

**ASSESSMENT OF HEAVY METAL CONTAMINATION IN
VEGETABLES FIELD OF BOGURA DISTRICT: OVERVIEW
OF POLLUTION INDICES AND RISK ASSESSMENT**

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OF POLLUTION INDICES AND RISK ASSESSMENT**

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CERTIFICATE

This is to certify that the thesis entitled “**ASSESSMENT OF HEAVY METAL CONTAMINATION IN VEGETABLES FIELD OF BOGURA DISTRICT: OVERVIEW OF POLLUTION INDICES AND RISK ASSESSMENT**” submitted to the Department of Agricultural Chemistry, Faculty of Agriculture, Sher-e-Bangla Agricultural University, Dhaka, in partial fulfillment of the requirements for the degree of **MASTERS OF SCIENCE (M.S.) in AGRICULTURAL CHEMISTRY**, embodies the result of a piece of bonafide research work carried out by **SADIA SAMMA**, Registration No. **19-10334** under my supervision and guidance. No part of the thesis has been submitted for any other degree or diploma.

I further certify that any help or source of information, received during the course of this investigation has been duly acknowledged.

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The Author

Assessment of heavy metal contamination in vegetables field of Bogura district: overview of pollution indices and risk assessment

ABSTRACT

The current study focused on quantifying hazardous heavy metals (Pb, Cu, and Cr) in soil-vegetables (potato, tomato, pepper, cauliflower, and cabbage) systems in different Upazilla of Bogura district in Bangladesh to assess their effect on human health and the environment. The instrumental detection method was validated according to linearity, determination co-efficient, limit of detection (LOD), limit of quantification (LOQ), accuracy, and precision. All the vegetables and their field soil had Cu concentrations lower than the maximum permissible limit set by BfSA, FAO/WHO. The concentration of Cr and Pb in vegetables exceeded the maximum allowable limit, but their field soil in different Upazilla of Bogura District did not exceed the maximum allowable limit as per FAO/WHO. For contamination factor (CF), only Pb severely contaminated the vegetables ($CF > 6$). The contamination degree (CD) data also showed that all vegetables had severe contamination. The pollution load index (PLI) results indicated vegetable field soil of Kahaloo, and Bogura Sadar had the highest PLI, while the vegetables produced in Nandigram and Dupchanchia had the lowest PLI. The bioaccumulation factor (BF) values were found < 1 in all vegetables for Cu, Cr, and Pb indicating trace metal transfer from field soil to consumable parts. Furthermore, the potential ecological risk factor (ERi) by single metal and risk index (PERI) results revealed lower ecological risks in vegetable fields. Health risk assessment showed that all vegetable samples were unsafe for human consumption as the carcinogenic health risk ($CHR > 10^{-4}$) and non-carcinogenic health risk ($HI > 1$) quotients seem more than the safe level in all vegetable samples. Findings show that heavy metal concentrations were high in vegetable fields endangering the environment and consumer health. Actions should be taken to reduce heavy metal pollution in the vegetable fields of different Upazilla of Bogura district to protect the environment and the consumer's health.

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ABBREVIATIONS AND ACRONYMS

Cu	=	Copper
Cr	=	Chromium
Pb	=	Lead
AAS	=	Atomic Absorption Spectrophotometer
%	=	Percentage
LOD	=	Limit of Detection
LOQ	=	Limit of Quantification
RSD	=	Relative Standard Deviation
IARC	=	International Agency for Research on Cancer
etc.	=	Etcetera
mm	=	Mili meter
g	=	Gram (s)
Min	=	Minutes
L	=	Liter
Max	=	Maximum
Min	=	Minimum
ml	=	Mili Litre
M.S.	=	Master of Science
No.	=	Number
SAU	=	Sher-e-Bangla Agricultural University
SD	=	Standard Deviation
°C	=	Degree Celsius
BDL	=	Below Detectable Limit
MAL	=	Maximum Allowable Limit
BFSA	=	Bangladesh Food Safety Authority
mg	=	Milligram
kg	=	Kilogram
CF	=	Contamination Factor
CD	=	Contamination Degree
PLI	=	Pollution Load Index
BF	=	Bioaccumulation Factor
FAO	=	Food and Agriculture Organization of The United Nations
USA EPA	=	United States of America Environmental Protection Agency
WHO	=	World Health Organization
ERi	=	Ecological Risk Factor
PERI	=	Potential Ecological Risk Index
RfD	=	Reference Dose
EDI	=	Expected Daily Intake
HQ	=	Hazard Quotient
HI	=	Hazard Index
CSF	=	Cancer Slope Factor
ILCRi	=	Incremental Lifetime Cancer Risk
CHR	=	Cancer Health Risk

CHAPTER I

INTRODUCTION

Rapid urbanization and industrialization, combined with poor environmental planning, frequently lead to the factory and waste water effluents being released into water sources (Lokeshwari and Chandrappa, 2006; Singh et al., 2011). Heavy metal pollution in soil and water has thus become a global issue due to intensive agriculture, fast urbanization, industrialization, and mining operations (Krishna et al., 2013; Singh et al., 2011). Heavy metal contamination could be spread to non-polluted crop fields by contaminated irrigation water from several polluted sources or by the routine application of fertilizer and agrochemicals containing heavy metals (Chanda et al., 2011). Heavy metals are trace elements that are required in small amounts for plant and animal development (Shahriar et al., 2021). They are pretty hazardous environmental contaminants because of their potential toxicity, abundance, and source of accumulation by crops. The contribution of effluent from waste water treatment plants, industry, mining, power stations, and agricultural activities can be linked to the increase of heavy metals in soil (Guevara-Riba et al., 2004). Furthermore, many human activities contribute to the accumulation of heavy metals in soil, which are then transmitted to edible plant portions (Taghipour and Mosaferi, 2013). Heavy metal contamination of the environment by industrial, agricultural, and residential activities poses significant obstacles to the safe use of agricultural land. When crops are cultivated on contaminated soils or in contaminated water, the total yield decreases and the consumable portion becomes contaminated, having a severe effect on human health (Fytianos et al., 2001; Solidum et al., 2012). Heavy metals are toxic pollutants in food and the environment because they are non-biodegradable and have extended biological half-lives.

Vegetables, rich in vitamins, minerals, and bioactive substances, are widely considered healthful and eaten by people all over the world. Vegetable eating improves immunity and decreases the risk of heart and age-related illnesses (Iso, 2018; Zhao et al., 2021). They are many contaminant types that can be identified on

and within the tissue of raw vegetables (Bigdeli and Seilsepour, 2008). However, consuming vegetables contaminated with heavy metals may pose a risk to human health; therefore, heavy metal contamination of vegetables is one of the most crucial parts of food quality assurance (Khan et al., 2009). Bogura is an important agricultural and industrial hub of Bangladesh. The district is well known for intensive agriculture especially vegetable production as well as numerous numbers of agrochemical and battery industries. Fruits and vegetables grown in other industrial regions of the country exceeded the levels of heavy metals such as lead, cadmium, arsenic, mercury, and nickel (Ahmad and Goni, 2010b; Laboni et al., 2022). Vegetables grown on contaminated soil could pose a threat to the health of a substantial number of people. Moreover, even in trace doses, frequent exposure to several heavy metals such as Pb, Cd, Cr, and Ni can pose substantial health hazards (Guo et al., 2010). Numerous investigations have demonstrated that certain vegetables can accumulate high levels of toxic metals in the soil or water (Garcia et al., 1981).

The present study was, therefore, undertaken with the following objectives:

1. To determine the amount of heavy metals in some vegetables and their corresponding field soil of different Upazilla of Bogura District,
2. To evaluate the contamination, bioaccumulation and ecological risk factors on vegetables and their corresponding field soil by heavy metals,
3. To assess both carcinogenic and non-carcinogenic health risk of heavy metals in vegetables produced in different Upazilla of Bogura District.

CHAPTER II

REVIEW OF LITERATURE

This chapter's goal is to review past research works relevant to the current study. In this chapter, an attempt has been made to provide a concise summary of research data evaluating the presence of toxic metallic components in the soil, and vegetables collected from different Upazilla of Bogra, Bangladesh. In Bangladesh, research on plants and soil pollution in near the urban areas is still in its infancy, and there is a dearth of relevant literature. As a result of the fact that a review of the relevant literature establishes a connection between past and present research related to the problem, it enables an investigation to reach a satisfactory conclusion. Presented below are the most pertinent studies that have been done in the recent past concerning this research program.

2.1. Overview of heavy metal pollution

According to Nazir et al. (2015), heavy metals are a diverse group of compounds whose chemical characteristics and biological activities vary considerably. Heavy metal pollution in water and the environment is a big problem, especially in medium-sized cities in developing countries. The main causes are irrigation with contaminated water, the use of fertilizers, metal-based pesticides, and industrial emissions and effluents in the environment.

According to Mora et al. (2009), heavy metals present a unique difficulty for cleanup because they do not biodegrade like organic contaminants. Plants are utilized to remediate polluted soils because of their hyper accumulation properties, which allow them to trap and store toxic metals in their bio matter (Farooq et al., 2012).

Bioremediation is a costly remediation procedure, yet developing countries often lack the resources to do such research. As a result, heavy metal contamination has been identified as one of the significant concerns impacting developing countries, and its impacts are manifested primarily in humans and ecosystems. Suffice it to say that some heavy metals are needed for the human body's metabolism. Heavy metals

such as copper, cadmium, chromium, and lead are highly hazardous in nature, even at low concentrations (Momodu and Anyakora, 2010).

2.2. Heavy metal concentration in vegetable field soil

Kansal and Sing (1983) reported that the increasing availability of metals in sewage-irrigated soils may have contributed to the higher metal content in diverse crops. Soils irrigated with sewage had a greater buildup of toxic metals in crops than soils in other Punjab towns.

Guttormsen et al., (1995) observed that vegetables, especially leafy crops, cultivated in soils contaminated with heavy metals have a higher accumulation of heavy metals than those produced in unpolluted soil (Guttormsen et al., 1995).

Adhikari et al., (1998) indicate an increase in the concentration of trace metals Cd, Pb, Zn, Cu, Mn, and Fe in surface soils irrigated with raw sewage and industrial effluents.

According to Sawidis et al., (2001) soil contamination has resulted from a wide range of human activities, including the improper disposal of municipal trash, effluents and emissions, military testing, and agriculture activities. When plants decompose, heavy metals absorbed by the plants are redistributed, enriching the soil once more with contaminants.

According to Sharma et al. (2006), uptake through roots is dependent on numerous variables, including the soluble amount of toxic metals in the soil, soil pH, plant physiological phases, crop type, fertilizers, and soil.

Heavy metals emitted by vehicles can concentrate in surface soils, and their accumulation over time might result in anomalous enrichment, so contaminating surface soils with metals (Fong et al., 2008).

The amount of heavy metals in soils and vegetables growing in and around an industrial region in Bangladesh has been estimated. In contaminated irrigation water, the metal amount was found to be Fe >Cu >Zn >Cr >Pb>Ni >Cd, and a similar pattern was identified in arable soils: Fe>Zn >Ni >Cr >Pb>Cu>Cd. Cu, Fe, and Cd concentrations in groundwater and Cd levels in soil were well over the permissible

threshold. Except for Cd, the accumulation of heavy metals in examined vegetables was below the acceptable maximum tolerated limits established by the Joint FAO/WHO Expert Committee on Food Additives (Ahmad and Goni, 2010a).

Alamgir et al. (2015) examined the concentration of toxic metals in the urban environment by measuring the amounts of Cd, Cu, Pb, Mn, Ni, and Zn in 21 roadside soil samples from Chittagong city. The mean contents of Cd, Cu, Pb, Mn, Ni, and Zn in the urban soils studied are 2.43, 32.63, 7.33, 160.79, 860.33, and 139.30 mg/kg, respectively. Similar levels of Cu, Cd, Pb, Mn, and Zn were found in urban soils in several different cities around the globe. The Ni value was significantly higher than the maximum permitted quantity (60 mg/kg), indicating contamination. Stepwise multiple regression revealed that soil parameters accounted for 37 to 42% of the variation in Cd, Cu, and Pb concentration, but only 16% of the variation in Ni content. The primary sources of Ni pollution in Chittagong can be attributed to human activity.

2.3. Heavy metal concentration in vegetables

Lark et al. (2002) showed that the metal content of vegetables watered continuously with sewage water alone is consistently greater than the metal content of vegetables when irrigation with domestic wastewater was switched with tube well water roughly ten years earlier.

According to Aboaba et al. (2004), it is essential to assess the metal content of vegetables from health, food nutrition, and crop yield technological standpoint. The accumulation of metals in edible plant parts varies according to the composition of the soil and the absorption rate of each plant. A sufficient intake of key components and nutrients is needed for overall health and peak human performance.

According to Al-Jassir et al. (2005), crop accumulation of heavy metals may be absorbed through roots from polluted soils or deposited on foliar surfaces. Toxic metals from industrial and automotive pollutants may be deposited on the foliar surfaces of crops during production, transport, and marketing.

Lokeshwari and Chandrappa (2006) conducted a study to determine the level of heavy metal pollution of agricultural vegetation caused by irrigation with lake water containing sewage. Several heavy metals have been evaluated in water, soil, and plant samples, including cadmium, lead, and mercury. Using an atomic absorption spectrophotometer, one may detect the elements iron, zinc, copper, nickel, chromium, lead, and cadmium.

The results demonstrate the presence of heavy metals in grains and vegetables that exceeds Indian regulations. Significant metal transfer factors from soil to plants are observed for Zn, Cu, Pb, and Cd. Comparing the concentrations of toxic metals in water, soil, and vegetation to their corresponding natural levels revealed that lake water had a greater impact on vegetation than soil (Guo et al., 2010).

Bigdeli et al., (2008) observed crops cultivated at adversely affected locations in Sahre Rey may collect and absorb metal concentrations that are likely to be hazardous to human health. This study analyzed the metal content of several vegetables in Sahre Rey, Iran, with a focus on their toxicological effects. According to the data, discrepancies in metal absorption by vegetables are attributable to plant susceptibility to toxic metals and vegetable species. The lead levels in all vegetable samples exceeded the maximum permissible level, whereas Cd pollution was detected in radish, cress, dill, spinach, and eggplant. Data revealed that the Zn content of Celery, Mint, Dill, Spinach and Green pepper exceeded the permissible range. There wasn't any evidence that vegetables were contaminated with Cu.

According to Sharma and Prasad (2009), toxic metals concentrate in edible parts (leaves and roots). Vegetables frequently contain cadmium, copper, arsenic, chromium, lead, zinc, cobalt, and nickel, among other heavy metals. Some of them, when present in minute amounts, are micronutrients. However, they can offer a considerable risk to human health, leading to a variety of chronic disorders, especially at high concentrations or with extended dietary exposure.

Ghosh et al. (2011) analyzed the eight roadside marketplaces and two regular markets for vegetables that were distinguished by purchase. The present analysis was limited to sites -1 through 4. Six of thirteen vegetables had a higher metal pollution index at sites 3 and 4. All sites had Lead (Pb) concentrations that were several times

greater than the PFA limit. Site 4 has a considerably higher Pb content than all other locations (P0.001).

Laboni et al. (2022) determine the lead (Pb), cadmium (Cd), chromium (Cr), and nickel (Ni) contents in 72 vegetable samples. Most of the examined vegetables included Pb, Cd, Cr, and Ni, with 79.17%, 44.444%, and 1.39% of samples exceeding the FAO/WHO maximum allowed limit (MAL) for Pb, Cd, and Ni, respectively.

2.4. Environmental pollution by heavy metals

According to Kibria et al. (2012), the levels of heavy metals in the soils of the tested sites are high, with Cd exceeding the worldwide natural background concentration. The mean total concentrations of Cd, Pb, Zn, Cu, Mn, and Fe in the 0-15 cm depth of the research region ranged from 0.08 to 2.39, 13.96 to 50.29, 14.73 to 21.12, 27.07 to 59.13, 116.25 to 326.63, and 1523 to 2798 mg/kg, respectively. The metals content in the 15-30 cm depth ranged from 0.01 to 1.98 mg/kg, 8.96 to 33.29 mg/kg, 51.44 to 267.31 mg/kg, 18.63 to 43.79 mg/kg, 68.89 to 271.74 mg/kg, and 1126 to 2054 mg/kg, respectively. Total and 0.1 N HCl extractable Cd, Pb, Zn, Cu, Mn, and Fe concentrations in wastewater-irrigated soils were considerably greater than in control soils. The total Cd, Pb, Zn, and Cu concentrations of surface soil in areas watered with waste water were higher than normal. Cd, Pb, Zn, Cu, Mn, and Fe concentrations in rice, radish, and aurum plant sections ranged from 0.02 to 16.65 mg/kg, 0.08 to 35.55 mg/kg, 0.84 to 102.75 mg/kg, 0.86 to 32.67 mg/kg, and 3.23 to 485.23 mg/kg, respectively. Cd, Pb, Zn, Cu, Mn, and Fe bioaccumulation coefficients in plants varied from 0.20 to 13.91, 0.008 to 0.72, 0.006 to 1.60, 0.03-0.64, 0.01 to 0.73, and 0.002 to 0.18, respectively. Bangladesh requires the establishment of soil quality criteria for heavy metals to foresee human-induced soil pollution.

According to Aktaruzzaman et al. (2013), the deposition of heavy metals in environmental matrices poses a risk to living systems due to their absorption by plants and subsequent incorporation into the food chain. Along the Dhaka-Aricha Road, soil and leafy vegetable samples were analyzed for the content of heavy

metals to evaluate their potential ecological risk. According to the Potential Ecological Risk Index, Cd posed a very significant risk to the local ecosystem due to its greater Risk Factor of >320, and according to the Transfer Factor, Pb and Cd were determined to be the highest accumulators among the tested metals. According to the findings of this study, the bioconcentration of toxic metals along the Dhaka-Aricha Road posed a significant threat to the ecosystem.

Soil contamination has been produced by environmental pollution, whilst wastewater irrigation has resulted in a substantial addition of non-essential materials to agricultural lands (Deribachew et al., 2015).

Assessing the influence on the environment of heavy metals (As, Cd, Cr, Pb, Ni, and Zn) in soil-rice systems near the Buriganga River in Bangladesh was the topic of a prior study. The mean amounts of As, Cd, Cr, Ni, and Zn in soil exceeded FAO/WHO tolerable levels, and the metal pollution index (MPI) indicated that all rice field soil samples were more polluted (MPI₃₀) than water and rice grain samples. According to the sum of pollution index (SPI) by examined metals, rice grains from Kamrangirchor (29.36), Dhakauddan (28.75), and Bosila (18.08) were badly contaminated. Bio-concentration factors (BCFs) and Transfer factors (TF_{grain/soil}) in rice grains were arranged as follows: Cd (6.034) > Zn (1.752) > Pb (0.697) Ni (0.666) > Cr (0.135) > As (0.037), and Cd (1.150) > Zn (0.421) > Ni (0.112) Pb (0.072) > Cr (0.015) > As (0.034), respectively, Except for the water in Kamrangirchar and Keraniganj rice fields, the prospective ecological risk index (RI) revealed that none of the other rice fields' soil and water samples posed a serious ecological risk (RI₁₁₀) due to the presence of several harmful heavy metals (Kabir et al., 2022).

2.5. Health risk assessment

Khan et al. (2009) analyzed the public health risk of toxic metals via the consumption of vegetables, which is one of the issues caused by the increased usage of fertilizers and pesticides to meet the rising need for human food production. Health risk assessment for toxic metals of the populations is a very effective method since such an assessment would be valuable in providing information regarding any harm posed by heavy metal contamination in vegetables. Researchers utilize many

methodologies for assessing health risks. These approaches include the daily intake of metals (DIM), the daily dietary index (DDI), and the Provisional tolerated daily intake (PTDI), as well as the health evaluation methods. The approaches for assessing health risks include the hazard quotient (HQ) and the health risk index (HRI).

Vegetables contaminated with heavy metals may contribute to the development of numerous chronic diseases. According to Kribek et al. (2010), the uptake of heavy metals in soft tissues disrupts normal physiological functioning and generally exerts its toxicity by building complexes with organic molecules.

Their toxicity can impair or diminish mental and sensory nervous system function, reduce energy levels, and cause harm to the blood, lungs, kidneys, liver, and other essential organs. Long-term exposure can lead to slow-moving, degenerative changes in the muscles, nerves, and bones that can cause muscular dystrophy and multiple sclerosis (Liang et al., 2011).

An assessment of human health risks is one way for determining the potential health effects of heavy metals. A complete health risk assessment approach includes four distinct phases: (1) hazard identification, (2) exposure assessment, (3) dose-response assessment, and (4) risk characterization. This method is used to classify the possible adverse health effects on humans as either non-carcinogenic or carcinogenic in response to environmental hazards (USEPA, 2022).

Dietary exposure is one of how humans are exposed to heavy metals; therefore, to determine the dose intake, the estimated daily intake (EDI) and hazards quotient (HQ) of heavy metals for vegetables are calculated using equations for the hazard quotient and oral toxicity reference dose (RfD) values provided by the US EPA (USEPA, 2004).

Non-carcinogenic compounds are presumed to have a threshold; a level below that no negative health effects will be noticed, when the dose-response phase of a risk assessment requires the use of a reference dose (RfD). It is assumed that carcinogens have no lethal level. Consequently, there is no safe limit for exposure to carcinogenic substances, as this assumption suggests there is a threat of cancer development even at low levels (Hamwiinga, 2017).

If HQ is less than 1, the exposed people are considered to be safe. In general, the oral Reference Dose (RfD) is an estimate (with uncertainty spanning maybe an increasing pattern) of a long-term exposure to the human population (including sensitive subgroups) that is unlikely to pose a meaningful lifetime risk of adverse effects. The RfD is stated in mg/kg/day and is predicated on the premise that limits exist for certain hazardous consequences, such as cellular necrosis (Liu et al., 2013).

The use of contaminated industrial effluent for agricultural cultivation in China has been linked to a 36% increase in liver failure (oversized liver) and a 100% increase in both cancer and fetal deformity, according to Chinese studies (Rajankar et al., 2014). In Japan, Itai-Itai sickness, a disorder of the bones and kidneys, was linked to persistent cadmium contamination of paddy water from the Jizu River, resulting in both non-carcinogenic and carcinogenic deleterious health effects (Liu et al., 2013).

According to Laboni et al. (2022), the presence of heavy metals in commonly consumed vegetables poses a significant risk to public health. She assessed the number of heavy metals in frequently eaten watercress (WC), alligator weed (AW), red amaranth (RA), spinach (SP), cauliflower (CF), and eggplant (EP) grown in industrial areas (such as the Narsingdi district) of Bangladesh to evaluate the potential health risks. The estimated daily intake (EDI) of a single heavy metal was below the maximum tolerated daily intake for that metal (MTDI). The incremental lifetime cancer risk (ILCR) values of Cd in all samples surpassed the threshold limit (ILCR > 10⁻⁴) for both adults and children, showing that eating contaminated vegetables poses a lifetime cancer risk. The target hazard quotient (THQ) for each heavy metal was less than one (except for Ni in a few samples), consumers usually exposed to a single heavy metal face no non-cancer risk. However, the hazard index (HI) values of toxic metals in polluted WC and AW for both adults and children were larger than one. In the meantime, WC, AW, and SP samples for children have surfaced as potential health hazards for residents of the researched locations.

CHAPTER III

MATERIALS AND METHODS

A study was conducted from December 2020 to March 2022 to determine the levels of heavy metals in five vegetables (potato, tomato, pepper, cauliflower, and cabbage) and their field of soils field collected from Bogura District. The laboratory analysis was carried out at the Food Safety Laboratory of the Department of Agricultural Chemistry, Sher-e-Bangla Agricultural University, Dhaka. This chapter discusses procedures used for vegetable harvesting, soil sampling, laboratory preparation/analysis, and statistical data analysis.

3.1 Study area

The study was conducted in the Bogura district one of the fastest-growing and industrialized districts in northern Bangladesh. Bogura is a district in Rajshahi Division, located between 24° 50' 59.99" North latitude and 89° 21' 59.99" East longitude. The district has a total area of 71.56 km². The district is bordered by the districts of Gaibanda, Joypurhat, Naogaon, Natore, and Sirajganj. Different types of land topography and soil can be found there, from river-eroded regions to the high Barind Tract. The area is split up into several different Agricultural Ecological Zones (AEZs), including the Tista Meander Floodplain (AEZ-3), Karatoa-Bangali Floodplain (AEZ-4), Lower Atrai Basin (AEZ-5), Active Brahmaputra-Jamuna Floodplain (AEZ-7), Active Ganges Floodplain (AEZ-10), High Ganges River Floodplain (AEZ-11), Low Ganges River Floodplain (AEZ-12), Level Barind (AEZ-27). The climate and soil in this area are ideal for cultivating a wide variety of food crops, especially grains and vegetables. In recent years, growing vegetables and spices appear to be more beneficial for farmers in the 12 Upazilas of the Bogra district than cultivating paddy and other crops. But, overpopulation, rapid industrialization, and urbanization in the last 30 years have made Bogra district very vulnerable to environmental pollution. Our Agriculture has a strategic interest in the management and protection of water and soil against heavy metal contamination. However, environmental management of the water and soil in these industrial areas is extremely challenging and expensive, but it is an absolute necessity for safe food production and general public health.

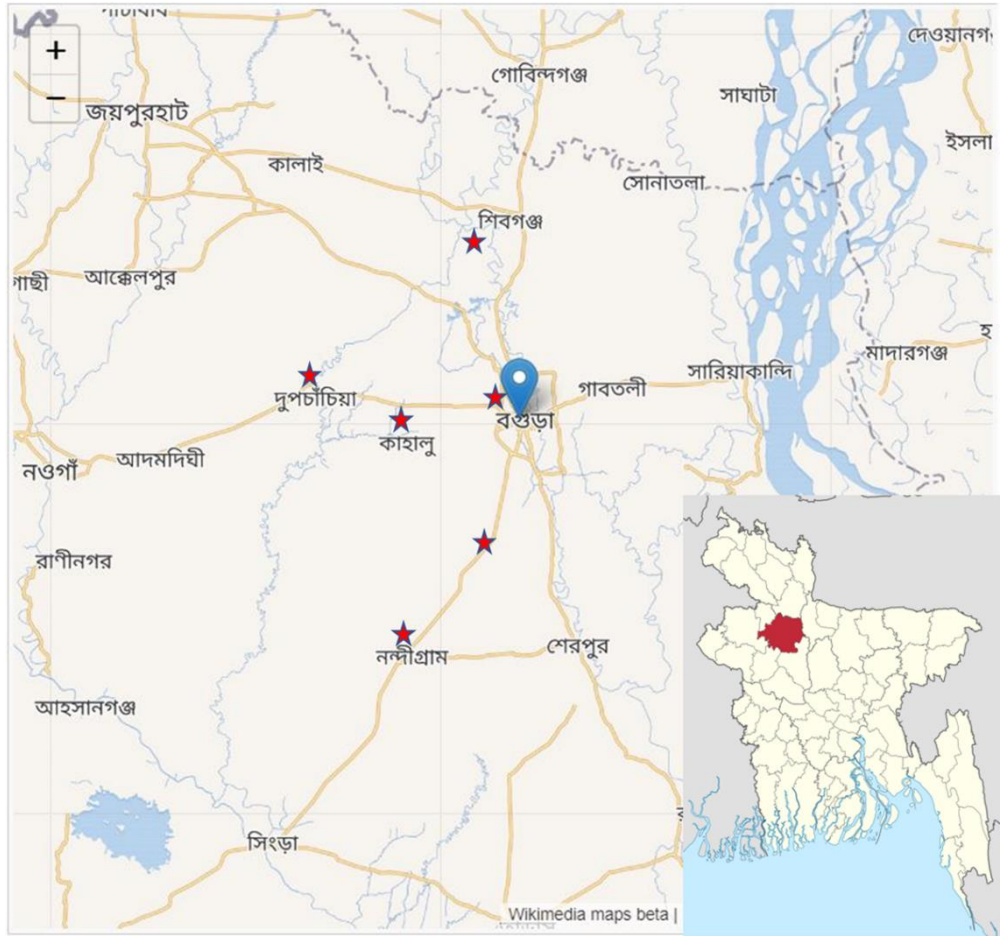


Figure 1. Sampling sites in the study area

3.2. Sampling sites

Vegetables and soil samples were taken from the vegetable fields of six Upazilla (Kahaloo, Bogura Sadar, Shajahanpur, Shibganj, Nandigram, and Dupchanchia) in Bogra district, Bangladesh (Figure 1). Table 1 shows sample lists with sampling locations.

Table 1. Name of studied samples and sampling sites

Sampling sites	Sample name
1. Kahaloo	1. Potato
2. Bogura Sadar	2. Potato soil
3. Shajahanpur	3. Tomato
4. Shibganj	4. Tomato soil
5. Nandigram	5. Chili pepper
6. Dupchanchia	6. Pepper soil
	7. Cauliflower
	8. Cauliflower soil
	9. Cabbage
	10. Cabbage soil

3.3. Study design

This study was a cross-sectional examination. It was done at a single time or over a relatively short time. The study sample was collected from December 2020 to March 2021 to give an "overview" of the output. Six Upazilla in the Bogura district's vegetable fields were chosen to have soil samples and vegetable samples collected.

3.4. Chemicals and reagents

Heavy metal standards (>95%) of Pb, Cu, and Cr for AAS analysis were bought from scharlau chemicals (Spain) through Bangladesh Scientific and Chemical Company Pvt. Ltd., Dhaka, Bangladesh. Analytical grade and EMSURE grade nitric acid, perchloric acid, distilled water, and hydrogen peroxide were bought from Kuri & Company (Pvt.) Limited, Dhaka, Bangladesh.

3.5. Collection of vegetable samples

Potato, tomato, chili pepper, cabbage, and cauliflower samples were randomly collected from farmers' vegetable fields in six Upazilas (Kahaloo, Bogura Sadar, Shajahanpur, Shibganj, Nandigram, and Dupchanchia) of Bogura district. Vegetables were collected at the harvesting stage from the farmer's field. Each vegetable sample was collected three times and mixed to make representative samples for sample preparation from every distinct sampling site. Vegetable samples were rinsed with

distilled water immediately after harvest and kept in ziplock polybags then transferred to the Food safety laboratory of Sher-e-Bangla Agricultural University, Dhaka, Bangladesh.

Table 2. Name of the vegetable samples collected for the study

Sl. No.	Local name	English Name	Type	Scientific Name	Family
1	Alu	Potato	Root vegetable	<i>Solanum tuberosum</i>	Solanaceae
2	Tomato	Tomato	Fruit vegetable	<i>Solanum lycopersicum</i>	Solanaceae
3	Morich	Chili pepper	Fruit vegetable	<i>Capsicum annuum</i>	Solanaceae
4	Badhakopi	Cabbage	Leaf vegetable	<i>Brassica oleracea</i>	Brassicaceae
5	Fulkopi	Cauliflower	Flower vegetable	<i>Brassica oleracea</i>	Brassicaceae

3.6. Collection of soil sample

Five vegetable fields (potato, tomato, chili pepper, cauliflower, and cabbage) in six upazillas (Kahaloo, Bogura Sadar, Shajahanpur, Shibganj, Nandigram, and Dupchanchia) of Bogura district were selected as sampling sites. Three surface soil (0-15 cm) samples were taken at the same location as each vegetable sample and mixed to 1.5 kg volume from each site of each vegetable field. A total of 30 soil samples from six upazillas of the Bogura district were collected and mixed in a clean ziplock bag to create a representative sample for sample preparation.

3.7. Sample preparation

Vegetable samples were dried in a lab oven at 60–70°C for three days to get a constant dry weight. Samples of dry vegetables were ground with a grinder and stored in ziplock poly bags. Soil samples were cleaned by hand to remove rocks, gravel, roots, leaves, and other debris. Before laboratory analysis, soil samples were air dried for three days at 30 - 40 °C and then passed through a 2 mm sieve. Before acid digestion, the 30 sieved soil samples and 30 ground vegetable samples were stored in closed plastic containers.

3.8. Digestion of vegetables and soil samples

1.0 gram of dry grinded vegetables and 1.0 gram of finely ground soil were placed into separate glass digestion tubes and pre-digested with 20 ml di-acid mixture (2:1, HNO₃:HClO₄). The following day, digest preparations were heated for two hours at 180-220°C. Plant and soil matter was completely digested as a result of this procedure. All samples were allowed to cool for 30 minutes after digestion, then diluted to 100 ml with deionized water, mixed with a vortex mixer (10 seconds), and filtered through Whatman 42 filter papers. The samples were stored in a refrigerator (4-7 °C) in clearly marked plastic bottles. An atomic absorption spectrophotometer was used to determine heavy metals in digestion samples of vegetables and soil.

3.9. Instruments

Analytik Jena's novAA 400P Atomic Absorption Spectrophotometer was used to determine the total content of copper, chromium, lead, and cadmium in the vegetable and soil digest (Analytik Jena NovAA 400P, 2012, country of origin: Germany). The hollow cathode lamps in AAS were used for estimations in a wide range of situations, depending on the element that was being analyzed. The concentration of heavy metals was reported in parts per million (mg/kg). Table 3 shows the detailed instrumental conditions for determining copper, chromium, lead, and cadmium.

Table 3. Instrumental conditions for determination of Cu, Cr, Pb, and Cd.

Element	Cu	Cr	Pb
Wavelength (nm)	324.8	357.9	217.0
Slit (nm)	1.2	0.2	1.2
Lamp	HCL	HCL	HCL
Lamp current (mA)	2	4	2
Flame	Air-Ac	Air-Ac	Air-Ac
Air/Ac flow (L/min)	50	100	65
Burner Head (mm)	100	100	100
Burner height (mm)	6	8	6
Read time (seconds)	3	3	3

3.10. Quality control of the method

The method was validated by method linearity, determination coefficient, accuracy, and precision. The linearity and determination coefficient was determined by generating calibration curves with standard solutions ranging in concentration from 0.0 to 1.0 mg/L. All analyses were carried out in triplicate. The Analytical Jena Aspect LS software is used to quality control and validate the analytical method. The spiked QC samples were used to figure out the percent recovery and relative standard deviation at a 95% confidence level (Al-Weher, 2008; Borošová et al., 2002).

3.11. Quantification of heavy metals

The standard curve was constructed by graphing the absorbance reading on the Y axis against multiple concentrations of each standard metal solution on the X axis. The concentration of metal in the vegetable and soil samples was then estimated by placing the AAS reading on the standard curve. The concentration of mineral contents is calculated from the Instrumental standard curve according to the following formula (1):

$$x = (y - b)/m \dots\dots\dots (1)$$

Where, x = concentration of metals in the sample, y = instrumental absorbance for metals of known and unknown concentration, m = slope from the standard curve of plotting the absorbance against concentration, b = intercept from the standard curve of plotting the absorbance against concentration

The following equation (2) calculates the final concentration of metals in the vegetables and soils samples collected from different Upazilla of Bogura district:

$$\text{Metal (mg/kg)} = \frac{C \times V \times D}{W} \dots\dots\dots (2)$$

Where, C = Concentration of the sample from calibration curve, V = total volume of digest (ml), D = dilution factor, W = Weight of sample (g)

3.12. Assessment of pollution indices

The levels of the heavy metals copper (Cu), lead (Pb), and chromium (Cr) were measured in both the vegetables and the soil samples taken from the fields where the vegetables were grown. The Pollution Index is a powerful tool for processing,

evaluating, and communicating raw environmental data to decision-makers, managers, experts, and the general public. The contamination factor is a reflection of the pollution characteristics that were found in the researched location. The contamination factor (CF) was proposed to indicate single metal pollution at specific sites, whereas the contamination degree (CD), pollution load index (PLI), and potential ecological risk index (PERI) proposed by Hakanson (1980) were assessed as indices for diagnosing multi-metal pollution.

3.12.1. Contamination factor (CF)

The single metal contamination factor (CF_i) is the ratio of the single metal concentration in a biotic or abiotic medium to the regulatory standard set by national or international organizations like the Bangladesh Food Safety Authority (BSFA), the World Health Organization (WHO), and the United States Environmental Protection Agency (EPA) (USEPA). Using equation 3, the single metal contamination factor (CF) was computed (Hakanson, 1980a).

$$CF_i = C_{\text{vegetable or soil}} / C_{\text{BFSA or FAO}} \dots\dots\dots (3)$$

Where $C_{\text{vegetable or soil}}$ is the metal concentration in the vegetables or soil sample. $C_{\text{BFSA or FAO}}$ is the value of the regulatory limit of heavy metals by the Bangladesh Food Safety Authority or FAO. The contamination factor (CF) values were divided into four broad categories: $CF < 1$ (low contamination), $1 < CF < 3$ (moderate contamination), $3 < CF < 6$ (significant contamination), and $CF > 6$ (severe contamination) (Hakanson, 1980b).

3.12.2. Ecological risk factor (ER_i)

The ecological risk factor (ER) is introduced to evaluate the contamination of soils by a single toxic compound. The following equation (4) was used to determine the ecological risk factor (ER) for single metal pollution at different sites (Hakanson, 1980b).

$$ER_i = TR \times CF_i \dots\dots\dots (4)$$

TR is the biological toxic response factor for each element. For Cu and Pb, it is 5, and for Cr, it is 2 (Hakanson, 1980b). The ecological risk factor (ER_i) is classified using the following categories: $ER_i < 40$ (low ecological risk factor); $40 \leq ER_i < 80$

(moderate ecological risk factor); $80 \leq ER_i < 160$ (significant ecological risk factor); $160 \leq ER_i < 320$ (high ecological risk factor); and $ER_i \geq 320$ (severe ecological risk factor). The formula indicates the degree of heavy metal toxicity and ecological sensitivity to heavy metal contamination.

3.12.3. Contamination degree (CD)

The concentration degree (CD) is the sum of the contamination factors (CF) of the heavy metals that have been measured at specific sites. It measures the overall contamination level at the study location. The following equation (5) was used to determine the contamination degree (CD) at different sites (Islam et al., 2015):

$$CD = \sum CF \dots\dots\dots (5)$$

The contamination degree (CD) values were divided into four broad categories: $CF < 8$ (low degree contamination), $8 < CF < 16$ (moderate degree contamination), $16 < CF < 32$ (significant degree contamination), and $CF > 32$ (severe degree contamination) (Ogundele et al., 2020b). The Cd is intended to provide a measurement of the degree of overall contamination in certain samples at a certain sampling site.

3.12.4. Pollution load index (PLI)

The pollution load index (PLI) is used to measure the total heavy metal concentrations in vegetables and soils, which were computed using the geometrical mean of all metal concentrations. Using equation (6), the single pollution load index (PLI) was computed (Varol, 2011).

$$PLI = (CF_1 \times CF_2 \times CF_3 \times \dots \times CF_n)^{1/n} \dots\dots\dots (6)$$

where CF represents the contamination factor and n represents the total number of parameters. This pollution load index gives a quick and easy way to analyze the level of heavy metal pollution. If PLI is greater than 1, there is pollution; however, if PLI is less than 1, there is no metal pollution (Varol, 2011). The PLI provided an evaluation of the sample's overall toxicity and was the consequence of the contribution of four hazardous elements.

3.12.5. Bioaccumulation factor for plants (BF)

The bioaccumulation factor (BF) is the ratio of metal concentrations in plants (the total of each plant's concentration of heavy metals) to soil concentrations. The bioaccumulation factor (BF) was calculated using the following equation (8) (Rashed, 2010):

$$BF = (C_1/C_2) \dots\dots\dots (7)$$

Where, C_1 and C_2 are the mean concentrations of metal in vegetables and soil, respectively. If the bioaccumulation factor (BF) is greater than 1, then the vegetables are shown to be heavy metal accumulators, whereas a bio-accumulation factor less than 1 indicates that the vegetable is an excluder of heavy metal accumulation.

3.12.6. Potential ecological risk index (PERI)

Swedish scientist Hakanson established the Potential Ecological Risk Index (PERI) in 1980; it has since been used to assess the risk posed by multi-heavy metals in soils. The technique was widely employed and had a significant international impact (Hakanson, 1980b). The potential ecological risk index (PERI) is also introduced to evaluate the degree of contamination of different toxic compounds in soils. The potential ecological risk index for multiple heavy metals in soil is calculated by the sum of the single potential ecological risk factor together. Guo et al. (2010) formulated the equations (7) used to calculate PER, which are as follows (Guo et al., 2010).

$$PERI = \sum_{i=1}^m ER_i \dots\dots\dots (8)$$

The potential ecological risk index (PERI) is classified using the following categories: $PERI < 150$ (low potential ecological risk index); $150 \leq PERI < 300$ (moderate potential ecological risk index); $300 \leq PERI < 600$ (high potential ecological risk index); and $PERI \geq 600$ (severe potential ecological risk index) (Guo et al., 2010; Ogundele et al., 2020a). It indicates the potential ecological risk posed by the total contamination and represents the biological community's sensitivity to the poisonous element.

3.13. Assessment of Health risk

Human health risk assessment based on heavy metal contamination in food and the environment reveals negative health impacts. This study assessed cancer and non-cancer health risks posed to children and adults in Bangladesh by consuming vegetables contaminated with heavy metals to develop consumer health protection strategies. The Environmental Protection Agency of the United States (USEPA) proposed the equation for the assessment of non-carcinogenic and carcinogenic health risks associated with exposure to heavy metals contamination in foods (USEPA, 2001).

3.13.1. Non-carcinogenic health risk

The health risk to consumers is measured by comparing the estimated daily intake (EDI) with the oral reference dose (RfD), which is set by regulatory bodies like FAO/WHO. The following is an assessment of the EDI values for different heavy metals, as provided by the US Environmental Protection Agency (EPA).

$$EDI = \frac{C \times IR \times EF \times ED}{BW \times AT} \dots\dots\dots (9)$$

Where, C is the heavy metal concentration in the vegetable (mg/kg), IR is the ingestion rate of vegetable (0.1673 kg/person/day) (Laboni et al., 2022), EF is the exposure frequency (365 days/year), ED is the exposure duration (70 years), BW is the bodyweight of the exposed individual (60 kg), and AT is the time over which the dose is averaged (365 days/year X the number of exposure years, average 70 years) (Kabir et al., 2022; Laboni et al., 2022).

The non-carcinogenic hazard for a single metal in a vegetable was characterized by the hazard quotient (HQ) computed using equation 10, and the non-carcinogenic hazard for multiple metals was calculated using equation 11.

$$HQ_i = EDI / RfD_i \dots\dots\dots (10)$$

$$HI = \sum_{i=1}^n HQ_i \dots\dots\dots (11)$$

Where $RfDi$ represents the standard oral reference dose for metal i . The reference dose (mg/kg/day) represents the maximum risk posed to humans by a lifetime of daily exposure. The $RfDi$ values for Pb, Cr, and Cu are 0.0035, 0.003, and 0.04 (mg/kg/day), respectively (USEPA, 2021). The sum of HQ for multiple heavy metals is HI. If the HQ and HI values are greater than one, exposed persons are likely to suffer negative health impacts. Therefore, if both the HQ and HI values are less than one, the heavy metal is deemed safe for human health (Li, et al., 2014).

3.13.2. Carcinogenic health risk

The Incremental Lifetime Cancer Risk (ILCR i) is calculated to assess the potential cancer risk resulting from the consumption of carcinogenic single heavy metals via foods, as estimated below (equation 12).

$$ILCRi = EDI \times CSF \dots\dots\dots (12)$$

EDI is for estimated daily intake of heavy metals (mg/kg BW/day) and CSF stands for cancer slope factor (mg/kg/day)⁻¹. The cancer slope factor CSF for heavy metals Cr, and Pb is 0.5, and 0.0085 (kg/day/mg), respectively.

The CHR is used to calculate the total cancer risk associated with the intake of different heavy metals from a certain food type.

$$CHR = \sum_{i=1}^n ILCRi \dots\dots\dots (13)$$

The CHR is the sum of carcinogenic health risks of individual heavy metals i . Thus, the cancer risk could be expressed as no significant carcinogenic health risk (ILCR i or CHR < 10⁻⁶); acceptable carcinogenic health risk (10⁻⁶ < ILCR i or CHR < 10⁻⁴); or unacceptable carcinogenic health risk (ILCR i or CHR > 10⁻⁴).

3.14. Statistical analysis

Th Analytica Jena Aspect LS software was used to detect, calculate, and statistical analysis of the amount of heavy metals in soil and vegetable samples at 95% level of significance. MS Excel 2016 version was employed to generate descriptive statistics about the amounts of heavy metals. Results were reported as mean concentration with standard deviation (SD).

CHAPTER IV

RESULTS AND DISCUSSION

This chapter shows the results of analyses of heavy metals in soil and vegetables collected from several Upazila in the Bogura district. Several metals were examined, including copper, lead, and chromium.

4.1. Method Validation

Heavy metal analysis in soil and vegetables depends on a validated procedure to ensure precise findings. Method validation ensures that results are accurate and precise for their intended application.

4.1.1. Linearity and determination co-efficient (R^2)

The linearity and determination co-efficient of the method was assessed from the calibration curve of different heavy metals. A calibration curve was obtained by plotting the absorbance against the concentrations of the standards. For every analytical run, a new calibration curve was created for each metal. The linearity of the calibration curves ranges between 0.998 and 0.9993 (determination coefficient), confirming the calibration's linearity. The standards were set or put through the same instrumentation conditions at different times. Figure 2 showed the good linearity for Cu, Cd, and Pb with determination co-efficient (R^2) higher than 0.998 (Figure 2). These standard calibration curves were used to calculate the amount of heavy metals in plant and soil samples.

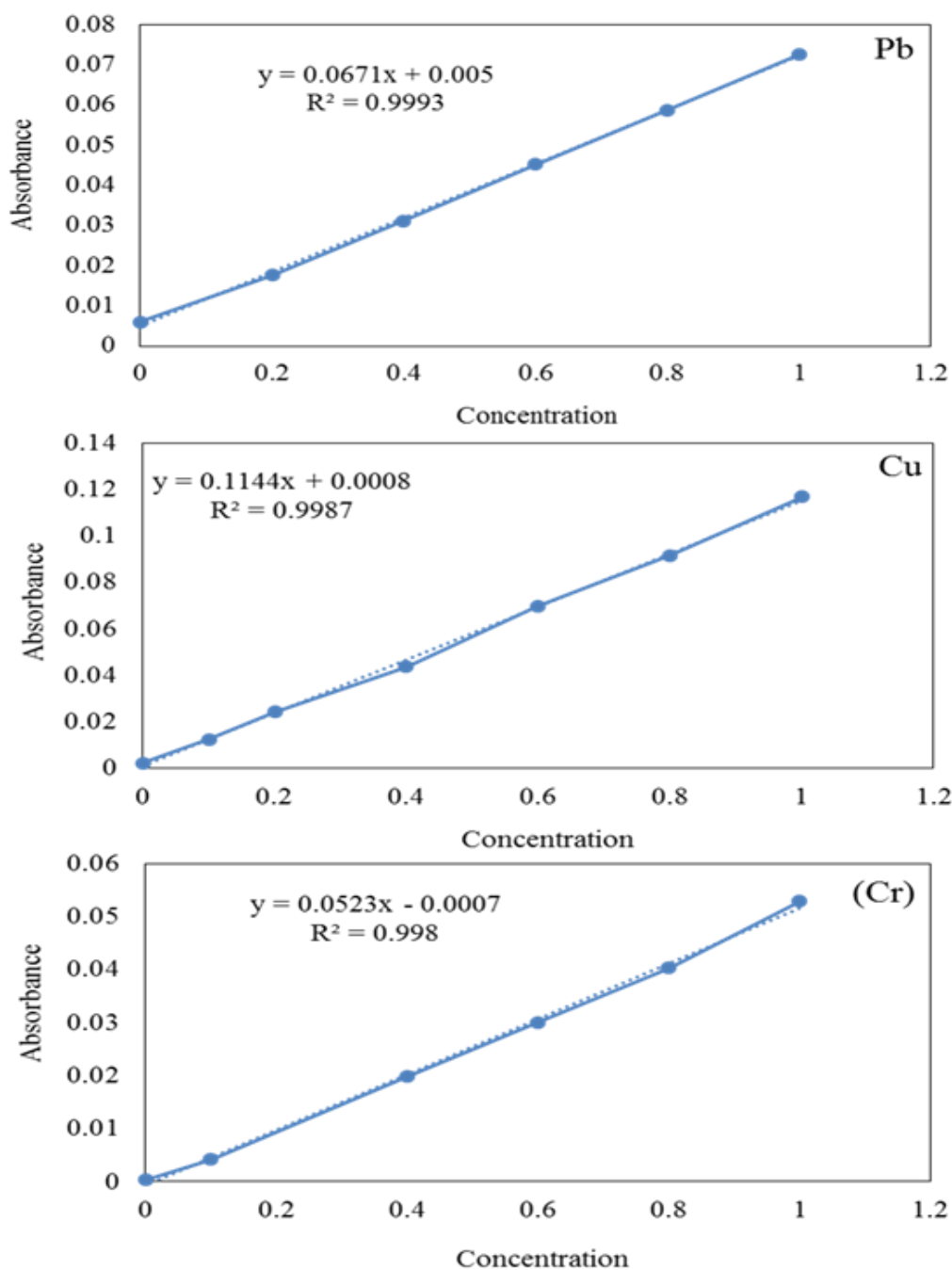


Figure 2. The standard curve of heavy metals shows linearity and determination coefficient

4.1.2. Limit of detection (LOD), quantification (LOQ), and sensitivity

The Analytica Jena Aspect LS software was used for quality control and validation of the analytical method. The instrumental limit of detection (LOD), the limit of quantification, and sensitivity were calculated from the calibration curve by

Analytical Jena Aspect LS AAS software. The methods sensitivity (0.0381 to 0.0835 mg/L) and LODs (0.0354 to 0.0676 mg/L) amount is much lower for qualitative analysis as well as the LOQs (0.1298 to 0.2529 mg/L) amount of the methods is also good enough for the quantitative determination of heavy metals in plant and soil samples (Table 4).

Table 4. Instrumental validation parameters

Metal element	Cu	Cr	Pb
Method sensitivity (mg/L)	0.0381	0.0835	0.07084
Method LOD (mg/L)	0.0354	0.0676	0.0375
Method LOQ (mg/L)	0.1298	0.2529	0.1431
Spiked sample (mg/L)	0.2	0.6	0.4
Mean Accuracy (%)	102.3	100.8	94.7
Precision (%)	2.9	2.3	1.7

4.1.3. Accuracy and precision of the method

This research focused on precision and accuracy for quality assurance purposes. Repeatability for precision is the variation that a single analyst sees on a single instrument. Recovery for accuracy refers to the amount of analyte spiked into a sample before its examination and digestion. This procedure was carried out in triplicate for each run and each metal. The precision is indicated by relative standard deviations (RSDs) that are between 1.7 and 2.9 %. The accuracy is shown by a recovery rate between 94.7 and 102.3 % (Table 4). The method showed good recovery with the lowest relative standard deviation for quantitative analysis of heavy metals in plants and soil samples.

4.2. Heavy metal accumulation in the vegetable field

4.2.1. Copper (Cu) accumulation in vegetable fields of Bogura district

Copper concentrations in different vegetables and soils collected from several Upazilla vegetable fields in the Bogura district varied significantly (Figure 3). In terms of Cu concentration in different vegetables (potato, tomato, pepper, cauliflower and cabbage), it is ranged from 8.26 to 11.38 mg/kg, 8.34 to 18.55

mg/kg, 7.79 to 15.56 mg/kg, 3.86 to 9.13 mg/kg, and 5.01 to 16.36 mg/kg (Table 5). In the case of vegetable field soil (potato soil, tomato soil, pepper soil, cauliflower soil, and cabbage soil), it ranged from 11.02 to 18.81 mg/kg, 7.68 to 18.77 mg/kg, 10.75 to 25.41 mg/kg, 8.99 to 18.81 mg/kg, and 12.88 to 19.06 mg/kg respectively (Table 5). The result indicated that the highest Cu accumulation in vegetables was found (18.55 mg/kg) in tomatoes while the lowest Cu accumulation in vegetables was found (3.86 mg/kg) in cauliflower. The results also indicated highest Cu accumulation was found (25.41 mg/kg) in pepper field soil while the lowest Cu accumulation was found (7.68 mg/kg) in tomato field soil (Table 5).

The mean Cu accumulation in vegetables grown in several Upazilla in the Bogura district was found in the order of tomato > pepper > cabbage > potato > cauliflower. In vegetable fields soil in several Upazilla of the Bogura district, the mean accumulation of Cu was found in the order of cabbage field soil > pepper field soil > cauliflower field soil > potato field soil > tomato field soil (Figure 3 & Table 5). As shown in Table 5, the concentration of Cu in vegetables and the soil of their fields in different Upazilla of Bogura District not exceeded the maximum allowable limit as per FAO/WHO (Ogundele et al., 2015). The status of Cu in this study is within the limits, and all of the vegetables have Cu levels that are lower than the toxic limit and safe for human to eat. This is similar to the findings above.

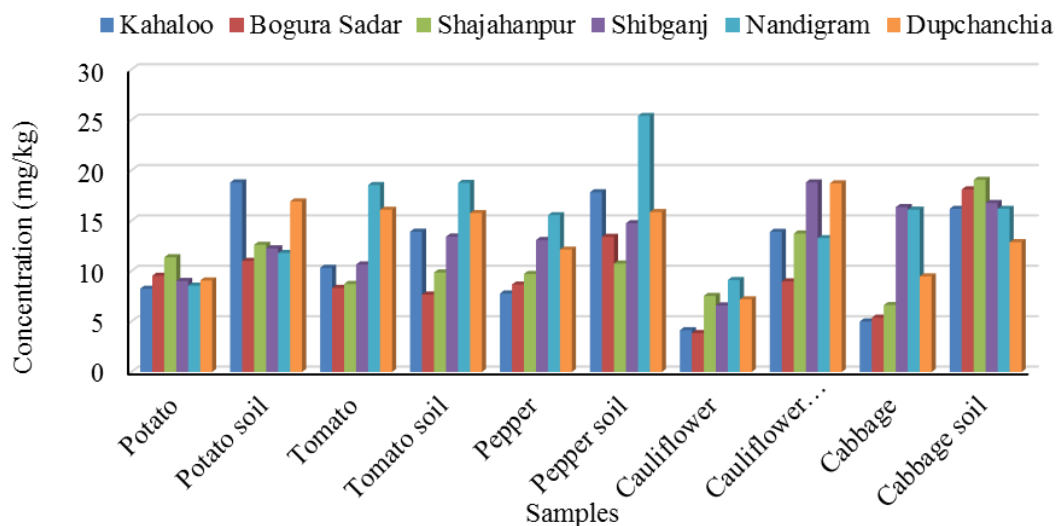


Figure 3. Copper (Cu) accumulation in vegetables and their field soils in different Upazilla of Bogura district.

Table 5. Summary of concentrations (mg/kg) of heavy metals and transfer factor (TF) in vegetables and surface soil from the vegetable fields in several Upazilla of Bogura district.

Metal	Concentration (mg/kg)	Potato	Potato soil	Tomato	Tomato soil	pepper	pepper soil	Cauliflower	Cauliflower Soil	Cabbage	Cabbage Soil
Cu	Mean	9.31	13.91	12.12	13.24	11.17	16.34	6.42	14.58	9.83	16.54
	Min	8.26	11.02	8.34	7.68	7.79	10.75	3.86	8.99	5.01	12.88
	Max	11.38	18.81	18.55	18.77	15.56	25.41	9.13	18.81	16.36	19.06
	BF (mean)	0.67		0.92		0.68		0.44		0.59	
	SD	1.11	3.17	4.20	3.99	2.95	5.04	2.05	3.72	5.20	2.12
Cr	Mean	1.6	41.3	1.6	29.9	0.7	43.5	1.8	70.2	1.3	44.8
	Min	BDL	20.4	BDL	16.8	BDL	12.0	BDL	26.2	BDL	21.3
	Max	3.8	90.9	5.4	72.3	2.8	88.9	3.8	110.7	3.1	64.3
	BF (mean)	0.04		0.05		0.02		0.03		0.03	
	SD	1.75	26.48	2.04	21.17	1.19	26.89	1.72	37.30	1.35	16.69
Pb	Mean	12.41	21.47	13.88	22.51	11.81	21.09	13.73	21.39	13.27	22.41
	Min	5.97	10.42	3.12	10.56	4.61	13.49	4.76	16.42	6.93	13.43
	Max	25.77	31.38	21.69	33.03	20.58	35.57	22.84	26.09	23.28	35.64
	BF (mean)	0.58		0.62		0.56		0.64		0.59	
	SD	7.11	7.43	6.33	8.14	5.99	8.40	7.64	3.33	7.21	8.58

BDL= below the detectable limit

4.2.2. Chromium (Cr) accumulation in vegetable fields of Bogura district

Chromium concentrations in different vegetables and soils collected from several Upazilla vegetable fields in the Bogura district varied significantly (Figure 4). In terms of Cr concentration in different vegetables (potato, tomato, pepper, cauliflower, and cabbage), it is ranged from below the detectable limit to 3.78 mg/kg, below the detectable limit to 5.44 mg/kg, below the detectable limit to 2.75 mg/kg, below detectable limit to 3.76 mg/kg, and below the detectable limit to 3.1 mg/kg, respectively (Table 5). In the case of vegetable field soil (potato soil, tomato soil, pepper soil, cauliflower soil, and cabbage soil), it ranged from 20.42 to 90.92 mg/kg, 16.79 to 72.28 mg/kg, 11.95 to 88.86 mg/kg, 26.17 to 110.70 mg/kg, and 21.3 to 64.3 mg/kg respectively (Table 5). The result indicated that the highest Cr accumulation in vegetables was found (5.44 mg/kg) in tomatoes. The results also indicated highest Cr accumulation was found (110.70 mg/kg) in cauliflower field soil while the lowest Cr accumulation was found (11.95 mg/kg) in pepper field soil (Table 5). The mean Cr accumulation in vegetables grown in several Upazilla in the Bogura district was found in the order of cauliflower > tomato > potato > cabbage > pepper. In vegetable fields soil in several Upazilla of the Bogura district, the mean accumulation of Cr was found in the order of cauliflower field soil > cabbage field soil > pepper field soil > potato field soil > tomato field soil (Table 5 and Figure 4). As shown in Table 5, the concentration of Cr in vegetables exceeds the maximum allowable limit as per WHO but the soil of their fields in different Upazilla of Bogura District did not exceed the maximum allowable limit as per FAO/WHO (Ogundele et al., 2015). The level of Cr in the vegetables collected for this study is alarming, or toxic to health. Farming techniques that can contaminate the soil with Cr should be made more aware to farmers.

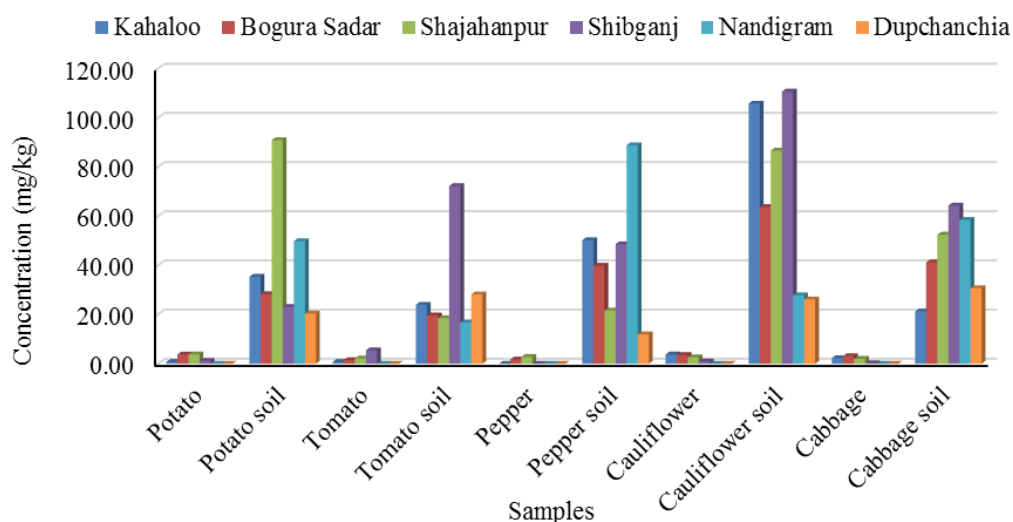


Figure 4. Chromium (Cr) accumulation in vegetables and their field soil in different Upazilla of Bogura district.

4.2.3. Lead (Pb) accumulation in vegetable fields of Bogura district

Lead concentration in different vegetables and soils collected from several Upazilla vegetable fields in the Bogura district varied significantly (Figure 5). In terms of Pb concentration in different vegetables (potato, tomato, pepper, cauliflower and cabbage) ranged from 5.97 to 25.77 mg/kg, 3.12 to 21.69 mg/kg, 4.61 to 20.58 mg/kg, 4.76 to 22.84 mg/kg, and 6.93 to 23.28 mg/kg, respectively (Table 5). In the case of vegetable field soil (potato soil, tomato soil, pepper soil, cauliflower soil, and cabbage soil), it ranged from 10.42 to 31.38 mg/kg, 10.56 to 33.03 mg/kg, 13.49 to 35.57 mg/kg, 16.42 to 26.09 mg/kg, and 13.43 to 35.64 mg/kg respectively (Table 5). The result indicated that the highest Pb accumulation in vegetables was found (25.77 mg/kg) in potatoes while the lowest Pb accumulation in vegetables was found (3.12 mg/kg) in tomatoes. The results also indicated highest Pb concentration was found (35.64 mg/kg) in cabbage field soil while the lowest Pb concentration was found (10.42 mg/kg) in potato field soil (Table 5). The mean Pb accumulation in vegetables grown in several Upazilla in the Bogura district was found in the order of tomato > cauliflower > cabbage > potato > pepper. In vegetable fields soil in several Upazilla of the Bogura district, the mean concentration of Pb was found in the order of tomato field soil > cabbage field soil > potato field soil > cauliflower field soil > pepper field soil (Table 5 and Figure 5). As shown in Table 5, the accumulation of Pb in vegetables exceeds the maximum allowable limit as per WHO

but the soil of their fields in different Upazilla of Bogura District did not exceed the maximum allowable limit as per FAO/WHO (Ogundele et al., 2015). The result revealed conclusively that vegetables exceed the maximum permissible Pb level. Farmers should be made aware of farming techniques that can result in Pb pollution.

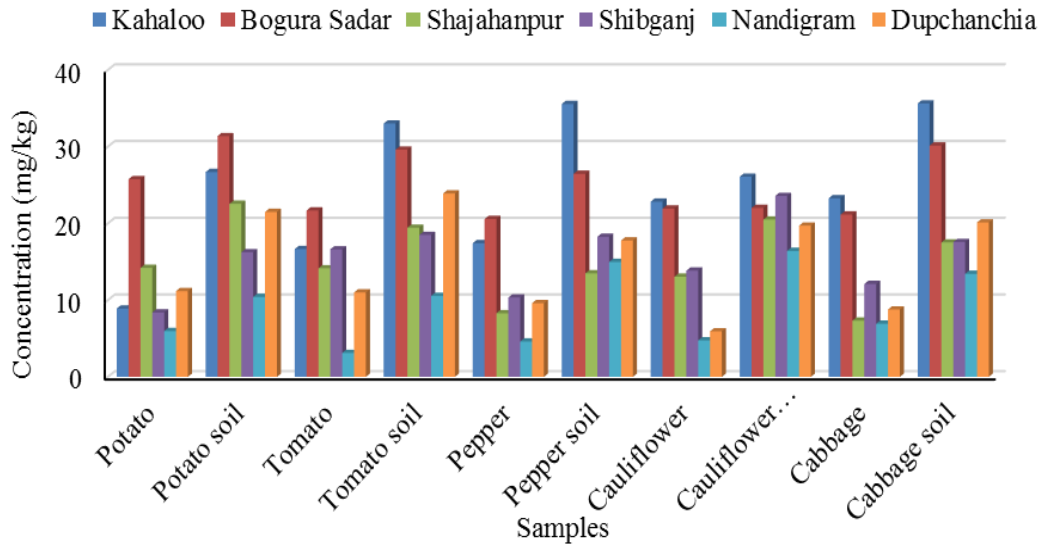


Figure 5. Lead (Pb) accumulation in vegetables and their field soil in different Upazilla of Bogura district.

4.3. Environmental pollution by heavy metals

4.3.1. Contamination factor (CF)

The single metal contamination factor (CF_i) is the ratio of the single metal concentration in a biotic or abiotic medium to the regulatory standard set by national or international organizations like the Bangladesh Food Safety Authority (BSFA), the World Health Organization (WHO), and the United States Environmental Protection Agency (USEPA). The contamination factor (CF) for single metal is important in determining pollution by individual heavy metals in soil, and vegetable samples. A CF < 1 value indicates low contamination, a 1 < CF < 3 value indicates moderate pollution, 3 < CF < 6 value indicates significant pollution, but a CF > 6 value implies severe pollution (Hakanson, 1980b). Similarly, CF = 1 denotes a critical state, making the samples involved important for environmental monitoring. In terms of vegetable contamination, only Pb severely contaminated the vegetables (CF > 6)

produced in different Upazilla of the Bogura district, while the $CF < 1$ and $1 < CF < 3$ values for Cu and Cr indicated low to moderate contamination in vegetables produced in the Bogura district (Figure 6). In the case of vegetable field soil samples, contamination factor ($CF < 1$) values for Cu, Cr, and Pb revealed low contamination in all vegetable field soil collected from different Upazilla of the Bogura district (Figure 6). So, cultivated vegetables in different Upazilla were severely contaminated by Pb.

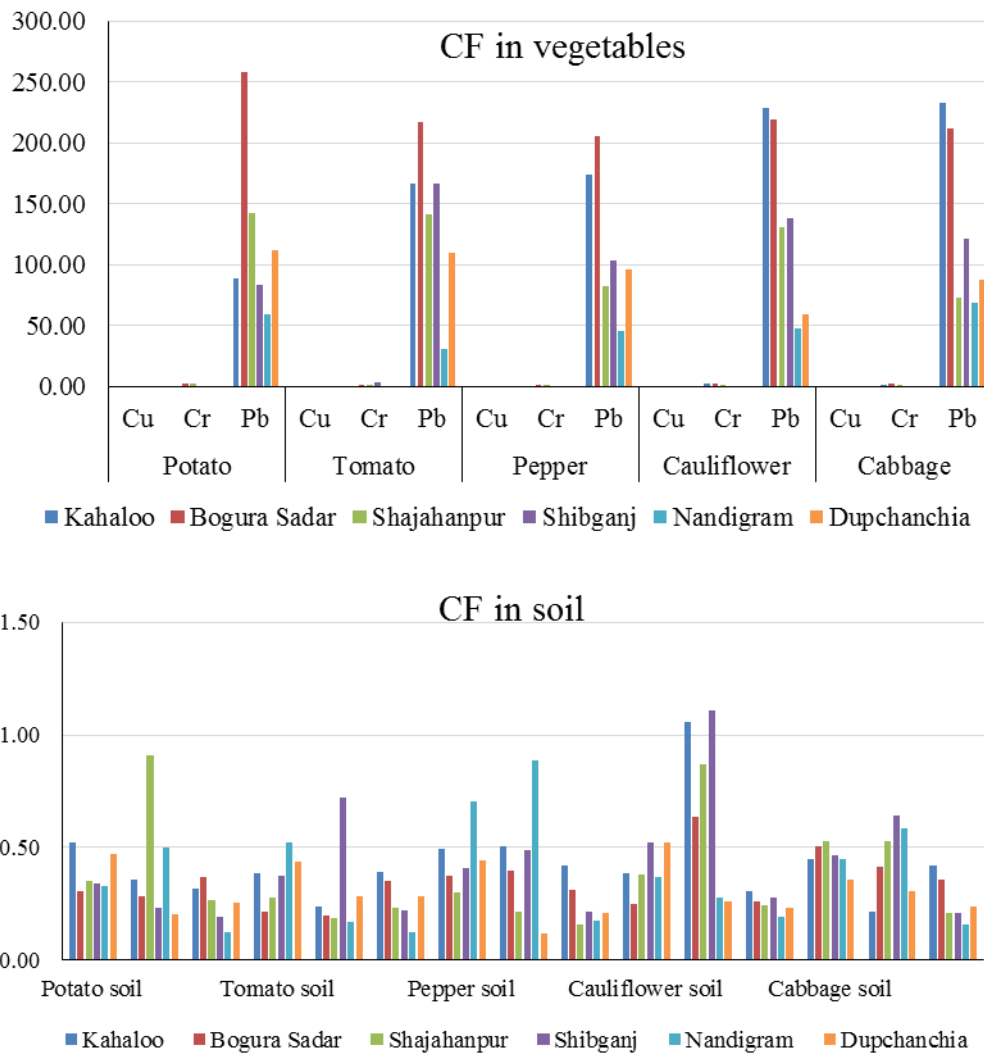


Figure 6. Contamination factor (CF) of heavy metals in vegetables (A) and their field soils (B) in different Upazilla of Bogura district.

4.3.2. Contamination degree (CD)

Contamination degree (CD) is the sum of the contamination factors (CF) of the heavy metals that have been measured at specific sites. The aim of the contamination degree (CD) is to provide an assessment of the overall level of contamination in specific samples at a certain sampling site. The contamination degree (CD) values varied significantly between vegetable field soils, and vegetable samples collected from the study area (Figure 7). All the vegetable samples (potato, tomato, pepper, cauliflower, and cabbage) collected from all the locations of Bogura district had severe degree contamination, while all the vegetable fields soil had low degree contamination (Figure 7). The highest degree of contamination was found in vegetables produced in Bogura Sadar and Kahaloo, while the lowest degree of contamination was found in the vegetables produced in Nandigram and Dupchanchia (Figure 7). The contamination degree results revealed that, although all vegetable fields soil of different Upazilla of Bogura district had a low degree of contamination but all the vegetables produced in the fields of different Upazilla of Bogura district had a severe degree of contamination.

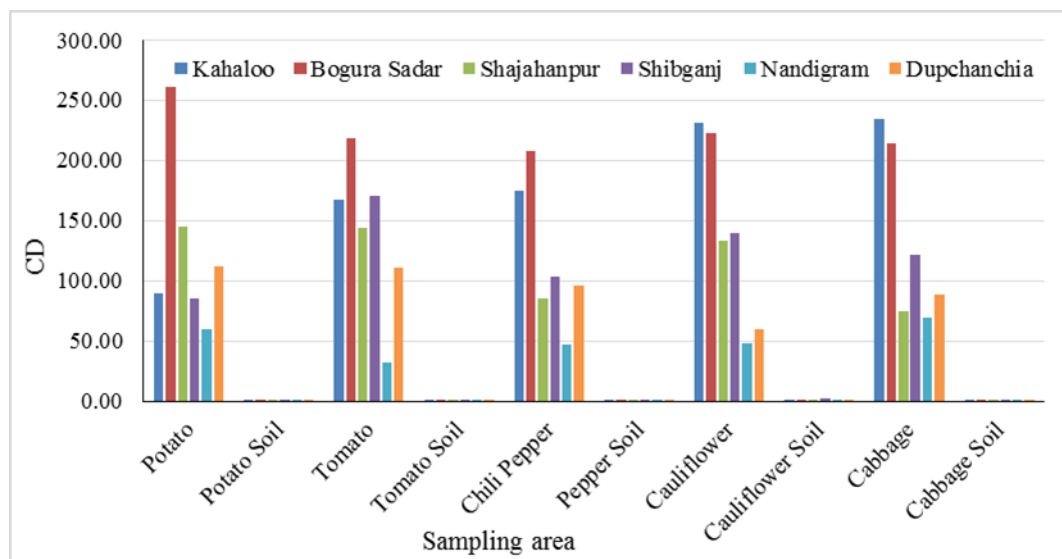


Figure 7. Contamination degree (CD) of heavy metals in vegetables and their field soils in different Upazilla of Bogura district.

4.3.3. Pollution load index (PLI)

The pollution load index (PLI) is used to measure the total heavy metal concentrations in vegetables and soils. This was done by taking the geometric mean of all metal concentrations. The PLI provided an evaluation of the sample's overall toxicity and was the consequence of the contribution of four hazardous elements. The pollution load index (PLI) values varied significantly between soil, and vegetable samples collected from the study area (Figure 8). According to Figure 8, the pepper field soils in Kahaloo had the highest PLI values. On the other hand, PLI was low in all vegetables compared to their field soil. Therefore, the vegetable field soil of Kahaloo and Bogura Sadar had the highest pollution load index, while the vegetables produced in Nandigram and Dupchanchia had the lowest pollution load index.

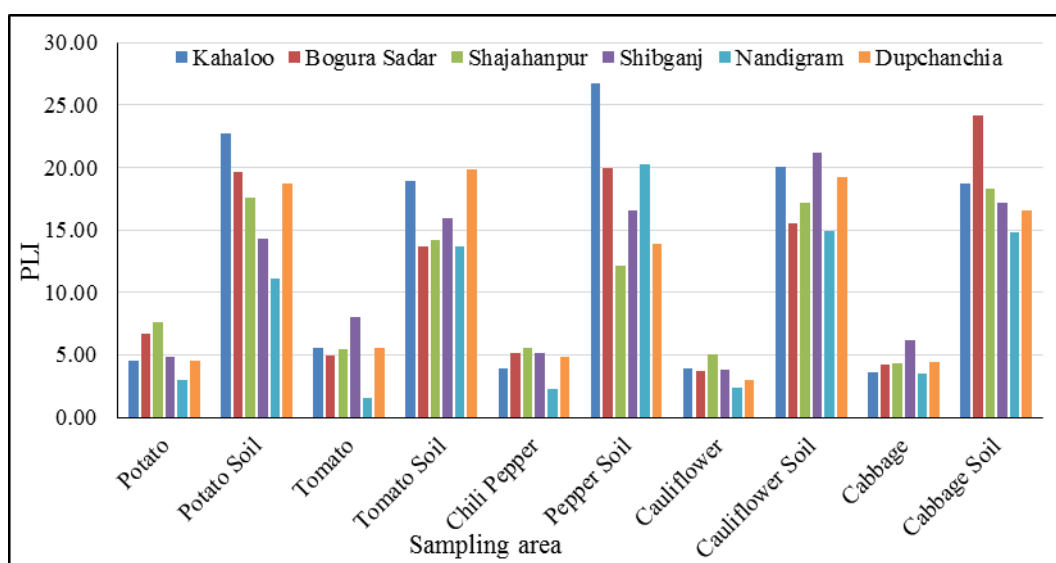


Figure 8. Pollution load index (PLI) of heavy metals in vegetables and their field soil of different Upazilla of Bogura district.

4.3.4. Bioaccumulation factor for plants (BF)

Copper, chromium, and lead concentrations in the vegetables were lower than in their field soil. Heavy metals (Cu, Cr, and Pb) were found in high concentrations in the field soil of several Bogura district Upazilla vegetable fields. The bioaccumulation factors (BF) for copper, chromium, and lead in potatoes, tomatoes,

pepper, and cabbage were significantly different. In the case of Cu, the highest bioaccumulation factor (BF) was found (0.92) for tomatoes, while the lowest BF (0.44) was found for Cu in cauliflower (Table 5). For Cr, the highest bioaccumulation factor (BF) was found (0.05) for tomato, while the lowest BF (0.02) was found for Cr in pepper (Table 1). Moreover, the highest bioaccumulation factor (BF) for Pb was found (0.64) in cauliflower, while the lowest BF was found (0.56) in pepper (Table 5). The bioaccumulation factor (BF) values were found <1 in all vegetables (potato, tomato, pepper, cauliflower, and cabbage) for Cu, Cr, and Pb. As evidenced by the BF values for different metals in vegetables, it is important to highlight that vegetable plants translocate trace amounts of metals (Cu, Cr, and Pb) to the consumable parts in comparison to the metal amount in the vegetable field soil. Our research showed that hyper accumulation is often metal-selective and that diffusion limits at the soil level lower the overall potential of some plants accumulating particular metals, Cu, Cr, and Pb being probable ones.

4.3.5. Ecological risk factor (ERi)

The ecological risk factor (ER) is designed to assess soil contamination by a single toxic compound. The ecological risk factor (RFi) for soil samples collected from study areas of Bogura district were summarized in Figure 9. According to the ecological risk factor (ERi) values for individual Cu, Cr, and Pb present in five vegetables (potato, tomato, pepper, cauliflower, and cabbage) field soil sampled in six study sites (Kahaloo, Bogura Sadar, Shajahanpur, Shibganj, Nandigram, and Dupchanchia) posed low ecological risk factor since their ERi values for Cu, Cr and Pb were all less than 40. The highest ecological risk factor (ERi) is posed by Copper, followed by Pb and Cr in five vegetable field soils in Kahaloo, Bogura Sadar, Shajahanpur, Shibganj, Nandigram, and Dupchanchia Upazilla of Bogura district.

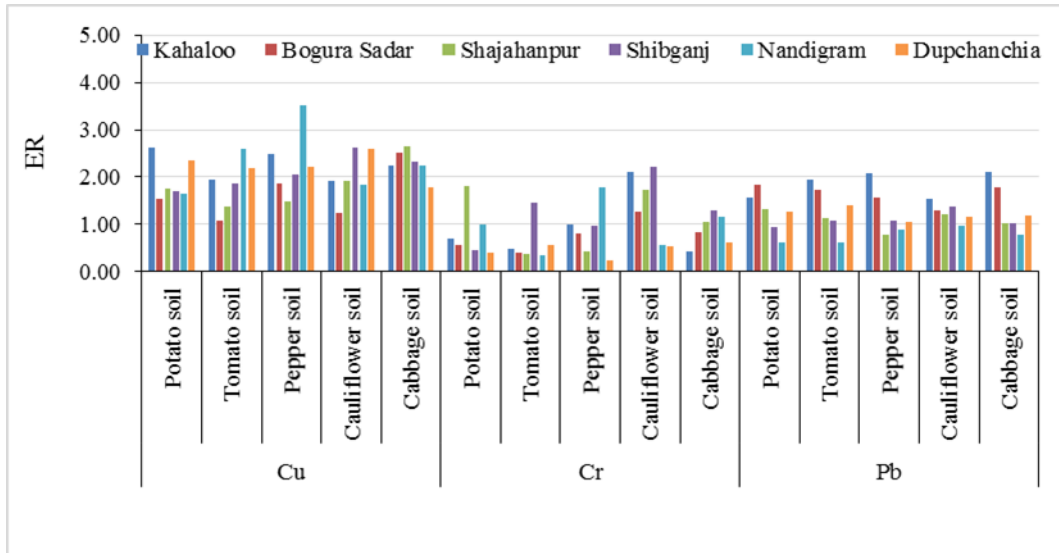


Figure 9. Ecological risk factors by Cu, Cr, and Pb in vegetable field soil of different Upazilla of Bogura district.

4.3.6. Potential ecological risk index (PERI)

The potential ecological risk index is widely used to evaluate soil quality. The potential ecological risk index (PERI) is also introduced to evaluate the degree of contamination of different toxic compounds in soils. The potential ecological risk index (PERI) for soil samples collected from study areas of Bogura district were summarized in Figure 10. According to the potential ecological risk index (PERI) values for all Cu, Cr, and Pb present in the soil sample, six study sites posed low ecological concerns since their PERI values were all less than 150. Biological community sensitivity to heavy metals as a result of excessive toxic metal contamination is reflected in ecological risk. The overall ecological risk assessment result of this experiment reveals that the multiple toxic heavy metals have lower ecological risks in vegetable fields in different Upazilla of the Bogura district.

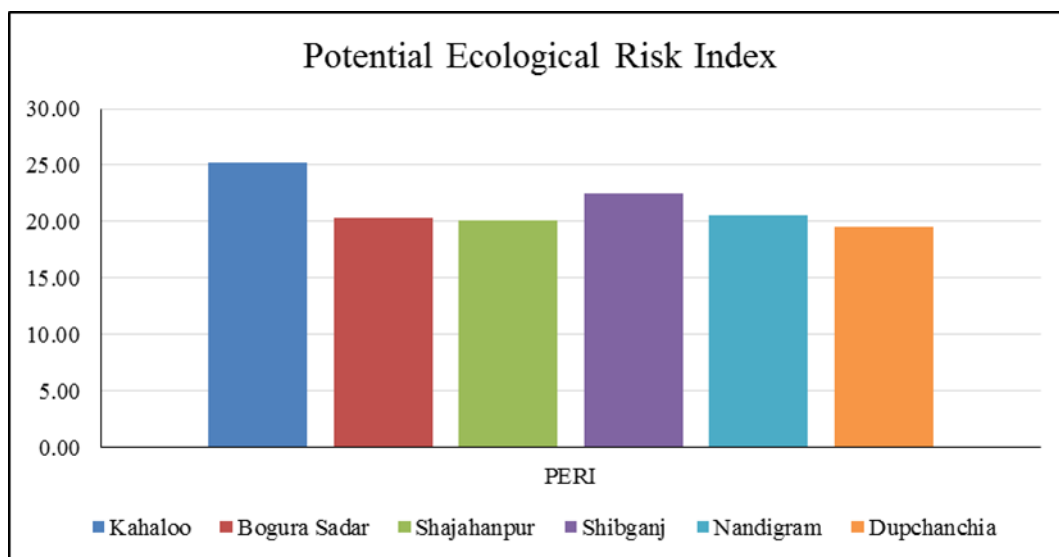


Figure 10. Potential ecological risk index by multi heavy metals in vegetable fields of different Upazilla of Bogura district.

4.4. Health risks of heavy metals in vegetables

The assessment of human health risks based on heavy metal contamination in food and the environment finds adverse health impacts. To establish consumer health protection policies, this study investigated cancer and non-cancer health hazards posed to adults in Bangladesh by consuming heavy metal-contaminated vegetables produced in the Bogura district. The United States Environmental Protection Agency (USEPA) proposed the equation for assessing the non-carcinogenic and carcinogenic health risks linked with heavy metal pollution in foods.

4.4.1. Non-carcinogenic health risk:

The non-carcinogenic hazard quotient (HQ_i) of individual heavy metals and the cumulative non-carcinogenic hazard index (HI) from numerous metals were explored through vegetable consumption (potato, tomato, pepper, cauliflower, and cabbage) for adults in the study region, as shown in Table 6. In the case of Cu, a higher than the safe level of hazard quotient (HQ_i > 1) was observed in tomato, pepper and cabbage produced in the Kahaloo, Dupchanchia, Nandigram, and Shibganj areas of the Bogura district. The result indicates that all other vegetables produced in the six Upazilla were within the safe limit (HQ < 1) for Hazard Quotient from Cu. In the case of Cr, the potato, tomato, pepper, cauliflower, and cabbage

samples collected from Shibganj, Shajahanpur, Bogura Sadar, and Kahaloo areas were found to have a higher target hazard quotient ($HQ > 1$) than the safe level, except for potato, tomato and pepper produced in Kahaloo, and pepper, cauliflower and cabbage produced in Shibganj Upazilla of Bogura district (Table 6). For Pb, vegetables produced in all study areas showed 2 to 20 times the target hazard quotient ($HQ > 1$) for adults. The hazard quotients of ($HQ > 1$), Pb were much higher for adults than other heavy metals investigated in potato, tomato, pepper, cauliflower, and cabbage produced in different Upazilla of Bogura district due to its high-level Pb content and very low RfD (Table 6).

After quantitative analysis and calculated investigation, the cumulative hazard indexes from multiple metals were found much higher ($HI > 1$) than the safe limit for vegetable consumption from the study areas. It indicates that intake of these heavy metals through the consumption of vegetables poses a considerable non-cancer risk. The cumulative non-carcinogenic health risk associated with vegetable consumption were assigned to Bogura Sadar > Kahaloo > Shajahanpur > Shibganj > Dupchanchia > Nandigram in decreasing order of producing areas. According to this study, heavy metals may pose a health hazard to the consumer of vegetables produced in different Upazilla of the Bogura district. So, action is needed to reduce heavy metal pollution in the vegetable fields of different Upazilla of Bogura district to protect the consumer's health.

4.4.2. Carcinogenic health risk

In this experiment, Cr, and Pb were evaluated to determine the risk of cancer associated with vegetable (potato, tomato, pepper, cauliflower, and cabbage) consumption. The International Agency for Research on Cancer (IARC) classifies Cr, and Pb as carcinogenic agents and chronic exposure to low concentrations of these metals can cause a variety of cancers (Järup, 2003; Tani and Barrington, 2005). The US-Environmental Protection Agency recommends that the cancer risk could be expressed as no significant carcinogenic health risk ($ILCRi$ or $CHR < 10^{-6}$); acceptable carcinogenic health risk ($10^{-6} < ILCRi$ or $CHR < 10^{-4}$); or unacceptable carcinogenic health risk ($ILCRi$ or $CHR > 10^{-4}$) (USEPA, 2001). Table 7 presents the calculated carcinogenic health risk ($ILCRi$) due to individual single metal and

cumulative cancer risk for multiple metals (CHR) for Cr and Pb through the studied vegetable consumption. The carcinogenic health risk (ILCRi) for Pb exceeded the unacceptable cancer risk limit ($>10^{-4}$) in all of the all-vegetable samples collected from six upazillas of the Bogura district. As exceeded the unacceptable cancer risk limit ($>10^{-4}$) for Cr in vegetables only produced in Bogura Sadar and Shajahanpur Upazillas of Bogura district posed unacceptable cancer risk for Cr in vegetables (Table 7). As a result, Pb is the most abundant carcinogen in the research area, and efforts should be made to limit Pb exposure to the environment to protect the population from cancer risk. Therefore, the cumulative carcinogenic health risk for multiple metals (CHR) of all the analyzed vegetable samples collected from different upazillas of Bogura district exceeded the unacceptable cancer risk level ($>10^{-4}$). Among all the vegetable samples analyzed, vegetables produced in Bogura Sadar have the highest risk of cancer ($3.65E+01$), while vegetables produced in Nandigram have the lowest risk of cancer ($8.33E+00$). The CHR values for Cr and Pb in vegetables produced showed the following order: Bogura Sadar > Kahaloo > Shibganj > Shajahanpur > Dupchanchia > Nandigram (Table 7).

Table 6. Non-carcinogenic health risk (HQi and HI) for the Adult population through the consumption of vegetables produced in the study area.

HQi	Potato			Tomato			Pepper			Cauliflower			Cabbage			HI
	Cu	Cr	Pb	Cu	Cr	Pb	Cu	Cr	Pb	Cu	Cr	Pb	Cu	Cr	Pb	
Kahaloo	0.58	0.67	7.10	1.31	0.69	13.27	0.54	0.00	13.89	0.29	3.49	18.20	0.35	2.10	18.55	81.02
Bogura Sadar	0.67	3.48	20.53	0.77	1.38	17.28	0.61	1.57	16.40	0.27	3.28	17.48	0.38	2.86	16.87	103.80
Shajahanpur	0.79	3.51	11.32	0.88	1.98	11.27	0.68	2.56	6.60	0.53	2.42	10.40	0.46	1.83	5.84	61.08
Shibganj	0.63	1.10	6.69	0.86	5.06	13.24	0.91	0.00	8.25	0.46	0.85	11.03	1.14	0.21	9.65	60.07
Nandigram	0.60	0.00	4.76	0.82	0.00	2.49	1.08	0.00	3.67	0.64	0.00	3.79	1.12	0.00	5.52	24.49
Dupchanchia	0.63	0.00	8.91	1.18	0.00	8.77	0.85	0.00	7.66	0.50	0.00	4.73	0.66	0.00	6.99	40.88

Table 7. Carcinogenic health risk (CHR) for the Adult population through the consumption of vegetables produced in the study area.

ICLE	Potato		Tomato		Pepper		Cauliflower		Cabbage		CHR
	Cr	Pb	Cr	Pb	Cr	Pb	Cr	Pb	Cr	Pb	
Kahaloo	0.00	2.92	0.00	5.47	0.00	5.72	0.02	7.49	0.01	7.64	2.93E+01
Bogura Sadar	0.02	8.45	0.01	7.12	0.01	6.75	0.02	7.20	0.02	6.94	3.65E+01
Shajahanpur	0.02	4.66	0.01	4.64	0.02	2.72	0.01	4.28	0.01	2.40	1.88E+01
Shibganj	0.01	2.76	0.03	5.45	0.00	3.40	0.01	4.54	0.00	3.97	2.02E+01
Nandigram	0.00	1.96	0.00	1.02	0.00	1.51	0.00	1.56	0.00	2.27	8.33E+00
Dupchanchia	0.00	3.67	0.00	3.61	0.00	3.15	0.00	1.95	0.00	2.88	1.53E+01

CHAPTER V

SUMMARY AND CONCLUSION

The study was conducted to investigate the status of heavy metals (Cu, Cr, and Pb) in five vegetables (potato, tomato, pepper, cauliflower, and cabbage) collected from six different Upazilas (Kahaloo, Bogura Sadar, Shahjahanpur, Shibganj, Nandigram, and Dupchanchia) of Bogura district, Bangladesh. Heavy metal concentrations in five different vegetables collected from the Bogura District were tested using an Atomic Absorption Spectrophotometer (AAS). The detection method was validated according to linearity, determination co-efficient, LOD, LOQ, accuracy, and precision of the instrumental situation. The method showed good linearity with the highest determination coefficient as well as good recovery with the lowest relative standard deviation for quantitative analysis of heavy metals in vegetables and soil samples. The potential environmental contamination of vegetable field soil and health concerns posed by heavy metals to consumers through vegetable intake were calculated.

The concentrations of Cu in vegetables are quite variable, ranging from 3.86 to 18.55 mg/kg. The results showed that vegetable field soil contained higher Cu than vegetables. All the vegetables and their field soil had Cu concentrations lower than the maximum permissible limit set by BFSA, FAO/WHO. The mean Cu accumulation in vegetables grown in several Upazilas in the Bogura district was found in the order of tomato > pepper > cabbage > potato > cauliflower, while for Cu accumulation in vegetable field soil the order of cabbage field soil > pepper field soil > cauliflower field soil > potato field soil > tomato field soil. The Cr concentration in vegetables ranged from below the detectable limit to 5.44 mg/kg, while its concentration in vegetable field soil ranged from 11.95 to 110.70 mg/kg in different Upazilas of the Bogura district. The mean Cr accumulation in vegetables grown in several Upazilas in the Bogura district was found in the order of cauliflower > tomato > potato > cabbage > pepper. In vegetable fields soil in several upazillas of the Bogura district, the mean concentration of Cr was found in the order of cauliflower field soil > cabbage field soil > pepper field soil > potato field soil > tomato field soil. The concentration of Cr in vegetables exceeds the maximum

allowable limit as per WHO but the soil of their fields in different upazillas of Bogura District did not exceed the maximum allowable limit as per FAO/WHO. The heavy metal Pb concentrations in vegetables are variable, ranging from 3.12 to 25.77 mg/kg. while its concentration in vegetable field soil ranged from 10.42 to 35.64 mg/kg in different Upazilla of Bogura district. The mean Pb accumulation in vegetables grown in several Upazilas in the Bogura district was found in the order of tomato > cauliflower > cabbage > potato > pepper. In vegetable fields soil in several upazillas of the Bogura district, the mean accumulation of Pb was found in the order of tomato field soil > cabbage field soil > potato field soil > cauliflower field soil > pepper field soil. The concentration of Pb in vegetables exceeds the maximum allowable limit as per WHO but the soil of their fields in different upazillas of Bogura District did not exceed the maximum allowable limit as per FAO/WHO.

To assess environmental pollution, in this study we calculated contamination factor (CF), Contamination degree (CD), Pollution load index (PLI), Bioaccumulation factor for plants (BF), Ecological risk factor (ER_i), and Potential ecological risk index (PERI) from the detected amount of heavy metals in vegetables and their field soil. For contamination factor (CF), in terms of vegetable contamination, only Pb severely contaminated the vegetables (CF>6) produced in different Upazilas of Bogura district, while the CF<1 and 1<CF<3 values for Cu and Cr indicated low to moderate contamination in vegetables produced in Bogura district. In the case of vegetable field soil samples, contamination factor (CF<1) values for Cu, Cr, and Pb revealed low contamination in all vegetable field soil collected from different Upazilas of the Bogura district. Moreover, the contamination degree (CD) results also revealed that, although all vegetable fields soil of different Upazilas of Bogura district had a low degree of contamination but all the vegetables produced in the fields of different Upazilas of Bogura district had a severe degree of contamination. The pollution load index (PLI) is used to measure the total heavy metal concentrations in vegetables and soils. Therefore, the vegetable field soil of Kahaloo and Bogura Sadar had the highest pollution load index, while the vegetables produced in Nandigram and Dupchanchia had the lowest pollution load index. The bioaccumulation factor (BF) values were found <1 in all vegetables (potato, tomato, pepper, cauliflower, and cabbage) for Cu, Cr, and Pb. As evidenced by the BF values for different metals in vegetables, it is important to highlight that vegetable plants

translocate trace amounts of metals (Cu, Cr, and Pb) to the consumable parts compared to the metal amount in the vegetable field soil. Furthermore, the potential ecological risk factor (ERi) by single metal and risk index (PERI) by multiple metals is widely used to evaluate soil quality. According to the ecological risk factor (ERi) values for individual Cu, Cr and Pb present in five vegetable field soil sampled in six study sites posed a low ecological risk, since their ERi values for Cu, Cr, and Pb were all less than 40. According to the potential ecological risk index (PERI) values for all Cu, Cr, and Pb present in the soil sample, six study sites posed low ecological concerns since their PERI values were all less than 150. The overall ecological risk assessment result of this experiment reveals that the individual and multiple toxic heavy metals have lower ecological risks in vegetable fields in different Upazilas of the Bogura district.

The assessment of human health risks based on heavy metal contamination in vegetables produced in different Upazillas of Bogura district to find adverse health impacts to consumers. The non-carcinogenic hazard quotient (HQi) of individual heavy metals and the cumulative non-carcinogenic hazard index (HI) from multi-metals were explored through vegetables consumption (potato, tomato, pepper, cauliflower, and cabbage) for adults in the study region. In the case of Cu, a higher than the safe level of hazard quotient ($HQ_i > 1$) was observed in pepper and cabbage produced in the Nandigram, and Shibganj areas of the Bogura district. In the case of Cr, the potato, tomato, pepper, cauliflower, and cabbage samples collected from Shibganj, Shajahanpur, Bogura Sadar, and Kahaloo areas were found to have a higher target hazard quotient ($HQ > 1$) than the safe level, except for potato, tomato and pepper produced in Kahaloo, and pepper, cauliflower and cabbage produced in Shibganj Upazila of Bogura district. For Pb, vegetables produced in all study areas showed 2 to 20 times the target hazard quotient ($HQ > 1$) for adults. The hazard quotients of ($HQ > 1$), Pb were much higher for adults than other heavy metals investigated in potato, tomato, pepper, cauliflower, and cabbage produced in different Upazilas of Bogura district due to its high-level Pb content and very low RfD. The cumulative hazard indexes from multiple metals were found much higher ($HI > 1$) than the safe limit for vegetable consumption from the study areas. It indicates that intake of these heavy metals through the consumption of vegetables poses a considerable non-cancer risk. In this experiment, Cr, and Pb were evaluated

to determine the risk of cancer associated with vegetable (potato, tomato, pepper, cauliflower, and cabbage) consumption. The calculated carcinogenic health risk (ILCRi) due to individual single metal and cumulative cancer risk for multiple metals (CHR) for Cr and Pb through the studied vegetable consumption. The carcinogenic health risk (ILCRi) for Pb exceeded the unacceptable cancer risk limit ($>10^{-4}$) in all of the all-vegetable samples collected from six upazillas of the Bogura district. As exceeded the unacceptable cancer risk limit ($>10^{-4}$) for Cr in vegetables only produced in Bogura Sadar and Shajahanpur Upazillas of Bogura district posed unacceptable cancer risk for Cr in vegetables. the cumulative carcinogenic health risk for multiple metals (CHR) of all the analyzed vegetable samples collected from different upazillas of the Bogura district exceeded the unacceptable cancer risk level ($>10^{-4}$). The CHR values for Cr and Pb in vegetables produced showed the following order: Bogura Sadar > Kahaloo > Shibganj > Shajahanpur > Dupchanchia > Nandigram. According to this study, heavy metals may pose a carcinogenic and non-carcinogenic health hazard to the consumer of vegetables produced in different Upazilas of Bogura district. So, action is needed to reduce heavy metal pollution in the vegetable fields of different Upazilas of Bogura district to protect the consumer's health.

CHAPTER VI

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