore, organometallic salts of different heavy metals such as chromium (Cr) cadmium (Cd), copper (Cu), lead (Pb), etc. are extensively used as coloring agents and mordant during leather dyeing and in other post-tanning and finishing operations to

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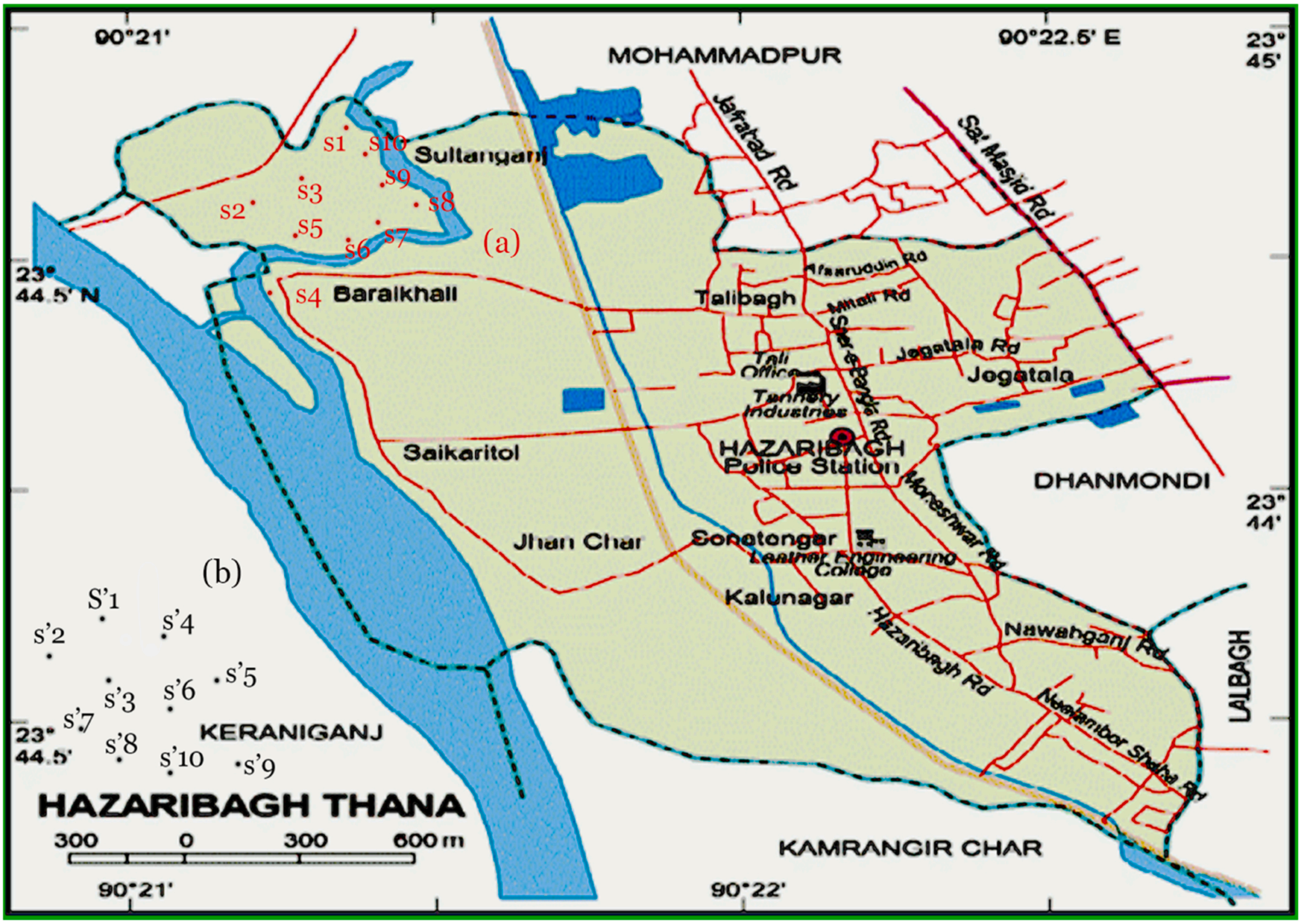

Fig. 1. Locations of the (a) Tannery effluent contaminated and (b) Non-contaminated agricultural reference soil sites.
Adapted upon modification from Ali et al. [43].

make the leather suitable for making various leather products such as bags, shoes, wallets, upholstery furniture, etc. [8,9]. As a result, a substantial amount of different heavy metals like Cr, Cd, Cu, Ni, Pb, Ba, As, Fe, etc. are discharged with tannery wastewaters [10]. Besides, the dumping of tannery solid wastes in land filling, use of treated tannery effluents as irrigation water, and burning of leather wastes are some common pathways of heavy metal transmission into natural resources [11–13].

The ultimate fate of these metals is to deposit into the soil and water, might get transferred to different plant tissues (stem, root, leaf, etc.) as some of them act as essential micro-nutrients for plant's growth, and finally introduced to human food chain if those plants are consumed [14, 15]. Previous studies have stated that heavy metal-containing industrial wastewaters are responsible for bringing about the toxicological effect on natural elements (soil, water) and other living species [16–18]. Singh and Kalamdhad [19] showed that heavy metals (e.g., Cu, Ni, Cd, Zn, Cr, and Pb) reduce the physicochemical parameters (e.g., organic matter, clay contents, and pH) of soil and show toxic symptoms towards soil living biota by decreasing soil microorganism (leguminous bacteria) number that hamper their enzymatic and metabolic activities, and stimulate the formation of reactive oxygen species (ROS), which causes lethal consequences on other non-vertebrates (earthworms) and reptiles (snakes, lizards, rats) living in soil, and in fishes and other aquatic species when they mix up with surface water through agricultural runoff. Moreover, the bioavailability of some heavy metals (Cu, Zn) causes a degradation in the aquaculture population through physical deformation in water species and cellular damages in both aquatic flora and fauna [20]. Apart from environmental pollution, some of the heavy metals are also reported as the topmost hazardous elements that result in deleterious influences on plant materials and human health by hampering normal cellular activities. Scholars have observed that the plants grown around industrial zones or waste dumping yards uptake more heavy metals or trace elements as compared to those plants grown around nonindustrial areas; store them in their vegetative and reproductive parts; and exhibit toxicity symptoms e.g., necrotic lesions, and chlorosis as a result of prolonged exposure [21,22]. These heavy metals can easily accumulate to different plant organs (e.g., roots, leaves, seeds) and show toxic effects in long-term exposure. For instance, the toxicity of chromium and cadmium to plant brings about less seed germination, alteration in different organs (root, stem, leaves), shackles in development and metabolic performance, inhibit photosynthesis ratio through chlorosis, hinder the transportation of nutrients and water, and advances cellular decay [23,24]. Although copper, zinc, lead is documented as essential micronutrients for plant's development, the excess deposition of these metals causes extreme phytotoxic effects like stunting, leaf discoloration, necrosis, curling of young leaves, death of leaf tips, lipid peroxidation, inhibition in ATP production during respiration, DNA damage due to excessive ROS generation and many more [25–27]. Consequently, consumption of metal contaminated vegetables can cause a negative impact in the food chain and may extend a negative influence on human health which can be an obstacle to ensuring safer foods and also achieving the UN's Sustainable Development Goal-2 (Zero hunger), Goal-3 (Good health and well-being) and Goal-12 (Responsible consumption and production) for many countries [28].

Many toxicological reports have proclaimed that the daily intake of metal contaminated vegetables at an excess rate can cause carcinogenic, mutagenic, neurotoxic, and teratogenic effects in the human body that result in severe health problems; like anemia, hypertension, malnutrition, cancer, organ disability, infertility, cardiovascular diseases, metabolic and psychological disorder which may lead to death at prolonged exposure [29–31]. The risk factors related to heavy metals' contamination in food mainly arise from agro-based products like rice, crops, and vegetables and have affected many regions around the globe, exclusively in developing countries like Bangladesh [32,33]. Although vegetables are recognized as one of the most popular daily diets due to their enrichment with nutritious elements (e.g., fiber, minerals, and vitamins), the metal uptake rate of leafy vegetables was found to be the highest among all crops and act as a bio-indicator of soil pollution [34, 35]. Therefore, particular studies on metal contamination in leafy vegetables must be prioritized to draw attention to such health risks and raise public awareness for its remedy since only a few publications are available on this issue in the context of Bangladesh [29,36–38].

Concerning this susceptible situation, an attempt had been taken to determine the amount of selected heavy metals in common leafy vegetables cultivated on tannery effluents contaminated soil and agricultural land soil to investigate the gateway of heavy metal accumulation in the human body. Specifically, this study was envisioned at

a) Assessment of metal content in both tannery effluent affected soil and agricultural land soil.
b) Measurement of the metal translocation rate from soil to plant.
c) Evaluation of the potential health risk factors of metal uptake through vegetable consumption with respect to risk indices (e.g., daily metal intake, health risk index, non-carcinogenic risk).

2. Experimental

2.1. Study area

The study was conducted with soils collected from canal side at the downstream of Hazaribagh tannery area under Dhaka South City Corporation (DSCC) and Keraniganj agricultural area, Bangladesh. The areas are located at a latitude in between 23°43′ and 23°44′ N and longitude in between 90°21′ and 90°22.5′ E [39]. Approximately 185 of the total 220 tanneries had been situated around 4 km^2 areas, with an average daily production of 220 metric tons of finished leathers after their foundation (1965) to continuous development, before the tanneries were completely relocated to Hemayetpur, Savar by the fiscal year 2017–18 [40]. During production days, the tanneries daily produced around 20000 m^3 of tannery effluents from various chemical processes, and they were directly discharged to the Buriganga River that contributed hazardous impact on river ecosystem and water quality for several decades [41,42].

The sampling points for soil collection are shown in Fig. 1. The main discharge point of tannery effluents located near the Beribadh (a) was selected for collecting contaminated soil samples. It was under the direct influence of tannery effluents for several decades and directly linked with the Buriganga River by a narrow canal. In the same way, reference soil samples were collected from an agricultural land located at Keraniganj (b), outskirts of the city which was comparatively contamination free than the site (a).

2.2. Preparation of soil bed and cultivation of vegetables

Ten soil samples (n = 10) were collected from each of the tannery effluent contaminated area and non-contaminated agricultural area. About 10 kg soil was collected from each point up to 15 cm depth (0–15 cm) with a stainless-steel auger. A minimum distance of 5 m was maintained between two sample points. All the soil samples from each category i.e., tannery effluent contaminated area and non-contaminated agricultural area were dried, grinded, mixed, and homogenized separately before preparing the seed beds. Thirty parallel seed beds were prepared with those tannery effluent contaminated soil and non-contaminated agricultural soils in labeled plastic pots prior to cultivating the leafy vegetables (number of set, n = 5/vegetable). Seeds of different vegetable plants were collected from Bangladesh Agricultural Development Corporation (BADC) in labeled plastic bottles. The collection bottles were dipped overnight into diluted nitric acid solution, washed with running water for several times to reduce surplus acid, and dried properly to disinfect them before collecting the seeds.

Six leafy vegetables viz., Stem amaranths (*Amaranthus lividus),* Spinach *(Spinacia oleracea),* Red amaranths *(Amaranthus gangeticus),* Jute mallows *(Corchorus capsularis),* Water spinach *(Ipomoea aquatica),* and Malabar spinach *(Basella alba)* were cultivated in the collected soils in labeled pots following pot farming method, suggested by Dobson [44]. No chemical fertilizer or plant protection product was used in cultivation of vegetables to avoid external heavy metal contamination. In addition, only harvested rainwater was used during watering plants. All plants were cultivated in the same manner under natural environment. A proper care was taken during cultivation of the vegetables and preparing edible parts of vegetable for analysis.

2.3. Sample preparation

Non-soil particles like stones, wooden pieces, debris, etc. were removed from each soil sample manually. About 100 g of soil sample was dried at 95 °C in an air woven for 48 h and ground into powders. Soil powders were sieved with a nylon mesh (2 mm) to remove non-granular matters. The samples were then homogenized and stored in a labeled polythene sampling bags for wet digestion at − 15 °C and to prevent any chemical change.

300 g edible parts of cultivated vegetable species were collected randomly and packed in polyethylene bags. The collected edible top parts and leaves were then washed with running water and subsequently dipped into diluted HCl (0.01 N) for few minutes. Then the samples were thoroughly washed with deionized water for removing airborne pollutants and cut into small pieces with a sharp stainless-steel knife. The cut pieces were completely oven dried at 70–80 °C, crushed, sieved, and stored in airtight labeled plastic containers until experiment.

2.4. Sample digestion

The digestion of each vegetable and soil sample was carried out following the standard method. A known amount (0.5 g) of each vegetable and soil samples was digested on a hot plate (Jeo Tech TM-14SB) at 80 °C with 14 ML concentrated acid mixture (HNO_3: H_2SO_4: $HClO_4$ = 5:

Table 1
Instrumental operating condition for metal analysis.

Metals	Wavelength (nm)	Slit width (nm)	Lamp type	Lamp current (mA)	Detection Level (mg/L)	Range
Cr	357.87	0.7	HCL	7	0.31	5.0
Zn	213.86	0.7	EDL/HCL	5	0.084	1.0
Ni	232.00	0.2	Iron containing ML	4	1.7	2.0
Cd	228.80	0.7	EDL/HCL	4	0.11	2.0
Cu	324.75	0.7	Nickel/Iron containing ML	4	0.45	5.0

Table 2
Analytical results obtained on certified reference material IAEA 433.

Metals	Certificate Value (mg/kg)	Observed Value (mg/kg)	Precision CV (%)	Recovery (%)
Cr	136	133	2.205882	97.79412
Zn	101	93	7.920792	92.07921
Cu	30.8	29	5.844156	94.15584
Ni	39.4	36.01	8.604061	91.39594
Cd	0.153	0.145	5.228758	94.77124

1: 1) to achieve a transparent solution. The solutions were filtered with Whattman No. 41 filter paper and the further diluted to 25 ML with DI water and stored at ambient temperature.

2.5. Metal analysis

2.5.1. Instrument and operating condition

An Atomic Absorption Spectrophotometer (Model: Varian AA240FS) was subjected to measure the concentration of heavy metals in soil and vegetable samples. The functional parameters viz. alignment of lamp and burner, adjustment of slit width, and wavelength were optimized by following the instrument instruction to obtain results at the highest signal intensity. The flame condition was kept favorable by using acetylene and controlling the airflow rate. The operating conditions for the quantification of Cr, Zn, Ni, Cd, and Cu in AAS were recorded as showed in Table 1.

2.5.2. Machine calibration

The metal assessment in examined samples was conducted by using the calibrated curves for each heavy metal. Standard solution of each metal was procured from Merck, India. Standard solutions were diluted with deionized water to make appropriate working standard solution for metal detection. Samples were aspirated through a nebulizer and the absorbance was measured with a blank reference. The calibration curve was obtained using standard samples (containing 0.5, 1.0, 1.5, 2.0, and 2.5 mg/L standard solutions for each metal). The correlation coefficient had determined, and the samples had to be diluted many folds to keep the results in the analytical range.

2.5.3. Quality assurance and quality control

Assessment of contamination and reliability of data was done as a part of quality control measure. Blank, quality control standard samples were examined after every ten samples for ensuring that obtained results are within the range. The Certified Reference Materials (CRM) for every metal obtained from the International Atomic Energy Agency (IAEA) were used to check for accuracy and precision of analysis of each metal in soil matrix and for vegetables samples spike samples are used to check recovery. A good agreement was observed between analytical results and certified values of the CRM, with the percentage recoveries ranging from 91% to 97%. The precision of the analytical procedures expressed as the coefficient of variation (CV) was within 10% for all the metals analyzed by AAS (Table 2). All analyses were carried out in triplicate, and the results were expressed by the mean value.

2.6. Data analysis

2.6.1. Pollution load index

The pollution load index (PLI) is used to determine the soil contamination factor by each heavy metal [45]. The following equation was used to calculate PLI value of the contaminated site [46]:

$$\mathrm{PLI} = \frac{C_c}{C_r} \tag{1}$$

Here, C_c= heavy metal's concentration in contaminated soil and C_r = heavy metal's concentration in reference soil. The categorization of PLI values is given below [46]:

PLI 0–1 = no pollution; PLI 1–2 = uncontaminated to moderately contaminated; PLI 2–3 = moderately contaminated; PLI 3–4 = moderately contaminated to highly contaminated; PLI 4–5 = highly contaminated; and PLI > 5 = very highly contaminated.

2.6.2. Metal transfer factor

The metal transfer factor (MTF) represents the possible bioavailability of a particular metal at a specific plant part [47]. It is the ratio of metal concentration in plant tissue (mg/kg Dw) e.g., stem, leaf, root, etc. to metal concentration in soil (mg/kg Dw) [48]. Metal transfer factor of Cr, Zn, Ni, Cd, and Cu was calculated by using the following equation [49]:

$$\mathrm{MTF} = \frac{C_{Plant}}{C_{Soil}} \tag{2}$$

Here, C_{plant}= metal concentration in plant tissue (mg/kg Dw), C_{soil}= metal concentration in soil (mg/kg Dw). The translocation of each metal from soil to plant was measured for all vegetable samples that were grown in both the contaminated and non-contaminated agricultural soil.

2.6.3. Metal pollution index

The metal pollution index (MPI) is the assessment of the overall metal load in each vegetable growing in at a particular site. It is also known as the geometric mean of the total concentration of all metals in edible parts of the vegetables. To calculate MPI of the vegetable samples (grown in both the contaminated and non-contaminated agricultural soil), the following equation was used [13]:

$$\mathrm{MPI} = (C_1 \times C_2 \times . \times C_n)^{\frac{1}{n}} \tag{3}$$

Here, C_n = concentration of 'n' number of metals in the samples (Here, n = 5).

2.6.4. Health risk assessment

2.6.4.1. Daily Intake of Metals.

Daily intake of metals (DIM) represents the relative phyto-availability of metals [50]. The following equation was used to determine the daily intake of metals through vegetable consumption [51]:

$$DIM = \frac{\left(C_{metal} \times C_{factor} \times C_{\mathrm{int}\ ake}\right)}{B_{weight}} \tag{4}$$

Where, C_{metal}= the concentration of heavy metals in vegetables (mg/kg), C_{factor}= conversion factor to convert the fresh weight of vegetables to dry weight, C_{intake}= ingestion rate.

In this study, the ingestion rate for leafy vegetables was considered 0.0385 kg/person/day [52], conversion factor (0.085) was used for the selected vegetable samples [53], and B_{weight}= the average body weight, considered 70 kg (adults) [54].

2.6.4.2. Health Risk Index.

Health risk index (HRI) represents the susceptible health risk through continuous exposure to heavy metal that further leads to a chronic hazard [55]. The index is a numerical value where higher numbers represent higher risk. The following equation was used to calculate HRI for each leafy vegetable [56]:

$$HRI = \frac{DIM}{R_f D} \tag{5}$$

The R_fD values for Cr, Zn, Ni, Cd and Cu were 1.5, 0.3, 0.02, 0.001, 0.04 mg/kg/day respectively [57]. The HRI value less than 1 indicates population safety whereas it may turn to a concerning matter if HRI value is in between 1 and 5 [58].

Health risk index may be carcinogenic and non-carcinogenic risk.

Table 3
Concentration of heavy metals (Mean ± SD) in soil samples (mg/kg Dw).

Metals	Sites		Permissible levels in soil*	Permissible levels in soil**	t-test***	PLI
	Effluent contaminated soil	Non-contaminated agricultural soil				
Cr	738.62 ± 0.06	48.96 ± 0.11	100	100	11.4	15.09
Zn	99.13 ± 0.003	64.21 ± 0.09	300	200	3.29	1.543
Ni	17.62 ± 0.40	10.35 ± 0.5	50	50	1.88	1.702
Cd	7.21 ± 0.10	3.86 ± 0.02	3	1.5	2.04	1.87
Cu	22.28 ± 0.005	17.20 ± 1.08	140	60	1.33	1.29

* WHO (2001) [63],
** DOE (2015) [64],
*** $p < 0.001$.

Non-carcinogenic risk is represented as Hazard quotient (HQ) [59]. It is the ratio of the potential exposure to a substance and the level at which no adverse effects are expected. The HQ is calculated according to the following equation [60]:

$$HQ = \frac{EDI}{R_f D} \tag{6}$$

$$EDI = \frac{C \times IR \times EF \times ED}{BW \times AT} \tag{7}$$

Here, C= metal concentration in vegetable (mg/kg Dw), IR= Ingestion rate of leafy vegetables (0.0385 kg/person/day), EF=Exposure frequency (365 days/year), ED= Exposure duration= 72 years (adults) [61]. $R_f D$= oral reference dose, BW= average body weight (70 kg), AT= average exposure time for non-carcinogenic effects= (365 days/year × number of exposure years (72)) = 26280 days.

The following equation was used to estimate hazard index (HI) [60]:

$$HI = \sum_{k=1}^{n} HQ_K \tag{8}$$

The HI value < 1 indicates less health hazard, while the value of 1–5 indicates a concerned health hazard level and HI> 10 indicates chronic health hazard [62].

2.7. Statistical analysis

The recorded data were statistically analyzed by SPSS version 12. Descriptive analysis was represented to narrate the scenario of soil pollution and metal translocation in plants as compared to standards. Paired t-tests were carried out to determine the significant differences between heavy metals concentration in soil and vegetable samples of both sites. A one-way analysis of variance (ANOVA-1) test was conducted to estimate the homogeneity of the recorded data and validation of results.

3. Results and discussion

3.1. Heavy metals in soil

Heavy metal concentrations in soil collected from Keranigonj agricultural land and downstream of Hazaribagh tannery area (Veribadh canal side) were presented in Table 3. The metal content in the soil of Hazaribagh canal side was higher than that of Keranigonj agricultural land site and the concentration of Cr (738.62 ± 0.06 mg/kg Dw) and Cd (7.21 ± 0.10 mg/kg Dw) was beyond the permissible limit. However, the metal content in agricultural soil was found less than the threshold limit except Cd which was slightly higher [63,64]. The descending order of metal contents was Cr>Zn>Ni>Cu>Cd for contaminated soil and Zn>Cr>Cu>Ni>Cd for agricultural soil. The PLI pointed out a significant variation in the accumulation of chromium in contaminated soil (Table 3) and exceeded the tolerable limit in following order Cr>Cd>Ni>Zn>Cu. The quantity of chromium and cadmium, including their PLI in the soil of Hazaribagh area was higher than any other metal which was similar to the findings of previous research works [65–67]. This is because of the deposition of abundant chromium from the discharged tanning effluents, as 90% tanning industries use basic chromium sulfate for tanning process [6,68]. The extensive application of cadmium containing dyes and pigments in leather dyeing and finishing is the main basis of cadmium deposition in the soil of Hazaribagh, since these coloring components impart a vibrant impression on dyed substrates [69]. It can be understood from t-test values, that the metal content values obtained from both sides are not significantly different.

Table 4
Heavy metal values (Mean ± SD) in vegetables (mg/kg Dw).

Vegetables	Cr	Zn	Ni	Cd	Cu
Tannery effluents contaminated soil					
Stem Amaranths	125.20 ± 0.76	29.73 ± 0.39	4.28 ± 0.56	0.19 ± 0.69	2.95 ± 0.77
Spinach	168.99 ± 0.26	36.24 ± 0.33	5.49 ± 0.82	0.83 ± 0.57	4.03 ± 0.37
Red Amaranths	132.96 ± 0.33	30.93 ± 0.45	4.34 ± 0.95	0.26 ± 0.22	3.39 ± 0.92
Jute Mallows	139.65 ± 0.70	33.36 ± 0.48	4.79 ± 0.78	0.46 ± 0.28	3.67 ± 0.66
Water Spinach	154.96 ± 0.88	34.48 ± 0.27	5.14 ± 0.29	0.42 ± 0.15	3.54 ± 0.84
Malabar Spinach	159.32 ± 0.93	34.64 ± 0.64	5.27 ± 0.38	0.32 ± 0.71	3.83 ± 0.76
Non-contaminated agricultural soil					
Stem Amaranths	1.26 ± 0.39	13.12 ± 0.68	2.12 ± 0.60	0.0051 ± 0.88	2.83 ± 0.66
Spinach	2.03 ± 0.20	14.51 ± 0.50	3.13 ± 0.31	0.013 ± 0.26	4.12 ± 0.23
Red Amaranths	1.41 ± 0.65	13.31 ± 0.79	2.45 ± 0.62	0.005 ± 0.11	3.13 ± 0.59
Jute Mallows	1.64 ± 0.89	15.72 ± 0.62	2.57 ± 0.77	0.007 ± 0.95	3.49 ± 0.76
Water Spinach	1.91 ± 0.72	14.81 ± 0.65	3.04 ± 0.56	0.008 ± 0.77	4.03 ± 0.85
Malabar Spinach	1.75 ± 0.61	15.38 ± 0.28	2.76 ± 0.37	0.007 ± 0.84	3.97 ± 0.30
Threshold limits[63]	2.30	50	10	0.02	40
F value*	9.91	6.01	4.99	1.667	2.96

* $p < 0.01$, that depicted that the variables show no significant difference.

3.2. Heavy metals in vegetables

Heavy metal contents in vegetables cultivated on the soil collected from the contaminated and non-contaminated agricultural sites were presented in Table 4. Analysis of heavy metal contents showed a noticeable variation in the vegetable samples. Metal accumulation in vegetables grown in contaminated soil was found higher than that of the reference agricultural soil. Total heavy metals contents in vegetables cultivated in both soil was found in descending order as follows: spinach>water spinach>Malabar spinach>jute mallows>red amaranths>stem amaranths.

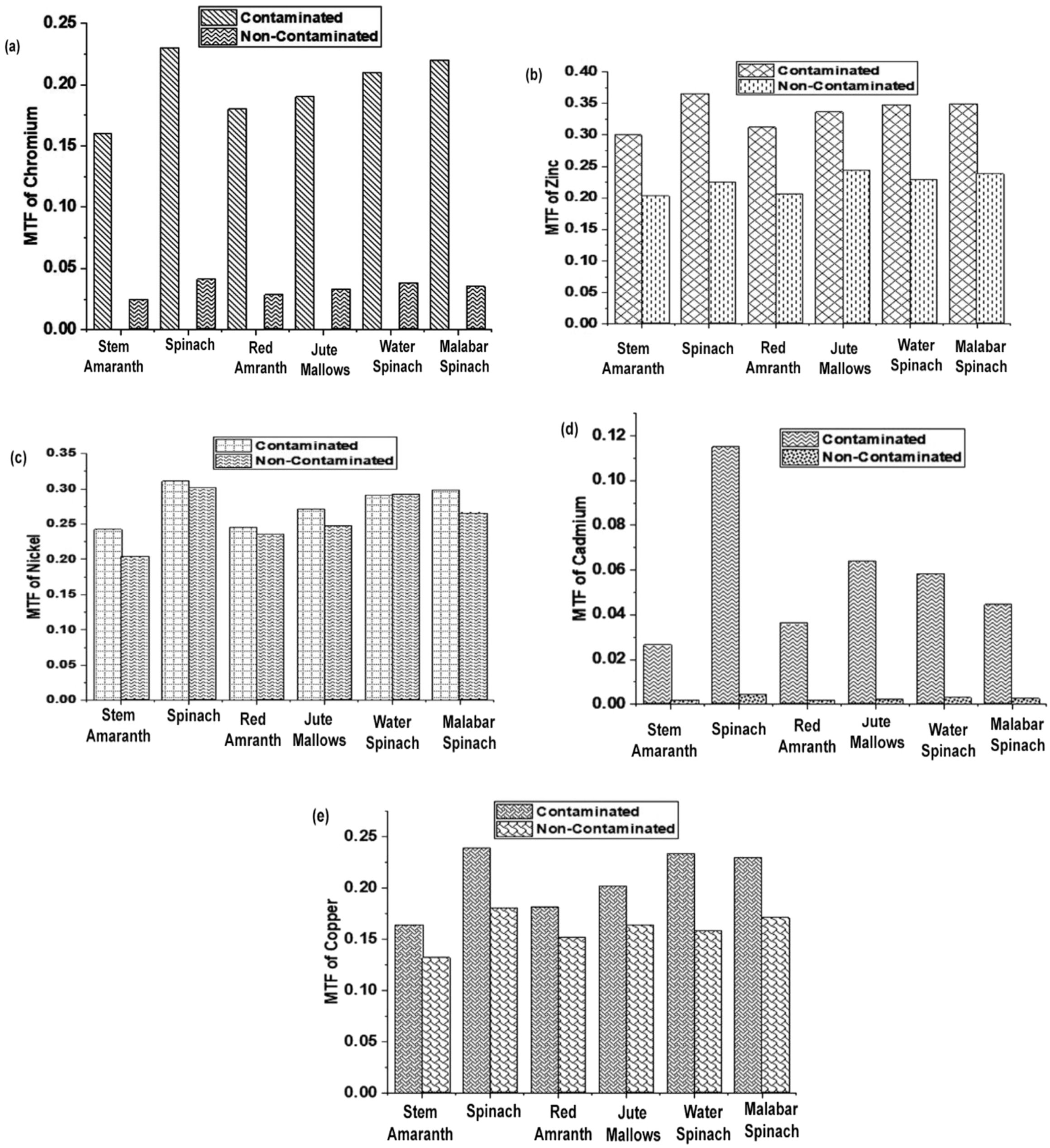

Fig. 2. Metal Transfer Factor of (a) Cr, (b) Zn, (c) Ni, (d) Cd and (e) Cu in vegetables grown in both the tannery effluent contaminated soil and non-contaminated agricultural soil.

Chromium and Cadmium contents of the vegetables grown in contaminated soil were higher than the threshold limit (2.30 mg/kg Dw for chromium and 0.02 mg/kg Dw for cadmium) value recommended by World Health Organization (WHO) [63]. Among these vegetables the highest chromium content was found in spinach (168.99 ± 0.26 mg/kg Dw) followed by water spinach>Malabar spinach>jute mallows>red amaranths>stem amaranths. The cadmium content was found highest in jute mallows (0.46 ± 0.28 mg/kg Dw) followed by water spinach>Malabar spinach>spinach>red amaranths>stem amaranths. The overall metal content in vegetables cultivated in contaminated source was in the order of Cr>Zn>Ni>Cd>Cu.

On the other hand, the accumulation of Zn in the vegetables cultivated in the reference soil was found higher compared to other metals. The highest Zn content was found in jute Mallows (15.72 ± 0.62 mg/kg Dw) followed by Malabar spinach>water spinach>spinach>red amaranths>stem amaranths. The accumulation of heavy metals in vegetables grown on reference agricultural soil were in the order of Zn>Cr>Cu>Ni>Cd and were within the permissible limit.

3.3. Metal translocation in plants from soil

Metal transfer from soil to plants is a principal factor of heavy metal exposure in human food chain and describes the possible availability of any specific trace element in any plant part. The Metal Transfer Factor (MTF) of heavy metals (Cr, Zn, Ni, Cd and Cu) of cultivated vegetables in both the contaminated and agricultural soil was represented in Fig. 2.

The metal translocation was observed higher in vegetables grown in contaminated soil than those of the referenced soil from the vivid analysis. Among these vegetables, the MTF was higher in spinach and Malabar spinach. The MTF in spinach was found in the order of Cr (0.41)>Zn (0.37)>Cd (0.32)>Ni (0.31)>Cu (0.18) and in Malabar spinach the MTF was in the order Cr (0.40)>Zn (0.35)>Cd (0.35)>Ni

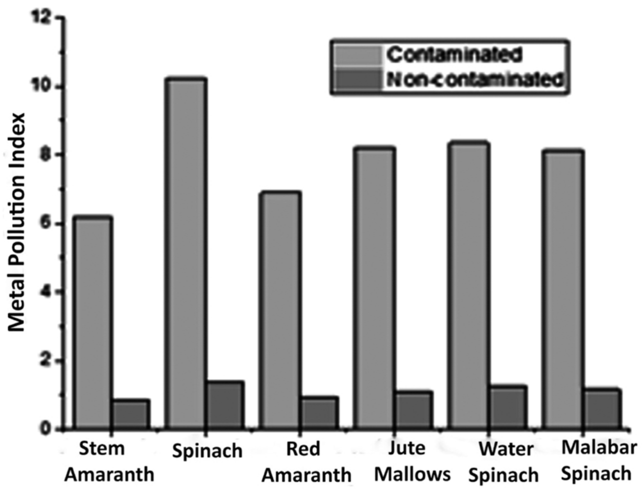

Fig. 3. Metal Pollution Index of vegetables grown in both the tannery effluent contaminated and non-contaminated soil.

(0.29)>Cu (0.17). However, MTF in other vegetables was in the order: water spinach>jute mallows>red amaranths>stem amaranths. The translocation of metals in vegetables was observed in the following order: Cr>Zn>Cd>Ni>Cu.

The MTF in vegetables grown in reference soil was found highest in spinach followed by water spinach>Malabar spinach>jute mallows>red amaranths>stem amaranths in the following metal accumulation order: Zn>Cr>Cu>Ni>Cd.

3.4. Metal pollution index

Metal Pollution Index (MPI) is a precise and reliable method that is used for the regular monitoring of heavy metal pollution by quantification of metal contents in crops grown in contaminated sites [38]. MPI value of each vegetable was measured that showed difference in plant species along with sampling sites.

The vegetables grown in contaminated soil had the higher metal accumulation and uptake capacity than those grown in non-contaminated soil. Hence, the MPI values will be higher in the vegetables cultivated on tannery effluent affected soil (Fig. 3). Among the vegetables grown in contaminated soil, the highest MPI was found in spinach (10.24), followed by Malabar spinach (8.36), water spinach (8.13) the least was found in stem amaranths (6.16). Since each vegetable possesses higher MPI values (>5), they are remarked as highly contaminated foodstuffs which may be responsible for sever, acute and chronic diseases upon long-term consumption [70,71].

On the other hands, the MPI found among the vegetables grown in non-contaminated soil was in the order of spinach (1.37)>water spinach (1.25)>Malabar spinach (1.17)>jute mallows (1.09)>red amaranths (0.94)>stem amaranths (0.87). The observed MPI values showed consistency in variance among vegetables grown in both soil samples.

This study was conducted in the same location and no pesticides were applied. Therefore, it was found a very good agreement between the higher MPI value and excess heavy metal load in the vegetables grown in contaminated soil.

3.5. Health risk assessment

3.5.1. Daily intake of metal

Heavy metals contamination in vegetable is an outcome of soil contamination and an obstacle of having quality foods for life. The risk of metal exposure in human body depends on the metal concentration in the respective food. Daily intake of metal (DIM) is an index to determine the exposure of heavy metal in body by feeding [72]. DIM values were observed higher in vegetables grown in tannery effluent contaminated soil than those of non-contaminated agricultural soil (Table 5). Among vegetables cultivated in contaminated soil, the DIM rate for metals was observed as Cr>Zn>Ni>Cd>Cu. DIM was found highest for Cr (7.90E-03) in spinach followed by Malabar spinach (7.45E-03). In spinach the DIM values for metals were found to be in the order of Cr (7.90E-3)>Zn (1.69E-3)>Ni (0.26E-3)>Cu (0.19E-3)>Cd (3.9E-05) and

Table 5
Daily intake of metals (mg/kg/Dw-day) from vegetables grown in both tannery effluent contaminated soil and non-contaminated agricultural soil.

Vegetables	Cr	Zn	Ni	Cd	Cu
Tannery effluents contaminated soil					
Stem Amaranths	585E-05	139E-05	20E-05	8.9E-05	14E-05
Spinach	790E-05	169E-05	26E-05	3.9E-05	19E-05
Red Amaranths	622E-05	145E-05	20E-05	1.2E-05	16E-05
Jute mallows	653E-05	156E-05	22E-05	2.2E-05	17E-05
Water Spinach	724E-05	161E-05	24E-05	2.0E-05	17E05
Malabar spinach	745E-05	162E-05	25E-05	1.5E-05	18E-05
Non-contaminated agricultural soil					
Stem Amaranths	5.9E-05	61E-05	9.9E-05	240E-05	13E-05
Spinach	9.5E-05	68E-05	15E-05	610E-05	19E-05
Red Amaranths	6.6E-05	62E-05	11E-05	250E-05	15E-05
Jute mallows	7.7E-05	73E-05	12E-05	300E-05	16E-05
Water Spinach	8.9E-05	69E-05	14E-05	410E-05	19E-05
Malabar spinach	8.2E-05	72E-05	13E-05	340E-05	19E-05
Maximum tolerable limit	0.02[a]	11(8)[b]	0.5[b]	0.068[c]	10[c]

[a] [75]
[b] [73];
[c] [74];

Table 6
HRI of HM for adults via vegetables consumption grown in both the tannery effluents contaminated soil and non-contaminated agricultural soil.

Vegetables	Cr	Zn	Ni	Cd	Cu
Tannery effluents contaminated soil					
Stem Amaranths	19500E-04	46.33E-04	100E-04	80E-04	37E-04
Spinach	26300E-04	56.47E-04	128E-04	390E-04	51E-04
Red Amaranths	20700E-04	48.20E-04	102E-04	120E-04	43E-04
Jute mallows	21800E-04	51.99E-04	112E-04	220E-04	46E-04
Water Spinach	24200E-04	53.73E-04	120E-04	200E-04	45E-04
Malabar spinach	24800E-04	53.98E-04	123E-04	150E-04	48E-04
Non-contaminated agricultural soil					
Stem Amaranths	39.3E-04	20.45E-04	49.56E-04	2.40E-04	35.76E-04
Spinach	63.3E-04	22.61E-04	73.16E-04	6.10E-04	52.06E-04
Red Amaranths	44.0E-04	20.74E-04	57.27E-04	2.50E-04	39.55E-04
Jute mallows	51.1E-04	24.50E-04	60.07E-04	3.00E-04	44.10E-04
Water Spinach	59.6E-04	23.08E-04	71.06E-04	4.10E-04	50.92E-04
Malabar spinach	54.6E-04	23.97E-04	64.52E-04	3.40E-04	50.16E-04

Table 7
HQ and HI of heavy metals for adults via vegetables consumption grown in both the tannery effluent contaminated soil and non-contaminated agricultural soil.

Vegetables	Hazard quotient (HQ)					HI
	Cr	Zn	Ni	Cd	Cu	
Tannery effluents contaminated soil						
Stem Amaranths	22.95	0.05451	0.1177	0.182	0.040563	23.35
Spinach	30.98	0.06644	0.15098	0.152	0.055413	31.41
Red Amaranths	24.38	0.05671	0.11935	0.048	0.046613	24.65
Jute mallows	25.60	0.06116	0.13173	0.084	0.050463	25.93
Water Spinach	28.41	0.06321	0.14135	0.077	0.048675	28.74
Malabar spinach	29.21	0.06351	0.14493	0.059	0.052663	29.53
Non-contaminated agricultural soil						
Stem Amaranths	0.00046	0.024	0.058	0.0009	0.039	0.1227
Spinach	0.00074	0.027	0.086	0.0024	0.057	0.1725
Red Amaranths	0.00051	0.025	0.068	0.001	0.043	0.1363
Jute mallows	0.00060	0.029	0.071	0.0012	0.048	0.1493
Water Spinach	0.00070	0.027	0.084	0.0016	0.055	0.1685
Malabar spinach	0.00064	0.028	0.076	0.0013	0.054	0.1607

comparatively lower DIM values were found in stem amaranths in the order of Cr (5.85E-03)>Zn (1.39E-03)>Ni (0.20 E-03)>Cu (0.14E-03)> Cd (4.6E-05).

Besides, the DIM values for spinach grown in referenced soil was observed in the order Zn (68E-54)>Cr (24E-5)>Cu (19E-5)>Ni (15E-5)>Cd (6.1E-6). However, the DIM was found in such order Malabar spinach>water spinach>jute mallows>red amaranths>stem amaranths. The order of metal ingestion rate from vegetables grown in non-contaminated agricultural soil was found Zn>Cr>Cu>Ni>Cd. The variance of TM concentration in vegetable samples is the main cause for the difference of DIM values that is influenced by factors like metal accumulation, HM concentration in soil, plant speciation, etc.

3.5.2. Health risk index

Health risk index evaluates the possibility of any health hazard due to consumption of contaminated food. HRI values of heavy metals for adults via vegetables consumption which was grown in the contaminated soil were higher than those of grown in non-contaminated agricultural soil (Table 6). Among these vegetables, the contribution to highest HRI value was found for chromium in spinach (2.63). However, the HRI values of chromium for each vegetable grown in contaminated soil exceeded 1 since excess accumulation of chromium from tannery effluents to vegetable plants. Therefore, consumption of these vegetables could pose threat to human health.

HRI values for all studied metals in the vegetables grown non-contaminated agricultural soil did not exceed1, which ensure no further harm in consumption of these vegetables as daily diet.

HQ and HI values were measured to determine the possibility of non-carcinogenic health risk of the dwellers of the study sites upon vegetable consumption and the values were shown in Table 7. Based on the analyses, it was found that the HQ value of chromium crossed 10 for the vegetables grown in polluted soil, which revealed the possibility of chronic hazard symptoms in human body upon vegetable consumption. On the other hand, the HQ values of each heavy metal in the vegetables that were cultivated in non-contaminated agricultural soil were found less than 1. Therefore, there might be no harm to consume those vegetables.

Carcinogenicity depends on the changes in cell's DNA due to genetic motive and environmental influences, e.g., lifestyle factors (nutrition, tobacco and alcohol use, physical inactivity, etc.), naturally occurring exposures (ultraviolet light, radon gas, infectious agents, etc.), medical treatments (radiation and medicines including chemotherapy, hormone drugs, drugs that suppress the immune system, etc.), workplace exposures, household exposures, pollution, etc. In this research carcinogenic risk was not evaluated due to lack of time for further extensive research. Authors would like to suggest for further investigations to evaluate the carcinogenic risks for the consumption of heavy metals contaminated vegetables.

4. Conclusion

Metal contamination in vegetable has become a matter of great concern in many countries as the assurance to have good quality and safer foods are getting interest among people. In this study, the possible scenario of metal contamination by tannery effluents and their adverse effect on human health was illustrated. The article was particularly focused on non-carcinogenic health risk assessment. It was found that among the metal contents, chromium content is the highest in the soil around the industrial zone. Cultivation of leafy vegetables in tannery effluent contaminated soil results in crucial contamination with heavy metals. It was also observed that the leafy vegetables have the high tendency to accumulate heavy metals that is quite alarming for human consumption. Therefore, these vegetables are not suitable for cultivating in tannery effluent contaminated soil. The research inspires and enables the adoption of suitable strategies to manage and mitigate heavy metals from tannery effluents to the safer level before discharge to the environment for the safety of human health.

CRediT authorship contribution statement

Sobur Ahmed: Conceptualization, Methodology, Investigation, Editing, Writing – original draft. **Fatema-Tuj-Zohra:** Writing- Introduction, Literature review and Conclusions. **Meem Muhtasim Mahdi:** Conceptualization, Methodology, Formal analysis, Data collection, Writing – original draft. **Dr. Md. Nurnabi:** Resources, Supervision, Writing – review & editing. **Dr. Md. Zahangir Alam:** Supervision, Resources, Methodology, Writing – review & editing. **Dr. Tasrina Rabia Choudhury:** Visualization, Data analysis, Writing – review & editing. All authors have read and agreed to the published version of the manuscript.

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.

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