HUMAN HEALTH RISK ASSESSMENT THROUGH QUANTITATIVE DETERMINATION OF HEAVY METALS IN VEGETABLES FROM THE SOUTHWEST PARTS OF BANGLADESH

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CERTIFICATE

This is to certify that the thesis entitled **“HUMAN HEALTH RISK ASSESSMENT THROUGH QUANTITATIVE DETERMINATION OF HEAVY METALS IN VEGETABLE FROM SOUTHWESTERN PARTS OF BANGLADESH”** submitted to the Department of Agricultural Chemistry, Faculty of Agriculture, Sher-e-Bangla Agricultural University, Sher-e-Bangla Nagar, Dhaka in partial fulfillment of the requirements for the degree of **Master of Science (M.S.)** in **Agricultural Chemistry**, embodies the result of a piece of bonafide research work carried out by **KEYA ROY, Registration No. 15-06809** 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, as has been availed of during the course of this investigation has been duly acknowledged by the Author.

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The Author

HUMAN HEALTH RISK ASSESSMENT THROUGH QUANTITATIVE DETERMINATION OF HEAVY METALS FROM VEGETABLE IN THE SOUTHWESTERN PART OF BANGLADESH[1]

ABSTRACT

A study was conducted to assess the human health risk through the quantitative determination of heavy metals (Pb, Cu, Cd and Cr) from vegetables in the southwest part of Bangladesh. Samples of vegetables and soils were collected from nine upazila of three districts- Satkhira (Assasuni, Kaliganj, Shyamnagar); Jashore (Jikragacha, Monirampur Sorsha); Khulna (Koyra, Paikgacha, Dumuria).Vegetable species e.g., Spinach, Tomato, Calabash, Cauliflower, Kohlrabi, Cabbage, Red Amaranth, and Bean. The highest Pb and Cd concentration were found in the Assasuni, Satkhira which were higher than the FAO/WHO permissible limits (0.1 mg kg^{-1} for Pb and 0.05 mg kg^{-1} for Cd) in most of the vegetables indicating might pose higher risk. The highest Cr concentration was found in Assasuni, Satkhira and the lowest in Khulna and Jashore which were lower than the FAO/WHO permissible limits (2.30 mg kg^{-1}) except for a few vegetables in Satkhira that indicated Cr did pose a high risk. The highest Cu concentration was found in Kaliganj, Satkhira, and the lowest was found in Shyamnagar, Satkhira. The trend of Transfer Factor for heavy metals was in order: Cu>Cd>Pb>Cr. The highest Sediment Pollution Index value was found in Spinach (258.98) in Assasuni, Satkhira, and the lowest value was found in Kohlrabi in Dumuria, Khulna. The highest Metal Pollution Index 9.40 mg kg^{-1} was found in Assasuni, Satkhira and the lowest 0.105 mg kg^{-1} was found in Dumuria, Khulna. The cumulative non-carcinogenic health risk associated with vegetable consumption were assigned to Assasuni > Kaliganj > Shyamnagar in Satkhira; Monirampur > Sorsha >Jikragacha in Jashore and Paikgacha > Koyra >Dumuria in Khulna. The cumulative cancer risk for multiple metals for Pb, Cd and Cr of all studied locations was higher than the unacceptable limit ($>10^{-4}$) which indicates heavy metals may pose a carcinogenic and non-carcinogenic health hazard . So, steps were needed to alleviate heavy metal pollution in the vegetable fields to protect the consumer's health.

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ABBREVIATIONS USED

%	Percent
&	And
/	Per
a.i.	Active ingredient
e.g.	Example Gratia (For example)
etc.	Etcetera (and all)
et al.	et alia (and other)
F. cal.	Calculated value of F
F. tab.	Tabulated value of F F- test
Fig.	Figure
Hrs.	Hours
i.e.	id est. (That is) INAA International Neutron Activation Analysis
Kg	Kilogram
IFAD	Liter
Min.	Minimum
Max.	Maximum
Mg	Milligram
Ml	Milliliter
N.S.	Non – significant
Ppm	parts per million
CRD	Complete Randomized Design Rs.
S.D.	Standard deviation SE(9±)
SR	Serial
Viz.	vide licet (namely)
˚C	Degree Centigrade / Degree Celsius
C.D	Critical Difference
Cm	Centimeter
CV	Coefficient of Variation
Df	Degree of Freedom
ECV	Environmental Coefficient of Variation EMS
ESP	Error Sum of Products
ESS	Error Sum of Squares
G	Gram
Mm	Millimeter
AAS	Atomic absorption spectrophotometer
NAFDC	National Agency for Food and Drug Administration control PFA
CFU	Colony form unit
MSS	Mean Sum of Squares WHO

DIM	Daily intake of metals
DDI	Daily dietary intake
RSS	Replication Sum of Squares
SEPA	State environmental protection administration. SS
Tr SS	Treatment Sum of Squares
TSS	Total Sum of Squares
HQ	Hazard Quotients
HRI	Health risk index
TMDI	Theoretical maximum daily intake
ICMSF	International Commission on Microbiological Specifications for Food.
MPI	Metal pollution index
SPI	Sediment pollution Index
TF	Transfer factor
Cu	Cuprous (Copper)
Cd	Cadmium
Pb	Lead
Ni	Nickel
Mg	Magnesium
Mn	Manganese
Zn	Zinc
Cr	Chromium

CHAPTER I

INTRODUCTION

The fresh edible parts of herbaceous plant roots, stems, leaves, or fruits are referred to as vegetables. Around the world, vegetables are used in daily food. It is a crucial source of the vitamins and minerals needed to recover from various human ailments. They are also regarded as a low-cost source of energy. 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 such as heart and age-related illnesses (Iso, 2018; Zhao et al., 2021). According to studies from Kansas State University, consuming a variety of foods high in antioxidants can help prevent numerous chronic diseases as well as slow the aging process. Numerous free antioxidants have been linked to decreased incidence of heart disease and cancer death (USDA Agricultural Research Service). It serves as a crucial buffer for the acidic components created during digestion (Thompson and Kelly, 1990).

Metals, on the other hand, are elements that exist as positive ions in chemical compounds or as cations (+charged ions) in solution. Because vegetables are a crucial part of the human diet, their contamination with heavy metals (lead, arsenic, cadmium, copper, chromium, and nickel) cannot be understated. Heavy metals are dangerous environmental and food contaminants that are not biodegradable and have lengthy biological half-lives. They are among a number of significant pollutants that are present in the surface and tissue of fresh vegetables (Bigdeli and Seilsepour, 2008). Due to their high toxicity, abundance, and source of accumulation by many crops, they are very harmful environmental contaminants. The contribution of effluent from waste water treatment plants, industry, mining, power plants, surgical procedures, and agriculture can be used to explain the rise in heavy metals in soil (Guevara-Riba et al., 2004). In the environment, heavy metals are incredibly persistent. Due to their inability to degrade through both thermal and biological means, they can easily build up to dangerous amounts (Akguc et al., 2008). However, consuming heavy metal-contaminated vegetables may pose a risk to one's health; as a result, they are among the most crucial parts of food quality assurance (Radwan and Salama, 2006; Khan etal., 2009). The extraction and distribution of mineral compounds from their natural reserves have accompanied the global expansion of industry in the period of industrialization. Metals are not biodegradable and can undergo biomagnifications in living tissues, like many other environmental contaminants. Plants either take in and accumulate heavy metals through their roots or foliar surfaces (Sawidis et al., 2001).

A large population may be at risk from eating vegetables grown in contaminated soil, which could have negative health implications. Numerous studies have demonstrated that some vegetables have the capacity to accumulate significant amounts of harmful metals from the soil or water (Garcia et al., 1981). The surface and tissue of fresh vegetables can include a variety of significant types of pollutants, including heavy metals. Eat more fruits and vegetables, and most individuals will benefit. Consuming a lot of fruits and vegetables keeps the body healthy and disease-free. Macro and microelements are the two primary categories of minerals, depending on consumption. Calcium, Phosphorus, Sodium, Potassium, Magnesium ,Sulfur and Chloride are macro minerals, whereas iron, Copper, Cobalt, Iodine, Zinc, Manganese, Molybdenum, Fluoride, Chromium, Selenium, and are microminerals. The macro minerals must be present in concentrations larger than 100 mg/dL, whereas the microminerals must be present in concentrations less than 100 mg/dL (Murray et al., 2000). Vegetables' ability to absorb those trace elements varies depending on the variety, irrigation, cross-cultural interactions, maturity, genetics, age, and environmental factors (such as soil types, climate, season, water source, and fertilizer use) of plants as well as handling and processing techniques (Pennington and Calloway, 1973). Heavy metals have been identified as significant vegetable pollutants by a number of studies. Vegetables may become contaminated with heavy metals as a result of irrigation with contaminated water. It is important to test and analyze these vegetable items to make sure that the levels of these contaminants comply with the established international standards due to their potential toxicity, persistent nature, and cumulative behavior, as well as the intake of fruits and vegetables. In the majority of wealthy countries, regular heavy metal content monitoring programs have been in place for many years. But there isn't much information on heavy metals available in poor nations like our own. Because of their toxicity, bioaccumulation, and bio magnification in the food chain, heavy metal contamination of vegetables poses a severe hazard. Examples of these metals include arsenic (As), cadmium (Cd), mercury (Hg), cobalt (Co), nickel (Ni), lead (Pb), and others (Eisler, 1994). Untreated wastewater includes water discharged after usage from homes, laundry facilities, storm drains, sewage systems, and slaughterhouses. A product originates from domestic sources and by others. A murky, diluted, watery solution with both mineral and organic materials is typically sewage. Detergents, soap, human waste, metal, glass, garbage, sewage sludge, etc. are also included. Domestic sewage and other pollutants are known to enter aquatic bodies, such as ponds, lakes, streams, and rivers, untreated or only partially treated. In other words, sewage primarily consists of biodegradable pollutants like human waste, animal waste, and some dissolved organic compounds (such as urea, carbohydrates, etc.). It also contains organic salts like nitrates and phosphates from detergents, as well as sodium, potassium, calcium, and chloride ions. The majority of the sewage's biodegradable pollutants dissolve quickly through natural processes, but when they collect in significant amounts, which occurs when their input into the environment exceeds the nature's capacity for breakdown or disposal, issues result (Kannan et al., 2003).

These metals can be extremely harmful to people's health, especially at concentrations that are higher than what the body actually needs. Therefore, metals in food sources must be under control to ensure the safety of the general public's health. These metals may enter the food chain via various biochemical processes, where they may then bio magnify at various tropic levels and ultimately endanger human health. Another major environmental problem is heavy metal contamination of plants. They can be found everywhere in the environment through a variety of natural and human-made processes. Metals may accumulate to dangerous concentrations and harm the environment in certain environmental circumstances. While Arsenic, Cadmium, Chromium, Mercury, Nickel, and Lead are cumulative poisons, organisms need metals like Iron, Copper, Zinc, and Manganese for metabolic functions. They are reportedly extremely toxic, according to reports (Ellen et al., 1990). Intoxications with lead have been linked to issues with the kidney and liver, the central nervous system, the reproductive organs, and anemia (Lokeshwari and Chandrappa, 2006). Our population is made up largely of people who are aware that vegetables should be a part of every meal. Despite the fact that vegetables are more economical than foods of animal origin for a large portion of the population, ignorance of their significance in fulfilling daily micronutrient requirements is one of the main causes of the widespread incidence of malnutrition. On the other hand, due to a lack of scientific understanding, a lack of cultivation land, certain individuals abusing the environment, Wawa pesticides, fertilizers, etc., which are substantial source of heavy metals, vegetables grown in those conditions must be contaminated. The study is necessary because many people eat the seven vegetables that are grown there.

It is clear that heavy metal pollution may have a catastrophic impact on both agricultural output and human health. However, there is no such study to understand the heavy metals such as (Cr, Cd, Pb, and Cu) content in winter vegetable samples collected from three districts of southwest parts of Bangladesh. Therefore, the following objectives were set for the current study:

Objectives of the research work:

1. To determine concentration of heavy metals (Pb, Cd, Cr and Cu) in different vegetables
2. To interpret the results from the level of contamination
3. To assess the probabilistic human health risks of those heavy metals .

CHAPTER II

REVIEW OF THE LITERATURE

A review of the literature helps to compare the past and present research works related to the study which helps an investigation to draw a satisfactory conclusion for this research work. The most relevant studies, which have been conducted in the recent past related to the present research work, are presented below.

Laboni et al. (2023) conducted a study to determine the quantity of heavy metals in widely consumed watercress (WC), alligator weed (AW), red amaranth (RA), spinach (SP), cauliflower (CF), and eggplant (EP) cultivated in industrial areas (e.g., Narsingdi district) of Bangladesh to assess the potential health hazards. Atomic absorption spectroscopy (AAS) served to determine the concentrations of lead (Pb), cadmium (Cd), chromium (Cr), and nickel (Ni) in vegetable samples (n = 72). The contents of Pb, Cd, Cr, and Ni were found in most of the analyzed vegetables, whereas 79.17%, 44.44%, and 1.39% samples exceeded the FAO/WHO maximum allowable concentration (MAC) for Pb, Cd, and Ni, respectively. The estimated daily intake (EDI) of single heavy metal was below the corresponding maximum tolerable daily intake (MTDI). The incremental lifetime cancer risk (ILCR) values of Cd in all samples exceeded the threshold limit ($ILCR > 10^{-4}$) for both adults and children, indicating lifetime cancer risk due to the consumption of contaminated vegetables.

Kabir et al., 2022 conducted a study focused on quantifying hazardous heavy metals (As, Cd, Cr, Pb, Ni, and Zn) in soil-rice systems near the Buriganga River in Bangladesh to assess their impact on human health and the environment. The mean concentrations of As, Cd, Cr, Ni, and Zn in soil exceeded FAO/WHO acceptable limits, and the metal pollution index (MPI) indicated that all soil samples collected from the rice fields were severely polluted (MPI>30) than water and rice grain samples. According to the sum of pollution index (SPI) by studied metals, rice grains collected from Kamrangirchor (29.36), Dhakauddan (28.75), and Bosila (18.08) were severely polluted. Mean Bio-concentration factors (BCFs) and Transfer factors (TFgrain/soil) in rice grains were in the following order: 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 indicating higher accumulation of Cd in rice grain than others toxic heavy metal.

Ali et al. (2018) was conducted a study to assess the levels of toxic metals like arsenic (As), chromium (Cr), cadmium (Cd), and lead (Pb) in water and sediments of the Pasur River in Bangladesh. The ranges of Cr, As, Cd, Pb in water were 25.76-77.39, 2.76-16.73, 0.42-2.98 and 12.69-42.67 μg/L and in sediments were 20.67-83.70, 3.15-19.97, 0.39-3.17 and 7.34-55.32 mg/kg. The level of studied metals in water samples exceeded the safe limits of drinking water, indicating that water from this river is not safe for drinking and cooking. Certain indices, including pollution load index (PLI) and contamination factor (C^i_f) were used to assess the ecological risk. The PLI indicated progressive deterioration of sediments by the studied metals. Potential ecological risks of metals in sediment indicated low to considerable risk. However, the C^i_f values of Cd ranged from 0.86 to 8.37 revealed that the examined sediments were strongly impacted by Cd. Considering the severity of potential ecological risk (PER) for single metal (E^i_r), the descending order of contaminants was Cd > Pb > As > Cr. According the results, some treatment scheme must formulate and implement by the researchers and related management organizations to save the Pasur River from metals contamination.

Hasan et al. (2016) stated his study that the distribution of heavy metals (Pb, Ni, Fe, Mn, Cd, Cu) in the surface water of Bengal Coast at the southern part of Bangladesh. We also examined the common water quality parameters to discuss the impacts of pollution. It was revealed that the majority of the heavy metals have been introduced into the Bengal marine from the riverine inflows that are also affected by the impact of industrial, ship breaking yard, gas production plant and urban wastes. The concentration of heavy metals was measured using atomic absorption spectroscopy (AAS) instrument. Heavy metals concentrations were found to decrease in a sequence of Fe > Mn > Pb > Cu > Cd > Ni. Results showed that heavy metal concentrations in the marine surface water generally exceed the criteria of international marine water quality.

Alamgir et al. (2015) assessed the distribution of heavy metals in the urban environment; concentrations of Cd, Cu, Pb, Mn, Ni and Zn were measured on 21 topsoil samples collected from roadside soils of Chittagong city. The heavy metal concentrations were determined by flame atomic absorption spectrometry after digesting the soils with nitric acid perchloric acid. Mean Cd, Cu, Pb, Mn, Ni and Zn concentrations of the investigated urban soils are 2.43, 32.63, 7.33, 160.79, 860.33, 139.30 mg kg-1 respectively. Compared to urban soils of some other cities in the world Cu, Cd, Pb, Mn and Zn concentrations were somewhat similar. Ni concentration largely exceeded the maximum allowable concentration (60 mg/kg) indicating high contamination. Stepwise multiple regression indicated that soil properties were responsible for 37 to 42% variation in Cd, Cu and Pb content and in case of Ni it was only 16%. The main sources of Ni contamination in Chittagong city can be considered anthropogenic sources.

Tasrina et al. (2015) carried out an experiment and resulted that the Hg in the sampling station was below the detection limit (<0.03 mg/ kg) and the concentration of Ni, Cu, Cd, Pb, Cr, Co were below the permissible limits recommended by Indian Standard Awashthi and European Union.

Aktaruzzaman et al. (2013) conducted a study to investigate the heavy metals concentration in soils and leafy vegetable samples along the Dhaka Aricha Road to assess their potential ecological risk. Concentrations of all the tested heavy metals except Cd in soil samples were below the permissible level. The mean concentration of Cd was found 3.99±1.85 mg kg-1. Concentrations of all the tested heavy metals except Cd and Cr in vegetables samples were lower than recommended level. Mean concentration of Cd and Cr were found 1.00 ± 0.68 mg kg-1 and 2.32±0.84 mg kg-1, respectively. Based on the Potential Ecological Risk Index, Cd posed very high risk to the local ecosystem due to its higher Risk Factor, >320 and based on Transfer Factor of Pb and Cd were found higher accumulator among the tested metals. The results of present study revealed that the bio concentration of heavy metals along the Dhaka Aricha Road posed high risk to the ecosystem. Considering the Transfer Factor of Cd and Pb it can be suggested that plants and leafy vegetables grow in the soil near Dhaka Aricha Road should not be used as food or feed.

Bvenura and Afolayan (2012) conducted a study to examine the accumulation of some essential (copper, manganese and zinc) and toxic metals (lead and cadmium) in cultivated vegetables – Brassica oleracea (cabbage), Daucuscarota (carrot), Allium cepa (onion), Spinacia oleracea (spinach) and Solanum lycopersicum (tomato). The vegetables were locally cultivated in home gardens in Alice, a small town in the Eastern Cape Province of South Africa. Samples of these vegetables were randomly collected from residential areas, dried, digested and analyzed for the heavy metals using inductively coupled plasma optical emission spectrometry. The concentrations of heavy metals in the vegetables were in the range of 0.01 mg/kg – 1.12 mg/kg dry weight for cadmium, 0.92 mg/kg – 9.29 mg/kg for copper, 0.04 mg/kg – 373.38 mg/kg for manganese and 4.27 mg/kg– 89.88 mg/kg for zinc. Lead was undetectable in all the samples. Results of analysis of soils from the area revealed that cadmium in soil was in the range of 0.01 mg/kg – 0.08 mg/kg, copper levels were 4.95 mg/kg – 7.66 mg/kg, lead levels were 5.15 mg/kg – 14.01 mg/kg and zinc levels were 15.58 mg/kg – 53.01 mg/kg. The concentration of manganese was the highest of all the metals, ranging between 377.61 mg/kg and 499.68 mg/kg, at all three residential sites. Although the concentrations in soils and vegetables of the critical heavy metals, such as lead and cadmium, may not pose a threat (according to FAO/WHO standards), the concentration of manganese was very high in spinach and soils, whilst that of zinc exceeded safe levels in spinach, onions and tomatoes.

Guerra et al. (2012) mentioned in their paper that the ingestion of vegetables containing heavy metals is one of the main ways in which these elements enter the human body. Once entered, heavy metals are deposited in bone and fat tissues, overlapping noble minerals. Slowly released into the body, heavy metals can cause an array of diseases. This study aimed to investigate the concentrations of cadmium, nickel, lead, cobalt and chromium in the most frequently consumed foodstuff in the São Paulo State, Brazil and to compare the heavy metal contents with the permissible limits established by the Brazilian legislation. A value of intake of heavy metals in human diets was also calculated to estimate the risk to human health. Vegetable samples were collected at the São Paulo General Warehousing and Centers Company, and the heavy metal content was determined by atomic absorption spectrophotometry. All sampled vegetables presented average concentrations of Cd and Ni lower than the permissible limits established by the Brazilian legislation. Pb and Cr exceeded the limits in 44 % of the analyzed samples. The Brazilian legislation does not establish a permissible limit for Co contents.

Kibria et al. (2012) conducted a study to see the effects of Chittagong city wastewater irrigation on the heavy metal contamination of soils and their uptake by plants, soil and plant samples. Result showed that, mean total Cd, Pb, Zn, Cu, Mn and Fe content in 0-15 cm depth of the study area ranged between 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 15-30 cm depth was in the ranges 0.01 to 1.98, 8.96 to 33.29, 51.44 to 267.31, 18.63 to 43.79, 68.89 to 271.74 and 1126 to 2054 mg /kg, respectively. Total and 0.1 N HCl extractable Cd, Pb, Zn, Cu, Mn and Fe contents of soils were significantly higher in wastewater irrigated location than those in the control location. Total Cd, Pb, Zn and Cu contents of surface soil in waste water irrigated locations were above the normal ranges of these metals for soils. Concentration of Cd, Pb, Zn, Cu, Mn and Fe in different plants (plant parts of rice, radish and aurum) varied from 0.02 to 16.65, 0.08 to 35.55, 0.84 to 102.75, 0.86 to.32.67, 0.95 to 185.50 and 3.23 to 485.23 mg /kg, respectively. Bioaccumulation coefficient of Cd, Pb, Zn, Cu, Mn and Fe in plants ranged from 0.20 to 13.91, 0.008 -0.72, 0.006 -1.60, 0.03-0.64, 0.01 -0.73 and 0.002 -0.18, respectively.

Ghosh et al. (2011) have studied the eight road side markets and two organized markets were demarcated for vegetables through purchasing. The present study was focused on site -1 to site-4 only. Six vegetables out of thirteen showed higher metal pollution index in site 3 and site-4. All sites showed several fold higher concentration of Lead (Pb), than the permissible PFA limit. Site-4 contains significantly higher concentration of Pb ($P<0.001$) than all other sites. The present study has generated data on heavy metal pollution in and around Ranchi city, Capital of Jharkhand and associated risk assessment for consumer’s exposure to the heavy metals.

Iqbal et al. (2011) conducted a study to assess the accumulation of heavy metals (Ni, Cu, Cd, Cr, and Pb) in agricultural soils and their uptake in spring seasonal plants being irrigated by industrial waste water. In Ethiopia, from the increasing human population, uncontrolled urbanization and inadequate sanitation infrastructure cause serious quality degradation of surface waters.

Naser et al. (2011) reported that heavy metal content in different leafy vegetables varies significantly. The content varies with time of harvesting and stage of maturity of crops. The Cd and Cr contents in leafy vegetables in this study were detected higher while Pb and Ni were within the permissible limits as per the WHO standard but all the metals were within the maximum allowable level as per PFA, 1954, India. The magnitude of time dependence of plant metal concentration variations differed among crop species and metals. Heavy metal content gradually increased at the early growing stage and fall during later stages of growth. The significant differences ($P < 0.01$) were observed between the mean metal concentrations in the three vegetables species. The Pb and Co concentrations in amaranth were found higher compared to those found in spinach and red amaranth. Spinach exhibited higher levels of Cd and Cr than those of other vegetables. However, the three vegetables did not differ significantly in its Ni concentration. The order of heavy metal level in different vegetables was Cd<Co<Pb<Ni<Cr. In vegetable species in respect of heavy metal concentration Cd, Ni, and Cr was highest in spinach and amaranth showed highest concentration in Pb and Co. The highest correlation between soil-plant was found for Cd, while the lowest for Ni. Metal concentrations in the vegetables studied were found lower than the maximum allowable level in India but the concentrations of Cd and Cr were higher than the allowable levels set by the World Health Organization (WHO).

Singh et al. (2010) assessed the risk to human health by heavy metals (Cd, Cu, Pb, Zn, Ni and Cr) through the intake of locally grown vegetables, cereal crops and milk from wastewater irrigated site. The higher values of metal pollution index and health risk index indicated heavy metal contamination in the wastewater irrigated site that presented a significant threat of negative impact on human health. Rice and wheat grains contained less heavy metal as compared to the vegetables, but health risk was greater due to higher contribution of cereals in the diet. The study suggests that wastewater irrigation led to accumulation of heavy metals in food stuff causing potential health risks to consumers.

Khan et al. (2009) assessed the health risk of heavy metals for the population via consumption of vegetables which is one of the problems that arise due to the increased uses of fertilizers and other chemicals to meet the higher demands of food production for human consumption. Health risk assessment for heavy metals of the population is a very good technique because such assessment would be useful to give information about any threat regarding heavy metals contamination in vegetables. For health risk assessment different methods are used by different researchers. These methods include the daily intake of metals (DIM), daily dietary index (DDI), Provisional tolerable daily intake (PTDI), along with the methods used for the health assessment. The health risk assessment methods include hazard quotient (HQ) and health risk index (HRI).

Bigdeli et al. (2008) conducted a study to analyze the metal contents of some vegetables in Sahre Rey-Iran with emphasis on their toxicological implications. Recently matured leaf and fruit samples of Shahrerey vegetable farms were sampled and analyzed to determine heavy metals. Data showed that metal uptake differences by the vegetables are attributed to plant differences intolerance to heavy metals and vegetable species. The lead concentration in all vegetable samples was more than maximum permitted concentrations, while Cd, pollution was observed in radish, Cress, Dill, spinach and eggplant. Data showed that Zn concentration in Celery, Mint, Dill, Spinach and Green pepper were more than Zn permitted level. There was no evidence about Cu contamination in vegetables. Data also showed that the intake of most of the metals constitutes less than the TMDI (theoretical maximum daily intake) at present and hence health risk is minimal. But with increase in vegetable consumption by the community the situation could worsen in the future. Treatment of industrial effluents and phyto-extraction of excess metals from polluted environments could reduce health risk. In turn, industrial or municipal wastewater is mostly used for irrigation of crops mainly in periurban ecosystem. This is because wastewater is easily available coupled with disposal problems and scarcity of fresh water.

Lokeshwari and Chandrappa (2006) conducted a study to assess the extent of heavy metal contamination of vegetation due to irrigation with sewage-fed lake water on agricultural land. The results show the presence of some of heavy metals in rice and vegetables, beyond the limits of Indian standards. Metal transfer factor from soil to vegetation are found significant for Zn, Cu, Pb and Cd. Comparing the results of heavy metals in water, soil and vegetation with their respective natural levels, it is observed that impact of lake water on vegetation was found to be more than the soil.

Murtaza and Monir (2006) mentioned their paper that, the wastewater irrigation practices give very good crop yields because wastewater contains large amounts of organic material and some inorganic elements essential for plant growth. But it may also contain large amounts of non-essential heavy metals which can be transferred to animal and human beings through food chain.

Sharma et al. (2006) revealed that uptake through roots depends on many factors such as the soluble content of heavy metals in soil, soil pH, plant growth stages as well as type of crops, fertilizers and soil.

Jassir et al. (2005) have reported that uptake of heavy metals by crops may be done through absorption from contaminated soils through roots or by deposition on foliar surfaces. Deposition of heavy metals from industrial and vehicular emissions on crops foliar surfaces may occur during production, transportation and marketing.

Mapanda et al. (2005) have revealed that there is an increase in the heavy metal contents when soil irrigated with waste water, also reported that in less industrialized countries including Zimbabwe, Zambia and Nigeria have shown that heavy metal contamination can have significant environmental and health impacts. Also, auto emissions from over-aged vehicles are common in Ghana, as in many parts of sub-Saharan Africa. The main sources of pollution that enter surface water bodies are industries, municipal solid waste and oily wastes from garages and fuel stations. Most of the water resources are gradually becoming contaminated due to the addition of foreign materials from the surroundings. These include organic matter of plant and animal origin, land surface washing and industrial and sewage effluents.

Aboaba and Ugoji (2004) reported that it is important to determine the metal contents of vegetables from health, food nutrition perspective and for crop yield technology point of view. Metal accumulation in edible portions of plants varies and depends on both soil composition and rate of uptake by each plant. For good health and optimum human performance, adequate intake of essential elements and nutrients is crucial.

Minhas and Samra (2004) have reported that the problem of microbial pollution becomes more serious with the vegetables, because many of them are being consumed raw. However, the extent of the pollution decreases if the vegetable's edible plant parts are above the ground, while it increases if they are near the ground.

Iannelli et al. (2002) stated in their paper that heavy metals persist in soil which then reaches down into the groundwater and may induce enhanced antioxidant enzymatic activities in plants or become adsorbed with solid soil particles.

Lark et al. (2002) reported that the content of the metals are always higher for the vegetables which have been continuously irrigated with the sewage water alone as compared to the vegetables where irrigation with the sewage water was replaced with tube well water some ten year before. The comparative study of the metal contents in the soils of the two kinds of fields gives roughly the differential accumulation of the metals in the last ten years, which results in corresponding much higher content of metals in the vegetables. Vegetables can absorb metals from soil as well as from deposits on the parts of the vegetables exposed to the air from polluted environments (Haiyan and Stuanes, 2003).

Anonymous (2001) studied the high concentration of heavy metals in vegetables grown in the vicinity of industries and identify leafy vegetables at greatest risk of accumulation elevated concentrations. The national environment protection (Assessment investigation levels (ML) measures identifies environmental investigation levels (ML) also exist for Cd and Pb in vegetable crops (fresh weight basis), and are applied by the Australian and New Zealand food authority (ANZFA). Broad leafy vegetables and herbs accumulated greater concentrations of most heavy metals.

Sawidis et al. (2001) mentioned in their paper that, when plants decay, heavy metals that had been taken into the plants are redistributed so the soil is then again enriched with the pollutants.

WHO (2000) reported that the lack of resources for effective wastewater treatment facilities in most developing countries has contributed to large volumes of wastewater generated especially in urban areas remaining untreated. The report also showed estimates of median levels of treated wastewater in Asia to be about 35% and 14% in Latin America and Caribbean, respectively but an abysmal 0% in sub-Saharan Africa

(SSA). Hence, large amounts of untreated wastewater being discharged into urban drainage systems and other natural waterways are used by farmers in these countries.

Adhikari et al. (1998) stated in their paper that increasing concentration of trace metals Cd, Pb, Zn,Cu, Mn, and Fe in surface soils irrigated with untreated sewage and industrial effluents. They also stated that, if these effluents are continuously used for irrigation for long periods of time then it may result in toxic levels for plants and animal health.

Wescott (1997) conducted a study on direct and indirect wastewater use in agriculture occurs in most developing countries. Direct wastewater irrigation practices are normally centered near large metropolitan areas but only a small percentage of the wastewater generated is used directly. Rather, indirect use of wastewater prevails in most developing countries. Indirect use occurs when treated, partially treated or untreated wastewater contaminate surface water that supply irrigation water to agriculture. Indirect wastewater reuse poses health and environmental problems of the same nature and magnitude as those associated with direct wastewater use in agriculture.

Sugiyama (1994) mentioned his paper that, water contamination by heavy metals in some areas is practically inevitable due to natural process (weathering of rocks) and anthropogenic activities (industrial, agricultural and domestic effluents)

Gouttormsun et al. (1995) reported that those vegetables particularly leafy crops, grown in heavy metals contaminated soils have a higher concentration of heavy metals than those grown in uncontaminated soil.

Kansal and sing (1983) mentioned in their paper that the higher content of metals in various crops was possibly due to the greater availability of these metals in sewage-irrigated soils. Soils under sewage irrigation to observed higher accumulation of heavy metals in plants in soils of other towns of Punjab.

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CHAPTER III

MATERIALS AND METHODS

3.1 Sampling site and location:

All samples were collected from 3 different districts of the Southern western region of Bangladesh. A total of six locations, three locations from each district, viz., Assasuni, Kaliganj, and Shyamnagar of Satkhira; Jikragacha, Monirampur and Sorsha of Jashore; Koyra, Paikgacha, and Dumuria of Khulna districts (Figure 1)

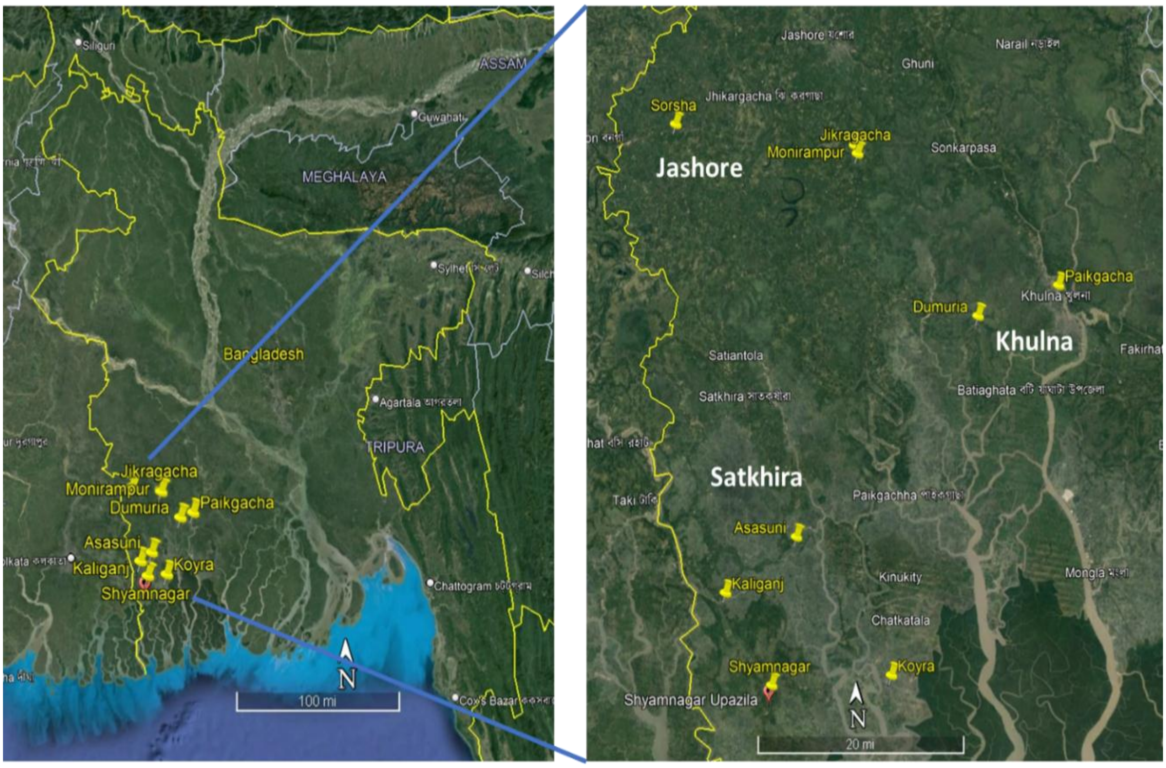

Figure 1. A map showing the study area

3.2 Collection of vegetables and soil samples

Eight species of vegetables e.g., Spinach, Tomato, Calabash, Couliflower, Kohlrabi, Cabbage, Red Amaranth, and Bean, and their soil was collected randomly from each selected location (Table 1). The samples were put into individual polyethylene bag with proper labeling and tagging and brought to the laboratory under the Department of Agricultural Chemistry, Sher-e-Bangla Agricultural University, Dhaka for chemical analyses. The data were obtained from Atomic Absorption Spectrophotometer. 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.

Table 1. Name of the vegetables samples collected for the study

Sl No.	Local Name	English Name	Scientific Name	Type	Family
1	পালংশাক	Spinach	*Spinacia oleracea*	Leaf vegetable	Amaranthaceae
2	টমেটো	Tomato	*Solanum lycopersicum*	Fruit vegetable	Solanaceae
3	লাউ	Calabash	*Lagenaria siceraria*	Fruit vegetable	Cucurbitaceae
4	ফুলকপি	Couliflower	*Brassica oleracea*	Flower vegetable	Brassicaceae
5	ওলকপি	Kohlrabi	*Brassica oleracea*	Stem vegetable	Brassicaceae
6	বাধাকপি	Cabbage	*Brassica oleracea*	Leaf vegetable	Brassicaceae
7	লালশাক	Red Amaranth	*Amaranthus cruentus*	Leaf vegetable	Amaranthaceae
8	শিম	Bean	*Phaseolus vulgaris*	Fruit vegetable	Leguminaceae

3.3 Sample preparation

The study samples were collected mechanically using hand gloves and carefully packed into polyethylene bags which are permanently labeled and the plant body was brought to the laboratory safely from the study area of six different districts from July, 2021 to June, 2022 in clean, pre-sterilized sampling bags in order to avoid contamination. To determine the total heavy metal content (Lead, Chromium, Cadmium, and Copper) the 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 ºC 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.

3.4 Chemical Analysis

3.4.1 Preparation of plant extract

To achieve a consistent weight, vegetable samples were divided into leaves, shoots, and fruits before being dried in an oven at 70°C. Samples that had been oven-dried were pulverized in a Wiley Hammer Mill, put through 40 mesh screens, well mixed, and kept in plastic vials. In a digestive tube, precise 1g oven-dried samples of several vegetables were collected. Di-acid combination (HClO4 and HNO3= 2:1) in the amount of 10 ml was taken in a digestion tube, allowed to sit for 20 minutes, and then moved to a digestion chamber where it was heated at 100ºC for the remaining time. To avoid foaming, the temperature was progressively raised to 365°C (in 50ºC stages). The solution was allowed to digest until the yellowish tint changed to a white color. After that, the heating chamber was opened, and the digesting tubes were taken out to cool to room temperature. The digestion tubes were carefully filled with around 20 ml of de-ionized water, and the contents were filtered through Whatman No. 40 filter paper into a 100 ml volumetric flask. The volume was then filled to the appropriate level with distilled water. At Straje water, the samples were kept in containers with prominent markings.

3.4.2 Instrumental information

Analytik Jena's novAA400P Atomic Absorption Spectrophotometer was used to determine the total content of copper, chromium, lead, and cadmium in the vegetable and soil digest (Analytik Jena's 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 2) shows the detailed instrumental conditions for determining copper, chromium, lead, and cadmium.

Table 2. Instrumental conditions for determination of Cu, Cr, Pb, and Cd.

Element	Cu	Cr	Pb	Cd
Wavelength (nm)	324.8	357.9	217.0	228.9
Slit (nm)	1.2	0.2	1.2	0.7
Lamp	HCL-Cu	HCL-Cr	HCL-Pb	HCL-Cd
Lamp current (mA)	2	4	2	2
Flame	Air-Ac	Air-Ac	Air-Ac	Air-Ac
Burner Head (mm)	100	100	100	100
Burner height (mm)	6	8	6	6
Read time (seconds)	3	3	3	3

3.4.3 Determination of Lead, Cadmium, Chromium and Copper

Total Lead concentration was determined from the digest by Analytic Jena's novel 400P Atomic Absorption Spectrophotometer at 217.00 nm (country of origin: Germany).

The construction of the standard curve involved plotting the absorbance values on the Y axis against various concentrations of each standard metal solution on the X axis. The estimation of metal concentration in the water, soil and rice samples by using the AAS reading in conjunction with the standard curve.

The following equation calculates the final concentration of metals in water, soil, and rice samples collected from different crop fields in the study sites.

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

Where C is the concentration of the sample from the calibration curve, V is the total volume of digest (ml), D is the dilution factor, and W is the weight of the sample (g).

3.5. Statistical Analysis

Data were compiled and tabulated in proper form and were subjected to statistical analysis. The Microsoft Excel was used for statistical analysis.

3.5.1 Some pollution index (SPI)

The pollution index (Pi) is the ratio of metal concentration in a biotic or abiotic medium to that of the regulatory Standard of International bodies such as World Health Organization (WHO), United States Environmental Protection Agency (USEPA) Food and Agriculture Organization (FAO) (Jamali *et al*., 2007).

Mathematically, Pi is expressed as:

$Pi = C_{plant}/C_{FAO/WHO\text{-}standard}$

Where P iis the individual pollution index of study metal,
C_{plant} is the concentration of the metal in plant.
$C_{FAO/WHO\text{-}standard}$ is the value of the regulatory limit of the heavy metal by FAO/WHO

Sum of some pollution index (SPI) described by Qingjie *et al*. (2008) was used for the present application.

SPI = PiPb + PiCr + PiCd + PiCu

Where, Pi = Single factor pollution index of heavy metals

3.5.2 Metal pollution index (MPI)

To examine the overall heavy metal concentrations of vegetables, the metal pollution index (MPI) was computed by Usero *et al*., 1997. This index was obtained by calculating the geometrical mean of concentrations of all the metals.

$\text{MPI (mg /kg)} = (C_1 \times C_2 \times C_3 \times \ldots\ldots\ldots.. \times C_n)^{1/n}$

Where, C_n = Concentration of metal n in the sample

3.5.3. Health risk assessment

Health risk assessment was computed based on the average contents of carcinogenic and non carcinogenic metals determined in the vegetable and fruit sample using the United States Environment Protection Agency human health risk assessment models. This study evaluated both cancer and non-cancer risks of heavy metals through vegetable and fruit consumption to take safety measures.

3.5.3.1. Non-carcinogenic risks calculation

The target hazard quotient (THQ) was used to assess the non-carcinogenic risk of metal intake by residents in vegetables (Liu et al., 2021).

THQ is calculated as follows, $THQ = CDI/RfD$.
CDI is the Chronic daily intake calculation of trace elements
CDI is calculated as follows:
$CDI= (EDI\times C\times EF\times ED)/ (BW\times AT)$

Where, the EDI is the daily metal intake caused by eating vegetables, the average daily intake of vegetables by adults in Bangladesh was 0.202 kg/person/day (HIES, 2023), C represents the concentration of metals (mg/g), EF represents the exposure frequency (365 days of the whole year), ED denotes the average exposure duration for adults (71.9 years), AT is the period of exposure for non-carcinogenic effects (365 × ED), BW is the average weight of adults (18 years old and above) 60 kg (BBS, 2015)

And 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, Cd, Cr, and Cu are 0.0036, 0.001, 0.003, and 0.04 (mg/kg/day), respectively (USEPA, 2021 and Health Canada, 2007).

The THQ of the trace elements determined for each vegetable is added to generate the hazard index (HI). The HI assumes that consuming a specific vegetable would expose a person to a number of potentially hazardous substances at once. Even though each component's THQ is less than unity separately, the combined effect of consumption may negatively impact health. 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). HI is calculated using the subsequent formula:

HI = THQ1 + THQ2 + THQ3 + ……….. + THQn

Where n is the total number of each trace element in vegetable species.

3.5.3.2. Carcinogenic risk calculation

For cancer risk analysis, the lifetime Carcinogenic risk (CR) was calculated by multiplying the daily dose by the cancer slope factor (CSF) derived from the response–dose curve for toxicant ingestion.

CR is calculated as CR = CDI × CSF.

Where, CDI is the Chronic daily intake calculation of trace elements and CSF is the cancer slope factor (CSF). The CHR is the sum of carcinogenic health risks of individual heavy metals.

Based on the USEPA Integrated Risk Information System (USEPA, 2010) database, the CSF value was 0.0085 for Pb, 0.38 for Cd mg kg-1 day-1, 0.5 for Cr mg/ kg per day. (Naseri et al., 2021; Nduka et al., 2019)

If CR or CHR > 1×10^{-4}, it indicates that the risk of developing cancer over a human lifetime is a 1 in 10,000 carcinogenic risk. If 1×10^{-6} < CR or CHR < 1×10^{-4}, it indicates an acceptable level of carcinogenic risk. If CR or CHR < 1×10^{-6}, it indicates a negligible carcinogenic risk (Quispe et al., 2021)

CHAPTER IV

RESULTS AND DISCUSSION

4.1. Lead (Pb) concentration in vegetable samples

The mean value of lead (Pb) concentration of eight different vegetable species is shown in (Figure 2) and Appendix I. The highest Pb concentration was found in the Bean at 8.34 mg/kg collected from the Assasuni of Satkhira district and the Pb concentration is 0.00 in all of the vegetables collected from Dumuria except bean and spinach of Khulna district.

In Assasuni of Satkhira district, the lowest Pb concentration was found in the cabbage, 3.09 mg/kg and the highest concentration was found in beans, 8.34 mg/kg. The mean value of Pb concentration (3.09-8.34 mg/kg) of eight studied vegetables was higher than the FAO/WHO permissible limits (0.1 mg/kg). In Kaliganj of Satkhira district, the lowest Pb concentration was found in the calabash, 0.51 mg/kg and the highest concentration was found in spinach, 7.43 mg/kg which is also higher than the FAO/WHO limit. In Shyamnagar of Satkhira district, the lowest Pb concentration was found in the Red Amaranth, 0.32 mg/kg and the highest concentration was found in cabbage, 8.07 mg/kg which is also higher than the FAO/WHO limit.

In Jikragacha of Jashore district, the lowest Pb concentration was found in the tomato (0.54 mg/kg) and the highest concentration was found in spinach (7.18 mg/kg). The mean Pb concentration (0.54-7.18 mg/kg) of eight studied vegetables was higher than the FAO/WHO permissible limits (0.1 mg/kg). In Monirampur of Jashore district, the lowest Pb concentration was found in the Tomato (1.57 mg/kg) and the highest concentration was found in calabash (4.76 mg/kg) which is also higher than the FAO/WHO limit.

In Sorsha of Jashore district, the Pb concentration is 0.00 in tomato, calabash, couliflower, and red amaranth, and the highest concentration was found in spinach, (5.39 mg/kg). The Pd concentration in spinach, kohlrabi, cabbage, and bean was higher than the FAO/WHO limit whereas the concentration was lower than the FAO/WHO limit in Tomato, Calabash, Cauliflower, and Red amaranth.

In Koyra of Khulna district, the Pb concentration is 0.00 in tomato and cauliflower, and the highest concentration was found in spinach (3.44 mg/kg). In Koyra, the Pd concentration was higher than the FAO/WHO limit in spinach, kohlrabi, cabbage, bean, calabash, and red amaranth whereas the concentration was lower than the FAO/WHO limit in tomato and cauliflower. In Paikgacha of Khulna district, the Pb concentration is 0.00 in cauliflower, kohlrabi, red amaranth and bean, and the highest concentration was found in cabbage (2.67 mg/kg) . The Pd concentration was higher than the FAO/WHO limit in Spinach, Cabbage, Calabash, and Tomato whereas the concentration was lower than the FAO/WHO limit in cauliflower, kohlrabi, red amaranth and bean. In Dumuria of Khulna district, the Pb concentration is 0.00 in all of the studied vegetables except spinach (1.47 mg/kg) and bean (1.66 mg/kg). The Pd concentration was lower than the FAO/WHO limit in tomato cauliflower, kohlrabi, cabbage, calabash, and red amaranth whereas the concentration was higher than the FAO/WHO limit in spinach, and bean.

From these results`, we found that the value of Pb concentration was higher than the FAO/WHO permissible limits (0.1 mg/kg) in most of the vegetables in the studied location indicating Pb might pose risk from vegetable consumption.

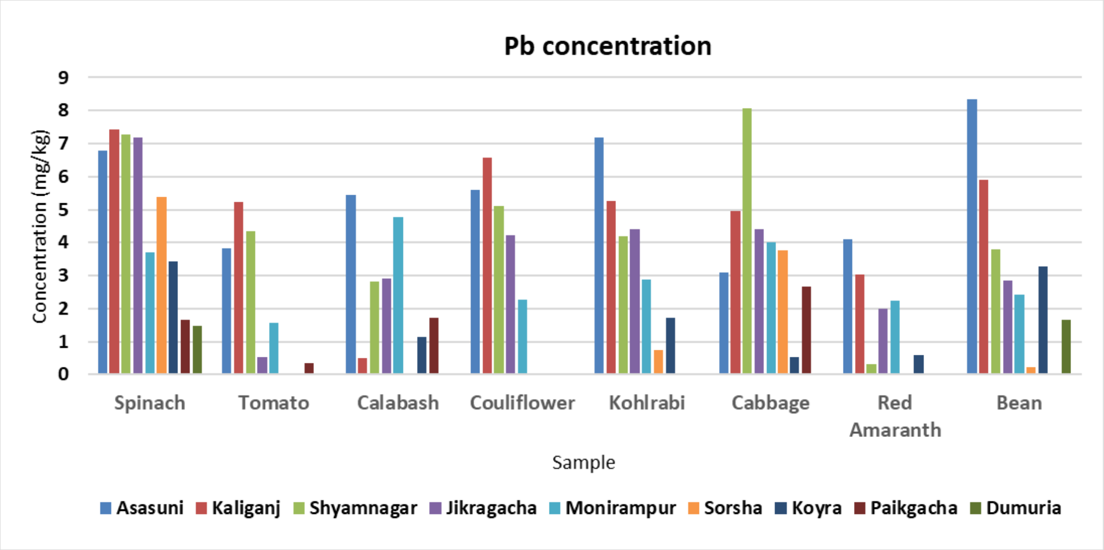

Figure 2. Lead (Pb) concentration found in the studied vegetable samples from locations

4.2. Cadmium (Cd) concentration in the studied vegetable samples

The mean Cadmium (Cd) concentration of eight different vegetable species is shown in (Figure 3) and Apendix II. The highest Cd concentration was found in the Spinach (9.34mg/kg) from the Asasuni of Satkhira district, and the lowest Cd concentration was found in Red Amaranth (0.17 mg/kg) from Jikragacha of the Jashore district.

In Asasuni of Satkhira district, the lowest Cd concentration was found in the Red Amaranth (0.59 mg/kg) and the highest concentration was found in spinach (9.34 mg/kg). The Cd concentration was higher (0.23-6.62 mg/kg) than the FAO/WHO limit (0.05 mg/kg) in all eight vegetables. In Kaliganj of Satkhira district, the lowest Cd concentration was found in the Kohlrabi, 0.23 mg/kg and the highest concentration was found in spinach, 6.62 mg/kg. The Cd concentration was higher (1.59-9.34 mg/kg) than the FAO/WHO limit (0.05 mg/kg) in all eight vegetables. In Shyamnagar of Satkhira district, the lowest Cd concentration was found in the Red Amaranth, 0.26 mg/kg and the highest concentration was found in cabbage (5.27 mg/kg). The Cd concentration was higher (0.26-5.27 mg/kg) than the FAO/WHO limit (0.05 mg/kg) in all eight vegetables.

In Jikragacha of Jashore district, the lowest Cd concentration was found in the red amarant, 0.17 mg/kg and the highest concentration was found in spinach, 2.97 mg/kg. The Cd concentration was higher than the FAO/WHO limit (0.05 mg/kg) in all eight vegetables. In Monirampur of Jashore district, the lowest Cd concentration was found in the Cabbage (0.28 mg/kg) and the highest concentration was found in spinach (3.67 mg/kg). The Cd concentration was higher than the FAO/WHO limit (0.05 mg/kg) in all eight vegetables. In Sorsha of Jashore district, the lowest Cd concentration was found in beans(2.12 mg/kg)and the highest concentration was found in spinach, 4.19. The Cd concentration was higher than the FAO/WHO limit (0.05 mg/kg) in all eight vegetables.

In Koyra of Khulna district, the lowest Cd concentration was found in cabbage (0.29 mg/kg) and the highest concentration was found in spinach (4.31 mg/kg). The Cd concentration was higher than the FAO/WHO limit (0.05 mg/kg) in all eight vegetables. In Paikgacha of Khulna district, the lowest Cd concentration was found in red amaranth 2.38 mg/kg and the highest concentration was found in spinach, 3.99 mg/kg. The Cd concentration was higher than the FAO/WHO limit (0.05 mg/kg) in all eight vegetables. In Dumuria of Khulna district, the lowest Cd concentration was found in kohlrabi (0.21 mg/kg) and the highest Cd concentration was found spinach (4.26 mg/kg). The Cd concentration was higher than the FAO/WHO limit (0.05 mg/kg) in all eight vegetables.

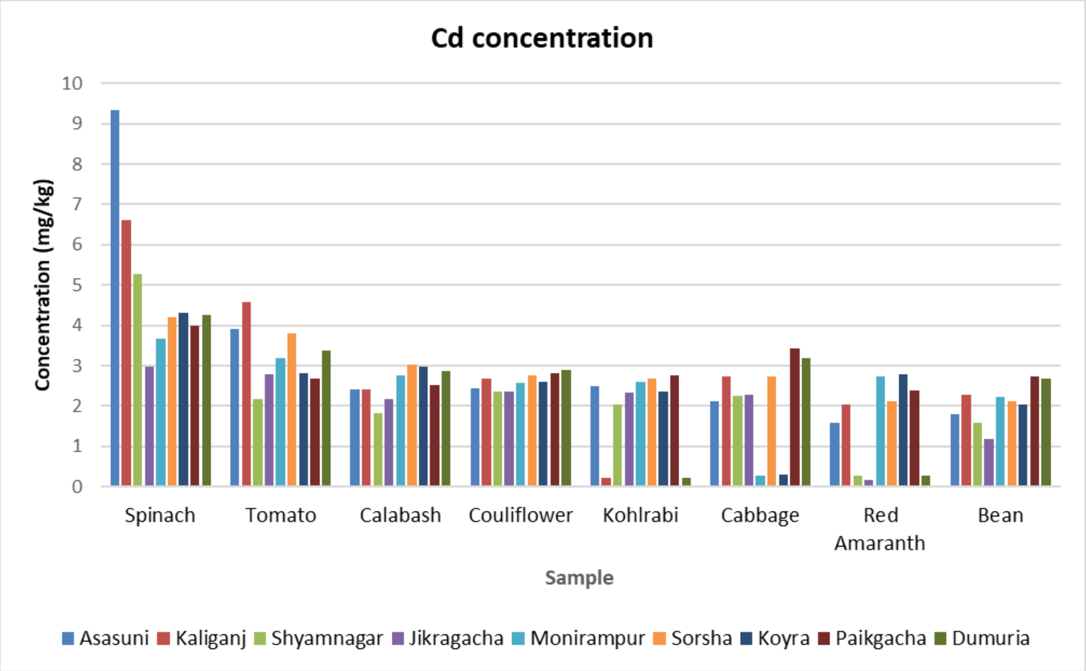

Figure 3. Cadmium Cd concentration found in the studied vegetable samples from locations

From these results, we found that the mean value of Cd concentration in vegetables of all of the studied locations was higher than the FAO/WHO permissible limits (0.05 mg/kg) indicating Cd might pose high risk from vegetable consumption.

4.3. Chromium (Cr) concentration in the studied vegetable samples

The mean value of Chromium (Cr) concentration of eight different vegetable species is shown in figure 4 and Appendix III. The highest Cr concentration was found in the Spinach, 9.45 mg/kg from the Assasuni of Satkhira district and the lowest concentration is 0.00 mg/kg in all of the vegetables collected from the Khulna district and almost all of the vegetables from the Jashore district except spinach and tomato.

In Assasuni of Satkhira district, the lowest Cr concentration was found in tomato (0.29 mg/kg) and the highest concentration was found in spinach (9.45 mg/kg). The Cr concentration was higher than the FAO/WHO limit (2.3 mg/kg) in spinach, calabash, cauliflower, kohlrabi, and bean whereas the concentration was lower than the FAO/WHO limit (2.3mg/kg) in Tomato, Cabbage, and Red Amaranth. In Kaliganj of Satkhira district, the lowest Cr concentration was found in the red amaranth, 0.00 mg/kg and the highest concentration was found in tomato (4.08 mg/kg). The Cr concentration was higher than the FAO/WHO limit in Tomato, Calabash, and kohlrabi whereas the concentration was lower than the FAO/WHO limit (2.3 mg/kg) in spinach, cabbage, cauliflower, red amaranth, and bean. In Shyamnagar of Satkhira district, the lowest Cr concentration was 0.00 mg/kg in cauliflower, red amaranth, and bean, and the highest concentration was found in spinach, 2.16 mg/kg. The Cr concentration was lower than the FAO/WHO limit (2.3 mg/kg) in all vegetables.

In Jikragacha of Jashore district, did not found any Cr concentration in the studied vegetables except in the spinach and tomato which was 1.83 mg/kg and 1.08 mg/kg respectively. The Cr concentration was lower than the FAO/WHO limit (2.3 mg/kg) in all vegetables. In Monirampur of Jashore district, did not found any Cr concentration in the studied vegetables except in the Spinach, 0.08 mg/kg. The Cr concentration was lower than the FAO/WHO limit (2.3 mg/kg) in all vegetables. In Sorsha of Jashore district did not found any Cr concentration in the studied vegetables.

There is no Cr concentration found in the studied vegetable samples from three locations viz., Koyra, Paikgacha and Dumuria of the Khulna district.

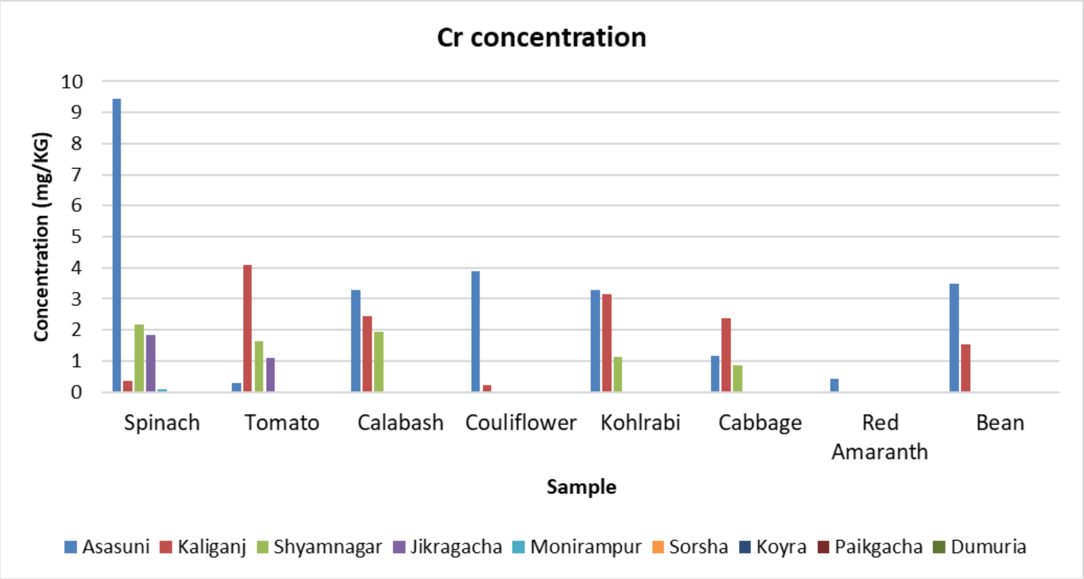

Figure 4 Chromium (Cr) concentration found in the studied vegetable samples from locations

From these results, we found that the mean Cr concentration in vegetables of all of the studied locations were lower than the FAO/WHO permissible limits (2.3 mg/kg) except few vegetables from Satkhira which indicated Cr did not pose a high risk from vegetable consumption.

4.4. Copper (Cu) concentration in the studied vegetable samples

The mean Copper (Cu) concentration of the eight different vegetable species is shown in (Figure 5) and Appendix IV. The highest Cu concentration was found in the tomato, 25.55 mg/kg from the Kaliganj of the Satkhira district, and the lowest Cu concentration was found in kohlrabi 0.26 mg/kg from Shyamnagar of the Satkhira district. The Cu concentration was lower than the FAO/WHO limit (40 mg/kg) in all vegetables which indicated Cu did not pose a high risk from vegetable consumption.

In Assasuni of the Satkhira district, the lowest Cu concentration was found in the cabbage, 1.56 mg/kg and the highest concentration was found in Calabash, 19.3 mg/kg. In Kaliganj of Satkhira district, the lowest Cu concentration was found in the red amaranth, 0.48 mg/kg and the highest concentration was found in tomato, 25.55 mg/kg. In Shyamnagar of Satkhira district, the lowest Cu concentration was found in the kohlrabi, 0.26 mg/kg and the highest concentration was found in tomato, 21.17 mg/kg.

In Jikragacha of the Jashore district, the lowest Cu concentration was found in the red amaranth, 0.58 mg/kg and the highest concentration was found in spinach, 19.43 mg/kg. In Monirampur of the Jashore district, the lowest Cu concentration was found in the red amaranth 4.34 mg/kg and the highest concentration was found in spinach, 20.25 mg/kg. In Sorsha, the lowest Cu concentration was also found in red amaranth, 4.85 mg/kg and the highest concentration was found in spinach, 20.57 mg/kg.

In Koyra of Khulna district, the lowest Cu concentration was found in cabbage, 0.91 mg/kg, and the highest concentration was found in calabash, 17.32 mg/kg. In Paikgacha of Khulna district, the lowest Cu concentration was found in tomato 4.16 mg/kg and the highest concentration was found in calabash, 16.18 mg/kg. In Dumuria of Khulna district, the lowest Cu concentration was found in kohlrabi, 4.81 and the highest concentration was found in tomato, 17.08 mg/kg.

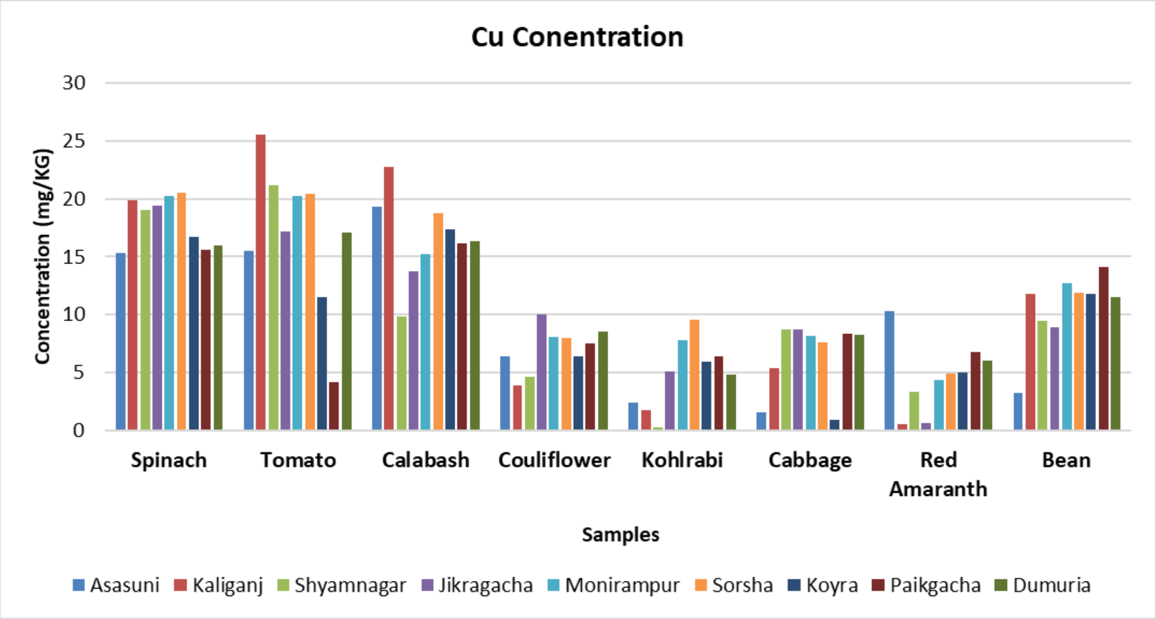

Figure 5. Copper concentration found in the studied vegetable samples from locations

4.5. Heavy metal transfer from soil to vegetables:

The soil-to-plant transfer is one of the key components of human exposure to metals through the food chain. Transfer Factor (TF) is a parameter to describe the transfer of trace elements from soil to plant body. In the present study, the transfer factor of different heavy metal from soil to vegetables is presented in (Table 3). The TF value ranges are Pb: 0.20 mg/kg-0.73 mg/kg, Cd: 0.24 mg/kg- 0.74 mg/kg, Cr: 0.01mg/kg-0.23 mg/kg and Cu: 0.69 mg/kg -2.68 mg/kg . Metals with high TF are easily transferred to crops, unlike metals with low TF. The trend of TF for heavy metals in studied vegetable samples was in order: Cu>Cd>Pb>Cr.

The mobility of metals from soil to plants is a function of the physical and chemical properties of the soil and of the vegetable species and is altered by innumerable environmental and human factors (Zurera et al. 1989).

Table 3. Transfer factor of heavy metal from soils into vegetable samples

	Transfer factor (mg/kg)			
Sample	**Lead (Pb)**	**Cadmium (Cd)**	**Chromium (Cr)**	**Copper (Cu)**
Spinach	0.73	0.74	0.23	2.68
Tomato	0.26	0.48	0.12	2.52
Calabash	0.32	0.38	0.13	2.46
Cauliflower	0.39	0.39	0.07	1.04
Kohlrabi	0.43	0.29	0.12	0.72
Cabbage	0.52	0.32	0.07	0.95
Red Amaranth	0.20	0.24	0.01	0.69
Bean	0.47	0.31	0.08	1.57

4.6. Some pollution index (SPI)

The some pollution index of each of the Heavy metals was calculated to assess the pollution degree of different heavy metals in vegetables.

The Sediment pollution index (SPI) of the eight different vegetable species is shown in Figure 6 and Appendix V. The highest SPI value was found in the spinach, 258.98 from the Assasuni of the Satkhira district, and the lowest SPI value was found in kohlrabi from Dumuria of the Khulna district.

In Assasuni of the Satkhira district, the lowest SPI was found in the red amaranth, 73.34 and the highest SPI was found in spinach, 258.98. In Kaliganj of Satkhira district, the lowest SPI was found in the calabash, 54.93 and the highest SPI was found in spinach, 207.04. In Shyamnagar of Satkhira district, the lowest SPI was found in the red amaranth, 8.48 and the highest SPI was found in Spinach, 179.32 .

In Jikragacha of the Jashore district, the lowest SPI was found in the red amaranth, 23.41 and the highest concentration was found in spinach, 132.18 . In Monirampur of the Jashore district, the lowest SPI was found in the Cabbage 46 and the highest SPI was found in Spinach, 110. In Sorsha, the lowest SPI was found in Red amaranth, 42.72 and the highest SPI was found in spinach, 137.83 .

In Koyra of Khulna district, the lowest SPI was found in cabbage, 11.02 , and the highest SPI was found in spinach, 120.69 . In Paikgacha of Khulna district, the lowest SPI was found in Red amaranth, 47.77 and the highest SPI was found in spinach, 96.34 . In Dumuria of Khulna district, the lowest SPI was found in kohlrabi, 4.32 and the highest SPI was found in spinach, 99.94 .

The highest SPI value was found in the spinach, 258.98 from the Assasuni of the Satkhira district, and the lowest SPI value was found in Kohlrabi from Dumuria of the Khulna district.the value was risk for the human health (Jamali *et al*., 2007).

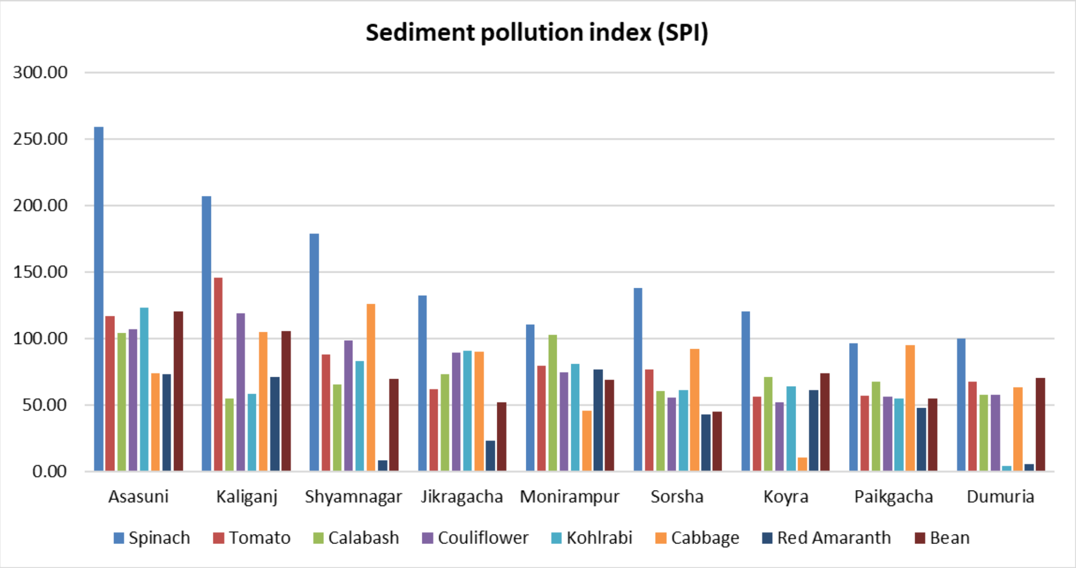

Figure 6. Some pollution index (SPI) in the studied locations

4.7. Metal pollution index

Metal pollution index metal pollution in samples is reliably estimated using the metal pollution index (MPI) in (Figure 7). demonstrates the metal pollution index of vegetable samples from each site (Appendix-VI). The highest metal pollution index 9.40 mg/kg was found in the vegetable samples from Assasuni of Satkhira district and the lowest MPI 0.105 mg/kg was found in Dumuria of Khulna district.

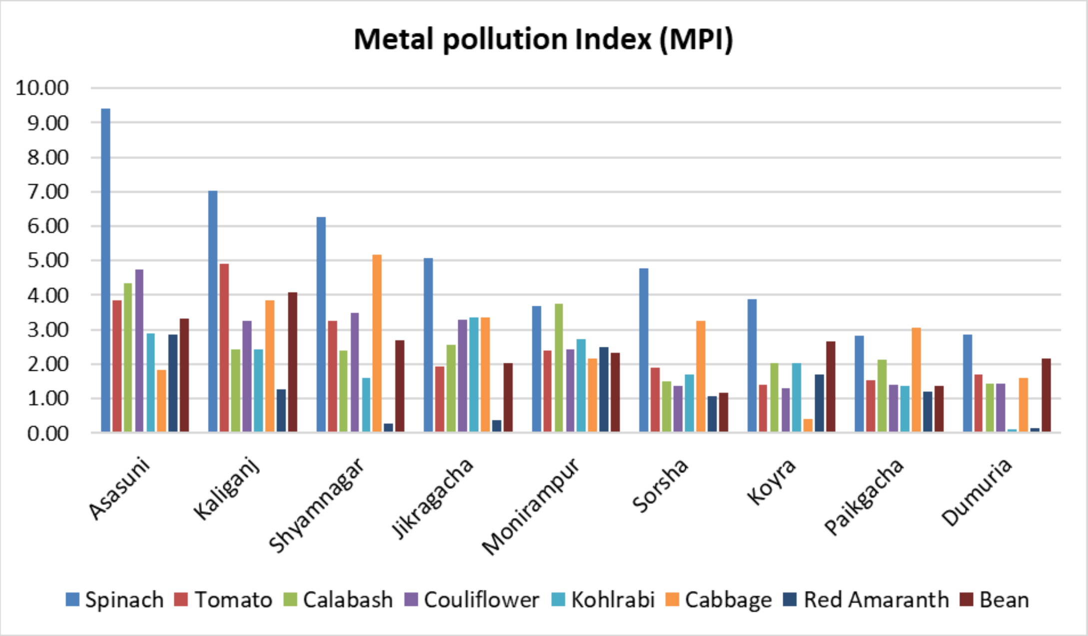

Figure 7. Metal pollution index (MPI) (mg/kg)of vegetable samples from the studied location

In Assasuni of the Satkhira district, the lowest MPI was found in the cabbage (1.85 mg/kg) and the highest was found in spinach, 9.40 mg/kg. In Kaliganj of Satkhira district, the lowest MPI was found in the red amaranth, 1.26 mg/kg and the highest was found in spinach, 7.03 mg/kg. In Shyamnagar of Satkhira district, the lowest SPI was found in the red amaranth, 0.29 mg/kg and the highest was found in spinach, 6.28 mg/kg.

In Jikragacha of the Jashore district, the lowest MPI was found in the red amaranth, 0.38 mg/kg and the highest concentration was found in spinach, 5.08 mg/kg. In Monirampur of the Jashore district, the lowest MPI was found in the cabbage 2.15 mg/kg and the highest was found in calabash, 3.76 mg/kg. In Sorsha, the lowest SPI was found in red amaranth, 1.07 mg/kg and the highest was found in spinach,4.79 mg/kg.

In Koyra of Khulna district, the lowest MPI was found in cabbage, 0.41 mg/kg, and the highest was found in spinach, 3.88 mg/kg. In Paikgacha of Khulna district, the lowest MPI was found in red amaranth, 1.19 mg/kg and the highest was found in cabbage, 3.05 mg/kg. In Dumuria of Khulna district, the lowest MPI was found in kohlrabi, 0.11 mg/kg and the highest was found in spinach, 2.87 mg/kg.

The highest metal pollution index 9.40 mg/kg was found in the vegetable samples from Assasuni of Satkhira district and the lowest MPI 0.105 mg/kg was found in Dumuria of Khulna district. The MPI was higher than the general level and step should be taken. (Usero *et al*., 1997)

4.8. Human health risks assessment

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 Satkhira, Jashore and Khulna 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.

4.8.1. Non-carcinogenic health risk:

The non-carcinogenic hazard quotient (HQi) of individual heavy metals and the cumulative non-carcinogenic hazard index (HI) from numerous metals were explored through vegetable consumption for adults in the locations of three districts as shown below

In case of Satkhira districts, Pb concentration in all of the studied vegetable form Assasuni, Kaliganj and Shyamnagar area of this district was higher than the safe level of hazard quotient (HQ > 1) except the calabash from Kaliganj (0.48), and red amaranth from Shyamnagar (0.30) (Figure 8). On the other hand, the Cd concentration in all of the studied vegetable form Assasuni, Kaliganj and Shyamnagar area of this district was higher than the safe level of hazard quotient (HQ> 1) except the Kohlrabi from Kaliganj, and Red Amaranth from Shyamnagar which was lower than the safe level of hazard quotient (HQ< 1) (Figure 8). The Cr concentration in all of the studied vegetable form Assasuni, Kaliganj and Shyamnagar area of this district was higher than the safe level of hazard quotient (HQ > 1) except the tomato and red amaranth from Assasuni; spinach, couliflower and red amaranth from Kaliganj; couliflower, cabbage, red amarant and bean from Shyamnagar upazila which is lower than the safe level of hazard quotient (HQ< 1) (Figure 8). The Cu concentration in the couliflower, kohlrabi, cabbage, red amaranth and bean form Assasuni, Kaliganj and Shyamnagar area was lower than the safe level of hazard quotient (HQ< 1) except the spinach, tomato and calabash from all studied location higher than the safe level of hazard quotient (HQ> 1) (Figure 8).

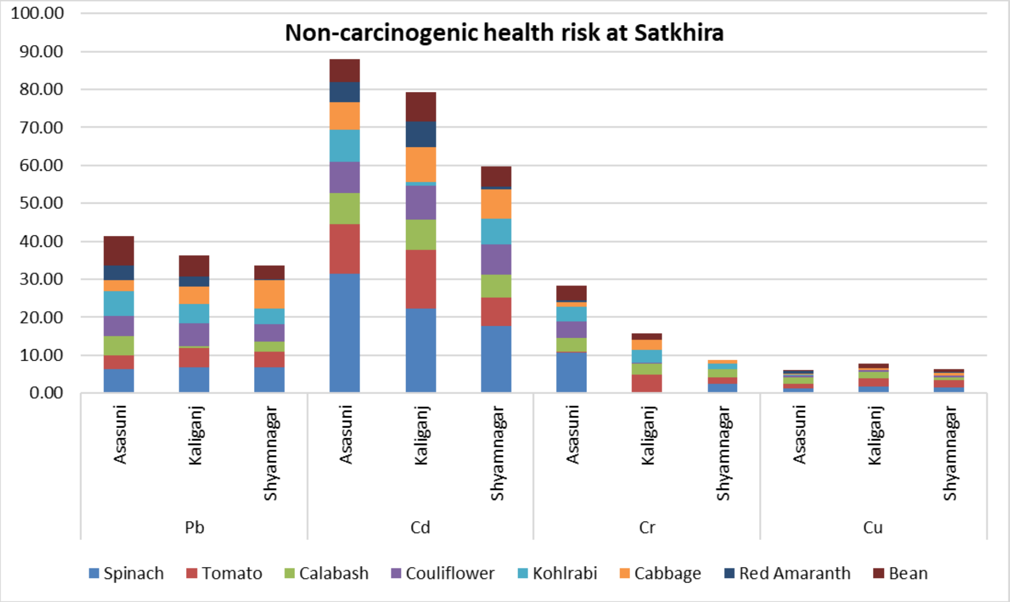

Figure 8. Non-carcinogenic health risk (mg/kg/day) (HQ and HI) for the adult population through the consumption of vegetables produced in the Satkhira district

After quantitative analysis and calculated investigation , the cumulative hazard indexes from multiple metals from the Satkhira district 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 Assasuni > Kaliganj > Shyamnagar in decreasing order of producing areas. According to this study, heavy metals may pose a health hazard to the consumer of vegetables produced in studied locations of the Satkhira district. So, action is needed to reduce heavy metal pollution in the vegetable fields of different Asasuni, Kaliganj and Shyamnagar area of the Satkhira district to protect the consumer's health.

In case of Jashore districts, Pb concentration in all of the studied vegetable form Jikragacha and Monirampur of this district was higher than the safe level of hazard quotient (HQ > 1) except the tomato from Jikragacha whereas the all the studied vegetable from Sorsha were lower than the safe level of hazard quotient (HQ< 1) except spinach and cabbage which was higher than safe limit (Figure 9). On the other hand, the Cd concentration in all of the studied vegetable form studied locations were higher than the safe level of hazard quotient (HQ> 1) except the red amaranth, bean from

Jikragacha and cabbage from Monirampur which was lower than the safe level of hazard quotient (HQ< 1) (Figure 9). The Cr concentration in all of the studied vegetable form all studied locations were lower than the safe level of hazard quotient (HQ< 1) except the spinach and tomato from Jikragacha which is higher than the safe level of hazard quotient (HQ> 1) (Figure 9). the cu concentration of couliflower, kohlrabi, cabbage, red amaranth and bean from all the studied location was lower than the safe level of hazard quotient (HQ> 1) except the Bean from Monirampur and Sorsha were higher than the safe limit whereas Cu concentration in the spinach, tomato and calabash from all the studied locations were higher than the safe level of hazard quotient (HQ >1) (Figure 9.).

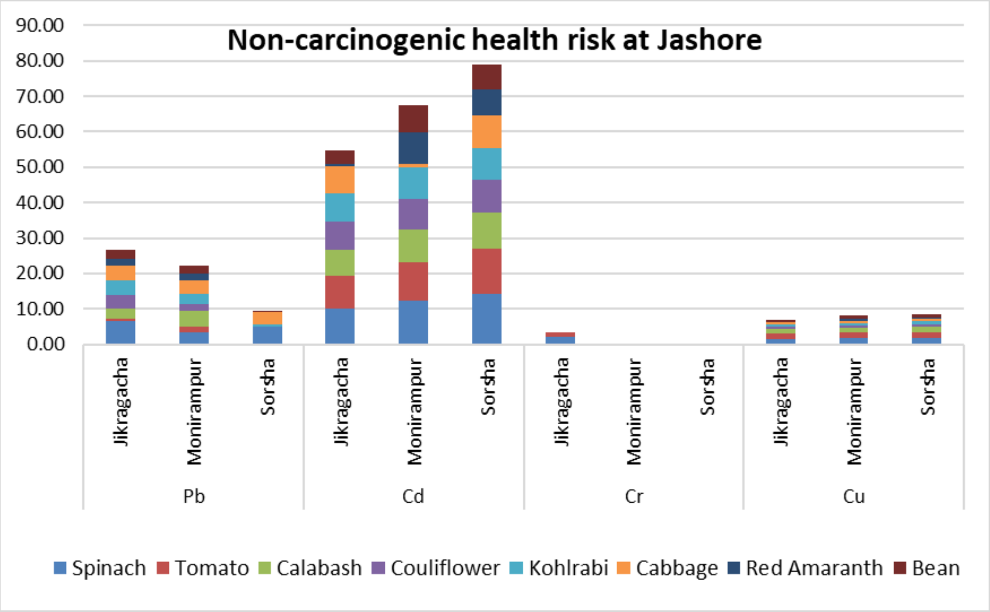

Figure 9. Non-carcinogenic health risk (HQ and HI) for the Adult population through the consumption of vegetables produced in the Jashore district

After quantitative analysis and calculated investigation, the cumulative hazard indexes from multiple metals from the Jashore district 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 Monirampur > Sorsha > Jikragacha in decreasing order of producing areas. According to this study heavy metals may pose a health hazard to the consumer of vegetables produced in studied locations of the Jashore district. So, action is needed to reduce heavy metal pollution in the vegetable fields of Monirampur, Sorsha and Jikragacha area of the Jahsore district to protect the consumer's health.

In case of Khulna districts, Pb concentration in tomato, couliflower, and red amaranth form all locations, calabash from Dumuria, kohlrabi from Paikgacha and Dumuria, cabbage from Koyra and Dumuria bean from Paikgacha were lower than the safe level of hazard quotient (HQ < 1) (Figure 10). On the other hand, the Cd concentration in all of the studied vegetable form studied locations were higher than the safe level of hazard quotient (HQ> 1) except the kohlrabi from Dumuria, cabbage from Koyra and red amaranth from Paikgacha were lower than the safe level of hazard quotient (HQ< 1) (Table 10). The Cr concentration in all of the studied vegetable form all studied locations was not detected (Figure 10). The cu concentration of spinach and calabash from all studied locations and bean from Paikgacha were lower than the safe level of hazard quotient (HQ> 1) whereas Cu concentration in the tomato from Koyra and Paikgacha, couliflower, kohlrabi, cabbage, and red amaranth from all studied location and bean from the Koyra and Dumira were lower than the safe level of hazard quotient (HQ<1) (Figure 10).

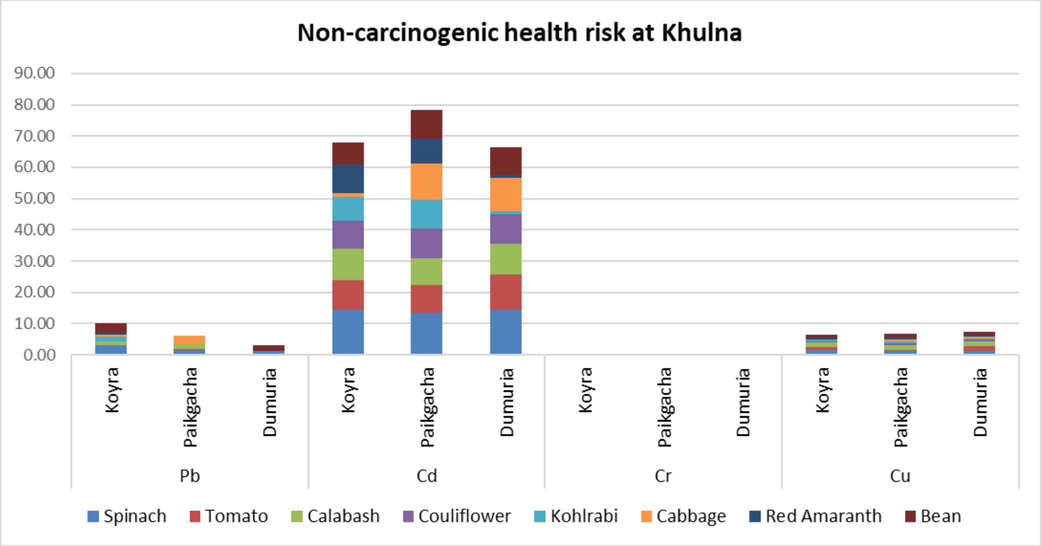

Figure 10. Non-carcinogenic health risk(mg/kg/day) (HQ and HI) for the adult population through the consumption of vegetables produced in th Khulna district

After quantitative analysis and calculated investigation, the cumulative hazard indexes from multiple metals from the Khulna district 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 Paikgacha > Koyra > Dumuria in decreasing order of producing areas. According to this study heavy metals may pose a health hazard to the consumer of vegetables produced in studied locations of the Khulna district. So, action is needed to reduce heavy metal pollution in the vegetable fields of the studied area of the Khulna district to protect the consumer's health. (USEPA, 2021 and Health Canada, 2007).

4.8.2. Carcinogenic health risk:

In this experiment, Pb, Cd and Cr were evaluated to determine the risk of cancer associated with vegetables, spinach, tomato, calabash. cauliflower, kohlrabi, cabbage, red amaranth and bean consumption. The US-Environmental Protection Agency recommends that the cancer risk could be expressed as no significant carcinogenic health risk (CR or CHR<10^{-6}); acceptable carcinogenic health risk (10^{-6}< CR or CHR < 10^{-4}); or unacceptable carcinogenic health risk (CR or CHR > 10^{-4}) (USEPA, 2001).

In the case of the Satkhira district, the calculated carcinogenic health risk (ILCRi) due to individual single metals is given in (Figure 11). The carcinogenic health risk (ILCRi) for Pb from all vegetables was higher than the unacceptable limit (>10^{-4}) except calabash from Kaliganj and Shyamnagar, cabbage from Assasuni and red amaranth from Shyamnagar upazila which were below the unacceptable limit (>10^{-4}). The carcinogenic health risk (ILCRi) for Cd from all vegetables exceeded the unacceptable limit (>10^{-4}). The carcinogenic health risk (ILCRi) for Cr from all vegetables exceeded the unacceptable limit (>10^{-4}) except cauliflower from Shyamnagar, red amaranth from Kaliganj and Shyamnagar and bean from Shyamnagar which were below the unacceptable limit (>10^{-4}). The cumulative cancer risk for multiple metals (CHR) for Pb, Cd and Cr of three locations, Assasuni, Kaliganj and Shyamnagar upazila of Satkhira district were 0.07716 mg/kg/day, 0.05505 mg/kg/day and 0.03676 mg/kg/day respectively which are also higher than the unacceptable limit (>10^{-4}).

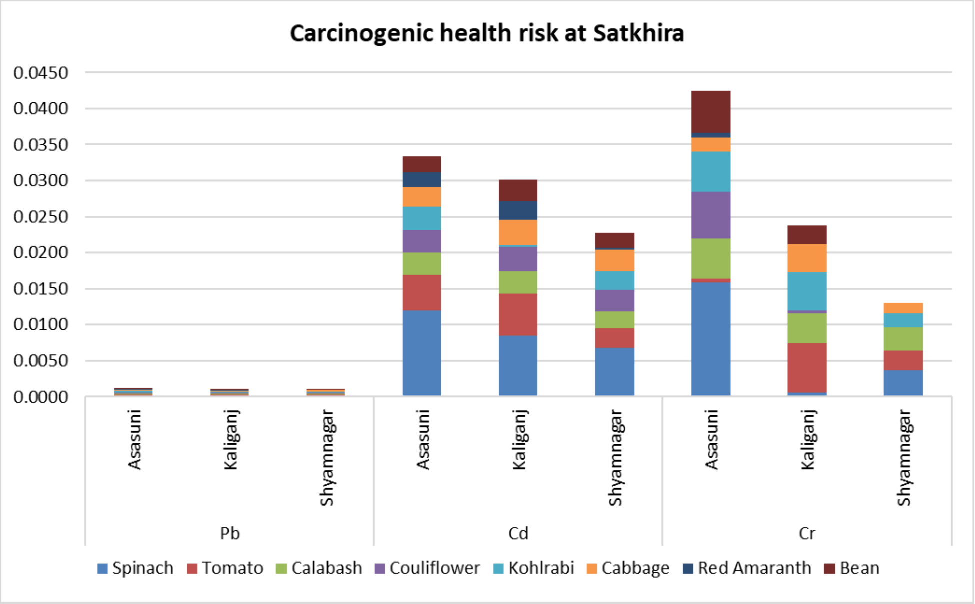

Figure 11. Carcinogenic health risk (mg/kg/day) for the Adult population through the consumption of vegetables produced in the Satkhira district study area

In the case of the Jashore district, the calculated carcinogenic health risk (CR) due to individual single metals is given in (Figure 12). The carcinogenic health risk (CR) for Pb from all vegetables was lower than the unacceptable limit ($>10^{-4}$) except Spinach and cabbage from all studied locations, calabash from Monirampu, couliflower, and kohlrabi from Jikragacha upazila which were higher than the unacceptable limit ($>10^{-4}$). The carcinogenic health risk (CR) for Cd from all vegetables higher the unacceptable limit ($>10^{-4}$). The carcinogenic health risk (CR) for Cr from all vegetables lower the unacceptable limit ($>10^{-4}$) except Spinach from Jikragacha and Monirampur and tomato from Jikragacha which were higher the unacceptable limit ($>10^{-4}$). The cumulative cancer risk for multiple metals (CHR) for Pb, Cd and Cr of three locations, Jikragacha, Monirampur and Sorsha of Satkhira district were 0.0265 mg/kg/day, 0.0265 mg/kg/day and 0.0303mg/kg/day respectively which are also higher than the unacceptable limit ($>10^{-4}$).

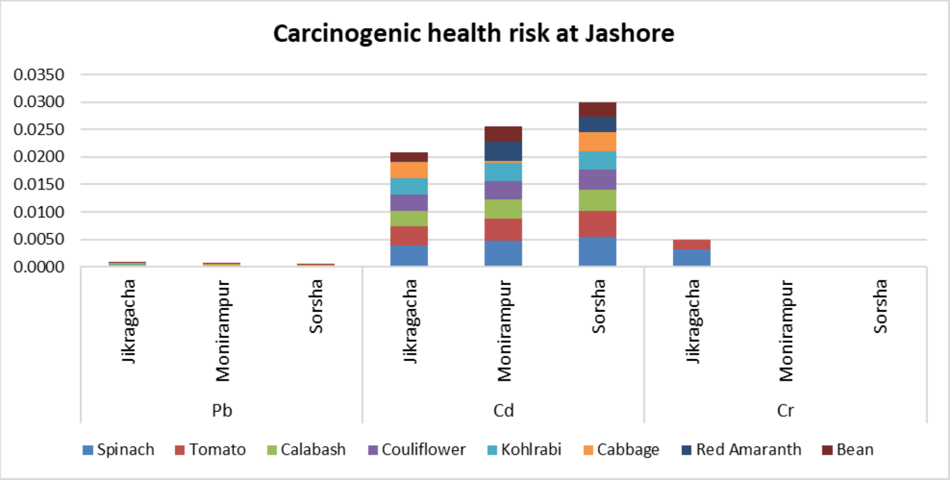

Figure 12. Carcinogenic health risk (mg/kg/day) for the adult population through the consumption of vegetables produced in the Jashore district study area

In the case of the Khulna district, the calculated carcinogenic health risk (CR) due to individual single metals is given in (Figure 13). The carcinogenic health risk (CR) for Pb and Cr from all vegetables was lower than the unacceptable limit ($>10^{-4}$) whereas the carcinogenic health risk (CR) for Cd from all vegetables was higher the unacceptable limit ($>10^{-4}$). The cumulative cancer risk for multiple metals (CHR) for Pb, Cd and Cr of three locations, Koyra, Paikgacha and Dumuriaha of Satkhira district were 0.02608 mg/kg/day, 0.02997 mg/kg/day and 0.02534 mg/kg/day respectively which are also higher than the unacceptable limit ($>10^{-4}$).

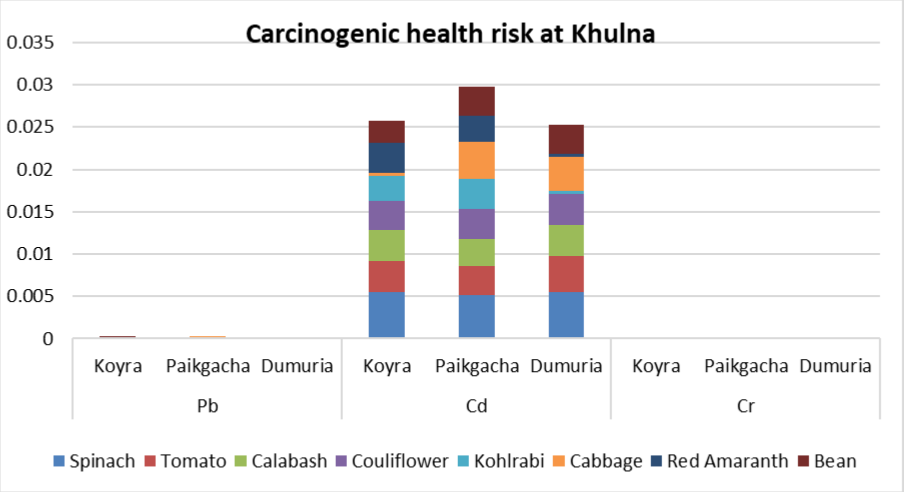

Figure 13. Carcinogenic health risk (mg/kg/day) for the adult population through the consumption of vegetables produced in the Khulna district

The cumulative cancer risk for multiple metals (CHR) for Pb, Cd and Cr of three locations, Assasuni, Kaliganj and Shyamnagar upazila of Satkhira district were 0.07716 mg/kg/day, 0.05505 mg/kg/day and 0.03676 mg/kg/day respectively which are also higher than the unacceptable limit (>10-4).The cumulative cancer risk for multiple metals (CHR) for Pb, Cd and Cr of three locations, Jikragacha, Monirampur and Sorsha of Satkhira district were 0.0265 mg/kg/day, 0.0265 mg/kg/day and 0.0303 mg/kg/day respectively which are also higher than the unacceptable limit (>10-4).The cumulative cancer risk for multiple metals (CHR) for Pb, Cd and Cr of three locations, Koyra, Paikgacha and Dumuriaha of Satkhira district were 0.02608 mg/kg/day, 0.02997 mg/kg/day and 0.02534 mg/kg/day respectively which are also higher than the unacceptable limit (>10-4). (USEPA, 2001).

CHAPTER V

SUMMARY AND CONCLUSION

Samples of vegetables and soil were collected from nine different locations in the southwest part (Assasuni, Kaliganj, and Shyamnagar of Satkhira;Jikragacha, Monirampur and Sorsha of Jashore; Koyra, Paikgacha, and Dumuria of Khulna districts) to detect and quantify metal residues. Eight species of vegetables e.g., spinach, tomato, calabash, couliflower, kohlrabi, cabbage, red amaranth, and bean, were collected in this study. Vegetables were collected at the stage of harvesting from farmers field.

The highest Pb concentration was found in the bean at 8.34 mg/kg collected from the Assasuni of Satkhira district and the Pb concentration is 0.00 in most of the vegetables (except spinach and bean) collected from Dumuria and Paikgacha (except spinach and tomato) of Khulna district. The mean Pb concentration was higher than the FAO/WHO permissible limits (0.1 mg/kg) in most of the vegetables in the studied location of Satkhira and Jashore district indicating Pb might pose risk from vegetable consumption.

The highest Cd concentration was found in the Spinach, 9.34mg/kg from the Assasuni of Satkhira district, and the lowest Cd concentration was found in Red Amaranth 0.17 mg/kg from Jikragacha of the Jashore district. The mean Cd concentration in vegetables of all of the studied locations was higher than the FAO/WHO permissible limits (0.05 mg/kg) indicating Cd might pose high risk from vegetable consumption.

The highest Cr concentration was found in the Spinach, 9.45 mg/kg from the Assasuni of Satkhira district and the Cr concentration is 0.00 in all of the vegetables collected from the Khulna district and almost all of the vegetables from the Jashore district. The mean Cr concentration in vegetables of all of the studied locations was lower than the FAO/WHO permissible limits (2.3 mg/kg) except few vegetables from Satkhira which indicated Cr did not pose a high risk from vegetable consumption.

The highest Cu concentration was found in the Tomato, 25.55 mg/kg from the Kaliganj of the Satkhira district, and the lowest Cu concentration was found in Kohlrabi 0.26 mg/kg from Shyamnagar of the Satkhira district. The Cu concentration was lower than the FAO/WHO limit (40 mg/kg) in all vegetables which indicated Cu did not pose a high risk from vegetable consumption.

The transfer factor TF value ranges are Pb: 0.20-0.73 mg/kg, Cd: 0.24- 0.74 mg/kg, Cr: 0.01-0.23 mg/kg and Cu: 0.69-2.68 mg/kg. Metals with high TF are easily transferred to crops unlike metals with low TF. The trend of TF for heavy metals in studied vegetable samples was in order: Cu > Cd > Pb > Cr.

The highest SPI value was found in the spinach, 258.98 from the Assasuni of the Satkhira district, and the lowest SPI value was found in Kohlrabi from Dumuria of the Khulna district.The highest metal pollution index 9.40 mg/kg was found in the vegetable samples from Assasuni of Satkhira district and the lowest MPI 0.105 mg/kg was found in Dumuria of Khulna district.

The cumulative non-carcinogenic health risk associated with vegetable consumption were assigned to Assasuni > Kaliganj > Shyamnagarin Satkhira district; Monirampur > Sorsha > Jikragacha in Jashore district and Paikgacha > Koyra > Dumuria in Khulna district decreasing order of producing areas. In decreasing order of producing areas.

The cumulative cancer risk for multiple metals (CHR) for Pb, Cd and Cr of three locations, Assasuni, Kaliganj and Shyamnagar upazila of Satkhira district were 0.07716 mg/kg/day, 0.05505 mg/kg/day and 0.03676 mg/kg/day respectively which are also higher than the unacceptable limit (>10-4).The cumulative cancer risk for multiple metals (CHR) for Pb, Cd and Cr of three locations, Jikragacha, Monirampur and Sorsha of Satkhira district were 0.0265 mg/kg/day, 0.0265 mg/kg/day and 0.0303 mg/kg/day respectively which are also higher than the unacceptable limit (>10-4).The cumulative cancer risk for multiple metals (CHR) for Pb, Cd and Cr of three locations, Koyra, Paikgacha and Dumuriaha of Satkhira district were 0.02608 mg/kg/day, 0.02997 mg/kg/day and 0.02534 mg/kg/day respectively which are also higher than the unacceptable limit (>10-4).

Following this study, heavy metals may pose a carcinogenic and non-carcinogenic health hazard to the consumer vegetables produced in different of this districts. So step should be needed to alleviate heavy metal pollution in the vegetable fields to protect the consumer’s health.

CHAPTER VI
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CHAPTER VII

APPENDICES

Appendix I. Amount of Pb concentration in different vegetables collected from study areas

	Satkhira			**Jashore**			**Khulna**		
Sample	Asasuni	Kaliganj	Shyamnagar	Jikragacha	Monirampur	Sorsha	Koyra	Paikgacha	Dumuria
Spinach	6.79	7.43	7.28	7.18	3.69	5.39	3.44	1.65	1.47
Tomato	3.82	5.23	4.34	0.54	1.57	0	0	0.35	0
Calabash	5.43	0.51	2.83	2.92	4.76	0	1.13	1.73	0
Couliflower	5.61	6.57	5.11	4.22	2.27	0	0	0	0
Kohlrabi	7.18	5.26	4.19	4.41	2.87	0.74	1.72	0	0
Cabbage	3.09	4.94	8.07	4.4	4.02	3.76	0.52	2.67	0
Red Amaranth	4.11	3.03	0.32	2	2.24	0	0.58	0	0
Bean	8.34	5.91	3.78	2.86	2.41	0.23	3.29	0	1.66

Appendix II. Amount of Cd concentration in different vegetables collected from study areas

	Satkhira			**Jashore**			**Khulna**		
Sample	Asasuni	Kaliganj	Shyamnagar	Jikragacha	Monirampur	Sorsha	Koyra	Paikgacha	Dumuria
Spinach	9.34	6.62	5.27	2.97	3.67	4.19	4.31	3.99	4.26
Tomato	3.91	4.57	2.18	2.79	3.19	3.81	2.81	2.69	3.37
Calabash	2.41	2.41	1.81	2.18	2.75	3.02	2.96	2.51	2.87
Couliflower	2.45	2.67	2.37	2.35	2.58	2.76	2.61	2.81	2.89
Kohlrabi	2.49	0.23	2.04	2.33	2.61	2.68	2.35	2.75	0.21
Cabbage	2.13	2.73	2.25	2.29	0.28	2.74	0.29	3.42	3.18
Red Amaranth	1.59	2.04	0.26	0.17	2.73	2.13	2.79	2.38	0.27
Bean	1.79	2.27	1.59	1.18	2.23	2.12	2.03	2.73	2.69

Appendix III. Amount of Cr concentration in different vegetables collected from study areas

	Satkhira			**Jashore**			**Khulna**		
Sample	Asasuni	Kaliganj	Shyamnagar	Jikragacha	Monirampur	Sorsha	Koyra	Paikgacha	Dumuria
Spinach	9.45	0.36	2.16	1.83	0.08	0	0	0	0
Tomato	0.29	4.08	1.65	1.08	0	0	0	0	0
Calabash	3.28	2.45	1.93	0	0	0	0	0	0
Couliflower	3.89	0.22	0	0	0	0	0	0	0
Kohlrabi	3.28	3.15	1.13	0	0	0	0	0	0
Cabbage	1.15	2.36	0.85	0	0	0	0	0	0
Red Amaranth	0.41	0	0	0	0	0	0	0	0
Bean	3.49	1.53	0	0	0	0	0	0	0

Appendix IV. Amount of Cu concentration in different vegetables collected from study areas

	Satkhira			**Jashore**			**Khulna**		
Sample	Asasuni	Kaliganj	Shyamnagar	Jikragacha	Monirampur	Sorsha	Koyra	Paikgacha	Dumuria
Spinach	15.32	19.88	19.01	19.43	20.25	20.57	16.72	15.58	15.96
Tomato	15.51	25.55	21.17	17.16	20.23	20.43	11.51	4.16	17.08
Calabash	19.3	22.77	9.87	13.71	15.2	18.79	17.32	16.18	16.36
Couliflower	6.34	3.83	4.62	10	8.09	7.92	6.34	7.47	8.49
Kohlrabi	2.36	1.71	0.26	5.12	7.74	9.52	5.94	6.38	4.81
Cabbage	1.56	5.39	8.67	8.74	8.17	7.59	0.91	8.32	8.21
Red Amaranth	10.27	0.48	3.32	0.58	4.34	4.85	5.03	6.76	6.04
Bean	3.18	11.77	9.41	8.86	12.74	11.85	11.82	14.14	11.47

Appendix V. Sediment pollution Index (SPI) of different study areas

	Spinach	Tomato	Calabash	Couliflower	Kohlrabi	Cabbage	Red Amaranth	Bean
Asasuni	258.98	116.91	104.41	106.95	123.09	74.04	73.34	120.80
Kaliganj	207.04	146.11	54.93	119.29	58.61	105.16	71.11	105.46
Shyamnagar	179.32	88.25	65.59	98.62	83.20	126.29	8.48	69.84
Jikragacha	132.18	62.10	73.14	89.45	90.83	90.02	23.41	52.42
Monirampur	110.43	80.01	102.98	74.50	81.09	46.00	77.11	69.02
Sorsha	137.83	76.71	60.87	55.40	61.24	92.59	42.72	45.00
Koyra	120.69	56.49	70.93	52.36	64.35	11.02	61.73	73.80
Paikgacha	96.34	57.40	67.90	56.39	55.16	95.31	47.77	54.95
Dumuria	99.94	67.83	57.81	58.01	4.32	63.81	5.55	70.69

Appendix VI. Metal pollution Index (MPI) of different study areas

	Spinach	Tomato	Calabash	Couliflower	Kohlrabi	Cabbage	Red Amaranth	Bean
Asasuni	9.40	3.865	4.355	4.75	2.885	1.845	2.85	3.335
Kaliganj	7.03	4.9	2.43	3.25	2.43	3.835	1.26	4.09
Shyamnagar	6.28	3.26	2.38	3.495	1.585	5.16	0.29	2.685
Jikragacha	5.08	1.935	2.55	3.285	3.37	3.345	0.375	2.02
Monirampur	3.68	2.38	3.755	2.425	2.74	2.15	2.485	2.32
Sorsha	4.79	1.905	1.51	1.38	1.71	3.25	1.065	1.175
Koyra	3.88	1.405	2.045	1.305	2.035	0.405	1.685	2.66
Paikgacha	2.82	1.52	2.12	1.405	1.375	3.045	1.19	1.365
Dumuria	2.87	1.685	1.435	1.445	0.105	1.59	0.135	2.175

Appendix VII. Vegetable digestion

Appendix VIII. Vegetable sample extraction

Appendix IX.Data collection from AAS machine